THE SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL ANTICANCER AGENTS

A Thesis submitted in part fulfilment of the requirements of the degree of Doctor of Philosophy

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Dedicated to my mum and dad

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And finally, my biggest thank you of all is to my family: mum, dad, Claire and John who have given me their unconditional love and support from the very beginning, so much more than I could have ever expected. Thank you.

ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BMN	Benzylidenemalononitrile
bp	Boiling point
br.	Broad (NMR spectroscopy)
cat.	Catalyst
CI	Chemical Ionisation
cmpd	Compound
CNS	Central nervous system
d	Day(s)
d	Doublet (NMR spectroscopy)
dec.	Decomposed (mp)
DEPT	Distortionless Enhancement by Polarisation Transfer
DMF	*N,N*-dimethylformamide
DNA	Deoxyribonucleic acid
EI	Electron Impact
ERK	Extracellular signal-regulated kinase
GI_{50}	Concentration required to reduce growth by 50%
GST	Glutathione *S*-transferase
h	Hour(s)
IARC	International Agency for Research on Cancer
IR	Infrared
JNK	c-Jun N-terminal kinase
lit.	Literature value
LSPD	Long range selective proton decoupling
m	Multiplet (NMR spectroscopy)
m	Medium (IR spectroscopy)
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MEK	MAP/ERK kinase
mp	Melting point
ms	Mass spectrometry
NMR	Nuclear magnetic resonance
PKC	Protein kinase C
q	Quartet (NMR spectroscopy)

quat.	Quaternary (NMR spectroscopy)
Rf	Retention factor
RNA	Ribonucleic acid
rt	Room temperature
s	Strong (IR spectroscopy)
s	Singlet (NMR spectroscopy)
SAPK	Stress-activated protein kinase
SDS	Sodium dodecyl sulfate
ser	Serine
SRB	Sulforhodamine B
t	Triplet (NMR spectroscopy)
THF	Tetrahydrofuran
thr	Threonine
TLC	Thin layer chromatography
tyr	Tyrosine
UV	Ultraviolet
w	Weak (IR spectroscopy)

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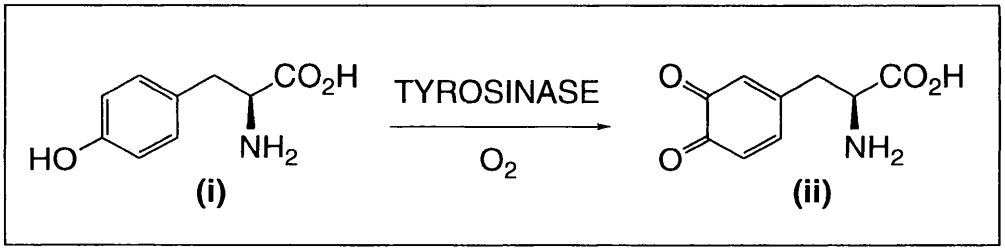
CHAPTER 2 MALIGNANT MELANOMA AND ANTIMELANOMA AGENTS

CHAPTER 3 CELL SIGNALLING AND MAP KINASE INHIBITORS

SUMMARY

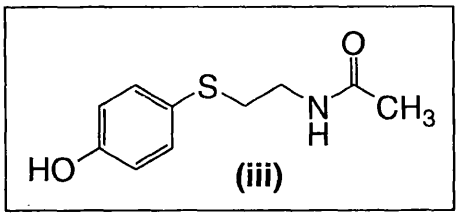
Two separate cancer topics are included in our studies of the synthesis and biological evaluation of novel anticancer agents. These topics are synthesis of antimelanoma agents to combat malignant melanoma and synthesis of MAP kinase inhibitors designed to interfere with cell signalling pathways.

In the chemotherapeutic approach towards malignant melanoma treatment a selective approach can be adopted in drug design. Melanin is synthesised within specialised cells called melanocytes and malignant melanoma results when the control of melanocytic cell division is disturbed leading to excessive melanin production. The target strategy behind malignant melanoma drug design exploits specifically Tyrosinase, an enzyme crucial to melanin synthesis and unique to the melanocyte. Tyrosinase catalyses the conversion of L-tyrosine **(i)** into dopaquinone **(ii)**.



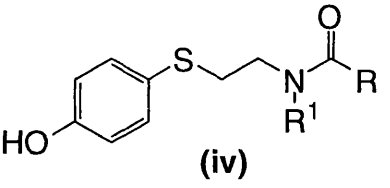
(i) **(ii)**

Design strategies concentrate on phenolic prodrugs modelled on the natural substrate **(i)** of the oxidase enzyme. Tyrosinase catalyses the conversion of the phenolic prodrug into the corresponding *o*-quinone. These *o*-quinones are the mediators of cytotoxicity believed to impair the melanocytes' metabolism by combining with sulfhydryl-containing entities crucial to the cell cycle such as DNA polymerase. Alternatively the *o*-quinones may participate in a cyclic redox process resulting in the production of active oxygen species. The parent drug in the antimelanoma field, **(iii)**, has been found to possess significant activity. An extensive programme of research was performed by Neil Lant at the University of Glasgow into the structure activity relationships of related compounds.



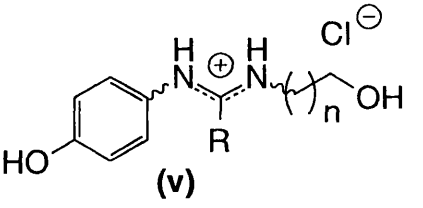
(iii)

Our programme of research involved the synthesis of three sets of target compounds modelled on the lead compound designed as substrates for Tyrosinase. These tertiary amides **(iv)**, amidine salts **(v)** and tyrphostins **(vi)** were successfully prepared and biologically evaluated for *in vitro* antimelanoma activity and relative substrate activity for Tyrosinase.



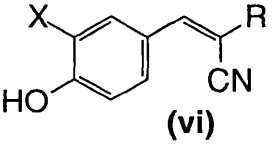
(iv)

R = Me, Ph, *cyclo*C_6H_{11}
R^1 = Me, Et, Bn



(v)

n = 1, 2
R = Me, Et



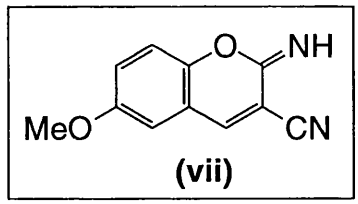
(vi)

X = H, OH, OMe
R = CN, CO_2Et, $CONH_2$, $C(NH_2)=C(CN)_2$

The lipophilic tertiary amides **(iv)** proved to be the most successful as potential antimelanoma agents with the *N*-benzyl methyl analogue in particular exhibiting good levels of cytotoxicity and selectivity for Tyrosinase-containing melanoma cell lines with GI_{50} values in the range 5-30 μM. Furthermore this compound was a Tyrosinase substrate. Although the amidine salts indicated some levels of cytotoxicity and selectivity their cytotoxicity is believed to be the result of a non-Tyrosinase mediated mechanism of action. In general this biological behaviour exhibited by the amidines **(v)** was mirrored by the tyrphostins **(vi)**. In conclusion we believe that the combination of the lipophilic nature of the tertiary amides and the close structural resemblance to the parent compound **(iii)** provided the series **(iv)** with better antimelanoma activity than the other two series **(v)** and **(vi)**.

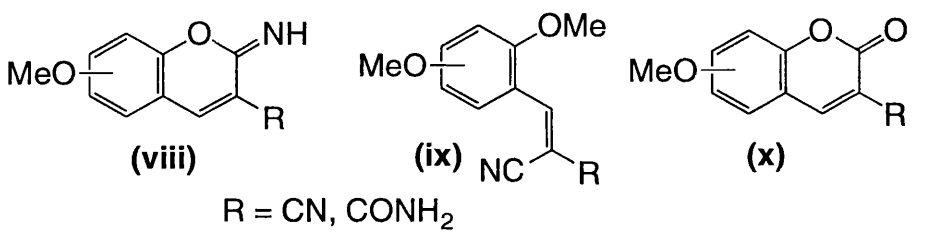
The second area of cancer chemotherapy research investigated is concerned with cell signalling pathways and inhibition of mitogen-activated protein (MAP) kinases which will prevent division of cancer cells. In our studies

into MAP kinase inhibitors we employed 2-iminolactone derivative **(vii)** as our lead which had been identified from a preliminary screen within the Robins research group. This compound is modelled on serine, an amino acid residue phosphorylated by the MAP kinase enzyme.



(vii)

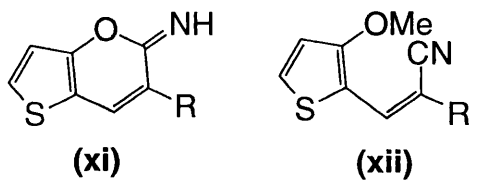
Our programme of research first investigated the optimum position of the methoxy substituent on the benzene ring of the lead compound with the series **(viii)** prepared. The corresponding open chain derivatives **(ix)** and the coumarins **(x)** were also prepared.



(viii) **(ix)** **(x)**

R = CN, $CONH_2$

The *in vitro* inhibitory assay results for the 2-iminolactones **(viii)** and tyrphostins **(ix)** indicated worthwhile candidates for a more detailed study into MAP kinase inhibitory behaviour. However, the coumarins **(x)** did not exhibit inhibitory behaviour. In response to the biological study results the preparation of thiophene bioisosteric replacements **(xi)** and **(xii)** of the 2-iminolactone **(viii)** and tyrphostins **(ix)** was proposed. Only the thiophene tyrphostins **(xii)** were successfully prepared and the MAPK inhibitory activity shown by the thiophene tyrphostins appeared to support the strategy behind their design.



(xi) **(xii)**

R = CN, $CONH_2$

CHAPTER 1

CANCER - A GENERAL OVERVIEW

1.1 CANCER - AN EPIDEMIC

The World Health Organisation reported that an estimated 10 million cases of cancer occurred world-wide in 1998. Furthermore they predicted a continuation of this onslaught with numbers rising to 14.7 million over the next 20 years.[1] The current situation is that the proportion of fatalities from cancer in the population is increasing simply because fewer people are dying from other illnesses.[2]

Many factors combine to ensure the prevalence of this epidemic which is especially high in the industrialised nations. Among the causative factors are increased life expectancy, growing urbanisation, industrialisation and changes in lifestyle. Although the incidence in the number of cancer cases (and deaths) could be significantly lowered through changes in factors such as lifestyle and diet, preventative measures for many cancers remain either unknown or not applicable.[2]

In these cancer-stricken times, comfort must be taken from the technological advances and scientific progress which continue to be made by the present science community. These developments in turn enable a greater understanding of epidemiological diseases such as cancer and ultimately should facilitate the progress towards prevention, treatment and cure for most, if not all, cancers by sometime in the 21st century.

1.2 THE AETIOLOGY OF CANCER

The term carcinogen is a very loosely used one and by definition encompasses any agent which confers an increase in cancer risk. This is a vast topic and under this heading of aetiology of cancer some of the main causative factors of cancer will be presented.

1.2.1 CHEMICALS

The cancer-causing potential of chemicals was first recognised over 200 years ago by the English physician Pott who linked soot to the incidence of scrotal cancer in chimney sweeps.[3] The carcinogen involved, a polycyclic aromatic hydrocarbon, was not identified until much later. This exemplifies the workplace as one source of chemical carcinogens, with other sources including pollutants in air and water, foodstuffs and natural products.[4] A monograph series published by the International Agency for Research on Cancer (IARC)[5] is one of many publications that details chemicals with carcinogenic potential for employers, workers and the public in general to consult.

The polycyclic aromatic hydrocarbons like those found in the chimney soot are far more abundant today as air pollutants. With the onset of industrialisation these chemicals are prevalent in exhaust fumes and factory emissions.[4] A very well publicised chemical carcinogen of modern times is asbestos,[6] with pneumoconiosis or asbestosis affecting workers in that area. Furthermore, it is believed that exposure to this chemical increases the individual's risk of lung cancer and mesothelioma.[6]

These are just a few representatives of the vast number of chemical carcinogens known. However, many chemical cancer causing agents found in some sources are present in very low levels such that the attributable risk is considered to be negligible, e.g. some food colourants.

1.2.2 RADIATION

Following Roentgen's discovery of X-rays in 1895 the first reports of radiation carcinogenesis emerged. The cancer appeared on the hands of pioneering radiologists and later cases of leukaemia were to emerge.[7] The carcinogenic nature of ionising and ultraviolet (UV) radiation has been well documented since these early reports. Radiation cancer risk studies were carried out on individuals including patients irradiated for medical treatment and the Hiroshima and Nagasaki atomic bomb survivors.[8] These studies confirmed the radiation exposure/cancer risk correlation. Some of the cancers reported with an increased risk of development following exposure include leukaemia and cancer of the breast, lung and stomach.[9]

UV light radiation is a well known carcinogen.[10] The correlation between malignant melanoma and UV light is an established phenomenon with overexposure to this form of radiation thought to increase the cancer risk.[11] The

use of UV artificial light sources such as sun lamps and beds confirms these findings.[12] The UV light/melanoma topic is discussed in greater detail in Chapter 2.

1.2.3 LIFESTYLE

Of all the causative factors in cancer the one responsible for the most cancer deaths is cigarette smoking.[13] So great are the number of deaths linked to smoking that cigarette companies are legally bound to advertise the fatal implications of this habit on their packaging. As well as lung cancer, other tobacco-related cancers include cancer of the oral cavity, pharynx, urinary bladder and pancreas.[13]

The American Institute for Cancer Research is devoted to cancer prevention through changes in diet and nutrition. This research body reports reductions in some cancers of 60 to 70% by changes in lifestyle such as diet, weight and physical activity.[1] The pioneering work in this area by Tannenbaum[14] demonstrated correlations between animal diets and risk of breast cancer with increased carcinogenicity found in animals consuming a diet high in fat and calories compared to those adhering to normal diets.

1.2.4 VIRUSES

Cancer-causing viruses can be divided according to their genetic makeup such that DNA- and RNA-containing tumour viruses exist.[15] Retroviruses are the oncogenic viruses with an RNA genome exclusively. The first reported human retrovirus, human T-lymphotropic virus-type 1 (HTLV-1) infects mature T-lymphocytes which results in adult T-cell leukaemia-lymphoma (ATL). ATL incurs high mortality rates and is geographically restricted with the highest incidence found in Japan and the Caribbean.[16] Consider the second group of oncogenic viruses. Examples of these DNA-containing tumour viruses are more plentiful, e.g. the Epstein-Barr virus (EBV). This infects B lymphoma cells producing lymphoproliferative diseases including Burkitt's lymphoma and infectious mononucleosis.[17] Other DNA-containing tumour viruses such as human papilloma virus (HPV) and herpes simplex virus-type 2 (HSV-2) both produce cervical cancer.

1.2.5 CANCER-CAUSING GENES

Genes are well established as a causative factor of many cancers. The two main types of these genes are oncogenes and tumour suppressor genes.

1.2.5.1 Oncogenes (and proto-oncogenes)[18]

Oncogenes are the main cancer-causing genes. When expressed these genes encode protein products which bring about changes to a normal cell characteristic of malignancy. Proto-oncogenes are genes found in every cell in the human body which can be converted into oncogenes, e.g. by mutation or deletion which then enables them to assume an oncogenic function. In normal cells proto-oncogenes encode proteins which play important roles in cellular processes such as proliferation and growth.[19] Therefore the net effect of the proto-oncogene/oncogene transformation is that proteins are encoded with aberrant cellullar functions and the transformed cell proliferates in an uncontrolled manner, characteristic of a cancer cell.[20] Proto-oncogenes and their role in malignant transformations of normal cell are discussed in greater detail in section 3.4.

1.2.5.2 Tumour suppressor genes

Tumour suppressor genes play a regulatory role in normal cell growth. These genes are activated on demand to halt the cell growth process. However, when these genes are lost or inactivated, their inhibitory regulation of cell growth is thus removed whereupon malignancy results.[21] Wilms' tumour gene is a common suppressor gene. Deletions or mutations to this gene on the short arm of chromosome 11 are attributed to Wilms' tumour, the most prevalent childhood kidney cancer.[22] The *CDKN2* tumour suppressor gene is the most important familial malignant melanoma gene.[23] This gene and its relationship with malignant melanoma is discussed further in section 2.4.3

1.3 THE CELL CYCLE[24]

Cell division plays a role of immeasurable importance in the life processes of various organisms. Just consider for example the unicellular organism Amoeba which reproduces by cell division or that multicellular organisms require cell division for growth and repair.[25]

The cell cycle comprises of an ordered sequence of definitive events within the dividing cell. This cycle is made up of the mitotic (M) phase and interphase with 90% of cycle time devoted to interphase. In G_1, G_2 and S, the three sub phases of interphase, the cell grows (G_1 and G_2 sub phases) and chromosomes are copied (only in S subphase). On reaching the mitotic phase of the cycle the cell is divided whereupon two identical daughter cells are produced.[26]

The crucial point for the cell cycle occurs within G_1 phase at the restriction point. A trigger at this point is required for procession into the S phase. However, the cell can depart from the cycle at this point into the G_0 phase, the phase in which most human cells are found. The proliferative nature of cells vary such that some cells, for example marrow cells rapidly divide, whereas other cells like those of the nerves or muscle, never divide. However, certain cells including lung and liver cells, have the potential to enter the cell cycle when required which is usually only for repair purposes.[26]

1.3.1 CANCER CELL DIVISION

Cancer cell proliferation is uncontrolled with the transformed cell failing to respond to the regulatory mechanisms for normal growth control.[27] The malignant transformation of normal cells to cancer cells is under genetic control with the genes which are controlling cell proliferation being active beyond control and apparently unable to be switched off. The mechanism which maintains the genes in this active state holds the key to fully understanding cancerous cell production and the genetics behind its control.[27]

1.4 THE TREATMENT OF CANCER

1.4.1 SURGERY AND RADIOTHERAPY

Cancer in its many forms can be treated with a variety of therapeutic agents which include surgery, radiotherapy, chemotherapy and immunotherapy.

The use of surgery in cancer treatment is relatively diverse and very much dependent on the nature of the cancer, e.g. surgery is fundamental for use in biopsies and localised excisions but limited in the successful treatment of many advanced neoplastic cancers.[28] Common to most cancer therapies, surgery is regularly supplemented with other approaches.

Radiotherapy is a potentially very effective treatment of cancer, hampered only by its ability to damage normal cells. Irradiation sources include X-rays, γ-rays and radioactive isotopes of elements such as radium and cobalt.[29] This therapeutic agent is common in adjuvant treatment for post-surgical patients to limit the risk of recurrence. In the treatment of Hodgkin's disease, radiotherapy and chemotherapy (see section 1.4.2) in combination or as single agents provide one of the most successful treatments of a cancer regardless of the stage of the disease.[30] Computerised tomography[31] and NMR imaging[32] advances have enabled improvements in surgery and radiotherapy by providing a more defined image of the cancer. These imaging modems enable a precise treatment of infected tissues with normal tissues affected as little as possible.

1.4.2 CHEMOTHERAPY[33, 34]

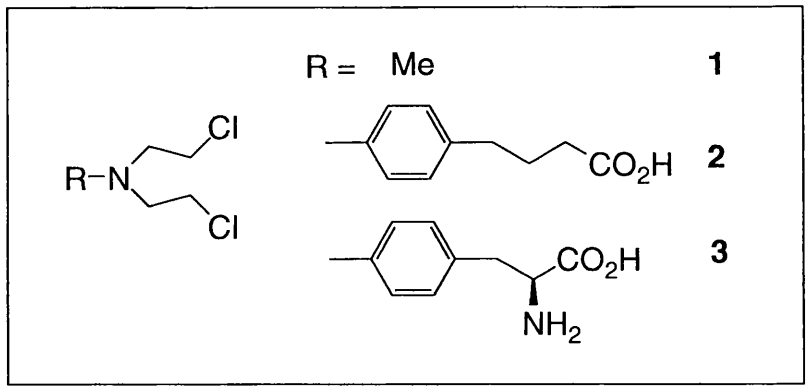
The vast range and number of cancer chemotherapeutic agents in clinical use today alone means that this topic cannot be discussed here in great detail. However, a representative discussion of the major classes of anticancer drugs will follow describing alkylating agents, antimetabolites, topoisomerase II inhibitors and antimitotic agents.

1.4.2.1 Alkylating agents

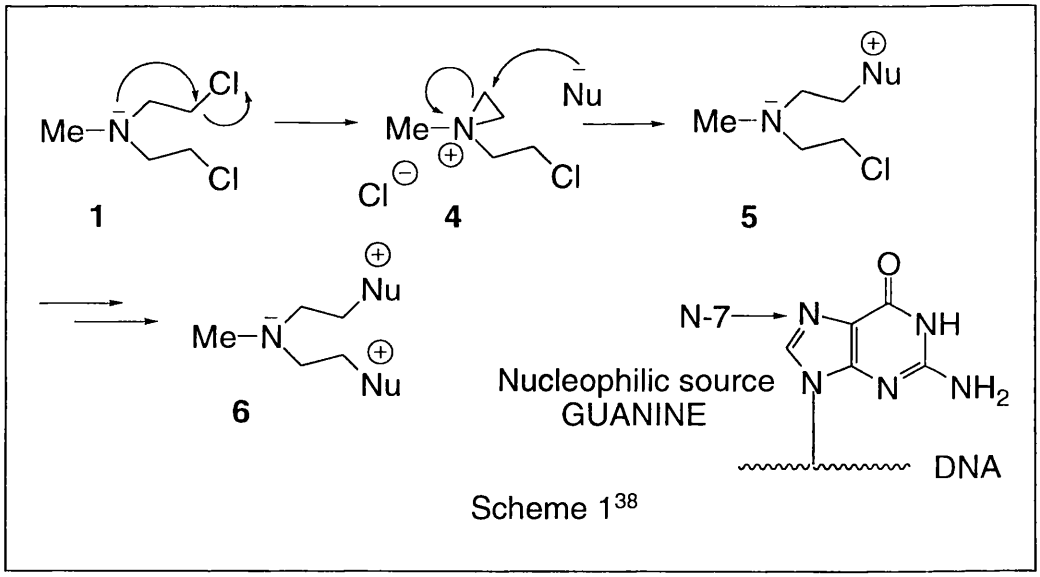
This class of chemotherapeutic agents react chemically with the cancer cells' own DNA. On rendering the DNA structurally and functionally redundant, cell death occurs. The alkylating agents can be classified into nitrogen mustards, platinum complexes and nitrosoureas.

1.4.2.1.1 Nitrogen mustards[35]

The tertiary amine methyl bis(2-chloroethyl)amine (mechlorethamine) **1** was the first clinical cancer chemotherapeutic agent used and still finds employment today as part of a drug combination regimen to treat Hodgkin's disease.[36] Chlorambucil **2** and melphalan **3**, both structural analogues of mechlorethamine are widely used antitumour agents in combination or as single agents. Notably melphalan is used as an adjuvant to surgery in primary breast cancer treatment.[37]



The mechanism of cytotoxicity for these three nitrogen mustards is the same with the bis(2-chloroethyl)amino moiety providing the structural feature required.[35] The nitrogen mustards function as alkylating agents by crosslinking the double strands of DNA. Cell death ensues as a crosslinked DNA species cannot unwind and replicate to complete the cell cycle. A simplified illustration for this cytotoxic mechanism of the action of nitrogen mustards using mechlorethamine **1** is shown in Scheme 1.

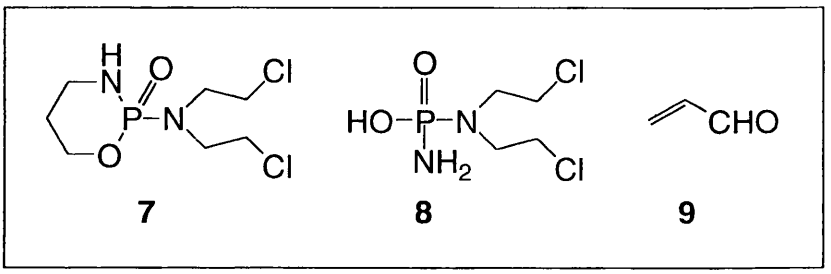


Scheme 1[38]

On forming the aziridine species **4**, the nitrogen mustard is set up for nucleophilic attack. For cytotoxicity leading to cell death DNA provides the source of nucleophilicity. Nucleotide bases on the DNA, e.g. guanine provide the nucleophilic site (N-7 position) for attack of the aziridine. The monoadduct product **5** can result in the mispairing of bases or strand breakage of the DNA; however the cell can invariably repair this damage. On the other hand, the monoadduct can be further involved in a repeat crosslinking process with a final

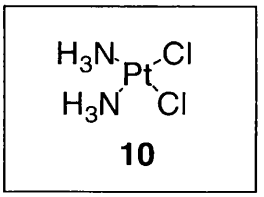
adduct **6** linking either the same DNA strand (intrastrand crosslink) or as is more commonly found linking complementary DNA strands (interstrand crosslink).[35]

The leading alkylating agent, cyclophosphamide **7** is not itself active, requiring metabolic activation before it can exert cytotoxicity against the cancer. Amongst the cyclophosphamide metabolites produced are phosphoramide mustard **8** and acrolein **9** and these are believed to be the cytotoxic species with the phosphoramide mustard **8** behaving as a DNA crosslinking species. Cyclophosphamide exhibits broad spectrum anticancer activity which is reflected in its wide use in the treatment of cancers such as acute lymphocytic leukaemia, breast carcinoma and Hodgkin's disease.[39]



7 **8** **9**

1.4.2.1.2 Platinum complexes

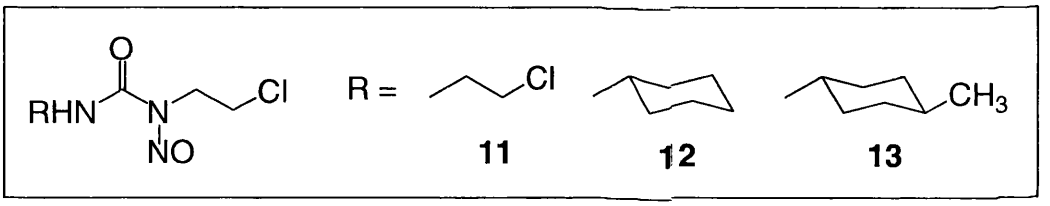


10

Of the platinum complexes cisplatin **10** exhibits the best antitumour effects with activity against several human cancers when used alone or in combination drug therapy.[40] Structure activity studies identified the *cis*-arrangement of ligands in cisplatin to be imperative for activity.[40] The cytotoxicity of this platinum complex is believed to arise from DNA interactions whereby the main adducts form from intrastrand DNA crosslinks.[41] Carboplatin, iproplatin and tetraplatin are among the most potent of 2000 or so analogues of cisplatin synthesised. However, they do not improve on cisplatin itself as they fail to reduce levels of toxicity or resistance of tumours found with repeated cisplatin drug use.[40]

1.4.2.1.3 Nitrosoureas

Random screens performed by the National Cancer Institute in 1959 prompted the first work into nitrosoureas as potential chemotherapeutic agents.[42] Later 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) **11** emerged as one of the first nitrosoureas with anticancer potential, followed by lomustine (CCNU) **12** and its 4-methyl homologue, semustine (MeCCNU) **13**.[43] The replacement of the chloroethyl moiety in BCNU **11** with a cyclohexyl ring in CCNU **12** and MeCCNU **13** was found to improve lipophilicity thus greatly assisting absorption. This enhanced lipophilicity enabled penetration of the blood-brain barrier with central nervous system tumours able to be reached.[44] Although all three nitrosoureas are employed in drug combination chemotherapy, each of them are found to exhibit significant activity in the treatment of Hodgkin's disease[44] and brain tumours. DNA is the major target of the nitrosoureas with evidence of interstrand crosslinking and single strand breaks reported.[43]



11 **12** **13**

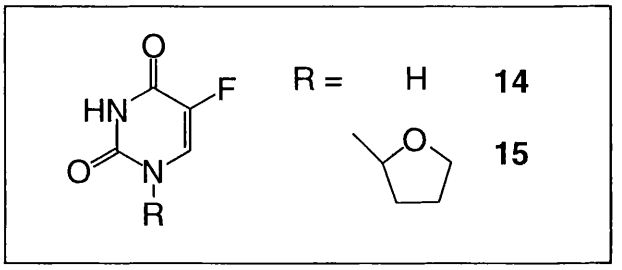
1.4.2.2 Antimetabolites

Antimetabolites target the metabolic pathways of the cancer cells. These agents usually take the form of synthetic analogues of the normal metabolites with the prime objective being to halt the uncontrolled cell growth in the cancer patient.[45] In effect, antimetabolites assume the role of an imposter in the metabolic pathways of cancer cells. The most successful antimetabolite drugs are found to affect the pathways leading to the production of DNA-related components.[45] The main antimetabolites include fluoropyrimidines, thiopurines and antifolates.

1.4.2.2.1 Fluoropyrimidines

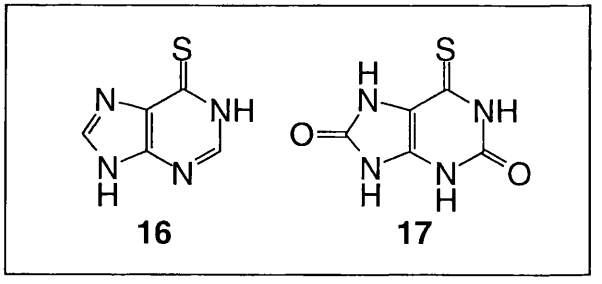
The lead fluoropyrimidine, 5-fluorouracil (5-FU) **14** was designed as a pyrimidine base mimic. The cytotoxicity of 5-FU results from DNA-directed action of the drug due to incorporation of 5-FU into DNA. This fluoropyrimidine as a single chemotherapeutic agent is most commonly used in the treatment of

solid tumours, in particular breast and gastrointestinal tract carcinomas. However, it is employed to a greater extent in combination drug therapy.[45] For example, 5-FU in a three-drug regimen with doxorubicin (see section 1.4.2.3) and cyclophosphamide is used to treat advanced breast cancer.[46] Derivatisation of fluoropyrimidines has been found to be biologically very useful. One such example is the prodrug ftorafur **15** which exhibits antitumour activity against a range of solid tumours.[47]



1.4.2.2.2 Thiopurines

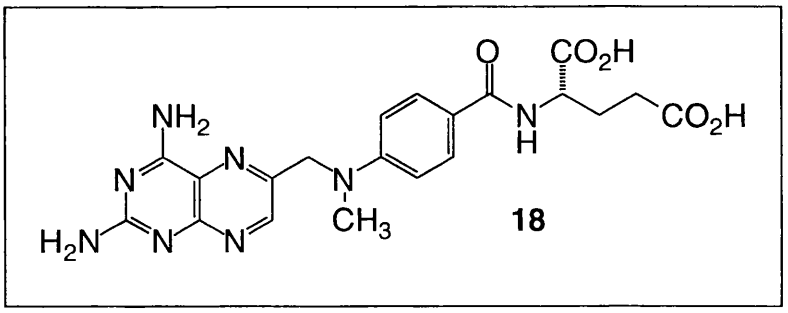
Of the unnatural purines investigated for anticancer activity, 6-mercaptopurine **16** and 6-thioguanine **17** proved to be among the most promising.[48] However, 6-mercaptopurine **16** proved to be the most potent of the two antimetabolites exhibiting activity against acute childhood leukaemia.[49] The tumour response with 6-mercaptopurine **16** has been correlated with conversion of the drug into the corresponding ribonucleotide.[48]



1.4.2.2.3 Antifolates

The folates are coenzymes involved in many biochemical transformations. Dihydrofolate reductase (DHFR) is among the many folate-utilising enzymes and plays a key role in pathways leading to the formation of nucleic acids.[50] Methotrexate (MTX), **18** a classical antifolate, potently inhibits DHFR resulting in cytotoxicity. Used in the treatment of choriocarcinoma and

acute lymphocytic leukaemia,[51] MTX **18** is also employed in various combination drug regimens. However major limitations in MTX drug use arise via toxic side effects and the development of resistance to the drug by tumour cells.[51] These are problems commonly faced by many antitumour agents.

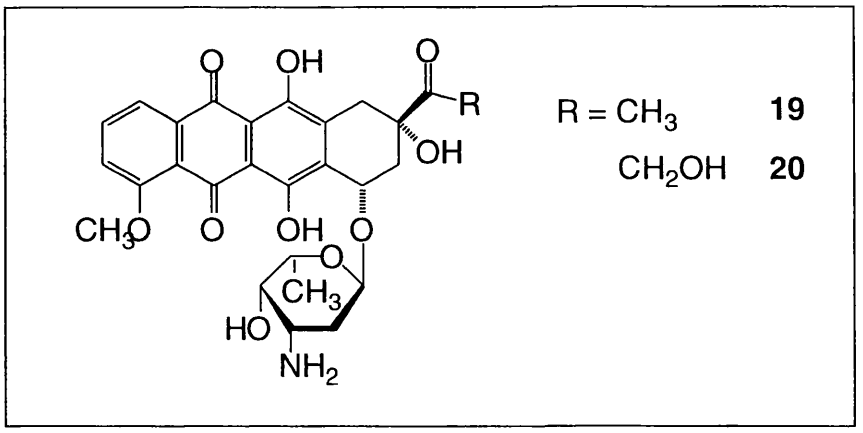


18

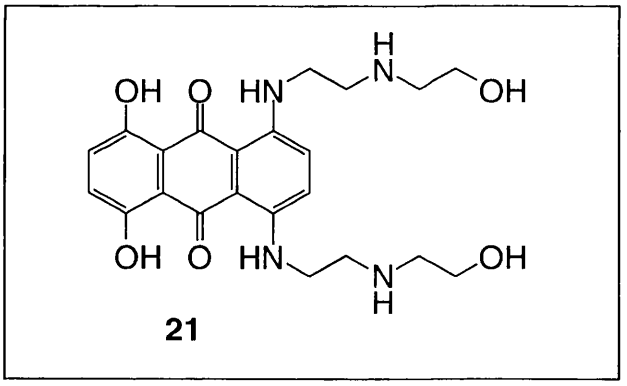
1.4.2.3 Topoisomerase II inhibitors

DNA topoisomerase II is a homodimeric protein responsible for the control of double stranded DNA topology. When required these enzymes function by first breaking the paired DNA strands and then later reforming the breaks e.g. during transcription whereby the DNA returns to its original strand-paired state.[52] This characteristic topoisomerase II action has seen these enzymes implicated in facilitating the cytotoxicity of intercalating drugs. These agents bind tightly to DNA but this alone does not correlate with the cytotoxicity observed.[53] A complex mechanism is proposed whereby the intercalators inhibit the breakage-reunion reaction of the topoisomerase II.[54] Cell death arises from lethal breaks in the double stranded DNA.[54]

The anthracycline antibiotics are DNA intercalating agents believed to target topoisomerase II enzymes to elicit their anticancer activity. Isolated from a *Streptomyces* species, the anthracyclines include Daunomycin (daunorubicin) **19** and Adriamycin (doxorubicin) **20** which were the first anthracycline antibiotics to be used in tumour chemotherapy. Notably Daunomycin **19** predominates in the treatment of acute leukaemia. However the side effects of toxicity and alopaecia are standard.[55] The functional diversity of the anthracyclines is of particular interest because in addition to inhibiting topoisomerase II, these chemotherapeutic agents chelate transition metal ions and generate free radicals within the tumour cells.[55]



The synthesis of anthracycline analogues has provided little improvement on anticancer activity. However, the synthetic anthraquinone mitoxantrone **21** although not strictly an anthracycline analogue is structurally quite similar. While it demonstrates similar cytotoxicity,[56] mitoxantrone **21** reduced overall toxicity suggesting perhaps that the toxicity in the anthracyclines may be attributable to the varied functionality they exhibit. Metastatic breast cancer, leukaemias and renal cell carcinoma are among the cancers with which mitoxantrone has produced responses.[56]

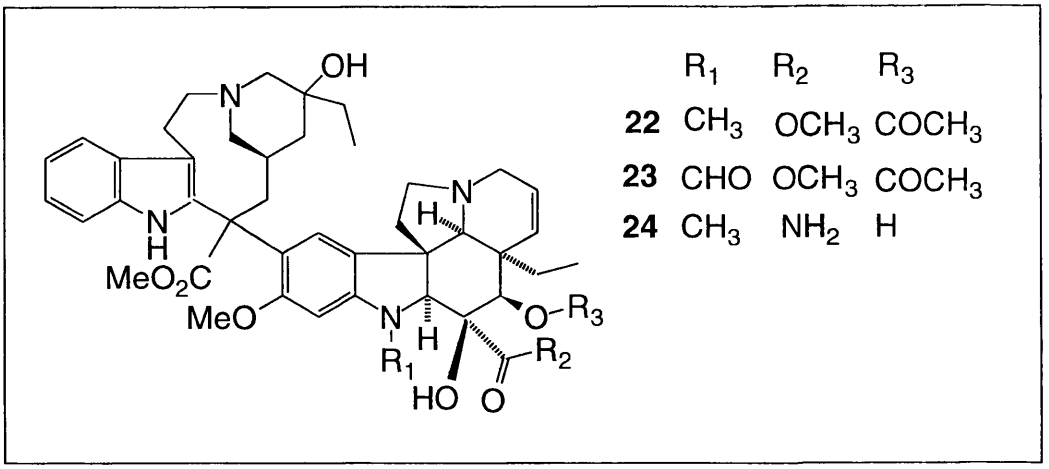


21

1.4.2.4 Antimitotic agents

As the name infers this class of compounds act during the mitotic phase of the cell cycle. In normal cells spindle apparatus operates in this mitotic phase by controlling the division of chromosomal material, a process crucial to successful cell division. The spindle is constructed from microtubule proteins which co-exist in a balanced state with tubulin.[57] Antimitotic agents disturb this delicate microtubule/tubulin balance and by preventing spindle formation halt cell growth.[57]

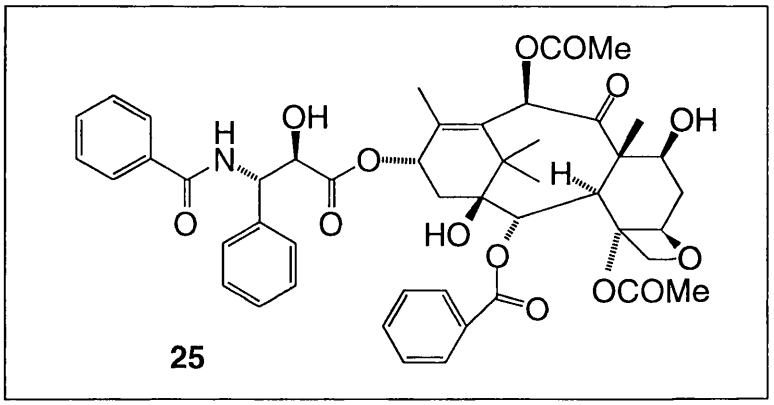
1.4.2.4.1 *Catharanthus* (Vinca) alkaloids



	R_1	R_2	R_3
22	CH_3	OCH_3	$COCH_3$
23	CHO	OCH_3	$COCH_3$
24	CH_3	NH_2	H

Vinblastine **22** and vincristine **23** isolated from the Madagasian periwinkle plant *Catharanthus roseus* are amongst the best known Vinca alkaloids.[58] These antimitotic agents function by binding to dimeric tubulin which perturbs the balanced microtubule/tubulin state. Inhibition of spindle construction and function results, followed by cell death.[59] Vinblastine **22** and vincristine **23** are used to treat rapidly growing cancers such as leukaemia but neurotoxic side effects are common.[59] This toxicity along with multidrug resistance has hampered the rapid progression with further natural or synthetic Vinca alkaloids.[58] However, vindesine **24** is a more recently identified antimitotic agent used to treat leukaemia.[60]

1.4.2.4.2 Paclitaxel (Taxol)



25

Unlike the Vinca alkaloids, the antimitotic agent paclitaxel (Taxol) **25** disturbs the delicate microtubule balance by catalysing microtubule formation.[61] The equilibrium becomes heavily unbalanced in favour of the microtubule

protein and hence cell death ensues due to incompletion of the mitotic phase of the cell cycle. The isolation of Taxol from the stem bark of the Pacific yew *Taxus brevifolia* revealed an antimitotic agent with potent anticancer properties.[62] This discovery prompted the quest for a synthetic source of this very scarce natural product. However, it was over 20 years before the first total synthesis emerged from the groups of Nicolaou[63] and Holton[64] closely followed by Danishefsky.[65] From the endeavours of these workers and others, chemistry crucial for the semi-synthetic production of Taxol was revealed. Initially approved for ovarian cancer treatment,[66] this potent drug exhibits significant activity against breast, lung and head and neck cancers.[67] The literature continues to be saturated with reported uses of Taxol. Whether on its own or in combination chemotherapy, this exceptionally promising agent is employed in treatment of the advanced metastatic forms of many cancers.

1.4.3 IMMUNOTHERAPY[68]

The immune system removes any agent or agents it regards as foreign to the human body by eliciting a specific immunological response. The potential for an immunological role in cancer therapy requires tumour cells to have target molecules recognised by antibodies or cells involved in the immune process.[69] Current approaches to immunotherapy include monoclonal antibodies and biological response modifiers.

1.4.3.1 Monoclonal antibodies

The use of monoclonal antibodies in cancer treatment is very limited. These antibodies are employed to recognise antigens specifically associated with the tumour cells.[70] Some antigenic tumours include melanoma ovarian and colon cancers. Problems which limit this form of immunotherapy can arise from immunological responses removing the antibodies from the system.[70] However, monoclonal antibody-directed therapy enjoys a far greater potential in cancer therapy. In effect, this therapy links the antibody to a drug thus directing the drug to the tumour cell surface.[71] Shortages of specific antibodies and insufficient quantities of drug delivered have produced problems.[71] However, promising results with experimental leukaemias or ascites tumour have been observed.[72]

1.4.3.2 Biological response modifiers[73]

The biological response modifiers which are mostly polypeptides elicit their anticancer behaviour through regulating the immunological responses of the cancer patient.[73] The most promising biological response modifiers are the interferons. Interferons are usually produced by the body in response to viral infection and display inhibition of cell proliferation.[73] Gene cloning techniques have been employed to produce these immunotherapeutic agents and among the most successful are the interferons IFN-α and interleukin-2 (IL-2). IFN-α is used to treat malignancies including advanced lymphoma and renal-cell cancers. Interleukin (IL-2) is particularly effective against melanoma and kidney cancer.[74] However, the problem with these interferons and other biological response modifiers is that the mechanism of action remains unclear.

CHAPTER 2

MALIGNANT MELANOMA AND ANTIMELANOMA AGENTS

2.1 SKIN CANCER - A MISUNDERSTOOD TERM

Skin cancer is a familiar term to the population in general, however, its true nature is not well understood. Unknown to many, skin cancer or cutaneous malignancy comprises three forms: basal cell carcinoma, squamous cell carcinoma and malignant melanoma.[11] Despite its comparative rarity malignant melanoma is the potential killer of the three forms.[11] However non-melanoma skin cancer (basal cell carcinoma and squamous cell carcinoma) is one of the most common cancers to affect men and women.[2] With mortality rates very low and tumours only rarely known to metastasise, sufferers of non-malignant melanoma can be treated in a very low key manner in general practice or as hospital outpatients.

The skin cancer discussed in depth from this point on is the comparatively rare but potentially lethal form, namely malignant melanoma.

2.2 TYPES OF MALIGNANT MELANOMA

There are four main types of malignant melanoma, namely superficial spreading, nodular, lentigo and acral melanomas.[75] The superficial spreading and nodular melanomas generally occur in younger people, whereas lentigo malignant melanoma is most likely to affect the faces of the elderly. The acral-lentiginous melanoma is a malignancy of the skin which affects the palms and soles.[75]

Of the four types, the superficial spreading and nodular melanomas are the most common and aggressive types. Between them they constitute approximately 90% of all malignant melanoma cases and most recent epidemiological studies concentrate only on these types.[76]

2.3 MALIGNANT MELANOMA - AN EPIDEMIC

Considering that malignant melanoma is the cause of only 1% of all new cancer cases,[77] why then is there so much concern associated with this particular cancer? With this in mind, consider the following very sobering aspects of the disease which should illustrate the reasoning behind such widespread concern.

- At present the incidence of malignant melanoma continues to escalate among the fair-skinned populations of the world.[76]

- The major aetiological factor attributed to the cancer is known, namely overexposure of skin to strong sunlight (ultraviolet light with wavelength 290-320 nm, UV-B).[10,11]

- This cancer can be cured but only if recognised in its early stages of development before the onset of metastasis.[78] Otherwise no curative therapy for advanced forms exists.

- The age at onset is earlier than in most other cancers. Over the period 1986-1995 it was the third most common cancer in the 15-34 age group.[79]

So in summary, unlike most other cancers, melanoma is potentially curable and the major cause known. It would therefore appear that through early identification of the cancer and simple education with regard to sun exposure the incidence and early age onset of the disease could be reduced.

2.4 RISK FACTORS AND THE DEVELOPMENT OF MALIGNANT MELANOMA

As already mentioned the main aetiological factor associated with skin cancer is exposure to solar radiation.[10,11] However, the risk of developing malignant melanoma can be further increased by a number of contributing factors or risk factors. These principal risk factors can be illustrated through discussion of skin type, naevi and genetics and family history.

2.4.1 SKIN TYPE

Susceptibility to malignant melanoma is considered to be related to skin pigmentation,[77] i.e. those phenotypes with naturally occurring brown or black skin are at a far lower risk of developing malignant melanoma than fair-skinned individuals who have a tendency to burn or freckle rather than tan. It has also been proposed that episodes of severe sunburn and general overexposure to the sun can cause malignant melanoma [80]

2.4.2 NAEVI

Benign pigmented melanocytic naevi or "moles" as they are more commonly known, can be present from around birth (congenital naevi) or acquired later in life (common or acquired naevi). Both types of naevi are considered to be risk factors for malignant melanoma.[81]

Dysplastic naevi are acquired naevi. The risk of malignant melanoma has been reported as proportional to the number of these naevi[80] with large numbers proving to increase the significance of the risk factor. However, as solar exposure increases the number of naevi it remains unclear whether UV radiation promotes conversion of naevi into melanoma or acts as some sort of initiator.[77]

The other type of mole, congenital naevi, have been used as a powerful tool in public awareness campaigns to promote self-recognition of the early signs of the cancer.[82] Warning signs of malignant melanoma manifest themselves in featural changes of these moles, from changes in mole size, outline and colour to bleeding and itching.[11]

2.4.3 EARLY HISTORY AND GENETICS

Case studies identified the link between family history and melanoma risk with an individual's risk of developing malignant melanoma doubled if they have at least one first-degree relative with the cancer.[83] Statistically this represents approximately 2% of melanoma sufferers.[84] Furthermore, it would appear that genetic susceptibility and sun exposure are likely to act multiplicatively on melanoma risk.[83]

The search for the hereditary genetic component of malignant melanoma is an ongoing one. While 5-10% of malignant melanoma cases occur in a hereditary form,[85] a number of familial cancer genes have been cloned and characterised, most of which prove to be involved in sporadic cancers as well as hereditary cancers.[86] The most important familial melanoma gene reported to date is the $p16^{INK4}$(*CDKN2*) MTS1 gene found on chromosome 9p21.[87] *CDKN2* possesses the hall marks of a tumour suppressor gene and is lost or mutated in many cell lines including melanoma.[85,88] Such *CDKN2* mutations have been reported in approximately 20-30% of all familial melanoma cases.[89]

2.5 BRESLOW THICKNESS AND THE STAGES OF MELANOMA

Malignant melanoma originates within the melanocyte where under conditions of uncontrolled melanocytic cell division excessive production of melanin occurs. This results in malignant melanoma appearing as dark naevi or lesions on the skin. However, the most feared aspect of melanoma is metastasis, the spread of the cancer, as metastasis can spread the cancer to distant sites within the body including the brain, lungs and gastrointestine.[78] It is of no surprise then that chances of survival are far greater when the malignancy is treated in the early stages of development, before metastasis.[78]

Of paramount importance in prognosis is the vertical thickness of the tumour known as the Breslow thickness.[90] Quite simply "the thinner the better" best describes tumour prognosis for recovery. Breslow thickness along with the anatomical level of local invasion are used to characterise malignant melanoma into four stages of development[91] as detailed below.

STAGE I

- Tumour less than I.5 mm thick and/or invades papillary-reticular dermal interface.
- No regional lymph node or distant metastasis.

STAGE II

- Tumour between I.5 and 4 mm thick and/or invades reticular dermis.
- No regional lymph node or distant metastasis.

STAGE III

- Tumour more than 4 mm thick with invasions as far as subcutaneous tissue.
- Metastasis in any regional node(s).

- No distant metastasis.

STAGE IV

- Mestasis in regional lymph node(s) and distant metastasis in skin tissue, subcutaneous tissue or lymph node(s) beyond the regional lymph nodes.

Having detected the cancer's stage of development, the appropriate treatment can be selected. However, treatment choice is greatly diminished for advanced metastatic tumours in the later stages of advancing metastasis.

2.6 TREATMENT OF MALIGNANT MELANOMA

The therapeutic forms of malignant melanoma treatment include surgery, radiotherapy, chemotherapy and biological therapy. As already implied, the applicability of each treatment is very much dependent on the stage of development of the cancer. Each of the four treatments will be discussed in turn.

2.6.1 SURGERY

Surgery is the most effective treatment of the early stages of melanoma.[92] Furthermore, studies reported that surgical margins need be no greater than 2 cm for the excision of melanomas of intermediate thickness (0.75 to 4.0 mm).[93] This significantly reduces the need for skin grafting with most patients treated on an outpatient basis. Surgery faces a far more limited role in the treatment of advanced melanoma. However, isolated metastases found in the lungs, gastrointestinal tract, bone or occasionally the brain, may be palliated by resection with some improvements in survival resulting.[94]

2.6.2 RADIOTHERAPY

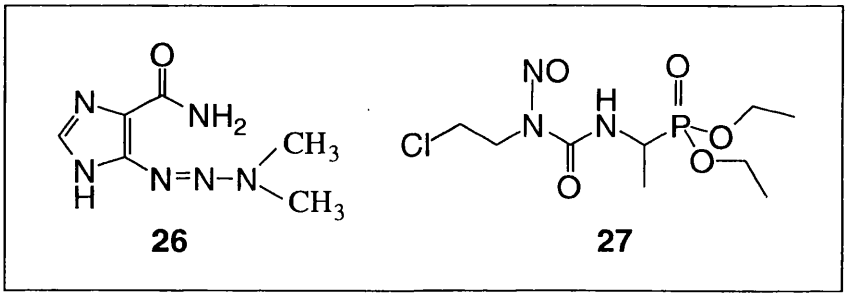
Radiation therapy does not play a curative role in treatment of melanoma patients. However, this treatment is not redundant as radiotherapy provides symptomatic relief for sufferers with metastases including those affecting the central nervous system and bone.[95]

2.6.3 CHEMOTHERAPY

The chemotherapeutic approach to melanoma treatment can be divided into the use of drugs as single agents or as part of a combination regimen.

2.6.3.1 Single agents

From the early 1970s dacarbazine (DTIC) **26** has remained the drug of choice for single agent treatment of metastatic melanoma. DTIC **26** displays response rates around 20%.[96] Before the discovery of DTIC **26**, some of the single agents in use included hydroxyurea, procarbazine and alkylating agents such as cyclophosphamide.[97] Their use was discontinued due to low response rates. Later cisplastin[98] and the Vinca alkaloid family (vindesine[99] and vinblastin[100]) demonstrated antitumour activity; in particular response rates of 10 to 15% were exhibited for cisplatin treatment.[98] The potential of these single agents was recognised by incorporating these drugs in combination chemotherapy regimens. To date, the nitrosourea family continues to produce encouraging single agents for melanoma treatment. Earlier nitrosourea examples include carmustine (BCNU), lomustine (CCNU) and semustine (MeCCNU)[96] which reported antitumour potency analogous to DTIC. However severe toxicological problems hampered further use of nitrosoureas such as CCNU.[96]



26 **27**

Fotemustine **27** is a relatively new nitrosourea with response rates reported in the region of DTIC.[101] Such was the antitumour potential of fotemustine **27**, it was incorporated immediately into combination therapeutic regimens with DTIC **26** and further studies for it as a single agent should be imminent. Many of these chemotherapeutic agents, their uses and mechanisms of action are discussed in section 1.4.2.

2.6.3.2 Combination chemotherapy

In an attempt to improve on the antitumour activity of single agents, combination chemotherapy employs two and three combinations of drugs to treat metastatic melanoma. However, results have proven to be quite disappointing with the response rates observed for DTIC **26** never surpassed.

Combination chemotherapy regimens involving DTIC and the nitrosoureas BCNU, CCNU and MeCCNU displayed some potential but not comparable to the results from DTIC alone.[102] Similarly two and three drug combinations of vinblastine, vindesine and cisplatin with DTIC were to produce analogous observations in their results.[103] Encouraging results were obtained very recently in a study using DTIC and fotemustine. Although response rates were mediocre (11%), minimal side effects and no lung toxicity were observed. This provides a palliative treatment for advanced metastic melanoma.[104]

To date however, DTIC maintains the lead single chemotherapeutic agent for malignant melanoma and as yet no conclusive evidence has been reported to demonstrate combination chemotherapy as a superior mode of drug treatment.

2.6.4 BIOLOGIC THERAPY

2.6.4.1 Interferons

The first evidence of effective adjuvant therapy for metastatic malignant melanoma treatment emerged recently using interferons.[105] These immunotherapeutic agents provide a novel source of anticancer agents (see section 1.4.3.2). The Eastern Co-operative Oncology Group (ECOG) trial 1684 reported using interferon alfa-2b (IFN-α2b) at maximally tolerated doses for one year.[106] Overall survival was improved by approximately 1 year with estimated overall 5-year survival rates of 46% (compared to 37% for those who received no adjuvant therapy). Furthermore, the toxic side effects were tolerated by the majority of patients.[106]

2.6.4.2 Other immunotherapies

One target in the development of immunological cancer vaccines are the ganglioside antigens found expressed on the cell surface of human malignant melanoma.[107] The ganglioside GM2 antigen vaccine (GM2 vaccine) induces antibody responses with a high percentage of patients and on combining the GM2 vaccine with *Bacillus calmette-guerin* (BCG), in the GM2/BCG vaccine, improvements in overall recovery and relapse times occurred.[108] To investigate these findings further, GM2 vaccine and Interferon IFN combination trials are expected.

Another approach in specific tumour immunotherapy arises from the discovery of T-cell recognised tumour associated antigens (TAA).[109] This has enabled the development of T-cell antigens vaccines which have been used in clinical trials for the past few years.[110]

To summarise the treatments of malignant melanoma discussed: although surgery enables treatment of early stage melanoma with relative ease and minimal disruption to the patient, advanced metastatic melanoma remains resistant to most therapies. However the reported encouraging responses from some chemotherapy and especially the emerging immunotherapies can surely provide hope for sufferers and provide concrete foundations for further work in this cancer field.

2.7 PREVENTATIVE MEDICINE?

When we consider the overwhelming contribution sun exposure plays in the incidence of skin cancer, never has prevention been such an attractive therapy. In an attempt to change habits with regard to sun exposure public awareness campaigns have been implemented world-wide from Australia to the UK.[111] These campaigns have a common theme whereby tanned skin is no longer to be considered a sign of beauty, wealth or status. Re-education tactics include promoting the use of sunscreens, avoidance of midday sun and special care of young children's skin whilst in the sun.[112] To enable the earliest treatment of this cancer, prompt identification through self-examination of moles is also encouraged (see section 2.4.2).

Sadly however, on reflection, the white skinned individual's urge for the perfect tan has had alarming consequences on a scale which could never have been predicted. It can only be hoped then that in this trend-following world, styles will revert back in time to the Victorian era when white almost porcelain-like skin was desired to portray the image of upper class status, wealth and social standing!

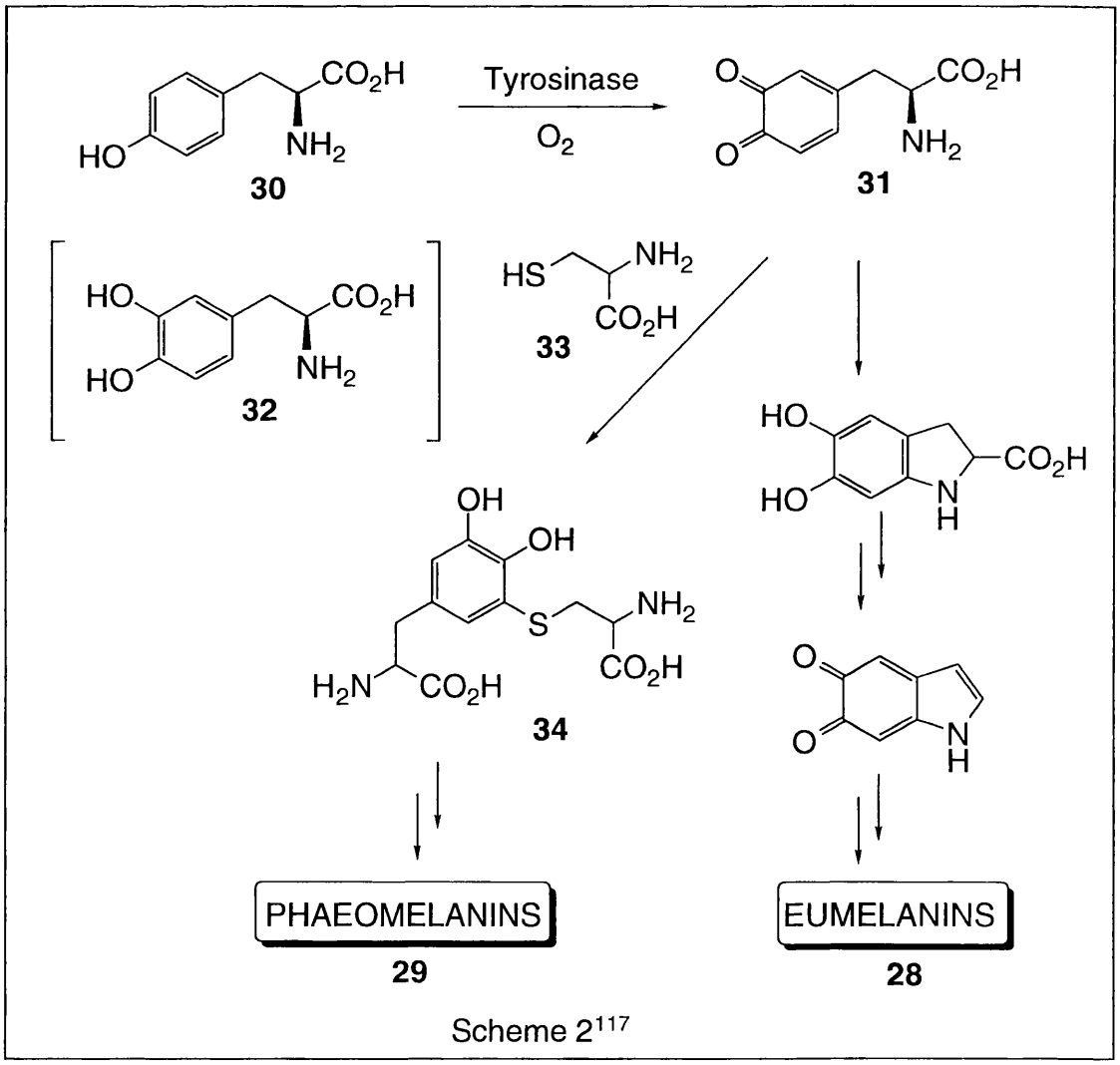
2.8 THE MELANIN PIGMENTARY SYSTEM

Malignant melanoma results from the aberration of melanocytic cell division which leads to excessive melanin production.[113] To understand the biological process of pigmentation itself[114] the functional components of the melanin pigmentary system should be examined. The more important

components include Tyrosinase, the melanosome, keratinocytes, melanocytes and melanin itself.

2.8.1 MELANIN

This brown-black pigment of the skin is synthesised within the melanocyte on the melanosome (a sub-cellular organelle). The virtual insolubility of melanin made elucidation of the biosynthetic pathway very difficult.[115] Scheme 2 depicts a very simplified version of this pathway. Two types of pigment, eumelanins **28** and phaeomelanins **29** (coloured brown-black and reddish respectively) are both seen to originate from tyrosine **30**, the amino acid starting material of the complex pathway.[116] The photoprotective role played by melanin is exemplified when one considers the ease with which a light-skinned individual burns compared to their naturally dark-skinned counterpart, e.g. Negro.[115]



Scheme 2[117]

2.8.2 TYROSINASE

This aerobic oxidase enzyme is unique to the melanocyte cell where it plays a crucial role in melanisation.[114] The enzyme catalyses the conversion of tyrosine **30** into dopaquinone **31**, a reaction believed to proceed via the catechol L-dopa **32**.[118] However, recent studies indicate that L-dopa arises from the disproportionation of dopaquinone and acts as an activator of the Tyrosinase enzyme.[119] This also explains the unusual kinetic properties found with Tyrosinase oxidation of tyrosine and other phenols.[119] Dopaquinone **31** then reacts further to yield eumelanin **28** and on combining with sulfur-rich cysteine **33**, phaeomelanin **29** is produced (via cysteinyldopa **34** and subsequent intermediates) (Scheme 2).[114] The Tyrosinase activity is subject to regulatory control including negative feedback inhibition by melanin and control from intracellular cyclic AMP.[120] Perhaps of greater relevance to this discussion is the increase in tyrosinase activity caused by ultraviolet light.[121]

2.8.3 MELANOSOME

The synthesis of melanin occurs within this subcellular organelle.[116] Four stages of this melanisation take place.[122] In stages I and II the melanosome changes from a spherical to an oval shape. The development of melanin synthesis proceeds from a partial state in stage III to completion by stage IV. The few melanosomes seen in pale-skinned individuals are found to be in stages I and II only. However, in Negroes for example, mostly stage IV melanosomes are present. The resultant effects of overexposure to UV light in the paler Caucasians can be observed with stage III melanosomes appearing following exposure.[122]

2.8.4 MELANOCYTES[113]

Within these specialised cells melanosomes and the melanocyte enzyme Tyrosinase can be found.[113] Melanocytes are found in the mucous membranes, nervous system and eye (ureal tract and retinal epithelium) as well as the skin. Malignancy can arise in all these sites except the retinal pigment epithelium.[113] No correlation between melanocyte number and skin colour is thought to exist. However, exposure to UV light can increase the number of melanocytes.[116]

2.8.5 KERATINOCYTES

The transfer of melanin from the melanocyte to the keratinocyte is essential in the pigmentation process.[122] Within the keratinocyte, while the larger melanosomes function individually, the smaller melanosomes congregate to maintain their functional purpose.[122]

2.8.6 EPIDERMAL MELANIN UNIT

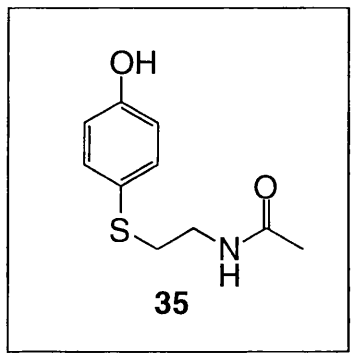
This functional unit is formed when the components of the melanin pigmentary system combine and interact functionally. The epidermal melanin unit is made up of one melanocyte surrounded by 20 to 36 keratinocytes.[116,123] The transfer of melanin from the melanocyte to the keratinocytes is believed to be crucial for pigmentation. Additional major factors in determining skin colour include the stage of melanisation, and the size and number of melanosomes.[116,123]

2.9 STRATEGY FOR SPECIFIC DRUG DESIGN

2.9.1 TYROSINASE - THE KEY TARGET[124]

The target strategy for malignant melanoma drug design is to exploit specifically Tyrosinase, an enzyme crucial to melanin synthesis. As Tyrosinase is unique to the melanocytes this provides a facilitative modem for distinguishing the melanocyte from non-melanocytic cells.[124]

Phenolic prodrugs modelled on tyrosine **30** (see Scheme 2), the natural substrate of the enzyme can be accepted by Tyrosinase. However, catecholic compounds were removed promptly from drug design programmes as toxicity arose from oxidation via a non-enzymatic route.[125] The lead compound to emerge from this area of malignant melanoma drug design which specifically targets Tyrosinase was compound **35**.[126,127]



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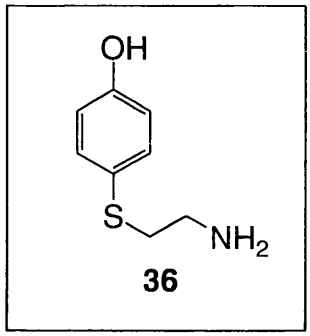
2.9.2 THE JOURNEY OF DISCOVERY TO PARENT DRUG 35

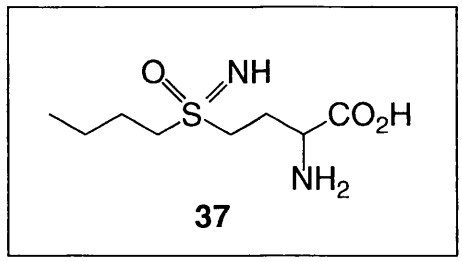
The ground breaking progress in the search for new melanocytic agents was made by Miura and co-workers who prepared a series of phenolic thioether compounds.[128] The novel introduction of sulfur aimed to increase lipophilicity. In this early work the most potent compound **36** was found to possess significant *in vivo* and *in vitro* antimelanoma activity.[128] However Padgette and co-workers reported[129] that phenylaminoethyl sulfides including compound **36** were substrates for monoamine oxidase (MAO) and the action of this enzyme produced the corresponding aldehyde. Further to these findings it was later revealed that the potent melanocytic activity of compound **36** was completely lost on inhibition of MAO.[130]



36

To circumvent this catabolic enzyme's action, the preliminary lead compound **36** was derivatised and it was from this work that the *N*-acetyl derivative **35** emerged displaying marked improvements over previous compounds.[126] This compound displayed *in vivo* melanocytic behaviour which included remarkably high depigmentation of black mice hair follicles while *in vitro* inhibition of B16F10 murine melanoma cell lines was equally as successful.[127]

Alena and co-workers furthered this study[127] by combining lead compound **35** with buthionine sulfoximine (BSO) **37** whereupon enhanced *in vitro* and *in vivo* antimelanoma effects of the lead compound were found. BSO facilitates this improved activity by depleting melanocytes of glutathione, a component involved in the pathway of phaeomelanin synthesis.



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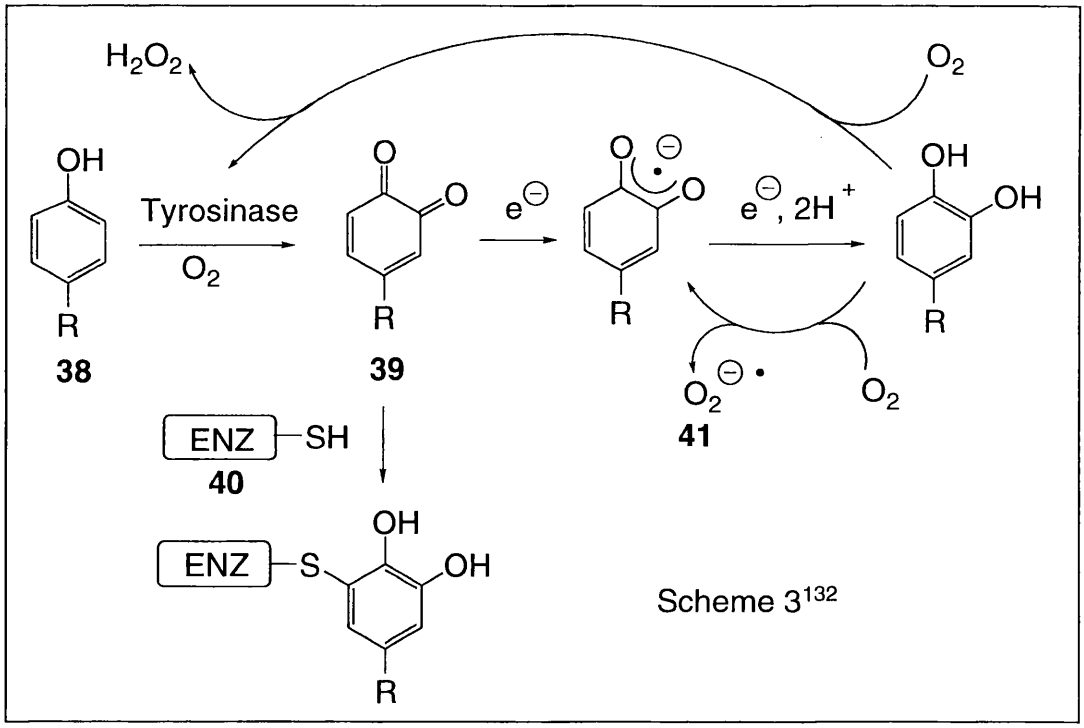
2.9.3 PHENOLIC PRODRUG MECHANISM OF ACTION[131]

Within the melanocytic cells the targeted enzyme Tyrosinase catalyses the conversion of inactive phenol prodrug **38** into the corresponding *o*-quinone **39**. These active oxygenated species mediate cytotoxicity and impair the melanocytes' metabolism by combining with sulfhydryl containing entities **40** such as DNA polymerase or membrane ion pumps which are crucial to the cell cycle. Alternatively the *o*-quinone may participate in a cyclic redox process whereupon active oxygen species **41** are produced.



Scheme 3[132]

2.9.4 THE RESEARCH STORY SO FAR

The significant *in vivo* antimelanoma activity exhibited by compound **35** inspired the beginning of a comprehensive drug design and synthesis programme within the Robins research group. Preliminary investigative work

was performed by McKeown[133] followed immediately by the extensive research of Lant.[134] These workers modified systematically the structure of lead compound **35** in an attempt to reveal the pharmacophoric moieties of the drug. The detailed studies of McKeown and Lant provided a wealth of compounds which can be represented in the summary below (Diagram 1).

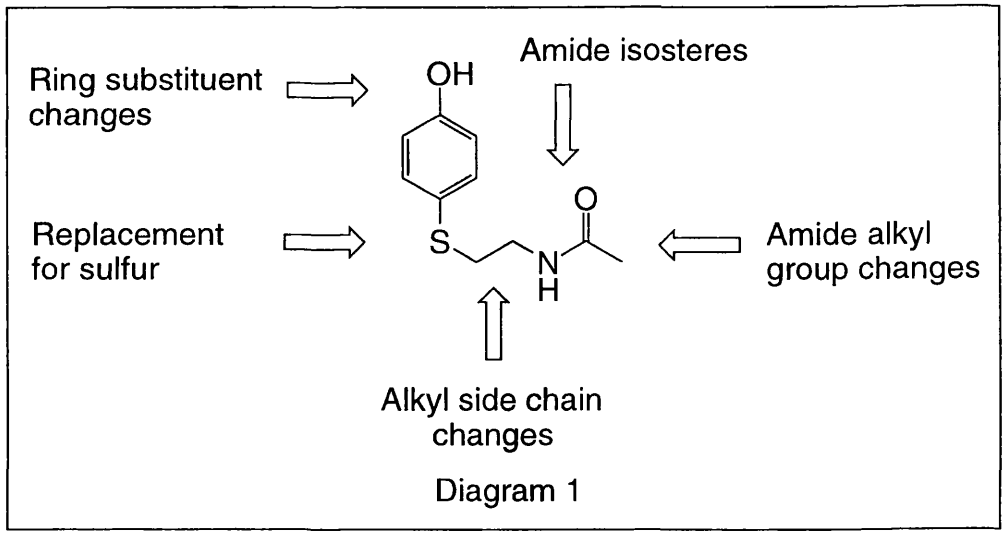
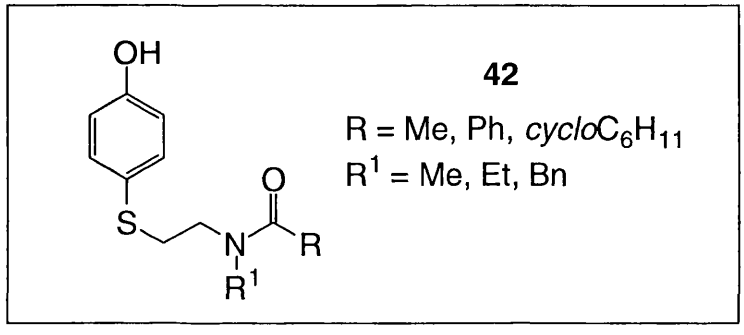


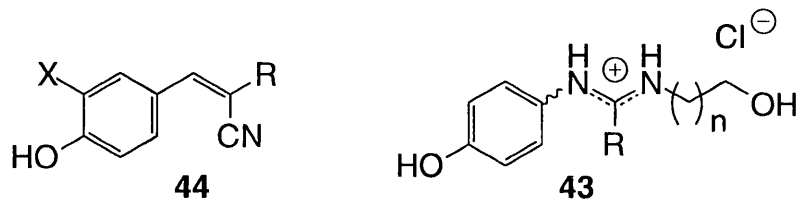
Diagram 1

2.9.5 PROPOSED WORK

A major structural feature of the parent drug which remained untouched throughout the before-mentioned investigations was the secondary amide group functionality. One programme of structural modifications proposed for this project involved the production of a series of tertiary amide derivatives of the parent drug. The series **42** would also incorporate variations in the amide alkyl group some of which had previously showed enhanced activity.[134] Through this modification of two structural features the series of compounds **42** were anticipated at the very least to improve the lipophilic nature of the drug, remove the hydrogen bonding possibilities of the amide group and furthermore enhance the antimelanoma activity.



This programme of research also included two additional structural attacks on the parent compound. The first area of this additional work involved the synthesis of a series of amidine salts **43**. These compounds introduced a novel structural feature to the antimelanoma agents with this amidine group. The effect of this more water-soluble moiety on activity was hoped to be beneficial with enhanced biological activity anticipated. The third area of work was concerned with the development of a range of tyrphostins **44**. These phenolic compounds have already shown interesting antiproliferative activity[136] and possess similarity to the structure of the substrates of Tyrosinase.



44

X = H, OH, OMe
R = CN, CO_2Et, $CONH_2$, $C(NH_2)=C(CN)_2$

43

n = 1, 2
R = Me, Et, Ph, *cyclo*C_6H_{11}

Following the completion of each of the three programmes of research, the *in vitro* melanocytotoxicity of the target compounds and their potential as Tyrosinase substrates would be investigated. The results from these biological studies will then enable the test compounds' potential as antimelanoma agents to be assessed. Concurrently the hypothesis behind the design of the three compounds series' will also be explored. The synthesis and biological evaluation of these potential antimelanoma agents is discussed in Chapter 4.

CHAPTER 3

CELL SIGNALLING AND MAP KINASE INHIBITORS

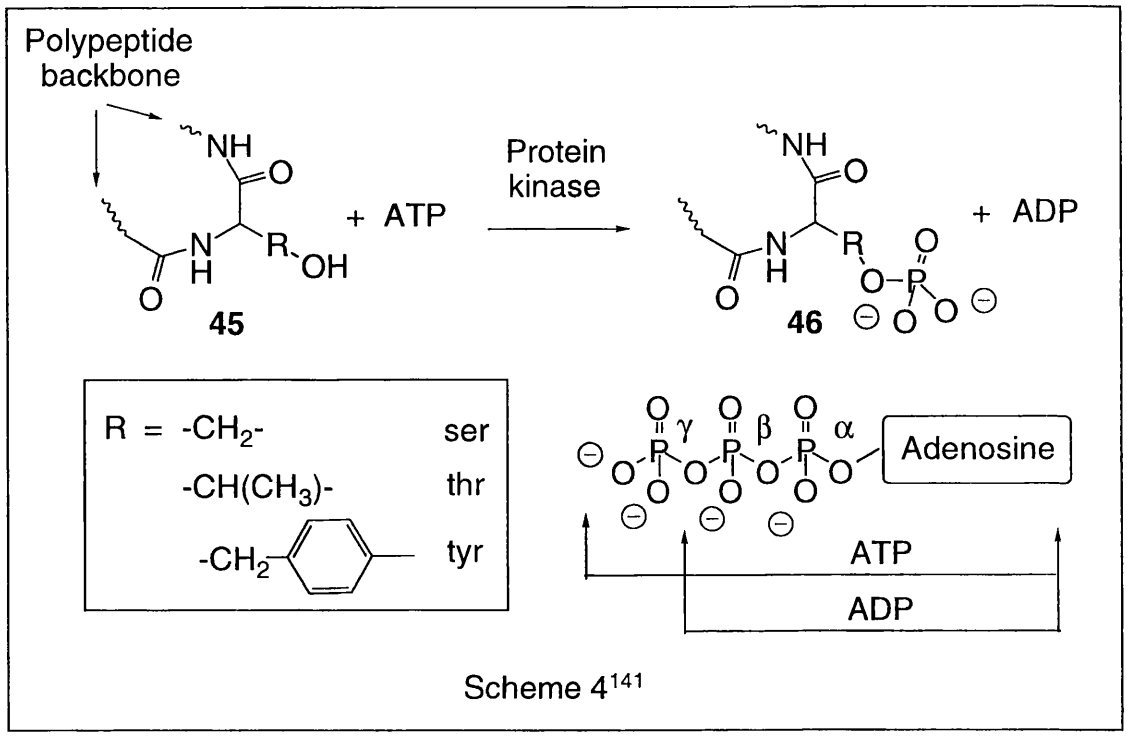
3.1 PROTEIN KINASES AND CELL SIGNALLING PATHWAYS

The successful transduction of signals from the cell surface to the nucleus is crucial to cellular function. These signals are transferred via a complicated sequence of events within pathways termed cell signalling pathways.[137] Protein kinases play a vital regulatory role within these transduction pathways whereby they phosphorylate target proteins at specific sites in the pathways. The importance of protein kinases and their protein phosphorylating function to the cell is exemplified by the number of protein kinase genes present in human genetic makeup. It is estimated that as many as 2000 protein kinase genes are possessed by man and the protein kinases are considered one of the largest families of proteins and genes.[138]

3.1.1 PROTEIN KINASE SUPERFAMILY

Over the past 20 years the discovery of new protein kinases has progressed at such an incredibly fast rate that these protein kinases are now described as a superfamily.[139,140] The rate of discovery was a direct result of implementing developing molecular cloning techniques and the realisation that many oncogenes encode protein kinases.[139] The members of the superfamily are related through structure and function. There is a common structural feature present in the catalytic kinase region of the protein where approximately 250 to 350 amino acids are found. The three dimensional catalytic core results from the folding of the 12 subdomains of the protein.[140]

Consider the phosphorylation function common to the superfamily of kinases (Scheme 4). This involves the enzymatic-catalysed transfer of the γ-phosphate of ATP to the alcohol (serine/threonine) or phenol (tyrosine) group of the protein **45** whereupon the phosphate monoester **46** and ADP are formed.[140]



Scheme 4[141]

Although the superfamily proteins share similar three dimensional core structures and phosphorylate via a common mechanism, two main subdivisions of the family exist: the protein serine/threonine (ser/thr) kinases and the protein tyrosine (tyr) kinases.[139] A method for distinguishing the two protein groups from one another exists in examination of the major autophosphorylation site in the catalytic region. Discovery of a tyrosine residue in the presence of acidic amino acids identifies a protein tyrosine kinase.[139]

The detailed discussion from this point will concentrate on the protein serine/threonine kinases with particular emphasis on one member of this family, the mitogen-activated protein (MAP) kinases.

3.1.2 MITOGEN-ACTIVATED SER/THR KINASE PATHWAYS

An area of ongoing research and great interest involves the cell signalling pathways in which mitogen-activated (ser/thr) kinases participate. Insulin, growth factors and cytokines, the mediators of these pathways,[142,143] bind to specific receptors on the cell surface. These receptors are transmembrane tyrosine kinases or are associated with intracellular tyrosine kinases.[143] Binding produces tyrosine phosphorylation of the receptors which triggers the intracellular signalling pathway. In the cascade of events which follows many ser/thr proteins kinases are activated and a veritable flood of

ser/thr phosphorylation events ensues.[143] Mitogen-activated protein (MAP) kinases are a family of ser/thr kinases involved in such pathways.

3.2 MAP KINASE FAMILY

3.2.1 PIONEERING WORK

The pioneering work in the MAP kinase field of research was carried out by Sturgill and Ray[144] who were investigating insulin-promoted ser/thr phosphorylation of polypeptides. They employed a protein phosphatase-1 regulatory protein, Inhibitor-2 (I-2), as a substrate to probe for kinase activity in insulin-treated 3T3-LI cells. A ser/thr phosphorylated polypeptide extracted from the I-2 isolate was identified as microtubule-associated protein-2 (MAP-2).[144] The MAP-2 kinase then isolated from the insulin-treated 3T3-LI cells was identified as the insulin-promoted ser/thr phosphorylating enzyme.[144]

These early observations of Sturgill and Ray generated further interest with two observations. Firstly via a collaboration with Erikson and Maller[145] they demonstrated phosphorylation and activation of Rsk enzymes S6 kinase II by MAP-2 kinase and secondly that MAP-2 kinase activation required both tyrosine and threonine residues on the MAP-2 kinase polypeptide to be phosphorylated.[145]

This pioneering work discovered MAP-2 kinase, which was later renamed mitogen-activated protein (MAP) kinase.[146] MAP kinase is now recognised to play a vital role in cell signalling by converting tyrosine phosphorylation into serine/threonine phosphorylation essential for regulation of pathways within the cell.[147]

3.2.2 MAP KINASES IN YEAST

The role of MAP kinases in yeast cell signalling pathways has been well explored with many pathways elucidated. It is believed that the distinct similarities in mammalian and yeast MAP kinases should enable the application of the understanding of yeast pathways to those in mammals.[148]

Yeast MAP kinases are known to participate in at least four distinct pathways. For example, in the mating pathway of *Saccharomyces cerevisiae*, two gene products FUS3[149] and KSSI[150] are kinases which function at G_1 in

the yeast cell cycle thereby arresting growth. Such are the similarities in mammalian MAP kinases and yeast kinases that sequences encoding newly identified mammalian kinases are compared to those of known yeast kinases.[148]

3.2.3 MAP KINASE SUBFAMILIES

Boulton and co-workers dominated the discovery of extracellular signal-regulated kinases (ERKs), an insulin-activated MAP kinase subfamily.[151] ERK-1 was the first ERK family member to be purified and cloned. This 44kDa insulin-activated MAP kinase was found to possess remarkable homology (56%) to the FUS3 and KSS1 yeast genes. Using the same molecular cloning techniques two other ERK subfamily members were discovered, ERK-2 and ERK-3.[152] ERK-2 was found to correspond to the original kinase isolated by Sturgill and Ray (MAP-2 kinase). ERK-2 possessed the same identity with the yeast genes (56%) as ERK-1. However, ERK-3 was found to possess homology to a lower extent (37%) thereby implicating ERK-3 as belonging to a separate subfamily.[152]

A second MAP kinase subfamily was uncovered by Kyriakis and Arruch.[153] The p54 SAP kinase was purified from rat liver following cycloheximide injection. This MAP kinase subfamily also exhibits homology with FUS3 and KSSI (42%). The p54 SAP and ERK subfamilies are all highly expressed in the mammalian brain. This along with the homology the subfamilies exhibit towards the MAP kinases involved in the arrest of yeast cell growth indicates the potential role played by MAP kinases in post mitotic cells. The MAP kinases discussed here can be divided into 3 main subfamilies of ERK-I and ERK-2 in the first subfamily and the other two subfamilies composed of ERK-3 and the p54 SAP kinases.[154] However, such is the pace of discovery in this area that novel subfamilies will continue to emerge.

3.3 MAP KINASE PATHWAYS

As is the case for many signalling pathways, the prime concern for the MAP kinase pathways is to transport the extracellular signal successfully to the predetermined destination within the cell which more often than most is the nucleus of the cell.[148] The cellular instructions contained within the signal must be conserved throughout the biochemical cascade of events. In MAP kinase pathways where the signal instructions are directed towards transcription of

specific genes, expression of these genes controls a variety of cell functions which include proliferation.[155]

Fundamental to the MAP kinase (MAPK) pathways is a functional triad of serine/threonine protein kinases: MAPK is activated through threonine/tyrosine phosphorylation by MAPKK, a dual-specificity protein kinase which is in turn activated via serine phosphorylation by MAPKKK, the third member of the MAPK pathway core.[147]

Four distinct MAP kinase pathways have been identified in yeast and three in mammals but progress is such that more are destined to be uncovered. Perhaps the best way to discuss the mammalian MAP kinase pathways is through an example using the most well-known ras/ERK MAPK pathway (see Diagram 2).

3.3.1 RAS/ERK MAP KINASE COMPONENTS

Within the known ras/ERK MAPK pathway the components corresponding to the functional triad are ERK (MAPK), MEK (MAPKK) and raf (MAPKKK).[147] Each of these components will now be discussed as well as ras which functions as the link between the extracellular signal and the MAPK cascade (Diagram 2).

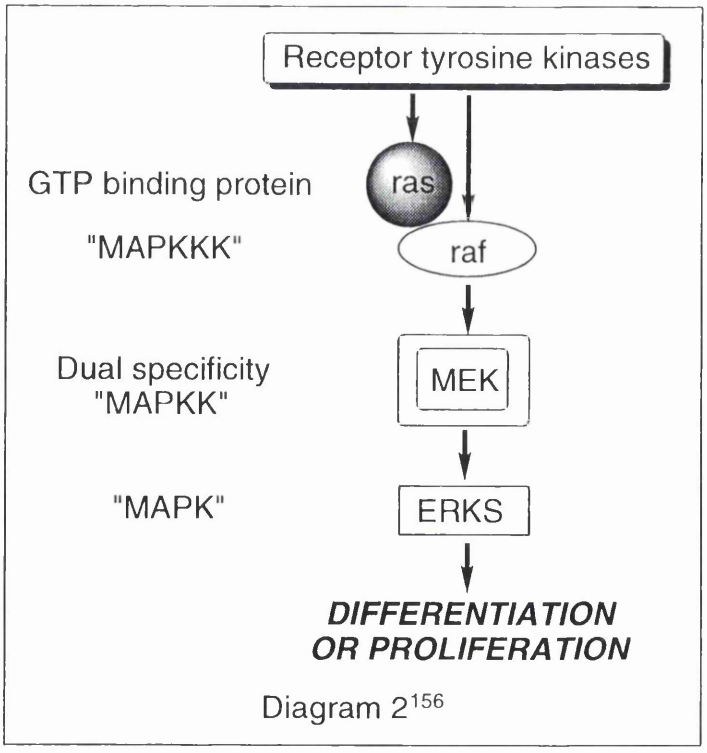


Diagram 2[156]

3.3.1.1 MEK

The entity controlling activation of MAP kinase (later known to be a MAP kinase kinase) was first discovered by Ann and co-workers.[157] They revealed that this activator could reactivate ERK-1 and ERK-2 MAP kinases previously inactivated by treatment with phosphatase. Further to these findings Gomez and Cohen[158] reported that MAP kinase kinase (MKK) required serine/threonine phosphorylation to function and later the ERK-specific MKK gene was identified and named *mek* (**M**AP/**E**RK **k**inase).[159] The dual specific nature of MEK unfolded from the discovery that phosphorylation of both tyrosine and threonine residues is required for activation of the ERK MAP kinases (ERK-1 and ERK-2).[160]

3.3.1.2 Raf

The immediate upstream activator of MEK in the ras/ERK pathway is raf. This kinase has been shown to activate MEK by phosphorylation of two adjacent serine/threonine (ser/thr) residues, an activation mechanism consistent to MAPKKs alike.[161] Raf was identified as the upstream kinase of MEK and has been likened to protein kinase C (PKC), another ser/thr kinase.[162] Although structural and operational similarities exist between the two kinases, the operational aspects for PKC are understood to a far greater extent. Raf is believed to be regulated by multiple mechanisms of control.[162]

3.3.1.3 Ras

Ras is a small (21 kDa) guanine nucleotide binding protein.[163] This G-protein plays a pivotal role in the ras/ERK pathways by linking receptor tyrosine kinase activation to the intracellular MAP kinase pathway (see Diagram 2). Ras is positioned immediately upstream of raf in this pathway and is only active in a GTP-bound state as guanine nucleotide binding controls the activity of the G-protein.[164] However, raf is not directly activated by binding to the GTP-bound ras. It is proposed that raf bound to ras positions raf at the membrane, whereupon an additional as yet undefined event follows resulting in raf kinase activation.[163]

3.3.1.4 ERKs

MAPK is the effector of the MAPK pathway and many MAP kinases have the potential to regulate gene expression by targeting transcription factors in the final phosphorylation of the pathway.

In the ras/ERK pathway the transcription factor target is TCF (ternary complex factor) in particular the SAP-1 and Elk-1 family members.[165] Found within the nucleus the ERK-1 and ERK-2 MAP kinases activate these TCFs by phosphorylating ser/thr residues on the SAP-1 and Elk-1. This phosphorylation is reportedly crucial to stimulation of the c-fos gene transcription process which follows.[165] The ras/ERK MAPK pathway represents how a signal produced from extracellular mitogen-activation of a tyrosine kinase receptor is then involved in a ser/thr cascade of intracellular phosphorylation events to deliver the signal to its destination in the nucleus where the signal's instructions can be implemented.[155]

3.3.2 JNK/SAPK MAPK PATHWAY

The predeterminant for the activation of the ERKs (ERK-1 and -2) and p54 SAP MAP kinases is dual ser/thr and tyr phosphorylation.[166] However, the modulator for this process is quite different for each of the two MAPK subfamilies. The ERKs' activator MEK was reported as unable to activate bacterially expressed p54 and in a variety of cell lines, the mitogenic activators of the ERKs which act via ras were functionally redundant with the p54 MAPK.[166] However, in response to cellular stress the p54 kinases were strongly activated and hence the name stress-activated protein kinases (SAPK) was introduced. Other SAPK activators include heat-shock, ultraviolet light and tumour necrosis factor (TNf-α). It is believed that this activation is not coupled to ras and the MAPK pathway.[166]

The SAPKs target the transcription factor c-jun and phosphorylate ser63 and ser73 in the amino-terminal activation domain. SAPKs stimuli were shown to stimulate phosphorylation and activation of c-jun and the SAPKs are believed to be the main c-jun kinases (JNK).[166]

In summary, this JNK/SAPK pathway represents a distinct MAPK cell signalling pathway. Activated in response to stress stimuli the pathway targets

proliferation.[166,167] However, as yet not all of the upstream components of the cascade have been identified but of interest are the similarities found in yeast reported with the stress-activated HOG1 MAP kinase pathway.[167]

3.4 MAPK PATHWAYS AND CANCER

A great many genes have been implicated in the malignant transformation of cells leading to the development of cancer.[20] A large number of these genes are oncogenes or cancer-causing genes as they are sometimes called. These oncogenes facilitate the aberration of normal cell behaviour by disturbing the regulatory systems central to the cell, e.g. the cell signalling pathways.[20] More than 20 oncogenes have been identified and these arise from mutation, amplification or altered expression of normal genes called proto-oncogenes found within the cell.[19] The protein products encoded by the proto-oncogenes are components of cell proliferation and differentiation processes such as growth factors, growth factor receptors, signal transduction machinery and transcription factors.[20,168] Therefore, in theory aberration can occur potentially at many points along the pathway.

For the MAPK pathways oncogenic components identified include the protein products encoded by the ras, raf, jun and fos oncogenes.[19] These transformed genes facilitate the malignant transformations of cells which employ the deregulated MAPK signalling pathways.

The G-protein ras is a key player in MAPK pathways especially the ras/ERK MAPK pathway. The ras oncogenes were discovered following lengthy research into the existence of dominant oncogenes in human tumours.[169] The first such gene transfer assays were performed by Harvey[170] and Kirsten[171] on strains of rat sarcoma viruses. In human cancers ras oncogenes are the most prevalent family found and about 10% of the most common human cancers contain these transformed ras genes.[172] The genetic transformation occurs via a single point mutation within the phosphate binding region, whereupon the mutant ras protein is locked in the activated GTP-bound state. Loss of G-protein control results in continued signal transduction and ultimately uncontrolled cell growth.[172]

A further oncogenic MAPK pathway component is mutated raf protein.[173] In normal raf the N-terminal domain of the protein functions to suppress kinase activity.[174] Transformation of the gene involves the deletion of this domain,

whereupon high levels of deregulated kinase activity are exhibited by the oncogene raf.[173]

This very brief outline, depicts the powerful role played by genetics in cell function regulated by enzymes such as MAPK. Identification of the components in such signal transduction pathways and the genes encoding these functional entities is the key to a thorough understanding of cell proliferation in normal and malignant states. Such a wealth of information is pivotal to the development of targets for anti cancer treatment.

3.5 MAPK INHIBITION

For the protein kinase superfamily the protein tyrosine kinases dominate the area of research devoted to inhibitors. During the past 5 years there has been a veritable explosion in this area with drastic improvements in the potency and specificity of protein tyrosine kinase inhibitors reported.[175] However, the situation for protein serine/threonine kinase inhibitors is not quite as awe-inspiring. While some members of this family such as protein kinase C (PKC) have been studied in depth with resultant potent and selective inhibitors reported,[176,177] the MAP kinase family remains untouched with respect to successful inhibition of members of this enzyme group. Encouraging reports however have involved inhibition of other member of the MAPK pathway such as MEK, the upstream kinase activator of MAP kinase.[177,178]

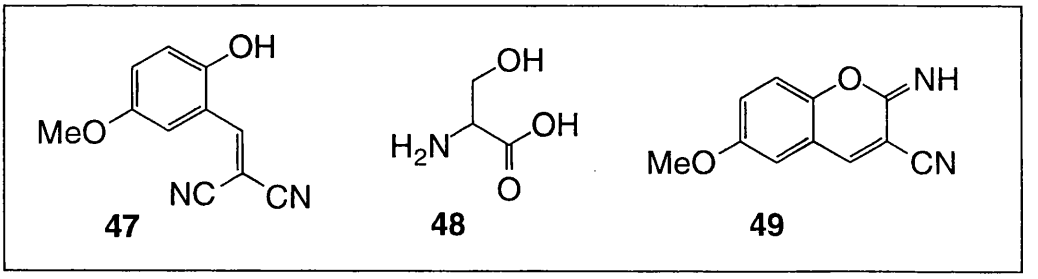
At present progress in the area of cell signalling is so rapid that revelations regarding MAPK inhibitors must be imminent. With biological probes developing and understanding of cellular pathways improving, interest in this field of research continues apace.

3.6 MODEL FOR MAPK INHIBITORS IN THIS WORK

Very limited work has been carried out within the Robins research group in the area of MAPK inhibitors. However, a preliminary biological screen[179] of a series of compounds prepared provided encouraging results with a lead compound identified possessing potential for further work in the development of novel MAP kinase inhibitors.

This compound **47** was modelled on serine **48**, an amino acid phosphorylated on MAPK substrates by the enzyme's action. The structure is a

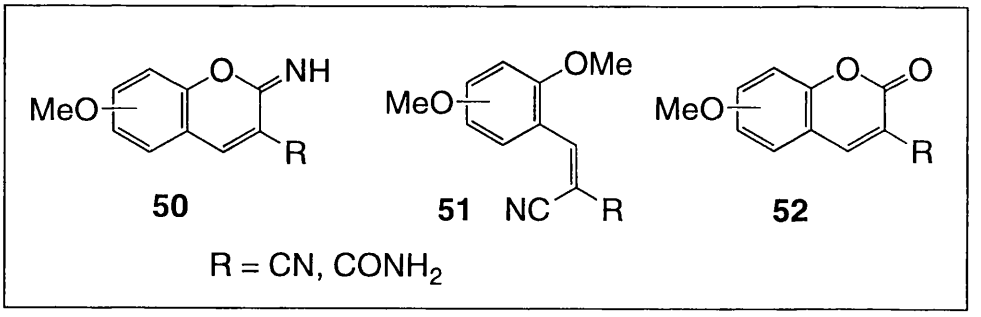
conformationally restricted form of serine incorporating an electron rich benzene ring. Furthermore, it has been reported that such compounds exist in an iminolactone form **49** rather than the open form of **47**[180] and require careful handling due to the sensitive nature of these compounds.[181,182]



The research proposal to develop the lead compound **49** within this project involved preparation of three sets of compounds.

1 A series of iminolactones **50** derived from **49** would investigate ring substitution position.

2 A series of compounds fixed in the open form **51** might enhance the serine-like moiety within the structure further.



3 The iminolactones **50** would be employed as starting materials for the facile conversion into the corresponding lactones **52**. These compounds are coumarins, a family of compounds known to possess a variety of interesting biological activities.[183]

On completing these synthetic targets, the three sets of compounds would be biologically evaluated for MAP kinase inhibitory potential. The synthesis and biological test results are discussed in Chapter 5.

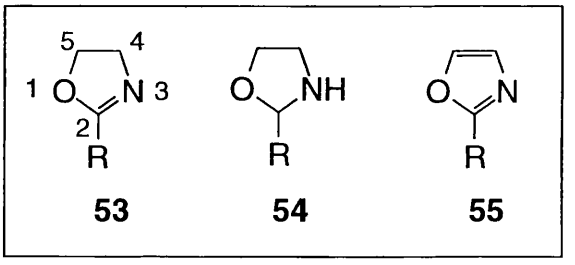
CHAPTER 4

SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTIMELANOMA AGENTS

4.1 PHENOLIC THIOETHER TARGET COMPOUNDS

4.1.1 OXAZOLINES[184]

The century-old 2-oxazoline ring system[185] **53** has evolved well beyond its established origin as a masked carboxylic acid moiety and the stability of this heterocyclic compound is such that it remains resistant to most reagents with the exception of mineral and Lewis acids.[186] Indeed, the 2-oxazolines **53** provide synthetic precursors for various other heterocycles[187] including oxazolidines **54** and oxazoles **55**.



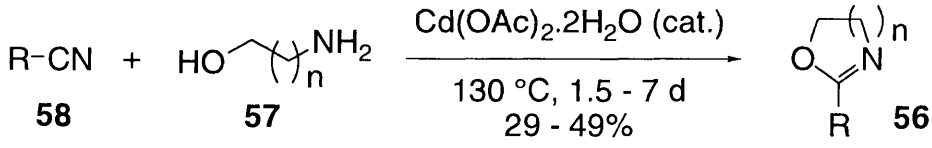
53 **54** **55**

The functionality of the 2-oxazoline and its derivatives enables these compounds to penetrate into diverse fields of interest from pharmaceuticals and agriculture to protective coatings and plasticizers.[187] However, to the synthetic organic chemist the 2-oxazoline ring provides a system with potential for modifications at the 2-, 4- and 5-positions. With these levels of structural variety possible the resultant chiral and achiral 2-oxazolines possess a whole host of synthetic uses[184,188] which include applications as protecting groups, analytical probes and monomers in polymerisation processes. This synthetic utility of 2-oxazolines extends to the preparation of conformationally constrained peptides which are of increasing importance to the medicinal chemist. Moreover, optically active oxazolines enjoy participation in asymmetric syntheses and play the role of recoverable ligands in asymmetric catalytic processes.[184]

The variety of the structural modifications possible on the 2-oxazoline ring system is reflected in the wealth of synthetic routes reported alone for these compounds.[184] However, as the 2-oxazolines involved in our work are a simple form featuring substitution at the 2-position only, the synthesis of these 2-oxazolines is very straightforward. Therefore the synthesis of 2-oxazolines detailed in section 4.1.1.1 is not to be considered representative of the synthetic area as a whole.

4.1.1.1 Synthesis of oxazolines

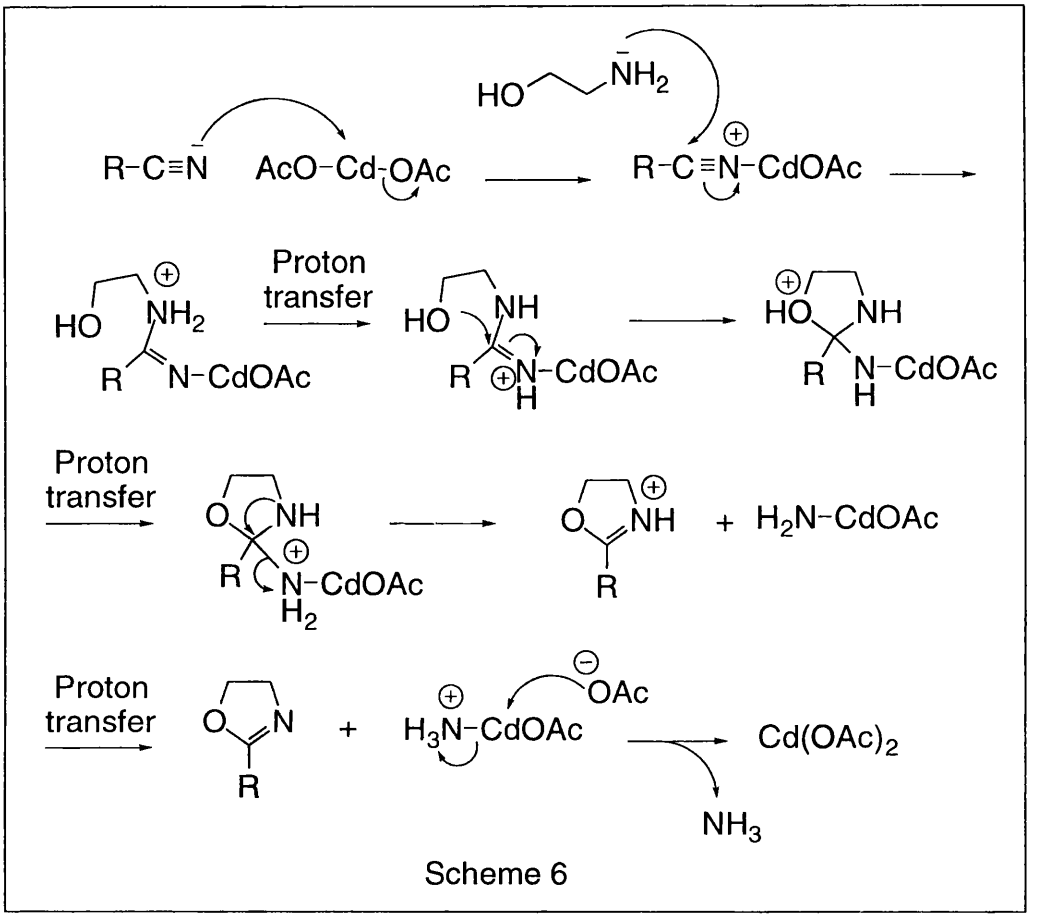
The 2-oxazolines **56** were prepared in a single step catalytic procedure[189] from ethanolamine **57** and the appropriate nitrile **58**. Following fractional distillation of the solvent-free reaction mixture, the products were obtained in moderate yields (see scheme 5). This cadmium acetate-catalysed synthesis was found to be of particular use in our work with its application extended to the preparation of oxazines required for application in section 4.2. For the oxazine series (**56**, n=2) rather than ethanolamine, 3-amino-1-propanol was used which maintained the continued use of cheap, readily available materials. The structures of these known compounds **59** to **64** were confirmed by the spectroscopic data collected. This information together with the boiling points recorded were found to be in agreement with the findings reported in the literature.



n	R	Product	Yield
1	Et	**59**	34%
	cycloC_6H_{11}	**60**	42%
2	Me	**61**	49%
	Et	**62**	43%
	cycloC_6H_{11}	**63**	37%
	Ph	**64**	29%

Scheme 5

A mechanism is proposed[134] for this catalytic preparation of 2-oxazolines and oxazoles for a general case in scheme 6. Notice that although cadmium acetate was our catalyst of choice, soluble heavy-metal salts including zinc acetate and zinc chloride are also recommended.[189]

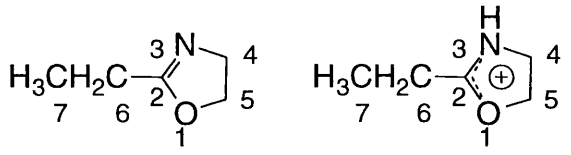


Scheme 6

4.1.1.2 Reactivity of 2-oxazolines

For our purposes the 2-oxazolines were employed as an electrophilic source poised for ring opening attack by selected nucleophilic sources. The chemical nature of this heterocyclic species is such that the 2- and 5-ring positions are potentially most susceptible to nucleophilic attack. Fazio confirmed this with spectroscopic investigations he performed on the neutral **59** and cationic **65** forms of 2-ethyl-2-oxazoline.[190] The oxazolinium cation formed by protonation in ring opening reactions was created in the study through Lewis acid coordination. In table 1 the ^{13}C NMR values found for the two ethyl-2-oxazoline species can be seen. The deshielding of the 2- and 5-ring carbons is very clear and identifies the electrophilicity of these two positions. This

therefore confirms the 2- and 5-ring oxazoline positions as the potential sites for attack by an incoming nucleophile.

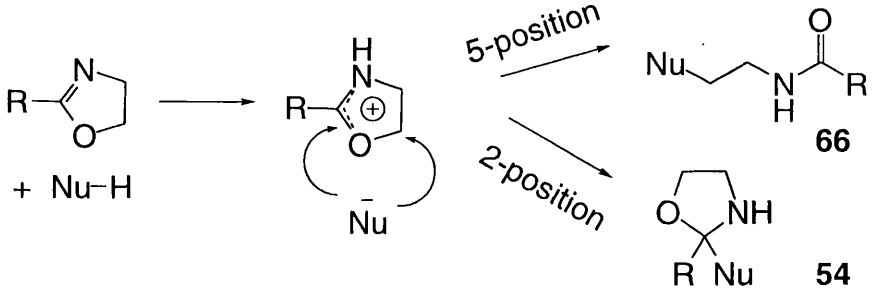


59 **65**

	^{13}C NMR Shift		
Carbon	δ **59**	δ **65**	δ **59** - δ **65**
2	168.4	182.3	-13.9
4	55.0	45.7	+9.3
5	67.4	73.8	-6.4
6	21.5	21.2	+0.3
7	10.5	8.0	+2.5

Table 1

Consider the 2-oxazoline ring opening reaction presented in a generalised form below.



Scheme 7

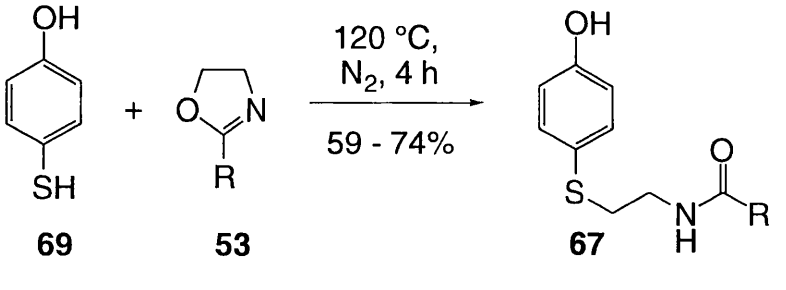
The oxazolidine **54** and carboxamide **66** are the products of opening the oxazoline ring at the 2- and 5-positions, respectively.[190] The pathway taken, and therefore the product obtained, are controlled by the nucleophilic source involved. Later we illustrate this effect with the use of aryl thiols and primary aryl amine salts - two very different nucleophiles.

4.1.2 SYNTHESIS OF THE PHENOLIC THIOETHER TARGET COMPOUNDS

The strategy behind our melanoma drug design together with the three programmes of research we proposed are outlined in section 2.9. The first of the three programmes of research involved the preparation of a series of phenolic thioether compounds which incorporated a tertiary amide into the alkyl side arm. However, before embarking on the synthesis of this first set of target compounds, the simple parent compounds **67** from which they originate had to be prepared. The parent compounds were to be included in the biological studies performed on the target compounds to provide base values for comparison purposes. In fact, one of these compounds, **35**, is the lead drug in the antimelanoma field on which our compounds are modelled.

4.1.2.1 The Wehrmeister reaction

The Wehrmeister reaction[191] was employed to prepare the two parent compounds **35** and **68** and this reaction is an example of the exclusive opening of the 2-oxazoline ring system at the 5-position (discussed in section 4.1.1.2). Under neat heating at reflux and catalytic conditions, *p*-hydroxybenzenethiol **69** and 2-oxazoline **53** were combined to afford the carboxamide **67**. Notice the nucleophilic supremacy demonstrated by the thiol over the phenol within the *p*-hydroxythiophenol reagent **69**. The Wehrmeister reaction will play a pivotal role in the three step synthesis of the phenolic thioether target compounds in section 4.1.2.2.



69 **53** **67**

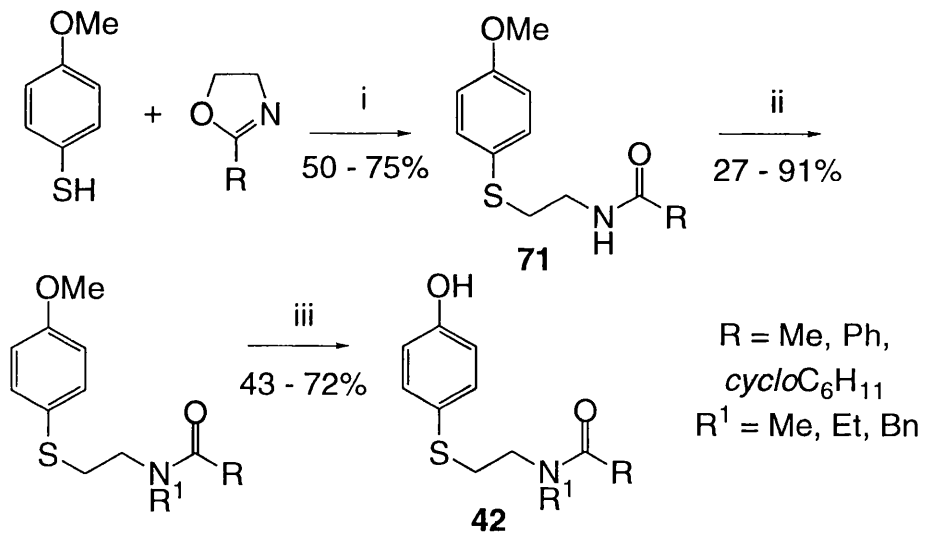
Substrate	R	Product	Yield
70	Me	**35**	74%
60	*cyclo*C_6H_{11}	**68**	59%

Scheme 8

The reaction was found to proceed smoothly with both products obtained in high yields. In the preparation of compound **68**, the 2-cyclohexyl-2-oxazoline **60** was provided by the cadmium acetate catalysed reaction[189] presented in section 4.1.1.2 while commercially available oxazoline **70** was used to prepare compound **35**. The sharp melting points and spectroscopic data of **35** and **68** were in agreement with the literature findings for these known compounds.

4.1.2.2 Overall route to general phenolic thioether target compounds

The series of phenolic thioether compounds **42** were of a structurally similar nature which enabled the devising of one scheme for the preparation of all nine target compounds. In the three step synthesis, the first step provided the basic starting compounds **71** which would be transformed over the following two steps. Each step will be discussed in turn.



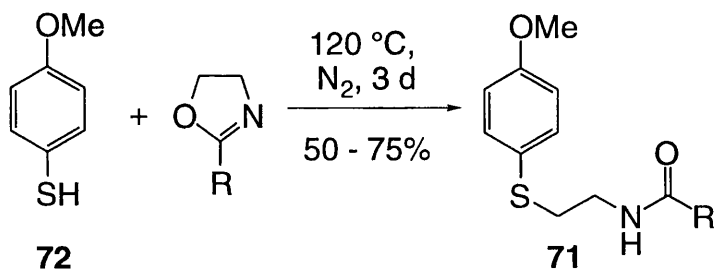
i. 130 °C, N_2, 3 d
ii. NaH; R^1X, THF, 40 °C; 80 °C, 15 h - 4 d
iii. BBr_3, DCM,–78 °C;–78 °C→ rt, 2 h - 3 d

Scheme 9

4.1.2.2.1 Step1: Oxazoline ring-opening

The first step was crucial to the synthesis as the products **71** incorporate the basic structural skeleton required for the final target compounds. The oxazoline ring-opening Wehrmeister reaction[191] was employed with the

nucleophilic source provided by 4-methoxybenezenethiol **72**. As expected the reaction furnished the carboxamide product **71**.



72 **71**

Substrate	R	Product	Yield
70	Me	**73**	75%
60	*cyclo*C_6H_{11}	**74**	65%
76	Ph	**75**	50%

70 and **76** commercially available

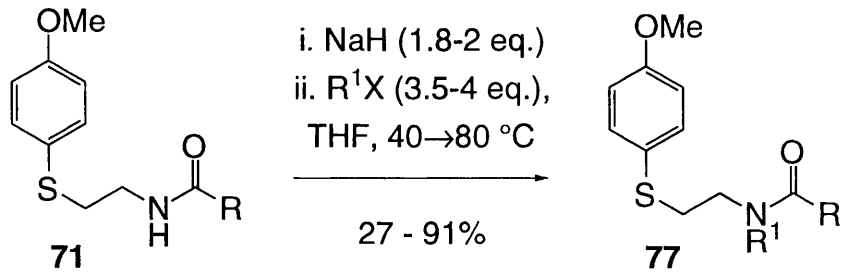
Scheme 10

The reaction produced compounds **73**, **74** and **75** in high yields, with sharp melting points and analytical purity. Spectral confirmation of the products' formation centred around the amide functionality with IR observations including characteristic absorption frequencies in the 1630-1645 cm^{-1} region for the carbonyl bond and the NH stretching vibrations at *ca.* 3300 cm^{-1}. The ^{13}C NMR spectra supported these findings with the carbonyl carbon signal at *ca.* δ 170. As expected the *p*-disubstituted benzene ring produced ^{1}H NMR signals characteristic of an AA'BB' system at *ca.* δ 6.8 and 7.3. The corresponding signals on ^{13}C NMR spectra resonated at *ca.* δ 115 and 134.

4.1.2.2.2 Step 2: *N*-Alkylation of secondary amides

The straightforward conversion[192] of secondary amide **71** into the corresponding tertiary amide **77** was facilitated using sodium hydride and three alkylating sources; methyl iodide, ethyl bromide and benzyl bromide. As a result, nine products were obtained from the three starting compounds. Preparative attempts using isopropyl bromide as an alkylator proved to be unsuccessful even under forcing conditions. This result was attributed to the hindered nature of the secondary alkyl halide. On completion of the successful reactions the sodium halide side product was removed by filtration from the

reaction mixture and the solution worked-up in a standard manner[192] to yield the crude amide. Following purification by column chromatography, the nine compounds were isolated as oils in a range of yields (mean value 60%) and the structures were confirmed by full spectroscopic characterisation. A discussion of the most pertinent features of these spectroscopic results will follow.



R	Substrate	R^1X	Product	Yield
Me	**73**	MeI	**78**	91%
Me	**73**	EtBr	**79**	90%
Me	**73**	BnBr	**80**	52%
cycloC_6H_{11}	**74**	MeI	**81**	81%
cycloC_6H_{11}	**74**	EtBr	**82**	27%
cycloC_6H_{11}	**74**	BnBr	**83**	38%
Ph	**75**	MeI	**84**	68%
Ph	**75**	EtBr	**85**	52%
Ph	**75**	BnBr	**86**	41%

Scheme 11

NMR spectroscopy provided the data of greatest interest. However, the IR spectra are deserving of a mention as the successful conversion of secondary into tertiary amide was confirmed by the absence of any absorption frequencies for the amide NH bonds. The interest in the NMR spectra arose from the doubling of signals observed on both the ^1H and ^{13}C NMR spectra thus indicating that the tertiary amides exist in rotameric forms. Integration of peak heights on the ^1H NMR spectra enabled the rotameric ratio for each compound to be established. A range of ratios for the minor:major rotamer signals from 1:1.2 to 1:1.5 for the nine compounds was calculated. The ^1H and ^{13}C NMR spectra of compound **81** were chosen to exemplify this NMR phenomenon exhibited by the tertiary amide series (see diagrams 3 and 4).

Diagram 3 ^{1}H NMR spectrum for compound **81**

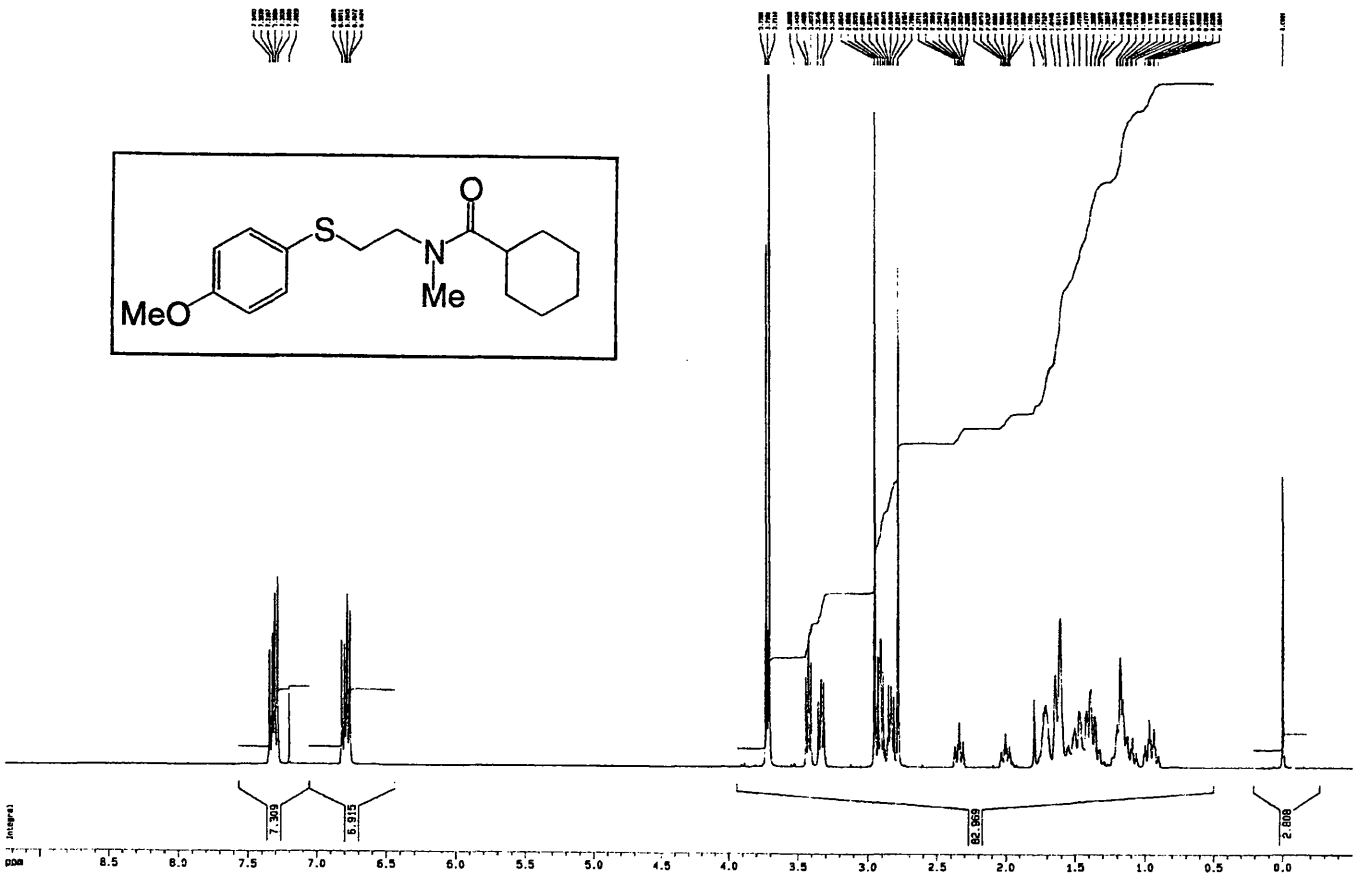
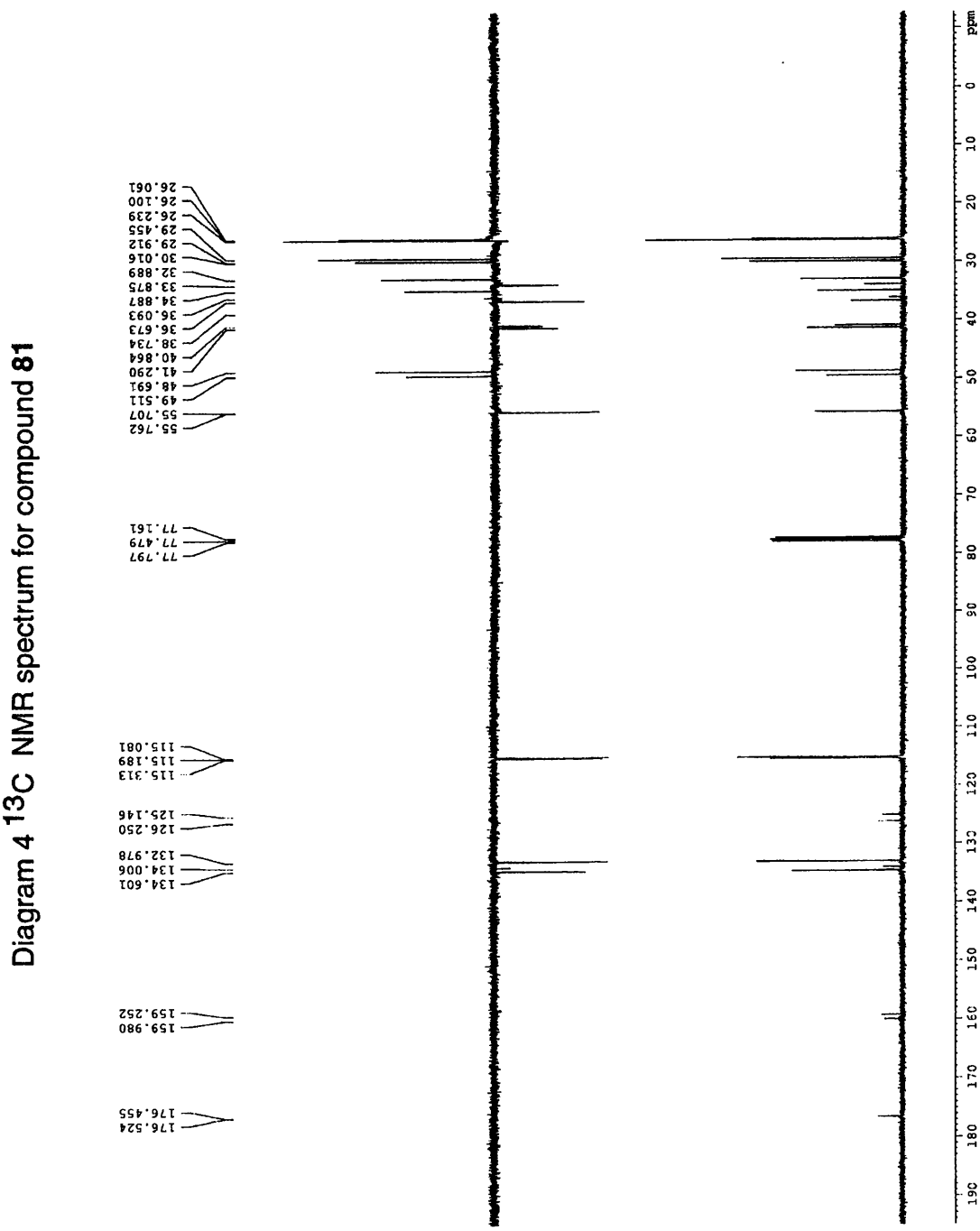


Diagram 4 ^{13}C NMR spectrum for compound **81**

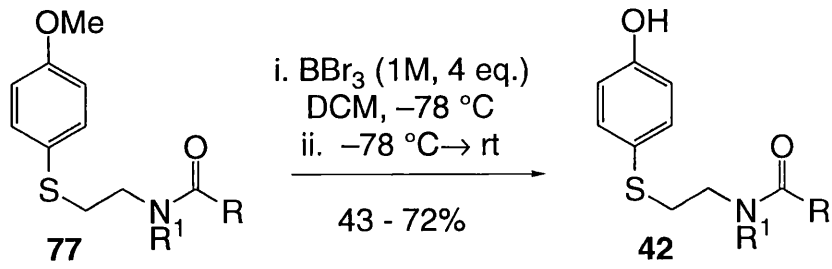


From the ^{1}H NMR spectrum of compound **81** (diagram 3) a ratio of 1:1.2 for the minor:major rotamers was established. The signals for the cyclohexyl moiety were evident in the upfield region δ 0.90 to 2.34. In particular the methine proton produced a pair of triplet of triplets at δ 2.00 and 2.34. Notice the coupling constant values of 11.6 and 3.3 Hz for each signal consistent with *cis*- and *trans*-vicinal coupling expected for such a six-membered saturated ring system. This ^{1}H NMR spectrum demonstrates pairing of signals with beautiful clarity such that even the pairs of rotameric signals for the AA'BB' system in the downfield region δ 6.76-7.33 show distinct separation. The ^{13}C NMR spectrum (diagram 4) maintains this illustrative pleasure by exhibiting a paired signal for the deshielded carbonyl carbon at δ 176.4 and 176.5, a feature not preserved throughout the nine compound series.

The three phenyl compounds **84**, **85** and **86** were found to produce broad pairs of signals in their ^{1}H NMR spectra. This broadening of signals may be due to an electronic effect with perhaps conjugation of the phenyl ring with the amide causing the change in amide bond character.

4.1.2.2.3 Step 3: Methyl ether deprotection

The final step in the three step synthesis of the phenolic thioether target compounds **42** employed a boron tribromide-mediated reaction to deprotect the methyl phenyl ethers **77** (see scheme 12). Preliminary investigations into reagents for this synthetic transformation included the chlorotrimethyl silane/sodium iodide reagent[193] and boron trichloride.[194] The former proved rather disappointing with very poor yields obtained and although boron trichloride was a successful reagent its bromide counterpart proved to be the better boron-based Lewis acid for the ether cleavage.



R	R^1	Substrate	Product	Yield
Me	Me	**78**	**87**	77%
Me	Et	**79**	**88**	60%
Me	Bn	**80**	**89**	76%
cycloC_6H_{11}	Me	**81**	**90**	68%
cycloC_6H_{11}	Et	**82**	**91**	43%
cycloC_6H_{11}	Bn	**83**	**92**	53%
Ph	Me	**84**	**93**	88%
Ph	Et	**85**	**94**	53%
Ph	Bn	**86**	**95**	72%

Scheme 12

For all nine compounds the low temperature (-78 °C) deprotection proceeded in a satisfactory manner. Following an ice/water-mediated quench of the reaction, standard work-up conditions[194] were employed. Eight of the nine compounds required purification by column chromatography and all of the purified products were obtained in moderate to high yields with a mean value of 66%. The phenolic compounds provided sharp melting points and were fully characterised.

The successful deprotection of most phenyl ethers is a transformation easily followed by spectroscopic analysis and this was found to be the case here with the phenol group providing the most important feature in the IR spectra. Characteristic OH absorption frequencies were found in the 3400-2500 cm^{-1} region and at *ca.* 1270 cm^{-1} for the respective stretching and bending OH vibrational modes. Trade mark phenolic behaviour also prevailed in the UV spectra with the main absorption band common to all nine compounds found at *ca.* λ 253 nm (ε 6000-10120). This UV activity was in agreement with the findings reported by Lant[134] for compounds containing the same chromophore.

NMR spectra produced the rotameric observations expected for these tertiary amides. The NMR of phenol **90** are included here (diagrams 5 and 6).

Diagram 5 ^{1}H NMR spectrum for compound **90**

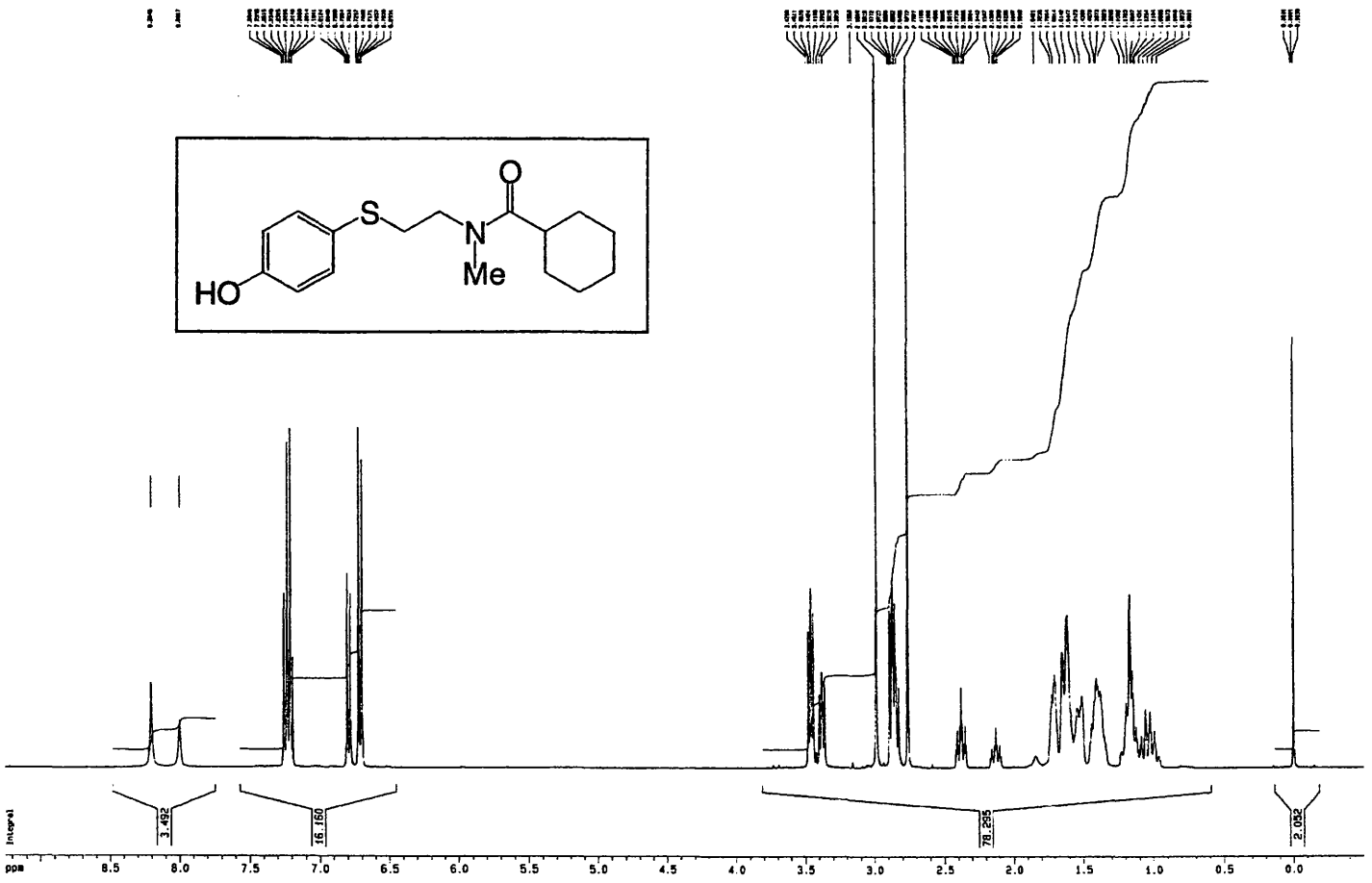
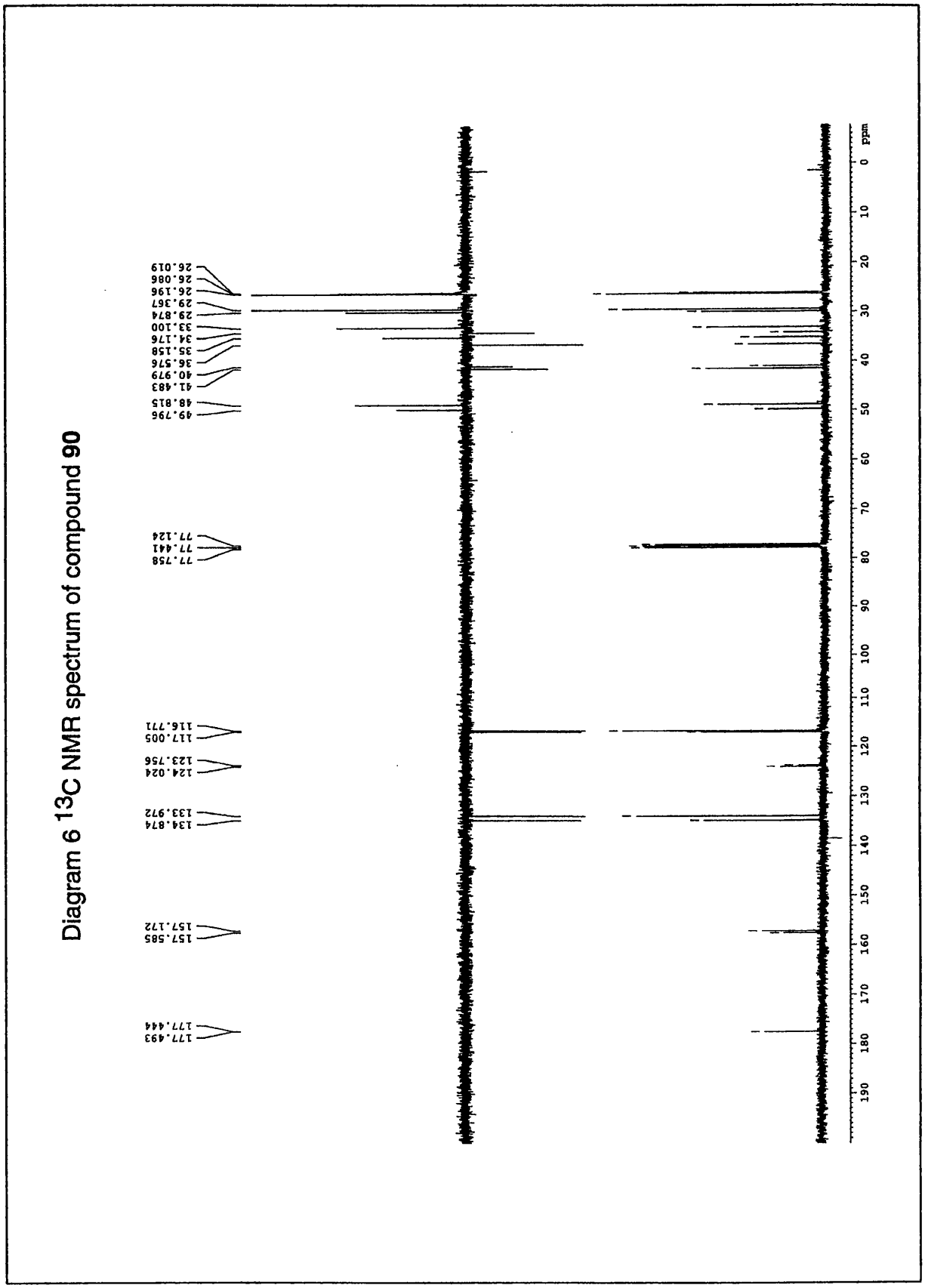


Diagram 6 ^{13}C NMR spectrum of compound **90**



These spectra provide another demonstration of very clearly paired signals. Consider the ^{1}H NMR spectrum (diagram 5); the successful deprotection is quite apparent through the absence of the methyl ether signal at δ 3.6-3.8 while the phenol produces a pair of broad singlets in the characteristic downfield region δ 7.8-9.1. However, it should be noted that such paired phenol signals are not a feature conserved throughout the series of nine target compounds. The ^{13}C NMR spectrum (diagram 6) **90** was found to demonstrate the same evidence for the deprotection through the absence of the methyl ether signal at *ca.* δ 55. A continuation of this exploration into the NMR behaviour of these rotameric compounds found changes in the rotamer ratios occurred on deprotecting the phenol. For example, a 1.3 fold increase in the ratio of minor to major rotamers is found on deprotecting **81** to afford **90**.

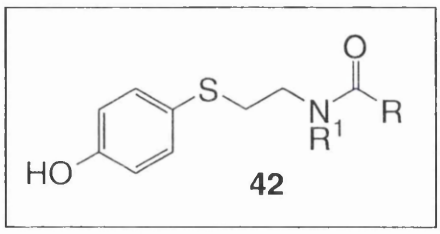
4.1.3 BIOLOGICAL EVALUATION OF PHENOLIC THIOETHER COMPOUNDS

4.1.3.1 *In vitro* 96 hour sulforhodamine growth inhibition assay

The first of two investigations into the biological activity of the phenolic thioether compounds was performed by Dr Lloyd Kelland of The Institute of Cancer Research, Surrey.[195] In this *in vitro* evaluation the test compounds were incubated in a 96 hour sulforhodamine B (SRB) growth inhibition assay where the inhibitory activities of the compounds were assessed against six melanoma cell lines (B008, B0010, G361, HT144, SKMeL24 and SKMeL2) and an ovarian cancer cell line (SKOV-3) which acted as the non-melanoma control. The SKMeL24 is a specialised melanoma cell line which lacks the biochemical machinery required to convert the phenolic prodrugs into the corresponding *ortho*-quinones. This non melanotic cell line provides an indication of cytotoxicity produced by a mechanism of action independent of Tyrosinase. Since we designed our compounds to target Tyrosinase this specific cell line was an important feature of the assay. The cytotoxicity of the compound was assessed from the size of the GI_{50} value quoted for the assay. The GI_{50} value is the concentration of drug which causes 50% inhibition of cell growth. Ideally we required a test compound to demonstrate cytotoxicity and selectivity for melanoma cell lines containing Tyrosinase. To do so we wanted low GI_{50} values for the five Tyrosinase-containing melanoma cell lines together with high values for the non melanotic and ovarian cancer cell lines.

4.1.3.1.1 Results

As we discussed earlier in section 4.1.2 the parent N-H compounds **35**, **68** and **96** were included in the assay to provide comparison results. The assay results for all the test compounds can be seen in the following three tables which house separately the results for the methyl, cyclohexyl and phenyl analogues prepared. Compound **96** was kindly supplied by Lant.[134]



42

Table 2 GI_{50} values (μM) for compounds **42** (**R** = **Me**) in 96 h SRB assay

Cmpd	R^1	Cell line						
		B008	B0010	G361	HT144	SKMeL2	SKMeL24	SKOV-3
35	**H**	>100	>100	>100	>100	>100	>100	>100
87	**Me**	78	>100	>100	>100	>100	>100	>100
88	**Et**	33.5	>100	70	92	92	>100	>100
89	**Bn**	5	30	12	17	17.5	>100	>100

Table 3 GI_{50} values (μM) for compounds **42** (**R** = ***cyclo*C$_6$H$_{11}$**) in 96 h SRB assay

Cmpd	R^1	Cell line						
		B008	B0010	G361	HT144	SKMeL2	SKMeL24	SKOV-3
68	**H**	29	>100	77	>100	61	>100	>100
90	**Me**	7.8	41	90	13.5	14.5	>100	51
91	**Et**	14.5	74	39	53	49	>100	>100
92	**Bn**	14	24.5	14	20	32	>100	53

Table 4 GI_{50} values (μM) for compounds **42** (**R** = **Ph**) in 96 h SRB assay

Cmpd	R^1	Cell line						
		B008	B0010	G361	HT144	SKMeL2	SKMeL24	SKOV-3
96	**H**	>100	>100	>100	52	>100	>100	>100
93	**Me**	34	110*	69	93	105*	>100	>100
94	**Et**	24	85	43	63	73	>100	>100
95	**Bn**	16	34	15.5	30	37	>100	56.5

* Obtained by extrapolation
results of particular interest

4.1.3.1.2 General observations

In general most of the compounds exhibited improved cytotoxicity over the lead compound **35**. Attention must be drawn to compounds **89**, **91** and **94**, three potentially exciting new compounds. In particular the *N*-benzylmethyl analogue **89** exhibited better selectivity and cytotoxicity than our group has seen before. This compound produced low GI_{50} values against the melanotic cell lines and this together with a high GI_{50} value for the SKMeL24 cell line demonstrated the good selectivity possessed by compound **89** for the Tyrosinase-containing cell lines. This significant improvement in cytotoxicity and selectivity was facilitated by the simple action of converting the parent drug **35** into the corresponding *N*-benzyl compound **89**. Compounds **91** and **94** also demonstrated similiar selectivity but the cytotoxicity levels were not as high. Selectivity problems were found with cyclohexyl compounds **90** and **92** as well as phenyl compound **95**. Because these three compounds showed low GI_{50} values against the SKOV-3 cell line as well as the melanotic ones, selectivity appeared to be lower.

4.1.3.1.3 Conclusions

The lead compound **89** from this assay cannot be disputed. It exhibited good levels of cytotoxicity and some desirable selectivity. This level of biological activity was followed by **91** and **94** but with less potency.

In general *N*-alkylation of the simple parent compounds was found to improve cytotoxicity greatly and in most cases selectivity for the melanoma cell lines. This improved cytotoxicity could be attributed to the lipophilic character of the test compounds. These tertiary amides would be expected to be more

lipophilic than the corresponding secondary amides and as such the lipophilicity may increase drug absorption and therefore distribution in the tissues. However, lipophilicity alone cannot be used to explain the improvement in both cytotoxicity and selectivity observed because these attributes were not common to all the tertiary amide compounds tested. It would appear that some sort of balance is required between the size of the two substituents (R and R^1) in the compounds found to produce the desirable antimelanoma activity *in vitro*. Consider the lead compound **89**, an *N*-benzylmethyl derivative. Selectivity was lost when the other *N*-benzyl compounds **92** and **95** were created. Could this reduced selectivity be attributed to too much steric bulk offsetting the proposed delicate balance between the size of the two substituents required for the success found with compound **89**. In addition it was found on replacing the *N*-benzyl group in compound **89** with *N*-ethyl that an overall loss in cytotoxicity and selectivity occurred. Therefore we would propose that the suggested balance between the two substituents could have been disturbed again but this time as a result of too little steric bulk.

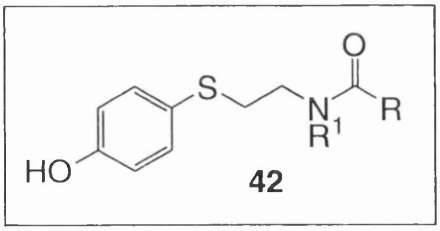
4.1.3.2 *In vitro* mushroom Tyrosinase spectrophotomeric assay

To widen the biological evaluation of our potential antimelanoma compounds a spectrophotomeric enzyme assay[196] was employed to investigate the relative substrate activity of these compounds for the enzyme Tyrosinase. The assay was performed by Miss Isabel Freer of the Tissue Culture Unit, University of Glasgow. As Tyrosinase of mammalian origin was not available to us, commercially available mushroom Tyrosinase was used instead. Like mammalian Tyrosinase, the mushroom enzyme is a copper-containing aerobic oxidase which catalyses the conversion of L-Tyrosine into dopaquinone.[197] However it must be remembered that any observations made and conclusions that may be drawn from the assay are to be used only as a guide to how the test compounds might behave with mammalian Tyrosinase. Nonetheless, we hoped the results would compliment the *in vitro* SRB inhibition assay results (section 4.1.3.1).

The mushroom Tyrosinase assay used UV spectroscopy at wavelength 280 nm to follow the production of *ortho*-quinone from the phenolic test compound. The spectrophotomeric assay measurements enabled us to determine the relative substrate activity of the test compound by comparing the relative activity of the unnatural substrate when it replaced the natural substrate in the assay at the same concentration. Obviously L-tyrosine, the natural

substrate of Tyrosinase, would produce a value of 100% relative substrate activity. The assay results can be seen in table 5.

4.1.3.2.1 Results and discussion



42

Table 5 Relative substrate activities (%) of compound **42** for mushroom Tyrosinase

		R^1			
		H	**Me**	**Et**	**Bn**
R = Me	Compound	**35**	**87**	**88**	**89**
	% Relative substrate activity	4.5	7.9	17.5	20.7
R = cyclohexyl	Compound	**68**	**90**	**91**	**92**
	% Relative substrate activity	37.8	7.1	16.8	0
R = Ph	Compound	**96**	**93**	**94**	**95**
	% Relative substrate activity	6.4	17.8	28.3	0

results of particular interest

From the results the best substrate appears to be **68** and this compound had showed modest cytotoxity against some cell lines in the SRB inhibitory assay. The *N*-ethyl compounds **88**, **91** and **94** were the next best substrates in the assay. However, **93** and **89** were also found to produce values in this range. These Tyrosinase assay results can be used as an indication that the compounds are substrates for Tyrosinase and may exhibit their cytotoxicity by Tyrosinase-mediated oxidation. Surprising results were found for the *N*-benzyl compounds **92** and **95** with 0% relative substrate activities recorded in the assay, an indication that these compounds may be poor substrates for the enzyme.

4.1.3.3 Correlation of assay results

The results appeared to compliment the *in vitro* assay observations. As was already discussed (section 4.1.3.1) the most successful candidate from the *in vitro* assay was compound **89** followed by **91** and **94**. These compounds

showed good cytotoxicity and selectivity for Tyrosinase-containing cell lines. The Tyrosinase assay results complimented these findings with **89**, **91** and **94** being among the test compounds which showed good values for relative substrate activity, an indication of their potential as Tyrosinase substrates. The inhibitory assay findings had also revealed **90**, **92** and **95** to be cytotoxic but what is more important is that they lacked selectivity. Perhaps this lack of selectivity can be explained by the Tyrosinase assay results which found both **92** and **95** as poor potential substrates for the enzyme with zero values for relative substrate activity recorded.

4.1.3.3.1 Conclusions on biological test results

Armed with the results of the two assays we propose that compounds **89**, **91** and **94** are cytotoxic and selective for the Tyrosinase-containing melanoma cell lines and that the cytotoxicity might be the result of Tyrosinase-mediated oxidation of those phenolic test compounds. Furthermore we propose that the lack of selectivity exhibited by compounds **90**, **92** and **95** could be the result of cytotoxicity produced from a non-Tyrosinase mediated mechanism of action.

4.2 AMIDINE SALTS TARGET COMPOUNDS

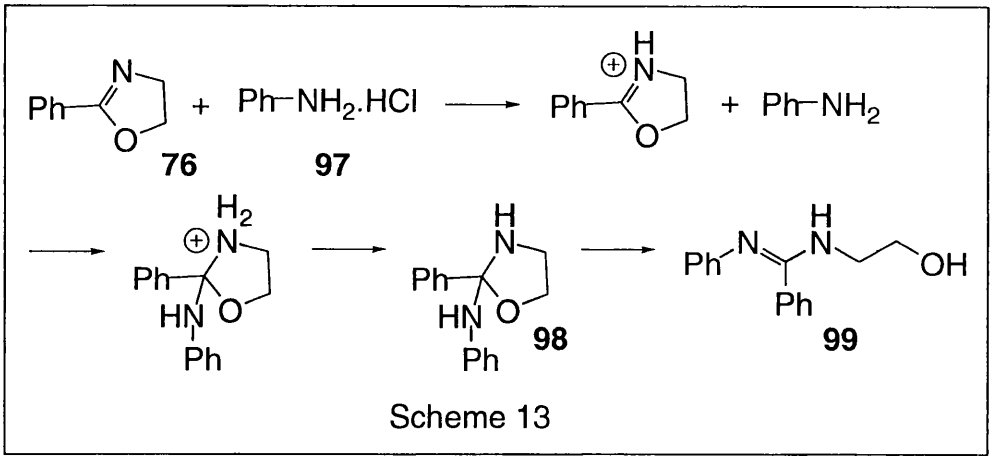
The second programme of research we proposed for the antimelanoma field involved the preparation of a series of amidine salts. This would introduce a novel structural feature to the phenolic thioether-based compounds already prepared. As outlined in section 2.9.5, we anticipated that the amidine moiety might confer some hydrophilic character to these compounds, an effect which we hoped would be beneficial in the biological assay results. Moreover, in preparing these compounds the ring opening of 2-oxazolines and oxazines by aryl amine salts would be investigated, a reaction very much dependent on the nucleophilic reagent employed (see section 4.1.1.2).

4.2.1 AMINES AND 2-OXAZOLINE RING-OPENING REACTIONS

In sections 4.1.2.1 and 4.1.2.2 we illustrated the reaction between aryl thiols and 2-oxazolines, which is an exclusive ring opening reaction at the oxazoline 5-position. However, on substituting the aryl thiol with an amine or amine salt far less clear-cut results are obtained.

Fazio[190] identified carboxamide products from the reaction between 2-oxazolines and a selection of secondary aliphatic and aromatic amines. Although primary aromatic amines provided a mixture of products, these too were afforded from oxazoline ring opening at the 5-position.[190] On the other hand, Magosch[198] found that 1,2- and 1,3-alkylene diamines provided oxazolidine products characteristic of 2-position attack on oxazolines. These findings were also mirrored by Fazio using other primary alkyl amines.[190] However the reaction pathway taken changed on introducing an oxazoline substituted at the 2-position with the bulky tertiary butyl group. As a consequence ring opening at the 5-position was forced to occur and the carboxamide product was obtained.[190]

Our work followed the investigations of Kormendy *et al*[199] into the reaction of aryl amine salts **97** and 2-position opening of oxazoline ring systems **76** (see scheme 13). In this amine addition reaction the oxazolidine **98** can afford the corresponding amidine **99**. However, purely on the premise of IR data Kormendy *et al*[199] concluded that they had isolated the oxazolidine **98**. On the basis of investigative work performed by Lant[134] it would appear Kormendy *et al* had misassigned the product and so we employed the reported experimental protocol to prepare a series of amidine salts from aryl amine salts and a selection of 2-oxazolines and oxazines.

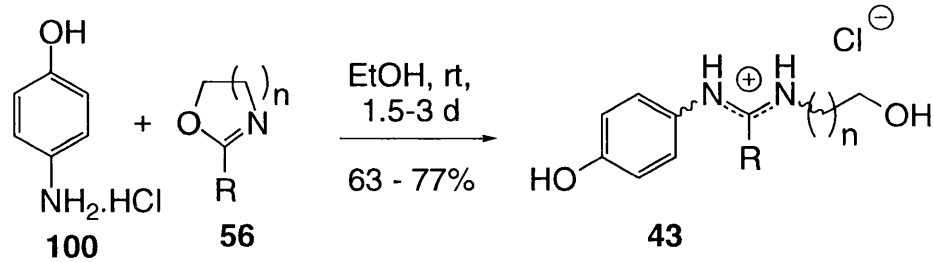


Scheme 13

4.2.2 SYNTHESIS OF AMIDINE SALTS

The synthesis of the amidine salt target compounds **43** was a straightforward, single step procedure performed at room temperature (scheme 14). The experimental conditions employed to couple the 4-aminophenol hydrochloride **100** to a series of 2-oxazoline and oxazines **56** were those

reported by Kormendy *et al.*[199] The oxazine reagents (**56**, n=2) were introduced to extend the alkyl side chain length by one carbon in the product. The synthesis of these compounds and the 2-oxazolines is detailed in section 4.1.1.1 using a cadmium acetate catalysed procedure[189] to combine the appropriate nitrile and aminoalcohol under neat reflux conditions. The alkyl groups in the phenolic thioether series were also introduced into the amidine series through the use of oxazolines and oxazines substituted with these alkyl groups.



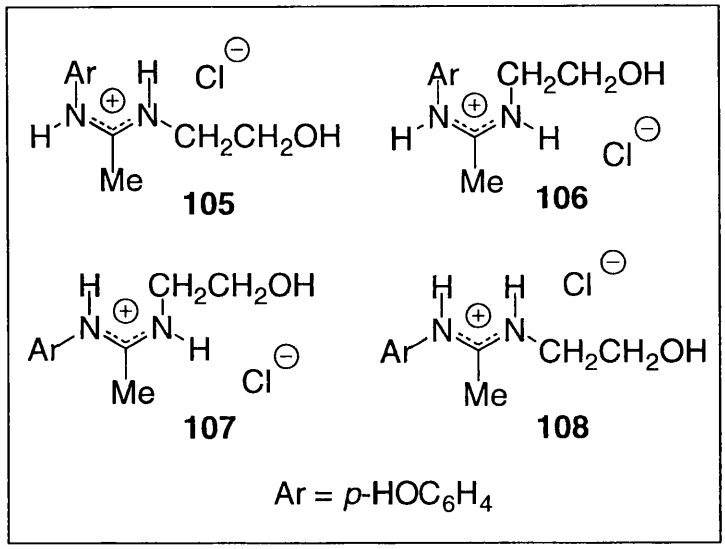
n	R	Substrate	Product	Yield
1	Me	**70**	**101**	77%
1	Et	**59**	**102**	69%
2	Me	**61**	**103**	63%
2	Et	**62**	**104**	68%

Scheme 14

In this series only the amidine salts prepared from the methyl and ethyl substituted ring systems were isolated. The four products (**101** to **104**) were easily isolated from the reaction mixture through dropwise addition of chilled diethyl ether. The filtered products were recrystallised affording the purified products in high yields (mean value of 70%) with sharp melting points. Combustion data confirmed the molecular formulae for the hydrochloride salts with elemental analyses recorded within 0.1% of the theoretical values. Meanwhile EIMS for the four compounds confirmed the molecular ion for the free base and a common base peak (*m/z* 109) was identified as the *p*-hydroxyanilinium fragment ion.

However, following numerous attempts the preparation of the amidine salts containing cyclohexyl and phenyl moieties proved to be unsuccessful. These findings were attributed to attack at the 2-position of the oxazoline being blocked by the bulk of the substituent positioned there. This is analogous to

Fazio's report[190] on the reaction of primary alkyl amines and 2-oxazolines which suffered from a similar handicap from bulk preventing attack at the oxazoline's 2-position (see section 4.2.1). Nevertheless, the four target compounds isolated provided interesting NMR observations which indicated that the amidine salt was present as geometric isomers.



In theory four geometric isomers of the amidine salt are possible. Consider the example shown for amidine **101**. Using simple molecular modelling the more thermodynamically favourable conformation for the geometric isomers can be visualised. In the conformation where the aromatic and amidine moieties are arranged coplanar, the steric interference would be greatest for **105** and **106** and therefore we propose that the product is composed of geometric isomers **107** and **108**.

On the ^{1}H and ^{13}C NMR spectra, pairs of peaks were observed for the two isomeric product forms and the NMR spectra of the simplest amidine salt **101** are included (diagrams 7 and 8).

Diagram 7 ^{1}H NMR spectrum for compound **101**

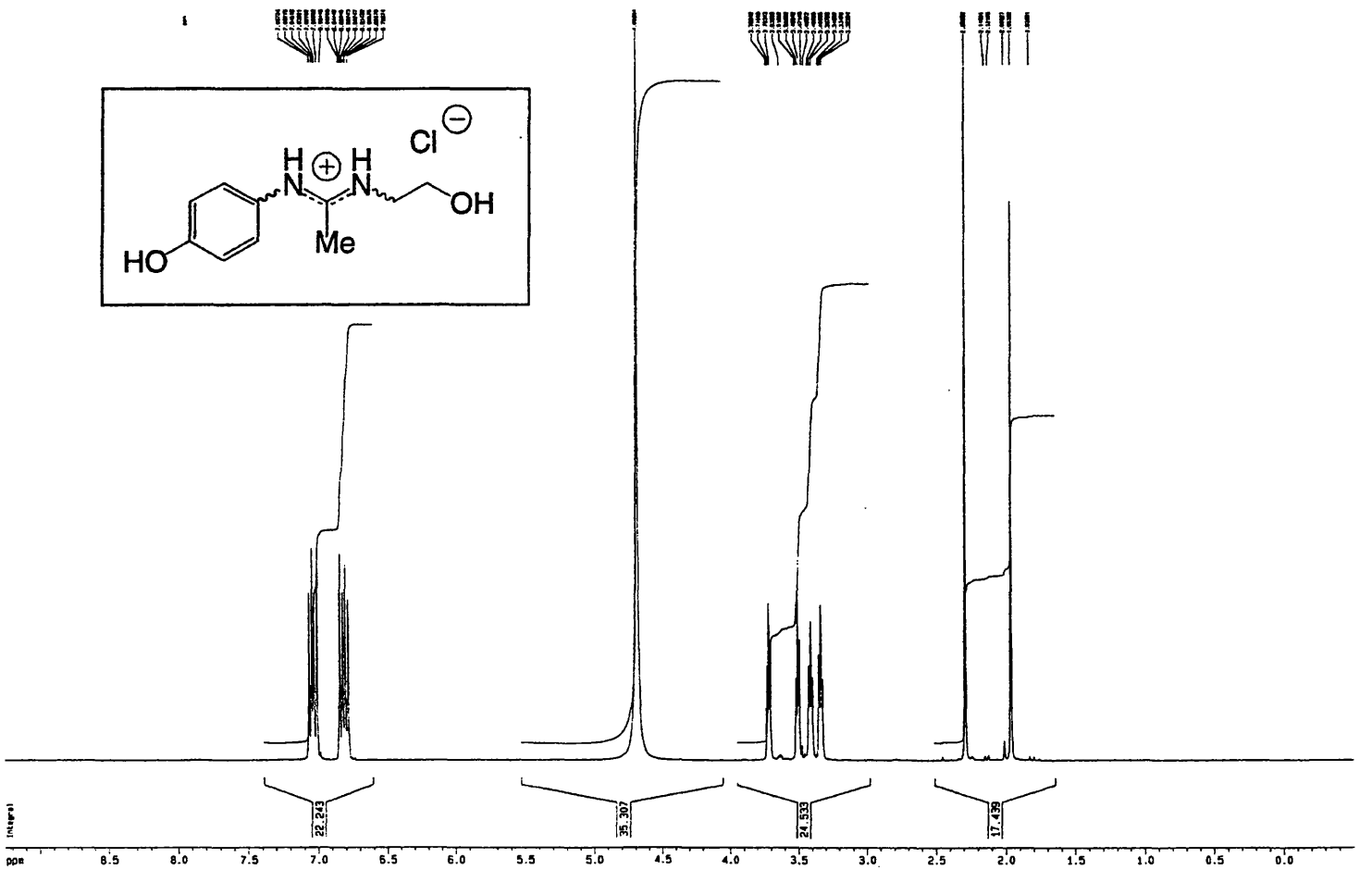
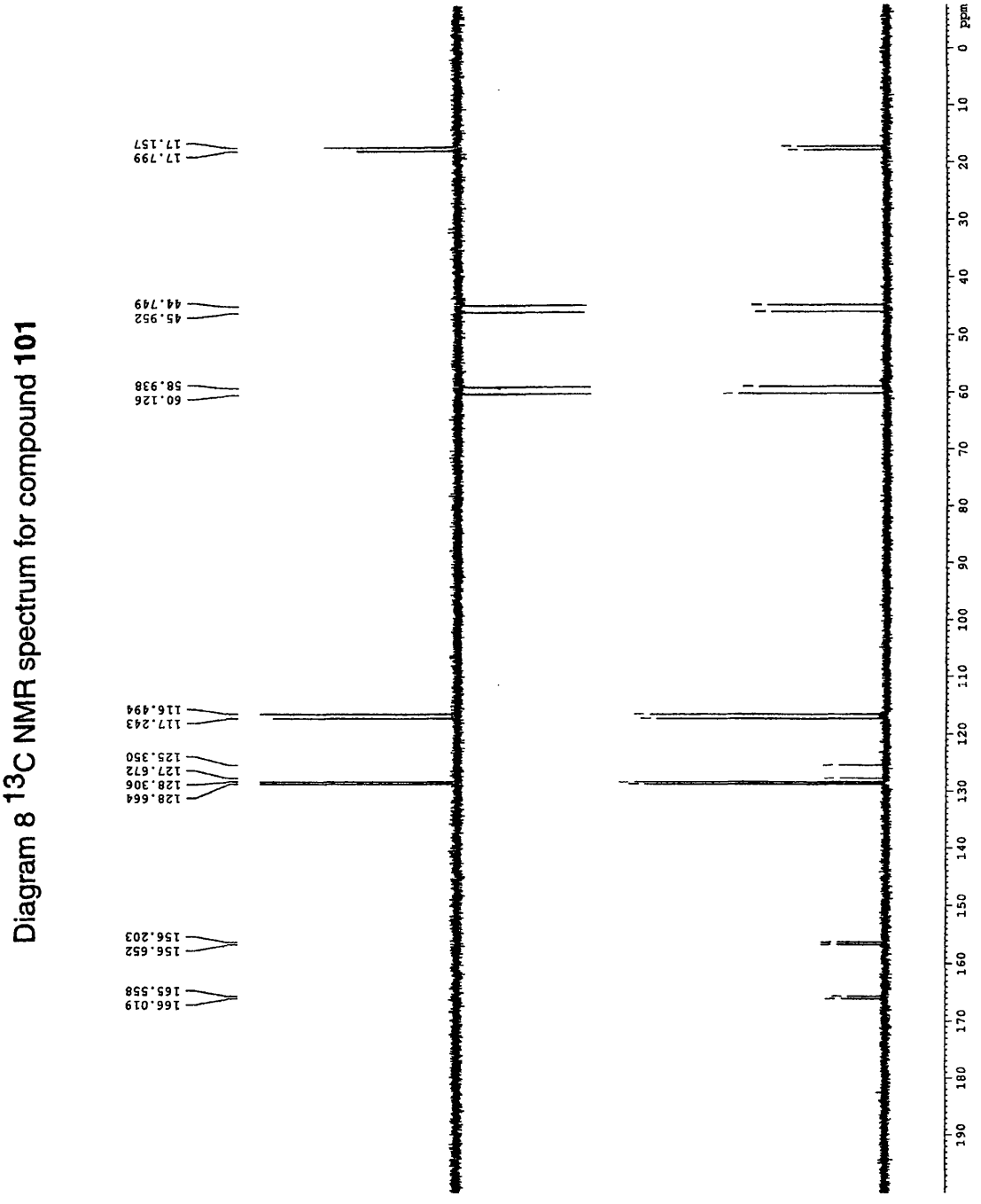


Diagram 8 ^{13}C NMR spectrum for compound **101**



Integrating the peak heights of the signals on the ^{1}H NMR spectrum (diagram 7) revealed the two geometric isomers to be present in a 1:1 ratio. In fact this was found to be the case for all four compounds prepared. The ^{1}H NMR spectra is simplified by the absence of OH and NH signals which the NMR solvent D_2O exchanged (HOD peak is very obvious at δ 4.69). The methyl group can be clearly seen as a pair of sharp singlets at δ 1.96 and 2.29. The two methylene groups were observed as two pairs of triplets in the δ 3.2-3.8 region with vicinal coupling behaviour exhibited by the protons in each of the two groups. The ^{13}C NMR spectrum (diagram 8) was also beautifully clear with all the isomeric pairs of signals readily identified. The pair of signals for the methylene carbon alpha to the hydroxyl group resonated downfield at δ 58.9 and 60.1 and can be compared to the paired signals for the less deshielded methylene carbon alpha to the amidine moiety which was found to resonate at δ 44.7 and 46.0.

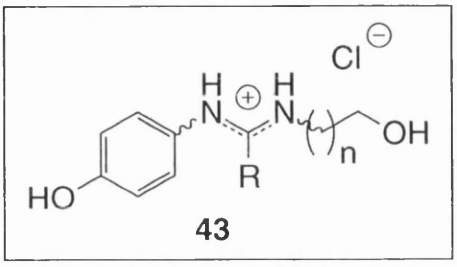
The IR spectra yielded evidence for the more prominent features of the amidine salts with strong absorption bands at *ca.* 1650 cm^{-1} observed for the C=N stretching vibration and characteristic NH stretching bands in the 3200-2800 cm^{-1} region. The phenolic OH absorption bands for bending vibrations were sharp at *ca.* 1270 cm^{-1} while the stretching vibration found some overlap with the NH absorption bands in the *ca.* 3400 cm^{-1} region.

These phenolic compounds were anticipated to possess UV activity and all four compounds recorded main absorption bands at *ca.* λ 224 nm (ε 16700-9600). The *p*-substitution of the phenol with an amidine group appears to have extended the conjugation of the phenol chromophore and therefore the amidine moiety is considered to be behaving as a complementary auxochrome. The UV activity of the amidine salts can be compared to phenol (λ_{max} 211 nm, ε 6200[200]) which demonstrates this observed UV behaviour.

4.2.3 BIOLOGICAL EVALUATION OF AMIDINE SALTS

4.2.3.1 Results and discussion

The amidine salts were evaluated biologically using the same 96 hour sulforhodamine (SRB) inhibitory assay used to investigate the activity of the phenolic thioether compounds in section 4.1.3.1. The results from this *in vitro* assessment of the cytotoxicity and selectivity of test compounds **43** are shown in tables 6 and 7.



43

Table 6 GI_{50} values (μM) for compounds **43** (**n** = **1**) in 96 h SRB assay

Cmpd	R	Cell line B008	B0010	G361	HT144	SKMeL2	SKMeL24	SKOV-3
101	**Me**	28	45	51	42	60	110*	>100
102	**Et**	15.5	32.5	43	22	58	>100	>100

* Obtained by extrapolation
results of particular interest

Table 7 GI_{50} values (μM) for compounds **43** (**n** = **2**) in 96 h SRB assay

Cmpd	R	Cell line B008	B0010	G361	HT144	SKMeL2	SKMeL24	SKOV-3
103	**Me**	>100	>100	>100	>100	>100	>100	>100
104	**Et**	>100	66	>100	78	>100	>100	>100

From the test results it would appear that the two carbon chain feature in the amidine salts (table 6) improves the inhibitory activity of these test compounds. In particular the ethyl analogue **102** proved to be the most potent and selective for the Tyrosinase-containing cell lines. Although the corresponding methyl compound **101** was found to be inhibitory, the modest level of toxicity it showed against the non melanotic SKMeL24 cell line does question the selectivity of the compound. Compounds **103** and **104** which contain a three carbon chain (table 7) did not demonstrate any notable activity against the panel of cell lines used in the study. Nevertheless, all four amidine salts were further investigated by their inclusion in the mushroom Tyrosinase assay detailed in section 4.1.3.2. The results from this study into the compounds' potential as Tyrosinase substrates are shown in table 8.

Table 8 Relative substrate activities (%) of compounds **43** for mushroom Tyrosinase

		R	
		Me	**Et**
n = 1	Compound	**101**	**102**
	% Relative substrate activity	0.1	0
n = 2	Compound	**103**	**104**
	% Relative substrate activity	0	1.3

The trend previously observed for the phenolic thioether compounds between the cell line testing and enzymatic assay results was not detected for the amidine salts. Extremely low values of less than 1% for relative substrate activity were found for all the amidines including the two compounds **101** and **102** that had shown inhibitory activity in the cell line tests. The results shown in table 8 indicate that the amidine test compounds are poor substrates for Tyrosinase.

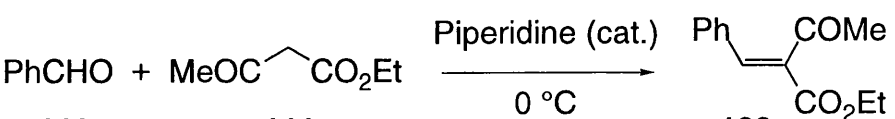
4.2.3.2 Conclusions

We can conclude that while the *in vitro* cell tests indicated that the amidine **102** is cytotoxic and selective towards cell lines containing Tyrosinase, the enzyme assay indicated that the cytotoxic nature of the compound is the effect of a non-Tyrosinase mediated mechanism of action. Perhaps then these results reflect the hydrophilic character of the amidine salts, an indication that these compounds may not be absorbed as readily into the tissues and as such are unable to exert their antimelanoma activity. Furthermore we believe that the amidine salts may be structurally too dissimilar from the original lead compound.

4.3 TYRPHOSTIN TARGET COMPOUND SERIES

4.3.1 THE KNOEVENAGEL CONDENSATION REACTION[22]

The Knoevenagel condensation is a fundamental carbon-carbon bond forming reaction regularly used by synthetic chemists. Early investigations into this type of reaction by Knoevenagel in 1894[202] were followed two years later with the reaction shown in scheme 15.[203] This piperidine-catalysed production of the benzylidene-1,3-dicarbonyl **109** from benzaldehyde **110** and ethyl acetoacetate **111** remains a good illustration of the traditional Knoevenagel condensation.



110 **111** **109**

Scheme 15

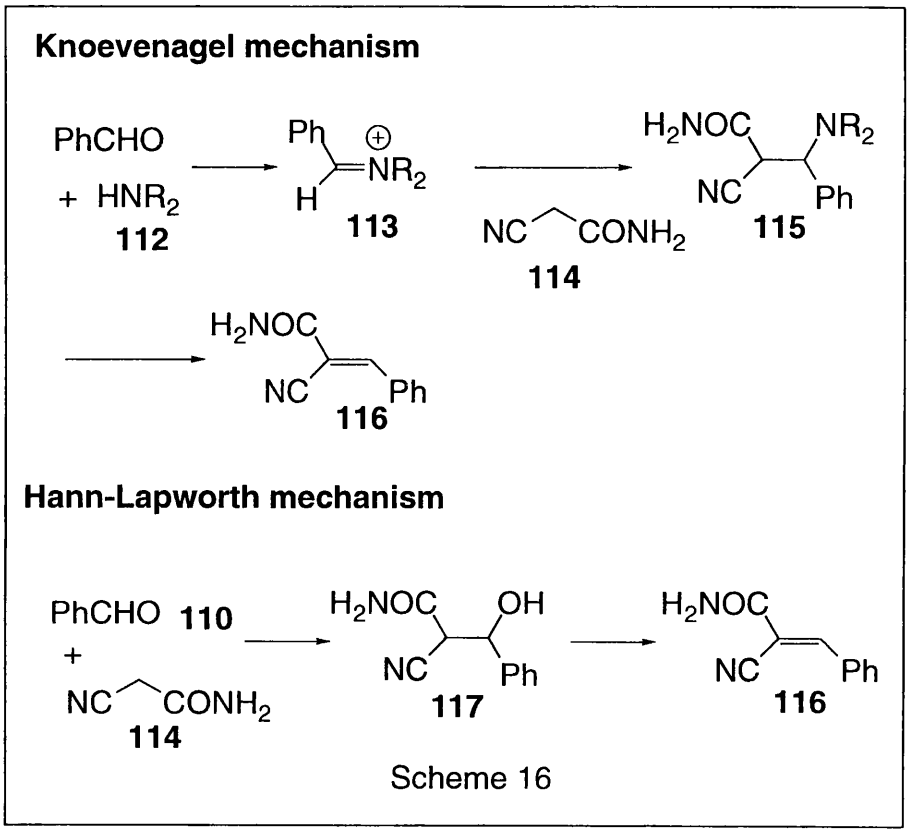
The limited reactivity of ketones in the reaction restricts the source of carbonyl compounds to aldehydes.[201] However the range of aldehydes in existence is far from restricted. Similarly, a large range of active methylene compounds are available with the presence of two electron withdrawing moieties flanking the methylene group being the only structural requisite.[201] This great variety in the two reactant sources therefore creates a potentially abundant supply of Knoevenagel products, many of which assume the role of crucial intermediates in synthetic transformations.

The homogeneous conditions of this condensation reaction most commonly employ as a catalyst a weak base such as an amine or ammonium salt or Lewis acids such as $ZnCl_2$[204] and $TiCl_4$/base.[205] However, more recently the nature of the Knoevenagel catalyst has been changing, a timely reminder of the economic and environmental pressures to which modern synthetic chemists must respond. The desire for high yielding reactions under milder conditions with no side products has prompted the application of heterogeneous catalysis to the Knoevenagel reaction. Through the use of inorganic solid supports low cost, recyclable catalytic sources are available with some recent examples including rare-earth exchanged NaY zeolite[206] and caesium/sodium cation-exchanged mesoporous MCM-41.[207] As well as the use of these synthetic zeolites, aluminium oxide[208] and xonotlite or xonotlite doped with potassium *tert*-butoxide[209] are also established heterogeneous catalytic sources while the inorganic solid potassium fluoride[210] is an alternative base. These heterogeneous catalysts are useful to enable industrial application of the reaction.

4.3.1.1 Mechanism for Knoevenagel reaction

Two mechanistic pathways are proposed for the Knoevenagel reaction and it is the base employed which decides the mechanism to be taken. The Knoevenagel mechanism[211] proposed by the reaction's founder occurs under the more traditional conditions when a primary or secondary amine **112** is

employed. Knoevenagel proposed the formation first of an imine **113** which on reacting with the active methylene compound **114** affords an intermediate **115**. Following elimination on **115**, the desired alkene product **116** is obtained. In the presence of a tertiary amine the Hann-Lapworth mechanism[212] is proposed. This is a shorter mechanism and assumes the formation of β-hydroxy compound **117** directly from the two reactants **114** and **110** followed by dehydration to yield the Knoevenagel product **116** as before.



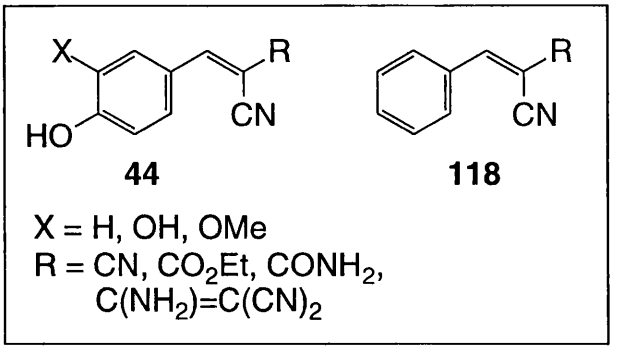
Scheme 16

Scheme 16 details the Knoevenagel product of benzaldehyde **110** and 2-cyanoacetamide **114**. As depicted here and in accordance with the literature findings consistently reported for such a reaction, the geometric isomer formed is assumed to be in the *E*-form. This is the less sterically hindered and more thermodynamically stable form.

4.3.2 SYNTHESIS OF TYRPHOSTIN TARGET COMPOUNDS

The third and final programme of research we proposed for the antimelanoma area in section 2.9.5 involved the preparation of a tyrphostin series **44**. In a manner similar to the amidine series we would move away from the phenolic thioether structure common to the previous antimelanoma

compounds. Although the third compound series **44** would maintain the phenolic character we planned to introduce a *p*-substituent with alkene character. In fact these tyrphostins compounds **44** are the Knoevenagel products of phenolic benzaldehydes and malononitrile derivatives.



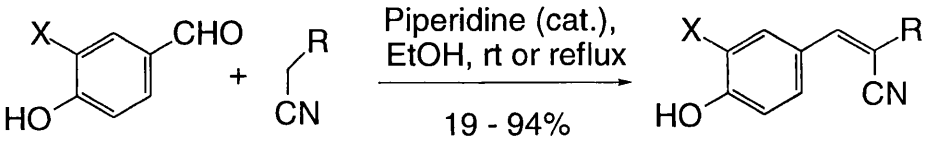
44 **118**

X = H, OH, OMe
R = CN, CO_2Et, $CONH_2$, $C(NH_2)=C(CN)_2$

The tyrphostins **44** were included in the target antimelanoma compounds as they had already been found to possess antiproliferative activity.[136,213] Gazit made this discovery in compounds which incorporated the benzylidene malononitrile (BMN) nucleus **118**. He coined the name "tyrphostin" for these compounds, a name which describes many compounds even those which deviate from the BMN structure. Moreover, it was the hydroxybenzylidenemalononitriles which were found to possess the most distinctive activity.[213] Therefore, we planned to investigate whether our tyrphostin series **44** would exhibit selectivity for melanoma by behaving as unnatural substrates for the target enzyme Tyrosinase.

4.3.2.1 Synthesis results

The tyrphostins **44** were prepared under traditional Knoevenagel conditions of homogeneity with piperidine providing the catalytic source (see Scheme 17).[213] Piperidine is one of the more popular bases employed for a simple Knoevenagel transformation.[201] Three groups of tyrphostins were prepared, each derived from the aldehyde **119** employed with four malononitrile derived compounds **120** providing the active methylene reactants. In total twelve target compounds **121** to **132** were prepared. Notably, the reactions with *p*-hydroxybenzaldehyde and vanillin favoured room temperature reaction conditions whereas for the preparation of the catechol compounds reflux conditions were preferred (scheme 17). Following completion of the reaction, product precipitation was achieved (only if required) by the addition of water to the cooled ethanolic reaction mixture.



119 **120** **44**

X	R	Product	Conditions	Yield
H	CN	**121**	rt	81%
H	CO_2Et	**122**	rt	58%
H	$CONH_2$	**123**	rt	61%
H	$C(NH_2)=C(CN)_2$	**124**	rt	19%
OH	CN	**125**	reflux	59%
OH	CO_2Et	**126**	reflux	43%
OH	$CONH_2$	**127**	reflux	30%
OH	$C(NH_2)=C(CN)_2$	**128**	reflux	25%
OMe	CN	**129**	rt	81%
OMe	CO_2Et	**130**	rt	94%
OMe	$CONH_2$	**131**	rt	64%
OMe	$C(NH_2)=C(CN)_2$	**132**	rt	29%

Scheme 17

The recrystallised products were brightly coloured compounds with sharp melting points. The three malononitrile dimer reaction products **124**, **128** and **132** recorded the lowest yields of the series at less than 30%. However, the remaining products were obtained in moderate to high yields with a mean yield of 64%.

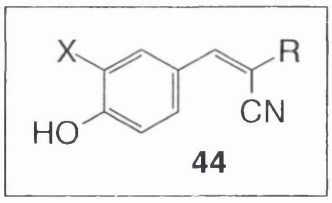
The eight compounds from the phenol and catechol tyrphostin series **121** to **128** were known compounds and their melting points and spectroscopic data were in agreement with those reported in the literature. On the other hand, the vanillin-derived series **129** to **132** required full characterisation which confirmed their structures. The prominent nitrile, alkene and phenol features of these compounds were easily identified by IR spectroscopy. The series of bands in the 1500-1600 cm^{-1} region were characteristic of the alkene moieties while the strong nitrile and phenol stretching frequencies could be observed at *ca.* 2200 cm^{-1} and *ca.* 3400 cm^{-1}, respectively. The NMR spectra for the four compounds were straightforward with successful reactions confirmed on ^1H NMR spectra by the absence of the vanillin aldehyde proton at *ca.* δ 10 and the appearance of the alkene proton singlet at *ca.* δ 8.2. Similarly on ^{13}C NMR

spectra the nitrile group common to all four compounds afforded a signal at *ca.* δ 117. However, it was the UV spectra for these highly conjugated compounds which produced the results of greatest interest. The alkene moiety creates an electronic compliment for the phenol within these tyrphostins. This delocalised, electron-withdrawing group behaves as an auxochrome by creating a shift in the absorption maximum of the phenol chromophore from λ_{max} 211 nm[200] to *ca.* λ_{max} 365 nm in the target compounds. This red shift towards longer wavelength is also visually observed by the bright colouring characteristic of the compounds.

4.3.3 BIOLOGICAL EVALUATION OF TYRPHOSTINS

4.3.3.1 Results and discussion

The tyrphostins were biologically evaluated in the same way as the phenolic thioether compounds and amidine salts. The results from the *in vitro* SRB inhibitory cell line testing of the tyrphostins are shown in tables 9, 10 and 11.



44

Table 9 GI_{50} values (μM) for compounds **44** (**X** = **H**) in 96 h SRB assay

Cmpd	R	Cell line B008	B0010	G361	HT144	SKMeL2	SKMeL24	SKOV-3
121	**CN**	47	27.5	33	38	49	>100	71
122	**CO_2Et**	59	45	54	54	54	>100	95
123	**$CONH_2$**	81	26.5	53	53	50	>100	>100
124	**dimer**	>100	>100	>100	>100	>100	>100	>100

results of interest

dimer = $C(NH_2)=C(CN)_2$

Table 10 GI_{50} values (μM) for compounds **44** (**X** = **OH**) in 96 h SRB assay

Cmpd	R	Cell line						
		B008	**B0010**	**G361**	**HT144**	**SKMeL2**	**SKMeL24**	**SKOV-3**
125	**CN**	43.5	20	20.5	30	53	>100	>100
126	**CO_2Et**	>100	>100	88	100	>100	>100	>100
127	**$CONH_2$**	>100	>100	>100	>100	>100	>100	>100
128	**dimer**	41	23	21	29	56	>100	>100

results of interest

dimer = $C(NH_2)=C(CN)_2$

Table 11 GI_{50} values (μM) for compounds **44** (**X** = **OMe**) in 96 h SRB assay

Cmpd	R	Cell line						
		B008	**B0010**	**G361**	**HT144**	**SKMeL2**	**SKMeL24**	**SKOV-3**
129	**CN**	>100	82	85	82	>100	>100	>100
130	**CO_2Et**	>100	>100	>100	>100	>100	>100	>100
131	**$CONH_2$**	>100	>100	>100	>100	>100	>100	>100
132	**dimer**	>100	>100	>100	>100	>100	>100	>100

dimer = $C(NH_2)=C(CN)_2$

Overall no real patterns of inhibitory activity were found for the tyrphostins with only compounds **123**, **125** and **128** found to exhibit modest cytotoxic and selective behaviour towards the Tyrosinase cell lines. Compounds **121** and **122** were found to be moderately potent but with less selectivity as cytotoxicity was shown against the SKOV-3 ovarian cancer cell line. Furthermore very low levels of cytotoxicity were demonstrated against most cell lines by the four tyrphostins derived from vanillin, **129** to **132** (table 11). Despite these generally disappointing results, to complete the biological evaluation process all the tyrphostins were included in the mushroom Tyrosinase enzymatic assay. The results from the Tyrosinase assay are shown in table 12.

Table 12 Relative substrate activities (%) of compounds **44** for mushroom Tyrosinase

		R			
		CN	**$CONH_2$**	**CO_2Et**	**dimer**
X = H	Compound	**121**	**122**	**123**	**124**
	% Relative substrate activity	6.1	0	2.0	0.3
X = OH	Compound	**125**	**126**	**127**	**128**
	% Relative substrate activity	5.0	14.1	6.0	12.1
X = OMe	Compound	**129**	**130**	**131**	**132**
	% Relative substrate activity	2.5	1.4	1.1	0

Of the three compounds, **123**, **125** and **128**, which demonstrated moderate inhibitory activity in the cell line tests, only compound **128** produced a significant result in the Tyrosinase assay. This was only a modest value for relative substrate activity and suggests there would be some Tyrosinase-mediated cytotoxicity by the compound. All of the other tyrphostins apart from **126** exhibited low values for relative substrate activity.

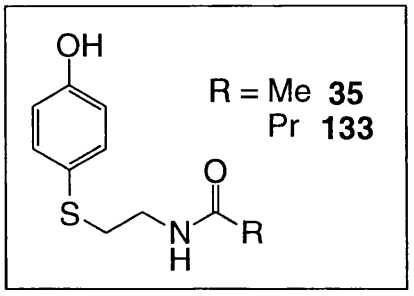
4.3.3.2 Conclusions

When considering compounds for their potential antimelanoma activity the tyrphostins would not be a good choice. Perhaps the mushroom Tyrosinase assay provided the most useful information regarding the biological activity of these compounds. Indeed the cytotoxicity exhibited by these compounds could be attributed to a non-Tyrosinase mediated mechanism of action. The results from the biological evaluation of the tyrphostins would also appear to confirm the findings from the amidines' test results which led us to propose that moving too far away from the structure of the phenolic thioether parent compound **35** appeared to reduce the biological characteristics required for specific antimelanoma activity.

4.4 RECENT DEVELOPMENTS

Following their early discoveries into the antimelanoma potency of **35**,[127] Jimbow and co-workers recently proposed to manipulate the lipophilic character of the drug in an attempt to improve its activity.[214] This compound change was achieved by preparing the propionyl derivative of the lead compound. In recent

studies this new phenolic thioether compound **133** was found to produce very interesting *in vitro* and *in vivo* results.[214,215]



From the *in vitro* studies compound **133** was found to be both a better substrate for Tyrosinase[215] and more melanocytotoxic than **35**.[214] Furthermore, the *in vivo* investigations revealed that the new compound significantly inhibits melanoma tumour growth and increases the life span of the melanoma-bearing study group of C57 black mice.[214] *In vivo* melanocytotoxicity of the drug was confirmed by its depigmentation of mice hair follicles with potency comparable to **35**.[214] These *in vivo* findings established **133** as a new phenolic thioether lead compound for melanogenesis-based antimelanoma agents.

These recent findings by Jimbow and co-workers were quite surprising to us as Lant prepared this propionyl derivative **133** during his research programme of work and following the inclusion of **133** in an *in vitro* SRB inhibition growth study (see table 13) this compound was not considered to possess significant antimelanoma activity.

Table 13 GI_{50} values (μM) for compound **133** in 96 h SRB assay

Cell line						
B008	**B0010**	**G361**	**HT144**	**SKMeL2**	**SKMeL24**	**SKOV-3**
82	>100	>100	>100	>100	>100	>100

The results from the assay shown in table 13 indicate that compound **133** exhibits only modest levels of cytotoxicity and selectivity.

4.5 CONCLUSIONS AND FUTURE PLANS

The biological evaluation of the target compounds prepared enabled us to assess their potential as antimelanoma agents. The most obvious conclusion

must be that the phenolic thioether compound series afforded the candidates with the most potential. However, both the tyrphostin and amidine salt series did produce a range of cytotoxicty values with some selectivity observed. Indications of their non-Tyrosinase mediated cytotoxicty were not too surprising and accordingly we proposed that the structures of these two series may be too far removed from the parent compound **35** to enable them to exhibit specific antimelanoma activity. Furthermore, we believed the lipophilicity of the tertiary amide compounds together with a certain combination of alkyl groups may contribute to the activity exhibited by many members of that series.

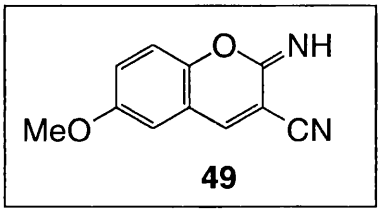
The phenolic thioether compounds proved particularly successful in the *in vitro* studies with levels of potency and selectivity for melanotic cell lines observed. We believe that the more attractive candidates from the series merit further biological evaluation. Hence we suggest that the collaboration with Professor Rona MacKie, Department of Dermatology[216] should be re-established. This collaboration previously provided an *in vivo* evaluation of a selection of compounds prepared by Lant.[134] Moreover *in vivo* assessment of the melanocytotoxicity and antimelanoma activity of these compounds is imperative to complete their biological evaluation and should be carried out with compounds **89**, **91** and **94**.

CHAPTER 5

SYNTHESIS AND BIOLOGICAL EVALUATION OF MAP KINASE INHIBITORS

5.1 BACKGROUND

The inhibition of MAP kinases in cell signalling pathways is the second area of cancer research which our studies embrace. In Chapter 3 this area was discussed in some detail together with the proposed research work (section 3.6). There we introduced the lead compound **49**. This 2-iminolactone or 2-iminochromene derivative as it is also known, provided a model for our synthetic investigations into compounds with MAP kinase inhibitory potential.

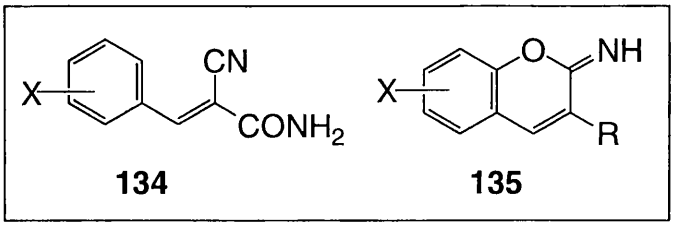


49

5.2 2-IMINOLACTONE TARGET COMPOUNDS

5.2.1 2-IMINOLACTONE RING SYSTEM

The 2-iminolactone ring system enjoys positions of importance in both biological and chemical fields. Originally used in the design of conformationally constrained mimics of active compounds such as **134**, the bicyclic iminolactone compounds **135** and many of their derivatives are now established as a source of biologically active compounds in their own right.[217,218] Behaviour which they exhibit includes significant antitumour activity.[218]

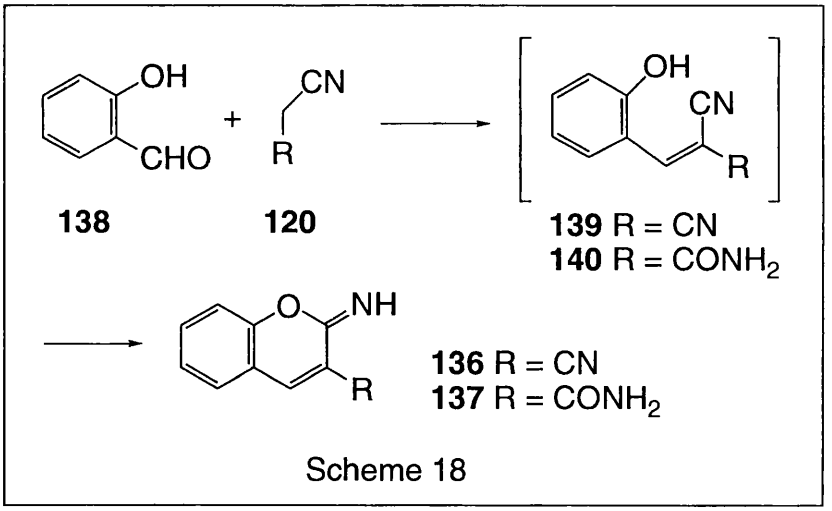


134 **135**

Furthermore, the functionality of these 2-iminolactone compounds enables them to provide the structural base for the synthesis of polycyclic compounds such as substituted benzopyranopyridines and benzopyranopyridopyrimidines.[219]

5.2.2 STRUCTURE ELUCIDATION OF 2-IMINOLACTONES

Schiemenz implemented infrared-based product characterisation to formulate 2-iminolactones **136** and **137** correctly as the products obtained from the Knoevenagel condensation of salicylaldehyde **138** and malononitrile derivative **120** (scheme 18).[180] Previously the products were assigned the isomeric open chain structure **139** and **140**, which is the traditional type of Knoevenagel product.[182] IR spectroscopy was crucial to confirming the misassignment of the products **136** and **137**. In particular the characteristic nitrile stretch at *ca.* 2260 cm^{-1} for the amide **140** in the open chain form should have been obvious on the IR spectrum. However, this was not present and bands attributed to NH groups were observed in the 3500-3300 cm^{-1} region thus confirming the cyclic iminolactone structure **137**.



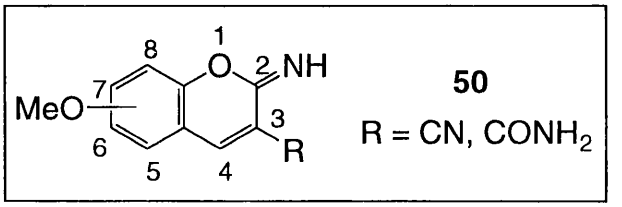
Scheme 18

Iminolactone formation also provides information for the stereospecific nature of this carbon-carbon bond forming reaction as the nitrile group must be *cis* to the phenyl group in **140** to enable cyclisation affording the iminolactone **137**. This *trans*-stereospecific nature of the Knoevenagel condensation reaction is discussed later in section 5.3.2.2.

5.2.3 SYNTHESIS OF 2-IMINOLACTONE TARGET COMPOUNDS

5.2.3.1 Strategy for synthesis

Our first synthetic proposal as outlined in section 3.6 involved an investigation into the optimum position of the methoxy substituent on the benzene ring of the lead compound **49**. In order to do this a series of iminolactone compounds **50** featuring methoxy substitution on the 5-, 6-, 7- and 8-benzene ring positions were prepared as well as the unsubstituted compounds. Although the lead compound **49** featured a nitrile group, in keeping with the reported finding on the biological activity of iminolactones (see section 5.2.1) we also planned to prepare the corresponding amide derivatives.

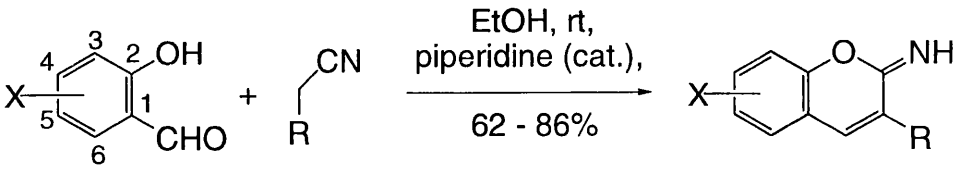


50

R = CN, $CONH_2$

5.2.3.2 Synthesis

The *o*-hydroxyaldehyde **141** and malononitrile-derived compound **120** were condensed under the mild reaction conditions of room temperature and a catalytic amount of piperidine[182] (scheme 19). For each reaction the 2-iminolactone product **135** was found to precipitate readily from the alcohol reaction mixture. However, the literature reports that these isolated crude products cannot be recrystallised unchanged.[181,182] Therefore in order to obtain analytical purity the compounds were triturated in cold alcohol. Further handling difficulties were encountered due to the powerful sternutative and irritant properties of these bicyclic compounds.



141 **120** **135**

X	R	Product	Yield
H	CN	**136**	62%
H	$CONH_2$	**137**	75%
3-OMe	CN	**142**	84%
3-OMe	$CONH_2$	**143**	86%
4-OMe	CN	**144**	86%
5-OMe	CN	**49**	62%
5-OMe	$CONH_2$	**145**	74%
6-OMe	CN	**146**	67%
6-OMe	$CONH_2$	**147**	74%

Scheme 19

The products were obtained in good yields (mean value 74%) and their structures were confirmed from the spectroscopic data collected. Compounds **136**, **137**, **143** and **145** were identified as known compounds and the data recorded for these four compounds were found to be in agreement with the findings reported in the literature. As already mentioned in section 5.2.2 perhaps the most important spectroscopic features of the iminolactones are found on the IR spectra as the characteristic nitrile band at *ca.* 2300 cm^{-1} was absent for the amide iminolactones and this together with the NH stretching vibrations in the 3400-3200 cm^{-1} region, confirmed the cyclic iminolactone structure. From the ^{1}H NMR spectra of the iminolactones the imine proton signal was observed at *ca.* δ 8.4 while on the ^{13}C NMR spectra the nitrile group carbons of the 3-cyanonitriles were found to resonate at *ca.* δ 116.

5.2.4 BIOLOGICAL EVALUATION OF 2-IMINOLACTONE TARGET COMPOUNDS

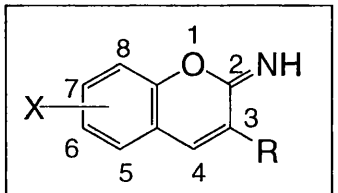
5.2.4.1 *In vitro* JNK MAP kinase assay

The 2-iminolactones prepared were assessed for their MAP kinase inhibition by Dr David Gillespie at the Beatson Institute for Cancer Research,

Glasgow.[220] This investigative assay was designed as a screen to identify the best compounds as candidates for a more detailed study. The MAP kinase enzyme employed in the assay was the c-Jun *N*-terminal kinase (JNK), a component of the stress-activated protein kinase (SAPK) pathway which regulates c-Jun transcriptional activity (The JNK/SAPK MAPK pathway was discussed in section 3.3.2.). The principles behind this assay[221] involved the use of a recombinant GST-c-Jun protein (purified from *E.coli*) bound to Sepharose beads. This behaved as a combined affinity matrix and substrate for the JNK MAP kinases. Cell extracts were prepared from primary avian fibroblasts treated with anisomycin to provide the source of activated endogenous JNK to be incubated with the GST-c-Jun beads. The assay followed standard experimental protocol[221] and reactions were performed for each 2-iminolactone test compound at concentrations of 500, 50 and 5 micromolar. SDS gel electrophoresis was used to resolve the bead-bound reaction mixtures and following electroblotting, the labelling of the GST-c-Jun from each reaction was visualised by autoradiography.

5.2.4.2 Results

Although the results from the assay were determined by visually analysing the autoradiography plates, this qualitative approach still enabled iminolactones that exhibited inhibitory activity to be identified. Test compounds which failed to show any inhibitory activity were easily identified from the autoradiograph by a stain of intensity comparable to that exhibited by the experimental control. The autoradiography results for the 2-iminolactones **135** are represented in table 14.



135

Table 14 JNK MAPK inhibitory assay results for 2-iminolactones **135**

X	R	Cmpd	Cmpd conc. (μM) 5	50	500
H	CN	**136**	●	●	○
H	$CONH_2$	**137**	●	●	◐
8-OMe	CN	**142**	●	●	●
8-OMe	$CONH_2$	**143**	●	●	●
7-OMe	CN	**144**	●	●	◐
6-OMe	CN	**49**	●	●	○
6-OMe	$CONH_2$	**145**	●	●	○
5-OMe	CN	**146**	●	●	○
5-OMe	$CONH_2$	**147**	●	●	●
Experiment control			●	●	●

○ clearly inhibitory activity ◐ modest inhibitory activity
● no inhibitory activity

At first glance it is clear that at the lower concentrations of 5 and 50 μM none of the 2-iminolactones exhibited inhibitory activity. As the next concentration is 500 μM we cannot assess the inhibitory behaviour within the 50 and 500 μM range. The model compound **49** showed clear inhibitory activity at 500 μM concentration as did the corresponding amide iminolactone **145**. Compound **146** was the third iminolactone with methoxy ring substitution to show such inhibitory activity. The simplest iminolactones **136** and **137** both exhibited activity; however it was the 3-cyanonitrile **136** which exhibited better activity. The modest inhibitory activity of **137** was also shown by **144**. The only compounds which did not show inhibitory activity at any concentration were **142** and **143**, the two 8-methoxy substituted iminolactones and iminolactone **147**.

5.2.4.3 Trends

As we would have anticipated the model compound **49** exhibited clear inhibitory activity as did the amide iminolactone **145**. An additional two compounds, **136** and **146**, were also clearly seen to inhibit. Like the lead compound **49**, these two iminolactones were also cyanonitriles. Finally, the two compounds, **137** and **144**, which were found to possess less potent inhibitory

activity were amide and nitrile iminolactones which featured no substitution on the benzene ring and methoxy group at the 5-position respectively.

5.3 TYRPHOSTIN TARGET COMPOUNDS

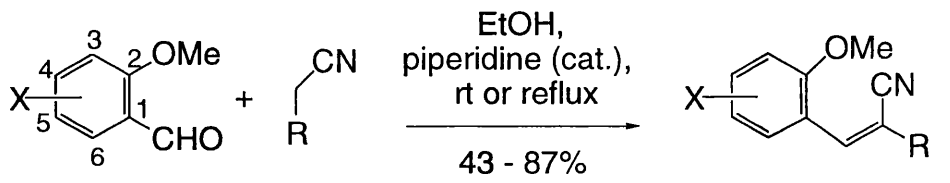
5.3.1 BACKGROUND

Following the proposal of an investigative study into the cyclic 2-iminolactones it was considered to be a good idea to explore the synthesis of the corresponding open chain derivatives. These compounds would provide analogues of the iminolactones. Prepared in exactly the same manner as their cyclic counterparts this series would employ *o*-methoxybenzaldehyde (anisaldehyde) rather than *o*-hydroxybenzaldehyde (salicylaldehyde) to ensure no cyclisation onto the nitrile group could occur. This second series of potential MAP kinase inhibitors are tyrphostins **148** which can be prepared easily under Knoevenagel reaction conditions. A phenolic series of tyrphostins was prepared during the antimelanoma research programme and so in sections 4.3.1 and 4.3.2 the history, mechanistics and alternative reaction conditions of the Knoevenagel condensations have already been presented.

5.3.2 SYNTHESIS OF TYRPHOSTIN TARGET COMPOUNDS

5.3.2.1 Synthesis

The open chain olefinic compounds **148** were prepared under piperidine-catalysed Knoevenagel reaction conditions.[213] The anisaldehyde **149** was combined with malononitrile or 2-cyanoacetamide **120** to afford a series of amide and nitrile tyrphostins **150** to **159** (scheme 20). Room temperature reaction conditions were sufficient for most of the compounds with only **151** and **159** requiring conditions of reflux. Unlike their cyclic iminolactone counterparts these open chain compounds were far easier to handle and following isolation of the products from the reaction mixture the tyrphostins were recrystallised from a selection of alcohols to afford the purified products in good yields (mean value 67%).



149 **120** **148**

X	R	Product	Conditions	Yield
H	CN	**150**	rt	43%
H	$CONH_2$	**151**	reflux	46%
3-OMe	CN	**152**	rt	68%
3-OMe	$CONH_2$	**153**	rt	70%
4-OMe	CN	**154**	rt	80%
4-OMe	$CONH_2$	**155**	rt	80%
5-OMe	CN	**156**	rt	57%
5-OMe	$CONH_2$	**157**	rt	81%
6-OMe	CN	**158**	rt	87%
6-OMe	$CONH_2$	**159**	reflux	61%

Scheme 20

Infrared spectroscopy confirmed the open chain structure of these compounds. Unlike the iminolactones the derivatives prepared from 2-cyanoacetamide produced the nitrile stretch at *ca.* 2220 cm^{-1} on the IR spectra. Furthermore there were no bands observed in the 3400-3200 cm^{-1} range common to iminolactone NH stretching vibrations. The success of the condensation reaction was also observed on the NMR spectra. On the ^{1}H NMR spectra of the tyrphostins the olefinic proton produced a singlet at *ca.* δ 8.4 which replaced the starting material aldehyde proton signal at *ca.* δ 10.5. Similarly on the ^{13}C NMR spectra the olefinic methylene carbon of the products was found to resonate at *ca.* δ 150 (compared to *ca.* δ 190 for the aldehyde carbonyl carbon). The NMR spectra obtained for the amide target compounds indicated that the product was present as a single geometric isomer and on considering the thermodynamics of the geometric isomers it is assumed that the amide group is positioned *trans* to the phenyl group. This stereospecific nature of the Knoevenagel reaction was briefly mentioned in section 4.3.1.1. In fact the stereochemistry of the Knoevenagel products has been reported in the literature

and in particular **151** along with several other compounds relevant to our work have been investigated.

5.3.2.2 NMR Studies into stereoselective Knoevenagel reaction products

NMR spectroscopy has been used by many groups to confirm the stereochemistry of Knoevenagel condensation products.[222,223] We followed these examples in order to confirm the *trans* stereochemistry we had assigned to tyrphostins prepared in sections 4.3.2.1 and 5.3.2.1. A ^{13}C NMR long-range selective proton decoupling (LSPD) experiment was performed on compound **151** chosen to represent the tyrphostins prepared during the course of our research. In the coupled ^{13}C NMR experiment the cyano and amide carbons were each found to produce a doublet with coupling constants of *J* 14.3 Hz and *J* 6.2 Hz respectively (see diagram 9).

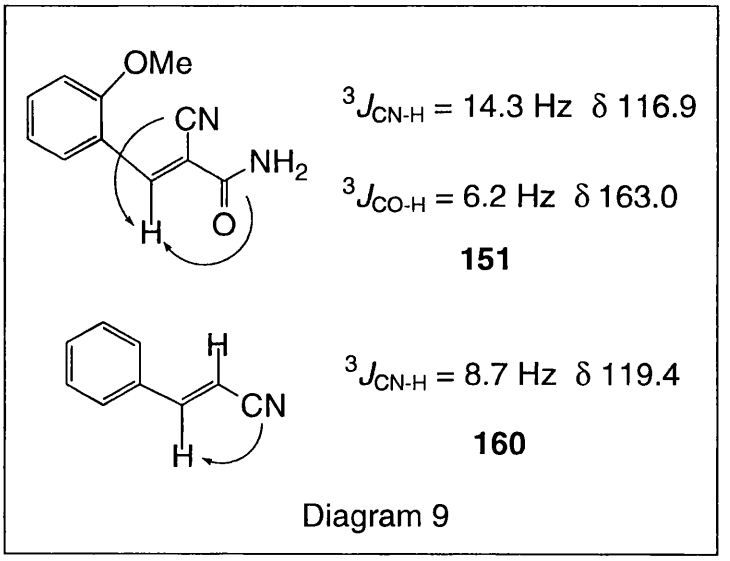


Diagram 9

The LSPD experiment which followed irradiated the olefinic proton in **151** whereupon the coupling behaviour exhibited by the two carbons mentioned was removed. This identified three-bond heteronuclear coupling between the cyano and amide carbons with the olefinic proton and the *J* values were in agreement with the reported values for this compound ($^3J_{CN-H}$ 14.2 Hz and $^3J_{CO-H}$ 6.6 Hz).[222] Indeed, these long distance heteronuclear coupling constants recorded for **151** confirmed the *trans*-stereochemistry assigned to this compound. Furthermore, the cyano carbon *J* value of 14.3 Hz is characteristic of *trans* positioned coupling nuclei and similarly the *cis*-arranged olefinic proton and amide group were confirmed from the smaller *J* value of 6.2 Hz. Confirmation for assigning *trans* stereochemistry to compound **151** on the basis of *J* values

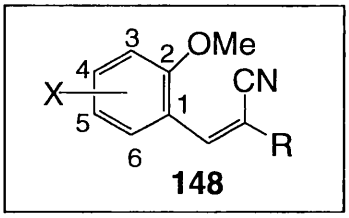
was obtained from considering the three-bond coupling constants for the cyano group and olefinic proton in cinnamonitrile **160** (see diagram 9). In this compound, where the olefinic proton and cyano carbon are arranged *cis*, $^{3}J_{CN\text{-}H}$ 8.7 Hz was recorded. This provides a *J* value for *cis*-coupled nuclei to compare with the values recorded for the coupling nuclei of interest within **151**.[223]

Other ^{13}C NMR LSPD experiments as well as X-ray crystallographic studies have been used to elucidate the *trans* stereochemistry found in various 3-, 4- and 5-phenolic substituted tyrphostins with amide and ester groups present in the olefinic side arm.[223] Many of these compounds possess the same or very similar structures to the tyrphostin target compounds which we prepared during our MAP kinase inhibitory and antimelanoma work. These reports[222,223] together with the NMR study performed on compound **151** are good evidence for the *trans* stereochemistry we assigned to our products.

5.3.3 BIOLOGICAL EVALUATION OF TYRPHOSTIN TARGET COMPOUNDS

5.3.3.1 Results

The tyrphostin target compounds were biologically evaluated using the same JNK MAPK inhibitory assay employed to assess the inhibitory potential of the 2-iminolactone compounds in section 5.2.4. The autoradiography results are represented in table 15.



148

Table 15 JNK MAPK inhibitory assay results for tyrphostins **148**

X	R	Cmpd	Cmpd conc. (μM) 5	50	500
H	CN	**150**	●	●	◐
H	$CONH_2$	**151**	●	●	◐
3-OMe	CN	**152**	●	○	○
3-OMe	$CONH_2$	**153**	●	●	◐
4-OMe	CN	**154**	●	●	●
4-OMe	$CONH_2$	**155**	●	●	●
5-OMe	CN	**156**	●	●	●
5-OMe	$CONH_2$	**157**	●	●	◐
6-OMe	CN	**158**	●	●	●
6-OMe	$CONH_2$	**159**	●	●	◐
Experiment control			●	●	●

○ clearly inhibitory activity ◐ modest inhibitory activity
● no inhibitory activity

As was found with the 2-iminolactones, no inhibitory behaviour was exhibited by the tyrphostins at the lower concentrations of 5 and 50 μM except for compound **152** which showed almost complete inhibitory activity at 50 μM. This identified the malononitrile-derived tyrphostin **152** as the most active of the series mirroring the inhibitory activity of the best iminolactones (see section 5.2.4.2). The other five active tyrphostins displayed inhibitory activity of only modest proportions. Among these compounds were **150** and **151**, the two compounds possessing the simplest benzene ring substitution and the cyanoacetamide-derived tyrphostin **153**. The 2,5- and 2,6-dimethoxy substituted tyrphostins (**157** and **159**) were the other two JNK MAPK inhibitory compounds identified.

5.3.3.2 Trends

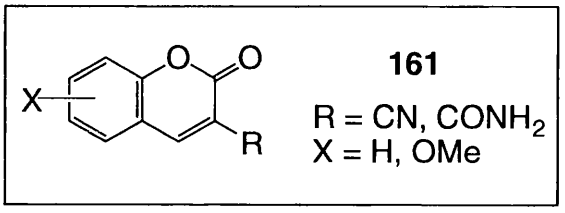
Compound **152** was identified as the lead tyrphostin in the JNK MAPK inhibitory assay. Although a further five tyrphostins exhibited inhibitory behaviour it was of more modest potency. The main pattern observed among these less active compounds was that four of the five compounds were

cyanoacetamide-derived tyrphostins. These active compounds featured 2-, 2,3-, 2,5- and 2,6-patterns of methoxy substitution on their benzene rings. Compound **150** was the only malononitrile-derived tyrphostin among the less potent JNK MAPK inhibitory compounds.

5.4 3-COUMARIN TARGET COMPOUNDS

5.4.1 TARGET COMPOUND DESIGN STRATEGY

The 3-substituted coumarins **161** were the third series modelled on the lead compound **49**. These target compounds were included in the design of potential MAP kinase inhibitors for the following reasons: their structural closeness to the 2-iminolactone ring system; their ease of preparation from the corresponding 2-iminolactone; and the diverse activities that the coumarin family as a whole are known to possess.



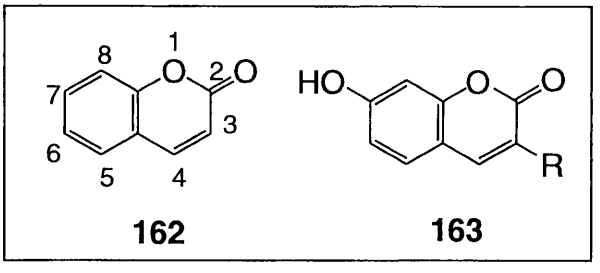
161

R = CN, $CONH_2$
X = H, OMe

5.4.2 COUMARINS[183]

5.4.2.1 Naturally occurring coumarins

Coumarin is the commonly used name for compounds which are derived from or incorporate the skeleton **162**. This aryl lactone **162** was first isolated from tonka beans in 1820[224] and synthesised some 50 years later by Perkin.[225]



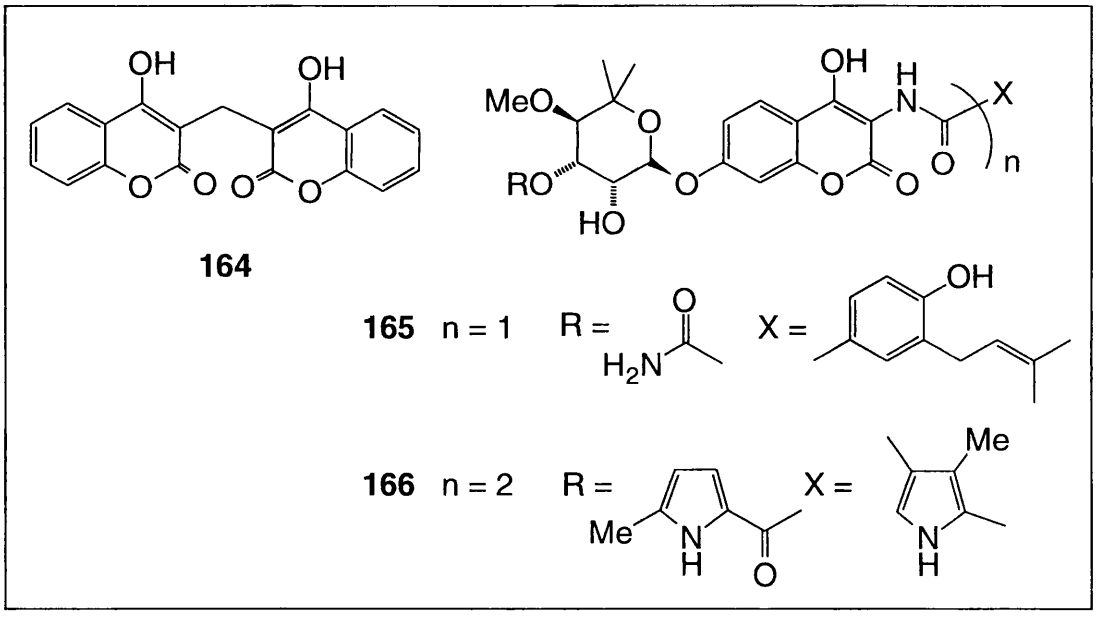
162 **163**

Most naturally occurring coumarins have been isolated from higher plants of the Umbelliferae and Rutaceae families and although their role in plant

metabolism is not fully understood, it is believed that coumarins may behave as growth regulators. Out of the nearly 800 natural coumarins identified, only 35 are found to lack an oxygen function at C-7. As a result therefore 7-hydroxycoumarin **163**, also commonly referred to as umbelliferone, is regularly found to be the basic framework of complex coumarins.

5.4.2.2 Properties of coumarins

Among the many coumarins of natural origins some notable examples are dicoumarol (3,3'-methylenebis(4-hydroxycoumarin)) **164**, the blood anticoagulant[226] and the antibiotics novobiocin **165** and coumermycin A_1 **166**.[227] Notice that dicoumarol **164** does not feature any substituents on the benzene ring, in particular the most commonly observed oxygenation at C-7. However, the two antibiotics incorporate the umbelliferone structural base with the main structural difference between them arising from the three methylpyrrolyl units in coumermycin A_1 **166**.



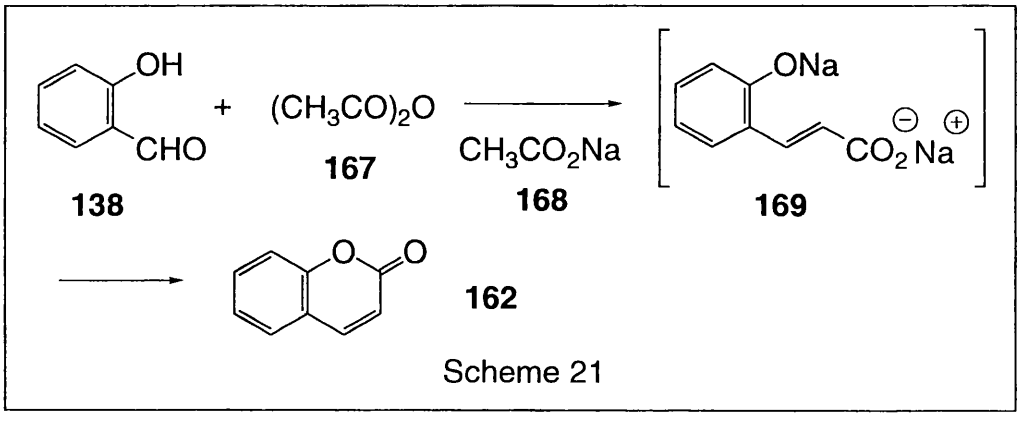
The fluorescence possessed by many natural coumarins affords extensive uses for these compounds and their synthetic counterparts. This important physical property allows 7-hydroxy and 7-aminocoumarins which contain an aryl or heteroaryl group at C-3 to be employed as laser dyes and fluorescent brightening agents in detergents, paper and textiles which mask yellowing of white materials.[228,229] Further exploitation is possible with some of

the structurally more simple coumarin derivatives such as 7-hydroxy-4-methylcoumarin which is used in fluorimetric enzyme assays.[229]

As can be seen the coumarins, whether of natural or synthetic origins, enjoy a whole host of applications based on the compounds' wealth of biological and physical properties. The coumarins mentioned here and their applications described represent a small cross-section of a far larger area of interest.

5.4.2.3 Syntheses of coumarins

As already mentioned in section 5.4.2.1, Perkin in 1868 was the first to synthesise coumarin **162**. This classical reaction combined salicylaldehyde **138** with acetic anhydride **167** and anhydrous sodium acetate **168**. The intermediate sodium salt **169** spontaneously cyclised to the final product **162** (scheme 21).[225]

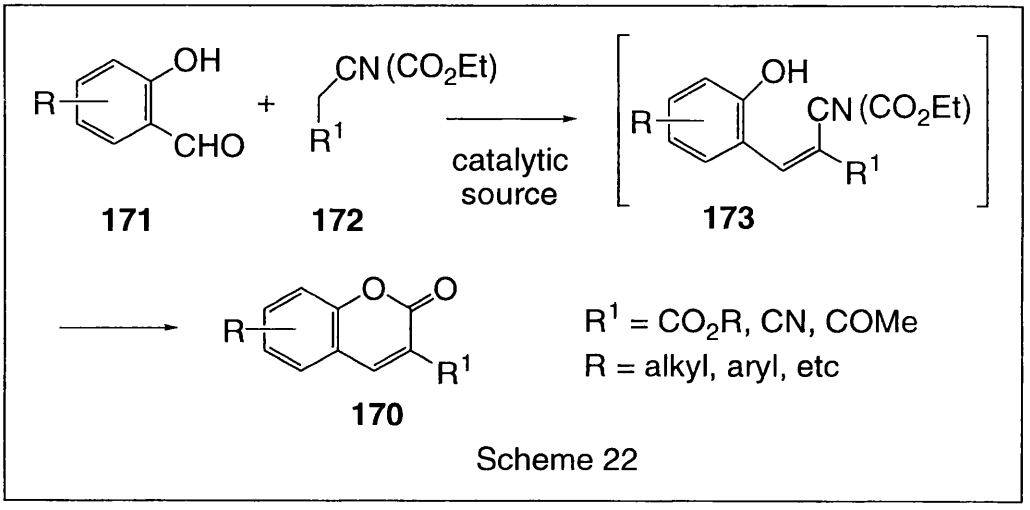


Scheme 21

This synthesis is limited to those coumarins which possess no substitution in the pyrone ring. Indeed the need for structurally different coumarins for both chemical and biological applications is reflected in the ongoing investigations into synthetic approaches to these compounds.

Substitution in the C-7 position is important to this family of compounds (see section 5.4.2.1) and Wittig[230] and Pechmann[231] chemistry in particular find common applications in the preparation of these coumarins. In order to prepare 3- and 4-substituted coumarins the requisite substituents should be in place before construction of the pyrone ring. Accordingly the Reformatsky reaction[232] is often used for preparing such coumarins with substituents positioned on carbon-4 of the coumarin pyrone ring. However, as we designed 3-substituted coumarins in our research programme our synthetic interest lies

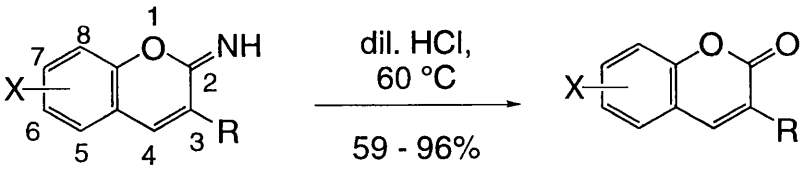
more towards the preparation of these compounds. The majority of syntheses reported for such compounds (**170**) can be generalised as Knoevenagel-based one pot procedures. These tend to employ substituted salicylaldehyde **171** and active methylene compounds **172** derived from malononitrile under catalytic conditions of homo- or hetero-geneity (see scheme 22).[232,233] In fact these syntheses where the intermediate compound **173** is not isolated are almost identical to those outlined in section 4.3.1 for the Knoevenagel preparation of tyrphostins. The pressures of trying to attain environmentally and ecologically friendly reaction conditions for the preparation of these 3-coumarins have seen the use of inorganic solid support catalysts[233] and solid-phase syntheses[234] to mention two synthetic conditions applied.



Scheme 22

5.4.3 SYNTHESIS OF 3-COUMARIN TARGET COMPOUNDS

The 3-substituted coumarins **161** were prepared in a facile acid hydrolysis reaction which employed the freshly-prepared 2-iminolactones **135** (see section 5.2.3) as starting materials (scheme 23). On warming the aqueous solution of dilute hydrochloric acid and 2-iminolactone **135**, the corresponding lactone **161** was quickly afforded. Unlike the 2-iminolactone starting materials, the products **174** to **182** provided no handling difficulties and the 3-coumarins were recrystallised and obtained in high yields (mean value 84%).



135 **161**

Substrate	X	R	Product	Yield
136	H	CN	**174**	86%
137	H	$CONH_2$	**175**	89%
142	8-OMe	CN	**176**	89%
143	8-OMe	$CONH_2$	**177**	59%
144	7-OMe	CN	**178**	91%
49	6-OMe	CN	**179**	66%
145	6-OMe	$CONH_2$	**180**	96%
146	5-OMe	CN	**181**	96%
147	5-OMe	$CONH_2$	**182**	82%

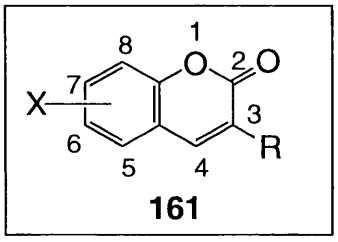
Scheme 23

The structural similarity between the iminolactones and lactones was most obvious from comparing the compounds' NMR spectra where the only difference observed was the absence of the imine proton signal at *ca.* δ 8.3 on the 3-coumarins' ^1H NMR spectra. IR spectroscopy also supported the success of the iminolactone hydrolysis with the NH stretching bands of the starting material common to the 3400-3200 cm^{-1} region being replaced by the carbonyl absorption at *ca.* 1700 cm^{-1} region on the IR spectra of the coumarins. This observation was more prominent for the 3-cyanonitrile coumarins as the corresponding amide compounds' carbonyl stretch masked the new lactone IR band. Further data of interest were the melting points of the 3-coumarins which were found to be consistently higher than those of the corresponding 2-iminolactones. The coumarin melting points were generally greater than 220 °C compared to *ca.* 140 to 180 °C for the 2-iminolactones.

5.4.4 BIOLOGICAL EVALUATION OF 3-COUMARIN TARGET COMPOUNDS

5.4.4.1 Results

The 3-coumarins, the final set of target compounds, were assessed for their inhibitory potential in the JNK MAPK assay using the same *in vitro* conditions employed to investigate the 2-iminolactones and tyrphostins. In an identical manner as before the autoradiography results obtained are represented in tabular form (table 16).



161

Tables 16 JNK MAPK inhibitory assay results for coumarins **161**

X	R	Cmpd	Cmpd conc. (μM) 5	50	500
H	CN	**174**	⬬	⬬	⬬
H	$CONH_2$	**175**	⬬	⬬	⬬
8-OMe	CN	**176**	⬬	⬬	⬬
8-OMe	$CONH_2$	**177**	⬬	⬬	⬬
7-OMe	CN	**178**	⬬	⬬	⬬
6-OMe	CN	**179**	⬬	⬬	⬬
6-OMe	$CONH_2$	**180**	⬬	⬬	⬬
5-OMe	CN	**181**	⬬	⬬	⬬
5-OMe	$CONH_2$	**182**	⬬	⬬	⬬
Experiment control			⬬	⬬	⬬

⬭ clearly inhibitory activity ⬬ modest inhibitory activity
⬬ no inhibitory activity

At the 5, 50 and 500 μM compound concentrations none of the lactones were found to exhibit inhibitory activity in the assay. Therefore we would

conclude that the 3-substituted coumarins did not provide candidates for further investigations into their JNK MAPK inhibitory activity.

5.5 OVERALL RESULTS FROM BIOLOGICAL EVALUATION OF TARGET COMPOUNDS

5.5.1 CORRELATION OF RESULTS

The results from this investigative inhibitory assay allowed us to conclude that the 2-iminolactones provided the most number of inhibitory compounds while the structurally very similar 3-substituted coumarins did not afford inhibitory compounds. However, the tyrphostins which were designed as analogues of the iminolactones provided one potent inhibitory compound and a very simplistic structural relationship was tentatively assigned between the 2-iminolactones and their open chain derivatives. This structural relationship was identified for three of the potent inhibitory iminolactones **136**, **49** and **146** which were 3-cyanonitrile iminolactones featuring no substitution, 6-methoxy and 5-methoxy substitution patterns on the benzene ring of these bicyclic compounds. Of the tyrphostins modelled on these three iminolactones it was the cyanoacetamide derivatives **151**, **157** and **159** which were found to possess inhibitory activity.

5.5.2 CONCLUSIONS

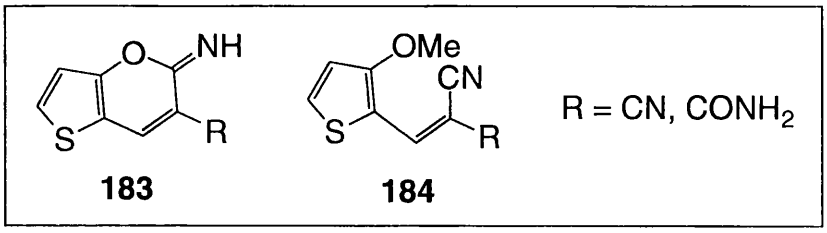
As mentioned in section 5.2.4 the *in vitro* biological evaluation of the target compounds was designed as a screen to identify candidates which showed significant MAP kinase inhibitory activity. Dr Gillespie proposed to investigate the active compounds in a more detailed study. From the results shown in sections 5.2.4, 5.3.3 and 5.4.4 the compounds for further evaluation would be those 2-iminolactones and tyrphostins found to exhibit JNK MAPK inhibitory activity at 500 μM concentration.

5.6 THIOPHENE ISOSTERE TARGET COMPOUNDS

5.6.1 SYNTHETIC PROPOSAL

Following the results from the biological evaluation of our target compounds where the most active compounds were electron rich aromatic derivatives, an extension of the original research programme was undertaken.

This design strategy involved the preparation of thiophene isosteric replacements of the 2-iminolactones and tyrphostins which were found to exhibit MAP kinase inhibitory activity (see section 5.5.2). The target compounds **183** and **184** featured 2,3-ring substitution patterns on the bicyclic iminolactones **183** and their open chain derivatives **184**. This approach where electron-rich heterocyclic compounds are prepared as analogues of aromatic compounds to investigate structure-activity relationships is an approach commonly employed in medicinal chemistry research.

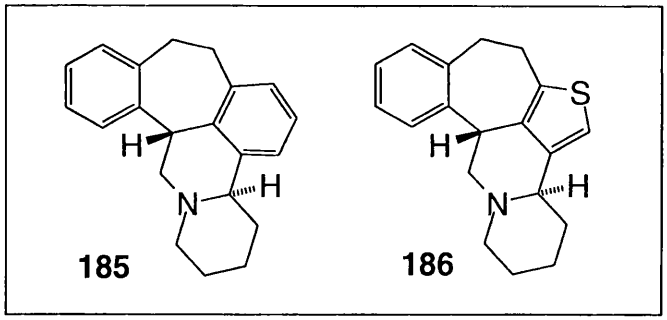


183 **184**

5.6.2 SOME BIOLOGICALLY ACTIVE THIOPHENE SYSTEMS

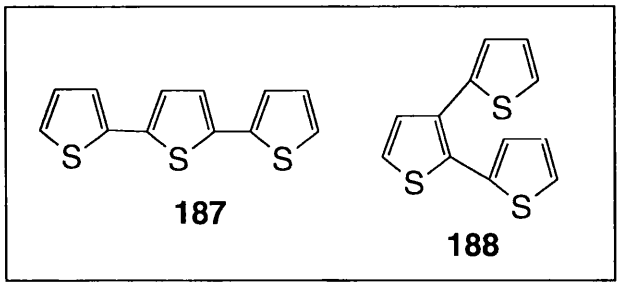
Thiophene derivatives are pharmacologically active compounds with applications which include central nervous system (CNS) and cardiovascular drugs. They are also employed in metabolic and infectious disease therapies. When we consider that infectious diseases include bacterial, fungal and viral infections the scope of thiophene derivatives in biological applications is very wide indeed.[235]

The use of thiophenes in biologically active compounds can be illustrated with the following examples. Consider taclamine **185** and QM-7184 **186**, both pyschotropic agents.[236] While taclamine is used to treat anxiety its thiophene isostere QM-7184 provides drug therapy for schizophrenia.[236] This demonstrates the change in pharmacological activity produced on preparing the simple thiophene isostere of compound **185**.



185 **186**

Quite separate from the use of thiophene as a benzene isostere in drug design is the importance of the substitution patterns on the thiophene ring of active compounds. This can be exemplified with two terthienyl isomers **187** and **188** used in infectious disease therapy. The α-terthienyl **187** exhibits antifungal and nematicidal behaviour while isomer **188** is a cytotoxic agent.[235,237]



187 **188**

5.6.3 THIOPHENE TYRPHOSTIN TARGET COMPOUNDS

5.6.3.1 3-Methoxythiophene

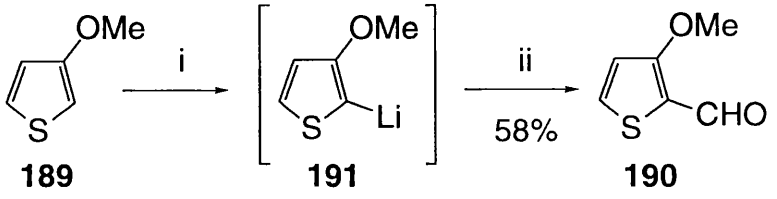
The two thiophene tyrphostin target compounds were prepared before the iminolactones. The differences and resultant difficulties in the syntheses of these thiophene tyrphostins compared to their benzene counterparts became apparent immediately. The synthesis of the benzene-based tyrphostins described in section 5.3.2.1 used cheap, commercially-available anisaldehyde as starting material in the Knoevenagel condensation reaction. However, for the thiophene isosteric compounds the corresponding starting material possessing the aldehyde and methoxy groups in adjacent positions was not available. Therefore, the 2,3-disubstituted thiophene had to be synthesised.

The preparation and reactivity of 3-methoxythiophene systems have been reported in the literature[238,239] and it is possible to prepare 3-methoxythiophene from 3-bromothiophene in a cupric oxide-catalysed reaction with sodium methylate in methanol.[239] However on consideration of the economics we preferred to employ commercially available 3-methoxythiophene in our synthesis.

5.6.3.2 Formylation of 3-methoxythiophene

The key to the carbon-2 formylation of 3-methoxythiophene **189** is the metalation of this compound or more specifically *ortho*-lithiation.[238,239] The

metalation of 3-methoxythiophene **189** with *n*-butyllithium was found to occur exclusively at position 2 (scheme 24). Aldehyde **190** formation in the 2-position substantiated the formation of the 2-thienyllithium species **191** which was treated with *N,N*-dimethylformamide at 0 °C to afford the final product **190**. On warming the reaction mixture slowly to room temperature and quenching with water, the mixture was worked up in a standard manner. Following purification by column chromatography, the recrystallised product was obtained in 58% yield.



189 **191** **190**

i *n*BuLi (1.6 M, 1 eq.), rt; reflux
ii DMF (1 eq.), 0 °C; 0 °C → rt

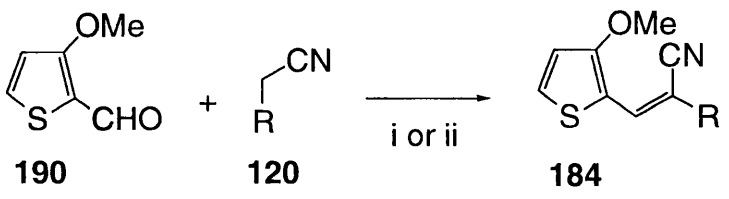
Scheme 24

The combustion data confirmed the elemental composition of the formylated 3-methoxythiophene **190** and high resolution mass spectroscopy identified the compound's accurate mass. However, it was NMR spectroscopy that provided the conclusive evidence for the aldehyde group introduction into the 2-position. The ^{1}H NMR spectrum confirmed the presence of two thiophene ring protons in the downfield region (δ 6.8-7.6) in the form of two doublets and the large *J* value of 5.2 Hz was consistent with *ortho* coupled protons. The ^{13}C NMR spectrum confirmed two aromatic CH carbons (at δ 116.2 and 135.6) together with the deshielded aldehyde carbon at δ 181.7. IR spectroscopy further confirmed the successful formylation with a strong absorption at 1643 cm^{-1} characteristic of an aryl carbonyl stretch.

The metalation of 3-*t*-butoxythiophenes has also been reported in the literature[240] and the *o*-lithiations of these compounds along with their 3-methoxy counterparts we have dealt with demonstrates a very powerful method for selectively introducing 2-position substituents such as acetyl, carbethoxy and alkyl groups to 3-alkoxythiophene compounds. In fact the important role played by the methoxy group in the thiophene 3-position is reflected in the metalation of 3-methylthiophene where lithiation is directed to the 5-position,[238] a site electronically favoured by the sulfur and the least hindered position.

5.6.3.3 Synthesis of thiophene tyrphostin target compounds

Preparation of the thiophene compounds **184** was not as straightforward as the previous syntheses of tyrphostins (see section 4.3.2 and 5.3.2). The reaction conditions employed for the compounds **192** and **193** were different (see scheme 25).



190 **120** **184**

i EtOH, rt, neat piperidine (cat.)
ii EtOH, reflux

R	Product	Conditions	Yield
$CONH_2$	**192**	i	72%
CN	**193**	ii	68%

Scheme 25

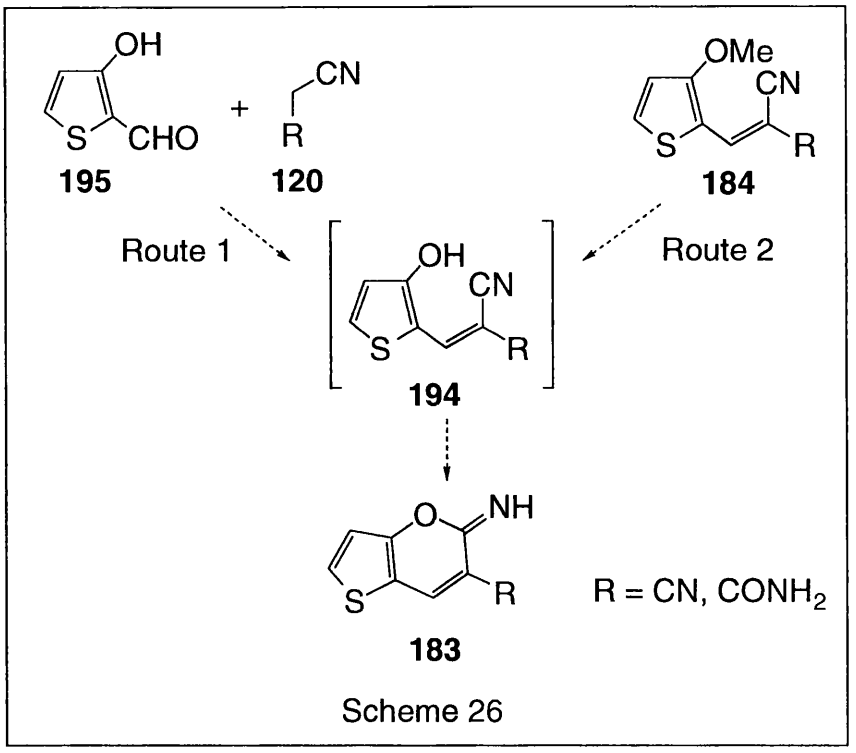
The thiophene compounds **184** were the condensation products of the freshly prepared 2-formyl-3-methoxythiophene **190** (section 5.6.3.2) and active methylene compound **120** (malononitrile or 2-cyanoacetamide) (see scheme 25). The synthesis of **192** which employed 2-cyanoacetamide proceeded smoothly under Knoevenagel reaction conditions of neat piperidine catalysis and ethanol at room temperature. The product **192** was recrystallised from isopropanol to provide the target compound in 72% yield. However, the second thiophene tyrphostin **193** was not as easy to prepare. Standard piperidine-catalysed Knoevenagel reaction conditions were not conducive to obtaining the product in an acceptable yield and therefore different reaction conditions where the thiophene aldehyde and malononitrile were condensed under reflux conditions were employed. These catalyst-free reaction conditions were reported by Henrio and co-workers[241] who performed the condensation reaction with 2-formyl-3-*t*-butoxythiophene and malononitrile. The recrystallised product **193** was obtained in 68% yield and the yellow needles were found to be particularly light sensitive requiring storage in the dark.

The success of the syntheses of the two tyrphostins **192** and **193** was most obvious from their NMR spectra. The ^{1}H NMR spectra identified the products from the olefinic proton signal at *ca.* δ 8.1 and the absence of the aldehyde proton signal at δ 9.91 of the starting material. Similarly on the ^{13}C NMR spectra the aldehyde's carbon signal which resonated downfield at δ 181.7 was absent and replaced with the nitrile carbon signal at *ca.* δ 144 for both **192** and **193** and the carbonyl carbon at δ 164.6 for compound **192**. NMR spectroscopy confirmed the synthesis of a single geometric isomer for the amide **192** which we assigned *trans* stereochemistry on thermodynamically favoured grounds and in accordance with the stereoselective nature of Knoevenagel condensation products which was discussed in detail in sections 4.3.1.1 and 5.3.2.2. On the IR spectra the success of the reaction could also be observed with the presence of a characteristic nitrile stretch at *ca.* 2200 cm^{-1} for both condensation products as well as the carbonyl vibrational band at 1704 cm^{-1} for the amide **192**.

5.6.4 THIOPHENE IMINOLACTONE TARGET COMPOUNDS

5.6.4.1 Two synthetic routes proposed

Following the successful synthesis of the thiophene tyrphostin target compounds in section 5.6.3.3, the corresponding iminolactones **183** were to be prepared. We designed and investigated two different synthetic routes towards this target (scheme 26). It was anticipated that both routes would proceed via the 2-hydroxy open chain compound **194** which would spontaneously cyclise to form the bicyclic iminolactone compound **183** as their benzene counterparts were found to do in section 5.2.



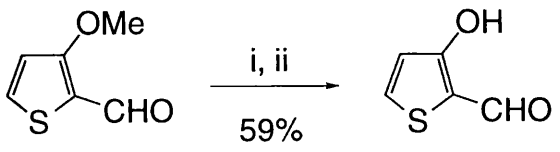
Scheme 26

However, the thiophene iminolactone compounds **183** were not formed. The attempted syntheses of these target compounds by routes 1 and 2 will now be presented in turn.

5.6.4.2 Attempted synthesis of iminolactones using route 1

5.6.4.2.1 Synthesis of 2-formyl-3-hydroxythiophene

The 3-hydroxy-2-formyl thiophene **195** required as starting material in the first proposed synthetic route (route 1, scheme 26) was prepared from the corresponding 3-methoxy compound **190.** This thienyl methyl ether deprotection allowed us to apply synthetic experience gained from an analogous procedure performed on a series of aryl methyl ether compounds during the antimelanoma research programme of work (section 4.1.2). As we had done previously, the Lewis acid boron tribromide was employed to facilitate this deprotection. This mild reaction was performed under conditions of low temperature and the methyl ether protection group was readily removed to afford the desired compound (scheme 27).



190 **195**

i BBr_3 (1 M in DCM, 3 eq.), -78 °C
ii -78 °C → rt

Scheme 27

Purification provided the 3-hydroxythiophene compound **195** in a good yield (59%) with a sharp melting point and the spectroscopic data were found to be in agreement with the selected data reported in the literature. IR spectroscopy confirmed the presence of the hydroxyl group with a broad band in the region of 3100-2593 cm^{-1} which we believed to be indicative of intramolecular hydrogen bonding. The relatively low value for the carbonyl absorption of 1613 cm^{-1} provided further evidence suggesting the existence of this interaction between the thiophene 2- and 3-ring substituents. NMR spectroscopy confirmed the successful deprotection through the absence of the methyl ether signal at δ 3.92 and δ 59.2 on the respective ^1H and ^{13}C NMR spectra.

5.6.4.2.2 Attempted synthesis of iminolactone

The first route designed to obtain the iminolactone compounds involved condensing 2-formyl-3-hydroxythiophene **195** and the appropriate malononitrile derivative **120** (malononitrile or 2-cyanoacetamide). However, various reaction conditions were investigated and applied but without success. The standard Knoevenagel condensation reaction conditions which we had successfully employed in section 4.3.2 and 5.3.2 were used with the catalytic source of piperidine (neat and diluted in ethanol) as well as aqueous potassium hydroxide. However under room temperature and refluxing reaction conditions neither of these bases was successful in catalysing the reaction. Furthermore the catalyst-free conditions used to condense 3-formyl-2-methoxythiophene and 2-cyanoacetamide in section 5.6.3.3 proved to be equally unsuccessful.

Naturally this synthetic outcome was disappointing but surprising too as we assumed that the 2-formyl-3-hydroxythiophene **195** would be a reactive species with respect to the aldehyde and hydroxyl groups enabling iminolactone

synthesis with ease. However, on reflection, perhaps the intramolecular hydrogen-bonded character of this compound may have reduced the reactivity of the thiophene and this may have adversely affected the outcome of the reaction.

5.6.4.3 Attempted synthesis of iminolactones using route 2

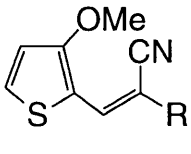
The second route designed to synthesise the thiophene iminolactone target compounds like the first route featured a thienyl methyl ether deprotection reaction. In fact within these methoxythiophene tyrphostins **184** the olefinic bond holds the cyano group in close proximity to the methoxy group such that it was anticipated that on demethylation the unmasked hydroxyl group would cyclise on to the nitrile carbon providing the bicyclic target compound **183** (see scheme 26, section 5.6.4.1).

We investigated boron tribromide and pyridinium hydrochloride as demethylating reagents to mediate the reaction in question but to no avail. Following numerous attempts, our synthesis of the thiophene iminolactone target compounds **183** by either condensing the hydroxythiophene and malononitrile derivative or in a demethylative ring closure reaction of the appropriate 2-methoxythiophene tyrphostin proved to be unsuccessful. As a result we could only supply the two methoxythiophene tyrphostin compounds **192** and **193** for biological evaluation by Dr David Gillespie.

5.6.5 BIOLOGICAL EVALUATION OF THIOPHENE TYRPHOSTIN TARGET COMPOUNDS

5.6.5.1 Results

The thiophene tyrphostins **184** were biologically evaluated for JNK MAPK inhibitory activity and the *in vitro* assay conditions employed are detailed in section 5.2.4. The autoradiography results are presented in table 17.



184

Table 17 JNK MAPK inhibitory assay results for tyrphostins **184**

R	Cmpd	Cmpd conc. (μM) 5	50	500
$CONH_2$	**192**	●	◐	○
CN	**193**	●	◐	○
Experiment control		●	●	●

○ clearly inhibitory activity ◐ modest inhibitory activity
● no inhibitory activity

From the results it is obvious that both the thiophene tyrphostins **192** and **193** showed some inhibitory activity at 50 μM concentration and complete inhibition at 500 μM.

5.6.5.2 Conclusions

The inhibitory activity shown by the thiophene tyrphostins would appear to support the strategy behind their design (see section 5.6.1). The thiophene compounds were modelled on the corresponding electron rich benzene-based compounds found to possess inhibitory activity in sections 5.2.4, 5.3.3 and 5.4.4. We therefore propose future investigations could continue this use of electron rich heterocyclic compounds to prepare more isosteres of the active compounds e.g. using pyrroles and furans.

Following the successful biological evaluation of all the target compounds prepared, those possessing JNK MAPK inhibitory behaviour have been identified. As mentioned earlier in section 5.2.4 we now await a more detailed study involving these inhibitory compounds.

CHAPTER 6

EXPERIMENTAL

6.1GENERAL

Reagents were purchased from Aldrich Chemical Company (Gillingham, UK) or Lancaster Synthesis (UK) and were used without further purification. Organic solvents were obtained from Rhône-Poulenc Rorer and were dried, as necessary, using the procedures described in Leonard, Lygo and Procter.[242] Melting points were determined in open capillaries using Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM200-SY spectrometer operating at 200 MHz and 50 MHz respectively, or where stated, on a Bruker DPX/400 spectrometer operating at 400 MHz and 100 MHz respectively. ^{13}C NMR spectra were assigned with the aid of Distortionless Enhancement by Polarisation Transfer (DEPT)-edited spectra and ^{1}H NMR coupling constants were calculated and reported in Hz. The numbering schemes shown are used for ease of assigning the NMR spectra and do not refer to the system of nomenclature. Mass spectra (MS) were recorded on a Jeol JMS700 high resolution mass spectrometer and percentage figures refer to relative intensity as a percentage of the base peak. MS were obtained using electron-impact ionisation (EI) mode or, where stated, chemical ionisation (CI) mode. Infrared (IR) spectra were recorded on a JASCO FT/IR 410 spectrometer. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-1601 spectrophotometer and combustion data were obtained from a Carlo-Erba 1106 elemental analyser. Retention factors (R_f) were obtained by analytical Thin Layer Chromatography (TLC) on Merck aluminium-backed silica plates of 0.25 mm thickness and chromatograms were visualised using UV conditions at 254 nm or using a variety of common stains prepared using the procedures described in Leonard, Lygo and Procter.[242] All column chromatography was carried out on silica gel (particle size 70-230 mesh).

6.2 EXPERIMENTAL FOR CHAPTER 4

6.2.1 GENERAL PREPARATIVE METHODS 1 AND 2

General method 1

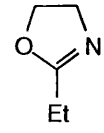
The method of Gazit and co-workers[213] was modified as follows. To a stirred solution of hydroxybenzaldehyde (1 equiv.) and malononitrile derivative (1 equiv.) in ethanol (*ca.* 0.5 mL/mmol), neat piperidine (a few drops) was added. The reaction mixture was heated at reflux until TLC indicated the reaction to be complete whereupon the mixture was cooled to room temperature. If precipitation of product was not observed dropwise addition of water was employed. The precipitated product was then filtered, dried under suction filtration and recrystallised from an appropriate solvent.

General method 2

General method 2 followed the same experimental protocol outlined for general method 1 with the exception that the reaction mixture was stirred at room temperature rather than heating under reflux.

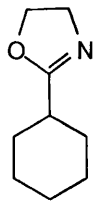
6.2.2 EXPERIMENTAL DATA

4,5-Dihydro-2-ethyloxazole **59**



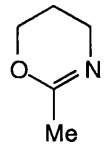
Compound **59** was prepared in 34% yield on a 36.3 mmol scale by the method of Witte and Seeliger[189] and gave bp 130-134 °C (760 mmHg) (lit.,[243] 124-126 °C/760 mmHg). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[134]

4,5-Dihydro-2-cyclohexyloxazole **60**



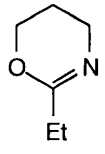
Compound **60** was prepared in 42% yield on a 45.8 mmol scale by the method of Witte and Seeliger[189] and gave bp 60-65 °C (1.5 mmHg) (lit.,[244] 125 °C/15 mmHg). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[134,245]

5,6-Dihydro-2-methyl-*4H*-1,3-oxazine **61**



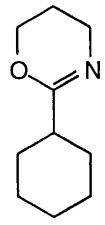
Compound **61** was prepared in 49% yield on a 48.7 mmol scale by the method of Witte and Seeliger[189] and gave bp 25-30 °C (1.5 mmHg) (lit.,[246] 132-133 °C/760 mmHg). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[246]

5,6-Dihydro-2-ethyl-*4H*-1,3-oxazine **62**



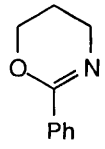
Compound **62** was prepared in 43% yield on a 36.3 mmol scale by the method of Witte and Seeliger[189] and gave bp 38-40 °C (1.5 mmHg) (lit.,[243] 70-71 °C/43 Torr). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[243]

5,6-Dihydro-2-cyclohexyl-*4H*-1,3-oxazine **63**



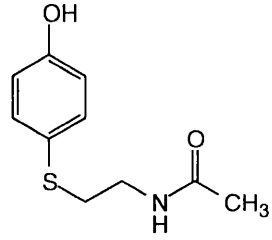
Compound **63** was prepared in 37% yield on a 23.1 mmol scale by the method of Witte and Seeliger[189] and gave bp 88-90 °C (1.5 mmHg) (lit.,[134] 74-76 °C/2 mmHg). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[134]

5,6-Dihydro-2-phenyl-*4H*-1,3-oxazine **64**



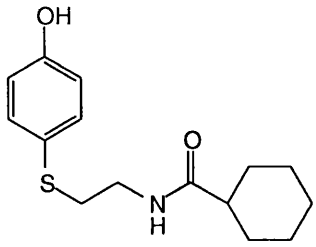
Compound **64** was prepared in 29% yield on a 38.8 mmol scale by the method of Witte and Seeliger[189] and gave bp 80-82 °C (2 mmHg) (lit.,[247] 115 °C/1.5 mmHg). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[248]

N-{2-[(4-Hydroxyphenyl)thio]ethyl}acetamide **35**



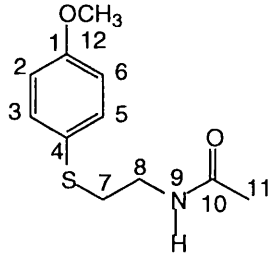
Compound **35** was prepared in 74% yield on a 48.2 mmol scale by the method of Padgette and co-workers[129] and gave mp 120-122 °C (EtOAc) (lit.,[129] 123-125 °C). The spectroscopic data were in agreement with the IR, high resolution mass spectroscopy and ^{1}H NMR data reported.[133]

N-{2-[(4-Hydroxyphenyl)thio]ethyl}cyclohexanecarboxamide **68**



Compound **68** was prepared in 59% yield on a 25.5 mmol scale by the method of Padgette and co-workers[129] and gave mp 92-93 °C (EtOAc) (lit.,[134] 94-95 °C). The spectroscopic data were in agreement with the IR, high resolution mass spectroscopy and ^{1}H NMR data reported.[134]

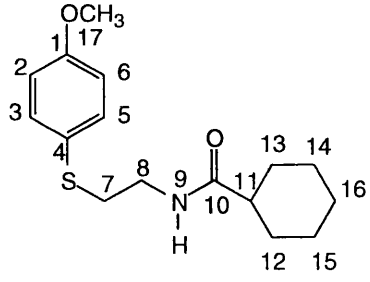
N-{2-[(4-Methoxyphenyl)thio]ethyl}acetamide **73**



The method of Padgette and co-workers[129] was used. A mixture of 2-methyl-2-oxazoline (1.38 mL, 1.38 g, 16.3 mmol) and 4-methoxybenzenethiol (2.00 mL, 2.28 g, 16.3 mmol) was heated at reflux neat for 3 d under N_2. The reaction was cooled to 0 °C whereupon scratching induced crystallisation. The product was purified by column chromatography, eluant ethyl acetate, and the title compound **73** (2.74 g, 12.2 mmol, 75%) was recrystallised from ethyl acetate to provide white needles, mp 100-101 °C (Found: C, 58.48; H, 6.74; N, 6.14. $C_{11}H_{15}NO_2S$ requires C, 58.67; H, 6.67; N, 6.22%); ν_{max} (KBr disc)/cm^{-1} 3288m (NH), 2951w and 2936w (CH), 1636s (CO), 1562m and 1496m (C=C), 1441m (CH), 1249m and 1030m (C-O) and 815m (CH); δ_H (400 MHz; $CDCl_3$) 1.87 (3 H, s, 11-H), 2.86 (2 H, t, *J* 6.4 Hz, 7-H), 3.32 (2 H, m, 8-H), 3.72 (3 H, s, 12-H), 5.88 (1 H, br. s, 9-H), 6.78 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 7.29 (2 H, AA'BB', d, *J* 8.8 Hz, 3,5-H); δ_C (100 MHz; $CDCl_3$) 23.6 (11-CH_3), 36.0 (7-CH_2), 38.9 (8-CH_2), 55.7 (12-CH_3), 115.2 (2,6-CH), 125.2 (4-quat. C), 134.0 (3,5-CH), 159.7 (1-quat. C) and 170.5 (10-CO); *m/z* (EI) 225.0823 ($M^{+\bullet}$.

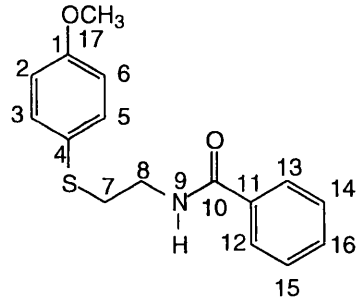
$C_{11}H_{15}NO_2S$ requires 225.0822); *m/z* (%) 225 (36.2), 166 (100), 139 (21.3), 135 (9.9), 109 (11.3), 86 (52.5), 77 (7.8) and 63 (5.0).

N-{2-[(4-Methoxyphenyl)thio]ethyl}cyclohexanecarboxamide **74**



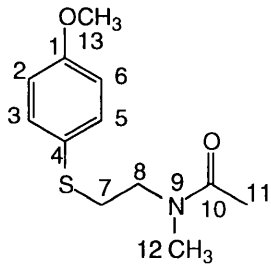
Compound **74** was prepared from oxazoline **60** (500 mg, 3.27 mmol) and 4-methoxybenzenethiol (0.40 mL, 458 mg, 3.27 mmol) by the method used to prepare compound **73**. Purification by column chromatography, eluant diethyl ether-hexane (2:1), and subsequent recrystallisation from ethyl acetate provided compound **74** (627 mg, 2.14 mmol, 65%) as pale tan crystals, mp 68-71 °C (Found: C, 65.28; H, 7.78; N, 4.69. $C_{16}H_{23}NO_2$ requires C, 65.53; H, 7.85; N, 4.78%); ν_{max} (KBr disc)/cm^{-1} 3313m (NH), 2927m and 2851m (CH), 1632s (CO), 1539m and 1493m (C=C), 1591w (NH), 1438w (CH), 1236m and 1027m (C-O) and 818m (CH); δ_H (400 MHz; $CDCl_3$) 1.21 (6 H, m, 14 to 16-H), 1.70 (4 H, m, 12,13-H), 1.95 (1 H, m, 11-H), 2.86 (2 H, t, *J* 6.2 Hz, 7-H), 3.32 (2 H, m, 8-H), 3.72 (3 H, s, 17-H), 5.85 (1 H, br. s, 9-H), 6.78 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 7.29 (2 H, AA'BB', d, *J* 8.8 Hz, 3,5-H); δ_C (100 MHz; $CDCl_3$) 26.1 (14 to 16-CH_2), 30.0 (12,13-CH_2), 36.1 (7-CH_2), 38.7 (8-CH_2), 45.8 (11-CH), 55.7 (17-CH_3), 115.2 (2,6-CH), 125.3 (4-quat. C), 134.0 (3,5-CH), 159.7 (1-quat. C) and 176.5 (10-CO); *m/z* (EI) 293.1447 ($M^{+\bullet}$. $C_{16}H_{23}NO_2S$ requires 293.1444); *m/z* (%) 293 (19.5), 166 (100), 154 (36.9), 139 (17.7), 83 (17.7), 77 (31.9) and 55 (18.1).

N-{2-[(4-Methoxyphenyl)thio]ethyl}benzamide **75**



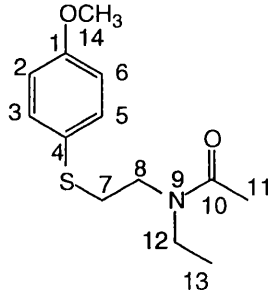
Compound **75** was prepared from 2-phenyl-2-oxazoline (0.54 mL, 600 mg, 4.08 mmol) and 4-methoxybenzenethiol (0.50 mL, 572 mg, 4.08 mmol) by the method used to prepare compound **73**. Purification by column chromatography, eluant diethyl ether-hexane (2:1), and subsequent recrystallisation from ethyl acetate provided the title compound **75** (588 mg, 2.05 mmol, 50%) as a cream powder, mp 63-65 °C (Found: C, 66.77; H, 5.90; N, 4.73. $C_{16}H_{17}NO_2$ requires C, 66.90; H, 5.92; N, 4.88%); ν_{max} (KBr disc)/cm^{-1} 3315m (NH), 1644s (CO), 1541m and 1494m (C=C), 1454m (CH), 1243s and 1029m (C-O) and 810s, 695s and 676s (CH); δ_H (400 MHz; $CDCl_3$) 2.97 (2 H, t, *J* 6.3 Hz, 7-H), 3.53 (2 H, m, 8-H), 3.69 (3 H, s, 17-H), 6.57 (1 H, br. s, 9-H), 6.75 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H), 7.32 (4 H, m, 3,5-H and 14,15-H), 7.40 (1 H, m, 16-H) and 7.62 (2 H, m, 12,13-H); δ_C (100 MHz; $CDCl_3$) 35.9 (7-CH_2), 39.6 (8-CH_2), 55.7 (17-CH_3), 115.3 (2,6-CH), 125.2 (4-quat. C), 127.3 (14,15-CH), 128.9 (12,13-CH), 131.9 (16-CH), 134.1 (3,5-CH), 134.7 (11-quat. C), 159.7 (1-quat. C) and 167.8 (10-CO); *m/z* (EI) 287.0977 ($M^{+\bullet}$. $C_{16}H_{17}NO_2S$ requires 287.0974); *m/z* (%) 287 (25.4), 166 (100), 14.8 (34.6), 139 (9.2), 105 (55.8), 77 (46.3) and 51 (9.5).

N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-methylacetamide **78**



A modified version of the method of Orito and co-workers[192] was used. To a stirred suspension of sodium hydride (60% dispersion in mineral oil, 427 mg, 10.7 mmol) in tetrahydrofuran (20 mL), at rt, was added dropwise *N*-acetamide **73** (1.20 g, 5.33 mmol) in tetrahydrofuran (20 mL) followed by methyl iodide (1.16 mL, 2.65 g, 18.7 mmol). The reaction was slowly heated to 40 °C. After hydrogen gas evolution ceased the reaction was maintained at a gentle reflux for 24 h. On cooling to rt white precipitates of sodium iodide were removed by suction filtration and the reaction mixture concentrated *in vacuo*. The oily residue was dissolved in chloroform (30 mL), washed with 10% sodium thiosulfate solution (2 x 15 mL) and water (2 x 15 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo*. Purification by column chromatography, eluant ethyl acetate, provided the title compound **78** (1.16 g, 4.85 mmol, 91%) as a yellow oil. ν_{max} (thin film)/cm^{-1} 3002w and 2933w (CH), 1646s (CO), 1593m and 1495s (C=C), 1246s and 1030m (C-O) and 828m (CH); δ_H (400 MHz; $CDCl_3$) [1.95 (1.2 H, s) and 2.02 (1.8 H, s) 11-H], [2.85 (1.2 H, s) and 2.98 (1.8 H, s) 12-H], [2.94 (0.8 H, t, *J* 7.4 Hz) and 3.01 (1.2 H, t, *J* 7.0 Hz) 7-H], [3.42 (0.8 H, t, *J* 7.6 Hz) and 3.51 (1.2 H, t, *J* 7.2 Hz) 8-H], [3.78 (1.2 H, s) and 3.80 (1.8 H, s) 13-H], 6.86 (2 H, m, 2,6-H) and 7.37 (2 H, m, 3,5-H); δ_C (100 MHz; $CDCl_3$) 21.5 and 22.2 (11-CH_3), 32.9 and 34.3 (7-CH_2), 33.5 and 37.6 (12-CH_3), 48.3 and 50.7 (8-CH_2), 55.7 and 55.7 (13-CH_3), 115.0 and 115.2 (2,6-CH), 125.2 and 126.2 (4-quat. C), 132.9 and 134.2 (3,5-CH), 159.2 and 159.8 (1-quat. C) and 170.7 and 171.0 (10-CO); *m/z* (EI) 239.0980 ($M^{+\bullet}$. $C_{12}H_{17}NO_2S$ requires 239.0980); *m/z* (%) 239 (19.3), 166 (100), 139 (15.0), 135 (8.9), 100 (85.7), 86 (5.7), 77 (5.0) and 58 (14.6).

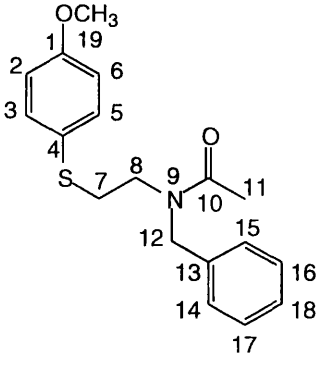
N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-ethylacetamide **79**



Compound **79** was prepared from compound **73** (1.80 g, 8.00 mmol), ethyl bromide (2.1 mL, 3.05 g, 28.0 mmol) and sodium hydride (60% dispersion in mineral oil, 576 mg, 14.4 mmol) by the method used to prepare compound

78. Purification by column chromatography, eluant ethyl acetate-hexane (4:1), provided title compound **79** (1.82 g, 7.21 mmol, 90%) as a yellow oil. ν_{max} (thin film)/cm^{-1} 2971w and 2933w (CH), 1639s (CO), 1593m and 1495s (C=C), 1459m and 1442m (CH), 1246m and 1030m (C-O) and 829m (CH); δ_H (400 MHz; $CDCl_3$) [0.98 (1.2 H, t, *J* 7.1 Hz) and 1.03 (1.8 H, t, *J* 7.2 Hz) 13-H], [1.84 (1.2 H, s) and 1.95 (1.8 H, s) 11-H], [2.84 (0.8 H, t, *J* 7.8 Hz) and 2.94 (1.2 H, t, *J* 7.4 Hz) 7-H], 3.23 (2 H, m, 12-H), [3.31 (0.8 H, t, *J* 7.8 Hz) and 3.38 (1.2 H, t, *J* 7.4 Hz) 8-H], [3.70 (1.8 H, s) and 3.71 (1.2 H, s) 14-H], 6.78 (2 H, m, 2,6-H) and 7.30 (2 H, m, 3,5-H); δ_C (100 MHz; $CDCl_3$) 13.4 and 14.5 (13-CH_3), 21.7 and 21.8 (11-CH_3), 32.9 and 34.8 (7-CH_2), 40.8 and 44.7 (12-CH_2), 46.2 and 48.4 (8-CH_2), 55.7 and 55.8 (14-CH_3), 115.0 and 115.2 (2,6-CH), 125.1 and 125.2 (4-quat. C), 132.7 and 134.4 (3,5-CH), 159.1 and 159.9 (1-quat. C) and 170.2 and 170.6 (10-CO); *m/z* (EI) 253.1134 ($M^{+\bullet}$. $C_{13}H_{19}NO_2S$ requires 253.1132); *m/z* (%) 253 (20.6), 166 (100), 151 (9.2), 139 (20.6), 114 (93.6), 100 (6.4), 72 (11.3) and 58 (87.2).

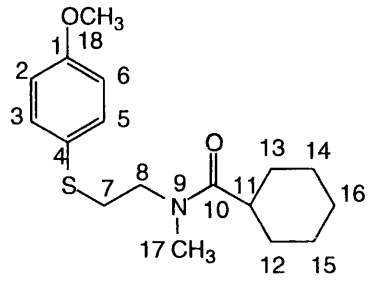
N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-benzylacetamide **80**



Compound **80** was prepared from compound **73** (225 mg, 1.00 mmol), benzyl bromide (0.42 mL, 599 mg, 3.50 mmol) and sodium hydride (60% dispersion in mineral oil, 72 mg, 1.80 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant diethyl ether, provided title compound **80** (165 mg, 0.52 mmol, 52%) as a cloudy yellow oil. ν_{max} (thin film)/cm^{-1} 2930w (CH), 1645s (CO), 1592w and 1494s (C=C), 1464m (CH), 1245s and 1029m (C-O) and 827m and 729m (CH); δ_H (400 MHz; $CDCl_3$) [1.94 (1.3 H, s) and 1.99 (1.7 H, s) 11-H], [2.74 (0.9 H, t, *J* 8.0 Hz) and 2.94 (1.1 H, t, *J* 7.2 Hz) 7-H], [3.26 (0.9 H, t, *J* 7.8 Hz) and 3.43 (1.1 H, t, *J* 7.4 Hz) 8-H], [3.70 (1.7 H, s) and 3.73 (1.3 H, s) 19-H], 4.43 (2 H, s, 12-H), 6.76 (2 H, m, 2,6-H), 7.05 (2 H, m, 3,5-H) and 7.24 (5 H, m, 14 to 18-H); δ_C (100 MHz;

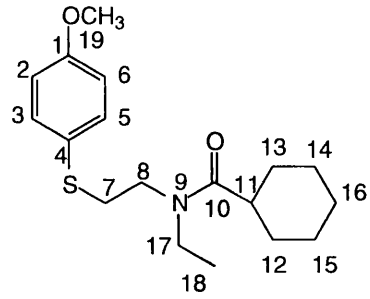
$CDCl_3$) 20.3 and 20.7 (11-CH_3), 31.4 and 32.9 (7-CH_2), 45.5 and 46.5 (8-CH_2), 47.3 and 52.3 (12-CH_2), 54.3 and 54.4 (19-CH_3), 113.7 and 113.8 (2,6-CH), 123.6 and 124.8 (4-quat. C), 126.4 and 126.7 (18-CH), 125.4 and 127.2 (16,17-CH), 127.6 and 127.9 (14,15-CH), 131.3 and 133.3 (3,5-CH), 135.7 and 136.5 (13-quat. C), 157.8 and 158.5 (1-quat. C) and 169.4 and 170.1 (10-CO); *m/z* (EI) 315.1296 ($M^{+\bullet}$. $C_{18}H_{21}NO_2S$ requires 315.1299); *m/z* (%) 315 (14.9), 272 (1.4), 176 (67.4), 166 (89.4), 139 (19.9), 120 (32.6), 91 (100) and 65 (6.4).

N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-methylcyclohexanecarboxamide **81**



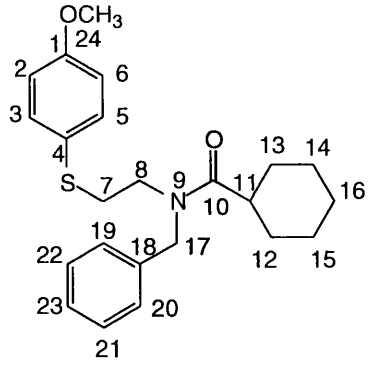
Compound **81** was prepared from compound **74** (610 mg, 2.08 mmol), methyl iodide (0.45 mL, 1.03 g, 7.29 mmol) and sodium hydride (60% dispersion in mineral oil, 167 mg, 4.16 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant ethyl acetate-hexane (1:1), provided title compound **81** (520 mg, 1.69 mmol, 81%) as a clear yellow oil. ν_{max} (thin film)/cm^{-1} 2929s and 2853m (CH), 1638s (CO), 1593m and 1495s (C=C), 1463m (CH), 1246s and 1031m (C-O) and 828m (CH); δ_H (400 MHz; $CDCl_3$) 0.90-1.64 (10 H, m, 12 to 16-H), [2.00 (0.4 H, tt, *J* 11.6 and 3.6 Hz) and 2.34 (0.6 H, tt, *J* 11.6 and 3.3 Hz) 11-H], [2.79 (1.4 H, s) and 2.95 (1.6 H, s) 17-H], [2.83 (0.9 H, t, *J* 7.6 Hz) and 2.91 (1.1 H, t, *J* 7.2 Hz) 8-H], [3.71 (1.9 H, s) and 3.73 (1.6 H, s) 18-H], [6.76 (1.1 H, AA'BB', d, *J* 8.8 Hz) and 6.81 (0.9 H, AA'BB', d, *J* 8.8 Hz) 2,6-H] and [7.29 (1.1 H, AA'BB', d, *J* 8.8 Hz) and 7.33 (1.1 H, AA'BB', d, *J* 8.8 Hz) 3,5-H]; δ_C (100 MHz; $CDCl_3$) 26.0 and 26.1 (14 to 16-CH_2), 29.4 and 29.9 (12,13-CH_2), 32.9 and 34.9 (7-CH_2), 33.9 and 36.1 (17-CH_3), 40.8 and 41.3 (11-CH), 48.7 and 49.5 (8-CH_2), 55.7 and 55.8 (18-CH_3), 115.1 and 115.2 (2,6-CH), 125.1 and 126.2 (4-quat. C), 133.0 and 134.6 (3,5-CH), 159.2 and 160.0 (1-quat. C) and 176.4 and 176.5 (10-CO); *m/z* (EI) 307.1605 ($M^{+\bullet}$. $C_{17}H_{25}NO_2S$ requires 307.1604); *m/z* (%) 307 (8.6), 166 (100), 139 (15.0), 111 (5.0), 83 (37.9) and 55 (16.4).

N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-ethylcyclohexanecarboxamide **82**



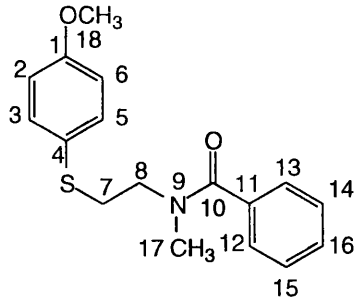
Compound **82** was prepared from compound **74** (1.80 g, 6.14 mmol), ethyl bromide (1.60 mL, 2.34 g, 21.5 mmol) and sodium hydride (60% dispersion in mineral oil, 442 mg, 11.1 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant diethyl ether-hexane (3:1), provided title compound **82** (524 mg, 1.63 mmol, 27%) as a yellow oil. ν_{max} (thin film)/cm^{-1} 2929s and 2854m (CH), 1638s (CO), 1593m and 1494s (C=C), 1450m and 1427m (CH), 1246s and 1031m (C-O) and 828m (CH); δ_H (400 MHz; $CDCl_3$) [0.97 (1.7 H, t, *J* 7.1 Hz) and 1.06 (1.3 H, t, *J* 7.1 Hz) 18-H], 1.07-1.70 (10 H, m, 12 to 16-H), [1.91 (0.4 H, tt, *J* 11.4 and 3.6 Hz) and 2.30 (0.6 H, tt, *J* 11.6 and 3.3 Hz) 11-H], [2.80 (0.9 H, t, *J* 8.0 Hz) and 2.91 (1.1 H, t, *J* 6.4 Hz) 7-H], 3.21-3.30 (2 H, m, 17-H), [3.21-3.30 (0.9 H, m) and 3.36 (1.1 H, t, *J* 7.4 Hz) 8-H], [3.71 (1.7 H, s) and 3.73 (1.3 H, s) 19-H], [6.77 (1.1 H, AA'BB', d, *J* 8.8 Hz) and 6.81 (0.9 H, AA'BB', d, *J* 8.4 Hz) 2,6-H] and [7.29 (1.1 H, AA'BB', d, *J* 8.8 Hz) and 7.34 (0.9 H, AA'BB', d, *J* 8.8 Hz) 3,5-H]; δ_C (100 MHz; $CDCl_3$) 13.5 and 15.3 (18-CH_3), 25.9 and 26.2 (14 to 16-CH_2), 29.9 and 30.0 (12,13-CH), 33.0 and 35.5 (7-CH_2), 41.2 (11-CH), 41.0 and 43.6 (17-CH_2), 46.4 and 47.2 (8-CH_2), 55.7 and 55.8 (19-CH_3), 115.3 and 115.2 (2,6-CH), 125.1 and 126.3 (4-quat. C), 132.7 and 134.8 (3,5-CH), 159.2 and 160.0 (1-quat. C) and 176.0 and 176.4 (10-CO); *m/z* (EI) 321.1765 ($M^{+\bullet}$. $C_{18}H_{27}NO_2S$ requires 321.1767); *m/z* (%) 321 (14.9), 293 (1.4), 182 (100), 166 (96.5), 139 (16.3), 111 (5.7), 83 (39.7) and 58 (29.8).

N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-benzylcyclohexanecarboxamide **83**



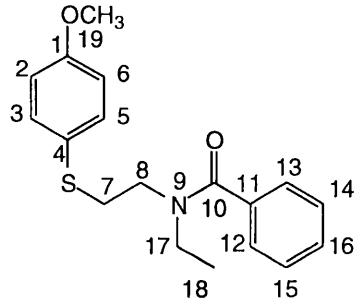
Compound **83** was prepared from compound **74** (300 mg, 1.02 mmol), benzyl bromide (0.43 mL, 613 mg, 3.57 mmol) and sodium hydride (60% dispersion in mineral oil, 74 mg, 1.85 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant hexane-diethyl ether (1:1), provided title compound **83** (149 mg, 0.39 mmol, 38%) as a pale yellow oil. ν_{max} (thin film)/cm^{-1} 2928s and 2854m (CH), 1641s (CO), 1593m and 1494s (C=C), 1450m and 1424m (CH), 1246s and 1030m (C-O) and 828s and 699s (CH); δ_H (400 MHz; $CDCl_3$) 0.97 (4 H, m, 12,13-H), 1.13 (6 H, m, 14 to 16-H), [2.02 (0.6 H, tt, *J* 11.2 and 3.5 Hz) and 2.35 (0.4 H, tt, *J* 11.6 and 3.3 Hz) 11-H], [2.72 (1.1 H, t, *J* 8.0 Hz) and 2.90 (0.9 H, t, *J* 7.3 Hz) 7-H], [3.23 (1.1 H, t, *J* 8.0 Hz) and 3.39 (0.9 H, t, *J* 7.3 Hz) 8-H], [3.68 (1.4 H, s) and 3.69 (1.6 H, s) 24-H], [4.44 (1.1 H, s) and 4.47 (0.9 H, s) 17-H], [6.73 (0.9 H, AA'BB', d, *J* 8.8 Hz) and 6.78 (1.1 H, AA'BB', d, *J* 8.8 Hz) 2,6-H], 7.01 (2 H, m, 3,5-H) and 7.20 (5 H, 19 to 23-H); δ_C (100 MHz; $CDCl_3$) 24.6 and 24.7 (14 to 16-CH_2), 28.5 and 28.6 (12,13-CH_2), 31.4 and 33.7 (7-CH_2), 39.6 and 39.9 (11-CH), 45.4 and 45.5 (8-CH_2), 47.3 and 51.1 (17-CH_2), 54.3 and 54.3 (24-CH_3), 113.7 and 113.9 (2,6-CH), 123.6 and 124.8 (4-quat. C), 125.4 and 126.9 (21,22-CH), 126.3 and 126.6 (23-CH), 127.5 and 127.8 (19,20-CH), 133.4 and 136.2 (3,5-CH), 136.2 and 137.1 (18-quat. C), 157.7 and 158.6 (1-quat. C) and 175.2 and 175.7 (10-CO); *m/z* (EI) 383.1917 ($M^{+\bullet}$. $C_{23}H_{29}NO_2S$ requires 383.1914); *m/z* (EI) (%) 383 (12.8), 272 (2.5), 244 (81.6), 230 (4.3), 217 (2.1), 166 (83.7), 120 (26.9), 83 (100) and 55 (20.6).

N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-methylbenzamide **84**



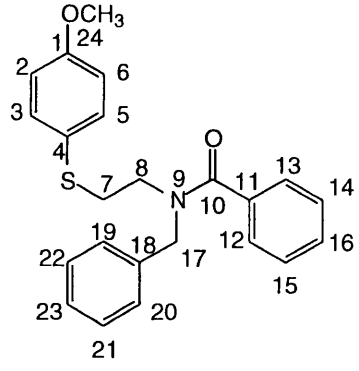
Compound **84** was prepared from compound **75** (660 mg, 2.30 mmol), methyl iodide (0.57 mL, 1.31 g, 9.20 mmol) and sodium hydride (60% dispersion in mineral oil, 166 mg, 4.15 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant diethyl ether-hexane (4:1), provided title compound **84** (475 mg, 1.58 mmol, 68%) as a yellow oil. ν_{max} (thin film)/cm^{-1} 2929w and 2836w (CH), 1634s (CO), 1593m and 1494s (C=C), 1444m (CH), 1246s and 1028m (C-O) and 827m and 712m (CH); δ_H (400 MHz; $CDCl_3$) [2.78 (0.9 H, br. t) and 3.07 (1.1 H, br. t) 7-H], [2.88 (1.7 H, br. s) and 2.95 (1.3 H, br. s) 17-H], [3.33 (0.9 H, br. t) and 3.61 (1.1 H, br. t) 8-H], 3.70 (3 H, s, 18-H), [6.63 (0.9 H, AA'BB', br. d) and 6.78 (1.1 H, AA'BB', br. d, AA'BB') 2,6-H], [7.01 (0.9 H, AA'BB', br. d) and 7.19-7.36 (1.1 H, AA'BB', br. d) 3,5-H], 7.21-7.36 (5 H, br. m, 12 to 16-H); δ_C (100 MHz; $CDCl_3$) 31.5 and 32.6 (7-CH_2), 32.1 and 37.6 (17-CH_3), 46.6 and 49.9 (8-CH_2), 54.3 (18-CH_3), 113.7 and 113.9 (2,6-CH), 123.5 and 124.5 (4-quat. C), 125.5 and 126.0 (14,15-CH), 127.3 and 127.4 (12,13-CH), 128.3 and 128.6 (16-CH), 131.9 and 132.3 (3,5-CH), 135.2 (11-quat. C), 158.0 and 158.1 (1-quat. C) and 170.5 and 170.9 (10-CO); *m/z* (EI) 301.1138 ($M^{+\bullet}$. $C_{17}H_{19}NO_2S$ requires 301.1139); *m/z* (%) 301 (23.9), 166 (100), 162 (74.3), 139 (9.3), 105 (99.3), 77 (49.6) and 51 (6.4).

N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-ethylbenzamide **85**



Compound **85** was prepared from compound **75** (287 mg, 1.00 mmol) ethyl bromide (0.26 mL, 381 mg, 3.50 mmol), and sodium hydride (60% dispersion in mineral oil, 72 mg, 1.80 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant hexane-ethyl acetate (3:2), provided title compound **85** (164 mg, 0.52 mmol, 52%) as a yellow oil. ν_{max} (thin film)/cm^{-1} 2969w and 2935w (CH), 1633s (CO), 1593m and 1494m (C=C), 1464m and 1443m (CH), 1246s and 1029m (C-O) and 828m and 707m (CH); δ_H (400 MHz; $CDCl_3$) [0.95 (1.8 H, br. t) and 1.11 (1.2 H, br. t) 18-H], [2.75 (0.8 H, br. t) and 3.09 (1.2 H, br. t) 7-H], [3.19 (1.2 H, br. q) and 3.29 (0.8 H, br. q) 17-H], [3.42 (0.8 H, br. t) and 3.55 (1.2 H, br. t) 8-H], 3.69 (3 H, s, 19-H), [6.62 (0.8 H, AA'BB', br. d) and 6.78 (1.2 H, AA'BB', br. d) 2,6-H], [6.99 (0.8 H, AA'BB', br. d) and 7.19-7.34 (1.2 H, AA'BB', br. d) 3,5-H] and 7.19-7.34 (4 H, br. m, 12 to 16-H); δ_C (100 MHz; $CDCl_3$) 12.0 and 13.2 (18-CH_3), 31.5 and 32.8 (7-CH_2), 39.1 and 47.5 (17-CH_2), 43.8 and 43.9 (8-CH_2), 54.3 and 54.5 (19-CH_3), 113.7 and 113.9 (2,6-CH), 125.3 (14,15-CH), 127.4 (12,13-CH), 128.1 (4-quat.C), 128.3 (16-CH), 131.5 and 132.3 (3,5-CH), 135.6 (11-quat. C), 157.9 (1-quat. C) and 170.8 (10-CO); *m/z* (EI) 315.1291 ($M^{+\bullet}$. $C_{18}H_{21}NO_2S$ requires 315.1289); *m/z* (%) 315 (16.3), 176 (66.7), 166 (100), 139 (8.5), 105 (100), 77 (39.7) and 51 (4.3).

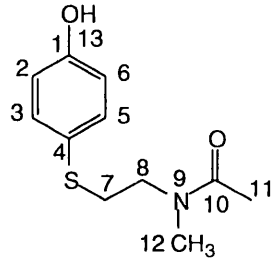
N-{2-[(4-Methoxyphenyl)thio]ethyl}*N*-benzylbenzamide **86**



Compound **86** was prepared from compound **75** (1.0 g, 3.48 mmol), benzyl bromide (1.66 mL, 2.38 g, 13.9 mmol) and sodium hydride (60% dispersion in mineral oil, 251 mg, 6.28 mmol) by the method used to prepare compound **78**. Purification by column chromatography, eluant diethyl ether-hexane (2:1), provided title compound **86** (541 mg, 1.44 mmol, 41%) as a pale yellow oil. ν_{max} (thin film)/cm^{-1} 2938w and 2835w (CH), 1633s (CO), 1494s and 1593m (C=C), 1420m (CH), 1247s and 1029s (C-O) and 827m and 700s

(CH); δ_H (400 MHz; $CDCl_3$) [2.67 (0.9 H, br. t) and 3.05 (1.1 H, br. t) 7-H], [3.25 (0.9 H, br. t) and 3.52 (1.1 H, br. t) 8-H], 3.70 (3 H, s, 24-H), [4.45 (1.1 H, br. s) and 4.63 (0.9 H, br. s) 17-H], [6.62 (0.9 H, AA'BB', br. d) and 6.74 (1.1 H, AA'BB', br. d) 2,6-H], [6.99 (1.1 H, AA'BB', br. d) and 7.19-7.31 (0.9 H, AA'BB', br. d) 3,5-H] and 7.19-7.31 (10 H, br. m, 12 to 16-H and 19 to 23-H); δ_C (100 MHz; $CDCl_3$) 32.8 and 33.9 (7-CH_2), 45.5 and 48.4 (8-CH_2), 54.3 and 60.8 (17-CH_2), 55.7 (24-CH_3), 115.1 (2,6-CH), 126.8 (23-CH), 127.2 (14,15-CH), 127.4 (20,21-CH), 128.6 (4-quat. C), 128.9 (12,13-CH), 129.2 (19,20-CH), 130.0 (16-CH), 132.9 (3,5-CH), 133.9 (11-quat. C), 136.6 (18-quat. C), 159.4 (1-quat. C) and 172.7 (10-CO); *m/z* (EI) 377.1451 ($M^{+\bullet}$. $C_{23}H_{23}NO_2S$ requires 377.1453) *m/z* (%) 377 (8.6), 328 (4.3), 272 (2.5), 238 (43.6), 224 (6.1), 166 (3.6), 139 (11.1), 105 (100), 91 (40.7) and 77 (32.1).

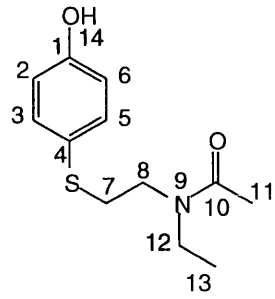
N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-*me*thylacetamide **87**



To a solution of acetamide **78** (600 mg, 2.51 mmol) in dichloromethane (20 mL), at -78 °C, was added dropwise boron tribromide (1 M in DCM, 10.0 mL, 10.0 mmol). After stirring at -78 °C for 1 h the reaction mixture was allowed to slowly warm to rt whereupon stirring was continued for a further 2 h. The reaction mixture was poured into ice/water (40 mL) and the product extracted with chloroform (3 x 20 mL). The combined organic extracts were washed with saturated sodium bicarbonate (2 x 15 mL) and brine (2 x 10 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo.* Purification by column chromatography, eluant ethyl acetate, followed by recrystallisation from ethyl acetate provided the title compound **87** (434 mg, 1.93 mmol, 77%) as white needles, mp 95-97 °C (Found: C, 58.57; H, 6.60; N, 6.18. $C_{11}H_{15}NO_2S$ requires C, 58.67; H, 6.67; N, 6.22%); λ_{max} (MeOH)/nm 254 (ε/dm^3 mol^{-1} cm^{-1} 7000); ν_{max} (KBr disc)/cm^{-1} 3078-2605m (OH), 1609s (CO), 1574s and 1495s (C=C), 1447s (CH), 1277s (OH), 1240s (C-O) and 827s (CH); δ_H (400 MHz; $CDCl_3$) [1.91 (1.0 H, s) and 1.97 (2.0 H, s) 11-H], [2.73 (1.0 H, s) and 2.93 (2.0 H, s) 12-H], [2.87 (0.7 H, t, *J* 7.2 Hz) and 2.91 (1.3 H, t, *J* 7.2 Hz) 7-H], [3.83

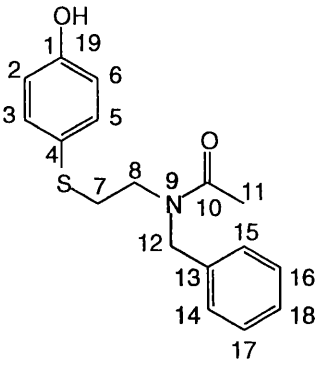
(0.7 H, t, *J* 7.2 Hz) and 3.49 (1.3 H, t, *J* 7.2 Hz) 8-H], 6.76 (2 H, m, 2,6-H) and [7.22 (0.7 H, AA'BB', d, *J* 8.8 Hz) and 7.26 (1.3 H, AA'BB', d, *J* 8.8 Hz) 3,5-H]; δ_C (100 MHz; $CDCl_3$) 20.1 and 20.5 (11-CH_3), 31.1 and 33.4 (7-CH_2), 32.2 and 36.0 (12-CH_3), 47.2 and 49.5 (8-CH_2), 115.3 and 115.6 (2,6-CH), 122.2 and 122.5 (4-quat. C), 132.2 and 133.4 (3,5-CH), 155.9 and 156.3 (1-quat. C) and 170.5 and 170.9 (10-CO); *m/z* (EI) 225.0823 ($M^{+\bullet}$. $C_{11}H_{15}NO_2S$ requires 225.0823); *m/z* (%) 225 (18.5), 166 (17.8), 152 (100), 125 (12.8), 100 (71.9), 83 (13.5) and 58 (14.2).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-ethylacetamide **88**



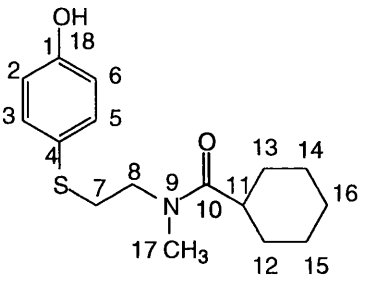
Compound **88** was prepared from **79** (450 mg, 1.78 mmol) and boron tribromide (1 M in DCM, 7.10 mL, 7.10 mmol) by the method used to prepare compound **87**. Purification by column chromatography, eluant ethyl acetate, and recrystallisation from benzene provided the title compound **88** (254 mg, 1.06 mmol, 60%) as white needles, mp 68-70 °C (Found: C, 59.86; H, 6.75; N, 5.69. $C_{12}H_{17}NO_2S$ requires C, 60.25; H, 7.11; N, 5.86%); λ_{max} (MeOH)/nm 254 (ε/dm^3 mol^{-1} cm^{-1} 7360); ν_{max} (KBr disc)/cm^{-1} 3088-2608m (OH), 2964m and 2932m (CH), 1611s (CO), 1576s and 1493s (C=C), 1434s (CH), 1271s (OH) and 828m (CH); δ_H (400 MHz; $CDCl_3$) [0.98 (0.9 H, t, *J* 7.1 Hz) and 1.08 (2.1 H, t, *J* 7.2 Hz) 13-H], [1.84 (0.9 H, s) and 1.97 (2.1 H, s) 11-H], [2.84 (0.6 H, t, *J* 7.5 Hz) and 2.93 (1.4 H, t, *J* 7.5 Hz) 7-H], [3.21 (0.6 H, q, *J* 7.0 Hz) and 3.25 (1.4 H, q, *J* 7.2 Hz) 12-H], [3.33 (0.6 H, t, *J* 7.4 Hz) and 3.44 (1.4 H, t, *J* 7.6 Hz) 8-H], 6.75 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H), 7.24 (2 H, m, 3,5-H) and [8.69 (0.3 H, br. s) and 9.09 (0.7 H, br. s) 14-H]; δ_C (100 MHz; $CDCl_3$) 13.2 and 14.4 (13-CH_3), 21.3 and 21.6 (11-CH_3), 32.6 and 35.1 (7-CH_2), 40.9 and 44.8 (12-CH_2), 46.8 and 48.5 (8-CH_2), 116.7 and 116.9 (2,6-CH), 123.5 and 124.0 (4-quat. C), 133.2 and 135.0 (3,5-CH), 157.1 and 157.7 (1-quat. C) and 171.4 and 171.9 (10-CO); *m/z* (EI) 239.0983 ($M^{+\bullet}$. $C_{12}H_{17}NO_2S$ requires 239.0986); *m/z* (%) 239 (15.5), 237 (0.7), 152 (100), 114 (78.9), 100 (7.7), 72 (10.6) and 58 (79.6).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-benzylacetamide **89**



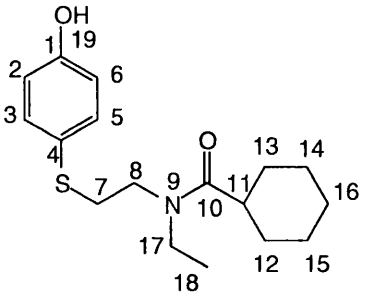
Compound **89** was prepared from **80** (400 mg, 1.27 mmol) and boron tribromide (1 M in DCM, 5.08 mL, 5.08 mmol) by the method used to prepare compound **87**. Purification by column chromatography, eluant diethyl ether, and recrystallisation from chloroform/hexane provided the title compound **89** (291 mg, 0.97 mmol, 76%) as white crystals, mp 90-92 °C (Found: C, 67.70; H, 6.49; N, 4.46. $C_{17}H_{19}NO_2S$ requires C, 67.77; H, 6.31; N, 4.65%); λ_{max} (MeOH)/nm 253 (ε/dm^3 mol^{-1} cm^{-1} 6800); ν_{max} (KBr disc)/cm^{-1} 3100-2500m (OH), 2933m (CH), 1606s (CO), 1494s and 1476s (C=C), 1433s (CH), 1360m (CH),1284s (C-O) and 835s and 695s (CH); δ_H (400 MHz; $CDCl_3$) [1.92 (1.2 H, s) and 2.01 (1.8 H, s) 11-H], [2.74 (0.8 H, t, *J* 7.3 Hz) and 2.92 (1.2 H, t, *J* 6.9 Hz) 7-H], [3.30 (0.8 H, t, *J* 7.2 Hz) and 3.48 (1.2 H. t, *J* 6.9 Hz) 8-H], [4.41 (0.8 H, s) and 4.45 (1.2 H, s), 12-H], 6.75 (2 H, AA'BB', d, *J* 8.0 Hz, 2,6-H), 7.03 (2 H, AA'BB', d, *J* 6.0 Hz, 3,5-H), 7.17-7.26 (5 H, m, 14 to 18-H) and [8.40 (0.4 H, br. s) and 8.76 (0.6 H, br. s) 19-H]; δ_C (100 MHz; $CDCl_3$) 21.5 and 21.8 (11-CH_3), 32.6 and 34.6 (7-CH_2), 47.4 and 53.6 (12-CH_2), 48.0 and 48.8 (8-CH_2), 116.8 and 117.0 (2,6-CH), 123.5 and 124.2 (4-quat. C), 126.8 and 128.6 (16,17-CH), 128.0 and 128.3 (18-CH), 129.1 and 129.5 (14,15-CH), 133.3 and 135.1 (3,5-CH), 136.4 and 137.3 (13-quat. C), 157.0 and 157.7 (1-quat. C) and 171.9 and 173.0 (10-CO); *m/z* (EI) 301.1134 ($M^{+\bullet}$. $C_{17}H_{19}NO_2S$ requires 301.1132); *m/z* (%) 301 (13.4), 258 (1.4), 176 (54.9), 152 (93.7), 120 (42.3), 106 (4.2), 91 (100), 65 (8.8) and 63 (1.4).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-methylcyclohexanecarboxamide **90**



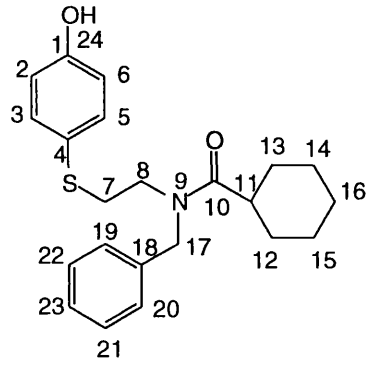
Compound **90** was prepared from **81** (400 mg, 1.30 mmol) and boron tribromide (1 M in DCM, 5.46 mL, 5.46 mmol) by the method used to prepare compound **87**. Purification by column chromatography, eluant diethyl ether, and recrystallisation from diethyl ether provided the title compound **90** (259 mg, 0.88 mmol, 68%) as cream crystals, mp 90-92 °C (Found: C, 65.55; H, 7.81; N, 4.73. $C_{16}H_{23}NO_2S$ requires C, 65.53; H, 7.85; N, 4.78%); λ_{max} (MeOH)/nm 253 (ε/dm^3 mol^{-1} cm^{-1} 3960); ν_{max} (KBr disc)/cm^{-1} 3155m (OH), 2929m and 2852m (CH), 1615s (CO), 1580s and 1497s (C=C), 1447m (CH), 1269m (OH), 1234m (C-O) and 835m (CH) ; δ_H (400 MHz; $CDCl_3$) 0.96-1.72 (10 H, m, 12 to 16-H), [2.13 (0.4 H, tt, *J* 11.6 and 3.2 Hz) and 2.38 (0.6 H, tt, *J* 11.6 and 3.2 Hz) 11-H], [2.76 (1.2 H, s) and 2.99 (1.8 H, s) 17-H], 2.83-2.89 (2 H, m, 7-H), [3.38 (0.8 H, t, *J* 7.4 Hz) and 3.46 (1.2 H, t, *J* 7.4 Hz) 8-H], [6.71 (1.2 H, AA'BB', d, *J* 8.8 Hz) and 6.80 (0.8 H, AA'BB', d, *J* 8.8 Hz) 2,6-H], [7.22 (0.8 H, AA'BB', d, *J* 8.4 Hz) and 7.25 (1.2 H, AA'BB', d, *J* 8.4 Hz) 3,5-H] and [8.00 (0.4 H, br. s) and 8.20 (0.60 H, br. s) 18-H]; δ_C (100 MHz; $CDCl_3$) 26.0 and 26.1 (14 to 16-CH_2), 29.3 and 29.9 (12,13-CH_2), 33.1 and 35.1 (7-CH_2), 34.2 and 36.6 (17-CH_3), 41.0 and 41.5 (11-CH), 48.8 and 49.8 (8-CH_2), 116.8 and 117.0 (2,6-CH), 123.7 and 124.0 (4-quat. C), 134.0 and 134.9 (3,5-CH), 157.2 and 157.6 (1-quat. C) and 177.4 and 177.5 (10-quat. C); *m/z* (EI) 293.1448 ($M^{+\bullet}$. $C_{16}H_{23}NO_2S$ requires 293.1447); *m/z* (%) 293 (13.7), 170 (86.3), 152 (100), 142 (17.3), 125 (15.1), 83 (48.9) and 55 (20.5).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-ethylcyclohexanecarboxamide **91**



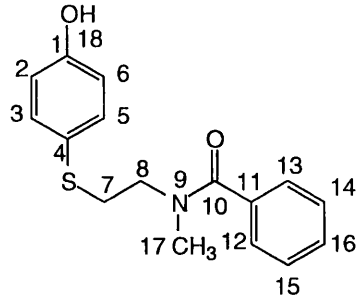
Compound **91** was prepared from **82** (380 mg, 1.18 mmol) and boron tribromide (1 M in DCM, 5.92 mL, 5.92 mmol) by the method used to prepare compound **87**. Purification by column chromatography, eluant ethyl acetate-hexane (3:1), and recrystallisation from benzene provided the title compound **91** (154 mg, 0.50 mmol, 43%) as white crystals, mp 103-104 °C ; λ_{max} (MeOH)/nm 253 (ε/dm^3 mol^{-1} cm^{-1} 5990); ν_{max} (KBr disc)/cm^{-1} 3100-2900m (OH), 2933m and 2855m (CH), 1600s (CO), 1583s and 1499s (C=C), 1431m (CH), 1272s (OH), 1226m (C-O) and 833m (CH); δ_H (400 MHz; $CDCl_3$) [0.97 (1.5 H, t, *J* 6.8 Hz) and 1.09 (1.5 H, t, *J* 7.0 Hz) 18-H], 0.95-1.17 (6 H, m, 14 to 16-H), 1.33-1.70 (4 H, m, 12,13-H), [2.02 (0.9 H, br. t, *J* 11.6 Hz) and 2.33 (1.1 H, br. t, *J* 11.4 Hz) 11-H], [2.81 (0.9 H, t, *J* 7.6 Hz) and 2.89 (1.1 H, t, *J* 7.4 Hz) 7-H], 3.32-3.20 (2 H, m, 17-H), [3.32 (0.9 H, t) and 3.41 (1.1 H, t, *J* 7.4 Hz) 8-H], [6.70 (1.1 H, AA'BB', d, *J* 8.2 Hz) and 6.81 (0.9 H, AA'BB', d, *J* 8.2 Hz) 2,6-H], [7.22 (1.1 H, AA'BB', d, *J* 8.3 Hz) and 7.26 (0.9 H, AA'BB', d, *J* 8.2 Hz) 3,5-H] and [8.32 (0.4 H, br. s) and 8.40 (0.6 H, br. s) 19-H]; δ_C (100 MHz; $CDCl_3$) 13.4 and 15.3 (18-CH_3), 26.0 and 26.1 (14 to 16-CH_2), 29.8 and 29.9 (12,13-CH_2), 33.4 and 35.7 (7-CH_2), 41.1 and 41.3 (17-CH_2), 41.3 and 43.7 (11-CH), 46.8 and 47.5 (8-CH_2), 116.8 and 117.0 (2,6-CH), 123.4 and 123.9 (4-quat. C), 133.9 and 135.1 (3,5-CH), 157.2 and 157.8 (1-quat. C) and 176.9 and 177.4 (10-CO); *m/z* (EI) 307.1609 ($M^{+\bullet}$. $C_{17}H_{25}NO_2S$ requires 307.1612); *m/z* (%) 307 (14.2), 182 (100), 232 (0.7), 152 (93.6), 125 (17.0), 111 (8.5), 83 (56.0) and 58 (46.1).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-benzylcyclohexanecarboxamide **92**



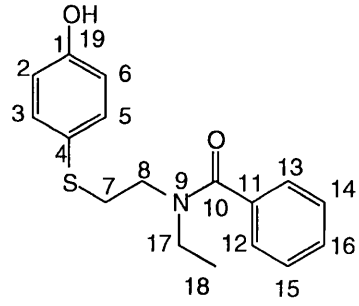
Compound **92** was prepared from **83** (265 mg, 0.69 mmol) and boron tribromide (1 M in DCM, 2.77 mL, 2.77 mmol) by the method used to prepare compound **87**. Purification by column chromatography, eluant diethyl ether-hexane (2:1), provided the title compound **92** (135 mg, 0.37 mmol, 53%) as a pale tan powder, mp 117-120 °C; λ_{max} (MeOH)/nm 256 (ε/dm^3 mol^{-1} cm^{-1} 10120); ν_{max} (KBr disc)/cm^{-1} 3100-2900m (OH), 2954m and 2958m (CH), 1618s (CO), 1583s and 1495s (C=C), 1436s (CH), 1263s (OH), 1146m (C-O) and 836m and 696m (CH); δ_H (400 MHz; $CDCl_3$) 0.95-1.18 (4 H, m, 12,13-H), 1.41-1.68 (6 H, m, 14 to 16-H), [2.11 (0.5 H, tt, *J* 11.4 Hz) and 2.39 (0.6 H, tt, *J* 11.6 and 3.2 Hz) 11-H], [2.73 (1.0 H, t, *J* 7.8 Hz) and 2.88 (1.0 H, t, *J* 7.4 Hz) 7-H], [3.28 (1.0 H, t, *J* 7.8 Hz) and 3.43 (1.0 H, t, *J* 7.4 Hz) 8-H], [4.44 (1.0 H, s) and 4.51 (1.0, s) 17-H], [6.69 (1.0 H, AA'BB', d, *J* 6.8 Hz) and 6.77 (1.0 H, AA'BB', d, *J* 8.8 Hz) 2,6-H], 7.01 (2 H, m, 3,5-H), 7.14-7.29 (5 H, m, 19 to 23-H) and [7.77 (0.5 H, br. s) and 7.84 (0.5 H, br. s) 24-H]; δ_C (100 MHz; $CDCl_3$) 24.6 and 24.7 (14,15,16-CH_2), 28.4 and 28.5 (12,13-CH_2), 31.8 and 33.8 (7-CH_2), 39.7 and 40.1 (11-CH), 45.6 and 45.8 (8-CH_2), 47.5 and 51.1 (17-CH_2), 115.4 and 115.6 (2,6-CH), 122.3 and 123.0 (4-quat. C), 125.4 and 126.8 (21,22-CH), 126.4 and 126.7 (23-CH), 127.6 and 128.0 (19,20-CH), 132.3 and 133.7 (3,5-CH), 135.6 and 136.4 (18-quat. C), 155.5 and 156.1 (1-quat. C) and 176.1 and 176.7 (10-CO); *m/z* (EI) 369.1761 ($M^{+\bullet}$. $C_{22}H_{27}NO_2S$ requires 369.1760); *m/z* (%) 369 (13.5), 244 (100), 218 (46.1), 205 (1.4), 152 (94.3), 120 (51.1), 91 (73.1), 83 (53.2) and 55 (33.3).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-methylbenzamide **93**



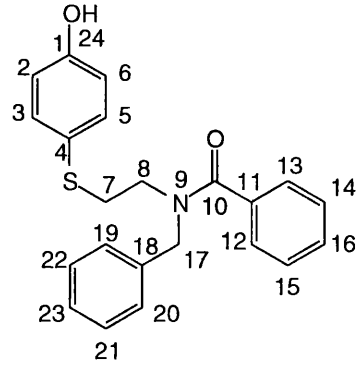
Compound **93** was prepared from **84** (300 mg, 1.00 mmol) and boron tribromide (1 M in DCM, 4.0 mL, 4.0 mmol) by the method used to prepare compound **87**. Recrystallisation from ethyl acetate provided the title compound **93** (253 mg, 0.88 mmol, 88%) as white crystals, mp 121-122 °C (Found: C, 66.73; H, 5.87; N, 4.79. $C_{16}H_{17}NO_2S$ requires C, 66.90; H, 5.92; N, 4.88%); λ_{max} (MeOH)/nm 253 (ε/dm^3 mol^{-1} cm^{-1} 9380); ν_{max} (KBr disc)/cm^{-1} 3100-2600m (OH), 2918m and 2859m (CH), 1608s (CO), 1574s and 1494s (C=C), 1440s (CH), 1236s (C-O) and 831m and 698m (CH); δ_H (400 MHz; $CDCl_3$) [2.80 (0.6 H, t, *J* 7.2 Hz) and 3.04 (1.4 H, t, *J* 7.0 Hz) 7-H], 2.92 (3 H, s, 17-H), [3.36 (0.6 H, t, *J* 7.2 Hz) and 3.66 (1.4 H, t, *J* 7.0 Hz) 8-H], [6.56 (0.6 H, AA'BB', d, *J* 8.2 Hz) and 6.69 (1.4 H, AA'BB', d, *J* 8.4 Hz) 2,6-H], [6.98 (0.6 H, AA'BB', d, *J* 8.1 Hz) and 7.22-7.33 (1.4 H, AA'BB', d) 3,5-H], 7.22-7.33 (5 H, m, 12 to 16-H) and 7.83 (1 H, br. s, 18-H); δ_C (100 MHz; $CDCl_3$) 32.2 and 37.5 (17-CH_3), 31.7 and 33.2 (7-CH_2), 46.9 and 50.1 (8-CH_2), 115.4 (2,6-CH), 122.5 (4-quat. C), 125.5 and 125.9 (14,15-CH), 127.5 (16-CH), 128.6 and 129.0 (12,13-CH), 132.6 and 132.9 (3,5-CH), 134.5 and 137.1 (11-quat. C), 155.8 (1-quat. C) and 171.4 (10-CO); *m/z* (EI) 287.0977 ($M^{+\bullet}$. $C_{16}H_{17}NO_2S$ requires 287.0974); *m/z* (%) 287 (12.0), 163 (4.2), 152 (80.9), 136 (10.6), 105 (100), 77 (45.8) and 51 (7.7).

N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-ethylbenzamide **94**



Compound **94** was prepared from **85** (880 mg, 2.79 mmol) and boron tribromide (1 M in DCM, 11.2 mL, 11.2 mmol) by the method used to prepare compound **87**. Purification by column chromatography, eluant ethyl acetate-hexane (3:2), and recrystallisation from ethyl acetate provided the title compound **94** (441 mg, 1.47 mmol, 53%) as white needles, mp 120-122 °C (Found: C, 67.58; H, 6.32; N, 4.56. $C_{17}H_{19}NO_2S$ requires C, 67.77; H, 6.31; N, 4.65%); λ_{max} (MeOH)/nm 253 (ε/dm^3 mol^{-1} cm^{-1} 7780); ν_{max} (KBr disc)/cm^{-1} 3109-2792m (OH), 3077m and 2934m (CH), 1604s (CO), 1572s and 1495s (C=C), 1431m (CH), 1269s (OH) and 829w (CH); δ_H (400 MHz; $CDCl_3$) [1.00 (2.1 H, t, *J* 7.0 Hz) and 1.22 (0.9 H, br. t) 18-H], [2.74 (0.6 H, br. t) and 3.07 (1.4 H, t, *J* 7.2 Hz) 7-H], [3.24 (1.4 H, q, *J* 6.9 Hz) and 3.31 (0.6 H, br. q) 17-H], [3.41 (0.6 H, br. t) and 3.60 (1.4 H, t, *J* 7.2 Hz) 8-H], [6.53 (0.6 H, d, *J* 7.5 Hz) and 6.67 (1.4 H, AA'BB', d, *J* 8.3 Hz) 2,6-H], [6.92 (0.6 H, AA'BB', d, *J* 7.4 Hz) and 7.22-7.45 (1.4 H, AA'BB', d) 3,5-H], 7.22-7.45 (5 H, m) and 8.05 (1 H, br. s, 19-H); δ_C (100 MHz; $CDCl_3$) 11.9 and 13.1 (18-CH_3), 31.7 and 33.3 (7-CH_2), 39.4 and 47.7 (17-CH_2), 43.8 and 44.3 (8-CH_2), 115.4 and 115.5 (2,6-CH), 121.9 and 122.7 (4-quat. C), 125.2 (14,15-CH), 127.5 (12,13-CH), 128.5 and 128.7 (16-CH), 132.3 and 132.9 (3,5-CH), 134.8(11-quat. C), 155.7 (1-quat. C) and 171.2 and 171.6 (10-CO); *m/z* (EI) 301.1135 ($M^{+\bullet}$. $C_{17}H_{19}NO_2S$ requires 301.1133); *m/z* (%) 301 (15.5), 176 (36.9), 152 (68.1), 150 (12.8), 105 (100), 77 (36.9) and 51 (4.9).

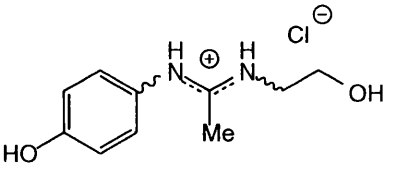
N-{2-[(4-Hydroxyphenyl)thio]ethyl}*N*-benzylbenzamide **95**



Compound **95** was prepared from **86** (346 mg, 0.92 mmol) and boron tribromide (1 M in DCM, 3.67 mL, 3.67 mmol) by the method used to prepare compound **87**. Recrystallisation from ethyl acetate provided the title compound **95** (241 mg, 0.66 mmol, 72%) as white crystals, mp 101-102 °C (Found: C, 72.59; H, 5.79; N, 3.83. $C_{22}H_{21}NO_2S$ requires C, 72.73; H, 5.71; N, 3.86%);

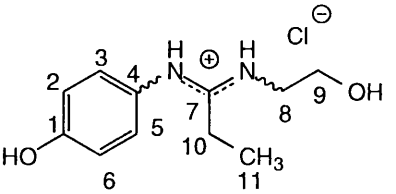
λ_{max} (MeOH)/nm 254 (ε/dm^3 mol^{-1} cm^{-1} 9700); ν_{max} (KBr disc)/cm^{-1} 3400-3000m (OH), 2949m (CH), 1613s (CO), 1598s and 1498s (C=C), 1465m (CH), 1277s (OH), 1360m (CH), 1158m (C-O) and 826m and 698m (CH); δ_H (400 MHz; $CDCl_3$) [2.68 (0.7 H, br. t) and 3.02 (1.3 H, t, *J* 7.0 Hz) 7-H], [3.26 (0.7 H, br. t) and 3.55 (1.3 H, t, *J* 7.0 Hz) 8-H], [4.48 (1.3 H, s) and 4.61 (0.7 H, s) 17-H], 6.36 (1 H, br. s, 24-H), [6.54 (0.6 H, AA'BB', d, *J* 7.7 Hz) and 6.67 (1.4 H, AA'BB', d, *J* 8.2 Hz) 2,6-H], [6.90 (0.6 H, AA'BB', d, *J* 7.7 Hz) and 7.02 (1.4 H, AA'BB', d, *J* 7.0 Hz) 3,5-H] and 7.16-7.34 (10 H, m, 12 to 16-H and 19 to 23-H); δ_C (100 MHz; $CDCl_3$) 31.4 and 32.9 (7-CH_2), 44.3 and 47.1 (8-CH_2), 52.8 (17-CH_2), 116.7 (2,6-CH), 123.0 (4-quat. C), 125.3 (23-CH), 125.7 and 126.0 (14,15-CH), 126.0 (21,22-CH), 126.8 and 127.2 (12,13-CH), 127.6 and 127.9 (19,20-CH), 128.6 (11-quat. C), 129.0 (16-CH), 134.4 and 135.1 (18-quat. C), 155.4 (1-quat. C) and 171.9 (10-CO); *m/z* (EI) 363.1296 ($M^{+\bullet}$. $C_{22}H_{21}NO_2S$ requires 363.1299); *m/z* (%) 363 (10.2), 258 (2.1), 238 (37.3), 212 (20.4), 152 (80.3), 125 (7.0), 105 (100), 91 (35.9), 77 (33.5) and 65 (4.9).

N-(2-Hydroxyethyl)-*N'*-(4-hydroxyphenyl)ethanamidine hydrochloride **101**



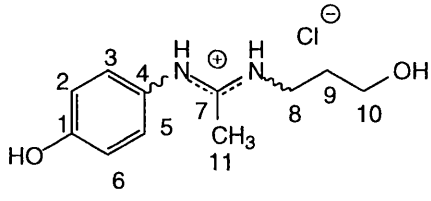
Compound **101** was prepared in a 77% yield on a 13.7 mmol scale by the method of Kormendy and co-workers[199] and gave mp 154-155 °C (*i*PrOH) (lit.,[134] 155-156 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[134]

N-(2-Hydroxyethyl)-*N'*-(4-hydroxyphenyl)propionamidine hydrochloride **102**



The method of Kormendy and co-workers[199] was used. To a solution of 4-aminophenol hydrochloride (735 mg, 5.05 mmol) in ethanol (20 mL) was added oxazoline **59** (500mg, 5.05 mmol). The reaction mixture was stirred at rt for 3 d whereupon chilled diethyl ether was added drowise to precipitate the product. The precipitate was filtered and dried under suction. The title compound **102** (850 mg, 3.48 mmol, 69%) was recrystallised from isopropanol to provide cream crystals, mp 158-161 °C (Found: C, 53.90; H, 6.88; N, 11.52. $C_{11}H_{17}N_2O_2Cl$ requires C, 53.99; H, 6.95; N, 11.45%); λ_{max} (H_2O)/nm 223 (ε/dm^3 mol^{-1} cm^{-1} 16670); ν_{max} (thin film)/cm^{-1} 3468m (OH), 3172-2920s (NH), 1639vs (C=N), 1585w (NH), 1516s (C=C), 1265m (OH) and 850w (CH); δ_H (400MHz, D_2O) 1.23 (3 H, t, *J* 7.6 Hz, 11-H) and 0.95 (3 H, t, *J* 7.6 Hz, 11-H), 2.32 (2 H, q, *J* 7.6 Hz, 10-H) and 2.63 (2 H, q, *J* 7.6 Hz, 10-H), 3.37 (2 H, t, *J* 5.2 Hz, 8-H) and 3.43 (2 H, t, *J* 5.2 Hz, 8-H), 3.52 (2 H, t, *J* 5.2 Hz, 9-H) and 3.73 (2 H, t, *J* 5.2 Hz, 9-H), 6.83 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 6.86 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 7.07 (2 H, m, 3,5-H); δ_C (100MHz, D_2O) 10.1 and 10.5 (11-CH_3), 23.7 and 24.4 (10-CH_2), 44.6 and 45.6 (8-CH_2), 59.0 and 60.0 (9-CH_2), 116.5 and 117.3 (3,5-CH), 125.3 and 127.3 (4-quat. C), 128.4 and 128.9 (2,6-CH), 156.3 and 156.7 (1-quat. C) and 169.7 and 169.9 (7-quat. C); *m/z* (EI) 208.1212 (M^+-HCl. $C_{11}H_{16}N_2O_2$ requires 208.1211); *m/z* (%) 208 (7.8), 189 (2.5), 163 (2.8), 148 (8.5), 134 (4.6), 109 (100), 99 (29.8), 80 (35.5), 69 (31.2) and 54 (16.3).

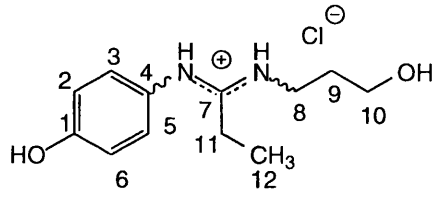
N-(3-Hydroxypropyl)-*N'*-(4-hydroxyphenyl)ethanamidine hydrochloride **103**



Compound **103** was prepared from oxazine **61** (612 mg, 6.18 mmol) and 4-aminophenol hydrochloride (900 mg, 6.18 mmol) by the method used to prepare compound **102**. Recrystallisation from isopropanol provided **103** (950 mg, 3.89 mmol, 63%) as a white powder, mp 185-187 °C (Found: C, 53.88; H, 7.01; N, 11.41. $C_{11}H_{17}N_2O_2Cl$ requires C, 53.99; H, 6.95; N, 11.45%); λ_{max} (H_2O)/nm 225 (ε/dm^3 mol^{-1} cm^{-1} 9580); ν_{max} (thin film)/cm^{-1} 3409m (OH), 3225-2923s (NH), 1649vs (C=N), 1520s and 1448s (C=C), 1280m (OH), 1068m (C-O) and 847w (CH); δ_H (400MHz, D_2O) 1.60 (2 H, m, 9-H) and 1.80 (2 H, m,

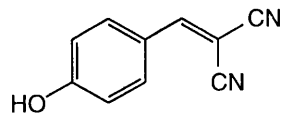
9-H), 1.92 (3 H, s, 11-H) and 2.25 (3 H, s, 11-H), 3.30 (2 H, m, 8-H), 3.45 (2 H, t, *J* 6.0 Hz, 10-H) and 3.57 (2 H, t, *J* 6.2 Hz, 10-H), 6.78 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 6.81 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 7.01 (2 H, AA'BB', d, *J* 8.4 Hz, 3,5-H) and 7.02 (2 H, AA'BB', d, *J* 8.8 Hz, 3,5-H); δ_C (100MHz, D_2O) 16.6 and 17.7 (11-CH_3), 29.5 and 30.8 (9-CH_2), 39.5 and 41.6 (8-CH_2), 58.9 and 59.2 (10-CH_2), 116.4 and 117.2 (3,5-CH), 125.4 and 127.7 (4-quat. C), 128.3 and 128.7 (2,6-CH), 156.1 and 156.6 (1-quat. C) and 164.8 and 165.3 (7-quat. C); *m/z* (EI) 208.1210 (M^+-HCl. $C_{11}H_{16}N_2O_2$ requires 208.1209); *m/z* (%) 208 (26.5), 177 (15.6), 164 (17.0), 134 (51.8), 109 (100), 99 (28.4), 80 (25.5), 65 (9.2) and 57 (6.4).

N-(3-Hydroxypropyl)-*N'*-(4-hydroxyphenyl)propionamidine hydrochloride **104**



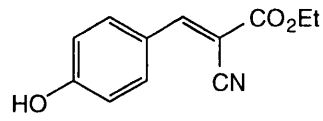
Compound **104** was prepared from oxazine **62** (600 mg, 5.31 mmol) and 4-aminophenol hydrochloride (773 mg, 5.31 mmol) by the method used to prepare compound **102**. Recrystallisation from isopropanol provided **104** (935 mg, 3.62 mmol, 68%) as a pale pink powder, mp 131-133 °C (Found: C, 55.74; H, 7.33; N, 10.79. $C_{12}H_{19}N_2O_2Cl$ requires C, 55.71; H, 7.35; N, 10.83%); λ_{max} (H_2O)/nm 223 (ε/dm^3 mol^{-1} cm^{-1} 11070); ν_{max} (thin film)/cm^{-1} 3238-2883s (OH, NH), 1650vs (C=N), 1517s (C=C), 1271m (OH), 1068m (C-O) and 850w (CH); δ_H (400 MHz, D_2O) 0.94 (3 H, t, *J* 7.6 Hz, 12-H) and 1.23 (3 H, t, *J* 7.6 Hz, 12-H), 1.64 (2 H, m, 9-H) and 1.83 (2 H, m, 9-H), 2.30 (2 H, q, *J* 7.6 Hz, 11-H) and 2.61 (2 H, q, *J* 7.6 Hz, 11-H), 3.36 (2 H, m, 8-H), 3.48 (2 H, t, *J* 5.9 Hz, 10-H) and 3.60 (2 H, t, *J* 6.2 Hz, 10-H), 6.83 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 6.86 (2 H, AA'BB', d, *J* 8.8 Hz, 2,6-H) and 7.05 (2 H, AA'BB', d, *J* 8.6 Hz, 3,5-H) and 7.07 (2 H, AA'BB', d, *J* 8.6 Hz, 3,5-H); δ_C (100 MHz, D_2O) 10.1 and 10.6 (12-CH_3), 23.5 and 24.3 (9-CH_2), 29.5 and 31.0 (11-CH_2), 39.5 and 41.5 (8-CH_2), 59.0 and 59.4 (10-CH_2), 116.5 and 117.3 (3,5-CH), 125.3 and 127.3 (4-quat. C), 128.4 and 129.0 (2,6-CH), 156.3 and 156.7 (1-quat. C) and 168.9 and 169.2 (7-quat. C); *m/z* (EI) 222.1367 (M^+-HCl. $C_{12}H_{18}N_2O_2$ requires 222.1365); *m/z* (%) 222 (8.2), 204 (9.9), 178 (5.7), 148 (28.4), 136 (7.1), 109 (100), 108 (14).

2-Cyano-3-(4-hydroxyphenyl)propenonitrile **121**



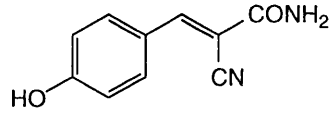
Compound **121** was prepared in 81% yield on a 4.10 mmol scale by general method 2 and gave mp 181-182 °C ($CHCl_3$) (lit.,[213] 180 °C). The spectroscopic data were in agreement with the IR and ^1H NMR data reported.[249]

Ethyl 2-Cyano-3-(4-hydroxyphenyl)propenoate **122**



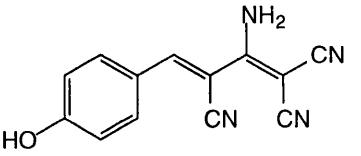
Compound **122** was prepared in 58% yield on a 4.09 mmol scale by general method 2 and gave mp 170-171 °C (^{i}PrOH) (lit.,[249] 169 °C). The spectroscopic data were in agreement with the IR and ^1H NMR data reported.[249]

2-Cyano-3-(4-hydroxyphenyl)propenamide **123**



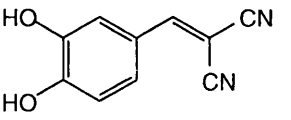
Compound **123** was prepared in 61% yield on a 4.10 mmol scale by general method 2 and gave mp 247-248 °C ($CHCl_3$/MeOH) (lit.,[213] 250 °C). The spectroscopic data were in agreement with the IR and ^1H NMR data reported.[249]

3-Amino-2,4-dicyano-5-(4-hydroxyphenyl)penta-2,4-dienonitrile **124**



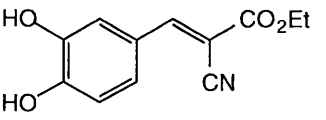
Compound **124** was prepared in 19% yield on a 4.09 mmol scale by general method 2 and gave mp 223-224 °C (MeOH/EtOH) (lit.,[213] 225 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[249]

2-Cyano-3-(3,4-dihydroxyphenyl)propenonitrile **125**



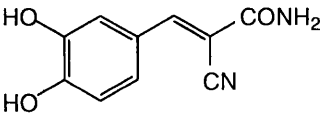
Compound **125** was prepared in 59% yield on a 7.97 mmol scale by general method 1 and gave mp 185 °C (decomp.) (EtOH) (lit.,[249] 220 °C (decomp.)). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[249]

Ethyl 2-cyano-3-(3,4-dihydroxyphenyl)propenonitrile **126**



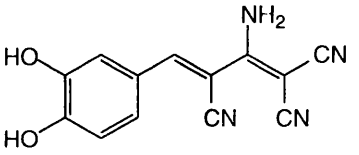
Compound **126** was prepared in 43% yield on a 3.62 mmol scale by general method 1 and gave mp 162-165 °C (EtOH/H_2O) (lit.,[249] 162 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[249]

2-Cyano-3-(3,4-dihydroxyphenyl)propenamide **127**



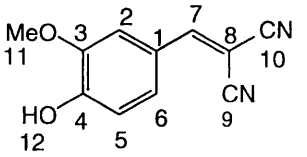
Compound **127** was prepared in 30% yield on a 3.62 mmol scale by general method 1 and gave mp 225-226 °C (*i*PrOH) (lit.,[249] 221 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[249]

3-Amino-2,4-dicyano-5-(3, 4-dihydroxyphenyl)penta-2,4-dienonitrile **128**



Compound **128** was prepared in 25% yield on a 3.62 mmol scale by general method 1 and gave mp 227-229 °C (EtOH) (lit.,[249] 229 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[249]

2-Cyano-3-(3-methoxy-4-hydroxyphenyl)propenonitrile **129**

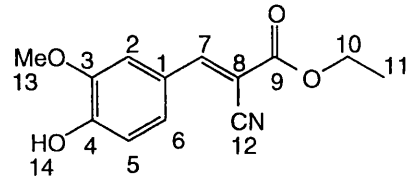


Compound **129** was prepared from vanillin (2.00 g, 13.1 mmol) and malononitrile (868 mg, 13.1 mmol) by general method 2. Recrystallisation from isopropanol provided the title compound **129** (2.14 g, 10.7 mmol, 81%) as pale yellow needles, mp 130-131 °C (lit.,[250] 133.5-134.5 °C). The spectroscopic data were in agreement with the IR and combustion data reported.[180,250]

New data: λ_{max} (MeOH)/nm 246, 371 and 438 (ε/dm^3 mol^{-1} cm^{-1} 6060, 14050 and 5590); δ_H (400 MHz, $(CD_3)_2SO$)) 3.79 (3 H, s, 11-H), 6.97 (1 H, d, *J* 8.3 Hz, 5-H), 7.47 (1 H, d, *J* 7.9 Hz, 6-H), 7.61 (1 H, s, 2-H) and 8.20 (1 H, s, 7-

H); δ_C (100 MHz, $(CD_3)_2SO$)) 55.8 (11-CH_3), 75.3 (8-quat.C), 113.3 (5-CH), 114.6 and 115.4 (9,10-CN), 116.4 (6-CH), 123.4 (1-quat.C), 128.0 (2-CH), 148.2 (4-quat.C), 154.2 (3-quat.C) and 161.0 (7-CH); *m/z* (EI) 200.0587 ($M^{+\bullet}$. $C_{11}H_8N_2O_2$ requires 200.0588); *m/z* (%) 200 (100), 185 (12.0), 157 (58.5), 143 (2.8), 129 (16.9), 102 (22.5), 83 (8.5), 75 (7.0) and 51 (5.0).

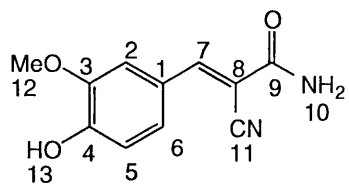
Ethyl 2-cyano-3-(3-methoxy-4-hydroxyphenyl)propenonitrile **130**



Compound **130** was prepared from vanillin (2.00 g, 13.1 mmol) and ethyl isocyanoacetate (1.40 mL, 1.49 g, 13.1 mmol) by general method 2. Recrystallisation from isopropanol provided the title compound **130** (3.06 g, 12.39 mmol, 94%) as a pale yellow powder, mp 105-107 °C (lit.,[251] 107 °C).

New data: λ_{max} (MeOH)/nm 245 and 360 (ε/dm^3 mol^{-1} cm^{-1} 8160 and 19430); ν_{max} (KBr disc)/cm^{-1} 3374m (OH), 2986w (CH), 2941w (CH), 2220m (CN), 1702s (CO), 1573s and 1509s (C=C), 1277s (CH) and 1174w (C-O); δ_H (400 MHz, $(CD_3)_2SO$)) 1.29 (3 H, t, *J* 7.1 Hz, 12-H), 3.82 (3 H, s, 13-H), 4.27 (2 H, q, *J* 7.1 Hz, 11-H), 6.94 (1 H, d, *J* 8.3 Hz, 5-H), 7.58 (1 H, dd, *J* 2.0 and 8.4 Hz, 6-H), 7.73 (1 H, d, *J* 2.0 Hz, 2-H) and 8.18 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 14.3 (12-CH_3), 55.9 (13-CH_3), 62.2 (11-CH_2), 97.3 (8-quat.C), 114.3 (5-CH), 116.3 (6-CH), 116.9 (9-CN), 123.2 (1-quat.C), 127.5 (2-CH), 148.1 (4-quat.C), 153.1 (3-quat.C), 155.2 (7-CH) and 162.9 (10-CO); *m/z* (EI) 247.0847 ($M^{+\bullet}$. $C_{13}H_{13}NO_4$ requires 247.0850); *m/z* (%) 247 (100), 219 (27.5), 202 (15.5), 170 (30.3), 158 (7.0), 114 (9.2), 103 (6.3), 76 (5.6) and 51 (2.0).

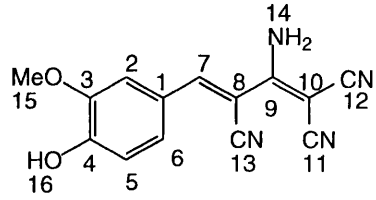
2-Cyano-3-(3-methoxy-4-hydroxyphenyl)propenamide **131**



Compound **131** was prepared from vanillin (2.00 g, 13.1 mmol) and 2-cyanoacetamide (1.11 g, 13.1 mmol) by general method 2. Recrystallisation from methanol provided the title compound **131** (1.82 g, 8.35 mmol, 64%) as yellow crystals, mp 206-207 °C (lit.,[252] 209 °C). The spectroscopic data were in agreement with the IR and combustion data reported.[180,252]

New data: λ_{max} (MeOH)/nm 243 and 356 (ε/dm^3 mol^{-1} cm^{-1} 8710 and 16590); δ_H (400 MHz, $(CD_3)_2SO$)) 3.82 (3 H, s, 12-H), 6.94 (1 H, d, *J* 8.3 Hz, 5-H), 7.46 (1 H, dd, *J* 1.5 and 8.3 Hz, 6-H), 7.66 (1 H, d, *J* 1.4 Hz, 2-H), 7.68 (2 H, 2 br. s, 11-H) and 8.07 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 55.9 (12-CH_3), 101.8 (8-quat.C), 113.5 (5-CH), 116.3 (6-CH), 117.8 (9-CN), 123.6 (1-quat.C), 126.3 (2-CH), 148.1 (4-quat.C),151.2 (7-CH), 151.8 (3-quat.C) and 164.0 (10-CO); *m/z* (EI) 218.0691 ($M^{+\bullet}$. $C_{11}H_{10}N_2O_3$ requires 218.0691); *m/z* (%) 218 (100), 202 (11.9), 170 (10.9), 158 (11.9), 114 (10.6), 76 (9.9) and 51 (6.3).

3-Amino-2,4-dicyano-5-(3-methoxy-4-hydroxyphenyl)penta-2,4-dienonitrile **132**



Compound **132** was prepared from vanillin (2.00 g, 13.14 mmol) and malononitrile dimer (1.74 g, 13.14 mmol) by general method 2. Recrystallisation from ethanol provided the title compound **132** (999 mg, 3.76 mmol, 29%) as an orange powder, mp 220-221 °C ; λ_{max} (EtOH)/nm 253 and 372 (ε/dm^3 mol^{-1} cm^{-1} 9350 and 11960); ν_{max} (KBr disc)/cm^{-1} 3396m (OH), 3396-3212m (NH), 2959w (CH), 2215m (CN), 1574m and 1512s (C=C), 1292s (CH) and 1131m (C-O); δ_C (100 MHz, $(CD_3)_2SO$)) 55.9 (15-CH_3), 97.2 (11-quat.C), 113.3 (5-CH), 115.4 (8-quat.C), 116.1 and 116.2 (9,12,13-CN), 116.4 (6-CH), 123.4 (1-quat.C), 127.0 (2-CH), 148.2 (4-quat.C), 152.6 (3-quat.C), 153.7 (7-CH) and 166.6 (10-quat.C); *m/z* (EI) 266.0805 ($M^{+\bullet}$. $C_{14}H_{10}N_4O_2$ requires 266.0806); *m/z* (%) 266 (100), 251 (12.0), 240 (47.2), 235 (19.7), 195 (15.5), 168 (28.9), 157 (6.3), 141 (14.1), 130 (5.6), 114 (12.0), 76 (7.7) and 51 (6.3).

6.3 EXPERIMENTAL FOR CHAPTER 5

6.3.1 GENERAL PREPARATIVE METHODS 3 TO 6

General method 3

The method of Baker and Howes[182] was modified as follows. To a stirred solution of hydroxybenzaldehyde (1 equiv.) and malononitrile derivative (1 equiv.) in ethanol (*ca.* 1 mL/mmol), neat piperidine (a few drops) was added. The reaction mixture was stirred at room temperature until TLC indicated the reaction to be complete whereupon the crude product was filtered, washed with cold ethanol and dried under suction. The iminolactone product required careful handling as it could not be recrystallised unchanged[181,182] and subsequently purification was achieved by trituration in cold ethanol.

General method 4

The method of Gazit and co-workers[213] was modified as follows. To a stirred solution of methoxybenzaldehyde (1 equiv.) and malononitrile derivative (1 equiv.) in ethanol (*ca.* 4 mL/mmol), neat piperidine (a few drops) was added. The reaction mixture was heated at reflux until TLC indicated the reaction to be complete whereupon the mixture was cooled to room temperature. If precipitation of product was not observed dropwise addition of water was employed. The precipitated product was then filtered, dried under suction and recrystallised from an appropriate solvent

General method 5

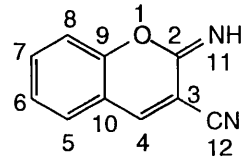
General method 5 followed the same experimental protocol outlined for general method 4 with the exception of stirring the reaction mixture at room temperature rather than heating under reflux.

General method 6

The method of Cabello and workers[181] was used. A solution of iminolactone in dilute hydrochloric acid (4 M, *ca.* 15 mL/mmol) was heated at 60 °C. The reaction's progress was monitored by TLC and on reaching completion, the mixture was cooled to room temperature. The precipitated product was filtered, dried under suction and recrystallised from an appropriate solvent.

6.3.2 EXPERIMENTAL DATA

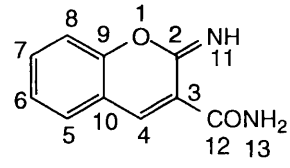
2-Imino-*2H*-1-benzopyran-3-carbonitrile **136**



Compound **136** was prepared from salicylaldehyde (0.50 mL, 573 mg, 4.69 mmol) and malononitrile (310 mg, 4.69 mmol) by general method 3. Trituration in cold ethanol provided the title compound **136** (495 mg, 2.91 mmol, 62%) as a yellow powder, mp 160 °C (decomp.) (lit.,[181] mp 160 °C (decomp.)). The spectroscopic data were in agreement with the IR and combustion data reported.[181,182]

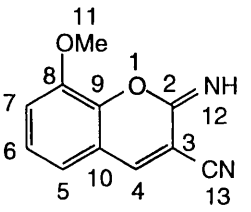
New data: δ_H (200 MHz; $(CD_3)_2SO$)) 7.12-7.34 (2 H, m, 6,8-H), 7.59-7.66 (2 H, m, 5,7-H), 8.40 (1 H, s, 11-H) and 8.89 (1 H, s, 4-H); δ_C (50 MHz; $(CD_3)_2SO$)) 104.2 (3-quat. C), 115.1 (9-CN), 117.5 (10-quat. C), 115.6 (8-CH), 124.3 (6-CH), 129.7 (7-CH), 134.2 (5-CH), 148.0 (4-CH), 151.6 (9-quat. C) and 153.9 (2-quat. C); *m/z* (CI/NH_3) 171.0559 (MH^+. $C_{10}H_6N_2O$ + H^+ requires 171.0560); *m/z* (CI/NH_3) (%) 171 (100), 152 (1.0), 136 (2.5), 113 (4.0), 96 (39.4) and 86 (2.0).

2-Imino-*2H*-1-benzopyran-3-carboxamide **137**



Compound **137** was prepared in 75% yield on a 4.92 mmol scale by general method 3 and gave mp 175-176 °C (lit.,[253] 178 °C). The spectroscopic data were in agreement with the IR and ^1H and ^{13}C NMR data reported.[180,254]

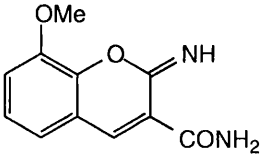
8-Methoxy-2-imino-*2H*-1-benzopyran-3-carbonitrile **142**



Compound **142** was prepared from *o*-vanillin (600 mg, 3.94 mmol) and malononitrile (261 mg, 3.94 mmol) by general method 3. Trituration in cold ethanol provided the title compound **142** (660 mg, 3.30 mmol, 84%) as a mustard yellow powder, mp 176-177 °C (lit.,[219] 172-174 °C). The spectroscopic data were in agreement with the IR and combustion data reported.[219]

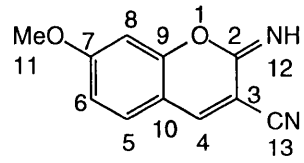
New data: δ_H (400 MHz; $(CD_3)_2SO$)) 3.88 (3 H, s, 11-H), 7.13 (1 H, dd, *J* 1.4 and 7.8 Hz, 7-H), 7.20 (1 H, t, *J* 8.0 Hz, 6-H), 7.31 (1 H, dd, *J* 1.2 and 8.0 Hz, 5-H), 8.35 (1 H, s, 12-H) and 8.95 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.4 (11-CH_3), 105.1 (3-quat. C), 115.6 (13-CN), 117.1 (7-CH), 118.2 (10-quat. C), 121.0 (6-CH), 124.5 (5-CH), 138.0 (9-quat. C), 146.4 (8-quat. C), 147.5 (4-CH) and 151.4 (2-quat. C); *m/z* (EI) 200.0585 ($M^{+\bullet}$. $C_{11}H_8N_2O_2$ requires 200.0584); *m/z* (%) 200 (100), 185 (14.2), 157 (19.9), 129 (14.9), 102 (14.2), 84 (23.8), 79 (14.2) and 63 (13.5).

8-Methoxy-2-imino-*2H*-1-benzopyran-3-carboxamide **143**



Compound **143** was prepared from *o*-vanillin (600 mg, 3.94 mmol) and 2-cyanoacetamide (332 mg, 3.94 mmol) by general method 3. Trituration in cold ethanol provided the title compound **143** (735 mg, 3.37 mmol, 86%) as a grey powder, mp 166-167 °C (lit.,[255] 195 °C). The spectroscopic data were in agreement with the IR and ^1H and ^{13}C NMR data reported.[254,255]

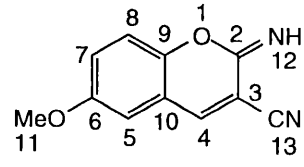
7-Methoxy-2-imino-*2H*-1-benzopyran-3-carbonitrile **144**



Compound **144** was prepared from 4-methoxysalicylaldehyde (300 mg, 1.97 mmol) and malononitrile (130 mg, 1.97 mmol) by general method 3. Trituration in cold ethanol provided the title compound **144** (340 mg, 1.70 mmol, 86%) as a yellow powder, mp 143-145 °C (lit.,[182] 162-165 °C).

New data: ν_{max} (KBr disc)/cm^{-1} 3436m and 3286m (NH), 3025w and 2938w (CH), 2222m (CN), 1617s and 1553m (C=C), 1278m (C-O) and 852m (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.91 (3 H, s, 11-H), 6.81 (1 H, d, *J* 2.1 Hz, 8-H), 6.93 (1 H, dd, *J* 2.4 and 8.6 Hz, 6-H), 7.57 (1 H, d, *J* 8.6 Hz, 5-H), 8.34 (1 H, s, 12-H) and 8.75 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.4 (11-CH_3), 100.5 (3-quat. C), 100.9 (8-CH), 111.2 (10-quat. C), 111.3 (6-CH), 116.1 (13-CN), 131.1 (5-CH), 147.2 (4-CH), 152.1 (9-quat. C), 156.0 (7-quat. C) and 164.5 (2-quat. C); *m/z* (EI) 200.0587 ($M^{+\bullet}$. $C_{11}H_8N_2O_2$ requires 200.0588); *m/z* (%) 200 (100), 173 (68.3), 158 (30.2), 149 (4.3), 130 (5.8), 102 (10.8), 84 (8.6), 63 (5.8) and 57 (3.6).

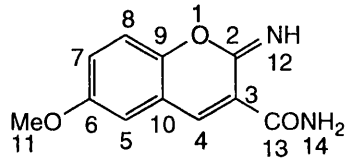
6-Methoxy-2-imino-*2H*-1-benzopyran-3-carbonitrile **49**



Compound **49** was prepared from 5-methoxysalicylaldehyde (0.41 mL, 500 mg, 3.28 mmol) and malononitrile (217 mg, 3.28 mmol) by general method 3. Trituration in cold ethanol provided the title compound **49** (405 mg, 2.03 mmol, 62%) as a yellow powder, mp 184-185 °C. ν_{max} (KBr disc)/cm^{-1} 3456m and 3344m (NH), 2924w (CH), 2218m (CN), 1573m and 1495m (C=C) and 1211s (C-O); δ_H (400 MHz; $(CD_3)_2SO$)) 3.80 (3 H, s, 11-H), 7.15 (3 H, m, 5,7,8-H), 8.28 (1 H, s, 12-H) and 8.74 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 55.9 (11-CH_3), 104.7 (3-quat. C), 112.2 (7-CH), 115.4 (13-CN), 116.7 (5-CH), 121.0 (8-CH), 146.9 (4-CH), 148.1 (9-quat. C), 151.9 (6-quat. C) and 155.3 (2-

quat. C); *m/z* (EI) 200.0595 ($M^{+\bullet}$. $C_{11}H_8N_2O_2$ requires 200.0604); *m/z* (%) 200 (100), 185 (22.7), 173 (10.3), 158 (16.5), 130 (6.2), 102 (11.3), 78 (48.3), 63 (45.4) and 61 (7.2).

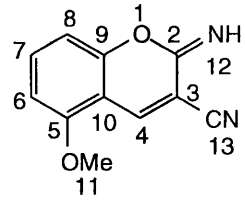
6-Methoxy-2-imino-*2H*-1-benzopyran-3-carboxamide **145**



Compound **145** was prepared from 5-methoxysalicylaldehyde (0.20 mL, 250 mg, 1.64 mmol) and 2-cyanoacetamide (138 mg, 1.64 mmol) by general method 3. Trituration in cold ethanol provided the title compound **145** (266 mg, 1.22 mmol, 74%) as a pale yellow powder, mp 193-194 °C (lit.,[218] mp 192-194 °C). The spectroscopic data were in agreement with the ^1H and ^{13}C NMR data reported.[254]

New data: ν_{max} (KBr disc)/cm^{-1} 3280s (NH, NH_2), 2950-2759w (CH), 1681s (CO), 1601m and 1573s (C=C), 1287m (C-O) and 853m (CH); *m/z* (EI) 218.0690 ($M^{+\bullet}$. $C_{11}H_{10}N_2O_3$ requires 218.0689); *m/z* (%) 218 (54.6), 201 (100), 186 (19.1), 158 (21.3), 148 (3.5), 130 (5.0), 102 (4.3), 79 (48.2), 63 (47.5) and 61 (7.1).

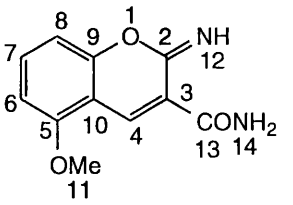
5-Methoxy-2-imino-*2H*-1-benzopyran-3-carbonitrile **146**



Compound **146** was prepared from 5-methoxysalicylaldehyde (600 mg, 3.94 mmol) and malononitrile (260 mg, 3.94 mmol) by general method 3. Trituration in cold ethanol provided the title compound **146** (528 mg, 2.64 mmol, 67%) as a salmon pink powder, mp 182-183 °C. ν_{max} (KBr disc)/cm^{-1} 3433w and 3276m (NH), 2231m (CN), 1673s and 1608s (C=C), 1272m (C-O) and 785s (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.90 (3 H, s, 11-H), 6.73 (1 H, d, *J* 8.4 Hz, 8-

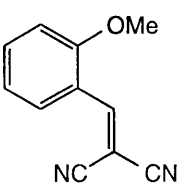
H), 7.52 (1 H, t, *J* 8.4 Hz, 7-H), 8.21 (1 H, s, 12-H) and 8.77 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.8 (11-CH_3), 102.7 (3-quat. C), 106.6 (8-CH), 107.7 (11-CN), 108.1 (6-CH), 115.7 (10-quat. C), 135.6 (7-CH), 141.3 (4-CH), 151.7 (9-quat. C), 154.8 (5-quat. C) and 156.7 (2-quat. C); *m/z* (EI) 200.0586 ($M^{+\bullet}$. $C_{11}H_8N_2O_2$ requires 200.0587); *m/z* (%) 200 (100), 173 (24.1), 158 (13.5), 135 (18.4), 102 (12.1), 76 (5.0), 63 (2.8) and 52 (2.8).

5-Methoxy-2-imino-*2H*-1-benzopyran-3-carboxamide **147**



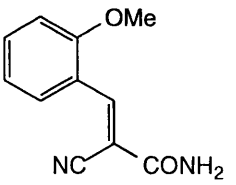
Compound **147** was prepared from 5-methoxysalicylaldehyde (395 mg, 2.60 mmol) and 2-cyanoacetamide (218 mg, 2.60 mmol) by general method 3. Trituration in cold ethanol provided the title compound **147** (420 mg, 1.93 mmol, 74%) as a cream solid, mp 210-212 °C. ν_{max} (KBr disc)/cm^{-1} 3268-3073m (NH, NH_2), 2945w and 2843w (CH), 1672s (CO), 1603s and 1571m (C=C), 1288m (C-O) and 773m (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.96 (3 H, s, 11-H), 6.78 (1 H, d, *J* 8.2 Hz, 8-H), 6.87 (1 H, d, *J* 8.3 Hz, 6-H), 7.50 (1 H, t, *J* 8.3 Hz, 7-H), 7.76 (1 H, s, 4-H), 8.39 (2 H, 2 br. s, 14-H) and 9.51 (1 H, s, 12-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.7 (11-CH_3), 106.2 (8-CH), 107.6 (6-CH), 108.6 (3-quat. C), 119.4 (10-quat. C), 134.2 (7-CH), 135.5 (4-CH), 154.8 (9-quat. C), 155.6 (5-quat. C), 157.1 (2-quat. C) and 163.3 (13-quat. C); *m/z* (EI) 218.0690 ($M^{+\bullet}$. $C_{11}H_{10}N_2O_3$ requires 218.0689); *m/z* (%) 218 (20.6), 201 (30.5), 187 (3.5), 175 (10.6), 149 (14.2), 137 (6.4), 96 (5.7), 79 (97.9), 63 (100) and 61 (14.9).

2-Cyano-3-(2-methoxyphenyl)propenonitrile **150**



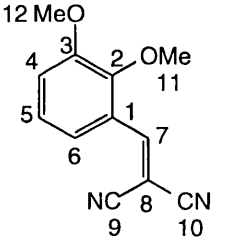
Compound **150** was prepared in 43% yield on a 5.88 mmol scale by general method 5 and gave mp 80-81 °C (^{i}PrOH) (lit.,[250] 84-85 °C). The spectroscopic data were in agreement with the IR and ^{1}H and ^{13}C NMR data reported.[222]

2-Cyano-3-(2-methoxyphenyl)propenamide **151**



Compound **151** was prepared from *o*-anisaldehyde (800 mg, 5.88 mmol) and 2-cyanoacetamide (494 mg, 5.88 mmol) by general method 4. Recrystallisation from ethyl acetate/methanol provided the title compound **151** (550 mg, 2.72 mmol, 46%) as pale yellow crystals, mp 157-158 °C (lit.,[222] 151-153 °C).The spectroscopic data were in agreement with the IR and ^{1}H and ^{13}C NMR data reported.[222]

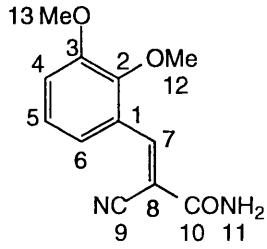
2-Cyano-3-(2,3-dimethoxyphenyl)propenonitrile **152**



Compound **152** was prepared from 2,3-dimethoxybenzaldehyde (1.0 g, 6.02 mmol) and malononitrile (398 mg, 6.02 mmol) by general method 5. Recrystallisation from methanol provided the title compound **152** (881 mg, 4.12 mmol, 68%) as pale yellow needles, mp 98-100 °C (Found: C, 67.14; H, 4.63; N, 13.01. $C_{12}H_{10}N_2O_2$ requires C, 67.29; H, 4.67; N, 13.08%); ν_{max} (KBr disc)/cm^{-1} 3048-2841w (CH), 2225m (CN), 1570s and 1481s (C=C), 1281s (C-O) and 751m (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.86 and 3.87 (two s, 6 H, 11- and 12-H), 7.29 (1 H, d, *J* 8.0 Hz, 4-H), 7.40 (1 H, dd, *J* 1.2 and 8.0 Hz, 5-H), 7.60 (I H, dd, *J* 1.2 and 8.0 Hz, 6-H) and 8.51 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 56.4 and 62.0 (11 and 12-CH_3), 83.2 (8-quat. C), 113.4 and 114.6

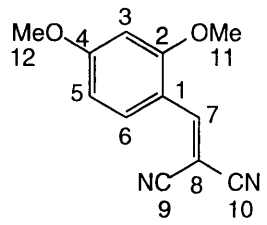
(9,10-CN), 119.2 (4-CH), 119.6 (5-CH), 125.0 (6-CH), 125.3 (1-quat. C), 149.2 and 152.9 (2 and 3-quat. C) and 156.7 (7-CH); *m/z* (EI) 214.0743 ($M^{+\bullet}$. $C_{12}H_{10}N_2O_2$ requires 214.0744); *m/z* (%) 214 (100), 199 (66.0), 184 (4.3), 171 (18.4), 169 (11.3), 130 (15.6), 101 (14.9), 84 (5.7), 75 (5.0) and 51 (3.5).

2-Cyano-3-(2,3-dimethoxyphenyl)propenamide **153**



Compound **153** was prepared from 2,3-dimethoxybenzaldehyde (1.0 g, 6.02 mmol) and 2-cyanoacetamide (506 mg, 6.02 mmol) by general method 5. Recrystallisation from isopropanol provided the title compound **153** (981 mg, 4.23 mmol, 70%) as a lemon fluffy solid, mp 164-166 °C (Found: C, 62.02; H, 5.31; N, 12.04. $C_{12}H_{12}N_2O_3$ requires C, 62.07; H, 5.17; N, 12.07%); ν_{max} (KBr disc)/cm^{-1} 3470s and 3142m (NH), 2947w (CH), 2216m (CN), 1750s (CO), 1603m and 1576m (C=C), 1277s (C-O) and 745m (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.81 and 3.85 (6 H, two s, 12 and 13-H), 7.25 (1 H, d, *J* 8.0 Hz, 4-H), 7.29 (1 H, dd, *J* 1.2 and 8.4 Hz, 5-H), 7.62 (1 H, dd, *J* 1.2 and 7.6 Hz, 6-H), 7.91 (2 H, two br. s, 11-H) and 8.34 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 56.3 and 61.6 (12 and 13-CH_3), 108.3 (8-quat. C), 116.6 (4-CH), 117.2 (9-CN), 119.8 (5-CH), 124.8 (6-CH), 126.1 (1-quat. C), 145.9 (7-CH), 148.8 and 152.9 (2 and 3-quat. C) and 162.9 (10-quat. C); *m/z* (EI) 232.0849 ($M^{+\bullet}$. $C_{12}H_{12}N_2O_3$ requires 232.0850); *m/z* (%) 232 (21.3), 201 (100), 186 (7.1), 174 (9.2), 159 (8.5), 149 (5.3), 102 (4.3), 84 9 (12.1) and 51 (4.3).

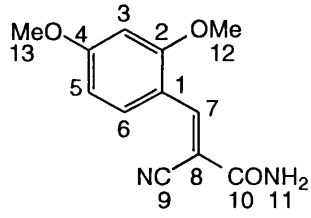
2-Cyano-3-(2,4-dimethoxyphenyl)propenonitrile **154**



Compound **154** was prepared from 2,4-dimethoxybenzaldehyde (200 mg, 1.20 mmol) and malononitrile (80 mg, 1.20 mmol) by general method 5. Recrystallisation from isopropanol provided the title compound **154** (206 mg, 0.96 mmol, 80%) as yellow crystals, mp 139-141 °C (lit.,[256] 142.2-142.6 °C). The spectroscopic data were in agreement with the ^{1}H NMR and combustion data reported.[256]

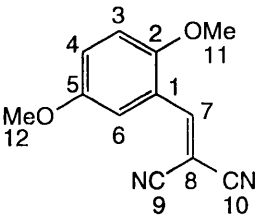
New data: ν_{max} (KBr disc)/cm^{-1} 2924w (CH), 2223m (CN), 1609m and 1563m (C=C) and 1281s (C-O); δ_C (100 MHz; $(CD_3)_2SO$)) 56.5 (12-CH_3), 56.6 (11-CH_3), 76.1 (8-quat. C), 98.6 (3-CH), 108.1 (5-CH), 113.4 (1-quat. C), 114.6 and 115.6 (9,10-CN), 130.8 (6-CH), 154.1 (7-CH), 161.7 (4-quat. C) and 167.2 (2-quat. C); *m/z* (EI) 214.0747 ($M^{+\bullet}$. $C_{12}H_{10}N_2O_2$ requires 214.0751); *m/z* (%) 214 (100), 199 (4.9), 186 (10.7), 171 (9.9), 149 (30.7), 128 (8.9), 121 (21.8), 101 (8.9), 89 (4.9) and 63 (3.9).

2-Cyano-3-(2,4-dimethoxyphenyl)propenamide **155**



Compound **155** was prepared from 2,4-dimethoxybenzaldehyde (200 mg, 1.20 mmol) and 2-cyanoacetamide (101 mg, 1.20 mmol) by general method 5. Recrystallisation from acetone provided the title compound **155** (140 mg, 0.60 mmol, 80%) as pale yellow crystals, mp 198-200 °C (Found: C, 62.09; H, 5.18; N, 12.08. $C_{12}H_{12}N_2O_3$ requires C, 62.07; H, 5.17; N, 12.07%); ν_{max} (KBr disc)/cm^{-1} 3461m and 3356m (NH_2), 2931w (CH), 2210m (CN), 1667s (CO), 1612m and 1570s (C=C) and 1274s (C-O); δ_H (400 MHz; $(CD_3)_2SO$)) 3.86 (3 H, s, 12-H), 3.89 (3 H, s, 13-H), 6.68 (1 H, s, 3-H), 6.72 (1 H, d, *J* 8.8 Hz, 4-H), 7.70 (2 H, 2 br. s, 11-H), 8.10 (1 H, d, *J* 8.0 Hz, 6-H) and 8.36 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 56.1 (12-CH_3), 56.4 (13-CH_3), 98.7 (3-CH), 102.4 (8-quat. C), 106.9 (5-CH), 113.6 (1-quat. C), 117.7 (9-CN), 129.9 (6-CH), 145.1 (7-CH), 160.8 (2-quat. C), 163.4 (4-quat. C) and 165.0 (10-CO); *m/z* (EI) 232.0836 ($M^{+\bullet}$. $C_{12}H_{12}N_2O_3$ requires 232.0825); *m/z* (%) 232 (61.3), 201 (100), 186 (6.9), 173 (6.9), 158 (7.9), 149 (5.9), 124 (4.4), 121 (6.9), 109 (5.9), 102 (4.9), 77 (3.9), 76 (3.9), 63 (2.9) and 51 (1.9).

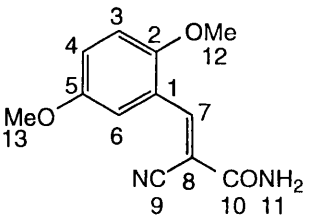
2-Cyano-3-(2,5-dimethoxyphenyl)propenonitrile **156**



Compound **156** was prepared from 2,5-dimethoxybenzaldehyde (200 mg, 1.20 mmol) and malononitrile (80 mg, 1.20 mmol) by general method 5. Recrystallisation from isopropanol provided the title compound **156** (146 mg, 0.68 mmol, 57%) as bright yellow crystals, mp 105-106°C (lit.,[256] 107.4-108.2 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[256,257]

New data: δ_C (100 MHz, $(CD_3)_2SO$)) 55.9 and 56.7 (11,12-CH_3), 81.8 (8-quat. C), 112.7 (4-CH), 113.7 and 114.7 (9,10-CN), 114.0 (3-CH), 120.3 (1-quat. C), 123.1 (6-CH), 153.0 and 153.6 (2- and 5-quat. C) and 155.7 (7-CH); *m/z* (EI) 214.0740 ($M^{+\bullet}$. $C_{12}H_{10}N_2O_2$ requires 214.0738); *m/z* (%) 214 (100), 199 (91.1), 184 (9.4), 171 (10.4), 158 (2.9), 143 (3.5), 128 (7.4), 116 (11.4), 101 (7.9), 89 (4.9), 75 (3.9) and 54 (2.9).

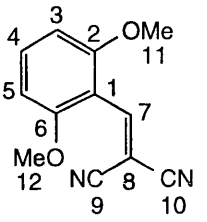
2-Cyano-3-(2,5-dimethoxyphenyl)propenamide **157**



Compound **157** was prepared from 2,5-dimethoxybenzaldehyde (100 mg, 0.60 mmol) and 2-cyanoacetamide (51 mg, 0.60 mmol) by general method 5. Recrystallisation from ethanol provided the title compound **157** (120 mg, 0.48 mmol, 81%) as fluorescent yellow crystals, mp 193-194 °C (Found: C, 62.01; H, 5.14; N, 12.02. $C_{12}H_{10}N_2O_3$ requires C, 62.07; H, 5.17; N, 12.07%); ν_{max} (KBr disc)/cm^{-1} 3391m (NH), 2966-2923w (CH), 2213m (CN), 1698s (CO), 1585m and 1497s (C=C) and 1228s (C-O); δ_H (400 MHz; $(CD_3)_2SO$)) 3.75 (3 H, s, 12-H), 3.84 (3 H, s, 13-H), 7.12 (1 H, d, *J* 9.1 Hz, 3-H), 7.17 (1 H, dd, *J* 9.1 and 2.8 Hz, 4-H), 7.60 (1 H, d, *J* 2.6 Hz, 6-H), 7.85 (2 H, 2 br. s, 11-H) and 8.38

(1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 55.8 and 56.6 (12,13-CH_3), 106.9 (8-quat. C), 113.1 and 113.4 (4,6-CH), 116.9 (9-CN), 120.1 (3-CH), 121.1 (1-quat. C), 145.7 (7-CH), 153.0 and 153.1 (2- and 5-quat. C) and 162.9 (10-CO); *m/z* (EI) 232.0841 ($M^{+\bullet}$. $C_{12}H_{10}N_2O_3$ requires 232.0834); *m/z* (%) 232 (53.5), 201 (100), 186 (4.9), 174 (31.7), 159 (8.9), 130 (8.8), 102 (6.9), 76 (5.9) and 63 (3.0).

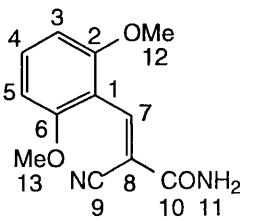
2-Cyano-3-(2,6-dimethoxyphenyl)propenonitrile **158**



Compound **158** was prepared from 2,6-dimethoxybenzaldehyde (450 mg, 2.71 mmol) and malononitrile (179 mg, 2.71 mmol) by general method 5. Recrystallisation from ethanol provided the title compound **158** (502 mg, 2.35 mmol, 87%) as pale yellow needles, mp 143-144 °C (lit.,[257] 148 °C). The spectroscopic data was in agreement with the combustion data reported.[257]

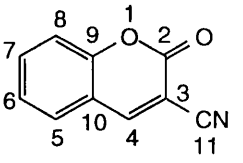
New data: ν_{max} (KBr disc)/cm^{-1} 2982-2845w (CH), 2226m (CN), 1598s and 1482m (C=C) and 1270m (C-O); δ_H (400 MHz; $(CD_3)_2SO$)) 3.35 (6 H, s, 11,12-H), 6.79 (2 H, d, *J* 8.5 Hz, 3,5-H), 7.59 (1 H, t, *J* 8.5 Hz, 4-H) and 8.23 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 56.2 (11,12-CH_3), 87.8 (8-quat. C), 104.5 (3,5-CH), 109.7 (1-quat. C), 113.3 and 115.3 (9,10-CN), 136.6 (4-CH), 154.2 (7-CH) and 159.3 (2,6-quat. C); *m/z* (EI) 214.0744 ($M^{+\bullet}$. $C_{12}H_{10}N_2O_2$ requires 214.0745); *m/z* (%) 214 (100), 195 (8.6), 186 (12.1), 156 (7.9), 149 (66.4), 128 (10.7), 91 (12.1), 78 (4.6) and 63 (5.0).

2-Cyano-3-(2,6-dimethoxyphenyl)propenamide **159**



Compound **159** was prepared from 2,6-dimethoxybenzaldehyde (1.1 g, 6.62 mmol) and 2-cyanoacetamide (557 mg, 6.62 mmol) by general method 4. Recrystallisation from ethanol provided the title compound **159** (940 mg, 4.05 mmol, 61%) as cream crystals, mp 135-137 °C; ν_{max} (KBr disc)/cm^{-1} 3440m, 3396m and 3177m (NH_2), 2978-2841w (CH), 2212m (CN), 1692s (CO), 1599s and 1477s (C=C) and 1262s (C-O); δ_H (400 MHz; $(CD_3)_2SO$)) 3.84 (6 H, s, 12,13-), 6.76 (2 H, d, *J* 8.5 Hz, 3,5-H), 7.46 (1 H, t, *J* 8.4 Hz, 4-H), 7.78 (2 H, 2 br. s, 11-H) and 8.08 (1 H, s, 7-H); δ_C (100 MHz, $(CD_3)_2SO$)) 56.0 and 56.1 (12,13-CH_3), 104.4 (3,5-CH), 110.4 (1-quat. C), 116.2 (9-CN), 133.6 (4-CH), 148.6 (7-CH), 158.5 (2,6-quat. C) and 163.1 (10-CO); *m/z* (EI) 232.0847 ($M^{+\bullet}$. $C_{12}H_{10}N_2O_3$ requires 232.0846); *m/z* (%) 232 (15.8), 201 (100), 186 (12.9), 158 (5.0), 149 (4.3), 130 (2.9), 92 (4.0), 77 (2.5) and 63 (1.4).

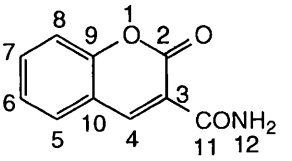
2-Oxo-*2H*-1-benzopyran-3-carbonitrile **174**



Compound **174** was prepared from iminolactone **136** (225 mg, 1.32 mmol) by general method 6. Recrystallisation from ethanol provided the title compound **174** (193 mg, 1.13 mmol, 86%) as cream crystals, mp 184-185 °C (lit.,[182] mp 184-185 °C (EtOH)). The spectroscopic data were in agreement with the IR and combustion data reported.[181,182]

New data: δ_H (400 MHz; $(CD_3)_2SO$)) 7.49 (2 H, m, 6,8-H), 7.81 (2 H, m, 5,7-H) and 8.95 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 102.5 (3-quat. C), 114.9 (11-CN), 117.9 (10-quat. C), 117.1 (8-CH), 125.8 (6-CH), 130.3 (5-CH), 135.8 (7-CH), 153.8 (4-CH), 154.4 (9-quat. C) and 157.2 (2-quat. C); *m/z* (EI) 171.0319 ($M^{+\bullet}$. $C_{10}H_5NO_2$ requires 171.0317); *m/z* (%) 171 (100), 144 (7.9), 143 (75.7), 114 (33.2), 113 (10.0), 89 (12.1), 88 (4.3), 63 (7.9) and 62 (7.1).

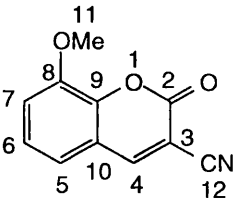
2-Oxo-*2H*-1-benzopyran-3-carboxamide **175**



Compound **175** was prepared from iminolactone **137** (390 mg, 2.07 mmol) by general method 6. Recrystallisation from ethanol provided the title compound **175** (350 mg, 1.85 mmol, 89%) as white fluffy needles, mp 264-265 °C (lit.,[253] 258-260 °C). The spectroscopic data were in agreement with the IR and ^{1}H NMR data reported.[258]

New data: δ_C (100 MHz; $(CD_3)_2SO$)) 116.5 (8-CH), 118.8 (3-quat. C), 119.6 (10-quat. C), 125.4 (6-CH), 130.6 (5-CH), 134.4 (7-CH), 148.1 (4-CH), 154.4 (9-quat. C), 160.7 (2-quat. C) and 162.9 (11-quat. C).

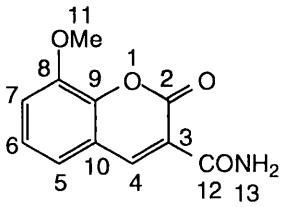
8-Methoxy-2-oxo-*2H*-1-benzopyran-3-carbonitrile **176**



Compound **176** was prepared from iminolactone **142** (200 mg, 1.00 mmol) by general method 6. Recrystallisation from methanol provided the title compound **176** (165 mg, 0.89 mmol, 89%) as yellow fluffy needles, mp 224-225 °C (lit.,[259] 222-223 °C). The spectroscopic data were in agreement with the IR and combustion data reported.[259]

New data: δ_H (400 MHz; $(CD_3)_2SO$)) 3.93 (3 H, s, 11-H), 7.33 (1 H, dd, *J* 1.4 and 7.8 Hz, 7-H), 7.40 (1 H, m, 6-H), 7.49 (1 H, dd, *J* 1.4 and 8.1 Hz, 5-H) and 8.92 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.6 (11-CH_3), 102.7 (3-quat. C), 114.9 (12-CN), 117.8 (7-CH), 118.3 (10-quat. C), 121.1 (6-CH), 125.8 (5-CH), 143.6 (9-quat. C), 146.8 (8-quat. C), 154.0 (4-CH) and 156.9 (2-quat. C); *m/z* (EI) 201.0424 ($M^{+\bullet}$. $C_{11}H_7NO_3$ requires 201.0422); *m/z* (%) 201 (100), 186 (9.9), 158 (22.8), 130 (22.1), 102 (21.0), 76 (9.9) and 52 (7.2).

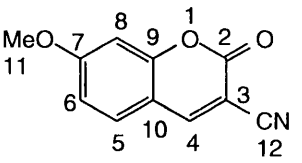
8-Methoxy-2-oxo-*2H*-1-benzopyran-3-carboxamide **177**



Compound **177** was prepared from iminolactone **143** (320 mg, 1.47 mmol) by general method 6. Recrystallisation from methanol provided the title compound **177** (189 mg, 0.86 mmol, 59%) as a pale yellow solid, mp 239-241 °C (lit.,[255] 247 °C). The spectroscopic data was in agreement with the combustion data reported.[255]

New data: ν_{max} (KBr disc)/cm^{-1} 3460m and 3392m (NH_2), 3040-2947w (CH), 1710s (CO), 1611s and 1591s (C=C), 1281s (C-O) and 799s (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.94 (3 H, s, 11-H), 7.36 (1 H, m, 6-H), 7.42 (1 H, d, *J* 8.0 Hz, 7-H), 7.50 (1 H, d, *J* 7.6 Hz, 5-H), 8.00 (2 H, 2 br. s, 13-H) and 8.82 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.5 (11-CH_3), 116.4 (7-CH), 119.3 (3-quat. C), 119.7 (10-quat. C), 121.5 (5-CH), 125.3 (6-CH), 143.9 (9-quat. C), 146.6 (8-quat. C), 148.3 (4-CH), 160.4 (2-quat C) and 162.8 (12-quat. C); *m/z* (EI) 219.0533 ($M^{+\bullet}$. $C_{11}H_9NO_4$ requires 219.0535); *m/z* (%) 219 (100), 230 (30.1), 176 (15.6), 148 (9.9), 133 (14.2), 105 (11.3), 90 (7.1), 78 (10.6) and 52 (7.1).

7-Methoxy-2-oxo-*2H*-1-benzopyran-3-carbonitrile **178**

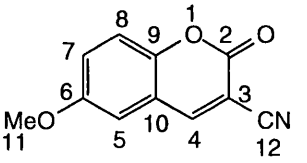


Compound **178** was prepared from iminolactone **144** (350 mg, 1.75 mmol) by general method 6. Recrystallisation from methanol/acetone provided the title compound **178** (320 mg, 1.59 mmol, 91%) as pale yellow needles, mp 217-219 °C (lit.,[259] 218-219 °C). The spectroscopic data were in agreement with the IR and combustion data reported.[259]

New data: δ_H (400 MHz; $(CD_3)_2SO$)) 3.90 (3 H, s, 11-H), 7.05 (1 H, d, *J* 8.3 Hz, 6-H), 7.10 (1 H, s, 8-H), 7.71 (1 H, d, *J* 8.6 Hz, 5-H) and 8.8 (1 H, s, 4-

H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.8 (11-CH_3), 97.8 (3-quat. C), 101.3 (6-CH), 111.6 (10-quat. C), 114.2 (8-CH), 115.4 (12-CN), 131.6 (5-CH), 153.5 (4-CH), 156.9 (9-quat. C), 157.7 (7-quat. C) and 165.7 (2-quat. C); *m/z* (EI) 201.0425 ($M^{+\bullet}$. $C_{11}H_7NO_3$ requires 201.0424); *m/z* (%) 201 (100), 173 (50.4), 158 (44.0), 148 (14.2), 130 (10.6), 102 (13.5), 79 (11.3), 70 (17.0) and 57 (14.9).

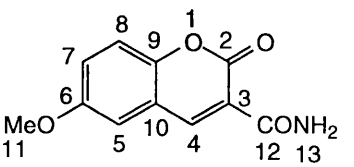
6-Methoxy-2-oxo-*2H*-1-benzopyran-3-carbonitrile **179**



Compound **179** was prepared from iminolactone **49** (81 mg, 0.40 mmol) by general method 6. Recrystallisation from ethanol provided the title compound **179** (54 mg, 0.29 mmol, 66%) as brown crystals, mp 223-224°C (lit.,[260] 226-228 °C). The spectroscopic data were in agreement with the IR and combustion data reported.[260]

New data: δ_H (400 MHz; $(CD_3)_2SO$)) 3.82 (3 H, s, 11-H), 7.31 (1 H, d, *J* 3.2 Hz, 5-H), 7.38 (1 H, dd, *J* 9.2 and 3.2 Hz, 7-H), 7.44 (1 H, d, *J* 8.8 Hz, 8-H) and 8.85 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.2 (11-CH_3), 102.7 (3-quat. C), 111.7 (5-CH), 115.0 (12-CN), 118.2 (10-quat. C), 118.3 (7-CH), 123.5 (8-CH), 148.9 (9-quat. C), 153.4 (4-CH), 156.4 (6-quat. C) and 157.3 (2-quat. C); *m/z* (EI) 201.0426 ($M^{+\bullet}$. $C_{11}H_7NO_3$ requires 201.0426); *m/z* (%) 201 (100), 186 (25.2), 158 (35.3), 143 (3.6), 130 (13.7), 102 (12.2), 77 (7.9), 63 (8.6) and 57 (2.9).

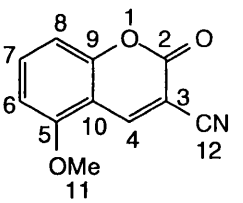
6-Methoxy-2-oxo-*2H*-1-benzopyran-3-carboxamide **180**



Compound **180** was prepared from iminolactone **145** (230 mg, 1.06 mmol) by general method 6. Recrystallisation from methanol provided the title compound **180** (224 mg, 1.02 mmol, 96%) as pale yellow crystals, mp 228-230

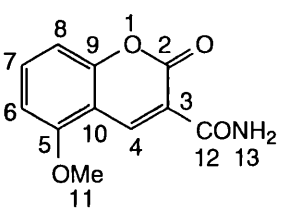
°C (Found: C, 60.19; H, 3.90; N, 6.19. $C_{11}H_9NO_4$ requires C, 60.27; H, 4.11; N, 6.39%); ν_{max} (KBr disc)/cm^{-1} 3419m and 3258w (NH_2), 1716s (CO), 1623m and 1570s (C=C), 1278m (C-O) and 829m (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.83 (3 H, s, 11-H), 7.35 (1 H, dd, *J* 3.0 and 9.1 Hz, 7-H), 7.45 (1 H, d, *J* 9.1 Hz, 8-H), 7.54 (1 H, d, *J* 3.0 Hz, 5-H), 8.01 (2 H, 2 br. s, 13-H) and 8.85 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.2 (11-CH_3), 112.2 (7-CH), 117.6 (8-CH), 119.2 (3-quat. C), 119.7 (10-quat. C), 122.3 (5-CH), 148.0 (4-CH), 148.9 (9-quat. C), 156.3 (6-quat. C), 160.8 (2-quat. C) and 162.9 (12-quat. C); *m/z* (EI) 219.0532 ($M^{+\bullet}$. $C_{11}H_9NO_4$ requires 219.0533); *m/z* (%) 219 (100), 203 (23.1), 176 (12.1), 161 (14.2), 133 (13.5), 119 (6.4), 78 (6.5) and 63 (3.6).

5-Methoxy-2-oxo-*2H*-1-benzopyran-3-carbonitrile **181**



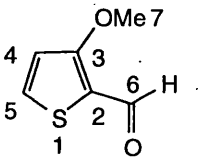
Compound **181** was prepared from iminolactone **146** (200 mg, 1.00 mmol) by general method 6. Recrystallisation from ethanol provided the title compound **181** (177 mg, 0.96 mmol, 96%) as tan crystals, mp 196-197 °C. ν_{max} (KBr disc)/cm^{-1} 3060-2849w (CH), 2234m (CN), 1727s (CO), 1604s and 1480s (C=C), 1266s (C-O) and 785m (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.95 (3 H, s, 11-H), 7.02 (2 H, d, *J* 8.4 Hz, 6,8-H), 7.73 (1 H, t, *J* 8.4 Hz, 7-H) and 8.82 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 57.1 (11-CH_3), 100.4 (3-quat. C), 107.3 (8-CH), 108.4 (12-CN), 109.0 (7-CH), 115.0 (10-quat. C), 137.2 (6-CH), 148.1 (4-CH), 155.1 (9-quat. C), 157.1 (5-quat. C) and 157.2 (2-quat. C); *m/z* (EI) 201.0426 ($M^{+\bullet}$. $C_{11}H_7NO_3$ requires 201.0427); *m/z* (%) 201 (100), 173 (24.1), 158 (27.7), 130 (14.2), 102 (13.5), 76 (6.4) and 57 (7.1).

5-Methoxy-2-oxo-*2H*-1-benzopyran-3-carboxamide **182**



Compound **182** was prepared from iminolactone **147** (200 mg, 0.92 mmol) by general method 6. Recrystallisation from ethanol provided the title compound **182** (165 mg, 0.75 mmol, 82%) as white crystals, mp 266-267 °C (Found: C, 60.09; H, 4.08; N, 6.21. $C_{11}H_9NO_4$ requires C, 60.27; H, 4.11; N, 6.39%); ν_{max} (KBr disc)/cm^{-1} 3406m and 3160m (NH_2), 1720s (CO), 1610s and 1567s (C=C), 1259m (C-O) and 791s (CH); δ_H (400 MHz; $(CD_3)_2SO$)) 3.98 (3 H, s, 11-H), 6.70 (1 H, t, *J* 8.4 Hz, 7-H), 7.02 (1 H, d, *J* 8.3 Hz, 8-H), 7.05 (1 H, d, *J* 8.4 Hz, 6-H), 7.97 (2 H, 2 br. s, 13-H) and 8.89 (1 H, s, 4-H); δ_C (100 MHz; $(CD_3)_2SO$)) 56.9 (11-CH_3), 106.8 (8-CH), 108.6 (6-CH), 109.1 (3-quat. C), 117.5 (10-quat. C), 135.8 (7-CH), 142.4 (4-CH), 155.3 (9-quat. C), 157.3 (5-quat. C), 160.6 (2-quat. C) and 162.8 (12-quat. C); *m/z* (EI) 219.0532 ($M^{+\bullet}$. $C_{11}H_9NO_4$ requires 219.0533); *m/z* (%) 219 (100), 203 (67.6), 201 (18.5), 173 (13.5), 134 (16.4), 105 (6.4), 78 (7.8) and 51 (3.6).

2-Formyl-3-methoxythiophene **190**

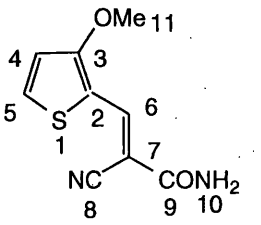


To a stirred solution of 3-methoxythiophene (0.87 mL, 1.0 g, 8.67 mmol) in tetrahydrofuran (10 mL), at rt, was gradually added *n*-butyllithium (1.6 M in hexanes, 5.47 mL, 8.76 mmol). Following this, the reaction mixture was gently heated at reflux for 3.5 h. On cooling to 0 °C, a solution of *N,N*-dimethylformamide (0.70 mL, 656 mg, 8.98 mmol) in tetrahydrofuran (10 mL) was added dropwise and stirring continued at 0 °C for 1 h. The mixture was then warmed to rt and the reaction progress monitored by TLC. After stirring at rt for a further 15 h the reaction was quenched by pouring the mixture into water (50 mL) and the product was extracted with ethyl acetate (3 x 30 mL). The organic extracts were combined, washed with brine (2 x 30 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification by column chromatography, eluant hexane-ethyl acetate (1:1), and subsequent recrystallisation from isopropanol provided the title compound **190** (717 mg, 5.05 mmol, 58%) as yellow needles, mp 80-82 °C (lit.,[261] 82 °C).

New data: Found C, 50.73; H, 4.14. $C_6H_6SO_2$ requires C, 50.70; H, 4.23%); ν_{max} (KBr disc)/cm^{-1} 3102m and 3089m (CH), 1643vs (CO), 1538s (C=C) and 1215s and 1078s (C-O); δ_H (400 MHz; $CDCl_3$) 3.92 (3 H, s, 7-H),

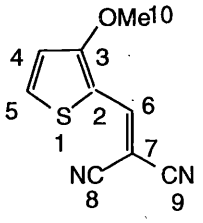
6.80 (1 H, d, *J* 5.2 Hz, 4-H), 7.58 (1 H, d, *J* 5.2 Hz, 5-H) and 9.91 (1 H, s, 6-H); δ_C (100 MHz; $CDCl_3$) 59.2 (7-CH_3), 116.2 (4-CH), 121.5 (2- quat. C), 135.6 (5-CH), 165.5 (3-quat. C) and 181.7 (6-CH); *m/z* (EI) 142.0089 ($M^{+\bullet}$. $C_6H_6SO_2$ requires 142.0090); *m/z* (%) 142 (100), 124 (30.4), 111 (25.7), 96 (22.1), 84 (84.3), 70 (7.1) and 51 (21.4).

2-Cyano-3-[2-(3-methoxythienyl)]propenamide **192**



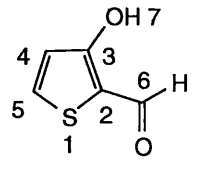
To a stirred solution of compound **190** (150 mg, 1.06 mmol) and 2-cyanoacetamide (89 mg, 1.06 mmol) in ethanol (10 mL), at rt, neat piperidine (2 drops) was added. Stirring was maintained at rt for 6 h whereupon dropwise addition of water to the filtrate caused the product to precipitate from the solution. The product was filtered, dried under suction and recrystallised from isopropanol to provide the title compound **192** (158 mg, 0.76 mmol, 72%) as yellow crystals, mp 201-203 °C (Found: C, 51.84; H, 3.79; N, 13.31. $C_9H_8N_2O_2S$ requires C, 51.92; H, 3.85; N, 13.46%); ν_{max} (KBr disc)/cm^{-1} 3467s (NH_2), 3140-3079m (CH_3), 2198m (CN), 1704s (CO), 1565vs and 1521s (C=C), 1361s and 1313s (CH_3), 1052m (C-O) and 764m (CH); δ_H (400 MHz; $CDCl_3$) 4.00 (3 H, s, 11-H), 7.23 (1 H, d, *J* 5.6 Hz, 4-H), 7.63 (2 H, 2 br. s, 10-H), 8.10 (1 H, d, *J* 5.6 Hz, 5-H) and 8.27 (1 H, s, 6-H); δ_C (100 MHz; $CDCl_3$) 59.7 (11-CH_3), 97.5 (7-quat. C), 113.5 (8-CN), 116.7 (4-CH), 117.8 (2-quat. C), 135.5 (5-CH), 139.9 (6-CH), 163.3 (3-quat. C) and 164.6 (9-quat. C); *m/z* (EI) 208.0307 ($M^{+\bullet}$. $C_9H_8N_2O_2S$ requires 208.0307); *m/z* (%) 208 (78.0), 177 (100), 175 (11.3), 150 (16.3), 121 (12.1), 109 (16.7), 83 (39.7) and 63 (18.4).

2-Cyano-3-[2-(3-methoxythienyl)]propenonitrile **193**



The method of Henrio and co-workers[241] was used. To a stirred solution of compound **190** (250 mg, 1.76 mmol) in ethanol (5 mL), at rt, was added a solution of malononitrile (116 mg, 1.76 mmol) in ethanol (5 mL). The mixture was then heated at reflux for 15.5 h. On cooling to 0 °C the precipitated product was filtered, dried under suction and recrystallised from ethanol to provide the title compound **193** (226 mg, 1.19 mmol, 68%) as yellow needles, mp 142-143 °C (Found: C, 56.67; H, 3.13; N, 14.65. $C_9H_6N_2OS$ requires C, 56.84; H, 3.16 ; N, 14.74%); ν_{max} (KBr disc)/cm^{-1} 3100-2945m (CH_3), 2219s (CN), 1569vs and 1512vs (C=C), 1332s and 1242s (CH_3) and 1070s (C-O); δ_H (400 MHz; $CDCl_3$) 3.96 (3 H, s, 10-H), 6.83 (1 H, d, *J* 5.6 Hz, 4-H), 7.72 (1 H, dd, *J* 5.6 and 0.8 Hz, 5-H) and 7.95 (1 H, d, *J* 0.8 Hz, 6-H); δ_C (100 MHz; CDCl3) 58.3 (10-CH_3), 71.6 (7-quat. C), 113.0 (2-quat. C), 113.7 (4-CH), 113.8 and 113.9 (8,9-CN), 136.1 (5-CH), 146.5 (6-CH) and 164.5 (3-quat. C); *m/z* (EI) 190.0200 ($M^{+\bullet}$. $C_9H_6N_2OS$ requires 190.0199); *m/z* (%) 190 (100), 175 (43.8), 147 (20.6), 121 (37.0), 94 (15.7), 83 (7.1) and 45 (17.8).

2-Formyl-3-hydroxythiophene **195**



To a stirred solution of compound **190** (200 mg, 1.41 mmol) in dichloromethane (10 mL), at -78 °C, was added boron tribromide (1 M in DCM, 4.22 mL, 4.22 mmol). After stirring at -78 °C for 5 h, the reaction mixture was allowed to slowly warm to rt and stirring was continued overnight. The reaction was quenched by pouring the mixture into ice/water (50 mL) and the product was extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were washed with aqueous sodium hydroxide (2 M, 3 x 15 mL) and the

resulting aqueous layers were combined, acidified to *ca.* pH 3 then extracted with ethyl acetate (3 x 50 mL). The organic extracts were dried over anhydrous magnesium sulfate, concentrated *in vacuo.* Purification by column chromatography, eluant hexane-ethyl acetate (1:1), provided the title compound **195** (0.83 mmol, 106 mg, 59%) as a pale pink powder, mp 88-90 °C (lit.,[262] 88-89.5 °C). The spectroscopic data were in agreement with the ^{1}H NMR and combustion data reported.[262]

New data: ν_{max} (KBr disc)/cm^{-1} 3100-2593m (OH, H-bonded), 1613s (CO), 1449s and 1411s (C=C), 1312s (OH) and 1021s (C-O); δ_C (100 MHz; $CDCl_3$) 114.6 (2-quat. C), 118.6 (4-CH), 135.4 (5-CH), 165.3 (3-quat. C) and 184.7 (6-CH); *m/z* (EI) 142.0089 ($M^{+\bullet}$. $C_6H_6SO_2$ requires 142.0090); *m/z* (%) 142 (100), 124 (30.4), 111 (25.7), 96 (22.1), 84 (84.3), 70 (7.1) and 51 (21.4).

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