The Synthesis and Evaluation of Anti-Melanoma Drugs

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

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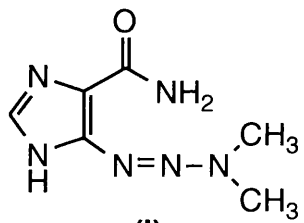
Neil Lant
Glasgow, April 1998

Summary

Although chemotherapy provides a potentially systemic cure for cancer, its application is hampered by a lack of exploitable differences between tumour cells and those which make up normal tissue. Even cells within the same tumour exhibit differing sensitivities to cytotoxic drugs, depending on factors such as their location in the cell cycle or distance from a blood supply. A further problem is increasing resistance of tumour cells to anticancer drugs.

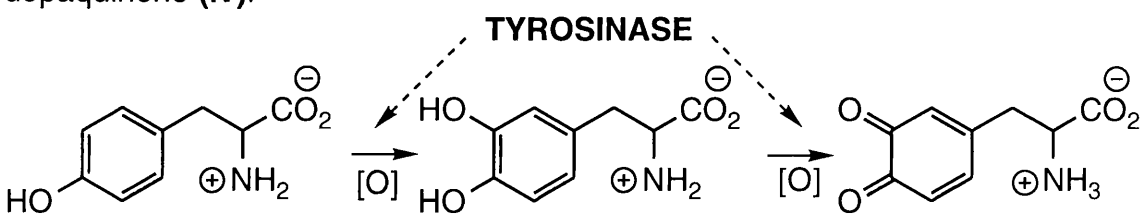
Chemotherapeutic drugs commonly target cell division but many of the common malignancies such as cancer of the breast, colon and lung are "solid" tumours containing a low proportion of dividing cells. Moreover, drugs which inhibit cell proliferation can cause many unpleasant side-effects by affecting normal regions of fast-dividing cells too.

The treatment of advanced malignant melanoma is one area where current chemotherapy affords very low success rates. Dacarbazine **(i)** is the current drug of choice, yet it only gives a positive response in 20% of patients.



(i)

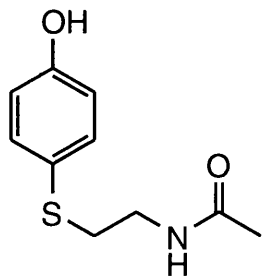
In the search for new anti-melanoma compounds, efforts are being made to exploit features which distinguish melanoma cells from other cell types in the body. One such difference is that melanoma cells generally continue to synthesise the pigment melanin by enzymes contained within the melanosome organelle. A key enzyme in the melanin biosynthetic pathway is Tyrosinase which catalyses the conversion of tyrosine **(ii)** to dopa **(iii)** and then dopaquinone **(iv)**.



(ii) **(iii)** **(iv)**

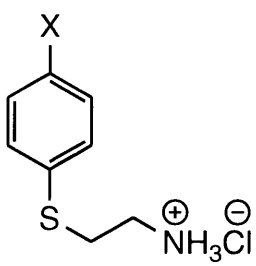
One approach towards achieving selective treatment of melanoma is to design phenolic prodrugs which could be selectively oxidised to quinones by Tyrosinase in melanoma cells. The quinone product could then cause cell death

by attacking essential thiol-containing enzymes. Phenol **(v)** was previously prepared and shown to possess interesting anti-melanoma activity. As little work had been done to optimise its activity, we started a programme to probe its structure-activity relationship in some detail.



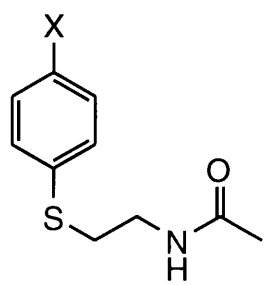
(v)

We initially prepared a series of 37 related compounds, represented by the general structures **(vi)**, **(vii)**, **(viii)** and **(ix)**. These were evaluated by Professor R.M. MacKie of the Department of Dermatology, University of Glasgow and Dr L.R. Kelland of the Institute of Cancer Research, Surrey. Compounds of type **(viii)** containing two methyl groups α– to the nitrogen (R^1, R^2) and a bulky group at the acyl position (R^3) were far more cytotoxic than the parent compound **(v)**, although some toxicity towards non-melanoma cell lines was observed. Compounds without a phenol group did not cause significant cytotoxicity, apart from phenyl esters which are likely to have been converted into phenols by hydrolysis. The most promising compounds exhibited GI_{50} values down to 5 μM for melanoma cell lines and showed some selectivity over non-melanoma cell types (e.g. GI_{50} > 40 μM for the SKOV-3 ovarian line).



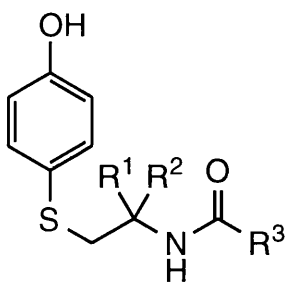
(vi)

X = Cl, H, OH



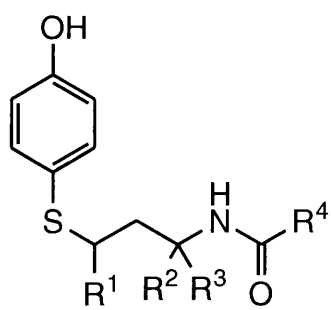
(vii)

X = OH, H, OCH_3, CH_3, Cl, OAc



(viii)

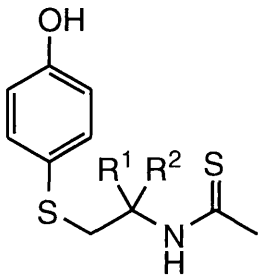
R^1, R^2 = H, alkyl, (jointly) cycloalkyl
R^3 = H, alkyl, cycloalkyl, aryl



(ix)

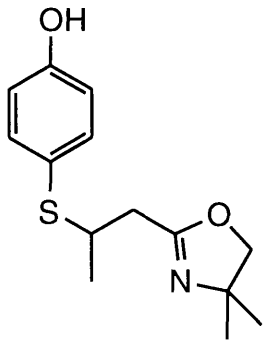
R^1, R^2, R^3 = H, CH_3
R^4 = CH_3, Cy

Another area of investigation was to prepare a series of compounds to evaluate the effects of replacing the amide group of **(v)** with other functional groups. Thioamides of the general structure **(x)** and the heterocyclic derivative **(xi)** showed the highest levels of toxicity during biologically evaluation by Dr Kelland and the Ares Serono Group.



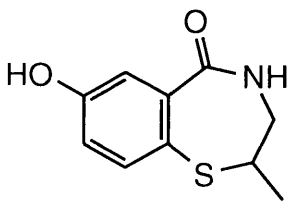
R^1,R^2 = H, CH_3

(x)



(xi)

We evaluated the effects of constraining conformation by the preparation and testing of bicyclic derivatives such as **(xii)**. Analogues of **(v)** with general structure **(xiii)** containing an inverted amide group were also prepared. Related compounds which do not contain a sulfide group, represented by general structure **(xix)**, were prepared for comparison.



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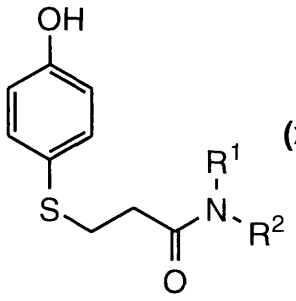
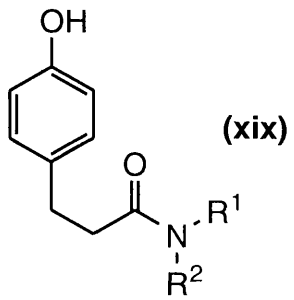


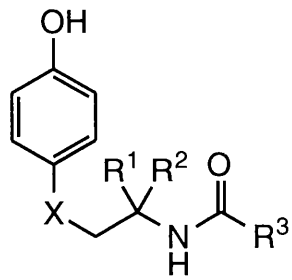
(xiii)



(xix)

R^1,R^2 = H, alkyl, (together) cycloalkyl

In order to study the biological effects of varying the side-chain bridging group, a series of eleven compounds of general structure **(xx)** were prepared and compared in terms of lipophilicity, electronic properties, steric effects and biological activity. It was found that selenide analogues exhibited greater anti-melanoma activity than their corresponding sulfides. This was attributed to greater lipophilicity on the basis of their measured partition coefficients.



(xx)

X = SO_2, C=O, S=O, CH_2, Se, O, NH_2

R^1, R^2, R^3 = H, alkyl

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Abbreviations

ADP	Adenosine Diphosphate
b.p.	boiling point
br	broad
CI	Chemical Ionisation
COSHH	Control of Substances Hazardous to Health
d	doublet (NMR spectroscopy)
d	day(s)
dec.	decomposed (m.p.)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DEPT	Distortionless Enhancement by Polarisation Transfer
DME	1,2-dimethoxyethane
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide.
DNA	deoxyribonucleic acid
EI	Electron Impact
GI_{50}	concentration required to reduce growth by 50%
h	hour(s)
HMPA	hexamethyl phosphoramide
IR	infra red
lit.	literature value
m	multiplet (NMR spectroscopy)
min	minute(s)
m.p.	melting point
MS	mass spectrometry
NMR	nuclear magnetic resonance
q	quartet (NMR spectroscopy)
RNA	ribonucleic acid
r.t.	room temperature
s	singlet (NMR spectroscopy)
SAR	Structure - Activity Relationship
t	triplet (NMR spectroscopy)
TBAF	tetrabutyl ammonium fluoride
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid

$\sim IC_{50}$

TLC	Thin Layer Chromatography
UV	ultraviolet

1

Cancer: Causes and Cures

1.1 The Cancer Problem

In the industrialised nations, cancer is arguably the most feared of diseases. Statistically there is good reason for this; around one third of people in the developed world will develop cancer; one fifth will die from it. To the public at large, cancer is often considered to be a single condition differing only in the part of the body it affects. More specifically, it is a complex class of around one hundred diseases which vary widely in the cell type from which they are derived.

As there are so many different types of cancer, progress towards our understanding of the condition has been slow. The search for its cure has proved to be hard but by no means hopeless; the many successes in cancer treatment include testicular cancer, certain ovarian cancers, Hodgkin's disease, Ewing's sarcoma and acute lymphocytic childhood leukaemia. Unfortunately, progress towards curative therapy of the more common cancers of the lung, breast, prostate and colon has been comparatively slow.

1.2 The Origin of Cancer

In 1914, Boveri suggested that the origin of cancer may be malfunction of the cell's genetic material. It is now understood that almost all cancers originate from the aberration of the DNA sequence of a *single* cell.[1] However, a single mutation is not sufficient to cause cancer; this will occur only in the combination of several rare biochemical accidents. The likelihood of these accidents occurring is determined by a variety of internal and external factors.

The generation of cancer (carcinogenesis) is closely correlated to the induction of DNA changes (mutagenesis) in the following four classes of agents.

1.2.1 Chemical Mutagens

The evidence linking certain chemicals with cancer has been accumulating since 1761, when John Hill noted the high incidence of nasal

cancer among snuff users. This was followed by numerous observations linking particular substances to specific types of cancer, often in an occupational environment where the effects of a particular agent could be seen throughout the workforce. Since the early twentieth century, animal testing has supplemented our knowledge of the structurally diverse chemicals which are known to give rise to DNA mutation. Some examples of mutagenic compounds, their type of exposure and typical sites of human cancer are shown in Table 1.[2]

Table 1 Selected Mutagenic Compounds

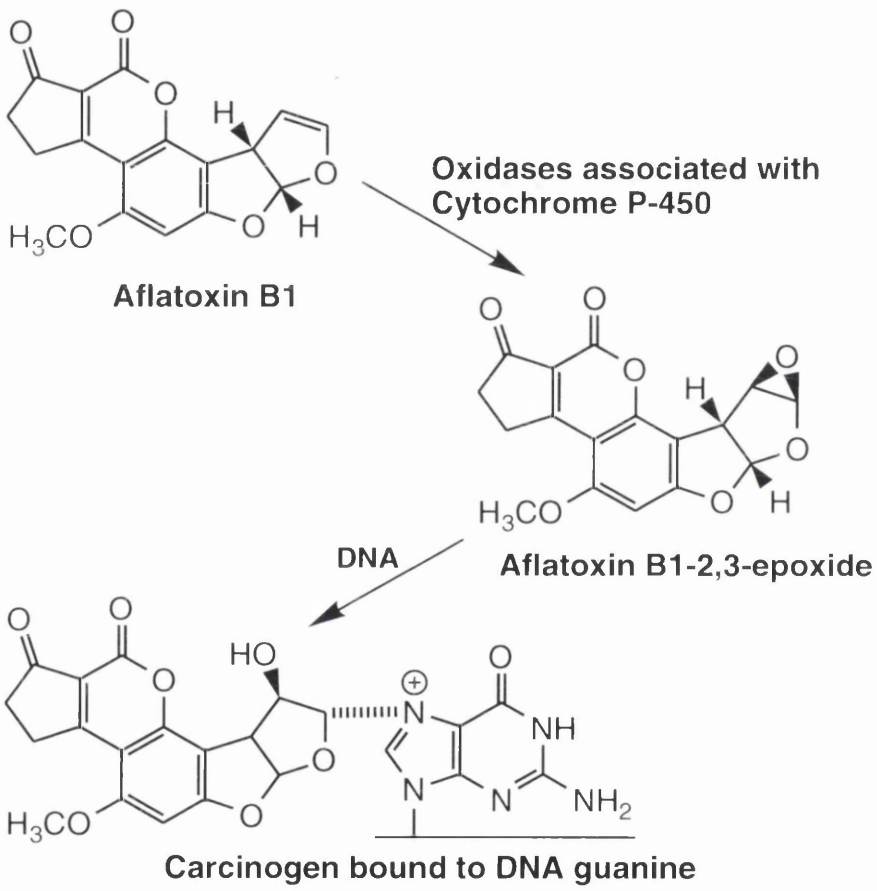
Chemical	Type of Exposure			Cancer Site
	Occupational	Medical	Food	
Aflatoxin			X	Liver
Benzene	X			Marrow
Benzidine	X			Bladder
Busulfan		X		Marrow
Chlornaphazine		X		Bladder
Cyclophosphamide		X		Bladder
Melphalan		X		Marrow
Mustard gas	X			Larynx, lung
Oxymetholone		X		Liver
Phenacetin		X		Kidney
Vinyl Chloride	X			Liver

The use of all hazardous chemicals in all types of employment is now strictly controlled by COSHH regulations and earlier government legislation. This has led to a reduction in occupational exposure to mutagens yet we are still at risk from other sources such as air pollution, cigarette smoke, certain prescribed drugs and even some types of food.

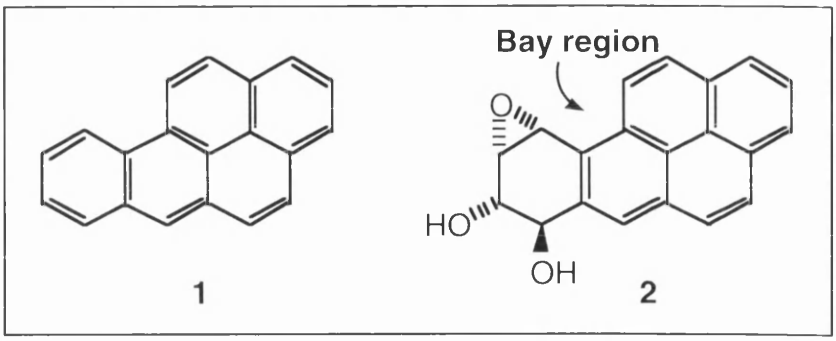
The mechanism of genetic mutation by chemicals commonly involves some form of simple change to the nucleotide sequence, often by alkylation of the nucleophilic sites of DNA bases. This mechanism is analogous to the action of the alkylating class of anticancer drugs; indeed many anticancer drugs are known to be carcinogens (*vide infra*). In many cases it is a daughter of an

inactive parent compound which is the active mutagen, frequently resulting from metabolism by the cellular oxidase enzymes associated with cytochrome P-450. One such example is aflatoxin B1, a toxin from the mould *Aspergillus flavus oryzae* which grows on grain and nuts which have been stored under warm humid conditions. When contaminated food is eaten, metabolites of aflatoxin are involved in mutations to the *p53* gene by the mechanism shown in Scheme 1.[3] This is thought to be an important contributing factor to the incidence of liver cancer in tropical regions.

Scheme 1 Metabolic Activation of Aflatoxin B1



A similar oxidation-alkylation mechanism is evident with certain polycyclic aromatic hydrocarbons (PAHs), for example benzo[a]pyrene **1**. The metabolic activation of PAHs involves a series of oxidative reactions which result in the formation of diol epoxides such as **2**. Jerina and Daly have postulated that a bay region adjacent to the epoxide is instrumental to the mutagenicity of these agents.[4]



PAHs are known to result from incomplete fossil fuel combustion, making them an important constituent of air pollution. Their presence in whisky and barbecued food is more insidious, unlike their predominance in the deadly cocktail of over 50 carcinogens which are found in tobacco smoke.

1.2.2 Ionising Radiation

The harmful nature of X-rays was evident shortly after their discovery in 1895 by Röntgen. The initial symptoms of skin blistering and reddening were followed, several years later, by relatively high incidence of skin cancers, leukaemia and brain tumours amongst radiologists. As with chemical carcinogens, debate still continues about the "safe" levels of exposure to the high-energy electromagnetic radiation of X-rays, γ-rays and particles arising from radionuclei.

The high energy associated with ionising radiation causes widespread damage through bond-breaking processes within the cell. Absorption of dissipated energy by water is critically important in mutagenesis; ionisation ultimately leads to the generation of hydroxyl radicals, hydrogen radicals and solvated free electrons. On interaction with DNA, these cause single and double strand breaks together with base deletions and rearrangements.[5] Genetic mutation can also arise when these forms of damage are improperly rectified by the error-prone DNA repair processes of the cell.

The different forms of ionising radiation vary in the way they dissipate energy on contact with tissue; the concept of linear energy transfer (LET) is used to measure this factor. High LET forms of radiation, such as α-particles and neutrons, rapidly release their energy over a short distance causing large amounts of DNA damage and cell death. Low LET radiation, including γ-rays, and X-rays, is markedly less cytotoxic.

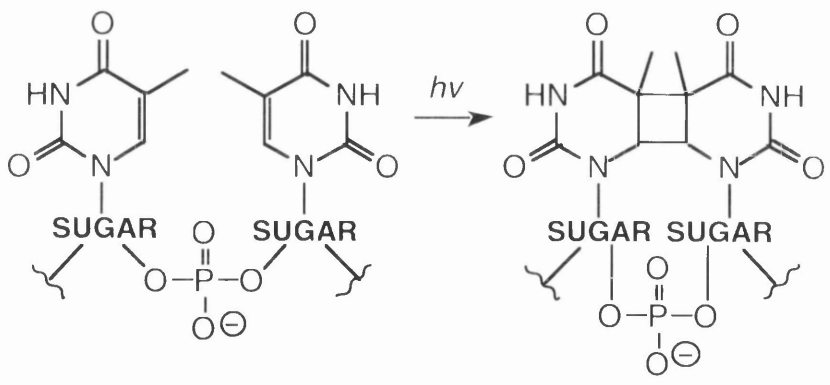
In common with mutagenic chemicals, the consequences of ionising radiation may not take effect until many years after exposure The estimated latent periods for lung, skin, brain, thyroid and intestinal cancers are all greater than twenty years.[6]

1.2.3 Ultraviolet Radiation

The lower energy and low penetration of ultraviolet (UV) radiation accounts for the observation that its effects are limited to the skin. Initially, these effects appear to be quite pleasant; increased melanin formation is induced thus giving the sought-after "healthy-looking" bronzed complexion. Unfortunately, a side-effect of this can be the induction of skin cancers, the most rapidly increasing forms of cancer in the developed world.

UV radiation is grouped into three wavelength bands; UVA (>320 nm), UVB (290-320 nm) and UVC (200-290 nm). Of these, UVB is the most important in carcinogenesis and is also the band largely filtered out of solar radiation by the ozone layer. In contrast to high-energy ionising radiation, UV radiation is not thought to cause genetic mutation through widespread nucleic acid bond-breaking processes. Its activity appears to be associated with the generation of excited states in DNA bases which leads to mutagenic photochemical reactions, such as the dimerisation of pyrimidine bases as exemplified in Scheme 2.[7] Thymine-cytosine and cytosine-cytosine dimers can form in a similar manner.

Scheme 2 Photodimerisation of Pyrimidines



The role of UV radiation in the incidence of a deadly form of cancer known as malignant melanoma will be discussed in more detail in chapter 2.

1.2.4 Viruses[8]

Viruses are parasitic infective agents which require host cells for their survival and reproduction. Structurally, they consist of genetic material (DNA or RNA) encased in a protein capsid or membrane envelope which allows movement from one cell to another. On entry to a cell, viruses deposit their nucleic acid and utilise the host's biochemical machinery to create typically hundreds of progeny which can leave to infect other cells. In many cases, viral infection will cause the cell to burst open (lysis) thus accelerating further infection. Viruses are responsible for a large number of human diseases such as cold sores, chicken pox, the common cold and polio, yet their role in human carcinogenesis has proved to be more elusive.

Viral carcinogenesis can be mediated through both RNA- and DNA-containing viruses. RNA viruses infect cells and use reverse transcription to produce DNA which can then cause mutation by incorporation into the host's genome. This form of carcinogenesis is common in other animals but has little impact on human cancer where DNA-containing viruses predominate. To induce carcinogenesis, the latter viruses rely on the rare occurrence that their DNA does not replicate in the host, but is satisfactorily incorporated into the cell's chromosomes. If the inserted DNA somehow activates the host's machinery for replication, cancer can result.

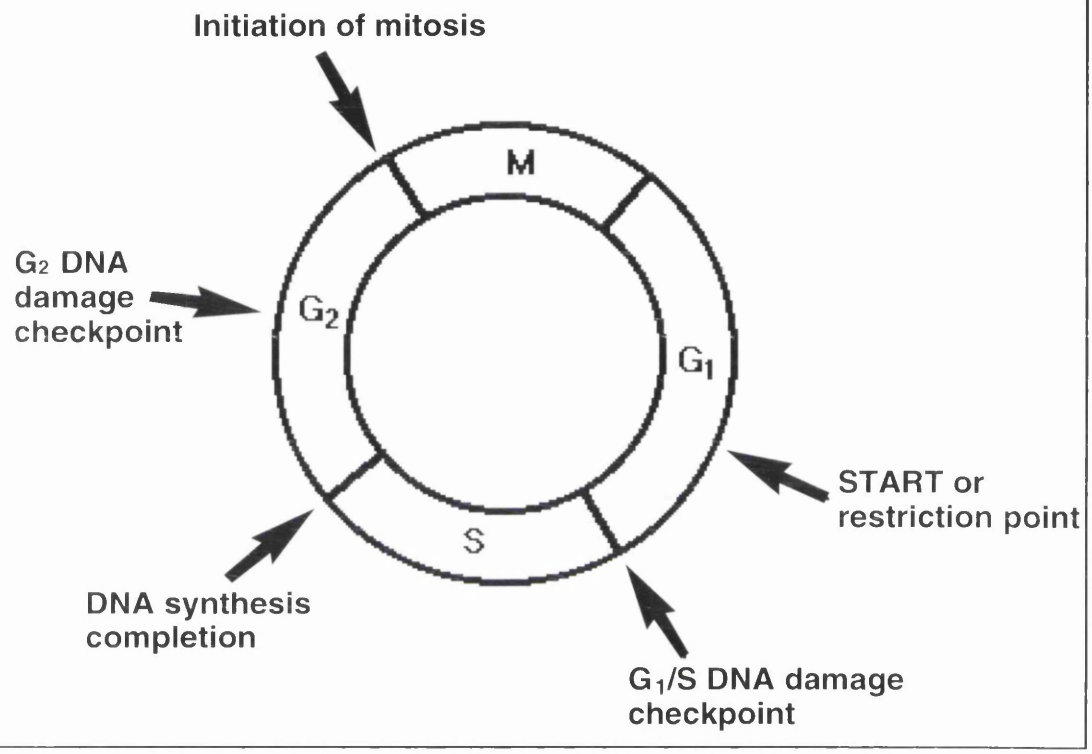
Viruses are thought to be involved in about 15% of human cancers,[9] including forms of cervical cancer (papillomavirus), liver cancer (hepatitis-B virus) and Burkitt's lymphoma (Epstein-Barr virus). The study of animal tumour viruses has provided valuable clues to the mechanisms of cancer in general.

1.3 Cell Proliferation and Cancer

The process of cell proliferation is controlled by the cell cycle, a well-defined sequence of events which directs DNA duplication and mitosis (cell division). It was thought that cells continuously synthesise DNA between mitoses until the 1950s when it was found that the cell cycle has four distinct phases as shown in Figure 1.[10] Firstly, cells enter G_1 where RNA and proteins are synthesised and preparations are made to begin DNA synthesis. This is followed by the S phase, where DNA is replicated and DNA-packing proteins (histones) are synthesised. Thirdly, in G_2 phase, the replicated DNA is complexed with protein and preparations are made for cell division. Finally, in M phase, the cell nucleus and cytoplasm divide in the process of mitosis. The two daughter cells can then begin the cycle, or in the absence of correct signals or nutrients, enter a resting G_0 phase. Checkpoints are present at sites around the

cell cycle to ensure that the tasks of one phase are complete before transition to the next. The time taken for one complete cycle, T_C, is usually within the range 15 to 120 hours; a notable exception is liver cells, which sometimes exhibit T_C values of over one year.[11]

Figure 1 The Cell Cycle



Tumours were long considered to be masses of rapidly dividing cells and we would therefore expect the T_C of a cancer cell to be somewhat shorter than that of normal cells. In reality, tumour cells complete an average cell cycle in a slightly greater time than normal, implying that if T_C was the only factor, tumours would grow at a similar rate to surrounding tissue. However, cell replication is normally strictly controlled to replace lost cells in order to maintain a constant cell number. In tumours, these control mechanisms are lost so the net cell number does increase with the rate of increase being affected by the extent of cell kill mechanisms such as the immune system and apoptosis (programmed cell death).

In spite of the monocellular origin of cancer, tumours are only detectable once thay have matured to a late stage in their life. When palpable, they will contain over 10^9 cells and aberrant cells may have already metastasised by contamination of the lymphatic and circulatory systems. The growing tumour will be expanding in size, starving other tissue of nutrients and space thus causing damage to blood vessels and ultimately, the vital organs of the body. Once the

disease has spread away from the primary tumour, the prognosis is generally very poor. Effective treatment of cancer therefore depends on early detection and rapid therapy. The various types of cancer treatment will be discussed in the following section.

1.4 Treatment of Cancer[12]

As soon as cancer is detected, a programme of therapy is rapidly implemented to maximise the chance of survival. If localised, the cancer is usually surgically removed and adjuvant therapy is given to kill residual tumour cells. Radiotherapy is commonly used to treat cancer cells which remain in the tissue which surrounded the excised tumour; however, the encasement of malignant cells inside a region of hypoxia (oxygen deficiency) can sometimes render them radioresistant. Moreover, radiotherapy is not effective against undetected metastases which may have developed in other parts of the body.

Chemotherapy provides a potentially systemic cure for cancer; however its application is hampered by the lack of exploitable differences between tumour cells and those present in normal tissue. Even cells within the same tumour exhibit differing sensitivities to cytotoxic drugs, depending on factors such as their location in the cell cycle or distance from blood supply. A further problem is the increasing resistance of tumour cells to anticancer drugs, a situation which is being addressed by combined treatment with a cocktail of two or three agents. Chemotherapeutic drugs commonly target cell division but many of the common malignancies such as cancer of the breast, colon and lung are "solid" tumours with a low proportion of dividing cells; these cancers are less susceptible to treatment with anticancer agents.

Despite these problems, chemotherapy has achieved remarkable successes in certain types of cancer. The different types of anticancer drugs and their mechanisms of action will now be briefly surveyed.

1.5 Chemotherapeutic Agents[13]

Over the past fifty years, many thousands of compounds have been evaluated for potential as anticancer drugs; the fruits of this research are the forty chemotherapeutic agents which are in common use today. Hopes of discovering an across-the-board drug to combat all types of the disease have been all but abandoned with most effort now being directed towards the design of medicines for particular forms of cancer.

For our purposes, the compounds which are in current use can be conveniently categorised into five areas: alkylating agents, antimetabolites,

noncovalent DNA binding drugs, inhibitors of chromatin function and drugs affecting endocrine function. Each of these classes of agent will be discussed separately.

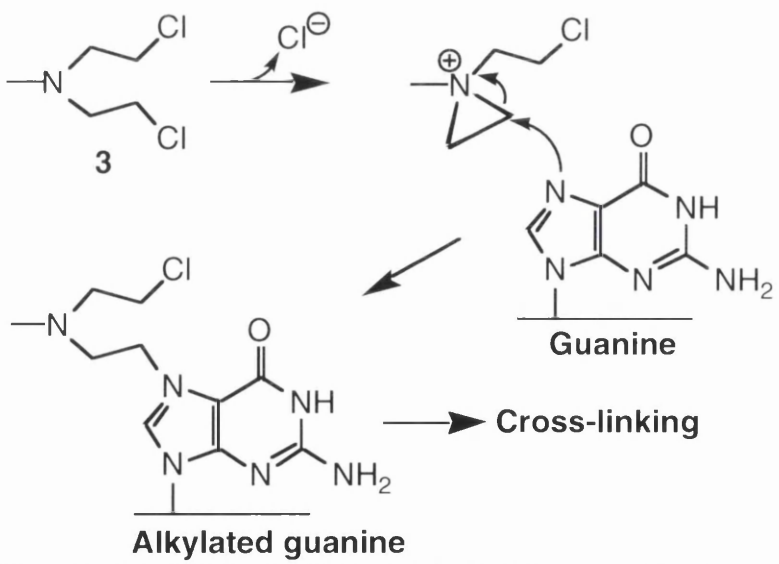
1.5.1 Alkylating Agents

The alkylating class of drug forms adducts with the nucleic acid bases thus interfering with DNA synthesis. Some of these compounds contain two reactive moieties which enable them to cross-link the complementary strands of DNA and thus prevent uncoiling of the strands during replication. Alkylating agents are the largest family of clinically used cancer drugs; three structural types are discussed here.

1.5.1.1 Nitrogen Mustards

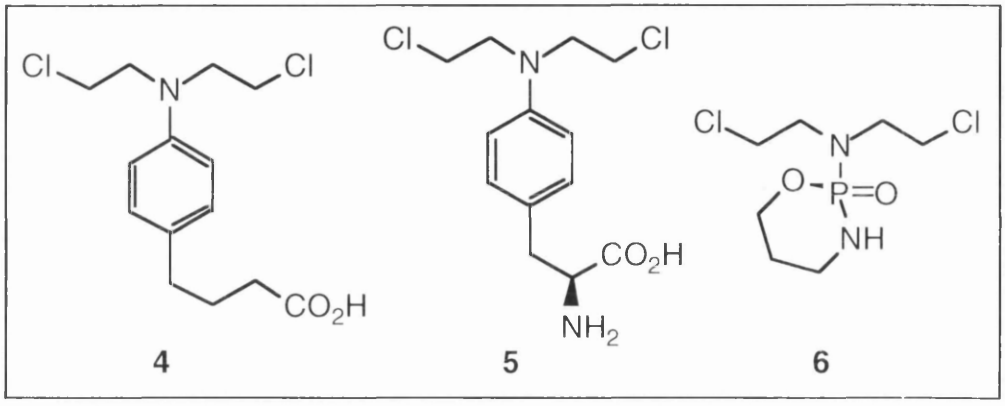
The structure and activity of the archetype mustard mechlorethamine **3** demonstrates the function of this type of agent (Scheme 3).[14] Intramolecular reaction, with concomitant expulsion of chloride leads to an electrophilic aziridinium ion. On reaction with the nucleophilic sites of DNA such as the N-7 of guanine, genetic mutation will result. Cross-linking occurs when the remaining chloroethyl group reacts with the complementary strand of DNA.

Scheme 3 Biological Action of Nitrogen Mustard



Mechlorethamine **3** now has only very limited application in modern chemotherapy. It is highly toxic, corrosive, acts for only a very short period of time and requires intravenous infusion. In order to alleviate these problems,

chlorambucil **4** and melphalan **5** were developed by British scientists in the 1950s. The electron-withdrawing effects of the aromatic ring of these agents has pronounced effects on the rate of aziridinium ion formation and alkylation thus extending the lifetime of the drug. This improves absorption of the drug and allows the compounds to be orally administered.



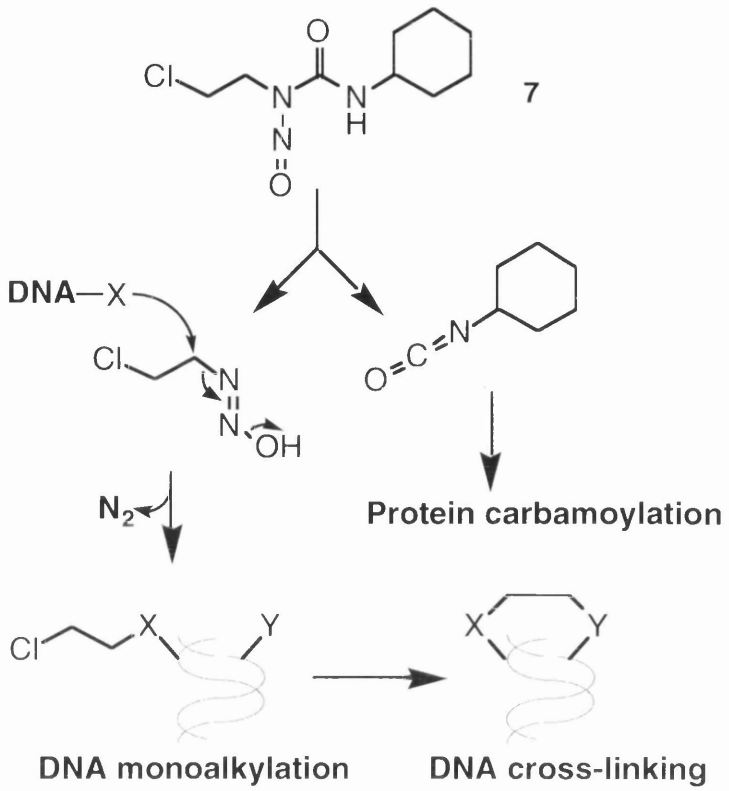
4 **5** **6**

Cyclophosphamide **6** was designed as a prodrug which would utilise the high concentrations of phosphoramidases at tumour sites to release the active drug at the required site of action. In practice, the drug was found not to be metabolised by tumour cells, actually undergoing initial metabolic activation by the liver. Further transformation affords an active phosphoramide drug and the side-product acrolein which is responsible for the bladder toxicity associated with cyclophosphamide chemotherapy. This compound remains to be an important drug, often used in combination with methotrexate and 5-fluorouracil for the treatment of a variety of cancers.

1.5.1.2 Nitrosoureas

Nitrosoureas, such as cyclohexylchloroethylnitrosourea (CCNU) **7**, are a success resulting from the U.S. National Cancer Institute's drug development programme. They have widespread alkylating activity and are known to inhibit DNA polymerase thus preventing the repair of DNA strand breaks. In this class the drugs are lipophilic and are able to cross the blood-brain barrier which has allowed their successful application to cerebral cancers. The mechanism of their activity is not as clear as that of the nitrogen mustards but it is known that their action is mediated by both alkylation and carbamoylation. Interestingly, despite the presence of only one chloroethyl group in CCNU, this and other nitrosoureas are known to be potent DNA cross-linkers. The simplified mechanism shown in Scheme 4 accounts for this.[15]

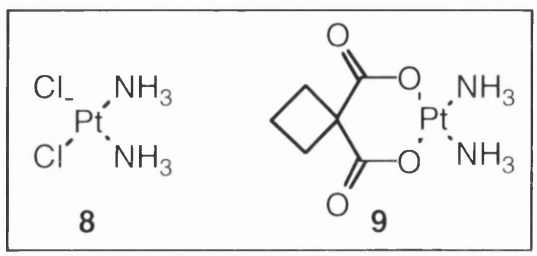
Scheme 4 Nitrosoureas: Mode of Action



The major clinical application of nitrosoureas is in the treatment of lymphomas. The bone marrow toxicity associated with them has precluded their inclusion in combination chemotherapeutic regimes.

1.5.1.3 Platinum Compounds

In 1965 Rosenburg *et al.* serendipitously discovered that the electrolysis products from a platinum electrode caused inhibition of bacterial division.[16] Four years later, the same research group observed the anticancer activity of the square planar complex cisplatin **8**,[17] now a major cytotoxic drug which has found particular application against testicular and ovarian cancers. Many analogues have been prepared in the hope of producing compounds which lack the severe toxicity of cisplatin. Of these, carboplatin **9** is now in clinical use and offers reduced side-effects towards the kidneys and nervous system.



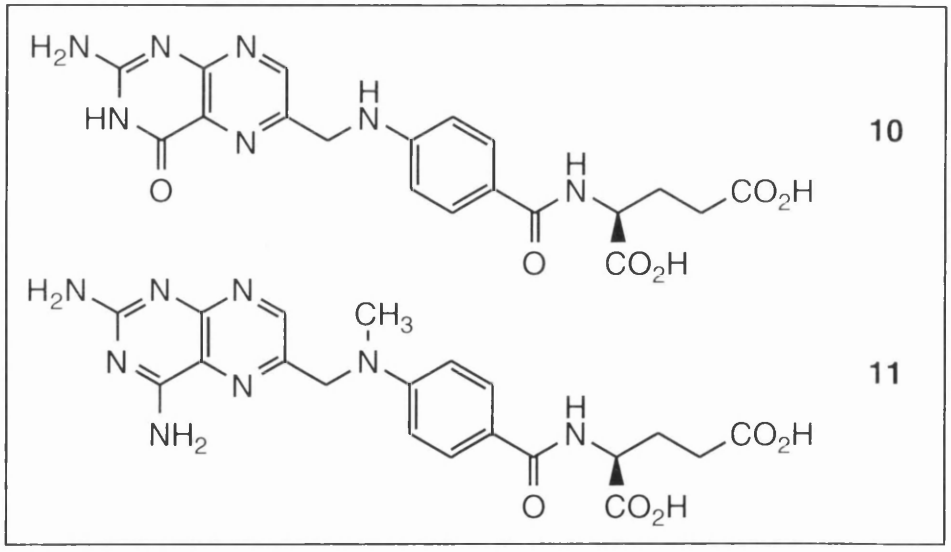
The action of cisplatin and its analogues is known to involve metallation of the bases of DNA nucleotides; a *cis* pair of labile ligands has been found to be essential. However, unlike the nitrogen mustards, the platinum anticancer drugs are thought to exert their effects by formation of crosslinks between bases on the *same* strand of DNA. This is thought to cause bending of the duplex towards the major groove resulting in inhibition of DNA replication.[18]

1.5.2 Antimetabolites

In preparation for mitosis, an accumulation of nucleic acid and protein is required to furnish the daughter cells. Antimetabolites are designed to interfere with this process by closely mimicking natural compounds thus causing enzyme inhibition or the accumulation of false components into nucleic acids. Two different types of inhibitor are discussed here.

1.5.2.1 Folate Inhibitors

Folic acid **10** is an essential nutrient which undergoes a two-step conversion by dihydrofolate reductase (DHFR) into 7,8-dihydrofolate and thence 5,6,7,8-tetrahydrofolate. The latter is required to generate several co-factors which are essential to purine biosynthesis and the formation of thymidine monophosphate (dTMP). Methotrexate **11** potently inhibits DHFR thus causing depletion of cellular tetrahydrofolate; this blocks nucleoside formation leading to a halt in DNA synthesis.

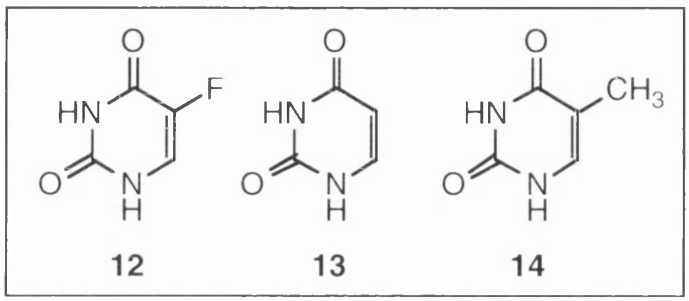


The administration of methotrexate is highly effective towards the treatment of choriocarcinoma. It has also found application in the treatment of acute leukaemia and many solid tumours.

1.5.2.2 Pyrimidine Antagonists

The development of this class of metabolite arose from an observation that rat hepatomas required increased quantities of the RNA pyrimidine base uracil **13** over normal liver cells. 5-Fluorouracil (5-FU) **12** was prepared and evaluated to test the theory that increased uracil uptake could be exploited to gain selectve chemotherapy.

5-FU is a close analogue of uracil **13** and thymidine **14**, the corresponding base in DNA. It is known to act by several mechanisms; each of which can result in cytotoxicity.[19]



5-FU can cause mutation from rapid metabolism and incorporation into DNA and RNA. Another important effect is due to its metabolite 5-fluoro-dUMP, which inhibits thymidylate synthase and thereby slows down DNA production by depletion of suitable thymine-containing precursors.[20]

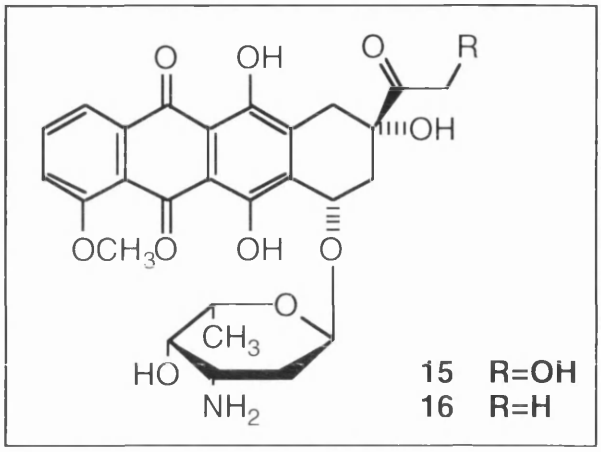
5-FU is used to treat several solid tumours, principally those of the breast and gastrointestinal tract. Application is often dependent on combination treatment with other anticancer agents or with certain non-toxic agents which are known to potentiate its activity.

1.5.3 Noncovalent DNA Binding Drugs

Most examples of this class of anticancer drug are derived from natural sources and also exhibit antibiotic activity. Structurally, they all contain a planar polycyclic moiety which is thought to interact with DNA in a different fashion to the alkylating agents. Activity is mediated through intercalation of the complementary strands of DNA in addition to other cytotoxic effects. Two of the clinically used groups will be discussed in this section.

1.5.3.1 Anthracycline Antibiotics

These antibiotics, typified by doxorubicin **15** and daunorubicin **16**, were isolated in the 1960s from different strains of *Streptomyces* fungi. They are characterised by a tetracyclic structure linked to the aminosugar daunosamine *via* a glycosidic bond.

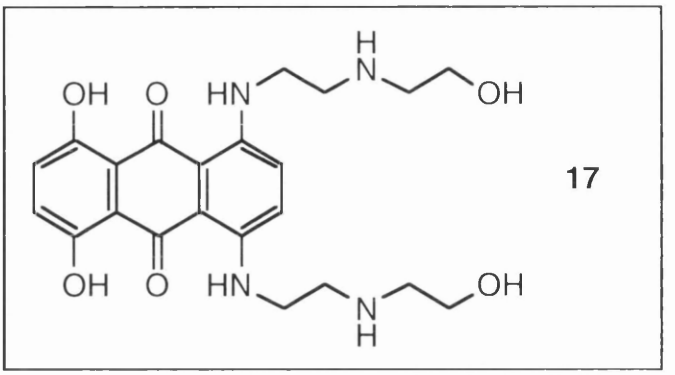


The mechanism of action of these agents has still not been fully elucidated but they are known to initiate their effects by tight intercalation between the strands of DNA. X-Ray crystallography of DNA adducts of anthracyclines has revealed that this is mediated through strong interaction between the hydrophobic planar faces of DNA bases and the tetracyclic core of the antibiotic. Once bound, the activity of these drugs involves inhibition of topoisomerase II,[21] a group of enzymes which normally direct strand breakage, uncoiling and annealing during DNA replication (see section 1.5.4.1). Another significant mode of action appears to involve the formation of iron chelates which are able to produce free radical and active oxygen species within the cell; these too can initiate DNA strand breaks and thus contribute to the overall cytotoxicity of anthracycline antibiotics.

Daunorubicin was initially found to have good activity towards leukaemias. Doxorubicin is more versatile and is an effective drug for the treatment of a wide variety of solid tumours.

1.5.3.2 Anthracenediones

Mitozantrone **17** is the most important member of this class of synthetic anticancer drugs. Structurally, it bears resemblance to the anthracyclines (*vide supra*) but does not contain a sugar conjugate.



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The anthracenediones act by a similar mechanism to that of the anthracycline antibiotics; interference with DNA strand reunion by topoisomerase II inhibition is believed to be the principal response. In contrast to the antibiotics, mitozantrone does not initiate the formation of free radicals which accounts for its lower toxicity to the heart.

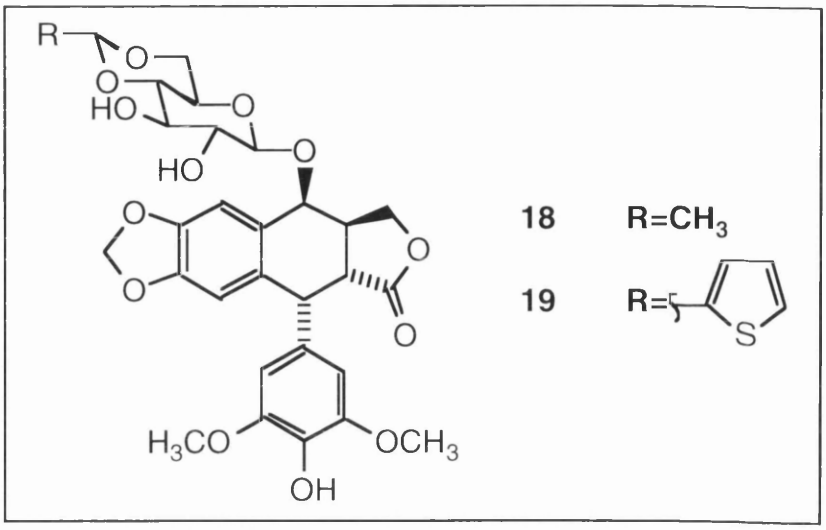
Clinically, the anthracenediones are employed to treat forms of lymphoma, leukaemia and advanced breast cancer.

1.5.4 Inhibitors of Chromatin Function

Throughout the cell cycle, chromosomes are required to undergo a series of major conformational changes in order to facilitate DNA replication and mitosis. Various enzymes are involved in these changes, particularly in the function of chromatin, in which DNA is decondensed and complexed with packing proteins called histones prior to replication. Two groups of agent have been found to be cytotoxic to proliferating cells by interfering with the enzymic manipulation of chromosomes.

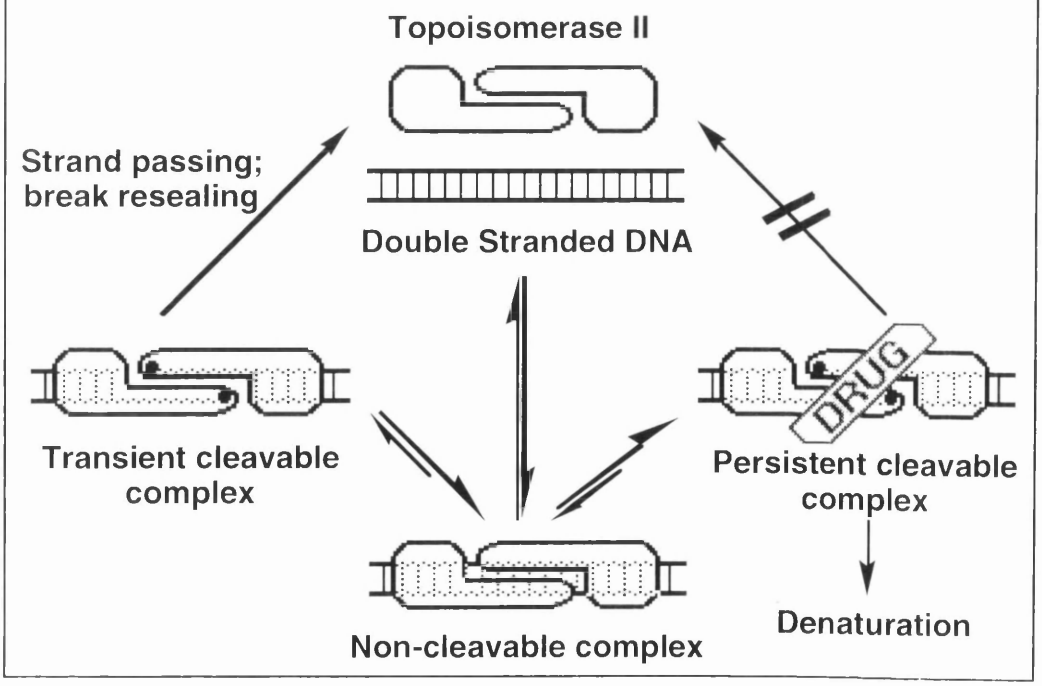
1.5.4.1 Topoisomerase Inhibitors

The two groups of topoisomerase enzymes (types I and II) are involved in the untwisting of DNA portions to make them available for vital functions such as transcription and replication. Of the commonly used drugs, cancer chemotherapy by inhibition of topoisomerase II is more common, although further agents are in development which are known to target the type-I enzymes. Important topoisomerase II inhibitors include the podophyllotoxin derivatives etoposide **18** and teniposide **19.**



The normal function of topoisomerase II involves initial non-covalent interaction with the two strands of DNA resulting in a non-cleavable complex, so called because dissociation of the enzyme-DNA complex at this stage would not result in strand breaks. After cleavage of the strands and covalent bonding to the free 5'-ends of the nucleic acid chains, a transient cleavable complex results. Figure 2[22] shows how etoposide inhibits topoisomerase II action by stabilising the cleavable complex thus preventing the completion of transcription or replication and the regeneration of the free enzyme and DNA.[23] The drug-stabilised persistent cleavable complex may then be denatured, giving rise to a DNA strand break.

Figure 2 DNA Damage by Inhibition of Topoisomerase II



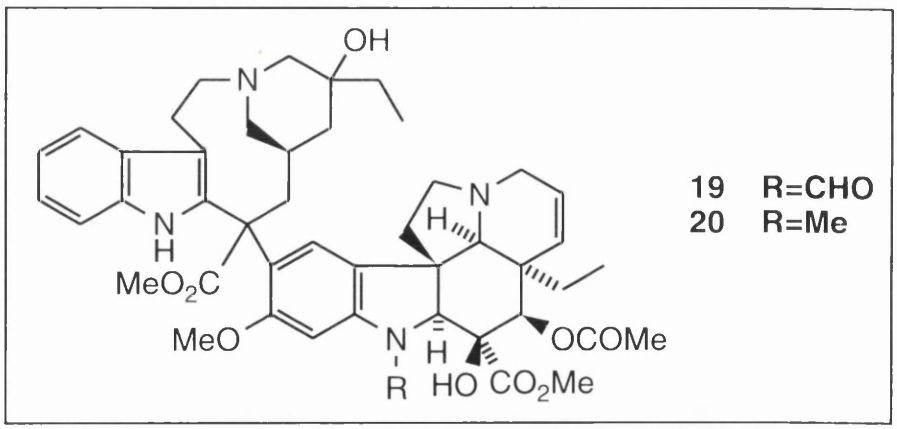
Another effect of these drugs appears to be the prevention of protein kinase p34 activation.[24] This kinase is usually activated during the G_2 phase of the cell cycle and has a pivotal role in the initiation of mitosis. The observation that etoposide treatment can confine cells to G_2 corroborates this theory.[25]

Etoposide has proved to be particularly useful in the chemotherapy of lung cancer and testicular cancer; combinational therapy with cisplatin and bleomycin is now the standard treatment for the latter condition. Teniposide has found utility in the treatment of adult brain tumours and acute lymphocytic leukaemia.

1.5.4.2 Microtubule Inhibitors

Microtubules are polymeric proteins which fulfil a variety of essential roles within the cell, predominantly in functions relating to cellular shape and movement. During mitosis, cellular microtubules form a spindle which directs the complex processes of chromosomal division which precede cytokinesis (cytoplasm cleavage). To function correctly, microtubules exist in a sensitive equilibrium with dimeric tubulin; by interfering with this balance, several natural products have been found to exert useful anticancer properties.

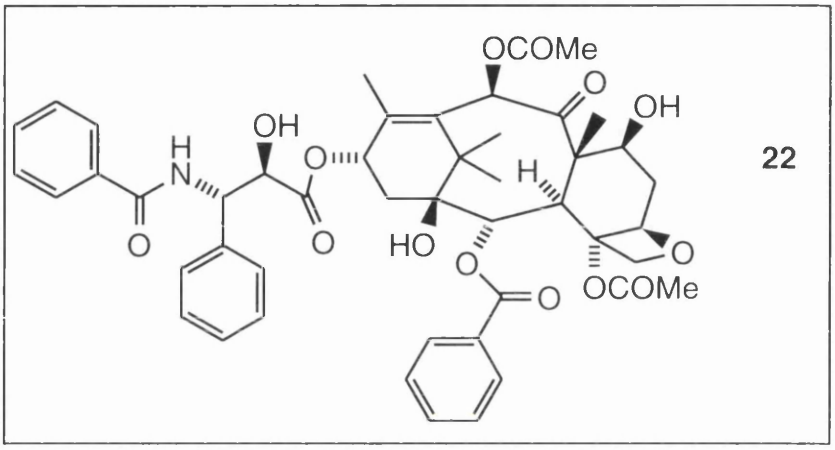
The vinca alkaloids vincristine **20** and vinblastine **21** were isolated from the periwinkle *Catharanthus rosea.* They are potent drugs which act by specifically complexing free tubulin dimers thereby causing depletion of the polymeric microtubules which form essential components of the cell. The anticancer properties are predominantly due to the destruction of the mitotic spindle which prevents the completion of cell division.



Vincristine has been applied to conditions such as acute leukaemias, lymphomas and breast cancer but its use is hampered by side-effects towards

the heart and nervous system. Vinblastine is comparatively less neurotoxic and has found application in lymphoma and cancers of the reproductive system.

The terpenoid derivative paclitaxel (Taxol®) **21** is a more recently discovered microtubule antagonist which has provoked much interest amongst cancer biologists and organic chemists; its total synthesis has been completed by the groups of Nicolaou,[26] Holton[27] and Danishefsky.[28] The compound was originally isolated from the bark of the yew *Taxus brevifolia* but is now generally obtained by partial synthesis from 10-deacetyl baccatin III, obtained from the needles of a related plant.



In contrast to the vinca alkaloids, the mechanism of paclitaxel's activity stems from its ability to tilt the tubulin-microtubule balance in the favour of microtubule formation. This causes depletion of tubulin dimers, the construction of abnormal bundles of microtubules and the stabilisation of the microtubule-containing components of the cell.[29]

Paclitaxel is being used investigationally in the treatment of breast and ovarian cancers. Difficulties such as bone marrow depression and poor solubility have led to the search for more suitable analogues.

1.5.5 Drugs Affecting Endocrine Function[30]

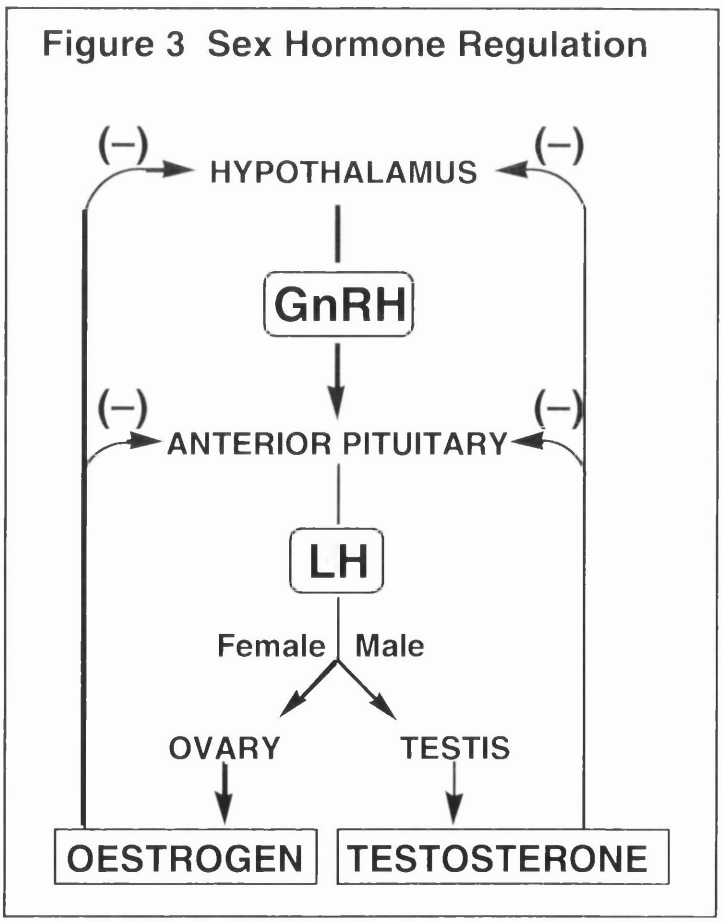
Cell proliferation in sexually differentiated tissues such as the endometrium, prostate and breast can be strongly influenced by the levels of related hormones in the body. In order to utilise this observation in chemotherapy, we must understand how a type of cancer is affected by a particular hormone. Tumours are termed hormone-responsive if increased hormone concentration gives rise to increased proliferation, hormone-dependent if growth is inhibited. In this section, the use of drugs affecting endocrine function will be illustrated by their application to the treatment of prostate cancer.

1.5.5.1 Prostate Cancer

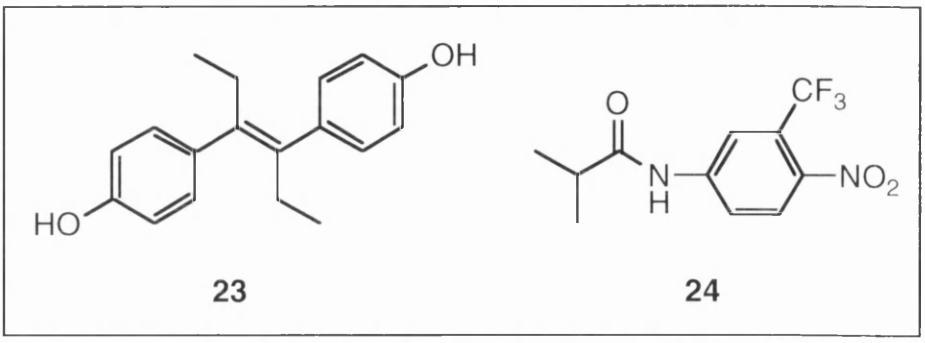
Control of cell proliferation in the prostate is affected by the levels of testosterone in the body; this hormone-dependent response may therefore be exploited chemotherapeutically to control the growth of cancer within this organ. Treatment involves the combinational use of drugs to inhibit both testosterone synthesis and action.

Testosterone is secreted by the Leydig cells of the testes in a process controlled by gonadotrophin releasing hormone (GnRH) and leuteinising hormone (LH), as shown in Figure 3. Feedback inhibition by the sex hormones controls GnRH and LH production. Therefore administration of oestrogens such as diethylstilbestrol **23** to prostate cancer patients decreases GnRH and LH formation which leads to the required fall in testosterone levels. As abnormally high oestrogen concentrations cause several undesired effects to the male body, another strategy involves the use of GnRH agonists to block GnRH receptors in the anterior pituitary. This prevents the release of LH, and hence, secretion of testosterone. Leuprolide and goserelin are now used clinically to mimic the decapeptide GnRH; the drugs are also decapeptides and work by competitive inhibition of the GnRH receptors in the anterior pituitary.

Figure 3 Sex Hormone Regulation



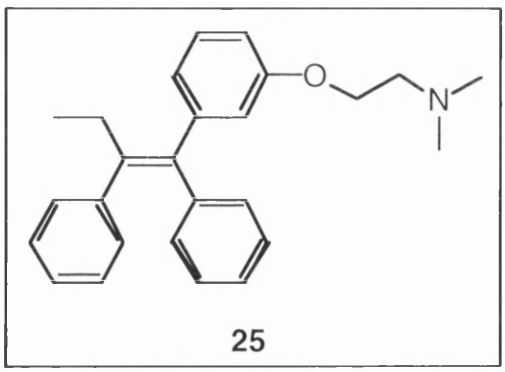
In addition to the inhibition of testosterone synthesis, adjuvant therapy is employed to block the effects of its active metabolite dihydrotestosterone. Flutamide **24** is a clinically employed antagonist which competitively inhibits androgenic receptors thus leading to a reduction in prostate growth.



23 **24**

Unfortunately, chemotherapy by androgen-suppression is never curative in cases of prostate cancer. However, in such a disease for which there is no particularly effective cytotoxic drug, hormone therapy can stabilise the condition thus providing a welcome relief from the symptoms of cancer.

A major success of hormonal anticancer therapy has been in the treatment of adenocarcinoma of the breast, a common cancer amongst European women. The drug of choice is the antioestrogen tamoxifen **25**, which is employed in early breast cancer and in the treatment of metastatic disease in post-menopausal women. In comparison to classical anticancer agents, administration of tamoxifen is relatively free of side-effects yet gives a similar therapeutic response rate to ablative removal of the oestrogen source by ovariectomy.[31]



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1.6 The Search for New Anticancer Drugs

The preceding sections have provided an overview of cancer and its treatment, with particular emphasis on the anticancer drugs and their mechanisms of action. The discovery of these compounds has occurred by a variety of means such as screening of chemicals and natural products, targeted synthesis of designed drugs, analogue studies of active compounds and, of course, serendipity. In future, drugs are likely to arise from all these areas, although more emphasis will be placed on the rational design of drugs to exploit the subtleties of cancer cells which differentiate them from normal cells. As advances in molecular biology emerge, they are accompanied by often elaborate chemotherapeutic approaches towards selective toxicity against cancer cells. Recent developments have included the use of enzyme-antibody conjugates to target cancers and selectively convert inactive prodrugs into cytotoxic drugs at the tumour sites. This regime is known as antibody-directed catalysis (ADC)[32] or antibody-directed prodrug therapy (ADEPT) and elegantly highlights the importance of progress towards better drug-delivery systems to compliment the discovery of new types of anticancer agent. In addition to chemotherapeutic advances, exciting developments have been made towards the search for a genetic cure for cancer through gene therapy. Ideally, this might involve the beneficial use of viruses to insert tumour suppressor genes into the DNA of cancer cells; proliferation could then give rise to cells which fight the cancer rather than propagate it.

It is hoped that in the 21st century, these and other sophisticated therapies will come to replace the harsh drugs which can make cancer chemotherapy such a traumatic experience. In the meantime, it must be borne in mind that epidemiologists estimate that 80% of cancers arise from preventable causes. It is even thought that certain foods can help prevent cancer; however, educating those most at risk to eat broccoli rather than deep-fried foods is not a simple task.

Chapter 2 details how the factors which differentiate malignant melanoma tumours from normal tissue may be exploited to generate selective chemotherapy towards that form of cancer. Chapters 3-6 describe the synthesis and properties of some new phenols which have been designed to utilise this strategy.

2

Melanoma: The Search for a Cure

2.1 Introduction

The rational development of new anticancer agents is driven by the search for biochemical features which differentiate tumour cells from their normal healthy counterparts. In the case of malignant melanoma, one such feature is the highly pigmented nature of most tumours, a characteristic that arises due to their continued production of melanin. Efforts that have been made towards the use of the enzymes of the melanin biosynthetic pathway in melanoma chemotherapy are discussed in this chapter.[32]

2.1.1 Skin Cancer

The unique position of the skin at the interface between the body and the outside world makes this, the largest organ, also the most vulnerable to the harmful effects of UV radiation. Those races most at risk have been furnished with skin containing large quantities of melanin, a pigment that absorbs the energy of solar radiation thus preventing it from inducing the DNA mutations that can lead to skin tumours. However, with increasing foreign travel, emigration and the use of sun-beds, greater numbers of people are becoming exposed to UV radiation at levels unsuitable to their skin type. Preventative measures, such as the use of sunscreens, are often neglected in the pursuit of a "beautiful" bronzed complexion. An unfortunate consequence is the increased incidence of skin cancers in all white-skinned populations of the world.

Non-melanoma skin cancers, such as basal cell carcinoma and squamous cell carcinoma are normally restricted in growth and do not readily metastasise. In these conditions, which make up the vast majority of skin cancers, surgery is a highly effective cure and affords success rates of greater than 95%.[33] This is in stark contrast to malignant melanoma of the skin, an extremely aggressive condition that is highly metastatic and difficult to treat satisfactorily.

2.1.2 The Melanoma Problem

Cutaneous malignant melanoma is a relatively rare condition, accounting for less than 3% of cancer cases in the industrialised nations.[34] In common with other skin cancers, the incidence of melanoma is rising rapidly in all areas of the world from which accurate data are available. Independent statistics from Norway, Australia and Scotland show that the occurrence of the condition in these areas is doubling every decade.[35] Mortality is also rising, albeit at a lower rate.

Efforts are being made to establish the genetic factors which can lead to the disease, but as yet, the root causes of melanoma are still to be confirmed. In common with the other types of skin cancer, much evidence points to UV radiation as the major preventable cause of melanoma. The following observations add weight to this hypothesis.

- Due to lower protection from the sun, melanoma is predominant amongst white-skinned people. For example, the incidence of melanoma in Queensland, Australia is 150 times that observed in Osaka, Japan.[36]

- Israeli orthodox Jews wear head coverings and dark clothing all year round which protects them from the sun and may explain their significantly lower rates of melanoma when compared to their non-orthodox counterparts.[37]

- Case control studies have revealed that instances of severe sunburn are a risk factor in the development of melanoma.[38]

- Women are five times more likely than men to develop melanoma on their legs, which may be due to clothing such as skirts and dresses providing inadequate protection from the sun. [38]

- Pale-skinned individuals who have fair hair and tend to burn rather than generate a protective tan on sun exposure are at greatest risk from melanoma.[39]

Despite the significant evidence that UV radiation is a principal cause of melanoma, some puzzling observations suggest that other factors are important too. For example, the incidence of melanoma in non-exposed regions of the anatomy such as the oesophagus, genitalia and the soles of the feet would appear to rule out carcinogenesis mediated by solar radiation in these cases. However, speculation about other possible causes of melanoma such as dietary

factors, fluorescent light, tobacco, arsenic and female reproductive factors have been met with controversy. [40]

Current knowledge suggests that the initiation of melanoma only requires episodic exposure to high levels of UV radiation. Moreover, the likelihood of developing the cancer appears to be determined more by the presence of freckles or moles rather than any environmental factors. For example, the occurrence of greater than 100 facial freckles in an individual has been reported by Holman and Armstrong to be associated with a 20-fold increase in the risk of developing melanoma.[41]

In the search for the cause and cure of melanoma, it must be borne in mind that there are several pathological forms of this disease. The four principal types of melanoma and their distinguishing features are outlined in Table 2.[42]

Table 2 Clinical Features of Common Melanoma Types

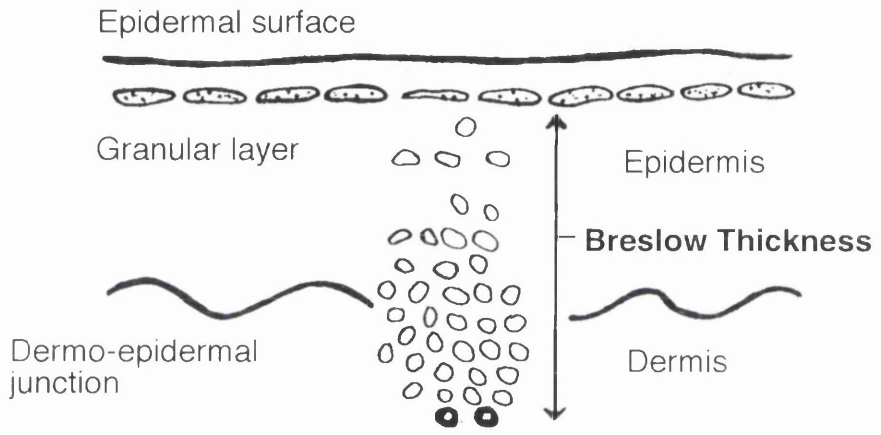
Clinical feature	Superficial spreading melanoma	Nodular melanoma	Lentigno maligna melanoma	Acral lentiginous melanoma
Frequency in whites	~70%	~10%	~10%	~10%
Typical site	All surfaces	All surfaces	Exposed areas: especially the face	Unexposed areas: e.g. soles of feet
Typical age at diagnosis	44	53	65	65
Shape of tumour	Distinctly palpable	Palpable: spheroid	Flat	Flat
Colour	Mosaic of brown, black and tan	Uniform bluish/black	Black, brown and tan	Black, brown and tan
Size of tumour	2.5 cm	1-2 cm	4-7 cm	3 cm
Growth pattern	2 phase Radial (1-12 yr) Vertical (Wks/Months)	1 phase Vertical (from outset)	2 phase. Radial (5-20 yr) Vertical (Wks/Months)	2 phase. Radial (1-10 yr) Vertical (Wks/Months)

Further study is needed to establish whether different causal factors are responsible for the different types of melanoma shown in Table 2. Clearly, lentigo maligna melanoma is more likely to be UV-dependent in origin than acral lentiginous melanoma which develops in sites generally protected from the sun. Despite the varying properties of these tumours, the prognosis in every case is dependent on one main factor; the degree of skin invasion.

Melanoma can be treated satisfactorily provided that metastasis to other tissues has not occurred. Spread of melanoma cells to other areas of the body arises by transport of aberrant cells through the lymphatic or circulatory systems. The likelihood of invasion to blood or lymphatic vessels, and hence the chance of survival from melanoma, is governed by the level to which

melanoma cells have invaded through the skin. In order to assess the prognosis of an individual melanoma case, the distance between the overlying granular layer of the epidermis to the deepest identifiable tumour cell is measured as shown in Figure 4.[43] This measurement, known as the Breslow thickness, is the principal factor used to postulate the likelihood of survival after surgical removal of the primary tumour.[43] For example, a Breslow thickness of greater than 3 mm implies a 5-year survival rate of less than 40%. In contrast, tumours possessing Breslow thicknesses of below 1 mm have good prognosis and are associated with 5-year survival rates of greater than 90%.[44]

Figure 4 Calculation of Tumour Thickness



2.1.3 Surgical Treatment

The extent of invasion and metastasis is used to classify melanoma cases into three clinical stages.[45]

Stage I	No clinically detectable lymph node metastasis.
Stage II	Clinically detectable lymph node metastasis.
Stage III	Distant metastases.

Melanoma is initially treated by surgical removal of the primary tumour and 2-5 cm of surrounding tissue; the clinical stage is used to determine the quantity of healthy tissue that must be excised. Local lymph nodes, which can mediate metastasis, are also removed in clinical stages II and III where pathological examination reveal that these have been contaminated by tumour cells. Some surgeons also carry out lymphadenectomy on stage I patients as microscopic examination of excised lymph nodes is useful in confirming the

prognosis. Even with appropriate surgery and additional therapy, the 5-year survival rate for stage III patients is almost 0%.[45]

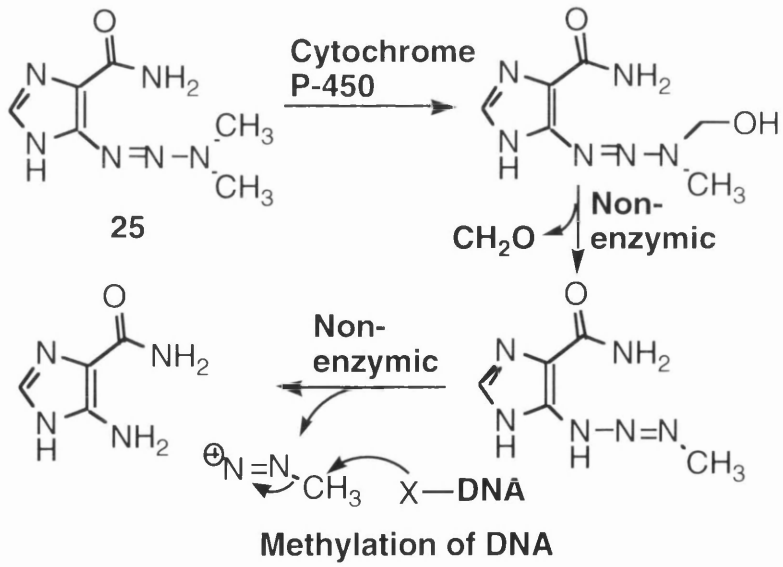
Radiotherapy is usually limited to certain cases of the rarer lentigo maligna melanoma form of the disease. Occasionally, this can also be applied to tumours on the extremities which lie away from internal organs that could be harmed by the therapy. However, these applications account for only a small proportion of melanoma cases with surgery being the best available treatment for the vast majority of these cancers.[46]

2.1.4 Current Chemotherapy

The lack of an effective drug to treat disseminated melanoma severely hampers the management of this disease.[47] All traditional cytotoxic agents afford low response rates, both as single agents or in combination. However, as there is no alternative, chemotherapeutic agents such as dacarbazine (DTIC) **25** are employed in advanced melanoma therapy. DTIC gives a positive effect in 20% of patients, although very few long-term cures arise from treatment. Nevertheless, single-agent DTIC is superior to any other drug regime in the fight against melanoma.[48]

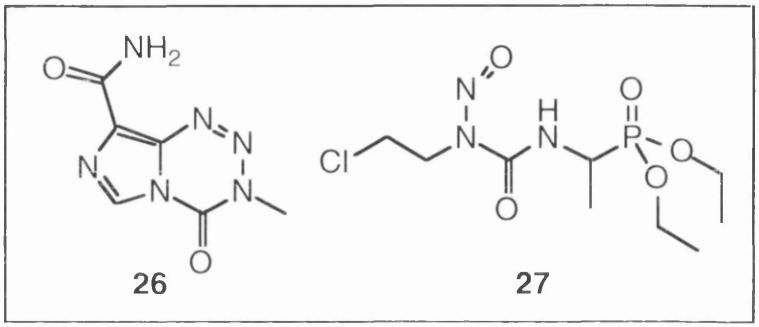
The anticancer activity of DTIC mainly arises by the mechanism shown in Scheme 5.[49] Metabolism by cytochrome P-450 in the liver followed by loss of formaldehyde ultimately leads to expulsion of methyldiazonium ions which mutate DNA and RNA by methylating nucleophilic sites such as guanine N-7.

Scheme 5 DTIC Metabolism



Of the newer anticancer agents, two compounds possess interesting activity against melanoma. In contrast to DTIC, temozolamide **26** can be administered orally and is not light sensitive. Stage II clinical trials have shown that this drug gives a comparable response rate to DTIC but offers greater convenience.[50] As temozolamide is an analogue of the active metabolite of DTIC, hepatic oxidation is not required for biological action.

In the last few years fotemustine **27** has emerged as the most promising new anti-melanoma agent. In addition to its unique ability to treat melanoma metastases in the brain,[51] this nitrosourea is as effective as DTIC in treating metastases in other areas. Phase III clinical trials may well lead to this compound being established as the drug of choice for the treatment of metastatic melanoma.



26 **27**

2.1.5 Immunotherapy

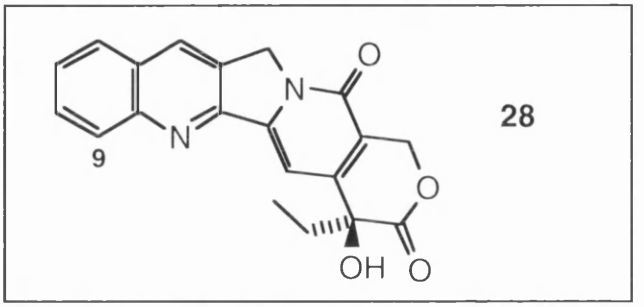
Interferons are naturally occurring proteins that are produced by the body to combat viruses and a variety of other foreign agents. Due to their potent effects on the immune system, interferons have been widely evaluated for antitumour activity.[52,53] One subspecies, IFN-α2, has been found to have useful properties against melanoma and was approved for this purpose by the United States F.D.A. in December 1995. Clinical trials revealed that its use in post-surgical therapy lead to a 42% improvement in relapse-free survival rate. Unfortunately, combination therapy employing IFN-α2 in conjunction with cytotoxic drugs such as DTIC has yielded mixed results, a situation that needs to be addressed by more comprehensive trials. Further work is also required to optimise the method and time-scale of interferon administration in order to maximise its activity towards melanoma.

2.1.6 New Strategies

Genetic therapy is currently being investigated in the fight against melanoma and clinical applications may soon be available.[48] Several strategies

are currently being studied, such as the transfer of genes which may lead to increased expression of interferons.[48]

Nature's storehouse of biologically active organic compounds continues to provide new leads in the fight against cancer. The alkaloid (+)-camptothecin **28** and its 9-nitro derivative have shown promising activity against melanoma cell lines.[54] Further studies are underway to evaluate fully their activity *in vivo*.



28

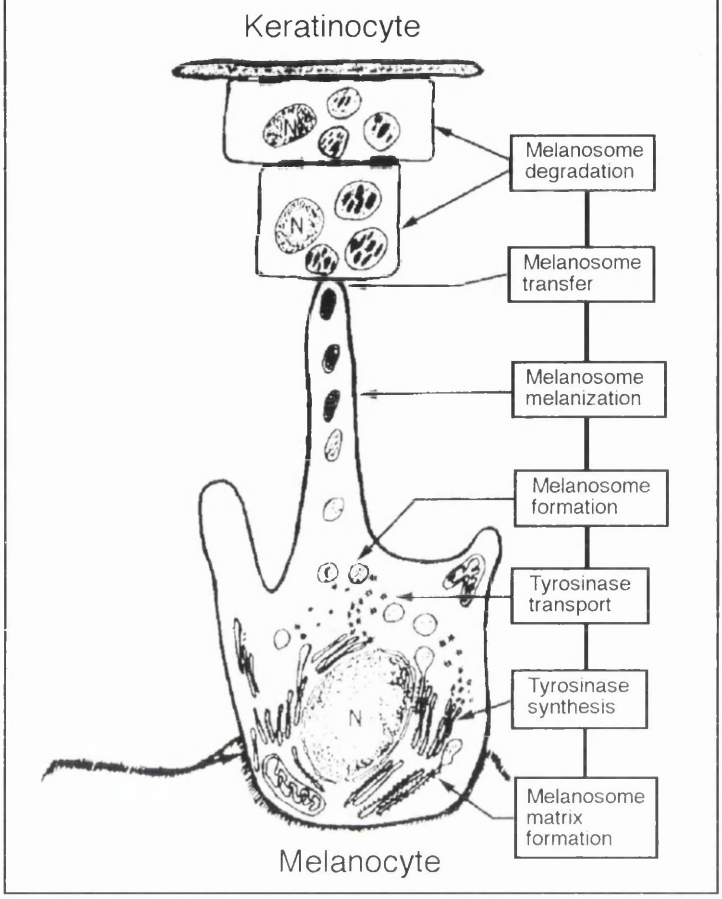
Increased melanin synthesis is a major characteristic that differentiates melanoma cells from normal healthy tissue. Attempts are being made to exploit this trait by utilising certain enzymes of the melanin synthetic pathway to generate active drugs at melanoma sites selectively. In the remainder of this chapter this rationale is explained in more detail, beginning with a discussion of melanin biosynthesis.

2.2 Melanin Biosynthesis

2.2.1 The Melanocyte

Melanoma tumours arise by aberration of pigment-producing cells called melanocytes (Figure 5).[55] These cells are associated with keratinocytes in the epidermis, a covering of epithelial tissue that forms the outermost layer of the skin. Melanin synthesis takes place by a complex series of reactions in the highly specialised melanosome organelle. As melanosomes are especially active in most melanoma cells, their unique biochemistry has been targeted as a strategy to exert cytotoxic effects selectively at these tumours.[56] Such chemotherapy would be particularly useful in the treatment of inoperable or even undetectable secondary tumours.

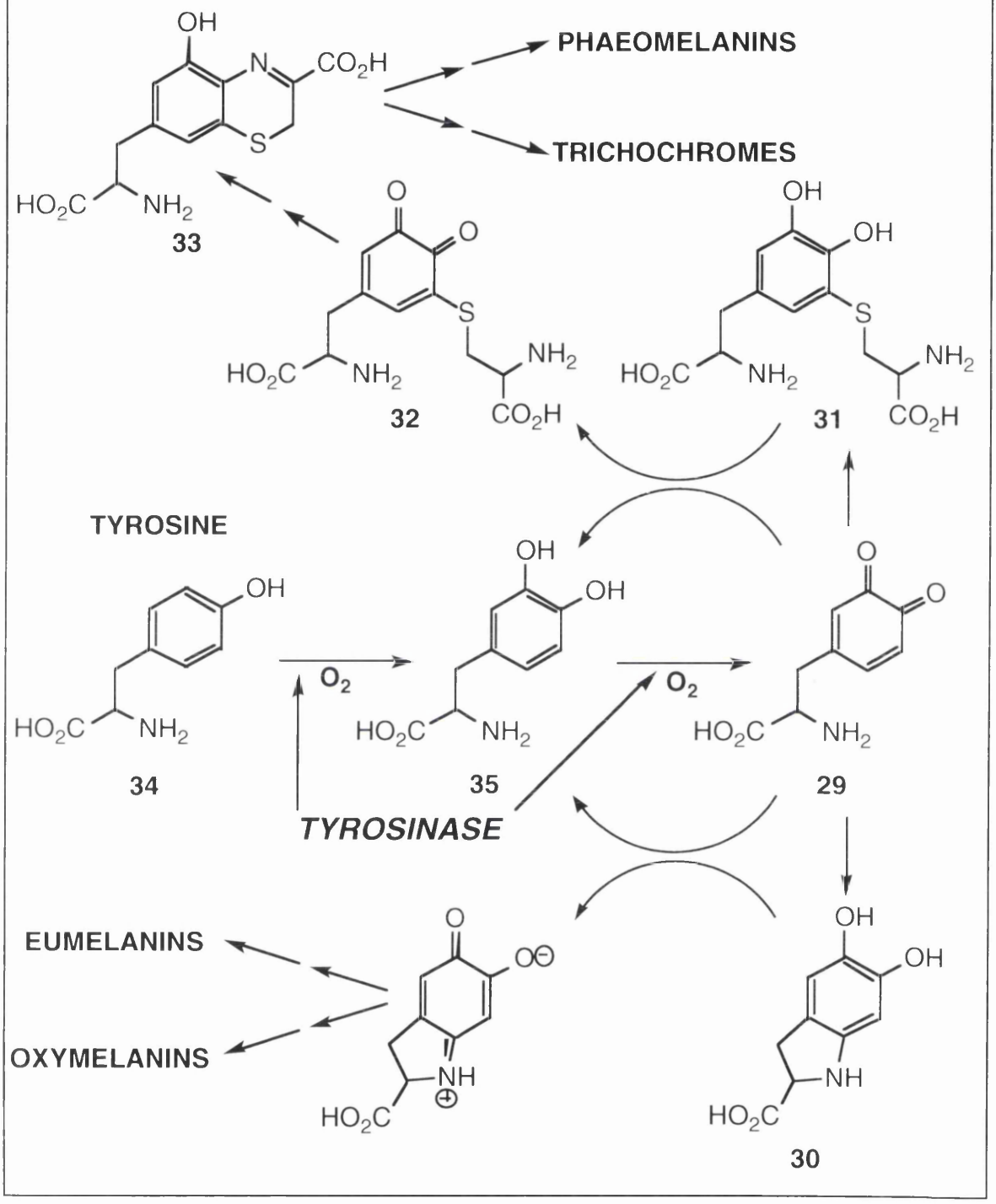
Figure 5 The Melanocyte



2.2.2 Biosynthetic pathway

The different colours of hair and skin pigmentation result from the blending of phaeomelanins, trichochromes, eumelanins and oxymelanins. These complex polymers arise from a cascade of enzyme-catalysed and spontaneous reactions from dopaquinone **29.** Scheme 6[57] shows how this substrate can undergo cyclisation to give cyclodopa **30** which affords, after further metabolism, the brown/black eumelanins and oxymelanins. Alternatively, dopaquinone can react with cysteine to give 5-*S*-cysdopa **31** and the minor product 2-*S*-cysdopa. These are then oxidised by dopaquinone to the corresponding cysdopaquinones (e.g. **32**) which after further elaboration *via* the benzothiazinylalanines (e.g. **33**), furnish the yellow/red phaeomelanins and trichochromes. The common substrate for all branches of melanin biosynthesis is tyrosine **34**. It is converted into dopa **35** and thence into dopaquinone **29** by the copper enzyme Tyrosinase.

Scheme 6 Early Stages of Melanin Biosynthesis



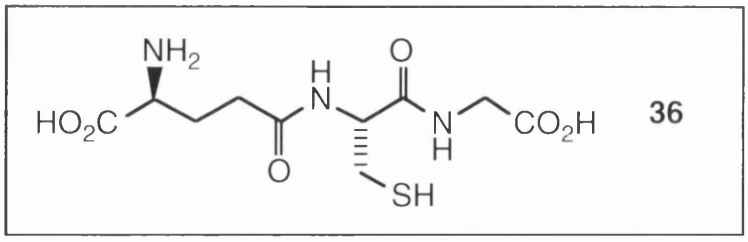
2.2.3 Tyrosinase

As shown in Scheme 6, Tyrosinase fulfils a pivotal role in melanogenesis by its unique ability to act as both phenol *ortho*-oxidase and catechol dehydrogenase by the conversion of tyrosine and dopa **35** into dopaquinone. The latter step also participates in a tandem shuttling mechanism where dopaquinone oxidises its daughter metabolites cysdopa and cyclodopa. This

reactivity of Tyrosinase towards phenols is akin to potassium nitrosodisulfonate (Frémy's salt), the stable radical compound that is widely used in the chemical laboratory to convert phenols into *ortho*-quinones.[58]

The enzyme is found exclusively in the melanosome thus making it an excellent biochemical marker for sites of melanin synthesis. Its essential role in melanogenesis, mediating an early sequence of conversions, ensures that high concentrations of the enzyme are always available. For this reason, Tyrosinase has attracted interest as a catalyst to aid the selective delivery of drugs to melanoma tumours. To achieve this goal, suitable Tyrosinase substrates must be designed which can be metabolised into powerful cytotoxic agents.

Scheme 6 showed how the Tyrosinase product dopaquinone is converted by endocyclisation, reaction with cysteine or reconversion into dopa. These processes help maintain low dopaquinone concentrations in order to suppress the toxicity that is associated with *ortho*-quinones.[56] Furthermore, other mechanisms are employed by melanocytes to prevent damage by these species such as the compartmentalisation of melanin biosynthesis into the melanosome organelle. This segregates dopaquinone thus preventing it from interfering with the essential cellular processes that occur in the cytosol or other organelles. The tripeptide glutathione **36** augments this by "mopping-up" any dopaquinone that may escape from the melanosome. Its action relies on nucleophilic attack on the quinone by the thiol group of the peptide's cysteine residue in a process that is directly analogous to the formation of cysdopa **31** (*vide supra*). In addition, the products from glutathione addition to dopaquinone undergo further conversion thus allowing them to rejoin melanin biosynthesis. This economical mechanism was long thought to be a major pathway to the synthesis of melanin. However, Karg *et al.* recently concluded that the role of glutathione is essentially protective, having little significance in normal melanogenesis.[59]



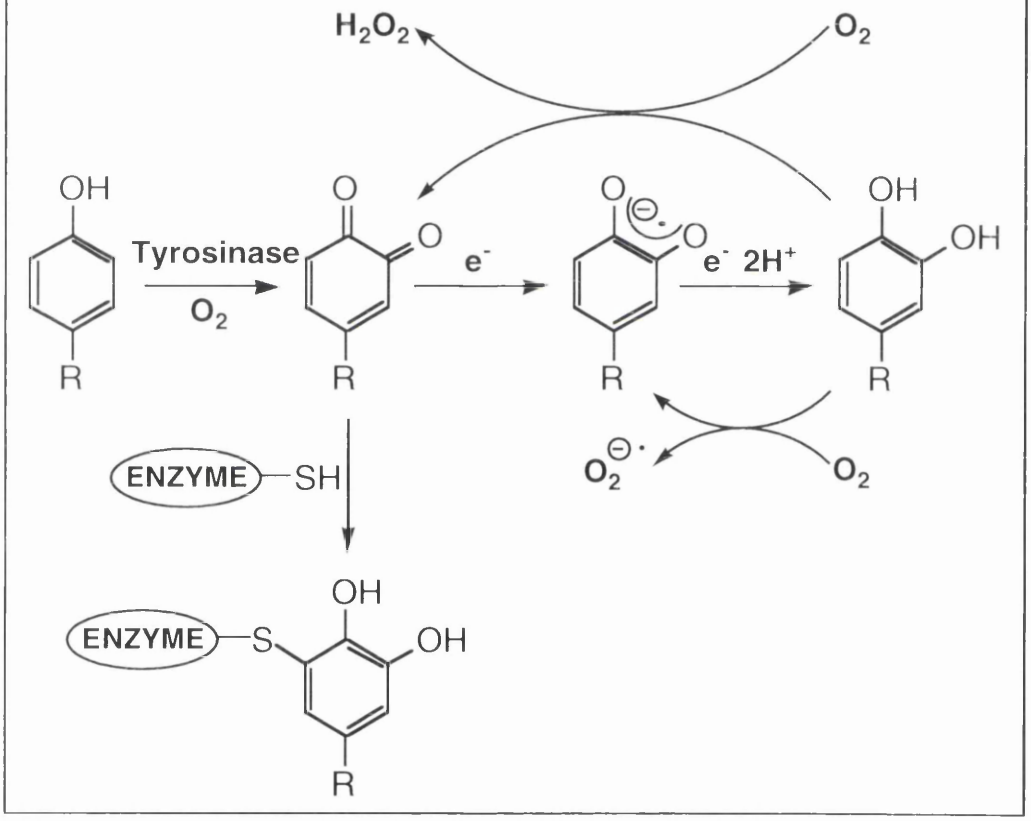
36

2.3 Drug Design

The search for a suitable Tyrosinase-catalysed drug regime for melanoma is aimed towards the selective generation of cytotoxic *ortho*-quinones at tumour sites. Dopaquinone is rapidly metabolised, therefore

alternative substrates must be designed that will be transformed into more stable quinones which could percolate from the melanosome and exert their cytotoxicity by attacking vital cellular components. As phenols and catechols are both potential substrates for Tyrosinase, both have been investigated for anti-melanoma activity. However, as early studies revealed that catechols cause indiscriminate toxicity through non-enzymic auto-oxidation,[60] most work in this area has been directed towards the synthesis and evaluation of phenols. Concurrently, studies have been made to reveal the toxic effects of resulting *ortho*-quinones. Scheme 7[57] illustrates how these are thought to be caused by the generation of active oxygen species and by alkylation of thiol-containing enzymes.

Scheme 7 Phenol Prodrugs: Mechanism of Action



In order to utilise this rationale in selective anti-melanoma activity, the designed prodrug would ideally have the following properties.

- Low toxicity before bio-conversion.
- Selective oxidation, exclusively by Tyrosinase.
- Sufficient water solubility to facilitate distribution in the bloodstream.
- Appropriate lipophilicity to allow diffusion into melanocytes.
- Favourable enzyme kinetics towards Tyrosinase.

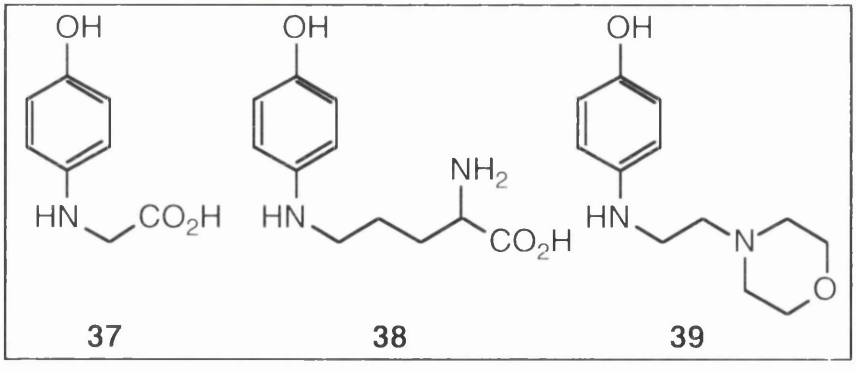
Effective biological activity is also reliant on the evolved drug possessing the following characteristics.

- Long lifetime.
- Capable of diffusion into the cytosol.
- High toxicity towards the cell

Three groups of tyrosine analogues have been prepared and evaluated towards meeting these criteria.[57] The results obtained from each structural type are discussed in the following sections.

2.3.1 4-Aminophenols

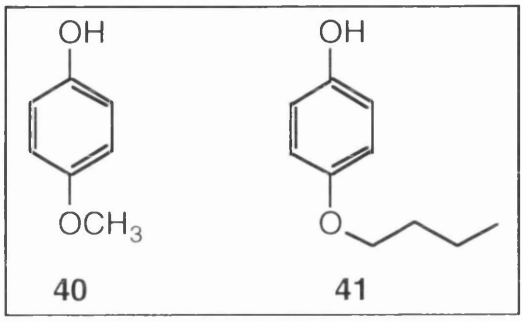
The evaluation of 4-aminophenols as anti-melanoma agents stems from reports that susceptibility to oxidation by Tyrosinase is controlled by the electronic properties of the substrate, in addition to steric factors.[61] Accordingly, the powerful electron-releasing amino group was incorporated into a range of tyrosine analogues in the hope that it would potentiate Tyrosinase activity. Studies by Kern *et al.*[62] and Mascagna *et al.*[63] have highlighted several active compounds such as *N*-4-hydroxyphenylglycine **37**, *N*-4-hydroxyphenylornithine **38** and *N*-2-morphilinoethyl-4-aminophenol **39**.



Mascagna *et al.* showed that oxidation of **37** by Tyrosinase leads to the formation of muconic semialdehyde derivatives through cleavage of the intermediate *ortho*-quinone. Furthermore, the same study concluded that non-Tyrosinase mediated oxidation competes leading to the generation of free aminophenol and glyoxylic acid.[64] The latter mechanism may account for the poor selectivity that is associated with these analogues.

2.3.2 4-(Alkylthio)phenols and 4-(Alkyloxy)phenols

Research in this area continues to be based on simple hydroquinone derivatives such as 4-hydroxyanisole **40**. Clinical trials showed that **40** was well-tolerated but its use was hampered by poor pharmacokinetics and non-selective activity.[56] In order to address these problems, Naish-Byfield *et al.* studied analogues containing a longer ether side-chain, such as **41**, and found that these exhibited different biological effects to **40**.[65] Furthermore, the observed toxicity of the compounds could not be satisfactorily correlated with the rate of oxidation by Tyrosinase.



40 **41**

More recently, the same group elegantly employed pulse radiolysis to generate *ortho*-quinones rapidly from analogues of **40** in order to measure their reactivity towards thiols such as cysteine and glutathione.[66] These studies confirmed that *ortho*-quinones derived from 4-(alkylthio)-phenols are 5-10 times more reactive towards thiols than those arising from 4-(alkyloxy)-phenols.[67] Quantitative Structure-Activity Relationship (QSAR) was used to forecast accurately the reactivity of *ortho*quinones towards selected thiols thus providing a useful numerical tool in the rational design of further analogues.[68]

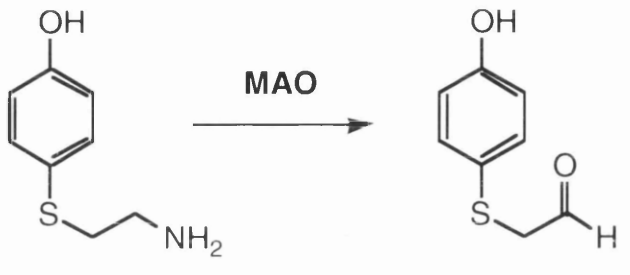
The consequences of varying the bridging atom in simple 4-hydroxyanisole analogues were recently studied in more detail by Riley *et al.*[69] They confirmed that good biological activity depends on the presence of a heteroatom substituent such as sulfur, oxygen or selenium at the 4-position of the phenol.

2.3.3 Analogues of 4-*S*-Cysteaminylphenol

The potential of 4-*S*-cysteaminylphenol **42** in the chemotherapy of malignant melanoma was initially reported by Jimbow and co-workers.[70] Preliminary findings revealed that the drug effectively caused depigmentation of new hair growth in mice (80%)[71] and significantly extended the life-span of melanoma-bearing mice.[72] Promisingly, examination of non-melanotic tissue by

electron microscopy showed no signs of damage, thus providing evidence for selectivity towards melanocytes.[73] However, further evaluation revealed a significant primary toxicity which resulted in a low LD_{50} value. Independent studies by May and co-workers inferred that non-selective action may be due to the enzyme monoamine oxidase (MAO).[74] They showed that **42** is an excellent substrate for MAO leading to the formation of the toxic aldehyde **43**, as shown in Scheme 8.

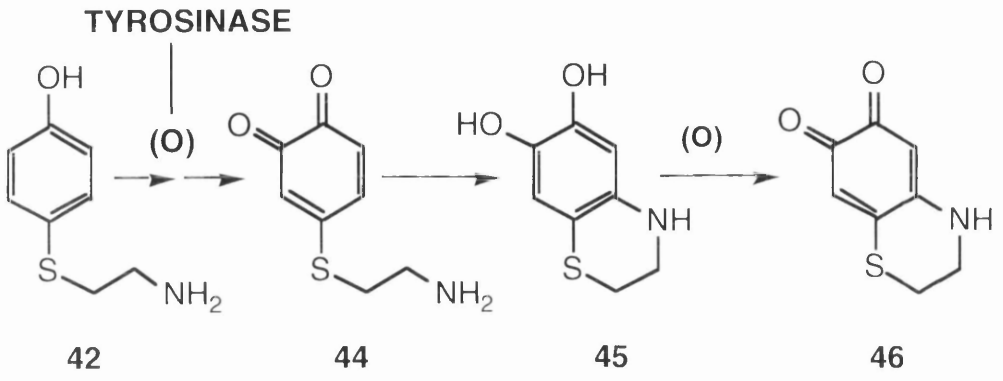
Scheme 8



42 **43**

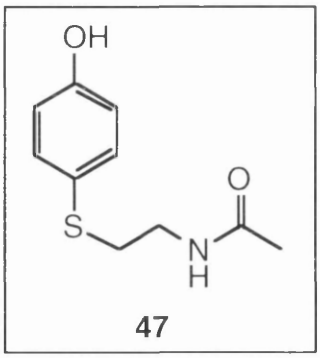
Herman *et al.* revealed that the major side-effect of **42** administration was antihypertensivity through interaction with dopamine β-hydroxylase.[75] Although these problems have precluded clinical interest in 4-SCAP, its mechanism of depigmentation continues to attract attention. Novel biomimetic studies by Mascagna *et al.*[76] provided evidence that it is oxidised by Tyrosinase to give a transient *ortho*-quinone **44**. Scheme 9 shows how this is rapidly attacked intramolecularly to afford **45** which, after auto-oxidation, gives dihydro-4*H*-1,4-benzothiazine-6,7-dione **46** in a similar manner to the previously discussed endocyclisation of dopaquinone. Hasegawa *et al.* recently confirmed that **46** is the active metabolite of **42**, providing evidence through reaction with thiol-containing peptides. Hence, **46** is likely to exert the cytotoxic effects of **42** through alkylation of essential sulfhydryl-containing enzymes.[77]

Scheme 9 4-*S*-CAP Metabolism



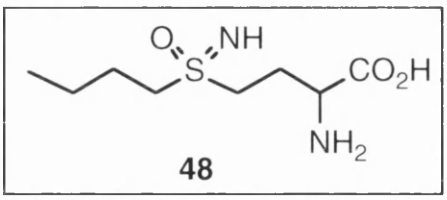
42 **44** **45** **46**

Alena *et al.* prepared several derivatives of **42** in order to prevent the troublesome MAO-mediated toxicity.[7 8] Of these, *N*-acetyl-4-*S*-cysteaminylphenol **47** proved to have the greatest de-pigmentation and anti-melanoma activity. In addition, this new compound selectively reduces the size of melanoma tumours and leads to a reduction in the size of lung melanoma metastases in mice.[78]



47

The biological activity of **47** analogues also involves formation of *ortho*-quinones, although endocyclisation is not possible due to the lack of a free amino group. As discussed in section 2.3.3, the melanocyte uses glutathione to prevent cellular damage by excess dopaquinone. As glutathione is non-selective in its action, it will also de-activate quinones derived from compounds such as **47**. Therefore, **47** must be surmounting this response to generate its anticancer activity. Alena *et al.* provided further evidence of glutathione's protective role by observing that the effects of **47** were greatly increased by co-treatment with D,L-buthionine-(*S,R*)-sulfoximine **48**.[79] The latter compound blocks glutathione synthesis by inhibition of γ-glutamyl cysteine synthetase and thus potentiates the activity of **47**.



48

Recent work by Thomas *et al.* has shown that **47** also elicits a non-Tyrosinase mediated cytotoxic effect through inhibition of poly-(ADP-ribose) polymerase.[80] As the evaluation of only a few analogues of **47** has been reported in the literature, there is an urgent need for a comprehensive chemistry-driven programme to examine the viability of melanoma treatment using this type of compound.

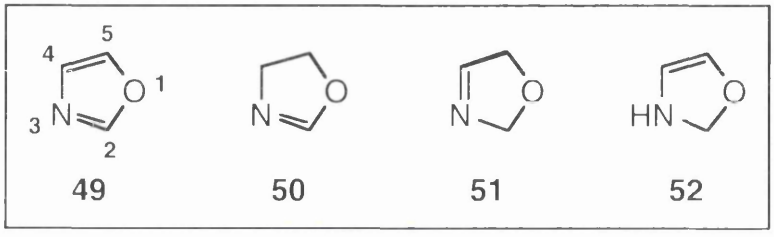
Chapters 4-7 constitute a study directed towards meeting that important goal through the design, synthesis and biological evaluation of over 70 target compounds. Most of the functionality present in **47** has been systematically manipulated in order to probe the structure-activity relationship of this type of agent. In Chapter 3 nucleophilic ring-opening reactions of oxazolines are discussed, a continuing theme throughout the thesis.

3

The Wehrmeister Reaction

3.1 Introduction

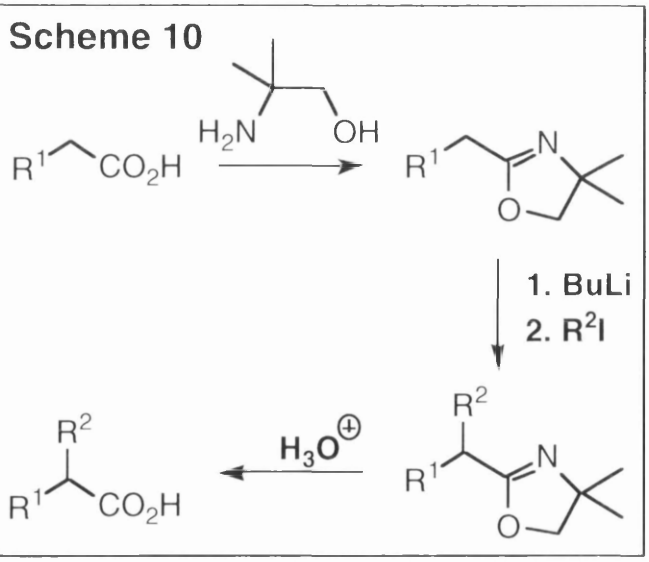
Oxazolines are dihydro derivatives of the oxazole **49** heteroaromatic system. Three regioisomeric types are known: 2-oxazoline **50**, 3-oxazoline **51** and 4-oxazoline **52**. Of these, only 2-oxazolines (4,5-dihydro-oxazoles) have found useful application in organic chemistry.



49 **50** **51** **52**

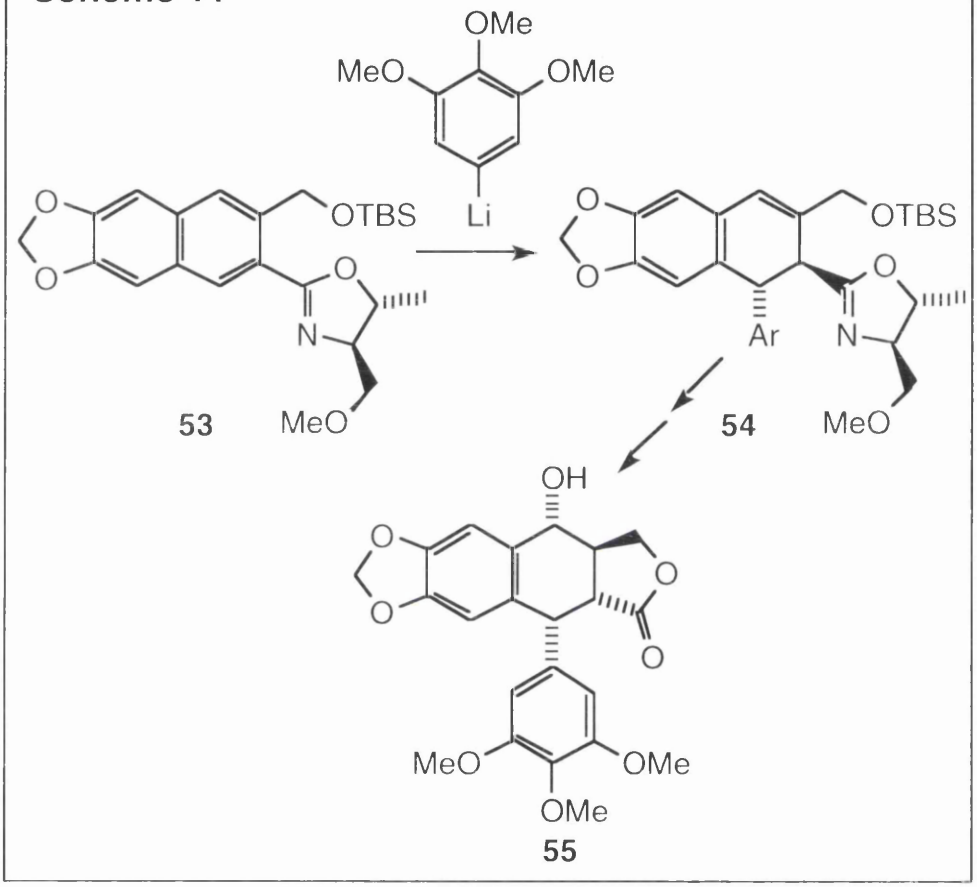
Substituted 2-oxazolines are particularly useful in their ability to act as latent or protected carboxyl or carbonyl groups. For example, carboxylic acids can be readily alkylated at the α-position indirectly by the procedure shown in Scheme 10.[81] The alkylation step utilises the base-stability of the heterocycle whereas the final deprotection step exploits its sensitivity to acid.

Scheme 10



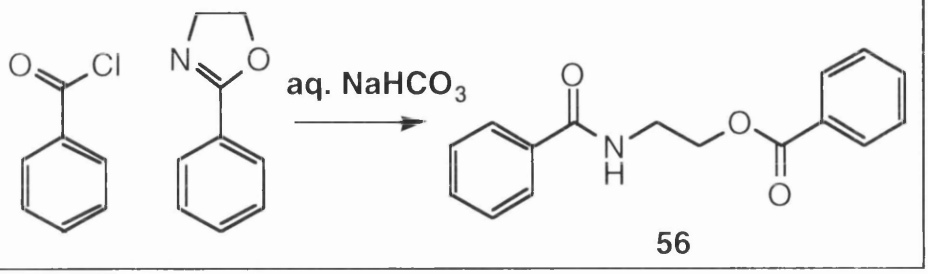
Pioneering work by Meyers and co-workers has led to the widespread use of 2-oxazolines in asymmetric synthesis. Readily available homochiral amino alcohols can be used to cause chiral induction after the formation of optically active auxiliaries. Andrews *et al.* used this methodology in the total synthesis of (-)-podophylleotoxin **55** (Scheme 11).[82] The key step involved aryl addition to the chiral oxazoline **53** to furnish **54** in 92% diastereomeric excess.

Scheme 11



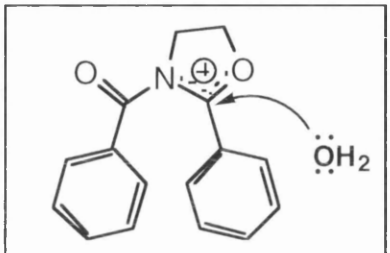
The numerous applications of 2-oxazolines and their methods of preparation have been comprehensively reviewed.[83-87] The purpose of this chapter is to focus on the use of this heterocycle in the synthesis of carboxamides through nucleophilic ring-opening reactions. This area of organic chemistry has developed from the initial observation by Goldberg and Kelly that treatment of benzoyl chloride with 2-phenyl-2-oxazoline in aqueous sodium bicarbonate affords amide **56** in good yield (Scheme 12).[88]

Scheme 12



56

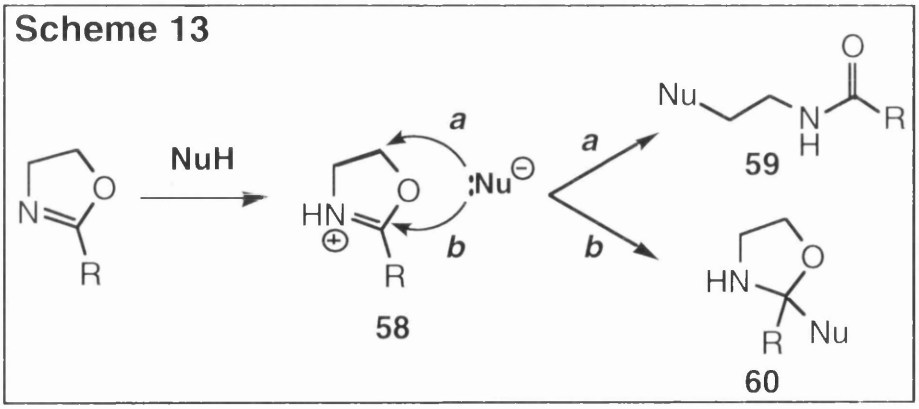
The mechanism of the reaction was studied by Fry[89] and also more recently by Tomalia and Paige[90] through the use of functionalised aromatic oxazolines and acid chlorides. It is known to progress through nucleophilic attack on the intermediate cation **57** by water.



57

Scheme 13 illustrates how similar cations **58** arise when oxazolines are treated with acidic substrates such as phenols or thiophenols. In principle, these ions can undergo nucleophilic attack at the 2-position to afford the oxazolidine **60** or attack at the 5-position with concomitant ring-opening to give carboxamide **59**.

Scheme 13



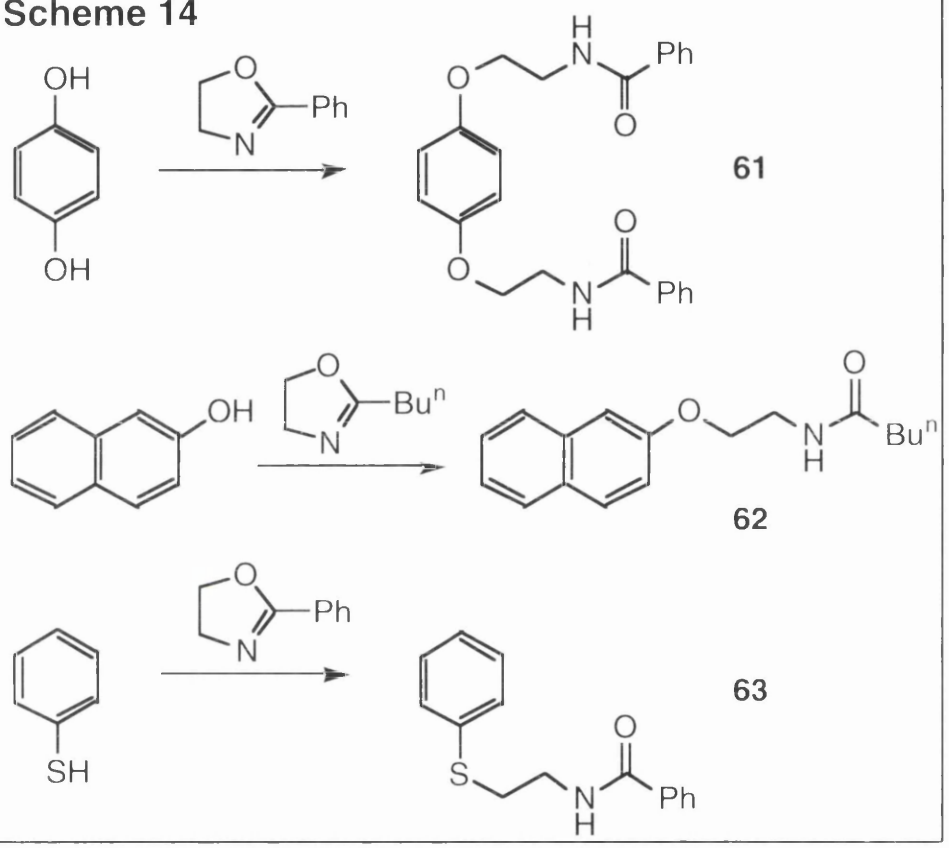
58 59 60

The use of nucleophilic oxazoline ring-opening reactions in synthesis will be discussed in the following sections.

3.2 Wehrmeister Reaction

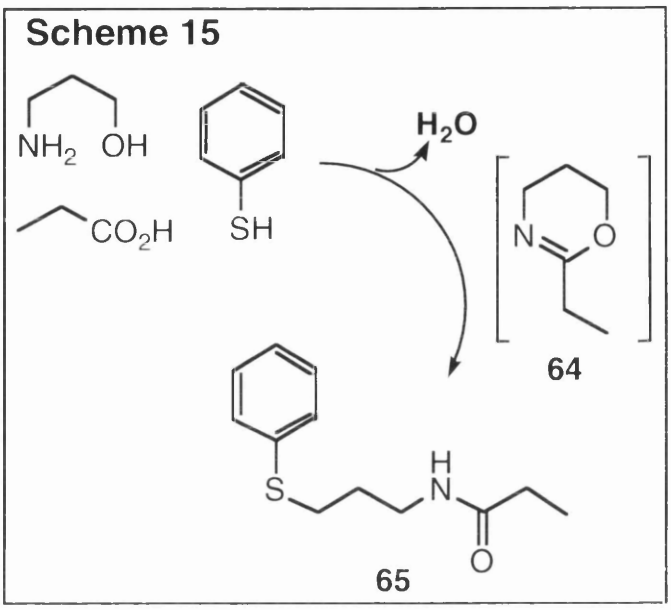
In 1959, Jäger first reported the use of phenols and thiophenols to effect ring-opening reactions of oxazolines.[91] Several examples were given in the patent, including the high-yielding syntheses of amides **61**, **62** and **63** (Scheme 14).

Scheme 14

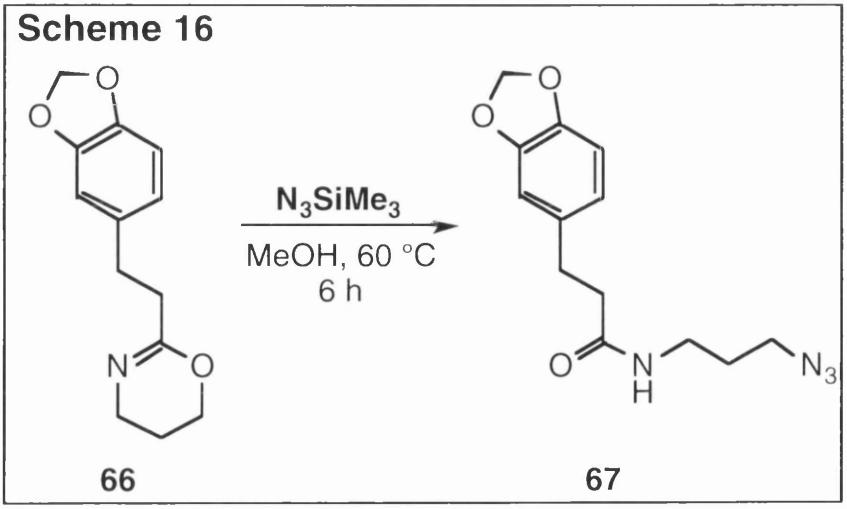


Independently, Wehrmeister published similar findings in 1963 although studies were confined to the use of thiophenols.[92] Perhaps unfairly, this method of preparing carboxamides has become known as the Wehrmeister reaction on the basis of this later work.

In a subsequent report Wehrmeister stated that isolation of the intermediate oxazoline was not necessary.[93] The same study also extended the utility of the reaction to the preparation of amides with one extra carbon between the sulfur and nitrogen in the side chain. For example, 3-amino-1-propanol was treated with molar equivalent quantities of thiophenol and propanoic acid in toluene with azeotropic distillation of water to give amide **65** in a one pot procedure. Scheme 15 shows the course of the reaction, presumably *via* the 5,6-dihydro-oxazine **64**. Evidence that a cyclic intermediate is involved was provided by the observation that 4-amino-1-propanol does not react in a similar fashion.[93]

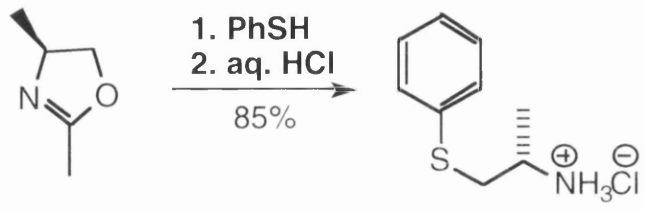


In 1984, Saito *et al.* published a communication claiming to have discovered a new method of preparing carboxamides through the use of 2-oxazolines.[94] The experimental procedure reported was essentially the same as that of Jäger[91] and Wehrmeister[92] yet no citation to the earlier work was made. However, the paper of Saito *et al.*[94] does complement the previous studies by establishing that selenophenols are also good substrates for the Wehrmeister reaction. Moreover, it was concluded that 2-oxazolines can successfully undergo nucleophilic ring-opening by azide or chloride ions through the use of azidotrimethylsilane or chlorotrimethylsilane, respectively. As an example, the 5,6-dihydro-oxazine **66** was used to prepare substituted azide **67** in 74% yield as shown in Scheme 16. The product was required as a building block in the synthesis of kukoamine A.[94]



The Wehrmeister reaction has predominantly been employed in the area of medicinal chemistry. May and co-workers used a commercially available homochiral amino alcohol to prepare (*S*)-2,4-dimethyl-2-oxazoline **68**.[95] Treatment of this with thiophenol gave, after hydrolysis, the potent antihypertensive agent **69** as shown in Scheme 17. The (*R*) enantiomer of **69** was prepared by a similar route and was found to have no antihypertensive activity due to its poor affinity for dopamine β-monooxygenase.

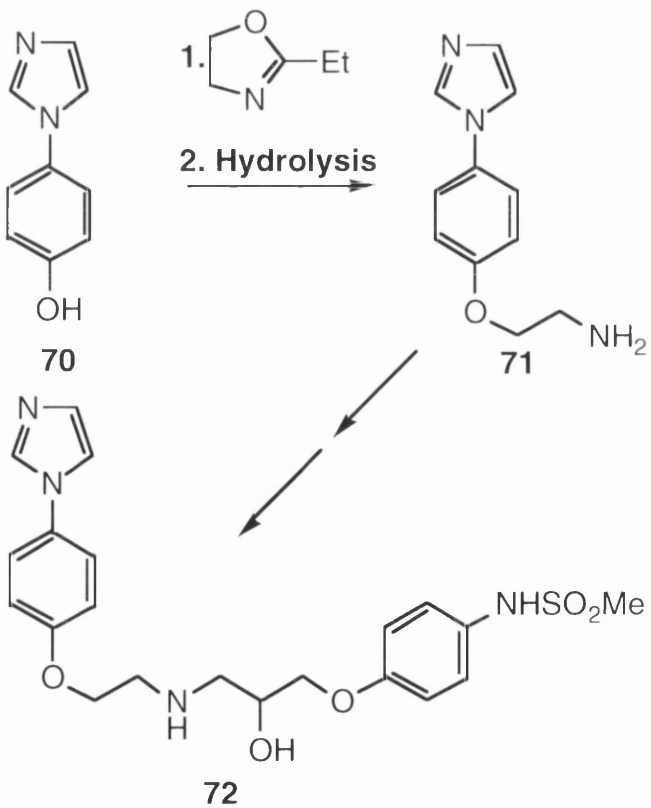
Scheme 17



68 **69**

Morgan and co-workers recently used the nucleophilic ring-opening of 2-ethyl-2-oxazoline by phenol **70** to synthesise novel antiarrhythmic agents such as **72** (Scheme 18).[96,97] Acid hydrolysis of the amide from the Wehrmeister reaction furnished the required amine intermediate **71** in good yield.

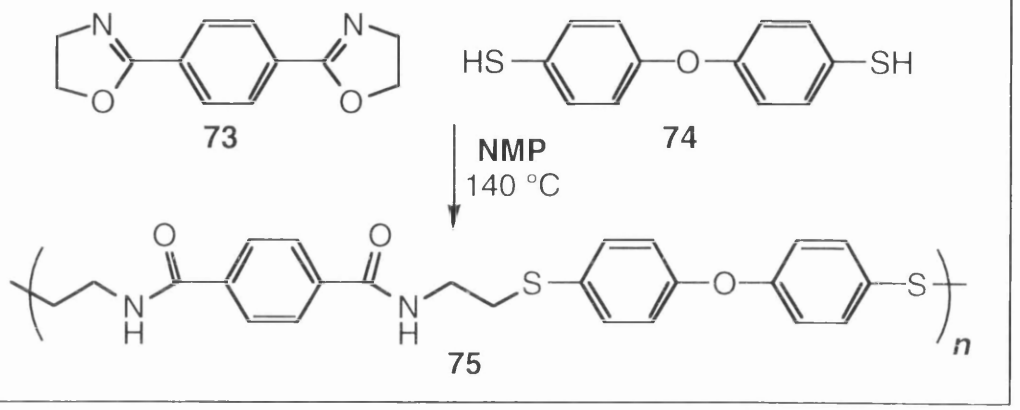
Scheme 18



70 **71** **72**

The Wehrmeister reaction has also been applied to polymerisation reactions through the use of bis-oxazolines and dithiols. Scheme 19 shows how Nishikubo *et al.* prepaped polyamide **75** in almost quantative yield by heating a mixture of co-monomers **73** and **74** in *N*-methylpyrrolidine (NMP).[98]

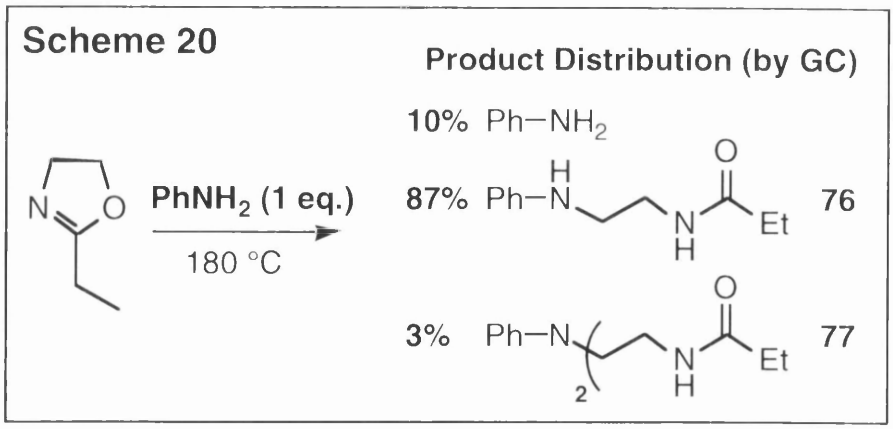
Scheme 19



3.3 Ring-Opening by Aromatic Amines

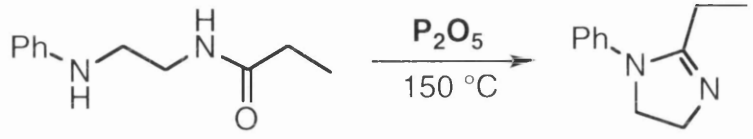
Treatment of 2-oxazolines with aromatic amines or their hydrochloride salts is known to give a mixture of products. Fazio reported that the treatment of aniline with an equimolar amount of 2-ethyl-2-oxazoline under acid catalysis gave a mixture of mono and bis(amidoethylated) compounds **76** and **77** in the ratios shown in Scheme 20.[99]

Scheme 20



Seeliger *et al.* previously employed this reaction to prepare intermediates for the synthesis of substituted imidazolines.[84] Scheme 21 shows how dihydroimidazole **78** was prepared in good yield by dehydration of carboxamide **76** using phosphorus pentoxide.

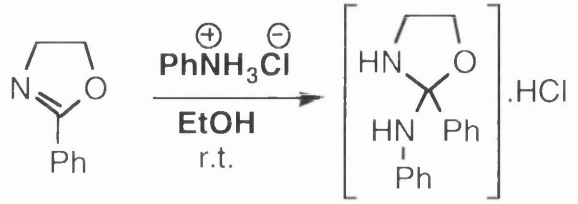
Scheme 21



76 **78**

Treatment of oxazolines with aromatic amines has also been reported to generate alternative products by nucleophilic addition to the 2-position of the heterocycle. Kormendy *et al.* prepared amino-oxazolidine **79** by treatment of aniline hydrochloride with one equivalent of 2-phenyl-2-oxazoline under the conditions shown in Scheme 22.[100]

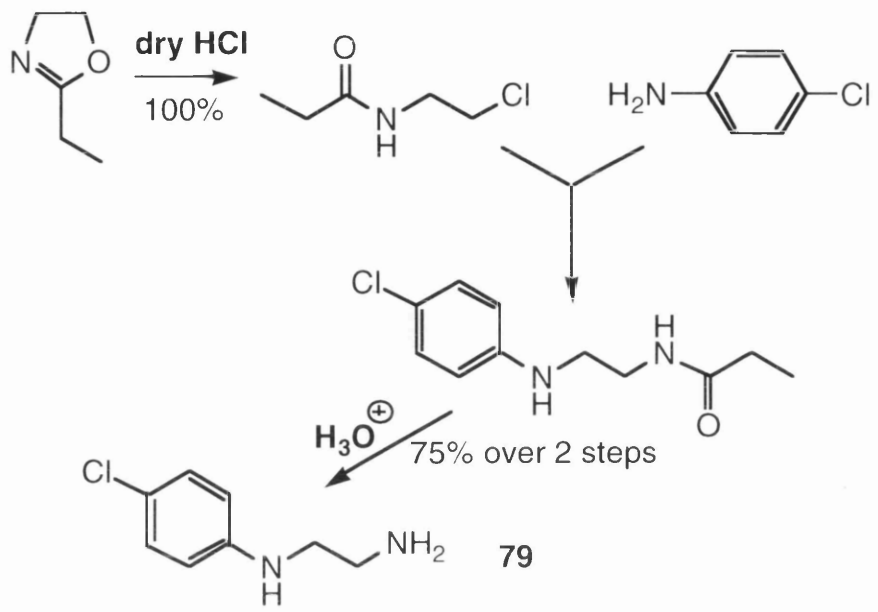
Scheme 22



79

Studies by Hünig have revealed that amino-oxazolidines and carboxamides are the kinetic and thermodynamic products, respectively, from the reaction of oxazolines with aromatic amines.[101] However, the reversible nature of aminooxazolidine formation can, under the correct conditions, allow amides to be preferentially formed using this procedure. Nonetheless, Poindexter devised an alternative strategy to avoid this side-reaction whereby oxazolines were treated with anhydrous hydrogen chloride to prepare *N*-chloroethyl carboxamides.[102] These were reacted with aromatic amines to give amides and (after hydrolysis) diamines in good yield. Scheme 23 shows how the process was exemplified by the preparation of aromatic diamine **80** thus offering an alternative to the use of highly-toxic aziridine, the classical reagent for ethanediamine synthesis.[102]

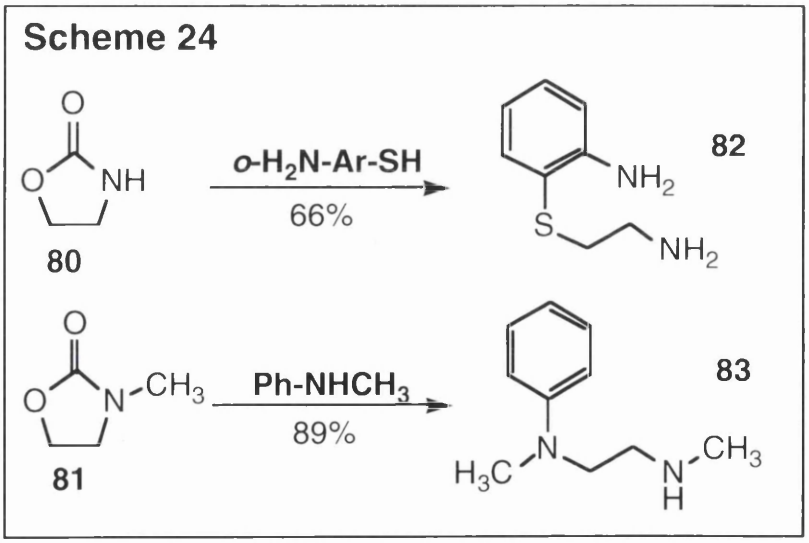
Scheme 23



3.4 New Methods

Despite the success of the above methodology in the synthesis of ethanediamines, a more convenient method for their preparation was recently disclosed by Poindexter *et al.*[103] It was found that phenols, thiophenols and aromatic amines could be effectively aminoethylated using 2-oxazolidinone **80** and its *N*-alkyl derivatives such as **81**. Scheme 24 illustrates the application of this new strategy to the syntheses of amines **82** and **83**.

Scheme 24



Unlike ring-opening of oxazolines, this reaction could not be extended to the use of oxazolidinones bearing substituents on the ring carbon atoms.[103]

Therefore both of these reaction types are of complementary value to the synthetic organic chemist.

The use of oxazoline ring-opening reactions in the synthesis of some new phenolic anticancer drugs will be described in the following chapters.

4

Synthesis of New Phenolic Sulfides

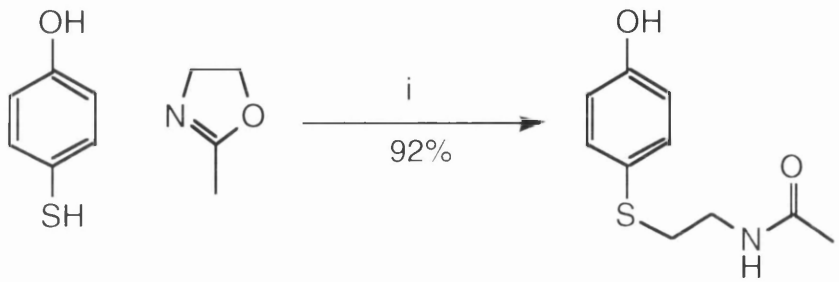
4.1 Introduction

Our interest in the area of anti-melanoma drugs developed from discussions with Professor R.M. MacKie of Glasgow University's department of Dermatology. Her group required samples of 4-*S*-cysteaminylphenol **42** and *N*-acetyl-4-*S*-cysteaminylphenol **47** for their own studies and we were keen to assist by synthesising these drugs. As discussed in the previous chapter, these compounds have provoked much attention through their ability to mediate selective melanoma chemotherapy by targeting melanin biosynthesis. However, despite comprehensive biological studies of **47**, our literature search revealed very little by way of medicinal chemistry in this area. Towards meeting this need, some preliminary work was carried out within our group as part of the Ph.D. studies of Paul McKeown.[104] The interesting biological results obtained in that study provided the basis for the chemistry discussed in this chapter.

4.2 Synthesis of Compound 47

The phenol **47** was initially prepared by Padgette *et al.* for evaluation as an antihypertensive agent.[105] By the use of the Wehrmeister reaction, 4-hydroxythiophenol was treated with 2-methyl-2-oxazoline to afford the desired amide in 92% yield (Scheme 25). The high yield reported demonstrates the high selectivity for thiophenols over phenols in the ring-opening reactions of oxazolines.

Scheme 25

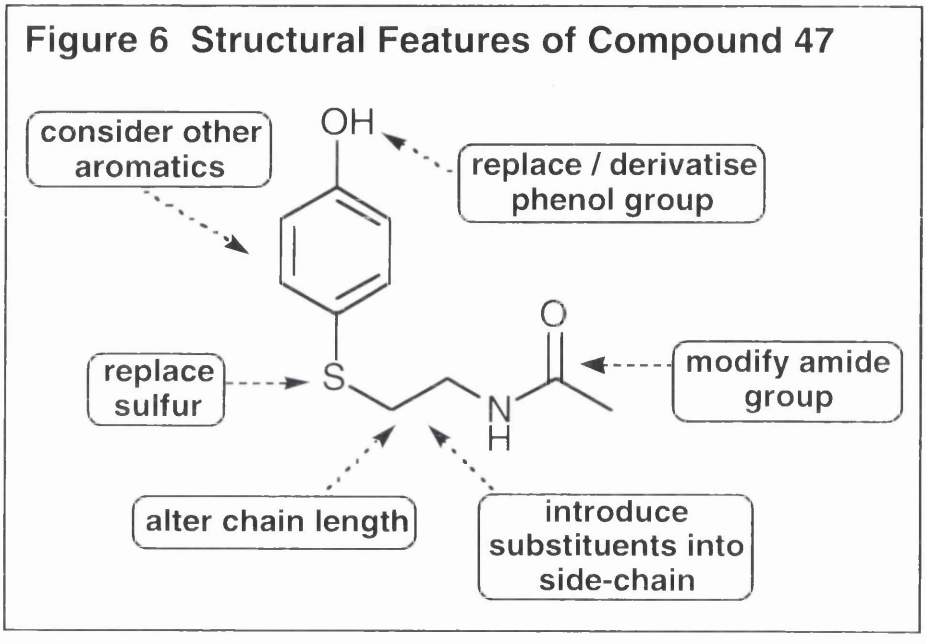


i. Neat, 120 °C, 2 h, N_2.

47

We began work in this area by preparing **47** in 87% yield by the procedure of Padgette *et al.*[105] Our spectroscopic data corresponded well with those reported in the literature.[105] At this point we decided which structural changes would be required in order to probe the structure-activity relationship (SAR) of **47**. As the drug is believed to act by mimicking tyrosine, we were particularly keen to justify structural features such as the amide and sulfide groups. These and other proposed areas of investigation are illustrated in Figure 6.

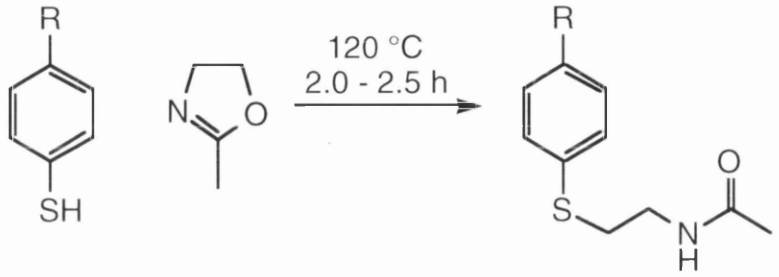
Figure 6 Structural Features of Compound 47



4.3 The Phenol Group

As **47** is thought to exert its cytotoxic effects through oxidation by Tyrosinase, it seemed likely that the phenol group would be essential to anti-melanoma activity. To help confirm this theory, a series of amides **84-87** were prepared by treatment of the appropriate thiophenols with 2-methyl-2-oxazoline (Scheme 26).

Scheme 26

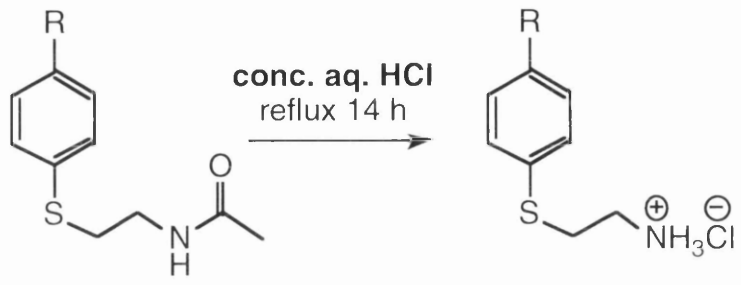


Product	R	Time (h.)	Yield
84	H	2.0	82%
85	OCH_3	2.5	76%
86	CH_3	2.5	82%
87	Cl	2.0	64%

Two of the four products shown in Scheme 26 have been previously reported in the literature. Bewick *et al.* prepared sulfide **84** by a Wehrmeister reaction although different reaction conditions were used.[106] By heating the two reagents together in refluxing acetonitrile with a catalytic amount of *p*-toluenesulfonic acid, they obtained an 85% yield of **84**. Our own analytical data were in good accordance with those reported.[106] McCormack and McElhinney prepared **86** by a multi-step route and gave a similar m.p. to that of our own sample.[107] As no spectroscopic data were given, full data are presented in chapter 7.

In addition to analogues containing an amide group, we were also interested in preparing some analogues of 4-*S*-cysteaminylphenol **42**. The parent compound **42** was prepared by the acid-catalysed hydrolysis of **47**, as described by Padgette *et al.*[105] Known amines **88** and **89** were also prepared in a similar manner by hydrolysis of **84** and **87** (Scheme 27).

Scheme 27

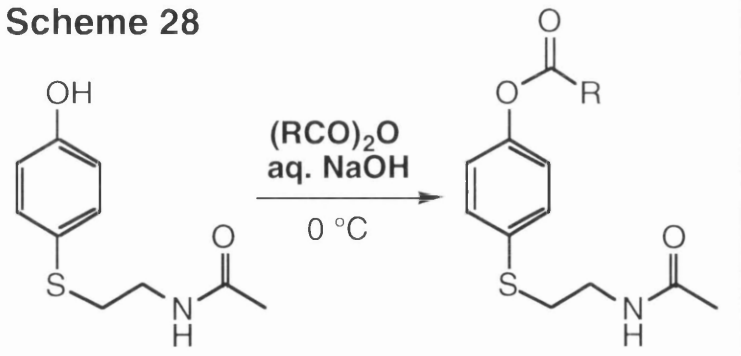


Product	R	Yield
42	OH	88%
88	H	65%
89	Cl	69%

The biological evaluation of the products shown in Schemes 25-27 revealed that only those compounds containing a phenol group were significantly active.[108] Of course, this did not prove that the toxicity of **42** and **47** was mediated through metabolism by Tyrosinase as some indiscriminate primary toxicity of phenols may have been responsible for the observed cell growth inhibition. However, on the basis of this result we decided to concentrate future work on phenols

We were also interested in functonalising the phenol moiety present in **47** with the possibility that active drug would be released after metabolism in the body. To investigate this, the general procedure of Furniss *et al.*[109] was employed to prepare two ester derivatives **90** and **91** in good yield by treatment of **47** with the corresponding carboxylic anhydride (Scheme 28).

Scheme 28



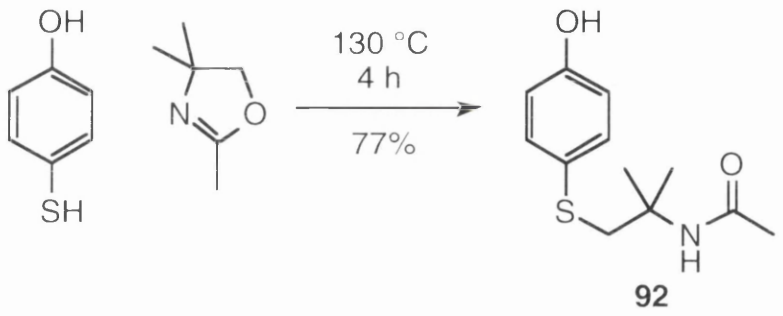
Product	R	Yield
90	Me	52%
91	Et	62%

The esters showed comparable *in vitro* activity to the parent phenol **47**; therefore it seemed likely that hydrolysis was taking place within the cell. Within our group, McKeown exploited this observation by conjugating **47** to the polymer poly(ethylene glycol) *via* ester linkages.[110] The resulting adduct showed excellent water-solubility and retained good anti-cancer activity.

4.4 Side-Chain Variations

Although the phenol group is the presumed pharmacophore of **47**, we were keen to examine the effects of introducing variations to its side-chain. New analogues with different shape and lipophilicity to **47** would be expected to demonstrate variation in cytotoxicity and in kinetics towards Tyrosinase. Initially, we used 4-hydroxythiophenol and the commercially available 2,4,4-trimethyl-2-oxazoline to prepare **92** in 77% yield (Scheme 29).

Scheme 29

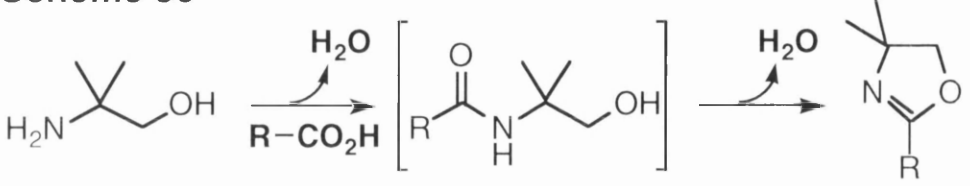


92

Compared to **47**, the *in vitro* evaluation of **92** revealed significantly higher anti-cancer activity and thus underlined the need for the synthesis of further analogues. It was decided to retain the new dimethyl functionality and examine the effects of varying the amide alkyl group. In order to synthesise that type of compound using a Wehrmeister reaction, samples of the corresponding 2-oxazolines were required.

The classical method of oxazoline synthesis involves the cyclo-condensation of an amino alcohol with a carboxylic acid (Scheme 30). Removal of water by azeotropic distillation is required to afford good yields of product.

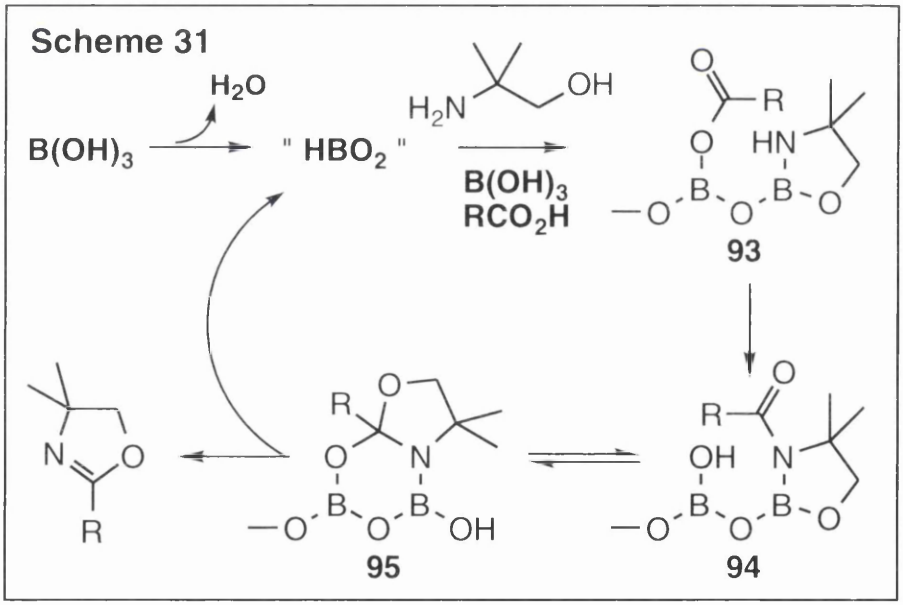
Scheme 30



The success of this procedure is known to be dependent on a number of factors. As Dean and Stark conditions are used, volatile substrates are often removed from the reaction mixture before condensation has taken place. In a similar way, volatile 2-oxazoline products sometimes co-distil with the hydrocarbon solvent once they have formed thus adding further complications. In addition, a major problem that arises when using this procedure is isolation of the intermediate amido alcohol (Scheme 30). For many substrates, this intermediate is not effectively dehydrated under the reaction conditions and a separate cyclisation step using thionyl chloride is often required to furnish the desired 2-oxazoline.

Barton *et al.* reported that the problem of amido-alcohol cyclisation could be circumvented by addition of boric acid to the reaction mixture.[111] However, this method is only useful in the preparation of 2-oxazolines lacking labile functionality due to the harsh conditions involved. In particular, it has found value in the protection of carboxyl groups in steroid systems.[112] The mechanism of boric acid catalysis suggested by Barton *et al.* is given in Scheme 31.[111]

Scheme 31

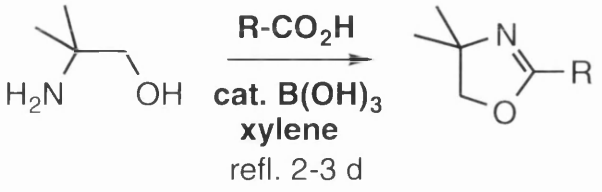


On moderate heating, HBO_2 is generated which reacts with the amino alcohol and carboxylic acid to give **93**. In this intermediate, the amine is suitably placed to interact with the now activated carbonyl group, leading to **94**. This is converted through bicyclic intermediate **95** thus furnishing the desired oxazoline and re-generating HBO_2.

We employed this method to synthesise oxazolines **96-101** as shown in Scheme 32. Our analytical data for compounds **96-98** corresponded well with

those reported in the literature.[111,113-116] Novel 2-oxazolines **99-101** were fully characterised and gave the expected spectroscopic data.

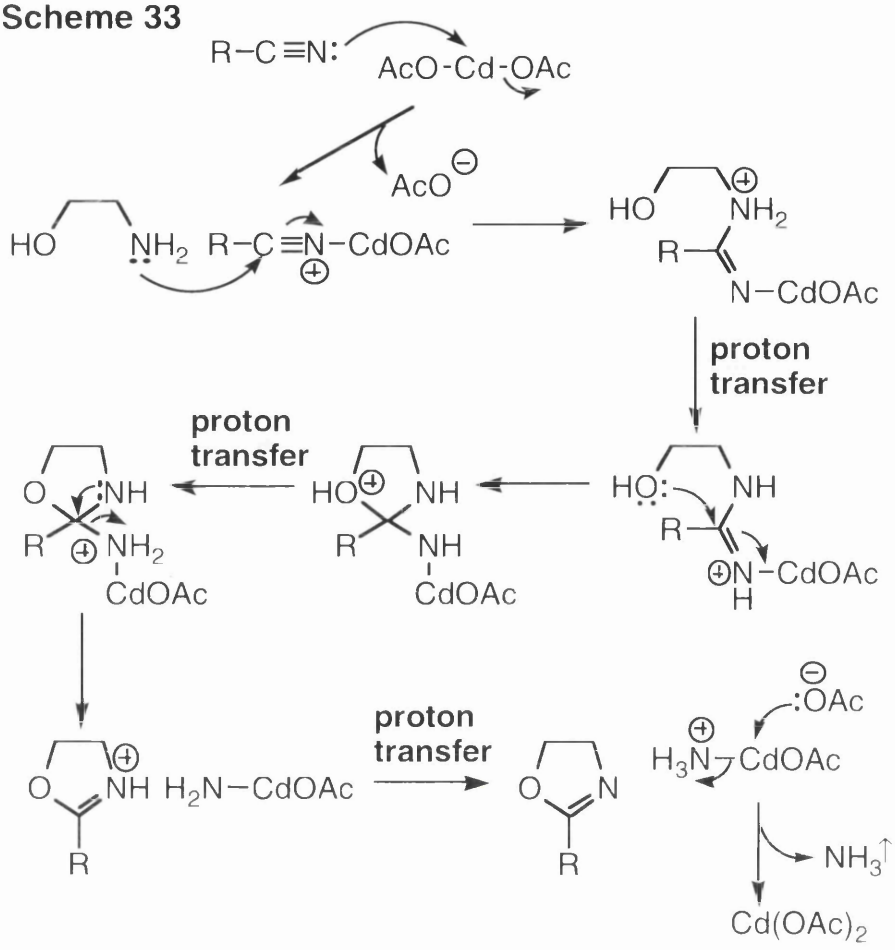
Scheme 32



Product	R	Time (d.)	Yield
96	*cyclo*-C_6H_{11}	2.0	75%
97	$(CH_2)_6CH_3$	2.0	84%
98	$(CH_2)_2$-*cyclo*-C_6H_{11}	2.0	58%
99	$(CH_2)_3$-*cyclo*-C_6H_{11}	3.0	52%
100	$(CH_2)_4$-*cyclo*-C_6H_{11}	3.0	54%
101	*cyclo*-C_5H_9	3.0	62%

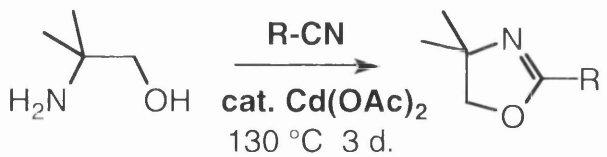
Witte and Seeliger first demonstrated the Lewis acid-catalysed synthesis of 2-oxazolines from nitriles and amino alcohols.[117] The reported procedure is particularly convenient as no additional solvent is required when aliphatic nitriles are used. Moreover, the progress of reaction can be easily followed by monitoring the evolution of the by-product ammonia. This proved to be an extremely reliable method of oxazoline synthesis, limited only by the availability of nitrile substrates. A proposed mechanism of cadmium acetate-catalysed oxazoline formation from nitriles is given in Scheme 33.

Scheme 33



Two known 2-oxazolines, **102** and **103**, were prepared using this methodology (Scheme 34). The spectroscopic data for these compounds were in good agreement with those reported in the literature.[115,116,118,119]

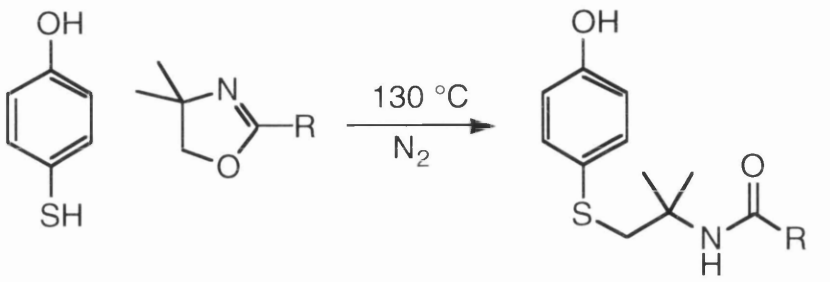
Scheme 34



Product	R	Yield
102	Pr^n	60%
103	Pr^i	76%

The oxazolines shown in Schemes 32 and 34 were used to prepare novel sulfides **104-111** by treatment with 4-hydroxythiophenol (Scheme 35).

Scheme 35



Oxazoline	Product	R	Time (h.)	Yield
96	**104**	*cyclo*-C_6H_{11}	4.0	75%
97	**105**	$(CH_2)_6CH_3$	4.0	74%
98	**106**	$(CH_2)_2$-*cyclo*-C_6H_{11}	7.0	73%
99	**107**	$(CH_2)_3$-*cyclo*-C_6H_{11}	7.0	65%
100	**108**	$(CH_2)_4$-*cyclo*-C_6H_{11}	4.0	58%
101	**109**	*cyclo*-C_5H_9	4.0	72%
102	**110**	Pr^n	4.0	61%
103	**111**	Pr^i	4.0	69%

Padgette reported that the Wehrmeister reaction of 4-hydroxythiophenol with 2-methyl-2-oxazoline was essentially complete after a 2 hour reaction time at 120 °C. We found that for 2-oxazolines substituted at the 4-position, a combination of higher temperature and extended reaction time was required to give an acceptable yield of product.

Together with similar compounds prepared by McKeown,[120] the eight products shown in Scheme 35 were evaluated *in vitro* by Dr L.R. Kelland of the Institute of Cancer Research, Surrey. Table 3 shows the growth inhibition data for compounds **104-111** and the parent compound **47**. The GI_{50} values quoted refer to the concentration of drug required to cause 50% growth inhibition in a 96 hour sulforhodamine B (SRB) growth inhibition assay. Activities were evaluated against four melanoma cell lines (B0010, SK-Mel-24, SK-Mel-2 and G361) and for comparison the ovarian SKOV-3 line. Selective anti-melanoma activity would be expected to cause low GI_{50} values for the melanoma lines together with high values for the ovarian cancer cell line.

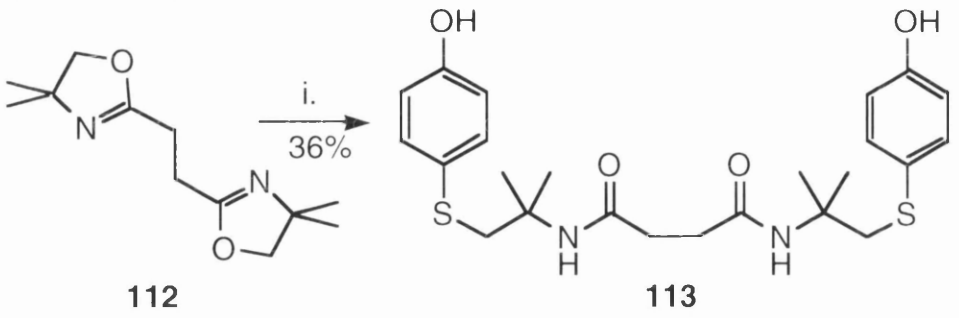
Table 3 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line				
Compound	**B0010**	**SK-Mel-24**	**SK-Mel-2**	**G361**	**SKOV-3**
47	>100	>100	>100	>100	>100
104	21	24	19	14	25
105	>100	>100	>100	>100	>100
106	58	83	71	26	56
107	61	>100	>100	54	>100
108	54	87	55	52	51
109	70	48	56	37	>100
110	84	74	56	35	>100
111	>100	100	100	67	>100

Several conclusions can be drawn from the *in vitro* test data given in Table 3. Firstly, most compounds are considerably more cytotoxic than the parent drug **47**. Although promising, this is only of real value if selectivity is increased too. Compound **104** exhibited the greatest degree of growth inhibition, yet it appears to be toxic towards non-melanoma cells too. We initially thought this increased activity might be largely due to higher lipophilicity yet other compounds with large hydrophobic groups such as **105** gave high GI_{50} values. Moreover, it was found that a reduction in growth inhibition resulted when we further decreased the polarity of **105** through addition of methylene groups to the amide alkyl group (compounds **106** - **108**). Promisingly, sulfides **109** and **110** showed good selectivity for the melanoma lines over SKOV-3 which prompted us to design a further round of related compounds for screening.

Initially, we decided to prepare a bifunctional compound containing two phenolic moieties. By the procedure of Furuta *et al.*[121] bis-oxazoline **112** was prepared in 70% yield and treated with two equivalents of 4-hydroxythiophenol (Scheme 36). A double Wehrmeister reaction afforded the novel dimeric compound **113** in 36% yield after column chromatography and recrystallisation. Due to its symmetry, the aliphatic region of **113** gave rise to 3 singlets in its ^1H NMR spectrum (D_6-DMSO). Similarly, the DEPT-edited ^{13}C NMR spectra of **113** showed nine peaks, as reported in section 7.2.2.

Scheme 36

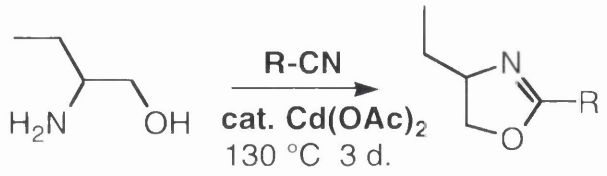


112 **113**

i. ***p*-HO-Ar-SH**, neat, 130 °, 4 h.

Another area of investigation involved further changes to the alkyl region between the sulfur and nitrogen atoms in **47**. As the introduction of methyl groups to this region had proved successful, analogues containing other functionality were required to define further the SAR of these agents. To this end, the general procedure of Witte and Seeliger[117] was employed to prepare two new ethylated 2-oxazolines **114** and **115** (Scheme 37) as racemates for use as substrates in the Wehrmeister reaction.

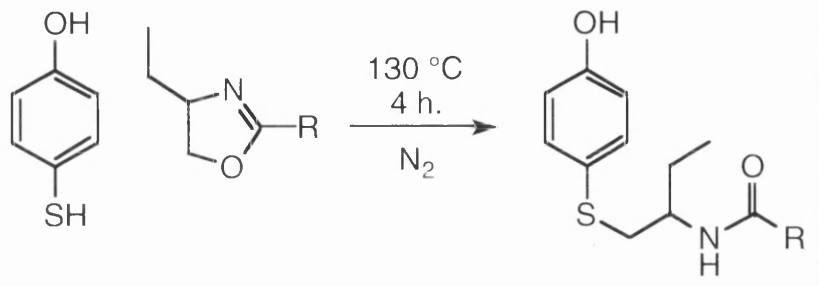
Scheme 37



Product	R	Yield
114	Et	79%
115	*cyclo*-C_6H_{11}	55%

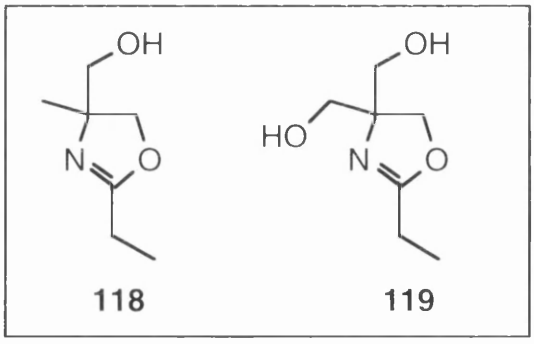
The moderate yield of **115** was probably caused by steric factors impeding the reactivity of cyclohexanecarbonitrile. Complex ^1H NMR spectra were obtained for both products, due to the stereogenic centre at the 4-position of the heterocycle. However, oxazoline formation was confirmed by the presence of a peak at *ca.* δ 170 in the ^{13}C NMR spectra of the products. This was corroborated by the characteristic C=N stretching band at *ca.* 1665 cm^{-1} in the IR spectra of **114** and **115**. Both of these oxazolines readily reacted with 4-hydroxythiophenol under the standard conditions to give the required novel sulfides **116** and **117** as shown in Scheme 38.

Scheme 38



Oxazoline	Product	R	Yield
114	**116**	Et	79%
115	**117**	*cyclo*-C_6H_{11}	65%

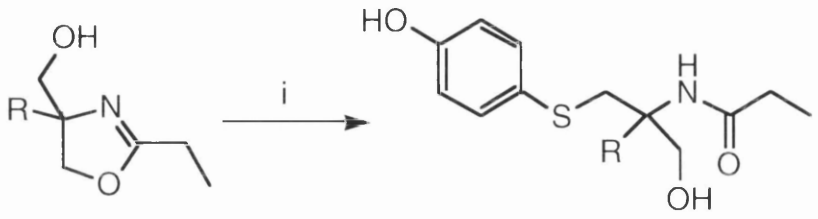
In addition to lipophilic substituents, we were also keen to introduce polar groups in the side-chain. Known 2-oxazolines **118** and **119** were prepared as described by Wehrmeister.[122] As only b.p. data for **118** are reported in the literature,[122] this compound was characterised by the standard spectroscopic techniques. The m.p. and ^{1}H NMR spectral data for **119** accorded well with those in the literature.[122,123]



118 **119**

Treatment of oxazolines **118** and **119** with 4-hydroxythiophenol under the standard conditions[92] did not result in any detectable amide formation. As extended reaction times did not seem to resolve this problem, an alternative procedure was sought that would increase the rate of nucleophilic addition. In particular, it was thought that the use of polar aprotic solvents might enhance ring-opening by solvation of the troublesome hydroxymethyl group(s). Accordingly, required novel amides **120** and **121** were prepared in low yield through the use of DMF as reaction solvent (Scheme 39).

Scheme 39

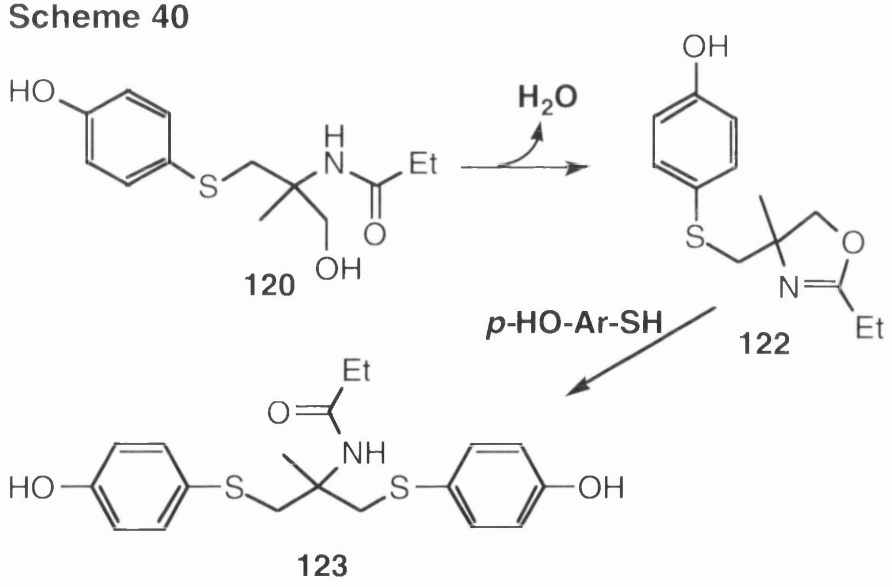


i. ***p*-HO-Ar-SH**, DMF, 7 h., 130 °C, N_2

Oxazoline	Product	R	Yield
118	**120**	Me	30%
119	**121**	CH_2OH	21%

The low yields encountered may be explained by further reaction of the products. For example, compound **120** may undergo dehydration to give the oxazoline **122** which could feasibly react with another thiophenol molecule to give the amide **123** (Scheme 40). This type of mechanism was suggested by Wehrmeister to account for a similar type of reaction,[93] although Dean-Stark conditions were used in that case which would promote the initial dehydration step.

Scheme 40



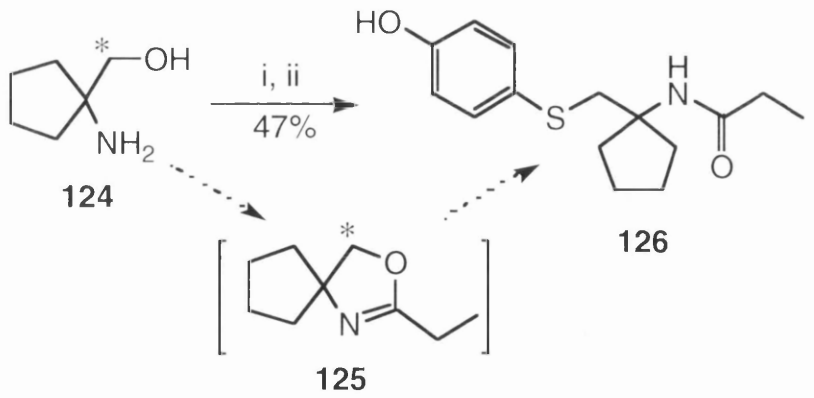
If further hydroxymethylated analogues are required, attempts should be made to enhance ring-opening and to suppress product decomposition. As this would be difficult to achieve through control of reaction temperature and duration, the use of a protecting group may be more suitable. Meyers and Yamamoto[123] have previously prepared acetals of dihydroxylated oxazolines

such as **119**. Such a protecting group could be removed under mild conditions after Wehrmeister reaction with 4-hydroxythiophenol.

In almost all cases, a rigorously pure sample of each 2-oxazoline was employed in our Wehrmeister reactions. Although only technical grade 4-hydroxythiophenol is commercially available, satisfactory yields of each carboxamide were produced in most cases. Nonetheless, we sometimes avoided oxazoline isolation in cases where substrates were expensive or in short supply.

As an example, amide **126** was required for testing in order to examine the effect of introducing a cycloalkyl group into the side-chain. We envisaged that this could be prepared from spirocyclic oxazoline **125** by the use of a Wehrmeister reaction. Due to the expense of commercially available amino alcohol **124**, it was decided to prepare the required intermediate **125** and use it without any purification. Accordingly, cycloleucinol **124** was treated with propanonitrile and cadmium diacetate dihydrate as shown in Scheme 41. The progress of reaction was conveniently followed using ^{1}H NMR spectroscopy by monitoring the appearance of a singlet at δ 4.00 arising from the protons on C-5 (*) of the oxazoline **125**. By comparison with the singlet at δ 3.37 arising from the analogous protons (*) in cycloleucinol **124**, integration was used to estimate the extent of reaction. After three days, formation of **125** was essentially complete, therefore one equivalent of 4-hydroxythiophenol was added before the mixture was heated for a further 5 h under nitrogen. Flash chromatography of the crude product mixture gave the required product **126** in 47% yield (Scheme 41).

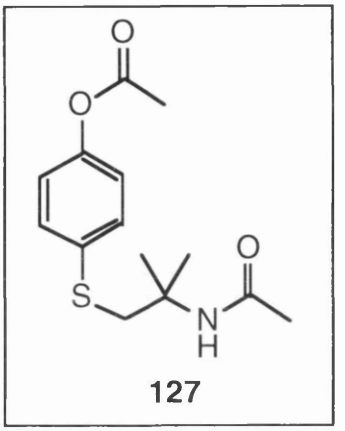
Scheme 41



i. **Pr^{n}CN**, **cat. Cd(OAc)$_2$**, 3 d., 120 °C.
ii. ***p*-HO-Ar-SH**, 5 h., 130 °C, N_2.

As preliminary testing had revealed that phenyl esters had anti-melanoma activity, the acetate **127** was prepared from sulfide **92** in 92% yield

by a published general procedure.[109] We required this ester to complete a set of compounds for *in vitro* biological evaluation.



127

The results from biological evaluation of the amides containing hydroxylated side chains showed that these were poor growth inhibitors of each cell line tested. Treatment with a 25 μM solution of the compounds in ethanol resulted in the low percentage growth inhibition values shown in Table 4. These results are likely to be due to poor lipophilicity as much as any other factor. As **120** only contains one hydroxymethyl group, the observation that this compound shows better growth inhibition data than its more polar analogue **121** would appear to corroborate this.

Table 4 25μM 96H SRB Assay. Growth Inhibition (%)

	Cell Line				
Compound	**B0010**	**SK-Mel-24**	**SK-Mel-2**	**G361**	**SKOV-3**
120	2.4	20.4	0	19.7	2.9
121	0	0	0	5.5	1.1

Table 5 shows how *in vitro* evaluation of the less polar amides revealed low levels of cytotoxicity when compared to the preceding round of test data (Table 3). In particular, the bifunctional compound **113** and the diethylated compound **116** were surprisingly inactive. Phenol **117** showed promising levels of cytotoxicity with apparent selectivity for the melanoma lines but the IC_{50} values for this compound were somewhat higher than those previously determined for its isomer **104**. Compound **126** was synthesised to examine the effects of introducing a bulky cycloalkyl group into the central section of side chain. From the data in Tables 3 and 5, it would seem that the greatest activity results when this type of group is placed at the amide terminus of the compound.

The prodrug **127** showed some activity, but we decided that the GI_{50} values were too high to warrant further study of phenyl esters as potential anti-melanoma agents.

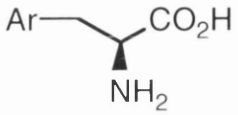
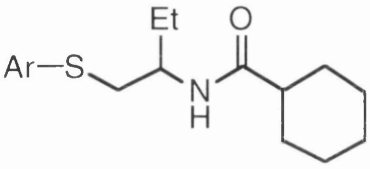
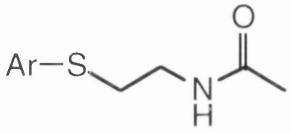
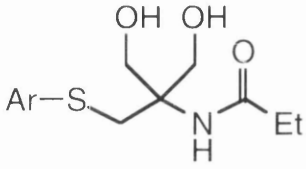
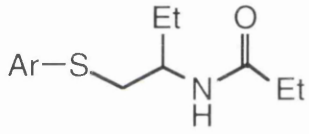
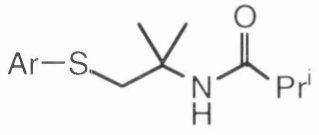
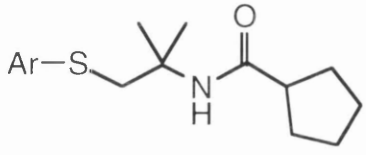
Table 5 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line				
Compound	**B0010**	**SK-Mel-24**	**SK-Mel-2**	**G361**	**SKOV-3**
113	>100	>100	>100	>100	>100
116	>100	>100	>100	>100	>100
117	70	54	52	44	91
126	n.d.	>100	62	96	>100
127	>100	57	72	40	>100

n.d. = not determined

We believed that the wide spectrum of results obtained might have resulted from a combination of lipophilicity variations and the ability to act as substrates for Tyrosinase. Therefore, in order to gain further information we wished to determine whether these new compounds were substrates for the enzyme Tyrosinase. It was reasoned that selectivity for melanoma cell lines would be enhanced if good activity towards this enzyme was obtained. Therefore to investigate this, an established assay[124] was employed to measure metabolism of the substituted phenols by commercially available mushroom Tyrosinase. It was envisaged that this would give a guide to corresponding activities towards human Tyrosinase, which is not readly available from suppliers. The rates of *ortho*-quinone formation were determined by UV spectroscopy and calculated as a percentage relative to that obtained for L-tyrosine. Observed activities for a representative selection of compounds are shown in Table 6. Interestingly, the activity of compounds towards Tyrosinase appears to bear no correlation to their ability to inhibit cell growth. Compound **109**, for example, is a potent inhibitor of cell proliferation yet its activity towards mushroom Tyrosinase is very low. Conversely, phenol **119** is a good substrate for Tyrosinase but shows little toxicity to cells *in vitro*. As discussed earlier, the poor lipophilicity of compounds such as **119** may account for their poor biological activity.

Table 6 Tyrosinase Activity Data

Compound	Side Chain Structure	% Relative Activity
Tyr		100
115		10.5
47		9.7
119		5.6
114		4.6
111		3.8
109		< 0.2

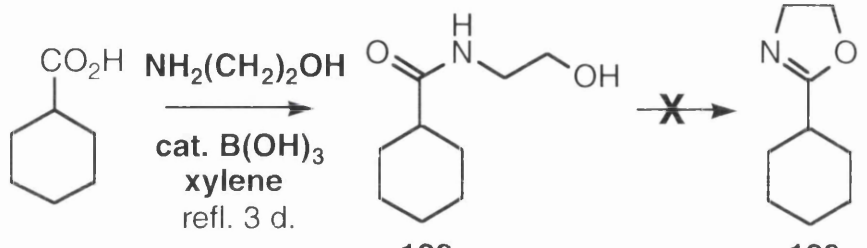
From the results of the Tyrosinase assays we concluded that the growth-inhibiting properties of compounds such as **109** may be due to increased non-Tyrosinase mediated toxicity. Compounds containing a dimethyl group in the side-chain seemed to exhibit the lowest Tyrosinase activities and often high levels of indiscriminate cytotoxicity; therefore it was decided to exclude that structural feature from the following round of analogues.

4.5 Variations to the Amide Alkyl Group

In an attempt to increase Tyrosinase activity and explore the role of lipophilicity in this class of drug, we planned a series of compounds which

would vary only in the structure of the amide alkyl group. In this way, lipophilic analogues of **47** could be prepared that might also show good activity towards Tyrosinase. To achieve this, a series of 2-oxazolines was required that would possess no substituents at the 4- or 5-positions of the ring. Early attempts at preparing these compounds using the boric acid catalysed conditions of Barton *et al.*[111] resulted in isolation of the intermediate amido alcohol, as exemplified by the formation of **128** rather than the desired oxazoline **129** (Scheme 42).

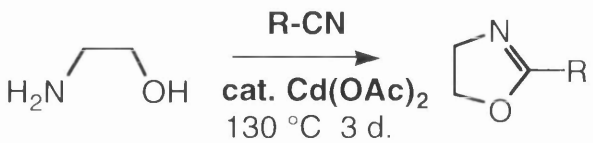
Scheme 42



128 **129**

Poindexter[125] prepared 2-cyclohexyl-2-oxazoline **120** by a 3-step procedure involving initial formation of amido alcohol **128**. He reported that treatment of this alcohol with thionyl chloride gave the corresponding alkyl chloride which could be cyclised using aqueous sodium hydroxide. However, for convenience it was decided to prepare **129** and several other 2-oxazolines **130-134** from their corresponding nitriles by the general procedure of Witte and Seeliger[117] (Scheme 43).

Scheme 43

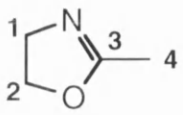


Product	R	Yield
130	Et	65%
131	Pr^i	67%
132	Pr^n	50%
133	Bu^n	65%
134	Bu^t	43%
129	*cyclo*-C_6H_{11}	43%

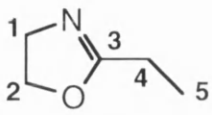
Our characterisation data for oxazolines **129-134** accorded well with those reported in the literature.[117,125-131] and additional new data are provided

in section 7.2.2. The new ^{13}C NMR spectral data for these compounds and 2-methyl-2-oxazoline **135**[132] are presented in Table 7.

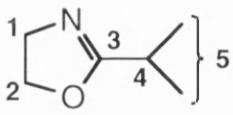
Table 7 ^{13}C NMR Spectroscopy of 2-Oxazolines



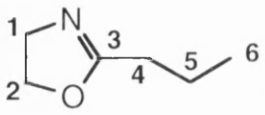
135



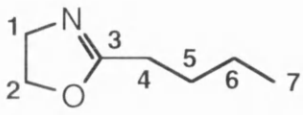
130



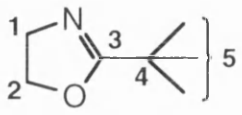
131



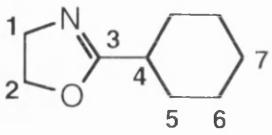
132



133



134



129

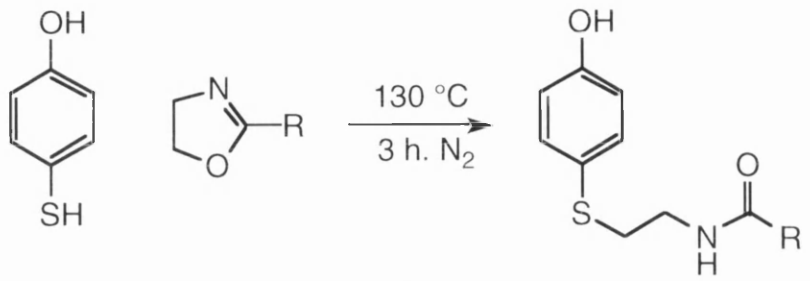
^{13}C NMR Chemical Shifts (δ)

	Carbon Number						
	1	**2**	**3**	**4**	**5**	**6**	**7**
135	53.8	66.5	164.4	12.8			
130	54.1	67.0	169.3	21.1	10.0		
131	54.1	67.0	172.4	27.9	19.5		
132	54.1	66.7	168.0	29.5	19.1	13.4	
133	54.1	66.8	168.4	27.8	27.4	22.1	13.5
134	54.0	67.2	174.3	63.4	27.5		
129	54.0	66.8	171.5	37.2	29.7	25.5	25.7

The Table shows that the chemical shifts of the two ring methylene groups are essentially unaffected by changes to the 2-alkyl substituent. Conversely, the resonant frequency of the imidate carbon is highly diagnostic and conveys valuable information. Due to inductive effects, a methyl or methylene group at the 2-position results in shifts of less than δ 170 for the imidate carbon and if a quaternary carbon is adjacent to the ring then the value is generally around δ 175. Intermediate values of approximately δ 172 result when a methine carbon is located at the same position.

The six oxazolines **129-134** were smoothly converted into the required sulfides **136-141** by treatment with 4-hydroxythiophenol (Scheme 44).

Scheme 44



Oxazoline	Product	R	Yield
130	**136**	Et	87%
131	**137**	Pr^i	72%
132	**138**	Pr^n	79%
133	**139**	Bu^n	74%
134	**140**	Bu^t	64%
129	**141**	*cyclo*-C_6H_{11}	81%

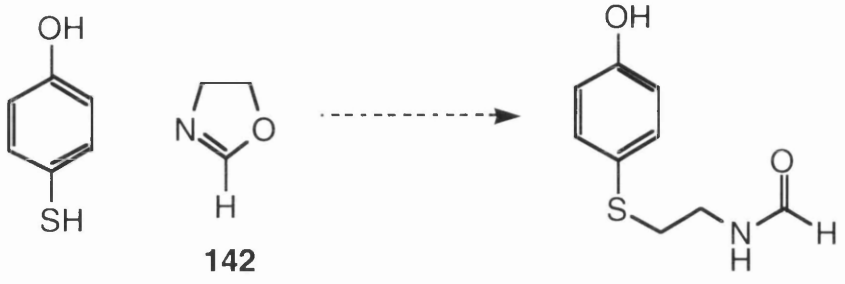
The novel sulfides **136-141** all gave the expected analytical and spectroscopic data. ^1H NMR spectroscopy of the products at ~1 mol dm^{-3} concentration in D_6-DMSO revealed chemical shifts of about δ 9.9 for their phenolic protons thus indicating strong hydrogen bonding. Spin-spin coupling between the amide proton and the methylene protons of the adjacent carbon was also evident.

Salient features of the solid state IR spectra of the products were the strong amide I absorption at ~1640 cm^{-1} and the slightly weaker amide II band at ~1562 cm^{-1}. The characteristic bands at ~1600 cm^{-1}, ~1580 cm^{-1} and ~1500 cm^{-1}, indicative of an aromatic system, were also identified. A weaker band at ~820 cm^{-1} was assigned to the C-H out-of-plane deformation of protons in the *para*-disubstituted aromatic system.

The EI mass spectra of these compounds show a relatively strong molecular ion peak and a base peak at *m/z* 152. The lack of any peaks between these two may reflect a propensity of these compounds to undergo McLafferty rearrangement resulting in cleavage between the amide nitrogen atom and the adjacent methylene group.

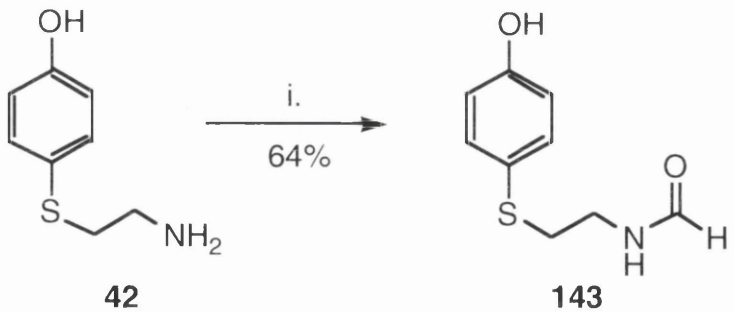
We were also interested in extending the series of compounds to include the formamide derivative **143**. As before, this might be prepared by a Wehrmeister reaction of 4-hydroxythiophenol with 2-oxazoline **142** (Scheme 45).

Scheme 45



2-Oxazoline **142** is not commercially available but a 3-step procedure for its synthesis has been reported by Smith and Atigadda.[133] As this would involve a total of four steps to prepare **143**, we decided to explore a more convenient approach using the previously prepared amine **42** (section 3.3). Treatment of **42** with a refluxing mixture of excess ethyl formate provided the required amide in 64% yield after flash chromatography. As **42** is almost insoluble in ethyl formate, a small quantity of ethanol was added to the reaction mixture to aid dissolution (Scheme 46).

Scheme 46

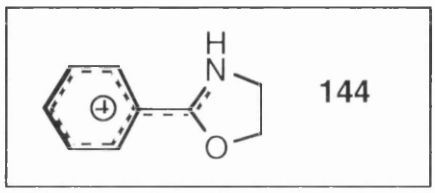


i. **HCO_2Et**, **EtOH**, 14 h, refl.

The structure of product **143** was confirmed by ^1H NMR spectroscopy with the resonance at δ 8.06 being assigned to the formamide C-H proton. Its ^{13}C NMR spectrum in D_6-DMSO revealed a doubling of the amide quaternary carbon peak due to geometric isomerism, a phenomenon that was not apparent with the other analogues in the set. The possibility that this additional peak was caused by formylation of the phenol was ruled out on the basis of IR spectroscopy, UV spectroscopy, mass spectrometry and combustion analysis data.

In order to complete our desired set of compounds, we required a sample of the substituted benzamide **145**. We initially considered preparing this

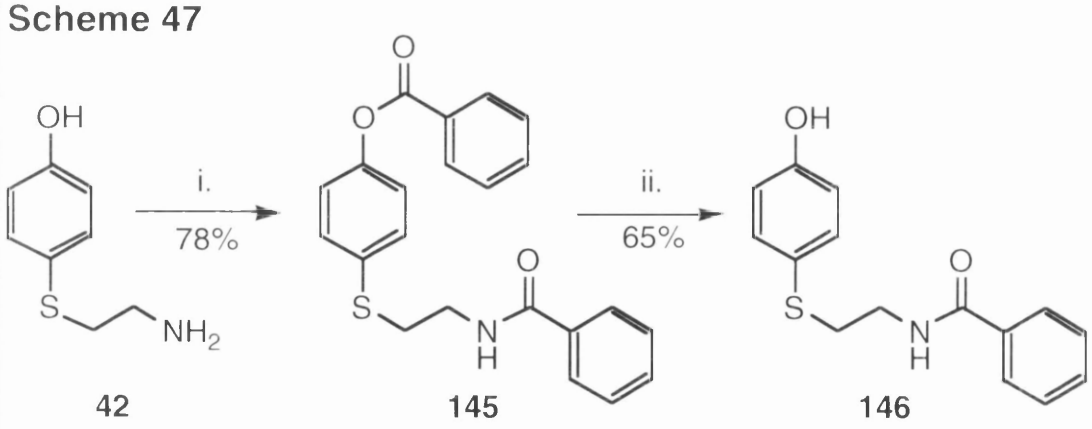
by the Wehrmeister reaction; however the reaction of 4-hydroxythiophenol with 2-aryl-2-oxazolines is reported to proceed in low yield under the forcing conditions required.[134] This is possibly due to nucleophilic addition being hampered by delocalisation of the intermediate oxazolinium cation **144**.



144

As an alternative, we prepared **146** in 51% yield over 2 steps from amine **42**. Standard Schotten-Baumann conditions[135] were employed to generate **145** in good yield before selective cleavage of the ester using butylamine.[136] The second step was carried out according to the procedure of Bell,[136] although the suggested solvent of benzene was replaced by THF. This method of ester deprotection under mild conditions is believed to be selective for phenolic esters. Its main disadvantage is the generation of a molar equivalent of *N*-butylbenzamide as a by-product.

Scheme 47



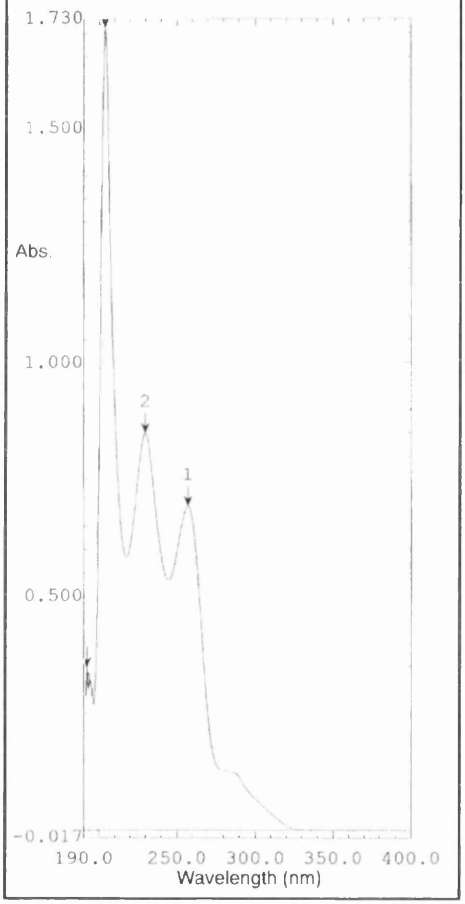
42 **145** **146**

i. **BzCl**, **aq. NaOH**, 5 min, r.t.
ii. **Bu^{n}NH$_2$**, THF, 20 h, r.t.

Compound **146** gave the expected spectroscopic properties, and thus completed our desired set of 8 simple analogues of **47**. In order to measure their lipophilicity, we now required the evaluation of their partition coefficients. This could allow us to highlight compounds which show increased activity due to factors other than lipid solubility. Naish-Byfield *et al.* employed hexadecane and pH 7.4 Phosphate Buffered Saline (PBS) as the two phases in their evaluation of the lipophilicity of a range of phenols.[65] However, this system proved to be unsuitable for **47** and its analogues due to virtual insolubility in the

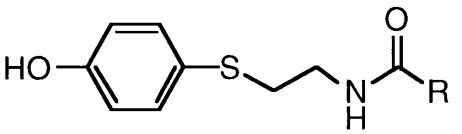
non-polar phase. We found that more informative log *P* values could be obtained by the use of 1-octanol and PBS. Electronic spectroscopy was used to determine the relative concentrations of each phenol in the PBS layer before and after extraction by 1-octanol. The UV spectra of these compounds is typified by the spectrum of parent compound **47** in ethanol (Figure 7). The presence of *para*-disubstituted non-complementary auxochromes account for the absorption maxima at 228 nm (ε9000) and 255 nm (ε7300) from the benzene ring.

Figure 7



The absorbance of the peak at ~228 nm was used in the measurement of the partition coefficients *P* for compounds **47**, **136-141**, **143** and **146** (Table 8).

Table 8 1-Octanol: PBS Partition Coefficients



Compound	R	*P*	log *P*
143	H	3.96	0.60
47	Me	2.03	0.31
136	Et	1.99	0.30
137	Pr^i	5.63	0.75
138	Pr^n	3.60	0.56
139	Bu^n	3.02	0.48
140	Bu^t	7.85	0.89
141	*cyclo*-C_6H_{11}	7.18	0.86
146	Ph	13.47	1.13

Apart from the unexpectedly high log *P* value for formamide derivative **143**, the results broadly confirm that increasing the size of the alkyl group leads to greater lipophilicity. We were therefore keen to establish whether the *in vitro* growth inhibition data followed a similar trend. As before, the compounds were dissolved in ethanol and treated with a panel of human melanoma cell lines and the ovarian carcinoma line SKOV-3. The results, expressed as GI_{50} (μmol), are shown in Table 9.

Table 9 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
143	>100	>100	>100	>100	>100	>100	>100
47	>100	>100	>100	>100	>100	>100	>100
136	>100	>100	>100	>100	>100	>100	>100
137	>100	>100	>100	>100	>100	>100	>100
138	82	>100	>100	>100	>100	>100	>100
139	47	>100	>100	>100	84	>100	>100
140	67	>100	>100	>100	>100	>100	>100
141	29	>100	77	>100	61	>100	>100
146	>100	>100	>100	52	>100	>100	>100
145	90	>100	>100	>100	76	>100	>100

These analogues obviously cause comparatively low levels of cytotoxicity to the cell types examined. However, certain compounds do show

significantly higher activity than the lead compound **47**. This is illustrated by comparing the percentage growth inhibition data of **47** with those of *n*-butyl-substituted amide **139** and cyclohexyl-substituted amide **141** (Table 10).

Table 10 25μM 96H SRB Assay. Growth Inhibition (%)

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
47	3.14	4.1	0.35	0	6.3	0	0
139	22.4	4.1	10.7	6.4	9.1	0.9	0.2
141	45.6	15.9	19.3	10.6	24.3	1.3	0.4

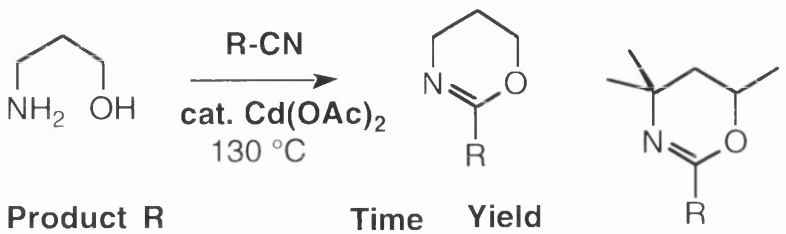
Not only does **141** have far greater activity than **47**, it shows promising selectivity over the non-melanoma control (SKOV-3) too. In addition, good selectivity for Tyrosinase-containing cells is actually corroborated by the poor activity observed towards the SK-Mel-24 melanoma line. This cell type is non-melanotic and therefore does not contain the required biochemical machinery to generate *ortho*-quinones from these phenolic prodrugs.

Although some of the compounds discussed in section 3.4 showed greater cytotoxicity than **141**, they showed little selectivity. Therefore **141** may be a suitable compromise that exhibits moderate activity together with greater selectivity for melanoma. Furthermore, the solubility of **141** in saline up to 100 mM is an added advantage.

4.6 Extension of the Side-Chain

A two-atom linkage between sulfur and nitrogen atoms has been a feature of all the analogues previously discussed. In order to broaden our investigation, it was decided to prepare a series of compounds with a three-carbon linking unit for comparison. We postulated that the extended chain would further enhance lipophilicity and might also lead to better Tyrosinase activity by further separating the bulky amide group from the aromatic moiety. To this end, three 5,6-dihydro-oxazines **147-149** were highlighted as potential substrates for Wehrmeister reaction with 4-hydroxythiophenol. Compounds **147** and **148** were prepared from acetonitrile and cyclohexanecarbonitrile, respectively, by the procedure of Witte and Seeliger[117] (Scheme 48). Owing to its widespread use in the Meyers aldehyde synthesis,[136,137] dihydro-oxazine **149** is commercially available.

Scheme 48



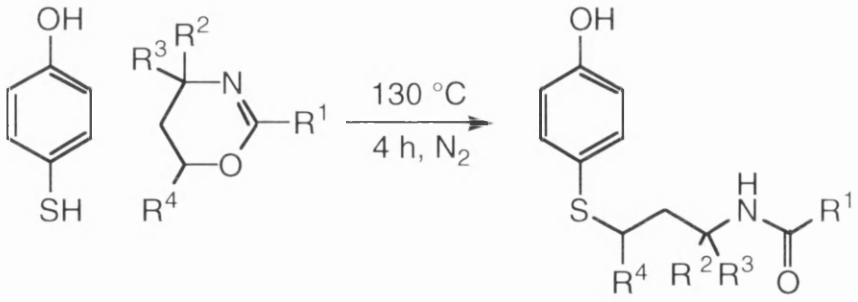
Product	R	Time	Yield
147	Me	2 d	59%
148	*cyclo*-C_6H_{11}	4 d	32%

149

Our spectroscopic data for 5,6-dihydro-2-methyl-4*H*-1,3-oxazine **147** corresponded well with those described by Toshimitsu *et al.*[138] Similar data were acquired for the hitherto unknown dihydro-oxazine **148**. Its IR spectrum revealed a strong absorption at 1671 cm^{-1} due to C=N stretching. As this value is very close to the C=N stretching frequency in analogous 2-oxazolines, this technique cannot be satisfactorily used to differentiate dihydro-oxazines from 2-oxazolines. In our experience, ^{13}C NMR spectroscopy is more informative as the chemical shift of the imidate carbon resonance of 2-oxazolines is typically *ca.* 8 ppm greater than that of corresponding 5,6-dihydro-4*H*-1,3-oxazines.

Treatment of compounds **147-149** with 4-hydroxythiophenol under the standard conditions afforded the required novel sulfides **150-152** (Scheme 49).

Scheme 49



Substrate	Product	R^1	R^2	R^3	R^4	Yield
147	**150**	Me	H	H	H	63%
148	**151**	*cyclo*-C_6H_{11}	H	H	H	58%
149	**152**	Me	Me	Me	Me	75%

All three new products **150-152** were fully characterised and gave the expected analytical data. The presence of a stereogenic centre in the side-chain of **152** was clearly evident from its ^1H NMR NMR spectrum. This revealed complex multiplicity due to the diastereotopic nature of the protons in the

methylene group and separate singlets for the two methyl substituents on the carbon atom adjacent to its amide nitrogen atom.

Table 11 shows the results from the *in vitro* screening of phenols **150-152** in terms of GI_{50} values (μM).

Table 9 GI_{50} Values (μM) for 96H SRB Assay

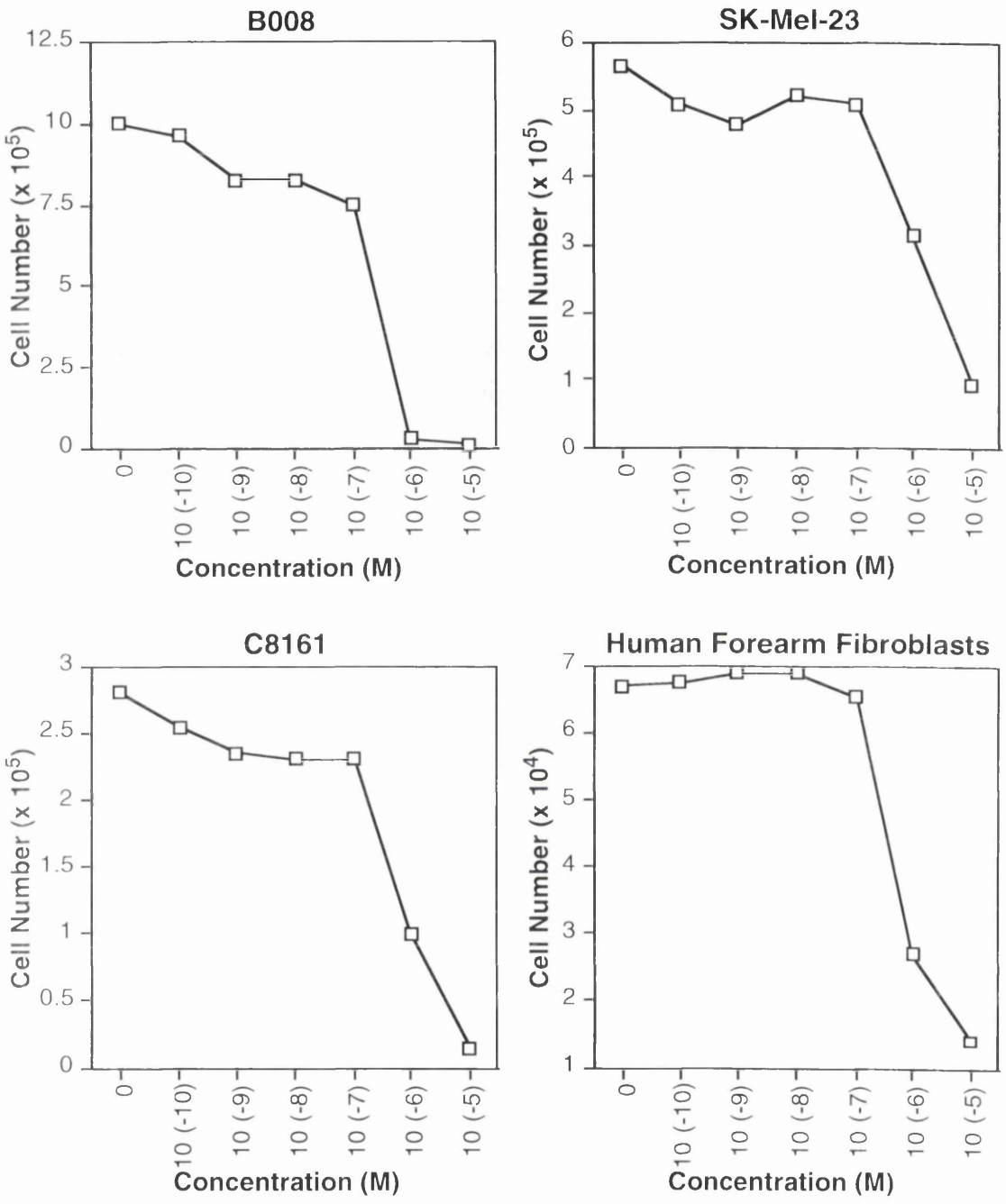
	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
150	60	>100	>100	>100	>100	>100	>100
151	5.3	34.5	11	70	11	>100	54
152	48	>100	90	>100	>100	>100	>100

The GI_{50} values for **150** and **152** show that the introduction of methyl groups to an extended side-chain appears to cause no significant changes in biological activity. However, compound **151** shows good activity towards all of the Tyrosinase-containing melanoma lines in addition to low toxicity towards the non-melanotic SK-Mel-24 melanoma cell type. Its moderate toxicity towards the ovarian carcinoma SKOV-3 line is rather disappointing. The increased activity of **151** over the other compounds may be largely due to greater lipophilicity.

4.7 Further Biological Evaluation

The *in vitro* screening discussed in this chapter was performed at the Institute of Cancer Research by Dr L.R. Kelland and co-workers. These data enabled us to highlight compounds which warranted further investigation both *in vitro* and *in vivo*. As an example, the data in Table 3 showed that compound **104** was highly cytotoxic towards the cell lines examined, exhibiting GI_{50} values as low as 15 mM. Further testing was required to give more information about the selectivity of this phenol at various concentrations. Professor R.M. MacKie kindly agreed to undergo these *in vitro* experiments, and the results for phenol **104** are illustrated in Figure 8. The effects of the drug at six concentrations from 10^{-10} M to 10^{-5} M were measured against four cell types: B008 (melanoma), C8161 (melanoma), SK-Mel-23 (highly pigmented melanoma) and human fibroblasts (non-cancer).

Figure 8 Further Evaluation of Compound 104



For these assays, it is the change in cell number rather than the actual cell number that is important when comparing the graphs. We were particularly interested in the effects of **104** on the heavily pigmented SK-Mel-23 line, as this contains high levels of Tyrosinase and would therefore be expected to be particularly susceptible to these compounds. However, the data suggest that this cell line is actually more resistant to **104** than the other two melanoma lines. More importantly, high toxicity towards the non-tumour fibroblasts is observed at the micromolar concentrations required to kill melanoma cells. Therefore, we conclude from the combined *in vitro* experiments that there is a non-selective

action associated with phenol **104**, which may operate together with a Tyrosinase-mediated response.

Preliminary *in vivo* studies with **104** were carried out at the laboratories of Ares-Serono in Italy. Male mice were injected intraperitoneally with 245 μg/kg of **104** in a mixed vehicle of water, ethanol and propylene glycol. This dose was selected to give approximate maximal drug concentrations of twenty times the active concentration *in vitro* (20 μM). Major acute toxicity was observed together with undesired toxic effects towards the white blood cells. Further studies are needed to ascertain whether these effects are caused by a primary toxicity of phenols or by a mode of action specific to structures such as **104**.

Initial screening of compounds such as **141** indicate that they are more selective than **104** towards melanoma cell lines. In order to confirm this observation, comprehensive *in vitro* assays are underway at the U.S. National Cancer Institute.

A concurrent study was aimed at determining the importance of the amide group present in **47** and its analogues. The results from this work are discussed in the following chapter.

5

Amide Group Replacements

5.1 Introduction

The conclusion that the non-selective toxicity of **42** was due to oxidation by monoamine oxidase led to the synthesis and evaluation of the substituted acetamide **47** by Alena and co-workers.[78] As hydrolysis of the amide group in **47** is not essential for anti-melanoma activity, we wished to investigate the biological properties of analogues containing other groups at this position. A further aim was to determine whether inversion of the amide geometry would give rise to any changes in biological activity.

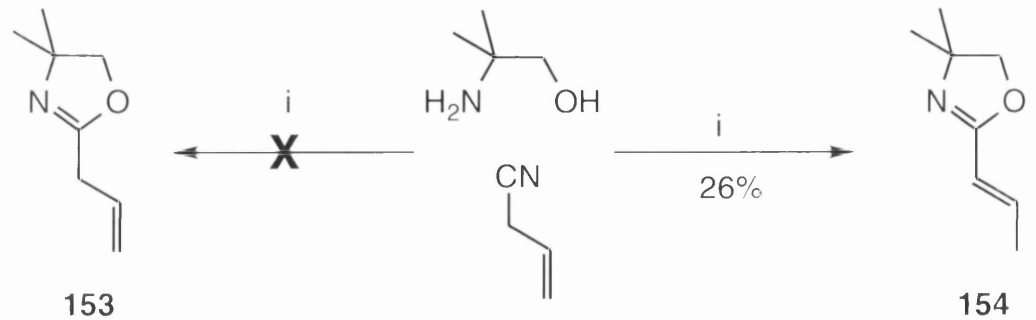
Our interest in isosteric replacements for the amide group in **47** stemmed from investigations into the reactivity of thiophenols towards 2-alkenyl-2-oxazolines, as discussed in the following section.

5.2 Wehrmeister Reaction - Further Studies

5.2.1 2-Alkenyl-2-oxazolines

In an attempt to prepare the olefinic 2-oxazoline **153** we heated allyl cyanide with 2-amino-2-methyl-1-propanol and a catalytic quantity of cadmium diacetate, as shown in Scheme 50. Although fractional distillation of the viscous product mixture did not result in isolation of the desired product **153**, a 26% yield of the isomeric oxazoline **154** was obtained.

Scheme 50



153 **154**

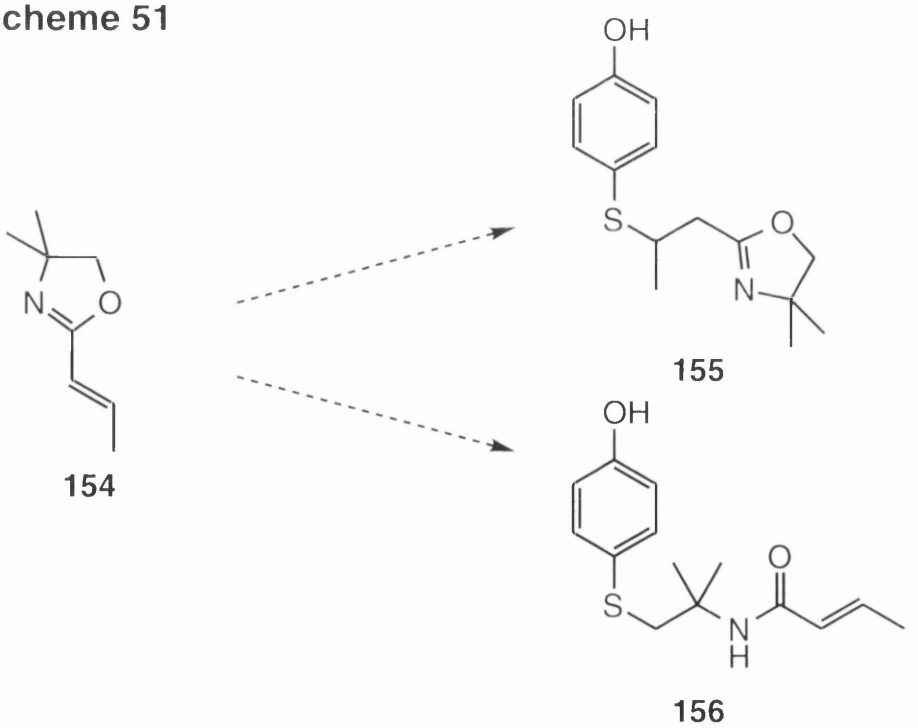
i. **$Cd(OAc)_2$ (cat.)**, neat, 130 °C, 2 d

Our ^{1}H NMR and IR spectroscopic data for **154** were in accordance with those reported by Alvhäll *et al.*[115] They synthesised **154** in 44% yield by cyclo-condensation of *N*-2-butenoyl-2-amino-2-methyl-1-propanol with thionyl chloride. Recently, Dahuront and Langlois reported an 88% yield of **154** through the use of phosphorus oxychloride as the dehydrating agent.[139]

The thermal isomerisation of βγ-unsaturated 2-alkenyl-2-oxazolines into αβ-unsaturated analogues was also discussed in the paper by Alvhäll *et al.*[115] They reported that βγ-unsaturated 2-alkenyl-2-oxazolines could not be purified by chromatography or distillation due to extensive decomposition. Moreover, they concluded that 2-alkenyl-2-oxazolines in general could not be satisfactorily prepared at elevated temperatures due to their propensity to undergo polymerisation. The high reactivity of αβ-unsaturated 2-alkenyl-2-oxazolines was recently exploited by Elliott and Kruiswijk in their stereocontrolled syntheses of substituted piperidines by aza-Diels-Alder reactions.[140]

We decided to use our sample of **154** to further our study of the Wehrmeister reaction. Due to the ambident nature of the oxazoline, we envisaged that treatment of **154** with 4-hydroxythiophenol could potentially lead to two different products. Conjugate addition to the alkene would yield oxazoline **155** whereas nucleophilic ring-opening would give rise to the amide **156** (Scheme 51).

Scheme 51

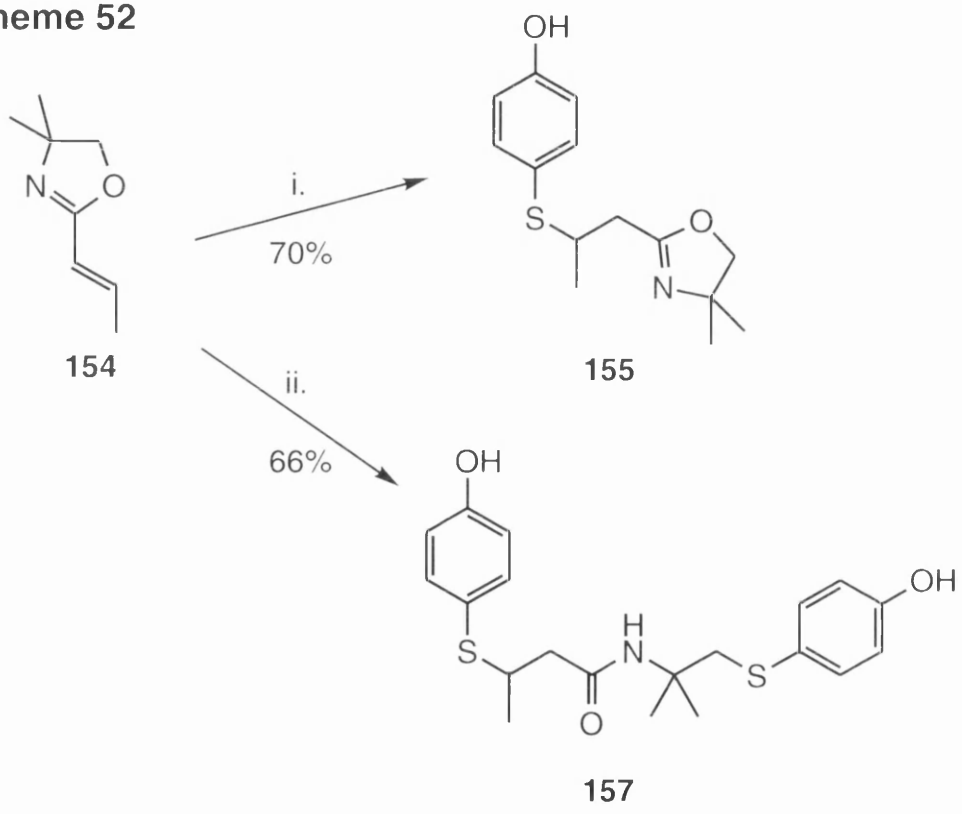


Treatment of **154** with one equivalent of 4-hydroxythiophenol for 2 h at 120 °C gave oxazoline **155** in 70% yield after chromatography (Scheme 52).

The presence of the stereogenic centre in the product was confirmed by the non-identical nature of the oxazoline methyl carbon atoms, as revealed by DEPT-edited ^{13}C NMR spectroscopy. The solid state IR spectrum of **155** was dominated by a strong absorption at 1654 cm^{-1}, likely to arise from oxazoline C=N stretching.

In order to determine whether **155** would be reactive towards an additional equivalent of thiophenol, oxazoline **154** was treated with two equivalents of 4-hydroxythiophenol. The amide product **157** was obtained in 66% yield, presumably by Wehrmeister reaction of the intermediate product **155** (Scheme 52).

Scheme 52



i. ***p*-HO-Ar-SH (1 equiv.)**, neat, 120 °C, 2 h
ii. ***p*-HO-Ar-SH (2 equiv.)**, neat, 120 °C, 2 h

These experiments illustrate the strong chemoselectivity of thiophenols towards Michael-type addition in preference to nucleophilic ring-opening. This is likely to be due to the more favourable energy stabilisation that is associated with such soft acid - soft base interactions.

5.2.2 Biological Evaluation

The *in vitro* biological screening of compounds **155** and **157** revealed high levels of cytotoxicity against every cell line tested (Table 10).

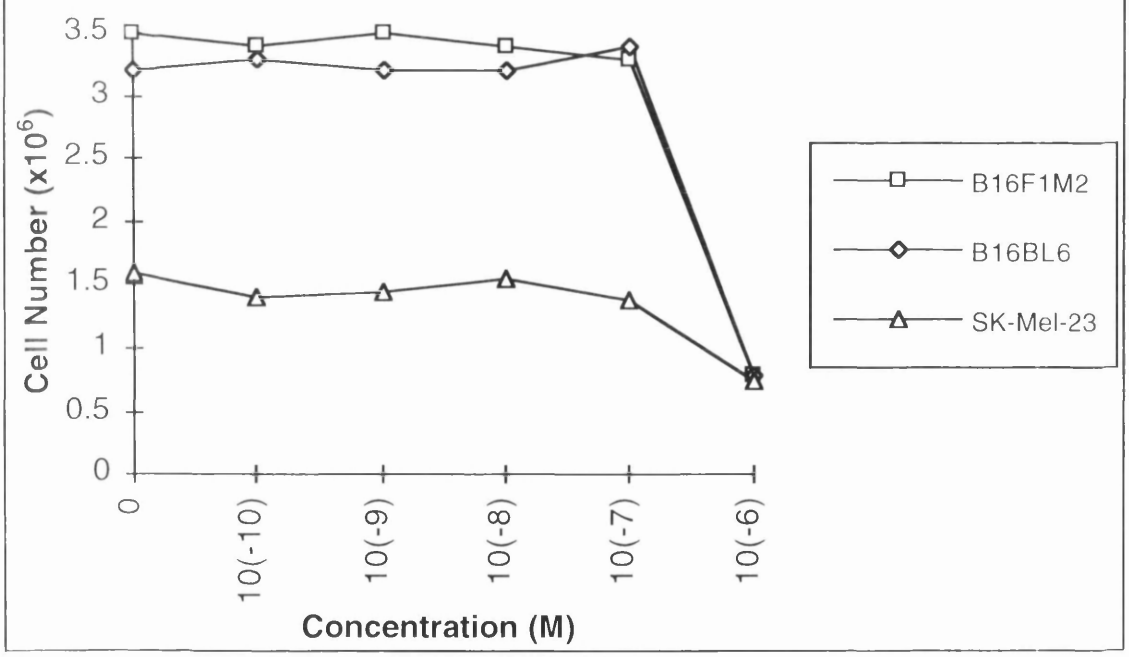
Table 10 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
155	n.d.	7.2	12	n.d.	9.6	n.d.	21
157	3.2	18	7.7	13	17	20	31

n.d. = not determined

Although no evidence of selectivity for melanoma cells was obtained from our initial studies, we were prompted to evaluate **155** more comprehensively on the basis of its interesting structure and high toxicity. The effects of **155** against the highly pigmented SK-Mel-23 human melanoma line and the murine B16F1M2 and B16BL6 melanoma lines were measured by Professor R.M. MacKie. The cells were cultured for 4 days in the presence of six concentrations of **155** before harvesting and determination of total cell number. The variation in cell number for the three cell types at the six concentrations is illustrated in Figure 9.

Figure 9 Further Evaluation of Compound 155

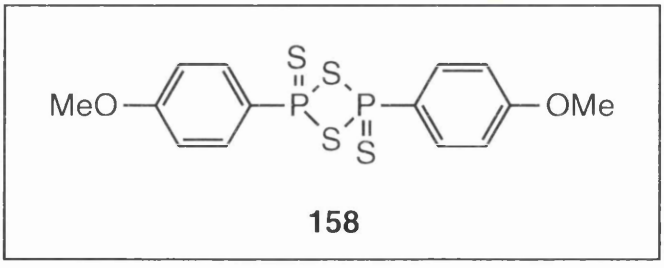


These data show that compound **155** causes no significant toxicity towards the three cell lines at sub-micromolar concentrations. In particular, cytotoxicity appears to be Tyrosinase-independent as the highly pigmented SK-Mel-23 line is no more susceptible to the drug than the other lines. This result corroborated the poor selectivity observed in our initial *in vitro* testing and prompted us to evaluate analogues of **47** containing other amide replacements. We initially focused on the synthesis of thioamides in order to increase lipophilicity without severely affecting molecular shape.

5.3 Thioamides

5.3.1 Synthesis of Thioamides

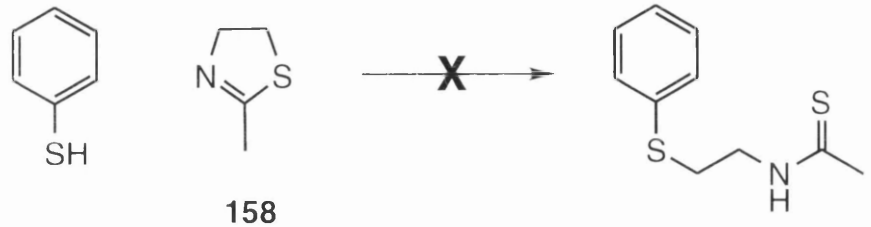
The classical method of preparing thioamides involves the thionation of carboxamides with phosphorus pentasulfide. However, the application of this procedure is often hampered by incompatibility with functional groups and an inability to suppress the decomposition of the products into nitriles and hydrogen sulfide.[141] Although P_4S_{10} is still frequently employed in the synthesis of thioamide-containing heterocycles, it has been superseded by Lawesson reagent **158** in most other thionation reactions.[142]



158

We initially attempted to prepare thioamides such as **159** by treating thiophenol with 2-methyl-2-thiazoline (Scheme 53). No product formation was detected by the use of standard Wehrmeister reaction experimental conditions,[92] even with extended heating times.

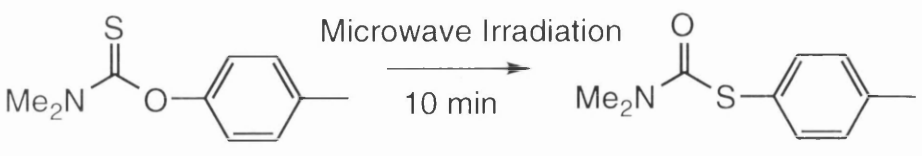
Scheme 53



158 159

From such simple experiments, it is not possible to conclude that 2-thiazolines do not react with thiophenols *per se*, but we can infer that reactivity is far lower than with 2-oxazolines. This difference can be rationalised on thermodynamic grounds by considering the bond-breaking and bond-forming processes that are involved. In both cases, formation of a new double bond is followed by cleavage of a carbon-nitrogen double bond and the feasibility of the process will be governed by the relative stability of the oxazolinium or thiazolinium anionic intermediate relative to the ring-opened product. Reactivity of 2-oxazolines with thiophenols involves cleavage of an imidate functionality to yield a stable amide product containing a resonance-stabilised pπ-pπ bonded carbonyl group. In contrast, with thiazolines a carbon-nitrogen double bond would have to be sacrificed in order to gain a relatively unstable pπ-dπ bonded carbon-sulfur double bond. This higher thermodynamic stability of multiple bonds between first row elements relative to those between first and second row elements is sometimes useful in synthesis. One example is the isomerisation of thionocarbamates which is usually effected thermally at temperatures of 200 °C or above or alternatively by microwave irradiation. Recently, Villemin *et al.* prepared thiophenols by hydrolysis of products such as **161**, obtained by isomerisation of thionocarbamate **160** (Scheme 54).[143]

Scheme 54



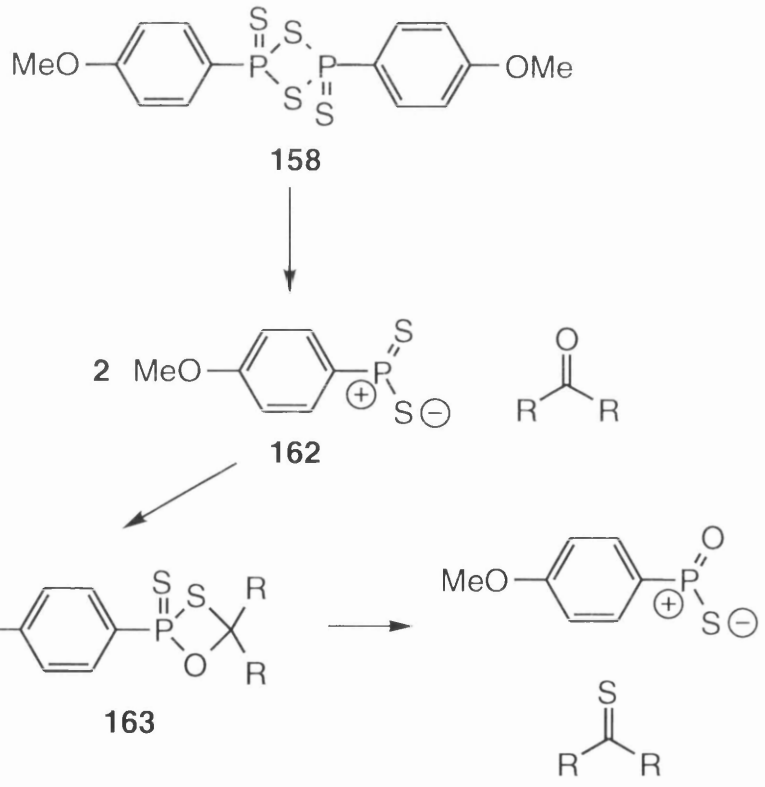
160 161

Scheibye *et al.* have studied the Lawesson reagent-mediated thionation of amides which also contain a phenol group.[144] They reported that only low to moderate yields of the corresponding thioamide were obtained by heating the

substrates with 0.5 equivalents in HMPA. The low yields were attributed to a side-reaction between the phenol functional group and Lawesson Reagent, although the use of HMPA is thought to suppress this side-reaction by coordinating to the phenol. The ability of HMPA to form complexes with amino- and hydroxy-aromatics is well known.[145]

In analogy to the Wittig reaction, the driving force for these thionation reactions is the formation of strong phosphorus-oxygen bonds. Furthermore, it is thought that the active substrate is not actually Lawesson Reagent **158** itself, but a highly reactive dithiophosphine ylid **162**. Such dipolar species have been detected by ^{31}P NMR spectroscopy in solutions of Lawesson Reagent.[146] The mechanism proposed by Lawesson and co-workers also involves the Wittig-type intermediate **163**, as shown in Scheme 55.[147]

Scheme 55

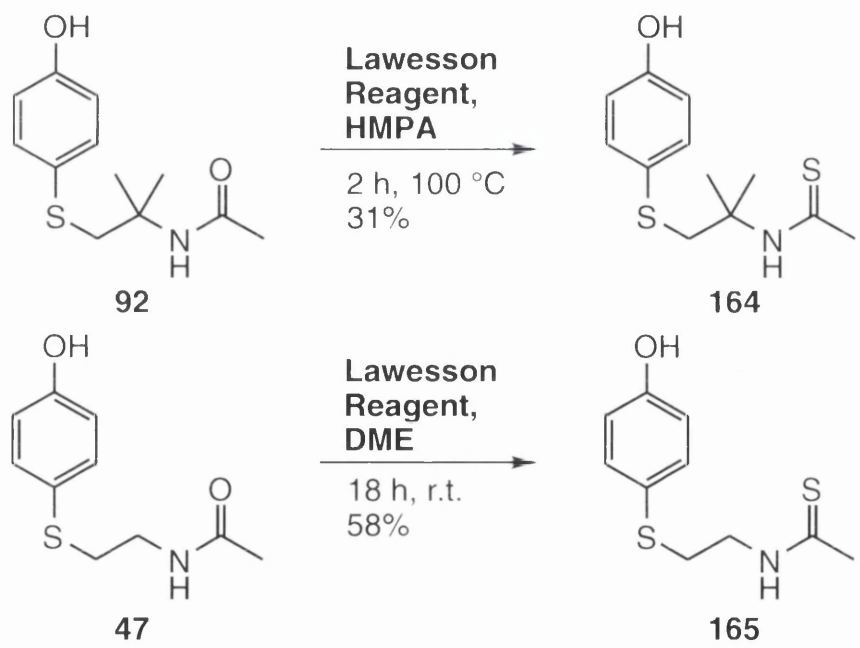


We used the conditions described by Scheibye *et al.*[144] to prepare **164** from the amide **92** in 31% yield (Scheme 56). Despite the poor yield of **164**, it was readily separated from side-products by column chromatography due to its low polarity. Although the yield may have been improved by the use of a protective group for the phenol, we wished to find an alternative method of thionation to preclude use of the highly toxic HMPA solvent.

Taylor *et al.*[148] recently reported a procedure for the preparation of thioamides from carboxamides using Lawesson reagent in DME at room temperature. We were therefore keen to establish whether these milder and less hazardous conditions could be applied to substrates containing a phenol group. To this end, a 58% yield of **165** was obtained by stirring **47** with 0.5 equivalents of Lawesson Reagent, in accordance with the published conditions[148] (Scheme 56).

On the basis of these two experiments it is not possible to conclude that DME is intrinsically a better solvent for these thionation reactions as different substrates and reaction temperatures were employed. The better yield obtained in the preparation of **165** may have been due the lower temperature enhancing the chemoselectivity of Lawesson reagent towards the amide group versus the phenol group. Another possibility is that DME coordinatively protects the phenol group more satisfactorily than HMPA.

Scheme 56



The IR spectra of **164** and **165** show, *inter alia*, absorption peaks at ~1540 cm^{-1} and ~1270 cm^{-1}. These are characteristic of thioamides and arise from the N-H bend and C=S stretching modes. Whilst the N-H bend (~1540 cm^{-1}) is at a similar frequency to that recorded for analogous carboxamides, the thiocarbonyl stretching frequency (~1270 cm^{-1}) is considerably lower than the frequency of the amide I absorption in the parent carboxamides (1640 cm^{-1}). This difference can be explained by the relatively weak nature of the

thiocarbonyl bond relative to the strongly pπ-pπ bonded carbonyl group of the carboxamides.

Another major spectroscopic disparity between the thioamides and their parent amides is the chemical shift of the carbon atom in the (thio)carbonyl group, as revealed by ^{13}C NMR spectroscopy. Due to poor orbital overlap, the π-bonding electrons in thioamides groups are not as delocalised as those of amides, which causes deshielding of the thioamide sp^2-hybridised carbon atom. This results in a ^{13}C NMR chemical shift of ~δ 200, some 30 ppm higher than that of the corresponding amide.

5.3.2 Biological Evaluation

GI_{50} values from the screening of thioamides **164** and **165** against the usual panel of cell lines are shown in Table 11. The results show that **164**, which contains a branched side-chain, is considerably more cytotoxic than its parent amide. From the data obtained, it is not possible to conclude whether **165** is a better cell growth inhibitor than its precursor **47**.

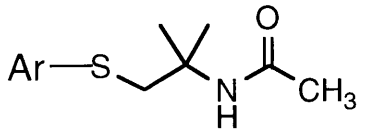
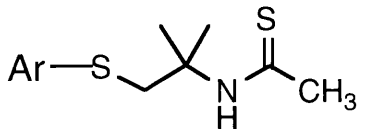
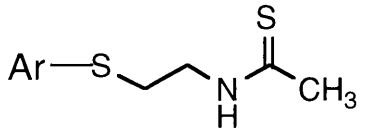
Table 11 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
92	*29*	*>100*	*52*	*95*	*>100*	*79*	*>100*
164	7.2	44	16.5	33.5	>100	22	67
47	*n.d.*	*>100*	*>100*	*n.d.*	*>100*	*>100*	*>100*
165	48	>100	>100	>100	>100	>100	>100

n.d. = not determined

Although **164** is clearly a very toxic compound, its effects are not significantly selective for the melanoma cell lines. Moreover, a high level of growth inhibition was measured against the melanoma line SK-Mel-24 which does not use Tyrosinase. Therefore, it is likely that the increased activity is simply due to the greater lipophilicity of the thioamide functional group. In order to quantify this increased lipid-solubility, we determined the 1-octanol:water partition coefficients of **164** and **165** and compared these with the value for **92** (Table 12).

Table 12 1-Octanol: Water Partition Coefficients

Compound		*P*	log *P*
92		2.76	0.44
164		8.54	0.93
165		3.55	0.55

These data show that **165** is apparently more lipophilic than **92**, yet its GI_{50} values are higher. This may indicate that the high toxicity of compounds with two methyl groups in the side-chain is due to factors other than greater lipid solubility. As discussed in Section 4.5, the cause of non-selectivity may arise due to low activity towards Tyrosinase.

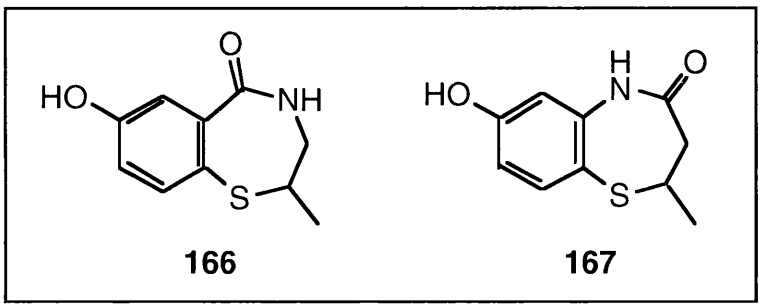
An important feature of drugs with high lipophilicity is their poor solubility in saline. In contrast to amides such as **47** which are soluble in saline to concentrations of 100 μM, the thioamides **164** and **165** are virtually insoluble. As the administration of such non-polar compounds would inevitably be problematic, we decided not to prepare any further thioamides for testing.

5.4 Constriction of Conformation

In the lead compound **47** and its analogues, the side-chain can rotate freely around the sulfide bridging group. As binding to the target enzyme Tyrosinase will require the adoption of a specific conformation, a drug that is locked in this conformation could be expected to exhibit higher activity. In order to prepare analogues of **47** with fewer degrees of freedom, several bicyclic compounds were prepared.

5.4.1 Derivatives of 1,5-benzothiazepin-5(4*H*)-one

2,3-Dihydro-7-hydroxy-2-methyl-1,5-benzothiazepin-5(4*H*)-one **166** and the isomeric system **167** were chosen as initial synthetic targets as they incorporate the structural features of **47** into a more rigid framework. A methyl group was included as, at the time, it was thought that cytotoxicity was increased by the presence of this group (see section 5.2.2).

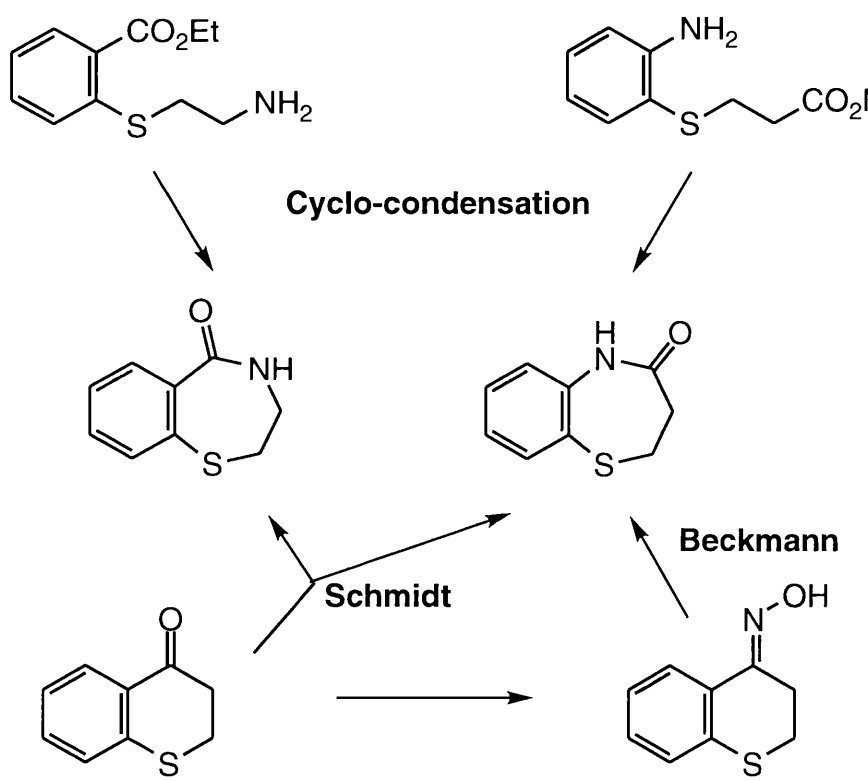


166 **167**

The main literature synthetic routes to these heterocyclic systems are illustrated in Scheme 57 and include the following reaction types.

- Amide bond-forming cyclocondensation reactions.[149,150]
- Beckmann rearrangement of thiochroman-4-one oximes.[151]
- Schmidt reactions of thiochroman-4-ones.[152]

Scheme 57



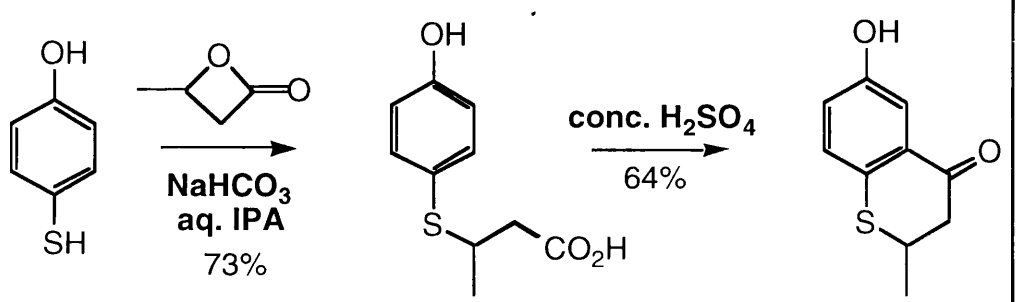
As we needed to incorporate phenol and methyl groups, a ring-expansion reaction seemed to be the most convenient approach. We started by attempting to prepare the novel carboxylic acid **168** by a nucleophilic ring-opening of β-butyrolactone with 4-hydroxythiophenol. Initially we used sodium hydroxide as base, according to the literature procedure of Griesbeck and

Seebach.[153] Several products were detected by TLC, possibly due to side-reactions from deprotonation of the phenol in addition to the thiophenol. An alternative method by Clayton *et al.*[154] uses sodium hydride and would therefore be expected to be similarly non-selective.

In order to enhance selectivity, we modified the procedure of Griesbeck and Seebach[153] by replacing sodium hydroxide with sodium carbonate and this furnished a 71% yield of **168** after recrystallisation (Scheme 58). The product gave the expected spectroscopic and analytical data, and was identical to a sample prepared by acid hydrolysis of **155** (Section 5.2).

Using the procedure of Robillard *et al.*,[155] compound **168** was smoothly converted into the novel thiochroman-4-one **169** by brief treatment with concentrated sulfuric acid (Scheme 58). This Friedel-Crafts cyclisation reaction gave **169** as a yellow powder in 64% yield.

Scheme 58



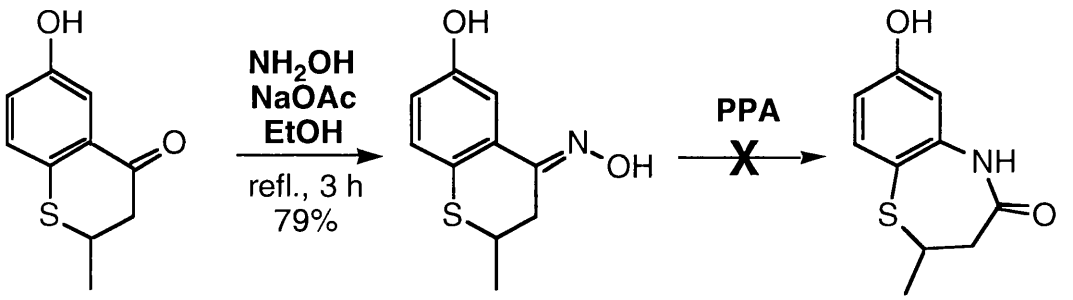
168 **169**

The yellow coloration of **169** is a consequence of an absorption maximum at 381 nm (ε2600), as determined by UV-visible spectroscopy. This arises due to the n→π* transition of the carbonyl group being shifted towards the visible region by the sulfide and phenol substituents of the benzene ring. The presence of the ketone group in **169** was confirmed by solid state IR spectroscopy which revealed an absorption band at 1662 cm^{-1}, likely to arise from C=O stretching. This compares to the analogous band at 1714 cm^{-1} for the carboxylic acid precursor **168**.

Compound **169** was converted into the novel oxime **170** by treatment with hydroxylamine hydrochloride under standard conditions[156] (Scheme 59). The product was isolated as a fawn powder and was found to contain only one oxime geometric isomer by the absence of any doubling of signals in its ^{13}C NMR spectrum. Literature precedent[156] suggests that (*E*)-oximes are preferentially formed by thiochroman-4-one oximes, hence **170** has been tentatively assigned to that geometry. It was intended that the structure of the amide product **167** after Beckmann rearrangement of **170** would be used to

prove its structure. However, this conversion could not be performed satisfactorily due to the formation of many inseparable products after treatment with freshly prepared polyphosphoric acid (Scheme 59).

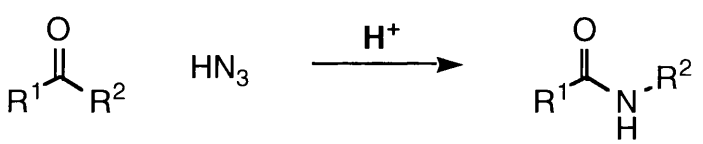
Scheme 59



169 **170** **167**

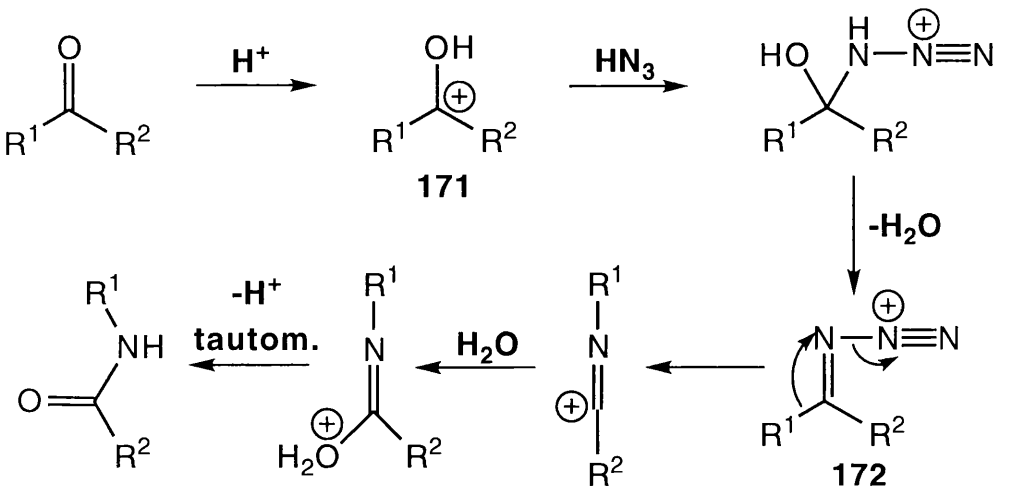
Concentrated sulfuric acid was similarly unsatisfactory in effecting the Beckmann rearrangement, therefore an alternative strategy was sought. As it seemed likely that the phenol group of **170** was causing side reactions, we initially considered protecting this group prior to the Beckmann rearrangement. However, as **170** *and* its regioisomer were required, we envisaged that a Schmidt reaction of thiochromanone **169** would effectively furnish both of these compounds in one reaction. When applied to ketones, this reaction uses hydrazoic acid to provide a method of inserting NH between the carbonyl group and one R group, as shown in Scheme 60.

Scheme 60



The mechanism[166,167] is believed to proceed *via* initial formation of carbocation **171**. Nucleophilic attack by hydrazoic acid, followed by dehydration, migration of the R group and concomitant loss of nitrogen affords intermediate **172**. Hydration and tautomerisation leads to the amide product, as shown in Scheme 61.[166,167]

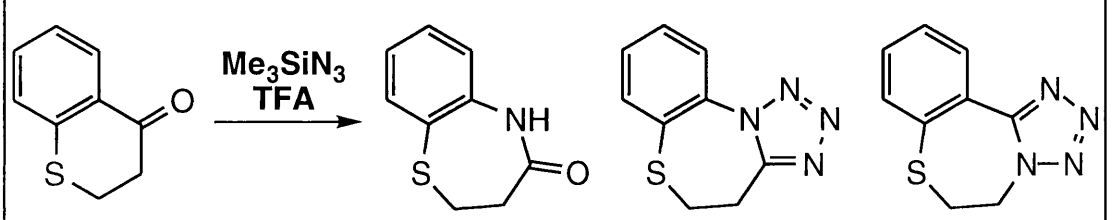
Scheme 61



The migratory aptitude of the R group is obviously critical to the useful application of this reaction in synthesis. Generally, aliphatic ketones react more rapidly than those containing aromatic groups and with aryl alkyl ketones it is usually the aryl group that migrates to the nitrogen atom. However, the alkyl group is known to migrate preferentially in aryl alkyl ketones containing a bulky alkyl group or with cyclic aryl alkyl ketones containing electron-withdrawing groups in the *ortho* or *para* positions of the benzene ring.[168]

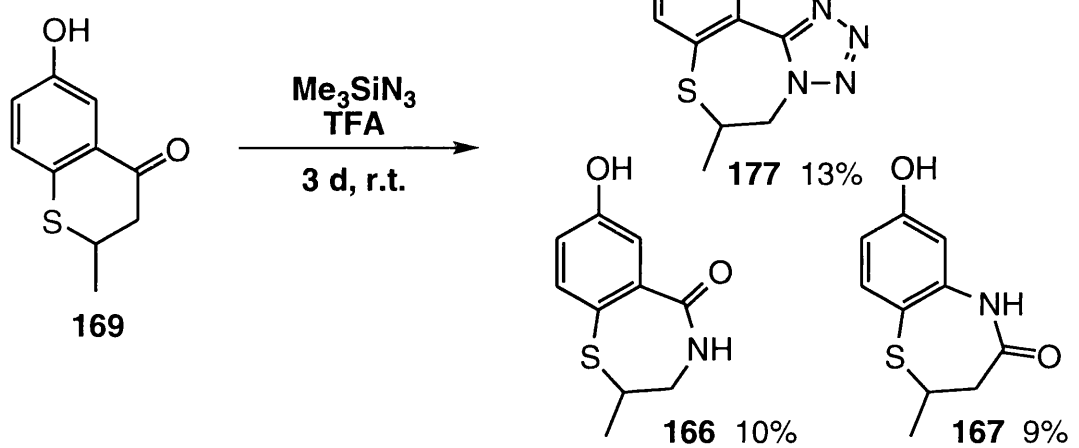
Due to its toxicity, hydrazoic acid is normally generated *in situ* by treatment of sodium azide with glacial acetic acid and sulfuric acid is typically added as a catalyst for the Schmidt reaction. Azidotrimethylsilane, in conjunction with trifluoroacetic acid (TFA), has also been developed as a non-explosive azide source for the Schmidt reaction[169] and this methodology was recently applied to thiochroman-4-ones by Kaye and Mphahele.[170] They found that reaction of the parent system **173** led to the formation of 2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one **174** in addition to the regioisomeric tetrazoles **175** and **176** (Scheme 62).

Scheme 62



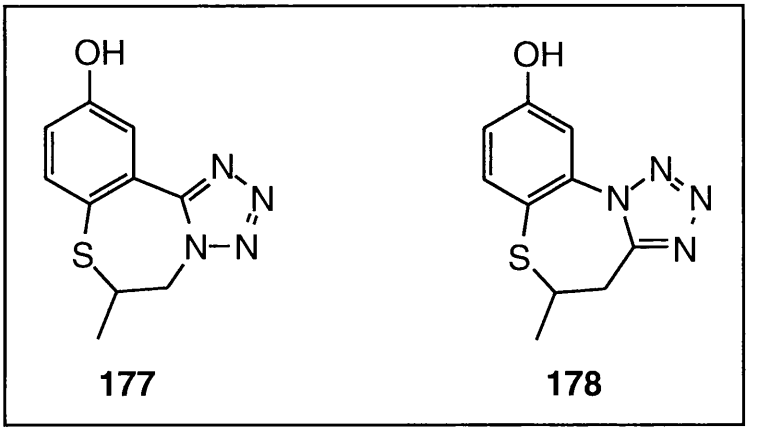
We applied the same conditions[170] to thiochroman-4-one **169** and isolated the tetrazole **177** and the two regioisomeric benzothiazepinones **166** and **167** from the product mixture by flash chromatography (Scheme 63). The other regioisomeric tetrazole was also formed in the reaction and co-eluted with **177** during chromatography. However, a pure sample of the major isomer **177** was obtained by crystallisation of the mixture.

Scheme 63



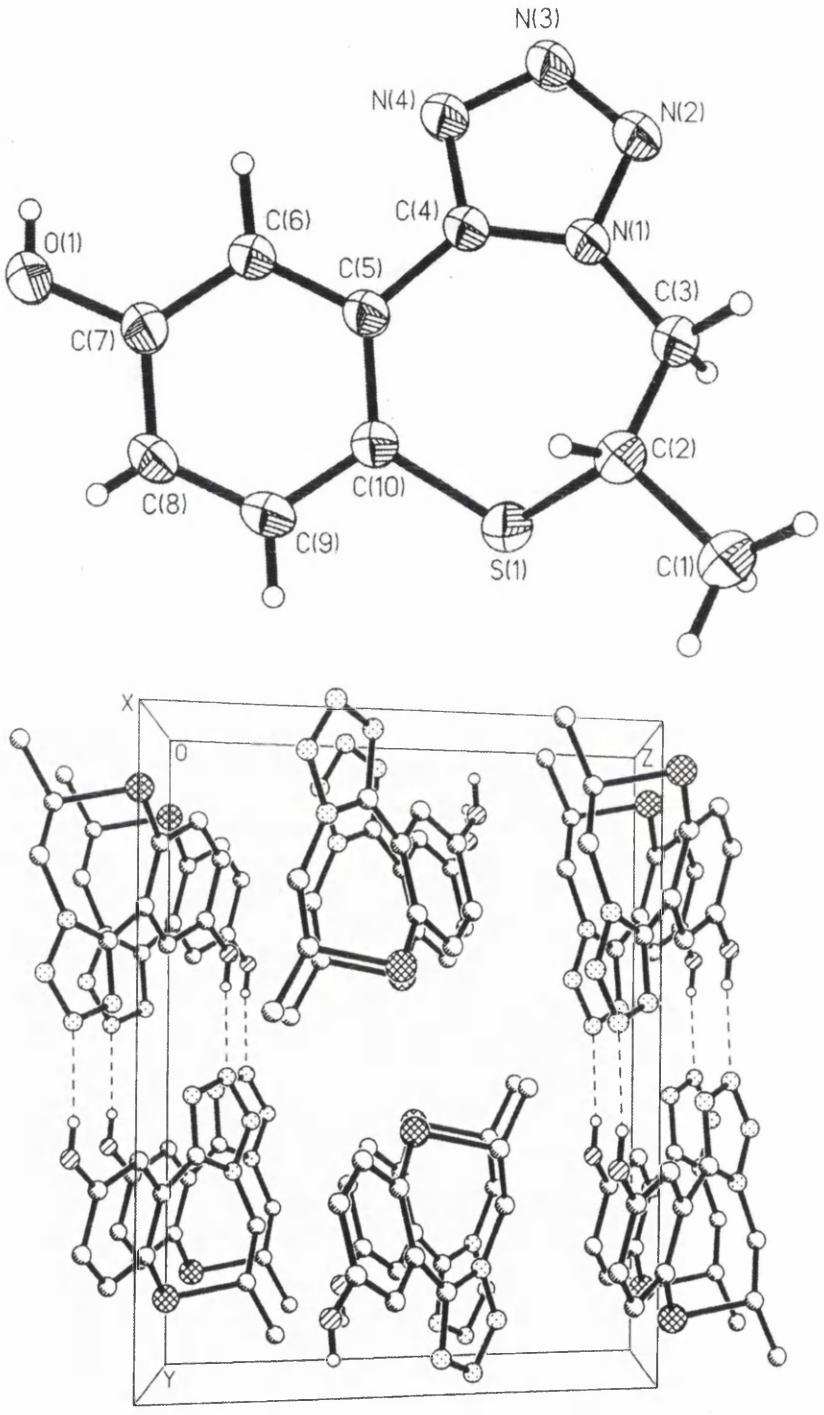
The formation of tetrazoles such as **177** arises due to reaction of a late cationic intermediate with an additional equivalent of hydrazoic acid rather than an equivalent of water[171] (cf. Scheme 61). The product **177** was readily differentiated from the required products **166** and **167** by the lack of a amide carbonyl stretching band at ~1650 cm^{-1} in its IR spectrum. In addition, ^{13}C NMR spectroscopy (D_6-DMSO) revealed that the imine-type carbon present in **177** resonated at δ 158, somewhat lower δ than the corresponding amide carbon atoms present in **166** (δ 171) and **167** (δ 179).

The isolated tetrazole **177** was initially differentiated from its regioisomer **178** on the basis of ^1H NMR spectroscopy. In the isolated product, the diastereotopic protons of the methylene group account for the doublet of doublets at δ 4.38 and the doublet of doublets at δ 4.83. According to literature data, chemical shifts for the analogous methylene group protons in **178** would be significantly (~1 ppm) lower and these were identified in the ^1H NMR spectrum of the mixture of **177** and **178** before crystallisation.



In collaboration with McCormack,[172] unequivocal proof of the structure of **177** was provided by X-ray single crystal structural analysis which revealed that the compound crystallised into the monoclinic $P2_{1/C}$ space group. The ORTEP diagrams (Figure 10) show important hydrogen bonding between the phenol group and a tetrazole nitrogen N(3) atom which was calculated to have a 2.09 Å bond length and an O-H-N angle of 173.2 °.

Figure 10 X-Ray Crystal Structure of Compound 177



The two regioisomeric benzothiazepinones **166** and **167** were differentiated by ^{1}H NMR spectroscopy, with coupling between the methylene group and the N-H group being used to identify **166**.

5.4.2 Biological Evaluation

The three products from the Schmidt reaction, thiochromanone **169**, oxime **170** and the carboxylic acid **168** were all evaluated for anti-melanoma activity using the same cell lines and growth inhibition assay as before (Table 13).

Table 13 GI_{50} Values (μM) for 96H SRB Assay

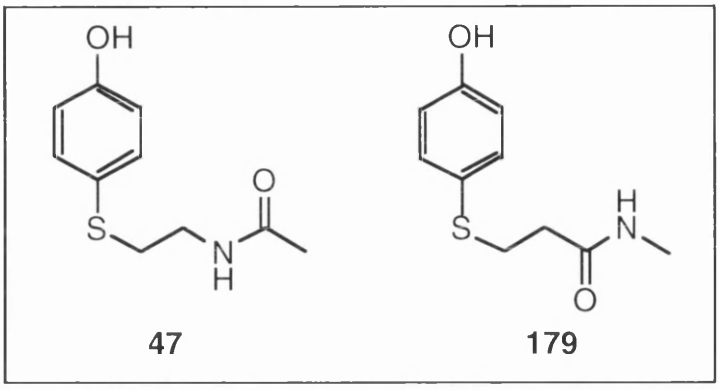
	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
166	>100	>100	>100	>100	>100	>100	>100
167	>100	>100	88	>100	>100	>100	>100
177	>100	>100	>100	>100	>100	>100	>100
169	>100	>100	>100	>100	>100	>100	>100
170	>100	>100	72	>100	>100	>100	>100
168	>100	>100	>100	>100	>100	>100	>100

The test results for these compounds suggest that they are of very low toxicity and apparently have no potential in anticancer chemotherapy. However, due to their structural resemblance to the benzodiazepine class of anxiolytic drugs, the new benzothiazepinones prepared may have other biological activity. Therefore, it may prove useful to evaluate these compounds for effects towards the central nervous system.

On the basis of the low cytotoxicity of these compounds, we decided to examine the effects of inverting the amide group present in the lead compound **47**. The results of this work are discussed in the following section.

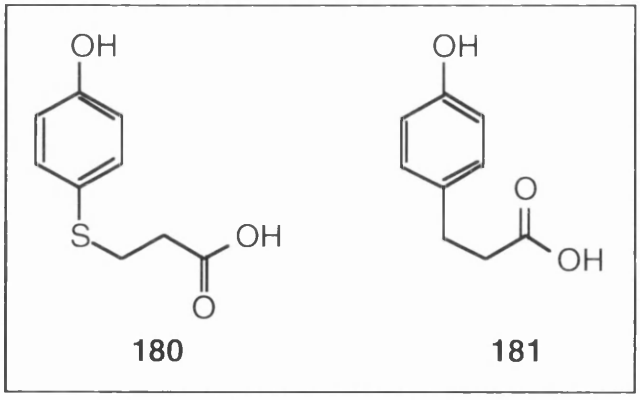
5.5 Inversion of the Amide Group

The anti-melanoma effects of **47** have been attributed to its structural similarity to tyrosine, which enables it to be oxidised by Tyrosinase to an active quinone drug. As regioisomeric analogues such as **179** have apparently not been evaluated for anti-melanoma activity, we were eager to prepare some of these compounds for testing.



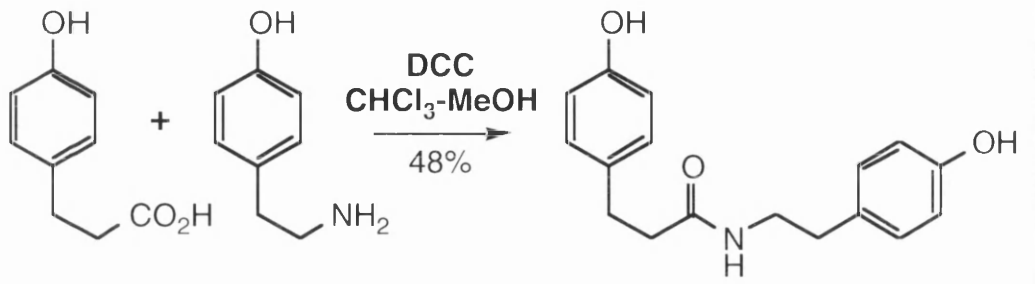
5.5.1 Derivatives of Phloretic Acid

It was presumed that **179** could be synthesised by amination of the carboxylic acid **180**, provided that competing reactivity by the phenol was minimal. In order to develop an effective preparative procedure, we decided to utilise the commercially available phloretic acid **181** as a model system for the amide-forming reactions. As **181** does not possess a sulfide group, we envisaged that the products formed would also be worthy of biological evauation on the basis of their tyrosine-like structure.



Direct aminations of **181** using DCC have been previously reported in the literature. For example, Herbert and Kattah treated **181** with an equivalent of tyramine **182** and excess DCC to afford the product **183** in 48% yield (Scheme 64).[173] However, we found that this method could not be applied to the synthesis of simpler analogues of phloretic acid due to rapid precipitation of the salt arising from protonation of the amines by the acid.

Scheme 64

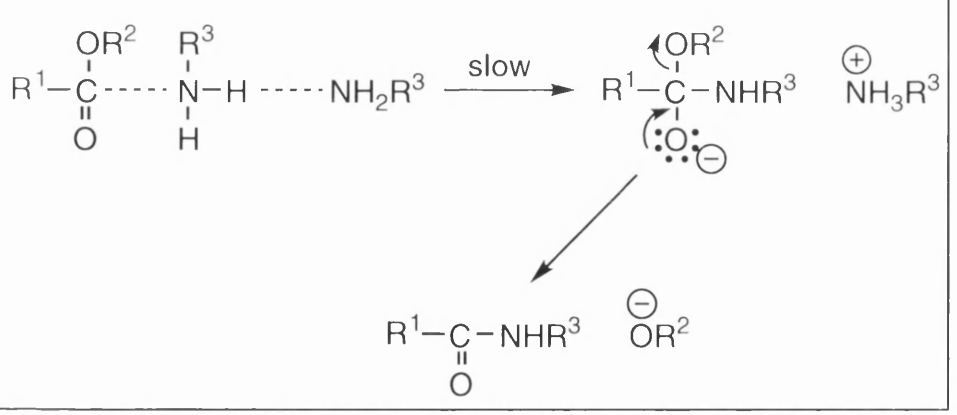


181 **182** **183**

Prompted by a preparation of the *para*-nitrophenyl ester of **181** by Williams and Salvadori,[174] we implemented a one-pot procedure whereby this labile ester was generated *in situ* by DCC coupling, prior to treatment by an amine. This method proved to be an effective method of derivatising **181**, and was found to be amenable to the coupling of both primary and secondary amines.

The mechanism of the acylation of amines by esters requires two molecules of amine in the rate determining step, as shown in Scheme 65.[175-177] Although this additional catalytic quantity of base is often provided by the use of additional amine substrate, an alternative is to use a non-nucleophilic base such as DBU.[178] With *para*-nitrophenyl esters, the acidic *para*-nitrophenol by-product effectively removes one equivalent of base by salt formation. Therefore, in that case the reaction is base-promoted rather than base-catalysed and means that two full equivalents of amine are required, of which one equivalent may be provided by DBU.

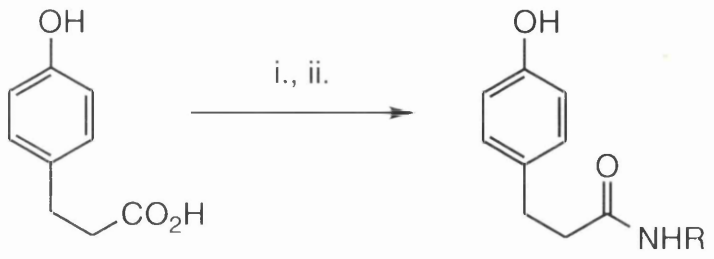
Scheme 65



Our one-pot procedure involved the initial treatment of **181** with *para*-nitrophenol and DCC in ethyl acetate solution. After stirring the mixture for 1 h at room temperature or 0 °C, the amine and DBU were added before stirring for

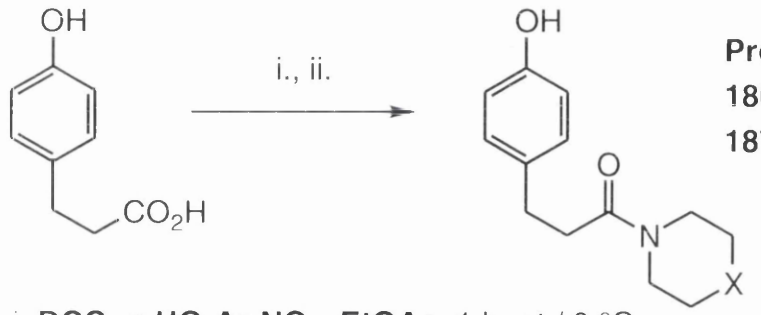
a further 14 h at room temperature. The required novel products **184**-**187** were isolated in yields of 39-65% after work-up and flash chromatography (Scheme 66).

Scheme 66



Product	R	Yield
184	Me	49%
185	Pr^n	43%

i. **DCC, *p*-HO-Ar-NO_2, EtOAc,** 1 h, 0 °C.
ii. **NH_2R, DBU,** 14 h, r.t.



Product	X	Yield
186	CH_2	65%
187	O	39%

i. **DCC, *p*-HO-Ar-NO_2, EtOAc,** 1 h, r.t./ 0 °C.
ii. **Piperidine/Morpholine, DBU,** 14 h, r.t.

All four products gave the expected analytical and spectroscopic data. For the tertiary amides, complex ^1H and ^{13}C NMR spectra were recorded due to the lack of rotation about the amide carbon-nitrogen bond which results in chemical (and magnetic) inequivalence between the ring methylene group protons of **186** and **187**.

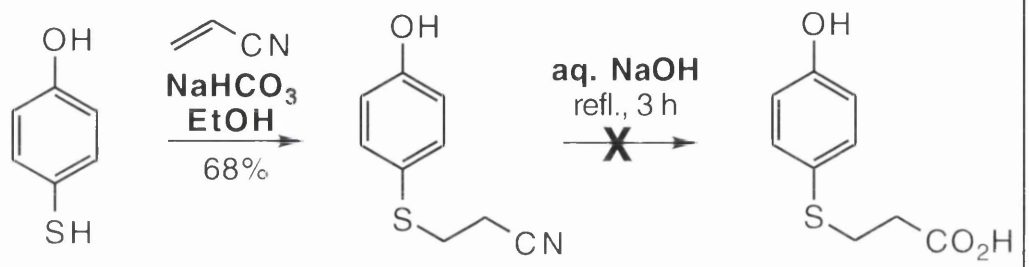
5.5.2 Sulfide-containing analogues

Although only moderate yields were obtained, the successful preparation of these compounds prompted us to prepare the analogous sulfur-containing products by the same method. For this, a sample of the carboxylic acid **180** was needed and we initially planned to prepare this by hydrolysis of nitrile **188**.

We prepared **188** by treatment of 4-hydroxythiophenol with acrylonitrile, by a slightly modified literature procedure (Scheme 64).[179] Our product gave a similar m.p. to that reported by Inoue *et al.*,[179] and as no other analytical or spectroscopic information were reported in the literature, ^1H NMR, ^{13}C NMR,

MS, IR and microanalytical data are presented in section 7.3. As attempted base-mediated hydrolysis of **188** failed (Scheme 67), possibly due to cleavage of the sulfide group, an alternative route to **180** was sought.

Scheme 67

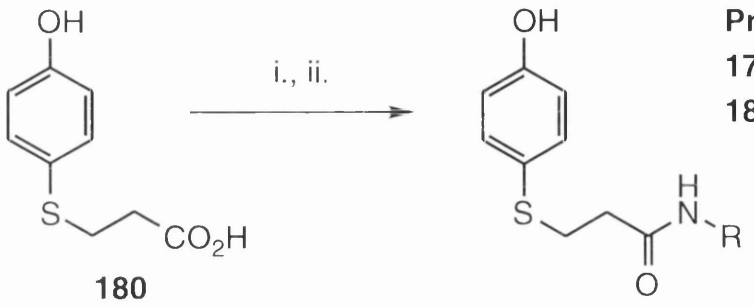


188 **180**

Although carboxylic acid **180** has not been reported in the literature covered by *Chemical Abstracts* for over 25 years, it was mentioned in three earlier publications.[180-182] Akagi and Aoki prepared **180** by reaction of 4-hydroxythiophenol with 3-bromopropanoic acid.[180,181] However, Buck and Sehring used a different approach by fusing 4-hydroxythioanisole with chloroacetic acid to furnish a 67% yield of the required acid **180**.[182]

We prepared **180** in 53% yield by treatment of 4-hydroxythiophenol with acrylic acid, according to a general procedure reported by Hogeveen and Montanari.[183] The product was subjected to the same one-pot procedure as before to afford the required amides **(179, 189-191)** in yields of 47-65% (Scheme 68).

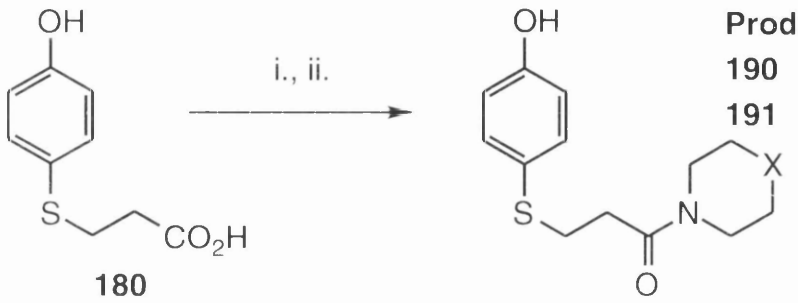
Scheme 68



Product	R	Yield
179	Me	61%
189	Pr^n	60%

180

i. **DCC, *p*-HO-Ar-NO_2, EtOAc,** 1 h, r.t..
ii. **NH_2R, DBU,** 14 h, r.t.



Product	X	Yield
190	CH_2	65%
191	O	47%

180

i. **DCC, *p*-HO-Ar-NO_2, EtOAc,** 1 h, r.t./ 0 °C.
ii. **Piperidine/Morpholine, DBU,** 14 h, r.t.

All four amides are new compounds, and they were fully characterised by the usual range of analytical and spectroscopic techniques. A major difference between the amides derived from phloretic acid and those containing a sulfide group was evident from their partition coefficients. As expected. the log *P* values (Table 14) show that the compounds containing a piperidine group are more lipophobic than those with a morpholine ring and those with an *N*-methyl group are more lipophobic than analogues with an *N*-propyl substituent. As compounds **179** and **189** - **191** are simply the phloretic acid derivatives **184** - **187** with a sulfur atom inserted into the side chain, these data also illustrate that the addition of the sulfide group has large effects in terms of lipophilicity. This, coupled with the vastly different electronic effects towards the benzene ring imposed by a sulfide group versus a methylene group, and different steric effects, would all be expected to cause much variation in cytotoxic activity. Also of interest is the observation that compound **189** is apparently more lipophilic than its regioisomer **47** (log *P* =0.31, Table 14), on the basis of the 1-octanol:PBS (pH 7.4 phosphate buffered saline) partition coefficients.

Table 14 1-Octanol: PBS Partition Coefficients

Phloretic Acid Derivatives			Sulfides		
Compound	***P***	**log *P***	**Compound**	***P***	**log *P***
184	2.67	0.43	**179**	5.57	0.75
185	3.87	0.59	**189**	14.4	1.2
186	20.4	1.3	**190**	9.53	0.98
187	2.28	0.36	**191**	5.67	0.75

5.5.3 Biological Evaluation

The four amides **184-187** were evaluated for anti-melanoma activity against the usual panel of cell lines (Table 15).

Table 15 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
47	*>100*	*>100*	*>100*	*>100*	*>100*	*>100*	*>100*
184	>100	>100	>100	>100	>100	>100	>100
185	>100	>100	>100	>100	>100	>100	>100
186	>100	>100	>100	>100	>100	>100	>100
187	>100	>100	>100	>100	>100	>100	>100

These data show that all of the phloretic acid derivatives exhibit low levels of cytotoxicity towards all of the cell lines tested. The final compounds **179, 189-191** essentially comprise these phloretic acid derivatives with a sulfur atom inserted at the benzene ring bridging position. Although these sulfides have been submitted to the Institute of Cancer Research for evaluation, the results were not available at the time of thesis submission.

The sulfide group of **47** undoubtedly has large effects on polarity, conformation, Tyrosinase binding and drug oxidation. Therefore, in order to examine the biological effects of introducing other groups to this bridging position, a further round of analogues was prepared for testing. The results from that study are discussed in the following chapter.

6

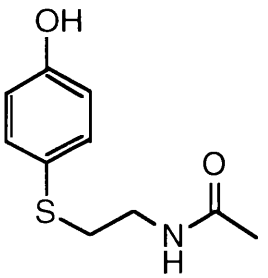
Sulfide Group Replacements

6.1 Introduction

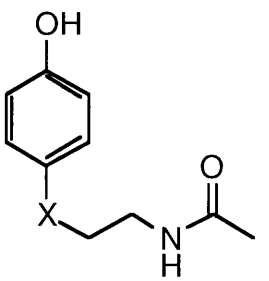
Passi and Nazzaro-Porro[61] concluded that the susceptibility of simple phenols to undergo oxidation by Tyrosinase is a function of the electron-releasing properties of the bridging atom. However, if this was the only factor controlling Tyrosinase oxidation, the natural substrate tyrosine would show little activity towards the enzyme. Other considerations such as bond angle, steric hindrance, lipophilicity and hydrogen-bonding ability are also critical.

In order to examine whether the sulfur atom of **47** is essential to its biological activity, we planned to make the series of compounds **192-198** shown in Figure 11.

Figure 11 Planned Sulfide Replacements



47



Compound	X
192	S=O
193	SO_2
194	O
195	Se
196	CH_2
197	C=O
198	NH

Although the compounds **192-198** are of similar structure, diverse procedures are required for their syntheses. For simplicity, we began work by preparing analogues with an oxidised sulfur atom, as discussed in the following section. The results from the biological evaluation of all the compounds covered in this chapter will be discussed in section 6.8.

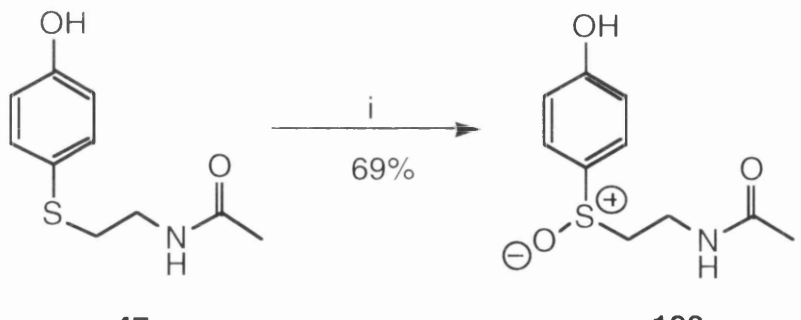
6.2 Sulfoxide / Sulfone

We envisaged that the introduction of a sulfoxide group to the bridging position of **47** would create a compound of lower activity due to decreased lipophilicity and deactivation of the benzene ring. However, the compound was required to confirm this hypothesis and was readily available by oxidation of the parent sulfide **47**.

In selecting a suitable oxidising agent to be used in the preparation of **192**, we needed a reagent that would selectively oxidise the sulfide without further oxidising the product or affecting the other functionality present. Although there are several reagents such as sodium permanganate[184] which oxidise sulfoxides in preference to sulfides, many more reagents further oxidise sulfoxide products sufficiently slowly to permit their isolation in high yield.

Accordingly, sodium periodate was employed to oxidise **47** to the required **192** in 69% yield (Scheme 69), according to the general procedure of Hiskey and Harpold.[185] This method was found to be particularly effective as the sodium iodate by-product conveniently precipitated from the ethanol reaction solvent.

Scheme 69

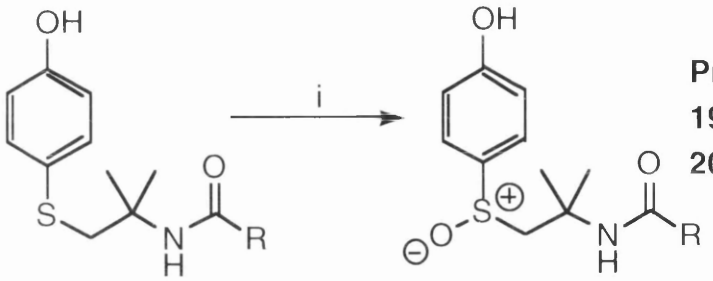


47 **192**

i. **aq. $NaIO_4$ (1.1 eq.), EtOH,** 15 h, 0 °C → r.t.

If the biological activity of **192** was governed mainly by polarity rather than the electronic or steric effects of the sulfoxide group, we envisaged that increasing lipophilicity would give rise to active compounds. Therefore, more lipophilic sulfoxides **199** and **200** were prepared by oxidation of sulfides **92** and **105** by the same procedure[185] (Scheme 70).

Scheme 70



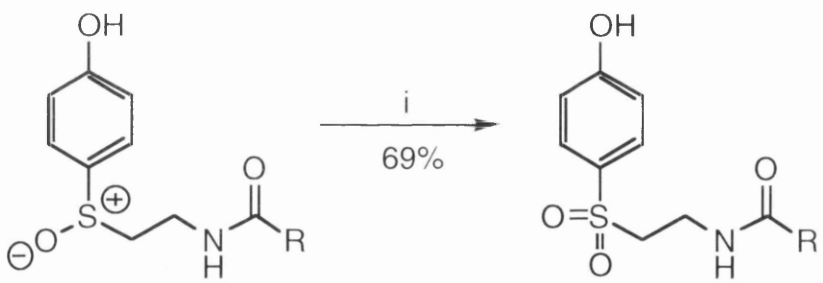
Product	R	Yield
199	Me	72%
200	$(CH_2)_6CH_3$	73%

i. **aq. $NaIO_4$ (1.1 eq.), EtOH,** 15 h, 0 °C → r.t.

Although sodium periodate is occasionally used to prepare sulfones from sulfides,[65] the second oxidation step is sometimes troublesome. In an attempted preparation of **193**, sulfide **47** was treated with two equivalents of sodium periodate under standard conditions.[185] However, sulfoxide **192** was the only product detected by TLC after 24 hours stirring at room temperature. Rather than attempt to drive the reaction by increased temperatures, we decided to seek an alternative oxidising agent.

Trost and Curran[186] developed the use of potassium peroxymonosulfate (Oxone®) as a convenient crystalline reagent for the preparation of sulfones from either sulfides or sulfoxides. By use of the original general procedure,[186] sulfoxide **200** was treated with an excess of Oxone® to furnish the required sulfone **193** in 69% yield after flash chromatography (Scheme 71).

Scheme 71



200 **193**

i. **Oxone® (1.5 eq.), MeOH,** 2 h, 0 °C → r.t.

The structure of product **193** was confirmed by ^1H NMR spectroscopy which revealed a far simpler spectrum than that of its chiral precursor **200**, as a consequence of removing the sulfoxide stereogenic centre. The IR spectrum (KBr disc) of **200** showed an absorption at 1024 cm^{-1}, likely to arise from sulfoxide S-O stretching. A peak at this frequency was not observed in the IR

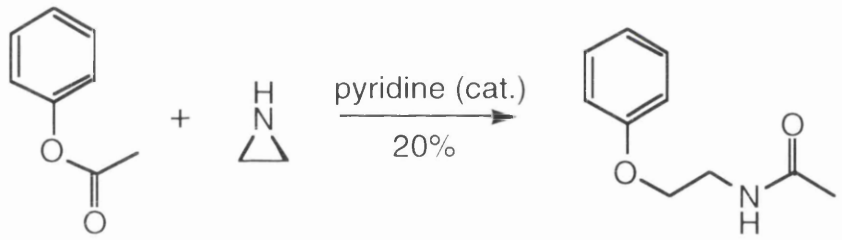
spectrum of **193**. However, it did reveal absorptions at 1304 and 1140 cm^{-1} which are typical values for sulfone S=O stretching modes.

6.3 Ethers

Our interest in the preparation of ether analogue **194** stemmed from a paper by Cooksey *et al.*[67] which reported that *ortho*-quinones with alkylthio substituents were oxidised approximately 5-10 times faster than analogous compounds bearing alkoxy substituents. Therefore, coupled with the lower lipophilicity of ethers, we would expect **194** to exhibit lower anti-melanoma activity than **47**. We wished to confirm this theory by preparing **194** in order to compare its biological activity with that of **47**.

Most literature methods for the preparation of substituted acetamides such as **194** are generally less convenient than the simple oxazoline ring-opening procedure known as the Wehrmeister reaction (Section 3.2). One such example is the formation of **201** by reaction of phenyl acetate with aziridine, as reported by Funahashi[187] (Scheme 72).

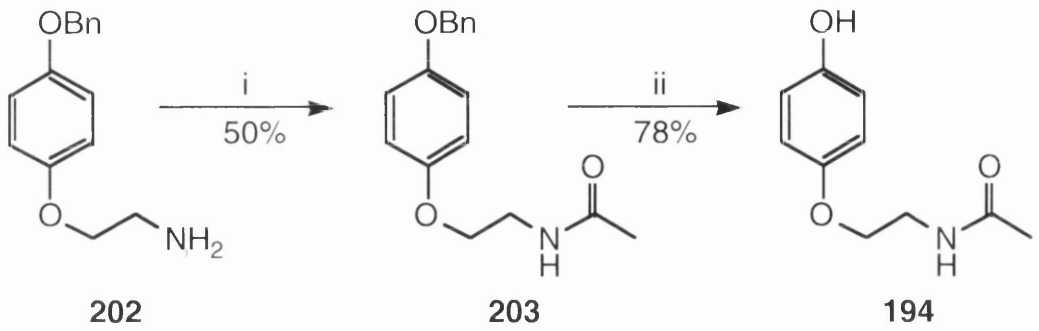
Scheme 72



201

As this method requires the use of the highly toxic aziridine, autoclave conditions and affords a low yield of product, we decided to evaluate other procedures to prepare the ethers **201** and **194**. We were particularly intrigued by the only literature synthesis of **194**, as reported by Howe *et al.*[188] (Scheme 73). This method is further hampered by the three additional steps needed to prepare the precursor **202** from hydroquinone

Scheme 73

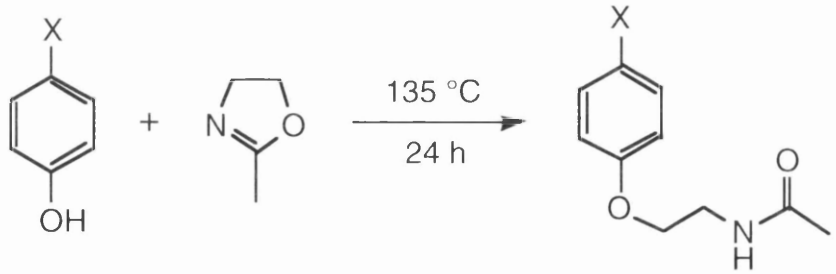


202 **203** **194**

i. **Ac_2O,** 18 h, r.t.
ii. **H_2, Pd-C, AcOH,** 60 °C.

As the patent of Jäger[91] reported that oxazoline ring-opening could be effected by phenols, we wished to determine whether this method could be employed to prepare **194** and **201**. In several trial experiments, it became apparent that more forcing conditions were required, compared to the analogous reaction with thiophenols. As these harsher conditions seemed to cause some decomposition, driving the reactions to completion led to severe problems in product isolation. Therefore, compromise conditions were employed to give a low yield of product with easier isolation (Scheme 74).

Scheme 74



Product	X	Yield
201	H	30%
194	OH	24%

Although the yields are rather disappointing, they are greater than those reported from the literature procedures shown in Schemes 72 and 73. In addition, the oxazoline method is more convenient and less hazardous. In the case of **194**, an increased yield could be obtained by using a mono-protected hydroquinone for the Wehrmeister reaction. However, cleanly protecting only one hydroxyl group of hydroquinone in high yield would not be easy on the basis of chemoselectivity. For example, Stoddart and co-workers prepared the

monobenzyl ether of hydroquinone in 63% yield by treatment with benzyl chloride.[189]

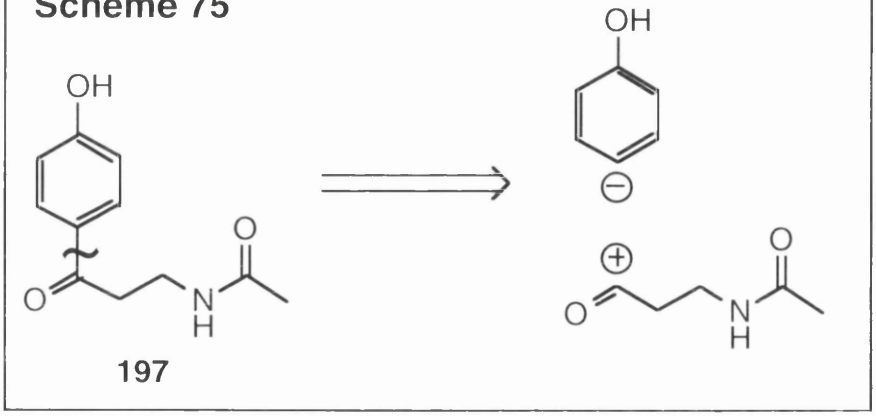
The products **194** and **201** gave spectroscopic data in accordance with those previously reported[187,188] and additional data are presented in Section 7.4.

6.4 Ketone

Replacing the sulfide group of **47** with a carbonyl group to give compound **197** would be expected to cause wholesale changes to the electronic and steric properties of the molecule. In particular, the ketone group is likely to adopt co-planarity with the aromatic system which would have major effects on the molecular conformation.

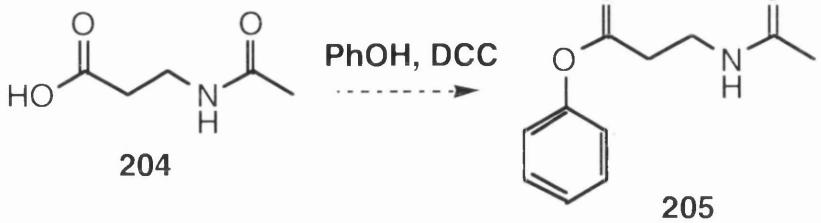
In planning a retrosynthesis of compound **197**, we envisaged that the best disconnection would be between the benzene ring and carbonyl group with the aromatic group as the nucleophilic component (Scheme 75).

Scheme 75



Although this disconnection would normally suggest a Friedel-Crafts acylation process, the presence of the *para*-hydroxyl group makes the Fries rearrangement an option. Such an approach would require the preparation of **205** for use as the substrate ester. In principle, this could be achieved by condensing *N*-acetyl-β-alanine **204** with phenol using DCC as the coupling agent (Scheme 76).

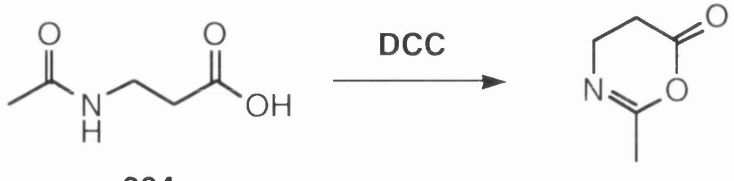
Scheme 76



204 **205**

The amide **204** was prepared in 77% yield by the general procedure of Black and Boscacci,[190] and gave m.p.[191] and NMR spectroscopic data[192] similar to those reported in the literature. Due to the insolubility of **204** in many organic solvents, DCC coupling with phenol was attempted in a mixture of ethyl acetate : pyridine (5:1 v/v) at 0 °C. Although product formation was detected by TLC, complete removal of phenol was not obtained. Analysis of the product mixture by ^{1}H NMR spectroscopy revealed the presence of the required product **205** and unreacted phenol. Curiously, the peaks corresponding to the methylene and methyl groups of **205** seemed to have shifted position. It became apparent that **204** had undergone a known[190] dehydrative cyclisation to give 2-methyl-5,6-dihydro-4*H*-1,3-oxazin-6-one **206** (Scheme 77).

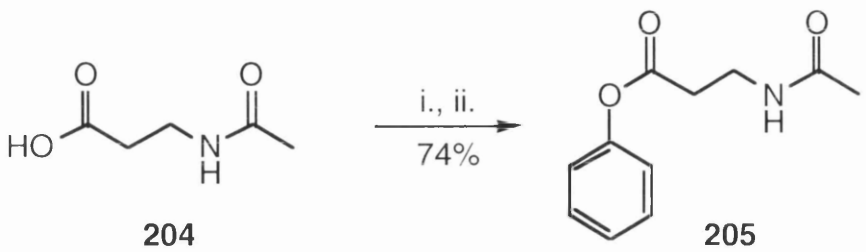
Scheme 77



204 **206**

It was found that with increased reaction temperatures, this side-product **206** is able to react with the residual phenol in the reaction mixture to give the required product. Hence, treatment of **204** with phenol in refluxing ethyl acetate - pyridine afforded ester **205** in moderate yield (Scheme 78).

Scheme 78

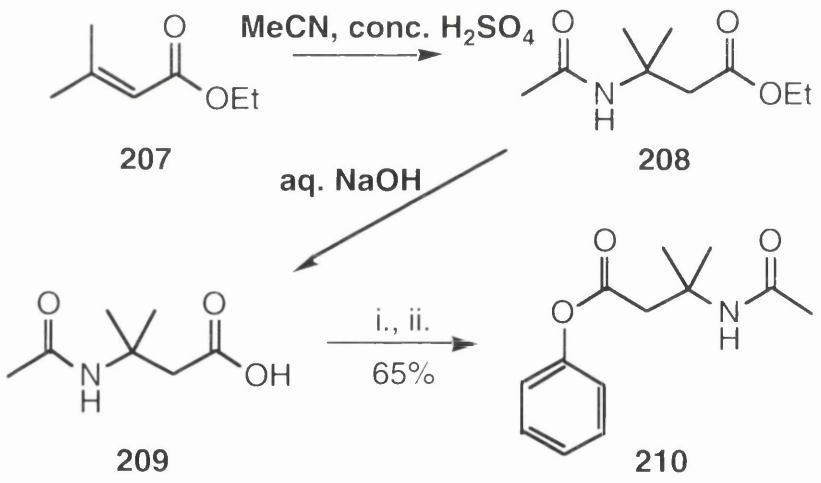


204 **205**

i. **PhOH, EtOAc-py (5:1), DCC,** 0 °C, 4 h.
ii. Reflux, 4 h.

In a similar manner, the novel ester **210** was prepared from carboxylic acid **209**, as shown in Scheme 79. The procedure of Eugster *et al.*[193] was employed to prepare **209** *via* a Ritter reaction of **207** with acetonitrile and subsequent hydrolysis of the ester intermediate **208**.

Scheme 79

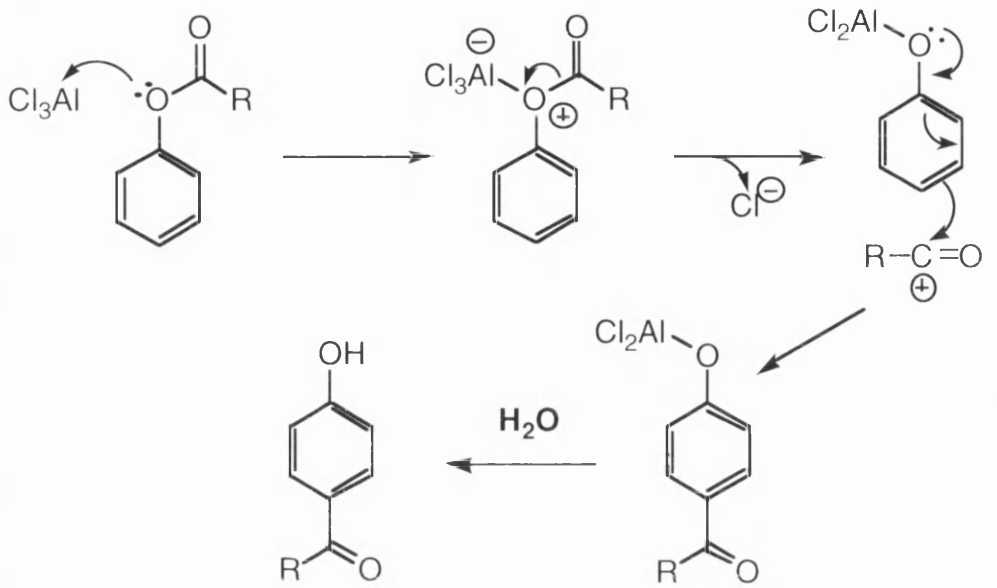


207 **208** **209** **210**

i. **PhOH, EtOAc-py (9:1), DCC,** 0 °C, 4 h.
ii. Reflux, 2 h.

The Lewis acid catalysed Fries Reaction offers an excellent method for preparing phenolic ketones, provided that the regiochemistry of the migration can be controlled. Generally, the reaction under aluminium chloride catalysis is believed to proceed *via* the mechanism shown in Scheme 80.[194] Initially, the Lewis acid attacks the ester bridging oxygen which promotes acylium ion release. The phenol group then directs acylium ion electrophilic attack towards the *ortho* or *para* positions of the aromatic system. As the liberated phenoxy group remains associated with aluminium until the quench, at least one molar equivalent of 'catalyst' is required for the reaction.

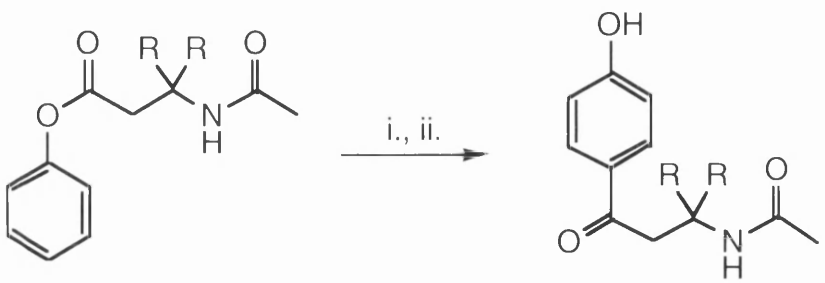
Scheme 80



As the *ortho-para* product ratio is dependent on the solvent, temperature, amount of aluminium chloride used and the nature of the alkyl residue, it is often possible to obtain the required isomer by optimising the first three variables. For example, increasing the reaction temperature generally increases the percentage of *ortho* isomer produced.[194]

We decided to employ the general protocol of Harwood and co-workers,[195] which involved the use of nitromethane[196] as the reaction solvent, a large excess of aluminium chloride and a low reaction temperature. Under these conditions, esters **205** and **210** were converted into the ketones **197** and **211** in low yield after flash chromatography (Scheme 81).

Scheme 81



i. **$AlCl_3$ (5 equiv.), $MeNO_2$,** 0 °C → r.t., 20 min., ii. 40 °C.

Substrate	R	Product	Time (h.)	Yield
205	H	**197**	24	32%
210	Me	**211**	4	42%

Although a low yield of **211** was obtained under the shorter reaction time, no *ortho* product was detected which simplified product isolation. This *para* selectivity is likely to be due to steric effects caused by the bulk of the side-chain dimethyl substituents. In contrast, a separation of **197** from its *ortho* isomer was required after the extended reaction time. Attempts to improve this selectivity through the use of lower temperatures were unsuccessful due to the consequent reduction in reaction rate.

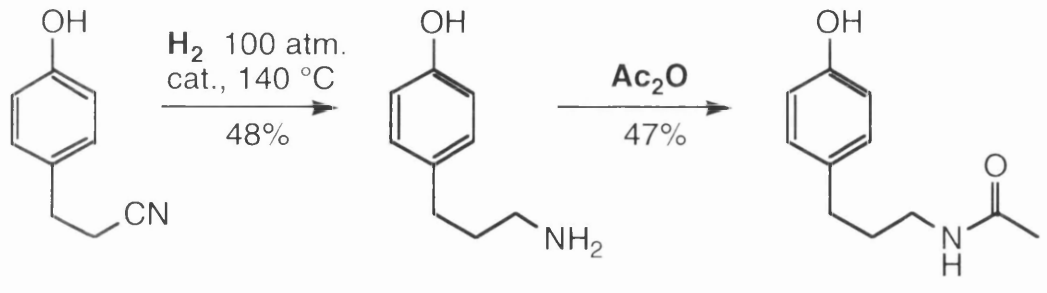
Both novel products **197** and **211** were fully characterised by the usual range of analytical techniques, with ^1H NMR spectroscopy proving to be particularly useful in determining the aromatic substitution pattern.

6.5 Methylene

Although the Tyrosinase-mediated oxidation of phenols is potentiated by electron-withdrawing substituents *para* to the hydroxyl group,[61] we wished to evaluate the effects of introducing a methylene group to that position in order to produce a more tyrosine-like analogue of **47**. We envisaged that the greater structural similarity of **196** might increase drug selectivity through better activity towards Tyrosinase.

A synthesis of compound **196** has been previously reported by Eckardt *et al.*[197] (Scheme 82). They hydrogenated 3-(4-hydroxyphenyl)propionitrile **212** to give the corresponding amine **213** which was acetylated to afford **196** in 23% yield over two steps.

Scheme 82

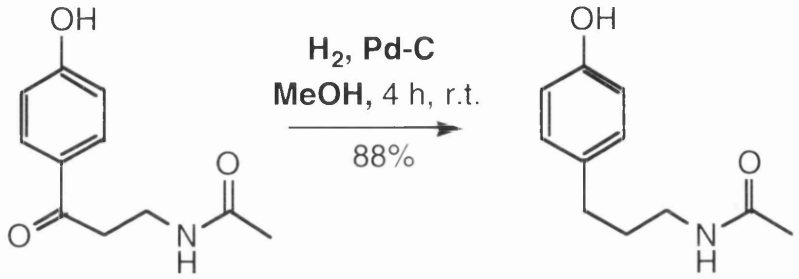


212 **213** **196**

Rather than follow this procedure, we decided to prepare **196** by reduction of ketone **197**. This conversion was achieved in high yield by catalytic hydrogenation using palladium on carbon, as shown in Scheme 83. Progress of the reaction was conveniently followed by TLC, which revealed rapid

conversion into the alcohol followed by a slower second reduction step to give final product **196**.

Scheme 83



197 **196**

The product **196** gave a similar m.p. to the literature[197] value and as no spectroscopic data were reported by Eckardt *et al.*,[197] additional analytical data are presented in Section 7.4. The functional group conversion was clearly evident by comparing the ^{13}C NMR chemical shifts of the side-chain bridging carbon atom of **197** (δ 197) and **198** (δ 33). Spectroscopic proof that complete reduction had been effected was provided by the DEPT-edited ^{13}C NMR spectrum of **196** which revealed three methylene carbon atoms.

6.6 Selenide

According to a recent study by Riley *et al.*,[69] the simple selenides **216** and **217** are oxidised *in vitro* by Tyrosinase significantly faster than their analogous sulfides **214** and **215** (Figure 12).

Figure 12

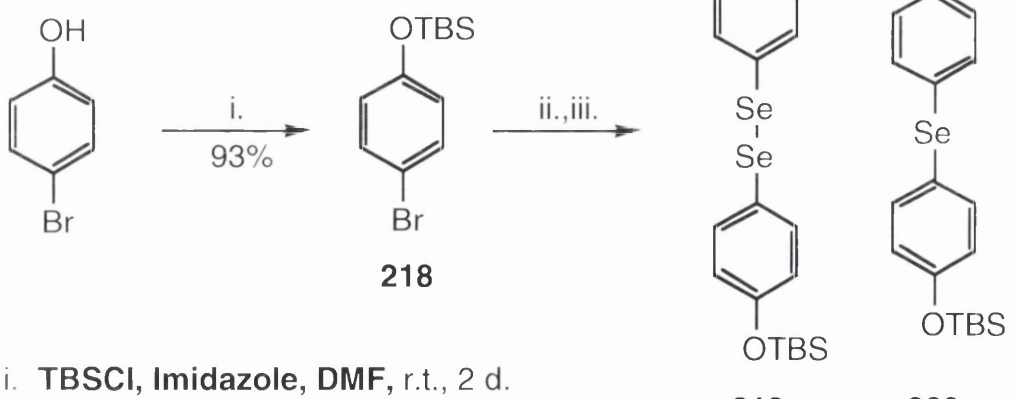
Sulfide	R	COR	Selenide	R	COR
214	Me	0.47	216	Me	0.64
215	Pr^n	0.83	217	Pr^n	1.66

COR = Comparative Rate of Oxidation by Tyrosinase, relative to 4-hydroxyanisole. Riley *et al.*[69]

Moreover, as the lipophilicity of selenides is generally greater than analogous sulfides, we could anticipate an increase in anti-melanoma activity for selenide **195** relative to the lead compound **47**. In order to evaluate this theory, the synthesis of novel selenide **195** (Figure 11) was planned.

As discussed in Section 3.2, Saito *et al.*[94] reported that the Wehrmeister Reaction could be extended to oxazoline ring-opening by selenophenols. Although only one example was given (employing commercially available selenophenol), we envisaged that the reaction could be used to prepare the required compound **195**. To this end, the procedure of Riley *et al.*[69] was employed to prepare the diselenide **219** from 4-bromophenol *via* treatment of Grignard reagent **218** with elemental selenium (Scheme 84). Although the problem of mono- and poly-selenide formation is discussed in the paper,[69] no attempt was made to isolate the required diselenide from the crude product mixture. We found that flash chromatography was partly effective in purifying diselenide **219**, although separation of the monoselenide by-product **220** could not be achieved using this technique. This difficulty in separating monoselenides from diselenides using standard separation methods is well known[198] and is a major disadvantage of this synthetic method. In the coupling of the Grignard reagent derived from **218** with selenium, we estimated the ratio of products **219 : 220** (Scheme 84) by ^{1}H NMR spectroscopy.

Scheme 84



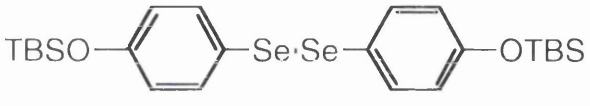
i. **TBSCl, Imidazole, DMF,** r.t., 2 d.
ii. **Mg, DBE, Et_2O,** refl., 5 h.
iii. **Se,** r.t., 12 h.

Monoselenides are often separated from diselenides by treatment with sodium borohydride. This converts diselenides into their corresponding selenols which can be removed by extraction using alkali. However, Riley *et al.*[69] treated a crude mixture containing **219** with sodium borohydride, followed by a large

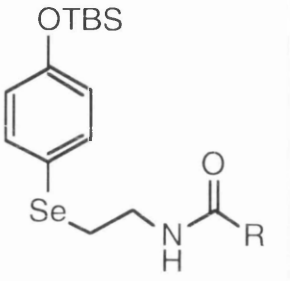
excess of methyl iodide to give **216** after deprotection; the analogous selenide **217** was prepared in a similar fashion.

By including an acidification in the work-up of the reduction, we modified this procedure to liberate the required selenophenol **221** in crude form. The impure mixture of **221** and selenide **220** was extracted into ether, rapidly dried under nitrogen and evaporated to dryness. The residue was immediately treated with a 2-oxazoline in order to facilitate a Wehrmeister reaction. By this protocol, selenides **222** and **223** were prepared using 2-methyl-2-oxazoline and 2-*tert*-butyl-2-oxazoline, respectively (Scheme 85). Extremely careful practical work was found to be essential in order to protect the selenol intermediate from air oxidation and to prevent its release into the laboratory (harmful and stench hazards).

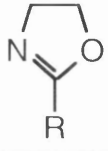
Scheme 85



219
*and monoselenide impurity **220***



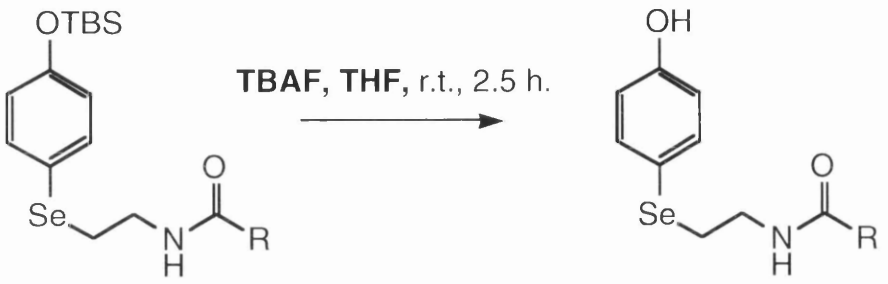
i. **$NaBH_4$, EtOH,** 0 °C → r.t., 30 min.
ii. **aq. HCl.**
iii.



R	Product	Yield
Me	**222**	48%
Bu^t	**223**	52%

After purification by column chromatography, novel products **222** and **223** were fully characterised by the usual range of spectroscopic techniques. Although the acetamide derivative **222** was isolated as an oil, the *tert*-butyl substituted amide **223** was found to be a solid of m.p. 75-76 °C. Both compounds were readily deprotected using TBAF in THF to afford the required phenols **195** and **224**, as shown in Scheme 86.

Scheme 86



R	Product	Yield
Me	**197**	87%
Bu^t	**224**	86%

The novel compounds **197** and **224** gave very similar ^1H and ^{13}C NMR and IR spectral data to their respective sulfide analogues **47** and **140**. Indeed, the IR spectra were virtually identical. However, their elemental composition was confirmed by high resolution mass spectrometry and combustion analysis.

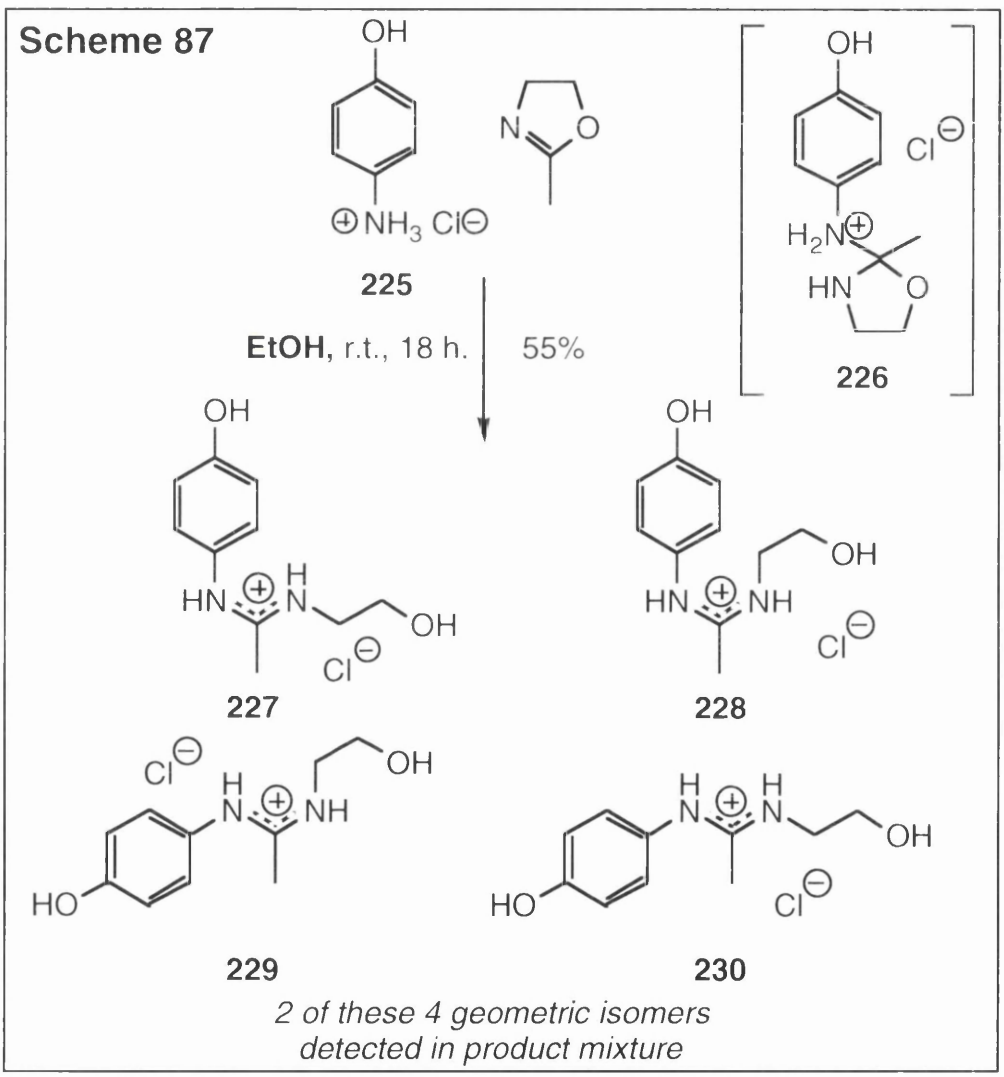
6.7 Amine

Our main reasons for replacing the sulfide group of **47** with an amine group were two-fold. Firstly, we believed that compounds with an amine group *para* to the phenol group would be rapidly oxidised by Tyrosinase. Secondly, the amine functionality opens up the possibility of producing water soluble hydrochloride salt derivatives.

We envisaged that compound **198** could be prepared by ring-opening of 2-methyl-2-oxazoline by a primary aromatic amine. However, as discussed in section 3.3, the reactivity of oxazolines towards amines is completely different to that towards phenols, selenides or thiols. The reactivity of oxazolines towards arylamine hydrochlorides is similarly complex, with amino-oxazolidines and carboxamides being reported as products. As Kormendy *et al.*[100] claimed that amino-oxazolidines could be prepared in good yield by treatment of oxazolines with arylamine hydrochlorides (Scheme 22), we started work in this area by evaluating their published protocol. To this end, 4-hydroxyaniline hydrochloride **225** was treated with 2-methyl-2-oxazoline under the conditions shown in Scheme 87. After stirring the reaction mixture overnight, off-white crystals were filtered off and analysed. Interestingly, on the basis of its IR spectrum, it became apparent that the solid was not the expected amino-oxazolidine **226**. A strong absorption was recorded at 1646 cm^{-1}, suggesting a multiple bonded structure; possibly a carbon-nitrogen double bond.

Another theory was that reaction had proceeded *via* a Wehrmeister-type ring cleavage to afford the carboxamide **198** as its hydrochloride salt. However, this option was ruled out by ^{13}C NMR spectroscopy by the absence of an amide carbonyl peak in the region δ180-170. Moreover, the ^{1}H and ^{13}C NMR spectra of the precipitate indicated that two compounds were present. However the two components seemed to be geometric isomers from the pairing of peaks. When the IR and NMR spectroscopic data were combined with comfirmation of molecular mass by CI-MS, it seemed likely that the observed product was an amidine salt. This could result from spontaneous cleavage of initially formed oxazolidine **226**, potentially giving rise to four isomeric amidine salts **227** - **230** (Scheme 87).

Scheme 87



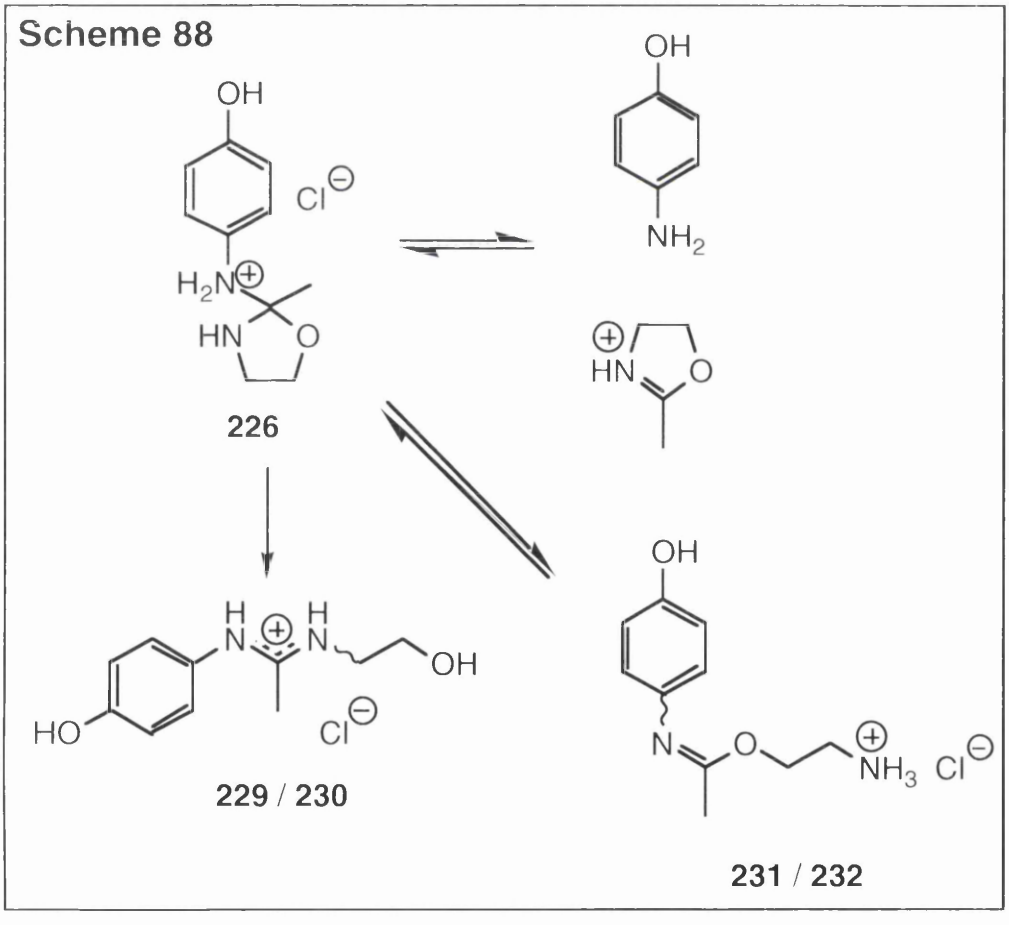
2 of these 4 geometric isomers detected in product mixture

By simple molecular modelling, it seems that the compounds **227** and **228** would contain greatest steric repulsions in a thermodynamically favourable conformation where the aromatic ring and the amidine group are co-planar. Therefore, we may *tentatively* assign the product mixture to be composed of

229 and **230**. By integration of the ^1H NMR methyl group singlets, approximately equal amounts of the two isomers were formed in the reaction.

Although a 55% yield of product was recovered from the reaction mixture, this was only the material which crystallised out of solution during the experiment. This could mean that the true yield of the reaction is higher, and this may indeed be a good synthetic method for the preparation of amidine salts. However, we must bear in mind that the crystallisation of amidine salt from the reaction mixture may have led to a series of equilibria being driven towards the formation of that particular product, and that this solubility difference may not be generally applicable. It is likely that cleavage of the presumed oxazolidine intermediate **226** could lead to either amidine salts such as **229** and **230** or the imidate **231**. A high yield of amidine salts may require the crystallisation of the required product from the reaction mixture together with an equilibrium process converting isomeric imidates **231** and **232** back into the oxazolidine intermediate **226**. This process is illustrated in Scheme 88.

Scheme 88

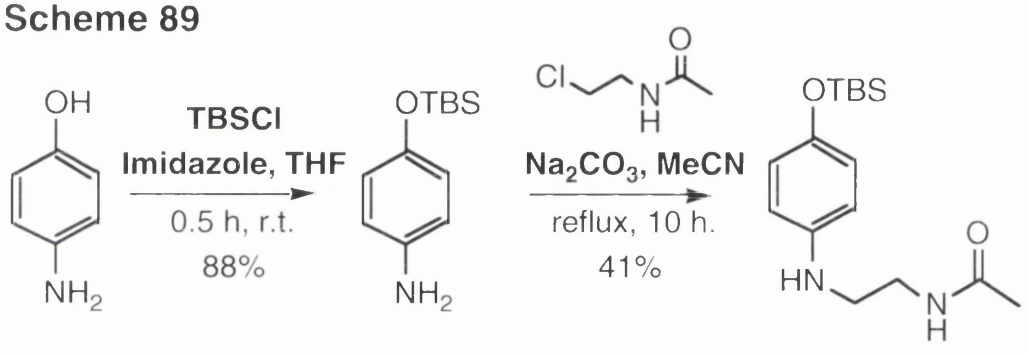


Our conclusions from the reaction between oxazolines and arylamine hydrochlorides appears to cast doubt on the earlier observations by Kormendy *et al.*[100] that amino-oxazolidines were the main product from the reaction. However, as our attempts to reproduce their original procedure using aniline

hydrochloride and 2-phenyl-2-oxazoline have been unsuccessful, further work is needed to confirm this.

Towards preparing compound **198**, we treated 4-aminophenol **225** with the commercially available *N*-(2-chloroethyl)acetamide according to the published general procedure of Poindexter.[102] However, a complex mixture of products resulted and we reasoned that this may have been due to deprotonation and consequent alkylation of the phenol group of **225.** To prevent this, we protected 4-aminophenol **225** as its *tert*butyldimethylsilyl ether **233** using the procedure of Swenton *et al.*[199] (Scheme 89). Compound **233** was then alkylated by the literature[102] general conditions to afford secondary amine **234** in 41% yield, as shown in Scheme 89. We believe that this low yield may have been due to further alkylation of product **234** by *N*-(2-chloroethyl)acetamide. In addition, some *N*-(2-chloroethyl)acetamide may have been removed by base-mediated elimination to give its corresponding alkene.

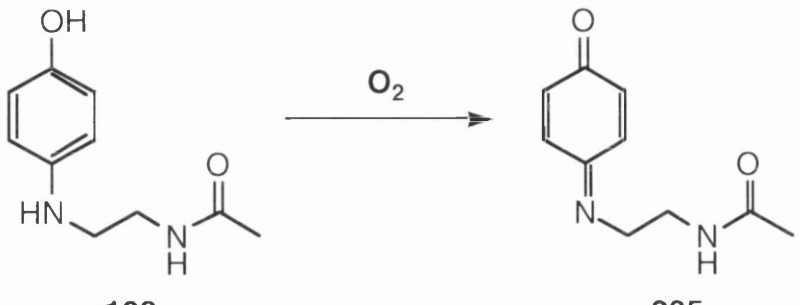
Scheme 89



225 **233** **234**

In an attempt to prepare **198** as its free base, we attempted to deprotect **234** using TBAF. Although the reaction seemed to proceed cleanly by TLC, extensive decomposition was encountered during purification. We envisaged that this may have been due to air oxidation of **198** to give *para*-aminoquinone **235** (Scheme 90). Such a process would be auto-catalysed through deprotonation of the phenol by the amine group.

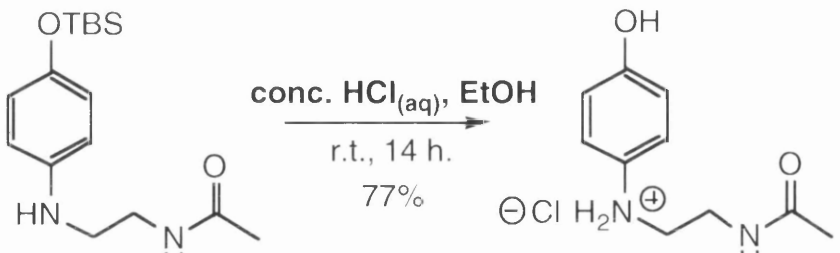
Scheme 90



198 **235**

In order to suppress this process, it seemed sensible to prepare **198** directly as its hydrochloride salt. We achieved this by deprotecting **234** using ethanolic hydrogen chloride. After stirring at room temperature overnight, the reaction mixture was simply evaporated to dryness, leaving a solid residue. Crystallisation furnished the required hydrochloride salt **198** in good yield (Scheme 91).

Scheme 91



234 **198**

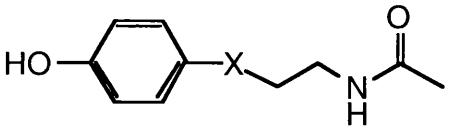
Novel compound **198** was characterised by the usual range of spectroscopic techniques. Although it showed good stabilty in water, decomposition was evident in alkaline solutions. For example, after dissolving the compound at 100 μM concentration in pH 7.4 phosphate buffer, the colour of the resulting solution changed from pale pink to light brown over 24 hours. Monitoring of this decomposition by UV spectroscopy revealed the appearance of a new absorption maximum at 372 nm, which may be due to formation of the aminoquinone **235** by the process shown in Scheme 90.

6.8 Partition Coefficients / Biological Evaluation

6.8.1 UV Spectroscopy and Partition Coefficients

The UV spectroscopy of *para*-substituted phenols was discussed in Section 3.5. It was stated that the absorption frequency of the principal wavelength maximum depended on the electronic nature of the substituent. If the substituent is electron withdrawing relative to the electron releasing phenol, then the absorption will take place at longer wavelength. This trend is illustrated by the UV spectra of the compounds prepared in this chapter with principal absorption maxima ranging from 280 nm for the *para*-hydroxy aromatic ketone **197** to 225 nm for the *para*-hydroxy aromatic ether **194**. As before (Section 3.5), these absorption maxima were used quantitatively to evaluate partition coefficients for the compounds **192** - **198**, thus allowing us to compare their lipophilicities relative to the lead compound **47**. The calculated partition coefficients for the 1-octanol : phophate buffered saline (pH 7.4) system are given in Table 16.

Table 16 1-Octanol: PBS Partition Coefficients



Compound	R	*P*	log *P*
192	SO	0.205	-0.69
193	SO_2	0.362	-0.44
198	$\oplus NH_2$	0.978	-0.01
194	O	1.24	0.09
197	C=O	1.70	0.23
47	S	2.03	0.31
196	CH_2	2.09	0.32
195	Se	3.42	0.53

6.8.2 Biological Evaluation

The data presented in Table 16 clearly show that the nature of the side-chain bridging group has a major impact on the overall lipophilicity of the compound. However, for a drug to be available to the cells in a physiological system, we still require appreciable water solubility. Therefore, we may expect

compounds with a "compromise" lipophilicity to be the most successful as these could be soluble in saline and be readily absorbed through cell membranes.

In the context of this type of anti-melanoma prodrug, other factors are important too. Clearly, the compounds must be substrates for Tyrosinase to permit their bio-conversion into active *ortho*-quinones. Moreover, an electron-releasing group such as an ether at the bridging position helps facilitate this process. However, this type of group is polar and hence lowers lipophilicity which can reduce uptake into cells!

These observations can help us predict that certain compounds are not likely to be successful anti-melanoma drugs. For example, the sulfoxide **192** is very polar and will therefore not be readily taken up by cells. In addition, its bridging group is electronegative which is likely to deactivate the compound from Tyrosinase-mediated oxidation. Considering these two factors, it is unlikely that **192** will exhibit significant anti-melanoma activity.

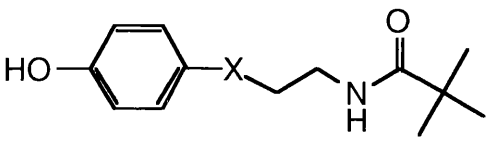
All of the phenols discussed in Sections 6.2-6.6 were evaluated *in vitro* against the usual panel of cell lines by Dr L.R. Kelland, Institute of Cancer Research. The results, expressed in terms of GI_{50} values, are given in Table 17.

Table 17 GI_{50} Values (μM) for 96H SRB Assay

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
Sulfoxide							
192	>100	>100	>100	>100	>100	>100	>100
199	>100	>100	>100	>100	>100	>100	>100
200	>100	>100	>100	>100	>100	>100	>100
Sulfone							
193	>100	>100	>100	>100	>100	>100	>100
Ether							
194	>100	>100	>100	>100	>100	>100	>100
Sulfide							
47	*>100*	*>100*	*>100*	*>100*	*>100*	*>100*	*>100*
Ketone							
197	>100	>100	>100	>100	>100	>100	>100
211	>100	>100	>100	>100	>100	>100	>100
Methylene							
196	>100	>100	>100	>100	>100	>100	>100
Selenide							
195	>100	>100	>100	>100	>100	>100	>100
224	48	>100	80	>100	>100	>100	>100

These data show that all of these compounds have relatively low toxicity towards the cell lines tested, when compared to the more lipophilic phenols discussed in Chapter 4. The only compound with GI_{50} values below 100μM was the *tert*-butyl substituted selenide **224**. From the partition coefficient measurements discussed in Section 8.6.1, selenides have greater lipophilicity. This is a likely explanation for its greater cytotoxicity of **224** relative to sulfide analogue **140**, as shown by the more informative percentage cell survival data at 100 μM (Table 18).

Table 18 Cell Survival (%Control) at 100μM in 96h SRB assay



	X
140	S
224	Se

	Cell Line						
Cmpd	**B008**	**B0010**	**G361**	**HT144**	**SK-Mel-2**	**SK-Mel-24**	**SKOV-3**
140	37.7	80.1	56.9	86.8	66.7	89.1	89.2
224	17.3	62.9	44.3	75.6	60.1	101.3	85.9

The observation that compounds such as **224** show cytotoxicity towards the pigmented melanoma cell lines and inactivity towards the non-pigmented line SK-Mel-24 may indicate toxicity via Tyrosinase-mediated oxidation. However, as toxicity is evident towards the ovarian cell line SKOV-3 too, these compounds clearly exert some non-specific biological effects.

6.9 Final Comments and Future Work

Although we have not prepared compounds which specifically focus their effects on melanoma, it must be borne in mind that the currently used drugs for this condition afford only low success rates. Further work is therefore required to determine whether the goal of Tyrosinase-mediated drug delivery at tumour sites can be achieved.

In order to gain a better picture of the selectivity and potential of the new compounds prepared in the course of this research, 36 have been accepted for evaluation by the U.S. National Cancer Institute. The results will be useful in planning where further research efforts are best directed.

During the course of this work, some interesting observations have been made in the area of 2-oxazoline chemistry. Further work is required to study the reactivity of oxazolines towards aromatic amines and their salts in order to clarify and extend the small amount of literature in this area.

The Wehrmeister reaction has been extensively used throughout this work. An example was given (Section 6.3) where a 5 step literature procedure was reduced to one step through the use of this technique. Another area of future work could be to extend this reaction to more complex 2-oxazolines or other nucleophilic species.

7

Experimental to Chapters 4-6

7.1 General

Reagents were purchased from Aldrich Chemical Company (Gillingham, UK) or Lancaster Synthesis (UK) and were used without further purification. Light petroleum refers to the fraction with boiling range 40-60 °C. Melting points (m.p.) were determined in open capillaries using a 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, at 360 MHz and 90 MHz respectively on a Bruker AM 360 spectrometer. *J* Values are given in Hz. ^{13}C NMR spectra were assigned with the aid of Distortionless Enhancement by Polarisation Transfer (DEPT)-edited spectra.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 AEI MS12 or MS902 spectrometers; percentage figures refer to relative intensity as a percentage of the base peak. MS were obtained using electron-impact ionisation (EI) mode or, if stated, chemical ionisation (CI) mode. Infra-red (IR) spectra were obtained on a Perkin Elmer PU 9800 FT-IR spectrophotometer. Ultra-violet (UV) spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Combustion analysis was carried out on 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; chromatograms were visualised using UV conditions at 254 nm or by staining with iodine. Flash chromatography and dry-column flash chromatography refer to the procedures described by Still *et al.*[200] and Harwood,[201] respectively. All column chromatography was carried out on silica gel (particle size 70-230 mesh).

7.2 Experimental to Chapters 4

7.2.1 General Procedures for the preparation of oxazolines

General Procedure 1, as reported by Barton *et al.*[111]

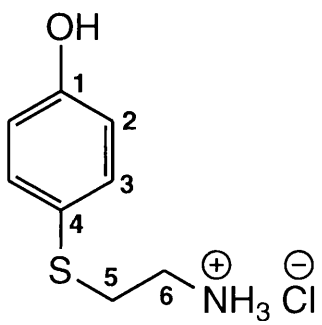
The carboxylic acid (1 equivalent), the amino alcohol (2 equivalents) and boric acid (0.05 equivalents) were heated in refluxing xylene for 2-3 d. After concentration of the product mixture under reduced pressure, fractional distillation afforded the 4,5-dihydro-oxazine as a colourless oil.

General Procedure 2, as reported by Witte and Seeliger.[117]

The nitrile (1 equivalent) was heated with the amino alcohol (1 equivalent) and cadmium diacetate dihydrate (0.025 equivalents) at 130 °C for 1-3 d. Distillation of the product mixture gave the 4,5-dihydro-oxazine as a colourless oil.

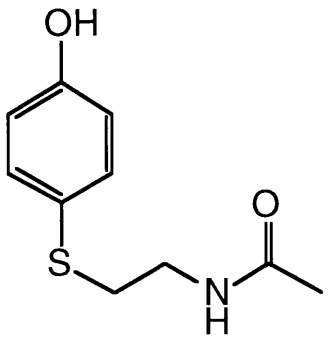
7.2.2 Experimental Data

2-[(4-Hydroxyphenyl)thio]ethylamine hydrochloride (42)



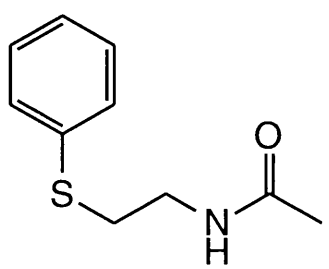
The title compound was prepared in 88% yield on a 10 mmol scale by the procedure of Padgette *et al.*[74] and gave m.p. 128-129 °C (from EtOAc-MeOH), (lit.,[74] 128-129 °C). The ^{1}H NMR, IR and MS spectral data were in good agreement with those reported.[74]
New data: δ_C (D_2O) 156.7 (C-1), 135.3 (C-3), 123.1 (C-4), 117.3 (C-2), 39.9 (C-6) and 33.4 (C-5).

***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]ethanamide (47)**



The title compound was prepared in 87% yield by the method of Padgette *et al.*[74] and gave m.p. 124-125 °C (from EtOAc), (lit.,[74] 123-125 °C). The ^{1}H NMR, ^{13}C NMR, IR and MS spectral data were in good agreement with those reported by McKeown.[202]

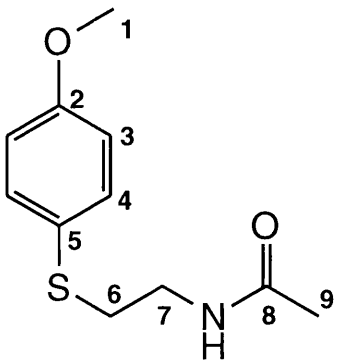
***N*-[2-(Phenylthio)ethyl]ethanamide (84)**



A mixture of thiophenol (3.30 g, 30.0 mmol) and 4.5-dihydro-2-methyloxazole (2.55 g, 30.0 mmol) was stirred under N_2 for 2 h at 120 °C. Cooling to r.t. gave a white precipitate which was recrystallised to afford sulfide **84** (4.81 g, 82%) as white crystals, m.p. 90-92 °C (from EtOAc), (lit.,[203] 89-91 °C). The ^{1}H NMR, ^{13}C NMR, IR and MS spectral data were in good agreement with those reported in the literature.[106]

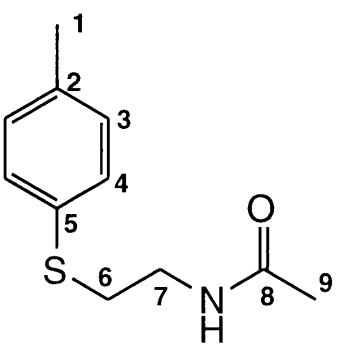
New data: R_f 0.40 (EtOAc); (Found: C, 61.55; H, 6.69; N, 7.11%. $C_{10}H_{13}NOS$ requires C, 61.51; H, 6.71; N, 7.17%).

***N*-[2-[(4-Methoxyphenyl)thio]ethyl]ethanamide (85)**



A mixture of 4-methoxythiophenol (2.35 g, 17.0 mmol) and 4.5-dihydro-2-methyloxazole (1.45 g, 18.0 mmol) was stirred under N_2 for 2.5 h at 120 °C. Cooling to r.t. gave a white precipitate which was recrystallised to afford *sulfide* **85** (3.10 g, 76%) as white crystals, m.p. 109-110 °C (from EtOAc); R_f 0.44 (EtOAc); ν_{max} (KBr) 3291 (N-H), 1638 (amide I), 1597, 1561 (amide II), 1497 and 816 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.36 (2 H, AA'BB', ^3J= 8.8, 4-H), 6.85 (2 H, AA'BB', ^3J= 8.8, 3-H), 6.05 (1 H, br s, N-H), 3.79 (3 H, s, 1-H), 3.39 (2 H, m, 7-H), 2.93 (2 H, t, ^3J= 6.5, 6-H) and 1.95 (3 H, s, 9-H); δ_C ($CDCl_3$) 170.2 (C-8), 159.2 (C-2), 133.6 (C-4), 124.8 (C-5), 114.7 (C-3), 55.3 (C-1), 38.4 (C-7), 35.5 (C-6) and 23.2 (C-9); *m/z* 225 (M^+, 23.6), 166 (94.6), 151 (34.2), 139 (31.9), 135 (25.3) and 86 (100%); (Found: C, 58.71; H, 6.73; N, 6.11%. $C_{11}H_{15}NO_2S$ requires C, 58.64; H, 6.71; N, 6.22%).

***N*-[2-[(4-Methylphenyl)thio]ethyl]ethanamide (86)**

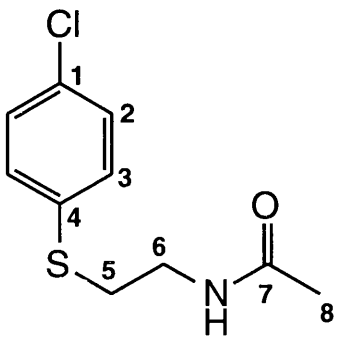


A mixture of 4-methylthiophenol (3.72 g, 30.0 mmol) and 4.5-dihydro-2-methyloxazole (2.55 g, 30.0 mmol) was stirred under N_2 for 2.5 h at 120 °C. Cooling to r.t. gave a white precipitate which was recrystallised to afford the title compound **86** (5.12 g, 82%) as white crystals, m.p. 76-78 °C (from EtOAc); (lit.,[107] 73-75 °C).

New data: R_f 0.30 (EtOAc); ν_{max} (KBr) 3289 (N-H), 1638 (amide I), 1561 (amide II), and 803 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.28 (2 H, AA'BB',

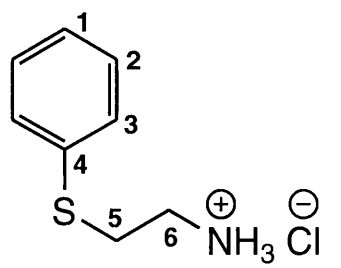
^3J= 8.9, 4-H), 7.12 (2 H, AA'BB', ^3J= 8.9, 3-H), 6.08 (1 H, br s, N-H), 3.41 (2 H, m, 7-H), 3.00 (2 H, t, ^3J= 6.5, 6-H), 2.32 (3 H, s, 1-H) and 1.94 (3 H, s, 9-H); δ_C ($CDCl_3$) 170.2 (C-8), 136.9 (C-2), 131.0 (C-5), 130.6 (C-4), 129.9 (C-3), 38.6 (C-7), 34.2 (C-6), 23.2 (C-9) and 21.0 (C-1); *m/z* 209 (M^+, 16.1), 150 (100), 135 (79.0), 123 (11.9) and 105 (11.3%); (Found: C, 63.23; H, 7.39; N, 6.49%. $C_{11}H_{15}NOS$ requires C, 63.12; H, 7.22; N, 6.69%).

_N_-[2-[(4-Chlorophenyl)thio]ethyl]ethanamide (87)



A mixture of 4-chlorothiophenol (5.91 g, 40.9 mmol) and 4.5-dihydro-2-methyloxazole (3.40 g, 40.0 mmol) was stirred under N_2 for 2 h at 120 °C. Cooling to r.t. gave a white precipitate which was recrystallised to afford *sulfide* **87** (5.91 g, 64%) as white crystals, m.p. 95-97 °C (from EtOAc); R_f 0.36 (EtOAc); ν_{max} (KBr) 3285 (N-H), 1638 (amide I), 1561 (amide II) and 812 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_4-methanol) 7.36 (2 H, AA'BB', ^3J= 6.1, 3-H), 7.28 (2 H, AA'BB', ^3J= 6.1, 2-H), 3.35 (2 H, t, ^3J= 6.8, 6-H), 3.03 (2 H, t, ^3J= 6.8, 5-H) and 1.70 (3 H, s, 8-H); δ_C (D_4-methanol) 173.3 (C-7), 136.0 (C-4), 133.0 (C-1), 131.7 (C-3), 130.1 (C-2), 40.0 (C-6), 33.5 (C-5) and 22.5 (C-8); *m/z* 229 (M^+, 12.0), 170 (79.4), 135 (83.9), 108 (26.9) and 30 (100%); (Found: C, 52.46; H, 5.22; N, 6.02%. $C_{10}H_{12}ClNOS$ requires C, 52.28; H, 5.27; N, 6.10%).

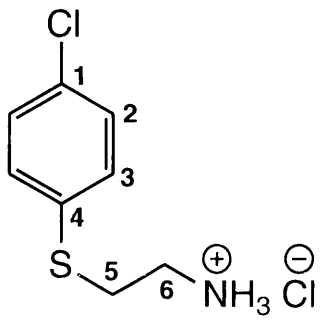
2-(Phenylthio)ethylamine hydrochloride (88)



The title compound was prepared in 65% yield on a 6 mmol scale by the general procedure of Padgette *et al.*[74] and gave m.p. 105-108 °C (from EtOAc-

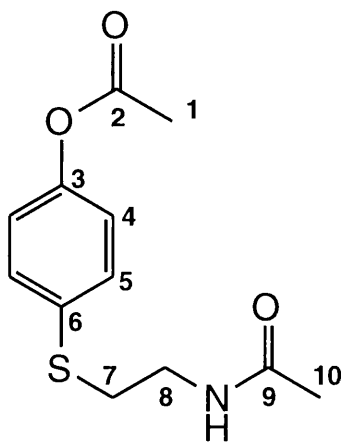
MeOH), (lit.,[103] 106-110 °C). The ^{1}H NMR[103] and IR[93] spectral data were in good agreement with those reported in the literature.
New data: δ_C (D_2O) 133.7 (C-4), 131.3 (C-2), 130.3 (C-3), 128.3 (C-1), 38.8 (C-6) and 31.4 (C-5); *m/z* 153 [M^+ (free base), 2.9], 124 (20.0), 109 (13.8), 91 (7.3), 77 (24.3) and 30 (100%); (Found: C, 50.54; H, 6.54; N, 7.29%. $C_8H_{12}ClNS$ requires C, 50.65; H, 6.38; N, 7.38%).

2-[(4-Chlorophenyl)thio]ethylamine hydrochloride (89)



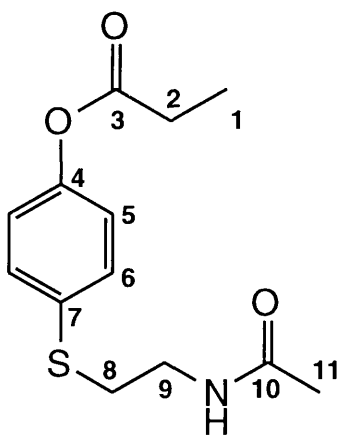
The title compound was prepared in 69% yield on a 6 mmol scale by the general procedure of Padgette *et al.*[74] and gave m.p. 165-166 °C (from EtOH), (lit.,[204] 170-172 °C).
New data: ν_{max} (KBr) 3011 (N-H str.), 1593, 1574, 1506, 1482 (N-H bend) and 818 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_2O) 7.26 (2 H, AA'BB', ^3J= 9.0, 3-H), 7.17 (2 H, AA'BB', ^3J= 9.0, 2-H) and 3.08-2.91 (4 H, m, 5-H and 6-H); δ_C (D_2O) 133.8 (C-4), 132.7 (C-2), 132.2 (C-1), 130.2 (C-3), 38.7 (C-6) and 31.6 (C-5); *m/z* 187 [M^+ (free base), 3.2], 158 (13.8), 143 (3.8), 108 (9.9), 75 (10.8) and 30 (100%); (Found: C, 42.79; H, 5.04; N, 6.02%. $C_8H_{11}Cl_2NS$ requires C, 42.87; H, 4.95; N, 6.25%).

***N*-[2-[(4-Ethanoyloxyphenyl)thio]ethyl]ethanamide (90)**



The general procedure of Furniss *et al.*[109] was employed as follows: Ice (10 g) was added to a solution of compound **47** (1.55 g, 7.30 mmol) in 3 mol dm^{-3} aq. NaOH (10 cm^3). After 5 min, acetic anhydride (0.92 g, 0.85 cm^3, 9.01 mmol) was added and the mixture swirled for 5 min. whereupon the crude precipitated product was collected by filtration and recrystallised to afford *ester* **90** (0.95 g, 52%) as white crystals, m.p. 79-80 °C (from EtOAc); R_f 0.25 (EtOAc); ν_{max} (KBr) 3291 (N-H), 1752 (C=O), 1638 (amide I), 1561 (amide II), and 843 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.38 (2 H, AA'BB', ^3J= 6.6, 5-H), 7.02 (2 H, AA'BB', ^3J= 6.6, 4-H), 6.08 (1 H, br s, N-H), 3.43 (2 H, m, 8-H), 3.03 (2 H, t, ^3J= 6.4, 7-H), 2.30 (3 H, s, 1-H) and 1.97 (3 H, s, 10-H); δ_C ($CDCl_3$) 170.4 (C-9), 169.4 (C-2), 149.4 (C-3), 132.3 (C-6), 131.1 (C-5), 122.4 (C-4), 38.7 (C-8), 34.0 (C-7), 23.2 (C-10) and 21.1 (C-1); *m/z* 253 (M^+, 5.1), 211 (5.9), 194 (12.7), 152 (59.0), 86 (36.4) and 43 (100%); (Found: C, 56.94; H, 5.98; N, 5.54%. $C_{12}H_{15}NO_3S$ requires C, 56.90; H, 5.97; N, 5.53%).

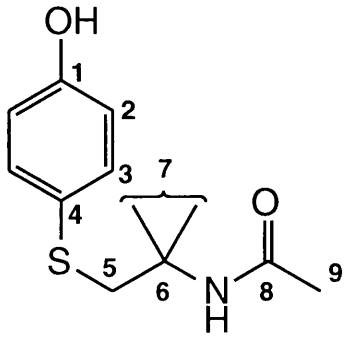
_N_-[2-[(4-Propanoyloxyphenyl)thio]ethyl]ethanamide (91)



The general procedure of Furniss *et al.*[109] was employed as follows: Ice (10 g) was added to a solution of compound **47** (1.50 g, 7.10 mmol) in 3 mol dm^{-3} aq. NaOH (10 cm^3). After 5 min, propanoic anhydride (1.32 g, 1.30 cm^3, 10.1 mmol) was added and the mixture swirled for 5 min whereupon the crude precipitated product was collected by filtration and recrystallised to afford *ester* **91** (1.18 g, 62%) as white crystals, m.p. 97-99 °C (from EtOAc); R_f 0.32 (EtOAc); ν_{max} (KBr) 3291 (N-H), 1750 (C=O), 1638 (amide I), 1561 (amide II), and 806 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.40 (2 H, AA'BB', ^3J= 8.7, 6-H), 7.04 (2 H, AA'BB', ^3J= 8.7, 5-H), 6.02 (1 H, br s, N-H), 3.42 (2 H, m, 9-H), 3.03 (2 H, t, ^3J= 6.5, 8-H), 2.58 (2 H, q, ^3J= 7.6, 2-H), 1.94 (3 H, s, 11-H) and 1.26 (3 H, t, ^3J= 7.6, 1-H); δ_C ($CDCl_3$) 172.9 (C-10), 170.3 (C-3), 149.6 (C-4), 132.1 (C-7), 131.2 (C-6), 122.4 (C-5), 38.7 (C-9), 34.1 (C-8), 27.7 (C-2), 23.2 (C-11) and 9.0 (C-1); *m/z* 267 (M^+, 1.8), 208 (2.3), 152 (34.4), 107 (8.4), 86 (36.4) and 29

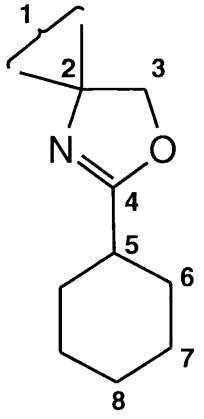
(100%); (Found: C, 58.31; H, 6.29; N, 5.14%. $C_{13}H_{17}NO_3S$ requires C, 58.41; H, 6.41; N, 5.24%).

_N_-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]ethanamide (92)



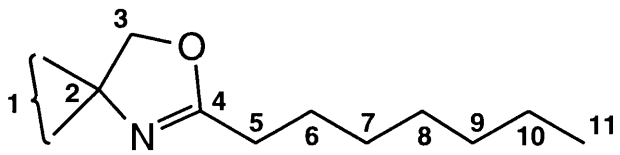
A mixture of 4-hydroxythiophenol (5.35 g, 42.4 mmol) and 5,6-dihydro-2,4,4-trimethyloxazole (5.28 g, 46.7 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **92** (7.77 g, 77%) as white crystals, m.p. 98-99 °C (from EtOAc-hexane); R_f 0.31 (EtOAc); ν_{max} (KBr) 3358 (N-H), 1646 (amide I), 1582, 1542 (amide II), 1494 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.83 (1 H, br s, OH), 7.29 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.96 (1 H, br s, NH), 6.77 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.38 (2 H, s, 5-H), 1.75 (3 H, s, 9-H) and 1.36 (6 H, s, 7-H); δ_C (D_6-acetone) 170.7 (C-8), 157.7 (C-1), 134.1 (C-3), 126.9 (C-4), 116.8 (C-2), 54.7 (C-6), 45.8 (C-5), 26.4 (C-7) and 23.7 (C-9); *m/z* 239 (M^+, 4.6), 180 (39.8), 165 (3.7), 140 (5.1), 100 (14.4) and 59 (100%); (Found: M^+, 239.0976; C, 60.28; H, 7.16; N, 5.78%. $C_{12}H_{17}NO_2S$ requires *M*, 239.0980; C, 60.22; H, 7.16; N, 5.85%).

2-Cyclohexyl-4,5-dihydro-4,4-dimethyloxazole (96)



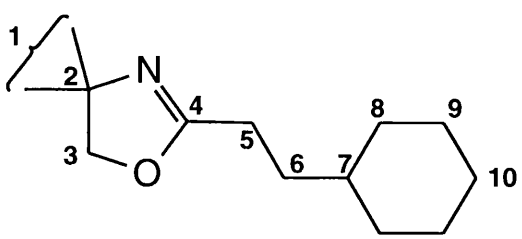
The title compound **96** was prepared in 75% yield on a 80 mmol scale using general procedure 1 (2 d) and gave b.p. 122-123 °C/25 mmHg, (lit.,[113] 95-97 °C/14 mmHg). The IR spectral data were in good agreement with those reported in the literature.[113]
New data: δ_H ($CDCl_3$) 3.80 (2 H, s, 3-H), 2.17 (1 H, m, 5-H), 1.92-0.95 (10 H, m, 6-H, 7-H and 8-H) and 1.18 (6 H, s, 1-H); δ_C ($CDCl_3$) 169.3 (C-4), 78.6 (C-3), 66.4 (C-2), 37.3 (C-5), 29.7 (C-6), 28.2 (C-1), 25.7 (C-8) and 25.5 (C-7).

2-Heptyl-4,5-dihydro-4,4-dimethyloxazole (97)



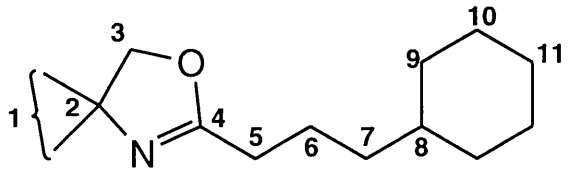
The title compound **97** was prepared in 84% yield on a 150 mmol scale using general procedure 1 (2 d) and gave b.p. 78 °C/2.0 mmHg, (lit.,[114] 110 °C/12 mmHg). The IR, ^1H NMR and ^{13}C NMR spectral data were in good agreement with those reported in the literature.[114,115]
New data: *m/z* 197 (M^+, 0.7), 126 (33.7), 113 (100), 98 (20.7) and 58 (48.4%); (Found: M^+, 197.1771; $C_{12}H_{23}NO$ requires *M*, 197.1780).

2-(2-Cyclohexylethyl)-4,5-dihydro-4,4-dimethyloxazole (98)



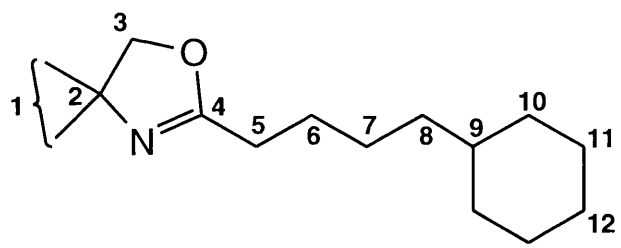
The title compound **98** was prepared in 58% yield on a 40 mmol scale using general procedure 1 (2 d) and gave b.p. 107-110 °C/2.0 mmHg, (lit.,[116] 96 °C/0.8 mmHg). The ^1H NMR and IR spectral data were in good agreement with those reported in the literature.[116]
New data: δ_C ($CDCl_3$) 166.4 (C-4), 78.8 (C-3), 66.7 (C-2), 37.3 (C-7), 33.4 (C-5), 32.9 (C-8), 28.3 (C-1), 26.5 (CH_2) 26.2 (C-9) and 25.7 (CH_2); *m/z* 209 (M^+, 7), 180 (17), 126 (93), 113 (100), 98 (13) and 83 (9%)

2-(3-Cyclohexylpropyl)-4,5-dihydro-4,4-dimethyloxazole (99)



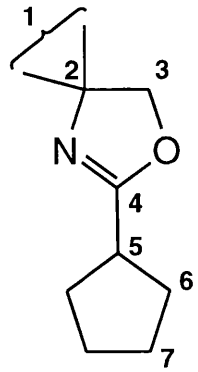
General procedure 1 was employed as follows: A mixture of 4-cyclohexylbutanoic acid (8.90 g, 52.2 mmol), 2-amino-2-methyl-1-propanol (9.30 g, 104 mmol) and boric acid (0.30 g, 4.9 mmol) was heated in refluxing xylene (150 cm^3) for 3 d. On cooling to r.t. the reaction mixture was concentrated *in vacuo* and distilled to afford *oxazoline* **99** (6.06 g, 52%) as a colourless oil, b.p. 115-117 °C/2.0 mmHg; ν_{max} (neat) 2924 (C-H), 1669 (C=N), 1449 and 996 cm^{-1}; δ_H ($CDCl_3$) 3.85 (2 H, s, 3-H), 2.17 (2 H, t, ^3J= 7.4, 5-H), 1.68-1.50 (7 H, m, 7-H; 8-H and 9-H), 1.22 (6 H, s, 1-H) and 1.20-0.80 (8 H, m, 6-H, 10-H and 11-H); δ_C ($CDCl_3$) 166.2 (C-4), 78.8 (C-3), 66.7 (C-2), 37.2 (C-8), 36.9 (C-7), 33.2 (C-5 and C-9), 28.4 (C-1), 26.6 (C-6), 26.3 (C-10) and 23.4 (C-11); *m/z* 223 (M^+, 2), 208 (8), 180 (9), 126 (24), 113 (100) and 98 (9%); (Found: M^+, 223.1919; $C_{14}H_{25}NO$ requires *M*, 223.1936).

2-(4-Cyclohexylbutyl)-4,5-dihydro-4,4-dimethyloxazole (100)



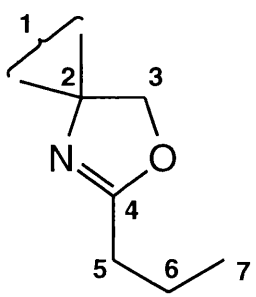
General procedure 1 was employed as follows: A mixture of 4-cyclohexylpentanoic acid (9.92 g, 53.8 mmol), 2-amino-2-methyl-1-propanol (9.60 g, 108 mmol) and boric acid (0.30 g, 4.9 mmol) was heated in refluxing xylene (150 cm^3) for 3 d. On cooling to r.t. the reaction mixture was concentrated *in vacuo* and distilled to afford *oxazoline* **100** (6.95 g, 54%) as a colourless oil, b.p. 125-127 °C/2.0 mmHg; ν_{max} (neat) 2924 (C-H), 1669 (C=N), 1449 and 996 cm^{-1}; δ_H ($CDCl_3$, 360 MHz) 3.89 (2 H, s, 3-H), 2.23 (2 H, t, ^3J= 7.5, 5-H), 1.61-1.49 (7 H, m, 8-H, 9-H and 10-H), 1.28-0.80 (10 H, m, 6-H, 7-H, 11-H and 12-H) and 1.18 (6 H, s, 1-H); δ_C ($CDCl_3$) 166.1 (C-4), 78.9 (C-3), 66.8 (C-2), 37.4 (C-9), 37.0 (C-8), 33.4 (C-5 and C-10), 28.4 (C-1), 28.2 (CH_2), 26.7 (CH_2), 26.4 (CH_2) and 26.3 (CH_2); *m/z* 237 (M^+, 3), 222 (8), 184 (8), 126 (38), 113 (100) and 58 (34%); (Found: M^+, 237.2095; $C_{15}H_{27}NO$ requires *M*, 237.2093).

2-Cyclopentyl-4,5-dihydro-4,4-dimethyloxazole (101)



General procedure 1 was employed as follows: A mixture of cyclopentane carboxylic acid (8.96 g, 8.51 cm^3, 78.5 mmol), 2-amino-2-methyl-1-propanol (14.0 g, 157 mmol) and boric acid (0.40 g, 6.5 mmol) was heated in refluxing xylene (150 cm^3) for 3 d. On cooling to r.t. the reaction mixture was concentrated *in vacuo* and distilled to afford *oxazoline* **101** (8.14 g, 62%) as a colourless oil, b.p. 104-105 °C/20 mmHg; ν_{max} (neat) 2964 (C-H), 1663 (C=N), 1191, 1146 (C-O) and 998 cm^{-1}; δ_H ($CDCl_3$) 3.74 (2 H, s, 3-H), 2.54 (1 H, m, 5-H), 1.86-1.33 (8 H, m, 6-H and 7-H) and 1.10 (6 H, s, 1-H); δ_C ($CDCl_3$) 169.1 (C-4), 78.7 (C-3), 38.1 (C-5), 30.3 (C-6), 28.2 (C-1) and 25.6 (C-7); *m/z* 167 (M^+, 13), 152 (49), 139 (46), 126 (100), 96 (23), 81 (10) and 69 (29%); (Found: M^+, 167.1316; $C_{10}H_{17}NO$ requires *M*, 167.1310).

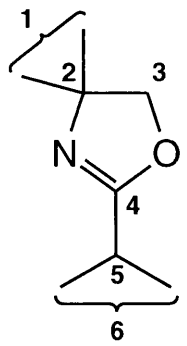
4,5-Dihydro-4,4-dimethyl-2-propyloxazole (102)



The title compound **102** was prepared in 60% yield on a 120 mmol scale using general procedure 2 (3 d) and gave b.p. 150-151 °C/760 mmHg, (lit.,[118] 152 °C/760 mmHg). The IR, ^1H NMR and ^{13}C NMR spectral data were in good agreement with those reported in the literature.[115,118]

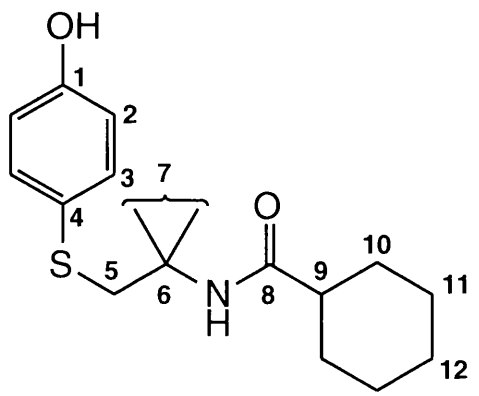
New data: *m/z* 141 (M^+, 1.0), 126 (80.3), 113 (94.0), 98 (51.9), 83 (69.6), 70 (70.2), 58 (29.1) and 41 (100%); (Found: M^+, 141.1143; $C_8H_{15}NO$ requires *M*, 141.1154).

4,5-Dihydro-4,4-dimethyl-2-(1-methylethyl)oxazole (103)



The title compound **103** was prepared in 76% yield on a 98 mmol scale using general procedure 2 (3 d) and gave b.p. 136-137 °C/760 mmHg, (lit.,[205] 137 °C/760 mmHg). The IR, ^{1}H NMR and ^{13}C NMR spectral data were in good agreement with those reported in the literature.[115,116,119]
New data: *m/z* 141 (*M*$^+$, 15.8), 126 (100), 111 (51.0), 96 (48.3), 70 (43.6) and 43 (50.0%); (Found: *M*$^+$, 141.1141; $C_8H_{15}NO$ requires *M*, 141.1154).

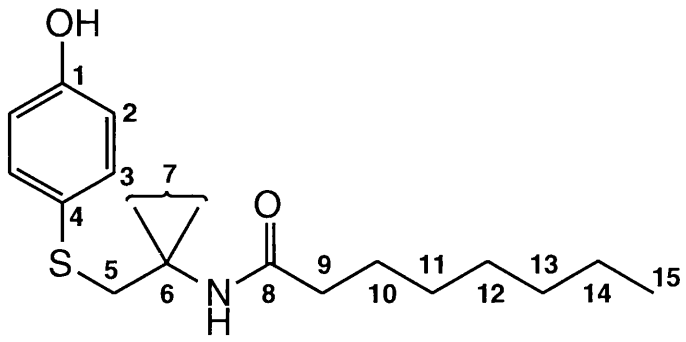
***N*-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]cyclohexanecarboxamide (104)**



A mixture of 4-hydroxythiophenol (0.85 g, 6.74 mmol) and oxazoline **96** (1.22 g, 6.74 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **104** (1.55 g, 75%) as white crystals, m.p. 153-154 °C (from hexane-EtOAc); R_f 0.65 (EtOAc); ν_{max} (KBr) 3357 (N-H), 1638 (amide I), 1600, 1583, 1541 (amide II), 1495 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 9.18 (1 H, br s, OH), 7.05 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.85 (1 H, br s, NH), 6.54 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.13 (2 H, s, 5-H), 1.85 (1 H, m, 9-H), 1.45 (2 H, m, 10-H), 1.11 (6 H, s, 7-H) and 1.05 (4 H, m, 11-H and 12-H); δ_C (D_6-acetone) 176.1 (C-8), 157.5 (C-1), 133.4 (C-3), 126.1 (C-4), 116.6 (C-2), 53.8 (C-6), 45.9 (C-9), 45.5 (C-5), 30.0 (C-10), 26.8 (C-7),

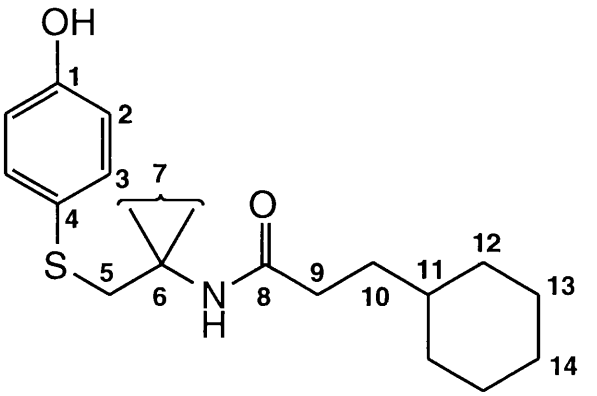
26.3 (C-12) and 26.1 (C-11); *m/z* 307 (M^+, 3.3), 180 (86.8), 139 (7.2), 125 (31.9), 97 (12.2) and 58 (100%); (Found: M^+, 307.1593; C, 66.58; H, 8.03; N, 4.54%. $C_{17}H_{25}NO_2S$ requires *M*, 307.1606; C, 66.41; H, 8.20; N, 4.56%).

_N_-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]octanamide (105)



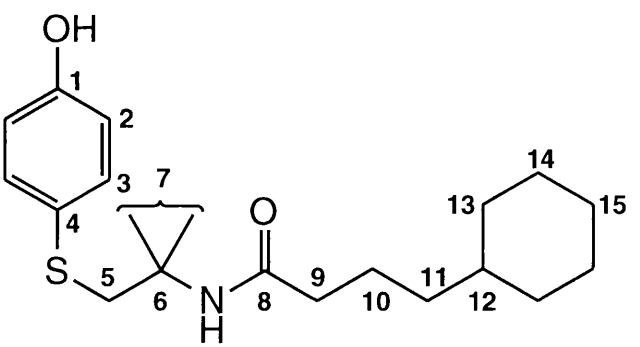
A mixture of 4-hydroxythiophenol (1.70 g, 13.5 mmol) and oxazoline **97** (2.66 g, 13.5 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **105** (3.24 g, 74%) as white crystals, m.p. 96-97 °C (from hexane-EtOAc); R_f 0.52 (2:1 hexane-EtOAc); ν_{max} (KBr) 3322 (N-H), 1630 (amide I), 1556 (amide II), 1494 and 822 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.94 (1 H, br s, OH), 7.26 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.75 (2 H, AA'BB', ^3J= 8.6, 2-H), 5.60 (1 H, br s, NH), 3.33 (2 H, s, 5-H), 2.00 (2 H, t, ^3J= 7.2, 9-H), 1.60-1.10 (10 H, m, 10-H, 11-H, 12-H, 13-H and 14-H), 1.38 (6 H, s, 7-H) and 0.84 (3 H, t, ^3J= 5.6, 15-H); δ_C ($CDCl_3$) 174.2 (C-8), 156.6 (C-1), 133.4 (C-3), 125.1 (C-4), 116.3 (C-2), 54.6 (C-6), 45.8 (C-5), 37.5 (C-9), 31.6 (C-13), 29.0 (C-10 or C-12), 28.9 (C-12 or C-10), 26.9 (C-7), 25.7 (C-11), 22.5 (C-14) and 14.1 (C-15); *m/z* 323 (M^+, 2.6), 198 (3.3), 180 (67.4), 125 (16.4), 97 (4.8) and 58 (100%); (Found: M^+, 323.1892; C, 66.84; H, 9.04; N, 4.25%. $C_{18}H_{29}NO_2S$ requires *M*, 323.1919; C, 66.83; H, 9.04; N, 4.33%).

***N*-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]-3-cyclohexylpropanamide (106)**



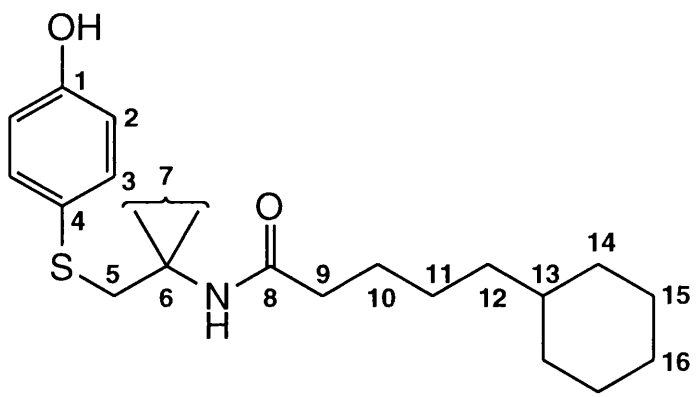
A mixture of 4-hydroxythiophenol (1.25 g, 9.9 mmol) and oxazoline **98** (2.07 g, 9.9 mmol) was stirred under N_2 for 7 h at 130 °C. Upon standing overnight at r.t., the oily reaction mixture solidified to give a white precipitate which was crystallised to afford *sulfide* **106** (2.41 g, 73%) as white crystals, m.p. 129-130 °C (from EtOAc); R_f 0.43 (1:1 hexane-EtOAc); ν_{max} (KBr) 3342 (N-H), 1637 (amide I), 1598, 1584, 1543 (amide II), 1495 and 832 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.74 (1 H, br s, OH), 7.29 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.86 (1 H, br s, NH), 6.78 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.39 (2 H, s, 5-H), 2.06 (2 H, t, ^3J= 7.5, 9-H), 1.75-0.90 (13 H, m, 10-H, 11-H, 13-H and 14-H) and 1.36 (6 H, s, 7-H); δ_C (D_6-acetone) 173.6 (C-8), 157.5 (C-1), 133.9 (C-3), 127.0 (C-4), 116.8 (C-2), 54.5 (C-6), 46.2 (C-5), 38.0 (C-11), 34.9 (C-9), 33.8 (C-10), 33.8 (C-13), 27.0 (C-12), 27.0 (C-7) and 26.9 (C-14); *m/z* 335 (M^+, 2.2), 210 (3.0), 180 (67.6), 125 (21.9), 95 (9.2) and 58 (100%); (Found: M^+, 335.1907; C, 68.20; H, 8.84; N, 4.18%. $C_{19}H_{29}NO_2S$ requires *M*, 335.1919; C, 68.02; H, 8.71; N, 4.17%).

***N*-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]-4-cyclohexylbutanamide (107)**



A mixture of 4-hydroxythiophenol (1.00 g, 7.93 mmol) and oxazoline **99** (1.77 g, 7.93 mmol) was stirred under N_2 for 7 h at 130 °C. Upon cooling to r.t., the crude product was purified by flash chromatography on silica gel eluting with hexane - EtOAc (3:2) followed by crystallisation to afford *sulfide* **107** (1.79 g, 65%) as white crystals, m.p. 110 °C (from EtOAc-hexane); R_f 0.49 (1:1 EtOAc-hexane); ν_{max} (KBr) 3334 (N-H), 1632 (amide I), 1586, 1546 (amide II), 1496 and 822 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 7.28 (2 H, AA'BB', ^3J= 8.5, 3-H), 6.93 (1 H, br s, NH), 6.78 (2 H, AA'BB', ^3J= 8.5, 2-H), 3.40 (2 H, s, 5-H), 2.04 (2 H, t, ^3J= 7.7, 9-H), 1.80 - 1.05 (15 H, m, 10-H, 11-H, 13-H, 14-H and 15-H) and 1.36 (6 H, s, 7-H); δ_C (D_6-acetone) 173.8 (C-8), 157.7 (C-1), 133.9 (C-3), 126.8 (C-4), 116.8 (C-2), 54.7 (C-6), 46.1 (C-5), 38.2 (C-12), 37.7 (CH_2), 37.6 (CH_2), 34.0 (CH_2), 27.3 (CH_2), 27.0 (CH_2), 27.0 (C-7) and 23.9 (CH_2); *m/z* 349 (M^+, 2.4), 224 (2.7), 210 (3.3), 180 (68.5), 125 (14.3), 107 (3.3) and 58 (100%); (Found: M^+, 349.2051; C, 68.67; H, 8.75; N, 3.94%. $C_{20}H_{31}NO_2S$ requires *M*, 349.2075; C, 68.73; H, 8.94; N, 4.01%).

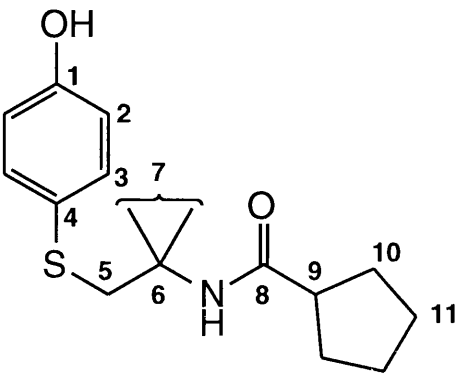
_N_-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]-5-cyclohexylpentanamide (108)



A mixture of 4-hydroxythiophenol (1.44 g, 11.4 mmol) and oxazoline **100** (2.71 g, 11.4 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **108** (2.42 g, 58%) as white crystals, m.p. 125-126 °C (from EtOAc-hexane); R_f 0.49 (1:1 EtOAc-hexane); ν_{max} (KBr) 3340 (N-H), 1636 (amide I), 1584, 1550 (amide II), 1494 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_4-methanol) 7.05 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.50 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.12 (2 H, s, 5-H), 1.81 (2 H, t, ^3J= 7.1, 9-H), 1.50 - 0.70 (17 H, m, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H and 16-H) and 1.14 (6 H, s, 7-H); δ_C (D_4-methanol) 175.9 (C-8), 157.9 (C-1), 134.5 (C-3), 127.2 (C-4), 116.9 (C-2), 55.2 (C-6), 46.4 (C-5), 38.8 (C-12), 38.4 (CH_2), 37.7 (CH_2), 34.5 (CH_2), 27.8 (CH_2), 27.5 (CH_2), 27.5 (CH_2), 27.3 (C-7) and 27.1 (CH_2); *m/z* 363 (M^+, 3.0),

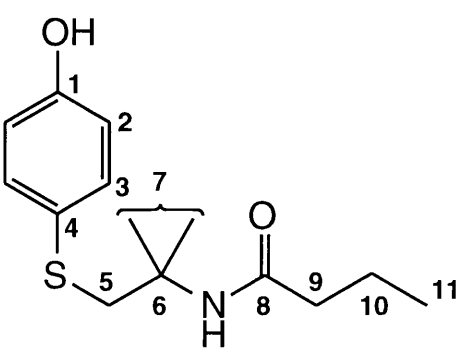
224 (3.7), 180 (82.4), 139 (3.6), 125 (14.4) and 58 (100%); (Found: M^+, 363.2230; C, 69.34; H, 9.21; N, 3.86%. $C_{21}H_{33}NO_2S$ requires *M*, 363.2232; C, 69.38; H, 9.15; N, 3.85%).

N-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]cyclopentanecarboxamide (109)



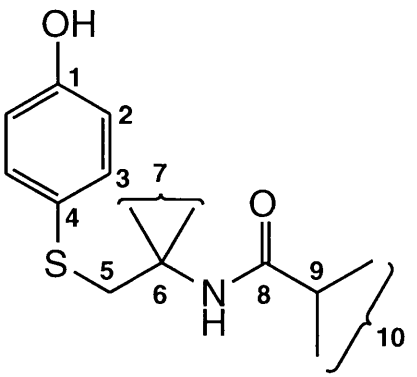
A mixture of 4-hydroxythiophenol (2.35 g, 18.6 mmol) and oxazoline **101** (3.12 g, 18.6 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **109** (3.93 g, 72%) as white crystals, m.p. 143-144 °C (from aq. EtOH); R_f 0.31 (1:1 hexane-EtOAc); ν_{max} (KBr) 3342 (N-H), 1639 (amide I), 1600, 1584, 1544 (amide II), 1495 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.44 (1 H, br s, OH), 7.11 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.60 (2 H, AA'BB', ^3J= 8.7, 2-H), 6.56 (1 H, br s, NH), 3.21 (2 H, s, 5-H), 2.36 (1 H, m, 9-H), 1.60-1.22 (8 H, m, 10-H and 11-H) and 1.17 (6 H, s, 7-H); δ_C (D_6-acetone) 176.5 (C-8), 157.5 (C-1), 133.8 (C-3), 127.1 (C-4), 116.8 (C-2), 54.5 (C-6), 46.2 (C-9), 46.2 (C-5), 31.0 (C-10), 27.0 (C-7) and 26.7 (C-11); *m/z* 293 (M^+, 4.6), 180 (74.7), 168 (3.4), 154 (6.4), 125 (14.5) and 58 (100%); (Found: M^+, 293.1451; C, 65.58; H, 8.02; N, 4.83%. $C_{16}H_{23}NO_2S$ requires *M*, 293.1449; C, 65.49; H, 7.90; N, 4.77%).

N-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]butanamide (110)



A mixture of 4-hydroxythiophenol (2.49 g, 19.7 mmol) and oxazoline **102** (2.79 g, 19.7 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling, the reaction mixture was dissolved in ethanol (10 cm^3) and diluted by slow addition of water (20 cm^3) to give a fine precipitate which was filtered and washed with diethyl ether. Crystallisation afforded *sulfide* **110** (3.24 g, 61%) as white crystals, m.p. 99-100 °C (from EtOAc); R_f 0.28 (2:1 hexane-EtOAc); ν_{max} (KBr) 3241 (N-H), 1635 (amide I), 1599, 1585, 1546 (amide II), 1496 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.86 (1 H, br s, OH), 7.27 (2 H, AA'BB', ^3J= 8.5, 3-H), 6.77 (2 H, AA'BB', ^3J= 8.5, 2-H), 5.57 (1 H, br s, NH), 3.34 (2 H, s, 5-H), 1.97 (2 H, t, ^3J= 7.2, 9-H), 1.53 (2 H, tq, ^3J= 7.2 and 7.3, 10-H), 1.39 (6 H, s, 7-H) and 0.88 (3 H, t, ^3J= 7.3, 11-H); δ_C ($CDCl_3$) 174.0 (C-8), 156.6 (C-1), 133.5 (C-3), 125.1 (C-4), 116.3 (C-2), 54.6 (C-6), 45.8 (C-5), 39.3 (C-9), 26.9 (C-7), 19.1 (C-10) and 13.6 (C-11); *m/z* 267 (M^+, 7.6), 180 (75.7), 165 (4.5), 142 (4.2). 125 (10.5) and 58 (100%); (Found: M^+, 267.1293; C, 62.86; H, 7.73; N, 5.23%. $C_{14}H_{21}NO_2S$ requires *M*, 267.1293; C, 62.89; H, 7.92; N, 5.24%).

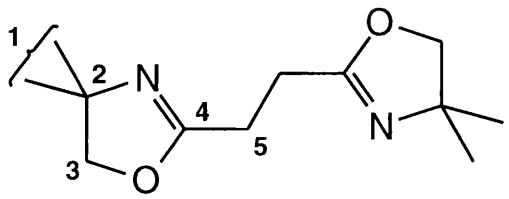
_N_-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]-2-methylpropanamide (111)



A mixture of 4-hydroxythiophenol (1.64 g, 13.0 mmol) and oxazoline **103** (1.84 g, 13.0 mmol) was stirred under N_2 for 4 h at 130 °C. Upon standing overnight at r.t., the oily reaction mixture solidified to give a white precipitate which was crystallised to afford *sulfide* **111** (2.41 g, 69%) as white crystals, m.p. 139-140 °C (from hexane-EtOAc); R_f 0.31 (2:1 hexane-EtOAc); ν_{max} (KBr) 3329 (N-H), 1642 (amide I), 1599, 1582, 1556 (amide II), 1497 and 831 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$+D_6-DMSO) 9.16 (1 H, br s, OH), 7.24 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.74 (2 H, AA'BB', ^3J= 8.6, 2-H), 6.38 (1 H, br s, NH), 3.29 (2 H, s, 5-H), 2.29 (1 H, septet, ^3J= 6.8, 9-H), 1.36 (6 H, s, 7-H) and 1.05 (6 H, d, ^3J= 6.8, 10-H); δ_C ($CDCl_3$+D_6-DMSO) 176.7 (C-8), 156.4 (C-1), 132.8 (C-3), 125.1 (C-4), 115.9 (C-2), 53.5 (C-6), 45.9 (C-5), 35.2 (C-9), 26.4 (C-7) and 19.4 (C-

10); *m/z* 267 (M^+, 5.3), 180 (68.6), 165 (4.9), 142 (4.3), 125 (13.4) and 58 (100%); (Found: M^+, 267.1282; C, 62.94; H, 7.94; N, 5.24%. $C_{14}H_{21}NO_2S$ requires *M*, 267.1293; C, 62.89; H, 7.92; N, 5.24%).

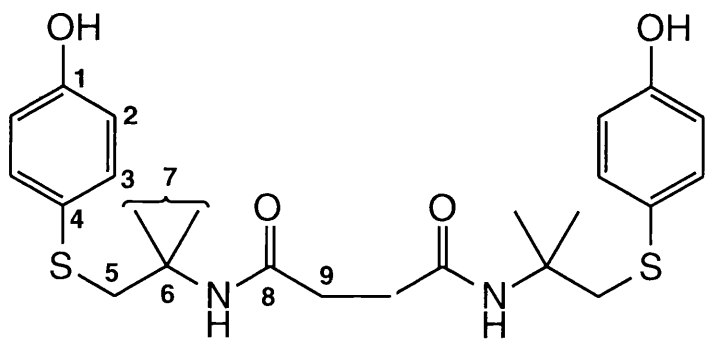
2,2'-(1,2-Ethanediyl)bis[4,5-dihydro-4,4-dimethyloxazole] (112)



Using the procedure of Furuta *et al.*[121] on a 50 mmol scale, the title compound was prepared in 70% yield as white crystals, m.p. 82-83 °C (from EtOAc). The ^{1}H NMR spectral data were in good agreement with those reported in the literature.[121]

New data: ν_{max} (KBr) 2970 (C-H), 1661 (C=N), 1464, 1366, 1148 (C-O) and 983 cm^{-1}; δ_C ($CDCl_3$) 164.5 (C-4), 79.1 (C-3), 67.0 (C-2), 28.4 (C-1) and 24.7 (C-5); *m/z* 224 (M^+, 6.4), 209 (49.1), 169 (21.9), 137 (100) and 110 (10.3%); (Found: M^+, 224.1517; $C_{12}H_{20}N_2O_2$ requires *M*, 224.1525).

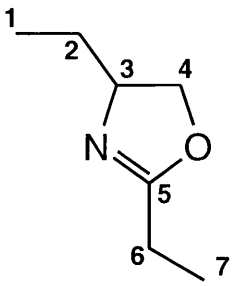
***N-bis*-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]succinamide (113)**



A mixture of 4-hydroxythiophenol (500 mg, 3.97 mmol) and bis-oxazoline **112** (440 mg, 1.99 mmol) was stirred under N_2 for 4 h at 130 °C. Cooling to r.t., followed by flash chromatography on silica gel eluting with EtOAc furnished, after crystallisation, *title compound* **113** (340 mg, 36%) as white crystals, m.p. 179-180 °C (from EtOAc); R_f 0.6 (EtOAc); ν_{max} (KBr) 3330 (N-H), 1643 (amide I), 1600, 1583, 1551 (amide II) and 829 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.35 (2 H, br s, OH), 7.35 (2 H, br s, NH), 7.04 (4 H, AA'BB', ^3J= 8.0, 3-H), 6.54 (4 H, AA'BB', ^3J= 8.0, 2-H), 3.10 (4 H, s, 5-H), 2.03 (4 H, s, 9-H) and 1.09 (12 H, s, 7-H); δ_C (D_6-DMSO) 171.5 (C-8), 156.5 (C-1), 132.6 (C-3), 125.3 (C-4), 116.1 (C-2), 53.4 (C-6), 44.9 (C-5), 31.8 (C-9), and 26.5 (C-7); *m/z*

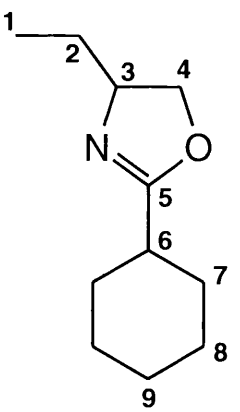
476 (M^+, 2.8), 337 (6.3), 297 (15.0), 280 (26.9) and 181 (100%); (Found: M^+, 476.1840. $C_{24}H_{32}N_2O_4S_2$ requires M, 476.1803).

2,4-Diethyl-4,5-dihydro-oxazole (114)



General procedure 2 was employed as follows: A mixture of propanonitrile (3.15 g, 57.2 mmol), (±)-2-amino-1-butanol (5.10 g, 57.2 mmol) and cadmium diacetate dihydrate (0.380 g, 1.43 mmol) was stirred for 3 d at 130 °C. Distillation of the crude product gave *oxazoline* **114** (5.78 g, 79%) as a colourless oil, b.p. 62-66 °C/25 mmHg; ν_{max} (neat) 2964 (C-H), 1670 (C=N), 1215, 1183 (C-O) and 1015 cm^{-1}; δ_H ($CDCl_3$) 4.09 (1 H, dd, ^2J= -8.4, ^3J= 7.6, 4-H_a), 3.83 (1 H, m, 3-H), 3.66 (1 H, dd, ^2J= -7.6, ^3J= 7.6, 4-H_b), 2.10 (2 H, q, ^3J= 7.5, 6-H), 1.39 (2 H, m, 2-H), 1.00 (3 H, t, ^3J= 7.5, 7-H) and 0.80 (3 H, t, ^3J= 7.4, 1-H); δ_C ($CDCl_3$) 168.2 (C-5), 71.6 (C-4), 67.1 (C-3), 28.4 (C-2), 21.3 (C-6), 10.2 (C-7) and 9.6 (C-1); *m/z* 127 (M^+, 7.0), 98 (89.2), 82 (57.4), 70 (100), 56 (33.1) and 43 (19.3%); (Found: M^+, 127.0995; $C_7H_{13}NO$ requires M, 127.0997).

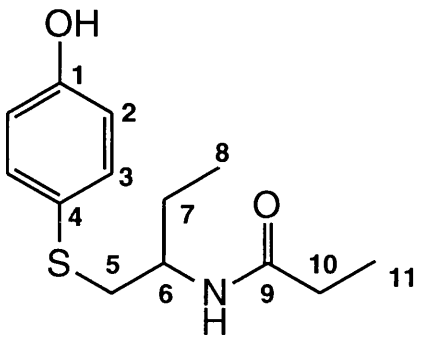
2-Cyclohexyl-4-ethyl-4.5-dihydro-oxazole (115)



General procedure 2 was employed as follows: A mixture of cyclohexane carbonitrile (4.83 g, 44.2 mmol), (±)-2-amino-1-butanol (3.94 g, 44.2 mmol) and cadmium diacetate dihydrate (0.392 g, 1.47 mmol) was stirred for 3 d at 130 °C. Distillation of the crude product gave *oxazoline* **115** (4.37 g, 55%) as a colourless oil, b.p. 95-97 °C/15 mmHg; ν_{max} (neat) 3380, 2932, 1664 (C=N),

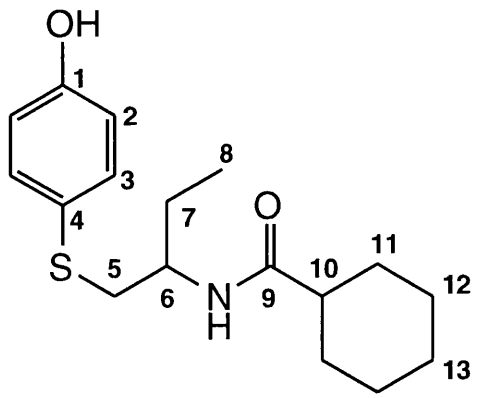
1450 and 1020 (C-O) cm^{-1}; δ_H ($CDCl_3$) 4.13 (1 H, dd, ^2J= -7.6, ^3J= 9.1, 4-H_a), 3.91 (1 H, m, 3-H), 3.72 (1 H, dd, ^2J= -7.6, ^3J= 7.6, 4-H_b), 2.18 (1 H, m, 6-H), 1.84-1.11 (12 H, m, 2-H, 7-H, 8-H and 9-H) and 0.81 (3 H, t, ^3J= 7.5, 1-H); δ_C ($CDCl_3$) 170.5 (C-5), 71.3 (C-4), 66.9 (C-3), 37.3 (C-6), 29.8 (C-7), 28.3 (C-2), 25.7 (C-9), 25.5 (C-8) and 9.5 (C-1); *m/z* 181 (M^+, 12), 168 (32), 152 (26), 128 (28), 126 (27), 83 (65) and 58 (100%); (Found: M^+, 181.1474; $C_{11}H_{19}NO$ requires *M*, 181.1467).

N-[1-[[(4-Hydroxyphenyl)thio]methyl]propyl]propanamide (116)



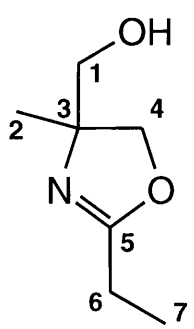
A mixture of 4-hydroxythiophenol (1.40 g, 11.1 mmol) and oxazoline **114** (1.41 g, 11.1 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **116** (2.23 g, 79%) as white crystals, m.p. 105-106 °C (from hexane-EtOAc); R_f 0.43 (EtOAc); ν_{max} (KBr) 3340 (N-H), 1640 (amide I), 1596, 1582, 1548 (amide II), 1496 and 828 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.79 (1 H, br s, OH), 7.14 (2 H, AA'BB', ^3J= 8.2, 3-H), 6.63 (2 H, AA'BB', ^3J= 8.2, 2-H), 5.43 (1 H, br d, ^3J= 8.3, NH), 3.80 (1 H, m, 6-H), 2.77 (1 H, dd, ^2J= -13.6, ^3J= 5.2, 5-H_a), 2.65 (1 H, dd, ^2J= -13.6, ^3J= 6.7, 5-H_b), 1.99 (2 H, q, ^3J= 7.4, 10-H), 1.50 (1 H, m, 7-H_a), 1.26 (1 H, m, 7-H_b), 0.94 (3 H, t, ^3J= 7.4, 11-H) and 0.69 (3 H, t, ^3J= 7.2, 8-H); δ_C ($CDCl_3$) 175.0 (C-9), 157.0 (C-1), 134.1 (C-3), 123.3 (C-4), 116.3 (C-2), 50.3 (C-6), 40.4 (C-5), 29.8 (C-10), 26.6 (C-7), 10.2 (C-11) and 10.0 (C-8); *m/z* 253 (M^+, 7.4), 180 (58.5), 165 (4.9), 139 (5.9), 125 (11.1) and 58 (100); (Found: M^+, 253.1155; C, 61.69; H, 7.47; N, 5.55%. $C_{13}H_{19}NO_2S$ requires *M*, 253.1136; C, 61.63; H, 7.56; N, 5.53%).

***N*-[1-Ethyl-2-[(4-hydroxyphenyl)thio]ethyl]cyclohexanecarboxamide (117)**



A mixture of 4-hydroxythiophenol (0.52 g, 4.2 mmol) and oxazoline **115** (0.75 g, 4.2 mmol) was stirred under N_2 for 4 h at 130 °C. Upon standing overnight at r.t., the oily reaction mixture solidified to give a white precipitate which was crystallised to afford *sulfide* **117** (0.83 g, 65%) as white crystals, m.p. 124-125 °C (from EtOAc); R_f 0.26 (EtOAc); ν_{max} (KBr) 3325 (N-H), 1640 (amide I), 1600, 1579, 1543 (amide II), 1496 and 828 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.59 (1 H, br s, OH), 7.26 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.74 (2 H, AA'BB', ^3J= 8.7, 2-H), 5.45 (1 H, br d, ^3J= 8.7, NH), 3.91 (1 H, m, 6-H), 2.87 (1 H, dd, ^2J= -14.0, ^3J= 5.4, 5-H_a), 2.75 (1 H, dd, ^2J= -14.0, ^3J= 6.9, 5-H_b), 1.98 (1 H, m, 10-H), 1.85-1.05 (12 H, m, 7-H, 11-H, 12-H and 13-H) and 0.80 (3 H, t, ^3J= 7.4, 8-H); δ_C ($CDCl_3$) 177.3 (C-9), 157.2 (C-1), 134.5 (C-3), 123.3 (C-4), 116.3 (C-2), 49.6 (C-6), 46.0 (C-10), 40.6 (C-5), 29.7 (C-11), 26.7 (C-7), 25.5 (C-12 and C-13) and 10.3 (C-8); *m/z* 307 (M^+, 7.0), 180 (100), 139 (9.6), 125 (17.3) and 97 (7.5); (Found: M^+, 307.1590; C, 66.51; H, 8.37; N, 4.56%. $C_{17}H_{25}NO_2S$ requires *M*, 307.1606; C, 66.41; H, 8.20; N, 4.56%).

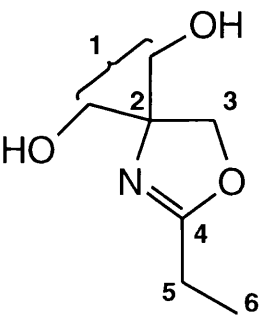
2-Ethyl-4,5-dihydro-4-hydroxymethyl-4-methyloxazole (118)



Using the procedure of Wehrmeister[122] on a 59 mmol scale, the title compound was prepared in 89% yield as a colourless oil, b.p. 115-117 °C/25 mmHg, (lit.,[122] 82-83 °C/10 mmHg).

New data: ν_{max} (neat) 1662 (C=N), 1462, 1454, 1378, 1222, 1186, 1152, 1060 and 1002 cm^{-1}; δ_H (D_4-methanol) 4.13 (1 H, d, ^2J= -8.4, 1-H_a), 3.70 (1 H, d, ^2J= -8.4, 1-H_b), 3.23 (2 H, m, 4-H), 2.10 (2 H, q, ^3J= 7.6, 6-H), 1.04 (3 H, s, 2-H) and 0.98 (3 H, t, ^3J= 7.6, 7-H); δ_C (D_4-methanol) 171.6 (C-5), 76.2 (C-4), 71.9 (C-3), 68.5 (C-1), 23.7 (C-2), 22.4 (C-6) and 10.7 (C-7); *m/z* 143 (M^+, 0.1), 112 (100), 84 (42.1), 56 (56.9) and 41 (9.1%); (Found: M^+, 143.0946; $C_7H_{13}NO_2$ requires *M*, 143.0946).

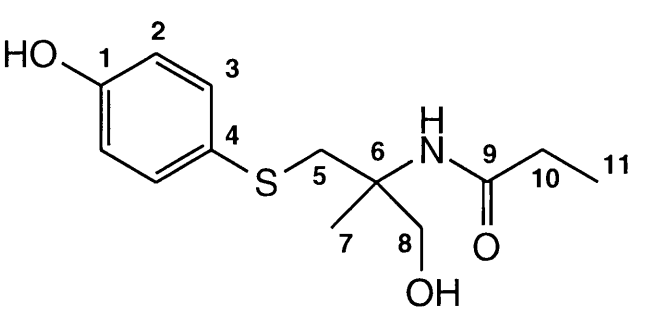
2-Ethyl-4,5-dihydro-4,4-di(hydroxymethyl)oxazole (119)



Using the procedure of Wehrmeister on a 50 mmol scale, the title compound was prepared in 66% yield as white crystals, m.p. 82-83 °C (from EtOAc), (lit.,[122] 82-83 °C). The ^1H NMR spectral data were in good agreement with those reported by Meyers and Yamamoto.[123]

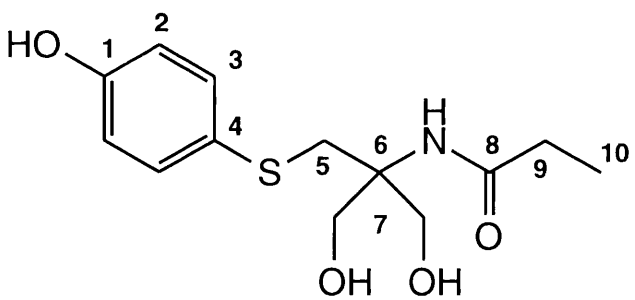
New data: ν_{max} (KBr) 3420 (O-H), 1665 (C=N) and 1032 (C-O) cm^{-1}; δ_C ($CDCl_3$) 171.6 (C-4), 75.7 (C-2), 71.9 (C-3), 64.8 (C-1), 21.7 (C-5) and 10.4 (C-6); *m/z* 160 ((*MH*)$^+$, 0.4), 128 (27.5), 98 (41.6), 82 (7.4), 72 (9.2), 56 (38.9) and 29 (100%); (Found: (*MH*)$^+$, 160.0972; $C_7H_{13}NO_3$ requires *MH*, 160.0974).

***N*-[1-Hydroxymethyl-2-[(4-hydroxyphenyl)thio]-1-methylethyl]propanamide (120)**



A solution of 4-hydroxythiophenol (1.00 g, 7.93 mmol) and oxazoline **118** (1.19 g, 8.33 mmol) in anhydrous DMF (2 cm^3) was stirred under N_2 for 7 h at 120 °C. After cooling to r.t., the reaction mixture was dissolved in EtOAc (50 cm^3), washed with water (10 x 10 cm^3) and brine (10 cm^3). The organic portion was dried ($MgSO_4$) and concentrated *in vacuo* to give an oil which was purified by flash chromatography on silica gel eluting with EtOAc. Crystallisation of the resulting oil afforded *sulfide* **120** (0.63 g, 30%) as white crystals, m.p. 98-100 °C (from EtOAc); R_f 0.42 (EtOAc); ν_{max} (KBr) 3424, 3342 (N-H), 1640 (amide I), 1600, 1585, 1542 (amide II), 1497 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.59 (1 H, br s, 1-OH), 7.30 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.86 (1 H, br s, NH), 6.78 (2 H, AA'BB', ^3J= 8.7, 2-H), 4.85 (1 H, dd, ^3J= 6.7 and 5.3, 8-OH), 3.68 (1 H, dd, ^2J= -11.1, ^3J= 6.8, 8-H_a), 3.60 (1 H, dd, ^2J= -11.1, ^3J= 5.3, 8-H_b), 3.52 (1 H, d, ^2J= -13.1, 5-H_a), 3.20 (1 H, d, ^2J= -13.1, 5-H_b), 2.08 (2 H, q, ^3J= 7.6, 10-H), 1.28 (3 H, s, 7-H) and 1.00 (3 H, t, ^3J= 7.6, 11-H); δ_C (D_6-acetone) 175.1 (C-9), 157.5 (C-1), 134.0 (C-3), 126.8 (C-4), 116.8 (C-2), 68.4 (C-8), 59.2 (C-6), 42.4 (C-5), 30.2 (C-10), 22.3 (C-7) and 10.1 (C-11); *m/z* 269 (M^+, 3.9), 196 (59.3), 182 (8.4), 139 (9.3), 126 (64.3) and 74 (100%); (Found: M^+, 269.1082; C, 57.97; H, 7.08; N, 5.20%. $C_{13}H_{19}NO_3S$ requires *M*, 269.1086; C, 57.97; H, 7.11; N, 5.20%).

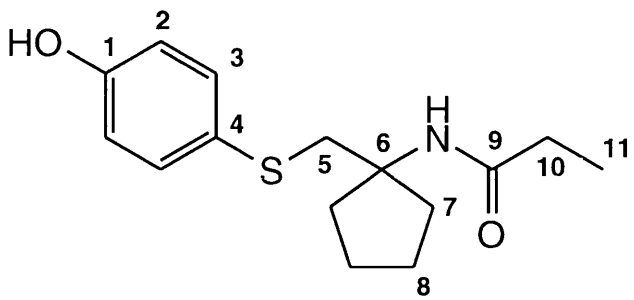
_N_-[1,1-Di(hydroxymethyl)-2-[(4-hydroxyphenyl)thio]ethyl]propanamide (121)



A solution of 4-hydroxythiophenol (1.50 g, 11.9 mmol) and oxazoline **119** (2.08 g, 13.1 mmol) in anhydrous DMF (5 cm^3) was stirred under N_2 for 7 h at 120 °C. After cooling to r.t., the reaction mixture was dissolved in EtOAc (100 cm^3), washed with water (10 x 15 cm^3) and brine (15 cm^3). The organic portion was dried ($MgSO_4$) and concentrated *in vacuo* to give an oil which was purified by flash chromatography on silica gel eluting with EtOAc. Crystallisation of the resulting oil afforded *sulfide* **121** (0.72 g, 21%) as white crystals, m.p. 117-118 °C (from EtOAc); R_f 0.3 (EtOAc); ν_{max} (KBr) 3273 (O-H), 1652 (amide I), 1601, 1585, 1564 (amide II), 1495 and 829 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_4-methanol) 7.10 (2 H, AA'BB', ^3J= 6.7, 3-H), 6.52 (2 H, AA'BB', ^3J= 6.7, 2-H),

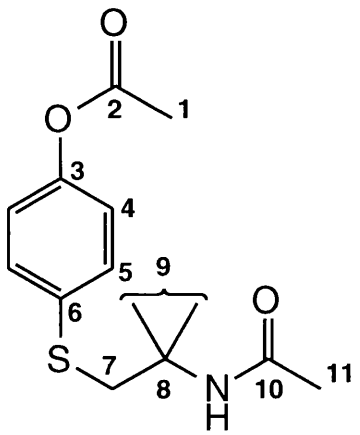
3.62 (2 H, d, ^2J= -11.1, 7-H$_a$), 3.54 (2 H, d, ^2J= -11.1, 7-H$_b$), 3.13 (2 H, s, 5-H), 1.87 (2 H, q, ^3J= 7.6, 9-H) and 0.82 (3 H, t, ^3J= 7.6, 10-H); δ_C (D_4-methanol) 177.5 (C-8), 158.0 (C-1), 134.7 (C-3), 126.7 (C-4), 116.9 (C-2), 63.4 (C-7), 62.8 (C-6), 38.3 (C-5), 30.3 (C-9) and 10.1 (C-10); *m/z* 285 (M^+, 4.6), 234 (6.5), 212 (49.9), 139 (19.8), 126 (70.3) and 57 (100%); (Found: M^+, 285.1030; C, 54.76; H, 6.60; N, 4.88%. $C_{13}H_{19}NO_4S$ requires *M*, 285.1035; C, 54.72; H, 6.71; N, 4.91%).

_N_-[2-[(4-Hydroxyphenyl)thio]-1,1-(propamethylene)ethyl]propanamide (126)



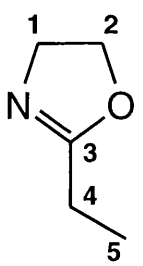
A mixture of 1-amino-1-(hydroxymethyl)cyclopentane (0.43 g, 3.7 mmol), propanonitrile (0.21 g, 0.27 cm^3, 3.7 mmol) and cadmium diacetate dihydrate (25 mg, 94 µmol) was stirred under gentle reflux (oil bath 120 °C) for 3 d. Upon cooling to r.t., the crude intermediate was transferred to a flask containing 4-hydroxythiophenol (0.47 g, 3.73 mmol) and stirred under N_2 for 5 h at 130 °C to give a viscous yellow oil. Purification by flash chromatoraphy on silica gel eluting with EtOAc-hexane (1:2 then 1:1) followed by crystallisation of the resulting oil furnished *title compound* **126** (0.49 g, 47%), as white crystals, m.p. 140-142 °C (from hexane-EtOAc); R_f 0.27 (1:1 hexane-EtOAc); ν_{max} (KBr) 3345 (N-H), 1636 (amide I), 1599, 1585, 1546 (amide II) and 831 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.34 (1 H, br s, OH), 7.38 (1 H, br s, NH), 7.06 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.54 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.19 (2 H, s, 5-H), 1.95 - 1.75 (4 H, m, 7-H$_a$ and 10-H), 1.62 - 1.25 (6 H, m, 7-H$_b$ and 8-H) and 0.76 (3 H, t, ^3J= 7.6, 11-H); δ_C (D_6-DMSO) 173.1 (C-9), 156.4 (C-1), 132.5 (C-3), 125.3 (C-4), 116.0 (C-2), 63.9 (C-6), 42.7 (C-5), 36.9 (C-7), 28.9 (C-10), 23.4 (C-8) and 10.0 (C-11); *m/z* 279 (M^+, 4.6), 206 (47.7), 140 (19.0), 125 (16.4) and 84 (100%); (Found: M^+, 279.1285; C, 64.22; H, 7.67; N, 5.04%. $C_{15}H_{21}NO_2S$ requires *M*, 279.1293; C, 64.48; H, 7.58; N, 5.01%).

_N_-[2-[(4-Ethanoyloxyphenyl)thio]-1,1-dimethylethyl]ethanamide (127)



The general procedure of Furniss *et al.*[109] was employed as follows: Ice (10 g) was added to a solution of compound **92** (0.860 g, 3.59 mmol) in 3 mol dm^{-3} aq. NaOH (10 cm^3). After 5 min, acetic anhydride (0.54 g, 0.50 cm^3, 5.29 mmol) was added and the mixture swirled for 5 min. whereupon the crude precipitated product was collected by filtration and recrystallised to afford *ester* **127** (0.92 g, 92%) as white crystals, m.p. 71 °C (from EtOAc-hexane); R_f 0.43 (EtOAc); ν_{max} (KBr) 3395 (N-H), 1741 (C=O), 1633 (amide I), 1550 (amide II) and 838 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 7.27 (2 H, AA'BB', ^3J= 8.8, 5-H), 6.90 (2 H, AA'BB', ^3J= 8.8, 4-H), 6.80 (1 H, br s, NH), 3.37 (2 H, s, 7-H), 2.08 (3 H, s, 1-H), 1.59 (3 H, s, 11-H) and 1.23 (6 H, s, 9-H); δ_C (D_6-acetone) 170.2 (C-10), 169.6 (C-2), 150.1 (C-3), 135.5 (C-6), 131.2 (C-5), 123.1 (C-4), 54.4 (C-8), 43.8 (C-7), 27.1 (C-9), 23.8 (C-11) and 21.0 (C-1); *m/z* 281 (M^+, 2.3), 222 (14.9), 180 (23.7), 100 (19.1) and 59 (100%); (Found: M^+, 281.1076; C, 59.89; H, 6.79; N, 4.92%. $C_{14}H_{19}NO_3S$ requires *M*, 281.1086; C, 59.76; H, 6.81; N, 4.97%).

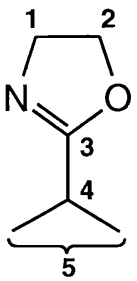
2-Ethyl-4,5-dihydro-oxazole (130)



The title compound **130** was prepared in 65% yield on a 120 mmol scale using general procedure 2 (3 d) and gave b.p. 128-129 °C/760 mmHg, (lit.,[126] 124-126 °C/760 mmHg). The IR, ^1H NMR and MS spectral data were in good agreement with those reported in the literature.[127]

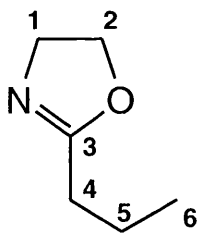
New data: δ_C ($CDCl_3$) 169.3 (C-3), 67.0 (C-2), 54.1 (C-1), 21.1 (C-4) and 10.0 (C-5).

4,5-Dihydro-2-(1-methylethyl)oxazole (131)



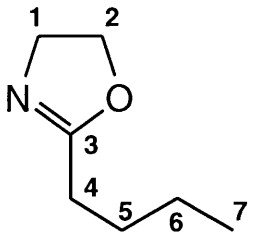
The title compound **131** was prepared in 67% yield on a 120 mmol scale using general procedure 2 (3 d) and gave b.p. 136-137 °C/760 mmHg, (lit.,[128] 64 °C/50 mmHg). The IR, ^1H NMR and MS spectral data were in good agreement with those reported in the literature.[127,128]
New data: δ_C ($CDCl_3$) 172.4 (C-3), 67.0 (C-2), 54.1 (C-1), 27.9 (C-4) and 19.5 (C-5).

4,5-Dihydro-2-propyloxazole (132)



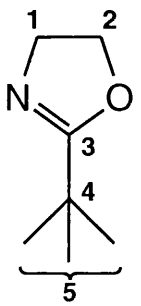
The title compound **132** was prepared in 50% yield on a 120 mmol scale using general procedure 2 (3 d) and gave b.p. 148-150 °C/760 mmHg, (lit.,[129] 148-149 °C/760 mmHg). The IR, ^1H NMR and MS spectral data were in good agreement with those reported in the literature.[127]
New data: δ_C ($CDCl_3$) 168.0 (C-3), 66.7 (C-2), 54.1 (C-1), 29.5 (C-4), 19.1 (C-5) and 13.4 (C-6).

2-Butyl-4,5-dihydro-oxazole (133)



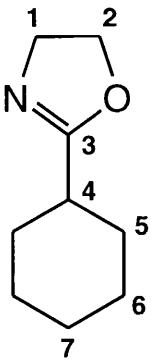
The title compound **133** was prepared in 65% yield on a 60 mmol scale using general procedure 2 (3 d) and gave b.p. 173-174 °C/760 mmHg, (lit.,[130] 174-175 °C/760 mmHg). The IR spectral data were in good agreement with those reported in the literature.[130]
New data: δ_H ($CDCl_3$) 4.01 (2 H, t, ^3J= 8.5, 2-H), 3.52 (2 H, t, ^3J= 8.5, 1-H), 2.08 (2 H, t, ^3J= 7.9, 4-H), 1.41 (2 H, m, 5-H), 1.25 (2 H, m, 6-H) and 0.83 (3 H, t, ^3J= 7.3, 7-H); δ_C ($CDCl_3$) 168.4 (C-3), 66.8 (C-2), 54.1 (C-1), 27.8 (C-4), 27.4 (C-5), 22.1 (C-6) and 13.5 (C-7); *m/z* 127 (M^+, 5), 98 (31), 85 (100), 57 (31) and 41 (35%).

4,5-Dihydro-2-(1,1-dimethylethyl)oxazole (134)



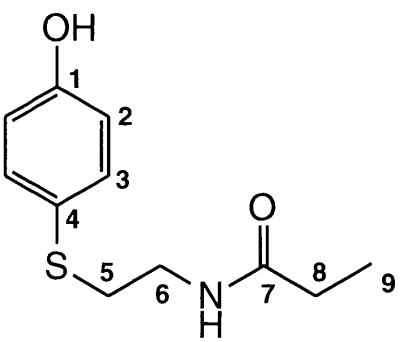
The title compound **134** was prepared in 43% yield on a 25 mmol scale using general procedure 2 (3 d) and gave b.p. 138-141 °C/760 mmHg, (lit.,[131] 142-143 °C/760 mmHg). The IR, ^1H NMR and MS spectral data were in good agreement with those reported in the literature.[127]
New data: δ_C ($CDCl_3$) 174.7 (C-3), 67.2 (C-2), 63.4 (C-4), 54.0 (C-1) and 27.5 (C-5).

2-Cyclohexyl-4,5-dihydro-oxazole (129)



The title compound **129** was prepared in 43% yield on a 60 mmol scale using general procedure 2 (3 d) and gave b.p. 130-132 °C/25 mmHg, (lit.,[125] 120 °C/15 mmHg). The IR spectral data were in good agreement with those reported by Witte and Seeliger.[117]
New data: δ_H ($CDCl_3$) 4.08 (2 H, t, ^3J= 8.4, 2-H), 3.69 (2 H, t, ^3J= 8.4, 1-H), 2.15 (1 H, m, 4-H) and 1.84-1.06 (10 H, m, 5-H, 6-H and 7-H); δ_C ($CDCl_3$) 171.5 (C-3), 66.8 (C-2), 54.1 (C-1), 37.2 (C-4), 29.7 (C-5), 25.7 (C-7) and 25.5 (C-6).

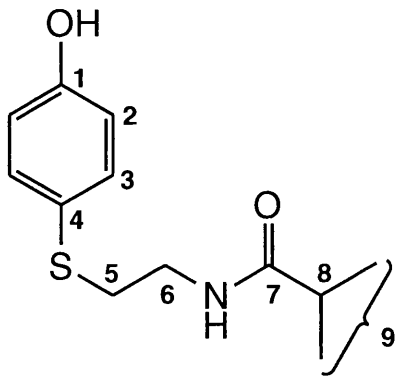
***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]propanamide (136)**



A mixture of 4-hydroxythiophenol (3.10 g, 24.6 mmol) and oxazoline **130** (2.43 g, 2.48 cm^3, 24.6 mmol) was stirred under N_2 for 3 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **136** (4.82 g, 87%) as white crystals, m.p. 81-82 °C (from hexane-EtOAc); R_f 0.34 (EtOAc); ν_{max} (KBr) 3290 (N-H), 1641 (amide I), 1601, 1591, 1562 (amide II), 1496 and 820 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.82 (1 H, br s, OH), 8.14 (1 H, br m, NH), 7.46 (2 H, AA'BB', ^3J= 8.1, 3-H), 6.94 (2 H, AA'BB', ^3J= 8.1, 2-H), 3.36 (2 H, m, 6-H), 3.01 (2 H, t, ^3J= 6.9, 5-H), 2.25 (2 H, q, ^3J= 7.3, 8-H) and 1.16 (3 H, t, ^3J= 7.3, 9-H); δ_C (D_6-DMSO) 173.2 (C-7), 157.1 (C-1), 133.4 (C-3), 123.3 (C-4), 116.4 (C-2), 38.6 (C-6), 34.6 (C-5), 28.7 (C-8) and 10.1 (C-9); *m/z* 225 (M^+, 19), 152 (100), 125 (12), 100 (30). and 57 (17%); (Found: M^+, 225.0825;

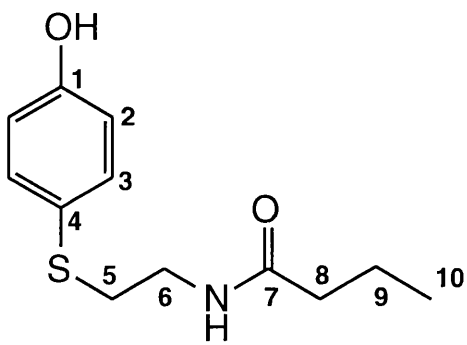
C, 58.62; H, 6.63; N, 6.14%. $C_{11}H_{15}NO_2S$ requires *M*, 225.0823; C, 58.64; H, 6.71; N, 6.22%).

N-[2-[(4-Hydroxyphenyl)thio]ethyl]-2-methylpropanamide (137)



A mixture of 4-hydroxythiophenol (2.31 g, 18.3 mmol) and oxazoline **131** (2.07 g, 18.3 mmol) was stirred under N_2 for 3 h at 130 °C. Cooling to r.t., followed by dry-column flash chromatography on silica gel eluting with EtOAc furnished, after crystallisation, sulfide **137** (3.14 g, 72%) as white crystals, m.p. 115-116 °C (from hexane-EtOAc); R_f 0.52 (EtOAc); ν_{max} (KBr) 3331 (N-H), 1641 (amide I), 1602, 1580, 1549 (amide II), 1495 and 835 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.65 (1 H, br s, OH), 7.95 (1 H, br t, ^3J= 5.4, NH), 7.31 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.79 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.21 (2 H, m, 6-H), 2.86 (2 H, m, 5-H), 2.36 (1 H, sept, ^3J= 6.9, 8-H) and 1.02 (6 H, d, ^3J= 6.9, 9-H); δ_C (D_6-DMSO) 176.3 (C-7), 157.0 (C-1), 133.3 (C-3), 123.2 (C-4), 116.2 (C-2), 38.4 (C-6), 34.4 (C-5), 34.1 (C-8) and 19.6 (C-9); *m/z* 239 (M^+, 17), 152 (100), 125 (13) and 114 (30%); (Found: M^+, 239.0984; C, 60.41; H, 7.27; N, 5.86%. $C_{12}H_{17}NO_2S$ requires *M*, 239.0980; C, 60.22; H, 7.16; N, 5.85%).

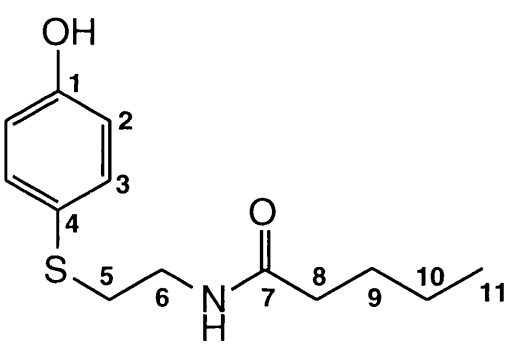
N-[2-[(4-Hydroxyphenyl)thio]ethyl]butanamide (138)



A mixture of 4-hydroxythiophenol (2.17 g, 17.2 mmol) and oxazoline **132** (1.95 g, 17.2 mmol) was stirred under N_2 for 3 h at 130 °C. Upon cooling to r.t., the

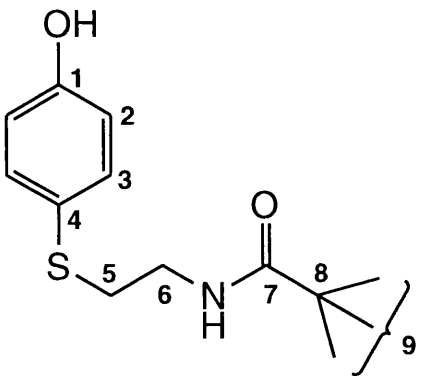
oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **138** (3.24 g, 79%) as white crystals, m.p. 85-86 °C (from hexane-EtOAc); R_f 0.45 (EtOAc); ν_{max} (KBr) 3284 (N-H), 1636 (amide I), 1602, 1590, 1560 (amide II), 1496 and 820 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.65 (1 H, br s, OH), 8.00 (1 H, br t, ^3J= 5.4, NH), 7.30 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.79 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.20 (2 H, m, 6-H), 2.85 (2 H, t, ^3J= 5.9, 5-H), 2.06 (2 H, t, ^3J= 7.2, 8-H), 1.53 (2 H, m, 9-H) and 0.88 (3 H, t, ^3J= 7.3, 10-H); δ_C (D_6-DMSO) 172.2 (C-7), 157.0 (C-1), 133.3 (C-3), 123.1 (C-4), 116.2 (C-2), 38.4 (C-6), 37.4 (C-8), 34.5 (C-5), 18.8 (C-9) and 13.7 (C-10); *m/z* 239 (M^+, 16), 152 (100), 125 (12) and 114 (29%); (Found: M^+, 239.0989; C, 59.94; H, 7.10; N, 5.76%. $C_{12}H_{17}NO_2S$ requires *M*, 239.0980; C, 60.22; H, 7.16; N, 5.85%).

N-[2-[(4-Hydroxyphenyl)thio]ethyl]pentanamide (139)



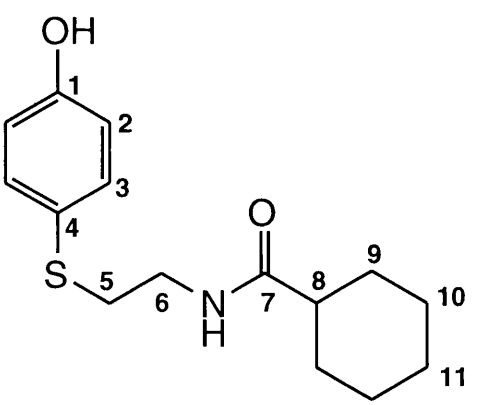
A mixture of 4-hydroxythiophenol (2.00 g, 15.9 mmol) and oxazoline **133** (2.02 g, 15.9 mmol) was stirred under N_2 for 3 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **139** (2.99 g, 74%) as white crystals, m.p. 91-92 °C (from hexane-EtOAc); R_f 0.52 (EtOAc); ν_{max} (KBr) 3286 (N-H), 1636 (amide I), 1602, 1591, 1563 (amide II), 1497 and 820 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.60 (1 H, br s, OH), 7.94 (1 H, br t, ^3J= 5.2, NH), 7.26 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.74 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.16 (2 H, m, 6-H), 2.80 (2 H, t, ^3J= 7.5, 5-H), 2.03 (2 H, t, ^3J= 7.1, 8-H), 1.45 (2 H, m, 9-H), 1.25 (2 H, m, 10-H) and 0.83 (3 H, t, ^3J= 7.1, 11-H); δ_C (D_6-DMSO) 172.6 (C-7), 157.2 (C-1), 133.4 (C-3), 123.3 (C-4), 116.4 (C-2), 38.6 (C-6), 35.4 (C-8), 34.6 (C-5), 27.6 (C-9), 22.1 (C-10) and 14.0 (C-11); *m/z* 253 (M^+, 14), 152 (100), 128 (27), 125 (11) and 107 (5%); (Found: M^+, 253.1130; C, 61.52; H, 7.47; N, 5.35%. $C_{13}H_{19}NO_2S$ requires *M*, 253.1136; C, 61.63; H, 7.56; N, 5.35%).

***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]-2,2-dimethylpropanamide (140)**



A mixture of 4-hydroxythiophenol (1.70 g, 13.5 mmol) and oxazoline **134** (1.71 g, 13.5 mmol) was stirred under N_2 for 3 h at 130 °C. Cooling to r.t., followed by flash chromatography on silica gel eluting with hexane-EtOAc (3:2) afforded, after crystallisation, sulfide **140** (2.20 g, 64%) as white crystals, m.p. 93-94 °C (from hexane-EtOAc); R_f 0.60 (EtOAc); ν_{max} (KBr) 3380 (N-H), 1626 (amide I), 1602, 1578, 1539 (amide II), 1495 and 824 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.62 (1 H, br s, OH), 7.65 (1 H, br t, ^3J= 5.5, NH), 7.30 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.79 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.21 (2 H, m, 6-H), 2.85 (2 H, m, 5-H) and 1.10 (9 H, s, 9-H); δ_C (D_6-DMSO) 177.6 (C-7), 157.0 (C-1), 133.1 (C-3), 123.3 (C-4), 116.2 (C-2), 39.0 (C-6), 38.1 (C-8), 34.1 (C-5) and 27.5 (C-9); *m/z* 253 (M^+, 17), 152 (100), 128 (34), 125 (17). and 57 (26%); (Found: M^+, 253.1139; C, 61.61; H, 7.74; N, 5.34%. $C_{13}H_{19}NO_2S$ requires *M*, 253.1136; C, 61.63; H, 7.56; N, 5.53%).

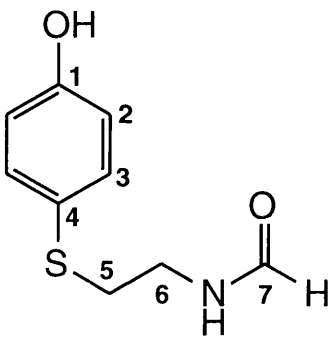
***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]cyclohexanecarboxamide (141)**



A mixture of 4-hydroxythiophenol (2.00 g, 15.9 mmol) and oxazoline **129** (2.43 g, 15.9 mmol) was stirred under N_2 for 3 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford the *sulfide* **141** (3.58 g, 81%) as white crystals,

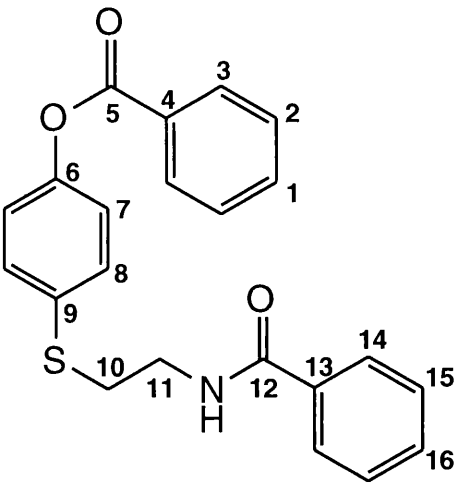
m.p. 94-95 °C (from hexane-EtOAc); R_f 0.58 (EtOAc); ν_{max} (KBr) 3227 (N-H), 1647 (amide I), 1602, 1581, 1547 (amide II), 1495 and 832 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.65 (1 H, br s, OH), 7.89 (1 H, br t, ^3J= 5.6, NH), 7.28 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.78 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.19 (2 H, m, 6-H), 2.83 (2 H, m, 5-H), 2.09 (1 H, m, 8-H), 1.70 (4 H, m, 9-H) and 1.42 - 1.09 (6 H, m, 10-H and 11-H); δ_C (D_6-DMSO) 175.4 (C-7), 157.0 (C-1), 133.3 (C-3), 123.2 (C-4), 116.2 (C-2), 44.1 (C-8), 38.4 (C-6), 34.5 (C-5), 29.3 (C-9), 25.6 (C-11) and 25.4 (C-10); *m/z* 279 (M^+, 11), 152 (100), 125 (11) and 83 (18%); (Found: M^+, 279.1298; C, 64.56; H, 7.75; N, 4.95%. $C_{15}H_{21}NO_2S$ requires *M*, 279.1293; C, 64.48; H, 7.58; N, 5.01%).

_N_-[2-[(4-Hydroxyphenyl)thio]ethyl]methanamide (143)



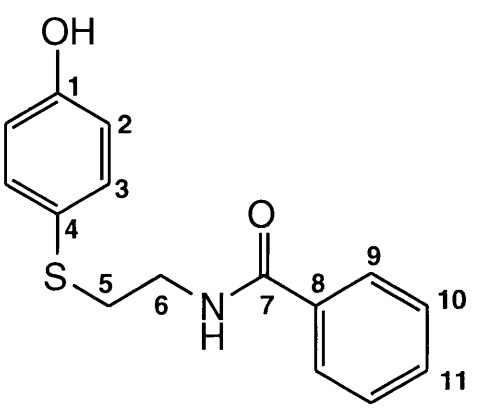
A solution of 2-[(4-hydroxyphenyl)thio]ethylamine **42** (1.00 g, 18.3 mmol) in ethanol (5 cm^3) and ethyl formate (20 cm^3) was heated under reflux for 12 h. On cooling to r.t., the reaction mixture was concentrated *in vacuo* to give a colourless oil which was purified by dry-column flash chromatography on silica gel, eluting with EtOAc, to afford *carboxamide* **143** (0.75 g, 64%) as a white powder, m.p. 91-92 °C (from hexane-EtOAc); R_f 0.24 (EtOAc); ν_{max} (KBr) 3323 (N-H), 1642 (amide I), 1601, 1582, 1562 (amide II), 1496, 1270, 1223 and 819 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 8.21 (1 H, br s, NH), 8.06 (1-H, s, 7-H), 7.31 (2 H, AA'BB', ^3J= 8.5, 3-H), 6.79 (2 H, AA'BB', ^3J= 8.5, 2-H), 3.25 (2 H, m, 6-H) and 2.87 (2 H, t, ^3J= 6.7, 5-H); δ_C (D_6-DMSO) 161.4 and 161.3 (C-7), 157.2 (C-1), 133.5 (C-3), 122.7 (C-4), 116.3 (C-2), 36.9 (C-6) and 34.5 (C-5); *m/z* 197 (M^+, 34), 152 (100), 139 (20), 125 (14), 95 (13) and 72 (27%); (Found: M^+, 197.0517; C, 54.77; H, 5.68; N, 6.98%. $C_9H_{11}NO_2S$ requires *M*, 197.0510; C, 54.80; H, 5.62; N, 7.10%).

***N*-[2-[(4-Benzoyloxyphenyl)thio]ethyl]benzamide (145)**



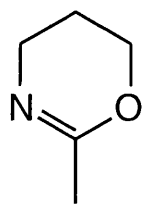
To a cooled (0 °C) solution of 2-[(4-hydroxyphenyl)thio]ethylamine hydrochloride **42** (0.42 g, 2.0 mmol) in aq. NaOH (2 mol dm^{-3}, 10 cm^3) was added benzoyl chloride (0.69 g, 0.57 cm^3, 4.9 mmol). The reaction mixture was shaken vigorously for 5 min before collection of the precipitated crude product by filtration. Recrystallisation furnished *benzamide* **145** (0.60 g, 78%) as white crystals, m.p. 127-128 °C (from hexane-EtOAc); R_f 0.70 (EtOAc); ν_{max} (KBr) 3318 (N-H), 1734 and 1728 (ester C=O), 1644 (amide I), 1602, 1579, 1542 (amide II), 1492, 1313, 1287, 703 and 695 cm^{-1}; δ_H ($CDCl_3$) 8.19 (2 H, m, 3-H), 7.76 (2 H, m, 14-H), 7.66 (1 H, m, 1-H), 7.58 - 7.32 (7 H, m, 2-H, 8-H, 15-H and 16-H), 7.16 - 6.95 (3 H, m, 7-H and N-H), 3.60 (2 H, m, 11-H) and 3.12 (2 H, t, ^3J= 6.3, 10-H); δ_C ($CDCl_3$) 167.7 (C-12), 165.1 (C-5), 149.6 (C-6), 134.1 (aryl quat. C), 133.8 (aryl quat. C), 132.4 (aryl quat. C), 131.6 (aryl quat. C), 131.1 (aryl *C*-H), 130.2 (aryl *C*-H), 129.2 (aryl quat. C), 128.6 (aryl *C*-H), 128.5 (aryl *C*-H), 127.0 (aryl *C*-H), 122.5 (C-7), 39.2 (C-11) and 33.8 (C-10); *m/z* 377 (M^+, 8), 256 (45), 148 (12), 105 (100) and 77 (32%); (Found: M^+, 377.1086;. $C_{22}H_{19}NO_3S$ requires *M*, 377.1086).

***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]benzamide (146)**



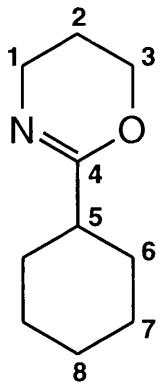
A solution of amide **145** (1.00 g, 2.74 mmol) and 1-butylamine (2.00 g, 2.70 cm^3, 27.3 mmol) in anhydrous THF (20 cm^3) was stirred at r.t. for 20 h. The reaction mixture was concentrated *in vacuo* and the residue partitioned between EtOAc (60 cm^3) and 2 mol dm^{-3} aq. NaOH (40 cm^3). The aqueous layer was adjusted to pH 2 using 5 mol dm^{-3} hydrochloric acid and extracted by ethyl acetate (2 x 30 cm^3). The two organic extracts from this stage were combined and washed with brine (30 cm^3), dried over $MgSO_4$ and concentrated *in vacuo* before purification by dry-column flash chromatography on silica gel using hexane-ethyl acetate (1:1) as eluent to afford *benzamide* **146** (0.49 g, 65%) as white crystals, m.p. 83-84 °C (from hexane-EtOAc); R_f 0.63 (EtOAc); ν_{max} (KBr) 3372 (N-H), 1633 (amide I), 1600, 1578, 1545 (amide II), 1494, 829 (*para*-disubstituted benzene) and 710 (monosubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.92 (1 H, br s, OH), 7.69 (2 H, m, 9-H), 7.46 - 7.36 (3 H, m, 10-H and 11-H), 7.29 (2 H, AA'BB', ^3J= 8.4, 3-H), 6.78 (2 H, AA'BB', ^3J= 8.4, 2-H), 6.70 (1 H, br s, NH), 3.58 (2 H, m, 6-H) and 3.00 (2 H, t, ^3J= 6.2, 5-H); δ_C ($CDCl_3$) 168.3 (C-7), 156.7 (C-1), 134.3 (C-3), 133.7 (C-8), 131.9 (C-11), 128.7 (C-9), 126.9 (C-10), 123.2 (C-4), 116.5 (C-2), 39.2 (C-6) and 35.5 (C-5); *m/z* 273 (M^+, 15), 152 (100), 148 (27), 105 (57) and 77 (35%); (Found: M^+, 273.0818; C, 65.92; H, 5.56; N, 5.04%. $C_{15}H_{15}NO_2S$ requires *M*, 273.0823; C, 65.91; H, 5.53; N, 5.12%).

5,6-Dihydro-2-methyl-4*H*-1,3-oxazine (147)



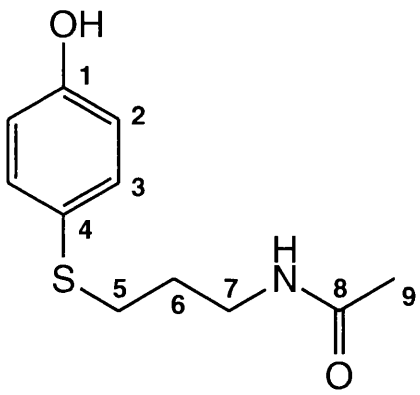
The title compound **147** was prepared in 59% yield on a 120 mmol scale using general procedure 2 (2 d) and gave b.p. 135-137 °C/760 mmHg, (lit.,[206] 132-133 °C/760 mmHg). The IR, ^1H NMR and ^{13}C NMR spectral data were in good agreement with those reported in the literature.[206]
New data: *m/z* 99 (M^+, 64), 72 (15), 56 (66), 42 (100) and 31 (32%)

2-Cyclohexyl-5,6-dihydro-4*H*-1,3-oxazine (148)



General procedure 2 was employed as follows: A mixture of cyclohexane carbonitrile (2.83 g, 25.9 mmol), 3-amino-1-propanol (1.95 g, 26.0 mmol) and cadmium diacetate dihydrate (0.189 g, 0.86 mmol) was stirred for 3 d at 130 °C. Distillation of the crude product gave *dihydrooxazine* **148** (1.39 g, 32%) as a colourless oil, b.p. 74-76 °C/2 mmHg; ν_{max} (neat) 2930, 1671 (C=N), 1245 and 1122 (C-O) cm^{-1}; δ_H ($CDCl_3$) 3.94 (2 H, m, 3-H), 3.16 (2 H, m, 1-H), 1.82 (1 H, m, 5-H) and 1.75-0.80 (12 H, m, 2-H, 6-H, 7-H and 8-H); δ_C ($CDCl_3$) 163.3 (C-4), 64.5 (C-3), 43.9 (C-5), 41.9 (C-1), 29.8 (C-6), 25.8 (C-2), 25.8 (C-7) and 21.7 (C-8); *m/z* 167 (M^+, 68), 138 (62), 112 (100) and 83 (17%); (Found: M^+, 167.1317; $C_{10}H_{17}NO$ requires *M*, 167.1310).

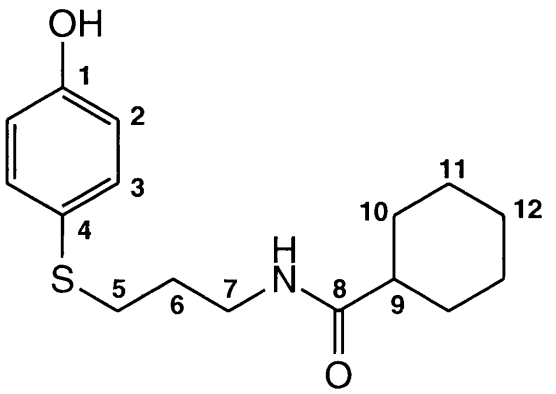
***N*-[3-[(4-Hydroxyphenyl)thio]propyl]ethanamide (150)**



A mixture of 4-hydroxythiophenol (1.92 g, 15.2 mmol) and 5,6-dihydro-2-methyl-4*H*-1,3-oxazine **147** (1.51 g, 15.2 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was purified by dry-column suction flash chromatography on silica gel eluting with hexane-EtOAc (1:1) then EtOAc to furnish *sulfide* **150** (2.17 g, 63%) as white crystals, m.p. 76-77 °C (from hexane-EtOAc); R_f 0.26 (EtOAc); ν_{max} (KBr) 1620 (amide I), 1597, 1584, 1569 (amide II), 1494, 1263, 1226 and 838 (*para*-disubstituted benzene) cm^{-1}; δ_H

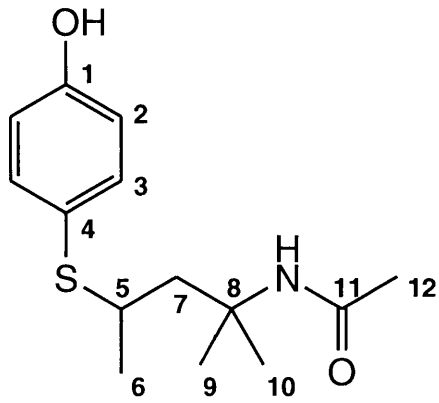
(D_6-DMSO) 9.64 (1 H, br s, OH), 7.93 (1 H, br m, NH), 7.26 (2 H, AA'BB', ^3J= 8.5, 3-H), 6.78 (2 H, AA'BB', ^3J= 8.5, 2-H), 3.14 (2 H, m, 7-H), 2.81 (2 H, t, ^3J=-7.5, 5-H), 1.83 (3 H, s, 9-H) and 1.63 (2 H, m, 6-H); δ_C (D_6-DMSO) 169.3 (C-8), 156.8 (C-1), 133.0 (C-3), 123.5 (C-4), 116.2 (C-2), 37.6 (C-7), 32.4 (C-5), 29.0 (C-6) and 22.7 (C-9); *m/z* 225 (M^+, 32), 125 (23), 100 (100), 79 (19), 58 (24) and 43 (24%); (Found: M^+, 225.0824; C, 58.74; H, 6.81; N, 6.14%. $C_{11}H_{15}NO_2S$ requires *M*, 225.0823; C, 58.64; H, 6.71; N, 6.22%).

_N_-[3-[(4-Hydroxyphenyl)thio]propyl]-1-cyclohexylmethanamide (151)



A mixture of 4-hydroxythiophenol (1.73 g, 13.7 mmol) and 2-cyclohexyl-5,6-dihydro-4*H*-1,3-oxazine **148** (2.29 g, 13.7 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was purified by flash chromatography on silica gel eluting with hexane-EtOAc (2:1) to furnish *sulfide* **151** (2.33 g, 58%) as white crystals, m.p. 84-85 °C (from hexane-EtOAc); R_f 0.58 (EtOAc); ν_{max} (KBr) 3321 (N-H), 1620 (amide I), 1597, 1578, 1546 (amide II), 1268, 1223 and 842 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.80 (1 H, br s, OH), 7.23 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.63 (2 H, AA'BB', ^3J= 8.6, 2-H), 6.15 (1 H, br t, ^3J= 5.6, NH), 3.32 (2 H, m, 7-H), 2.77 (2 H, t, ^3J=-7.0, 5-H), 2.07 (1 H, m, 9-H), 1.74 (6 H, m, 6-H and 10-H) and 1.50-1.10 (6 H, m, 11-H and 12-H); δ_C ($CDCl_3$) 177.5 (C-8), 156.6 (C-1), 133.7 (C-3), 124.4 (C-4), 116.2 (C-2), 45.5 (C-8), 38.5 (C-7), 33.6 (C-5), 29.6 (C-10), 28.9 (C-6) and 25.6 (C-11 and C-12); *m/z* 293 (M^+, 18), 168 (100), 125 (13), 83 (29) and 55 (15%); (Found: M^+, 293.1440; C, 65.29; H, 7.92; N, 4.66%. $C_{16}H_{23}NO_2S$ requires *M*, 293.1449; C, 65.50; H, 7.90; N, 4.47%).

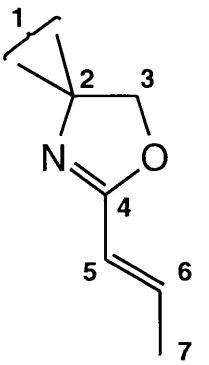
***N*-[3-[(4-Hydroxyphenyl)thio]-1,1-dimethylbutyl]ethanamide (152)**



A mixture of 4-hydroxythiophenol (1.72 g, 13.6 mmol) and 5,6-dihydro-2,4,4,6-tetramethyl-4*H*-1,3-oxazine (1.93 g, 13.6 mmol) was stirred under N_2 for 4 h at 130 °C. Upon cooling to r.t., the oily reaction mixture was triturated using a glass rod to give a white precipitate which was crystallised to afford *sulfide* **152** (2.72 g, 75%) as white crystals, m.p. 171-172 °C (from acetone); R_f 0.20 (1:1 EtOAc-hexane); ν_{max} (KBr) 3098 (N-H), 1628 (amide I), 1598, 1579, 1541 (amide II) and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 9.70 (1 H, br s, OH), 7.47 (1 H, br s, NH), 7.30 (2 H, AA'BB', ^3J= 8.4, 3-H), 6.80 (2 H, AA'BB', ^3J= 8.4, 2-H), 3.08 (1 H, m, 5-H), 2.07 (1 H, dd, ^2J=- 14.2, ^3J= 6.4, 7-H_a), 1.85 (4 H, m, 7-H_b and 12-H), 1.27 (3 H, s, 9-H), 1.25 (3 H, s, 10-H) and 1.18 (3 H, d, ^3J= 6.6, 6-H); δ_C (D_6-DMSO) 169.1 (C-11), 157.5 (C-1), 135.7 (C-3), 122.8 (C-4), 116.1 (C-2), 52.6 (C-8), 44.6 (C-7), 40.4 (C-5), 27.9 (C-9), 27.4 (C-10), 23.8 (C-12) and 22.8 (C-6); *m/z* 267 (M^+, 2.5), 142 (50.3), 125 (17.8), 100 (30.4), 83 (30.2) and 59 (100%); (Found: M^+, 267.1281; C, 62.95; H, 7.93; N, 5.16%. $C_{14}H_{21}NO_2S$ requires *M*, 267.1293; C, 62.89; H, 7.92; N, 5.24%).

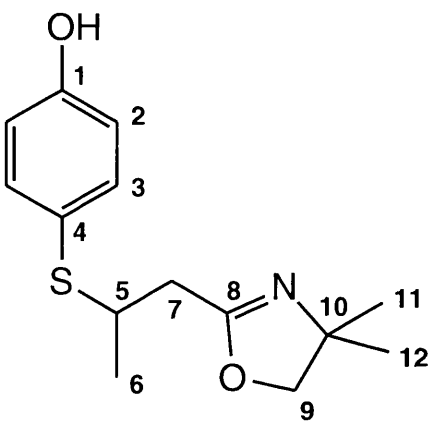
7.3 Experimental to Chapter 5

4,5-Dihydro-4,4-dimethyl-2-[(*E*)-1-propenyl]oxazole (154)



The title compound **154** was prepared in 26% yield on a 40 mmol scale using general procedure 2 (2 d) and gave b.p. 72-74 °C/25 mmHg, (lit.,[115] 62-65 °C/18 mmHg). The ^{1}H NMR and IR spectral data were in good agreement with those reported in the literature.[115]
New data: δ_C ($CDCl_3$) 161.2 (C-4), 138.6 (C-5), 119.1 (C-6), 78.4 (C-3), 66.7 (C-2), 28.1 (C-1) and 18.1; *m/z* 139 (M^+, 15.7), 124 (100), 96 (47.3), 68 (99.8) and 41 (90.6%).

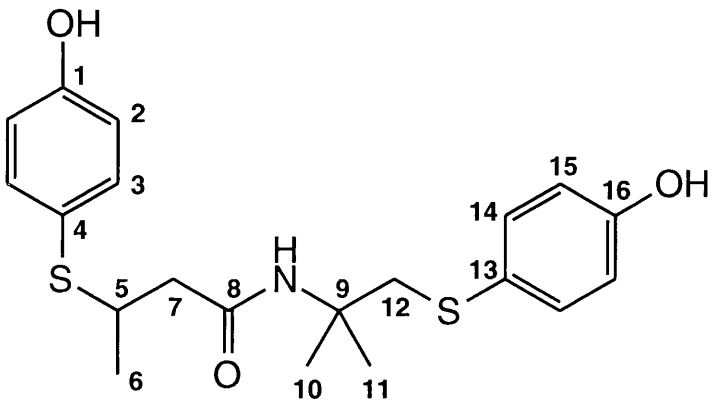
4,5-Dihydro-4,4-dimethyl-2-[2-(4-hydroxyphenyl)thio]propyloxazole (155)



A mixture of 4-hydroxythiophenol (0.85 g, 6.7 mmol) and oxazoline **154** (0.94 g, 6.7 mmol) was stirred under N_2 for 2 h at 120 °C. Upon cooling to r.t., the oily reaction mixture was subjected to flash chromatography on silica gel using EtOAc-light petroleum (1:1) as eluent to afford *sulfide* **155** (1.25 g, 70%) as a white powder, m.p. 115-116 °C (from EtOAc-hexane); R_f 0.44 (EtOAc); ν_{max} (KBr) 1654 (C=N), 1594, 1576, 1494 and 834 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 10.26 (1 H, br s, OH), 7.24 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.67 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.97 (2 H, m, 9-H), 3.32 (1 H, m, 5-H), 2.60 (1 H, dd, ^2J=

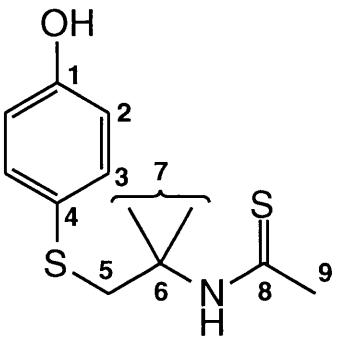
-14.7, ^3J= 6.1, 7-H$_a$), 2.34 (1 H, dd, ^2J= -14.7, ^3J= 8.8, 7-H$_b$), 1.31 (3 H, s, 11-H), 1.28 (3 H, s, 12-H) and 1.25 (3 H, d, ^3J= 7.0, 6-H); δ_C ($CDCl_3$) 166.0 (C-8), 157.9 (C-1), 136.8 (C-3), 120.8 (C-4), 116.1 (C-2), 79.4 (C-9), 66.7 (C-10), 40.9 (C-5), 35.4 (C-9), 28.2 (CH_3), 28.1 (CH_3) and 20.4 (C-6); *m/z* 265 (*M*$^+$, 19.1), 232 (21.1), 153 (19.2), 140 (47.2) and 113 (100%); (Found: *M*$^+$, 265.1129; C, 63.23; H, 7.12; N, 5.26%. $C_{14}H_{19}NO_2S$ requires *M*, 265.1136; C, 63.37; H, 7.22; N, 5.28%).

_N_-[1,1-Dimethyl-2-[(4-hydroxyphenyl)thio]ethyl]-3-[(4-hydroxyphenyl)thio]butanamide (157)



A mixture of 4-hydroxythiophenol (1.35 g, 10.7 mmol) and oxazoline **154** (0.745 g, 5.35 mmol) was stirred under N_2 for 6 h at 120 °C. Upon cooling to r.t., the oily reaction mixture was subjected to flash chromatography on silica gel using light petroleum-EtOAc (3:1) as eluent to afford *sulfide* **157** (1.38 g, 66%) as white crystals, m.p. 116-117 °C (from EtOAc-hexane); R_f 0.66 (EtOAc); ν_{max} (KBr) 3350 (N-H), 1650 (amide I), 1598, 1580, 1536 (amide II) and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.73 (2 H, br s, 1-OH and 16-OH), 7.30 (4 H, m, 3-H and 14-H), 7.11 (1 H, br s, N-H), 6.80 (4 H, m, 2-H and 15-H), 3.40 (2 H, m, 12-H), 2.39 (1 H, dd, ^2J= -14.1, ^3J= 5.5, 7-H$_a$), 2.13 (1 H, dd, ^2J= -14.1, ^3J= 9.2, 7-H$_b$), 1.37 (3 H, s, 10-H), 1.36 (3 H, s, 11-H) and 1.22 (3 H, d, ^3J= 6.7, 6-H); δ_C (D_6-acetone) 171.5 (C-8), 158.6 (aryl C-OH), 157.5 (aryl C-OH), 137.1 (aryl CH), 134.0 (aryl CH), 126.9 (aryl C-S), 123.4 (aryl C-S), 116.9 (aryl CH), 116.8 (aryl CH), 55.0 (C-9), 46.1 (C-12), 44.7 (C-7), 41.6 (C-5), 27.0 (C-10 and C-11) and 21.0 (C-6); *m/z* 391 (*M*$^+$, 5.5), 252 (7.1), 180 (78.1), 153 (23.7) and 12.5 (39.3%); (Found: *M*$^+$, 391.1261. $C_{14}H_{19}NO_2S$ requires *M*, 391.1276).

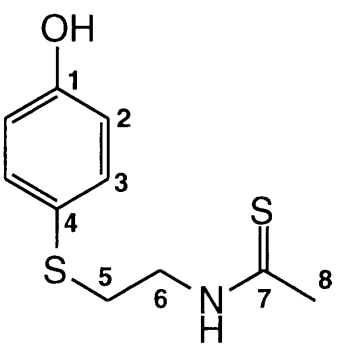
***N*-[2-[(4-Hydroxyphenyl)thio]-1,1-dimethylethyl]thioethanamide (164)**



CAUTION! All experiments involving the use of HMPA must be confined to a well-ventilated fume hood.

A solution of amide **92** (2.39 g, 10.0 mmol) and Lawesson Reagent (2.43 g, 6.01 mmol) in HMPA (10 cm^3) was stirred under N_2 for 2 h at 100 °C. Upon cooling to r.t., the mixture was poured into water (50 cm^3) and extracted using EtOAc (3 x 50 cm^3). The combined extracts were washed with brine (50 cm^3), concentrated *in vacuo* and the residue flash chromatographed on silica gel with hexane-EtOAc (7:3) as eluent to give *thioamide* **164** (0.79 g, 31%) as a white powder, m.p. 95-96 °C; R_f 0.65 (EtOAc); ν_{max} (KBr) 3290 (N-H), 1598, 1582, 1543 (amide II), 1494, 1269 (amide I) and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.28 (2 H, AA'BB', ^3J= 8.7, 3-H), 7.05 (1 H, br s, OH), 6.72 (2 H, AA'BB', ^3J= 8.7, 2-H), 5.87 (1 H, br s, NH), 3.50 (2 H, s, 5-H), 2.29 (3 H, s, 9-H) and 1.54 (6 H, s, 7-H); δ_C ($CDCl_3$) 200.4 (C-8), 155.5 (C-1), 133.9 (C-3), 125.9 (C-4), 116.3 (C-2), 59.0 (C-6), 44.4 (C-5), 26.7 (C-9) and 25.9 (C-7); *m/z* 255 (M^+, 10.3), 180 (100), 165 (12.6), 125 (31.0) and 59 (51.7%); (Found: M^+, 255.0755; C, 56.25; H, 6.92; N, 5.29%. $C_{12}H_{17}NOS_2$ requires *M*, 255.0752; C, 56.43; H, 6.71; N, 5.48%).

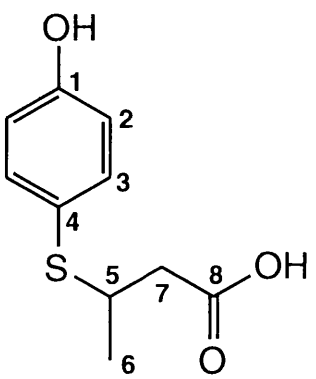
***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]thioethanamide (165)**



A solution of amide **47** (1.16 g, 5.49 mmol) and Lawesson Reagent (1.93 g, 2.80 mmol) in DME (30 cm^3) was stirred under N_2 for 18 h at r.t. The reaction

mixture was concentrated *in vacuo*, dissolved in EtOAc (50 cm^3) and washed with saturated aq. $NaHCO_3$ (30 cm^3), water (30 cm^3) and brine (30 cm^3). The organic portion was then dried ($MgSO_4$), and concentrated to give an oil which was purified by dry-column flash chromatography on silica gel eluting with hexane-EtOAc (1:1) to give *thioamide* **165** (0.720 g, 58%) as a white powder, m.p. 118-119 °C; R_f 0.63 (EtOAc); ν_{max} (KBr) 3286 (N-H), 1602, 1581, 1566 (amide II), 1492, 1224 (amide I) and 827 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$-D_4-methanol) 7.23 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.70 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.66 (2 H, t, ^3J= 6.5, 6-H), 2.96 (2 H, t, ^3J= 6.45, 5-H) and 2.39 (3 H, s, 8-H); δ_C ($CDCl_3$-D_4-methanol)) 200.9 (C-7), 156.6 (C-1), 134.1 (C-3), 123.1 (C-4), 116.1 (C-2), 45.1 (C-6), 33.4 (C-5) and 33.0 (C-8); *m/z* 227 (M^+, 36), 152 (100), 102 (71), 81 (8) and 59 (43%); (Found: M^+, 227.0435; C, 52.79; H, 5.94; N, 6.03%. $C_{10}H_{13}NOS_2$ requires *M*, 227.0439; C, 52.83; H, 5.76; N, 6.16%).

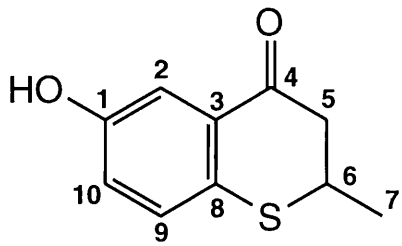
3-[(4-Hydroxyphenyl)thio]butanoic acid (168)



A stirred mixture of 4-hydroxythiophenol (1.76 g, 13.9 mmol), anhydrous K_2CO_3 (3.85 g, 27.9 mmol) and de-oxygenated 50% (v/v) aq. 2-propanol (20 cm^3) was cooled to 0 °C under N_2 before addition of ß-butyrolactone (1.19 g, 1.13 cm^3, 13.9 mmol) over 10 min. After stirring for 2 h at r.t., the reaction mixture was concentrated to *ca.* 10 cm^3 *in vacuo*, diluted with saturated aq. $NaHCO_3$ (150 cm^3) and washed with EtOAc (3 x 25 cm^3). The aq. portion was acidified to pH 1 with 5 mol dm^{-3} hydrochloric acid, extracted using EtOAc (3 x 70 cm^3), dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless oil which was crystallised to furnish *carboxylic acid* **168** (2.15 g, 73%) as white crystals, m.p. 92 °C (from $CHCl_3$); R_f 0.54 (5:1 EtOAc-MeOH); ν_{max} (KBr) 3360 (O-H), 1714 (C=O), 1600, 1586, 1494 and 836 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.47 (1 H, br s, 1-OH), 7.20 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.69 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.22 (1 H, m, 5-H), 2.42 (1 H, m, 7-H_a), 2.22 (1 H, m, 7-H_b) and 1.08 (3 H, m, 6-H); δ_C (D_6-acetone) 172.7 (C-8), 158.7 (C-1), 137.5 (C-3), 123.0 (C-4), 116.8 (C-2), 41.8 (C-7), 41.1 (C-5) and 21.1 (C-6); *m/z* 212

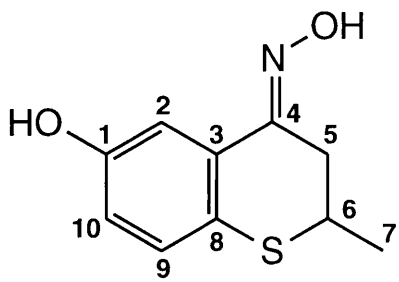
(M^+, 29.3), 153 (12.2), 126 (100) and 97 (68.3%); (Found: M^+, 212.0510; C, 56.34; H, 5.97%. $C_{10}H_{12}O_3S$ requires *M*, 212.0507; C, 56.32; H, 6.14%).

2,3-Dihydro-6-hydroxy-2-methyl-(4*H*)-1-benzothiopyran-4-one (169)



A solution of carboxylic acid **168** (1.50 g, 7.06 mmol) in conc. aq. sulfuric acid (25 cm^3) was stirred at r.t. for 0.5 h whereupon the reaction mixture was poured onto ice (200 g). The precipitated product was collected by filtration, dissolved in EtOAc (20 cm^3) and filtered through a short column of silica gel. Concentration of the filtrate *in vacuo* afforded *title compound* **169** (0.87 g, 64%) as a yellow powder, m.p. 133-134 °C; R_f 0.62 (EtOAc); ν_{max} (KBr) 3394 (O-H), 1662 (C=O), 1596, 1474, 1430 and 1214 cm^{-1}; δ_H (D_6-acetone) 8.67 (1 H, br s, OH), 7.51 (1 H, d, ^4J= 2.7, 2-H), 7.15 (1 H, d, ^3J= 8.5, 9-H), 7.01 (1 H, dd, ^3J= 8.5, ^4J= 2.7, 10-H), 3.63 (1 H, m, 6-H), 2.97 (1 H, dd, ^2J= -16.6, ^3J= 3.1, 5-H_a), 2.65 (1 H, dd, ^2J= -16.6, ^3J= 11.4, 5-H_b) and 1.37 (3 H, d, ^3J= 6.8, 7-H); δ_C (D_6-acetone) 194.6 (C-4), 155.9 (C-1), 129.7 (C-9), 122.8 (C-10), 114.8 (C-2), 41.6 (C-5), 37.2 (C-6) and 20.6 (C-7); *m/z* 194 (M^+, 58.2), 179 (13.1), 152 (100), 124 (47.4) and 95 (11.5%); (Found: M^+, 194.0386; C, 61.88; H, 5.22%. $C_{10}H_{10}O_2S$ requires *M*, 194.0401; C, 61.83; H, 5.19%).

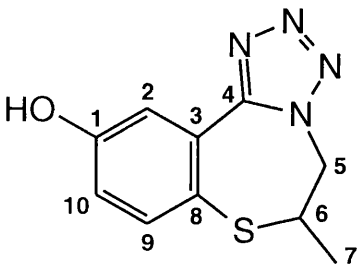
(*E*)-Oxime of 2,3-Dihydro-6-hydroxy-2-methyl-(4*H*)-1-benzothiopyran-4-one (170)



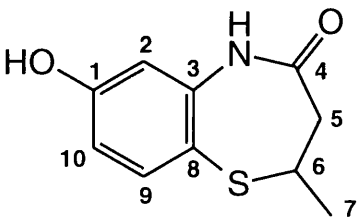
A mixture of **169** (1.49 g, 7.67 mmol), sodium acetate (6.0 g, 82 mmol) and hydroxylamine hydrochloride (3.0 g, 43 mmol) was heated in refluxing EtOH (30 cm^3) for 3 h. On cooling to r.t., the reaction mixture was concentrated *in vacuo,* diluted with water (50 cm^3) and extracted using CH_2Cl_2 (3 x 50 cm^3). The combined organic extracts were washed with water (50cm^3), dried (Na_2SO_4)

and evaporated *in vacuo* to afford *oxime* **170** (1.27 g, 79%) as tan crystals, m.p. 137 °C (from EtOH); R_f 0.60 (EtOAc); ν_{max} (KBr) 1598, 1566, 1470, 1438, 1316 and 1224 cm^{-1}; δ_H (D_6-acetone) 10.49 (1 H, br s, N-OH), 8.33 (1 H, br s, 1-OH), 7.48 (1 H, d, ^4J= 2.6, 2-H), 7.04 (1 H, d, ^3J= 8.5, 9-H), 6.78 (1 H, dd, ^3J= 8.5, ^4J= 2.6, 10-H), 3.37 (1 H, dd, ^2J= -17.3, ^3J= 3.5, 5-H_a), 3.25 (1 H, m, 6-H), 2.53 (1 H, dd, ^2J= -17.3, ^3J= 10.7, 5-H_b) and 1.35 (3 H, d, ^3J= 6.6, 7-H); δ_C (D_6-acetone) 155.8 (C-4), 153.0 (C-1), 132.2 (C-3), 129.9 (C-9), 126.0 (C-8), 118.0 (C-10), 112.6 (C-2), 36.2 (C-6), 34.9 (C-5) and 20.7 (C-7); *m/z* 209 (M^+, 100), 192 (41.3), 176 (13.7), 165 (12.4) and 150 (39.2%); (Found: M^+, 209.0495; C, 57.26; H, 5.31; N, 6.61%. $C_{10}H_{11}NO_2S$ requires *M*, 209.0510; C, 57.40; H, 5.30; N, 6.69%).

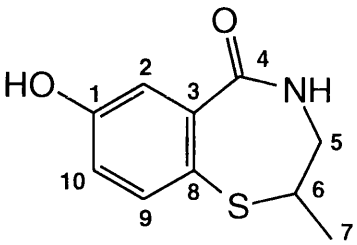
Schmidt Reaction of Thiochroman-4-one 169



177



167



166

Azidotrimethylsilane (1.21 g, 1.40 cm^3, 10.50 mmol) was added dropwise to a stirred solution of thiochroman-4-one **169** (1.36 g, 7.00 mmol) in trifluoroacetic acid (15 cm^3) under N_2 at r.t. After stirring for 3 d, the reaction mixture was concentrated *in vacuo*, taken into EtOAc (400 cm^3) and washed with saturated aq. $NaHCO_3$ (400 cm^3) and 1 mol dm^{-3} hydrochloric acid (100 cm^3) before extraction using cold 2 mol dm^{-3} aq. NaOH (100 cm^3). The aqueous layer was acidified to pH 1 using iced 2 mol dm^{-3} hydrochloric acid and extracted with EtOAc (2 x 100 cm^3). The combined organic extracts were washed with brine (50 cm^3) and evaporated to give a pale purple oil which was purified by flash chromatography on silica gel, eluting with hexane-EtOAc (3:1 to 1:1) to afford the following three *compounds*.

5,6-Dihydro-10-hydroxy-6-methyltetrazolo[1,5-*d*][1,4]benzothiazepine (177) (210 mg, 13%) as pale pink crystals, m.p. 179-180 °C; R_f 0.3 (1:1 EtOAc-hexane); ν_{max} (KBr) 1595, 1479, 1423, 1400, 1291 and 1232 cm^{-1}; δ_H (D_6-DMSO) 10.38 (1 H, br s, OH), 7.56 (1 H, d, ^3J= 8.5, 9-H), 7.34 (1 H, d, ^4J= 2.7, 2-H), 7.02 (1 H, dd, ^3J= 8.5, ^4J= 2.7, 10-H), 4.83 (1 H, dd, ^2J= -14.5, ^3J= 5.4, 5-H_a), 4.38 (1 H, dd, ^2J= -14.5, ^3J= 7.6, 5-H_b), 4.01 (1 H, m, 6-H) and 1.36 (3 H, d, ^3J= 6.6, 7-H); δ_C (D_6-DMSO) 158.4 (C-1), 154.4 (C-4), 136.4 (C-9), 130.0 (C-3),

120.5 (C-8), 119.1 (aryl CH), 117.3 (aryl CH), 52.3 (C-5), 46.3 (C-6) and 20.0 (C-7); *m/z* 234 (M^+, 100), 191 (54.6), 164 (54.7), 150 (39.0), 123 (12.1), 106 (13.2) and 56 (50.4%); (Found: M^+, 234.0567; C, 51.31; H, 4.18; N, 23.94%. $C_{10}H_{10}N_4OS$ requires *M*, 234.0575; C, 51.27; H, 4.30; N, 23.91%).

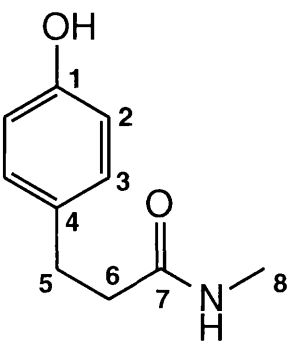
2,3-Dihydro-7-hydroxy-2-methyl-1,5-benzothiazepin-4(5*H*)-one (167)

(139 mg, 9%) as white crystals, m.p. 197 °C (dec.); R_f 0.45 (EtOAc); ν_{max} (KBr) 3100, 1653 (amide I), 1605, 1576, 1500, 1472 and 1305 cm^{-1}; δ_H (D_6-DMSO) 7.35 (1 H, d, ^3J= 8.1, 9-H), 6.64-6.56 (2 H, m, 2-H and 10-H), 3.75 (1 H, m, 6-H), 2.50 (1 H, dd, ^2J= -12.3, ^3J= 6.0, 5-H_a), 2.15 (1 H, dd, ^2J= -12.3, ^3J= 8.5, 5-H_b) and 1.28 (3 H, d, ^3J= 6.6, 7-H); δ_C (D_6-DMSO) 179.4 (C-4), 158.9 (C-1), 143.8 (C-3), 136.5 (C-9), 114.4 (C-8), 112.9 (aryl CH), 110.0 (aryl CH), 44.7 (C-6), 41.6 (C-5) and 23.2 (C-7); *m/z* 209 (M^+, 46.7), 166 (11.5), 141 (16.4) and 70 (100%); (Found: M^+, 209.0509; C, 57.36; H, 5.27; N, 6.61%. $C_{10}H_{11}NO_2S$ requires *M*, 209.0510; C, 57.40; H, 5.30; N, 6.69%).

2,3-Dihydro-7-hydroxy-2-methyl-1,4-benzothiazepin-5(4*H*)-one (166)

(142 mg, 10%) as white crystals, m.p. 193 °C (dec.); R_f 0.31 (EtOAc); ν_{max} (KBr) 3200, 1647 (amide I), 1563, 1460, 1410, 1294 and 1221 cm^{-1}; δ_H (D_6-DMSO) 10.00 (1 H, br s, OH), 8.42 (1 H, br m, NH), 7.28 (1 H, d, ^3J= 8.3, 9-H), 6.96 (1 H, d, ^4J= 2.7, 2-H), 6.84 (1 H, dd, ^3J= 8.3, ^4J= 2.7, 10-H), 3.47 (1 H, m, 6-H), 3.17 (1 H, m, 5-H_a), 2.73 (1 H, m, 5-H_b) and 1.17 (3 H, d, ^3J= 6.6, 7-H); δ_C (D_6-DMSO) 170.6 (C-4), 158.3 (C-1), 142.4 (C-8), 135.6 (C-9), 117.8 (aryl CH), 117.6 (C-3), 116.1 (aryl CH), 46.3 (C-6), 45.9 (C-5) and 19.5 (C-7); *m/z* 209 (M^+, 100), 180 (73.2), 165 (50.2), 151 (28.5), 137 (17.6) and 124 (18.9%); (Found: M^+, 209.0510; C, 57.36; H, 5.19; N, 6.55%. $C_{10}H_{11}NO_2S$ requires *M*, 209.0510; C, 57.40; H, 5.30; N, 6.69%).

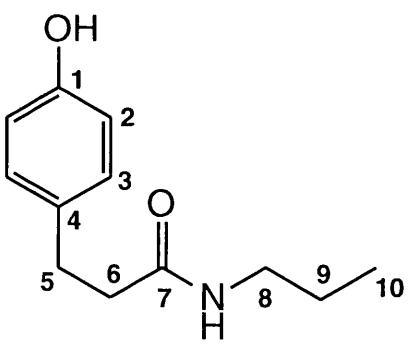
***N*-Methyl-3-(4-hydroxyphenyl)propanamide (184)**



DCC (1.96 g, 9.50 mmol) was added, with stirring, to a cooled (0 °C) solution of 3-(4-hydroxyphenyl)propanoic acid (1.75 g, 10.5 mmol) and 4-nitrophenol (1.32

g, 9.50 mmol) in EtOAc (40 cm^3). After stirring for 1 h at 0 °C, the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added 8.03 mol dm^{-3} ethanolic methylamine (3.50 cm^3, 28.1 mmol) and DBU (1.45 g, 1.42 cm^3, 9.52 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (40 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 30 cm^3), saturated aq. sodium bicarbonate solution (30 cm^3) and brine (30 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil which was subjected to flash chromatography on silica gel using EtOAc then EtOAc-MeOH (19:1) as eluent. Crystallisation of the resulting colourless oil afforded *amide* **184** (0.83 g, 49%) as white crystals, m.p. 99-100 °C (from hexane-EtOAc); R_f 0.25 (EtOAc); ν_{max} (KBr) 3364 (N-H), 1652 (amide I), 1612, 1595, 1560 (amide II), 1516, 1226 and 832 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$+D_6-DMSO) 8.57 (1 H, br s, OH), 6.82 (2 H, AA'BB', ^3J= 8.5, 3-H), 6.57 (2 H, AA'BB', ^3J= 8.5, 2-H), 6.50 (1 H, br s, NH), 2.67 (2 H, t, ^3J= 8.4, 5-H), 2.54 (3 H, d, ^3J= 4.7, 8-H) and 2.23 (2 H, t, ^3J= 8.4, 6-H); δ_C ($CDCl_3$+D_6-DMSO) 173.3 (C-7), 155.3 (C-1), 131.6 (C-4), 129.1 (C-3), 115.3 (C-2), 38.5 (C-5), 30.9 (C-6) and 26.0 (C-8); *m/z* 179 (M^+, 97), 147 (6), 120 (62), 107 (100). 91 (11) and 77 (17%); (Found: M^+, 179.0950; C, 67.02; H, 7.36; N, 7.91%. $C_{10}H_{13}NO_2$ requires *M*, 179.0946; C, 67.02; H, 7.31; N, 7.82%).

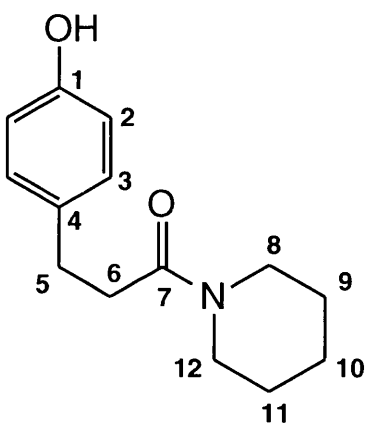
_N_-Propyl-3-(4-hydroxyphenyl)propanamide (185)



DCC (2.06 g, 9.98 mmol) was added, with stirring, to a cooled (0 °C) solution of 3-(4-hydroxyphenyl)propanoic acid (1.87 g, 11.3 mmol) and 4-nitrophenol (1.39 g, 9.99 mmol) in EtOAc (50 cm^3). After stirring for 1 h at 0 °C, the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added propylamine (1.18 g, 1.66 cm^3, 20.0 mmol) and DBU (1.52 g, 1.49 cm^3, 9.98 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (100 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 50 cm^3), saturated aq. sodium bicarbonate solution (50 cm^3) and brine (50 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil which was subjected to flash chromatography on silica gel using

EtOAc-hexane (2:1) as eluent. Crystallisation of the resulting colourless oil afforded *amide* **185** (0.81 g, 43%) as white crystals, m.p. 71-72 °C (from hexane-EtOAc); R_f 0.44 (EtOAc); ν_{max} (KBr) 3302 (N-H), 1642 (amide I), 1613, 1595, 1566 (amide II), 1514, 1458, 1246 and 829 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$+D_6-DMSO) 8.69 (1 H, br s, OH), 6.89 (2 H, AA'BB', ^3J= 8.3, 3-H), 6.77 (2 H, AA'BB', ^3J= 8.3, 2-H), 6.30 (1 H, br m, NH), 3.04 (2 H, m, 8-H), 2.75 (2 H, t, ^3J= 7.9, 5-H), 2.33 (2 H, t, ^3J= 7.9, 6-H), 1.35 (2 H, m, 9-H) and 0.74 (3 H, t, ^3J= 7.3, 10-H); δ_C ($CDCl_3$+D_6-DMSO) 173.0 (C-7), 155.3 (C-1), 131.4 (C-4), 129.1 (C-3), 115.4 (C-2), 41.2 (C-8), 39.7 (C-5), 31.0 (C-6), 22.6 (C-9) and 11.3 (C-10); *m/z* 207 (M^+, 95), 149 (10), 120 (63), 107 (100). 77 (14) and 60 (17%); (Found: M^+, 207.1263; C, 69.34; H, 8.11; N, 6.67%. $C_{12}H_{17}NO_2$ requires *M*, 207.1259; C, 69.54; H, 8.27; N, 6.76%).

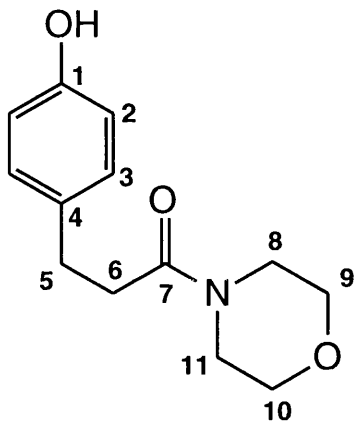
_N_-[3-(4-Hydroxyphenyl)propanoyl]piperidine (186)



DCC (2.09 g, 10.1 mmol) was added, with stirring, to a solution of 3-(4-hydroxyphenyl)propanoic acid (1.85 g, 11.1 mmol) and 4-nitrophenol (1.41 g, 10.1 mmol) in EtOAc (60 cm^3). After stirring for 1 h at r.t., the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added piperidine (1.72 g, 20.2 mmol) and DBU (1.54 g, 1.51 cm^3, 10.1 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (40 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 30 cm^3), saturated aq. sodium bicarbonate solution (30 cm^3) and brine (30 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil which was subjected to flash chromatography on silica gel using EtOAc then EtOAc-MeOH (15:1) as eluent. Crystallisation of the resulting colourless oil afforded *amide* **186** (1.52 g, 65%) as white crystals, m.p. 117-118 °C (from hexane-$CHCl_3$); R_f 0.45 (EtOAc); ν_{max} (KBr) 1603 (C=O), 1515, 1264, 1244, 1096 and 828 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.27 (1 H, br s, OH), 6.99 (2 H, AA'BB', ^3J= 8.4, 3-H), 6.78 (2 H, AA'BB', ^3J= 8.4, 2-H), 3.54 (2 H, m, 8-H), 3.30 (2 H, m,

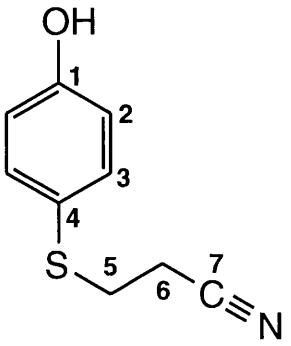
12-H), 2.84 (2 H, m, 5-H), 2.58 (2 H, m, 6-H) and 1.68-1.22 (6 H, m, 9-H, 10-H and 11-H); δ_C ($CDCl_3$) 171.4 (C-7), 155.2 (C-1), 131.8 (C-4), 129.3 (C-3), 115.5 (C-2), 46.7 (C-12), 43.0 (C-5), 35.5 (C-8), 30.9 (C-6), 26.3 (CH_2), 25.5 (CH_2) and 24.3 (C-10); *m/z* 233 (M^+, 78), 126 (100), 120 (29), 107 (44). 84 (34) and 77 (12%); (Found: M^+, 233.1409; C, 71.97; H, 8.07; N, 5.90%. $C_{14}H_{19}NO_2$ requires *M*, 233.1416; C, 72.07; H, 8.21; N, 6.00%).

_N_-[3-(4-Hydroxyphenyl)propanoyl]morpholine (187)



DCC (2.27 g, 11.0 mmol) was added, with stirring, to a cooled (0 °C) solution of 3-(4-hydroxyphenyl)propanoic acid (12.0 g, 12.3 mmol) and 4-nitrophenol (1.53 g, 11.0 mmol) in EtOAc (40 cm^3). After stirring for 1 h at 0 °C, the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added morpholine (2.87 g, 2.87 cm^3, 33.0 mmol) and DBU (1.67 g, 1.65 cm^3, 11.0 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (40 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 30 cm^3), saturated aq. sodium bicarbonate solution (30 cm^3) and brine (30 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil which was subjected to flash chromatography on silica gel using EtOAc as eluant. Crystallisation of the resulting colourless oil afforded *amide* **187** (1.02 g, 39%) as white crystals, m.p. 132-133 °C (from hexane-$CHCl_3$); R_f 0.34 (EtOAc); ν_{max} (KBr) 1607 (C=O), 1512, 1262, 1243, 1113 and 827 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.02 (1 H, br s, OH), 7.00 (2 H, AA'BB', ^3J= 7.9, 3-H), 6.77 (2 H, AA'BB', ^3J= 7.9, 2-H), 3.59 (4 H, br s, 9-H and 10-H), 3.45 (2 H, m, 8-H), 3.32 (2 H, m, 11-H), 2.86 (2 H, t, ^3J= 7.1, 5-H) and 2.57 (2 H, t, ^3J= 7.1, 6-H); δ_C ($CDCl_3$) 171.6 (C-7), 155.2 (C-1), 131.6 (C-4), 129.4 (C-3), 115.5 (C-2), 66.7 (CH_2), 66.3 (CH_2), 46.1 (C-11), 42.1 (C-5), 35.0 (C-8) and 30.8 (C-6); *m/z* 235 (M^+, 100), 128 (24), 120 (56), 107 (71). 88 (25) and 57 (22%); (Found: M^+, 235.1201; C, 66.14; H, 7.19; N, 5.77%. $C_{13}H_{17}NO_3$ requires *M*, 235.1208; C, 66.36; H, 7.28; N, 5.95%).

3-[(4-Hydroxyphenyl)thio]propanonitrile (188)

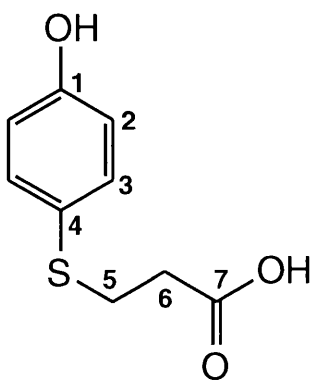


The procedure of Ito and co-workers[179] was adapted as follows.

A mixture of 4-hydroxythiophenol (2.05 g, 16.2 mmol), anhydrous sodium bicarbonate (1.36 g, 16.2 mmol) and acrylonitrile (1.03 g, 1.28 cm^3, 19.4 mmol) was stirred under N_2 in refluxing ethanol (4 cm^3) for 6 h. Upon cooling to r.t., the mixture was diluted with water (100 cm^3) and extracted using chloroform (2 x 100 cm^3). The combined extracts were washed with brine (50 cm^3), dried ($MgSO_4$) and concentrated *in vacuo* to give a white solid. Purification by flash chromatography on silica gel [hexane-EtOAc (1:1)] gave, after crystallisation, the title compound **188** (1.96 g, 68%) as white crystals, m.p. 70-71 °C (from benzene-hexane), (lit., 72-73 °C).[179]

New data: R_f 0.88 (EtOAc); ν_{max} (KBr) 2266 (CN), 1598, 1580, 1493, 1272, 1222 and 830 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.33 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.80 (2 H, AA'BB', ^3J= 8.6, 2-H), 6.39 (1 H, br s, OH), 2.96 (2 H, t, ^3J= 7.3, 5-H) and 2.54 (2 H, t, ^3J= 7.3, 6-H); δ_C ($CDCl_3$) 156.5 (C-1), 135.4 (C-3), 122.7 (C-4), 118.3 (C-7), 116.6 (C-2), 31.7 (C-5) and 18.2 (C-6); *m/z* 179 (M^+, 100), 139 (97), 125 (36) 95 (14) and 81 (8%); (Found: M^+, 179.0408; C, 60.38; H, 5.06; N, 7.68%. C_9H_9NOS requires *M*, 179.0405; C, 60.31; H, 5.06; N, 7.81%).

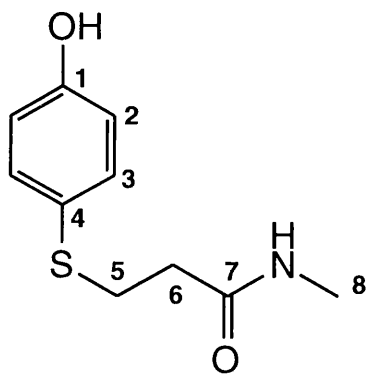
3-[(4-Hydroxyphenyl)thio]propanoic acid (180)



A mixture of 4-hydroxythiophenol (6.85 g, 54.3 mmol) and acrylic acid (3.91 g, 54.3 mmol) was stirred at 100 °C under N_2 for 12 h. On cooling to r.t., the reaction mixture precipitated to give a white plug of solid which was taken into acetone (50 cm^3) and loaded onto silica gel by evaporation of the solution *in vacuo*. Flash chromatography on silica gel, eluting with hexane-diethyl ether afforded the title compound **180** (5.72 g, 53%), m.p. 117-119 °C (from hexane-diethyl ether).

New data: R_f 0.31 (EtOAc); ν_{max} (KBr) 1715, 1694 (C=O), 1598, 1587, 1494, 1426, 1241 and 818 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 7.32 (2 H, AA'BB', ^3J= 6.8, 3-H), 6.84 (2 H, AA'BB', ^3J= 6.8, 2-H), 3.03 (2 H, t, ^3J= 7.2, 5-H) and 2.56 (2 H, t, ^3J= 7.2, 6-H); δ_C (D_6-acetone) 173.3 (C-7), 158.1 (C-1), 135.0 (C-3), 117.0 (C-2), 34.6 (C-6) and 31.7 (C-5); *m/z* 198 (M^+, 100), 139 (47), 125 (42) 97 (15) and 45 (21%); (Found: M^+, 198.0353; C, 54.53; H, 5.19%. $C_9H_{10}O_3S$ requires *M*, 198.0351; C, 54.53; H, 5.08%).

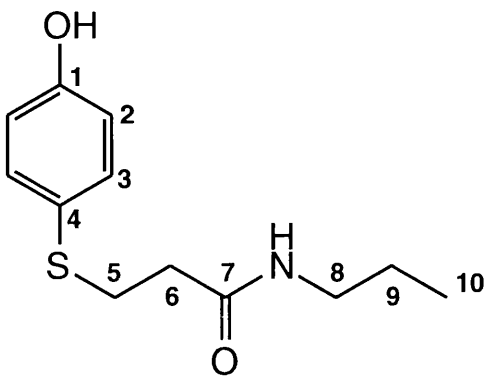
_N_-Methyl-3-[(4-hydroxyphenyl)thio]propanamide (179)



DCC (1.79 g, 8.68 mmol) was added, with stirring, to a solution of 3-[(4-hydroxyphenyl)thio]propanoic acid (1.72 g, 8.68 mmol) and 4-nitrophenol (1.32 g, 9.49 mmol) in EtOAc (50 cm^3). After stirring for 1 h at r.t., the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added 8.03 mol dm^{-3} ethanolic methylamine (2.16 cm^3, 17.36 mmol) and DBU (1.32 g, 1.30 cm^3, 8.68 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (70 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 30 cm^3), saturated aq. sodium bicarbonate solution (30 cm^3) and brine (30 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow solid which was dry-column flash chromatographed on silica gel [hexane:EtOAc (1:1) then EtOAc] to furnish *amide* **179** (1.12 g, 61%) as white crystals, m.p. 86-87 °C (from $CHCl_3$-MeOH); R_f 0.27 (EtOAc); ν_{max} (KBr) 3384 (N-H), 1644 (amide I), 1601, 1587, 1570 (amide II), 1496, 1254 and 820 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 9.82 (1 H, br s, OH), 7.28 (3 H, m, 3-H and N-H), 6.81 (2 H, AA'BB', ^3J= 7.9, 2-H), 3.04 (2 H, t, ^3J= 7.2, 5-H), 2.70

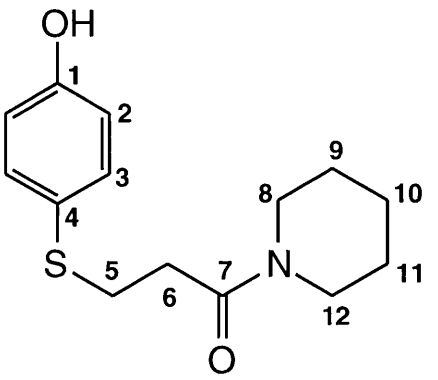
(3 H, d, ^3J= 4.7, 8-H) and 2.42 (2 H, t, ^3J= 7.2, 6-H); δ_C (D_6-acetone) 172.4 (C-7), 158.1 (C-1), 134.6 (C-3), 125.0 (C-4), 117.0 (C-2), 36.7 (C-6), 32.3 (C-5) and 26.2 (C-8); *m/z* 211 (*M*$^+$, 100), 152 (11), 139 (13), 125 (14) and 86 (54%); (Found: *M*$^+$, 211.0666; C, 56.64; H, 6.17; N, 6.42%. $C_{10}H_{13}SNO_2$ requires *M*, 211.0667; C, 56.85; H, 6.20; N, 6.63%).

_N_-Propyl-3-[(4-hydroxyphenyl)thio]propanamide (189)



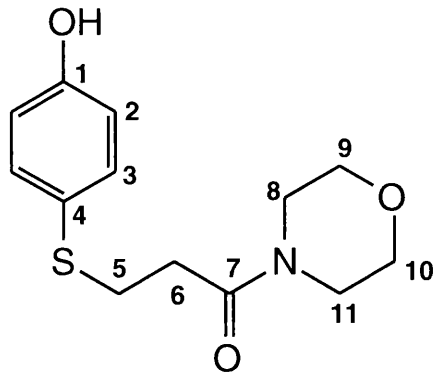
DCC (1.24 g, 6.00 mmol) was added, with stirring, to a solution of 3-[(4-hydroxyphenyl)thio]propanoic acid (1.16 g, 5.85 mmol) and 4-nitrophenol (0.85 g, 6.14 mmol) in EtOAc (50 cm^3). After stirring for 1 h at r.t., the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added propylamine (0.60 g, 0.97 cm^3, 11.7 mmol) and DBU (0.89 g, 0.87 cm^3, 5.85 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (50 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 20 cm^3), saturated aq. sodium bicarbonate solution (20 cm^3) and brine (20 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow solid which was flash chromatographed on silica gel [hexane:EtOAc (1:1)] to furnish *amide* **189** (0.84 g, 60%) as white crystals, m.p. 95-96 °C (from hexane-EtOAc); R_f 0.42 (EtOAc); ν_{max} (KBr) 3382 (N-H), 1639 (amide I), 1604, 1590, 1558 (amide II), 1496, 1434, 1255 and 820 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 9.91 (1 H, br s, OH), 7.38 (1 H, br s, NH), 7.27 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.81 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.14 (2 H, m, 8-H), 3.04 (2 H, t, ^3J= 7.5, 5-H), 2.42 (2 H, t, ^3J= 7.5, 6-H), 1.51 (2 H, m, 9-H) and 0.88 (3 H, t, ^3J= 7.4, 10-H); δ_C (D_6-acetone) 171.7 (C-7), 158.1 (C-1), 134.6 (C-3), 124.9 (C-4), 117.0 (C-3), 41.7 (C-8), 36.8 (C-6), 32.4 (C-5), 23.5 (C-9) and 11.7 (C-10); *m/z* 239 (*M*$^+$, 100), 152 (14), 125 (24), 114 (68) and 97 (7%); (Found: *M*$^+$, 239.0989; C, 60.17; H, 7.18; N, 5.89%. $C_{12}H_{17}SNO_2$ requires *M*, 239.0980; C, 60.22; H, 7.16; N, 5.85%).

***N*-[3-[(4-hydroxyphenyl)thio]propanoyl]piperidine (190)**



DCC (1.12 g, 5.45 mmol) was added, with stirring, to a solution of 3-[(4-hydroxyphenyl)thio]propanoic acid (1.08 g, 5.45 mmol) and 4-nitrophenol (0.86 g, 6.2 mmol) in EtOAc (40 cm^3). After stirring for 1 h at r.t., the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added piperidine (0.928 g, 1.08 cm^3, 10.9 mmol) and DBU (0.83 g, 0.82 cm^3, 5.5 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (50 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 25 cm^3), saturated aq. sodium bicarbonate solution (25 cm^3) and brine (25 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow solid which was flash chromatographed on silica gel [hexane-EtOAc (1:1) then EtOAc] to furnish *amide* **190** (0.94 g, 65%) as white crystals, m.p. 101-102 °C (from hexane-$CHCl_3$); R_f 0.47 (EtOAc); ν_{max} (KBr) 1620 (C=O), 1601, 1583, 1497, 1444, 1264, 1219 and 829 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.32 (1 H, br s, OH), 7.00 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.57 (2 H, AA'BB', ^3J= 8.4, 2-H), 3.30 (2 H, m, 12-H), 3.08 (2 H, m, 8-H), 2.86 (2 H, t, ^3J= 7.6, 5-H), 2.37 (2 H, t, ^3J= 7.6, 6-H) and 1.31 (6 H, m, 9-H, 10-H and 11-H); δ_C ($CDCl_3$) 170.1 (C-7), 156.6 (C-1), 133.5 (C-3), 124.1 (C-4), 116.3 (C-2), 46.8 (C-8), 43.1 (C-12), 33.2 (C-6), 31.5 (C-5), 26.3 (CH_2), 25.4 (CH_2) and 24.2 (CH_2); *m/z* 265 (M^+, 72), 152 (11), 140 (100), 125 (17) and 112 (22%); (Found: M^+, 265.1135; C, 63.27; H, 7.12; N, 5.22%. $C_{14}H_{19}SNO_2$ requires *M*, 265.1136; C, 63.37; H, 7.22; N, 5.28%).

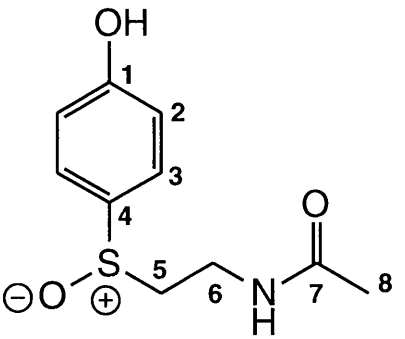
***N*-[3-[(4-hydroxyphenyl)thio]propanoyl]morpholine (191)**



DCC (1.56 g, 7.57 mmol) was added, with stirring, to a solution of 3-[(4-hydroxyphenyl)thio]propanoic acid (1.50 g, 7.57 mmol) and 4-nitrophenol (1.16 g, 8.32 mmol) in EtOAc (50 cm^3). After stirring for 1 h at r.t., the pale yellow reaction mixture was filtered through Celite® and to the filtrate was added morpholine (1.65 g, 1.65 cm^3, 18.9 mmol) and DBU (1.15 g, 1.13 cm^3, 7.57 mmol). Stirring for 14 h at r.t. gave a bright yellow solution which was diluted with EtOAc (70 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 30 cm^3), saturated aq. sodium bicarbonate solution (30 cm^3) and brine (30 cm^3). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow solid which was dry-column flash chromatographed on silica gel [EtOAc then EtOAc-MeOH (9:1)] to furnish *amide* **191** (0.96 g, 47%) as white crystals, m.p. 137-138 °C (from EtOAc); R_f 0.30 (EtOAc); ν_{max} (KBr) 1625 (C=O), 1601, 1583, 1497, 1428, 1267, 1219, 1114 (ether C-O), 1030 and 823 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$+D_4-methanol) 7.18 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.69 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.55 (6 H, m, 9-H, 10-H and 11-H), 3.28 (2 H, m, 8-H), 2.98 (2 H, t, ^3J= 7.6, 5-H) and 2.48 (2 H, t, ^3J= 7.6, 6-H); δ_C ($CDCl_3$+D_4-methanol) 170.4 (C-7), 156.6 (C-1), 133.7 (C-3), 123.7 (C-4), 116.0 (C-2), 66.5 (CH_2), 66.3 (CH_2), 45.7 (C-8), 41.7 (C-11), 32.7 (C-6) and 31.2 (C-5); *m/z* 267 [$(MH)^+$, 100], 152 (17), 142 (97), 125 (25) and 114 (24%); (Found: M^+, 267.0922; C, 58.35; H, 6.52; N, 5.03%. $C_{13}H_{17}SNO_3$ requires *M*, 267.0929; C, 58.41; H, 6.41; N, 5.24%).

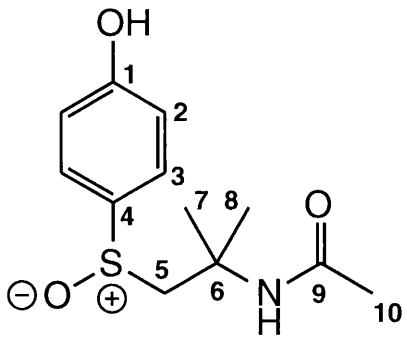
7.4 Experimental to Chapter 6

N-[2-[(4-Hydroxyphenyl)sulfinyl]ethyl]ethanamide (192)



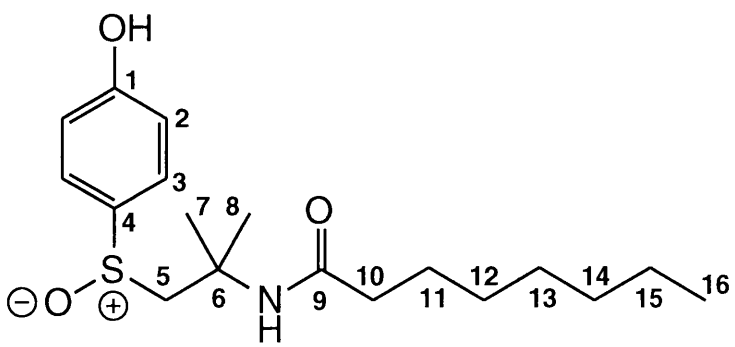
A solution of $NaIO_4$ (1.67 g, 7.81 mmol) in water (15 cm^3) was added over 5 min to a stirred, cooled (0 °C), solution of sulfide **47** (1.50 g, 7.10 mmol) in EtOH (100 cm^3). Allowing the reaction mixture to warm to r.t., stirring was continued for a further 15 h. Removal of precipitated sodium iodate by filtration through Celite® before concentration *in vacuo* gave a yellow solid which was purified by flash chromatography on silica gel eluting with EtOAc-methanol (6:1). Crystallisation afforded *sulfoxide* **192** (1.12 g, 69%) as pale yellow crystals, m.p. 137-138 °C (from EtOAc-MeOH); R_f 0.41 (5:1 EtOAc-MeOH); ν_{max} (KBr) 1657 (amide I), 1563 (amide II), 1497, 1278, 1024 (S=O) and 828 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$+D_4-methanol) 7.48 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.95 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.43 (2 H, m, 6-H), 2.89 (2 H, m, 5-H) and 1.82 (3 H, s, 8-H); δ_C ($CDCl_3$+D_4-methanol) 171.8 (C-7), 160.5 (C-1), 130.7 (C-4), 126.2 (C-3), 116.4 (C-2), 55.6 (C-5), 33.5 (C-6) and 22.2 (C-8); *m/z* (CI) 228 [(*MH*)$^+$, 100], 212 (6) and 86 (45%); (Found: (*MH*)$^+$, 228.0694; C, 52.93; H, 5.79; N, 6.16%. $C_{10}H_{13}NO_3S$ requires *MH*, 228.0694; C, 52.85; H, 5.77; N, 6.16%).

***N*-[2-[(4-Hydroxyphenyl)sulfinyl]-1,1-dimethylethyl]ethanamide (199)**



A solution of $NaIO_4$ (1.33 g, 6.20 mmol) in water (15 cm^3) was added over 5 min to a stirred, cooled (0 °C), solution of sulfide **92** (1.35 g, 5.64 mmol) in EtOH (120 cm^3). Using the same procedure as for **192** gave a yellow solid which was crystallised to afford *sulfoxide* **199** (1.03 g, 72%) as pale yellow crystals, m.p. 198-199 °C (from EtOAc); R_f 0.3 (8:1 EtOAc-MeOH); ν_{max} (KBr) 3243 (N-H), 1641 (amide I), 1586, 1498, 1034 (S=O) and 827 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-DMSO) 10.13 (1 H, br s, OH), 8.00 (1 H, br s, NH), 7.45 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.97 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.46 (1 H, d, ^2J= 13.3, 5-H_a), 2.96 (1 H, d, ^2J= 13.3, 5-H_b), 1.86 (3 H, s, 10-H), 1.45 (3 H, s, 7-H) and 1.36 (3 H, s, 8-H); δ_C (D_6-DMSO) 169.7 (C-9), 159.8 (C-1), 134.3 (C-4), 125.8 (C-3), 116.1 (C-2), 66.8 (C-5), 52.2 (C-6), 27.6 (C-7 and C-8) and 23.5 (C-10); *m/z* 239 (M^+ - O, 0.8), 180 (7.9), 141 (11.8), 114 (69.1) and 72 (100%); (Found: C, 56.18; H, 6.88; N, 5.34%. $C_{12}H_{17}NO_3S$ requires C, 56.45; H, 6.71; N, 5.49%).

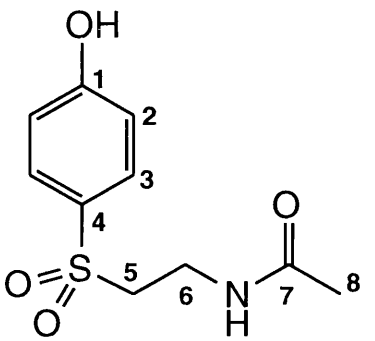
***N*-[2-[(4-Hydroxyphenyl)sulfinyl]-1,1-dimethylethyl]octanamide (200)**



A solution of $NaIO_4$ (0.32 g, 1.5 mmol) in water (3 cm^3) was added over 5 min. to a stirred, cooled (0 °C), solution of sulfide **105** (0.46 g, 1.4 mmol) in EtOH (20 cm^3). Using the same procedure as for **192** gave a yellow solid which was purified by flash chromatography on silica gel eluting with EtOAc. Crystallisation afforded *sulfoxide* **200** (0.35 g, 73%) as pale yellow crystals, m.p. 139 °C (from

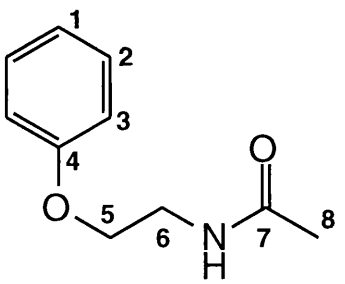
EtOAc); R_f 0.35 (EtOAc); ν_{max} (KBr) 3251 (N-H), 1638 (amide I), 1585, 1561 (amide II), 1500, 1029 (S=O) and 835 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 9.75 (1 H, br s, OH), 7.43 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.95 (2 H, AA'BB', ^3J= 8.6, 2-H), 6.59 (1 H, br s, NH), 3.47 (1 H, d, ^2J= 13.6, 5-H_a), 2.99 (1 H, d, ^2J= 13.6, 5-H_b), 2.23 (2 H, t, ^3J= 7.2, 10-H), 1.75 - 1.10 (10 H, m, 11-H, 12-H, 13-H, 14-H and 15-H) and 0.82 (3 H, t, ^3J= 6.6, 16-H); δ_C ($CDCl_3$) 174.8 (C-9), 160.5 (C-1), 132.8 (C-4), 125.8 (C-3), 116.1 (C-2), 66.9 (C-5), 53.4 (C-6), 37.3 (C-10), 31.6 (C-14), 29.1 (C-11 or C-13), 28.9 (C-13 or C-11), 28.4 (C-7), 27.5 (C-8), 25.8 (C-12), 22.5 (C-15) and 14.0 (C-16); *m/z* 322 (M^+ - O, 0.7), 198 (100), 180 (8.4) and 141 (20.8%); (Found: C, 63.72; H, 8.74; N, 4.10%. $C_{18}H_{29}NO_3S$ requires C, 63.68; H, 8.61; N, 4.13%).

_N_-[2-[(4-Hydroxyphenyl)sulfonyl]ethyl]ethanamide (193)



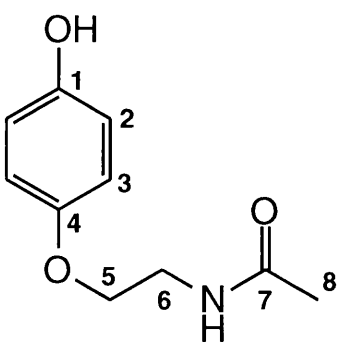
To a cooled (0 °C) solution of sulfoxide **200** (0.85 g, 3.74 mmol) in methanol (40 cm^3) was added a solution of potassium peroxymonosulfate (Oxone® monopersulfate compound, 3.45 g, 5.61 mmol) in water (10 cm^3). The resulting cloudy slurry was stirred for 2 h at r.t., concentrated to *ca.* 25 cm^3 *in vacuo* then diluted with water (50 cm^3) and extracted with EtOAc (4 x 50 cm^3). The combined organic extracts were washed with water (50 cm^3) and brine (50 cm^3) dried ($MgSO_4$) and evaporated to give a white solid which was purified by flash chromatography on silica gel using ethyl acetate-methanol (10:1) as eluent to afford, after crystallisation, *sulfone* **193** (0.63 g, 69%) as white crystals, m.p. 152-153 °C (from EtOAc-MeOH); R_f 0.19 (EtOAc); ν_{max} (KBr) 3329 (N-H), 1636 (amide I), 1553 (amide II), 1304 (SO_2), 1140 (SO_2) and 840 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 9.80 (1 H, br s, OH), 7.77 (2 H, AA'BB', ^3J= 8.8, 3-H), 7.34 (1 H, br s, NH), 7.03 (2 H, AA'BB', ^3J= 8.8, 2-H), 3.50 (2 H, m, 6-H), 3.34 (2 H, m, 5-H) and 1.82 (3 H, s, 8-H); δ_C (D_6-acetone) 171.2 (C-7), 163.2 (C-1), 131.2 (C-3), 130.8 (C-4), 116.7 (C-2), 55.6 (C-5), 30.7 (C-6) and 22.7 (C-8); *m/z* 243 (M^+, 44), 201 (12), 184 (12), 157 (17), 141 (22) and 86 (100%); (Found: M^+, 243.0544; C, 49.28; H, 5.24; N, 5.73%. $C_{10}H_{13}NO_4S$ requires *M*, 243.0565; C, 49.37; H, 5.39; N, 5.76%).

***N*-[2-(Phenyloxy)ethyl]ethanamide (201)**



A mixture of phenol (1.10 g, 11.7 mmol) and 4,5-dihydro-2-methyloxazole (0.99 g, 0.99 cm^3, 11.7 mmol) was stirred under reflux (oil bath temperature 135 °C) for 24 h. On cooling to r.t., the resulting brown mixture was dissolved in EtOAc (40 cm^3) and washed with 1 mol dm^{-3} hydrochloric acid (2 x 20 cm^3), 1 mol dm^{-3} aq. NaOH (2 x 20 cm^3), saturated aq. $NaHCO_3$ (20 cm^3) and brine (20 cm^3). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to afford, after crystallisation, the title compound **201** (0.63 g, 30%) as white crystals, m.p. 83-84 °C (from hexane-EtOAc), [lit.,[187] 88-89 °C (from cyclohexane)]. The ^1H NMR spectral data were in good agreement with those reported by Funahashi.[187] New data: R_f 0.35 (EtOAc); ν_{max} (KBr) 3316 (N-H), 1652 (amide I), 1554 (amide II), 1249, 764 and 891 (monosubstituted benzene) cm^{-1}; δ_C ($CDCl_3$) 170.5 (C-7), 158.4 (C-4), 129.5 (C-2), 121.1 (C-1), 114.3 (C-3), 66.5 (C-5), 39.0 (C-6) and 23.1 (C-8); *m/z* 179 (M^+, 2), 120 (14), 94 (12), 86 (100) and 77 (28%); (Found: M^+, 179.0946. $C_{10}H_{13}NO_2$ requires *M*, 179.0946).

***N*-[2-[(4-Hydroxyphenyl)oxy]ethyl]ethanamide (194)**

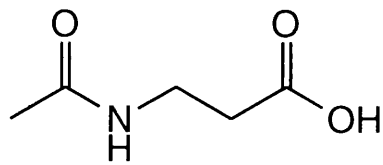


A mixture of hydroquinone (1.80 g, 11.7 mmol) and 4,5-dihydro-2-methyloxazole (1.81 g, 1.81 cm^3, 21.3 mmol) was stirred under reflux (oil bath temperature 135 °C) for 24 h. On cooling to r.t., the resulting brown mixture was dissolved in EtOAc (100 cm^3) and extracted using 2 mol dm^{-3} aq. NaOH (50 cm^3). The aq. portion was washed with EtOAc (100 cm^3), acidified (conc.

hydrochloric acid) and then extracted with EtOAc (3 x 80 cm^3). The combined organic extracts were washed [saturated aq. $NaHCO_3$ (80 cm^3) and brine (80 cm^3)], dried ($MgSO_4$) and concentrated *in vacuo* before purification by flash chromatography on silica gel using hexane-EtOAc (3:1) as eluent to afford the title compound **194** (0.81 g, 24%) as white crystals, m.p. 133-134 °C (from hexane-EtOAc), (lit.,[188] 132-134 °C).

New data: R_f 0.2 (EtOAc); ν_{max} (KBr) 3355 (N-H), 1624 (amide I), 1550 (amide II), 1514, 1478, 1223 and 834 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.38 (1 H, br s, OH), 7.68 (1 H, br s, NH), 6.77 (4 H, s, 2-H and 3-H), 3.95 (2 H, t, ^3J= 5.6, 5-H), 3.56 (2 H, m, 6-H) and 1.96 (3 H, s, 8-H); δ_C (D_6-acetone) 170.6 (C-7), 152.1 and 151.8 (C-1 and C-4), 115.9 and 115.6 (C-2 and C-3), 67.2 (C-5), 39.1 (C-6) and 22.1 (C-8); *m/z* 195 (M^+, 4), 136 (4), 110 (23), 86 (100), 81 (9) and 65 (14%); (Found: M^+, 195.0905. $C_{10}H_{13}NO_3$ requires *M*, 195.0895).

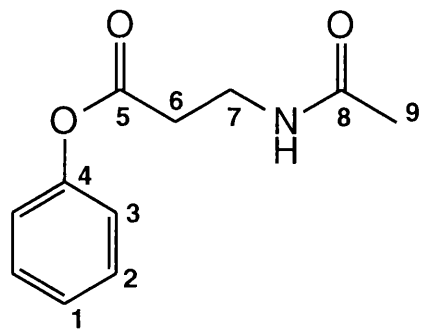
3-(Ethanoylamino)propanoic acid (204)



The title compound was prepared in 77% yield by the general method of Black and Boscacci[190] and gave m.p. 77-79 °C (from acetone), (lit.,[191] 78-80 °C). The ^1H NMR, ^{13}C NMR and IR spectral data were in good agreement with those reported.[191,192]

New data: *m/z* 131 (M^+, 3.6), 88 (22.3), 70 (9.8), 55 (4.8) and 43 (100%).

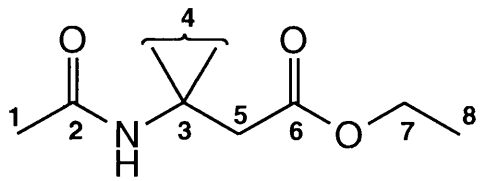
Phenyl 3-(ethanoylamino)propanoate (205)



A stirred solution of carboxylic acid **204** (5.00 g, 38.1 mmol), phenol (3.60 g, 38.1 mmol) and pyridine (10 cm^3) in EtOAc (50 cm^3) was cooled to 0 °C before addition of DCC (7.86 g, 31.0 mmol) in one portion. The mixture was stirred

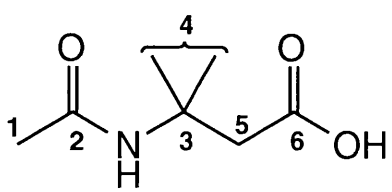
under N_2 at 0 °C for 4 h, filtered through Celite® and then heated under N_2 at reflux for a further 2 h whereupon the mixture was concentrated *in vacuo* to give a pale yellow oil. This was taken into EtOAc (150 cm^3), washed with cold 1 mol dm^{-3} hydrochloric acid (2 x 30 cm^3) and brine (30 cm^3), dried ($MgSO_4$) and then concentrated *in vacuo*. Purification by flash chromatography on silica gel eluting with EtOAc-hexane (3:1) afforded *ester* **205** (5.87 g, 74%) as a white powder, m.p. 57-58 °C; R_f 0.26 (EtOAc); ν_{max} (KBr) 3281 (N-H), 1756 (ester C=O), 1643 (amide I), 1561 (amide II), 1195, 1166, 1156, 765 and 714 (monosubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.34 (2 H, m, 2-H), 7.20 (1 H, m, 1-H), 7.04 (2 H, m, 3-H), 6.55 (1 H, br s, N-H), 3.53 (2 H, m, 7-H), 2.74 (2 H, t, ^3J= 6.0, 6-H) and 1.89 (3 H, s, 9-H); δ_C ($CDCl_3$) 171.2 (C=O), 170.5 (C=O), 150.3 (C-4), 129.4 (C-2), 126.0 (C-1), 121.4 (C-3), 34.9 (CH_2), 33.4 (CH_2) and 23.1 (C-9); *m/z* (CI) 195 [$(M+NH_4)^+$, 100], 208 [$(MH)^+$, 30], 114 (4) and 52 (3%); (Found: $(MH)^+$, 208.0974; C, 63.74; H, 6.29; N, 6.75%. $C_{11}H_{13}NO_3$ requires *MH*, 208.1003; C, 63.76; H, 6.32; N, 6.76%).

Ethyl 3-acetylamino-3-methylbutanoate (208)



The title compound **208** was prepared in 48% yield on a 50 mmol scale using the method of Eugster *et al.*[193] and gave IR and ^1H NMR spectral data in good agreement with those reported in the literature.[193,207]
New data: δ_C ($CDCl_3$) 171.2 (C=O), 170.2 (C=O), 60.0 (C-7), 51.8 (C-3), 43.5 (C-5), 26.8 (C-4), 23.8 (C-1) and 14.0 (C-8); *m/z* 187 (M^+, 6.4), 142 (13.6), 130 (37.1), 100 (29.8) and 59 (100%).

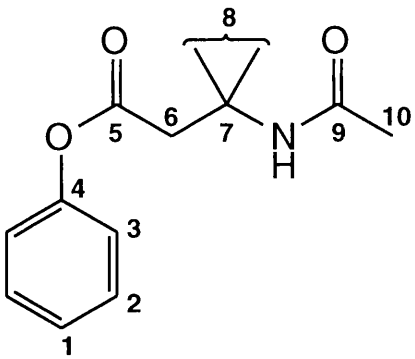
3-Acetylamino-3-methylbutanoic acid (209)



The title compound **209** was prepared in 71% yield on a 50 mmol scale using the method of Eugster *et al.*[193] and gave m.p. 135-136 °C (from acetone), (lit., 135-136 °C). The IR and ^1H NMR spectral data were in good agreement with those reported in the literature.[190,193]

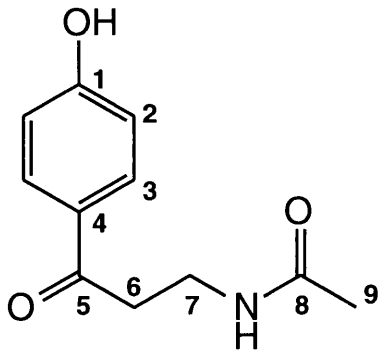
New data: R_f 0.2 (EtOAc); δ_C ($CDCl_3$) 172.6 (C=O), 170.3 (C=O), 52.1 (C-3), 43.5 (C-5), 27.3 (C-4) and 23.8 (C-1); *m/z* 159 (M^+, 10.6), 102 (47.6), 84 (30.0), 72 (12.5) and 59 (100%).

Phenyl 3-ethanoylamino-3-methylbutanoate (210)



A stirred solution of carboxylic acid **209** (0.800 g, 5.03 mmol), phenol (0.472 g, 5.02 mmol) and pyridine (1 cm^3) in EtOAc (9 cm^3) was cooled to 0 °C before addition of DCC (1.04 g, 5.03 mmol) in one portion. The mixture was stirred under N_2 at 0 °C for 4 h, filtered through Celite® and then heated under N_2 at reflux for a further 2 h whereupon the mixture was concentrated *in vacuo* to give a pale yellow oil. This was taken into EtOAc (100 cm^3), washed with cold 1 mol dm^{-3} hydrochloric acid (2 x 20 cm^3) and brine (20 cm^3), dried ($MgSO_4$) and then concentrated *in vacuo*. Purification by flash chromatography on silica gel eluting with EtOAc-hexane (1:1) afforded *ester* **210** (0.77 g, 65%) as a white powder, m.p. 65-66 °C; R_f 0.50 (EtOAc); ν_{max} (KBr) 1749 (ester C=O), 1644 (amide I), 1554 (amide II), 1495, 1435, 758 and 692 (monosubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.31 (2 H, m, 2-H), 7.15 (1 H, m, 1-H), 7.00 (2 H, m, 3-H), 5.74 (1 H, br s, N-H), 2.98 (2 H, s, 6-H), 1.86 (3 H, s, 10-H) and 1.44 (3 H, s, 8-H); δ_C ($CDCl_3$) 170.0 (C=O), 170.0 (C=O), 150.3 (C-4), 129.4 (C-2), 125.9 (C-1), 121.5 (C-3), 52.1 (C-7), 43.2 (C-6), 27.3 (C-8) and 24.3 (C-10); *m/z* 178 [$(M\text{-}57)^+$, 1.9], 142 (39.6), 100 (22.1), 92 (23.6), 83 (89.4) and 58 (100%); (Found: C, 66.27; H, 7.41; N, 6.17%. $C_{13}H_{17}NO_3$ requires C, 66.36; H, 7.28; N, 5.95%).

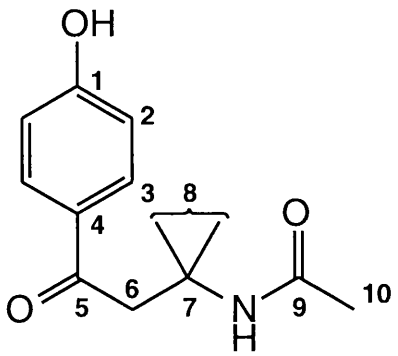
***N*-[3-(4-Hydroxyphenyl)-3-oxopropyl]acetamide (197)**



CAUTION! Nitromethane is known to explode when heated; the use of a blast shield throughout this experiment is recommended.

The general procedure of Harwood and co-workers[195] was adapted as follows. Ester **205** (1.08 g, 5.20 mmol) was dissolved in nitromethane (15 cm^3) and cooled to -10 °C with stirring under N_2. A solution of anhydrous aluminium chloride (3.50 g, 26.1 mmol) in nitromethane (15 cm^3) was added dropwise over 5 min and the mixture allowed to warm to r.t. over 20 min before being heated at 40 °C for 24 h. The mixture was then warmed to r.t., quenched with ice-cold 5 mol dm^{-3} hydrochloric acid (70 cm^3) and extracted with EtOAc (2 x 80 cm^3). The combined organic portions were washed with brine (50 cm3), dried ($MgSO_4$) and concentrated *in vacuo.* Purification by flash chromatography on silica gel eluting with EtOAc-hexane (1:1) afforded *ketone* **197** (0.35 g, 32%) as white crystals, m.p. 161-162 °C (from hexane-EtOAc); R_f 0.49 (acetone); ν_{max} (KBr) 3371 (N-H), 1655 (ketone C=O and amide I), 1608, 1545 (amide II), 1218 and 843 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 9.46 (1 H, br s, OH), 7.69 (2 H, AA'BB', ^3J= 8.8, 3-H), 7.24 (1 H, br s, N-H), 6.92 (2 H, AA'BB', ^3J= 8.8, 2-H), 3.53 (2 H, m, 7-H), 3.15 (2 H, t, ^3J= 6.2, 6-H) and 1.88 (3 H, s, 9-H); δ_C (D_6-acetone) 197.3 (C-5), 170.4 (C-8), 162.9 (C-1), 131.3 (C-3), 130.0 (C-4), 116.1 (C-2), 38.5 (C-7), 35.4 (C-6) and 22.9 (C-9); *m/z* 207 (M^+, 29), 164 (6), 148 (10), 121 (100), 86 (22) and 65 (16%); (Found: M^+, 207.0908; C, 63.53; H, 6.27; N, 6.62%. $C_{11}H_{13}NO_3$ requires *M*, 207.0895; C, 63.76; H, 6.32; N, 6.76%).

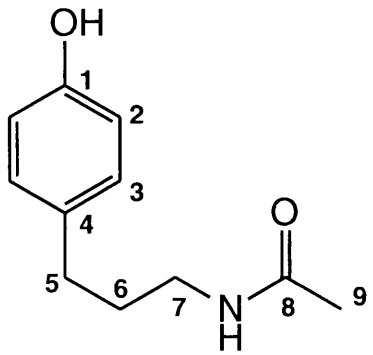
***N*-[3-(4-Hydroxyphenyl)-1,1-dimethyl-3-oxopropyl]ethanamide (211)**



CAUTION! Nitromethane is known to explode when heated; the use of a blast shield throughout this experiment is recommended.

The general procedure of Harwood and co-workers[195] was adapted as follows. Ester **210** (0.56 g, 2.38 mmol) was dissolved in nitromethane (7.5 cm^3) and cooled to -10 °C with stirring under N_2. A solution of anhydrous aluminium chloride (1.59 g, 11.9 mmol) in nitromethane (7.5 cm^3) was added dropwise over 5 min and the mixture allowed to warm to r.t. over 20 min before being heated at 40 °C for 4 h. The mixture was then warmed to r.t., quenched with ice-cold 5 mol dm^{-3} hydrochloric acid (40 cm^3) and extracted with EtOAc (3 x 50 cm^3). The combined organic portions were concentrated *in vacuo*, taken into 2 mol dm^{-3} aq. NaOH (50 cm^3), washed with diethyl ether (2 x 20 cm^3), acidified to pH 1 and then extracted using EtOAc (3 x 50 cm^3). The combined extracts were washed with saturated aq. $NaHCO_3$ (3 x 50 cm^3) and brine (3 x 30 cm^3), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography on silica gel eluting with EtOAc-hexane (1:1) afforded *ketone* **211** (0.24 g, 42%) as white crystals, m.p. 177-178 °C (from hexane-EtOAc); R_f 0.31 (EtOAc); ν_{max} (KBr) 3364 (N-H), 1688 (ketone C=O), 1653 (amide I), 1600, 1577, 1552 (amide II) and 1516 cm^{-1}; δ_H (D_6-acetone) 9.30 (1 H, br s, OH), 7.78 (2 H, AA'BB', ^3J= 8.8, 3-H), 6.87 (1 H, br s, N-H), 6.77 (2 H, AA'BB', ^3J= 8.8, 2-H), 3.30 (2 H, s, 6-H), 1.66 (3 H, s, 10-H) and 1.29 (6 H, s, 8-H); δ_C (D_6-acetone) 197.7 (C-5), 170.3 (C-9), 162.7 (C-1), 131.5 (C-3), 131.3 (C-4), 115.9 (C-2), 53.1 (C-7), 45.7 (C-6), 27.8 (C-8) and 23.9 (C-10); *m/z* 235 (M^+, 6.0), 176 (20.8), 161 (12.4), 136 (13.1) and 121 (100%); (Found: M^+, 235.1199; C, 66.20; H, 7.22; N, 5.95%. $C_{13}H_{17}NO_3$ requires *M*, 235.1208; C, 66.36; H, 7.28; N, 5.95%).

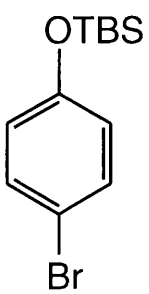
_N_-[3-(4-Hydroxyphenyl)propyl]acetamide (196)



A mixture of ketone **197** (100 mg, 0.48 mmol), 10% palladium on activated carbon (25 mg) and MeOH (20 cm^3) was stirred under a slight positive pressure of hydrogen gas for 3 h at r.t. The catalyst was then removed by filtration over Celite® and washed with MeOH (10 cm^3); evaporation of the combined filtrates gave the title compound **196** (95 mg, 88%) as white crystals, m.p. 93-95 °C (from hexane-EtOAc) (lit.,[197] 93-95 °C).

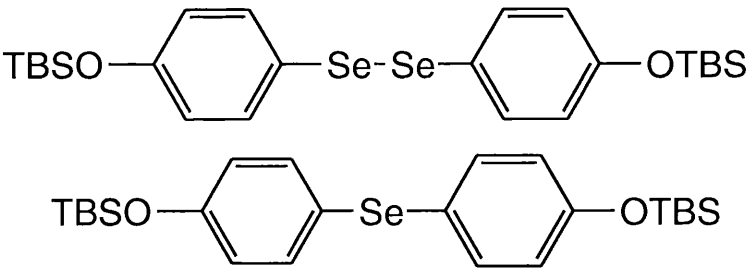
New data: R_f 0.54 (acetone); ν_{max} (KBr) 3316 (N-H), 1638 (amide I), 1596, 1570 (amide II), 1514, 1249 and 856 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 8.04 (1 H, br s, OH), 7.00 (1 H, br s, N-H), 6.89 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.60 (2 H, AA'BB', ^3J= 8.6, 2-H), 3.04 (2 H, m, 7-H), 2.40 (2 H, t, ^3J= 7.3, 5-H), 1.72 (3 H, s, 9-H) and 1.60 (2 H, m, 6-H); δ_C (D_6-acetone) 170.0 (C-8), 156.4 (C-1), 133.4 (C-4), 130.0 (C-3), 115.9 (C-2), 39.4 (C-7), 32.9 (CH_2), 32.6 (CH_2) and 22.9 (C-9); *m/z* 193 (M^+, 56), 134 (48), 107 (37), 73 (100) and 43 (27%); (Found: M^+, 193.1108; $C_{11}H_{15}NO_2$ requires *M*, 193.1103).

4-Bromo-*O*-(*tert*-butyldimethylsilyl)phenol (218)



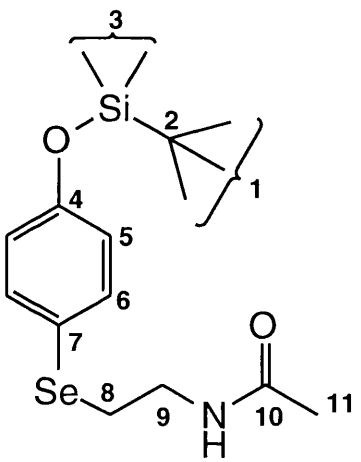
The title compound **218** was prepared in 93% yield on a 70 mmol scale by the procedure of Riley *et al.*[69] and gave b.p. 122-124 °C/1.0 mmHg (lit., 67-68 °C/0.01 mmHg). The IR, MS, ^1H NMR and ^{13}C NMR spectral data were in good agreement with those reported.[69,208,209]

***Bis*[4-[(*tert*-butyldimethylsilyl)oxy]phenyl] diselenide (219)**



The procedure of Riley *et al.*[69] on a 40 mmol scale, followed by flash chromatography on silica gel with hexane as eluent, afforded a ~3:1 mixture of diselenide **219** (~20% yield) and selenide **220** (~61% yield) as an orange oil. The mixture was used in the following step without further purification.

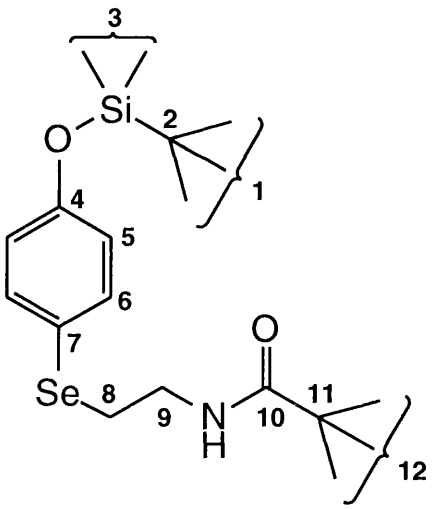
***N*-[2-[[4-[(*tert*-Butyldimethylsilyl)oxy]phenyl]seleno]ethyl]ethanamide (222)**



Sodium borohydride (0.200 g, 5.2 mmol) was added over 15 min to a solution of crude diselenide **219** (1.8 g mixture, 2.1 mmol diselenide) in ethanol (6 cm^3) at 0 °C under a stream of N_2. Allowing the mixture to warm to r.t., stirring was continued for a further 30 min to give a colourless solution which was quenched by addition of 0.5 mol dm^{-3} hydrochloric acid (20 cm^3) and extracted with 1:1 diethyl ether-hexane (3 x 15 cm^3). The combined extracts were dried ($CaCl_2$) and concentrated *in vacuo* to give an oil which was immediately treated with 4,5-dihydro-2-methyloxazole (1.00 g, 11.7 mmol) and stirred under N_2 for 4 h at 130 °C. Flash chromatography of the crude product on silica gel, eluting with EtOAc-hexane (1:1), gave *selenide* **222** (0.75 g, 48%) as a clear oil, R_f 0.40 (EtOAc); ν_{max} (neat) 3386 (N-H), 1653 (amide I), 1586, 1553 (amide II), 1489 and 840 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.26 (2 H, AA'BB', ^3J= 8.6, 6-H), 6.70 (2 H, AA'BB', ^3J= 8.6, 5-H), 6.24 (1 H, br s, NH), 3.42 (2 H, m, 9-H), 2.88 (2 H, t, ^3J= 6.6, 8-H), 1.87 (3 H, s, 11-H), 0.93 (9 H, s, 1-H) and 0.15 (6

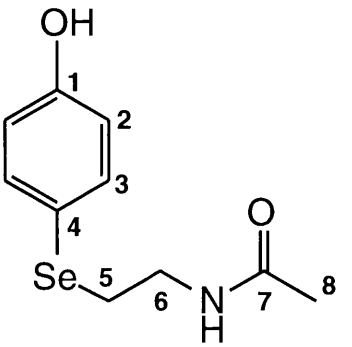
H, s, 3-H); δ_C ($CDCl_3$) 170.1 (C-10), 155.6 (C-4), 135.5 (C-6), 121.0 (C-5), 119.5 (C-7), 39.4 (C-9), 28.1 (C-8), 25.6 (C-1), 23.1 (C-11), 18.1 (C-2) and -4.5 (C-3); *m/z* 373 (M^+, 10), 257 (3), 215 (4), 116 (24) and 86 (100%); (Found: M^+, 373.0986. $C_{16}H_{27}NO_2SeSi$ requires *M*, 373.0976).

_N_-[2-[[4-[(*tert*-Butyldimethylsilyl)oxy]phenyl]seleno]ethyl]-2,2-dimethylpropanamide (223)



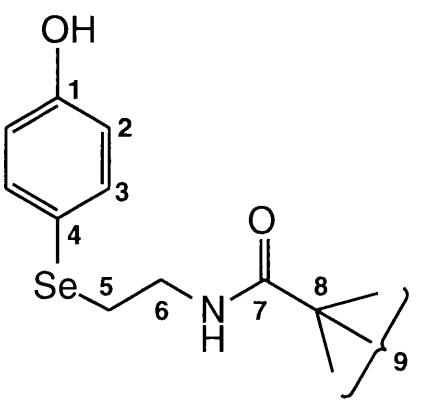
Sodium borohydride (0.460 g, 12.2 mmol) was added over 15 min to a solution of crude diselenide **219** (3.0 g mixture, 3.5 mmol diselenide) in ethanol (20 cm^3) at 0 °C under a stream of N_2. Allowing the mixture to warm to r.t., stirring was continued for a further 0.5 h to give a colourless solution which was quenched by addition of 0.5 mol dm^{-3} hydrochloric acid (40 cm^3) and extracted with 1:1 diethyl ether-hexane (3 x 20 cm^3). The combined extracts were dried ($CaCl_2$) and concentrated *in vacuo* to give an oil which was immediately treated with 4,5-dihydro-2-(1,1-dimethylethyl)oxazole (1.00 g, 7.9 mmol) and stirred under N_2 for 4 h at 130 °C. Flash chromatography of the crude product on silica gel, eluting with hexane-EtOAc (4:1), gave *selenide* **223** (1.52 g, 52%) as a white solid, m.p. 75-76 °C; R_f 0.75 (EtOAc); ν_{max} (KBr) 3330 (N-H), 1638 (amide I), 1584, 1534 (amide II), 1487 and 841 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 7.38 (2 H, AA'BB', ^3J= 8.6, 6-H), 6.73 (2 H, AA'BB', ^3J= 8.6, 5-H), 6.09 (1 H, br s, NH), 3.45 (2 H, m, 9-H), 2.92 (2 H, t, ^3J= 6.4, 8-H), 1.14 (9 H, s, 12-H), 0.96 (9 H, s, 1-H) and 0.17 (6 H, s, 3-H); δ_C ($CDCl_3$) 178.3 (C-10), 155.6 (C-4), 135.4 (C-6), 121.1 (C-5), 119.3 (C-7), 39.0 (C-9), 38.6 (C-11), 28.4 (C-8), 27.5 (C-12), 25.6 (C-1), 18.1 (C-2) and -4.5 (C-3); *m/z* (CI) 416 [$(MH)^+$, 7], 276 (3), 227 (6), 164 (42), 147 (100) and 130 (6%); [Found: $(MH)^+$, 416.1542; C, 55.13; H, 7.98; N, 3.29%. $C_{19}H_{33}NO_2SeSi$ requires *MH*, 416.1523; C, 55.05; H, 8.02; N, 3.38%].

***N*-[2-[(4-Hydroxyphenyl)seleno]ethyl]ethanamide (197)**



A solution of silyl ether **222** (2.30 g, 6.18 mmol) in anhydrous THF (60 cm^3) was treated with TBAF (1 mol dm^{-3} solution in THF, 15.5 cm^3, 15.5 mmol). After being stirred for 2.5 h at r.t., the mixture was diluted with saturated aq. NH_4Cl (30 cm^3) and extracted using EtOAc (3 x 50 cm^3). The combined extracts were washed with brine (50 cm^3), dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless oil. Dry-column flash chromatography on silica gel, eluting with EtOAc, gave *selenide* **197** (1.39 g, 87%) as a white solid, m.p. 110-111 °C; R_f 0.28 (EtOAc); ν_{max} (KBr) 3330 (N-H), 1635 (amide I), 1678, 1493, 1448 and 838 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_6-acetone) 9.88 (1 H, br s, OH), 7.47 (1 H, br s, NH), 7.41 (2 H, AA'BB', ^3J= 8.7, 3-H), 6.77 (2 H, AA'BB', ^3J= 8.7, 2-H), 3.40 (2 H, m, 6-H), 2.88 (2 H, t, ^3J= 6.6, 5-H) and 1.86 (3 H, s, 8-H); δ_C (D_6-acetone) 171.1 (C-7), 158.4 (C-1), 136.7 (C-3), 118.3 (C-4), 117.2 (C-2), 40.5 (C-6), 28.3 (C-5) and 22.8 (C-8); *m/z* 259 (M^+, 11), 200 (8), 173 (9), 120 (23), 107 (16) and 86 (100%); (Found: M^+, 259.0093; C, 46.52; H, 5.06; N, 5.27%. $C_{10}H_{13}NO_2Se$ requires *M*, 259.0111; C, 46.52; H, 5.08; N, 5.43%).

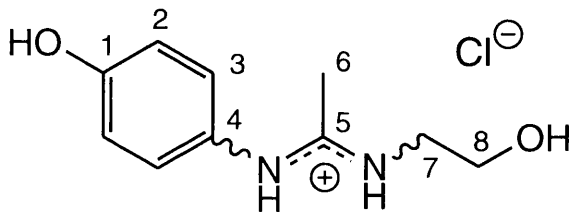
***N*-[2-[(4-Hydroxyphenyl)seleno]ethyl]-2,2-dimethylpropanamide (224)**



A solution of silyl ether **223** (0.600 g, 1.45 mmol) in anhydrous THF (30 cm^3) was treated with TBAF (1 mol dm^{-3} solution in THF, 2.89 cm^3, 2.89 mmol). After being stirred for 2.5 h at r.t., the mixture was diluted with saturated aq. NH_4Cl

(30 cm^3) and extracted using EtOAc (3 x 30 cm^3). The combined extracts were washed with brine (30 cm^3), dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless oil. Dry-column flash chromatography on silica gel, eluting with EtOAc-hexane (1:1), gave *selenide* **224** (0.37 g, 86%) as a white solid, m.p. 98-99 °C; R_f 0.50 (1:1 EtOAc-hexane); ν_{max} (KBr) 3374 (N-H), 1626 (amide I), 1600, 1576, 1539 (amide II), 1278 and 825 (*para*-disubstituted benzene) cm^{-1}; δ_H ($CDCl_3$) 8.52 (1 H, br s, OH), 7.36 (2 H, AA'BB', ^3J= 8.6, 3-H), 6.77 (2 H, AA'BB', ^3J= 8.6, 2-H), 6.28 (1 H, br m, NH), 3.44 (2 H, m, 6-H), 2.87 (2 H, t, ^3J= 6.5, 5-H) and 1.48 (9 H, s, 9-H); δ_C ($CDCl_3$) 179.5 (C-7), 157.1 (C-1), 136.1 (C-3), 116.7 (C-2 and C-4), 39.3 (C-6), 38.7 (C-8), 28.0 (C-5) and 27.4 (C-9); *m/z* (CI) 302 [$(MH)^+$, 45], 227 (9), 164 (12), 147 (96) and 130 (100%); [Found: $(MH)^+$, 302.0656; C, 52.09; H, 6.38; N, 4.74%. $C_{13}H_{19}NO_2Se$ requires *MH*, 302.0659; C, 52.00; H, 6.38; N, 4.66%].

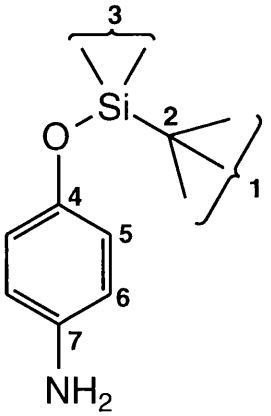
_N_-2-Hydroxyethyl-_N'_-(4-hydroxyphenyl)ethanimamide hydrochloride. (Two isomers of 227 - 230).



Mixture of two of the four isomers

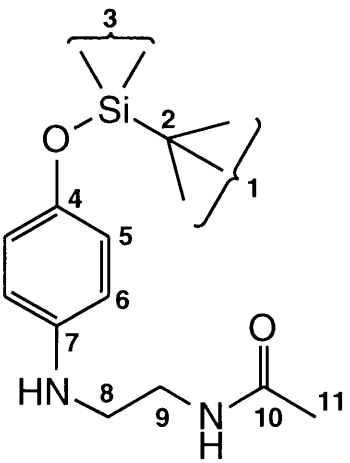
To a solution of 4-hydroxyaniline hydrochloride **225** (1.90 g, 13.1 mmol) in EtOH (50 cm^3) was added 2-methyl-2-oxazoline (1.11 g, 13.0 mmol) before the mixture was stirred for 14 h at r.t.. The *product* (1.67 g, 55%) was then filtered from the product mixtures as white crystals, m.p. 155-156 °C; ν_{max} (KBr) 1646 (C=N), 1518, 1269, 1222, 1060 and 844 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_2O) 6.93 (4 H, m, 3-H), 6.70 (4 H, m, 2-H), 3.63 (2 H, t, 8-H), 3.42 (2 H, t, 8-H), 3.32 (2 H, t, 7-H), 3.24 (2 H, t, 7-H), 2.20 (3 H, s, 6-H) and 1.84 (3 H, s, 8-H); δ_C (D_2O) 166.4 (C-5), 165.9 (C-5), 157.1 (C-3), 156.7 (C-3), 129.1 (C-1), 128.7 (C-1), 128.1 (C-4), 125.8 (C-4), 117.7 (C-2), 117.0 (C-2), 60.6 (C-8), 59.5 (C-8), 46.5 (C-7), 45.4 (C-7), 18.4 (C-6) and 17.8 (C-6); *m/z* 194 [M^+ (free base), 10], 134 (9), 122 (28), 109 (100) and 80 (42%); [Found: M^+ (free base), 194.1059. $C_{10}H_{15}N_2O_2Cl$ requires *M* (free base), 194.1055].

4-[(*tert*-Butyldimethylsilyl)oxy]aniline (233)



The procedure of Swenton *et al.*[199] was employed on a 20 mmol scale to give the crude product as a brown oil. Purification by flash chromatography on silica gel, eluting with hexane-ethyl acetate (3:1) afforded the title compound **223** in 88% yield as a yellow oil. The IR, MS and ^{1}H NMR spectral data were in good agreement with those reported in the literature.[199]
New data: δ_C ($CDCl_3$) 148.1 (C-7), 140.3 (C-4), 120.6 (C-5), 116.3 (C-6), 25.7 (C-1), 16.1 (C-2) and -4.5 (C-3).

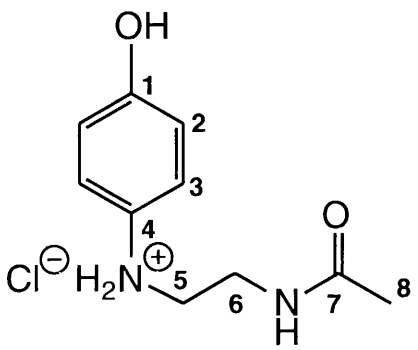
***N*-[2-[[4-(*tert*-Butyldimethylsilyl)oxyphenyl]amino]ethyl]acetamide (234)**



To a solution of 4-[(*tert*-butyldimethylsilyl)oxy]aniline **233** (4.00 g, 17.9 mmol and *N*-(2-chloroethyl)ethanamide (2.18 g, 1.81 cm^3, 17.9 mmol) in acetonitrile (15 cm^3) was added sodium carbonate (3.78 g, 35.8 mmol). The mixture was stirred under N_2 at reflux for 10 h to give a brown mixture which was filtered, concentrated *in vacuo* and flash chromatographed on silica gel using EtOAc then EtOAc-MeOH (9:1) as eluent to furnish *amine* **234** (2.25 g, 41%) as a cream solid, m.p. 62-63 °C; R_f 0.34 (EtOAc); ν_{max} (KBr) 3323 (N-H), 1626 (amide I), 1521 (amide II), 1255, 920 and 841 (*para*-disubstituted benzene) cm$^-$

^{1}; δ_H ($CDCl_3$) 6.70 (2 H, AA'BB', ^3J= 8.8, 5-H), 6.57 (2 H, AA'BB', ^3J= 8.8, 6-H), 6.13 (1 H, br s, amide NH), 3.47 (3 H, m, amine N-H and 9-H), 3.22 (2 H, m, 8-H), 1.98 (3 H, s, 11-H), 0.97 (9 H, s, 1-H) and 0.15 (6 H, s, 3-H); δ_C ($CDCl_3$) 170.9 (C-10), 148.2 (C-7), 141.6 (C-4), 120.8 (C-5), 114.5 (C-6), 45.1 (C-9), 39.1 (C-8), 25.7 (C-1), 23.2 (C-11), 16.1 (C-2) and -4.5 (C-3); *m/z* (CI) 309 [(*MH*)$^+$, 100], 241 (7), 224 (3), 148 (7) and 141 (6%).

_N_-[2-[(4-Hydroxyphenyl)amino]ethyl]acetamide hydrochloride (198)



To a solution of conc. hydrochloric acid (1.0 g) in EtOH (30 cm^3) was added amine **234** (0.900 g, 2.91 mmol) before the mixture was stirred for 14 h at r.t.. The resulting pale brown solution was evaporated to dryness *in vacuo* to give a brown solid which was recrystallised to afford the *title compound* **198** (0.520 g, 77%) as white needles, m.p. 197-198 °C (from EtOAc-MeOH); ν_{max} (KBr) 3248 (N-H), 1646 (amide I), 1629, 1571, 1535 (amide II), 1516 and 842 (*para*-disubstituted benzene) cm^{-1}; δ_H (D_2O) 7.11 (2 H, AA'BB', ^3J= 9.0, 3-H), 6.75 (2 H, AA'BB', ^3J= 9.0, 2-H), 3.31 (4 H, m, 5-H and 6-H) and 1.74 (3 H, s, 8-H); δ_C (D_2O) 175.5 (C-7), 157.5 (C-1), 127.2 (C-4), 124.8 (C-3), 117.6 (C-2), 51.3 (C-5), 36.3 (C-6) and 22.7 (C-8); *m/z* 194 [M^+ (free base), 20], 135 (19), 122 (100), 94 (12) and 65 (5%); [Found: M^+ (free base), 194.1056; C, 51.68; H, 6.78; N, 11.94%. $C_{10}H_{15}N_2O_2Cl$ requires *M* (free base), 194.1055; C, 52.06; H, 6.55; N, 12.14%).

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