Novel hypoxia-selective prodrugs of radiosensitisers and chemosensitisers

Parveen, Ifat

Award date:
2001

Awarding institution:
University of Bath

Novel hypoxia-selective prodrugs of radiosensitisers and chemosensitisers

submitted by

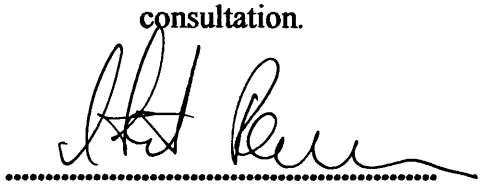
Ifat Parveen

for the degree of PhD

of the University of Bath

2001

The research work in this thesis has been carried out in the Department of Pharmacy and Pharmacology, under the supervision of Dr Michael D. Threadgill and Dr William J. D. Whish.



3/10/01

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ABSTRACT

Effective treatment of cancer is limited by the use of surgery, radiotherapy and chemotherapy. Surgery and radiotherapy are only successful if the disease is localised and after it has become widespread these forms of treatment can only offer (in some cases) symptomatic control. Chemotherapeutic drugs have been developed with the aim to target proliferating cells. However, because of the similarity of healthy cells and normal cells, such agents are unable to kill cancer cells selectively. Poly(ADP-ribose) polymerase (PARP) an enzyme found in most eukaryotic cells, appears to be involved in DNA excision repair. Inhibitors of PARP have been shown to potentiate the cytotoxicity of both DNA-damaging chemotherapeutic agents and radiotherapy. The aim of this project was to design a prodrug system, bearing a trigger moiety consisting of a nitro group, which when activated under hypoxic conditions would release potent PARP inhibitors selectively in solid tumours.

5-Bromo-2-((1-methyl-2-nitroimidazole-5-yl)methyl)isoquinolin-1,2-one was synthesised by a convergent synthesis. 5-Bromoisoquinolin-1-one, synthesised by an efficient novel route, was alkylated with 5-chloromethyl-1-methyl-2-nitroimidazole / 5-bromomethyl-1-methyl-2-nitroimidazole in the presence of base. Biomimetic reduction caused the release of 5-bromoisoquinolin-1-one. Currently, 5-aminoisoquinolin-1-one is of interest as a PARP inhibitor in haemorrhagic shock and renal dysfunction. 5-Chloromethyl-1-methyl-2-nitroimidazole reacted efficiently with the anion derived from 5-(Bocamino)isoquinolin-1-one to give 5-amino-2-((1-methyl-2-nitroimidazole-5-yl)methyl)isoquinolin-1-one, after deprotection.

Previous studies have shown that N-oxides can be bioreduced under hypoxic conditions. A prodrug was designed based on N-oxide bioreduction. Attempts were made to synthesise quinoline-8-carboxamide N-oxide and the analogue 2-phenylquinoline-8-carboxamide N-oxide. Although the compounds were not achieved, several advances in quinoline chemistry were made.

Curcumin is of particular interest as a cancer chemopreventative agent. Lithium-bromine exchange of 1-bromo-4-(1-ethoxyethoxy)-3-methoxybenzene with

butyllithium followed by quenching with O^2HCNMe_2 and acidic work up gave [^{2}H]-vanillin in 27% yield, based on O^2HCNMe_2. Condensation with pentane-2,4-dione gave [$^2H_2$]-curcumin. Adaptation of the procedures to use of $OH^{14}CNMe_2$ gave [^{14}C]-curcumin *via* [^{14}C]-vanillin in 81% chemical yield, 1.20 MBq, 81% radiochemical yield, specific radioactivity 12.7 MBq mmol^{-1}.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. Mike Threadgill and the late Dr. Bill Whish for their support, encouragement and enthusiasm throughout the research years.

I would like to thank Mr David Wood and Mr Harry Hartell for NMR spectra and Mr Chris Cryer for mass spectra.

Many thanks to my postdoctoral and postgraduate colleagues in particular Shane, Penny, Jackie, Andy, Sula, Dharshini and Norina for their help and advice.

I would also like to acknowledge Association for International Cancer Research for generous financial support.

Finally, a special thank you to my friend Barry for all his love and support and to all my family in particular my mum, dad and brother Sajj for their love and support throughout the PhD period.

CONTENTS

Abstract		ii
Acknowledgements		iv
Contents		v
List of Figures Schemes and Tables		vii
Abbreviations		ix

INTRODUCTION

1.0	Hypoxia	1
1.1	Direct and Indirect Measurements of Tumour Hypoxia	3
1.2	The Mechanism of Action of Radiation Therapy	5
1.3	Radiosensitisers	7
1.4	Chemosensitisers	10
1.5	Bioreductive Drugs	15
2.0	Poly(ADP-ribose) Polymerase	22
2.1.1	The DNA Binding Domain (DBD)	24
2.1.2	The Automodification Domain	25
2.1.3	The Catalytic Domain	26
2.2.1	Mechanism of Action of PARP	26
2.2.2	Modification of the Chromatin Structure	27
2.3	Poly(ADP-ribose) Glycohydrolase (PARG)	27
2.4	Mono- and Poly(ADP-ribosyl)ation	28
2.5	Functions of PARP	28
2.5.1	Role of PARP in Viral Replication	28
2.5.2	Role of PARP in Apoptosis	29
2.5.3	Role of PARP in Inflammation	30
2.5.4	Role of PARP in Ischaemia-Reperfusion Injury	31
2.5.5	Role of PARP in DNA Repair	32

3.0	Inhibitors of Poly(ADP-ribose) Polymerase	35
3.1.1	Chemosensitisation	35
3.1.2	Radiosensitisation	36
3.2	PARP Inhibitors Developed to Date	38
4.0	Curcumin	46
5.0	Aims and Objectives	52
6.0	Results and Discussion	56
6.1	Critical 2-Nitroimidazole Intermediates	60
6.2	Critical 5-Bromoisoquinolin-1-one Intermediates	66
6.3	Reductively Triggered Release from Prodrug (118)	72
6.4	Synthesis of Prodrug (127)	76
6.5	Attempted Synthesis of Prodrug (129)	79
6.6	Analogues of Prodrug (118)	81
6.7	Other Routes to 1,5-Disubstituted-2-Nitroimidazoles	83
6.8	Critical 2-Nitrothiophene Intermediates	87
6.9	Quinoline-8-Carboxamides and Quinoline-N-oxides	89
7.0	Synthesis of ^{14}C-labelled Curcumin (235)	103
8.0	Experimental	113
9.0	References	161

List of Figures, Schemes and Tables

Figure 1.	Diagramatic representation of a solid tumour.	3
Figure 2.	Illustrates the radicals generated during the interaction of radiation with water and the organic molecules DNA and RNA.	6
Figure 3.	Identification of the three domains of PARP.	23
Figure 4.	Constituents of Curcuma longa L.	48
Figure 5.	SAR necessary in the development of PARP inhibitors.	52
Figure 6.	SAR necessary for the bioreducible nitroimidazole moiety.	53
Figure 7.	Illustrates the general structure of tumour hypoxia selective prodrugs.	54
Figure 8.	Typical HPLC chromatograms from the reductively triggered drug release study.	75
Figure 9.	Demonstrates the structure of the carboxamide when it is 'in-plane' and when twisted 'out-of-plane'.	91

Scheme 1.	Schematic representation of poly(ADP-ribosyl)ation	38
Scheme 2.	Proposed mechanism of bioreductively triggered release of drugs from general nitroimidazole prodrug.	54
Scheme 3.	Reported synthesis of 2-nitroimidazoles.	57
Scheme 4.	Synthesis of 1-methyl-2-nitroimidazoles.	59
Scheme 5.	Reported synthesis of 5-bromoisoquinolin-1-one.	66
Scheme 6.	Synthesis of 5-bromoisoquinolin-1-one and prodrug (118).	67
Scheme 7.	Reductively activated release of 5-bromoisoquinolin-1-one (108).	72
Scheme 8.	Synthesis of 5-amino-2-((1-methyl-2-nitroimidazol-5-yl)methyl)isoquinolin-1-one.	76
Scheme 9.	Synthesis of quinazolin-1-one.	80
Scheme 10.	Attempted synthesis of analogue (141).	81
Scheme 11.	Attempted synthesis of 2-nitroimidazole-5-methanol.	84
Scheme 12.	Attempted synthesis of prodrugs (161), (162) and (163).	87
Scheme 13.	Synthesis of naphthalene-1-carboxamide	92
Scheme 14.	Synthesis of quinoline-8-carboxamide.	92
Scheme 15.	Attempted synthesis of 8-bromoquinoline N-oxide.	93
Scheme 16.	Proposed mechanism for the synthesis of quinoline-8-carboxamide N-oxide by directed oxidation / hydrolysis.	94
Scheme 17.	Attempted synthesis of quinoline-8-carboxamide and 2-phenylquinoline-8-carboxamide.	97
Scheme 18.	Schematic representation of the attack of the Grignard reagent on 8-methylquinoline N-oxide.	99
Scheme 19.	Schematic representation of the attack of hydrogen peroxide on quinoline-2-phenylcarboxaldehyde.	101
Scheme 20.	Synthesis of ^{14}C-labelled curcumin.	104
Table 1	Optimisation studies for the synthesis of (223).	106
and		
Table 2.		108

ABBREVIATIONS

3-AB	3-aminobenzamide
ADEPT	antibody directed enzyme-prodrug therapy
ADP-ribose	adenosine diphosphoribose
5-AIQ	5-aminoisoquinolin-1-one
APC	adenomatous polyposis coli
Aq.	aqueous
Ar	argon
ATP	adenosine trisphosphate
Boc	*tert*-butoxycarbonyl
bp	boiling point
BRCT	BRCA1 C-terminus
Bu^t	*tert*-butyl
CCNU	1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea
***m*-CPBA**	*meta*-chloroperoxybenzoic acid
Conc.	concentrated
COX2	cyclooxygenase 2
d	days
Da	Daltons
DBD	deoxyribonucleic acid binding domain
DCM	dichloromethane
dGTP	2'-deoxyguanosine 5'-triphosphate
DIBAL	diisobutylaluminium hydride
DMBA	dimethylbenz (a) anthracene
DME	1,2-dimethoxyethane
DMF	*N,N*-dimethylformamide
DMFDMA	*N,N*-dimethylformamide dimethylacetal
DMS	dimethyl sulfate
DNA	deoxyribonucleic acid
DNABER	deoxyribonucleic acid-base-excision-repair complex

DSBs	double stranded breaks
EI	electron impact
FAB	fast atom bombardment
F1	zinc finger 1
F2	zinc finger 2
GDEPT	gene-directed enzyme-prodrug-therapy
GSα	GTP-binding protein
HHC	hexahydrocurcumin
HMG	high mobility group
HN_2	*N,N*-bis(2-chloroethyl)methylamine
HPLC	high performance liquid chromatography
IC_{50}	concentration causing 50% inhibition
IKKs	I Kappa B Kinase
IN	virus-encoded integrase protein
IR	infrared
K_i	inhibition constant
LET	linear energy transfer
MDZ	misonidazole
Me	methyl
mNBA	*meta*-nitrobenzyl alcohol
MMC	mitomycin C
mp	melting point
MS	mass spectroscopy
MTIC	5-(3-methyltriazen-1-yl)imidazole-4-carboxamide
m/z	mass to charge ratio (mass spectroscopy)
NAD^+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NBS	*N*-bromosuccinimide
NLS	nuclear location signal
NMR	nuclear magnetic resonance
NOS	nitric oxide synthetase

PARG	poly(adenosine diphosphoribose) glycohydrolase
PARP	poly(adenosine diphosphoribose) polymerase
PARS	poly(adenosine diphosphoribose) polymerase
PGHS	prostaglandin H synthetase
Pr^i	isopropyl
RNA	ribonucleic acid
S_N	nucleophilic substitution
SSBs	single stranded breaks
TBAF	*tetra*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THC	tetrahydrocurcumin
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	temozolomide
TP	topotecan
Ts	tosyl
UV	ultraviolet

In the figures and schemes, the charges on N ($^+$) and O ($^-$) of the quinoline-N-oxides have been omitted for clarity.

INTRODUCTION

1.0 *HYPOXIA*

Cancer is one of the most common causes of mortality today, with one person in three estimated to develop the disease sometime during their lifetime. Currently, cancer therapy involves the use of three forms of treatment, surgery, radiotherapy and chemotherapy. There are many problems, however, associated with the use of each of the above forms of treatments.

In the case of surgery and radiotherapy these forms of treatments are only successful if the disease is localised, so that it can either be completely removed by surgery or treated by radiation therapy. However, once the disease has become widespread, these forms of treatment can only offer in some cases symptomatic control but are unable to cure the disease.

In the case of chemotherapy, the majority of the drugs that have been developed target the DNA of cells, which are proliferating. This consequently limits their use as selective agents due to the similarity between normal and cancer cells and, therefore, many of these drugs tend to be toxic to the bone marrow and gastrointestinal tract. However, chemotherapeutic agents have been successful in rapidly growing cancers such as childhood tumours and Hodgkin's disease[1]. Nevertheless, the majority of human cancers are the relatively slow-growing solid tumours, including carcinomas of the lung, colon and breast, and so this is where the problem is, because such cancers respond poorly to existing chemotherapeutic drugs.

Over the last century, many studies have been carried out in order to identify differences between malignant and normal cells[2]. Any biochemical characteristics that are unique to malignant cells can be exploited in the treatment of cancer. Although a number of metabolic differences between normal and malignant cells have been reported, few, if any, have proved to be either unique to cancer cells or useful in the development of

selective chemotherapeutic agents. Nevertheless, one such characteristic of solid tumours which is thought to be useful in the treatment of cancer is the presence of hypoxic cells.

Thomlinson and Gray[3] first reported the presence of hypoxic cells in tumours in 1955. Analysis of the distribution of blood vessels, the viable tumour tissue and necrosis in human bronchogenic carcinoma in terms of the utilisation and distribution of molecular oxygen within the tissue revealed that the viable cells on the edge of the necrotic regions in these tumours were, in fact, severely hypoxic. Further evidence in support of this was provided by Moulder and Rockwell[4-5] who reported that the majority of solid cancers contained hypoxic cells and that these often contributed about 10-20% of the total viable tumour cell population. Furthermore, hypoxic tumour cells have been reported to exist in an environment exhibiting oxygen concentrations from 0.01-5% O_2[6].

In solid tumours, hypoxic cells develop as a result of the unbalanced growth of the tumour cells and the vascular components that are required in order to provide an adequate blood supply[6,7]. As a result of this, cells are depleted of molecular oxygen and various other nutrients. At a distance of about 150 μm from a capillary, cells tend to be well-oxygenated. At greater distances, however, tissue oxygen tension decreases with distance from the capillary and eventually falls to a level which is insufficient for cell division to take place[6,7]. As a consequence, these oxygen-deprived cells die and this forms the necrotic region. Viable hypoxic cells tend to occur in the interface regions between the well-oxygenated tissue and the necrotic regions[6,7].

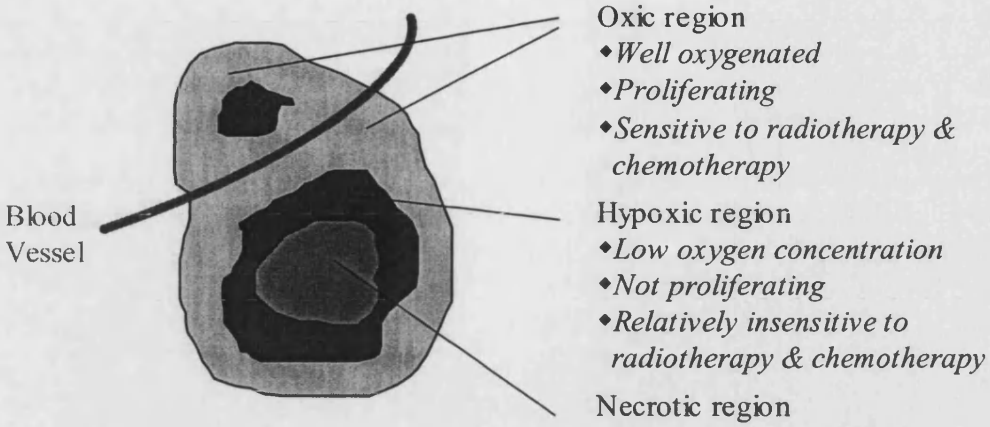


Figure 1: Diagramatic representation of a solid tumour

1.1 DIRECT AND INDIRECT MEASUREMENTS OF TUMOUR HYPOXIA

There are several methods that can be used in order to measure oxygen in tumours and tissues, which include vascular density measurements, the use of oxygen electrodes, redox-sensitive markers of hypoxia and metabolically activated chemical markers[8].

Vascular Density

Poor vascularisation of tumours means that oxygen is poorly delivered to cells resulting in chronically hypoxic tumour cells. Indirect methods of measuring tumour hypoxia can be achieved by taking measurements of the density of the tumour vasculature. These measurements can be taken wherever a biopsy is obtained from a human tumour and histological sections made. However, this method is extremely time-consuming and, in addition, ignores the possibility of acutely hypoxic cells[8].

Oxygen Electrodes

Oxygen electrodes have been used to directly measure oxygen concentrations in human tumours. Even though oxygen electrodes have been designed to be as accurate as

possible, they require the use of very small diameter[8]. However, the smallest needle developed is still large in comparison to the diameter of a tumour cell and, therefore, when inserted into a tumour, an oxygen electrode could be sampling a volume containing both hypoxic and well-oxygenated cells[8].

The Use of Spectroscopy in Redox-Sensitive Markers of Hypoxia

Near-infrared spectroscopic measurements of haemoglobin oxygenation have been used to provide non-invasive information on the oxygenation of the tissue but, because the reporter molecule is localised in the vasculature, it is unable to give information regarding the status of cells which are hypoxic[8]. Furthermore, these redox-related changes are reversible upon reoxygenation on exposure to air and, therefore, cannot be used in biopsy material.

Metabolically Activated Chemical Markers

Nitro-aromatic compounds, such as misonidazole, can be metabolically reduced to the nitro radical anion, which, in aerobic cells, is reoxidised by molecular oxygen to inhibit further metabolism[8]. However, under hypoxic conditions, reactive metabolites are generated and these effectively bind to cellular molecules, therefore labelling hypoxic cells. However, although the majority of nitro-aromatic compounds have been successful as probes for hypoxic cells *in vitro*, few have been successful in studies *in vivo*[8].

Studies with isotopically-labelled 2-nitroimidazoles have also been used as hypoxia markers. However, the use of large amounts of radioactivity for long periods of time limits the clinical usefulness of this technique[8]. However, more recent studies have used 2-nitroimidazoles with immunohistochemically detectable side chains, and the use of such compounds (for example NITP) has shown hypoxia-specific staining in experimental tumours[8,9].

1.2 THE MECHANISM OF ACTION OF RADIATION THERAPY

Over the last century, radiation therapy has been used in the treatment of human cancers. Radiotherapy involves the use of X-rays or γ-rays. Both of these types of ionising radiation cause the ejection of a fast-moving electron from the target molecule, resulting in the formation of an ion radical[10]. This species, in turn, reacts with other molecules, resulting in the formation of ions and free radicals. It is the ions and free radicals that are produced from the interaction of radiation with water that interact with organic molecules such as DNA and RNA, thus resulting in cellular damage[10]. When oxygen is present, a wider range of ions and free radicals are formed, hence more DNA damage occurs. When the DNA is damaged directly by radiation, radical cationic and anionic sites are produced. In the presence of oxygen, the oxygen molecules are capable of abstracting an electron from the radical anions resulting in radical cations that have a long enough life to cause DNA damage. In the absence of oxygen, however, the electron from the radical anion tends to combine with the radical cation, therefore no DNA damage occurs. In both of the above cases, DNA damage is enhanced in the presence of oxygen[10,11].

Radicals produced from radiation therapy:

$$H_2O \rightarrow H_2O^+ + e^-$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH^\bullet$$

$$e^- + H_2O \rightarrow e^- \rightarrow OH^- + H^\bullet$$

$$e^- + H_3O^+ \rightarrow H_2O + H^\bullet$$

$$2OH^\bullet \rightarrow H_2O_2$$

$$2H^\bullet \rightarrow H_2 + OH^\bullet \rightarrow H_2O + H^\bullet$$

$$H_2O_2 + H^\bullet \rightarrow H_2O + OH^\bullet$$

Reaction with O_2:

$$H^\bullet + O_2 \rightarrow HO_2^\bullet \rightarrow H_2O_2 + O_2$$

$$HO_2 + e^- \rightarrow HO_2^- + H^+ \rightarrow H_2O$$

Reaction with organic molecules:

$$X + OH^\bullet \rightarrow XOH^\bullet$$

$$X + OH^\bullet \rightarrow X^\bullet + H_2O$$

$$X^\bullet + O_2 \rightarrow XO_2^\bullet \rightarrow X + O_2^\bullet$$

$$XO_2^\bullet + X^1H \rightarrow XO_2H + X^1$$

Figure 2: Illustrates the radicals generated during the interaction of radiation with water and the organic molecules DNA and RNA (these are represented by X)[10]

Oxygen, therefore, modifies the number and character of free radical reactions and thus increases the DNA damage produced by a given dose of radiation. As a consequence of this, the cell-survival curve for aerobic cells is enhanced by a factor of about three in comparison to that for severely hypoxic cells. Radiosensitisation can occur at relatively low concentrations of oxygen, and a concentration of 0.25% oxygen effectively shifts the dose-response curve half-way towards the fully oxygenated cells[1].

1.3 RADIOSENSITISERS

As previously mentioned, the presence of hypoxic cells within tumours makes them extremely resistant to radiation therapy. In fact, mammalian cells that have been irradiated in air have slopes that are three times as steep as the radiation dose-response curves for cells that are irradiated under severely hypoxic conditions[1]. When a tumour is irradiated, the oxygenated radiosensitive cells are readily killed consequently, increasing the oxygen-diffusion path-length. As a result of this, hypoxic cells are reoxygenated, and can, therefore, enter the cell cycle and allow the tumour to regrow[1,7].

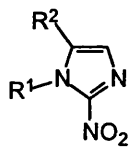
Hypoxic cell radiosensitisers are drugs that have been specifically designed to be given to patients with solid tumours, shortly before undergoing radiation therapy. These compounds are capable of interacting with short-lived free radicals that are produced during radiation, thus enhancing cell killing. Oxygen itself is known to be an effective radiosensitiser and hypoxic cell radiosensitisers act to restore (or at least to some extent) the radiosensitivity of hypoxic tumour cells, which are otherwise resistant to cell killing by radiation therapy[1,7]. Radiosensitising drugs are therefore oxygen substitutes that are capable of diffusing into hypoxic tumour cells and sensitise them *via* a similar mechanism to oxygen[1,7]. However, the important difference is that these drugs are not rapidly metabolised when diffusing through the tumour cells. As a result of this, they are able to penetrate further into the hypoxic regions of tumour cells even those that are some distance away from the nearest microcapillary[1,7]. In normal tissues, the well-oxygenated cells are difficult to sensitise further therefore such drugs are effectively selective towards hypoxic cells[1,7].

The notion that hypoxic cell radiosensitisers could be therapeutically useful first arose in 1969, when Adams and Cooke[12] observed that the more powerful oxidising agents were the most efficient sensitisers. The ability of a compound to sensitise hypoxic cells towards the lethal effects of ionising radiation has been demonstrated to be dependant on the one-electron redox potential[13]. Hypoxic cell radiosensitisation occurs with the transfer of one electron at a radiation-induced lesion, resulting in fixation of the

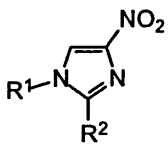
chemical damage. Compounds that exhibit an E^1_7 reduction potential in the range of -300 to -500 mV have been found to be the most effective sensitisers. Compounds with reduction potentials outside this range have been found to be either too toxic or ineffective as radiosensitisers[14,15]. The aim in the development of hypoxic cell radiosensitisers is to design compounds that can be used at doses that produce maximum radiosensitisation of the hypoxic cells. In addition, patient groups (or subgroups) that are likely to respond to hypoxic cell radiosensitisers or to other effects of improving tumour oxygenation need to be identified.

The earliest compounds that were evaluated as radiosensitisers were the class of nitro-heterocyclic compounds. Although work was carried out on a number of compounds including nitroimidazoles[1,7,14-16], nitrofurans[14,15,17], nitrobenzenes[14] and nitrothiophenes[18] much of the attention focused on nitroimidazoles because they appeared to show increased selective toxicity towards hypoxic cells in comparison to the well-oxygenated cells.

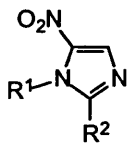
From the various studies carried out, 2-nitroimidazoles were found to be the most effective radiosensitisers with reduction potentials in the range of -250 to -400 mV. When the nitro group was changed from position 2 to position 5 or 4, the electron-affinity of the nitroimidazoles decreased[14]. This was demonstrated by Adams *et al*[14] with nitroimidazoles RGW-601 **(1)**, RGW-611 **(2)** and nimorazole **(3)**.



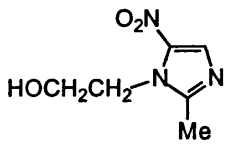
1



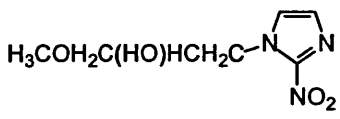
2



3



4



5

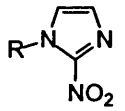
$R^1 = (CH_2)_2NC_4H_8OA$ (where A = morphilino group)
$R^2 = H$

One of the first hypoxic cell radiosensitisers reported to enhance the lethal effects of radiation selectively to hypoxic cells was metronidazole **(4)**. However, high concentrations of this compound were required to sensitise the cells *in vitro* and, therefore, an alternative compound was sought[7,19,20]. Misonidazole (MDZ, **5**) was the next imidazole compound to be developed and this was found to be superior to metronidazole both *in vitro* and *in vivo* studies[7,19-21]. Investigations carried out with mouse tumours revealed that the radiation response was increased significantly to single large doses of irradiation with MDZ[22]. However, the clinical studies carried out with this compound have been disappointing. This was due to the neurotoxicity that was induced in patients that received the drug at therapeutic concentrations. The poor results that were observed have also been attributed to the fact that majority of these clinical trials were performed on the heterogenous populations of tumours and therefore subgroups that may have shown an effect could have been masked by groups that were not affected. As a result of the neurotoxicity observed with MDZ, two analogues were developed; these were etanidazole (SR2508, **6**) and pimonidazole (Ro-03-8799, **7**)[7,16]. Etanidazole exhibited hydrophilic properties that would not allow the drug to cross the blood brain barrier and this was thought to make it less neurotoxic. Pimonidazole, on the other hand, was developed to be a more effective radiosensitiser and was achieved because the molecule was uncharged at acid pH; therefore, it was proposed that the drug would concentrate in the tumours. Both of these compounds underwent clinical evaluation and proved to be promising, as tumour concentrations of both of these drugs have been achieved at five times greater than those which were achieved with MDZ. This increases the sensitizer enhancement ratio (SER) in a twenty-fraction radiotherapy regime of 1.1-1.2 for misonidazole to approximately 1.5 for each of the two analogues. However, this was still below the therapeutically required maximum of about 2.5[7,16,22].

The next compound to be developed was the DNA-targeted radiosensitiser RSU 1069 **(8)**. In the side chain of RSU 1069 was incorporated an aziridine moiety that behaved as a monofunctional alkylating agent. This compound, therefore, had the potential to behave both as an alkylating agent and a radiosensitiser. Unfortunately, the drug failed clinically because of the reactivity of the aziridine group, which led to emesis[7,16]. In an

attempt to decrease the side-effect but retain its radiosensitising activity, the aziridine group was replaced by substituted acetohydroxamic acids[23] and β-haloethylamines[24]. In the acetohydroxamic acids developed, KIH-802 **(9)** was found to be the most potent analog, displaying significant activity *in vivo* in SCCVII tumours. However, studies revealed that this compound caused neurotoxicity[7,16]. Work carried out with β-haloethylamines resulted in two compounds, RB 6145 **(10)** and its derivative PD 130908 **(11)**. Both of these compounds were found to be equal in radiosensitising activity to RSU 1069, but were less toxic[7,16].

A number of non-nitro heterocyclic compounds have also been evaluated as hypoxic cell radiosensitisers and found to be metabolised quickly and therefore were inactive *in vivo*[16,25].



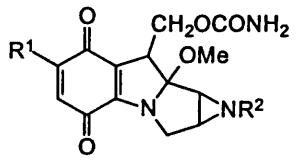
6 R = $CH_2NHCO(CH_2)_2OH$
7 R = $CH_2CH(OH)CH_2C_5H_5N$
8 R = $CH_2CH(OH)CH_2N(CH_2)_2$
9 R = $(CH_2)CONHOK$
10 R = $(CH_2)CH(OH)CH_2NH(CH_2)_2Br.HBr$
11 R = $(CH_2)_3NH(CH_2)_2Br.HBr$

1.4 CHEMOSENSITISERS

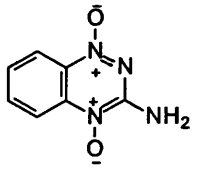
Currently chemotherapeutic drugs that are in clinical use for the treatment of cancer derive from the toxicity of the drugs to those cells, which are actively traversing the cell cycle. Unfortunately, the toxicity of such agents to proliferating cells of normal tissues limits their use. Cycle-active agents are ineffective against quiescent tumour cells which, at the time of treatment, may not be actively cycling but are capable of undergoing proliferation at a later stage, thus allowing the tumour to regrow[1,6,7,19]. The fact that hypoxic cells may have prolonged cell-cycle times or may be blocked in their progression through the G_1 phase makes these cells relatively resistant to chemo-

therapeutic drugs that are directed towards proliferating cells[1,6,7,19]. Furthermore, pharmacodynamic considerations such as the drugs not diffusing into the tumour tissue, or that they are unstable or rapidly metabolised cause further complications in the treatment of tumours. Because hypoxic cells are located in poorly vascularised regions makes drug accessibility very problematic, as these cells are usually located some distance away from the nearest microcapillary[1,6,7,19]. In some cases, molecular oxygen may be necessary for the energy requirement of a drug mechanism and, without it, the drug would be unable to exert its cytotoxic effect[1,6,7,19]. Furthermore, hypoxic cells tend to exist in an environment which is deficient in nutrients and is also relatively acidic. These differences in the cellular microenvironment of hypoxic cells may further affect the proliferation patterns of such cells[1,6,7,19].

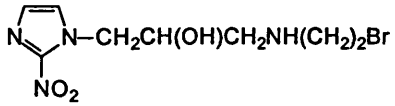
The aim of chemotherapeutic drugs is to eradicate the tumour, with no or minimal toxic side effects to the host. In order to develop chemotherapeutic agents, various characteristics of tumours need to be considered such as whether the cells are proliferating, quiescent, clonogenic, non-clonogenic, well-oxygenated, slightly hypoxic or severely hypoxic[1,6,7,19]. In addition to this, both cellular and environmental factors need to be considered as they may contribute to the transition state of cells between subpopulations. Even though hypoxic cells are resistant to many chemotherapeutic agents, certain anticancer drugs have been found to be selectively toxic towards hypoxic cells. These include nitroimidazoles such as MDZ, RSU-1069, quinones such as mitomycin C (MMC, **12**) and benzotriazine N-oxides such as SR 4233 **(13)**.



12 $R^1 = NH_2$, $R^2 = H$
16 $R^1 = NH_2$, $R^2 = Me$
17 $R^1 = NCHNMe_2$, $R^2 = Me$



13



14 R = *S* form
15 R = *R* form

MDZ was initially developed as a radiosensitiser but, in addition, showed chemo-sensitising properties. This drug and many other nitroimidazoles, were found to be selectively toxic towards hypoxic tumour cells[1,19,26]. Because hypoxic cells contain low oxygen concentrations, nitroimidazoles are capable of undergoing reductive metabolism to highly reactive electrophilic toxic agents[1,6,7,19]. The activation process is thought to be catalysed by flavoproteins such as NADPH cytochrome P450, NADPH cytochrome c reductase and xanthine oxidase. Therefore MDZ when activated by the reductase enzymes generates the first nitro-radical anion metabolite. This species under hypoxic conditions is reduced further to the nitroso, the hydoxylamine metabolite and eventually the amine. The reduction products that are formed during this process have been reported to bind to DNA, RNA and proteins, in addition to non-protein thiols such as glutathione[26,27].

A number of biochemical alterations have also been reported to occur by MDZ intermediates including disturbances in electron transport pathways[28], reduction of non-protein sulfhydryls[29], inhibition of nucleotide uptake and DNA synthesis[30], DNA single and double stranded breaks[30], and increased cell-cycle times[1].

In well-oxygenated cells, however, reductive metabolism of the drug leads to the formation of the nitro-radical anion. This species is very reactive towards oxygen, and under these conditions is oxidised back to the parent molecule.

MDZ has also been reported to sensitise mammalian cells to the cytotoxic effects of alkylating agents such as 1-(2-chloroethyl)-3-cyclohexyl1-nitrosourea (CCNU). It is thought that some of the chemosensitisation may be due to enhanced drug-induced cross-linking and also inhibition of CCNU metabolism by MDZ[31-33]. In addition, the depletion of cellular thiols by MDZ may also contribute to the chemopotentiation of the alkylating agents[34].

RSU-1069 has been reported to be about 80-fold more selectively toxic towards hypoxic cells than oxic cells[35,36]. This drug was found to be superior to MDZ and is also an

effective radiosensitiser[16]. However, the high gastrointestinal toxicity observed in non-tumour-bearing mice limited the clinical usefulness of this compound. The lower toxicity of RSU 1069 was achieved by using a prodrug of this compound, such as RB6145. RB 6145 is one of the most effective compounds *in vivo* that have been reported in this class. In the side-chain of RB 6145 is a β-carbon that exhibits chiral properties and so the compound can exist in two enantiomeric forms, which are PD-144871 (*S*) **(14)** and PD-144872 (*R*) **(15)**. However, both of these compounds have been found to be unsuitable for clinical use as they exhibited emesis side-effects[37].

MMC is a bioreductive-alkylating agent which has been shown to be selectively toxic towards hypoxic cells[1,19,38,39]. Toxicity of this drug is induced with the formation of DNA-DNA cross-links and MMC monoadducts in DNA. This drug is biotransformed to the alkylating species *via* the one-electron reduction of the drug to a semiquinone, which, under hypoxic conditions, activates the C-1 position of the aziridine ring, resulting in the binding of the drug to DNA[1,19]. After the initial covalent attachment of MMC to DNA at position N-2 of guanine in DNA, the drug is thought to undergo further activation to produce a second alkylation site at the C-10 position. The bifunctionally alkylated MMC has recently been reported to fit snugly into the minor groove with minimal deformation of the helix and this feature is thought to make MMC cross-links difficult to recognize and repair *in vivo*[40].

The activation of MMC is thought to be catalysed by several enzymes including xanthine oxidase and NADPH cytochrome P-450 reductase and toxicity of this drug is observed following the one- or two-electron reduction product. In well-oxygenated cells, it appears that the oxygen prevents further reduction of the MMC to the alkylating species by reacting with the one-electron reduction product to form both the parent compound and the superoxide radical[1,6,7,19]. The magnitude of the differential toxicity however, is dependant on the substrate specificities for the various reductases and on which reductive pathway occurs in the tumour cell line[1,6,7,19].

Under aerobic conditions, MMC has been shown to be activated chemically by mild acid. Under these conditions, the DNA adduct is formed at the N-7 of guanine which is different to that formed following reductive activation (N-2 of guanine). Similarly, studies carried out in cell culture have reported that the activity of this drug is enhanced under acidic conditions and this leads to increased interstrand cross-linking by the drug[1,7,41]. This may prove to be significant with hypoxic tumour cells, because they may exist in an acidic environment and this feature of such cells may be useful in enhancing the activity of the drug.

In this class of compounds, different toxicity is observed for compounds with different structures. For example, substitution at the aziridinyl nitrogen with a methyl group results in the analogue porfiromycin **(16)**. This drug has been reported to exhibit greater selective toxicity to hypoxic cells than MMC. The cytotoxicity of the drug is thought to be associated with the DNA cross-links that are formed[42]. Modification at position-7 of MMC, from the amino group to the amidine group results in compound BMY 25282 **(17)**, which shows increased selective toxicity towards aerobic cells in comparison to hypoxic cells[43]. This demonstrates that modification of the structure of MMC can alter the selectivity of these drugs.

The N-oxides such as the benzotriazine-N-oxide, tirapazamine (SR 4233), are also bioreductive alkylating agents that exhibit high selectivity for hypoxic cells. In fact, in some cases, differential toxicity ratios in some mouse cell lines are as high as 200-fold. These types of compounds have an absolute requirement for one-electron reduction in order for toxicity to be expressed. The mechanism of action is different to that reported for nitro compounds, in that it is the N-oxide radical anion species which causes toxicity by means of reacting directly with cellular macromolecules. It has been reported that hydrogen is abstracted from bases in DNA resulting in extensive single and double stranded breaks[44,45].

In well-oxygenated cells, however, back-oxidation of the nitroxide radical occurs, thereby reducing the number of toxic reactions that can occur. The enzymes involved in

the bioreductive activation of tirapazamine are P450 reductase and P450 itself to give the toxic one-electron reduced product. The two-electron reductase DT diaphorase was also found to metabolise tirapazamine and it was thought that this constituted a detoxification reaction, as the toxic nitroxide radical is by-passed[1,6].

1.5 BIOREDUCTIVE DRUGS

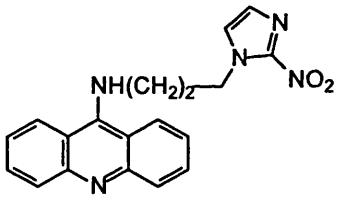
Another method of overcoming the resistance of hypoxic cells to both radiotherapy and chemotherapy is to eliminate the hypoxic cell population completely. This may be achieved by the use of bioreductive drugs. It has been proposed that bioreductive drugs would provide a bystander effect, by releasing a diffusible cytotoxin on activation by reductases, to utilise the hypoxic cells present in solid tumours. Bioreductive drugs can be divided into three domains that are the trigger, the effector and the linker. The trigger part of bioreductive compounds is responsible for activation of the prodrug and is also required to inhibit the reduction by free oxygen when activated by reductases. If tumour-specific enzymes activate the drug, the trigger needs to be highly selective and if the drug is activated by radiation the trigger has to have potential as a reducing agent. Compounds that behave as triggers include nitro-aromatic compounds, quinones, aromatic N-oxides (as previously mentioned), nitrofuranyl prodrugs[46] and transition metal complexes[47,48].

Bioreductive drugs are prodrugs that are capable of distributing efficiently to the hypoxic regions of tumours, thereby undergoing selective metabolism in these areas to cytotoxic species. Prodrugs must therefore have minimal toxicity to aerobic cells, be able to withstand metabolism under these conditions and should possess good solubility characteristics[47].

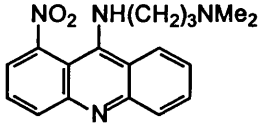
In order to improve aqueous solubility of a compound, basic functionalities (cationic charges) have been incorporated into a given drug. It was found that, in addition to providing solubilising potential, the basic functionalities also increased potency in cell cultures by inducing accumulation in cells. However, compounds that are positively

charged (weak bases) have usually proved to be disappointing as bioreductive drugs *in vivo*[47]. Examples of such compounds include NLA-1 **(18)**, nitracine **(19)** and 5-NQ **(20)**. It is thought that these compounds lack *in vivo* activity because the basic compounds may be sequestered into the low pH cellular organelles, such as lysosomes, or it may be due to the reversible binding to DNA that occurs with these types of compounds. In both of these cases, the concentration of the free drug is lowered, thereby reducing the rates of passive diffusion through the tissue. A further problem, which may be encountered with these drugs, is that weak bases may be excluded from cells where the surrounding medium is at a low pH[47].

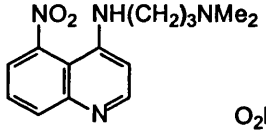
The incorporation of anionic groups (weak acids) to improve solubility characteristics has also been studied and it has been demonstrated that such compounds show preferential uptake in cells that exist in low external pH environments[47]. A recent study, which was carried out with 2,4-dinitrobenzamide mustards, demonstrated that the acid derivative **(21)** cytotoxicity was increased, by reducing the extracellular pH environment[47].



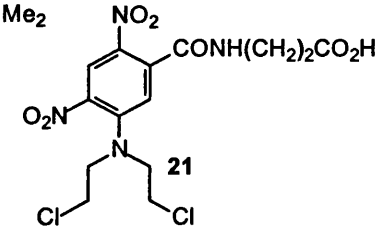
18



19



20



21

Bioreductive drugs can be activated by various enzyme activation systems. Most of the drugs that have been developed to date are activated by endogenous reducing enzymes, which are present in all cells. This process is common to nitro-aromatic compounds, transition metal complexes and to some quinone type compounds. In all of these cases, the mechanism of action results in the formation of a one-electron product, which can either be oxidised back to the parent compound in well-oxygenated cells, or can be reduced further to cytotoxic species in hypoxic cells. Recently, however, much of the

attention has been focused on the activation of bioreductive drugs by endogenous tumour-specific enzymes. For example quinones such as MMC have been selectively activated by DT diaphorase, *via* the two-electron reduction, thereby passing the one-electron reduction product.

These studies have been extended to the use of tumour specific exogenous enzymes, which are capable of activating bioreductive drugs, by deliberately introducing foreign enzymes into the cell population. One such approach is the antibody-directed enzyme-prodrug therapy (ADEPT)[49], which uses tumour-specific antibodies in order to locate the foreign enzyme. A similar approach is the gene-directed enzyme-prodrug therapy (GDEPT)[47], which involves the use of a foreign gene in order to generate the enzyme selectively.

Bioreductive drugs can also be activated by ionising radiation *via* the reducing species formed during the radiolysis of water. The advantage of this approach is that selectivity can be achieved by restricting the radiation field. This method of drug activation results in the formation of the one-electron reduced species and is independent of the expression levels of the reductive enzymes in tumour cells[47].

As previously mentioned, there is much evidence available to show that there are many compounds of diverse structure including the nitro aromatic compounds, quinones and N-oxides that can effectively kill mammalian hypoxic tumour cells. The similarity between all these compounds is that they all have to undergo enzyme-mediated reductive activation in order to express toxicity. Reductases that are involved in this process include xanthine oxidase, DT diaphorase, cytochrome P450 and NADPH-dependant cytochrome P450 reductase. The extent to which bioreduction of a drug will take place depends upon the ability of that drug to behave as a substrate for the intracellular reductase(s) and the expression levels of these enzymes within a particular tumour cell type[1,6,7,47]. All these factors contribute to the differential cytotoxic efficiency observed in various cell lines and furthermore, they contribute to the extent of cytotoxicity observed between the oxic and hypoxic cells.

Bioreductive drugs that are hypoxia-selective can also be combined with other treatments in therapy to target the surviving aerobic cell populations. The antitumour effectiveness of tirapazamine and RB6145 / RSU1069 has been demonstrated when these agents are used in combination with single high doses of X-rays. Radiation therapy kills the well-oxygenated cells, whilst administration of the bioreductive drug following irradiation reduces the number of hypoxic cells, thus improving tumour response[1,6,7,15,22].

A further method for inducing tumour hypoxia and therefore increasing bioreductive drug activity is to inhibit the action of the enzyme nitric oxide (NO) synthetase. Nitric oxide is the endogenous 'endothelial-relaxation' factor that is responsible for controlling or influencing the various processes involved in the function of normal endothelium[50,51]. It is thought that prodrugs that yield NOS inhibitors after hypoxia-mediated reductive activation maybe useful in the treatment of cancers[52].

Due to the assumption that hypoxic cells limit the effectiveness of both radiotherapy and chemotherapy, much of the earlier work was focused on designing experiments to eliminate hypoxic cells either by killing or by eliminating the selective protection of hypoxic cells[7,53]. Experiments involving irradiation schedules, minimising the importance of hypoxic cells have been proposed and tested[1,7,54]. Attempts to improve tumour oxygenation by the use of radiotherapy combined with hyperbaric oxygen[7,55,56], with oxygen and carbogen breathing[57,58], and with oxygenated perfluorochemical emulsions[59] have been developed. In addition, patients with low haemoglobin levels have been transfused prior to radiotherapy in order to improve tumour oxygenation[60,61]. An alternative approach reported that deliberate regional hypoxia was used to make the tumour and normal tissue equally hypoxic so that they were uniformly radioresistant[62]. Experiments involving hypoxic cell radiosensitisers[7,16] have been reported, and high LET radiotherapy has also been developed[63]. Many other approaches including adjunctive therapy with bioreductive alkylating agents[1,15,22], nitroheterocyclic

radiosensitisers[16], and hypothermia[64] have also been developed in order to exploit the selective toxicity of these agents to hypoxic cells.

The majority of the studies that have been carried out over the last fifty years to target hypoxic regions of solid tumours have focused on the development of electron-affinic radiosensitisers and bioreductively activated cytotoxins.

Naylor *et al*[65], in 1990, developed novel nitrofuran-carboxamides and -carboxylates as radiosensitisers and bioreductively activated cytotoxins. As previously mentioned, nitroheterocyclic compounds act as radiosensitisers. This is a fast free-radical process that is dependent upon the one-electron reduction potential. Furthermore, nitro compounds are capable of undergoing reductive metabolism to form highly potent cytotoxins. This process tends to occur in hypoxic tissues therefore, it is thought that such compounds may be specific to poorly oxygenated solid tumours. Although radiosensitisation and bioreductive activation occurs *via* different mechanisms both effects are related to the compounds reduction potential. This to some extent provides a basis for selective cancer chemotherapy. A series of 5-nitrofuran-2- and -3-carboxamides with various alkylating side chains have been developed which have been found to be effective as radiosensitisers of Chinese hamster cells (V79) *in vitro*. However, these compounds were found to be ineffective against KHT sarcomas in C3H mice.

Jenkins *et al*[24] in 1990 reported the development of a prodrug of RSU1069, which was the haloethylamine RB6145. This compound as previously mentioned was found to be superior to RSU1069, but less toxic and was able to act as both a radiosensitiser and a cytotoxin.

Similarly, Naylor *et al*[66], in 1992, developed a series of 2-nitroimidazole compounds with various side chains containing aziridinyl and or oxiranyl groups to behave as both a radiosensitiser and a cytotoxin. Although some of the compounds developed were of similar activity to RSU1069, none were taken forward into clinical evaluation.

More recently however, work has been carried out on indolequinone type compounds as hypoxia selective cytotoxins and radiosensitisers[67].

From the various studies carried out, it can be seen that much work has been carried out to find the most therapeutically beneficial hypoxic cell radiosensitiser and cytotoxin. However, little attention has been focused on exploiting the physiological difference in oxygen concentration between the normal and hypoxic tumour tissues.

Sykes *et al*[68,69], in 1995, carried out a number of studies on a bioreductively triggered system which was based on 2-nitroarylamides. It was demonstrated that, when activated, aminoaniline mustard alkylating agents were selectively released from the 2-nitroarylamide. Similarly, they reported the release of N-hydroxylactam and an amine-bearing mustard *via* a cyclisation reaction when the nitroamide is activated.

Even more recently, Berry *et al*[70] demonstrated the 5-nitrofuran-2-ylmethyl group as a bioreductively activated prodrug system. Similarly, Everett *et al*[71] reported the release of aspirin and salicylic acid from the 2-nitroimidazol-5-methylene moiety. In both of the above cases, the release mechanism is very similar in that, following reduction, the nitro moiety is reduced to the amino group. The lone pair of electrons on the amino group allow fragmentation and the drug is selectively released. In the case of the nitroimidazole compounds, the drug is attached to the trigger moiety *via* the 5-ylmethylene group.

It was hypothesised that upon selective bioreduction in hypoxic tumour tissue, the trigger part of the prodrug would release therapeutic drugs only in that tissue. This was thought to greatly improve the selectivity of the biodistribution of these types of compounds. Many studies have been carried out on 1-substituted 2-nitroimidazoles and it has been demonstrated that such agents are selectively bioreduced in poorly vascularised hypoxic tumour tissues[72,73]. In this study, 1-substituted 2-nitroimidazole compounds were selected as the bioreducible moiety because they tend to show greater

metabolic stability in normal tissues and they also exhibit a wide range of redox potentials. These types of compounds are widely used in medicines and many of them have been found to be effective radiosensitisers and cytotoxins.

2.0 *POLY(ADP-RIBOSE) POLYMERASE*

Poly(ADP-ribose) polymerase (PARP) (also known as poly(ADP-ribose) synthetase (PARS)) is a multifunctional homodimeric enzyme that is found in all eukaryotic cells ranging from protozoa to mammals[74]. 95% of the enzyme is found in the nucleus, where it is tightly bound to chromatin. Each mammalian cell nucleus has been reported to contain approximately one million copies of PARP. However, terminally differentiated cells, such as mature granulocytes, epidermal cells and intestinal cells, lack this enzyme. PARP was purified as early as 1977 from rat liver nuclei[75].

Human PARP comprises of a single polypeptide chain of 1014 amino acid residues, of molecular weight ~113 KDa. The location of the gene encoding human PARP has been recently identified in chromosome 1q41-q42[76]. When PARP is activated, the enzyme catalyses the transfer of ADP-ribose units from its substrate nicotinamide adenine dinucleotide (NAD^+) to a number of acceptor proteins. These may be nuclear proteins involved in chromatin architecture and DNA metabolism (heteromodification) or PARP itself (automodification)[74]. Despite all the research on PARP in the last 30 years, its precise biological role is not yet clear. However, it is known that PARP plays an important role in the repair of DNA damage and it is this property of PARP that has led researchers to target this enzyme by means of chemosensitisers and radiosensitisers.

Interestingly, since PARP was discovered, it had been accepted that there was only one type of PARP in each species. However, this view has now been challenged by the discovery of novel poly(ADP-ribosyl)ating enzymes[77-79]. These new PARPs have been classified into two subgroups according to their size. Type II PARPs are smaller than the generally accepted PARP and, in some cases, show nuclear localisation[77]. These PARPs have been found to be present in organisms such as archaea[79], plants[77] and mammals[80]. Type III PARPs, on the other hand, are large proteins containing ankyrin repeats and a PARP catalytic domain[78]. The only member of this subgroup to be reported is the human protein tankyrase[78]. This is likely to be found in higher eukaryotes[78]. Currently, very little information is available on the new-found PARPs. At this moment, it is assumed that they

contribute only modestly to the poly(ADP-ribosyl)ation potential of higher eukaryotes. Their discovery has led to the concept that they maybe involved in specific nuclear functions requiring only limited levels of poly(ADP-ribosyl)ation. Its thought that these new PARPs were not previously detected because their activities were masked by the very active and abundant PARP1. However, in this thesis, only the active abundant PARP1 of 113 KDa will be discussed.

In 1984, Kameshita *et al*[81] identified the three functional domains of PARP. These are the N-terminal DNA-binding domain, the central automodification domain and the C-terminal catalytic domain. These were separated from each other by limited proteolysis with α-chymotrypsin and papain. In short, cleaving the enzyme gave two fragments of M_r 54 KDa and 66 KDa. The 54 KDa fragment was found to contain the substrate-binding site, as it was protected by the addition of the substrate NAD^+, or its analogue nicotinamide. The 66 KDa fragment was further cleaved into two fragments of M_r 46 KDa and 22 KDa. The 46 KDa fragment was found to be bound to the DNA-cellulose column, with the 22 KDa fragment having little affinity for the DNA ligand. These results suggested that there were three domains, the first one being the NAD^+-binding site, the second being the DNA-binding site and the third domain behaving as the site(s) for accepting poly(ADP-ribose)[81]. Each of the domains will now be discussed in detail.

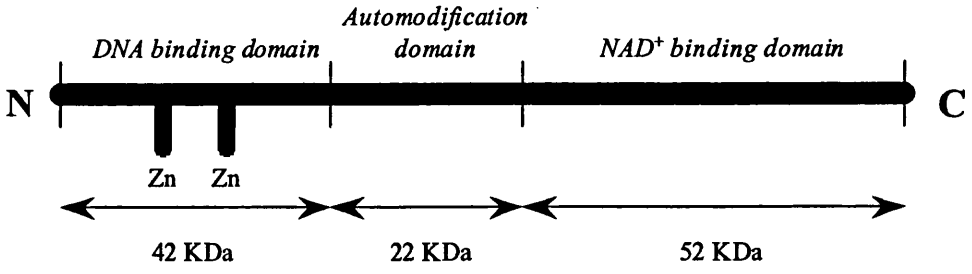


Figure 3: Identification of the three domains of PARP[81]

2.1.1 THE DNA BINDING DOMAIN (DBD)

The N-terminal DNA-binding domain of M_r 46 KDa extends from methionine-1 to threonine-373 in human PARP[82,83]. This domain contains two putative Zn fingers[84-86] in the N-terminal region and two helix-turn-helix motifs[87,88]. Furthermore, the DBD also contains a high proportion of basic residues, which are thought to be involved in the interaction of the enzyme with the DNA.

PARP is a metalloenzyme, in which each Zn is bonded to one histidine and three cysteine residues in the form of Cys-X_2-Cys-28-30-His-X_2-Cys. The first Zn finger (F1), beginning with Cys-21 and ending with Cys-56, consists of 28 residues and is electrically neutral (three basic and three acidic residues). The second Zn finger (F2) starts at Cys-125 and ends at Cys-162. This finger comprises of 30 residues and is basic overall (seven basic and five acidic residues).

PARP is activated by the presence of DNA strand breaks. Footprinting experiments[89] have shown that PARP prefers to bind symmetrically at DNA strand breaks, subsequently covering seven or eight nucleotide residues on each side of the break. At the site of a single stranded break (SSB), a V-shaped bend was observed when PARP (located at the kink) bound to the DNA break[90]. Many studies have been carried out in order to clarify the role of the two Zn fingers of PARP[84-86]. Ikejima *et al*[91] reported that both Zn fingers were required for PARP catalytic activity at SSBs, whereas activation by double stranded DNA breaks (DSBs) only required the first Zn finger. Therefore, F1 is thought to be responsible for PARP activation whereas F2 is responsible for the detection of DNA strand breaks. These results however, were in contrast to those of de Murcia *et al*[92] who reported that F2 was necessary for PARP catalytic activity in SSBs, whereas F1 was responsible for the binding of the enzyme to the breaks.

The DBD also contains a bipartite nuclear location signal (NLS), which is responsible for the transportation of PARP into the nucleus. 95% of the enzyme is found in the nucleus. The NLS is able to target proteins larger than 40 to 60 KDa to the cell nucleus. It is a short amino acid sequence rich in basic residues (207-226) of

the form KRK-X_{11}-KKKSKK. Site-directed mutagenesis[93] revealed that the two basic clusters (D1 and D2) are both necessary for the transportation of PARP.

A further feature present in the DBD region was the presence of a 36 KDa fragment (derived from limited proteolysis)[94] extending between amino acids 233 and 525. This domain contains the whole automodification domain and part of the DBD[80,82]. Analysis of this domain revealed the presence of two helix-turn-helix motifs at residues ~ 200-220 and 280-285. It is thought that this helix-turn-helix motif is able to mediate strong interactions between DNA and proteins[80].

2.1.2 THE AUTOMODIFICATION DOMAIN

This 22 KDa central automodification domain of PARP is located between residues 374 and 525[82,83]. Analysis of this region revealed that this domain is basic and comprises of most of the 15 glutamic acid residues that are thought to be involved in PARP automodification[80,85,95]. Interestingly, antibodies directed against this region were found to inhibit half of the automodification reaction, thus confirming that at least half of the residues that serve as acceptors are located in this domain.

The automodification reaction was initially thought to be intramolecular. This assumption suggested that the direction of chain elongation would occur at the enzyme-proximal end. It was difficult however, to envisage a single polypeptide chain catalysing the simultaneous ADP-ribose chain elongation of 15 to 28 units in an intramolecular mechanism[96]. However, Mendoza-Alvarez and Alvarez-Gonzalez[96] reported that ADP-ribose chain elongation occurred at the protein-distal end of the polymer. These results indicated that the automodification reaction was, in fact, intermolecular and the two monomeric units of PARP function simultaneously as catalyst and acceptor molecules.

This domain also comprises of a BRCT (BRCA1 C-terminus) domain that extends from amino acids 384 to 479[97]. This domain comprises of about 95 (weakly conserved) amino acids found in proteins that have been shown to regulate cell-cycle checkpoints and DNA repair[80]. These BRCT domains are thought to be protein-protein interactions that allow BRCT containing proteins to have strong and specific

associations[98]. Recently BRCT-mediated interactions between the BRCT domain of ligase III and the second (C-terminal) BRCT domain of XRCC1 have been identified and found to be responsible for the formation of a DNA-base-excision-repair (BER) complex[99,100].

2.1.3 THE CATALYTIC DOMAIN

The 54 KDa catalytic domain is located in the C-terminal region of the enzyme and extends from residues 526 to 1014 in human PARP[82,83,101]. The catalytic activity of this domain is independent of DNA strand breaks. The basal activity is the same as that of full length PARP. This domain comprises the 'minimal catalytic domain' which catalyses the initiation, elongation and branching of (ADP-ribose) polymers, in the absence of DNA strand breaks. Simonin *et al*[102] demonstrated that the loss of the last 45 amino acids in the C-terminal end of this domain would abolish activity. In human PARP, residues 859-908 are conserved and comprise the area known as the 'PARP signature'.

The catalytic domain of PARP appears to be very similar in structure to mono(ADP-ribosyl)ating enzymes. Studies of both PARP and mono(ADP-ribosyl)transferases have suggested that residue Glu-988 is necessary for PARP activity. Mutation studies[103] of this residue resulted in a decrease of the elongation of the polymer 2000-fold, and, in addition, reduced (although to a lesser extent) the initiation of new chains of ADP-ribose. The structure of the catalytic domain of chicken PARP complexed to an inhibitor has been elucidated by X-ray diffraction[104]. It was found that the active site appeared to consist of a β-α-loop-β-α motif that was present also in mono(ADP-ribosyl)transferases and is thought to be responsible for NAD^+ binding. Furthermore by photoinsertion[105] of a NAD^+ analogue it was shown that NAD^+ associates with Lys-893 and Trp-1014. However, Trp-1014 was not necessary for PARP activity, as was demonstrated in mutation studies[80].

2.2.1 MECHANISM OF ACTION OF PARP

DNA damage by chemotherapy or radiotherapy results in the activation of PARP[80]. In response to this damage, PARP catalyses the transfer of ADP-ribose from its

substrate NAD^+ to nucleophilic acceptor sites on DNA-associated proteins[74,80]. This maybe PARP itself (automodification), histones (heteromodification), HMG proteins, topoisomerases, DNA polymerases and ligases[74,80]. This results in the release of nicotinamide. PARP then transfers and links the ADP-ribose portion to the mono(ADP-ribosyl)ated protein *via* a (1"→2') glycosidic bond to synthesise a straight chain[74,80]. Branching may occur at every 30-50 residues[95,106] with a ribosyl (1"'→2") bond causing branch formation. PARP then appears to lose its affinity for DNA. The precise mechanism is not yet known but it has been suggested that electrostatic repulsions occur between the highly negatively charged poly(ADP-ribose) chains resulting in dissociation of PARP from the DNA strands. The (ADP-ribose) polymers are broken down by the enzyme poly(ADP-ribose) glycohydrolase (PARG) to ADP-ribose units. These are removed from the protein by ADP-ribosyl protein lyase, subsequently returning PARP to its original form[74,80].

2.2.2 MODIFICATION OF THE CHROMATIN STRUCTURE

The two enzymes PARP and PARG have been reported to modify the chromatin structure during DNA repair[80]. It has been proposed that, when DNA is damaged, PARP binds to the strand breaks resulting in the activation of the enzyme. This in turn, results in the rapid shuttling of PARP from DNA strand breaks. After the histones are modified (H1, H2B, H2A, H3 and H4) the chromatin structure opens. The enzyme PARG then enters the nucleus, resulting in a rapid turnover of the polymer. The chromatin structure remains in a decondensed state. Finally, DNA damage is repaired; polymer levels are reduced due to the presence of PARG and chromatin returns to its original conformation. Similarly, Althaus[107] also postulated that a shuttle mechanism for chromatin exists in which relaxation of its structure facilitates DNA BER.

2.3 POLY(ADP-RIBOSE) GLYCOHYDROLASE (PARG)

This is the most important enzyme required for the breakdown of poly(ADP-ribose) chains. It is a monomeric protein of M_r 59 KDa[80]. It has been reported that PARG prefers to breakdown longer chains (>20 ADP-ribose residues) and that they are

degraded rapidly, as opposed to smaller chains, which are degraded much less efficiently[80,108].

2.4 MONO- AND POLY(ADP-RIBOSYL)ATION

Living organisms are able to modify their protein structures and functions by ADP-ribosylation reactions. The two main types of ADP-ribosylation reactions that occur are mono(ADP-ribosyl)ation and poly(ADP-ribosyl)ation[109].

Mono(ADP-ribosyl) transferases are enzymes that are able to transfer a single unit of ADP-ribose from NAD^+ to acceptor proteins. These enzymes have been found in both eukaryotes and procaryotes[106,109]. Bacterial toxins that are mono(ADP-ribosyl) transferases are capable of modifying specific acceptor sites on their target proteins. Diphtheria toxin and *pseudomonas* exotoxin A[106,109] have been shown to mono(ADP-ribosyl)ate a modified histidine residue of elongation factor 2. Cholera toxin A and *Escherichia coli* heat-labile toxin[106,109] specifically modify an arginine residue of a GTP-binding protein (GSα) and the pertussin toxin[106,109] carries out mono(ADP-ribosyl)ation of a cysteine residue.

2.5 FUNCTIONS OF PARP

The physiological function of PARP and poly(ADP-ribosyl)ation is still under heavy debate. However, with the use of pharmacological inhibitors of PARP, the enzyme has been shown to regulate various processes including cellular differentiation[74,80], viral replication[110], apoptosis[80,111-115], inflammation and ischaemia reperfusion injuries[80,116,117]. The role of PARP in some of these processes will now be discussed.

2.5.1 ROLE OF PARP IN VIRAL REPLICATION

Gåken *et al*[110] established that inhibition of PARP blocked the entrance of the infected retrovirus into mammalian cells. From the various methods used to inhibit PARP, it was demonstrated that inhibition occurred in the step in which the retroviral DNA is introduced into the host genome[110]. It seems that PARP acts with the virus-

encoded integrase protein (IN) in order to incorporate the viral genome into the host genome.

2.5.2 ROLE OF PARP IN APOPTOSIS

Apoptosis is an active form of cell death that occurs in both normal and tumour cells. It can be triggered physiologically and pathologically when the cells are under mild stress[80]. It is different to the passive cell death that results from high doses of damage to the cell, known as necrosis. When under mild stress, apoptosis can be blocked by inhibitors, subsequently increasing cell survival[80,111]. Apoptosis is thought by many scientists to be a mechanism useful in the process of eliminating potentially mutant cells that have been sub-lethally hit by a DNA-damaging agent. It is thought that PARP may then translate this DNA damage into a message of cell suicide. In general, apoptosis has attracted a lot of interest. This is because it is thought that dysregulation of apoptosis may lead to diseases such as cancer, arthritis and diabetes. Unfortunately, the exact events involved in apoptosis are not yet fully known[80,111].

It is, however, known that, for the apoptosis process to be completed, it requires ATP[80.112-115]. When genotoxic agents damage DNA, PARP is activated. As PARP uses its substrate NAD^+ in poly(ADP-ribosyl)ation reactions, these levels are significantly reduced, thus depleting the ATP levels in cells and the dGTP levels. As a result those cells which are in the apoptotic stages cannot complete their course of action due to the lack of ATP, resulting in the apoptotic cells switching to the necrotic pathway. Necrotic cells have been found to be the main cause of cellular damage associated with ischaemia and inflammation[80,116,117]. Because of these effects, apoptosis is the favoured means of cell death. Interestingly, it has been demonstrated that, even in cases of little cellular damage, PARP still uses large amounts of NAD^+, thus depleting the levels of ATP drastically.

Recently, there has been evidence[80,115] put forward suggesting that eukaryotic cells have developed a mechanism to protect themselves from switching over to the necrotic pathway. It was shown that about 30 min after the induction of apoptosis, death proteases known as caspases cleave PARP in its bipartite NLS ($DEVD^{214}$ /

G^{215}) to give a 24 KDa DNA-binding fragment and an 89 KDa catalytic fragment[80,115]. This inactivates the catalytic activity of PARP, thus preventing the apoptotic cells turning necrotic. This mechanism, however only appears to function in the presence of moderate levels of DNA damage.

Many studies[112,113,116] have demonstrated that reactive free radical species and peroxynitrite induce apoptosis in various cell types. It seems that sudden exposure to high concentrations cause cell necrosis, whereas sustained exposure or low levels of peroxynitrite induce apoptosis. Nitric oxide, a precursor of peroxynitrite, in low concentrations has been shown to inhibit apoptosis, whereas exposure to high levels of NO has been reported to cause apoptosis.

From the various experiments[118,119] carried out with PARP inhibitors, the results appear to be conflicting. The PARP inhibitors 3-aminobenzamide (3-AB) and nicotinamide have been shown to reduce NO-induced apoptosis. However, in the case of raw murine macrophages, these inhibitors were reported to be ineffective in blocking apoptosis[118,119]. It is thought that the reason for this maybe because NO-induced apoptosis is mediated through a mechanism which is different to that of potent DNA single-strand-breaking agents, such as peroxynitrite.

2.5.3 ROLE OF PARP IN INFLAMMATION

Peroxynitrite and hydroxyl radicals are also known to induce various forms of inflammation[116,117]. As previously mentioned, they are potent DNA strand-breaking reagents that activate PARP. In response to DNA damage, PARP depletes the concentration of its substrate NAD^+, which, in turn, depletes ATP levels and dGTP levels, subsequently slowing down glycolysis, electron-transport and ATP formation. As a consequence of this cell necrosis and cell dysfunction are known to occur. In addition to this, PARP also appears to modulate the course of inflammation by regulating the expression of a number of genes, which includes collagenase and the inducible nitric oxide synthase[116,117]. PARP inhibitors are therefore thought to be useful by protecting against cell death under these conditions[116,117].

Experiments[116,117] *in vivo* have demonstrated that PARP inhibition by 3-AB delayed the onset of streptozotocin- and alloxan-induced diabetes. Similarly, with the use of the nicotinamide inhibitor, the extent of obesity-associated diabetes was reduced[120]. Inhibition with 3-AB has also been shown to reduce oedema formation, and suppress the development of rheumatoid arthritis in rodent models[121,122].

2.5.4 ROLE OF PARP IN ISCHAEMIA-REPERFUSION INJURY

The fact that hydroxyl radicals and peroxynitrite trigger PARP activation has also been well established in the pathogenesis of ischaemia-reperfusion injury. For example, peroxynitrite generation has been demonstrated in the reperfused heart[123], kidney[124], brain[125], and lung[126]. In such cases, inhibition of peroxynitrite generation by inhibiting NO biosynthesis has been shown to reduce reperfusion injury, modestly[125]. Even though these reactive species are exerting cytotoxic effects, the activation of PARP appears to result in major cellular injury.

Recently, a number of studies[127-129] have shown that inhibition of PARP results in protective effects in animal models of stroke[127], myocardial ischaemia-reperfusion[128] and splanchnic ischaemia-reperfusion[129].

However, it is also known that severe haemorrhage and resuscitation leads to organ ischaemia. Oxygen-derived free-radicals are generated upon reperfusion leading to DNA strand breaks and subsequently PARP activation. 3-AB has previously been shown to reduce the circularatory effect and the organ dysfunction / injury by inhibition of PARP[130,131]. Due to the solubility problems encountered with 3-AB, an alternative PARP inhibitor was required. Recently, McDonald *et al*[132] reported that 5-aminoisoquinolinone (5-AIQ, a potent PARP inhibitor) abolished the multiple organ injury caused by severe haemorrhage and resuscitation. Following this work, McDonald and co-workers extended their studies to the effect of 5-AIQ on cardiac myoblasts. They found that inhibition of PARP in cardiac myoblasts resulted in the reduction of the myocardial infarct size in rats, *in vivo*. These results highlight the potential of such inhibitors in conditions associated with myocardial ischaemia and reperfusion (C. Thiemermann and M. D. Threadgill, personal communication).

2.5.5 ROLE OF PARP IN DNA REPAIR

In living cells, DNA repair is known to occur *via* several mechanisms. Although the enzyme PARP is known to be involved in DNA BER[133], its precise mechanism remains to be elucidated. However, previous studies have demonstrated that poly(ADP-ribosylation) is not involved in the excision of the damaged bases[80,134] nor in the resynthesis of DNA after excision[80,135]. Some models have been proposed to explain PARP's role in DNA repair:

(1) The Recruiting Model

As PARP is one of the first enzymes to be aware of DNA damage, it is thought to be in the perfect position to recruit the DNA (base excision) repair enzymes to the sites of damage. This theory has been supported by several authors who have identified a BER complex comprising of PARP, XRCC1, DNA ligase III and DNA polymerase β[80,99,100,136]. As XRCC1 has been poly(ADP-ribosyl)ated *in vitro*, it is plausible that PARP may regulate the activity of the complex by modifying XRCC1 activity *in vivo*[100,136]. This would subsequently prevent it from reacting with other components of the complex. It is not known whether other components of the complex are poly(ADP-ribosyl)ated. The stoichiometry for this complex is thought important for regulation of PARP activity. When XRCC1 and DNA ligase III are in excess over PARP, they were shown to inhibit PARP activity, *in vitro*[80]. DNA ligase III is thought to inhibit *via* a competitive mechanism as both enzymes share similar zinc fingers. It has been proposed that DNA repair factors maybe recruited by PARP through modification of the chromatin proteins. As a result of this, the long chains of poly(ADP-ribose) would be able to target repair enzymes to DNA damaged sites much faster than if they had to find their own way. The recruiting model implies that PARP acts before / and or after the excision of damaged bases[80].

(2) The Anti-Recombination Model

This model was proposed by Satoh and Lindahl[137], who reported that PARP inhibits DNA repair, thus preventing DNA recombination. The poly(ADP-ribosyl)ation of neighbouring proteins and PARPs association with DNA breaks would allow PARP

to protect DNA ends from nuclease / and or DNA strand exchange activities necessary for recombination. This theory was supported in $PARP^{-/-}$ double-knockout animals, which revealed that recombination was stimulated in the absence of PARP, resulting in increased incidence of lymphomas[138].

Recently, PARP was found to be present in a multi-protein complex that is able to perform DNA recombination activities[80,139]. Interestingly, three of the components of this complex including numatrin/B23[140], nucleolin/C23[141] and PARP itself have been shown to be modified by poly(ADP-ribosyl)ation. It has been proposed that, by poly(ADP-ribosyl)ating its components, the enzyme may be able to regulate the activity of the complex.

(3) The Chromatin-Dependent Model

The fact that histones are poly(ADP-ribosyl)ated in response to DNA damage suggested that PARP plays an important role in the repair of DNA, when the DNA is structured in chromatin[80,142,143]. When PARP is activated by DNA damage, the chromatin structure opens because of poly(ADP-ribosyl)ation of histones resulting in the disruption of the chromatin structure, which is necessary for the repair of highly condensed chromatin. This is consistent with the fact that, in the absence of PARP, DNA repair in the condensed chromatin structure is less efficient than the repair in the open chromatin structure[80]. This is because DNA proteins and transcription factors are able to access the site of damage. In a similar fashion, PARP would be able to facilitate DNA repair by alleviating steric congestion caused by packed chromatin, subsequently allowing DNA repair factors access.

(4) The Signalling Model

It has been reported that PARP could stimulate DNA repair and ensure genomic stability by signalling the presence of DNA lesions to effectors downstream involved in co-ordinating the cellular responses to DNA damage[80,144,145]. It's thought that the modification of PARP and histones at sites of DNA damage could act as a strong signal activating signalling molecules and or repair machinery. This model was proposed following the observation that poly(ADP-ribosyl)ation reactions are

involved in p53 regulatory functions. It has been reported that PARP inhibition suppressed the accumulation of p53, in response to ionising radiation[80]. p53 is known to be a key factor of G_1 arrest and is also involved, to a lesser extent, in G_2 arrest. In the absence of PARP activity, lesions may remain undetected before DNA replication, resulting in further damage during the S phase, subsequently arresting at the G_2 phase. It is thought that induction of the p53-induced cell cycle by poly(ADP-ribosyl)ation may allow the necessary time required for these lesions to be repaired[80].

However, it is not yet known how PARP regulates p53. It is thought that, as p53 is able to interact strongly and non-covalently with ADP-ribose polymers, it is plausible that poly(ADP-ribosyl)ation reactions at DNA damaged sites may provide a strong signal to direct signalling factors or p53 to the sites of damage in the nucleus[80]. The breakdown of these poly(ADP-ribose) polymers by the enzyme PARG would allow p53 to perform efficiently its signalling functions at the site of damage. PARP is also known to directly interact with p53 and it has been proposed that this interaction may regulate p53, either by modification of its functional properties or by recruiting them to the sites of DNA damage[80].

3.0 *INHIBITORS OF POLY(ADP-RIBOSE) POLYMERASE*

With the discovery that poly(ADP-ribose) polymerase appears to be involved in DNA excision repair, came the notion that inhibitors of PARP may be capable of potentiating the cytotoxicity of both DNA-damaging agents and radiotherapy. Such compounds are of particular interest because they are prime targets for the design of chemosensitisers and radiosensitisers in the treatment of cancer.

Many studies have been reported that have used either monofunctional alkylating agents or ionising radiation to determine PARP's involvement in the repair of DNA damage[74,146].

3.1.1 CHEMOSENSITISATION

Chemosensitisers are compounds that sensitise tumour cells to the cytotoxic effects of DNA-damaging drugs. Thus, inhibitors of PARP are thought to be useful in chemotherapy.

Durkacz *et al*[147], in 1980, demonstrated, with the use of 3-AB, that PARP inhibition potentiated cytotoxicity of the alkylating agent dimethyl sulfate (DMS) in L1210 mouse leukaemia lymphoblast cells. Similar results were also reported by other studies[74,146,147] using alkylating agents such as DMS and N-methyl-N-nitrosourea to potentiate DNA damage in various cell lines. More recent studies include that of Weltin *et al*[148], who reported that 6(5H)-phenanthridinone, in combination with the alkylating agent N,N-bis(2-chloroethyl)methylamine (HN_2) significantly reduced the proliferation of RDM4 murine lymphoma cells. This demonstrates the involvement of PARP in the proliferation of lymphoma cells.

Griffin *et al*[149], in 1998, enhanced the cytotoxicity of the alkylating agent 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) 3.5-fold against L1210 murine leukaemia cells by inhibition of PARP. This was achieved by the novel 8-hydroxy-2-methylquinazolin-4-one (NU 1025) inhibitor. The cytotoxicity of the alkylating

agent was potentiated in a concentration-dependant manner and the PARP inhibitor was found to be 50-fold more potent as a chemopotentiator than 3-AB.

More recently White *et al*[150] found that, *in vitro*, the novel compound 2-(4-hydroxyphenyl)-1H-benzimidazole-4-carboxamide was able to potentiate the cytotoxicity of both temozolomide (TM) and topotecan (TP) against A2780 cells 2.8 and 2.9 fold, respectively. These results have proved promising in the use of such compounds as chemosensitisers. Following this work, Delaney *et al*[151] extended these studies to human tumour cell lines (lung, colon, ovary and breast cancer). It was reported that both compounds NU 1025 and NU 1085 were able to enhance the cytotoxicity of TM and TP in the various tumour cell lines and furthermore they were used at concentrations, which were not toxic to the cell.

Although these recent *in vitro* studies seem promising, activities *in vivo* remain to be seen. The problem with many of these compounds is that they lack specificity and, consequently, when inhibitors are used *in vivo* studies, they tend to cause other cellular disruptions[146]. This makes it difficult to determine if the sensitisation observed is due to PARP inhibition or to other cellular defects. For example, 3-AB has been used *in vivo* to potentiate the cytotoxicity against Erhlich ascites tumour for bleomycin and cisplatin[146,152,153]. At the doses which have been used, it is not known whether the cytotoxicity is due to PARP inhibition or whether it is the hypothermic effect observed when such doses of inhibitors are used.

3.1.2 RADIOSENSITISATION

Radiosensitisers are compounds that sensitise tumour cells to radiotherapy by inhibiting PARP. Currently, the problem with radiotherapy is that it involves the use of daily low X-ray doses to minimise the normal tissue damage and also to allow tumour reoxygenation between doses. This has proved problematic, because extensive repair has been known to occur between X-ray doses. Inhibitors of PARP are therefore thought to be useful as radiosensitisers, because of their ability to inhibit the DNA repair mechanism.

As in the case of chemosensitisers, many studies have been carried out in order to potentiate the cytotoxicity of cells to X-ray and γ-radiation[74,146,149,154]. Earlier studies used nicotinamide and 3-AB to enhance DNA damage. Both of these inhibitors have been shown to potentiate damage in plateau V79 Chinese hamster cells but not in log phase cells[146]. However, earlier studies involving PARP inhibitors only reported a slight increase in cell killing, or in some cases none at all[11,74,146]. This was thought to be due to the insufficient time allowed for the inhibitor to be taken up by the cells, or the low concentrations of the inhibitors used[11,74,146]. Furthermore, it was found that following irradiation the effect of the inhibitor should be measured within two hours, as this was the time when PARP was thought to be most active[11,146]. Following these observations, studies were carried out using higher inhibitor concentrations, for shorter periods of time and consequently, these were found to be more successful[11,146].

More recently, Griffin *et al*[149] reported that the resistance modifying agent NU 1025 enhanced the cytotoxicity of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide 1.4 fold, against L1210 murine leukaemia cells, following γ-radiation. This compound was found to be an effective radiopotentiator as well as being an effective chemopotentiator.

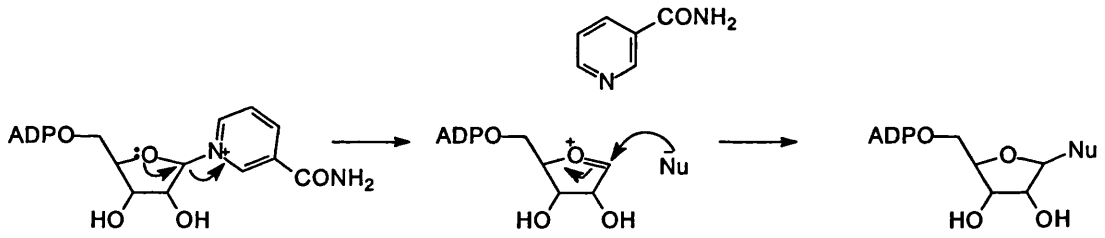
Sebolt-Leopold and Scavone[155] and Elliot *et al*[156] reported that, *in vivo*, the PARP inhibitor 3,4-dihydro-5-methylisoquinolin-1-one (PD128763), in combination with radiotherapy, produced a 1.4-1.6 increase in cell kill in SCC7, RIF-1 and KHT murine tumours, thus increasing growth delay by 10-15 days. This combination of radiation therapy and a PARP inhibitor resulted in tumour regressions, whereas radiotherapy alone was reported to result in only a minor growth delay. This emphasised the importance of such compounds in the treatment of radiotherapy.

From the various studies carried out on PARP inhibitors, it can be seen that they have been developed with two main aims: The first one is to understand the enzymes physiological role in DNA-repair but, more importantly, the second aim is to potentiate the cytotoxic effects of both radiotherapy and chemotherapy. However, this has proved difficult because a PARP inhibitor targeted specifically to the enzyme is yet to be achieved. Current PARP inhibitors tend to lack tissue-selectivity

and possess poor solubility characteristics. These factors will be discussed with respect to known inhibitors of PARP.

3.2 PARP INHIBITORS DEVELOPED TO DATE

Most PARP inhibitors that have been developed to date are competitive, reversible inhibitors that bind to the NAD^+ binding-site of the enzyme. As previously mentioned, when activated, the enzyme catalyses the cleavage of the ribose-nicotinamide bond in NAD^+, with the initial transfer of the ADP-ribosyl fragment to a nucleophilic centre on a protein acceptor, which may be PARP itself (automodification) or target proteins such as histones (heteromodification)[146]. Cleavage of the ribose-nicotinamide in NAD^+ bond results in the formation of an oxonium ion, facilitated by the lone pair of electrons on the adjacent oxygen. Following initiation, the process can be repeated through elongation (*via* combination of the 2'-OH of ribose (attached to adenine) with the 1-position of the ribose attached to nicotinamide)[146]. Branching can occur every thirty to fifty residues (*via* combination of ribose 1-position initially attached to nicotinamide, with the 2'-OH of another ADP-ribose polymer)[146].

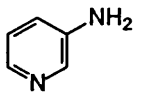


Scheme 1: Schematic representation of poly(ADP-ribosyl)ation[146]

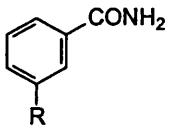
Nicotinamide **(22)** was the first compound that was found to inhibit PARP, not surprisingly. Unfortunately, there were many problems associated with the use of nicotinamide as a PARP inhibitor. As well as being a weak inhibitor of the enzyme, it was only slightly soluble, lacked specificity and behaved as a substrate for NAD^+-metabolising enzymes.

Consequently an alternative compound was required. Interestingly, most of the inhibitors that have been developed are mimics of the nicotinamide unit. The next compound to be analysed as a PARP inhibitor was benzamide. Benzamide **(23)** is structurally very similar to nicotinamide. It was first shown by Shall[157] in 1975 to be an effective inhibitor of PARP. Furthermore, it lacked a ring nitrogen and was unable to undergo metabolism. Unfortunately, due to its poor solubility characteristics, this compound could not be used as a PARP inhibitor.

By introducing polar substituent onto the aromatic ring of benzamide, Purnell and Whish[158] hoped to increase the aqueous solubility of the compound. It was found that substitution at the 3-position of the ring improved solubility of the compound, without affecting its activity against the enzyme. Subsequently, 3-amino- **(24)**, 3-hydroxy **(25)** and 3-methoxybenzamide **(26)** were found to be potent inhibitors of PARP with relatively good solubility characteristics. Compounds **(24)** and **(26)** were found to be competitive inhibitors of PARP, exhibiting K_i values of less than 2μM. Interestingly, alkylation of the carboxamide group or substitution with a carboxylic acid reduced significantly or abolished enzymic activity. However, acylation of the 3-amino group of **(24)** only slightly reduced enzymic activity but poor solubility was observed.



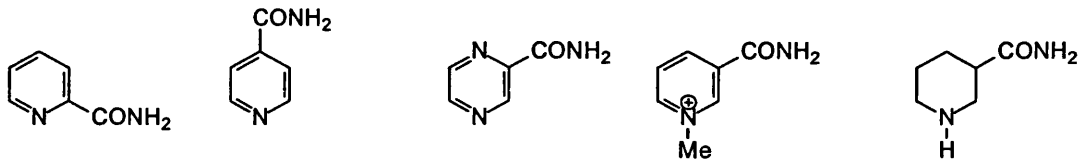
22



23 R = H
24 R = NH_2
25 R = OH
26 R = OCH_3

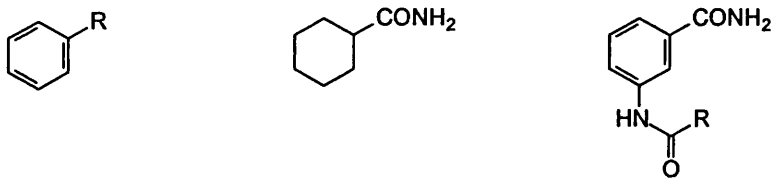
In 1982, Sims *et al*[159] evaluated the PARP inhibitory activity in a number of compounds including the nicotinamide and benzamide derivatives. It was demonstrated that the presence of a carbamoyl group was necessary for significant activity, although the position of this substituent was found to be less important. Both picolinamide **(27)** and isonicotinamide **(28)** were found to have similar enzymic activities to nicotinamide. When an additional ring nitrogen was introduced this appeared to be tolerated and, subsequently, pyrazinamide **(29)** was found to be only slightly less potent than nicotinamide. However, alkylation of the ring nitrogen **(30)**,

or reduction of the aromatic ring **(31)** reduced inhibitory activity significantly or abolished activity.



27 **28** **29** **30** **31**

Sestilli *et al*[160,161] and Cantoni *et al*[162] provided further information on the structure and activity relationships of the benzamide series. They reported that, when the carbamoyl substituent was modified, this had a crucial effect on the activity. Subsequently, the alkylamide **(32)**, sulfonamide **(33)** and thioamide **(34)** analogues appeared to be much less active than benzamide, whereas benzoic acid and 3-aminobenzoic acid were found to be inactive. However, it was found that reduction of the aryl ring to give cyclohexanecarboxamide **(35)** abolished activity. Interestingly, although alkylation of the 3-amino group of **(24)** had little effect on inhibitory activity, acylation of the amino group enhanced inhibitory activity significantly; subsequently 3-acetamido- **(36)** and 3-propanamidobenzamide **(37)** were found to be potent inhibitors of PARP. In support of this evidence was Purnell and Whish[158] who had previously reported that 3-acetamidobenzamide and its analogue, 3-succinylaminobenzamide **(38)**, were excellent inhibitors of the porcine thymus enzyme. However, the introduction of a halogen abolished activity, thus chloroacetylbenzamide **(39)** was found to be inactive.

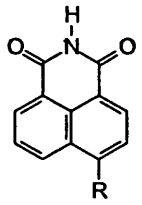


32 R = CONHMe
33 R = SO_2NH_2
34 R = $CSNH_2$

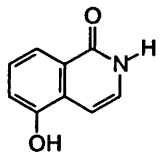
35

36 R = Me
37 R = CH_2Me
38 R = $CH_2CH_2CO_2H$
39 R = CH_2Cl

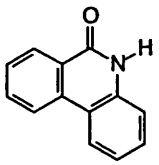
Banasik *et al*[163] evaluated over one hundred and fifty compounds of various structures for activity against calf thymus PARP and mono(ADP-ribose)transferases. They reported that 3-hydroxybenzamide was found to be the most potent PARP inhibitor of the benzamide series, which was in contrast to the report of Purnell and Whish[158]. It was suggested that this discrepancy may have been due to differences in the assay conditions employed, such as the substrate concentration and the enzyme preparation. Nevertheless, the most important observation made during these studies was again the presence of a carbamoyl substituent incorporated within a ring system. This appeared to be very important for PARP activity and subsequently 1,8-naphthalimide derivatives **(40, 41)**, 5-hydroxyisoquinolin-1-one **(42)**, phenanthridinone **(43)** and quinazolinone **(44)** were found to be very potent inhibitors of PARP. These results provided essential structure-activity relationship (SAR) information necessary for PARP inhibitors. It was found that a 6-membered aryl or heteroaryl ring was necessary for activity. Any modifications made resulted in inactivity as was observed for nipecotamide **(31)**, and cyclohexanecarboxamide **(35)** and claimed for thiophene-3-carboxamide **(45)**. However, it was suggested that polyaromatic heterocyclic systems such as phenanthrene, isoquinoline and quinazoline may be associated with potent inhibitory activity. In addition, a carbonyl group conjugated to the arene appeared to be essential for activity. On this basis and the fact that a carboxamide group in the benzamide and nicotinamide series is essential for activity, it was deduced that these compounds were occupying the nicotinamide binding site of PARP and that the 3-substituent was situated within the ribose nucleoside-binding domain. These compounds were able to serve as competitive inhibitors as they are unable to undergo C-N bond cleavage. It was also observed that, in the benzamide series, the only compounds that appeared to be active were those that contained an unsubstituted carbamoyl NH_2 group. This indicated that they may be making important hydrogen-bond interactions with an amino-acid donor within the enzyme's active site.



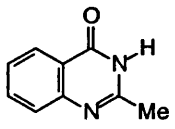
40 R = H
41 R = NH_2



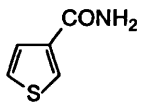
42



43

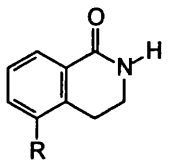


44

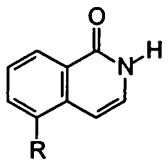


45

In 1991, Suto *et al*[164] provided evidence supporting this hypothesis, by designing compounds that constrained the carbamoyl group into either the 'syn' or 'anti' conformation. This was achieved *via* two ways: In the first case, 'rigid' benzamide analogues were prepared in which the orientation of the carboxamide group was constrained into a ring system – (dihydro)isoquinolin-1-ones. The second approach involved introducing substituents at the 2-position of the ring – 2,3-disubstituted benzamides. In was found that, the 5-substituted (dihydro)isoquinolin-1-one compounds, where the carboxamide was constrained in the 'anti' conformation, resulted in highly potent compounds, thus confirming this to be the biologically active conformation. Also, positioning of the substituent on the aromatic ring proved crucial for activity. When the substituent was placed on the 6-, 7- or 8-positions of the ring, inhibitory activity was lost, indicating that the 5-position was important for activity. Of this series, 5-methoxy- (**46**, IC_{50} 0.42 μM), 5-hydroxy- (**47**, IC_{50} 0.10 μM) and 5-aminodihydroisoquinolinone (**48**, IC_{50} 0.41μM) were found to be potent PARP inhibitors. Similarly, 5-methoxy- (**49**, IC_{50} 0.58 μM), 5-hydroxy- (**50**, IC_{50} 0.15 μM), and 5-aminoisoquinolinone (**51**, IC_{50} 0.28 μM) were also found to be potent inhibitors of PARP. In the second approach, the 2,3-disubstituted analogues were found to be completely inactive, because the biologically inactive 'syn' conformation predominated in these compounds.

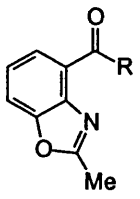


46 R = OMe
47 R = OH
48 R = NH_2

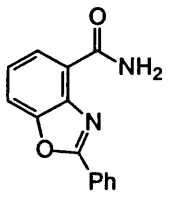


49 R = OMe
50 R = OH
51 R = NH_2

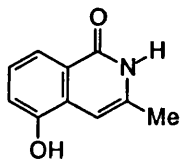
Following these studies, Griffin *et al*[165] extended this work to benzoxazole-4-carboxamide compounds, on the basis that intramolecular hydrogen-bond interactions resulting between the amide NH and the oxazole nitrogen in the electron-rich heterocycle would afford a good donor carbonyl group constrained in the biologically active 'anti' conformation for association with the NAD^+-binding site. This theory was reported to be achieved in the benzoxazole-4-carboxamides **(52)** and **(54)**, which proved to be potent inhibitors of PARP, exhibiting IC_{50} values of 9.5 μM and 2.1 μM, respectively. These results highlight the fact that the intramolecular hydrogen-bond between the carboxamide and the oxazole nitrogen in **(52)** and **(54)** constrained the carboxamide in the biologically active conformation and, furthermore, the compounds possessed good donor properties due to the electron rich heterocycle. In the case of compound **(53)**, the methyl group was occupying a site where a second hydrogen-bond to PARP was required, hence the compound was inactive. It was also noted that the presence of a phenyl group next to the oxazole oxygen appeared to enhance inhibitory activity significantly. In the quinazolinone series, 8-hydroxy-2-methylquinazolinone **(55)** was found to be the most potent PARP inhibitor, exhibiting an IC_{50} value of 0.44 μM. These results were similar to those of Suto *et al*[164] who reported that the active conformation was the 'anti' conformation.



52 R = NH_2
53 R = NHMe

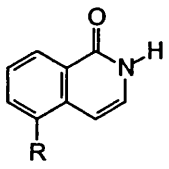


54

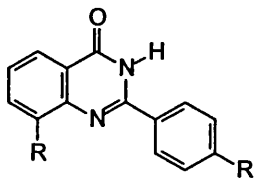


55

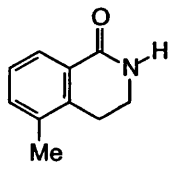
Following this work in 1998, Griffin *et al*[149] reported the activity of various quinazolin-4-one inhibitors. The 8-methyl **(56)** and 8-hydroxy **(57)** substituents were reported to enhance inhibitory activity in comparison to the 8-methoxy group **(58)**. The 2-phenylquinazolinones were found to be slightly less potent than the 2-methylquinazolinones, however, the introduction of an electron-donating or electron-with-drawing group on the 4'-substituent appeared to increase potency significantly. This was particularly evident in the 8-methylquinazolinone series, where compounds **(59)**, **(60)**, **(61)**, **(62)** and **(63)** had IC_{50} values of 0.13-0.27μM[146].



56 R = Me
57 R = OH
58 R = OMe



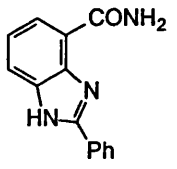
59 R = NO_2, X = Me
60 R = CN, X = Me
61 R = OMe, X = Me
62 R = NH_2, X = Me
63 R = OH, X = Me



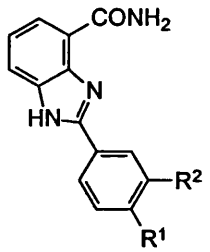
64

Recent studies[104,149,166] have shed light onto the crystal structures of PD128763 **(64)** and NU 1025 **(55)** bound to the catalytic domain of PARP. From the crystal structures, it can be seen that all the inhibitors bind in the same 'anti' orientation and complexes are formed between the two hydrogen bonds occurring between the carbonyl and NH (or NH_2) groups and the peptide backbone of Gly-863[146]. The third hydrogen bond occurs between the carbonyl group of the inhibitor and the side chain of Ser-904. In the case of NU1025, a further hydrogen bond interaction was evident between the 8-hydroxyl substituent and the hydroxyl group of Tyr-907.

White *et al*[150], in 2000, expanded these studies to the development of benzimidazole-4-carboxamides as PARP inhibitors. 2-Phenyl-1H-benzimidazole-4-carboxamide **(65)** was found to be a potent PARP inhibitor, exhibiting a K_i value of 15nM. Derivatives of this compound were developed with various substituents on the phenyl ring. From the analysis of these compounds they found that many of them exhibited K_i values for PARP inhibitory activity <10 nM, with 2-(4-hydroxymethylphenyl)-1H-benzimidazole-4-carboxamide **(66)** exhibiting a K_i value of 1.6 nM and being the most potent inhibitor of PARP reported to date.



65



66 $R^1 = CH_2OH$
67 $R^2 = OCH_3$

The structure-activity relationships were also studied using the complex formed between 2-(3-methoxyphenyl)-1H-benzimidazole-4-carboxamide (**67**, K_i = 6 nM) and the catalytic fragment of chicken PARP[150]. It was found that the carboxamide group of compound **(67)** formed three important hydrogen bonds. It was observed that the carbonyl oxygen on the inhibitor accepted two hydrogen bonds, one from the Gly 863 polypeptide amide NH and the other from the side chain oxygen of Ser 904. The amide NH of compound **(67)** on the same side of the carbonyl group formed a hydrogen bond donor to the Gly-863 carbonyl oxygen. The benzimidazole part of compound **(67)** appeared to lie in a deep pocket formed by residues Phe 897, Ala 898, Lys 903 and Glu 988. The benzimidazole was reported to be buried between two tyrosine residues-Tyr 896 and 907. Tyr 907 appeared to be coplanar with the benzimidazole, whereas Tyr 896 was rotated about 37° from coplanarity[150]. The 2-(3-methoxyphenyl) group was found to be located above a large, solvent-containing part of the enzyme and was, therefore, not bound to the enzyme's active site. Both faces of the 2-phenyl ring appeared to be exposed to the solvent. The 3-methoxy group was also found to be exposed to the solvent but appeared to be partially shielded from the solvent by the presence of a Gln 763 side chain. The 3-methoxy and 4-carboxamide groups appeared to be centred over the enzyme cavity. The unsubstituted 4- and 5- positions were oriented towards the surface of the enzyme cavity. The 3-methoxy position and 4-position appeared to have space available around them, which was much less for positions 6, 7 and 8[150].

Other compounds that have been of interest as PARP inhibitors are thymidine and thymidine analogues[167] and thiophenecarboxamide compounds[168]. In the case of thiophenecarboxamides, Shinkwin *et al*[168] reported that the benzene ring of the benzamide compounds could be replaced by a heterocyclic thiophene ring without significantly affecting PARP inhibitory activity.

4.0 *CURCUMIN*

Scientists for many years have been aware that the onset of cancer is a gradual, stepwise process that may unfold over the course of several years. Carcinogenesis is the result of a prolonged accumulation of injuries at various biological levels, and includes both genetic and biochemical changes in cells. At each of the biological levels, there is an opportunity to prevent, slow down or stop the healthy cells from becoming malignant. Chemoprevention is therefore an attempt to intervene in the early stages of carcinogenesis by using natural and / or synthetic compounds.

The concept of chemoprevention first arose in the 1960s and 1970s when Wattenberg[169] demonstrated that compounds, particularly those associated with fruits and vegetables such as indoles and isothiocyanates, could inhibit tumours induced in animals. In the mid 1970's this phenomenon was termed "cancer chemoprevention". Since then, many scientists all over the world have contributed to this concept as a strategy for reducing human tumours. Chemoprevention is therefore the process of inhibiting, delaying or reversing carcinogenesis by the use of natural or non-toxic synthetic compounds[170].

To date, over six hundred compounds can be considered as chemopreventive agents, including inhibitors of the initiation process, such as phenols, aromatic isothiocyanates, flavones and glutathione and inhibitors of the post-initiation stages, such as β-carotene, tamoxifen, retinoids, protease inhibitors, terpenes and prostaglandin inhibitors. These agents can either be natural or synthetic compounds.

In order for a compound to be classed as a chemopreventive agent, it has to meet several criteria including, (1) availability (the compound has to be available in large quantities either naturally or synthetically). (2) Cheap (the compound has to be relatively cheap as it may be consumed daily over a long period of time). (3) Oral administration (the compound should be in a form, which is easy to administer orally). (4) Non-toxic (the compound should be non-toxic, or have very little side effects, and (5) a known mechanism of action (the compounds mechanism of action should be known so that it can be targeted specifically to the problem area). In

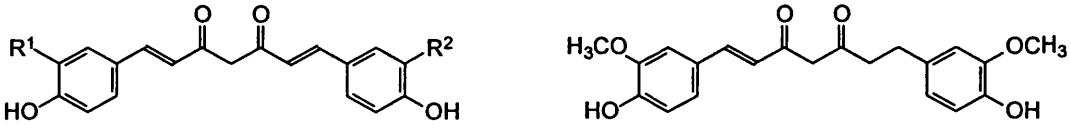
general, natural compounds are preferred over synthetic compounds because they have been used over many decades and have little known side-effects[171].

In order to understand how chemopreventive agents exert their activity, it is important to appreciate the steps involved in epithelial carcinogenesis. Carcinogenesis occurs in three stages: the first one being the initiation stage, the second is the promotion stage and the third is the progression stage. In the case of initiation, this process is rapid and involves the direct binding of the carcinogen to the DNA, resulting in DNA damage. The mutation that results is irreversible. In the second stage of the process, promotion occurs which involves the clonal expansion of the initiated cells. These cells are induced by agents acting as mitogens and this stage is usually reversible. Following promotion, the final progression stage occurs. This is generally an extension of the promotion stage and results from it, as the cellular damage is further enhanced[172]. Following this stage genotypically and phenotypically altered cells result. Both of the promotion and progression stages of carcinogenesis are prolonged. Depending on which stage the chemopreventive agent affects, they can be divided into tumour 'blocking' agents or tumour 'suppressing' agents. Tumour 'blocking' agents usually interfere with the initiation stage of carcinogenesis so that it can counteract the cancer[172]. These agents generally, tend to reduce the accumulation of initiating mutations. Tumour 'suppressing' agents tend to act on both the promotion and progression stages of carcinogenesis and work to suppress the development of the initiated cell to a full-blown tumour. This can be achieved by scavenging free-radicals thereby behaving as antioxidants, inhibiting arachidonic acid metabolism, hormone and growth factor activity and oncogene activity. These agents are also known to suppress the tumour by modulating the cellular transduction pathways[172].

A class of dietary compounds that have been evaluated as chemopreventive and chemoprotective agents are the polyphenols. One such member of this class which is under intense investigation, is curcumin. Curcumin is the major constituent of the spice turmeric. Turmeric is prepared from the dried rhizomes of Curcuma longa L, which tends to be found in the tropical regions of Asia. Turmeric has long been used as a spice in foods and gives the characteristic yellow colour. More importantly, however, it has been used in herbal medicines in countries such as China, India and

Indonesia to combat cancer, inflammation, alleviate fever and to treat sprains and bruises.

Three major phenolic diarylheptanoids have been isolated from *Curcuma longa* L, including curcumin **(68)**, demethoxycurcumin **(69)** and bisdemethoxycurcumin **(70)**. However, recently, a further compound **(71)** has been reported to be isolated from this naturally occurring herb. Compound **(71)** was isolated and identified by Satyanarayana and Ravindranath[173].



68 $R^1 = R^2 = OMe$
69 $R^1 = H$, $R^2 = OMe$
70 $R^1 = R^2 = H$

71

Figure 4. Constituents of Curcuma longa L[173]

Curcumin (diferuloylmethane) is the yellow pigment in turmeric and has had widespread use in the food, cosmetic and pharmaceutical industries. It was first isolated in 1870 but its structure was not elucidated until as late as 1910. Curcumin has been reported to possess strong anticarcinogenic effects in various tissues of animals. It was reported to possess anti(tumour promoting) activity by Huang *et al*[174], who demonstrated that curcumin applied topically to the skin of mice inhibited tumour promotion. Subsequently, further investigations carried out reported that curcumin was able to inhibit benzo[*a*]pyrene-induced forestomach tumours[175], azoxymethane-induced crypts in rat colon[176] and DMBA-induced hyperplastic nodules in cultured rat mammary gland tissue[177]. As well as possessing antitumour activity, curcumin also exhibits antiflammatory activity[178]. Recently, curcumin has also been shown to be a modest inhibitor of HIV-1 and HIV-2 proteases[179]. In addition, interestingly curcumin has also been reported to be an inhibitor of PARP[171]. Its inhibitory activity was found to be similar to that of 3-AB. As PARP is known to play an important role in DNA repair, curcumin may be useful in the treatment of radiotherapy and chemotherapy. However, currently curcumin is of

great interest as a chemopreventive agent for cancer, and in particular carcinoma of the colon[172,180-182].

In the western countries, colorectal cancer is the major cause of deaths[172,180-182]. However, epidemiological data have suggested that modification of the diet may reduce these deaths by up to 90%. At the biochemical level, curcumin has been shown to inhibit cyclooxgenase 2 (COX2) activity, indirectly. This was achieved through the inhibition of phosphorylation of IκB, inhibition of NFκB activation and inhibition of the COX2 expression.

COX2, an inducible isoform of prostaglandin H synthase (PGHS), is known to mediate prostaglandin synthesis during inflammation, and in colon tumours is known to be overexpressed. COX2 is therefore thought to play an important role in colon carcinogenesis. Experiments involving the pharmacological inhibition, or genetic knock-out of COX2 have been shown to protect against development of colonic tumours in rats exposed to the colon carcinogen azoxymethane, or mice harbouring a germline knockout mutation of the adenomatous polyposis coli (APC) tumour suppressor gene[183,184]. In colonic epithelial cells, overexpression of COX2 is thought to promote tumour development by causing cellular alterations and also by resisting apoptosis. However, non-steroidal antiflammatory agents have been used to inhibit COX2 activity and this has been shown to reduce the risk of sporadic colon cancer[181]. Unfortunately, due to the serious side-effects that are known to occur with such agents, this means that they cannot be used to suppress human tumours.

Recently, Plummer *et al*[182] reported that in human colon epithelial cells, curcumin was successful in inhibiting COX2 induction by the colon tumour promoters, tumour necrosis factor α, or fecapentaene-12. It was found that COX2 induction by oxidative stress or inflammatory cytokines could be mediated by the nuclear factor kappa B (NF-κB). In addition to curcumin inhibiting NF-κB activation, it was also found to prevent phosphorylation of IκB by inhibiting the activity of the IKKs. This feature makes curcumin an attractive candidate for the prevention of colon cancer.

The concentrations that have been used in order to bring about the pharmacological effects of curcumin *in vitro* have been reported[182] to be in the range of 10-100 μM. However, it has been demonstrated that the bioavailability of curcumin in rodents, following oral administration, is poor. This suggested that maybe one or more of the metabolites of curcumin are involved in its pharmacological activity.

Although the metabolism of curcumin in humans is unknown, a number of studies[185,186] have addressed the absorption, metabolism and tissue distribution of curcumin, following oral administration of 400, 80 and 10 mg of [^{3}H]-curcumin in rats[187]. Measurements of the radioactivity in the plasma levels have shown that curcumin was poorly absorbed from the gut. These studies therefore suggested that curcumin was transformed during absorption from the intestines. It was reported that the transformed product(s), which was found to be more polar than curcumin entered the serosal side. Holder *et al*[187] in 1978 reported that, following i.v. administration of 50 mg Kg^{-1} [^{3}H]-curcumin in rats, most of the radioactivity present in the bile was found in the form of glucuronide conjugates of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC), although some curcumin was found in the bile.

Following this work, the biotransformation of curcumin in mice was reported by Pan *et al*[188]. It was reported that, following i.p. administration of curcumin (0.1g Kg^{-1}) to mice, approximately 2.25 μg mL^{-1} of curcumin was found in the plasma in the first 15 minutes. One hour later, curcumin was found to be present in the intestines, spleen, liver and kidneys (177.04, 26.06, 26.90 and 7.51 μg g^{-1})[188]. Analysis of the metabolites indicated that when administered, the curcumin was reduced by UDP-glucuronosyl transferases, subsequently giving curcumin-glucuronoside, dihydrocurcumin-glucuronoside, THC-glucuronoside and THC. As THC was found to be one of the major metabolites of curcumin, studies have been carried out to elucidate its biological function. Sugiyama *et al*[189] recently reported it to be a stronger antioxidant than curcumin *in vitro*. If this is the case it is thought that THC may show similar pharmacological activities as curcumin, therefore may itself be useful as a chemopreventive agent.

As previously mentioned, the absorption and metabolism of curcumin is not yet known in humans. However, an ongoing collaborative study on the uptake of this compound with [^{14}C]-labelled curcumin is currently underway.

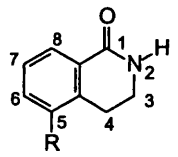
5.0 *AIMS AND OBJECTIVES*

HYPOXIA SELECTIVE PRODRUGS

The aim of this project was to build on the earlier studies, in order to exploit the lower oxygen concentration in solid tumours, by developing a prodrug system which when activated under hypoxic conditions will release inhibitors of PARP, selectively in solid tumours. Prodrugs that selectively release therapeutic drugs in hypoxic tumour tissues would, therefore, behave as tumour-selective radiosensitisers.

Inhibitors of PARP that were selected in this project were 5-bromo- and 5-aminoisoquinolinone. From the previous studies carried out, it is known that 5-bromo- and 5-aminoisoquinolinone are particularly active[70,164] and, consequently, were chosen as the drugs for delivery. Isoquinolinone has previously been shown to be released from the nitrofuranylmethyl derivative when activated chemically. Other inhibitors that were thought to be useful as PARP inhibitors were quinoline-8-carboxamide N-oxides.

From the previous studies carried out on PARP inhibitors, the structural activity requirements (SAR) necessary for PARP inhibitory activity have been reported.



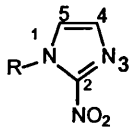
72

Figure 5. SAR necessary in the development of PARP inhibitors[146]

- It is essential that the conformation of the amide be in the 'anti' orientation. It consists of a secondary arene-carboxamide that has an absolute requirement for the N-H to be 'syn' to the C=O bond.

- At the 3 and 4 positions of the isoquinoline-1-one ring, a bulky substituent is permitted.
- The R group cannot be an electron withdrawing group.
- At the 6, 7 and 8 positions of the isoquinoline-1-one ring no substituent is permitted.

In this study 1-substituted-2-nitroimidazole was selected as the bioreducible moiety because, as previously mentioned, these compounds show greater metabolic stability in normal tissues and they exhibit a wide range of redox potentials. From the literature studies on the nitroimidazole compounds, the SAR for such compounds has been identified[7].



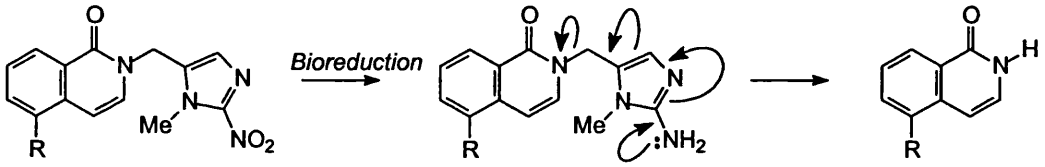
73

Figure 6. SAR necessary for the bioreducible nitroimidazole moiety[7]

- The nitro group in the 2-position of the imidazole ring largely determines the electron affinity, hence the radiosensitising properties. In general, the more negative the reduction potential, the less electron affinic the compound. As the nitro group is changed from position 2 to 5 to 4, the electron-affinity of the nitroimidazoles decreases.
- The side chain R determines the solubility characteristics in water and lipids, hence the pharmacokinetics. The prodrug must be highly soluble in water and lipids so that it is able to diffuse through a nonvascularised cell mass in order to reach the hypoxic cells, which can be located as far as 200 μm from the nearest capillary.
- Nitroimidazole type compounds show metabolic stability in normal tissues and can have a wide range of redox potentials. Previous studies[1,7,13-15] have suggested that a redox potential between –250 mV to –400 mV is required for effective radiosensitisation, bioreductive metabolism and efficient back oxidation by molecular oxygen.

PROPOSED PRODRUG SYSTEM

It is proposed that, in hypoxic tumour tissue, the nitroimidazole will be metabolically reduced by cellular reductases to the aminoimidazole. The available lone pair of electrons on the nitrogen will then promote fragmentation to release the drug selectively in the tumour tissue. This is demonstrated schematically below.



Scheme 2. Proposed mechanism of bioreductively triggered release of drugs from general nitroimidazole prodrug.

However, in aerobic cells, the nitroimidazole will only be reduced by the cellular reductases to the initial nitro radical anion metabolite. This species is very reactive towards oxygen and, in oxygenated cells, will be oxidised back to the parent compound.

The aim of this project was therefore to (i) synthesise 2-nitroimidazolyl-5-methyl derivatives of isoquinolinone compounds, in addition to other PARP inhibiting drugs, (ii) to optimise the solubility characteristics of the 2-niroimidazolyl-5-methyl moiety, (iii) to optimise release characteristics of the 2-niroimidazolyl-5-methyl moiety, (iv) to synthesise prodrugs of general structure **(76)** and to synthesise the novel PARP inhibitor quinoline-8-carboxamide N-oxide **(182)**.

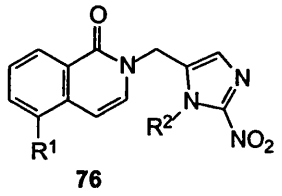


Figure 7. Illustrates the general structure of tumour hypoxia selective prodrugs.

CURCUMIN

As previously mentioned, curcumin is of particular interest as a chemopreventive agent in the treatment of various forms of cancer. However, the metabolism of this compound following administration in humans is unknown. Nevertheless, studies carried out with mice have reported that bioavailability of curcumin, following oral administration, is poor. The aim of this part of the project was to synthesise ^{14}C-labelled curcumin **(235)** so that, in an ongoing collaborative study, its biodistribution and pharmacology, could be evaluated.

6.0 *RESULTS AND DISCUSSION*

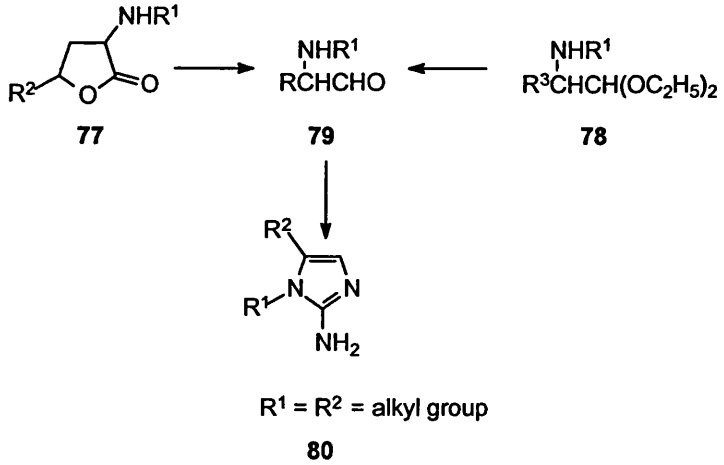
The first synthetic target was the (nitroimidazolylmethyl)isoquinolinone **(119)**. This carries the 2-nitroimidazol-5-ylmethyl trigger unit linked to the potent PARP inhibitor 5-bromoisoquinolin-1-one[70,164]. In this first target, the N^1 nitrogen of the imidazole is masked with a simple methyl group; in later targets, this nitrogen substituent could be modified to incorporate other functions, such as water-solubilising groups.

As a synthetic target, the molecule can be divided conveniently into two parts. Alkylations of the anions derived from isoquinolinones have been reported[70] to be efficient in introducing alkyl and aralkyl groups at the heterocyclic nitrogen. Indeed, 5-bromoisoquinolin-1-one has been alkylated[70] with 4-methoxybenzyl chloride in the presence of base to give 5-bromo-2-(4-methoxybenzyl)isoquinoline-1-one[70]. For the present target, this reaction represents a very good point for retrosynthetic disconnection, as it would lead to two similar-sized fragments and thus the forward synthesis would be convergent. Thus the synthetic problem becomes the synthesis of two fragments, a 2-nitro-5-(halomethyl)imidazole (or an analogous electrophile) and 5-bromoisoquinolin-1-one.

Although these structurally simple compounds appear easy to synthesise they have proved difficult for the chemist. Direct nitration of imidazole and its derivatives introduces the nitro group into either the 4- or 5-positions of the ring, when they are free[190]. However, even if the 4- and 5-positions are occupied, electrophilic substitution does not take place at the 2-position[190].

Synthetic strategies have been reported in which 2-nitroimidazoles can be obtained from the corresponding 2-aminoimidazoles[190]. In literature studies[190], there are only few examples of the oxidation of an amino group at the 2-position. These include oxidation with peroxytrifluoroacetic acid and microbial oxidation. Alternatively, the amino group can be replaced with nitro by diazotisation, followed by a Gattermann reaction with sodium nitrite in the presence of copper[190-192].

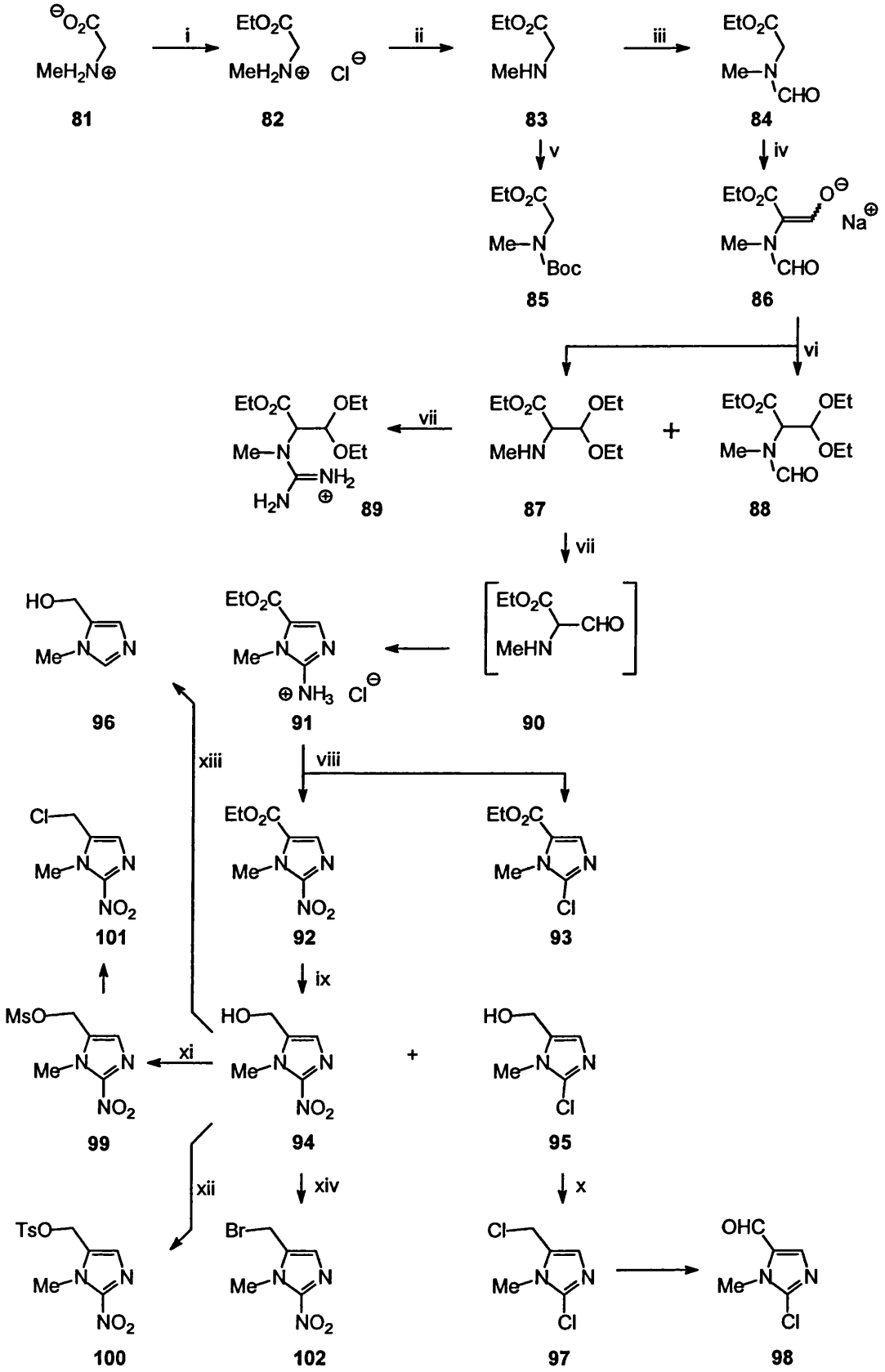
It was Lancini *et al*[191] in 1969 who first reported the synthesis of a number of 1,5-disubstituted-2-nitroimidazoles by diazotisation and Gattermann reaction on the corresponding 2-aminoimidazoles. The aminoimidazoles were prepared from either amino acetals or lactones. In the first reported route[191], hydrolysis of alkylamino acetals (prepared from the treatment of α-bromo acetals with monoalkylamines or α-amino acetals with bromo esters) followed by condensation of the aminoaldehyde with cyanamide afforded the aminoimidazole compound. In the second route, Akabori reduction[191] of α-alkylaminolactones (prepared from bromolactones), followed by condensation with cyanamide also gave the required aminoimidazole. However, the reported procedures acquired the use of expensive chemicals, tedious work-up requirements and afforded low yields. As a result, an alternative route was preferred.



Scheme 3. Reported synthesis of 2-nitroimidazoles[191].

In 1972, Cavalleri *et al*[192] reported a synthetic route to 1-methyl-2-nitro-5-hydroxymethylimidazole **(94)**. Their synthetic procedure started from ethyl 2-amino-1-methylimidazole-5-carboxylate which undergoes diazotisation in tetra-fluoroboric acid at low temperatures, followed by reaction of the diazonium salt with nitrite in the presence of copper (by the procedure reported by Lancini *et al*[191]), which resulted in the corresponding 1-methyl-2-nitroimidazole-5-carboxylate **(92)**. Selective reduction of the ester of **(92)** with lithium borohydride was reported to give the 5-hydroxymethyl derivative.

As compound **(94)** was recognised as a key intermediate in the synthesis of 5-chloromethyl-1-methyl-2-nitroimidazole **(101)**, this was the preferred route. Furthermore, this procedure involved the use of readily available starting materials. However, low yields were reported in Cavalleri's procedure[192] for compounds **(92)** and **(94)**. In attempts to optimise the reported yields, the experimental procedures were evaluated and optimised in order to bring forth a good yield of the key compound **(101)** for elaboration with the PARP inhibitory isoquinolinones.



Scheme 4. Synthesis of 1-methyl-2-nitroimidazoles. *Reagents:* i, EtOH / $SOCl_2$, ii, K_2CO_3, iii, Ac2O / NaO_2CH, iv, NaOEt / EtOCHO, v, EtOCHO / NEt_3 / di-*t*-butyl dicarbonate, vi, EtOH / HCl, vii, aq. HCl / cyanamide, viii, H_2O / HBF_4 / $NaNO_2$ / Cu, ix, $LiBH_4$ (solid) / THF, x, $SOCl_2$, xi, dry pyridine / $MeSO_2Cl$, xii, dry pyridine / TsCl, xiii, $LiBH_4$ (solution), xiv, dry DMF / Br_2 / PPh_3.

6.1 CRITICAL 2-NITROIMIDAZOLE INTERMEDIATES

Sarcosine **(81)** was converted to its ethyl ester **(82)** using thionyl chloride and ethanol. In the next step of the reaction, it was essential to protect the nitrogen, to ensure that formylation would take place at the methylene of compound **(82)** and not at the nitrogen. Compound **(82)** was protected as the formamide. In initial experiments, following a common procedure for protection, the free base of the ester was extracted and was treated with ethyl formate in the presence of base. Although the reaction was successful, N-formylsarcosine ethyl ester **(84)** was obtained in a poor yield of 25%. The low yield was attributed to the fact that the majority of the free base was lost in the aqueous layer. However, treatment of compound **(83)** with the electrophile appeared to go to completion, according to TLC analysis.

As a result, the reaction was repeated but without initially extracting the free base. However, in this procedure, compound **(84)** was obtained in a lower yield of 11%. At this stage, it was thought that perhaps it was due to the presence of solvent (ethanol) that was causing problems by diluting the reaction mixture, consequently slowing down the rate of reaction. As a result, the reaction was repeated but without the use of solvent. This led to compound **(84)** being obtained in a yield of 18%. Although this procedure improved the yield slightly, it was still extremely low yielding and inefficient. The low yields obtained questioned the reactivity of the electrophile in use. Schmidt and Geiger[193] reported the synthesis of compound **(84)** by treatment of the ester hydrochloride salt **(82)** with the more reactive electrophilic formylating reagent acetic formic anhydride. This reaction gave compound **(84)** in a much-improved yield of 70%.

Due to the delocalisation of the lone pair of electrons on nitrogen into the carbonyl of the amide, there is partial double bond character in the C-N amide bond, leading to restricted rotation. Correspondingly, signals for the *cis* and *trans* rotamers of **(84)** were observed in the ^{1}H NMR spectrum at 25°C. This was particularly evident in the signals for the NMe and NCH_2 protons. The two rotamers existed in a 1:2 ratio. As the sample is heated, the rate of rotation about this bond should increase and become rapid on the NMR time-scale. The signals should then coalesce. However, at 90°C the rotameric signals appeared to get closer together and even more so at 120°C, but

the signals did not merge, indicating a high energy barrier to rotation. In addition to the protective function of the formyl group, its presence also enhances the next formylation step at the methylene of compound **(84)**. This is due to the electron-withdrawing effect of the formyl group on the nitrogen resulting in a shift of electron density and a slightly electropositive nitrogen.

Alternative Boc protection of the nitrogen of **(82)** was also investigated. Initially, the free base was extracted and allowed to react with di-*t*-butyl dicarbonate to give N-Boc-sarcosine ethyl ester **(85)** in 97% yield. However, the one-pot reaction between the hydrochloride **(82)** and di-*t*-butyl dicarbonate, in the presence of excess tertiary amine base, went to completion and gave compound **(85)** in 97% yield. For this N-Boc compound, the NMR spectrum showed the presence of rotamers in a 1:1 ratio, reflecting the greater bulk of the Bu^tO group, compared to that of the H for the formyl compound.

With the nitrogen of **(84)** protected with formyl, the methylene was deprotonated with sodium ethoxide; the anion reacted with ethyl formate to give ethyl 2-(N-formyl-N-methylamino)-3-oxopropanoate as its sodium enolate salt. The newly introduced aldehyde was protected as the diethyl acetal by prolonged treatment at reflux with ethanol saturated with HCl. These conditions also served to remove the N-formyl group by ethanolysis. The driving force for the cleavage of the formamide was the formation of the salt of the product amine. The ethyl 3,3-diethoxy-2-methylaminopropanoate **(87)** was obtained in 41% yield.

During the optimisation of this procedure, the method reported in the literature[191] was investigated. In this, the N-formyl enolate salt **(86)** was merely stirred with ethanolic HCl at ambient temperature. ^1H NMR indicated the presence of two product compounds. Compound **(87)** was present in addition to the formylated acetal **(88)**, suggesting that complete removal of the formyl group with HCl had been unsuccessful. Attempts to separate the two by column chromatography failed as the crude mixture decomposed on silica gel.

The aldehyde was unmasked using aqueous hydrochloric acid and the amino-imidazole **(91)** was formed by condensation with cyanamide. In the initial

experiment, the literature procedure[192] was followed. It was reported that unmasking of the aldehyde by 10% hydrochloric acid at 60°C could be achieved after 2 h. However, this was not the case and it was not known whether the hydrolysis or the condensation step was unsuccessful but ^{1}H NMR indicated that a large amount of the acetal was present. Furthermore, during the reaction it was found that **(90)** could not be monitored *via* TLC as it had a similar Rf value to the starting compound **(87)**. In order to overcome the hydrolysis problem, compound **(87)** was treated with hydrochloric acid at 60°C for a longer period of 12 h. Although it was known that, if the mixture was left heating for a prolonged period, this may have resulted in the hydrolysis of the ester. Despite this, the reaction was treated with aqueous hydrochloric acid for 12 h at 60°C and, following very slow recrystallisation from aqueous propan-2-ol, the aminoimidazole **(91)** was obtained in 55% yield. ^{1}H NMR confirmed the presence of this compound, as the imidazole ring proton was observed at δ7.76 as a singlet. Furthermore, a broad peak was observed at δ8.26 corresponding to the amino group. In D_2O, the imidazole 4-H remained, whereas the amino group protons were exchanged.

Kirby[194] reported an alternative synthetic route to 2-aminoimidazoles which involved the treatment of the acetal with S-methylisothiuronium sulfate, followed by unmasking of the aldehyde with 10% hydrochloric acid and a condensation reaction to give the aminoimidazole. However, treatment of the acetal **(87)** with S-methylisothiuronium sulfate failed to give compound **(89)**, as ^{1}H NMR indicated that the compound had decomposed. Treatment of the acetal **(87)** with cyanamide was also tried in an attempt to form the intermediate compound **(89)**. However, again the reaction was unsuccessful and only decomposed material was recovered.

In the next stage of the reaction, the amino group was diazotised and a copper-catalysed Gattermann reaction introduced the required 2-nitro group in ethyl-1-methyl-2-nitroimidazole-5-carboxylate **(92)**. In this reaction, a suspension of the diazonium tetrafluoroborate in aqueous sodium nitrite was treated with copper powder. The solution was made acidic by treating with aqueous hydrochloric acid in order to retain any starting aminoimidazole compound **(91)**. The 2-nitro compound **(92)** was extracted with ethyl acetate. ^{1}H NMR indicated the presence of two compounds, which included the desired ethyl-1-methyl-2-nitroimidazole-5-

carboxylate **(92)** and the side product ethyl-1-methyl-2-chloroimidazole-5-carboxylate **(93)**, obtained in 48% and 18% yields, respectively. This latter 2-chloro compound **(93)** was thought to have been formed during the work-up procedure in which the nucleophilic chloride ions substitute the nitro group. In subsequent reactions, aqueous sulfuric acid was used in place of the hydrochloric acid to prevent this nucleophilc substitution from occurring, as the sulfate ions are much less nucleophilic than the chloride ions. Under these reaction conditions, compound **(92)** was obtained in a higher yield of 63% and the 2-chloro compound **(93)** was obtained in only 4% yield.

Compound **(92)** in tetrahydrofuran was reduced by addition of solid lithium borohydride to its solution in tetrahydrofuran. The excess lithium borohydride was decomposed by addition of aqueous hydrochloric acid. This resulted in formation of two compounds, the first being the desired 1-methyl-2-nitro-5-hydroxymethylimidazole **(94)**, obtained in 36% yield, and the side compound 1-methyl-2-chloro-5-hydroxymethylimidazole **(95)** was obtained as a crude mixture. Compound **(94)** was obtained pure following column chromatography. However, column chromatography and recrystallisation failed to purify compound **(95)**. The 2-chloro analogue was thought to have occurred due to the chloride ions in aqueous hydrochloric acid being nucleophilic resulting in the substitution of the nitro group. To prevent this from occurring, in subsequent reactions, glacial acetic acid was used to decompose the excess lithium borohydride, as the acetic acid is a much weaker acid than hydrochloric acid. In ^{1}H NMR, the peaks for the chloro analogue **(95)** were slightly more upfield than those observed for compound **(94)**. This was due to the less deshielding effect of the chloro substituent. Under these reaction conditions, compound **(94)** was obtained in a higher yield of 60%.

Interestingly, the reaction was repeated with commercial solution of lithium borohydride in tetrahydrofuran and it was found that over-reduction occurred, which resulted in the cleavage of the nitro group. This is the first report of the reductive removal of the nitro group with lithium borohydride. ^{1}H NMR provided evidence for the formation of compound **(96)**, which was obtained in 91% yield. The two imidazole ring protons were observed at δ6.76 and δ7.53 as singlets, each integrating

for one proton. The imidazole 2-H was assigned to the signal at δ7.53, *i.e.* downfield due to the electron-withdrawing effect of the heteroaromatic ring nitrogen.

Attempts to substitute the hydroxy of compound **(94)** selectively using thionyl chloride failed. However, the novel compound 2-chloro-5-chloromethyl-1-methylimidazole **(97)** was obtained. As the hydroxide ion is a poor leaving group, it cannot be displaced by nucleophiles in S_N2 reactions. However, reaction of the alcohol with thionyl chloride converts the hydroxy into a much better leaving group which is substituted by nucleophilic attack of the chloride ion. The nucleophilic chloride ions in solution also substitute the nitro group at the 2-position of the imidazole ring. Compound **(97)** was oxidized in air to the aldehyde compound **(98)**. No further reactions were carried out with thionyl chloride as this reagent was found to be too reactive for the nitroimidazole type compounds. ^{1}H NMR provided evidence for the formation of compound **(98)** as the CH_2 previously observed at δ4.55 was absent. Furthermore, the appearance of an aldehyde peak at δ9.65 further supported this assignment.

Compound **(94)** was treated with methanesulfonyl chloride, following a common procedure[195] to form compound **(99)**. However, this failed but provided the equally equivalent useful electrophile 5-chloromethyl-1-methyl-2-nitroimidazole **(101)** in 70% yield. It was thought that the mesylate **(99)** was formed initially but was then substituted by chloride. Use of methanesulfonic anhydride would have avoided the formation of the chloromethyl compound but was not investigated, as the chloromethylimidazole **(101)** proved to be a suitable electrophile for further synthesis. Similar treatment of **(94)** with *p*-toluenesulfonyl chloride also afforded compound **(101)** but in a lower yield of 31%.

Larger halogens, such as bromine, are better leaving groups. If problems were encountered with the chloro group when attaching the drug to the imidazole, an alternative leaving group, such as the bromide in **(102)**, would be considered. 5-Bromomethyl-1-methyl-2-nitroimidazole **(102)** was synthesised by treatment of compound **(94)** with bromine and triphenylphosphine[196]. Compound **(102)** was obtained in 17% yield. The reaction proceeds *via* a Mitsunobu-like mechanism. Initially, the triphenylphosphine attacks the bromine molecule and forms a

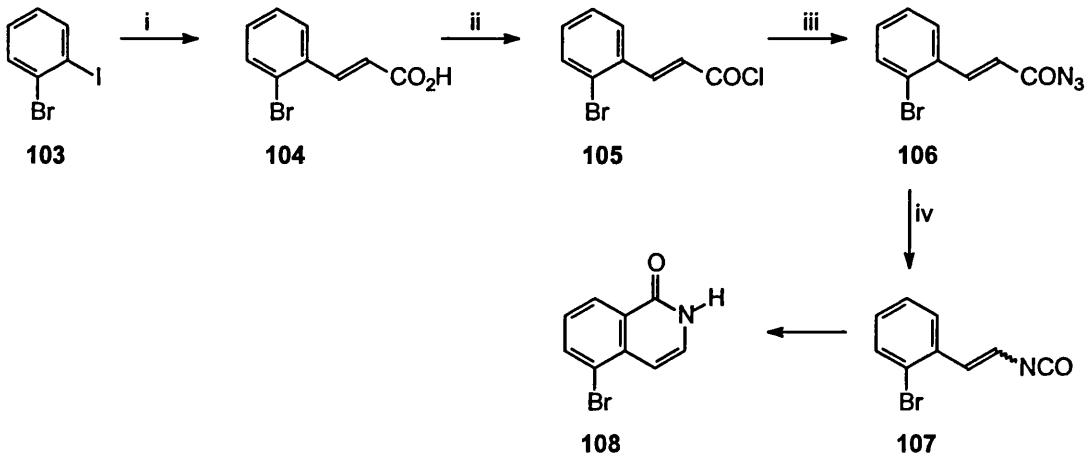
bromotriphenylphosphinium adduct. The nucleophilic oxygen attacks the electropositive phosphorus and eliminates the bromide ion. The nucleophilic bromide ion in turn attacks the electropositive carbon, hence eliminating the triphenylphosphine oxide group.

Having achieved the first part of the synthetic target, the potent PARP inhibitor 5-bromoisoquinolin-1-one was synthesised.

6.2 CRITICAL 5-BROMOISOQUINOLIN-1-ONE INTERMEDIATES

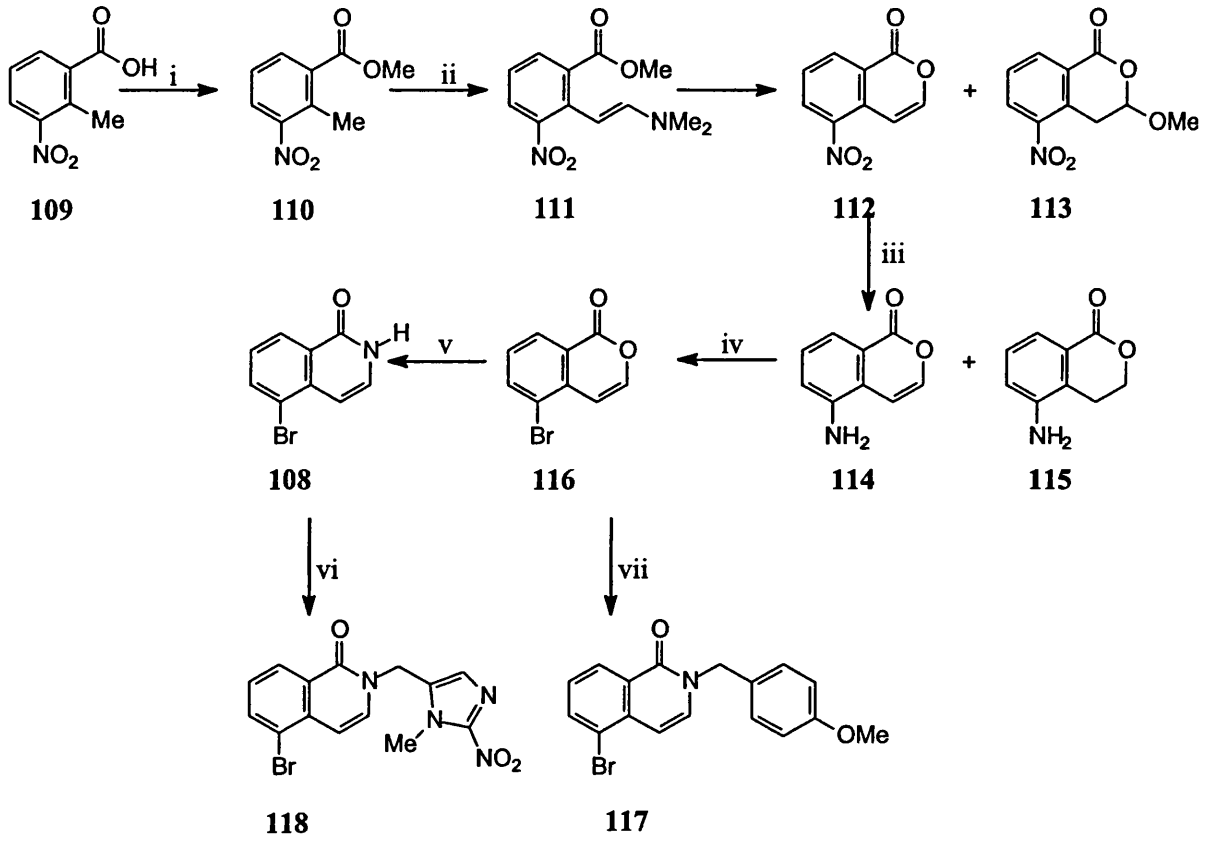
5-Substituted isoquinolin-1-ones have been reported[70,164] to be potent PARP inhibitors. As previously mentioned, PARP is an enzyme which plays an important role in initiating the excision repair of DNA following damage induced by radiation therapy and / or chemotherapeutic drugs. Of the reported compounds, 5-bromo-isoquinolin-1-one was found to be particularly active[70,164] and was, therefore, chosen as the first drug for delivery.

Synthesis of 5-bromoisoquinolin-1-one has previously been reported by Berry *et al*[70]. The reported synthetic strategy initially involved the formation of the bromo-cinnamic acid **(104)**, which was prepared from 2-bromoiodobenzene **(103)** in an iodine-selective Heck reaction. 2'-Bromocinnamic acid was converted into the acid chloride **(105)** and then to the acid azide **(106)** by reaction with sodium azide. Curtius rearrangement of the acid azide at >260°C, with subsequent cyclisation, was reported to furnish 5-bromoisoquinolin-1-one in 10% yield. As a consequence of the poor yield, new routes were developed for the synthesis of 5-bromoisoquinolin-1-one **(108)**.



Scheme 5. Reported synthesis of 5-bromoisoquinolin-1-one[70]. *Reagents:* i, $H_2C{=}CHCO_2H$ / $Pd(OAc)_2$ / NEt_3 / EtCN, ii, $SOCl_2$ / DMF, iii, NaN_3 / H_2O / 1,4-dioxane, iv, heat, Ph_2O.

Masanori *et al*[197] recently reported a simple synthetic route to 5-aminoisocoumarin. In our laboratory, it was proposed that diazotisation of 5-aminoisocoumarin with sulfuric acid and sodium nitrite and treatment with copper (I) bromide / potassium bromide, followed by treatment with ammonia and 2-methoxyethanol would afford 5-bromoisoquinolin-1-one. The new synthetic route employed for the formation of 5-bromoisoquinolin-1-one is outlined below.



Scheme 6. Synthesis of 5-bromoisoquinolin-1-one and prodrug **(118)**. *Reagents:* i, MeOH / $SOCl_2$, ii, DMF / DMFDMA, iii, THF / aq. HCl / Pd/C / H, iv, H_2O / $NaNO_2$ / aq. H_2SO_4 / CuBr / KBr, v, 2-methoxyethanol / NH_3, vi, DMF / lithium bis(trimethylsilyl)amide / 5-chloromethyl-1-methyl-2-nitroimidazole / NaI, vii, 2-methoxyethanol / 4-methoxybenzylamine.

2-Methyl-3-nitrobenzoic acid **(109)** was converted to its methyl ester **(110)** using thionyl chloride and methanol. Compound **(110)** was condensed with dimethylformamide dimethylacetal in boiling DMF. Evaporation gave crude **(111)**.

In the ^{1}H NMR, the $N(CH_3)_2$ was observed as a singlet at δ2.83. The two alkenic protons were each observed as doublets at δ6.34 and δ5.66. The alkene proton adjacent to the aromatic ring was observed further upfield than the adjacent alkenic proton because the enamine electrons push into this proton. The alkene is shown to exist as the *E*-stereoisomer because the coupling constant (J = 13.6 Hz) is typical of a *trans* isomer.

Cyclisation of the enamine **(111)** on silica gave compound **(112)** in 39% yield. Further elution gave **(113)** in 10% yield. Initially, the enamine tautomerises to give the iminium compound. Nucleophilic oxygen of water attacks at the electropositive carbon of the iminium and pushes electrons into the electropositive nitrogen to give a hemiaminal type compound. Proton transfer and delocalisation of the hydroxy group results in the aldehyde. Tautomerism to the enol, followed by transesterification / cyclisation gave 5-nitroisocoumarin. However, in the case of compound **(113)** it is thought that the nucleophilic oxygen of methanol attacks at the aldehyde electropositive carbon, resulting in the hemiacetal compound. Transesterification now gives the side product compound **(113)**.

In subsequent runs to compound **(112)**, the enamine **(111)** was left to cyclise overnight on silica. Column chromatography and recrystallisation gave **(112)** in 60% yield. In ^{1}H NMR, the 4-H proton was split into a double doublet by the adjacent 3-proton and the 8-proton. A small coupling constant of 0.5 Hz was evident between the 4 and 8 protons. Here a five-bond coupling is observed which is unusual; however, everything is in plane and the coupling is W-like. The 3-H was split into a doublet by the adjacent 4 proton and was observed at δ7.44. The 8-H was observed as a double double doublet due to *ortho* coupling being observed between the 7-proton, *meta* coupling being observed between the 6 proton and a five bond W-like coupling being observed between the 4 proton. The signal was observed further downfield at δ8.65, due to the deshielding effect of the adjacent carbonyl group.

Evidence for the formation of compound **(113)** was obtained by the presence of the OCH_3 group in ^{1}H NMR, which was observed as a singlet at δ3.54. The methylene protons at position 4 of the aromatic ring were observed as a doublet at δ3.59. The

methine proton was split into a triplet by the methylene protons and observed at δ5.52. The methylene and methine protons have a vicinal coupling constant of 3.3 Hz.

Generally, it is known that nitroaromatic compounds can be reduced by catalytic hydrogenation (palladium/carbon, hydrogen). Treatment of 5-nitroisocoumarin **(112)** with palladium/carbon and hydrogen for 6 hours gave 5-aminoisocoumarin **(114)** in 99% yield. However, in some runs, catalytic hydrogenation resulted in over reduction. This was due to exposure of 5-nitroisocoumarin **(112)** to hydrogen for a longer period of time (12 h), which consequently reduced the double bond of the isocoumarin ring to give 5-amino-3,4-dihydroisocoumarin **(115)** in 91% yield. However, Bellamy and Ou[198] reported the use of an alternative reducing agent (tin(II) chloride) which was selective and would not reduce the double bonds. Although reduction was achieved, compound **(114)** was obtained in a lower yield of 77%. Evidence for the formation of compound **(114)** was provided by ^{1}H NMR whereby a broad singlet was observed at δ3.90 corresponding to the amino group. Furthermore, the 6-proton adjacent to the amino group becomes more upfield due to the shielding effect of the amino group in comparison to 5-nitroisocoumarin **(112)** where the 6-proton was deshielded and was evident at δ8.30 due to the adjacent electron-withdrawing nitro group. In the case of compound **(115)** the 3-H_2 and 4-H_2 were each observed as triplets at δ4.46 and δ2.74.

Diazotisation of compound **(114)** with aqueous sulfuric acid, sodium nitrite, followed by treatment with copper bromide and potassium bromide gave 5-bromoisocoumarin **(116)** in 35% yield. The aromatic amine is thought to undergo reaction with cold nitrous acid in sufuric acid solution to give the aryl diazonium sulfate species. The nitrous acid is generated in *situ* by reaction of the sodium nitrite with the acid. The resulting diazonium salt is very reactive and can be displaced by nucleophilic bromide ions. Such substitution reactions are thought to proceed *via* an aryl cation typically through an S_N1 mechanism. This is known as the Sandmeyer reaction. However, it has also been reported that some reactions proceed *via* a free-radical mechanism[199]. In ^{1}H NMR the disappearance of the broad NH_2 peak provided evidence for the formation of compound **(116)**. Furthermore, the isocoumarin 6-H

was shifted further downfield due to the negative inductive effect of the bromide substituent and consequently the signal is evident at δ7.95. MS data provided further evidence for the formation of this compound, whereby the molecular ion in the FAB^+ spectrum was evident at 227/225. 5-Bromoisocoumarin **(116)** was converted to 5-bromoisoquinolin-1-one **(108)** in a one-pot process by treatment with ammonia in boiling 2-methoxyethanol. Recrystallisation furnished compound **(108)** in 71% yield. The ^1H NMR spectrum was consistent with that reported in the literature[70], in which the N-H was observed at δ11.55 as a broad singlet integrating for 1 H.

Berry *et al*[70] reported that deprotonation of 5-bromoisoquinolin-1-one **(108)** with lithium bis(trimethylsilyl)amide, followed by alkylation with 1-chloromethyl-4-methoxybenzene gave 5-bromo-2-(4-methoxyphenylmethyl)isoquinolin-1-one **(117)**. In the present work, this compound was also formed by condensation of 5-bromo-isocoumarin **(116)** with 4-methoxybenzylamine, demonstrating the generality of this method of formation of isoquinolinones.

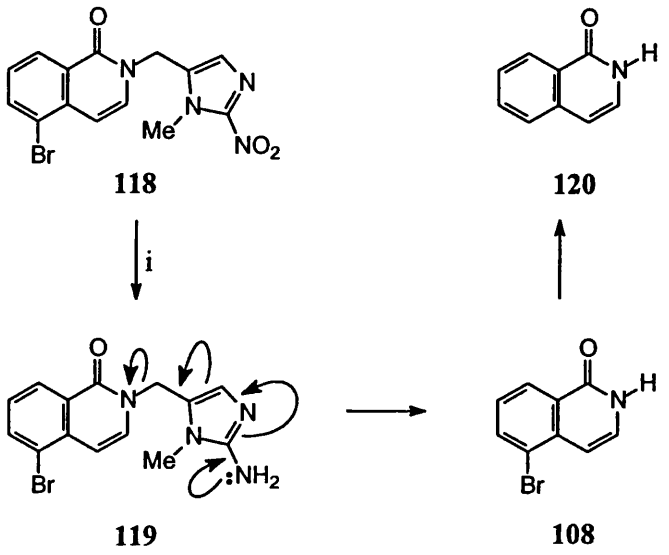
In the present work, 5-bromoisoquinolin-1-one **(108)** was deprotonated with lithium bis(trimethylsilyl)amide and the anion was allowed to react with 5-chloromethyl-1-methyl-2-nitroimidazole **(101)** to give the first target prodrug 5-bromo-2-((1-methyl-2-nitroimidazol-5-yl)methyl)isoquinolin-1-one **(118)** in 85% yield. In the ^1H NMR the spectrum, the CH_2 peak was shifted slightly further downfield at δ5.23 (previously observed at δ4.55 in **(101)** due to the deshielding effect of the isoquinoline ring nitrogen. The 4-H was observed as a double doublet at δ6.93 due to coupling being observed between the 4- and 8-protons. The isoquinoline 3-H was split into a doublet by the adjacent 4-H and was observed at δ7.14. The imidazole 4-H was evident as a singlet at δ7.23. The 7-H was split into a triplet by the 6- and 8-protons and was observed at δ7.38. The 6-H was evident as a double doublet at δ7.93 due to coupling being observed between the adjacent *ortho* 7-H and the *meta* 8-H. The 8-H was split into a double double doublet by the adjacent *ortho* 7-H, the *meta* 6-H and the *para* 4-H and was observed at δ8.39.

Similarly, the anion derived from 5-bromoisoquinolin-1-one **(108)** was treated with 5-bromomethyl-1-methyl-2-nitroimidazole **(102)**. The target prodrug **(118)** was obtained in a lower yield of 65%.

The routes described above allow the synthesis of **(118)** in excellent overall yields through convergent sequences. Interestingly, the corresponding nitrofuranylmethyl-isoquinolinone reported by Berry *et al*[70] had to be assembled stepwise, with alkylation of isoquinoline-1-one with chloromethylfuran, followed by a difficult regioselective nitration.

6.3 REDUCTIVELY TRIGGERED RELEASE FROM PRODRUG (118)

A number of experiments were conducted to reduce the nitroimidazole prodrug **(118)** to the aminoimidazole **(119)**, to study the release of the potent PARP inhibitor 5-bromoisoquinolin-1-one **(108)**. The mechanism by which this is predicted to occur is outlined below.



Scheme 7. Reductively activated release of 5-bromoisoquinolin-1-one **(108)**. i, NH_4Cl / MeOH / H_2O / Zn dust or $NaBH_4$ / Pd/C / Pr^iOH / H_2O.

There are several ways in which a nitro group can be reduced selectively to the amino compound[70,71,197,198,200]. Everett *et al*[71] demonstrated the use of γ-radiolysis and pulse radiolysis which selectively reduce nitro compounds to nitro radical anions. Other methods include the use of hydrogen and catalyst. However, such agents are not particularly selective and are known to cleave at benzylic positions, such as the imidazole-CH_2-N unit. However, tin (II) chloride and titanium trichloride have been reported to be selective for nitro compounds[70,197,198]. In addition, sodium borohydride and palladium / carbon have also been reported to be selective for aromatic compounds. Berry *et al*[70] reported the reduction of nitrofuranylmethylderivatives using a mild method for selective chemical reduction of the nitro group. Sodium borohydride in the presence of palladium / carbon and tin (II) chloride reducing systems were reported to selectively release the drug through

the intermediate aminofuran. Reduction of the nitro of the prodrug 5-nitrofuran-2-ylmethyl *N*-[3-(1,2-dicarba-*closo*-dodecarboran-(12)-yl)propyl]carbamate was selectively reduced by tin (II) chloride; carboranylpropylamine was obtained in 40% yield. As these systems proved successful and selectively reduced the nitro group of the nitrofuran moiety, these systems were investigated for the selective reduction of the nitro of prodrug **(118)**.

Treatment of the prodrug **(118)** in aqueous propan-2-ol with excess sodium borohydride and palladium on carbon resulted in the complete consumption of the prodrug within 10 min, as demonstrated by HPLC. A peak representing the 5-bromoisoquinolin-1-one was observed at a retention time of 5.5 min. However, in addition, two further peaks were observed with retention times of 4.5 min and 4.9 min. Control experiments were carried out, in which 5-bromoisoquinolin-1-one in propan-2-ol was treated with sodium borohydride / palladium / carbon system. From HPLC analysis it appeared that the peak corresponding to the 5-bromoisoquinolin-1-one had been completely converted to the peak exhibiting a retention time of 4.9 min. This suggested that this compound was, in fact, a product of the delivered drug. Analysis of this compound by HPLC, ^{1}H NMR, and MS provided evidence that this compound was the 5-unsubstituted isoquinoline-1-one compound **(120)**. This suggested that hydrogenolysis of the aryl bromide was taking place with the palladium / carbon and sodium borohydride reducing agents. These reducing systems were reported to be selective to nitro compounds and hydrogenolysis with such reagents has not previously been reported. However, recently Madani[201] has shown reduction of an alkyne with this reagent system. This, therefore, demonstrates that the reported reductants are not as selective as originally reported. Evidence for the formation of **(120)** was provided by ^{1}H NMR whereby; the 5-H was observed as a multiplet at δ7.48. The 6- and 7-protons were also observed as a multiplet at δ7.68.

Treatment of the prodrug **(118)** with palladium / carbon in aqueous propan-2-ol also afforded the debrominated product. This may have been due to hydrogen being adsorbed onto the metal surface of the compound during manufacture. Other possibility by which it is thought that the 5-unsubstituted isoquinoline-1-one **(120)** may have formed, is that either the 5-bromoisoquinolin-1-one **(108)** was initially released from the prodrug and further degradation of **(108)** gave the unsubstituted

analogue **(120)**, or that initially the bromine of the prodrug **(118)** was cleaved, and then releases the unsubstituted analogue **(120)**.

The nitro group of the prodrug **(118)** was also reduced by treatment with tin (II) chloride. From HPLC analysis it appeared that the nitro group of the prodrug appeared to be reduced completely in less than 5 min. Following reaction times of 1 h and 2 h, a number of peaks were observed. However, no peak was observed for 5-bromoisoquinolin-1-one. Therefore, a control experiment was carried out in which 5-bromoisoquinolin-1-one was treated with tin (II) chloride. Analysis of HPLC data indicated that the tin (II) chloride had no effect on the drug. These results suggested that the peaks observed may have been due to the amino or intermediate hydroxylaminoimidazole species. It was thought that failure of tin (II) chloride to release the drug **(108)** may have been due to the tin complexing as a Lewis acid to the amine which made it no longer an electron-donor.

Other reducing systems, which were considered were sodium dithionite and diisobutylaluminium hydride (DIBAL). However, treatment of the prodrug with sodium dithionite failed to release the drug. It was thought that the reducing agent may have reacted with the compound as the prodrug was consumed but the products could not be identified. In the case of DIBAL, solubility problems of the prodrug in toluene prevented the use of this reagent.

Varghese and Whitmore[202] demonstrated selective reduction of the nitro group in misonidazole by treatment with zinc dust in aqueous ammonium chloride. This system was, therefore, thought appropriate for reduction of the nitro group. Initially, control experiments were carried out with the prodrug **(118)**, 5-bromoisoquinolin-1-one **(108)**, 1-methyl-2-nitro-5-hydroxymethylimidazole **(94)** and both zinc and ammonium chloride individually. The results indicated that none of the compounds were affected by either of the reagents alone. However, treatment of the prodrug with both zinc and ammonium chloride in propan-2-ol afforded the slow release of 5-bromoisoquinolin-1-one. In addition, a small peak for the 5-unsubstituted isoquinolin-1-one was observed. A small number of other peaks were also evident in the chromatogram, which were thought to be the reduction products of the nitroimidazole species.

It was noted that under these reducing conditions the prodrug was completely consumed after 20 min. At this stage of the reaction, only a small peak was observed for the delivered drug 5-bromoisoquinolin-1-one. However, at shorter time periods, major peaks were observed in the HPLC chromatogram. These were thought to be the reduction products of the nitro group of the prodrug.

After a period of 1 h, the peak corresponding to 5-bromoisoquinolin-1-one had increased significantly. After 1 and 2 days, the 5-bromoisoquinolin-1-one **(108)** and the 5-unsubstituted isoquinolinone **(120)** peaks were strongly present in addition to peaks corresponding to the reduced nitroimidazole species. This is represented by the chromatogram below.

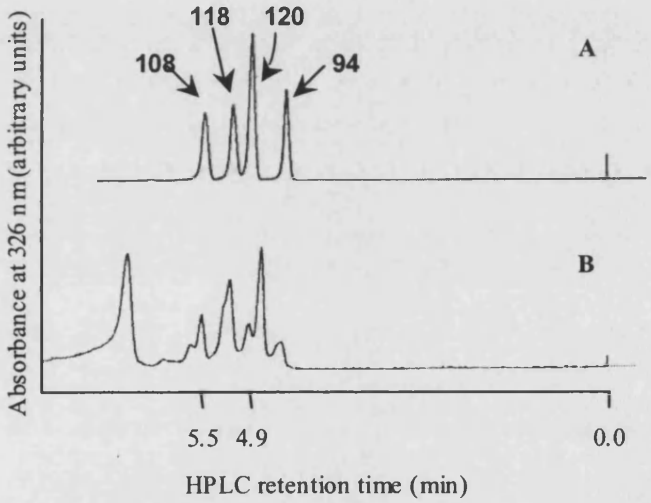
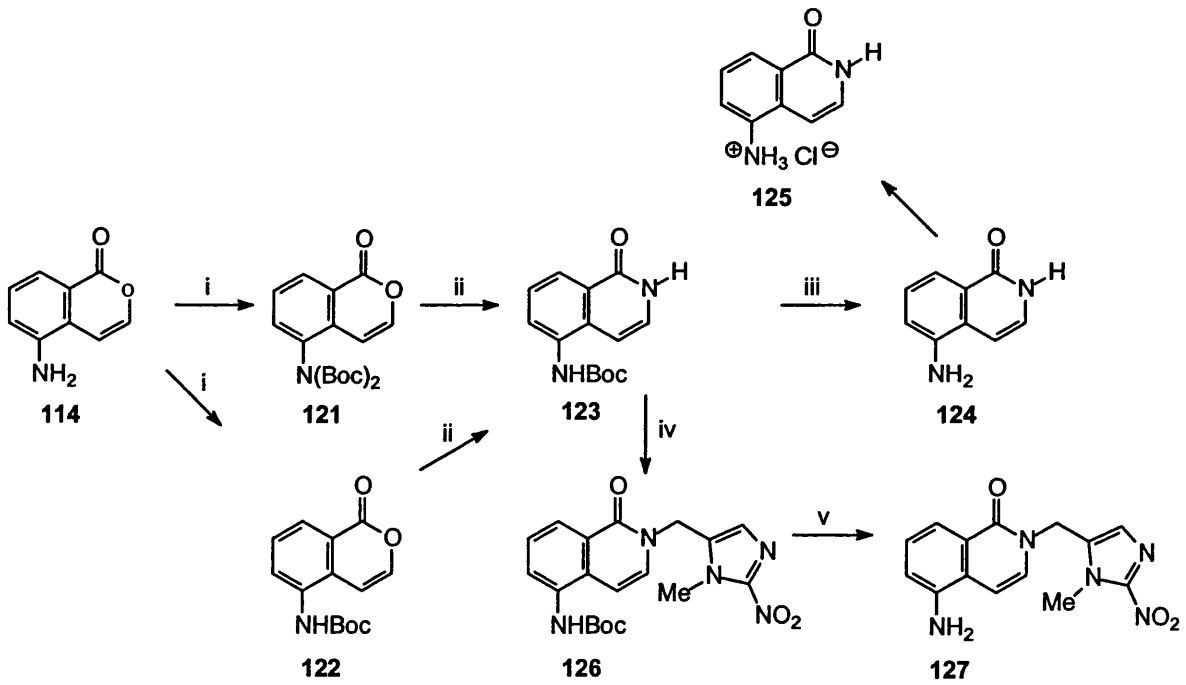


Figure 8. Typical HPLC chromatograms from the reductively triggered drug release study. **A**. Synthetic standards; **B**. **118** + Zn / aq. NH_4Cl (24 h).

6.4 SYNTHESIS OF PRODRUG (127)

McDonald *et al*[132] recently reported that 5-aminoisoquinolin-1-one **(124)** (previously shown to be a potent PARP inhibitor by Suto *et al*[164]) was successful in abolishing the multiple organ injury caused by severe haemorrhage and resuscitation. However, even more recently, they demonstrated[203,204] that inhibition of PARP by 5-aminoisoquinolin-1-one reduces myocardial infarct size in rats. Furthermore, **(124)** has been shown to be effective in the kidney[205]. As 5-aminoisoquinolin-1-one was proving to be extremely useful as a PARP inhibitor, this was the next drug that would be considered in the attachment to 5-hydroxymethyl-1-methyl-2-nitroimidazole trigger moiety. The synthetic route employed for this compound is outlined below.



Scheme 8. Synthesis of 5-amino-2-((1-methyl-2-nitroimidazol-5-yl)methyl)isoquinolin-1-one. *Reagents:* i, $CHCl_3$ / di-*t*-butyl dicarbonate, ii, 2-methoxyethanol / NH_3, iii, TFA / $CHCl_3$ / HCl, iv, dry DMF / lithium bistrimethylsilylamide / 1-methyl-2-nitro-5-chloromethylimidaole / NaI, v, TFA.

5-Aminoisocoumarin **(114)** was treated with 3.0 equivalents of di-*t*-butyl dicarbonate in the presence of base for 4 d. Interestingly, these conditions led to the introduction of two Boc groups on the exocyclic nitrogen. Compound **(121)** was obtained in 58%

yield. The ^{1}H NMR spectrum was consistent with this structure in that the two *t*-butyl groups were observed at δ1.83 as a singlet.

When 5-aminoisocoumarin **(114)** was treated with a lower excess of di-*t*-butyl dicarbonate (1.5 equivalents) for 4 d, it gave the mono-Boc compound **(122)** in 56% yield. Now the ^{1}H NMR spectrum showed only one *t*-butyl group at δ1.45. Furthermore, the NH was observed at δ6.50 as a broad singlet. The mass spectrum gave a molecular ion at 262, some 100 Da less than that of **(121)**.

For the synthesis of 5-aminoisoquinolin-1-one **(123)**, it was not necessary which route was taken in order to obtain the desired compound. Both compounds **(121)** and **(122)** were converted to the isoquinolinone compound **(123)** following treatment with 2-methoxyethanol and ammonia. Both procedures afforded **(123)** in a yield of 71% and 76%, respectively. In ^{1}H NMR the presence of the NH next to the Boc group was evident as a broad singlet at δ6.61, and the NH adjacent to the carbonyl was observed as a broad singlet at δ10.60.

Deprotection of **(123)** with trifluoroacetic acid followed by treatment with chloroform and HCl gave 5-aminoisoquinolin-1-one hydrochloride **(125)** in 79% yield. Evidence for the formation of **(125)** was provided by ^{1}H NMR whereby a broad singlet was observed at δ4.02 corresponding to the three amino protons of the salt.

Having achieved the key intermediates **(123)** for the second target, alkylation of **(123)** at the heterocyclic nitrogen of the isoquinoline ring was achieved following treatment with base and **(101)**. Although there was a possibility that the imidazole trigger could attach at the NH adjacent to the Boc group, it was thought that steric hindrance would prevent this. 5-(1,1-Dimethylethoxycarbonylamino)-2-((1-methyl-2-nitroimidazol-5-yl)methyl)isoquinolin-1-one **(126)** was obtained in 54% yield. In the ^{1}H NMR spectrum the *t*-Bu group was observed as a singlet at δ1.53. The NCH_3 of the imidazole was observed as a singlet at δ4.05. The CH_2 attached to the N-2 of the isoquinoline ring was observed as a singlet at δ5.29 further downfield than when

it was attached to the chloro group δ4.63 in **(101)**. This was due to the deshielding effect of the heteroaromatic ring nitrogen.

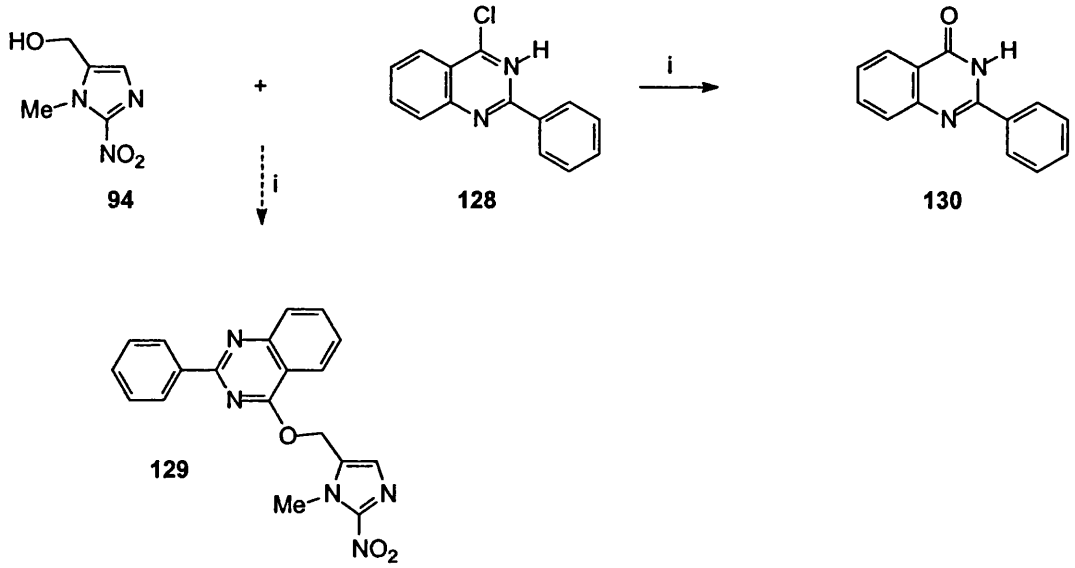
Removal of the Boc group was achieved by treatment with trifluoroacetic acid. Extraction of the free base gave compound **(127)** in 80% yield. Evidence for the formation of **(127)** was provided by ^{1}H NMR, whereby the NH_2 was evident as a broad singlet at δ1.58. Furthermore, the peak for the Boc group that was previously present at δ1.53 was no longer evident.

5-Aminoisoquinolin-1-one is a potent PARP inhibitor shown to be useful in haemorrhagic shock and renal dysfunctions[132,203-205]. This was attached to the 2-nitroimadzole trigger **(101)** with the aim that when reductively activated it will release the potent PARP inhibitor 5-aminoisoquinolin-1-one selectively in the hypoxic tumour tissue. Incorporation of the 5-aminoisoquinolin-1-one drug furthermore enhanced the aqueous solubility of the prodrug, which is essential in hypoxic tumour tissues because of the poorly vascularised blood capillary network. Compound **(127)** will be evaluated *in vitro* and *in vivo* as a prodrug of 5-aminoisoquinolin-1-one.

6.5 ATTEMPTED SYNTHESIS OF PRODRUG (129)

Several authors have reported by using a variety of models and *ab initio* calculations that the carboxamide moiety in the *anti* conformation is essential for PARP inhibitory activity and furthermore the carboxamide can be restricted into the *anti* conformation in heterocyclic aromatic compounds. One group of heteroaromatic compounds that are currently of interest are the quinazolin-4-ones. Griffin *et al*[146] recently reported the synthesis of a number of quinazolinones and found them to be extremely potent PARP inhibitors. Of this series, 8-hydroxy-2-methylquinazolin-4-one exhibited an IC_{50} of 0.4 μM and was found to enhance the cytotoxicity of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC, a metabolite of dacabazine and of temozolomide) and γ-radiation 3.5- and 1.4-fold, respectively, against L1210 cells.

Miyashita *et al*[206] reported the synthesis of 4-(arylmethyloxy)quinazolines using 4-chloroquinazoline and an arylmethanol. Formation of the arylquinazolines was thought to involve a nucleophilic substitution reaction between the aryl methoxide ion and 4-chloroquinazoline. Although the alcohol used by Miyashita *et al*[206] was different to that used in the present work, it was proposed that treatment of 4-chloro-2-phenylquinazoline **(128)** with 5-hydroxymethyl-1-methyl-2-nitroimidazole **(84)** may give prodrug **(129)**. The reaction mechanism was thought to proceed *via* a nucleophilic substitution reaction between the aryl methoxide ion of **(84)** and 2-phenylquinazoline **(128)**, linking the 2-phenylquinazoline to the nitroimidazole *via* an ether linkage.



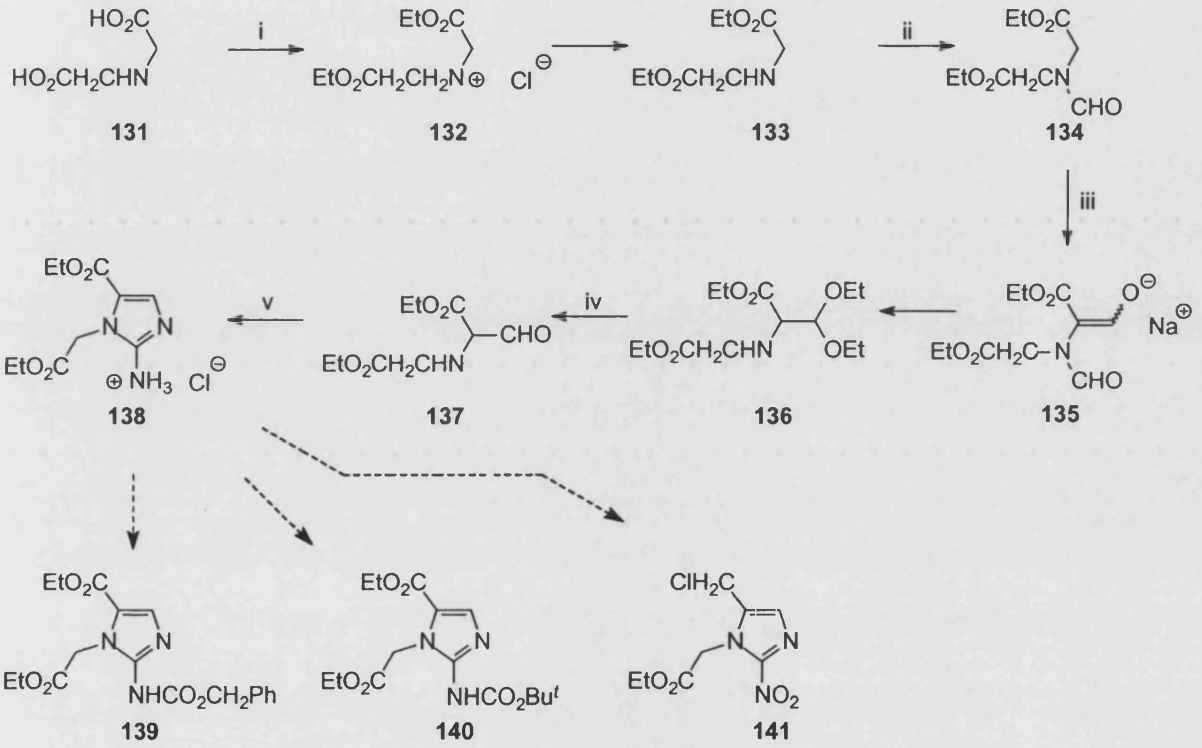
Scheme 9. Synthesis of quinazolin-1-one. *Reagents:* i, NaH.

Attempts to synthesise prodrug **(129)** by treatment of 4-chloro-2-phenylquinazoline **(128)** with 1-methyl-2-nitro-5-hydroxymethylimidazole **(94)** in the presence of base (sodium hydride) failed. However, ^{1}H NMR showed that 2-phenylquinazolin-1-one **(130)** had formed *via* a hydrolysis reaction. Although, the reaction was performed under dry conditions and under argon it was thought that maybe the starting quinazoline **(128)** was wet. However, repetition of this reaction with dry quinazoline also failed and again **(130)** was observed in ^{1}H NMR. Interestingly, the starting nitroimidazole compound **(94)** was absent in the ^{1}H NMR spectrum, suggesting that it may have decomposed under the reaction conditions.

Treatment of the quinazoline **(128)** with the 2-nitroimidazole **(94)** in the presence of lithium bis(trimethysilyl)amide also failed, as ^{1}H NMR indicated only the presence of 2-phenylquinazolin-1-one. Evidence for the formation of **(130)** was provided by ^{1}H NMR, whereby the NH of the quinazolinone ring was observed at δ10.20 as a broad singlet. No peaks corresponding to the starting nitroimidazole compound **(94)** were evident in ^{1}H NMR.

6.6 ANALOGUES OF PRODRUG (118)

In the first target, the N^1 nitrogen of the imidazole was masked with a simple methyl group. However, in this advanced target, the nitrogen substituent was modified to incorporate a methyl ester to provide a site for elaboration to enhance aqueous solubility of the compound. The synthetic route employed for the analogue **(141)** is outlined below.



Scheme 10. Attempted synthesis of analogue **(141)**. *Reagents:* i, EtOH / $SOCl_2$, ii, Ac_2O / NaO_2CH, iii, NaOEt / EtOCHO, iv, aq. HCl, v, cyanamide.

Iminodiacetic acid **(131)** was converted to its diethyl ester **(132)** quantitatively by treatment with thionyl chloride and ethanol. The nitrogen of compound **(132)** was protected as the formamide, as before, using acetic formic anhydride, to give diethyl N-formyliminodiacetate **(134)** in 86% yield.

The methylene of compound **(134)** was deprotonated with sodium ethoxide and the anion reacted with ethyl formate to give the sodium enolate salt **(135)**. The aldehyde was protected as the diethyl acetal by prolonged treatment at reflux with boiling

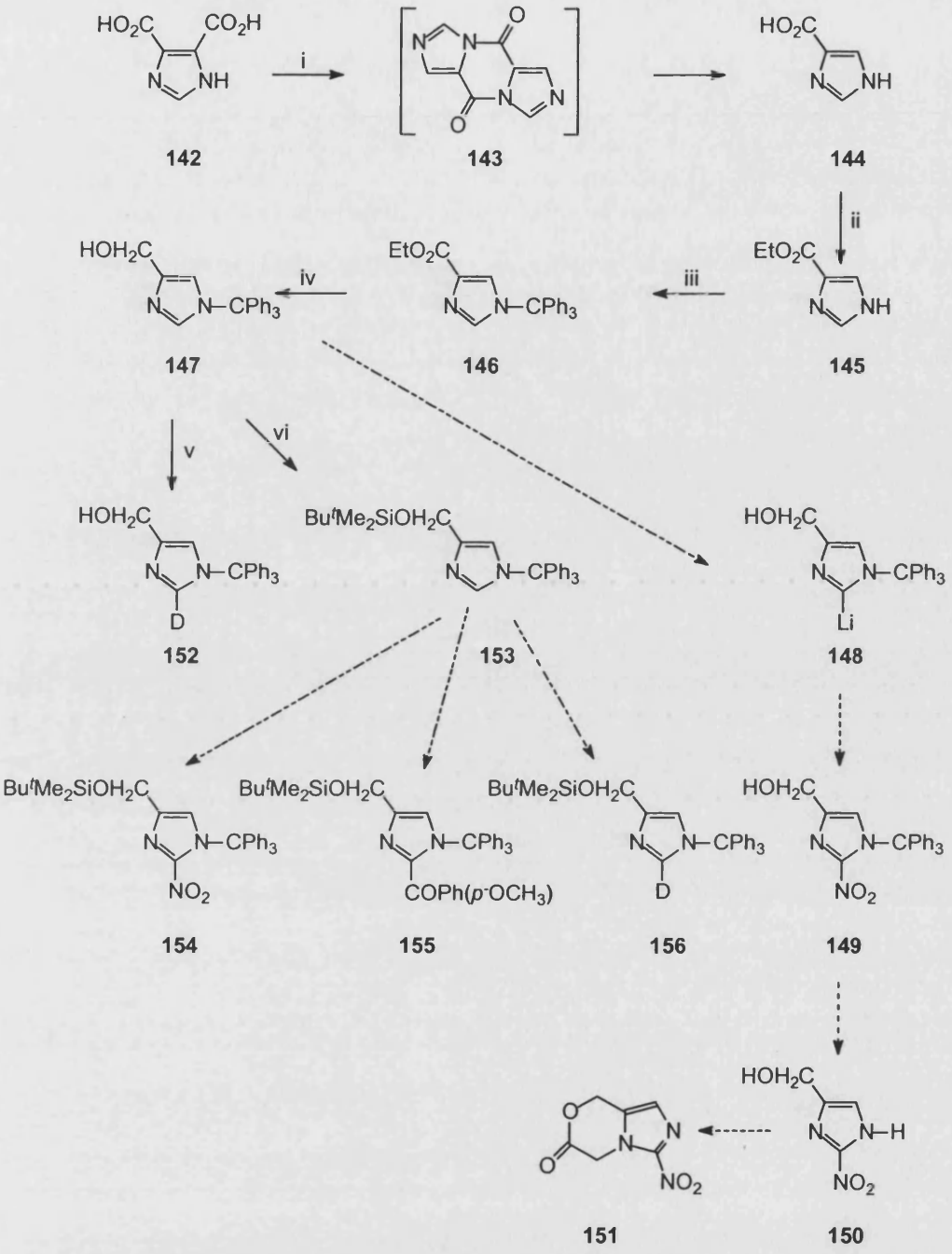
ethanol saturated with HCl. The acetal **(136)** was obtained in 62% yield. In the ^1H NMR spectrum the ethyl ether groups are magnetically nonequivalent, with the methyl resonances at δ1.17 and δ1.20. The ester CH_3 groups were observed slightly further downfield at δ1.24 and δ1.27. The protons of the CH_2 of the ether groups show geminal coupling as well as vicinal couplings, demonstrating that the two protons of these methylenes are also magnetically inequivalent. The CH_2 protons were observed as double quartets at δ3.54, δ3.55, δ3.71 and δ3.72. The CH_2 of the $CHCO_2CH_2$ group also showed both geminal and vicinal couplings and were each observed as double quartets at δ4.12 and δ4.19. The CH_2 of the other ester $CH_2CO_2CH_2CH_3$ was split into a quartet by the adjacent CH_3 group and was observed at δ4.15. Geminal couplings were also observed for the inequivalent NCH_2 protons, which were observed as doublets at δ3.39 and δ3.47.

The aldehyde was unmasked by treatment with aqueous hydrochloric acid and the aminoimidazole **(138)** was formed by condensation with cyanamide. However, it was not possible to isolate compound **(138)**. Attempted recrystallisation from propan-2-ol gave a material identifiable as a trans esterification product. However, accurate mass spectrum data of the crude material confirmed the presence of compound **(138)** as a peak of molecular weight 242 corresponded to the molecular ion of **(138)**.

Since the aminoimidazole may be highly polar and / or unstable under the isolation conditions, attempts were made to mask the exocyclic amine. The crude mixture was treated with benzyl chloroformate and, separately, with di-*t*-butyl dicarbonate. TLC indicated that the protected compounds **(139)** and **(140)** had formed but they were unstable to silica during column chromatography.

6.7 OTHER ROUTES TO 1,5-DISUBSTITUTED-2-NITROIMIDAZOLES

Davis *et al*[207] reported a novel synthetic strategy for 1-unsubstituted-2-nitroimidazoles. A synthetic route to the key compound 2-nitroimidazole-4-methanol **(150)** involved 8 steps. Although there may be a problem by attaching the alkylating agent to the hydroxy group, it can be directed towards the adjacent nitrogen. This was predicted to occur by the selective functionalisation of **(150)** at the N-1 position, which would give access to the bioreducible masking agent **(151)**. The scheme adapted for the synthesis of compound **(150)** is outlined overleaf.



Scheme 11. Attempted synthesis of 2-nitroimidazole-5-methanol. *Reagents:* i, Ac_2O / heat / H_2O, ii, EtOH / $SOCl_2$ or EtOH / H_2SO_4, iii, NEt_3 / chlorotriphenylmethane, iv, $LiAlH_4$, v, DMF / TBDMSCl, imidazole, vi, 1,2-dimethoxyethane / BuLi / D_2O.

The strategy towards the 1-unsubstituted analogue **(150)** was markedly different to that for 1,5-disubstituted-2-nitroimidazoles. Imidazole-4,5-dicarboxylic acid **(142)**

was decarboxylated by heating with acetic anhydride and water to give the intermediate tricycle **(143)**. This was hydrolysed with water to give imidazole-4-carboxylic acid **(144)** in 49% yield. ^{1}H NMR showed a single peak at δ7.70 (integrating for 1-H) and a single peak at δ8.50 (integrating for 1-H) corresponding to the 5-H and 2-H, respectively, of the imidazole ring. Esterification of compound **(144)** was achieved by treatment with ethanol and thionyl chloride or with ethanol and concentrated H_2SO_4 to give ethyl imidazole-4-carboxylate.

The imidazole N-H was masked prior to the critical lithiation at the 2-position of the ring; this was achieved by tritylating the less sterically hindered nitrogen to yield ethyl 1-tritylimidazole-4-carboxylate **(146)**. Reduction of compound **(146)** with lithium aluminium hydride gave 1-tritylimidazole-4-methanol **(147)** in 93% yield.

Davis *et al*[207] reported that treatment of compound **(147)** with two equivalents of butyllithium in tetrahydrofuran at -78°C formed the alkoxide in the side chain and introduced lithium at position 2 of the imidazole ring. Furthermore, it was reported that quenching with propyl nitrate and detritylation gave the nitroimidaole **(150)** in 29% yield.

It was known that the formed anion was easily quenched by moisture, therefore the reaction was carried out under nitrogen or argon. As the nitration step was unsuccessful, a number of reasons for failure were considered. It was noted that butyllithium may be decomposing in tetrahydrofuran, hence the solvent 1,2-dimethoxyethane was used.

Due to the problems encountered with the lithiation of compound **(147)**, it was proposed that masking of the hydroxy group at position 4 of the ring by silylation may enhance lithiation at the C-2 position. A procedure for the silylation step reported by Bond *et al*[208] was followed and compound **(153)** was obtained in 99% yield. However, attempts to nitrate compound **(153)** by treatment with butyllithium and propyl nitrate failed, as only starting materials were recovered as shown in ^{1}H NMR.

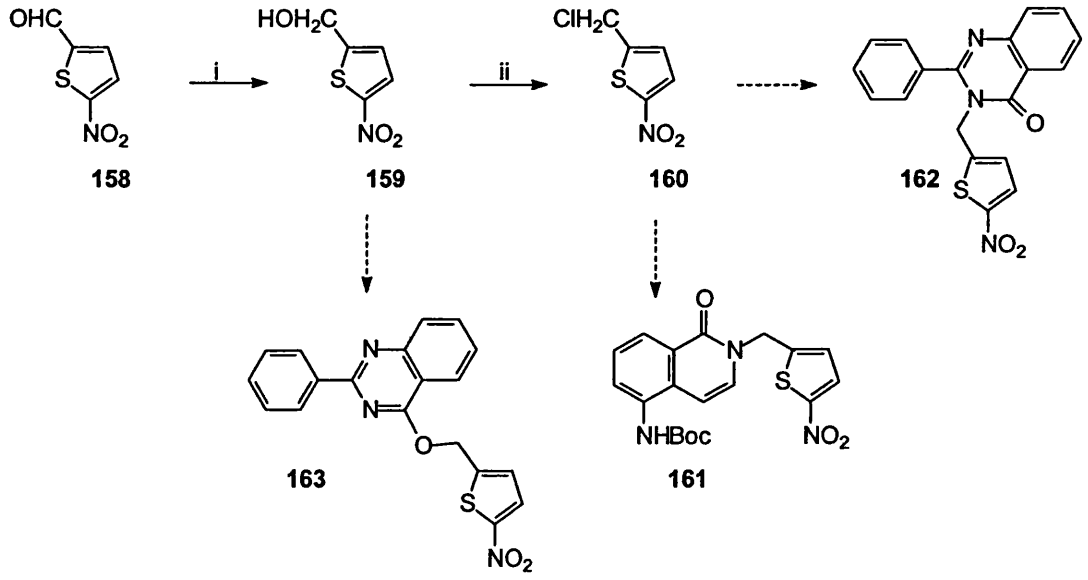
Attempts were carried out to lithiate the silylated imidazole **(153)** with butyllithium at -79°C, followed by quenching with various agents, including the electrophile 4-methoxybenzaldehyde and D_2O, to give compounds **(155)** and **(156)**. However, the reactions failed and only starting materials were evident in the ^1H NMR spectra.

Most lithiation reactions take place at low temperature conditions (-79°C) and Davis *et al*[207] reported that the lithiation of the imidazole **(147)** was achieved at -79°C. In contrast to these conditions, Bond *et al*[208] reported that lithiation at the 2-position of the imidazole ring required heating at 40°C for 4 h. Failure to achieve lithiation at -79°C led to the employment of the reaction conditions reported by Bond *et al*[208], even though Bond nitrated different nitroimidazole compounds to those in the present work. Consequently, the reaction mixture containing compound **(147)**, 1,2-dimethoxyethane and butyllithium was heated to 45°C for 4 h under argon. Quenching with D_2O gave crude **(152)**. The ^1H NMR spectrum indicated that the intensity of the proton at δ7.30 corresponding to the 2-H proton of the imidazole ring had decreased by half, indicating that at least 50% lithiation had taken place. Accurate MS data confirmed that compound **(152)** was present, as a molecular ion at 341 was evident.

Although the lithiation reaction reported by Davis *et al*[207] used mild ambient conditions, it was found during these studies that harsh conditions were required to lithiate at the 2- position of the imidazole ring. This was demonstrated in the reaction between the imidazole **(147)** and butyllithium in which the reaction mixture was heated at 45°C for 4 h. Quenching with D_2O gave crude **(152)**. ^1H NMR spectrum indicated that at least 50% lithiation had taken place as the intensity of the 2-proton had decreased.

6.8 CRITICAL 2-NITROTHIOPHENE INTERMEDIATES

Recently, there has been widespread interest in the use of nitrothiophene type compounds as radiosensitisers, bioreductively activated cytotoxins and inhibitors of PARP[18,168]. A number of authors[18,168] have previously reported the synthesis of a variety of nitrothiophene compounds with various allkylating agents that have been shown to be effective as radiosensitisers. Therefore, the next synthetic target was the nitrothienylmethylisoquinolin-1-one **(161)**. This consists of the 2-nitrothiophene-5-ylmethyl trigger unit linked to the (masked) potent PARP inhibitor 5-aminoisoquinolin-1-one **(124)**. Attachment of 5-chloromethyl-2-nitrothiophene **(160)** to a quinazolin-1-one was also attempted to synthesise prodrug **(162)**. The route investigated for the synthesis of the prodrugs **(161)** and **(162)** is outlined below.



Scheme 12. Attempted synthesis of prodrugs **(161)**, **(162)** and **(163)**. *Reagents:* i, THF / $NaBH_4$, ii, $SOCl_2$.

2-Nitrothiophene-5-carboxaldehyde **(158)** is a readily available and cheap starting material and is the starting point in the synthesis of the key intermediate 5-chloromethyl-2-nitrothiophene. Reduction of compound **(158)** with sodium borohydride gave 5-hydroxymethyl-2-nitrothiophene **(159)** in excellent yield. Reaction of compound **(159)** by treatment with thionyl chloride gave 5-

chloromethyl-2-nitrothiophene **(160)** in very high yield. In the ^{1}H NMR spectrum, the CH_2 was split into a doublet by long-range coupling to the 4-H.

Alkylation of the isoquinolinone **(123)** was attempted with 5-chloromethyl-2-nitrothiophene **(160)** in dimethylformamide. As no reaction occurred at 20°C, the mixture was heated to 50°C for 12 h and boiled under reflux for 5 h. ^{1}H NMR analysis indicated only the presence of the starting material **(123)** and no peaks were evident for the nitrothiophene compound. This suggested that the nitrothiophene compound may have decomposed under the harsh conditions employed.

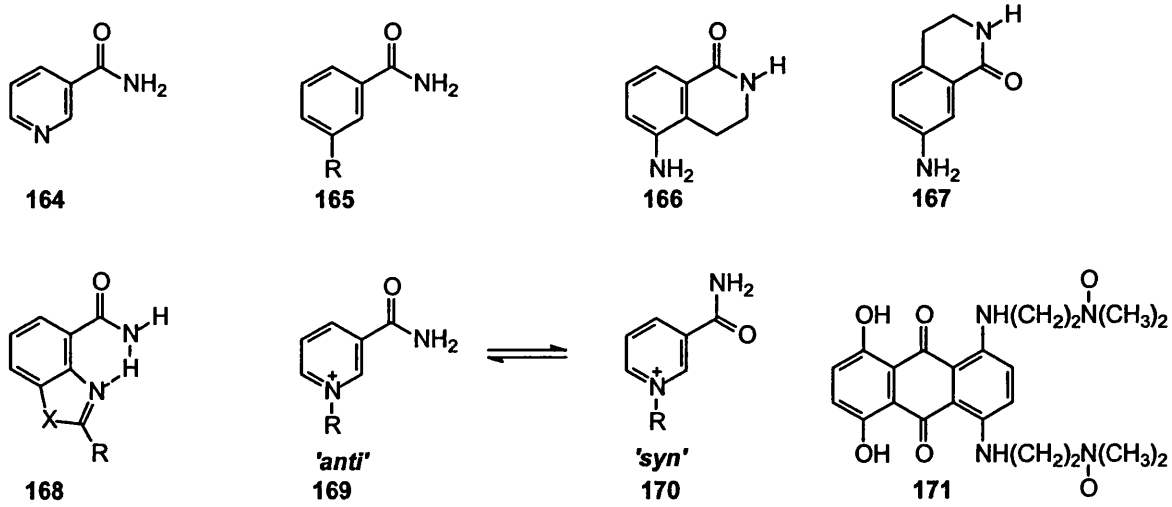
Synthesis of prodrug **(162)** was also attempted by treatment of 2-phenylquinazolin-1-one **(128)** with 5-chloromethyl-2-nitrothiophene **(160)** in the presence of lithium bis(trimethysilyl)amide. No reaction occurred at 20°C, consequently, the mixture was heated to 50°C for 48 h and at 70°C for 24 h. ^{1}H NMR only indicated the presence of quinazolin-1-one **(130)**.

As mentioned earlier, Miyashita *et al*[206] reported a synthetic route to 4-(arylmethyloxy)quinazolines using 4-chloroquinazoline and aryl methanol in the presence of base. Following the reported procedure, attempts were made in order to synthesise prodrug **(163)** using 4-chloro-2-phenylquinazoline **(128)** and 5-hydroxymethyl-2-nitrothiophene **(159)**. However, the reaction was unsuccessful and only quinazolin-1-one **(130)** was obtained *via* the hydrolysis reaction. In the ^{1}H NMR spectrum, no signals were evident for 5-hydroxymethyl-2-nitrothiophene suggesting that the compound had decomposed.

6.9 QUINOLINE-8-CARBOXAMIDES AND QUINOLINE N-OXIDES

As mentioned in the introductory chapter, N-oxides, such as SR 4233, have been shown to be bioreductive agents that exhibit high selective toxicity for hypoxic cells. These types of compound have an absolute requirement for one-electron reduction in order for toxicity to be expressed. The mechanism of action is thought to be different to that reported for the nitroaromatic compounds in that it is the N-oxide radical species that causes toxicity by means of reacting directly with cellular macromolecules. This is thought to be achieved by hydrogen being abstracted from bases in DNA, which results in extensive DNA single and double stranded breaks.

It was proposed that rather than having a nitroimidazole based prodrug, we can have an N-oxide type prodrug. Previous studies have shown for example, that SR4233 **(13)**[6,37] and AQ4N **(171)**[209] are bioreduced. As the N-oxides can be bioreduced, a prodrug was designed based on N-oxide bioreduction.



164 165 166 167 168 169 170 171

Sims *et al*[159] found that the carboxamide function was critical for PARP inhibition. Furthermore, it was shown that the carboxamide function could be incorporated into a heteroaromatic ring without affecting its activity against the enzyme.

Banasik *et al*[163] substantiated this evidence by synthesising compounds in which the carbamoyl substituent is incorporated within a ring system. This resulted in highly potent PARP inhibitors and 1,8-naphthalimide derivatives, 5-hydroxyisoquinolin-1-

one, phenanthridinone and quinazolin-4-one compounds were all found to be active. Based on the above and the fact that a carboxamide group in the benzamide and nicotinamide series is essential for activity, it was thought that such compounds were occupying the nicotinamide binding site of PARP and that the 3-substituent was incorporated in the ribose nucleoside-binding domain. Furthermore, it was observed in the benzamide series that the active compounds were those that contained a primary amide. This indicated that important hydrogen bond interactions were occurring with an amino acid donor within the enzyme's active site.

In fact, recent studies[104,149,150,166] have shed light onto the crystal structures of PD128763 and NU1025 bound to the catalytic domain of PARP. From the crystal structures it can be seen that all the inhibitors bind in the same 'anti' orientation and complexes are formed between the two hydrogen bonds occurring between the carbonyl and NH (or NH_2) groups and the peptide backbone of Gly-863. The third hydrogen bond is thought to occur between the carbonyl group of the inhibitor and the side chain of Ser-904.

Suto *et al*[164] demonstrated the 'anti' theory by using 2,3-disubstituted benzamides and 5-substituted dihydroisoquinolinone compounds. In the former case, the analogues were found to be completely inactive as the 'syn' conformation was adopted. However, in the case of dihydroisoquinolinones where the carboxamide was constrained in the 'anti' conformation, the compounds were found to be extremely potent.

Griffin *et al*[165] extended these studies to benzoxazole-4-carboxamide compounds. It was reported that intramolecular hydrogen bond interactions occurred between the amide NH and the oxazole nitrogen. This afforded a donor carbonyl group constrained in the biologically active 'anti' conformation. However, even more recently, White *et al*[150] extended these studies to the benzimidazole-4-carboxamides and found 2-phenyl-1H-benzimidazole-4-carboxamide (**65**, K_i 15nM) to be particularly active against PARP. A number of analogues were developed with various substituents on the phenyl ring. Many of them exhibited Ki values for PARP inhibitory activity <10 nM, with 2-(4-hydroxymethylphenyl)-1H-benzimidazole-4-carboxamide **(66)** exhibiting a K_i value of 1.6 nM and being the most potent inhibitor

of PARP reported to date. The concept was similar to that of the benzoxazole-4-carboxamide compounds in that the carboxamide group was restricted *via* an intramolecular hydrogen bond[150,164].

These studies demonstrate that the 'anti' / 'syn' conformation can be controlled by either constraining the carboxamide within a heteroaromatic ring system or by constraining the carboxamide *via* an intramolecular hydrogen bond. In both benzoxazole-4-carboxamides and benzimidazole-4-carboxamide the design is similar in that they consist of a 6 / 5 fused ring. However, the compounds that were synthesised in our laboratory were 6 / 6 fused rings. Based on the above studies, it was postulated that a quinoline-N-oxide compound would demonstrate this concept as it was thought that in the presence of the N-oxide group the carboxamide would be tipped out of plane where as in the absence of the oxygen the compound would be in plane. The carbonyl twisted out of plane destroys the pharmacophore and with appropriate substituents at the 2-position it was thought to keep the carbonyl in plane.

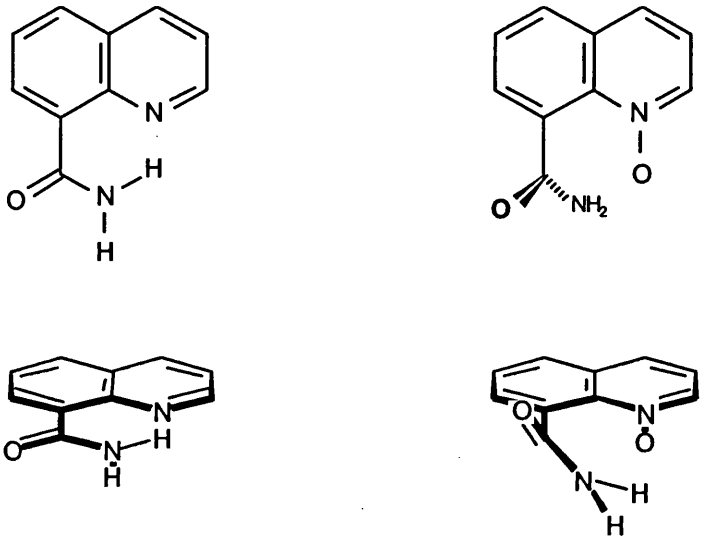
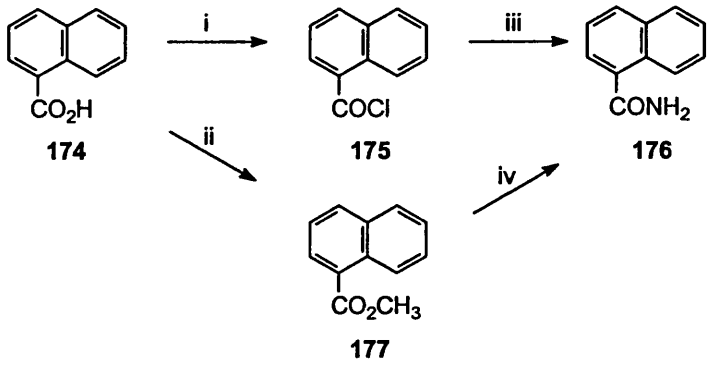


Figure 9. Demonstrates the structure of the carboxamide when it is 'in-plane' and when twisted 'out-of-plane'.

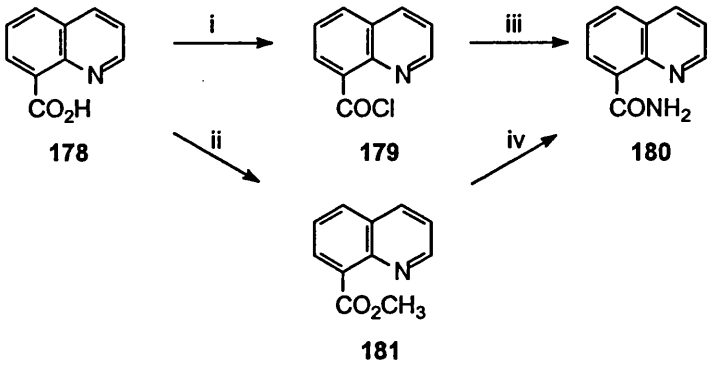
To establish whether this postulated H-bond is real and whether the substituents at the N-1 position would twist the carboxamides out of plane, a number of quinoline and quinoline N-oxide compounds were synthesised.

Naphthalene-1-carboxylic acid **(174)** was converted to its acid chloride **(175)** following treatment with thionyl chloride. Treatment of **(175)** with ammonia and chloroform gave naphthalene-1-carboxamide **(176)** in 87% yield. An alternative route for synthesis, involved initially converting the acid **(174)** to its methyl ester **(177)** by treatment with thionyl chloride and methanol. Treatment with methanol / conc. ammonia gave the carboxamide **(176)** in a lower yield of 53%.



Scheme 13. Synthesis of naphthalene-1-carboxamide. *Reagents:* i, $SOCl_2$, ii, MeOH / $SOCl_2$, iii, $CHCl_3$ / NH_3, iv, MeOH / conc. NH_3.

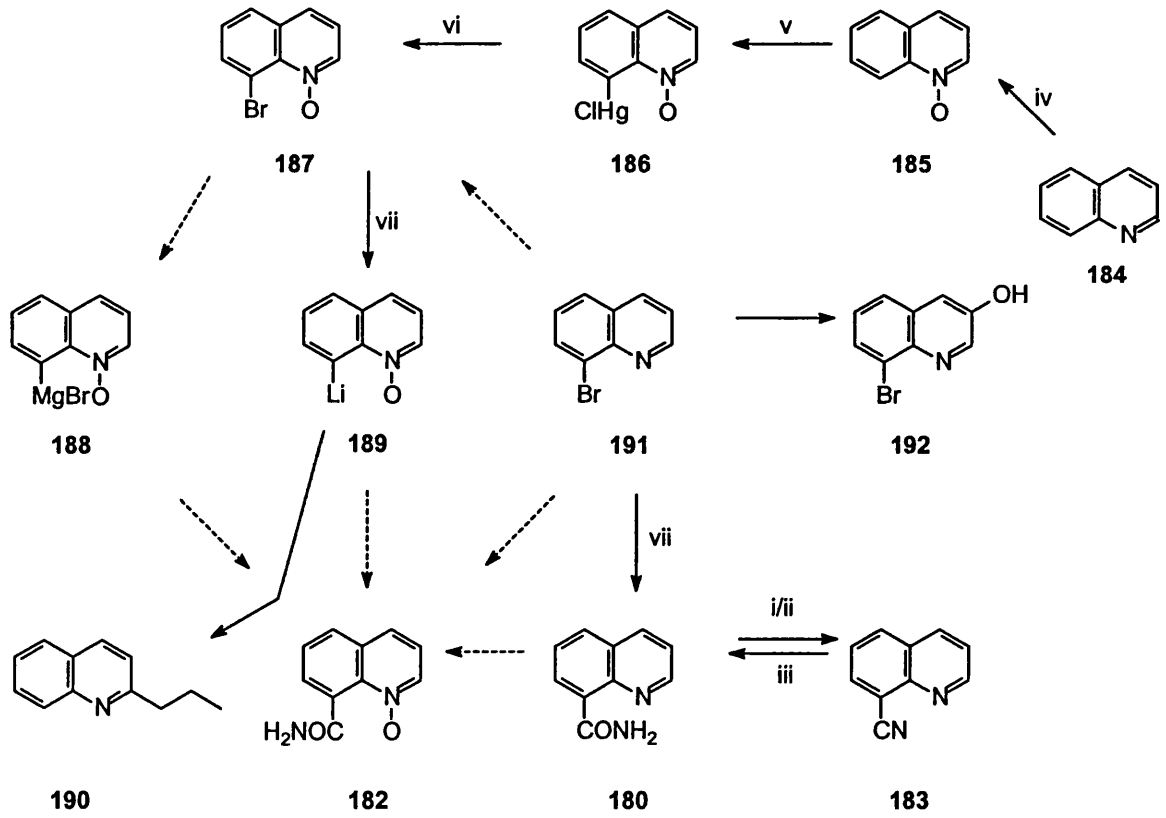
Quinoline-8-carboxylic acid **(178)** was converted to its acid chloride **(179)** by treatment with thionyl chloride. Treatment of the acid chloride **(179)** with chloroform and ammonia gave quinoline-8-carboxamide **(180)** in 90% yield. An alternative route for synthesis involved initially converting the acid **(178)** to its methyl ester **(181)** and subsequent reaction with ammonia to give the carboxamide **(180)** in a slightly lower yield.



Scheme 14. Synthesis of quinoline-8-carboxamide. *Reagents:* i, $SOCl_2$, ii, MeOH / $SOCl_2$, iii, $CHCl_3$ / NH_3, iv, MeOH / conc. NH_3.

In the ^1H NMR spectrum of naphthalene-8-carboxamide, one N-H was observed at δ7.56 and the other at δ7.99. The NH_2 signals are close together. However, in quinoline-8-carboxamide, one of the N-H signals was evident at δ6.04 whereas the other N-H was observed much further downfield at δ10.94. This study demonstrates that the carboxamide has a very restricted conformation and that the N-H at δ10.94 is tightly hydrogen-bonded.

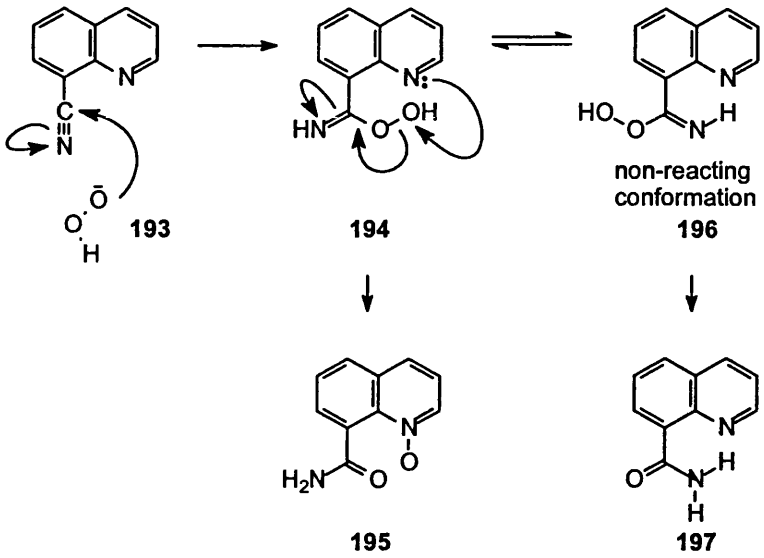
A number of strategies were employed to approach the synthesis of quinoline-8-carboxamide N-oxide **(182)**[210-214]. The synthetic routes employed are outlined below.



Scheme 15. Attempted synthesis of 8-bromoquinoline N-oxide. *Reagents:* i/ii, $SOCl_2$ or TsCl, iii, EtOH / NaOH / H_2O_2, iv, AcOH, H_2O_2, v, $(CH_3CO_2)_2Hg$ / NaCl, vi, H_2O / Br_2 / KBr, vii, THF / BuLi / trimethylsilyl isocyanate.

Attempts to oxidise **(180)** directly to the quinoline-8-carboxamide N-oxide **(182)** with *m*-chloroperoxybenzoic acid also failed and only the starting compound and

decomposed material were recovered. It was thought that failure of the reaction may have been due to ring nitrogen not being nucleophilic enough.



Scheme 16. Proposed mechanism for the synthesis of quinoline-8-carboxamide N-oxide by directed oxidation / hydrolysis.

It is generally known that nitriles are formed following dehydration of corresponding amides. It was thought that, as direct oxidation of quinoline-8-carboxamide **(180)** was unsuccessful, it may be possible to carry out a directed oxidation whereby 8-cyanoquinoline **(183)** undergoes treatment with hydrogen peroxide. Treatment of quinoline-8-carboxamide with thionyl chloride gave 8-cyanoquinoline **(183)** in 80% yield. Similar treatment with *p*-toluenesulfonyl chloride gave **(183)** in a higher yield of 97% and with less decomposed material. Direct oxidation of 8-cyanoquinoline **(183)** with concomitant hydrolysis by treatment with sodium hydroxide and hydrogen peroxide failed, giving only quinoline-8-carboxamide **(180)**. Failure of this reaction was thought to be due to the wrong conformation being adopted, hence preventing the reaction from occurring. The proposed mechanism is outlined above.

The results obtained demonstrate that a direct oxidation of quinoline-8-carboxamide **(180)** was unsuccessful in yielding the carboxamide N-oxide **(182)**. Furthermore, directing oxidation of the cyanoquinoline **(183)** to the N-oxide compound **(182)** also failed. Consequently, an alternative route was proposed[210,211]. Brzezinski[211] claimed

that 8-bromoquinoline N-oxide could be converted to its Grignard reagent; reaction with formaldehyde introduced a 1-carbon unit to give 8-hydroxymethylquinoline N-oxide. Similarly, it was proposed that formation of the Grignard reagent and treatment with trimethylsilyl isocyanate would give the N-oxide **(182)** directly from **(187)**.

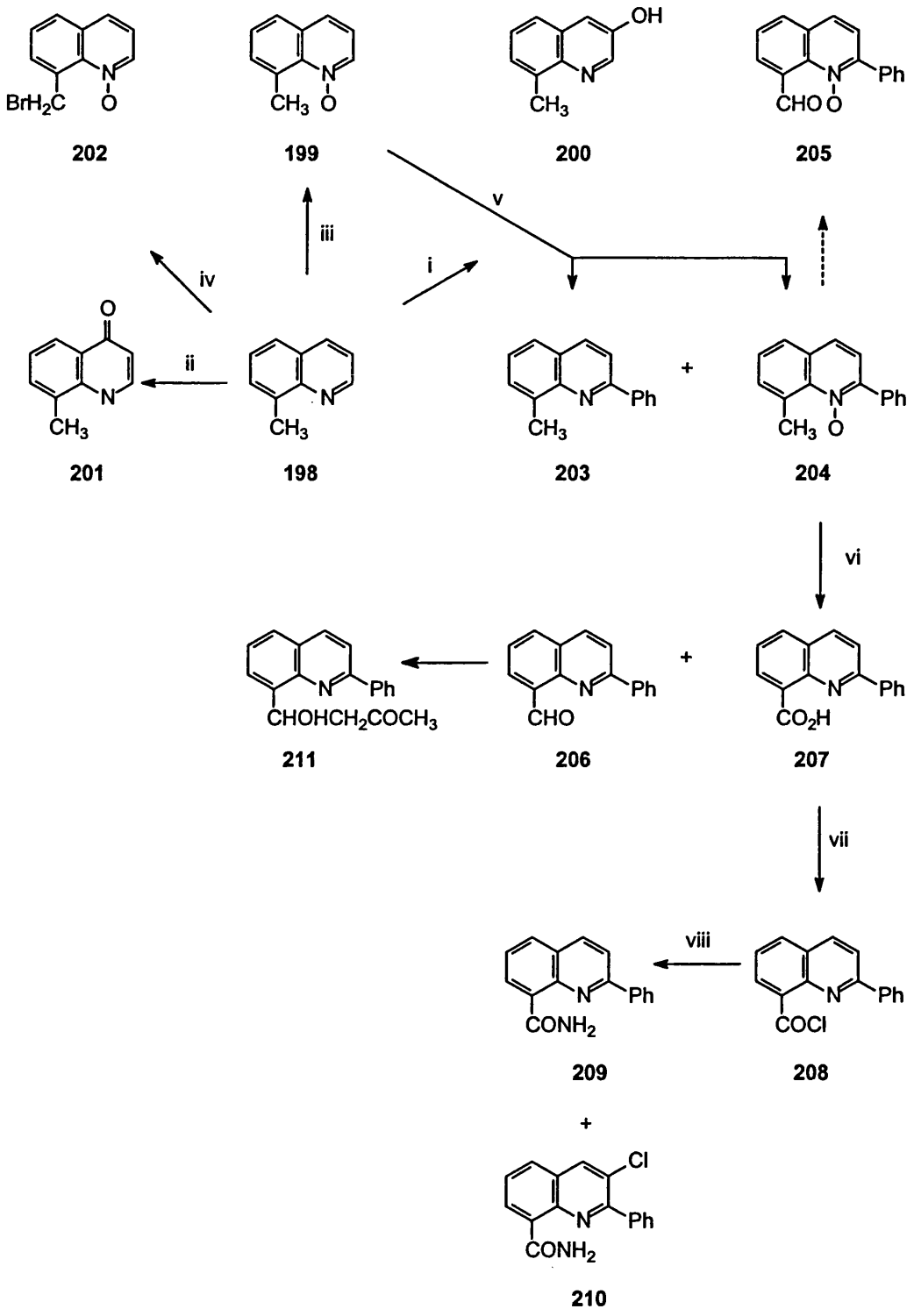
Quinoline **(184)** was oxidised to the N-oxide **(185)** by treatment with hydrogen peroxide and acetic acid, in very high yield. In the ^{1}H NMR spectrum of quinoline **(184)** the 8-proton was evident at δ7.70 where as in the N-oxide **(185)** the 8-proton moved sharply downfield to δ8.76. This was due to the deshielding effect of the adjacent oxygen. However, when making the N-oxide the 2-proton shifts slightly upfield from δ8.93 in **(184)** to δ8.55 in **(185)**.

Mercuration at the 8-position by treatment with mercuric (II) acetate and sodium chloride gave the chloromercuri compound **(186)** in 83% yield. Electrophilic replacement of the chloromercuri group by bromine gave 8-bromoquinoline N-oxide **(187)** in 29% yield. In the ^{1}H NMR spectrum the 2-H was observed downfield at δ8.75 due to the deshielding effect of the adjacent N-oxide group. Further evidence to support the formation of **(187)** was provided by MS data whereby the molecular ion was evident at 224/226 and loss of hydrogen bromide gave an ion at 144. Treatment of 8-bromoquinoline N-oxide **(187)** in ether with magnesium and trimethylsilyl isocyanate failed to give the N-oxide **(182)** and ^{1}H NMR only indicated the presence of the starting materials, suggesting that the Grignard reagent (RMgBr) had not formed. Furthermore, solubility problems were encountered with the use of diethyl ether as the solvent and this factor may have contributed to the failure of the reaction.

An alternative route[212] to incorporate the carboxamide group into the 8-position without affecting the N-oxide moiety was considered. It was proposed that halogen→lithium exchange and treatment with trimethylsilyl isocyanate may afford the desired compound **(182)**. Initially, the hypothesis was tested with 8-bromoquinoline **(191)**. Treatment of **(191)** with butyllithium followed by quenching with trimethylsilyl isocyanate gave quinoline-8-carboxamide in 79% yield.

However, treatment of 8-bromoquinoline N-oxide **(187)** with butyllithium and quenching with trimethylsilyl isocyanate failed to give the N-oxide **(182)**, but furnished quinoline-8-carboxamide in 77% yield. It was thought that the reason for failure was partly due to the fact that 8-bromoquinoline N-oxide **(187)** is hygroscopic and therefore picks water easily from the atmosphere, consequently making the lithiation reaction more difficult. Furthermore, the use of the base butyllithium appeared to cleave the oxygen of the N-oxide group, suggesting that such compounds may be unstable under these basic conditions.

Nakashima and Suzuki[213] reported the direct oxidation of 8-methylquinoline with hydrogen peroxide and acetic acid. It was reported that 3-hydroxy-8-methylquinoline and 8-methylquinoline N-oxide were obtained in 14% and 8% yield. It was proposed that similarly, it may be possible to oxidise 8-bromoquinoline directly with hydrogen peroxide and acetic acid to give the N-oxide **(187)**. However, following treatment with hydrogen peroxide and acetic acid, ^{1}H NMR indicated the presence of only 8-bromo-3-hydroxyquinoline **(192)**, which was obtained in 86% yield. Evidence for the formation of **(192)** was provided by the fact that the 3-proton was no longer evident in ^{1}H NMR. However, Hamm and Philipsborn[214] reported the use of an alternative oxidising agent. It was reported that 8-methylquinoline was oxidised to 8-methylquinoline N-oxide in 54% yield by treatment with peroxybenzoic acid. However, in subsequent runs, attempts to oxidise 8-bromoquinoline **(191)** with *m*-chloroperoxybenzoic acid failed and ^{1}H NMR only indicated the presence of the starting material and decomposed material.

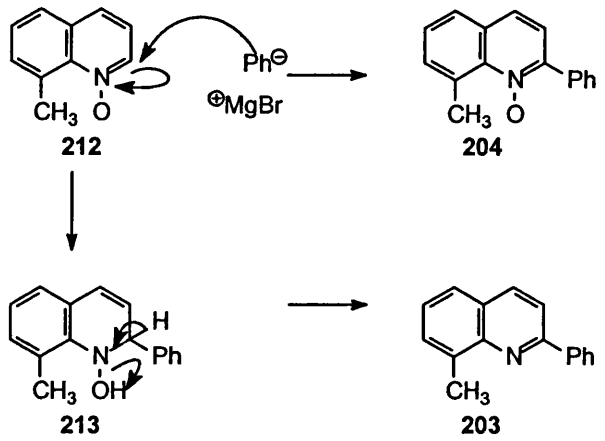


Scheme 17. Attempted synthesis of quinoline-8-carboxamide and 2-phenyl-quinoline-8-carboxamide. *Reagents:* i, AcOH / H_2O_2, ii, $CHCl_3$ / SeO_2, iii, $CHCl_3$ / *m*-CPBA, iv, CCl_4 / NBS / dibenzoyl peroxide, v, THF / PhMgBr / NH_4Cl / PbO_2 / C_6H_6, vi, EtOH / NaOH / H_2O_2, vii, $SOCl_2$ or oxalyl chloride, viii, $CHCl_3$ / NH_3.

As it was proving difficult to synthesise the desired quinoline-8-carboxamide N-oxide **(182)** by the above synthetic routes, an alternative route was sought. This was to oxidise an 8-methyl unit in the N-oxide ring system. It was proposed that oxidation of 8-methylquinoline to the N-oxide, followed by oxidation of the methyl group to an acid and subsequent formation of the amide would provide a route for the synthesis of quinoline-8-carboxamide N-oxide **(182)**. Attempts to oxidise 8-methylquinoline **(198)** directly to the N-oxide **(199)** by hydrogen peroxide in acetic acid failed to give the N-oxide but gave 8-methyl-3-hydroxyquinoline **(200)** in 93% yield. Evidence for the formation of **(200)** was provided by ^{1}H NMR whereby the 3-H was no longer present and coupling was observed between the 2 and 4 protons. Furthermore, the OH signal was evident at δ10.25. However, treatment of **(198)** with *m*-chloroperoxybenzoic acid afforded 8-methylquinoline N-oxide **(199)** in 95% yield. This represents a major advance on the reported 54% using peroxybenzoic acid[214]. In the ^{1}H NMR spectrum of 8-methylquinoline **(198)**, the Me group was evident at δ2.83. However, in the N-oxide **(199)** the Me group moves downfield to δ3.19 due to the deshielding effect of the adjacent oxygen. Also evident in the spectrum is that the 2-proton moves slightly upfield going from 8-methylquinoline **(198)** at δ8.97 to δ8.39 in the N-oxide **(199)**. This is consistent with the data obtained for quinoline N-oxide **(185)** where the 2-proton similarly shifted upfield going from quinoline **(184)** to the N-oxide **(185)**.

Having achieved the synthesis of 8-methylquinoline N-oxide **(199)**, attempts were made to oxidise the 8-methyl group to the aldehyde with selenium dioxide. Oxidation of 8-methylquinoline **(198)** with selenium dioxide failed to give the aldehyde but gave 8-methylquinolin-4-one **(201)** in 82% yield. The ^{1}H NMR spectrum of the crude product mixture also indicated a trace amount of 8-methylquinoline **(198)**. This demonstrates that either the N-oxide **(199)** is thermally unstable or that selenium dioxide is behaving as a reducing agent. ^{1}H NMR was consistent with compound **(201)** as the 4-H was no longer evident. Oxidation of **(199)** with potassium permanganate also failed to oxidise the methyl group and only decomposed material was evident in the ^{1}H NMR spectrum. Therefore, an alternative route was sought.

Aromatic compounds can be brominated under free radical conditions by treatment with N-bromosuccinimide and a radical initiation. This was proposed to provide a route to quinoline-8-carboxamide N-oxide. However, treatment of **(199)** with N-bromosuccinimide gave mainly the starting material. Nevertheless, MS data revealed trace amounts of the desired compound **(202)**.



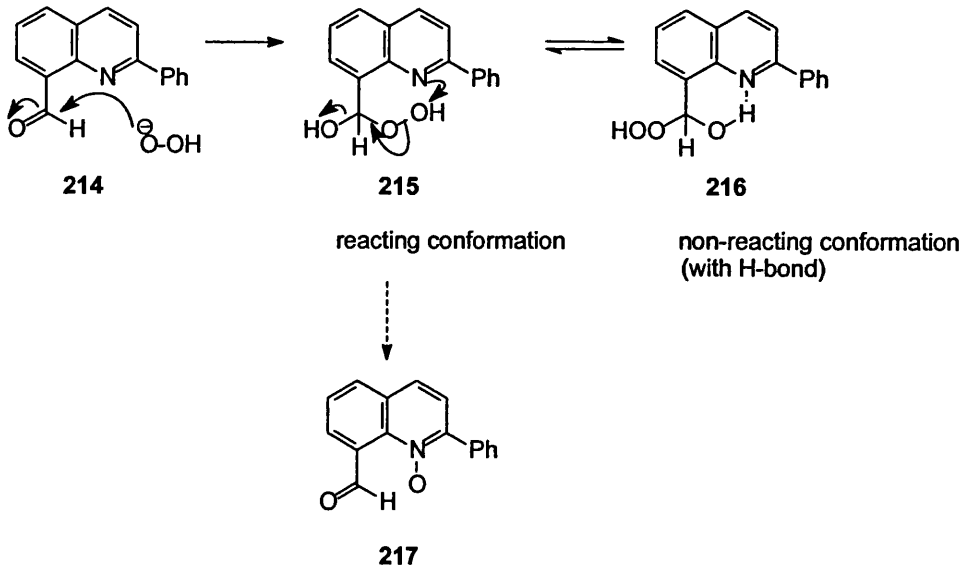
Scheme 18. Schematic representation of the attack of the Grignard reagent on 8-methylquinoline N-oxide. *Reagents:* i, THF / PhMgBr / C_6H_6 / PbO_4.

In 1980, Colonna *et al*[215] reported a synthetic route to 2-phenylquinoline N-oxides in that treatment of 8-methylquinoline N-oxide with phenylmagnesium bromide results in nucleophilic attack at the C-2 position to give the corresponding 2-phenylquinoline N-oxide. The analogue 2-phenylquinoline-8-carboxamide N-oxide was of interest in ^{1}H NMR studies because the 2-phenyl would occupy the region of space as the 2-aryl substituent in the benzimidazole-4-carboxamide[147]. Furthermore, the phenyl group was proposed to improve the potency of the compound. Treatment with Grignard gives **(213)**. However, the intermediate can be oxidized in *situ* with lead (IV) dioxide to give the N-oxide **(204)**. Column chromatography gave 8-methyl-2-phenylquinoline **(203)** in 48% yield and the desired novel 8-methy-2-phenylquinoline N-oxide **(204)** in 29% yield. In the ^{1}H NMR spectrum of 8-methyl-2-phenylquinoline **(203)**, the Me group was evident at δ2.89; however, the signal moves further downfield in the N-oxide **(204)** to δ3.21 due to the deshielding effect of the adjacent oxygen attached to the heteroaromatic ring nitrogen. Also evident in the ^{1}H NMR spectrum was that the 2- and 6-protons of the phenyl group at position 2

of the heteroaromatic ring in **(203)** were at δ8.23 but in the N-oxide **(204)** they had shifted slightly upfield to δ7.84. The data for compounds **(203)** / **(204)** are consistent with that reported for 8-methylquinoline / 8-methylquinoline N-oxide in that the 8-methyl group is shifted downfield in the N-oxide compounds. Similarly, in the ^{13}C NMR spectrum, the CH_3 of **(203)** was observed at δ_C18.36, whereas the CH_3 of **(204)** with the N-oxide was observed further downfield at δ_C25.51. Evidence for the formation of the N-oxide compound was further substantiated by MS in which the molecular ion was observed at 236.

Direct oxidation of 8-methyl-2-phenylquinoline **(203)** with *m*-chloroperoxybenzoic acid to introduce the N-oxide into the compound failed and ^1H NMR only indicated the presence of the starting compound. It was thought that steric hindrance from the C-2 phenyl group may have prevented the oxidation from taking place.

Attempts to oxidise the CH_3 of the N-oxide **(204)** with selenium dioxide to the aldehyde compound **(205)** also failed. Column chromatography of the crude indicated the presence of a number of compounds. Analysis of the ^1H NMR spectrum found them to be 8-methyl-2-phenylquinoline **(203)** in 16% yield, followed by the novel 2-phenylquinoline-8-carboxaldehyde **(206)** obtained in 40% yield and 2-phenylquinoline-8-carboxylic acid **(207)** obtained in 29% yield. It was thought that in the reaction the selenium dioxide was converting to selenium metal, which was thought to be responsible for reducing the compound. Evidence for the formation of the aldehyde **(206)** was provided by ^1H NMR, whereby the CH_3 peak had disappeared and a CHO peak was observed at δ11.67, which was very low field for an aldehyde. This indicates that the aldehyde is in-plane and deshielded. Furthermore, the 7-H was shifted downfield to δ8.34 due to the electron-withdrawing effect of the aldehyde. Evidence for the formation of the acid **(207)** was obtained by the fact that no CHO signal was observed in ^1H NMR and furthermore, the acid signal was evident at δ17.00 as a broad singlet, the very pronounced deshielding indicating intramolecular H-bonding to the quinoline ring nitrogen.



Scheme 19. Schematic representation of the attack of hydrogen peroxide on quinoline-2-phenylcarboxaldehyde.

It was proposed that it may be possible to form the N-oxide compound by a directed oxidation of the aldehyde **(206)** with hydrogen peroxide. However, treatment with the above reagents failed to give the N-oxide **(205).** It was thought that the reaction may have failed due to the wrong conformation being adopted, or that the heteroaromatic ring nitrogen is not nucleophilic enough hence preventing the oxidation from taking place. This was demonstrated earlier with 8-cyanoquinoline.

Treatment of the aldehyde **(206)** with sodium hydroxide and hydrogen peroxide in acetone also failed to give the N-oxide compound **(205)** and instead gave the novel compound **(211)** in 83% yield, in an aldol reaction with the solvent. However, it is an interesting compound to have for comparison with the carboxamide compounds.

Since the acid compound **(207)** was obtained, it was thought that the 2-phenylquinoline-8-carboxamide may be useful in ^{1}H NMR studies. Consequently, **(207)** was converted into the acid chloride **(208)** by treatment with thionyl chloride. The acid chloride was then treated with ammonia. Two compounds were evident in the ^{1}H NMR spectrum. One was the desired 2-phenylquinoline-8-carboxamide **(209)**, whereas the other appeared to be missing the quinoline 3-H. Recrystallisation gave **(210)** in 20% yield and **(209)** in 65% yield. It was found that thionyl chloride

gave an unexpected oxidation, chlorinating at an unusual position. Evidence for the formation of compound **(209)** was obtained by ^{1}H NMR whereby one of the N-H groups was observed at δ6.36 and the other N-H was evident further downfield at δ11.18. This was consistent with quinoline-8-carboxamide where similar chemical shifts were observed for the N-H_2 protons. This study demonstrates that the carboxamide has a very restricted conformation and that the N-H observed at δ11.18 is hydrogen bonded. In compound **(210)** the 4 proton was evident as a singlet at δ8.42. Further evidence was provided by MS data, whereby the molecular ion was observed at 285/283. In subsequent runs, to prevent the formation of the chloro analogue, compound **(207)** was treated with the less oxidative oxalyl chloride. Treatment of the acid chloride with ammonia gave 2-phenylquinoline-8-carboxamide **(209)** in 94% yield.

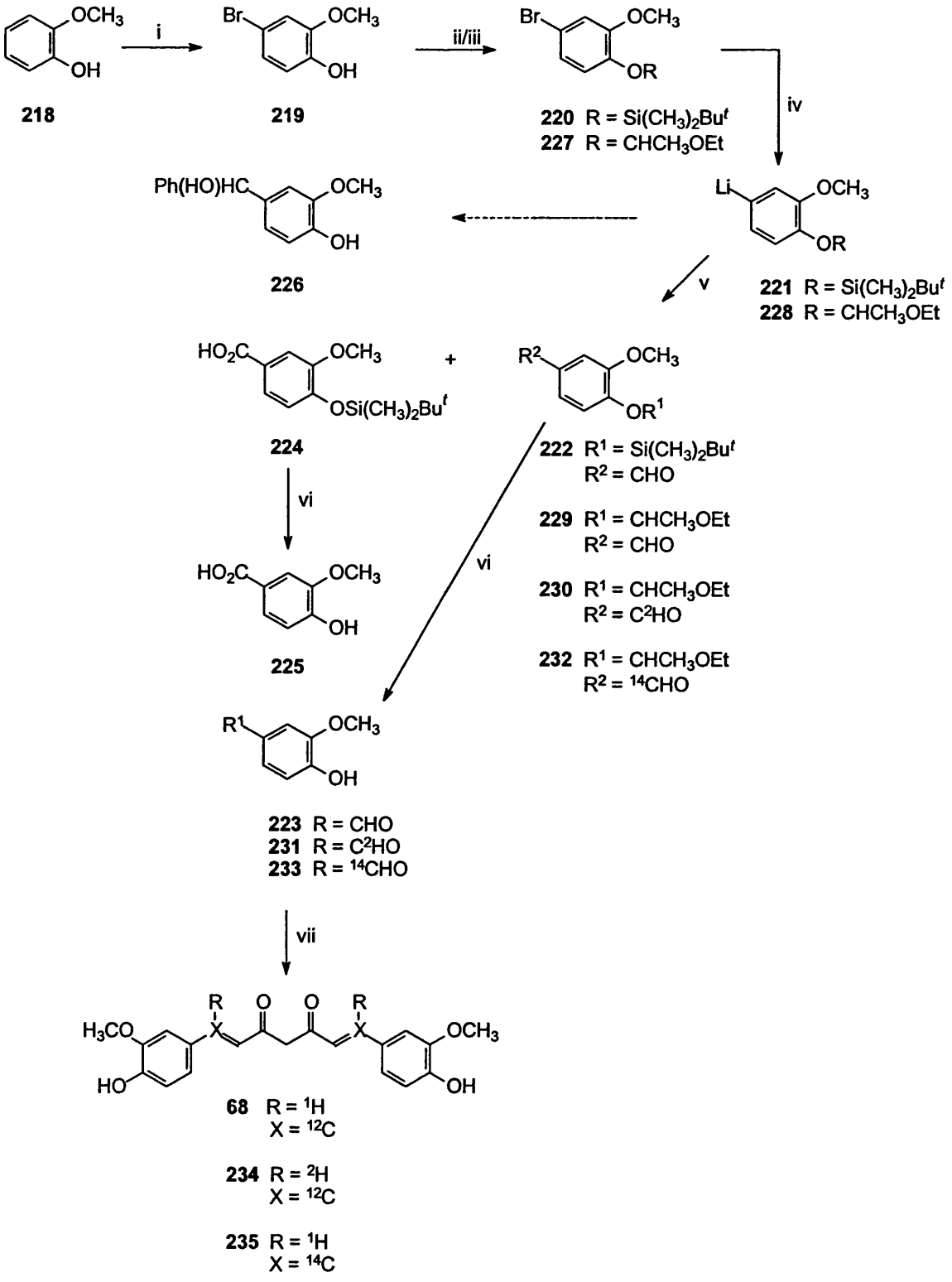
In this section of the project, although the synthesis of the target was not achieved, several advances in quinoline chemistry were made. It was found that 8-methyl-quinoline N-oxide could be obtained in excellent yield by treatment with *m*-chloroperoxybenzoic acid. In addition, the synthesis of the analogue 8-methyl-2-phenylquinoline N-oxide was found to highly sterically hinder the N-oxide. Formation of 2-phenylquinoline-8-carboxamide by treatment of the acid **(207)** with thionyl chloride gave unexpected oxidation at the 3-position. During these studies the intramolecular hydrogen-bond in quinoline-8-carboxamide and 2-phenyl-quinoline-8-carboxamide was demonstrated. ^{1}H NMR studies showed that when the carboxamide was in plane, one of the N-H were found to be hydrogen-bonded resulting in the signal being very low field. Although the attempted directed oxidations with 8-cyanoquinoline and 2-phenylquinoline-8-carboxaldehyde failed to give the N-oxide compounds, it was thought that this may have been due to the reacting materials participating in the intramolecular hydrogen-bond, which prevented the formation of the N-oxide compounds.

7.0 SYNTHESIS OF ^{14}C-LABELLED CURCUMIN (235)

Curcumin is currently of interest as a chemopreventive and chemoprotective agent against cancer. Studies carried out with mice have demonstrated that curcumin is poorly adsorbed from the gut[185-188]. In an attempt to elucidate its metabolism, biodistribution and pharmacological activity in human trials, ^{14}C-labelled curcumin was required so that the compound may be monitored in the urine by isotope ratio mass spectroscopy.

The isotopic label was incorporated into the heptatriene core of curcumin, which was thought to ensure its retention during metabolic processes. The majority of the synthetic routes published in the synthesis of unlabelled curcumin have involved the condensation of 4-hydroxy-3-methoxybenzaldehyde **(223)** with pentane-2,4-dione under acidic or basic conditions[216-220]. It was proposed that the isotope be located in the aldehyde component of compound **(223)** as opposed to incorporating it in the pentane-2,4-dione moiety, because the required intermediate could be synthesised from a one-carbon unit. Furthermore, the labelled intermediates 4-hydroxy-3-methoxybenz-[^{2}H]-aldehyde and 4-hydroxy-3-methoxybenz-[^{14}C]-aldehyde may be of use in biochemical studies.

The procedure followed to synthesise unlabelled curcumin was that reported by Babu and Rajasekharan[219]. This was the chosen route for synthesis as the route allowed for the incorporation of the isotope in the heptatriene core of curcumin. The synthetic route employed is outlined on the following page.



Scheme 20. Synthesis of ^{14}C-labelled curcumin. *Reagents:* i, $CHCl_3$ / Br_2, ii, DMF / TBDMSCl / imidazole, iii, $CHCl_3$ / ethoxyethene / pyridinium4-methylbenzene sulfonate, iv, THF / BuLi, v, DMF or $DCON(CD_3)_2$ or $H^{14}CON(CH_3)_2$, vi, aq. H_2SO_4 / TBAF or aq. HCl, vii, DMF / pentane-2,4-dione / B_2O_3 / AcOH / 1,2,3,4-tetrahydroquinoline.

Treatment of 2-methoxyphenol **(218)** with bromine and chloroform by the procedure reported by Mabic and Lepoittevin[221], gave 4-bromo-2-methoxyphenol **(219)** in 98% yield.

Although synthesis of regiospecifically ring [^{2}H]-labelled vanillins have been reported[222-224], whereby the 2-H was incorporated either by direct $^1H \rightarrow ^2H$ exchange or by replacement of a halogen, it was preferred to carry out a halogen-lithium exchange on an oxygen-protected 4-bromo-2-methoxyphenol followed by quenching with anhydrous (labelled) dimethylformamide.

In earlier reactions, the oxygen of compound **(219)** was protected as its $OSiMe_2Bu^t$ derivative. This was achieved following treatment of compound **(219)** with *t*-butyldimethylchlorosilane, which afforded the silylated compound **(220)** in 84% yield.

Comp. No.	Mass	Dry THF (mL)	BuLi	Stirring time at -78°C	Dry DMF	$CHCl_3$ (mL)	TBAF (mL)	% Yield
220	330 mg, 1.0 mmol	1.0	*n*-BuLi 0.6 mL, 0.9 mmol (0.9 eq.)	2	0.5 mL, 6.0 mmol (6.0 eq.)	1.0	0.5	21
220	230 mg, 0.7 mmol	1.0	*t*-BuLi 0.43 mL, 0.65 mmol (0.9 eq.)	2	0.34 mL, 4.4 mmol (6.0 eq.)	1.0	0.5	52
220	483 mg, 1.5 mmol	1.0	*t*-BuLi 1.0 mL, 1.5 mmol (1.0 eq.)	2	0.11 mL, 1.4 mmol (0.9 eq.)	1.0	0.5	2
220	230 mg, 0.7 mmol	1.0	*t*-BuLi 0.5 mL,	2	0.085 mL, 1.1 mmol	1.0	0.5	49

Comp. No.	Mass	Dry THF (mL)	BuLi	Stirring time at -78°C	Dry DMF	$CHCl_3$ (mL)	TBAF (mL)	% Yield
			0.7 mmol (1.0 eq.)		(1.5 eq.)			
220	1.0 g, 3.2 mmol	5.0	*t*-BuLi 2.1 mL, 3.2 mmol (1.0 eq.)	2	0.75 mL, 9.6 mmol (3.0 eq.)	1.0	0.5	49
220	276 mg, 0.87 mmol	1.0	*n*-BuLi 1.65 mL, 2.6 mmol (3.0 eq.)	2	0.20 mL, 2.6 mmol (3.0 eq.)	1.0	0.5	64

Table 1. Optimisation studies for the synthesis of **(223)**.

Treatment of compound **(220)** with butyllithium, unlabelled dry dimethylformamide, followed by acid hydrolysis and deprotection with *tetra*butylammonium fluoride gave 3-methoxy-4-hydroxybenzaldehyde (vanillin) **(223)**. However, many problems were encountered during the synthesis of **(223)**.

Initially, it was thought that 0.9 / 1.0 equivalents of butyllithium was sufficient for complete lithiation to take place. However, this was not the case and treatment of compound **(220)** with butyllithium (0.9 equivalents) in the presence of excess dimethylformamide only gave compound **(223)** in 21% yield. Repetition of the above reaction but with the more reactive *t*-butyllithium and using similar ratios of reacting agents gave the benzaldehyde **(223)** in 51% yield. However, it was noted with the use of the more reactive *t*-butyllithium that a number of further compounds were evident on TLC. In subsequent runs a further compound **(225)** was identified in ^{1}H NMR. It was thought that use of aqueous sulfuric acid was oxidising the aldehyde to the acid and crude **(225)** was obtained. To prevent the formation of the acid derivative **(225)**, in further reactions, aqueous hydrochloric acid was used during the work-up procedure.

Due to the very low yields obtained it was needed to be determined whether the compound was lithiating efficiently or whether the lithio compound reacted. This was tested by carrying out a bromine→lithium exchange of compound **(220)** with the *t*-butyllithium (fixed at 1.0 equivalents), followed by quenching with benzaldehyde. Aliquots were taken out every hour to see whether the compound was lithiating. However, no lithiation occurred, as determined by TLC and ^{1}H NMR. This suggested that the bromine→lithium exchange was not taking place with ratio of base used.

In an attempt to increase the yield of compound **(223)**, the temperature conditions for lithiation were investigated and found that the optimum lithiation temperature with *t*-butyllithium was -79°C. An alternative lithiating reagent was also investigated. Treatment of compound **(220)** with methyllithium (1.0 equivalents) at -79°C and DMF (1.0 equivalents) failed and only starting material was recovered.

Another problem that was encountered during the synthesis of **(223)** was separating the starting compound from the intermediate silylated compound **(222)**. This was due to both compounds having very similar Rf values. However, although time-consuming, this problem was overcome by the use of column chromatography carried out under gravity. With the silylated compound **(220)**, the acid used (aqueous hydrochloric acid / aqueous sulfuric acid) was unsuccessful in removing the protecting group completely, therefore the crude had to be treated with *tetra*-butylammonium fluoride. It was noted that to incorporate the isotope into the aldehyde from a one-carbon unit, it was important to optimise the yield based on dimethyformamide as opposed to the bromoarene. Hence, the problems encountered with the use of the starting silylated compound **(220)** suggested that an alternative protecting group was required.

Recently, Ralph *et al*[225] reported the synthesis of 4-hydroxy-3-[^{12}C, $^{2}H_3$]methoxy-benzaldehyde from the starting 4-bromo-2-[$^{2}H_3$]methoxyphenol which was protected as an acetal. This reaction was reported to give the isotopically labelled benzaldehyde in a yield of 78%. The reaction employed the use of butyllithium (3.0

equivalents) and dimethylformamide (3.0 equivalents). Treatment of compound **(220)** using the above ratios gave **(223)** in 64% yield.

Comp. No.	Mass	Dry THF (mL)	BuLi	Stirring time at -78°C	Dry DMF (mL)	% Yield
227	377 mg, 1.4 mmol	1	1.3 mL, 2.1 mmol (1.5 eq.)	1	0.16 mL, 2.10 mmol (1.5 eq.)	0
227	574 mg, 2.1 mmol	3.0	*n*-BuLi 3.9 mL, 6.3 mmol (3.0 eq.)	1	0.33 mL, 4.2 mmol (2.0 eq.)	55
227	511 mg, 1.9 mmol	3.0	*n*-BuLi 3.5 mL, 5.6 mmol (3.0 eq.)	1	0.43 mL, 5.6 mmol (3.0 eq.)	70
227	653 mg, 2.4 mmol	3.5	*n*-BuLi 4.45 mL, 7.1 mmol (3.0 eq.)	2	0.37 mL, 4.8 mmol (2.0 eq.)	64
227	413 mg, 1.5 mmol	2.0	*n*-BuLi 2.8 mL, 4.5 mmol (3.0 eq.)	4	0.2 mL, 3.0 mmol (2.0 eq.)	45

Table 2. Optimisation studies for the synthesis of **(223)**.

4-Bromo-2-methoxyphenol **(219)** was protected as the acetal by treatment with ethoxyethene and pyridinium-4-methylbenzenesulfonate. 4-Bromo-4-(1-ethoxyethoxy)-2-methoxybenzene **(227)** was obtained in 99% yield.

Bromine→lithium exchange with butyllithium at -79°C gave the anion **(228)** and quenching with unlabelled dimethylformamide gave compound **(229)**. Acid hydrolysis (aqueous hydrochloric acid) cleaved both the intermediate iminium and the protecting acetal to give the desired benzaldehyde **(223)**.

An optimisation study was carried out with the acetal **(227)**. Initially, **(227)** was treated with 1.5 equivalents of butyllithium and quenched with 1.5 equivalents of anhydrous DMF. However, the reaction was unsuccessful and ^{1}H NMR indicated the presence of starting material only. This study demonstrated that higher base and formamide ratios were required in order for the reaction to be successful.

In subsequent runs, compound **(227)** was treated with 3.0 equivalents of butyllithium and a 2.0 equivalents of anhydrous DMF. Compound **(223)** was obtained in 55% yield. As the compound was only lithiated for 1 h at -79°C, it was proposed that this lithiating time period may be insufficient for complete lithiation to take place. Consequently, reactions were carried out with the butyllithium fixed at 3.0 equivalents and the dimethylformamide fixed at 2.0 equivalents and the lithiating reaction times were varied from 2 to 4 h. Lithiating the mixture for 2 h at -79°C gave **(223)** in 64% yield. However, increasing the lithiation time to 4 h resulted in a lower yield of 45%. Therefore, the optimum lithiation time was in fact 2 h and it was thought that at longer lithiating periods the reaction was slowly being quenched.

Increasing the ratio of dimethylformamide enhanced the yield of compound **(223)**. Treatment of compound **(227)** with butyllithium (3.0 equivalents) and dimethylfomamide (3.0 equivalents) gave the aldehyde **(223)** in 70% yield.

Based on these optimisation studies it was observed that an excess of the alkyl lithium was required. With the butyllithium fixed at 3.0 equivalents, it was found that by varying the amount of the dimethylformamide (from 2.0 to 3.0 equivalents) afforded a higher yield based on compound **(227)**. However, dimethylformamide fixed at 2.0 equivalents afforded the potentially more isotopically efficient yield based on dimethylformamide. Furthermore, it was established that the optimum time for bromine→lithium exchange was 2 h at -79°C resulting in a maximum yield of

32% of 4-hydroxy-3-methoxybenzaldehyde **(223)** based on dimethylformamide and 64% based on the bromoarene.

Application of the optimised lithiation conditions and quenching with $O^2HCN(C^2H_3)_2$ (2.0 equivalence ratio) afforded 4-hydroxy-3-methoxybenz-[^{2}H]-aldehyde **(231)** in 55% yield based on the bromoarene and 27% based on the dimethylformamide. ^{1}H NMR spectroscopy further confirmed the formation of the ^{2}H-compound **(231)**, as no aldehyde peak was evident in ^{1}H NMR. ^{13}C NMR also provided further evidence for the formation of **(231)**, whereby the C^2HO was observed as a triplet at δ190.64, with a coupling constant of $^1J_{C\text{-}D}$ 26.4 Hz (^{2}H has spin $I = 1$).

Similarly, treatment of compound **(227)** with butyllithium (3.0 equivalents), followed by quenching with $OH^{14}CNMe_2$ and hydrolysis of the intermediate **(232)** gave 4-hydroxy-3-methoxybenz-[^{14}C]-aldehyde **(233)** in 45% yield and 8.1% radiochemical yield. The lower efficiency of isotopic incorporation was due to problems encountered in the delivery of $OH^{14}CNMe_2$ from the container in which it was purchased to the reaction mixture.

In the condensation of unlabelled 4-hydroxy-3-methoxybenzaldehyde **(223)** with pentane-2,4-dione it was important that the bis-enol(ate) of the β-diketone was formed, in order to prevent the reaction from taking place at the central CH_2. In attempts to avoid this, a number of studies have complexed the dione with boric acid (H_3BO_3)[217,219] or with borate esters ($B(OR)_3$)[216,226]. The procedure reported by Babu and Rajasekharan[219] was adapted. It was a simple procedure and was reported to afford unlabelled compound **(68)** in a yield of 78%.

In initial experiments, unlabelled 4-hydroxy-3-methoxybenzaldehyde **(223)** was treated with pentane-2,4-dione and boric acid. Decomplexation with aqueous acetic acid gave *E,E*-1,7-di(4-hydroxy-3-methoxyphenyl)-3-hydroxyhepta-1,3,6-trien-5-one (curcumin) **(68)** in 57% yield. It was thought that the yield maybe further enhanced by initially forming the complex between the boric acid and pentane-2,4-dione, followed by addition of the benzaldehyde **(223)**. However, in subsequent runs, compound **(68)** was obtained in a lower yield of 40%. Although, it was reported in

the literature[219] that boric acid was more reactive than boron oxide, studies were also carried out with boron oxide. It was found that complexation of boron oxide with pentane-2,4-dione, followed by addition of compound **(223)** gave compound **(68)** in a higher yield of 77%. The heptatriene 4 proton was observed as a singlet at δ6.06. The heptatriene alkenic 2 and 6 protons were observed as a doublet at δ6.76 due to coupling being observed between the adjacent 1 and 7 protons. The coupling constant was large (J = 15.6Hz) which was indicative of a *trans* stereoisomer. The 1- and 7-alkenic heptatriene protons were observed at δ7.56 as a doublet due to coupling to the adjacent 2- and 6-protons. These protons were shifted slightly further downfield in comparison to the alkenic 2- and 6-protons because of the deshielding effect of the aromatic ring. The two OH signals were observed as a broad singlet at δ9.70.

Application of the optimised conditions in the reaction of the isotopically labelled compound **(231)** gave *E,E*-1,7-di(4-hydroxy-3-methoxyphenyl)-3-hydroxy-1,7-di-[^{2}H]-hepta-1,3,6-trien-5-one **(234)** in 43% yield. ^{1}H NMR provided evidence for the formation of **(234)**, as the heptatriene 1 and 7 protons were no longer evident in the spectrum. Further evidence for the formation of compound **(234)** was obtained by MS in which (FAB$^+$) *m/z* 372 corresponded to the weight of the dideuterated compound.

Treatment of the radiolabelled 4-hydroxy-3-methoxybenz-[^{14}C]-aldehyde **(233)** with the boron oxide and pentane-2,4-dione complex, followed by treatment with 1,2,3,4-tetrahydroquinoline and acetic acid, gave the radiolabelled compound **(235)** in 81% yield. It was thought that the prolonged reaction time of 24 h at 110°C attributed to the high radiochemical yield obtained.

So far, the studies carried out with the radiolabelled ^{14}C-curcumin **(235)** by our collaborators is in agreement with related studies in that curcumin may be useful in the chemoprevention of human intestinal malignancies related to *APC* mutations. In a recent paper submitted by Perkins *et al*[227], it was reported that a daily dose of 17g of curcumin was required for efficacy in humans. This was based on studies carried out with mice, which demonstrated that a dietary concentration of 0.2 and 0.5% of

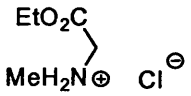
curcumin reduced ademona multiplicity by 39 and 40% respectively, in comparison to untreated mice. For curcumin to be effective as a chemopreventative agent, concentrations of ~ 100 nmole / g was required. When extrapolated to the humans it was found that the dose of curcumin required for efficacy was ~17 g / person / day.

8.0 *EXPERIMENTAL*

General Procedures

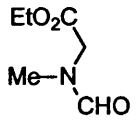
All melting points were determined using a Reichert-Jung Thermo Galen Kofler block. ^{1}H NMR spectra were recorded on either JEOL EX400 Varian Mercury 400 or JEOL GX270 spectrometers. Thin layer chromatography (TLC) was performed on precoated silica plates (Merck TLC aluminium silica sheets 60 F_{254}, Art no. 5554). Visualisation was made by dipping in potassium permanganate solution (for detection of esters) / phosphomolybdic acid in methanol (for detection of alcohols) / ninhydrin (for detection of amines) (followed by heating) or by U.V. light (for detection of aromatic compounds), where appropriate. Preparative thin layer chromatography was performed on silica plates (Merck TLC silica gel 60 F_{254}, 20 x 20 cm, layer thickness 2 mm, Art no. 5717). Elemental analysis (CHN) was carried out at the University of Bath microanalysis service. Mass spectra were recorded at the University of Bath mass spectrometry service, on VG 7070 double focussing and VG Autospec instruments. Infra-red spectra were performed on a Perkin-Elmer 782 FT-IR spectrometer. HPLC analysis was performed using a Kromasil 10C18 semi-preparative column and a JASCO PU-986 preparative pump with methanol as the eluent and flow rate at 5 mL min^{-1}. UV detection was at 326 nm by a JASCO UV-975 detector and an injection volume of 20 μL was used. Chemicals were purchased from the Aldrich / Sigma, Fluka, Lancaster or Acros chemical companies. Tetrahydrofuran, 1,2-dimethoxyethane and diethyl ether were obtained in "anhydrous" form and dried by distillation from sodium and benzophenone indicator. Solutions in organic solvents were dried over magnesium sulfate or sodium sulfate. Experiments were conducted at ambient temperature, except when noted.

SARCOSINE ETHYL ESTER HYDROCHLORIDE (82)



To sarcosine **(81)** (50.0 g, 0.56 mol) in EtOH (1000 mL) was added thionyl chloride (123 mL, 1.69 mol). The mixture was stirred for 24 h. Evaporation and washing with Et_2O (1000 mL) gave **(82)** (86.0 g, 100%) as white crystals: mp 129-131°C (no literature mp reported); ^{1}H NMR ($CDCl_3$) δ 1.31 (3 H, t, J = 7.0 Hz, CH_2CH_3), 2.85 (3 H, s, NCH_3), 3.89 (2 H, s, NCH_2), 4.29 (2 H, q, J = 7.0 Hz, CH_2CH_3), 9.73 (2 H, s, N^+H_2); ν_{max} (KBr) 3400, 2770 and 1730 cm^{-1}; MS (FAB$^+$) *m/z* 235 (2 M + H), 118 (M + H).

N-FORMYLSARCOSINE ETHYL ESTER (84)



PROCEDURE (1)

Compound **(82)** (170.0 g, 1.11 mol) was dissolved with heating in 99% formic acid (138 mL, 3.7 mol) and was added to a hot solution of sodium formate (83.0 g, 1.22 mol) in 99% formic acid (111 mL, 2.9 mol). The solution was stirred for 4 h then filtered. The solid was washed with Ac_2O (500 mL, 5.32 mol) which was then added slowly to the formic acid solution. The mixture was heated for 1 h and the solvent was evaporated. Distillation gave **(84)** (113.3 g, 70%) as a colourless liquid $bp_{0.1}$ 95°C (lit[193] $bp_{0.2}$ 93-95°C): TLC Rf = 0.4 (EtOAc); ν_{max} (KBr) 1730 and 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$; Rotamer 1) δ 1.29 (3 H, t, CH_3CH_2, J = 7.1 Hz), 3.05 (3 H, s, NCH_3), 4.10 (2 H, s, NCH_2), 4.26 (2 H, q, CH_2CH_3, J = 7.1 Hz), 8.13 (1 H, s, CHO); ^{1}H NMR ($CDCl_3$; Rotamer 2) δ 1.30 (3 H, t, CH_3CH_2, J = 7.1 Hz), 2.94 (3 H, s, NCH_3), 3.99 (2 H, s, CH_2N), 4.21 (2 H, q, CH_2CH_3, J = 7.1 Hz), 8.05 (1 H, s, CHO). Rotamers 1 and 2 were evident in the ratio 1:2.

PROCEDURE (2)

Compound **(82)** (154.8 g, 1.0 mol) was dissolved with heating in 99% formic acid (126 mL, 3.5 mol) and was added to a hot solution of sodium formate (75.0 g, 1.1 mol) in 99% formic acid (100 mL, 2.7 mol). The solution was stirred for 4 h then filtered. The solid was washed with Ac_2O (457 mL, 4.8 mol) which was then added slowly to the formic acid solution. The mixture was heated for 1 h and the solvent was evaporated. The oil, in EtOAc, was washed with aq. $NaHCO_3$ and brine. Drying, filtration and evaporation gave **(84)** (69.8g, 48%) as a colourless oil, with properties as above.

PROCEDURE (3)

To **(82)** (98.1 g, 0.64 mol) in water (450 mL), was added sodium carbonate (~10.0 g) until the pH reached 11. The solution was extracted (EtOAc) and the extracts were washed with brine, dried and filtered. Evaporation gave **(83)** (21.6 g, 29%) as a colourless oil. ^1H NMR ($CDCl_3$) δ 1.05 (3 H, t, J = 7.3 Hz, CH_3CH_2), 3.12 (2 H, s, NCH_2), 3.96 (2 H, q, J = 7.3 Hz, CH_3CH_2). Ethyl formate (205 mL, 2.55 mol) and triethylamine (5 mL, 0.04 mol) were added to **(83)** (21.6 g, 0.18 mol). The solution was stirred for 24 h, then boiled under reflux for 24 h and the solvents were evaporated. The oil, in $CHCl_3$, was washed with aq. HCl (2 M) and brine. Drying, filtration and evaporation yielded **(84)** (23.2 g, 87%) as a colourless oil, with properties as above.

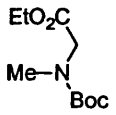
PROCEDURE (4)

To **(82)** (85.0 g, 0.55 mol) was added EtOH (500 mL), ethyl formate (170 mL, 2.1mol) and triethylamine (116 mL, 0.83 mol). The mixture was stirred for 48 h, then boiled under reflux for 10 h. After evaporation the oil, in EtOAc, was washed with aq. HCl (2M), and brine. Drying, filtration and evaporation yielded **(84)** (9.23 g, 11%) as a colourless oil, with properties as above.

PROCEDURE (5)

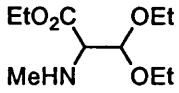
To **(82)** (87.8 g, 0.57 mol) was added ethyl formate (600 mL, 7.5 mol) and triethylamine (159 mL, 1.14 mol). The mixture was stirred for 48 h. After evaporation the oil, in EtOAc, was washed with aq. HCl (2M), and brine. Drying, filtration and evaporation yielded **(84)** (15.1 g, 18%) as a colourless oil, with properties as above.

N-(1,1-DIMETHYLETHOXYCARBONYL)SARCOSINE ETHYL ESTER (85)



To **(82)** (103.0 g, 0.67 mol) was added H_2O (2000 mL) and sodium carbonate (~10.0 g) until the pH reached 11. The solution was extracted (EtOAc). The extract was washed with brine, dried and filtered. Evaporation gave **(83)** (7.21 g, 9%) as a colourless oil. To **(83)** (7.21 g, 62 mmol) was added dropwise di-*t*-butyl dicarbonate (13.5 g, 62 mmol) in CH_2Cl_2 (20 mL). The solution was stirred for 24 h. Evaporation gave **(85)** (13.0 g, 97%) as a pale yellow oil: TLC Rf = 0.7 (EtOAc); $\nu_{max.}$ (KBr) 1750, 1700 cm^{-1}; ^1H NMR ($CDCl_3$; rotamer 1) δ 1.27 (3 H, t, J = 7.1 Hz, CH_3CH_2), 1.48 (9 H, s, Bu^t), 2.94 (3 H, s, NCH_3), 3.89 (2 H, s, NCH_2), 4.20 (2 H, q, J = 7.1 Hz, CH_2CH_3); ($CDCl_3$; rotamer 2) δ 1.29 (3 H, t, J = 7.1 Hz, CH_3CH_2), 1.48 (9 H, s, Bu^t), 2.92 (3 H, s, NCH_3), 3.97 (2 H, s, NCH_2), 4.19 (2 H, q, J = 7.1 Hz, CH_2CH_3). Rotamers 1 and 2 were evident in the ratio 1:1; MS (FAB^+) *m/z* 218.1395 (M + H) ($C_{10}H_{20}N_1O_4$ requires 218.1392), 118 (M + H – Boc).

ETHYL 3,3-DIETHOXY-2-METHYLAMINOPROPANOATE (87)



PROCEDURE (1)

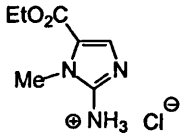
To **(84)** (113.3 g, 0.78 mol) and ethyl formate (187 mL, 2.28 mol) in dry Et_2O (228 mL) at 0°C was added a suspension of sodium ethoxide (58.5 g, 0.86 mol) in dry Et_2O (685 mL) dropwise with stirring. After 1 h at 0°C, the mixture was diluted with dry Et_2O (500 mL) and was allowed to stand overnight at 4°C. The Et_2O was decanted. The residual sodium enolate **(86)** was treated with EtOH (1370 mL), saturated with HCl and was stirred for 6 h. The mixture was further saturated with HCl and was boiled under reflux for 10 h. Aq. potassium carbonate was added until the pH reached 13. Extraction with EtOAc, washing with brine, drying, filtering, evaporation and Kugelrohr distillation gave **(87)** (69.5 g, 41%) as a pale yellow oil $bp_{0.15}$ 120°C (lit[193] $bp_{0.05}$ 61-62°C): TLC Rf = 0.2 (EtOAc / hexane 3:1); ν_{max} 3390 and 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (3 H, t, J = 7.0 Hz, OCH_2CH_3), 1.20 (3 H, t, J = 7.1 Hz, OCH_2CH_3), 1.28 (3 H, t, J = 7.1 Hz, $CO_2CH_2CH_3$), 2.39 (3 H, s, NCH_3), 3.34 (1 H, d, J = 6.0 Hz, $NCHCO_2$), 3.50-3.60 (2 H, m) and 3.65–3.80 (2 H, m) (2 × OCH_2CH_3), 4.235 (1 H, q, J = 7.0 Hz) and 4.238 (1 H, q, J = 7.1 Hz, $CO_2CH_2CH_3$,), 4.61 (1 H, d, J = 6.0 Hz, OCHO); MS (FAB^+) *m/z* 220 (M + H), 174 (M – EtOH).

PROCEDURE (2)

To **(84)** (99.8 g, 0.69 mol) and ethyl formate (161 mL, 2.0 mol) in dry Et_2O (200 mL) at 0°C was added a suspension of sodium ethoxide (51.5 g, 0.76 mol) in dry Et_2O (300 mL) dropwise with stirring. After 1 h at 0°C, the mixture was diluted with dry Et_2O (300 mL) and was allowed to stand overnight at 4°C. The Et_2O was decanted. The residual sodium enolate **(86)**, was treated with EtOH (1200 mL) saturated with HCl and was stirred for 6 h. The mixture was further saturated with HCl for 6 h. Aq. potassium carbonate was added until the pH reached 13. Extraction with EtOAc,

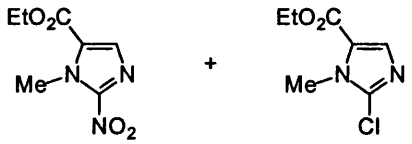
washing with brine, drying, filtering, evaporation and Kugelrohr distillation gave **(87)** and **(88)**. The crude oil in EtOH (200 mL) was saturated with HCl for 3 h and was boiled under reflux for 3 h. Aq. potassium carbonate was added until the pH reached 13. Extraction with EtOAc, washing with brine, drying, filtering, evaporation and Kugelrohr distillation gave **(87)** (33.9 g, 22%) as a pale yellow oil, with properties as above.

ETHYL 2-AMINO-1-METHYLIMIDAZOLE-5-CARBOXYLATE HYDROCHLORIDE (91)



Compound **(87)** (1.6 g, 7.3 mmol) in aq. HCl (10%, 21 mL) was stirred at 60°C for 12 h. After cooling to –10°C, the pH was brought to 4.5 with aq. NaOH (10%) and cyanamide (0.8 g, 19 mmol) was added. The mixture was stirred at 60°C for 2 h, maintaining the pH at 4.5 by addition of either aq. HCl (10%) or aq. NaOH (10%) and the solvents evaporated. The residue was triturated with Et_2O (100 mL) to remove unreacted cyanamide and was extracted thrice with EtOH (300 mL) saturated with HCl. Evaporation and recrystallisation (propan-2-ol) gave **(91)** (0.82 g, 55%) as pale buff crystals: mp 205-210°C (lit.[192] mp 200-205°C); TLC Rf = 0.5 (CH_2Cl_2 / MeOH / H_2O / AcOH 25:10:2:1); ν_{max} (KBr) 3380 and 1660 cm^{-1}; ^1H NMR (D_2O) δ 1.27 (3 H, t, J = 7.0 Hz, CH_3CH_2), 3.64 (3 H, s, NCH_3), 4.26 (2 H, q, J = 7.0 Hz, CH_2), 7.76 (1 H, s, 4-H), 8.26 (3 H, brs, N^+H_3); MS (FAB^+) *m/z* 377/375 (2 M + 2 H + Cl), 349 (2 M + H), 170 (M + H), 142 (M + H - C_2H_4).

ETHYL 1-METHYL-2-NITROIMIDAZOLE-5-CARBOXYLATE (92) AND ETHYL 1-METHYL-2-CHLOROIMIDAZOLE-5-CARBOXYLATE (93)



PROCEDURE (1)

Sodium nitrite (1.17 g, 17 mmol) in H_2O (4 mL) was added dropwise at -20°C to a stirred mixture of **(91)** (3.19 g, 15.5 mmol), H_2O (9.5 mL) and aq. HBF_4 (40%, 10.8 mL). Stirring was continued at -15°C for 50 min. The solution was added to a well-stirred mixture of sodium nitrite (10.8 g, 0.16 mol), copper powder (3.28 g, 0.05 mmol) and H_2O (300 mL). After 6 h, the copper was filtered off and the filtrate was brought to pH 2 with aq. H_2SO_4 (10%). The evaporation residue, in EtOAc, was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(92)** (1.95 g, 63%) as pale yellow crystals: mp 59-61°C (lit.[192] mp 60°C); TLC Rf = 0.3 (EtOAc / hexane 1:3); ^1H NMR ($CDCl_3$) δ 1.41 (3 H, t, J = 7.1 Hz, CH_3CH_2), 4.35 (3 H, s, NCH_3), 4.40 (2 H, q, J = 7.1 Hz, CH_2), 7.75 (1 H, s, 4-H). Further elution gave **(93)** (113 mg, 4%) as a colourless oil: TLC Rf = 0.5 (EtOAc); $\nu_{max.}$ (KBr) 2980, 1715 and 770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (3 H, t, J = 7.1 Hz, CH_3CH_2O), 3.82 (3 H, s, NCH_3), 4.25 (2 H, q, J = 7.1 Hz, CH_2), 7.20 (1 H, s, 4-H); MS (FAB^+) *m/z* 191.0399 (M + H) ($C_7H_{10}{}^{37}ClN_2O_2$ requires 191.0401), 189.0437 (M + H) ($C_7H_{10}{}^{35}ClN_2O_4$ requires 189.0431).

PROCEDURE (2)

Sodium nitrite (0.86 g, 12.4 mmol) in H_2O (3 mL) was added dropwise at -20°C to a stirred mixture of **(91)** (2.34 g, 11.4 mmol), H_2O (6.9 mL) and aq. HBF_4 (40%, 8.1 mL). Stirring was continued at -15°C for 50 min. The solution was added to a well-stirred mixture of sodium nitrite (7.9 g, 0.11 mol), copper powder (2.12g, 38.0 mmol) and H_2O (217 mL). After 2 h, the copper was filtered off and the filtrate was brought

to pH 2 with aq. HCl (10%). The evaporation residue, in EtOAc, was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(92)** (1.08 g, 48%) as pale yellow crystals, with properties as above. Further elution gave **(93)** (0.4g, 18%) as a colourless oil, with properties as above.

5-HYDROXYMETHYL-1-METHYL-2-NITROIMIDAZOLE (94) AND 5-HYDROXYMETHYL-1-METHYL-2-CHLOROIMIDAZOLE (95)

PROCEDURE (1)

To **(92)** (0.86 g, 4.3 mmol) in anhydrous THF (130 mL) was added solid $LiBH_4$ (0.19 g, 9.0 mmol). The solution was stirred for 48 h, then cooled to 0°C. The excess $LiBH_4$ was decomposed with AcOH (1.0 mL). Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(94)** (0.41 g, 60%) as pale yellow crystals: mp 139-141°C (lit.[192] mp 140°C); TLC Rf = 0.2 (EtOAc); ν_{max} (KBr) 3220, 1530 and 1355 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 3.91 (3 H, s, CH_3), 4.55 (2 H, d, J = 5.0 Hz, CH_2), 5.50 (1 H, t, J = 5.0 Hz, OH), 7.11 (1 H, s, 4-H).

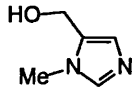
PROCEDURE (2)

To **(92)** (330 mg, 1.7 mmol) in anhydrous THF (50 mL) was added solid $LiBH_4$ (40 mg, 1.8 mmol). The solution was stirred for 20 h, after which more solid $LiBH_4$ (33 mg, 1.52 mmol) was added. The solution was stirred for a further 48 h, cooled to 0°C and the excess $LiBH_4$ was decomposed with aq. HCl (10%, 1.0 mL). Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(94)** (110 mg, 42%) as pale yellow crystals, with properties as above. Further elution gave crude **(95)** (42 mg, 17%): ^1H NMR ($(CD_3)_2SO$) δ 3.55 (3H, s, CH_3), 4.40 (2 H, d, J = 5.2 Hz, CH_2), 5.15 (1 H, t, J = 5.2 Hz, OH), 6.8 (1 H, s, 4-H).

PROCEDURE (3)

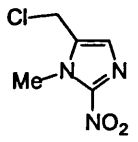
To **(92)** (1.31 g, 7.0 mmol) in anhydrous THF (200 mL) was added solid $LiBH_4$ (0.29 g, 13.3 mmol). The solution was stirred for 48 h, then cooled to 0°C. The excess $LiBH_4$ was decomposed with aq. HCl (10%, 1.0 mL). Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(94)** (0.37g, 36%) as pale yellow crystals, with properties as above. Further elution gave crude **(95)** (96mg, 10%), with the above properties.

5-HYDROXYMETHYL-1-METHYLIMIDAZOLE (96)



To **(92)** (1.56 g, 7.8 mmol) in anhydrous THF (235 mL) was added $LiBH_4$ (2.0 M solution in THF, 4.3 mL, 8.6 mmol). The solution was stirred for 24 h, then cooled to 0°C. The excess $LiBH_4$ was decomposed with AcOH (1.5 mL). Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(96)** (0.80 g, 91%) as off-white crystals: mp 114-116°C (lit.[228] mp 113-114°C); ν_{max} (KBr) 3400 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 3.60 (3 H, s, NCH_3), 4.41 (2 H, s, CH_2), 6.76 (1 H, s, 4-H), 7.53 (1 H, s, 2-H); MS (FAB^+) *m/z* 113.0718 (M + H) ($^{12}C_5H_8N_2O$ requires 113.0715), 95 (M – OH).

5-CHLOROMETHYL-1-METHYL-2-NITROIMIDAZOLE (101)



PROCEDURE (1)

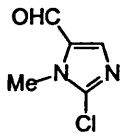
To **(94)** (50 mg, 0.32 mmol) in dry pyridine (1.0 mL) at 0°C was added methanesulfonyl chloride (50 mg, 0.48 mmol) and the solution was stirred for 3 h. The evaporation residue, in $CHCl_3$, was washed with aq. $CuSO_4$, aq. $NaHCO_3$ and brine and was dried and filtered. Evaporation gave **(101)** (39 mg, 70%) as yellow

crystals: mp 94-96°C (decomp.); TLC Rf = 0.8 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.10 (3 H, s, NCH_3), 4.63 (2 H, s, CH_2), 7.20 (1 H, s, 4-H); MS *m/z* (EI) 177.0131 (M) ($^{12}C_5H_6N_3O_2{}^{37}Cl$ requires 177.0119), 175.0158 (M) ($^{12}C_5H_6N_3O_2{}^{35}Cl$ requires 175.0149), 140 (100%) (M - Cl).

PROCEDURE (2)

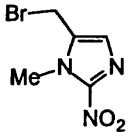
To **(94)** (50 mg, 0.32 mmol) in dry pyridine (1.0 mL) at 0°C, was added 4-methylbenzenesulfonyl chloride (115 mg, 0.60 mmol) and the solution was stirred for 6 h. The evaporation residue, in $CHCl_3$, was washed with aq. $CuSO_4$, aq. $NaHCO_3$ and brine and was dried and filtered. Evaporation gave **(101)** (17.1 mg, 31%) as pale yellow crystals, with properties as above.

1-METHYL-2-NITROIMIDAZOLE-5-CARBOXALDEHYDE (98)



To **(94)** (50 mg, 0.32 mmol) was added thionyl chloride (1.0 mL, 13.7 mmol) and the solution was stirred for 3 h. Evaporation gave crude **(98)** (44 mg, 96%) as a colourless oil: TLC Rf = 0.5 (EtOAc / hexane 1:1); ν_{max} 3340, 1740 and 760 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.93 (3 H, s, NCH_3), 7.69 (1 H, s, 4-H), 9.65 (1 H, s, CHO); MS (FAB^+) *m/z* 145.0211 (M + H) ($C_5H_5{}^{35}ClN_2O$ requires 145.0169).

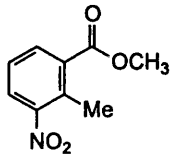
5-BROMOMETHYL-1-METHYL-2-NITROIMIDAZOLE (102)



To **(94)** (50 mg, 0.32 mmol) in dry DMF (1.0 mL) was added bromine (53 mg, 330 μmol) and triphenylphosphine (90 mg, 343 μmol) and the mixture was stirred for 16 h. The evaporation residue, in EtOAc, was washed with H_2O, brine, dried and filtered. Evaporation and preparative TLC (EtOAc / hexane 1:1) gave **(102)** (12 mg,

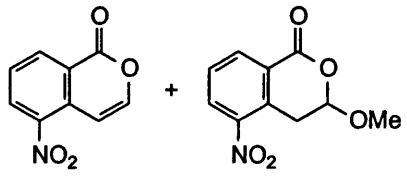
17%) as pale yellow crystals: mp 84-87°C (decomp.); TLC Rf = 0.8 (EtOAc); ^{1}H NMR ($CDCl_3$) δ3.98 (3 H, s, NCH_3), 4.41 (2 H, CH_2), 7.13 (1 H, s, imidazole 4-H); MS (EI) *m/z* 220.9562 (M) ($C_5H_6{}^{81}BrN_3O_2$ requires 220.9623), 218.9582 (M) ($C_5H_6{}^{79}BrN_3O_2$ requires 218.9643), 140 (M - Br).

METHYL 2-METHYL-3-NITROBENZOATE (110)



To **(109)** (74.8 g, 0.41 mol) in MeOH (1500 mL) was added thionyl chloride (50 mL, 0.7 mol). The mixture was boiled under reflux for 20 h. Evaporation gave **(110)** (78.6 g, 98%) as pale yellow crystals: mp 64-66°C (lit.[197] mp 64-65°C); TLC Rf = 0.7 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.61 (3 H, s, $ArCH_3$), 3.93 (3 H, s, H_3CO), 7.38 (1 H, t, *J* = 8.0 Hz, 5-H), 7.84 (1 H, d, *J* = 8.0 Hz, 6-H), 7.98 (1 H, d, *J* = 8.0 Hz, 4-H).

5-NITROISOCOUMARIN (112) AND 3-METHOXY-5-NITRO-3,4-DIHYDROISOCOUMARIN (113)



PROCEDURE (1)

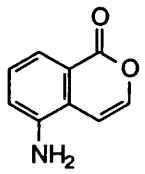
To **(110)** (50.0 g, 0.26 mol) in DMF (153 mL) was added dimethylformamide dimethylacetal (120 mL). The solution was heated at 120°C for 20 h. Evaporation gave the crude enamine **(111)** as a black tar: ^{1}H NMR ($CDCl_3$) δ 2.83 (6 H, s, $N(CH_3)_2$), 5.66 (1 H, d, *J* = 13.6 Hz, NC=CH), 6.34 (1 H, d, *J* = 13.6 Hz, NCH=C), 7.09 (1 H, t, *J* = 7.9 Hz, 7-H), 7.70 (1 H, d, *J* = 7.9 Hz, 6-H), 7.72 (1 H, d, *J* = 7.9 Hz, 8-H). Chromatography (hexane / EtOAc 10:1) gave **(112)** (5.0 g, 10%) as pale yellow crystals. Further elution gave crude **(112)** and **(113)**. Recrystallisation (hexane / EtOAc 10:1) gave **(112)** (24.8 g, 50%) as pale yellow crystals: mp 172-173°C (lit.[197] mp 173-174°C); TLC Rf = 0.2 (EtOAc / hexane 1:10); ν_{max} (KBr) 1720,

1540 and 1340 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 (1 H, dd, J = 6.1, 0.5 Hz, 4-H), 7.44 (1 H, d, J = 6.1 Hz, 3-H), 7.68 (1 H, t, J = 8.1 Hz, 7-H), 8.50 (1 H, dd, J = 8.1, 1.3 Hz, 6-H), 8.65 (1 H, ddd, 8.1, 1.3, 0.5 Hz, 8-H); MS (FAB^+) *m/z* 192 (M + H).

PROCEDURE (2)

To **(110)** (78.6 g, 0.4 mol) in DMF (240 mL) was added dimethylformamide dimethylacetal (185 mL). The solution was heated at 120°C for 20 h. Evaporation gave the crude enamine **(111)** as a black tar. Column chromatography (hexane / EtOAc 10:1) gave **(112)** (29.9 g, 39%) as pale yellow crystals, with properties as above. Further elution gave **(113)** (9.3 g, 10%) as pale yellow crystals: mp 112-113°C (lit.[197] mp 111.5-112.5°C); TLC Rf = 0.15 (EtOAc / hexane 1:10); ^1H NMR ($CDCl_3$) δ 3.54 (3 H, s, CH_3), 3.59 (2 H, d, J = 3.1 Hz, 4-H_2), 5.52 (1 H, t, J = 3.1 Hz, 3-H), 7.59 (1 H, t, J = 8.2 Hz, 7-H), 8.30 (1 H, dd, J = 8.2, 1.2 Hz, 6-H), 8.44 (1 H, d, J = 8.2, 1.2 Hz, 8-H); MS (FAB^+) *m/z* 447 (2 M + H), 224 (100%) (M + H), 192 (M + H - MeOH), 173 (M – H_2O).

5-AMINOISOCOUMARIN (114)



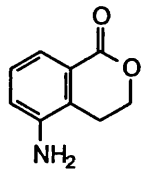
PROCEDURE (1)

To **(112)** (2.98 g, 15.6 mmol) in THF (44 mL) was added aq. HCl (2M, 8 mL). Pd/C (10%, 370 mg,) was added and the flask was filled with hydrogen for 6 h, after which the mixture was filtered through Celite and the solvent was evaporated. The residue, in CH_2Cl_2, was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation gave **(114)** (2.49 g, 99%), as pale yellow crystals: mp 185-187°C (lit.[197] mp 194-195°C); TLC Rf = 0.4 (EtOAc / hexane 1:1); ^1H NMR ($CDCl_3$) δ 3.90 (2 H, brs, NH_2), 6.37 (1 H, dd, J = 5.9, 0.5 Hz, 4-H), 6.96 (1 H, dd, J = 7.9, 1.1 Hz, 6-H), 7.19 (1 H, d, J = 5.9 Hz, 3-H), 7.25 (1 H, t, J = 7.9 Hz, 7-H), 7.68 (1 H, ddd, J = 7.9, 1.1, 0.5 Hz, 8-H); ν_{max} (KBr) 3360, 3300 and 1690 cm^{-1}; MS (FAB^+) *m/z* 162 (M + H).

PROCEDURE (2)

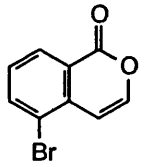
To **(112)** (3.88 g, 20.3 mmol) in EtOH (50 mL) was added $SnCl_2$ (11.6 g, 60.9 mmol). The solution was heated at 70°C for 1 h, after which it was added to ice. The pH was brought to 7-8 by addition of aq. $NaHCO_3$, (5%). Extraction with EtOAc, washing with brine, drying, filtering and evaporation gave **(114)** (2.5 g, 77%) as pale yellow crystals, with properties as above.

5-AMINO-3,4-DIHYDROISOCOUMARIN (115)



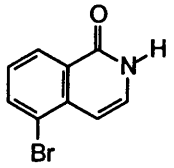
To **(112)** (1.0 g, 5.2 mmol) in THF (15 mL) was added aq. HCl (2 M, 3.0 mL). Pd/C (10%, 124 mg,) was added and the flask was exposed to hydrogen for 12 h, after which the mixture was filtered through Celite and the solvent was evaporated. The residual solid, in CH_2Cl_2, was washed with aq. $NaHCO_3$ and brine, was dried and was filtered. Evaporation gave **(115)** (0.78 g, 91%) as pale yellow crystals: mp 138-140°C; TLC Rf = 0.2 (EtOAc / hexane 1:1); ν_{max} (KBr) 3350, 3305 and 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.74 (2 H, t, J = 6.1 Hz, 4-H_2), 4.46 (2 H, t, J = 6.1 Hz, 3-H_2), 6.86 (1 H, dd, J = 7.9, 1.1 Hz, 6-H), 7.13 (1 H, t, J = 8.1 Hz, 7-H), 7.50 (1 H, dd, J = 8.1, 1.1 Hz, 8-H); MS (FAB^+) *m/z* 164.0719 (M + H) ($^{12}C_9H_9N_1O_2$ requires 164.0712); Found: C, 53.8; H, 5.03; N, 6.86; $C_9H_9NO_2$.HCl requires C, 54.15; H, 5.05; N, 7.02.

5-BROMOISOCOUMARIN (116)



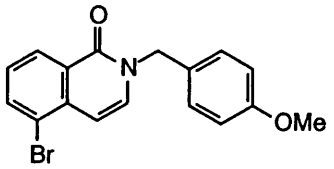
To **(114)** (2.49 g, 15.5 mmol) was added aq. H_2SO_4 (2M, 70 mL) and the solution cooled to 0°C. A solution of sodium nitrite (1.07 g, 15.5 mmol) in H_2O (5 mL) was added, maintaining the temperature < 5°C throughout. KBr (3.65 g, 30.9 mmol) and CuBr (4.45 g, 30.9 mmol) were added slowly to the solution. The solution was stirred for a further 2 h. The evaporation residue, in EtOAc, was washed with H_2O, brine, dried and filtered. Evaporation, column chromatography (hexane / EtOAc 5:1) and recrystallisation (hexane / EtOAc) gave **(116)** (1.22 g, 35%) as white crystals: mp 113-115°C; TLC Rf = 0.9 (EtOAc); ν_{max} (KBr) 1735 and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.87 (1 H, dd, J = 6.0, 0.7 Hz, 4-H), 7.23 (1 H, d, J = 6.0 Hz, 3-H), 7.39 (1 H, t, J = 7.9 Hz, 7-H), 7.95 (1 H, dd, J = 7.9, 1.1 Hz, 6-H), 8.29 (1 H, ddd, J = 7.9, 1.1, 0.7 Hz, 8-H); MS (FAB^+) *m/z* 226.9534 (M + H) ($C_9H_5O_2{}^{81}Br$ requires 226.9531), 224.9551 (M + H) ($C_9H_5O_2{}^{79}Br$ requires 224.9551); Found: C, 48.0; H, 2.23, $C_9H_5O_2Br$ requires C, 48.04; H, 2.24%.

5-BROMOISOQUINOLIN-1-ONE (108)



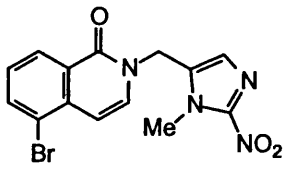
Compound **(116)** (0.60 g, 2.7 mol) in 2-methoxyethanol (50 mL) was saturated with NH_3 for 1 h. The solution was boiled under reflux for 8 h. Evaporation and recrystallisation (MeCN) gave **(108)** (428 mg, 71%) as white crystals: mp 220-222°C (lit.[70] mp 242-244°C); TLC Rf = 0.6 (EtOAc); ν_{max} (KBr) 3400, 1720 and 690 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 6.66 (1 H, d, J = 7.9 Hz, 4-H), 7.35 (1 H, dd, J = 8.1, 7.7 Hz, 7-H), 7.42 (1 H, d, J = 7.9 Hz, 3-H), 8.03 (1 H, d, J = 7.7 Hz, 6-H), 8.21 (1 H, d, J = 8.1 Hz, 8-H), 11.55 (1 H, brs, NH); MS (FAB^+) *m/z* 377 (M + H + mNBA), 224 (M + H).

5-BROMO-2-(4-METHOXYPHENYLMETHYL)ISOQUINOLIN-1-ONE (117)



Compound **(116)** (100 mg, 0.44 mmol) in 2-methoxyethanol (1.0 mL) was boiled under reflux with 4-methoxybenzylamine (63 mg, 0.44 mmol) for 24 h. Evaporation, column chromatography (hexane / EtOAc 5:1), and recrystallisation (Et_2O) gave **(117)** (40 mg, 27%) as white crystals: mp 97-100°C (lit.[70] mp 98-100°C); TLC Rf = 0.8 (EtOAc / hexane 1:1); ν_{max} (KBr) 1640, 1610 and 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.79 (3 H, s, CH_3), 5.15 (2 H, s, CH_2), 6.82 (1 H, d, J = 7.6 Hz, 4-H), 6.87 (2 H, d, J = 8.5 Hz, Ph 3,5-H_2), 7.18 (1 H, d, J = 7.6 Hz, 3-H), 7.27 (2 H, J = 8.5 Hz, Ph 2,6-H_2), 7.33 (1 H, t, J = 8.0 Hz, 7-H), 7.87 (1 H, dd, J = 8.0, 1.5 Hz, 6-H), 8.43 (1 H, ddd, J = 8.0, 1.5, 0.5 Hz, 8-H).

5-BROMO-2-((1-METHYL-2-NITROIMIDAZOL-5-YL)METHYL)-ISOQUINOLIN-1-2-ONE (118)



PROCEDURE (1)

Lithium bis(trimethylsilyl)amide (1.0 M in THF, 340 μL, 340 μmol) was stirred with **(108)** (58 mg, 260 μmol) in dry DMF (1.0 mL) for 2 h. Compound **(101)** (37 mg, 210 μmol) in dry DMF (1.0 mL) and NaI (2 mg) were added and the mixture was stirred for 2 d. The evaporation residue, in EtOAc, was washed with H_2O and brine and was dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:2) gave **(118)** (66 mg, 85%) as pale yellow crystals: mp 208-210°C; TLC Rf = 0.8 (EtOAc / hexane 1:1); ν_{max} (KBr) 1635, 1610, 1525, 1350 and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.99 (3 H, s, NCH_3), 5.23 (2 H, s, CH_2), 6.93 (1 H, dd, J = 8.4, 0.5 Hz, isoquinoline 4-H), 7.14 (1 H, d, J = 8.4 Hz, isoquinoline 3-H), 7.23 (1 H, s, imidazole 4-H), 7.38 (1 H, t, J = 8.1 Hz, isoquinoline 7-H), 7.93 (1H, dd, J = 8.1, 1.1

Hz, isoquinoline 6-H), 8.39 (1 H, ddd, J = 8.2, 1.1, 0.5 Hz, isoquinoline 8-H); MS (EI) *m/z* 363.9983 (M) ($C_{14}H_{11}{}^{81}BrN_4O_3$ requires 362.0015), 362, 345, 318, 316, 140; Found: C, 46.4; H, 3.09; N, 15.0; $C_{14}H_{11}BrN_4O_3$ requires C, 46.30; H, 3.05; N, 15.43%.

PROCEDURE (2)

Lithium bis(trimethylsilyl)amide (1.0 M in THF, 35 μL, 35 μmol) was stirred with **(108)** (5 mg, 23.5 μmol) in dry DMF (0.5 mL) for 2 h. Compound **(102)** (4 mg, 18.1 μmol) in dry DMF (0.5 mL) and NaI (2 mg) were added and the mixture was stirred for 5 d. The evaporation residue, in EtOAc, was washed with H_2O, brine, dried and filtered. Evaporation and preparative TLC (EtOAc / hexane 1:2) gave **(118)** (4.3 mg, 65%) as pale yellow crystals, with the above properties.

REDUCTIVELY ACTIVATED RELEASE OF 5-BROMOISOQUINOLIN-1-ONE (108)

PROCEDURE (1)

To **(94)** / **(108)** / **(118)** (0.5 mg) and NH_4Cl (0.5 mg) in MeOH (0.95 mL) and H_2O (0.05 mL) was added Zn dust (10 mg). The mixture was stirred. At various time points, aliquots (100 μL) were removed, filtered through glass wool and analysed by HPLC.

PROCEDURE (2)

To **(94)** / **(108)** / **(118)** (0.5 mg) in MeOH (1.0 mL) was added $SnCl_2$ (1.3 mg). The mixture was stirred. At various time points aliquots (100 μL) were removed, filtered through glass wool and analysed by HPLC.

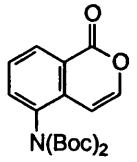
PROCEDURE (3)

To **(94) / (108) / (118)** (5.0 mg) and Pd/C (10%, 5.0 mg) in Pr^iOH (2.0 mL) and H_2O (0.04 mL) was added $NaBH_4$ (2.0 mg). The mixture was stirred. At various time points aliquots (100 μL) were removed, filtered through glass wool and analysed by HPLC.

PROCEDURE (4)

To **(94) / (108) / (118)** (2.2 mg) in Pr^iOH (1.0 mL) was added Pd/C (10%, 12 mg). The mixture was stirred. At various time points, aliquots (100 μL) were removed, filtered through glass wool and analysed by HPLC. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(120)** as colourless crystals: mp 209-211°C (lit.[229] mp 211-214°C); TLC Rf = 0.2 (EtOAc / hexane 1:1); ^1H NMR ($(CD_3)_2SO$); δ 6.55 (1 H, d, J = 7.3 Hz, 4-H), 7.16 (1 H, dd, J = 7.0, 5.5 Hz, 3-H), 7.48 (1 H, m, 5-H), 7.68 (2 H, m, 6,7-H_2), 8.17 (1 H, d, J = 8.1 Hz, 8-H), 11.24 (1 H, brs, N-H); MS (FAB^+) *m/z* 299 (M + nNBA), and 146 (M + H).

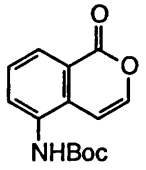
5-(N,N-BIS(1,1-DIMETHYLETHOXYCARBONYL)AMINO)ISOCOUMARIN (121)



To **(114)** (100 mg, 0.62 mmol) in CH_2Cl_2 (1.0 mL) was added DMF (0.5 mL) and triethylamine (10 μL). The solution was cooled to 0°C and di-*t*-butyl dicarbonate (403 mg, 1.84 mmol) in CH_2Cl_2 (1.0 mL) was added slowly. The solution was stirred for 4 d. The evaporation residue in EtOAc, was washed with H_2O, brine, dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:4) gave **(121)** (130 mg, 58%) as white crystals: mp 165-167°C; TLC Rf = 0.8 (EtOAc / hexane 1:1); $\nu_{max.}$ (KBr) 3415, 1720 and 635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (18 H, s, 2 x Bu^t), 6.47 (1 H, dd, J = 5.9, 0.6 Hz, 4-H), 7.32 (1 H, d, J = 5.9 Hz, 3-H), 7.53 (1 H, d, J = 7.9 Hz, 7-H), 7.54 (1 H, dd, J = 7.9, 1.3 Hz, 6-H), 8.29 (1 H, ddd, J = 7.9, 1.3, 0.6 Hz, 8-H); MS (FAB^+) *m/z* 362.1617 (M + H) ($C_{19}H_{24}N_1O_6$ requires

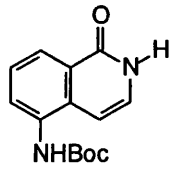
362.1604), 361.1544 (M) ($C_{19}H_{23}N_1O_6$ requires 361.1525), 262 (M + H – Boc), 206 (M + H – Boc - Bu^t).

5-(1, 1-DIMETHYLETHOXYCARBONYLAMINO)ISOCOUMARIN (122)



To **(114)** (0.70 g, 4.4 mmol) in CH_2Cl_2 (3.0 mL) was added DMF (3.5 mL) and triethylamine (70 μL). The solution was cooled to 0°C and di-*t*-butyl dicarbonate (1.4 g, 6.4 mmol), in CH_2Cl_2 (3.0 mL) was added and the solution was stirred for 4 d. The evaporation residue, in EtOAc, was washed with H_2O, brine, dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:4) gave **(122)** (635 mg, 56%) as pale yellow crystals: mp 188-190°C; TLC Rf = 0.7 (EtOAc / hexane 1:1); ν_{max} (KBr) 3320, 1720 and 1682 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (9 H, s, Bu^t), 6.49 (1 H, d, J = 5.9 Hz, 4-H), 6.50 (1 H, brs, N-H), 7.22 (1 H, d, J = 5.9 Hz, 3-H), 7.42 (1 H, t, J = 7.9 Hz, 7-H), 7.97 (1 H, d, J = 7.9 Hz, 6-H), 8.02 (1 H, d, J = 7.9 Hz, 8-H); MS (FAB$^+$) *m/z* 262.1085 (M + H) ($C_{14}H_{16}N_1O_4$ requires 262.1079); 261.1008 (M) ($C_{14}H_{15}N_1O_4$ requires 261.1001), 206 (M – Bu^t); Found C, 64.20; H, 5.83; N, 5.35 $C_{14}H_{15}N_1O_4$ requires C, 64.36; H, 5.75; N, 5.36.

5-(1,1-DIMETHYLETHOXYCARBONYLAMINO)ISOQUINOLIN-1-ONE (123)



PROCEDURE (1)

To **(121)** (68 mg, 0.19 mmol) was added 2-methoxyethanol (10 mL). The solution was saturated with NH_3 for 1 h after which it was boiled under reflux for 24 h. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(123)** (35 mg, 71%) as pale yellow crystals: mp >230°C; TLC Rf = 0.5 (EtOAc); ν_{max} (KBr) 3320, 1690 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.54 (9 H, s, (Bu^t), 6.58 (1 H, d, J = 7.3 Hz, 4-H), 6.61 (1 H, brs, N*H*Boc), 7.22 (1 H, d, J = 7.3 Hz, 3-H), 7.49 (1 H, t, J = 8.1 Hz, 7-H), 8.04 (1 H, d, J = 8.1 Hz, 6-H), 8.22 (1 H, d, J = 8.1 Hz, 8-H), 10.60 (1 H, brs, N-H); MS (FAB^+) *m/z* 261.1235 (M + H) ($C_{14}H_{17}N_2O_3$ requires 261.1239); 260.1169 (M) ($C_{14}H_{16}N_2O_3$ requires 260.1161); Found C, 63.5; H, 6.28; N, 10.58; $C_{14}H_{16}N_2O_3$. 0.25 H_2O C, 63.60; H, 6.22, N, 10.20.

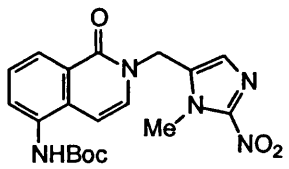
PROCEDURE (2)

To **(122)** (376 mg, 1.4 mmol) was added 2-methoxyethanol (10 mL). The solution was saturated with NH_3 for 1 h after which it was boiled under reflux for 24 h. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(123)** (283 mg, 76%) as pale yellow crystals, with properties as above.

5-AMINOISOQUINOLIN-1-ONE HYDROCHLORIDE (125)

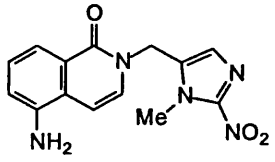
HCl was passed through **(124)** (50 mg, 0.19 mmol) in $CHCl_3$ (20 mL) for 1 h. Filtration gave **(125)** (30 mg, 79%) as white crystals: mp >230°C (no literature mp reported); ν_{max} (KBr) 3140 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.02 (3 H, s, N^+H_3), 6.63 (1 H, d, J = 7.4 Hz, 4-H), 7.31 (1 H, d, J = 7.4 Hz, 3-H), 7.47 (1 H, t, J = 7.8 Hz, 7-H), 7.70 (1 H, d, J = 7.8 Hz, 6-H), 8.09 (1 H, d, J = 7.8 Hz, 8-H), 11.50 (1 H, brs, N-H); MS (FAB^+) *m/z* 161 (M + H), 143 (M – H_2O).

5-(1,1-DIMETHYLETHOXYCARBONYLAMINO)-2-((1-METHYL-2-NITROIMIDAZOL-5-YL)METHYL)ISOQUINOLIN-1-ONE (126)



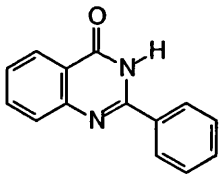
Lithium bis(trimethylsilyl)amide (1.0 M in THF, 280 μL, 0.28 mmol) was stirred with **(123)** (45 mg, 0.17 mmol) in dry DMF (1.0 mL) for 2 h. Compound **(101)** (39.5 mg, 0.22 mmol) in dry DMF (1.0 mL) and NaI (2 mg) were added and the mixture was stirred for 2 d. The evaporation residue, in EtOAc, was washed with H_2O and brine and was dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(126)** (37 mg, 54%) as a yellow oil: TLC Rf = 0.7 (EtOAc); ^1H NMR ($CDCl_3$) δ 1.53 (9 H, s, Bu^t), 4.05 (3 H, s, NCH_3), 5.29 (2 H, s, CH_2), 6.58 (1 H, brs, N-H), 6.60 (1 H, d, J = 7.7 Hz isoquinoline 4-H), 7.07 (1 H, d, J = 7.7 Hz, isoquinoline 3-H), 7.21 (1 H, s, imidazole 4-H), 7.52 (1 H, t, J = 7.9 Hz, isoquinoline 7-H), 7.98 (1 H, d, J = 7.9 Hz isoquinoline 6-H), 8.24 (1 H, d, J = 7.9 Hz, isoquinoline 8-H); MS (FAB^+) *m/z* 400.1624 (M + H) ($C_{19}H_{22}N_5O_5$ requires 400.1621), 399.1551 (M) ($C_{19}H_{21}N_5O_5$ requires 399.1543).

5-AMINO-2-((1-METHYL-2-NITROIMIDAZOL-5-YL)METHYL)-ISOQUINOLIN-1-ONE (127)



To **(126)** (10 mg, 0.03 mmol) was added trifluoroacetic acid (1.0 mL). The solution was stirred for 20 min. After evaporation, the oil, in EtOAc, was washed with aq. K_2CO_3 (2 M) and brine and was dried and filtered. Evaporation gave **(127)** (6.0 mg, 80%) as yellow crystals: mp >230°C; TLC Rf = 0.3 (EtOAc); ν_{max} 3360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (2 H, brs, NH_2), 4.05 (3 H, s, NCH_3), 5.30 (2 H, s, CH_2), 6.51 (1 H, d, J = 7.9 Hz, isoquinoline 4-H), 7.00 (1 H, dd, J = 7.7, 1.1 Hz, isoquinoline 6-H), 7.01 (1 H, d, J = 7.9 Hz, 3-H), 7.22 (1 H, s, imidazole 4-H), 7.34 (1 H, t, J = 8.1 Hz, isoquinoline 7-H), 7.88 (1 H, d, J = 8.1 Hz, isoquinoline 8-H); MS (FAB^+) *m/z* 300.1087 (M + H) ($C_{14}H_{14}N_5O_3$ requires 300.1097), 299.1010 (M) ($C_{14}H_{13}N_5O_3$ requires 299.1018); ^1H NMR (CF_3CO_2D) δ 4.52 (3 H, s, NCH_3), 5.68 (2 H, s, CH_2), 7.19 (1 H, t, J = 7.3 Hz, isoquinoline 4-H), 7.78 (1 H, dd, J = 7.3 Hz, isoquinoline 3-H), 7.83 (1 H, d, J = 7.3 Hz, 7-H), 7.86 (1 H, s, imidazole 4-H), 8.11 (1 H, d, J = 7.3 Hz, isoquinoline 6-H), 8.62 (1 H, d, J = 7.3 Hz, isoquinoline 8-H); MS (FAB^+) *m/z* 301.1136 (M) ($C_{14}H_{13}{}^2HN_5O_3$ requires 301.1159).

2-PHENYLQUINAZOLIN-4(3H)-ONE (130)



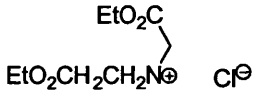
PROCEDURE (1)

To **(94)** (78 mg, 0.5 mmol), **(128)** (92 mg, 0.38 mmol) and NaI (2 mg) in dry DMF (1 mL) was added lithium bis(trimethylsilyl)amide (1.0 M in THF, 625 μL, 0.61 mmol) under Ar. The solution was stirred for 12 h at 20°C and at 50°C for 24 h. The evaporation residue, in EtOAc, was washed with H_2O, aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(130)** (65 mg, 77%) as pale yellow crystals: mp 238-240°C (lit.[230] mp 237-239°C); TLC Rf = 0.6 (EtOAc / hexane 1:1); ^{1}H NMR ($CDCl_3$) δ 7.56 (4 H, m, Ph 3,4,5-H_3 + 6-H), 7.84 (2 H, m, 7,8-H_2), 8.23 (3 H, m, Ph 2,6-H_2 + 5-H), 10.20 (1 H, s, NH).

PROCEDURE (2)

To **(94)** (85 mg, 0.5 mmol) and **(128)** (100 mg, 0.4 mmol) was added lithium bis(trimethylsilyl)amide (1.0 M in THF, 420 μL, 0.42 mmol) and the mixture was boiled under reflux for 10 h, under Ar. The evaporation residue, in EtOAc, was washed with H_2O, aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(130)** (70 mg, 76%) as pale yellow crystals, with properties as above.

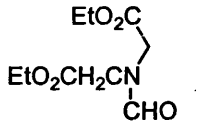
DIETHYL IMINODIACETATE HYDROCHLORIDE (132)



To **(131)** (10.5 g, 0.08 mol) in EtOH (200 mL) was added thionyl chloride (35 mL, 0.5 mol). The mixture was stirred for 24 h. Evaporation and washing with Et_2O (500 mL) gave **(132)** (17.8 g, 100%) as a buff gum: ^{1}H NMR ($CDCl_3$) δ 1.31 (6 H, t,

J = 7.0 Hz, 2 x CH_2CH_3), 3.49 (4 H, s, 2 x NCH_2), 4.22 (4 H, q, J = 7.0 Hz, 2 x CH_2CH_3); MS (FAB+) *m/z* 379 (2 M + H), 190 (M + H), 116 (M – CH_2CO_2Et).

DIETHYL N-FORMYLIMINODIACETATE (134)



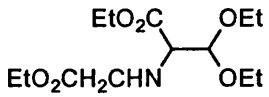
PROCEDURE (1)

Compound **(132)** (34.5 g, 0.15 mol) was dissolved with heating in 99% formic acid (19 mL, 0.5 mol) and was added to a hot solution of sodium formate (11.5 g, 0.17 mol) in 99% formic acid (15 mL, 0.41 mol). The solution was stirred for 4 h then filtered. The solid was washed with Ac_2O (70 mL, 0.73 mol) which was then added slowly to the formic acid solution. The mixture was boiled under reflux for 1 h and the solvent was evaporated. Distillation gave **(134)** (28.5 g, 86%) as a colourless liquid $bp_{0.1}$ 100°C: TLC Rf = 0.3 (EtOAc); ν_{max} 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (3 H, t, J = 7.1 Hz, CH_2CH_3), 1.28 (3 H, t, J = 7.1 Hz, CH_2CH_3), 4.13 (4 H, s, 2 x NCH_2), 4.24 (2 H, q, J = 7.1 Hz, CH_2CH_3), 4.25 (2 H, q, J = 7.1 Hz, CH_2CH_3), 8.12 (1 H, s, NCHO); MS (EI+) *m/z* 218.0979 (M + H) ($C_9H_{16}N_1O_5$ requires 218.1028), 217.0942 (M) ($C_9H_{15}N_1O_5$ requires 217.0950), 189 (M – C_2H_4).

PROCEDURE (2)

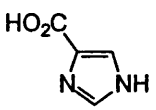
Compound **(132)** (20.0 g, 0.09 mol) was dissolved with heating in 99% formic acid (12 mL, 0.3 mol) and was added to a hot solution of sodium formate (6.9 g, 0.1 mol) in 99% formic acid (10 mL, 0.2 mol). The solution was stirred for 4 h then filtered. The solid was washed with Ac_2O (41 mL, 0.4 mol) which was then added slowly to the formic acid solution. The mixture was heated for 1 h and the solvent was evaporated. The oil, in EtOAc, was washed with aq. $NaHCO_3$ and brine. Drying, filtration and evaporation gave **(134)** (15.5g, 81%) as a colourless oil, with properties as above.

ETHYL 3,3-DIETHOXY-2-(ETHOXYCARBONYLMETHYLAMINO) PROPANOATE (136)



To **(134)** (26.2 g, 0.12 mol) and ethyl formate (26 mL, 0.33 mol) in dry Et_2O (55 mL) at 0°C was added a suspension of sodium ethoxide (9.0 g, 0.13 mol) in dry Et_2O (160 mL) dropwise with stirring. After 1 h at 0°C, the mixture was diluted with dry Et_2O (500 mL) and allowed to stand overnight at 4°C. The Et_2O was decanted. The residual sodium enolate **(135)**, was treated with EtOH (320 mL) saturated with HCl and was stirred for 6 h. The mixture was further saturated with HCl and boiled under reflux for 10 h. Aq. potassium carbonate was added until pH reached 13. Extraction with EtOAc, washing with brine, drying, filtering, evaporation and Kugelrohr distillation gave **(136)** (21.9 g, 62%) as a pale yellow oil $bp_{0.15}$ 210°C: TLC Rf = 0.5 (EtOAc); ν_{max} 3320, 1730 and 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (3 H, t, J = 7.0 Hz, OCH_2CH_3), 1.20 (3 H, t, J = 7.0 Hz, OCH_2CH_3), 1.24 (3 H, t, J = 7.0 Hz, $CO_2CH_2CH_3$) 1.27 (3 H, t, J = 7.0 Hz, $CO_2CH_2CH_3$), 2.25 (1 H, brs, NH), 3.39 (1 H, d, J = 18.0 Hz), and 3.47 (1 H, d, J = 18.0 Hz) (NCH_2), 3.52 (1 H, d, J = 5.7 Hz, $NCHCO_2$), 3.54 (1 H, dq, J = 14.0, 7.0 Hz), 3.55 (1 H, dq, J = 14.0, 7.0 Hz), 3.71 (1 H, dq, J = 14.0, 7.0 Hz), 3.72 (1 H, dq, J = 14.0, 7.0 Hz) (2 x OCH_2), 4.12 (1 H, dq, J = 14.0, 7.0 Hz) and 4.19 (1 H, dq, J = 14.0, 7.0 Hz) ($CHCO_2CH_2$), 4.15 (2 H, q, J = 7.0 Hz, $CH_2CO_2CH_2$), 4.66 (1 H, d, J = 5.7 Hz, OCHO); MS (FAB^+) *m/z* 292.1763 (M + H) ($C_{13}H_{26}N_1O_6$ requires 292.1760), 246 (M – OEt).

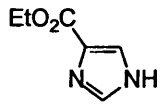
IMIDAZOLE-4-CARBOXYLIC ACID (144)



Compound **(142)** (5.67 g, 36.4 mmol) in Ac_2O (213 mL) was boiled under reflux for 2 d. The solid formed was removed by filtration and the filtrate was evaporated. Recrystallisation (EtOH / Et_2O 3:2) gave **(144)** (2.0 g, 49%) as pale buff crystals: mp

260-265°C (lit.[207] mp 279-281°C); TLC Rf = 0.4 (CH_2Cl_2 / MeOH / H_2O / AcOH 25:10:2:1); ^1H NMR (D_2O) δ 7.70 (1 H, s, 5-H), 8.49 (1 H, s, 2-H).

ETHYL IMIDAZOLE-4-CARBOXYLATE (145)



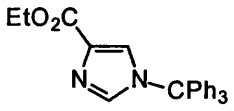
PROCEDURE (1)

Compound **(144)** (5.54 g, 49 mmol) in EtOH (500 mL) was boiled under reflux with thionyl chloride (30 mL, 410 mmol) for 5 h. Evaporation gave **(145)** (6.2 g, 90%) as pale buff crystals: mp 155-157°C (lit.[207] mp 156-158°C); TLC Rf = 0.2 (EtOAc / hexane 1:2); ^1H NMR (D_2O) δ 1.19 (3 H, t, J = 7.0 Hz, CH_3), 3.66 (2 H, q, J = 7.0 Hz, CH_2), 7.52 (1 H, s, 5-H), 7.66 (1 H, s, 2-H).

PROCEDURE (2)

To **(144)** (4.82 g, 43 mmol) in EtOH (90 mL) was added conc. H_2SO_4 (5 mL). The mixture was stirred and refluxed for 40 h, under N_2. The solution was cooled in an ice bath and neutralised to pH 8 with aq. NaOH (10%, 20 mL). Evaporation and recrystallisation (H_2O) gave **(145)** (5.15 g, 85%) as pale buff crystals, with properties as above.

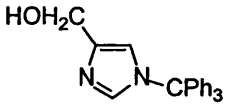
ETHYL 1-(TRIPHENYLMETHYL)IMIDAZOLE-4-CARBOXYLATE (146)



Compound **(145)** (6.81 g, 48.6 mmol) in dry DMF (162 mL) was stirred with triethylamine (21 mL) and chlorotriphenylmethane (13.9 g, 49.6 mmol) for 16 h. The evaporation residue, in $CHCl_3$, was washed with aq. $NaHCO_3$ and brine and was dried and filtered. Recrystallisation (EtOH / H_2O 3:1) gave **(146)** (0.52 g, 3%) as

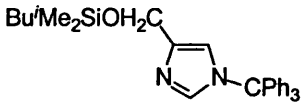
pale yellow crystals. Evaporation of the filtrate and column chromatography (EtOAc / hexane 1:2) gave **(146)** (11.3 g, 61%) as pale yellow crystals: mp 167-169°C (lit.[207] mp 168-169°C); TLC Rf = 0.6 (EtOAc / hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 1.37 (3 H, t, J = 7.0 Hz, CH_3), 4.35 (2 H, q, J = 7.0 Hz, CH_2), 7.10 (6 H, m, 3 × Ph 2,6-H_2), 7.35 (9 H, m, 3 × Ph 3,4,5-H_3), 7.46 (1 H, d, J = 1.4 Hz, 5-H), 7.58 (1 H, d, J = 1.4 Hz, 2-H).

1-(TRIPHENYLMETHYL)IMIDAZOLE-4-METHANOL (147)



To a suspension of $LiAlH_4$ (1.25 g, 30 mmol) in dry THF (108 mL) at 0°C was added a solution of **(146)** (10.8 g, 28 mmol) in dry THF (108 mL) over 15 min with stirring. The mixture was stirred at 20°C for 1 h, then cooled to 0°C. H_2O (1.25 mL) was added slowly, followed by aq. NaOH (10%, 1.85 mL) and H_2O (3.0 mL). The mixture was stirred for 30 min then filtered. The filter cake was washed with hot THF and extracted with hot EtOH. The combined ethanolic extracts and THF filtrate were cooled to give, after filtration and drying, **(147)** (8.9 g, 93%) as white crystals: mp 238-241°C (lit.[207] mp 239-240°C); TLC Rf = 0.3 (EtOAc / MeOH 5:1); ^{1}H NMR ($CDCl_3$) δ 4.51 (2 H, s, CH_2), 6.71 (1 H, d, J = 1.5 Hz, 5-H), 7.10 (6 H, m, 3 × Ph 2,6-H_2), 7.35 (9 H, m, 3 × Ph 3,4,5-H_3), 7.35 (1 H, d, J = 1.5 Hz, 2-H); MS (FAB^+) *m/z* 341 (M + H), 243 (CPh_3).

4-(*t*-BUTYLDIMETHYLSILYLOXY)METHYL-1-(TRIPHENYLMETHYL)-IMIDAZOLE (153)



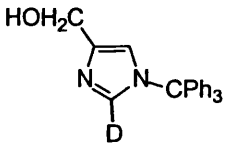
PROCEDURE (1)

To **(147)** (2.15 g, 6.3 mmol) and imidazole (1.03 g, 15.1 mmol) in dry DMF (8.2 mL) was added *t*-butyldimethylchlorosilane (1.14 g, 7.6 mmol). The solution was heated at 45°C for 14 h, under N_2. Saturated aq. Na_2CO_3 was added and the mixture was extracted with Et_2O. The extract was washed with brine, dried and filtered. Evaporation gave **(153)** (2.84 g, 99%), as colourless crystals: mp 81-83°C (lit.[208] mp 79-80°C); TLC Rf = 0.7 (EtOAc / MeOH 5:1); ^{1}H NMR ($CDCl_3$) δ 0.01 (6 H, s, $Si(CH_3)_2$), 0.83 (9 H, s, Bu^t), 4.67 (2 H, s, CH_2), 6.71 (1 H, s, 5-H), 7.10 (6 H, m, 3 × Ph 2,6-H_2), 7.35 (9 H, m, 3 × Ph 3,4,5-H_3), 7.36 (1 H, s, 2-H); MS (FAB+) *m/z* 909 (2 M + H), 477 (M + Na), 456.2554 (M + H) ($C_{28}H_{35}N_2OSi$ requires 456.2552), 455.2531 (M) ($C_{29}H_{34}N_2OSi$ requires 455.2519).

PROCEDURE (2)

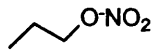
To **(147)** (0.99 g, 2.9 mmol) and imidazole (0.43 g, 6.0 mmol) in dry DMF (10.0 mL) was added *t*-butyldimethylchlorosilane (0.45 g, 3.0 mmol). The mixture was stirred for 5 d. The evaporation residue, in $CHCl_3$, was washed with saturated aq. Na_2CO_3, H_2O, brine, dried and filtered. Evaporation and column chromatography (hexane / EtOAc 3:1) gave **(153)** (1.03 g, 78%) as colourless crystals, with properties as above.

2-DEUTERIO-1-(TRIPHENYLMETHYL)IMIDAZOLE-4-METHANOL (152)



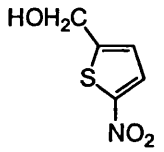
To **(147)** (250 mg, 0.74 mmol) in DME (2.0 mL) was added BuLi (1.6 M in hexanes; 460 μL, 0.7 mmol). The solution was heated at 45°C for 4 h and at 20°C for 12 h, under Ar. D_2O (0.1 mL) in DME (0.5 mL) was added and the solution was stirred for 1 h. The evaporation residue, in EtOAc, was washed with H_2O, brine, dried and filtered. Evaporation gave crude **(152)**: ^1H NMR ($(CD_3)_2SO$) δ 4.33 (2 H, d, J = 5.0 Hz, CH_2), 4.90 (1 H, t, J = 5.0 Hz, OH), 6.72 (1 H, s, 5-H), 7.10 (6 H, m, 3 x Ph 2, 6-H_2), 7.30 (0.5 H, d, J = 1.4 Hz, 2-H), 7.41 (9 H, m, 3 x Ph 3,4,5-H_3); MS (FAB^+) *m/z* 342.1718 (M + H) ($C_{23}H_{20}{}^2HN_2O$ requires 342.1717), 341.1662 (M) ($C_{23}H_{19}{}^2HN_2O$ requires 341.1638), 243 (CPh_3).

PROPYL NITRATE (157)



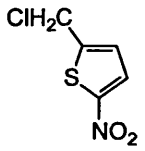
Concentrated nitric acid (10 mL) at 0°C was added slowly to concentrated sulfuric acid (10 mL) at 0°C. Propanol (7 mL) was very slowly added dropwise to the ice-cooled solution. The solution was vigorously stirred and the temperature kept <3 °C. The ester was decanted and immediately washed with aq. Na_2CO_3 (10 mL), H_2O (10 mL), dried ($CaCl_2$) and filtered to yield **(157)** (5 mL) as a colourless liquid: ^1H NMR ($CDCl_3$) δ 1.01 (3 H, t, J = 7.5 Hz, CH_3), 1.76 (2 H, m, $CH_3CH_2CH_2$), 4.42 (2 H, t, J = 6.8 Hz, CH_2ONO_2).

5-HYDROXYMETHYL-2-NITROTHIOPHENE (159)



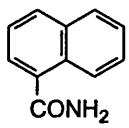
To **(158)** (500 mg, 3.2 mmol) in dry MeOH (100 mL) was added $NaBH_4$ (360 mg, 9.5 mmol). The solution was stirred for 1 h. The excess $NaBH_4$ was decomposed with glacial AcOH (1.0 mL). The evaporation residue, in EtOAc, was washed with H_2O, $NaHCO_3$, brine, dried and filtered. Evaporation gave **(159)** (500 mg, 99%) as a buff oil: TLC Rf = 0.4 (EtOAc / hexane 1:1); ^1H NMR ($CDCl_3$) δ 4.59 (2 H, s, CH_2), 6.94 (1 H, dt, J = 4.0, 0.7 Hz, 4-H), 7.83 (1 H, d, J = 4.0 Hz, 3-H).

5-CHLOROMETHYL-2-NITROTHIOPHENE (160)



To **(159)** (500 mg, 3.1 mmol) was added thionyl chloride (1.0 mL, 13.7 mmol). The solution was stirred for 2 h. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(160)** (550 mg, 99%) as a buff oil: TLC Rf = 0.9 (EtOAc / hexane 1:1); ^1H NMR ($CDCl_3$) δ 4.73 (2 H, d, J = 0.8 Hz, CH_2), 7.05 (1 H, dd, J = 4.0, 0.8 Hz, 4-H), 7.80 (1 H, d, J = 4.0 Hz, 3-H).

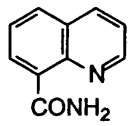
NAPHTHALENE-1-CARBOXAMIDE (176)



To **(174)** (2.25g, 13.1 mmol) was added thionyl chloride (60 mL, 0.82 mol) and the solution was stirred for 24 h. Evaporation gave **(175)** (2.5 g, 100%) as pale buff oil; TLC Rf = 0.2 (EtOAc / hexane 1:1); ^1H NMR ($CDCl_3$) δ 7.53 (1 H, t, J = 7.8 Hz, 3-H), 7.55 (1 H, dt, J = 7.8, 1.3 Hz, 7-H), 7.65 (1 H, ddd, J = 8.6, 6.9, 1.7 Hz, 6-H), 7.88 (1 H, dd, J = 8.2, 1.7 Hz, 8-H), 8.08 (1 H, d, J = 8.2 Hz, 4-H), 8.53 (1 H, dd, J =

7.3, 1.3 Hz, 5-H), 8.73 (1 H, d, J = 9 Hz, 2-H). Compound **(175)** (2.5 g, 13.1 mmol) in $CHCl_3$ (50 mL) was saturated with NH_3 for 1 h. Filtration gave **(176)** (1.95 g, 87%) as pale buff crystals: mp 198-200°C (lit.[231] mp 203-204°C); TLC Rf = 0.2 (EtOAC / hexane 1:1); ν_{max} 3310, 3140 and 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.74 (4 H, m, 3,5,6,7-H_4, N-H), 7.96 (1 H, d, J = 5.5 Hz, 4-H), 7.99 (1 H, brs, NH), 8.00 (1 H, d, J = 8.1 Hz, 8-H), 8.30 (1 H, d, J = 9.9 Hz, 2-H); MS (FAB^+) *m/z* 343 (2 M + H), 172 (M + H).

QUINOLINE-8-CARBOXAMIDE (180)



PROCEDURE (1)

To **(178)** (385 mg, 2.2 mmol) was added thionyl chloride (10.0 mL, 0.14 mol). The solution was boiled under reflux for 12 h. Following evaporation the oil, in $CHCl_3$ (50 mL), was saturated with NH_3 for 1 h. Filtration gave **(180)** (0.34 g, 90%) as pale buff crystals: mp 170-172°C (lit.[232] mp 171-173°C); TLC Rf = 0.2 (EtOAc / CH_2Cl_2 1:2); ^1H NMR ($CDCl_3$) δ 6.04 (1 H, brs, NH), 7.44 (1 H, dd, J = 8.4, 4.4 Hz, 3-H), 7.63 (1 H, t, J = 8.1 Hz, 6-H), 7.94 (1 H, dd, J = 8.4, 1.8 Hz, 4-H), 8.23 (1 H, dd, J = 8.4, 1.8 Hz, 5-H), 8.81 (1 H, dd, J = 7.3, 1.8 Hz, 7-H), 8.88 (1 H, dd, J = 4.4, 1.8 Hz, 2-H), 10.94 (1 H, brs, NH).

PROCEDURE (2)

To **(178)** (0.5 g, 2.9 mmol) in MeOH (200 mL) was added thionyl chloride (20 mL, 0.28 mol). The solution was stirred for 24 h. To the evaporation oil, in MeOH (25 mL), was added conc. NH_3 (25 mL) at 0°C. Filtration and column chromatography (EtOAc / hexane 2:1) gave **(180)** (380 mg, 77%), as pale buff crystals with properties as above.

PROCEDURE (3)

To **(191)** (0.25 g, 1.2 mmol) in dry THF (1 mL) at –79°C was added BuLi (1.6 M in hexanes, 0.83 mL, 1.3 mmol). After 30 min trimethylsilylisocyanate (0.5 mL, 3.7 mmol) was added. The solution was stirred for a further 15 min at –79°C and for 12 h at 20°C. The evaporation residue, inCH_2Cl_2, was washed with H_2O, brine, dried and filtered. Evaporation and column chromatography (CH_2Cl_2 / EtOAc 2:1) gave **(180)** (164 mg, 79%) as pale buff crystals, with properties as above.

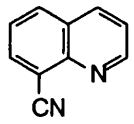
PROCEDURE (4)

To **(187)** (0.5 g, 2.2 mmol) in dry THF (2 mL) at –79°C was added BuLi (1.6 M in hexanes, 1.65 mL, 2.5 mmol). After 30 min trimethylsilylisocyanate (1.0 mL, 7.4 mmol) was added. The solution was stirred for a further 15 min at –79°C and for 12 h at 20°C. The evaporation residue, in CH_2Cl_2, was washed with H_2O, brine, dried and filtered. Evaporation and column chromatography (CH_2Cl_2 / EtOAc 2:1) gave **(180)** (295 mg, 77%) as pale buff crystals, with properties as above: In some runs was obtained crude 2-butylquinoline **(190)** (21.7 mg, 19%): TLC Rf = 0.6 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.89 (3 H, t, J = 7.3 Hz, CH_3), 1.37 (2 H, sextet, J = 7.3 Hz, CH_2CH_3), 1.72 (2 H, qn, J = 7.3 Hz, $CH_2CH_2CH_3$), 2.90 (2 H, t, J = 7.3 Hz, $CH_2CH_2CH_2CH_3$), 7.21 (1 H, dd, J = 8.4, 5.0 Hz, 3-H), 7.40 (1 H, m, 6-H), 7.60 (1 H, m, 7-H), 7.70 (1 H, d, J = 8.1 Hz, 5-H), 7.97 (1 H, d, J = 8.4 Hz, 4-H), 7.98 (1 H, d, J = 8.1 Hz, 8-H).

PROCEDURE (5)

To **(183)** (60 mg, 0.4 mmol) in EtOH (1 mL) was added aq. NaOH (20.0 μL, 0.5 M) and 27% H_2O_2 (20.0 μL). The solution was heated at 50°C for 1 h, then neutralised with aq. HCl (1.0 mL, 2M). The evaporation residue, in EtOAc, was washed with brine, dried and filtered. Evaporation and column chromatography (EtOAc / CH_2Cl_2 1:2) gave **(180)** (65 mg, 97%), as pale yellow crystals with properties as above.

8-CYANOQUINOLINE (183)



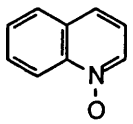
PROCEDURE (1)

To **(180)** (146 mg, 0.85 mmol) was added thionyl chloride (2.0 mL, 27.0 mmol). The solution was boiled under reflux for 1 h. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(183)** (105 mg, 80%), as pale yellow crystals: mp 85-86°C (lit.[233] mp 87.5-88.3°C); TLC Rf = 0.7 (EtOAc); ν_{max} 2240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.57 (1 H, dd, J = 8.4, 4.2 Hz, 3-H), 7.63 (1 H, dd, J = 8.2, 7.1 Hz, 6-H), 8.09 (1 H, dd, J = 8.2, 1.6 Hz, 4-H), 8.14 (1 H, dd, J = 7.1, 1.3 Hz, 5-H), 8.27 (1 H, dd, J = 8.4, 1.3 Hz, 7-H), 9.11 (1 H, dd, J = 4.2, 1.6 Hz, 2-H).

PROCEDURE (2)

To **(180)** (100 mg, 0.6 mmol) in pyridine (110 μL) was added 4-methyl-benzenesulfonyl chloride (110 mg, 0.6 mmol). The solution was stirred for 3 h. The evaporation residue, in EtOAc, was washed with aq. HCl (2 M), aq. $NaHCO_3$, brine, dried and filtered. Evaporation gave **(183)** (87 mg, 97%) as pale yellow crystals, with properties as above.

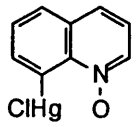
QUINOLINE-N-OXIDE (185)



To **(184)** (50.2 g, 0.4 mol) in glacial AcOH (430 mL) was added aq. H_2O_2 (140 mL, 30%). The solution was boiled under reflux for 24 h. The evaporation residue, in EtOAc, was washed with aq. Na_2CO_3, brine, dried and filtered. Evaporation gave **(185)** (53.5 g, 95%), as buff hygroscopic crystals: TLC Rf = 0.5 (MeOH); ν_{max} 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (1 H, dd, J = 8.4, 6.1 Hz, 3-H), 7.65 (1 H, ddd, J =

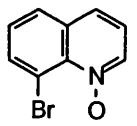
8.2, 7.0, 1.3 Hz, 6-H), 7.75 (1 H, d, J = 8.6 Hz, 4-H), 7.77 (1 H, ddd, J = 8.6, 7.0, 1.5 Hz, 7-H), 7.87 (1 H, d, J = 8.2 Hz, 5-H), 8.55 (1 H, dd, J = 6.1, 0.9 Hz, 2-H), 8.76 (1 H, d, J = 8.6 Hz, 8-H); MS (FAB^+) *m/z* 291 (2 M + H), 146 (M + H).

8-(CHLOROMERCURI)QUINOLINE-N-OXIDE (186)



To **(185)** (20.0 g, 0.14 mol) in glacial AcOH (20 mL) was added $Hg(OAc)_2$ (44.1 g, 0.14 mol). The mixture was boiled under reflux for 5 h. The residue was poured into brine (200 mL) and the solid formed was filtered and washed with H_2O (200 mL) to give **(186)** (43.5 g, 83%), as pale buff crystals: mp >230°C (lit.[210] mp 235°C (decomp); ^1H NMR ($(CD_3)_2SO$) δ 7.58 (1 H, dd, J = 8.4, 5.9 Hz, 3-H), 7.78 (1 H, dd, J = 8.4, 7.1 Hz, 6-H), 8.02 (1 H, d, J = 7.1 Hz, 7-H), 8.05 (1 H, d, J = 8.4 Hz, 4-H), 8.11 (1 H, d, J = 8.2 Hz, 5-H), 8.76 (1 H, dd, J = 5.9, 1.0 Hz, 2-H); MS (FAB^+) *m/z* 381 (M + H) isotope cluster at 381, 346 (M - Cl), 145 (M – HgCl), 129 (M – HgClO).

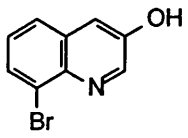
8-BROMOQUINOLINE-N-OXIDE (187)



Bromine (0.51 g, 3.13 mol) and KBr (0.41 g, 3.4 mmol) in H_2O (2 mL) was slowly added to **(186)** (1.08 g, 2.9 mmol) with vigorous stirring. Aq. NaOH (500 mL, 10%) was added to the mixture. The evaporation residue, in $CHCl_3$, was washed with brine, dried and filtered. Evaporation and column chromatography (EtOAc) gave **(187)** (183 mg, 29%), as pale buff crystals: mp 210-212°C (lit.[210] mp 102-104°C); TLC Rf = 0.3 (EtOAc); ^1H NMR ($(CD_3)_2SO$) δ 7.58 (1 H, dd, J = 8.4, 6.0 Hz, 3-H), 7.78 (1 H, dd, J = 8.0, 7.0 Hz, 6-H), 8.02 (1 H, d, J = 7.0 Hz, 7-H), 8.04 (1 H, d, J = 8.4 Hz, 4-H), 8.10 (1 H, d, J = 8.0 Hz, 5-H), 8.75 (1 H, d, J = 6.0 Hz, 2-H); MS (EI^+) *m/z* 225.9641 (M + H) ($C_9H_7{}^{81}BrNO$ requires 225.9691), 224.9613 (M) ($C_9H_6{}^{81}Br$

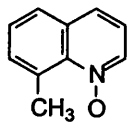
NO requires 224.9612), 223.9669 (M + H) ($C_9H_7{}^{79}BrNO$ requires 223.9711), 222.9634 (M) ($C_9H_6{}^{79}BrNO$ requires 222.9633), 144 (M – Br), 128 (M – BrO).

8-BROMO-3-HYDROXYQUINOLINE (192)



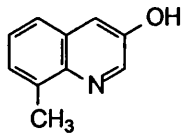
To **(191)** (202 mg, 0.97 mmol) in glacial AcOH (1 mL) was added aq. H_2O_2 (1.0 mL, 30%). The solution was boiled under reflux for 24 h. The evaporation residue, in $CHCl_3$, was washed with aq. $NaHCO_3$ and brine and was dried and filtered. Evaporation and column chromatography (EtOAc) gave **(192)** (188 mg, 86%), as off-white crystals: mp 224-226°C; TLC Rf = 0.5 (EtOAc); ^1H NMR ($CDCl_3$) δ 7.36 (1 H, t, J = 8.0 Hz, 6-H), 7.51 (1 H, d, J = 2.7 Hz, 4-H), 7.67 (1 H, dd, J = 8.1, 1.1 Hz, 5-H), 7.90 (1 H, dd, J = 8.8, 1.1 Hz, 7-H), 8.79 (1 H, d, J = 2.7 Hz, 2-H); MS (FAB^+) *m/z* 225.9672 (M + H) ($C_9H_7{}^{81}Br$ NO requires 225.9691), 223.9699 (M + H) ($C_9H_7{}^{79}BrNO$ requires 223.9711); Found: C, 46.9; H, 2.95; N, 5.64; $C_9H_6BrNO_3.0.25$ H_2O requires C, 46.38; H, 3.03; N, 6.01%.

8-METHYLQUINOLINE-N-OXIDE (199)



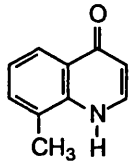
To **(198)** (1.4 g, 9.8 mmol) in $CHCl_3$ (30 mL) was added 3-chloroperoxybenzoic acid (3.35 g, 19.4 mmol). The solution was stirred for 48 h. The evaporation residue, in $CHCl_3$, was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (EtOAc / MeOH 5:1) gave **(199)** (1.48g, 95%) as white crystals: mp 34-35°C (lit.[214] mp 36-38°C); TLC Rf = 0.3 (EtOAc / MeOH 5:1); ν_{max} 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.19 (3 H, s, CH_3), 7.18 (1 H, dd, J = 8.3, 6.1 Hz, 3-H), 7.44 (4 H, m) (4,5,6,7-H_4), 8.39 (1 H, dd, J = 6.0, 1.1 Hz, 2-H); MS (FAB^+) *m/z* 319 (2 M + H), 160 (M + H), 142 (M – H_2O).

8-METHYL-3-HYDROXYQUINOLINE (200)



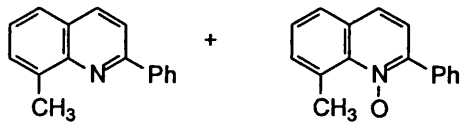
To **(198)** (240 mg, 1.7 mmol) in glacial AcOH (2 mL) was added aq. H_2O_2 (1.0 mL, 30%). The solution was boiled under reflux for 24 h. The evaporation residue, in $CHCl_3$, was washed with aq. $NaHCO_3$ and brine and was dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(200)** (247 mg, 93%) as pale yellow crystals: mp 209-211°C (lit.[213] mp 211°C); TLC Rf = 0.9 (EtOAc); ν_{max} 2900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.66 (3 H, s, CH_3), 7.36 (2 H, m, 5,6-H_2), 7.47 (1 H, d, J = 2.6 Hz, 4-H), 7.62 (1 H, m, 7-H), 8.60 (1 H, d, J = 2.6 Hz, 2-H), 10.25 (1 H, brs, OH); MS (FAB^+) *m/z* 160.0717 (M + H) ($C_{10}H_{10}NO$ requires 160.0762), 159.0682 (M) ($C_{10}H_9NO$ requires 159.0684).

8-METHYLQUINOLIN-4-ONE (201)



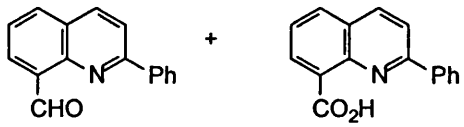
To **(198)** (164 mg, 1.1 mmol) was added SeO_2 (260 mg, 2.3 mmol). The mixture was heated at 200°C for 5 min. The residue, in $CHCl_3$ was washed with H_2O, brine, dried and filtered. Evaporation and column chromatography (EtOAc / MeOH 5:1) gave **(201)** (150 mg, 82%), as pale yellow crystals. mp 223-225°C; TLC Rf = 0.9 (EtOAc); ^1H NMR ($CDCl_3$) δ 2.45 (3 H, s, CH_3), 6.61 (1 H, d, J = 9.5 Hz, 3-H), 7.08 (1 H, dd, J = 8.4, 7.5 Hz, 6-H), 7.30 (1 H, d, J = 7.5 Hz, 5-H), 7.37 (1 H, d, J = 8.4 Hz, 7-H), 7.75 (1 H, d, J = 9.5 Hz, 2-H); MS (FAB^+) *m/z* 160 (M + H).

8-METHYL-2-PHENYLQUINOLINE (203) AND 8-METHYL-2-PHENYLQUINOLINE N-OXIDE (204)



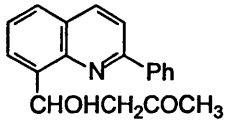
To **(199)** (1.07 g, 7.5 mmol) in dry THF (20 mL) was added PhMgBr (1 M in THF, 7.5 mL, 7.5 mmol) in dry THF (5 mL). The solution was stirred for 5 h. The reaction mixture was poured into aq. NH_4Cl (100 mL, 10%), extracted with $CHCl_3$, washed with brine, dried and filtered. The evaporation residue, in dry benzene (15 mL), was treated with lead dioxide (PbO_2, 2.1 g, 8.8 mmol) and the mixture was stirred for 2 h. Filtration, evaporation and column chromatography (CH_2Cl_2) gave **(203)** (0.785 g, 48%), as a pale yellow oil: TLC Rf = 0.9 (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 2.89 (3 H, s, CH_3), 7.31 (1 H, tt, J = 7.4, 1.9 Hz, Ph 4-H), 7.41 (3 H, m, Ph 3,5-H_2), 7.48 (1 H, dd, J = 7.9, 1.6 Hz, 7-H), 7.55 (2 H, m, 5,6-H_2), 7.82 (1 H, d, J = 8.6 Hz, 3-H), 8.09 (1 H, d, J = 8.6 Hz, 4-H), 8.23 (1 H, dd, J = 7.0, 1.9 Hz, Ph 2,6-H_2); ^{13}C NMR ($CDCl_3$) δ 18.36 (CH_3), 118.41, 125.60, 126.23, 127.37, 127.46, 127.67, 128.97, 129.43, 129.89, 137.11, 137.87, 140.03, 147.32, 155.68. Further elution gave **(204)** (0.452 g, 29%), as pale yellow crystals: mp >230°C; TLC Rf = 0.1 (CH_2Cl_2); ν_{max} 1210 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.21 (3 H, s, CH_3), 7.39 (1 H, d, J = 8.6 Hz, Ph 3-H), 7.48 (7 H, m, Ph 3,4,5-H_3, and 4,5,6,7-H_4), 7.84 (2 H, dd, J = 8.2, 1.6 Hz, Ph 2,6-H_2); ^{13}C NMR ($CDCl_3$) δ 25.51 (CH_3), 123.09, 125.39, 126.72, 127.83, 128.26, 129.09, 129.37, 131.62, 133.70, 134.12; MS (FAB $^+$) *m/z* 471 (2 M + H), 236 (M + H), 218 (M – H_2O); Found: C, 81.50; H, 5.57; N, 5.98; $C_{16}H_{13}NO$ requires C, 81.70; H, 5.53; N, 5.98%.

2-PHENYLQUINOLINE-8-CARBOXALDEHYDE (206) AND 2-PHENYL-QUINOLINE-8-CARBOXYLIC ACID (207)



To **(204)** (200 mg, 0.9 mmol) was added SeO_2 (0.59 g, 5.3 mmol). The paste was heated at 200°C for 45 mins. The melt was extracted with hot $CHCl_3$, washed with H_2O and brine and was dried and filtered. Evaporation and column chromatography (CH_2Cl_2 / hexane 20:1) gave **(203)** (33 mg, 16%) as a pale yellow oil, with properties as above. Further elution gave **(206)** (85 mg, 40%) as white crystals: mp 95-96°C; TLC Rf = 0.4 (CH_2Cl_2); ν_{max} 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (3 H, m, Ph 3,4,5-H_3), 7.66 (1 H, dd, J = 8.1, 7.3 Hz, 6-H), 8.04 (1 H, d, J = 8.8 Hz, 3-H), 8.11 (1 H, dd, J = 8.1, 1.5 Hz, 5-H), 8.29 (2 H, m, Ph 2,6-H_2), 8.32 (1 H, d, J = 8.8 Hz, 4-H), 8.34 (1 H, dd, J = 7.3, 1.5 Hz, 7-H), 11.67 (CHO); MS (FAB +) *m/z* 387 (M + H + mNBA), 234.0923 (M + H) ($C_{16}H_{12}NO$ requires 234.0919), 233.0829 (M) ($C_{16}H_{11}NO$ requires 233.0841). Further elution gave **(207)** (66 mg, 29%) as white crystals: mp 155-157°C (lit.[234] mp 158-159°C); TLC Rf = 0.2 (CH_2Cl_2); ν_{max} 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.58 (3 H, m, Ph 3,4,5-H_3), 7.70 (1 H, t, J = 8.1 Hz, 6-H), 8.20 (4 H, m, Ph 2, 6-H_2, 3, 5-H_2), 8.45 (1 H, d, J = 8.5 Hz, 4-H), 8.80 (1 H, dd, J = 8.1, 1.4 Hz, 7-H), 17.00 (1 H, brs, CO_2H); MS (FAB+) 250 (M + H), 232 (M – H_2O), 205 (M – CO_2H).

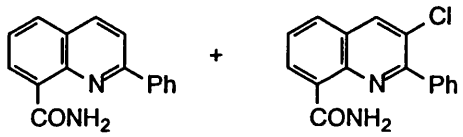
(+/-)-8-(1-HYDROXY-3-OXOBUTYL)-2-PHENYLQUINOLINE (211)



To **(206)** (48 mg, 0.21 mmol) was added acetone (1 mL), H_2O_2 (30%, 0.2 mL) and NaOH (80 mg, 2.0 mmol). The solution was stirred for 72 h. The evaporation residue, in EtOAc, was washed with aq. HCl (2 M), aq. $NaHCO_3$ and brine and was dried and filtered. Evaporation and column chromatography (CH_2Cl_2) gave **(211)** (50 mg, 83%) as white crystals. mp 115-116°C; TLC Rf = 0.9 (EtOAc); ^1H NMR

($CDCl_3$) δ 2.22 (3 H, s, CH_3), 3.25 (2 H, m, CH_2), 5.87 (1 H, q, J = 8.2 Hz, CH), 6.07 (1 H, brs, OH), 7.52 (3 H, m, Ph 3,4,5-H_3), 7.69 (1 H, dd, J = 7.4, 0.8 Hz, 3-H), 7.75 (1 H, dd, J = 8.2, 1.2 Hz, 4-H), 7.93 (1 H, d, J = 8.6 Hz, 5-H), 8.12 (2 H, m, Ph 2,6-H_2), 8.28 (1 H, d, J = 8.6 Hz, 7-H); ^{13}C NMR ($CDCl_3$) δ 31.38 (CH_3), 52.89 (CH_2), 70.98, 118.89, 126.48, 127.25, 127.54, 127.74, 129.24, 129.97, 138.10, 139.02, 139.64, 145.83, 155.62, 168.58, 208.31 (CO); MS (FAB+) *m/z* 292.1346 (M + H) ($C_{19}H_{18}NO_2$ requires 292.1338), 291.1244 (M) ($C_{19}H_{17}NO_2$ requires 291.1259), 274 (M – H_2O), 234 (M – CH_3COCH_3); Found: C, 78.0; H, 5.91; N, 4.69; $C_{19}H_{17}NO_2$ requires C, 78.32; H, 5.88; N, 4.81%.

2-PHENYLQUINOLINE-8-CARBOXAMIDE (209) AND 4-CHLORO-2-PHENYLQUINOLINE-8-CARBOXAMIDE (210)



PROCEDURE (1)

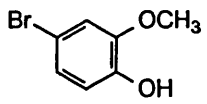
To **(207)** (200 mg, 0.8 mmol) was added thionyl chloride (2.0 mL, 27 mmol). The solution was boiled under reflux for 24 h. The evaporation residue, in $CHCl_3$ (20 mL) was saturated with NH_3 for 1 h. Filtration gave **(209)** (130 mg, 65%) as pale yellow crystals: mp 212-214°C (lit.[234] mp 210-212°C); TLC Rf = 0.4 (EtOAc / hexane 1:1); ν_{max} 3310, 3120 and 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.36 (1 H, brs, NH), 7.55 (3 H, m, Ph 3,4,5-H_3), 7.66 (1 H, dd, J = 7.8, 7.4 Hz, 6-H), 7.92 (1 H, d, J = 8.6 Hz, 3-H), 8.00 (1 H, dd, J = 7.8, 1.8 Hz, 5-H), 8.02 (2 H, dd, J = 8.2, 1.6 Hz, Ph 2,6-H_2), 8.34 (1 H, d, J = 8.6 Hz, 4-H), 8.88 (1 H, dd, J = 7.4, 1.8 Hz, 7-H), 11.18 (1 H, brs, NH); ^{13}C NMR δ_C 119.09, 126.04, 127.56, 129.04, 129.81, 132.05, 134.40, 138.30, 139.01, 145.36, 157.13, 167.61 (CO); MS (FAB$^+$) *m/z* 249.1036 (M + H) ($C_{16}H_{13}N_2O$ requires 249.1028), 248.0952 (M) ($C_{16}H_{12}N_2O$ requires 248.0950), 232 (M + H – NH_2), 205 (M – $CONH_2$). Concentration of the filtrate and recrystallisation (EtOAc / hexane 1:1) gave **(210)** (45 mg, 20%), as pale yellow crystals: mp 226-228°C; TLC Rf = 0.3 (EtOAc / hexane 1:1); ν_{max} 3380 and 1690 cm^{-1}; ^{1}H NMR

($CDCl_3$) δ 6.06 (1 H, brs, NH), 7.64 (3 H, m, Ph 3,4,5-H_3), 7.73 (1 H, dd, J = 8.2, 7.4 Hz, 6-H), 7.82 (2 H, m, Ph 2,6-H_2), 7.98 (1 H, dd, J = 8.2, 1.7 Hz, 5-H), 8.42 (1 H, s, 4-H), 8.88 (1 H, dd, J = 7.4, 1.7 Hz, 7-H), 10.67 (1 H, brs, NH); MS (FAB^+) *m/z* 285.0616 (M + H) ($C_{16}H_{12}{}^{37}ClN_2O$ requires 285.0609), 283.0652 (M + H) ($C_{16}H_{12}{}^{35}ClN_2O$ requires 283.0638), 282.0560 (M) ($C_{16}H_{12}{}^{35}ClN_2O$ requires 282.0560), 268, 266 (M – NH_2), 241, 239 (M – $CONH_2$).

PROCEDURE (2)

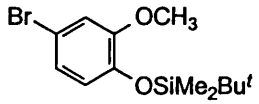
To **(207)** (150 mg, 0.6 mmol) in CH_2Cl_2 (10 mL) was added oxalyl chloride (230 mg, 1.8 mmol). After evaporation the oil, in $CHCl_3$ (20 mL) was saturated with NH_3 for 1 h. Filtration, evaporation and column chromatography (EtOAc / hexane 1:1) gave **(209)** (140 mg, 94%) as pale yellow crystals, with properties as above.

4-BROMO-2-METHOXYPHENOL (219)



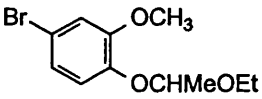
To **(218)** (19.9 g, 0.16 mmol) in dry $CHCl_3$ (50 mL) at 0°C was added Br_2 (25.6 g, 0.16 mmol) in dry $CHCl_3$ (50 mL) slowly. The solution was stirred at 0°C for 2 h. Evaporation and Kugelrohr distillation gave **(219)** (32.0 g, 98%) as a colourless oil $bp_{0.15}$ 137°C (lit[235] $bp_{0.05}$ 120°C): TLC Rf = 0.7 (hexane); ν_{max} 3500 and 630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.85 (3 H, s, CH_3), 5.67 (1 H, s, OH), 6.79 (1 H, d, J = 7.4 Hz, 6-H), 6.96 (1 H, d, J = 2.0 Hz, 3-H), 6.97 (1 H, dd, J = 7.4, 2.0 Hz, 5-H).

4-BROMO-1-(1,1-DIMETHYLETHYLDIMETHYLSILYLOXY)-2-METHOXYBENZENE (220)



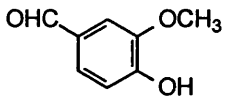
To **(219)** (10.8 g, 0.05 mol) in dry DMF (10 mL) was added imidazole (7.6 g, 0.11 mol) and *t*-butylchlorodimethylsilane (16.2 g, 0.11 mol). The mixture was stirred for 3 d. The evaporation residue in $CHCl_3$, was washed with H_2O and brine and was dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(220)** (14.1 g, 84%) as a pale yellow oil: TLC Rf = 0.8 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.14 (6 H, s, $Si(CH_3)_2$), 0.98 (9 H, s, Bu^t), 3.79 (3 H, s, OCH_3), 6.71 (1 H, dd, J = 8.1, 0.6 Hz, 5-H), 6.94 (1 H, dd, J = 8.1, 2.2 Hz, 6-H) 6.95 (1 H, dd, J = 2.2, 0.6 Hz, 2-H).

4-BROMO-1-(1-ETHOXYETHYL)-2-METHOXYBENZENE (227)



To **(219)** (13.63 g, 67 mmol) and ethoxyethene (25 mL) in CH_2Cl_2 (13 mL) was added pyridinium 4-methylbenzenesulfonate (60 mg) and the solution was stirred for 16 h. The evaporation residue, in EtOAc, was washed with aq. $CuSO_4$, aq. $NaHCO_3$ and brine and was dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:5) gave **(227)** (18.23g, 99%) as a colourless oil: TLC Rf = 0.7 (EtOAc / hexane 1:1); ν_{max} 690 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$) δ 1.08 (3 H, t, J = 7.1 Hz, CH_2CH_3), 1.35 (3 H, d, J = 5.3 Hz, CH_3), 3.48 (1 H, dq, J = 9.5, 7.1 Hz) and 3.68 (1 H, dq, J = 9.5, 7.1 Hz) (CH_2CH_3), 3.78 (3 H, s, OCH_3), 5.33 (1 H, q, J = 5.3 Hz, CH), 6.98 (1 H, d, J = 8.6 Hz, 5-H) 7.04 (1 H, dd, J = 8.6, 2.0 Hz, 6-H), 7.16 (1 H, d, J = 2.0 Hz, 2-H); MS (FAB$^+$) *m/z* 298 (M + Na), 276.0191 (M + H) ($C_{11}H_{15}{}^{81}BrO_3$ requires 276.0184), 275.0159 (M) ($C_{11}H_{14}{}^{81}BrO_3$ requires 275.0106), 274.0195 (M + H) ($C_{11}H_{15}{}^{79}BrO_3$ requires 274.0205), 273.0121 (M) ($C_{11}H_{14}{}^{79}BrO_3$ requires 273.0126), 259 (M + H - Me), 202 (M – EtOCHMe).

4-HYDROXY-3-METHOXYBENZALDEHYDE (223)



GENERAL PROCEDURE

To **(220)** in dry THF (1.0 mL) at –78°C was added the lithiating reagent (*t*-BuLi (1.5 M in pentanes) / *s*-BuLi (1.3 M in cyclohexanes) / n-BuLi (1.6 M in hexanes)) under argon and the mixture was stirred at –78°C for 1 / 2 / 4 h. Dry DMF was added and the mixture was stirred at –78°C for 2 h and at 20°C for a further 16 h. The evaporation residue, in EtOAc was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation gave **(222)** and **(224)**. ^1H NMR ($CDCl_3$) δ 0.17 (6 H, s, $Si(CH_3)_2$), 1.01 (9 H, s, Bu^t), 3.87 (3 H, s, OCH_3), 6.96 (1 H, d, J = 8.1 Hz, 5-H), 7.38 (2 H, m, 2,6-H_2), 9.83 (1 H, s, CHO). To the residue, in $CHCl_3$ (1.0 mL) was added TBAF (1.0 M in THF, 0.5 mL) and the solution stirred for 12 h. Evaporation and column chromatography (EtOAc) gave **(223)**: mp 78-80°C (lit.[236] mp 76.5-78°C); TLC Rf = 0.4 (EtOAc / hexane 1:1); ν_{max} 3160 and 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.97 (3 H, s, OCH_3), 6.22 (1 H, s, OH), 7.05 (1 H, d, J = 8.6 Hz, 5-H), 7.43 (2 H, m, 2,6-H_2), 9.83 (1 H, s, CHO); MS (FAB^+) *m/z* 305 (2 M + H), 153 (M + H), 135 (M – H_2O), (FAB^-) 151 (M – H).

(As the above procedure using the following quantities)

Comp. No.	Mass	Dry THF (mL)	BuLi	Stirring time at -78°C	Dry DMF	$CHCl_3$ (mL)	TBAF (mL)	% Yield
220	330 mg, 1.0 mmol	1.0	*n*-BuLi 0.6 mL, 0.9 mmol (0.9 eq.)	2	0.5 mL, 6.0 mmol (6.0 eq.)	1.0	0.5	21

Comp. No.	Mass	Dry THF (mL)	BuLi	Stirring time at -78°C	Dry DMF	$CHCl_3$ (mL)	TBAF (mL)	% Yield
220	230 mg, 0.7 mmol	1.0	*t*-BuLi 0.43 mL, 0.65 mmol (0.9 eq.)	2	0.34 mL, 4.4 mmol (6.0 eq.)	1.0	0.5	52
220	483 mg, 1.5 mmol	1.0	*t*-BuLi 1.0 mL, 1.5 mmol (1.0 eq.)	2	0.11 mL, 1.4 mmol (0.9 eq.)	1.0	0.5	2
220	230 mg, 0.7 mmol	1.0	*t*-BuLi 0.5 mL, 0.7 mmol (1.0 eq.)	2	0.085 mL, 1.1 mmol (1.5 eq.)	1.0	0.5	49
220	1.0 g, 3.2 mmol	5.0	*t*-BuLi 2.1 mL, 3.2 mmol (1.0 eq.)	2	0.75 mL, 9.6 mmol (3.0 eq.)	1.0	0.5	49
220	276 mg, 0.87 mmol	1.0	*n*-BuLi 1.65 mL, 2.6 mmol (3.0 eq.)	2	0.20 mL, 2.6 mmol (3.0 eq.)	1.0	0.5	64

GENERAL PROCEDURE

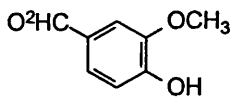
To **(227)** in dry THF (1.0 mL) at –78°C was added the lithiating reagent (*t*-BuLi (1.5 M in pentanes) / *s*-BuLi (1.3 M in cyclohexanes) / n-BuLi (1.6 M in hexanes)) under argon and the mixture was stirred at –78°C for 1 / 2 / 4 h. Dry DMF was added and the mixture was stirred at –78°C for 2 h and at 20°C for a further 16 h. The evaporation residue, in EtOAc was washed with aq. $NaHCO_3$, brine, dried and

filtered. Evaporation and column chromatography (EtOAc / hexane 1:4) gave **(223)** with the above properties.

(As the above procedure using the following quantities)

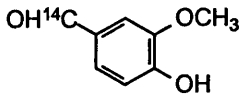
Comp. No.	**Mass**	**Dry THF (mL)**	**BuLi**	**Stirring time at -78°C**	**Dry DMF (mL)**	**% Yield**
227	377 mg, 1.4 mmol	1	1.3 mL, 2.1 mmol (1.5 eq.)	1	0.16 mL, 2.10 mmol (1.5 eq.)	0
227	574 mg, 2.1 mmol	3.0	*n*-BuLi 3.9 mL, 6.3 mmol (3.0 eq.)	1	0.33 mL, 4.2 mmol (2.0 eq.)	55
227	511 mg, 1.9 mmol	3.0	*n*-BuLi 3.5 mL, 5.6 mmol (3.0 eq.)	1	0.43 mL, 5.6 mmol (3.0 eq.)	70
227	653 mg, 2.4 mmol	3.5	*n*-BuLi 4.45 mL, 7.1 mmol (3.0 eq.)	2	0.37 mL, 4.8 mmol (2.0 eq.)	64
227	413 mg, 1.5 mmol	2.0	*n*-BuLi 2.8 mL, 4.5 mmol (3.0 eq.)	4	0.2 mL, 3.0 mmol (2.0 eq.)	45

4-HYDROXY-3-METHOXYBENZ-[^{2}H]-ALDEHYDE (231)



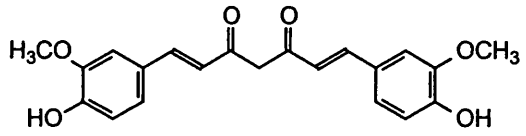
To **(227)** (525 mg, 1.9 mmol) in dry THF (3.0 mL) was added BuLi (1.6 M in hexanes; 3.6 mL, 5.6 mmol) at –78°C, under Ar. The solution was stirred at -78°C for 2 h. Di-[$^2H_3$]-methyl-[^{2}H]-formamide (281 mg, 3.5 mmol) was added and the solution was stirred at –78°C for 2 h and at 20°C for a further 16 h, under Ar. Aq. HCl (3 M, 7.0 mL) was added under Ar and the solution was stirred for 30 min. The evaporation residue in EtOAc was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:4) gave **(223)** (161 mg, 55%) as off-white crystals: mp 72-75°C (lit.[236] mp 76.5-78°C) (unlabelled material); TLC Rf = 0.4 (EtOAc / hexane 1:1); ^{1}H NMR ($CDCl_3$) δ 3.91 (3 H, s, OCH_3), 7.01 (1 H, d, J = 8.6 Hz, 5-H), 7.40 (2 H, m, 2, 6-H_2); ^{13}C NMR ($CDCl_3$) δ 55.97 (CH_3), 108.95 (2-C), 114.46 (5-C), 127.37 (6-C), 129.59 (1-C), 147.24 (4-C), 151.86 (3-C), 190.64 (t, J = 26.4 Hz, C^{2}HO).

4-HYDROXY-3-METHOXYBENZ-[^{14}C]-ALDEHYDE (233)



To **(227)** (141.2 mg, 0.51 mmol) in dry THF (1.5 mL) was added BuLi (1.6 M in hexanes; 1.0 mL, 1.6 mmol) at –78°C, under Ar and the solution was stirred at -78°C for 2.5 h. Dimethyl-[^{14}C]-formamide (3.0 mg, 41 μmol, 18.5 MBq) and dry DMF (57 mg, 776 μmol) in dry THF (750 μL) were added and the solution was stirred at -78°C for 2 h and at 20°C for a further 12 h, under Ar. Aq. HCl (2 M, 2.0 mL) was added under Ar and the solution was stirred for 30 min. The evaporation residue, in EtOAc, was washed with aq. $NaHCO_3$, brine, dried and filtered. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(233)** (35.6 mg, 45% chemical yield; 1.49 MBq, 8.1% radiochemical yield, specific activity 6.36 MBq mmol^{-1}) as a pale buff solid chromatographically identical to **(223)**.

***E,E*-1,7-DI(4-HYDROXY-3-METHOXYPHENYL)-3-HYDROXYHEPTA-1,3,6-TRIEN-5-ONE (68)**



PROCEDURE (1)

To **(223)** (1.53 g, 10 mmol) and pentane-2,4-dione (0.5 mL, 5.0 mmol) in dry DMF (1 mL) was added H_3BO_3 (1.0 g, 16.3 mmol) and the mixture was heated for 5 mins at 120°C. A mixture of 1,2,3,4-tetrahydroquinoline (0.1 mL, 0.8 mmol) and glacial AcOH (0.3 mL) in DMF (1 mL) was added and the mixture was heated for 8 h at 120°C. After cooling, aq. AcOH (50 mL, 20%) was added and the mixture was stirred for 1 h at 20°C. Evaporation and column chromatography (EtOAc / hexane 1:2) gave **(68)** (1.05 g, 57%) as orange crystals: mp 184-186°C (lit.[237] mp 185-186°C); TLC Rf = 0.9 (EtOAc); ν_{max} 3500 and 1630 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ3.84 (6 H, s, 2 x CH_3), 6.06 (1 H, s, 4-H), 6.76 (2 H, d, J = 15.6 Hz, 2, 6-H_2), 6.84 (2 H, d, J = 7.8 Hz, 2 x Ar 5-H), 7.15 (2 H, d, J = 7.8 Hz, 2 x Ar 6-H), 7.33 (2 H, s, 2 x Ar 2-H), 7.56 (2 H, d, J = 15.6 Hz, 1, 7-H_2), 9.70 (2 H, brs, 2 x ArOH); δc 55.80 (2 x CH_3), 100.97 (4-C), 111.53, 115.87, 121.22, 123.17, 126.49 (br 2,6-C_2), 140.81 (br 1,7-C_2), 148.13, 149.49, 183.30 (3,5-C_2); MS (FAB+) *m/z* 369 (M + H).

PROCEDURE (2)

To H_3BO_3 (1.0 g, 16.2 mmol) in dry DMF (1 mL) was added pentane-2,4-dione (0.5 mL, 5.0 mmol). The mixture was heated for 10 mins at 120°C. The mixture was added to **(223)** (1.53 g, 10 mmol). A mixture of 1,2,3,4-tetrahydroquinoline (0.1 mL, 0.8 mmol) and glacial AcOH (0.3 mL) in DMF (1 mL) was added and the mixture was heated for 20 h at 100°C. After cooling, aq. AcOH (50 mL, 20%) was added and the mixture stirred for 1 h at 20°C. Evaporation and column chromatography

(EtOAc / hexane 1:2) gave **(68)** (0.75 g, 40%) as orange crystals, with properties as above.

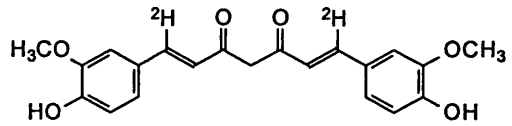
PROCEDURE (3)

To B_2O_3 (1.34 g, 19.3 mmol) in dry DMF (1 mL) was added pentane-2,4-dione (0.5 mL, 5.0 mmol). The mixture was heated for 15 min at 120°C. The mixture was added to **(223)** (1.53 g, 10 mmol). A mixture of 1,2,3,4-tetrahydroquinoline (0.1 mL, 0.8 mmol) and glacial AcOH (0.3 mL) in DMF (1 mL) was added and the mixture was heated for 10 h at 110°C. After cooling, aq. AcOH (50 mL, 20%) was added and the mixture stirred for 1 h at 20°C. Evaporation and column chromatography (EtOAc / hexane 1:2) gave **(68)** (1.2 g, 65%) as orange crystals, with properties as above.

PROCEDURE (4)

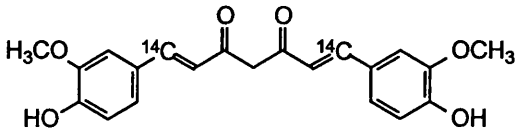
To B_2O_3 (1.2 g, 17.2 mmol) in dry DMF (1 mL) was added pentane-2,4-dione (0.5 mL, 5.0 mmol). The mixture was heated for 15 min at 120°C. Compound **(223)** (1.53 g, 10 mmol) in dry DMF (1.0 mL) was added and the mixture stirred for 15 mins at 120°C. A mixture of 1,2,3,4-tetrahydroquinoline (0.1 mL, 0.8 mmol) and glacial AcOH (0.3 mL) in DMF (1 mL) was added and the mixture was heated at 120°C for 45 min and at 110°C for 10 h. After cooling, aq. AcOH (50 mL, 20%) was added and the mixture stirred for 1 h at 20°C. Evaporation and column chromatography (EtOAc / hexane 1:2) gave **(68)** (1.43 g, 77%) as orange crystals, with properties as above.

_E,E_-1,7-DI(4-HYDROXY-3-METHOXYPHENYL)-3-HYDROXY-1,7-DI-[^{2}H]-HEPTA-1,3,6-TRIEN-5-ONE ([$^2H_2$]-CURCUMIN) (234)



Pentane-2,4-dione (43 mg, 430 μmol) was heated with B_2O_3 (117 mg, 1.7 mmol) in dry DMF (90 μL) at 120°C for 15 min. Compound **(231)** (134 mg, 876 μmol) in dry DMF (300 μL) was added and the mixture was stirred for 15 min at 120°C. 1,2,3,4-Tetrahydroquinoline (10.6 mg, 80 μmol) and glacial AcOH (26 mg, 437 μmol) were added and the mixture was stirred for 8.5 h at 110°C. Aq. AcOH (20%, 5 mL) was added and the mixture was stirred for 16 h at 20°C. Evaporation and column chromatography (EtOAc / hexane 1:1) gave **(234)** (70 mg, 43%) as an orange-brown solid: mp 183-185°C (lit.[237] mp 185-186°C (unlabelled material)); TLC Rf = 0.9 (EtOAc); ^{1}H NMR ($(CD_3)_2SO$) δ 3.84 (6 H, s, 2 x CH_3), 6.06 (1 H, s, 4-H), 6.75 (2 H, d, J = 15.6 Hz, 2, 6-H_2), 6.82 (2 H, d, J = 8.1 Hz, 2 x Ar 5-H), 7.16 (2 H, dd, J = 8.1, 1.5 Hz, 2 x Ar 6-H), 7.33 (2 H, d, J = 1.5 Hz, 2 x Ar 2-H), 9.67 (2 H, brs, 2x ArOH); δc 55.46 (2 x CH_3), 101.46 (4-C), 111.97, 116.33, 121.60, 123.71, 126.88, 140.92 (br 1, 7-C_2), 148.54, 149.89, 183.67 (3, 5-C_2); ν_{max} 3400 and 1580 cm^{-1}; MS (FAB+) *m/z* 372.1544 (M +H) ($^{13}C_1{}^{12}C_{20}{}^2H_2{}^1H_{19}{}^{16}O_6$ requires 372.1464), 371.1489 (M + H) ($^{12}C_{21}{}^2H_2{}^1H_{19}{}^{16}O_6$ requires 371.1464), 370.1406 (M) ($^{12}C_{21}{}^2H_2{}^1H_{18}{}^{16}O_6$ requires 370.1386).

_E,E_-1,7-DI(4-HYDROXY-3-METHOXYPHENYL)-3-HYDROXY-1-[^{14}C]-HEPTA-1,3,6-TRIEN-5-ONE ([^{14}C]-CURCUMIN) (235)



Pentane-2,4-dione (117 mg, 117 μmol) was heated with B_2O_3 (28.0 mg, 402 μmol) in dry DMF (223 μL) at 120°C for 15 min. Compound **(233)** (35.4 mg, 233 μmol, 1.48 MBq) in dry DMF (500 μL) was added and the mixture was stirred for 15 min at

120°C. 1,2,3,4-Tetrahydroquinoline (2.7 mg, 20 μmol) and glacial AcOH (7.9 mg, 131 μmol) were added and the mixture was stirred for 24 at 110°C h. Aq. AcOH (20%, 1.0 mL) was added and the mixture was stirred for 24 h at 20°C. Evaporation and column chromatography (EtOAc / hexane 1:4) gave **(235)** (34.7 mg, 81% chemical yield; 1.20 MBq, 81% radiochemical yield, specific radioactivity 12.7 MBq $mmol^{-1}$) as an orange-brown solid chromatographically identical to **(68)**.

9.0 *REFERENCES*

1. Kennedy, K. A.; Teicher, B. A.; Rockwell, S.; Sartorelli, A. C. *Biochem. Pharmacol.* **1980**, *29*, 1.
2. Vaupel, P.; Kallinowski, F.; Okunieff, P. *Cancer Res.* **1989**, *49*, 6449.
3. Thomlinson, R. H.; Gray, L. H. *Br. J. Cancer.* **1955**, *9*, 539.
4. Moulder, J. E.; Rockwell, S. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 695.
5. Moulder, J. E.; Rockwell S. *Cancer Metastasis Rev.* **1987**, *5*, 313.
6. Kennedy, K. A. *Anticancer Drug Des.* **1987**, *2*, 181.
7. Hall, E. J. Radiobiology for the Radiobiologist (3rd edition), J. B. Lippincott Company, Philadelphia, **1988**.
8. Hodgkiss, R. J. Review Article: Gray Laboratory Annual Report, **1993** http://www.graylab.ac.uk/lab/reviews/hypoxia.html, cited 11/7/01.
9. Hodgkiss, R. J.; Jones, G. W.; Long, A.; Middleton, R. W.; Parrick, J.; Stratford, M. R. L.; Wardman, P.; Wilson G. D. *J. Med. Chem.* **1991**, *34*, 2268.
10. Creasey, W. A. *Cancer, An Introduction*, Oxford University Press, New York, **1981**.
11. Watson, C. Y. PhD Thesis, University of Bath, **1997**.
12. Adams, G. E.; Cooke, M. S. *Int. J. Radiat. Biol.* **1969**, *15*, 457.
13. Beveridge, A. J.; Williams, M.; Jenkins, T. C. *J. Chem. Soc., Faraday Trans.*, **1996**, *92*, 763.
14. Adams, G. E.; Clarke, E. D.; Flockhart, I. R.; Jacobs, R. S.; Sehmi, D. S.; Stratford, I. J.; Wardman, P.; Watts, M. E. *Int. J. Radiat. Biol.* **1979**, *35*, 133.
15. Fielden, E. M.; Adams, G, E.; Cole, S.; Naylor, M. A.; O'Neill, P.; Stephens, M. A.; Stratford, I. J. *Int. J. Radiat. Oncol. Biol. Phys.* **1992**, *22*, 707.
16. Suto, M. J. *Ann. Rep. Med. Chem.* **1991**, 151.
17. Chapman, J. D.; Reuvers, A. P.; Borsa, J.; Perkau, A.; McCalla, D. R. *Cancer Res.* **1972**, *32*, 2616.
18. Threadgill, M. D.; Webb, P.; O'Neill, P.; Naylor, M. A.; Stephens, M. A.; Stratford, I. J.; Cole, S.; Adams, G. E.; Fielden, E. M. *J. Med. Chem.* **1991**, *34*, 2112.
19. Adams, G. E.; Stratford, I. J. *Biochem. Pharmacol.* **1986**, *35*, 71.

20. Adams, G. E.; Stratford, I. J.; Wallace, R. G.; Wardman, P.; Watts, M. E. *J. Natl. Cancer Inst.* **1980**, *64*, 555.
21. Denekamp, J.; Hirst, D. G.; Stewart, F. A.; Terry, N. H. A. *Br. J. Cancer.* **1980**, *41*, 1.
22. Brown, J. M.; Hall, E. J.; Hirst, D. G.; Kinsella, T. J.; Kligerman, M. M.; Mitchell, J. B.; Travis, E. J.; Valeriote, F. *Am. J. Clin. Oncol.* **1988**, *11*, 288.
23. Hori, H.; Murayama, C.; Mori, T.; Shibamoto, Y.; Abe, M.; Onoyama, Y.; Inayama, S. *Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *16*, 1029.
24. Jenkins, T. C.; Naylor, M. A.; O'Neill, P.; Threadgill, M. D.; Cole, S.; Stratford, I. J.; Adams, G. E.; Fielden, E. M.; Suto, M. J.; Steir, M. A. *J. Med. Chem.* **1990**, *33*, 2603.
25. Shenoy, M. A.; Singh, B. B. *Int. J. Radiat. Biol.* **1985**, *48*, 315.
26. Taylor, Y. C.; Rauth, A. M. *Cancer Res.* **1978**, *38*, 2745.
27. Heimbrook, D. C.; Sartorelli, A.C. *Mol. Pharmacol.* **1986**, *29*, 168.
28. Varnes, M. E.; Biaglow, J. E. *Int. J. Radiat. Oncol. Biol. Phys.* **1982**, 8, 683.
29. Russo, A., Carmichael, J.; Friedman, N. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1347.
30. Olive, P. L. *Br. J. Cancer*, **1979**, *40*, 89.
31. Mulcahy, R. T. *Cancer Res.* **1986**, *46*, 2892.
32. Mulcahy, R. T. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *8*, 1389.
33. Lee, F. Y. F.; Workman, P. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1383.
34. Siemann, D. W. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1585.
35. Hill, R. P.; Gulyas, S.; Whitmore, G. F. *Br. J. Cancer* **1986**, *53*, 743.
36. Stratford, I. J.; Walling, J. M.; Silver, A. R. J. *Br J. Cancer* **1986**, *53*, 339.
37. Adams, G. E.; Stratford, I. J. *Int. J. Radiat. Oncol. Biol. Phys.***1994**, *29*, 231.
38. Kirkpatrick, D. L. *Pharmacol. Ther.* **1989**, *40*, 383.
39. Keyes, S. R.; Fracasso, P. M.; Heimbrook, D. C.; Rockwell, S.; Sligar, S. G.; Sartorelli, A. C. *Adv. Enzyme Reg.* **1985**, *23*, 291.
40. Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G.; Nakanishi, K. *Science* **1987**, *235*, 1204.
41. Kennedy, K. A.; McGurl, J. D.; Leondaridis, L.; Alabaster, O. *Cancer Res.* **1985**, *45*, 3541.
42. Fracasso, P. M.; Sartorelli, A. C. *Cancer Res.* **1986**, *46*, 3939.
43. Pritsos, C. A.; Sartorelli, A. C. *Cancer Res.*. **1986**, *46*, 3528.

44. Laderoute, K. R.; Rauth, A. M. *Biochem. Pharmacol.* **1986**, *35*, 3417.
45. Zeman, E. M.; Brown, J. M.; Lemmon, M. J.; Hirst, V. K.; Lee, W. W. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1239.
46. Mahmud, N. P.; Garrett, S. W.; Threadgill, M. D. *Anticancer Drug Des.* **1998**, *131*, 655.
47. Denny, W. A.; Wilson, W. R.; Hay, M. P. *Br. J. Cancer* **1996**, *74*, S32.
48. Ware, D. C.; Palmer, B. D.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **1993**, *36*, 1839.
49. Bagshawe K. D. *Drug Devel. Res.* **1995**, *34*, 220.
50. Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109.
51. Chinje, E. C.; Stratford, I. J. *Essays in Biochem.* **1997**, *32*, 61.
52. Ulhaq, S.; Naylor, M. A.; Chinje, E. C.; Threadgill, M. D.; Stratford, I. J. *Anticancer Drug Des.* **1997**, *12*, 61.
53. Suit, H. D. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 101.
54. Holsti, L. R.; Salno, M.; Elkind, M. M. *Br. J. Cancer* **1978**, *37*, 307.
55. Churchill-Davidson, I.; Sanger, C.; Thomlinson, R. H. *Br. J. Radiol.* **1957**, *30*, 406.
56. Dische, S. *Br. J. Radiol.* **1979**, *51*, 888.
57. Inch, W.; McCredie, J. A.; Sutherland, R. M. *Cancer* **1970**, *25*, 926.
58. Evans, N. T. S.; Naylor, P. F. D. *Br. J. Radiol.* **1963**, *36*, 418.
59. Rockwell, S. *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *11*, 97.
60. Bush, R. S.; Jenkin, R. D. T.; Allt, W. E. C.; Beale, F. A.; Bean, H.; Dembo, A. J.; Pringle, F. R. *Br. J. Cancer* **1976**, *37*, 302.
61. Overgaard, J.; Hansen, M. S.; Jorgensen, R.; Hansen, M. H. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 515.
62. Baker, D. J.; Lindop, P. J.; Morgan, W. G.; Skeggs, D. B. L.; Whittle, R. J. M.; Williams, I. G. *Br. J. Radiol.* **1966**, 39, 908.
63. Withers, H. R. *Radiol.* **1973**, *108*, 131.
64. Overgaard, J. *Br. J. Radiol.* **1981**, *54*, 245.
65. Naylor, M. A.; Stephens, M. A.; Cole, S.; Threadgill, M. D.; Stratford, I. J.; O'Neill, P.; Fielden, E. M.; Adams, G. E. *J. Med. Chem.* **1990**, *33*, 2508.
66. Naylor, M. A.; Threadgill, M. D.; Webb, P.; Stratford, I. J.; Stephens, M. A.; Fielden, E. M.; Adams, G. E. *J. Med. Chem.* **1992**, *35*, 3573.

67. Everett, S. A.; Naylor, M. A.; Nolan, J.; Patel, K. B.; Wardman, P. *Anticancer Drug Des.* **1998**, *13*, 635.

68. Sykes, B. M.; Atwell, G. J.; Denny, W. A.; O'Connor, C. J. *J. Phys. Org. Chem.* **1995**, *8*, 587.

69. Sykes, B. M.; Atwell, G. J.; Denny, W. A.; McLennan, D. J.; O'Connor, C. J. *J. Chem. Soc., Perkin Trans. 2* **1995**, 337.

70. Berry, J. M.; Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. *J. Chem. Soc. Perkin Trans. 1* **1997**, 1147.

71. Everett, S. A.; Naylor, M. A.; Patel, K. B.; Stratford, M. R. L.; Wardman, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1267.

72. Parliament, M. B.; Chapman, J. D.; Urtasun, R. C. McEwan, A. J.; Golberg, L.; Mercer, J. R.; MAannan, R. H.; Wiebe, L. I. *Br. J. Cancer* **1992**, *65*, 90.

73. Long, A.; Parrick, J.; Hodgkiss, R. T. *Synthesis* **1991**, 709.

74. Shall, S. *Adv. Radiat. Biol.* **1984**, *11*, 1.

75. Okayama, H..; Edson, C. M.; Fukoshima, M.; Ueda, K.; Hayaishi, O. *J. Biol. Chem.* **1977**, *252*, 7000.

76. Lindahl T.; Satoh, M. S.; Poirier, G. G.; Klungland, A. *Trends Biochem. Sci.* **1995**, *20*, 405.

77. Babichuk, E.; Cottrill, P. B.; Storozhenko, S.; Fuangthong, M.; Chen, Y.; O'Farrell, M. K.; van Montagu, M.; Inze, D.; Kushnir, S. *Plant J.* **1998**, *15*, 635.

78. Smith, S.; Giriat, I.; Schmitt, A.; de Lange, T. *Science* **1998**, *282*, 1484.

79. Faraone-Menella, M. R.; Gambacorta, A.; Nicolaus, B.; Farina, B. *Biochem. J.* **1998**, *335*, 441.

80. D'Amours, D.; Desnoyers, S.; D'Silva, D.; Poirier, G. G. *Biochem. J.* **1999**, *342*, 249.

81. Kameshita, I.; Matsuda, Z.; Taniguchi, T.; Shizuta, Y. *J. Biol. Chem.* **1984**, *259*, 4770.

82. Kurosaki, T.; Ushiro, H.; Mitsuuchi, Y.; Suzuki, S.; Matsuda, M.; Matsuda, Y.; Katunuma, N.; Kangawa, K.; Matsuo, H.; Hirose, T.; Inayama, S.; Shizuta, Y. *J. Biol. Chem.* **1987**, *262*, 15990.

83. Alkhatib, H. M.; Chen, D.; Cherney, B.; Bhatia, K.; Notario, B.; Giri, C.; Stein G.; Slattery, E.; Roeder, R. G.; Smulson, M. E. *Proc. Natl. Acad. Sci. USA.* **1987**, *84*, 1224.

84. Mazen, A.; Ménissier-de Murcia, J. M.; Molinete, M.; Simonin, F.; Gradwohl, G.; Poirier, G. G.; de Murcia, G. *Nucleic Acids Res.* **1989**, *17*, 4689.

85. Cherney, B.; McBride, O. W.; Chen, D.; Alkahatib, H.; Bhatia, K.; Hensley, P.; Smulson, M. E. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 8370.

86. Berg, J. M. *J. Biol. Chem.* **1990**, *265*, 6513.

87. Saito, I.; Hatakeyama, K.; Kido, T.; Ohkubo, H.; Nakanishi, S.; Ueda, K. *Gene* **1990**, *90*, 249.

88. Brennan, R. G.; Matthews, B. W. *J. Biol. Chem.* **1989**, *264*, 1903.

89. Gradwohl, G.; Ménissier-de Murcia, J.; Molinete, M.; Simonin, F.; Koken, M.; Hoeijmakers, J. H. J.; de Murcia, G. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 2990.

90. Le Cam, E; Ménissier-de Murcia, J.; Cognet, J. A. H.; Barbin, A.; Sarantoglou, V.; Revet, B.; Delain, E.; de Murcia, G. *J. Mol. Biol.* **1994**, *235*, 1062.

91. Ikejima, M.; Noguchi, S.; Yamashita, R.; Ogura, T.; Sugimura, T.; Gill, D. M.; Miwa, M. *J. Biol. Chem.* **1990**, *265*, 21907.

92. Ménissier-de Murcia, J.; Molinete, M.; Gradwohl, G.; Simonin, F.; de Murcia, G. *J. Mol. Biol.* **1989**, *210*, 229.

93. de Murcia, G.; Schreiber, V.; Molinete, M.; Saulier, B.; Poch, O.; Masson, M.; Niedergang, C.; Ménissier-de Murcia, J. *Mol. Cell. Biochem.* **1994**, *138*, 15.

94. Buki, K. G.; Kun, E. *Biochemistry* **1988**, *27*, 5990.

95. Kawaichi, M.; Ueda, K.; Hayaishi, O. *J. Biol. Chem.* **1981**, *256*, 9483.

96. Mendoza-Alvarez, H.; Alvarez-Gonzalez, R. *J. Biol. Chem.* **1993**, *268*, 22575.

97. Bork, P.; Hofmann, K.; Bucher, P.; Neuwald, A. F.; Altschul, S. F.; Koonin, E. V. *FASEB J.* **1997**, *11*, 68.

98. Zhang, X.; Morera, S.; Bates, P. A.; Whitehead, P. C.; Coffer, A. I.; Hainbucher, K.; Nash, R. A.; Sternberg, M. J. E.; Lindahl, T.; Freemont, P. S. *EMBO J.* **1998**, *17*, 6404.

99. Kubota, Y.; Nash, R. A.; Klungland, A.; Schar, P.; Barnes, D. E.; Lindahl, T. *EMBO J.* **1996**, *15*, 6662.

100. Masson, M.; Niedergang, C.; Schreiber, V.; Muller, S.; Ménissier-de Murcia, J.; de Murcia, G. *Mol. Cell. Biol.* **1998**, *18*, 3563.

101. Masson, M.; Rolli, V.; Dantzer, F.; Trucco, C.; Schreiber, V.; Fribourg, S.; Molinete, M.; Ruf, A.; Miranda, E. A.; Niedergang, C.; Hunting, G.; Gowans, B.; Schulz, G. E.; Ménissier-de Murcia, J.; de Murcia, G. *Biochimie* **1995**, *77*, 456.

102. Simonin, F.; Ménissier-de Murcia, J.; Poch, O.; Muller, S.; Gradwohl, G.; Molinete, M.; Penning, C.; Keith, G.; de Murcia, G. *J. Biol. Chem.* **1990**, *265*, 19249.

103. Marsischky, G. T.; Wilson, B. A.; Collier, R. J. *J. Biol. Chem.* **1995**, *270*, 3247.

104. Ruf, A.; de Murcia, G.; Schulz, G. E. *Biochemistry* **1998**, *37*, 3893.

105. Kim, H.; Jacobson, M. K.; Rolli, V.; Ménissier-de Murcia, J.; Reinbolt, J.; Simonin, F.; Ruf, A.; Schultz, G.; de Murcia, G. *Biochem. J.* **1997**, *322*, 469.

106. Ueda, K.; Hayaishi, O. *Ann. Rev. Biochem.* **1985**, *54*, 73.

107. Althaus, F. R. *J. Cell Sci.* **1992**, *102*, 663.

108. Hatakeyama, K.; Nemoto, Y.; Ueda, K.; Hayaishi, O. *J. Biol. Chem.* **1986**, *261*, 14902.

109. Alvarez-Gonzalez, R. *Mol. Cell. Biochem.* **1994**, *138*, 213.

110. Gåken, J. A.; Tavassoli, M.; Gan, S. U.; Vallian, S.; Giddings, I.; Darling, D. C.; Galea-Lauri, J.; Thomas, M. G.; Abedi, H.; Schreiber, V.; Ménissier-de Murcia, J.; Collins, M. K. L.; Shall, S.; Farzaneh, F. *J. Virology* **1996**, *70*, 3992.

111. Nosseri, C.; Coppola, S.; Ghibelli, L. *Exp. Cell Res.* **1994**, *212*, 367.

112. Duriez, P. J.; Shah, G. M. *Biochem. Cell. Biol.* **1997**, *75*, 337.

113. Oliver, F. J.; Ménissier-de Murcia, J.; de Murcia, G. *Am. J. Human Genet.* **1999**, *64*, 1282.

114. Patel, T.; Gores, G. J.; Kaufmann, S. H. *FASEB J.* **1996**, *10*, 587.

115. Greidinger, E. L.; Miller, D. K.; Yamin, T. T.; Casciola-Rosen, L.; Rosen, A. *FEBS Lett.* **1996**, *390*, 299.

116. Szabo, C. *Eur. J. Pharmacol.* **1998**, *350*, 1.

117. Szaba, C.; Dawson, V. L. *Trends Pharmacol. Sci.* **1998**, *19*, 287.

118. Kuo, M. L.; Chau, Y. P.; Wang, J. H.; Shiah, S. G. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 502.

119. Messmer, U. K.; Brune, B. *Arch. Biochem. Biophys.* **1996**, *327*, 1.

120. Shimabukuro, M.; Ohneda, M.; Lee, Y.; Unger, R. H. *J. Clin. Invest.* **1997**, *100*, 290.

121. Cuzzocrea, S.; Zingarelli, B.; Gilad, E.; Hake, P.; Salzmann, A. L.; Szabo, C. *Eur. J. Pharmacol.* **1998**, *342*, 67.

122. Szabo, C. Virag, L.; Cuzzocrea, S.; Scott G. S.; Hake, P.; O'Connor, M. P.; Zingarelli, B.; Salzmann, A.; Kun, E. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3867.

123. Matheis, G.; Sherman, M. P.; Buckberg, G. D.; Haybron, D. M.; Young, H. N.; Ignarro, L. *J. Am. J. Physiol.* **1992**, *262*, H616.

124. Yu, L.; Gengaro, P. E.; Niederberger, M.; Burke, T. J.; Schrier, R. W. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 1691.

125. Keller, J. N.; Kindy, M. S.; Holtsberg, F. W.; St. Claire, D. K.; Yen, H. C.; Germeyer, A.; Steiner, S. M.; Bruce-Keller, A. J.; Hutchins, J. B.; Mattson, M. P. *J. Neurosci.* **1998**, *18*, 687.

126. Ischiropoulos, H.; Al-Mehdi, A. B.; Fischer, A. B. *Am. J. Physiol.* **1995**, *269*, L158.

127. Takahashi, K.; Greenberg, J. H.; Jackson, P.; Maclin, K.; Zhang, J. *J. Cerebr. Blood Flow Metab.* **1997**, *17*, 1137.

128. Thiemermann, C.; Bowes, J.; Myint, F. P.; Vane, J. R. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 679.

129. Cuzzocrea, S.; Zingarelli, B.; Costantino, G.; Szabo, A.; Salzman, A. L.; Caputi, A. P.; Szabo, C. *Br. J. Pharmacol.* **1997**, *121*, 1065.

130. Szabo, A.; Hake, P.; Salzman, A. L.; Szabo, C. *Shock* **1998**, *10*, 347.

131. McDonald, M. C.; Mota-Filipe, H.; Thiemermann, C. *Br. J. Pharmacol.* **1999**, *128*, 1339.

132. McDonald, M. C.; Mota-Filipe, H.; Wright, J. H.; Abdelrahman, M.; Threadgill, M. D.; Thompson, A. S.; Thiemermann, C. *Br. J. Pharmacol.* **2000**, *130*, 843.

133. Molinete, M.; Vermeulen, W.; Burkle, A.; Ménissier-de Murcia, J.; Kupper, J. H.; Hoeijmakers, J. H.; de Murcia, G. *EMBO J.* **1993**, *12*, 2109.

134. Cleaver, J. E.; Bodell, W. J.; Morgan, W. F.; Zelle, B. *J. Biol. Chem.* **1983**, *258*, 9059.

135. Cleaver, J. E. *Cancer Res.* **1985**, *45*, 1163.

136. Caldecott, K. W.; Aoufouchi, S.; Johnson, P.; Shall, S. *Nucleic Acids Res.* **1996**, *24*, 4387.

137. Satoh, M. S.; Lindahl, T. *Nature* **1992**, *356*, 356.

138. Morrison, C.; Smith, G. C. M.; Stingl, L.; Jackson, S. P.; Wagner, E. F.; Wang, Z. Q. *Nature Genet.* **1997**, *17*, 479.

139. Borggrefe, T.; Wabl, M.; Akhmedov, A. T.; Jessberger, R. *J. Biol. Chem.* **1998**, *273*, 17025.

140. Ramsamooj, P.; Notario, V.; Dritschilo, A. *Radiat. Res.* **1995**, *143*, 158.

141. Leitinger, N.; Wesierska-Gadek, J. *J. Cell. Biochem.* **1993**, *52*, 153.

142. Poirier, G. G.; de Murcia, G.; Niedergang, C.; Jonsstra-Bilen, J.; Mandel, P. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 3423.

143. Thraves, P. J.; Kasid, U.; Smulson, M. E. *Cancer Res.* **1985**, *45*, 386.

144. Whitacre, C. M.; Hashimoto, H.; Tsai, M. L.; Chatterjee, S.; Berger, S. J.; Berger, N. A. *Cancer Res.* **1995**, *55*, 3697.

145. Vaziri, H.; West, M. D.; Allsopp, R. C.; Davison, T. S.; Wu, Y.; Arrowsmith, C. H.; Poirier, G. G.; Benchimol, S. *EMBO J.* **1997**, *16*, 6018.

146. Griffin, R. J.; Curtin, N. J.; Newell, D. R.; Golding, B. T.; Durkacz, B. W.; Calvert, A. H. *Biochimie* **1995**, *77*, 408.

147. Durkacz, B. W.; Omidiji, O.; Gray, D. A.; Shall, S. *Nature* **1980**, *283*, 593.

148. Weltin, D.; Marchal, J.; Dufour, D.; Potworoswski, E.; Oth, D.; Bischoff, P. *Oncol. Res.* **1994**, 6, 399.

149. Griffin, R. J.; Srinivasan, S.; Bowman, K.; Calvert, A. H.; Curtin, N. J.; Newell, D. R.; Pemberton, L. C.; Golding, B. T. *J. Med. Chem.* **1998**, *41*, 5247.

150. White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. *J. Med. Chem.* **2000**, *43*, 4084.

151. Delaney, C. A.; Wong, L.; Kyle, S.; White, A. W.; Calvert, A. H.; Curtin, N. J.; Durkacz, B. W.; Hostomsky, Z.; Newell, D. R. *Clin. Cancer Res.* **2000**, *6*, 2860.

152. Kato, T.; Suzumura, Y.; Fukushima, M. *Anticancer Res.* **1988**, *8*, 239.

153. Chen, G.; Pan, Q. C. *Cancer Chemother. Pharmacol.* **1988**, *22*, 303.

154. Ben-Hur, E.; Utsumi, H.; Elkind, M. M. *Br. J. Can.* **1984**, *49*, 39.

155. Sebolt-Leopold, J. S.; Scavone, S. V. *Int. J. Radiat. Oncol. Biol. Phys.* **1992**, *22*, 619.

156. Elliot, W. L.; Sebolt-Leopold, J. S.; Leopold, W. R.; Siemann, D. W. *Proc. Am. Assoc. Can. Res.* **1990**, *31*, 412.

157. Shall, S. *J. Biochemistry (Tokyo)* **1975**, *77*, 2p.

158. Purnell, M. R.; Whish, W. J. D. *Biochem. J.* **1980**, *185*, 775.

159. Sims, J. L.; Sikorski, G. W.; Catino, D. M.; Berger, S. J.; Berger, N. A. *Biochemistry* **1982**, *21* 1813.

160. Sestili, P.; Balsamini, C.; Spadoni, G.; Cattabeni, F.; Cantoni, O. *Pharmacol. Res. Commun.* **1988**, *20*, 613.

161. Sestili, P.; Spadoni, G.; Balsamini, C.; Scovassi, I.; Cattabeni, F.; Duranti, E.; Cantoni, O.; Higgins, D.; Thomson, C. J. *Cancer Res. Clin. Oncol.* **1990**, *116*, 615.

162. Cantoni, O.; Sestili, P.; Spadoni, G.; Balsamini, C.; Cucchiarini, L.; Cattabeni, F. *Biochem. Int.* **1987**, *15*, 329.

163. Banasik, M.; Komura, H.; Shimoyama, M.; Ueda, K. *J. Biol. Chem.* **1992**, *267*, 1569.

164. Suto, M. J.; Turner, W. R.; Arundel-Suto, C. M.; Werbel, L. M.; Sebolt-Leopold, J. S. *Anticancer Drug Des.* **1991**, *7*, 107.

165. Griffin, R. J.; Pemberton, L. C.; Rhodes, D.; Bleasdale, C.; Bowman, K.; Calvert, A. H.; Curtin, N. J.; Durkacz, B. W.; Newell, D. R.; Porteous, J. K.; Golding, B. T. *Anticancer Drug Des.* **1995**, *10*, 507.

166. Ruf, A.; Ménissier-de Murcia, J.; de Murcia, G.; Schulz, G. E. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7481.

167. Pivazyan, A. D.; Birks, E. M.; Wood, T. G.; Lin, T.; Prusoff, W. H. *Biochem. Pharmacol.* **1992**, *44*, 947.

168. Shinkwin, A. E.; Whish, W. J. D.; Threadgill, M. D. *Bioorg. Med. Chem.* **1999**, *7*, 297.

169. Wattenberg, L. W. *Cancer Res.* **1966**, *26*, 1520.

170. Pezzuto, J. M. *Biochem. Pharmacol.* **1997**, *53*, 121.

171. Sharma, S.; Stutzman, J. D.; Kelloff, G. J.; Steele, V. E. *Cancer Res.* **1994**, *54*, 5848.

172. Gescher, A.; Pastorino, U.; Plummer, S. M.; Manson, M. M. *Br. J. Clin. Pharmacol.* **1998**, *45*, 1.

173. Satyanarayana, M. N.; Ravindranath, V. *Photochem.* **1980**, *19*, 2031.
174. Huang, M.; Smart, R. C.; Wong, C.; Conney, A. H. *Cancer Res.* **1988**, *48*, 5941.
175. Nagabhushan, M.; Bhide, S. V. *J. Am. Coll. Nutr.* **1992**, *11*, 192.
176. Rao, C. V.; Simi, B.; Reddy, B. S. *Carcinogenesis* **1993**, *14*, 2219.
177. Mehta, R. G.; Moon, R. C. *Anticancer Res.* **1991**, *11*, 593.
178. Ammon, H. P. T.; Wahl, M. A. *Plant Medica* **1991**, *57*, 1.
179. Sui, Z.; Salto, R.; Li, J.; Craik, C.; Ortiz de Montellano, P. R. *Bioorg. Med. Chem.* **1993**, *1*, 415.
180. Kelloff, G. F.; Boone, C. W.; Crowell, J. A.; Steele, V. E.; Lubet, R.; Sigman, C. C. *Cancer Epidemiology Biomarkers and Prevention* **1994**, *3*, 85.
181. Rao, C. V.; Rivenson, A.; Simi, B.; Reddy, B. S. *Cancer Res.* **1995**, *55*, 259.
182. Plummer, S. M.; Holloway, K. A.; Manson, M. M.; Munks, R. J. L.; Kaptein, A.; Farrow, S.; Howells, L. *Oncogene* **1999**, *18*, 6013.
183. Kawamori, T.; Rao, C. V.; Seibert, K.; Reddy, B. S. *Cancer Res.* **1998**, *58*, 409.
184. Oshima, M.; Dinchuk, J. E.; Kargman, S. L.; Oshima, H.; Hancock, B.; Kwong, E.; Trzaskos, M.; Evans, J. F.; Taketo, M. M. *Cell* **1996**, *87*, 803.
185. Ravindranath, V.; Chandrasekhara, N.; *Toxicol.* **1981**, *20*, 251.
186. Ravindranath, V.; Chandrasekhara, N.; *Toxicol.* **1981**, *22*, 337.
187. Holder, G. M.; Plummer, J. L.; Ryan, A. J. *Xenobiotica* **1978**, *8*, 761.
188. Pan, H.; Huang, T.; Lin, J. K. *Drug Metab. Disposit.* **1999**, *27*, 486.
189. Sugiyama, Y.; Kawakishi, S.; Osawa, T. *Biochem. Pharmacol.* **1996**, *52*, 519.
190. Breccia, A.; Cavalleri, B.; Adams, G. E. *Nitroimidazoles Chemistry, Pharmacology and Clinical Application*, Plenum Press, New York and London, **1982**.
191. Lancini, G. C.; Lazzari, E.; Arioli, V,; Bellani, P.; *J. Med. Chem.* **1969**, *12*, 775.
192. Cavalleri, R.; Ballotta, A.; Lancini, G. C. *J. Hetercyclic Chem.* **1972**, *9*, 979.
193. Schmidt, U.; Geiger, F.; *Justus Liebigs Ann. Chem.* **1963**, *664*, 168.
194. Kirby, M. J. *Final Year Project Report for Bachelor of Pharmacy Degree*, University of Bath, **1992**.
195. Desai, T.; Gigg, J.; Gigg, R. *Aust. J. Chem.* **1996**, *49*, 305.

196. Wiley, G. A.; Hershkowitz, R. L.; Rein, B. M.; Chung, B. C. *J. Am. Chem. Soc.* **1963**, *86*, 964.

197. Somei, M.; Karasawa, Y.; Shoda, T.; Kaneko, K. *Chem. Pharm. Bull.* **1981**, *1*, 249.

198. Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, *25*, 839.

199. Abeywickrema, A. N.; Beckwith, A. L. J. *J. Org. Chem.* **1987**, *52*, 2568.

200. Neilson, T.; Wood, H. C. S.; Wylie, A. G.; *J. Chem. Soc.* **1962**, 371.

201. Madani, H. MPhil Thesis, University of Bath, **2001**.

202. Varghese, A. J.; Whitmore, G. F. *Cancer Res.* **1980**, *40*, 2165.

203. Chatterjee, P. K.; Pederson, H.; Chatterjee, B. E.; McDonald, M. C.; Thompson, A. S.; Threadgill, M. D. *Br. J. Pharmacol.* **2001**, *133*, 123P, Suppl. 5.

204. Threadgill, M. D.; Wayman, N.; McDonald, M. C.; Thiemermann, C.; Parveen, I.; Thompson, A. S. *Abstract 117, ADPR 2001 International Symposium on ADP-ribosylation*, New York, **2001**.

205. Chatterjee, P. K.; Pederson, H.; McDonald, M. C.; Thompson, A. S.; Threadgill, M. D.; Thiemermann, C. *Abstract 119, ADPR 2001 International Symposium on ADP-ribosylation*, New York, **2001**.

206. Miyashita, A.; Matsuda, H.; Iijima, C.; Higashino, T. *Chem. Pharm. Bull.* **1992**, *40*, 43.

207. Davis, D. P.; Kirk, K. L.; Cohen, L. A. *J. Heterocyclic Chem.* **1982**, *19*, 253.

208. Bond, R. F.; Bredenkamp, M. W.; Holzapfel, C. W. *Synth. Commun.* **1989**, *19*, 2551.

209. Patterson, L. H.; McKeown, S. R. *Br. J. Cancer* **2000**, *83*, 1589.

210. Ukai, T.; Yamamoto, Y.; Hirano, S. *Yakugaku Zasshi* **1953**, *73*, 823.

211. Brzezinski, B. *Pol. J. Chem.* **1983**, *57*, 249.

212. Suggs, J. W.; Pearson, G. D. N. *J. Org. Chem.* **1980**, *45*, 1514.

213. Nakashima, T.; Suzuki, I. *Chem. Phar. Bull.* **1969**, *17*, 2293.

214. Hamm, P.; Philipsborn, W. V. *Helv. Chim. Acta.* **1971**, *54*, 2363.

215. Colonna, M.; Greci, L.; Poloni, M. *J. Hetercyclic Chem.* **1980**, *17*, 293.

216. van Baar, B. L. M.; Rozendal, J.; van der Goot, H. *J. Mass Spectrom.* **1998**, *33*, 319.

217. Syu, W.; Shen, C.-C.; Don, M.-J.; Ou, J.-C.; Lee, G.-H.; Sun, C.-M. *J. Nat. Prod.* **1998**, *61*, 1531.

218. Nurfina, A. N.; Reksohadiprodjo, M. S.; Timmermann, H.; Jenie, U. A.; Sugiyanto, D.; van der Goot, H. *Eur. J. Med. Chem.* **1997**, *32*, 320.
219. Babu, K. V. D.; Rajasekharan, K. N. *Org. Prep. Proc. Int.* **1994**, *26*, 674.
220. Mann, G.; Beyer, L.; Arrieta, A. *Z. Chem.* **1987**, *27*, 172.
221. Mabic, S.; Lepoittevin, J.-P. *Tetrahedron Lett.* **1995**, *36*, 1705.
222. Freudenberg, K.; Jovanovic, V. *Chem. Ber.* **1963**, *96*, 2178.
223. Hill, R. A.; Kirby, G. W.; O'Loughlin, G. J.; Robins, D. J. *J. Chem. Soc., Perkin Trans. 1*, **1993**, 1967.
224. Costa, A.; Saa, J. M. *Tetrahedron Lett.* **1987**, *28*, 5551.
225. Ralph, J.; Zhang, Y.; Ede, R. M. *J. Chem. Soc., Perkin Trans. 1*, **1998**, 2609.
226. Roughley, P. J.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2379.
227. Perkins, S.; Verschoyle, R. D.; Hill, K.; Parveen, I.; Threadgill, M. D.; Sharma, R. A.; Williams, M. L.; Steward, P.; Gescher, A. J. submitted for publication.
228. Martin, P. K.; Matthews, R.; Rapoport, H.; Thyagarajan, G. *J. Org. Chem.* **1968**, *33*, 3758.
229. Caramella, P.; Bandiera, T.; Albini, F. M.; Gamba, A.; Corsaro, A.; Perrini, G. *Tetrahedron* **1988**, *44*, 4917.
230. Trepanier, D. L.; Sunder, S. *J. Heterocyclic Chem.* **1975**, *12*, 321.
231. Amin, J. H.; de Mayo, P. *Tetrahedron Lett.* **1963**, *24*, 1585.
232. Prijs, B.; Gall, R.; Hinderling, R.; Erlenmeyer, H. *Helv. Chim. Acta* **1954**, *37*, 90.
233. Anderson, B. A.; Bell, E. C.; Ginah, F. O.; Harn, N. K.; Pagh, L. M.; Wepsiec, J. P. *J. Org. Chem.* **1998**, *63*, 8224.
234. Elderfield, R. C.; Gernsler, W. J. Bembry, T. H.; Williamson, T. A.; Weisl, H. *J. Am. Chem. Soc.* **1946**, *68*, 1589.
235. Wittcoff, H.; Reigel, C. *J. Am. Chem. Soc.* **1946**, *68*, 1913.
236. Hansson, C.; Wickberg, B. *Synthesis* **1976**, 191.
237. Matthes, H. W. D.; Luu, B.; Ourisson, G. *Phytochem.* **1980**, *19*, 2643.