NOVEL INHIBITORS OF POLY ADENINE DIPHOSPHATE RIBOSE POLYMERASE TO POTENTIATE DNA REACTIVE DRUGS

A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

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To my Parents

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Preface

Declaration

This dissertation records the work carried out at the University of Newcastle-upon-Tyne between October 1991 and July 1994 and is original except where acknowledged by reference.

No portion of this work is being, or has been, submitted for a degree, diploma, or any other qualification at any other university.

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Finally, I would like to thank my parents for their love and support.

List of Abbreviations

Ac	Acetyl
ADP	Adenine diphosphate
AIBN	Azobisisobutyronitrile
ATP	Adenine triphosphate
^{t}Bu	*tert*butyl
^{i}Bu	*iso*butyl
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulphoxide
DTT	Dithiothreitol
EGTA	Ethylenebis(oxyethylenenitrilo)tetraacetic acid
EI	Electron impact
EtOH	Ethanol
FAB	Fast atom bombardment
h	Hours
KDa	Kilodaltons
Me	Methyl
m.p.	Melting point
NMR	Nuclear magnetic resonance
PADPRP	Poly adenine diphosphate ribose polymerase
PBS	Phosphate buffered saline
Ph	Phenyl
PPTS	*para*-Pyridinium toluenesulphonic acid
Pr	Propyl
TCA	Trichloroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV	Ultra violet

ABSTRACT

Poly (adenine diphosphate-ribosyl)ation of a variety of nuclear proteins is the immediate response in most eukaryotic cells to DNA strand breaks. This modification is catalysed by the chromatin bound enzyme Poly (ADP-ribose) polymerase (PADPRP). The enzyme is thought to be intimately involved in several cellular processes including cell differentiation, gene expression, transformation of oncogenes and repair of DNA damage. As a consequence, inhibitors of PADPRP are able to potentiate the cytotoxicity of many anti-tumour agents whose actions include DNA damage, and as such these inhibitors are potential resistance-modifying agents for use in cancer therapy.

In order to probe the active site of the enzyme a series of potential mimics (**i**) of NAD^+ were synthesised from readily available 3-hydroxybenzamide.

i ii iii

The conformation of the amide bond is considered to be important and for increased potency the amide carbonyl should be *anti* with respect to the nicotinamide C2-C3 bond. Based on this reported observation a series of novel inhibitors were synthesised, which include a series of benzoxazole-4-carboxamide analogues (**ii**) and 8-hydroxy-2-(substituted)quinazolin-4-one analogues (**iii**). The structure of the benzoxazole analogues are such that the amide is anchored into the required conformation by an

intramolecular hydrogen bond between the carboxamide N-H and the benzoxazole nitrogen.

The benzyloxybenzamide analogues had comparable potency to 3-hydroxybenzamide against PADPRP. However, both the benzoxazole-4-carboxamide and 8-hydroxy quinazolin-4-one series of analogues exhibited outstanding inhibitory activity against PADPRP. The most potent of the benzoxazole-4-carboxamide analogues (**ii**, R = phenyl) had an IC_{50} value of 2.1 μM. Exceptional PADPRP inhibitory activity was observed in the 8-hydroxyquinazolin-4-one (**iii**) series, where R = CH_3 (IC_{50} = 0.4 μM) and R = 4-nitrophenyl (IC_{50} = 0.2 μM). Further *in vitro* evaluation has shown that 8-hydroxy-2-methylquinazolin-4[3*H*]-one potentiates cytotoxicity in temozolomide treated cells.

CHAPTER ONE

1.0 INTRODUCTION

Cancer is the second most common cause of death after cardiovascular disease in developed countries, accounting for nearly 1 in 5 deaths.[1] The reported incidence of cancer has increased dramatically as other once fatal diseases, such as smallpox, have been virtually eradicated. As life expectancy increases so do the number of deaths linked to cancer.

Presently there are three major weapons against cancer: surgery, radiotherapy and chemotherapy, which can either be used alone or in combination. Surgery and radiotherapy are normally used to treat primary tumours. Chemotherapy is used to treat both primary and secondary metastatic cancers. Unfortunately, chemotherapy frequently fails because the tumour becomes resistant to the administered drug.

1.1. Resistance in Cancer Chemotherapy

Resistance may be either intrinsic or acquired.[2-7]

Intrinsic or primary resistance is when cells in the tumour do not respond to initial chemotherapy.

Acquired or secondary resistance arises when tumour cells respond initially to therapy but eventually tumour growth resumes. This type of resistance may be due to either a genetic or epigenetic change in these tumour cells.

Mechanisms of Resistance

Primary resistance is not completely understood as it is difficult to replicate under laboratory conditions. Therefore, it is assumed that the mechanisms of intrinsic resistance are similar to those of acquired resistance. Acquired resistance has been widely studied and there are many proposed mechanisms of acquired drug resistance which include:

Altered membrane transport: Many drugs are dependent on a cellular transport system for cell entry. Therefore, loss or inactivation of this system may lead to resistance. Also in the cell membrane there are a variety of plasma membrane proteins (P-glycoproteins) which are responsible for the export of lipophilic drugs from the cell. Overexpression of P-glycoproteins, particularly P-170, causes the efflux of large hydrophobic drugs, which results in non-specific multidrug resistance.

Altered drug activation: Many drugs are introduced into the body as prodrugs, that is, they require metabolic activation before their cytotoxic potential can be realised. For many prodrugs the activator is an enzyme. Thus, a decrease in the activating enzyme means that the drug will remain in the inactive prodrug form.

Altered drug-target complex: This encompasses a wide range of resistance mechanisms, which include:

a. A specific mutation in the target enzyme causing decreased affinity of the enzyme for the drug.

b. Overexpression of the target enzyme which will swamp the drug, making it difficult to achieve complete inhibition.

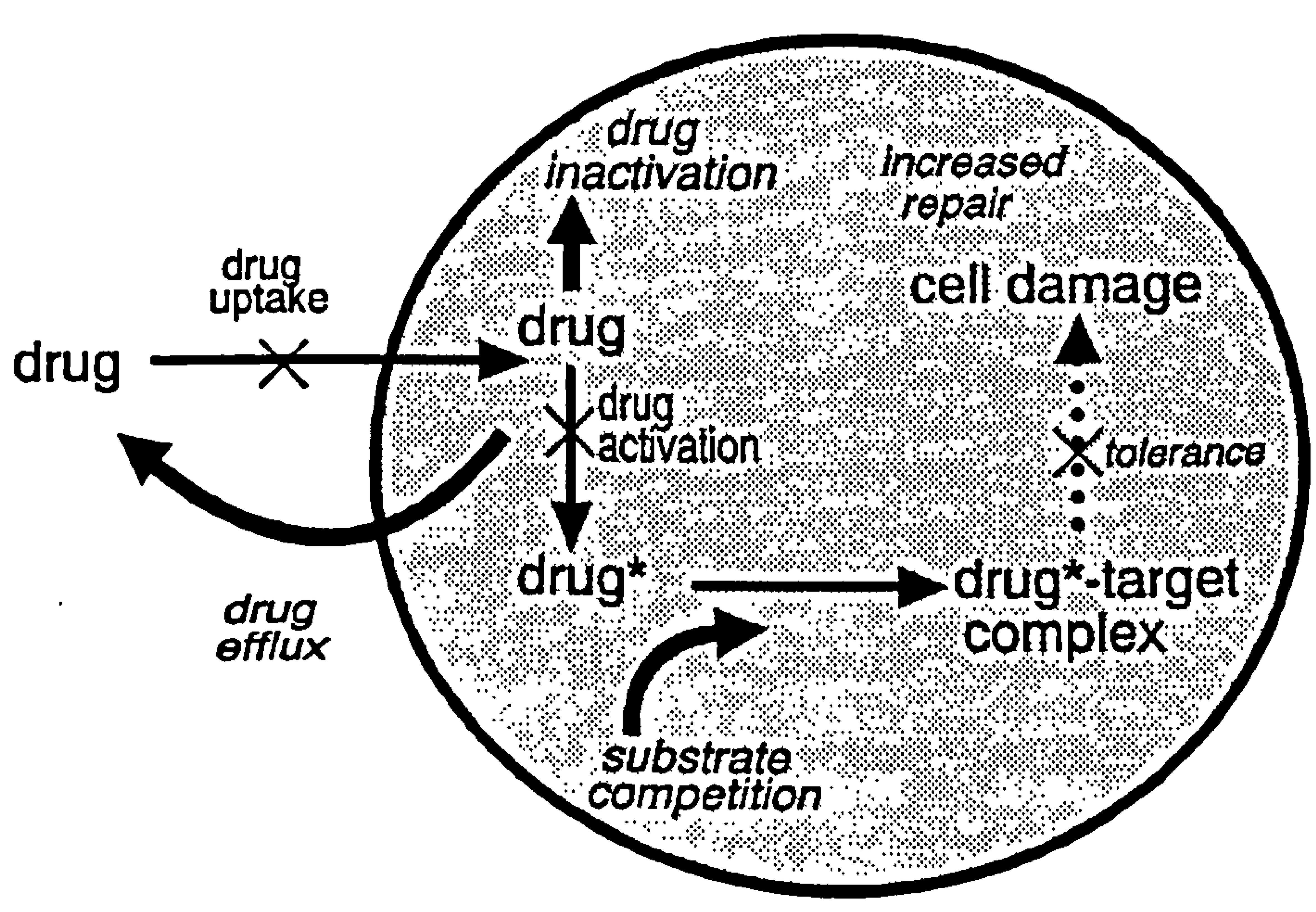
c. An increase in the normal substrate which will decrease the likelihood of the drug binding to the target enzyme.

Increased tolerance to the drug: Any route which allows the cell to circumvent the effect caused by the drug falls in this category. An example of this is the role of nucleoside transport in antimetabolite chemotherapy. Nucleoside uptake allows the cell to bypass blocks in the *de novo* purine and pyrimidine synthesis and evade the DNA synthesis block.

Enhanced DNA repair: Many antitumour drugs eventually kill the cell by DNA damage. The cell may counteract this increased DNA damage by increasing the amount of DNA repair enzymes present, thus, reducing the amount of damage done by the carcinostatic drug. There are multiple mechanisms for DNA repair, and several hundred genes are involved in this process.

As there are a number of anticancer drugs which interact with DNA, such as by intercalation and alkylation, it is likely that DNA repair is of significant value in the development of resistance.

Figure: 1.1.1. Cartoon showing simplified mechanisms of biochemical drug resistance



1.2. DNA Repair

Carcinogenic agents are involved in the disruption or mutation of DNA. After DNA damage the cell has three options: the damaged region may be repaired, deleted or retained. If the lesion is retained then the cell may die, or possibly become neoplastic. Generally, the cell will implement the appropriate repair mechanism and repair the damage.[2]

Mechanisms of Repair

DNA repair mechanisms fall into two broad categories: reversal of damage and excision repair.

Reversal of Damage:

O^6-Methylguanine DNA methyl transferase (MGMT) reverses DNA damage by removing covalent alkyl groups from the O^6 position of guanine, transferring the alkyl moiety to a cysteine residue in the active site, and hence repairing DNA.

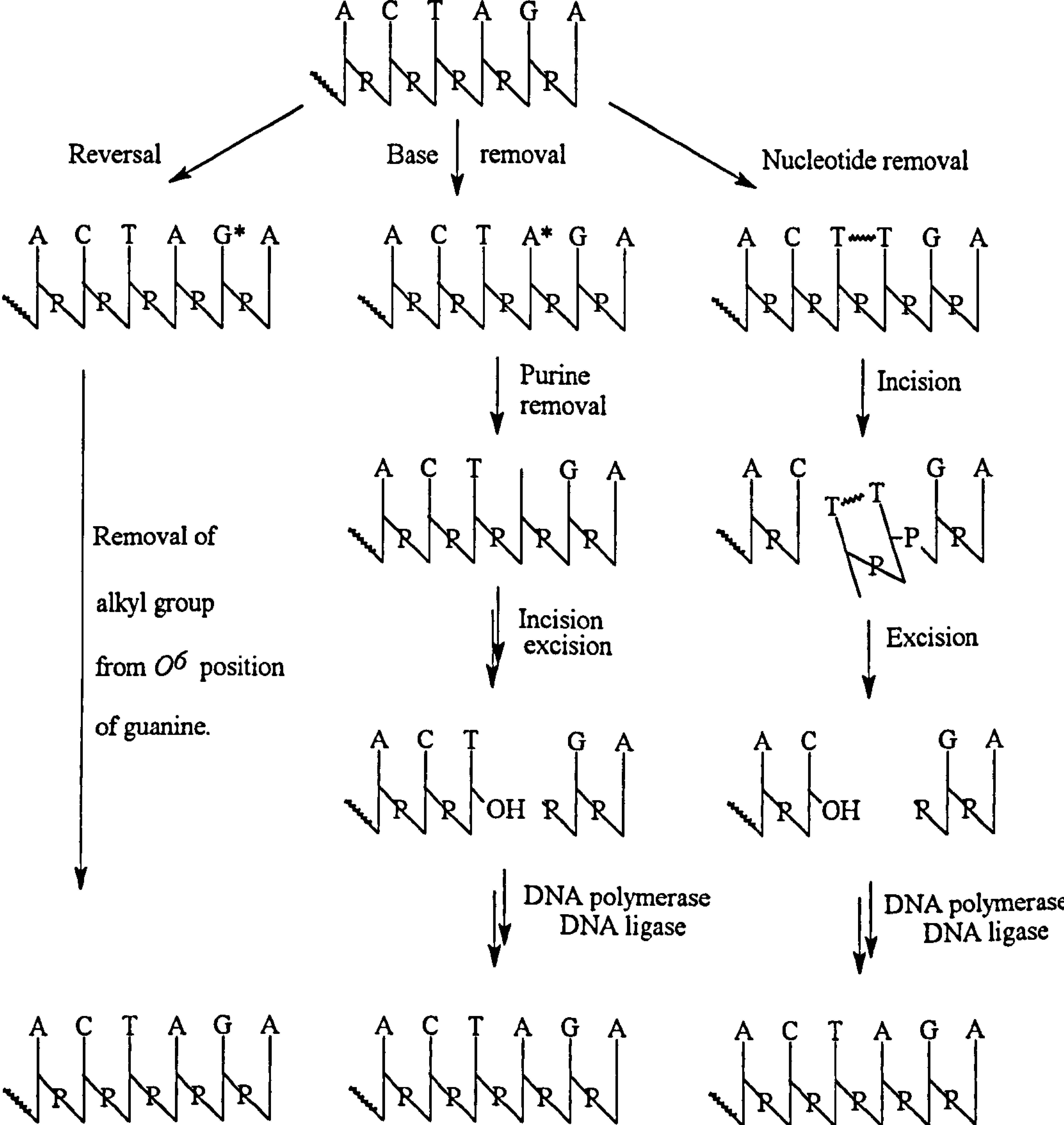
DNA ligase seals single DNA strand breaks which have no missing nucleotides and juxtaposed 3'-hydroxy and 5'-phosphate termini. Ligation has also been indicated as the final step in excision repair mechanisms.

Excision of Damage

Base excision repair and *nucleotide excision* repair mechanisms cover a host of different glycosylases, endonucleases and exonucleases, all of which are involved in the incision or excision of damaged DNA. In some cases, when only the base has been damaged, such as by alkylation of purines, then *glycosylases* excise the base by hydrolysis of the *N*-glycosidic bond. However, if the DNA has been altered by a change in conformation, such as occurs in the formation of pyrimidine dimers, then the damaged section will be removed as part of a small oligonucleotide.

Regardless of the method of excision the damage has not been repaired until the missing nucleotides are replaced. DNA *polymerases* replace the missing segment of DNA and the strand is re-annealed *via* the action of DNA *ligases*.

Figure: 1.2.1 Simplified mechanisms for DNA repair



There are many enzymes and proteins which have been indicated in post-incision events during excision repair. Although the principal proteins have been mentioned, there are other enzymes which may be intimately involved in repair of DNA *in vivo*. One of these is Poly (adenine diphosphate ribose)polymerase (PADPRP). Whilst PADPRP is apparently neither required for incision nor is involved in repair synthesis, inhibition of this enzyme nevertheless produces a synergistic increase in cell kill after treatment of cells with radiation or monofunctional alkylating agents.[8,9]

1.3. Resistance Modification

Resistance to chemotherapy is probably one of the most important therapeutic problems in medical oncology. Whilst there are many mechanisms of resistance and thus, many ways of overcoming resistance, exploitation of the increased DNA repair mechanisms in the cell may be one method of resistance modification. Research is currently underway into inhibition of the most studied of the repair proteins, MGMT. Treatment with an alkylating agent, whilst inhibiting MGMT, would render the cell incapable of repair, thus causing cell death. This strategy may be applied to, and indeed, has been applied to other DNA repair proteins. In ovarian cancer the drug aphidicolin, a potent inhibitor of both α and β DNA polymerases, can restore tumour sensitivity to L-phenylanine mustard and cisplatin in otherwise drug-resistant tumours.[4]

Similarly, suppression of PADPRP may have beneficial effects, since PADPRP is activated by DNA damage, particularly single strand breaks rather than DNA mutations. It is an ideal target for overcoming resistance in otherwise unresponsive tumours, by potentiating DNA damaging agents.

1.4. Poly (Adenine Diphosphate Ribose) Polymerase (PADPRP)

Poly (adenine diphosphate ribosyl)ation is a significant post translational modification involving the covalent attachment of long negatively charged homopolymers to a variety of nuclear proteins, particularly histones. This dramatically alters the chromatin structure, thus affecting functions such as gene expression,[10-12] DNA replication,[13] DNA repair,[14-16] differentiation[10, 11, 14] and apoptosis.[17, 18]

Historical Background

The enzyme poly (ADP-ribose) polymerase [EC 2.4.2.30] was discovered in the early 1960's by Mandel *et al*,[19] who observed that [^{14}C Adenine] ATP was incorporated into an acid soluble fraction of hen liver nuclei, and that this phenomenon could be stimulated by addition of nicotinamide mononucleotide. It was later confirmed by the same group, that the isolated product was a homopolymer of repeating (ADP) ribose units.[20] Since this discovery there has been much interest in PADPRP as to its function in the eukaryotic cell and its effects on DNA and gene expression.[9-11, 18, 21, 22]

Enzyme Structure

PADPRP is a highly conserved nuclear enzyme of 116 Kda (1014 amino acids) and is present in most eukaryotic cells, including insects and lower order eukaryotes. By limited proteolysis the enzyme was found to contain three major domains.[23]

The *46Kda amino terminal domain* is highly charged and contains two homologous sequences (2-97 and 106-207) which form two zinc fingers. It is these zinc fingers which are responsible for binding the enzyme to DNA strand breaks.[24, 25]

The *22Kda central domain* contains the automodification sequence and is rich in lysine and glutamic acid. Glutamic acid residues have been shown to be the primary automodification sites, hence there is a perfect coincidence between the number of glutamic acid residues in this domain and the number of automodification sites identified.

The *54Kda carboxyl terminal domain* is the most highly conserved domain containing the carboxyl terminal region of PADPRP. The NAD^+ binding site is situated within a strictly conserved region in this domain This region has 99 % conservation between vertebrates and 92 % conservation between all species, and is known as the 'PADPRP signature'.[26]

1.5. Biosynthesis of the Polymer

PADPRP catalyses the transfer of ADP ribose from NAD^+ (Figure: 1.5.1.) to a nuclear protein acceptor, generating mono, oligo and poly (ADP)ribose tails (Figure: 1.5.2).

Figure. 1.5.1. Nicotinamide adenine dinucleotide

Figure 1.5.2. Metabolism of (ADP) ribose polymers

ADP ribosyl protein lyase

Protein acceptor

Poly (ADP-ribose) Polymerase

Branched chain

Poly (ADP-ribose) Glycohydrolase

Phosphodiesterase

Poly (ADP-ribose) Polymerase

Mechanism of Action

Cleavage of the nicotinamide-ribose bond may be facilitated by donation of the adjacent ribose oxygen electron lone pair. This type of assistance has been well documented for the lysozyme mediated hydrolysis of bacterial cell walls. Lysozymes, are widespread in biological tissues and secretions. Their exact function is still not clear, but their mechanism of action has been extensively studied.[27] Phillips proposed a mechanism based on structural studies of lysozyme and a knowledge of acetal hydrolysis.[28]

After the lysozyme has bound to the bacterial cell wall, the glycoside involved in the hydrolysis reaction is distorted into a sofa conformation, and Glu-35 donates a proton to the exocyclic oxygen. Cleavage of the C-O-R_1 moiety is assisted by donation from the neighbouring oxygen forming a resonance stabilised oxonium species. During hydrolysis the negatively charged Asp-52 acts to stabilise the oxonium species through a charge-charge interaction. Finally, water is added and Glu-35 becomes reprotonated, with subsequent release of the hemiacetal from the enzyme active site completing the process (Figure: 1.5.3.).

This reaction mechanism can be considered because PADPRP hydrolyses the nicotinamide-ribose bond in a manner comparable to that of lysozyme hydrolysis. Slama and Simmons proposed that cleavage of the pyridinium ribose bond is facilitated by an analogous assistance from the ribose oxygen.[29, 30] Such assistance may be achieved by the ribose adopting an envelope conformation, thus maximising orbital overlap from a lone pair of electrons in the pseudoaxial position on oxygen with the σ^* orbital on carbon to form an sp^2 hybridised oxonium species. This would facilitate C-N bond cleavage with loss of nicotinamide (Figure: 1.5.4.).

Figure: 1.5.3. Proposed mechanism of lysozyme hydrolysis

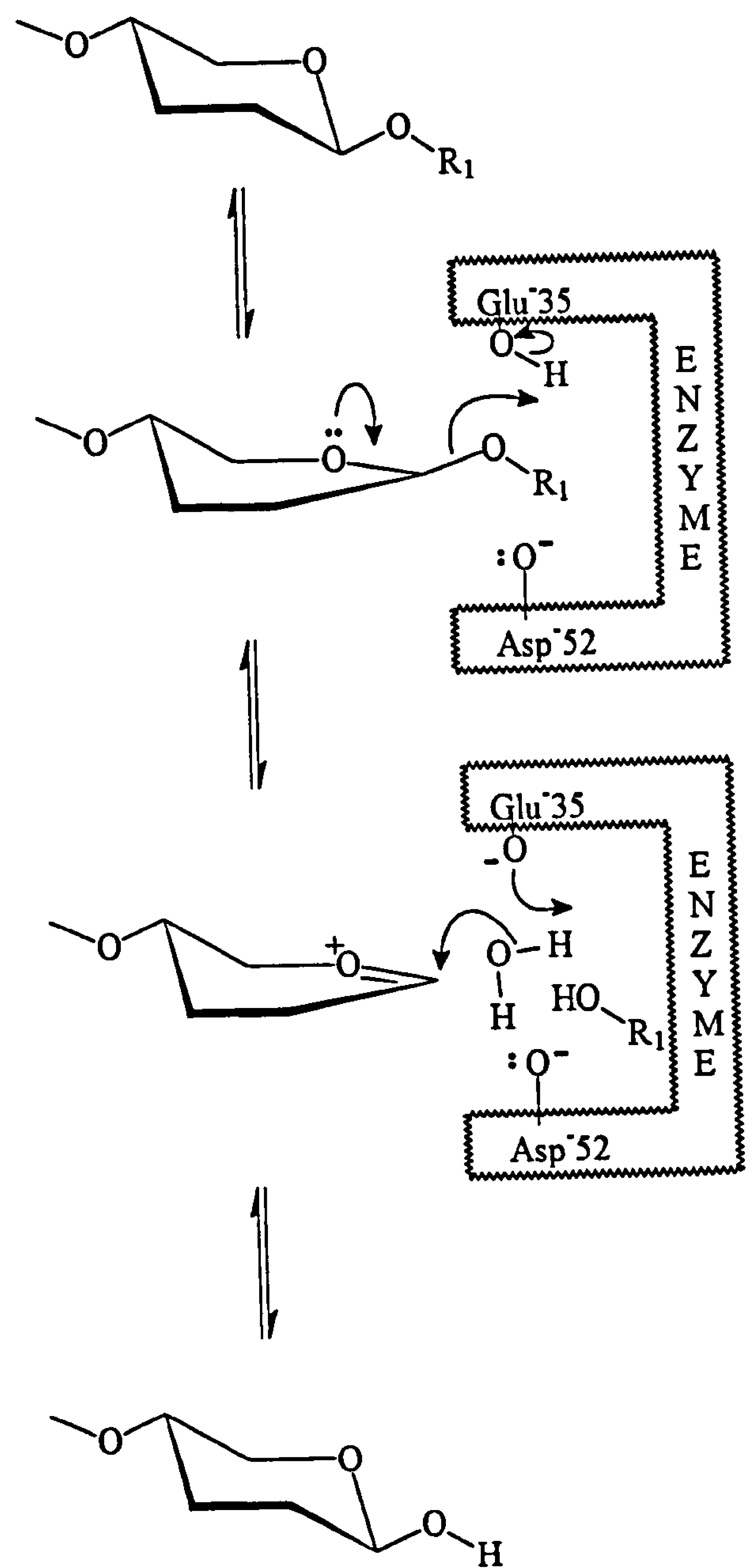


Figure 1.5.4. Proposed S_N1 mechanism of PADPRP-catalysed NAD^+ cleavage

Schuber *et al* have been exploring the mechanism of NAD^+ glycohydrolases, and have chemical evidence in favour of a stabilised oxonium intermediate in the NAD^+ glycohydrolase catalysed reactions.[31] The authors compared the effects of solvent on the rate of spontaneous solvolysis of NAD^+ with that of enzyme catalysed solvolysis, in order to determine whether NAD^+ glycohydrolase stabilises the ADP-

ribosyl species. Their findings confirm the work of Jencks and Young [31] in that the oxonium ion would be too unstable to exist as a free solvent-equilibrated intermediate, and that in the example of non-catalysed solvolysis there may be an enforced preassociated mechanism where the oxonium species is stabilised by the leaving nicotinamide and the attacking solvent molecules in an S_N2 type reaction. Conversely, the enzyme catalysed solvolysis studies indicate that the mechanism of oxonium ion formation is by an S_N1 style mechanism in which the oxonium ion is stabilised by the glycohydrolase. The stabilisation effects are considerable because the lifetime of the oxonium-enzyme species is long enough to allow the diffusion of nicotinamide out of the active site before nucleophilic attack at the carbon centre, because the stereochemistry of solvolysis is with retention of configuration.[31]

Catabolism of the Polymer

There are two main enzymes involved in the breakdown of (ADP) ribose polymers. PADPRP *glycohydrolase* degrades the ribose-ribose bond in both linear and branched chains from the 2' position,[32-32] and a eukaryotic *phosphodiesterase* attacks the pyrophosphate bond in the polymers.[35, 36] Once the long (ADP-ribose) polymers have been degraded, (ADP)ribose protein *lyase* is responsible for removal of the final (ADP)ribose moiety from an automodified polymerase, thus releasing the enzyme and allowing it to reassociate with other DNA strand breaks.[37]

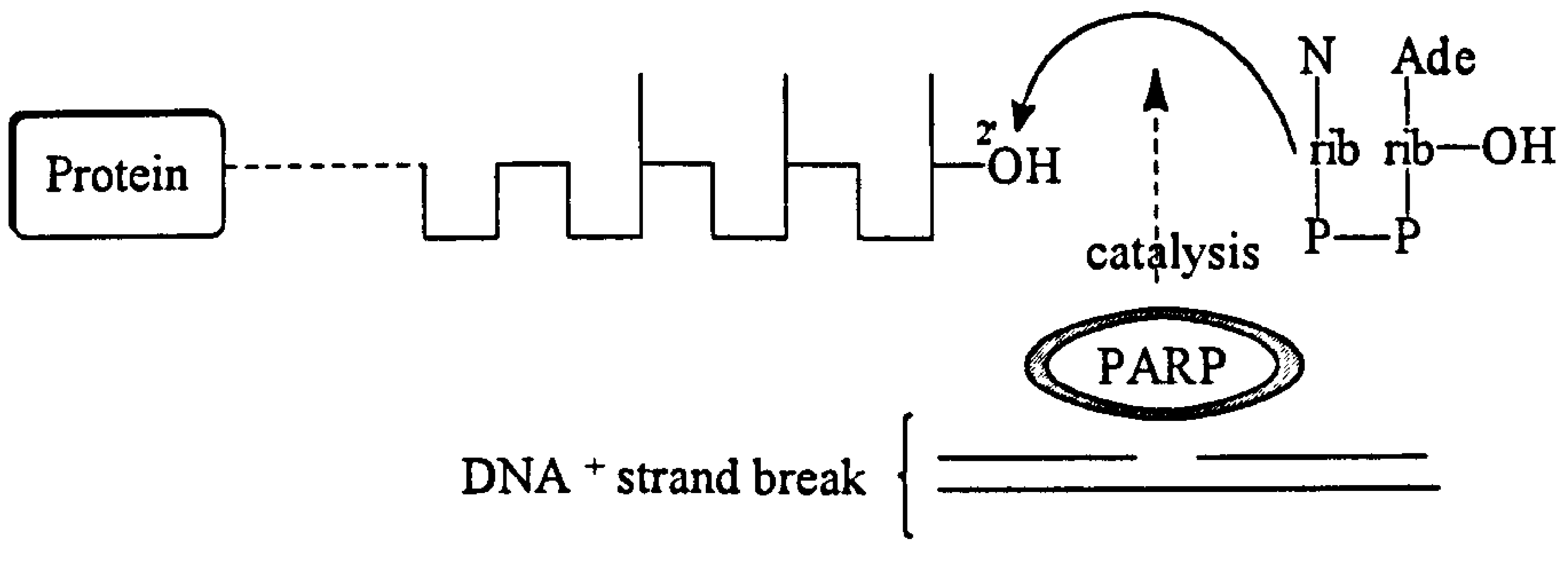
1.6. Mechanisms of Polymer Growth

There are two possible mechanisms for the progressive addition of the polymer tail, either distal or proximal depending upon the acceptor protein. If the acceptor is any other protein but PADPRP itself then distal addition may occur. However, if PADPRP is the acceptor, and as such is being automodified, then the proximal model may possibly take precedence.

Distal Addition

Polymerisation of a single ADP ribose residue added to the free 2' OH will build up the polymer. This kind of polymer addition occurs for (ADP ribosyl)ation of most nuclear protein acceptors.[9]

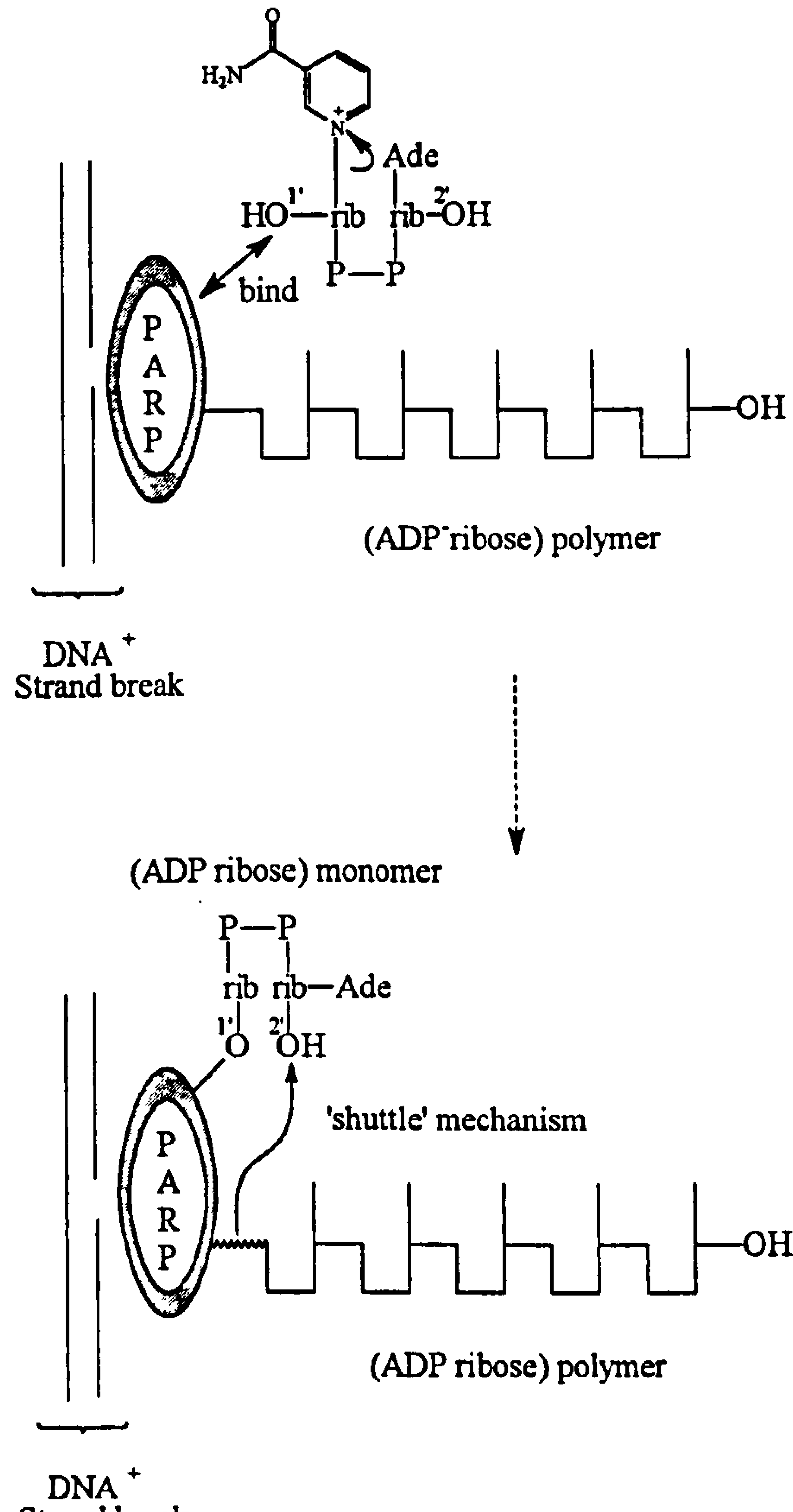
Figure: 1.6.1 Distal addition



Proximal Addition

If PADPRP underwent automodification *via* a distal type mechanism, then a second PADPRP and glycohydrolase may compete for the same 2'-OH site. The proximal model shows polymer growth *via* the addition of the ADP-ribose unit initially binding from the 1'-OH position of NAD^+ to the NAD^+ binding site in PADPRP. Subsequent transfer of the existing polymer to the monomer's 2'-OH position builds up the homo polymer tail, thus forming a shuttle type mechanism between two different sites on one polymerase.[38, 39]

Figure: 1.6.2. Proximal addition



As yet the exact mechanism is not known and these postulated mechanisms are still under considerable debate. Alvarez-Mendoza *et al.* have postulated that two PADPRP monomers are required for the progression of automodification, which infers that it is an intermolecular process rather than an intramolecular process.[40]

1.7. Activation and Binding of PADPRP

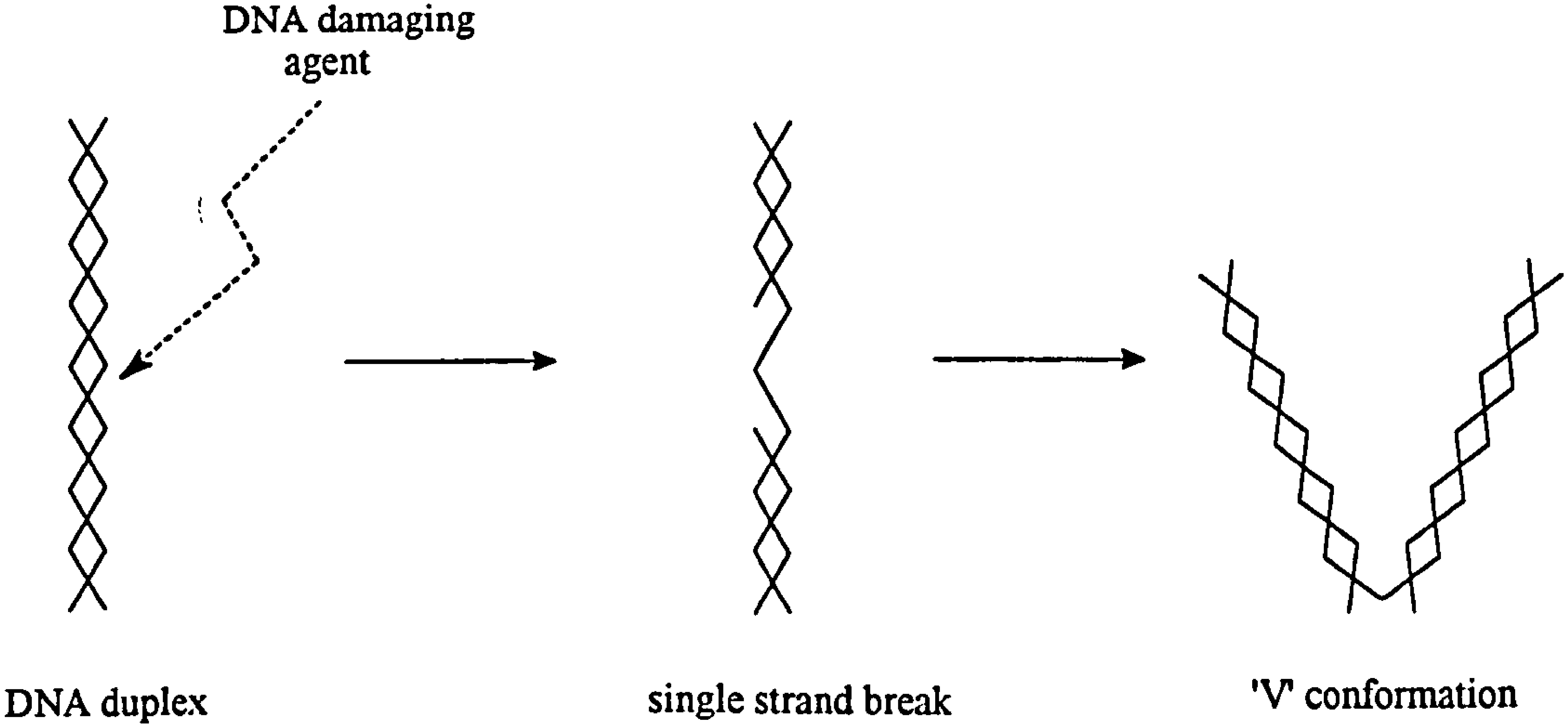
PADPRP has been shown to bind specifically to DNA strand breaks, but with no apparent sequence specificity.[41, 42] Ménissier de Murcia *et al* found, *via* an elegant series of experiments, that the two zinc fingers located in the amino terminal domain are responsible for the polymerase binding to DNA. Specific site mutagenesis in the gene which codes for PADPRP, to remove one of the zinc fingers, results in association with DNA double strand breaks only. Loss of both zinc fingers completely, prevents DNA binding and blocks activation of the enzyme by DNA strand breaks.[42]

Although PADPRP binds to DNA strand breaks in a zinc dependent manner,[41] the actual mechanism of binding and ribosylation is still unclear. Recently, de Murcia and Ménissier de Murcia highlighted the binding specificity of PADPRP during automodification.[26]

Automodification of PADPRP

When single strand breaks occur in the DNA duplex a certain amount of flexibility is introduced, and the structure adopts a 'V' conformation (Figure: 1.7.1). PADPRP binds specifically to the centre of the V, as observed by electron microscopy,[43] in a manner reminiscent of specific PADPRP binding to DNA cruciforms[44] and crossovers[45] (Figure: 1.7.2). The authors propose that the specific angle formed by the V may assist PADPRP binding. Also, the nick-induced flexibility may enable two PADPRP monomers to associate, one either side of the V shaped DNA strand break, thus initiating automodification. The possible role of automodified PADPRP in the cell nucleus has been investigated recently.

Figure: 1.7.1. V conformation adopted by DNA with a single strand break

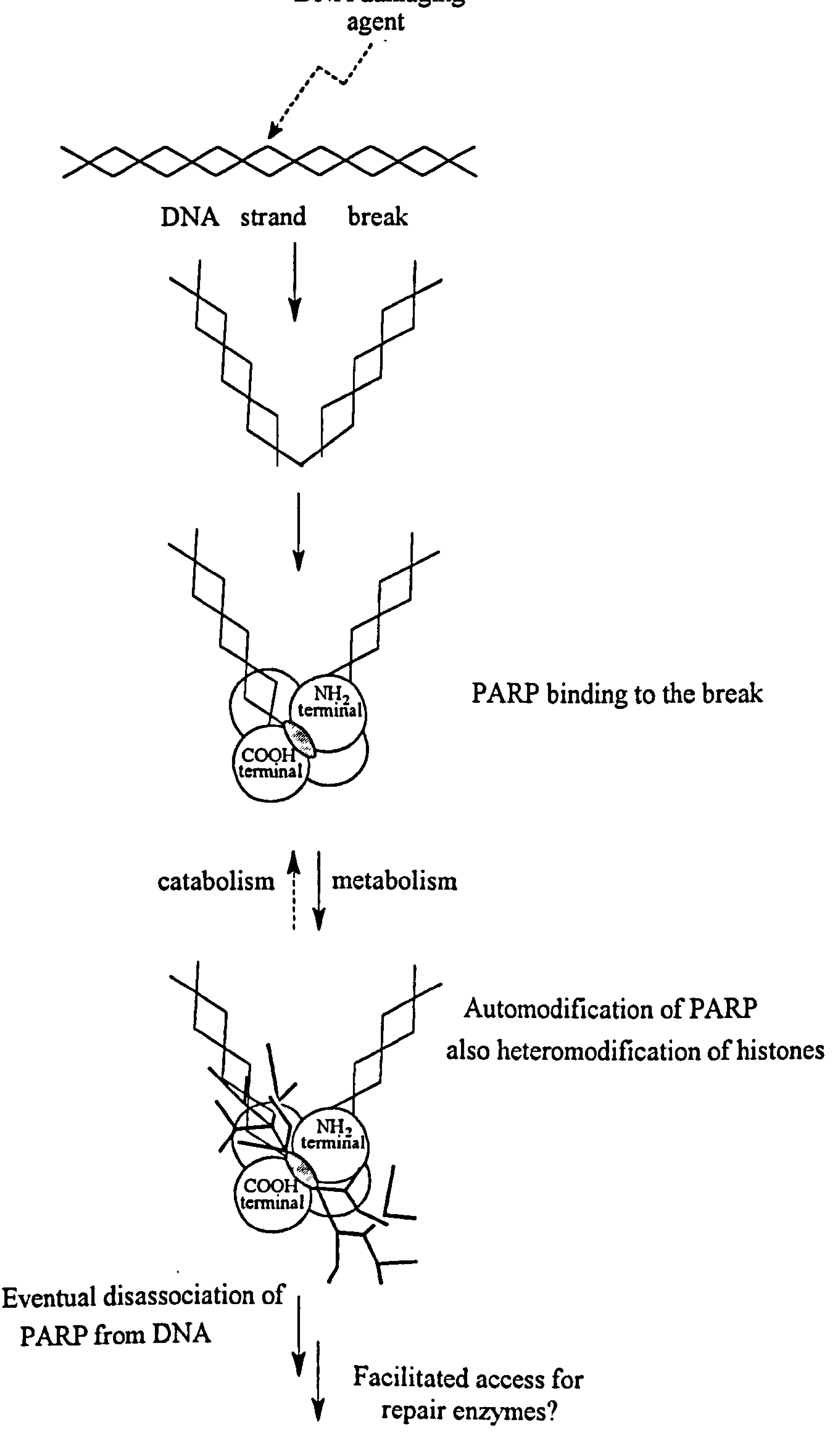


1.8. Biological Function of Poly (ADP ribosyl)ation

Automodification and heteromodification of nuclear proteins, such as histones, generally inhibits their function. As PADPRP is intimately associated with chromatin the effects of such inhibition have been indicated in DNA replication,[13] DNA repair,[14-16] cell survival,[17, 18] gene expression and transformation.[10, 11]

Before the DNA can be accessed by the many proteins and enzymes, the chromatin needs to be unravelled. A cell nucleus 8μm in diameter contains on average 5 billion base pairs which total approximately 1.8 m in length; hence the DNA must be organised into several tiers. Initially the DNA is organised into nucleosomes which condense to form chromatin.

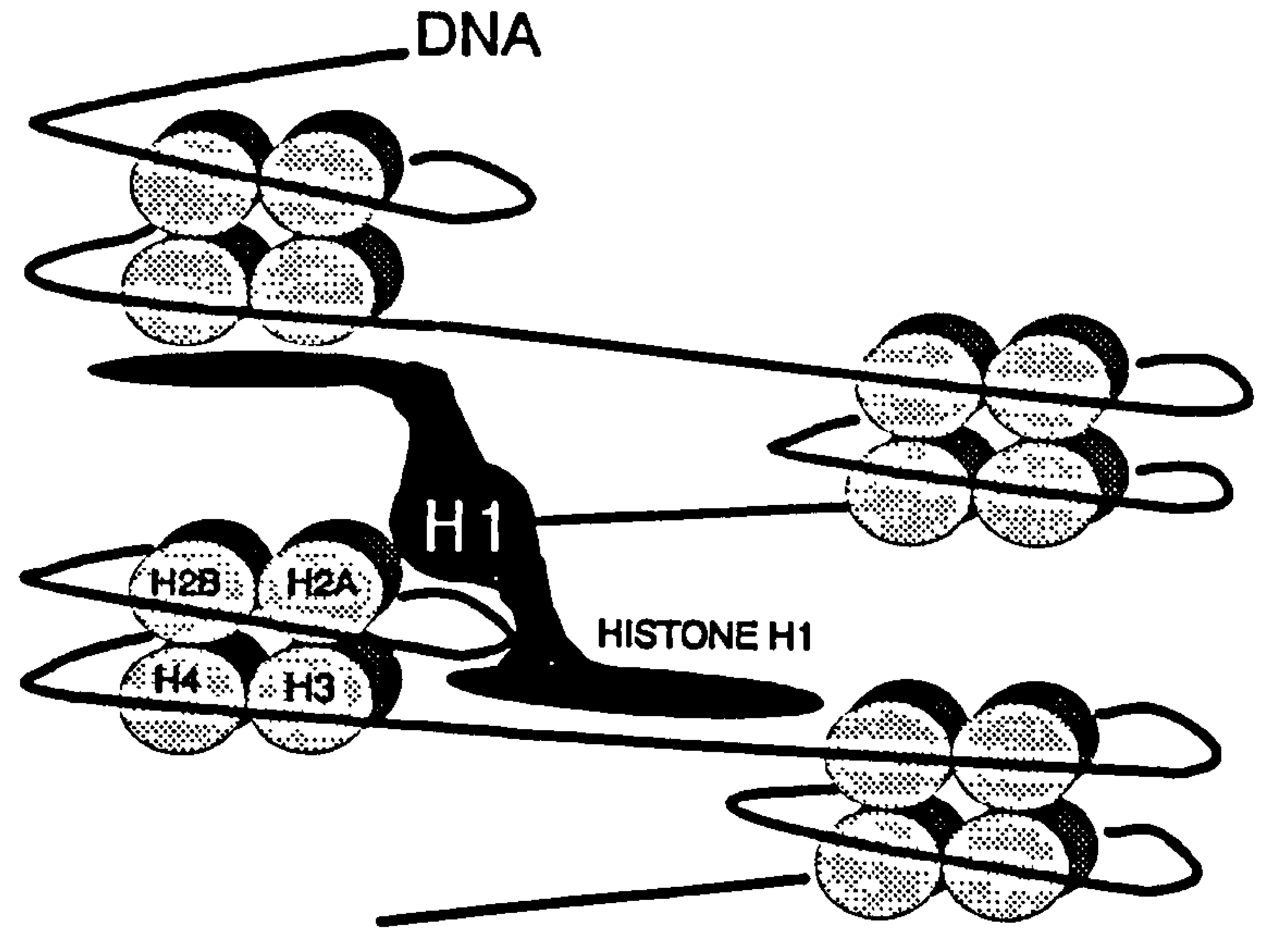
Figure: 1.7.2. Binding of two PADPRP monomers to a DNA strand break[26]



Nucleosomes consist of nine nuclear proteins known as histones. Histones are basic proteins and can be classified according to amino acid content, marginally lysine rich (H2A and H2B), marginally arginine rich (H3 and H4) or exceptionally lysine rich (H1). All the histones appear to be significantly conserved between eukaryotic species, which implies that they have a specific role. Histones H2A and H2B form dimers whilst 2 x (H3 and H4) form tetramers. These building blocks form the core of the nucleosome, with the DNA being wrapped around the surface of the octamer.

The histone H1 seals the nucleosome where the DNA enters and leaves, thus protecting 166 base pairs of DNA. The nucleosomes are further condensed to form chromatin. Chromatin is held together by the interaction of histone H1 with the core histones of neighbouring nucleosomes.

Figure: 1.8.1. Representation of chromatin



The structure and modification of chromatin largely determines replication, repair, gene expression and other genomic processes. Histones, the nuclear matrix and indeed PADPRP itself are all acceptors for the (ADP) ribose polymer, but the combined effects of (ADPribosyl)ation on these proteins have yet to be elucidated. (ADP ribosyl)ation of histone H1 is particularly important since this protein not only closes its own nucleosome, but is responsible for the condensed state of the chromatin. Poirer *et al* observed that poly (ADP ribosyl)ation of histone H1 did relax chromatin, and this was confirmed by the inability of chromatin to condense to higher order structures *in vitro*, after poly (ADP ribosyl)ation.[46, 47]

Automodification of PADPRP to form long homopolymeric chains may cause the histones to be pulled off the DNA and to associate with the polymer-polymerase-DNA complex, especially as histones H3 and H4, have an equal or higher affinity for (ADP) ribose branched polymers than for DNA alone.[26] Once the polymers reach a critical size the automodified PADPRP dissociates from the DNA strand break and the polymer is degraded. The histones, having lost the electrostatic attraction become reassociated with the DNA. This is known as histone shuttling. Heteromodification of histones adds a large negative charge which causes them to be repelled from DNA. Eventually PADPRP glycohydrolase degrades the (ADP) ribose polymers on the histones, thus reducing the negative charge. Consequently the histones become reassociated with DNA to reform the original structure of the nucleosome. This loosening of the chromatin structure may allow access of repair enzymes by either heteromodification or histone shuttling and automodification.[48-50]

Figure: 1.8.2a. Possible involvement of automodified PADPRP in histone shuttling[49]

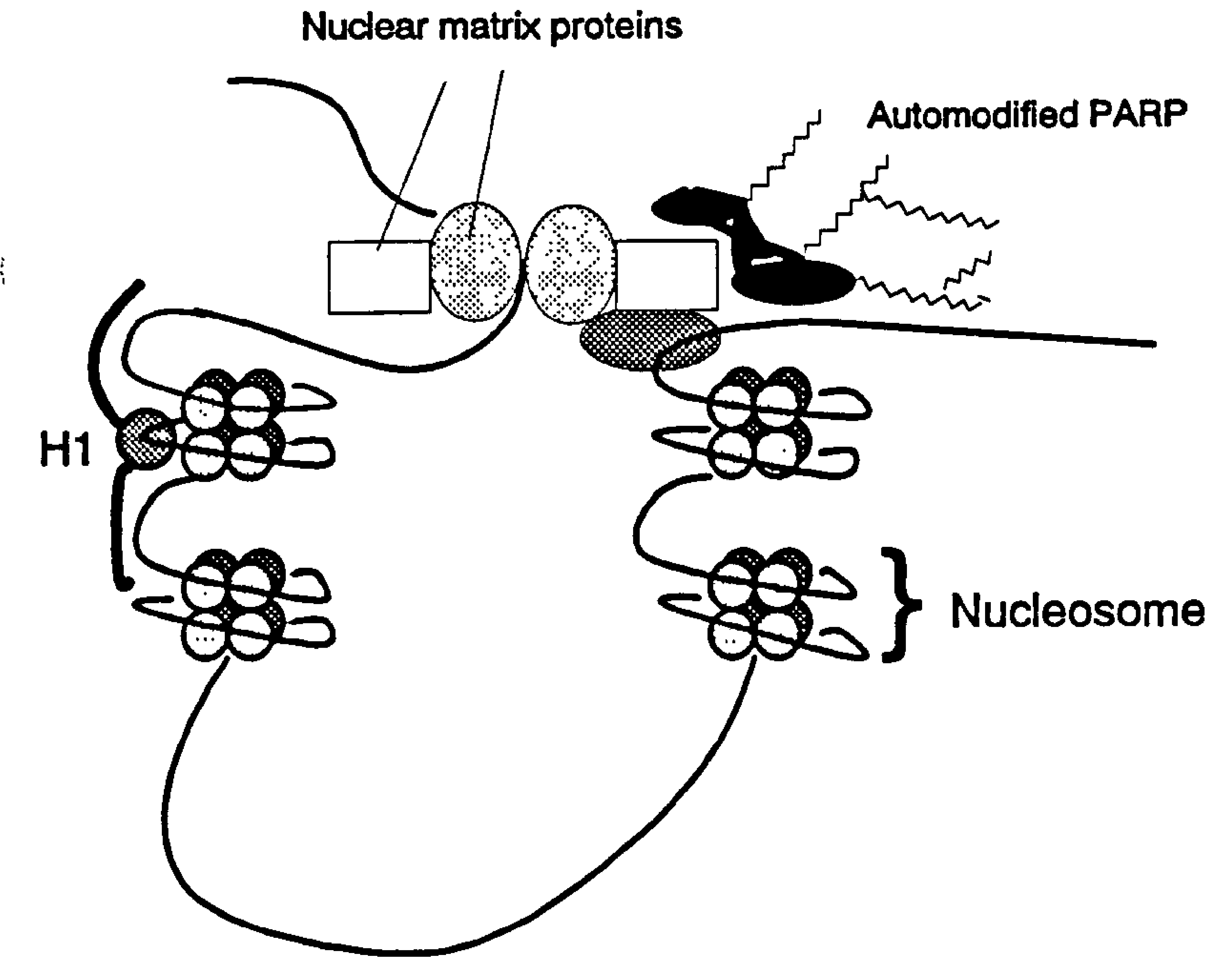
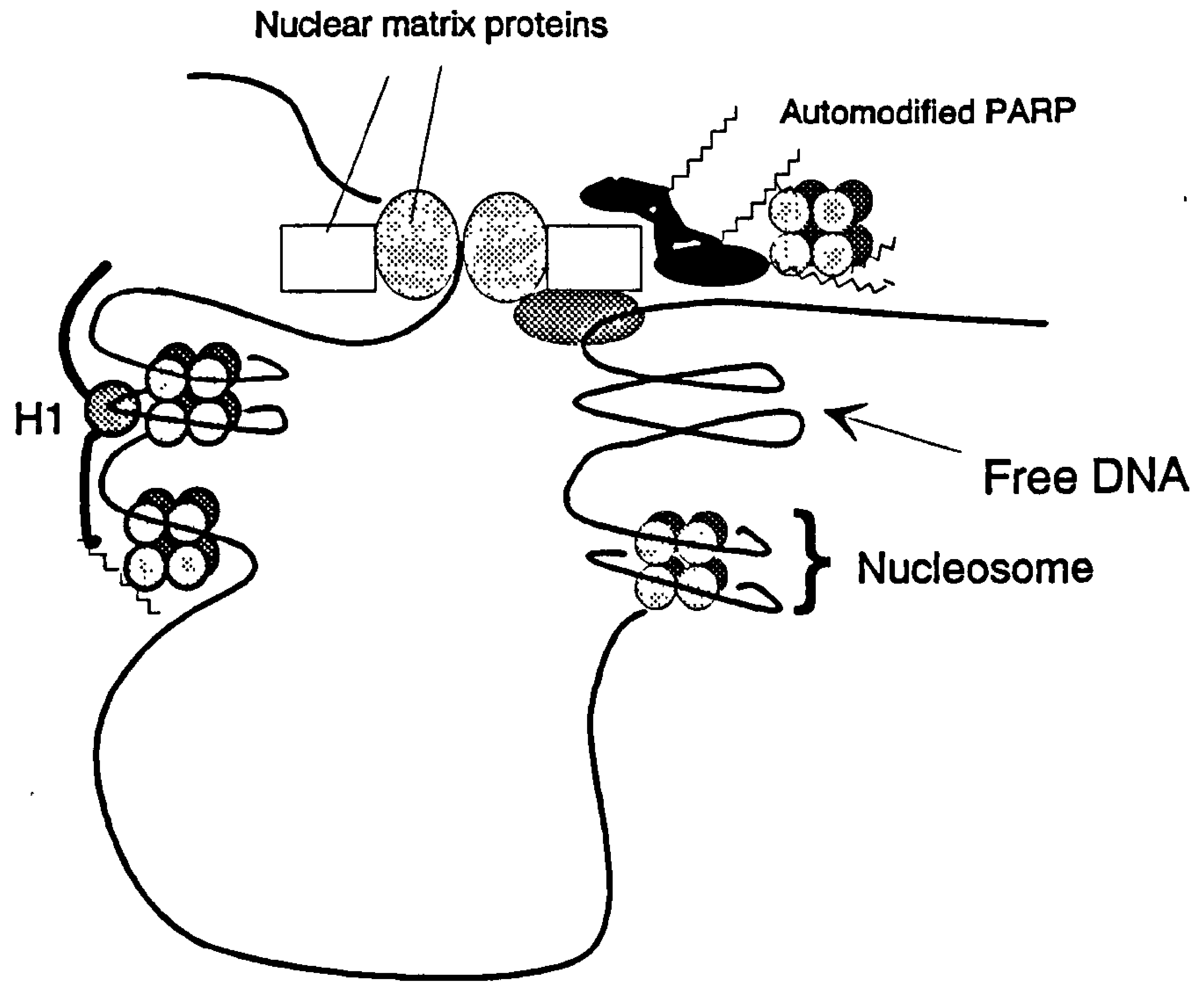


Figure: 1.8.2b. Automodified PADPRP releasing histones from the nucleosome



Poly (ADP ribosyl)ation of other nuclear enzymes may assist this DNA repair. Topoisomerases and high mobility group proteins (HMG) are associated with regulating transcriptionally active chromatin, and ribosylation of these proteins renders them inactive thus causing cessation of transcription, recombination, replication *etc.*[51, 52] PADPRP is activated by DNA strand breaks, therefore, for ribosylation to occur there must be local DNA damage. By inactivating the enzymes involved in DNA replication the cell has time to repair the damage.

1.9. Inhibition of PADPRP

Most research into PADPRP inhibitors have centred on the nicotinamide unit of NAD^+, with benzamide being a classical NAD^+ mimetic. Inhibitors of PADPRP were initially used to elucidate the function and physiological effects of the enzyme. These inhibitors included nicotinamide, 5-methylnicotinamide,[53] thymidine,[54] theophylline and caffeine.[55] Unfortunately these inhibitors also cause other cellular dysfunctions. For example, nicotinamide affects NAD^+ biosynthesis and depletes cellular phosphoribosyl diphosphate pools, leading to decreased nucleotide synthesis.[56] Thymidine is thought to inhibit DNA synthesis[57] and xanthines are known to inhibit many enzymes, including the enzyme cyclic phosphodiesterase, and other cell functions.[58-60] Thus, if these inhibitors are utilised to elucidate PADPRP's biological function, any significant effects that result cannot be directly attributed to inhibition of this enzyme alone.

Figure: 1.9.1. Classical PADPRP inhibitors

Nicotinamide

5-Methyl nicotinamide

Thymidine

Theophylline

Caffeine

Benzamide has been shown to exhibit good inhibitory activity against PADPRP.[61] The increased inhibitory activity, when compared to nicotinamide, can be attributed to the lack of a ring nitrogen, which confers stability to NAD^+ metabolising enzymes.

Benzamide

Benzamide, however, has low aqueous solubility due to its hydrophobic nature, and this has caused problems in physiological buffer solutions.

Purnell and Whish introduced substituted benzamides as potential PADPRP inhibitors.[56] They screened a series of benzamide analogues for inhibitory activity, and these included compounds substituted at the 3-position with amino, methoxy, bromo, hydroxy and nitro groups (Figure: 1.9.2.).

Figure: 1.9.2. 3-Substituted benzamides as PADPRP inhibitors

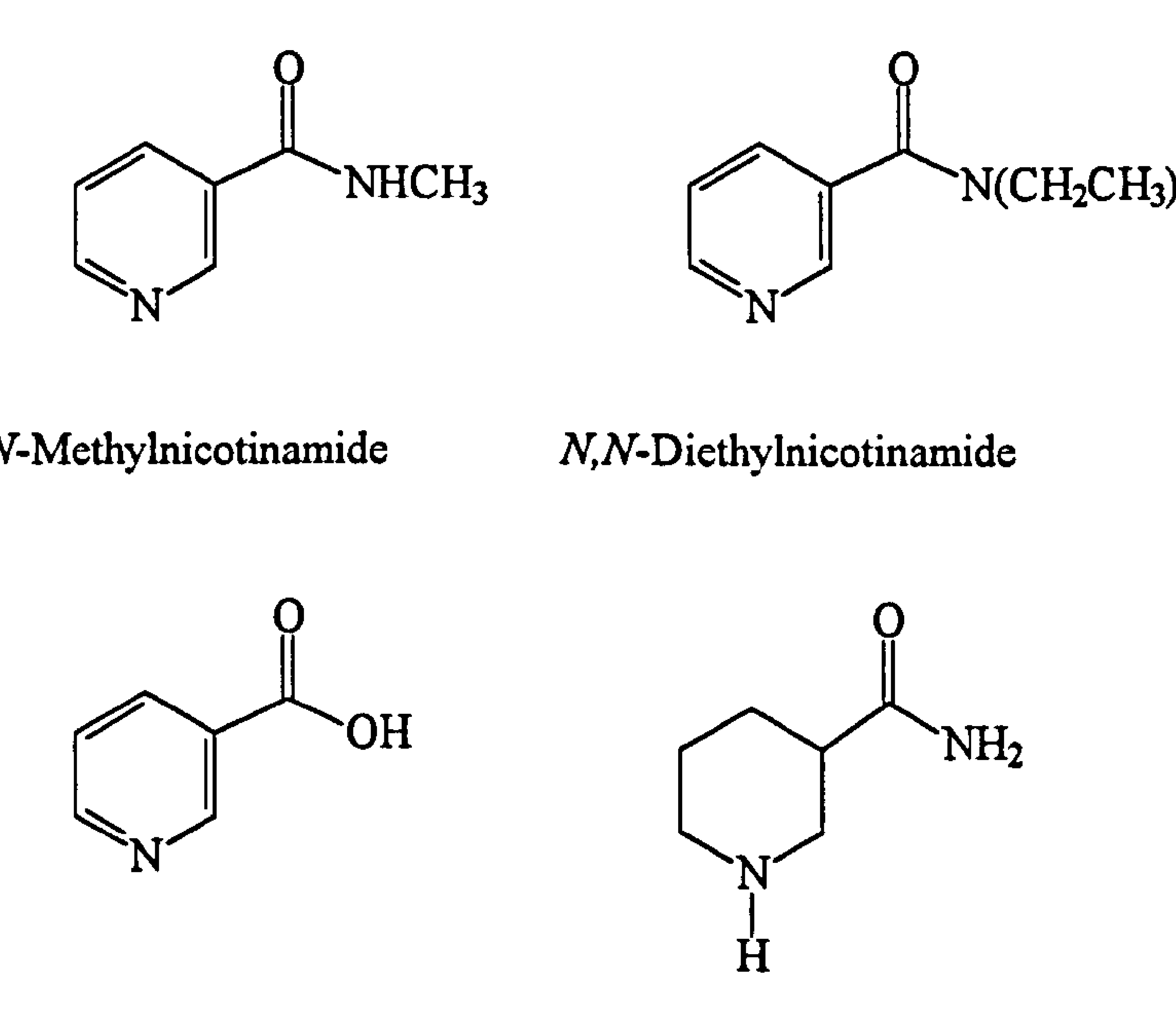
$R = NH_2$
$R = OCH_3$
$R = Br$
$R = OH$
$R = NO_2$
$R = NHCOCH_3$

With the exception of 3-nitrobenzamide, the observed inhibitory activity was very high and all, including 3-nitrobenzamide, were considerably more potent than nicotinamide. Acylation of 3-aminobenzamide to form 3-acetamidobenzamide afforded one of the better PADPRP inhibitors.

Sims *et al* screened a selection of nicotinamides, benzamides, pyrazinamides and purine analogues for their PADPRP inhibitory activity. Of the nicotinamides screened nicotinamide and picolinamide were the most potent.[62] Structural modifications to the parent compound resulted in either decreased or complete loss

of activity. Notably, modification of the amide had the most dramatic effect. The inhibitory activity decreased dramatically on mono- or di-substitution of the amide. Conversion to nicotinic acid also resulted in total loss of activity. Inactivity was similarly observed in an analogue which had a piperazine rather than an aromatic ring (Figure: 1.9.3.).

Figure: 1.9.3. Structural alteration of known PADPRP inhibitors



Classical bioisosteres of benzamide evaluated include those bearing other heteroatoms, for example sulfur. Cantoni *et al* examined three sulfur-containing analogues in order to elucidate PADPRP's dependency on dipole moments, hydrogen bond strength and steric hindrance of the amide.[63] Benzenesulfonamide and thiobenzamide were chosen for their obvious structural similarity to benzamide, and thiophene-3-carboxamide as a direct isostere of benzamide, with the divalent sulfur replacing the vinylic CH=CH.

Figure: 1.9.4. Sulfur containing PADPRP inhibitors

Benzenesulfonamide   Thiobenzamide   Thiophene carboxamide

In vitro evaluation showed that benzenesulfonamide and thiobenzamide had no inhibitory activity, but thiophene carboxamide exhibited weak inhibitory activity against PADPRP.

In an attempt to find more specific PADPRP inhibitors Banasik *et al* screened over two hundred compounds to determine inhibitory effects on PADPRP and mono (ADP)ribose polymerase.[64] All of the potent inhibitors, with the exception of the benzamides were polyaromatic. 4-Amino-1,8-naphthalimide was the most active against PADPRP with phenanthridinone having the same order of potency. The distinguishing feature of these inhibitors is that the carbonyl is restricted as part of a ring system conjugated to an aromatic ring. This reinforces the idea that a potent PADPRP inhibitor requires a carbonyl group pointing in the correct direction for binding to the enzyme. It was noted, however, that alkylation of the imide nitrogen in (*N*-2-chloroethyl)-4-amino-1,8-naphthalimide resulted in complete loss of activity. In view of previous literature reports, this was not surprising since it is believed that at least one amide proton is required to bind to the enzyme active site, thus conferring inhibitory activity (Table: 1.9.A.).[62, 65]

Table: 1.9.A. Illustrative examples of known inhibitors

structure	compound	IC_{50} μM [200 μM NAD^{+}]
	4-amino-1,8-naphthalimide	0.18
	6[5*H*]-phenanthridinone	0.3
	2-nitro-6[5*H*]-phenanthridinone	0.35
	(*N*-2-chloroethyl) 4-amino-1,8-naphthalimide	1800
	3-hydroxybenzamide	1.9
	3-methylbenzamide	19
	2-methylbenzamide	1500
	4-methylbenzamide	1800

Among the benzamide analogues the authors found 3-hydroxybenzamide to be quite potent against PADPRP, whilst movement of the methyl substituent around the ring caused a severe loss of activity.

After examining the proposed mechanism of NAD^+ cleavage by ADP-ribosyl transferases, Slama and Simmonds synthesised a carbanicotinamide analogue of the co-enzyme NAD^+. Since enzymatic cleavage is thought to be assisted by electron donation (see p 11), replacement of the ribose oxygen by the isosteric CH_2 affords a compound which should bind in a similar manner to NAD^+, but whose nicotinamide bond will be significantly more stable to cleavage.[29]

The carbanicotinamide analogue initially evaluated for inhibitory activity was a mixture of diastereoisomers. Later studies, however, showed that the unnatural diastereoisomer (ψ carba NAD) was responsible for the observed inhibition and the natural isomer (carba NAD) had no effect on PADPRP.[30] Although there are no clear reasons for this selectivity, the authors propose that the presence of the isosteric CH_2 in the dihydroxypentane ring in ψ carba NAD distorts the nucleotide into a conformation preferred for binding, whereas for the natural isomer the distortion is unfavourable for binding. Oppenheimer subsequently supported this theory by NMR studies (Figure: 1.9.5.).[66]

Figure: 1.9.5. Structural isomers of NAD^+

carba NAD

ψ carba NAD

Further research into the preparation of the carba NAD includes the synthesis of a radiolabelled NAD, which may prove useful in determining NAD binding.[67]

1.10. Clinical use of PADPRP Inhibitors

As PADPRP is involved in DNA repair, its inhibition can interfere with the repair of DNA lesions. Research has shown that inhibitors of PADPRP may enhance the cytotoxicity of DNA damaging agents.[8, 9, 18] Enhancement in the destruction of cells, by the administration of a PADPRP inhibitor with a DNA damaging agent, has tremendous implications for the treatment of cancer, particularly for tumours which have become resistant to standard chemotherapy. Concomitant dosing of a

PADPRP inhibitor with a DNA damaging agent may circumvent the mechanism of resistance, thus causing cytotoxicity in a tumour, resistant by virtue of enhanced PADPRP associated DNA repair.

One of the earliest reports regarding the use of PADPRP inhibitors as resistance modifying agents in cancer chemotherapy was performed by Smulson *et al.*[68] Mice were injected with L1210 murine leukaemia cells intraperitoneally, and after 2 days were administered with either *N*-methyl-*N*-nitrosourea (MNU) alone or co-administered with nicotinamide. Those animals treated with MNU alone had an increased survival time in comparison to controls, but co-administration with nicotinamide increased the survival time over mice treated with MNU alone.[68] Subsequent studies investigated the effects of PADPRP inhibitors on whole cell systems *in vitro*. Inhibitors of PADPRP have potentiated the cytotoxic effects of many DNA damaging agents (Table: 1.10.A.).

Table: 1.10.A.

DNA damaging agent	cell line	inhibitor and concentration	modifying effect	author
X-rays	Chinese hamster ovary	3-AB 20 mM	+	BenHur [69]
γ-rays	mouse embryo BALB 3T3	benzamide 1-5 mM	+	Kasid *et al*[70]
Dimethyl sulphate	human fibroblast	3-AB 5 mM	++	James and Lehmann[71]
Methylnitroso-urea	mouse lymphoma (L1210)	nicotinamide 2 mM	+++	Nduka[12]
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	mouse C3H/10T1/2	3-MB 1 mM	++	Jacobson *et al*[72]

3-AB, 3-Aminobenzamide; 3-MB, 3-Methoxybenzamide.
+, small effect; ++, moderate effect; +++, large effect.

In an attempt to improve these results Suto *et al* designed a series of novel 3,4-dihydroisoquinolinones which were evaluated for radiopotentiation activity.[73]

Figure: 1.10.1. 3,4-dihydroisoquinolinone as PADPRP inhibitors

3,4-dihydroisoquinolinones

The rational for the design of these novel inhibitors was based on the NAD^+ binding domain of PADPRP. 3-Substituted benzamides are believed to bind to PADPRP in the same fashion as the nicotinamide moiety of NAD^+. The authors proposed that the orientation of the amide was important in the association of the substrate / inhibitors with the enzyme. This has since been corroborated by comparison of PADPRP with glycohydrolases, which are other NAD^+ dependent enzymes. *Ab initio* studies show that in free solution the amide of NAD^+ is not restricted. However, on binding to the glycohydrolase enzyme the amide moiety preferentially adopts the *anti* conformation with respect to the 1,2-bond.[74, 75]

anti *syn*

There are two conformations the amide can adopt, either *syn* or *anti* to the 1,2-bond. The amide was constrained into one of these conformations by either of two

methods. The *anti* conformation was achieved *via* the introduction of an ethane bridge. This restriction produced a series of either 5- or 7- substituted 3,4-dihydroisoquinolinones.[73]

The second method involved introducing a substituent into the 2-position to form 2,3-disubstituted benzamide analogues, thus, forcing the amide into a *syn* conformation (Figure: 1.10.2).

On *in vitro* evaluation, the 2,3-disubstituted benzamide analogues were essentially completely inactive. Conversely, the dihydroisoquinolinones were exceptionally potent, with the 5-substituted dihydroisoquinolinones proving much more potent than nicotinamide. Interestingly, as the substituents were moved around the ring from 5- to 8-, the relative potencies decreased, with 8-hydroxy-3,4-dihydroxyisoquinolinone being the least active in the series, although still more active than nicotinamide.

Figure: 1.10.2. Benzamide analogues to determine the effects of substitution in the 2-position

VS

$R = OCH_3$, $R' = CH_3$,
$R = OH$ $R' = CH_2CH_3$,
$R' = CH_2CH_2CH_3$,
$R' = CH_2CHCH_2$

Previous work with PADPRP inhibitors, specifically 3-aminobenzamide, showed that inhibition of the enzyme during *X*-ray irradiation caused DNA damage, but that this damage was small and species specific. Studies showed that, after X-ray irradiation, some cells exhibited enhanced DNA damage if treated with 5-substituted isoquinolinones.

PD128763

Further to this, Elliott *et al* developed the most potent published PADPRP inhibitor to date, PD128763. This compound enhanced DNA damage in cells which had been exposed to X-rays. When examined *in vivo*, treatment of sarcoma with PD128763, in combination with radiotherapy, potentiated a 20 day tumour growth delay and caused 50% regression in tumour size with little side effects.[76]

1.11. Structure-Activity Requirements for Optimal PADPRP Inhibition

In reviewing reported studies regarding PADPRP inhibitors certain structural similarities have emerged, which suggest an optimal structure for specific and potent PADPRP inhibition.

Planarity and aromaticity are pre-requisite structural features for potent PADPRP inhibition. Saturation of the ring system causes loss of activity, and 1,4-dihydrobenzamide analogues are also inactive.

A *carbonyl group*, as part of the skeleton or attached to the aromatic system is desirable for inhibition. An increase in potency is observed if the carbonyl is part of an amide with at least one hydrogen present on the amide nitrogen. Inhibitory activity is considerably reduced on substitution of the C=O by C=S and completely lost when the amide is replaced by a carboxylic acid or a ketone.

The *conformation* of the amide has a significant influence on potency with the *anti* conformation being more favourable for inhibition than the *syn* conformation, relative to the 1,2-bond in benzamide (Figure: 1.11.1.).

The *removal of heteroatoms* from the ring may improve potency, since benzamide is more potent than nicotinamide.

Figure 1.11.1 Minimal structure for potent PADPRP inhibition

Substitution is tolerated at the 3-position but not at the 4-, 5- or 6- positions. PADPRP will tolerate groups at the 2- position provided the conformation of the amide remains unaffected. The presence of a *non-cleavable* bond at the 3-position increases inhibitory activity, as confirmed by Slama *et al* [67] This is particularly relevant if the proposed mechanism of action is correct.

1.12. Method of PADPRP Assay

It is not surprising that only general trends rather than specific conclusions can be drawn concerning the role of PADPRP in the cell and the structural requirements for its inhibition. The discrepancies between results may be due to the different cell lines used, the stage in the cell cycle in which exposure to the inhibitor occurred, timing of inhibitor addition and concentrations used. There are many different methods for screening for PADPRP inhibitors. The two more commonly used methods utilise permeabilised cells or pure enzyme.

Therefore, it should be noted that quotation of reported inhibitory activities *per se* provides little information for comparison purposes. Table: 1.16.A. highlights the inter-assay differences when evaluating benzamide between some of the more commonly referenced papers.

Table: 1.12.A. Inter-assay differences when evaluating benzamide

author	method of assay	[NAD^+]	Expression of results
Banasik *et al* [64]	pure enzyme	200 μM [ade-U-^{14}C]	Inhibition as % of control 92 % at [1.0 mmol]
Sims *et al* [62]	permeabilised cell	[adenine-^{3}H]	Inhibition as % of control 96 % at [2.0 mmol]
Purnell and Whish [56]	isolated porcine thymus nuclei	50 μM [adenine-^{3}H]	Inhibition as % of control 96 % at [50 μM]
Cantoni *et al* [63]	pure enzyme	111 μM [^{32}P]	Inhibition as % of control 75 % at [50 μM]

1.13. Summary

As PADPRP is involved in the post incision repair of damaged DNA there are many theories as to its role in the cell, which include:

PADPRP blocking sites of DNA damage, thus preventing the DNA polymerases from transcribing aberrant DNA. These stalling tactics may provide sufficient time for the DNA damage to be repaired, thus allowing the DNA polymerases a complete template for replication. Alternatively, PADPRP binding to the damaged site with subsequent automodification, could be an emergency signal to alert the cell to DNA damage.

However, inhibition of PADPRP has been shown to retard DNA repair in DNA-damaged cells and concomitant dosing with a DNA damaging agent whilst inhibiting PADPRP has been shown to potentiate cell death. This potentiation of

cell death by PADPRP inhibition may provide a new potent resistant modifying agent to improve chemotherapy.

This thesis details the research into novel inhibitors of Poly (adenine diphosphophate ribose) polymerase to potentiate DNA reactive drugs. The analogues detailed have been designed to achieve inhibition by mimicing PADPRP's cofactor NAD^+.

CHAPTER TWO

Development of Analogues of 3-Hydroxybenzamide

2.0 Introduction

3-Substituted benzamides, particularly 3-aminobenzamide, are probably the most widely accepted PADPRP inhibitors, and benzamides appear to be good NAD^+ mimics, binding to the nicotinamide region in the enzyme active site. There has been little research into other aspects of PADPRP binding including the effects of the ribose moiety and phosphate backbone mimics of NAD^+ on potency. The work detailed in this chapter aims to elucidate the effects of larger hydrophobic groups and long chain spacer groups on inhibitory activity.

Results and Discussion

2.1. Synthesis of 3-Hydroxybenzamide

3-Hydroxybenzamide was chosen as the parent compound because the phenolic OH provides a suitable 'handle' for the introduction of differing functionalities. As 3-hydroxybenzamide is not commercially available, an efficient preparation of this compound was required, and studies centred on a synthetic strategy beginning with 3-hydroxybenzoic acid **1**.

Treatment of **1** with ethyl chloroformate and triethylamine gave the mixed anhydride and also protected the phenolic-OH. Subsequent addition of liquid ammonia at -78 °C afforded 3-hydroxybenzamide **2** in moderate yield, together with ethyl carbamate (urethane) **3**, which is a carcinogen.

Figure: 2.1.1 Synthesis of 3-hydroxybenzamide

To circumvent this toxic by-product the mixed anhydride reaction was repeated using benzyl chloroformate. The major product, this time, was not the required 3-hydroxybenzamide but benzyl carbamate, with **1** remaining unchanged. As this procedure was problematic a simpler method was sought.

benzyl carbamate

One of the simplest methods for amide formation is *via* amination of an acid chloride. This approach cannot be applied to **1** directly owing to the presence of the phenolic OH. Therefore, **1** was treated with acetic anhydride at 0 °C, yielding 3-acetoxybenzoic acid **4**, which was converted into the acid chloride by refluxing in thionyl chloride. Addition of aqueous ammonia furnished 3-hydroxybenzamide **2** with concomitant deprotection of the phenol. This methodology was first attempted by Rhodes.[84]

Figure: 2.1.2 Improved synthesis of 3-hydroxybenzamide

Ac_2O, NaOH; HCl, 0 °C; 56 %
i. $SOCl_2$; ii. NH_3 (aq); 37 %
1 → 4 → 2

2.2. 3-Benzyloxybenzamide Analogues

Benzylation of **2** should provide information as to whether PADPRP tolerates large hydrophobic moieties. Suitably substituted phenyl groups may occupy the NAD^+ binding site in a similar manner to the ribose group of NAD^+. Base catalysed alkylation of **2** using potassium carbonate and benzyl bromide in refluxing acetonitrile furnished 3-benzyloxybenzamide **5** (BOB) in good yield (Figure: 2.2.1).

Figure: 2.2.1 Synthesis of 3-benzyloxybenzamide

$C_6H_5CH_2Br$, K_2CO_3; CH_3CN, reflux, 16h, 65 %
2 → 5

A series of 3-benzyloxybenzamide analogues was prepared. The substituents included in this series were intended to determine the tolerance of PADPRP to

electronic effects, hydrophobicity, polarity and increasing chain length (Figure: 2.2.2).

Figure: 2.2.2 Benzyloxybenzamide analogues

O, NH_2, OH, 2 — RCH_2Br, K_2CO_3, CH_3CN, reflux → O, NH_2, OR

6 R = 4-methoxybenzyl
7 R = 4-nitrobenzyl
8 R = 4-fluorobenzyl
9 R = 4-bromobenzyl
10 R = 4-trifluoromethylbenzyl
11 R = 4-cyanobenzyl
12 R = 3-nitrobenzyl
13 R = 2-nitrobenzyl
14 R = 3,4-dioxymethylenebenzyl
15 R = cinnamyl
16 R = phenethyl

The introduction of a 4-methoxybenzyl and a 4-nitrobenzyl moiety was intended to determine the effects of strong electron donating and electron withdrawing groups, respectively, on inhibitory activity against PADPRP. Fluorine was introduced as it is a classical bioisostere for hydrogen, being of a similar size but with a different electronic effect. The bromo-substituent in **9** was introduced to increase the lipophilicity of the compound, which may increase diffusion across the cell membrane. Another bioisostere prepared was 4-cyano-BOB **11**, as the nitrile moiety is a pseudohalide and would also be a precursor to a carboxylic acid group.

By moving the substituents around the benzyl ring *i.e.* 2-nitro **13**, 3-nitro **12** and 4-nitro-BOB **7**, any electron withdrawing effects would be determined. Similarly, the introduction of a strong electron donating group, for example in 3-(2,3

methylenedioxybenzyloxy)benzamide **14**, may increase inhibitory activity. Phenethyl and cinnamyl derivatives (**15** and **16**) were intended to determine whether the chain length between the benzamide and phenyl moieties was important. The cinnamyl group also possesses an unsaturated bond which provides a useful handle for further functionalisation.

Another interesting analogue required was 3-(4-azidobenzyloxy)benzamide **17**. Azido groups are particularly important for photoaffinity labelling active drugs. They are also pseudohalides having similar physiochemical properties to bromo substituents.

2.3 3-(4-Azidobenzyloxy)benzamide

p-Toluidine served as the starting material for the synthesis of 4-azidobenzyl bromide as reported by Mornet *et al.*[79] *p*-Toluidine was diazotised and the azide introduced under standard conditions yielding 4-azidotoluene **18**. Free radical bromination of **18**, using *N*-bromosuccinimide with azoisobutyronitrile (AIBN) as the free radical initiator, afforded 4-azidobenzylbromide **19**. Base catalysed alkylation of **2** with **19** yielded 3-(4-azidobenzyloxy)benzamide **17** in 55 % yield (Figure: 2.3.1).

Figure: 2.3.1 Preparation of 3-(4-azidobenzyloxy)benzamide

CH_3 / NH_2 → i. $NaNO_2$, HCl (6 M), 0°C; ii. NaN_3 → CH_3 / N_3 **18**

NBS, AIBN, benzene, reflux

CH_2Br / N_3 **19** → **2**, K_2CO_3, CH_3CN → O, NH_2, N_3, O **17**

A major obstacle encountered during the determination of inhibitory activity was the low solubility of the analogues in physiological buffers. To overcome this problem two compounds were targeted, namely 4-amino-BOB **20** and 4-carboxy-BOB **21**. The amino moiety in **20** is a polar group which may increase the solubility of the compound in aqueous systems, and preparation of the hydrochloride salt should be water soluble. Similarly, the 4-carboxylic acid analogue **21** should also exhibit an increased solubility either as the free acid or the sodium salt.

2.4. 3-(4-Aminobenzyloxy) benzamide

The standard procedure for the preparation of the BOB analogues was by nucleophilic displacement of a halide from the appropriate benzyl bromide as starting material. However, 3-(4-aminobenzyloxy) benzamide **20** could not be prepared from 4-aminobenzyl bromide because this compound is insufficiently stable. 4-Nitrobenzyl bromide had already been used to synthesise 3-(4-nitrobenzyloxy)benzamide **7**, so the latter was used as a source for the amine **20**. Reduction of **7** by hydrogenation was attempted, since the rate of nitro group reduction may be more rapid than the rate of debenzylation. Compound **7** was added to a palladium-carbon catalyst suspended in methanol under a hydrogen atmosphere, and the reaction monitored by TLC. Although reduction of the nitro group occurred, so did debenzylation and the isolated products were **2** and *p*-toluidine.

The reaction was repeated using a microhydrogenator, which enabled the reduction to be conducted with a defined volume of gas.[80] One equivalent of hydrogen was taken up and a mixture of products was obtained. It is likely that the rate of debenzylation may be enhanced by the presence of the electron withdrawing 4-nitro group, so that it occurs as rapidly, if not faster than reduction of the nitro group. Since this method was unsuccessful further methods were sought.

Amino groups can also be introduced by the preferential reduction of azides under very mild conditions, therefore 4-azidobenzyloxybenzamide **17** was utilised. A well documented and mild procedure for the reduction of azides is the Staudinger reaction.[81] The Staudinger reaction utilises the nucleophilicity of triphenylphosphine to convert the azide to an iminophosphorane, which decomposes on treatment with water to form the amino group and triphenylphosphine oxide as a by-product.

Figure: 2.4.1. Mechanism of the Staudinger reduction

This approach was utilised for the reduction of **17** in anhydrous ether. On work up there was no product isolated in the organic phase, but trace amounts of product were found in the aqueous phase. The reaction was repeated, but this time was quenched with 0.1 M aqueous HCl in order to form the hydrochloride salt of the required amine. Although **22** was isolated, the yield was very low, and an alternative approach was therefore investigated.

Figure: 2.4.2. Synthesis of 3-(4-aminobenzyloxy)benzamide hydrochloride

O NH$_2$ N$_3$ — TPP; Et$_2$O; HCl (aq) → O NH$_2$ NH$_2$.HCl

17 22

A method reported by Rosen *et al* was the reduction of an azide with subsequent acylation of the resulting amino group.[82] Compound **17** was dissolved in an excess of thioacetic acid and stirred at ambient temperature until the reaction was complete. Removal of the excess of thioacetic acid yielded 3-(4-*N*-acetylaminobenzyloxy)benzamide **23** (Figure 2.4.3).

Figure: 2.4.3. Synthesis of 3-(4-*N* acetylaminobenzyloxy)benzamide

O NH$_2$ N$_3$ — CH_3COSH; 96 h; 64 % → O NH$_2$ NHCOCH$_3$

17 23

Whilst this method furnished **23** in moderate yield the reaction was slow and deprotection of the *N*-acetylamino functionality to form the free amine species added yet another step to the synthetic pathway.

An alternative procedure examined was the mild and selective reduction of azides by dithiothreitol (DTT)[83]. A possible mechanism for this reduction involves intramolecular attack by the δ thiol to yield a dithiane by-product which was easily removed (Figure: 2.4.5).

Figure: 2.4.4. Mechanism of azide reduction by dithiothreitol

Figure: 2.4.5. Synthesis of 3-(4-aminobenzyloxy)benzamide

2.5. 3-(4-Carboxybenzyloxy)benzamide

4-Chloromethylbenzoic acid was the starting material used. Acid catalysed esterification of 4-chloromethylbenzoic acid in refluxing methanol furnished methyl (4-chloromethyl)benzoate **24**. The methyl ester was required for increased solubility and easier manipulation. Compound **24** was used for the base catalysed alkylation of **2** to yield 3-(4-carboxymethylbenzyloxy)benzamide **25** (Figure: 2.5.1).

Figure: 2.5.1. Synthesis of 3-(4-carboxymethylbenzyloxy)benzamide

Cl
COOH
CH_3OH / H^+
reflux
99 %
Cl
$COOCH_3$
24
2
K_2CO_3
CH_3CN
55 %
O
NH_2
$COOCH_3$
O
25

Stirring **25** in a solution of 0.1 M sodium hydroxide hydrolysed the methyl ester affording the sodium salt, and subsequent acidification to pH 3 yielded the free acid **21** (Figure: 2.5.2).

Figure: 2.5.2. Synthesis of 3-(4-carboxybenzyloxy)benzamide

O
NH_2
$COOCH_3$
O
25
i. NaOH
CH_3OH / H_2O
ii. H^+ to pH 3
O
NH_2
COOH
O
21

2.6 3-Alkoxybenzamide Analogues

Novel PADPRP inhibitors designed to mimic the phosphate backbone and adenosyl group of NAD have previously been prepared. These inhibitors encompassed a series of 3-(bromoalkoxy)benzamide and 3-(purinylalkoxy)benzamide analogues.[84] When examined *in vitro* these analogues exhibited some inhibitory activity, but whether this potency was determined by chain length, or by the moieties attached, or a combination of both was not ascertained. To clarify this, a simple homologous series of inhibitors without any pendant functional groups was required to determine the effect of chain length on potency.

$n = 5, 8, 12$

3-(bromoalkoxy)benzamides

$n = 5, 8, 12$

$R = Cl, NH_2$

3-(purinylalkoxy)benzamides

The homologous series included analogues ranging from butyl to octyl. The butyl **26** and pentyl **27** derivatives were synthesised *via* base catalysed alkylation of **2** with the corresponding alkyl iodides (Figure: 2.6.1).

Figure: 2.6.1. Synthesis of 3-butyl and 3-pentyloxybenzamide

$I(CH_2)_nCH_3$, K_2CO_3, CH_3CN, reflux

26 n = 3 butyl

27 n = 4 pentyl

The hexyl **28**, heptyl **29** and octyl **30** derivatives were prepared, by Day (University of Newcastle upon Tyne, unpublished results), from the corresponding mesylates because the respective alkyl halides were not commercially available. The mesylates were prepared by treating the respective alcohols with methanesulphonyl chloride in the presence of triethylamine. Subsequent base catalysed alkylation of **2** with the appropriate mesylate afforded the corresponding alkoxybenzamide (Figure: 2.6.2).

Figure: 2.6.2. General synthesis of 3-hexyloxybenzamide, 3-heptyloxybenzamide and 3-octyloxybenzamide analogues

$CH_3SO_3(CH_2)_nCH_3$, K_2CO_3, CH_3CN, reflux

28 n = 5 hexyl

29 n = 6 heptyl

30 n = 7 octyl

CHAPTER THREE

Development of 2-Substituted Derivatives of 3-Hydroxybenzamide

3.0 Introduction

Suto *et al* proposed that the conformation of the amide group of 3-substituted benzamides was partially responsible for potency, and that the active conformation has the carbonyl bond *anti* to the 1,2 bond.[73] The most potent PADPRP inhibitors reported contain an amide conformationally restricted in a bicyclic system, notably PD128763, which has potent activity both *in vitro* and *in vivo*.[76]

This chapter details research into an alternative means of restraining the amide *via* a hydrogen bond between the amide and an electron-donating moiety in the 2-position, thus producing effective PADPRP inhibitors.

Results and Discussion

3.1. Benzoxazole-4-carboxamides

Certain benzoxazole derivatives are of biological interest owing to their anti-inflammatory activity, the most notable example being benoxaprofen. Benzoxazole-containing natural products of biological interest include Calcinomycin.[87]

Calcinomycin

Benoxaprofen

There are various methods for the preparation of benzoxazole, and these have been the subject of many reviews, but *o*-amino phenol is one of the most versatile starting materials.[88-95]

Figure: 3.1.1. General synthesis of benzoxazoles

RCOOH

RCOCl

$(RCO)_2O$

$RCONH_2$

$RCHO / Pb(OAc)_4$

RCN

RC(OR')=NH

In most reported procedures the products possess a substituent in the 5-position of the phenyl ring. Recently, Goldstein and Dambek detailed the synthesis of a series

of methyl benzoxazole-4-carboxylates, as intermediates for natural products, starting from 3-hydroxyanthranilic acid **31**.[96]

31 **32**

Whilst **31** is commercially available, the cost of the quantity required to complete a series of inhibitors was prohibitive. Therefore, the immediate precursor, 3-hydroxy-2-nitrobenzoic acid **32**, which is considerably cheaper, was used as the starting material.

3.2. Starting Material

The carboxylic acid group of 3-hydroxy-2-nitrobenzoic acid **32** was protected as the methyl ester **33** by acid catalysed-esterification, since **32** is only soluble in polar solvents such as methanol or DMSO, thus causing manipulation problems. The ester was also prepared in order to prevent any unwanted side reactions occurring during further steps. Catalytic hydrogenation of the nitro group afforded methyl 2-amino-3-hydroxybenzoate **34**.

Figure: 3.2.1 Preparation of methyl-2-amino-3-hydroxybenzoate; general starting material for benzoxazole synthesis

CH_3OH, H^+ reflux, 98%; Pd / C (cat), H_2 CH_3OH, 100%

32 **33** **34**

3.3. 2-Methylbenzoxazole-4-carboxamide

The first analogue targeted was 2-methylbenzoxazole-4-carboxamide. Initial studies centred on the use of acid chlorides to form the oxazole moiety in a one pot procedure.[96] Treatment of **34** with acetyl chloride, triethylamine and the acid catalyst pyridinium-*p*-toluenesulfonate (PPTS) in refluxing *m*-xylene afforded a small amount of the required methyl 2-methylbenzoxazole carboxylate **35**, which was isolated by column chromatography. As this procedure was low yielding another route was explored.

Figure: 3.3.1 Preparation of methyl 2-methylbenzoxazole-4-carboxylate

O
OCH3
NH2
OH
34
CH_3COCl
NEt_3
PPTS
m-xylene
reflux, 6%
O
OCH3
N
O
CH3
35

An alternative method for the production of benzoxazoles is from the reaction of aminophenols with iminoethers. The formation of iminoethers from nitriles is known as the Pinner reaction and has been well documented.[87] Treatment of the nitrile with an alcohol in the presence of hydrogen chloride affords the HCl salt of the iminoether. Thus, ethyl acetimidate hydrochloride **36** was obtained by acid catalysed ethanolysis of acetonitrile under anhydrous conditions at 0 °C.

Figure: 3.3.2. Pinner synthesis to form ethyl acetimidate hydrochloride

$H_3C-C\equiv N^+H$ + $HOCH_2CH_3$ → $H_3C-C(=NH.HCl)OCH_2CH_3$

36

A model study for the preparation of 2-methylbenzoxazole was performed using **36**. 2-Aminophenol was refluxed with imidate **36** in anhydrous ethanol for 5h. The dry conditions are important as imidates are susceptible to hydrolysis to the corresponding amide. The reaction between the aminophenol and the imidate to create the oxazole moiety is dependent on the electrophilicity of the carbonyl group as the initial step is attack from the amino group at the electrophilic centre. Subsequent ring closure followed by dehydration afforded 2-methylbenzoxazole **37** cleanly and in good yield.

Figure: 3.3.3. Model studies for benzoxazole formation

36, CH_3CH_2OH, reflux

37

Encouraged by this success, imidate **36** was refluxed in anhydrous ethanol with **34** until the reaction was complete. The product, methyl 2-methylbenzoxazole-4-carboxylate **35** was again isolated cleanly and in good yield.

Figure: 3.3.4. Preparation of methyl 2-methylbenzoxazole-4-carboxylate

34 → 35 (36, CH_3CH_2OH, reflux)

Attempts at the preparation of the 2-methylbenzoxazole-4-carboxamide by the direct reaction of ammonia with **35** at high pressure were unsuccessful. Under these reaction conditions an interesting rearrangement took place, resulting in the formation of another series of potent PADPRP inhibitors (see Chapter 4).

Figure: 3.3.5. Attempted preparation of 2-methylbenzoxazole-4-carboxamide

35 → 39 (not formed), 69 (NH_3 (liq), 20 bar pressure, 55 °C)

An alternative approach involved hydrolysis of the ester to the acid, followed by conversion into the acid chloride, which when treated with ammonia should afford the carboxamide.

Base catalysed hydrolysis of carboxylate **35** with 0.1 M sodium hydroxide at 40 °C gave the sodium salt as expected, but acidification to liberate the free acid occurred with concomitant ring opening. This ring opening side reaction was surprising since the C2 position of benzoxazoles is not especially susceptible to nucleophilic attack. However, in strong acid solution protonation of the oxazole moiety presumably facilitates hydrolysis, which leads to ring opening.

Figure: 3.3.6. Formation of 2-methylbenzoxazole carboxylic acid

35

0.1M NaOH
40 °C
HCl

NaOH

HCl/H_2O

On a small scale, formation of the ring opened product could be reduced by cooling the reagents and solvents to 4 °C. On a larger scale, however, ring opening still occurred and as a result this method required further optimisation.

To avoid the ring opening side reaction the ester **35** was hydrolysed with one equivalent of sodium hydroxide. The salt obtained was isolated and redissolved in water with cooling to 4 °C. Dropwise acidification using 0.05 M hydrochloric acid was closely monitored (pH meter) and an extraction was performed with cold solvent with each pH unit change observed. The optimum pH for formation of the free acid, without ring opening, was found to be 4.5. By this approach 2-methylbenzoxazole-4-carboxylic acid **38** was isolated in a reasonable yield.

Figure: 3.3.7. Preparation of 2-methylbenzoxazole-4-carboxylic acid

O
ONa
N
O
CH_3

0.05 M HCl
pH = 4.5
4 °C

O
OH
N
O
CH_3

38

The acid **38** was converted into the amide *via* aminolysis of the corresponding acid chloride and the 2-methylbenzoxazole-4-carboxamide **39** product was purified by recrystallisation from ethyl acetate/petrol.

Figure: 3.3.8. Synthesis of 2-methylbenzoxazole-4-carboxamide

O
OH
N
O
CH_3

i. $SOCl_2$
DMF
THF
ii. NH_3 (aq)

O
NH_2
N
O
CH_3

38 **39**

A comparison of the spectroscopic data for 2-methylbenzoxazole-4-carboxamide **39** and the rearranged product 8-hydroxy-2-methylquinazolinone **69** are highly informative. In the ^{1}H NMR of benzoxazole **39** (Figure: 3.3.9) H_7 was shifted downfield from H_6, whereas in the quinazolinone spectrum (Figure: 3.3.10) H_7 is upfield from H_6, which may be attributed to electron donation from the hydroxy group. In ^{13}C NMR of the benzoxazole carboxamide **34**, the carbonyl carbon resonated at 174.5 ppm (Figure: 3.3.11), whereas the quinazolinone carbonyl carbon **69**, resonated at 162.1 ppm (Figure: 3.3.12).

Substantial evidence to distinguish the isomers was obtained by measuring their UV spectra in neutral and basic solutions. The differences in the UV spectra for benzoxazole **39** before, and after addition of the base were minimal (Figure: 3.3.13). Conversely, for quinazolinone **69** there was a significant shift to a longer wavelength on addition of base, corresponding to increased conjugation expected on deprotonation of the phenol (Figure: 3.3.14).

The final, and conclusive analysis, which confirmed the respective structures, was *X*-ray crystallography. The crystal structure of 2-methylbenzoxazole-4-carboxamide (Figure: 3.3.15) highlights the presence of the H-bond between N(1) and H(2a), with the bond being 2.09 Å in length. This was considerably longer than a normal N-H bond (*ca* 1.05 Å in length),[97] and is typical of a hydrogen bond.

Once the synthesis of amide **39** had been optimised, the benzoxazole carboxylic acid **38** proved a suitable precursor for another interesting analogue, namely 2-methylbenzoxazole-4-*N*-methylcarboxamide **40**. This compound may provide an insight as to whether PADPRP is tolerant to substitution of the amide protons with other groups. 2-Methylbenzoxazole-4-*N* methylcarboxamide **40** was prepared from the acid chloride of **38** by treatment with methylamine (Figure: 3.3.16).

Figure: 3.3.16. Synthesis of 2-methyl benzoxazole-4-*N* methylcarboxamide

i. $SOCl_2$ DMF THF; ii. CH_3NH_2 (33 % in EtOH)

38 → **40**

3.4. 2-Phenylbenzoxazole-4-carboxamide

Repeating the Pinner reaction with benzonitrile in acidic ethanol afforded the ethyl benzimidate hydrochloride salt **41** .

Figure: 3.4.1. Synthesis of ethyl benzimidate hydrochloride

CH_3CH_2OH, HCl 0°C

41

Heating aminophenol **34** and imidate **41** in ethanol under reflux for 16 h afforded methyl 2-phenylbenzoxazole-4-carboxylate **42**. The ester was stirred at ambient temperature in aqueous ammonia to produce the amide **43** with no rearrangement to the quinazolinone. The stability observed could possibly be due to the steric bulk of the phenyl ring restricting access of ammonia to the C2 centre. Alternatively, or additionally, conjugation of the phenyl with the benzoxazole system may reduce the electrophilicity of the C2 centre.

Figure:3.3.9 [1]NMR for 2-methylbenzoxazole-4-carboxamide 39 ($CDCl_3$)

Figure: 3.3.10 ¹NMR for 8-Hydroxyquinazolin-4[3*H*]-one 69 (d_6-DMSO)

PPM 174.471 166.457 150.804 139.371 125.440 124.338 123.277 113.905 77.734 77.103 76.457 28.416

Figure: 3.3.11 ^{13}C NMR of 2-methylbenzoxazole-4-carboxamide 39 ($CDCl_3$)

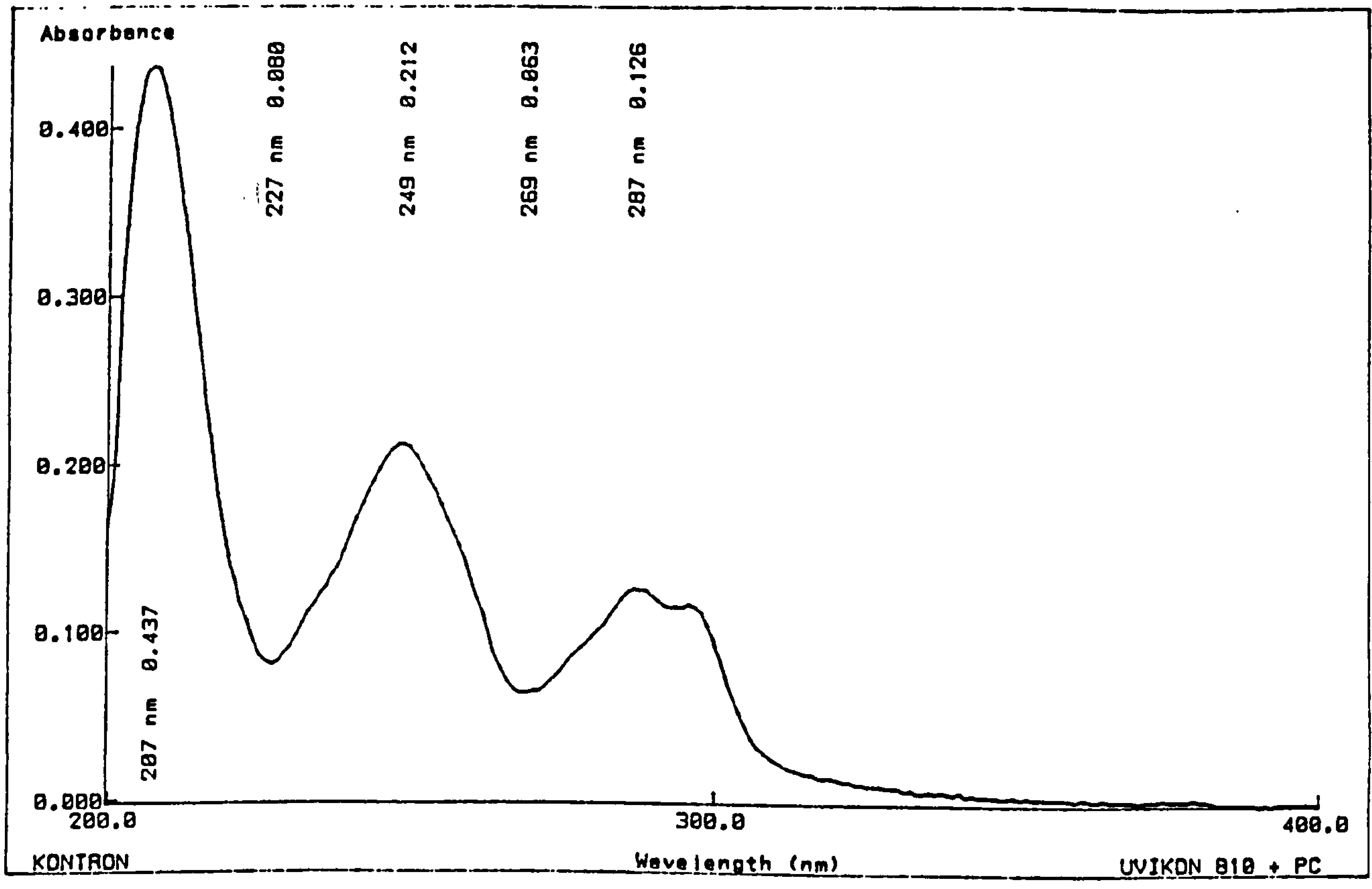
O NH$_2$ N O CH$_3$

C=O

200 180 160 140 120 100 80 60 40 20 0
PPM

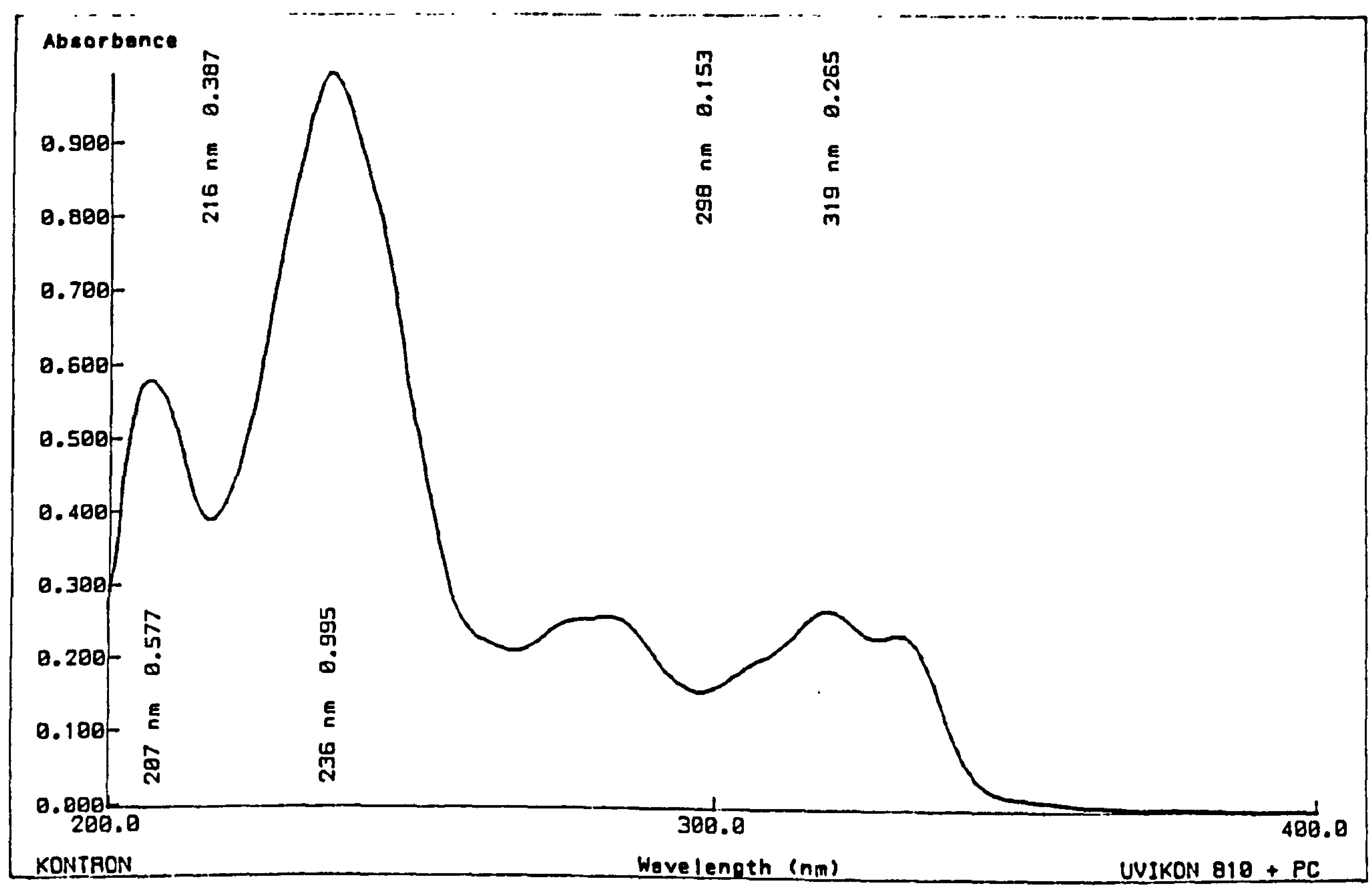
Figure: 3.3.12 ^{13}C NMR for 8-Hydroxyquinazolin-4[3*H*]-one 69 (d_6-DMSO)

Figure: 3.3.13 UV spectra for 2-methylbenzoxazole-4-carboxamide 39 (ethanol)



Absorbance
230 nm 0.121
248 nm 0.231
270 nm 0.073
288 nm 0.134
With 0.1 M NaOH
1.000
209 nm 1.562
200.0
300.0
400.0
KONTRON
Wavelength (nm)
UVIKON 810 + PC

Figure: 3.3.14 UV spectra of 8-Hydroxyquinazolin-4[3*H*]-one 69 (ethanol)



Absorbance
With 0.1 M NaOH
281 nm 0.864
328 nm 0.189
1.000
207 nm 1.269
200.0
300.0
400.0
KONTRON
Wavelength (nm)
UVIKON 810 + PC

Figure: 3.3.15. X-ray crystal structure of 2-methylbenzoxazole-4-carboxamide (crystallographic data in Appendix 1)

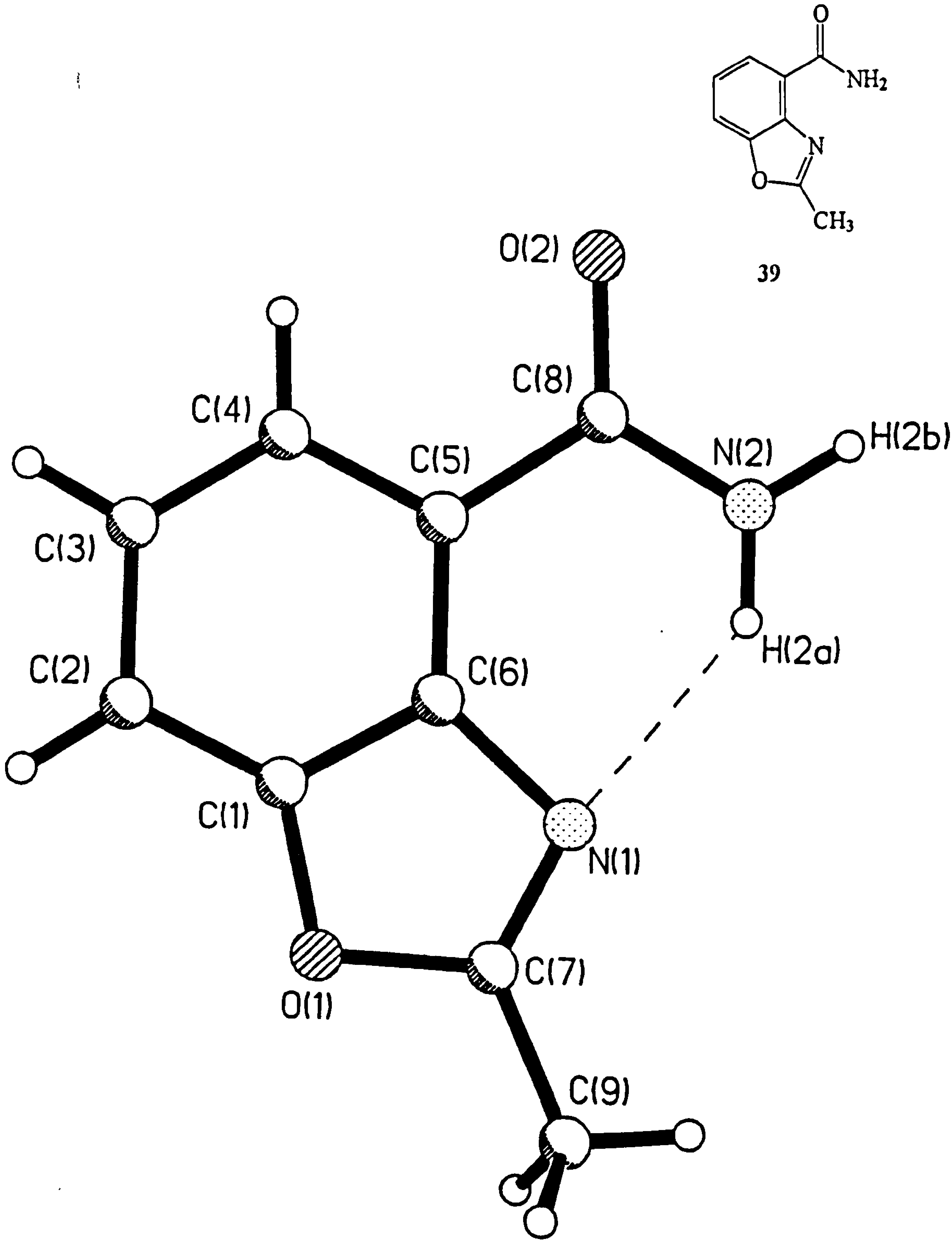


Figure: 3.4.2. Synthesis of 2-phenylbenzoxazole-4-carboxamide

3.5. 2-*tert*-Butylbenzoxazole-4-carboxamide

Unfortunately, the Pinner reaction was unsuccessful with ^{t}butyronitrile, as the steric effect of the butyl group will reduce the rate of alcohol attack on the nitrile carbon. Therefore, the alternative acid chloride method was utilised. The hydroxyanthranilic ester **34** was refluxed with ^{t}butylcarbonyl chloride, triethylamine and PPTS in *m*-xylene for 16 h, and the product, methyl 2-^{t}butylbenzoxazole-4-carboxylate **44**, was isolated by column chromatography. Conversion to the amide **45** was again achieved by stirring ester **44** in aqueous ammonia at ambient temperature. The C2 centre was again stabilised in a manner comparable to the phenyl derivative. The steric effect of the ^{t}butyl group restricting access of ammonia to the C2 centre stopped any rearrangement or ring opening side reactions occuring.

Figure: 3.5.1 Synthesis of 2-^{t}butylbenzoxazole-4-carboxamide

3.6. Substituted phenylbenzoxazole-4-carboxamides

The two analogues required initially were 2-(4-methoxyphenyl)benzoxazole-4-carboxamide and 2-(4-nitrophenyl)benzoxazole-4-carboxamide since the 4-nitro and 4-methoxy moieties will indicate whether electronic effects cause a difference in potency. Initial attempts at preparing the respective imidates by the Pinner procedure were unfruitful. A second method reported by Schaefer and Peters, details the preparation of imidates from nitriles *via* base catalysis.[98] Although this method details the synthesis of a wide variety of aliphatic and aromatic imidates, those nitriles which have electronegative groups react at a faster rate. This is not surprising considering the enhanced electrophilicity at the nitrile carbon. Unfortunately, no product was isolated when this method was attempted.

Whilst the method reported by Goldstein and Dambek worked, the yields were poor and optimisation of this method was necessary. Although the preparation of benzoxazole carboxylates involved a one pot reaction, the mixture of products obtained suggested that the reaction would proceed more efficiently if performed stepwise.

The first step involved acylation of the nitrogen with the substituted acid chloride, and initial attempts yielded only 50 % of the required product. This was possibly

due to the amine becoming protonated as the reaction progressed. Addition of pyridine as a proton acceptor and acylation catalyst, afforded the amides **46** and **47** in excellent yield, particularly if the acid chloride and pyridine were stirred together before addition of the aminophenol starting material **34**.

Figure: 3.6.1. General synthesis of methyl 2-(4-substitutedbenzoylamino)-3-hydroxybenzoate

Acid catalysed cyclisation to the oxazole moiety was achieved by refluxing the methyl-2-(4-substitutedbenzoylamino)-3-hydroxybenzoates **46** and **47** with triethylamine and PPTS in *m*-xylene for 16 h, the products being isolated by column chromatography.

Since conversion of ester **35** to amide **39** had proved successful, this method was again employed for compounds **48** and **49** which were each hydrolysed under basic conditions, and the reaction was acidified to pH = 4.5. This yielded the free acid **51**, but unfortunately ring opening of the oxazole occurred with ester **48** hence compounds **50** and **52** were not completed. Aminolysis of the acid chloride from **51** yielded 2-(4-methoxyphenyl)benzoxazole-4-carboxylate **53**.

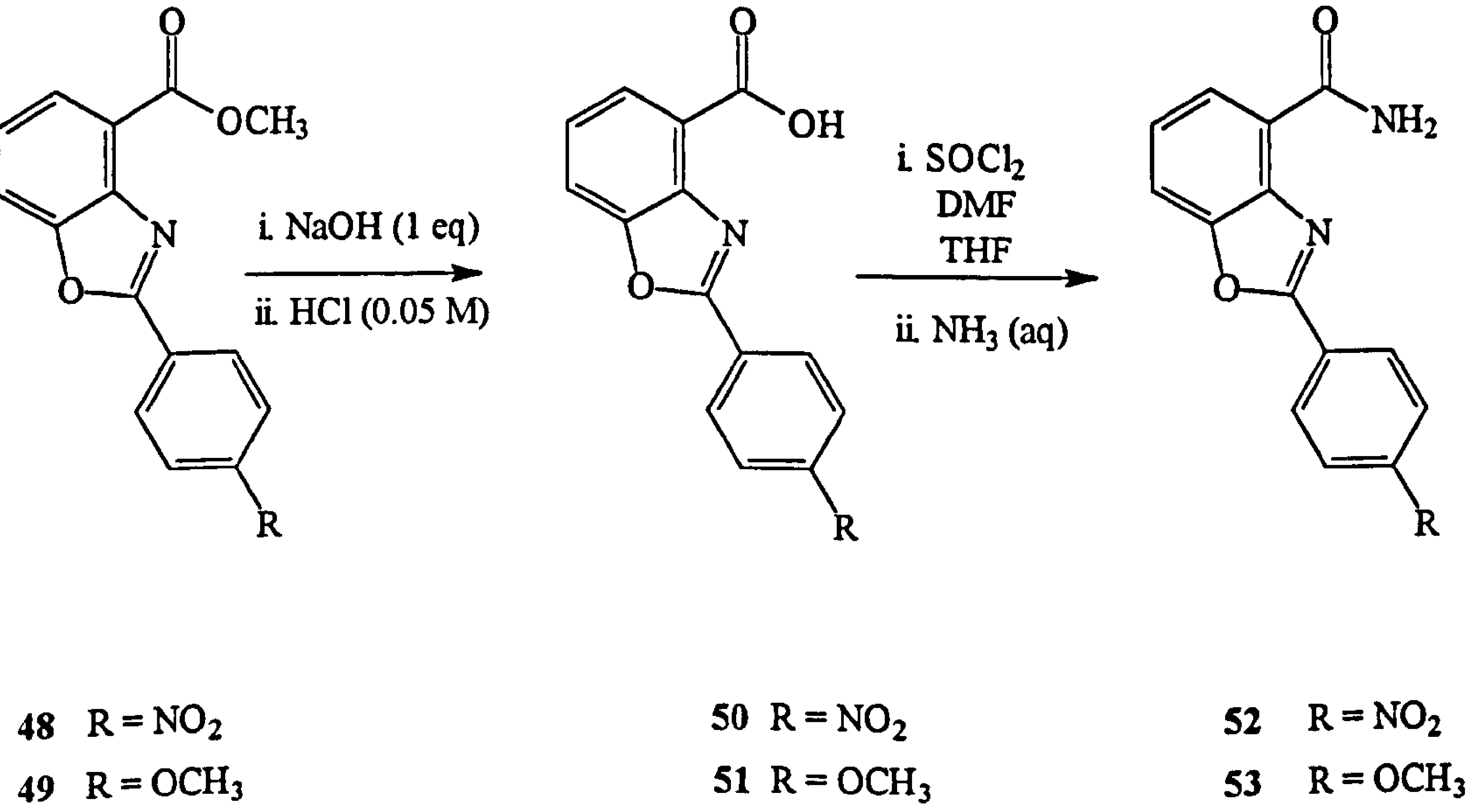
Figure: 3.6.2. Synthesis of methyl 2 (4-substituted phenyl)benzoxazole carboxylate derivatives

PPTS
m-xylene
reflux
16 h

46 R = NO_2
47 R = OCH_3

48 R = NO_2
49 R = OCH_3

Figure: 3.6.3. Synthesis of 2 (4-substituted phenyl)benzoxazole-4-carboxamide derivatives



3.7. 2-(4-Carboxyphenyl)benzoxazole-4-carboxamide

Solubility problems are frequently encountered during biological evaluation, particularly with some of the more hydrophobic analogues. Therefore, 2-(4-carboxylphenyl)benzoxazole-4-carboxamide was required since this should be a water soluble benzoxazolecarboxamide analogue. Preparation of this analogue began from the usual hydroxyanthranilic ester starting material **34**. The acid chloride required for the acylation step was prepared by stirring monomethyl*tere*phthalic acid in thionyl chloride. Acylation of methyl 2-amino-3-hydroxybenzoate **34** with monomethyl*tere*phthalic acid chloride afforded methyl 2-(4-methylcarboxybenzoylamino)-3-hydroxybenzoate **54**, which was subsequently cyclised to methyl-2-(4-methylcarboxyphenyl)benzoxazole-4-carboxylate **55**.

Figure: 3.7.1. Preparation of methyl 2-(4-methycarboxyphenyl)benzoxazole-4-carboxylate

^1H NMR analysis of the diester **55** showed the two methyl groups to be marginally different, about 0.2 ppm apart. Selective deprotection of the diester **55** was required, as it was necessary to prepare the 4-carboxamide before conversion of the phenyl ester to the free acid, in order to prevent formation of the diamide. Hydrolysis of **55** using sodium hydroxide yielded a salt which on acidification gave the diacid. Alternative methods tried included the use of zinc chloride.

Zinc chloride should act as a Lewis acid and coordinate to the ester and the oxazole nitrogen, thus enhancing the electrophilicity of the carbonyl and facilitating hydrolysis. This reaction was attempted at a variety of pH's without success, hence an alternative route to selective deprotection was investigated, where the amide was introduced initially, thus avoiding the need for selective deprotection. The alternative procedure began by protecting the phenolic OH of 3-hydroxy-2-nitrobenzoic acid as an acetoxy group to afford **57**. 3-Hydroxy-2-nitrobenzamide **58** was prepared *via* aminolysis of the ^{i}butyl mixed anhydride and hydrogenation of **58**, using a carbon on palladium catalyst under a hydrogen atmosphere, yielded 2-amino-3-hydroxybenzamide 59 in excellent yield.

Figure: 3.7.2. Attempted selective deprotection of the diester

O
OCH₃
N
O
COOCH₃
55

$ZnCl_2$ / H_2O (crossed out)

O
OH
N
O
COOCH₃
56

Acylation of 2-amino-3-hydroxybenzamide **59** with monomethyl*tere*phthalic acid chloride afforded 3-hydroxy-2-(4-carboxymethylbenzoyl)aminobenzamide **60**. Cyclisation to the target benzoxazole has proven to be problematical, and insufficient time remained to enable further investigation of this reaction.

Figure: 3.7.4. Synthesis of 2-(4-carboxyphenyl)benzoxazole-4-carboxylate

Reagents: i. Ac_2O, NaOH, 0 °C, [H^+ work up]; ii. ^{i}BuOCOCl, NEt_3, THF, 0 °C; NH_3 (aq.); iii. Pd/C cat., H_2, CH_3OH; iv $CH_3OCOC_6H_4COCl$, pyridine, THF; v. NEt_3, PPTS, *m*-xylene, reflux; vi ester hydrolysis.

3.8. 2,3-Methylenedioxybenzamide

2,3-Methylenedioxybenzamide was required on the basis that the oxygen in the 2-position may act as an electron donor, thus, increasing the negativity at the carbonyl oxygen and enhancing inhibitory activity. Also it may form an intramolecular hydrogen bond with an amide proton. Since the first reported methylation of catechol by Fittig and Remson[99] there have been numerous alternative methods published,[100, 101] including the use of strong bases in aprotic solvents with bronze,[102] or cupric oxide[103] catalysts to enhance reaction rates. Phase transfer

catalysis of catechols may provide a mild and high yielding method for alkylation, but reaction times may be slow.[104]

Initial attempts at the synthesis of 2,3-methylenedioxybenzamide using several of the above procedures were unsuccessful; in most cases the starting material remained unchanged after extended reaction at high temperatures. There was some concern as to the detrimental affects of a carboxylic acid *ortho* to the reacting moiety, because the electron withdrawing properties of the carboxylic acid deactivates the adjacent hydroxy group, decreasing the nucleophilicity of the anion, thus slowing the rate of reaction.

However, Perkins and Trikojus reported a simple method for the preparation of **63** by refluxing diiodomethane and potassium hydroxide with 2,3-dihydroxybenzoic acid in methanol/water.[105] The corresponding acid chloride was prepared by stirring **62** with thionyl chloride; subsequent addition of aqueous ammonia furnishing the target compound **63** in excellent yield.

Figure: 3.8.1 Synthesis of 2,3-dioxymethylenebenzamide

CH_2I_2, KOH, CH_3OH / H_2O, reflux → **62**; i. $SOCl_2$, ii. NH_3 (aq) → **63**

3.9. 2-Fluoro-3-hydroxybenzamide

The synthesis of 2-fluoro-3-hydroxybenzamide **68** was attempted as fluorine is a strongly electronegative species which forms hydrogen bonds readily. Therefore, fluorine substituted in the 2-position of 3-hydroxybenzamide should also constrain the amide into the *anti* conformation *via* an intramolecular hydrogen bond. A well documented method for preparing aromatic fluorides from aryl amines is the Balz-Schiemann decomposition of a diazonium tetrafluoroborate salt.[106,107] However, certain substituents are known to affect adversely the decomposition of the diazonium species, (particularly hydroxy and carboxy moieties). Consequently, 3-methoxy-2-nitrobenzoic acid was used as the starting material, with the methoxy group being deprotected as the final step. 3-Methoxy-2-nitrobenzoic acid was converted into the amide **64** by aminolysis of the corresponding acid chloride. Catalytic reduction of **64** yielded 2-amino-3-methoxybenzamide **65**. Diazotisation of **65** in a solution of fluoroboric acid with sodium nitrite at 0 ^oC produced the tetrafluoroborate salt **66**. Decomposition of this intermediate should have given the fluoro species **67**, but instead afforded in a complex mixture of reaction products which were inseparable by preparative TLC. Further attempts at decomposing **66** in different fluorinated solvents such as trifluoroethanol were unsuccessful.

Figure: 3.9.1. Attempted synthesis of 2-fluoro-3-hydroxybenzamide

O
OH
NO_2
OCH_3

i. $SOCl_2$
reflux
ii. NH_3 (aq)
88 %

O
NH_2
NO_2
OCH_3
64

Pd /C (cat)
H_2; CH_3OH
99 %

O
NH_2
NH_2
OCH_3
65

$NaNO_2$
HBF_4
H_2O
62 %

O
NH_2
N_2BF_4
OCH_3
66

decomposition

O
NH_2
F
OCH_3
67

HBr

O
NH_2
F
OH
68

CHAPTER FOUR

Rearrangement of Benzoxazole-4-carboxamides to 8-Hydroxyquinazolin-4-ones

4.0 Introduction

Certain quinazolinones have been shown to have important cancer chemotherapeutic properties. The most notable is CB3717, which has potent inhibitory activity against the enzyme thymidylate synthase (TS).[109, 110] TS uses 5,10-methylenetetrahydrofolic acid as a cofactor to catalyse the conversion of deoxyuridine monophosphate to deoxythymidylate by reductive methylation.

5,10-Methylenetetrahydrofolic acid

CB 3717

TS inhibition can cause a block in the *de novo* synthesis of deoxythymidylate which, in the absence of exogenous thymine, leads to a 'thymine-less cell death'.

There are many known methods for the preparation of quinazolinones, [111-115] some of which are summarised in Figure 4.0.1.

Figure: 4.0.1 General synthesis of quinazolinones

a. (2-aminobenzamide: $CONH_2$, NH_2) —$EtOCHNR_1$→

b. (2-aminobenzonitrile: CN, NH_2) —$EtOCHNR_1$ or R_1COCl→

c. (methyl anthranilate: CO_2CH_3, NH_2) —$ClC(NH)R_2.HCl$ ($R_1 = H$)→

d. (N-acyl-2-nitrobenzamide: O, O, N, R_1, R_2, NO_2) —3CO, $Ru_3(CO)_{12}$→

e. (N-acyl-2-azidobenzamide: O, O, N, R_1, R_2, N_3) —PPh_3→

(quinazolinone product: O, N, R_1, N, R_2)

A general approach to the preparation if quinazolinones begins with an *ortho* substituted aromatic ring. In examples a, b, and c, (Figure:4.0.1) acylation of the amino group followed by cyclisation formed the quinazolinone skeleton. These methods are the most versatile and widely used ways of preparing quinazolinone derivatives.[111,112] However, there are more elegant, although not necessarily more efficient ways, of preparing these compounds. Example d (Figure:4.0.1) shows the catalytic reductive *N*-heterocyclisation of nitrobenzoyl amides under a carbon monoxide atmosphere to form quinazolinones.[113] Whilst this is one of the first ruthenium-catalysed syntheses of quinazolinones, the use of an expensive catalyst, and with only moderate yields, makes this a less versatile approach to these derivatives.[114] Conversely, example e (Figure:4.0.1) exhibits an efficient high yielding synthesis of quinazolinones *via* an intramolecular aza-Wittig reaction.[115]

Results and Discussion

4.1. The Rearrangement

The rearrangement of benzoxazole-4-carboxamide to 8-hydroxyquinazolin-4-[3*H*]-one was chanced upon under reaction conditions employed to form an amide from an ester. The rearrangement was first observed when methyl 2-methylbenzoxazole-4-carboxylate **42** was reacted with ammonia under high pressure. Under these conditions the product isolated was 8-hydroxy-2-methylquinazolin-4[3*H*]-one **69** rather than the expected benzoxazole-4-carboxamide. The structure of 8-hydroxyquinazolin-4-[3*H*]-one was confirmed by *X*-ray crystallography.

Figure: 4.1.1. X-ray crystal structure of 8-hydroxy-2-methylquinazolin-4[3*H*]-one

(crystallographic data in appendix 2)

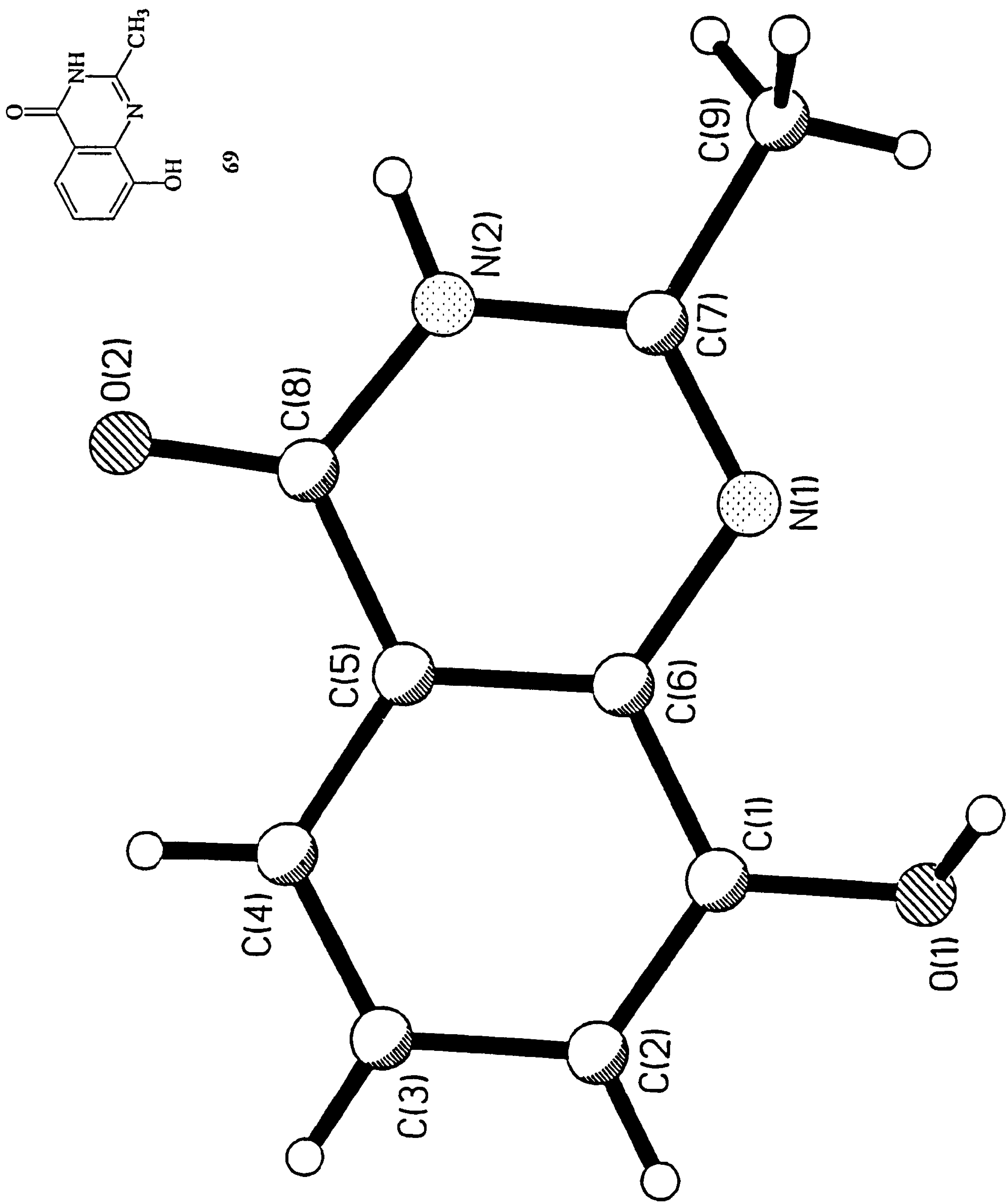


Figure: 4.1.2 Formation of 8-hydroxy-2-methylquinazolin-4[3*H*]-one from methyl 2-methylbenzoxazole-4-carboxylate

$$\text{42} \xrightarrow[\text{20 bar pressure, 55 °C}]{NH_3\text{ (liquid)}} \text{69}$$

Similar conditions were employed for the preparation of 8-hydroxy-2-(4-nitrophenyl)quinazolin-4[3*H*]-one **70** from methyl 2-(4-nitrophenyl)benzoxazole-4-carboxylate **57**.

Figure: 4.1.3 Preparation of 8-hydroxy-2-(4-nitrophenyl)quinazolin-4[3*H*]-one.

$$\text{57} \xrightarrow[\text{20 bar pressure, 55 °C}]{NH_3\text{ (liquid)}} \text{70}$$

A further example of the rearrangement, but under milder reaction conditions, was the preparation of 8-hydroxy-2-methyl-3-methylquinazolin-4[3*H*]-one **71** from methyl-2-methylbenzoxazole-4-carboxylate **42** by stirring with methylamine (33 % in ethanol) at ambient temperature.

Figure: 4.1.4. Preparation of 8-hydroxy-2-methyl-3-methylquinazolin-4[3*H*]-one

Treatment of methyl 2-(4-methoxyphenyl)benzoxazole-4-carboxylate **58** with ammonia under pressure did not afford the quinazolinone. The product isolated was the benzoxazole-4-carboxamide **54**.

Figure: 4.1.5. Preparation of 2-(4-methoxyphenyl)benzoxazole-4-carboxamide

4.2 Putative mechanism of benzoxazole rearrangement

The probable mechanism of the benzoxazole rearrangement may be deduced from the few examples cited. The rearrangement depends upon the electrophilicity of the C2 centre. That is, if the substituent in the 2-position is a good electron donor or sterically bulky the rearrangement does not occur. However, if the C2 carbon is relatively electron deficient then the rearrangement may take place.

In compounds **69** and **70** the rearrangements took place at relatively high pressure. The formation of **71**, however, was at ambient temperature with methylamine as the nucleophile. The ease with which this rearrangement occurred was not unexpected considering that methylamine is a stronger nucleophile than ammonia.

Therefore, the initial phase of the rearrangement may entail nucleophilic attack at the C2 centre to form a ring opened intermediate, analogous to the acid catalysed ring opening of benzoxazoles discussed in Chapter 3 (Figure: 4.2.1).

Figure: 4.2.1 Postulated mechanism of ring opening

Ring closure may occur in either of two ways:

A. attack from the amidine nitrogen to the carbonyl;

B. attack from the amide to the iminium centre.

In either case the mechanism may be facilitated by proton transfer (Figure: 4.2.2).

Figure: 4.2.2. Postulated mechanisms of ring closure

In the proposed mechanisms it was assumed that aminolysis of the carboxylic ester occurred before ring opening. Although likely, this may not be the case, and the unchanged ester may participate in the rearrangement. If this is so then there can only be one mechanism of ring closure, *i.e.* attack from the amidine nitrogen onto the carbonyl with subsequent loss of methanol.

Figure: 4.2.3. Alternative mechanism for the rearrangement

CHAPTER FIVE

5.0 Enzyme Inhibition Studies

5.0.1. Permeabilised Cell Assay

The analogues were evaluated using a permeabilised cell assay by the author and Bowman (Cancer Research Unit, Newcastle upon Tyne). The cell membrane was permeabilised using hypoxic shock and cold conditions. The permeabilised cells were added to a tube containing a solution of drug, NAD^+ cocktail and oligonucleotide, and incubated for exactly 5 mins.[116,117] Inhibitor activity was determined by measuring the degree of ^{32}P incorporation into an acid insoluble pellet. The degree of inhibition was calculated as a percentage of control.

5.1. Inhibition of PADPRP by Benzyloxybenzamide Analogues

3-Hydroxybenzamide **2** has been used as a reference system throughout the *in vitro* evaluation. There was no decrease in inhibitory activity when benzyloxybenzamide **5** was tested in the permeabilised cell assay which suggested that PADPRP was tolerant to larger hydrophobic moieties (Table 5.1.A.). In general, the analogues with electron donating groups have exhibited marginally increased inhibitory activity, for example **6** and **14**. Conversely analogues with electron withdrawing substituents were less active, see **7**, **10** and **12**. These results may be useful when elaborating more active PADPRP inhibitors, for example 8-hydroxyquinazolinones.

Table: 5.1.A. Percentage PADPRP inhibition by benzyloxybenzamide analogues

No	R (position)	compound	% inhibition at 10μM
3	-	3-hydroxybenzamide	35
5	(4) H	3-benzyloxybenzamide	36
6	(4) OCH_3	3-(4-methoxybenzyloxy)benzamide	60
7	(4) NO_2	3-(4-nitrobenzyloxy)benzamide	26
8	(4) F	3-(4-fluorobenzyloxy)benzamide	14
9	(4) Br	3-(4-bromobenzyloxy)benzamide	26
10	(4) CF_3	3-(4-trifluoromethyl benzyloxy)benzamide	3
11	(4) CN	3-(4-cyanobenzyloxy)benzamide	23
12	(3) NO_2	3-(3-nitrobenzyloxy)benzamide	12
13	(2) NO_2	3-(2-nitrobenzyloxy)benzamide	insoluble
14	(3,4) OCH_2O	3-(3,4-dioxymethylenebenzyloxy) benzamide	50
15	n = 3	3-cinnamyloxy benzamide	23
16	n = 2	3-phenethyloxy benzamide	30
17	N_3	3-(4-azidobenzyloxy)benzamide	43
20	(4) NH_2	3-(4-aminobenzyloxy)benzamide	40
21	(4) COOH	3-(4-carboxybenzyloxy)benzamide	0
23	(4) CH_3CO	3-(4-*N*-acetylamino benzyloxy)benzamide	49
25	(4) $COOCH_3$	3-(4-methylcarboxy benzyloxy)benzamide	insoluble

Several of the analogues evaluated were insoluble in the assay medium, so to overcome this 3-(carboxybenzyloxy)benzamide **21** was prepared. Whilst **21** was more soluble in the physiological buffers the *in vitro* evaluation was poor. Compound **21** possesses an electron withdrawing carboxy group which possibly reduces the % inhibition, but may also non-specifically bind to other proteins and further lower the degree of PADPRP inhibition.

Extension of the chain length has been explored, initially by preparing phenethylBOB **16** and cinnamylBOB **15**. A short extension of chain length was tolerated by PADPRP without compromising potency, but this still required further exploration.

5.2 Inhibition of PADPRP by Alkoxybenzamide Analogues

Table: 5.2.A. PADPRP inhibition by alkoxybenzamide analogues

O
NH$_2$
O
(CH$_2$)$_n$
CH$_3$

No	n	compound	% inhibition at 10 μM
26	3	3-butyloxybenzmide	23
27	4	3-pentyloxybenzamide	18
28	5	3-hexyloxybenzamide	15
29	6	3-heptyloxybenzamide	25
30	7	3-octyloxybenzamide	insoluble

The trend towards a decrease in inhibitory activity was observed as chain length increased. This decreasing activity may be due to the insolubility of the analogues, because as chain length increased, solubility decreased. The lack of activity may also be due to the formation of a hydrophobic tail created by the long chains coiling up, thus preventing the drug from binding to or indeed entering the cofactor binding region of the enzyme.

5.3 Inhibition of PADPRP by 2-Substituted Benzoxazole Analogues

Table: 5.3.A. PADPRP inhibition by 2-Substituted Benzoxazole Analogues

No	R_1	R_2	compound	% inhibition at 10 μM
39	$CONH_2$	CH_3	2-methylbenzoxazole-4-carboxamide	95 (IC_{50} 9.8 μM)
45	$CONH_2$	$C(CH_3)_3$	2-^{t}butylbenzoxazole-4-carboxamide	87 (IC_{50} 8.4 μM)
43	$CONH_2$	Ph	2-phenylbenzoxazole-4-carboxamide	82 (IC_{50} 2.1 μM)
53	$CONH_2$	$PhOCH_3$	2-(4-methoxyphenyl)benzoxazole-4-carboxamide	Insoluble
52	$CONH_2$	$PhNO_2$	2-(4-nitrophenyl)benzoxazole-4-carboxamide	Synthesis not completed
40	$CONHCH_3$	CH_3	2-methylbenzoxazole-4-*N*-methylcarboxamide	18 at 100 μM
38	CO_2H	CH_3	2-methylbenzoxazole-4-carboxylic acid	0
51	CO_2H	$PhOCH_3$	2-(4-methoxyphenyl)benzoxazole -4-carboxylic acid	0
37	H	CH_3	2-methylbenzoxazole	0

A series of novel inhibitors were designed where the amide group was restricted into the *anti* conformation by an intramolecular hydrogen bond (Table 5.3.A.). 2-Methylbenzoxazole-4-carboxamide **39** was the simplest benzoxazole prepared, and the inhibitory activity was promising with an IC_{50} of 9.8 μM. Further analogues were made, namely, 2-phenylbenzoxazole-4-carboxamide **43** and 2-^{t}butylbenzoxazole-4-carboxamide **45**, in order to determine the effects of hydrophobicity and steric bulk on potency. Compound **45** exhibited good activity, which was comparable with 3-hydroxybenzamide **2**, but compound **43** was the most active benzoxazole analogue of the series. It is possible that PADPRP possesses a hydrophobic pocket with which the 2-substituted moiety associates. Alternatively, only the amide moiety binds to the active site, and the higher electron density at the carbonyl functionality enhances the binding affinity, thus increasing inhibitory activity.

Further evidence has been obtained for the requirement of an amide group for good inhibitory activity. Removal of the amide as for 2-methylbenzoxazole **37**, abolished inhibitory activity, as did replacing the amide with a carboxylic acid as in **38** and **51**. A very interesting result was obtained with 2-methylbenzoxazole-4-*N*-methylcarboxamide **40**, where one of the amide hydrogens was replaced by a methyl group, resulting in a severe loss in inhibitory activity. Because the methyl group is present there may be reduced hydrogen bonding in the active site of the enzyme, hence loss of activity.

Hydrogen bonding with the enzyme

Hydrogen bonding blocked by the CH_3 group

5.4 Inhibition of PADPRP by 8-hydroxy-2-[substituted] quinazolin-4[3*H*]ones

The most potent PADPRP inhibitors prepared were the product of an interesting rearrangement. Although the natural product 2-methylquinazoline-4[3*H*]one has already been reported as a PADPRP inhibitor,[118] the derivatives 8-hydroxy-2-(substituted)quinazolin-4[3*H*]ones **69-71** have not.

Table: 5.4.A Inhibition of PADPRP by 8-hydroxy-2-[substituted] quinazolin-4[3*H*]ones

No	R_1	R_2	compound	% inhibition at 10 μM
69	H	CH_3	8-hydroxy-2-methylquinazolin-4[3*H*]one	92 (IC_{50} = 0.4 μM)
70	H	$PhNO_2$	8-hydroxy-2-(4-nitro phenyl)quinazolin-4[3*H*]one	92 (IC_{50} = 0.2 μM)
71	CH_3	CH_3	8-hydroxy-2-methyl-4-*N*-methylquinazolin-4-one	5

Compounds **69** and **70** were outstandingly active *in vitro* with IC_{50} values of 0.4 and 0.2 μM respectively. Further experiments were performed to determine the effect of a PADPRP inhibitor, specifically **69**, on whole cells, and whether by inhibiting PADPRP cell death could be attained.

5.5 Cytotoxicity Testing

Temozolomide (8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4-(3*H*)-one), is a member of the antitumour imidazotetrazinone series which are currently undergoing clinical trials as novel antitumour agents.[119-120]

Figure: 5.5.1. Known monofunctional DNA alkylators

R_1HN O N N N N N R_2 O

$R_1 = H$ $R_2 = (CH_2)_2Cl$ Mitozolomide
$R_1 = H$ $R_2 = CH_3$ Temozolomide

Initially temozolomide is activated *in situ* by nucleophilic attack at the ring C4 carbonyl. From this point the action of temozolomide could progress by successive S_N2 mechanisms (Figure: 5.5.2). Athough there is little evidence for either mechanism, the reactive species initially after activation is structurally similar to 5-(3,3-dimethyltriazen-1-yl)-imidazole-4-carboxamide (DTIC) which is a precursor of a potent alkylating agent. These mechanisms may occur in a laboratory under specific chemical conditions, however, it is probably a different situation *in vivo*.

Figure: 5.5.2. Possible mechanisms of temozolomide activation

activation

activation

DNA base

DNA base

DTIC

Potentiation Studies

Temozolomide is a good DNA monofunctional alkylating agent, whose effects can be compromised by DNA repair mechanisms. Inhibition of a repair mechanism, such as PADPRP, may result in an increased level of DNA damage and hence increased cytotoxicity.

Clonogenic assays were performed to determine if 8-hydroxy-2-methylquinazolin-4-[3*H*]-one **69** was cytotoxic and, more importantly, whether it potentiated cytotoxicity in temozolomide treated cells. The assays determined the effects of inhibitor on the ability of cells to grow and divide to form colonies. In the control assay, the cells have no inhibitor added, and the numbers of colonies are directly proportional to the number of cells plated out. However, in assays which have had inhibitors added, and if the inhibitors are cytotoxic, the numbers of colonies will be

considerably reduced. The number of colonies were counted and expressed as a percentage of the control. Figure:5.5.3 shows that increasing concentrations of quinazolinone 69 alone are relatively non cytotoxic upto 1 mM, and even at concentrations as high as 2 mM the clonogenic potential was only reduced by about 50 %. It should be noted that the concentrations of quinazolinone 69 used in the clonogenic assays are non-cytotoxic (below 0.5 mM), and any potentiation effects observed can be directly attributed to the inhibition of PADPRP by quinazolinone 69.

Figure:5.5.3. Cytoxicity of 8-hydroxy-2-methylquinazolin-4[3*H*]-one

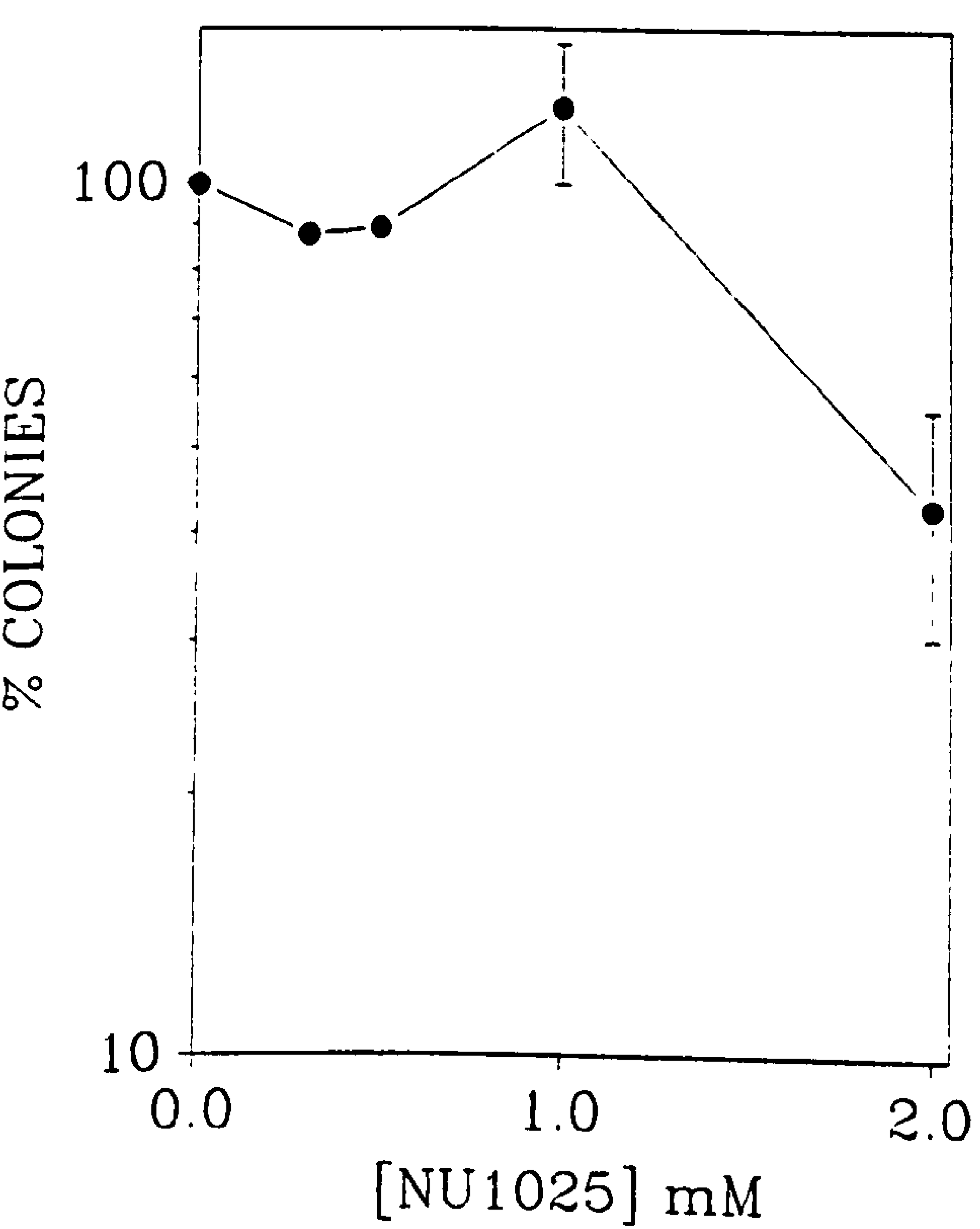
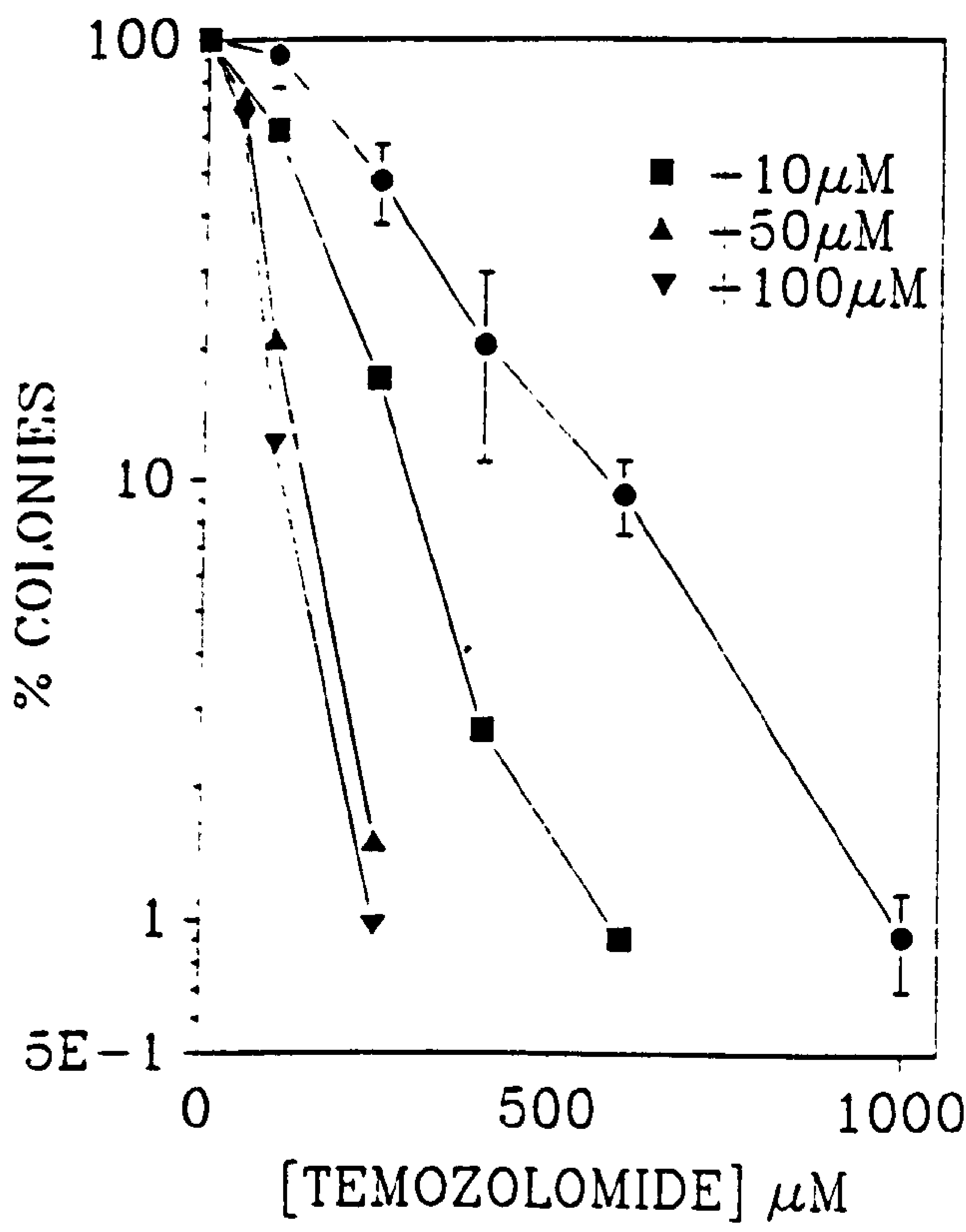


Figure: 5.5.4 Synergistic enhancement of temozolomide by 8-hydroxy-2-methylquinazolin-4[3*H*]-one (69)



[69]	IC_{10} Temozolomide alone	IC_{10} Temozolomide + 69	Dose enhancement factor
10 μM	618	367	1.4
50 μM	470	125	4.0
100 μM	426	96	5.2

As can be seen from Figure:5.5.4, treatment of the cells for 16 h with 250 μM temozolomide alone caused a 50 % reduction in colonies and to reduce the number of colonies to 99 % of the original required 1000 μM temozolomide (-•-). Co-incubation of L1210 cells with 250 μM temozolomide and 10 μM **69** caused an 85 % reduction in colonies (-▪-). A greater proportion of cell kill was observed at a higher concentration of **69** but the same concentration of temozolomide (250 μM); at 50 μM the number of colonies was reduced by 99 %(-◆-), and similarly for 100 μM **69**. The effect observed is dose related, and this data can be expressed as dose enhancement by examining the concentration of drug required to reduce the number of cells to 10 % of original (IC_{10}) for both temozolomide alone, and for the co-incubated cells. The IC_{10} for temozolomide alone is 618 μM, but on co-incubation with 10 μM **69** the IC_{10} was reduced to 367 μM of temozolomide, which is a dose enhancement of 1.4; at 50 μM **69** the IC_{10} was reduced to 125 μM of temozolomide which is a 4-fold enhancement and, similarly at 100 μM **69** there was a 5-fold enhancement.

It should be noted that these results are still preliminary and the large error between the temozolomide IC_{10}'s can be attributed, in part, to the condition of the cells used in the assay. If the cells are not in perfect condition this will be reflected in the variability of the results. Further work indicates that the dose enhancement factor may be considerably increased.

Summary

Some of the novel PADPRP inhibitors designed and synthesised have proved to be extremely active in vitro and show good potentiation of monofunctional alkylating agents. *O*-Alkylation of 3-hydroxybenzamide produces inhibitors that are comparable to the parent inhibitor. Of the benzyloxybenzamide analogues, those compounds with electron donating substituents enhanced activity slightly, whereas electron withdrawing substituents retarded inhibition. Whether there is a tentative structure activity relationship is difficult to say, but these results reinforce the idea that a good PADPRP inhibitor requires a strong electronegative carbonyl for binding to the enzyme active site.

BOB

A further method of improving the inhibitory activity against PADPRP was *via* restriction of the amide bond into the *anti* conformation. This was achieved by the formation of a series of benzoxazole-4-carboxamides where the amide was conformationally restricted into the required conformation by an intramolecular hydrogen bond. This hydrogen bond was confirmed by NMR spectroscopy and *X*-ray crystallography.

benzoxazole-4-carboxamide

The benzoxazole analogues prepared were significantly more active than 3-hydroxybenzamide, with 2-phenylbenzoxazole-4-carboxamide being the most potent inhibitor having an IC_{50} of 2.1 μM. From these results it can be concluded that constraining the amide into the *anti* conformation does increase inhibitory activity.

The necessity of at least one amide proton has been confirmed. In the benzoxazole series a substantial loss of activity was observed when the amide was monosubstituted, and activity was abolished when the amide or indeed the carbonyl are replaced or removed. It may be concluded that substitution of the amide will be tolerated so long as the amide conformation remains unaffected.

A novel series of potent PADPRP inhibitors were prepared as the product of an interesting rearrangement. The quinazolin-4[3*H*]-ones obtained have an amide as part of a ring system and are outstandingly active, however, substitution of the remaining amide proton caused loss of inhibitory activity. The inhibitors, particularly 8-hydroxy-2-methylquinazolin-4[3*H*]-one have been shown to potentiate the effects of temozolomide with a dose enhancement factor of upto 5.

8-hydroxy-2-substituted quinazolin-4[3*H*]-one

Whilst the *in vitro* data is still preliminary, these compounds may prove to be a useful lead for improved resistance modifing agents in cancer chemotherapy.

Future Investigations

The most obvious approach would be to continue the quinazolinone series of analogues. The synthetic targets should include preparation of 8-hydroxy-2-phenylquinazolin-4[3*H*]-one and 8-hydroxy-2-(4-methoxyphenyl)quinazolin-4[3*H*]-one. Another valuable analogue would be 8-hydroxy-2-(4-azidophenyl)quinazolin-4[3*H*]-one since it would be predicted that this analogue would have the same order of activity as 8-hydroxy-2-methyl)quinazolin-4[3*H*]-one, providing a useful tool for radiolabelling studies

R = H
R = OCH_3
R = N_3

Completion of the outstanding analogues should also be considered, the compounds 2-(4-nitrophenyl)benzoxazole-4-carboxamide and 2-(4-carboxymethylphenyl)-benzoxazole-4-carboxamide may shed more light on the mode of binding and confirm any putative structure activity relationships.

Although the rearrangement of the benzoxazole to the quinazolinone occurred *via* an interesting rearrangement, these conditions do not provide an efficient synthesis to these novel potent PADPRP inhibitors. Therefore, alternative routes to this compound should be explored.

CHAPTER SIX

Experimental

7.1 Biological assay

Hypotonic buffer: 9 mM Hepes; 4.5 mM dextran; 4.5 mM $MgCl_2$; 5 mM DTT added prior to use, pH adjusted to 7.8.

Isotonic buffer: 40 mM Hepes; 130 mM KCl; 4 % dextran; 2 mM EGTA; 2.3 mM $MgCl_2$; 225 mM sucrose; 2.5 mM DTT added prior to use, pH adjusted to 7.8.

NAD cocktail: NAD diluted with water to 600 µM. To this ^{32}P NAD (2-5 µl/ml) was added.

Exponentially growing L1210 cells (~ 8 x 10^6/ml) were spun down 1,500 rpm for 5 min. The pellet was resuspended in PBS and respun. The pellet was resuspended at 3 x 10^7 cells/ml in cold hypotonic buffer and left on ice for 30 min. 9 Volumes of ice cold isotonic buffer were added and the cells checked for number and permeabilisation with a haemocytometer and trypan blue as a stain.

The cells were prewarmed at 26 °C for 7 min. before use and the assay was performed at 26 °C in prewarmed tubes.

To each tube was added:

Oligonucleotide 5 µM (200 µg/ml); NAD cocktail 50 µl; DMSO + drug 8µl; water 37 µl. The assay was started by the addition of cell suspension 300 µl. The assay tubes were shaken at 26 °C for 5 min. The reaction was stopped by addition of ice cold 10 % TCA 10 %, NaPPi solution and the tubes put on ice for 1 h.

The contents of the tubes were filtered through Whatman GF/C filters (rough side up), which were rinsed 6 times with 1 % TCA, 1 % NaPPi solution. The filters were dried, tranferred to vials and the β emmision counted.

7.2 Synthesis

All NMR spectra were run on a Bruker Spectrospin AC 200E spectrometer, the solvent used was $CDCl_3$ unless stated otherwise; *J* values are given in hertz and δ values are stated in p.p.m. Mass spectra were obtained on an AE1 MS9 or Kratos MS80 in EI mode (unless otherwise stated) or on a Kratos machine using MNBA matrix in FAB mode. Infrared spectra were obtained using a Nicolet 205X spectrophotometer as KBr discs. Elemental analysis were determined with a Carlo-Erba Strumentazione model 1106 elemental analyser. Thin layer chromatography was carried out using TLC aluminium sheets pre-coated with Kieselgel 60 F_{254}, 0.2 mm and column chromatography was performed using Kieselgel 60.

All solvents were distilled: Ethanol and methanol were dried using Mg/I_2 according to 'Vogel', 4th Edn., p 269, and stored over 3 Å molecular sieves. Diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Dichloromethane was pre-dried over K_2CO_3 and distilled from CaH_2. Acetonitrile was pre-dried over K_2CO_3, distilled from CaH_2 and stored over 3 Å molecular sieves. Pyridine was distilled from CaH_2 and stored over KOH pellets. Benzene was static dried over alumina. The petrol used was in the boiling range 40-60 °C.

Experimental For Chapter Two

3-Acetoxybenzoic acid 4

3-Hydroxybenzamide (1.0 g, 7.3 mmol) was added to an ice cooled solution of sodium hydroxide (0.61 g, 15.25 mmol in 10 ml water). Acetic anhydride (0.81 g, 9.93 mmol) was added with crushed ice (2 g), and the solution stirred at 0 °C for 1 h. Acidification with hydrochloric acid (3 ml, 6M) yielded a white precipitate which was extracted into dichloromethane (3 x 30 ml). The organics were combined, dried, filtered and the solvent removed under reduced pressure to yield a white solid which recrystallised from boiling water.(0.75 g, 56%).

m.p. 122-125°C; ν_{max}/cm^{-1} 3020, 2883, 2662, 2548, 1761, 1680, 1585, 1485, 1454, 1412, 1373; *m/z* 180 (30%, M^+), 138 (100%, M^+-$COCH_3$), 121 (82%, M^+-$COCH_3OH$), 110, 92, 81, 65, 43 (98%, $[COCH_3]^+$); δ_H d_6-DMSO 2.3 (s, 3H, CH_3), 7.45 (m, 1H), 7.6 (m, 1H), 7.7 (m, 1H), 7.9 (m, 1H), 13.3 (br.s, 1H, OH); δ_C d_6-DMSO 26.09, 127.90, 131.65, 131.87, 135.07, 137.49, 155.72, 171.79; Found M^+ 180.0420 $C_9H_8O_4$ requires 180.0422.

3-Hydroxybenzamide **2**

Compound 4 (0.5 g, 2.8 mmol) was dissolved in thionyl chloride (1.74 ml, 20 mmol) and the mixture refluxed for 4 h. The excess thionyl chloride was removed *via* vacuum distillation to yield a yellow oil. The oil was added dropwise to an ice cooled solution of ammonia (35 %, 20 ml). The solution was reduced in volume by evaporation and the title product **2** allowed to crystallise out of solution. The product was collected and recrystallised from water to yield colourless plates (0.14 g, 37%).

m.p. 156-158°C; ν_{max}/cm^{-1} 3402, 3244, 1653, 1614, 1597, 1506, 1450, 1402, 1352; *m/z* (EI) 137 (96 %, M^+), 121 (100 %, M^+-NH_2), 93 (10 %, M^+-CO), 65, 44; δ_H d_6-DMSO 7.0 (m, 1H), 7.4 (m, 4H), 9.8 (br.s, 1H); δ_C d_6-DMSO 114.81, 118.30, 118.41, 129.48, 136.11, 157.15, 168.33; Anal. found C 61.48, H 14.97, N 10.27, $C_7H_7NO_2$ requires C 61.29, H 15.15, N 10.22 %.

Preparation of substituted benzyloxybenzamide - General Procedure A.

Aryl bromide (1 mmol, 1 eq.) was added to a solution of 3-hydroxybenzamide (0.137 g, 1 mmol, 1 eq) and potassium carbonate (0.138 g, 1 mmol) in anhydrous acetonitrile (10 ml) and refluxed for 5h. Once the reaction mixture had cooled the acetonitrile was removed under reduced pressure and the solids suspended in water (20 ml). The organics were extracted into dichloromethane (3 x 30 ml), dried and

concentrated under reduced pressure to yield a solid. Recrystallisation from ethyl acetate / petrol yielded the product as a solid.

3-(Benzyloxy)benzamide 5

The title product was prepared using benzyl bromide (0.137 g, 1.0 mmol) as the electrophile in general procedure A. Compound **5** was isolated as a white powder (0.253 g, 83 %).

m.p. 131-132 °C; υ_{max}/cm^{-1} 3317, 3119, 1635, 1610, 1595, 1419, δ_H d_6-DMSO 5.25 (s, 2H, OCH_2Ph), 7.25 (m, 1H), 7.4 (m, 9H, NH), 8.15 (s, 1H); δ_C d_6-DMSO 69.65, 113.99, 118.12, 120.28, 128.04, 128.22, 128.78, 129.70, 136.05, 137.23, 158.58, 167.90; Anal. found C 74.41, H 5.53, N 5.94, required for $C_{14}H_{13}NO_2$ C 73.98, H 5.77, N 6.17 %

3-(4-Methoxybenzyloxy)benzamide 6

The title product was prepared using 4-methoxybenzyl bromide (0.201 g, 1.0 mmol) as the electrophile in general procedure A. Compound **6** was isolated as a white powder (0.162 g, 63 %).

m.p. 156-158 °C; δ_H d_6-DMSO 3.85 (s, 3H, OCH_3), 5.18 (s, 2H, OCH_2Ph), 7.05 (d, 2H, J 7.6), 7.25 (m, 9H), 8.05 (s, 1H). δ_C d_6-DMSO 55.53, 69.25, 112.97, 117.38, 120.0, 128.23, 129.62, 129.25, 131.25, 136.05, 159.46, 167.96; Anal. found C 70.33, H 5.79, N 5.33, $C_{15}H_{15}NO_3$ requires C 70.01, H 5.88, N 5.45 %.

3-(4-Nitrobenzyloxy)benzamide 7

The title product was prepared using 4-nitrobenzyl bromide (0.184 g, 1.0 mmol) as the electrophile in general procedure A. Compound 7 was isolated as a white powder (0.156 g, 55 %).

m.p.146-149 °C; υ_{max}/cm^{-1} 3474, 3356, 1651, 1624, 1601, 1585, 1518, 1495, 1444, 1400; *m/z* 272 (5 %, M^+), 137 (100 %, $[OHC_6H_4CONH_2]^+$), 77, 51; δ_H d_6-DMSO 5.43 (s, 2H, OCH_2Ph), 7.27 (m 1H), 7.53 (m, 4H), 7.83 (d, 2H, J 7.8), 8.07 (s, 1H), 8.39 (d, 2H, J 8.1); δ_C d_6-DMSO 68.46, 114.04, 118.15, 120.59, 123.96, 128.53, 129.79, 136.16, 145.17, 147.34, 158.14, 167.77; Anal. found C 61.58, H 4.32, N 9.94, $C_{14}H_{12}N_2O_4$ requires C 61.75, H 4.44, N 10.29 %.

3-(4-Fluorobenzyloxy)benzamide 8

The title product was prepared using 4-fluorobenzyl bromide (0.189 g, 1.0 mmol) as the electrophile in general procedure A. Compound **8** was isolated as white needles (0.142 g, 58 %).

m.p. 161-162°C; υ_{max}/cm^{-1} 3366, 3171, 1658, 1626, 1603, 1583, 1518. *m/z* 245 (20 %, M^+), 109 (100%, $[CH_2C_6H_4F]^+$), 91, 77; δ_H *d$_6$*-DMSO 5.23 (s, 2H, OCH_2Ph), 7.5 (m, 9H), 8.11 (m, 1H); δ_C *d$_6$*-DMSO 68.914, 113.951, 115.405, 118.124, 120.329, 129.329, 130.255, 133.487, 136.056, 158.464, 164.546, 167.853; Anal. found C 67.79, H 4.81, N 5.56, $C_{14}H_{12}FNO_2$ requires C 67.2, H 4.8, N 5.6 %.

3-(4-Bromobenzyloxy)benzamide 9

The title product was prepared using 4-bromobenzyl bromide (0.250 g, 1.0 mmol) as the electrophile in general procedure A. Compound **9** was isolated as a white powder (0.253 g, 83 %).

m.p. 160-161°C; υ_{max}/cm^{-1} 3323, 3146, 1670, 1622, 1601, 1581, 1489, 1462, 1446; *m/z* 307 (63%, MH^+), 262, 212, 169, (100%, $[BrC_6H_4CH_2]^+$), 101, 90; δ_H *d$_6$*-DMSO 5.26 (s, 2H, OCH_2Ph), 7.3 (d, 1H, *J* 6.6), 7.6 (m, 9H); δ_C *d$_6$*-DMSO

68.83, 114.03, 118.12, 120.40, 121.25, 129.70, 130.10, 131.69, 136.09, 136.72, 158.37, 167.84; Anal. found C 54.61, H 3.66, N 4.47, $C_{14}H_{12}BrNO_2$ requires C 55.26, H 3.29, N 4.47 %.

3-(4-Trifluoromethylbenzyloxy)benzamide **10**

The title product was prepared using bromotrifluoro-4-xylene (0.239 g, 0.155 ml, 1.0 mmol) as the electrophile in general procedure A. Compound **10** was isolated as a white solid (0.219 g, 74 %).

m.p. 177-179 ^{o}C; *m/z* 295 (38 %, M^+), 159 (100%, $[CH_2C_6H_4CF_3]^+$), 135, 109, 91, 77; δ_H d_6-DMSO 5.1 (s, 2H, OCH_2Ph), 7.3 (m, 1H), 7.6 (m, 4H), 7.8 (d, 2H, *J* 6.8), 7.9 (d, 2H, *J* 6.5), 8.1 (s, 1H); δ_C d_6-DMSO 68.72, 114.01, 118.12, 120.51, 125.62, 125.69, 128.3, 129.0, 129.77, 136.13, 142.13, 158.29, 167.85; Anal. found C 61.05, H 3.94, N 5.06, $C_{15}H_{12}F_3NO_2$, requires C 61.02, H 4.06, N 4.75 %.

3-(4-Cyanobenzyloxy)benzamide **11**

The title product was prepared using bromo-4-toluonitrile (0.196 g, 1.0 mmol) as the electrophile in general procedure A. Compound **11** was isolated as a white powder (0.211 g, 84 %).

m.p. 156-158 ^{o}C; υ_{max}/cm^{-1} 3362, 3179, 2228, 1736, 1647, 1626, 1601, 1585, 1510, 1491, 1452, 1442, 1421, 1402, 1383; *m/z* 252 (18%, M^+), 153, 116 (75%, $[CNC_6H_4CH_2]^+$), 89, 70, 61, 43 (100%, $[O{=}C{=}NH]^+$); δ_H d_6-DMSO 5.3 (s, 2H, OCH_2Ph), 7.2 (m, 1H), 7.43 (m, 4H), 7.7 (d, 2H, *J* 6.2), 7.9 (d, 2H, *J* 6.5), 8.1 (s, 1H); Anal. found C 71.27, H 4.96, N 10.67, $C_{15}H_{12}F_3N_2O_2$ requires C 71.43, H 4.76, N 11.11 %.

3-(3-Nitrobenzyloxy)benzamide **12**

The title product was prepared using 4-bromobenzyl bromide (0.250 g, 1.0 mmol) as the electrophile in general procedure A. Compound **9** was isolated as a white powder (0.253 g, 83 %).

m.p. 178-179°C; υ_{max}/cm^{-1} 3368; 3196, 1651, 1626, 1612, 1601, 1583, 1518, 1491, 1452; *m/z* 272 (3.8%; M^+), 181, 136 (100%, M^+-$CH_2C_6H_4NO_2$), 89, 78; δ_H d_6-

DMSO 5.5 (s, 2H, OCH_2Ph), 7.14 (m, 1H), 7.4 (m, 4H), 7.62 (m, 2H), 8.0 (br.s, 1H), 8.02 (m, 1H); δ_C d_6-DMSO 66.78, 113.85, 118.17, 120.75, 125.18, 129.49, 129.54, 132.67, 134.33, 136.13, 147.78, 158.11, 167.77; Anal. found C 61.53, H 4.11, N 9.98, $C_{14}H_{12}N_2O_4$ requires C 61.75, H 4.44, N 10.29 %.

3-(2-Nitrobenzyloxy)benzamide **13**

The title product was prepared using 2-nitrobenzyl bromide (0.216 g, 1.0 mmol) as the electrophile in general procedure A. Compound **13** was isolated as a yellow powder (0.173 g, 64 %).

m.p. 154-156 ^oC; υ_{max}/cm^{-1} 3379, 3198, 2846, 1678, 1647, 1622, 1610, 1581, 1518, 1495; *m/z* 272 (3.8%, M^+), 248, 217, 196, 181, 136 (100%); δ_H d_6-DMSO 5.2 (s, 2H, OCH_2Ph), 7.20 (m, 1H), 7.45 (m, 5H), 7.6 (m, 2H), 8.0 (br.s, 1H), 8.1 (d, 1H, *J* 7.8); δ_C d_6-DMSO. 73.78, 114.85, 119.23, 122.75, 125.28, 129.49, 129.24, 129.54, 132.47, 134.34, 136.24, 147.64, 158.31, 167.77; Anal. found C 61.49, H 4.42, N 10.11, $C_{14}H_{12}N_2O_4$ requires C 61.75, H 4.44, N 10.29 %.

3-(3,4-Methylenedioxyphenylmethyloxy)benzamide **14**

The title product was prepared using (3,4-methylenedioxy)benzyl chloride (0.207 g, 1.0 mmol) as the electrophile in general procedure A. Compound **14** was recrystallised from water and isolated as a white powder (0.265 g, 97 %).

m.p. 141-142 ^{o}C; *m/z* 271 (6 %; M^+), 136 (100%, $[OHC_6H_4CONH_2]^+$), 105, 77; δ_H d_6-DMSO 5.1 (s, 2H, OCH_2Ph), 6.1 (s, 2H, OCH_2O), 7.1 (m, 3H) 7.25 (m 1H), 7.5 (m, 4H), 8.05 (m, 1H); δ_C d_6-DMSO 69.023, 101.36, 108.41, 108.81, 114.01, 118.19, 120.22, 121.90, 129.61, 136.02, 147.21, 147.64, 158.55, 167.89; Anal. found C 66.18, H 4.47, N 4.94, $C_{15}H_{13}NO_4$ requires C 66.42, H 4.78, N 4.94 %.

3-Cinnamyloxybenzamide **15**

The title product was prepared using (3-chloropropenyl)benzene (0.152 g, 0.138 ml, 1.0 mmol) as the electrophile in general procedure A. Compound **15** was isolated as a white powder (0.114 g, 45 %).

m.p. 131-133 ^{o}C; υ_{max}/cm^{-1} 3381, 3196, 2914, 2864, 1630, 1612, 1597, 1579, 1489, 1444, 1392, 1373, 1329; *m/z* 254 (28%, MH^+), 137 (39%, $[CONH_2C_6H_4OH]^+$), 117 (100%, $[CH_2CHCHC_6H_5]^+$), 91 (50%), 77; δ_H d_6-DMSO 4.88 (d, 2H, $OCH_2CHCHPh$, *J* 5.4), 6.65 (dt, 1H, $OCH_2CHCHPh$, *J* 5.6,

16), 6.89 (d, 1H, $OCH_2CHCHPh$, *J* 16), 7.2 (m, 1H), 7.40 (m, 9H), 8.10 (br.s, 1H); δ_C d_6-DMSO 73.43, 118.86, 122.97, 125.17, 130.04, 131.75, 133.17, 133.96, 134.66, 137.69, 141.02, 141.39, 163.43, 172.88; Anal. found C 74.68, H 6.37, N 5.79, $C_{16}H_{15}NO_2$ requires C 74.65, H 6.27, N 5.81 %.

3-Phenethylbenxyloxybenzamide **16**

The title product was prepared using (2-bromoethyl)benzene (0.185 g, 0.136 ml, 1.0 mmol) in general procedure A. Compound **16** was isolated as a white powder (0.034 g, 14 %).

m.p. 132-1341 °C; υ_{max}/cm^{-1} 3327, 3154, 2955, 2930, 2883, 1666, 1630, 1599, 1583, 1491, 1450, 1402, 1387; *m/z* 241 (9%, M^+), 105 (100%, $[OCH_2CH_2HC_6H_4]^+$), 91 (60%), 91, 77; δ_H d_6-DMSO 3.08 (t, 2H, OCH_2CH_2Ph, *J* 7.0), 4.1 (t, 2H, OCH_2CH_2Ph, *J* 7.0), 6.00 (br.s, 2H), 6.99 (m, 1H), 7.20 (m, 8H); δ_C d_6-DMSO 68.35, 70.24, 113.90, 118.09, 119.92, 123.67, 129.22, 129.59, 129.91, 135.95, 139.00, 158.81, 167.96; Anal found C 74.68, H 6.37, N 5.79, $C_{15}H_{15}NO_2$ requires C 74.65, H 6.27, N 5.81 %.

3-(4-Azidobenzyloxy)benzamide **17**

The title product was prepared using 4-azidobenzylbromide **19** (0.2 g, 1.0 mmol) as the electrophile in general procedure A. Compound **17** was isolated as a yellow solid (0.136 g, 51 %).

m.p. 167-169 ^{o}C; υ_{max}/cm^{-1} 3341, 3155, 2121, 2094, 1664, 1631, 1610, 1583, 1506, 1450; *m/z* 269 (6%, MH^+), 252 (6.1%, M^+-NH_2), 233 (M^+-$CONH_2$), 167 (M^+-N_2), 137 (38 %, $[OHC_6H_4CONH_2]^+$), 104 (100%, $[N_3C_6H_4CH_2]^+$), 93, 77; δ_H d_6-DMSO 5.27 (s, 2H, OCH_2Ph), 7.27 (m, 3H), 7.5 (m, 6H), 8.12 (s, 1H); δ_C d_6-DMSO 69.09, 114.04, 118.16, 119.52, 120.35, 129.73, 129.88, 134.11, 136.09, 139.33, 158.48, 167.88; Anal. found C 62.66, H 4.51, N 20.89, $C_{14}H_{12}N_4O_2$ requires C 62.14, H 4.30, N 20.7 %.

*4-Azidotoluene*18 [79]

p-Toluidine (1.0 g, 9.3 mmol, 1 eq.) was added to a solution of hydrochloric acid at 0 oC and stirred until dissolved. Sodium nitrite (0.709 g, 10.28 mmol, 1.1 eq.) in 3 ml water) was added dropwise over 15 min. and the solution stirred at 0 ^{o}C for 30 min. Sodium azide (2.43 g, 2.43 mmol, 4.0 eq.) was added slowly over a period of 30 min [CARE use glass spatula], and the reaction stirred for 1 h. Water was

added (100 ml) and sodium hydrogen carbonate was added until the pH = 6. The organics were extracted into dichloromethane (3 x 50 ml), combined, dried, filtered and the solvent removed under reduced pressure to yield a yellow oil. Flash chromatography using petrol as the eluent yielded **18** as an amber oil (0.77 g, 62 %).

m/z 134 (6%, MH^+), 104 (93 %, M^+-N_2), 90, 78 (100% $[C_6H_6]^+$); δ_H 2.5 (s, 3H, CH_3), 7.02 (d, 2H, *J* 6.4), 7.9 (d, 2H, *J* 6.4).

4-Azidobenzyl bromide **19** [79]

CH_2Br

N_3

N-Bromosuccinimide (0.44 g, 2.48 mmol) and azoisobutyronitrile (0.035 g, 0.214 mmol) were added to a solution of **18** (0.3 g, 2.2 mmol) in benzene [**CARE** potent carcinogen], and the mixture refluxed for 7 h. The solvent was removed by vacuum distillation and the remaining oil dissolved in diethyl ether (50 ml). The ethereal phase was washed with water (3 x 20 ml). The organics were dried, filtered and the solvent removed under reduced pressure to yield an oil which was purified by column chromatography using petrol as the eluent. Compound **19** was isolated as a yellow oil (0.27 g, 57 %).

δ_H 4.67 (s, 2H, CH_2), 7.02 (d, 2H, *J* 8.3), 7.66 (d, 2H, *J* 8.2).

3-(4-Aminobenzyloxy)benzamide **20** [83]

Triethylamine (0.302 g, 0.415 ml, 2.99 mmol, 4 eq.) and dithiothreitol (0.460 g, 2.99 mmol, 4 eq.) were added to a solution of 3-(4-azidobenzyloxy)benzamide **17** (0.2 g, 0.746 mmol) in dry methanol (20 ml). The contents were protected from light, and the reaction stirred at r.t. for 1 h. The solvents were removed under reduced pressure and the remaining solid resuspended in water (20 ml). The organics were extacted into dichloromethane (3 x 30 ml), dried, filtered and the solvent removed under reduced pressure. Recrystallisation from ethyl acetate and petrol yielded **20** as a pale yellow powder (0.099 g, 55 %).

m.p. 149-151 ^oC; υ_{max}/cm^{-1} 3385, 3177, 1643, 1597, 1577, 1522, 1493, 1448, 1417, 1390, 1375; *m/z* 242 (4%, M^+), 225 (9%, M^+-NH_2), 198 (30%) 137 (95%, $[OHC_6H_4CONH_2]^+$), 121 (100%, $[OHC_6H_4CO]^+$), 106 (88%, $[NH_2C_6H_4CH_2]^+$), 92, 77; δ_H d_6-DMSO 4.99 (s, 2H, NH_2), 5.2 (s, 2H, OCH_2Ph), 6.65 (d, 2H, *J* 6.9), 7.2 (d, 2H, *J* 7.1), 7.45 (m, 5H), 8.05 (s, 1H); δ_C d_6-DMSO 70.24, 113.90, 118.09, 119.92, 123.67, 125.36, 129.59, 129.91, 135.95, 149.0, 158.81, 167.96; Anal. found C 68.99, H 5.71, N 11.42, $C_{14}H_{14}N_2O_2$ requires C 69.42, H 5.78, N 11.57 %.

3-(4-N Acetyl-4-aminobenzyloxy)benzamide 23 [82]

Compound 17 (0.05 g, 0.187 mmol) was dissolved in thiolacetic acid (1.0 ml) and stirred for 96 h. On completion the solvent was removed under vacuum distillation to yield a yellow solid. The crude material was purified by column chromatography using 9 : 1 dichloromethane : methanol as the eluent. The sample was triturated with water, then recrystallised from ethyl acetate and petrol to yield a yellow powder (0.033 g, 64 %).

m.p. 198-199 ^{o}C; *m/z* 285 (2%; MH^+), 148 (92%, $[CH_2C_6H_4NHCOCH_3]^+$), 137 (10%, $[OHC_6H_4CONH_2]^+$), 121 (20%, $[OHC_6H_4CO]^+$), 106 (100%, $[NH_2C_6H_4CH_2]^+$), 92, 78; δ_H d_6-DMSO 2.19 (s, 3H, $COCH_3$), 5.2 (s, 2H, OCH_2Ph), 7.3 (d, 1H),7.6 (m, 9H), 10.1 (s, 1H, $NHCOCH_3$); δ_C d_6-DMSO 24.33, 69.50, 113.99, 118.09, 119.19, 120.19, 128.78, 129.66, 131.54, 136.03, 139.36, 158.59, 167.88, 168.65; Anal. found C 67.56, H 5.21, N 9.75, $C_{16}H_{16}N_2O_3$ requires C 67.82, H 5.34, N 9.89 %.

3([4-Methyl carboxyl]benzyloxy)benzamide **25**

The title product was prepared using methyl (4-chloromethyl)benzoate (0.184 g, 1.0 mmol) as the electrophile in general procedure A. Compound **25** was isolated as a white powder (0.156 g, 55 %).

m.p. 162-163 °C; ν_{max}/cm^{-1} 3331, 3154, 2959, 1722, 1666, 1630, 1601, 1581, 1489, 1450, 1435, 1417; *m/z* 285 (48%; M^+), 254 (38%, M^+-OCH_3), 149 (100%, $[CH_3OCOC_6H_4CH_2]^+$), 121 (75%,$[OHC_6H_4CO]^+$); δ_H d_6-DMSO 3.9 (s, 3H, CH_3), 5.25 (s, 2H, OCH_2Ph), 7.25 (d, 1H, *J* 5.7), 7.4 (m, 5H), 7.6 (d, 2H, *J* 8.2), 7.9 (d, 2H, *J* 8.3); δ_C d_6-DMSO 52.41, 68.94, 114.01, 118.09, 120.45, 127.79, 129.29, 129.69, 136.09, 142.78, 158.33, 159.25, 166.31, 167.82; Anal. found C 67.19, H 5.16, N 4.78, $C_{16}H_{15}NO_4$ requires C 67.37, H 5.30, N 4.91 %.

3-(4-carboxylbenzyloxy)benzamide 21

Sodium hydroxide (0.014 g, 3.5 mmol) in water (5 ml) was added to a solution of 3([4-methylcarboxyl]benzyloxy)benzamide **25** (0.1 g, 3.5 mmol) in methanol (5 ml). The solution was warmed to 40 °C and stirred for 5 h. The solvents were removed

under reduced pressure and the remaining solid dissolved in water (15 ml). The solution was cooled to about 4 °C and acidified using ice cold hydrochloric acid (1.0 M). The organics were extracted into ethyl acetate (3 x 30 ml), dried, filtered and the solvent removed under reduced pressure to yield **25** as a white solid (0.073 g, 77 %).

m.p. > 230 °C; ν_{max}/cm^{-1} 3435, 3194, 3435-2903, 2525, 1680, 1612, 1581, 1468; *m/z* 270 (30%; M^+-H), 135 (100%, $[HOCOC_6H_4CH_2]^+$), 121 (75%,$[OHC_6H_4CO]^+$), 107, 90, 77; δ_H d_6-DMSO 5.39 (s, 2H, OCH_2Ph), 7.25 (d, 1H, *J* 5.2), 7.3 (m, 6H), 8.15 (m, 3H), 13.05 (br.s, 1H); Found M^+ 271.0836, $C_{15}H_{13}NO_4$ requires 271.0845.

3-(n-Butyloxy)benzamide 26

3-hydroxybenzamide (0.137 g, 1.0 mmol) was dissolved in anhydrous acetonitrile (10 ml) and to this was added potassium carbonate (0.138 g, 1.0 mmol) and 1-iodobutane (0.184 g, 1.0 mmol) and the mixture refluxed for 20 h. The acetonitrile was removed under reduced pressure and the remaining solid dissolved in water (10 ml). The organics were extracted into dichloromethane (3 x 20 ml), combined, dried, filtered and the solvent removed under reduced pressure. The product was sublimed to yield **26** as a white solid (0.037 g, 20 %).

m.p. 114-116°C; ν_{max}/cm^{-1} 3341, 3161, 2959, 2934, 2874, 1666, 1613, 1601, 1585, 1491, 1450, 1400; *m/z* 193 (65 %, M^+), 137 (93 %, $[OHC_6H_4CONH_2]^+$), 121 (100 %, $[OHC_6H_4CO]^+$), 92, 80, 65; δ_H d_6-DMSO 0.91 (t, 3H, $OCH_2CH_2CH_2CH_3$, *J* 7.3), 1.48 (m, 2H, $OCH_2CH_2CH_2CH_3$ *J* 7.3), 1.77 (m, 2H,

$OCH_2CH_2CH_2CH_3$, *J* 7.2, 6.4), 3.98 (t, 2H, $OCH_2CH_2CH_2CH_3$, *J* 6.4), 5.9 (br.d, 2H, NH_2), 7.04 (dt, 1H, *J* 2.5, 1.2), 7.34 (m, 3H); δ_C d_6-DMSO 13.85, 19.25, 31.27, 67.99, 113.24, 118.78, 119.03, 129.63, 134.73, 159.46, 169.43; Anal. found C 68.53, H 7.98, N 7.26 $C_{11}H_{15}NO_2$ requires C 68.35, H 7.83, N 7.25 %.

3-(n-Pentyloxy)benzamide 27

Potassium carbonate (0.138 g, 1.0 mmol) and 1-iodopentane (0.198 g, 1.0 mmol) were added to a solution of 3-hydroxybenzamide (0.137 g, 1.0 mmol) in anhydrous acetonitrile (10 ml) and refluxed for 25 h. The acetonitrile was removed under reduced pressure and the remaining solid dissolved in water (10 ml). The organics were extracted into dichloromethane (3 x 20 ml), combined, dried , filtered and the solvent removed under reduced pressure. The product was sublimed to yield **27** as a white powder (0.077 g, 37 %).

m.p. 116-118 °C; ν_{max}/cm^{-1} 3360, 3173, 2955, 2939, 2870, 1658, 1626, 1603, 1583, 1491, 1450; *m/z* (EI) 207 (40 %, M^+), 137 (100 %, $[OHC_6H_4CONH_2]^+$) 121 (85 %, $[OHC_6H_4CO]^+$), 92, 70, 65; δ_H d_6-DMSO 0.91 (t, 3H, $OCH_2CH_2CH_2CH_2CH_3$, *J* 7.2), 1.42 (m, 4H, $OCH_2CH_2CH_2CH_2CH_3$ *J* 7.3), 1.78 (m, 2H, $OCH_2CH_2CH_2CH_2CH_3$, *J* 7.0, 6.6), 3.98 (t, 2H, $OCH_2CH_2CH_2CH_2CH_3$, *J* 6.6), 5.5-6.1 (br.d, 2H), 7.04 (dt, 1H, *J* 2.5, 1.6), 7.33 (m, 3H); δ_C d_6-DMSO 14.06, 22.48, 28.19, 28.92, 68.27, 113.23, 118.70, 119.04, 129.62, 134.76, 159.44, 169.50; Anal. found C 69.38, H 8.47, N 6.47 $C_{12}H_{17}NO_2$ requires C 69.54, H 8.27, N 6.76 %.

General Procedure B: Preparation of alkylmesityl groups.

Triethylamine (9.0 mmol) was added to a solution of alkyl alcohol (6.0 mmol) in dichloromethane (10 ml). This was cooled to 0 ^{o}C and methanesulfonyl chloride (6.0 mmol) was added dropwise over 10 min. The reactants were stirred at 0 ^{o}C for 2 h. The organic phase was washed sequentially with cold water (10 ml), cold hydrochloric acid (10 %, 10 ml), saturated sodium hydrogen carbonate solution (10 ml) and saturated sodium chloride solution (10 ml). The organics were dried, filtered and the solvent removed under reduced pressure.

Hexan-1-ol O mesylate

H_3C SO_2CH_3

The product was formed with hexan-1-ol (0.612 g, 0.752 ml) being utilised in general procedure B. The title compound was isolated as an oil (0.570g, 40 %).

Heptan-1-ol O mesylate

SO_2CH_3
H_3C

The product was formed with heptan-1-ol (0.697 g, 0.849 ml) being utilised in general procedure B. The title compound was isolated as a yellow oil (0.450g, 39 %).

Octan-1-ol O-mesylate

The product was formed with octan-1-ol (0.781 g, 0.944 ml) being utilised in general procedure B. The title compound was isolated as an oil (0.432 g, 37 %).

3-(n-hexyloxy)benzamide **28**

Potassium carbonate (0.138 g, 1.0 mmol) and hexan-1-ol *O*-mesylate (0.26 g, 1.4 mmol) were added to a solution of 3-hydroxybenzamide (0.137 g, 1.0 mmol) in anhydrous acetonitrile (10 ml) and refluxed for 25 h. The acetonitrile was removed under reduced pressure and the remaining solid suspended in water (10 ml). The organics were extracted into dichloromethane (3 x 20 ml), combined, dried , filtered and the solvent removed under reduced pressure. The product was isolated *via* flash chromatography using 19 : 1 dichloromethane : methanol as the eluent. The product was sublimed to yield **28** a white solid (0.208 g, 94 %).

m.p. 114-115 °C; ν_{max}/cm^{-1} 3364, 3173, 2955, 2939, 2870, 1657, 1626, 1603, 1583, 1491, 1450, 1392; *m/z* (EI) 221 (63 %, M^+), 137 (100 %, $[OHC_6H_4CONH_2]^+$) 121 (95 %, $[OHC_6H_4CO]^+$),109, 92; δ_H d_6-DMSO 0.81 (t, 3H, $OCH_2CH_2(CH_2)_3CH_3$, *J* 7.2), 1.41 (m, 6H, $OCH_2CH_2(CH_2)_3CH_3$ *J* 7.3), 1.78 (m, 2H, $OCH_2CH_2(CH_2)_3CH_3$), 4.01 (t, 2H, $OCH_2CH_2(CH_2)_3CH_3$, *J* 6.6), 5.7-6.1 (bd, 2H), 7.04 (m, 1H), 7.33 (m, 3H); δ_C d_6-DMSO 14.077, 22.63, 25.72, 29.18,

31.59, 68.29, 113.24, 118.79, 119.04, 129.63, 132.01, 159.20, 169.39, Anal.. found C 70.64, H 8.94, N 6.03 $C_{13}H_{19}NO_2$ requires C 70.56, H 8.65, N 6.33 %.

3-(n-Heptyloxy)benzamide **29**

Potassium carbonate (0.248 g, 1.79 mmol) and heptan-1-ol *O*-mesylate (0.339 g, 1.75 mmol) were added to a solution of 3-hydroxybenzamide (0.239 g, 1.75 mmol) in anhydrous acetonitrile (10 ml) and refluxed for 36 h. The acetonitrile was removed under reduced pressure and the remaining solid suspended in water (10 ml). The organics were extracted into dichloromethane (3 x 20 ml), combined, dried, filtered and the solvent removed under reduced pressure. The product was recrysallised from water and ethanol to yield **29** as a white solid (0.152 g, 37%).

m.p. 110-111°C; ν_{max}/cm^{-1} 3362, 3173, 2992, 2856, 1658, 1626, 1603, 1583, 1450, 1392; *m/z* (EI) 235 (53 %, M^+), 137 (100 %, $[OHC_6H_4CONH_2]^+$) 121 (80 %, $[OHC_6H_4CO]^+$),109, 93; δ_H d_6-DMSO 0.86 (t, 3H, $OCH_2CH_2(CH_2)_4CH_3$, *J* 7.7), 1.21 (m, 8H, $OCH_2CH_2(CH_2)_4CH_3$), 1.76 (m, 2H, $OCH_2CH_2(CH_2)_4CH_3$), 3.97 (t, 2H, $OCH_2CH_2(CH_2)_4CH_3$, *J* 6.5), 5.8-6.2 (br.d, 2H), 7.03 (m, 1H), 7.29 (m, 3H); δ_C d_6-DMSO 14.14, 22.711, 26.05, 29.22, 29.36, 31.86, 68.29, 113.24, 118.83, 119.04, 129.64, 134.62, 159.47, 169.36; Anal. found C 69.15, H 8.86, N 5.47 $C_{14}H_{21}NO_2$ requires C 71.46, H 8.99, N 5.95 % sample calculated to contain 0.5 M water.

3-(n-Octyloxy)benzamide **30**

Potassium carbonate (0.138 g, 1.0 mmol) and octan-1-ol *O*-mesylate (0.23 g, 1.1 mmol) were added to a solution of 3-hydroxybenzamide (0.137 g, 1.0 mmol) in anhydrous acetonitrile (10 ml), and refluxed for 30 h. The acetonitrile was removed under reduced pressure and the remaining solid dissolved in water (10 ml). The organics were extracted into dichloromethane (3 x 20 ml), combined, dried , filtered and the solvent removed under reduced pressure. The product was recrysallised from water and ethanol to yield **30** as a white solid (0.182 g, 71%).

m.p. 109-110°C; ν_{max}/cm^{-1} 3366, 3173, 2955, 2937, 2922, 2856, 1932, 1658, 1626, 1603, 1583, 1491, 1469, 1450; *m/z* 249 (78 %, M^+), 137 (98 %, $[OHC_6H_4CONH_2]^+$), 121 (93 %,$[OHC_6H_4CO]^+$); δ_H d_6-DMSO 0.86 (t, 3H, $OCH_2CH_2(CH_2)_5CH_3$, *J* 6.7), 1.3 (m, 10H, $OCH_2CH_2(CH_2)_5CH_3$), 1.8 (m, 2H, $OCH_2CH_2(CH_2)_5CH_3$), 3.97 (t, 2H, $OCH_2CH_2(CH_2)_5CH_3$, *J* 6.6), 5.7-6.2 (bd, 2H), 7.03 (m, 1H), 7.28 (m, 3H); δ_C d_6-DMSO 14.14, 22.64, 26.02, 29.07, 29.22, 31.82, 68.29, 113.23, 118.80, 119.02, 129.63, 135.1, 159.47, 169.35; Anal. found C 72.04, H 9.55, N 5.43 $C_{15}H_{23}NO_2$ requires C 72.25, H 9.30, N 5.62 %.

Experimental for Chapter Three

Methyl-3-hydroxy-2-nitrobenzoate **33**

3-Hydroxy-2-nitrobenzoic acid (5.0 g, 27.3 mmol) was dissolved in methanol (50 ml). Anhydrous hydrogen chloride gas was bubbled through the solution until saturated and the mixture refluxed for 16 h. Removal of solvent under reduced pressure yielded a beige solid which was dissolved in water (100 ml). Sodium hydrogen carbonate (*ca* 5 g) neutralised the solution and the organics were extracted into ethyl acetete (3 x 50 ml), combined and washed sequentially with saturated brine solution (50 ml), then water (20 ml). The organics were dried, filtered and the solvent removed under reduced pressure to yield methyl-3-hydroxy-2-nitrobenzoate **33** as a beige solid (4.64 g, 86 %).

m.p. 112-114 °C; υ_{max}/cm^{-1} 3298, 3076, 2963, 2853, 1963, 1884, 1694, 1614, 1583, 1537, 1479, 1452, 1439; *m/z* 197 (98 %, M^+), 165 (100 %, M^+-OCH_3), 136 (12 %), 121, 107, 92; δ_H 3.9 (s, 3H, CH_3), 7.04 (dd, 1H, *J* 1.3, 6.1), 7.2 (dd, 1H, *J* 1.1, 6.0), 7.5 (t, 1H, *J* 6.5); δ_C 53.46, 120.75, 121.99, 130.63, 132.33, 135.88, 154.88, 166.64; Found M^+ 197.0339 $C_8H_7NO_5$ requires 197.0324.

Methyl-2-amino-3-Hydroxybenzoate **34** [96]

O
OCH_3
NH_2
OH

Palladium on carbon catalyst (0.5 g) was added to a nitrogen charged flask, this was suspended in methanol (150 ml) and methyl 3-hydroxy-2-nitrobenzoate **33** (4.0 g, 20.3 mmol) was added. The flask was evacuated, then charged with hydrogen. The mixture was stirred vigorously for 5 h.

The flask was again evacuated, then opened to the atmosphere. The mixture was filtered through a celite pad to remove the catalyst and the pad washed in portions with methanol (4 x 30 ml). The methanol was removed under reduced pressure to yield **34** as a brown solid (3.34 g, 99 %).

δ_H $CDCl_3$ 3.85 (s, 3H, CH_3), 5.57 (br.s, 2H, NH_2), 6.48 (t, 1H, *J* 8), 6.80 (dd, 1H, *J* 1.0, 6.8), 7.46 (dd, 1H, *J* 1.1, 7.1).

Ethylacetimidate hydrochloride **36**

NH.HCl
H_3C OCH_2CH_3

Anhydrous hydrogen chloride gas was bubbled through a of solution acetonitrile (0.263 ml, 5.0 mmol) in dry ethanol (0.69 ml, 15.0 mmol) until saturated and the solution stirred for 48 h. The white solid formed **36**, was collected and dried.

m.p. 126-128 °C; υ_{max}/cm^{-1} 4432, 2982, 2694, 1956, 1643, 1603, 1560, 1479, 1456; *m/z* 88 (30 %, MH^+-HCl), 60 (3 %, MH^+-CH_2CH_3), 42 (100 %, $[CH_3CN]^+$), 36, 29; δ_H d_6-DMSO 1.38 (t, 3H, *J* 7.0, CH_2CH_3), 2.44 (s, 3H, CH_3), 4.57 (q, 2H, *J* 8.0, CH_2CH_3); Found M^+-HCl 87.0707, C_4H_9NO requires 87.0684.

Methyl 2-methylbenzoxazole-4-carboxylate **35**

Ethylacetimidate hydrochloride **36** (0.615 g, 5.0 mmol) was added to a nitrogen charged flask containing methyl-2-amino-3-hydroxybenzoate **34** (0.835 g, 5.0 mmol) in anhydrous ethanol. The mixture was refluxed for 24 h. The solvent was removed under reduced pressure and the remaining solid dissolved in water (20 ml). The organics were extracted into ethyl acetate (3 x 15 ml), combined, dried, filtered and the solvent removed under reduced pressure. Recrystallisation from ethyl acetate/ petrol yielded **35** as a cream solid (0.84 g, 85 %).

m/z 253 (M^+, 100 %), 222 (98 %, M^+-OCH_3), 195 (97 %, M^+-$COOCH_3$), 166, 140, 103, 89, 77; δ_H 4.0 (s, 3H, CH_3), 7.35 (t, 1H, *J* 8.2), 7.45 (m, 3H), 7.75 (dd, 1H, *J* 1.0, 7.1), 8.0 (dd, 1H, *J* 1.2, 6.9) 8.3 (m, 2H); δ_C 13.86, 114.97, 122.02, 124.48, 126.57, 127.12, 128.27, 128.93, 141.72, 165.90; Found M^+ 191.0579, $C_{10}H_9NO_3$ requires 191.0582.

2 Methylbenzoxazole carboxylic acid **38**

Sodium hydroxide (0.052 g, 1.3 mmol) in water (5 ml) was added to a solution of methyl 2-methylbenzoxazole-4-carboxylate (0.25 g, 1.3 mmol)) in methanol (5 ml). The solution was warmed to 40 °C and stirred for 5 h. The solvents were removed under reduced pressure and the remaining solid dissolved in water (15 ml). The solution was cooled to about 4 °C and acidified using ice cold hydrochloric acid (0.05 M) until pH = 4.5. The organics were extracted immediately into ice cold ethyl acetate. (3 x 30 ml), dried, filtered and the solvent removed under reduced pressure to yield **38** as a yellow solid (0.187 g, 81 %).

m.p.> 230 °C. υ_{max}/cm^{-1} 2554, 1979, 1676, 1620, 1603, 1570, 1491, 1429; *m/z* 177 (40 %, M$^+$), 160 (25 %, M$^+$-OH), 133 (100%, M$^+$-COOH), 107, 91, 77; δ_H d_6-DMSO 2.8 (s, 3H, CH_3), 7.5 (m, 1H), 7.97 (m, 2H), 13.0 (br.s, 1H, OH). δ_C 14.66, 114.95, 122.80, 124.50, 126.55, 141.01, 151.57, 165.85, 166.52. Found M$^+$ 177.0426, $C_9H_7NO_3$ requires 177.0425.

2-Methylbenzoxazole-4-carboxamide 39

Dry thionyl chloride (0.066 g, 0.040 ml, 0.616 mmol)) was added to a solution of 2-methylbenzoxazole-4-carboxylic acid (0.1 g, 0.56 mmol) in anhydrous THF with a catalytic amount of DMF, and the reaction stirred for 2 h under a nitrogen atmosphere. Aqueous ammonia (2 ml) was added and the reaction stirred for a further 15 min. The solvents were removed under reduced pressure and the product recrysallised from *iso*propanol. The title compound **39** was isolated as a cream solid. (0.83 g, 84 %).
m.p. 192-193 °C; υ_{max}/cm^{-1} 3375, 3200, 1965, 1878, 1674, 1626, 1595, 1574, 1431; *m/z* 176 (97 %, M^+), 160 (100 %, M^+-NH_2), 133 (93 %, M^+-$CONH_2$), 106, 91, 78, 68; δ_H 2.86 (s, 3H, CH_3), 5.8 (br.s, 1H, NH), 7.39 (t, 1H, *J* 8.0), 7.6 (dd, 1H, *J* 1.1, 8.0), 8.1 (dd, 1H, *J* 1.1, 8.1), 8.8 (br.s, 1H, NH); Anal. found C 61.2, H 4.41, N 15.53, $C_9H_9N_2O_2$ requires C 61.34, H 4.58, N 15.91 %.

2-Methylbenzoxazole 4-(N methyl)carboxamide 40

Dry thionyl chloride (0.111 g, 0.068 ml, 0.931 mmol) was added to a solution of 2-methylbenzoxazole-4-carboxylic acid **38** (0.15 g, 0.846 mmol) in anhydrous THF with

a catalytic amount of DMF, and the reaction stirred for 2 h. Aqueous methylamine (excess, 2 ml) was added and the reaction stirred for a further 15 min. The solvents were removed under reduced pressure and the product **40** recrysallised from *iso*propanol as a white solid (0.117 g, 73 %).

υ_{max}/cm^{-1} 3344, 2932, 1658, 1601, 1570, 1481, 1425; *m/z* 190 (71 %, M^+), 174 (18 %), 160 (76 %, M^+-$NHCH_3$), 133 (100 %, M^+-$CONHCH_3$), 116, 107 (70 %), 91,74; δ_H 2.65 (s, 3H, CH_3), 3.6 (d, 3H, $NHCH_3$), 7.35 (t, 1H, *J* 8.0), 7.56 (dd, 1H, *J* 1.1, 7.1), 8.12 (dd, 1H, *J* 1.1, 6.7), 8.8 (br.s, 1H, NH); Anal. found C 63.46, H 5.50, N 14.50, $C_{10}H_{10}N_2O_2$ requires C 63.15, H 5.30, N 14.73 %.

Ethyl benzimidate hydrochloride **41**

NH.HCl

OCH_2CH_3

Benzonitrile (10.28 ml, 100.0 mmol) was added to dry ethanol (15 ml, 300 mmol) and anhydrous hydrogen chloride gas was bubbled through the solution until saturated. The solution was stirred for 48 h ay ambient temperature. The white solid formed **41**, was collected and dried (9.63 g, 52 %).

m.p. 126-128 ^oC; υ_{max}/cm^{-1} 2856, 1915, 1631, 1601, 1585, 1541, 1506, 1454, 1441, 1387, 1363, 1327, 1305; *m/z* 148 (29 %, M^+-HCl), 121 (38 %, M^+-CH_2CH_3), 105 (100 %, $[PhNH]^+$), 77 (51 %, $[Ph]^+$), 51, 42, 36; δ_H d_6DMSO 1.56 (t, 3H, *J* 7.1, CH_2CH_3), 4.77 (q, 2H, *J* 8.0, CH_2CH_3), 7.5 (m, 2H), 7.6 (m, 1H), 8.2 (m, 2H); Anal. found C 58.13, H 6.43 N 7.36, $C_9H_{12}NOCl$ requires C 58.23, H 6.51, N 7.54 %.

Methyl 2-phenylbenzoxazole-4-carboxylate **42**

Ethyl benzimidate hydrochloride **41** (0.222 g, 1.19 mmol) was added to a nitrogen charged flask containing methyl 2-amino-3-hydroxybenzoate **34** (0.1 g, 0.59 mmol) in anhydrous ethanol (20 ml). The mixture was refluxed for 24 h. The solvent was removed under reduced pressure and the remaining solid dissolved in water (20 ml). The organics were extracted into ethyl acetate (3 x 15 ml), combined, dried, filtered and the solvent removed under reduced pressure. Recrystallisation from ethyl acetate/ petrol yielded **42** as a yellow solid (0.127 g, 85 %).

m.p. 191-193 °C; υ_{max}/cm^{-1} 2999, 2915, 1714, 1610, 1550, 1485, 1452, 1435, 1421, 1356, *m/z* 253 (M^+, 100 %), 222 (98 %, M^+-OCH_3), 195 (97 %, M^+-COOCH), 166, 140, 103, 89, 77; δ_H 4.0 (s, 3H), 7.35 (t, 1H, *J* 8.2), 7.45 (m, 3H), 7.75 (dd, 1H, *J* 1.0, 7.1), 8.0 (dd, 1H, *J* 1.2, 6.9) 8.3 (m, 2H); δ_C 152.55, 114.97, 122.02, 124.48, 126.57, 127.12, 128.27, 128.93, 132.16, 141.72, 151.46, 165.90, 171.34; Found M^+ 253.0716, $C_{15}H_{11}NO_3$ requires 253.0739.

2-Phenylbenzoxazole-4-carboxamide **43**

Methyl 2-phenylbenzoxazole-4-carboxylate **42** (0.1 g, 0.56 mmol) was added to a solution of methanolic ammonia (2 ml, $^{V}/v$). This was stirred for 12 h at ambient temperature. The solvent was removed under reduced pressure to yield a solid which was recrystallised from ethyl acetate / petrol affording **43** as a white solid (0.83 g, 84 %).

m.p. 199-201 °C; υ_{max}/cm^{-1} 3383, 3165, 1676, 1606, 1595, 1552, 1491, 1483, 1452, 1425, 1392; *m/z* 238 (100 %, M^+), 222 (68 %, M^+-NH_2), 195 (98 %, M^+-CO), 143 (10 %), 104, 91, 77, 63; δ_H 6.01 (br.s, 1H, NH), 7.43 (t, 1H, *J* 8), 7.57 (m, 3H), 7.74 (dd, 1H, *J* 1, 7), 8.23 (dd, 1H, *J* 1.1, 7.3), 8.27 (m, 2H), 8.97 (br.s, 1H, NH); Anal. found C 70.41, H 4.24 N 11.77, $C_{14}H_{10}N_2O_2$ requires C 70.58, H 4.23, N 11.76 %.

Methyl-2-^{tert}butylbenzoxazole-4-carboxylate 44

^{t}Butylcarbonyl chloride (0.865 ml, 7.19 mmol), triethylamine (1.0 ml, 7.19 mmol) and pyridinium-4-toluene sulphonate (5 mol %) were added to a solution of 3-hydroxyanthranillic ester **34** (1.0 g, 5.99 mmol) in *m*-xylene (50 ml) and refluxed for 26 h. The *m*-xylene was removed under reduced pressure to yield a brown solid which was suspended in water. The organics were extracted into ethyl acetate (3 x 30 ml), combined, dried, filtered and the solvent removed under reduced pressure to yield a yellow solid. The product was isolated by flash column chromatography using 1 : 4 ethyl acetate : petrol as the eluent, and recrysallised from ethyl acetate / petrol to yield 44 as a yellow solid (0.77 g, 59 %).

υ_{max}/cm^{-1} 2970, 2874, 1927, 1867, 1811, 1709, 1618, 1597, 1562, 1487, 1464, 1431, 1419, 1373, 1363; *m/z* 233 (64 %, M^+), 218 (50 %, M^+-CH_3), 202 (43 %, M^+-CH_3), 186 (100 %, M^+-CH_3), 173, 160, 146 (58 %, M^+-OCH_3), 117, 91; δ_H 1.5 (s, 9H, $C(CH_3)_3$), 3.9 (s, 3H, CH_3), 7.2 (t, 1H, *J* 8), 7.6 (dd, 1H, *J* 1.0, 7.1), 7.9 (dd, 1H, *J* 1.1,6.7); Anal. found C 67.10, H 6.76 N 5.94, $C_{13}H_{15}NO_3$ requires C 66.94, H 6.48, N 5.94 %.

2-^{tert}Butylbenzoxazole-4-carboxamide 45

Methyl 2-^{t}butylbenzoxazole-4-carboxylate 44 (0.2 g, 1.19 mmol) was added to a solution of methanolic ammonia (2 ml). This was stirred for 12 h. at ambient temperature. The solvent was removed under reduced pressure to yield a solid which was recrystallised from ethyl acetate / petrol affording 45 as white plates (0.18g, 69 %).

υ_{max}/cm^{-1} 3395. 3163, 2972, 1687, 1626, 1595, 1562, 1489, 1462, 1427, 1385, 1367, 1352; *m/z* 281 (98 %, M^{+}), 186 (96 %, M^{+}-CH$_3$), 175, 160, 146, 133, 113, 63; δ_H 1.44 (s, 9H, C(CH$_3$)$_3$), 5.9 (br.s, 1H, NH), 7.34 (t, 1H, *J* 8), 7.59 (dd, 1H, *J* 1.1, 7.0), 78.06 (dd, 1H, *J* 1.1, 6.7), 8.0 (br.s, 1H, NH); δ_C 28.41, 34.37, 113.90, 123.27, 124.33, 125.44, 139.37, 150.80, 166.45, 174.47; Anal. found C 66.01, H 6.52, N 12.52, $C_{12}H_{14}N_2O_2$ requires C 66.02, H 6.47, N 12.84 %.

General procedure C:- Acylation of methyl-2-amino-3-hydroxybenzoate 34.

Pyridine (7.19 mmol, 1.2 eq) was added to a nitrogen charged flask containing a solution of acid chloride (7.19 mmol, 1.2 eq) dissolved in anhydrous THF (50 ml). Methyl-2-amino-3-hydroxybenzoate 34 (1.0 g, 5.99 mmol, 1.0 eq) was added in one portion and stirred for 2 h. at r.t. The solvents were removed under reduced pressure to yield a solid. Water (50 ml) was added and the organics were extracted into ethyl acetate (3 x 30 ml), dried and the solvent removed under reduced pressure. The product was recrystallised from boiling ethyl acetate / petroleum ether.

Methyl 2-(4-nitrobenzoyl)amino-3-hydroxybenzoate 46

The product was achieved using 4-nitrobenzoyl chloride (1.33 g, 7.19 mmol) in general procedure C. The product 46 was isolated as a brown solid (1.47 g, 77 %). m.p. 175-176 °C; υ_{max}/cm^{-1} 3443, 3113, 1697, 1649, 1604, 1552, 1527, 1493, 1468; *m/z* 316 (14 %, M+), 167 (25 %,$[NHCOC_6H_4NO_2]^+$ 104, 97, 92; δ_H 3.91 (s, 3H, CH_3), 7.25 (m, 2H), 7.6 (dd, 1H, *J* 1.5, 5.9) 8.2 (d, 2H, *J* 7.9), 8.3 (d, 2H, *J* 7.9), 9.12, (s, 1H, NH), 10.10 (br.s, 1H, OH); δ_C 53.07, 119.57, 123.41, 124.19, 126.42, 126.70, 128.35, 129.18, 138.41, 150.14, 157.25, 165.23, 169.53; Anal. found C 56.69, H 3.85 N 8.77, $C_{15}H_{12}N_2O_6$ requires C 56.96, H 3.82, N 8.85 %.

Methyl-2-(4-methoxybenzoyl)amino-3-hydroxybenzoate 47

The product was prepared using 4-methoxybenzoyl chloride (1.23 g, 7.19 mmol) in general procedure C. Compound 47 was isolated as a yellow solid (1.22 g, 67 %). m.p. 177-179 °C; υ_{max}/cm^{-1} 3453, 3209, 3072, 2839, 1691, 1643, 1606, 1564, 1541, 1508, 1471, 1402, 1332; *m/z* 301 (20 %, M^+), 270 (5 %, M^+-OCH_3), 135 (100 %,$[NHCOC_6H_4OCH_3]^+$), 107, 92, 77; δ_H 3.87 (s, 3H, OCH_3), 3.93 (s, 3H, $COCH_3$),

6.99 (d, 2H, J 7.1), 7.14 (t, 1H, J 7.9), 7.26 (dd, 1H, J 1.7, 6.6), 7.64 (dd, 1H, J 1.6, 6.1), 8.0 (d, 2H, J 8.2), 10.3 (s, 1H, NH), 11.96 (br.s, 1H, OH); Found M^+ 301.0956. $C_{16}H_{15}NO_5$ requires 301.0950.

Methyl-2-(4-methylcarboxybenzoyl)amino-3-hydroxybenzoate 54

The product was prepared using 4-methoxybenzoyl chloride (1.23 g, 7.19 mmol) in general procedure C. Compound 54 was isolated as a yellow solid (1.22 g, 67 %). m.p. 177-179 °C; υ_{max}/cm^{-1} 3426 - 2594, 1724, 1693, 1639, 1601, 1541, 1504, 1464, 1435; m/z 329 (85 %, M^+), 297 (45 %, M^+-$COCH_3$), 163 (100 %, $[COC_6H_4COOCH_3]^+$), 135 (78 %, $[C_6H_4COOCH_3]^+$), 120 (36 %), 104, 76; δ_H 3.93 (s, 6H, 2 x $COCH_3$), 7.2 (m, 2H), 7.6 (dd, 1H, J 2.0, 7.9), 8.10 (m, 4H), 9.9 (s, 1H, NH), 12.10 (br.s, 1H, OH); Found M^+ 329.0912. $C_{17}H_{15}NO_6$ requires 329.0899.

General Procedure D: Cyclisation to form the oxazole moiety

To a solution of methyl-2-substituted benzoxazole-4-carboxylate (1.0 g, 1mol eq) in *m*-xylene (50 ml) was added triethylamine (1.2 eq) and pyridinium-4-toluene sulphonate (5 mol %). The mixture was refluxed for 16 h. The *m*-xylene was removed under reduced pressure to yield a brown solid which was suspended in water. The organics were extracted into ethyl acetate (3 x 30 ml), combined, dried, filtered and the solvent removed under reduced pressure to yield a solid. The product was isolated by

flash chromatography using 1 : 4 ethyl acetate : petrol as eluent and recrysallised from ethyl acetate and petrol.

Methyl-2-(4-nitrophenyl)benzoxazole-4-carboxylate **48**

The product was prepared from **46** (1.0 g, 3.16 mmol) using general procedure D. Compound **48** was isolated as a brown solid (0.71 g, 79 %).

m.p. 213-215 ^oC; υ_{max}/cm^{-1} 3055, 2957, 1726, 1620, 1604, 1556, 1522, 1489, 1462, 1446, 1421; *m/z* 298 (80 %, M^+), 160 (43 %,), 267 (100 %, M^+-OCH_3), 240 (40 %, M^+-$COOCH_3$), 229, 221, 163, 140 107 (87 %), 88, 79, 63; δ_H 4.05 (s, 3H, CH_3), 7.4 (t, 1H, *J* 8.0), 7.83 (dd, 1H, *J* 1.2, 7.1), 8.0 (dd, 1H, *J* 1.1, 6.7), 8.3 (d, 2H, *J* 8.1), 8.5 (d, 2H, *J* 8.3); Anal. found C 61.17, H 4.53 N 15.50, $C_{15}H_{10}N_2O_5$ requires C 61.36, H 4.54, N 15.50 %.

Methyl 2-(4-methoxyphenyl)benzoxazole-4-carboxylate **49**

The product was prepared from **47** (1.0 g, 3.32 mmol) using general procedure D, and the title compound **49** was isolated as a beige solid (0.70 g, 75 %).

m.p. 103-104 °C; υ_{max}/cm^{-1} 3063, 3024, 2978, 2955, 2831, 1720, 1614, 1502, 1419, 1356, 1323, 1300; *m/z* 283 (48 %, M^+), 252 (38 %, M^+-OCH_3), 225 (58 %, M^+-$COOCH_3$), 133, 92, 77, 63 (100 %); δ_H 3.8 (s, 3H, OCH_3), 4.03 (s, 3H, $COCH_3$) 6.9 (dd, 2H, *J* 2.1, 4.9), 7.35 (t, 1H, *J* 8.0), 7.74 (dd, 1H, *J* 1.1, 7.0), 7.99 (dd, 1H, *J* 1.1, 6.9), 8.28 (dd, 2H, *J* 2.2, 4.7); δ_C 52.46, 55.48, 114.32, 114.63, 119.05, 121.61, 123.82, 126.87, 130.11, 142.10, 151.41, 162.81, 164.91, 165.92; Found M^+ 283.0854. $C_{16}H_{13}NO_4$ requires 283.0845.

Methyl 2-(4-carboxymethylphenyl)benzoxazole-4-carboxylate 55

The product was prepared from 54 (1.0 g, 3.03 mmol) using general procedure D, and the title compound 55 was isolated as a yellow solid (0.63 g, 67 %).

m.p. 105-107 °C; υ_{max}/cm^{-1} 2957, 1940, 1738, 1720, 1620, 1603, 1577, 1556, 1498, 1437, 1423; *m/z* 311 (83 %, M$^+$), 280 (100 % M$^+$-OCH$_3$), 253 (58 %, M$^+$-COOCH$_3$), 220, 163, 125; δ_H 3.8 (s, 3H, OCH$_3$), 4.03 (s, 3H, COCH$_3$), 6.9 (dd, 2H, *J* 2.1, 4.9), 7.35 (t, 1H, *J* 8.0), 7.74 (dd, 1H, *J* 1.1, 7.0), 7.99 (dd, 1H, *J* 1.1, 6.9), 8.28 (dd, 2H, *J* 2.2, 4.7); Found M$^+$ 311.0800. $C_{17}H_{13}NO_5$ requires 311.0793.

2-(4-Methoxyphenyl)benzoxazole-4-carboxylate 51

Sodium hydroxide (0.014 g, 0.353 mmol) in water (5 ml) was added to a solution of methyl 2-(4-methoxyphenyl)benzoxazole-4-carboxylate 49 (0.1 g, 0.353 mmol) in methanol (5 ml). The solution was warmed to 40 °C and stirred for 5 h. The solvents

were removed under reduced pressure and the remaining solid dissolved in water (15 ml). The solution was cooled to about 4 °C and acidified using ice cold hydrochloric acid (0.05 M) until pH = 4.5. The organics were extracted immediately into ice cold ethyl acetate. (3 x 30 ml), dried, filtered and the solvent removed under reduced pressure. The title product **51** was isolated as a white solid (0.071 g 75 %).

m.p. 187-188 °C; υ_{max}/cm^{-1} 3029, 1743, 1628, 1608, 1581, 1560, 1500, 1481, 1458, 1433, 1423, 1398, 1356, 1329; *m/z* 269 (91 %, M$^+$), 225 (100 %, M$^+$-CO_2), 182 (18 %),182, 154, 135, 113, 92; δ_H d_6DMSO 3.98 (s, 3H, CH_3), 7.28 (d, 2H, *J* 8.9), 7.57 (t, 1H, *J* 8.0), 7.98 (dd, 1H, *J* 1.0, 7.2), 8.12 (dd, 1H, *J* 1.1, 7.1) 8.10 (d, 2H, *J* 8.9), 13.2 (br.s, 1H, OH); Anal. found C 66.50, H 4.08 N 5.08, $C_{15}H_{11}NO_4$ requires C 66.90, H 4.12, N 5.20 %.

2-(4-Methoxyphenyl)benzoxazole-4-carboxamide **53**

Dry thionyl chloride (1.1 eq) was added to a solution of 2-methylbenzoxazole-4-carboxylic acid **51** (0.1 g, 0.371 mmol) in anhydrous THF and the reaction stirred for 2 h. Aqueous ammonia (excess) was added and the reaction stirred for a further 15 min. The solvents were removed under reduced pressure and the product recrysallised from *iso*propanol, yielding **53** as a yellow solid (0.077 g 78 %).

m.p. 221-223 °C; υ_{max}/cm^{-1} 3370, 3298, 3169, 2924, 2845, 1680, 1610, 1599, 1562, 1502, 1462, 1441, 1377, 1354, 1356; *m/z* 268 (93 %, M$^+$), 252 (52 %, M$^+$-NH_2),

237 (5 %, M^+-OCH_3), 225 (100 %, M^+-CO), 209, 182, 135, 126 (20 %), 119, 97, 91; δ_H 3.89 (s, 3H, CH_3), 6.03 (br.s, 1H, NH), 7.01 (d, 2H, *J* 4.9), 7.41 (t, 1H, *J* 7.8), 7.71 (dd, 1H, *J* 1.0, 7.1), 8.16 (dd, 1H, *J* 1.1, 6.8) 8.21 (d, 2H, *J* 4.9), 9.02 (br.s, 1H, NH); Anal. found C 66.08, H 4.38, N 10.05, $C_{15}H_{12}N_2O_3$ requires C 67.14, H 4.51, N 10.45 %.

3-Acetoxy-2-nitrobenzoic acid 57

3-hydroxy-2-nitrobenzoic acid (0.1 g, 0.546 mmol) was added to a cooled solution of sodium hydroxide (0.2 M, 3 ml). Upon dissolution, acetic anhydride (0.114 ml) was added with crushed ice (1.0 g), this was left to warm to ambient temperature then stirred for 16 h. The solution was neutralised with hydrochloric acid (6 M, 5 ml) and the organics extracted into ethyl acetate (3 x 20 ml). The organics were dried, filtered and the solvent removed under reduced pressure to yield a yellow solid 57 (0.112 g, 91 %).

υ_{max}/cm^{-1} 3300, 3092, 2897, 2683, 1977, 1776, 1705, 1614, 1583, 1543, 1527, 1469, 1454, 1419, 1352; *m/z* 183 (63 %, M^+-$COCH_3$), 165 (42 %, -H_2O), 121 (55 %, M^+-CO_2), 107, 91, 81, 63, 52, 43 (100 %, $[HCOCH_3]^+$); δ_H d_6-DMSO 2.1 (s, 3H, CH_3), 7.5 (m, 3H), 11.35 (br.s, 1H OH); Found M^+-$COCH_3$ 183.0160. $C_7H_5NO_5$ requires 183.0168.

3-Hydroxy-2-nitrobenzamide 58

Triethylamine (0.6 ml, 4.31 mmol) was added to a solution of 3-acetoxy-2-nitrobenzoic acid **57** (1.0 g, 3.92 mmol) in anhydrous THF (150 ml) at 0 °C under a nitrogen atmosphere. *Iso*butyl chloroformate (0.61 ml, 4.70 mmol) was added dropwise to the solution. This was stirred for 1 h at 0 °C. On addition of excess aqueous ammonia (3.0 ml) the solution turned dark yellow, and the reaction stirred for a further 4 h. The solvent was removed under reduced pressure and the remaining solid dissolved in water (100 ml). The organics were extracted into ethyl acetate (3 x 50 ml), dried, filtered and the solvent removed under reduced pressure to yield a yellow solid. The title compound was isolated *via* flash chromatography using 1 : 9 methanol : dichloromethane as the eluent. The title compound **58** was isolated as a cream solid (0.41 g, 58 %).

m.p. 123-126 °C; υ_{max}/cm^{-1} 3366, 3182, 1658, 1622, 1604, 1583, 1541, 1469, 1454, 1394, 1338; *m/z* 182 (M^+, 57 %), 165 (100 %, M^+-NH_2), 121 (56 %), 92, 63; δ_H d_6-DMSO 7.25 (dt, 2H, *J* 1.1, 6.6), 7.5 (t, 1H, *J* 8.7), 7.73 (br.s, 1H), 8.2 (br.s, 1H), 11.2 (br.s, 1H); Found M^+ 182.0332. $C_7H_6N_2O_4$ requires 182.0327.

2-Amino-3-hydroxybenzamide 59

To a flask charged with nitrogen was added a palladium on carbon catalyst (0.2 g), this was then suspended in methanol (100 ml) and 3-hydroxy-2-nitrobenzamide **58** (1.0 g, 5.49 mmol) was added in one portion. The flask was evacuated, then charged with hydrogen and the mixture stirred vigorously for 5 h.

The flask was again evacuated, then opened to the atmosphere. The mixture was filtered through a celite pad which was washed in portions with methanol (4 x 30 ml). The methanol was removed under reduced pressure to yield **59** as a red solid (0.79 g, 94 %).

m.p. 193-195 °C; *m/z* 152 (48 %, M^+), 135 (57 %, M^+-OH), 105 (55 %), 87, 74, 62, 44 (100 %, $[CONH_2]^+$); δ_H d_6-DMSO 6.16 (br.s, 2H, NH_2), 6.45 (t, 1H, *J* 7.8), 6.86 (dd, 1H, *J* 1.2, 6.4), 7.18 (dd, 2H, *J* 1.2, 6.9), 7.7 (br.s, 1H, NH), 9.6 (br.s, 1H, NH); δ_C d_6-DMSO 114.447, 116.026, 119.704, 139.948, 145.168, 171.936; Anal. found M^+ 152.0583. $C_7H_8N_2O_2$ requires 152.0585.

2-([4-methylcarboxybenzoyl]amino)-3-hydroxybenzamide **60**

Mono methyl*tere*phthalic acid (1.0 g, 5.46 mmol) was dissolved in excess thionyl chloride (10 ml) and stirred for 2 h at r.t. Any remaining thionyl chloride was removed by vacuum distillation to yield a white solid (0.931 g, 86 %), which was used immediately. To a flask charged with nitrogen containing anhydrous THF (50 ml) was added the freshly prepared acid chloride (0.718 g, 3.62 mmol, 1.1 eq) and pyridine (3.62 mmol), this was stirred for 15 min. 3-Hydroxyanthranilic amide **59** (0.5 g, 3.29 mmol) was added in one portion, and the reaction stirred for a further 2 h. The solvent was removed under reduced pressure and the remaining solid dissolved in water (100 ml). The organics were extracted into ethyl acetate (3 x 50 ml), dried, filtered and the solvent removed under reduced pressure to yield a brown solid. The title compound **60** was purified *via* flash chromatography using 1 : 9 methanol : dichloromethane as the eluent, which yielded **60** as a brown solid (0.63 g, 61 %).

υ_{max}/cm^{-1} 3397, 3339, 3248, 1946, 1726, 1711, 1657, 1641, 1616, 1581; *m/z* 314 (6 %, M^+), 163 (98 %, $[HONH_2C_6H_3CONH_2]^+$), 155 (100 %,$[NHCOC_6H_4OCH_3]^+$), 149, 35, 105, 91, 77; δ_H 4.02 (s, 3H, $COOCH_3$), 7.22 (m, 3H), 7.6 (br.s, 1H, NH), 7.86 (br.s, 1H, NH), 8.0 (d, 4H, *J* 7.9), 10.3 (s, 1H, NH), 11.96 (br.s, 1H, OH); Found M^+ 314.0906. $C_{16}H_{14}N_2O_5$ requires 314.0902.

2,3-methylenedioxybenzoic acid 62

2,3-Dihydroxybenzoic acid (0.5 g, 3.25 mmol) was suspended in water (10 ml), and potassium hydroxide was added (0.542 g, 10.4 mmol), followed by diiodomethane (0.968 ml,) in methanol (1.43 ml). The mixture was refluxed with vigorous stirring for 16 h. On cooling the mixture was acidifed with concentrated hydrochloric acid until pH = 2, extracted into ethyl acetate, dried, filtered and the solvent removed under vacuum to yield a brown powder (0.338 g, 63 %).

m.p. 224-226 °C (lit m.p. 227 °C); υ_{max}/cm^{-1} 3424-3061, 1954, 1871, 1678, 1633, 1597, 1502, 1479, 1454; *m/z* 166 (48 %, M$^+$), 138 (31 %, M$^+$-CO), 121 (35 %, M$^+$-H_2O), 109, 74, 65 (100 %); δ_H d_6-DMSO 6.2 (s, 2H, CH_2), 6.9 (t, 1H, *J* 8.0), 7.2 (dd, 1H, *J* 1.3, 6.4), 7.3 (dd, 1H, *J* 1.3, 6.8); δ_C d_6-DMSO 57.184, 116.226, 119.997, 129.848, 131.787, 150.799, 165.795.

2,3-Methylenedioxybenzamide 63

2,3-Methylenedioxybenzoic acid (0.2 g, 1.20 mmol) was dissolved in anhydrous THF (10 ml) and thionyl chloride (0.156 g, 0.096 ml, 1.33 mmol) was added. This was

stirred for 2 h, then excess concentrated ammonia (2 ml) was added and the reaction stirred for a futher 15 min. The solvents were removed under reduced pressure, and the product sublimed to yield a white powder (0.184 g, 93 %)

m.p. 176-177 °C (lit m.p. 176 °C); υ_{max}/cm^{-1} 3474, 3240, 3186, 2918, 2851, 1675, 1657, 1614, 1591, 1500, 1477; *m/z* 165 (100 %, M^+), 149 (31 %, M^+-NH_2), 136 (41 %, M^+-CO), 65; δ_H d_6-DMSO 6.2 (s, 2H, CH_2), 7.02 (t, 1H, *J* 8.0), 7.2 (dd, 1H, *J* 1.2, 6.4), 7.28 (br.s, 1H, NH), 7.38 (dd, 1H, *J* 1.3, 6.6), 7.78 (br.s, 1H, NH); δ_C d_6-DMSO 101.89, 111.51, 116.69, 121.73, 121.79,145.84, 147.99, 164.76; Anal. found C 58.03, H 4.41, N 8.32, $C_8H_7NO_3$ requires C 58.17, H 4.27, N 8.48 %.

3-Methoxy-2-nitrobenzamide 64

O
NH2
NO2
OCH3

Thionyl chloride (5 ml) was added to a dry, nitrogen purged flask containing 3-methoxy-2-nitrobenzoic acid (1.0 g, 5.07 mmol). The mixture was refluxed for 4 h. Excess thionyl chloride was removed by vacuum distillation to yield a yellow solid. The solid was dissolved in anhydrous THF (30 ml), and aqueous ammonia was added dropwise forming a white solid. After stirring for 30 min. the solvents were removed under reduced pressure to yield a white solid which was recrystallised from hot ethyl acetate and petrol to afford **64** as a white solid (0.878 g, 88 %).

m.p. 209-210 °C; υ_{max}/cm^{-1} 3348, 3179, 2984, 2951, 1672, 1626, 1610, 1579, 1537, 1468, 1437, 1394, 1305; *m/z* 196 (100 %, M^+), 180 (64 %, M^+-NH_2), 149 (35 %), 106 (35 %), 92, 76 (70 %); δ_H d_6-DMSO 3.9 (s, 3H, CH_3), 7.45 (dd, 1H, *J* 1.1, 6.5), 7.5 (dd, 1H, *J* 1.1, 7.5), 7.7 (t, 1H, *J* 6.8), 7.83 (br.s, 1H, NH), 8.3 (br.s, 1H, NH); δ_C

d_6-DMSO 57.18, 116.22, 119.99, 129.84, 131.78, 150.79, 165.79; Found M^+ 196.0495. $C_8H_8N_2O_4$ requires 196.0484.

2-Amino-3-methoxybenzamide **65**

To a flask charged with nitrogen was added palladium on carbon catalyst (0.20 g), this was suspended in methanol (30 ml) and 3-methoxy-2-nitrobenzamide (0.5 g, 2.55 mmol) was added. The flask was evacuated, then charged with hydrogen. The mixture was left to stir vigorously for 5 h. The flask was evacuated, then opened to the atmosphere. The mixture was filtered through a celite pad and the pad washed with methanol (4 x 10 ml). The solvent was removed under reduced pressure to yield a white solid **65** (0.426 g, 99 %).

m.p. 146-147 °C; *m/z* 196 (100 %, M^+, 180 (64 %,M^+-NH_2), 149 (35 %), 106 (35 %), 92, 76 (70 %); δ_H d_6-DMSO 3.9 (s, 3H, OCH_3), 6.3 (br.s, 2H, NH_2), 6.57 (t, 1H, *J* 8.0), 6.98 (dd, 1H, *J* 1.1, 6.9), 7.19 (br.s, 1H, NH), 7.27 (dd, 1H, *J* 1.1, 7.0), 7.83 (br.s, 1H, NH); δ_C d_6-DMSO 57.18, 116.22, 119.99, 129.84, 131.78, 150.79, 165.79; Found M^+ 166.0748, $C_8H_{10}N_2O_2$ requires 166.0742.

3-Methoxy-2-diazonium tetrafluoroborate benzamide **66**

Fluoroboric acid (1 ml) [CARE] was added to a solution of 2-amino-3-methoxybenzamide **65** (0.2 g, 1.02 mmol) in water (1 ml). Sodium nitrite (0.07 g, 1.02 mmol in 0.2 ml water) was added dropwise and the solution stirred for 1 h. The precipitate formed **66** was collected and dried (0.159g, 62 %).

m.p. 177-178 °C; υ_{max}/cm^{-1} 3474, 3366, 3335, 3154, 3154, 2957, 1668, 1618, 1593, 1549, 1469, 1437, 1414; *m/z* (FAB) 178 (100 %, M^+); δ_H d_6-DMSO 4.39 (s, 3H, OCH_3), 8.05 (dd, 2H), 8.4 (t, 1H, *J* 8.6) 8.62 (br.s, 1H, NH), 8.97 (br.s, 1H, NH).

Experimental for Chapter Four

8-Hydroxy-2-methylquinazolin-4[3H]-one **69**

Methyl-2-methylbenzoxazole-4-carboxylate **35** (0.1 g, 0.523 mmol) was placed in an autoclave, to this was added excess liquid ammonia (30 ml). The autoclave was sealed and set to 55°C, 20 bar pressure and left for 20 h. Once the autoclave had cooled, the material was removed and the excess ammonia evaporated. The product was recrystallised from propan-2-ol to yield **69** a cream solid (0.058 g, 63 %).

υ_{max}/cm^{-1} 3177, 3036, 2999, 2882, 2808, 1670, 1626, 1577, 1512, 1473; *m/z* 176 (100%, M^+), 160 (43 %), 148 (25 %), 134, 107 (87 %), 88, 79, 63; δ_H d_6-DMSO 2.47 (s, 3H, CH_3), 7.25 (dd, 1H, *J* 1.5 6.2), 7.35 (t, 1H, *J* 7.7), 7.6 (dd, 1H, *J* 1.5, 6.2); δ_C d_6-DMSO 21.75, 115.72, 118.45, 121.76, 126.54, 138.28, 152.59, 152.87, 162.07; Anal. found C 61.17, H 4.53 N 15.50, required for $C_9H_8N_2O_2$ C 61.36, H 4.54, N 15.50 %.

8-Hydroxy-2-(4-nitrophenyl)quinazolin-4[3H]-one 70

Methyl-2-(4-nitrophenyl)benzoxazole-4-carboxylate **48** (0.20 g, 0.671 mmol) was placed in an autoclave, to this was added excess liquid ammonia (50 ml). The autoclave was sealed and set to 55°C, 20 bar pressure. This was left for 20hr. Once the autoclave had cooled, the material was removed and the excess ammonia evaporated. The product was recrystallised from propan-2-ol to yield **70** a yellow solid (0.098 g, 52 %).

υ_{max}/cm^{-1} 3410, 1686, 1522, 1471, 1385, 1352. *m/z* 283 (35%, M^+), 267 (70%, M^+-NH_2), 253 (20%), 240, (30%, M^+-CO) 221, 97, 85, 69, 57 (100%); δ_H d_6-DMSO 7.38 (dd, 1H, *J* 1.5, 6.2), 7.51 (t, 1H, *J* 7.7), 7.7 (dd, 1H, *J* 1.5, 6.2) 9.95 (br.s, 1H, NH), 12.85 (brs, 1H, OH); δ_C d_6-DMSO 116.03, 119.08, 122.35, 123.77, 128.35, 129.70, 137.53, 138.70, 148.80, 149.21, 153.72, 162.41; Anal. found C 58.22, H 2.97 N 14.27, required for $C_{14}H_9N_3O_4$ C 59.37, H 3.202, N 14.835 % (calculated to contain 0.3 M water).

8-Hydroxy-2-methyl N methylquinazolin-4-one 71

To a solution of methyl-2-methyl benzoxazole carboxylate **35** (0.05 g, 0.262 mmol) in ethanol (5 ml) was added methylamine (33% in ethanol, 0.2 ml). This was stirred at rt for 24h. The solvents were removed under reduced pressure and the solid sublimed to yield the title product **71** as a white powder (0.042 g, 85 %).

υ_{max}/cm^{-1} 3387, 1687, 1657, 1599, 1583; *m/z* 190 (87%, M$^+$), 148 (15%,), 133 (18 %), 111, 85, 69, 56 (100%); δ_H d_6-DMSO 2.58 (s, 3H, NCH_3), 3.58 (s, 3H, CH_3), 7.2 (dd, 1H, *J* 1.4, 6.8), 7.29 (t, 1H, *J* 7.8), 7.68 (dd, 1H, *J* 1.3, 6.4); δ_C d_6-DMSO 23.56, 31.17, 116.50, 117.05, 120.11, 127.16, 135.76, 150.92, 153.73, 161.56. Anal. found C 62.13, H 5.15 N 14.40, required for $C_{10}H_{10}N_2O_2$ C 63.15, H 5.29, N 14.73 %.

Appendix 1: Crystallographic data for 2-methylbenzoxazole-4-carboxamide

Table 1. Crystal data, structure solution and refinement for rjg2.

Identification code	rjg2
Chemical formula	$C_9H_8N_2O_2$
Formula weight	176.17
Temperature	160(2) K
Radiation and wavelength	MoKα, 0.71073 Å
Crystal system, space group	orthorhombic, $Pna2_1$
Unit cell dimensions	a = 7.3482(10) Å α = 90°
	b = 15.712(2) Å β = 90°
	c = 6.9114(8) Å γ = 90°
Volume	797.9(2) $Å^3$
Z	4
Density (calculated)	1.467 g/cm^3
Absorption coefficient μ	0.107 mm^{-1}
F(000)	368
Reflections for cell refinement	35 (θ range 11.46 to 12.45°)
Crystal colour	colourless
Crystal size	0.39 × 0.34 × 0.29 mm
Data collection method	Stoe-Siemens diffractometer,
	ω/θ scans with on-line profile fitting
θ range for data collection	2.59 to 24.97°
Index ranges	$-8 \le h \le 8$, $-18 \le k \le 18$, $-8 \le \ell \le 8$
Standard reflections	5 every 60 minutes
Intensity decay of standards	0%
Reflections collected	2648
Independent reflections	1399 (R_{int} = 0.0103)
Reflections with $I>2\sigma(I)$	1380
Absorption correction	none
Structure solution	direct methods
Refinement method	full-matrix least-squares on F^2
Weighting parameters a, b	0.0353, 0.1069
Data / restraints / parameters	1399 / 1 / 126
Goodness-of-fit on F^2	1.089
Final R indices [$I>2\sigma(I)$]	R1 = 0.0222, wR2 = 0.0586
R indices (all data)	R1 = 0.0228, wR2 = 0.0596
Absolute structure parameter	0.1(9)
Extinction coefficient	0.042(3)
Largest and mean shift/esd	-0.001 and 0.000
Largest diff. peak and hole	.157 and -.119 $eÅ^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for rjg2. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	1624(2)	6795.2(8)	10074(2)	24.0(3)
O(1)	1289.4(11)	6049.7(5)	9085.1(14)	26.2(2)
C(2)	2312(2)	6888.4(9)	11918(2)	28.4(3)
C(3)	2485(2)	7725.0(9)	12550(2)	30.4(3)
C(4)	2005(2)	8413.1(8)	11375(2)	27.2(3)
C(5)	1338(2)	8307.4(8)	9505(2)	22.2(3)
C(6)	1143.6(14)	7464.3(7)	8864(2)	20.9(3)
N(1)	498.6(13)	7125.8(6)	7112(2)	22.2(2)
C(7)	610(2)	6310.7(7)	7335(2)	23.6(3)
C(9)	94(2)	5628.5(8)	5974(2)	30.3(3)
N(2)	215(2)	8915.8(7)	6522(2)	28.9(3)
C(8)	867(2)	9066.9(7)	8297(2)	23.5(3)
O(2)	1101.5(12)	9797.4(5)	8915(2)	31.8(2)

Table 3. Bond lengths (Å) and angles (°) for rjg2.

C(1)-O(1)	1.378(2)	C(1)-C(2)	1.379(2)
C(1)-C(6)	1.389(2)	O(1)-C(7)	1.371(2)
C(2)-C(3)	1.391(2)	C(3)-C(4)	1.398(2)
C(4)-C(5)	1.392(2)	C(5)-C(6)	1.404(2)
C(5)-C(8)	1.497(2)	C(6)-N(1)	1.405(2)
N(1)-C(7)	1.292(2)	C(7)-C(9)	1.476(2)
N(2)-C(8)	1.338(2)	C(8)-O(2)	1.237(2)
O(1)-C(1)-C(2)	127.86(11)	O(1)-C(1)-C(6)	107.44(11)
C(2)-C(1)-C(6)	124.69(12)	C(7)-O(1)-C(1)	104.35(9)
C(1)-C(2)-C(3)	115.11(12)	C(2)-C(3)-C(4)	121.68(12)
C(5)-C(4)-C(3)	122.43(12)	C(4)-C(5)-C(6)	116.20(12)
C(4)-C(5)-C(8)	120.29(11)	C(6)-C(5)-C(8)	123.51(11)
C(1)-C(6)-C(5)	119.88(13)	C(1)-C(6)-N(1)	108.54(10)
C(5)-C(6)-N(1)	131.58(11)	C(7)-N(1)-C(6)	104.53(10)
N(1)-C(7)-O(1)	115.13(11)	N(1)-C(7)-C(9)	128.85(13)
O(1)-C(7)-C(9)	116.01(11)	O(2)-C(8)-N(2)	122.09(12)
O(2)-C(8)-C(5)	120.96(11)	N(2)-C(8)-C(5)	116.93(11)

Appendix 2: Crystallographic data for 8-hydroxy-2-methylquinazolin-4[3*H*]-one

Table 1. Crystal data, structure solution and refinement for rjgl.

Identification code	rjgl
Chemical formula	$C_9H_8N_2O_2$
Formula weight	176.17
Temperature	297(2) K
Radiation and wavelength	MoKα, 0.71073 Å
Crystal system, space group	monoclinic, C2/c
Unit cell dimensions	a = 24.568(10) Å α = 90°
	b = 4.856(2) Å β = 131.00(2)°
	c = 18.149(8) Å γ = 90°
Volume	1634.0(12) $Å^3$
Z	8
Density (calculated)	1.432 g/cm^3
Absorption coefficient μ	0.104 mm^{-1}
F(000)	736
Reflections for cell refinement	36 (θ range 11.03 to 12.42°)
Crystal colour	colourless
Crystal size	0.48 × 0.26 × 0.05 mm
Data collection method	Stoe-Siemens diffractometer, ω/θ scans with on-line profile fitting
θ range for data collection	2.97 to 22.52°
Index ranges	$-22 \le h \le 26$, $-5 \le k \le 5$, $-19 \le \ell \le 19$
Standard reflections	5 every 60 minutes
Intensity decay of standards	1%
Reflections collected	1450
Independent reflections	1068 (R_{int} = 0.0141)
Reflections with $I>2\sigma(I)$	769
Absorption correction	none
Structure solution	direct methods
Refinement method	full-matrix least-squares on F^2
Weighting parameters a, b	0.0524, 1.2790
Data / restraints / parameters	1064 / 0 / 121
Goodness-of-fit on F^2	1.087
Final R indices [$I>2\sigma(I)$]	R1 = 0.0357, wR2 = 0.0939
R indices (all data)	R1 = 0.0584, wR2 = 0.1189
Extinction coefficient	0.0044(11)
Largest and mean shift/esd	0.001 and 0.000
Largest diff. peak and hole	.199 and -.144 $eÅ^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for rjgl. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	6176.5(13)	2773(5)	8032(2)	48.2(7)
O(1)	5862.6(11)	4391(4)	8279(2)	63.7(6)
C(2)	6907.8(14)	2914(6)	8582(2)	56.1(7)
C(3)	7253.8(14)	1280(6)	8369(2)	59.4(8)
C(4)	6862.6(13)	-507(6)	7593(2)	53.2(7)
C(5)	6113.9(12)	-639(5)	7011(2)	42.2(6)
C(6)	5760.2(12)	1023(5)	7218(2)	41.7(6)
N(1)	5010.1(10)	1026(4)	6635.8(14)	44.1(6)
C(7)	4642.2(13)	-673(5)	5903(2)	41.9(6)
C(9)	3840.4(13)	-819(6)	5238(2)	54.1(7)
N(2)	4958.6(10)	-2399(4)	5688.1(13)	45.2(6)
C(8)	5684.5(12)	-2483(5)	6187(2)	44.4(6)
O(2)	5925.4(9)	-4046(4)	5918.1(12)	57.2(6)

Table 3. Bond lengths (Å) and angles (°) for rjgl.

C(1)-O(1)	1.367(3)	C(1)-C(2)	1.370(4)
C(1)-C(6)	1.403(3)	C(2)-C(3)	1.390(4)
C(3)-C(4)	1.372(4)	C(4)-C(5)	1.398(3)
C(5)-C(6)	1.405(3)	C(5)-C(8)	1.443(3)
C(6)-N(1)	1.399(3)	N(1)-C(7)	1.300(3)
C(7)-N(2)	1.362(3)	C(7)-C(9)	1.491(3)
N(2)-C(8)	1.372(3)	C(8)-O(2)	1.243(3)
O(1)-C(1)-C(2)	119.0(2)	O(1)-C(1)-C(6)	121.0(2)
C(2)-C(1)-C(6)	119.9(2)	C(1)-C(2)-C(3)	121.2(2)
C(4)-C(3)-C(2)	120.1(2)	C(3)-C(4)-C(5)	119.5(3)
C(4)-C(5)-C(6)	120.6(2)	C(4)-C(5)-C(8)	121.1(2)
C(6)-C(5)-C(8)	118.3(2)	N(1)-C(6)-C(1)	118.9(2)
N(1)-C(6)-C(5)	122.5(2)	C(1)-C(6)-C(5)	118.5(2)
C(7)-N(1)-C(6)	117.3(2)	N(1)-C(7)-N(2)	122.6(2)
N(1)-C(7)-C(9)	121.4(2)	N(2)-C(7)-C(9)	116.0(2)
C(7)-N(2)-C(8)	124.6(2)	O(2)-C(8)-N(2)	120.4(2)
O(2)-C(8)-C(5)	125.0(2)	N(2)-C(8)-C(5)	114.6(2)

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