Design, Synthesis and SAR Evaluation of Novel Benzoxa-[2,1,3]-diazole Amino Acid Hydrazides Against *Mycobacterium Tuberculosis*

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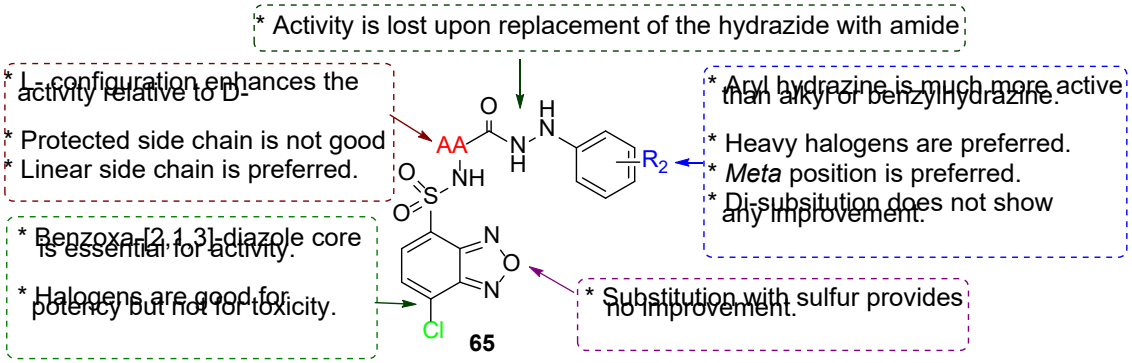
Abstract

The major challenges of tuberculosis (TB) treatment are the emergence of the drug-resistant strains and the higher risk of hepatotoxicity with prolonged treatment. Therefore, efforts to effectively control TB require the discovery and development of new therapeutic options possessing new mechanisms of action. This project focused on the design and synthesis of novel agents targeting *Mycobacterium tuberculosis* (*Mtb*). We have successfully synthesised 170 hydrazides with 40 of those intermediates being converted to benzoxa-[2,1,3]-diazole substituted amino acid hydrazides **65**. The resulting compounds were screened against susceptible and resistant *Mtb* strains utilising a Resazurin Microtiter Assay (REMA). A subsequent structure activity relationship (SAR) strategy investigated the structural modification of the benzo-[2,1,3]-diazole peptidomimetics architecture **65** as the main focus of the research presented in this thesis. The findings from this SAR study indicate that an increased size of the amino acid side chain, incorporation of heavy halogens at the *meta* position of the aryl hydrazine, the L-configuration of the amino acid and the benzoxa-[2,1,3]-diazole moiety each play a key role in improving the antitubercular activity.



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List of Publications arising from this research

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2. Brown, A. K.; Aljohani, A. K. B.; Gill, J. H.; Steel, P. G.; Sellars, J. D. Identification of Novel Benzoxa-[2,1,3]-Diazole Substituted Amino Acid Hydrazides as Potential Anti-Tubercular Agents. *Molecules*, **2019**, *24* (4), 811. **(Chapter 2 and 3)**

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Abbreviations

SAR	structure activity relationship
REMA	Resazurin Microtiter Assay
WHO	World Health Organisation
PCR	Polymerase chain reaction
MDR-TB	Multi drug-resistant tuberculosis
XDR-TB	Extensively drug-resistant tuberculosis
DOTS	Directly Observed Therapy Short-course
AG	Arabinogalactan
AMPs	Antimicrobial peptides
ATP	Adenosine triphosphate
AP	Atmospheric Pressure
Aq	Aqueous
Ar	Aromatic
BCG	Bacillus Calmette–Guérin
Bn	Benzyl
br	Broad
°C	Degrees Celsius
$CDCl_3$	Deuterated chloroform
CI	Chemical Ionisation
cm	Centimetres
d	Doublet
DCM	Dichloromethane
dd	Doublet doublet
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EI	Electron Ionisation
EMB	Ethambutol
eq	Equivalents
ESI	Electroscopy
EtOAc	Ethyl acetate
EtOH	Ethanol
Et_2O	Diethyl ether
g	Grams
h	Hours
HIV	Human Immunodeficiency Virus
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
IR	Infrared
INH	Isoniazid
LAM	Lipoarabinomannan
LC-MS	Liquid chromatography mass spectrometry
LTBI	Latent TB infection
FDA	Food and Drug Administration
J	Coupling constant
$MgSO_4$	Magnesium sulphate
M	Molar

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m	Multiplet
mins	Minutes
MIC	Minimum Inhibitory Concentration
Mg	Milligram
µg	Microgram
mL	Milliliter
MHz	Megahertz
mmol	Millimolar
m.p.	Melting Point
Mtb	*Mycobacterium tuberculosis*
$NaHCO_3$	Sodium bicarbonate
NH_4Cl	Ammonium chloride
NMR	Nuclear Magnetic Resonance
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
ppm	Parts per million
Ph	Phenyl
q	Quartet
Rf	Retention Factor
RIF	Rifampicin
RNA	Ribonucleic acid
Rt	Room Temperature
s	Singlet
t	Triplet
TB	Tuberculosis
THF	Tetrahydrofuran
DIPEA	*N*,*N*-diisopropylethylamine
TEA	Triethylamine
DCC	*N*,*N'*-dicyclohexylcarbodiimide
HOBt	1-hydroxybenzotriazole
DMTMM	4-4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
MeCN	Acetonitrile
ATP	Adenosine triphosphate
TST	Tuberculin skin test
TLC	Thin Layer Chromatography
δ	Chemical shift
°	Degrees

Originality Statement

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at University of Newcastle or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by others, with whom I have worked at University of Newcastle or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.'

Signed: **Ahmed Khalaf B. Aljohani**

Date: **2nd January 2022**

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1 Introduction

This thesis describes research concerning i.) design of new chemical entities with promising antitubercular activity and selectivity towards *Mtb*[1,2], ii.) the synthesis of a new library of novel benzoxa-[2,1,3]-diazole substituted amino acid hydrazides **65**[1,2] and iii.) structure activity relationship studies of novel benzoxa-[2,1,3]-diazole peptidomimetics **65**[1,2]. The following section will focus on the epidemiology of the bacteria, with emphasis on background, recent epidemiological reports, resistance and treatment. Section 2 will present the successful synthesis and characterisation of novel antitubercular agents based on the benzoxa-[2,1,3]-diazole substituted amino acid hydrazides **65**, with Section 3 presenting current progress towards our SAR investigations. The penultimate Section 4 will highlight the findings obtained from both Sections 2 and 3; the synthesis and SAR analysis of the resulting intermediates and final compounds. Finally, Section 5 will conclude the work presented by looking at prospects for future exploitation of the results arising from this thesis. Section 6 will detail the experimental procedures.

1.1 Tuberculosis epidemiology

According to the World Health Organisation's Global Tuberculosis Report 2019, Tuberculosis (TB) is recognised as a communicable disease, is responsible for a major cause of ill health and is one of the top ten causes of death worldwide from a single infectious agent.[3] In 2018, there were an estimated ten million people (range 9.0-11.1 million) reported to have become infected with TB, which is equivalent to 132 cases per 100 000 population, whilst ten percent of these cases were associated with other co-morbid disease such as HIV/AIDS. In addition, there are high mortality rates caused by TB with 1.2 million HIV negative patient deaths and 251,000 HIV positive patient deaths reported in 2018.[3] Globally, the estimated number of new cases of TB has been relatively stable at around 10 million per year since 2014, whilst the HIV-positive TB incidence rates have increased to nearly double. Contrary to this, the mortality rates of HIV-positive TB cases has steadily declined by 64% (Table 1).[3,4,5,6,7]

Table 1: Global incidence, HIV/TB co-infection and Mortality of TB in last 5 years.[3,4,5,6,7]

Year of incidence	TB Incidence	HIV-pos. TB Incidence	Mortality	
			HIV-neg.	HIV-pos.
2014	9.6 mil	1.2 mil	1.1 mil	390,000
2015	10.4 mil	1.2 mil	1.4 mil	400,000
2016	10.4 mil	1.0 mil	1.3 mil	374,000
2017	10.0 mil	920,000	1.3 mil	300,000
2018	10.0 mil	862,000	1.2 mil	251,000

Geographically, in 2018 the WHO identified the global regions worst affected by TB as South-East Asia, Africa and the Western Pacific, with countries such as India characterised as the highest TB burden country (27%, 199,000) followed by China (9%, 610,000), Indonesia and the Philippines (6%, 554,000) amongst others (Table 2).

Table 2: Global incidence, HIV/TB co-infection and Mortality of TB.[3]

WHO region	TB Incidence*		HIV-pos TB Incidence	Mortality	
		% of Global total		HIV-neg.	HIV-pos.
Africa	2.45 mil	24%	615,000	397,000	211,000
The Americas	289,000	3%	29,000	17,000	5,900
Eastern Mediterranean	810,000	8%	6,900	77,000	2,200
Europe	259,000	3%	30,000	23,000	4,400
South-East Asia	4.37 mil	44%	140,000	637,000	21,000
Western Pacific	1.84 mil	18%	41,000	90,000	6,500
Global total	10 mil	100%	862,000	1.24 mil	251,000

* Incidence is a number of new cases arising during a defined period.

Many studies have revealed the emergence of multi drug-resistant tuberculosis (MDR-TB) is clearly linked to mismanagement of front line treatments for drug-sensitive TB.[8,9] Additionally, structural weaknesses in health care systems and socioeconomic factors are associated with the sharper rise of MDR-TB, for example, repeated use of short-course TB treatments, inadequate dosing, inconsistent drug supply and poor patient adherence due to complicated regimens and prolonged treatment periods.[9,10] In addition, studies have revealed the emergence of primary MDR-TB infection, arising in areas with poor TB control programmes and community transmission, which further complicates and hampers the global control of TB.[8,10]

For these reasons, rapid detection and appropriate therapeutic plans are essential to save the lives of TB patients and preclude the spread of MDR-TB.

One such diagnostic test is the drug susceptibility test, or phenotypic method, which is used to diagnose MDR-TB or XDR-TB. It is undertaken by culturing *Mtb* isolated from the sputum of an infected person in the presence of a front-line TB treatment, such as isoniazid, however, this method is time consuming and expensive. More recently, a fully automated test has been devised which utilises a polymerase chain reaction (PCR) based technique to identify gene mutations, arising from resistance markers, in *Mtb* cultured from an infected individual.[10]

With detection technology improving and becoming quicker and cheaper, the reported incidence rates have increased in countries including India, the Russian Federation and China which have contributed to an estimated 45% of the global total MDR-TB cases in existence in 2018, with an estimated 484,000 new MDR and rifampicin resistant (RR) TB cases reported (Figure 1).[3] In order to control and reduce incidence numbers, the design of an appropriate MDR-TB management plan is essential, especially in high MDR-TB burden countries.[9] These treatment plans can include: utilising an assured quality treatment and optimised individualised treatment regimens based on drug susceptibility testing results and patient medical history to enhance the cure rate of MDR-TB.[9]

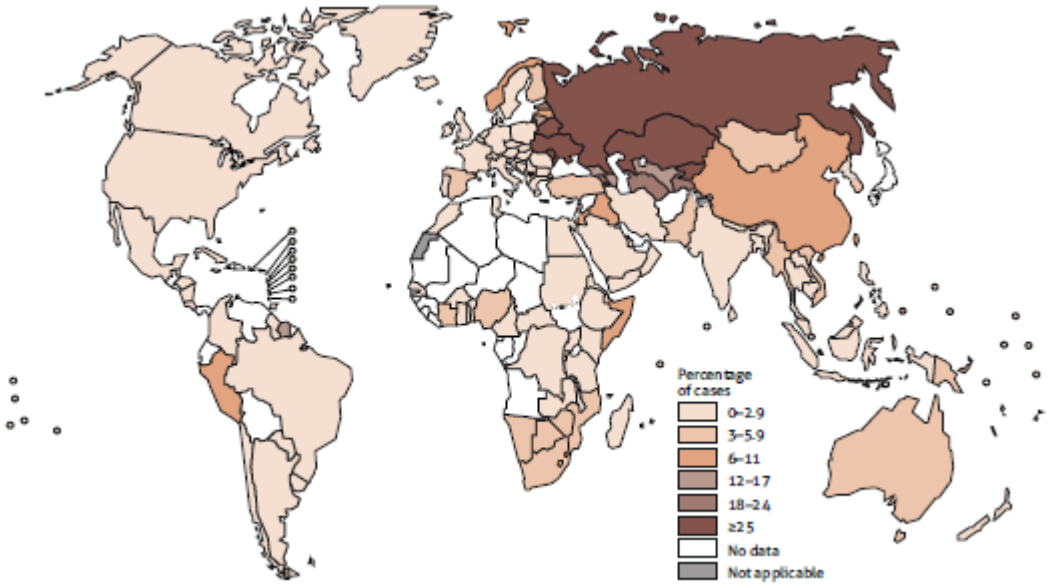


Figure 1: The global percentage of new MDR/RR-TB cases (Adapted from *Global tuberculosis report, 2019* [3])

Alongside MDR-TB, cases are extensively drug-resistant TB (XDR-TB) which continues to add to the patient burden. Brought about predominantly by a lack of drug management, bacteria which are extensively resistant do not respond to any of the antitubercular agents, hence the treatment regimen for XDR-TB is limited. In 2019, WHO reported that XDR-TB cases totalled 13,068, arising mainly in India, China and Russia (Table 3).[3] WHO also declared that the treatment success rate reached by only 39% for existing patients with XDR-TB, therefore, XDR-TB is considered a growing challenge in the controlling of TB around the world.[3]

Table 3: Global MDR/RR-TB incidence, and XDR-TB Incidence.[3]

	MDR/RR-TB Incidence		**XDR-TB Incidence**	
WHO region		% of Global Total		% of Global Total
Africa	24,712	13%	727	6%
The Americas	4,759	3%	149	1%
Eastern Mediterranean	5,584	3%	122	1%
Europe	48,739	26%	7,899	60%
South-East Asia	75,964	41%	3,580	27%
Western Pacific	27,014	14%	591	5%
Global total	186,772	100%	13,068	100%

Consequently, the growing spread of XDR-TB has increased the demand even further for significant development of new treatments for TB, with new mechanisms of action.

1.2 Mycobacterium Tuberculosis

1.2.1 Life cycle of TB

In 2019, the World Health Organisation (WHO) estimated that nearly a third of the earth's population is infected with *Mycobacterium tuberculosis* (*Mtb*) based on the community spread of pulmonary TB infection, mostly in developing countries.[3] Infection with *Mtb* usually begins when an exposed individual inhales contaminated water droplets containing *Mtb* which lodge in the pulmonary alveoli, where it is primarily phagocytosed by alveolar macrophages. After macrophage uptake, a long-standing *Mtb* - macrophage interaction is developed and leads to one of three outcomes: *Mtb* eradication (Route A), active infection (Route B) or dormancy (Route C) (Figure 3).[11]

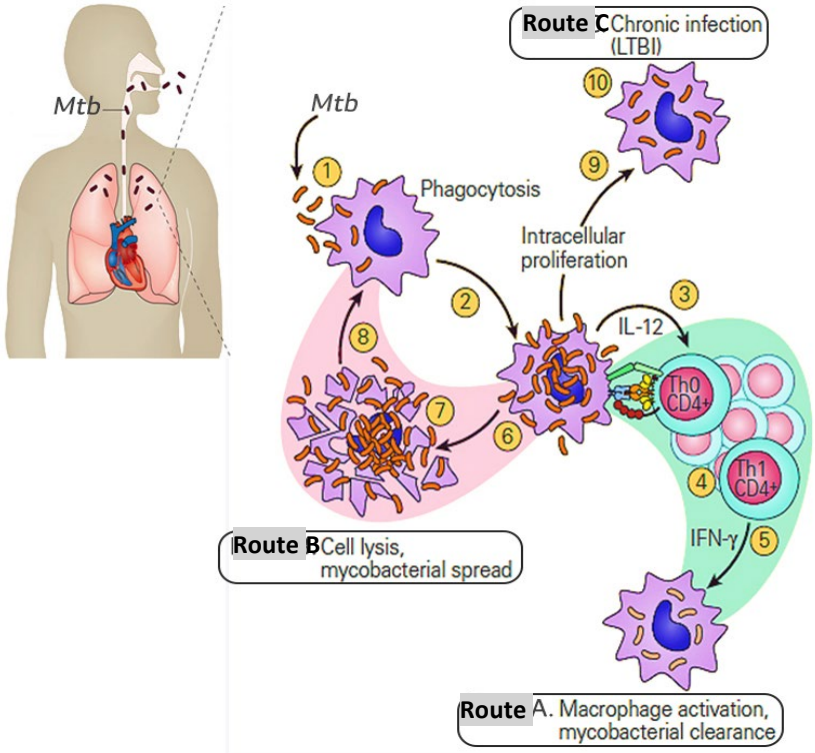


Figure 3: The three possible outcomes of *Mtb* - macrophage interaction (Adapted from Russell *et al.* 2010 [12])

Because of the broad and quick effect, an innate immune system is capable of preventing infection and microbial spread in the body. For instance, a strong and rapid pulmonary immune response is mainly responsible for the ability of the host to eradicate *Mtb* (Route A, Figure 3).[12] During the initial interaction between *Mtb* and the immune cells, macrophages release pro-inflammatory cytokines to trigger recruiting additional immune cells including dendritic cells, monocytes and neutrophils into the site of infection. Then, migration of infected dendritic cells to adjacent lymph nodes, where rapid expansion occurs in order to fight the infection.[13] Upon Interleukin-12 (IL-12), 18 (IL-18) and 27 (IL-27) production by infected cells, natural killer and T cells become active and directly exert cellular cytotoxicity through releasing perforin and granzyme or granulysin. They also have an indirect role to control intracellular infection by secreting interferon-γ (IFN-γ) which is essential for macrophage activation.[14] Once activated, they release tumour necrosis factor-α (TNF-α) to exert their antimicrobial activity via a variety of mechanisms including phagosomal acidification, production of antimicrobial effectors (nitric oxide and reactive oxygen intermediates) and apoptosis of infected macrophages.[13]

Notwithstanding this, the susceptibility of patients to contracting TB with co-morbidities and or who are immunocompromised with, for example HIV, is much greater in these populations when compared against the general population. This synergic effect is due predominantly to a decrease the $CD4^+$ effector T-cells which are responsible for the antimicrobial activities of infected phagocytes. Consequently, several studies have reported the higher TB mortality rate amongst HIV-positive patients.[15] Nevertheless, the emergence of active TB occurs among small groups of healthy people (5 – 10%) due to the increase in inhibitory cytokine (Interleukin-10) levels, which alters the balance between inflammatory and immunopathological responses. Higher level of Interleukin-10 can suppress the antimicrobial mechanisms in the macrophage through inhibiting secretion of pro-inflammatory mediators (IFN-γ, TNF-α and IL-12) and hence, associated with uncontrolled intracellular *Mtb* replication. *Mtb* is characteristically capable of surviving and replicating within the host macrophage cells and can reach a point where the bacterial load becomes too great although all the required cellular immune components exist. At this threshold, cell death occurs and *Mtb* will be disseminated eventually through the respiratory tract and is characterised

as active pulmonary TB (Route B, Figure 3). Additionally, *Mtb* can enter the bloodstream to infect other organs, leading to other forms of TB infection.[16]
Having said that, the majority of infected individuals remain healthy and harbour the infection in a latent form during their lifetime, whilst they can develop TB at some point, 90% of the cases remain dormant. Dormancy occurs when the host immune system fails to eliminate the bacteria completely, and consequently a sequence of events primes the immune system to recruit the required components to form a granuloma (Route C, Figure 3). By reducing their metabolic activity, *Mtb* can adapt to this intracellular environment present in the granuloma where hypoxia, acidity and low levels of nutrients exist.[12] In this stage, the granuloma can segregate and prevent spreading of the infection into the airways, and hence, an equilibrium between *Mtb* and host contributes to establishment of the dormancy state. However, if the individual becomes immunocompromised, the equilibrium will shift towards the dormant *Mtb* which can regrow and then induce the granuloma to rupture, which leads to spreading viable *Mtb* into the whole body and lung in particular, thereby developing the reactivation.[12] If untreated, half of those active TB cases die. Thus, the development of new anti-mycobacterial agents faces a significant challenge because of low permeability of both the mycobacterial cell wall and the latency of TB in granuloma's.

1.2.2 The structure of mycobacterial cell wall

Pulmonary TB infection is caused by *Mtb*, which is a member of the mycobacteriaceae species. *Mtb* is usually defined by its cell wall which has characteristics of both gram-negative and gram-positive bacteria. As gram-positive bacteria contain several layers of peptidoglycan interspersed with unique teichoic acids whilst gram negative bacteria are characterised by a thin layer of peptidoglycan surrounded by an outer membrane of lipoprotein (Figure 4).[17] Moreover, *Mtb* cell wall is considered a major determinant of virulence, and thus, it deserves more attention.

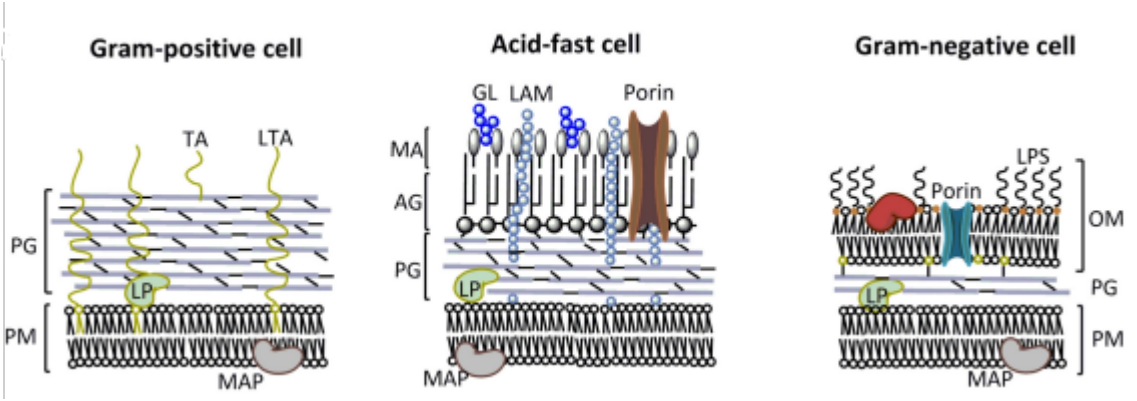


Figure 4: Differences in cell wall structure of the gram-positive, mycobacteria and gram-negative bacteria. Arabinogalactan (AG); glycolipid (GL); lipoarabinomannan (LAM); lipoprotein (LP); lipopolysaccharide (LPS); lipoteichoic acid (LTA); mycolic acid (MA); membrane-associated protein (MAP); outer membrane (OM); peptidoglycan (PG); plasma membrane (PM); teichoic acid (TA) (Adapted from Fullam *and* Young, 2021.[18])

Structurally, the cell wall of mycobacteria is comprised of an asymmetric bilayer which surrounds the plasma membrane (Figure 5). The unique inner compartment of the *Mtb* cell wall has a characteristic hydrophobic fraction (over 60% of cell wall content as compared to 20% in gram-negative bacteria) possessing cord factor and wax-D and mycolic acids which act as stimulators to the host's immune system. The outer mycolic acids and multi-layered peptidoglycan covalently join to the arabinogalactan, a branched polysaccharide, to form the mycolic acid-arabinogalactan-peptidoglycan complex (MAPc). This complex is essential for the viability and the persistence of *Mtb*, particularly, in the latency stage as *Mtb* can survive inside of macrophages whilst also conferring distinctive immunostimulatory properties.[19,20] Interestingly, the outer membrane, known as the capsule, can be impermeable to antimicrobial agents used for gram negative bacteria. In addition to saturated lipopolysaccharides, the capsule contains proteins, glycoconjugates and high content of lipoglycans including phosphatidyl-*myo*-inositol mannosides, lipomannan and lipoarabinomannan in particular.[21]

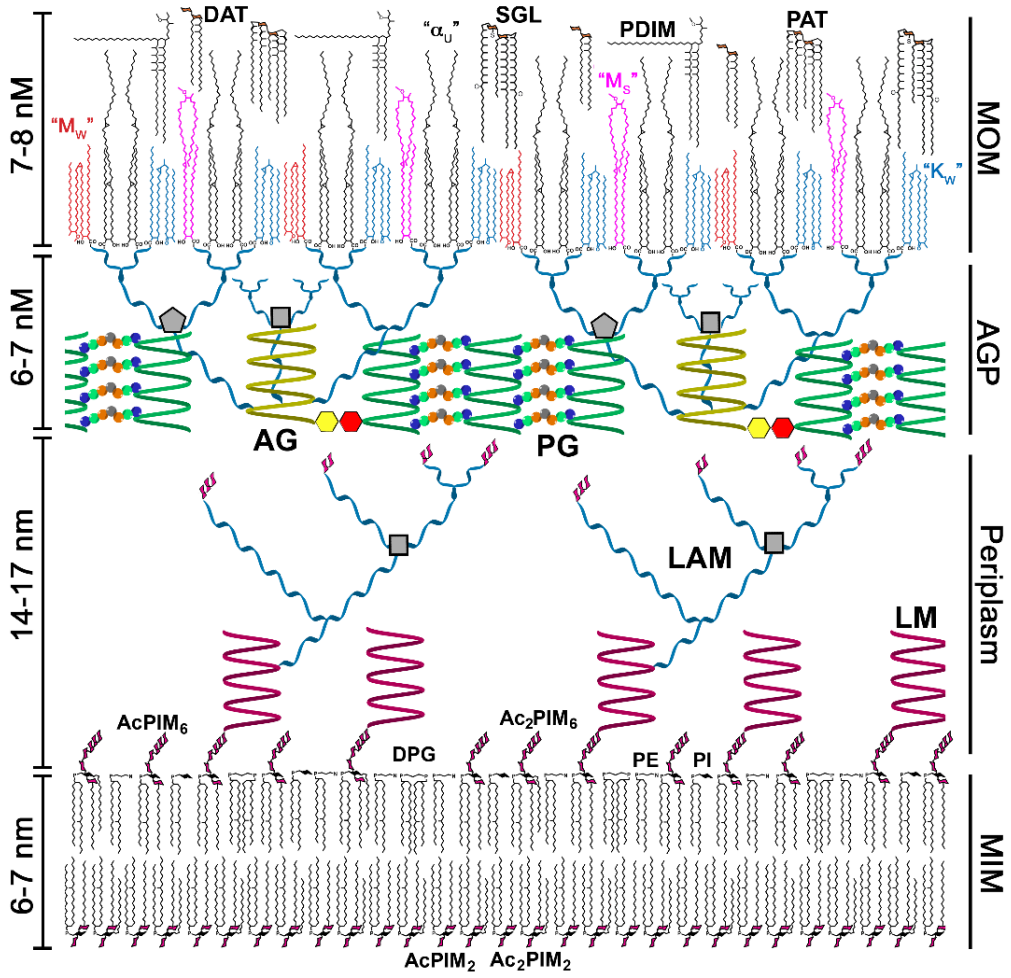


Figure 5: The cell wall structure of mycobacterial species. Phosphatidyl-myo-inositol mannosides (PIMs); lipomannan (LM); lipoarabinomannan(LAM); mannosylated lipoarabinomannan (ManLAM), peptidoglycan (PG); arabinogalactan (AG); mycolic acids (MA); trehalose monomycolate (TMM); trehalose dimycolate (TDM); diacyltrehalose (DAT); polyacyltrehalose (PAT); phthiocerol dimycocerosate (PDIM); sulfoglycolipid (SGL); mycobacterial outer membrane (MOM); arabinogalactan–peptidoglycan (AGP); mycobacterial inner membrane (MIM); diphosphatidylglycerol (DPG); phosphatidylethanolamine (PE); phosphatidylinositol (PI).

The mycobacterial peptidoglycan is made of repeating *N*-acetylglucosamines joined to *N*-acetylmuramic acid via peptide bonds situated on the plasma membrane. It serves to maintain cell wall rigidity, integrity and for virulence. Consequently, it is an excellent target for antibacterial drug discovery.[22]

Another essential component for the cell wall integrity is arabinogalactan which is composed of branched arabinans and linear galactan. Moreover, it is essential for mycobacteria replication and survival in the infected host as it links both the peptidoglycan and the mycolic acid to form an integral arrangement of the cell

wall via a phosphoryl-N-acetylglucosaminosyl-rhamnosyl and arabinan anchors, respectively.[22]

Mycolic acids are a remarkable macromolecular component of the mycobacterial cell which shield the bacteria from environmental stress such as antimicrobial pressure or adaptive immune responses. They also play a major role in regulating the permeability, acid-fast staining, viability and *Mtb* virulence. Mycolic acids are a strong hydrophobic component containing long chain fatty acids synthesised by two fatty acid synthase (FAS) systems which are attractive targets for the rational design of new antitubercular agents (Figure 6).

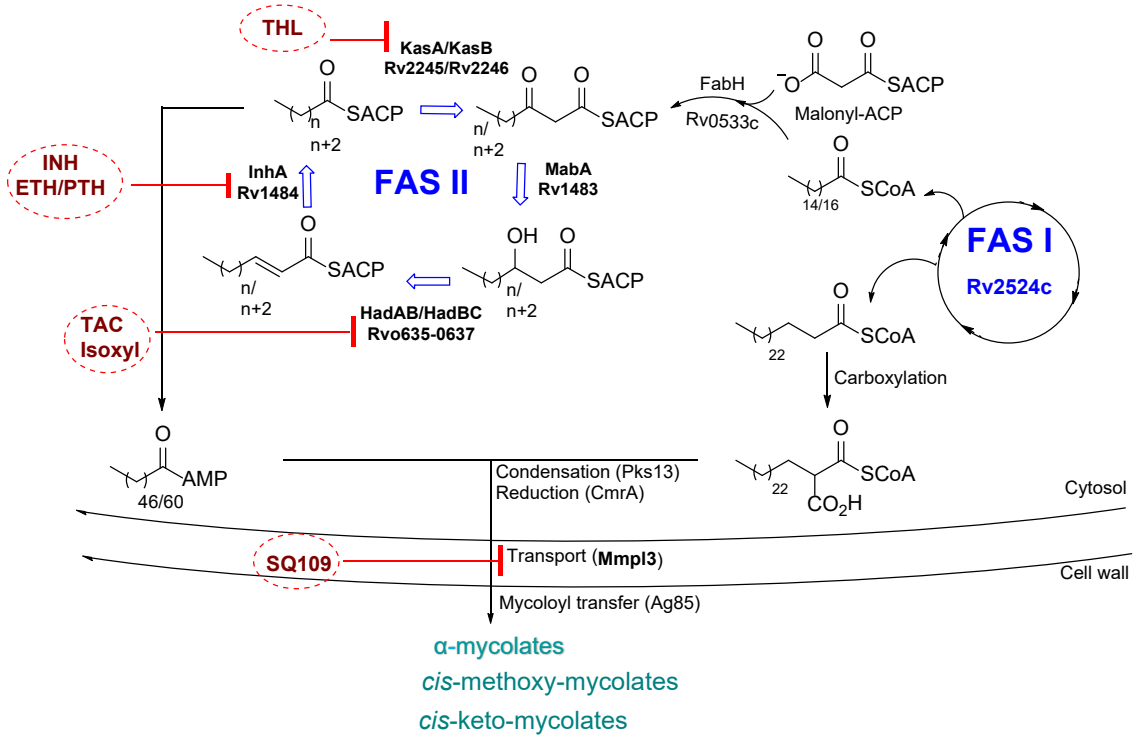


Figure 6: A general description of mycolic acid biosynthesis which can be targeted by different antitubercular agents. Isoniazid (INH) and thioamides (ETH/PTH) are InhA inhibitors; thiolactomycin (THL) is KasA inhibitor; thioacetazone (TAC) and isoxyl are dehydration step inhibitors and SQ109 inhibits the transport activity of MmpL3. (Adapted from Vilchèze, 2020 [22])

FAS 1 produces medium chain fatty acid (C20-C26), while FAS 2 produces a long meromycolic chain (C60-C90) with some modifications (*cis*/*trans* cyclopropanations, keto or methoxy groups) which are introduced during an elongation process.[22]

Phosphatidyl-*myo*-inositol mannosides (PIMs) are unique glycolipids which can interfere with proximal T-cell receptor signalling, thereby inhibiting $CD4^+$ effector T-cell activation. They are not only important in cell wall permeability and integrity, but also essential in the regulation of cell septation and division.[22]

Lipomannan and lipoarabinomannan are glycolipids. In addition to their role in the integrity of cell wall, they show a pathogenic role when they release in the macrophage as they exert different immunomodulatory activities including inhibiting phagosomal maturation and shifting the cytokine response from pro- to anti-inflammatory.[24]

The complexity of the mycobacterial cell wall structure provides for bacterial survival in harsh conditions, along with a strong impermeability to several antimicrobial agents, particularly β-lactams (penicillins and cephalosporins) due to several factors such as the presence of β-lactamase and low affinity penicillin binding proteins.[25] It is also associated with mycobacterial pathogenicity and hence it remains a component of interest for developing novel antimycobacterial agents which target crucial enzymes involved in mycobacterial cell wall biosynthesis. In this context, it is comprised of three main components which are common target sites for attack by current antitubercular agents (Figure 7).[22] More importantly, the cell wall inhibitors are targeting novel protein targets, including DprE1 and MmpL3 which were discovered by a drug to target screening strategy, in addition to well-known targets (InhA and peptidoglycan synthesis).[26] Recently published a study highlighting the ability of the mycobacterial cell wall inhibitors such as isoniazid **1** to induce the bactericidal activity against dormant *Mtb*. So, the resistance may be some other mechanism.[27]

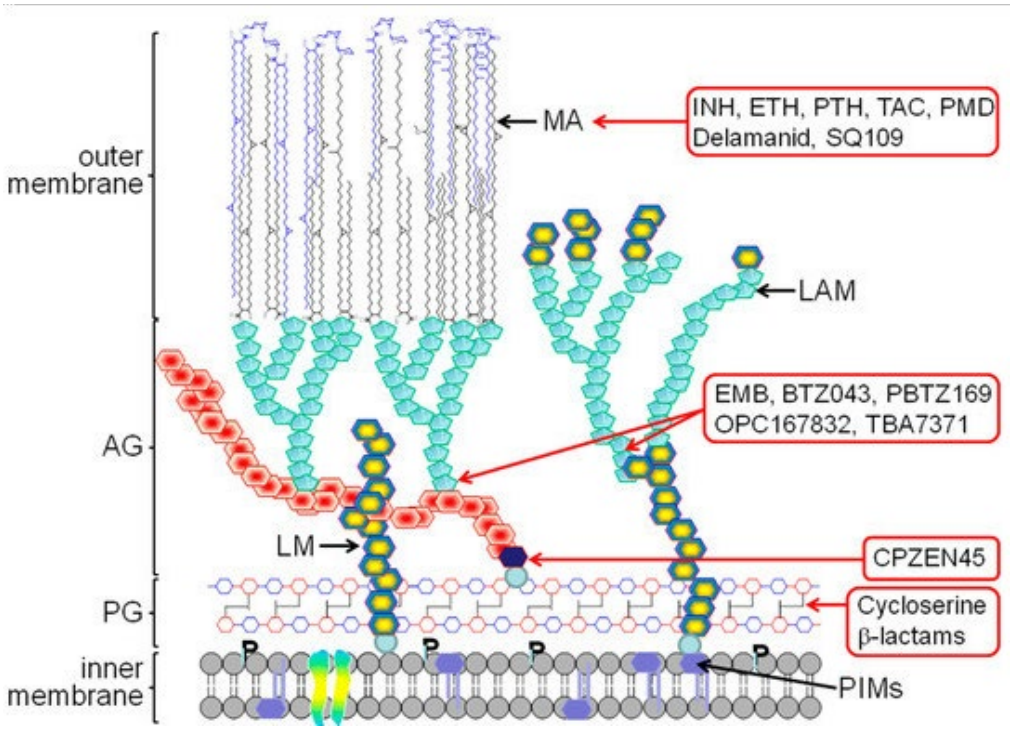


Figure 7: The main components of *Mtb* cell wall including Phosphatidyl-myo-inositol mannosides (PIMs); lipomannan (LM); lipoarabinomannan(LAM), peptidoglycan (PG); arabinogalactan (AG); mycolic acids (MA) are targeted by antitubercular agents. (Adapted from Abrahams and Besra, 2018 [22])

1.3 Anti-tubercular Agents and Their Targets

Notwithstanding the issues arising from *Mtb* epidemiology, TB is recognized as a treatable infection, as according to the World Health Organisation's Global Tuberculosis Report 2019, an estimated 58 million cases (2000 – 2018) were treated successfully. An early effective therapeutic intervention for TB is the use of the Bacillus Calmette–Guérin (BCG) vaccine in children. This has demonstrated excellent results, however it does not provide total protection.[3]

1.3.1 Vaccination

The utilisation of vaccination to improve the protective immunity against TB has a major role to play in order to interrupt *Mtb* transmission and ultimately to eradicate TB. In the 19th century, the Bacillus Calmette-Guerin (BCG) vaccine which is a live attenuated form of *M. bovis*, was introduced as a part of the TB control program.[28] Since the introduction of BCG vaccine, around 4 billion people around the world have been vaccinated, with an efficacy from 0 – 80% in children up to the age of 16 compared to adults when administered later in life. Unfortunately, this variation in BCG efficacy has emerged because of the genetic

and nutritional differences among human populations.[28] Additional factors are the variation in BCG strains and prior exposure to environmental mycobacteria which could be detected using the tuberculin skin test (TST) especially in endemic areas.[28] The immunisation route can also influence the protective effect of BCG and hence the mucosal route is more efficient to provide a high level of protection against disseminated and pulmonary TB with an accelerated production of IFN-γ, compared to intravenous route.[29] However, oral BCG vaccination was initially used, until 73 accidental deaths occurred in Lübeck, Germany following orally administrated BCG vaccine which was contaminated with virulent *Mtb*[30] so, the vaccine is now administered via the intradermal route.

Notwithstanding this, the BCG vaccine provides incomplete protection against the development of adult pulmonary TB because this age group of adults is associated with higher risk of prior exposure with environmental mycobacteria. In 2002, Brandt L. *et al.* demonstrated the negative impact of sensitization with environmental mycobacteria on the protective efficacy of BCG vaccine against pulmonary TB as sensitized mice with cocktails of mycobacteria initiated antimycobacterial responses which inhibit the multiplication of BCG and reduce its activity.[31] Furthermore, the lack of evidence for long-term BCG immunisation could also contribute to ongoing incidence of pulmonary TB.[32] Therefore, evaluating the protective efficacy of BCG vaccine against *Mtb* still remains a controversial issue along with the global BCG vaccination policy since the early evaluation of the BCG vaccination.[28]

Notwithstanding the differences in protection and routes of administration, the immunological mechanisms of parenteral BCG and poor lung protection is still under investigation to determine a new strategy to overcome the immunological gap. This gap is believed to arise from a lack of airway luminal anti-TB T cells, which can afford a delay in pulmonary T-cell responses to *Mtb* exposure particularly at the early stages of infection, whilst a large population of anti-TB T-cells, generated by the parenteral BCG immunization, reside in peripheral tissues.[33] Consequently, there is clearly a strong demand for the development of novel and effective TB vaccines against all stages of TB infection in all age groups to reduce the global incidence of TB in general, and drug-resistant TB in particular which remains paramount to achieve the goal of the End TB Strategy by 2035.[3] In order to achieve such MDR-TB elimination, the significant challenge facing TB

vaccine developers is to design a safe and effective TB vaccine to prevent spreading the drug-resistant TB strains which relies on less effective and more toxic TB treatments.[34]

1.3.2 Anti-tubercular drugs

Consequently, treatment with small molecule drugs is necessary with front line treatments such as isoniazid **1**, pyrazinamide **2**, ethambutol **3** or rifampicin **4** (Figure 8).

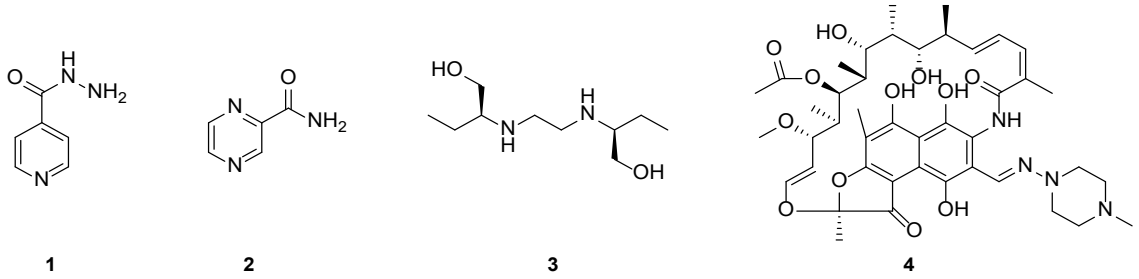


Figure 8: Front line treatments for TB treatment.

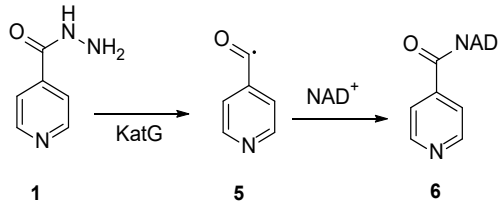
Generally, each anti-tubercular agent has a specific target and mechanism of resistance and can be classified according to their treatment order (Table 4).[3,35,36]

Anti-tubercular agents	Bacterial target	Mechanism of resistance	Treatment order
Isoniazid (INH) **1**	Mycolic acid biosynthesis inhibition	Mutations in InhA, KatG, aphC and Ndh.[37]	Front line
Rifampicin (RIF) **4**	RNA synthesis inhibition	Mutation in rpoB	
Pyrazinamide (PAZ) **2**	Membrane potential disruption	Mutations in pncA	
Ethambutol (EMB) **3**	Arabinogalactan biosynthesis inhibition	Mutation in embB	
Aminoglycosides	Protein synthesis inhibition	Mutation in 16S rRNA	Second line
Fluoroquinolones	DNA synthesis inhibition	Mutation in gyrA and gyrB	
Ethionamide (ETH) **16** / Prothionamide (PTH) **17**	Mycolic acid biosynthesis inhibition	Mutation in ethA and inhA.[37]	
D-Cycloserine (DCS) **18**	Peptidoglycan biosynthesis inhibition	Overexpression of airA	

Para-aminosalicyclic acid (PAS) **20**	Folic acid and iron metabolism inhibition	Mutation in thyA	
Thiacetazone (TAC)	Mycolic acid cyclopropanation inhibition	Mutation in ethA	
Clarithromycin	Protein synthesis inhibition	Mutation in ermA, ermB and ermC	
Bedaquiline (BDQ) **22**	Mycobacterial ATP synthesis inhibition	Mutation in atpE and rv0678	Third line[39]
Delamanid (DLM) **25** / Pretomanid (Pa) **26**	Mycolic acid synthesis inhibition	Mutation in ddn and fdg1	
Clofazimine	Mycobacterial DNA replication inhibition	Mutation in rv0678	
Linezolid (LZD) **23**	Protein synthesis inhibition	Mutation in 23S rRNA	

1.3.3 First-line anti-tuberculosis drugs

The most common treatment of drug-susceptible TB is with isoniazid **1** (INH, known as isonicotinic acid hydrazide), which elicits its anti-mycobacterial activity through inhibiting mycolic acid biosynthesis. This enfeebles the permeability of the mycobacterial cell wall leading to destruction of the cell integrity and ultimately its death.[40] INH **1** has a bactericidal activity against rapidly dividing mycobacteria, but it is not as effective as rifampicin **4** against a non or slow growing mycobacteria, particularly those in granuloma.[41] INH **1** is a prodrug which is converted by Mycobacterial catalase-peroxidase (KatG) to isonicotonic acyl radical **5** which forms a nicotinoyl-NAD adduct **6**. Consequently, disrupting fatty acid chain elongation when the active metabolite binds tightly to the enzyme enoyl acyl carrier protein reductase (InhA) which is a precursor for mycolic acid biosynthesis (Scheme 1).[40]



Scheme 1: conversion of isoniazid **1** to its active metabolite **5** through KatG enzyme.[40]

Rifampicin (RIF) **4** is one member of the front-line TB treatment which is a potent and broad spectrum antibacterial agent. It shows an equivalent level of antimycobacterial activity against both fast and slow growing mycobacteria through supressing the mycobacterial RNA polymerase (RNAP) which is required for transcription. RIF **4** contains naphthoquinones that attach in a pocket of RNAP β subunit through forming hydrogen bonds between hydroxyl groups of naphthoquinone and amino acids of enzyme (Figure 9). This sterically inhibits the process of RNA elongation and thus leads to cell death.[42] Due to the broad spectrum nature of rifampicin **4**, it is usually combined with other antitubercular agents to reduce the chance of resistance. For long-term TB treatment, the use of rifampicin **4** with isoniazid **1** is more common than pyrazinamide **2** due to their potential to cause severe hepatotoxicity.[43]

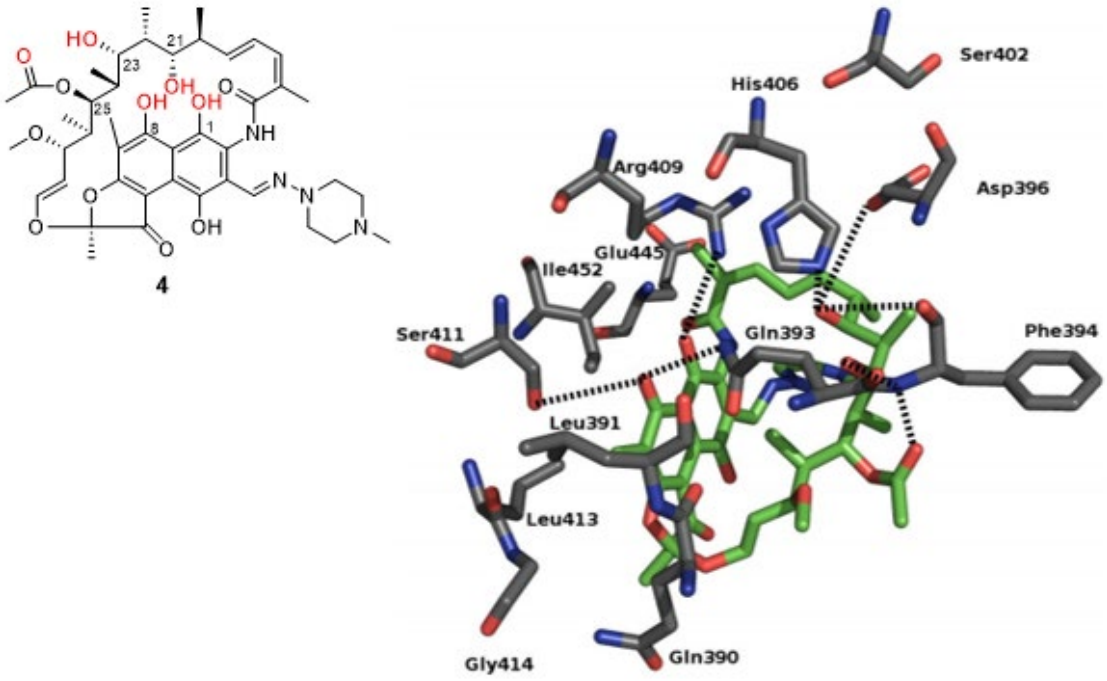


Figure 9: According to the crystal structure of rifampicin **4** in complex with the Thermus aquaticus core DNA dependent RNAP, rifampicin 4 binding involves hydrogen bonding interactions between the hydroxyl groups at C-1, C-8, C-21 and C-23 and the carbonyl oxygen of the C-25 acetoxy group with the amino acid residues Arg409, Ser411, Gln393, His406, Asp396 and Phe394. (Adapted from Campbell *et al,* 2001 [42])

Pyrazinamide (PAZ) **2**, a nicotinamide analogue, is uniquely effective in treating drug susceptible TB and MDR-TB and also reduces the total duration of treatment when used in combination with rifampicin **4** and isoniazid **1**.[44] The clinical trials

which were undertaken in East Africa by the British Medical Research Council tuberculosis units, demonstrated that use PAZ with rifampicin **4** showed a synergetic antimycobacterial effect and shortened TB treatment period from 12 to 9 months. Furthermore, lower doses of pyrazinamide **2** decreased the probability of hepatotoxicity.[45] PAZ **2** is a prodrug and shows powerful sterilizing activity only against non-growing *Mtb* when it is converted to its active form **7** (pyrazinoic acid, POA) by a nicotinamidase-peroxidase enzyme known as pyrazinamidase encoded by the *pncA* gene in the mycolic acids (Figure 10).[44] Due to its activity at acidic pH, PAZ **2** is ideal for killing intracellular *Mtb* that mainly reside within infected macrophages. However, it is only used in the first two months as longer use does not show additional benefit. This is presumably due to increased the acidity in the lesions during acute inflammation, which is decreased after two months treatment.[44]

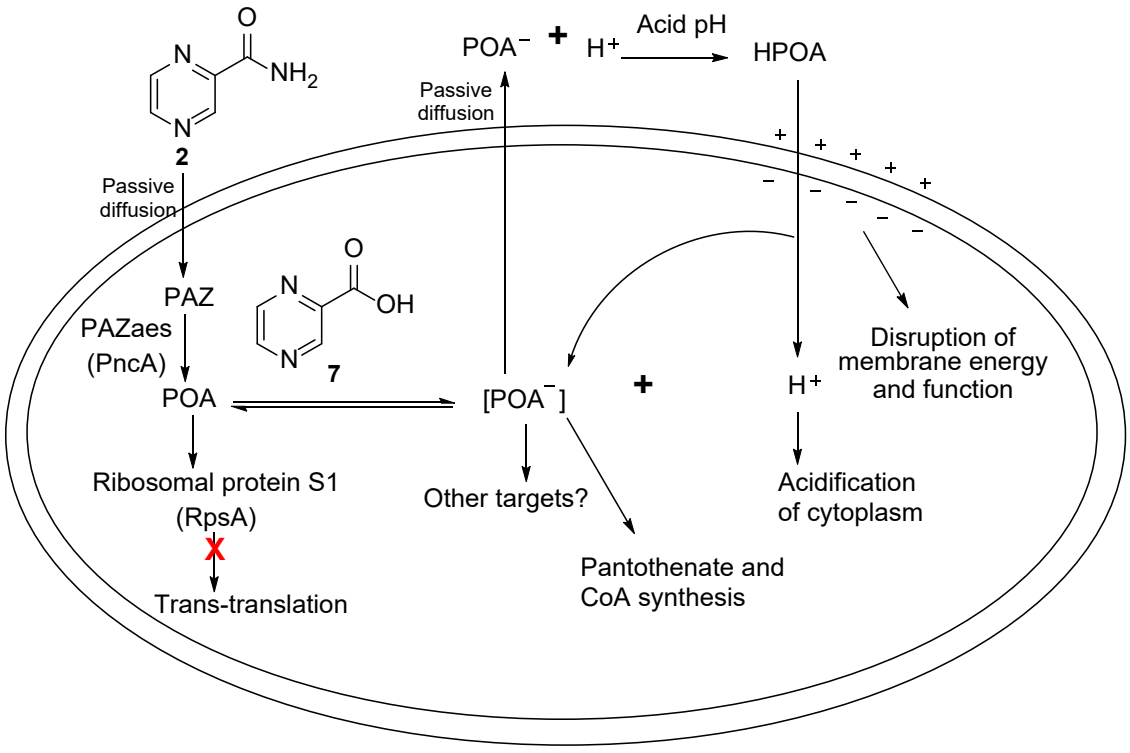


Figure 10: The conversion of pyrazinamide **2** (PZA) to its active pyrazinoic acid **7** (POA) in acidic environment. (Adapted from Zhang *et al,* 2013 [44])

Ethambutol (EMB) **3** is a member of front-line TB treatments that reduce the global burden of TB infection caused by *Mtb* due to its bacteriostatic activity against growing drug susceptible and monoresistant strains. EMB **3** interferes with mycobacterial cell wall biosynthesis by inhibiting the action of arabinosyl

transferase (EmbB) which is involved in the synthesis of arabinan for the arabinogalactan complex. EMB **3** is used in combination with front line TB treatments to improve their potency and reduce the length of TB therapy.[46] Although EMB **3** is effective in preventing the emergence of drug resistance, it is not a preferred TB treatment option in young children due to its ocular toxicity.[35]

1.3.4 MDR-TB

Despite the successes of front-line TB treatments, resistance to one or more of these effective drugs (most often, isoniazid or rifampicin) is a global challenge for TB control. Therefore, the use of second-line TB treatments is an alternative option for MDR-TB cases, but this is challenging as treatments include drugs that are more toxic, expensive and require longer administration, e.g. for at least a 24-month treatment course. Consequently, drug susceptibility testing (DST) for first- and second-line drugs is recommended to design the appropriate MDR-TB treatment regimen and thus improve treatment outcomes.[10]

1.3.5 Second-line antituberculosis drugs

Aminoglycosides

Streptomycin (STR) **8**, kanamycin (KAN) **9**, amikacin (AMK) **10** and capreomycin (CAP) **11** are common second line TB treatments which are used to reduce mortality due to MDR-TB (Figure 11).

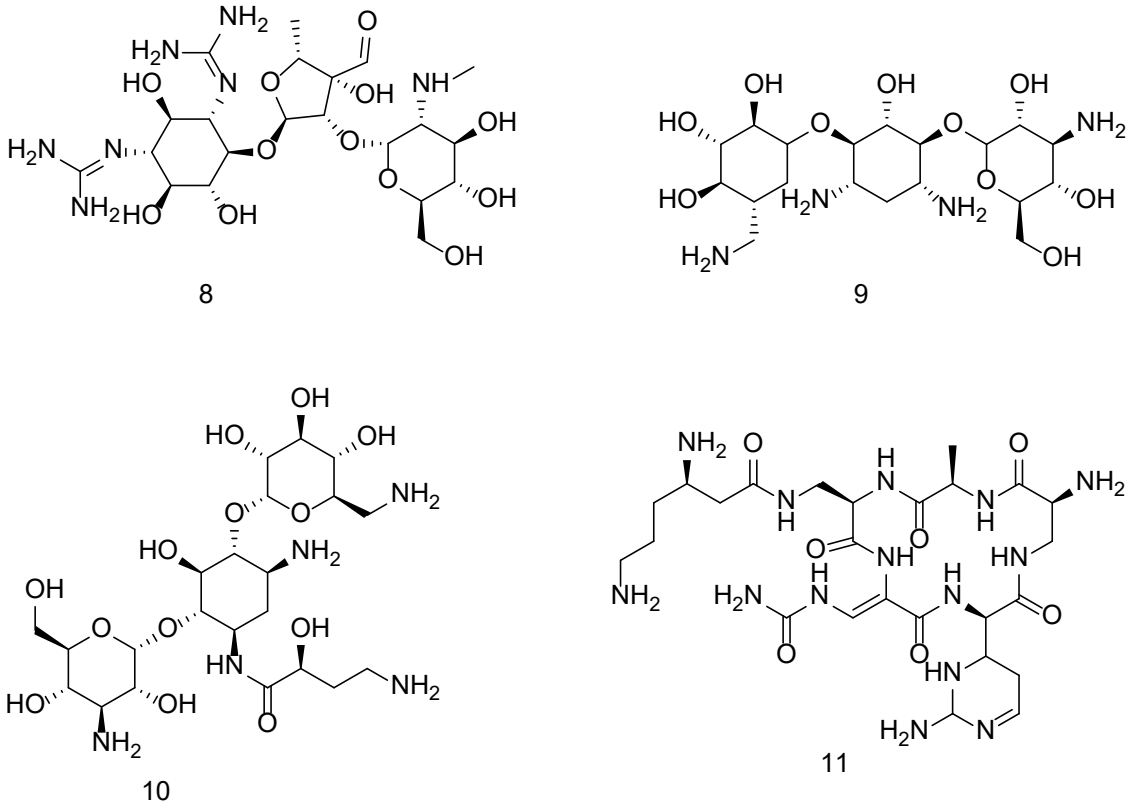


Figure 11: Structures of representative aminoglycosides, including streptomycin **8**, kanamycin **9**, amikacin **10** and capreomycin **11**.

Most are protein synthesis inhibitors, which usually act as bacteriostatic agents, but some act as bactericidal agents due to their irreversible inhibition of bacterial protein synthesis.[47] Their mechanism of action, after crossing the bacterial cell envelope is to induce a ribosomal misreading on the messenger RNA template through binding to the 30S subunit of bacterial ribosomes. The ribosomes are present in both human and bacterial cells and as a result, long term TB treatment with aminoglycosides can show ototoxicity and nephrotoxicity.[48] Under anaerobic and low pH conditions, such as in the granuloma where *Mtb* resides in its latent state, antitubercular activity of aminoglycosides is depressed as penetration of drug to bacterial cell is reduced due to a reduction in energy-dependent transport.[48]

Streptomycin **8** is derived from *Streptomyces griseus* and effective against TB and is administered parenterally due to poor gastrointestinal tract absorption. Even though the drug susceptibility testing (DST) shows susceptibility, extensive Streptomycin **8** treatment is not recommended in order to avoid higher rates of Streptomycin **8** resistance in patients with MDR-TB.

Kanamycin (KAN) **9** or Amikacin (AMK) **10** can be used instead as the initial choice of injectable agent because they have little cross-resistance with Streptomycin **8**, less toxicity and are cheaper than Capreomycin (CAP) **12**.[49] Although, KAN **9** and AMK **10** have similar structures, AMK **10** is most efficacious and has a lower minimum inhibitory concentration (MIC). J. A. Dijkstra et al. (2018) reported the possible differences in MICs between AMK **10**, KAN **9** and CAP **11** against wild-type *Mtb* which showed 2, 4 and 8 μg/mL, respectively.[50] The similarity in structure between KAN **9** and AMK **10**, leads to cross-resistance and so CAP **11**, a cyclic peptide, is recommended for the treatment of MDR-TB, however, CAP **11** can also have cross-resistance with amikacin/kanamycin in the presence of the *rrs* gene mutation.[49] F. A. Sirgel et al. (2011) examined the MICs of AMK **10** and CAP **11** against *Mtb* isolates with the ribosomal (rrs) A1401G mutation and showed >20 and 15 μg/mL, respectively. Therefore, this gene mutation is responsible for a higher level of resistance to AMK **10**, with only a minor impact on the CAP **11** MIC.[51]

Fluoroquinolones

Fluoroquinolone (FQ) use is recommended by the World Health Organization for TB cases including resistance or intolerance to front-line TB treatments because of their ability to eliminate both active and persistent *Mtb*, particularly in the case of moxifloxacin **12**.[52] Moreover, the fluoroquinolones such as levofloxacin **13** have less risk of hepatotoxicity than isoniazid **1**.[53] The fluoroquinolones elicit antibacterial activity through binding to the bacterial topoisomerase II (DNA gyrase) and IV which are critical in DNA supercoiling process. Consequently, fluoroquinolones inhibit DNA replication and transcription, resulting in bacterial death. Fluoroquinolones have different affinity to both target enzymes. For example, levofloxacin **13** shows greater antibacterial effect on gram-positive bacteria due to their greater affinity to bacterial DNA gyrase than norfloxacin.[47] By binding to both enzymes, moxifloxacin **12** is advantageous in preventing the high-level resistance if one of targeted enzymes being mutated.[54]

Based on results from a large clinical trial, the use of newer fluoroquinolones, such as moxifloxacin **12** and levofloxacin (*L*-isomer of ofloxacin) **13**, are the most valuable as substitute agents for MDR-TB than their older counterparts,

especially ciprofloxacin **14** and ofloxacin **15**, because they have better pharmacokinetic and pharmacodynamic and lower minimum inhibitory concentrations (MICs) against wild-type *Mtb* (Figure 12).[52]

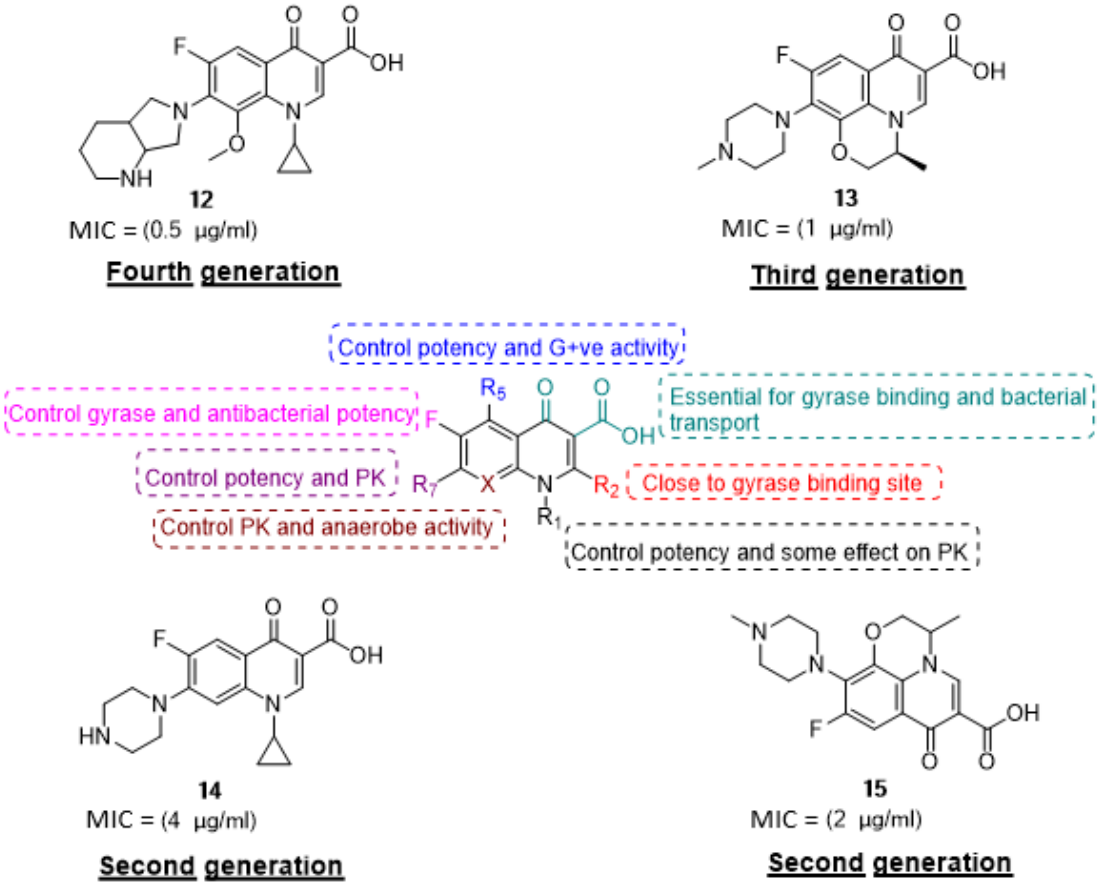


Figure 12. The old and new generations of fluoroquinolones include moxifloxacin **12**, levofloxacin **13**, ciprofloxacin **14** and ofloxacin **15**.

For instance, moxifloxacin **12** is more broad-spectrum than the earlier generation agents and effective against ofloxacin-resistant *Mtb* strains. It is also used for prophylaxis of those exposed to MDR-TB based on its excellent tissue penetration to alveolar macrophages.[55] Furthermore, moxifloxacin **12** can be used to treat MDR-TB, if the drug susceptibility testing does not show resistance against pyrazinamide **2**, fluoroquinolones or aminoglycosides, and the patient does not have TB treatment history.[55] However, the use of fluoroquinolones is not recommended for children, pregnant and lactating women due to their potential for serious side effects such as cartilage toxicity and mutagenesis.[52]

Thioamides

The oral second line treatments for MDR-TB are thioamides including ethionamide **16** (ETH) and prothionamide **17** (PTH) which are structural analogues of isonicotinic acid hydrazide **1** (Figure 13).

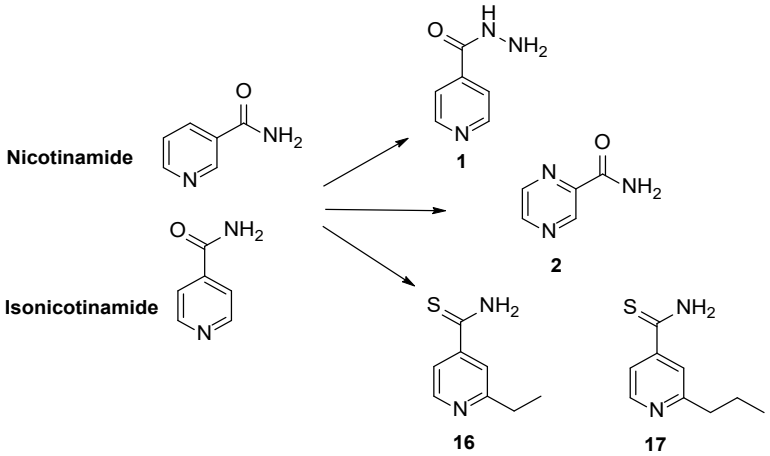


Figure 13: All isoniazid **1**, pyrazinamide **2**, ethionamide **16**, prothionamide **17** are derived from nicotinamide or isonicotinamide.

They exhibit bactericidal activity against growing *Mtb* when the pro-drugs are exposed to enzymatic activation by monooxygenase EthA. Once activated, they form covalent adducts with nicotinamide adenine dinucleotide (NAD) binding the same target of INH **1** (InhA enzyme) resulting in inhibition of mycolic acid biosynthesis. Therefore, the gene mutation in *inhA,* which encodes the primary target of both INH **1** and thioamides **16**, **17** is responsible for potential co-resistance whilst KatG mutant strains resistant to INH **1** remain to be susceptible to thioamides **16**, **17** because thioamides **16**, **17** require a different activator enzyme (EthA) (Figure 14).[56] Based on their ability to cross the blood brain barrier, thioamides **16**, **17** are also recommended to manage drug-susceptible TB meningitis.[57]

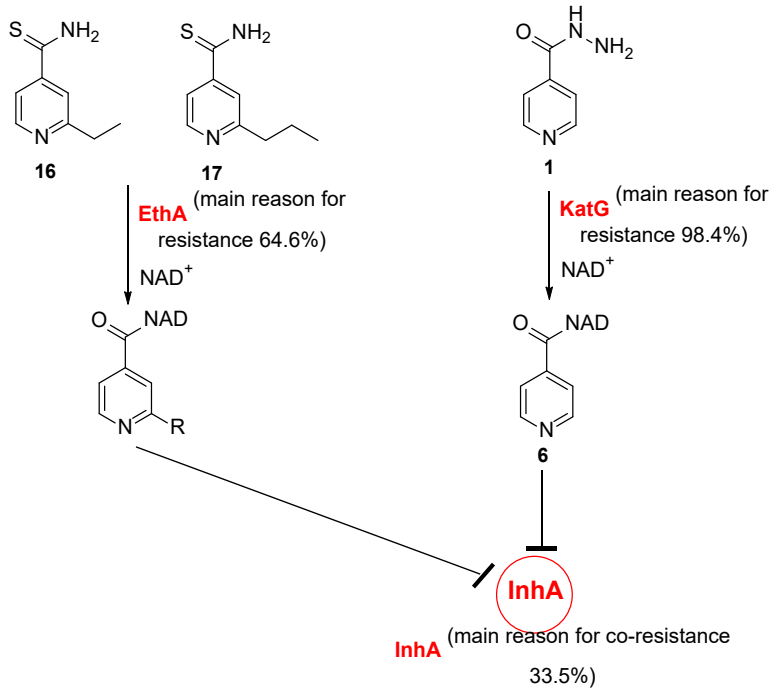


Figure 14: The mechanism of resistance of thioamides **16**, **17** and isoniazid **1.** (Adapted from Islam *et al,* 2019 [56])

Amino acid

D-Cycloserine **18** (DCS) is an oral second line TB treatment which is used only for MDR-TB due to its neurological toxicity.[58] DCS **18** is a broad-spectrum bacteriostatic agent which targets the action of essential enzymes such as D-alanine racemase (Alr) and D-alanine:D-alanine ligase (Ddl) involved in synthesis of cell wall peptidoglycan (Figure 15).

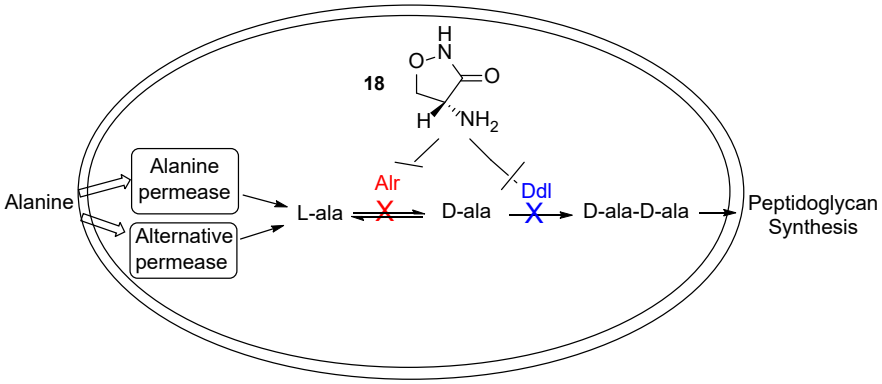


Figure 15: D-Cycloserine **18** impairs alanine conversion leading to disrupting peptidoglycan biosynthesis. (Adapted from Gallagher *et al,* 2019 [59])

The targeting of D-alanine racemase blocks the interconversion process of L-alanine to D-alanine which acts as a substrate for D-alanine ligase. Due to inability of D-alanine to be incorporated into the peptidoglycan, the susceptibility

of bacteria such as methicillin-resistant strains of *S. aureus* (MRSA), to β-lactams is increased by DCS **18**.[59] However, mutation of *alrA* gene in D-alanine racemase causes resistance to DCS **18** but no cross-resistance with other antitubercular agents.[58]

Sulfonamides

Para-aminosalicyclic acid **20** (PAS), a structural analogue of folate precursor (p-aminobenzoic acid) **19**, is more potent antitubercular agent than sulfonamides **21**, however, it is used only for MDR-TB due to its poor efficacy and tolerability (Figure 16).[60]

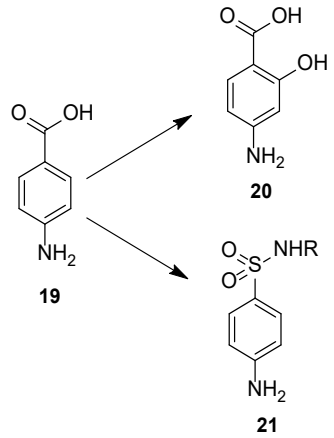


Figure 16: The chemical structure of p-aminobenzoic acid **19** and its structural analogues such as p-aminosalicyclic acid **20** and sulfonamides **21**.

The bacteria cannot use an external source of folic acid and thus PAS **20** shows bacteriostatic activity via targeting the folic acid biosynthesis. PAS **20** is a prodrug which is activated by dihydropteroate synthase (DHPS) and dihydrofolate synthase (DHFS) thus interferes with dihydrofolate reductase (DHFR) enzymatic activity (Figure 17).[60] Furthermore, PAS **20** may block iron uptake, thus inhibiting the synthesis of mycobactin which is a mycobacterial cell wall component.[61]

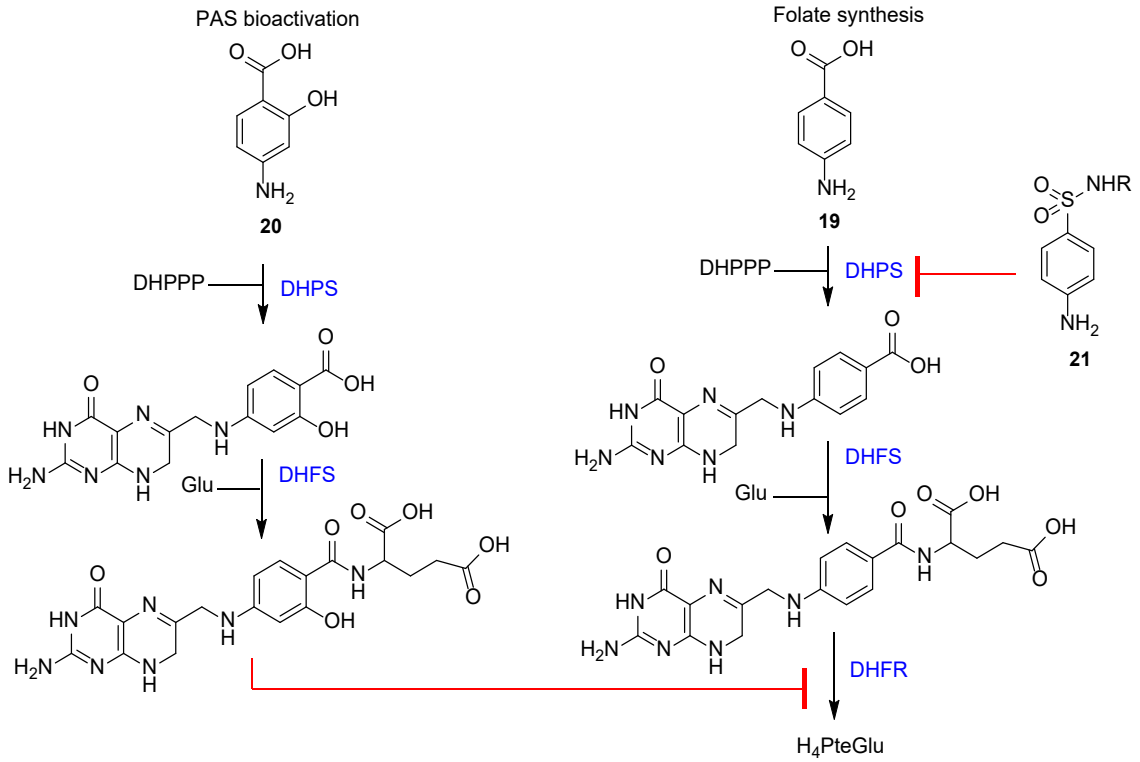


Figure 17: Incorporation of p-aminosalicyclic acid **20** instead of p-aminobenzoic acid **19** into folate pathway and antifolate action of sulfonamides **21**. (Adapted from Thiede *et al,* 2016 [62])

1.3.6 XDR-TB

The inappropriate/ inaccurate diagnosis of MDR-TB and use of second line TB treatment increases the risk of resistance to more antitubercular agents leading to the development of extensively drug resistant TB (XDR-TB). It is challenging because drug resistant TB requires longer treatments (over two years) and occurrence of serious adverse effects have a negative impact on patient compliance. Moreover, lack of systematic drug susceptibility tests (DST), which are not always available in developing countries, contributes to inappropriate therapy selection, and therefore poor treatment outcomes. With no new treatments for 50 years, recently a plethora of new treatments have become available which are reserved as third line treatments. Bedaquiline (BDQ) **22**, linezolid (LZD) **23** and delamanid (DLM) **25** / pretomanid (Pa) **26**, were recently approved in a combination regimen (B-Pa-L) for XDR-TB by the US Food and Drug Administration (FDA). According to the Nix-TB trial at three South African sites, which enrolled 109 cases with XDR-TB and who were treated with BPaL regimen for six months. After the end of treatment, a successful outcome was

attained in 98 patients who completed 26 weeks although the majority of surviving participants around 81% suffered from a peripheral neuropathy reported on treatment. For this reason, most cases required a linezolid dose adjustment or allowable interruptions of up to 35 consecutive days but no permanently discontinued during period of treatment.[63]

1.3.7 Third-line antituberculosis drugs

Diarylquinolines

Bedaquiline (BDQ) **22** represents the first agent of diarylquinolines to be bactericidal against both dormant and growing *Mtb* via inhibiting adenosine triphosphate (ATP) synthesis (Figure 18). Herein, BDQ **22** interferes with the essential enzyme for ATP synthesis, the mycobacterial ATP synthase, but not with mammalian ATP synthase, thereby resulting in disrupting the energy production and subsequent bacterial death. BDQ **22** binds to ATP synthase through the transmembrane oligomeric subunit C, and therefore, BDQ **22** resistance arises from the mutation in *atpE* gene encoding this subunit. Furthermore, resistance is enhanced with mutations in a transcriptional repressor (*rv0678*) resulting in the upregulation of the MmpS5-MmpL5 efflux pump which is involved in non-target-based resistance to bedaquiline.[64]

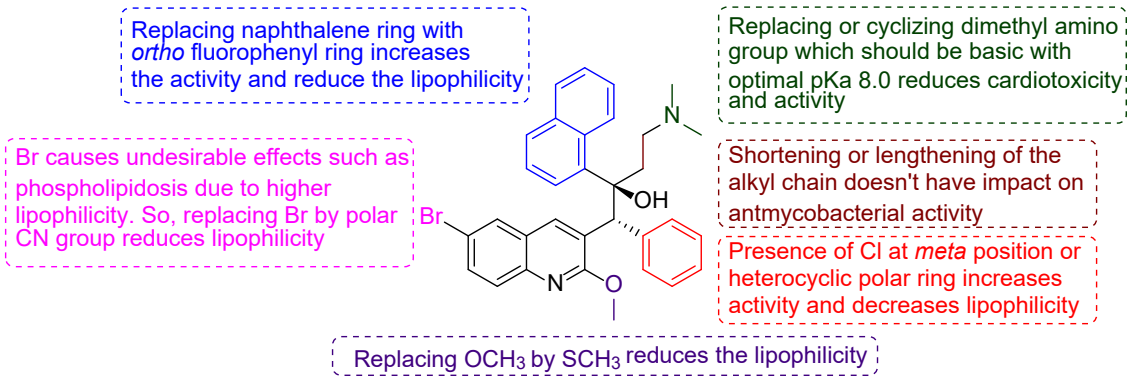


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Figure 18: The chemical structure for bedaquiline (BDQ) **22**. (Adapted from Kumar *et al,* 2015 [65])

Oxazolidinones

Linezolid (LZD) **23**, the only FDA-approved oxazolidinone for clinical use, has bacteriostatic activity against drug-resistant gram-positive bacteria, particularly, methicillin-resistant strains of *Staphylococcus aureus* (MRSA) (Figure 19).

Furthermore, LZD **23** showed significant activity against all *Mtb* strains (MIC_{90} values ≤ 0.5 μg/mL) as it inhibits an early step of bacterial protein synthesis by binding to ribosomal 50S subunits, surrounded by 23S rRNA nucleotides. Thus, mutation of 23S rRNA can cause LZD **23** resistance, with poor MIC_{90} values ≥ 16 μg/mL. However, in case of the prolonged use of LZD **23**, a regular dosage adjustment is required because it can inhibit the mitochondrial protein synthesis leading to serious hematologic and neurologic toxicities.[64] Therefore, Pfizer has developed sutezolid **24**, a thiomorpholinyl analogue of linezolid **23**, which showed significant bactericidal activity in a murine model of TB and a better safety profile in comparison with linezolid **23** (Figure 19). Both linezolid **23** and sutezolid **24** showed bactericidal activity against dormant *Mtb* and so oxazolidinone is gaining great interest as novel antitubercular class.[66]

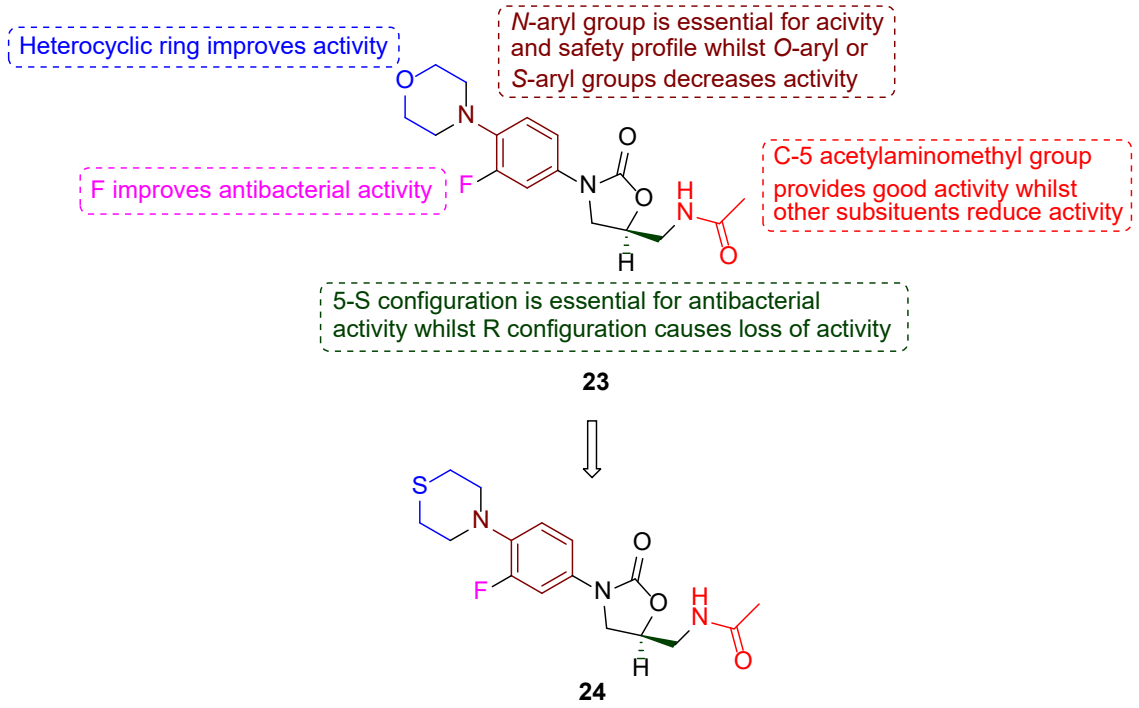


Figure 19: The chemical structure for linezolid (LZD) **23** and sutezolid **24**. (Adapted from Kumar *et al,* 2015 [65])

Nitroimidazoles

Nitroimidazoles including delamanid **25** (DLM) and pretomanid **26** (Pa), are a new class of XDR-TB treatment, with potent activity against all strains of replicating and nonreplicating *Mtb* but DLM **25** (nitroimidazo-oxazole) is 20 times more potent than Pa **26** (nitroimidazo-oxazine) (MIC_{90} values 0.006 / 0.024 μg/mL and 0.015 / 0.25 μg/mL, respectively).[64] In comparison to Pa **26**, the next generation

of nitroimidazoles such as TBA354 **27**, (nitroimidazo-oxazine) showed greater activity against replicating and nonreplicating *Mtb* (MIC_{90} values 0.006 / 0.006 µg/mL) (Figure 20)**.**[67] In 2016, TB Alliance announced discontinuation of the human clinical trials of TBA354 **27** because of its toxicity and pharmacokinetic data.[68]

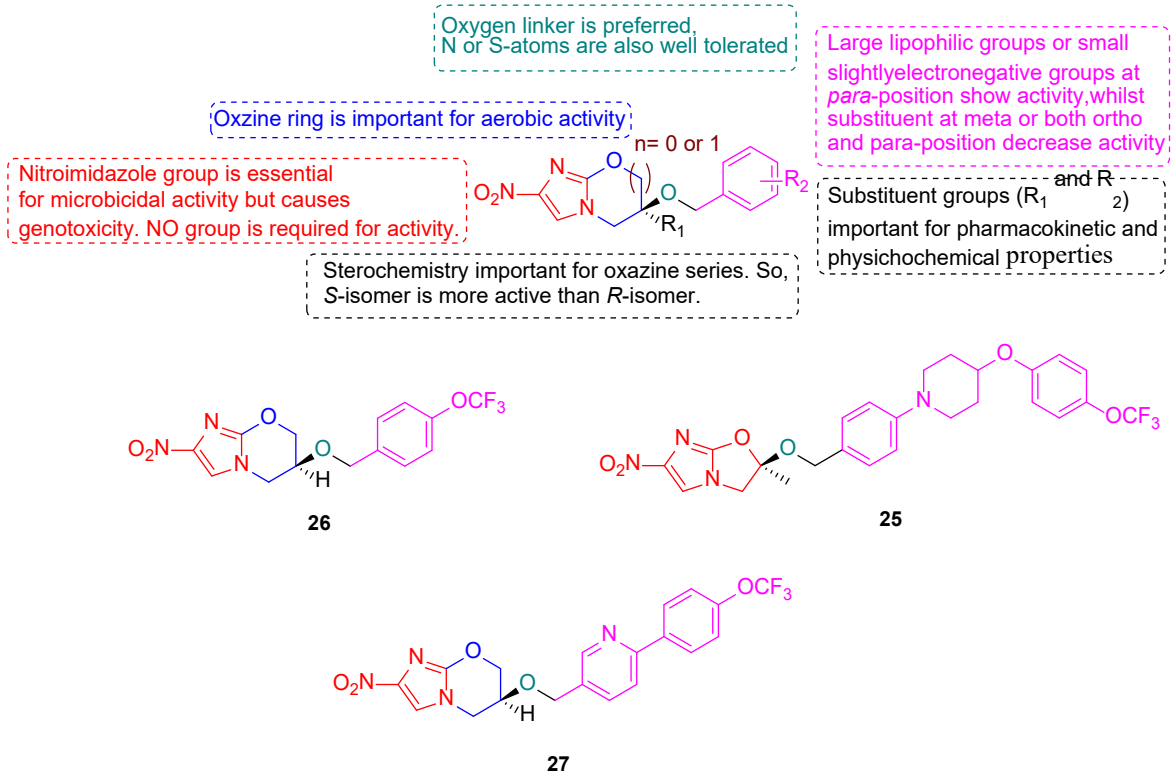


Figure 20: The chemical structure for the nitroimidazole class, which includes delamanid **25** (DLM), pretomanid **26** and TBA354 **27.** (Adapted from Kumar *et al,* 2015 [65])

Because of inhibition of the biosynthesis of mycolic acids, their main active metabolite (desnitro-imidazooxazole) exhibits significant bactericidal and sterilizing activity when **25**/**26** are being activated by a deazaflavin-dependent nitroreductase (ddn) (Rv3547).[64] Once activated, they show also anaerobic activity against nonreplicating *Mtb* through intracellular reactive nitrogen species release (Figure 21).[69] Therefore, the gene mutation in *ddn*, which encodes the activating enzyme (ddn), and in *fgd1* (Rv0407) are associated with resistance to nitroimidazoles.[64] Since 2013, DLM **25** was approved for clinical use by the European Medicines Agency (EMA) although the use of nitroimidazole

antituberculosis agents for long term treatment may be associated with cardiac problems such as QT prolongation which can cause fatal arrhythmias.[64]

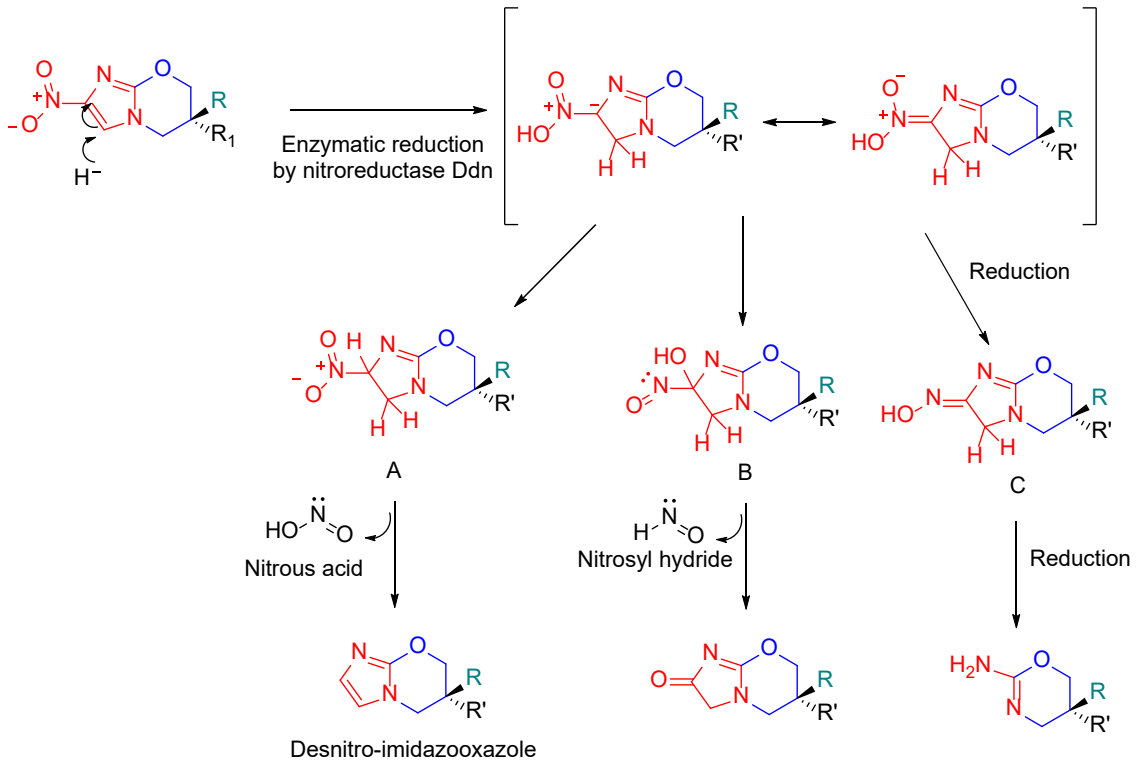


Figure 21: The anaerobic activity of nitroimidazooxazines class against nonreplicating *Mtb* by intracellular reactive nitrogen species release. (Adapted from Singh *et al,* 2008 [70])

1.4 New antimicrobial treatments

As the rise in drug-resistant infections is presented as the main challenge to current antibiotic therapies due to their ability to inactivate conventional treatments, new classes of antibiotic are required with new biological targets to offer a broad spectrum of antimicrobial activity. A selection of these new antimicrobial agents and their modes of action will be discussed in the following subsections.

1.4.1 Antimicrobial peptides

Antimicrobial peptides (AMPs) are derived from wide variety of living organisms which constitute an essential component of their innate immune defense and thus, called host defense peptides (HDPs). Unlike most peptide-based antibiotics of bacterial origin (polymyxins) or fungal origin (β-lactams), the ribosomally synthesized AMPs have been found to have broad spectrum and rapid microbiocidal activity due to their versatility.[71] The antimicrobial activity of these

peptides can be affected by several factors such as sequence, charge, conformation and structure, size, hydrophobicity and amphipathic stereo geometry.[72] Moreover, AMPs can overcome the bacterial resistance through targeting the bacterial cell wall and not specific receptors based on antimicrobial peptide self-association to the cell membrane.[71] Herein, AMPs were found to cause osmotic lysis of the bacterial cell by forming transmembrane channels which resulted from electrostatic interaction between the bacterial, anionic membranes (lipopolysaccharide or phospholipid for gram-negative and teichoic acids for gram-positive) and cationic peptides (containing excess lysine and arginine) (Figure 22). This peptide-lipid interaction contributes to increase the selectivity of AMPs towards bacterial targets over the host cells as the differences in the lipid composition of cell membrane between bacterial and eukaryote host cell membrane represent the targets for AMPs.[73]

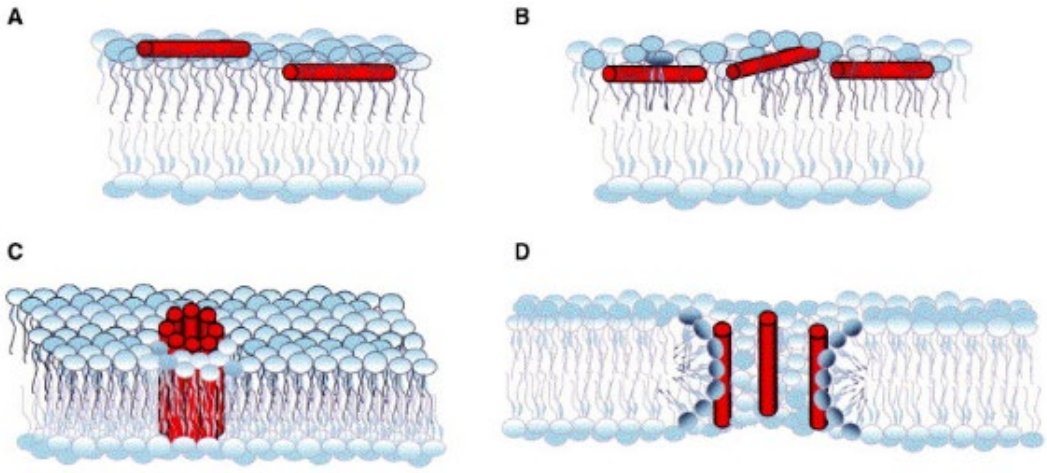


Figure 22: Damaging cell wall through forming transmembrane ion channels.
(Adapted from Sato and Feix, 2006 [73])

Besides having immunomodulatory properties, AMPs act as immune modulators which make them especially interesting agents and it is believed that they can stimulate the innate immune response through different mechanisms including inducing pro-inflammatory cytokine production or inhibiting bacterial lipopolysaccharide (endotoxine) (Figure 23).[74]

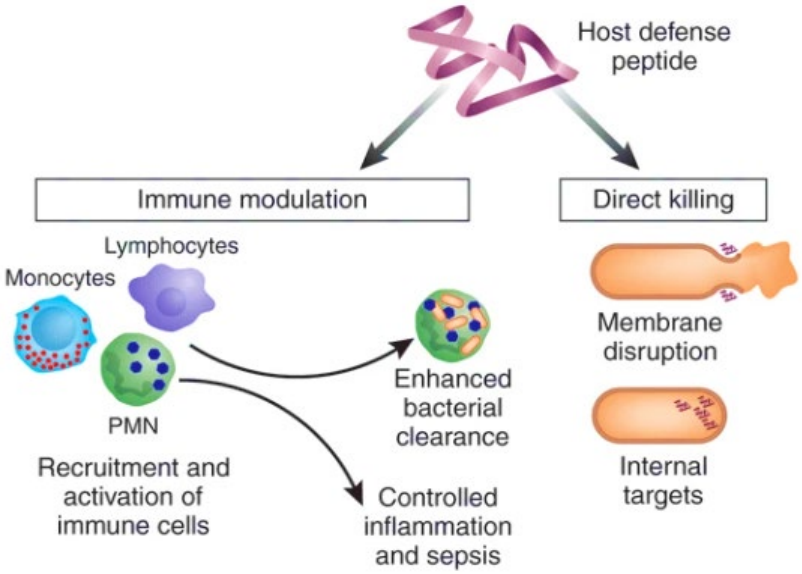
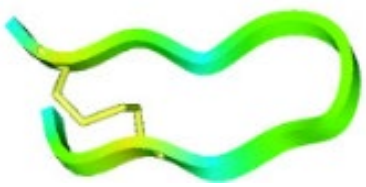
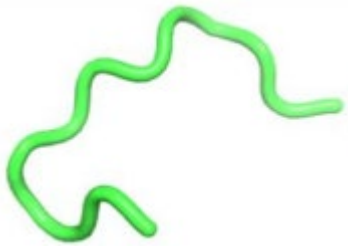


Figure 23: Both direct antimicrobial activity and immune modulatory are used by AMPs. (Adapted from Hancock and Sahl, 2006 [74])

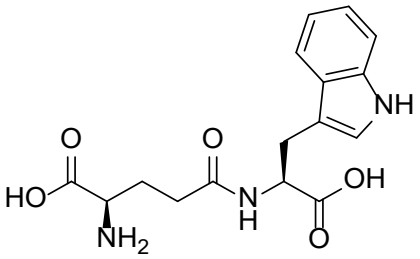
In the database, more than 2,000 AMPs with variable length and amino acid composition have been classified based on their secondary structure into four main classes: β-sheet, α-helical, loop and extended peptides (Table 5).[75]

Class	**Properties**[72]	**Examples**
β-sheet peptide	Composed of at least two antiparallel β-strands stabilized by two to four interchain disulfide bridges between these strands due to containing cysteine residue.	Human β-defensin 2
α-helical peptide	Representing around 30 – 50% of all AMPs. Composed of 12 - 40 amino acids particularly, helix stabilising residues such as alanine, leucine, and lysine.	Magainin 2
Loop peptides	Composed of 20 – 46 amino acids with only a single disulfide bridge.	Bactenecin 

Extended peptide	Rich in specific amino acids such as glycine, proline, tryptophan, arginine and histidine.	Indolicidin 

The natural AMPs have been investigated for more than 30 years. Unfortunately, they are associated with several shortcomings including incomplete understanding of their mechanism of action, reduced activity in higher pH and the high cost of production as compared to conventional antimicrobial agents. On top of this, pharmacokinetic and pharmacodynamic issues, for instance, AMPs show poor absorption after oral administration and cannot cross the blood-brain barrier because they are large and strongly cationic peptides. Moreover, they are susceptible to the rapid proteolytic degradation in both the bloodstream and the gastrointestinal tract which is a significant factor in bioavailability issue.[76]

Based on the described shortcomings, the development of peptidomimetic analogues which are fragments or derivatives of natural AMPs, have been an area of great interest. They can display their advantages over their natural peptides and can be used in medicine development because of their structural simplicity with low synthetic cost and ease of optimization.[77] For instance, Dipeptide γ-*D*-glutamyl-*L*-tryptophan **28**, γ-glutamyl derivatives, boosts immune response by stimulating T-lymphocyte and secretion of interferon-γ and interleukin-2 to promote bacterial clearance. Based on clinical outcomes, this molecule **28** was very effective in combination with standard TB treatment, therefore, it is a prospective antitubercular agent and its future demand in the pharmaceutical industry is expected (Figure 24).[78]

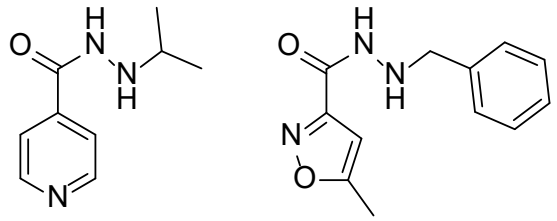


28

Figure 24: The chemical structure of γ-*D*-glutamyl-*L*-tryptophan **28**.[78]

1.4.2 Antimicrobial hydrazides

The continuing demand for new and effective antimicrobial agents with novel mode of action is a never-ending task to tackling the development and spread of the antimicrobial resistance. In continuation of designing of new compounds possessing antimicrobial activity based on hydrazide skeleton, such as isonicotinylhydrazide (INH) **1** which is one of the attractive backbones in antibacterial research today due to its chemical reactivity and biological activities. Based on the aforementioned facts, isoniazid **1** therapy is considered a cornerstone of TB treatment although it has two major drawbacks. Prolonged treatment with isoniazid **1** causes hepatotoxicity because it is metabolized by acetylation and dehydrazination and produces an *N*-acetylhydrazine metabolite which is responsible for the hepatotoxic effect.[79] The second is the action of *N*-arylaminoacetyltransferases (NATs), which are present in both mycobacterial and mammalian cells, to deactivate INH **1** by acetylation reaction at $-NH_2$ group. So, the blockage of $-NH_2$ group by alkyl group, as in example the *N*-isopropylated form of isoniazid (Iproniazid) **29**, has been developed primarily as antituberculosis agent and based on its mood-elevating effect so used as antidepressant. Later, it was withdrawn due to the high hepatotoxicity. Other examples of blocking the $-NH_2$ group include isocarboxazid **30** which exhibits less hepatotoxicity, and is thus still available for clinical use as an antidepressant (Figure 25).[80]



29 **30**

Figure 25: The chemical structure of Iproniazid **29** and Isocarboxazid **30**.

Subsequent chemical modification of blocking *N*-acetylation of INH **1** was demonstrated by synthesis INH hydrazide-hydrazones **31** (Schiff's bases derivatives) that led to more effective and less hepatotoxic derivatives, such as ftivazide **32**, saluzide **33** and salizide **34** (Figure 26).[81]

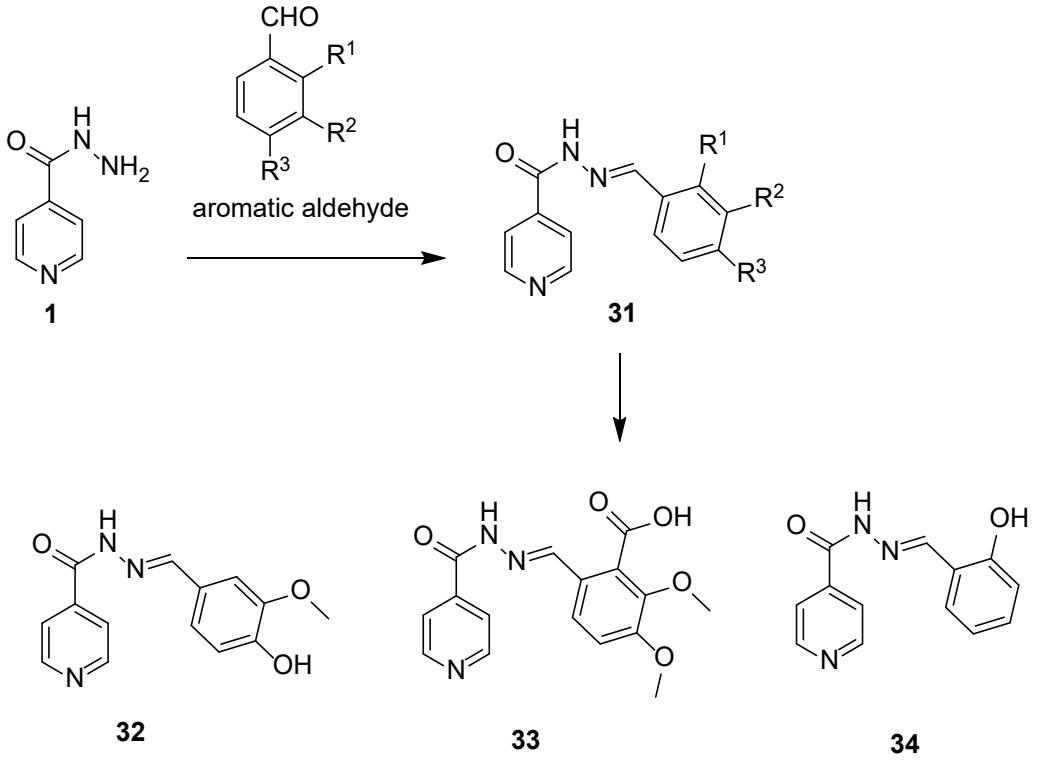


Figure 26: The synthetic route of Schiff bases of isoniazid **31** such as ftivazide **32**, saluzide **33** and salizide **34** via reacting INH **1** with aromatic aldehyde. (Adapted from Villamizar-Mogotocoro *et al,* 2020 [81])

They act as an optimal transport vehicle for INH **1** moiety inside both mycobacterial and mammalian cells (Figure 27).[81] However, all the efforts of re-designing INH **1**, that have been undertaken, in order to develop a more effective analogue against INH resistant *Mtb* strains, have unfortunately met without much success.[82]

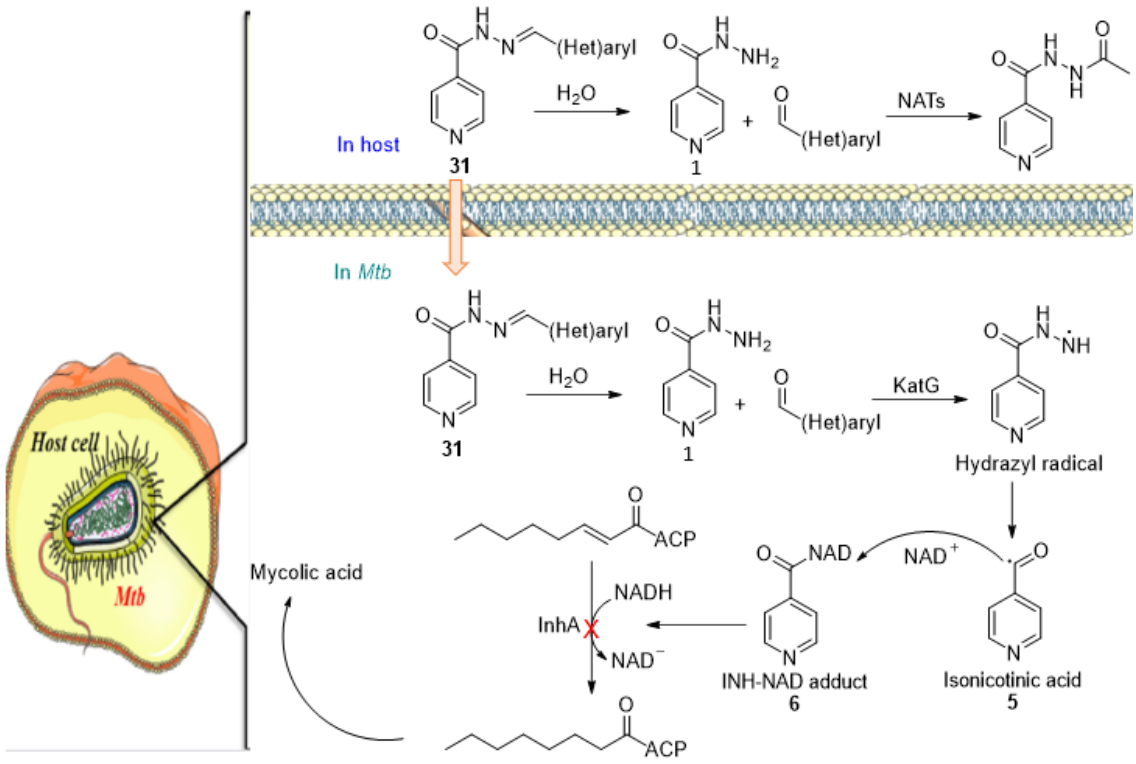


Figure 27: The proposed mechanism of action of Schiff bases of isoniazid **31**. (Adapted from Villamizar-Mogotocoro *et al*, 2020 [81])

The use of the hydrazide-hydrazone skeleton **35** as a starting material has long attracted attention for new antibiotic development. Furthermore, widely used antimicrobial agents such as nitrofurazone **36**, furazolidone **37** and nitrofurantoin **38** further support the interest towards the synthesis of hydrazide-hydrazones agents exhibiting wide range of antimicrobial activity (Figure 28).[83]

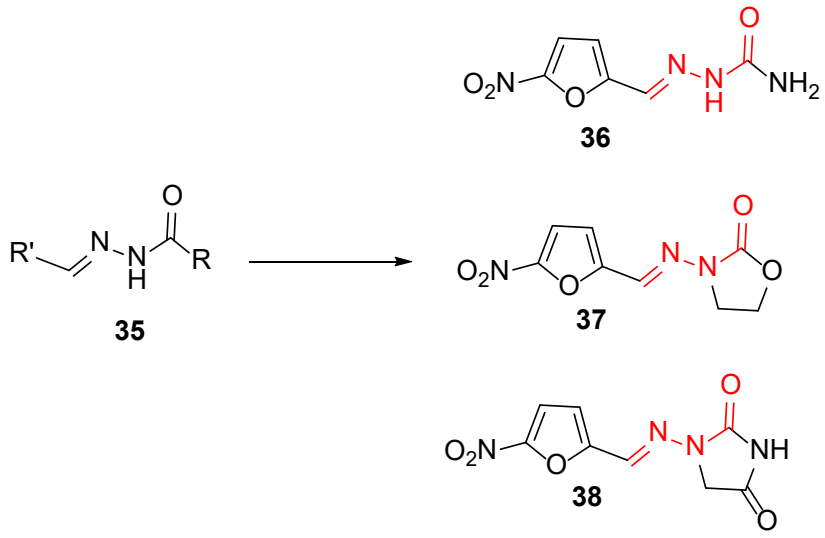


Figure 28: Examples of current antimicrobial agents containing hydrazide-hydrazone skeleton **35** such as nitrofurazone **36**, furazolidone **37** and nitrofurantoin **38**.[83]

In the light of literature, there were recent attempts to develop new antitubercular compounds containing hydrazide-hydrazone moiety such as 4-fluorobenzoic acid hydrazide **39**,[84] *trans*-cinnamic acid hydrazides **40**,[85] fluorine containing hydrazones **41** [86] and sudoterb **42** [79] (Figure 29).

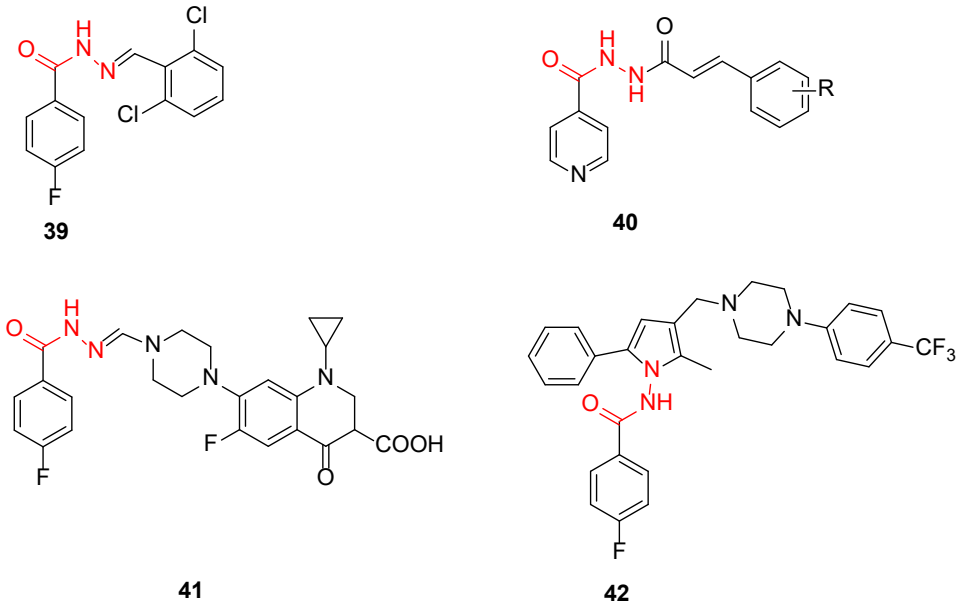


Figure 29: Examples of hydrazide-hydrazones possessing antimycobacterial activity.

1.4.3 Antimicrobial aryl-oxadiazoles

Despite increased research efforts to discover new antibiotics, treatments for the resistant infections still remain a challenging task, as conventional antibiotics are often ineffective. In recent decades, literature studies reveal a vast number of nitrogen containing heterocyclic compounds that display better biological activity than non-nitrogen compounds.[87] It is not surprising, therefore that the system of aryl-oxadiazoles, five membered aromatic heterocycles, have been investigated with a view to their use in the medicinal chemistry. It is known that this system of aryl-oxadiazoles contains four regioisomers including 1,2,3-, 1,2,4-, 1,3,4- or 1,2,5-oxadiazoles which have different charge distributions (Figure 30).[88]

Figure 30: Oxadiazole regioisomers.[88]

However, 1,2,4- or 1,3,4-oxadiazoles are widely studied and exhibit wide spectrum of biological activities. Therefore, they are present in several drugs **43**, **44**, **45**, **46** and **47** in clinical use containing oxadiazole in their backbone (Figure 31).[89]

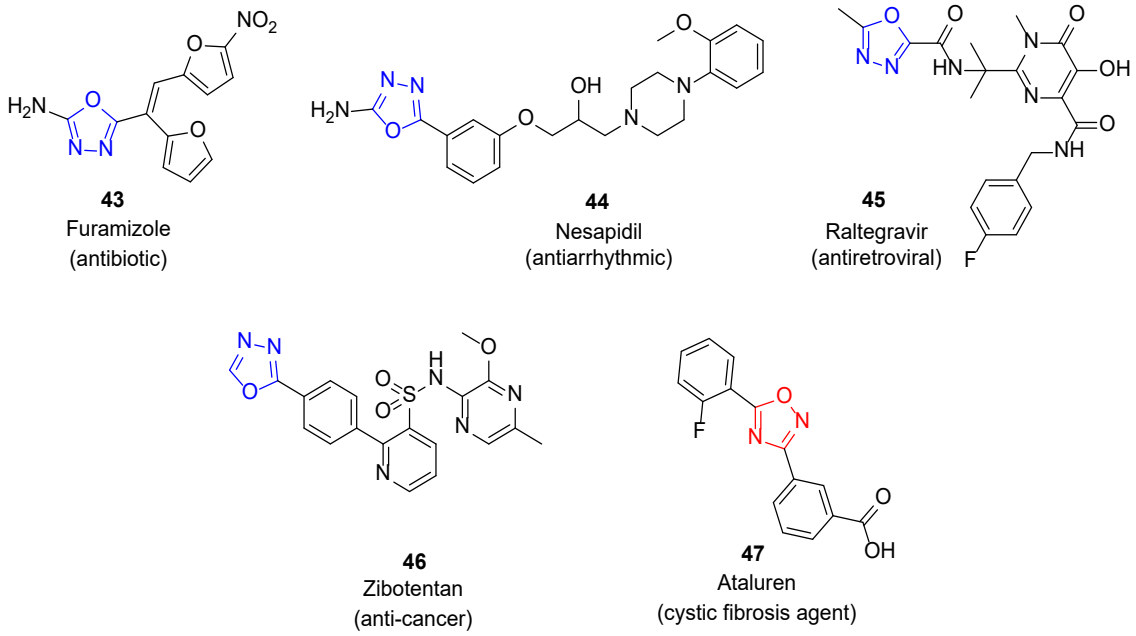
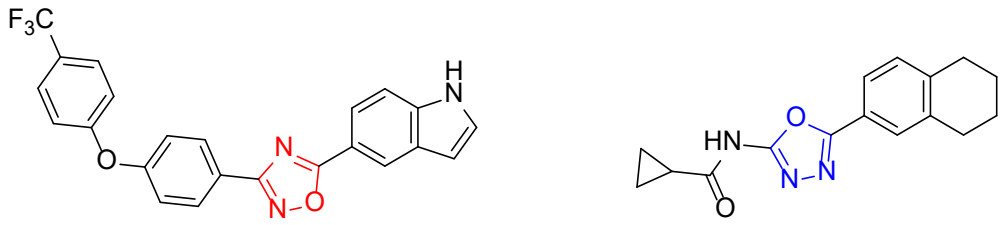


Figure 31: Chemical structure of marketed medicines containing oxadiazole units.[89]

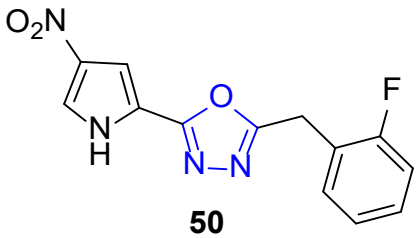
Due to the ongoing problem of bacterial resistance to β-lactam antibiotics, the scoring of series of 1,2,4-oxadiazoles against the crystal structure of a penicillin-binding protein through an *in-silico* analysis was carried out. They, particularly compound **48**, showed antibacterial activity against gram positive bacterial strains including vancomycin-resistant, linezolid-resistant and methicillin-resistant *Staphylococcus aureus* (Figure 27).[90] In 2018, Zheng *et al*, reported that the 1,3,4-oxadiazoles, particularly compound **49**, showed bactericidal activity against S. *aureus* by interfering with biofilm formation which is essential for staphylococcal pathogenesis. The same research group suggested the presence of the toxophoric –N=C–O– linkage group may be responsible for antibacterial activity by reacting with the nucleophilic centers of the bacterial cells (Figure 32).[91]



48 **49**

Figure 32: Compound **48** was efficacious against MRSA strain in a mouse model.[90] Compound **49** demonstrated potent antibacterial activity against S. *aureus*.[91]

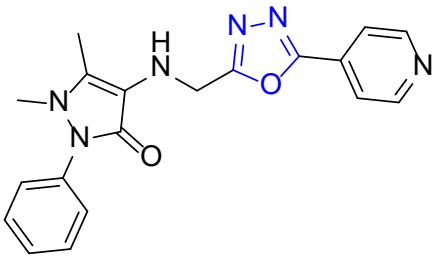
In connection to the antitubercular studies, the continued emergence of resistance to TB treatments has posed a challenge for treating MDR-TB and XDR-TB. Thus, the aryl-oxadiazoles have attracted many research groups to develop new TB treatments. For instance, the antitubercular activity of 2-(4-nitro-pyrrol-2-yl)-5-aryl-1,3,4- oxadiazole derivatives were screened against *Mtb* ($H_{37}Rv$) and promising compound **50** showed lowest MIC value (0.46 μM) which is close to INH **1** (0.40 μM) (Figure 33).[92]



50

Figure 33: Compound **50** showed promising antitubercular activity.[92]

In an other antitubercular activity investigation of novel 1,3,4- oxadiazoles, compounds were screened against two strains of *Mtb* ($H_{37}Rv$ and isoniazid-resistant) and compound **51** exhibited the highest antitubercular activity (0.78 μM and 1.52 μM, respectively) (Figure 34).[93]



51

Figure 34: Compound **51** showed promising antitububercular activity against wild-type and isoniazid mono-resistant *Mtb*.[93]

1.5 Antimicrobial Resistance

With a rise in drug resistant bacteria, MDR-TB is one of more infections developed by treatment-resistant strains. It is similar to malaria, methicillin-resistant S. *aureus* (MRSA) or vancomycin-resistant *enterococci* (VRE) infections, which have become serious global health threats in this century. The resistance to at least 4 of the core TB treatments brings significant challenges. Understanding these mechanisms allows for new TB treatments to be developed.[94]

1.5.1 INH resistance or resistance to front line drugs

Despite the potent antimycobacterial effect of INH **1**, INH **1** resistance is considered as a surrogate marker of MDR-TB. *Mtb* uses several mechanisms including inactivating enzymes and the overexpression of genes which encode either the target enzyme or an enzyme that has a critical role in drug activation, to resist the action of INH **1**. A primary mechanism of INH **1** resistance arises from mutation in *katG* gene that encodes the katG enzyme and alters its activity toward INH **1**, resulting in fewer isonicotonic acyl radicals **5** being generated. Once activated, the second most common mutation occurs in the *inhA* gene which is associated with reducing the affinity of active metabolite **5** to the target enzyme (InhA) (Figure 35).[95] A recent study reported that that low-level resistance to INH **1** is provided by gene mutations in *acpM* and *kasA* that encode two intracellular targets malonyl acyl carrier protein (AcpM) and a β-ketoacyl-ACP synthase (KasA) which are involved in process of fatty acid elongation (Figure 35). Additionally,

active metabolite **5** has been shown to inhibit dihydrofolate reductase, a key enzyme for nucleic acid synthesis, therefore, there is correlation between INH **1** resistance and mutation in the *dfrA* gene, but this is still not proven.[96]

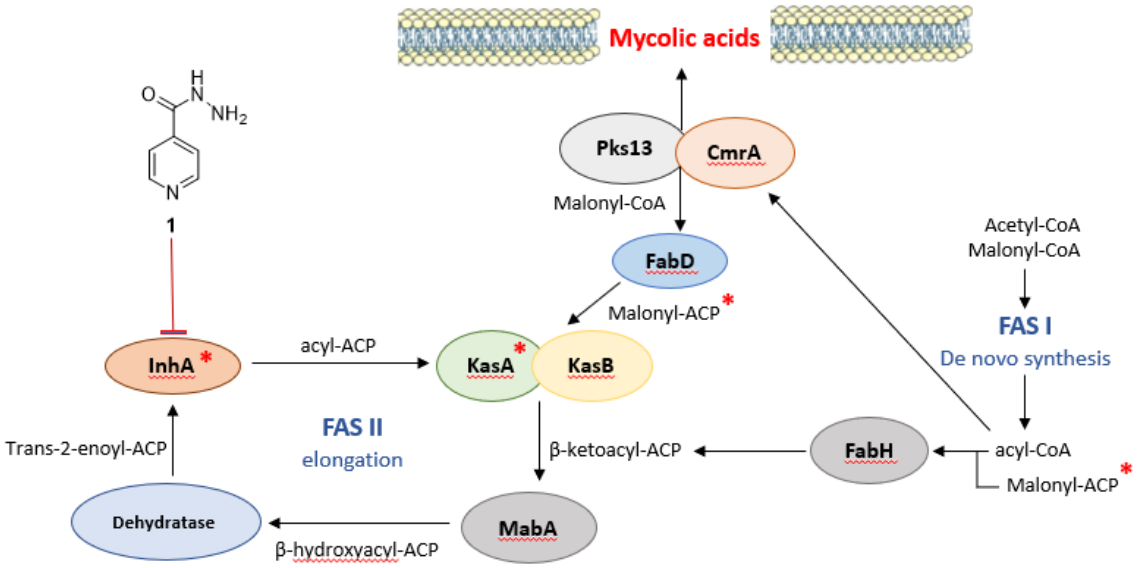
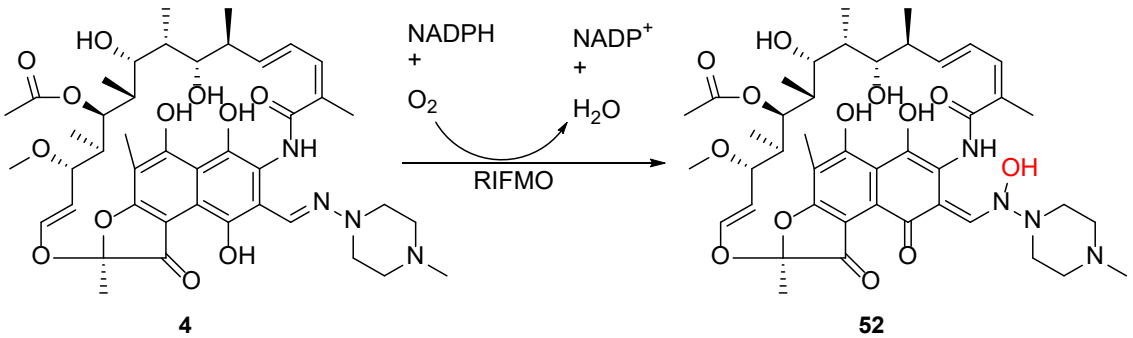


Figure 35: The genetic mutations are responsible for INH **1** resistance where (*) indicates to targeted genes including *inhA*, *acpM* and *kasA*.

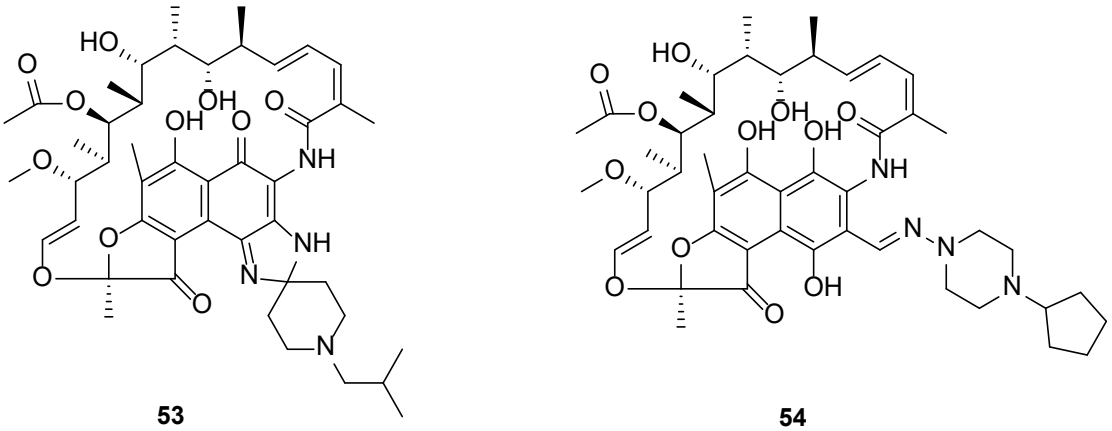
Notwithstanding this, *Mtb* is becoming resistant to rifampicin **4** because of extensive usage of RIF **4** and its derivatives. The bacterial resistance to both RIF **4** and INH **1** has been observed among greater than 90% of MDR-TB strains as this is responsible for worse TB treatment outcomes. Whilst not fully understood, rifampicin monooxygenase (RIFMO) could be contributing to the decreased anti-tubercular activity of rifampicin **4** by the *N*-hydroxylation of rifampicin **4** to 2'-*N*-hydroxy-4-oxo-rifampicin **52** (Scheme 2).[97]



Scheme 2: Conversion of Rifampicin **4** to its inactive metabolite **52** through RIFMO enzyme.[97]

The major rifampicin-resistant strains of *Mtb* emerge from gene mutation in molecular target. The harbor mutations within 81 base pair of *rpoB* gene can

cause a structural change in β-subunit of RNA polymerase which reduces the binding affinity to RIF **4**. As a result of this, most laboratories examine the 81 base pair region to determine RIF **4** resistance by using molecular methods.[95] Cross resistance occurs in conjunction with other rifamycins such as rifabutin **53** and rifapentine **54** and this is important for TB treatment in patients co-infected with HIV (Figure 36). In this case, rifabutin **53** is more preferred than RIF **4** because it is a weak inducer of the cytochrome P450 that doesn't increase the metabolism of antiretroviral therapy.[95]

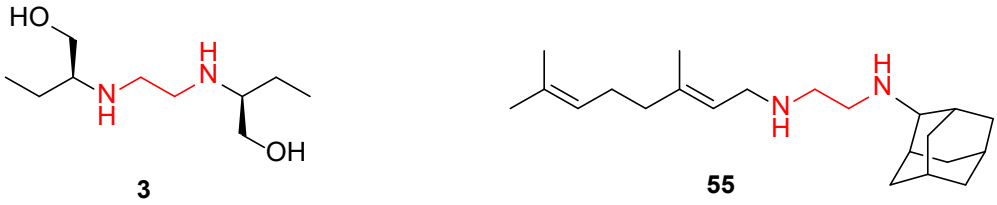


53 **54**

Figure 36: Other rifamycins such as rifabutin **53** and rifapentine **54**.[95]

In *Mtb*, PAZ **2** is activated by pyrazinamidase encoded by the *pncA* gene whilst pyrazinamidase usually loses its activity in most PAZ **2**-resistant *Mtb* strains because of the structural gene mutation in *pncA*. In addition to *pncA* regulation, pyrazinamide-resistant *Mtb* strains showed other resistance mechanisms including pyrazinamide uptake and pyrazinoic acid efflux.[95] The resistance to PAZ **2** increases the risk of treatment failure in cases with drug-susceptible or drug-resistant TB because it provides synergistic effects when it is included in MDR-TB regimen, especially, with bedaquiline **22** and pretomanid **26**.[98]

Not unexpectedly, Ethambutol **3** is not only part of front-line treatment, but is structurally related to the new antitubercular agent SQ 109 **55** (Figure 37).[99] Unfortunately, several studies have shown EMB **3** resistance is commonly linked to gene mutations in *embB* which encodes its target enzyme.[96] Interestingly, drug-resistant strains of *Mtb*, including EMB **3**-resistant *Mtb* strains are susceptible to SQ109 **55** with no likelihood of cross-resistance with EMB **3**. Therefore, SQ109 **55** is suggested to be an ideal alternative for EMB **3**.[99]



3 **55**

Figure 37: Process to develop new antitubercular agent (SQ109 **55**) based on 1,2-ethylenediamine.[99]

The action and mechanisms of resistance to front-line TB treatments are still not fully understood, although the genetic mutations that are associated with each form of drug resistance have been detected by effective approaches such as whole-genome sequencing analysis. This is because TB treatments are usually tested against a limited number of the *Mtb* complex genotypes. In addition to detection of genetic mutations, further investigation about drug-resistant TB is required because other important information including the level of resistance, cross-resistance to other agents, relatedness of strains, and virulence, are increasingly being used to control the spreading of antibiotic resistance, particularly in the context of TB.[100]

1.6 Conclusion

In this chapter, facts about global TB epidemiology, themes related to *Mtb*, TB vaccination and treatments as well as increasing emergence of drug resistant forms of TB, especially MDR-TB and XDR-TB have been outbred. TB treatment research has been in decline since the 1960s due to introduction of INH **1** and RIF **4** until the emergence of drug-resistant TB in the end of the 20th century. Due to the increased prevalence of MDR-TB and XDR-TB, the objective of the current research is to design and synthesis novel antitubercular agents with new molecular targets to tackle the continuing global burden of this infection and the efficacy and cost limitations associated with current therapies. The hydrazide functional group compound of interest in this work could be easily modified to afford benzoxa-[2,1,3]-diazole substituted amino acid hydrazides as potential antitubercular agents.

1.7 Previous work in the group

Previous studies in the group have generated a small library of diverse bioactive molecules (~100) to ascertain their utility as potential antibacterials. A Resazurin Microtiter Assay (REMA)[101] was undertaken at a fixed concentration (128 μg/mL) against a range of drug susceptible bacteria including gram positive, gram negative and mycolata bacteria.[1] Whilst many compounds demonstrated little activity at these high concentrations, several classes were successful at retarding bacterial growth. Analysis of the compounds revealed a variety of architectures including flavones **56**, purines **57**, benzo-[2,1,3]-diazoles **58**, quinolines **59**, indolin-2-ones **60** and thiocoumarins **61** that provided positive results at this concentration (Figure 38).

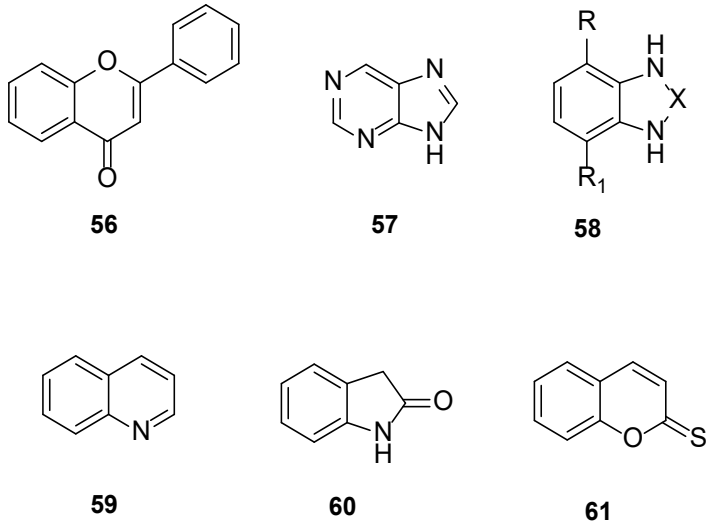


Figure 38: The chemical structure of flavone **56**, purine **57**, benzo-[2,1,3]-diazole **58**, quinoline **59**, indolin-2-one **60** and thiocoumarin **61**.

Further analysis of the library revealed a class of molecules demonstrating activity against only mycolata bacteria. These molecules possess a core comprising a benzo-[2,1,3]-diazole that to the best of our knowledge, have been utilised as anti-cancer treatments[102] and as herbicides[103] in only a small number of studies. Consequently, we focused on this pharmacophore and undertook another REMA assay on a reduced set of organisms to determine the minimum inhibitory concentrations of these molecules, and to determine their utility for subsequent investigations (Table 6).

Table 6: Minimum inhibitory concentration (MIC) results against gram positive, negative and mycolata bacteria for our library.

EMB	RIF	INH																			

The results reveal a mix of activity against the tested organisms, with simple substituted benzo-[2,1,3]-diazole compounds leading to broad organism activity and potency, mirroring the initial screening results (Entries **58a-58d**, Table 6). Pleasingly, benzo-[2,1,3]-diazole molecules **62** possessing a sulfonamide peptidomimetic yielded far greater specificity and activity against mycolata bacteria including *Mtb* (Entry **63a**, **64a-b** and **65a**, Table 6).

In order to explore this architecture further, subsequent experiments were undertaken to produce a variety of benzo-[2,1,3]-diazoles **65a-g** and **64a-b** to understand their activity against *Mtb* (Figure 39).

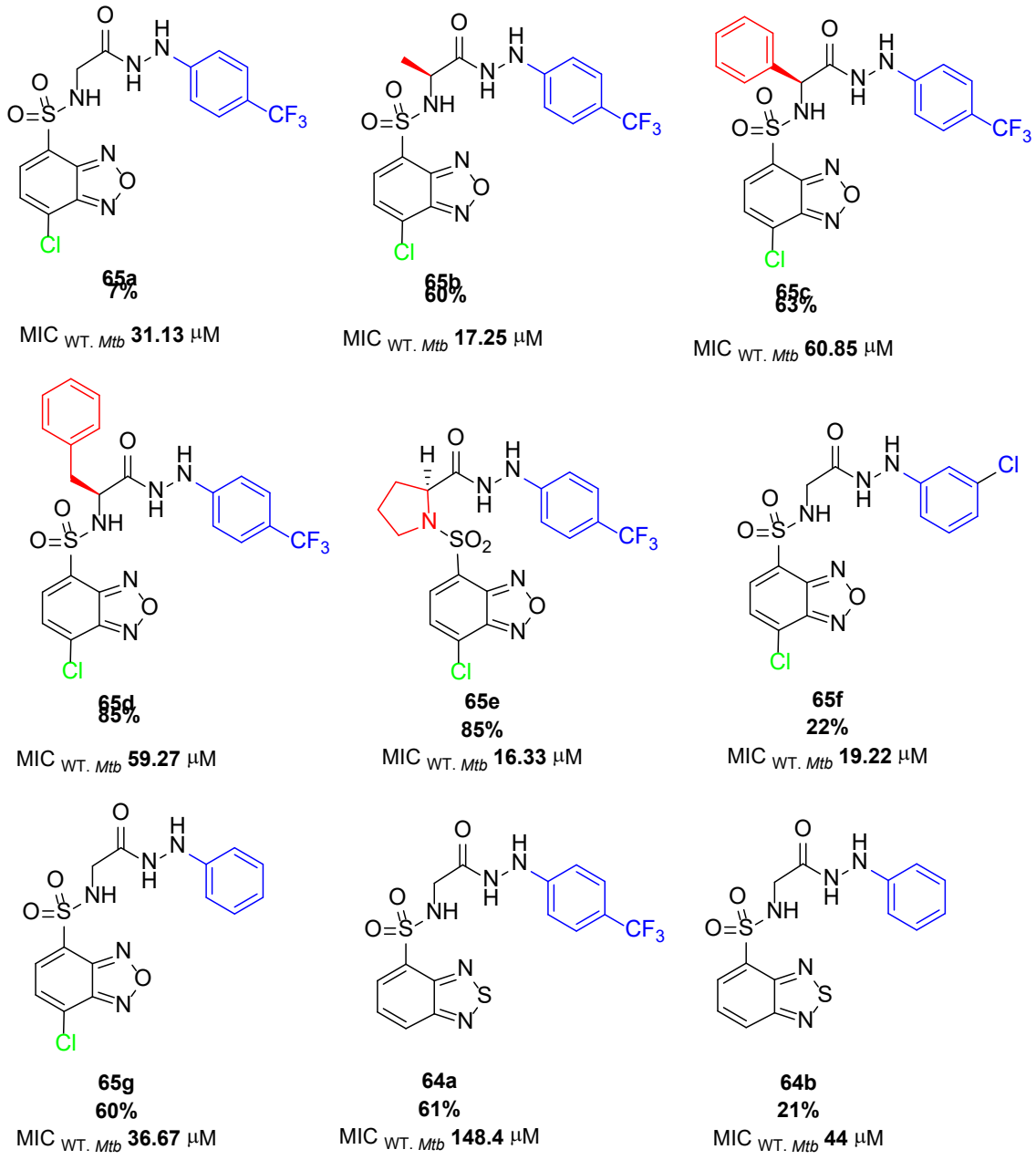


Figure 39: A series of benzo-[2,1,3]-diazoles **65a-g** and **64a-b** showed different MIC value against WT. *Mtb*. Where (%) indicates to an actual yield of resulting compound.

In this small series of compounds, we retained the hydrazine and benzo-[2,1,3]-diazole component and explored the amino acid. It can be seen for compounds **65b-65d**, an increase in MIC was observed, due to the increased size of the amino acid side chain. Curiously, a similar level of activity is observed with **65e** as **65b**, suggesting that there may be a bend or kink which can be exploited by these substrates. Notwithstanding this, retaining unsubstituted amino acids and benzo-[2,1,3]-diazole component allowed us to explore the hydrazine fragment. Interestingly, the presence of a chloro-substituent at the *meta*-position as seen in **65f** produced a lower MIC than **65g** which is substituent-free suggesting that space in this fragment is available for exploitation. Finally, retaining the unsubstituted amino acid and hydrazine whilst exploring the benzo-[2,1,3]-diazole indicated a further shift in MIC as replacement of benzoxa-[2,1,3]-diazoles in **64a**-**64b** by benzothia-[2,1,3]-diazole to investigate their antimycobacterial activity showed similar or lowers levels of inhibitory activity towards *Mtb* (*cf*. **65a** vs **64a**) and (*cf*. **65g** vs **64b**).[1]

Aims and Objectives

As discussed previously (*cf.* section 1.3.2), the requirement of highly toxic and less effective TB treatments with longer duration to treat MDR-TB or XDR-TB is a principal factor for continuous slow rise in TB infection. One of the strategies to tackle the problematic exacerbation of drug-resistant TB is an identification of new chemical entity possessing new mechanism of action which will open a new TB therapeutic avenue.

In previous work from within the group, the benzo-[2,1,3]-diazole peptidomimetics architecture **65** has demonstrated significant selectivity and mycobacterial activity. Therefore, the overall aim of this thesis is to to generate a structure activity relationship study with these substrates to further understand which components of the molecules are crucial for activity against *Mtb*.

Subsequent chemical investigations were undertaken to produce a variety of new library of novel benzoxa-[2,1,3]-diazole substituted amino acid hydrazides **65** to understand their activity against *Mtb*. We wanted to explore how the structural modification may influence the inhibition of mycobacterial growth through

screening the resulting compounds against susceptible and resistant *Mtb* strains utilising a Resazurin Microtiter Assay (REMA).

We approached the SAR analysis for the benzo-[2,1,3]-diazole peptidomimetics **65** to investigate the chemical structure to biological activity of this architecture further and to accelerate trends toward improved understanding of the molecular modifications which can improve the antitubercular activity and leads to a potent lead compound. The cytotoxicity of the final compounds **65** and their intermediates **67** was also examined on mammalian cell lines.

2 Synthesis of benzo-[2,1,3]-diazoles peptidomimetics

2.1 Introduction

In an effort to continue the previous work on benzo-[2,1,3]-diazole peptidomimetics, an SAR investigation was initiated to explore these novel molecules. After initial investigation into these novel antitubercular agents, the aim is to contribute more analogues of the lead compounds **65** to determine which parts are necessary for activity and ultimately improve their potency, affinity and reduce toxicity.

To begin with, a retrosynthetic analysis of the molecule **65** was undertaken. The initial disconnection breaks the sulfonamide bond of **65** to produce the commercially available benzoxa-[2,1,3]-diazole **66** and the amino acid hydrazide **67** or **68**. Further disconnection of the amino acid hydrazide **67** or **68** at the hydrazide bond to produce commercially available aryl hydrazines **69** and *N*-protected amino acids **70** (Figure 40).

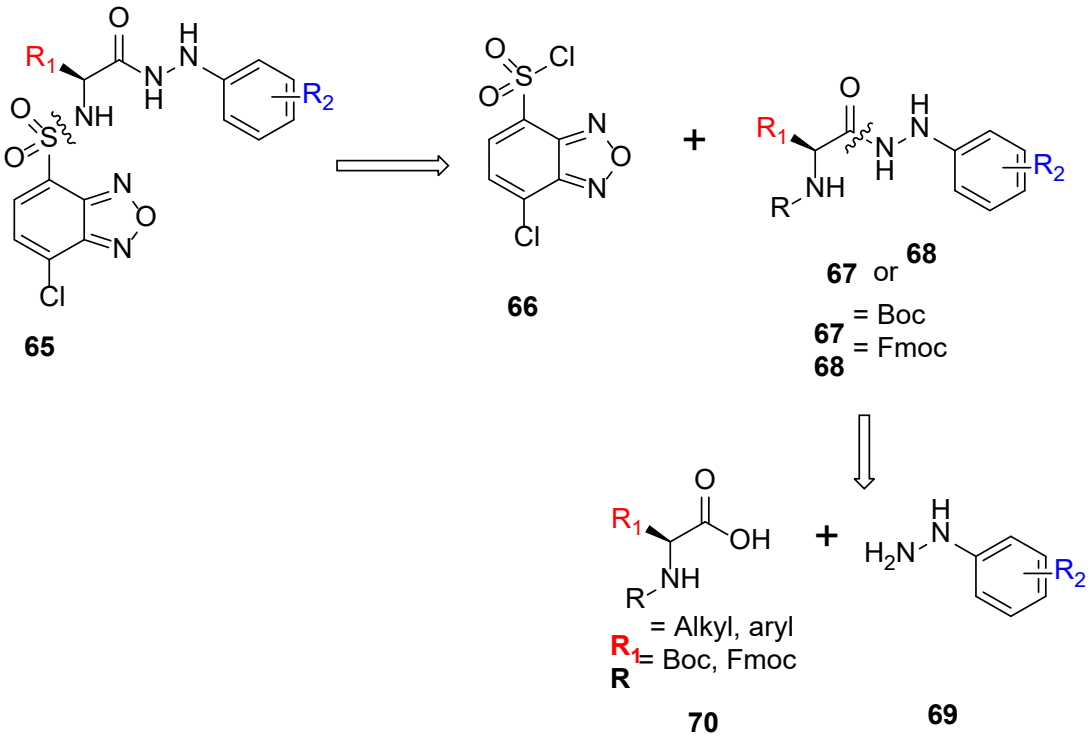


Figure 40: Splitting the architecture of final compound **65** into its substrates.

In order to produce the target compounds, an initial peptide coupling reaction between a variety of *N*-protected amino acids **70** with a large number of aryl hydrazines **69** would give rise to hydrazides **67** and **68**. Following successful

coupling, deprotection of intermediates **67** or **68** will produce the free amine and allow for its reaction with the benzoxa-[2,1,3]-diazole sulfonyl chloride **66** to deliver the final benzoxa-[2,1,3]-diazole products **65**.

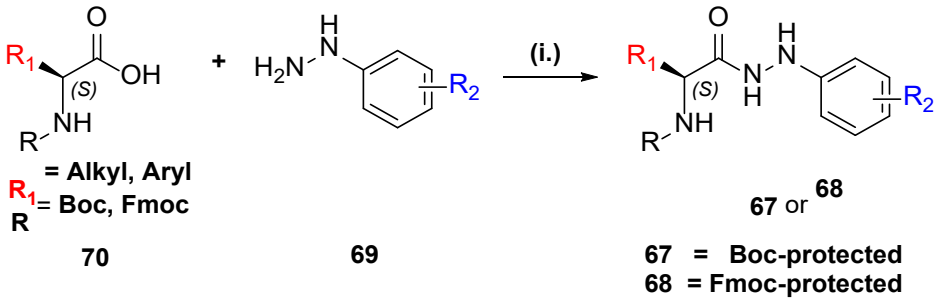
Whilst the use of Boc protected amino acids **67** will predominate, there was be a requirement to use Fmoc protected amino acids **68**. For when reactive side chains are present on the amino acid, an orthogonal process will be required to deprotect the backbone amine whilst having the side chain protection remain intact.

Consequently, both of the hydrazides **67** and **68** and the final benzo-[2,1,3]-diazole products **65** will be screened against *Mtb* utilising the REMA assay to allow for comparative analysis.

2.1.1 Synthesis of Amino acid hydrazides

To get started, the first goal was to synthesise a library of hydrazides **67** and **68.** To carry out the initial SAR investigation, we wished to explore the use of a variety of substituted aryl hydrazines **69** with the full range of amino acids **70**. To focus the study on the amino acids **70** we chose to limit the number of aryl hydrazines **69** based on the activity shown in the previous study.[1] Therefore, we looked at utilising predominantly electron withdrawing groups, donating groups and their position around the aromatic ring. Specifically, trifluoromethyl, halogens (including fluoride, chloride, bromide), nitro, nitrile, methanesulfonyl, and alkyl groups such as methyl, and isopropyl.

We began by synthesising the hydrazides **67** and **68** following the established procedure within the group. This required the coupling of amino acids **70** with aryl hydrazines **69** using standard peptide coupling reagents *N*,*N*′-dicyclohexylcarbodiimide (DCC) **71** and 1-hydroxybenzotriazole HOBt **72** to afford the desired compound **67** and **68** (Scheme 3).[1]



Scheme 3: Reagents and conditions: i.) DCC, HOBt, THF, 2-4 h, (47- 95%), r.t.[1]

Following the outlined procedure (*cf.* Scheme 3), the desired hydrazides **67** and **68** were synthesised successfully, however, the carbodiimides lead to the formation of a number of by-products.[104] As expected, this coupling method gave rise to a very poorly water-soluble by-product (1,3-dicyclohexylurea) **73** that was difficult to separate from the product (Figure 42).[105]

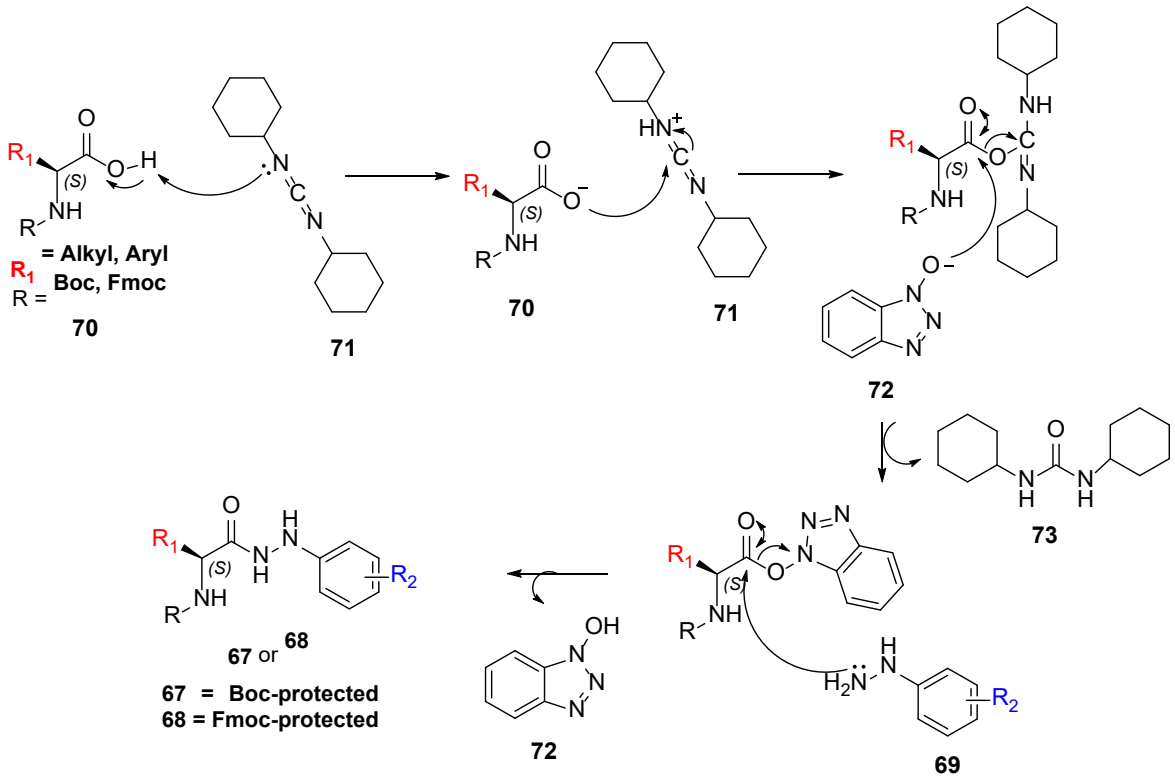


Figure 42: DCC **71** / HOBt **72** coupling mechanism forming insoluble by-product DCU **73**.

Many attempts were made to remove DCU **73**, initially by quenching the reaction once it had reached completion with n-hexane and then cooling the sample overnight in fridge. The precipitated DCU **73** was then removed by filtration,[106] however, some of the DCU **73** remained in the sample. Whilst it was suspected that small amounts of DCU contamination might be tolerated in the subsequent

coupling reaction, it was decided to repeat the reaction and carry on the synthesis without rigorous purification. Unfortunately, this approach was unsuccessful with subsequent coupling to the sulfonyl chloride **66** producing a complex mixture of products. Consequently, the experiment was repeated and after extraction of the crude product and following n-hexane precipitation and filtration of DCU, flash chromatography was used to obtain a pure hydrazide. After extensive purification, the desired hydrazide **67q** was isolated in 29% yield, when DCC **71** and HOBt **72** (*cf.* Scheme 3) were used as amide coupling reagents (Figures 43 and 44).

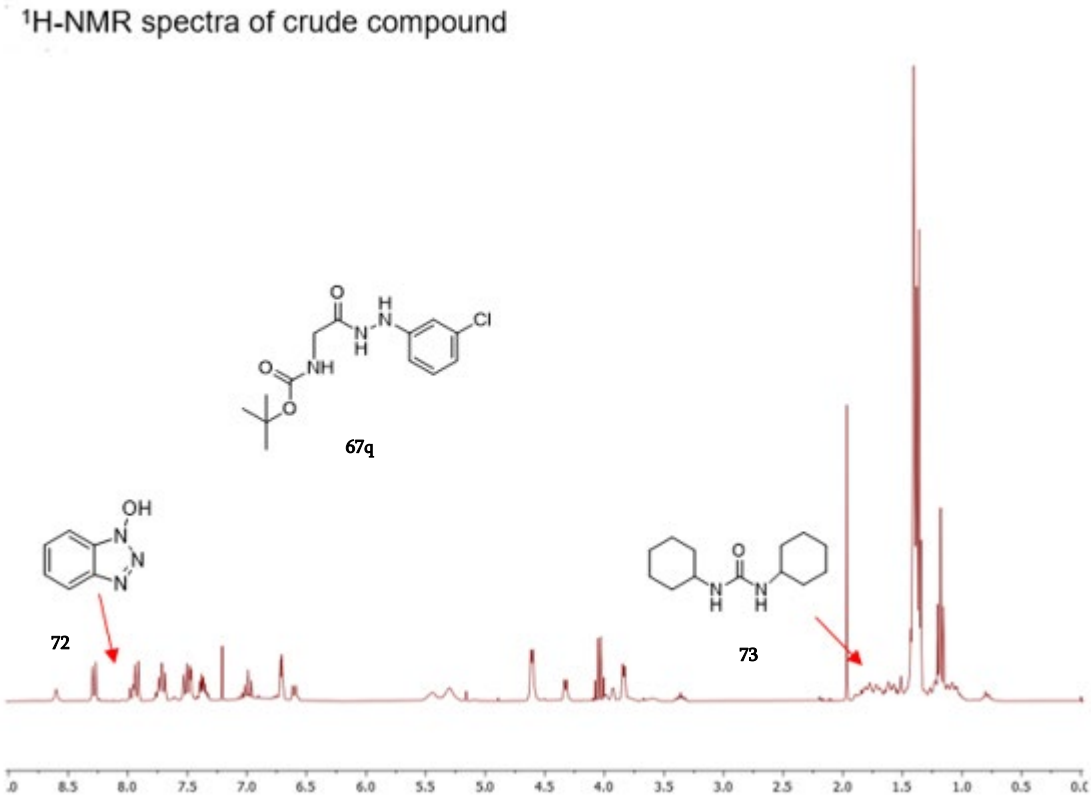


Figure 43: ^{1}H NMR spectra of crude hydrazide **67q** showing the excess of HOBt **72** and DCU **73** by-product which were still shown in ^{1}H NMR spectra of filtrated hydrazide **67q**.

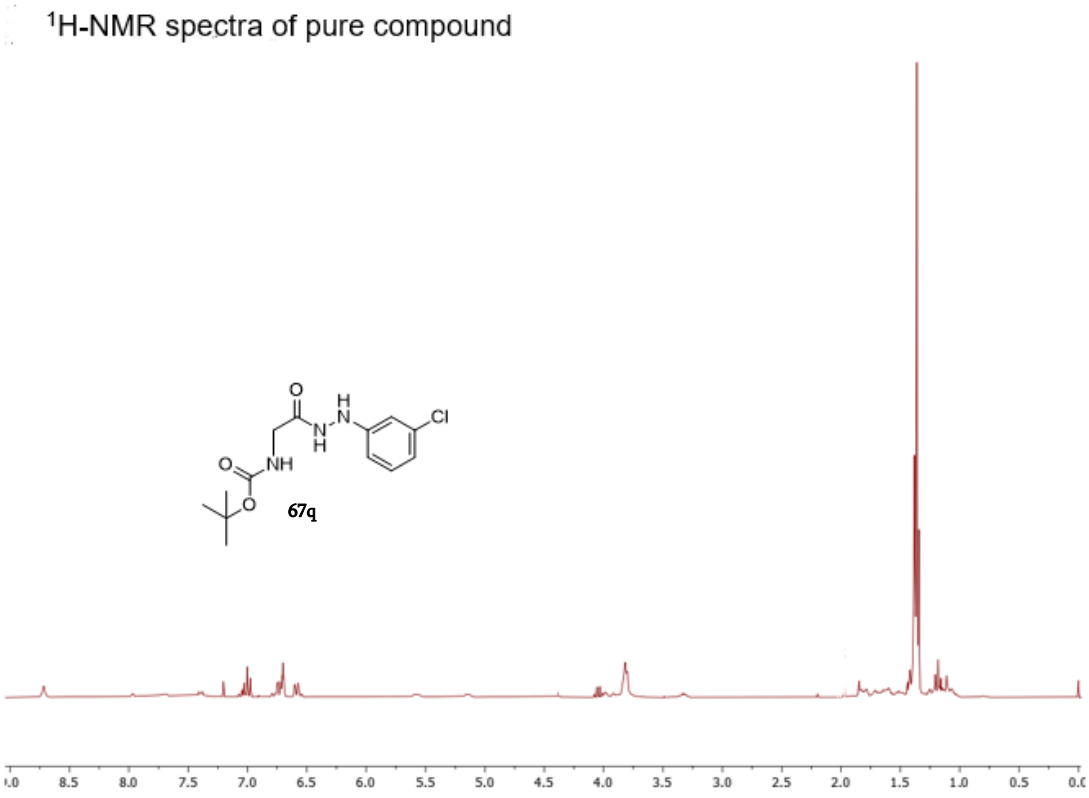


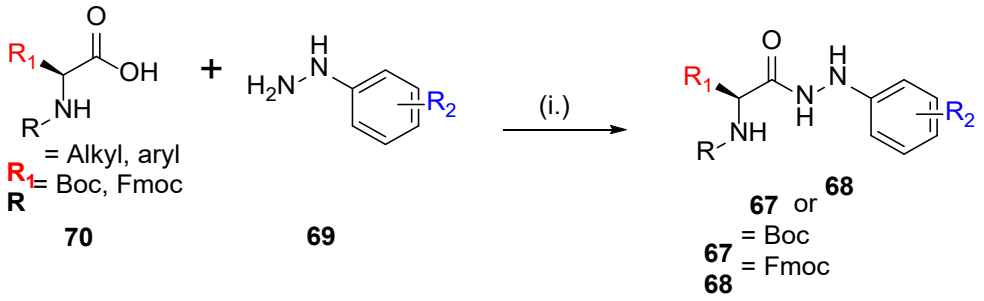
Figure 44: ^{1}H NMR spectra of pure hydrazide **67q** which had successfully been purified by using flash chromatography.

Synthesis of the hydrazide **67q** was confirmed by ^{1}H NMR and MS with peaks at 7.11 (Arylhydrazine-*H*), 6.84 (Arylhydrazine-*H*), 6.79 (Arylhydrazine-*H*), 6.67 (3-Arylhydrazine-*H*), 3.88 (glycine-CH_2), and 1.47 (Boc), ppm in the ^{1}H NMR spectrum for the aromatic hydrazine and amino acid components.

Whilst this method was able to provide pure products, the requirement of filtration and chromatography resulted in the preclusion of the rapid synthesis of a library of hydrazides. These steps also reduced the overall yield resulting in insufficient amounts of compound for sulfonamide coupling; thus, an alternative strategy was sought.

To circumvent this problem, we looked in the literature for an alternative coupling partner. 4-4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium (DMTMM) **74** has been highlighted as an effective activating agent for amide coupling reactions and for difficult couplings e.g. proline.[107] Also, DMTMM has been shown to reduce the risk of racemisation of the chiral amino acids.[107]

With this in mind, the reaction was repeated but this time the coupling of the free amine of aryl hydrazine **69** to carboxylic acid of amino acids **70** was attempted using DMTMM **74** and *N*,*N*-diisopropylethylamine (DIPEA) **75** in THF (Scheme 4).[107]



Scheme 4: Reagents and conditions: i.) DMTMM, DIPEA, THF, 6-8 h, (59- 87%), r.t.

N,*N*-diisopropylethylamine (DIPEA) **75** was added to deprotonate the carboxylic acid and/or neutralise the aryl hydrazine hydrochlorides **69** where the salts were present.[107]

Unlike DCC, this coupling method gave rise to a non-toxic triazinone by-product **76**, which is insoluble in diethyl ether, thus the minor traces were removed through trituration (Figure 45).

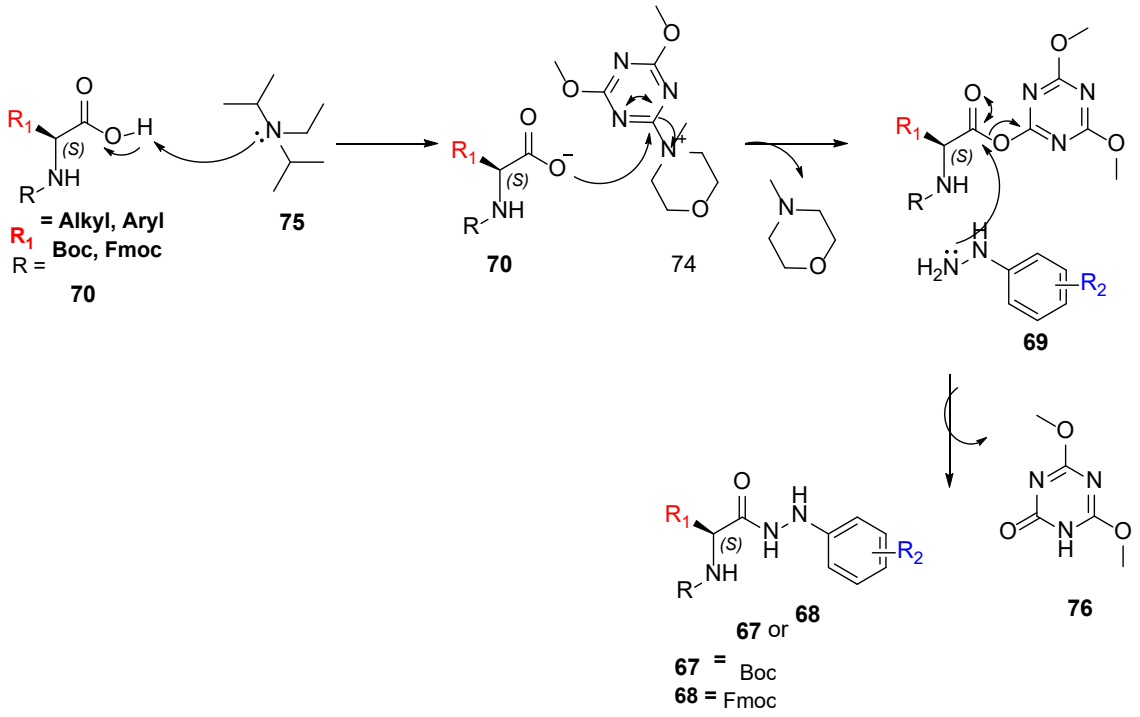
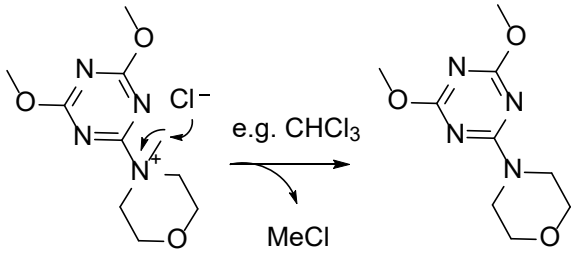


Figure 46: DMTMM **74** / DIPEA **75** coupling mechanism forming insoluble a triazinone by-product **76**.

Although it worked very well as an amide coupling reagent in our hands, this triazine chemistry has limitations such as stability in organic solution and high

cost.[104,108] According to the qualitative stability studies on DMTMM **74**, it undergoes demethylation at the morpholinium nitrogen by 50% in 15 minutes when suspended in DMF and approximately 2 hours in DMSO. While this self-immolative degradation in chloroform would take 3 hours to be completed (Figure 47).[108,109]



74 74**a**

Figure 47: Self-immolative degradation of DMTMM Cl **74** into **74a** in organic solution.[107]

Nevertheless, in THF it was found to be stable with only 13% **74a** detected after significant time around 13 hours.[108,109] This self-immolative degradation will of course have a negative impact on the stoichiometry of the reaction. The production of chloromethane will also have ramifications for the yield and purity of the desired compound as it could methylate carboxylate substrates (giving the corresponding esters) or alkylate elsewhere in the substrate or product.[107]

Another drawback is that, in some cases, the excess triazinone by-product **76** needed to be removed by flash chromatography. Consequently, this method is time consuming and requires more resource to produce the valuable hydrazides. After extensive purification, the desired hydrazide **67EI** was isolated in 60% yield, when DMTMM **74** (*cf.* Scheme 4) was used as an amide coupling reagent (Figure 48).

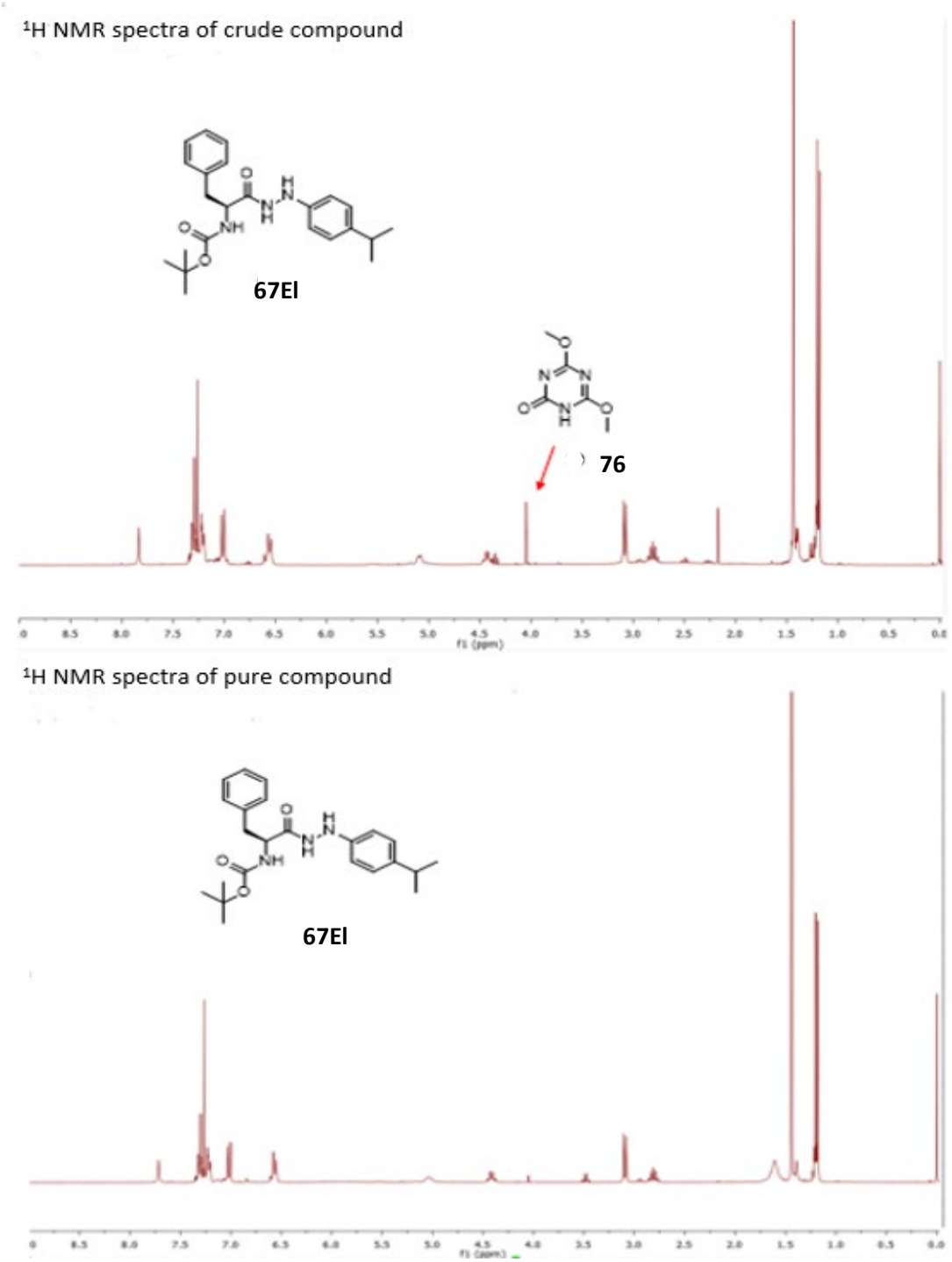
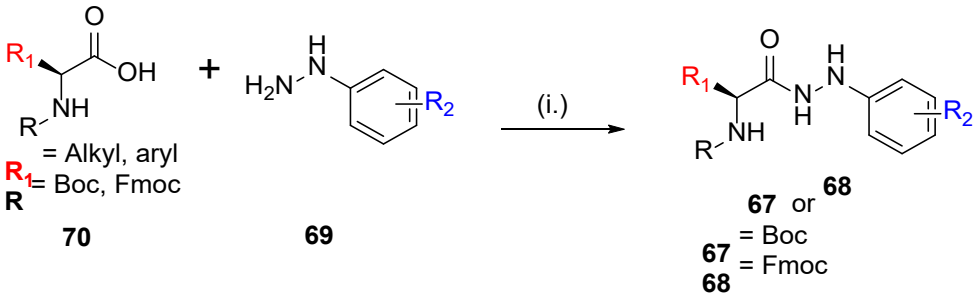


Figure 48: Example ^{1}H NMR spectra of crude hydrazide **67EI** showing the excess triazinone by-product **76** that was successfully removed using flash chromatography.

Synthesis of the hydrazide **67EI** was confirmed by ^{1}H NMR and MS with peaks at 7.33 – 7.30 (phenylalanine-*H*), 7.23 – 7.20 (phenylalanine-*H*), 7.03 – 7.00 (Arylhydrazine-*H*), 6.58 – 6.55 (Arylhydrazine-*H*), 4.45 (phenylalanine-C*H*), 3.10 (phenylalaine-CH_2), 2.86 (isopropyl-C*H*), 1.44 (Boc), 1.20 – 1.18 (isopropyl-CH_3) ppm in the ^{1}H NMR for the aromatic hydrazine and amino acid components.

Consequently, a change of coupling conditions was considered to increase the brevity of the process of hydrazide synthesis. We focused again on peptide coupling reagents and found that utilising 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) **77** led to a more efficient coupling method and higher yielding synthesis, eliminating the need for flash chromatography (Scheme 5).



Scheme 5: Reagents and conditions: i.) HBTU, DIPEA, THF, 5-6 h, (47- 96%) r.t.

Whilst HBTU **77** is an economical alternative coupling reagent, the tetramethyl urea (TMU) **78** by-product can be removed during work-up, is highly water soluble and has poor solubility in reduced polarity organic solvents such as diethyl ether. In addition to a quicker purification process, HBTU presents a low risk of racemisation (Figure 49).[110]

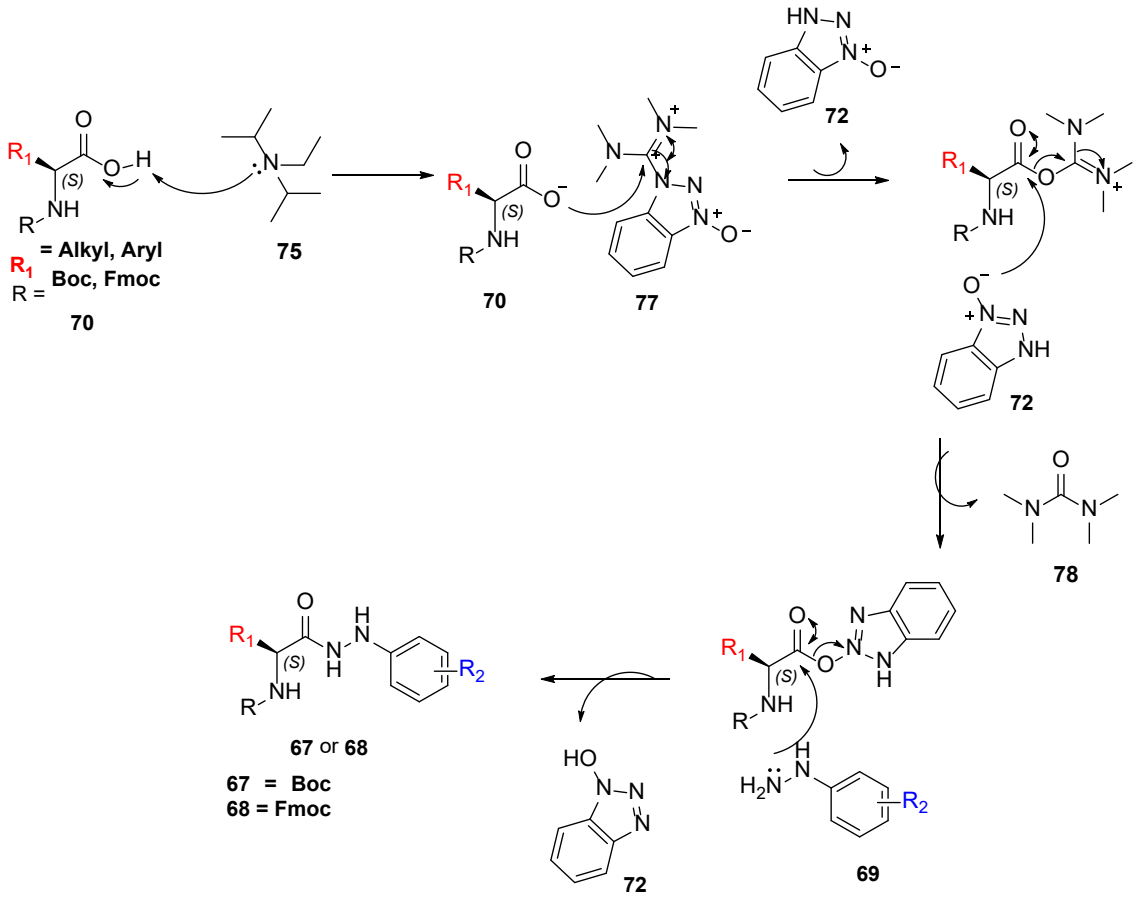
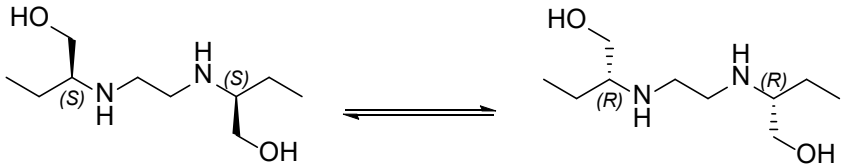


Figure 49: HBTU **77** / DIPEA **75** amide coupling mechanism forming a water-soluble by-product tetramethyl urea **78**.[110]

2.1.1.1 Enantiomeric purity determination

With HBTU working well to produce the hydrazides **67** and **68**, the configurational integrity of these products must be determined. The duality, good yield and the preservation of optical purity is important as the biological activity of chiral compounds depend strongly on the stereochemical configurations. Recently, more than 50% of medications in clinical use are chiral compounds.[111]

Chirality is important as differing enantiomers of drug molecules may exhibit marked differences in pharmacodynamics and pharmacokinetics. For example, ethambutol (EMB) **3** which is a member of front-line TB treatments, shows a tuberculostatic activity via the (S,S)-isomer while its (R,R)-isomer produces optical neuritis, which does lead to blindness (Figure 50).[111]



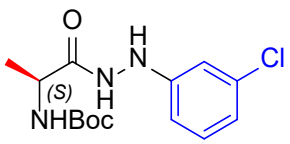
3 (*S*,*S*)-(+)-ethambutol

3 (*R*,*R*)-(-)-ethambutol

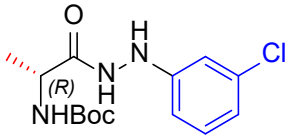
Figure 50: Concerning the absolute stereochemistry of the enantiomeric (-)-(*R*,*R*) and (+)-(*S*,*S*)-ethambutol.[112]

Although enantiomers of chiral compounds have the same chemical structure, they exhibit different optical activity. One of these enantiomers will rotate plane-polarized light counter clockwise and be laevorotatory and is by convention given a minus (-) sign, while the other which has a clockwise rotation will be dexorotatory and is given a plus sign (+).[111] With the potential for unexpected racemisation of the chiral carbon of the amino acid **70** in the first step of the synthesis upon activation of the α-carboxylic acid with HBTU, it was important to investigate the chiroptical properties of the obtained hydrazides. In order to do this, a polarimeter was used to measure the specific optical rotation ($[\alpha]_D$) of hydrazides **67r** with *S* enantiomer of alanine and **67n** with *R* enantiomer of alanine.[111] Consequently, the hydrazides **67r** and **67n** were dissolved (5 mg in 1 mL of methanol) and analysed with an Optical Activity PolAAR 2001 automatic polarimeter. The hydrazides **67r** and **67n** produced specific optical rotation ($[\alpha]_D$) of (-) **35** and (+) **33.4** respectively. This result confirms that the chiral properties of the enantiomers has not been compromised during the coupling step.

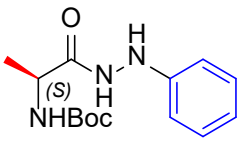
To further confirm the specific optical rotation, another hydrazide pair **67b** with S and **67An** with R enantiomers of alanine was also performed. **67b** and **67An** were synthesized utilizing DMTMM **74** as coupling reagent with a good resistance to racemization. The $[\alpha]_D$ values were measured at higher concentration (10 mg/mL) for both returning values of (-) **47.5** and (+) **45.8** respectively, again confirming that this coupling route provides optically pure compounds (Figure 51).



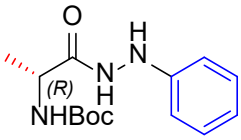
67r
(-) 0.175



67n
(+) 0.167



67b
(-) 0.475



67An
(+) 0.458

Figure 51: The corresponding hydrazides **67r, 67n**, **67b** and **67An** with their observed optical rotation values.

Taking the analysis one step further, chiral HPLC was utilized to further confirm the chiral purity of hydrazides **67** prior to their biological evaluation. Consequently, the hydrazides **67r** with S and **67n** with R enantiomers of alanine were ran separately through the chiral HPLC system using a Daicel Chiralpak® IB column (1 mL/ minute, 94:5:1 acetonitrile: methanol: 0.1% formic acid) to confirm the obtained hydrazides **67** were contained only as a single enantiomer (Figure 52 and 53).

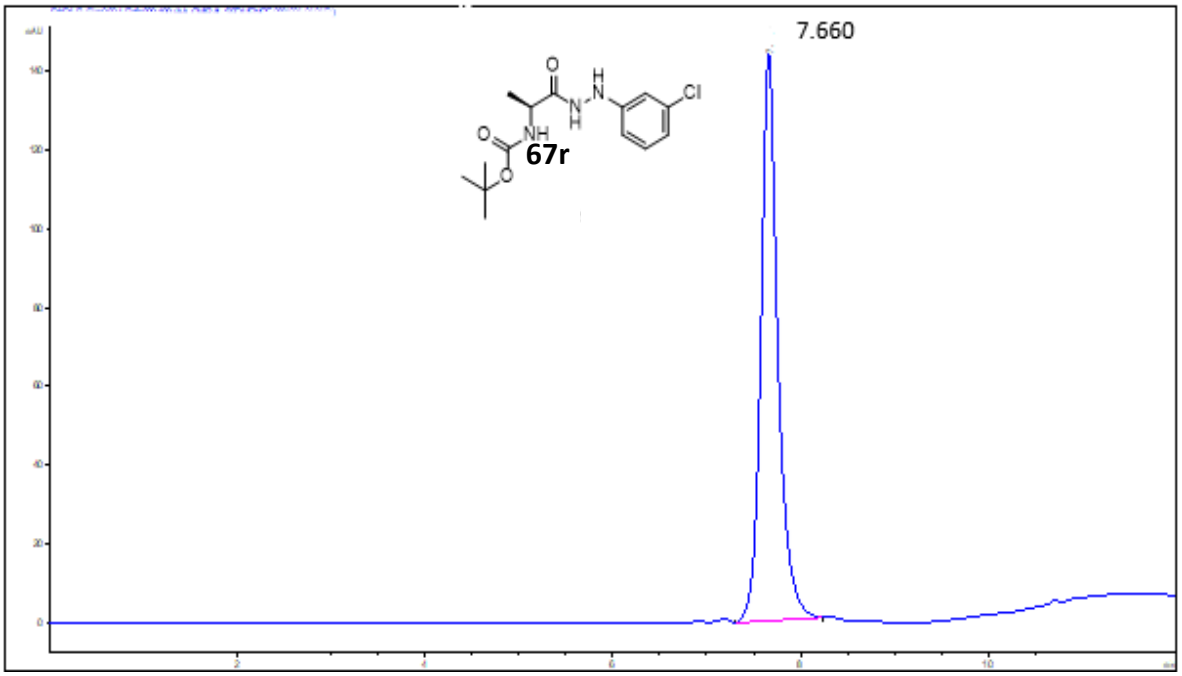


Figure 52: chiral HPLC of hydrazides **67r** indicating the presence the only one single peak, Daicel Chiralpak® IB column, 4.6 x 250 mm, 5µm particle size, acetonitrile: methanol: 0.1% formic acid (94:5:1), 1 mL/min, UV at 260 nm.

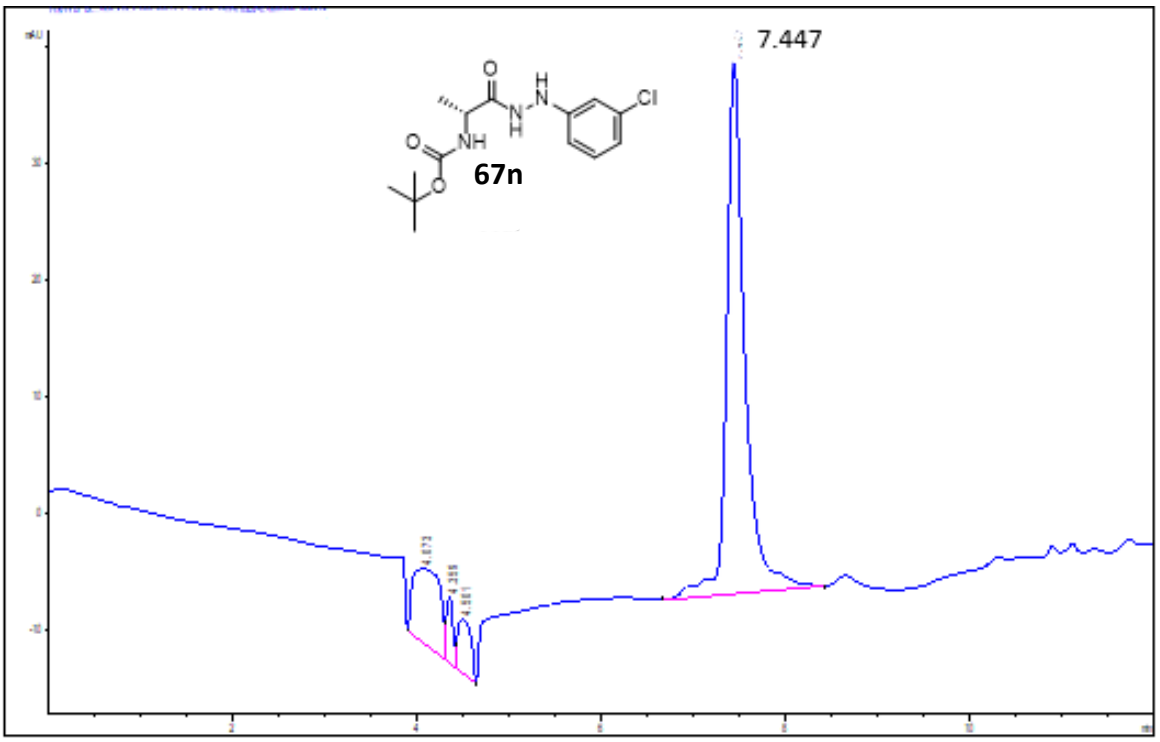
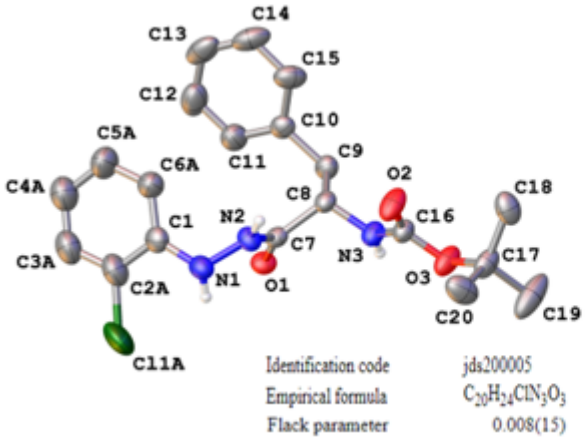
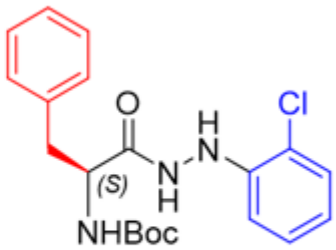


Figure 53: chiral HPLC of hydrazides **67n** indicating the presence the only one single peak, Daicel Chiralpak® IB column, 4.6 x 250 mm, 5µm particle size, acetonitrile: methanol: 0.1% formic acid (94:5:1), 1 mL/min, UV at 260 nm.

With sufficient evidence to confirm that the coupling step does not racemise the chiral centre, we were fortunate enough to be able to obtain a crystal structure for compound **67Aw** containing L-phenylalanine by slow evaporation from a dichloromethane solution. Analysis of the crystal structure led to the determination of the unambiguous structure of hydrazide **67Aw** and assignment of absolute structure, with the flack parameter determined to be 0.008(15) (Figure 54).[113]



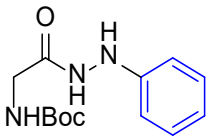


67Aw

Figure 54: Crystal structure determination of non-centrosymmetric hydrazide **67Aw** with flack parameter close to zero indicating high confidence.

2.1.1.2 Generation of a Library of Amino acid hydrazides

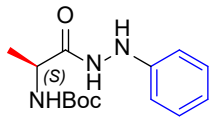
Having established coupling conditions that would allow for enantiomerically pure hydrazides to be created, subsequent studies focused on synthesis of a series of hydrazides **67** and **68** to evaluate their structure activity relationship. Initially the decision was made to vary the *L*-amino acids **70a-t** whilst utilising a simple hydrazine, phenylhydrazine **69a** to assess the synthesis on the full scope of substrates. Additionally, in our previous studies, hydrazide **67a** was synthesised, screened and returned an MIC of 90.46 μM against wildtype *Mtb* (Figure 55).[1] During the course of our research it was observed that some of the resulting compounds had been produced as a mixture of rotamers, especially when with a Boc-Gly-OH unit. This was not investigated until the final compounds were produced and the results are presented in section 2.1.2.6.



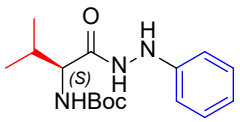
67a
55%
4:1
90.46 μM

Figure 55: Structure of unsubstituted amino acid hydrazide **67a** with a percent of actual yield, rotamer ratio and MIC value against the wild-type *Mtb*.[1]

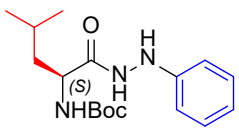
Consequently, the first ten compounds produced were Boc-protected hydrazides **67a-j** and the Fmoc-protected hydrazides **68a-j** (Figure 56). Both were characterized as previously described and afforded in an acceptable yield.



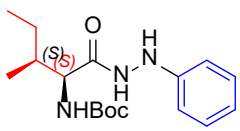
67b
33%



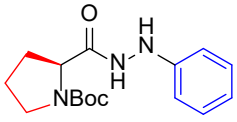
67c
24%
4:1



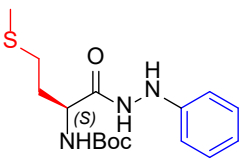
67d
19%



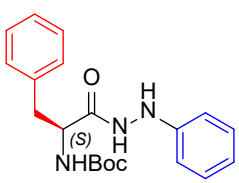
67e
93%



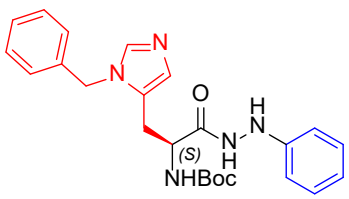
67f
52%



67g
23%



67h
50%
5:1



67i
70%

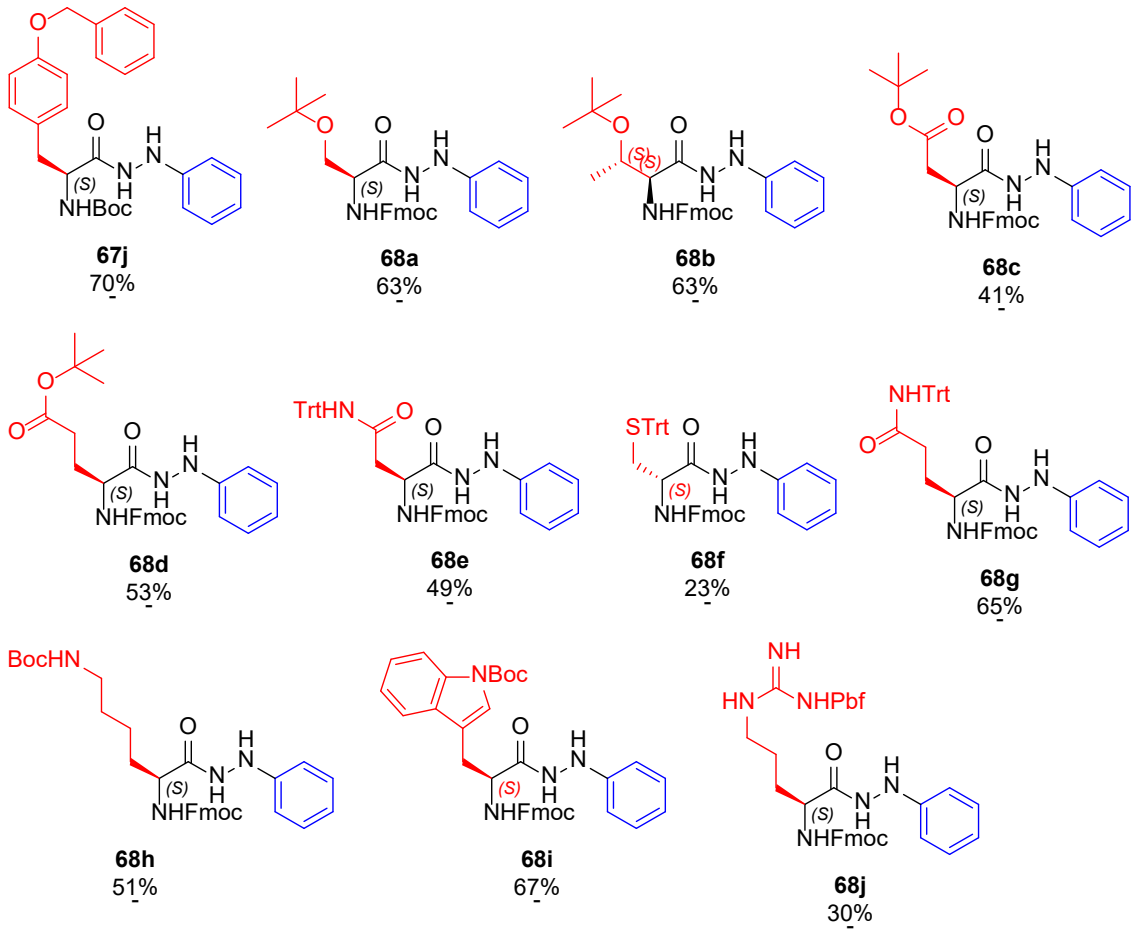


Figure 56: The corresponding Boc-protected hydrazides **67a-j** and Fmoc-hydrazides **68a-j** where (%) a percent of actual yield and (-) lack of rotamer ratio.

In addition to the L-amino acids, three unnatural L-amino acids were also coupled with phenylhydrazine to assess the contribution of modified side chains in improving the potency of the resulting hydrazides **67k-m** (Figure 57).

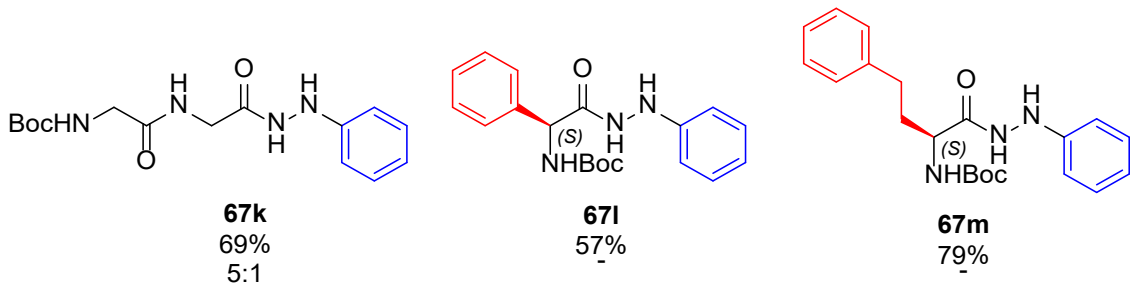


Figure 57: The corresponding hydrazides **67k-m** containing unnatural L-amino acids where (%) a percent of actual yield and (-) lack of rotamer ratio.

In view of the role of the chirality in the development of antibiotics [114] and in order to expand the scope of the SAR study, we produced the hydrazides **67n-p** with amino acids in D- configuration to compare their antitubercular activity to that of the hydrazides **67b**, **67g** and **67h** containing natural L-amino acids to determine the most active enantiomer (Figure 58).

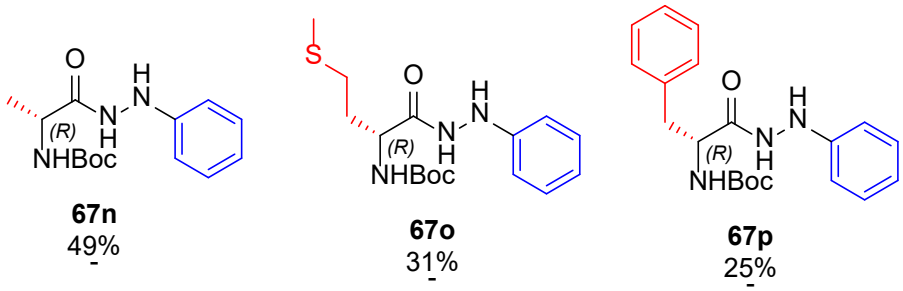


Figure 58: The corresponding hydrazides **67n-p** containing D-amino acids where (%) a percent of actual yield and (-) lack of rotamer ratio.

Electron withdrawing groups

Completion of this initial series saw attention turn to changes to the hydrazine to realise the aim of the project. We next investigated substitution of the aromatic ring with halogens, a common modification in medicinal chemistry.[115] Their incorporation, especially the lighter fluorine and chlorine atoms, enhance permeability by increasing log P and allow the molecule to occupy deeper hydrophobic regions of the target protein.[115]

Moreover, as shown in previous work and from work ongoing within the group, the presence of chlorine in the *meta* position with enlarging side chains of the L-amino acid fragment, provides promising antitubercular activity (Figure 59).[1,2]

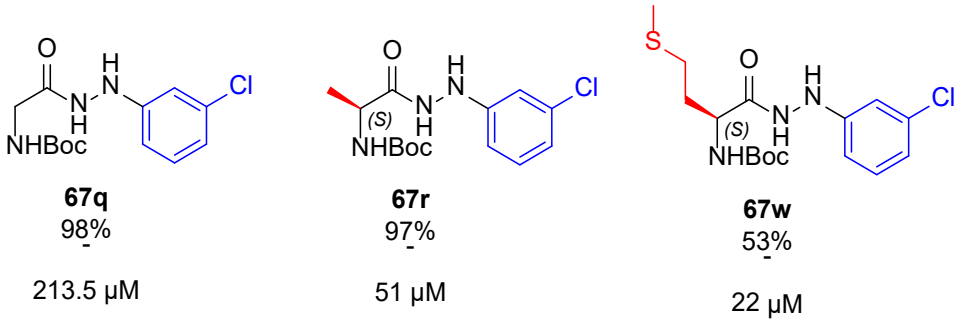


Figure 59: Results of synthesis of substituted amino acid hydrazides **67q,r,w** and their MIC values.[1,2]

So, to expand this area and investigate the impact of chlorine at the *meta* position of the aryl hydrazine fragment **69**, synthesis of hydrazides was expanded to

include all L-amino acids, three selected unnatural L-amino acids, and D-amino acids (Figure 60).

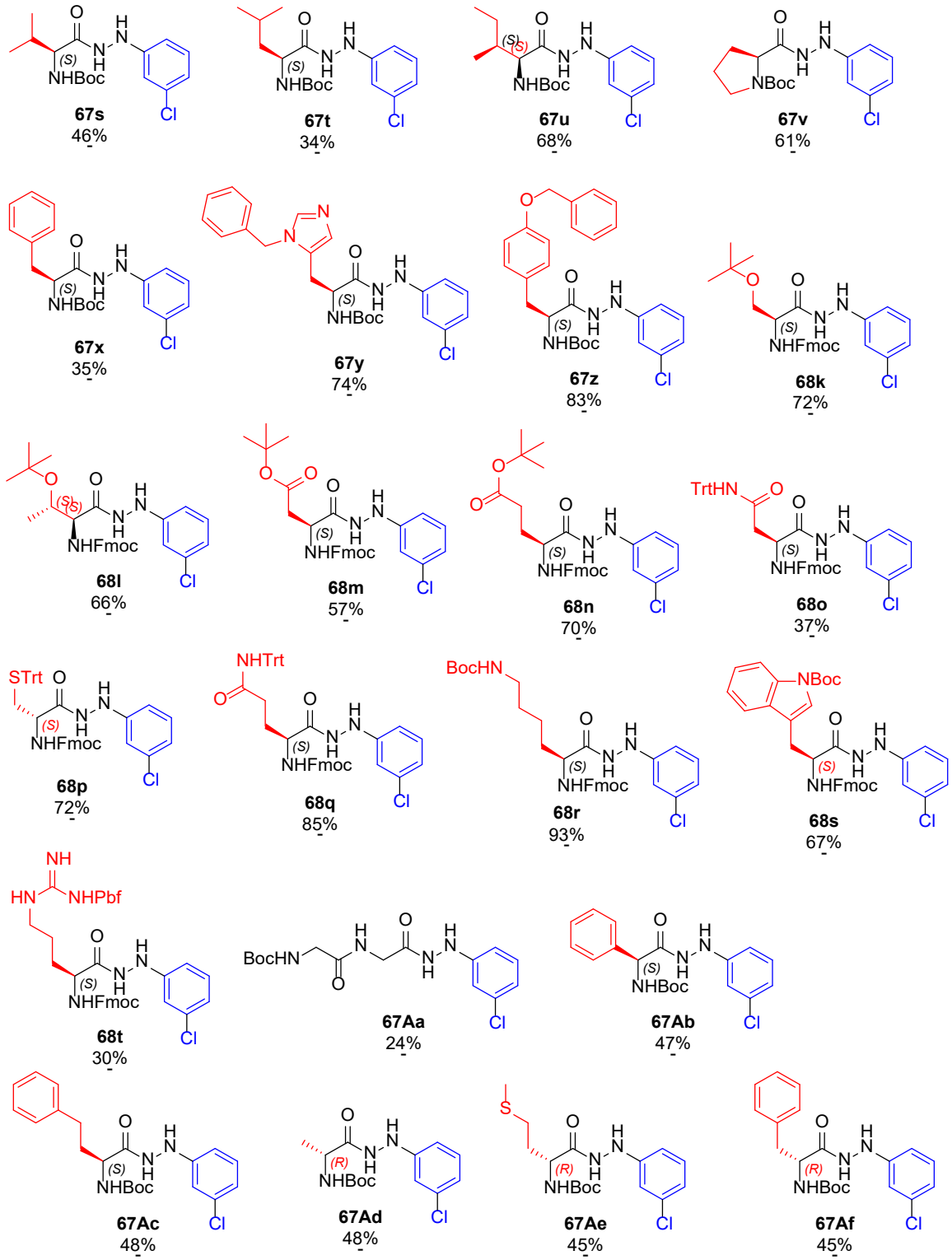


Figure 60: The chlorinated Boc-*N*-hydrazides **67q-z**, Fmoc-*N*-hydrazides **68k-t**, Boc-*N*-hydrazides **67Aa-Ac** containing unnatural *L*-amino acids and Boc-*N*-hydrazides **67Ad-Af** containing *D*-amino acids at the *meta* position where (%) a percent of actual yield and (-) lack of rotamer ratio.

With promising MIC results for chlorinated hydrazides **67r, w** from our previous studies, exploration of mono-substituted Boc-hydrazides with halogens attached was something to be explored further. Consequently, we initiated a study to explore the 2- and 4-positions of aryl hydrazines with halogens, initially chloride, as previously known examples exhibited promising antitubercular activity (Figure 61).[1]

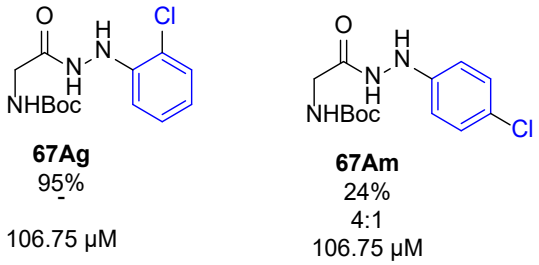


Figure 61: Results of synthesis of substituted amino acid hydrazides **67Ag** and **67Am** and their MIC values where (%) a percent of actual yield and (-) lack of rotamer ratio.[1]

Therefore, the *ortho* chlorinated Boc-hydrazides **67Ag-Ak** and *para* substituted analogues **67x** and **67Am-Ar** were obtained in good to moderate yields (Figure 62).

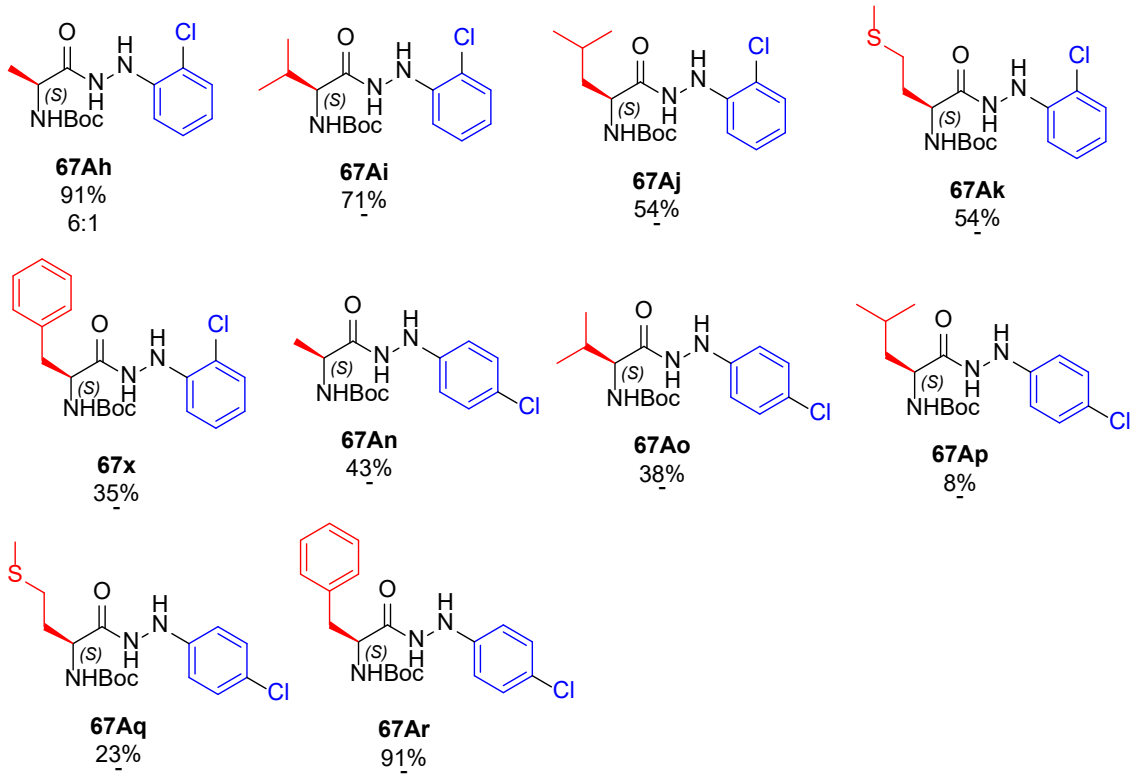


Figure 62: The *ortho* chlorinated Boc-*N*-hydrazides **67Ag-Al** and the *para* chlorinated **67Am-Ar** where (%) a percent of actual yield and (-) lack of rotamer ratio.

In addition to mono-substitution, di-substitution of the aromatic rings was something we wished to explore. Therefore, the synthesis of di-*ortho* chlorinated hydrazides was achieved with a variety of amino acids (Figure 63).

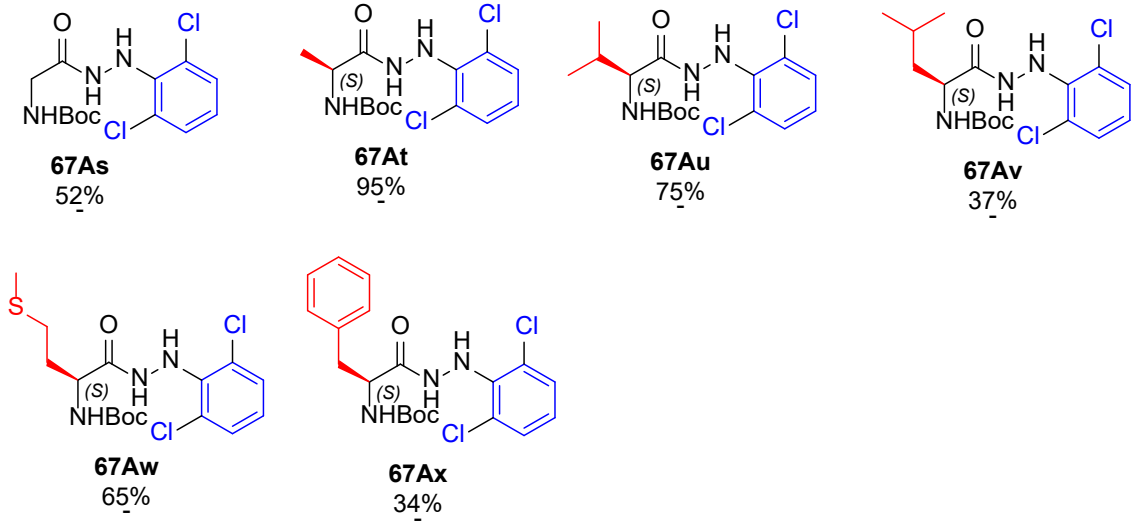


Figure 63: The di-*ortho* chlorinated Boc-protected hydrazides **67As-Ax** where (%) a percent of actual yield and (-) lack of rotamer ratio.

Other halogens, such as fluorine are contained within drugs such as fluoroquinolones (*cf.* section 1.3.5) that are of particular interest as the strong electron-withdrawing effect or isosteric similarity to a proton contribute to enhanced antibacterial activity.[116] In previous work from within the group, the use of trifluoromethyl moieties demonstrated good antitubercular activity (Figure 64).[1]

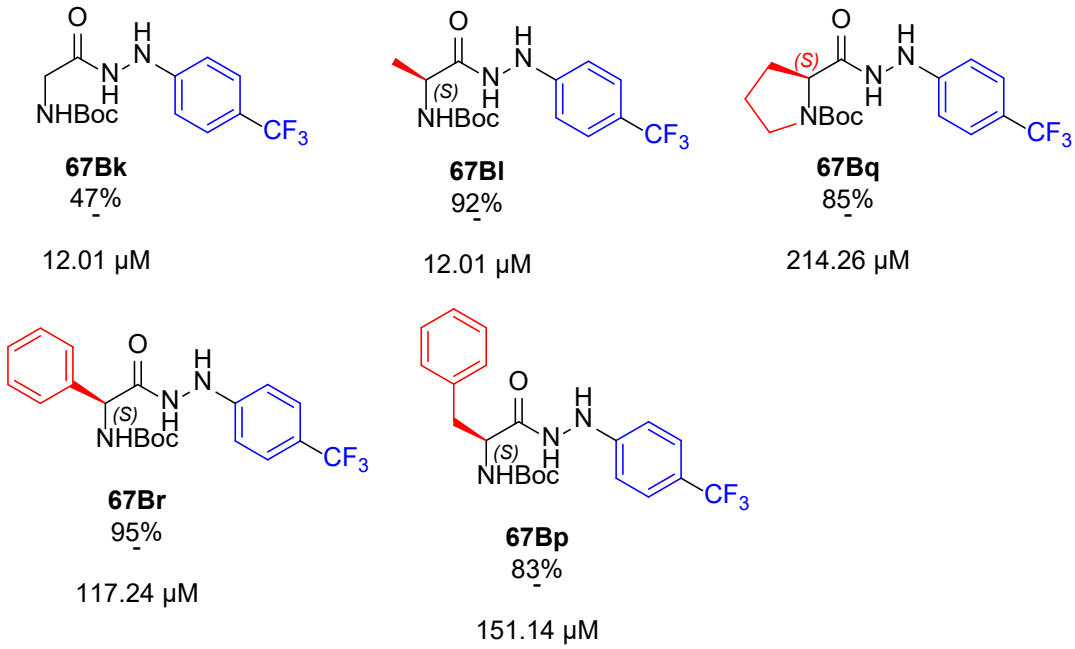


Figure 64: Results of synthesis of substituted amino acid hydrazides **67Bk, 67Bl, 67Bq, 67Br** and **67Bp** and their MIC values where (%) a percent of actual yield and (-) lack of rotamer ratio.[1]

Consequently, hydrazides of the remaining amino acids containing the trifluoromethyl group in the *para* position were synthesised for further investigation (Figure 65).

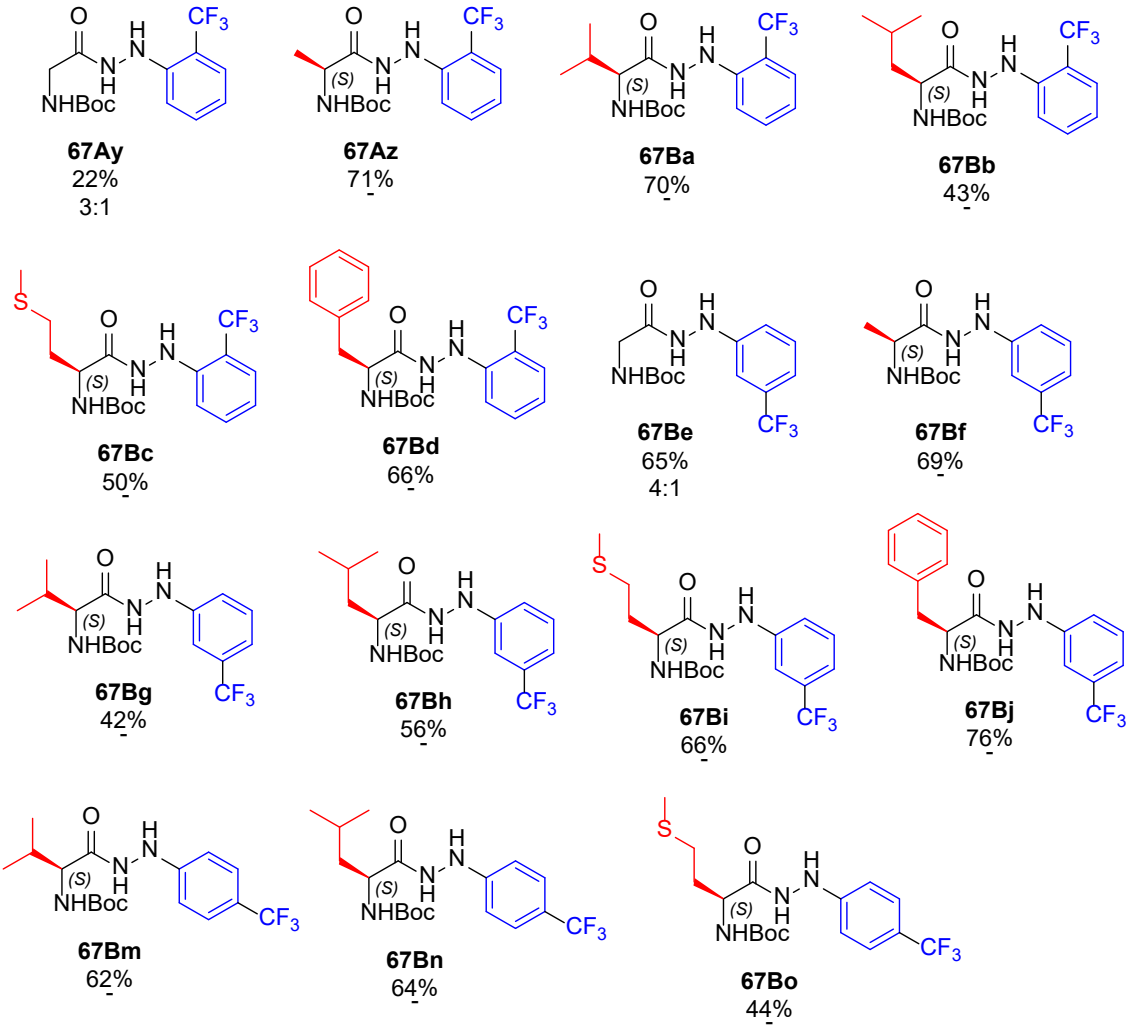


Figure 65: The corresponding boc-hydrazides **67Ay-Bp** containing trifluoromethyl group on aryl hydrazine where (%) a percent of actual yield and (-) lack of rotamer ratio.

Furthermore, like with the halogens, synthesis of disubstituted trifluoromethylated hydrazides **67Bs-Bx** at *meta* positions was undertaken to understand the relationship between mono and di-substitution of the aromatic ring (Figure 66).

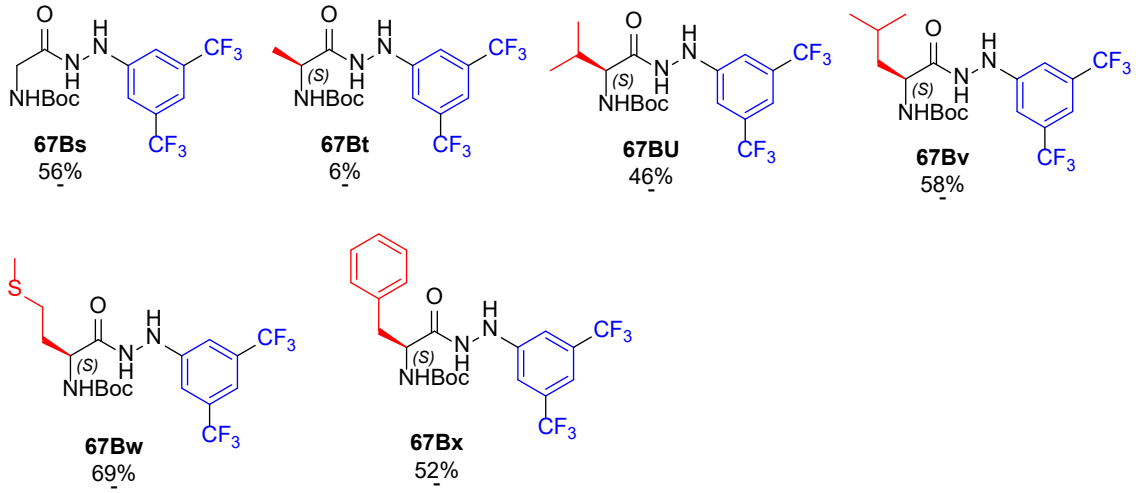


Figure 66: The corresponding boc-hydrazides **67Bs-Bx** containing two trifluoromethyl groups on aryl hydrazine.

As previously stated, introduction of fluorine into bioactive molecules has the potential to improve their potency.[115] In order to further investigate this, it was decided to synthesise the fluorinated Boc-hydrazide **67By-Cd** possessing fluorine in the 2, 4- positions. Initial attempts to synthesise this in previous work utilising DMTMM as a coupling reagent yielded the product in poor yield. However, the revised synthetic route utilising HBTU was able to improve the yield significantly and produce a small library of hydrazides (Figure 67).

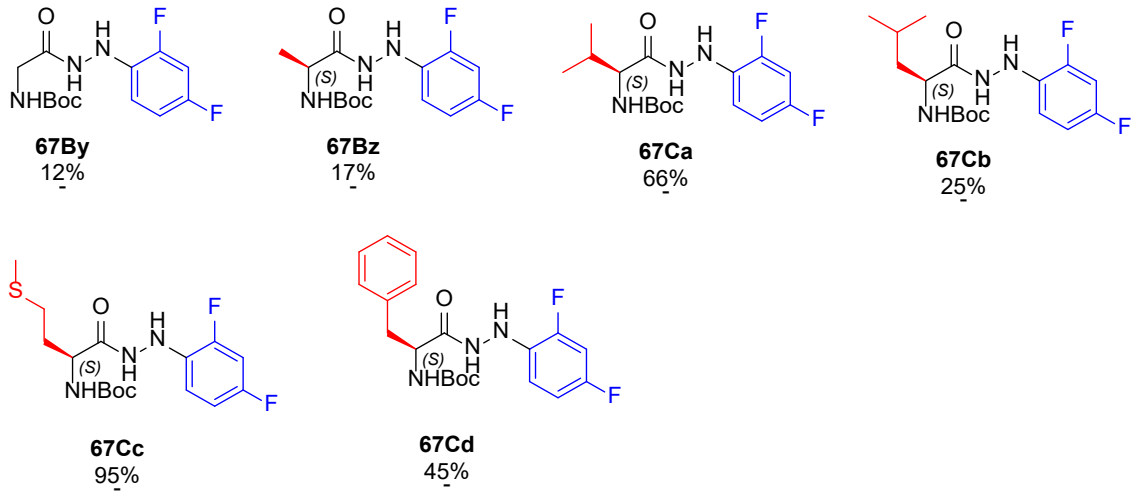
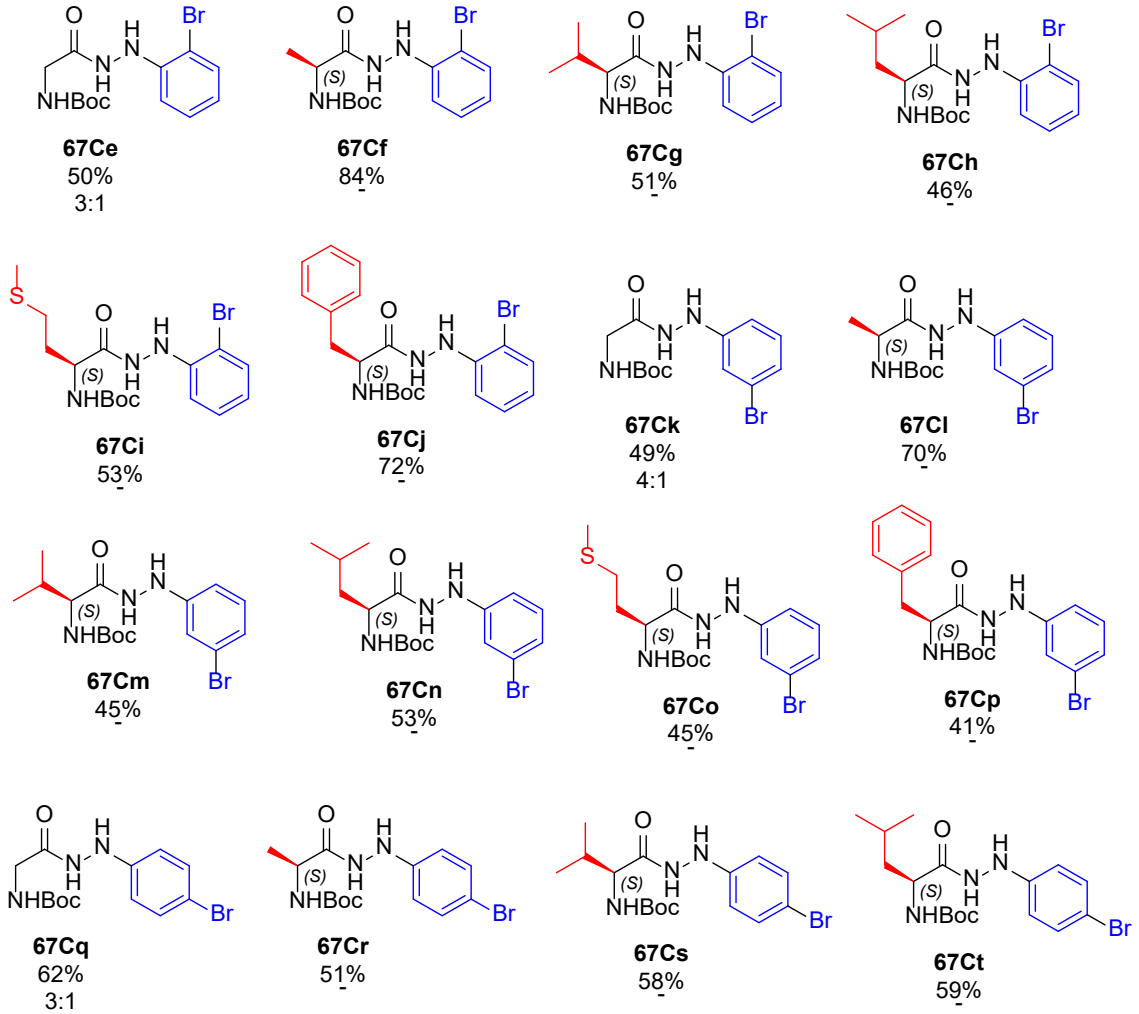


Figure 67: The corresponding boc-hydrazides **67By-Cd** containing two fluorine atoms on aryl hydrazine.

The success of bedaquiline **22** (*cf.* section 1.3.7) has generated significant interest in the use of bromine in drug molecules, therefore, it was decided to synthesise the brominated hydrazides at all three aromatic positions, mirroring the chlorine analogues. However, the presence of heavy halogen like bromine is not considered a privileged substituent in medicinal chemistry due to safety issues.[117] Bromine-containing drugs, which are targeted at the lungs, have been associated with pulmonary toxicity because they have an extremely long half-life and low water solubility. Nevertheless, the brominated hydrazides **67Ce-Cv**, were obtained to investigate their potency against *Mtb* because the potency of antitubercular drugs is often positively associated with their lipophilicity (Figure 68).



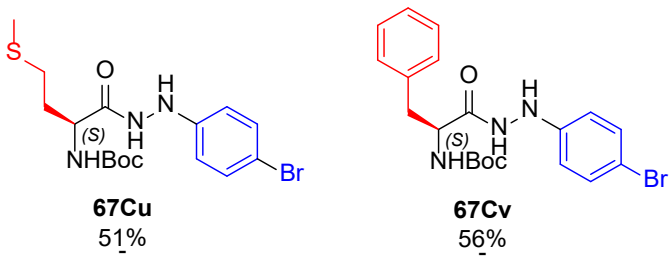


Figure 68: The corresponding boc-hydrazides **67Ce-Cv** containing bromine at different positions.

Having sufficient coverage of the halogens, it was decided to incorporate a nitrile functionality onto the C-4 position of the aryl hydrazine **69**. The nitrile moiety is considered a substituent of choice in SAR studies because of its polarity, directionality, and low molecular weight. As a result of these properties, introduction of a nitrile group is an important chemical motif found in drug candidates (Figure 69).[118]

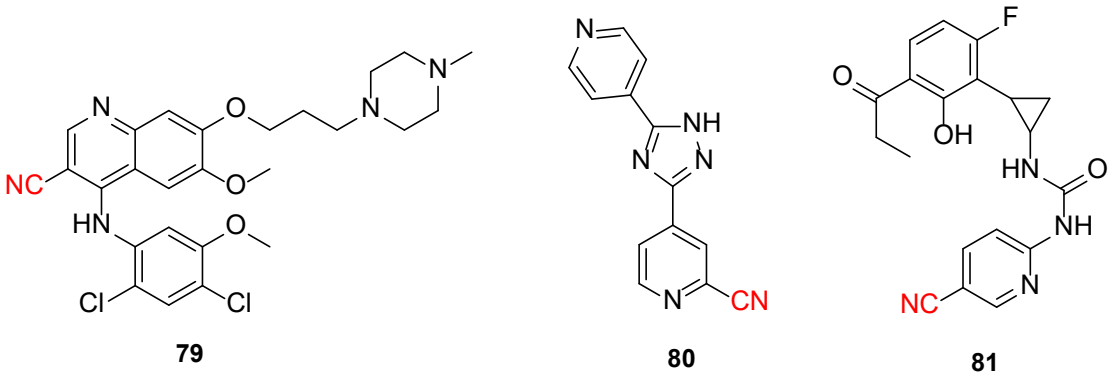
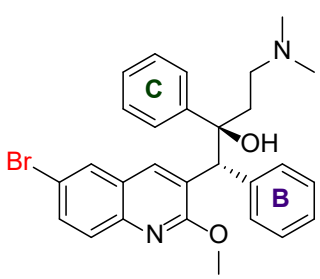
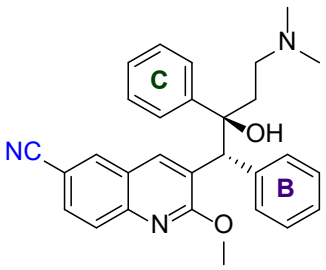


Figure 69: The nitrile-containing drugs: bosutinib **79**, anti-cancer; topiroxostat **80**, anti-gout; MIV-150 **81**, anti-HIV.

Moreover, as exemplified by Tong *et al.*, the high lipophilicity of bedaquiline (BDQ) **22** may contribute to its induction of phospholipidosis, potent inhibition of hERG channel, resulting in QT prolongation and tissue overproportioned accumulation which leads to a long terminal elimination half-life. These findings suggest that less lipophilic bedaquiline analogues would be of potential interest, to improve its pharmacokinetic properties. Consequently, 6-cyano analogues **83** demonstrated a reduction in lipophilicity and potentially safer diarylquinolines, and had only modest effects on MIC values (Figure 70).[119]



82



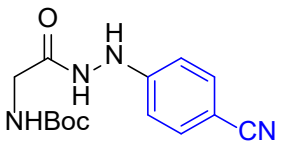
83

		clogP	MIC_{90} (mg/ml)		clogP	MIC_{90} (mg/ml)
B = Ph	C = 3-F	6.22	0.23		4.86	0.69
B = 2-F, 3-OMe	C = 3-F	6.22	0.10		4.87	0.18
B = 2-F, 3-OMe	C = 3-OMe	6.00	0.10		4.64	0.09
B = C = 2,3-diOMe		4.99	0.20		3.64	0.34

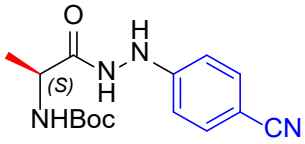
clogP lowered
MIC_{90} retained

Figure 70: The bedaquiline analogues **82** and 6-cyano analogues **83** with their clogP and MIC_{90} values.[119]

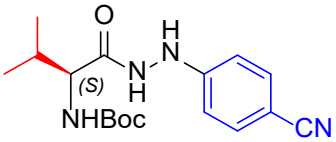
Encouraged by this, the *para* substituted nitrile hydrazides **67Cw-Db** were afforded in good yields following the standard procedure to evaluate their antitubercular activity in comparison to the other electron withdrawing hydrazides (e.g. **67Bp**) (Figure 71).



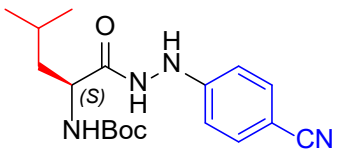
82 %
5:1
67Cw



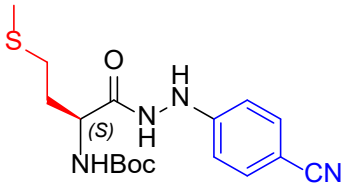
78 %
67Cx



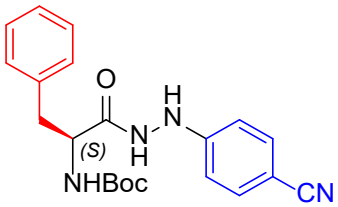
52 %
67Cy



63 %
67Cz



35 %
67Da



68 %
67Db

Figure 72: The corresponding Boc-hydrazides **67Cw-Db** containing nitrile on aryl hydrazine.

Whilst the nitrile moiety provides a polar, directional substituent with electron withdrawing properties, literature searches have shown that there is a great deal of interest in nitro compounds that have emerged as potent molecules against replicating and non-replicating *Mtb*.[67,120,121] One example that is commonly reported in the literature is the nitroimidazoles (*cf.* section 1.3.7) such as delamanid **25** and pretomanid **26** that have shown potent intracellular activity in the dormancy stage due to the reductive activation of the nitro group which results in the production of nitric oxide.[67,120,121] From a medicinal chemistry perspective, the structural modifications may improve activity, therefore, it was decided to synthesise hydrazides **67Dc-Dh** containing a nitro group at *meta* position for further investigation (Figure 72).

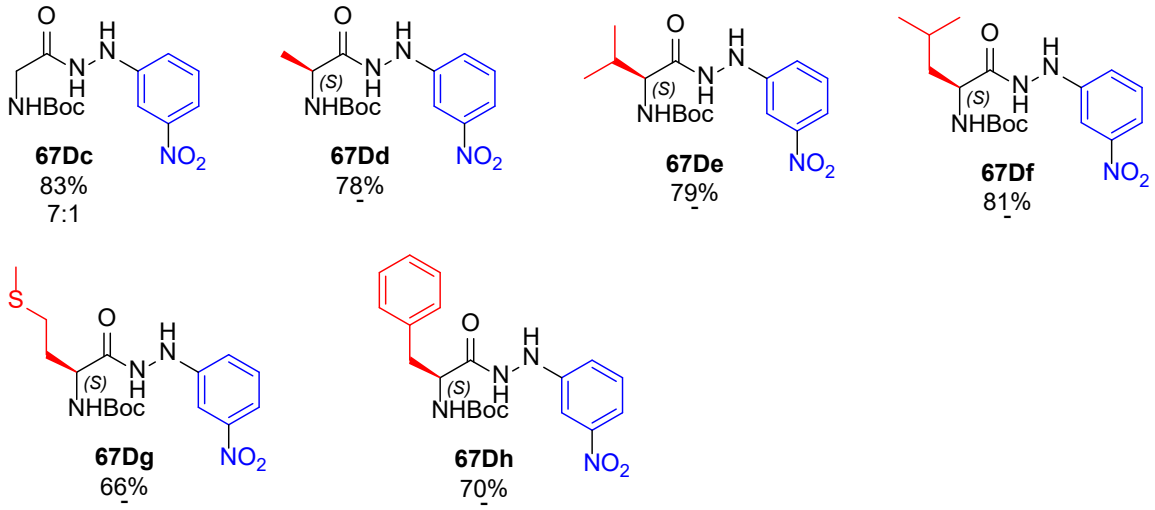
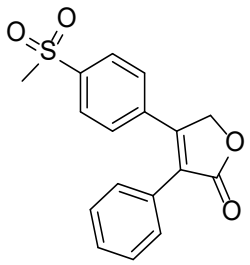


Figure 72: The corresponding Boc-hydrazides **67Dc-Dh** containing nitro on aryl hydrazine.

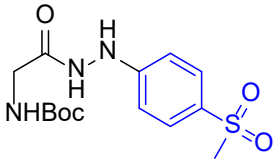
Along the same lines, incorporating another powerful electron withdrawing group such as a sulfonyl group has the potential to affect the antitubercular activity of these molecules. In 1999, rofecoxib **84**, a common member of cyclooxygenase 2 (COX-2) selective nonsteroidal anti-inflammatory drugs (NSAID), was approved to treat chronic or acute pain conditions such as osteoarthritis, rheumatoid arthritis and migraine. Nevertheless, it was withdrawn from the market as the long term use of rofecoxib **84** increased the risk of cardiovascular thrombotic events. According to the SAR analysis for rofecoxib **84**, a methylsulfonyl moiety confers the inhibitory potency and selectivity against the COX-2 enzyme (Figure 73).



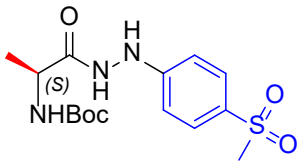
84

Figure 73: The chemical structure of rofecoxib **84** which is one of methylsulfonyl drug containing.

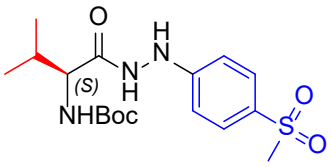
The methylsulfonyl group had not been investigated further in previous work. Hence, the desired hydrazides **67Di-Dn** were obtained in good yields utilising HBTU to couple the components to assess the effect of the presence of the methylsulfonyl moiety on MIC (Figure 74).



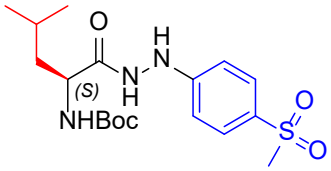
67Di
84%



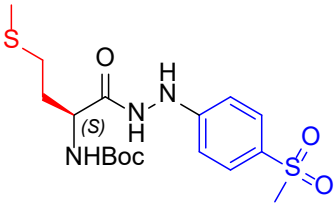
67Dj
99 %
5:1



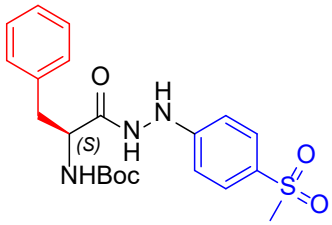
67Dk
59 %



67Dl
54 %



67Dm
61 %



67Dn
36 %

Figure 74: The corresponding Boc-hydrazides **67Di-Dn** containing methylsulfonyl on aryl hydrazine.

Electron donating groups

To this point, electron withdrawing components were the focus, however, we also wanted to explore the effect of electron donating groups. Our initial starting point was to consider the incorporation of a methyl group. The importance of a methylation strategy has been documented in drug design, and shown to enhance the pharmacological activity, selectivity, solubility, metabolism and pharmacokinetic/pharmacodynamic properties of drug candidates. Indeed, substitution with a single non-bioisosteric group led to a sharp increase in the potency, for example, methylated safinamide **85a**, which is a monoamine oxidase type B inhibitor, resulting in a >20-fold raise of the potency and nearly 1000-fold enhancement in selectivity (Figure 75).[122]

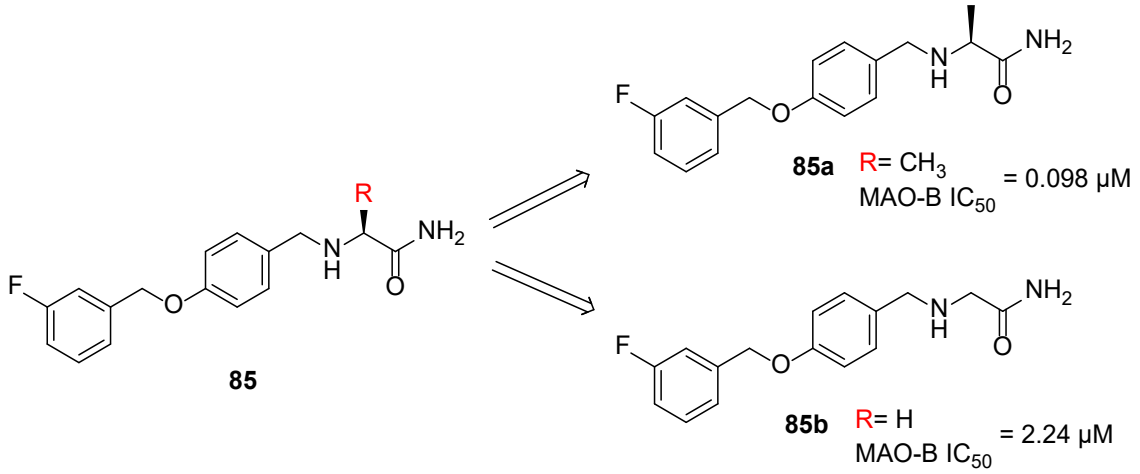


Figure 75: The methylated safinamide **85a**.

To study this effect, substituted hydrazides with a methyl group at all three aromatic positions were produced following the standard procedure in good yield following the standard procedure with HBTU (Figure 76).

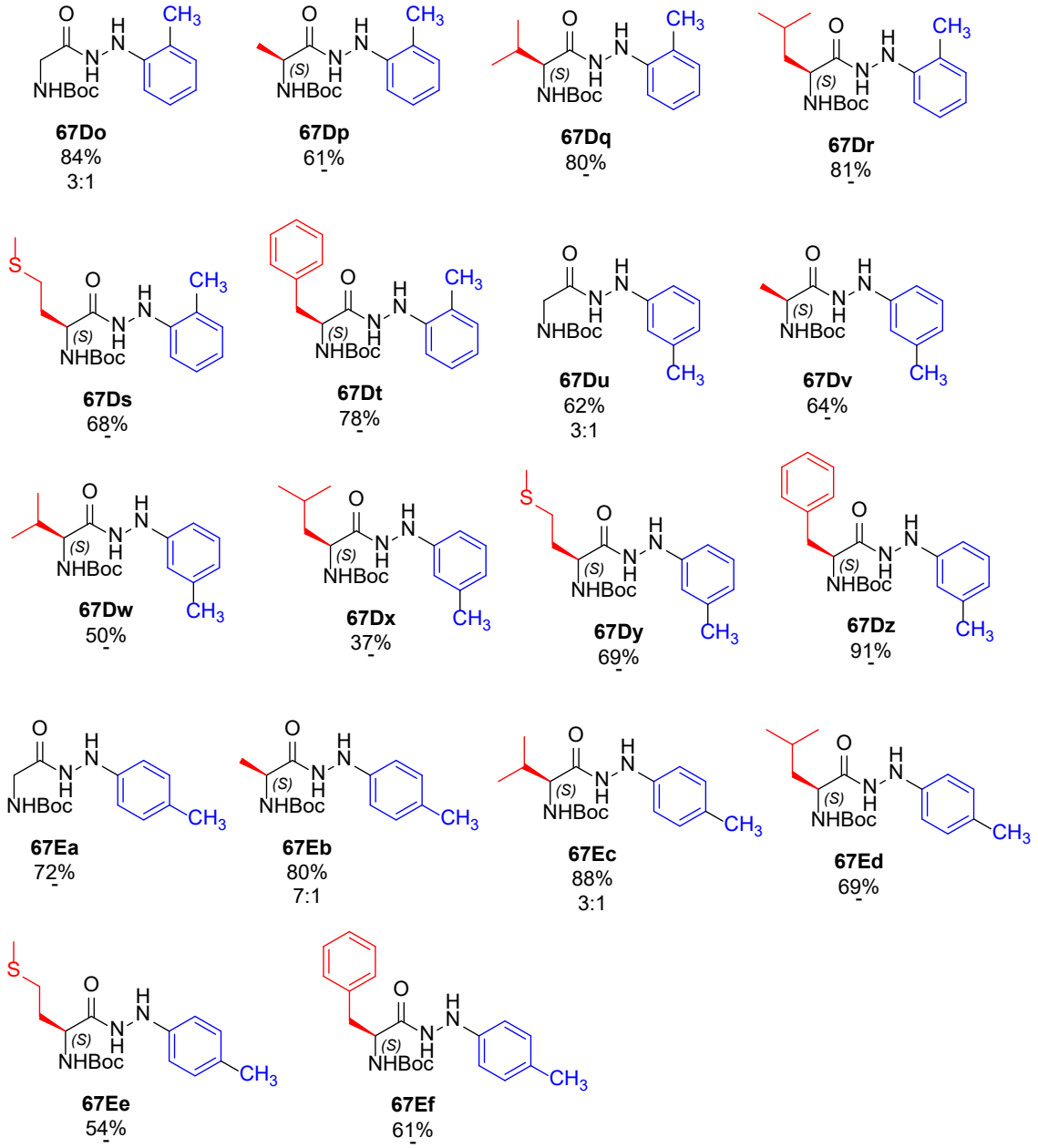


Figure 76: The substituted boc-hydrazides **67Dn-Ef** with a methyl group on aryl hydrazine.

To explore the electron donating character of other alkyl groups, attention turned to the incorporation of larger alkyl groups, like isopropyl. Incorporation of bulky groups is considered one way to enhance the selectivity of a drug to a specific target. For example, the isopropyl group of atenolol **86**, β-blocker medication for hypertension, allows it to be more selective toward β_1-receptors while the t-butyl group of salbutamol **87,** bronchodilator agent, is very important for selective interaction with the β_2-receptor (Figure 77).[123]

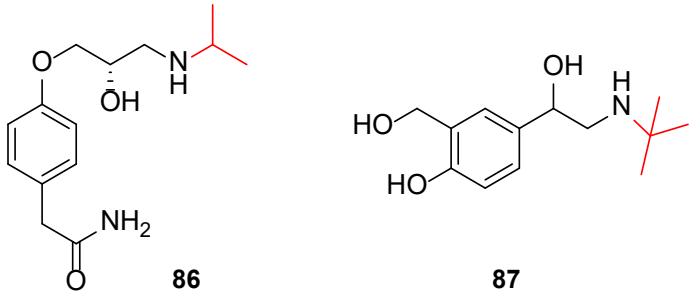


Figure 77: The chemical structure of atenolol **86** and salbutamol **87**.

Therefore, the introduction of an isopropyl group at the *para* position on the aryl hydrazine **69** would be of interest. The desired hydrazides **67Eg-El** were obtained again following the standard procedure in an acceptable yield (Figure 78).

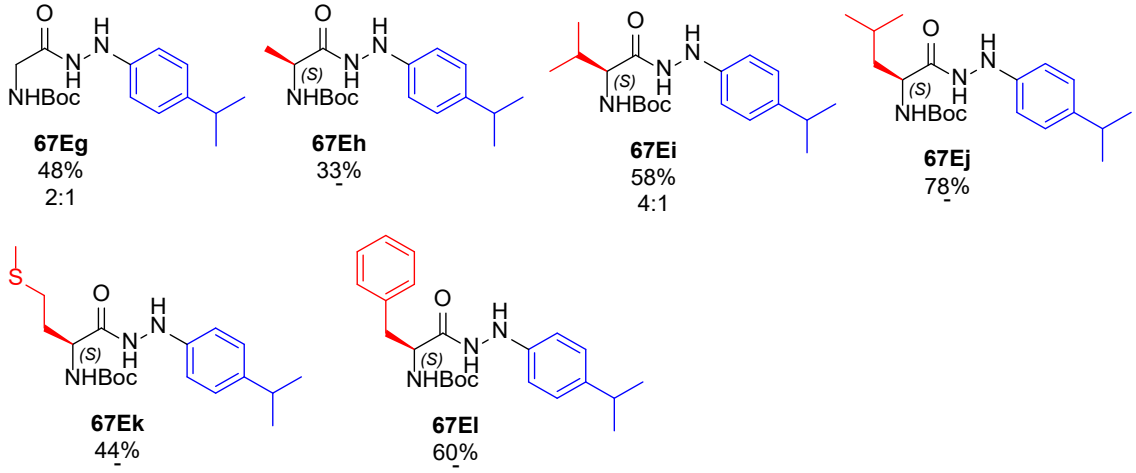
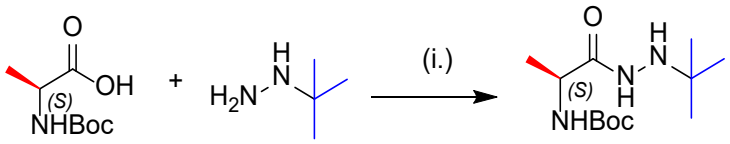


Figure 78: The corresponding boc-hydrazides **67Eg-El** containing isopropyl on aryl hydrazine.

The present work in the group is focused on the determination of the fundamental structure-activity relationship (SAR) of benzo-[2,1,3]-diazoles amino acid hydrazides with the incorporation of aromatic hydrazines.[1]

Nevertheless, to explore the requirement for an aromatic hydrazine, the use of alkyl hydrazines to form hydrazides with amino acids was of interest. Previously the group had failed to successfully synthesise the hydrazides **89** (*cf.* Scheme 6) as during synthesis with DCC and HOBt it was believed that the secondary nitrogen reacted with the activated carboxylic acid due to the lack of hinderance afforded without the aromatic ring and increase nucleophilicity provided from the electron donating alkyl group.

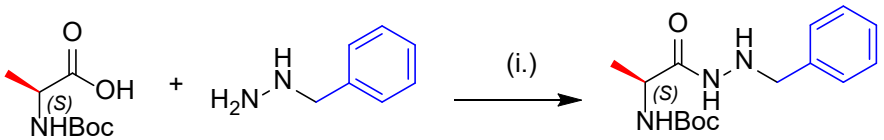
Nevertheless, synthesis of an alkyl hydrazide was attempted using DMTMM coupling conditions, in the belief that the fast and less sterically hindered process would allow for the hydrazide to be formed. Pleasingly, with the use of tert-butylhydrazine **88a** and Boc-L-alanine **70b** and DMTMM, we were able to successfully synthesise the desired hydrazide **89** in acceptable yield (Scheme 6).



70b **88a** **89**

Scheme 6: Reagents and conditions: i.) DMTMM, DIPEA, THF, 5-6 h, (40%), r.t.

In a parallel thought process, we chose to use benzyl hydrazine **90** for another hydrazide to explore if the aromatic ring needs to be present close to the amine or whether it can be moved further away. For this synthesis, benzyl hydrazine **90** also coupled with Boc-L-alanine **70b** and DMTMM to afford the desired hydrazide **91**, except this time in a poor yield (Scheme 7).

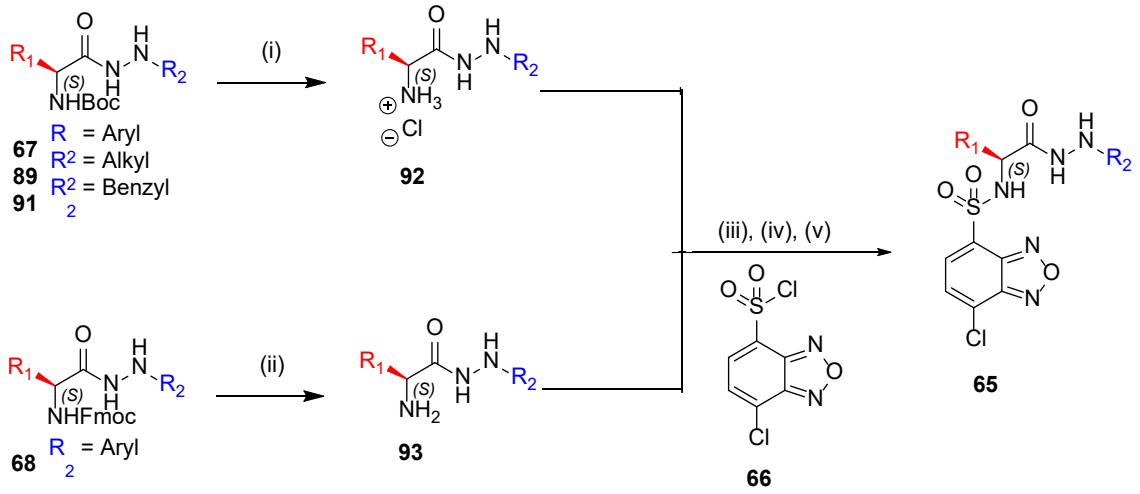


70b **90** **91**

Scheme 7: Reagents and conditions: i.) DMTMM, DIPEA, THF, 5-6 h, (27%), r.t.

2.1.2 The conversion of intermediates into the final benzoxa-[2,1,3]-diazole peptidomimetic

Research thus far has focused on the synthesis of the amino acid hydrazides and now attention must turn to the synthesis of benzo-[2,1,3]-diazoles **65**. To undertake this investigation, synthesis of novel benzoxa-[2,1,3]-diazoles was successfully achieved in reasonable yields via the formation of sulfonamide bonds between the synthesized *N*-protected hydrazides **67**, **68**, **89** or **91** and sulfonyl chloride **66.** Initially, deprotection of the hydrazides **67**, **68**, **89** or **91** allowed reaction with the sulfonyl chloride **66** to deliver the final benzoxa-[2,1,3]-diazole products **65** (Scheme 8).



Scheme 8: Synthesis of benzoxa-[2,1,3]-diazole peptidomimetics **65** from either Boc/Fmoc protected amino acid hydrazides; Reagents and conditions: i) 4M HCl in dioxane, 90 min, r.t.; (ii) DBU, octanethiol in THF, 2 h, r.t.; (iii) **66**, Et_3N, THF, 5 - 6 h, (64 - 85%), r.t.; (iv) **66**, DIPEA, THF, 4 h, (34 - 55%), r.t.; (v) **66**, pyridine, MeCN, 4 h, (39 - 72%), r.t.

In this way a library of final compounds **65** could be synthesised in a straightforward manner by exploiting a key set of protection/deprotection and coupling strategies.

2.1.2.1 The Boc deprotection

As has been shown in previous work, 4 M HCl in dioxane has been used to deprotect the Boc amino acid hydrazides but, in previous syntheses the deprotected hydrazide had been treated as an intermediate and not isolated or purified before continuing. This made for difficult purification and separation of the impurities at the final stage and so to make a comprehensive library of compounds we needed to address this.[1]

It was realized that the intermediate hydrochloride salt of the amino acid hydrazide could be isolated following dissolution in methanol and precipitation with diethyl ether as an antisolvent. [124] The isolation step removed any impurities following the deprotection enabling the *N*-terminal hydrazide **92** to be utilised in the final sulfonamide coupling. An example of this is the Boc-hydrazide **67Em** that was reacted with 4M HCl in dioxane. The resulting product **92a** was precipitated by using methanol and diethyl ether to deliver a pure quaternary ammonium salt (Figure 79).

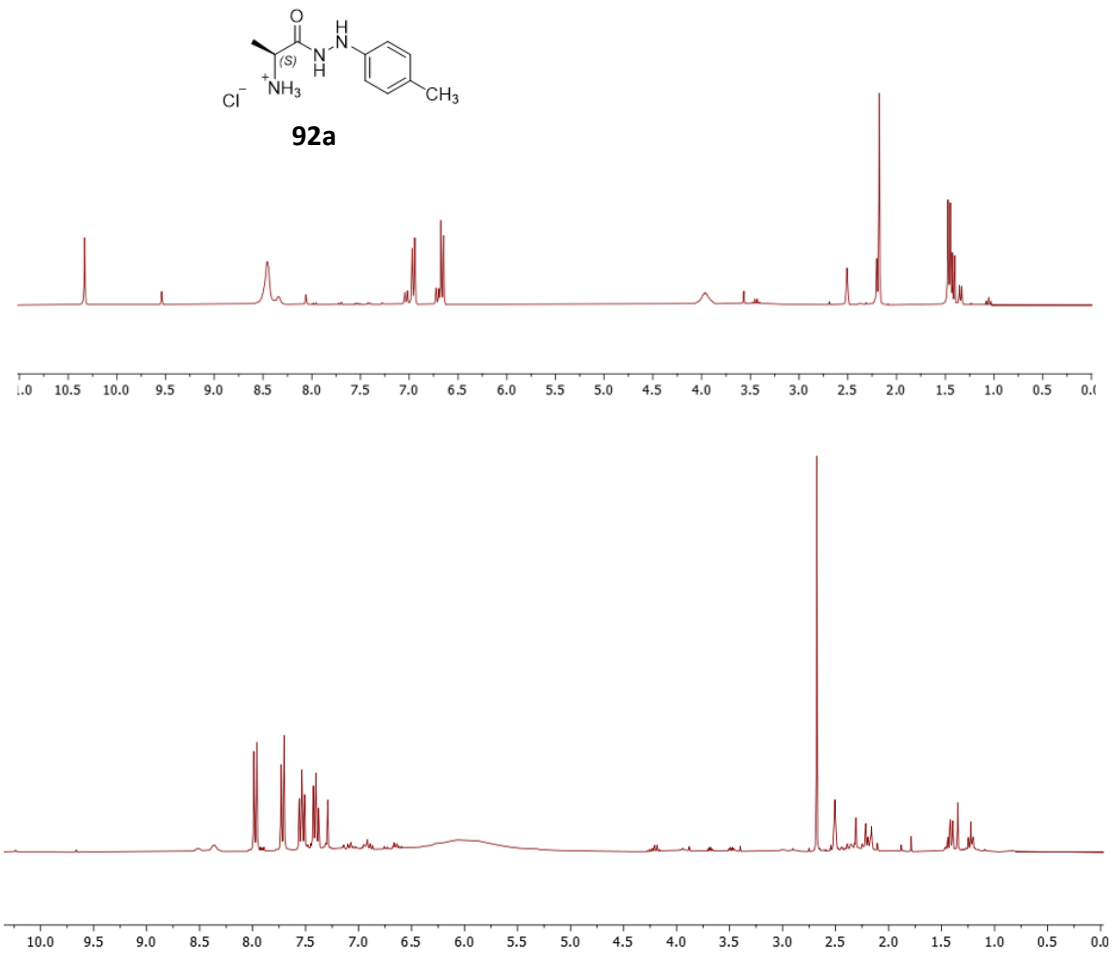


Figure 79: ^{1}H NMR spectra of *N*-hydrazide **67Em** (top) showed the absence of a Boc group which had successfully been removed by using 4 M HCl in dioxane whilst, ^{1}H NMR spectra of liquid fraction (bottom) showed the impurities which had successfully been removed by using the precipitation process.

2.1.2.2 The Fmoc deprotection

Alongside the Boc protected hydrazides, we also made Fmoc (9-fluorenylmethoxycarbony-) protected hydrazides. Fmoc groups are base-labile and acid-stable; thus it is frequently used in an orthogonal protection strategy for amino acids that have reactive side chains requiring protection through subsequent synthetic steps.

Consequently, Fmoc-protected hydrazides **68** were used in a trial deprotection reaction to generate free *N*-terminal hydrazide **93** by stirring with 20% piperidine **94** in THF overnight. Unfortunately, analysis of the crude reaction mixture revealed the presence of an undesired product **96** that arose through reaction of the desired product **93** with the benzofulvene by-product **95** (Figure 80).

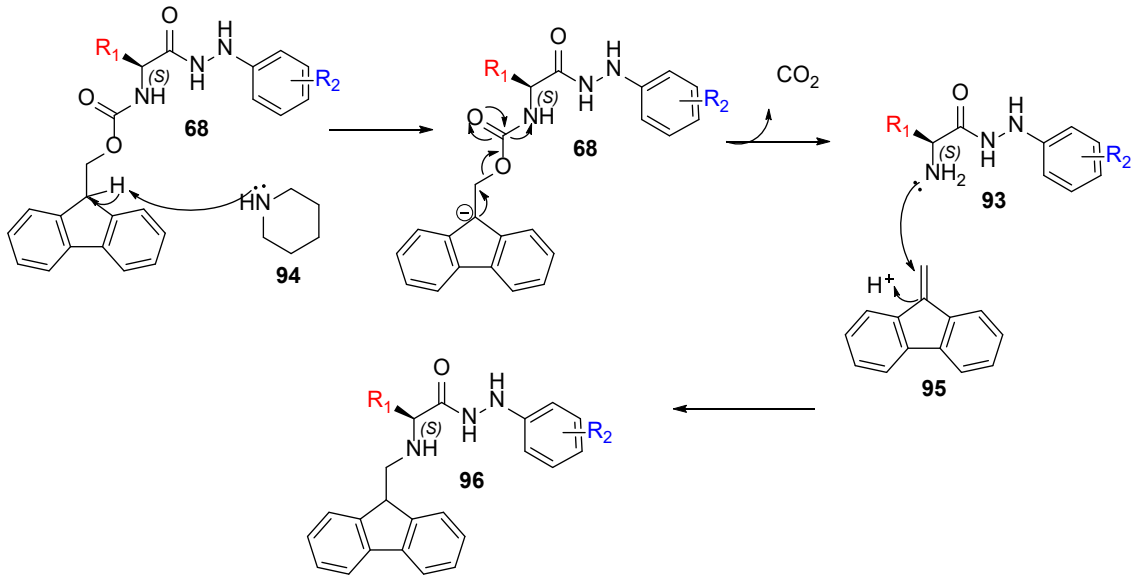


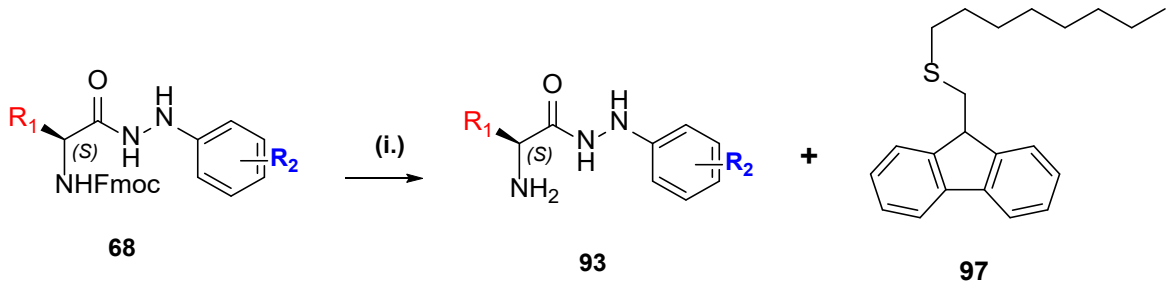
Figure 80: Fmoc deprotection mechanism using piperidine **94**.

To explore this further and attempt to isolate the free amine, other bases where investigated. Firstly, 4-methylpiperidine was utilised in THF to afford the free amine of hydrazide **93**.[125] Although this basic deprotection condition was successful (loss of Fmoc observed), the benzofulvene by-product **95** was the primary product.

Consequently, it was clear that the standard deprotection method was not going to enable the production of the desired product and so further investigation was required.

Upon reviewing the literature, a paper by Sheppeck *et al.* demonstrated the effective use of the base 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) in a catalytic

quantity with a high equivalence of octanethiol as a Michael donor to outcompete the reaction of the amine nucleophile for the benzofulvene by-product **95** would produce the by-product **97** and the desired free amine in a solution phase reaction (Scheme 9).[126]



68 **93** **97**

Scheme 9: (i.) DBU, octanthiol in THF, 2 h, r.t.

Consequently, we set about repeating this study and to our delight, we were able to isolate the free amine of the Fmoc protected amino acid hydrazides. One example of this is the Fmoc-protected hydrazide **68Ab** that was treated with a catalytic amount of DBU and octanethiol in THF for 2 hours under N_2 and then precipitated with methanol and diethyl ether to afford the free *N*-terminal hydrazide **93a**. The analysis of the ^{1}H NMR spectra of the precipitate showed a detectable amount of the octanethiol alongside the desired free *N*-hydrazide **93a** (Figure 81).

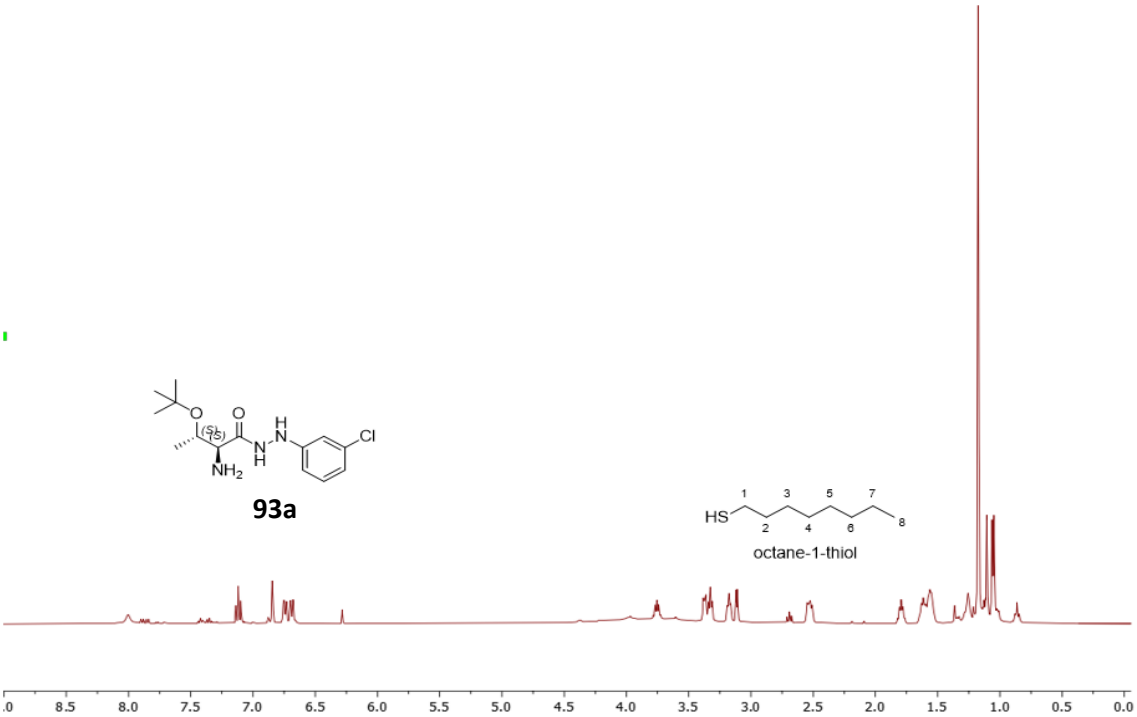


Figure 81: ^{1}H NMR spectra of *N*-protected hydrazide **68Ab** showed the presence of Fmoc group which had successfully been removed by using DBU/octanethiol in THF.

Unfortunately, we were not able to purify away the octanethiol completely from the reaction product and so in this instance we opted to carry on with the synthesis of benzoxa-[2,1,3]-diazole peptidomimetics carrying this impurity through.

2.1.2.3 The sulfonamide bond formation

Sulfonamide bond formation is one of the most used transformations in the synthesis of pharmaceutical compounds displaying a wide range of biological activities (Figure 82). For example, glibenclamide **98** has found use as a hypoglycaemic agent, E7070 **99** as an anticancer agent, amprenavir **100** is used in HIV therapy and furosemide **101** as a diuretic.[127,128]

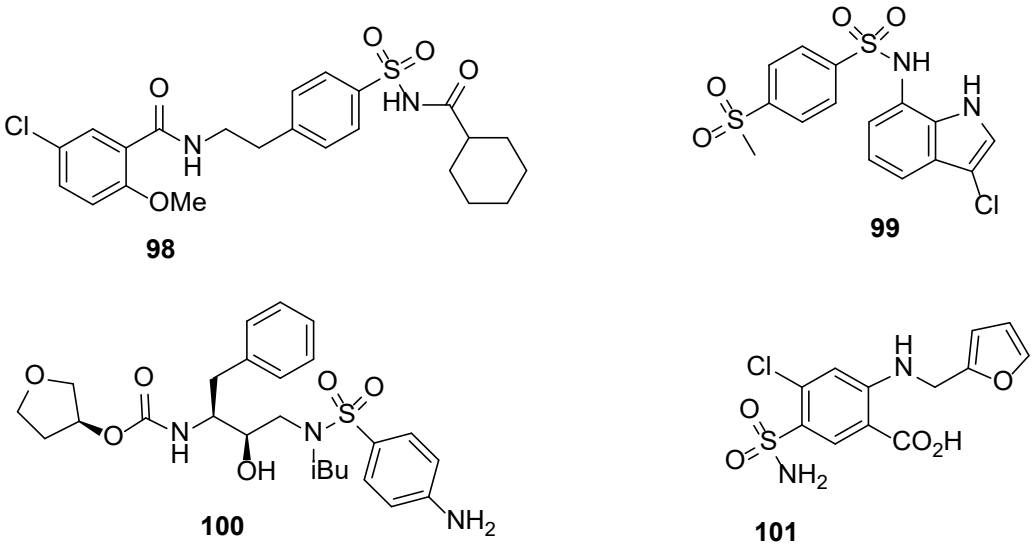


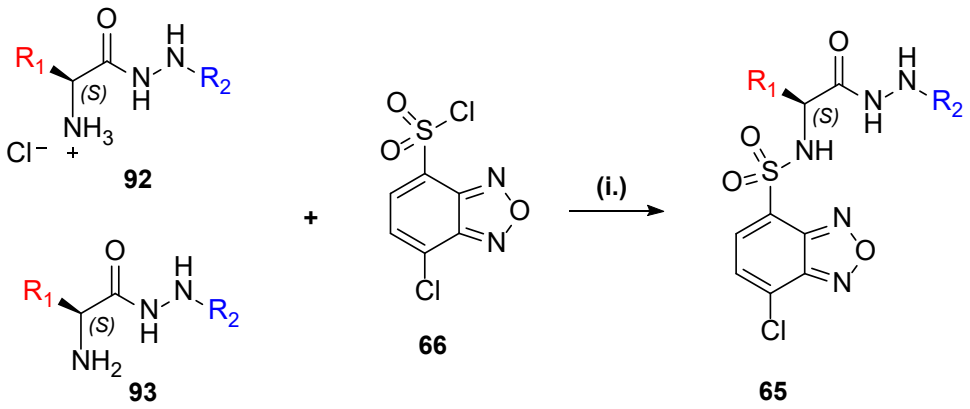
Figure 82: The chemical structure of therapeutics that display a wide range of biological activities.

The sulfonamide bond greater resistance to metabolic catabolism due to its structural similarity to the peptide bond in enzymatic hydrolysis, and its more acidic hydrogen giving rise to the possibility of a stronger H-bonding are some of the reasons why these functional groups appear in drug molecules.[129] For these reasons, there are a plethora of methods for sulfonamide bond formation.[130] The

most frequently used procedures involve the reaction of sulfonyl chlorides with primary or secondary amines.

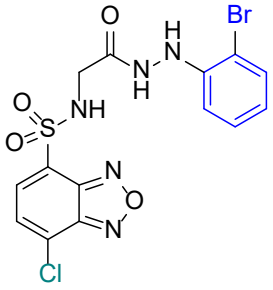
2.1.2.4 Generation of a Library of Benzoxa-[2,1,3]-diazole peptidomimetics

To develop of our novel benzoxa-[2,1,3]-diazoles **65**, we were able to utilise coupling conditions described in previous work. We reasoned the use of tertiary amines such as triethylamine or diisopropylethylamine would be required to neutralise the HCl salt of the hydrazide hydrochloride and the hydrochloride formed during the reaction.[1] Therefore, substituted *N*-hydrazides **92** or **93** and sulfonyl chlorides **66** where mixed in the presence of 3 equivalents of triethylamine in THF to produce the desired products **65** (Scheme 10).[1]

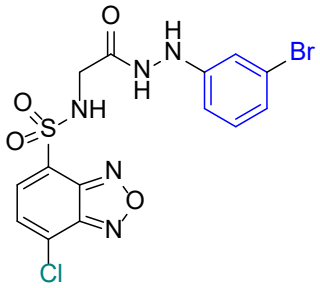


Scheme 10: Reagents and conditions: i.) Et_3N, THF, 4-5 h, (26- 97%),

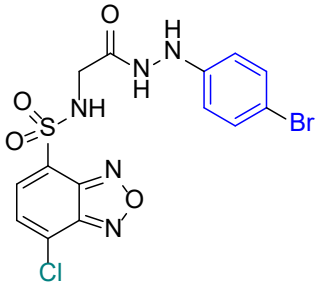
With the synthesis outlined, we began by deprotecting the protected *N*-hydrazides produced previously and then reacted these with the corresponding benzoxadiazole sulphonylchloride to afford the desired compound **65h-Al** in fair to very good yields (Figure 83).



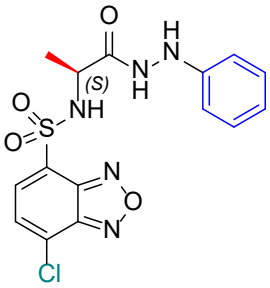
65h
48%
4:1



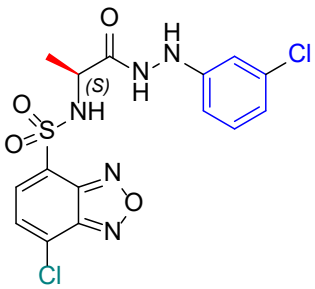
65i
68%
5:1



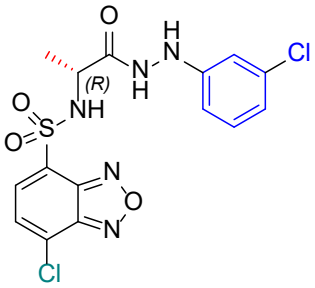
65j
76%
4:1



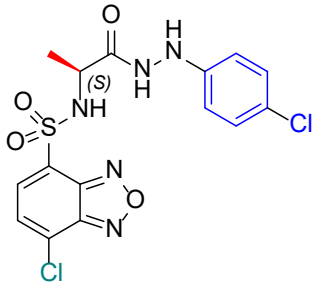
65k
26%



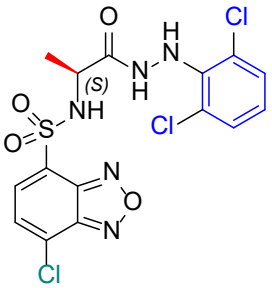
65l
48%



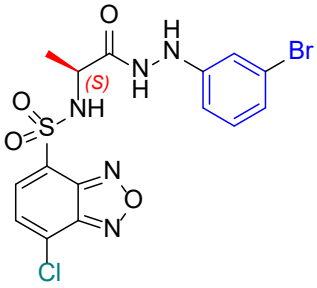
65m
86%



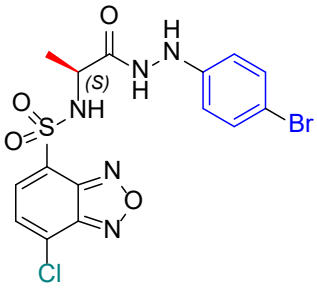
65n
54%



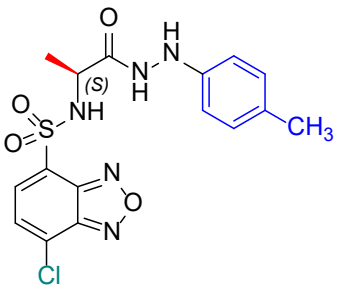
65o
97%



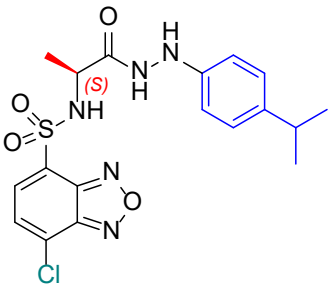
65p
79%



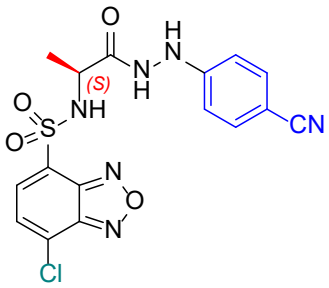
65q
67%



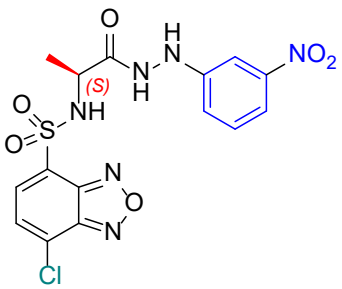
65r
86%



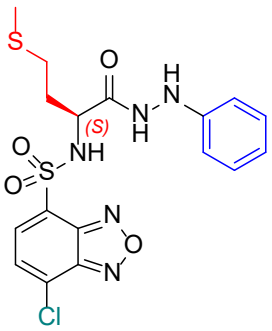
65s
68%



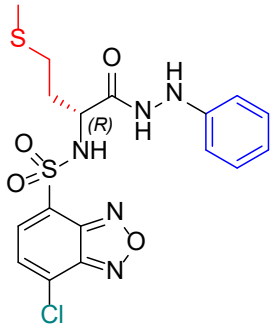
65t
62%



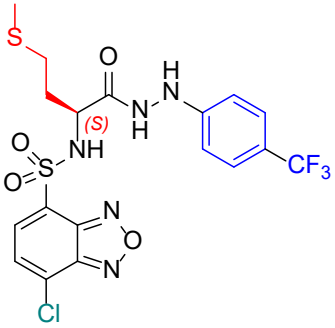
65u
56%



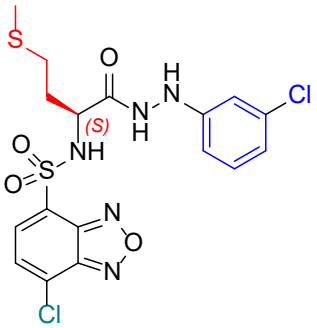
65v
53%



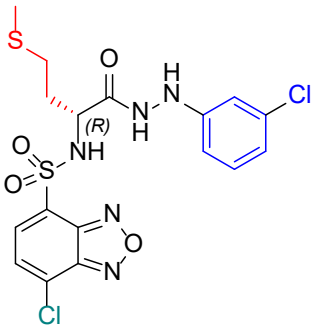
65w
72%



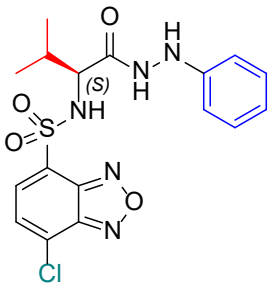
65x
43%



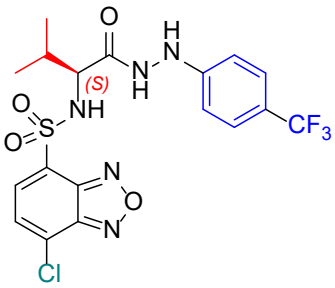
65y
69%



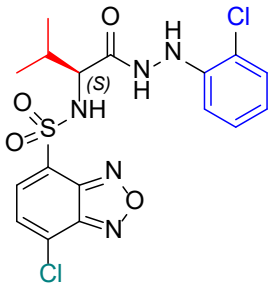
65z
56%



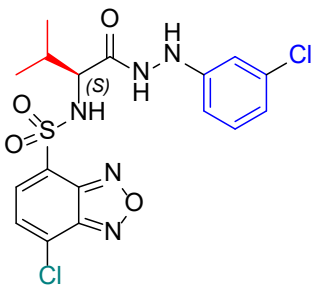
65Aa
72%



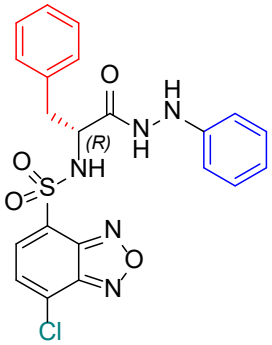
65Ab
61%



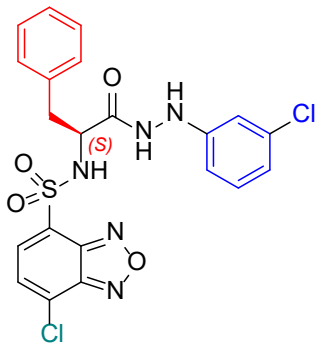
65Ac
72%



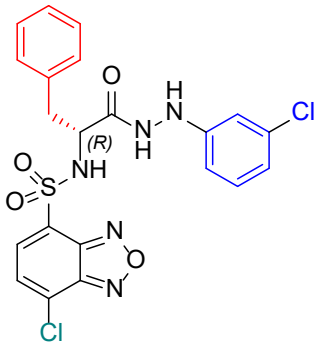
65Ad
66%



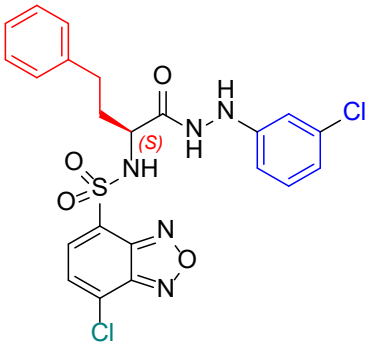
65Ar
61%



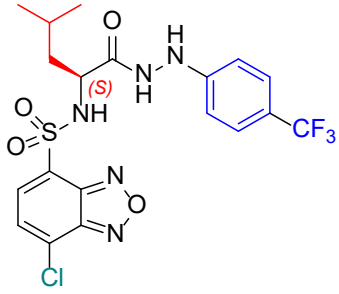
65Af
68%



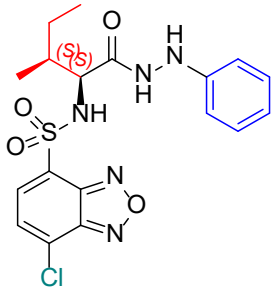
65Ag
64%



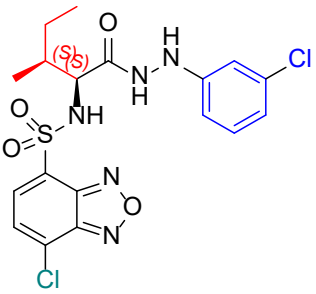
65Ah
45%



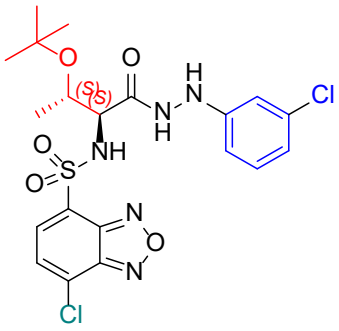
65Ai
59%



65Aj
68%



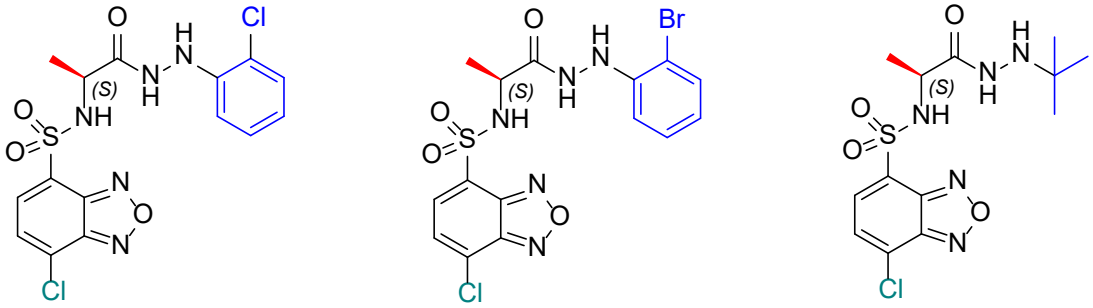
65Ak
54%



65Al
61%

Figure 83: The resulting sulfonamidopeptides **65h-Al** which had been successfully synthesized in presence of triethylamine with very good yields.

In attempts to improve the yield, other tertiary amines such as diisopropylethylamine (DIPEA) were examined. Unfortunately, using DIPEA in place of triethylamine saw no improvement on the efficiency of the system, and in fact the yield of the desired compounds **65Am-Ao** was somewhat disappointing (Figure 84). As compared to (*cf.* Figure 83), low yields reported for these compounds **65Am-Ao**, necessitating switch to alternative bases such as pyridine.



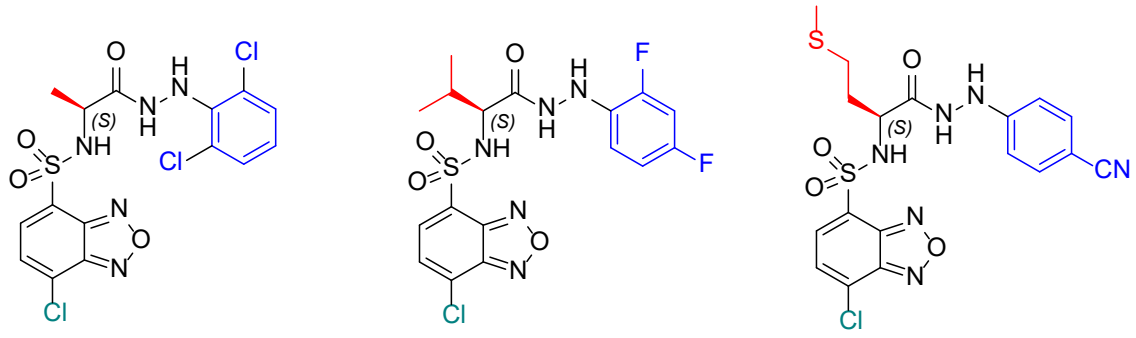
65Am
14%
3:1

65An
26%
3:1

65Ao
50%

Figure 84: The resulting sulfonamidopeptides **65Am-Ao** which had successfully been synthesized in presence of DIPEA **75** with low yields.

Less frequently seen is the replacement of the tertiary amine with pyridine **102** (*cf*. section 2.1.2.3). In the optimized reaction condition utilising HBTU, the reactions were attempted in the presence of a slight excess of pyridine **102**.[128,132] Pleasingly, this work demonstrated that pyridine was successful in producing benzoxa-[2,1,3]-diazoles **65Ap-Ar** in acceptable yields, however, not better than with triethylamine (Figure 85).



65Ap
39%
6:1

65Aq
54%

65Ar
72%

Figure 85: The resulting sulfonamidopeptides **65Ap-Ar** that were successfully synthesized in presence of pyridine **102** with acceptable yields.

In summary, this work has identified a convenient procedure for the synthesis of benzoxa-[2,1,3]-diazoles **65** from substituted *N*-hydrazides **92** or **93**. Our studies show that the conversion to benzoxa-[2,1,3]-diazoles **65** was accomplished in a lower yield with DIPEA **75** and pyridine **102**, than with triethylamine. So, optimisation of the reaction base or solvent failed to improve the overall yield.

2.1.2.5 Enantiomeric purity determination

As this synthesis utilises a base, racemisation of the chiral centre at the amino acid is a possibility and needed to be checked. Therefore, to determine if this was the case, we utilised the same resolution techniques (described previously) to determine the enantiomeric purity of our novel benzo-[2,1,3]-diazoles **65**. Chiral HPLC (Chiralcel-OD, MeCN: 0.1% formic acid/MeOH: 0.1% formic acid) produced the electronic circular dichroism (ECD) spectra of the corresponding enantiomers. In the case of synthesized *L*-benzo-[2,1,3]-diazoles **65l** and *D*-benzo-[2,1,3]-diazoles **65m** we confirmed that the ECD spectra of each contained only a single enantiomer (Figure 86 and 87).

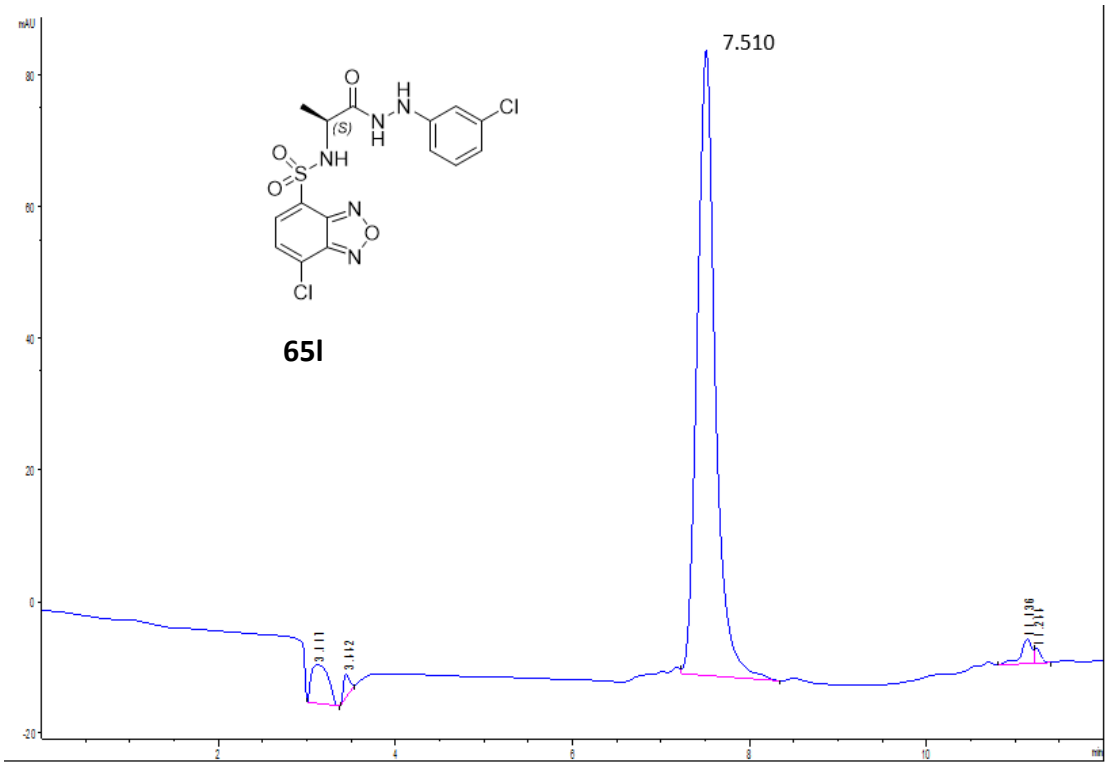


Figure 86: chiral HPLC of *L*-benzo-[2,1,3]-diazole **65l** indicating the presence of a single peak, Daicel Chiralpak® IB column, 4.6 x 250 mm, 5 µm particle size, acetonitrile: methanol: 0.1% formic acid (94:5:1), 1 mL/min, UV at 282 nm.

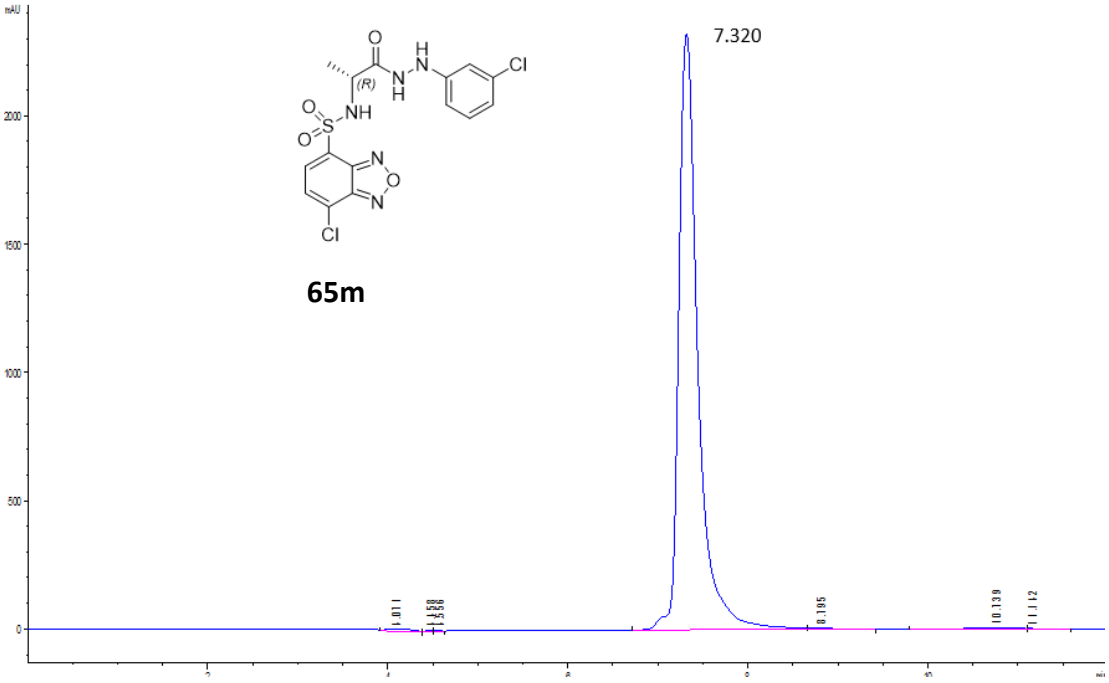


Figure 87: chiral HPLC of *D*-benzo-[2,1,3]-diazole **65m** indicating the presence of a single peak, Daicel Chiralpak® IB column, 4.6 x 250 mm, 5 µm particle size, acetonitrile: methanol: 0.1% formic acid (94:5:1), 1 mL/min, UV at 282 nm.

2.1.2.6 Determination of the rotameric mixture of benzoxa-[2,1,3]-diazole peptidomimetics

As alluded to earlier, in some cases we observed the presence of another compound in the ^{1}H NMR which we surmised was a rotamer. To determine if this was the case, we undertook a variable temperature ^{1}H NMR study of the benzoxa-[2,1,3]-diazole **65g** to determine this. Dissolving **65g** in DMSO-d_6 and beginning with a room temperature experiment, we then raised the temperature of the probe in 10 ºC increments and once stable, an ^{1}H NMR was recorded. Measurements were taken up to 90 ºC and then the sample returned to room temperature where a final ^{1}H NMR spectrum was taken for analysis (Figure 88).

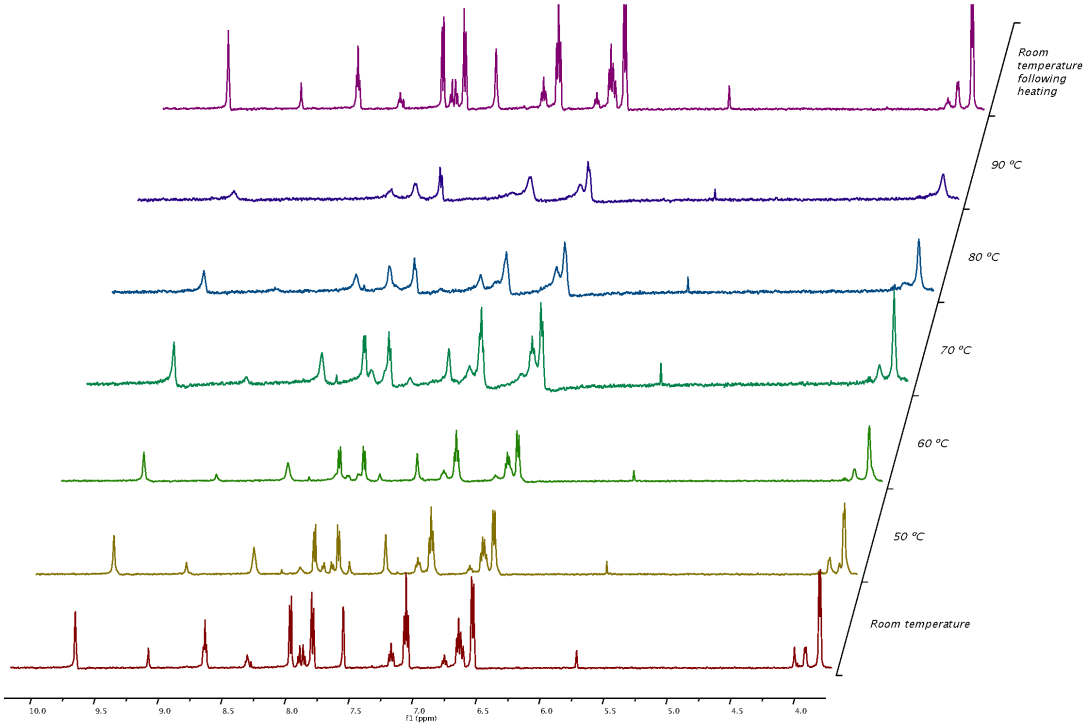
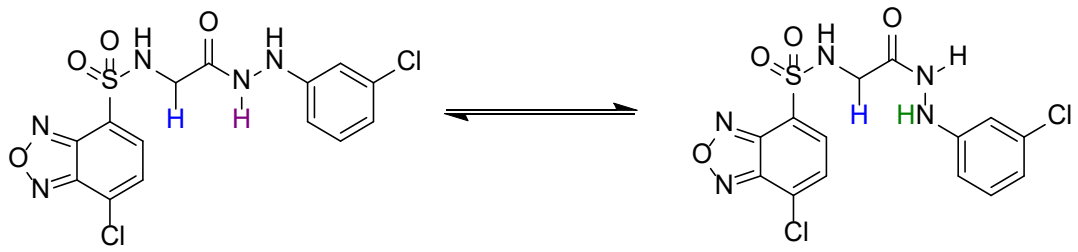


Figure 88: Variable temperature ^{1}H NMR of benzoxa-[2,1,3]-diazole **65g**

The result of the experiment determined that the second signals in the ^{1}H NMR are from a rotamer of the compound. The ratio of the two compounds at the beginning of the experiment is 4:1 and then as the temperature was raised through 50 ºC the signals begin to coalesce (nothing changed before this temperatre) until we get to 90 ºC where this is complete. Pleasingly when the sample was returned to room temperature the ^{1}H NMR produced was in complete corroboration with the NMR before the experiments were undertaken and the rotamer ratio returns to 4:1.

In order to determine the conformation of the major rotamer of our benzoxa-[2,1,3]-diazoles we initiated a study utilising unassigned nuclear Overhauser effect spectroscopy (NOESY) experiment. This experiment records the through-space dipolar interactions between protons nearby in three-dimensional (3D) space. To determine the configuration, we utilised the benzoxa-[2,1,3]-diazole peptidomimetic **65f** to determine which hydrazine NH proton coupled to the CH_2 protons and ultimately determine the cis/trans conformation of the major rotamer (Scheme 11).



65f (trans) **65f** (cis)

Scheme 11: The possible orientations of **65f** around the amide bond to arise trans and cis rotamers.

The NOESY spectra showed the CH_2 peak at 3.95 ppm (highlighted with blue) coupled to the amide proton (highlighted with purple) N*H* at 9.79 ppm (Figure 89).

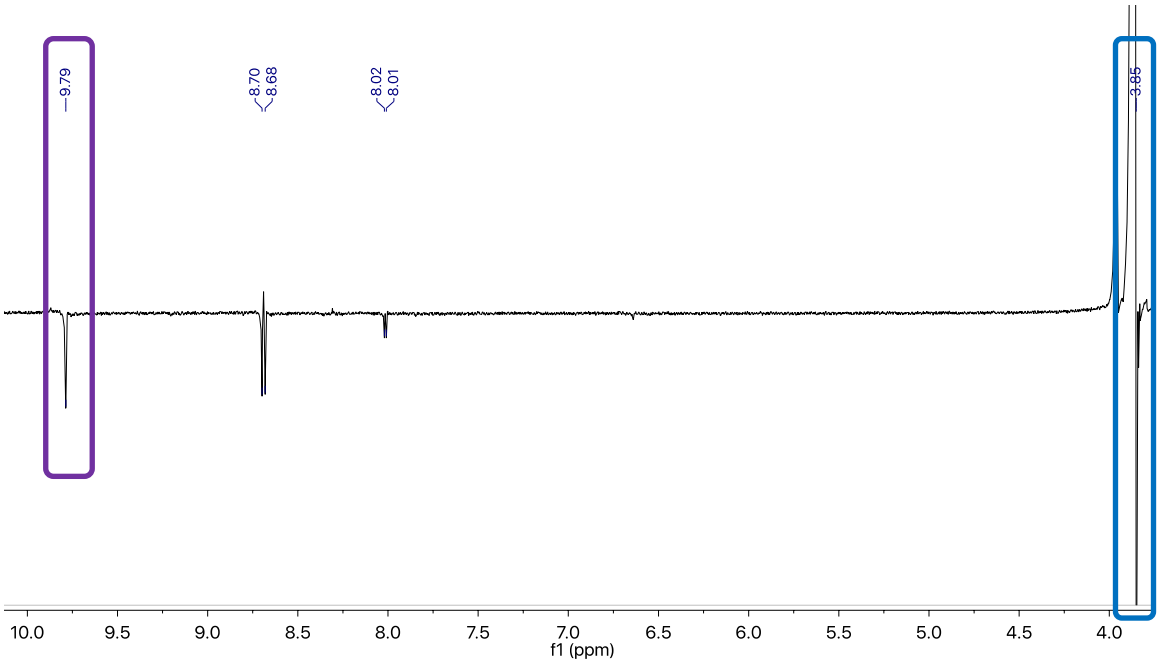


Figure 89: 2D NOESY of **65f** displaying the through space coupling of hydrogens to verify **trans** is present as the major rotamer.

Therefore, ^{1}H-^{1}H analysis confirmed the trans orientation of **65f** as the major rotamer as would be expected from the steric repulsion from the aryl hydrazine and the benzoxa-[2,1,3]-diazole. Consequently, knowing we have a mixture of compounds will have an impact on activity as one of the rotamers is likely to have the correct orientation for stronger interactions with the target.

3 Structure-activity-relationship studies of benzo-[2,1,3]-diazoles peptidomimetics

Benzoxa-[2,1,3]-diazole peptidomimetics **65** were designed as promising antitubercular agents. Previously, a limited number of simple substituted benzo-[2,1,3]-diazole peptidomimetics **65a-g** have been shown to exhibit promising antitubercular activity and selectivity against *mycolata* over Gram-positive and Gram-negative bacteria (*cf.* section 1.7).[1] Based on this preliminary study, further investigation into the antitubercular activity of benzo-[2,1,3]-diazole peptidomimetics **65** is warranted. Therefore, the substituted benzoxa-[2,1,3]-diazole peptidomimetics **65** were chosen as the partner to amino acid hydrazides **67**, **68**, **89** and **91** for further investigation, via an SAR study to understand the importance of amino acids **70** and the aryl hydrazine **69** on antitubercular activity. To undertake this, the methods of synthesis outlined (*cf.* section 2.1.1) in this study provided the capability to produce a variety of hydrazides **67**, **68**, **89** and **91** and some of these intermediates were converted to the benzoxa-[2,1,3]-diazole peptidomimetics **65h-Al** (*cf.* section 2.1.2). *In vitro* screening was undertaken to evaluate the antitubercular potency of both intermediates and final compounds against wild-type *Mtb*, isoniazid-resistant *Mtb* strains and rifampicin-resistant *Mtb* via a REMA assay.

3.1.1 Antimycobacterial susceptibility testing

The colorimetric redox indicator technique has been developed and endorsed by the WHO to test drug susceptibility in non-reference laboratories.[131] The Resazurin microtiter assay (REMA) is a colorimetric drug-susceptibility method that uses a redox indicator, Resazurin **105**. Palomino *et al.* (2002) demonstrated that REMA is a simple, rapid and inexpensive technique to determine minimal inhibitory concentrations (MIC) of bioactive molecules.[132] This colorimetric assay works through irreversible reduction of resazurin (purple) **105** to resofurin (pink) indicating mycobacterial growth (Figure 90).

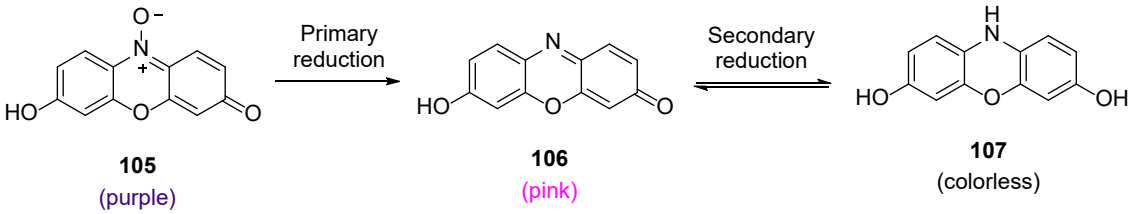


Figure 90: Irreversible reduction of resazurin **105** to resorufin **106** due to mycobacterial growth. Further reduction may be observed which shows the formation of dihydroresorufin **107**.

The redox potential of resorufin can be observed visually for colour changes and read by a spectrophotometer at an excitation of 530 nm with an emission of 590 nm. In brief, the *Mtb* strain is diluted with Middlebrook 7H9GC medium and added to 96 well plates along with compounds that have been serially diluted across the plate. The plates are incubated at 37 ºC for seven days. Then, the resazurin **105** is added into the plates and tests are read ~48 hours later by observing the color change (Figure 91).

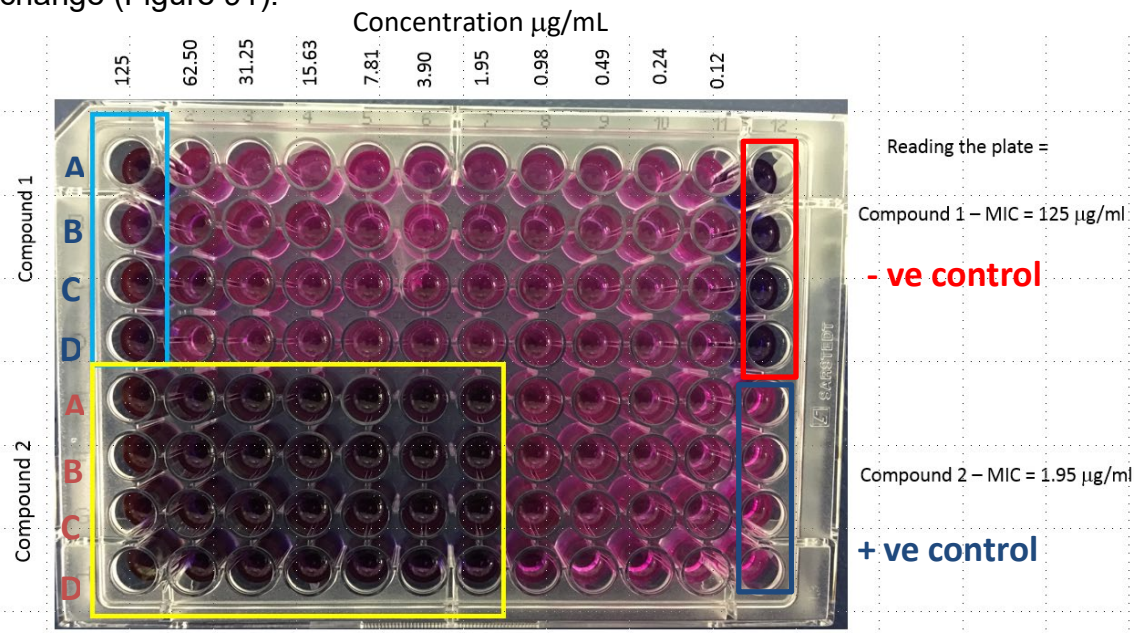


Figure 91: Example of REMA plate set up.

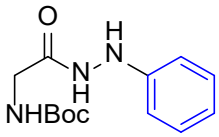
The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a drug which has no colour change for the resazurin (purple) while the maintenance of a dark blue colour is indicative of the ability of compounds to provide 95-100% inhibition in mycobacterial growth. Therefore, we can see that compound 2 has a minimum inhibitory concentration of 1.95 µg/mL (Figure 91).[131]

3.1.2 The SAR exploration of amino acid hydrazide

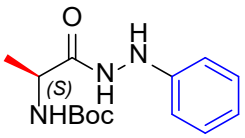
The first part of this project was successful, to generate a library of substituted and unsubstituted hydrazides with good yields. Modification of the amino acids or aryl hydrazines within the hydrazides **67**, **68**, **89** and **91** would provide the opportunity to explore their structure activity relationships against *Mtb* strains. Consequenly, to assess their anti-tubercular activity we chose to use wild-type *Mtb*, isoniazid-resistant *Mtb* and rifampicin-resistant *Mtb* in a REMA assay to allow for a comparative analysis of their minimum inhibitory concentrations.

To begin it was envisaged that utilising the unsubstituted aryl hydrazides coupled to the amino acids would allow for the comparison of amino acid side chains and their antitubercular activity. It was demonstrated that increasing the side chain of the amino acid would increase the log *P* and enhance membrane permeability but also provide some insight into the optimum structure required for antitubercular activity. Therefore, the antimycobacterial activity of unsubstituted Boc-*N*-hydrazides **67a-j** and Fmoc-*N*-hydrazides **68a-j** was assessed *in vitro* against wild-type *Mtb*, isoniazid-resistant *Mtb* strain and rifampicin-resistant *Mtb* at concentrations of 128 –0.125 μg/mL, and converted to μM (Appendix, Table A1).

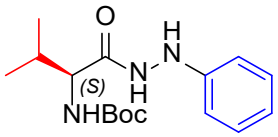
Gratifyingly, the biological analysis provided evidence for the importance of the size of side chain as the MIC result of the unsubstituted hydrazide **67a** with no side chain against the susceptible *Mtb* was disappointing, with no activity against the more-resistant strains. It was observed that the small side chain of the unsubstituted hydrazide **67b** improved the potency two-fold compound to **67a** against the wild-type *Mtb*, but still with no observable activity against the mono-resistant strains. The continued increase in the size of side chain of the unsubstituted Boc-*N*-hydrazides **67a-h** indicated that an improved cell wall penetration can be attributed to the large side chain, owing to the increase in lipophilicity. It is very clear that the linear and larger hydrophobic side chains were important for the antitubercular activity and in line with what we postulated. As a result, the most potent unsubstituted hydrazide was found to be **67h** which contained an *L*-phenylalanine (Figure 92).



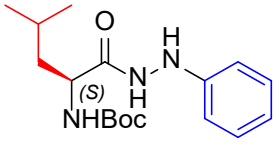
67a
241.22 µM wt. *Mtb*
- µM INH^R *Mtb*



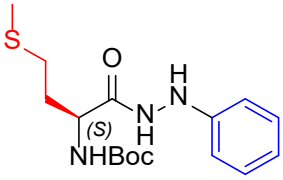
67b
114.56 µM wt. *Mtb*
- µM INH^R *Mtb*



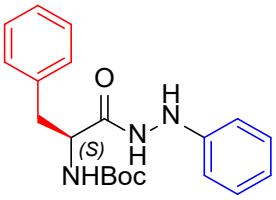
67c
13.01 µM wt. *Mtb*
208.20 µM INH^R *Mtb*



67d
12.44 µM wt. *Mtb*
199.12 µM INH^R *Mtb*



67g
5.89 µM wt. *Mtb*
188.54 µM INH^R *Mtb*



67h
2.81 µM wt. *Mtb*
90.03 µM INH^R *Mtb*

Figure 92: The improvement of MIC results against the wild-type *Mtb* and INH^R *Mtb* as increase in the side chain of the unsubstituted hydrazides **67a-h.**

To gain more insight into the role of the size of side chain in this regard, *L*-histidine or *L*-tyrosine with protected side chain groups were utilised, a significantly diminished antitubercular activity was seen. We believe these results from the incorporation of a much larger side chain hindering the molecules binding (*cf.* **67h** vs **67i**) and (*cf.* **67h** vs **67j**) (Appendix, Table A1).

A discouraging observation was that while **67h** showed a high level of potency against susceptible *Mtb* comparable to the current TB treatment on the market (Ethambutol **3** MIC 4 µM and Ethionamide **16** MIC 6 µM),[2] the unsubstituted Fmoc-*N*-hydrazides **68a-j** produced a limited antitubercular activity against the wild-type and resistant *Mtb* strains. Slightly more promising results were observed that also supported our believe in the critical role of the linear and larger hydrophobic side chain (*cf.* **68a** vs **68b**) and (*cf.* **68c** vs **68d**) (Appendix, Table A1).

Also, further variations we wanted to investigate were whether replacing the *L*-amino acid with unnatural *L*-amino acids or *D*-amino acids affected antitubercular activity. These variations could elucidate some key features including the modified side chains and configuration that may improve the antimycobacterial performance and tackling of the antimycobacterial resistance (Appendix, Table A2).

Owing to the investigation of structural modification of the amino acids, the dipeptide of glycine (glycylglycine) **67k** was utilised to lengthen the hydrazide in an attempt to explore the proximity requirements of the aryl group. Regrettably, **67k** lost all its ability to inhibit the growth of *Mtb* (*cf.* **67a** vs **67k** Table A2). This suggests that the target site requires a single amino acid to be present.

As shown in Figure 92, the promising MIC results of the unsubstituted hydrazides **67a-h** increases with the size of side chain. We explored substitution with homophenylalanine (**67m**). In view of the lengthening of the alkyl chain, further distance between phenyl and chiral carbon doesn't have a positive impact on MIC result (*cf.* **67h** vs **67m**) (Appendix, Table A2).

Antimicrobial peptides (*cf.* section 1.4.1) have been reported in the literature using both *L*- and *D*- amino acids that showed a similar antibacterial activity against Gram-positive and Gram-negative bacteria.[133] Furthermore, there is limited data on their antitubercular activity. For these reasons, strong interest lies in the synthesis and evaluation of hydrazides that contain both *L*- and *D*- amino acids against susceptible and resistant *Mtb* strains. To investigate the ability of configuration to affect activity, the poor MIC results of the hydrazides **67n-p** suggest a negative impact of *D*-configuration on the antimycobacterial activity as no observable activity against all *Mtb* strains as compared to the hydrazides with *L*- configuration (Figure 93). Thereby it is perhaps unsurprising that optimal activity requires an *L*-configuration.

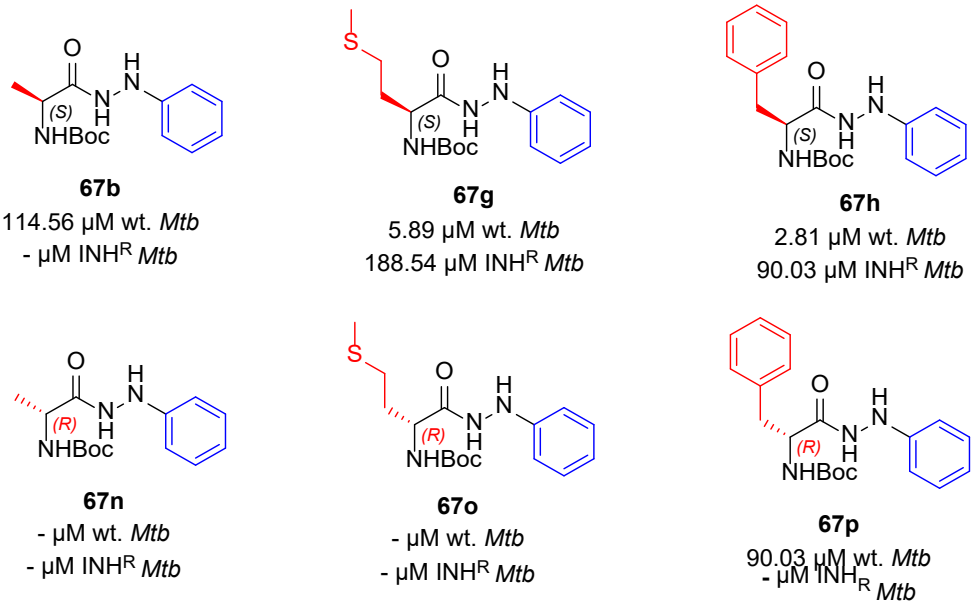
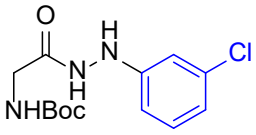


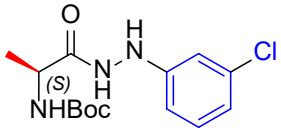
Figure 93: The negative impact of *D*-configuration on the antimycobacterial activity; where a (-) indicates to a lack of inhibition activity.

To support the investigation discussed above, the substituted Boc-*N*-hydrazides **67q-Af** and Fmoc-*N*-hydrazides **68k-t** were obtained from utilizing the same amino acid as seen in (Appendix, Table A1 and A2), but in this case the coupling reaction was carried out with a 3-chlorophenylhydrazine to confirm the effect of size of side chain and configuration of amino acid on MIC results. We also wanted to examine whether the involvement of substituents in aryl hydrazines affected their potency. Therefore, MIC results of the antimycobacterial susceptibility testing of the substituted Boc-*N*-hydrazides **67q-Af** and Fmoc-*N*-hydrazides **68k-u** were obtained (Appendix, Table A3).

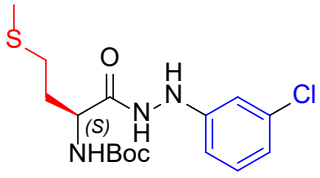
The initial investigation confirmed the existence of a direct association between the size of linear hydrophobic side chains of the amino acid and a good MIC result. This being so, the MIC results of the substituted hydrazides against the wild-type *Mtb* strain were improved as an increase of the length of hydrophobic side chain (Figure 94). Considering above the MIC results of unsubstituted hydrazides **67a, b, g** against the wild-type *Mtb* strain, a clear improvement in potency was observed as compared to *meta* chlorinated hydrazides **67q, r, w**.



67q
13.34 µM wt. *Mtb*



67r
3.19 µM wt. *Mtb*

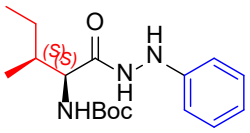


67w
2.67 µM wt. *Mtb*

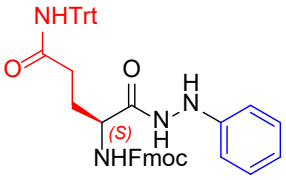
Figure 94: The improvement of MIC results against the wild-type *Mtb* as increase in the side chain of the substituted hydrazides **67q, r, w**.

By assessing the impact of the configuration, a specific replacement of *L*-amino acid with *D*-amino acid component also impaired antimycobacterial activity of the substituted hydrazides **67Ad-Af** (*cf.* **67Ad** vs **67r**), (*cf.* **67Ae** vs **67w**) and (*cf.* **67Af** vs **67x**) (Appendix, Table A3). Unlike the unsubstituted hydrazides **67n-p** previously discussed (*cf.* Figure 93), the substituted hydrazides **67Ad-Af** showed better activity than unsubstituted hydrazides **67n-p**. It is believed that the unwanted effect of *D*-configuration on MIC could be improved by incorporation a substituent on aryl hydrazine **69**.

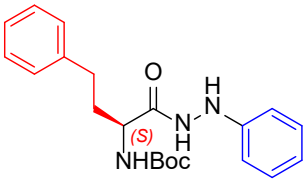
in order to expand the scope of the positive effect of a substituent on the potency was demonstrated by replacement of phenylhydrazine (Appendix, Table A1 and A2). with 3-chlorophenylhydrazine in the structures (Appendix, Table A3) which resulted in an improved antitubercular activity. The substituted hydrazides showed a potent activity as the amino acid fragment remained constant with introducing the chlorine group; this substitution led to a vast improvement in MIC results when compared to unsubstituted hydrazides (Figure 95).



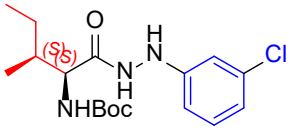
67e
- µM wt. *Mtb*



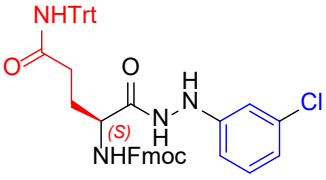
68g
- µM wt. *Mtb*



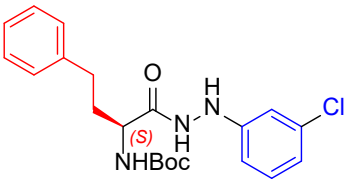
67m
86.61 µM wt. *Mtb*



67u
44.96 µM wt. *Mtb*



68q
10.88 µM wt. *Mtb*



67Ac
23.40 µM wt. *Mtb*

Figure 95: The positive impact of substituent on the antimycobacterial activity.

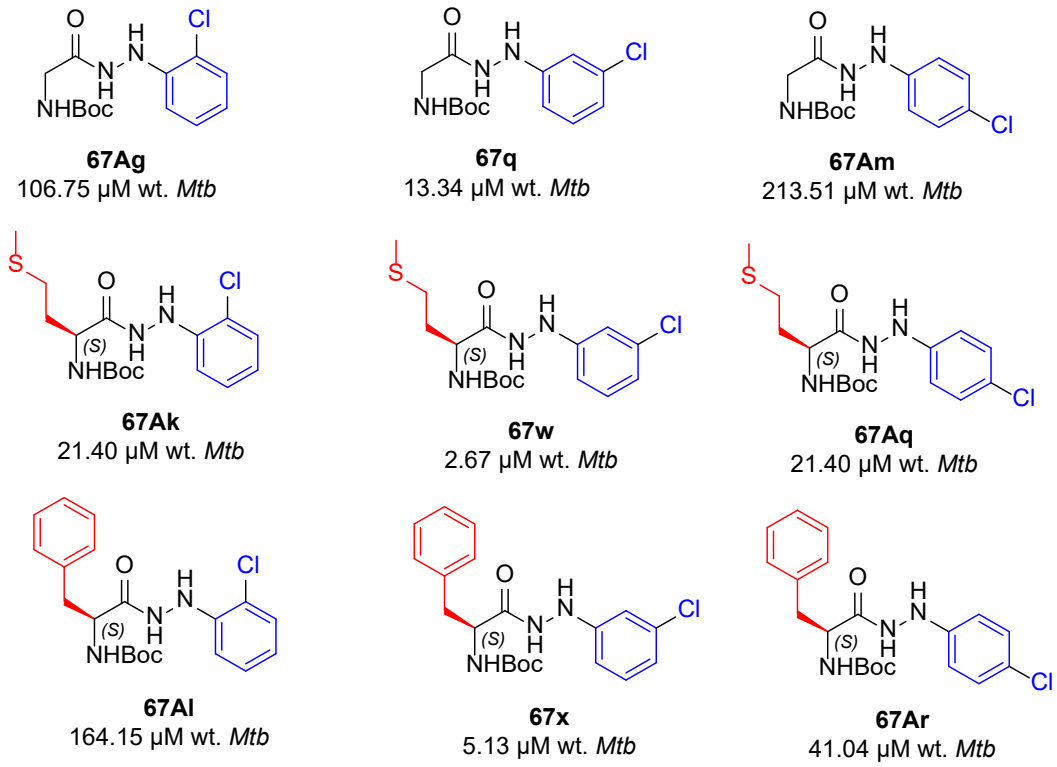
As observed in (Appendix, Table A3), the chlorinated hydrazide **67w** showed higher activity against the wild-type *Mtb*. We explored the impact of the size of the carbamate group on the antimycobacterial activity; an introduction of the Fmoc group instead of the Boc group was utilized. This alteration resulted in reduced potency (*cf.* **67w** vs **68u**).

Consequently, this study employed a similar feature comparison to attempt to understand the key structural features including, the size of side chains, configuration, the incorporation of substituents and the size of carbamate group that has been shown to have a significant effect on the antitubercular activity of these intermediates. More specifically, the findings from the initial screening revealed that the incorporation of the synthetic amino acids with side chains or with D-configuration, the unsubstituted aryl hydrazine and the bulky carbamate group were explored to improve the potency, but they showed an undesired influence on the MIC results. This being so, it was decided to focus on the six *L*-amino acids that showed promising MIC values and coupled with different substituted aryl hydrazines to investigate the most active hydrazide, and to explore the impact of the nature of substituent and its position on the antitubercular activity of intermediates.

Due to the promising MIC results of the chlorinated hydrazides **67q-z**, it was decided to conduct a simple comparison of the structures of hydrazides **67Ag-Ar** which revealed a couple of key differences in activity: i) the increase of the linear lipophilic side chain; ii) the position of the chlorine group. Therefore, the MIC results of *in vitro* antitubercular screening of the chlorinated Boc-*N*-hydrazides **67Ag-Ar** at different positions on aryl hydrazines were obtained (Appendix, Table A4).

In search of more potent antitubercular agents, MIC results (Appendix, Table A4) confirmed the critical role of the size of side chain as the *ortho* chlorinated hydrazides **67Ag-Al** and *para* position **67Am-Ar** with large side chains providing much improved inhibition capability compared to those with no side chain (*cf.* **67Ag** vs **67Ak**) and (*cf.* **67Am** vs **67Aq**). The *para* chlorinated hydrazide **67An**, with the smallest side chain, showed the lowest MIC against the wild-type *Mtb*

(1.59 µM). It is clear from the three examples given in (Figure 96) that the trend is that *meta* is the preferred position for activity against the wild-type *Mtb* as compared to other positions.



67Ag
106.75 µM wt. *Mtb*

67q
13.34 µM wt. *Mtb*

67Am
213.51 µM wt. *Mtb*

67Ak
21.40 µM wt. *Mtb*

67w
2.67 µM wt. *Mtb*

67Aq
21.40 µM wt. *Mtb*

67Al
164.15 µM wt. *Mtb*

67x
5.13 µM wt. *Mtb*

67Ar
41.04 µM wt. *Mtb*

Figure 96: The chlorinated hydrazides at *meta* position showed much better antitubercular activity against the wild-type *Mtb* than other positions.

Although the chlorinated Boc-*N*-hydrazides at the *meta* position have been highly effective against the wild-type *Mtb*, they are less effective against the resistant *Mtb* strains than the *para* chlorinated Boc-*N*-hydrazides (Figure 97).

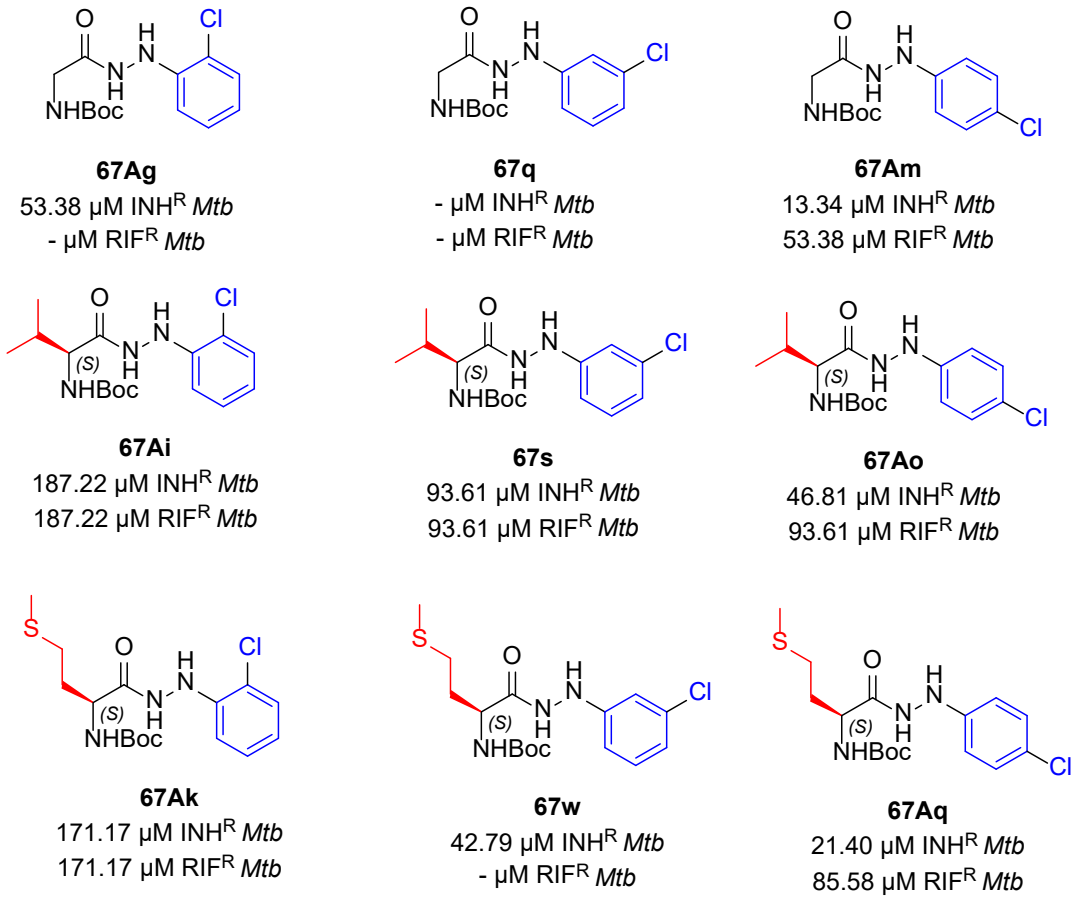


Figure 97: The chlorinated hydrazides at *para* position showed much better antitubercular activity against the resistant *Mtb* than other positions.

We decided to investigate the incorporation of two chlorine groups. For this reason, the di-*ortho* chlorinated Boc-*N*-hydrazides **67As-Ax** were obtained and screened to examine their antimycobacterial activity and compared to the mono chlorinated Boc-*N*-hydrazides (Appendix, Table A5).

In comparison, MIC data analysis was carried out on mono or di- chlorinated Boc-*N*-hydrazides and it was found that the di-chlorinated Boc-*N*-hydrazides provided a better potency against the wild-type *Mtb* than *ortho* or *para* chlorinated Boc-*N*-hydrazides but not greater than the *meta* chlorinated Boc-*N*-hydrazides (Figure 98).

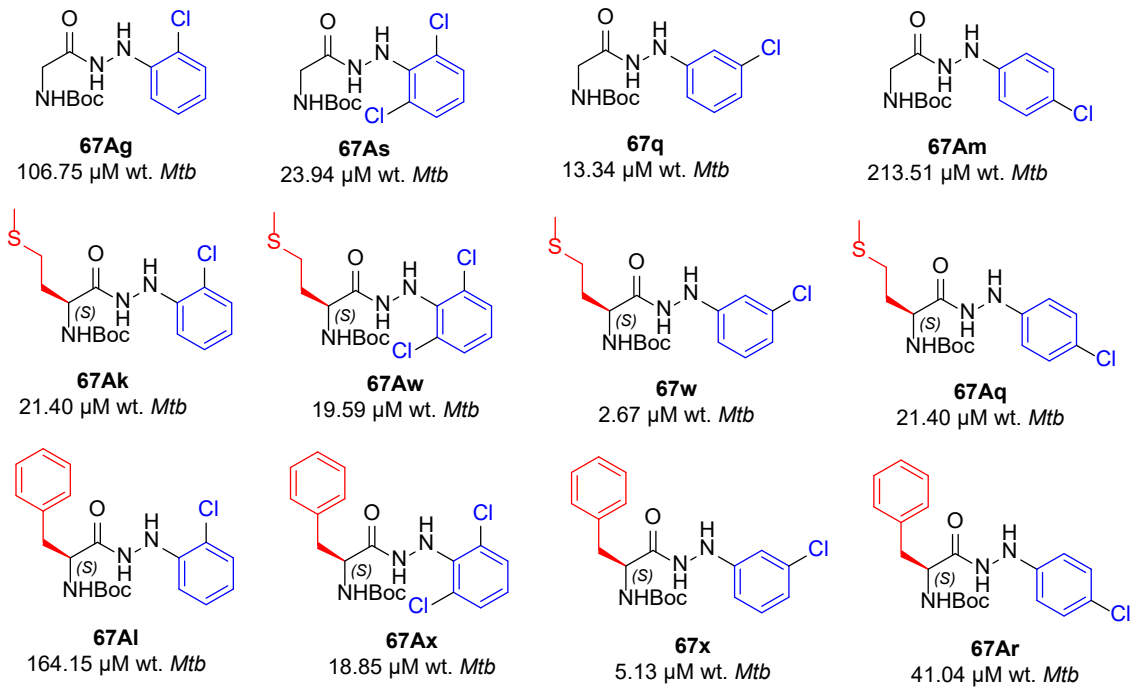


Figure 98: The MIC comparison between the di-chlorinated hydrazides and mono-chlorinated hydrazides.

The di-chlorinated Boc-*N*-hydrazides exhibited reduced activity against resistant strains as compared to the mono-chlorinated Boc-*N*-hydrazides. This led us to further investigations with other substituents at different positions.

To achieve this, exchanging the chlorine moiety for a trifluoromethyl group was assessed. The introduction of the trifluoromethyl group into medicinal agents has been increasingly popular and served as a promising strategy in lead optimization to enhance drug potency. It is certainly true that fluorine has desirable properties, such as enhancing binding energy to target proteins, small atomic size, moderate electron withdrawing effect and lipophilicity.[134] Therefore, the trifluoromethylated Boc-*N*-hydrazides **67Ay-Bp** were screened to investigate the impact of the nature of substituent on MIC (Appendix, Table A6).

No significant improvements were observed in drug potency based on the alteration of −Cl to −CF_3 (Figure 99).

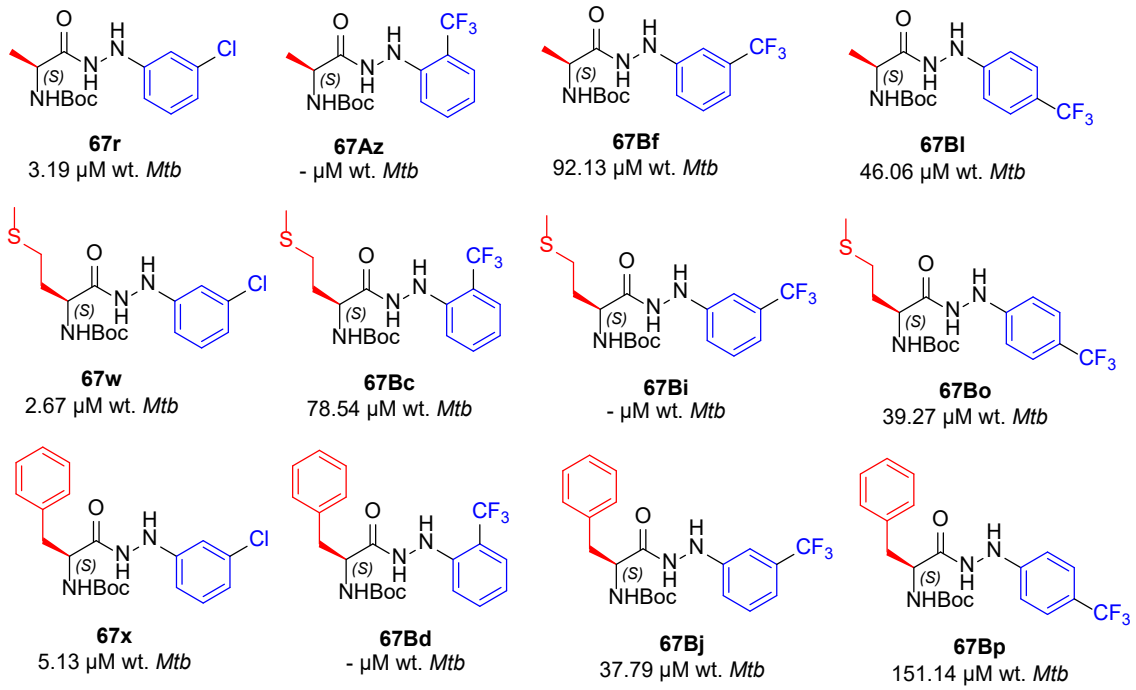


Figure 99: The negative impact of replacement of -Cl to -CF_3 on MIC results against the susceptible *Mtb*.

Moreover, the position of the trifluoromethyl group on the aryl hydrazine was examined and it did not ensure an improvement of antitubercular activity on average (Appendix, Table A6). Overall, the introduction of trifluoromethyl group has no advantage on potency as compared to the chlorine group.

As it appeared that the MIC data of the trifluoromethylated Boc-*N*-hydrazides **67Ay-Bp** was disappointing, therefore, the involvement of two trifluoromethyl groups was also examined. For this reason, the di-*meta* trifluoromethylated Boc-*N*-hydrazides **67Bs-Bx** were obtained and screened to examine their antimycobacterial activity as compared to the mono trifluoromethylated Boc-*N*-hydrazides **67Ay-Bp** (Appendix, Table A7).

Unfortunately, this failed to provide any increase in activity (Appendix, Table A7). We are still inspired by the continuous success of fluorinated drugs as 13 new fluoro-pharmaceuticals were approved by the FDA in 2019.[135] Owing to this, the incorporation of other fluorinated functional groups, such as a fluorine atom was explored.[135] therefore, the activity of di-fluorinated hydrazides **67By-Cd** were investigated (Appendix, Table A8). Given this precedent, it was demonstrated that the di-fluorinated Boc-*N*-hydrazide **67By-Cd** showed no more activity than the di-trifluoromethylated Boc-*N*-hydrazides **67Bs-Bx**.

Common to all TB treatments, high lipophilicity is positively associated with enhanced potency against *Mtb* strains (*cf.* section 2.1.1.2). Because of this, introduction of a bulky halogen substituent such as bromine that could be an invaluable substituent to improve the MIC of these intermediates. For this reason, the brominated hydrazides, **67Ce-Cv**, were obtained and screened to compare their potency against *Mtb* strains with the previous substituents such as chlorine, trifluoromethyl and fluorine and to investigate the effect of the electronic nature of the substituent on antimycobacterial activity (Appendix, Table A9).

The promising change observed relates to the introduction of a bromine group which has shed some light onto the utility of the bulky substituents. The MIC results showed that the brominated hydrazides **67Ce-Cv**, particularly, at the *ortho* and *para* positions containing the higher lipophilic substituent exhibited excellent efficacy against the wild-type *Mtb* strain (Figure 100). In addition to the positive correlation between potency (MIC) toward *Mtb* strains and drug lipophilicity, the MIC data demonstrated that changing the substituent on aryl hydrazine as a useful tool in the search for potentially potent hydrazides (Appendix, Table A1 – A9).

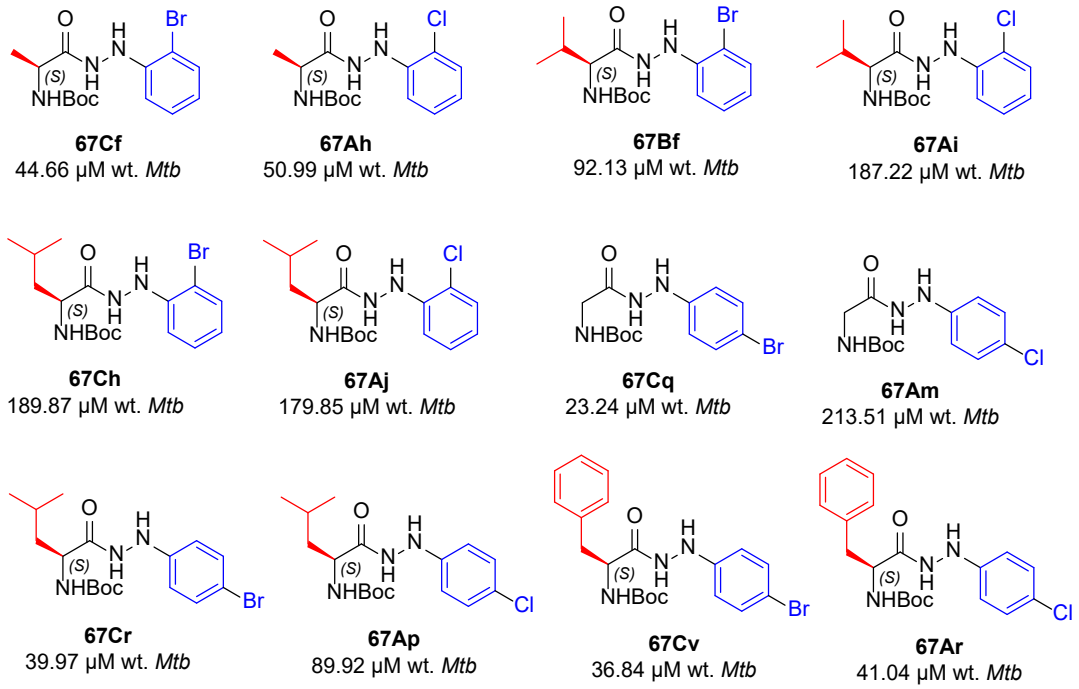


Figure 100: The positive impact of incorporation -Br on MIC results against the susceptible *Mtb*.

Meanwhile, the brominated hydrazides **67Ce-Cv** were shown to have greater activity against the isoniazid resistant *Mtb* strains over other substituted hydrazides. This observed potency was comparable to that of ethambutol **3** and ethionamide **16** (Figure 101).[2]

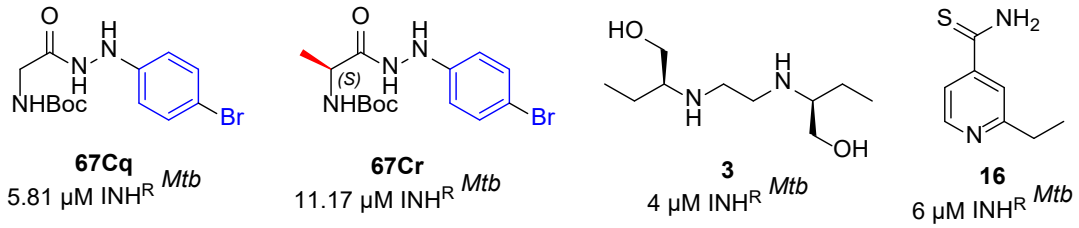


Figure 101: The MIC results of the brominated hydrazides against the resistant INH *Mtb* as compared to current TB treatments.

To confirm the correlation between drug potency and lipophilicity, the substitution of a bulky lipophilic group (bromine) with a less lipophilic substituent such as a nitrile group was proposed. For this reason, the inhibiting capability of cyanated hydrazides **67Cw-Db** was investigated (Appendix, Table A10).

With the MIC analysis in mind, a brief search in the literature suggests that this substitution reduces lipophilicity as mentioned previously (*cf.* section 2.1.1.2).[119]

As shown in (Appendix, Table A10) the cyanation of hydrazides does not seem to be a way of improving drug potency in the TB treatments.

Contrary to this, the antitubercular activity of the hydrazides **67Dc-Dh** containing a nitro group, that is also a less lipophilic substituent, was evaluated against replicating *Mtb* strains. Moreover, the nitro moiety plays a vital role in anaerobic activity of nitroimidazoles including delamanid **25** and pretomanid **26** (*cf.* section 1.3.7). For this reason, the obtained hydrazides **67Dc-Dh** were screened with the MIC results of *in vitro* antitubercular screening revealing a significant decrease in activity against all replicating *Mtb* strains (Appendix, Table A11).

Also involvement of the less lipophilic methylsulfonyl group in the hydrazides had not been investigated further in previous work. This group was also incorporated into hydrazides **67Di-Dn** at the *para* position (Appendix, Table A12).

Unfortunately, MIC results were also disappointing raising concerns about retained antitubercular activity of hydrazides with incorporating a less lipophilic substituent such as a nitrile, nitro or methylsulfonyl (Appendix, Table A10 - A12). For this reason, interest lies in the MIC investigation of hydrazides with higher lipophilic substituents.

As a consequence of these observations, the methylated hydrazides **67Do-Ef** would be of interest to enhance the potency of intermediates (Appendix, Table A13). A small alkyl substituent has the potential to improve potency, when compared to the other less lipophilic groups. During the MIC analysis, it was observed that the *para* methylated hydrazides **67Eb** and **67Ee** were able to induce a very potent antitubercular activity although the *meta* methylated hydrazides **67Du-Dz** showed, in general, better inhibitory activity than other positions. Nevertheless, it is important to highlight that this substituent does not provide better MIC results than chlorinated hydrazides (Figure 102).

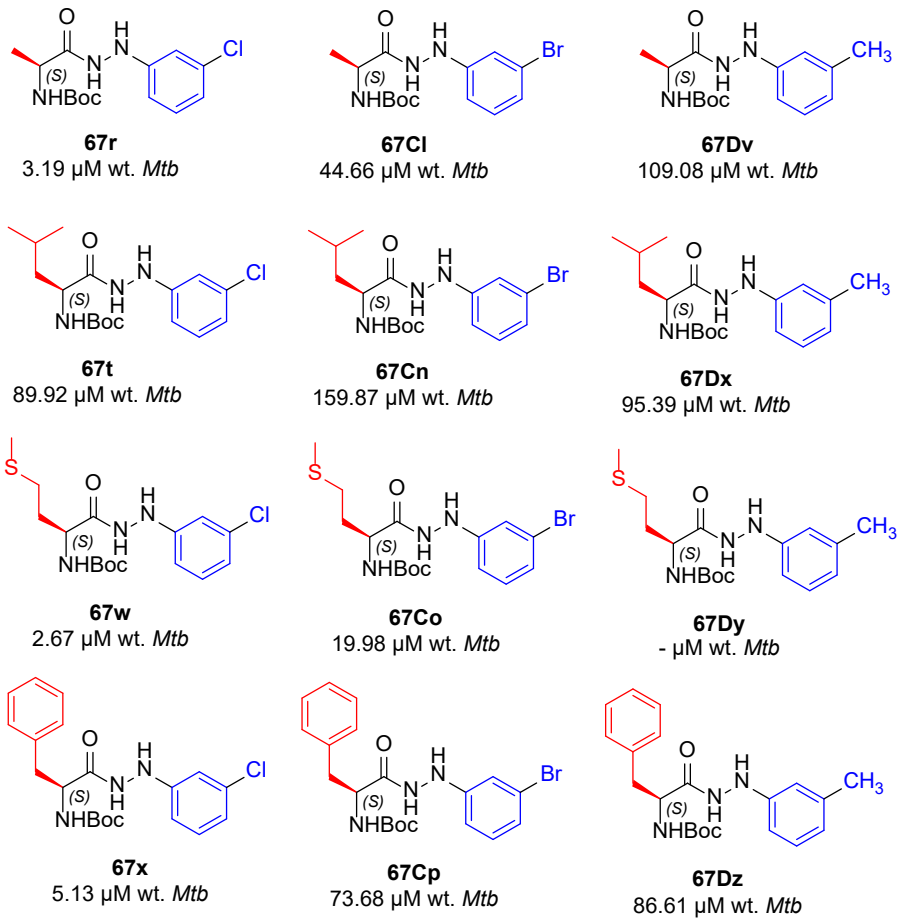


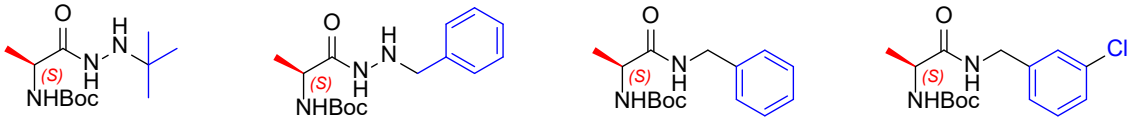
Figure 102: No positive impact of incorporating -CH_3 instead of -Cl on MIC results against the susceptible *Mtb*.

It is important to highlight that the antitubercular activity of the methylated hydrazides (Appendix, Table A13) against the resistant strains was not as good as the chlorinated hydrazides (Appendix, Table A3 – A4) and the brominated hydrazides (Appendix, Table A9).

For the purpose of this SAR study, the range of the substituted hydrazides was expanded, across a number of modified aryl hydrazines, seeking more bulky alternatives to the methyl group that would provide analogues with greater potency. We chose the isopropyl group as it features enhanced cell penetration and has the potential to occupy deeper hydrophobic regions of the drug target. Owing to this, the hydrazides **67Eg-El** containing isopropyl groups at the *para* position were obtained and screened (Appendix, Table A14).

The MIC results demonstrate that incorporation of a larger alkyl substituent slightly improves inhibitory activity as compared to the methylated Boc-*N*-hydrazides (*cf.* **67Eg** vs **67Ea**), (*cf.* **67Ei** vs **67Ec**) and (*cf.* **67El** vs **67Ef**) (Appendix, Table A14).

Now it is worthy of saying that in this SAR work, the use of aryl hydrazines is associated with higher potency, which can be modulated by exchanging the aryl hydrazine with other units, such as alkyl hydrazine, benzyl hydrazine, benzyl amine or 3-chloro benzylamine. Owing to this, it could provide access to a range of structurally diverse amino acid hydrazides and examine if and how these changes affected the pharmacological and physicochemical properties of the resulting compounds (Figure 103).



89
- µM wt. *Mtb*
- µM INH^R *Mtb*
- µM RIF^R *Mtb*

91
- µM wt. *Mtb*
- µM INH^R *Mtb*
- µM RIF^R *Mtb*

114
- µM wt. *Mtb*
- µM INH^R *Mtb*
- µM RIF^R *Mtb*

114a
- µM wt. *Mtb*
- µM INH^R *Mtb*
- µM RIF^R *Mtb*

Figure 103: The negative impact of exchanging of the aryl hydrazine **69** with alkyl hydrazine **88**, benzyl hydrazine **90**, benzyl amine or 3-chloro benzylamine on antitubercular activity.

Unfortunately, this modulation led to the loss of antimycobaterial activity of the amino acid hydrazides. The involvement of alkyl hydrazine **89** and benzyl hydrazine **91** proved ineffective as no activity was observed. Moreover, the importance of the hydrazide moiety was confirmed as the incorporation of benzyl amine **114** failed to show any activity against all *Mtb* strains and even with 3-chloro benzylamine **114a**. For these reasons, the use of alkyl hydrazines, benzyl hydrazines, benzyl amine or 3-chloro benzylamine does not seem to be a way of enhancing drug potency. Therefore, aryl hydrazines would remain constant to preserve the hydrazide pharmacophore and the results have demonstrated that substitution can be achieved at three sites around these molecules (Figure 104).

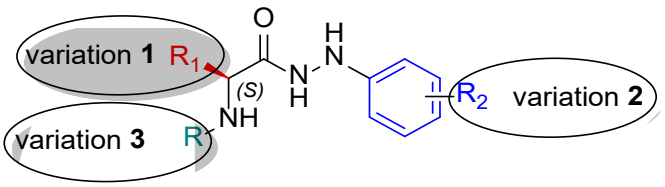


Figure 104: The core building block structure of hydrazide.

The data collected so far allowed for an SAR study to investigate the most active components of the protected hydrazides. Depending on their structural composition,these have been shown to possess varying degrees of potency towards susceptible and resistant *Mtb* strains. The lack of activity related to the alkyl hydrazine and the benzyl hydrazine indicate the aryl hydrazine is essential for activity. Taken together, it is possible that the substantial changes observed in MIC results relate to the amino acid itself, most likely due to the presence of some bioactive functional groups including phenol **70j** indole **70s**, thioether **70g** sulfhydryl **70p**, guanidinyl **70t** and imidazole **70i**. Therefore, the MIC results confirmed that the single protected amino acid did not interfere with mycobacterial growth (Appendix, Table A15).

The screening profile of amino acids revealed that the protected amino acids showed no activity but have the potential to be a novel component when to designing novel TB treatments. Based on these investigations, the promising antitubercular activity came from the hydrazide core, that are designed to act as potential antimycobacterial intermediates.

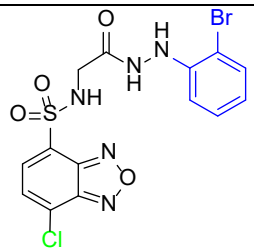
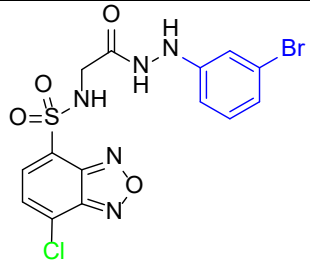
3.1.3 The SAR Exploration of Benzoxa-[2,1,3]-diazole

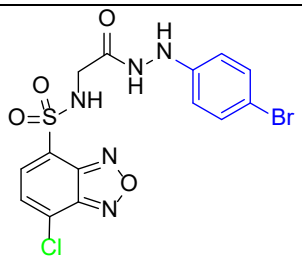
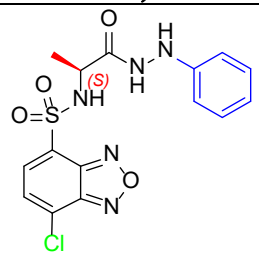
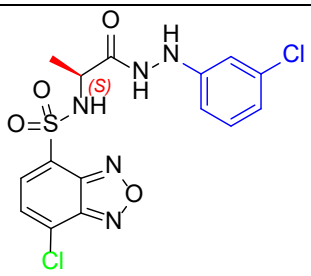
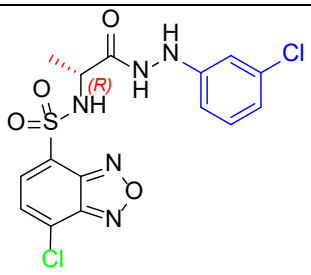
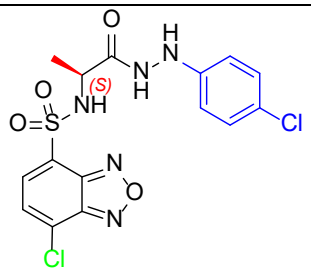
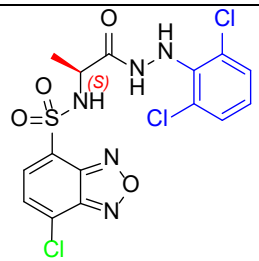
Promising antitubercular activity has been achieved screening many hydrazides, and there are very active examples discussed throughout this report. Due to their efficacy, the substituted Boc-*N*-hydrazide, mainly with higher lipophilic substituents, still inspire drug design. The conversion of these intermediates into the final compounds **65** could improve their potency and binding affinity as the benzoxa-[2,1,3]-diazole core appears to be essential for activity and selectivity (*cf.* section 1.7).[1] Thus, it would be expected that the designed benzoxa-[2,1,3]-diazole peptidomimetics **65** tend to better occupy the active target site, including a deeper pocket.

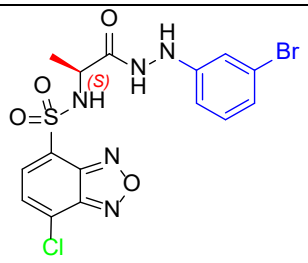
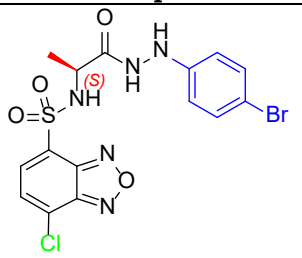
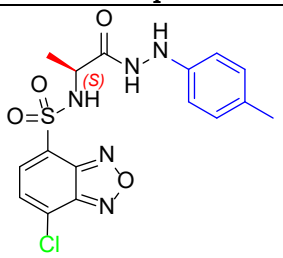
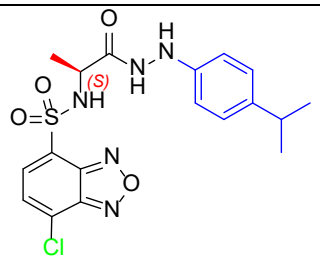
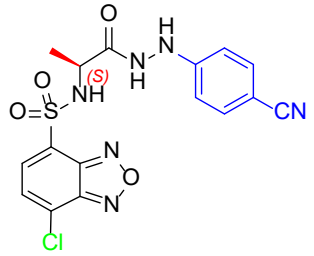
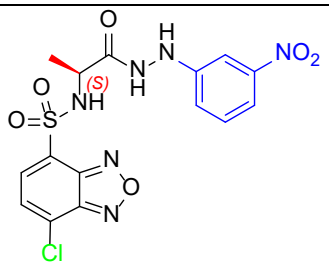
Thereby, this conjugation provides a good opportunity for enhancing the potency of potential drug candidates. Therefore, the efficacy of the designed benzoxa-[2,1,3]-diazole peptidomimetics **65** required further assessment.

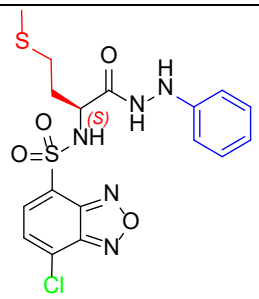
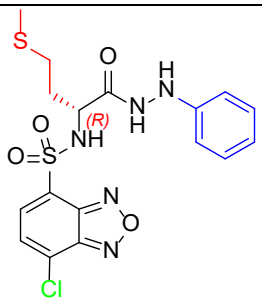
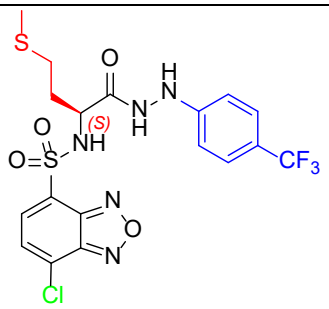
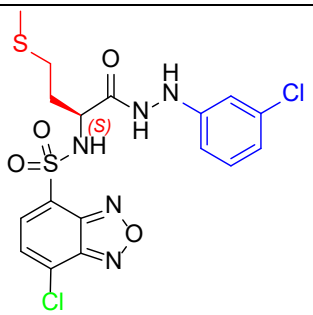
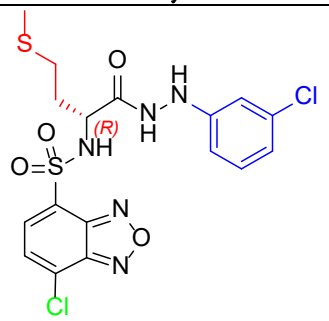
As a result, the final benzoxa-[2,1,3]-diazoles **65h-Ar** were screened in the same way against the drug-susceptible, mono-resistant and double-resistant *Mtb* strains (Table 7).

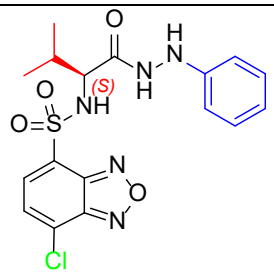
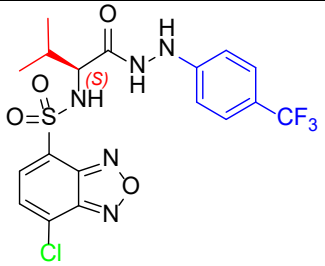
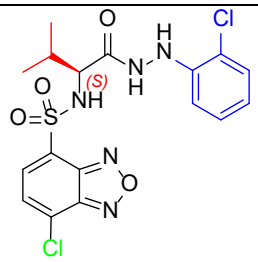
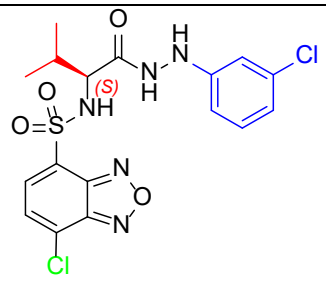
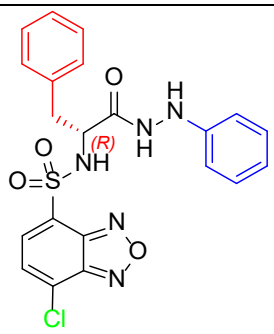
Table 7: The MIC results of the antimycobacterial susceptibility testing of the benzoxa-[2,1,3]-diazole peptidomimetics **65h-Ar**; where a (-) indicates to a lack of inhibition activity.

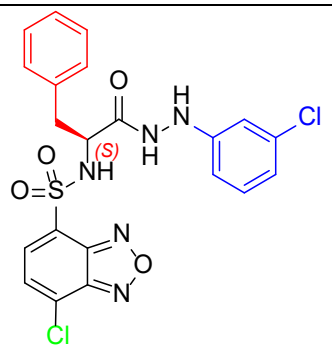
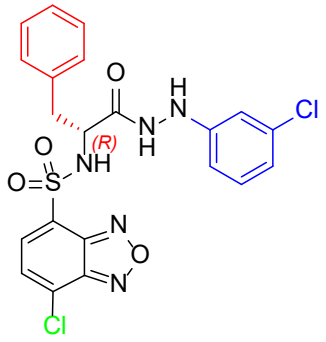
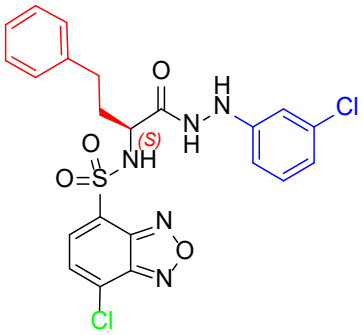
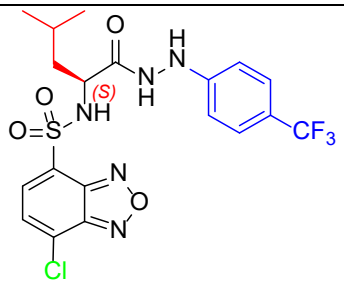
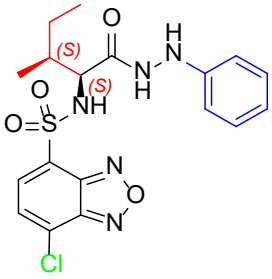
		MIC (µM)			
Entry	Structure	WT. *Mtb* mc²7902	INH. resistant *Mtb* mc²8245	INH./ RIF. resistant *Mtb* mc²8250	INH./ RIF. resistant *Mtb* mc²8258
1	 **65h**	34.73	34.73	34.73	34.73
2	 **65i**	34.73	34.73	34.73	34.73

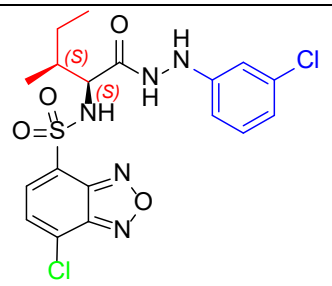
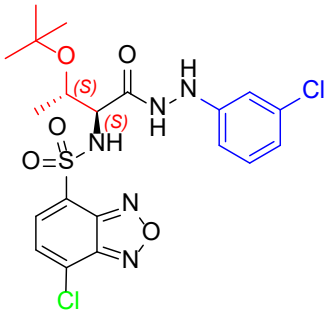
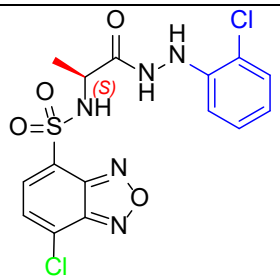
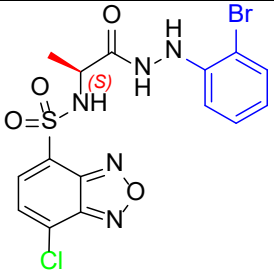
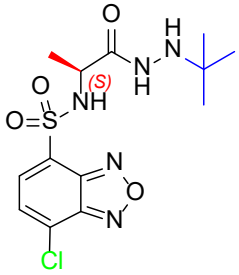
3	 **65j**	69.46	69.46	69.46	69.46
4	 **65k**	161.69	161.69	161.69	161.69
5	 **65l**	74.37	74.37	148.75	74.37
6	 **65m**	-	-	-	-
7	 **65n**	74.37	74.37	74.37	74.37
8	 **65o**	-	-	-	-

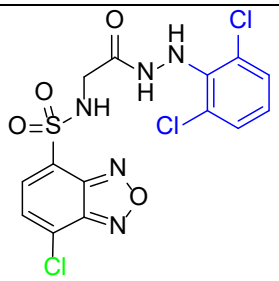
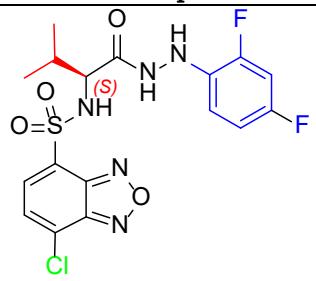
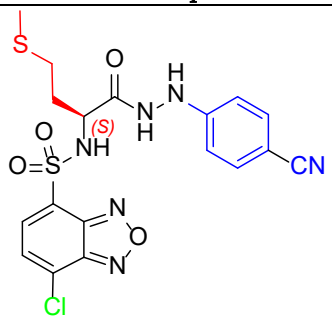
9	 **65p**	67.41	67.41	67.41	67.41
10	 **65q**	67.41	67.41	67.41	67.41
11	 **65r**	78.08	156.15	78.08	78.08
12	 **65s**	73.08	146.15	146.15	146.15
13	 **65t**	76.04	76.04	76.04	76.04
14	 **65u**	72.59	72.59	72.59	72.59

15	 **65v**	140.37	-	-	-
16	 **65w**	-	-	-	-
17	 **65x**	122.15	122.15	122.15	122.15
18	 **65y**	65.26	65.26	65.26	65.26
19	 **65z**	65.26	32.63	65.26	65.26

20	 **65Aa**	150.99	-	-	-
21	 **65Ab**	130.12	130.12	130.12	130.12
22	 **65Ac**	69.82	69.82	69.82	69.82
23	 **65Ad**	69.82	69.82	69.82	69.82
24	 **65Ae**	-	-	-	-

25	 **65Af**	31.60	31.60	31.60	31.60
26	 **65Ag**	126.39	63.20	126.39	126.39
27	 **65Ah**	61.49	61.49	61.49	61.49
28	 **65Ai**	63.25	63.25	63.25	63.25
29	 **65Aj**	-	-	-	-

30	 **65Ak**	135.50	67.75	135.50	135.50
31	 **65Al**	123.94	123.94	123.94	123.94
32	 **65Am**	74.37	74.37	37.19	37.19
33	 **65An**	67.41	67.41	33.70	33.70
34	 **65Ao**	-	-	-	-

35	 **65Ap**	-	-	-	-
36	 **65Aq**	-	-	-	-
37	 **65Ar**	-	-	-	-

In most cases, introducing the benzoxa-[2,1,3]-diazole moiety demonstrated the expected increment in antitubercular activity against the wild-type and particularly mono-resistant and double resistant *Mtb* as compared to their intermediates (*cf.* **65u** vs **67Dd**), (*cf.* **65z** vs **67Ae**), (*cf.* **65Ab** vs **67Bm**), (*cf.* **65Ac** vs **67Ai**), (*cf.* **65Ad** vs **67s**), (*cf.* **65Ag** vs **67Af**) and (*cf.* **65Ai** vs **67Bn**).

The biological data agreed with the results (discussed in section 3.1.2) as consequence of the promising change in MIC results that were observed following introduction of the substituted aryl hydrazine instead of phenylhydrazine. For example, using different halogen substituents on the aryl hydrazine showed a significant effect on MIC (*cf.* **65p** vs **65k**), (*cf.* **65y** vs **65v**), (*cf.* **65Ad** vs **65Aa**), (*cf.* **65Ag** vs **65Ae**) and (*cf.* **65Ak** vs **65Aj**). Likewise, changing the position of the substituent, particularly, at the *meta* position resulted in a noticeable decrease in MIC (*cf.* **65i** vs **65j**).

In light of these results, we examined the activities of designed benzoxa-[2,1,3]-diazole peptidomimetics bearing di-halogenated aryl hydrazines. Regrettably, the involvement of di-chlorinated aryl hydrazine **65o**, **65Ap** or di-fluorinated aryl hydrazine **65Aq** led to a significant loss of activity to inhibit the growth of *Mtb*. This finding raises concerns about the feasibility of addition more than a substituent on aryl hydrazine. In particular, we were interested to show how presence of a double substituent on aryl hydrazine may also reduce the antimycobacterial activity of both intermediates (*cf.* Table A5, Table A7 and Table A8) and final compounds **65o**, **65Ap** and **65Aq**. Consequently, this leads to the belief that the target site requires a single substituent to be present.

The effect of the linear and larger hydrophobic side chains on the antitubercular activity is somewhat conflicting. In the results (*cf.* Figure 92 and 94), the adaptation of the amino acid generally resulted in a decrease in MIC as a consequence of enlarging the side chain. Owing to this, an increase in the length and size of side chain gave more promising MIC results of the chlorinated final compounds (Figure 105). There is also agreement with the earlier findings (cf. Figure 95). It was found that involvement of homophenylalanine **65Ah** did not show any improvement in MIC result (*cf.* **65Af** vs **65Ah**).

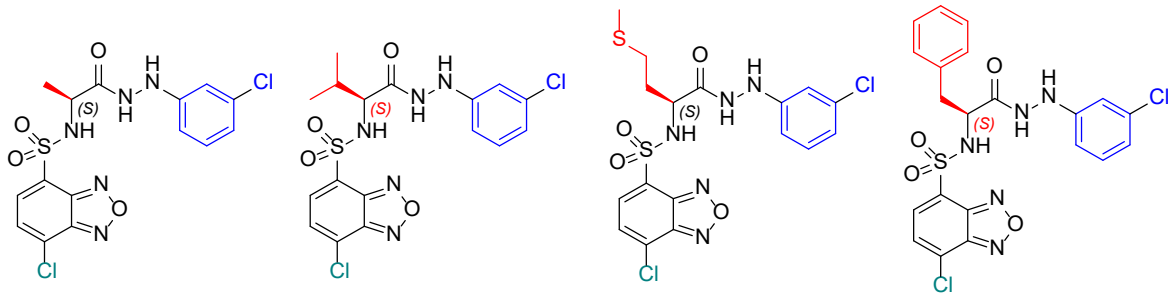


Figure 105: The improvement of MIC results against the wild-type *Mtb* as increase in the side chain of the chlorinated final compounds.

Despite their structural similarities, the MIC data of the final compounds bearing bromine substituent, have an advantage in potency as compared to the chlorine group (*cf.* **65An** vs **65Am**), (*cf.* **65p** vs **65l**) and (*cf.* **65q** vs **65n**). However, in this case, the fluorinated compounds **65x** and **65Ab** provided a poorer activity against all *Mtb* strains. Taken together, this leads to the conclusion that the involvement

of high lipophilic substituents could be desirable to increase the potency of benzoxa-[2,1,3]-diazole peptidomimetics.

As indicated in Table 7, the poor MIC results of final compounds **65l**, **65w** and **65Ae** also demonstrated the negative impact of D-configuration on the antimycobacterial activity with no observable activity against all *Mtb* strains.

The substitutions to the aryl hydrazine **69** were applied using different electron donating groups such as alkyl groups, and electron withdrawing groups such as halogens to investigate the effect these substitutions have on the biological activity of benzoxa-[2,1,3]-diazole peptidomimetics **65** against the wild-type, mono-resistant and double-resistant *Mtb*. As seen in table 7, compounds **65n** and **65q** having 4-chlorophenylhydrazine and 4-bromophenylhydrazine in their structures were determined to have significantly higher antitubercular potential against all *Mtb* strains than compound **65r** and **65s** having 4-methylophenylhydrazine and 4-isopropylphenylhydrazine (*cf.* **65n** vs **65r**) and (*cf.* **65q** vs **65s**). However, in the compounds **65t** and **65u** containing strong electron withdrawing groups namely cyano and nitro substituent, no meaningful changes were observed in MIC results most probably because of their reduced lipophilicity.

To investigate the ability of exchanging the aryl hydrazine **69** with alkyl hydrazine **88** to affect the activity, this modulation proved ineffective as no activity was observed (*cf.* **65k** vs **65Ao**).

According to the obtained MIC data (Table 7), the final compound **65Af** possessed significant antitubercular activity against the susceptible, mono-resistant and double-resistant *Mtb* strains with a MIC value of 31.60 µM

Finally, the designed benzoxa-[2,1,3]-diazole peptidomimetics **65**, having large linear side chain amino acids, L-configuration and halogenated aryl hydrazine at *meta* position, gave rise to the most potent structures.

4 Conclusion

The continuing demand for effective TB treatments with novel modes of action is particularly awaited in order to combat the development and spread of the multi- and extensively drug-resistant *Mtb* strains. Owing to this, attention turned to a drug - to - target strategy because it may shorten the time scale for TB drug discovery. The focus of this strategy is to assess new chemical entities to identify the most potent and selective inhibitor to *Mtb* growth.[136]

Our previous studies demonstrated that benzo-[2,1,3]-diazole peptidomimetics **65** have excellent activity and selectivity against *Mtb*.[1] Based on these findings, the present work was mainly focused on the determination of the fundamental structure-activity relationship of this architecture **65** as promising antitubercular agents. Throughout this study, a rational series of derivatives based around the benzo-[2,1,3]-diazole peptidomimetics **65** was synthesised in an attempt to investigate antimycobacterial activity against wild-type, INH-resistant, and INH/RIF-resistant *Mtb* strains through a Resazurin Microtiter Assay (REMA). A number of conclusions could be derived from this work. Firstly, the section of chemistry explored in this work provides a good framework for the amide and sulfonamide bond formations to generate a new library of intermediates **67**, **68**, **89** and **91** and final compounds **65**. With many problems to contend with, the synthesis of hydrazides highlighted that a change of amide coupling conditions was important. Replacement of DCC /HOBt with alternative coupling partners including DMTMM and HBTU proved successful. The revised synthetic route employing HBTU was quicker, higher yielding and more reliable for the synthesis of hydrazides. Once synthesised, the enantiomeric purity of resulting intermediates **67** was determined through a variety of techniques, including polarimetry, chiral HPLC, and the Flack parameter of a single crystal structure.

The final step involved the Boc or Fmoc deprotection of intermediates to yield the free amine hydrazide **92** and **93** to allow for their reaction with the benzoxa-[2,1,3]-diazole sulfonyl chloride **66** to deliver the final benzoxa-[2,1,3]-diazole peptidomimetic **65**. The enantiomeric purity of the final compounds **65** was also confirmed by chiral HPLC and the major rotamer of our benzoxa-[2,1,3]-diazoles (trans) was determined utilising a nuclear Overhauser effect spectroscopy

(NOESY) experiment. During the course of the MIC analysis, it was observed that the conjugation of the hydrazides **67**, **68** and **89** with benzoxa-[2,1,3]-diazole moiety **66** provided a compound with good level of activity against susceptible, mono-resistant and double-resistant *Mtb* strains although it appears to have observable cytotoxicity. The findings from this SAR study indicated that an increased the size of side chain of L-amino acids **70** showed much better antitubercular activity against *Mtb*. Alkyl hydrazine **88**, benzyl hydrazine **90**, larger carbamate group (Fmoc group), less lipophilic substituents on aryl hydrazine **69** (nitro, nitrile and methylsulfonyl), the di-substituted aryl hydrazine **69** and D-configuration did not show any improvement on MIC results. Whilst, the size of side chain (linear unreactive side chains), incorporation of heavy halogens (bromide and chloride), L-configuration, mono-substitution at *meta* position and the benzoxa-[2,1,3]-diazole moiety **66** play a key role in improving the antimycobacterial activity.

The final conclusion drawn was that the structural modification of this architecture **65** at three sites (the hydrazine **69**, amino acid **70**, and the benzoxa-[2,1,3]-diazole **66**) would considerably improve the antitubercular activity and reduce the cellular cytotoxicity of the promising compounds whilst not affecting their mycobacterial selectivity, making them attractive architectures for further exploitation as novel antitubercular agents (Figure 106).

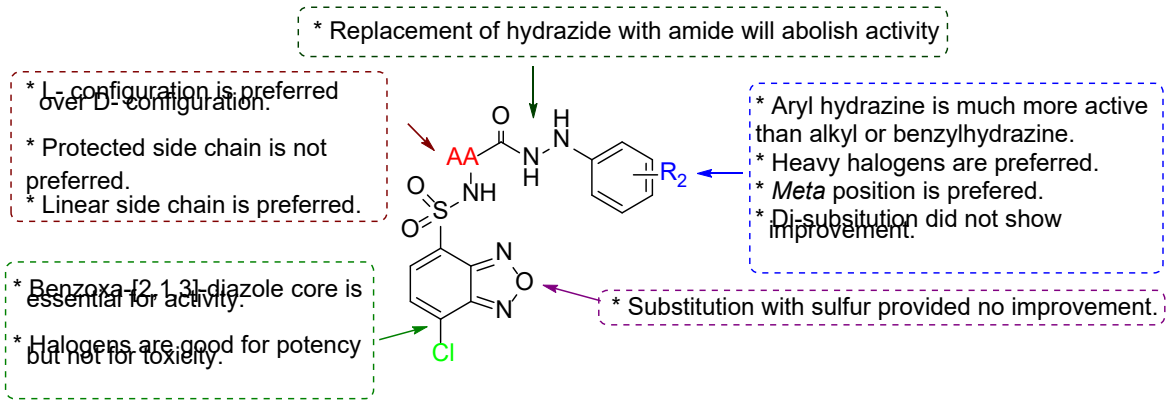


Figure 106: SAR conclusions structural modification of the benzoxa-[2,1,3]-diazole peptidomimetics architecture **65** is to develop a novel TB treatment.

5 Future work and additional studies

5.1 The incorporation of the functionalized amino acids

Pleased by the initial results but with no time remaining, a fruitful strategy for investigating the efficacy of incorporation of the functionalized amino acids was proposed. This strategy started with azido-modified unnatural amino acids as only the three derivatives Boc-3-azido-*L*-alanine **70Ab**, Boc-6-azido-*L*-norleucine **70Ac** and Boc-4-azido-*L*-phenylalanine **70Ad** (Figure 107) are available commercially.

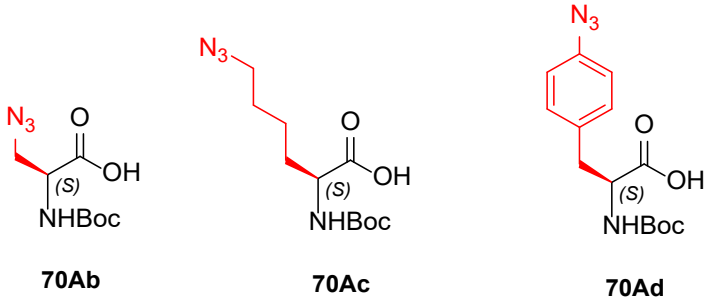
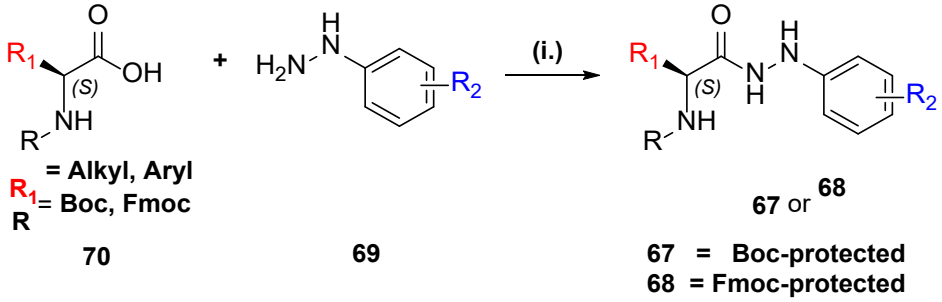


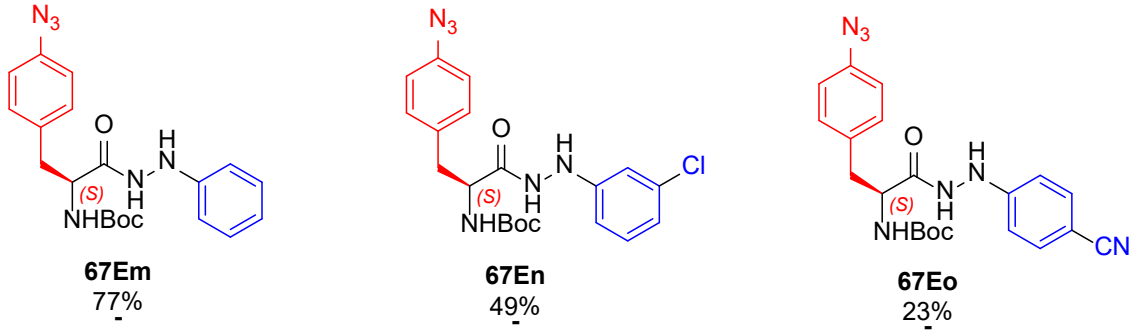
Figure 107: The chemical structures of azido-modified unnatural amino acids.

The synthetic approach that was followed to obtain the hydrazides **67Em-Eo** containing azido derivatives of amino acids would again be undertaken to investigate the efficacy of more azido derivatives (Scheme 4)[107].



Scheme 4: Reagents and conditions: i.) DMTMM, DIPEA, THF, 6-8 h, (59- 87%), r.t.

Due to impurities and rapid decomposition, particularly with the alkyl azide derivatives, this methodology is time-consuming and limited to the aryl azide derivatives which led to difficulties in obtaining the pure hydrazides **67** containing the alkyl azide residues. As a result of this, it was decided to focus on coupling the Boc-4-azido-*L*-phenylalanine **70Ad** with three different aryl hydrazines **69** to afford the hydrazides **67Em-Eo** (Figure 108).



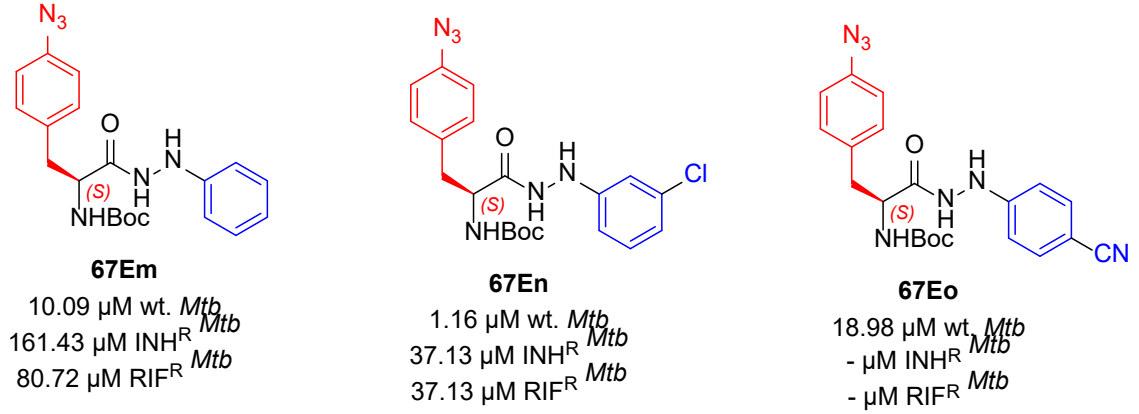
67Em
77%

67En
49%

67Eo
23%

Figure 108: The chemical structures of the hydrazides **67Em-Eo** containing azido derivatives of amino acids.

To undertake this investigation, the impact of incorporation of the azido derivatives of amino acids was evaluated through screening these resulting hydrazides **67Em-Eo** against the susceptible and resistant *Mtb* strains (Figure 109).



67Em
10.09 µM wt. *Mtb*
161.43 µM INH^R *Mtb*
80.72 µM RIF^R *Mtb*

67En
1.16 µM wt. *Mtb*
37.13 µM INH^R *Mtb*
37.13 µM RIF^R *Mtb*

67Eo
18.98 µM wt. *Mtb*
- µM INH^R *Mtb*
- µM RIF^R *Mtb*

Figure 109: The positive impact of incorporation of the azido derivatives of amino acids on antitubercular activity.

Based on the promising results, introducing Boc-4-azido-*L*-phenylalanine **70Ad** demonstrated a further decrease in MIC. In comparison, the hydrazides **67Em-Eo**, azido-containing derivatives exhibited the best antitubercular activity as the resulting hydrazides (discussed in section 3.1.2) were less potent against all the *Mtb* strains.

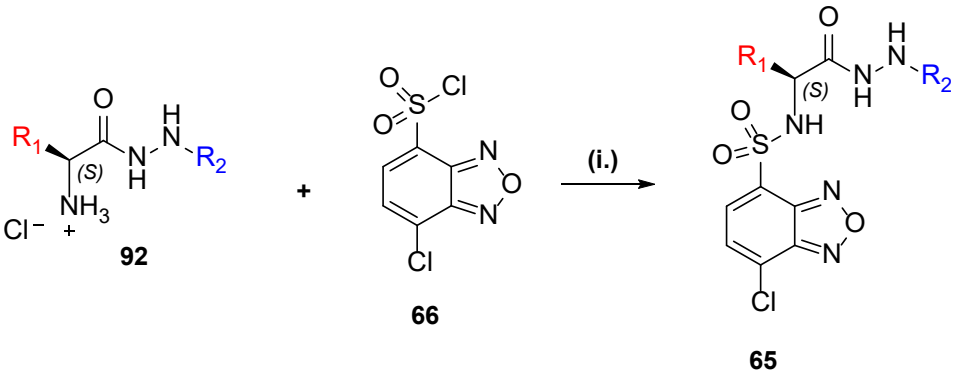
In fact, when the azido group is attached to the side chain of amino acids, the drug affinity increased as well as the potency greatly enhanced particularly, in the case of cyanated Boc-*N*-hydrazides (*cf*. **67Db** vs **67Eo**).

In regard to the conversion of these intermediates into the final compounds **65**, the hydrazide **67En**, which possesses the lowest MIC value, was converted into

the final benzo-[2,1,3]-diazole **65As**. Due to instability of the azide group in the strong acidic conditions, this step consumed a long time to accomplish even with different acids in different concentration (Table 8).

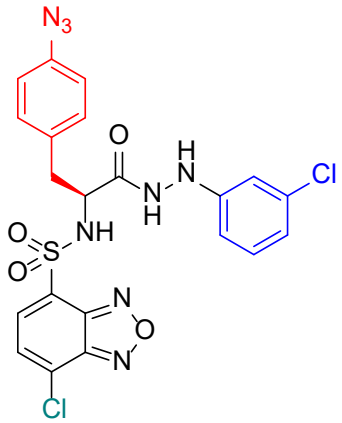
Entry	Condition	Yield %
1	4 M HCl in dioxan, 30 minutes at rt.	-
2	25% v/v TFA in DCM, 2 h at rt.	-
3	50% v/v TFA in DCM, 30 minutes at rt.	-
4	1 M HCl in H_2O, 2 h at rt.	-
5	2 eq. v/v 4 M HCl in dioxan in Et_2O, 18 h at rt.	-
6	15% v/v aq. HCl in DCM, 1 h at rt.	95 %

Following this is the deprotection of intermediate, which will allow reacting with sulfonyl chloride **66** to deliver the final benzo-[2,1,3]-diazole **65As** (Scheme 12).



Scheme 12: Reagents and conditions: i.) pyridine, MeCN, 4-5 h, (39- 72%), r.t.

The antitubercular activity of benzo-[2,1,3]-diazole **65As** was examined against all *Mtb* strains (Figure 110).

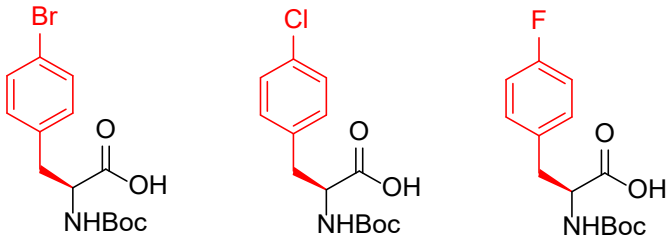


65As 58.46 µM wt. *Mtb*
35 % 29.23 µM INH^R *Mtb*
58.46 µM INH/RIF^R *Mtb*
29.23 µM INH/RIF^R *Mtb*

Figure 110: The yield and MIC results of the benzo-[2,1,3]-diazoles **65As** containing azido derivatives of amino acids.

The incorporation of the functionalized amino acids for future work

As mentioned previously, there is a positive correlation the lipophilicity and potency of the resulting compounds, therefore, the natural amino acid fragment had been changed to a functionalized unnatural amino acid. Out of the amino acid derivatives used it appears that those with Boc-4-azido-*L*-phenylalanine **70Ad** proved to be more potent, hence why it may be worth exploring more phenyl substituted amino acids to carry forward to investigate a new lead compound. As a result of this SAR study, the future aim of this project will continually evaluate the impact of the functionalized amino acids, therefore, there are a number of functionalized derivatives of amino acids such as Boc-4-bromo-*L*-phenylalanine **70Ae**, Boc-4-chloro-*L*-phenylalanine **70Af**, Boc-4-fluoro-*L*-phenylalanine **70Ag** (Figure 111). All of this information points to the conclusion that more functionalized derivatives of amino acids need more study alongside changing the substituted aryl hydrazine.



70Ae **70Af** **70Ag**

Figure 111: The chemical structure of functionalized derivatives of amino acids **70Ae-Ag**

5.2 Modification of the benzoxa-[2,1,3]-diazole moiety to produce a better inhibitor effect for future work

It is also possible to incorporate new substituted sulfonyl chloride moieties **69b-e** (Figure 112) by changing the substituents in selected positions. This strategy could improve the potency of a new series of modified benzo-[2,1,3]-diazole peptidomimetics.

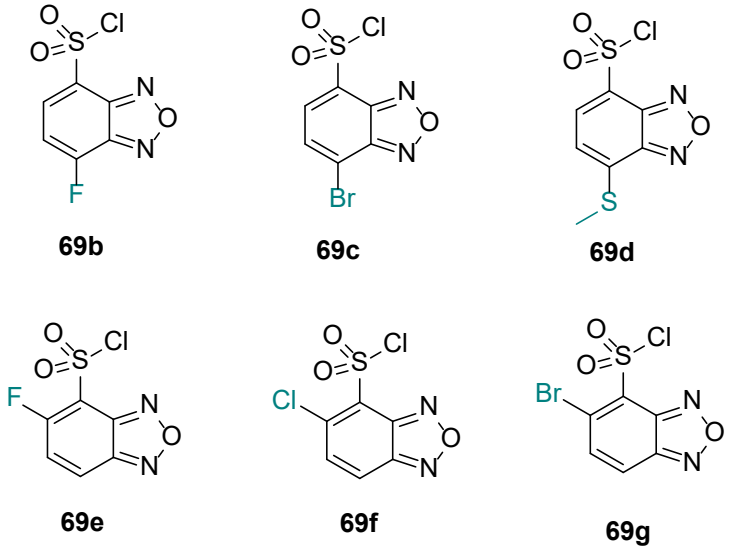


Figure 112: The chemical structure of new substituted sulfonyl chloride moieties **69b-g**

Once the final sulfonamide coupling is successfully achieved, the strategy will be to evaluate the pharmacological effect of the new series of modified benzo-[2,1,3]-diazole against the susceptible and resistant *Mtb* strains. The SAR investigation of these derivatives would be useful as it would uncover the role of the substituent at different position in **69**. Further synthesis and MIC analysis in the future would determine which substituted sulfonyl chloride moiety **69** has a better activity against all *Mtb* strains.

5.3 Proteomic investigation for future work

The major limitation of this explorative study was that a mechanism underlying the inhibitory effect of the compounds being tested was not investigated. Future work would need to address this shortfall. In an attempt to identify the drug target, the designing of chemical probes related to hydrazides **67** and benzo-[2,1,3]-diazole derivatives **65** is a future aim of this project. This will help us to understand the toxic and medicinal properties of these bioactive compounds. In proteomic studies, there are two valuable techniques for studying the interaction of drugs with their target proteins. These include photoaffinity labelling and activity-based protein profiling (ABPP).

5.3.1 Photoaffinity probe design

The most common photoactivatable probes utilized in photoaffinity labelling (PAL) studies are benzophenone **108**, aryl azide **109** and alkyl diazirine **110** which can be activated by UV light (Figure 113).

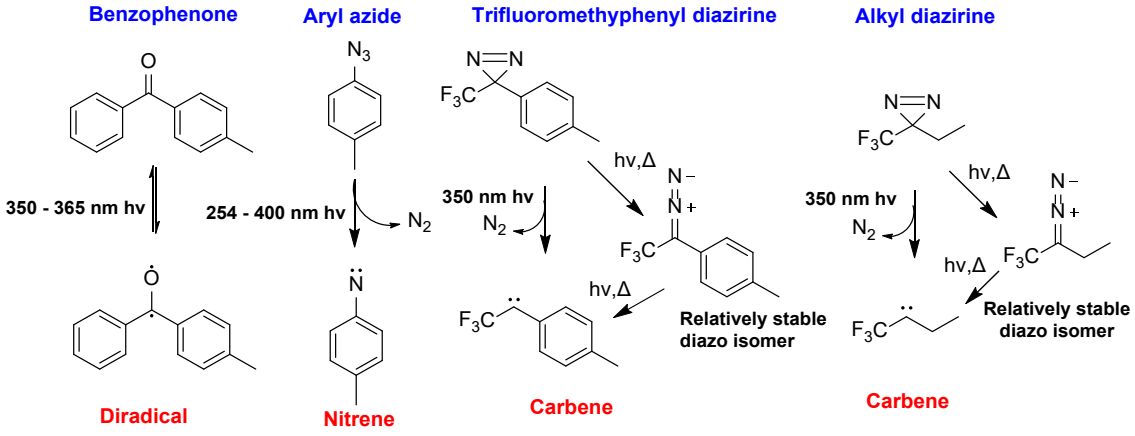


Figure 113: The mode of action of different UV-photoactivating groups.[137]

These covalently crosslink to the drug target protein which permits the use of analytical tools such as electrophoresis and MS to investigate the structure of protein receptor-drug complexes.[138] This study begun with incorporating the azido-modified unnatural amino acids **70Ab-Ad** (discussed in section 5.1) In the PAL study, we present a significantly improved procedure for the synthesis of hydrazide **67Em-Eo** and benzo-[2,1,3]-diazole **65As** probes containing the aryl azide group, which was incubated with all *Mtb* strains and then bound specifically

to the pocket of receptor due to their affinity. Although azides have been widely used in nitrene transfer reactions, the photolysis of these azide groups may produce imines as byproducts. The exposure of these probes to light and heat could induce this decomposition. Furthermore, these probes can react with other reagents and displace the azide group to form new substituted molecules due to their similar behaviour to halogens.[139] We can therefore conclude that these azido-modified unnatural amino acid **70Ab-Ad** showed poor stability and very restricted suitability for *in vivo* application. Another conclusion which can be drawn from these data is that the approach of activity-based protein profiling (ABPP) could be useful for protein target identification purpose.

5.3.2 Biotinylated probe design

In ABPP experiments biotinylated probes (biotin-tagged) are incubated with substances such as avidin, streptavidin or neutravidin beads, followed by the digestion step of enriched proteins with trypsin and LC-MS/MS analysis (Figure 114).[140]

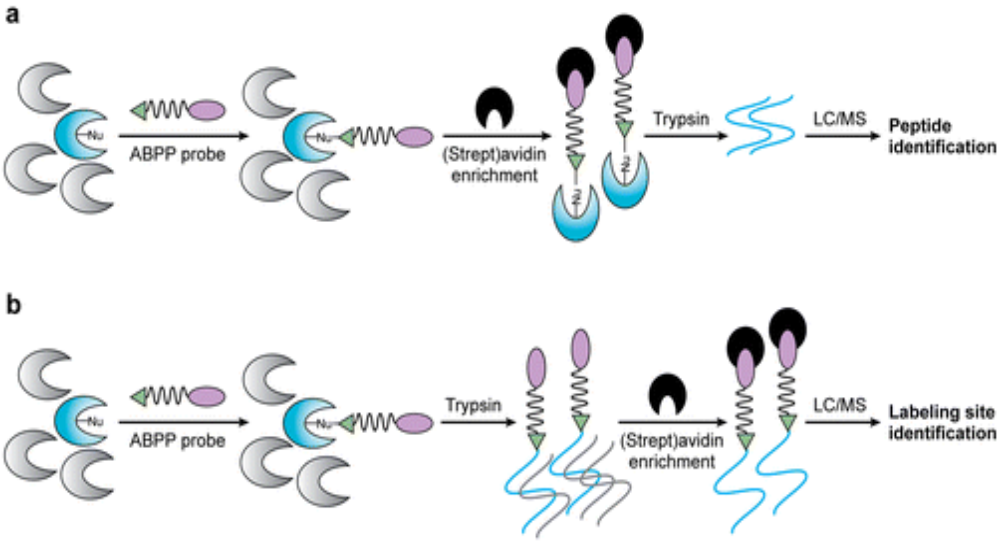
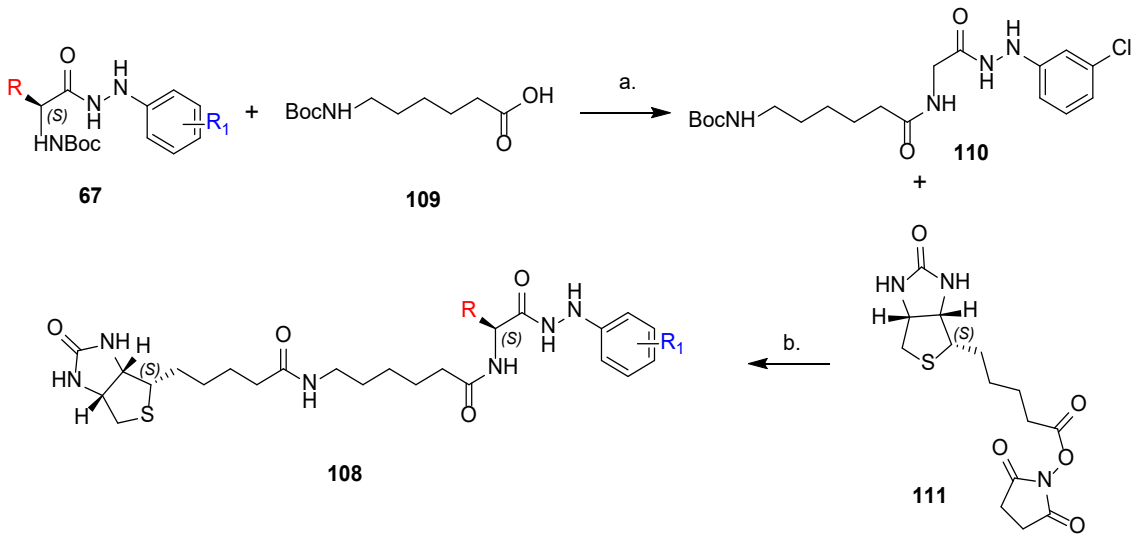


Figure114: Incubation process of biotinylated probes in activity-based protein profiling (ABPP).[140]

5.3.2.1 Synthesis of biotinylated hydrazide probe

Herein, we describe the design and synthesis of a biotinylated affinity probe **108** which is composed of the hydrazide component **67** of interest tethered via a lipophilic linker **109** to a biotin-tag. This could potentially be used for identifying the molecular target of the active compound **67**.

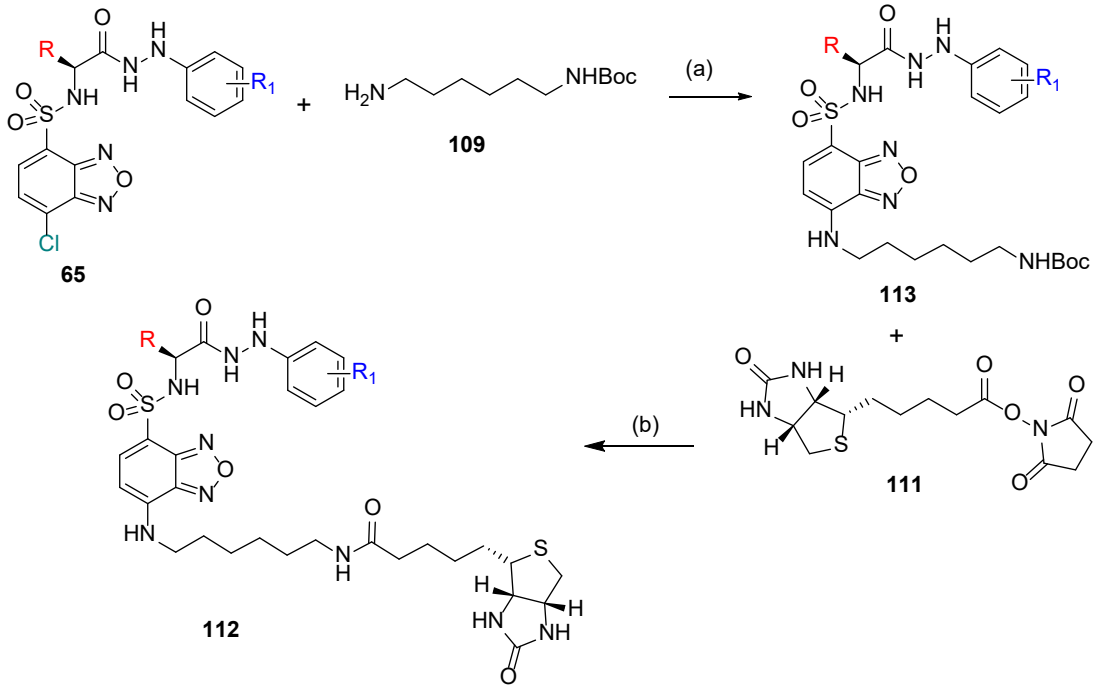
We look at a four-step synthesis to derive the biotinylated hydrazide probe **108** which will be incubated with *Mtb* cells and interact with target proteins in their native environment inside a living cell.[141] This synthesis process will be accomplished via multiple peptide coupling reactions between the lead hydrazide **67** with Boc-6-aminohexanoic acid **109** to give rise to intermediate **110**. Following this is the deprotection of intermediate **110** which will allow reacting with biotin-NHS **111** to deliver the biotinylated hydrazide probe **108** (Scheme 13).



Scheme 13: Reagents and conditions (a.) HBTU, DIPEA, THF, 6-7 h, r.t. (b.i.) 4 M HCl in dioxane, 1 h, r.t.; (b.ii.) **111**, DIPEA, THF, 6-7 h, r.t.

5.3.2.2 Synthesis of biotinylated benzoxa-[2,1,3]-diazole probe

As indicated above, the attachment of biotin to the lead benzo-[2,1,3]-diazole **65** provides a powerful tool in proteomic which is capable of identifying the targets of benzo-[2,1,3]-diazole peptidomimetics **65**. Here, we present a synthetic approach to design the biotinylated probe **112** which is composed of the bioactive benzo-[2,1,3]-diazole **65** of interest tethered via a lipophilic linker **109** to a biotin-tag. This could potentially be used for identifying the molecular target of the active compound **65** (Scheme 14).



Scheme 14: (a.) DIPEA, THF, r.t. (b.i.) 4 M HCl in dioxane, 1 h, r.t; (b.ii.). **111**, DIPEA, THF, r.t.

5.4 *In vitro* cytotoxicity evaluation

As stated already in the first paper which was published by Brown *et al*,[1] the MTT assay was applied within the group to determine the cytotoxicity of a limited examples of the intermediates **67** and the final benzo-[2,1,3]-diazole peptidomimetics **65** against the murine macrophage cell line RAW264.7 (Figure 115).

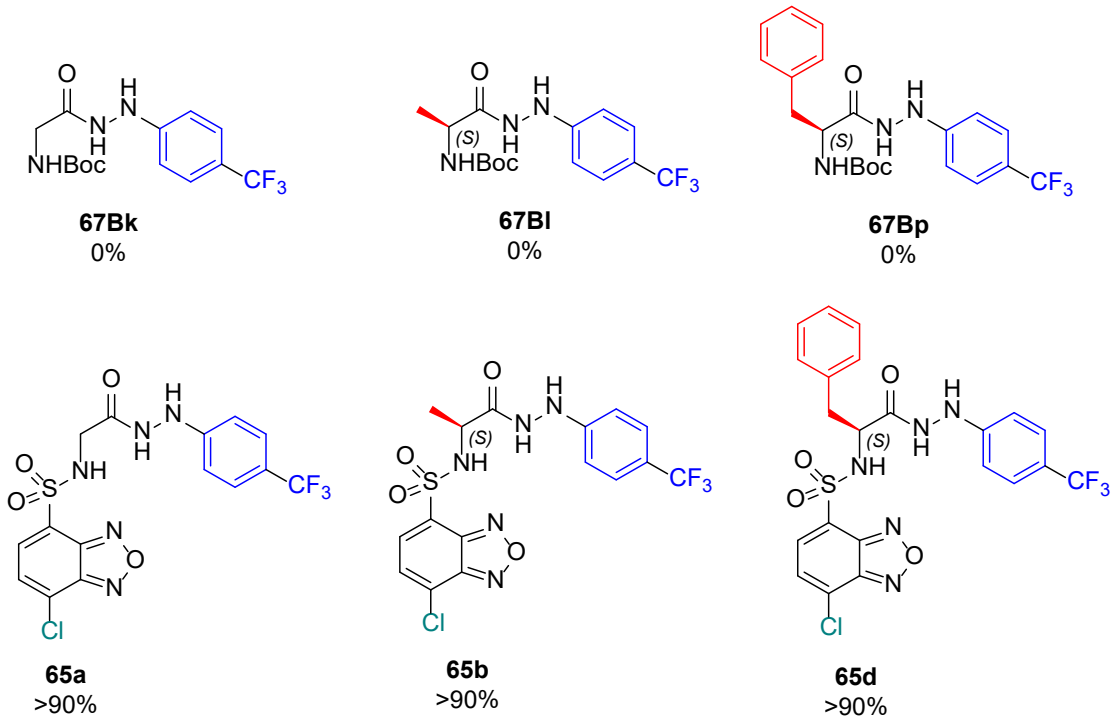


Figure 115: The percentage of cytotoxicity of a limited examples of the intermediates **67** and the final benzo-[2,1,3]-diazole peptidomimetics **65** against the murine macrophage cell line.

In vitro hepatotoxicity evaluation for future work

One of the main issues facing the long-term TB treatments is the rise in incidence of hepatotoxicity as a result of the lack of alternative TB treatments (discussed in section 1.3.3). However, drug-induced hepatotoxicity is difficult to predict and remains a significant cause of drug development failures. Predictive toxicology screening assays for identifying biomarkers and providing mechanistic evaluations enable identification of potential liabilities earlier in the lead optimization stage and can result in recommendations to mitigate these risks. In addition to that, the potential hepatotoxicity is, on the one hand, an important determinant of TB treatment pharmacology, leading to efficacy or failure of drug candidate during drug development. The hepatocytes

have a critical role in xenobiotics metabolism, treatments, which induces a severe hepatotoxicity, are frequently withdrawn from the market. For this reason, one of the future aims is to assess their cellular cytotoxicity against the rat B-13 hepatocyte progenitor cells via MTT assay.[142] As mentioned previously, it would be ideal to develop new antitubercular agents with novel mechanism of action that are effective against both the susceptible and resistant *Mtb* strains and possess less toxicity potential.

6 Experimental section

6.1 General Experimental Information

6.1.1 Analysis

All reactions were carried out under a nitrogen atmosphere in glassware dried in the oven overnight (>75 °C) or infrequently by a heat-gun. All solvents and reagents used as supplied direct from the chemical supplier unless stated otherwise. When utilising mixtures of solvents, the ratios refer to the volumes used. The use of 40-60 petrol refers to the fraction of alkanes boiling between 40 and 60 °C and was redistilled.

Analytical thin layer chromatography (TLC) was completed for all reactions using aluminium plates (silica gel 60Å F_{254}) and visualised by UV radiation at 254 nm, or via staining with ninhydrin. All flash chromatography was executed using 12 g pre-packed silica gel 40-63μ 60Å columns.

All ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded in deuterated solvents $CDCl_3$ or DMSO-d_6 on a Bruker Advance III HD 700 MHz, Jeol Lambda 500 MHz, Jeol ECS-400 MHz or Bruker Advance III 300 MHz NMR spectrometer instruments. Spectra analysis reported as the following; chemical shift δ (ppm) (number of equivalent protons, multiplicity, coupling constant *J* (Hz), assignment). All quoted chemical shift values are in parts per million relative to the standard tetramethylsilane (δH = 0.00 ppm) with all coupling constants stated to the nearest 1 Hz. Analysis and assignment of spectra supported by COSY, HSQC and HMBC experiments. Internal reference used dependent on solvent present using residual protic peaks of $CHCl_3$ to be δH = 7.26 or DMSO as δH = 2.50. Similarly, with ^{13}C NMR the internal reference was taken as the central resonance of $CDCl_3$ with δC = 77.0 ppm or DMSO with a value of δC = 39.5 ppm.

Melting points were measured using Stuart SMP_3 melting point apparatus. Infrared (IR) spectra were recorded on either a Varian Scimitar 800 FT-IR spectrometer or a Perkin Elmer Spectrum Two FT-IR spectrometer. High resolution mass spectra (HRMS) were provided by the Mass Spectrometry Service (University of Durham and University of Teesside). Specific optical

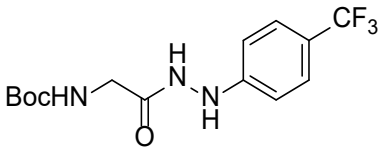
rotation ($[\alpha]_D$) was measured using an Optical Activity PolAAR 2001 automatic polarimeter.

6.1.2 Standard procedures

6.1.2.1 Synthesis of *N*-protected Hydrazides – General Procedure

Under a nitrogen atmosphere *N*-protected amino acid (1 equiv.) dissolved in THF (5 mL) at room temperature and treated with HOBt (2 equiv.) followed by DCC (1.2 equiv.). After stirring for 10 minutes at room temperature addition of *N*-aryl hydrazine (1.2 equiv.) continued stirring for another 2 hours at room temperature. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo* achieving the desired *N*-Boc hydrazides **67**.

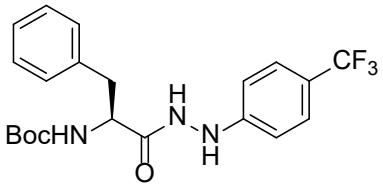
tert-Butyl (2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)ethyl) carbamate 67Bk



Following the general procedure outlined, *N*-Boc-glycine (0.50 g, 2.90 mmol) and 4-trifluorophenylhydrazine hydrochloride (0.61 g, 3.50 mmol) were transformed following flash chromatography (DCM/EtOH/NH_3 200:8:1) into the title compound which was isolated as a white solid (0.45 g, 47%); m.p. 185 – 187 °C; ν_{max} 3370 (NH), 3278 (NH), 3234 (NH), 3108, 3058, 2996, 1649 (C=O), 1615, 1518 (C=O), 1330, 1245, 1156, 1107, 1052, 828, 559 cm^{-1}; δ_H (700 MHz, DMSO-d_6) 9.78 (1H, s, Ar-N*H*NH), 8.32 (1H, s, Ar-NHN*H*), 7.40 (2H, d, *J* 9, Ar-*H*), 7.07 (1H, t, *J* 6, BocN*H*), 6.78 (2H, d, *J* 9, Ar-*H*), 3.60 (2H, d, *J* 6, NHCH_2), 1.37 (9H, s, (CH_3)$_3$COC(O)NH); δ_C (176 MHz, DMSO-d_6) 170.2 (*C*ONH), 156.6 ((CH$_3$)$_3$CO*C*(O)NH), 153.1 (Ar-*C*), 126.7 (Ar-*C*), 126.4 (Ar-*C*), 118.82 (Ar-*C*), 118.63 (Ar-*C*), 112.1 (Ar-*C*), 78.8 (NH*C*H$_2$), 42.7 ((CH$_3$)$_3$*C*O), 28.8 ((*C*H$_3$)$_3$CO);

δ_F (658 MHz, DMSO-d_6) -59.30; *m*/*z* (ES^+) 356 (MNa^+), 689 (2M + Na^+); HRMS (ES^+) Found MH^+, 334.1372 ($C_{14}H_{19}F_3N_3O_3$ requires 334.1373).

tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl) propan-2-yl)carbamate 67Bp

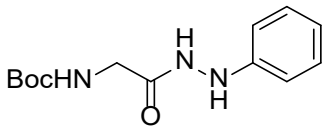


Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.25 g, 0.94 mmol) and 4-trifluorophenylhydrazine hydrochloride (0.30 g, 1.75 mmol) were transformed following flash chromatography into the title compound as a golden-brown solid (0.330 g, 83%); R_f 0.39 (DCM/EtOH/NH_3 [200:8:1]); m.p. 157 – 159 °C; ν_{max} 3324 (N-H), 3274 (N-H), 2931, 2851, 1686 (C=O), 1660 (C=O), 1520, 1328, 1156, 1103, 1067, 835, 716, 515 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.93 (1H, bs, N*H*), 7.39 (2H, d, *J* 8, Ar-*H*), 7.36 – 7.32 (3H, m, Ar-*H*), 7.26 – 7.22 (2H, m, Ar-*H*), 6.61 (2H, d, *J* 8, Ar-*H*), 6.18 (1H, bs, N*H*), 5.09 (1H, bd, *J* 7, N*H*), 4.46 (1H, q, *J* 8, CH_2CHCO), 3.12 (2H, d, *J* 8, CH_2CHCO), 1.47 (9H, s, $C(CH_3)_3$); δ_C (176 MHz, $CDCl_3$) 171.6 ($NHCHCO$), 150.3 ($NHCOO$), 135.96 (Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.2 (Ar-*C*), 126.4 (Ar-*C*), 126.4 (Ar-*C*), 125.2 (Ar-*C*), 123.6 (CF_3), 112.7 (Ar-*C*), 80.9 ($C(CH_3)_3$), 54.5 ($NHCHCO$), 33.5 (CH_2CHCO), 28.3 ($C(CH_3)_3$); δ_F (376 MHz, $CDCl_3$) -61.56 (3F, s, CF_3); *m*/*z* (ES^+) 424 (MH^+), 446 (MNa^+), 869 (2M + Na^+); HRMS (ES^+) Found MH^+, 424.1847 ($C_{21}H_{25}F_3N_3O_3$ requires 424.1843).

6.1.2.2 Synthesis of *N*-protected Hydrazides – General Procedure

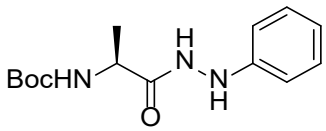
Under a nitrogen atmosphere *N*-protected amino acid (1 equiv.) dissolved in THF (5 mL) at room temperature and treated with DIPEA (1.5 equiv.) followed by DMTMM (1.2 equiv.). After stirring for 10 minutes at room temperature addition of N-Aryl / alkyl or benzyl hydrazine (1.1 equiv.) continued stirring for another 5 hours at room temperature. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo* achieving the desired *N*-Boc hydrazides **67**, **89** or **91**.

tert-Butyl (2-oxo-2-(2-phenylhydrazineyl)ethyl)carbamate 67a



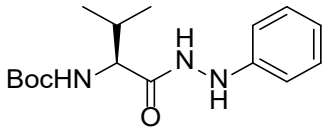
Following the general procedure outlined, *N*-Boc-glycine (0.20 g, 1.14 mmol) and phenylhydrazine (0.14 mL, 1.37 mmol) were transformed following trituration with $CHCl_3$ into the title compound which was isolated as a dark brown gummy solid (0.17 g, 55%) as a mixture of rotamers [4:1]; R_f 0.44 (DCM/EtOH/NH_3 200:8:1); m.p. 86 - 88 °C; υ_{max} 3281 (N-H), 2977, 2929 (C-H), 1678 (C=O), 1602 (C=O), 1495, 1366, 1248, 1160, 750, 692 cm^{-1}; all data provided for the major rotamer; δ_H (700 MHz, $CDCl_3$) 8.73 (1H, bs, CON*H*NH), 7.18 – 7.14 (2H, m, Ar-*H*), 6.85 (1H, m , Ar-*H*), 6.75 – 6.74 (2H, d, *J* 8, Ar-*H*), 5.64 (1H, bs , BocN*H*CH_2), 3.80 (2H, d, *J* 6, BocNHC*H*$_2$), 1.43 (9H, s, (C*H*$_3$)$_3$CO); δ_C (700 MHz, $CDCl_3$) 170.3 (*C*=ONHNH), 156.4 *(C*=ONH), 147.7 (*ipso*-Ar-*C)*, 129.15 (Ar-*C)*, 129.12 (Ar-*C)*, 120.9 (Ar-*C)*, 113.45 (Ar-*C)*, 113.29 (Ar-*C),* 80.4 ((CH$_3$)$_3$*C*O), 43.1 (BocNH*C*H$_2$), 28.1 (*C*H$_3$)$_3$CO); ***m/z*** (ES$^+$) 266 (MH$^+$); HRMS (ES$^+$) Found MH$^+$, 266.1515 ($C_{13}H_{20}N_3O_3$ requires 266.1505).

tert-Butyl (*S*)-(1-oxo-1-(2-phenylhydrazineyl)propan-2-yl)carbamate 67b



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and phenylhydrazine (0.13 mL, 1.27 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a light orange solid (0.09 g, 33%); R_f 0.26 (DCM/EtOH/NH_3 400:8:1); m.p. 129 - 132 °C; υ_{max} 3432, 3288, 3026 (N-H), 2982(C-H), 1688 (C=O), 1601 (C=O), 1492, 1454, 1369, 1243, 1155, 755, 701 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.78 (1H, bs, CON*H*NH), 7.20 – 7.14 (2H, m, Ar-*H*), 6.85 (1H, t, *J* 7, Ar-*H*), 6.81-6.71 (2H, m, Ar-*H*), 5.40 (1H, bs, BocN*H*CH), 4.31 (1H, m, BocNHC*H*), 1.43 (9H, s, (CH_3)$_3$CO), 1.33 (3H, d, *J* 6, NHCHCH_3); δ_C (500 MHz, $CDCl_3$) 173.2 (*C*=ONHNH), 155.8 *(C*=ONH), 147.8 (*ipso*-Ar-*C)*, 129.3 (Ar-*C)*, 129.1 (Ar-*C)*, 120.9 (Ar-*C)*, 115.5 (Ar-*C)*, 113.4 (Ar-*C)*, 80.4 ((CH$_3$)$_3$*C*O), 48.6 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 18.1 (NHCH*C*H$_3$); *m/z* (ES^+) 280 (MH^+); HRMS (ES^+) Found MH^+, 280.1674 ($C_{14}H_{22}N_3O_3$ requires 280.1661).

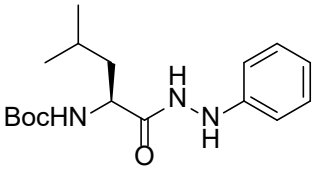
tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl) carbamate 67c



Following the general procedure outlined, *N*-Boc-*L*-valine (0.20 g, 0.92 mmol) and phenylhydrazine (0.10 mL, 1.01 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:8:1) into the title compound which was isolated as a reddish brown oil (0.07 g, 24%) as a mixture of rotamers [4:1]; R_f 0.44 (DCM/EtOH/NH_3 200:8:1); υ_{max} 3341, 3259 (N-H), 2960, 2925 (C-H), 1660 (C=O), 1521, 1307, 1250, 1168, 689 cm^{-1}; all data provided for the major rotamer; δ_H (700 MHz, $CDCl_3$) 8.00 (1H, bs, CON*H*NH), 7.23 – 7.20 (2H, t, *J* 7, Ar-*H*), 6.90 (1H, t, *J* 7, Ar-*H*), 6.85 – 6.83 (2H, d, *J* 7, Ar-*H*), 5.04 (1H, bd, *J* 9, BocN*H*), 3.96 (1H, m, BocNHC*H*), 2.18 (1H, m, CHC*H*(CH$_3$)$_2$), 1.47 (9H, s, (CH_3)$_3$CO), 1.00 (3H, d, *J* 6, CHCH(CH_3)$_2$), 0.98 (3H, d, *J* 6, CHCH(CH_3)$_2$); δ_C (700 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.9 (*C*=ONH), 147.7 (*ipso*-Ar-*C*), 129.0 (Ar-*C*), 128.7 (Ar-*C*),

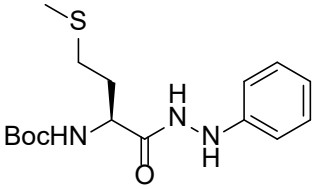
121.9 (Ar-*C*), 113.6 (Ar-*C*), 113.0 (Ar-*C*), 80.4 ($(CH_3)_3CO$), 58.9 (BocNH*C*H), 31.3 (CH*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 18.9 (CHCH($CH_3)_2$), 18.1 (CHCH($CH_3)_2$); ***m/z*** (ES^+) 308 (MH^+); HRMS (ES^+) Found MH^+, 308.1965 ($C_{16}H_{26}N_3O_3$ requires 308.1974).

tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-phenylhydrazinyl)pentan-2-yl)carbamate 67d



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.20 g, 0.87 mmol) and phenylhydrazine (0.09 mL, 0.95 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light orange solid (0.05 g, 19%) R_f 0.32 (n-hex/EtOAc 3:1); m.p. 110 - 114 °C; υ_{max} 3338, 3275 (N-H), 2963 (C-H), 1672 (C=O), 1520, 1367, 1257, 1167, 851, 694 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.20 (1H, bs, CON*H*NH), 7.23 – 7.19 (2H, t, *J* 7, Ar-*H*), 6.90 (1H, t, *J* 7, Ar-*H*), 6.84 (2H, d, *J* 7, Ar-*H*), 4.91 (1H, bd, *J* 8, BocN*H*), 4.20 (1H, m, BocNHC*H*), 1.77 – 1.67 (2H, m, CH*H*CH$(CH_3)_2$, CHHC*H*$(CH_3)_2$), 1.55 (1H, m, C*H*HCH$(CH_3)_2$), 1.47 (9H, s, (C*H*$_3)_3$CO), 0.97 (3H, d, *J* 6, CH_2CH(C*H*$_3)_2$), 0.95 (3H, d, *J* 6, CH_2CH(C*H*$_3)_2$); δ_C (400 MHz, $CDCl_3$) 129.2 (Ar-*C*), 121.5 (Ar-*C*), 113.8 (Ar-*C*), 51.2 (BocNH*C*H), 40.2 (*C*H_2CH$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 24.7 (CH_2*C*H$(CH_3)_2$), 22.8 (CH_2CH(*C*$H_3)_2$), 22.1 (CH_2CH(*C*$H_3)_2$); ***m/z*** (ES^+) 322 (MH^+); HRMS (ES^+) Found MH^+, 322.2122 ($C_{17}H_{28}N_3O_3$ requires 322.2131).

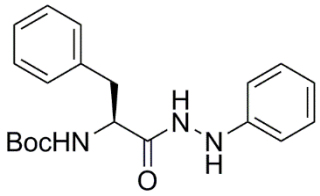
tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-phenylhydrazinyl)butan-2-yl) carbamate 67g



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and phenylhydrazine (0.09 mL, 0.88 mmol) were transformed following

trituration with Et_2O into the title compound which was isolated as a white solid (0.06 g, 23%); R_f 0.38 (DCM/EtOH/NH_3 200:8:1); m.p. 106 - 109 °C; υ_{max} 3316, 3258 (N-H), 2972 (C-H), 1685, 1656 (C=O), 1522, 1495, 1308, 1249, 1166, 1055, 764, 689 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.67 (1H, bs, CON*H*NH), 7.20 – 7.16 (2H, t, *J* 7, Ar-*H*), 6.88 (1H, t, *J* 7, Ar-*H*), 6.81 – 6.79 (2H, d, *J* 8, Ar-*H*), 5.39 (1H, bd, *J* 8, BocN*H*CH), 4.41 (1H, m, BocNHC*H*), 2.52 (2H, t, *J* 7, $CHCH_2C\mathit{H}_2S$), 2.16 – 2.04 (4H, m, $CH_2CH_2SC\mathit{H}_3$, $CHC\mathit{H}HCH_2S$), 1.93 (1H, m, $CHCH\mathit{H}CH_2S$),, 1.45 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (400 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.9 (*C*=ONH), 147.5 (*ipso*-Ar-*C*), 129.5 (Ar-*C*), 129.2 (Ar-*C*), 121.4 (Ar-*C*), 113.7 (Ar-*C*), 112.9 (Ar-*C*), 80.6 ($(CH_3)_3\mathit{C}O$), 52.0 (BocNH*C*H), 31.2 ($\mathit{C}H_2CH_2S\mathit{C}H_3$), 30.2 ($CH_2\mathit{C}H_2SCH_3$), 28.4 ($(\mathit{C}H_3)_3CO$), 15.4 ($CH_2CH_2S\mathit{C}H_3$); ***m/z*** ($ES^+$) 340 ($MH^+$); HRMS ($ES^+$) Found MH^+, 340.1690 ($C_{16}H_{25}N_3O_3S$ requires 340.1695).

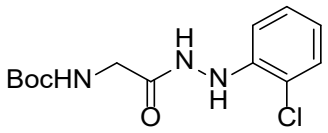
tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-phenylhydrazinyl)propan-2-yl) carbamate 67h



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.20 g, 0.75 mmol) and phenylhydrazine (0.09 mL, 0.91 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 400:8:1) into the title compound which was isolated as a brown gummy solid (0.11 g, 50%) as a mixture of rotamers [5:1]; R_f 0.31 (DCM/EtOH/NH_3 400:8:1); υ_{max} 3262 (N-H), 2978, 1669 (C=O), 1602 (C=O), 1494, 1455, 1366, 1249, 1162, 749, 692 cm^{-1}; all data provided for the major rotamer δ_H (500 MHz, $CDCl_3$) 8.45 (1H, bs, CON*H*NH), 7.28 – 7.24 (3H, m, Ar-*H*), 7.18-7.15 (3H, m, Ar-*H*), 7.12-7.09 (2H, t, *J* 7, Ar-*H*), 6.85-6.82 (1H, m, Ar-*H*), 6.53-6.51 (1H, d, *J* 7, Ar-*H*), 5.37 (1H, bs, BocN*H*CH), 4.54 (1H, m, BocNHC*H*), 3.03 (2H, m, Ar-CH_2), 1.41 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (500 MHz, $CDCl_3$) 171.8 (*C*=ONHNH), 155.7 *(C*=ONH), 147.5 (*ipso*-Ar-*C)*, 136.3 (*ipso*-Ar-*C)*, 129.5 (Ar-*C)*, 129.4 (Ar-*C)*, 129.1 (Ar-*C)*, 128.7 (Ar-*C)*, 126.9 (Ar-*C)*, 120.9 (Ar-*C)*, 115.4 (Ar-*C)*, 113.5 (Ar-*C)*, 112.9 (Ar-*C)*, 80.5 ($(CH_3)_3\mathit{C}O$), 54.4

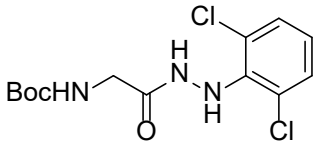
(BocNHCH), 38.4 (Ar-CH_2),28.3 ((CH_3)$_3$CO); *m/z* (ES^+) 356 (MH^+); HRMS (ES^+) Found MH^+, 356.1977 ($C_{20}H_{26}N_3O_3$ requires 356.1974).

tert-Butyl (2-(2-(2-chlorophenyl)hydrazineyl)-2-oxoethyl)carbamate 67Ag



Following the general procedure outlined, *N*-Boc-glycine (0.25 g, 1.43 mmol) and 2-chlorophenylhydrazine hydrochloride (0.31 g, 1.71 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a brown gum (0.24 g, 56%) as a [4:1] rotameric mixture; Rf: 0.21 (DCM/EtOH/NH_3 200:8:1). m.p 113 - 119°C. υ_{max} 3367 (NH) 3271 (NH), 1677 (C=O), 1586, 1494, 1253, 1366, 1248, 1155, 1042cm^{-1}; NMR data given for the major rotamer δ_H (700 MHz, DMSO-d_6) 1.40 (9H, s, C(C*H*$_3$)$_3$), 3.64 (2H, d, *J* 7, NHC*H*$_2$), 5.30 (1H, bs, N*H*), 6.75 (1H, t, Ar-*H*), 6.84 (1H, d, J 14 Ar-*H*), 7.12 (1H, dd, J 7, J 21, Ar-*H*), 7.28 (1H, d, J 7); δ_C (700 MHz, DMSO-d_6) 169.9 (*C*=O), 156.4 (*C*=O), 145.1 (*ipso*-Ar-*C*), 129.9 (Ar-*C*), 128.1, (Ar-*C*), 120.3 (Ar-*C*), 117.5 (Ar-*C*-Cl), 113.6 (Ar-*C*), 78.6 (O-*C*(CH$_3$)$_3$), 42.5 (NH*C*H$_2$), 28.7 (C(*C*H$_3$)$_3$); *m/z* (ES^+) 300 ([^{35}Cl] MH^+), 302 ([^{37}Cl] MH^+), 322 ([^{35}Cl] MNa^+), 324 ([^{37}Cl] MNa^+); HRMS (ES^+) Found MH^+, 300.1115 ($C_{13}H_{19}N_3O_3{}^{35}Cl$ requires 300.1115).

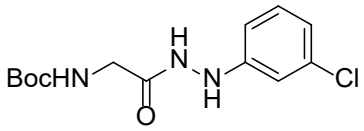
tert-Butyl (2-(2-(2,6-dichlorophenyl)hydrazinyl)-2-oxoethyl)carbamate 67As



Following the general procedure outlined, *N*-Boc-glycine (0.20 g, 1.14 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (0.29 g, 1.37 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.19 g, 52%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); m.p. 115 - 118 °C; υ_{max} 3468, 3309, 3209 (N-H), 1721, 1673 (C=O), 1505, 1448, 1361, 1266, 1169, 1054, 868, 765 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.43 (1H, bs, CON*H*NH), 7.25 - 7.23 (2H, d, *J* 7, Ar-*H*), 6.93 (1H, t, *J* 7, Ar-*H*), 6.76 (1H, bd, *J* 4, Ar-N*H*), 5.13 (1H, bs,

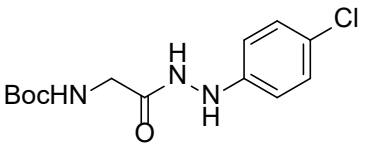
BocN*H*CH_2), 3.84 (2H, d, *J* 6, BocNHC*H*$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 168.7 (*C*=ONHNH), 140.8 (*ipso*-Ar-*C)*, 128.9 (Ar-*C*), 128.8 (Ar-*C*), 126.1 (*ipso*-Ar-*C*), 125.1 (*ipso*-Ar-*C*), 124.2 (Ar-*C*), 80.7 ((CH_3)$_3$*C*O), 43.1 (BocNH*C*H_2), 28.3 ((*C*H_3)$_3$CO); *m/z* (ES$^+$) 356 ([^{35,35}Cl]MNa$^+$), 358 ([^{35,37}Cl]MNa$^+$), 360 ([^{37,37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MNa$^+$, 356.0540 ($C_{13}H_{17}{}^{35,35}ClN_3O_3Na$ requires 356.0545).

tert-Butyl (2-(2-(3-chlorophenyl)hydrazinyl)-2-oxoethyl)carbamate 67q



Following the general procedure outlined, *N*-Boc-glycine (0.23 g, 1.32 mmol) and 3-chlorophenylhydrazine hydrochloride (0.28 g, 1.58 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a pale yellow solid (0.39 g, 99%); as a [3:1] rotameric mixture; R_f 0.39 (DCM/EtOH/NH_3 200:8:1); mp 96 – 104 °C; ν_{max} 3344, 3269 (NH), 3078 (Ar-CH), 2980, 2933 (CH), 1712, 1659 (C=O), 1513 (C=C), 1367 (C-N), 1154 (C-O) cm^{-1}; NMR data given for major rotamer δ_H (700 MHz, $CDCl_3$) 8.43 (1H, s, ArN*H*), 7.11 (1H, t, *J* 7, Ar-C*H*), 6.84 (1H, dd, *J* 7, 1, Ar-C*H*), 6.79 (1H, t, *J* 1, Ar-C*H*), 6.67 (1H, dd, *J* 7, 2, Ar-C*H*), 6.22 (1H, s, CON*H*NH) 5.38 (1H, s, N*H*CH_2), 3.88 (1H, d, *J* 5, NHC*H*$_2$), 1.47 (9H, s, C(C*H*$_3$)$_3$); δ_C (700 MHz, $CDCl_3$) 169.9 (*C*OO), 156.7 (CH*C*=O) 148.9 (1-Ar-*C*NH), 135.1 (3-Ar-*C*Cl), 130.2 (5-Ar-*C*H), 121.3 (6-Ar-*C*H), 113.4 (2-Ar-*C*H), 111.8 (4-Ar-*C*H*)*, 55.9 (*C*(CH_3)$_3$), 43.6 (NH*C*H_2), 28.3 (C(*C*H_3)$_3$); *m/z* (ES$^+$) 322 (MNa$^+$), 300 (MH$^+$), 200 (M-Boc$^+$); HRMS (ES$^+$) Found MH$^+$, 300.1115 ($C_{13}H_{19}{}^{35}ClN_3O_3$ requires 300.1120).

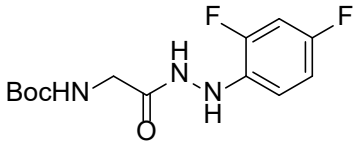
tert-Butyl-(2-(2-(4-chlorophenyl)hydrazinyl)-2-oxoethyl)carbamate 67Am



Following the general procedure outlined, *N*-Boc-glycine (0.20 g, 1.14 mmol) and 4-chlorophenylhydrazine hydrochloride (0.25 g, 1.37 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a

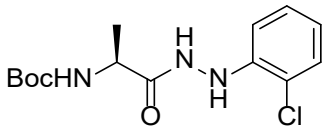
white solid (0.08 g, 24%) as a mixture of rotamers [4:1]; R_f 0.30 (DCM/EtOH/NH_3 200:8:1); m.p. 97 - 99 °C; ν_{max} 3273, 3119 (N-H), 2979 (C-H), 1656 (C=O), 1527, 1489, 1363, 1241, 1159, 815 cm^{-1}; NMR data given for major rotamer δ_H (400 MHz, $CDCl_3$) 8.45 (1H, bs, CON*H*NH), 7.15 – 7.13 (2H, d, *J* 8, Ar-*H*), 6.73 – 6.71 (2H, d, *J* 8, Ar-*H*), 6.25 (1H, bs, Ar-N*H*), 5.41 (1H, bs, BocN*H*CH_2), 3.86 (2H, d, *J* 5, BocNHC*H*$_2$), 1.46 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*=ONHNH), 156.5 *(C*=ONH), 146.3 (*ipso*-Ar-*C)*, 129.4 (Ar-*C*), 129.1 (Ar-*C*), 125.9 (*ipso*-Ar-*C*), 114.7 (Ar-*C*), 113.8 (Ar-*C*), 80.9 ((CH$_3$)$_3$*C*O), 43.3 (BocNH*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES^+) 322 ([^{35}Cl]MNa^+), 324 ([^{37}Cl]MNa^+); HRMS (ES^+) Found [^{35}Cl]MNa^+, 322.0932 ($C_{13}H_{18}N_3O_3{}^{35}ClNa$ requires 322.0934).

tert-Butyl-(2-(2-(2,4-difluorophenyl)hydrazinyl)-2-oxoethyl)carbamate 67By



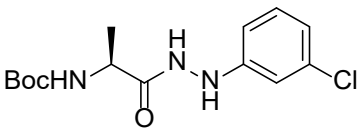
Following the general procedure outlined, *N*-Boc-glycine (0.20 g, 1.14 mmol) and 2,4-difluorophenylhydrazine hydrochloride (0.25 g, 1.37 mmol) were transformed following flash column chromatography (DCM/MeOH 9:1) into the title compound which was isolated as a brown oil (0.04 g, 12%) as a mixture of rotamers [4:1]; R_f 0.38 (DCM/EtOH/NH_3 200:8:1); ν_{max} 3281 (N-H), 2980 (C-H), 1855, 1679, 1611 (C=O), 1504, 1368, 1256, 1162, 1143, 960, 848 cm^{-1}; all data provided for the major rotamer; δ_H (400 MHz, $CDCl_3$) 8.50 (1H, bs, N*H*), 6.86 – 6.71 (3H, m, Ar-*H*), 5.39 (1H, bs, BocN*H*CH_2), 3.86 (2H, d, *J* 5, BocNHC*H*$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*=ONHNH), 155.8 *(C*=ONH), 132.2 (*ipso*-Ar-*C)*, 132.1 (*ipso*-Ar-*C)*, 114.3 (Ar-*C*), 110.9 (Ar-*C*), 104.2 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 43.2 (BocNH*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); δ_F (282 MHz, $CDCl_3$) -120.4 (Ar-*F*), -128.9 (Ar-*F*); *m/z* (ES^+) 324 (MNa^+); HRMS (ES^+) Found MNa^+, 324.1165 ($C_{13}H_{17}N_3O_3F_2Na$ requires 324.1136).

tert-Butyl (*S*)-(1-(2-(2-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl) carbamate 67Ai



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 2-chlorophenylhydrazine hydrochloride (0.23 g, 1.27 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a yellow solid (0.30 g, 91%); R_f 0.56 (DCM/EtOH/NH_3 100:8:1); m.p. 146 - 149 °C; υ_{max} 3453, 3284 (N-H), 2976 (C-H), 1693 (C=O), 1675 (C=O), 1492, 1455, 1371, 1247, 1160, 753, 658 cm^1; δ_H (700 MHz, $CDCl_3$) 8.36 (1H, bs, CON*H*NH), 7.26 (1H, dd, *J* 8, 2, Ar-*H*), 7.12 (1H, dt, *J* 7, 2, Ar-*H*), 6.85 (1H, dd, *J* 8, 2, Ar-*H*), 6.81 (1H, dt, *J* 7, 2, Ar-*H*), 6.42 (1H, bs, Ar-N*H*), 5.05 (1H, bs, BocN*H*CH), 4.29 (1H, m, BocNHC*H*), 1.46 (9H, s, (C*H*$_3$)$_3$CO) 1.41 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (700 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 155.9 *(C*=ONH), 143.7 (*ipso*-Ar-*C)*, 129.5 (Ar-*C)*, 127.6 (Ar-*C)*, 121.4 (Ar-*C)*, 119.6 (Ar-*C)*, 113.5 (Ar-*C)*, 80.7 ((CH$_3$)$_3$*C*O), 48.6 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 17.5 (NHCH*C*H$_3$); ***m/z*** (ES^+) 314 ([^{35}Cl]MH^+), 316 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 314.1285 ($C_{14}H_{21}{}^{35}ClN_3O_3$ requires 314.1271).

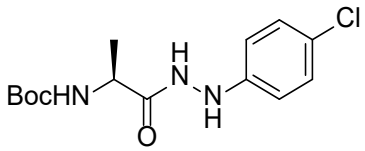
tert-Butyl (*S*)-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl) carbamate 67r



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.25 g, 1.32 mmol) and 3-chlorophenylhydrazine hydrochloride (0.28 g, 1.56 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a pale yellow solid (0.39 g, 94%)**;** R_f 0.19 (DCM:EtOH:NH_3 200:8:1)**;** mp 125 – 127 °C; ν_{max} 3428, 3278 (NH), 3078 (Ar-CH), 2979, 2933 (CH), 1678 (C=O), 1504, 1367, 1158 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.52 (1H, bs, CON*H*NH),

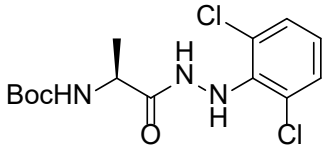
7.12 (1H, t, *J* 7, Ar-*H*), 6.85 (1H, d, *J* 7, Ar-*H*), 6.80 (1H, s, Ar-*H*), 6.70 (1H, d, *J* 7, Ar-*H*), 5.15 (1H, s, N*H*CH(CH_3)), 4.30 (1H, m, NHC*H*CH_3), 1.48 (9H, s, C(C*H*$_3$)$_3$), 1.40 (3H, d, *J* 7, NHCH(C*H*$_3$)); δ_C (700 MHz, $CDCl_3$) 172.9 (*C*=O), 155.9 (CH*C*=O) 149.1 (Ar-*C*), 135.1 (Ar-*C*), 130.2 (Ar-*C*), 121.1 (Ar-*C*Cl), 113.4 (Ar-*C*), 111.7 (Ar-*C*), 80.8 (*C*(CH_3)$_3$), 48.6 (NH*C*H(CH_3)), 28.3 (C(*C*H_3)$_3$), 17.5 (NHCHC*H*$_3$); *m/z* (ES^+) 314 (MH^+), 258 (M-tert-butyl$^+$); HRMS (ES^+) Found MH^+, 314.1259 ($C_{14}H_{21}{}^{35}ClN_3O_3$ requires 314.1271).

tert-butyl (*S*)-(1-(2-(4-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl) carbamate 67An



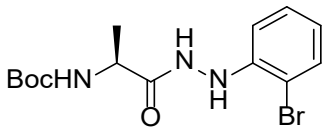
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.10 g, 0.53 mmol) and 4-chlorophenylhydrazine hydrochloride (0.10 g, 0.53 mmol) were transformed following work up with EtOAc into the title compound which was isolated as an orange solid (0.08 g, 46%)**;** R_f 0.23 (DCM:EtOH:NH_3 200:8:1)**;** mp 122 – 124 °C; ν_{max} 3428, 3278 (NH), 3078 (Ar-CH), 2979, 2933 (CH), 1678 (C=O), 1504, 1367, 1158 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.68 (1H, bs, CON*H*NH), 7.11 – 7.09 (2H, m, Ar-*H*), 6.69 – 6.67 (2H, m, Ar-*H*), 6.80 (1H, s, Ar-*H*), 6.70 (1H, d, *J* 7, Ar-*H*), 5.27 (1H, bs, N*H*CHCH_3), 4.28 (1H, m, NHC*H*CH_3), 1.44 (9H, s, C(C*H*$_3$)$_3$), 1.35 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (400 MHz, $CDCl_3$) 173.3 (*C*=O), 155.9 (CH*C*=O) 146.6 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 125.8 (Ar-*C*Cl), 114.7 (Ar-*C*), 80.8 (*C*(CH_3)$_3$), 48.7 (NH*C*H(CH_3)), 28.4 (C(*C*H_3)$_3$), 17.9 (NHCHC*H*$_3$); *m/z* (ES^+) 314 ([^{35}Cl]MH^+), 316 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 314.1193 ($C_{14}H_{21}{}^{35}ClN_3O_3$ requires 314.1181).

tert-Butyl (*S*)-(1-(2-(2,6-dichlorophenyl)hydrazinyl)-1-oxopropan-2-yl) carbamate 67At



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (0.27 g, 1.27 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a golden brown solid (0.35 g, 95%); R_f 0.48 (DCM/EtOH/NH_3 200:8:1); m.p. 98 - 101 °C; υ_{max} 3335, 3304 (N-H), 2983 (C-H), 16786, 1674 (C=O), 1521, 1449, 1317, 1248, 1164, 1162, 766, 627 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.68 (1H, bs, CON*H*NH), 7.26 - 7.24 (2H, d, *J* 8, Ar-*H*), 6.92 (1H, t, *J* 8, Ar-*H*), 6.74 (1H, bs, Ar-N*H*), 5.05 (1H, d, *J* 7, BocN*H*CH), 4.29 (1H, m, BocNHC*H*), 1.46 (9H, s, (C*H*$_3$)$_3$CO), 1.37 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (500 MHz, $CDCl_3$) 171.8 (*C*=ONHNH), 155.6 (*C*=ONH), 140.9 (*ipso*-Ar-*C)*, 128.9 (Ar-*C*), 125.9 (*ipso*-Ar-*C*), 124.0 (Ar-*C*), 80.5 ((CH$_3$)$_3$*C*O), 48.4 (BocNH*C*H), 28.4 ((*C*H$_3$)$_3$CO), 17.7 (NHCH*C*H$_3$); *m/z* (ES^+) 348 ([^{35,35}Cl]MH^+), 350 ([^{35,37}Cl]MH^+), 352 ([^{37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 348.0865 ($C_{14}H_{20}{}^{35,35}ClN_3O_3$ requires 348.0882).

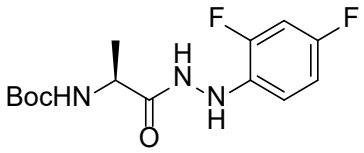
tert-Butyl (*S*)-(1-(2-(2-bromophenyl)hydrazineyl)-1-oxopropan-2-yl) carbamate 67Cf



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 2-bromophenylhydrazine hydrochloride (0.28 g, 1.27 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a light brown solid (0.32 g, 84%); R_f 0.5 (DCM/EtOH/NH_3 200:8:1); m.p. 112 - 114 °C; υ_{max} 3286 (N-H), 2983 (C-H), 1672 (C=O), 1491, 1453, 1366, 1247, 1160, 749, 647 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.66 (1H, bs, CON*H*NH), 7.41 (1H, dd, *J* 8, 1, Ar-*H*), 7.14 (1H, m, Ar-*H*), 6.83 (1H, dd, *J* 8, 1, Ar-*H*), 6.73 (1H,

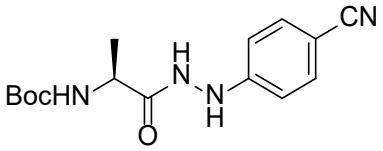
m, Ar-*H*), 6.39 (1H, d, *J* 3 Ar-N*H*), 5.24 (1H, d, *J* 6, BocN*H*CH), 4.33 (1H, m, BocNHC*H*), 1.44 (9H, s, (CH_3)$_3$CO) 1.39 (3H, d, *J* 7, NHCHCH_3); δ_C (700 MHz, $CDCl_3$) 172.8 (*C*=ONHNH), 155.9 *(C*=ONH), 144.6 (*ipso*-Ar-*C)*, 132.6 (Ar-*C)*, 128.3 (Ar-*C)*, 121.8 (Ar-*C)*, 113.7 (Ar-*C)*, 109.1 (Ar-*C)*, 80.6 ((CH$_3$)$_3$*C*O), 48.6 (BocNH*C*H), 28.4 ((*C*H$_3$)$_3$CO), 17.8 (NHCH*C*H$_3$); *m/z* (ES$^+$) 358 ([^{79}Br] MH$^+$), 360 ([^{81}Br] MH$^+$); HRMS (ES$^+$) Found [^{79}Br]MH$^+$, 358.0769 ($C_{14}H_{21}{}^{79}BrN_3O_3$ requires 358.0766).

tert-Butyl (*S*)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-1-oxopropan-2-yl) carbamate 67Bz



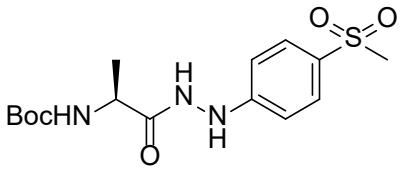
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 2,4-difluorophenylhydrazine hydrochloride (0.22 g, 1.27 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange gummy solid (0.06 g, 17%); R_f 0.29 (DCM/EtOH/NH_3 400:8:1); m.p. 91 - 94 °C; υ_{max} 3215 (N-H), 2989 (C-H), 1686, 1668 (C=O), 1508, 1319, 1252, 1161, 1135, 963, 853, 669 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.24 (1H, bs, CON*H*NH), 6.84 (1H, m, Ar-*H*), 6.80 (1H, m, Ar-*H*), 6.73 (1H, t, *J* 7, Ar-*H*), 6.12 (1H, bs, Ar-N*H*), 4.97 (1H, bd, *J* 7, BocN*H*CH), 4.23 (1H, m, BocNHC*H*), 1.47 (9H, s, (CH_3)$_3$CO), 1.40 (3H, d, *J* 7, NHCHCH_3); δ_C (700 MHz, $CDCl_3$) 171.2 (*C*=ONHNH), 155.9 (*C*=ONH), 132.3 (Ar-*C*), 115.4 (Ar-*C*), 110.8 (Ar-*C*), 103.9 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 21.1 (NHCH*C*H$_3$); δ_F (700 MHz, $CDCl_3$) -120.7 (Ar-*F*), -129.1 (Ar-*F*); *m/z* (ES$^+$) 338 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 338.1300 ($C_{14}H_{19}F_2N_3O_3Na$ requires 338.1292).

tert-Butyl (*S*)-(1-(2-(4-cyanophenyl)hydrazinyl)-1-oxopropan-2-yl) carbamate 67Cx



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 4-cyanophenylhydrazine hydrochloride (0.22 g, 1.27 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.25 g, 78%); R_f 0.21 (DCM/EtOH/NH_3 200:8:1); m.p. 115 - 121 °C; υ_{max} 3282 (N-H), 2220 (C≡N), 16780, 1603 (C=O), 1508, 1318, 1250, 1164, 1166, 830, 743, 537 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.40 (1H, bs, CON*H*NH), 7.48 - 7.46 (2H, d, *J* 9, Ar-*H*), 6.82 - 6.80 (2H, d, , *J* 9, Ar-*H*), 6.37 (1H, bd, *J* 3, Ar-N*H*), 5.00 (1H, d, *J* 7, BocN*H*CH), 4.25 (1H, m, BocNHC*H*), 1.47 (9H, s, (C*H*$_3$)$_3$CO), 1.42 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (500 MHz, $CDCl_3$) 172.9 (*C*=ONHNH), 153.9 (*C*=ONH), 151.4 (*ipso*-Ar-*C*), 133.6 (Ar-*C*), 119.5 (*C*≡N), 112.9 (Ar-*C*), 111.4 (Ar-*C*), 103.3 (Ar-*C*), 81.1 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 17.1 (NHCH*C*H$_3$); *m/z* (ES^+) 305 (MH^+); HRMS (ES^+) Found MH^+, 305.1617 ($C_{15}H_{21}N_4O_3$ requires 305.1614).

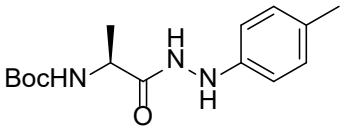
tert-Butyl (*S*)-(1-(2-(4-(methylsulfonyl)phenyl)hydrazinyl)-1-oxopropan-2-yl) carbamate 67Dj



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 4-(methylsulphonyl)phenylhydrazine hydrochloride (0.26 g, 1.12 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a light yellow solid (0.38 g, 99%) as a mixture of rotamers [5:1]; R_f 0.59 (DCM/EtOH/NH_3 200:8:1); m.p. 126-130 °C; υ_{max} 3326 (N-H), 2976 (C-H), 1718, 1688 (C=O), 1513, 1371. 1145, 829, 775, 516 cm^{-1}; NMR data given for the major rotamer δ_H (500 MHz, $CDCl_3$) 8.46 (1H, bs, CON*H*NH), 7.74 – 7.72 (2H, dd, *J* 6, 1, Ar-*H*), 6.88 – 6.86 (2H, dd, *J* 6, 1, Ar-*H*), 5.02 (1H, bs, BocN*H*CH), 4.26

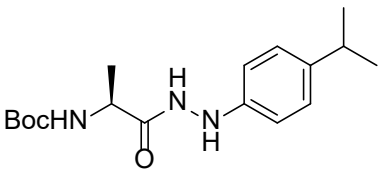
(1H, m, BocNHC*H*), 4.26 (3H, s, SO_2CH_3), 1.48 (9H, s, $(CH_3)_3CO$), 1.43 (3H, d, *J* 7, NHCHCH_3); δ_C (500 MHz, $CDCl_3$) 172.9 (*C*=ONHNH), 152.4 (*C*=ONH), 131.7 (*ipso*-Ar-*C*), 129.3 (Ar-*C*), 129.2 (Ar-*C*), 81.1 ($(CH_3)_3CO$), 48.8 (BocNH*C*H), 44.9 (SO_2CH_3), 28.3 ($(CH_3)_3CO$), 17.1 (NHCH*C*H_3); *m/z* (ES^+) 358 (MH^+); HRMS (ES^+) Found MH^+, 358.1450 ($C_{15}H_{24}N_3O_5S$ requires 358.1437).

tert-Butyl (*S*)-(1-oxo-1-(2-(p-tolyl)hydrazinyl)propan-2-yl)carbamate 67Eb



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 4-methylphenylhydrazine hydrochloride (0.20 g, 1.27 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a yellowish orange solid (0.25 g, 80%) as a mixture of rotamers [7:1]; R_f 0.5 (DCM/EtOH/NH_3 200:8:1); m.p. 112-114 °C; υ_{max} 3213 (N-H), 2987 (C-H), 1686 (C=O), 1664 (C=O), 1513, 1321, 1250, 1163, 805, 641 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.50 (1H, bs, CON*H*NH), 7.00 – 6.98 (2H, d, *J* 8, Ar-*H*), 7.71 – 6.69 (2H, d, *J* 8, Ar-*H*), 6.09 (1H, bs, Ar-N*H*), 5.23 (1H, bs, BocN*H*CH), 4.28 (1H, m, BocNHC*H*), 2.24 (3H, s, Ar-CH_3), 1.45 (9H, s, $(CH_3)_3CO$), 1.36 (3H, d, *J* 6, NHCHCH_3); δ_C (700 MHz, $CDCl_3$) 172.8 (*C*=ONHNH), 155.8 *(C*=ONH), 145.4 (*ipso*-Ar-*C)*, 130.7 (Ar-*C)*, 129.9 (Ar-*C)*, 129.6 (Ar-*C)*, 113.7 (Ar-*C)*, 112.9 (Ar-*C)*, 80.5 ($(CH_3)_3CO$), 48.6 (BocNH*C*H), 28.4 ($(CH_3)_3CO$), 20.6 (Ar-*C*H_3), 17.9 (NHCH*C*H_3); *m/z* (ES^+) 294 (MH^+); HRMS (ES^+) Found MH^+, 294.1829 ($C_{15}H_{24}N_3O_3$ requires 294.1818).

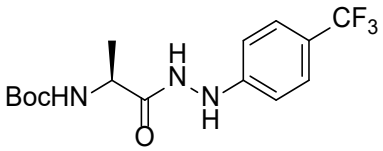
tert-Butyl (*S*)-(1-(2-(4-isopropylphenyl)hydrazinyl)-1-oxopropan-2-yl) carbamate 67Eh



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and 4-isopropylphenylhydrazine hydrochloride (0.20 g, 1.27 mmol) were transformed following flash column chromatography (DCM/EtOH 100:6) into the

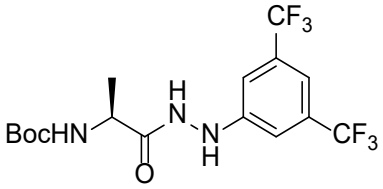
title compound which was isolated as a brown oil (0.11 g, 33%); R_f 0.46 (DCM/EtOH/NH_3 200:8:1); υ_{max} 3288 (N-H), 2959 (C-H), 1669, 1615 (C=O), 1512, 1365, 1248, 1052, 827, 754, 539 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.51 (1H, bs, CON*H*NH), 7.05 – 7.03 (2H, d, *J* 7, Ar-*H*), 6.74 – 6.72 (2H, d, *J* 7, Ar-*H*), 5.21 (1H, bs, BocN*H*CH), 4.29 (1H, m, BocNHC*H*), 2.81 (1H, m, C*H*(CH_3)$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 1.37 (3H, d, *J* 7, NHCHC*H*$_3$), 1.19 – 1.18 (6H, d, *J* 7, CH(C*H*$_3$)$_2$); δ_C (400 MHz, $CDCl_3$) 172.8 (*C*=ONHNH), 155.7 (*C*=ONH), 145.7 (*ipso*-Ar-*C*), 141.7 (*ipso*-Ar-*C*), 127.3 (Ar-*C*), 127.0 (Ar-*C*), 113.7 (Ar-*C*), 113.1 (Ar-*C*), 80.5 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 33.3 (*C*H(CH$_3$)$_2$), 28.4 ((*C*H$_3$)$_3$CO), 24.2 (CH(*C*H$_3$)$_2$), 24.0 (CH(*C*H$_3$)$_2$), 17.9 (NHCH*C*H$_3$); ***m/z*** (ES$^+$) 322 (MH$^+$); HRMS (ES$^+$) Found MH$^+$, 322.2136 ($C_{17}H_{28}N_3O_3$ requires 322.2131).

tert-Butyl (*S*)-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate 67BI



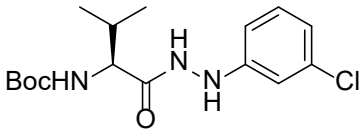
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.25 g, 1.32 mmol) and 4-trifluorophenyl hydrazine hydrochloride (0.30 g, 1.75 mmol) were transformed following flash chromatography (DCM/EtOH/NH_3 200:8:1) into the title compound as a dark brown solid (0.43 g, 93%); R_f 0.37 (DCM/EtOH/NH_3 [200:8:1]); m.p. 126 – 131 °C; υ_{max} 3321 (N-H), 2929, 2851, 1683 (C=O), 1664 (C=O), 1617, 1524, 1322, 1157, 1101, 1066, 830, 641 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.50 (1H, bs, N*H*), 7.46 (2H, d, *J* 8, Ar-*H*), 6.87 (2H, d, *J* 8, Ar-*H*), 6.34 (1H, bs, N*H*), 5.07 (1H, bd, *J* 6, N*H*), 4.40 (1H, m, CH_3C*H*), 1.50 (9H, s, C(C*H*$_3$)$_3$), 1.43 (3H, d, *J* 7, C*H*$_3$CH); δ_C (176 MHz, $CDCl_3$) 171.7 (NHCH*C*O), 158.7 (NH*C*OO), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.2 (Ar-*C*), 126.4 (*C*F$_3$), 112.8 (Ar-*C*), 80.9 (*C*(CH$_3$)$_3$), 54.6 (NH*C*HCO), 33.7 (CH*C*H$_3$), 28.3 (C(*C*H$_3$)$_3$); δ_F (376 MHz, $CDCl_3$) -61.55 (3F, s, C*F*$_3$); ***m/z*** (ES$^+$) 348 (MH$^+$), 370 (MNa$^+$), 717 (2M + Na$^+$); HRMS (ES$^+$) Found MNa$^+$, 370.1352 ($C_{15}H_{20}F_3N_3O_3Na$ requires 370.1349).

tert-Butyl (*S*)-(1-(2-(2,4-bis(trifluoromethyl)phenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate 67Bt



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and (3,5-bis(trifluoromethyl)phenyl)hydrazine hydrochloride (0.27 g, 0.95 mmol) were transformed following flash chromatography (DCM/EtOH/NH_3 400:8:1) into the title compound as a golden white solid (0.03 g, 6%); R_f 0.26 (DCM/EtOH/NH_3 400:8:1); m.p. 174 - 177 °C; υ_{max} 3331, 3267 (N-H), 2995 (C-H), 1669, 1623 (C=O), 1522, 1285, 1161, 1123, 1086, 878, 682 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.44 (1H, bs, CON*H*NH), 7.36 (1H, s, Ar-*H*), 7.21 (2H, s, Ar-*H*), 6.39 (1H, bs, Ar-N*H*), 4.94 (1H, bd, *J* 7, BocN*H*CH), 4.27 (1H, m, BocNHC*H*), 1.47 (9H, s, (CH_3)$_3$CO), 1.42 (3H, d, *J* 7, NHCHCH_3); δ_C (700 MHz, $CDCl_3$) 173.1 (*C*=ONHNH), 156.1 (*C*=ONH), 149.2 (*ipso*-Ar-*C*), 132.6 (1C, q, *J* 33, Ar-*C*F_3), 123.3 (1C, q, *J* 272, *ipso*-Ar-*C*CF_3), 114.4 (Ar-*C*), 112.9 (Ar-*C*), 81.2 ((CH_3)$_3$*C*O), 48.7 (BocNH*C*H), 28.2 ((*C*H_3)$_3$CO), 16.8 (NHCH*C*H_3); δ_F (300 MHz, $CDCl_3$) -63.1 (C*F*$_3$); *m/z* (ES^-) 414 (MH^-); HRMS (ES^-) Found (MH^-), 414.1251 ($C_{16}H_{18}F_6N_3O_3$ requires 414.1252).

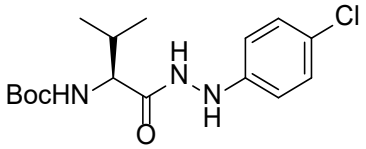
tert-Butyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl) carbamate 67s



Following the general procedure outlined, *N*-Boc-*L*-valine (0.30 g, 1.38 mmol) and 3-chlorophenylhydrazine hydrochloride (0.27 g, 1.52 mmol) were transformed following trituration with Et_2O into the title compound as a white solid (0.22 g, 46%); R_f 0.61 (DCM/EtOH/NH_3 200:8:1); m.p. 150 - 153 °C; ν_{max} 3323, 3259 (N-H), 2969 (C-H), 1685, 1663 (C=O), 1522, 1468, 1306, 1246, 1168, 858, 680 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.49 (1H, bs, CON*H*NH), 7.11 (1H, t, *J* 8, Ar-*H*), 6.84 –

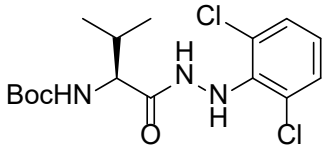
6.80 (2H, m, Ar-*H*), 6.69 (1H, dd, *J* 8, 2, Ar-*H*), 6.27 (1H, bd, *J* 3, Ar-N*H*), 5.19 (1H, bd, *J* 9, BocN*H*CH), 4.00 (1H, t, *J* 8, BocNHC*H*), 2.12 (1H, m, CHC*H*$(CH_3)_2$), 1.45 (9H, s, $(CH_3)_3CO$), 0.99 – 0.96 (6H, m, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 156.2 (*C*=ONH), 149.2 (*ipso*-Ar-*C)*, 134.9 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 121.1 (Ar-*C*), 113.6 (Ar-*C*), 111.7 (Ar-*C*), 80.5 ($(CH_3)_3CO$), 58.8 (BocNH*C*H), 30.5 (CH*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 19.4 (CHCH($CH_3)_2$), 18.2 (CHCH($CH_3)_2$); *m/z* (ES^+) 364 ($[^{35}Cl]MNa^+$), 366 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 364.1401 ($C_{16}H_{24}{}^{35}ClN_3O_3Na$ requires 364.1404).

tert-Butyl-(*S*)-(1-(2-(4-chlorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Ao



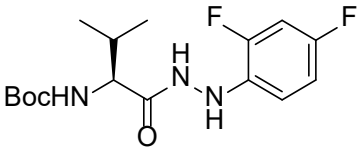
Following the general procedure outlined, *N*-Boc-*L*-valine (0.20 g, 0.92 mmol) and 4-chlorophenylhydrazine hydrochloride (0.14 g, 1.01 mmol) were transformed following trituration with Et_2O into the title compound as a yellow solid (0.12 g, 38%); R_f 0.32 (DCM/EtOH/NH_3 200:8:1); m.p. 146 - 150 °C; ν_{max} 3325, 3281 (N-H), 2967, 2870 (C-H), 1685, 1649 (C=O), 1522, 1487, 1382, 1245, 1167, 829, 643 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.35 (1H, bs, CON*H*NH), 7.15 - 7.13 (2H, d, *J* 8, Ar-*H*), 6.75 – 6.73 (2H, d, *J* 8, Ar-*H*), 6.19 (1H, bd, *J* 3, Ar-N*H*), 5.15 (1H, bd, *J* 8, BocN*H*CH), 3.97 (1H, t, *J* 8, BocNHC*H*), 2.14 (1H, m, CHC*H*$(CH_3)_2$), 1.45 (9H, s, $(CH_3)_3CO$), 0.98 – 0.97 (6H, d, *J* 6, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 156.2 (*C*=ONH), 146.5 (*ipso*-Ar-*C)*, 129.3 (Ar-*C*), 129.0 (Ar-*C*), 125.9 (*ipso*-Ar-*C*), 114.8 (Ar-*C*), 114.3 (Ar-*C*), 80.5 ($(CH_3)_3CO$), 58.8 (BocNH*C*H), 30.4 (CH*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 19.4 (CHCH($CH_3)_2$), 18.2 (CHCH($CH_3)_2$); *m/z* (ES^+) 364 ($[^{35}Cl]MNa^+$), 366 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 364.1412 ($C_{16}H_{24}{}^{35}ClN_3O_3Na$ requires 364.1404).

tert-Butyl (*S*)-(1-(2-(2,6-dichlorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Au



Following the general procedure outlined, *N*-Boc-*L*-valine (0.20 g, 0.92 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (0.24 g, 1.11 mmol) were transformed following trituration with Et_2O into the title compound as a light brown solid (0.26 g, 75%); R_f 0.43 (DCM/EtOH/NH_3 200:6:1); m.p. 123 - 126 °C; υ_{max} 3328 (N-H), 2975 (C-H), 1689, 1670 (C=O), 1521, 1449, 1300, 1244, 1168, 766, 654 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.45 (1H, bs, CON*H*NH), 7.25 - 7.22 (2H, d, *J* 7, Ar-*H*), 6.93 (1H, t, *J* 7, Ar-*H*), 6.77 (1H, bd, *J* 4, Ar-N*H*), 5.07 (1H, bd, *J* 8, BocN*H*CH), 3.96 (1H, t, *J* 7, BocNHC*H*), 2.13 (1H, m, CHC*H*$(CH_3)_2$), 1.43 (9H, s, CH_3)$_3$CO), 0.91 – 0.86 (6H, m, CHCH(CH_3)$_2$); δ_C (300 MHz, $CDCl_3$) 171.1 (*C*=ONHNH), 155.8 (*C*=ONH), 140.9 (*ipso*-Ar-*C*), 129.0 (Ar-*C*), 128.7 (Ar-*C*), 126.1 (*ipso*-Ar-*C*), 124.1 (Ar-*C*), 80.2 ((CH$_3$)$_3$*C*O), 58.3 (BocNH*C*H), 30.7 (CH*C*H$(CH_3)_2$), 28.3 ((*C*H$_3$)$_3$CO), 19.2 (CHCH(*C*H$_3$)$_2$), 17.8 (CHCH(*C*H$_3$)$_2$); *m/z* (ES$^+$) 398 ([^{35,35}Cl]MNa$^+$), 400 ([^{35,37}Cl]MNa$^+$), 402 ([^{37,37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MNa$^+$, 398.1015 ($C_{16}H_{23}{}^{35,35}ClN_3O_3Na$ requires 398.1014).

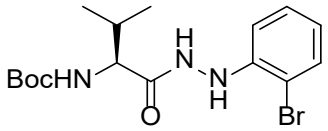
tert-Butyl-(*S*)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Ca



Following the general procedure outlined, *N*-Boc-*L*-valine (0.20 g, 0.92 mmol) and 2,4- difluorophenylhydrazine hydrochloride (0.20 g, 1.11 mmol) were transformed following work up with EtOAc into the title compound as an orange solid (0.21 g, 66%); R_f 0.52 (DCM/EtOH/NH_3 200:8:1); m.p. 125 - 128 °C; υ_{max} 3453, 3273 (N-H), 2975 (C-H), 1689, 1613 (C=O), 1501, 1371, 1258, 1162, 958, 846 cm^{-1}; δ_H (400 MHz, DMSO-d6) 9.82 (1H, bd, *J* 2, CON*H*NH), 7.62 (1H, bs, Ar-N*H*), 7.10 (1H, dt, *J* 9, 2, Ar-*H*), 6.89 - 6.76 (2H, m, Ar-*H*), 3.73 (1H, dd, *J* 8, BocNHC*H*),

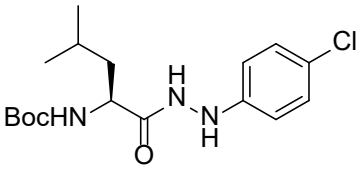
1.95 (1H, m, CHC*H*$(CH_3)_2$), 1.40 (9H, s, $(CH_3)_3CO$), 0.89 – 0.87 (6H, m, $CHCH(CH_3)_2$); δ_C (100 MHz, DMSO-d_6) 172.1 (*C*=ONHNH), 156.1 (*C*=ONH), 134.2 (*ipso*-Ar-*C)*, 114.7 (Ar-*C*), 111.0 (Ar-*C*), 110.8 (*ipso*-Ar-*C*), 104.3 (Ar-*C*), 104.1 (*ipso*-Ar-*C*), 78.6 ($(CH_3)_3CO$), 59.4 (BocNH*C*H), 30.1 ($CHCH(CH_3)_2$), 28.6 ($(CH_3)_3CO$), 19.7 ($CHCH(CH_3)_2$), 19.2 ($CHCH(CH_3)_2$); δ_F (282 MHz, DMSO-d_6) -124.1 (Ar-*F*), -128.4 (Ar-*F*); *m/z* (ES^+) 366 (MNa^+); HRMS (ES^+) Found MNa^+, 366.1589 ($C_{16}H_{23}N_3O_3F_2Na$ requires 366.1605).

tert-Butyl (*S*)-(1-(2-(2-bromophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl) carbamate 67Cg



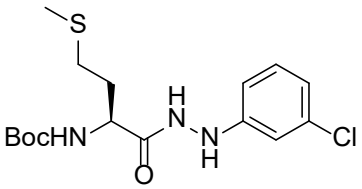
Following the general procedure outlined, *N*-Boc-*L*-valine (0.20 g, 0.92 mmol) and 2-bromophenylhydrazine hydrochloride (0.23 g, 1.02 mmol) were transformed following flash chromatography (DCM/EtOH/NH3 [200:8:1] into the title compound as an orange solid (0.18 g, 51%); R_f 0.52 (DCM/EtOH/NH_3 200:8:1); m.p. 120 - 125 °C; υ_{max} 3318 (N-H), 2960 , 2870, 1678 (C=O), 1650 (C=O), 1525, 1481, 1301,1170, 744 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.68 (1H, m, CON*H*NH), 7.47 (1H, d, *J* 8, Ar-H), 7.14 (1H, t, *J* 8, Ar-H), 6.86 (1H, d, *J* 8, Ar-H), 6.75 (1H, t, *J* 8, Ar-H), 6.46 (1H, bd, *J* 4, Ar-N*H*), 5.30 (1H, bs, BocN*H*CH), 4.05 (1H, t, *J* 8, BocNHC*H*), 2.13 (1H, m, CHC*H*$(CH_3)_2$), 1.45 (9H, s, $(CH_3)_3CO$), 1.01 (6H, m, $CHCH(CH_3)_2$); δ_C (700 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 156.2 (*C*=ONH), 144.7 (*ipso*-Ar-*C)*, 132.6 (Ar-*C*), 128.3 (Ar-*C*), 121.8 (*ipso*-Ar-*C*), 113.8 (Ar-*C*), 109.1 (Ar-*C*), 80.4 ($(CH_3)_3CO$), 58.8 (BocNH*C*H), 30.5 ($CHCH(CH_3)_2$), 28.4 ($(CH_3)_3CO$), 19.4 ($CHCH(CH_3)_2$), 19.3 ($CHCH(CH_3)_2$); *m/z* (ES^+) 386 ([^{79}Br] MH^+), 388 ([^{81}Br] MH^+); HRMS (ES^+) Found [^{79}Br] MH^+, 386.1107 ($C_{16}H_{24}{}^{79}BrN_3O_3$ requires 386.1079).

tert-Butyl (*S*)-(1-(2-(4-chlorophenyl)hydrazinyl)-4-methyl-1-oxopentan-2-yl) carbamate 67Ap



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.20 g, 0.87 mmol) and 4-chlorophenylhydrazine hydrochloride (0.15 g, 1.04 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.02 g, 8%); R_f 0.58 (DCM/EtOH/NH_3 200:8:1); m.p. 96 - 99 °C; ν_{max} 3307 (N-H), 2959 (C-H), 1652 (C=O), 1519, 1491, 1366, 1235, 1166, 822 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.23 (1H, bs, CON*H*NH), 7.16 – 7.14 (2H, d, *J* 8, Ar-*H*), 6.75 – 6.73 (2H, d, *J* 8, Ar-*H*), 6.10 (1H, bs, CONHN*H*), 4.93 (1H, bd, *J* 8, BocN*H*CH), 4.18 (1H, m, BocNHC*H*), 1.72 – 1.66 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.54 (1H, m, C*H*HCH$(CH_3)_2$), 1.47 (9H, s, $(CH_3)_3CO$), 0.97 – 0.95 (3H, d, *J* 6, $CH_2CH(CH_3)_2$) 0.94 – 0.92 (3H, d, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.6 (*C*=ONHNH), 146.5 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 125.9 (*ipso*-Ar-*C*), 114.8 (Ar-*C*), 80.7 ($(CH_3)_3CO$), 51.4 (BocNH*C*H), 40.2 ($CH_2CH(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 22.1 ($CH_2CH(CH_3)_2$); *m/z* (ES^+) 356 ($[^{35}Cl]MH^+$), 358 ($[^{37}Cl]MH^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 356.1694 ($C_{17}H_{27}N_3O_3{}^{35}Cl$ requires 356.1741).

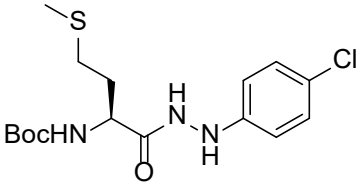
tert-Butyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67w



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 3-chlorophenylhydrazine hydrochloride (0.13 g, 0.88 mmol) were transformed following flash chromatography (DCM/EtOH/NH_3 400:8:1) and

(DCM/EtOH/NH_3 200:8:1) into the title compound which was isolated as a pale yellow solid (0.16 g, 53%); R_f 0.30 (DCM/EtOH/NH_3 200:8:1);); m.p. 94 - 97 °C; ν_{max} 3331, 3272 (N-H), 2985 (C-H), 1683, 1658 (C=O), 1521, 1163, 768 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.73 (1H, bs, CON*H*NH), 7.10 (1H, t, *J* 8, Ar-*H*), 6.85 (1H, d, *J* 8, Ar-*H*), 6.80 (1H, s, Ar-*H*), 6.67 (1H, d, *J* 8, Ar-*H*), 6.34 (1H, bs, Ar-N*H*), 5.42 (1H, bd, *J* 8, BocN*H*CH), 4.43 (1H, q, *J* 7, BocNHC*H*), 2.57 (2H, t, *J* 7, CH_2CH_2S), 2.13 – 2.09 (5H, m, C*H*HCH_2S, $CH_2CH_2SCH_3$, CH*H*CH_2S), 1.47 (9H, s, $(CH_3)_3CO$); δ_C (700 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 156.9 (*C*=ONH), 149.0 (*ipso*-Ar-*C*), 134.9 (Ar-*C*), 130.2 (Ar-*C*), 121.0 (Ar-*C*), 113.4 (Ar-*C*), 111.6 (Ar-*C*), 80.8 ($(CH_3)_3CO$), 51.9 (BocNH*C*H), 31.0 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$), 15.5 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 374 ($[^{35}Cl]MH^+$), 376 ($[^{37}Cl]MH^+$), 396 ($[^{35}Cl]MNa^+$), 398 ($[^{37}Cl]MNa^+$); HRMS (ES^+) found ($[^{35}Cl]MNa^+$), 396.1141 ($C_{16}H_{24}N_3O_3S^{35}ClNa$ requires 396.1125).

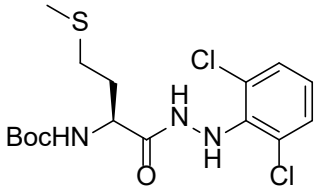
tert-Butyl (*S*)-(1-(2-(4-chlorophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Ar



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 4-chlorophenylhydrazine hydrochloride (0.13 g, 0.88 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange gum (0.07 g, 23%); R_f 0.35 (DCM/EtOH/NH_3 200:8:1); ν_{max} 3313, 3271 (N-H), 2977 (C-H), 1657 (C=O), 1519, 1489, 1305, 1249, 1159, 821 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.58 (1H, bs, CON*H*NH), 7.15 – 7.13 (2H, d, *J* 8, Ar-*H*), 6.74 – 6.72 (2H, d, *J* 8, Ar-*H*), 6.24 (1H, bs, Ar-N*H*), 5.34 (1H, bs, BocN*H*CH), 4.39 (1H, m, BocNHC*H*), 2.57 (2H, m, CH_2CH_2S), 2.13 – 2.06 (4H, m, C*H*HCH_2S, $CH_2CH_2SCH_3$), 1.97 (1H, m, CH*H*CH_2S), 1.45 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.0 (*C*=ONHNH), 155.9 (*C*=ONH), 146.4 (*ipso*-Ar-*C*), 129.4 (Ar-*C*), 129.1 (Ar-*C*), 125.9 (*ipso*-Ar-*C*), 114.8 (Ar-*C*), 114.2 (Ar-*C*), 80.8 ($(CH_3)_3CO$), 52.0 (BocNH*C*H), 30.9 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$),

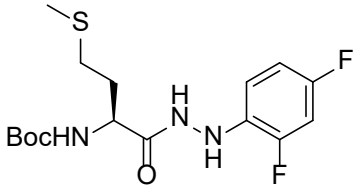
15.4 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 396 ($[^{35}Cl]MNa^+$), 398 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 396.1140 ($C_{16}H_{24}N_3O_3S^{35}ClNa$ requires 396.1125).

tert-Butyl (*S*)-(1-(2-(2,6-dichlorophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Aw



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (0.19 g, 0.88 mmol) were transformed following flash column chromatography (*n*-hex/EtOAc 3:1) into the title compound which was isolated as a yellow gum (0.21 g, 65%); R_f 0.40 (n-hex/EtOAc 3:1); m.p. 89 - 91 °C; ν_{max} 3342, 3286 (N-H), 2998, 2967, 2914 (C-H), 1673 (C=O), 1519, 1308, 1256, 1167, 767 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.73 (1H, bs, CON*H*NH), 7.25 (1H, d, *J* 8, Ar-*H*), 6.90 (1H, t, *J* 8, Ar-*H*), 6.75 (1H, d, *J* 4, Ar-*H*), 5.26 (1H, bd, *J* 8, BocN*H*CH), 4.40 (1H, m, BocNHC*H*), 2.50 (2H, t, *J* 8, $CHCH_2C\mathit{H}_2S$), 2.14 – 2.03 (4H, m, $CH_2CH_2SC\mathit{H}_3$, $CHC\mathit{H}HCH_2S$), 1.91 (1H, m, $CHCH\mathit{H}CH_2S$), 1.44 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 170.9 (*C*=ONHNH), 155.7 (*C*=ONH), 140.8 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 128.9 (Ar-*C*), 128.5 (Ar-*C*), 128.0 (Ar-*C*), 126.0 (Ar-*C*), 124.1 (Ar-*C*), 80.5 ($(CH_3)_3\mathit{C}O$), 51.7 (BocNH*C*H), 31.4 ($\mathit{C}H_2CH_2SCH_3$), 30.0 ($CH_2\mathit{C}H_2SCH_3$), 28.3 ($(\mathit{C}H_3)_3CO$), 15.3 ($CH_2CH_2S\mathit{C}H_3$); *m/z* (ES^+) 408 ($[^{35,35}Cl]MH^+$), 410 ($[^{35,37}Cl]MH^+$), 412 ($[^{37,37}Cl]MH^+$); HRMS (ES^+) Found $[^{35,35}Cl]MH^+$, 408.0916 ($C_{16}H_{24}N_3O_3S^{35,35}Cl$ requires 408.0915).

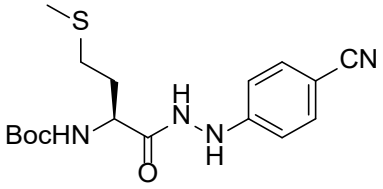
tert-Butyl-(*S*)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Cc



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 2,4-difluorophenylhydrazine hydrochloride (0.16 g, 0.88 mmol) were

transformed following flash column chromatography (*n*-hex/EtOAc 3:1) into the title compound which was isolated as an orange gummy solid (0.29 g, 95%); R_f 0.43 (DCM/EtOH/NH_3 200:8:1); m.p. 92 – 95 °C; ν_{max} 3317 (N-H), 2979 (C-H), 1665, 1611 (C=O), 1507, 1369, 1254, 1164, 958, 834 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.40 (1H, bs, CON*H*NH), 6.81 – 6.69 (2H, m, Ar-*H*), 6.65 (1H, t, *J* 8, Ar-*H*), 5.18 (1H, bs, BocN*H*CH), 4.33 (1H, q, *J* 7, BocNHC*H*), 2.51 (2H, m, $CHCH_2C\mathit{H}_2S$), 2.08 – 2.02 (4H, m, $CHC\mathit{H}HCH_2S$, $CH_2CH_2SC\mathit{H}_3$), 1.93 (1H, m, $CHCH\mathit{H}CH_2S$), 1.39 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.9 (*C*=ONH), 149.9 (*ipso*-Ar-*C*), 132.3 (*ipso*-Ar-*C*), 115.4 (Ar-*C*), 111.0 (Ar-*C*), 110.9 (*ipso*-Ar-*C*), 104.2 (Ar-*C*), 80.8 ($(CH_3)_3\mathit{C}O$), 51.9 (BocNH*C*H), 30.8 ($\mathit{C}H_2CH_2SCH_3$), 30.2 ($CH_2\mathit{C}H_2SCH_3$), 28.3 ($(\mathit{C}H_3)_3CO$), 15.4 ($CH_2CH_2S\mathit{C}H_3$); δ_F (282 MHz, $CDCl_3$) -120.5 (Ar-*F*), -129.0 (Ar-*F*); *m/z* (ES^+) 398 (MNa^+); HRMS (ES^+) Found MNa^+, 398.1348 ($C_{16}H_{23}N_3O_3F_2SNa$ requires 398.1326).

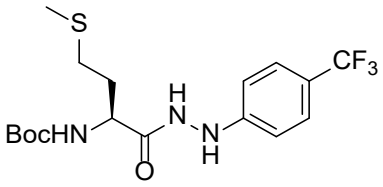
tert-Butyl (*S*)-(1-(2-(4-cyanophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Da



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 4-cyanophenylhydrazine hydrochloride (0.15 g, 0.88 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow gummy solid (0.10 g, 35%); R_f 0.41 (DCM/EtOH/NH_3 200:8:1); m.p. 57 - 60 °C; ν_{max} 3264 (N-H), 2979, 2218 (C-H), 2112 (C≡N), 1673, 1605 (C=O), 1505, 1366. 1249, 1161, 1047, 831, 545 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.39 (1H, bs, CON*H*NH), 7.49 – 7.47 (2H, d, *J* 8, Ar-*H*), 6.83 – 6.81 (2H, d, *J* 8, Ar-*H*), 6.38 (1H, bs, Ar-N*H*), 5.19 (1H, bd, *J* 7, BocN*H*CH), 4.37 (1H, q, *J* 7, BocNHC*H*), 2.59 (2H, m, $CHCH_2C\mathit{H}_2S$), 2.14 – 2.09 (4H, m, $CH_2CH_2SC\mathit{H}_3$, $CHC\mathit{H}HCH_2S$), 2.00 (1H, m, $CHCH\mathit{H}CH_2S$), 1.48 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (400 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 156.1 (*C*=ONH), 151.3 (Ar-*C*), 133.7 (*ipso*-Ar-*C*), 119.4 (*C*≡N), 113.1 (Ar-*C*), 103.5 (Ar-*C*), 81.4 ($(CH_3)_3\mathit{C}O$), 51.9 (BocNH*C*H), 30.3 ($\mathit{C}H_2CH_2SCH_3$), 30.2 ($CH_2\mathit{C}H_2SCH_3$), 28.3 ($(\mathit{C}H_3)_3CO$), 15.4 ($CH_2CH_2S\mathit{C}H_3$); *m/z*

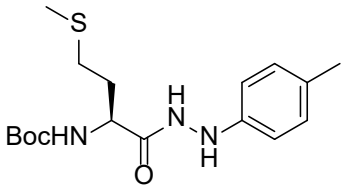
(ES$^+$) 365 (MH$^+$); HRMS (ES$^+$) Found MH$^+$, 365.1653 ($C_{17}H_{25}N_4O_3S$ requires 365.1647).

tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-(4-(trifluoromethyl)phenyl) hydrazineyl)butan-2-yl)carbamate 67Bo



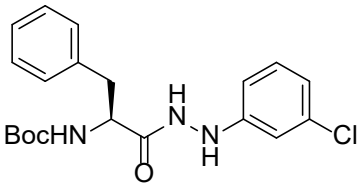
Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 4-trifluoromethylphenylhydrazine hydrochloride (0.19 g, 0.88 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a pale yellow solid (0.14 g, 44%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 102 - 108 °C; ν_{max} 3321 (N-H), 2984 (C-H), 1680 (C=O), 1662(C=O) 1515, 1327, 1306, 1156, 1102, 830, 589 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.82 (1H, bs, CON*H*NH), 7.41 (2H, d, *J* 8, Ar-*H*), 6.81 (2H, d, *J* 8, Ar-*H*), 6.53 (1H, bs, Ar-N*H*), 5.43 (1H, bd, *J* 8, BocN*H*CH), 4.44 (1H, q, *J* 7, BocNHC*H*), 2.56 (2H, t, *J* 7, CH_2CH_2S), 2.09 - 2.14 (5H, m, $CH_2CH_2SCH_3$,$CH_2CH_2CH_2S$), 1.49 (9H, s, $(CH_3)_3CO$); δ_C (700 MHz, $CDCl_3$) 172.4 (*C*=ONHNH), 156.1 (*C*=ONH), 150.5 (*ipso*-Ar-*C*), 133.0 (Ar-CF_3), 126.3 (1C, t, *J* 5, *ipso*-Ar-CCF_3), 125.2 (Ar-*C*), 120.0 (Ar-*C*), 123.7 (Ar-*C*), 112.7 (Ar-*C*), 80.9 ($(CH_3)_3CO$), 51.9 (BocNH*C*H), 30.9 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$), 15.4 ($CH_2CH_2SCH_3$); δ_F (700 MHz, $CDCl_3$) -61.8 (CF_3); *m/z* (ES$^+$) 408 (MH$^+$); HRMS (ES$^+$) Found MH$^+$, 408.1573 ($C_{17}H_{25}N_3O_3F_3S$ requires 408.1569).

tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-(p-tolyl)hydrazineyl)butan-2-yl) carbamate 67Ee



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.20 g, 0.80 mmol) and 4-methylphenylhydrazine hydrochloride (0.19 g, 0.88 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a reddish brown solid (0.16 g, 54%); R_f 0.26 (DCM/EtOH/NH_3 200:6:1); m.p. 96 - 99 °C; ν_{max} 3324 (N-H); 3201 (N-H), 2967 (C-H), 2919, 1682 (C=O), 1657 (C=O), 1513, 1290, 1162, 641 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.60 (1H, bs, CON*H*NH), 7.02 (2H, d, *J* 8, Ar-*H*), 6.73 (2H, d, *J* 8, Ar-*H*), 6.12 (1H, bs, Ar-N*H*), 5.41 (1H, bs, BocN*H*CH), 4.39 (1H, m, BocNHC*H*), 2.54 (2H, t, *J* 7, CH_2CH_2S), 2.26 (3H, s, Ar-CH_3), 2.09 - 2.11 (5H, m, $CH_2CH_2SCH_3$,CH_2CH_2S), 1.48 (9H, s, $(CH_3)_3CO$); δ_C (500 MHz, $CDCl_3$) 171.8 (*C*=ONHNH), 155.8 (*C*=ONH), 144.4 (*ipso*-Ar-*C*), 130.6 (Ar-*C*), 129.7 (Ar-*C*), 113.8 (Ar-*C*), 80.6 ($(CH_3)_3CO$), 52.0 (BocNH*C*H), 31.3 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.4 ($(CH_3)_3CO$), 20.6 (Ar-CH_3), 15.4 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 354 (MH^+); HRMS (ES^+) Found MH^+, 354.1860 ($C_{17}H_{28}N_3O_3S$ requires 354.1851).

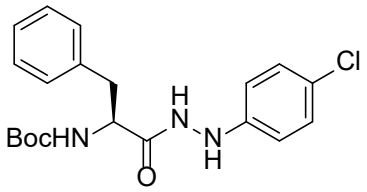
tert-Butyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl) carbamate 67x



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.20 g, 0.75 mmol) and 3-chlorophenylhydrazine hydrochloride (0.16 g, 0.91 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.10 g, 34%); R_f 0.79 (DCM/EtOH/NH_3 200:8:1]); m.p. 148 - 150 °C; υ_{max} 3339, 3238 (N-H), 2986, 1694 (C=O), 1674 (C=O), 1596, 1525, 1495, 1492, 1249, 1047, 862 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.83 (1H, bs, N*H*),

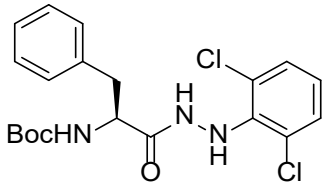
7.34 – 7.31 (2H, m, CH_2Ar-*H*), 7.29 (1H, m, Ar-*H*), 7.22 – 7.20 (2H, m, Ar-*H*), 7.06 (1H, t, *J* 8, Ar-*H*), 6.83 (1H, d, *J* 8, NHAr-*H*), 6.65 (1H, s, Ar-*H*), 6.48 (1H, d, *J* 8, Ar-*H*), 5.05 (1H, s, BocN*H*CH), 4.42 (1H, dt, *J* 8, BocNHC*H*), 3.09 (2H, d, *J* 7, C*H*$_2$Ph), 1.44 (9H, s, (C*H*$_3$)$_3$CO); δ_C (700 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 148.8 (*C*=ONH), 136.0 (Ar-*C)*, 134.9 (Ar-*C)*, 130.1 (Ar-*C)*, 129.3 (Ar-*C)*, 128.9 (Ar-*C)*, 127.3 (Ar-*C)*, 121.2 (Ar-*C)*, 113.4 (Ar-*C)*, 111.7 (Ar-*C)*, 81.0 (CH_3)$_3$*C*O), 54.5 (BocNH*C*H), 37.8 (*C*H_2Ph), 28.3 (*C*H_3)$_3$CO); ***m/z*** (ES^+) 390 ([^{35}Cl]MH^+), 392 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 390.1579 ($C_{20}H_{25}{}^{35}ClN_3O_3$ requires 390.1584).

tert-Butyl-(*S*)-(1-(2-(4-chlorophenyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Ar



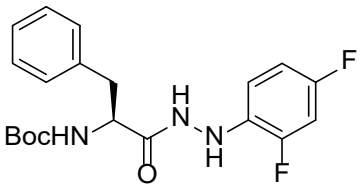
Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.20 g, 0.75 mmol) and 4-chlorophenylhydrazine hydrochloride (0.16 g, 0.91 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a yellow solid (0.27 g, 91%); R_f 0.31 (DCM/EtOH/NH_3 200:8:1); m.p. 139 - 141 °C; ν_{max} 3328, 3246 (N-H), 2976 (C-H), 1662 (C=O), 1518, 1489, 1296, 1165, 622 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.02 (1H, bs, CON*H*NH), 7.31 – 7.28 (3H, m, Ar-*H*), 7.21 – 7.19 (2H, d, *J* 7, Ar-*H*), 7.08 – 7.06 (2H, d, *J* 8, Ar-*H*), 6.47 – 6.45 (2H, d, *J* 8, Ar-*H*), 6.03(1H, bs, Ar-N*H*), 5.16 (1H, bd, *J* 8, BocN*H*CH), 4.45 (1H, m, BocNHC*H*), 3.08 (2H, d, *J* 7, Ar-CH_2), 1.43 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 155.6 (*C*=ONH), 146.1 (*ipso*-Ar-*C*), 136.1 (*ipso*-Ar-*C*), 129.5 (Ar-*C*), 129.4 (Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 128.8 (Ar-*C*), 128.7 (Ar-*C*), 128.6 (Ar-*C*), 127.2 (Ar-*C*), 125.9 (*ipso*-Ar-*C*), 114.8 (Ar-*C*), 80.8 ((CH_3)$_3$*C*O), 54.5 (BocNH*C*H), 37.9 (Ar-*C*H_2), 28.3 ((*C*H_3)$_3$CO); ***m/z*** (ES^+) 412 ([^{35}Cl]MNa^+), 414 ([^{37}Cl]MNa^+); HRMS (ES^+) Found [^{35}Cl]MNa^+, 412.1395 ($C_{20}H_{24}N_3O_3{}^{35}ClNa$ requires 412.1404).

tert-Butyl (*S*)-(1-(2-(2,6-dichlorophenyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Ax



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.20 g, 0.75 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (0.19 g, 0.91 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a pale yellow solid (0.11 g, 34%); R_f 0.27 (DCM/EtOH/NH_3 200:8:1); m.p. 147 – 150 °C; υ_{max} 3337, 3303 (N-H), 2967 (C-H), 1691, 1669 (C=O), 1525, 1495, 1454, 1292, 1248, 1168, 764, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.17 (1H, bs, CON*H*NH), 7.23 – 7.21 (5H, m, Ar-*H*), 7.12-7.10 (2H, m, Ar-*H*), 6.92 (1H, t, *J* 8, Ar-*H*), 6.75 (1H, bd, Ar-N*H*), 4.91 (1H, bs, BocN*H*CH), 4.39 (1H, m, BocNHC*H*), 3.03 (2H, m, Ar-CH_2), 1.40 (9H, s, (CH_3)$_3$CO); δ_C (500 MHz, $CDCl_3$) 170.3 (*C*=ONHNH), 155.4 (*C*=ONH), 140.7 (*ipso*-Ar-*C*), 136.1 (*ipso*-Ar-*C*), 129.2 (Ar-*C*), 128.8 (Ar-*C*), 128.7 (Ar-*C*), 127.3 (Ar-*C*), 126.3 (Ar-*C*), 124.2 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 54.3 (BocNH*C*H), 37.9 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 424 ([^{35,35}Cl]MH$^+$), 426 ([^{35,37}Cl]MH$^+$), 428 ([^{37,37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MH$^+$, 424.1199 ($C_{20}H_{24}N_3O_3{}^{35,35}Cl$ requires 424.1195).

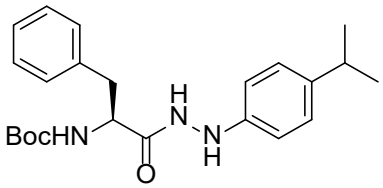
tert-Butyl (*S*)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Cd



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.20 g, 0.75 mmol) and 2,4-difluorophenylhydrazine hydrochloride (0.16 g, 0.91 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 400:8:1) into the title compound which was isolated as a light orange solid (0.13 g, 45%); R_f 0.35 (DCM/EtOH/NH_3 400:8:1); m.p. 132 – 138 °C; υ_{max} 3325 (N-H), 2974 (C-

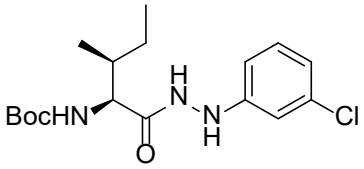
H), 1667, 1523, 1367, 1291, 1166, 960, 850, 703 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.84 (1H, bs, CON*H*NH), 7.33 – 7.28 (3H, m, Ar-*H*), 7.21-7.20 (2H, d, *J* 6, Ar-*H*), 6.78 (1H, dt, *J* 8, 2, Ar-*H*), 6.61 (1H, t, *J* 8, Ar-*H*), 6.40 (1H, m, Ar-*H*), 6.03 (1H, bs, Ar-N*H*), 5.07 (1H, bs, BocN*H*CH), 4.42 (1H, q, *J* 7, BocNHC*H*), 3.09 (2H, d, *J* 7, Ar-CH_2), 1.43 (9H, s, (CH_3)$_3$CO); δ_C (500 MHz, $CDCl_3$) 171.5 (*C*=ONHNH), 155.6 (*C*=ONH), 136.0 (*ipso*-Ar-*C*), 131.9 (*ipso*-Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.2 (Ar-*C*), 115.4 (Ar-*C*), 110.9 (Ar-*C*, dd, *J* 22, 4), 103.8 (Ar-*C*, dd, *J* 26, 22), 80.8 ((CH$_3$)$_3$*C*O), 54.6 (BocNH*C*H), 37.8 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); δ_F (500 MHz, $CDCl_3$) -120.7 (Ar-*F*), -129.2 (Ar-*F*); *m/z* (ES^+) 392 (MH^+); HRMS (ES^+) Found MH^+, 392.1785 ($C_{20}H_{24}N_3O_3F_2$ requires 392.1786).

tert-Butyl (*S*)-(1-(2-(4-isopropylphenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67El



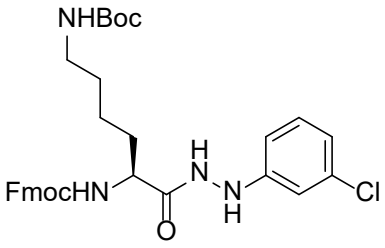
Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.30 g, 1.13 mmol) and 4-isopropylphenylhydrazine hydrochloride (0.23 g, 1.24 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.27 g, 60%); R_f 0.51 (DCM/EtOH/NH_3 200:8:1); m.p. 141 - 143 °C; ν_{max} 3319 (N-H), 2962 (C-H), 1688, 1656 (C=O), 1516, 1498, 1365, 1252, 1171, 825 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.72 (1H, bs, CON*H*NH), 7.33 – 7.30 (3H, m, Ar-*H*), 7.23 – 7.20 (2H, dd, *J* 7, 1, Ar-*H*), 7.03 – 7.00 (2H, d, *J* 8, Ar-*H*), 6.58 – 6.55 (2H, d, *J* 8, Ar-*H*), 5.04 (1H, bs, BocN*H*CH), 4.45 (1H, q, *J* 7, BocNHC*H*), 3.10 (2H, d, *J* 7, Ar-C*H*$_2$), 2.86 (1H, m, C*H*(CH$_3$)$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO), 1.20 – 1.18 (6H, d, *J* 6, CH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 155.6 (*C*=ONH), 145.4 (*ipso*-Ar-*C*), 136.4 (*ipso*-Ar-*C*), 129.4 (Ar-*C*), 128.7 (Ar-*C*), 128.6 (Ar-*C*), 128.4 (Ar-*C*), 127.0 (Ar-*C*), 126.9 (Ar-*C*), 113.7 (Ar-*C*), 80.4 ((CH$_3$)$_3$*C*O), 53.5 (BocNH*C*H), 38.4 (Ar-*C*H$_2$), 33.3 (*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.2 (CH(*C*H$_3$)$_2$); *m/z* (ES^+) 420 (MNa^+); HRMS (ES^+) Found MNa^+, 420.2262 ($C_{23}H_{31}N_3O_3Na$ requires 420.2258).

tert-Butyl ((2*S*,3*S*)-1-(2-(3-chlorophenyl)hydrazineyl)-3-methyl-1-oxopentan-2-yl)carbamate 67u



Following the general procedure outlined, *N*-Boc-*L*-isoleucine (0.20 g, 0.87 mmol) and 3-chlorophenylhydrazine hydrochloride (0.19 g, 1.04 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a pale yellow solid (0.21 g, 68%); R_f 0.61 (DCM/EtOH/NH_3 200:8:1); m.p. 117 - 119 °C; ν_{max} 3326, 3272 (N-H), 2964 (C-H), 1682, 1657 (C=O), 1519, 1470, 1243, 1168, 775 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.43 (1H, bs, CON*H*NH), 7.09 (1H, t, *J* 7, Ar-*H*), 6.84 (1H, d, *J* 7, Ar-*H*), 6.80 (1H, d, *J* 2, Ar-*H*), 6.69 (1H, d, *J* 7, Ar-*H*), 5.16 (1H, bd, *J* 8, BocN*H*CH), 4.01 (1H, t, *J* 8, BocNHC*H*), 1.88 (1H, m, NHCHC*H*), 1.61 (1H, m, CHC*H*HCH$_3$), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 1.16 (1H, m, CH(CH$_3$)CH*H*CH$_3$), 0.96 (3H, d, *J* 6, CH(C*H*$_3$)CH$_2$CH$_3$), 0.92 (3H, t, *J* 7, CH(CH$_3$)CH$_2$C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 156.1 (*C*=ONH), 149.2 (*ipso*-Ar-*C*), 135.0 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 121.1 (Ar-*C*), 113.6 (Ar-*C*), 111.8 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 57.8 (BocNH*C*H), 36.6 (NHCH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 24.8 (CH(CH$_3$)*C*H$_2$CH$_3$), 15.6 (CH(*C*H$_3$)CH$_2$CH$_3$), 11.1 (CH(CH$_3$)CH$_2$*C*H$_3$); *m/z* (ES$^+$) 378 ([^{35}Cl]MNa$^+$), 380 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 378.1539 ($C_{17}H_{26}N_3O_3{}^{35}ClNa$ requires 378.1560).

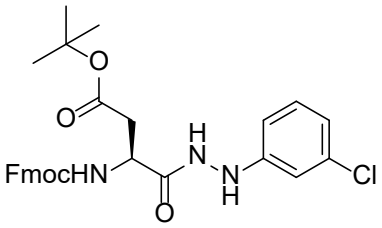
(9H-fluoren-9-yl)methyl tert-Butyl (6-(2-(3-chlorophenyl)hydrazinyl)-6-oxohexane-1,5-diyl)(*S*)-dicarbamate 68r



Following the general procedure outlined, *N*-Fmoc-*N*6-(tert-butoxycarbonyl)-*L*-lysine (0.20 g, 0.43 mmol) and 3-chlorophenylhydrazine hydrochloride (0.09 g, 0.51 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a pale yellow solid (0.24 g, 93%); R_f 0.50 (*n*-

hexane/EtOAc 4:1); m.p. 145 - 148 °C; ν_{max} 3288 (N-H), 2939 (C-H), 1683, 1647 (C=O), 1532, 1478, 1271, 1250, 1165, 737 cm^{-1}; δ_H (399 MHz, $CDCl_3$) 8.39 (1H, bs, CON*H*NH), 7.76 – 7.74 (2H, d, *J* 7, Ar-*H*), 7.57 – 7.55 (2H, d, *J* 7, Ar-*H*), 7.41 – 7.36 (2H, t, *J* 7, Ar-*H*), 7.31 – 7.28 (2H, d, *J* 7, Ar-*H*), 7.10 (1H, t, *J* 8, Ar-*H*), 6.84 – 6.77 (2H, m, Ar-*H*), 6.66 (1H, d, *J* 8, Ar-*H*), 6.18 (1H, bs, Ar-N*H*), 5.64 (1H, bs, FmocN*H*CH), 4.67 (1H, m, CO_2CH_2CH-Fluorene), 4.47 (1H, m, CO_2CH_2CH-Fluorene), 4.22 – 4.17 (2H, m, FmocNHC*H*, CO_2CH_2CH-Fluorene), 3.10 (2H, m, BocNHCH_2), 1.86 (1H, m, FmocNHCHC*H*H), 1.72 – 1.60 (3H, m, FmocNHCHCCH*H*, BocNHCH$_2$CH_2), 1.50 (2H, m, NHCHCH$_2$CH_2), 1.42 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 172.0 (*C*=ONHNH), 156.4 *(C*=ONH), 149.1 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 134.9 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 124.9 (Ar-*C*), 121.1 (Ar-*C*), 120.0 (Ar-*C*), 113.5 (Ar-*C*), 111.7 (Ar-*C*), 79.6 ((CH$_3$)$_3$*C*O), 67.2 (CO_2CH_2CH-Fluorene), 53.1 (FmocNH*C*H), 47.2 (*C*-Fluorene), 39.8 (BocNH*C*H$_2$), 31.4 (FmocNHCH*C*H$_2$), 29.6 (BocNHCH$_2$*C*H$_2$), 28.6 ((*C*H$_3$)$_3$CO), 22.5 (NHCHCH$_2$*C*H$_2$); *m/z* (ES^+) 615 ([^{35}Cl]MNa^+), 617 ([^{37}Cl]MNa^+); HRMS (ES^+) Found [^{35}Cl]MNa^+, 615.2349 ($C_{32}H_{37}{}^{35}ClN_4O_5Na$ requires 615.2350).

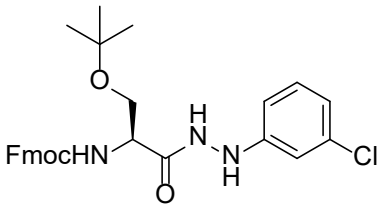
tert-Butyl(*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(2-(3-chlorophenyl)hydrazinyl)-5-oxopentanoate 68m



Following the general procedure outlined, *N*-Fmoc-*O*-(tert-butyl)-*L*-aspartic acid (0.30 g, 0.73 mmol) and 3-chlorophenylhydrazine hydrochloride (0.14 g, 0.80 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.22 g, 57%); R_f 0.72 (n-hex/EtOAc 4:1); m.p. 131 - 133 °C; υmax 3284 (N-H), 1692, 1667 (C=O), 1543, 1478, 1269, 1165, 1049, 737 cm-1; δ_H (300 MHz, DMSO-d_6) 9.94 (1H, bd, *J* 2, CON*H*NH), 8.05 (1H, bd, *J* 2, CON*H*CH), 7.91 – 7.89 (2H, d, *J* 7, Ar-*H*), 7.75 – 7.71 (2H, m, Ar-*H*), 7.45 – 7.40 (2H, td, *J* 7,1, Ar-*H*), 7.36 – 7.30 (2H, td, *J* 7, 1, Ar-*H*), 7.13 (1H, t, *J* 7, Ar-

H), 6.85 (1H, m, Ar-*H*), 6.76 (1H, m, Ar-*H*), 6.71 (1H, m, Ar-*H*), 4.49 (1H, m, FmocNHC*H*), 4.35 (2H, m, CO_2CH_2CH-Fluorene), 4.28 (1H, m, CO_2CH_2CH-Fluorene), 2.77 (1H, m, CHC*H*HCO_2), 2.59 (1H, m, CHCH*H*CO_2), 1.41 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, DMSO-d_6) 170.8 (*C*=ONHNH), 156.5 *(C*=ONH), 148.9 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 141.4 (*ipso*-Ar-*C)*, 135.0 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 127.9 (Ar-*C*), 127.2 (Ar-*C*), 124.9 (Ar-*C*), 121.2 (Ar-*C*), 120.1 (Ar-*C*), 113.5 (Ar-*C*), 111.8 (Ar-*C*), 82.3 ($(CH_3)_3CO$), 67.3 (CO_2CH_2CH-Fluorene), 50.2 (FmocNH*C*H), 47.2 (*C*-Fluorene), 36.9 ($CHCH_2CO_2$), 28.1 ($(CH_3)_3CO$); *m/z* (ES^+) 536 ($[^{35}Cl]MH^+$), 538 ($[^{37}Cl]MH^+$), 558 ($[^{35}Cl]MNa^+$), 560 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 536.1978 ($C_{29}H_{32}{}^{35}ClN_3O_5$ requires 536.1947).

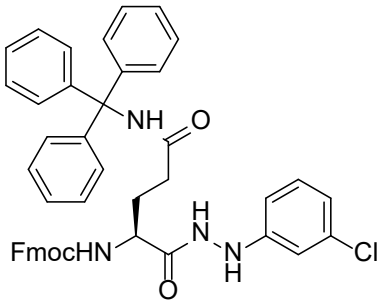
(9H-fluoren-9-yl)methyl (*S*)-(3-(tert-butoxy)-1-(2-(3-chlorophenyl)hydrazinyl) -1-oxopropan-2-yl)carbamate 68k



Following the general procedure outlined, *N*-Fmoc-*O*-(tert-butyl)-*L*-serine (0.30 g, 0.78 mmol) and 3-chlorophenylhydrazine hydrochloride (0.15 g, 0.86 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange solid (0.29 g, 72%); R_f 0.83 (*n*-hexane/EtOAc 4:1); m.p. 141 - 144 °C ; ν_{max} 3325, 3273 (N-H), 1687, 1667 (C=O), 1531, 1296, 1035, 756, 737 cm^{-1}; δ_H (399 MHz, $CDCl_3$) 8.12 (1H, bs, CON*H*NH), 7.77 – 7.75 (2H, d, *J* 7, Ar-*H*), 7.59 – 7.58 (2H, d, *J* 7, Ar-*H*), 7.43 – 7.37 (2H, q, *J* 7, 5, Ar-*H*), 7.33 – 7.29 (2H, m, Ar-*H*), 7.12 (1H, t, *J* 7, Ar-*H*), 6.87 – 6.84 (2H, m, Ar-*H*), 6.73 (1H, d, *J* 7, Ar-*H*), 6.10 (1H, bs, Ar-N*H*), 5.68 (1H, bd, *J* 5, FmocN*H*CH), 4.49 (2H, m, CO_2CH_2CH-Fluorene), 4.36 (1H, m, FmocNHC*H*), 4.23 (1H, t, *J* 6, CO_2CH_2CH-Fluorene), 3.84 (1H, m, FmocNHCHC*H*H), 3.49 (1H, m, FmocNHCHC*H*H), 1.23 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 170.7 (*C*=ONHNH), 156.1 (*C*=ONH), 148.9 (*ipso*-Ar-*C*), 143.7 (*ipso*-Ar-*C)*, 141.4 (*ipso*-Ar-*C)*, 135.1 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 124.9 (Ar-*C*), 121.2 (Ar-*C*), 120.1 (Ar-*C*), 113.5 (Ar-*C*), 111.7 (Ar-*C*), 74.6 ($(CH_3)_3CO$), 67.2 (CO_2CH_2CH-Fluorene), 61.5 (FmocNHCHCH_2), 54.1 (FmocNH*C*H), 47.2 (*C*-Fluorene), 27.5 ($(CH_3)_3CO$); *m/z*

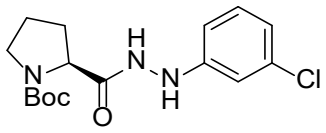
(ES$^+$) 530 ([^{35}Cl]MNa$^+$), 532 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 530.1819 ($C_{28}H_{30}N_3O_4{}^{35}ClNa$ requires 530.1823).

(9H-fluoren-9-yl)methyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-1,5-dioxo-5-(tritylamino)pentan-2-yl)carbamate 68q



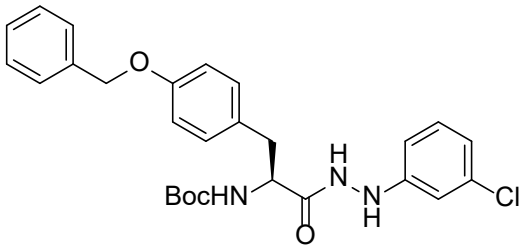
Following the general procedure outlined, *N*-Fmoc-*N*-(trityl)-*L*-glutamine (0.30 g, 0.49 mmol) and 3-chlorophenylhydrazine hydrochloride (0.09 g, 0.54 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.31 g, 85%); R_f 0.69 (n-hex/EtOAc 4:1); m.p. 115 - 118 °C; υmax 3246, 3062 (N-H), 1659 (C=O), 1598, 1489, 1231, 1035, 740, 698 cm-1; δ_H (300 MHz, $CDCl_3$) 8.56 (1H, bs, CON*H*NH), 7.73 – 7.71 (2H, d, *J* 7, Ar-*H*), 7.54 – 7.52 (3H, d, *J* 7, Ar-*H*), 7.38 – 7.33 (3H, t, *J* 7, Ar-*H*), 7.27 – 7.18 (15H, m, Ar-*H*), 7.04 (1H, m, Ar-*H*), 6.79 (1H, d, *J* 7, Ar-*H*), 6.66 (1H, s, Ar-*H*), 6.53 (1H, d, *J* 7, Ar-*H*), 5.98 (1H, bd, *J* 7, FmocN*H*CH), 4.39 (2H, m, CO_2CH_2CH-Fluorene), 4.18 – 4.04 (2H, m, CO_2CH_2CH-Fluorene, FmocNHC*H*), 2.49 (2H, m, $CHCH_2CH_2CO$), 2.03 (1H, m, CHC*H*HCH_2CO), 1.92 (1H, m, $CHCHHCH_2CO$); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONH(trityl)), 171.7 (*C*=ONHNH), 156.5 *(C*=ONH), 149.0 (*ipso*-Ar-*C)*, 144.3 (*ipso*-Ar-*C)*, 143.8 (*ipso*-Ar-*C)*, 143.7 (*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 134.9 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 128.7 (Ar-*C*), 128.0 (Ar-*C*), 127.8 (Ar-*C*), 127.2 (Ar-*C*), 127.1 (Ar-*C*), 125.1 (Ar-*C*), 120.9 (Ar-*C*), 120.0 (Ar-*C*), 113.4 (Ar-*C*), 111.6 (Ar-*C*), 70.7 (*C*-(trityl), 67.1 (CO_2CH_2CH-Fluorene), 52.7 (FmocNH*C*H), 47.2 (*C*-Fluorene), 33.3 ($CHCH_2CH_2CO$), 29.2 ($CHCH_2CH_2CO$); *m/z* (ES$^+$) 757 ([^{35}Cl]MNa$^+$), 759 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 757.2456 ($C_{45}H_{39}{}^{35}ClN_4O_4Na$ requires 757.2552).

tert-Butyl (*S*)-2-(2-(3-chlorophenyl)hydrazine-1-carbonyl)pyrrolidine-1-carboxylate 67v



Following the general procedure outlined, *N*-Boc-*L*-proline (0.30 g, 1.39 mmol) and 3-chlorophenylhydrazine hydrochloride (0.27 g, 1.53 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.29 g, 61%); R_f 0.75 (n-hexane/EtOAc 4:1); m.p. 158 - 160 °C; ν_{max} 3282 (N-H), 2977, 2874 (C-H), 1697, 1661 (C=O), 1598, 1406, 1395, 1162, 1133, 780, 770 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.84 (1H, bs, CON*H*NH), 7.14 (1H, t, *J* 8, Ar-*H*), 6.85 (1H, d, *J* 8, Ar-*H*), 6.80 (1H, s, Ar-*H*), 6.71 (1H, dd, *J* 8, 2, Ar-*H*), 6.12 (1H, bs, Ar-N*H*), 4.39 (1H, d, *J* 6, CH_2BocNC*H*), 3.45 (2H, m, C*H*$_2$BocNCH), 2.38 (1H, m, BocNCHC*H*$_2$), 1.94 (3H, m, BocNCHC*H*$_2$, CHCH$_2$C*H*$_2$), 1.52 (9H, s, (C*H*$_3$)$_3$CO) δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C)*, 135.1 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 120.9 (Ar-*C*), 113.4 (Ar-*C*), 111.8 (Ar-*C*), 81.0 ((CH$_3$)$_3$*C*O), 58.4 (CH$_2$BocN*C*H), 47.2 (*C*H$_2$BocNCH) 28.5 ((*C*H$_3$)$_3$CO), 27.6 (BocNCH*C*H$_2$), 24.8 (CHCH$_2$*C*H$_2$); *m/z* (ES$^+$) 362 ([^{35}Cl]MNa$^+$), 364 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 362.1243 ($C_{16}H_{22}{}^{35}ClN_3O_3Na$ requires 362.1247).

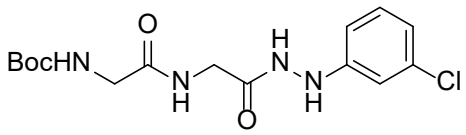
tert-Butyl(*S*)-(3-(4-(benzyloxy)phenyl)-1-(2-(3-chlorophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate 67z



Following the general procedure outlined, *N*-Boc-*O*-benzyl-*L*-tyrosine (0.20 g, 0.54 mmol) and 3-chlorophenylhydrazine hydrochloride (0.16 g, 0. 65 mmol) were transformed following trituration with DCM into the title compound which was isolated as a white solid (0.22 g, 83%); R_f 0.52 (DCM/EtOH/NH_3 200:8:1); m.p. 133 - 135 °C; ν_{max} 3347, 3243 (N-H), 2985 (C-H), 1676 (C=O), 1518, 1238, 1167, 1015, 748 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.91 (1H, bs, CON*H*NH), 7.45 – 7.33 (5H,

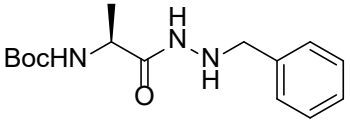
m, Ar-*H*), 7.13 – 7.10 (2H, d, *J* 8, Ar-*H*), 7.07 (1H, t, *J* 8, Ar-*H*), 6.94 – 6.91 (2H, d, *J* 8, Ar-*H*), 6.84 (1H, d, *J* 8, Ar-*H*), 6.66 (1H, s, Ar-*H*), 6.46 (1H, d, *J* 8, Ar-*H*), 6.04 (1H, bs, CONHN*H*), 5.09 – 5.02 (3H, m, BocN*H*CH, Ar-CH_2O), 4.42 (1H, q, *J* 7, BocNHC*H*), 3.04 (2H, m, Ar-CH_2CH), 1.44 (9H, s, (CH_3)$_3$CO); δ_C (75 MHz, $CDCl_3$) 171.8 (*C*=ONHNH), 157.9 (*C*=ONH), 148.8 (*ipso*-Ar-*C*), 130.0 (Ar-*C*), 130.2 (Ar-*C*), 128.2 (Ar-*C*), 128.1 (Ar-*C*), 127.5 (Ar-*C*), 121.2 (Ar-*C*), 115.2 (Ar-*C*), 113.4 (Ar-*C*), 111.8 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 70.0 (Ar-*C*H$_2$O), 54.6 (BocNH*C*H), 36.9 (Ar-*C*H$_2$CH), 28.3 ((*C*H$_3$)$_3$CO); ***m/z*** (ES$^+$) 518 ([^{35}Cl]MNa$^+$), 520 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 518.1838 ($C_{27}H_{30}N_3O_4{}^{35}ClNa$ requires 518.1823).

tert-Butyl (2-((2-(2-(3-chlorophenyl)hydrazinyl)-2-oxoethyl)amino)-2-oxoethyl)carbamate 67Aa



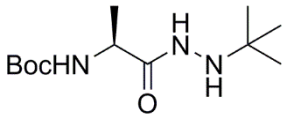
Following the general procedure outlined, *N*-Boc-glycylglycine (0.30 g, 1.29 mmol) and 3-chlorophenylhydrazine hydrochloride (0.25 g, 1.42 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.11 g, 24%); R_f 0.17 (*n*-hexane/EtOAc 4:1); m.p. 174 - 179 °C; ν_{max} 3314, 3274 (N-H), 3054, 2983 (C-H), 1653 (C=O), 1597, 1548, 1307, 1235, 1169, 947, 664 cm^{-1}; δ_H (399 MHz, DMSO-d6) 9.65 (1H, bs, CON*H*NH), 8.22 (1H, m, CON*H*CH$_2$), 7.15 (1H, t, *J* 7, Ar-*H*), 7.05 (1H, t, *J* 6, Ar-*H*), 6.72 (1H, s, Ar-*H*), 6.70 (1H, m, Ar-*H*), 3.82 (2H, t, *J* 6, CONHC*H*$_2$), 3.62 (1H, t, *J* 6, BocNHC*H*$_2$), 1.39 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, DMSO-d6) 170.4 (*C*=ONHNH), 169.3 *(C*=ONH), 156.4 *(C*=ONH), 151.3 (*ipso*-Ar-*C)*, 133.9 (*ipso*-Ar-*C*), 130.7 (Ar-*C*), 118.3 (Ar-*C*), 111.9 (Ar-*C*), 111.3 (Ar-*C*), 78.7 ((CH$_3$)$_3$*C*O), 43.9 (BocNH*C*H$_2$), 41.5 (CONH*C*H$_2$), 28.7 ((*C*H$_3$)$_3$CO); ***m/z*** (ES$^+$) 379 ([^{35}Cl]MNa$^+$), 381 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 379.1150 ($C_{15}H_{21}{}^{35}ClN_4O_4Na$ requires 379.1149).

tert-Butyl (*S*)-(1-(2-benzylhydrazinyl)-1-oxopropan-2-yl)carbamate 91



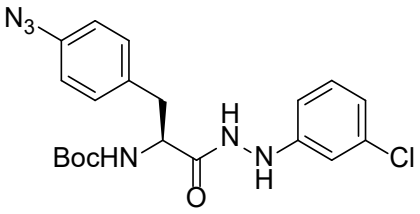
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and benzylhydrazine hydrochloride (0.25 g, 1.27 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light yellow solid (0.084 g, 27%); R_f 0.43 (DCM/EtOH/NH_3 100:8:1); m.p. 112 - 114 °C; υ_{max} 3324, 3000 (N-H), 2911 (C-H), 1689 (C=O), 1651 (C=O), 1523, 1491, 1249, 1162, 1053, 746, 701 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.58 (1H, bs, N*H*), 7.35 – 7.32 (5H, m, Ar-*H*), 7.28 (1H, m, Ar-*H*), 4.89 (1H, bs, BocN*H*CH), 4.09 (1H, m, BocNHC*H*), 3.97 (2H, s, CH_2Ar),1.42 (9H, s, (CH_3)$_3$CO) 1.33 (3H, d, *J* 7, NHCHCH_3); δ_C (700 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 155.4 *(C*=ONH), 137.3 (*ipso*-Ar-*C)*, 128.9 (Ar-*C)*, 128.8 (Ar-*C)*, 128.5 (Ar-*C)*, 127.6 (Ar-*C)*, 80.3 ((CH$_3$)$_3$*C*O), 55.8 (*C*H$_2$Ar), 48.8 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 18.1 (NHCH*C*H$_3$); *m/z* (ES^+) 294 (MH^+); HRMS (ES^+) Found MH^+, 294.1819 ($C_{15}H_{24}N_3O_3$ requires 294.1818).

tert-Butyl (*S*)-(1-(2-(tert-butyl)hydrazinyl)-1-oxopropan-2-yl)carbamate 89



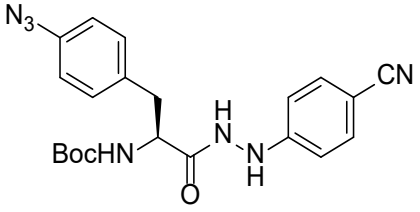
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.20 g, 1.06 mmol) and *tert*-butylhydrazine hydrochloride (0.16 g, 1.27 mmol) were transformed following work up with EtOAc into the title compound which was isolated as a white solid (0.11 g, 40%); m.p. 133 - 135 °C; υ_{max} 3380(N-H), 2973(C-H), 1681 (C=O), 1642 (C=O), 1530, 1365, 1248, 1168, 1044, 828, 764, 634 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.55 (1H, bs, N*H*), 4.95 (1H, bs, N*H*), 4.17 (1H, m, BocNHC*H*), 1.47 (9H, s, (CH_3)$_3$CO), 1.40 (3H, d, *J* 8, NHCHCH_3), 1.10 (9H, s, (CH_3)$_3$CNH); δ_C (500 MHz, $CDCl_3$) 171.4 (*C*=ONHNH), 155.6 *(C*=ONH), 80.4 ((CH$_3$)$_3$*C*O), 55.2 ((CH$_3$)$_3$*C*NH), 48.9 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO), 27.1 ((*C*H$_3$)$_3$CNH), 17.7 (NHCH*C*H$_3$); *m/z* (ES^+) 260 (MH^+); HRMS (ES^+) Found MH^+, 260.1985 ($C_{12}H_{26}N_3O_3$ requires 260. 1974).

tert-Butyl (*S*)-(3-(4-azidophenyl)-1-(2-(3-chlorophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate 67En



Following the general procedure outlined, *N*-Boc-*L*-4-azidophenylalanine (0.20 g, 0.65 mmol) and 3-chlorophenylhydrazine hydrochloride (0.14 g, 0.78 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a pale yellow solid (0.14 g, 49%); R_f 0.35 (DCM/EtOH/NH_3 200:8:1); m.p. 153 - 156 °C; υ_{max} 3326, 3242 (N-H), 2099 (N_3), 1672 (C=O), 1595, 1529, 1272, 1168, 1050, 861, 771 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.96 (1H, bs, N*H*), 7.21 – 7.20 (2H, d, *J* 8, Ar-*H*), 7.10 (1H, t, *J* 8, Ar-*H*), 7.01 – 7.00 (2H, d, *J* 8, Ar-*H*), 6.88 (1H, d, *J* 8, Ar-*H*), 6.64 (1H, s, Ar-*H*), 6.54 (1H, d, *J* 8, Ar-*H*), 6.07 (1H, bs, Ar-N*H*), 5.08 (1H, bd, *J* 8, BocN*H*CH), 4.43 (1H, q, *J* 7, BocNHC*H*), 3.09 (2H, m, Ar-C*H*$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO); δ_C (700 MHz, $CDCl_3$) 171.4 (*C*=ONHNH), 155.4 (*C*=ONH), 148.8 (*ipso*-Ar-*C*), 139.1 (*ipso*-Ar-*C*), 135.0 (*ipso*-Ar-*C*), 132.7 (*ipso*-Ar-*C*), 130.7 (Ar-*C*), 130.2 (Ar-*C*), 121.3 (Ar-*C*), 119.5 (Ar-*C*), 119.1 (Ar-*C*), 113.4 (Ar-*C*), 111.8 (Ar-*C*), 110.9 (Ar-*C*), 81.0 ((CH$_3$)$_3$*C*O), 54.4 (BocNH*C*H), 37.0 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 431 ([^{35}Cl]MH$^+$), 433 ([^{37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35}Cl]MH$^+$, 431.1592 ($C_{20}H_{24}N_6O_3{}^{35}Cl$ requires 431.1598).

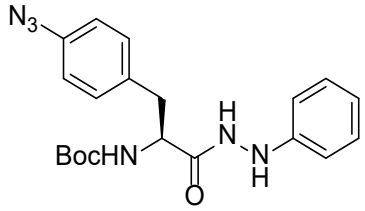
tert-Butyl (*S*)-(3-(4-azidophenyl)-1-(2-(4-cyanophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate 67Eo



Following the general procedure outlined, *N*-Boc-*L*-4-azidophenylalanine (0.20 g, 0.65 mmol) and 4-cyanophenylhydrazine hydrochloride (0.13 g, 0.78 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a pale yellow solid (0.06 g, 23%); R_f 0.31 (DCM/EtOH/NH_3 200:8:1); m.p. 171 - 174 °C; υ_{max} 3302 (N-H), 2218 (CN), 2104 (N_3), 1689, 1655 (C=O),

1507, 1368, 1293, 1163, 1050, 832, 542 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 7.83 (1H, bs, N*H*), 7.46 – 7.44 (2H, dd, *J* 8, 1, Ar-*H*), 7.22-7.20 (2H, dd, *J* 8, 1, Ar-*H*), 7.00-6.99 (2H, dd, *J* 8, 2, Ar-*H*), 6.65-6.64 (2H, d, *J* 8, Ar-*H*), 6.15 (1H, bs, Ar-N*H*), 4.96 (1H, bs, BocN*H*CH), 4.36 (1H, m, BocNHC*H*), 3.11 (1H, m, Ar-CH_2), 3.06 (1H, m, Ar-CH_2), 1.46 (9H, s, (CH_3)$_3$CO); δ_C (700 MHz, $CDCl_3$) 171.4 (*C*=ONHNH), 151.0 (*C*=ONH), 143.1 (*ipso*-Ar-*C*), 134.3 (*ipso*-Ar-*C*), 133.6 (Ar-*C*), 133.4 (Ar-*C*), 130.8 (Ar-*C*), 129.3 (Ar-*C*), 119.4 (Ar-*C*), 119.3 (Ar-*C*), 116.3 (Ar-*C*), 113.1 (Ar-*C*),.4 ((CH$_3$)$_3$*C*O), 54.3 (BocNH*C*H), 36.6 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES^+) 422 (MH^+); HRMS (ES^+) Found MH^+, 422.1948 ($C_{21}H_{24}N_7O_3$ requires 422.1941).

tert-Butyl (*S*)-(3-(4-azidophenyl)-1-oxo-1-(2-phenylhydrazinyl)propan-2-yl) carbamate 67Em

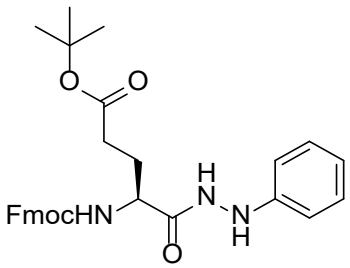


Following the general procedure outlined, *N*-Boc-*L*-4-azidophenylalanine (0.20 g, 0.65 mmol) and phenylhydrazine (0.07 mL, 0.72 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 120:6:1) into the title compound which was isolated as a brown gum (0.20 g, 77%); R_f 0.34 (DCM/EtOH/NH_3 200:8:1); m.p. 92 - 95 °C; υ_{max} 3300 (N-H), 2926 (C-H), 2111 (N_3), 1685, 1659 (C=O), 1505, 1366, 1289, 1166, 1048, 752, 691 cm^{-1}; δ_H (700 MHz, $CDCl_3$) 8.09 (1H, bs, N*H*), 7.18 – 7.14 (3H, m, Ar-*H*), 6.94 – 6.93 (2H, d, *J* 8, Ar-*H*), 6.89 – 6.87 (2H, m, Ar-*H*), 6.60 – 6.59 (2H, d, *J* 8, Ar-*H*), 5.15 (1H, bd, *J* 8, BocN*H*CH), 4.43 (1H, m, BocNHC*H*), 3.08 (1H, m, Ar-CH_2), 3.02 (1H, m, Ar-CH_2), 1.44 (9H, s, (CH_3)$_3$CO); δ_C (700 MHz, $CDCl_3$) 171.3 (*C*=ONHNH), 155.6 (*C*=ONH), 147.4 (*ipso*-Ar-*C*), 138.9 (*ipso*-Ar-*C*), 132.9 (*ipso*-Ar-*C*), 130.8 (Ar-*C*), 129.1 (Ar-*C*), 121.3 (Ar-*C*), 119.3 (Ar-*C*), 119.0 (Ar-*C*), 115.4 (Ar-*C*), 113.5 (Ar-*C*), 112.8 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 54.4 (BocNH*C*H), 37.4 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES^+) 397 (MH^+); HRMS (ES^+) Found MH^+, 397.1971 ($C_{20}H_{25}N_6O_3$ requires 397.1988).

6.1.2.3 Synthesis of *N*-protected Hydrazides – General Procedure

Under a nitrogen atmosphere *N*-protected amino acid (1 equiv.) dissolved in THF (5 mL) at room temperature and treated with DIPEA (1.2 equiv.) followed by HBTU (1.2 equiv.). After stirring for 10 minutes at room temperature addition of N-aryl hydrazine (1.1 equiv.) continued stirring for another 5 hours at room temperature. Reaction mixed with diethyl ether (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in vacuo achieving the desired *N*-Boc hydrazides **67** or *N*-Fmoc hydrazides **68**.

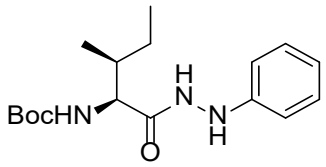
tert-Butyl (*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-oxo-5-(2-phenylhydrazineyl)pentanoate 68d



Following the general procedure outlined, *N*-Fmoc-*O*-(tert-butyl)-*L*-glutamic acid (0.40 g, 0.94 mmol) and phenylhydrazine (0.10 mL, 1.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.26 g, 53%); R_f 0.46 (DCM/EtOH/NH_3 400:6:1); m.p. 113 - 116 °C; υmax 3287 (N-H), 2969 (C-H), 1691, 1658 (C=O), 1539, 1497, 1257, 1152, 1085, 738, 692 cm-1; δ_H (300 MHz, $CDCl_3$) 8.38 (1H, bs, CON*H*NH), 7.79 – 7.76 (2H, d, *J* 7, Ar-*H*), 7.60 – 7.58 (2H, d, *J* 7, Ar-*H*), 7.44 – 7.39 (2H, t, *J* 7, Ar-*H*), 7.34 – 7.31 (2H, t, *J* 7, 1, Ar-*H*), 7.24 – 7.19 (2H, t, *J* 7, Ar-*H*), 6.93 (1H, t, *J* 7, Ar-*H*), 6.83 – 8.81 (1H, d, *J* 7, Ar-*H*), 5.85 (1H, bd, *J* 7, FmocN*H*CH), 4.46 (2H, m, CO_2CH_2CH-Fluorene), 4.39 (1H, m, CO_2CH_2CH-Fluorene), 4.25 (1H, m, FmocNHC*H*), 2.50 (2H, m, $CHCH_2CH_2CO_2$), 2.19 (1H, m, $CHCHHCH_2CO_2$), 2.06 (1H, m, $CHCHHCH_2CO_2$), 1.49 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.8 (*C*=OO(CH_3)$_3$), 171.5 (*C*=ONHNH), 156.8 *(C*=ONH), 147.6 (*ipso*-Ar-*C)*, 143.7

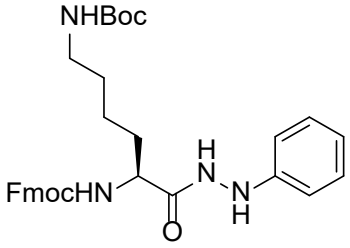
(*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 129.2 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 125.0 (Ar-*C*), 121.3 (Ar-*C*), 120.0 (Ar-*C*), 113.6 (Ar-*C*), 81.3 ($(CH_3)_3CO$), 67.2 (CO_2CH_2CH-Fluorene), 53.1 (FmocNH*C*H), 47.1 (*C*-Fluorene), 31.6 ($CHCH_2CH_2CO_2$), 28.1 ($(CH_3)_3CO$), 27.2 ($CHCH_2CH_2CO_2$); *m/z* (ES^+) 538 (MNa^+); HRMS (ES^+) Found MNa^+, 538.2327 ($C_{30}H_{33}N_3O_5Na$ requires 538.2312).

tert-Butyl ((2*S*,3*S*)-3-methyl-1-oxo-1-(2-phenylhydrazineyl)pentan-2-yl) carbamate 67e



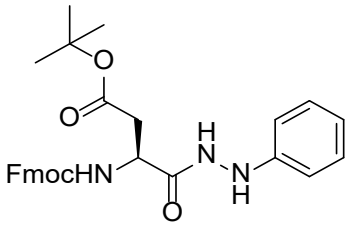
Following the general procedure outlined, *N*-Boc-*L*-isoleucine (0.30 g, 1.29 mmol) and phenylhydrazine (0.14 g, 1.43 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown solid (0.39 g, 93%); R_f 0.43 (DCM/EtOH/NH_3 200:6:1); m.p. 142 - 145 °C; ν_{max} 3320, 3270 (N-H), 2962 (C-H), 1686, 1658 (C=O), 1522, 1494, 1292, 1170, 769, 692 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.46 (1H, bs, CON*H*NH), 7.20 – 7.15 (2H, t, *J* 7, Ar-*H*), 6.89 (1H, t, *J* 7, Ar-*H*), 6.82 – 6.78 (2H, m, Ar-*H*), 6.20 (1H, bs, Ar-N*H*), 5.22 (1H, bd, *J* 9, BocN*H*CH), 4.05 (1H, t, *J* 8, BocNHC*H*), 1.89 (1H, m, NHCHC*H*), 1.55 (1H, m, $CH(CH_3)CHHCH_3$), 1.45 (9H, s, $(CH_3)_3CO$), 1.18 (1H, m, $CH(CH_3)CHHCH_3$), 0.94 – 0.86 (6H, m, $CH(CH_3)CH_2CH_3$, $CH(CH_3)CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 156.1 (*C*=ONH), 147.8 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 121.1 (Ar-*C*), 113.6 (Ar-*C*), 80.3 ($(CH_3)_3CO$), 57.8 (BocNH*C*H), 36.7 (NHCH*C*H), 28.3 ($(CH_3)_3CO$), 24.8 ($CH(CH_3)CH_2CH_3$), 15.6 ($CH(CH_3)CH_2CH_3$), 11.1 ($CH(CH_3)CH_2CH_3$); *m/z* (ES^+) 344 (MNa^+); HRMS (ES^+) Found MNa^+, 344.1966 ($C_{17}H_{27}N_3O_3Na$ requires 344.1945).

(9H-fluoren-9-yl)methyl tert-Butyl (6-oxo-6-(2-phenylhydrazineyl)hexane-1,5-diyl)(*S*)-dicarbamate 68h



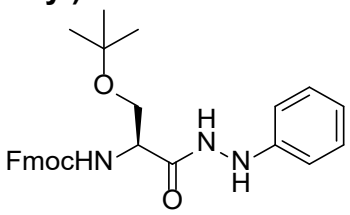
Following the general procedure outlined, *N*-Fmoc-N6-(tert-butoxycarbonyl)-*L*-lysine (0.40 g, 0.85 mmol) and phenylhydrazine (0.10 g, 0.94 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.24 g, 51%); R_f 0.37 (DCM/EtOH/NH_3 200:6:1); m.p. 125 - 128 °C; ν_{max} 3222, 3300 (N-H), 1687, 1655 (C=O), 1527, 1487, 1362, 1252, 1159, 764, 685 cm^{-1}; δ_H (399 MHz, $CDCl_3$) 8.34 (1H, bs, CON*H*NH), 7.76 – 7.74 (2H, d, *J* 7, Ar-*H*), 7.57 – 7.55 (2H, d, *J* 7, Ar-*H*), 7.41 – 7.36 (2H, t, *J* 7, Ar-*H*), 7.31 – 7.28 (2H, d, *J* 7, Ar-*H*), 7.20 – 7.15 (2H, t, *J* 7, Ar-*H*), 6.89 (1H, t, *J* 7, Ar-*H*), 6.79 – 6.76 (2H, d, *J* 7, Ar-*H*), 5.63 (1H, bd, *J* 7, FmocN*H*CH), 4.64 (1H, m, BocN*H*CH_2), 4.45 (2H, m, CO_2C*H*$_2$CH-Fluorene), 4.25 – 4.17 (2H, m, FmocNHC*H*, CO_2CH_2C*H*-Fluorene), 3.09 (2H, m, BocNHC*H*$_2$), 1.71 (1H, m, FmocNHCHC*H*H), 1.63 (1H, m, FmocNHCHCH*H*), 1.51 (2H, m, BocNHCH$_2$C*H*$_2$), 1.43 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 156.3 *(C*=ONH), 147.7 (*ipso*-Ar-*C)*, 143.7 (*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 129.2 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 125.0 (Ar-*C*), 121.3 (Ar-*C*), 120.0 (Ar-*C*), 113.6 (Ar-*C*), 79.4 ((CH$_3$)$_3$*C*O), 67.1 (CO_2*C*H$_2$CH-Fluorene), 53.4 (FmocNH*C*H), 47.2 (*C*-Fluorene), 39.7 (BocNH*C*H_2), 31.6 (FmocNHCH*C*H$_2$), 29.6 (BocNHCH$_2$*C*H$_2$), 28.5 ((*C*H$_3$)$_3$CO), 22.5 (NHCHCH$_2$*C*H$_2$); *m/z* (ES^+) 581 (MNa^+); HRMS (ES^+) Found MNa^+, 581.2764 ($C_{32}H_{38}N_4O_5Na$ requires 581.2762).

tert-Butyl(*S*)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(2-phenylhydrazineyl)butanoate 68c



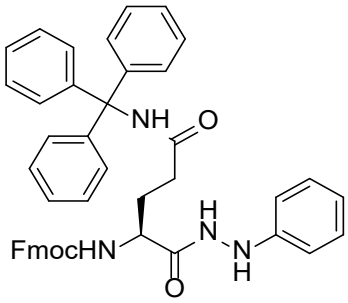
Following the general procedure outlined, *N*-Fmoc-*O*-(tert-butyl)-*L*-aspartic acid (0.40 g, 0.97 mmol) and phenylhydrazine (0.11 mL, 1.07 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.20 g, 41%); R_f 0.46 (DCM/EtOH/NH_3 200:6:1); m.p. 158 - 161 °C; υmax 3287 (N-H), 1690, 1656 (C=O), 1534, 1496, 1266, 1159, 1046, 738, 692 cm-1; δ_H (300 MHz, $CDCl_3$), 8.26 (1H, bs, CON*H*NH), 7.76 – 7.74 (2H, d, *J* 7, Ar-*H*), 7.58 – 7.55 (2H, d, *J* 7, Ar-*H*), 7.42 – 7.35 (2H, td, *J* 7, 3, Ar-*H*), 7.32 – 7.26 (2H, m, Ar-*H*), 7.20 – 7.15 (2H, t, *J* 7, Ar-*H*), 6.89 (1H, t, *J* 7, Ar-*H*), 6.80 – 6.77 (1H, d, *J* 7, Ar-*H*), 6.02 (1H, m, FmocN*H*CH), 4.62 (1H, m, FmocNHC*H*), 4.53 (2H, m, CO_2CH_2CH-Fluorene), 4.22 (1H, t, *J* 6, CO_2CH_2CH-Fluorene), 2.92 (1H, dd, *J* 16, 4, CHC*H*HCO_2), 2.70 (1H, dd, *J* 16, 6, CHCH*H*CO_2), 1.44 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.9 (*C*=O),170.7 (*C*=ONHNH), 156.2 *(C*=ONH), 147.6 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 143.5 (*ipso*-Ar-*C)*, 141.4 (*ipso*-Ar-*C*), 129.2 (Ar-*C*), 127.9 (Ar-*C*), 127.8 (Ar-*C*), 127.2 (Ar-*C*), 127.1 (Ar-*C*), 124.9 (Ar-*C*), 121.3 (Ar-*C*), 120.1 (Ar-*C*), 120.0 (Ar-*C*), 113.6 (Ar-*C*), 82.3 ((CH_3)$_3$*C*O), 67.3 (CO_2*C*H_2CH-Fluorene), 50.2 (FmocNH*C*H), 47.2 (*C*-Fluorene), 37.1 (CH*C*H_2CO_2), 28.1 ((*C*H_3)$_3$CO); *m/z* (ES^+) 524 (MNa^+), 1003 ($2M+H^+$), 1025 ($2M+Na^+$); HRMS (ES^+) Found MNa^+, 524.2174 ($C_{29}H_{31}N_3O_5Na$ requires 524.2156).

(9H-fluoren-9-yl)methyl (*S*)-(3-(tert-butoxy)-1-oxo-1-(2-phenylhydrazineyl)propan-2-yl)carbamate 68a



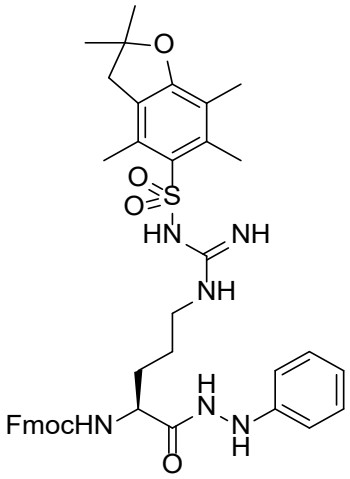
Following the general procedure outlined, *N*-Fmoc-*O*-(tert-butyl)-*L*-serine (0.40 g, 1.04 mmol) and phenylhydrazine (0.11 mL, 1.15 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a light brown solid (0.31 g, 63%); R_f 0.51 (DCM/EtOH/NH_3 200:6:1); m.p. 140 - 142 °C; ν_{max} 3388 (N-H), 2979 (C-H), 1693, 1651 (C=O), 1543, 1233, 11193, 1032, 756, 738 cm^{-1}; δ_H (300 MHz, DMSO-d6) 9.81 (1H, bd, *J* 2, CON*H*NH), 7.91 – 7.89 (2H, d, *J* 7, Ar-*H*), 7.77 – 7.76 (2H, m, Ar-*H*), 7.45 – 7.40 (2H, t, *J* 7, Ar-*H*), 7.36 – 7.31 (2H, t, *J* 7, Ar-*H*), 7.13 – 7.09 (2H, t, *J* 7, Ar-*H*), 6.80 – 6.77 (2H, d, *J* 7, Ar-*H*), 6.71 (1H, t, *J* 7, Ar-*H*), 4.31 – 4.21 (4H, m, CO_2CH_2CH-Fluorene, FmocNHC*H*, CO_2CH_2CH-Fluorene), 3.55 (2H, m, FmocNHCHCH_2), 1.18 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, DMSO-d6) 170.5 (*C*=ONHNH), 156.3 (*C*=ONH), 149.5 (*ipso*-Ar-*C*), 144.4 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 128.9 (Ar-*C*), 128.1 (Ar-*C*), 127.5 (Ar-*C*), 125.8 (Ar-*C*), 120.6 (Ar-*C*), 118.8 (Ar-*C*), 112.8 (Ar-*C*), 73.4 ($(CH_3)_3CO$), 66.4 (CO_2CH_2CH-Fluorene), 62.2 (FmocNHCHCH_2), 54.4 (FmocNH*C*H), 47.1 (*C*-Fluorene), 27.7 ($(CH_3)_3CO$); *m/z* (ES^+) 496 (MNa^+); HRMS (ES^+) Found MNa^+, 496.2253 ($C_{28}H_{31}N_3O_4Na$ requires 496.2207).

(9H-fluoren-9-yl)methyl (*S*)-(1,5-dioxo-1-(2-phenylhydrazineyl)-5-(tritylamino)pentan-2-yl)carbamate 68g



Following the general procedure outlined, *N*-Fmoc-*N*-(trityl)-*L*-glutamine (0.40 g, 0.66 mmol) and phenylhydrazine (0.07 g, 0.72 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.29 g, 65%); R_f 0.40 (DCM/EtOH/NH_3 200:6:1); m.p. 126 - 129 °C; υ_{max} 3247 (N-H), 1717, 1659 (C=O), 1533, 1495, 1230, 1046, 744, 699 cm-1; δ_H (300 MHz, DMSO-d_6) 9.75 (1H, bd, *J* 2, CON*H*NH), 8.61 (1H, bs, CON*H*-trityl), 7.92 – 7.89 (2H, d, *J* 7, Ar-*H*), 7.77 – 7.72 (3H, m, Ar-*H*), 7.45 – 7.40 (2H, t, *J* 7, Ar-*H*), 7.34 – 7.18 (15H, m, Ar-*H*), 7.13 – 7.08 (2H, t, *J* 7, Ar-*H*), 6.72 – 6.69 (3H, d, *J* 8, Ar-*H*), 4.34 (2H, m, CO_2CH_2CH-Fluorene), 4.27 (1H, m, CO_2CH_2CH-Fluorene), 4.08 (1H, m, FmocNHC*H*), 2.45 (2H, m, $CHCH_2CH_2CO$), 1.95 (2H, m, $CHCH_2CH_2CO$); δ_C (100 MHz, DMSO-d_6) 172.1 (*C*=ONH(trityl)), 171.8 (*C*=ONHNH), 156.5 *(C*=ONH), 149.7 (*ipso*-Ar-*C)*, 145.4 (*ipso*-Ar-*C)*, 144.4 (*ipso*-Ar-*C)*, 144.3 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 129.1 (Ar-*C*), 128.9 (Ar-*C*), 128.1 (Ar-*C*), 127.9 (Ar-*C*), 127.6 (Ar-*C*), 126.8 (Ar-*C*), 125.8 (Ar-*C*), 120.6 (Ar-*C*), 118.9 (Ar-*C*), 112.6 (Ar-*C*), 69.7 (*C*-(trityl), 66.2 (CO_2CH_2CH-Fluorene), 53.7 (FmocNH*C*H), 47.2 (*C*-Fluorene), 33.3 ($CHCH_2CH_2CO$), 28.3 ($CHCH_2CH_2CO$); *m/z* (ES^+) 723 (MNa^+); HRMS (ES^+) Found MNa^+, 723.2944 ($C_{45}H_{40}N_4O_4Na$ requires 723.2942).

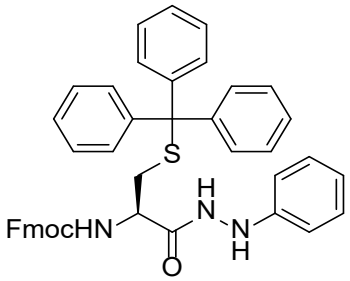
(9H-fluoren-9-yl)methyl (*S*)-(1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)-1-(2-phenylhydrazineyl) pentan-2-yl)carbamate 68j



Following the general procedure outlined, *N*-Fmoc-*N*-((2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl)-*L*-arginine (0.40 g, 0.62 mmol) and phenylhydrazine (0.07 mL, 0.68 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.14 g, 30%); R_f 0.20 (DCM/EtOH/NH_3 200:6:1); m.p. 137 - 140 °C; υmax 3319, 1727, 1664 (C=O), 1584, 1517, 1249, 1102, 1065, 735, 619 cm-1; δ_H (300 MHz, DMSO-d6) 9.82 (1H, bd, *J* 2, CON*H*NH), 7.91 – 7.89 (2H, d, *J* 7, Ar-*H*), 7.77 – 7.74 (2H, m, Ar-*H*), 7.45 – 7.40 (2H, t, *J* 7, Ar-*H*), 7.35 – 7.31 (2H, d, *J* 7, Ar-*H*), 7.14 – 7.09 (2H, t, *J* 7, Ar-*H*), 6.74 – 6.71 (2H, d, *J* 8, Ar-*H*), 6.46 (1H, bs, Ar-N*H*), 4.33 (2H, m, CO_2CH_2CH-Fluorene), 4.26 (1H, m, CO_2CH_2CH-Fluorene), 4.13 (1H, m, FmocNHC*H*), 3.12 (2H, m, CH_2NHCNHNH), 2.94 (2H, s, CH_2-benzofuran), 2.53 (3H, s, CH_3-benzofuran), 2.46 (3H, s, CH_3-benzofuran), 2.02 (3H, m, CH_3-benzofuran), 1.70 (2H, m, FmocNHCHCH_2), 1.54 (2H, m, $CHCH_2CH_2$), 1.40 (6H, s, $(CH_3)_2$-benzofuran); δ_C (100 MHz, DMSO-d6) 172.2 (*C*=ONHNH), 157.9 (*C*=NHNH) 156.6 *(C*=ONH), 149.7 (*ipso*-Ar-*C)*, 144.4 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 137.8 (*ipso*-Ar-*C*), 134.7 (*ipso*-Ar-*C*), 131.9 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 128.8 (Ar-*C*), 128.1 (Ar-*C*), 127.6 (Ar-*C*), 125.7 (Ar-*C*), 124.8 (Ar-*C*), 120.6 (Ar-*C*), 118.9 (Ar-*C*), 116.8 (*ipso*-Ar-*C*), 112.7 (Ar-*C*), 86.8 (*C*-benzofuran), 66.2 (CO_2CH_2CH-Fluorene), 53.5 (FmocNH*C*H), 47.2 (*C*-Fluorene), 42.9 (CH_2-benzofuran), 40.8 (CH_2NHCNHNH), 29.6 (FmocNHCHCH_2), 28.8

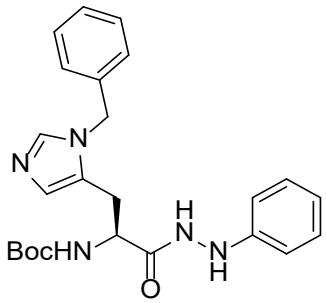
(CH_3)$_2$-benzofuran), 25,6 (CHCH$_2$CH$_2$), 19.5 (CH_3-benzofuran), 18.1 (CH_3-benzofuran),12.8 (CH_3-benzofuran); *m/z* (ES$^+$) 739 (MH$^+$), 761 (MNa$^+$); HRMS (ES$^+$) Found MH$^+$, 739.3268 ($C_{40}H_{47}N_6O_6S$ requires 739.3272).

(9H-fluoren-9-yl)methyl (*S*)-(1-oxo-1-(2-phenylhydrazineyl)-3-(tritylthio) propan-2-yl)carbamate 68f



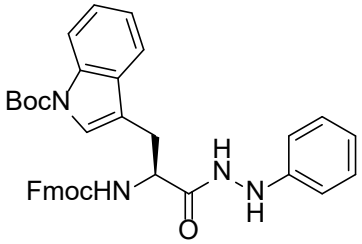
Following the general procedure outlined, *N*-Fmoc-*S*-(trityl)-*L*-cysteine (0.40 g, 0.68 mmol) and phenylhydrazine (0.07 mL, 0.75 mmol) were transformed following trituration with Et$_2$O into the title compound which was isolated as a brown oil (0.11 g, 23%); R$_f$ 0.35 (DCM/EtOH/NH$_3$ 200:6:1); υmax 3234, 3054, 3028 (N-H), 1689, 1602 (C=O), 1535, 1445, 1248, 1024, 741, 698 cm-1; δ_H (300 MHz, DMSO-d6) 9.92 (1H, bd, *J* 2, CON*H*NH), 8.60 (1H, bs, Ar-N*H*), 8.05 (1H, bd, *J* 2, FmocN*H*CH), 7.92 – 7.90 (2H, d, *J* 7, Ar-*H*), 7.76 – 7.73 (3H, dd, *J* 7, 2, Ar-*H*), 7.43 – 7.39 (3H, q, *J* 7, Ar-*H*), 7.24 – 7.19 (15H, m, Ar-*H*), 7.13 (1H, t, *J* 7, Ar-*H*), 6.73 – 6.70 (2H, m, Ar-*H*), 6.69 – 6.63 (2H, m, Ar-*H*), 4.43 (2H, m, CO$_2$C*H*$_2$CH-Fluorene), 4.29 – 4.22 (2H, m, CO$_2$CH$_2$C*H*-Fluorene, FmocNHC*H*), 2.83 (1H, m, CHC*H*HS-(trityl)), 2.59 (1H, d, *J* 4, CHCH*H*S-(trityl)); δ_C (100 MHz, DMSO-d6) 171.8 (*C*=ONHNH), 156.3 *(C*=ONH), 151.3 (*ipso*-Ar-*C)*, 145.2 (*ipso*-Ar-*C)*, 144.4 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 133.9 (*ipso*-Ar-*C*), 130.7 (Ar-*C*), 129.1 (Ar-*C*), 128.1 (Ar-*C*), 127.9 (Ar-*C*), 127.8 (Ar-*C*), 126.8 (Ar-*C*), 125.8 (Ar-*C*), 125.7 (Ar-*C*), 120.6 (Ar-*C*), 118.3 (Ar-*C*), 111.9 (*ipso*-Ar-*C*), 111.2 (Ar-*C*), 69.9 (*C*-(trityl), 66.4 (CO$_2$*C*H$_2$CH-Fluorene), 51.4 (FmocNH*C*H), 47.2 (*C*-Fluorene), 39.2 (CH*C*H$_2$S-(trityl)); *m/z* (ES$^+$) 698 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 698.2353 ($C_{43}H_{37}N_3O_3SNa$ requires 698.2448).

tert-Butyl (*S*)-(3-(1-benzyl-1H-imidazol-5-yl)-1-oxo-1-(2-phenylhydrazineyl) propan-2-yl)carbamate 67i



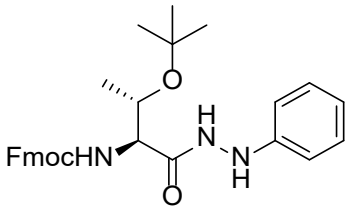
Following the general procedure outlined, *N*-Fmoc-*N*-(im)-benzyl-*L*-histidine (0.40 g, 1.16 mmol) and phenylhydrazine (0.13 mL, 1.27 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow gummy solid (0.36 g, 70%); R_f 0.20 (DCM/EtOH/NH_3 200:6:1); υmax 3274 (N-H), 2977, 2930 (C-H), 1676, 1602 (C=O), 14953, 1365, 1248, 1163, 1019, 751, 693 cm-1; δ_H (300 MHz, $CDCl_3$) 9.30 (1H, bs, CON*H*NH), 7.81 (1H, bs, Ar-N*H*), 7.38 – 7.34 (4H, m, Ar-*H*), 7.18 – 7.14 (3H, m, Ar-*H*), 7.12 (1H, s, Ar-*H*), 6.86 (1H, t, *J* 7, Ar-*H*), 6.79 (1H, s, Ar-*H*), 6.65 (1H, d, *J* 7, Ar-*H*), 6.32 (1H, bd, *J* 7, BocN*H*CH), 5.06 (2H, s, Ar-CH_2Im), 4.63 (1H, m, BocNHC*H*), 3.12 (2H, m, CHCH_2Im), 1.45 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 148. (*ipso*-Ar-*C)*, 136.3 (*ipso*-Ar-*C*), 135.2 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 129.0 (Ar-*C*), 128.6 (Ar-*C*), 128.3 (Ar-*C*), 127.7 (Ar-*C*), 120.8 (Ar-*C*), 117.9 (Ar-*C*), 113.5 (Ar-*C*), 80.1 ((CH$_3$)$_3$*C*O), 51.4 (Ar-*C*H$_2$Im), 30.0 (CH*C*H$_2$Im), 28.4 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 436 (MH$^+$), 458 (MNa$^+$); HRMS (ES$^+$) Found MH$^+$, 436.2355 ($C_{24}H_{29}N_5O_3$ requires 436.2343).

tert-Butyl (*S*)-3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-oxo-3-(2-phenylhydrazineyl)propyl)-1H-indole-1-carboxylate 68i



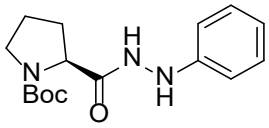
Following the general procedure outlined, *N*-Fmoc-*N*-(tert-butyl)-*L*-tryptophan (0.40 g, 0.76 mmol) and phenylhydrazine (0.08 mL, 0.84 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a white solid (0.31 g, 67%); R_f 0.62 (DCM/EtOH/NH_3 200:6:1); m.p. 163 - 166 °C; υmax 3277 (N-H), 2980 (C-H), 1732, 1706, 1687 (C=O), 1455, 1372, 1255, 1154, 1085, 747 cm-1; δ_H (300 MHz, DMSO-d6) 10.00 (1H, bd, *J* 2, CON*H*NH), 8.08 (1H, bd, *J* 8, FmocN*H*CH), 7.89 – 7.87 (3H, d, *J* 7, Ar-*H*), 7.83 (1H, d, *J* 7, Ar-*H*), 7.77 (1H, d, *J* 2, Ar-*H*), 7.67 – 7.63 (3H, t, *J* 6, Ar-*H*), 7.42 – 7.35 (3H, m, Ar-*H*), 7.31 – 7.23 (2H, m, Ar-*H*), 7.09 – 7. 04 (2H, t, *J* 7, Ar-*H*), 6.69 (1H, t, *J* 7, Ar-*H*), 6.61 – 6.59 (2H, d, *J* 7, Ar-*H*), 4.54 (1H, m, FmocNHC*H*), 4.23 – 4.18 (3H, m, CO_2CH_2CH-Fluorene, CO_2CH_2CH-Fluorene), 3.19 (1H, dd, *J* 14, 5, FmocNHCHC*H*H), 3.07 (1H, dd, *J* 14, 9, FmocNHCHCH*H*), 1.59 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, DMSO-d6) 171.6 (*C*=ONHNH), 156.4 (*C*=ONH), 149.5 (*ipso*-Ar-*C)*, 144.3 (*ipso*-Ar-*C)*, 144.1 (*ipso*-Ar-*C)*, 141.1 (*ipso*-Ar-*C)*, 135.2 (*ipso*-Ar-*C*), 130.6 (Ar-*C*), 129.4 (Ar-*C*), 129.0 (Ar-*C*), 128.1 (Ar-*C*), 127.8 (Ar-*C*), 127.5 (Ar-*C*), 125.7 (Ar-*C*), 124.9 (Ar-*C*), 124.8 (Ar-*C*), 122.9 (Ar-*C*), 121.8 (Ar-*C*), 120.6 (Ar-*C*), 120.1 (Ar-*C*), 118.9 (Ar-*C*), 116.9 (Ar-*C*), 115.1 (Ar-*C*), 112.6 (Ar-*C*), (Ar-*C*), 84.0 ($(CH_3)_3CO$), 66.3 (CO_2CH_2CH-Fluorene), 53.6 (FmocNH*C*H), 47.0 (*C*-Fluorene), 28.1 ($(CH_3)_3CO$); *m/z* (ES^+) 639 (MNa^+); HRMS (ES^+) Found MNa^+, 639.2583 ($C_{37}H_{36}N_4O_5Na$ requires 639.2503).

(9H-fluoren-9-yl)methyl ((2*S*,3*S*)-3-(tert-butoxy)-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl)carbamate 68b



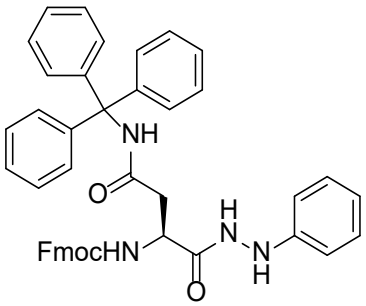
Following the general procedure outlined, *N*-Fmoc-*O*-(*tert*-butyl)-*L*-threonine (0.40 g, 1.01 mmol) and phenylhydrazine (0.09 mL, 1.11 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a reddish brown gummy solid (0.31 g, 63%); R_f 0.48 (DCM/EtOH/NH_3 200:6:1); υ_{max} 3317, 3252 (N-H), 2979 (C-H), 1692, 1652 (C=O), 1535, 1496, 1249, 1181, 1068, 757, 736 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.59 (1H, bs, CON*H*NH), 7.80 – 7.78 (2H, d, *J* 7, Ar-*H*), 7.74 – 7.71 (2H, d, *J* 7, Ar-*H*), 7.45 – 7.40 (2H, t, *J* 7, Ar-*H*), 7.35 – 7.31 (3H, t, *J* 7, Ar-*H*), 6.95 – 6.91 (1H, m, Ar-*H*), 5.99 (1H, bd, *J* 6, FmocN*H*CH), 4.47 (2H, d, *J* 7, CO_2CH_2CH-Fluorene), 4.36 (1H, m, FmocNHC*H*), 4.28 – 4.21 (2H, m, FmocNHCHC*H*, CO_2CH_2CH-Fluorene), 1.35 (9H, s, $(CH_3)_3CO$), 1.14 (3H, d, *J* 6, NHCHCHCH_3); δ_C (100 MHz, $CDCl_3$) 169.8 (*C*=ONHNH), 156.1 (*C*=ONH), 147.8 (*ipso*-Ar-*C*), 143.8 (*ipso*-Ar-*C)*, 143.8 (*ipso*-Ar-*C)*, 143.8 (*ipso*-Ar-*C)*, 141.4 (*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 129.2 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 125.1 (Ar-*C*), 121.4 (Ar-*C*), 120.0 (Ar-*C*), 113.8 (Ar-*C*), 75.9 ($(CH_3)_3CO$), 67.1 (CO_2CH_2CH-Fluorene), 66.6 (FmocNHCH*C*H), 58.2 (FmocNH*C*H), 47.2 (*C*-Fluorene), 28.3 ($(CH_3)_3CO$) 17.2 (NHCHCH*C*H_3); *m/z* (ES^+) 510 (MNa^+); HRMS (ES^+) Found MNa^+, 510.2423 ($C_{29}H_{33}N_3O_4Na$ requires 510.2421).

tert-Butyl (*S*)-2-(2-phenylhydrazine-1-carbonyl)pyrrolidine-1-carboxylate 67f



Following the general procedure outlined, *N*-Boc-*L*-proline (0.40 g, 1.86 mmol) and phenylhydrazine (0.20 mL, 2.04 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a light brown solid (0.29 g, 52%); R_f 0.40 (DCM/EtOH/NH_3 200:6:1); m.p. 151 - 153 °C; ν_{max} 3274 (N-H), 2972, 2874 (C-H), 1665, 1603 (C=O), 1538, 1404, 1398, 1162, 1134, 756, 698 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.69 (1H, bs, CON*H*NH), 7.17 – 7.12 (2H, t, *J* 7, Ar-*H*), 6.84 (1H, d, *J* 7, Ar-*H*), 6.77 – 6.74 (2H, dd, *J* 8, 1, Ar-*H*), 6.02 (1H, bs, Ar-N*H*), 4.31 (1H, m, CH_2BocNC*H*), 3.41 (2H, m, C*H*$_2$BocNCH), 2.31 (1H, m, BocNCHC*H*H), 1.87 (3H, m, BocNCHCH*H*, $CHCH_2CH_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 148.0 (*ipso*-Ar-*C)*, 129.1 (Ar-*C*), 120.0 (Ar-*C*), 113.6 (Ar-*C*), 80.5 ((CH_3)$_3$*C*O), 58.4 (CH_2BocN*C*H), 47.2 (*C*H_2BocNCH) 28.5 ((*C*H_3)$_3$CO), 27.3 (BocNCH*C*H_2), 24.6 ($CHCH_2$*C*H_2); ***m/z*** (ES$^+$) 328 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 328.1659 ($C_{16}H_{23}N_3O_3Na$ requires 328.1632).

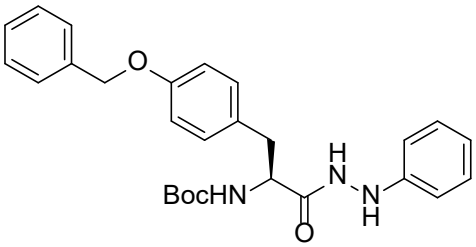
(9H-fluoren-9-yl)methyl (*S*)-(1,4-dioxo-1-(2-phenylhydrazineyl)-4-(tritylamino)butan-2-yl)carbamate 68e



Following the general procedure outlined, *N*-Fmoc-*N*-(trityl)-*L*-asparagine (0.40 g, 0.67 mmol) and phenylhydrazine (0.07 mL, 0.74 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.23 g, 49%); R_f 0.40 (DCM/EtOH/NH_3 200:6:1); m.p. 144 - 146 °C; υmax 3292 (N-H), 1696, 1655 (C=O), 1528, 1493, 1448, 1259, 1085, 839, 738 cm-1; δ_H (300 MHz, DMSO-d6) 9.83 (1H, bd, *J* 2, CON*H*NH), 8.59 (1H, bs,

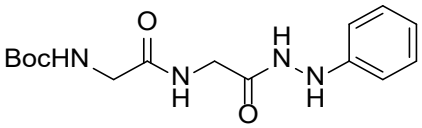
CON*H*-trityl), 7.92 – 7.90 (2H, d, *J* 7, Ar-*H*), 7.77 – 7.71 (4H, m, Ar-*H*), 7.46 – 7.39 (2H, q, *J* 7, Ar-*H*), 7.25 – 7.19 (15H, m, Ar-*H*), 7.13 – 7.09 (2H, t, *J* 7, Ar-*H*), 6.73 – 6.66 (3H, m, Ar-*H*), 4.45 (2H, m, CO_2CH_2CH-Fluorene), 4.31 – 4.22 (2H, m, FmocNHC*H*, CO_2CH_2CH-Fluorene), 2.84 (1H, m, FmocNHCHC*H*H), 2.62 (1H, dd, *J* 14, 4, FmocNHCHCH*H*); δ_C (100 MHz, DMSO-d6) 171.7 (*C*=ONHNH), 169.1 (*C*=NH-trityl), 156.2 (*C*=ONH), 149.7 (*ipso*-Ar-*C)*, 144.3 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 129.1 (Ar-*C*), 128.1 (Ar-*C*), 127.6 (Ar-*C*), 127.6 (Ar-*C*), 127.6 (Ar-*C*), 126.8 (Ar-*C*), 125.8 (Ar-*C*), 125.7 (Ar-*C*), 120.6 (Ar-*C*), 118.9 (Ar-*C*), 112.6 (Ar-*C*), 69.9 (*C*-(trityl), 66.4 (CO_2CH_2CH-Fluorene), 51.5 (FmocNH*C*H), 47.2 (*C*-Fluorene) 39.2 (FmocNHCH*C*H_2); *m/z* (ES^+) 687 (MH^+), 709 (MNa^+); HRMS (ES^+) Found MNa^+, 709.2783 ($C_{44}H_{38}N_4O_4Na$ requires 709.2785).

tert-Butyl (*S*)-(3-(4-(benzyloxy)phenyl)-1-oxo-1-(2-phenylhydrazineyl) propan-2-yl)carbamate 67j



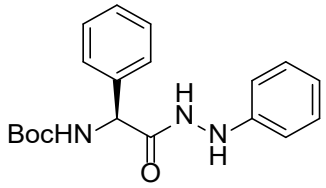
Following the general procedure outlined, *N*-Boc-*O*-benzyl-*L*-tyrosine (0.40 g, 1.08 mmol) and phenylhydrazine (0.12 mL, 1.19 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.35 g, 70%); R_f 0.37 (DCM/EtOH/NH_3 200:6:1); m.p. 130 - 133 °C; ν_{max} 3339, 3229 (N-H), 1689, 1665 (C=O), 1521, 1489, 1247, 1164, 1044, 731, 695 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.75 (1H, bs, CON*H*NH), 7.46 – 7.44 (2H, m, Ar-*H*), 7.42 (1H, m, Ar-*H*), 7.39 – 7.35 (2H, m, Ar-*H*), 7.17 – 7.14 (4H, m, Ar-*H*), 6.96 – 6.86 (4H, m, Ar-*H*), 6.65 (1H, d, *J* 7, Ar-*H*), 5.07 (2H, m, Ar-CH_2O), 4.44 (1H, q, *J* 7, BocNHC*H*), 3.07 (2H, dd, *J* 7, 3, Ar-CH_2CH), 1.47 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.4 (*C*=ONHNH), 158.0 (*C*=ONH), 147.4 (*ipso*-Ar-*C*), 136.9 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 129.1 (Ar-*C*), 128.6 (Ar-*C*), 128.0 (Ar-*C*), 127.5 (Ar-*C*), 121.3 (Ar-*C*), 115.2 (Ar-*C*), 113.3 (Ar-*C*), 70.1 (Ar-*C*H_2O), 28.3 ((*C*H_3)$_3$CO); *m/z* (ES^+) 462 (MH^+), 484 (MNa^+); HRMS (ES^+) Found MH^+, 462.2396 ($C_{27}H_{32}N_3O_4$ requires 462.2387).

tert-Butyl (2-oxo-2-((2-oxo-2-(2-phenylhydrazineyl)ethyl)amino)ethyl) carbamate 67k



Following the general procedure outlined, *N*-Boc-glycylglycine (0.40 g, 1.72 mmol) and phenylhydrazine (0.19 mL, 1.89 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.38 g, 69%) as a mixture of rotamers [5:1]; R_f 0.11 (DCM/EtOH/NH_3 200:6:1); m.p. 157 - 160 °C; ν_{max} 3296, 3235 (N-H), 2975 (C-H), 1662, 1646 (C=O), 1552, 1496, 1309, 1234, 1168, 946, 753 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, DMSO-d6) 9.59 (1H, bd, *J* 2, CON*H*NH), 8.17 (1H, bt, *J* 6, CON*H*CH_2), 7.70 (1H, bd, *J* 2, BocN*H*CH_2), 7.15 – 7.10 (2H, t, *J* 7, Ar-*H*), 6.73 – 6.70 (3H, dd, *J* 8, 2, Ar-*H*), 3.82 (2H, d, *J* 5, CONHCH_2), 3.62 (2H, d, *J* 5, BocNHCH_2), 1.38 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, DMSO-d6) 170.4 (*C*=ONHNH), 169.2 *(C*=ONH), 156.3 *(C*=ONH), 149.6 (*ipso*-Ar-*C)*, 129.1 (Ar-*C*), 118.9 (Ar-*C*), 112.7 (Ar-*C*), 78.6 ((CH$_3$)$_3$*C*O), 43.9 (BocNH*C*H_2), 41.5 (CONH*C*H_2), 28.7 ((*C*H$_3$)$_3$CO); ***m/z*** (ES^+) 323 (MH^+), 345 (MNa^+); HRMS (ES^+) Found MNa^+, 345.1548 ($C_{15}H_{22}N_4O_4Na$ requires 345.1533).

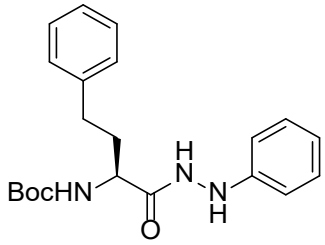
tert-Butyl (*S*)-(2-oxo-1-phenyl-2-(2-phenylhydrazineyl)ethyl)carbamate 67l



Following the general procedure outlined, *N*-Boc-*L*-phenylglycine (0.40 g, 1.59 mmol) and phenylhydrazine (0.17 mL, 1.75 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.31 g, 57%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); m.p. 155 - 158 °C; υmax 3327, 3273 (N-H), 17717, 1662 (C=O), 1508, 1497, 1369, 1256, 1159, 1027, 753, 697 cm-1; δ_H (300 MHz, DMSO-d6) 8.23 (1H, bs, CON*H*NH), 7.41 – 7.35 (4H, m, Ar-*H*), 7.26 (1H, m, Ar-*H*), 7.17 – 7.11 (2H, m, Ar-*H*), 7.05 (1H, t, *J* 7, Ar-*H*), 6.64 – 6.60 (2H, m, Ar-*H*), 5.41 (1H, m, CONHC*H*), 1.43 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, DMSO-d6) 170.8 (*C*=ONHNH), 155.4 *(C*=ONH), 147.5 (*ipso*-Ar-*C)*, 146.2 (*ipso*-

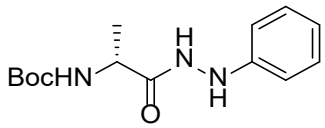
Ar-*C*), 137.4 (*ipso*-Ar-*C*), 129.2 (Ar-*C*), 129.1 (Ar-*C*), 129.1 (Ar-*C*), 128.8 (Ar-*C*), 128.6 (Ar-*C*), 127.9 (Ar-*C*), 127.2 (Ar-*C*), 113.4 (Ar-*C*), 112.9 (Ar-*C*), 80.6 ($(CH_3)_3CO$), 57.1 (BocNH*C*H), 28.3 ($(CH_3)_3CO$); *m/z* (ES^+) 364 (MNa^+); HRMS (ES^+) Found MNa^+, 364.1649 ($C_{19}H_{23}N_3O_3Na$ requires 364.1632).

tert-Butyl (*S*)-(1-oxo-4-phenyl-1-(2-phenylhydrazineyl)butan-2-yl)carbamate 67m



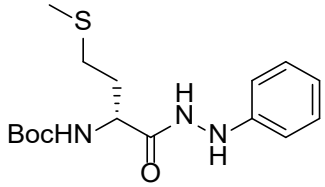
Following the general procedure outlined, *N*-Boc-*L*-homophenylalanine (0.40 g, 1.43 mmol) and phenylhydrazine (0.16 mL, 1.58 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a pale yellow solid (0.42 g, 79%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); m.p. 129 - 132 °C; υ_{max} 3292 (N-H), 1678, 1665 (C=O), 1518, 1495, 1366, 1278, 1164, 748, 693 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.44 (1H, bs, CON*H*NH), 7.40 (1H, m, Ar-N*H*), 7.19 – 7.16 (2H, m, Ar-*H*), 7.13 (1H, m, Ar-*H*), 7.10 – 7.04 (4H, m, Ar-*H*), 6.80 (1H, t, *J* 7, Ar-*H*), 6.71 – 6.68 (2H, dd, *J* 8, 1, Ar-*H*), 5.22 (1H, bd, *J* 8, CON*H*CH), 4.18 (1H, m, CONHC*H*), 2.61 (2H, t, *J* 7, CH_2CH_2-Ar), 2.11 (1H, m, CONHCHC*H*H), 1.90 (1H, m, CONHCHC*HH*), 1.38 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 155.9 *(C*=ONH), 147.7 (*ipso*-Ar-*C)*, 140.7 (*ipso*-Ar-*C)*, 129.6 (Ar-*C*), 129.4 (Ar-*C*), 129.1 (Ar-*C*), 128.6 (Ar-*C*), 128.4 (Ar-*C*), 128.3 (Ar-*C*), 126.2 (Ar-*C*), 121.1 (Ar-*C*), 115.4 (Ar-*C*), 113.5 (Ar-*C*), 112.9 (Ar-*C*), 80.6 ($(CH_3)_3CO$), 52.6 (BocNH*C*H), 33.6 (CONHCH*C*H_2), 31.8 (CH_2CH_2-Ar), 28.4 ($(CH_3)_3CO$); *m/z* (ES^+) 392 (MNa^+); HRMS (ES^+) Found MNa^+, 392.1964 ($C_{21}H_{27}N_3O_3Na$ requires 392.1945).

tert-Butyl (*R*)-(1-oxo-1-(2-phenylhydrazineyl)propan-2-yl)carbamate 67n



Following the general procedure outlined, *N*-Boc-*D*-alanine (0.40 g, 2.11 mmol) and phenylhydrazine (0.23 mL, 2.33 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.29 g, 49%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 82 - 85 °C; υmax 3433, 3289 (N-H), 1689, 1674 (C=O), 1539, 1493, 1370, 1244, 1156, 1022, 763, 702 cm-1; δ_H (300 MHz, $CDCl_3$) 8.21 (1H, bs, CON*H*NH), 7.24 – 7.19 (3H, m, Ar-*H*), 6.92 (1H, t, *J* 7, 1, Ar-*H*), 6.83 – 6.80 (1H, m, Ar-*H*), 5.02 (1H, bd, *J* 7, CON*H*CH), 4.31 (1H, m, CONHC*H*), 1.47 (9H, s, (C*H*$_3$)$_3$CO), 1.41 (1H, d, *J* 7, CONHCHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.1 (*C*=ONHNH), 155.8 *(C*=ONH), 147.8 (*ipso*-Ar-*C)*, 129.1 (Ar-*C*), 121.0 (Ar-*C*), 115.5 (Ar-*C*), 1113.5 (Ar-*C*), 112.9 (Ar-*C*), 80.4 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.3 ((*C*H$_3$)$_3$CO) 18.0 (CONHCH*C*H$_3$); *m/z* (ES^+) 302 (MNa^+); HRMS (ES^+) Found MNa^+, 302.1489 ($C_{14}H_{21}N_3O_3Na$ requires 302.1475).

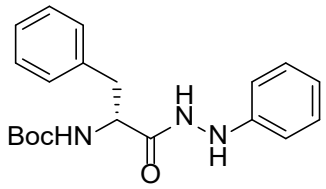
tert-Butyl (*R*)-(4-(methylthio)-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl)carbamate 67o



Following the general procedure outlined, *N*-Boc-*D*-methionine (0.40 g, 1.60 mmol) and phenylhydrazine (0.17 mL, 1.77 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.17 g, 31%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); m.p. 119 - 122 °C; υmax 3329, 3258 (N-H), 1685, 1655 (C=O), 1523, 1495, 1308, 1249, 1166, 1055, 764, 689 cm-1; δ_H (300 MHz, $CDCl_3$) 8.70 (1H, bs, CON*H*NH), 7.13 – 7.09 (2H, t, *J* 7, Ar-*H*), 6.82 (1H, t, *J* 7, Ar-*H*), 6.71 – 6.69 (2H, d, *J* 8, Ar-*H*), 6.18 (1H, bd, *J* 3, N*H*-Ar), 5.42 (1H, bd, *J* 5, CON*H*CH), 4.37 (1H, q, *J* 7, CONHC*H*), 2.46 (2H, t, *J* 7, CHCH$_2$C*H*$_2$S), 2.09 (1H, m, CHC*H*$_2$CH$_2$S), 1.99 (3H, s, CHCH$_2$CH$_2$SC*H*$_3$),1.89 (1H, m, CHC*H*$_2$CH$_2$S), 1.37 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 155.9 *(C*=ONH), 147.7 (*ipso*-Ar-*C)*, 129.2 (Ar-*C*), 121.1 (Ar-*C*),

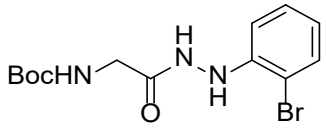
113.5 (Ar-*C*), 112.9 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 52.0 (BocNH*C*H), 31.4 (CONHCH*C*H$_2$), 30.1 (CH*C*H$_2$CH$_2$S), 28.4 ((*C*H$_3$)$_3$CO), 15.4 (CHCH$_2$CH$_2$S*C*H$_3$); *m/z* (ES$^+$) 362 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 362.1536 ($C_{16}H_{25}N_3O_3SNa$ requires 362.1509).

tert-Butyl (*R*)-(1-oxo-3-phenyl-1-(2-phenylhydrazineyl)propan-2-yl)carbamate 67p



Following the general procedure outlined, *N*-Boc-*D*-phenylalanine (0.40 g, 1.51 mmol) and phenylhydrazine (0.16 mL, 1.66 mmol) were transformed following trituration with Et$_2$O into the title compound which was isolated as a brown gum (0.14 g, 25 %); R$_f$ 0.35 (DCM/EtOH/NH$_3$ 200:6:1); υmax 3314 (N-H), 1688, 1667 (C=O), 1521, 1496, 1366, 1251, 1167, 749, 694 cm-1; δ_H (300 MHz, CDCl$_3$) 8.05 (1H, bs, CON*H*NH), 7.22 – 7.18 (3H, m, Ar-*H*), 7.12 – 7.09 (2H, m, Ar-*H*), 7.08 – 7.03 (2H, t, *J* 7, Ar-*H*), 6.78 (1H, t, *J* 7, Ar-*H*), 6.49 – 6.46 (2H, d, *J* 7, Ar-*H*), 5.16 (1H, bd, *J* 8, CON*H*CH), 4.42 (1H,m, CONHC*H*), 3.00 (1H, dd, *J* 7, 2, CONHCHC*H*$_2$), 1.35 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, CDCl$_3$) 171.6 (*C*=ONHNH), 155.6 *(C*=ONH), 147.5 (*ipso*-Ar-*C)*, 136.3 (*ipso*-Ar-*C)*, 129.4 (Ar-*C*), 129.1 (Ar-*C*), 128.8 (Ar-*C*), 127.0 (Ar-*C*), 121.0 (Ar-*C*), 113.5 (Ar-*C*), 112.9 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 54.5 (BocNH*C*H), 38.2 (CONHCH*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 378 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 378.1811 ($C_{20}H_{25}N_3O_3Na$ requires 378.1788).

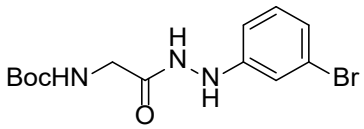
tert-Butyl (2-(2-(2-bromophenyl)hydrazineyl)-2-oxoethyl)carbamate 67Ce



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 2-bromophenylhydrazine hydrochloride (0.56 g, 2.51 mmol) were transformed following work up with Et$_2$O into the title compound which was isolated as a yellow oil (0.39 g, 50%) as a mixture of rotamers [3:1]; R$_f$ 0.46 (DCM/EtOH/NH$_3$ 200:6:1); ν_{max} 3345 (N-H), 2979 (C-H), 1703, 1653 (C=O), 1595, 1465, 1367, 1277, 1155,

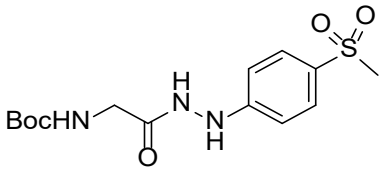
1023, 750 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.52 (1H, bs, CON*H*NH), 7.43 (1H, m, Ar-*H*), 7.16 (1H, td, *J* 8,1, Ar-*H*), 6.85 (1H, m, Ar-*H*), 6.77 (1H, td, *J* 7,1, Ar-*H*), 6.39 (1H, bd, *J* 3, Ar-N*H*), 5.45 (1H, bs, BocN*H*CH_2), 3.88 (2H, d, *J* 6, BocNHC*H*$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 169.8 (*C*=ONHNH), 156.5 *(C*=ONH), 144.5 (*ipso*-Ar-*C)*, 132.6 (Ar-*C)*, 128.3 (Ar-*C)*, 121.9 (Ar-*C)*, 113.7 (Ar-*C)*, 109.0 (*ipso*-Ar-*C)*, 80.8 ((CH$_3$)$_3$*C*O), 43.3 (BocNH*C*H$_2$), 28.4 (*C*H$_3$)$_3$CO); *m/z* (ES^+) 366 ([^{79}Br] MNa^+), 368 ([^{81}Br] MNa^+), 709 ([^{79,79}Br] 2M+Na^+), 711 ([^{79,81}Br] 2M+Na^+), 713 ([^{81,81}Br] 2M+Na^+); HRMS (ES^+) Found [^{79}Br]MH^+, 366.0438 ($C_{13}H_{18}{}^{79}BrN_3O_3Na$ requires 366.0424).

tert-Butyl (2-(2-(3-bromophenyl)hydrazineyl)-2-oxoethyl)carbamate 67Ck



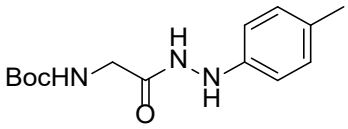
Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 3-bromophenylhydrazine hydrochloride (0.56 g, 2.51 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a brown oil (0.39 g, 49%) as a mixture of rotamers [4:1]; R_f 0.32 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3276 (N-H), 2979 (C-H), 1676 (C=O), 1595, 1474, 1367, 1249, 1157, 1047, 849, 768 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.63 (1H, bs, CON*H*NH), 7.01 (1H, s, Ar-*H*), 6.98 (1H, m, Ar-*H*), 6.92 (1H, m, Ar-*H*), 6.70 (1H, m, Ar-*H*), 6.40 (1H, bs, Ar-N*H*), 5.55 (1H, bs, BocN*H*CH_2), 3.84 (2H, d, *J* 6, BocNHC*H*$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.5 (*C*=ONHNH), 156.5 *(C*=ONH), 149.1 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 123.8 (*ipso*-Ar-*C*), 123.1 (Ar-*C*), 116.0 (Ar-*C*), 112.0 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 43.2 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO); *m/z* (ES^+) 366 ([^{79}Br] MNa^+), 368 ([^{81}Br] MNa^+), 709 ([^{79,79}Br] 2M+Na^+), 711 ([^{79,81}Br] 2M+Na^+), 713 ([^{81,81}Br] 2M+Na^+); HRMS (ES^+) Found [^{79}Br]MH^+, 366.0452 ($C_{13}H_{18}{}^{79}BrN_3O_3Na$ requires 366.0423).

tert-Butyl (2-(2-(4-(methylsulfonyl)phenyl)hydrazineyl)-2-oxoethyl) carbamate 67Di



Following the general procedure outlined, *N*-Boc-glycine (0.20 g, 1.14 mmol) and 4-(methylsulphonyl)phenylhydrazine hydrochloride (0.28 g, 1.26 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown oil (0.33 g, 84%); R_f 0.44 (DCM/EtOH/NH_3 200:6:1); m.p. 158 - 161 °C; ν_{max} 3359, 3291 (N-H), 1678, 1600 (C=O), 1506, 1285, 1139, 960, 773 cm^{-1}; δ_H (300 MHz, DMSO-d6) 9.89 (1H, bs, CON*H*NH), 8.59 (1H, bs, Ar-N*H*), 7.64 – 7.61 (2H, d, *J* 8, Ar-*H*), 7.15 (1H, bt, *J* 6, BocN*H*CH_2), 6.84 – 6.81 (2H, d, *J* 8, Ar-*H*), 3.63 (2H, d, *J* 6, BocNHC*H*$_2$), 3.08 (3H, s, SO_2C*H*$_3$), 1.40 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 169.9 (*C*=ONHNH), 156.4 *(C*=ONH), 153.9 (*ipso*-Ar-*C)*, 129.5 (Ar-*C)*, 128.9 (Ar-*C)*, 111.6 (Ar-*C)*, 78.6 ((CH$_3$)$_3$*C*O), 44.7 (SO_2*C*H$_3$), 42.5 (BocNH*C*H$_2$), 28.7 (*C*H$_3$)$_3$CO); ***m/z*** (ES^+) 366 ($MNah^+$); HRMS (ES^+) Found MNa^+, 366.1105 ($C_{14}H_{21}N_3O_5S$ requires 366.1094).

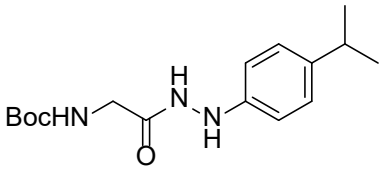
tert-Butyl (2-oxo-2-(2-(p-tolyl)hydrazineyl)ethyl)carbamate 67Ea



Following the general procedure outlined, *N*-Boc-glycine (0.30 g, 1.71 mmol) and 4-methylphenylhydrazine hydrochloride (0.29 g, 1.89 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown gum (0.35 g, 72%) as a mixture of rotamers [3:1]; R_f 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3289 (N-H), 2979, 2929 (C-H), 1676, 1615 (C=O), 1511, 1366, 1247, 1161, 730 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.36 (1H, bs, CON*H*NH), 6.93 – 6.91 (2H, d, *J* 8, Ar-*H*), 6.64 – 6.61 (2H, d, *J* 8, Ar-*H*), 5.79 (1H, bs, Ar-N*H*), 5.37 (1H, bs, BocN*H*CH_2), 3.76 (2H, d, *J* 5, BocNHC*H*$_2$), 2.17 (3H, s, Ar-C*H*$_3$), 1.38 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 169.9 (*C*=ONHNH), 156.4 *(C*=ONH), 145.3 (*ipso*-Ar-*C)*, 129.9 (Ar-*C)*, 129.7 (Ar-*C)*, 113.7 (Ar-*C)*, 112.8 (Ar-*C)*, 80.6 ((CH$_3$)$_3$*C*O), 43.2 (BocNH*C*H$_2$), 28.3

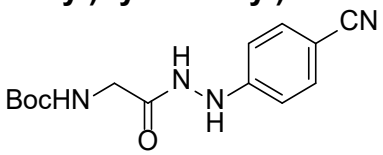
(CH_3)$_3$CO), 20.6 (Ar-CH_3); *m/z* (ES^+) 302 (MNa^+); HRMS (ES^+) Found MNa^+, 302.1492 ($C_{14}H_{21}N_3O_3Na$ requires 302.1481).

tert-Butyl (2-(2-(4-isopropylphenyl)hydrazineyl)-2-oxoethyl)carbamate 67Eg



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 4-isopropylphenylhydrazine hydrochloride (0.47 g, 2.51 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a brown solid (0.34 g, 48%) as a mixture of rotamers [2:1]; R_f 0.37 (DCM/EtOH/NH_3 200:6:1); m.p. 156 – 159 °C; ν_{max} 3357, 3263 (N-H), 2958(C-H), 1650, 1614 (C=O), 1512, 1473, 1367, 1243, 1166, 1051, 814 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.24 (1H, bs, CON*H*NH), 7.09 – 7.06 (2H, d, *J* 8, Ar-*H*), 6.77 – 6.74 (2H, d, *J* 8, Ar-*H*), 6.07 (1H, bs, Ar-N*H*), 5.31 (1H, bt, *J* 6, BocN*H*CH_2), 3.86 (2H, d, *J* 5, BocNHC*H*$_2$), 2.85 (1H, m, Ar-C*H*(CH_3)$_2$), 1.46 (9H, s, (C*H*$_3$)$_3$CO), 1.20 – 1.18 (6H, d, *J* 6, Ar-CH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 169.7 (*C*=ONHNH), 145.5 (*ipso*-Ar-*C)*, 141.9 (*ipso*-Ar-*C)*, 127.4 (Ar-*C)*, 127.1 (Ar-*C)*, 113.8 (Ar-*C)*, 112.8 (Ar-*C)*, 80.7 ((CH_3)$_3$*C*O), 43.2 (BocNH*C*H_2), 33.3 (Ar-*C*H(CH_3)$_2$), 28.4 (*C*H$_3$)$_3$CO), 24.1 (Ar-CH(*C*H$_3$)$_2$); *m/z* (ES^+) 330 (MNa^+); HRMS (ES^+) Found MNa^+, 330.1803 ($C_{16}H_{25}N_3O_3Na$ requires 330.1788).

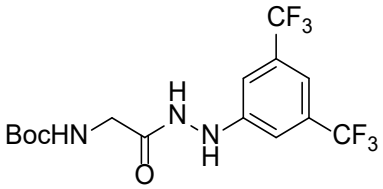
tert-Butyl (2-(2-(4-cyanophenyl)hydrazineyl)-2-oxoethyl)carbamate 67Cw



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 4-cyanophenylhydrazine hydrochloride (0.43 g, 2.51 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown solid (0.54 g, 82%) as a mixture of rotamers [5:1]; R_f 0.72 (*n*-hex/EtOAc 2:1); m.p. 113 – 116 °C; ν_{max} 3331, 3276 (N-H), 2219 (C≡N), 1683, 1605 (C=O), 1519, 1250, 1163, 833 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.33 (1H, bs, CON*H*NH), 7.49 – 7.47 (2H, d, *J* 8, Ar-*H*), 6.84 – 6.81 (2H, d, *J* 8,

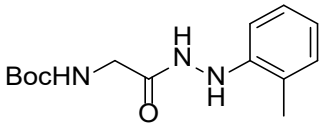
Ar-*H*), 6.45 (1H, bd, *J* 2, Ar-N*H*), 5.29 (1H, bt, *J* 6, BocN*H*CH_2), 3.90 (2H, d, *J* 6, BocNHC*H*$_2$), 1.46 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*=ONHNH), 151.3 *(C*=ONH), 133.7 (Ar-*C)*, 119.5 (*C*≡N), 112.9 (Ar-*C)*, 103.4 (*ipso*-Ar-*C)*, 81.3 ((CH$_3$)$_3$*C*O), 43.6 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO); *m/z* (ES^+) 313 (MNa^+); HRMS (ES^+) Found MNa^+, 313.1285 ($C_{14}H_{18}N_4O_3Na$ requires 313.1271).

tert-Butyl (2-(2-(3,5-bis(trifluoromethyl)phenyl)hydrazineyl)-2-oxoethyl) carbamate 67Bs



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 3,5-bis(trifluoromethyl)phenyl)hydrazine hydrochloride (0.70 g, 2.51 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown solid (0.52 g, 56%); R_f 0.56 (*n*-hex/EtOAc 4:1); m.p. 111 – 114 °C; ν_{max} 3342, 3237 (N-H), 1686, 1678 (C=O), 1524, 1387, 1280, 1124, 879 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.49 (1H, bs, CON*H*NH), 7.35 (1H, s, Ar-*H*), 7.20 (2H, s, Ar-*H*), 6.58 (1H, bs, Ar-N*H*), 5.30 (1H, bs, BocN*H*CH_2), 3.92 (2H, d, *J* 6, BocNHC*H*$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.4 (*C*=ONHNH), 149.9 *(C*=ONH), 132.7 (*ipso*-Ar-*C)*, 125.1 (*ipso*-Ar-*C)*, 121.4 (*C*F_3*)*, 114.3 (Ar-*C)*, 112.9 (Ar-*C)*, 81.3 ((CH$_3$)$_3$*C*O), 43.5 (BocNH*C*H$_2$), 28.2 (*C*H$_3$)$_3$CO); δ_F (300 MHz, $CDCl_3$) -63.2 (C*F*$_3$); *m/z* (ES^+) 424 (MNa^+); HRMS (ES^+) Found MNa^+, 424.1069 ($C_{15}H_{17}N_3O_3F_6Na$ requires 424.1066).

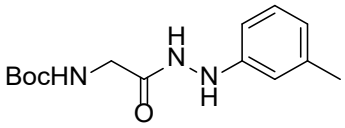
tert-Butyl (2-oxo-2-(2-(o-tolyl)hydrazineyl)ethyl)carbamate 67Do



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 2-methylphenylhydrazine hydrochloride (0.39 g, 2.51 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown solid (0.53 g, 84%) as a mixture of rotamers [3:1]; R_f 0.56 (*n*-hex/EtOAc 4:1); m.p.

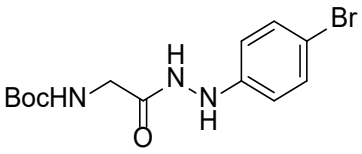
90 – 92 °C; ν_{max} 3299 (N-H), 1672 (C=O), 1529, 1368, 1286, 1161, 751 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.33 (1H, bs, CON*H*NH), 7.09 – 7.04 (2H, m, Ar-*H*), 6.83 – 6.78 (2H, m, Ar-*H*), 5.77 (1H, bs, Ar-N*H*), 5.38 (1H, bt, , *J* 5, BocN*H*CH_2), 3.88 (2H, d, *J* 5, BocNHC*H*$_2$), 2.21 (3H, s, Ar-C*H*$_3$), 1.46 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 169.6 (*C*=ONHNH), 155.9 *(C*=ONH), 145.3 (*ipso*-Ar-*C)*, 130.8 (Ar-*C)*, 126.9 (Ar-*C)*, 121.0 (Ar-*C)*, 112.1 (Ar-*C)*, 80.9 ((CH$_3$)$_3$*C*O), 43.4 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO), 17.0 (Ar-*C*H$_3$); *m/z* (ES^+) 302 (MNa^+); HRMS (ES^+) Found MNa^+, 302.1493 ($C_{14}H_{21}N_3O_3Na$ requires 302.1475).

tert-Butyl (2-oxo-2-(2-(m-tolyl)hydrazineyl)ethyl)carbamatecarbamate 67Du



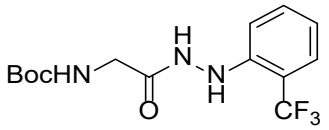
Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 3-methylphenylhydrazine hydrochloride (0.39 g, 2.51 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.33 g, 51%) as a mixture of rotamers [3:1]; R_f 0.37 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3276 (N-H), 2927 (C-H), 1656, 1606 (C=O), 1524, 1498, 1367, 1249, 1161, 777 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.48 (1H, bs, CON*H*NH), 7.10 (1H, t, *J* 7, Ar-*H*), 6.70 (1H, d, *J* 7, Ar-*H*), 6.59 (1H, s, Ar-*H*), 6.57 (1H, m, Ar-*H*), 6.16 (1H, bd, *J* 3, Ar-N*H*), 5.49 (1H, bs, BocN*H*CH_2), 3.84 (2H, d, *J* 5, BocNHC*H*$_2$), 2.25 (3H, s, Ar-C*H*$_3$), 1.45 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.0 (*C*=ONHNH), 156.4 *(C*=ONH), 147.7 (*ipso*-Ar-*C)*, 130.1 (Ar-*C)*, 129.0 (Ar-*C*), 122.0 (Ar-*C)*, 114.2 (Ar-*C)*, 110.6 (Ar-*C)*, 80.5 ((CH$_3$)$_3$*C*O), 43.2 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO), 21.5 (Ar-*C*H$_3$); *m/z* (ES^+) 581 (2M+Na^+); HRMS (ES^+) Found 2M+Na^+, 581.3073 ($C_{28}H_{42}N_6O_6Na$ requires 581.3058).

tert-Butyl (2-(2-(4-bromophenyl)hydrazineyl)-2-oxoethyl)carbamate 67Cq



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 4-bromophenylhydrazine hydrochloride (0.56 g, 2.51 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown oil (0.49 g, 62%) as a mixture of rotamers [3:1]; R_f 0.51 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3369, 3265 (N-H), 2985 (C-H), 1698, 1652 (C=O), 1525, 1488, 1369, 1242, 1159, 812 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.57 (1H, bs, CON*H*NH), 7.27 – 7.24 (2H, d, *J* 8, Ar-*H*), 6.66 – 6.63 (2H, d, *J* 8, Ar-*H*), 5.40 (1H, bs, BocN*H*CH_2), 3.84 (2H, d, *J* 6, BocNHC*H*$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.3 (*C*=ONHNH), 156.5 *(C*=ONH), 146.8 (*ipso*-Ar-*C*), 132.3 (Ar-*C*), 131.9 (*ipso*-Ar-*C*), 115.1 (Ar-*C*), 113.0 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 43.2 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO); ***m/z*** (ES^+) 366 ([^{79}Br]MNa^+), 368 ([^{81}Br]MNa^+); HRMS (ES^+) Found [^{79}Br]MNa^+, 366.0434 ($C_{13}H_{18}{}^{79}BrN_3O_3Na$ requires 366.0424).

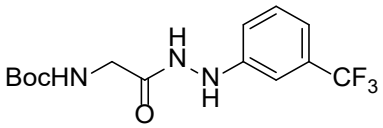
tert-Butyl (2-oxo-2-(2-(2-(trifluoromethyl)phenyl)hydrazineyl)ethyl) carbamate 67Ay



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 2-trifluoromethylphenylhydrazine hydrochloride (0.53 g, 2.51 mmol) were transformed following trituration with DCM into the title compound which was isolated as a pale yellow solid (0.17 g, 22%) as a mixture of rotamers [3:1]; R_f 0.46 (*n*-hex/EtOAc 4:1); m.p. 123 – 125 °C; ν_{max} 3335, 3194 (N-H), 1703, 1654 (C=O), 1535, 1322, 1276, 1101, 763 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.23 (1H, bs, CON*H*NH), 7.52 (1H, d, *J* 7, Ar-*H*), 7.41 (1H, t, *J* , Ar-*H*), 7.02 (1H, d, *J* 8, Ar-*H*), 6.99 (1H, t, *J* 7, Ar-*H*), 6.52 (1H, bs, Ar-N*H*), 5.26 (1H, bt, *J* 6, BocN*H*CH_2), 3.92 (2H, d, *J* 6, BocNHC*H*$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 169.7 (*C*=ONHNH), 145.20 (*ipso*-Ar-*C*), 133.1

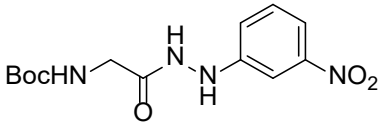
(Ar-*C)*, 126.6 (Ar-*C)*, 120.3 (Ar-*C)*, 113.5 (Ar-*C)*, 80.9 ((CH$_3$)$_3$*C*O), 43.4 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO); δ_F (300 MHz, CDCl$_3$) -61.8 (C*F*$_3$); ***m/z*** (ES$^+$) 356 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 356.1208 ($C_{14}H_{18}N_3O_3F_3Na$ requires 356.1193).

tert-Butyl (2-oxo-2-(2-(3-(trifluoromethyl)phenyl)hydrazineyl)ethyl) carbamate 67Be



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 3-trifluoromethylphenylhydrazine hydrochloride (0.53 g, 2.51 mmol) were transformed following work up with Et$_2$O into the title compound which was isolated as a dark orange solid (0.49 g, 65%) as a mixture of rotamers [4:1]; R$_f$ 0.44 (*n*-hex/EtOAc 4:1); m.p. 93 – 95 °C; ν_{max} 3272 (N-H), 1674, 1658 (C=O), 1525, 1339, 1252, 1116, 699 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, CDCl$_3$) 8.68 (1H, bs, CON*H*NH), 7.25 (1H, d, *J* 7, Ar-*H*), 7.10 (1H, d, *J* 7, Ar-*H*), 7.00 (1H, s, Ar-*H*), 6.94 (1H, dd, *J* 8, 2, Ar-*H*), 5.48 (1H, bs, BocN*H*CH$_2$), 3.88 (2H, d, *J* 5, BocNHC*H*$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, CDCl$_3$) 170.4 (*C*=ONHNH), 156.6 *(C*=ONH), 148.1 (*ipso*-Ar-*C*), 131.7 (*ipso*-Ar-*C)*, 129.6 (Ar-*C)*, 125.4 (*C*F$_3$*)*, 117.5 (Ar-*C)*, 116.4 (Ar-*C)*, 109.7 (Ar-*C)*, 80.9 ((CH$_3$)$_3$*C*O), 43.3 (BocNH*C*H$_2$), 28.2 (*C*H$_3$)$_3$CO); δ_F (300 MHz, CDCl$_3$) -62.8 (C*F*$_3$); ***m/z*** (ES$^+$) 356 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 356.1203 ($C_{14}H_{18}N_3O_3F_3Na$ requires 356.1193).

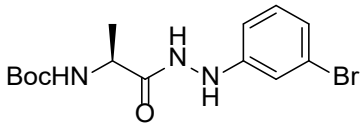
tert-Butyl (2-(2-(3-nitrophenyl)hydrazineyl)-2-oxoethyl)carbamate 67Dc



Following the general procedure outlined, *N*-Boc-glycine (0.40 g, 2.28 mmol) and 3-nitrophenylhydrazine hydrochloride (0.48 g, 2.51 mmol) were transformed following work up with Et$_2$O into the title compound which was isolated as a yellow solid (0.59 g, 83%) as a mixture of rotamers [7:1]; R$_f$ 0.39 (*n*-hex/EtOAc 4:1); m.p. 116 – 119 °C; ν_{max} 3334, 3237 (N-H), 1709, 1684 (C=O), 1516, 1336, 1289, 1163,

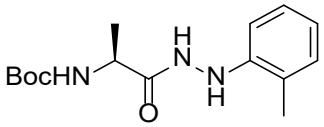
733 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.54 (1H, bs, CON*H*NH), 7.71 (1H, m, Ar-*H*), 7.61 (1H, t, *J* 2, Ar-*H*), 7.35 (1H, t, *J* 8, Ar-*H*), 7.11 (1H, m, Ar-*H*), 6.58 (1H, bd, *J* 2, Ar-N*H*), 5.40 (1H, bs, BocN*H*CH_2), 3.94 (2H, d, *J* 6, BocNHC*H*$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 170.4 (*C*=ONHNH), 156.5 *(C*=ONH), 149.1 (*ipso*-Ar-*C*), 149.0 (*ipso*-Ar-*C)*, 129.9 (Ar-*C)*, 119.2 (Ar-*C)*, 115.7 (Ar-*C)*, 107.6 (Ar-*C)*, 81.2 ((CH$_3$)$_3$*C*O), 43.5 (BocNH*C*H$_2$), 28.3 (*C*H$_3$)$_3$CO); *m/z* (ES^+) 333 (MNa^+); HRMS (ES^+) Found MNa^+, 333.1182 ($C_{13}H_{18}N_4O_5Na$ requires 333.1169).

tert-Butyl (*S*)-(1-(2-(3-bromophenyl)hydrazineyl)-1-oxopropan-2-yl) carbamate 67Cl



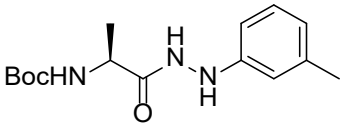
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.40 g, 2.11 mmol) and 3-bromophenylhydrazine hydrochloride (0.52 g, 2.33 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange gummy solid (0.53 g, 70%); R_f 0.31 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3425, 3278 (N-H), 2980 (C-H), 1689, 1676 (C=O), 1504, 1480, 1245, 1158, 778 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.73 (1H, bs, CON*H*NH), 7.01 (1H, s, Ar-*H*), 6.97 (1H, t, *J* 1, Ar-*H*), 6.91 (1H, t, *J* 1, Ar-*H*), 6.69 (1H, m, Ar-*H*), 6.35 (1H, d, *J* 3, Ar-N*H*), 5.33 (1H, m, BocN*H*CH), 4.29 (1H, t, *J* 7, BocNHC*H*), 1.45 (9H, s, (C*H*$_3$)$_3$CO) 1.37 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.4 (*C*=ONHNH), 155.9 *(C*=ONH), 149.2 (*ipso*-Ar-*C)*, 130.5 (Ar-*C)*, 123.7 (Ar-*C)*, 116.1 (Ar-*C)*, 112.0 (Ar-*C)*, 80.7 ((CH*3*)$_3$*C*O), 43.6 (BocNH*C*H), 28.4 ((*C*H*3*)$_3$CO), 17.9 (NHCH*C*H$_3$); *m/z* (ES^+) 380 ([^{79}Br] MNa^+), 382 ([^{81}Br] MNa^+), 737 ([^{79,79}Br] $2MNa^+$); 739 ([^{79,81}Br] $2MNa^+$), 741 ([^{81,81}Br] $2MNa^+$); HRMS (ES^+) Found [^{79}Br]MNa^+, 380.0591 ($C_{14}H_{20}{}^{79}BrN_3O_3Na$ requires 380.0580).

tert-Butyl (*S*)-(1-oxo-1-(2-(o-tolyl)hydrazineyl)propan-2-yl)carbamate 67Dp



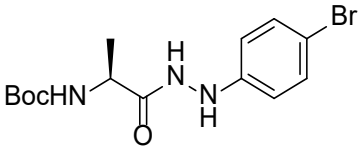
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.10 g, 0.54 mmol) and 2-methylphenylhydrazine hydrochloride (0.07 g, 0.57 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange solid (0.09 g, 61%); R_f 0.39 (DCM/EtOH/NH_3 200:6:1); m.p. 134 - 136 °C; ν_{max} 3351, 3279 (N-H), 2980 (C-H), 1673, 1606 (C=O), 1534, 1479, 1286, 1152, 752, 627 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.75 (1H, bs, CON*H*NH), 7.67 (1H, ddd, *J* 8, 16, 31, Ar-*H*), 7.05 (2H, m, Ar-*H*), 6.79 (1H, ddd, *J* 5, 7, 20, Ar-*H*), 5.39 (1H, bd, *J* 8, BocN*H*CH), 4.33 (1H, m, BocNHC*H*), 2.18 (3H, s, Ar-CH_3), 1.43 (9H, s, (CH_3)$_3$CO), 1.36 (3H, d, *J* 8, BocNHCHCH_3); δ_C (100 MHz, $CDCl_3$) 173.0 (*C*=ONHNH), 155.8 *(C*=ONH), 145.5 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 126.9 (*ipso*-Ar-*C*), 123.2 (*ipso*-Ar-*C*), 120.9 (Ar-*C*), 112.0 (Ar-*C*), 80.4 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.4 (*C*H$_3$)$_3$CO), 18.2 (Ar-*C*H$_3$), 17.0 (BocNHCH*C*H$_3$); *m/z* (ES$^+$) 332 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 332.1290 ($C_{15}H_{23}N_3O_3Na$ requires 332.1275).

tert-Butyl (*S*)-(1-oxo-1-(2-(m-tolyl)hydrazineyl)propan-2-yl)carbamate 67Dv



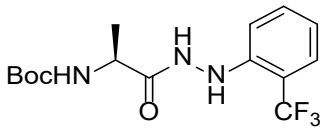
Following the general procedure outlined, *N*-Boc-*L*-alanine (0.30 g, 1.59 mmol) and 3-methylphenylhydrazine hydrochloride (0.28 g, 1.74 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a dark orange oil (0.30 g, 64%); R_f 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3313 (N-H), 2982 (C-H), 1683, 1660 (C=O), 1505, 1366, 1249, 1160, 1060, 779 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.61 (1H, bs, CON*H*NH), 7.06 (2H, m, Ar-*H*), 6.68 (1H, d, *J* 7, Ar-*H*), 6.58 (1H, d, *J* 9, Ar-*H*), 5.33 (1H, bs, BocN*H*CH), 4.30 (1H, m, BocNHC*H*), 2.25 (3H, s, Ar-CH_3), 1.45 (9H, s, (CH_3)$_3$CO), 1.35 (3H, d, *J* 7, BocNHCHCH_3); δ_C (100 MHz, $CDCl_3$) 173.1 (*C*=ONHNH), 155.8 *(C*=ONH), 147.9 (*ipso*-Ar-*C*), 129.1 (*ipso*-Ar-*C*), 122.1 (Ar-*C*), 114.3 (Ar-*C*), 110.7 (Ar-*C*), 80.5 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.5 (*C*H$_3$)$_3$CO), 21.6 (Ar-*C*H$_3$), 18.1 (BocNHCH*C*H$_3$); *m/z* (ES$^+$) 316 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 316.1643 ($C_{15}H_{23}N_3O_3Na$ requires 316.1632).

tert-Butyl (*S*)-(1-(2-(4-bromophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate 67Cr



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.40 g, 2.11 mmol) and 4-bromophenylhydrazine hydrochloride (0.52 g, 2.33 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.33 g, 51%); R_f 0.37 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3316, 3302 (N-H), 2987, 2937 (C-H), 1683, 1663 (C=O), 1520, 1486, 1320, 1249, 1161, 812 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.43 (1H, bs, CON*H*NH), 7.30 – 7.27 (2H, d, *J* 9, Ar-*H*), 6.69 – 6.66 (2H, d, *J* 8, Ar-*H*), 6.15 (1H, bs, Ar-N*H*), 5.12 (1H, bd, *J* 7, BocN*H*CH), 4.28 (1H, m, BocNHC*H*), 1.46 (9H, s, (C*H*$_3$)$_3$CO), 1.39 (3H, d, *J* 7, BocNHCHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.9 (*C*=ONHNH), 155.8 *(C*=ONH), 146.9 (*ipso*-Ar-*C*), 131.9 (Ar-*C*), 129.2 (*ipso*-Ar-*C*), 115.1 (Ar-*C*), 113.1 (*ipso*-Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 48.6 (BocNH*C*H), 28.3 (*C*H$_3$)$_3$CO), 17.6 (BocNHCH*C*H$_3$); *m/z* (ES^+) 380 ([^{79}Br]MNa^+), 382 ([^{81}Br]MH^+); HRMS (ES^+) Found [^{79}Br]MNa^+, 380.0589 ($C_{14}H_{20}{}^{79}BrN_3O_3Na$ requires 380.0580).

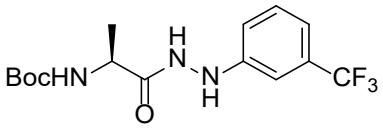
tert-Butyl (*S*)-(1-oxo-1-(2-(2-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate 67Az



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.40 g, 2.11 mmol) and 2-trifluoromethylphenylhydrazine hydrochloride (0.49 g, 2.33 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow gummy solid (0.52 g, 71%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3311 (N-H), 2982 (C-H), 1696, 1657 (C=O), 1512, 1487, 1322, 1252, 1158, 1097, 757 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.47 (1H, bs, CON*H*NH), 7.50 (1H, d, *J* 7, Ar-*H*), 7.38 (1H, t, *J* 7, Ar-*H*), 7.00 (1H, d, *J* 8, Ar-*H*), 6.97 (1H, t, *J* 7, Ar-*H*), 6.53 (1H, bs, Ar-N*H*), 5.16 (1H, bd, *J* 7, BocN*H*CH), 4.34 (1H, m, BocNHC*H*), 1.46 (9H, s, (C*H*$_3$)$_3$CO), 1.43 (3H, d, *J* 7, BocNHCHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.7 (*C*=ONHNH), 155.9 *(C*=ONH), 145.4 (*ipso*-Ar-*C*), 133.0 (Ar-*C*), 126.6 (Ar-*C*),

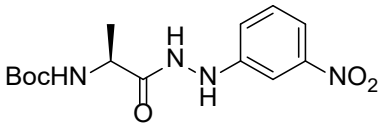
120.2 (Ar-*C*), 113.4 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.3 (*C*H$_3$)$_3$CO), 17.5 (BocNHCH*C*H$_3$); δ_F (300 MHz, CDCl$_3$) -61.8 (C*F*$_3$); *m/z* (ES$^+$) 370 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 370.1361 ($C_{15}H_{20}N_3O_3F_3Na$ requires 370.1349).

tert-Butyl (*S*)-(1-oxo-1-(2-(3-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate 67Bf



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.40 g, 2.11 mmol) and 3-trifluoromethylphenylhydrazine hydrochloride (0.49 g, 2.33 mmol) were transformed following work up with Et$_2$O into the title compound which was isolated as a yellow solid (0.51 g, 69%); R_f 0.28 (DCM/EtOH/NH$_3$ 200:6:1); m.p. 105 – 108 °C; ν_{max} 3328, 3265 (N-H), 2987 (C-H), 1683, 1668 (C=O), 1520, 1335, 1249, 1159, 1072, 785 cm^{-1}; δ_H (300 MHz, CDCl$_3$) 8.61 (1H, bs, CON*H*NH), 7.30 (1H, t, *J* 7, Ar-*H*), 7.12 (1H, d, *J* 7, Ar-*H*), 7.01 (1H, s, Ar-*H*), 6.96 (1H, dd, *J* 8, 2, Ar-*H*), 6.37 (1H, bs, Ar-N*H*), 5.16 (1H, bd, *J* 7, BocN*H*CH), 4.32 (1H, m, BocNHC*H*), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 1.40 (3H, d, *J* 7, BocNHCHC*H*$_3$); δ_C (100 MHz, CDCl$_3$) 173.2 (*C*=ONHNH), 155.9 *(C*=ONH), 148.3 (*ipso*-Ar-*C*), 131.7 (Ar-*C*F$_3$), 129.6 (Ar-*C*), 125.9 (Ar-*C*F$_3$), 117.6 (Ar-*C*), 116.5 (Ar-*C*), 109.8 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 48.7 (BocNH*C*H), 28.3 (*C*H$_3$)$_3$CO), 17.6 (BocNHCH*C*H$_3$); δ_F (300 MHz, CDCl$_3$) -62.8 (C*F*$_3$); *m/z* (ES$^+$) 370 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 370.1365 ($C_{15}H_{20}N_3O_5F_3Na$ requires 370.1349).

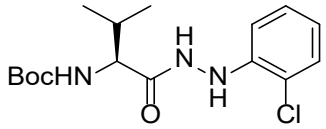
tert-Butyl (*S*)-(1-(2-(3-nitrophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate 67Dd



Following the general procedure outlined, *N*-Boc-*L*-alanine (0.40 g, 2.11 mmol) and 3-nitrophenylhydrazine hydrochloride (0.44 g, 2.33 mmol) were transformed following work up with Et$_2$O into the title compound which was isolated as a yellow gummy solid (0.53 g, 78%); R_f 0.23 (DCM/EtOH/NH$_3$ 200:6:1); ν_{max} 3345, 3328, 3242 (N-H), 3003 (C-H), 1706, 1672 (C=O), 1509, 1343, 1302, 1253, 1160, 734

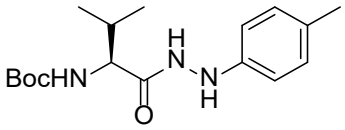
cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.54 (1H, bs, CON*H*NH), 7.72 (1H, dd, *J* 7, 1, Ar-*H*), 7.62 (1H, t, *J* 2, Ar-*H*), 7.36 (1H, t, *J* 8, Ar-*H*), 7.12 (1H, dd, *J* 8, 2, Ar-*H*), 6.43 (1H, bd, *J* 3, Ar-N*H*), 5.08 (1H, bd, *J* 7, BocN*H*CH), 4.35 (1H, m, BocNHC*H*), 1.48 (9H, s, (C*H*$_3$)$_3$CO), 1.44 (3H, d, *J* 7, BocNHCHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.1 (*C*=ONHNH), 155.9 *(C*=ONH), 149.1 (*ipso*-Ar-*C*), 129.9 (Ar-*C*), 119.3 (Ar-*C*), 117.9 (*ipso*-Ar-*C*), 115.8 (Ar-*C*), 107.7 (Ar-*C*), 80.9 ((CH$_3$)$_3$*C*O), 48.6 (BocNH*C*H), 28.3 (*C*H$_3$)$_3$CO), 17.2 (BocNHCH*C*H$_3$); *m/z* (ES^+) 347 (MNa^+); HRMS (ES^+) Found MNa^+, 347.1344 ($C_{14}H_{20}N_4O_5Na$ requires 347.1334).

tert-Butyl (*S*)-(1-(2-(2-chlorophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Ai



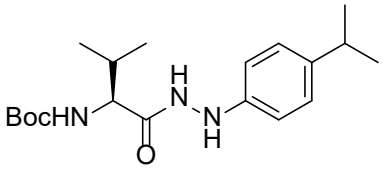
Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 2-chlorophenylhydrazine hydrochloride (0.36 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange solid (0.45 g, 71%); R_f 0.36 (DCM/EtOH/NH_3 200:6:1); m.p. 108 - 111 °C; ν_{max} 3318 (N-H), 2970 (C-H), 1661, 1651 (C=O), 1526, 1485, 1303, 1248, 1171, 745, 646 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.59 (1H, bs, CON*H*NH), 7.26 (1H, dd, *J* 8, 1, Ar-*H*), 7.09 (1H, td, *J* 8, 1, Ar-*H*), 6.88 (1H, dd, *J* 8, 1, Ar-*H*), 6.80 (1H, td, *J* 7, 1, Ar-*H*), 5.28 (1H, bd, *J* 8, BocN*H*CH), 4.02 (1H, dd, *J* 7, 1, BocNHC*H*), 2.13 (1H, m, CHC*H*(CH$_3$)$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO), 1.00 – 0.96 (6H, m, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.0 (*C*=ONHNH), 156.2 (*C*=ONH), 143.7 (*ipso*-Ar-*C*), 129.4 (Ar-*C*), 127.6 (Ar-*C*), 121.3 (Ar-*C*), 113.7 (Ar-*C*), 80.5 ((CH$_3$)$_3$*C*O), 58.8 (BocNH*C*H), 30.5 (CH*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 19.4 (CHCH(*C*H$_3$)$_2$), 18.3 (CHCH(*C*H$_3$)$_2$); *m/z* (ES^+) 364 ([^{35}Cl]MNa^+), 366 ([^{37}Cl]MNa^+), 705 ([^{35,35}Cl]2M+Na^+), 707 ([^{35,37}Cl]2M+Na^+), 709 ([^{37,37}Cl]2M+Na^+); HRMS (ES^+) Found [^{35}Cl]MNa^+, 364.1415 ($C_{16}H_{24}{}^{35}ClN_3O_3Na$ requires 364.1404).

tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-(p-tolyl)hydrazinyl)butan-2-yl)carbamate 67Ec



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 4-methylphenylhydrazine hydrochloride (0.32 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.52 g, 88%) as a mixture of rotamers [3:1]; R_f 0.39 (DCM/EtOH/NH_3 200:6:1); m.p. 126 - 129 °C; ν_{max} 3334, 3297 (N-H), 2970 (C-H), 1686, 1650 (C=O), 1523, 1365, 1245, 1162, 1020, 818, 646 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.65 (1H, bs, CON*H*NH), 7.00 – 6.97 (2H, d, *J* 8, Ar-*H*), 6.73 – 6.70 (2H, d, *J* 8, Ar-*H*), 5.36 (1H, bd, *J* 8, BocN*H*CH), 4.02 (1H, t, *J* 7, BocNHC*H*), 2.24 (3H, s, Ar-CH_3), 2.11 (1H, m, CHC*H*$(CH_3)_2$), 1.44 (9H, s, $(CH_3)_3CO$), 0.97 – 0.93 (6H, m, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 156.2 (*C*=ONH), 145.3 (*ipso*-Ar-*C*), 129.6 (Ar-*C*), 113.9 (Ar-*C*), 80.4 ($(CH_3)_3CO$), 58.8 (BocNH*C*H), 30.5 (CH*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 20.6 (Ar-CH_3), 19.3 (CHCH$(CH_3)_2$), 17.5 (CHCH$(CH_3)_2$); ***m/z*** (ES^+) 344 (MNa^+), 665 ($2M+Na^+$); HRMS (ES^+) Found MNa^+, 344.1967 ($C_{17}H_{27}N_3O_3Na$ requires 344.1945).

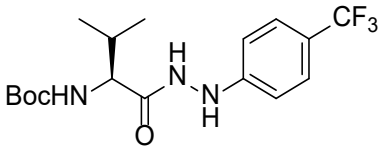
tert-Butyl (*S*)-(1-(2-(4-isopropylphenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Ei



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 4-isopropylphenylhydrazine hydrochloride (0.38 g, 2.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange solid (0.37 g, 58%) as a mixture of rotamers [4:1]; R_f 0.42 (DCM/EtOH/NH_3 200:6:1); m.p. 88 - 91 °C; ν_{max} 3328 (N-H), 2959 (C-H), 1687, 1657 (C=O), 1513, 1304, 1248, 1168, 830 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.88 (1H, bs, CON*H*NH), 7.04 – 7.01 (2H, d, *J* 8, Ar-*H*), 6.74 – 6.72 (2H, d, *J* 8, Ar-*H*), 5.44 (1H, bd, *J* 9, BocN*H*CH), 4.03 (1H, t, *J*

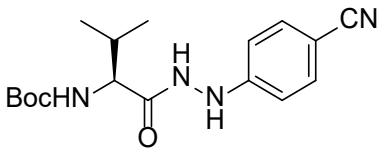
7, BocNHC*H*), 2.84 (1H, m, C*H*(CH_3)$_2$), 2.09 (1H, m, CHC*H*(CH_3)$_2$), 1.43 (9H, s, (C*H*$_3$)$_3$CO), 1.19 – 1.17 (6H, d, *J* 7, CH(C*H*$_3$)$_2$) 0.94 – 0.89 (6H, m, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 156.2 (*C*=ONH), 145.6 (*ipso*-Ar-*C*), 141.6 (*ipso*-Ar-*C*), 129.9 (Ar-*C*), 113.8 (Ar-*C*), 80.2 ((CH$_3$)$_3$*C*O), 58.7 (BocNH*C*H), 33.3 (*C*H(CH$_3$)$_2$), 30.8 (CH*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.2 (CH(*C*H$_3$)$_2$), 19.3 (CHCH(*C*H$_3$)$_2$), 18.2 (CHCH(*C*H$_3$)$_2$); ***m/z*** (ES$^+$) 372 (MNa$^+$), 721 (2M+Na$^+$); HRMS (ES$^+$) Found MNa$^+$, 372.2285 ($C_{19}H_{31}N_3O_3Na$ requires 372.2258).

tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazinyl) butan-2-yl)carbamate 67Bm



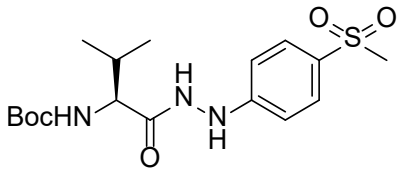
Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 4-trifluoromethylphenylhydrazine hydrochloride (0.43 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.43 g, 62%); R_f 0.41 (DCM/EtOH/NH_3 200:6:1); m.p. 114 - 116 °C; ν_{max} 3322, 3255 (N-H), 1683, 1661 (C=O), 1521, 1507, 1267, 1251, 1159 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.75 (1H, bs, CON*H*NH), 7.03 – 7.00 (2H, d, *J* 8, Ar-*H*), 6.80 – 6.77 (2H, d, *J* 8, Ar-*H*), 5.31 (1H, bd, *J* 8, BocN*H*CH), 4.02 (1H, m, BocNHC*H*), 2.11 (1H, m, CHC*H*(CH$_3$)$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO), 0.97 – 0.95 (6H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 156.2 (*C*=ONH), 146.5 (*ipso*-Ar-*C*), 143.1 (*ipso*-Ar-*C*), 122.1 (Ar-*C*), 114.2 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 58.8 (BocNH*C*H), 30.5 (CH*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 19.3 (CHCH(*C*H$_3$)$_2$), 18.2 (CHCH(*C*H$_3$)$_2$); δ_F (300 MHz, $CDCl_3$) -58.4 (C*F*$_3$); ***m/z*** (ES$^+$) 414 (MK$^+$); HRMS (ES$^+$) Found MK$^+$, 414.1632 ($C_{17}H_{24}N_3O_3F_3$ requires 414.1630).

tert-Butyl (*S*)-(1-(2-(4-cyanophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Cy



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 4-cyanophenylhydrazine hydrochloride (0.34 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.32 g, 52%); R_f 0.34 (DCM/EtOH/NH_3 200:6:1); m.p. 124 - 126 °C; ν_{max} 3288 (N-H), 2970(C-H), 2223 (C≡N), 1667, 1607 (C=O), 1507, 1366, 1244, 1168, 829, 648 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.71 (1H, bs, CON*H*NH), 7.44 – 7.41 (2H, d, *J* 8, Ar-*H*), 6.79 – 6.77 (2H, d, *J* 8, Ar-*H*), 6.66 (1H, bs, Ar-N*H*), 5.24 (1H, bd, *J* 9, BocN*H*CH), 4.02 (1H, t, *J* 8, BocNHC*H*), 2.13 (1H, m, CHC*H*$(CH_3)_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 0.99 – 0.97 (6H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 156.3 (*C*=ONH), 151.5 (*ipso*-Ar-*C*), 133.6 (Ar-*C*), 119.5 (*C*≡N), 112.9 (Ar-*C*), 103.0 (*ipso*-Ar-*C*), 80.7 ((CH$_3$)$_3$*C*O), 58.8 (BocNH*C*H), 30.4 (CH*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 19.4 (CHCH(*C*H$_3$)$_2$), 18.3 (CHCH(*C*H$_3$)$_2$); *m/z* (ES^+) 355 (MNa^+); HRMS (ES^+) Found MNa^+, 355.1748 ($C_{17}H_{24}N_4O_3Na$ requires 355.1741).

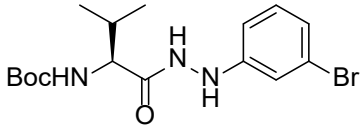
tert-Butyl (*S*)-(3-methyl-1-(2-(4-(methylsulfonyl)phenyl)hydrazineyl)-1-oxobutan-2-yl)carbamate 67Dk



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 4-(methylsulphonyl)phenylhydrazine hydrochloride (0.45 g, 2.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.42 g, 59%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 155 - 158 °C; ν_{max} 3299 (N-H), 2977 (C-H), 1668 (C=O), 1598, 1505, 1288, 1136, 1088, 769, 509 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.60 (1H, bs, CON*H*NH), 7.70 – 7.67 (2H, d, *J* 8, Ar-*H*), 6.85 – 6.82 (2H, d, *J* 8, Ar-*H*), 6.64 (1H, bs, Ar-N*H*), 5.20 (1H,

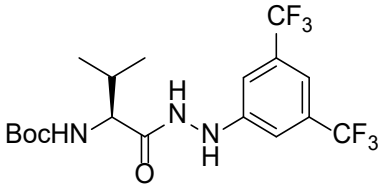
bd, *J* 8, BocN*H*CH), 4.03 (1H, dd, *J* 7, 1, BocNHC*H*), 3.00 (3H, s, SO_2CH_3), 2.19 (1H, m, CHC*H*$(CH_3)_2$), 1.46 (9H, s, $(CH_3)_3CO$), 1.01 – 0.98 (6H, dd, *J* 6, 4, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.4 (*C*=ONHNH), 156.2 (*C*=ONH), 152.4 (*ipso*-Ar-*C*), 131.4 (*ipso*-Ar-*C*), 129.1 (Ar-*C*),112.7 (Ar-*C*), 80.7 ($(CH_3)_3CO$), 58.9 (BocNH*C*H), 44.8 (SO_2CH_3), 30.4 (CH*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 19.4 (CHCH$(CH_3)_2$), 18.2 (CHCH$(CH_3)_2$); *m/z* (ES^+) 408 (MNa^+); HRMS (ES^+) Found MNa^+, 408.1575 ($C_{17}H_{27}N_3O_5SNa$ requires 408.1563).

tert-Butyl (*S*)-(1-(2-(3-bromophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Cm



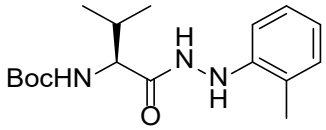
Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 3-bromophenylhydrazine hydrochloride (0.45 g, 2.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.32 g, 45%); R_f 0.29 (DCM/EtOH/NH_3 200:6:1); m.p. 107 - 110 °C; ν_{max} 3359, 3259 (N-H), 2972 (C-H), 1684, 1664 (C=O), 1523, 1471, 1306, 1246, 1169, 765, 649 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.55 (1H, bs, CON*H*NH), 7.03 (1H, d, *J* 7, Ar-*H*), 6.99 (1H, t, *J* 1, Ar-*H*), 6.96 (1H, m, Ar-*H*), 6.73 (1H, dt, *J* 7, 1, Ar-*H*), 6.28 (1H, bs, Ar-N*H*), 5.22 (1H, bd, *J* 8, BocN*H*CH), 4.02 (1H, dd, *J* 7, 1, BocNHC*H*), 2.17 (1H, m, CHC*H*$(CH_3)_2$), 1.45 (9H, s, $(CH_3)_3CO$), 0.99 – 0.97 (6H, d, *J* 6, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 156.2 (*C*=ONH), 149.3 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 123.9 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 116.4 (Ar-*C*), 112.2 (Ar-*C*), 80.5 ($(CH_3)_3CO$), 58.7 (BocNH*C*H), 30.6 (CH*C*H$(CH_3)_2$), 28.4 ($(CH_3)_3CO$), 19.4 (CHCH$(CH_3)_2$), 18.2 (CHCH$(CH_3)_2$); *m/z* (ES^+) 408 ($[^{79}Br]MNa^+$), 410 ($[^{81}Br]MNa^+$); HRMS (ES^+) Found $[^{79}Br]MNa^+$, 408.0903 ($C_{16}H_{24}N_3O_3{}^{79}BrNa$ requires 408.0893).

tert-Butyl (*S*)-(1-(2-(3,5-bis(trifluoromethyl)phenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Bu



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and (3,5-bis(trifluoromethyl)phenyl)hydrazine hydrochloride (0.57 g, 2.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.37 g, 46%); R_f 0.35 (DCM/EtOH/NH_3 200:6:1); m.p. 142 - 144 °C; ν_{max} 3337 (N-H), 1671, 1660 (C=O), 1524, 1373, 1277, 1167, 1121, 877, 682 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.55 (1H, bs, CON*H*NH), 7.34 (1H, s, Ar-*H*), 6.21 (2H, s, Ar-*H*), 6.58 (1H, bs, Ar-N*H*), 5.12 (1H, bd, *J* 8, BocN*H*CH), 4.02 (1H, d, *J* 7, BocNHC*H*), 2.18 (1H, m, CHC*H*$(CH_3)_2$), 1.45 (9H, s, $(CH_3)_3CO$), 1.02 – 0.99 (6H, d, *J* 6, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.6 (*C*=ONHNH), 156.2 (*C*=ONH), 149.1 (*ipso*-Ar-*C*), 132.7 (Ar-*C*F_3, t, *J* 33), 125.0 (*ipso*-Ar-*C*), 121.3 (*ipso*-Ar-*C*), 114.1 (Ar-*C*), 112.9 (Ar-*C*), 80.7 ($(CH_3)_3CO$), 58.7 (BocNH*C*H), 30.4 (CH*C*H$(CH_3)_2$), 28.2 ($(CH_3)_3CO$), 19.2 (CHCH(CH_3)$_2$), 18.1 (CHCH(CH_3)$_2$); δ_F (300 MHz, $CDCl_3$) -63.3 (C*F*$_3$); *m/z* (ES^+) 466 (MNa^+); HRMS (ES^+) Found MNa^+, 466.1547 ($C_{18}H_{23}N_3O_3F_6Na$ requires 466.1536).

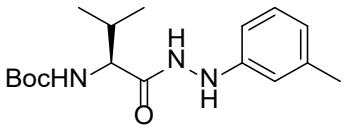
tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-(o-tolyl)hydrazineyl)butan-2-yl) carbamate 67Dq



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 2-methylphenylhydrazine hydrochloride (0.32 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a dark red gummy solid (0.47 g, 80%); R_f 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3319 (N-H), 2967 (C-H), 1679, 1643 (C=O), 1525, 1494, 1304, 1247, 1171, 747, 647 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.47 (1H, bs, CON*H*NH), 7.06 – 7.04 (2H, d, *J* 7, Ar-*H*), 6.83 – 6.81 (2H, d, *J* 7, Ar-*H*), 5.24 (1H, bd, *J* 9, BocN*H*CH), 4.04 (1H, dd, *J* 7, 1,

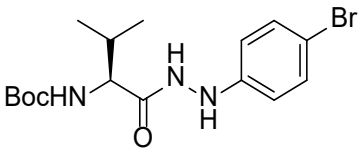
BocNHC*H*), 2.21 (3H, s, Ar-CH_3), 2.16 (1H, m, CHC*H*(CH_3)$_2$), 1.44 (9H, s, (CH_3)$_3$CO), 0.99 – 0.97 (6H, m, CHCH(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 171.7 (*C*=ONHNH), 156.1 (*C*=ONH), 145.5 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 126.8 (Ar-*C*), 123.2 (*ipso*-Ar-*C*), 120.9 (Ar-*C*), 112.2 (Ar-*C*), 80.3 ((CH_3)$_3$*C*O), 58.8 (BocNH*C*H), 30.6 (CH*C*H(CH_3)$_2$), 28.3 ((CH$_3$)$_3$CO), 19.4 (CHCH(*C*H$_3$)$_2$), 18.2 (CHCH(*C*H$_3$)$_2$), 17.0 (Ar-CH_3); *m/z* (ES^+) 344 (MNa^+); HRMS (ES^+) Found MNa^+, 344.1964 ($C_{17}H_{27}N_3O_3Na$ requires 344.1945).

tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-(m-tolyl)hydrazineyl)butan-2-yl) carbamate 67Dw



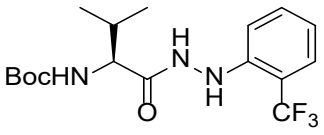
Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 3-methylphenylhydrazine hydrochloride (0.32 g, 2.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.29 g, 50%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 146 - 148 °C; ν_{max} 3325, 3269 (N-H), 2970 (C-H), 1685, 1664 (C=O), 1524, 1478, 1306, 1246, 1168, 693 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.87 (1H, bs, CON*H*NH), 7.07 (1H, t, *J* 7, Ar-*H*), 6.68 (1H, d, *J* 7, Ar-*H*), 6.59 – 6.57 (2H, m, Ar-*H*), 6.25 (1H, bd, *J* 3, Ar-N*H*), 5.41 (1H, bd, *J* 9, BocN*H*CH), 4.06 (1H, t, *J* 8, BocNHC*H*), 2.22 (3H, s, Ar-CH_3), 2.08 (1H, m, CHC*H*(CH_3)$_2$), 1.43 (9H, s, (CH_3)$_3$CO), 0.94 – 0.92 (6H, d, *J* 6, CHCH(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 171.2 (*C*=ONHNH), 156.1 (*C*=ONH), 147.9 (*ipso*-Ar-*C*), 138.9 (*ipso*-Ar-*C*), 128.9 (Ar-*C*), 121.9 (Ar-*C*), 114.4 (Ar-*C*), 110.7 (Ar-*C*), 80.1 ((CH_3)$_3$*C*O), 58.6 (BocNH*C*H), 30.9 (CH*C*H(CH_3)$_2$), 28.4 ((CH$_3$)$_3$CO), 21.5 (Ar-CH_3), 19.3 (CHCH(*C*H$_3$)$_2$), 18.2 (CHCH(*C*H$_3$)$_2$); *m/z* (ES^+) 344 (MNa^+); HRMS (ES^+) Found MNa^+, 344.1970 ($C_{17}H_{27}N_3O_3Na$ requires 344.1945).

tert-Butyl (*S*)-(1-(2-(4-bromophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl) carbamate 67Cs



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 4-bromophenylhydrazine hydrochloride (0.45 g, 2.03 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid (0.41 g, 58%); as a mixture of rotamers [9:1]; R_f 0.41 (DCM/EtOH/NH_3 200:6:1); m.p. 127 - 129 °C; ν_{max} 3323, 3284 (N-H), 2972 (C-H), 1686, 1651 (C=O), 1521, 1489, 1245, 1164, 826, 634 cm^{-1}; all data provided for the major rotamer; δ_H (300 MHz, $CDCl_3$) 8.33 (1H, bs, CON*H*NH), 7.31 – 7.28 (2H, d, *J* 9, Ar-*H*), 6.73 – 6.70 (2H, d, *J* 8, Ar-*H*), 6.28 (1H, bs, Ar-N*H*), 5.15 (1H, bd, *J* 8, BocN*H*CH), 4.00 (1H, dd, *J* 7, 1, BocNHC*H*), 2.18 (1H, m, CHC*H*$(CH_3)_2$), 1.48 (9H, s, $(CH_3)_3CO$), 1.01 – 0.98 (6H, m, CHCH$(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 155.9 (*C*=ONH), 146.9 (*ipso*-Ar-*C*), 131.9 (Ar-*C*), 115.3 (Ar-*C*), 80.4 ($(CH_3)_3CO$), 58.7 (BocNH*C*H), 30.3 (CH*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 19.4 (CHCH$(CH_3)_2$), 18.2 (CHCH$(CH_3)_2$); *m/z* (ES^+) 408 ($[^{79}Br]MNa^+$), 410 ($[^{81}Br]MNa^+$) ; HRMS (ES^+) Found $[^{79}Br]MNa^+$, 408.0906 ($C_{16}H_{24}N_3O_3{}^{79}BrNa$ requires 408.0893).

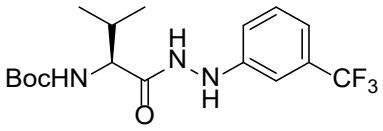
tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-(2(trifluoromethyl)phenyl)hydrazineyl) butan-2-yl)carbamate 67Ba



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 2-trifluoromethylphenylhydrazine hydrochloride (0.43 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.48 g, 70%); R_f 0.35 (DCM/EtOH/NH_3 200:6:1); m.p. 114 - 116 °C; ν_{max} 3327 (N-H), 2972 (C-H), 1679, 1652 (C=O), 1526, 1496, 1323, 1288, 1107, 1036, 753 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.44 (1H, bs, CON*H*NH), 7.49

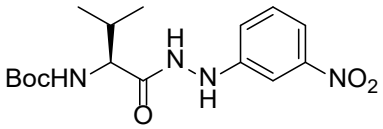
(1H, d, *J* 8, Ar-*H*), 7.35 (1H, t, *J* 7, Ar-*H*), 7.01 (1H, d, *J* 8, Ar-*H*), 6.93 (1H, t, *J* 7, Ar-*H*), 6.58 (1H, bs, Ar-N*H*), 5.22 (1H, bd, *J* 8, BocN*H*CH), 4.05 (1H, m, BocNHC*H*), 2.16 (1H, m, CHC*H*(CH_3)$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO), 1.03 – 0.98 (6H, m, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 156.2 (*C*=ONH), 145.4 (*ipso*-Ar-*C*), 132.9 (Ar-*C*), 126.5 (Ar-*C*), 120.1 (Ar-*C*), 117.7 (*ipso*-Ar-*C*), 113.5 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 58.8 (BocNH*C*H), 30.4 (CH*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 19.4 (CHCH(*C*H$_3$)$_2$), 18.3 (CHCH(*C*H$_3$)$_2$); δ_F (300 MHz, $CDCl_3$) -61.9 (C*F*$_3$); *m/z* (ES$^+$) 398 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 398.1685 ($C_{17}H_{24}N_3O_3F_3Na$ requires 398.1662).

tert-Butyl (*S*)-(3-methyl-1-oxo-1-(2-(3(trifluoromethyl)phenyl)hydrazineyl) butan-2-yl)carbamate 67Bg



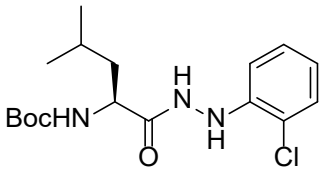
Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 3-trifluoromethylphenylhydrazine hydrochloride (0.43 g, 2.03 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a light brown solid (0.29 g, 42%); R_f 0.37 (DCM/EtOH/NH_3 200:6:1); m.p. 117 - 119 °C; ν_{max} 3322, 3252 (N-H), 1683, 1663 (C=O), 1524, 1333, 1120, 1162, 693 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.52 (1H, bs, CON*H*NH), 7.33 (1H, t, *J* 7, Ar-*H*), 7.15 (1H, d, *J* 7, Ar-*H*), 7.07 (1H, t, *J* 1, Ar-*H*), 7.00 (1H, dd, *J* 8, 2, Ar-*H*), 5.19 (1H, bd, *J* 9, BocN*H*CH), 4.06 (1H, dd, *J* 7, 1, BocNHC*H*), 2.19 (1H, m, CHC*H*(CH_3)$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO), 1.03 – 1.00 (6H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.4 (*C*=ONHNH), 156.2 (*C*=ONH), 148.3 (*ipso*-Ar-*C*), 129.6 (Ar-*C*), 122.2 (*ipso*-Ar-*C*), 117.6 (Ar-*C*), 116.6 (Ar-*C*), 110.0 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 58.8 (BocNH*C*H), 30.5 (CH*C*H(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 19.3 (CHCH(*C*H$_3$)$_2$), 18.1 (CHCH(*C*H$_3$)$_2$); δ_F (300 MHz, $CDCl_3$) -62.9 (C*F*$_3$); *m/z* (ES$^+$) 398 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 398.1677 ($C_{17}H_{24}N_3O_3F_3Na$ requires 398.1662).

tert-Butyl (*S*)-(3-methyl-1-(2-(3-nitrophenyl)hydrazineyl)-1-oxobutan-2-yl)carbamate 67De



Following the general procedure outlined, *N*-Boc-*L*-valine (0.40 g, 1.84 mmol) and 3-nitrophenylhydrazine hydrochloride (0.38 g, 2.03 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.51 g, 79%); R_f 0.49 (DCM/EtOH/NH_3 200:6:1); m.p. 135 - 137 °C; ν_{max} 3333, 3249 (N-H), 2975 (C-H), 1679, 1654 (C=O), 1521, 1481, 1302, 1244, 1159, 1017, 734 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.47 (1H, bs, CON*H*NH), 7.64 (1H, dd, *J* 8, 2, Ar-*H*), 7.57 (1H, t, *J* 2, Ar-*H*), 7.27 (1H, t, *J* 8, Ar-*H*), 7.05 (1H, dd, *J* 8, 2, Ar-*H*), 5.19 (1H, bd, *J* 8, BocN*H*CH), 4.06 (1H, dd, *J* 8, 1, BocNHC*H*), 2.12 (1H, m, CHC*H*$(CH_3)_2$), 1.39 (9H, s, (C*H*$_3)_3$CO), 0.96 – 0.94 (6H, d, *J* 6, CHCH(C*H*$_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 156.2 (*C*=ONH), 149.1 (*ipso*-Ar-*C*), 129.6 (Ar-*C*), 119.3 (Ar-*C*), 115.7 (Ar-*C*), 107.8 (Ar-*C*), 80.7 ((CH$_3)_3$*C*O), 58.8 (BocNH*C*H), 30.4 (CH*C*H$(CH_3)_2$), 28.3 ((*C*H$_3)_3$CO), 19.4 (CHCH(*C*H$_3)_2$), 18.1 (CHCH(*C*H$_3)_2$); *m/z* (ES^+) 375 (MNa^+); HRMS (ES^+) Found MNa^+, 375.1649 ($C_{16}H_{24}N_4O_5$ requires 375.1647).

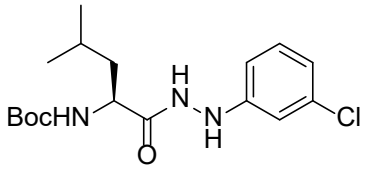
tert-Butyl (*S*)-(1-(2-(2-chlorophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Aj



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.30 g, 1.30 mmol) and 2-chlorophenylhydrazine hydrochloride (0.26 g, 1.43 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.25 g, 54%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 119 - 121 °C; ν_{max} 3334 (N-H), 2956 (C-H), 1722, 1658 (C=O), 1595, 1516, 1369, 1159, 837, 752 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.73 (1H, bs, CON*H*NH), 7.17 (1H, dd, *J* 7, 1, Ar-*H*), 7.02 (1H, td, *J* 8, 1, Ar-*H*), 6.78 (1H, dd, *J* 8, 1, Ar-*H*), 6.73 (1H, td, *J* 7, 1, Ar-*H*), 6.38 (1H, bs, CONHN*H*), 5.17 (1H, bd, *J* 8, BocN*H*CH),

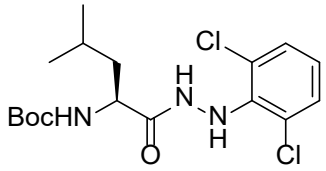
4.25 (1H, m, BocNHC*H*), 1.64 (2H, m, C*H*$_2$CH(CH$_3$)$_2$), 1.48 (1H, m, CH$_2$C*H*(CH$_3$)$_2$), 1.36 (9H, s, (C*H*$_3$)$_3$CO), 0.87 – 0.83 (6H, t, *J* 6, CH$_2$CH(C*H*$_3$)$_2$); δ_C (100 MHz, CDCl$_3$) 172.8 (*C*=ONHNH), 156.1 (*C*=ONH), 143.7 (*ipso*-Ar-*C*), 129.4 (Ar-*C*), 127.6 (Ar-*C*), 121.2 (Ar-*C*), 119.4 (*ipso*-Ar-*C*), 113.5 (Ar-*C*), 80.5 ((CH$_3$)$_3$*C*O), 51.5 (BocNH*C*H), 40.6 (*C*H$_2$CH(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.7 (CH$_2$*C*H(CH$_3$)$_2$), 22.7 (CH$_2$CH(*C*H$_3$)$_2$), 22.2 (CH$_2$CH(*C*H$_3$)$_2$); ***m/z*** (ES$^+$) 378 ([^{35}Cl]MNa$^+$), 380 ([^{37}Cl]MNa$^+$), 733 ([^{35,35}Cl]2M+Na$^+$), 735 ([^{35,37}Cl]2M+Na$^+$), 737 ([^{37,37}Cl]2M+Na$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 378.1567 ($C_{17}H_{26}{}^{35}ClN_3O_3Na$ requires 378.1555).

tert-Butyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-4-methyl-1-oxopentan-2-yl)carbamate 67t



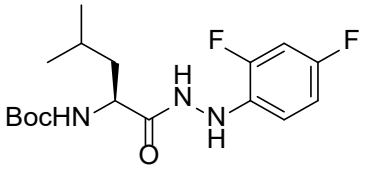
Following the general procedure outlined, *N*-Boc-*L*-leucine (0.30 g, 1.30 mmol) and 3-chlorophenylhydrazine hydrochloride (0.26 g, 1.43 mmol) were transformed following flash column chromatography (*n*-hex/EtOAc 4:1) into the title compound which was isolated as a brown solid (0.16 g, 34%); R_f 0.39 (*n*-hex/EtOAc 4:1); m.p. 123 - 125 °C; ν_{max} 3287 (N-H), 2959 (C-H), 1697, 1682 (C=O), 1594, 1475, 1387, 1367, 1163, 769, 685 cm^{-1}; δ_H (300 MHz, CDCl$_3$) 8.77 (1H, bs, CON*H*NH), 7.07 (1H, t, *J* 8, Ar-*H*), 6.82 (1H, d, *J* 8, Ar-*H*), 6.77 (1H, t, *J* 2, Ar-*H*), 6.66 (1H, dd, *J* 8, 2, Ar-*H*), 5.20 (1H, bd, *J* 8, BocN*H*CH), 4.27 (1H, m, BocNHC*H*), 1.69 – 1.61 (2H, m, CH*H*CH(CH$_3$)$_2$, CH$_2$C*H*(CH$_3$)$_2$), 1.54 (1H, m, C*H*HCH(CH$_3$)$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 0.94 – 0.89 (6H, m, CH$_2$CH(C*H*$_3$)$_2$); δ_C (100 MHz, CDCl$_3$) 173.1 (*C*=ONHNH), 156.1 *(C*=ONH), 149.1 (*ipso*-Ar-*C*), 134.9 (*ipso*-Ar-*C*), 130.1 (Ar-*C*), 128.6 (*ipso*-Ar-*C*), 120.8 (Ar-*C*), 113.3 (Ar-*C*), 111.6 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 51.5 (BocNH*C*H), 40.6 (*C*H$_2$CH(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.7 (CH$_2$*C*H(CH$_3$)$_2$), 22.87(CH$_2$CH(*C*H$_3$)$_2$), 22.1 (CH$_2$CH(*C*H$_3$)$_2$); ***m/z*** (ES$^+$) 378 ([^{35}Cl]MNa$^+$), 380 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 378.1572 ($C_{17}H_{26}N_3O_3{}^{35}ClNa$ requires 378.1572).

tert-Butyl (*S*)-(1-(2-(2,6-dichlorophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Av



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.30 g, 1.30 mmol) and 2,6-dichlorophenylhydrazine hydrochloride (0.30 g, 1.43 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown oil (0.19 g, 37%); R_f 0.40 (*n*-hex/EtOAc 4:1); ν_{max} 3307, 3264 (N-H), 2979, 2961 (C-H), 1679, 1656 (C=O), 1517, 1446, 1281, 1162, 768, 618 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.59 (1H, bs, CON*H*NH), 7.24 (1H, s, Ar-*H*), 7.22 (1H, s, Ar-*H*), 6.92 (1H, t, *J* 8, Ar-*H*), 6.76 (1H, bd, *J* 4, CONHN*H*), 4.95 (1H, bd, *J* 8, BocN*H*CH), 4.21 (1H, m, BocNHC*H*), 1.67 – 1.62 (2H, m, C*H*HCH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.50 (1H, m, CH*H*CH$(CH_3)_2$), 1.44 (9H, s, $(CH_3)_3CO$), 0.92 – 0.88 (6H, t, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.7 (*C*=ONH), 140.9 (*ipso*-Ar-*C*), 128.8 (Ar-*C*), 126.9 (*ipso*-Ar-*C*), 125.9 (*ipso*-Ar-*C*), 123.9 (Ar-*C*), 80.2 ($(CH_3)_3CO$), 51.2 (BocNH*C*H), 40.9 ($CH_2CH(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 24.6 ($CH_2CH(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 22.0 ($CH_2CH(CH_3)_2$); *m/z* (ES^+) 412 ($[^{35,35}Cl]MNa^+$), 414 ($[^{37,35}Cl]MNa^+$), 416 ($[^{37,37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35,35}Cl]MNa^+$, 412.1182 ($C_{17}H_{25}{}^{35,35}ClN_3O_3Na$ requires 412.1165).

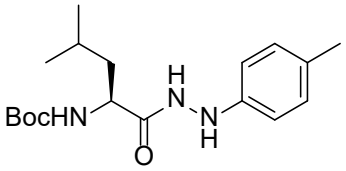
tert-Butyl (*S*)-(1-(2-(2,4-difluorophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Cb



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.30 g, 1.30 mmol) and 2,4-difluorophenylhydrazine hydrochloride (0.26 g, 1.43 mmol) were transformed following flash column chromatography (*n*-hex/EtOAc 4:1) into the title compound which was isolated as a brown oil (0.11 g, 25%); R_f 0.43 (*n*-hex/EtOAc 4:1); ν_{max} 3283 (N-H), 2960 (C-H), 1669 (C=O), 1506, 1469, 1367, 1251, 1163, 961 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.89 (1H, bs, CON*H*NH), 6.83 (1H,

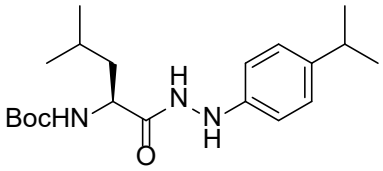
m, Ar-*H*), 6.76 (1H, m, Ar-*H*), 6.68 (1H, t, *J* 8, Ar-*H*), 6.22 (1H, bs, CONHN*H*), 5.31 (1H, bd, *J* 8, BocN*H*CH), 4.30 (1H, m, BocNHC*H*), 1.66 – 1.52 (3H, m, C*H*$_2$CH(CH$_3$)$_2$, CH$_2$C*H*(CH$_3$)$_2$), 1.43 (9H, s, (C*H*$_3$)$_3$CO), 0.94 – 0.90 (6H, t, *J* 6, CH$_2$CH(C*H*$_3$)$_2$); δ_C (100 MHz, CDCl$_3$) 173.1 (*C*=ONHNH), 156.1 (*C*=ONH), 132.4 (1C, dd, *J* 10, 3, Ar-*C*F), 115.3 (Ar-*C*), 110.9 (1C, dd, *J* 22, 3, Ar-*C*), 104.1 (1C, q, *J* 22, Ar-*C*), 80.5 ((CH$_3$)$_3$*C*O), 51.5 (BocNH*C*H), 40.6 (*C*H$_2$CH(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.7 (CH$_2$*C*H(CH$_3$)$_2$), 22.7 (CH$_2$CH(*C*H$_3$)$_2$), 22.1 (CH$_2$CH(*C*H$_3$)$_2$); δ_F (100 MHz, CDCl$_3$) -121.0 (Ar-*F*), -129.2 (Ar-*F*); *m/z* (ES$^+$) 380 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 380.1779 ($C_{17}H_{25}N_3O_3F_2Na$ requires 380.1756).

tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-(p-tolyl)hydrazineyl)pentan-2-yl) carbamate 67Ed



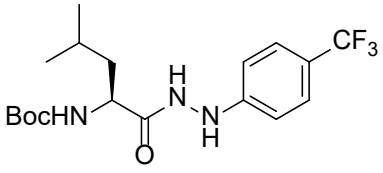
Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 4-methylphenylhydrazine hydrochloride (0.30 g, 1.90 mmol) were transformed following work up with Et$_2$O into the title compound which was isolated as a yellow gummy solid (0.39 g, 69%); R_f 0.46 (DCM/EtOH/NH$_3$ 200:6:1); ν_{max} 3379 (N-H), 2957, 2931 (C-H), 1675, 1614 (C=O), 1512, 1453, 1366, 1249, 1164, 1045, 814 cm^{-1}; δ_H (300 MHz, CDCl$_3$) 8.55 (1H, bs, CON*H*NH), 7.00 – 6.97 (2H, d, *J* 8, Ar-*H*), 6.71 – 6.68 (2H, d, *J* 8, Ar-*H*), 5.13 (1H, bs, BocN*H*CH), 4.23 (1H, m, BocNHC*H*), 2.24 (3H, s, Ar-C*H*$_3$), 1.69 – 1.59 (2H, m, CH*H*CH(CH$_3$)$_2$, CH$_2$C*H*(CH$_3$)$_2$), 1.53 (1H, m, C*H*HCH(CH$_3$)$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 0.94 – 0.90 (6H, t, *J* 6, CH$_2$CH(C*H*$_3$)$_2$); δ_C (100 MHz, CDCl$_3$) 172.7 (*C*=ONHNH), 155.9 (*C*=ONH), 145.5 (*ipso*-Ar-*C*), 130.4 (*ipso*-Ar-*C*), 129.8 (Ar-*C*), 129.6 (Ar-*C*), 113.7 (Ar-*C*), 113.1 (Ar-*C*), 80.4 ((CH$_3$)$_3$*C*O), 51.5 (BocNH*C*H), 40.7 (*C*H$_2$CH(CH$_3$)$_2$), 28.4 ((*C*H$_3$)$_3$CO), 24.7 (CH$_2$*C*H(CH$_3$)$_2$), 22.8 (CH$_2$CH(*C*H$_3$)$_2$), 22.1 (CH$_2$CH(*C*H$_3$)$_2$), 20.6 (Ar-*C*H$_3$); *m/z* (ES$^+$) 358 (MNa$^+$), 693 (2M+Na$^+$); HRMS (ES$^+$) Found MNa$^+$, 358.2119 ($C_{18}H_{29}N_3O_3Na$ requires 358.2101).

tert-Butyl (*S*)-(1-(2-(4-isopropylphenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Ej



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 4-isopropylphenylhydrazine hydrochloride (0.36 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown gummy solid (0.49 g, 78%); R_f 0.41 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3357, 3244, (N-H), 2957, 2933, 2870 (C-H), 1689, 1661 (C=O), 1511, 1467, 1366, 1254, 1168, 1048, 828 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.39 (1H, bs, CON*H*NH), 7.07 – 7.04 (2H, d, *J* 8, Ar-*H*), 6.75 – 6.72 (2H, d, *J* 8, Ar-*H*), 5.07 (1H, bd, *J* 8, BocN*H*CH), 4.22 (1H, m, BocNHC*H*), 2.86 (1H, m, Ar-C*H*$(CH_3)_2$), 1.72 – 1.62 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.54 (1H, m, C*H*HCH$(CH_3)_2$), 1.46 (9H, s, $(CH_3)_3CO$), 2.86 (6H, d, *J* 6, Ar-C*H*$(CH_3)_2$), 0.95 – 0.91 (6H, t, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.6 (*C*=ONHNH), 155.9 (*C*=ONH), 145.6 (*ipso*-Ar-*C*), 141.7 (*ipso*-Ar-*C*), 127.0 (Ar-*C*), 113.7 (Ar-*C*), 80.5 ($(CH_3)_3CO$), 51.5 (BocNH*C*H), 40.6 ($CH_2CH(CH_3)_2$), 33.3 (Ar-*C*H$(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 24.2 (Ar-CH$(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 22.1 ($CH_2CH(CH_3)_2$); *m/z* (ES^+) 386 (MNa^+), 749 ($2M+Na^+$); HRMS (ES^+) Found MNa^+, 386.2433 ($C_{20}H_{33}N_3O_3Na$ requires 386.2414).

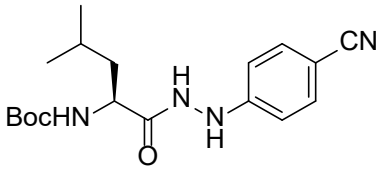
tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl) pentan-2-yl)carbamate 67Bn



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 4-trifluoromethylphenylhydrazine hydrochloride (0.40 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow gummy solid (0.43 g, 64%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1);

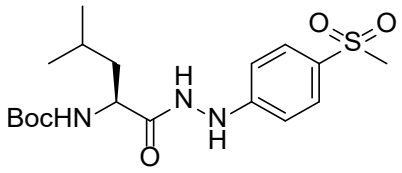
ν_{max} 2961 (C-H), 1684, 1657 (C=O), 1513, 1468, 1321, 1160, 1114, 1067, 835 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.84 (1H, bs, CON*H*NH), 7.39 – 7.37 (2H, d, *J* 8, Ar-*H*), 6.79 – 6.77 (2H, d, *J* 8, Ar-*H*), 5.20 (1H, bd, *J* 8, BocN*H*CH), 4.27 (1H, m, BocNHC*H*), 1.68 – 1.53 (3H, m, CH_2CH(CH_3)$_2$, CH_2C*H*(CH_3)$_2$), 1.45 (9H, s, (CH_3)$_3$CO), 0.93 – 0.88 (6H, t, *J* 6, CH_2CH(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 173.2 (*C*=ONHNH), 156.1 (*C*=ONH), 150.6 (*ipso*-Ar-*C*), 126.5 (Ar-*C*), 122.8 (*ipso*-Ar-*C*), 112.6 (Ar-*C*), 80.8 ((CH_3)$_3$*C*O), 51.5 (BocNH*C*H), 40.5 (*C*H_2CH(CH_3)$_2$), 28.3 ((*C*H_3)$_3$CO), 24.7 (CH_2*C*H(CH_3)$_2$), 22.7 (CH_2CH(*C*H_3)$_2$), 21.9 (CH_2CH(*C*H_3)$_2$); δ_F (300 MHz, $CDCl_3$) -61.6 (C*F*$_3$); *m/z* (ES^+) 412 (MNa^+); HRMS (ES^+) Found MNa^+, 412.1835 ($C_{18}H_{26}N_3O_3F_3Na$ requires 412.1818).

tert-Butyl (*S*)-(1-(2-(4-cyanophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Cz



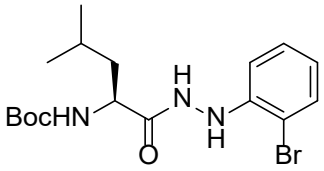
Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 4-cyanophenylhydrazine hydrochloride (0.32 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange solid (0.38 g, 63%); R_f 0.33 (DCM/EtOH/NH_3 200:6:1); m.p. 135 - 137 °C; ν_{max} 3315, 3253 (N-H), 2969 (C-H), 2214 (C≡N), 1679, 1609 (C=O), 1501, 1368, 1250, 1161, 1049, 826 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.72 (1H, bs, CON*H*NH), 7.44 – 7.41 (2H, d, *J* 8, Ar-*H*), 6.78 – 6.75 (2H, d, *J* 8, Ar-*H*), 6.66 (1H, bd, *J* 3, CONHN*H*), 5.13 (1H, bd, *J* 8, BocN*H*CH), 4.27 (1H, m, BocNHC*H*), 1.67 – 1.53 (3H, m, CH_2CH(CH_3)$_2$, CH_2C*H*(CH_3)$_2$), 1.46 (9H, s, (CH_3)$_3$CO), 0.95 – 0.93 (3H, d, *J* 6, CH_2CH(CH_3)$_2$), 0.92 – 0.90 (3H, d, *J* 6, CH_2CH(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 173.2 (*C*=ONHNH), 156.2 (*C*=ONH), 151.4 (*ipso*-Ar-*C*), 133.6 (Ar-*C*), 119.6 (*C*≡N), 112.9 (Ar-*C*), 102.9 (*ipso*-Ar-*C*), 80.9 ((CH_3)$_3$*C*O), 51.6 (BocNH*C*H), 40.4 (*C*H_2CH(CH_3)$_2$), 28.3 ((*C*H_3)$_3$CO), 24.7 (CH_2*C*H(CH_3)$_2$), 22.8 (CH_2CH(*C*H_3)$_2$), 21.9 (CH_2CH(*C*H_3)$_2$); *m/z* (ES^+) 369 (MNa^+); HRMS (ES^+) Found MNa^+, 369.1909 ($C_{18}H_{26}N_4O_3Na$ requires 369.1897).

tert-Butyl (*S*)-(4-methyl-1-(2-(4-(methylsulfonyl)phenyl)hydrazineyl)-1-oxopentan-2-yl)carbamate 67Dl



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 4-(methylsulphonyl)phenylhydrazine hydrochloride (0.42 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow gummy solid (0.37 g, 54%); R_f 0.45 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3399 (N-H), 1688, 1647 (C=O), 1597, 1524, 1303, 1291, 1138, 764 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.71 (1H, bs, CON*H*NH), 7.59 – 7.57 (2H, d, *J* 8, Ar-*H*), 6.73 – 6.71 (2H, d, *J* 8, Ar-*H*), 5.12 (1H, bd, *J* 8, BocN*H*CH), 4.19 (1H, m, BocNHC*H*), 2.92 (3H, s, SO_2CH_3), 1.64 – 1.48 (3H, m, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.39 (9H, s, $(CH_3)_3CO$), 0.89 – 0.87 (3H, d, *J* 6, $CH_2CH(CH_3)_2$), 0.86 – 0.84 (3H, d, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 173.2 (*C*=ONHNH), 156.1 (*C*=ONH), 152.4 (*ipso*-Ar-*C*), 131.1 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 112.5 (Ar-*C*), 80.8 ($(CH_3)_3CO$), 51.7 (BocNH*C*H), 44.8 (SO_2CH_3), 40.5 ($CH_2CH(CH_3)_2$), 28.4 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 21.9 ($CH_2CH(CH_3)_2$); *m/z* (ES^+) 422 (MNa^+); HRMS (ES^+) Found MNa^+, 422.1724 ($C_{18}H_{29}N_3O_5SNa$ requires 422.1720).

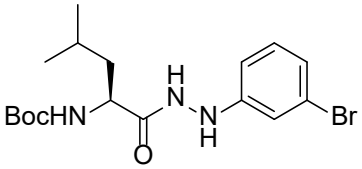
tert-Butyl (*S*)-(1-(2-(2-bromophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Ch



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 2-bromophenylhydrazine hydrochloride (0.43 g, 1.90 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange gummy solid (0.32 g, 46%); R_f 0.39 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3342, 3253 (N-H), 2959 (C-H), 1721, 1655 (C=O), 1514, 1364, 1253, 1157, 1020, 751 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.30 (1H, bs, CON*H*NH), 7.44 (1H, dd, *J* 7,1, Ar-*H*), 6.18 (1H, dt, *J* 8, 1, Ar-*H*), 6.86 (1H, dd, *J* 8,1, Ar-*H*), 6.78

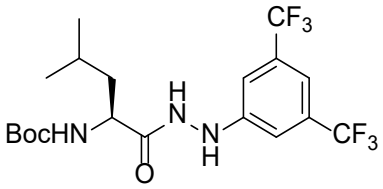
(1H, td, *J* 7,1, Ar-*H*), 6.40 (1H, bd, *J* 3, CONHN*H*), 4.97 (1H, bd, *J* 8, BocN*H*CH), 4.24 (1H, m, BocNHC*H*), 1.74 – 1.67 (3H, m, C*H*$_2$CH(CH$_3$)$_2$, CH$_2$C*H*(CH$_3$)$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO), 0.98 – 0.96 (3H, d, *J* 6, CH$_2$CH(C*H*$_3$)$_2$), 0.95 – 0.93 (3H, d, *J* 6, CH$_2$CH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.4 (*C*=ONHNH), 155.9 (*C*=ONH), 144.7 (*ipso*-Ar-*C*), 132.6 (Ar-*C*), 128.3 (Ar-*C*), 121.9 (Ar-*C*), 113.7 (Ar-*C*), 109.1 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 51.8 (BocNH*C*H), 40.3 (*C*H$_2$CH(CH$_3$)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.7 (CH$_2$*C*H(CH$_3$)$_2$), 22.7 (CH$_2$CH(*C*H$_3$)$_2$), 22.1 (CH$_2$CH(*C*H$_3$)$_2$); *m/z* (ES$^+$) 422 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 422.1063 ($C_{17}H_{26}N_3O_3{}^{79}BrNa$ requires 422.1050).

tert-Butyl (*S*)-(1-(2-(3-bromophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Cn



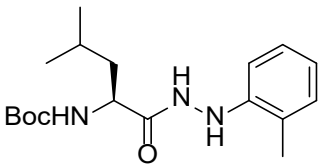
Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 3-bromophenylhydrazine hydrochloride (0.43 g, 1.90 mmol) were transformed following trituration with Et$_2$O into the title compound which was isolated as a light brown solid (0.36 g, 53%); R_f 0.36 (DCM/EtOH/NH$_3$ 200:6:1); m.p. 167 - 169 °C; ν_{max} 3283 (N-H), 2959, 2928 (C-H), 1697, 1681 (C=O), 1589, 1473, 1367, 1249, 1162, 768 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.43 (1H, bs, CON*H*NH), 7.04 (1H, d, *J* 7, Ar-*H*), 7.01 (1H, t, *J* 1, Ar-*H*), 6.95 (1H, t, *J* 1, Ar-*H*), 6.73 (1H, dt, *J* 7,1, Ar-*H*), 5.02 (1H, bd, *J* 8, BocN*H*CH), 4.25 (1H, m, BocNHC*H*), 1.71 – 1.66 (2H, m, CH*H*CH(CH$_3$)$_2$, CH$_2$C*H*(CH$_3$)$_2$), 1.55 (1H, m, C*H*HCH(CH$_3$)$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$CO), 0.97 – 0.95 (3H, d, *J* 6, CH$_2$CH(C*H*$_3$)$_2$), 0.94 – 0.92 (3H, d, *J* 6, CH$_2$CH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 172.9 (*C*=ONHNH), 156.1 (*C*=ONH), 149.3 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 123.9 (Ar-*C*), 123.1 (*ipso*-Ar-*C*), 116.3 (Ar-*C*), 112.2 (Ar-*C*), 80.7 ((CH$_3$)$_3$*C*O), 51.5 (BocNH*C*H), 40.4 (*C*H$_2$CH(CH$_3$)$_2$), 28.4 ((*C*H$_3$)$_3$CO), 24.7 (CH$_2$*C*H(CH$_3$)$_2$), 22.7 (CH$_2$CH(*C*H$_3$)$_2$), 22.1 (CH$_2$CH(*C*H$_3$)$_2$); *m/z* (ES$^+$) 422 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 422.1066 ($C_{17}H_{26}N_3O_3{}^{79}BrNa$ requires 422.1050).

tert-Butyl (*S*)-(1-(2-(3,5-bis(trifluoromethyl)phenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Bv



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and (3,5-bis(trifluoromethyl)phenyl)hydrazine hydrochloride (0.53 g, 1.90 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.46 g, 58%); R_f 0.46 (DCM/EtOH/NH_3 200:6:1); m.p. 112 - 114 °C;ν_{max} 3329, 3267 (N-H), 2972 (C-H), 1669, 1621 (C=O), 1522, 1498, 1382, 1277, 1163, 867 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.72 (1H, bs, CON*H*NH), 7.33 (1H, s, Ar-*H*), 6.65 (2H, s, Ar-*H*), 6.65 (1H, bs, CONHN*H*), 5.02 (1H, bd, *J* 7, BocN*H*CH), 4.25 (1H, m, BocNHC*H*), 1.69 – 1.62 (2H, m, CH*H*CH(CH_3)$_2$, CH_2C*H*(CH_3)$_2$), 1.54 (1H, m, C*H*HCH(CH_3)$_2$), 1.45 (9H, s, (C*H*$_3$)$_3$CO), 0.95 – 0.93 (3H, d, *J* 6, CH_2CH(C*H*$_3$)$_2$), 0.91 – 0.89 (3H, d, *J* 6, CH_2CH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 173.3 (*C*=ONHNH), 156.2 (*C*=ONH), 149.1 (*ipso*-Ar-*C*), 132.3 (*ipso*-Ar-*C*), 125.1 (*ipso*-Ar-*C*), 121.4 (*ipso*-Ar-*C*), 114.1 (Ar-*C*), 112.9 (Ar-*C*), 81.1 ((CH$_3$)$_3$*C*O), 51.6 (BocNH*C*H), 40.3 (*C*H_2CH(CH_3)$_2$), 28.2 ((*C*H$_3$)$_3$CO), 24.7 (CH_2*C*H(CH_3)$_2$), 22.4 (CH_2CH(*C*H$_3$)$_2$), 22.0 (CH_2CH(*C*H$_3$)$_2$); δ_F (300 MHz, $CDCl_3$) -63.2 (C*F*$_3$); *m/z* (ES^+) 480 (MNa^+); HRMS (ES^+) Found MNa^+, 480.1700 ($C_{19}H_{25}N_3O_3F_6Na$ requires 480.1692).

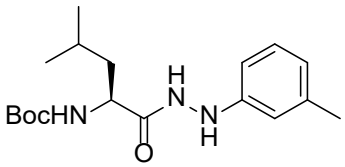
tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-(o-tolyl)hydrazineyl)pentan-2-yl) carbamate 67Dr



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 2-methylphenylhydrazine hydrochloride (0.30 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown gummy solid (0.47 g, 81%); R_f 0.42 (DCM/EtOH/NH_3 200:6:1);

ν_{max} 3281 (N-H), 2959, 2933 (C-H), 1667, 1608 (C=O), 1496, 1366, 1249, 1163, 1046, 748 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.57 (1H, bs, CON*H*NH), 6.98 – 6.96 (2H, d, *J* 7, Ar-*H*), 6.73 – 6.71 (2H, d, *J* 7, Ar-*H*), 5.13 (1H, bd, *J* 8, BocN*H*CH), 4.22 (1H, m, BocNHC*H*), 2.12 (3H, s, Ar-CH_3), 1.64 – 1.54 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.48 (1H, m, C*H*HCH$(CH_3)_2$), 1.37 (9H, s, $(CH_3)_3CO$), 0.88 – 0.84 (6H, t, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 156.0 (*C*=ONH), 145.6 (*ipso*-Ar-*C*), 130.4 (Ar-*C*), 126.8 (Ar-*C*), 123.2 (Ar-*C*), 120.8 (Ar-*C*), 112.1 (Ar-*C*), 80.4 ($(CH_3)_3CO$), 51.5 (BocNH*C*H), 40.7 ($CH_2CH(CH_3)_2$), 28.4 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 22.2 ($CH_2CH(CH_3)_2$), 16.9 (Ar-CH_3); *m/z* (ES^+) 358 (MNa^+); HRMS (ES^+) Found MNa^+, 358.2124 ($C_{18}H_{29}N_3O_3Na$ requires 358.2101).

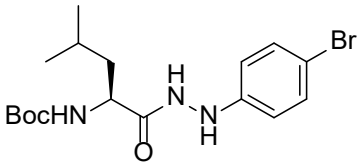
tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-(m-tolyl)hydrazineyl)pentan-2-yl)carbamate 67Dx



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 3-methylphenylhydrazine hydrochloride (0.30 g, 1.90 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.22 g, 37%); R_f 0.56 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3263 (N-H), 2959, 2929, 2872 (C-H), 1698, 1681, 1664 (C=O), 1526, 1366, 1280, 1163, 772 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.42 (1H, bs, CON*H*NH), 7.02 (1H, t, *J* 7, Ar-*H*), 6.62 (1H, d, *J* 7, Ar-*H*), 6.53 (2H, s, Ar-*H*), 6.06 (1H, bs, CONHN*H*), 5.05 (1H, bd, *J* 8, BocN*H*CH), 4.17 (1H, m, BocNHC*H*), 2.18 (3H, s, Ar-CH_3), 1.65 – 1.56 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.42 (1H, m, C*H*HCH$(CH_3)_2$), 1.38 (9H, s, $(CH_3)_3CO$), 0.88 – 0.83 (6H, t, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.8 (*C*=ONHNH), 155.9 (*C*=ONH), 147.8 (*ipso*-Ar-*C*), 138.9 (*ipso*-Ar-*C*), 129.0 (Ar-*C*), 122.0 (Ar-*C*), 114.3 (Ar-*C*), 110.6 (Ar-*C*), 80.4 ($(CH_3)_3CO$), 51.5 (BocNH*C*H), 40.7 ($CH_2CH(CH_3)_2$), 28.4 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 22.1 ($CH_2CH(CH_3)_2$), 21.5 (Ar-CH_3);

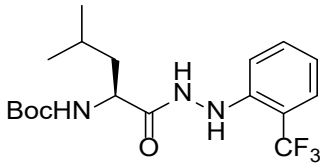
m/z (ES^+) 336 (MH^+); HRMS (ES^+) Found MH^+, 336.1901 ($C_{18}H_{30}N_3O_3$ requires 336.1904).

tert-Butyl (*S*)-(1-(2-(4-bromophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Ct



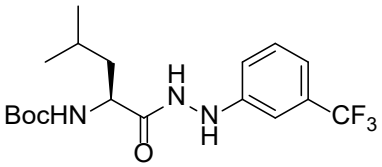
Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 4-bromophenylhydrazine hydrochloride (0.43 g, 1.90 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light orange solid (0.41 g, 59%); R_f 0.53 (DCM/EtOH/NH_3 200:6:1); m.p. 141 - 143 °C;ν_{max} 3307 (N-H), 2957 (C-H), 1688, 1653 (C=O), 1520, 1488, 1366, 1270, 1171, 821 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.66 (1H, bs, CON*H*NH), 7.26 – 7.24 (2H, d, *J* 8, Ar-*H*), 6.66 – 6.63 (2H, d, *J* 8, Ar-*H*), 6.24 (1H, bs, CONHN*H*), 5.16 (1H, bd, *J* 8, BocN*H*CH), 4.24 (1H, m, BocNHC*H*), 1.68 – 1.57 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.53 (1H, m, C*H*HCH$(CH_3)_2$), 1.45 (9H, s, $(CH_3)_3CO$), 0.94 – 0.89 (6H, t, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 172.9 (*C*=ONHNH), 156.0 (*C*=ONH), 147.0 (*ipso*-Ar-*C*), 131.9 (Ar-*C*), 115.1 (Ar-*C*), 112.9 (*ipso*-Ar-*C*), 80.6 ($(CH_3)_3CO$), 51.5 (BocNH*C*H), 40.6 ($CH_2CH(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 22.8 ($CH_2CH(CH_3)_2$), 22.1 ($CH_2CH(CH_3)_2$); *m/z* (ES^+) 422 ($[^{79}Br]MNa^+$), 424 ($[^{81}Br]MNa^+$); HRMS (ES^+) Found $[^{79}Br]MNa^+$, 422.1058 ($C_{17}H_{26}N_3O_3{}^{79}BrNa$ requires 422.1050).

tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-(2-(trifluoromethyl)phenyl)hydrazineyl) pentan-2-yl)carbamate 67Bb



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 2-trifluoromethylphenylhydrazine hydrochloride (0.40 g, 1.90 mmol) were transformed following flash column chromatography (DCM/EtOH/NH_3 200:8:1) into the title compound which was isolated as a yellow oil (0.29 g, 43%) R_f 0.32 (DCM/EtOH/NH_3 200:8:1); ν_{max} 3288 (N-H), 2961 (C-H), 1671, 1614 (C=O), 1524, 1322, 1276, 1164, 1109, 1035 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.18 (1H, bs, CON*H*NH), 7.36 – 7.31 (2H, t, *J* 8, Ar-*H*), 7.16 (1H, t, *J* 8, Ar-*H*), 6.87 (1H, d, *J* 8, Ar-*H*), 6.49 (1H, bs, CONHN*H*), 5.50 (1H, bd, *J* 8, BocN*H*CH), 4.34 (1H, m, BocNHC*H*), 1.59 – 1.47 (3H, m, C*H*$_2$CH(CH_3)$_2$, CH_2C*H*(CH_3)$_2$), 1.33 (9H, s, (C*H*$_3$)$_3$CO), 0.84 – 0.80 (6H, t, *J* 5, CH_2CH(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 173.0 (*C*=ONHNH), 156.1 (*C*=ONH), 145.4 (*ipso*-Ar-*C*), 132.9 (Ar-*C*), 126.3 (1C, *ipso*-Ar-*C*CF_3), 122.7 (Ar-*C*), 119.9 (Ar-*C*), 114.9 (1C, q, *J* 31, Ar-*C*F_3), 113.5 (Ar-*C*), 80.5 ((CH_3)$_3$*C*O), 51.4 (BocNH*C*H), 40.8 (*C*H_2CH(CH_3)$_2$), 28.3 ((*C*H$_3$)$_3$CO), 24.7 (CH_2*C*H(CH_3)$_2$), 22.6 (CH_2CH(*C*H_3)$_2$), 22.1 (CH_2CH(*C*H_3)$_2$); δ_F (300 MHz, $CDCl_3$) -61.9 (C*F*$_3$); *m/z* (ES^+) 412 (MNa^+); HRMS (ES^+) Found MNa^+, 412.1798 ($C_{18}H_{26}N_3O_3F_3Na$ requires 412.1819).

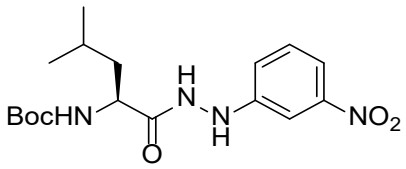
tert-Butyl (*S*)-(4-methyl-1-oxo-1-(2-(3-(trifluoromethyl)phenyl)hydrazineyl) pentan-2-yl)carbamate 67Bh



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 3-trifluoromethylphenylhydrazine hydrochloride (0.40 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was

isolated as a yellow oil (0.38 g, 56%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3327, 3278 (N-H), 2959 (C-H), 1680,1671, 1663 (C=O), 1519, 1333, 1249, 1112, 1071 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.93 (1H, bs, CON*H*NH), 7.15 (1H, d, *J* 7, Ar-*H*), 7.01 (1H, d, *J* 7, Ar-*H*), 6.92 (1H, s, Ar-*H*), 6.84 (1H, dd, *J* 7, 1, Ar-*H*), 6.46 (1H, bs, CONHN*H*), 5.18 (1H, bd, *J* 8, BocN*H*CH), 4.26 (1H, m, BocNHC*H*), 1.57 – 1.51 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.45 (1H, m, C*H*HCH$(CH_3)_2$), 1.35 (9H, s, $(CH_3)_3CO$), 0.84 (3H, d, *J* 6, $CH_2CH(CH_3)_2$), 0.81 (3H, d, *J* 6, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 173.4 (*C*=ONHNH), 156.1 (*C*=ONH), 148.4 (*ipso*-Ar-*C*), 132.1 (1C, q, *J* 31, Ar-CF_3), 129. 6 (Ar-*C*), 117.3 (Ar-*C*), 116.4 (Ar-*C*), 109.8 (Ar-*C*), 80.6 ($(CH_3)_3CO$), 51.5 (BocNH*C*H), 40.8 ($CH_2CH(CH_3)_2$), 28.2 ($(CH_3)_3CO$), 24.7 ($CH_2CH(CH_3)_2$), 22.5 ($CH_2CH(CH_3)_2$), 22.1 ($CH_2CH(CH_3)_2$); δ_F (300 MHz, $CDCl_3$) -62.8 (CF_3); *m/z* (ES^+) 412 (MNa^+); HRMS (ES^+) Found MNa^+, 412.1838 ($C_{18}H_{26}N_3O_3F_3Na$ requires 412.1818).

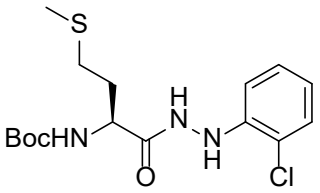
tert-Butyl (*S*)-(4-methyl-1-(2-(3-nitrophenyl)hydrazineyl)-1-oxopentan-2-yl) carbamate 67Df



Following the general procedure outlined, *N*-Boc-*L*-leucine (0.40 g, 1.73 mmol) and 3-nitrophenylhydrazine hydrochloride (0.36 g, 1.90 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.51 g, 81%); R_f 0.51 (DCM/EtOH/NH_3 200:6:1); m.p. 127 - 129 °C;ν_{max} 3329 (N-H), 2956 (C-H), 1665, 1619 (C=O), 1519, 1348, 1322, 1279, 1161, 733, 647 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.62 (1H, bs, CON*H*NH), 7.63 (1H, d, *J* 2, Ar-*H*), 7.53 (1H, s, Ar-*H*), 7.25 (1H, d, *J* 7, Ar-*H*), 7.04 (1H, m, Ar-*H*), 6.46 (1H, bs, CONHN*H*), 5.01 (1H, bs, BocN*H*CH), 4.20 (1H, m, BocNHC*H*), 1.65 – 1.57 (2H, m, CH*H*CH$(CH_3)_2$, $CH_2CH(CH_3)_2$), 1.51 (1H, m, C*H*HCH$(CH_3)_2$), 1.39 (9H, s, $(CH_3)_3CO$), 0.90 (3H, d, *J* 5, $CH_2CH(CH_3)_2$), 0.87 (3H, d, *J* 5, $CH_2CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 173.3 (*C*=ONHNH), 156.2 (*C*=ONH), 151.9 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 129.8 (Ar-*C*), 119.2 (Ar-*C*), 115.5 (Ar-*C*), 107.6 (Ar-*C*), 80.9 ($(CH_3)_3CO$), 51.5 (BocNH*C*H), 40.5 ($CH_2CH(CH_3)_2$), 28.3 ($(CH_3)_3CO$), 24.8

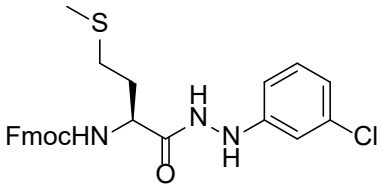
($CH_2CH(CH_3)_2$), 22.7 ($CH_2CH(CH_3)_2$), 22.1 ($CH_2CH(CH_3)_2$); *m/z* (ES^+) 389 (MNa^+); HRMS (ES^+) Found MNa^+, 389.1807 ($C_{17}H_{26}N_4O_5Na$ requires 389.1795).

tert-Butyl (*S*)-(1-(2-(2-chlorophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Ak



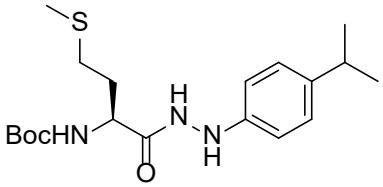
Following the general procedure outlined, *N*-Boc-*L*-methionine (0.30 g, 1.20 mmol) and 2-chlorophenylhydrazine hydrochloride (0.26 g, 1.33 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.25 g, 54%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); m.p. 119 - 121 °C; ν_{max} 3276 (N-H), 2986, 2918 (C-H), 1674 (C=O), 1489, 1367, 1249, 1161, 1034, 745 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.77 (1H, bs, CON*H*NH), 7.26 (1H, dd, *J* 7, 1, Ar-*H*), 7.12 (1H, td, *J* 7, 1, Ar-*H*), 6.86 (1H, dd, *J* 8, 1, Ar-*H*), 6.80 (1H, dd, *J* 7, 1, Ar-*H*), 6.48 (1H, bd, *J* 3, Ar-N*H*), 5.45 (1H, bd, *J* 8, BocN*H*CH), 4.47 (1H, m, BocNHC*H*), 2.58 (2H, t, *J* 7, CH_2CH_2S), 2.09 (3H, s, $CH_2CH_2SCH_3$), 2.01 (2H, m, CH_2CH_2S), 1.44 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 156.0 (*C*=ONH), 146.6 (*ipso*-Ar-*C*), 129.2 (Ar-*C*), 127.8 (Ar-*C*), 121.2 (*ipso*-Ar-*C*), 119.3 (*ipso*-Ar-*C*), 113.5 (Ar-*C*), 80.5 ($(CH_3)_3CO$), 52.0 (BocNH*C*H), 31.5 ($CH_2CH_2SCH_3$), 30.1 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$), 15.3 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 396 ($[^{35}Cl]MNa^+$), 398 ($[^{37}Cl]MNa^+$), 769 ($[^{35,35}Cl]2M+Na^+$) 771 ($[^{35,37}Cl]2M+Na^+$), 773 ($[^{37,37}Cl]2M+Na^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 396.1142 ($C_{16}H_{24}{}^{35}ClN_3O_3SNa$ requires 396.1119).

(9H-fluoren-9-yl)methyl (*S*)-(1-(2-(3-chlorophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 68u



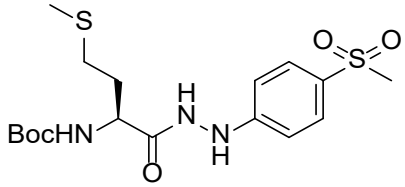
Following the general procedure outlined, *N*-Fmoc-*L*-methionine (0.40 g, 1.08 mmol) and 3-chlorophenylhydrazine hydrochloride (0.21 g, 1.19 mmol) were transformed following flash column chromatography (DCM/EtOH/NH3 200:6:1) into the title compound which was isolated as a light brown solid (0.28 g, 52%); R_f 0.42 (DCM/EtOH/NH_3 200:6:1); m.p. 186 - 189 °C; υmax 3278 (N-H), 1686, 1655 (C=O), 1541, 1476, 1304, 1265, 756, 740, 679 cm-1; δ_H (300 MHz, DMSO-d6) 9.93 (1H, bs, CON*H*NH), 8.07 – 8.06 (1H, bd, *J* 2, Ar-N*H*), 7.91 – 7.89 (2H, d, *J* 7, Ar-*H*), 7.75 – 7.72 (2H, m, Ar-*H*), 7.45 – 7.40 (2H, t, *J* 7, Ar-*H*), 7.35 – 7.31 (2H, t, *J* 7, Ar-*H*), 7.12 (1H, t, *J* 7, Ar-*H*), 6.73 – 6.63 (3H, m, Ar-*H*), 4.33 (2H, m, CO_2CH_2CH-Fluorene), 4.27 (1H, m, CO_2CH_2CH-Fluorene), 4.14 (1H, m, FmocNHC*H*), 2.56 (2H, m, $CHCH_2CH_2S$), 2.08 (3H, s, $CHCH_2CH_2SCH_3$),1.94 (2H, m, $CHCH_2CH_2S$); δ_C (100 MHz, DMSO-d6) 172.1 (*C*=ONHNH), 156.6 *(C*=ONH), 151.3 *(ipso*-Ar-*C*), 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 133.9 (*ipso*-Ar-*C*), 130.8 (Ar-*C*), 128.1 (Ar-*C*), 127.5 (Ar-*C*), 125.8 (Ar-*C*), 120.6 (Ar-*C*), 118.3 (Ar-*C*), 111.9 (Ar-*C*), 111.3 (Ar-*C*), 66.3 (CO_2CH_2CH-Fluorene), 53.0 (FmocNH*C*H), 47.2 (*C*-Fluorene), 31.9 ($CHCH_2CH_2S$), 30.2 ($CHCH_2CH_2S$), 15.1 ($CHCH_2CH_2SCH_3$); *m/z* (ES^+) 496 ($[^{35}Cl]MH^+$), 498 ($[^{37}Cl]MH^+$), 518 ($^{35}Cl]MNa^+$), 520 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 496.1456 ($C_{26}H_{27}{}^{35}ClN_3O_3S$ requires 496.1456).

tert-Butyl (*S*)-(1-(2-(4-isopropylphenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Ek



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.30 g, 1.20 mmol) and 2-chlorophenylhydrazine hydrochloride (0.25 g, 1.32 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange solid (0.22 g, 48%); R_f 0.36 (DCM/EtOH/NH_3 200:6:1); m.p. 147 - 149 °C; ν_{max} 3316 (N-H), 2959, 2919 (C-H), 1686, 1656 (C=O), 1514, 1456, 1365, 1251, 1167, 824, 536 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.61 (1H, bs, CON*H*NH), 7.05 (2H, d, *J* 8, Ar-*H*), 6.73 (2H, d, *J* 8, Ar-*H*), 6.15 (1H, bs, Ar-N*H*), 5.39 (1H, bd, *J* 8, BocN*H*CH), 4.40 (1H, q, *J* 8, BocNHC*H*), 2.81 (1H, Hept, *J* 7, C*H*$(CH_3)_2$), 2.53 (2H, m, CH_2CH_2S), 2.08 (3H, s, $CH_2CH_2SCH_3$), 1.94 (2H, m, CH_2CH_2S), 1.45 (9H, s, $(CH_3)_3CO$), 1.19 (6H, d, *J* 1, $CH(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 170.5 (*C*=ONHNH), 155.9 (*C*=ONH), 146.3 (*ipso*-Ar-*C*), 138.9 (*ipso*-Ar-*C*), 127.0 (Ar-*C*), 112.9 (Ar-*C*), 79.8 ($(CH_3)_3CO$), 60.3 (BocNH*C*H), 31.7 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$), 23.3 ($CH(CH_3)_2$), 15.3 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 404 (MNa^+); HRMS (ES^+) Found MNa^+, 404.1996 ($C_{19}H_{31}N_3O_3SNa$ requires 404.1978).

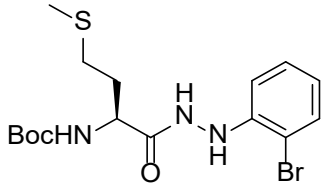
tert-Butyl (*S*)-(1-(2-(4-(methylsulfonyl)phenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Dm



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 4-(methylsulphonyl)phenylhydrazine hydrochloride (0.39 g, 1.77 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.41 g, 61%); R_f 0.43 (DCM/EtOH/NH_3 200:6:1); m.p. 141 - 143 °C; ν_{max} 3336, 3256 (N-H), 2984, 2915 (C-H), 1683, 1662 (C=O), 1598,

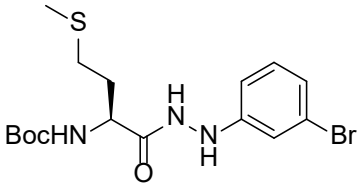
1322, 1285, 1129, 1091, 772 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.49 (1H, bs, CON*H*NH), 7.76 – 7.73 (2H, d, *J* 8, Ar-*H*), 6.90 – 6.87 (2H, d, *J* 8, Ar-*H*), 5.26 (1H, bd, *J* 7, BocN*H*CH), 4.44 (1H, m, BocNHC*H*), 3.02 (3H, s, SO_2CH_3), 2.63 (2H, t, *J* 6, CH_2CH_2S), 2.14 (3H, s, $CH_2CH_2SCH_3$), 2.06 (2H, m, CH_2CH_2S), 1.50 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 152.3 (*C*=ONH), 131.7 (*ipso*-Ar-*C*), 129.2 (Ar-*C*), 112.7 (Ar-*C*), 44.9 (SO_2CH_3), 30.2 ($CH_2CH_2SCH_3$), 28.4 ($(CH_3)_3CO$), 15.5 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 440 (MNa^+); HRMS (ES^+) Found MNa^+, 440.1296 ($C_{17}H_{27}N_3O_5SNa$ requires 417.1392).

tert-Butyl (*S*)-(1-(2-(2-bromophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Ci



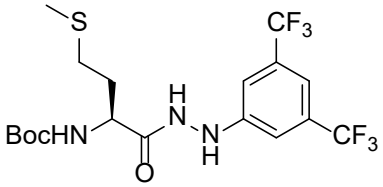
Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 2-bromophenylhydrazine hydrochloride (0.33 g, 1.77 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange oil (0.36 g, 53%); R_f 0.40 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3279 (N-H), 2977, 2918 (C-H), 1670 (C=O), 1594, 1486, 1366, 1249, 1160, 1022, 744 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.07 (1H, bs, CON*H*NH), 7.38 (1H, dt, *J* 8, 2, Ar-*H*), 7.09 (1H, m, Ar-*H*), 6.80 (1H, dd, *J* 8, *2,* Ar-*H*), 6.70 (1H, dt, *J* 8, 2, Ar-*H*), 6.44 (1H, bd, *J* 3, Ar-*H*), 5.64 (1H, bd, *J* 8, BocN*H*CH), 4.47 (1H, q, *J* 8, BocNHC*H*), 2.53 (2H, t, *J* 7, CH_2CH_2S), 2.06 (3H, s, $CH_2CH_2SCH_3$), 1.91 (2H, m, CH_2CH_2S), 1.42 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 155.9 (*C*=ONH), 144.4 (*ipso*-Ar-*C*), 132.7 (Ar-*C*), 128.1 (Ar-*C*), 121.5 (Ar-*C*), 113.8 (Ar-*C*), 108.6 (*ipso*-Ar-*C*), 81.1 ($(CH_3)_3CO$), 51.6 (BocNH*C*H), 31.0 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 27.8 ($(CH_3)_3CO$), 15.5 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 440 ($[^{79}Br]MNa^+$), 442 ($[^{81}Br]MNa^+$); HRMS (ES^+) Found $[^{79}Br]MNa^+$, 440.0622 ($C_{16}H_{24}{}^{79}BrN_3O_3SNa$ requires 440.0614).

tert-Butyl (*S*)-(1-(2-(3-bromophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Co



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.30 g, 1.20 mmol) and 3-bromophenylhydrazine hydrochloride (0.25 g, 1.32 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a pale yellow solid (0.23 g, 45%); R_f 0.41 (DCM/EtOH/NH_3 200:6:1); m.p. 112 - 114 °C; ν_{max} 3332, 3271 (N-H), 2991 (C-H), 1683, 1659 (C=O), 1522, 1473, 1245, 1164, 766 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.70 (1H, bs, CON*H*NH), 7.03 (3H, m, Ar-*H*), 6.70 (1H, m, Ar-*H*), 6.30 (1H, bd, *J* 4, Ar-*H*), 5.38 (1H, bd, *J* 8, BocN*H*CH), 4.40 (1H, q, *J* 8, BocNHC*H*), 2.55 (2H, t, *J* 7, CH_2CH_2S), 2.09 (3H, s, $CH_2CH_2SCH_3$), 1.93 (2H, m, CH_2CH_2S), 1.46 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.1 (*C*=ONHNH), 155.9 (*C*=ONH), 149.2 (*ipso*-Ar-*C*), 130.0 (Ar-*C*), 123.9 (Ar-*C*), 123.1 (Ar-*C*), 116.1 (Ar-*C*), 112.6 (*ipso*-Ar-*C*), 81.5 ($(CH_3)_3CO$), 53.2 (BocNH*C*H), 31.1 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.4 ($(CH_3)_3CO$), 15.4 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 440 ($[^{79}Br]MNa^+$), 442 ($[^{81}Br]MNa^+$); HRMS (ES^+) Found $[^{79}Br]MNa^+$, 440.0626 ($C_{16}H_{24}{}^{79}BrN_3O_3SNa$ requires 440.0614).

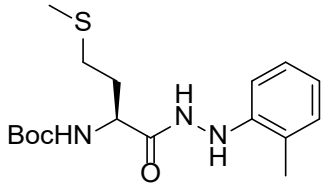
tert-Butyl (*S*)-(1-(2-(3,5-bis(trifluoromethyl)phenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Bw



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and (3,5-bis(trifluoromethyl)phenyl)hydrazine hydrochloride (0.49 g, 1.77 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a pale yellow solid (0.43 g, 69%); R_f 0.39 (DCM/EtOH/NH_3 200:6:1); m.p. 128 - 130 °C; ν_{max} 3331, 3271 (N-H), 2994 (C-H), 1679, 1666 (C=O), 1521, 1378, 1275, 1118, 877 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.50 (1H, bs, CON*H*NH), 7.36 (1H, s, Ar-*H*), 7.21 (2H, s, Ar-*H*), 6.45 (1H, bd, *J* 3, Ar-N*H*), 5.19

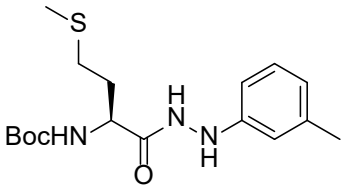
(1H, bd, *J* 7, BocN*H*CH), 4.44 (1H, q, *J* 7, BocNHC*H*), 2.62 (2H, t, *J* 7, $CH_2C\mathit{H}_2S$), 2.22 (1H, m, C*H*HCH_2S), 2.11 (3H, s, $CH_2CH_2SC\mathit{H}_3$), 2.05 (1H, m, CH*H*CH_2S), 1.47 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 149.1 (*ipso*-Ar-*C*), 132.8 (*ipso*-Ar-*C*), 132.4 (*ipso*-Ar-*C*), 114.3 (Ar-*C*), 112.7 (Ar-*C*), 30.2 ($\mathit{C}H_2CH_2SCH_3$), 28.2 ($(\mathit{C}H_3)_3CO$), 15.4 ($CH_2CH_2S\mathit{C}H_3$); δ_F (300 MHz, $CDCl_3$) -63.1 ($C\mathit{F}_3$); *m/z* (ES^+) 498 (MNa^+); HRMS (ES^+) Found MNa^+, 498.1265 ($C_{18}H_{23}N_3O_3F_6SNa$ requires 498.1257).

tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-(o-tolyl)hydrazineyl)butan-2-yl)carbamate 67Ds



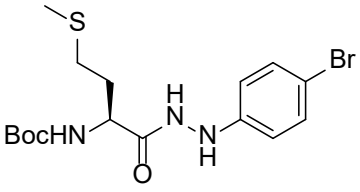
Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 2-methylphenylhydrazine hydrochloride (0.28 g, 1.77 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown oil (0.39 g, 68%); R_f 0.42 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3385 (N-H), 2977, 2918 (C-H), 1669, 1607 (C=O), 1495, 1366, 1248, 1161, 1048, 749 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.78 (1H, bs, CON*H*NH), 7.00 – 6.95 (2H, t, *J* 7, Ar-*H*), 6.75 – 6.69 (2H, t, *J* 8, Ar-*H*), 5.49 (1H, bd, *J* 8, BocN*H*CH), 4.40 (1H, m, BocNHC*H*), 2.47 (2H, t, *J* 7, $CH_2C\mathit{H}_2S$), 2.10 (3H, s, $CH_2CH_2SC\mathit{H}_3$), 2.05 (1H, m, C*H*HCH_2S), 1.99 (3H, s, Ar-CH_3), 1.89 (1H, m, CH*H*CH_2S), 1.36 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 171.8 (*C*=ONHNH), 155.9 (*C*=ONH), 145.5 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 126.9 (Ar-*C*), 123.2 (*ipso*-Ar-*C*), 120.9 (Ar-*C*), 112.1 (Ar-*C*), 80.5 ($(CH_3)_3\mathit{C}O$), 52.0 (BocNH*C*H), 31.4 ($\mathit{C}H_2CH_2SCH_3$), 30.1 ($CH_2\mathit{C}H_2SCH_3$), 28.4 ($(\mathit{C}H_3)_3CO$), 16.9 ($CH_2CH_2S\mathit{C}H_3$), 15.4 (Ar-$\mathit{C}H_3$); *m/z* (ES^+) 376 (MNa^+); HRMS (ES^+) Found MNa^+, 376.1685 ($C_{17}H_{27}N_3O_3SNa$ requires 376.1665).

tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-(m-tolyl)hydrazineyl)butan-2-yl)carbamate 67Dy



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 2-methylphenylhydrazine hydrochloride (0.28 g, 1.77 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a brown oil (0.39 g, 69%); R_f 0.49 (DCM/EtOH/NH_3 200:6:1); m.p. 132 - 134 °C; ν_{max} 3324, 3281, 3264 (N-H), 2971, 2918 (C-H), 1684, 1656 (C=O), 1523, 1498, 1304, 1243, 1164, 776 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.58 (1H, bs, CON*H*NH), 7.07 (1H, m, Ar-*H*), 6.70 (1H, d, *J* 7, Ar-*H*), 6.59 (1H, bd, *J* 1, Ar-N*H*), 5.40 (1H, bd, *J* 8, BocN*H*CH), 4.40 (1H, d, *J* 8, BocNHC*H*), 2.54 (2H, t, *J* 7, CH_2CH_2S), 2.26 (3H, s, $CH_2CH_2SCH_3$), 2.09 (3H, s, Ar-CH_3), 1.89 (2H, m, CH_2CH_2S), 1.45 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.1 (*C*=ONH), 147.3 (*ipso*-Ar-*C*), 139.2 (*ipso*-Ar-*C*), 129.0 (Ar-*C*), 122.1 (Ar-*C*), 114.3 (Ar-*C*), 110.6 (Ar-*C*), 80.6 ($(CH_3)_3CO$), 52.0 (BocNHCH), 31.3 ($CH_2CH_2SCH_3$), 30.1 ($CH_2CH_2SCH_3$), 28.4 ($(CH_3)_3CO$), 21.5 ($CH_2CH_2SCH_3$), 15.4 (Ar-CH_3); *m/z* (ES^+) 376 (MNa^+); HRMS (ES^+) Found MNa^+, 376.1685 ($C_{17}H_{27}N_3O_3SNa$ requires 376.1665).

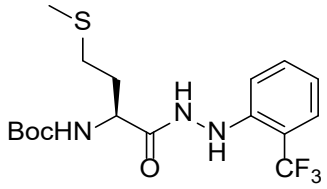
tert-Butyl (*S*)-(1-(2-(4-bromophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Cu



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 4-bromophenylhydrazine hydrochloride (0.39 g, 1.77 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown gummy solid (0.34 g, 51%); R_f 0.41 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3302 (N-H), 2979, 2918 (C-H), 1683, 1652 (C=O), 1518, 1488, 1377, 1251, 1163, 1003, 819 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.63 (1H, bs, CON*H*NH), 7.21 – 7.18

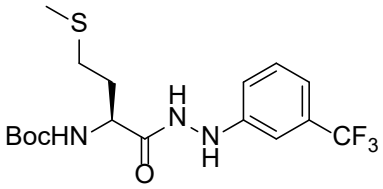
(2H, d, *J* 8, Ar-*H*), 6.60 – 6.57 (2H, d, *J* 8, Ar-*H*), 5.35 (1H, bd, *J* 8, BocN*H*CH), 4.34 (1H, m, BocNHC*H*), 2.48 (2H, t, *J* 7, CH_2CH_2S), 2.07 (1H, m, C*H*HCH_2S), 2.01 (3H, s, $CH_2CH_2SCH_3$), 1.92 (1H, m, CH*H*CH_2S), 1.38 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 155.9 (*C*=ONH), 146.9 (*ipso*-Ar-*C*), 131.9 (Ar-*C*), 115.1 (Ar-*C*), 113.1 (*ipso*-Ar-*C*), 80.8 ($(CH_3)_3CO$), 51.9 (BocNH*C*H), 31.1 ($CH_2CH_2SCH_3$), 30.2 ($CH_2CH_2SCH_3$), 28.4 ($(CH_3)_3CO$), 15.4 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 440 ([^{79}Br]MNa^+), 442 ([^{81}Br]MNa^+); HRMS (ES^+) Found [^{79}Br]MNa^+, 440.0628 ($C_{16}H_{24}{}^{79}BrN_3O_3SNa$ requires 440.0614).

tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-(2-(trifluoromethyl)phenyl) hydrazineyl)butan-2-yl)carbamate 67Bc



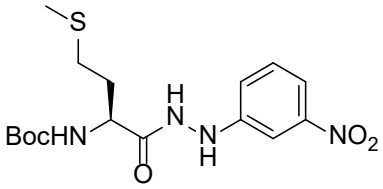
Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 2-trifluoromethylphenylhydrazine hydrochloride (0.38 g, 1.77 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown oil (0.33 g, 50%); R_f 0.53 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3378, 3312 (N-H), 2976 (C-H), 1676, 1614 (C=O), 1523, 1474, 1322, 1285, 1166, 1033, 767, 640 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.95 (1H, bs, CON*H*NH), 7.38 (1H, d, *J* 7, Ar-*H*), 7.23 (1H, m, Ar-*H*), 6.89 (1H, d, *J* 8, Ar-*H*), 6.81 (1H, t, *J* 7, Ar-*H*), 6.50 (1H, bs, Ar-N*H*), 5.54 (1H, bd, *J* 8, BocN*H*CH), 4.47 (1H, m, BocNHC*H*), 2.51 (2H, t, *J* 7, CH_2CH_2S), 2.00 (3H, s, $CH_2CH_2SCH_3$), 1.94 (2H, m, CH_2CH_2S), 1.35 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 156.1 (*C*=ONH), 145.3 (*ipso*-Ar-*C*), 133.0 (Ar-CF_3), 126.5 (1C, t, *J* 5, *ipso*-Ar-CCF_3), 122.7 (Ar-*C*), 120.0 (Ar-*C*), 113.4 (Ar-*C*), 111.9 (Ar-*C*), 80.7 ($(CH_3)_3CO$), 51.9 (BocNH*C*H), 31.2 ($CH_2CH_2SCH_3$), 30.1 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$), 15.3 ($CH_2CH_2SCH_3$); δ_F (300 MHz, $CDCl_3$) -61.8 (CF_3); *m/z* (ES^+) 408 (MNa^+); HRMS (ES^+) Found MH^+, 430.1383 ($C_{18}H_{24}N_3O_3F_3SNa$ requires 430.1386).

tert-Butyl (*S*)-(4-(methylthio)-1-oxo-1-(2-(3-(trifluoromethyl)phenyl) hydrazineyl)butan-2-yl)carbamate 67Bi



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 3-trifluoromethylphenylhydrazine hydrochloride (0.38 g, 1.77 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.43 g, 66%); R_f 0.51 (DCM/EtOH/NH_3 200:6:1); m.p. 125 - 127 °C; ν_{max} 3329, 3279 (N-H), 1681, 1667 (C=O), 1517, 1334, 1246, 1112, 1071, 784 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.82 (1H, bs, CON*H*NH), 7.29 (1H, t, *J* 7, Ar-*H*), 7.11 (1H, d, *J* 7, Ar-*H*), 7.02 (1H, s, Ar-*H*), 6.96 (1H, d, *J* 8, Ar-*H*), 6.46 (1H, bs, Ar-N*H*), 5.42 (1H, bd, *J* 8, BocN*H*CH), 4.47 (1H, q, *J* 7, BocNHC*H*), 2.57 (2H, t, *J* 7, CH_2CH_2S), 2.17 (1H, m, C*H*HCH_2S), 2.08 (3H, s, $CH_2CH_2SCH_3$), 2.00 (1H, m, CH*H*CH_2S), 1.45 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 156.0 (*C*=ONH), 148.2 (*ipso*-Ar-*C*), 131.7 (1C, q, *J* 32, Ar-*C*F_3), 129.7 (Ar-*C*), 125.8 (1C, q, *J* 271, *ipso*-Ar-*C*CF_3), 117.6 (Ar-*C*), 116.5 (Ar-*C*), 109.9 (Ar-*C*), 80.9 ($(CH_3)_3CO$), 52.0 (BocNH*C*H), 31.0 ($CH_2CH_2SCH_3$), 30.1 ($CH_2CH_2SCH_3$), 28.3 ($(CH_3)_3CO$), 15.3 ($CH_2CH_2SCH_3$); δ_F (300 MHz, $CDCl_3$) -61.8 (CF_3); *m/z* (ES^+) 408 (MNa^+); HRMS (ES^+) Found MH^+, 430.1383 ($C_{18}H_{24}N_3O_3F_3SNa$ requires 430.1386).

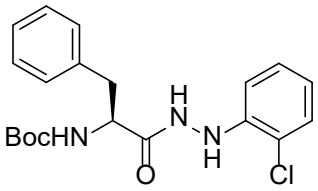
tert-Butyl (*S*)-(4-(methylthio)-1-(2-(3-nitrophenyl)hydrazineyl)-1-oxobutan-2-yl)carbamate 67Dg



Following the general procedure outlined, *N*-Boc-*L*-methionine (0.40 g, 1.60 mmol) and 3-nitrophenylhydrazine hydrochloride (0.33 g, 1.77 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow solid (0.41 g, 66%); R_f 0.29 (DCM/EtOH/NH_3 200:6:1); m.p.

124 - 126 °C; ν_{max} 3323 (N-H), 1678, 1665 (C=O), 1517, 1349, 1295, 1051, 734, 653 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.64 (1H, bs, CON*H*NH), 7.74 (1H, dd, *J* 8, 1, Ar-*H*), 7.64 (1H, t, *J* 2, Ar-*H*), 7.37 (1H, t, *J* 8, Ar-*H*), 7.14 (1H, dd, *J* 8, 1, Ar-*H*), 6.50 (1H, bs, Ar-N*H*), 5.31 (1H, bd, *J* 8, BocN*H*CH), 4.49 (1H, q, *J* 7, BocNHC*H*), 2.64 (2H, t, *J* 7, $CH_2C\mathit{H}_2S$), 2.24 (1H, m, C*H*HCH_2S), 2.14 (3H, s, $CH_2CH_2SC\mathit{H}_3$), 2.05 (1H, m, CH*H*CH_2S), 1.49 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.4 (*C*=ONHNH), 156.1 (*C*=ONH), 149.2 (*ipso*-Ar-*C*), 129.9 (Ar-*C*), 119.2 (Ar-*C*), 115.8 (Ar-*C*), 107.7 (Ar-*C*), 81.2 ($(CH_3)_3\mathit{C}O$), 52.0 (BocNH*C*H), 30.6 ($\mathit{C}H_2CH_2SCH_3$), 30.2 ($CH_2\mathit{C}H_2SCH_3$), 28.3 (($\mathit{C}H_3)_3CO$), 15.4 ($CH_2CH_2S\mathit{C}H_3$); *m/z* (ES^+) 407 (MNa^+); HRMS (ES^+) Found MNa^+, 407.0738 ($C_{16}H_{24}N_4O_5SNa$ requires 407.1360).

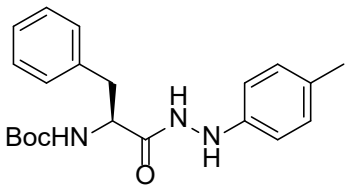
tert-Butyl (*S*)-(1-(2-(2-chlorophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Al



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.30 g, 1.13 mmol) and 2-chlorophenylhydrazine hydrochloride (0.22 g, 1.24 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a light brown gummy solid (0.41 g, 93%); R_f 0.58 (DCM/EtOH/NH_3 200:6:1); m.p. 135 - 137 °C; ν_{max} 3272 (N-H), 2978 (C-H), 1668 (C=O), 1596, 1492, 1366, 1249, 1161, 743, 698 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.82 (1H, bs, CON*H*NH), 7.33 – 7.29 (2H, m, Ar-*H*), 7.25 – 7.21 (3H, m, Ar-*H*), 7.04 (1H, t, *J* 7, Ar-*H*), 6.82 (1H, td, *J* 7, 1, Ar-*H*), 6.43 (1H, d, *J* 7, Ar-*H*), 6.33 (1H, bd, *J* 3, Ar-N*H*), 5.08 (1H, bd, *J* 8, BocN*H*CH), 4.48 (1H, q, *J* 7, BocNHC*H*), 3.12 (2H, d, *J* 7, Ar-CH_2), 1.44 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 171.3 (*C*=ONHNH), 143.3 (*ipso*-Ar-*C*), 136.1 (*ipso*-Ar-*C*), 131.6 (*ipso*-Ar-*C*), 129.4 (Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.6 (Ar-*C*), 127.2 (Ar-*C*), 121.4 (Ar-*C*), 113.6 (Ar-*C*), 28.3 (($\mathit{C}H_3)_3CO$); *m/z* (ES^+) 412 ($[^{35}Cl]MNa^+$), 414 ($[^{37}Cl]MNa^+$), 801 ($[^{35,35}Cl]2M+Na^+$),

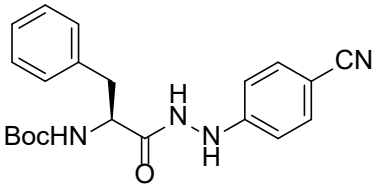
803 ([^{35,37}Cl]2M+Na$^+$), 805 ([^{37,37}Cl]2M+Na$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 412.1416 ($C_{20}H_{24}{}^{35}ClN_3O_3Na$ requires 412.1398).

tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-(p-tolyl)hydrazineyl)propan-2-yl) carbamate 67Ef



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.30 g, 1.13 mmol) and 4-methylphenylhydrazine hydrochloride (0.19 g, 1.24 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.26 g, 61%); R_f 0.43 (DCM/EtOH/NH_3 200:6:1); m.p. 116 - 118 °C; ν_{max} 3318 (N-H), 2962 (C-H), 1679, 1662, 1651 (C=O), 1526, 1485, 1303, 1249, 1172, 746, 647 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.09 (1H, bs, CON*H*NH), 7.28 – 7.26 (3H, t, *J* 6, Ar-*H*), 7.18 – 7.16 (2H, d, *J* 6, Ar-*H*), 6.94 – 6.92 (2H, d, *J* 7, Ar-*H*), 6.49 – 6.47 (2H, d, *J* 8, Ar-*H*), 5.94 (1H, bs, Ar-N*H*), 5.21 (1H, bd, *J* 8, BocN*H*CH), 4.47 (1H, m, BocNHC*H*), 3.06 (2H, m, Ar-CH_2), 2.23 (3H, s, Ar-CH_3), 1.41 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 155.6 (*C*=ONH), 145.1 (*ipso*-Ar-*C*), 136.3 (*ipso*-Ar-*C*), 129.6 (Ar-*C*), 129.4 (Ar-*C*), 128.8 (Ar-*C*), 127.07 (Ar-*C*), 113.7 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 54.5 (BocNH*C*H), 38.6 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO), 20.6 (Ar-*C*H$_3$); *m/z* (ES$^+$) 392 (MNa$^+$), 761 (2M+Na$^+$); HRMS (ES$^+$) Found MNa$^+$, 392.1966 ($C_{21}H_{27}N_3O_3Na$ requires 392.1945).

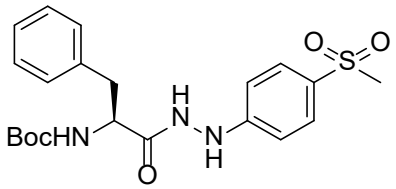
tert-Butyl (*S*)-(1-(2-(4-cyanophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Db



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 4-cyanophenylhydrazine hydrochloride (0.28 g, 1.66 mmol) were transformed following trituration with Et_2O into the title compound which was

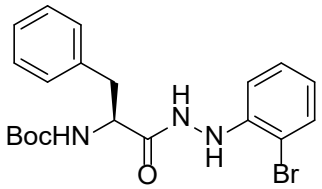
isolated as a light brown solid (0.39 g, 68%); R_f 0.48 (DCM/EtOH/NH_3 200:6:1); m.p. 138 - 140 °C; ν_{max} 3345, 3313, 3271 (N-H), 2986 (C-H), 2117 (C≡N), 1691, 1655 (C=O), 1529, 1485, 1270, 1165, 1047, 834, 704 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.83 (1H, bs, CON*H*NH), 7.42 – 7.39 (2H, d, *J* 8, Ar-*H*), 7.35 – 7.31 (3H, m, Ar-*H*), 7.23 – 7.20 (2H, dd, *J* 6, 1, Ar-*H*), 6.56 – 6.53 (2H, d, *J* 8, Ar-*H*), 6.18 (1H, bd, *J* 2, Ar-N*H*), 5.04 (1H, bd, *J* 7, BocN*H*CH), 4.45 (1H, q, *J* 7, BocNHC*H*), 3.11 (2H, m, Ar-CH_2), 1.45 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.5 (*C*=ONHNH), 151.1 (*ipso*-Ar-*C*), 133.6 (Ar-*C*), 129.4 (Ar-*C*), 128.9 (Ar-*C*), 127.4 (Ar-*C*), 113.0 (Ar-*C*), 28.3 ((*C*H_3)$_3$CO); *m/z* (ES^+) 403 (MNa^+); HRMS (ES^+) Found MNa^+, 403.1746 ($C_{21}H_{24}N_4O_3Na$ requires 403.1741).

tert-Butyl (*S*)-(1-(2-(4-(methylsulfonyl)phenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Dn



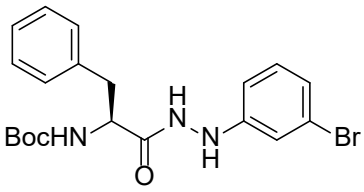
Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 4-(methylsulphonyl)phenylhydrazine hydrochloride (0.37 g, 1.66 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a white solid (0.24 g, 36%); R_f 0.41 (DCM/EtOH/NH_3 200:6:1); m.p. 124 - 126 °C; ν_{max} 3349, 3273 (N-H), 1713, 1668 (C=O), 1597, 1518, 1291, 1144, 766 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.88 (1zH, bs, CON*H*NH), 7.72 – 7.69 (2H, d, *J* 8, Ar-*H*), 7.37 (3H, m, Ar-*H*), 7.27 (2H, m, Ar-*H*), 6.67 – 6.65 (2H, d, *J* 8, Ar-*H*), 5.05 (1H, bd, *J* 8, BocN*H*CH), 4.48 (1H, q, *J* 7, BocNHC*H*), 3.14 (2H, d, *J* 7, Ar-CH_2), 3.02 (3H, s, SO_2C*H*$_3$), 1.48 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, DMSO-d_6) 172.1 (*C*=ONHNH), 155.8 (*C*=ONH), 153.7 (*ipso*-Ar-*C*), 138.2 (*ipso*-Ar-*C*), 129.8 (Ar-*C*), 128.8 (Ar-*C*), 128.7 (Ar-*C*), 126.8 (Ar-*C*), 111.6 (Ar-*C*), 78.7 ((CH$_3$)$_3$*C*O), 54.9 (BocNH*C*H), 44.8 (SO_2*C*H$_3$), 37.6 (Ar-*C*H$_2$), 28.6 ((*C*H$_3$)$_3$CO); *m/z* (ES^+) 456 (MNa^+); HRMS (ES^+) Found MNa^+, 456.0877 ($C_{21}H_{27}N_3O_5SNa$ requires 456.1564).

tert-Butyl (*S*)-(1-(2-(2-bromophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Cj



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 2-bromophenylhydrazine hydrochloride (0.37 g, 1.66 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown solid (0.47 g, 72%); R_f 0.38 (DCM/EtOH/NH_3 200:6:1); m.p. 131 - 133 °C; ν_{max} 3325, 3291 (N-H), 1679, 1661 (C=O), 1523, 1484, 1291, 1166, 1022, 745 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.62 (1H, bs, CON*H*NH), 7.36 (1H, d, *J* 7, Ar-*H*), 7.27 – 7.24 (2H, m, Ar-*H*), 7.20 – 7.17 (2H, m, Ar-*H*), 7.01 (1H, t, *J* 7, Ar-*H*), 6.69 (1H, t, *J* 7, Ar-*H*), 6.31 (2H, m, Ar-*H*), 5.47 (1H, bd, *J* 8, BocN*H*CH), 4.62 (1H, m, BocNHC*H*), 3.07 (2H, d, *J* 6, Ar-CH_2), 1.37 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.7 (*C*=ONHNH), 155.8 (*C*=ONH), 144.3 (*ipso*-Ar-*C*), 136.3 (*ipso*-Ar-*C*), 129.5 (Ar-*C*), 128.8 (Ar-*C*), 128.3 (Ar-*C*), 127.0 (Ar-*C*), 121.7 (Ar-*C*), 113.8 (Ar-*C*), 80.6 ((CH$_3$)$_3$*C*O), 54.3 (BocNH*C*H), 38.5 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 456 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 456.0911 ($C_{20}H_{24}{}^{79}BrN_3O_3Na$ requires 456.0893).

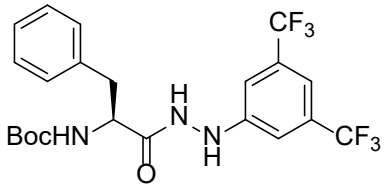
tert-Butyl (*S*)-(1-(2-(3-bromophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Cp



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.33 g, 1.23 mmol) and 3-bromophenylhydrazine hydrochloride (0.25 g, 1.35 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a pale orange solid (0.22 g, 41 %); R_f 0.94 (EtOAc); m.p. 129 - 131 °C; ν_{max} 3334, 3247 (N-H), 2987 (C-H), 1693, 1673 (C=O), 1525, 1487, 1320, 1248, 1165, 859, 678 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.07 (1H, bs, CON*H*NH), 7.29 (5H, m, Ar-*H*), 7.19 (2H, dd, *J* 7, 2, Ar-*H*), 6.97 (2H, d, *J* 4, Ar-*H*), 6.81 (1H, s, Ar-

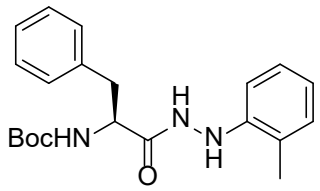
H), 6.49 (1H, bs, Ar-N*H*), 5.15 (1H, bd, *J* 8, BocN*H*CH), 4.45 (1H, q, *J* 7, BocNHC*H*), 3.07 (2H, t, *J* 7, Ar-CH_2), 1.43 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C*), 136.5 (*ipso*-Ar-*C*), 130.9 (Ar-*C*), 129.7 (Ar-*C*), 129.4 (Ar-*C*), 127.7 (Ar-*C*), 124.5 (Ar-*C*), 123.5 (Ar-*C*), 116.8 (Ar-*C*), 112.6 (Ar-*C*), 81.3 ((CH$_3$)$_3$*C*O), 54.9 (BocNH*C*H), 38.3 (Ar-*C*H$_2$), 28.8 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 456 ([^{79}Br]MNa$^+$), 458 ([^{81}Br]MNa$^+$); HRMS (ES$^+$) Found [^{79}Br]MNa$^+$, 456.0904 ($C_{20}H_{24}{}^{79}BrN_3O_3Na$ requires 456.0893).

tert-Butyl (*S*)-(1-(2-(3,5-bis(trifluoromethyl)phenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Bx



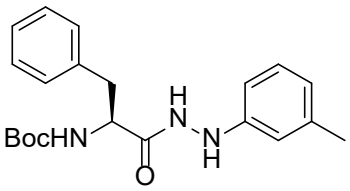
Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and (3,5-bis(trifluoromethyl)phenyl)hydrazine hydrochloride (0.47 g, 1.66 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a brown gummy solid (0.39 g, 52%); R_f 0.44 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3331, 3275 (N-H), 2964, 2936 (C-H), 1685, 1666 (C=O), 1524, 1468, 1379, 1276, 1121, 681 cm^{-1}; δ_H (300 MHz, i$CDCl_3$) 8.09 (1H, bs, CON*H*NH), 7.34 – 7.29 (4H, m, Ar-*H*), 7.21 – 7.18 (2H, *J* 7, 2, Ar-*H*), 7.10 (2H, s, Ar-*H*), 6.38 (1H, bd, *J* 3, Ar-N*H*), 5.06 (1H, bd, *J* 7, BocN*H*CH), 4.48 (1H, q, *J* 7, BocNHC*H*), 3.12 (2H, dd, *J* 6, 2, Ar-CH_2), 1.43 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 172.2 (*C*=ONHNH), 148.9 (*ipso*-Ar-*C*), 135.8 (*ipso*-Ar-*C*), 132.7 (*ipso*-Ar-*C*), 132.3 (*ipso*-Ar-*C*), 129.1 (Ar-*C*), 128.9 (Ar-*C*), 127.4 (Ar-*C*), 112.9 (Ar-*C*), 81.4 ((CH$_3$)$_3$*C*O), 28.2 ((*C*H$_3$)$_3$CO); δ_F (300 MHz, $CDCl_3$) -63.1 (C*F*$_3$); *m/z* (ES$^+$) 514 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 514.1542 ($C_{22}H_{23}N_3O_3F_6Na$ requires 514.1536).

tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-(o-tolyl)hydrazineyl)propan-2-yl)carbamate 67Dt



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.20 g, 0.75 mmol) and 2-methylphenylhydrazine hydrochloride (0.10 g, 0.83 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange gummy solid (0.22 g, 78%); R_f 0.95 (EtOAc); ν_{max} 3330, 3273 (N-H), 2977 (C-H), 1683, 1652 (C=O), 1522, 1496, 1294, 1165, 745 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.20 (1H, bs, CON*H*NH), 7.27 (3H, t, *J* 6, Ar-*H*), 7.20 (2H, d, *J* 6, Ar-*H*), 7.01 (1H, d, *J* 7, Ar-*H*), 6.96 (1H, t, *J* 7, Ar-*H*), 6.78 (1H, t, *J* 7, Ar-*H*), 6.36 (1H, d, *J* 7, Ar-*H*), 5.27 (1H, bd, *J* 8, BocN*H*CH), 4.51 (1H, q, *J* 7, BocNHC*H*), 3.08 (2H, t, *J* 7, Ar-CH_2), 2.16 (3H, s, Ar-CH_3), 1.41 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.5 (*C*=ONHNH), 155.8 (*C*=ONH), 145.2 (*ipso*-Ar-*C*), 136.3 (*ipso*-Ar-*C*), 130.5 (Ar-*C*), 129.5 (Ar-*C*), 128.9 (Ar-*C*), 127.2 (Ar-*C*), 126.9 (Ar-*C*), 123.2 (Ar-*C*), 121.0 (Ar-*C*), 112.2 (Ar-*C*), 80.7 ((CH$_3$)$_3$*C*O), 54.6 (BocNH*C*H), 38.3 (Ar-*C*H$_2$), 28.4 ((*C*H$_3$)$_3$CO), 17.1 (Ar-*C*H$_3$); ***m/z*** (ES^+) 392 (MNa^+); HRMS (ES^+) Found MNa^+, 392.1963 ($C_{21}H_{27}N_3O_3Na$ requires 392.1945).

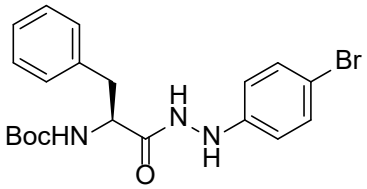
tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-(m-tolyl)hydrazineyl)propan-2-yl)carbamate 67Dz



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.33 g, 1.23 mmol) and 3-methylphenylhydrazine hydrochloride (0.16 g, 1.35 mmol) were transformed following work up with Et_2O into the title compound which was isolated as an orange oil (0.41 g, 91%); R_f 0.86 (EtOAc); ν_{max} 3327, 3285 (N-H), 1691, 1653 (C=O), 1518, 1495, 1250, 1164, 771, 699 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.14 (1H, bs, CON*H*NH), 7.27 (3H, t, *J* 7, Ar-*H*), 7.18 (2H, d, *J* 7, Ar-*H*), 7.01 (1H, t, *J* 7, Ar-*H*), 6.67 (1H, d, *J* 7, Ar-*H*), 6.46 (1H, s, Ar-*H*), 6.35 (1H, d, *J* 7, Ar-*H*),

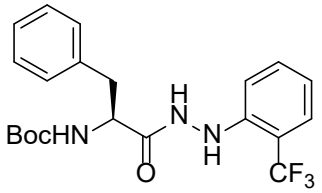
5.23 (1H, bd, *J* 8, BocN*H*CH), 4.48 (1H, q, *J* 7, BocNHC*H*), 3.05 (2H, t, *J* 8, Ar-C*H*$_2$), 2.23 (3H, s, Ar-C*H*$_3$), 1.41 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 155.7 (*C*=ONH), 147.5 (*ipso*-Ar-*C*), 139.0 (*ipso*-Ar-*C*), 136.4 (Ar-*C*), 129.5 (Ar-*C*), 129.1 (Ar-*C*), 128.9 (Ar-*C*), 127.1 (Ar-*C*), 122.3 (Ar-*C*), 114.4 (Ar-*C*), 110.7 (Ar-*C*), 80.4 ((CH$_3$)$_3$*C*O), 54.7 (BocNH*C*H), 38.2 (Ar-*C*H$_2$), 28.4 ((*C*H$_3$)$_3$CO), 21.6 (Ar-*C*H$_3$); *m/z* (ES$^+$) 392 (MNa$^+$); HRMS (ES$^+$) Found MNa$^+$, 392.1975 ($C_{21}H_{27}N_3O_3Na$ requires 392.1945).

tert-Butyl (*S*)-(1-(2-(4-bromophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Cv



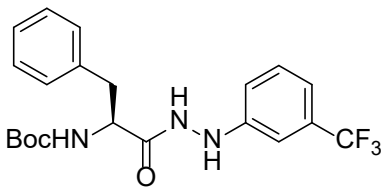
Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 4-bromophenylhydrazine hydrochloride (0.37 g, 1.66 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as an orange solid (0.37 g, 56%); R_f 0.37 (DCM/EtOH/NH_3 200:6:1); m.p. 127 - 129 °C; ν_{max} 3308 (N-H), 2984 (C-H), 1687, 1654 (C=O), 1521, 1488, 1366, 1252, 1167, 1003, 820 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.22 (1H, bs, CON*H*NH), 7.32 – 7.28 (3H, m, Ar-*H*), 7.23 – 7.18 (4H, m, Ar-*H*), 6.41 – 6.38 (2H, d, *J* 8, Ar-*H*), 5.27 (1H, bd, *J* 8, BocN*H*CH), 4.62 (1H, q, *J* 7, BocNHC*H*), 3.08 (2H, d, *J* 7, Ar-C*H*$_2$), 1.44 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.7 (*C*=ONHNH), 155.6 (*C*=ONH), 146.6 (*ipso*-Ar-*C*), 136.1 (*ipso*-Ar-*C*), 131.8 (Ar-*C*), 129.4 (Ar-*C*), 127.1 (Ar-*C*), 115.1 (Ar-*C*), 113.0 (Ar-*C*), 80.8 ((CH$_3$)$_3$*C*O), 54.4 (BocNH*C*H), 38.2 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 456 ([^{79}Br]MNa$^+$), 458 ([^{81}Br]MNa$^+$); HRMS (ES$^+$) Found [^{79}Br]MNa$^+$, 456.0897 ($C_{20}H_{24}{}^{79}BrN_3O_3Na$ requires 456.0893).

tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-(2-(trifluoromethyl)phenyl)hydrazineyl) propan-2-yl)carbamate 67Bd



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 2-trifluoromethylphenylhydrazine hydrochloride (0.35 g, 1.66 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow gummy solid (0.42 g, 66%); R_f 0.47 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3331, 3283 (N-H), 1665, 1618 (C=O), 1524, 1497, 1325, 1282, 1162, 1107, 751 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.66 (1H, bs, CON*H*NH), 7.37 (1H, m, Ar-*H*), 7.20 – 7.16 (3H, m, Ar-*H*), 7.13 – 7.02 (3H, m, Ar-*H*), 6.76 (1H, m, Ar-*H*), 6.36 (1H, m, Ar-*H*), 5.50 (1H, bd, *J* 8, BocN*H*CH), 4.60 (1H, m, BocNHC*H*), 3.00 (2H, m, Ar-C*H*$_2$), 1.27 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.8 (*C*=ONH), 145.1 (*ipso*-Ar-*C*), 136.2 (*ipso*-Ar-*C*), 132.9 (Ar-*C*F_3), 129.4 (Ar-*C*), 128.7 (Ar-*C*), 127.0 (Ar-*C*), 126.4 (1C, q, *J* 5, *ipso*-Ar-*C*CF_3), 122.7 (Ar-*C*), 119.9 (Ar-*C*), 117.7 (Ar-*C*), 113.5 (Ar-*C*), 111.9 (Ar-*C*), 80.7 ((CH$_3$)$_3$*C*O), 54.3 (BocNH*C*H), 38.5 (Ar-*C*H$_2$), 28.2 ((*C*H$_3$)$_3$CO); δ_F (300 MHz, $CDCl_3$) -61.9 (C*F*$_3$); *m/z* (ES^+) 424 (MNa^+); HRMS (ES^+) Found MNa^+, 446.1009 ($C_{21}H_{24}N_3O_3F_3Na$ requires 446.1662).

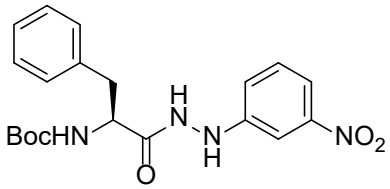
tert-Butyl (*S*)-(1-oxo-3-phenyl-1-(2-(3-(trifluoromethyl)phenyl)hydrazineyl) propan-2-yl)carbamate 67Bj



Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 3-trifluoromethylphenylhydrazine hydrochloride (0.35 g, 1.66 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.48 g, 76%); R_f 0.46 (DCM/EtOH/NH_3 200:6:1); m.p. 141 - 143 °C; ν_{max} 3338 (N-H), 1676 (C=O), 1519, 1334, 1249, 1161, 696 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.13 (1H, bs, CON*H*NH), 7.23 – 7.17 (3H, m, Ar-*H*), 7.15 – 7.10 (3H, m, Ar-*H*), 7.03 (1H, d, *J* 7, Ar-*H*), 6.85 (1H, s, Ar-*H*), 6.63 (1H, d, *J* 7,

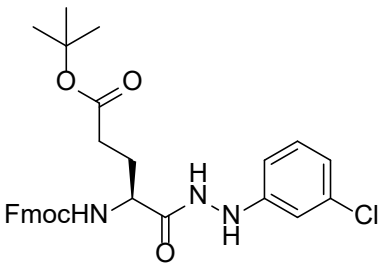
Ar-*H*), 5.12 (1H, bs, BocN*H*CH), 4.45 (1H, q, *J* 7, BocNHC*H*), 3.02 (2H, dd, *J* 7, 3, Ar-CH_2), 1.35 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 155.7 (*C*=ONH), 147.9 (*ipso*-Ar-*C*), 135.9 (*ipso*-Ar-*C*), 131.7 (1C, q, *J* 317, *ipso*-Ar-*C*F_3), 129.6 (Ar-*C*), 129.3 (Ar-*C*), 128.8 (Ar-*C*), 128.6 (Ar-*C*), 127.2 (Ar-*C*), 127.0 (Ar-*C*), 125.8 (Ar-*C*), 122.2 (1C, *ipso*-Ar-*C*CF_3), 117.6 (Ar-*C*), 116.4 (Ar-*C*), 110.0 (Ar-*C*), 80.9 ((CH$_3$)$_3$*C*O), 54.5 (BocNH*C*H), 37.9 (Ar-*C*H$_2$), 28.2 ((*C*H$_3$)$_3$CO); δ_F (300 MHz, $CDCl_3$) -62.7 (C*F*$_3$); *m/z* (ES^+) 424 (MNa^+); HRMS (ES^+) Found MNa^+, 446.1009 ($C_{21}H_{24}N_3O_3F_3Na$ requires 446.1662).

tert-Butyl (*S*)-(1-(2-(3-nitrophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl) carbamate 67Dh



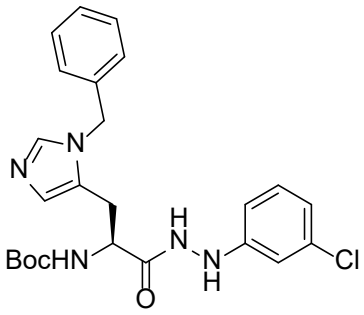
Following the general procedure outlined, *N*-Boc-*L*-phenylalanine (0.40 g, 1.51 mmol) and 3-nitrophenylhydrazine hydrochloride (0.31 g, 1.66 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a pale yellow solid (0.43 g, 70%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 134 - 136 °C; ν_{max} 3359, 3336, 3239 (N-H), 1694, 1664 (C=O), 1525, 1351, 1253, 1165, 1047, 733 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.13 (1H, bs, CON*H*NH), 7.73 (1H, dd, *J* 8, 1, Ar-*H*), 7.54 (1H, t, *J* 2, Ar-*H*), 7.35 – 7.30 (3H, m, Ar-*H*), 7.26 – 7.21 (2H, m, Ar-*H*), 6.89 (1H, d, *J* 8, Ar-*H*), 5.14 (1H, bd, *J* 8, BocN*H*CH), 4.53 (1H, q, *J* 7, BocNHC*H*), 3.02 (2H, d, *J* 7, 3, Ar-CH_2), 1.46 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.9 (*C*=ONHNH), 149.1 (*ipso*-Ar-*C*), 148.8 (*ipso*-Ar-*C*), 135.9 (*ipso*-Ar-*C*), 129.8 (Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.3 (Ar-*C*), 119.1 (Ar-*C*), 115.8 (Ar-*C*), 107.9 (Ar-*C*), 81.2 ((CH$_3$)$_3$*C*O), 51.1 (BocNH*C*H), 37.7 (Ar-*C*H$_2$), 28.3 ((*C*H$_3$)$_3$CO); *m/z* (ES^+) 423 (MNa^+); HRMS (ES^+) Found MNa^+, 423.1056 ($C_{20}H_{24}N_4O_5Na$ requires 423.1639).

tert-Butyl (*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(2-(3-chlorophenyl)hydrazinyl)-5-oxopentanoate 68n



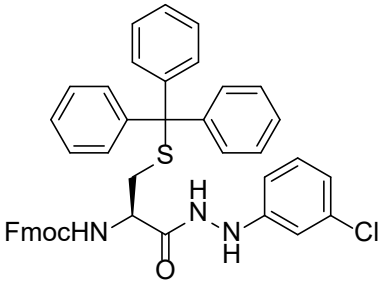
Following the general procedure outlined, *N*-Fmoc-*O*-(tert-butyl)-*L*-glutamic acid (0.30 g, 0.71 mmol) and 3-chlorophenylhydrazine hydrochloride (0.14 g, 0.78 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a yellow solid (0.27 g, 70%); R_f 0.75 (n-hex/EtOAc 4:1); m.p. 139 - 141 °C; υmax 3275 (N-H), 1686, 1659 (C=O), 1541, 1477, 1257, 1154, 1048, 735 cm-1; δ_H (300 MHz, $CDCl_3$) 8.31 (1H, bs, CON*H*NH), 7.76 – 7.74 (2H, d, *J* 7, Ar-*H*), 7.58 – 7.55 (2H, d, *J* 7, Ar-*H*), 7.41 – 7.36 (2H, t, *J* 7, Ar-*H*), 7.32 – 7.28 (2H, dt, *J* 7, 1, Ar-*H*), 7.09 (1H, t, *J* 7, Ar-*H*), 6.85 (1H, d, *J* 7, Ar-*H*), 6.79 (1H, s, Ar-*H*), 6.68 (1H, d, *J* 8, Ar-*H*), 5.82 (1H, bd, *J* 7, FmocN*H*CH), 4.46 (2H, m, CO_2CH_2CH-Fluorene), 4.37 (1H, m, CO_2CH_2CH-Fluorene), 4.22 (1H, m, FmocNHC*H*), 2.36 (2H, m, $CHCH_2CH_2CO_2$), 2.17 (2H, m, $CHCH_2CH_2CO_2$), 1.46 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.9 (C=OO(CH$_3$)$_3$), 171.7 (*C*=ONHNH), 156.5 *(C*=ONH), 148.9 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 135.0 (*ipso*-Ar-*C*), 130.3 (Ar-*C*), 127.3 (Ar-*C*), 127.1 (Ar-*C*), 124.9 (Ar-*C*), 121.2 (Ar-*C*), 120.0 (Ar-*C*), 113.6 (Ar-*C*), 111.7 (Ar-*C*), 81.4 ($(CH_3)_3CO$), 67.2 (CO_2CH_2CH-Fluorene), 53.1 (FmocNH*C*H), 47.1 (*C*-Fluorene), 31.6 ($CHCH_2CH_2CO_2$), 28.1 ($(CH_3)_3CO$), 27.3 ($CHCH_2CH_2CO_2$); ***m/z*** (ES^+) 572 ($[^{35}Cl]MNa^+$), 574 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 572.1934 ($C_{30}H_{32}{}^{35}ClN_3O_5Na$ requires 572.1923).

tert-Butyl (*S*)-(3-(1-benzyl-1H-imidazol-5-yl)-1-(2-(3-chlorophenyl) hydrazineyl)-1-oxopropan-2-yl)carbamate 67y



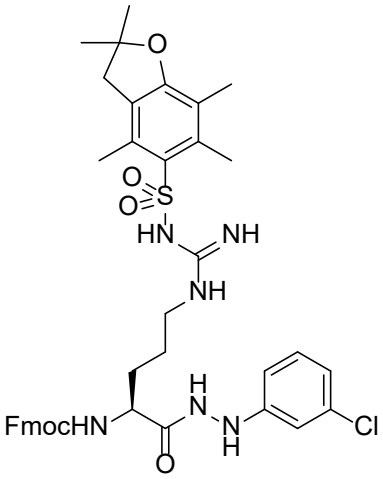
Following the general procedure outlined, *N*-Fmoc-*N*-(im)-benzyl-*L*-histidine (0.40 g, 1.16 mmol) and 3-chlorophenylhydrazine hydrochloride (0.23 g, 1.27 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a reddish brown oil (0.40 g, 74%); R_f 0.35 (DCM/EtOH/NH_3 200:6:1); υmax 3269 (N-H), 2979 (C-H), 1678 (C=O), 15497, 1479, 1366, 1249, 1160, 729 cm-1; δ_H (300 MHz, $CDCl_3$) 9.37 (1H, bs, CON*H*NH), 7.97 (1H, bs, Ar-N*H*), 7.38 – 7.32 (5H, m, Ar-*H*), 7.18 – 7.15 (2H, m, Ar-*H*), 7.06 (1H, t, *J* 7, Ar-*H*), 6.79 (1H, m, Ar-*H*), 6.68 (1H, t, *J* 2, Ar-*H*), 6.65 (1H, dd, *J* 8, 1, Ar-*H*), 6.32 (1H, bd, *J* 7, BocN*H*CH), 5.09 (2H, s, Ar-CH_2Im), 4.65 (1H, m, BocNHC*H*), 3.11 (2H, t, *J* 6, CHCH_2Im), 1.42 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C)*, 134.8 (*ipso*-Ar-*C*), 130.1 (Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.9 (Ar-*C*), 120.9 (Ar-*C*), 113.2 (Ar-*C*), 111.8 (Ar-*C*), 80.1 ((CH$_3$)$_3$*C*O), 52.1 (Ar-*C*H$_2$Im), 29.5 (CH*C*H$_2$Im), 28.4 ((*C*H$_3$)$_3$CO); ***m/z*** (ES$^+$) 470 ([^{35}Cl]MH$^+$), 472 ([^{37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35}Cl]MH$^+$, 470.1952 ($C_{24}H_{29}{}^{35}ClN_5O_3$ requires 470.1953).

(9H-fluoren-9-yl)methyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-1-oxo-3-(tritylthio)propan-2-yl)carbamate 68p



Following the general procedure outlined, *N*-Fmoc-*S*-(trityl)-*L*-cysteine (0.40 g, 0.68 mmol) and 3-chlorophenylhydrazine hydrochloride (0.15 g, 0.82 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a light brown solid (0.35 g, 72%); R_f 0.24 (n-hex/EtOAc 4:1); m.p. 90 - 93 °C; υmax 3271 (N-H), 1677 (C=O), 1597, 1486, 1445, 1219, 1033, 738, 699 cm-1; δ_H (300 MHz, $CDCl_3$) 7.76 – 7.73 (2H, d, *J* 7, Ar-*H*), 7.56 – 7.53 (2H, d, *J* 7, Ar-*H*), 7.43 – 7.39 (4H, m, Ar-*H*), 7.31 – 7.18 (15H, m, Ar-*H*), 7.06 (1H, t, *J* 7, Ar-*H*), 6.84 (1H, dd, *J* 7,2 , Ar-*H*), 6.74 (1H, s, Ar-*H*), 6.62 (1H, d, *J* 7, Ar-*H*), 5.92 (1H, bs, Ar-N*H*), 4.92 (1H, bd, *J* 7, FmocN*H*CH), 4.46 (2H, m, CO_2CH_2CH-Fluorene), 4.17 (1H, m, CO_2CH_2CH-Fluorene), 3.76 (1H, m, FmocNHC*H*), 2.68 (2H, m, CHCH_2S-(trityl)); δ_C (100 MHz, $CDCl_3$) 148.7 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 143.5 (*ipso*-Ar-*C)*, 141.4 (*ipso*-Ar-*C)*, 135.0 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 129.5 (Ar-*C*), 128.2 (Ar-*C*), 127.9 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 124.8 (Ar-*C*), 121.3 (Ar-*C*), 120.1 (Ar-*C*), 113.6 (Ar-*C*), 111.9 (Ar-*C*), 67.6 (*C*-(trityl), 67.1 (CO_2CH_2CH-Fluorene), 47.2 (*C*-Fluorene); *m/z* (ES^+) 732 ([^{35}Cl]MNa^+), 734 ([^{37}Cl]MNa^+); HRMS (ES^+) Found [^{35}Cl]MNa^+, 732.2071 ($C_{43}H_{36}ClN_3O_3S$Na requires 732.2058).

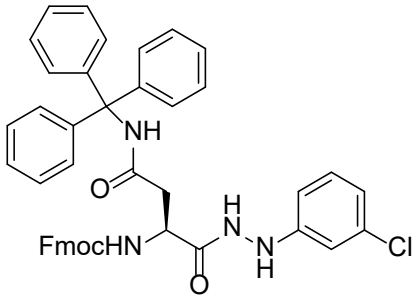
(9H-fluoren-9-yl)methyl (*S*)-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) pentan-2-yl)carbamate 68t



Following the general procedure outlined, *N*-Fmoc-*N*-((2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl)-*L*-arginine (0.40 g, 0.62 mmol) and 3-chlorophenylhydrazine hydrochloride (0.12 g, 0.68 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a light brown solid (0.14 g, 30%); R_f 0.32 (DCM/EtOH/NH_3 200:6:1); m.p. 120 - 122 °C; υmax 3282 (N-H), 2972, 2935 (C-H), 1684, 1658 (C=O), 1537, 1451, 1256, 1090, 758, 641 cm-1; δ_H (300 MHz, DMSO-d6) 9.89 (1H, bd, *J* 2, CON*H*NH), 8.06 (1H, bd, *J* 2, SO_2N*H*), 7.91 – 7.89 (2H, d, *J* 7, Ar-*H*), 7.75 – 7.71 (2H, t, *J* 6, Ar-*H*), 7.44 – 7.39 (2H, t, *J* 7, Ar-*H*), 7.35 – 7.30 (2H, d, *J* 7, Ar-*H*), 7.11 (1H, t, *J* 7, Ar-*H*), 6.74 – 6.70 (2H, m, Ar-*H*), 6.68 (1H, m, Ar-*H*), 6.45 (1H, bs, Ar-N*H*), 4.29 (2H, m, CO_2CH_2CH-Fluorene), 4.26 (1H, m, CO_2CH_2CH-Fluorene), 4.06 (1H, m, FmocNHC*H*), 3.09 (2H, m, CH_2NHCNHNH), 2.95 (2H, s, CH_2-benzofuran), 2.50 (3H, s, CH_3-benzofuran), 2.44 (3H, s, CH_3-benzofuran), 2.00 (3H, m, CH_3-benzofuran), 1.69 (2H, m, FmocNHCHCH_2), 1.49 (2H, m, $CHCH_2CH_2$), 1.40 (6H, s, $(CH_3)_2$-benzofuran); δ_C (100 MHz, DMSO-d6) 172.3 (*C*=ONHNH), 157.9 (*C*=NHNH) 156.5 *(C*=ONH), 151.3 (*ipso*-Ar-*C)*, 144.4 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 131.9 (Ar-*C*), 130.7 (Ar-*C*), 128.1 (Ar-*C*), 127.5 (Ar-*C*), 127.2 (Ar-*C*), 125.7 (Ar-*C*), 124.8 (Ar-*C*), 120.6 (Ar-*C*), 86.8 (*C*-benzofuran), 66.2 (CO_2CH_2CH-Fluorene), 53.7 (FmocNH*C*H), 47.1 (*C*-Fluorene), 42.9 (*C*H_2-benzofuran), 40.9 (*C*H_2NHCNHNH), 29.3 (FmocNHCH*C*H_2), 28.8 (CH_3)$_2$-

benzofuran), 25,2 (CHCH$_2$*C*H$_2$), 19.5 (*C*H$_3$-benzofuran), 18.1 (*C*H$_3$-benzofuran),12.8 (*C*H$_3$-benzofuran); *m/z* (ES$^+$) 773 ([^{35}Cl]MH$^+$), 775 ([^{37}Cl]MH$^+$), 795 ([^{35}Cl]MNa$^+$), 797 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MH$^+$, 773.2899 ($C_{40}H_{46}{}^{35}ClN_6O_6S$ requires 773.2883).

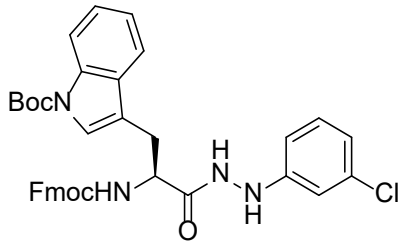
(9H-fluoren-9-yl)methyl (*S*)-(1-(2-(3-chlorophenyl)hydrazineyl)-1,4-dioxo-4 (tritylamino)butan-2-yl)carbamate 68o



Following the general procedure outlined, *N*-Fmoc-*N*-(trityl)-*L*-asparagine (0.40 g, 0.67 mmol) and 3-chlorophenylhydrazine hydrochloride (0.13 g, 0.74 mmol) were transformed following trituration with Et$_2$O into the title compound which was isolated as a white solid (0.19 g, 37%); R$_f$ 0.39 (DCM/EtOH/NH$_3$ 200:6:1); m.p. 168 - 171 °C; υmax 3304 (N-H), 1692, 1654 (C=O), 1527, 1489, 1259, 1033, 736, 701 cm-1; δ_H (300 MHz, DMSO-d6) 9.92 (1H, bd, *J* 2, CON*H*NH), 8.60 (1H, bs, CON*H*-trityl), 8.05 (1H, bd, *J* 2, CON*H*CH), 7.92 – 7.90 (2H, d, *J* 7, Ar-*H*), 7.76 – 7.74 (3H, d, *J* 7, Ar-*H*), 7.45 – 7.38 (3H, m, Ar-*H*), 7.23 – 7.19 (15H, m, Ar-*H*), 7.13 (1H, t, *J* 8, Ar-*H*), 6.73 (1H, m, Ar-*H*), 6.69 – 6.63 (2H, m, Ar-*H*), 4.43 (2H, m, CO$_2$C*H*$_2$CH-Fluorene), 4.32 – 4.25 (2H, m, FmocNHC*H*, CO$_2$CH$_2$C*H*-Fluorene), 2.83 (1H, m, FmocNHCHC*H*H), 2.59 (1H, d, *J* 4, FmocNHCHCH*H*); δ_C (100 MHz, DMSO-d6) 171.8 (*C*=ONHNH), 169.0 (*C*=NH-trityl), 156.3 (*C*=ONH), 151.3 (*ipso*-Ar-*C)*, 145.2 (*ipso*-Ar-*C)*, 144.4 (*ipso*-Ar-*C)*, 144.2 (*ipso*-Ar-*C)*, 141.2 (*ipso*-Ar-*C)*, 133.9 (*ipso*-Ar-*C*), 130.7 (Ar-*C*), 129.1 (Ar-*C*), 128.1 (Ar-*C*), 127.9 (Ar-*C*), 127.6 (Ar-*C*), 126.8 (Ar-*C*), 125.8 (Ar-*C*), 125.7 (Ar-*C*), 120.6 (Ar-*C*), 118.3 (Ar-*C*), 111.9 (Ar-*C*), 111.2 (Ar-*C*), 69.9 (*C*-(trityl), 66.4 (CO$_2$*C*H$_2$CH-Fluorene), 51.4 (FmocNH*C*H), 47.2 (*C*-Fluorene) 39.7 (FmocNHCH*C*H$_2$); *m/z* (ES$^+$) 743 ([^{35}Cl]MNa$^+$), 745 ([^{37}Cl]MNa$^+$), 1443 ([^{35,35}Cl]2M+H$^+$), 1445 ([^{35,37}Cl]2M+H$^+$), 1447 ([^{37,37}Cl]2M+H$^+$), 1463

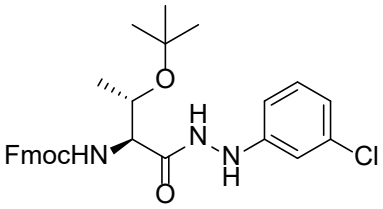
([^{35,35}Cl]2M+Na$^+$), 1465 ([^{35,37}Cl]2M+Na$^+$), 1467 ([^{37,37}Cl]2M+Na$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 743.2403 ($C_{44}H_{37}{}^{35}ClN_4O_4Na$ requires 743.2396).

tert-Butyl (*S*)-3-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(3-chlorophenyl)hydrazineyl)-3-oxopropyl)-1H-indole-1-carboxylate 68s



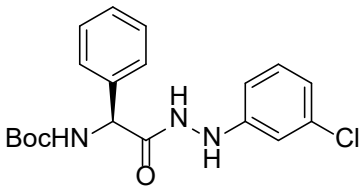
Following the general procedure outlined, *N*-Fmoc-*N*-(tert-butyl)-*L*-tryptophan (0.40 g, 0.76 mmol) and 3-chlorophenylhydrazine hydrochloride (0.15 g, 0.84 mmol) were transformed following Flash column chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gum (0.14 g, 27%); R_f 0.53 (DCM/EtOH/NH_3 200:6:1); υmax 3274 (N-H), 2975 (C-H), 1693, 1662 (C=O), 1597, 1452, 1369, 1269, 1158, 757, 735 cm-1; δ_H (300 MHz, DMSO-d6) 10.10 (1H, bd, *J* 2, CON*H*NH), 8.10 – 8.05 (2H, m, Ar-*H*), 7.89 – 7.86 (2H, d, *J* 7, Ar-*H*), 7.82 (1H, d, *J* 7, Ar-*H*), 7.66 – 7.63 (3H, t, *J* 5, Ar-*H*), 7.43 – 7.35 (3H, m, Ar-*H*), 7.28 – 7.20 (3H, m, Ar-*H*), 7.10 (1H, t, *J* 8, Ar-*H*), 6.70 – 6.67 (2H, m, Ar-*H*), 6.56 (1H, m, Ar-*H*), 4.50 (1H, m, FmocNHC*H*), 4.22 – 4.19 (3H, m, CO_2CH_2CH-Fluorene, CO_2CH_2CH-Fluorene), 3.14 (1H, dd, *J* 14, 5, FmocNHCHC*H*H), 3.07 (1H, dd, *J* 14, 9, FmocNHCHCH*H*), 1.59 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, DMSO-d6) 171.8 (*C*=ONHNH), 156.4 (*C*=ONH), 151.1 (*ipso*-Ar-*C)*, 149.5 (*ipso*-Ar-*C)*, 144.3 (*ipso*-Ar-*C)*, 144.1 (*ipso*-Ar-*C)*, 141.1 (*ipso*-Ar-*C)*, 135.2 (*ipso*-Ar-*C*), 133.9 (*ipso*-Ar-*C*), 130.9 (Ar-*C*), 130.5 (Ar-*C*), 128.1 (Ar-*C*), 127.5 (Ar-*C*), 125.7 (Ar-*C*), 124.8 (Ar-*C*), 124.7 (Ar-*C*), 122.9 (Ar-*C*), 120.6 (Ar-*C*), 119.9 (Ar-*C*), 118.3 (Ar-*C*), 116.8 (*ipso*-Ar-*C*), 115.2 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 84.0 ((CH_3)$_3$*C*O), 66.3 (CO_2*C*H_2CH-Fluorene), 53.5 (FmocNH*C*H), 47.0 (*C*-Fluorene), 28.1 ((*C*H_3)$_3$CO), 27.6 (FmocNHCH*C*H_2); *m/z* (ES$^+$) 550 (M-Boc+H$^+$).

(9H-fluoren-9-yl)methyl ((2*S*,3*S*)-3-(tert-butoxy)-1-(2-(3-chlorophenyl) hydrazinyl)-1-oxobutan-2-yl)carbamate 68l



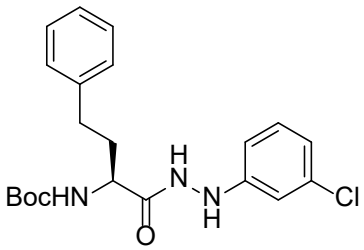
Following the general procedure outlined, *N*-Fmoc-*O*-(*tert*-butyl)-*L*-threonine (0.40 g, 1.01 mmol) and 3-chlorophenylhydrazine hydrochloride (0.19 g, 1.11 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a light brown solid (0.35 g, 66%); R_f 0.35 (n-hex/EtOAc 4:1); m.p. 88 - 90 °C; υmax 2973 (C-H), 1675 (C=O), 1598, 1478, 1190, 1077, 758, 739 cm-1; δ_H (300 MHz, $CDCl_3$) 8.47 (1H, bs, CON*H*NH), 7.77 – 7.74 (2H, d, *J* 7, Ar-*H*), 7.60 – 7.58 (2H, d, *J* 7, Ar-*H*), 7.42 – 7.37 (2H, t, *J* 7, Ar-*H*), 7.33 – 7.28 (2H, m, Ar-*H*), 7.14 (1H, t, *J* 7, Ar-*H*), 6.91 (1H, m, Ar-*H*), 6.86 (1H, s, Ar-*H*), 6.76 (1H, dd, *J* 8, 1, Ar-*H*), 5.91 (1H, bd, *J* 5, FmocN*H*CH), 4.44 (2H, m, CO_2CH_2CH-Fluorene), 4.32 (1H, m, FmocNHC*H*), 4.24 – 4.17 (2H, m, FmocNHCHC*H*, CO_2CH_2CH-Fluorene), 1.31 (9H, s, $(CH_3)_3CO$), 1.10 (3H, d, *J* 6, NHCHCHCH_3); δ_C (100 MHz, $CDCl_3$) 170.2 (*C*=ONHNH), 156.0 (*C*=ONH), 149.1 (*ipso*-Ar-*C*), 143.8 (*ipso*-Ar-*C)*, 143.6 (*ipso*-Ar-*C)*, 141.4 (*ipso*-Ar-*C)*, 141.3 (*ipso*-Ar-*C)*, 135.1 (*ipso*-Ar-*C*), 130.3 (Ar-*C*), 127.8 (Ar-*C*), 127.1 (Ar-*C*), 125.1 (Ar-*C*), 121.3 (Ar-*C*), 120.0 (Ar-*C*), 113.8 (Ar-*C*), 111.9 (Ar-*C*), 75.9 ($(CH_3)_3CO$), 67.1 (CO_2CH_2CH-Fluorene), 66.5 (FmocNHCH*C*H), 58.2 (FmocNH*C*H), 47.2 (*C*-Fluorene), 28.3 ($(CH_3)_3CO$) 17.2 (NHCHCH*C*H_3); *m/z* (ES^+) 544 ($[^{35}Cl]MNa^+$), 546 ($[^{37}Cl]MNa^+$), 1065 ($[^{35,35}Cl]2M+Na^+$), 1067 ($[^{35,37}Cl]2M+Na^+$), 1069 ($[^{37,37}Cl]2M+Na^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 544.2015 ($C_{29}H_{32}N_3O_4{}^{35}ClNa$ requires 544.1974).

tert-Butyl (*S*)-(2-(2-(3-chlorophenyl)hydrazinyl)-2-oxo-1-phenylethyl) carbamate 67Ab



Following the general procedure outlined, *N*-Boc-*L*-phenylglycine (0.40 g, 1.59 mmol) and 3-chlorophenylhydrazine hydrochloride (0.31 g, 1.75 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a pale yellow solid (0.28 g, 47%); R_f 0.45 (DCM/EtOH/NH_3 200:6:1); m.p. 146 - 149 °C; υmax 3302 (N-H), 1657 (C=O), 1597, 1486, 1362, 1251, 1170, 1051, 763, 696 cm-1; δ_H (300 MHz, DMSO-d6) 10.08 (1H, bd, *J* 2, CON*H*NH), 8.12 (1H, bd, *J* 2, CON*H*CH), 7. 51 – 7.49 (2H, m, Ar-*H*), 7. 40 – 7.33 (3H, m, Ar-*H*), 7.05 (1H, t, *J* 8, Ar-*H*), 6.68 (1H, m, Ar-*H*), 6.53 (1H, m, Ar-*H*), 6.51 (1H, m, Ar-*H*), 5.25 (1H, d, *J* 8, CONHC*H*), 1.42 (9H, s, (CH_3)$_3$CO); δ_C (100 MHz, DMSO-d6) 151.1 (*ipso*-Ar-*C)*, 133.9 (*ipso*-Ar-*C*), 130.6 (Ar-*C*), 128.8 (Ar-*C*), 128.3 (Ar-*C*), 127.8 (Ar-*C*), 118.3 (Ar-*C*), 111.2 (Ar-*C*), 78.7 ((CH$_3$)$_3$*C*O), 57.2 (BocNH*C*H), 28.6 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 398 ([^{35}Cl]MNa$^+$), 400 ([^{37}Cl]MNa$^+$), 773 ([^{35,35}Cl]2M+Na$^+$), 775 ([^{35,37}Cl]2M+Na$^+$), 777 ([^{37,37}Cl]2M+Na$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 398.1257 ($C_{19}H_{22}{}^{35}ClN_3O_3Na$ requires 398.1242).

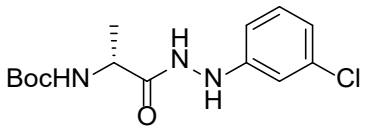
tert-Butyl (*S*)-(1-(2-(3-chlorophenyl)hydrazinyl)-1-oxo-4-phenylbutan-2-yl)carbamate 67Ac



Following the general procedure outlined, *N*-Boc-*L*-homophenylalanine (0.40 g, 1.43 mmol) and 3-chlorophenylhydrazine hydrochloride (0.28 g, 1.58 mmol) were transformed following work up with Et_2O into the title compound which was isolated as a light brown solid (0.28 g, 48%); R_f 0.45 (DCM/EtOH/NH_3 200:6:1); m.p. 112 - 115 °C; υmax 3287 (N-H), 2979 (C-H), 1676, 1661 (C=O), 1597, 1495, 1367, 1251, 1161, 768, 699 cm-1; δ_H (300 MHz, $CDCl_3$) 8.58 (1H, bs, CON*H*NH),

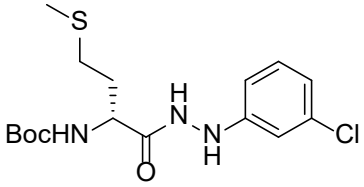
7. 27 – 7.25 (2H, m, Ar-*H*), 7. 21 (1H, m, Ar-*H*), 7.15 – 7.12 (2H, m, Ar-*H*), 7.06 (1H, t, *J* 8, Ar-*H*), 6.83 (1H, m, Ar-*H*), 6.77 (1H, t, *J* 2, Ar-*H*), 6.66 (1H, m, Ar-*H*), 5.27 (1H, bd, *J* 8, CON*H*CH), 4.20 (1H, m, CONHC*H*), 2.69 (2H, t, *J* 8, CH_2CH_2-Ar), 2.19 (1H, m, CONHCHC*H*H), 1.98 (1H, m, CONHCHCH*H*), 1.46 (9H, s, $(CH_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.5 (*C*=ONHNH), 156.0 *(C*=ONH), 149.1 (*ipso*-Ar-*C)*, 140.5 (*ipso*-Ar-*C)*, 135.0 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 128.6 (Ar-*C*), 128.4 (Ar-*C*), 128.3 (Ar-*C*), 126.3 (Ar-*C*), 121.0 (Ar-*C*), 113.4 (Ar-*C*), 111.7 (Ar-*C*), 80.8 ($(CH_3)_3CO$), 52.5 (BocNH*C*H), 33.3 ($CONHCHCH_2$), 31.8 (CH_2CH_2-Ar), 28.4 ($(CH_3)_3CO$); *m/z* (ES^+) 426 ($[^{35}Cl]MNa^+$), 428 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 426.1573 ($C_{21}H_{26}{}^{35}ClN_3O_3Na$ requires 426.1555).

tert-Butyl (*R*)-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl) carbamate 67Ad



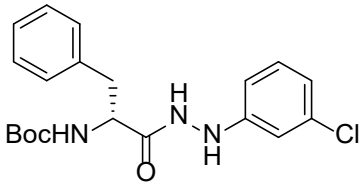
Following the general procedure outlined, *N*-Boc-*D*-alanine (0.40 g, 2.11 mmol) and 3-chlorophenylhydrazine hydrochloride (0.42 g, 2.33 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow gummy solid (0.32 g, 48%); R_f 0.42 (DCM/EtOH/NH_3 200:6:1); υmax 3428, 3279 (N-H), 1687 (C=O), 1598, 1484, 1245, 11159, 1023, 855 cm-1; δ_H (300 MHz, $CDCl_3$) 8.65 (1H, bs, CON*H*NH), 7.03 (1H, t, *J* 7, Ar-*H*), 6.76 (1H, m, Ar-*H*), 6.66 (1H, t, *J* 2, Ar-*H*), 6.58 (1H, m, Ar-*H*), 6.28 (1H, bd, *J* 3, N*H*-Ar), 5.24 (1H, m, CON*H*CH), 4.24 (1H, m, CONHC*H*), 1.38 (9H, s, $(CH_3)_3CO$), 1.30 (1H, d, *J* 7, $CONHCHCH_3$); δ_C (100 MHz, $CDCl_3$) 173.3 (*C*=ONHNH), 155.9 *(C*=ONH), 149.1 (*ipso*-Ar-*C)*, 134.9 (*ipso*-Ar-*C)*, 130.2 (Ar-*C*), 120.8 (Ar-*C*), 113.2 (Ar-*C*), 111.6 (Ar-*C*), 80.7 ($(CH_3)_3CO$), 48.6 (BocNH*C*H), 28.3 ($(CH_3)_3CO$) 17.9 ($CONHCHCH_3$); *m/z* (ES^+) 336 ($[^{35}Cl]MNa^+$), 338 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 336.1097 ($C_{14}H_{20}{}^{35}ClN_3O_3Na$ requires 336.1085).

tert-Butyl (*R*)-(1-(2-(3-chlorophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Ae



Following the general procedure outlined, *N*-Boc-*D*-methionine (0.40 g, 1.60 mmol) and 3-chlorophenylhydrazine hydrochloride (0.32 g, 1.77 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a yellow gummy solid (0.27 g, 45%); R_f 0.47 (DCM/EtOH/NH_3 200:6:1); υmax 3332, 3269 (N-H), 1683, 1659 (C=O), 1597, 1477, 1246, 1219, 1164, 736 cm-1; δ_H (300 MHz, $CDCl_3$) 8.76 (1H, bs, CON*H*NH), 7.03 (1H, t, *J* 8, Ar-*H*), 6.76 (1H, d, *J* 8, Ar-*H*), 6.69 (1H, t, *J* 2, Ar-*H*), 6.58 (1H, dd, *J* 8, 2, Ar-*H*), 6.32 (1H, bd, *J* 3, N*H*-Ar), 5.41 (1H, bd, *J* 8, CON*H*CH), 4.38 (1H, q, *J* 7, CONHC*H*), 2.49 (2H, t, *J* 7, $CHCH_2C\mathit{H}_2S$), 2.00 (3H, s, $CHCH_2CH_2SC\mathit{H}_3$),1.92 (2H, m, $CHC\mathit{H}_2CH_2S$), 1.38 (9H, s, $(C\mathit{H}_3)_3CO$); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*=ONHNH), 155.9 *(C*=ONH), 149.1 (*ipso*-Ar-*C)*, 134.9 (*ipso*-Ar-*C*), 130.2 (Ar-*C*), 120.9 (Ar-*C*), 113.3 (Ar-*C*), 111.6 (Ar-*C*), 80.8 ($(CH_3)_3\mathit{C}O$), 51.9 (BocNH*C*H), 31.2 ($CH\mathit{C}H_2CH_2S$), 30.2 ($CHCH_2\mathit{C}H_2S$), 28.4 ($(\mathit{C}H_3)_3CO$), 15.4 ($CHCH_2CH_2S\mathit{C}H_3$); *m/z* (ES^+) 396 ($[^{35}Cl]MNa^+$), 398 ($[^{37}Cl]MNa^+$); HRMS (ES^+) Found $[^{35}Cl]MNa^+$, 396.1136 ($C_{16}H_{24}{}^{35}ClN_3O_3SNa$ requires 396.1119).

tert-Butyl (*R*)-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Af



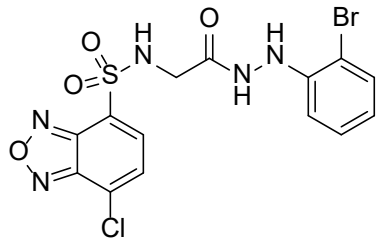
Following the general procedure outlined, *N*-Boc-*D*-phenylalanine (0.40 g, 1.51 mmol) and 3-chlorophenylhydrazine hydrochloride (0.29 g, 1.66 mmol) were transformed following trituration with Et_2O into the title compound which was isolated as a white solid gum (0.26 g, 45%); R_f 0.55 (DCM/EtOH/NH_3 200:6:1); m.p. 148 - 150 °C; υmax 3355, 3337, 3244 (N-H), 2987 (C-H), 1692, 1673 (C=O), 1596, 1525, 1166, 863, 772, 679 cm-1; δ_H (300 MHz, $CDCl_3$) 7.92 (1H, bs, CON*H*NH), 7.38 – 7.30 (3H, m, Ar-*H*), 7.24 – 7.21 (2H, m, Ar-*H*), 7.11 (1H, t, *J*

8, Ar-*H*), 6.87 (1H, dd, *J* 8, 1, Ar-*H*), 6.67 (1H, t, *J* 2, Ar-*H*), 6.51 (1H, d, *J* 8, Ar-*H*), 5.11 (1H, bd, *J* 8, CON*H*CH), 4.49 (1H, q, *J* 7, CONHC*H*), 3.12 (1H, d, *J* 7, CONHCHC*H*$_2$), 1.46 (9H, s, (C*H*$_3$)$_3$CO); δ_C (100 MHz, $CDCl_3$) 171.6 (*C*=ONHNH), 148.8 (*ipso*-Ar-*C)*, 136.0 (*ipso*-Ar-*C)*, 134.9 (*ipso*-Ar-*C*), 130.1 (Ar-*C*), 129.3 (Ar-*C*), 128.9 (Ar-*C*), 127.3 (Ar-*C*), 121.2 (Ar-*C*), 113.4 (Ar-*C*), 111.7 (Ar-*C*), 28.4 ((*C*H$_3$)$_3$CO); *m/z* (ES$^+$) 412 ([^{35}Cl]MNa$^+$), 414 ([^{37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 412.1410 ($C_{20}H_{24}{}^{35}ClN_3O_3Na$ requires 412.1398).

6.1.2.4 Synthesis of Benzoxa-[2,1,3]-diazole Peptidomimetics – General Procedure

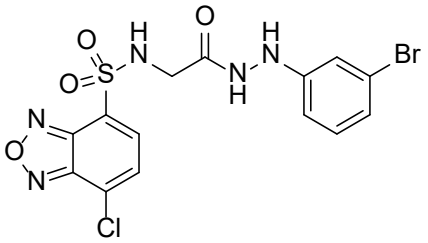
Under a nitrogen atmosphere *N*-Boc amino acid hydrazides (1.10 equiv.) were dissolved in 4 M HCl solution in dioxane (3 mL) and stirred at room temperature. After 90 minutes the mixture was evaporated, dried in *vacuo* and precipitated by using (EtOH / Et_2O). The quat was directly used in the next step. the resulting solid (1 equiv.) was suspended in THF (3 mL) and triethylamine (3 equiv.) was added. After stirring for 10 minutes at room temperature, the solution was treated with 7-chlorobenzoxa-[2,1,3]-diazole-4-sulfonyl chloride (1.10 equiv.) and continued stirring for 5 hours. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo*. The precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH3 [600:8:1], [400:8:1], [200:6:1]) afforded the desired sulfonamides **65**.

N-(2-(2-(2-bromophenyl)hydrazineyl)-2-oxoethyl)-7-chlorobenzo[c][1,2,5] oxadiazole-4-sulfonamide 65h



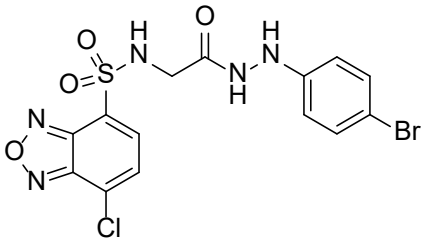
Following the general procedure outlined, *tert*-Butyl (2-(2-(2-bromophenyl)hydrazineyl)-2-oxoethyl)carbamate **67Ce** (0.27 g, 0.78 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.19 g, 48%) as a mixture of rotamers [4:1]; Rf 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3156 (N-H), 1660 (C=O), 1591, 1488, 1340, 1159, 1040, 948 cm^{-1}; all data provided for the major rotamer δ_H (300 MHz, DMSO-d_6) 9.93 (1H, bd, *J* 2, CON*H*NH), 8.73 (1H, bs, Ar-N*H*), 8.04 (1H, d, *J* 7, Ar-*H*), 7.93 (1H, bd, *J* 5, SO_2N*H*CH), 7.88 (1H, d, *J* 7, Ar-*H*), 7.42 (1H, dd, *J* 7, 1, Ar-*H*), 7.20 (1H, td, *J* 8, 1, Ar-*H*), 7.05 (1H, d, *J* 2, Ar-*H*), 6.62 (1H, dd, *J* 8, 1, Ar-*H*), 6.72 (1H, dt, *J* 7, 1, Ar-*H*), 3.88 (2H, s, SO_2NHCH_2); δ_C (300 MHz, DMSO-d_6) 168.1 (*C*=ONHNH), 149.2 (*ipso*-Ar-*C*), 145.9 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.3 (Ar-*C*), 132.8 (Ar-*C*), 131.3 (Ar-*C*), 128.7 (Ar-*C*), 125.4 (*ipso*-Ar-*C*), 120.8 (Ar-*C*), 113.5 (Ar-*C*), 107.3 (*ipso*-Ar-*C*), 44.2 (SNH*C*H_2); *m/z* (ES^+) 459 ([^{35}Cl, ^{79}Br]MH^+), 461 ([^{35}Cl, ^{81}Br]MH^+), 461 ([^{37}Cl, ^{79}Br]MH^+), 463 ([^{37}Cl, ^{81}Br]MH^+); HRMS (ES^+) Found ([^{35}Cl, ^{79}Br]MH^+), 459.8853 ($C_{14}H_{12}{}^{35}Cl^{79}BrN_5O_4S$ requires 459.9477).

N-(2-(2-(3-bromophenyl)hydrazineyl)-2-oxoethyl)-7-chlorobenzo[c][1,2,5] oxadiazole-4-sulfonamide 65i



Following the general procedure outlined, *tert*-Butyl (2-(2-(3-bromophenyl)hydrazineyl)-2-oxoethyl)carbamate **67Ck** (0.17 g, 0.49 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown solid (0.17 g, 68%) as a mixture of rotamers [5:1]; Rf 0.33 (DCM/EtOH/NH_3 200:6:1); m.p. 247 – 249 °C; ν_{max} 3312, 3263 (N-H), 1688, 1658 (C=O), 1589, 1520, 1342, 1159, 1039, 950 cm^{-1}; all data provided for the major rotamer δ_H (300 MHz, DMSO-d_6) 9.79 (1H, bd, *J* 2, CON*H*NH), 8.72 (1H, bt, *J* 5, SO_2N*H*CH), 8.03 (1H, dd, *J* 7, 1, Ar-*H*), 7.91 (1H, m, Ar-N*H*), 7.86 (1H, d, *J* 7, Ar-*H*), 7.08 (1H, t, *J* 8, Ar-*H*), 6.86 (1H, m, Ar-*H*), 6.80 (1H, t, *J* 2, Ar-*H*), 6.61 (1H, dd, *J* 8, 1, Ar-*H*), 3.87 (2H, d, *J* 6, SO_2NHCH_2); δ_C (300 MHz, DMSO-d_6) 168.1 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.9 (*ipso*-Ar-*C*), 134.1 (Ar-*C*), 131.2 (Ar-*C*), 128.9 (Ar-*C*), 125.4 (*ipso*-Ar-*C*), 122.5 (Ar-*C*), 121.4 (Ar-*C*), 114.7 (Ar-*C*), 111.5 (Ar-*C*), 44.2 (SNH*C*H_2); *m/z* (ES^+) 459 ([^{35}Cl, ^{79}Br]MH^+), 461 ([^{35}Cl, ^{81}Br]MH^+), 461 ([^{37}Cl, ^{79}Br]MH^+), 463 ([^{37}Cl, ^{81}Br]MH^+); HRMS (ES^+) Found ([^{35}Cl, ^{79}Br]MH^+), 461.8837 ($C_{14}H_{12}{}^{35}Cl^{79}BrN_5O_4S$ requires 459.9477).

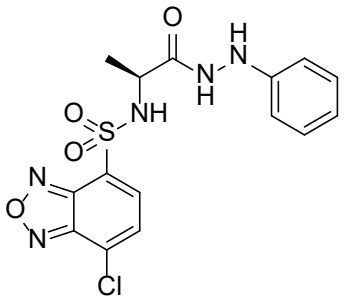
N-(2-(2-(4-bromophenyl)hydrazineyl)-2-oxoethyl)-7-chlorobenzo[c][1,2,5] oxadiazole-4-sulfonamide 65j



Following the general procedure outlined, *tert*-Butyl (2-(2-(4-bromophenyl)hydrazineyl)-2-oxoethyl)carbamate **67Cq** (0.22 g, 0.64 mmol) was

transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.25 g, 76%) as a mixture of rotamers [4:1]; Rf 0.39 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3302 (N-H), 1683 (C=O), 1524, 1488, 1340, 1156, 1042, 949 cm^{-1}; all data provided for the major rotamer δ_H (300 MHz, DMSO-d_6) 9.76 (1H, bd, *J* 2, CON*H*NH), 8.68 (1H, bt, *J* 5, SO_2N*H*CH), 8.02 (1H, d, *J* 7, Ar-*H*), 7.86 (1H, d, *J* 7, Ar-*H*), 7.80 (1H, bd, *J* 2, Ar-N*H*), 7.27 – 7.24 (2H, d, *J* 8, Ar-*H*), 6.56 – 6.53 (2H, d, *J* 8, Ar-*H*), 3.86 (2H, d, *J* 5, SO_2NHCH_2); δ_C (300 MHz, DMSO-d_6) 168.0 (*C*=ONHNH), 149.2 (*ipso*-Ar-*C*), 148.7 (*ipso*-Ar-*C*), 145.9 (*ipso*-Ar-*C*), 134.2 (Ar-*C*), 131.7 (Ar-*C*), 128.9 (Ar-*C*), 125.4 (*ipso*-Ar-*C*), 114.5 (Ar-*C*), 109.8 (Ar-*C*), 44.1 (SNH*C*H_2); *m/z* (ES^+) 459 ([^{35}Cl, ^{79}Br]MH^+), 461 ([^{35}Cl, ^{81}Br]MH^+), 461 ([^{37}Cl, ^{79}Br]MH^+), 463 ([^{37}Cl, ^{81}Br]MH^+); HRMS (ES^+) Found ([^{35}Cl, ^{79}Br]MH^+), 459.8880 ($C_{14}H_{12}{}^{35}Cl^{79}BrN_5O_4S$ requires 459.9477).

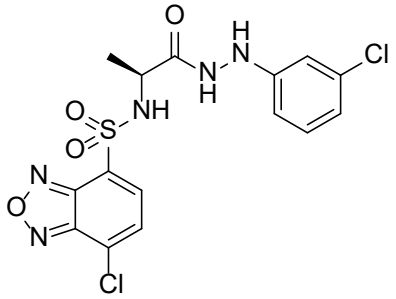
(*S*)-7-chloro-N-(1-oxo-1-(2-phenylhydrazineyl)propan-2-yl)benzo[c][1,2,5] oxadiazole-4-sulfonamide 65k



Following the general procedure outlined, *tert*-Butyl (S)-(1-oxo-1-(2-phenylhydrazineyl)propan-2-yl)carbamate **67b** (0.22 g, 0.79 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.09 g, 26%); Rf 0.34 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3083 (N-H), 2917, 2849 (C-H), 1703, 1601 (C=O), 1524, 1345, 1162, 1040, 946 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.75 (1H, bs, CON*H*NH), 8.80 (1H, bd, *J* 8, SO_2N*H*CH), 8.02 (1H, d, *J* 7, Ar-*H*), 7.83 (1H, d, *J* 7, Ar-*H*), 7.59 (1H, bs, Ar-N*H*), 7.11 – 7.06 (2H, td, *J* 7, 1, Ar-*H*), 6.72 (1H, t, *J* 7, Ar-*H*), 6.55 – 6.52 (1H, d, *J* 8, Ar-*H*), 4.22 (1H, m, SO_2NHC*H*), 1.30 (3H, d, *J* 7, NHCHCH_3); δ_C (300 MHz, DMSO-d_6) 171.3 (*C*=ONHNH), 149.3 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.4 (Ar-*C*), 131.2 (Ar-*C*), 129.1 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 119.1 (Ar-*C*),

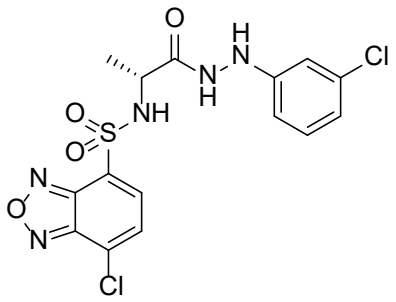
112.5 (Ar-*C*), 51.3 (SNH*C*H), 19.9 (NHCH*C*H_3); *m/z* (ES^+) 396 ([^{35}Cl]MH^+), 398 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 396.0027 ($C_{15}H_{15}{}^{35}ClN_5O_4S$ requires 396.0528).

(*S*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl)benzo [c][1,2,5]oxadiazole-4-sulfonamide 65l



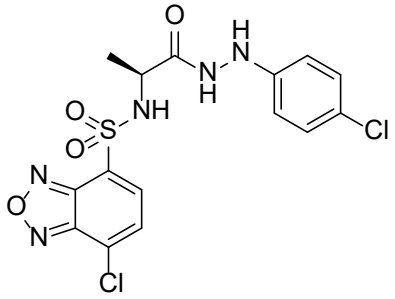
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate **67r** (0.12 g, 0.38 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.08 g, 48%); Rf 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3273 (N-H), 1674 (C=O), 1597, 1476, 1346, 1151, 1041, 947 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.86 (1H, bs, CON*H*NH), 8.83 (1H, bd, *J* 8, SO_2N*H*CH), 8.03 (1H, d, *J* 7, Ar-*H*), 7.94 (1H, bs, Ar-N*H*), 7.85 (1H, d, *J* 7, Ar-*H*), 7.14 (1H, t, *J* 8, Ar-*H*), 6.73 (1H, dd, *J* 7, 1, Ar-*H*), 6.63 (1H, t, *J* 2, Ar-*H*), 6.53 (1H, dd, *J* 8, 1, Ar-*H*), 4.25 (1H, m, SO_2NHC*H*), 1.29 (3H, d, *J* 7, NHCHCH_3); δ_C (300 MHz, DMSO-d_6) 171.5 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.3 (Ar-*C*), 133.9 (*ipso*-Ar-*C*), 131.2 (Ar-*C*), 130.8 (*ipso*-Ar-*C*), 128.9 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.0 (Ar-*C*), 51.3 (SNH*C*H), 19.8 (NHCH*C*H_3); *m/z* (ES^+) 430 ([^{35,35}Cl]MH^+), 433 ([^{35,37}Cl]MH^+), 435 ([^{37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 429.9606 ($C_{15}H_{14}{}^{35,35}Cl_2N_5O_4S$ requires 430.0139).

(*R*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65m



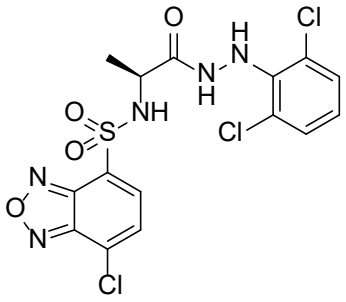
Following the general procedure outlined, *tert*-Butyl (R)-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate **67Ad** (0.12 g, 0.38 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.15 g, 86%); Rf 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3263 (N-H), 1666, 1648 (C=O), 1597, 1475, 1348, 1162, 1051, 947 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.86 (1H, bs, CON*H*NH), 8.83 (1H, bd, *J* 8, SO_2N*H*CH), 8.03 (1H, d, *J* 7, Ar-*H*), 7.93 (1H, bs, Ar-N*H*), 7.83 (1H, d, *J* 7, Ar-*H*), 7.14 (1H, t, *J* 8, Ar-*H*), 6.72 (1H, dd, *J* 7, 1, Ar-*H*), 6.63 (1H, t, *J* 2, Ar-*H*), 6.54 (1H, dd, *J* 8, 1, Ar-*H*), 4.26 (1H, m, SO_2NHC*H*), 1.30 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (300 MHz, DMSO-d_6) 171.5 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.3 (Ar-*C*), 133.9 (*ipso*-Ar-*C*), 131.2 (Ar-*C*), 130.8 (*ipso*-Ar-*C*), 128.9 (Ar-*C*), 125.6 (*ipso*-Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.0 (Ar-*C*), 51.3 (SNH*C*H), 19.8 (NHCH*C*H$_3$); *m/z* (ES$^+$) 430 ([^{35,35}Cl]MH$^+$), 433 ([^{35,37}Cl]MH$^+$), 435 ([^{37,37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MH$^+$, 429.9618 ($C_{15}H_{14}{}^{35,35}Cl_2N_5O_4S$ requires 430.0139).

(*S*)-7-chloro-N-(1-(2-(4-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65n



Following the general procedure outlined, *tert*-butyl (S)-(1-(2-(4-chlorophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate **67An** (0.17 g, 0.54 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.14 g, 54%); Rf 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3276 (N-H), 1679 (C=O), 1598, 1491, 1338, 1151, 948 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.82 (1H, bd, *J* 2, CON*H*NH), 8.82 8.83 (1H, bd, *J* 8, SO_2N*H*CH), 8.02 (1H, d , *J* 7, Ar-*H*), 7.85 (1H, d, *J* 7, Ar-*H*), 7.81 (1H, bd, *J* 2, Ar-N*H*), 7.14 – 7.11 (2H, d, *J* 8, Ar-*H*), 6.57 – 6.54 (2H, d, *J* 8, Ar-*H*), 4.20 (1H, m, SO_2NHC*H*), 1.29 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (300 MHz, DMSO-d_6) 171.4 (*C*=ONHNH), 149.2 (*ipso*-Ar-*C*), 148.4 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.4 (Ar-*C*), 131.3 (Ar-*C*), 128.9 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 122.4 (Ar-*C*), 113.9 (Ar-*C*), 51.3 (SNH*C*H), 19.9 (NHCH*C*H3); *m/z* (ES^+) 430 ([^{35,35}Cl]MH^+), 433 ([^{35,37}Cl]MH^+), 435 ([^{37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 429.9630 ($C_{15}H_{14}{}^{35,35}Cl_2N_5O_4S$ requires 430.0139).

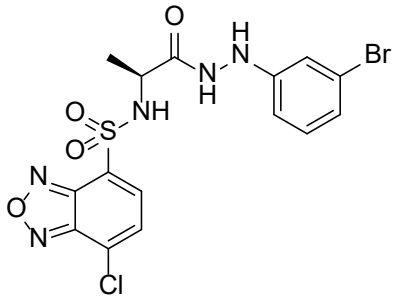
(*S*)-7-chloro-N-(1-(2-(2,6-dichlorophenyl)hydrazineyl)-1-oxopropan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65o



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(2,6-dichlorophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **67At** (0.12 g, 0.35 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the

flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.16 g, 97%); Rf 0.28 (DCM/EtOH/NH_3 200:6:1); ν_{max} 2920 (C-H), 1688, 1602 (C=O), 1524, 1496, 1216, 1153, 1051, cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.08 (1H, bd, *J* 2, CON*H*NH), 8.65 (1H, bd, *J* 8, SO_2N*H*CH), 7.90 (1H, d, *J* 7, Ar-*H*), 7.77 (1H, d, *J* 7, Ar-*H*), 7.27 – 7.24 (2H, d, *J* 8, Ar-*H*), 6.95 (1H, m, Ar-*H*), 6.70 (1H, bd, *J* 2, Ar-N*H*), 4.15 (1H, dd, *J* 7, 1, SO_2NHC*H*), 1.22 (3H, d, *J* 7, NHCHCH_3); δ_C (300 MHz, DMSO-d_6) 170.5 (*C*=ONHNH), 149.0 (*ipso*-Ar-*C*), 141.4 (*ipso*-Ar-*C*), 134.2 (Ar-*C*), 129.3 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 124.5 (Ar-*C*), 123.5 (Ar-*C*), 50.9 (SNH*C*H), 19.6 (NHCH*C*H_3); *m/z* (ES^+) 463 ([^{35,35,35}Cl]MH^+), 465 ([^{35,35,37}Cl]MH^+), 467 ([^{35,37,37}Cl]MH^+), 469 ([^{37,37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35,35}Cl]MH^+, 463.9211 ($C_{15}H_{12}{}^{35,35,35}Cl_3N_5O_4S$ requires 463.9749).

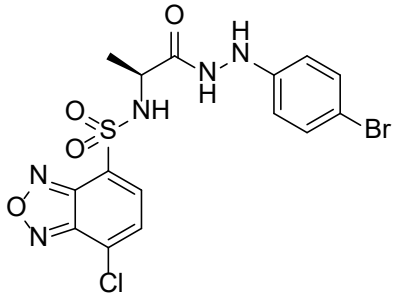
(*S*)-N-(1-(2-(3-bromophenyl)hydrazineyl)-1-oxopropan-2-yl)-7-chlorobenzo [c][1,2,5]oxadiazole-4-sulfonamide 65p



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-bromophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate **67Cl** (0.12 g, 0.34 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.13 g, 79%); Rf 0.33 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3261 (N-H), 1665 (C=O), 1595, 1474, 1347, 1149, 1040, 946 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.86 (1H, bd, *J* 2, CON*H*NH), 8.83 (1H, bd, *J* 8, SO_2N*H*CH), 8.02 (1H, d, *J* 7, Ar-*H*), 7.93 (1H, bd, *J* 2, Ar-N*H*), 7.84 (1H, d, *J* 7, Ar-*H*), 7.08 (1H, t, *J* 8, Ar-*H*), 6.86 (1H, m, Ar-*H*), 6.79 (1H, t, *J* 2, Ar-*H*), 6.57 (1H, dd, *J* 8, 1, Ar-*H*), 4.25 (1H, m, SO_2NHC*H*), 1.29 (3H, d, *J* 7, NHCHCH_3); δ_C (300 MHz, DMSO-d_6) 171.5 (*C*=ONHNH), 151.2 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.3 (Ar-*C*), 131.2 (Ar-*C*), 131.1 (Ar-*C*), 129.0 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 122.5 (*ipso*-Ar-*C*), 121.4 (Ar-*C*), 114.8 (Ar-*C*), 111.4 (Ar-*C*), 51.3 (SNH*C*H), 19.8

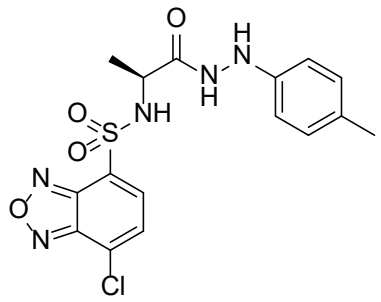
(NHCH*C*H$_3$); *m/z* (ES$^+$) 473 ([^{35}Cl, ^{79}Br]MH$^+$), 475 ([^{35}Cl, ^{81}Br]MH$^+$), 475 ([^{37}Cl, ^{79}Br]MH$^+$), 477 ([^{37}Cl, ^{81}Br]MH$^+$); HRMS (ES$^+$) Found ([^{35}Cl, ^{79}Br]MH$^+$), 473.9089 ($C_{15}H_{14}{}^{35}Cl^{79}BrN_5O_4S$ requires 473.9633).

(*S*)-N-(1-(2-(4-bromophenyl)hydrazineyl)-1-oxopropan-2-yl)-7-chlorobenzo [c][1,2,5]oxadiazole-4-sulfonamide 65q



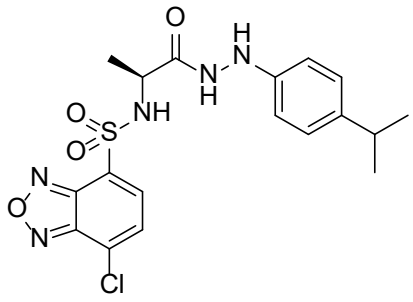
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(4-bromophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate **67Cr** (0.17 g, 0.48 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH$_3$ 200:6:1) into the title compound which was isolated as a brown oil (0.16 g, 67%); Rf 0.39 (DCM/EtOH/NH$_3$ 200:6:1); ν_{max} 3319 (N-H), 1678 (C=O), 1521, 1447, 1335, 1245, 1137, 1041, 945 cm^{-1}; δ_H (300 MHz, DMSO-d$_6$) 9.82 (1H, bs, CON*H*NH), 8.82 (1H, bd, *J* 8, SO$_2$N*H*CH), 8.03 (1H, d, *J* 7, Ar-*H*), 7.85 (1H, d, *J* 7, Ar-*H*), 7.26 – 7.23 (2H, d, *J* 8, Ar-*H*), 6.52 – 6.49 (2H, d, *J* 8, Ar-*H*), 4.23 (1H, m, SO$_2$NHC*H*), 1.29 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (300 MHz, DMSO-d$_6$) 171.4 (*C*=ONHNH), 149.2 (*ipso*-Ar-*C*), 148.8 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.4 (Ar-*C*), 131.7 (Ar-*C*), 131.2 (Ar-*C*), 129.1 (Ar-*C*), 128.9 (*ipso*-Ar-*C*), 125.5 (*ipso*-Ar-*C*), 114.5 (Ar-*C*), 109.9 (Ar-*C*), 51.3 (SNH*C*H), 19.9 (NHCH*C*H$_3$); *m/z* (ES$^+$) 473 ([^{35}Cl, ^{79}Br]MH$^+$), 475 ([^{35}Cl, ^{81}Br]MH$^+$), 475 ([^{37}Cl, ^{79}Br]MH$^+$), 477 ([^{37}Cl, ^{81}Br]MH$^+$); HRMS (ES$^+$) Found ([^{35}Cl, ^{79}Br]MH$^+$), 473.9099 ($C_{15}H_{14}{}^{35}Cl^{79}BrN_5O_4S$ requires 473.9633).

(*S*)-7-chloro-N-(1-oxo-1-(2-(p-tolyl)hydrazineyl)propan-2-yl)benzo[c][1,2,5] oxadiazole-4-sulfonamide 65r



Following the general procedure outlined, *tert*-Butyl (S)-(1-oxo-1-(2-(p-tolyl)hydrazinyl)propan-2-yl)carbamate **67Eb** (0.17 g, 0.58 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.23 g, 86%); Rf 0.34 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3312, 3097 (N-H), 1677, 1641 (C=O), 1512, 1450, 1335, 1137, 945 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.71 (1H, bs, CON*H*NH), 8.78 (1H, bd, *J* 8, SO_2N*H*CH), 8.01 (1H, d, *J* 7, Ar-*H*), 7.82 (1H, d, *J* 7, Ar-*H*), 7.40 (1H, bs, Ar-N*H*), 6.90 – 6.88 (2H, d, *J* 8, Ar-*H*), 6.45 - 6.43 (2H, d, *J* 8, Ar-*H*), 4.20 (1H, dd, *J* 7, 1, SO_2NHC*H*), 2.18 (3H, s, Ar-CH_3), 1.29 (3H, d, *J* 7, NHCHCH_3); δ_C (300 MHz, DMSO-d_6) 171.1 (*C*=ONHNH), 147.1 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.3 (Ar-*C*), 131.2 (Ar-*C*), 129.5 (Ar-*C*), 127.6 (Ar-*C*), 125.4 (*ipso*-Ar-*C*), 112.7 (Ar-*C*), 51.3 (SNH*C*H), 20.6 (Ar-*C*H_3), 20.0 (NHCH*C*H_3); *m/z* (ES^+) 410 ([^{35}Cl]MH^+), 412 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 410.0227 ($C_{16}H_{17}{}^{35}ClN_5O_4S$ requires 410.0685).

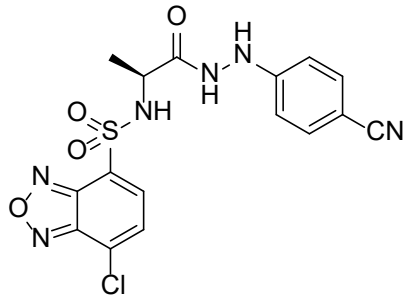
(*S*)-7-chloro-N-(1-(2-(4-isopropylphenyl)hydrazineyl)-1-oxopropan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65s



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(4-isopropylphenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **67Eh** (0.22 g, 0.68 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the

flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.23 g, 68%) as a mixture of rotamers [6:1]; Rf 0.36 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3270 (N-H), 2959 (C-H), 1674, 1615 (C=O), 1513, 1339, 1151, 1041, 947 cm^{-1}; all data provided for the major rotamer δ_H (300 MHz, DMSO-d_6) 9.73 (1H, bs, CON*H*NH), 8.79 (1H, bd, *J* 8, SO_2N*H*CH), 8.02 (1H, d, *J* 7, Ar-*H*), 7.81 (1H, d, *J* 7, Ar-*H*), 6.97 – 6.94 (2H, d, *J* 8, Ar-*H*), 6.47 - 6.44 (2H, d, *J* 8, Ar-*H*), 4.24 (1H, m, SO_2NHC*H*), 2.80 (1H, m, Ar-C*H*(CH_3)$_2$), 1.30 (3H, d, *J* 7, NHCHC*H*$_3$), 1.17 – 1.14 (6H, d, *J* 7, Ar-CH(C*H*$_3$)$_2$); δ_C (300 MHz, DMSO-d_6) 171.2 (*C*=ONHNH), 149.2 (*ipso*-Ar-*C*), 147.4 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 139.1 (*ipso*-Ar-*C*), 134.4 (Ar-*C*), 131.2 (Ar-*C*), 128.9 (Ar-*C*), 126.8 (Ar-*C*), 125.4 (*ipso*-Ar-*C*), 112.6 (Ar-*C*), 51.3 (SNH*C*H), 33.1 (Ar-*C*H(CH_3)$_2$), 24.7 (Ar-CH(*C*H_3)$_2$), 20.0 (NHCH*C*H_3); *m/z* (ES^+) 437 ([^{35}Cl]MH^+), 439 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 436.0531 ($C_{18}H_{21}{}^{35}ClN_5O_4S$ requires 437.0998).

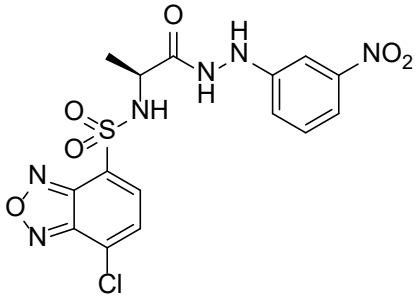
(*S*)-7-chloro-N-(1-(2-(4-cyanophenyl)hydrazineyl)-1-oxopropan-2-yl)benzo [c][1,2,5]oxadiazole-4-sulfonamide 65t



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(4-cyanophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **67Cx** (0.22 g, 0.72 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.22 g, 62%); Rf 0.41 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3278 (N-H), 2220 (C≡N), 1686, 1607 (C=O), 1511, 1336, 1154, 1041, 948 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.99 (1H, bs, CON*H*NH), 8.86 (1H, bs, SO_2N*H*CH), 8.53 (1H, bs, N*H*-Ar), 8.04 (1H, d, *J* 7, Ar-*H*), 7.89 (1H, d, *J* 7, Ar-*H*), 7.54 – 7.52 (2H, d, *J* 8, Ar-*H*), 6.65 – 6.62 (2H, d, *J* 8, Ar-*H*), 4.22 (1H, m, SO_2NHC*H*), 1.30 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (300 MHz, DMSO-d_6) 171.5 (*C*=ONHNH), 152.9 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.4 (Ar-*C*), 133.8 (Ar-*C*), 131.3 (Ar-*C*),

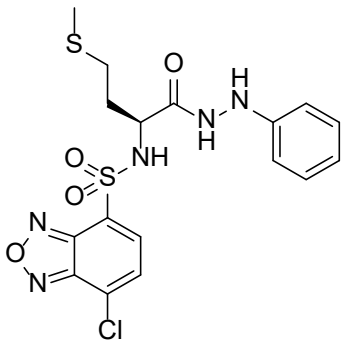
128.9 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 120.5 (*C*≡N), 112.0 (Ar-*C*), 99.5 (Ar-*C*), 51.3 (SNH*C*H), 19.7 (NHCH*C*H_3); *m/z* (ES^+) 421 ($[^{35}Cl]MH^+$), 423 ($[^{37}Cl]MH^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 421.0025 ($C_{16}H_{14}{}^{35}ClN_6O_4S$ requires 421.0481).

(*S*)-7-chloro-N-(1-(2-(3-nitrophenyl)hydrazineyl)-1-oxopropan-2-yl)benzo [c][1,2,5]oxadiazole-4-sulfonamide 65u



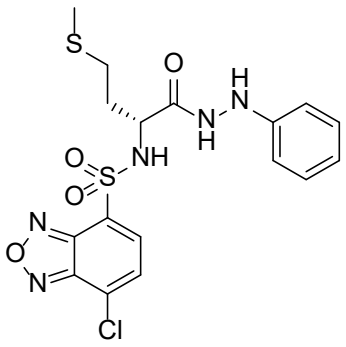
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-nitrophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate **67Dd** (0.22 g, 0.68 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.19 g, 56%); Rf 0.24 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3348, 3267 (N-H), 1669, 1652 (C=O), 1532, 1340, 1149, 946 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.0 (1H, bs, CON*H*NH), 8.86 (1H, bd, *J* 8, SO_2N*H*CH), 8.03 (1H, d, *J* 7, Ar-*H*), 7.83 (1H, d, *J* 7, Ar-*H*), 7.56 (1H, dd, *J* 7, 1, Ar-*H*), 7.43 (1H, t, *J* 2, Ar-*H*), 7.40 (1H, d, *J* 8, Ar-*H*), 7.02 (1H, dd, *J* 7, 1, Ar-*H*), 4.28 (1H, m, SO_2NHC*H*), 1.31 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (300 MHz, DMSO-d_6) 171.6 (*C*=ONHNH), 150.7 (*ipso*-Ar-*C*), 149.0 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.3 (Ar-*C*), 131.1 (Ar-*C*), 130.6 (Ar-*C*), 128.9 (Ar-*C*), 125.6 (*ipso*-Ar-*C*), 118.6 (Ar-*C*), 113.3 (Ar-*C*), 106.2 (Ar-*C*), 51.3 (SNH*C*H), 19.7 (NHCH*C*H_3); *m/z* (ES^+) 441 ($[^{35}Cl]MH^+$), 443 ($[^{37}Cl]MH^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 440.9915 ($C_{15}H_{14}{}^{35}ClN_6O_6S$ requires 441.0379).

(*S*)-7-chloro-N-(4-(methylthio)-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65v



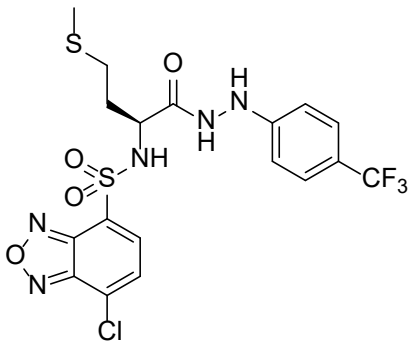
Following the general procedure outlined, *tert*-Butyl (S)-(4-(methylthio)-1-oxo-1-(2-phenylhydrazinyl)butan-2-yl)carbamate **67g** (0.22 g, 0.65 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.17 g, 53%); Rf 0.38 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3348, 3319 (N-H), 2855 (C-H), 1663, 1603 (C=O), 1524, 1494, 1332, 1154, 954 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.84 (1H, bd, *J* 2, CON*H*NH), 8.87 (1H, bs, N*H*-Ar), 8.05 (1H, d, *J* 7, Ar-*H*), 7.88 (1H, d, *J* 7, Ar-*H*), 7.63 (1H, bd, *J* 2, SO_2N*H*CH), 7.12 – 7.07 (2H, t, *J* 7, Ar-*H*), 6.72 (1H, t, *J* 7, Ar-*H*), 6.57 – 6.54 (2H, d, *J* 7, Ar-*H*), 4.11 (1H, m, SO_2NHC*H*), 2.43 (1H, m, CHCH_2C*H*HS), 2.32 (1H, m, CHCH_2CH*H*S), 1.92 (3H, s, CH_2CH_2SCH_3), 1.87 (2H, m, CHCH_2CH_2S); δ_C (300 MHz, DMSO-d_6) 170.5 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.9 (Ar-*C*), 131.9 (Ar-*C*), 129.1 (Ar-*C*), 128.5 (Ar-*C*), 125.6 (*ipso*-Ar-*C*), 119.0 (Ar-*C*), 112.5 (Ar-*C*), 54.4 (SNH*C*H), 32.3 (*C*H_2CH_2SCH_3), 29.8 (CH_2*C*H_2SCH_3), 14.8 (CH_2CH_2S*C*H_3); *m/z* (ES^+) 456 ([^{35}Cl]MH^+), 458 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 456.0096 ($C_{17}H_{19}{}^{35}ClN_5O_4S_2$ requires 456.0562).

(*R*)-7-chloro-N-(4-(methylthio)-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65w



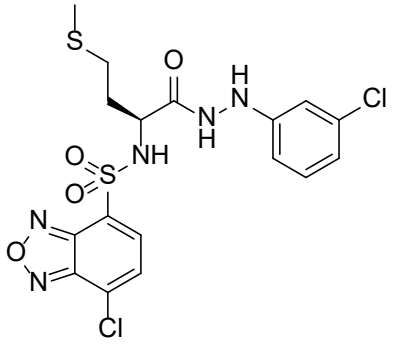
Following the general procedure outlined, *tert*-Butyl (R)-(4-(methylthio)-1-oxo-1-(2-phenylhydrazinyl)butan-2-yl)carbamate **67o** (0.12 g, 0.35 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.12 g, 72%); Rf 0.35 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3227 (N-H), 2853 (C-H), 1662 (C=O), 1524, 1331, 1153, 1099, 951 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.85 (1H, bs, CON*H*NH), 8.90 (1H, bd, *J* 8, N*H*-Ar), 8.06 (1H, d, *J* 7, Ar-*H*), 7.87 (1H, d, *J* 7, Ar-*H*), 7.12 – 7.07 (2H, t, *J* 7, Ar-*H*), 6.72 (1H, t, *J* 7, Ar-*H*), 6.57 – 6.55 (2H, d, *J* 7, Ar-*H*), 4.13 (1H, m, SO_2NHCH), 2.45 (1H, m, $CHCH_2CHHS$), 2.33 (1H, m, $CHCH_2CHHS$), 1.92 (3H, s, $CH_2CH_2SCH_3$), 1.88 (2H, m, $CHCH_2CH_2S$); δ_C (300 MHz, DMSO-d_6) 170.6 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.9 (Ar-*C*), 131.3 (Ar-*C*), 129.1 (Ar-*C*), 128.5 (Ar-*C*), 125.6 (*ipso*-Ar-*C*), 119.1 (Ar-*C*), 112.5 (Ar-*C*), 54.4 (SNH*C*H), 32.3 ($CH_2CH_2SCH_3$), 29.8 ($CH_2CH_2SCH_3$), 14.8 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 456 ($[^{35}Cl]MH^+$), 458 ($[^{37}Cl]MH^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 456.0105 ($C_{17}H_{19}{}^{35}ClN_5O_4S_2$ requires 456.0562).

(*S*)-7-chloro-N-(4-(methylthio)-1-oxo-1-(2-(4-(trifluoromethyl)phenyl) hydrazineyl)butan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65x



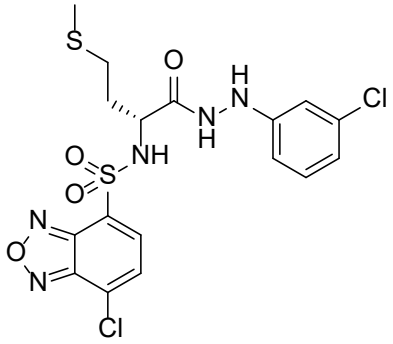
Following the general procedure outlined, *tert*-Butyl (S)-(4-(methylthio)-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)butan-2-yl)carbamate **67Bo** (0.32 g, 0.79 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a yellow gummy solid (0.20 g, 43%); Rf 0.40 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3259 (N-H), 1682, 1617 (C=O), 1524, 1323, 1156, 1108, 1065, 953 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.02 (1H, bs, CON*H*NH), 8.95 (1H, bd, *J* 7, N*H*-Ar), 8.31 (1H, bd, *J* 4, SO_2N*H*CH), 8.07 (1H, d, *J* 8, Ar-*H*), 7.90 (1H, d, *J* 8, Ar-*H*), 7.44 – 7.41 (2H, d, *J* 8, Ar-*H*), 6.68 – 6.65 (2H, d, *J* 8, Ar-*H*), 4.12 (1H, m, SO_2NHC*H*), 2.44 (1H, m, CHCH_2C*H*HS), 2.32 (1H, m, CHCH_2CH*H*S), 1.92 (3H, s, $CH_2CH_2SCH_3$), 1.89 (2H, m, CHCH_2CH_2S); δ_C (300 MHz, DMSO-d_6) 170.8 (*C*=ONHNH), 152.5 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.9 (Ar-*C*), 131.3 (Ar-*C*), 128.5 (*ipso*-Ar-*C*), 126.6 (Ar-*C*), 126.5 (Ar-*C*), 125.6 (Ar-*C*), 119.0 (1C, q, *J* 31, Ar-*C*F_3), 111.8 (Ar-*C*), 54.3 (SNH*C*H), 32.1 (*C*$H_2CH_2SCH_3$), 29.8 (CH_2*C*H_2SCH_3), 14.8 (CH_2CH_2S*C*H_3); δ_F (300 MHz, DMSO-d_6) -59.4 (C*F*$_3$); *m/z* (ES^+) 524 ([^{35}Cl]MH^+), 526 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 523.9919 ($C_{18}H_{18}{}^{35}ClF_3N_5O_4S_2$ requires 524.0436).

(*S*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65y



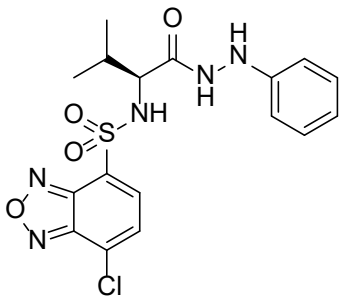
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-chlorophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate **67w** (0.22 g, 0.65 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.22 g, 69%); Rf 0.41 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3232, 3076 (N-H), 2919 (C-H), 1683 (C=O), 1597, 1525, 1340, 1156, 1023, 952 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.95 (1H, bs, CON*H*NH), 8.94 (1H, bd, *J* 8, SO_2N*H*CH), 8.06 (1H, d, *J* 7, Ar-*H*), 7.96 (1H, bs, N*H*-Ar), 7.88 (1H, d, *J* 7, Ar-*H*), 7.14 (1H, t, *J* 8, Ar-*H*), 6.74 (1H, dd, *J* 7, 1, Ar-*H*), 6.67 (1H, t, *J* 2, Ar-*H*), 6.56 (1H, dd, *J* 7, 1, Ar-*H*), 4.11 (1H, q, *J* 7, SO_2NHC*H*), 2.41 (1H, m, $CHCH_2CHHS$), 2.31 (1H, m, $CHCH_2CHHS$), 1.90 (3H, s, $CH_2CH_2SCH_3$), 1.86 (2H, m, $CHCH_2CH_2S$); δ_C (300 MHz, DMSO-d_6) 170.8 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.3 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.9 (Ar-*C*), 133.9 (*ipso*-Ar-*C*), 131.2 (Ar-*C*), 130.8 (Ar-*C*), 128.4 (*ipso*-Ar-*C*), 125.7 (*ipso*-Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 54.3 (SNH*C*H), 32.0 ($CH_2CH_2SCH_3$), 29.7 ($CH_2CH_2SCH_3$), 14.8 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 490 ($[^{35,35}Cl]MH^+$), 492 ($[^{35,37}Cl]MH^+$), 494 ($[^{37,37}Cl]MH^+$); HRMS (ES^+) Found $[^{35,35}Cl]MH^+$, 489.9696 ($C_{17}H_{18}{}^{35,35}Cl_2N_5O_4S_2$ requires 490.0172).

(*R*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65z



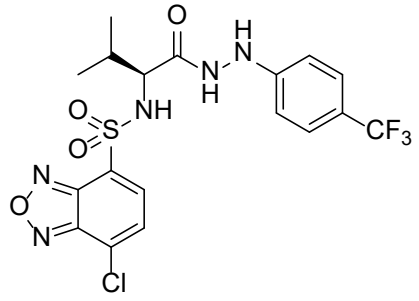
Following the general procedure outlined, *tert*-Butyl (R)-(1-(2-(3-chlorophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate **67Ae** (0.12 g, 0.32 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.09 g, 56%); Rf 0.41 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3357, 3322 (N-H), 3077, 2855 (C-H), 1665, 1600 (C=O), 1524, 1328, 1154, 952 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.96 (1H, bd, *J* 2, CON*H*NH), 8.06 (1H, d, *J* 7, Ar-*H*), 7.97 (1H, bd, *J* 2, SO_2N*H*CH), 7.88 (1H, d, *J* 7, Ar-*H*), 7.14 (1H, t, *J* 8, Ar-*H*), 6.74 (1H, dd, *J* 7, 1, Ar-*H*), 6.66 (1H, t, *J* 2, Ar-*H*), 6.56 (1H, dd, *J* 7, 1, Ar-*H*), 4.10 (1H, m, SO_2NHC*H*), 2.43 (1H, m, CHCH_2C*H*HS), 2.32 (1H, m, CHCH_2CH*H*S), 1.90 (3H, s, $CH_2CH_2SCH_3$), 1.86 (2H, m, CHCH_2CH_2S); δ_C (300 MHz, DMSO-d_6) 170.8 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.3 (*ipso*-Ar-*C*), 145.6 (*ipso*-Ar-*C*), 134.9 (Ar-*C*), 133.9 (*ipso*-Ar-*C*), 131.2 (Ar-*C*), 130.8 (Ar-*C*), 128.4 (*ipso*-Ar-*C*), 125.7 (*ipso*-Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 54.3 (SNH*C*H), 32.0 ($CH_2CH_2SCH_3$), 29.7 ($CH_2CH_2SCH_3$), 14.8 ($CH_2CH_2SCH_3$); *m/z* (ES^+) 490 ($[^{35,35}Cl]MH^+$), 492 ($[^{35,37}Cl]MH^+$), 494 ($[^{37,37}Cl]MH^+$); HRMS (ES^+) Found $[^{35,35}Cl]MH^+$, 489.9699 ($C_{17}H_{18}{}^{35,35}Cl_2N_5O_4S_2$ requires 490.0172).

(*S*)-7-chloro-N-(3-methyl-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl)benzo [c][1,2,5]oxadiazole-4-sulfonamide 65Aa



Following the general procedure outlined, *tert*-Butyl (S)-(3-methyl-1-oxo-1-(2-phenylhydrazineyl)butan-2-yl)carbamate **67c** (0.12 g, 0.39 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a light brown solid (0.13 g, 72%); Rf 0.39 (DCM/EtOH/NH_3 200:6:1); m.p. 212 - 215 °C; ν_{max} 1672, 1603 (C=O), 1524, 1496, 1340, 1161, 1041, 952 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.79 (1H, bs, CON*H*NH), 8.67 (1H, bd, *J* 9, SO_2N*H*CH), 8.06 (1H, d, *J* 7, Ar-*H*), 7.89 (1H, d, *J* 7, Ar-*H*), 7.06 – 7.01 (2H, t, *J* 7, Ar-*H*), 6.69 (1H, t, *J* 7, Ar-*H*), 6.47 – 6.45 (2H, d, *J* 7, Ar-*H*), 3.88 (1H, dd, *J* 7, 2, SO_2NHC*H*), 2.06 (1H, m, CHC*H*(CH_3)$_2$), 0.91 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$), 0.81 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (300 MHz, DMSO-d_6) 170.2 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.6 (Ar-*C*), 131.3 (Ar-*C*), 128.9 (Ar-*C*), 125.6 (*ipso*-Ar-*C*), 118.9 (Ar-*C*), 112.4 (Ar-*C*), 61.2 (SNH*C*H), 31.3 (CH*C*H(CH_3)$_2$), 19.6 (CHCH(*C*H_3)$_2$), 18.5 (CHCH(*C*H_3)$_2$); *m/z* (ES^+) 424 ([^{35}Cl]MH^+), 426 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 424.0449 ($C_{17}H_{19}{}^{35}ClN_5O_4S$ requires 424.0841).

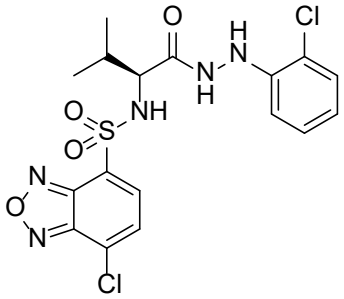
(*S*)-7-chloro-N-(3-methyl-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl) butan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ab



Following the general procedure outlined, *tert*-Butyl (S)-(3-methyl-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)butan-2-yl)carbamate **67Bm** (0.22 g, 0.59

mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a yellow oil (0.19 g, 61%); Rf 0.29 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3248 (N-H), 2969 (C-H), 1679, 1611 (C=O), 1507, 1342, 1253, 1152, 1025, 951 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.86 (1H, bd, *J* 2, CON*H*NH), 8.69 (1H, bs, N*H*-Ar), 8.06 (1H, d, *J* 7, Ar-*H*), 7.91 (1H, d, *J* 7, Ar-*H*), 7.06 – 7.03 (2H, d, *J* 8, Ar-*H*), 6.54 – 6.51 (2H, d, *J* 8, Ar-*H*), 3.84 (1H, d, *J* 7, SO_2NHC*H*), 2.04 (1H, m, CHC*H*(CH_3)$_2$), 0.89 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$), 0.78 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (300 MHz, DMSO-d_6) 170.3 (*C*=ONHNH), 149.1 (*ipso*-Ar-*C*), 148.6 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 140.8 (*ipso*-Ar-*C*), 134.6 (Ar-*C*), 131.3 (Ar-*C*), 128.9 (*ipso*-Ar-*C*), 125.5 (*ipso*-Ar-*C*), 122.2 (Ar-*C*), 112.9 (Ar-*C*), 61.2 (SNH*C*H), 31.2 (CH*C*H(CH_3)$_2$), 19.6 (CHCH(*C*H$_3$)$_2$), 18.5 (CHCH(*C*H$_3$)$_2$); δ_F (300 MHz, DMSO-d_6) -57.3 (C*F*$_3$); *m/z* (ES^+) 509 ([^{35}Cl]MNH_4^+), 511 ([^{37}Cl]MNH_4^+); HRMS (ES^+) Found [^{35}Cl]MNH_4^+, 508.0201 ($C_{18}H_{21}{}^{35}ClF_3N_6O_4S$ requires 509.0981).

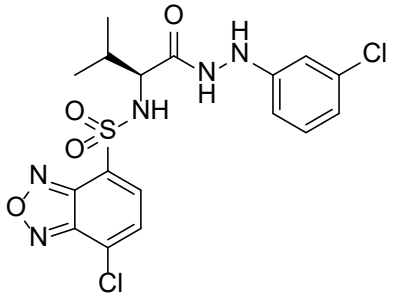
(*S*)-7-chloro-N-(1-(2-(2-chlorophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ac



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(2-chlorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate **67Ai** (0.12 g, 0.35 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.12 g, 72%); Rf 0.31 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3276 (N-H), 2962 (C-H), 1667 (C=O), 1522, 1442, 1344, 1159, 1037, 953 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.98 (1H, bs, CON*H*NH), 8.72 (1H, bd, *J* 9, SO_2N*H*CH), 8.07 (1H, d, *J* 7, Ar-*H*), 7.92 (1H, d, *J* 7, Ar-*H*), 7.27 (1H, dd, *J* 7, 1, Ar-*H*), 7.03 (1H, td, *J* 8, 1, Ar-*H*), 6.77 (1H, td, *J* 8, 1, Ar-*H*), 6.40 (1H, dd, *J* 8, 1, Ar-*H*), 3.86 (1H, dd, *J* 9, 2, SO_2NHC*H*), 2.05 (1H, m, CHC*H*(CH_3)$_2$), 0.91 (3H,

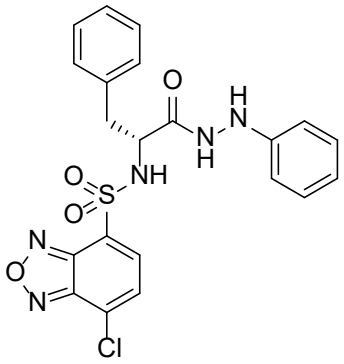
d, *J* 6, CHCH(C*H*$_3$)$_2$), 0.79 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (300 MHz, DMSO-d$_6$) 170.2 (*C*=ONHNH), 149.1 (*ipso*-Ar-*C*), 144.7 (*ipso*-Ar-*C*), 134.7 (Ar-*C*), 131.3 (Ar-*C*), 129.6 (*ipso*-Ar-*C*), 128.9 (Ar-*C*), 127.9 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 120.2 (Ar-*C*), 117.7 (*ipso*-Ar-*C*), 112.9 (Ar-*C*), 61.2 (SNH*C*H), 31.2 (CH*C*H(CH$_3$)$_2$), 19.6 (CHCH(*C*H$_3$)$_2$), 18.5 (CHCH(*C*H$_3$)$_2$); *m/z* (ES$^+$) 458 ([^{35,35}Cl]MH$^+$), 460 ([^{35,37}Cl]MH$^+$), 462 ([^{37,37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MH$^+$, 458.0037 ($C_{17}H_{18}{}^{35,35}Cl_2N_5O_4S$ requires 458.0452).

(*S*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ad



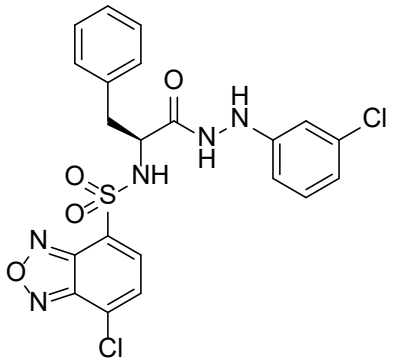
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-chlorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate **67s** (0.12 g, 0.35 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH$_3$ 200:6:1) into the title compound which was isolated as a brown gummy solid (0.11 g, 66%); Rf 0.31 (DCM/EtOH/NH$_3$ 200:6:1); ν_{max} 3368, 3328 (N-H), 1677, 1601 (C=O), 1524, 1485, 1332, 1164, 1039, 952 cm^{-1}; δ_H (300 MHz, DMSO-d$_6$) 9.88 (1H, bs, CON*H*NH), 8.74 (1H, bd, *J* 8, SO$_2$N*H*CH), 8.05 (1H, d, *J* 7, Ar-*H*), 7.90 (1H, d, *J* 7, Ar-*H*), 7.10 (1H, t, *J* 8, Ar-*H*), 6.72 (1H, dd, *J* 7, 1, Ar-*H*), 6.63 (1H, t, *J* 2, Ar-*H*), 6.47 (1H, dd, *J* 7, 1, Ar-*H*), 3.86 (1H, dd, *J* 7, 1, SO$_2$NHC*H*), 2.04 (1H, m, CHC*H*(CH$_3$)$_2$), 0.89 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$), 0.76 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (300 MHz, DMSO-d$_6$) 170.4 (*C*=ONHNH), 150.1 (*ipso*-Ar-*C*), 149.1 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.5 (Ar-*C*), 133.9 (*ipso*-Ar-*C*), 131.2 (Ar-*C*), 130.7 (Ar-*C*), 128.9 (*ipso*-Ar-*C*), 125.5 (*ipso*-Ar-*C*), 118.4 (Ar-*C*), 111.9 (Ar-*C*), 110.9 (Ar-*C*), 61.2 (SNH*C*H), 31.2 (CH*C*H(CH$_3$)$_2$), 19.6 (CHCH(*C*H$_3$)$_2$), 18.5 (CHCH(*C*H$_3$)$_2$); *m/z* (ES$^+$) 458 ([^{35,35}Cl]MH$^+$), 460 ([^{35,37}Cl]MH$^+$), 462 ([^{37,37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MH$^+$, 458.0037 ($C_{17}H_{18}{}^{35,35}Cl_2N_5O_4S$ requires 458.0452).

(*R*)-7-chloro-N-(1-oxo-3-phenyl-1-(2-phenylhydrazineyl)propan-2-yl)benzo [c][1,2,5]oxadiazole-4-sulfonamide 65Ae



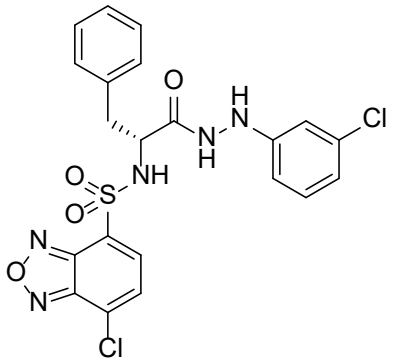
Following the general procedure outlined, *tert*-Butyl (R)-(1-oxo-3-phenyl-1-(2-phenylhydrazineyl)propan-2-yl)carbamate **67p** (0.22 g, 0.62 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a yellow solid (0.19 g, 61 %); Rf 0.27 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3385 (N-H), 1675 (C=O), 1599, 1496, 1343, 1161, 1045, 955 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.96 (1H, bd, *J* 2, CON*H*NH), 7.79 (1H, d, *J* 7, Ar-*H*), 7.77 (1H, bs, SO_2N*H*CH), 7.74 (1H, d, *J* 7, Ar-*H*), 7.15 – 7.09 (2H, t, *J* 7, Ar-*H*), 7.02 – 6.99 (2H, dd, *J* 7, 3, Ar-*H*), 6.84 – 6.82 (2H, dd, *J* 5, 1, Ar-*H*), 6.68 (1H, t, *J* 7, Ar-*H*), 6.66 – 6.64 (2H, d, *J* 7, Ar-*H*), 4.18 (1H, dd, *J* 10, 4, SO_2NHC*H*), 2.91 (1H, dd, *J* 13, 3, CHC*H*H-Ar), 2.74 (1H, dd, *J* 13, 2, CHCH*H*-Ar); δ_C (300 MHz, DMSO-d_6) 170.8 (*C*=ONHNH), 149.5 (*ipso*-Ar-*C*), 142.4 (*ipso*-Ar-*C*), 137.1 (*ipso*-Ar-*C*), 133.9 (Ar-*C*), 130.8 (Ar-*C*), 129.5 (Ar-*C*), 129.2 (Ar-*C*), 127.6 (Ar-*C*), 126.2 (Ar-*C*), 125.5 (Ar-*C*), 119.0 (Ar-*C*), 112.6 (Ar-*C*), 57.6 (SNH*C*H), 38.2 (CH*C*H_2-Ar); *m/z* (ES^+) 472 ($[^{35}Cl]MH^+$), 474 ($[^{37}Cl]MH^+$); HRMS (ES^+) Found $[^{35}Cl]MH^+$, 472.0429 ($C_{21}H_{19}{}^{35}ClN_5O_4S$ requires 472.0841).

(*S*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Af



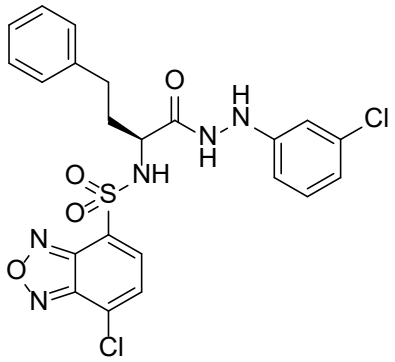
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-chlorophenyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamate **67x** (0.18 g, 0.46 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.16 g, 68%); Rf 0.30 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3283 (N-H), 1683 (C=O), 1596, 1523, 1157, 1079, 968 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.07 (1H, bd, *J* 2, CON*H*NH), 9.09 (1H, bd, *J* 8, SO_2N*H*CH), 8.14 (1H, bd, *J* 2, Ar-N*H*), 7.80 (1H, d, *J* 7, Ar-*H*), 7.74 (1H, d, *J* 7, Ar-*H*), 7.16 (1H, t, *J* 8, Ar-*H*), 7.00 – 6.97 (2H, dd, *J* 7, 2, Ar-*H*), 6.82 – 6.80 (2H, dd, *J* 5, 1, Ar-*H*), 6.75 – 6.72 (2H, m, Ar-*H*), 6.62 (1H, m, Ar-*H*), 4.17 (1H, m, SO_2NHC*H*), 2.91 (1H, dd, *J* 13, 3, CHC*H*H-Ar), 2.73 (1H, dd, *J* 13, 2, CHCH*H*-Ar); δ_C (300 MHz, DMSO-d_6) 170.9 (*C*=ONHNH), 151.1 (*ipso*-Ar-*C*), 148.9 (*ipso*-Ar-*C*), 144.7 (*ipso*-Ar-*C*), 136.9 (*ipso*-Ar-*C*), 133.9 (Ar-*C*), 130.7 (Ar-*C*), 129.5 (Ar-*C*), 128.2 (Ar-*C*), 127.5 (Ar-*C*), 126.2 (Ar-*C*), 125.6 (Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 57.5 (SNH*C*H), 37.9 (CH*C*H_2-Ar); *m/z* (ES^+) 506 ($[^{35,35}Cl]MH^+$), 508 ($[^{35,37}Cl]MH^+$), 510 ($[^{37,37}Cl]MH^+$); HRMS (ES^+) Found $[^{35,35}Cl]MH^+$, 506.0003 ($C_{21}H_{18}{}^{35,35}Cl_2N_5O_4S$ requires 506.0457).

(*R*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ag



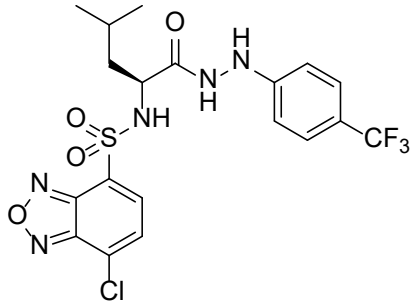
Following the general procedure outlined, *tert*-Butyl (R)-(1-(2-(3-chlorophenyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamate **67Af** (0.18 g, 0.46 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.15 g, 64%); Rf 0.32 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3265, 3029 (N-H), 1672 (C=O), 1597, 1477, 1155, 1042 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.07 (1H, bd, *J* 2, CON*H*NH), 8.14 (1H, bd, *J* 2, Ar-N*H*), 7.80 (1H, d, *J* 7, Ar-*H*), 7.74 (1H, d, *J* 7, Ar-*H*), 7.33 (1H, dd, *J* 4, 2, Ar-*H*), 7.17 (1H, t, *J* 8, Ar-*H*), 7.00 – 6.97 (2H, dd, *J* 7, 2, Ar-*H*), 6.82 – 6.80 (2H, dd, *J* 5, 1, Ar-*H*), 6.75 – 6.72 (2H, m, Ar-*H*), 6.62 (1H, m, Ar-*H*), 4.17 (1H, dd, *J* 10, 4, SO_2NHC*H*), 2.90 (1H, dd, *J* 13, 3, CHC*H*H-Ar), 2.73 (1H, dd, *J* 13, 2, CHCH*H*-Ar); δ_C (300 MHz, DMSO-d_6) 170.9 (*C*=ONHNH), 151.1 (*ipso*-Ar-*C*), 148.9 (*ipso*-Ar-*C*), 144.7 (*ipso*-Ar-*C*), 136.9 (*ipso*-Ar-*C*), 133.9 (Ar-*C*), 130.7 (Ar-*C*), 129.5 (Ar-*C*), 128.2 (Ar-*C*), 127.5 (Ar-*C*), 126.2 (Ar-*C*), 125.6 (Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 57.5 (SNH*C*H), 37.9 (CH*C*H_2-Ar); *m/z* (ES^+) 506 ([^{35,35}Cl]MH^+), 508 ([^{35,37}Cl]MH^+), 510 ([^{37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 506.0009 ($C_{21}H_{18}{}^{35,35}Cl_2N_5O_4S$ requires 506.0457).

(*S*)-7-chloro-N-(1-(2-(3-chlorophenyl)hydrazineyl)-1-oxo-4-phenylbutan-2-yl) benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ah



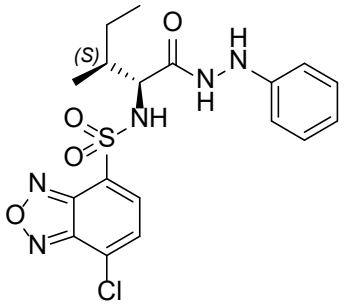
Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(3-chlorophenyl)hydrazinyl)-1-oxo-4-phenylbutan-2-yl)carbamate **67Ac** (0.22 g, 0.54 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.14 g, 45%); Rf 0.38 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3328, 3075 (N-H), 1669 (C=O), 1598, 1454, 1333, 1156, 1040, 947 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.96 (1H, bd, *J* 2, CON*H*NH), 8.03 (1H, d, *J* 7, Ar-*H*), 7.97 (1H, bd, *J* 2, SO_2N*H*CH), 7.83(1H, d, *J* 7, Ar-*H*), 7.24 – 7.22 (2H, d, *J* 7, Ar-*H*), 7.18 – 7.13 (2H, m, Ar-*H*), 7.08 – 7.05 (2H, m, Ar-*H*), 6.73 – 6.70 (2H, m, Ar-*H*), 6.67 (1H, t, *J* 2, Ar-*H*), 6.55 (1H, dd, *J* 7, 1, Ar-*H*), 4.12 (1H, m, SO_2NHC*H*), 2.69 (2H, m, CH_2C*H*$_2$-Ar), 1.98 (2H, m, C*H*$_2$$CH_2$-Ar); δ_C (300 MHz, DMSO-d_6) 170.8 (*C*=ONHNH), 151.1 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 141.1 (*ipso*-Ar-*C*), 134.5 (Ar-*C*), 133.9 (Ar-*C*), 131.2 (Ar-*C*), 130.8 (Ar-*C*), 128.8 (Ar-*C*), 128.5 (Ar-*C*), 126.4 (Ar-*C*), 125.6 (Ar-*C*), 118.5 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 55.4 (SNH*C*H), 34.7 (*C*$H_2$$CH_2$-Ar), 31.6 ($CH_2$*C*$H_2$-Ar); *m/z* ($ES^+$) 520 ([^{35,35}Cl]$MH^+$), 523 ([^{35,37}Cl]$MH^+$), 525 ([^{37,37}Cl]$MH^+$); HRMS ($ES^+$) Found [^{35,35}Cl]$MH^+$, 520.0156 ($C_{22}H_{20}{}^{35,35}Cl_2N_5O_4S$ requires 520.0613).

(*S*)-7-chloro-N-(4-methyl-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl) pentan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ai



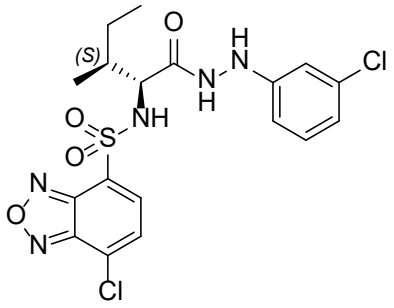
Following the general procedure outlined, *tert*-Butyl (S)-(4-methyl-1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)pentan-2-yl)carbamate **67Bn** (0.22 g, 0.57 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.18 g, 59%); Rf 0.27 (DCM/EtOH/NH_3 200:6:1); ν_{max} 2961 (C-H), 1682, 1617 (C=O), 1524, 1323, 1156, 1110, 1065, 950 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.88 (1H, bd, *J* 2, CON*H*NH), 8.84 (1H, bd, *J* 8, SO_2N*H*CH), 8.26 (1H, bd, *J* 2, Ar-N*H*), 8.06 (1H, d, *J* 7, Ar-*H*), 7.89 (1H, d, *J* 7, Ar-*H*), 7.42 – 7.40 (2H, d, *J* 8, Ar-*H*), 6.62 – 6.60 (2H, d, *J* 8, Ar-*H*), 4.09 (1H, m, SO_2NHC*H*), 1.57 – 1.48 (2H, m, C*H*HCH(CH_3)$_2$, CH_2C*H*(CH_3)$_2$), 1.45 (1H, m, CH*H*CH(CH_3)$_2$), 0.87 (3H, d, *J* 6, CH_2CH(C*H*$_3$)$_2$), 0.76 (3H, d, *J* 6, CH_2CH(C*H*$_3$)$_2$); δ_C (300 MHz, DMSO-d_6) 171.2 (*C*=ONHNH), 152.5 (*ipso*-Ar-*C*), 149.1 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.5 (Ar-*C*), 131.3 (Ar-*C*), 128.8 (*ipso*-Ar-*C*), 126.5 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 111.7 (Ar-*C*), 55.4 (SNH*C*H), 41.7 (*C*H_2CH(CH_3)$_2$), 24.4 (CH_2*C*H(CH_3)$_2$), 23.2 (CH_2CH(*C*H_3)$_2$), 21.4 (CH_2CH(*C*H_3)$_2$); δ_F (300 MHz, DMSO-d_6) -59.4 (C*F*$_3$); ***m/z*** (ES^+) 506 ([^{35}Cl]MH^+), 508 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 506.0444 ($C_{19}H_{20}{}^{35}ClF_3N_5O_4S$ requires 506.0877).

7-chloro-N-((3*S*)-3-methyl-1-oxo-1-(2-phenylhydrazineyl)pentan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Aj



Following the general procedure outlined, *tert*-Butyl ((2S,3S)-3-methyl-1-oxo-1-(2-phenylhydrazineyl)pentan-2-yl)carbamate **67e** (0.22 g, 0.68 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown oil (0.23 g, 68%); Rf 0.28 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3272 (N-H), 2968 (C-H), 1668, 1603 (C=O), 1524, 1496, 1338, 1159, 1041, 951 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.78 (1H, bs, CON*H*NH), 8.70 (1H, bd, *J* 9, SO_2N*H*CH), 8.05 (1H, d, *J* 7, Ar-*H*), 7.88 (1H, d, *J* 7, Ar-*H*), 7.60 (1H, bs, Ar-N*H*), 7.06 – 7.00 (2H, dd, *J* 7, 1, Ar-*H*), 6.69 (1H, t, *J* 7, Ar-*H*), 6.47 – 6.45 (2H, d, *J* 7, Ar-*H*), 3.88 (1H, dd, *J* 7, 1, SO_2NHC*H*), 1.82 (1H, m, NHCHC*H*), 1.45 (1H, m, CH(CH_3)C*H*HCH_3), 1.09 (1H, m, CH(CH_3)CH*H*CH_3), 0.87 (3H, d, *J* 6, CH(C*H*$_3$)CH_2CH_3), 0.76 (3H, t, *J* 7, CH(CH_3)CH_2C*H*$_3$); δ_C (300 MHz, DMSO-d_6) 170.1 (*C*=ONHNH), 149.4 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.6 (Ar-*C*), 131.3 (Ar-*C*), 128.9 (Ar-*C*), 128.9 (Ar-*C*), 125.5 (*ipso*-Ar-*C*), 118.9 (Ar-*C*), 112.4 (Ar-*C*), 59.9 (SNH*C*H), 37.3 (NHCH*C*H), 24.3 (CH(CH_3)*C*H_2CH_3), 15.7 (CH(*C*H_3)CH_2CH_3), 10.9 (CH(CH_3)CH_2*C*H_3); *m/z* (ES^+) 438 ([^{35}Cl]MH^+), 440 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 438.1011 ($C_{18}H_{21}{}^{35}ClN_5O_4S$ requires 438.1003).

7-chloro-N-((3*S*)-1-(2-(3-chlorophenyl)hydrazineyl)-3-methyl-1-oxopentan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ak

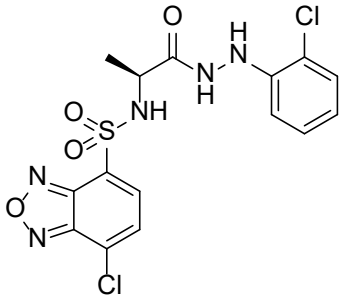


Following the general procedure outlined, *tert*-Butyl ((2S,3S)-1-(2-(3-chlorophenyl)hydrazineyl)-3-methyl-1-oxopentan-2-yl)carbamate **67u** (0.12 g, 0.34 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.09 g, 54%); Rf 0.30 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3265 (N-H), 2927 (C-H), 1699 (C=O), 1597, 1478, 1302, 1075, 993 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.90 (1H, bd, *J* 2, CON*H*NH), 8.05 (1H, d, *J* 7, Ar-*H*), 7.95 (1H, bd, *J* 2, SO_2N*H*CH), 7.89 (1H, d, *J* 7, Ar-*H*), 7.73 (1H, bd, *J* 2, Ar-N*H*), 7.09 (1H, t, *J* 8, Ar-*H*), 6.71 (1H, dd, *J* 8, 1, Ar-*H*), 6.63 (1H, t, *J* 2, Ar-*H*), 6.48 (1H, dd, *J* 8, 1, Ar-*H*), 3.87 (1H, d, *J* 7, SO_2NHC*H*), 1.81 (1H, m, NHCHC*H*), 1.40 (1H, m, CH(CH_3)C*H*HCH_3), 1.26 (1H, m, CH(CH_3)CH*H*CH_3), 0.85 (3H, d, *J* 6, CH(C*H*$_3$)CH_2CH_3), 0.72 (3H, t, *J* 7, CH(CH_3)CH_2C*H*$_3$); δ_C (300 MHz, DMSO-d_6) 170.4 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.1 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.6 (Ar-*C*), 133.9 (Ar-*C*), 131.2 (Ar-*C*), 130.6 (Ar-*C*), 128.9 (*ipso*-Ar-*C*), 125.6 (*ipso*-Ar-*C*), 118.4 (Ar-*C*), 111.9 (Ar-*C*), 111.1 (Ar-*C*), 59.9 (SNH*C*H), 37.1 (NHCH*C*H), 24.3 (CH(CH_3)*C*H_2CH_3), 15.7 (CH(*C*H_3)CH_2CH_3), 10.9 (CH(CH_3)CH_2*C*H_3); *m/z* (ES^+) 472 ([^{35,35}Cl]MH^+), 474 ([^{35,37}Cl]MH^+), 476 ([^{37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 472.0206 ($C_{18}H_{20}{}^{35,35}Cl_2N_5O_4S$ requires 472.0613).

6.1.2.5 Synthesis of Benzoxa-[2,1,3]-diazole Peptidomimetics – General Procedure

Under a nitrogen atmosphere *N*-Boc amino acid hydrazides (1.10 equiv.) were dissolved in 4 M HCl solution in dioxane (3 mL) and stirred at room temperature. After 90 minutes the mixture was evaporated, dried in *vacuo* and precipitated by using (EtOH / Et_2O). The quat was directly used in the next step. the resulting solid (1 equiv.) was suspended in THF (3 mL) and diisopropylethylamine (1.50 equiv.) was added. After stirring for 10 minutes at room temperature, the solution was treated with 7-chlorobenzoxa-[2,1,3]-diazole-4-sulfonyl chloride (1.10 equiv.) and continued stirring for 4 hours. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo*. The flash chromatography (DCM/EtOH/NH3 [600:8:1], [400:8:1], [200:8:1]) afforded the desired sulfonamides **65**.

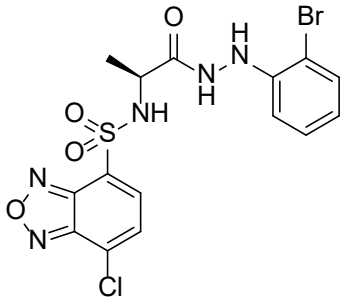
(*S*)-7-chloro-*N*-(1-(2-(2-chlorophenyl)hydrazinyl)-1-oxopropan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Am



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(2-chlorophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **67Ah** (0.05 g, 0.16 mmol) was transformed following flash chromatography (DCM/EtOH/NH_3 400:8:1) into the title compound which was isolated as a golden brown gummy solid (0.01 g, 14%) as a mixture of rotamers [3:1]; Rf 0.23 (DCM/EtOH/NH_3 400:8:1); δ_H (700 MHz, $CDCl_3$) 8.17 (1H, bs, CON*H*NH), 8.01 (1H, d , *J* 7, Ar-*H*), 7.51 (1H, d, *J* 7, Ar-*H*), 7.25 (1H, dd, *J* 8, 1, Ar-*H*), 7.13 (1H, dt, *J* 8, 1, Ar-*H*), 6.84 (1H, dt, *J* 8, 1, Ar-*H*), 4.38 (1H, m, SO_2NHCH), 1.40 (3H, d, *J* 7, NHCHCH_3); δ_C (700 MHz, $CDCl_3$)

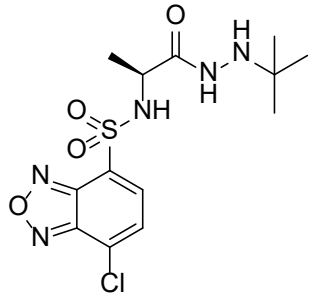
171.0 (*C*=ONHNH), 148.9 (*ipso*-Ar-*C*), 144.9 (*ipso*-Ar-*C*), 143.2 (*ipso*-Ar-*C*), 133.9 (Ar-*C*), 129.5 (Ar-*C*), 129.1 (Ar-*C*), 128.5 (Ar-*C*), 127.8 (Ar-*C*), 121.8 (Ar-*C*), 113.6 (Ar-*C*), 52.1 (SNH*C*H), 19.7 (NHCH*C*H_3); *m/z* (ES^+) 430 ([^{35,35}Cl]MH^+), 432 ([^{35,37}Cl]MH^+), 434 ([^{37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 430.0146 ($C_{15}H_{14}{}^{35,35}Cl_2N_5O_4S$ requires 430.0144).

(*S*)-N-(1-(2-(2-bromophenyl)hydrazinyl)-1-oxopropan-2-yl)-7-chlorobenzo [c][1,2,5]oxadiazole-4-sulfonamide 65An



Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(2-bromophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **67Cf** (0.10 g, 0.28 mmol) was transformed following flash chromatography (DCM/EtOH/NH_3 200:8:1) into the title compound which was isolated as a golden brown oil (0.03 g, 26%) as a mixture of rotamers [3:1]; R_f 0.3 (DCM/EtOH/NH_3 200:8:1); υ_{max} 3292 (N-H), 1668 (C=O), 1522, 1349, 1162, 947, 744, 635 cm^{-1}; all data provided for the major rotamer; δ_H (700 MHz, $CDCl_3$) 8.39 (1H, bs, N*H*), 8.02 (1H, d, *J* 7, Ar-*H*), 7.51 (1H, d, *J* 7, Ar-*H*), 7.43 (1H, dd, *J* 7, 1, Ar-*H*), 7.17 (1H, dt, *J* 7, 1, Ar-*H*), 6.80 (2H, m, Ar-*H*), 6.22 (1H, bd, *J* 7, Ar-N*H*), 4.40 (1H, m, SO_2NHC*H*), 1.41 (3H, d, *J* 7, NHCHC*H*$_3$); δ_C (700 MHz, $CDCl_3$) 171.2 (*C*=ONHNH), 148.9 (Ar-*C*), 144. 9 (Ar-*C*), 144.1 (*ipso*-Ar-*C*), 134.0 (Ar-*C*), 132.7 (Ar-*C*), 129.2 (Ar-*C*), 128.4 (Ar-*C*), 127.4 (*ipso*-Ar-*C*), 122.3 (Ar-*C*), 113.7 (Ar-*C*), 109.2 (Ar-*C*), 52.2 (SO_2NH*C*H), 19.6 (NHCH*C*H_3); *m/z* (ES^+) 473 ([^{79}Br^{35}Cl]MH^+), 475 ([^{79}Br ^{37}Cl]MH^+), 475 ([^{81}Br^{35}Cl]MH^+), 477 ([^{81}Br^{37}Cl]MH^+); HRMS (ES^+) Found [^{79}Br^{35}Cl]MH^+, 473.9633 ($C_{15}H_{14}N_5O_4S^{79}Br^{35}Cl$ requires 473.9638).

(*S*)-N-(1-(2-(tert-butyl)hydrazinyl)-1-oxopropan-2-yl)-7-chlorobenzo[c][1,2,5] oxadiazole-4-sulfonamide 65Ao

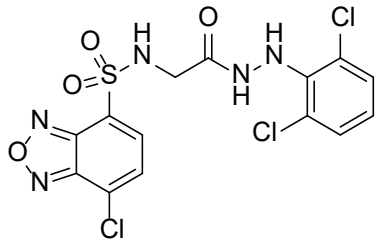


Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(tert-butyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **89a** (0.06 g, 0.75 mmol) was transformed following flash chromatography (DCM/EtOH/NH_3 200:8:1) into the title compound which was isolated as a light yellow solid (0.04 g, 50%); Rf 0.33 (DCM/EtOH/NH_3 200:8:1); m.p. 190 – 192 °C; υmax 3364 (N-H), 2972 (C-H), 1674 (C=O), 1466, 1350, 1171, 946, 745, 634 cm-1; δ_H (700 MHz, DMSO-d6) 9.23 (1H, bs, CON*H*NH), 7.70 (1H, bd, , *J* 8, SO_2N*H*CH), 7.98 (1H, d, *J* 7, Ar-*H*), 7.90 (1H, d, *J* 7, Ar-*H*), 4.10 (1H, m, SO_2NHC*H*), 2.09 (9H, s, (C*H*$_3$)$_3$CNH), 1.22 (3H, d, *J* 8, NHCHC*H*$_3$); δ_C (700 MHz, DMSO-d6) 170.0 (*C*=ONHNH), 149.2 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.4 (*ipso*-Ar-*C*), 131.2 (*ipso*-Ar-*C*), 128.9 (Ar-*C*), 125.5 (Ar-*C*), 79.6 ((CH$_3$)$_3$*C*NH), 51.3 (SNH*C*H), 27.2 ((*C*H$_3$)$_3$CNH) 20.2 (NHCHCH$_3$); *m/z* (ES$^+$) 376 ([^{35}Cl]MH$^+$), 378 ([^{37}Cl]MH$^+$); HRMS (ES$^+$) Found [^{35}Cl]MNa$^+$, 376.0836 ($C_{13}H_{19}{}^{35}ClN_5O_4S$ requires 376.0846).

6.1.2.6 Synthesis of Benzoxa-[2,1,3]-diazole Peptidomimetics – General Procedure

Under a nitrogen atmosphere *N*-Boc amino acid hydrazides (1.10 equiv.) were dissolved in 4 M HCl solution in dioxane (3 mL) and stirred at room temperature for 90 minutes. The solvent was removed in *vacuo*, the resulting solid (1 equiv.) was directly suspended in MeCN (5 mL) and pyridine (1.20 equiv.) was added. After stirring for 10 minutes at room temperature, the solution was treated with 7-chlorobenzoxa-[2,1,3]-diazole-4-sulfonyl chloride (1.20 equiv.) and continued stirring for 4 hours. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo*. The recrystalysation (EtOH / H_2O) or the flash chromatography (DCM/MeOH [100:5]) afforded the desired sulfonamides **65**.

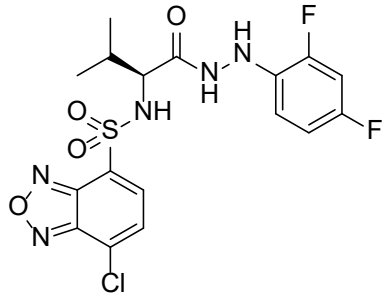
(*S*)-7-chloro-N-(2-(2-(2,6-dichlorophenyl)hydrazinyl)-2-oxoethyl)benzo [c][1,2,5]oxadiazole-4-sulfonamide 65Ap



Following the general procedure outlined, *tert*-Butyl (2-(2-(2,6-dichlorophenyl)hydrazinyl)-2-oxoethyl)carbamate **67As** (0.05 g, 0.15 mmol) was transformed following recrystalysation (EtOH / H_2O) into the title compound which was isolated as a light brown gummy solid (0.03 g, 39%) as a mixture of rotamers [6:1]; R_f 0.29 (DCM/EtOH/NH_3 400:8:1); ν_{max} 3344 (N-H), 2921, 2257 (C-H), 1676 (C=O), 1547, 1428, 1358, 1157, 1023, 996 cm^{-1}; all data provided for the major rotamer δ_H (400 MHz, DMSO-d_6) 10.01 (1H, bs, N*H*), 8.58 (1H, bt, *J* 6, CH_2NH), 7.92 (1H, d, *J* 7, Ar-*H*), 7.78 (1H, d, *J* 7, Ar-*H*), 7.27 (1H, d, *J* 8, Ar-*H*), 6.97 – 6.93 (2H, m, Ar-*H*), 3.77 (2H, d, *J* 6, SO_2NHCH_2); δ_C (100 MHz, DMSO-d_6) 172.5 (*C*=ONHNH), 167.3 (*ipso*-Ar-*C*), 145.8 (*ipso*-Ar-*C*), 141.3 (*ipso*-Ar-*C*), 134.0 (Ar-

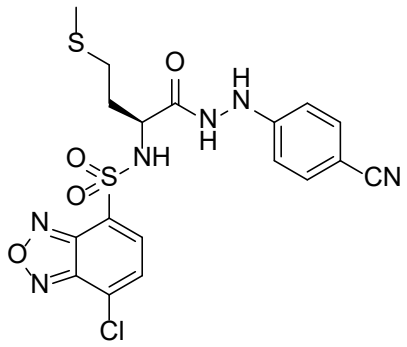
C), 131.1 (Ar-*C*), 129.4 (Ar-*C*), 128.8 (Ar-*C*), 125.3 (*ipso*-Ar-*C*), 124.3 (*ipso*-Ar-*C*), 123.3 (Ar-*C*), 43.5 (SNH*C*H_2); *m/z* (ES^+) 450 ([^{35,35,35}Cl]MH^+), 452 ([^{35,35,37}Cl]MH^+), 454 ([^{35,37,37}Cl]MH^+), 456 ([^{37,37,37}Cl]MH^+); HRMS (ES^+) Found [^{35,35,35}Cl]MH^+, 449.9609 ($C_{14}H_{11}N_5O_4S^{35,35,35}Cl$ requires 449.9597).

(*S*)-7-chloro-N-(1-(2-(2,4-difluorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Aq



Following the general procedure outlined, *tert*-Butyl(S)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate **67Ca** (0.05 g, 0.15 mmol) was transformed following flash column chromatography (DCM/MeOH 100:5) into the title compound which was isolated as an orange oil (0.04 g, 54%); R_f 0.70 (DCM/EtOH/NH_3 200:8:1); ν_{max} 3257 (N-H), 2962, 2928 (C-H), 1702, 1610 (C=O), 1506, 1354, 1268, 1159, 1026, 956 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 9.93 (1H, bs, N*H*), 8.69 (1H, bd, *J* 9, SO_2N*H*CH), 8.05 (1H, d, *J* 7, Ar-*H*), 7.91 (1H, d, *J* 7, Ar-*H*), 7.60 (1H, bs, N*H*), 7.11 (1H, dt, *J* 8, 2, Ar-*H*), 6.77 (1H, m, Ar-*H*), 6.49 (1H, m, Ar-*H*), 3.82 (1H, m, SO_2NHC*H*), 2.01 (1H, m, CHC*H*(CH_3)$_2$), 0.87 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$), 0.74 (3H, d, *J* 6, CHCH(C*H*$_3$)$_2$); δ_C (100 MHz, DMSO-d_6) 172.1 (*C*=ONHNH), 149.1 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.6 (Ar-*C*), 132.2 (*ipso*-Ar-*C*), 132.1 (*ipso*-Ar-*C*), 131.3 (Ar-*C*), 128.9 (*ipso*-Ar-*C*), 125.4 (*ipso*-Ar-*C*), 114.4 (Ar-*C*), 111.0 (Ar-*C*), 104.1 (Ar-*C*), 61.2 (SO_2NH*C*H), 31.2 (CH*C*H(CH_3)$_2$), 19.6 (CHCH(*C*H$_3$)$_2$), 18.5 (CHCH(*C*H$_3$)$_2$); δ_F (282 MHz, DMSO-d_6) -123.6 (Ar-*F*), -128.2 (Ar-*F*); *m/z* (ES^+) 460 ([^{35}Cl]MH^+); HRMS (ES^+) Found [^{35}Cl]MH^+, 460.0680 ($C_{17}H_{17}N_5O_4F_2S^{35}Cl$ requires 460.0658).

(*S*)-7-chloro-N-(1-(2-(4-cyanophenyl)hydrazineyl)-4-(methylthio)-1-oxobutan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ar

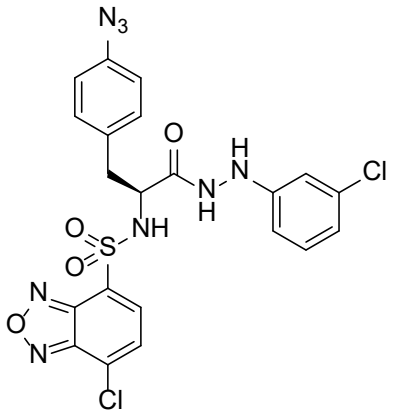


Following the general procedure outlined, *tert*-Butyl (S)-(1-(2-(4-cyanophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate **67Da** (0.04 g, 0.11 mmol) was transformed following recrystalysation (EtOH / H_2O) into the title compound which was isolated as a brown gummy solid (0.05 g, 72%); Rf 0.31 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3305, 3088 (N-H), 2227 (C≡N), 1679, 1606 (C=O), 1520, 1349, 1167, 1154, 1083, 947 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.07 (1H, bs, CON*H*NH), 8.96 (1H, bd, *J* 8, SO_2N*H*CH), 8.55 (1H, bs, N*H*-Ar), 8.06 (1H, d, *J* 7, Ar-*H*), 7.91 (1H, d, *J* 7, Ar-*H*), 7.53 – 7.51 (2H, d, *J* 8, Ar-*H*), 6.66 – 6.64 (2H, d, *J* 8, Ar-*H*), 4.09 (1H, m, SO_2NHC*H*), 2.42 (1H, m, CHCH_2C*H*HS), 2.31 (1H, m, CHCH_2CH*H*S), 1.89 (3H, s, $CH_2CH_2SCH_3$), 1.86 (2H, m, CHCH_2CH_2S); δ_C (300 MHz, DMSO-d_6) 168.8 (*C*=ONHNH), 150.9 (*ipso*-Ar-*C*), 147.1 (*ipso*-Ar-*C*), 143.5 (*ipso*-Ar-*C*), 132.9 (Ar-*C*), 131.7 (Ar-*C*), 129.2 (Ar-*C*), 126.2 (*ipso*-Ar-*C*), 123.6 (*ipso*-Ar-*C*), 118.6 (*C*≡N), 109.9 (*ipso*-Ar-*C*), 97.4 (*ipso*-Ar-*C*), 52.1 (SNH*C*H), 29.8 (*C*$H_2CH_2SCH_3$), 27.6 (CH_2*C*H_2SCH_3), 12.6 (CH_2CH_2S*C*H_3); *m/z* (ES^+) 481 ([^{35}Cl]MH^+), 483 ([^{37}Cl]MH^+); HRMS (ES^+) Found [^{35,35}Cl]MH^+, 481.0105 ($C_{18}H_{18}{}^{35}ClN_6O_4S_2$ requires 481.0514).

6.1.2.7 Synthesis of Benzoxa-[2,1,3]-diazole Peptidomimetics – General Procedure

Under a nitrogen atmosphere *N*-Boc-azido-amino acid hydrazides (1.10 equiv.) were dissolved in 15% v/v HCl in DCM (1.5 mL) and stirred at room temperature for a hour. The solvent was removed in *vacuo*, the resulting solid (1 equiv.) was directly suspended in MeCN (5 mL) and pyridine (1.20 equiv.) was added. After stirring for 10 minutes at room temperature, the solution was treated with 7-chlorobenzoxa-[2,1,3]-diazole-4-sulfonyl chloride (1.20 equiv.) and continued stirring for 19 hours. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo*. The recrystalysation (EtOH / H2O) afforded the desired sulfonamides **65**.

(S)-N-(3-(4-azidophenyl)-1-(2-(3-chlorophenyl)hydrazinyl)-1-oxopropan-2-yl) -7-chlorobenzo[c][1,2,5]oxadiazole-4-sulfonamide 65As



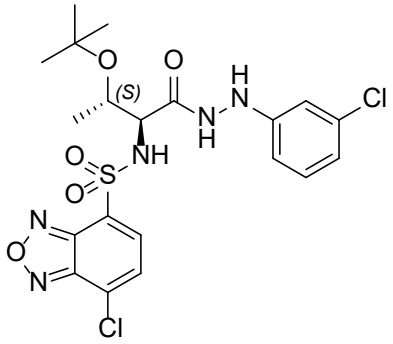
Following the general procedure outlined, *tert*-Butyl(S)-(3-(4-azidophenyl)-1-(2-(3-chlorophenyl)hydrazinyl)-1-oxopropan-2-yl)carbamate **67En** (0.07 g, 0.16 mmol) was transformed following recrystalysation (EtOH / H_2O) into the title compound which was isolated as a brown solid (0.03 g, 35%); R_f 0.41 (DCM/EtOH/NH_3 200:8:1); ν_{max} 3229, 3076 (N-H), 2925, 2856 (C-H), 2115 (N_3), 1677, 1596, 1346, 1288, 1156, 1024, 997 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 10.08 (1H, bs, N*H*), 9.10 (1H, bd, *J* 8, SO_2N*H*CH), 7.81 – 7.75 (2H, q, *J* 7, Ar-*H*), 7.17

(1H, t, *J* 8, Ar-*H*), 6.98 – 6.96 (2H, d, *J* 8, Ar-*H*), 6.74 – 6.72 (2H, m, Ar-*H*), 6.67 (1H, d, *J* 8, Ar-*H*), 6.46 – 6.44 (2H, d, *J* 8, Ar-*H*), 4.10 (1H, m, SO_2NHCH), 2.86 (1H, dd, *J* 13, 3, SO_2NHCH_2), 2.68 (1H, t, *J* 12, SO_2NHCH_2); δ_C (100 MHz, DMSO-d_6) 170.9 (*C*=ONHNH), 151.1 (*C*=ONH), 148.8 (*ipso*-Ar-*C*), 144.6 (*ipso*-Ar-*C*), 137.5 (*ipso*-Ar-*C*), 134.1 (Ar-*C*), 134.0 (Ar-*C*), 133.9 (Ar-*C*), 131.0 (Ar-*C*), 130.9 (Ar-*C*), 128.1 (*ipso*-Ar-*C*), 125.4 (*ipso*-Ar-*C*), 118.6 (Ar-*C*), 117.9 (Ar-*C*), 111.9 (Ar-*C*), 111.2 (Ar-*C*), 57.6 (SO_2NHCH), 37.1 (Ar-CH_2); *m/z* (ES^+) 547 ($[^{35,35}Cl]MH^+$), 549 ($[^{35,37}Cl]MH^+$), 551 ($[^{37,37}Cl]MH^+$); HRMS (ES^+) Found $[^{35,35}Cl]MH^+$, 547.0463 ($C_{21}H_{17}N_8O_4S^{35,35}Cl_2$ requires 547.0435).

6.1.2.8 Synthesis of Benzoxa-[2,1,3]-diazole Peptidomimetics – General Procedure

Under a nitrogen atmosphere *N*-Fmoc amino acid hydrazides (1.80 equiv.) were reacted with 1-octanethiol (7 equiv.) in THF (3 mL) and stirred at room temperature. After 10 minutes 1,8-diazabicyclo[5.4.0]undec-7-ene (1.10 equiv.) was added and continued stirring for 5 hours. The mixture was evaporated, dried in *vacuo* and precipitated by using (EtOH / Et_2O). The resulting solid (1 equiv.) was suspended in THF (4 mL) and Et_3N (3 equiv.) was added. After stirring for 10 minutes at room temperature, the solution was treated with 7-chlorobenzoxa-[2,1,3]-diazole-4-sulfonyl chloride (1.10 equiv.) and continued stirring for 5 hours. Reaction mixed with ethyl acetate (8 mL) and distilled water (5 mL). After separation of the two phases the organic layer washed again with distilled water (5 mL X 3 mL) followed by a wash with sat. aq. NH_4Cl (6 mL) then sat. aq. $NaHCO_3$ (6 mL) followed by brine (8 mL). Organic layer dried over $MgSO_4$, filtered, evaporated and dried in *vacuo*. The precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH3 [600:8:1], [400:8:1], [200:6:1]) afforded the desired sulfonamides **65**.

N-((2S,3S)-3-(tert-butoxy)-1-(2-(3-chlorophenyl)hydrazineyl)-1-oxobutan-2-yl)-7-chlorobenzo[c][1,2,5]oxadiazole-4-sulfonamide 65Al



Following the general procedure outlined, (9H-fluoren-9-yl)methyl ((2S,3S)-3-(tert-butoxy)-1-(2-(3-chlorophenyl)hydrazinyl)-1-oxobutan-2-yl)carbamate **68l** (0.18 g, 0.60 mmol) was transformed following precipitation (EtOAc / *n*-hexane) and then the flash chromatography (DCM/EtOH/NH_3 200:6:1) into the title compound which was isolated as a brown gummy solid (0.19 g, 61%); Rf 0.58 (DCM/EtOH/NH_3 200:6:1); ν_{max} 3276 (N-H), 2979, 2932 (C-H), 1729, 1688 (C=O), 1599, 1477, 1366, 11189, 1041, 950 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 9.64 (1H, bd, *J* 2, CON*H*NH), 8.49 (1H, bd, *J* 8, SO_2N*H*CH), 8.07 (1H, d, *J* 7, Ar-*H*), 7.97 (1H, bd, *J* 2, N*H*-Ar), 7.93 (1H, d, *J* 7, Ar-*H*), 7.09 (1H, t, *J* 7, Ar-*H*), 6.78 (1H, t, *J* 2, Ar-*H*), 6.70 (1H, dd, *J* 8, 1, Ar-*H*), 6.58 (1H, dd, *J* 8, 1, Ar-*H*), 4.07 (1H, m, SO_2NHC*H*), 3.85 (1H, m, SO_2NHCHC*H*), 1.10 (9H, s, (CH_3)$_3$CO), 1.04 (3H, d, *J* 6, NHCHCHCH_3); δ_C (300 MHz, DMSO-d_6) 168.5 (*C*=ONHNH), 151.0 (*ipso*-Ar-*C*), 149.2 (*ipso*-Ar-*C*), 145.5 (*ipso*-Ar-*C*), 134.6 (Ar-*C*), 133.9 (Ar-*C*), 131.3 (Ar-*C*), 130.5 (Ar-*C*), 128.9 (*ipso*-Ar-*C*), 125.6 (*ipso*-Ar-*C*), 118.4 (Ar-*C*), 112.1 (Ar-*C*), 111.3 (Ar-*C*), 74.5 ((CH$_3$)$_3$*C*O), 68.6 (SO_2NHCH*C*H), 60.2 (SO_2NH*C*H), 28.3 ((*C*H$_3$)$_3$CO) 18.9 (NHCHCH*C*H$_3$); *m/z* (ES$^+$) 538 ([^{35,35}Cl]MNa$^+$), 540 ([^{35,37}Cl]MNa$^+$), 540 ([^{37,37}Cl]MNa$^+$); HRMS (ES$^+$) Found [^{35,35}Cl]MNa$^+$, 538.0236 ($C_{20}H_{23}{}^{35,35}Cl_2N_5O_5SNa$ requires 538.0231).

6.2 General Experimental Information of Biological Assessment

6.2.1 Cell Lines

The mycobacterial strains and cell lines used in this study can be found in Table 9.

Strain	Genotype	Comments	Reference
Mtb mc²7000	ΔRD1(esx1):GFP Δ*panCD*	Deletion of esx1 or RD1 responsible for virulence factor secretion, ESAT-6 and CFP-10 replaced with GFP. Pantothenate auxotroph.	W. Jacobs, Albert Einstein College of Medicine.
Mtb mc²7902	Δ*leuCD* Δ*panCD* Δ*argB*	mc²6206 derived, arginine auxotroph	Vilcheze et al. 10.1128/mbio.00938-18
Mtb mc²8245	Δ*panCD* Δ*leuCD* Δ*argB* Δ2116169–2162530	mc²7902 derived, Δ2116169–2162530; INH-Resistant	Vilcheze et al. 10.1128/mbio.00938-18
Mtb mc²8250	Δ*panCD* Δ*leuCD* Δ*argB r poB* (H445Y) Δ2122397–2170320	mc²8247 derived, Δ2122397–2170320; INH-Resistant, *rpoB* His445 --> Lys; RIF resistant	Vilcheze et al. 10.1128/mbio.00938-18
Mtb mc²8257	Δ*panCD* Δ*leuCD* Δ*argB* *rpoB* (H445Y) *katG* (V1 A)	mc²8247 derived, *rpoB* His445 --> Lys; RIF resistant, *katG* Val1 --> Ala; INH-Resistant	Vilcheze et al. 10.1128/mbio.00938-18
Mtb AKB7002	ΔRD1(esx1):GFP Δ*panCD;* Kan ^{R} *rpoB* (H445Y)	As *Mtb* mc²7000 pMV261, generated spontaneous mutant, rifampicin-resistant, *rpoB* His445→Lys.	Brown, A.K. et al. *Molecules*, *25* (10), p.2387

Mtb AKB7021	ΔRD1(esx1):GFP Δ*panCD;* Kan ^{R} *katG* (S315N)	As *Mtb* mc^{2}7000 pMV261, generated spontaneous mutant, isoniazid-resistant, *katG* Ser315→Asp.	Brown, A.K. et al. *Molecules*, *25* (10), p.2387

6.2.2 Bacterial Growth Conditions

Mycobacterial species were cultured in either Middlebrook 7H9 broth or Middlebrook 7H10 agar media supplemented with albumin–dextrose–catalase (ADC) or oleic acid–albumin–dextrose–catalase (OADC) enrichments and 0.2% glycerol, 0.2% casamino acids, 24 µg/mL pantothenate, 1 µg/mL penicillin G, 10 µg/mL cyclohexamide, purchased from BD Biosciences. All reagents were purchased from Sigma-Aldrich, Gillingham, UK unless stated otherwise.

6.2.3 *In vitro* bacterial inhibitory assay

Minimum inhibitory concentrations (MICs) of the intermediates **67**, **68**, **89** and **91** and final compounds **65** on different mycobacterial strains were determined using broth micro-dilution assay in 96-well plates. Bacterial colonies from LJ slants were scraped and re-suspended in Middlebrook 7H9GC medium. Homogeneous mycobacterial suspension was prepared in sterile water, vortexed with glass beads (1-5 mm) for five minutes. After aerosol settlement, the colony-forming unit (CFU) of the suspension was determined by the measurement of optical density (OD) using the SmartSpecTM Plus spectrophotometer (BIO-RAD, CA, USA) at an excitation of 530 nm with an emission of 590 nm. The concentration of stock for each test compound is 10 mg/mL and then a 2-fold serial dilution was prepared (250 to 0.24 µg/mL) in Middlebrook 7H9 medium supplemented with OADC and glycerol as aforementioned. The correct mycobacterial strain suspension was added to an equal volume (100 µL) of test compounds solutions in each well to

obtain the final concentration of 1 x 107 CFU/mL for *Mtb* strains. The untreated bacterial suspension and sterile medium were used as positive and negative growth controls. The plates were then incubated at 37°C for 7 days in an oven incubator. A volume of 20 µL of 0.02% (w/v) resazurin dye was added to each well at the end of the incubation period. The color change was evaluated after ~48 h for *Mtb* strains. Resazurin assay is a colorimetric assay in which the viable cells reduce blue resazurin into fluorescent pink resorufin. MIC of each compound on the susceptible, mono-resistant and double-resistant *Mtb* strains was determined visually and defined as the lowest concentration remained in blue.

7 References

(1) Brown, A. K.; Aljohani, A. K. B.; Gill, J. H.; Steel, P. G.; Sellars, J. D. Identification of Novel Benzoxa-[2,1,3]-Diazole Substituted Amino Acid Hydrazides as Potential Anti-Tubercular Agents. *Molecules*, **2019**, *24* (4), 811.

(2) Brown, A. K.; Aljohani, A. K. B.; Alsalem, F. M. A.; Broadhead, J. L.; Gill, J. H.; Lu, Y.; Sellars, J. D. Identification of Substituted Amino Acid Hydrazides as Novel Anti-Tubercular Agents, Using a Scaffold Hopping Approach. *Molecules* **2020**, *25* (10), 2387.

(3) World Health Organization. *Global Tuberculosis Report 2019.*; World Health Organization: S.l., 2019.

(4) Organization, W. H. *Global Tuberculosis Report 2015*; World Health Organization, 2015.

(5) World Health Organization. *Global Tuberculosis Report 2016.*; World Health Organization: Geneva, 2016.

(6) Organisation mondiale de la santé. *Global Tuberculosis Report 2018*; 2018.

(7) World Health Organization. *Global Tuberculosis Report 2017.*; World Health Organization: Geneva, 2017.

(8) Laserson, K. F.; Osorio, L.; Sheppard, J. D.; Hernández, H.; Benítez, A. M.; Brim, S.; Woodley, C. L.; Hazbón, M. H.; Villegas, M. V.; Castaño, M. C.; Henriquez, N.; Rodriguez, E.; Metchock, B.; Binkin, N. J. Clinical and programmatic mismanagement rather than community outbreak as the cause of chronic, drug-resistant tuberculosis in Buenaventura, Colombia, 1998.

(9) Matteelli, A.; Roggi, A.; Carvalho, A. C. Extensively Drug-Resistant Tuberculosis: Epidemiology and Management. *Clin. Epidemiol.* **2014**, *6*, 111–118.

(10) Seung, K. J.; Keshavjee, S.; Rich, M. L. Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. *Cold Spring Harb. Perspect. Med.* **2015**, *5* (9).

(11) Bussi, C.; Gutierrez, M. G. Mycobacterium Tuberculosis Infection of Host Cells in Space and Time. *FEMS Microbiol. Rev.* **2019**, *43* (4), 341–361.

(12) Russell, D. G.; Barry, C. E.; Flynn, J. L. Tuberculosis: What We Don't Know Can, and Does, Hurt Us. *Science* **2010**, *328* (5980), 852–856.

(13) Korbel, D.S., Schneider, B.E. and Schaible, U.E., **2008**. Innate immunity in tuberculosis: myths and truth. *Microbes and Infection*, 10(9), pp.995-1004.

(14) Allen, M.; Bailey, C.; Cahatol, I.; Dodge, L.; Yim, J.; Kassissa, C.; Luong, J.; Kasko, S.; Pandya, S.; Venketaraman, V. Mechanisms of Control of Mycobacterium Tuberculosis by NK Cells: Role of Glutathione. *Front. Immunol.* **2015**, *62* (3).

(15) Mbow, M.; Santos, N. S. S.; Camara, M.; Ba, A.; Niang, A.; Daneau, G.; Wade, D.; Diallo, A. A.; Toupane, M.; Diakhaté, M.; Lèye, N.; Diaw, P. A.; Mboup, S.; Kestens, L.; Dieye, T. N. HIV and Tuberculosis Co-Infection Impacts T-Cell Activation Markers but Not the Numbers Subset of Regulatory T-Cells in HIV-1 Infected Patients. *Afr. J. Lab. Med.* **2013**, *2* (1).

(16) Cavalcanti, Y. V. N.; Brelaz, M. C. A.; Neves, J. K. de A. L.; Ferraz, J. C.; Pereira, V. R. A. Role of TNF-Alpha, IFN-Gamma, and IL-10 in the Development of Pulmonary Tuberculosis. *Pulm. Med.* **2012**.

(17) Maitra, A., Munshi, T., Healy, J., Martin, L.T., Vollmer, W., Keep, N.H. and Bhakta, S., **2019**. Cell wall peptidoglycan in Mycobacterium tuberculosis: An Achilles' heel for the TB-causing pathogen. *F FEMS Microbiol. Rev, 43* (5), 548-575.

(18) Fullam, E.; Young, R. J. Physicochemical Properties and Mycobacterium Tuberculosis Transporters: Keys to Efficacious Antitubercular Drugs? *RSC Med. Chem.* **2021**, *12* (1), 43–56.

(19) Sakamoto, K. The Pathology of *Mycobacterium Tuberculosis* Infection. *Vet. Pathol.* **2012**, *49* (3), 423–439.

(20) Chang, T.-W.; Lin, Y.-M.; Wang, C.-F.; Liao, Y.-D. Outer Membrane Lipoprotein Lpp Is Gram-Negative Bacterial Cell Surface Receptor for Cationic Antimicrobial Peptides. *J. Biol. Chem.* **2012**, *287* (1), 418–428.

(21) Schleifer, K. H. Classification of Bacteria and Archaea: Past, Present and Future. *Syst. Appl. Microbiol.* **2009**, *32* (8), 533–542.

(22) Vilchèze, C. Mycobacterial Cell Wall: A Source of Successful Targets for Old and New Drugs. *Appl. Sci.* **2020**, *10* (7), 2278.

(23) Abrahams, K. A.; Besra, G. S. Mycobacterial Cell Wall Biosynthesis: A Multifaceted Antibiotic Target. *Parasitology* **2018**, *145* (2), 116–133.

(24) Fukuda, T.; Matsumura, T.; Ato, M.; Hamasaki, M.; Nishiuchi, Y.; Murakami, Y.; Maeda, Y.; Yoshimori, T.; Matsumoto, S.; Kobayashi, K.; Kinoshita, T.; Morita, Y. S. Critical Roles for Lipomannan and Lipoarabinomannan in Cell Wall Integrity of Mycobacteria and Pathogenesis of Tuberculosis. *mBio*, **2013**, *4* (1), 472-12.

(25) Jilani, T. N.; Avula, A.; Zafar Gondal, A.; Siddiqui, A. H. Active Tuberculosis. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), **2020**.

(26) Shirude, P. S.; Chatterji, M.; Narayanan, S.; Iyer, P. S. Chapter Fourteen - Advancement of Cell Wall Inhibitors in Mycobacterium Tuberculosis*Present Address: Sai Life Sciences Ltd, Pune, India. In *Annual Reports in Medicinal Chemistry*; Desai, M. C., Ed.; Academic Press, **2014**, *49* (12), 209–220.

(27) Raghunandanan, S.; Jose, L.; Kumar, R. A. Dormant Mycobacterium Tuberculosis Converts Isoniazid to the Active Drug in a Wayne's Model of Dormancy. *J. Antibiot.* (*Tokyo*) **2018**, *71* (11), 939–949.

(28) Luca, S.; Mihaescu, T. History of BCG Vaccine. *Mædica*, **2013**, *8* (1), 53–58.

(29) Lyadova, I.V., Vordermeier, H.M., Eruslanov, E.B., Khaidukov, S.V., Apt, A.S. and Hewinson, R.G., **2001**. Intranasal BCG vaccination protects BALB/c mice against virulent Mycobacterium bovis and accelerates production of IFN-γ in their lungs. *Clinical & Experimental Immunology*, *126* (2), 274-279.

(30) Fox, G. J.; Orlova, M.; Schurr, E. Tuberculosis in Newborns: The Lessons of the "Lübeck Disaster" (1929–1933). *PLoS Pathog.* **2016**, *12* (1), 251-256.

(31) Brandt, L.; Cunha, J. F.; Olsen, A. W.; Chilima, B.; Hirsch, P.; Appelberg, R.; Andersen, P. Failure of the Mycobacterium Bovis BCG Vaccine: Some Species

of Environmental Mycobacteria Block Multiplication of BCG and Induction of Protective Immunity to Tuberculosis. *Infect. Immun.* **2002**, *70* (2), 672–678.

(32) Andersen, P. and Kaufmann, S.H., Novel vaccination strategies against tuberculosis. *Cold Spring Harbor perspectives in medicine*, **2014**, *4* (6), 852-857.

(33) Horvath, C. N.; Xing, Z. Immunization Strategies Against Pulmonary Tuberculosis: Considerations of T Cell Geography. In *The New Paradigm of Immunity to Tuberculosis*; Divangahi, M., Ed.; *Advances in Experimental Medicine and Biology*; Springer: New York, **2013**, 267–278.

(34) Dockrell, H.M., Towards new TB vaccines: What are the challenges?. *Pathogens and disease*, **2016**, *74* (4).

(35) Dookie, N.; Rambaran, S.; Padayatchi, N.; Mahomed, S.; Naidoo, K. Evolution of Drug Resistance in Mycobacterium Tuberculosis: A Review on the Molecular Determinants of Resistance and Implications for Personalized Care. *J. Antimicrob. Chemother.* **2018**, *73* (5), 1138–1151.

(36) Zhang, Y.; Yew, W.-W. Mechanisms of Drug Resistance in Mycobacterium Tuberculosis, **2015**. *The International Journal of Tuberculosis and Lung Disease*, *19* (11), 1276-1289.

(37) Vilcheze, C.; Jacobs, W. R. Resistance to Isoniazid and Ethionamide in Mycobacterium Tuberculosis: Genes, Mutations, and Causalities. *Microbiol. Spectr.* **2014**, *2* (4), 923–925.

(38) Shah, N.S., Wright, A., Bai, G.H., Barrera, L., Boulahbal, F., Martín-Casabona, N., Drobniewski, F., Gilpin, C., Havelková, M., Lepe, R. and Lumb, R., Worldwide emergence of extensively drug-resistant tuberculosis. Emerging infectious diseases, **2007**, *13* (3), 380-384.

(39) Center, C. I. T. Drug-Resistant Tuberculosis: A Survival Guide for Clinicians, 3rd Edition. 36.

(40) Timmins, G. S.; Deretic, V. Mechanisms of Action of Isoniazid. *Mol. Microbiol.* **2006**, *62* (5), 1220–1227.

(41) Koneman, E. W. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*; Lippincott Williams & Wilkins, **2006**.

(42) Campbell, E. A.; Korzheva, N.; Mustaev, A.; Murakami, K.; Nair, S.; Goldfarb, A.; Darst, S. A. Structural Mechanism for Rifampicin Inhibition of Bacterial RNA Polymerase. *Cell* **2001**, *104* (6), 901–912.

(43) Van Hest, R.; Baars, H.; Kik, S.; van Gerven, P.; Trompenaars, M.-C.; Kalisvaart, N.; Keizer, S.; Borgdorff, M.; Mensen, M.; Cobelens, F. Hepatotoxicity of Rifampin-Pyrazinamide and Isoniazid Preventive Therapy and Tuberculosis Treatment. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **2004**, *39* (4), 488–496.

(44) Zhang, Y.; Shi, W.; Zhang, W.; Mitchison, D. Mechanisms of Pyrazinamide Action and Resistance. *Microbiol. Spectr.* **2013**, *2* (4), 1–12.

(45) Fox, W.; Ellard, G. A.; Mitchison, D. A. Studies on the Treatment of Tuberculosis Undertaken by the British Medical Research Council

Tuberculosis Units, 1946–1986, with Relevant Subsequent Publications. *Int. J. Tuberc. Lung Dis.* **1999**, *3* (10), S231–S279.

(46) Shehzad, A.; Rehman, G.; Ul-Islam, M.; Khattak, W. A.; Lee, Y. S. Challenges in the Development of Drugs for the Treatment of Tuberculosis. *Braz. J. Infect. Dis.* **2013**, *17* (1), 74–81.

(47) Blanchard, J. S. Molecular Mechanisms of Drug Resistance in Mycobacterium Tuberculosis. *Annu. Rev. Biochem.* **1996**, *65* (1), 215–239.

(48) Xie, J.; Talaska, A. E.; Schacht, J. New Developments in Aminoglycoside Therapy and Ototoxicity. *Hear. Res.* **2011**, *281* (1), 28–37.

(49) Information, N. C. for B.; Pike, U. S. N. L. of M. 8600 R.; MD, B.; USA, 20894. *Treatment Strategies for MDR-TB and XDR-TB*; World Health Organization, **2014**.

(50) Dijkstra, J. A.; Laan, T. van der; Akkerman, O. W.; Bolhuis, M. S.; Lange, W. C. M. de; Kosterink, J. G. W.; Werf, T. S. van der; Alffenaar, J. W. C.; Soolingen, D. van. In Vitro Susceptibility of Mycobacterium Tuberculosis to Amikacin, Kanamycin, and Capreomycin. *Antimicrob. Agents Chemother.* **2018**, *62* (3).

(51) Sirgel, F. A.; Tait, M.; Warren, R. M.; Streicher, E. M.; Böttger, E. C.; van Helden, P. D.; Gey van Pittius, N. C.; Coetzee, G.; Hoosain, E. Y.; Chabula-Nxiweni, M.; Hayes, C.; Victor, T. C.; Trollip, A. Mutations in the *Rrs* A1401G Gene and Phenotypic Resistance to Amikacin and Capreomycin in *Mycobacterium Tuberculosis*. *Microb. Drug Resist.* **2012**, *18* (2), 193–197.

(52) Ginsburg, A. S.; Grosset, J. H.; Bishai, W. R. Fluoroquinolones, Tuberculosis, and Resistance. *Lancet Infect. Dis.* **2003**, *3* (7), 432–442.

(53) Silva, J. T.; San-Juan, R.; Fernández-Ruiz, M.; Aguado, J. M. Fluoroquinolones for the Treatment of Latent Mycobacterium Tuberculosis Infection in Liver Transplantation. *World J. Gastroenterol.* **2019**, *25* (26), 3291–3298.

(54) Saravolatz, L. D.; Leggett, J. Gatifloxacin, Gemifloxacin, and Moxifloxacin: The Role of 3 Newer Fluoroquinolones. *Clin. Infect. Dis.* **2003**, *37* (9), 1210–1215.

(55) Pranger, A. D.; van der Werf, T. S.; Kosterink, J. G. W.; Alffenaar, J. W. C. The Role of Fluoroquinolones in the Treatment of Tuberculosis in 2019. *Drugs* **2019**, *79* (2), 161–171.

(56) Islam, M. M.; Tan, Y.; Hameed, H. M. A.; Liu, Z.; Chhotaray, C.; Liu, Y.; Lu, Z.; Cai, X.; Tang, Y.; Gao, Y.; Surineni, G.; Li, X.; Tan, S.; Guo, L.; Cai, X.; Yew, W. W.; Liu, J.; Zhong, N.; Zhang, T. Detection of Novel Mutations Associated with Independent Resistance and Cross-Resistance to Isoniazid and Prothionamide in Mycobacterium Tuberculosis Clinical Isolates. *Clin. Microbiol. Infect.* **2019**, *25* (8), 1041.e1-1041.e7.

(57) Thee, S.; Garcia-Prats, A. J.; Donald, P. R.; Hesseling, A. C.; Schaaf, H. S. A Review of the Use of Ethionamide and Prothionamide in Childhood Tuberculosis. *Tuberculosis* **2016**, *97*, 126–136.

(58) Li, Y.; Wang, F.; Wu, L.; Zhu, M.; He, G.; Chen, X.; Sun, F.; Liu, Q.; Wang, X.; Zhang, W. Cycloserine for Treatment of Multidrug-Resistant Tuberculosis: A Retrospective Cohort Study in China. *Infect. Drug Resist.* **2019**, *12*, 721–731.

(59) Gallagher, L. A.; Shears, R. K.; Fingleton, C.; Alvarez, L.; Waters, E. M.; Clarke, J.; Bricio-Moreno, L.; Campbell, C.; Yadav, A. K.; Razvi, F.; O'Neill, E.; O'Neill, A. J.; Cava, F.; Fey, P. D.; Kadioglu, A.; O'Gara, J. P. An Alanine Permease Mutation or Exposure to D-Cycloserine Increases the Susceptibility of MRSA to β-Lactam Antibiotics. *bioRxiv*, **2019**, *22* (4) 616-619.

(60) Chakraborty, S.; Gruber, T.; Barry, C. E.; Boshoff, H. I.; Rhee, K. Y. Para-Aminosalicylic Acid Acts as an Alternative Substrate of Folate Metabolism in Mycobacterium Tuberculosis. *Science* **2013**, *339* (6115), 88–91.

(61) Minato, Y.; Thiede, J. M.; Kordus, S. L.; McKlveen, E. J.; Turman, B. J.; Baughn, A. D. Mycobacterium Tuberculosis Folate Metabolism and the Mechanistic Basis for Para-Aminosalicylic Acid Susceptibility and Resistance. *Antimicrob. Agents Chemother.* **2015**, *59* (9), 5097–5106.

(62) Thiede, J.; Kordus, S.; Turman, B.; Buonomo, J.; Aldrich, C.; Minato, Y.; Baughn, A. Targeting Intracellular P-Aminobenzoic Acid Production Potentiates the Anti-Tubercular Action of Antifolates. *Sci. Rep.* **2016**, *6*, 38083.

(63) Conradie, F.; Diacon, A. H.; Ngubane, N.; Howell, P.; Everitt, D.; Crook, A. M.; Mendel, C. M.; Egizi, E.; Moreira, J.; Timm, J.; McHugh, T. D.; Wills, G.; Bateson, A.; Hunt, R.; Van Niekerk, C.; Li, M.; Olugbosi, M.; Spigelman, M. Bedaquiline, Pretomanid and Linezolid for Treatment of Extensively Drug Resistant, Intolerant or Non-Responsive Multidrug Resistant Pulmonary Tuberculosis. *N. Engl. J. Med.* **2020**, *382* (10), 893–902.

(64) Lohrasbi, V.; Talebi, M.; Bialvaei, A. Z.; Fattorini, L.; Drancourt, M.; Heidary, M.; Darban-Sarokhalil, D. Trends in the Discovery of New Drugs for Mycobacterium Tuberculosis Therapy with a Glance at Resistance. *Tuberculosis* **2018**, *109*, 17–27.

(65) Kumar, D.; Negi, B.; Rawat, D. S. The Anti-Tuberculosis Agents under Development and the Challenges Ahead. *Future Med. Chem.* **2015**, *7* (15), 1981–2003.

(66) Zhang, M.; Sala, C.; Dhar, N.; Vocat, A.; Sambandamurthy, V. K.; Sharma, S.; Marriner, G.; Balasubramanian, V.; Cole, S. T. In Vitro and In Vivo Activities of Three Oxazolidinones against Nonreplicating Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **2014**, *58* (6), 3217–3223.

(67) Upton, A. M.; Cho, S.; Yang, T. J.; Kim, Y.; Wang, Y.; Lu, Y.; Wang, B.; Xu, J.; Mdluli, K.; Ma, Z.; Franzblau, S. G. In Vitro and In Vivo Activities of the Nitroimidazole TBA-354 against Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **2015**, *59* (1), 136–144.

(68) Showalter, H.D., Recent Progress in the Discovery and Development of 2-Nitroimidazooxazines and 6-Nitroimidazooxazoles to Treat Tuberculosis and Neglected Tropical Diseases. *Molecules*, **2020**, *25* (18), 4137.

(69) Manjunatha, U.; Boshoff, H. I. M.; Barry, C. E. The Mechanism of Action of PA-824. *Commun. Integr. Biol.* **2009**, *2* (3), 215–218.

(70) Singh, R.; Manjunatha, U.; Boshoff, H. I. M.; Ha, Y. H.; Niyomrattanakit, P.; Ledwidge, R.; Dowd, C. S.; Lee, I. Y.; Kim, P.; Zhang, L.; Kang, S.; Keller, T. H.; Jiricek, J.; Barry, C. E. PA-824 Kills Nonreplicating Mycobacterium

Tuberculosis by Intracellular NO Release. *Science* **2008**, *322* (5906), 1392–1395.

(71) Pushpanathan, M.; Gunasekaran, P.; Rajendhran, J. Antimicrobial Peptides: Versatile Biological Properties. *Int. J. Pept.* **2013**, *20* (4).

(72) Ebenhan, T.; Gheysens, O.; Kruger, H. G.; Zeevaart, J. R.; Sathekge, M. M. Antimicrobial Peptides: Their Role as Infection-Selective Tracers for Molecular Imaging, *BioMed research international*, **2014**, *52* (3), 381-390.

(73) Sato, H.; Feix, J. B. Peptide–Membrane Interactions and Mechanisms of Membrane Destruction by Amphipathic α-Helical Antimicrobial Peptides. *Biochim. Biophys. Acta BBA - Biomembr.* **2006**, *1758* (9), 1245–1256.

(74) Hancock, R. E. W.; Sahl, H.-G. Antimicrobial and Host-Defense Peptides as New Anti-Infective Therapeutic Strategies. *Nat. Biotechnol.* **2006**, *24* (12), 1551–1557.

(75) Hancock, R. E. W.; Lehrer, R. Cationic Peptides: A New Source of Antibiotics. *Trends Biotechnol.* **1998**, *16* (2), 82–88.

(76) Gordon, Y. J.; Romanowski, E. G.; McDermott, A. M. A Review of Antimicrobial Peptides and Their Therapeutic Potential as Anti-Infective Drugs. *Curr. Eye Res.* **2005**, *30* (7), 505–515.

(77) Lei, J.; Sun, L.; Huang, S.; Zhu, C.; Li, P.; He, J.; Mackey, V.; Coy, D. H.; He, Q. The Antimicrobial Peptides and Their Potential Clinical Applications. *Am. J. Transl. Res.* **2019**, *11* (7), 3919–3931.

(78) Suzuki, H.; Yamada, C.; Kato, K. γ-Glutamyl Compounds and Their Enzymatic Production Using Bacterial γ-Glutamyltranspeptidase. *Amino Acids* **2007**, *32* (3), 333–340.

(79) Elhakeem, M. A.; Taher, A. T.; Abuel-Maaty, S. M. Synthesis and Anti-Mycobacterial Evaluation of Some New Isonicotinylhydrazide Analogues. *Bull. Fac. Pharm. Cairo Univ.* **2015**, *53* (1), 45–52.

(80) Kumari, M.; Narang, R. Structure Activity Relationship and Mechanism of Action of Hydrazide Derivatives as Antimicrobial Molecule. *Journal of Chemical and Pharmaceutical Research*, **2016**, *8* (7), 823-836.

(81) Villamizar-Mogotocoro, A.-F.; Vargas-Méndez, L. Y.; Kouznetsov, V. V. Pyridine and Quinoline Molecules as Crucial Protagonists in the Never-Stopping Discovery of New Agents against Tuberculosis. *Eur. J. Pharm. Sci.* **2020**, *151*, 105374.

(82) Scior, T.; Garces-Eisele, S. J. Isoniazid Is Not a Lead Compound for Its Pyridyl Ring Derivatives,Isonicotinoyl Amides, Hydrazides, and Hydrazones: A Critical Review. *Curr. Med. Chem.* **2006**, *13* (18), 2205–2219.

(83) Popiołek, Ł. Hydrazide–Hydrazones as Potential Antimicrobial Agents: Overview of the Literature since 2010. *Med. Chem. Res.* **2017**, *26* (2), 287–301.

(84) Koçyiğit-Kaymakçıoğlu, B.; Elçin Oruç-Emre, E.; Unsalan, S.; Rollas, S. Antituberculosis Activity of Hydrazones Derived from 4-Fluorobenzoic Acid Hydrazide. *Med. Chem. Res.* **2009**, *18* (4), 277–286.

(85) Carvalho, S. A.; da Silva, E. F.; de Souza, M. V. N.; Lourenço, M. C. S.; Vicente, F. R. Synthesis and Antimycobacterial Evaluation of New Trans-Cinnamic Acid Hydrazide Derivatives. *Bioorg. Med. Chem. Lett.* **2008**, *18* (2), 538–541.

(86) Vavříková, E.; Polanc, S.; Kočevar, M.; Horváti, K.; Bősze, S.; Stolaříková, J.; Vávrová, K.; Vinšová, J. New Fluorine-Containing Hydrazones Active against MDR-Tuberculosis. *Eur. J. Med. Chem.* **2011**, *46* (10), 4937–4945.

(87) Arora, P., Arora, V., Lamba, H.S. and Wadhwa, D., Importance of heterocyclic chemistry: a review. International Journal of Pharmaceutical Sciences and Research, **2012**, *3* (9), 2947-50.

(88) Shukla, C. and Srivastava, S., Biologically active oxadiazole. Journal of Drug Delivery and Therapeutics, **2015**, *5* (6), 8-13.

(89) Paruch, K.; Popiołek, Ł.; Wujec, M. Antimicrobial and Antiprotozoal Activity of 3-Acetyl-2,5-Disubstituted-1,3,4-Oxadiazolines: A Review. *Med. Chem. Res.* **2020**, *29* (1), 1–16.

(90) Spink, E.; Ding, D.; Peng, Z.; Boudreau, M. A.; Leemans, E.; Lastochkin, E.; Song, W.; Lichtenwalter, K.; O'Daniel, P. I.; Testero, S. A.; Pi, H.; Schroeder, V. A.; Wolter, W. R.; Antunes, N. T.; Suckow, M. A.; Vakulenko, S.; Chang, M.; Mobashery, S. Structure–Activity Relationship for the Oxadiazole Class of Antibiotics. *J. Med. Chem.*, **2015**, *58* (3), 1380-1389

(91) Zheng, Z.; Liu, Q.; Kim, W.; Tharmalingam, N.; Fuchs, B. B.; Mylonakis, E. Antimicrobial Activity of 1,3,4-Oxadiazole Derivatives against Planktonic Cells and Biofilm of Staphylococcus Aureus. *Future Med. Chem.* **2018**, *10* (3), 283–296.

(92) Rane, R. A.; Bangalore, P.; Borhade, S. D.; Khandare, P. K. Synthesis and Evaluation of Novel 4-Nitropyrrole-Based 1,3,4-Oxadiazole Derivatives as Antimicrobial and Anti-Tubercular Agents. *Eur. J. Med. Chem.* **2013**, *70*, 49–58.

(93) Ahsan, M. J.; Samy, J. G.; Khalilullah, H.; Nomani, Md. S.; Saraswat, P.; Gaur, R.; Singh, A. Molecular Properties Prediction and Synthesis of Novel 1,3,4-Oxadiazole Analogues as Potent Antimicrobial and Antitubercular Agents. *Bioorg. Med. Chem. Lett.* **2011**, *21* (24), 7246–7250.

(94) Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial Resistance: A Global Multifaceted Phenomenon. *Pathog. Glob. Health* **2015**, *109* (7), 309–318.

(95) Somoskovi, A.; Parsons, L. M.; Salfinger, M. The Molecular Basis of Resistance to Isoniazid, Rifampin, and Pyrazinamide in Mycobacterium Tuberculosis. *Respir. Res.* **2001**, *2* (3), 164–168.

(96) Palomino, J. C.; Martin, A. Drug Resistance Mechanisms in Mycobacterium Tuberculosis. *Antibiotics* **2014**, *3* (3), 317–340.

(97) Liu, L.-K.; Abdelwahab, H.; Martin Del Campo, J. S.; Mehra-Chaudhary, R.; Sobrado, P.; Tanner, J. J. The Structure of the Antibiotic Deactivating, N-Hydroxylating Rifampicin Monooxygenase. *J. Biol. Chem.* **2016**, *291* (41), 21553–21562.

(98) Whitfield, M. G.; Soeters, H. M.; Warren, R. M.; York, T.; Sampson, S. L.; Streicher, E. M.; Helden, P. D. van; Rie, A. van. A Global Perspective on

Pyrazinamide Resistance: Systematic Review and Meta-Analysis. *PLOS ONE* **2015**, *10* (7), e0133869.

(99) Sacksteder, K. A.; Protopopova, M.; Barry, C. E.; Andries, K.; Nacy, C. A. Discovery and Development of SQ109: A New Antitubercular Drug with a Novel Mechanism of Action. *Future Microbiol.* **2012**, *7* (7), 823–837.

(100) Köser, C. U.; Ellington, M. J.; Peacock, S. J. Whole-Genome Sequencing to Control Antimicrobial Resistance. *Trends Genet.* **2014**, *30* (9), 401–407.

(101) Scalacci, N.; Brown, A. K.; Pavan, F. R.; Ribeiro, C. M.; Manetti, F.; Bhakta, S.; Maitra, A.; Smith, D. L.; Petricci, E.; Castagnolo, D. Synthesis and SAR Evaluation of Novel Thioridazine Derivatives Active against Drug-Resistant Tuberculosis. *Eur. J. Med. Chem.* **2017**, *127*, 147–158.

(102) Tahlan, S.; Kumar, S.; Kakkar, S.; Narasimhan, B. Benzimidazole Scaffolds as Promising Antiproliferative Agents: A Review. *BMC Chem.* **2019**, *13* (1), 66.

(103) Si, W.; Zhang, T.; Li, Y.; She, D.; Pan, W.; Gao, Z.; Ning, J.; Mei, X. Synthesis and Biological Activity of Novel Benzimidazole Derivatives as Potential Antifungal Agents. *J. Pestic. Sci.,* **2016**, *41* (1), 15–19.

(104) Sole, R.; Gatto, V.; Conca, S.; Bardella, N.; Morandini, A.; Beghetto, V. Sustainable Triazine-Based Dehydro-Condensation Agents for Amide Synthesis. *Molecules,* **2021**, *26* (1), 191-193.

(105) Zaletova, J., Dzurilla, M., Kutschy, P., Pazdera, P., Kováčik, V., Aldoelfi, J. and Bekešová, S., Synthesis of 4, 6-Disubstituted-2-(1H-indol-3-yl) benzothiazoles. *Collection of Czechoslovak chemical communications*, **2004**, 69 (2), 453-460.

(106) Wang, B.; Shan, D.; Wang, W.; Zhang, H.; Gudmundsson, O.; Borchardt, R. T. Synthesis of Coumarin-Based, Esterase-Sensitive Cyclic Prodrugs of Opioid Peptides with Enhanced Membrane Permeability and Enzymatic Stability. In *Peptidomimetics Protocols*; Humana Press: New Jersey, **1998**, 23 (7), 71–86.

(107) Falchi, A.; Giacomelli, G.; Porcheddu, A.; Taddei, M. 4-(4,6-Dimethoxy[1,3,5]Triazin-2-Yl)-4-Methyl-Morpholinium Chloride (DMTMM): A Valuable Alternative to PyBOP for Solid Phase Peptide Synthesis. *Synlett*, **2000**, *2000* (2), 275–277.

(108) Raw, S.A., An improved process for the synthesis of DMTMM-based coupling reagents. *Tetrahedron Letters*, **2009**, *50* (8), 946-948.

(109) Kunishima, M.; Kawachi, C.; Monta, J.; Terao, K.; Iwasaki, F.; Tani, S. 4-(4,6-Dimethoxy-1,3,5-Triazin-2-Yl)-4-Methyl-Morpholinium Chloride: An Efficient Condensing Agent Leading to the Formation of Amides and Esters. *Tetrahedron*, **1999**, *55* (46), 13159–13170.

(110) Dunetz, J. R.; Magano, J.; Weisenburger, G. A. Large-Scale Applications of Amide Coupling Reagents for the Synthesis of Pharmaceuticals. *Org. Process Res. Dev.* **2016**, *20* (2), 140–177.

(111) Nguyen, L. A.; He, H.; Pham-Huy, C. Chiral Drugs: An Overview. *Int. J. Biomed. Sci. IJBS* **2006**, *2* (2), 85–100.

(112) Blessington, B., Beiraghi, A., Lo, T.W., Drake, A. and Jonas, G.,. Chiral hplc-cd studies of the antituberculosis drug (+)-ethambutol. *Chirality*, 1992, *4* (4), 227-229.

(113) Parsons, S. Determination of Absolute Configuration Using X-Ray Diffraction. *Tetrahedron Asymmetry* **2017**, *28* (10), 1304–1313.

(114) Ananthi, N. Role of Chirality in Drugs. *Org. Med. Chem. Int. J.* **2018**, *5* (3).

(115) Hernandes, M. Z.; Cavalcanti, S. M. T.; Moreira, D. R. M.; de Azevedo Junior, W. F.; Leite, A. C. L. Halogen Atoms in the Modern Medicinal Chemistry: Hints for the Drug Design. *Curr. Drug Targets* **2010**, *11* (3), 303–314.

(116) Limban, C.; Chifiriuc, M. C. Antibacterial Activity of New Dibenzoxepinone Oximes with Fluorine and Trifluoromethyl Group Substituents. *Int. J. Mol. Sci.* **2011**, *12* (10), 6432–6444.

(117) Machado, D.; Girardini, M.; Viveiros, M.; Pieroni, M. Challenging the Drug-Likeness Dogma for New Drug Discovery in Tuberculosis. *Front. Microbiol.* **2018**, *9* (5).

(118) Elbert, B. L.; Farley, A. J. M.; Gorman, T. W.; Johnson, T. C.; Genicot, C.; Lallemand, B.; Pasau, P.; Flasz, J.; Castro, J. L.; MacCoss, M.; Paton, R. S.; Schofield, C. J.; Smith, M. D.; Willis, M. C.; Dixon, D. J. C–H Cyanation of 6-Ring N-Containing Heteroaromatics. *Chem. Weinh. Bergstr. Ger.* **2017**, *23* (59), 14733–14737.

(119) Tong, A. S. T.; Choi, P. J.; Blaser, A.; Sutherland, H. S.; Tsang, S. K. Y.; Guillemont, J.; Motte, M.; Cooper, C. B.; Andries, K.; Van den Broeck, W.; Franzblau, S. G.; Upton, A. M.; Denny, W. A.; Palmer, B. D.; Conole, D. 6-Cyano Analogues of Bedaquiline as Less Lipophilic and Potentially Safer Diarylquinolines for Tuberculosis. *ACS Med. Chem. Lett.* **2017**, *8* (10), 1019–1024.

(120) Kim, P.; Zhang, L.; Manjunatha, U. H.; Singh, R.; Patel, S.; Jiricek, J.; Keller, T. H.; Boshoff, H. I.; Barry, C. E.; Dowd, C. S. Structure-Activity Relationships of Antitubercular Nitroimidazoles. I. Structural Features Associated with Aerobic and Anaerobic Activities of 4- and 5-Nitroimidazoles. *J. Med. Chem.* **2009**, *52* (5), 1317–1328. https://doi.org/10.1021/jm801246z.

(121) Papadopoulou, M. V.; Bloomer, W. D.; Rosenzweig, H. S. The Antitubercular Activity of Various Nitro(Triazole/Imidazole)-Based Compounds. *Bioorg. Med. Chem.* **2017**, *25* (21), 6039–6048.

(122) Sun, S.; Fu, J. Methyl-Containing Pharmaceuticals: Methylation in Drug Design. *Bioorg. Med. Chem. Lett.* **2018**, *28* (20), 3283–3289.

(123) Harrold, M.W. and Zavod, R.M., Basic concepts in medicinal chemistry. **2014**, 988-989.

(124) Al Musaimi, O.; Jad, Y. E.; Kumar, A.; Collins, J. M.; Basso, A.; de la Torre, B. G.; Albericio, F. Investigating Green Ethers for the Precipitation of Peptides after Global Deprotection in Solid-Phase Peptide Synthesis. *Curr. Opin. Green Sustain. Chem.* **2018**, *11*, 99–103.

(125) Vergel Galeano, C. F.; Rivera Monroy, Z. J.; Rosas Pérez, J. E.; García Castañeda, J. E. Efficient Synthesis of Peptides with 4-Methylpiperidine as

Fmoc Removal Reagent by Solid Phase Synthesis. *J. Mex. Chem. Soc.* **2014**, *58* (4), 386–392.

(126) Sheppeck, J. E.; Kar, H.; Hong, H. A Convenient and Scaleable Procedure for Removing the Fmoc Group in Solution. *Tetrahedron Lett.* **2000**, *41* (28), 5329–5333.

(127) Supuran, C. T.; Innocenti, A.; Mastrolorenzo, A.; Scozzafava, A. Antiviral Sulfonamide Derivatives. *Mini Rev. Med. Chem.* **2004**, *4* (2), 189–200.

(128) Naredla, R. R.; Klumpp, D. A. Preparation of Sulfonamides from N-Silylamines. *Tetrahedron Lett.* **2013**, *54* (45), 5945–5947.

(129) Gennari, C.; Longari, C.; Ressel, S.; Salom, B.; Mielgo, A. Synthesis of Chiral Vinylogous Sulfonamidopeptides (vs-Peptides). *Eur. J. Org. Chem.* **1998**, *1998* (6), 945–959.

(130) Wilden, J. D. The Sulfonamide Motif as a Synthetic Tool. *J. Chem. Res.* **2010**, *34* (10), 541–548.

(131) Katawera, V.; Siedner, M.; Boum Ii, Y. Evaluation of the Modified Colorimetric Resazurin Microtiter Plate-Based Antibacterial Assay for Rapid and Reliable Tuberculosis Drug Susceptibility Testing. *BMC Microbiol.* **2014**, *14* (1), 259.

(132) Palomino, J.-C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. Resazurin Microtiter Assay Plate: Simple and Inexpensive Method for Detection of Drug Resistance in Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **2002**, *46* (8), 2720–2722.

(133) Domhan, C.; Uhl, P.; Kleist, C.; Zimmermann, S.; Umstätter, F.; Leotta, K.; Mier, W.; Wink, M. Replacement of L-Amino Acids by d-Amino Acids in the Antimicrobial Peptide Ranalexin and Its Consequences for Antimicrobial Activity and Biodistribution. *Molecules* **2019**, *24* (16), 2987.

(134) Abula, A., Xu, Z., Zhu, Z., Peng, C., Chen, Z., Zhu, W. and Aisa, H.A., Substitution effect of the trifluoromethyl group on the bioactivity in medicinal chemistry: statistical analysis and energy calculations. *Journal of Chemical Information and Modelling,* **2020**, *60* (12), 6242-6250.

(135) Inoue, M.; Sumii, Y.; Shibata, N. Contribution of Organofluorine Compounds to Pharmaceuticals. *ACS Omega* **2020**, *5* (19), 10633–10640.

(136) Grzelak, E. M.; Choules, M. P.; Gao, W.; Cai, G.; Wan, B.; Wang, Y.; McAlpine, J. B.; Cheng, J.; Jin, Y.; Lee, H.; Suh, J.-W.; Pauli, G. F.; Franzblau, S. G.; Jaki, B. U.; Cho, S. Strategies in Anti-Mycobacterium Tuberculosis Drug Discovery Based on Phenotypic Screening. *J. Antibiot. (Tokyo)* **2019**, *72* (10), 719–728.

(137) Murale, D. P.; Hong, S. C.; Haque, Md. M.; Lee, J.-S. Photo-Affinity Labeling (PAL) in Chemical Proteomics: A Handy Tool to Investigate Protein-Protein Interactions (PPIs). *Proteome Sci.* **2017**, *15* (1), 141-145.

(138) Robinette, D.; Neamati, N.; Tomer, K. B.; Borchers, C. H. Photoaffinity Labeling Combined with Mass Spectrometric Approaches as a Tool for Structural Proteomics. *Expert Rev. Proteomics* **2006**, *3* (4), 399–408.

(139) Bräse, S., Gil, C., Knepper, K. and Zimmermann, V., Organic azides: an exploding diversity of a unique class of compounds. *Angewandte Chemie International Edition*, **2005**, *44* (33), 5188-5240.

(140) Cravatt, B. F.; Wright, A. T.; Kozarich, J. W. Activity-Based Protein Profiling: From Enzyme Chemistry to Proteomic Chemistry. *Annu. Rev. Biochem.* **2008**, *77* (1), 383–414.

(141) Taldone, T.; Rodina, A.; Gomes, E. M. D.; Riolo, M.; Patel, H. J.; Alonso-Sabadell, R.; Zatorska, D.; Patel, M. R.; Kishinevsky, S.; Chiosis, G. Synthesis and Evaluation of Cell-Permeable Biotinylated PU-H71 Derivatives as Tumor Hsp90 Probes. *Beilstein J. Org. Chem.* **2013**, *9* (1), 544–556.

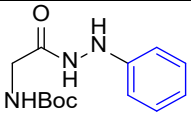
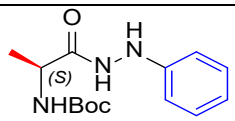
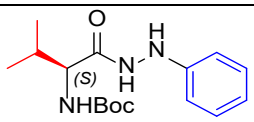
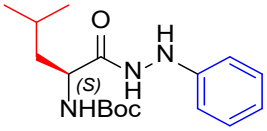
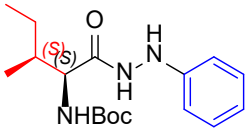
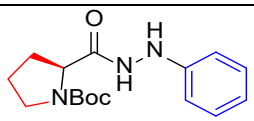
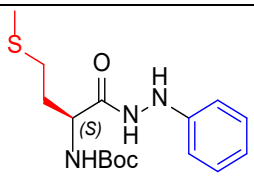
(142) Probert, P. M.; Leitch, A. C.; Dunn, M. P.; Meyer, S. K.; Palmer, J. M.; Abdelghany, T. M.; Lakey, A. F.; Cooke, M. P.; Talbot, H.; Wills, C.; McFarlane, W.; Blake, L. I.; Rosenmai, A. K.; Oskarsson, A.; Figueiredo, R.; Wilson, C.; Kass, G. E.; Jones, D. E.; Blain, P. G.; Wright, M. C. Identification of a Xenobiotic as a Potential Environmental Trigger in Primary Biliary Cholangitis. *J. Hepatol.* **2018**, *69* (5), 1123–1135.

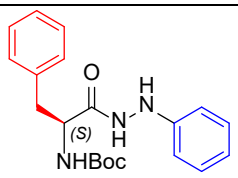
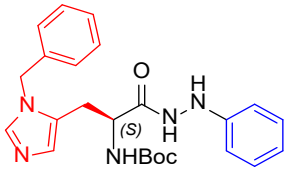
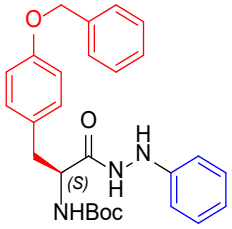
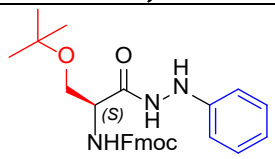
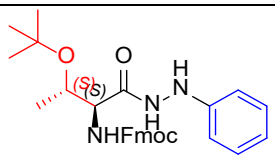
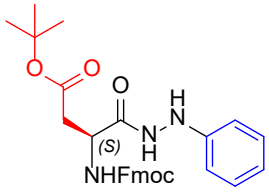
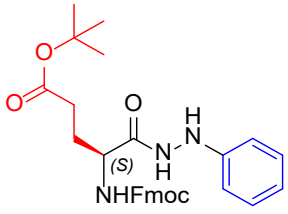
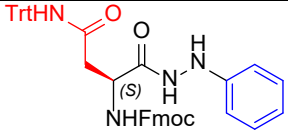
8 Appendix

8.1 The biological data for *in vitro* evaluating antitubercular activity

As observed for *in vitro* antitubercular screening, the MIC results of the Boc-*N*-hydrazides **67** and Fmoc-*N*-hydrazides **68 Ag-Ar** against wild-type *Mtb*, isoniazid-resistant *Mtb* strain and rifampicin-resistant *Mtb* were obtained.

Table A1: The MIC results of the antimycobacterial susceptibility testing of the unsubstituted Boc-*N*-hydrazides **67a-j** and Fmoc-*N*-hydrazides **68a-j**; where a (-) indicates to a lack of inhibition activity.

		MIC (μM)		
Entry	Structure	WT. *Mtb* mc²7000	INH. resistant *Mtb* mc²7021	RIF. resistant *Mtb* mc²7002
1	 **67a**	241.22	-	-
2	 **67b**	114.56	-	-
3	 **67c**	13.01	208.20	-
4	 **67d**	12.44	199.12	-
5	 **67e**	-	199.12	-
6	 **67f**	104.79	52.39	104.79
7	 **67g**	5.89	188.54	-

8	 **67h**	2.81	90.03	-
9	 **67i**	-	-	-
10	 **67j**	138.66	69.33	138.66
11	 **68a**	-	-	-
12	 **68b**	32.81	32.81	65.63
13	 **68c**	127.60	127.60	127.60
14	 **68d**	31.03	31.03	31.03
15	 **68e**	-	-	-

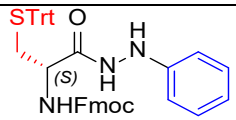
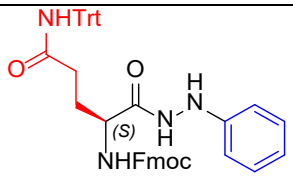
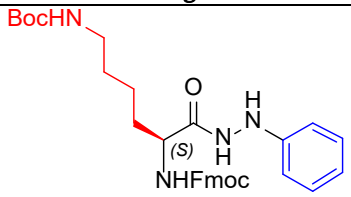
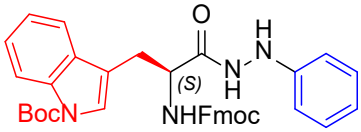
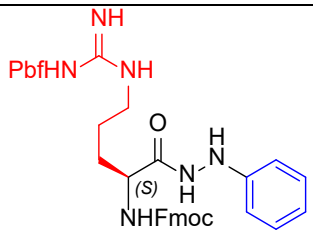
16	 **68f**	-	-	-
17	 **68g**	-	91.32	-
18	 **68h**	-	69.78	69.78
19	 **68i**	-	-	-
20	 **68j**	-	-	-

Table A2: The MIC results of the antimycobacterial susceptibility testing of the unsubstituted Boc-*N*-hydrazides **67k-p**; where a (-) indicates to a lack of inhibition activity.

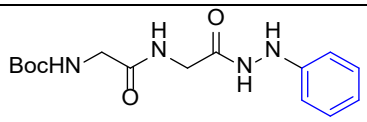
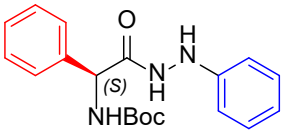
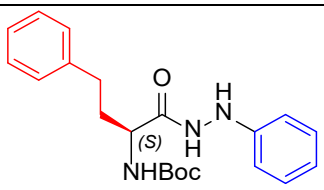
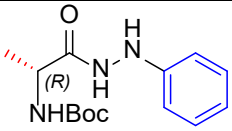
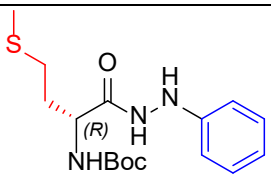
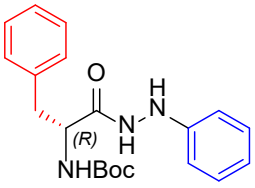
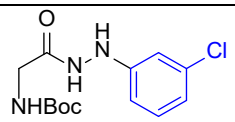
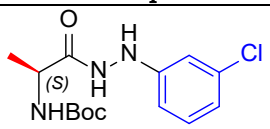
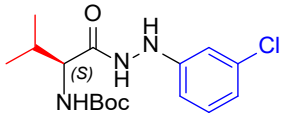
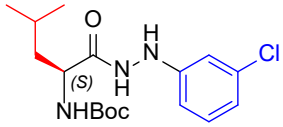
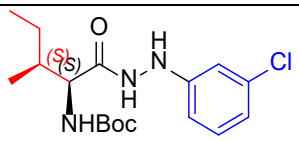
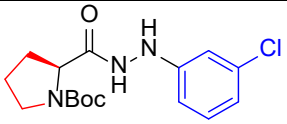
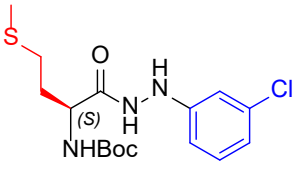
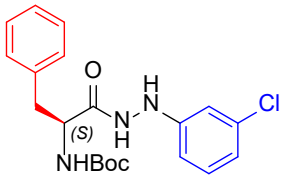
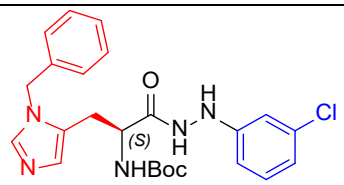
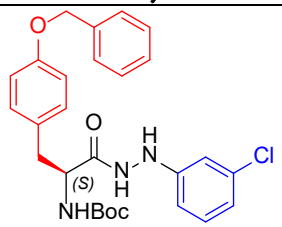
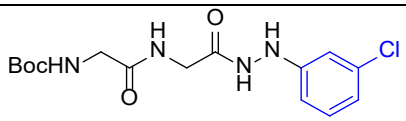
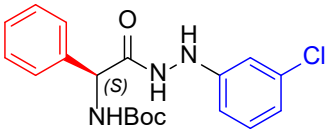
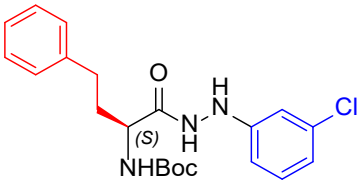
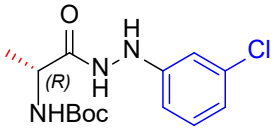
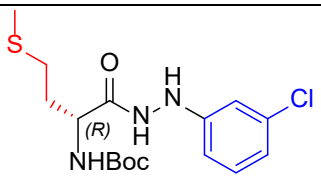
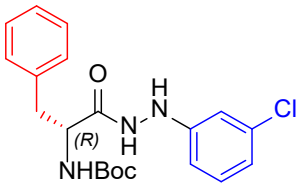
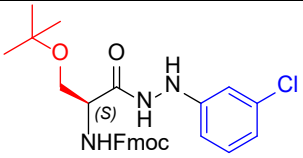
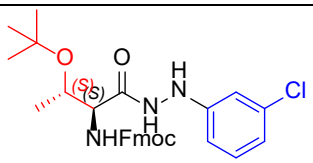
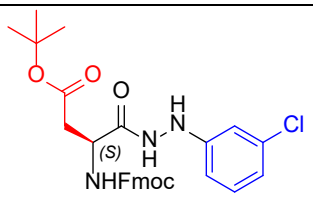
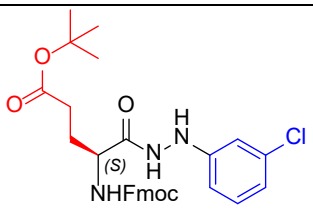
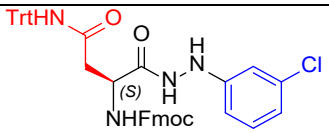
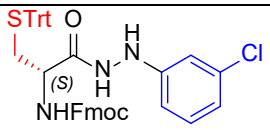
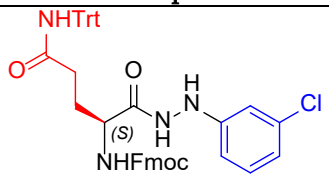
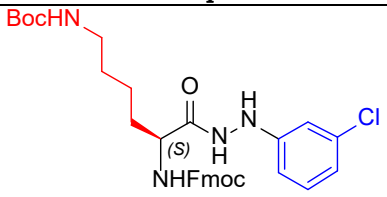
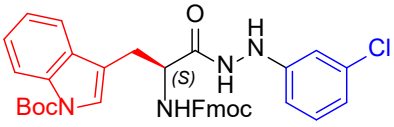
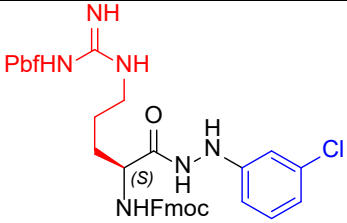
		MIC (µM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
21	 **67k**	-	289.25	-
22	 **67l**	57.28	-	-
23	 **67m**	86.61	173.22	173.22
24	 **67n**	-	-	-
25	 **67o**	-	-	-
26	 **67p**	90.03	-	-

Table A3: The MIC results of the antimycobacterial susceptibility testing of the substituted Boc-*N*-hydrazides **67q-Af** and Fmoc-*N*-hydrazides **68k-u**; where a (-) indicates to a lack of inhibition activity.

		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
27	 **67q**	13.34	-	-
28	 **67r**	3.19	12.75	101.98
29	 **67s**	93.61	93.61	93.61
30	 **67t**	89.92	179.85	89.92
31	 **67u**	44.96	44.96	89.92
32	 **67v**	94.17	47.08	94.17
33	 **67w**	2.67	42.79	-
34	 **67x**	5.13	82.08	41.04

35	 **67y**	34.04	17.02	34.04
36	 **67z**	129.03	64.52	129.03
37	 **67Aa**	95.13	95.13	95.13
38	 **67Ab**	85.14	42.57	85.14
39	 **67Ac**	23.40	23.40	46.81
40	 **67Ad**	101.98	101.98	101.98
41	 **67Ae**	85.58	85.58	85.58
42	 **67Af**	-	82.08	-
43	 **68k**	62.99	15.75	62.99

44	 **68l**	15.32	15.32	15.32
45	 **68m**	59.70	59.70	59.70
46	 **68n**	58.18	29.09	29.09
47	 **68o**	44.37	-	-
48	 **68p**	-	45.05	-
49	 **68q**	10.88	5.44	10.88
50	 **68r**	26.98	-	13.49
51	 **68s**	-	-	-
52		82.76	82.76	82.76

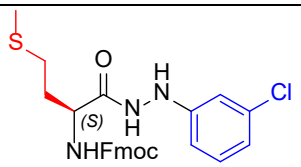
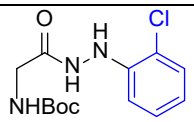
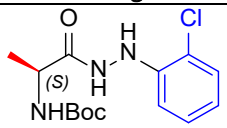
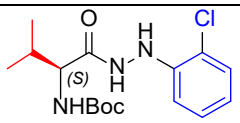
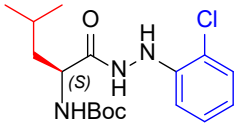
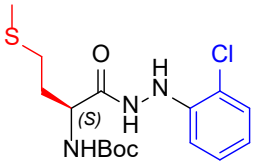
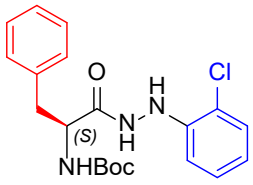
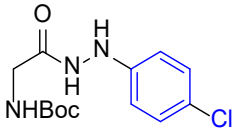
	68t			
53	 **68u**	32.26	129.03	32.26

Table A4: The MIC results of the antimycobacterial susceptibility testing of the chlorinated Boc-*N*-hydrazides **67Ag-Ar** at different positions on aryl hydrazines; where a (-) indicates to a lack of inhibition activity.

		MIC (µM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
54	 **67Ag**	106.75	53.38	-
55	 **67Ah**	50.99	101.98	-
56	 **67Ai**	187.22	187.22	187.22
57	 **67Aj**	179.85	179.85	179.85
58	 **67Ak**	21.40	171.17	171.17
59	 **67Al**	164.15	164.15	164.15
60		213.51	13.34	53.38

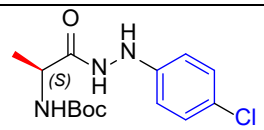
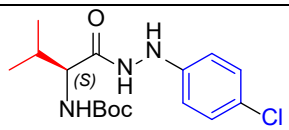
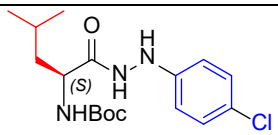
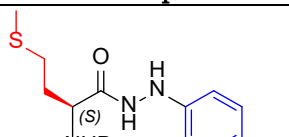
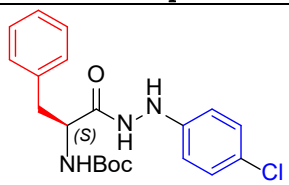
	67Am			
61	 **67An**	1.59	50.99	25.50
62	 **67Ao**	93.61	46.81	93.61
63	 **67Ap**	89.92	179.85	89.92
64	 **67Aq**	21.40	21.40	85.58
65	 **67Ar**	41.04	41.04	82.08

Table A5: The MIC results of the antimycobacterial susceptibility testing of the di-*ortho* chlorinated Boc-*N*-hydrazides **67As-Ax**; where a (-) indicates to a lack of inhibition activity.

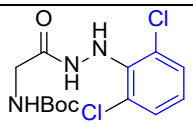
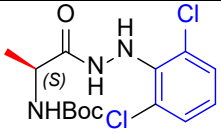
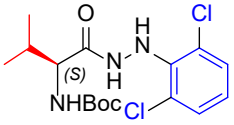
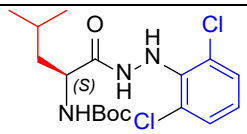
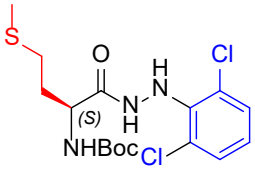
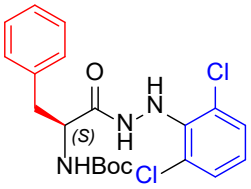
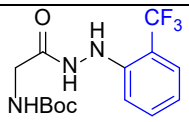
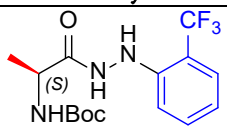
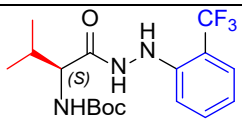
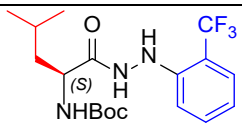
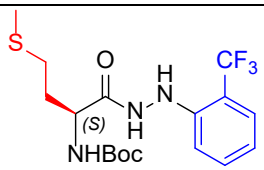
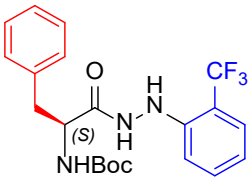
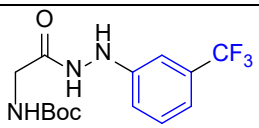
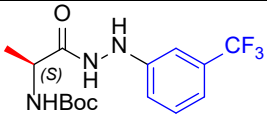
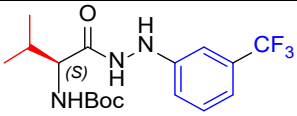
		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
66	 **67As**	23.94	191.50	191.50
67	 **67At**	45.95	-	-
68	 **67Au**	5.32	85.04	-
69	 **67Av**	81.99	163.97	81.99
70	 **67Aw**	19.59	156.73	-
71	 **67Ax**	18.85	150.83	-

Table A6: The MIC results of the antimycobacterial susceptibility testing of the trifluoromethylated Boc-*N*-hydrazides **67Ay-Bp**; where a (-) indicates to a lack of inhibition activity.

		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
72	 **67Ay**	96.01	192.01	192.01
73	 **67Az**	-	184.26	-
74	 **67Ba**	-	170.49	-
75	 **67Bb**	-	164.35	-
76	 **67Bc**	78.54	78.54	78.54
77	 **67Bd**	-	151.14	-
78	 **67Be**	-	48.00	96.01
79	 **67Bf**	92.13	92.13	92.13
80		-	85.24	-

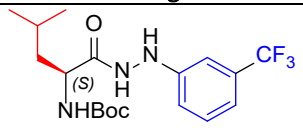
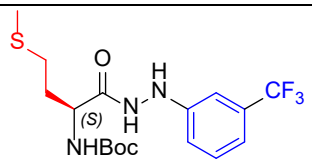
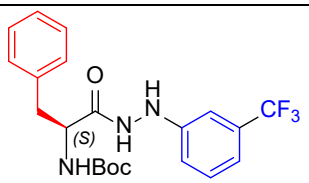
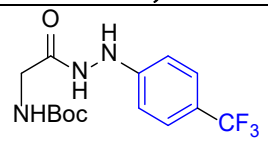
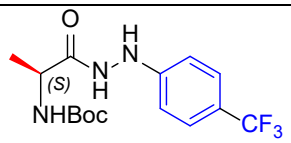
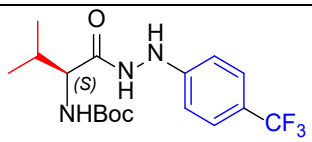
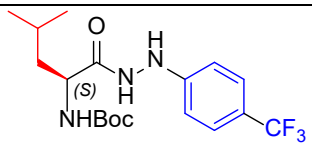
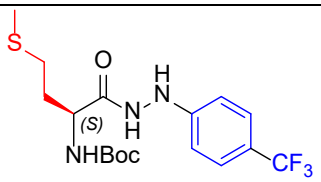
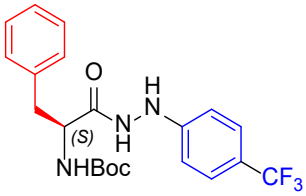
	67Bg			
81	 **67Bh**	20.54	41.09	41.09
82	 **67Bi**	-	-	-
82	 **67Bj**	37.79	37.79	75.57
83	 **67Bk**	12.01	NA	NA
84	 **67Bl**	46.06	23.03	92.13
85	 **67Bm**	-	-	-
86	 **67Bn**	82.17	-	82.17
87	 **67Bo**	39.27	39.27	39.27
88	 **67Bp**	151.14	NA	NA

Table A7: The MIC results of the antimycobacterial susceptibility testing of the di-*meta* trifluoromethylated Boc-*N*-hydrazides **67Bs-Bx**; where a (-) indicates to a lack of inhibition activity.

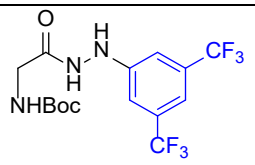
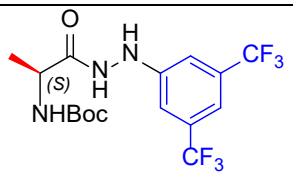
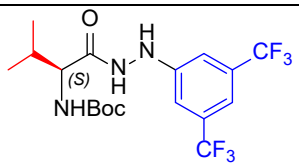
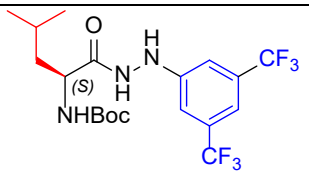
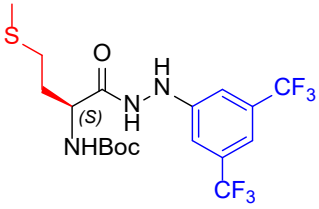
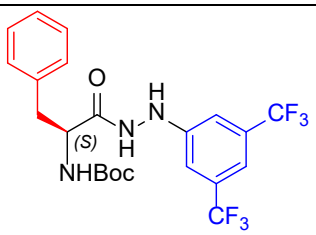
		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
89	 **67Bs**	79.74	79.74	79.74
90	 **67Bt**	19.26	-	-
91	 **67Bu**	NA	NA	NA
92	 **67Bv**	-	139.92	-
93	 **67Bw**	-	-	-
94	 **67Bx**	-	-	-

Table A8: The MIC results of the antimycobacterial susceptibility testing of the di-substituted hydrazides with fluorine **67By-Cd**; where a (-) indicates to a lack of inhibition activity.

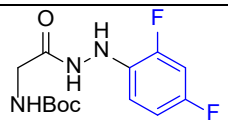
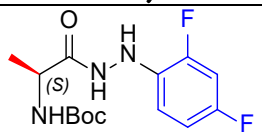
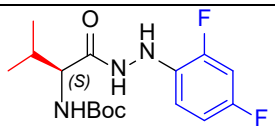
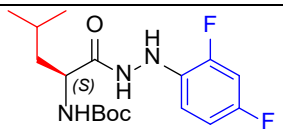
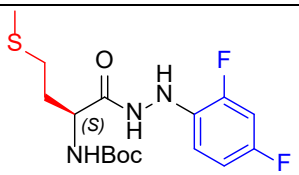
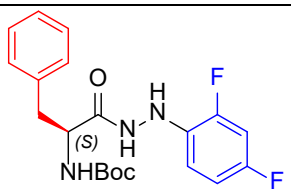
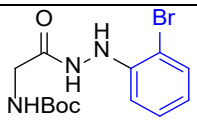
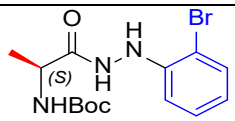
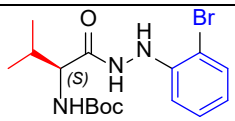
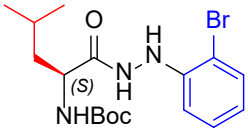
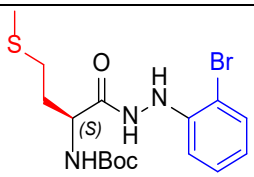
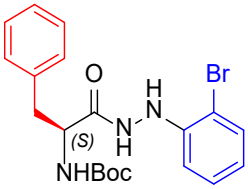
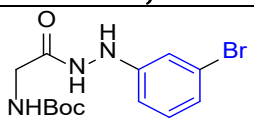
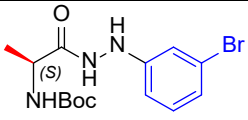
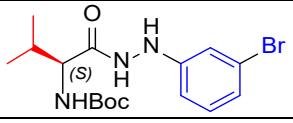
		MIC (µM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
95	 **67By**	106.21	26.55	106.21
96	 **67Bz**	25.37	-	-
97	 **67Ca**	-	-	-
98	 **67Cb**	-	179.07	-
99	 **67Cc**	-	-	-
100	 **67Cd**	10.22	-	-

Table A9: The MIC results of the antimycobacterial susceptibility testing of the brominated hydrazides **67Ce-Cv**; where a (-) indicates to a lack of inhibition activity.

		MIC (µM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
101	 **67Ce**	-	185.93	-
102	 **67Cf**	44.66	178.65	-
103	 **67Cg**	10.86	86.89	-
104	 **67Ch**	159.87	159.87	159.87
105	 **67Ci**	159.87	159.87	-
106	 **67Cj**	147.35	147.35	147.35
107	 **67Ck**	5.81	23.24	46.48
108	 **67Cl**	44.66	22.33	44.66
109		42.98	42.98	42.98

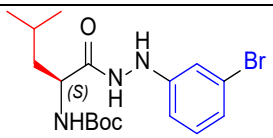
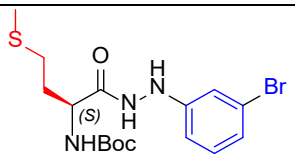
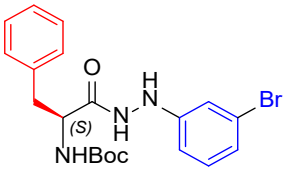
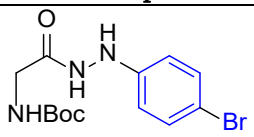
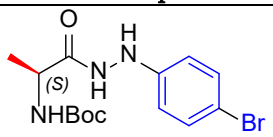
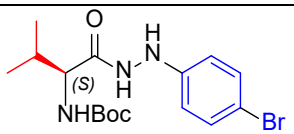
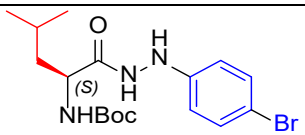
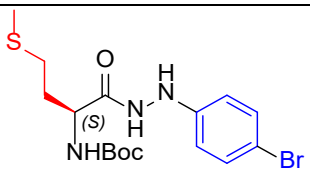
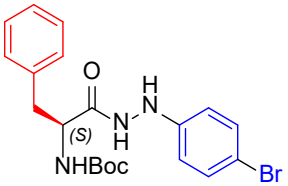
	67Cm			
110	 **67Cn**	159.87	19.98	39.97
111	 **67Co**	19.98	19.98	39.97
112	 **67Cp**	73.68	18.42	36.84
113	 **67Cq**	23.24	5.81	23.24
114	 **67Cr**	44.66	11.17	44.66
115	 **67Cs**	85.96	42.98	85.96
116	 **67Ct**	39.97	39.97	39.97
117	 **67Cu**	NA	NA	NA
118	 **67Cv**	36.84	36.84	18.42

Table A10: The MIC results of the antimycobacterial susceptibility testing of the cyanated hydrazides **67Cw-Db**; where a (-) indicates to a lack of inhibition activity.

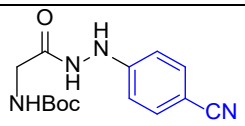
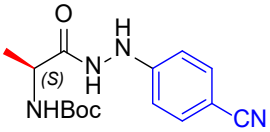
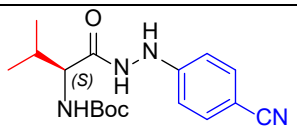
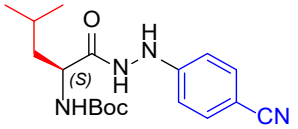
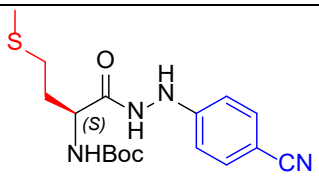
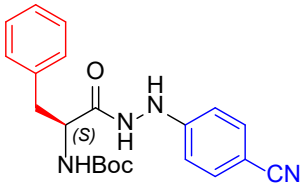
		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
119	 **67Cw**	110.22	110.22	220.45
120	 **67Cx**	26.29	-	-
121	 **67Cy**	-	-	-
122	 **67Cz**	-	184.74	184.74
123	 **67Da**	10.98	87.80	-
124	 **67Db**	-	-	-

Table A11: The MIC results of the antimycobacterial susceptibility testing of the 3-nitro hydrazides **67Dc-Dh**; where a (-) indicates to a lack of inhibition activity.

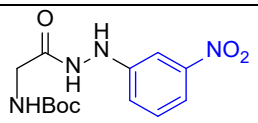
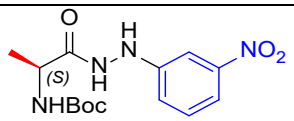
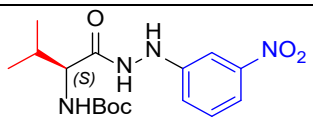
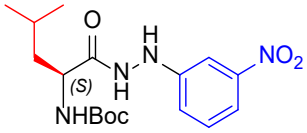
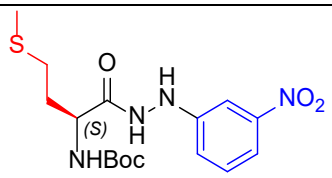
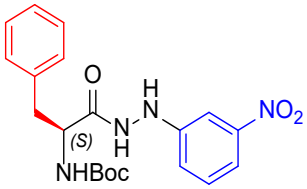
		MIC (µM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
125	 **67Dc**	-	-	-
126	 **67Dd**	98.66	98.66	98.66
127	 **67De**	-	-	-
128	 **67Df**	174.66	87.33	87.33
129	 **67Dg**	-	90.81	90.81
130	 **67Dh**	79.91	79.91	79.91

Table A12: The MIC results of the antimycobacterial susceptibility testing of the 4-methylsulfonyl hydrazides **67Di-Dn**; where a (-) indicates to a lack of inhibition activity.

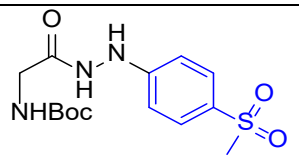
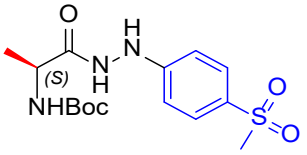
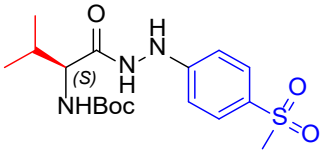
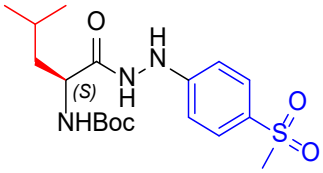
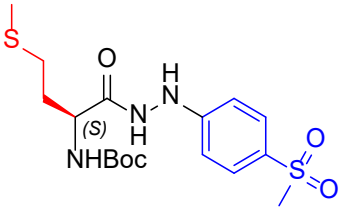
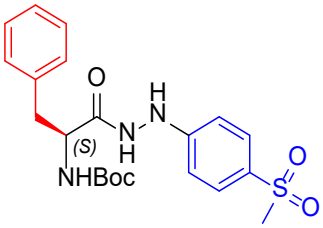
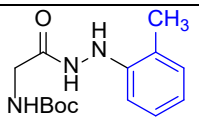
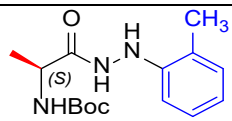
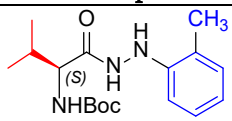
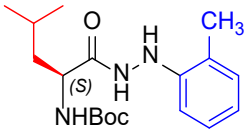
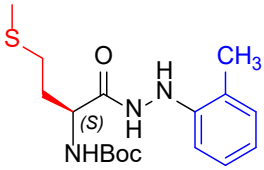
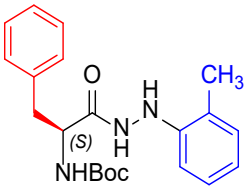
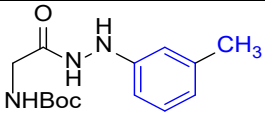
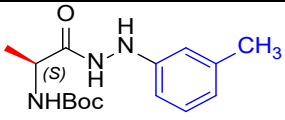
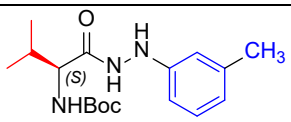
		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
131	 **67Di**	-	-	-
132	 **67Dj**	89.53	-	-
133	 **67Dk**	-	-	-
134	 **67Dl**	-	-	-
135	 **67Dm**	-	-	-
136	 **67Dn**	-	-	-

Table A13: The MIC results of the antimycobacterial susceptibility testing of the methylated hydrazides **67Do-Ef**; where a (-) indicates to a lack of inhibition activity.

		MIC (μM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
137	 **67Do**	229.11	229.11	-
138	 **67Dp**	109.08	109.08	-
139	 **67Dq**	99.56	199.12	199.12
140	 **67Dr**	190.79	190.79	190.79
141	 **67Ds**	181.06	-	-
142	 **67Dt**	173.22	86.61	173.22
143	 **67Du**	114.56	114.56	114.56
144	 **67Dv**	109.08	218.15	109.08
145		99.56	24.89	99.56

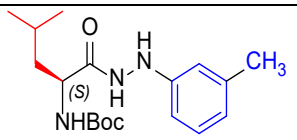
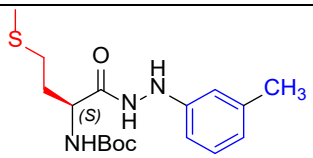
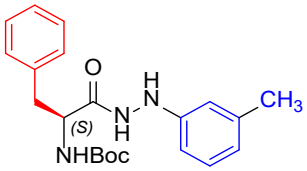
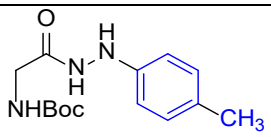
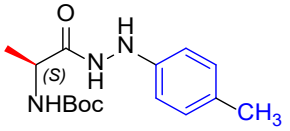
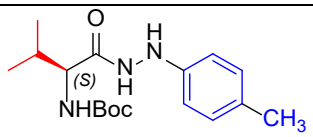
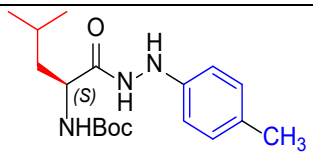
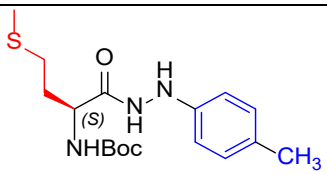
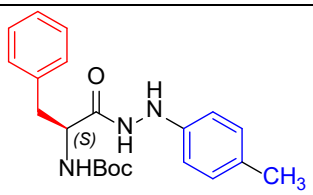
	67Dw			
146	 **67Dx**	95.39	95.39	190.79
147	 **67Dy**	-	181.06	181.06
148	 **67Dz**	86.61	86.61	86.61
149	 **67Ea**	-	229.11	229.11
150	 **67Eb**	3.41	109.08	109.08
151	 **67Ec**	199.12	199.12	199.12
152	 **67Ed**	190.79	95.39	95.39
153	 **67Ee**	1.41	45.26	181.06
154	 **67Ef**	173.22	173.22	173.22

Table A14: The MIC results of the antimycobacterial susceptibility testing of the 4-isopropyl hydrazides **67Eg-El**; where a (-) indicates to a lack of inhibition activity.

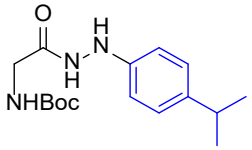
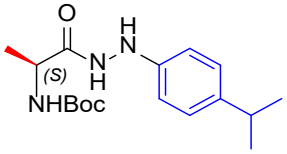
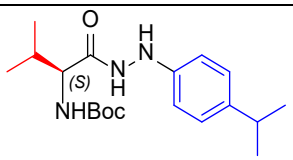
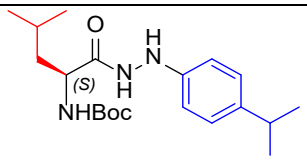
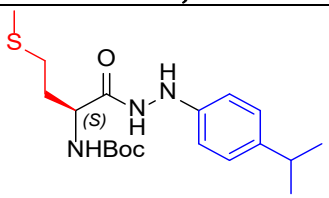
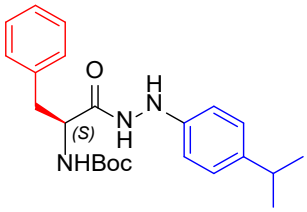
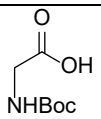
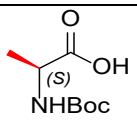
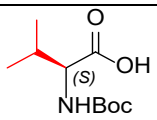
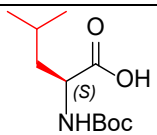
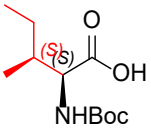
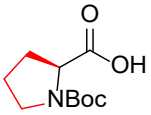
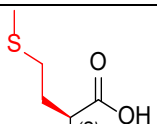
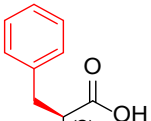
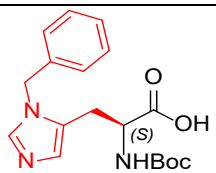
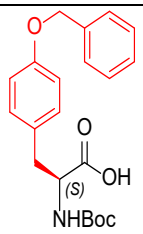
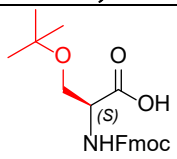
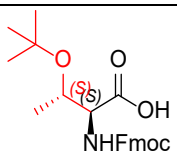
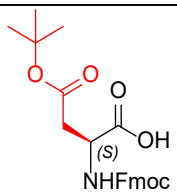
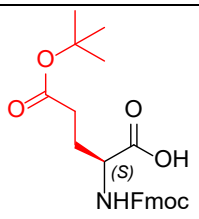
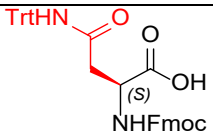
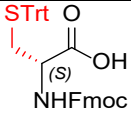
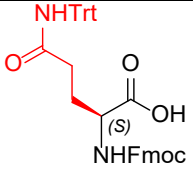
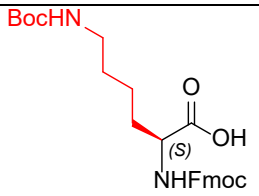
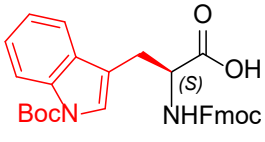
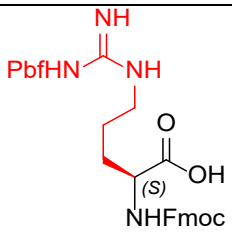
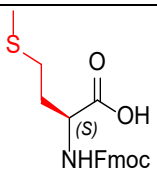
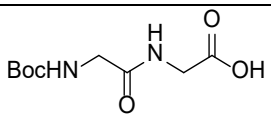
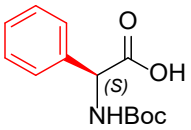
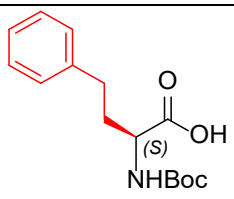
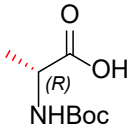
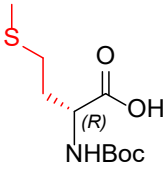
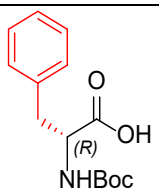
		MIC (μM)		
Entry	Structure	WT. *Mtb* mc²7000	INH. resistant *Mtb* mc²7021	RIF. resistant *Mtb* mc²7002
155	 **67Eg**	104.10	104.10	104.10
156	 **67Eh**	3.11	99.56	199.12
157	 **67Ei**	183.13	91.56	91.56
158	 **67Ej**	-	176.07	176.07
159	 **67Ek**	-	167.74	167.74
160	 **67El**	80.50	-	161.00

Table A15: The MIC results of the antimycobacterial susceptibility testing of the protected amino acids **70a-70Aa**; where a (-) indicates to a lack of inhibition activity.

		MIC (µM)		
Entry	**Structure**	**WT. *Mtb* mc²7000**	**INH. resistant *Mtb* mc²7021**	**RIF. resistant *Mtb* mc²7002**
165	 **70a**	-	-	-
166	 **70b**	-	-	-
167	 **70c**	-	-	-
168	 **70d**	-	-	-
169	 **70e**	-	-	-
170	 **70f**	-	-	-
171	 **70g**	-	-	-
172	 **70h**	-	-	-
173		-	-	-

	70i			
174	 70j	-	-	-
175	 70k	-	-	166.91
176	 70l	-	-	-
177	 70m	-	-	-
178	 70n	-	-	-
179	 70o	-	-	-
180	 70p	-	-	-
181	 70q	-	-	-

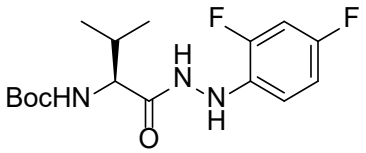
182	 **70r**	-	-	-
183	 **70s**	121.54	121.54	121.54
184	 **70t**	-	-	-
185	 **70u**	-	-	-
186	 **70v**	-	-	-
187	 **70w**	-	-	-
188	 **70x**	-	-	-
189	 **70y**	338.25	338.25	338.25
200	 **70z**	-	-	-

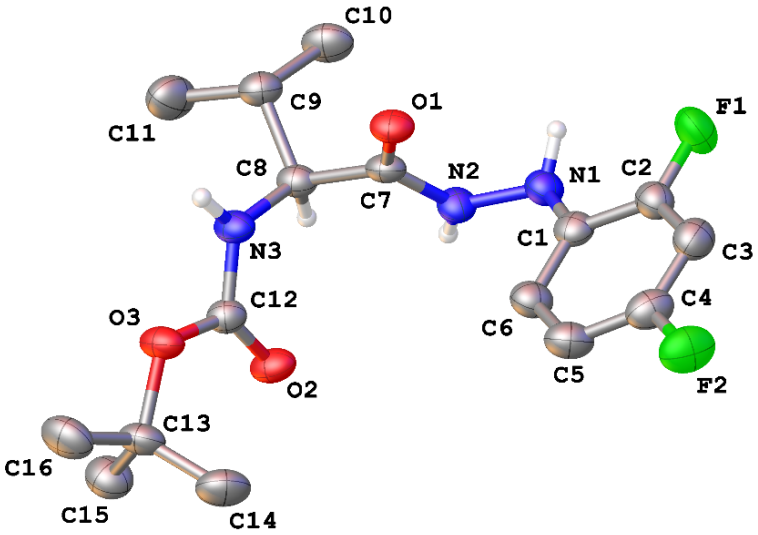
201	 **70Aa**	-	-	-

8.2 The Crystal data and structure refinement

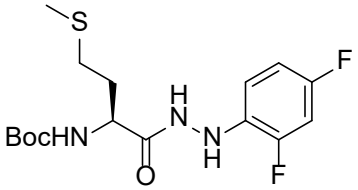
Crystal data and structure refinement for the resulting amino acid hydrazides.

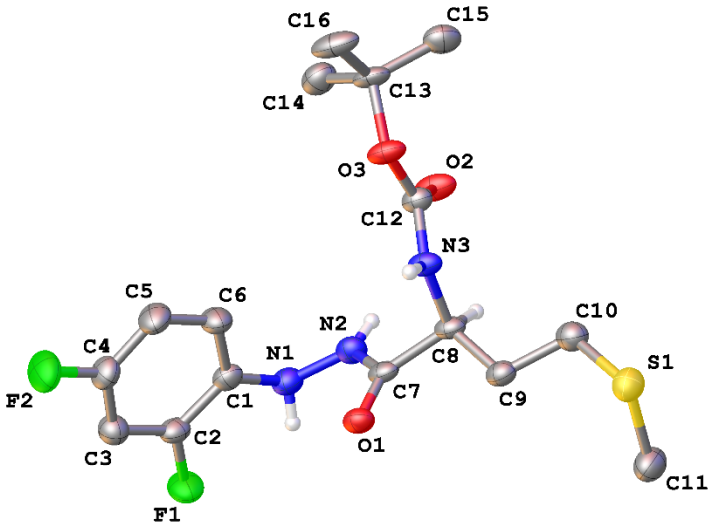
tert-Butyl-(S)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Ca



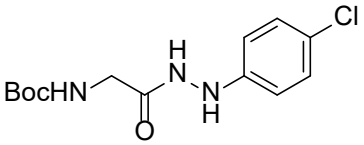


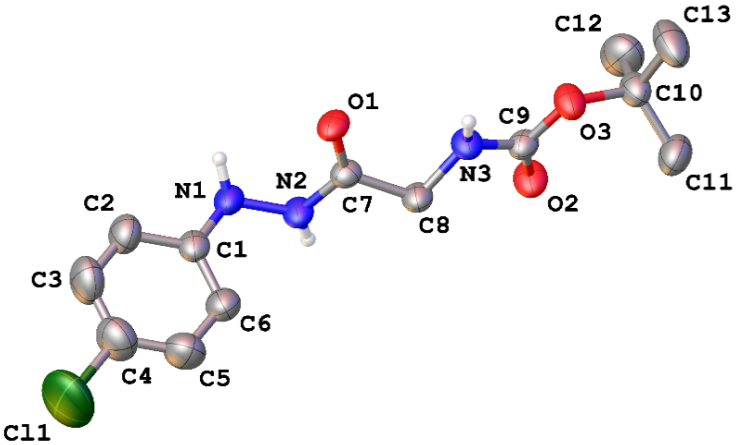
tert-Butyl-(S)-(1-(2-(2,4-difluorophenyl)hydrazinyl)-4-(methylthio)-1-oxobutan-2-yl)carbamate 67Cc





tert-Butyl-(2-(2-(4-chlorophenyl)hydrazinyl)-2-oxoethyl)carbamate 67Am

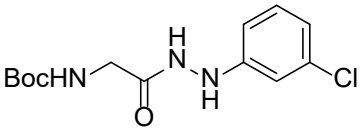


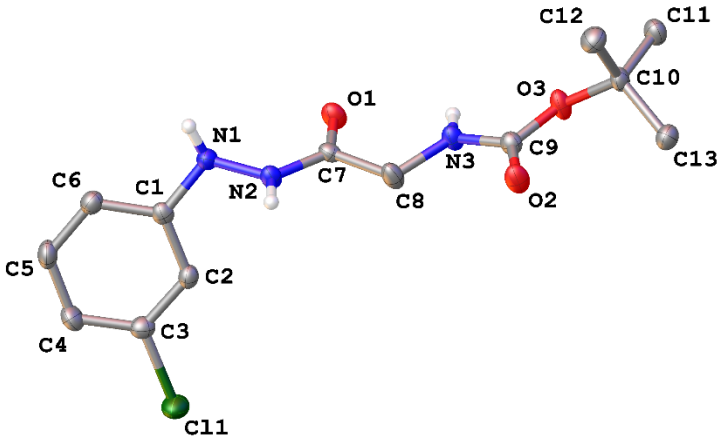


Compound	67Ca	67Cc	67Am
Empirical formula	$C_{16}H_{23}F_2N_3O_3$	$C_{16}H_{23}F_2N_3O_3S$	$C_{13}H_{18}ClN_3O_3$
Formula weight	343.37	375.43	299.75
Temperature/K	150.0(2)	150.0(2)	200.0(2)
Crystal system	orthorhombic	monoclinic	monoclinic
Space group	$P2_12_12_1$	$P2_1$	$P2_1/c$
a/Å	9.0949(2)	12.7631(5)	6.30898(15)
b/Å	10.4986(2)	5.04890(19)	18.1728(4)
c/Å	18.9737(5)	15.3008(7)	28.7539(10)
α/°	90	90	90
β/°	90	109.201(5)	92.489(3)
γ/°	90	90	90
Volume/Å^3	1811.68(7)	931.13(7)	3293.57(16)
Z	4	2	8
ρcalcg/cm^3	1.259	1.339	1.209
μ/mm^{-1}	0.857	1.901	2.150
F(000)	728.0	396.0	1264.0
Crystal size/mm^3	0.34 × 0.1 × 0.06	0.45 × 0.07 × 0.03	0.34 × 0.16 × 0.04
Radiation	CuKα (λ = 1.54184)	CuKα (λ = 1.54184)	CuKα (λ = 1.54184)
2Θ range for data collection/°	9.322 to 133.936	7.334 to 133.64	7.846 to 133.648
Index ranges	-8 ≤ h ≤ 10, -12 ≤ k ≤ 12, -22 ≤ l ≤ 22	-15 ≤ h ≤ 15, -5 ≤ k ≤ 5, -18 ≤ l ≤ 17	-4 ≤ h ≤ 7, -20 ≤ k ≤ 21, -33 ≤ l ≤ 34
Reflections collected	25502	7414	23718
Independent reflections	3206 [R_{int} = 0.0463, R_{sigma} = 0.0224]	2897 [R_{int} = 0.0441, R_{sigma} = 0.0439]	5839 [R_{int} = 0.0410, R_{sigma} = 0.0323]
Data/restraints/parameters	3206/0/232	2897/4/239	5839/442/422
Goodness-of-fit on F^2	1.046	1.065	1.034
Final R indexes [I>=2σ (I)]	R_1 = 0.0281, wR_2 = 0.0687	R_1 = 0.0382, wR_2 = 0.0899	R_1 = 0.0551, wR_2 = 0.1512
Final R indexes [all data]	R_1 = 0.0323, wR_2 = 0.0719	R_1 = 0.0440, wR_2 = 0.0954	R_1 = 0.0782, wR_2 = 0.1716

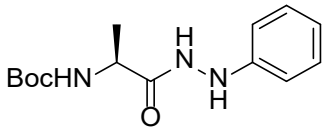
Largest diff. peak/hole/eÅ^{-3}	0.16/-0.13	0.23/-0.22	0.35/-0.31
Flack parameter	0.11(7)	0.00(3)	-

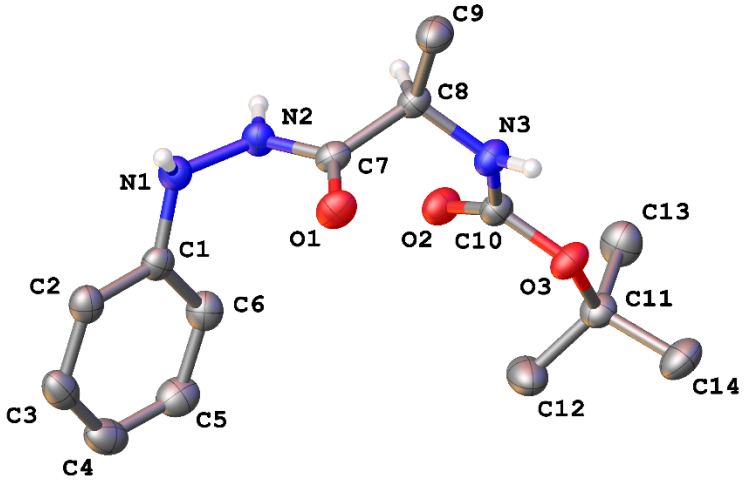
tert-Butyl (2-(2-(3-chlorophenyl)hydrazinyl)-2-oxoethyl)carbamate 67q



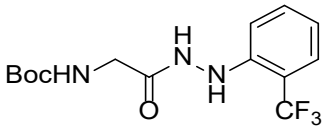


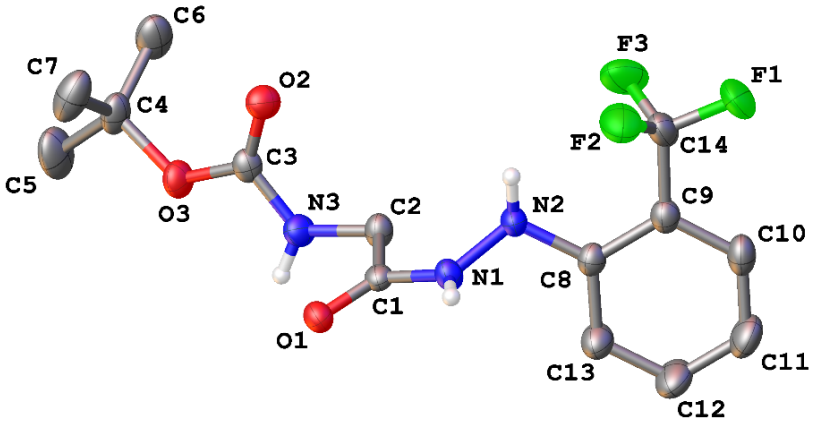
tert-Butyl (S)-(1-oxo-1-(2-phenylhydrazineyl)propan-2-yl)carbamate 67b





tert-Butyl (2-oxo-2-(2-(2-(trifluoromethyl)phenyl)hydrazineyl)ethyl) carbamate 67Ay

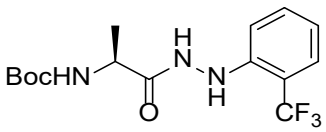


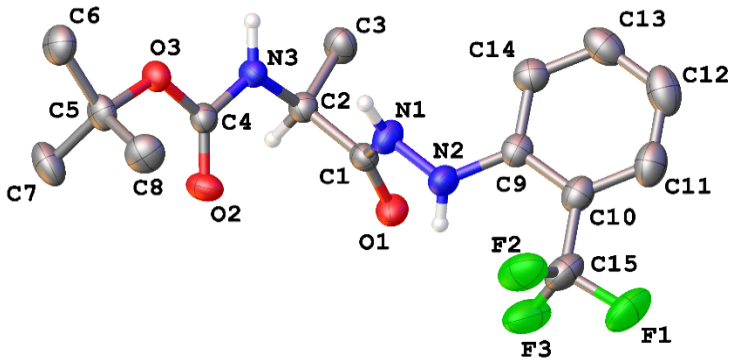


Compound	67q	67b	67Ay
Empirical formula	$C_{13}H_{18}ClN_3O_3$	$C_{14}H_{21}N_3O_3$	$C_{14}H_{18}F_3N_3O_3$
Formula weight	299.75	279.34	333.31
Temperature/K	150.0(2)	150.0(2)	150.0(2)
Crystal system	triclinic	orthorhombic	monoclinic
Space group	P-1	$P2_12_12_1$	$P2_1/n$
a/Å	5.6650(3)	9.1696(2)	7.3634(2)
b/Å	9.1347(4)	9.6329(3)	14.2093(4)
c/Å	14.1624(5)	17.2044(5)	15.6570(5)
α/°	91.485(3)	90	90
β/°	97.703(3)	90	102.288(3)
γ/°	102.737(4)	90	90
Volume/Å^3	707.23(5)	1519.66(7)	1600.64(8)
Z	2	4	4
ρcalcg/cm^3	1.408	1.221	1.383
μ/mm^{-1}	2.503	0.712	1.048
F(000)	316.0	600.0	696.0
Crystal size/mm^3	0.19 × 0.14 × 0.05	0.18 × 0.12 × 0.09	0.18 × 0.05 × 0.03

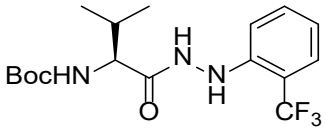
Radiation	CuKα (λ = 1.54184)	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	9.944 to 133.852	10.284 to 133.806	8.492 to 133.806
Index ranges	-6 ≤ h ≤ 6, -10 ≤ k ≤ 10, -16 ≤ l ≤ 16	-8 ≤ h ≤ 10, -11 ≤ k ≤ 11, -20 ≤ l ≤ 18	-6 ≤ h ≤ 8, -16 ≤ k ≤ 16, -18 ≤ l ≤ 18
Reflections collected	19294	10840	22137
Independent reflections	2502 [R_{int} = 0.0382, R_{sigma} = 0.0191]	2693 [R_{int} = 0.0523, R_{sigma} = 0.0380]	2835 [R_{int} = 0.0537, R_{sigma} = 0.0264]
Data/restraints/parameters	2502/0/194	2693/0/195	2835/0/221
Goodness-of-fit on F^2	1.026	1.068	1.043
Final R indexes [I>=2σ (I)]	R_1 = 0.0282, wR_2 = 0.0685	R_1 = 0.0362, wR_2 = 0.0907	R_1 = 0.0398, wR_2 = 0.1051
Final R indexes [all data]	R_1 = 0.0338, wR_2 = 0.0716	R_1 = 0.0409, wR_2 = 0.0963	R_1 = 0.0502, wR_2 = 0.1143
Largest diff. peak/hole/eÅ^{-3}	0.18/-0.19	0.13/-0.17	0.47/-0.34
Flack parameter	-	0.20(15)	-

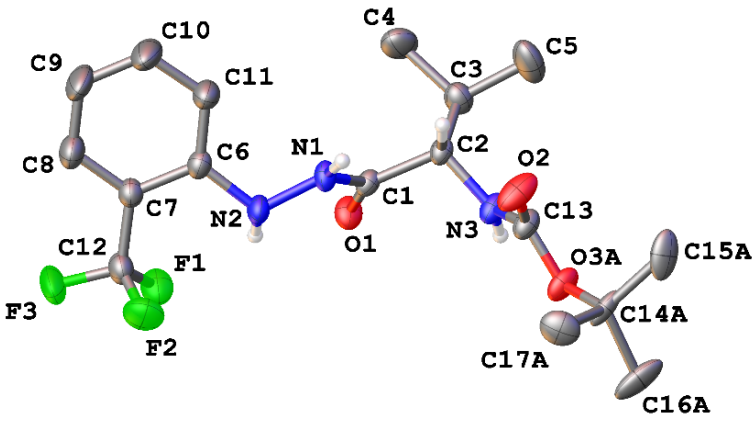
tert-Butyl (S)-(1-oxo-1-(2-(2-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate 67Az



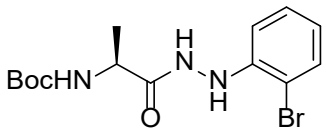


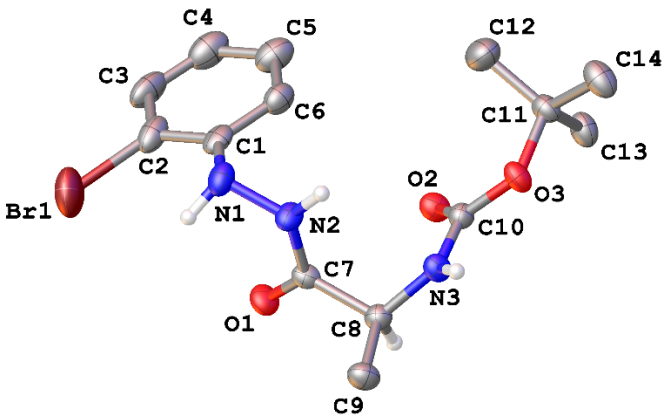
tert-Butyl (S)-(3-methyl-1-oxo-1-(2-(2(trifluoromethyl)phenyl)hydrazineyl) butan-2-yl)carbamate 67Ba





tert-Butyl (S)-(1-(2-(2-bromophenyl)hydrazineyl)-1-oxopropan-2-yl)carbamate 67Cf

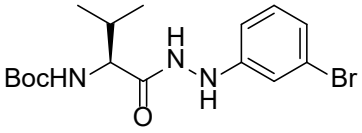


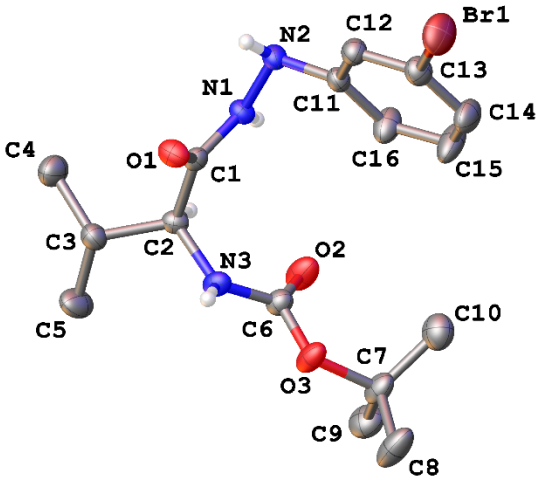


Compound	67Az	67Ba	67Cf
Empirical formula	$C_{15}H_{20}F_3N_3O_3$	$C_{17}H_{24}F_3N_3O_3$	$C_{29}H_{42}Br_2Cl_2N_6O_6$
Formula weight	347.34	375.39	801.40
Temperature/K	150.0(2)	150.0(2)	150.0(2)
Crystal system	Tetragonal	orthorhombic	orthorhombic
Space group	$P4_1$	$P2_12_12_1$	$P2_12_12_1$
a/Å	10.2512(2)	4.99930(10)	13.4621(3)
b/Å	10.2512(2)	19.5052(5)	16.4034(3)
c/Å	16.4278(7)	19.9425(5)	16.6639(3)
α/°	90	90	90
β/°	90	90	90
γ/°	90	90	90
Volume/Å^3	1726.35(10)	1944.64(8)	3679.79(12)
Z	4	4	4
ρcalcg/cm^3	1.336	1.282	1.447
μ/mm^{-1}	0.993	0.919	4.519
F(000)	728.0	792.0	1640.0
Crystal size/mm^3	0.26 × 0.2 × 0.06	0.28 × 0.15 × 0.06	0.38 × 0.08 × 0.06
Radiation	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)	CuKα (λ = 1.54184)
2Θ range for data collection/°	8.626 to 133.76	8.868 to 133.842	7.562 to 133.626
Index ranges	-12 ≤ h ≤ 11, -12 ≤ k ≤ 11, -19 ≤ l ≤ 18	-5 ≤ h ≤ 5, -23 ≤ k ≤ 23, -23 ≤ l ≤ 23	-13 ≤ h ≤ 16, -19 ≤ k ≤ 16, -19 ≤ l ≤ 14
Reflections collected	13849	20746	26356
Independent reflections	3026 [R_{int} = 0.0575, R_{sigma} = 0.0394]	3427 [R_{int} = 0.0625, R_{sigma} = 0.0373]	6503 [R_{int} = 0.0532, R_{sigma} = 0.0412]
Data/restraints/parameters	3026/1/231	3427/360/297	6503/373/447
Goodness-of-fit on F^2	1.064	1.079	1.052
Final R indexes [I>=2σ (I)]	R_1 = 0.0363, wR_2 = 0.0901	R_1 = 0.0374, wR_2 = 0.0924	R_1 = 0.0556, wR_2 = 0.1384
Final R indexes [all data]	R_1 = 0.0416, wR_2 = 0.0953	R_1 = 0.0419, wR_2 = 0.0966	R_1 = 0.0627, wR_2 = 0.1439

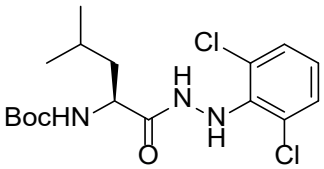
Largest diff. peak/hole/eÅ^{-3}	0.12/-0.13	0.15/-0.16	1.17/-0.82
Flack parameter	-0.14(11)	-0.07(8)	-0.023(15)

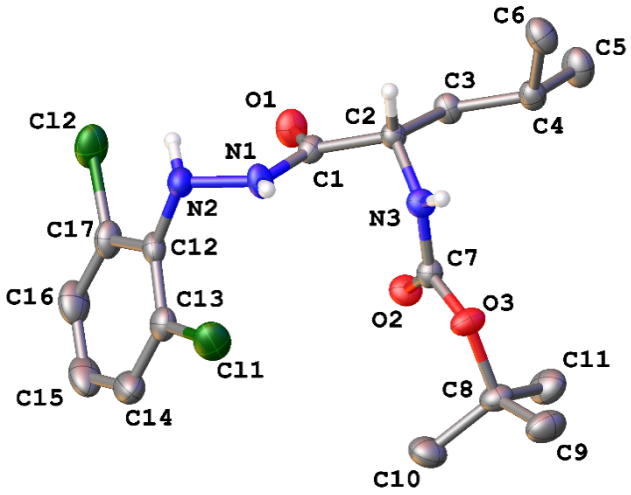
tert-Butyl (S)-(1-(2-(3-bromophenyl)hydrazineyl)-3-methyl-1-oxobutan-2-yl)carbamate 67Cm



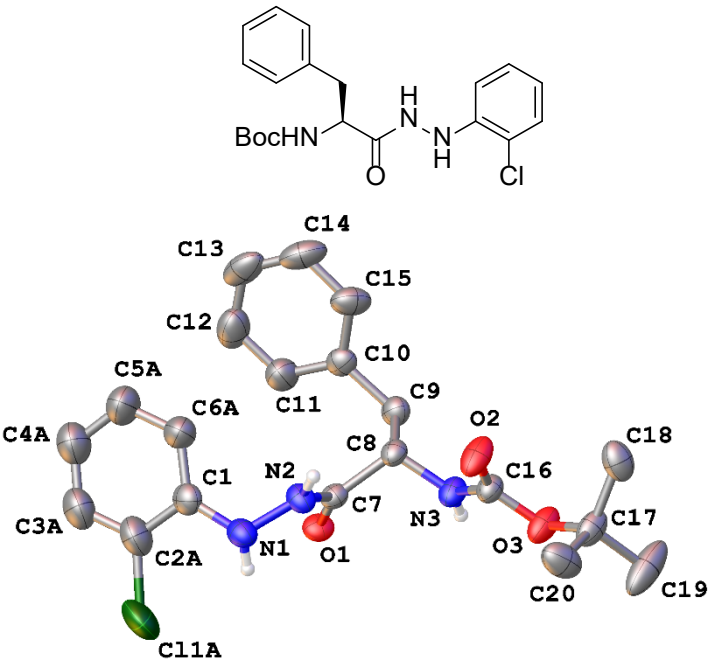


tert-Butyl (S)-(1-(2-(2,6-dichlorophenyl)hydrazineyl)-4-methyl-1-oxopentan-2-yl)carbamate 67Av

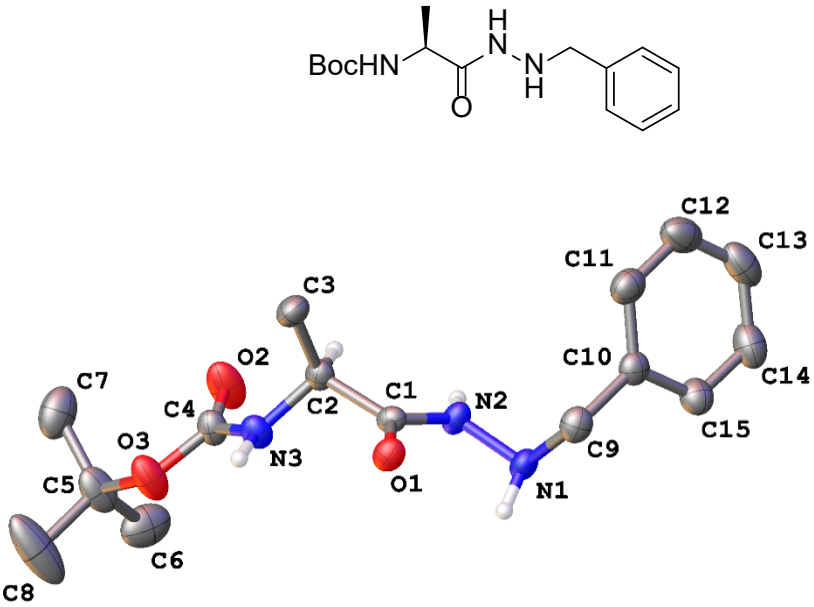




tert-Butyl (S)-(1-(2-(2-chlorophenyl)hydrazineyl)-1-oxo-3-phenylpropan-2-yl)carbamate 67Al



tert-Butyl (S)-(1-(2-benzylhydrazinyl)-1-oxopropan-2-yl)carbamate 91

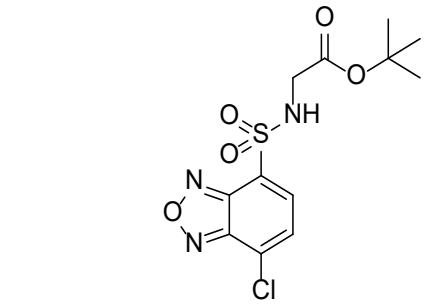


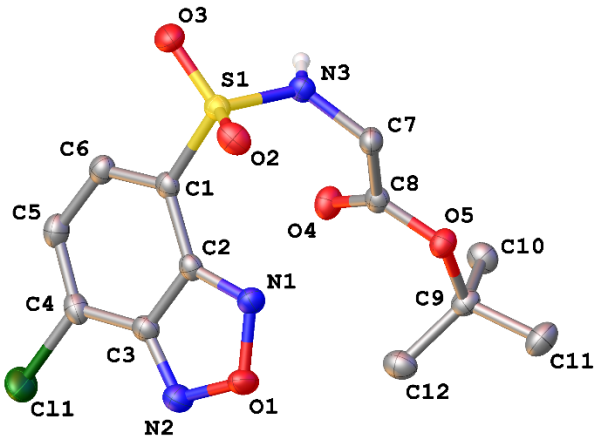
Compound	67Cm	67Av	67Al	91
Empirical formula	$C_{16}H_{24}BrN_3O_3$	$C_{17}H_{25}Cl_2N_3O_3$	$C_{20}H_{24}ClN_3O_3$	$C_{30}H_{46}N_6O_6$
Formula weight	386.29	390.30	389.87	586.73
Temperature/K	150.0(2)	150.0(2)	150.0(2)	150.0(2)
Crystal system	monoclinic	triclinic	orthorhombic	monoclinic
Space group	C2	P1	$P2_12_12_1$	$P2_1$
a/Å	25.7185(4)	9.2189(4)	5.0159(2)	10.1442(5)
b/Å	5.04190(10)	11.0840(4)	16.3832(7)	4.9901(3)
c/Å	15.0967(3)	11.7550(5)	24.6159(9)	15.7321(7)
α/°	90	62.645(4)	90	90
β/°	104.452(2)	71.907(4)	90	98.307(4)
γ/°	90	88.350(3)	90	90
Volume/Å^3	1895.65(6)	1004.81(8)	2022.85(14)	788.01(7)
Z	4	2	4	1
ρcalcg/cm^3	1.354	1.290	1.280	1.236
μ/mm^{-1}	3.099	3.076	1.876	0.709
F(000)	800.0	412.0	824.0	316.0
Crystal size/mm^3	0.2 × 0.09 × 0.04	0.21 × 0.18 × 0.14	0.32 × 0.11 × 0.05	0.43 × 0.1 × 0.03
Radiation	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)	CuKα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	7.098 to 133.61	8.99 to 133.526	7.182 to 133.716	8.81 to 133.146
Index ranges	-30 ≤ h ≤ 30, -5 ≤ k ≤ 5, -17 ≤ l ≤ 17	-10 ≤ h ≤ 10, -13 ≤ k ≤ 13, -13 ≤ l ≤ 13	-5 ≤ h ≤ 4, -19 ≤ k ≤ 19, -28 ≤ l ≤ 29	-12 ≤ h ≤ 12, -5 ≤ k ≤ 5, -18 ≤ l ≤ 18
Reflections collected	26168	26701	14161	10833
Independent reflections	3230 [R_{int} = 0.0504, R_{sigma} = 0.0228]	6773 [R_{int} = 0.0604, R_{sigma} = 0.0452]	3557 [R_{int} = 0.0553, R_{sigma} = 0.0450]	2639 [R_{int} = 0.0606, R_{sigma} = 0.0437]
Data/restraints/parameters	3230/4/222	6773/3/479	3557/425/311	2639/1/203
Goodness-of-fit on F^2	1.043	1.053	1.068	1.069

Final R indexes [I>=2σ (I)]	R_1 = 0.0261, wR_2 = 0.0665	R_1 = 0.0616, wR_2 = 0.1565	R_1 = 0.0431, wR_2 = 0.0953	R_1 = 0.0460, wR_2 = 0.1137
Final R indexes [all data]	R_1 = 0.0279, wR_2 = 0.0683	R_1 = 0.0671, wR_2 = 0.1647	R_1 = 0.0589, wR_2 = 0.1057	R_1 = 0.0558, wR_2 = 0.1256
Largest diff. peak/hole/eÅ^{-3}	0.31/-0.36	0.55/-0.39	0.26/-0.19	0.20/-0.19
Flack parameter	-0.020(11)	-0.01(2)	0.008(15)	-0.1(2)

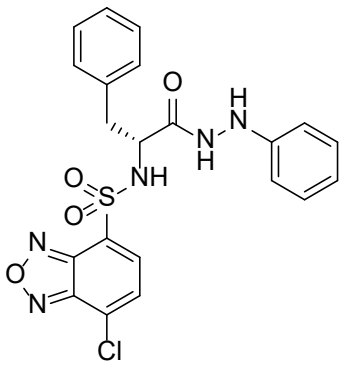
Crystal data and structure refinement for the resulting benzoxa-[2,1,3]-diazoles.

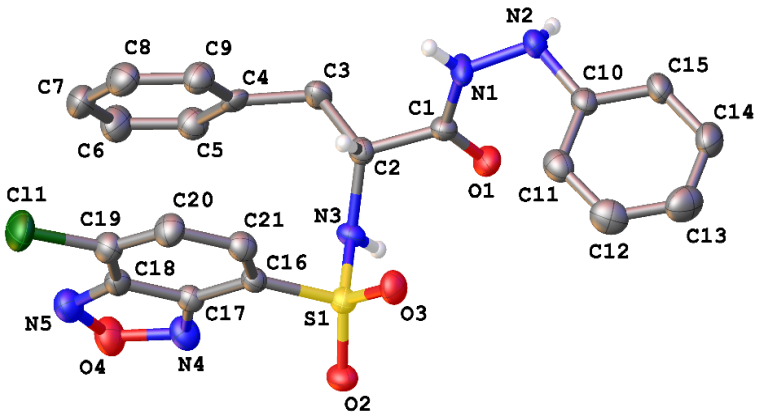
t-tert-Butyl ((7-chlorobenzo[c][1,2,5]oxadiazol-4-yl)sulfonyl)glycinate 63a





(R)-7-chloro-N-(1-oxo-3-phenyl-1-(2-phenylhydrazineyl)propan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide 65Ae





Compound	63a	65Ae
Empirical formula	$C_{12}H_{14}ClN_3O_5S$	$C_{21}H_{18}ClN_5O_4S$
Formula weight	347.77	471.91
Temperature/K	150.0(2)	150.0(2)
Crystal system	orthorhombic	triclinic
Space group	$Pca2_1$	P1
a/Å	11.2682(2)	7.4611(3)
b/Å	12.9378(3)	9.9630(4)
c/Å	10.1060(2)	14.5199(4)
α/°	90	87.046(3)
β/°	90	79.873(3)
γ/°	90	79.780(3)
Volume/Å^3	1473.32(6)	1045.43(7)
Z	4	2
ρcalcg/cm^3	1.568	1.499
μ/mm^{-1}	3.892	2.906
F(000)	720.0	488.0
Crystal size/mm^3	0.21 × 0.18 × 0.04	0.28 × 0.12 × 0.09
Radiation	CuKα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	6.832 to 133.55	9.022 to 133.668
Index ranges	$-13 \le h \le 11$, $-14 \le k \le 15$, $-11 \le l \le 12$	$-8 \le h \le 8$, $-11 \le k \le 11$, $-17 \le l \le 17$
Reflections collected	9999	27923
Independent reflections	2475 [R_{int} = 0.0372, R_{sigma} = 0.0305]	7049 [R_{int} = 0.0666, R_{sigma} = 0.0488]

Data/restraints/parameters	2475/1/206	7049/18/595
Goodness-of-fit on F^2	1.047	1.032
Final R indexes [I>=2σ (I)]	R_1 = 0.0263, wR_2 = 0.0608	R_1 = 0.0425, wR_2 = 0.1046
Final R indexes [all data]	R_1 = 0.0298, wR_2 = 0.0634	R_1 = 0.0484, wR_2 = 0.1112
Largest diff. peak/hole/eÅ^{-3}	0.21/-0.23	0.21/-0.35
Flack parameter	-0.004(10)	-0.008(14)