# Synthesis of Novel Anticancer Agents

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

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*" My head is tired, my hand sore. My eyes be'dimmed with overmuch looking at the white paper....."*

*William Caxton (1422-1491)*

*If you can meet with triumph and disaster*
*and treat those two imposters just the same.....*

*"If", Rudyard Kipling*

*"The man who thinks he knows something does not yet know as he ought to know"*

*1 Corinthians 8:2*


Nip over to the Finance Ministry, Ms Parker, and find out if we can afford to fight cancer!
MINISTRY OF HEALTH

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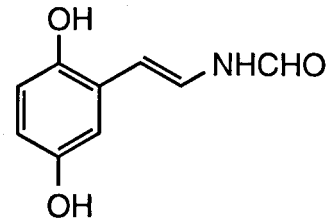
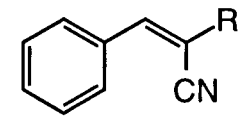
# Summary

Although traditional anticancer agents have been designed to disrupt the replicative process in the nucleus of a cell, much recent work has focused on events occurring earlier in the growth cycle. Among the gene products implicated in the onset of proliferative diseases are protein tyrosine kinases (PTKs), a family of enzymes which catalyse the phosphorylation of tyrosine residues by ATP. Several receptor molecules are known to contain internal PTK domains, including the epidermal growth factor receptor (EGFR), insulin receptor (InsR) and platelet derived growth factor receptor (PDGFR). Chemical agents which can inhibit the function of PTKs (known generically as 'tyrosine kinase inhibitors') have potential as anticancer agents. A large number of tyrosine kinase inhibitors has been obtained from both natural sources and chemical synthesis. Foremost among these is the natural product erbstatin (**i**) which has become the template for a series of synthetic analogues known as 'tyrphostins'. The common structural feature of most tyrphostins is the benzylidene malononitrile nucleus (**ii**, R=CN).



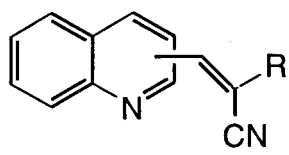

**(i)**



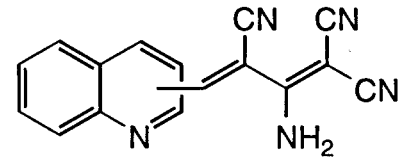

**(ii)**

Some compounds of this type inhibit the EGFR tyrosine kinase with $IC_{50}$ values in the low μM concentration range; several show the ability to discriminate between different members of the PTK family. *In vivo* antitumour activity has also been reported.

At our point of entry into this field, little work had been performed on heterocyclic tyrphostins and we were keen to become involved with this interesting area of research. To this end, a set of 24 tyrphostins based on quinoline were prepared, of which 18 were novel compounds. The general structure is depicted below (**iii**), with R being an electron-withdrawing group. Derivatives were prepared with R as nitrile, substituted diene, ester, amide, thioamide and carboxylic acid functions. The tyrphostins were tested against a range of cancer cell lines under the supervision of Dr Valerie Brunton at the Department of Medical Oncology, University of Glasgow. The most active compounds were quinoline derivatives substituted at either the 2- or 4- position with a diene side-arm bearing three nitrile groups and an amine function (**iv**). These compounds had $IC_{50}$ values against the EGFR kinase as low as 1.7 μM.



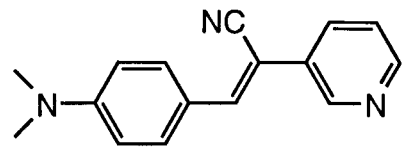

(iii)




(iv)

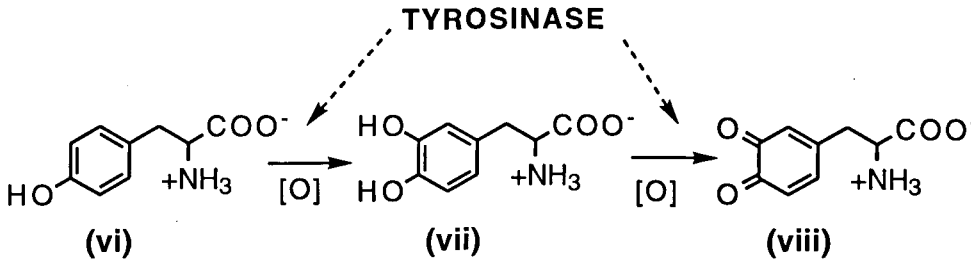
We considered the key structural features of (**iv**) to be the conjugated aliphatic side arm and the amine group, which could impart a partial negative charge onto the terminal nitrile groups by delocalisation. The importance of conjugation was tested by preparing 12 novel compounds containing aromatic or conjugated aliphatic side-arms, using both Knoevenagel and Wittig chemistry. Our work showed that tyrphostins incorporating straight-chain diene or triene side arms were particularly active. A further discovery was that the *cis*- nitrile moiety in structure (**iii**) could be replaced by another electron-withdrawing group or even hydrogen without loss of activity. Four tyrphostins with dimethylaminobenzene as the aromatic moiety were prepared to determine whether electron push into the side-arm was beneficial for activity. Three of these proved to be inactive.

To address the solubility problems encountered with tyrphostins, we developed three water-soluble tyrphostins which incorporated triethylene glycol and polyethyleneglycol moieties. The hydrochloride salt and N-oxide of (**iv**) were prepared, as well as two hydroxylated tyrphostins. The compounds prepared in this series were tested by Dr Alan McGown at the CRC Paterson Institute for Cancer Research, Manchester. Although no clear structure-activity relationship was observed, we succeeded in generating a further six compounds with $IC_{50}$ values of <5 μM and one (**v**) with an $IC_{50}$ value of 500 nm against the MCF-7 breast cancer cell line.




(v)

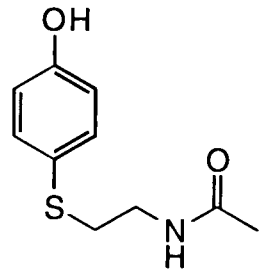
Another area of research which we have explored has been the development of chemotherapeutic agents for the treatment of malignant melanoma. This disease originates within melanocytes, the cells responsible for the synthesis of melanin pigments. Melanin synthesis is dependent upon tyrosinase, an enzyme which is unique to melanocytes and catalyses the oxidation of tyrosine (**vi**) to dopa (**vii**) and then dopa quinone (**viii**). The quinone then undergoes further transformations to produce melanin polymers.




**(vi)** **(vii)** **(viii)**

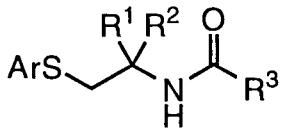
Quinones are electrophilic and readily react with any available nucleophiles, including those in DNA bases. By administering drugs which are substrates for tyrosinase, it may be possible to generate an excessive amount of quinone species which could poison the cell by alkylating DNA or inhibiting key enzymes such as DNA polymerase. This process may account for the well-established cytotoxic and depigmenting effects of phenols and catechols.

Amide (**ix**) was prepared previously both as an antihypertensive and antimelanoma agent. In preparing amide (**ix**) for biological evaluation, it became apparent that little had been done to optimise the activity of this compound.



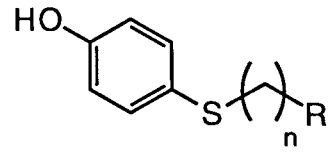

**(ix)**

We prepared a series of 17 structurally related compounds, represented by the general structures (**x**) and (**xi**), which were tested by Professor Rona Mackie of the Department of Dermatology, Glasgow University, and Dr Lloyd Kelland at the CRC Institute of Cancer Research, Royal Marsden NHS Trust. Of particular significance was the discovery that increasing the bulk of the side-arm, either $\alpha$–to nitrogen ($R^1$ and $R^2$) or at the acyl position ($R^3$) increased drug potency.



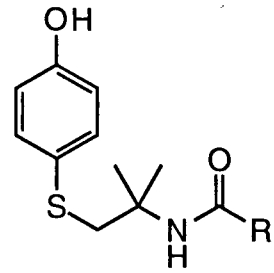
**(x)**

Ar = phenol, fluorobenzene
heterocyclic
$R^1$ , $R^2$ = H, alkyl
$R^3$ = alkyl, aryl



**(xi)**

n = 1 or 2
R = nitrile, acid, amide



**(xii)**

Three drugs in this class (**xii**, R = methyl, ethyl or $^t$butyl) showed a marked improvement in activity over the parent compound (**ix**) *in vitro* and *in vivo*. Optically active derivatives and analogues with improved water solubility have also been prepared.

# Table of Contents

# List of Figures

# Abbreviations

| | |
|---|---|
| **ADP** | Adenosine Diphosphate |
| **ATP** | Adenosine Triphosphate |
| **BOC** | $^{t}$Butoxycarbonyl |
| **b.p.** | boiling point |
| **br** | broad |
| **d** | doublet (NMR spectroscopy) |
| **d.** | decomposed (m.p.) |
| **DCC** | 1,3-Dicyclohexylcarbodiimide |
| **DCU** | Dicyclohexylurea |
| **DCM** | Dichloromethane |
| **DMAP** | 4-Dimethylaminopyridine |
| **DMF** | Dimethylformamide |
| **DMSO** | Dimethylsulfoxide |
| **DNA** | Deoxyribonucleic acid |
| **EGFR** | Epidermal growth factor receptor |
| **$GI_{50}$** | concentration required to reduce growth by 50% |
| **h** | hours |
| **$IC_{50}$** | concentration required to reduce a parameter to 50% of that in a control |
| **InsR** | Insulin receptor |
| **lit.** | literature value |
| **m** | medium intensity (IR spectroscopy) |
| **m** | multiplet (NMR spectroscopy) |
| **m.p.** | melting point |
| **min** | minute(s) |
| **ml** | millilitre(s) |
| **mmol** | millimole(s) |
| **mol** | mole(s) |
| **MPEG** | Polyethyleneglycol methyl ether |
| **MTEG** | Triethyleneglycol methyl ether |
| **PDGFR** | Platelet derived growth factor receptor |
| **PTK** | Protein tyrosine kinase |
| **PTSA** | *Para*-toluenesulfonic acid |

| | |
|---|---|
| **q** | quartet (NMR spectroscopy) |
| **s** | sharp (IR spectroscopy) |
| **s** | singlet (NMR spectroscopy) |
| **t** | triplet (NMR spectroscopy) |
| **v. br** | very broad |
| **w** | weak (IR spectroscopy) |

# 1

# Cancer - An Overview

## 1.1 The History of Cancer

A constant presence with us throughout the course of human evolution, cancer has a history as old as that of man himself. Egyptian medical records some 3,500 years old describe the incidence and symptoms of diseases which are now recognised as cancer. Archaeologists have unearthed human remains from this era which show the distinctive scarring common to victims of malignant melanoma. Through the ages, cancer has always been feared; its rise to global significance, however, has been a remarkably recent phenomenon. Mortality statistics taken from the American census of 1850 show that only one of every 190 deaths was attributable to cancer. The corresponding figure in 1986 was one in five.[1] This dramatic increase was due in no small part to a decrease in mortality from infectious diseases over the same period. Advances in nutritional expertise and public hygiene, coupled with the discovery of antibiotics, helped reduce the incidence of diseases like pneumonia and tuberculosis which had been the scourge of previous generations. Improved health meant increased longevity and by the end of the 20th century the average life-span in the developed nations had grown by almost 20 years. With the demise of infectious diseases and the demographic shift towards an older population it's not surprising that cancer, primarily a disease of the old and middle aged, should have assumed greater importance in our time.

## 1.2 Causes of Cancer

Cancers originate when a cell replicates itself using a faulty genetic programme; but how does the cell's remarkably ordered schedule become corrupted in the first place? The error must either arise from inside the confines of the body by some kind of intrinsic mechanism, or it must be the result of external factors acting upon the cell. The clear weight of evidence

indicates that external agents such as radiation, chemicals and viruses account for the incidence of most human cancers. This, in one sense, is fortuitous. Were cancer to arise spontaneously from intrinsic causes we would have little or no preventative measures with which to fight it. It might be argued that one's genetic make-up could constitute an intrinsic cause. The fact is that heredity only indicates a genetic predisposition to cancer. It is not, of itself, the cause of the disease.

As it is, science has been able to establish clear correlations between certain environmental factors and the incidence of cancer, which allow us to take sensible precautions against the disease. One estimate suggests that some 90% of all human cancers could be prevented with a fuller knowledge of these external factors.[2] The principle environmental causes of cancer are outlined below.

### 1.2.1 X-Rays

A few years after the discovery of X-rays in 1895, technicians working in the field started to develop cancer. Little was known about the medical effects of this new form of radiation; indeed, one technician who died from metastatic cancer was known to have tested the X-ray tubes he was manufacturing by projecting the beam onto the back of his hand. By 1908, animal experiments had proved that X-rays could cause cancer and that the associated risk was proportional to the degree of exposure.[3] Despite this discovery, doctors in many countries continued to use X-rays to treat a variety of non-malignant diseases. In Israel, a common treatment for ringworm of the scalp was to administer a dose of X-rays to the head and upper body. Israel now has the third highest incidence of thyroid cancer in the world.[4] Denmark, by contrast, where X-rays have never been used to treat non-malignant diseases, has one of the lowest rates of thyroid cancer in the world.[5]

Attempts to use high doses of X-rays in therapy for other diseases, such as tuberculosis, have also proved ill-fated. One group of women in this category proved to be ten times as likely to develop breast cancer after their radiation treatment. There is also clear evidence that embryos exposed to X-rays *in utero* have a greater likelihood of developing leukaemia in early childhood.[6]

Improved shielding and focusing, coupled with sparing use of this powerful technique, have made the risks from diagnostic X-rays extremely small. If used carefully, there is no reason why X-rays should not continue to find applications in medicine and the sciences.

### 1.2.2 Radioactive Elements

As with X-rays, the crippling effects of radioactive elements have been well documented over the years. Marie Curie, who co-discovered the elements polonium and radium, died from an anaemia induced by her repeated exposure to radioactive ores. Another famous example of radium poisoning early this century involved a group of 1000 American women who were employed to paint the luminous figures onto watch dials.[7] In licking the points of their brushes to maintain a fine point, these workers unwittingly ingested dangerous amounts of radium, the chemical which gave the paint its luminous glow. The incidence of bone cancer among these women was subsequently found to be abnormally high.

The long-term consequence for the survivors of the atomic bombs dropped on Hiroshima and Nagasaki has been a legacy of leukaemia and cancers of the breast, lung, stomach, thyroid, lymphatic system and uterus. More recently, the American government has disclosed alarming statistics regarding its own post-World War II nuclear testing. 26 explosions were carried out from 1951-1958 in the Nevada desert. The occurrence of infant leukaemia in neighbouring southern Utah increased during this period and fell back to the national average after testing stopped. A similar trend was witnessed among the soldiers who were involved in military exercises near the test area.[8]

The Chernobyl nuclear disaster in 1986 has been profoundly damaging, not only in the former Soviet Union, but much further afield. Livestock in England and Wales showed contamination levels well in excess of safe limits in the months following the accident. [9]

### 1.2.3 Ultraviolet Radiation

Exposure to ultraviolet (UV) radiation is the principal causal factor in malignant melanoma. Statistics from several countries show that the incidence of this disease has been doubling every decade: only lung cancer

in women has been growing at a faster rate.[10] UV radiation, in large doses, is a mutagen which can induce cancer of the skin. The body's natural protection against UV radiation is the pigment melanin, synthesised in dedicated cells close to the surface of the skin. A clear correlation exists between skin type and the incidence of malignant melanoma. These factors will be discussed further in chapter 5.

### 1.2.4 Chemicals

Relatively few people handle carcinogenic chemicals on a daily basis, except for a limited number in science, medicine and industry. Stringent new legislation in this country (Control Of Substances Hazardous to Health act) has helped reduce the risks to employees working in these areas. The main threat to public health from chemicals comes as a direct result of our lifestyle in the late 20th century. Overcrowded cities, dominated by the motor car, belch carcinogenic fumes into the atmosphere, making city-dwellers more likely to contract cancers of the respiratory tract. Dietary changes, particularly an increased intake of fat and processed foods, have also been detrimental to our health. Ultimately, though, the biggest risk comes from tobacco. Lung cancer is the leading cause of cancer deaths among men, accounting for 30.2% of the total number in England and Wales in 1990.[11] The West of Scotland has the fourth highest male mortality rate due to lung cancer in the world.[12] Although breast cancer still predominates in females, the increased social acceptability of women smoking has seen lung cancer grow at an alarming rate in the past 25 years.

Cigarette smoke is a lethal cocktail of some 6000 chemicals, 50 of which are known carcinogens including arsenic, benzanthracene, benzene, naphthylamine, nitrosamines and polonium-210. The effect of smoking on life expectancy is well researched. A 30 year-old man smoking 40 cigarettes a day can expect to lose, on average, eight years from his life-span, as well as increasing his risk of cardiovascular and respiratory disease.[13] Of the 110692 deaths due to smoking in the UK in 1988, as many were from coronary heart disease as were from cancer (29% each).[14] The number of smoking related deaths in Scotland that year was 10617 (17% of all deaths) and the estimated cost to the NHS was £ 68,786,000.[15]

### 1.2.5 Oncogenic Viruses

Viruses are microscopic parasites which invade cells and use the metabolic 'factory' there to reproduce their own kind. Many thousands of viral particles may be produced in a matter of hours, draining the host cell of its resources and possibly causing it to rupture. The newly-liberated virus particles then infect further cells, propagating the disease throughout the body. Herpes, the common cold, influenza, polio and measles are all viral in origin.

Oncogenic viruses, however, are more subtle in their action. After entering a cell, the viral DNA becomes incorporated into the host cell's chromosomes, thereby altering its genetic programme. The net result is that the transformed cell continues to reproduce without restraint, passing its mutated 'immortal' genes on to its progeny. The proportion of cancer deaths due to oncogenic viral infection is relatively small. The chief protagonists are Herpes Simplex II, implicated in cervical and penile cancer, and Epstein-Barr virus which is associated with certain lymphomas and nasal cancers.

## 1.3 Cell Growth and Cancer

The preceding section has given an outline sketch of the various causes of human cancer. The common thread which links them all is the ability to modify the genetic programme of a cell adversely. But what constitutes 'normal' growth, and how does a cancerous cell differ from a healthy one? This section explores those questions as a prelude to section 1.4, where the current forms of cancer chemotherapy are discussed.

### 1.3.1 The Growing Cell

Since the elucidation of the structure of DNA by Crick and Watson in 1953, the science of cell biology has come to rapid maturation. Many secrets of the nucleus have been uncovered, particularly with respect to the processes surrounding DNA synthesis. Recent science has been geared towards understanding the peripheral processes which govern cell function, now that the central events in the nucleus are relatively well understood.

For many years it was thought that cells were constantly synthesising DNA between mitoses. It is now known that the cell cycle consists of at least

four distinct phases, classified as growth (G), synthesis (S) and mitosis (M) (Figure 1).

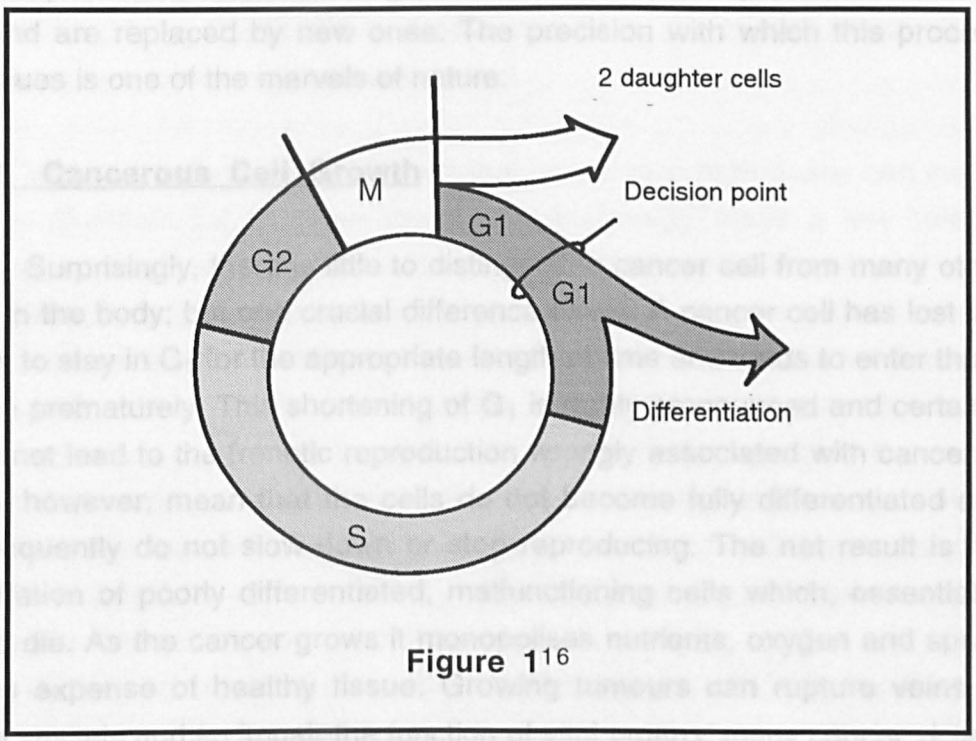



**Figure 1**[16]

$G_1$ is the period during which the nuclear material necessary for the synthesis of DNA is constructed, in preparation for the S phase. The replication of DNA takes place during this phase, followed by a period during which the nucleus prepares itself for mitosis ($G_2$). Separation into two daughter cells (M) completes the cell cycle. The time taken to complete one growth cycle depends entirely on the type of cell: liver cells take 400 days to replicate, whereas cells in the lining of the gut require only 13 hours.[17] The lengths of the S, $G_2$ and M phases are remarkably consistent across all cell types: typical values are 8, 3 and 0.7 hours respectively. Consequently, it is the length of time spent in the $G_1$ phase which determines the overall rate at which a cell replicates. This period is known as $G_1$ arrest.

One of the key properties of a healthy cell is the ability to 'decide' how long to remain in $G_1$ arrest. If, for example, part of the liver was surgically removed, the $G_1$ period of the liver cells would decrease from hundreds of days to a matter of hours. After the tissue had been repaired, the cells would revert to their normal $G_1$ period.

A further characteristic of normal cells is differentiation - the process by which they assume a particular role and function. In embryonic development, for instance, certain cells develop into organ tissue, while others form bone and cartilage. As cells start to adopt a more specialised

role, so their rate of reproduction slows down. In addition, cell-death is programmed into them during this process. Once adulthood has been reached, those tissues which regenerate exist in a steady state as old cells die and are replaced by new ones. The precision with which this process continues is one of the marvels of nature.

### 1.3.2 Cancerous Cell Growth

Surprisingly, there is little to distinguish a cancer cell from many other cells in the body; but one crucial difference exists. A cancer cell has lost the ability to stay in $G_1$ for the appropriate length of time and tends to enter the S phase prematurely. This shortening of $G_1$ is rarely pronounced and certainly does not lead to the frenetic reproduction wrongly associated with cancer. It does, however, mean that the cells do not become fully differentiated and consequently do not slow down or stop reproducing. The net result is the generation of poorly differentiated, malfunctioning cells which, essentially, fail to die. As the cancer grows it monopolises nutrients, oxygen and space at the expense of healthy tissue. Growing tumours can rupture veins or blood vessels and so impair the function of vital organs: many cancer victims die as a result of organ failure. If cancer cells enter the circulatory or lymphatic system, secondary colonies can develop in other sites around the body by a process known as metastasis. By this stage of the disease prognosis is very poor. Early diagnosis and treatment are therefore essential for the long-term prospects of the sufferer.

## 1.4 Current Cancer Therapies

A common misconception among the general public is that there is '*a* cure for cancer'. In reality it seems unlikely that a single 'blanket' cure will ever be found, given that more than 100 different types of cancer are known.[18] Current therapies have been successful in extending the life-spans of cancer patients, sometimes dramatically, but there is room for a great deal of improvement. Broadly speaking, three types of treatment are used in practice today: surgery, radiotherapy and chemotherapy.

Surgery has proven particularly useful in cases of malignant melanoma. This disease, which produces warty, dark-coloured lesions on the skin, can be detected early and treated before the cancer has a chance

to metastasise. Prognosis is good for patients who discover the disease in its early stages and have the tumour excised immediately (Chapter 5, Section 5.1.2). Since most cancers originate inside the body, however, it is often too late for surgery by the time the disease has been diagnosed. At this stage radiotherapy or chemotherapy is required.

It has already been mentioned that radiation in high doses can cause cancer (Section 1.2.1). Nevertheless, radiotherapy plays a key role in treating internal cancers, provided they are still well defined and non-metastatic. Both X-Rays and radioactive elements such as cobalt-60 can be used for this purpose. Although radiation harms healthy and diseased tissue, cells with a shorter cell cycle are particularly sensitive to its effects. Consequently, a cancer will be more susceptible to damage than the tissue from which it derives. It also means that rapidly dividing cells, such as those found in the lining of the stomach and in bone marrow, will be the hardest hit. The side effects of radiotherapy, vomiting, nausea, diarrhoea and hair loss, are a direct consequence of this.

Cells which are in $G_1$ arrest, particularly those with long $G_1$ phases, are less likely to be damaged by radiation than those in the S phase. Since at any one time a considerable fraction of cancer cells in a tumour will be in $G_1$, radiotherapy takes place in several sessions over a period of months. This allows resting cancer cells time to move into the S phase, as well as giving the patient a chance to recover from the side effects of the treatment. Radiotherapy is frequently used after surgery to destroy the small proportion of cancer cells still present. This can never be done with complete certainty though, and even one surviving cancer cell allows the possibility of recurrence in later years.

The final treatment in common use is chemotherapy, the administration of drugs which interfere with the processes of cell division. The drugs in question are largely non-selective and only have a therapeutic value for the same reasons as radiation, namely the increased turnover of cancer cells. As with radiotherapy, treatment is spread over several sessions and causes harmful side effects. Some cancers are known to be sensitive to particular chemical agents; methotrexate, for example, achieves cures in more than 60% of cases of choriocarcinoma.[19] Unfortunately, cancers also have the ability to develop resistance to drugs. Combination chemotherapy, where a cocktail of two or three different agents is administered, is one way of surmounting this problem. Chemotherapy may be used in combination

with surgery and radiotherapy and is the only real hope for patients with metastatic cancer.

Since the subject of this thesis is the development of novel anticancer drugs, it seems worthwhile to give a brief overview of the main classes of drugs currently in use. Section 1.5 reviews the mode of action of these drugs and Section 1.6 points the way forward to a more sophisticated chemotherapy.

## 1.5 Classes of Chemotherapeutic Agents[20, 21]

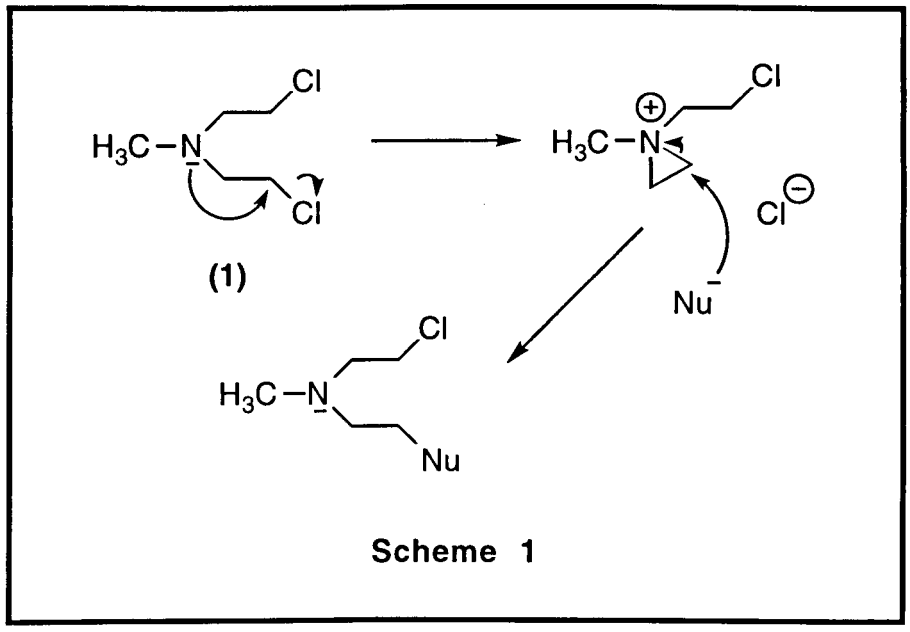
It is perhaps ironic that the discoveries which fuelled the search for chemotherapeutic agents over the past 50 years should have had their origin in the science of warfare. Mustard gas, the dreaded poison of the First World War, became the model upon which many of today's most successful anticancer agents have been based. Although thousands of chemicals have been synthesised and investigated for chemotherapeutic value over the past 50 years, only 40 or so are currently in clinical use. For our purposes these can be divided into four broad classifications: alkylating agents, antimetabolites, natural products and miscellaneous compounds.

### 1.5.1 Alkylating Agents

This chemically diverse range of compounds, of which nitrogen mustard (**1**, Scheme 1) is the parent, share the ability to form covalent bonds with nucleophilic centres in biological molecules. Alkylating agents can react with amine, phosphate, sulfhydryl and hydroxyl groups on crucial cellular molecules like nucleic acids, nucleotides and enzymes and so impair their normal function. The effects of alkylation are most lethal when the agent is bi-functional, that is, it has two active alkylating groups. Agents such as these can crosslink DNA and make it impossible for the individual strands of the double helix to 'unzip' during replication. The cell becomes locked in the S phase, cannot progress through the cell cycle and therefore dies. Monofunctional alkylating agents cannot form crosslinks, but they can damage the cell by confusing cytosine-guanine base-pairing during DNA synthesis.

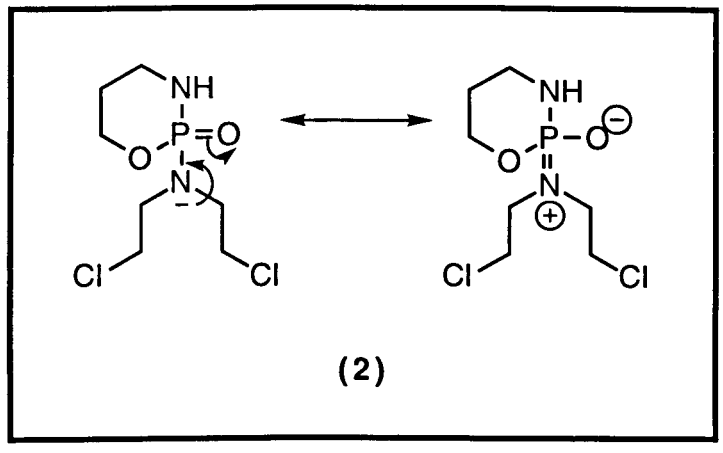
Nitrogen mustard exerts its toxicity by undergoing an intramolecular substitution reaction, expelling chloride ion to form an aziridine (Scheme 1).

This highly electrophilic group is an ideal alkylating agent, reacting readily with any good nucleophile. It is, however, too reactive to be of much clinical use.



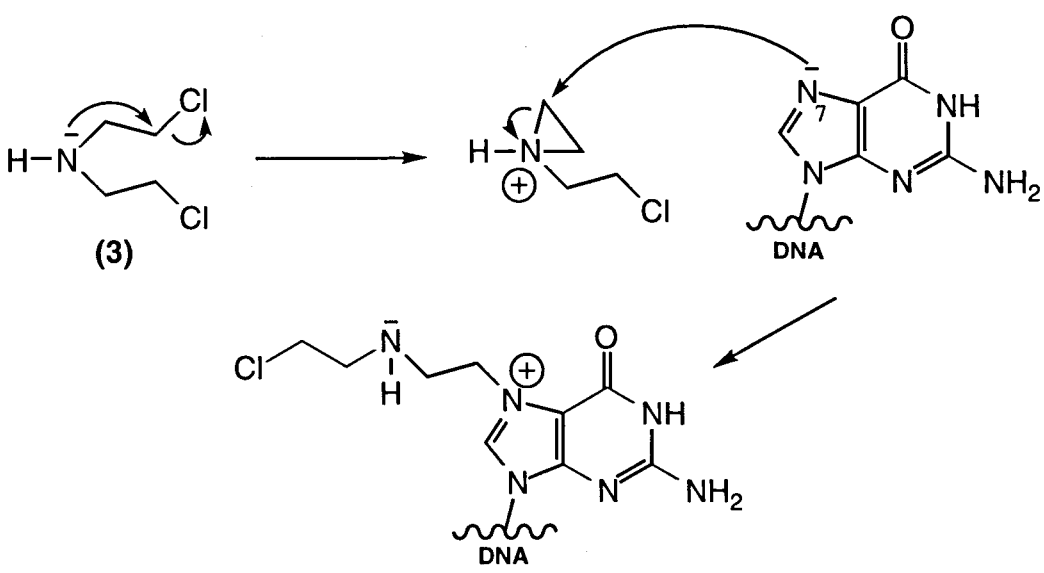

**Scheme 1**

Cyclophosphamide (**2**) is a closely related alkylating agent which is widely used in cancer therapy. It has been particularly useful in treating carcinoma of the breast and of the bronchus. The delocalisation of the nitrogen lone pair, coupled with the increased bulk due to the ring make this a more stable drug.



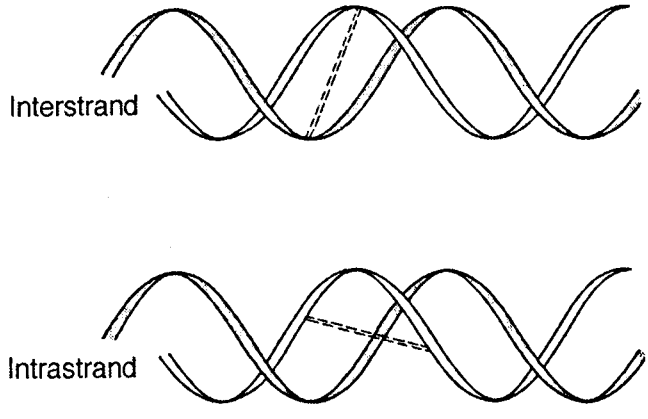

**(2)**

Although inactive itself, cyclophosphamide releases a number of cytotoxic compounds upon metabolism. Scheme 2 illustrates the reaction between a principal metabolite of cyclophosphamide, nor-nitrogen mustard (**3**), and a key nucleophilic site on DNA, N-7 of guanine.




**Scheme 2**

This single alkylation may lead to mispairing of bases or strand breakage. The second chloroethyl group could, of course, react with a further molecule of guanine on the opposite DNA strand to form a cross-link shown schematically in Figure 2.

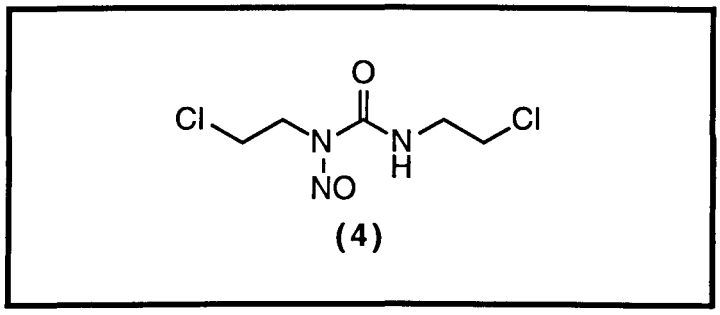



DNA crosslinking

**Figure 2**[22]

The success of cyclophosphamide has seen the development of other structurally related drugs, such as ifosfamide, melphalan and chlorambucil, each of which has found specific clinical applications. Alkylating agents which are administered directly in the form of an aziridine include thiotepa, particularly useful in cases of bladder cancer, and the related hexamethylmelamine.

A further useful series of drugs are the nitrosoureas, lipid soluble alkylating agents typified by bischloroethylnitrosourea (BCNU, **4**).




**(4)**

In addition to the alkylating properties associated with the chloroethyl moiety, metabolites of BCNU are also able to inhibit the enzyme DNA polymerase. Since these agents can pass the blood-brain barrier they have found some use in treating cerebral tumours, but their only real clinical application is in the management of lymphomas. A severe drawback to their usefulness is delayed-action toxicity to bone marrow.

### 1.5.2 Antimetabolites

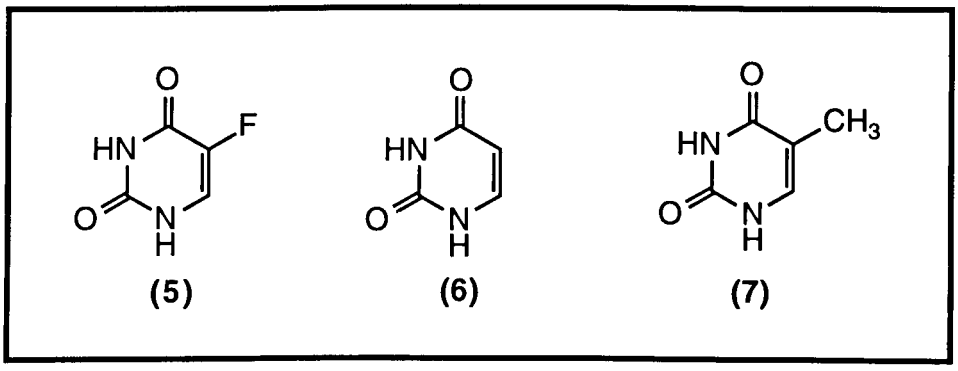
Before a cell can enter the S phase of its cycle it must build up a large reserve of the proteins required for the synthesis of nucleic acids. These proteins are themselves assembled from essential metabolites, such as amino acids, present in the cell. It is vital for cell function that these proteins are correctly synthesised: a single alteration in the primary structure of an enzyme, for instance, can render it totally ineffective.

Antimetabolites are chemicals which mimic natural metabolites and compete with them as enzyme substrates in protein synthesis. If incorporated into nuclear material, antimetabolites throw the normal recognition processes of the cell into confusion. Enzymes become dysfunctional or irreversibly inhibited, RNA cannot function properly and consequently the cell comes to a standstill. In one sense, these compounds are acting as metaphorical 'Trojan horses' - the consequence of

incorporating them in nuclear reactions doesn't become apparent until it's too late.

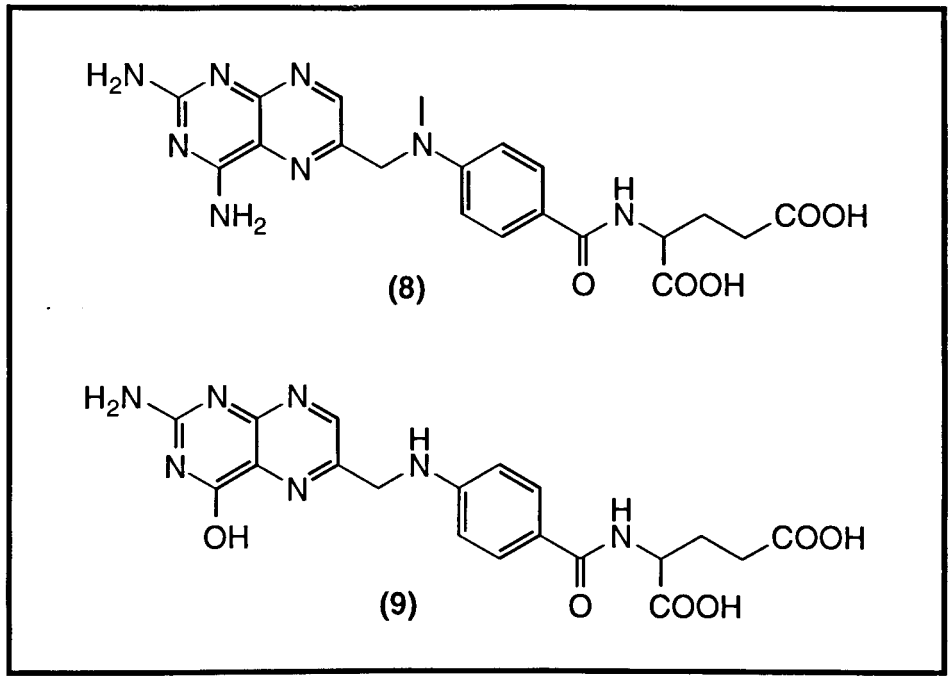
5-Fluorouracil (5-FU, **5**), a mimic for the pyrimidine bases uracil (**6**) and thymine (**7**), is one of the most important compounds of this genre. 5-FU is known to inhibit cell division in two ways. It blocks the enzyme thymidilate synthetase which is essential in the biosynthesis of pyrimidines. It can also become fraudulently incorporated into RNA in the form of 5-FU nucleotides and so cause confusion in base pairing during the process of transcription. The relative importance of the two mechanisms is the subject of much debate.

5-FU is used principally in the management of solid tumours and cancers of the breast and gastrointestinal tract. Its main side effects are bone marrow suppression and sickness which may be severe in the case of high doses.




Methotrexate (**8**) is an analogue of the vitamin folic acid (**9**), the reduced form of which plays a role in the synthesis of purines. The enzyme which effects this reduction, dihydrofolate reductase, is inhibited by methotrexate. Since the affinity of methotrexate for the enzyme is five orders of magnitude greater than that of dihydrofolic acid, the enzyme will preferentially reduce the antimetabolite. The resultant lack of tetrahydrofolic acid means that the cell cannot produce purines or pyrimidines, nor, therefore, DNA. Methotrexate is used in cases of acute leukaemia and is particularly efficacious in treating choriocarcinoma. The principal toxic effects are bone marrow suppression and gastrointestinal upset.

Other compounds in this class are cytosine arabinose, a nucleoside mimic used in the treatment of myeloid leukaemia in adults, 6-mercaptopurine, a purine antagonist, and 6-thioguanine which also inhibits purine synthesis.




### 1.5.3 Natural Products

Unlike the preceding two classes of anticancer compounds, natural products have no common mode of action: they are best dealt with on an individual basis.

#### 1.5.3.1 Vinca Alkaloids

The Vinca alkaloids, derived from the periwinkle plant *Vinca rosea*, inhibit cell growth by binding to the protein tubulin. During the period of mitosis called the metaphase, the doubled set of chromosomes orient themselves across the cell's equator in preparation for separation. An array of microtubules called the 'spindle' acts as a frame upon which the chromosomes position themselves. By binding to tubulin, Vinca alkaloids prevent the construction of microtubules and so halt the cell in this phase of mitosis.

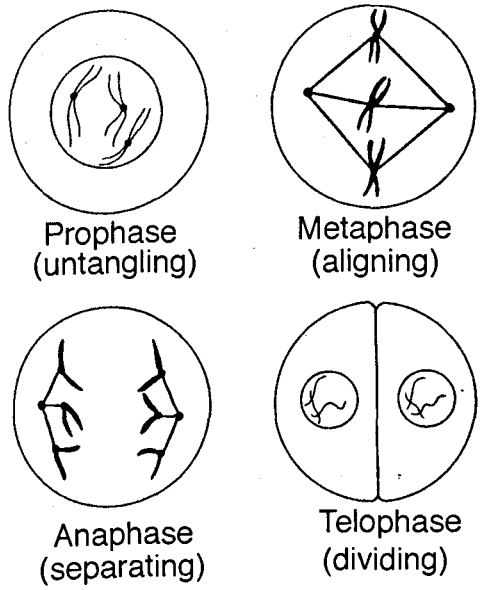
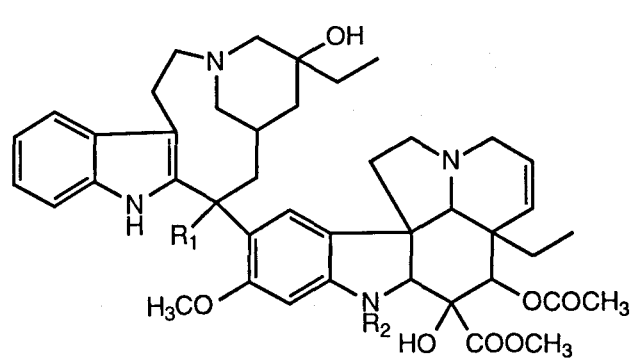



**Figure 3** [23]

The three alkaloids in this class, vincristine, vinblastine and vindesine, are all in clinical use. Vincristine (**10**) is of particular utility against leukaemias and lymphomas and is notably less damaging to the bone marrow than many other anticancer agents. Vinblastine (**11**) is used in the treatment of Hodgkin's disease and vindesine, the most recently introduced of these alkaloids, has performed well against malignant melanomas.

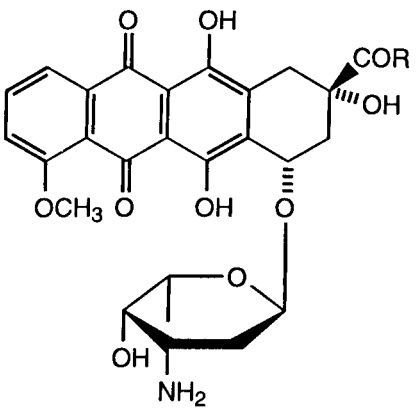



**(10)** $R_1$= $COOCH_3$ , $R_2$= CHO

**(11)** $R_1$= $COOCH_3$ , $R_2$= $CH_3$

### 1.5.3.2 Anthracycline Antibiotics

These drugs, produced by different strains of *Streptomyces*, emerged from the search for new antibiotics in the 1960s. Daunorubicin (**12**) and doxorubicin (**13**) show good activity against a broad range of solid tumours, but suffer from marked side effects, particularly bone marrow suppression.




**(12)** R= $CH_3$
**(13)** R= $CH_2OH$

The mode of action of these drugs is complex and at least five potential mechanisms for cytotoxicity have been proposed.

1) Intercalation (insertion with some form of binding) between the two strands of the DNA helix. It is believed that these compounds intercalate perpendicular to the long axis of the double helix, leading to partial unwinding of the DNA and loss of function.

2) Membrane binding. This alters the permeability of the cell and may change the pattern of ion transport.

3) Radical formation under enzymatic transformations, leading to indiscriminate alkylation.

4) Chelation of metal ions, so impairing cell function and possibly forming toxic organometallic compounds.

5) Direct alkylation.

Despite the excellent broad-spectrum toxicity of these agents, they find little use because of their dose-limiting side effects. The search for less debilitating isomers has yielded compounds like the anthracenediones mitoxantrone and bisantrene which are less toxic but have similar antitumour activity.

### 1.5.3.3 Non-Anthracycline Antibiotics

Although not as prolific as the anthracycline antibiotics, this group of compounds are also of value in cancer treatment. Actinomycin-D was identified in the 1940's as a product of the soil fungus *Actinomyces*. Its anticancer activity is thought to be due to its binding to guanine and subsequent intercalation between the DNA helix. Actinomycin-D is primarily used to treat paediatric tumours.

Bleomycin is a family of glycopeptides derived from *Streptomyces verticillis*, believed to exert toxicity by intercalating between the strands of DNA and forming free radicals which can alkylate or cause DNA strand breaks. The major use of bleomycin is in combination with other drugs in the treatment of testicular cancer and lymphomas. As with vincristine, bleomycin causes notably less damage to the bone marrow than most other anticancer agents.

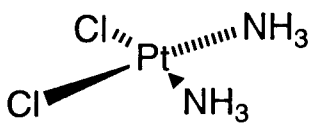
Mitomycin-C, again isolated from *Streptomyces* species, is believed to act as both an alkylating agent and a radical generator. Its principal use is in the treatment of gastrointestinal cancers.

### 1.5.4 Miscellaneous Anticancer Agents

Several anticancer drugs do not fit into any of the above classifications. Hydroxyurea, first synthesised over a century ago, inhibits ribonucleotide reductase, the enzyme which generates the deoxyribose nucleotides necessary for DNA synthesis. L-Asparaginase, itself an enzyme,

destroys leukaemic cells by depriving them of the L-asparagine they require for survival. Although interesting from a scientific perspective, neither of these drugs are widely used in practice.

Cisplatin (**14**), by contrast, is an important drug in the clinician's armoury. One of the few inorganic chemotherapeutic agents, cisplatin acts in a manner directly analogous to that of alkylating agents. The chloride ions can be displaced by nucleophiles on nucleic acids or proteins. N-7 of guanine is again the preferred site, although relatively few cisplatin molecules achieve DNA cross-linking. Interestingly, the *trans*-isomer appears to be completely unable to induce cross-strand linkages of the double helix.




**(14)**

Cisplatin finds applications in testicular cancer and in combination with other drugs in treatments for a variety of solid tumours. Its main drawbacks are severe nausea and vomiting, coupled with potential damage to the kidneys.

## 1.6 The Way Forward ?

The preceding sections have given a thumbnail sketch of cancer: its causes, character and cures. The past fifty years have seen undoubted progress in our understanding of the disease and the cancer patient may now be allowed a measure of hope; tempered, it must be said, with realism. What progress has been made is best described in terms of containment rather than cure, although mortality from Hodgkin's disease, leukaemia and testicular cancer has fallen markedly over this period.[24] The anticancer drugs in clinical use are, at best, 'healing poisons'. Acting indiscriminately against both cancerous and healthy tissue, their toxicity renders chemotherapy traumatic for the victim. This may seem a small price to pay for the chance of prolonging life, but it illustrates vividly the need for new,

less toxic, chemotherapeutic anticancer agents. The questions facing medicinal chemists in this area are essentially the same as they were 50 years ago. What distinguishes cancer cells from normal cells? How can this be exploited? Can cancer cells be targeted specifically?

Recent advances in the biological sciences give reasons for optimism as more and more mysteries of the cell are unravelled. Through an understanding of normal cell function we can come to a greater understanding of aberrant function, as in the case of cancer. This new knowledge enables us not only to apply principles of rational design to our existing anticancer agents, but also to identify completely new targets for drug development. The nucleus is no longer the sole focus for our efforts: the horizon has expanded to encompass the whole cell. It may well be that this shift of emphasis opens the doorway to a new generation of sophisticated, less harmful, anticancer drugs.

Chapters 2-4 of this thesis detail one approach to the rational design of a new breed of anticancer agent known as 'tyrphostins'.[25] These compounds are believed to inhibit cell growth by interrupting biochemical processes at the surface, rather than the nucleus, of the cell.

Chapter 5 and 6 describe how the unique properties of melanocytes, the melanin producing cells in the body, can be exploited to combat malignant melanoma.

# 2

# Cell Signalling and Cancer

It has been clear for some time that structural refinement of existing anticancer drugs is unlikely to bring much improvement in their therapeutic indices. Drugs which indiscriminately inhibit important reactions in the cell nucleus will always be handicapped by their extreme dose-limiting toxicity. Without denigrating the clinical uses of existing anticancer agents, it is evident that progress along the same lines will lead down a cul-de-sac. There is an urgent need for new strategies as we work towards a more refined cancer chemotherapy.

Fresh impetus has come as a direct result of the extraordinary progress being made in the fields of molecular biology and genetics. In particular, the burgeoning array of information related to cell growth has furnished the medicinal chemist with a host of new biochemical targets. This chapter highlights one of these new targets, a family of enzymes called protein tyrosine kinases (PTKs) and explains how PTK inhibition can arrest the growth of transformed cells.

## 2.1 Growth Factors and Cell Signalling

The science of growth factors and their involvement in cell signalling is still in its infancy, but rapid progress has been made since the discovery in 1954[26] of nerve growth factor, the protein from which this class of compounds derives its name. Initial studies in this area were stimulated by the observation that mammalian cells growing in culture required serum for good proliferation. The clear inference was that serum contained agents with the ability to promote cell growth. In subsequent years, these growth factors were isolated, characterised and synthesised and they are now well established as key-players in the regulation of cellular proliferation, differentiation and death. Growth factors are small proteins, typically 50 - 100 amino acid residues long. Figure 4 illustrates their mode of action schematically. Upon binding to specific cell surface receptors (1) the growth factor activates the internal catalytic domain of the receptor (2). This causes

a cascade of biochemical reactions (3) which culminates in cell growth and division (4).

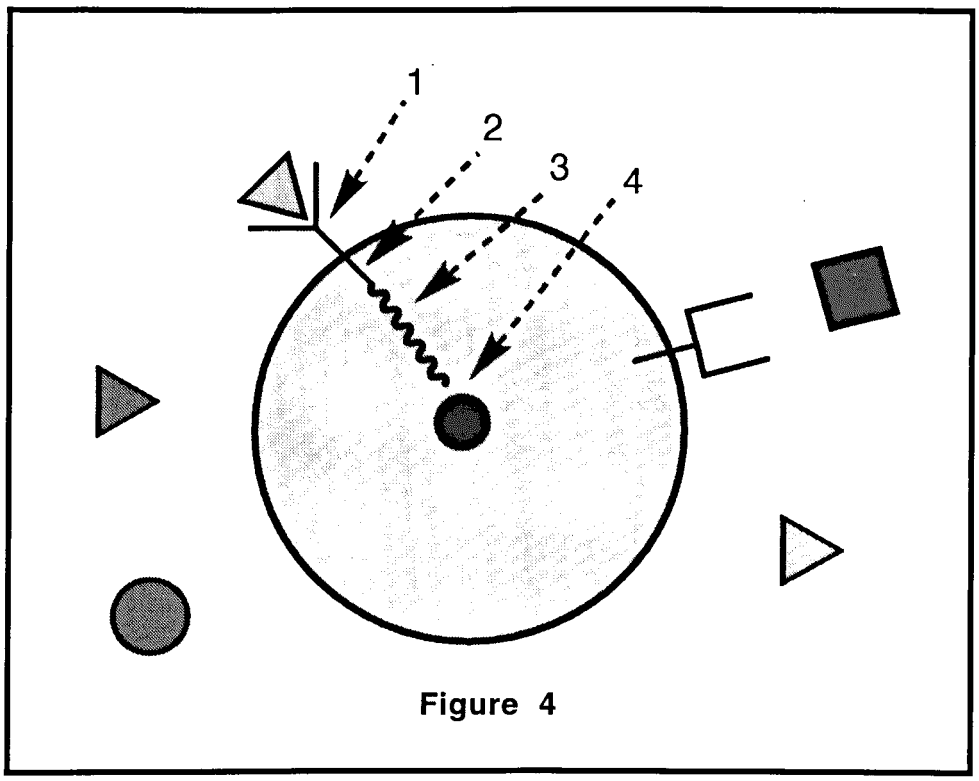



**Figure 4**

The functions of many growth factors and their receptors have been established and are well documented.[27] The exact sequence of events in the signalling cascade is the subject of much ongoing research.[28, 29]

There is enormous potential for exploiting these discoveries in fighting cancer. Given that the ultimate aim of any cancer therapy is to arrest the growth of transformed cells, there would seem to be a vast number of points along the signalling pathway at which the signal could be intercepted. Figure 5 illustrates the complexity of the network of cellular reactions involved in PTK mediated growth. Any of the signalling proteins represented could be viable targets for drug design. Indeed, choosing which target to focus on has become a major preoccupation for the pharmaceuticals industry.

## 2.2 Signalling Proteins and Cancer

The crucial observation which implicated growth factors in cancer was the discovery that cancer cells growing in tissue culture require less serum to support their growth than normal cells. The concept emerged that

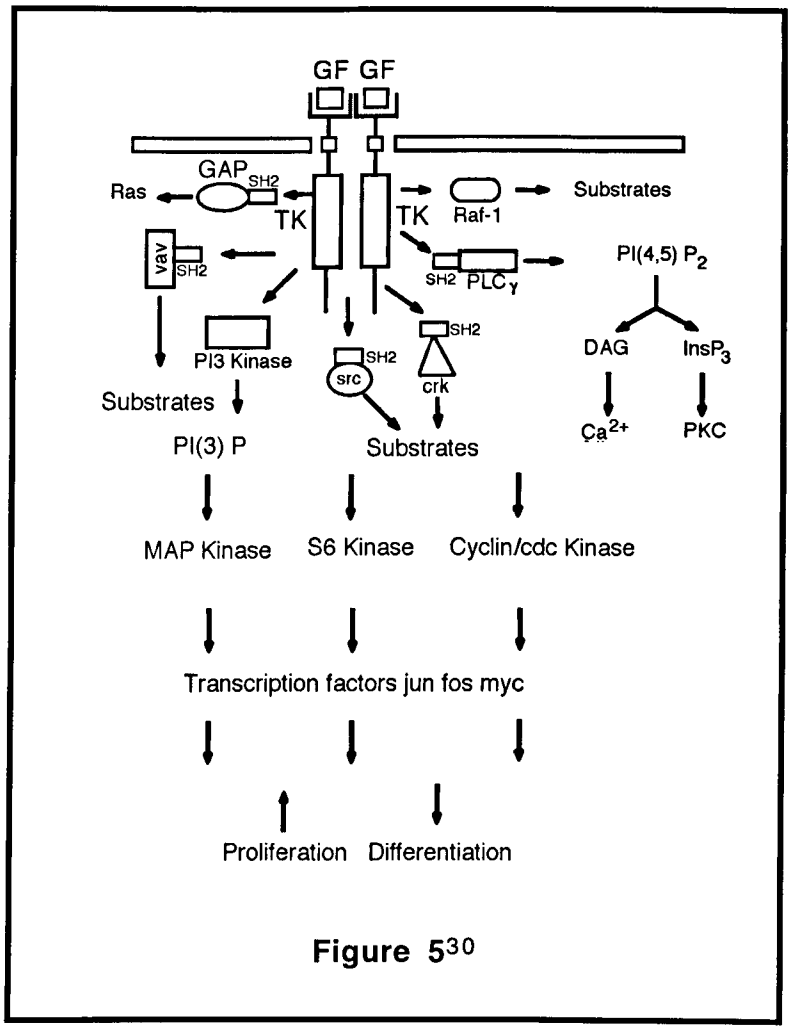



**Figure 5**[30]

Abbreviations: GF, growth factor; TK, tyrosine kinase; GAP, GTPase activating protein; SH2, *src* homology domain 2; $PLC_\gamma$, phospolipase $C_\gamma$; PI3-kinase, phosphatidylinositol-3'-kinase; $PI(4,5)P_2$, phosphatidyl inositol-4,5-biphosphate; $InsP_3$, inositol (1,4,5) triphosphate; PI(3)P, phosphatidylinositol-3-phosphate; DAG, diacylglycerol; PKC, protein kinase C.

transformed cells were overproducing growth factors, leading to uncontrolled growth either in themselves ('autocrine' growth) or neighbouring cells ('paracrine' growth). Figure 6 illustrates both types of growth.[31] The oncogene *sis* is one example of a gene which can induce autocrine growth. *Sis* encodes the B chain of platelet derived growth factor (PDGF) which modulates the growth of connective tissue cells. Simian sarcoma virus, derived from a monkey osteosarcoma, is known to overexpress this oncogene.[32] The excess PDGF generated interacts with both intracellular and surface receptors to stimulate proliferative growth.[33] Other oncogenes believed to act in this manner are *fgf-5, hst* and *int-2* which encode for fibroblast growth factor-like molecules. Interestingly the fidelity of the coding regions of these genes is preserved, indicating that

overexpression, rather than mutation, is the cause of their transforming nature.[31]

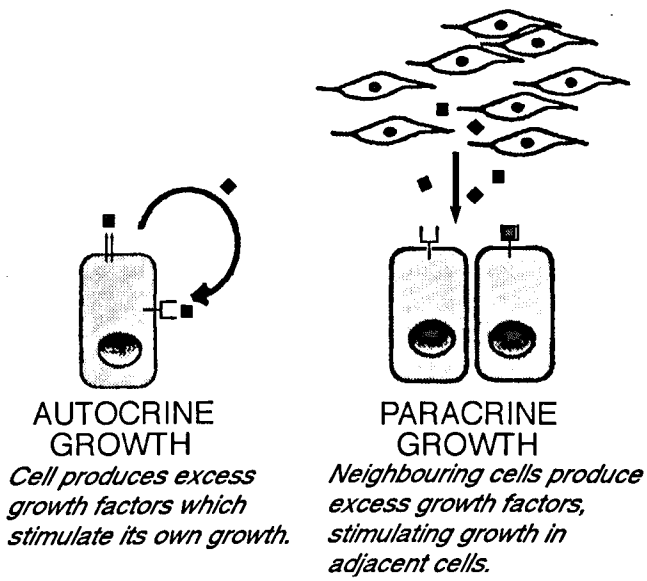



**Figure 6**[31]

Growth factor receptors are also known to be involved in the onset of cancer. Although overexpression of receptor-encoding genes is an established phenomenon, mutation of these genes to produce aberrant receptors is much more common. A frequent hallmark of mutated receptors is ligand independent signalling where the need for a growth factor is completely circumvented. In effect, these receptors can send constant growth signals to the nucleus of their own volition. The *erb-B* oncogene, which encodes a truncated form of the epidermal growth factor (EGF) receptor, forms part of the genome of the avian erythroblastosis virus. The EGF receptors encoded by *erb-B* have no external ligand binding domain and a shortened cytosolic C-terminus;[34] they are thought to induce cancer by ligand independent signalling.

The *erb-B2* oncogene (also known as HER-2) encodes for a 185 kD protein which is 44% homologous to the EGF receptor.[35] Unlike *erb-B*, this gene encodes for a fully developed receptor with external ligand binding and internal tyrosine kinase domains.[36] *Erb-B2* is structurally related to the rat oncogene *neu* which becomes transformed by point mutation of a single nucleotide. This mutation causes substitution of glutamic acid for valine-664 in the transmembrane segment of the *neu*-encoded receptor,[37] a change

which switches the receptor 'on' permanently. It is unclear whether or not *erb-B2's* oncogenic nature is due to mutation or overexpression, but point mutation of the equivalent valine in the normal *c-erb-B2* gene certainly induces transforming character.[38] The oncogenes *fms, trk* and *kit* are also known to encode for membrane receptors.

These examples are merely scratching the surface of what is now a vast area of research. When genes encoding for intracellular transducers and nuclear transcription factors are taken into account, it is apparent that at least half of all known oncogenes encode for signalling proteins.[39] Figure 7 highlights the diversity of oncogene products which present themselves as possible targets for drug development.

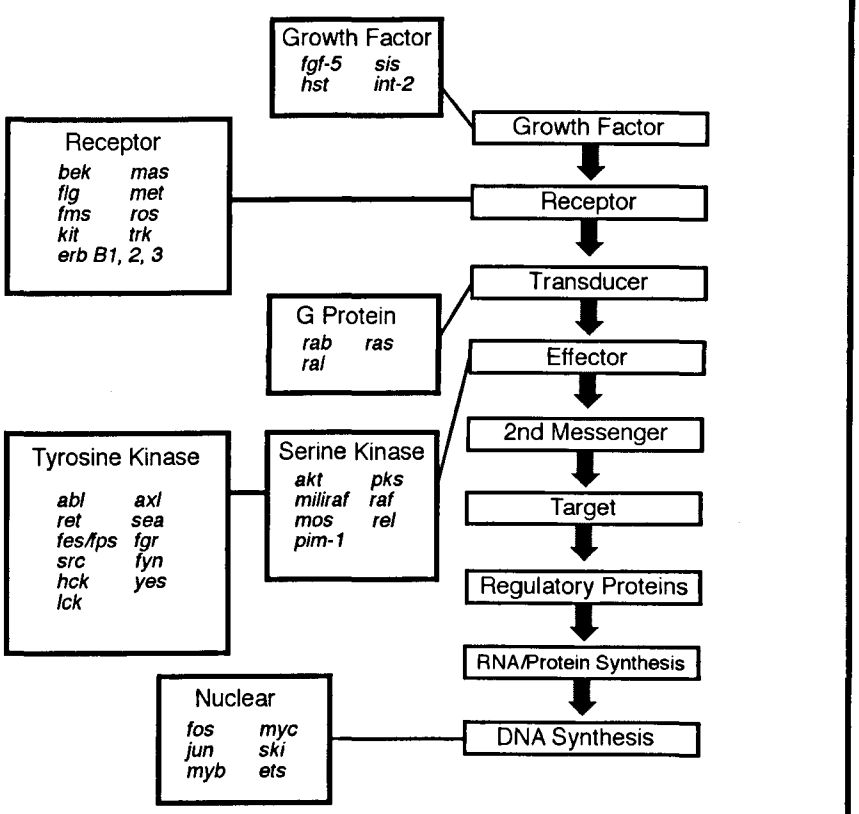



**Figure 7**[40]

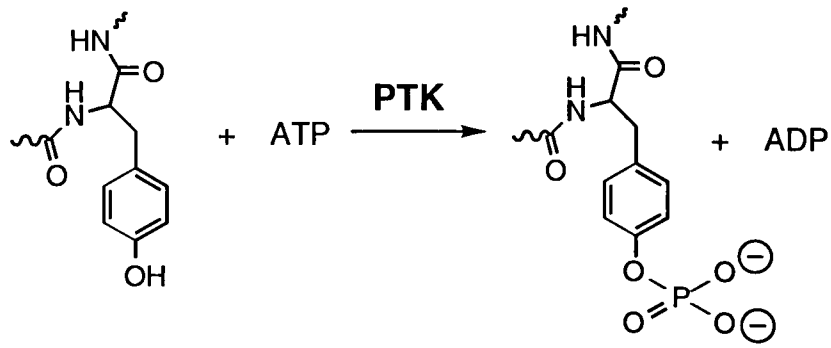
The potential for developing new cancer chemotherapies is obvious, even from this brief overview. Many questions about signalling pathway blockers remain, however. Will they simply be cytotoxic agents, no better than existing anticancer drugs? This is a valid question, given that normal and transformed cells share the same signalling pathways and it's unlikely that any selectivity could be built in to guide the drugs to the cancer cells. Despite this, the way the signalling network has evolved may work in our

favour. There is considerable redundancy within the cellular network: in other words, there are several ways for a biochemical signal to get from 'A' to 'B'. The cell has a natural ability to cope with changes in its environment and if a particular signalling pathway is blocked, others may be used in its place. This gives healthy cells a form of resilience. Cancerous cells share this resilience, but they frequently exhibit overexpression of a specific signalling pathway. Administration of a drug which inhibits that pathway should arrest the growth of transformed cells without significantly debilitating the healthy cells around it. Once the runaway growth of the transformed cells has been suppressed, it would be possible to eradicate them by administration of small doses of conventional anticancer drugs. Combinations of cytostatic and cytotoxic drugs such as that described above are widely used in clinical medicine.

A further question which needs to be addressed is *where* to arrest the growth signal. There are almost as many opinions on this issue as there are researchers in the field, but broadly speaking the answer lies in striking a balance between drug specificity and toxicity. Targets close to the cell membrane, including growth factors and receptors, allow a high degree of specificity because of their unique function. As described above, however, there is also the risk that redundancy in the system could allow the signal to be re-routed through alternative pathways. Inhibition further down the chain is likely to cause unacceptable toxicity as too many signal transduction pathways would be knocked out for the cell to function properly. Research is ongoing at almost every available junction in the signalling network, but for the purposes of this discussion it is necessary to focus on the area relevant to this research; protein tyrosine kinases.

## 2.3 Protein Tyrosine Kinases.

The term 'protein tyrosine kinase' encompasses a family of some 100 or so related enzymes with a pivotal role in normal cell division and abnormal cell proliferation.[41] PTKs are an integral part of many membrane bound receptors but can also be found as nonreceptor kinases in the cytoplasm. The function of PTKs is to catalyse the transfer of the γ-phosphate of ATP to the hydroxyl group of tyrosine on certain key proteins. This reaction is illustrated in Scheme 3.




**Scheme 3**

Overexpression of PTK activity is a common feature of many proliferative diseases including cancers, psoriasis, atherosclerosis and restenosis.[42]

## 2.3.1 Structure and Function of Receptor PTKs

Despite evolutionary differences, all members of the receptor PTK family share three conserved features: an extracellular ligand binding domain, a transmembrane helix which acts as an anchor and an internal PTK domain. A schematic diagram of a receptor PTK is given in Figure 8.

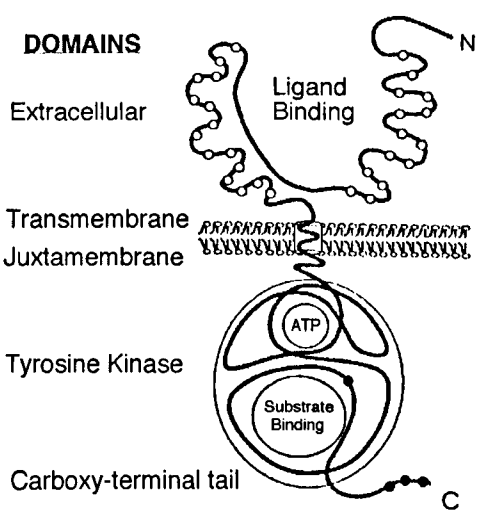



**Figure 8**[43]

Many of the common growth factor receptors such as epidermal growth factor receptor (EGFR), insulin receptor (InsR) and platelet derived

growth factor receptor (PDGFR) share these common features. The primary difference which distinguishes them from one another is the nature of their ligand binding domains (Figure 9). This enables receptors to be selective in accepting or rejecting proteins which attempt to bind to them.

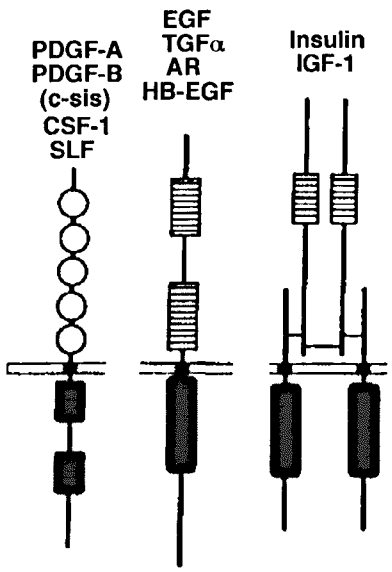



**Figure 9**[44]

Binding of a growth factor causes a conformational change in the receptor leading to dimerisation or oligomerisation. Subsequently, specific tyrosine residues in the receptor are phosphorylated by ATP, the reaction being catalysed by the receptor PTK.[45] It is uncertain whether this process involves self-phosphorylation or *trans*-phosphorylation but what is evident is that only a very few specific tyrosine residues participate. In the case of EGFR there are 4 known sites, all located at the carboxy terminal end of the receptor.[46] In effect this phosphorylation 'switches on' the receptor's latent tyrosine kinase activity. The phosphorylated tyrosine residues in the receptor have a high binding affinity for cytoplasmic proteins which contain non-catalytic domains of about 100 residues known as *src* homology regions (SH2). As a result, once the receptor has been activated, the phosphorylated receptor tyrosines can bind signalling proteins such as GAP, PI3-kinase, PLC and the serine/threonine kinase raf-1, drawing them from the cytoplasm towards the PTK domain of the receptor. The substrate proteins become activated in their signalling function by conformational change or a further

PTK catalysed phosphorylation, thus starting the 'knock on effect' which leads to mitosis.[30, 47, 48]

In summary, the binding of a growth factor activates a receptor's latent tyrosine kinase activity and causes a mitogenic signal through activation of cytoplasmic signalling proteins.

### 2.3.2 Inhibition of Receptor PTKs

As discussed above, the signalling reaction to be blocked is the phosphorylation of intracellular tyrosine residues by ATP, catalysed by receptor PTKs. The function of the enzyme is to hold the substrates in position while transferral of the γ-phosphate takes place. It is reasonable to expect the catalytic domain to consist of at least two specific binding sites, one for ATP and the other for the tyrosine residue. Peterli *et al.*[49] have postulated a transition state in the EGFR catalysed phosphorylation (Figure 10) where the γ phosphorus atom in ATP is pentacoordinated and oxygen atoms on the β and γ phosphates are complexed to bivalent metal ions, usually $Mn^{2+}$ or $Mg^{2+}$. Lysine 721 of the EGFR has been shown to participate in ATP binding. Point mutation of this residue arrests all EGF dependent cell functions.[50] This bi-substrate model was confirmed recently when the crystal structure of the insulin receptor's TK domain was elucidated.[51] In its unphosphorylated, inactive form, the catalytic site consists of an amino-terminal lobe, believed to bind ATP, and a nearby larger lobe containing the peptide binding site. Interestingly these 'pockets' are oriented away from one another in the inactive molecule, confirming that the TK activity of the receptor is latent until it is autophosphorylated. An extended sequence in the large lobe called the 'activation loop' contains the three tyrosines which become autophosphorylated during receptor activation. Crucially, one of these (Tyr 1162) sits directly in the active site, hydrogen bonded to Asp 1132. This precludes any substrate phosphorylation until Tyr 1162 is itself phosphorylated, bringing about the conformational change which frees the enzyme to express its PTK activity.

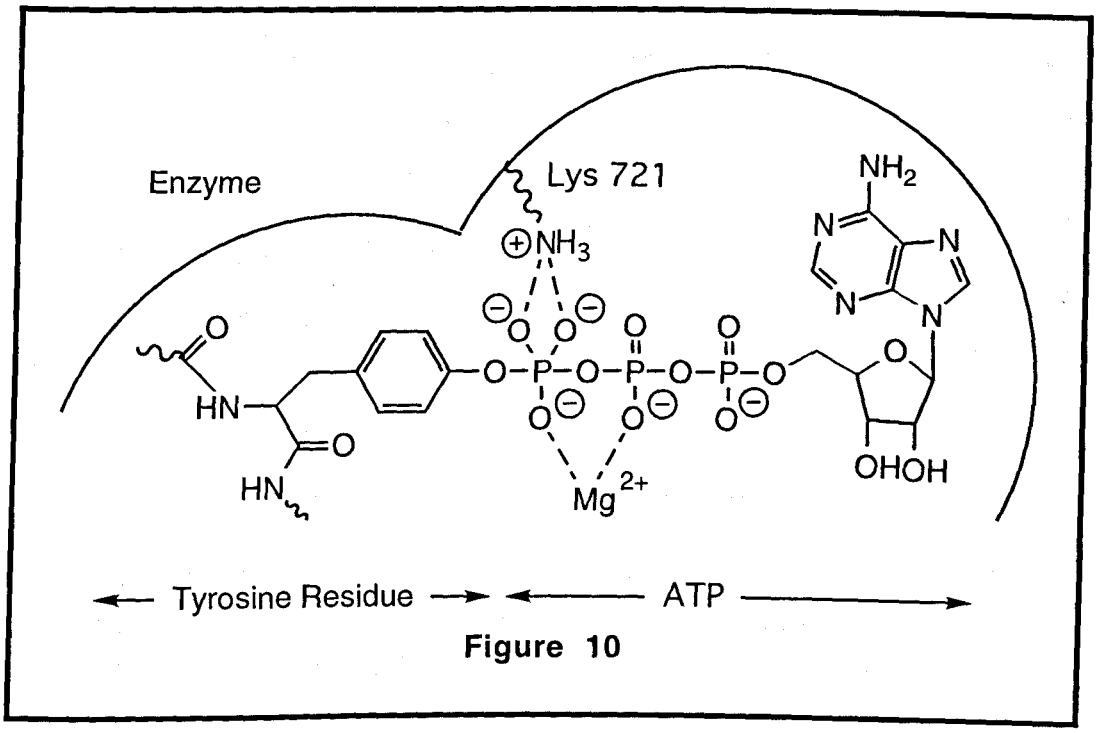
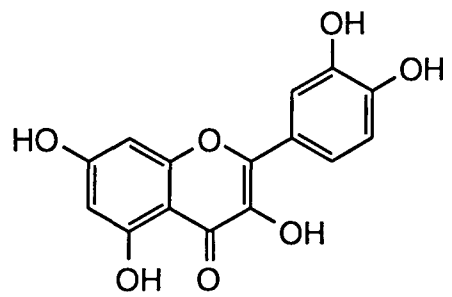



**Figure 10**

## 2.4 Natural Inhibitors of PTKs

By designing molecules which compete for one or other of the substrate sites it is possible to inhibit the catalytic effects of PTKs. Natural products have provided several lead compounds in this area. Quercetin (**15**), a flavone derived from plants, inhibits the growth of a number of tumour cell lines.[52] *In vitro* and *in vivo* studies show that the tyrosine phosphorylation activity of the pp60$^{v\text{-}src}$ protein product is abrogated in the presence of quercetin.[53] The same workers have established that quercetin competes with respect to the ATP subsite, a fact which accounts for its inhibition of other enzymes like cAMP-dependent kinase and PKC. Structural modifications to the basic structure have afforded analogues with improved selectivity.[54] Quercetin is also known to inhibit topoisomerase II and the transport of lactate, D-glucose and calcium ions in cells.

Genistein (**16**), a closely related isoflavone, is a potent inhibitor of tyrosine kinases with improved selectivity over quercetin. Although ATP competitive, genistein inhibits the pp60$^{v\text{-}src}$ and EGFR tyrosine kinases with $IC_{50}$ values of 6-7μM compared to values of >100μM for the cAMP and PKC kinases.[55] A significant complication with genistein is the fact that it inhibits topoisomerases I and II which stimulates double strand breaks in DNA.[56] As with quercetin, systemic toxicity is likely to be high and mitigates against the use of genistein *in vivo*.



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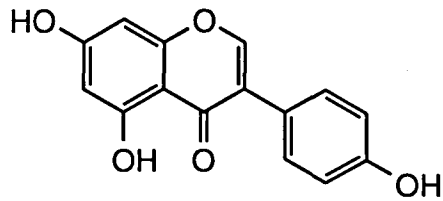
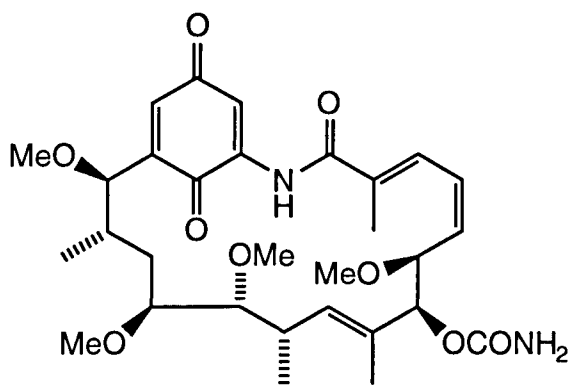


(16)

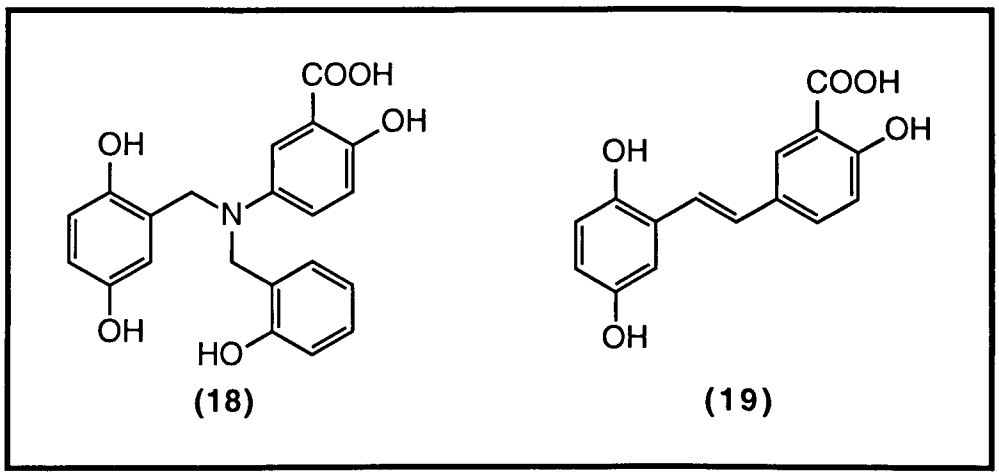
Herbimycin A (**17**) is a broad spectrum TK inhibitor with established *in vivo* activity, although there is some debate about its mode of action. This compound irreversibly blocks the TK activity of EGFR and the protein products of *src*, *bcr-abl* and *erb-B2*.[30] Inhibition can be prevented by the administration of sulfhydryl reagents such as mercaptoethanol, suggesting that the quinone moiety (which is easily attacked by nucleophiles) may be its key chemical feature.[57]



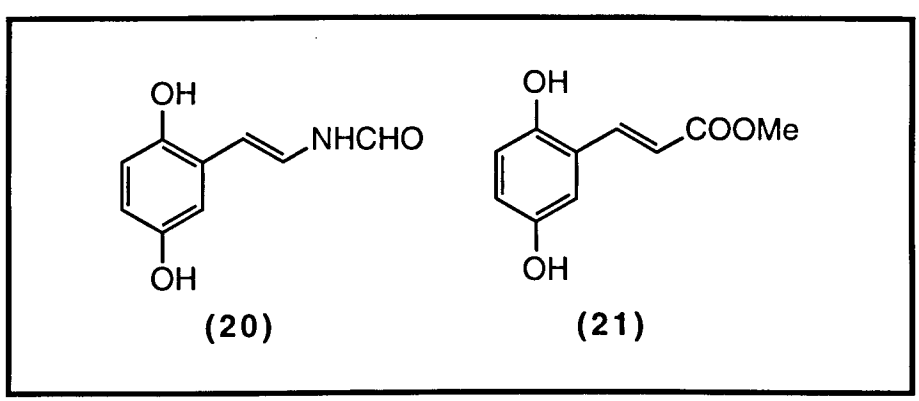
(17)

Lavendustin A (**18**), isolated from *Streptomyces griseolavendus* is a potent TK inhibitor with $IC_{50}$ values in the nanomolar region. It exhibits mixed inhibition characteristics, competing with both ATP and the peptide for occupation of a catalytic site.[58] Poor transport through the cell membrane has been overcome by minor structural modifications, including formation of the methyl ester.[59] Other analogues, incorporating only two of the three rings

in the parent compound, have also proved interesting. Compound (**19**) inhibits p56$^{lck}$ with an $IC_{50}$ value of 60 nM.[60]



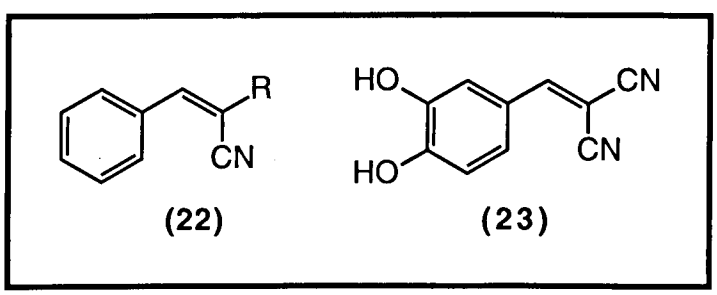

Erbstatin (**20**), also isolated from *Streptomyces*, has become the template for many synthetic TK inhibitors. This compound is the first of the natural products to inhibit by competing for the substrate site, and as such would seem to offer more potential for rational drug design. Initial studies suggested that erbstatin could selectively inhibit tyrosine kinases over serine/threonine kinases[61] but more recent work indicates that the compound inhibits both sets of enzymes in the same concentration range.[62] Erbstatin has been shown to inhibit a substantial range of tumour cell lines and phosphorylation events *in vitro*.[30] A mechanism has been proposed for the breakdown of erbstatin in serum, a process which occurs readily and requires the presence of oxygen and ferric ions. Analogues with improved stability have been synthesised: methyl 2,5-dihydroxycinnamate (**21**) is equipotent to erbstatin but is four times as stable in aqueous solution.[63]



## 2.5 Synthetic Inhibitors of PTKs

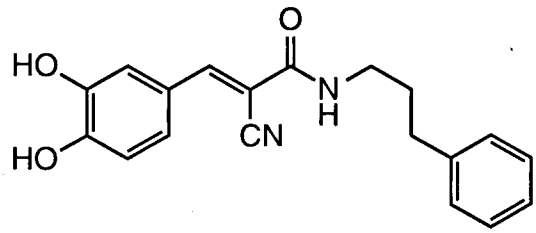
### 2.5.1 Tyrphostins

Tyrphostins are low molecular weight compounds designed to be substrate competitive inhibitors of tyrosine kinases. The earliest tyrphostins[25] mimicked erbstatin and consisted of variously substituted benzene rings bearing conjugated sidearms. (For the remainder of this thesis a 'tyrphostin' is defined as an aromatic or heteroaromatic group in conjugation with an exocyclic double bond , usually bearing a *cis*- nitrile moiety). A study of some 70 tyrphostins indicated that the substructure responsible for activity was the benzylidenemalononitrile group (**22**). Compound (**23**) proved to be three orders of magnitude more active against the EGFR than the InsR.[25]

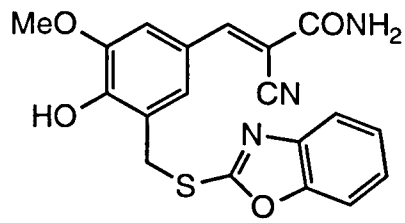



(**22**) (**23**)

The second wave of tyrphostins included heteroaromatic derivatives, which proved largely inactive, and conformationally constrained compounds which confirmed the importance of the *cis*- nitrile moiety.[64] Several of these constrained drugs were active at concentrations of less than 10 μM. A further set of amide tyrphostins showed some discrimination between the EGFR and *erb-B2* kinases. Compound (**24**), typical of the series, inhibited EGFR fifty times more effectively than *erb-B2*.[64] Introducing an *S*-aryl substituent on to the 5'-position of the benzene ring reversed this trend in selectivity. Compound (**25**) inhibited the *erb-B2* kinase preferentially, again by a factor of about 50.[65,66] The ability of tyrphostins to discriminate between normal and transforming *abl* proteins has also been demonstrated.[67]

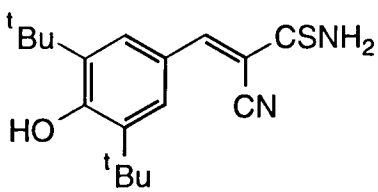



(24)




(25)

Recent work has uncovered compounds with even higher selectivity such as (**26**) which expresses a five hundred fold preference for inhibition of the *erb-B2* tyrosine kinase over the EGFR kinase.[68]



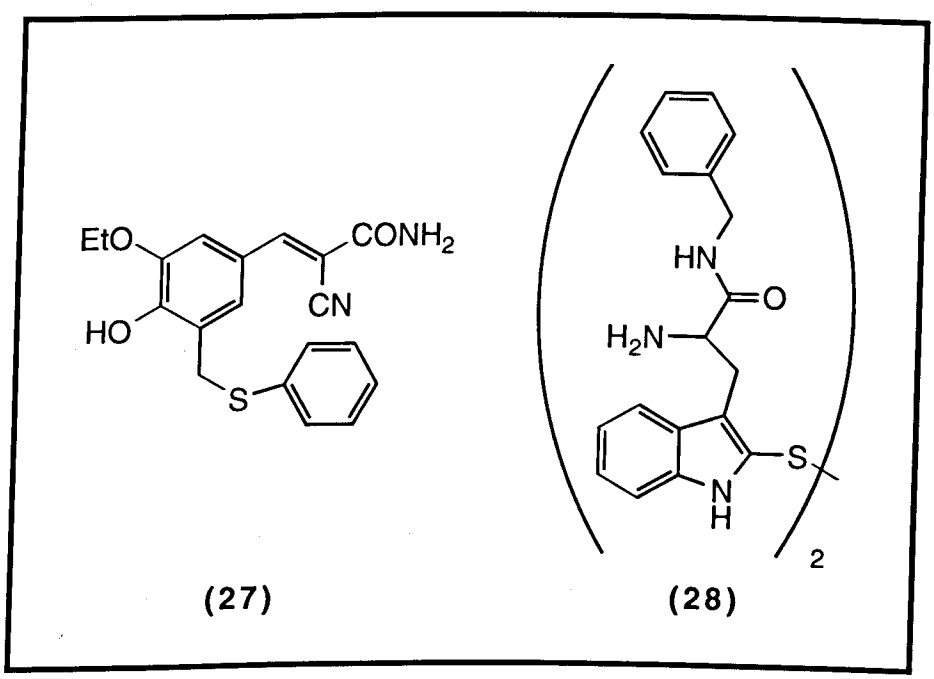

(26)

Although these results show promise, there are still many grey areas surrounding tyrphostins which require clarification. Originally purported to be non-competitive with respect to ATP, recent evidence suggests that most tyrphostins are mixed inhibitors of tyrosine kinases.[69] Indeed, there is some doubt as to whether their antiproliferative activity is due to inhibition of PTKs or other signalling proteins further downstream. The stability of tyrphostins has also been brought into question. In one study a tyrphostin degraded in serum to give a new product which was 10-fold more inhibitory to the PTK in question than the original compound.[70] Few *in vivo* results have been published for tyrphostins and these have not been overly convincing.[71] There is clearly some way to go before these compounds are sufficiently developed to be of any clinical use.

## 2.5.2 Other Synthetic PTK Inhibitors

The interest in TK inhibitors has grown so rapidly in the past decade that a comprehensive review is well beyond the scope of this thesis. Several excellent reviews have already been published.[28, 30, 39, 72] A brief outline of the main structures currently being developed in industry follows.

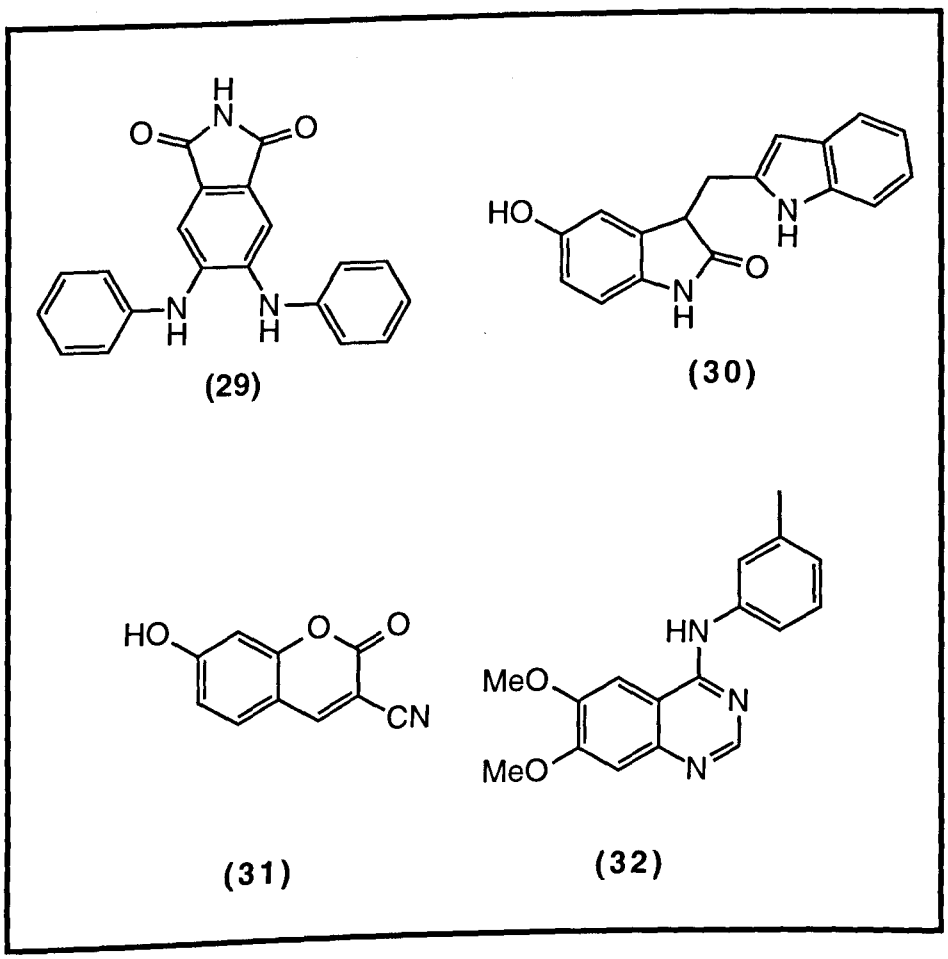
ST-638 (**27**), developed by the Kaneka corporation, is undergoing preclinical trials in Japan. The structure resembles early tyrphostins with the addition of the methylenethiophenyl moiety on the ring. The reported $IC_{50}$ value for this compound against the EGFR tyrosine kinase was 0.4 μM.[73] As yet no *in vivo* data have been forthcoming. The Warner-Lambert company has developed a series of 2-thioindole dimers, the best of which is PD 151514 (**28**). The series showed a clear structure-activity relationship depending on the substitution pattern of the indole ring and discrimination between different TKs was possible. Typical $IC_{50}$ values for the series were in the order of 1-5 μM.[74, 75] *In vivo* studies are currently in progress.




**(27)** **(28)**

Other compounds in development are Ciba Geigy's bis(arylamino) phthalimides (**29**),[76] Farmitalia's methylene-oxindoles (**30**)[77] and Biosignal's coumarin analogues (**31**).[78] By far the most potent TK inhibitors however, are Zeneca's substituted anilinoquinazolines,[79] typified by compound **32**, which exhibit $IC_{50}$ values as low as 5 nM.

Anilinoquinazolines have also been shown to inhibit growth of a human naso-pharyngeal cancer cell line at nM concentrations.




## 2.6 Rationale for Postgraduate Research Project

The Cancer Research group at Glasgow University entered the field of tyrosine kinase inhibitors in 1991 with the award of a PhD studentship to work in the area. This was quickly followed by two more in 1992. Our initial aim was to determine whether or not tyrphostins incorporating heterocyclic moieties could be as active as the polyhydroxylated benzene derviatives which led the field at that time. A limited study of heterocyclic tyrphostins had cast doubt on their potential[64] and we were keen to pursue this line of investigation. It quickly became apparent from our research that heteroaromatic tyrphostins could indeed show excellent activity. Imidazole and thiophene derivatives inhibited the EGFR with $IC_{50}$ values in the low μM concentration range. Incorporation of a nitro function onto the thiophene increased the activity even further. With these exciting results in hand, work

progressed to incorporate quinoline tyrphostins which formed the basis of most of this PhD work. The next two chapters detail the synthesis and biological evaluation of these compounds, charting the progress from initial structure-activity relationships to a more rational form of drug design.

# 3

# Synthesis and Biological Evaluation of Quinoline Tyrphostins: I

## 3.1 Introduction

The initial aim of this project was to synthesise a range of quinoline tyrphostins as quickly and simply as possible. Few heterocyclic tyrphostins had been reported[64,80] and it seemed expedient to build up a broad base of compounds from which to derive structure-activity relationships. The basic structure of quinoline tyrphostins could readily be disconnected to yield quinolinealdehydes and malononitrile derivatives as starting materials (Figure 11). The forward reaction corresponding to this disconnection is the Knoevenagel condensation.

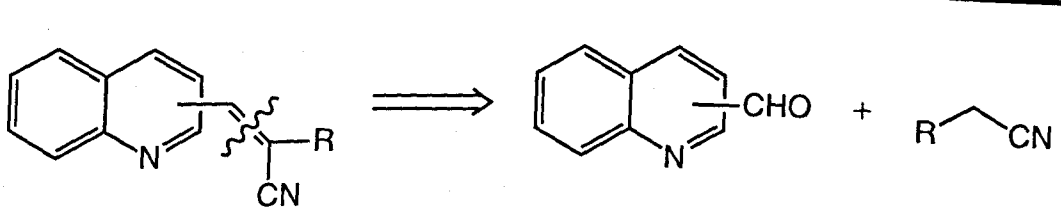
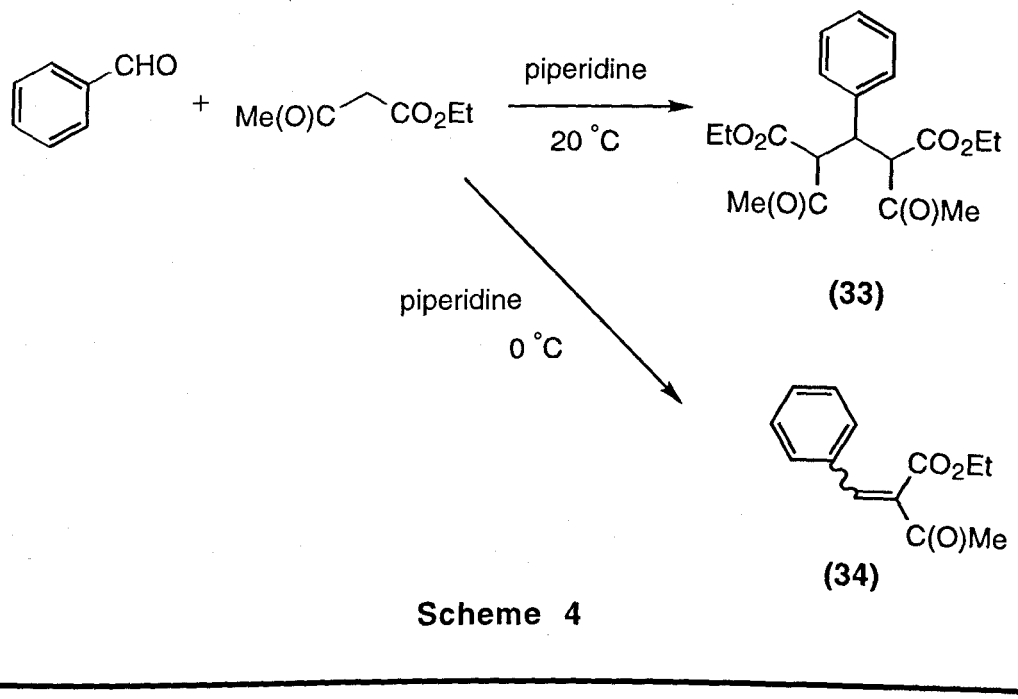



**Figure 11**

### 3.1.1 The Knoevenagel Condensation[81]

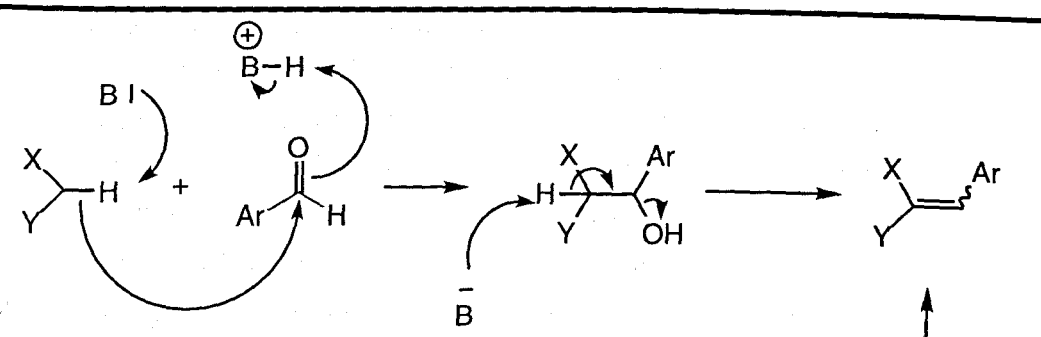
The Knoevenagel condensation, first reported in 1894, is the condensation of aldehydes and ketones with compounds containing activated (acidic) methylene groups with catalytic amounts of a weak base, often an amine. In 1896 Knoevenagel studied the reaction of benzaldehyde with ethyl acetoacetate in the presence of piperidine as catalyst (Scheme 4). At room temperature the bis adduct (**33**) was obtained, but at lower temperatures the elimination product (**34**) predominated.



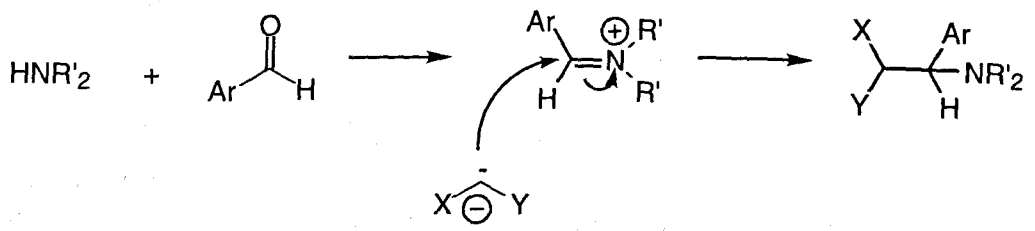

**Scheme 4**

The Knoevenagel reaction is now well established in the synthetic chemist's repertoire due to its wide applicability. The reagents for the reaction are generally inexpensive, a variety of solvents can be employed and reactions can often be performed at room temperature. Knoevenagel products are themselves useful synthetic intermediates and find many applications in the synthesis of dyes, natural products and drugs.

The mechanism of the Knoevenagel reaction depends strongly upon the nature of the catalyst. With primary and secondary amines the reaction proceeds via an iminium intermediate, a mechanism referred to as the Knoevenagel mechanism. Tertiary amines and stronger bases cause the reaction to proceed via the Hann-Lapworth mechanism, involving an hydroxy intermediate (Scheme 5). The end product is the same in both cases.




**Hann-Lapworth mechanism: Tertiary amines**




**Knoevenagel mechanism: Primary & Secondary amines**

**Scheme 5**

It is clear from this scheme that geometrical isomers can be obtained in the Knoevenagel reaction, provided that X and Y are different. The stereochemistry of the products (*E* or *Z*) is governed by the thermodynamic stability of the respective transition states. The lowest energy conformation has the bulkiest groups as far removed from each other as possible prior to elimination, leading to *trans* stereochemistry in the product. A wide variety of aldehydes and ketones undergo Knoevenagel condensation, although the use of ketones can be limited by their lower reactivity. Reactions with aromatic or $\alpha,\beta$– unsaturated aldehydes or ketones are particularly favourable due to the extension of conjugation in the product. The key chemical feature of the other reagent is an activated methylene group, that is, one which is bonded to two electron withdrawing groups, in which the hydrogen atoms are relatively acidic (pKa ~ 13). The reagents used most often are acyclic 1,3-dicarbonyls, malonates, acetoacetates, acetonitriles, acetylacetone and malonodinitrile. Cyclic compounds such as 1,3-cyclohexanediones have also been used. Although amines are the most common catalysts, phase transfer reagents, Lewis acids and potassium fluoride have also been employed.

The Knoevenagel condensation is strongly solvent dependent. Formation of the initial anion and its subsequent addition to the carbonyl (or iminium) function is favoured in highly polar solvents. The second step, a 1,2-elimination, is disfavoured by protic solvents, hence polar aprotic solvents such as DMF are preferred. Ambient conditions are often sufficient to allow the reaction to proceed, although removal of the water generated in the reaction, either azeotropically or over molecular sieves, can improve yields.

### 3.1.2 Application of the Knoevenagel Reaction in the Synthesis of Quinoline Tyrphostins.

The aldehydes required for our purposes, 2-, 3- and 4-quinoline carbaldehyde, and a range of compounds containing an activated methylene group were readily available from chemical suppliers. We chose to work with malononitrile (**35**), malononitrile dimer (**36**), methyl, ethyl, $^{n}$butyl and $^{t}$butyl cyanoacetates (**37-40**), cyanoacetamide (**41**) and thiocyanoacetamide (**42**). This gave a total of 24 compounds as shown in Figure 12.

| **Compounds with Activated Methylene Groups $RCH_2CN$** | | | |
|---|---|---|---|
| **R** | compound | **R** | compound |
| CN | **35** | $COO^{n}Bu$ | **39** |
| $C(NH_2)=C(CN)_2$ | **36** | $COO^{t}Bu$ | **40** |
| COOMe | **37** | $CONH_2$ | **41** |
| COOEt | **38** | $CSNH_2$ | **42** |

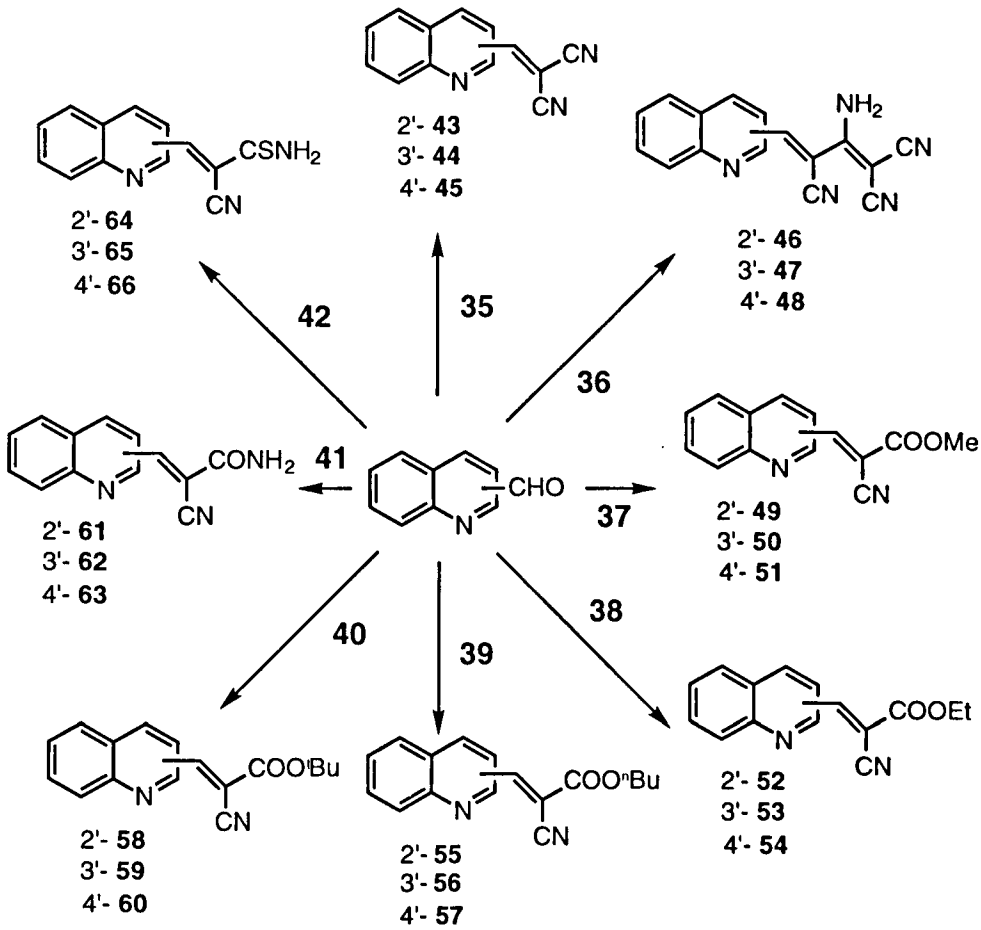


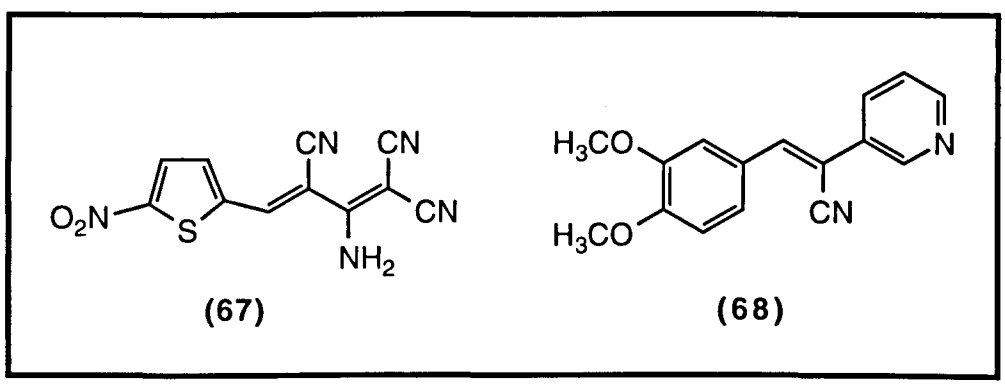

**Figure 12**

## 3.2 Synthesis and Characterisation of Quinoline Tyrphostins.

Literature precedent[25] and our own experience suggested that the reactions should proceed well at room temperature in ethanol with piperidine as catalyst. These conditions proved successful in all but two cases, **61** and **64**, which will be discussed later. Although most of the reagents were readily soluble in ethanol, the products tended to be insoluble, hence precipitation was a useful guide to the progress of the reaction. The precipitates could generally be recovered, washed and recrystallised with ease.

The structural similarity between these tyrphostins means that they have spectroscopic characteristics in common. These are summarised in Section 3.2.1. The only spectral data which are discussed in subsequent sections are those which are specific to the class of compound under scrutiny.

The issue of product stereochemistry has already been raised in Section 3.1.1. For each compound synthesised in this series, only one isomer was present by TLC and NMR. On the basis of thermodynamic arguments one would expect this to be the isomer with the nitrile group *cis* to the ring, which allows the bulkier groups to adopt the less sterically hindered *trans* arrangement. Although the configuration of these tyrphostins has not been determined experimentally, similar studies have been performed within our group. X-ray studies on 2,4-dicyano-3-amino-5-[2-(5-nitrothienyl)]-penta-2,4-dienonitrile (**67**) confirmed the *cis* relationship between the thiophene ring and the nitrile function.[82] Nuclear Overhauser experiments and $^{13}C$-$^{1}H$ coupling constants established that tyrphostin RG-13022 (**68**) preferentially formed the *cis* configuration.[83]




**(67)** **(68)**

## 3.2.1 General Spectroscopic Properties of Quinolines

The $^{13}C$, $^{1}H$, $^{14}N$ and $^{15}N$ NMR spectra of quinoline have all been determined and fully analysed.[84] The $^{1}H$ and $^{13}C$ NMR chemical shifts for quinoline in $CCl_4$ are shown in Figure 13.

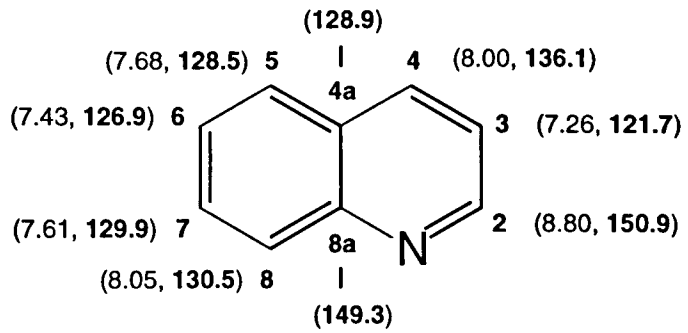



**Figure 13**
$^{1}H$ and $^{13}C$ NMR δ values of quinoline

The effect of the electronegative nitrogen is readily seen by comparing the spectra of naphthalene with those for quinoline. The nitrogen atom pulls electron density away from its nearest neighbours via the $\sigma$ system, so deshielding C-2 and C-8a. A similar effect is seen in the $\pi$ system. Resonance structures with a negative charge on nitrogen and a positive charge on the ring contribute to the overall electronic character of the compound. The ring is therefore electron deficient relative to benzene and several of its protons, particularly those at the 2- and 4- positions, are deshielded as a result.

The similarity in chemical shift for H-5 and H-8, and H-6 and H-7 makes it difficult to distinguish between them in some of the quinoline derivatives. Coupling constants prove particularly helpful in correctly assigning these protons. The *J* values for quinoline protons are illustrated in Figure 14.[84] Of particular note in Figure 14 is that the three-bond coupling between H-5 and H-6 is 8.2 Hz whereas that between H-7 and H-8 is 8.6 Hz. Assuming that these couplings stay fairly constant when quinoline is substituted in the pyridine ring, one can differentiate between protons 5 and 8 on the basis of the measured *J* values. Similarly, the four-bond coupling between H-5 and H-7 (1.4 Hz) is different to that between H-6 and H-8 (1.0 Hz) and helps distinguish between protons 6 and 7.

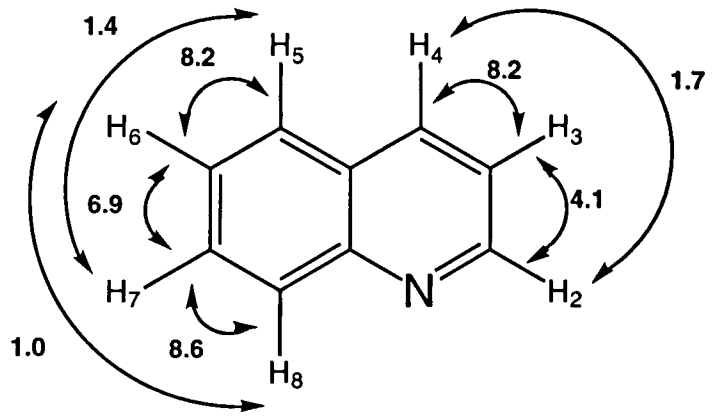



**Figure 14**
$^{1}$H NMR *J* values of quinoline (Hz)

The IR spectrum of quinoline is reproduced in Figure 15.[85] The spectrum shows two C-H stretching absorptions above 3000 $cm^{-1}$, three bands near 1600 $cm^{-1}$ and five in the region of 1300-1500 $cm^{-1}$ for the ring stretching modes (C=C and C=N). The 700-900 $cm^{-1}$ region shows bands for C-H wagging similar to those of naphthalene.

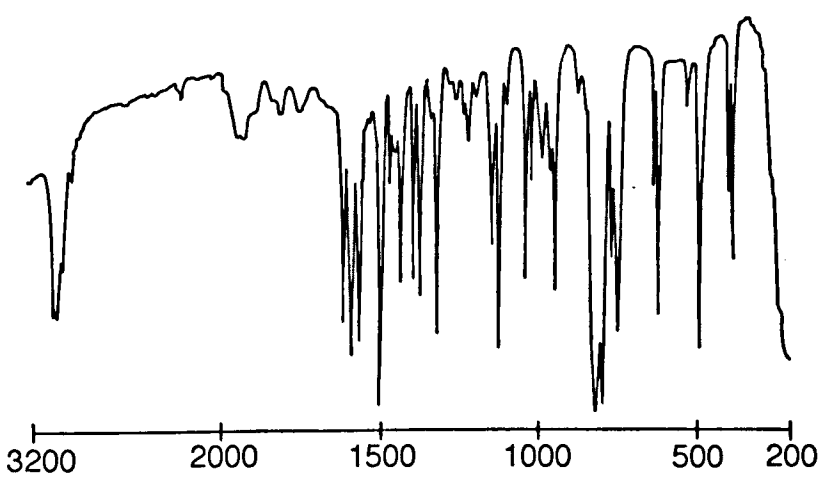



**Figure 15**
IR spectrum of quinoline

### 3.2.2 Derivatives with Malononitrile (43-45)

These compounds have previously been synthesised to study the effects of the conjugated side-arm on the UV spectrum of quinoline.[86] They have also been patented for use in the field of electrophotographic photoconductors.[87] Syntheses proceeded smoothly and rapidly to form the compounds **43**, **44** and **45** in moderate to good yields. **43** recrystallised well from ethyl acetate, whereas isopropanol proved better for **44** and **45**. The $^1$H NMR spectrum for each compound showed a singlet integrating for one proton in the region of $\delta$ 8.6-9.4, corresponding to the product's olefinic proton. The carbon bearing this proton gave a characteristic resonance at *ca.* $\delta$ 158 in each of the $^{13}$C NMR spectra. Two nitrile carbon resonances were seen in the region $\delta$ 112-114. The nitrile functions were also evident in the IR spectra, giving strong, well-defined absorptions at approximately 2220 cm$^{-1}$, the region associated with $\alpha,\beta$-unsaturated nitriles.

### 3.2.3 Derivatives with Malononitrile Dimer (46-48)

These compounds have also been prepared for UV studies.[86] Syntheses proceeded rapidly to produce **46, 47** and **48** as yellow compounds in moderate to good yields. All products were pure by TLC. Recrystallisation was attempted from ethanol, ethyl acetate, acetone, isopropanol and acetonitrile without success. Mixed solvent recrystallisations also failed. The compounds were, however, pure enough to proceed with characterisation.

The $^1$H NMR spectra showed two broad singlets in the range $\delta$ 9.2-9.5, commensurate with the amine function. The olefinic proton was clearly visible in each spectrum as a sharp singlet between $\delta$ 8.2 and 8.9. The conjugated sidearm produced some interesting features in the $^{13}$C NMR spectra. The carbon bonded to the nitrogen of the amine was consistently the farthest downfield, due to the two $\pi$-acceptor groups $\beta$ to it and the inductive effect of the nitrogen atom. These carbons came into resonance at *ca.* $\delta$ 165. The carbon bearing two nitrile groups was always the furthest upfield, occurring at around $\delta$ 50. Increased electron density due to delocalisation of the amine lone pair, coupled with the effects of two $\alpha$ nitrile groups accounts for its unusual upfield position. The IR spectra confirmed the presence of the primary amine function with two sharp, medium strength absorptions between 3190 and 3400 cm$^{-1}$. Sharp, well-defined absorptions in the region of 2200-2220 cm$^{-1}$ corresponded to the nitrile groups and

those in the region of 1643-1660 $cm^{-1}$ were due to carbon-carbon stretching of the alkene.

### 3.2.4 Derivatives with Cyanoacetates (49-60)

Compounds **49-60** have similar spectral properties which enables them to be dealt with together. The syntheses of these compounds generally proceeded smoothly to yield white or cream coloured products in moderate to good yields. Solubility of the products was good in alcohol, ethyl acetate and chlorofom. Ethyl acetate was the optimum solvent for recrystallisation and compounds **58-60** provided particularly good crystals.

A notable exception to the above was compound **57** which formed less readily and had to be purified by column chromatography.

The characteristic singlet for the olefinic proton was present in all of the $^1H$ NMR spectra (*ca.* $\delta$ 8.5-9.1). Each set of compounds showed the expected signals in the alkyl region for the particular ester function they contained. Compounds **49-51** gave a singlet integrating for three protons due to the methyl group of the ester, the resonance coming in the range $\delta$ 3.9-4.0. **52-54** showed the classic pattern for ethyl groups attached to oxygen, a downfield quartet integrating for two protons at *ca.* $\delta$ 4.4 and a triplet integrating for 3 protons at *ca.* $\delta$ 1.4. The $^3J$ value was 7.1 Hz as expected. The $^1H$ NMR spectra for **55-57** showed four distinct groupings for the four types of protons in the $^n$butyl group. These ranged from the most deshielded methylene, a triplet at *ca.* $\delta$ 4.4, to the terminal methyl group, a triplet centred on *ca.* $\delta$ 1.0. Each of the four groupings integrated for the correct number of protons. Compounds **58-60** showed a large singlet at *ca.* $\delta$ 1.6 due to the nine methyl protons of the $^t$butyl ester.

The carbonyl carbon of the ester function was a preserved feature of the $^{13}C$ NMR spectra of compounds **49-60**. The position of this, the most deshielded carbon, was consistently in the range $\delta$ 160-162. The alkyl groups of the ester were once again a distinctive structural motif in confirming the structure of the products. The deshielded methyl carbon in compounds **49-51** came relatively far downfield at *ca.* $\delta$ 54. With the ethyl esters **52-54**, the methyl carbon came at *ca.* $\delta$ 14 whereas the methylene resonated at *ca.* $\delta$ 63. The $^n$butyl compounds **55-57** showed four distinct peaks at *ca.* $\delta$ 67, 31, 19 and 13 for the three methylene and one methyl groups respectively. This pattern showed a direct correlation with distance from the oxygen atom. The quaternary carbon of the $^t$butyl groups in **58-60**,

deshielded by the oxygen atom, occurred relatively far downfield at *ca.* δ 84. The carbons of the three methyl groups came consistently at *ca.* δ 28.

The ester carbonyl carbons were clearly seen in all the IR spectra, absorbing strongly in the range 1720-1730 $cm^{-1}$. The C-O stretching mode was also present as a strong absorption between 1250 and 1270 $cm^{-1}$.

### 3.2.5 Derivatives with Cyanoacetamide (62 & 63)

Compounds **62** and **63** were prepared in moderate yields in the usual fashion, but compound **61** could not be prepared by this method. Neither extending the duration of the experiment to 24 h nor elevating the temperature to 40 °C assisted the reaction. At this stage in the project is was not considered worthwhile expending more effort on compound **61**.

**62** and **63** showed two broad singlets for the amide protons in the $^{1}H$ NMR spectra, coming in the region δ 7.9-8.3. The olefinic proton was evident in both spectra (δ 8.5-8.9). The carbonyl carbon of the amide was evident at *ca.* δ 162 in the $^{13}C$ NMR spectra. The N-H stretching modes in the IR spectra were poorly defined but both compounds showed a strong absorption at *ca.* 1700 $cm^{-1}$ for the amide's carbonyl group.

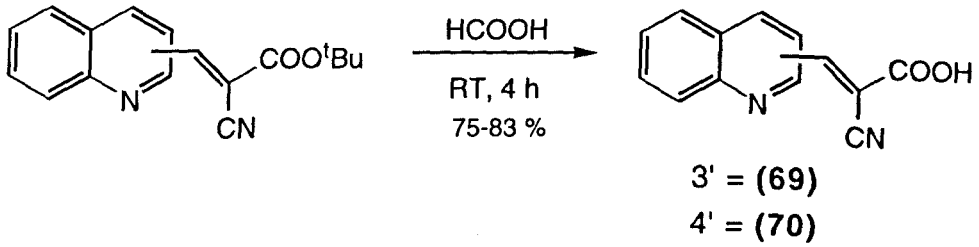
### 3.2.6 Derivatives with Thiocyanoacetamide (65 & 66)

Compounds **65** and **66** formed readily in moderate yields under the conditions already described. Attempts to form compound **64** by the same method inevitably resulted in an inseparable brown reaction mixture. An alternative preparation using alumina as catalyst also proved unsuccessful.[88]

The thioamide protons appeared as broad singlets well downfield in the $^{1}H$ NMR spectra (δ 10.0-10.5). The thiocarbonyl carbon at *ca.* δ 193 was the distinguishing feature of the $^{13}C$ NMR spectra. The IR spectra showed sharp absorptions above 3300 $cm^{-1}$, indicative of thioamide N-H stretching modes, and thiocarbonyl absorptions in the regions of 1616-1650 $cm^{-1}$ (amide II) and 1260-1270 $cm^{-1}$ (amide I).

### 3.2.7 Synthesis of Quinolinyl Propenoic Acid Derivatives

From compounds **59** and **60** it was a short step to hydrolyse the ester and generate acidic derivatives for testing. This transformation was effected by stirring the parent ester in formic acid for four hours (Scheme 6).[89]



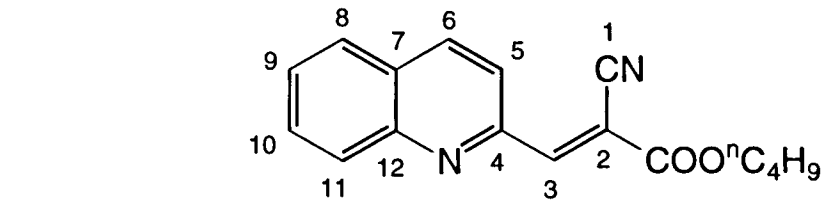

3' = **(69)**
4' = **(70)**

**Scheme 6**

The clearest indication of product formation in the $^1$H NMR spectra was the absence of the $^t$butyl methyl protons. The carboxylic acid proton was too broad to be seen. The $^{13}$C NMR spectra were notable for the carbonyl resonance at *ca.* $\delta$ 163 and the disappearance of the $^t$butyl carbon signals. The IR spectra showed a broad absorption well over 3000 cm$^{-1}$ and a slight lowering of the carbonyl frequency relative to that in the ester. Physical characteristics which confirmed the transformation were the lower $R_f$ values and higher melting points (due to H-bonding) of the products.

## 3.3 COSY NMR Studies on Compounds 50 and 55

In attempting to characterise the tyrphostins the main difficulty came in assigning the protons and carbons of the NMR spectra of the benzene ring in quinoline. Although splitting patterns helped with the $^1$H NMR spectra, the $^{13}$C NMR carbon signals were often too close together to assign with any confidence. To resolve this problem, 2D COSY NMR spectra were prepared, correlating the $^{13}$C and $^1$H NMR spectra for tyrphostins **50** and **55**. The COSY spectrum for **55** is reproduced in Figure 16. The y-axis shows the portion of the $^1$H NMR spectrum containing the aromatic protons. Assignments were made on the basis of chemical shift and coupling constants. For an explanation of the numbering system used, please see Section 7.2.2. The x-axis represents the section of the $^{13}$C NMR spectrum concerned, from $\delta$ 155-120 .




**(55)**

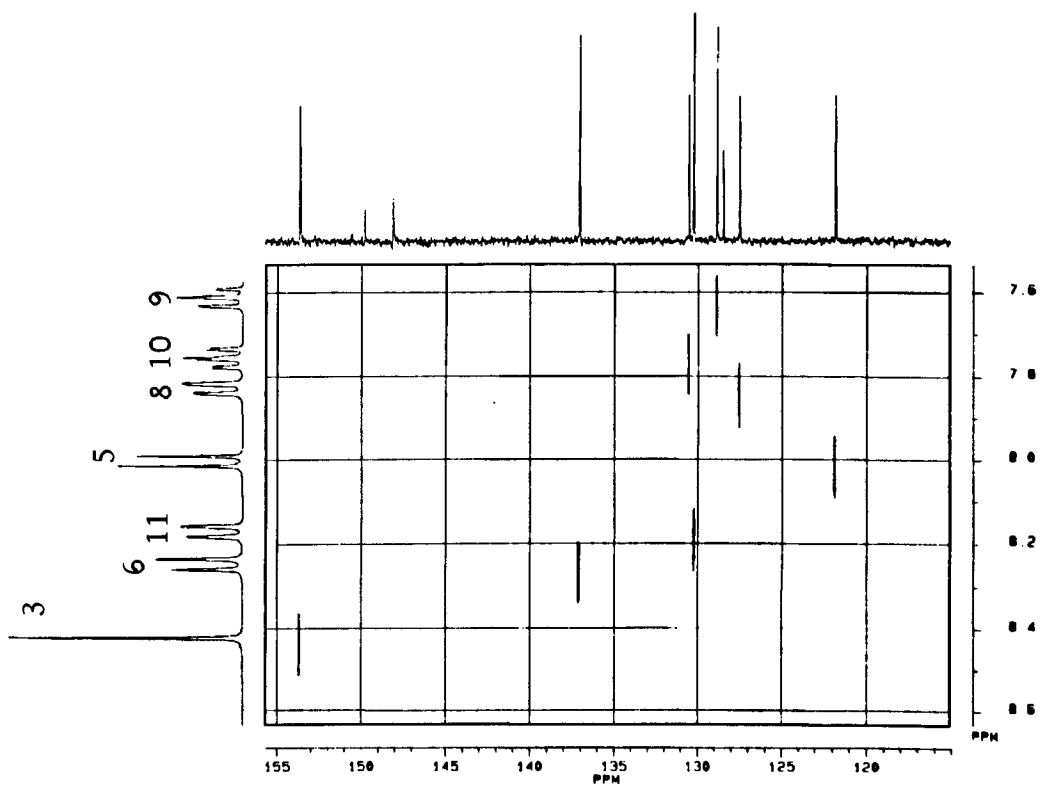



**Figure 16**

The peaks at δ 153.6 and 137.0 correspond to C-3 and -6 as expected. The peaks at δ 130.5, 130.2, 128.8 and 127.5 are due to C-10, -11, -9 and -8 respectively. This order could not have been ascertained without this NMR technique since C-10 and -11 differ by only 0.3 ppm. It is interesting that the order observed for the $^{13}C$ NMR spectra bears no resemblance to that seen in the $^{1}H$ NMR spectrum (H-11, -8, -10 and -9). The remaining peak due to C-5 is easily identified since it is always the most upfield quinoline C-H carbon.

A comparison of the chemical shift of carbons 8-11 for the 2-quinoline ester derivatives (**49**, **52**, **55** & **58**) shows enough consistency to suggest that the order C-10, -11, -9 and -8, is valid for this series. This only holds true because of the electronic similarity of the ester compounds and no inferences can be drawn about the correct order for the other tyrphostins without further 2D COSY NMR experiments.

A similar treatment for compound **50** gave the order C-10, -11, -8 and -9 which holds valid for the series of 3-quinoline esters (**50**, **53**, **56** & **59**).

## 3.4 Biological Testing of Quinoline Tyrphostins

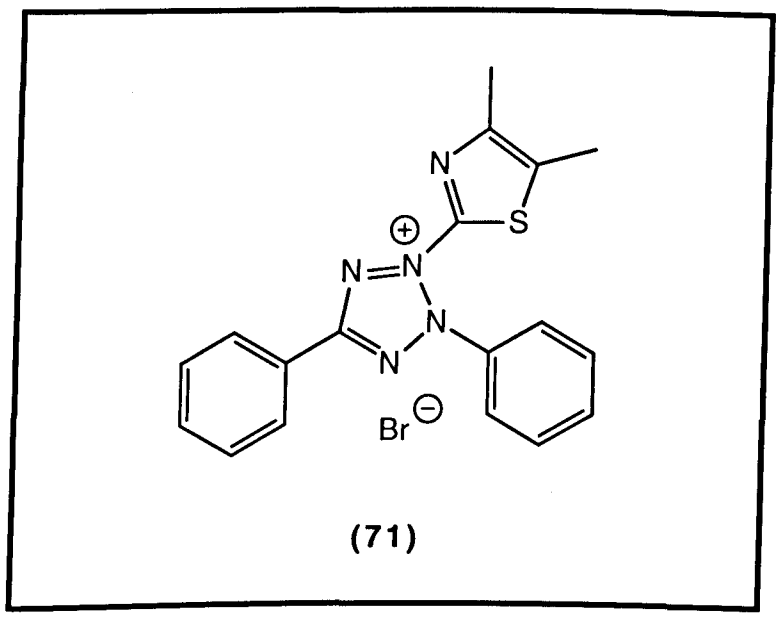
The initial biological testing of compounds **43** to **70** was carried out in collaboration with Professor Paul Workman and Dr Valerie Brunton at the Department of Medical Oncology at the CRC Beatson Laboratories in Glasgow. Our aim was to study the ability of the compounds to act both as antiproliferative and cytotoxic agents. To this end, the tyrphostins were subjected to two biological assays which are summarised below.

### 3.4.1 Biological Assays

EGFR receptor tyrosine kinase activity was measured in membrane fractions prepared from the HN5 cell line. The EGFR kinase activity was determined by measuring the EGF-dependent phosphorylation of a synthetic substrate, poly ($Glu_6$, $Ala_3$, $Tyr_1$) by [$\gamma$-$^{32}$P] ATP. The inhibitors were prepared as stock solutions and diluted in distilled water before administration to the cells in concentrations of 0.01 μM - 50 μM

Human squamous cell carcinoma or MCF-7 breast adenocarcinoma cells were used to assess the cytotoxicity of the tyrphostins. The cells were distributed into 96-well plates and incubated for five days. A range of inhibitor concentrations (0.01 μM - 50 μM) was then administered and after 24 h the growth medium and inhibitors were removed by suction and replaced by fresh medium. The remaining cells were allowed to grow over a period of three days after which time a small concentration of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT, **71**) was added to each well. MTT[90] is a water soluble salt which gives a yellow colouration in aqueous media. Under the action of dehydrogenase enzymes, the compound is converted into insoluble purple formazan by cleavage of the

tetrazolium ring. The change in colour (i.e. the absorbance) of the solution can therefore be taken as a measure of the concentration of living cells.



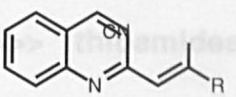
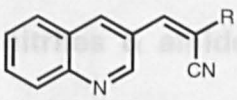
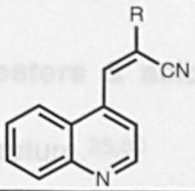
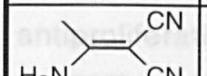

(71)

After addition of MTT the plates were incubated for a further four hours. Medium and residual MTT were removed by suction and the formazan crystals were dissolved in DMSO and buffer. The absorbance of the solution for each well was recorded and log concentration response curves were generated. $IC_{50}$ values were determined as the dose to inhibit MTT absorbance by 50% relative to a control.

### 3.4.2 Results of Biological Assays.

Table 1 records the $IC_{50}$ values for quinoline tyrphostins **43-70** in the assays previously described.

**Table 1**

| Ar / R | 2-QUINOLINES  | 3-QUINOLINES  | 4-QUINOLINES  |
|---|---|---|---|
| | No. $IC_{50}$ (μM) EGFRK MCF-7 | No. $IC_{50}$ (μM) EGFRK MCF-7 | No. $IC_{50}$ (μM) EGFRK MCF-7 |
| CN | **43** 58 >100 | **44** 400 76 | **45** 170 78 |
|  | **46** 1.7 77 | **47** 27 52 | **48** 4.7 55 |
| $CO_2Me$ | **49** 200 59 | **50** 450 >100 | **51** 250 16 |
| $CO_2Et$ | **52** 240 17 | **53** >500 >100 | **54** 330 - |
| $CO_2{}^nBu$ | **55** 50 57 | **56** >500 >100 | **57** 275 22 |
| $CO_2{}^tBu$ | **58** 145 14 | **59** >500 61 | **60** 325 1.6 |
| $CONH_2$ | | **62** >500 - | **63** 140 49 |
| $CSNH_2$ | | **65** 300 >100 | **66** 38 41 |
| COOH | | **69** 350 >100 | **70** 380 >100 |

Comparison of $IC_{50}$ values for the EGFR kinase antiproliferative assay show that for each R group, the order of potency was 2-quinoline > 4-quinoline > 3-quinoline. Compounds **46**, **47** & **48** incorporating the 2-aminoethene-1,1-dinitrile moiety (hereafter referred to as 'dimer', from malononitrile dimer) were the most potent tyrphostins having $IC_{50}$ values of 1.7, 27.0 and 4.7 μM respectively. Placing the structures in decending order of potency for this assay gives:

| 2-Quinolines | 3-Quinolines | 4-Quinolines |
|---|---|---|
| "dimer" | "dimer" | "dimer" |
| $^{n}$butyl ester | $CSNH_2$ | $CSNH_2$ |
| CN | COOH | $CONH_2$ |
| $CONH_2$ | CN | CN |
| $^{t}$butyl ester | Me ester | Me ester |
| Me ester | $CONH_2$ | $^{n}$butyl ester |
| Et Ester | $^{n}$butyl ester | $^{t}$butyl ester |
| | $^{t}$butyl ester | Et ester |
| | | COOH |

Broadly speaking this follows the trend

**"dimers" >>> thioamides > nitriles & amides > esters & acids**

which correlates well with previous observations in the literature.[25,80]

A wide range of $IC_{50}$ values was obtained for the cytotoxicity assay but there was no clear structure-activity relationship. Nor was there a direct correlation between cytotoxicity and the ability to inhibit EGFR in the antiproliferative assay. However these compounds are killing cells, it appears not to be solely via EGFR mediated pathways.

## 3.5 Further Studies on Compounds 46-48.

The success of the quinoline "dimer" compounds in the antiproliferative assays suggested that they should be the focus of our attention. As a result the compounds were dispatched to various other cancer groups for further study (see Section 3.6) and we set about investigating their properties further.

### 3.5.1 Stability Studies

It was somewhat ironic that compounds **46-48**, which were the most active tyrphostins, should have had the least desirable physical properties including poor solubility and difficulties with crystallisation. Another concern was the stability of the compounds in solution. When NMR samples of quinoline "dimers" were prepared in DMSO it was noted that they changed colour from yellow to green over the course of a few days. A simple NMR experiment confirmed that there was indeed a chemical change occurring in solution. A fresh solution of **48** (50 mg) in DMSO (1 ml) was prepared and its $^{1}H$ and $^{13}C$ NMR spectra recorded immediately. The sample was left sitting in daylight and its NMR spectra were recorded again after two hours and

then 24 hours. Over the two hour period no change was seen in the $^{1}H$ or $^{13}C$ NMR spectrum. After one day, however, identifiable peaks had appeared in the $^{1}H$ NMR spectrum and there was a distinct 'doubling up' of the peaks in the $^{13}C$ NMR spectrum. The $^{1}H$ NMR spectra for 0 hours and 24 hours into the experiment are shown in Figure 17.

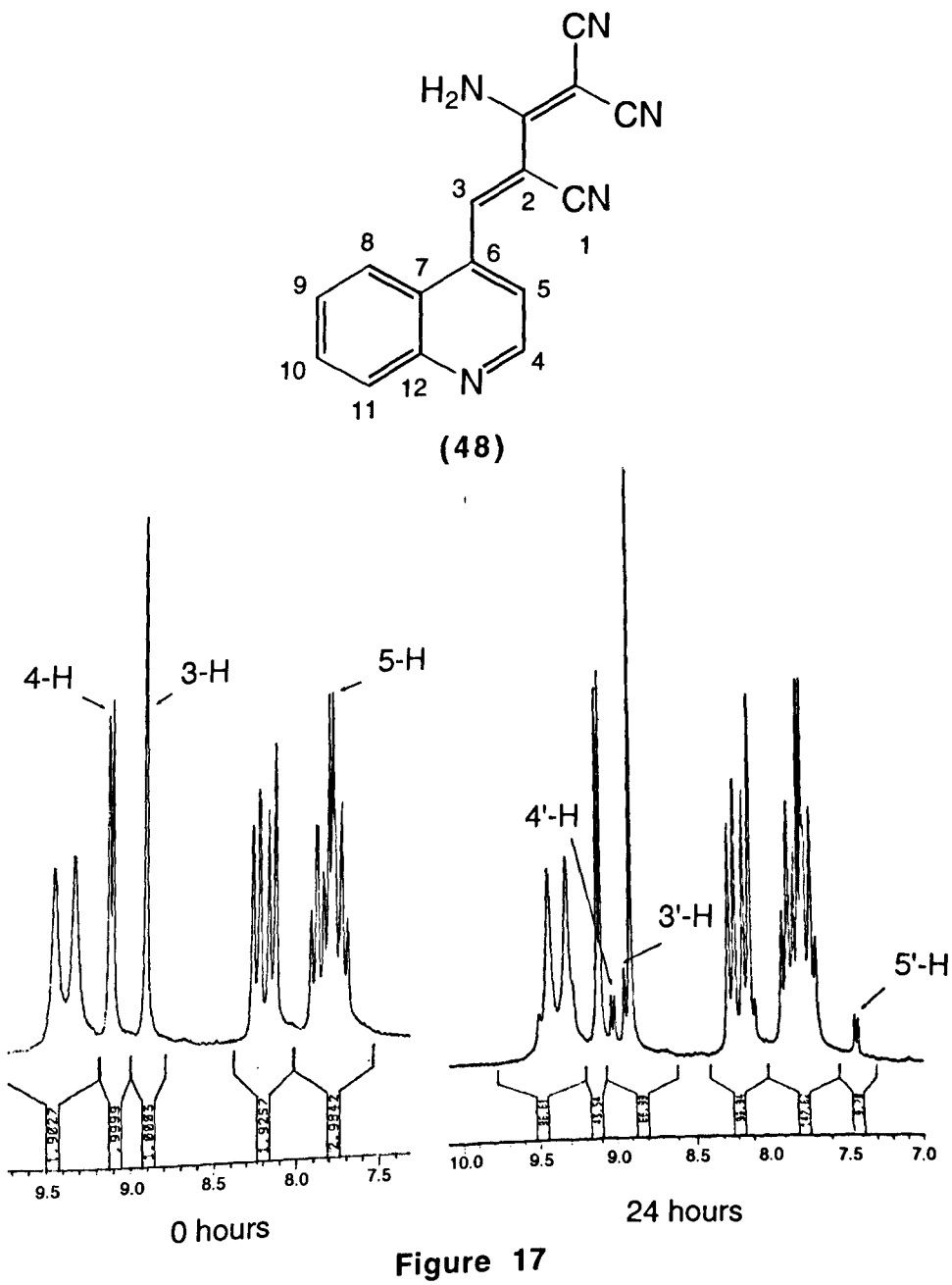
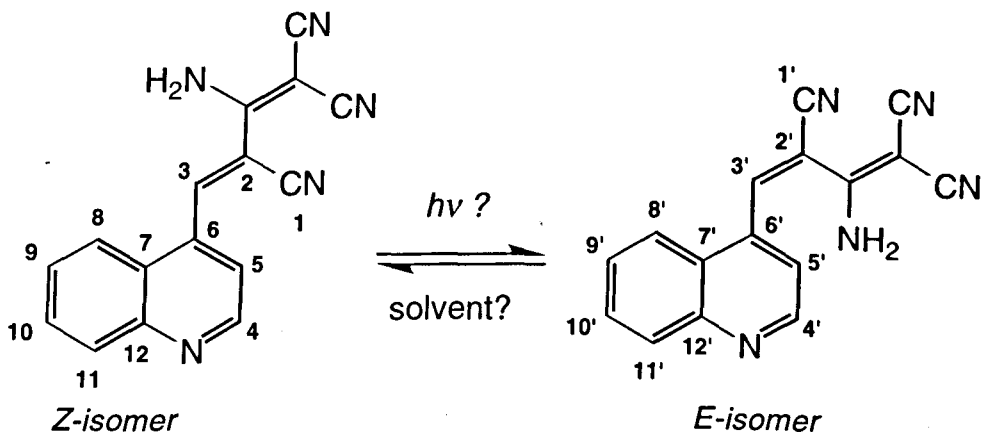



**Figure 17**

The peaks corresponding to protons 3, 4 and 5 are marked on the spectrum taken at 0 h. By 24 h a new product had formed with distinct

doublets at δ 9.02 and 7.42. It is clear from the coupling constants (4.4 Hz) that these are the equivalents of protons 4 and 5 in the new product (4' and 5'). In addition there is a small singlet at δ 8.94 for the new olefinic proton (3'-H). It is possible that a double bond isomerisation is taking place in solution whereby the *E*-and *Z*- forms of **48** are in equilibrium with one another (Figure 18).

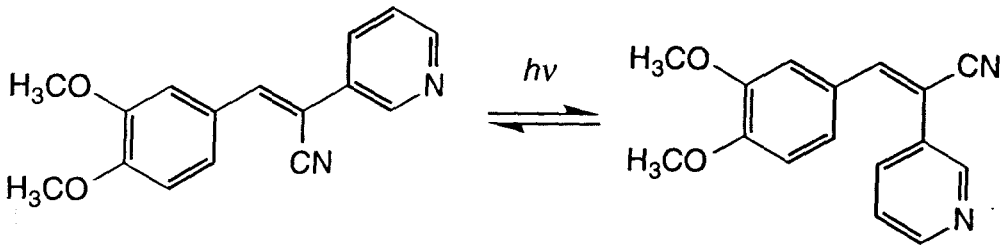



*Z-isomer* *E-isomer*

**Figure 18**

This theory may rationalise the observed upfield shift for proton 5. In the *E*- form of **48**, rotation about the bond joining C-2' to the carbon bearing the amine group can bring the amine nitrogen's lone pair close to proton 5'. A similar rotation in the *Z*- form cannot achieve the same effect. Hence proton 5' may experience slightly more shielding on average than proton 5, pushing the signal upfield.

Assuming that the initial sample of **48** was pure *Z*- isomer (see Section 3.2 for rationale), comparison of the integrals for protons 4 (43.54) and 5' (9.70) gives the ratio of *Z* : *E* after 24 h as 78 : 22 at this stage of the equilibration. These observations concur with previous studies on the tyrphostin RG-13022 (**68**) which is known to undergo a light-induced cis/trans isomerism (Figure 19).[71]




*Z-isomer* **(68)** *E-isomer*

**Figure 19**

Unpublished observations within our own group have shown that this phenomenon is both light and solvent dependent. Solutions of either geometric isomer of **68** kept at room temperature in the dark do not equilibrate, nor do solid samples when exposed to light. When a pure sample of either isomer is dissolved in $d_6$-DMSO and studied by NMR over a period of days the solution forms an equilibrium mixture of 78% *E*- and 22% *Z*- isomers, regardless of which isomer was initially chosen. In THF the ratio changes to 53% *E* and 47% *Z* at equilibrium.

These observations on compound **48**, although not comprehensive, are sufficient to suggest that it is equilibrating in solution. Thus compounds **46-48**, which have been prepared as the *Z*-isomers, should never be stored as stock solutions. For NMR experiments and biological assays the samples should be prepared and used immediately.

### 3.5.2 Decoupling Experiments on Compound 48

As previously mentioned (Section 3.2.1), elucidation of the proton NMR spectra for quinolines is straightforward apart for the benzene ring. Decoupling experiments were performed on **48** to enable the proton spectrum to be correctly assigned (Figure 20). These experiments were performed on a Bruker 360 MHz NMR machine.

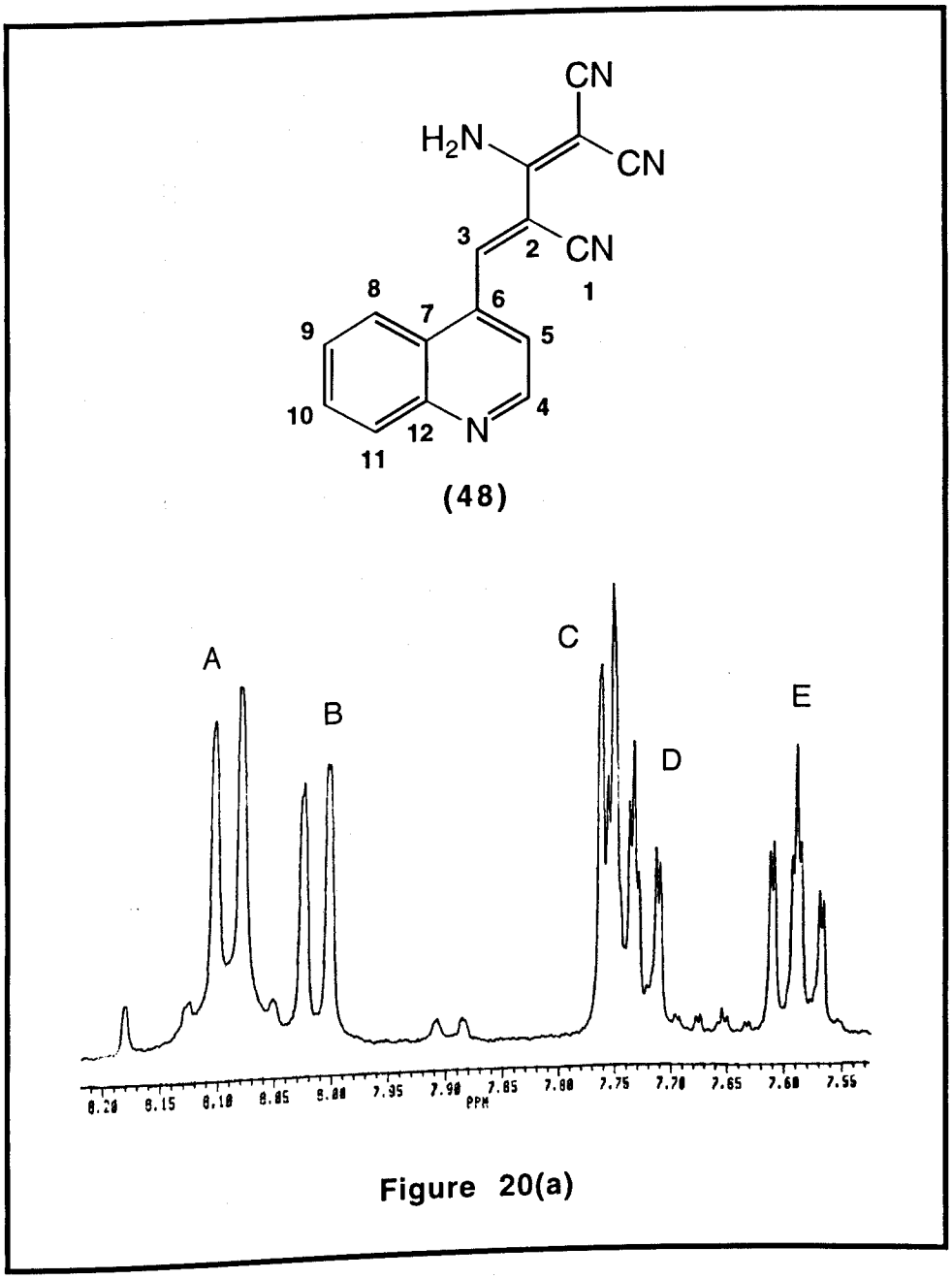



**Figure 20(a)**

Figure 20a shows detail of the region δ 7.55-8.15 before irradiation. The sample of **48** contained a small amount of 4-quinolinecarbaldehyde which accounts for the small peaks on the baseline. The region of the spectrum shown contains five protons, labelled A to E. A and B are either protons 8 or 11, split only once by a three-bond coupling to its neighbour. The complex pattern of C and D is due to proton 5, a doublet, superimposed upon either proton 9 or 10, which appears as a ddd system with two three-bond couplings and one four bond coupling. The remaining ddd multiplet is either proton 9 or 10.

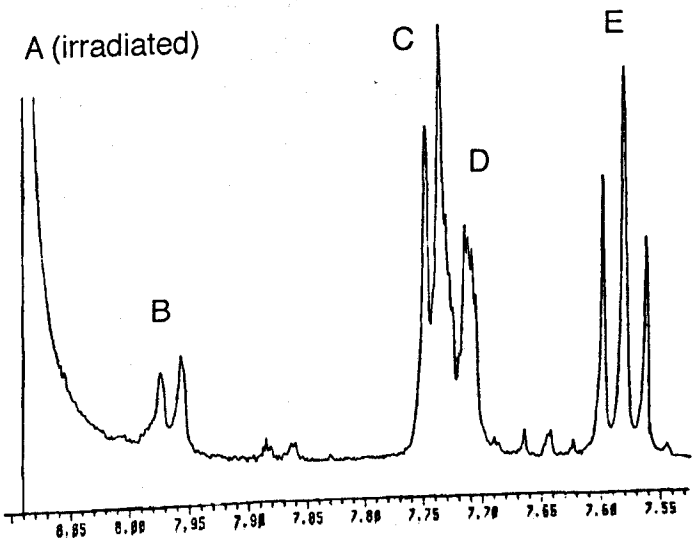



**Figure 20(b)**

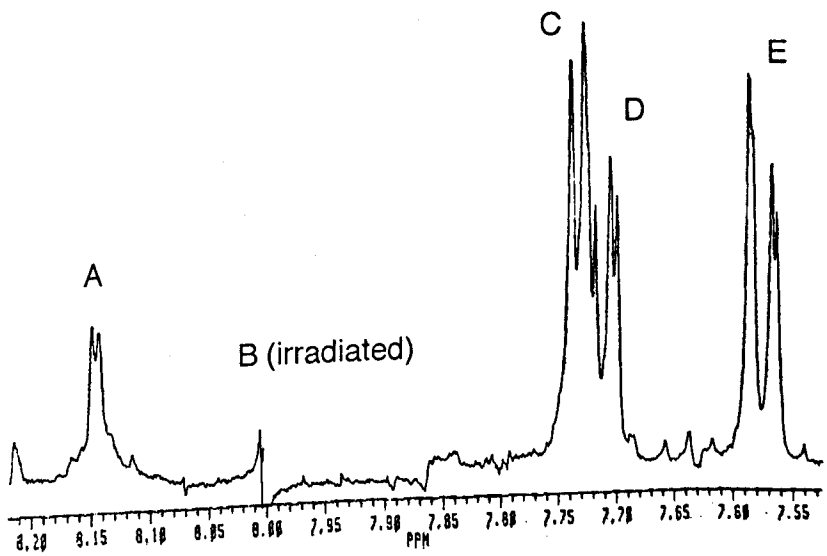



**Figure 20(c)**

Figure 20b shows the result of irradiating at δ 8.08, the chemical shift associated with proton A. Proton D collapses into a doublet with some long-range coupling and proton E loses its four-bond coupling.

Figure 20c shows the effects of irradiating at δ 8.01, the chemical shift of proton B. Proton E collapses to a doublet showing a little four-bond coupling.

It follows that protons A and D and B and E are adjacent neighbours. An examination of the coupling constants for these protons gives $^4J_D$= 1.3 Hz, $^4J_E$= 1.2 Hz, $^3J_A$= 8.1 Hz and $^3J_B$= 7.6 Hz. Assuming that the coupling constants will be similar to those of quinoline (Section 3.2.1), this leads to the conclusion that A is proton 11, B is proton 8, D is proton 10 and E is proton 9. This pattern is in agreement with the observed chemical shifts for quinoline itself.

## 3.6 Further Biological Evaluation of Tyrphostins 46-48

The promising results of **46-48** in the EGFR kinase assay (Section 3.4.2) suggested that it would be worth investigating these compounds further. Samples were dispatched to several cancer research groups, including the National Cancer Institute in the United States, to help get a fuller picture of their potential.

### 3.6.1 CRC Department of Medical Oncology. Further Testing

The ability of these tyrphostins to inhibit EGFR dependent growth suggested that they may be acting as EGFR inhibitors. We were keen to establish whether or not this was the case with intact cells, rather than specialised membrane preparations such as those prepared from HN5. Dr Brunton chose to examine the cytotoxic effects of **46** and **48** on a panel of squamous cell carcinomas with a wide range of EGFR expression. The $IC_{50}$ values for both compounds correlated well with the level of EGFR expression in the cell lines (Table 2).

| Cell Line | EGFR Number (x $10^6$/cell) | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | Compound 46 | Compound 48 |
| HN5 (Tongue) | 5.195 | **38.3** ± 7.5 | **8.9** ± 2.4 |
| A431 (Vulva) | 1.554 | **45.7** ± 7.5 | **6.3** ± 1.0 |
| CaSki (Cervix) | 0.785 | **69.0** ± 17.1 | **36.3** ± 3.8 |
| SiHa (Uterus) | 0.114 | **>100** | **>100** |

**Table 2**

The HN5 line, which has the highest number of receptors, was generally the most sensitive to the tyrphostins whereas the SiHa cell line, with a lower number of EGF receptors, was resistant to both inhibitors. This information suggests that these tyrphostins are inhibiting at some stage of the EGFR kinase signalling pathway. However, more detailed studies of the effects of tyrphostins **46-48** on HER14 cells revealed that treatment with compounds **46** and **47** did not reduce the levels of EGF receptor autophosphorylation, even at concentrations as high as 100 μM. Treatment with 100 μM of compound **48** arrested all EGF-induced tyrosine phosphorylation, presumably as a consequence of its toxicity at such high concentrations. All three compounds, however, specifically inhibited the EGF dependent phosphorylation of a 50 kDa protein. The function of this protein is not yet known but it may be involved in the mitogenic response to EGF.

In summary, it seems that although the compounds do not inhibit receptor autophosphorylation, they can inhibit subsequent phosphorylations of cytosolic proteins. This can be understood in the light of recent studies on the insulin receptor (Section 2.3.2). This work showed that the process of autophosphorylation caused a conformational change in the receptor's tyrosine kinase domain. It may be that these tyrphostins are unable to bind to the active site of the enzyme until this re-adjustment has occurred.

### 3.6.2 Testing at the Institute of Cancer Research, Surrey and the National Cancer Institute, USA.

Given the uncertainty about the mode of action of compounds **46-48**, it seemed expedient to check whether or not these agents were damaging DNA directly. Dr Lloyd Kelland at the CRC Institute of Cancer Research, Royal Marsden NHS trust, agreed to test compound **48** for us. In this assay the human ovarian cell line SKOV-3 was exposed to tyrphostin **48** for 2 h at 50 and 100 μM concentrations and then assessed for induction of DNA strand breaks. Dr Kelland found no evidence of DNA damage with this compound.

Compound **46** was submitted to the National Cancer Institute in the United States for *in vitro* testing against a panel of 60 human tumour cell lines. The mean $GI_{50}$ graph (corresponding to 50% growth inhibition, Figure 21) shows that the majority of non-small cell lung cancers were resistant to the drug, whereas most of the colon, central nervous system, melanoma and renal cell lines were relatively sensitive. The NCI did not consider these results sufficient to warrant progress to *in-vivo* testing.

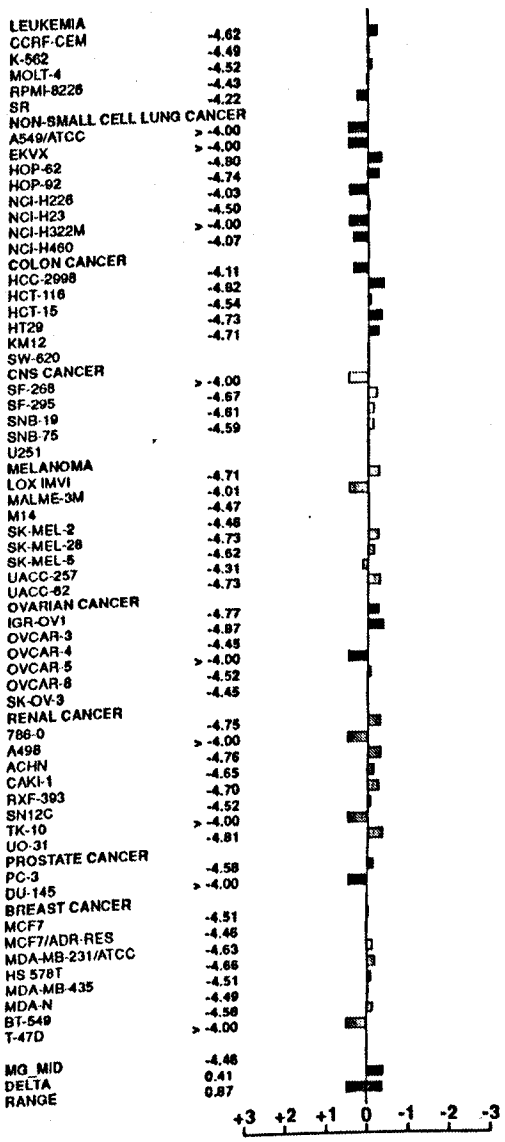


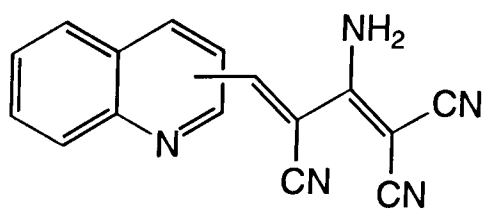

**Figure 21**

Test Data for compound **46** from the NCI screening programme. The vertical line represents the log of the mean $GI_{50}$ value based on all 60 cell lines. Bars to the right represent activity greater than the mean, (lower log ($GI_{50}$) while bars to the left represent activity lower than the mean (higher log ($GI_{50}$).

## 3.7 Structural Analysis of Lead Compounds 46-48.

By this stage of our work we had achieved our initial goal, in that we had shown that heterocyclic tyrphostins could match the activity of polyhydroxylated tyrphostins. This work is currently in press.[91] Our studies indicated that compounds **46-48** should be the model for the next generation of quinoline tyrphostins, despite questions over the exact nature of their biological action. But why were these compounds more active than the others? The important structural unit seemed to be the 2-aminoethene-1,1-dinitrile moiety which differed from the other substituents in two main ways: its extended conjugation and its electronic character.

None of the other tyrphostins contained two alkene double bonds in the side-chain. Did this simply make the molecules a better 'fit' in an enzyme substrate pocket, or did it significantly alter their chemical properties? Was the increased partial negative charge on the terminal nitrile groups due to delocalisation from the amine lone pair an important feature? In designing the next set of tyrphostins these were some of the questions we sought to answer. We also wanted to improve upon the poor physical properties of **46-48**, particularly with regards to solubility. These aims led to new, more varied chemistry and provided the basis for further work in this field.



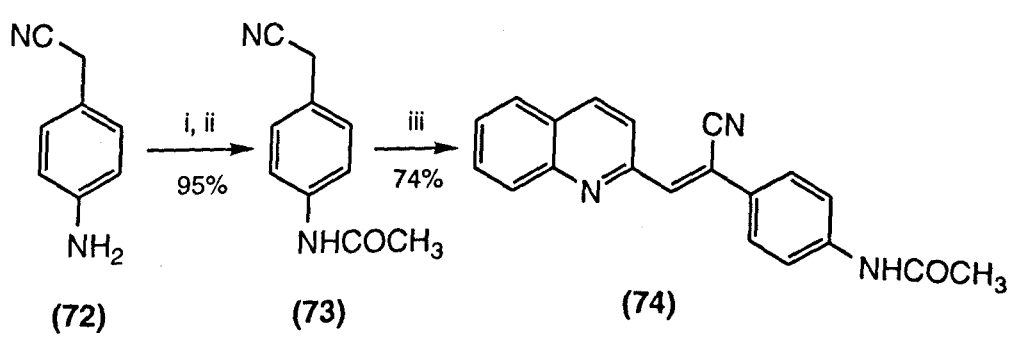
# 4

# Synthesis and Biological Evaluation of Quinoline Tyrphostins: II

Our work to this point had established that the quinoline "dimer" compounds **46-48** are the most potent anticancer agents in the series. One of the distinguishing features of these compounds was the extended conjugation in the side-arm. We decided to synthesise a range of conjugated quinoline tyrphostins to determine whether or not extended conjugation accounted for the superior activity of **46-48**. The simplest way to achieve this aim was to condense quinolinecarbaldehydes with activated methylene compounds containing aromatic groups and it was to this pursuit that we now turned.

## 4.1 Tyrphostins Incorporating Aromatic Substituents

Several aromatic tyrphostins were readily prepared by condensation of 2-quinolinecarbaldehyde with 4-aminobenzylcyanide, **72**, and derivatives (Schemes 7 & 8).



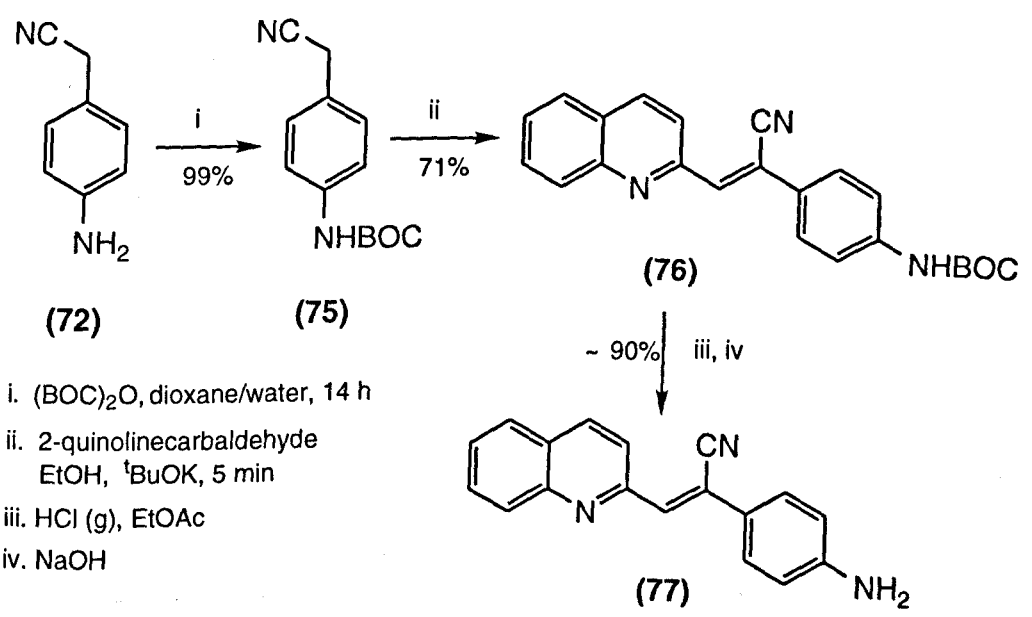

**(72)** **(73)** **(74)**

i. HCl (aq), stir ii. $(CH_3CO)_2O$, NaOAc iii. 2-quinolinecarbaldehyde, piperidine EtOH, 30 min

**Scheme 7**

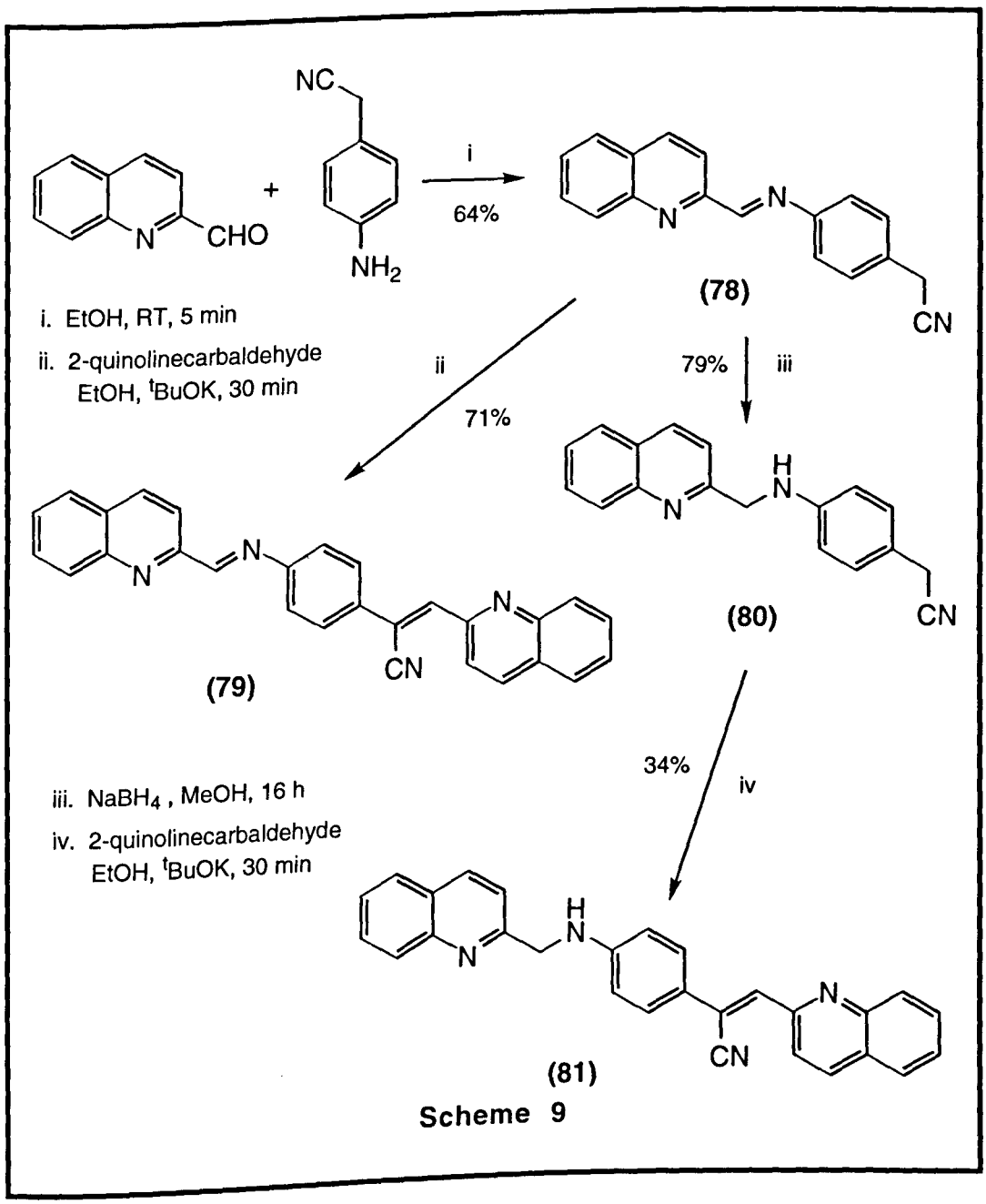
Amine **72** was acetylated with acetic anhydride[92] to give amide **73** in excellent yield. The $^1$H NMR spectrum for **73** showed a signal at δ 2.08 for the methyl protons and the $^{13}$C NMR spectrum showed the amide carbonyl carbon at δ 168.5. The melting point of **73** agreed with that previously reported.[93] Amide **73** condensed readily with 2-quinolinecarbaldehyde to form tyrphostin **74** under the standard Knoevenagel reaction conditions described in Section 7.2.1. The $^1$H NMR spectrum of compound **74** showed a new olefinic proton at δ 8.08 and a shift in the nitrile absorption in the IR spectrum (from 2247 to 2217 cm$^{-1}$) confirmed that it had moved into conjugation.

When **72** was treated with di-$^t$butyldicarbonate (BOC-O-BOC), it gave the carbamate **75** in almost quantitative yield (Scheme 8).[94] The $^t$butyl protons of **75** were present as a large singlet at δ 1.51 in the $^1$H NMR spectrum. The carbamate carbonyl carbon showed clearly in the $^{13}$C NMR spectrum (δ 152.7) and the C=O stretching mode was present at 1700 cm$^{-1}$ in the IR spectrum. Condensation of **75** with 2-quinolinecarbaldehyde proceeded rapidly to give compound **76** which was identified by the olefinic singlet at δ 8.12 in the $^1$H NMR spectrum and a conjugated nitrile absorption at 2214 cm$^{-1}$ in the IR spectrum. Carbamate **76** could be deprotected in good yield by bubbling HCl gas through a solution of the compound in ethyl acetate.




**Scheme 8**

The $^1H$ NMR spectrum of crude **77** showed that the $^tBOC$ group had been cleaved, although a trace of **76** was still present. The amine protons were present in the $^1H$ NMR spectrum as a new broad singlet at δ 5.16. Crude **77** was used in a subsequent reaction without further characterisation. Compound **77** could not be formed directly by condensing 2-quinolinecarbaldehyde with **72** because the competing reaction, imine formation, predominated (Scheme 9).




**Scheme 9**

The imine product, **78**, showed a singlet at δ 8.75 in the $^1H$ NMR spectrum for the proton attached to the imine carbon. The IR spectrum

contained a new absorption at 1624 $cm^{-1}$ corresponding to C=N stretching modes. Condensation of **78** with 2-quinolinecarbaldehyde failed with piperidine as catalyst, but an equivalent of potassium $^t$butoxide in ethanol (generating ethoxide ion) proved to be a good alternative giving **79** in good yield. Assignment of the $^1$H NMR spectrum of **79** was problematic because of the duplication of quinoline signals, but the two crucial singlets due to the imine C-H proton and the new olefinic proton were present at $\delta$ 8.70 and 8.01 respectively. The absorption at 2214 $cm^{-1}$ in the IR spectrum was commensurate with an $\alpha,\beta$-unsaturated nitrile function. Interestingly the UV spectrum of **79** showed an absorption maximum at 370 nm, indicating the presence of a highly conjugated chromophore.

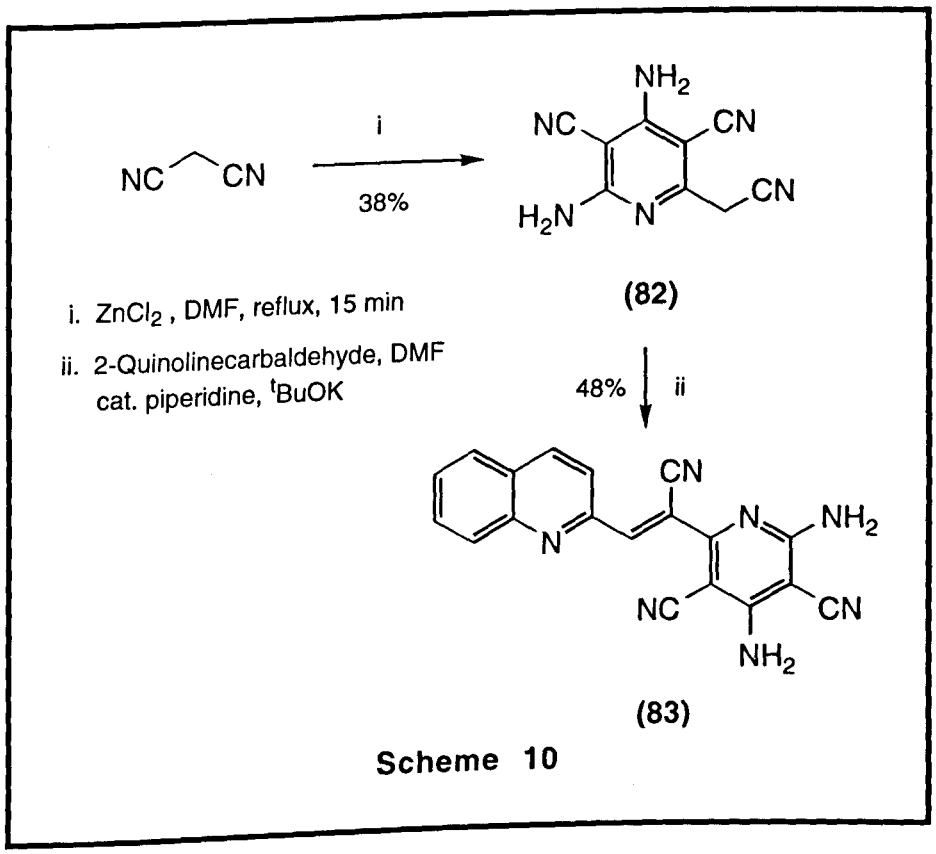
Several attempts were made to reduce the imine function of **79** with sodium borohydride.[95] MS of the crude products showed a significant fragment with an m/z ratio of 412 corresponding to the molecular weight of **81,** but this product could not be isolated by chromatography. An alternative route involved the reduction of imine **78** to give amine **80**, which was subsequently condensed with 2-quinolinecarbaldehyde affording amine **81** (Scheme 9). The $^1$H NMR spectrum of **80** showed a doublet integrating for two protons at $\delta$ 4.57, corresponding to the newly formed methylene group. The methylene carbon appeared at $\delta$ 49.1 in the $^{13}$C NMR spectrum and an N-H stretching absorption was apparent in the IR spectrum at 3348 $cm^{-1}$.

Initial attempts to react compound **80** with 2-quinolinecarbaldehyde gave a product with the correct molecular ion but a surprisingly low $R_f$ value. We believed that this was an iminium species formed by reaction of the aldehyde with the secondary amine function in **80**. To avoid this side reaction we preformed the anion of **80** by stirring a solution of **80** in ethanol with $^t$BuOK before addition of the aldehyde. This approach gave the desired product after chromatography. The IR spectrum of **81** showed the presence of amine N-H stretch (3387 $cm^{-1}$) and $\alpha,\beta$-unsaturated nitrile absorptions (2213 $cm^{-1}$).

## 4.2 Tyrphostins with Heteroaromatic Side-arms

A survey of the literature, particularly a review by Fatiadi on malononitrile derivatives,[96] suggested some interesting heterocyclic compounds as potential adducts with quinolinecarbaldehyde. The synthesis of a cyclic malononitrile trimer, **82**, had been reported[96] and it was reasoned that, given the right conditions, it should be possible to condense this with quinolinecarbaldehydes to give compounds structurally related to

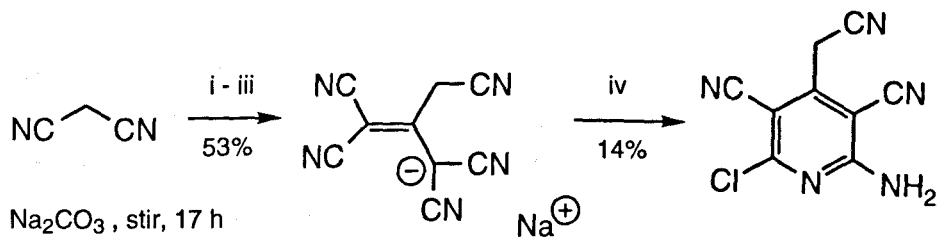
tyrphostins **46-48** (Scheme 10). Trimer **82** was prepared as described in the literature and its $^{1}H$ and $^{13}C$ NMR spectra correlated well with previous work.[97, 98]




**Scheme 10**

Condensation of **82** with 2-quinolinecarbaldehyde proved problematic, primarily due to solubility problems. Reactions in ethanol and isopropanol failed, but DMF gave satisfactory results. The product **83** showed a singlet at $\delta$ 8.23 for the new olefinic proton in the $^{1}H$ NMR spectrum and the presence of alkene C=C stretch (1662 $cm^{-1}$) and $\alpha,\beta$-unsaturated nitrile stretch (2208 $cm^{-1}$) in the IR spectrum.

The preparation of a similar substituted pyridine has been reported (Scheme 11).[96]

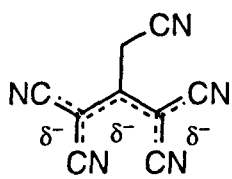



i. $Na_2CO_3$ , stir, 17 h
ii. HCl (conc.), extract EtOAc
iii. $NaHCO_3$ , stir, 2 h
iv. Acetone, HCl (conc.)

**(84)** **(85)**

**Scheme 11**

Pentanitrile **84** was prepared using the methodology of Kelly *et al.*[99] An interesting feature of the $^{13}C$ NMR spectrum was its simplicity. Only six different carbons were present. This was, of course, due to delocalisation of the lone pair which conferred symmetry on the molecule.




**(84)**

The IR spectrum of **84** showed a strong absorption at 2200 $cm^{-1}$ corresponding to $\alpha,\beta$-unsaturated nitriles. A smaller absorption at 2270 $cm^{-1}$ confirmed the presence of an unconjugated nitrile group.

Treatment of **84** with concentrated HCl in acetone afforded crude **85** after chromatography. The $^1H$ NMR spectrum of **85** showed two broad downfield singlets for the $NH_2$ protons and a singlet at $\delta$ 4.27 for the methylene group. Three distinct nitrile carbons were evident in the $^{13}C$ NMR spectrum and the correct molecular ion (m/z= 217) was present in the mass spectrum. Trinitrile **85** was used without further characterisation in an unsuccessful condensation reaction with 2-quinolinecarbaldehyde.

## 4.3 Tyrphostins with Conjugated Aliphatic Side-arms

Although there was merit in preparing aromatic derivatives, the fact remained that tyrphostins **46-48** contained an aliphatic and not an aromatic side-arm. The most straightforward way of testing the significance of the diene moiety to the activity of **46-48** was to prepare other tyrphostins containing this unit (Figure 22).

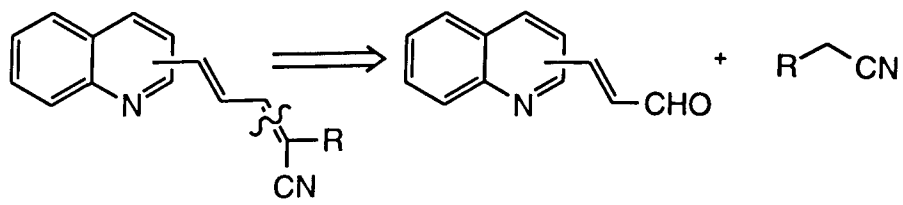
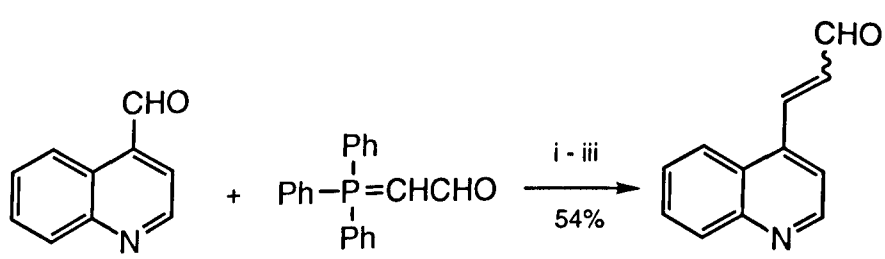



**Figure 22**

Disconnection of the second extraannular double bond led to those compounds containing activated methylene groups used previously, and quinolinyl propenal derivatives. A literature search for these substituted propenals provided surprisingly little information, apart from a useful synthesis of 3-(4-quinolinyl)-2-propenal using Wittig chemistry (Scheme 12).[100]




**(86)** **(87)**

i. $PhNO_2$, $N_2$, 24 h, RT ii. HCl, organic wash iii. $Na_2CO_3$

**Scheme 12**

Triphenylphosphoranylidene acetaldehyde (**86**) is commercially available and as a stabilised Wittig reagent would be expected to give *E*-

stereochemistry in the product. Reaction according to the literature procedure gave 3-(4-quinolinyl)-2-propenal (**87**) in moderate yield. The isomeric ratio was established by examination of the $^1H$ NMR spectrum of **87** (Figure 23). The coupling constant between protons $H_c$ and $H_b$, 15.9 Hz, indicates that they are in a *trans-* configuration across the double bond. Consequently the *E-* form of **87** is the major isomer. The percentage of each isomer was determined by comparing the integrals of the aldehyde protons Ha and Ha' which gave a ratio of 92% *E* to 8% *Z*.

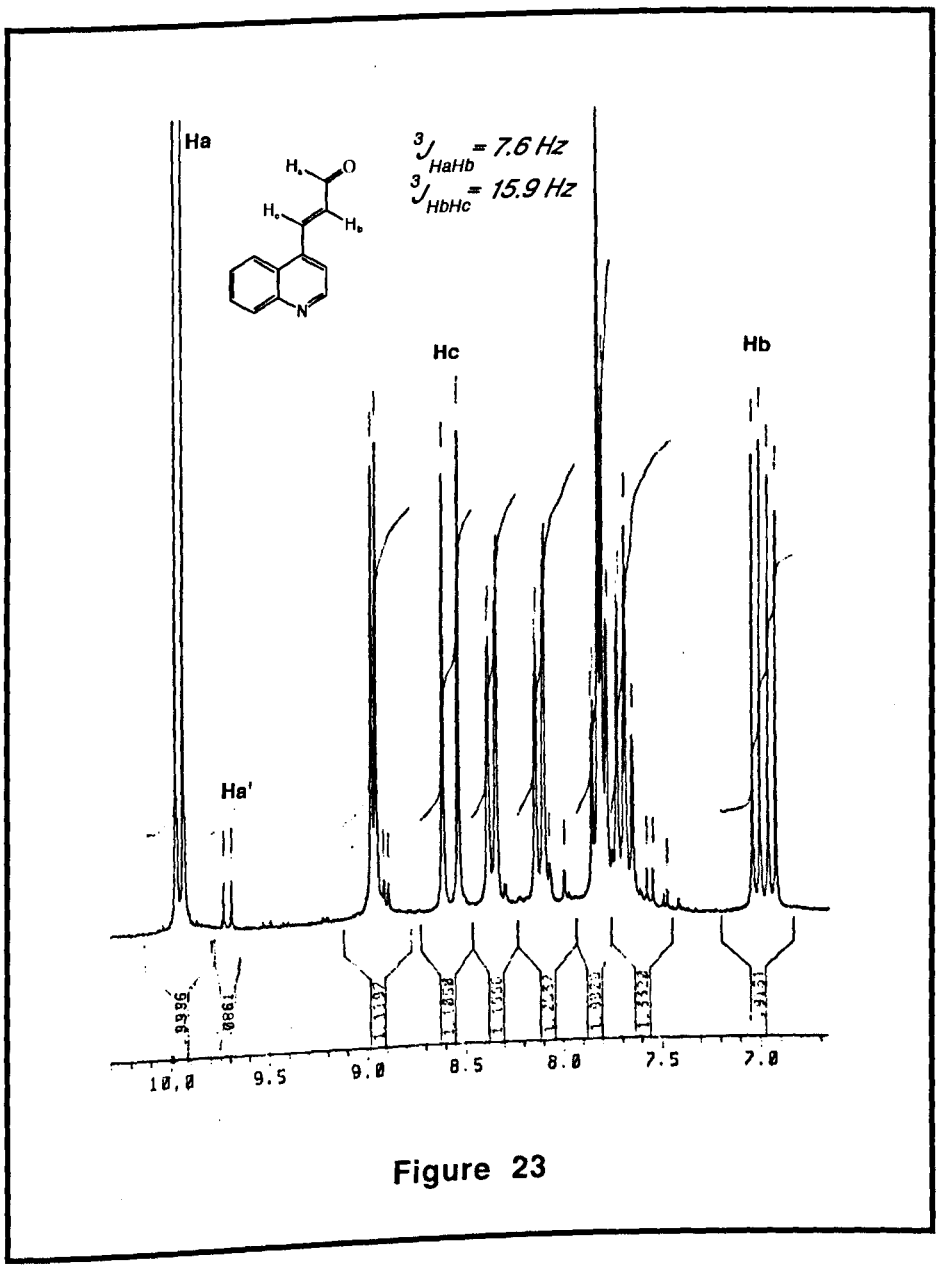
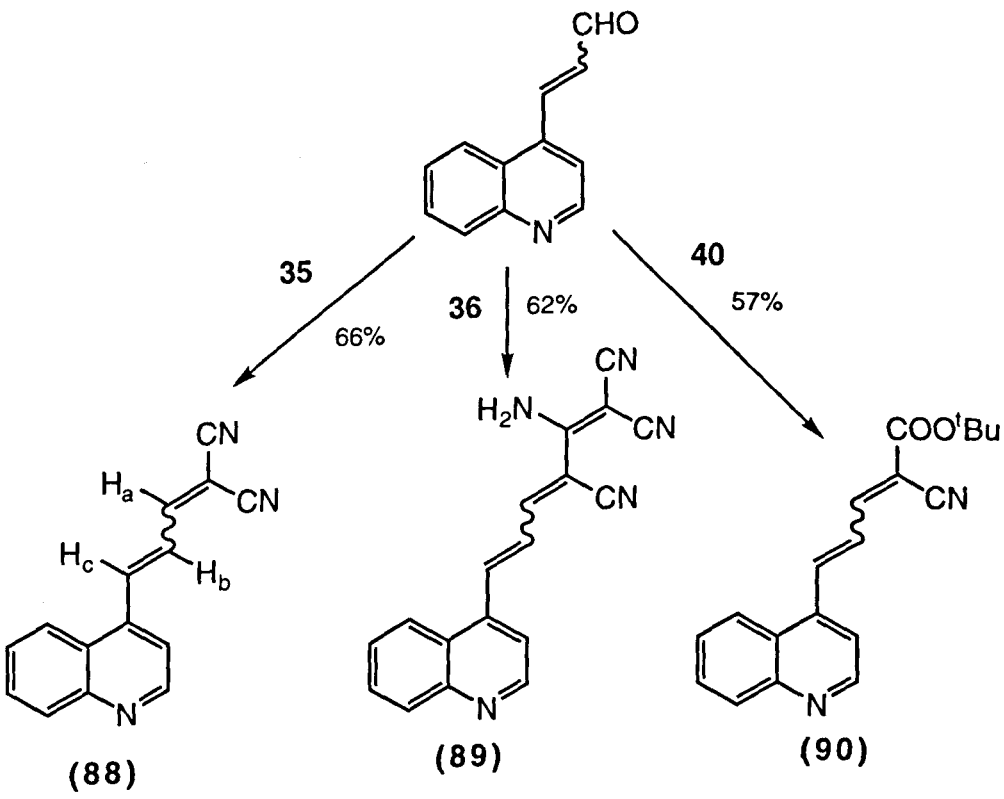



**Figure 23**

The $R_f$ values of the *E-* and *Z-* forms of **87** were so close as to make them virtually inseparable by column chromatography. Rather than separating the isomers by HPLC at this stage, we decided to progress with

aldehyde **87** as an *E/Z* mixture to determine whether or not derivatives with **87** were worth pursuing. Mixture **87** was condensed with malononitrile (**35**), malononitrile dimer (**36**) and $^t$butyl cyanoacetate (**40**) (Scheme 13).




**Scheme 13**

Although compound **88** could be made readily in good yield it was unstable and decomposed in a matter of hours upon standing. A further sample of **88** was prepared and kept in the dark but this also decomposed. It seems likely that **88** is moisture sensitive and if prepared again it should be rigorously dried and stored under anhydrous conditions. Compound **88** was not fully characterised but the spectra obtained were sufficient to establish its identity beyond doubt. The $^1$H NMR spectrum confirmed that the *E*- isomer of **88** predominated. The coupling constant for the protons of the first extraannular double bond ($H_c$ and $H_b$) was 15.0 Hz and the new olefinic proton ($H_a$) appeared as a doublet at δ 8.46 with a coupling constant of 11.4 Hz. The IR spectrum showed a sharp absorption at 3029 cm$^{-1}$ for alkene =C-H stretching and a strong band at 1603 cm$^{-1}$ for alkene C=C stretch. A large

peak for the molecular ion (m/z= 231) was seen in the low resolution mass spectrum.

Compound **89** was the first tyrphostin containing a triene side-arm to be prepared. Given that the starting material was stereochemically impure and that the stereochemistry of the Knoevenagel reaction could in theory lead to *E*- or *Z*- geometrical isomers, four geometrical isomers of **89** were possible from the reaction (Figure 24).

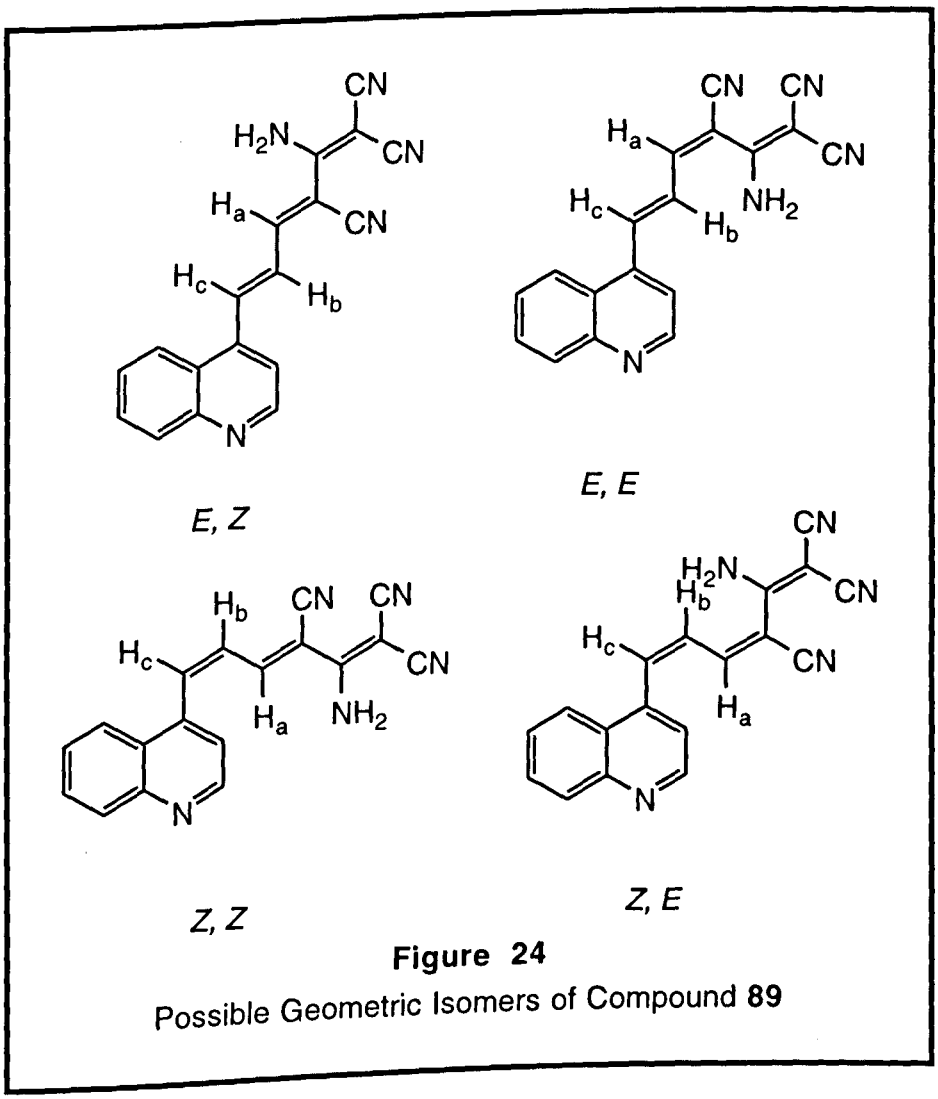



**Figure 24**

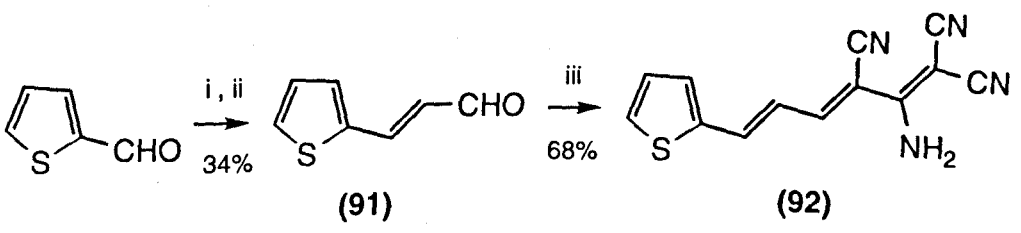
Possible Geometric Isomers of Compound **89**

The *Z*, *E*- and *Z*, *Z*- isomers could only account for 8% of the total reaction product because of the stereochemistry of the starting aldehyde. The unfavourable interaction of the 1,1-dinitrile-2-aminoethene moiety with $H_b$ in the *E*, *E*- isomer may mitigate against its formation. This isomer has considerably less rotational freedom that the *E*, *Z*- isomer which would appear to be the least sterically hindered and therefore the most energetically favourable isomer. The $^1H$ NMR spectrum of **89** confirmed the

presence of both the *E, Z-* and *E, E-* isomers. Evidence for the *Z, E-* and *Z, Z-* isomers could not be seen, presumably because the signals from these products were masked. Proton $H_b$ appears as a quartet, split by $H_a$ (11.2 Hz) and $H_c$ (15.0 Hz) and centred on δ 7.58. A much smaller quartet with exactly the same coupling constants can be seen, shifted about 0.1 ppm upfield. If the assumption about the relative stabilities of the *E* isomers is correct then the former quartet is due to the *E, Z-* isomer and the latter the *E, E-* isomer. This could be verified by carrying out nOe NMR studies on $H_b$ which should have a significant through-space interaction with the amine protons if **89** exists predominantly in the *E,E*-form. Alternatively, the isomers could be separated by HPLC and studied by X-ray diffraction provided that suitable crystals could be grown. Other spectral data which confirmed the structure of **89** were a strong absorption at 2215 $cm^{-1}$ for the nitrile groups and strong bands at 1647 and 1607 $cm^{-1}$ for C=C stretching modes in the IR spectrum.

The $^1H$ NMR spectrum for compound **90** showed predominantly one geometrical isomer which gave the same splitting pattern as **87** for the alkene protons. The $^{13}C$ NMR showed signals for the carbonyl carbon at δ 160.0 and the methyl carbons of the $^t$butyl group at δ 27.7. A strong absorption at 1724 $cm^{-1}$ in the IR spectrum confirmed the presence of the ester function.

Thiophene and nitrothiophene tyrphostins have formed some of the most potent anticancer agents under development within our group.[82, 101] We were keen to discover whether extended conjugation of the aliphatic side-arm would enhance the activity of thiophene-containing tyrphostins. 3-(2-Thienyl)propenal (**91**) was prepared[102] and condensed with malononitrile dimer as shown in Scheme (14).




**(91)** **(92)**

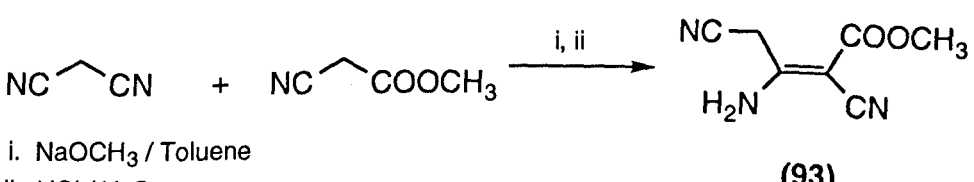
i. $CH_3CHO$ (40%), O °C, 3.5 h ii. $CH_3COOH$ iii. EtOH, malononitrile dimer, RT, 1 h

**Scheme 14**

The coupling constant for the alkene protons of **91** was 15.6 Hz, confirming that the distilled product consisted entirely of the *E*- isomer. An absorption at 1672 $cm^{-1}$ in the IR spectrum confirmed the presence of an $\alpha,\beta$–unsaturated aldehyde. Condensation of **91** with malononitrile dimer proceeded smoothly to give **92** which showed amine (3334 and 3217 $cm^{-1}$), nitrile (2212 $cm^{-1}$) and alkene (1658 $cm^{-1}$) absorptions in the IR spectrum. A new doublet with a coupling of 11.2 Hz was evident at δ 7.82 in the $^1H$ NMR spectrum which corresponded to the new olefinic proton.

## 4.4 Novel 'Dimer' Structures

A scan of the literature showed that many compounds similar in structure to malononitrile dimer had been synthesised. Several of these seemed worth preparing for our purposes. The reaction of malononitrile with methyl cyanoacetate to form a "crossed" dimer has been reported (Scheme 15).[103]




i. $NaOCH_3$ / Toluene
ii. HCl / $H_2O$

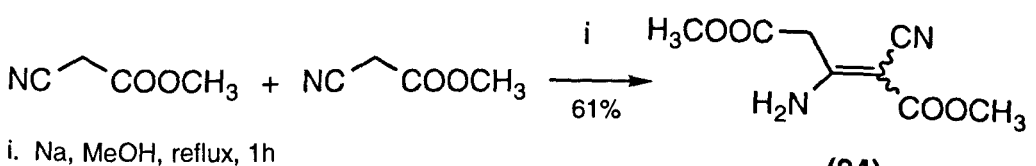
**(93)**

**Scheme 15**

Despite many attempts at this reaction the major product was always malononitrile dimer rather than the "crossed" dimer which was prepared in 61% yield according to the literature. In attempting to circumvent this problem we tried pre-forming the methylcyanoacetate anion before adding the malononitrile solution. This did not improve the yield of **93**, nor did adding the solution of malononitrile in methyl cyanoacetate dropwise over a period of ten minutes. By following the literature preparation on a large scale and after purification by chromatography a small amount of pure **93** was obtained. The $^1H$ NMR spectrum of **93** showed a broad singlet integrating for two protons at δ 9.10, corresponding to the amine protons. The only other resonances present were the methylene protons at δ 3.87 and the methyl protons at δ 3.72. The low resolution mass spectrum gave the correct molecular ion (m/z=165). Compound **93** was used without further

characterisation in an unsuccessful condensation reaction with 2-quinolinecarbaldehyde.

Attempts to form **94**, the dimer of methylcyanoacetate, proved more successful (Scheme 16).[104]

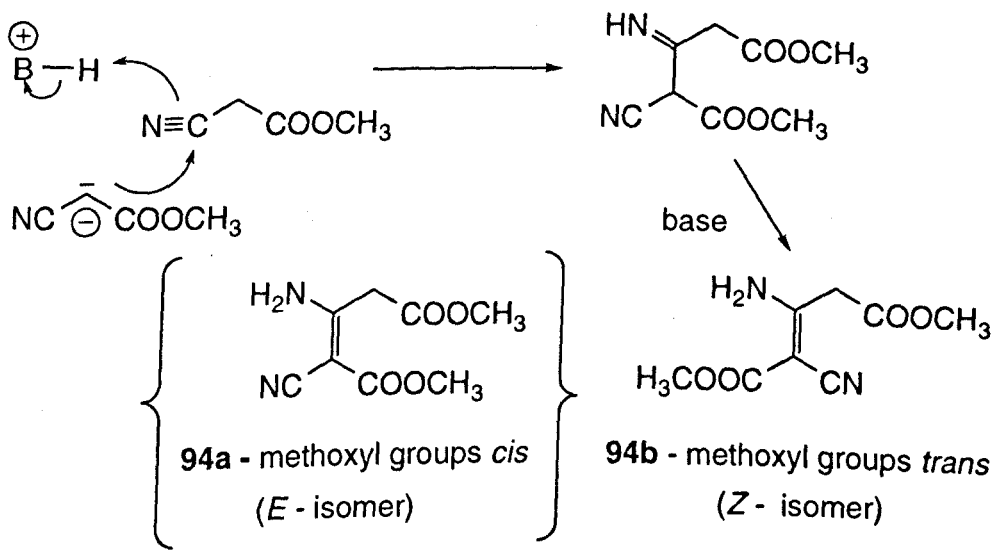



i. Na, MeOH, reflux, 1h

**(94)**

**Scheme 16**

The $^1H$ NMR spectrum of **94** confirmed the presence of a primary amine, showing two broad singlets at $\delta$ 9.13 and 8.94. N-H stretching modes were present in the IR spectrum at 3402 and 3290 $cm^{-1}$ as well as a strong absorption at 2206 $cm^{-1}$ for the conjugated nitrile. The configuration of **94** was of interest to us since in their paper, Junek and Wolny represented the dimer with the ester groups *cis* to one another (**94a**, Scheme 17). This seemed counter-intuitive on the basis of thermodynamic arguments, which would suggest that the bulkier groups should be as far removed from one another as possible leading to the *trans* isomer (**94b**).




**94a** - methoxyl groups *cis* (*E* - isomer)

**94b** - methoxyl groups *trans* (*Z* - isomer)

**Scheme 17**

One could also argue that the *Z*- isomer would have the added benefit of a stabilising hydrogen bonding interaction (Figure 25).

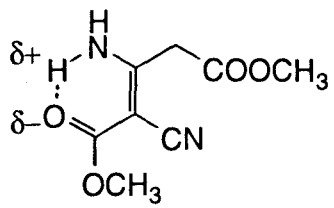



**Figure 25**
Stabilisation of *Z*- form of 94
by hydrogen bonding.

Given these observations, we believed that the structure in reference 104 had been wrongly assigned. To test this theory we performed nOe NMR studies on compound **94** to examine the interaction of the amine protons with those of the methoxyl groups. Molecular modelling of **94a** showed that the methoxyl group *trans* to the amine could not approach close enough to exhibit a nuclear Overhauser enhancement. The closest approach was of the order of 4-5 Å. In **94b**, however, several conformations of the $COOCH_3$ unit allowed the methoxyl group to come into proximity to the amine protons. This was also the case for the geminal methoxyl group in both **94a** and **b.** One would therefore expect to see only one nOe enhancement if **94a** predominated, and two if **94b** predominated. Our studies showed small but measurable enhancements for both methoxyl groups and the methylene group when the amine protons were irradiated. The nOe difference spectrum when the amine function is irradiated is reproduced in Figure 26. This indicated that the ester groups adopt a *trans* arrangement as represented in structure **94b**.

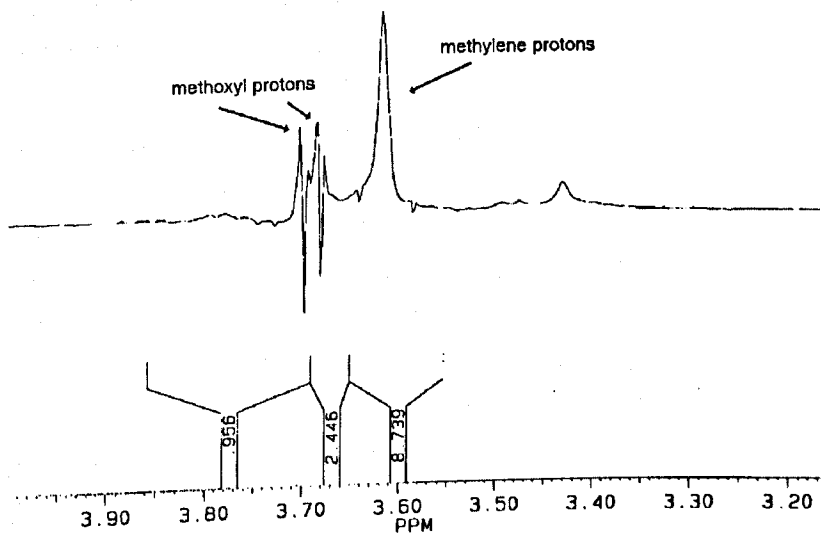
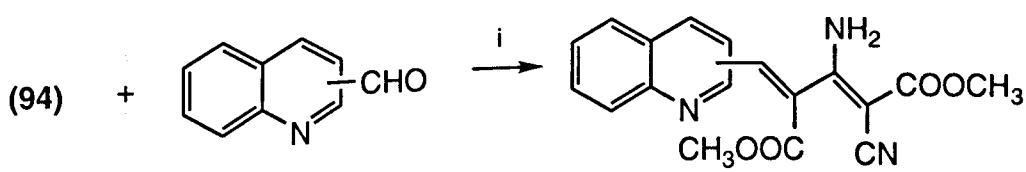



**Figure 26**

NOE difference spectrum for compound **94**
after irradiation of amine protons

Diester **94** was condensed with 2- and 4-quinolinecarbaldehyde under more forcing conditions than normal to give tyrphostins **95** and **96** in low to moderate yields (Scheme 18). These compounds were similar to tyrphostins **46** and **48** in that they possessed an electron-withdrawing group *cis* to the ring and an amine function which could confer a partial negative charge on the terminal groups of the side-arm by electronic delocalisation.




i. DMF, $^{t}$BuOK, 90 °C, 16-18 h

**2' - (95)** 46%
**4' - (96)** 16%

**Scheme 18**

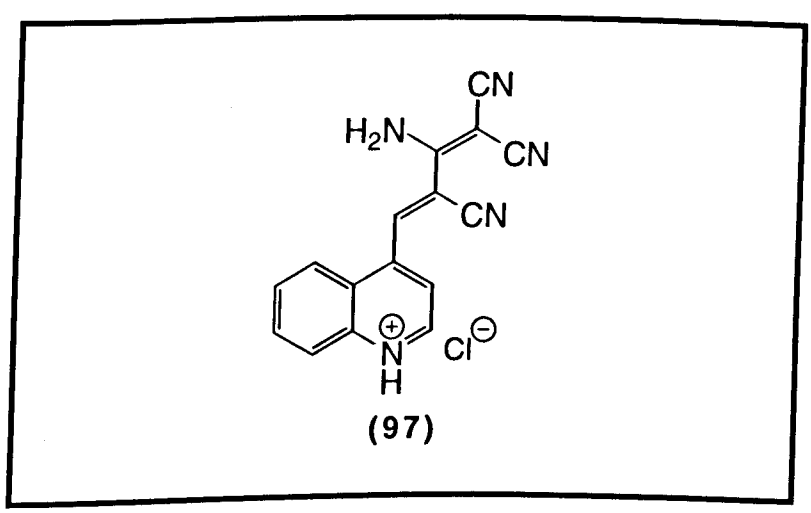
Both compounds showed the new olefinic proton as a singlet in the $^{1}$H NMR spectra (δ 8.32 and 8.46 respectively). The IR spectra showed

carbonyl absorption bands at 1720-1726 $cm^{-1}$ and strong alkene absorptions between 1620 and 1664 $cm^{-1}$.

## 4.5 Solubilisation of Tyrphostins

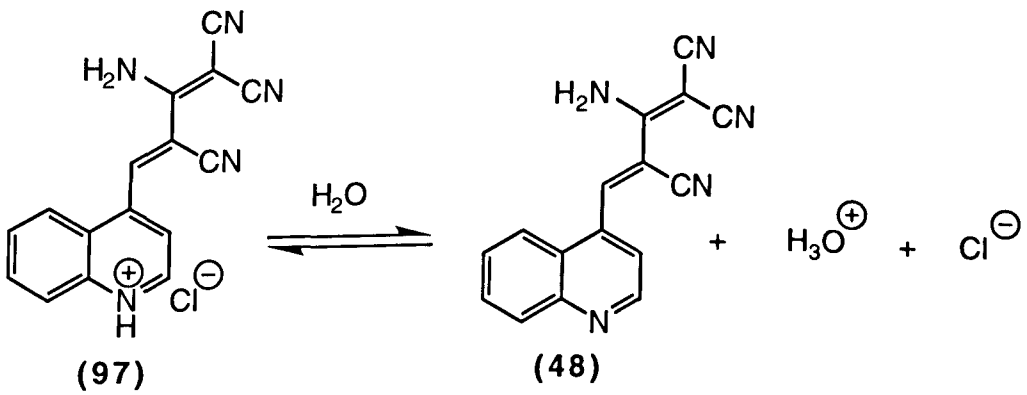
The hydrophobic nature of tyrphostins **46-48** was problematic, especially in terms of their potential as ingested or injected drugs. Empirical solubility tests on compound **46** showed that the maximum concentration achievable in 1% DMSO-water solution was only ~100 μM. To achieve concentrations greater than this required a larger proportion of DMSO which is undesirable for *in vivo* testing. In attempting to solve this problem the approaches available to us were to 'solubilise' existing tyrphostins or to design entirely new, more hydrophilic tyrphostins. Both methods were employed.

Many commercially available drugs are sold in the form of their hydrochloride salts to confer increased solubility in aqueous media. The quinoline moiety in compounds **46-48** provided a basic site for protonation by strong acid. By dissolving the compounds in ethanol and bubbling HCl gas through the solution, the hydrochloride salt (**97**) of compound **48** was obtained in almost quantitative yield. This preparation proved superior to methods using aqueous acids. The proton attached to the quinoline nitrogen gave a broad singlet at δ 10.49 in the $^1H$ NMR spectrum and the IR spectrum showed a broad absorption at 2430 $cm^{-1}$, indicating the presence of quaternary N-H stretching modes. As expected, the $R_f$ of **97** was very low when subjected to TLC with non-polar organic solvents as eluents.




**(97)**

Solubility tests were carried out on compound **97** to determine whether or not it showed improved solubility over compound **48**. 18.3 mg of **97** was dissolved in 337 µl of DMSO to give a 0.2 M solution. 10 µl of this was added to 990 µl of water, giving a 200 µM solution in 1% DMSO-water. Although initially transparent, the solution became cloudy within minutes, presumably because of precipitation of the free base (Scheme 19).




**Scheme 19**

The pKa of **97** has not been measured, but that of protonated quinoline is 4.94. Assuming that the pKa values for the two species are fairly similar, the extent of dissociation of **97** in dilute aqueous solution (pH 7) is given by the Henderson equation.

$$pH = pKa + \log [A^-]/[HA]$$

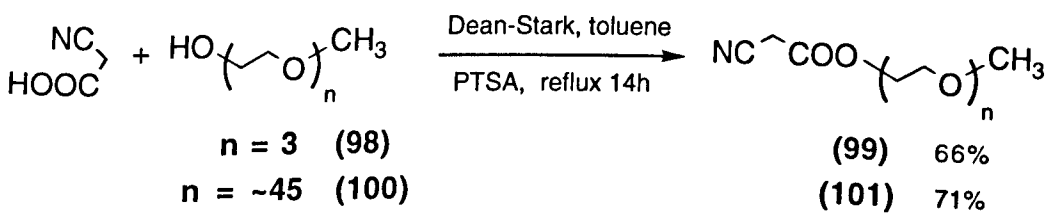
$$[A^-]/[HA] = \text{antilog } [pH\text{-}pKa]$$
$$= \text{antilog } [7\text{-}4.94]$$
$$= 114.8$$

This shows that at physiological pH, **97** exists primarily (~99%) as the free base. This degree of precipitation is clearly unacceptable.

An alternative strategy involves the solubilisation of drugs by the attachment of water-soluble groups. The most commonly used agents are polyethylene glycols (PEGs) which have been functionalised to react with nucleophilic or electrophilic centres. PEG-COOH, PEG-$NH_2$ and PEG-NCO and many similar derivatives are commercially available. The advantages of

using PEGs as solubilising agents are many; they are non-toxic, biocompatible and soluble, both in organic solvents and water. They are not biodegradeable and can therefore pass through the body without harm. Several well-known drugs such as penicillin V, aspirin, amphetamine, quinidine and atropine have been solubilised with PEGs.[105]

Applying this methodology to our work, we chose to work with the short-chain compound triethyleneglycol methyl ether (MTEG, **98**) and a polyethyleneglycol methyl ether with an average molecular weight of 2000 (MPEG, **100**). By esterifying these reagents with cyanoacetic acid, we were able to prepare water soluble compounds containing an activated methylene function (**99, 101**). These compounds would be exactly analogous to the cyanoacetates used previously to prepare tyrphostins (Scheme 20).




**n = 3 (98)**
**n = ~45 (100)**

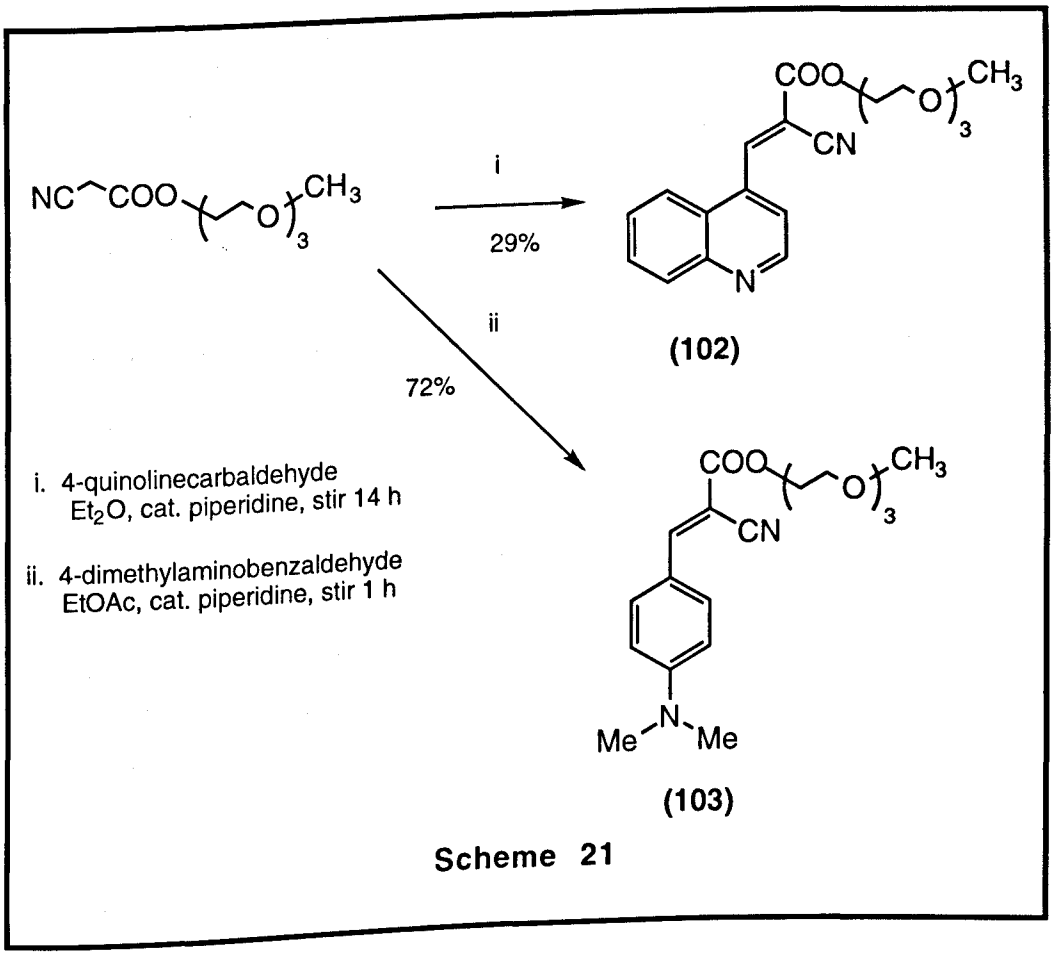
**(99)** 66%
**(101)** 71%

**Scheme 20**

Esterification of ethers **98** and **100** with cyanoacetic acid, with azeotropic removal of the water formed during the reaction, gave the products **99** and **101** in moderate yields. The $^{1}H$ NMR spectrum of compound **99** showed the correct number of protons for the product, most of which came into resonance in the region $\delta$ 3.34-3.56. The singlet due to the methoxyl protons was distinct at $\delta$ 3.20. The IR spectrum of **99** showed unconjugated nitrile (2261 $cm^{-1}$) and ester (1749 $cm^{-1}$) absorbances. Spectral analysis for compound **101** was more difficult because the large number of ethylene glycol units (~ 45) dwarfed the signals from the other functional groups. The $^{13}C$ NMR spectrum proved to be the most instructive, showing resonances for the carbonyl carbon, the nitrile carbon and the interposing methylene group at $\delta$ 165.3, 115.3 and 24.9, respectively.

Compound **99** was reacted with 4-quinolinecarbaldehyde and 4-dimethylaminobenzaldehyde to produce the novel solubilised tyrphostins **102** and **103** (Scheme 21). The rationale behind choosing 4-

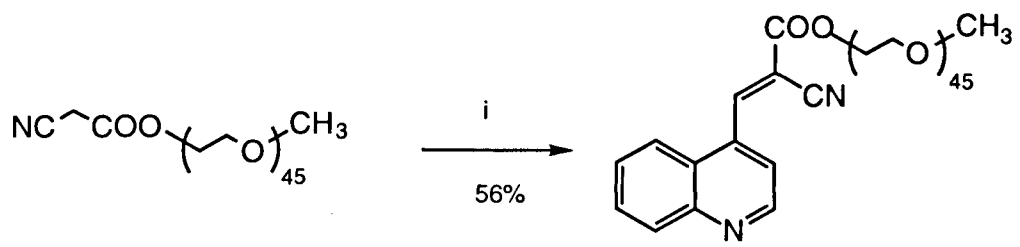
dimethylaminobenzaldehyde is explained in Section 4.7. Both compounds were readily soluble in water with gentle heating.




**Scheme 21**

Compounds **102** and **103** showed the characteristic singlet for the new olefinic proton at δ 8.86 and 7.87 in the $^1$H NMR spectra. The IR spectra showed absorptions corresponding to the conjugated nitrile (2212-2230 cm$^{-1}$), ester (1714-1734 cm$^{-1}$) and ether (1103-1188 cm$^{-1}$) functions.

The PEG solubilised tyrphostin **104** was prepared by reaction with 4-quinolinecarbaldehyde (Scheme 22).




i. 4-quinolinecarbaldehyde
EtOH, stir, 18 h

**(104)**

**Scheme 22**

As with compound **101**, spectral analysis was somewhat difficult because of the intense signals from the ethylene glycol units. Despite this, the quinoline signals were clearly seen in both the $^{1}H$ and $^{13}C$ NMR spectra. Comparison of the integrals for the quinoline protons with that for the methylene group attached to the ester (the only MPEG signal easily distinguished) confirmed that a small amount of starting material (~ 6%) was still present. IR spectroscopy confirmed the presence of an $\alpha,\beta$-unsaturated nitrile but also a trace of unconjugated nitrile from the starting material. The physical and chemical properties of **101** and **104** are dictated by the polymer chain, making separation difficult by any other method than HPLC. We felt that, at this stage of development, we should proceed with testing with a view to remaking and purifying compound **104** if the results proved interesting.

Electrospray mass spectrometry (ESMS) proved to be a useful tool in analysing compound **104** and other PEG solubilised anticancer agents (Section 6.7). This relatively mild technique is particularly useful for analysis of high molecular weight species such as proteins and polymers since it produces little fragmentation. Similar in principle to conventional mass spectrometry, the main difference is the method of introducing the compound into the analyser. A solution of the sample containing a small percentage of an electrolyte is injected into the spectrometer and carried towards the probe by a heated carrier gas, usually nitrogen. The liquid passes over a sharp-edged capillary which is at high voltage, causing formation of microdroplets. Spontaneous evaporation of solvent frees the charged ions, which accelerate towards the detector under the influence of the applied potential difference. The detector then measures and records the mass to charge ratio for each species present. The ESMS spectrum of compound **104** is

reproduced below (Figure 27). The breadth of the mass distribution reflects the variation in polymer length inevitable with compounds such as MPEG.

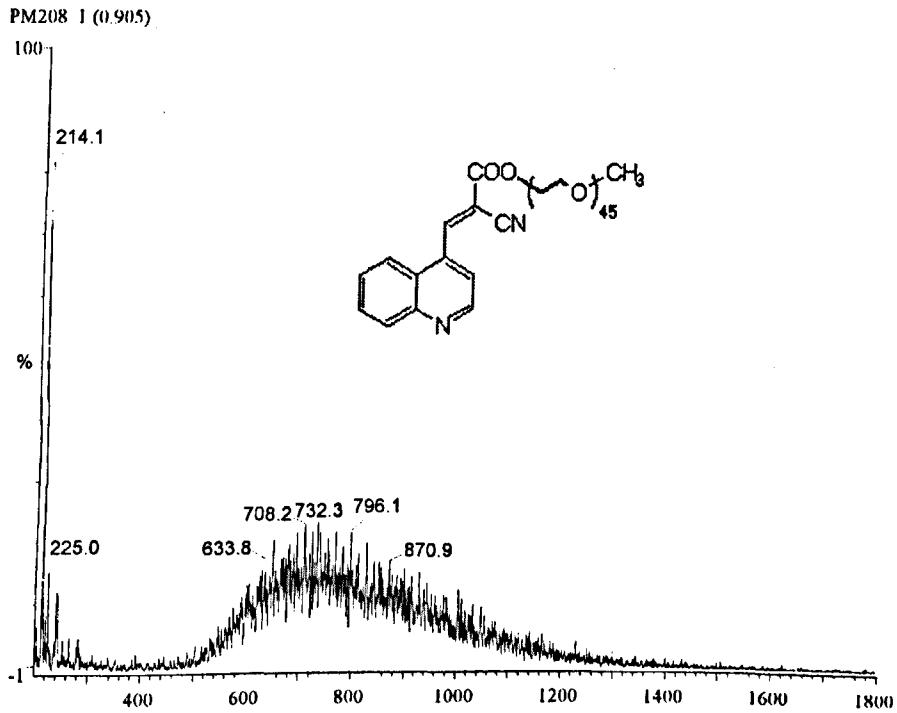


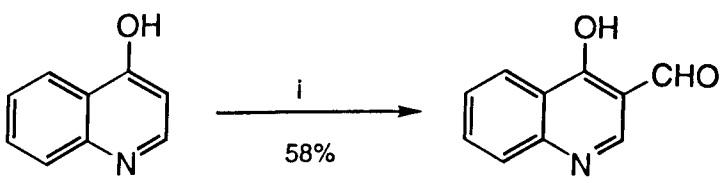

**Figure 27**

ESMS Spectrum of Compound **104**

When pure MPEG is subjected to ESMS, the distribution is centred on a mass/charge ratio of ~ 660 (Section 6.7, Figure 38) which indicates that most of the detected ions are triply charged. This is also true of the ESMS spectrum of **104**, although the skew in the graph indicates that doubly charged ions are also present. The centre of the distribution is at approximately 732 which corresponds to an overall molecular weight of 3 x 732 = 2196. The empirical formula of **104** is $C_{104}H_{190}N_2O_{47}$ which gives a molecular mass of 2218. This discrepancy equates to an error of 1%. Given the imprecision in estimating the centre of the distribution, it seems reasonable to conclude that the product **104** has indeed been formed.

## 4.6 Hydroxylated Quinoline Tyrphostins

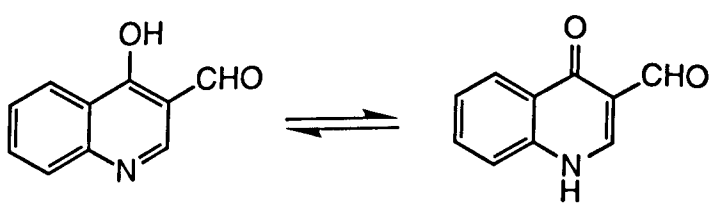
Previous work has shown that the activity of benzylidene-based tyrphostins increased as more hydroxyl substituents were introduced into the ring.[25,80] We were interested in preparing hydroxylated quinolines, not only to improve activity and solubility, but as a 'handle' for further functionalisation of the compounds. A literature search led to several hydroxylated quinoline aldehydes but most were substituted in the benzene ring which was not of direct interest to us at that time. Of more use to us was a preparation of 4-hydroxyquinoline-3-carbaldehyde (**105**) from 4-hydroxyquinoline (Scheme 23)[106]




i. NaOH, $CHCl_3/H_2O$, reflux, 5 h **(105)**

**Scheme 23**

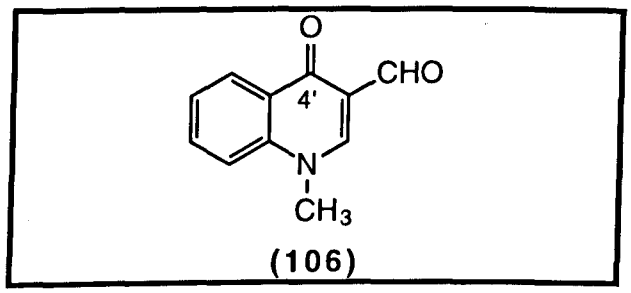
The formation of aldehyde **105** was confirmed by the presence of a singlet at δ 10.22 in the $^1H$ NMR spectrum due to the aldehydic proton and a resonance at δ 188.8 in the $^{13}C$ NMR spectrum from the carbonyl carbon. Compound **105** can tautomerise in solution, giving rise to 'keto' and 'enol' forms (Figure 27).




'enol' form 'keto' form

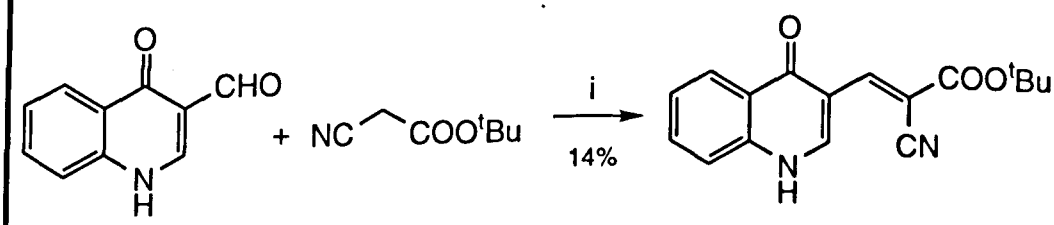
**Figure 28-** Tautomers of Compound **105**

In DMSO, only one set of signals for the compound was seen in the $^{13}C$ NMR spectrum, implying a preference for one tautomer in solution. The carbon at the 4' position of the quinoline ring came into resonance at δ 176.3 which is far enough downfield to indicate a carbonyl rather than an hydroxyl-bearing carbon. Further evidence comes from the work of Coppola *et al.* who prepared a series of non-tautomerising 2- and 4-quinolones.[107] When the quinolone nitrogen is substituted with a methyl group rather than a proton as in structure **106** , carbon 4' comes into resonance at δ 178.4, which is similar to the value observed for the analogous carbon in **105**.




**(106)**

The inference is that in DMSO solution, **105** prefers to exist in the 'keto' form, which is really a vinylogous amide. This observation concurs with studies on the tautomerism of 4-quinolone-3-carboxylic acid derivatives carried out by De la Cruz *et al.*[108] who concluded that these compounds preferentially exist in the 'keto' form in DMSO solution. The IR spectrum of **105** confirms the presence of vinylogous amide absorptions at 1616 and 1554 $cm^{-1}$, but also shows a broad absorption at 3444 $cm^{-1}$, indicating hydrogen-bonded OH. This observation, coupled with the presence of two distinct carbonyl absorptions at 1704 and 1682 $cm^{-1}$ would suggest that both tautomers are present in the solid state. Compound **105** was condensed with $^{t}$butyl cyanoacetate to give the novel tyrphostin **107** (Scheme 24).



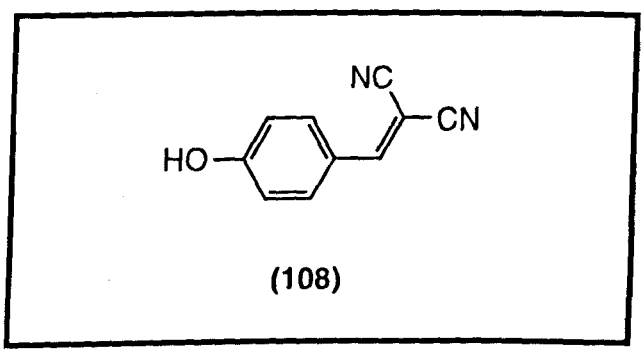

i) DMF, 16 h, RT

**(107)**

**Scheme 24**

The $^1H$ NMR spectrum showed the expected olefinic proton at δ 9.02 commensurate with the Knoevenagel product. The ester carbonyl carbon came at δ 161.9 in the $^{13}C$ NMR spectrum and gave a strong C=O absorption at 1719 $cm^{-1}$ in the IR spectrum. Strong absorptions at 1621 and 1553 $cm^{-1}$ were due to the vinylogous amide in **107**.

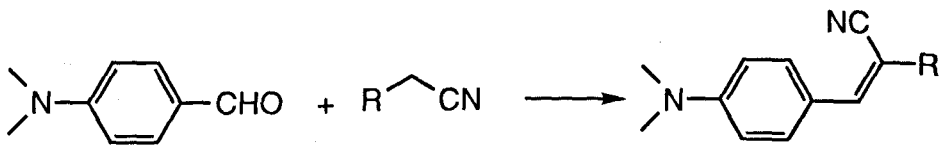
Compound **108**, an hydroxylated tyrphostin, has been prepared previously as a potential tyrosine kinase inhibitor. We were interested in preparing **108** not just as a tyrphostin but also as a potential antimelanoma agent. Phenols and catechols are known to possess significant activity against melanomas (Chapter 5). Knoevenagel condensation between 4-hydroxybenzaldehyde and malononitrile afforded **108** is good yield. The key spectral features of **108** were the broad O-H absorption at *ca.* 3350 $cm^{-1}$ in the IR spectrum and the presence of the characteristic olefinic singlet at δ 8.30 in the $^1H$ NMR spectrum.




**(108)**

## 4.7 Electron-Rich Tyrphostins

The electronic distribution in the side-arm of compounds **46-48** distinguished them from the other tyrphostins in the initial series. Delocalisation of the amine lone pair conferred a partial negative charge onto the terminal nitrile groups which may assist in binding to an enzyme. We reasoned that compounds containing the 4-dimethylaminobenzene moiety as the free base would be able to mimic this effect and so increase electron density on the terminal groups of the side-arms. Four new tyrphostins were prepared from dimethylaminobenzaldehyde as shown in Figure 29.




| R | COMPOUND |
|---|---|
| CN | **109** |
| $C(NH_2)=C(CN)_2$ | **110** |
| $CSNH_2$ | **111** |
| 3-pyridyl | **112** |

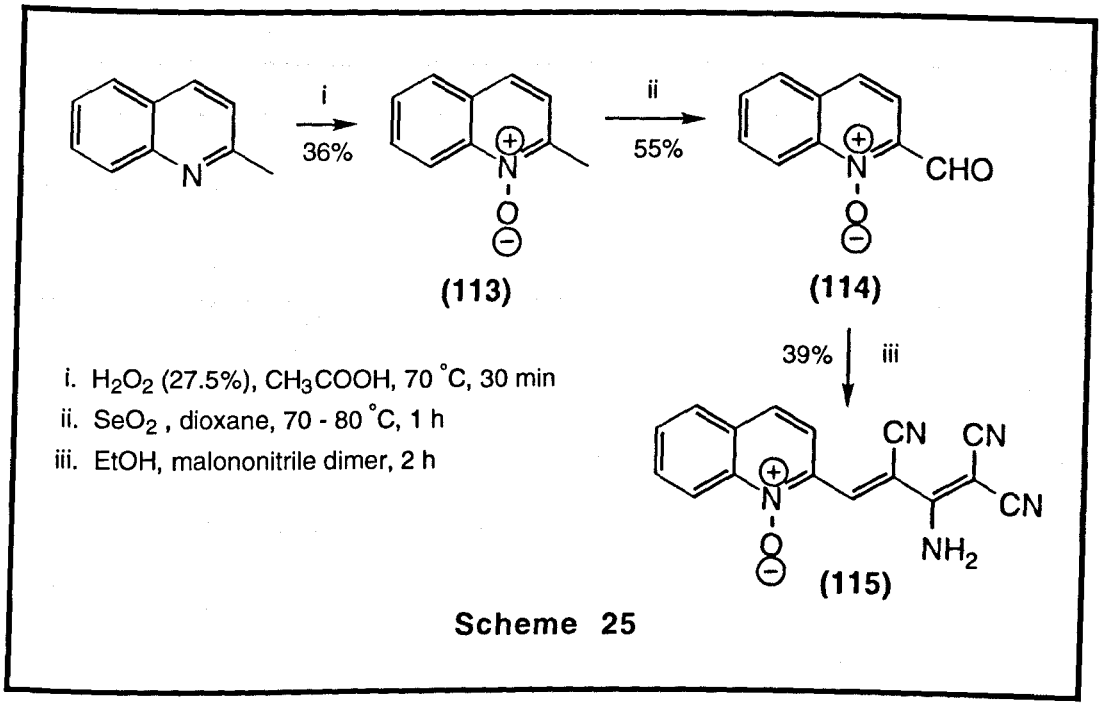
**Figure 29**

All of these compounds showed the characteristic singlet corresponding to the newly formed olefinic proton and the correct functionality as evidenced by the IR spectra.

## 4.8 A Bioreducible Tyrphostin

Bioreductive anticancer agents are a class of drugs which exploit a particular feature of solid tumours - oxygen deficiency. The demand for oxygen in proliferating tissue is such that the normal supply cannot keep pace with tumour growth. Cells which are poorly served by capillaries die; others, known as 'hypoxic' cells manage to survive on a sub-optimal oxygen supply. Under these conditions, reductase enzymes are often overexpressed and drugs which contain a reducible functional group such as a nitro, quinone or N-oxide moiety can be metabolised to their reduced forms. These reductions generate radical species which can alkylate DNA in a manner analogous to that described in Section 2.4. The ideal bioreductive agent would be a non-toxic prodrug which only becomes active upon reduction within the tumour.

We were interested in forming the N-oxide of our most potent tyrphostin **46** as a potential bioreductive agent. Attempts to form the N-oxide by direct oxidation seemed fruitless since epoxide formation would almost certainly be a competing reaction. An alternative scheme starting from quinaldine was proposed (Scheme 25).



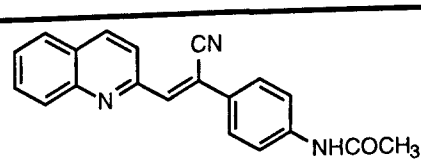
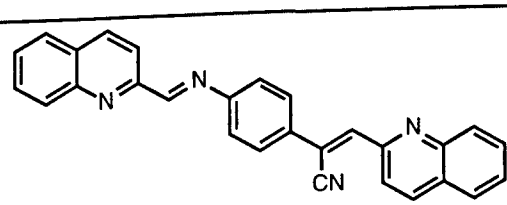
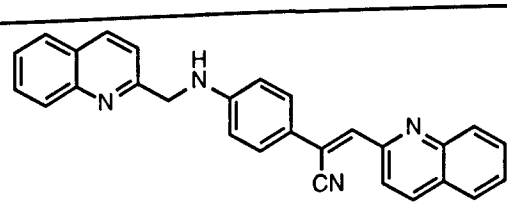
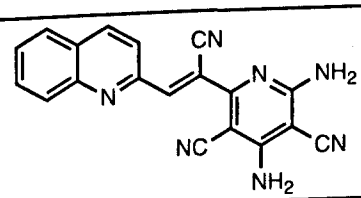
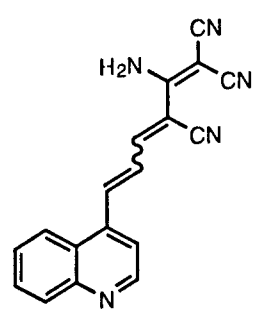
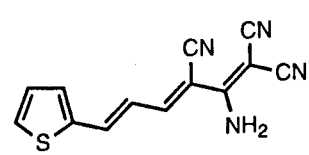

**Scheme 25**

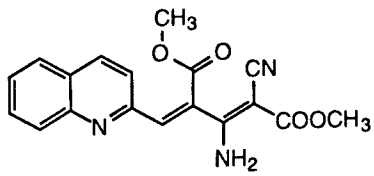
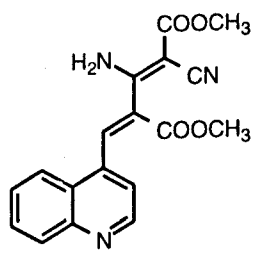
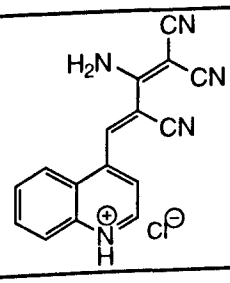
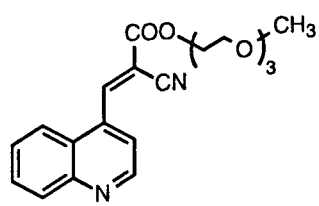
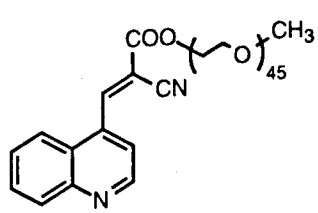
Oxidation of quinaldine with peracetic acid (generated *in situ*)[109] gave the N-oxide monohydrate **113**. The melting point of the product and the spectral data concurred with those in the literature. Further oxidation with selenium dioxide[110] afforded the aldehyde **114** in moderate yield. The carbonyl carbon of the aldehyde was evident at δ 186.5 in the $^{13}C$ NMR spectrum, as were C=O stretching modes (1694 $cm^{-1}$) in the IR spectrum. Condensation with malononitrile dimer gave **115**, the N-oxide of tyrphostin **46**. Both amine and olefinic protons were present in the $^{1}H$ NMR spectrum at δ 9.35, 9.30 and 8.43 respectively. The IR was notable for the strong nitrile absorption at 2216 $cm^{-1}$ and the broad absorption due to N-O stretching at 1262 $cm^{-1}$.

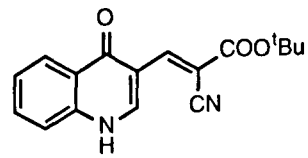
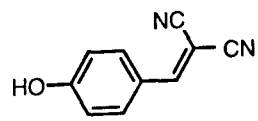
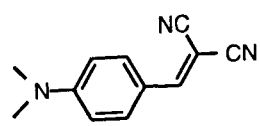
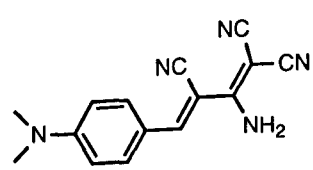
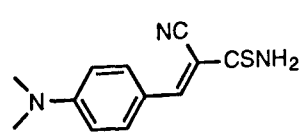
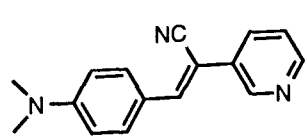
## 4.9 Biological Evaluation

The biological evaluation of the tyrphostins described in this chapter was carried out by Dr Alan McGown and colleagues at the CRC Paterson Institute for Cancer Research in Manchester. Cytotoxicity testing was performed on two breast cancer cell lines, MCF-7 and MCF-7/ADR which expresses a higher level of EGFR activity. The results are summarised in Table 3.

## Table 3

| COMPOUND | STRUCTURE | CYTOTOXICITY $IC_{50}$ (μM) | |
|---|---|---|---|
| | | MCF-7 | MCF-7/ADR |
| 74 |  | 25.9 | 11.2 |
| 79 |  | 31.2 | 44.4 |
| 81 |  | 31.5 | 3.0 |
| 83 |  | 13.4 | 14.0 |
| 89 |  | 4.0 | 5.7 |
| 92 |  | 12.3 | 9.1 |

| Table 3 (contd.) | | | |
|---|---|---|---|
| COMPOUND | STRUCTURE | CYTOTOXICITY $IC_{50}$ (μM) | |
| | | MCF-7 | MCF-7/ADR |
| 95 |  | 25.2 | 48.1 |
| 96 |  | 1.0 | 0.9 |
| 97 |  | 1.0 | 1.4 |
| 102 |  | 0.9 | 1.1 |
| 104 |  | 4.9 | 6.7 |

| Table 3 (contd.) | | | |
|---|---|---|---|
| COMPOUND | STRUCTURE | CYTOTOXICITY $IC_{50}$ (μM) | |
| | | MCF-7 | MCF-7/ADR |
| 107 |  | 83.4 | nd. |
| 108 |  | 147.1 | 14.1 |
| 109 |  | 101.5 | 97.5 |
| 110 |  | 108.8 | 38.0 |
| 111 |  | 194.8 | 112.6 |
| 112 |  | 0.5 | 0.5 |

### 4.9.1 Comment on Biological Evaluation

Compounds **74** and **79** showed only moderate activity against these MCF-7 cell lines. Interestingly, the reduced form of **79**, compound **81**, performed significantly better against the MCF-7/ADR line, suggesting that it may be inhibiting EGFR mediated growth. Compound **83** was more active than the other aromatic compounds but showed no selectivity between the cell lines. Better still was compound **89**, a triene, which inhibited with $IC_{50}$ values of 4.0 and 5.7 μM, figures comparable to those of the corresponding diene **48**. Significantly, **89** does not incorporate a *cis*-nitrile function which was previously believed to be important for activity.[25] Compound **92**, a thiophene with a triene side-arm, also performs well. It appears that for these compounds the important structural motif is the 1,1-dinitrile-aminoethene moiety in conjugation with the heterocycle, regardless of the presence or absence of the *cis*-nitrile functionality.

The novel dimers **95** and **96** show a surprising disparity in activity, the 4'-isomer out-performing the 2'-isomer by more than an order of magnitude. This trend is not evident for compounds **46** and **48**, suggesting that the extra steric bulk of the methyl ester functions may be detrimental to the binding properties of **95**. Tyrphostin **96** performs as well as compound **46** and **48** and shares with them the ability to feed electrons from the primary amine onto the terminal functional groups.

As expected, tyrphostin **97** exhibits good activity, showing a small improvement in $IC_{50}$ value compared to its free base **48**. This may simply be attributable to improved solubility. Compounds **102** and **104**, MTEG and MPEG solubilised esters showed a marked improvement over the ester derivatives previously synthesised. It seems likely that this may also be due to better uptake and transport through the cell membrane.

The hydroxylated tyrphostins **107** and **108** performed poorly, although **108** showed a tenfold increase in activity against the cell line expressing higher EGFR kinase activity. The dimethylaminobenzene derivatives were inactive in these assays with the exception of **112**, which proved to be the most potent tyrphostin in this series. Such was the interest in this compound that it was submitted to the University of Bradford's Clinical Oncology Unit for further evaluation by Professor J.A. Double. Compound **112** was tested against a panel of 5 cell lines for 96 hours *in vitro*. The $IC_{50}$ values are reported in Table 4.

| Table 4 | | | | |
|---|---|---|---|---|
| $IC_{50}$ values for Compound 112 (μM) | | | | |
| Breast MT-1 | Leukaemia K562 | Leukaemia WEHI-3B | Colon HT-29 | Colon DLD-1 |
| > 4 | 0.5 | 0.64 | 1.1 | 2.4 |

These data show that tyrphostin **112** exhibits slight selectivity for the leukaemic cell lines but possesses a fairly broad spectrum of activity. Given these facts and the lack of discrimination between MCF-7 and MCF-7/ADR it seems likely that **112** is not inhibiting solely by TK mediated routes.

Dr Ian Stratford at the MRC Radiobiology Unit, Didcot, agreed to test our N-oxide compound **115** and the parent structure **46** to determine whether there was sufficient difference in activity to warrant further investigation. Studies against three cell lines differing in their expression of EGFR kinase activity showed that the two compounds performed very similarly (Table5).

| Table 5 | | | |
|---|---|---|---|
| Cell Line | EGFRK activity (relative) | $IC_{50}$ Values (μM) 46 | 115 |
| MCF-7 | 1 | 60 | 70 |
| MDA-231 | 15 | 60 | 80 |
| MDA-468 | 640 | 60 | 90 |

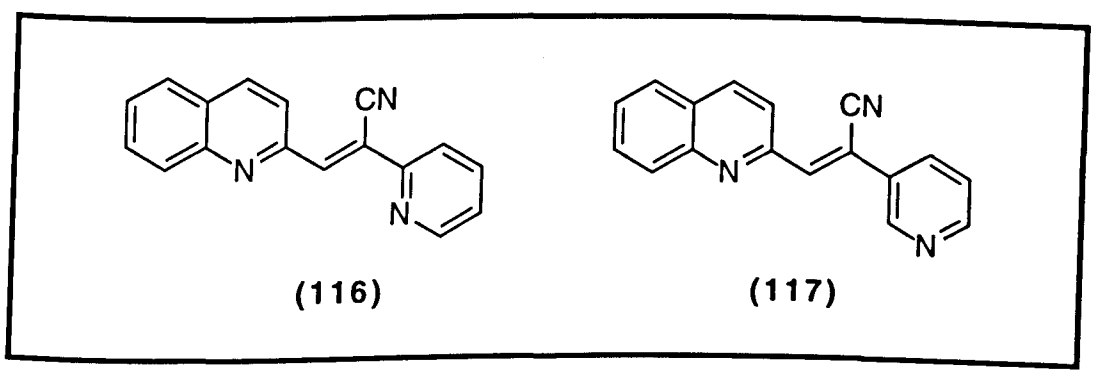
Given that we had been hoping for **115** to be inactive until reduced, it was evident that the compound would not be suitable for development as a bioreductive agent.

## 4.10 Discussion and Suggestions for Future Work

Our initial objective for this project was to establish whether or not heterocyclic tyrphostins, particularly quinolines, could match the activity of the polyhydroxylated benzene tyrphostins developed by Levitzki *et al.* This objective has been achieved. Of the eight most active tyrphostins, two exhibit $IC_{50}$ values of <5 μM against the EGFR tyrosine kinase (**46** & **48**) and six

inhibit MCF-7 growth with $IC_{50}$ values of ≤1 μM (**96**, **97**, **102** & **112**) or ≤5 μM (**89**, **104**).

Although the overall structure/activity pattern is still unclear, this work has established some important guidelines for further development. Much has been made of the 'dimer' structures **46** and **48**, in particular the diene side-arm which we felt was crucial to their activity. Our own work and that of others has shown that tyrphostins which incorporate 1,1-dinitrile-2-aminoethene moiety frequently, if not always, show good activity. Why is this? We believed that extended conjugation and the ability to delocalise charge onto the terminal nitrile groups were the crucial features which made this moiety biologically active. By preparing tyrphostins incorporating aromatic and heterocyclic groups in the side-arm, it became apparent that extension of conjugation, certainly through cyclic systems, was no guarantee of activity. Even compound **83**, which bears a close resemblance to **46** in terms of functionality, could not improve upon its activity. The pyridine-containing tyrphostin **112,** however, showed excellent activity against a variety of cell lines as described in the previous section. It seems likely, given that the steric bulk of the aromatic ring appears not to be beneficial, that the basic nitrogen of this heterocycle is of importance. The condensation products of 2-quinolinecarbaldehyde with 2- and 3-pyridylacetonitrile (**116**, **117**) have also been prepared.[91]

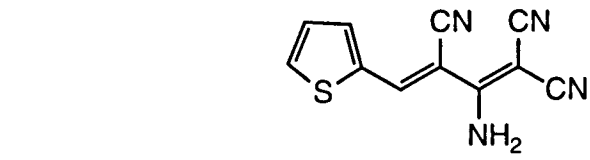



**(116)** **(117)**

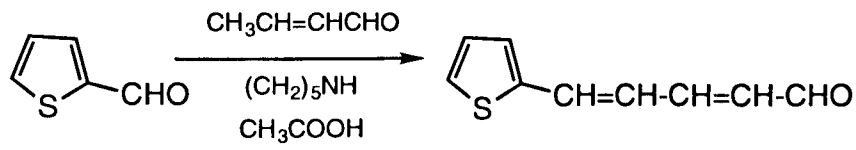
Both compounds show good activity against the MCF-7 cell line ($IC_{50}$ values of 5 and 8 μM respectively), although they inhibit the EGFR kinase only poorly ($IC_{50}$ values of >500 μM). These data add weight to the suggestion that tyrphostin **112** is not inhibiting by TK mediated pathways.

Tyrphostins incorporating an aliphatic triene side-arm (**89** and **92**) showed good activity, although this may be attributable to the fact that they both contain the 1,1-dinitrile-2-aminoethene moiety. Interestingly, compound

**92** shows a marked improvement in activity over its diene counterpart (**118**, $IC_{50}$ >50 against MCF-7).[80] A preparation of the highly conjugated thiophene aldehyde (**119**) has been reported[111] (Scheme 26) which may be worth condensing with malononitrile dimer to see whether further conjugation improves activity.




**(118)**



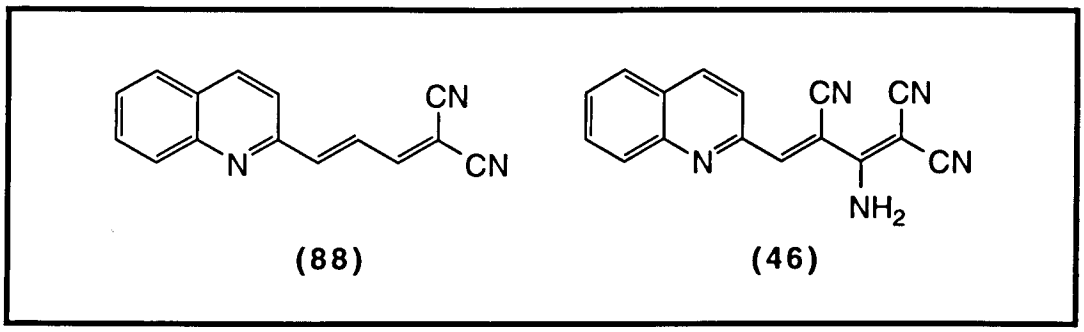

**(119)**

**Scheme 26**

The role of the extra double bond in modifying the activity of **102** is unclear. It may simply act as a 'spacer', causing the molecule to fit better into an enzyme pocket. It could, however, be altering the chemical properties of the compound. A highly conjugated group such as the side-arm of **102** could bond with nucleophilic centres on proteins or enzymes in a Michael reaction, and so inhibit cellular function. Conjugate addition of this type is known to account for the inhibitory properties of other anticancer agents such as Herbimycin A (Section 2.4). As an initial step towards establishing whether or not Michael-type conjugation is important to the efficacy of these compounds, their ability to react with sulfhydryl reagents such as ethanethiol or cysteine could be determined. Simple NMR experiments could provide this information. One would expect to see a direct relationship between the ability to conjugate and activity if this is an important mode of action of these drugs.

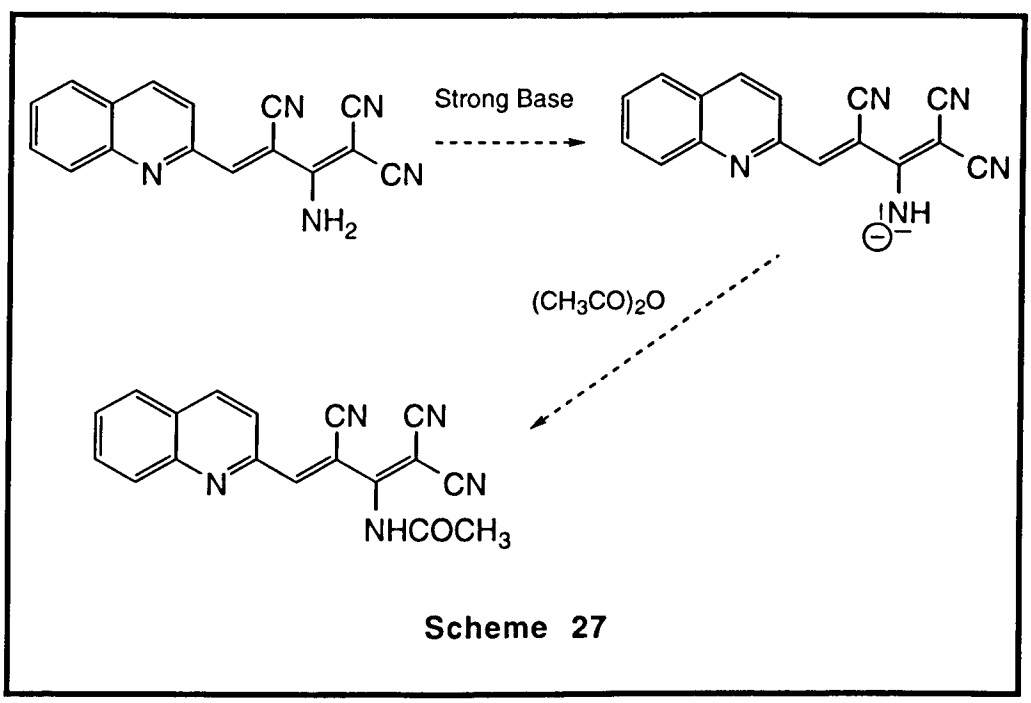
It would seem worthwhile preparing further tyrphostins with quinolinylpropenals to investigate the properties of these promising compounds more thoroughly. Would, for instance, incorporation of another double bond improve the activity of the less reactive tyrphostins previously synthesised? Of particular interest would be compound **88** which was

synthesised but decomposed rapidly and was not tested. This structure closely resembles that of **46**, apart from the *cis*-nitrile (which seems unimportant) and the amine moiety.




**(88)** **(46)**

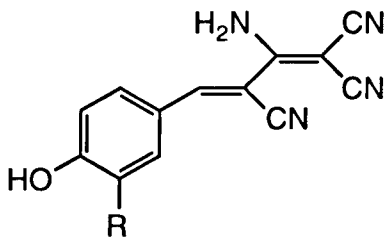
Comparing the activity of compound **88** with that of **46** would disclose information about the importance of the delocalised charge in the side-arm. An alternative method of achieving the same effect would be to attenuate the delocalisation by acetylating the amine (Scheme 27). It may prove difficult to effect this transformation given that the amine lone pair is not localised on nitrogen. It would be worthwhile developing this chemistry since the amine provides the only real structural handle for further modification of compounds **46** and **48**.




**Scheme 27**

The question of the electronic distribution in tyrphostins is an interesting one and evidence that it may have a bearing on their activity is found in several other publications. Levitzki *et al.* report that tyrphostin **120**,

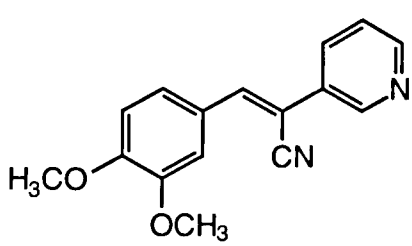
an hydroxylated benzene, inhibits the EGFR kinase with an $IC_{50}$ value of 0.125 μM.[25] When a nitro function is introduced *ortho* to the hydroxyl function, as in compound **121**, the activity decreases to 60 μM.



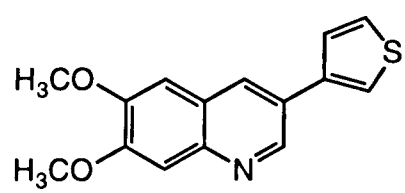

| COMPOUND | R | $IC_{50}$ (μM) |
|---|---|---|
| **120** | H | 0.125 |
| **121** | $NO_2$ | 60 |

The introduction of the $NO_2$ group changes the electronic distribution of the tyrphostin since the lone pair of the hydroxyl oxygen can delocalise into it. This has the effect of reducing the overall partial negative charge in the dimer side-arm and may account for the loss of activity.

Levitzki and others[112, 113] have developed potent TK inhibitors (**68**, **122**) incorporating methoxyl substituents which can delocalise into the sidearm in an exactly analogous manner.




**(68)**




**(122)**

Maguire *et al.*, who prepared a series of 63 3-substituted quinoline compounds as tyrosine kinase inhibitors, concluded that the presence of 6,7-dimethoxy groups on the quinoline ring was advantageous, if not essential, for good activity.[113]

Applying this thinking to our work, we prepared several tyrphostins incorporating the dimethylaminobenzene moiety which, in theory, could feed electrons into the side-arm. The pKa of protonated dimethylaminobenzene is of the order of 4, so at physiological pH one would expect it to exist largely as the free base. We were surprised to find that, with the notable exception of the pyridine derivative **112**, these compounds were inactive, which suggests that increasing the negative charge in the side arm actually diminishes activity. Indeed, compound **110**, which should have an abundance of negative charge at the nitrile terminal end, was among the least active tyrphostins in the series. Although electronic distribution does seem to play a part in determining activity, no clear structure-activity relationship has emerged. This warrants further investigation.

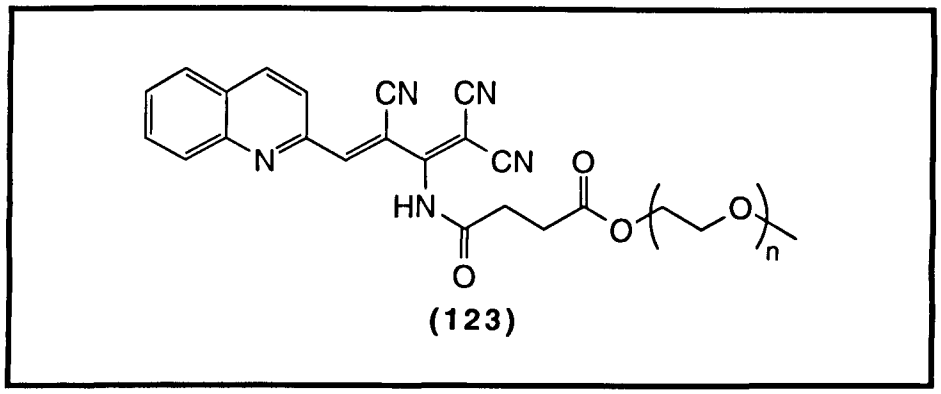
## Suggestions for Further Work

- The mode of action of tyrphostins **46** and **48** should be established, with a particular emphasis on discovering the nature of the 50 kDa protein they bind to (Section 3.6.1). We have had conflicting reports about the sensitivity of these compound to cell lines with different levels of EGFR kinase expression. This needs further clarification. The most active tyrphostins should be evaluated for inhibition of the EGFR kinase.

- More tyrphostins containing diene or triene side-arms should be prepared and evaluated as potential Michael acceptors as described previously.

- The importance of the amine function in **46-48** should be established by either removing it or disabling it. The chemistry of this amine should be investigated.

- The most active tyrphostins should be solubilised with MTEG, which is considerably easier to work with than MPEG. If the amine moiety in **46**, **48** and **96** can be employed, it may be possible to form amides (*e.g.* **123**) with suitably functionalised MTEG derivatives such as MTEG succinate (Chapter 6). The amide linkage may be metabolised to release the free amine *in vitro.*




**(123)**

An alternative approach would be to prepare quinolines with substituents in the benzene ring which would lend themselves to further functionalisation (such as hydroxyl, amine or carboxyl or ester groups, Figure 30). This would avoid compromising the integrity of the substituents in the pyridine ring.

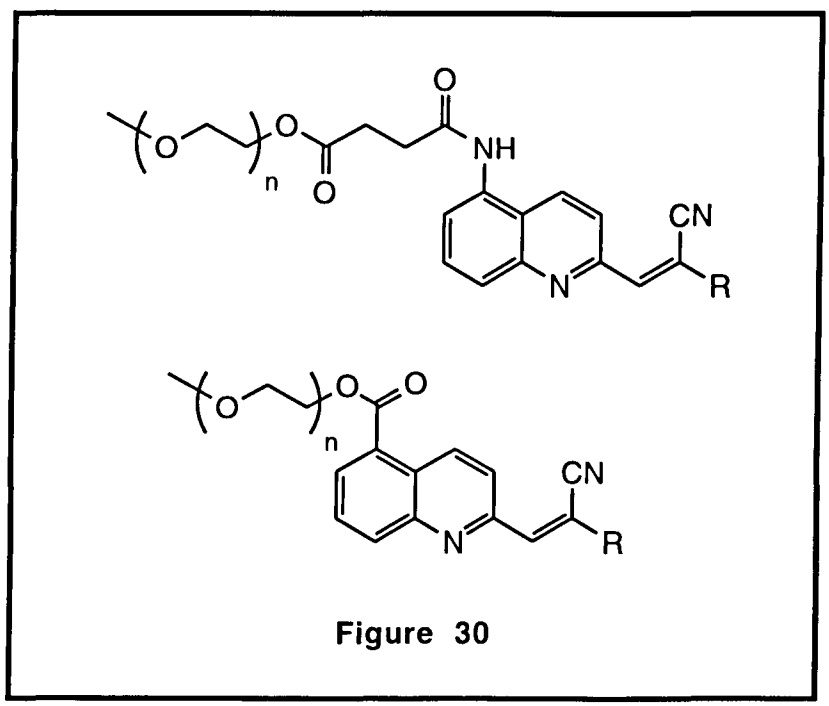
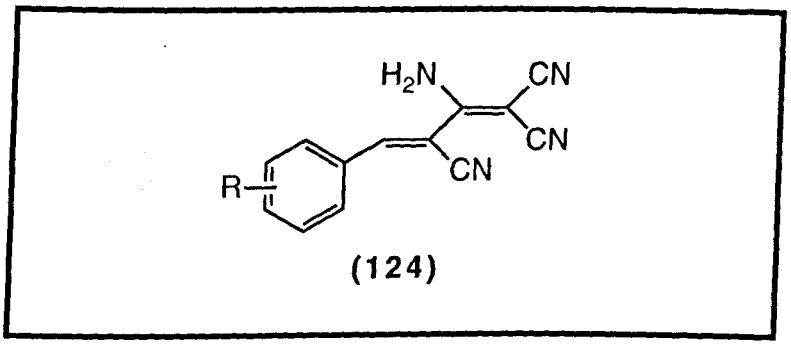



**Figure 30**

- The effects of electron-releasing and donating substituents on the activity of tyrphostins should be investigated. A simple benzene tyrphostin such as **124** could be substituted by a variety of functional groups with differing electronic properties. These could then be tested for cytotoxicity. The process should be repeated with other tyrphostin side-arms to determine whether a consistent pattern of activity is seen. The substituents which prove most beneficial to activity should be

incorporated into existing tyrphostins at the appropriate position to exert the correct 'pull' or 'push' of electrons.




(124)

# 5

# Malignant Melanoma: Causes and Cures.

## 5.1 Introduction

Despite progress in our understanding of the biochemistry of skin cancer and growing public awareness of the associated risk factors, malignant melanoma still poses many problems to the clinician and medicinal chemist alike.

As with all cancers, malignancy indicates that the normal growth patterns of the cell have been corrupted, allowing unrestrained division to occur. In malignant melanoma this manifests itself by the appearance of dark nevi or lesions on the skin caused by excessive production of melanin. While treatment in the early stages is possible by drug administration and surgery, little can be done for patients in the advanced stages of the disease. The lack of efficacious therapy for disseminated malignant melanoma is the driving force behind the drug development programmes described in this chapter.

### 5.1.1 The Epidemiology of Malignant Melanoma

The term 'skin cancer' covers a number of manifestations of the disease which vary in severity and presentation. Among these are the so-called 'non-melanotic' cancers such as basal cell carcinoma and squamous cell carcinoma which account for most cases (>75%) of skin cancer. These diseases, particularly basal cell carcinoma, have a relatively good prognosis since the cancers tend not to metastasise. Cure rates of 90-95% can regularly be achieved by surgery or radiotherapy. This is in sharp contrast to the more aggressive form of skin cancer, malignant melanoma, which is highly metastatic. By the time the disease has entered the lymph nodes surrounding the primary tumour, the likelihood of cure has dropped to only 15%. The median survival time for those presenting with fully disseminated disease is 6 months. Four main types of the disease are known: superficial

spreading melanoma, which accounts for 70% of cases; nodular melanoma, the most rapidly growing form; *lentigo maligna* melanoma, which tends to affect the elderly, and acral melanoma which occurs on the palms, soles and nails.[10, 114]

Although malignant melanoma only accounts for 2% of all cancers, its rate of incidence continues to rise at an alarming pace. Independent statistics from Scotland, Scandinavia and Australia show that the number of diagnoses of the disease is doubling every decade.[10] Mortality rates are also on the increase, albeit less rapidly thanks largely to earlier diagnosis. This elevation in the profile of the disease reflects several changes in society this century, the most marked of which is the amount of time we spend exposed to solar radiation. The link between sunlight and skin cancer has been known for over 100 years but it was first quantified in 1907 by William Dubreuilh, a French dermatologist who performed conclusive epidemiological studies on the subject. George Findlay, a British pathologist, confirmed and extended this work in 1928 by inducing skin cancer in mice exposed to UV radiation.[115] Although UV rays do not penetrate deeply into the skin, overexposure is known to cause mutagenesis and the onset of cancer. UVB (280-320 nm) has been identified as the most dangerous band. Depletion of the ozone layer has increased the amount of UVB reaching the earth's surface: one estimate suggests that a 1% depletion in the ozone layer would increase the amount of potentially damaging UVB reaching us by 2%.[116]

Despite these statistics, the sun-tan continues to be a Western symbol of health, wealth and happiness. In pursuit of brown skin, holidaymakers spend vast amounts of time soaking up the sun on the beach while taking few protective measures. Significantly, the group most at risk of contracting skin cancer are fair-skinned individuals who spend most of their time indoors but sunbathe to excess when on holiday.[117] Unsurprisingly, it's those countries where malignant melanoma is rife which have been leading the way in terms of public education programmes. Australia, where the incidence of skin cancer is three times the world average, has taken strides in raising public awareness with its recent 'Slip-Slap' campaign. The light-hearted advertisements encourage sunbathers to slip on a T-shirt or slap on some sun-cream. A strong emphasis is placed on protecting children from the sun, since early exposure seems to be a risk factor in developing the disease in later life.[118]

World-wide studies have established associations between sunlight and the incidence of malignant melanoma, several of which are outlined below.

- In a seminal paper of 1956,[119] Lancaster demonstrated that within groups of the same skin type, risk of death increased with proximity of residence to the equator. This pattern has been confirmed in more recent studies in North America[120], England and Wales[121] and the Scandinavian countries.[122, 123]

- Immigrants to countries with sunny climates are less likely to develop malignant melanoma than those of the same race born in that country. this indicates that childhood exposure may be a significant risk factor.[118]

- The likelihood of developing melanoma increases with one's educational and social status.[124]

- Women are more at risk from malignant melanoma than men. In the UK, female incidence is roughly twice than found in males. Much of this difference may be attributable to differences in dress codes. 47% of primary melanomas in women occur on the legs compared to only 10% of those in men.[125]

- Skin colour is a key factor in assessing an individual's chances of developing the disease. Incidence is 12 times higher in white-skinned people compared to black individuals with a similar lifestyle.[125] Interestingly, certain African tribes develop melanomas on the soles of their feet, the area least protected by melanin.[126]

- Within white-skinned races, those people with fair or red hair and blue eyes who do not tan readily are most at risk. A large number of moles on the individual's skin (> 40) may also indicate a genetic predisposition to the disease.

### 5.1.2 Prognosis and Current Therapy

The superficial nature of malignant melanoma makes it among the easiest of cancers to detect. Successful education programmes have alerted

the public to the classic signs of the disease: 'moles' which appear suddenly, change colour or start to itch or bleed. One such campaign in the west of Scotland increased the proportion of patients identifying the disease in its earliest stages by 17%.[127] The importance of identifying and treating the disease as soon as possible cannot be overstated since there is a clear correlation between tumour invasiveness and prognosis. The commonest clinical measurement of the extent of the disease is the Breslow thickness (Figure 31), named after a pathologist who saw the need to quantify the degree of tumour invasion as an aid to prognosis.[128] A section from the thickest part of the melanoma is excised and examined microscopically. The Breslow thickness is taken as the distance in millimetres from the granular layer in the epidermis to the deepest invasive tumour cell in the underlying tissue. The significance of this measurement is self-evident from Table 6.[129]

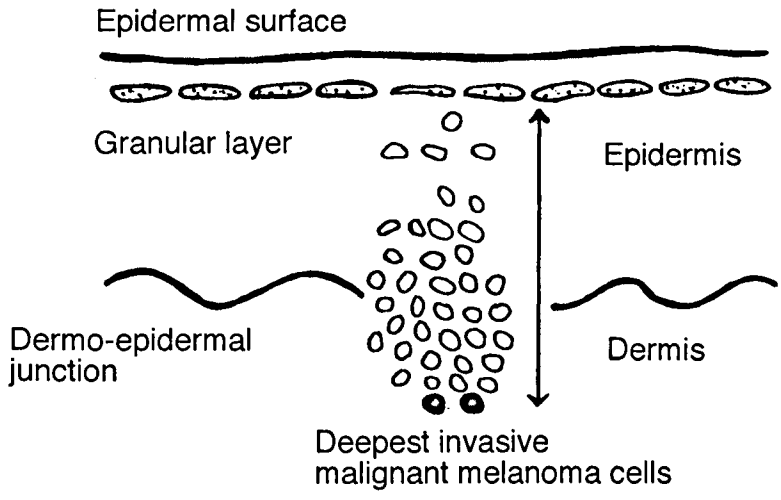



**Figure 31**[130]

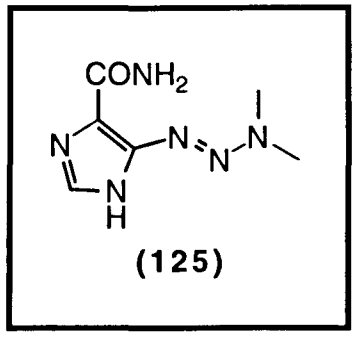
| Table 6 | |
|---|---|
| **Breslow Thickness/ mm** | **Actuarial 5-year survival rate (%)** |
| 0.0 - 0.75 | 93 |
| 0.76 - 1.50 | 82 |
| 1.51 - 3.00 | 67 |
| 3.01 - 4.50 | 57 |
| > 4.51 | 37 |

A variety of further classifications exist which depend on the extent to which the disease has metastasised. Broadly speaking, the progress of the cancer can be split into three stages.

I) Primary melanoma - no metastases.
II) Metastases in regional lymph nodes.
III) Disseminated disease.

Stage I tumours can be treated surgically, which involves excising the primary tumour to the appropriate depth with prophylactic removal of the surrounding tissue. A rule of thumb coined by the World Health Organisation is that for each mm of tumour depth, an area of diameter 1 cm should be excised around the primary tumour.[131] Five-year survival rates for patients with stage I tumours range from excellent (93% when Breslow thickness <1.5 mm) to moderate (38% when Breslow thickness >3.5 mm).[132]

Stage II tumours can also be treated by surgery but the degree of excision must be extended to include the regional lymph nodes as well. At the moment there is no satisfactory treatment for the advanced stage of the disease. Of the chemotherapeutic agents used, DTIC (dacarbazine, **125**) is the single most successful agent giving some degree of response in 22% of patents.[133] The anticancer activity of DTIC is ascribed to the formation of alkylating metabolites. [134]




**(125)**

Combination chemotherapy with DTIC can marginally improve response rates but its main advantage is in extending the duration of response. Despite this, most tumours are only susceptible to the drug for a matter of months. There is a clear need for novel anticancer agents for the treatment of advanced malignant melanoma.

Several classes of phenolic compounds have shown promise as antimelanoma agents. Acting in a non-classical fashion, these prodrugs use the biochemical 'machinery' of the melanocyte to generate toxic species *in*

*situ*. The subsequent sections of this chapter outline the normal function of the melanocyte and explain how its 'machinery', particularly the enzyme tyrosinase, can be used to advantage in fighting malignant melanoma.

## 5.2 Melanin: Function and Synthesis.

Melanin is the dark, pigmented heteropolymer which gives skin its colour. The synthesis of melanin takes place within melanosomes which are vesicles formed in dedicated cells called melanocytes (Figure 32). These pigment-producing cells are mainly located in the epidermis and the retina. The number of melanocytes is fairly consistent across all races; what differs is the amount of pigment they produce and disperse. This genetic variation gives rise to the different skin tones and colours with which we are familiar. Albinism is a congenital defect which precludes melanin synthesis, resulting in pale skin and white hair.

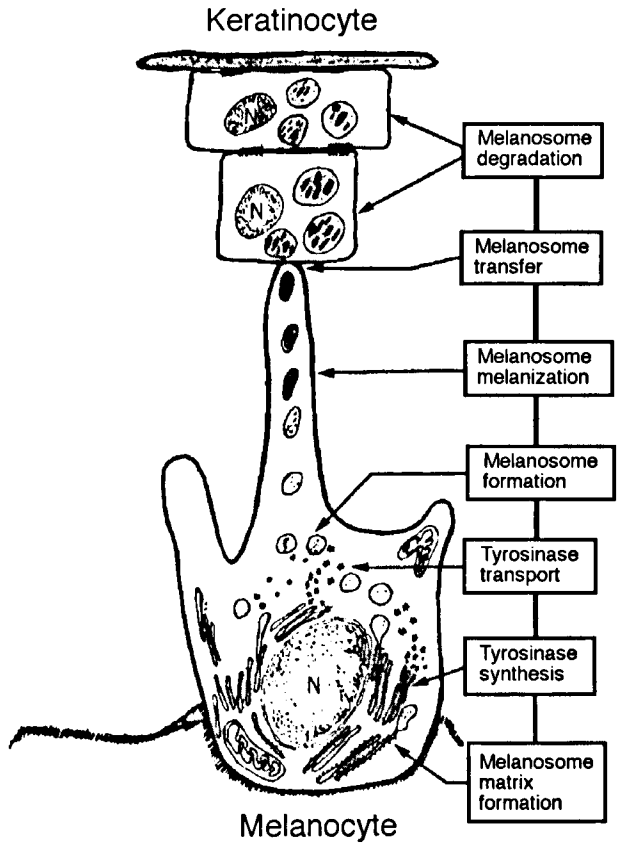
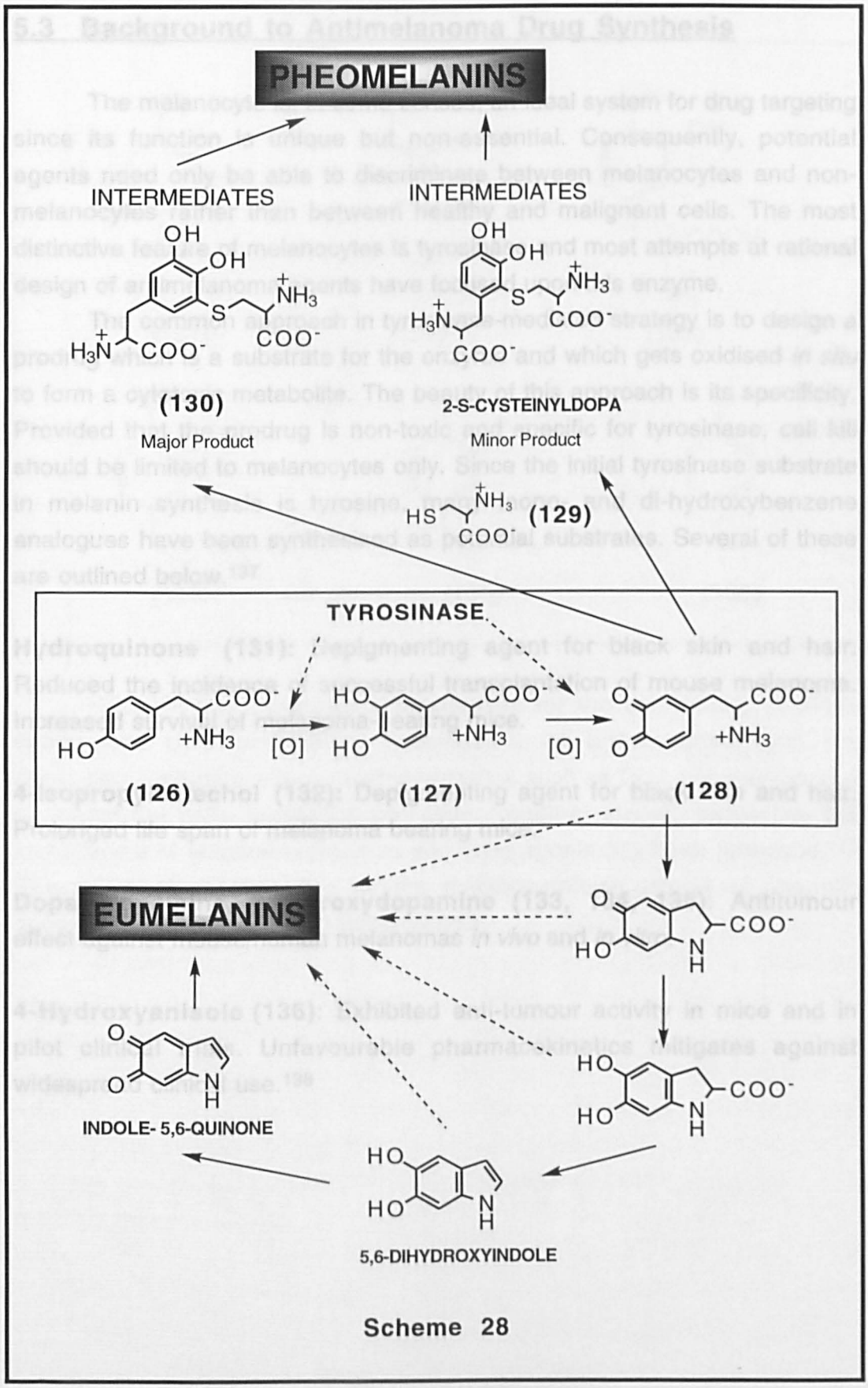



**Figure 32**[135]

Melanin is formed as a consequence of the oxidation of tyrosine **(126)**, catalysed by the enzyme tyrosinase, a process which occurs uniquely within melanocytes (Scheme 28). Melanin polymers are highly unsaturated and readily absorb the energy associated with UV radiation.

There are two main classes of integumentary melanin - brown/black eumelanin and yellow/red pheomelanin. In melanocytes, tyrosinase oxidises tyrosine to dihydroxyphenylalanine (dopa, **127**) and then dopaquinone **(128)** which is cyclised and oxidised further to give eumelanin. If dopaquinone encounters cysteine **(129)** or other related sulfhydryl compounds, pheomelanin is produced via cysteinyl dopas, among which 5-S-cysteinyl dopa **(130)** is the major isomer. Recent evidence indicates that pure pheomelanins or eumelanins rarely exist and that each melanin is best thought of as a heteropolymer containing varying degrees of cysteinyl or non-cysteinyl monomer units.[136]

The key biochemical feature of melanocytes is the cuproenzyme tyrosinase, which acts successively as a monophenol monooxygenase and then as an oxidase. The enzyme does not require a cofactor but uses the product of the hydroxylation, dopa, as the hydrogen donor. Tyrosinase is found only within melanocytes and as such can act as a specific biochemical marker for these cells.


PHEOMELANINS
INTERMEDIATES
INTERMEDIATES
(130)
Major Product
2-S-CYSTEINYLDOPA
Minor Product
(129)
TYROSINASE
[O]
[O]
(126)
(127)
(128)
EUMELANINS
INDOLE- 5,6-QUINONE
5,6-DIHYDROXYINDOLE

**Scheme 28**

## 5.3 Background to Antimelanoma Drug Synthesis

The melanocyte is, in some senses, an ideal system for drug targeting since its function is unique but non-essential. Consequently, potential agents need only be able to discriminate between melanocytes and non-melanocytes rather than between healthy and malignant cells. The most distinctive feature of melanocytes is tyrosinase and most attempts at rational design of antimelanoma agents have focused upon this enzyme.

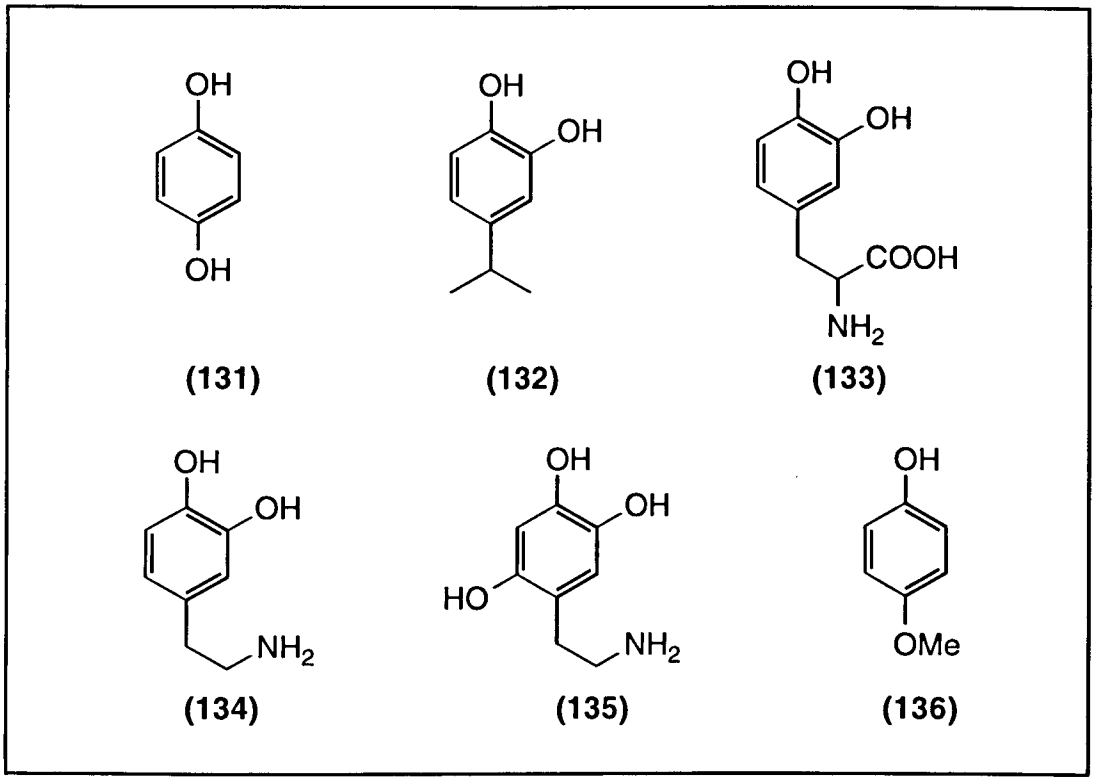
The common approach in tyrosinase-mediated strategy is to design a prodrug which is a substrate for the enzyme and which gets oxidised *in situ* to form a cytotoxic metabolite. The beauty of this approach is its specificity. Provided that the prodrug is non-toxic and specific for tyrosinase, cell kill should be limited to melanocytes only. Since the initial tyrosinase substrate in melanin synthesis is tyrosine, many mono- and di-hydroxybenzene analogues have been synthesised as potential substrates. Several of these are outlined below.[137]

**Hydroquinone (131)**: Depigmenting agent for black skin and hair. Reduced the incidence of successful transplantation of mouse melanoma. Increased survival of melanoma-bearing mice.

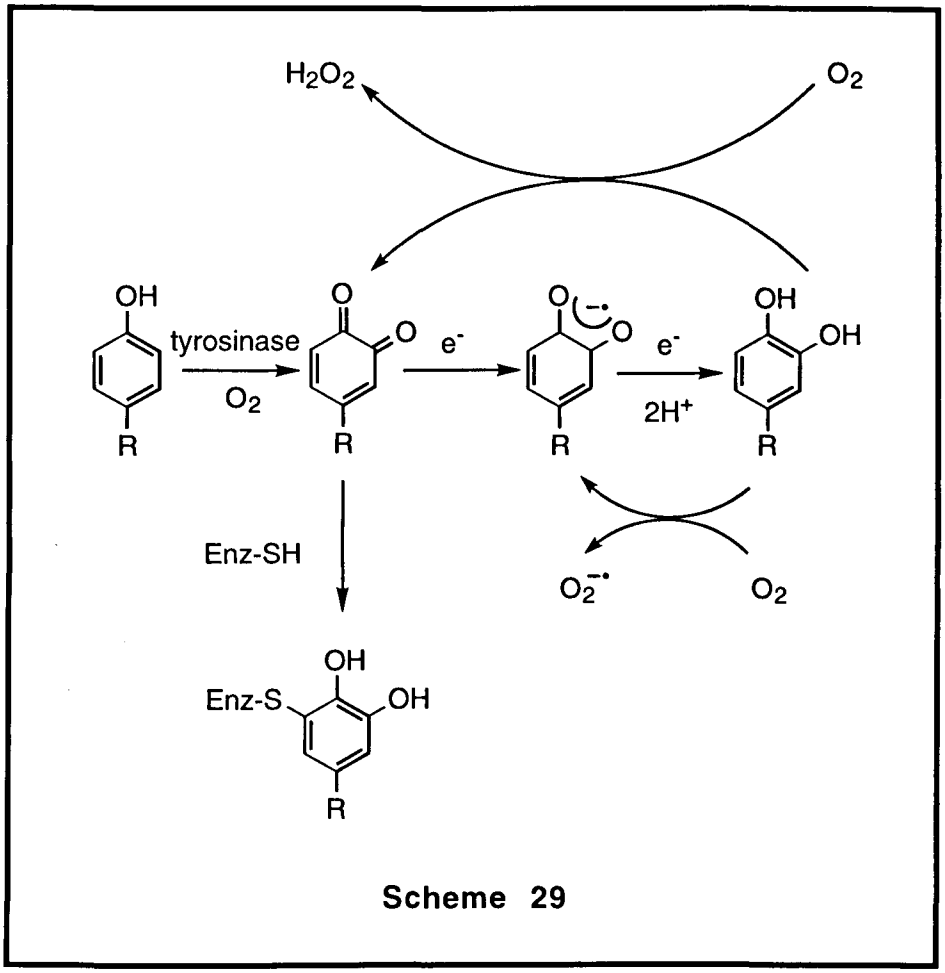
**4-Isopropylcatechol (132):** Depigmenting agent for black skin and hair. Prolonged life span of melanoma bearing mice.

**Dopa, dopamine, 6-hydroxydopamine (133, 134, 135)**: Antitumour effect against mouse/human melanomas *in vivo* and *in vitro.*

**4-Hydroxyanisole (136)**: Exhibited anti-tumour activity in mice and in pilot clinical trials. Unfavourable pharmacokinetics mitigates against widespread clinical use.[138]

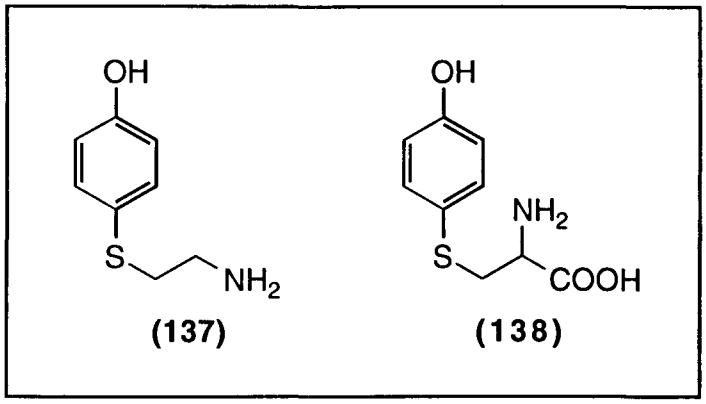



Wick[139] has proposed a mechanism for the cytotoxicity of these compounds. Upon oxidation by tyrosinase to the *ortho*-quinone form, the drugs can conjugate with sulfhydryl enzymes such as DNA polymerase and so inhibit the cell's replication processes. Another theory, suggesting the involvement of quinone radicals as alkylating agents has been proposed.[140] The proposed mechanisms for the melanocytotoxic effects of phenolic compounds are illustrated in Scheme (29).




**Scheme 29**

Despite their interesting biological effects, catechol-based drugs are unlikely to find much clinical use: instability and systemic toxicity due to non-specific oxidation are severe limitations. With this in mind, Miura *et al.* [137] have synthesised a range of sulfur-containing phenols (and catechols) as potential antimelanoma agents. Sulfur was incorporated to increase lipophilicity and hence improve uptake of the drug into the melanocyte. 4-S-Cysteaminylphenol **(137)** and 4-S-cysteinylphenol **(138)** proved to be the most successful compounds. Both increased the life span of melanoma-bearing mice and inhibited the growth of melanoma tissue. The results are summarised in Table 7. Significantly, the corresponding 2-S isomers of **137** and **138** did not show any significant antimelanoma effect.




(137) (138)

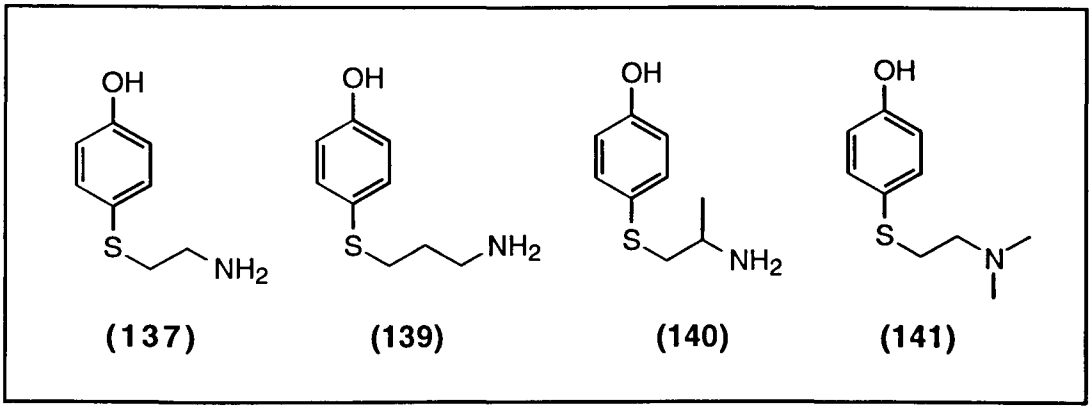
| Table 7 | | |
|---|---|---|
| COMPOUND | Increase in life span (% I.L.S.) | Growth Inhibition (% G.I.) |
| 137 | 48.7 | 64.2 |
| 138 | 15.4 | 20.3 |

Topical application of **137** and **138** on the skin of black guinea pigs caused marked depigmentation. This phenomenon was attributed to a decrease in the number of functioning melanocytes; a decrease in the number of melanosomes synthesised, and the destruction of membranous organelles within the melanocyte.[141]

A further study[142] showed that **137** and **138**, unlike their 2-S counterparts, were excellent substrates for mushroom tyrosinase and good substrates for mammalian tyrosinase. Given the difference in activity between the 2-S and 4-S isomers, these findings seemed to affirm a role for tyrosinase in generating cytotoxic species from hydroxybenzenes. This discovery added weight to Wick's quinone theory.[139] To this end, the ability of the quinone forms of **137** and **138** to conjugate with cysteine and cysteine residues was determined. With cysteine, both quinones performed better than the quinone derived from tyrosine itself. With cysteine residues in alcohol dehydrogenase, however, **137** was over five times as effective at conjugation as **138** which, it was suggested, could account for the difference in their activity (Table 7).

In 1990 the same group of workers were forced to review this theory after discovering that **137** was, in fact, being oxidised *in vitro* by monoamine oxidase (MAO).[143] This concurred with work carried out by Padgette *et al.*[144] which showed that phenyl aminoethyl sulfides were good substrates for

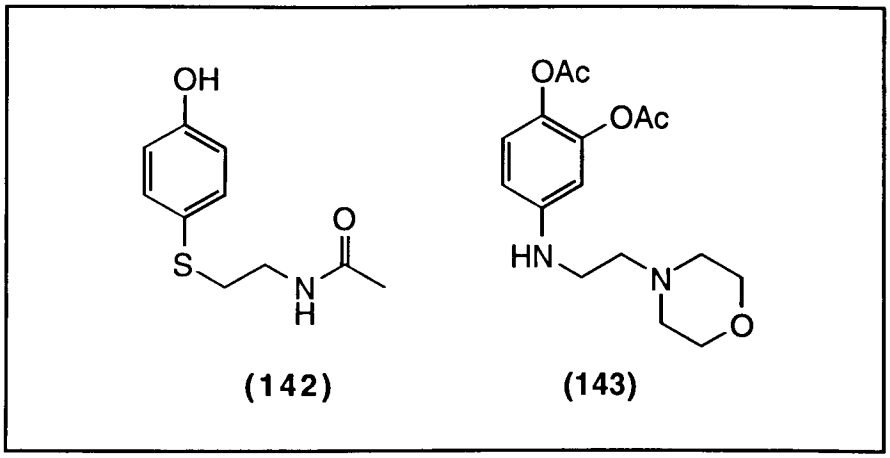
MAO. In this study, four phenolic derivatives were tested for *in vitro* antimelanoma effect.




**(137)** **(139)** **(140)** **(141)**

The compounds **137** and **139** proved to be much more cytotoxic than the other two analogues, but their activity was completely negated in the presence of semicarbazide, an inhibitor of plasma MAO. The activity of the second pair of amines was not modified by administration of semicarbazide, catalase or phenylthiourea, a tyrosinase inhibitor. In culture medium, the primary amine of **137** was shown to be oxidised to the aldehyde and then the acid. From this work it was concluded that the activity of the straight chain primary amines was attributable to the formation of oxidation products via MAO. The activity of the other two amines was, in part, attributable to tyrosinase but clearly involved other mechanisms.

In an attempt to diversify from straight-chain amines, Miura *et al.* synthesised the N-acetyl derivative (**142**) of **135** .[145] This compound, which is a substrate for mammalian tyrosinase but not for MAO, proved to be even more successful than its parent compound, exhibiting 74.4% growth inhibition of the B16 melanoma cell line in mice. When introduced subcutaneously into black mice, **142** showed 98% depigmentation of black hair follicles.[146] Further *in vivo* studies proved that **142** accumulated readily in melanotic tissue and that its antimelanoma activity could be enhanced if administered in combination with buthionine sulfoximine.[147] Human xenografts in nude mice were also sensitive to the reagent.[148] A recent *in vitro* study by the same authors has shown that a variety of cancer cell lines can be inhibited by **142** and its analogues, suggesting that non-tyrosinase mediated cytotoxicity may well be involved.[149]




(142) (143)

Thiophenols are by no means the only tyrosinase-activated prodrugs in development. Mascagna *et al.* have been studying *p*-aminophenols concurrently, several of which show good antimelanoma activity.[150] By introducing an amine function *para* to the phenolic hydroxyl group, the authors hoped to increase the susceptibility of their drugs to oxidation by tyrosinase. Prior work had established that phenols are more easily oxidised by tyrosinase when they contain electron donating substituents in the *para* position.[151] A lead compound from this study was the di-protected catechol **143** which exhibited an $IC_{50}$ of 15 μg/ml against the human HBL melanoma cell line. This class of compounds shows a fairly broad spectrum of activity across various cancer types, leading to the conclusion that their mode of action may not be entirely tyrosinase dependent.

## 5.4 Melanoma Research at Glasgow University

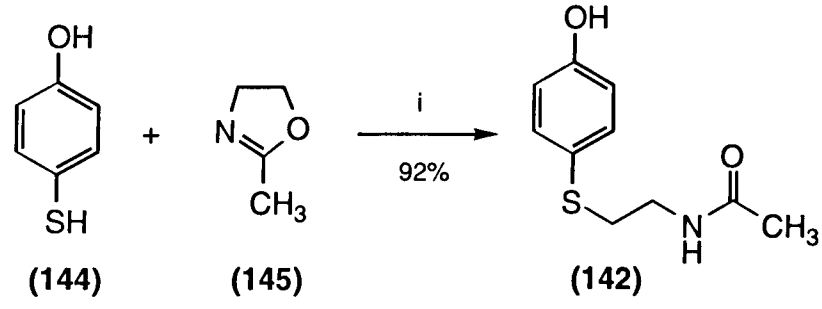
In 1993 the Cancer Research Group of Glasgow University chemistry department were approached by Professor Rona Mackie, a leading clinical dermatologist, and asked if we could provide a sample of compound **142** for her own studies. In the course of our literature search, it became clear that little progress had been made in refining or developing the structure of **142**. It appeared that the researchers concerned had hit upon a successful compound and were content to investigate its biological properties at length. It seemed to us, as medicinal chemists, that there was a great deal of work which could be done to develop this interesting class of compounds. Professor Mackie was glad to lend her support and experience to our efforts. This was the start of an ongoing programme which has seen us match, and go beyond our initial target compounds. Chapter 6 details the synthesis and biological evaluation of these novel antimelanoma agents.

# 6

# Synthesis and Biological Evaluation of Antimelanoma Agents.

## 6.1 Preparation, Characterisation and Development of Compound 142.

Our initial target in this area was the phenolic amide **142** which had previously been prepared by Padgette *et al.* as an antihypertensive agent.[144] The authors used a preparation involving the reaction of 4-hydroxythiophenol (**144**) with 2-methyl-2-oxazoline (**145**, Scheme 30).

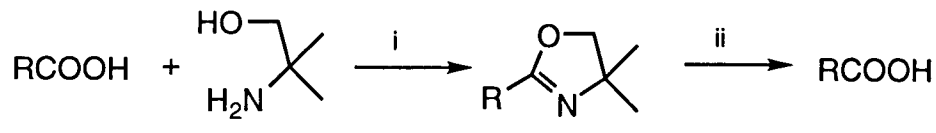



**(144)** **(145)** **(142)**

i. Reflux neat, 2h, $N_2$

**Scheme 30**

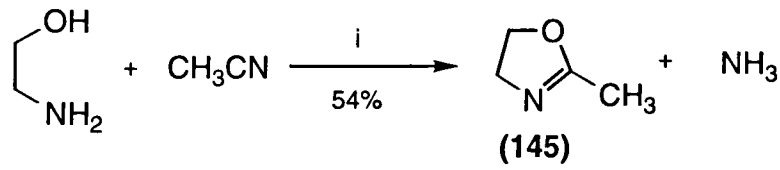
Oxazolines are five-membered heterocycles containing one double bond and are designated as 2-, 3- or 4- oxazolines depending on the position of that bond. 2-Oxazolines are the most common type. They are the dihydro derivatives of oxazoles and are commonly encountered as protecting groups for carboxylic acids (Scheme 31).[152]




i. Reflux, toluene, 70-80%  ii. HCl (aq), 90%

**Scheme 31**

They are also useful synthetic reagents in their own right and many are now commercially available. We prepared oxazoline **145** from ethanolamine and acetonitrile according to the method of Witte and Seeliger (Scheme 32).[153]




**(145)**

i. Reflux, $Cd(OAc)_2$, 120 °C

**Scheme 32**

The boiling point for **145** agreed with that recorded in the literature.[154] Reaction with 4-hydroxythiophenol under the conditions employed by Padgette *et al.* (Scheme 30), afforded **142** in excellent yield. The spectral data and melting point of **142** concurred with those in the literature.[144]

## 6.1.1 Crystal Structure of Compound 142

Compound **142** was recrystallised in a flat bottomed flask from analytical grade ethyl acetate, affording small, rhombic crystals. These were submitted to Dr K.W. Muir (University of Glasgow) for X-ray crystallographic analysis. The displacement ellipsoid structure of this compound is depicted overleaf (Figure 33).

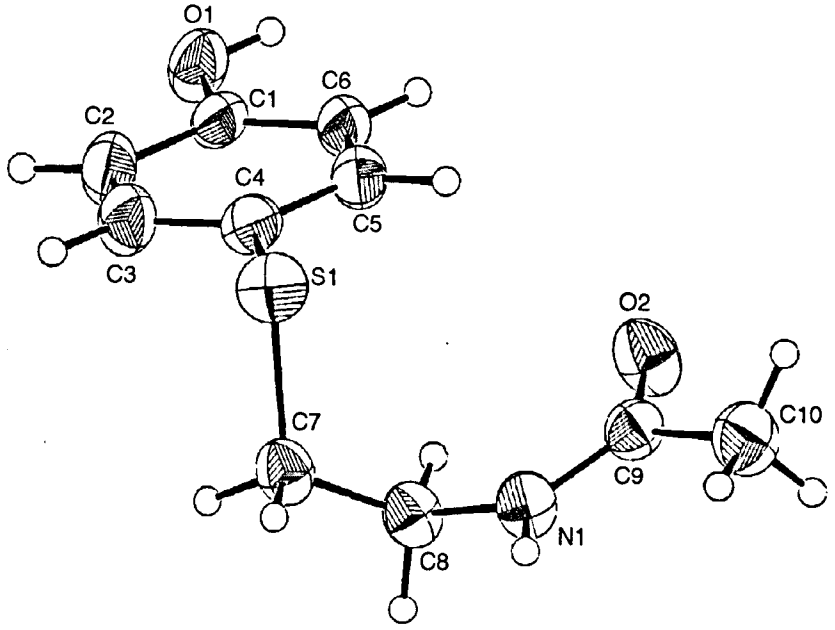



**Figure 33**

The molecule adopts a non-planar conformation, the crucial bond angles being the C(4)-S(1)-C(7) angle which is 99.81° and S(1)-C(7)-C(8) angle which is 114.7°. The C(4)-S(1)-C(7)-C(8) torsion angle is 71.5°. Crystallising in this form gives the molecule several stabilising hydrogen bonding interactions (Figure 34). The oxygen and hydrogen of the hydroxyl group participate in H-bonding with two different molecules. The hydroxyl oxygen binds to an adjacent amide hydrogen while the hydroxyl hydrogen associates with a carbonyl oxygen. The unit cell of the crystal is represented in Figure 35.

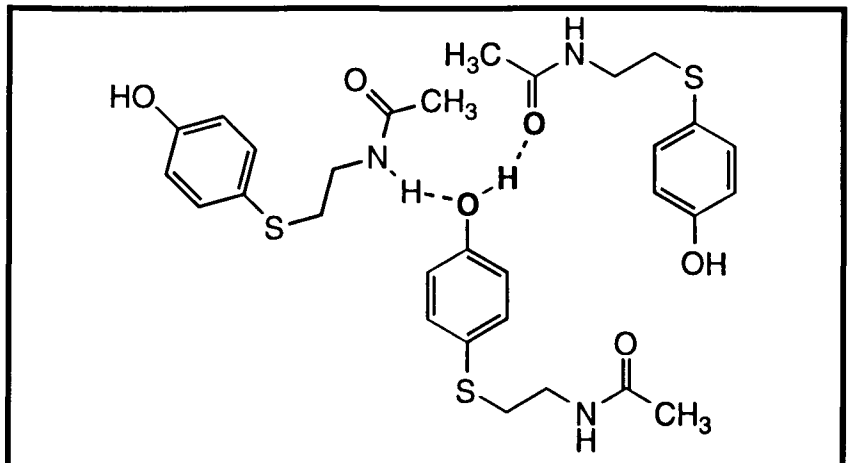



**Figure 34**

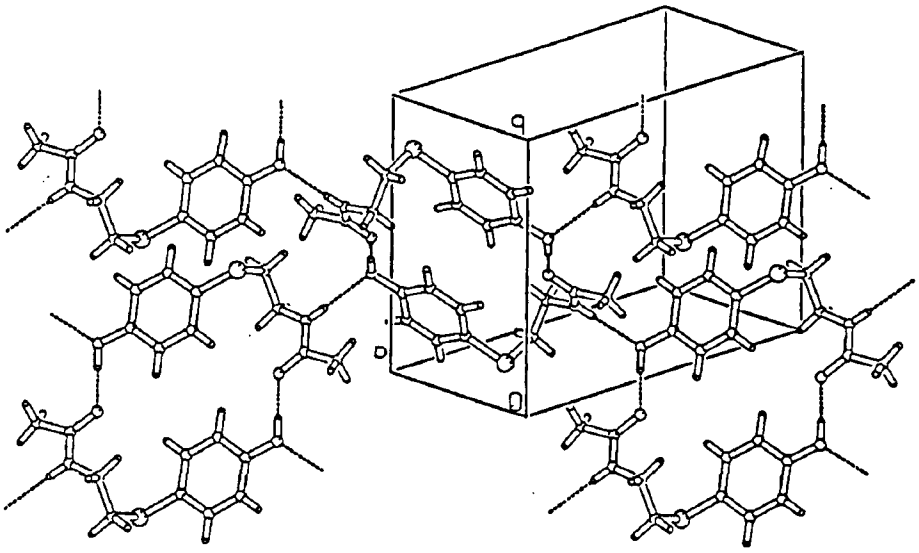


Figure 35

## 6.1.2 Proposed Structural Modifications to Compound 142

Having prepared **142** successfully, we set about modifying the structure in search of more active compounds. We pinpointed five areas, illustrated in Figure 36, which we considered worthy of investigation.

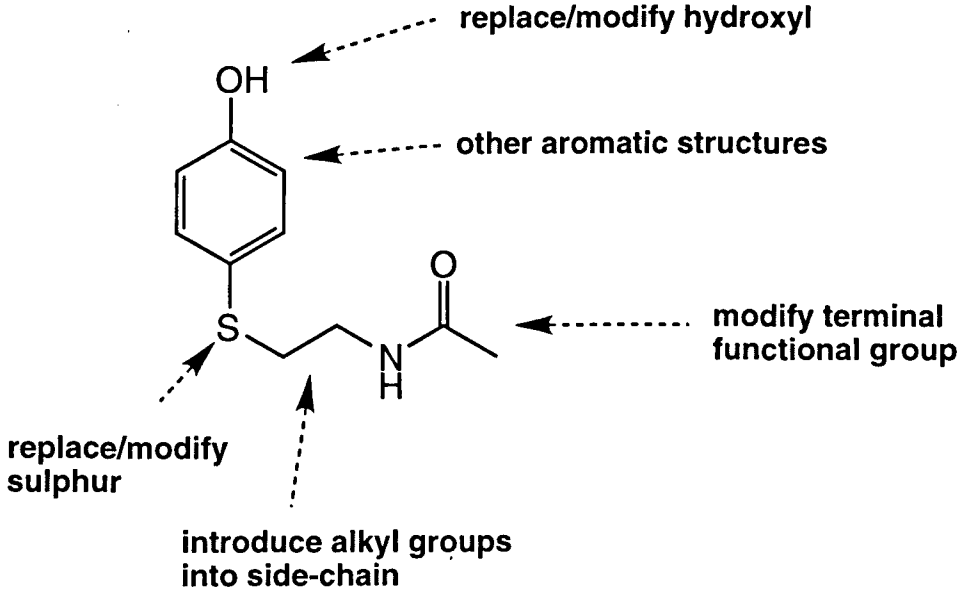



Figure 36

The rationale behind replacing the OH group was to test the hypothesis that phenols exert toxicity by oxidation to *ortho*-diols and then to *ortho*-quinone forms.[139] If this hypothesis is correct, we would expect to see a diminution of activity when OH is replaced by, for example, methyl or halide groups. We expected that the phenol would be the active part of the molecule, but to affirm this we decided to replace it with heterocyclic moieties. A negative result would again confirm the importance of the phenol.

Miura *et al.*[137] had incorporated sulfur in the structure of **142** to increase lipophilicity. We were interested in either replacing or oxidising this atom. The work of Mascagna *et al.*[150] suggested that the nature of the heteroatom was not crucial to the efficacy of these compounds. Finally, we wanted to explore the effects of introducing alkyl groups into the thioether side-chain and altering the nature of the terminal amide function.

Financial support from Professor Mackie (Department of Dermatology) enabled us to start this project in the summer of 1993 with the help of three undergraduate students, Neil Lant, Robert Campbell and Ian McBride. 23 novel compounds were prepared, including some where the hydroxyl moiety had been replaced by hydrogen, alkyl group and halides or derivatised as ethers and esters. A small number of compounds with different thioether side-chains were also prepared. The compounds were tested against murine melanoma cell lines *in vitro* by Professor Mackie. The conclusions from this work[155] can be summarised in three simple statements.

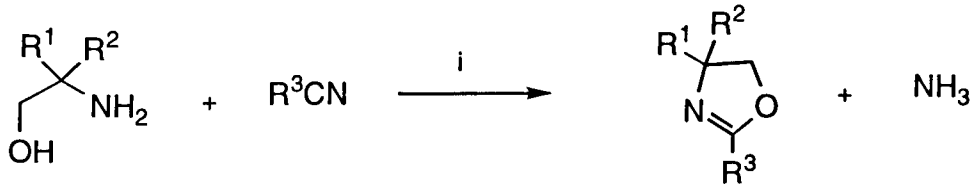
- *An hydroxyl, or latent hydroxyl function is essential for activity.*
- *Alkyl groups α-to the nitrogen improve activity.*
- *Larger terminal alkyl groups improve activity.*

We were interested to find that phenolic ester derivatives were active, whereas all the other compounds where the OH group had been replaced were much less. It seems likely that the esters are hydrolysed *in vitro* to generate the free phenol (this is what is meant by a 'latent' hydroxyl function). The discoveries from this work were a useful springboard for further development of phenolic antimelanoma agents.

## 6.2 Oxazoline Synthesis

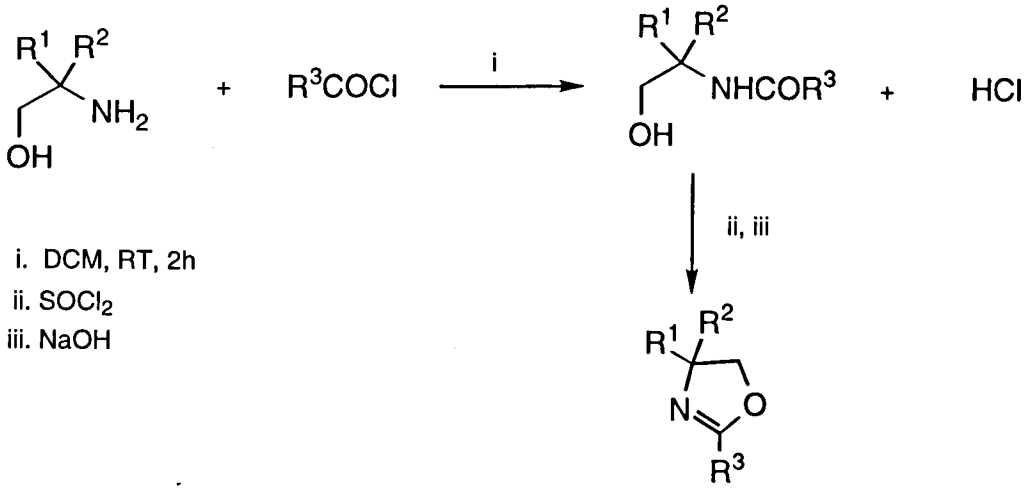
As a first priority, we concentrated our attention on increasing the bulk of the thioether side-arm. Given that we were preserving the phenolic portion of the molecule, the chemistry of Padgette *et al.* seemed eminently suitable for our purposes, provided that we could prepare the appropriately substituted oxazolines. A wide variety of preparative methods for oxazolines are available and have been well reviewed.[156,157] We employed two methods, which involved the reaction of nitriles[153] or acid chlorides[156] with 2-aminoalcohols. The general schemes for these reactions are shown below (Scheme 33).

**Method A:**




i. Reflux, Cd $(OAc)_2$, 120 °C

**Method B:**




i. DCM, RT, 2h
ii. $SOCl_2$
iii. NaOH

**Scheme 33** - $R^1$, $R^2$ and $R^3$ as specified in Table 8

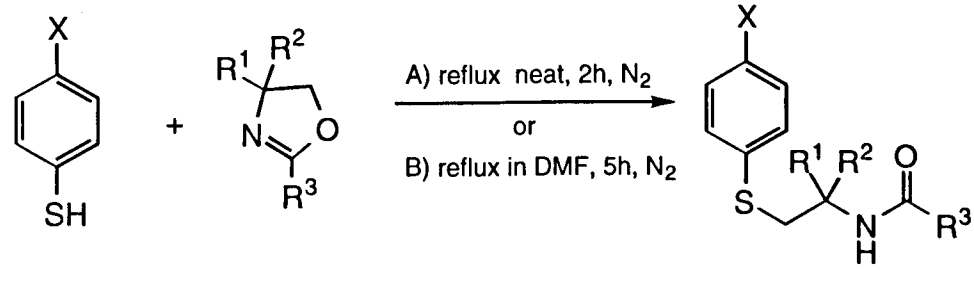
Five oxazolines were prepared using these methods (Table 8)

| Table 8 | | | | | |
|---|---|---|---|---|---|
| **LABEL** | **$R^1$** | **$R^2$** | **$R^3$** | **METHOD** | **% YIELD** |
| **145**[154] | H | H | $CH_3$ | A | 54 |
| **146**[158] | H | $CH_3$ | $CH_3$ | A | 49 |
| **147**[159] | $CH_3$ | $CH_3$ | $C_2H_5$ | A | 68 |
| **148**[160] | $CH_3$ | $CH_3$ | $^tBu$ | B | 69 |
| **149** | $CH_3$ | $CH_3$ | $C_6H_5$ | B | 70 |

Compounds **145**, **146**, **147** and **148** gave the correct boiling points according to the literature and showed the presence of imidic ester absorptions in the IR spectra (C=N at *ca.* 1674 $cm^{-1}$ and C-O at *ca.* 1230 $cm^{-1}$). Compound **149** showed aromatic and aliphatic C-H stretching modes in the IR spectrum at 3032 and 2968 $cm^{-1}$, respectively. The main features of the $^1H$ NMR spectrum of **149** were the five aromatic protons between δ 7.19 and 7.83 and the gem-dimethyl protons at δ 1.24.

## 6.3 Synthesis of Analogues of Compound 142

Six compounds were prepared by reaction of thiophenols with the various oxazolines described in Section 6.2. Five of these derivatives formed readily under the conditions described by Padgette, but **155** could only be prepared in DMF under more forcing conditions. The results are summarised in Scheme 34 and Table 9.




**Scheme 34** - X and R groups as specified in Table 9

| Table 9 | | | | | | |
|---|---|---|---|---|---|---|
| LABEL | X | $R^1$ | $R^2$ | $R^3$ | METHOD | % YIELD |
| 142 | OH | H | H | $CH_3$ | A | 41 |
| 150 | F | H | H | $CH_3$ | A | 72 |
| 151 | OH | $CH_3$ | H | $CH_3$ | A | 61 |
| 152* | OH | $CH_3$ | $CH_3$ | $CH_3$ | A | 52 |
| 153 | OH | $CH_3$ | $CH_3$ | Et | A | 63 |
| 154 | OH | $CH_3$ | $CH_3$ | $^t$Bu | A | 63 |
| 155 | OH | $CH_3$ | $CH_3$ | $C_6H_5$ | B | 19 |

* The oxazoline required in this preparation, 2,4,4-trimethyloxazoline, was purchased from Aldrich Chemicals.

The fluorine derivative **150** was easily identified by the $^{13}C/^{19}F$ coupling in the $^{13}C$ NMR spectrum. The observed coupling constants were 245, 8 and 22 Hz for the carbons at positions 4', 3' and 2' in the benzene ring. The amide carbonyl carbon came into resonance at δ 170.4. The IR spectrum of **150** showed amide stretching modes at 1637 and 1560 $cm^{-1}$ and a C-F absorption at 1242 $cm^{-1}$.

The racemic amide **151** showed an ABX system in the $^1H$ NMR spectrum for the diastereotopic methylene protons in the side-chain. The melting point and spectral data for **151** agreed with those previously recorded in the literature.[144]

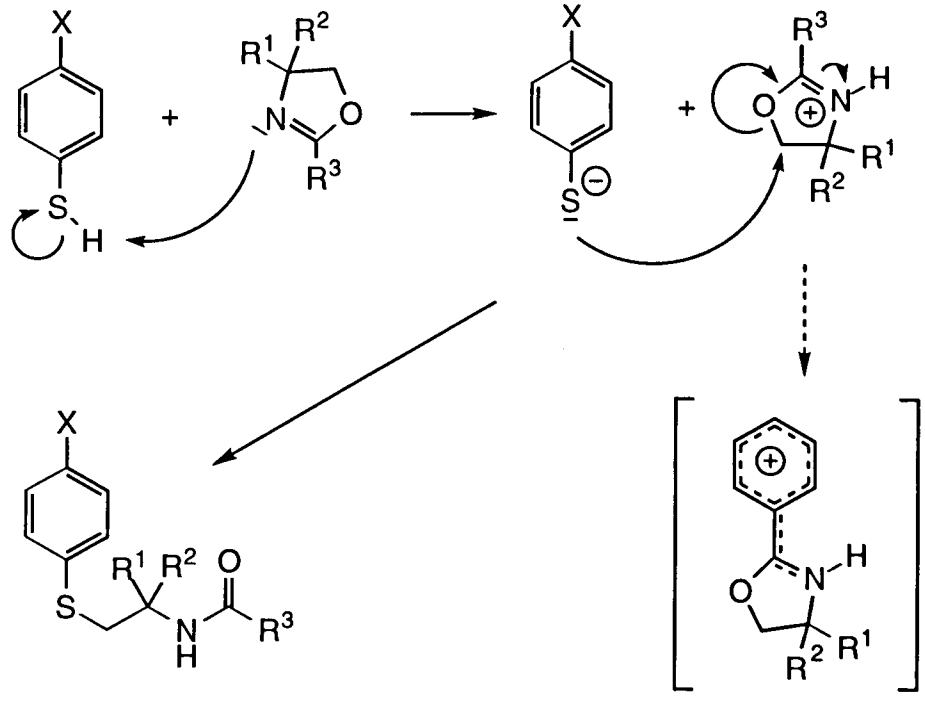
Compound **152** incorporated two methyl substituents in the side-chain which were present as a singlet integrating for six protons in the $^1H$ NMR spectrum. The IR spectrum showed N-H stretching at 3307 $cm^{-1}$ and amide absorptions at 1636 and 1559 $cm^{-1}$.

The propanamide **153** showed the required signals for the ethyl group in the $^1H$ NMR spectrum; the $^3J$ coupling constant between the methylene and methyl protons was 7.5 Hz. The methylene carbon adjacent to the amide carbonyl came into resonance at δ 30.2 in the $^{13}C$ NMR spectrum.

Compound **154** gave a large singlet at δ 1.06 integrating for 9 protons in the $^1H$ NMR spectrum. The $^t$butyl quaternary carbon came into resonance at δ 38.6 in the $^{13}C$ NMR spectrum. The IR spectrum showed

amide N-H and C=O stretching modes as well as strong aliphatic C-H absorptions at 2970 $cm^{-1}$.

The *N*-substituted benzamide **155** showed nine aromatic protons in the $^1H$ NMR spectrum and exhibited aromatic C-H stretching modes in the IR spectrum. Absorptions at 817 and 715 $cm^{-1}$ confirmed the presence of *para* disubstituted and monosubstituted benzene moieties. The poor yield of **155** may be understood by considering the mechanism of the reaction (Scheme 35). The first step is deprotonation of the thiol (pKa ~7) by the oxazoline nitrogen. The protonated oxazoline is then susceptible to nucleophilic attack at the 5' position, since this pushes electrons onto the positive nitrogen atom. When $R^3$ is a phenyl group, however, the positive charge can delocalise through the benzene ring, stabilising the intermediate and making it less likely to react with the thiolate anion.

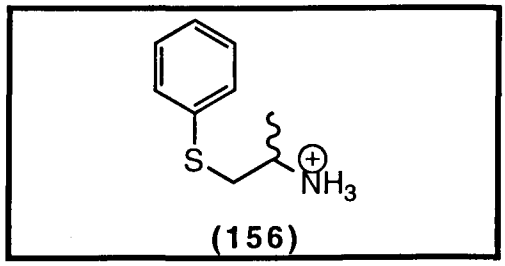


**Scheme 35**

## 6.4 Optically Active Antimelanoma Agents

In 1991, the same group of workers who had been studying **142** as an antihypertensive agent published an interesting paper in which they

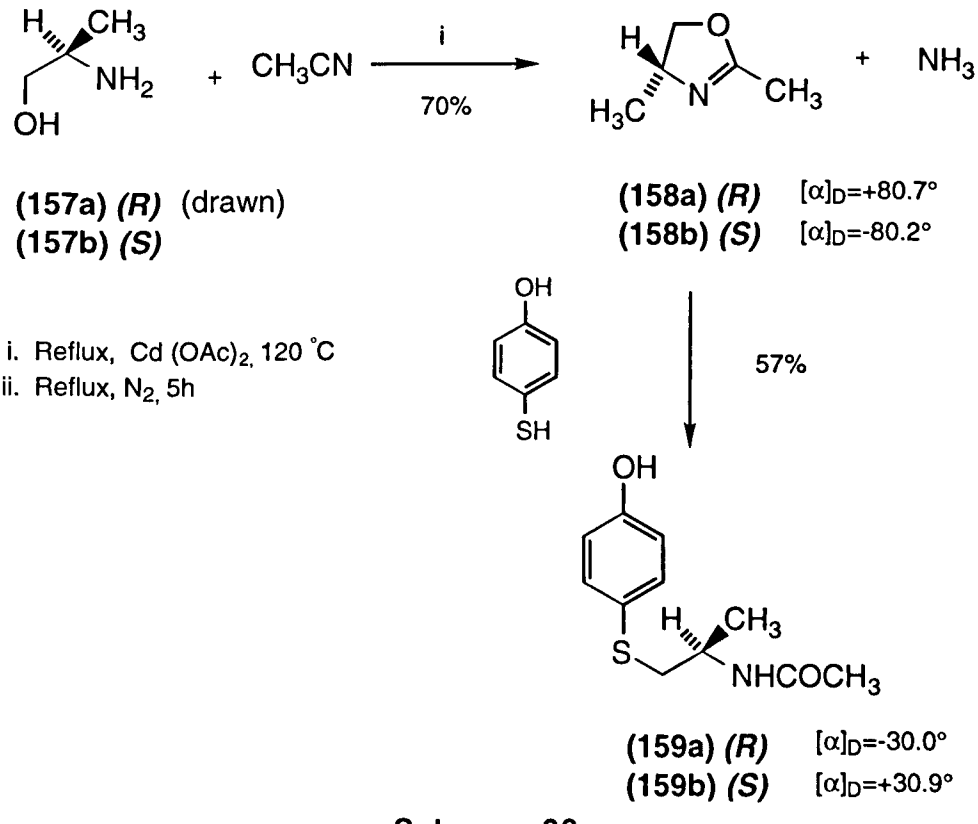
demonstrated an enantiomeric specificity in the antihypertensive activity of 1-(phenylthio)-2-aminopropane (**156**).[161]



**(156)**

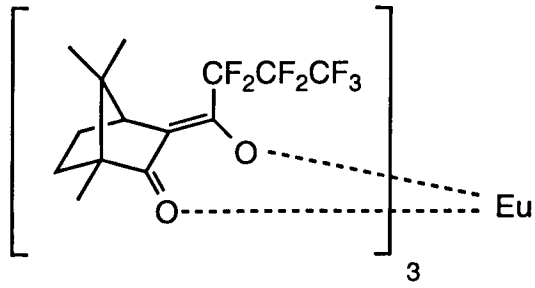
Their studies showed that the *(S)*- isomer of **156** was seven times more active then the *(R)*-isomer against the enzyme dopamine β-monooxygenase. We were interested in preparing the enantiomers of racemate **151** for testing to determine whether tyrosinase exhibited a preference for one stereoisomer.

Starting from chiral 2-aminopropanols (**157a** & **b**) we were able to prepare enantiomerically pure *(R)*-and *(S)*-*N*-{[2-(4-hydroxyphenyl)thio-1-methyl]ethyl}acetamides (**159a** & **b**, Scheme 36).[161]



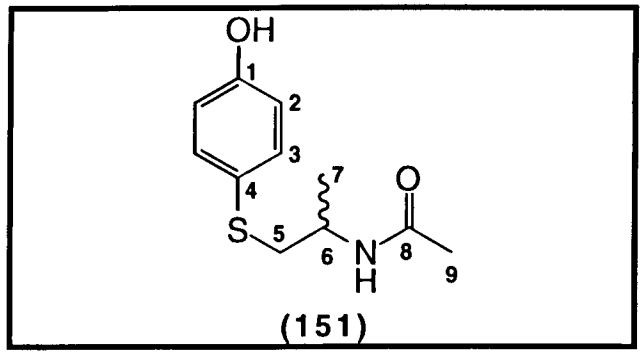

**Scheme 36**

Oxazolines **158a** and **b**[161,162] and products **159a** and **b** gave equal and opposite rotations but this was not sufficient proof that they were single enantiomers. Evidence to this effect was obtained by studying the $^1H$ NMR spectra of amides **159a** and **b** in the presence of the chiral shift reagent tris[3-(heptafluoropropylhydroxy methylene)-(-)-camphorato] europium (III) [$Eu(hfc)_3$, **160**].



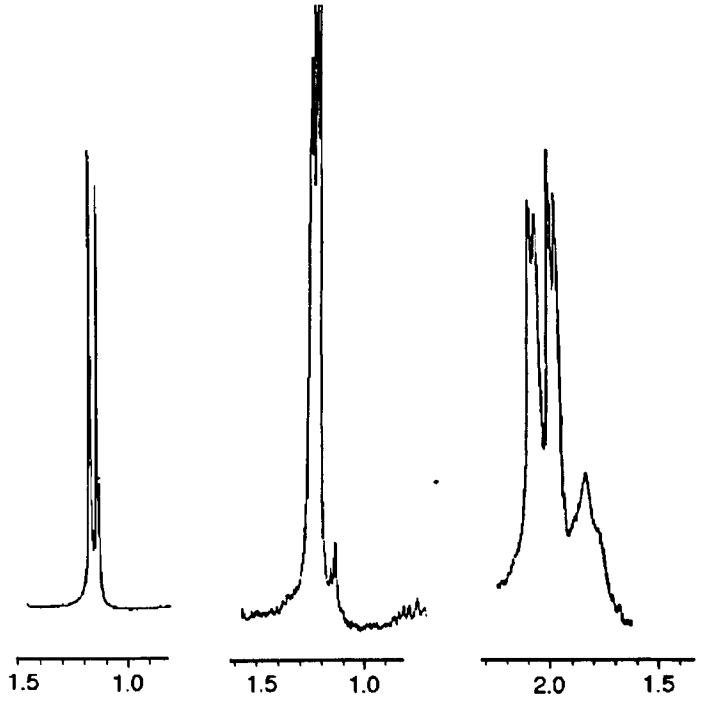

**(160)**

Figure 37a shows a section of the $^1H$ NMR spectrum of **151**, which is a racemic mixture of *(R)* - and *(S)*- enantiomers. The signal shown is due to the methyl group (labelled **7**) borne by the carbon $\alpha$- to nitrogen.



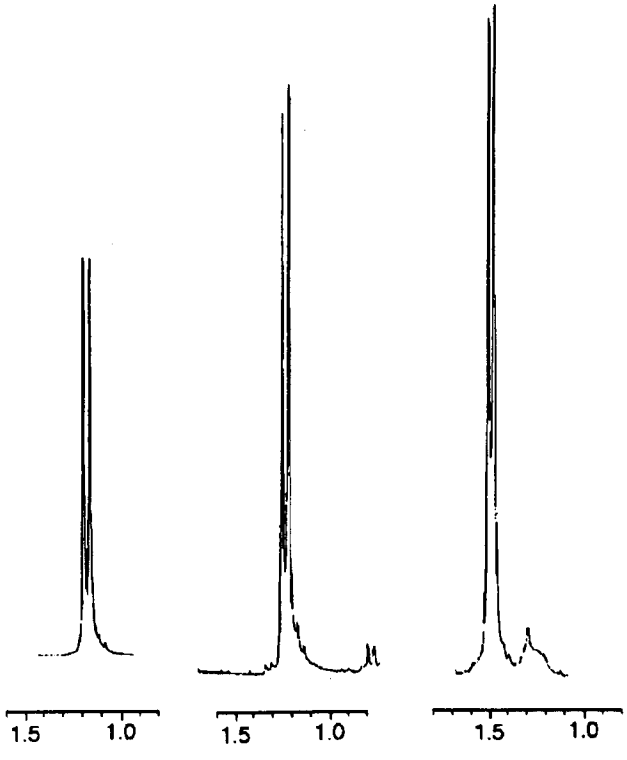

**(151)**

This signal is split into a doublet by a three bond coupling to the proton borne by carbon **6**. The *(R)*- and *(S)*- enantiomers are, of course, indistinguishable by NMR spectroscopy. Figures 37b and 37c show the same signal after addition of 1 mg and 10 mgs of $Eu(hfc)_3$. The shift reagent acts as a Lewis acid and forms a diastereomeric complex with both isomers of **151**. As the shift reagent is added the signal moves downfield under the influence of the paramagnetic europium atom and becomes resolved into two distinct doublets. The methyl groups are no longer equivalent and come into resonance at different chemical shifts. Although the spectrum becomes distorted, it is evident that the enantiomers are present in approximately equal quantities.




(a) (b) (c)

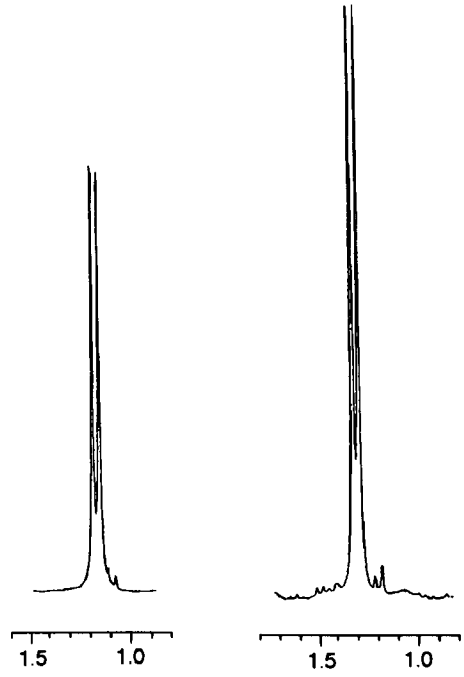
151 + 0 1 10 mg $Eu(hfc)_3$




(d) (e) (f)

159a + 0 2 6 mg $Eu(hfc)_3$

Figure 37

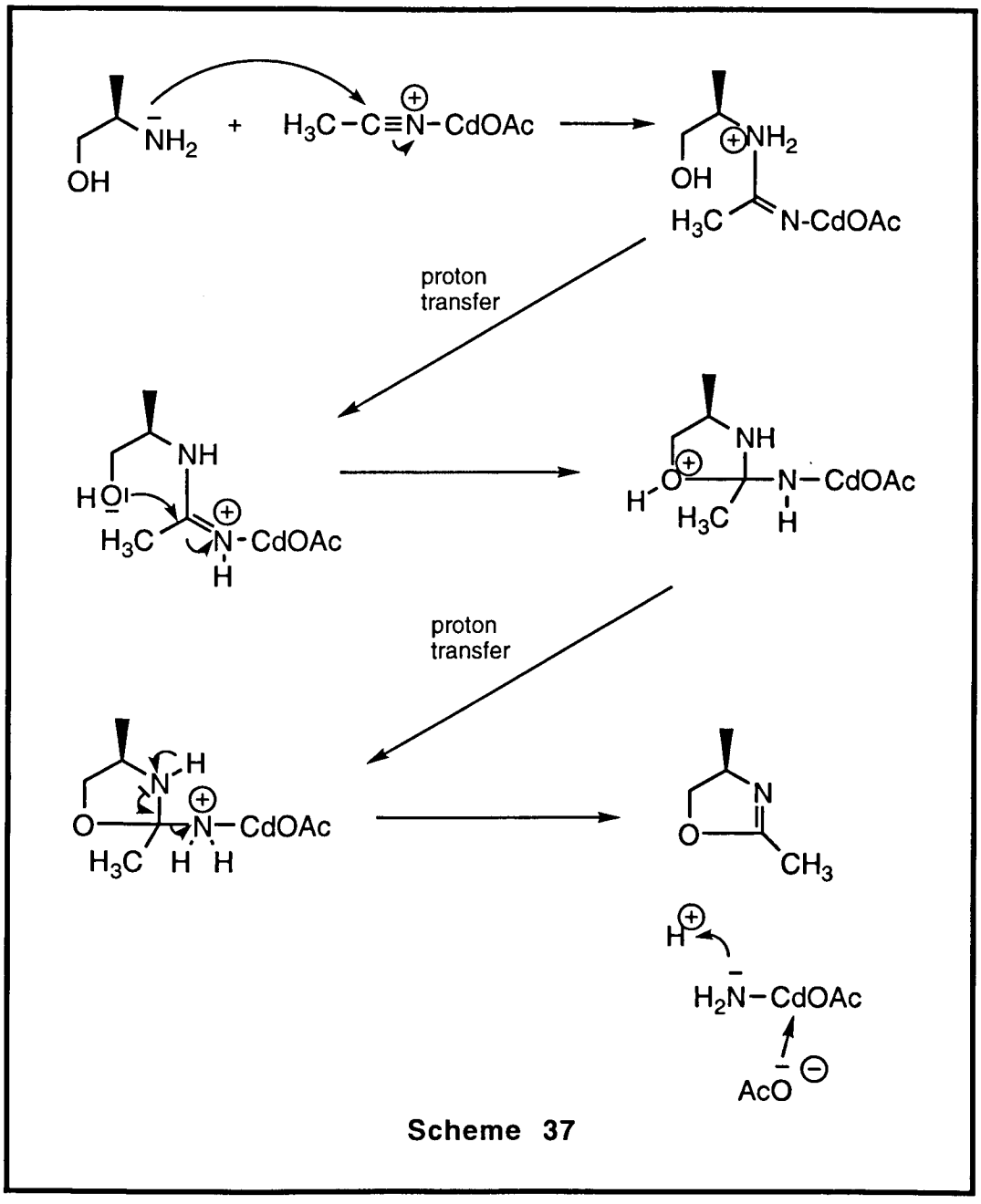



(g) (h)

**159b + 0 5 mg Eu(hfc)$_3$**

**Figure 37**

Figure 37d shows the $^1$H NMR spectrum for the same methyl group in the *(R)* -isomer **159a**. Even after addition of 6 mg of shift reagent the signal for the methyl group remains a clear doublet, indicating the presence of only one enantiomer. The same observation was made for **159b** (Figure 37g and 37h). Although these experiments do not prove that the absolute configurations assigned to **159a** and **159b** are correct, they indicate the presence of only one stereoisomer in each case. The proposed mechanism for the reaction is shown in Scheme 37.

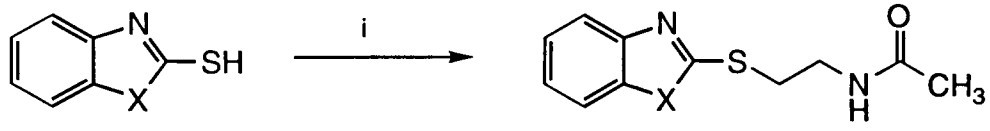



**Scheme 37**

If this mechanism is correct then the stereochemistry of the starting amino alcohol is preserved throughout the reaction. Labelling studies would be of use in determining the reaction pathway. If acetonitrile labelled with $^{15}N$ were reacted with 2-aminopropanol as in Scheme 32, one would expect to find none of the radioactive nitrogen in the oxazoline according to the proposed mechanism.

## 6.5 Novel Heterocyclic Derivatives

2-Methyl-2-oxazoline was reacted with three fused heterocyclic thiols to produce the novel compounds **161**, **162** and **163**. We thought it unlikely that the benzimidazole, benzoxazole and benzthiazole rings would be substrates for tyrosinase and were interested to discover whether any activity remained in the absence of the phenol moiety. The preparative details are summarised in Scheme 38 and Table 10.




i. 2-methyl-2-oxazoline, reflux 2h, $N_2$

**Scheme 38**

**Table 10**

| LABEL | X | % YIELD |
|---|---|---|
| **161** | N-H | 45 |
| **162** | O | 97 |
| **163** | S | 40 |

The benzimidazole derivative **161** showed some interesting spectral features. A broad absorption at 3155 $cm^{-1}$ in the IR spectrum indicated hydrogen-bonded N-H stretching modes which could either be from the N-H of the imidazole or the amide. Examination of the $^1H$ NMR spectrum showed that the coupling constant for the methylene protons of the side-chain was higher than normal by ~1-1.5 Hz. This suggested that the side chain may be twisting back on itself for the amide carbonyl to hydrogen bond with the imidazole N-H. Molecular modelling has shown that conformations which allow this interaction force the two methylene groups in the side chain to adopt a conformation with the protons eclipsed (so raising the observed coupling constant). If hydrogen bonding of this type is involved one would expect to see a concomitant lowering of the amide C=O stretching absorption by ~15-40 $cm^{-1}$. Although the main absorption is at 1666 $cm^{-1}$,

there is also a significant peak at 1626 $cm^{-1}$ which may be due to a hydrogen bonded carbonyl. Whether or not **161** crystallises in this form could be determined by X-ray studies.

The benzoxazole derivative **162** exhibited strong N-H and C=O stretching modes in the IR spectrum and a large absorption at 744 $cm^{-1}$ indicating an *ortho* disubstituted benzene moiety. An absorption for C-S stretching was also observed at 1236 $cm^{-1}$. The $^{13}C$ NMR spectrum showed two highly deshielded $sp^2$ carbons (δ 170.6 and 164.8) corresponding to the carbonyl carbon and position 2' of the benzoxazole ring. Two methylene carbons were also present in the spectrum.

Benzothiazole **163** showed similar spectral features to the other fused ring compounds, including N-H stretch (3298 $cm^{-1}$), amide carbonyl stretch (1642 $cm^{-1}$) and C-S stretching absorptions at 1236 $cm^{-1}$ in the IR spectrum.

## 6.6 Nitrile, Primary Amide and Carboxylic Acid Derivatives

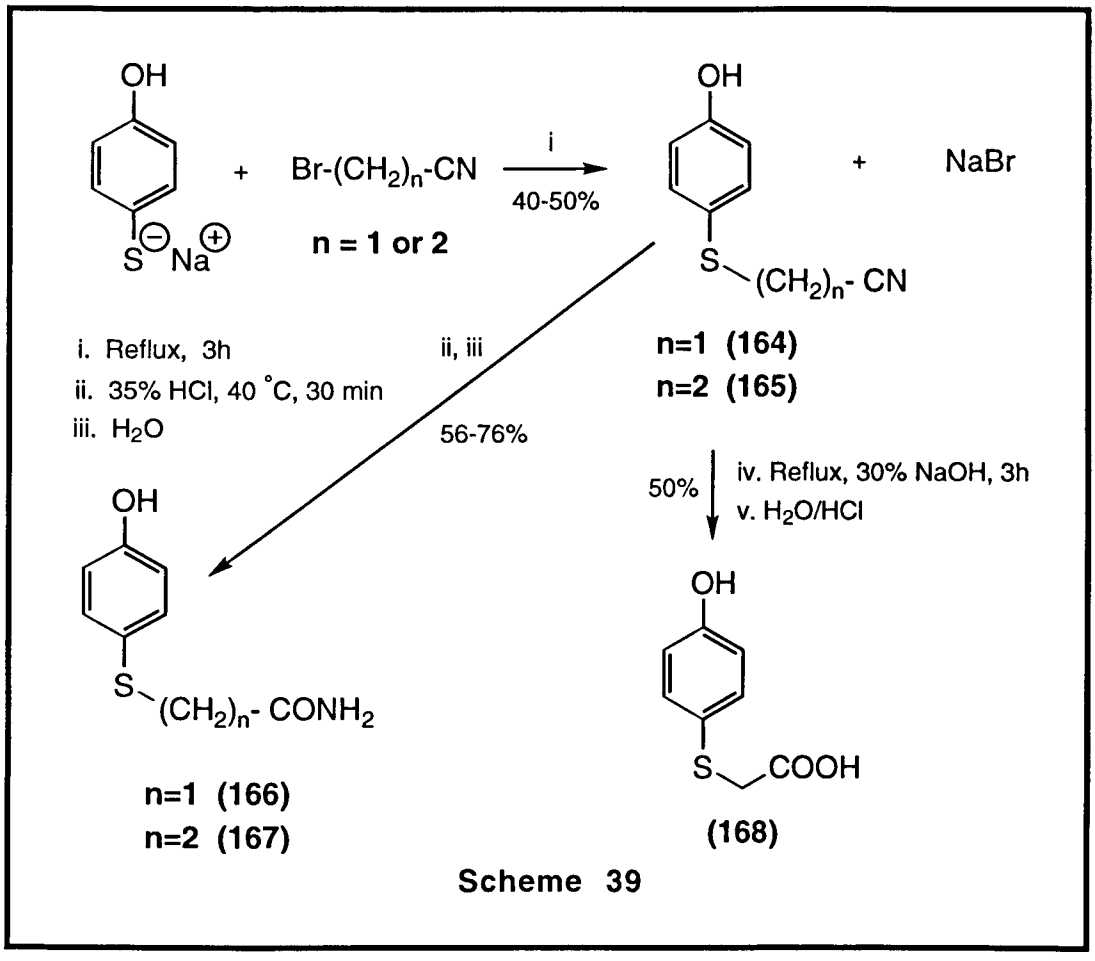
Having explored various modifications to the side-chain while preserving the secondary amide moiety, we now prepared compounds containing other functional groups. We considered the nitriles **165** and **164** to be good starting points and successfully hydrolysed these to form primary amide and acid groups (Scheme 39).

Nitrile **165** has been prepared previously[163] and the melting point and spectral data for our sample correlated well with those in the literature. The clearest indication of product formation was the aliphatic nitrile absorption at 2266 $cm^{-1}$ in the IR spectrum and the nitrile carbon resonance at δ 118.2 in the $^{13}C$ NMR spectrum. Compound **164** was prepared during the initial summer vacation work and was kindly donated by Robert Campbell.

Partial hydrolysis[164] of **165** and **164** led to the primary amides **167** and **166**. Both compounds showed two N-H stretching modes at *ca.* 3400 and 3180 $cm^{-1}$ and amide C=O stretching absorptions at *ca.* 1660 $cm^{-1}$. The carbonyl carbon came into resonance at *ca.* δ 171.

Complete hydrolysis[165] of **164** afforded acid **168**. The carboxylic acid OH group gave a very broad absorption at 3160 $cm^{-1}$ in the IR spectrum and the frequency of the carbonyl absorption increased by 23 $cm^{-1}$ to 1693 $cm^{-1}$

confirming the transformation from amide to acid. The carboxylic acid proton was also evident in the $^1H$ NMR spectrum as a broad signal at δ 12.56.




**Scheme 39**

## 6.7 Preparation of a Solubilised Antimelanoma Compound

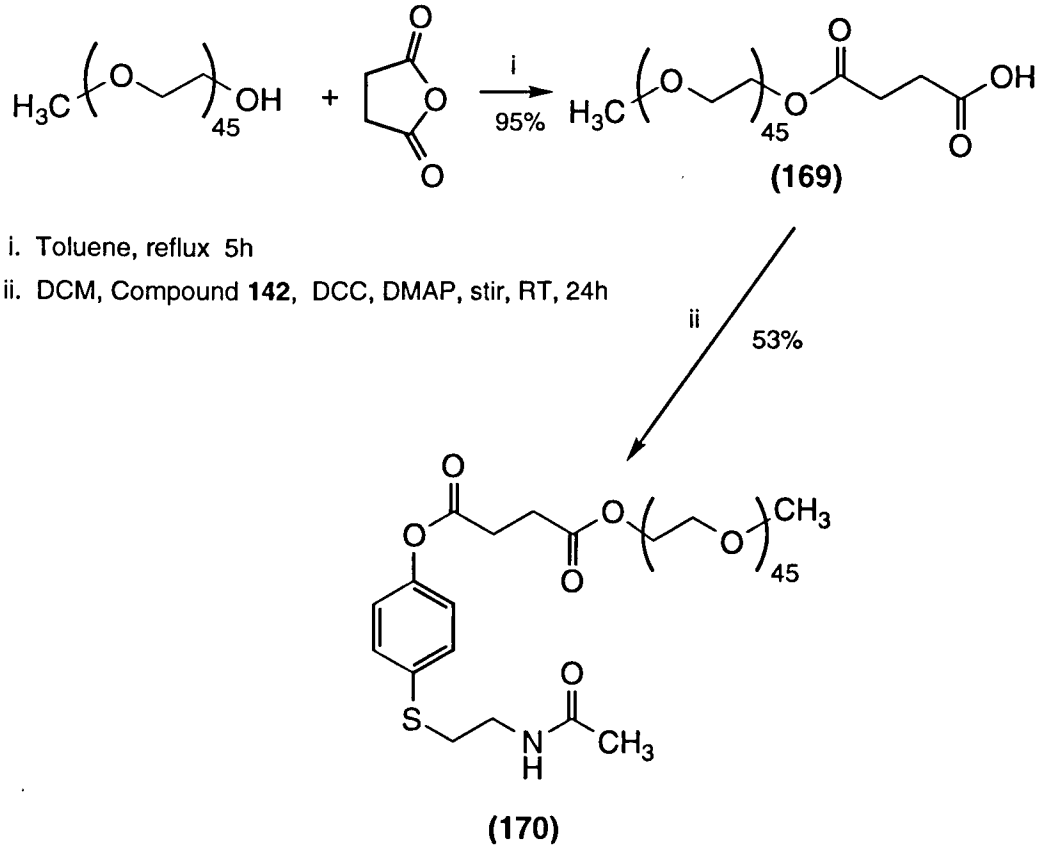
In attempting to prepare a solubilised version of **142** we made use of the hydroxyl group which was the most accessible functional 'handle'. We chose to esterify the phenol with MPEG succinate (**169**), formed by reaction of succinic anhydride with MPEG (Scheme 40).[105] The crude product of this reaction was purified from succinic anhydride by gel filtration chromatography but the $^1H$ NMR spectrum showed the presence of unreacted MPEG. Comparison of the integral for the polyethyleneglycol protons with that for the succinate protons gave the amount of starting material as ~16%. This figure was confirmed by titration of a solution of **169** with standard sodium hydroxide. 9.7 ml of 4.16 mM NaOH solution were required to neutralise a solution of 0.1 g **169** in 10 ml water. Since the

stoichiometry of the reaction between MPEG succinate and NaOH is 1:1, we can say:

$$\%\ \text{by weight of unreacted MPEG} = \frac{\text{no. moles } \mathbf{169} - \text{no. moles NaOH used}}{\text{no. moles } \mathbf{169}}$$

$$= \frac{0.1/2100 - (9.7/1000 \times 4.16/1000)}{0.1/2100} \times 100$$

$$= 15\%$$




**Scheme 40**

Crude **169** was esterified with **142** using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) to effect the reaction. Precipitation of dicyclohexylurea (DCU) confirmed that coupling had taken place and the $^{1}H$ NMR spectrum of the crude product **170** showed it to be approximately 75% of the desired product. The aromatic region of the $^{13}C$

NMR spectrum showed that the carbon at position 4' of the ring (*ortho-* to the ester function) had shifted downfield from δ 116.2 in **142** to δ 122.5 in **170**. This was because the carbonyl group in **170** lessened the shielding effect of the oxygen's lone pair on positions 4' and 2' in the ring. The IR spectrum, although dominated by aliphatic C-H and C-O stretching modes, showed absorptions for ester and amide carbonyl groups (1730 & 1660 $cm^{-1}$ respectively). Figure 38 shows the ESMS for MPEG, **169** and **170**. The distribution for MPEG shows that the polymer tends to lose three electrons under the ionising conditions employed. Although the peaks are flat and broad, there is a clear shift to the right as the average molecular weight increases from 2000 to 2112 to 2281. The expected m/z values for these three compounds are 666, 704 and 760 respectively.

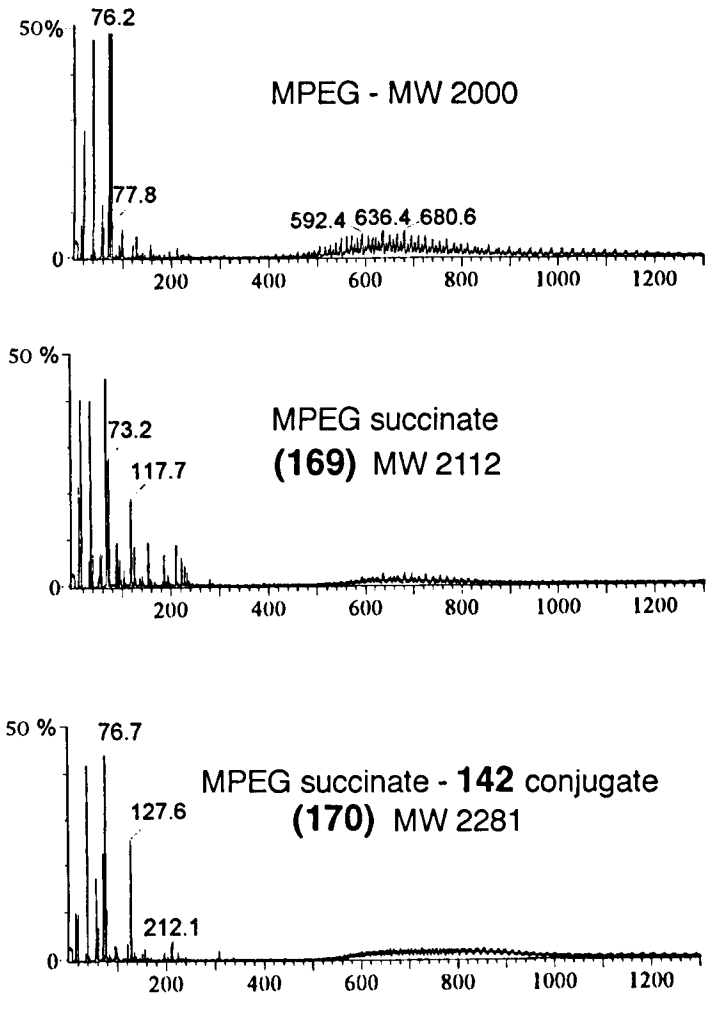



**Figure 38**

The ESMS spectrum for **170** is shown in clearer detail in Figure 39. The centre of the distribution lies somewhere between an m/z of 750-770 which is in the correct region for the desired product.

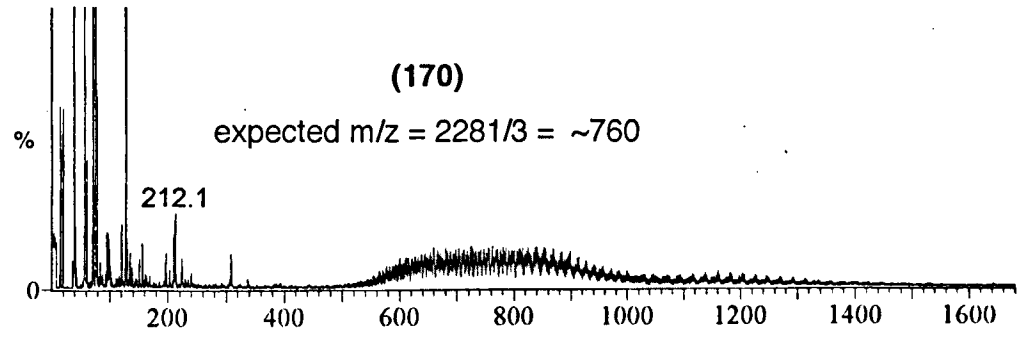



**Figure 39**

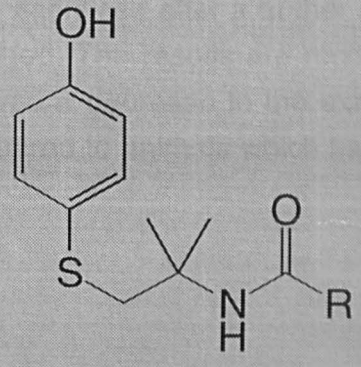
Solubility testing showed the conjugate to be readily soluble in water with gentle heating.

## 6.8 Biological Evaluation of Antimelanoma Compounds

The compounds described in this chapter were tested for us by Dr Lloyd Kelland at the CRC Institute of Cancer Research, Royal Marsden NHS Trust. Each compound was tested against six human melanoma cell lines and the human ovarian carcinoma SKOV-3 as a control. The cells were plated in 96-well plates at a concentration of 4000 cells/well and allowed to incubate overnight before the addition of the drugs at concentrations of 25, 10, 2.5 and 0.25 μM. The drugs were present for five days, after which time the cells were subjected to a sulforhodamine B (SRB) staining assay to determine the proportion of living cells. The results are expressed as the percentage of growth inhibition (%GI) observed at a concentration of 25 μM (Table 11). Large values indicate successful inhibition of cancer growth.

## Table 11
## Growth Inhibition of Antimelanoma Compounds

| No. | B008 | B0010 | HT144 | G631 | SK-Mel-2 | SK-Mel-24 | SKOV-3 |
|---|---|---|---|---|---|---|---|
| 142 | 13.5 | 4.1 | 0 | 2.9 | 0 | 5.6 | 1.7 |
| 150 | 7.4 | 6.7 | 0 | 0 | 2.6 | 0 | 6.6 |
| 151 | 16.9 | 1.3 | 0 | 14.1 | 0 | 7.9 | 1.4 |
| 152 | 67.0 | 7.0 | 3.9 | 38.4 | 5.7 | 26.4 | 13.1 |
| 153 | 79.9 | 20.4 | 10.7 | 34.6 | 2.7 | 26.2 | 12.4 |
| 154 | 65.7 | 41.0 | 37.4 | 55.6 | 32.4 | 25.8 | 22.2 |
| 159b | 26.0 | 7.0 | 0 | 11.5 | 0 | 14.7 | 1.2 |
| 159a | 8.3 | 2.4 | 0 | 12.6 | 5.8 | 0 | 2.2 |
| 163 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 165 | 9.0 | 6.7 | 0 | 14.4 | 0 | 5.2 | 3.5 |
| 167 | 11.7 | 3.1 | 0 | 7.3 | 0 | 10.8 | 6.7 |
| 166 | 4.0 | 0 | 0 | 5.2 | 0 | 0 | 0 |
| 168 | 3.8 | 20.4 | 0 | 0 | 0 | 4.2 | 0 |
| 170 | 15.6 | 1.4 | 0 | 1.6 | 2.7 | 0 | 0 |




| COMPOUND | R |
|---|---|
| 152 | Methyl |
| 153 | Ethyl |
| 154 | $^{t}$Butyl |

The success of compounds **152**, **153** and **154** (dark shading) in comparison with **142** (lighter shading) is noteworthy. **154** was the most consistently potent compound, inhibiting the growth of melanoma cells between five and ten times more effectively than **142**. As expected, the fluorobenzene derivative **150** and the benzothiazole **163** showed poor inhibition. The nitrile **165** exhibited similar inhibition to **142**, as did the primary amide **167** derived from it. The shorter chain amide **166** and its related acid **168** were relatively inactive.

The racemic compound **151** showed comparable activity to **142**, which is largely attributable to the influence of the *(S)* isomer **159b** which performs as well as, if not better than the *(R)* isomer **159a** in six out of the seven cell lines. It was encouraging that the solubilised compound **170** showed similar activity to **142**.

The *N*-substituted benzamide derivative **155** was not tested at this stage but was later screened against the same panel of cell lines. The compound did not inhibit effectively and gave $IC_{50}$ values of >50 μM in each case.

The fact that several of the most active compounds also inhibited the SKOV-3 ovarian carcinoma confirms that these compounds are able to exert toxicity by non-tyrosinase mediated routes.

Professor Rona Mackie contributed valuable information by testing the compounds against murine melanoma lines *in vitro* and *in vivo*. Both **152** and **153** out-performed **142** against the highly metastatic B16F-10 cell line *in vitro*, exhibiting %GI values of 79 and 81% compared to 51% for **142**. The *in vivo* testing was performed on C-57 black mice which were injected with B16F-1 or B16F-10 melanoma cells and given a dose of the appropriate drug at a concentration of 3 mg/g body weight seven days later. The mice were sacrificed after a further seven days and the tumours were excised and weighed. The results are recorded in Table 12. Empirical observations also showed a decrease in the extent of ulceration exhibited at the tumour sites compared to animals which had not been treated with the drug.

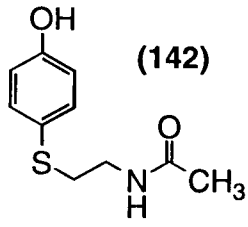
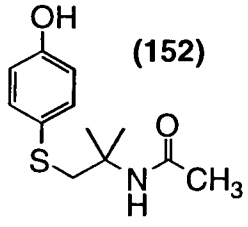
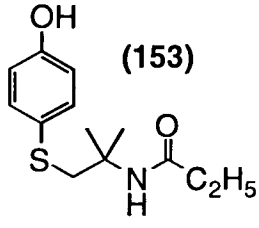
| COMPOUND | % Growth reduction in weight of tumour tissue | |
|---|---|---|
| | B16 F1 cell line | B16 F10 cell line |
|  (142) | 39 | 16 |
|  (152) | 43 | 45 |
|  (153) | 46 | 48 |

Table 12

## 6.9 Discussion and Suggestions for Future Work

Of our initial strategies in modifying the parent compound **142**, increasing the bulk in the side-chain while preserving the secondary amide has produced the most encouraging results. Altering the nature of the terminal functional group was not beneficial, and removing the phenol moiety brought a complete loss of activity. It is significant that drug potency seems to rise dramatically with the inclusion of two methyl groups in the side-chain. This is obvious from a comparison of the activity of **142** and **151** with **154**, **152** and **153**. Is there a limit to this effect? An obvious progression would be to increase the size of these groups to ethyl or propyl units, or possibly cyclic spiro compounds. The slight enantiomeric specificity

evidenced for **159b** suggests that it may be worth introducing different akyl groups at this position.

The nature of the terminal alkyl group also seems to have a bearing on activity. Taking the average %GI across all the cell lines, apart from SKOV-3, we find that **154** averages 42.9%, **153**, 29.1% and **152**, 24.7%. The only difference between these compounds is the nature of the terminal amide, which suggests that bulky groups are advantageous in this position. It is unclear whether this is a 'spacing' effect or whether it is to do with the lipophilicity of the compounds. This warrants further investigation, particularly as the phenyl derivative **155**, which one would expect to be active on the basis of these arguments, is actually a poor inhibitor. A more conclusive pattern could be established by preparing oxazolines from longer chain aliphatic acids such as butyric, isobutyric, hexanoic and palmitic acids and reacting these with 4-hydroxythiophenol. Alternatively, the parent compound **142** could be esterified by reaction with a long chain fatty acid to form a lipophilic equivalent of **170**. This work should be prioritised since the increase in activity provided by relatively minor structural alterations has been impressive. The X-ray structures of **152**, **153** and **154** should be elucidated to determine whether their crystal structures differ significantly from that of **142**.

Another area which could be explored is introducing chirality into the terminal amide group. This could readily be achieved by preparing oxazolines from chiral acids, acid chlorides or nitriles (Figure 41).

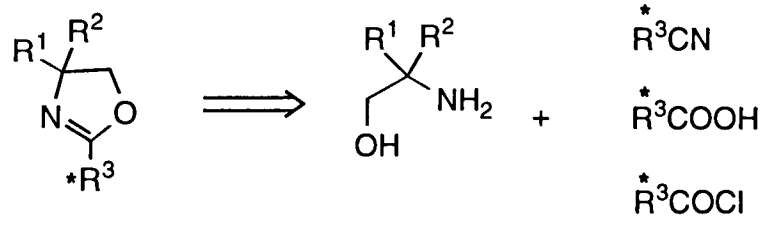
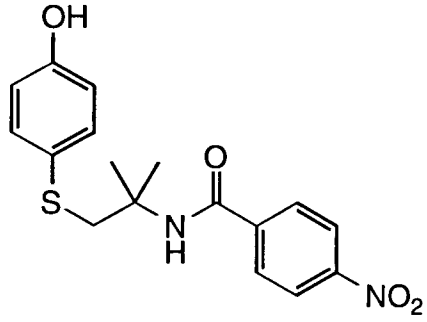
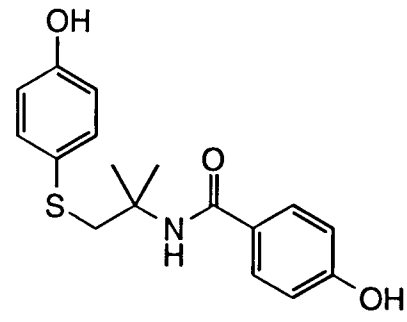



**Figure 41**

Although the phenyl derivative **155** was inactive, it would be instructive to prepare a small number of substituted phenyl derivatives to determine whether functionality in the ring alters activity. Two which would be worth pursuing are the substituted phenols **171** and **172**.



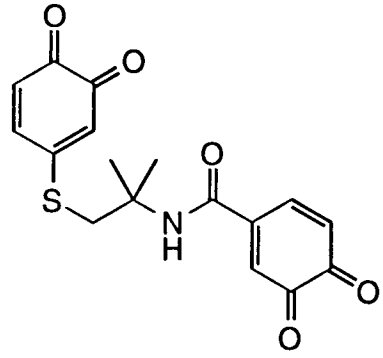

**(171)**




**(172)**

At one level, these compounds are interesting simply because of their different electronic character, but it is possible that each may exert an unusual mode of cytotoxicity. Most melanomas are solid tumours and, as has already been described (Section 4.8), under these conditions reductase enzymes are overexpressed. By incorporating a nitro group, while retaining the active phenol structural unit, it may be possible to take advantage of this factor in malignant tissue. This compound may have two modes of action. Where malignant melanocytes are functioning, the phenol could inhibit using the normal tyrosinase pathways and where hypoxic conditions are in evidence, the nitro group might be reduced to form cytotoxic radical species.

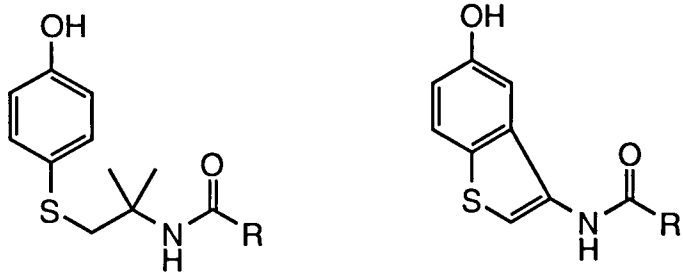
Incorporating two phenol moieties in the drug raises the possibility that both could be hydroxylated and oxidised by tyrosinase to form quinones such as **173**. Such species may have the ability to crosslink DNA.




**(173)**

The poor results for **150** and **163** confirm the importance of the phenol moiety and suggest that other aromatic groups may not be acceptable substitutes. It may be possible, however, to form ring-constrained analogues if suitably functionalised thianaphthenes can be prepared (e.g. **174**). Thianaphthenes substituted at the 2'- and 3'- positions are

commercially available and may provide useful building blocks for structures similar to that shown below.



(174)

**Summary of Future Work**

- The X-ray structures of **159b**, **159a**, **152**, **153** and **154** should be obtained.

- The ability of the most active antimelanoma agents to act as substrates for mammalian tyrosinase should be established.

- Labelling studies should be carried out to determine the mechanism of oxazoline formation as described by Witte and Seeliger (Scheme 32).

- The optimal substitution pattern for the side-chain should be determined, including an investigation of chirality $\alpha$– to the nitrogen and in the acyl position.

- The two novel substituted phenyl compounds **171** and **172** should be prepared and tested.

- Alkylation on the amide nitrogen to remove H-bonding and oxidation of the sulfur atom should be explored. The possibility of replacing sulfur with another heteroatom should be investigated.

- The chemistry of thianaphthenes should be researched and suitable compounds prepared.

# 7

# Experimental to Chapters 3-6

## 7.1 General Preamble to Experimental Section:

All chemicals were purchased from Aldrich Chemical Company (Gillingham, Dorset, UK). Organic solutions were dried with anhydrous $MgSO_4$ or $Na_2SO_4$ and solvents were removed under reduced pressure below 40 °C. Melting points (m.p.) were measured with a Gallenkamp apparatus and are uncorrected; the abbreviation 'd.' denotes compounds which decomposed before melting. $^1H$ and $^{13}C$ NMR spectra were obtained on a Bruker WP200-SY spectrometer operating at 200 MHz and 50 MHz respectively. All coupling constants quoted are measured in Hz. The numbering schemes shown are used for ease of assigning the NMR spectra. Mass spectra (MS) were recorded on AEI MS12 or MS902 spectrometers. Infra-red (IR) spectra were obtained for KBr discs on a Perkin Elmer 983 spectrophotometer or a Perkin Elmer PU 9800 FT-IR spectrophotometer. Ultra-violet (UV) spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Thin layer chromatography (TLC) was carried out on silica gel G plates of 0.25 mm thickness developed with petroleum ether (40-60 °C) - EtOAc (1:1) unless stated otherwise and compounds were visualised by UV and with iodine. Column chromatography was carried out on silica gel, 70-230 mesh with the same solvent system. Gel filtration chromatography was carried out on Sephadex LH-20 (10 g) with water as eluent.

## 7.2 Experimental to Chapter 3:

### 7.2.1 General Procedures

The majority of tyrphostins in this set were synthesised using the methodology employed by Gazit *et al.* [25]

***Method A:***[25] Quinolinecarbaldehyde (2-3 mmol) was dissolved in EtOH (2 ml) in a 25 ml round-bottomed flask. To the stirred solution was added an equimolar amount of the appropriate malononitrile derivative, solid compounds being pre-dissolved in EtOH (2 ml). A catalytic amount of EtOH-piperidine solution (10:1, 2 drops) was introduced and the reaction stirred at RT until a solid precipitate formed. The solid was filtered off, washed with petroleum ether (40-60 °C) and dried under suction.

In reactions where TLC indicated that the reaction had gone to completion but precipitation did not occur, water was added to the reaction mixture and the precipitated solid was filtered, dried and purified by column chromatography.

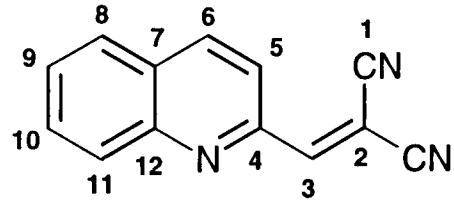
***Method B:***[89] The $^{t}$butyl cyanoacetate derivatives with 3- and 4-quinoline carbaldehyde (**59** & **60**) were stirred in formic acid at RT for 4 h. After this time the solution was evaporated to dryness and the solid products were purified from starting material by column chromatography.

### 7.2.2 Numbering System

The numbering system has been based on the quinolinyl propenenitrile unit which is common to all the structures in chapter 3 and to many in chapter 4. Numbering starts at the nitrile moiety and continues along the side arm, proceeding anti-clockwise around the quinoline rings. Where the quinoline has been substituted at the (formal) 3- or 4-position, the ring carbons keep the same number as they have in the 2-substituted compounds. This introduces a slight discontinuity in the flow of the numbering but allows easier comparison between structures.

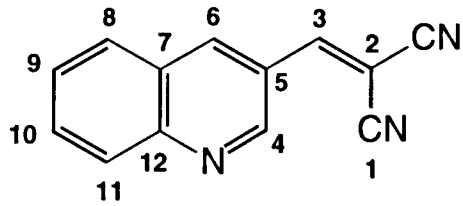
## 7.2.3 Experimental Data

### 2-Cyano-3-(2-quinolinyl)propenenitrile (43).




Compounds **43**, **44** and **45** have been prepared previously[86,87] but have only been reported in obscure journals. The spectral data for these compounds are presented in full here. Method A afforded nitrile **43** as a brown powder which was recrystallised to give orange needles (0.297 g, 68%), m.p. 168-170 °C (from EtOAc); $R_f$ 0.67; $\nu_{max}$ (KBr) 3050w (unsaturated C-H), 2220m (CN), 1590, 1500s (aromatic C=C & C=N) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.64 (1 H, s, 3-H), 8.52 (1 H, d, $^3J$= 8.4, 6-H), 8.02 (2 H, m, 8-H & 11-H), 7.78 (1 H, d, $^3J$= 8.5, 5-H), 7.84 (1 H, m, $^4J$= 1.6, 10-H) and 7.71 (1 H, m, $^4J$= 1.3, 9-H); $\delta_C$ ($D_6$-DMSO) 158.5 (C-3), 148.7 (C-4), 146.9 (C-12), 137.8 (C-6), 131.1 (quin C-H), 129.3 (quin C-H), 129.1 (quin C-H), 128.9 (C-7), 128.1 (quin C-H), 124.1 (C-5), 114.4 (C-1), 112.9 (CN) and 80.1 (C-2); m/z 205 ($M^+$, 100), 179 (12.5), 154 (35.2), 129 (25.7) and 101 (22.3%) (Found: $M^+$, 205.0629; C, 76.02; H, 3.49; N, 20.39%. $C_{13}H_7N_3$ requires *M*, 205.0639; C, 76.07; H, 3.44; N, 20.49%).

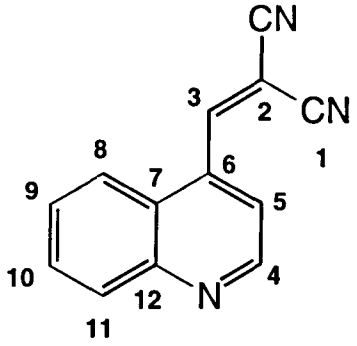
### 2-Cyano-3-(3-quinolinyl)propenenitrile (44).[86,87]




Method A afforded nitrile **44** as a brown powder, the reaction proceeding quickly without addition of catalyst. Recrystallisation afforded fawn coloured needles (0.334 g, 81%), m.p. 199-201 °C (from isopropanol); $R_f$ 0.52; $\nu_{max}$ (KBr) 3040w (unsaturated C-H), 2215m (CN), 1595 and 1490 (aromatic C=C) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.21 (1 H, d, $^4J$= 2.2, 4-H), 8.87 (1 H, d, $^4J$ ~ 1.9, 6-H), 8.69 (1 H, s, 3-H), 8.12-8.02 (2 H, m, 8-H & 11-H), 7.93 (1 H, ddd, 10-H) and 7.71 (1 H, ddd, 9-H); $\delta_C$ ($D_6$-DMSO) 158.8 (C-3), 149.9 (C-4), 148.8 (C-

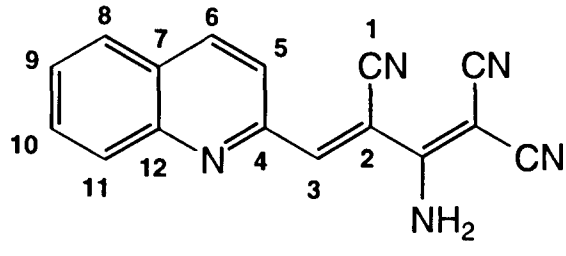
12), 138.8 (C-6), 133.1 (quin C-H), 129.7 (quin C-H), 128.9 (quin C-H), 128.8 (quin C-H), 126.3 (C-7), 124.8 (C-5), 113.9 (C-1), 113.1 (CN) and 83.4 (C-2); m/z 205 ($M^+$, 14.0), 151 (27.5), 127 (31.6), 99 (41.9) and 75 (95.3%) (Found: $M^+$, 205.0648; C, 76.01; H, 3.18; N, 20.43%. $C_{13}H_7N_3$ requires *M*, 205.0639; C, 76.07; H, 3.44; N, 20.49%).

**2-Cyano-3-(4-quinolinyl)propenenitrile (45).**[86,87]




Method A afforded nitrile **45** as a yellow powder which was recrystallised to give yellow needles (0.163 g, 40%), m.p. 145-146 °C (from isopropanol); $R_f$ 0.51; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2217m (CN), 1590 and 1500m (aromatic C=C) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.40 (1 H, s, 3-H), 9.12 (1 H, d, $^3J$= 4.5, 4-H), 8.22 (1 H, dd, $^3J$= 8.6, 11-H), 8.14 (1 H, dd, $^3J$= 8.6, 8-H), 7.97-7.83 (2 H, m, 5-H & 10-H) and 7.75 (1 H, ddd, 9-H); $\delta_C$ ($D_6$-DMSO) 158.9 (C-3), 150.4 (C-4), 147.9 (C-12), 136.3 (C-6), 130.6 (quin C-H), 129.7 (quin C-H), 128.2 (quin C-H), 124.4 (quin C-H), 124.3 (C-7), 120.2 (C-5), 113.2 (C-1), 112.2 (CN) and 89.8 (C-2); m/z 205 ($M^+$, 92.4), 178 (100.0), 151 (31.9), 100 (21.4) and 75 (34.1%) (Found: $M^+$, 205.0630; C, 75.72; H, 3.58; N, 20.31%. $C_{13}H_7N_3$ requires *M*, 205.0639; C, 76.07; H, 3.44; N, 20.49%).

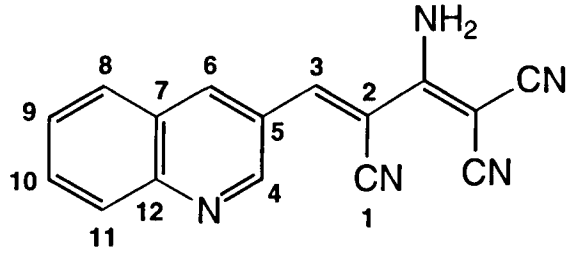
***(Z)*-2-Amino-4-(2-quinolinyl)-1,3-butadiene-1,1,3-tricarbonitrile (46).**[86]




Compounds **46**, **47** and **48** have been prepared previously but have only been reported in obscure journals. The spectral data for these compounds

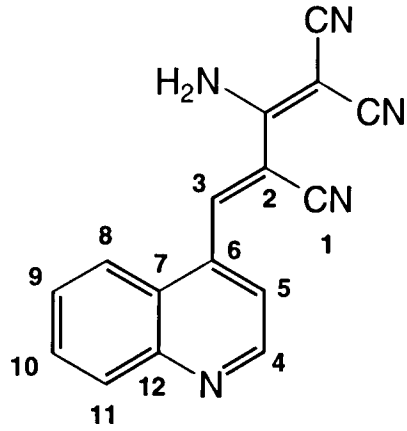
are presented in full here. Method A afforded nitrile **46** as a yellow solid (0.326 g, 70%), m.p. 180 °C (d.); $R_f$ 0.24; $\lambda_{max}$ (EtOH)/nm 203 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$ 49000), 230 (28000), 269 (31700) and 316 (18400); $\nu_{max}$ (KBr) 3320m ($NH_2$), 3190w, 2220, 2210 (CN), 1650s (alkene C=C) and 1610 (aromatic C=C) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.38 and 9.31 (2 H, 2 br s, $NH_2$), 8.62 (1 H, d, $^3J$= 8.4, 6-H), 8.29 (1 H, s, 3-H), 8.12 (2 H, m, 8-H & 11-H), 7.93 (1 H, m, $^4J$= 1.2, 10-H), 7.91 (1 H, d, $^3J$= 8.5, 5-H) and 7.77 (1 H, m, $^4J$= 1.0, 9-H); $\delta_C$ ($D_6$-DMSO) 165.7 (-*C* ($NH_2$)=C($CN)_2$), 150.3 (C-3), 149.5 (C-4), 147.1 (C-12), 137.8 (C-6), 131.0 (quin C-H), 129.1 (quin C-H), 128.8 (quin C-H), 128.4 (C-7), 128.1 (quin C-H), 123.6 (C-5), 115.6 (CN), 114.5 (2 x *C*N), 106.6 (C-2) and 50.2 (-C ($NH_2$)=*C*($CN)_2$); m/z 271 ($M^+$, 88.6), 245 (87.5), 206 (61.4), 179 (38.8) and 129 (100%) (Found: $M^+$, 271.0856; C, 70.56; H, 3.16; N, 25.62%. $C_{16}H_9N_5$ requires *M*, 271.0857; C, 70.83; H, 3.35; N, 25.83%).

**(*Z*)-2-Amino-4-(3-quinolinyl)-1,3-butadiene-1,1,3-tricarbonitrile (47).**[86]



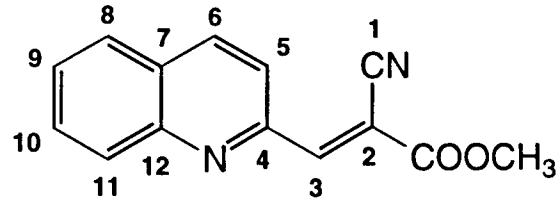

Method A afforded nitrile **47** as a yellow solid (0.261 g, 48%), m.p. 195 °C (d.); $R_f$ 0.51 (EtOAc); $\lambda_{max}$ (EtOH)/nm 205 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$ 64900), 231 (55300), 246 (48000) and 276 (43100); $\nu_{max}$ (KBr) 3414 and 3327 ($NH_2$), 2218, 2202m (CN), 1643s (alkene C=C), 1601 and 1491 (aromatic C=C) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.31 (1 H, d, $^4J$= 2.2, 4-H), 9.31 & 9.25 (2 H, 2 br s, $NH_2$), 8.97 (1 H, $^4J$= 2.0, 6-H), 8.39 (1 H, s, 3-H), 8.22-8.08 (2 H, m, 8-H & 11-H), 7.97 (1 H, ddd, $^4J$= 1.3, 10-H) and 7.77 (1 H, ddd, $^4J$= 1.0, 9-H); $\delta_C$ ($D_6$-DMSO) 165.0 (-*C* ($NH_2$)=C($CN)_2$), 150.8 (C-3), 150.2 (C-4), 148.6 (C-12), 137.8 (C-6), 136.3 (quin C-H), 129.6 (quin C-H), 129.0 (quin C-H), 128.2 (quin C-H), 126.7 (C-7), 125.1 (C-5), 115.5, 114.8, 114.7 (all CN), 104.3 (C-2) and 50.2 (-C ($NH_2$)=*C*($CN)_2$); m/z 271 ($M^+$, 78.7), 245 (44.8), 206 (72.1), 142 (95.9) and 115 (30.3%) (Found: $M^+$, 271.0847; C, 70.75; H, 3.12; N, 25.77%. $C_{16}H_9N_5$ requires *M*, 271.0857; C, 70.83; H, 3.35; N, 25.83%).

**(*Z*)-2-Amino-4-(4-quinolinyl)-1,3-butadiene-1,1,3-tricarbonitrile (48).**[86]




Method A afforded nitrile **48** as a yellow solid (0.379 g, 70%), m.p. 180 °C (d.); $R_f$ 0.55 (EtOAc); $\lambda_{max}$ (EtOH)/nm 212 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 85900), 252 (40100) and 307 (24900); $\nu_{max}$ (KBr) 3443 and 3335 ($NH_2$), 3177, 2228, 2220 (CN), 1660 (alkene C=C), 1608 and 1504 (aromatic C=C) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.46 and 9.35 (2 H, 2 br s, $NH_2$), 9.17 (1 H, d, $^3J$= 4.4, 4-H), 8.95 (1 H, s, 3-H), 8.29 (1 H, d, $^3J$= 8.1, 11-H), 8.19 (1 H, d, $^3J$= 8.2, 8-H), 7.96-7.88 (2 H, m, 5-H & 10-H) and 7.73 (1 H, ddd, 9-H); $\delta_C$ ($D_6$-DMSO) 164.2 (-*C* ($NH_2$)=C($CN)_2$), 151.0 (C-3), 150.9 (C-4), 147.9 (C-12), 137.6 (C-6), 130.7 (quin C-H), 129.8 (quin C-H), 128.0 (quin C-H), 125.1 (C-7), 124.9 (quin C-H), 120.3 (C-5), 115.8 (CN), 114.5 (C-1), 113.9 (CN), 109.9 (C-2) and 50.7 (-C ($NH_2$)=*C*($CN)_2$); m/z 271 (*M*$^+$, 100.0), 245 (84.6), 206 (53.4), 142 (32.7) and 75 (33.6%) (Found: *M*$^+$, 271.0856; C, 70.61; H, 3.16; N, 25.80%. $C_{16}H_9N_5$ requires *M*, 271.0857; C, 70.83; H, 3.35; N, 25.83%).

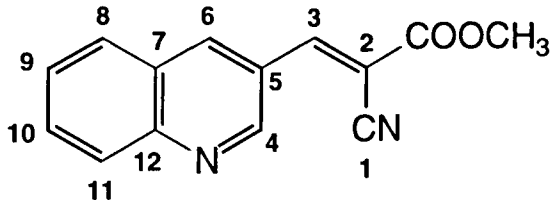
**Methyl *(E)*-2-cyano-3-(2-quinolinyl)propenoate (49).**




Method A afforded ester **49** as a cream solid which was recrystallised to give cream needles (0.132 g, 18%), m.p. 126-127 °C (from EtOAc); $R_f$ 0.68; $\nu_{max}$ (KBr) 3050w (unsaturated C-H), 2950w (aliphatic C-H), 2220m (CN), 1720s (ester C=O), 1625m (alkene C=C) and 1270 (C-O) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 8.48 (1 H, s, 3-H), 8.32 (1 H, d, $^3J$= 8.5, 6-H), 8.22 (1 H, dd, $^3J$= 8.5, 11-H),

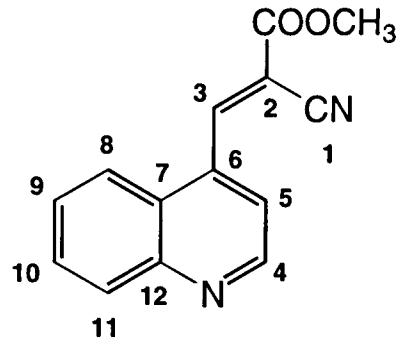
8.02 (1 H, d, $^3J$= 8.5, 5-H), 7.88 (1 H, dd, $^3J$= 8.4, 8-H), 7.81 (1 H, ddd, $^4J$= 1.6, 10-H), 7.66 (1 H, ddd, $^4J$= 1.3, 9-H) and 3.99 (3 H, s, $COOCH_3$); $\delta_C$ ($CDCl_3$) 162.6 ($COOCH_3$), 154.1 (C-3), 149.7 (C-4), 148.2 (C-12), 137.2 (C-6), 130.7 (C-10), 130.4 (C-11), 129.0 (C-9), 128.6 (C-7), 127.7 (C-8), 122.1 (C-5), 114.8 (C-1), 107.2 (C-2) and 53.7 ($COOCH_3$); m/z 238 ($M^+$, 11.3), 208 (70.4), 180 (100), 128 (61.5) and 101 (33.5%) (Found: $M^+$, 238.0740; C, 70.50; H, 4.32; N, 11.77%. $C_{14}H_{10}N_2O_2$ requires $M$, 238.0740; C, 70.56; H, 4.23; N, 11.76%).

**Methyl *(E)*-2-cyano-3-(3-quinolinyl)propenoate (50).**



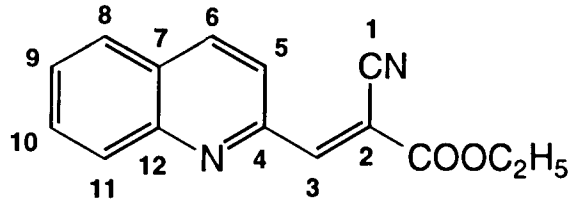

Method A afforded ester **50** as a white powder (0.367 g, 77%), m.p. 155-156 °C; $R_f$ 0.44; $\nu_{max}$ (KBr) 3050w (unsaturated C-H), 2960w (aliphatic C-H), 2220m (CN), 1730s (ester C=O), 1620m (alkene C=C) and 1270s (C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 9.13 (1 H, d, 4-H), 9.00 (1 H, d, $^4J$= 2.1, 6-H), 8.39 (1 H, s, 3-H), 8.11 (1 H, d, $^3J$= 8.4, 11-H), 7.90 (1 H, d, $^3J$= 8.0, 8-H), 7.82 (1 H, m, $^4J$= 1.4, 10-H), 7.60 (1 H, m, $^4J$= 1.0, 9-H) and 3.94 (3 H, s, $CH_3$); $\delta_C$ ($CDCl_3$) 162.4 ($COOCH_3$), 151.5 (C-4), 151.4 (C-3), 149.4 (C-12), 138.1 (C-6), 132.7 (C-10), 129.4 (C-11), 129.4 (C-8), 128.0 (C-9), 127.0 (C-7), 124.4 (C-5), 115.2 (C-1), 104.4 (C-2) and 53.6 ($COOCH_3$); m/z 238 ($M^+$, 51.6), 223 (38.2), 179 (44.2), 152 (100) and 75 (75.8%) (Found: $M^+$, 238.0743; C, 70.41; H, 4.02; N, 11.68%. $C_{14}H_{10}N_2O_2$ requires $M$, 238.0740; C, 70.56; H, 4.23; N, 11.76%).

**Methyl *(E)*-2-cyano-3-(4-quinolinyl)propenoate (51).**




Method A afforded ester **51** as a white crystalline solid (0.262 g, 36%), m.p. 140-141 °C (from EtOAc); $R_f$ 0.44; $\nu_{max}$ (KBr) 3050w (unsaturated C-H), 2950w (aliphatic C-H), 2220m (CN), 1720s (ester C=O), 1600m (aromatic C=C) and 1270s (C-O) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.13 (1 H, d, $^3J$= 4.5, 4-H), 9.10 (1 H, s, 3-H), 8.19 (1 H, d, $^3J$= 8.4, 11-H), 8.11 (1 H, d, $^3J$= 7.7, 8-H), 7.95-7.87 (2 H, m, 5-H & 10-H), 7.75 (1H, m, 9-H) and 3.98 (3 H, s, $CH_3$); $\delta_C$ ($D_6$-DMSO) 161.2 ($COOCH_3$), 152.1 (C-3), 150.3 (C-4), 147.8 (C-12), 137.3 (C-6), 130.4 (quin C-H), 129.8 (quin C-H), 127.9 (quin C-H), 124.6 (C-7), 124.3 (quin C-H), 120.3 (C-5), 114.4 (C-1), 110.7 (C-2) and 53.6 ($COOCH_3$); m/z 238 ($M^+$, 37.0), 207 (10.2), 179 (100), 152 (58.6) and 75 (17.2%) (Found: $M^+$, 238.0743; C, 70.46; H, 4.14; N, 11.67%. $C_{14}H_{10}N_2O_2$ requires *M*, 238.0740; C, 70.56; H, 4.23; N, 11.76%).

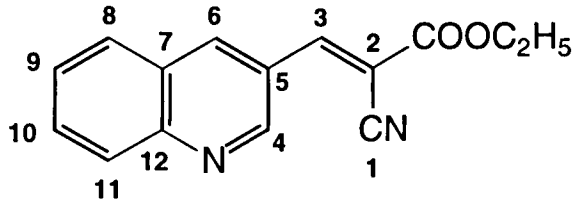
**Ethyl *(E)*-2-cyano-3-(2-quinolinyl)propenoate (52).**




Method A afforded ester **52** as a white powder which was recrystallised to yield cream coloured needles (0.349 g, 69%), m.p. 108-109 °C (from EtOAc); $R_f$ 0.73; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2980w (aliphatic C-H), 2220m (CN), 1730s (ester C=O), 1620m (alkene C=C) and 1250s (C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.47 (1 H, s, 3-H), 8.29 (1 H, d, $^3J$= 8.6, 6-H), 8.22 (1 H, dd, 11-H), 8.05 (1 H, d, $^3J$= 8.5, 5-H), 7.87 (1 H, dd, $^3J$= 8.2, $^4J$= 1.2, 8-H), 7.79 (1 H, ddd, $^4J$= 1.6, 10-H), 7.65 (1 H, ddd, $^4J$= 1.2, 9-H), 4.43 (2 H, q, $^3J$= 7.1, $COOCH_2CH_3$) and 1.43 (3 H, t, $^3J$= 7.1, $COOCH_2CH_3$); $\delta_C$ ($CDCl_3$) 162.0 ($COOCH_2CH_3$), 153.8 (C-3), 149.9 (C-4), 148.2 (C-12), 137.2 (C-6), 130.6

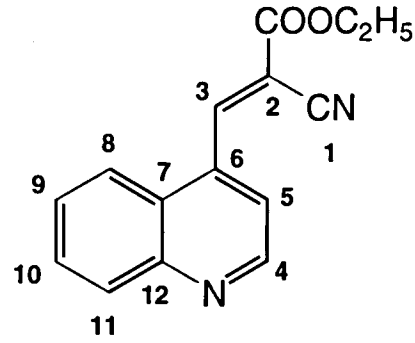
(C-10), 130.3 (C-11), 129.0 (C-9), 128.6 (C-7), 127.6 (C-8), 121.9 (C-5), 114.8 (C-1), 107.8 (C-2), 63.0 ($COOCH_2CH_3$) and 14.2 ($COOCH_2CH_3$); m/z 252 ($M^+$, 3.1), 208 (58.9), 180 (100) and 128 (42.5%) (Found: $M^+$, 252.0903; C, 71.18; H, 4.66; N, 11.03%. $C_{15}H_{12}N_2O_2$ requires *M*, 252.0896; C, 71.40; H, 4.79; N, 11.10%).

**Ethyl *(E)*-2-cyano-3-(3-quinolinyl)propenoate (53).**



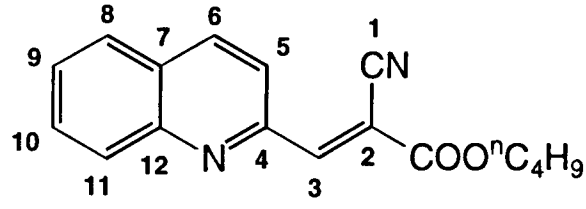

Method A afforded ester **53** as a white powder. Recrystallisation of the product yielded cream coloured needles (0.194 g, 76%), m.p. 173-174 °C (from EtOAc); $R_f$ 0.54; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2980w (aliphatic C-H), 2220m (CN), 1720s (ester C=O), 1605 (aromatic C=C) and 1250 (C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 9.19 (1 H, d, $^4J$= 2.4, 4-H), 9.07 (1 H, d, $^4J$= 2.3, 6-H), 8.42 (1 H, s, 3-H), 8.15 (1 H, dd, $^3J$= 8.4, $^4J$= 1.0, 11-H), 7.98 (1 H, dd, $^3J$= 8.2 $^4J$= 1.4, 8-H), 7.87 (1 H, ddd, $^4J$= 1.5, 10-H), 7.65 (1 H, ddd, $^4J$= 1.2, 9-H), 4.44 (2 H, q, $^3J$= 7.1, $COOCH_2CH_3$) and 1.44 (3 H, t, $^3J$= 7.1, $COOCH_2CH_3$); $\delta_C$ ($CDCl_3$) 162.0 ($COOCH_2CH_3$), 151.7 (C-4), 151.3 (C-3), 149.6 (C-12), 138.0 (C-6), 132.6 (C-10), 129.6 (C-11), 129.4 (C-8), 128.0 (C-9), 127.1 (C-7), 124.6 (C-5), 115.3 (C-1), 105.0 (C-2), 63.1 ($COOCH_2CH_3$) and 14.2 ($COOCH_2CH_3$); m/z 252 ($M^+$, 78.3), 208 (100), 179 (44.0) and 152 (49.5%) (Found: $M^+$, 252.0897; C, 71.29; H, 4.64; N, 10.97%. $C_{15}H_{12}N_2O_2$ requires *M*, 252.0896; C, 71.40; H, 4.79; N, 11.10%).

**Ethyl *(E)*-2-cyano-3-(4-quinolinyl)propenoate (54).**




Method A afforded ester **54** as a white powder. Recrystallisation of the product gave rhombic crystals (0.198 g, 39%), m.p. 97-98 °C (from EtOAc); $R_f$ 0.51; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2230m (CN), 1725s (ester C=O), 1620s (alkene C=C) and 1270s (C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 9.07 (1 H, d, $^3J$= 4.5, 4-H), 8.99 (1 H, s, 3-H), 8.23 (1 H, dd, $^3J$= 8.6, 11-H), 7.98 (1 H, dd, 8-H), 7.95 (1 H, dd, $^3J$= 4.5, 5-H), 7.84 (1 H, ddd, 10-H), 7.69 (1 H, ddd, 9-H), 4.47 (2 H, q, $^3J$= 7.2, $COOCH_2CH_3$) and 1.45 (3 H, t, $^3J$= 7.2, $COOCH_2CH_3$); $\delta_C$ ($CDCl_3$) 161.2 ($COOCH_2CH_3$), 150.7 (C-3), 150.1 (C-4), 148.5 (C-12), 136.3 (C-6), 130.6 (quin C-H), 130.4 (quin C-H), 128.2 (quin C-H), 125.4 (C-7), 122.9 (quin C-H), 120.1 (C-5), 114.1 (C-1), 110.3 (C-2), 63.4 ($COOCH_2CH_3$) and 14.1 ($COOCH_2CH_3$); m/z 252 ($M^+$, 52.0), 207 (16.5), 180 (43.1) and 179 (100%) (Found: C, 71.50; H, 4.76; N, 11.14%. $C_{15}H_{12}N_2O_2$ requires C, 71.40; H, 4.79; N, 11.10%).

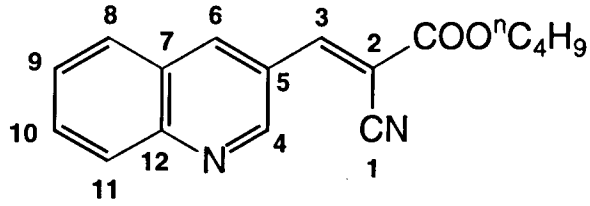
**$^n$Butyl *(E)*-2-cyano-3-(2-quinolinyl)propenoate (55).**




Method A afforded ester **55** as a white powder which was recrystallised to give large rhombic crystals (0.375 g, 45%), m.p. 71-72 °C (from EtOAc); $R_f$ 0.81; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2980w (aliphatic C-H), 2215m (CN), 1720s (ester C=O), 1630w (alkene C=C) and 1280s (C-O) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.60 (1 H, d, $^3J$= 8.5, 6-H), 8.56 (1 H, s, 3-H), 8.15-8.05 (3 H, m, 5-H, 8-H & 11-H), 7.92 (1 H, ddd, $^4J$= 1.4, 10-H), 7.77 (1 H, ddd, $^4J$= 1.2, 9-

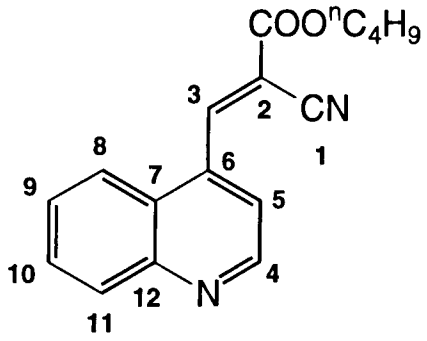
H), 4.35 (2 H, t, $^3J$= 6.6, COOC$H_2$-), 1.75 (2 H, m, COOCH$_2$C$H_2$-), 1.46 (2 H, m, -C$H_2$CH$_3$) and 0.98 (3 H, t, $^3J$= 7.2, C$H_3$); $\delta_C$ ($D_6$-DMSO) 161.9 (*C*OO(CH$_2$)$_3$CH$_3$), 153.6 (C-3), 149.8 (C-4), 148.1 (C-12), 137.0 (C-6), 130.5 (quin C-H), 130.2 (quin C-H), 128.8 (quin C-H), 128.4 (C-7), 127.5 (quin C-H), 121.8 (C-5), 114.6 (C-1), 107.7 (C-2), 66.7 (COO*C*H$_2$-), 30.4 (COOCH$_2$*C*H$_2$-), 18.9 (-*C*H$_2$CH$_3$) and 13.6 (-*C*H$_3$); m/z 280 (*M*$^+$, 10.5), 236 (15.8), 208 (49.8), 180 (100) and 128 (49.6%) (Found: *M*$^+$, 280.1216; C, 72.86; H, 5.81; N, 10.01%. $C_{17}H_{16}N_2O_2$ requires *M*, 280.1208; C, 72.82; H, 5.75; N, 10.00%).

**$^n$Butyl *(E)*-2-cyano-3-(3-quinolinyl)propenoate (56).**



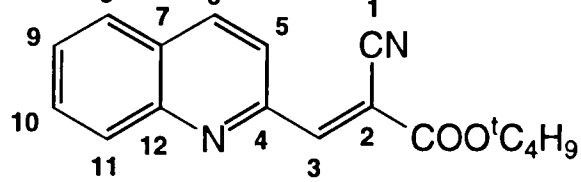

Method A gave ester **56** as a white powder. Recrystallisation of the product gave large rhombic crystals (0.400 g, 71%), m.p. 87-88 °C (from EtOAc); $R_f$ 0.68; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2980w (aliphatic C-H), 2220m (CN), 1720s (ester C=O), 1620s (alkene C=C) and 1280s (C-O) cm$^{-1}$; $\delta_H$ ($D_6$-acetone) 9.40 (1 H, d, $^4J$= 2.3, 4-H), 9.01 (1 H, d, $^4J$= 2.2, 6-H), 8.53 (1 H, s, 3-H), 8.10 (2 H, m, 8-H & 11-H), 7.91 (1 H, ddd, $^4J$= 1.4, 10-H), 7.70 (1 H, ddd, $^4J$= 1.2 9-H), 4.36 (2 H, t, $^3J$= 6.5, COOC$H_2$(CH$_2$)$_2$CH$_3$), 1.75 (2 H, m, COOCH$_2$C$H_2$CH$_2$CH$_3$), 1.49 (2 H, m, COO(CH$_2$)$_2$C$H_2$CH$_3$) and 0.98 (3 H, t, $^3J$= 7.2, COO(CH$_2$)$_3$C$H_3$); $\delta_C$ ($D_6$-acetone) 162.4 (*C*OO(CH$_2$)$_3$CH$_3$), 152.3 (C-3), 151.6 (C-4), 150.1 (C-12), 139.4 (C-6), 133.1 (C-10), 130.2 (C-11), 130.1 (C-8), 128.7 (C-9), 127.8 (C-7), 125.9 (C-5), 116.0 (C-1), 105.7 (C-2), 67.0 (COO*C*H$_2$(CH$_2$)$_2$CH$_3$), 31.2 (COOCH$_2$*C*H$_2$CH$_2$CH$_3$), 19.6 (COO(CH$_2$)$_2$*C*H$_2$CH$_3$) and 13.9 (COO(CH$_2$)$_3$*C*H$_3$); m/z 280 (*M*$^+$, 47.9), 224 (69.6), 207 (61.2), 180 (85.4) and 152 (69.4%) (Found: *M*$^+$, 280.1208; C, 72.93; H, 5.74; N, 10.08%. $C_{17}H_{16}N_2O_2$ requires *M*, 280.1208; C, 72.82; H, 5.75; N, 10.00%).

**$^{n}$Butyl *(E)*-2-cyano-3-(4-quinolinyl)propenoate (57).**




After 24 h reaction time using Method A, TLC indicated the formation of a new product with $R_f$ 0.63. This product was precipitated and purified by column chromatography to yield ester **57** as a fawn coloured solid (0.370 g, 53%), m.p. 42-43 °C; $R_f$ 0.63; $\nu_{max}$ (KBr) 3060w (unsaturated C-H), 2961w (aliphatic C-H), 2231m (CN), 1724s (ester C=O) and 1273s (C-O) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 9.07 (1 H, d, $^3J$= 4.5, 4-H), 8.99 (1 H, s, 3-H), 8.24 (1 H, d, $^3J$= 8.5, 11-H), 8.00-7.95 (2 H, m, 5-H and 8-H), 7.81 (1 H, m, 10-H), 7.68 (1 H, m, 9-H), 4.40 (2 H, t, $^3J$= 6.6, COOC*H*$_2$-), 1.76 (2 H, m, COOCH$_2$C*H*$_2$-), 1.50 (2 H, m, -C*H*$_2$CH$_3$) and 1.00, (3 H, t, $^3J$= 7.2, C*H*$_3$); $\delta_C$ ($CDCl_3$) 161.3 (C=O), 150.7 (C-4), 150.2 (C-3), 148.6 (C-12), 136.2 (C-6), 130.7 (quin C-H), 130.3 (quin C-H), 128.2 (quin C-H), 125.4 (C-7), 122.9 (quin C-H), 120.1 (C-5), 114.1 (C-1), 110.3 (C-2), 67.2 (COO*C*H$_2$-), 30.5 (COOCH$_2$*C*H$_2$-), 19.1 (-*C*H$_2$CH$_3$) and 13.7 (*C*H$_3$); m/z 280 ($M^+$, 9.2), 224 (21.1), 207 (22.9), 179 (89.7) and 152 (68.2%) (Found: $M^+$, 280.1212. $C_{17}H_{16}N_2O_2$ requires *M*, 280.1208).

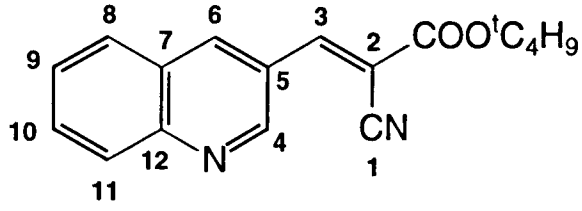
**$^{t}$Butyl *(E)*-2-cyano-3-(2-quinolinyl)propenoate (58).**




Method A afforded ester **58** as a cream precipitate which was recrystallised to give large rhombic crystals (0.432 g, 70%), m.p. 169-170 °C (from EtOAc); $R_f$ 0.84 (EtOAc); $\nu_{max}$ (KBr) 3060w (unsaturated C-H.), 2970w (aliphatic C-H), 2220m (CN), 1734s (ester C=O) and 1280s (C-O) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 8.39 (1 H, s, 3-H), 8.29 (1 H, d, $^3J$= 8.6, 6-H), 8.20 (1 H, d, $^3J$= 8.3, 11-H), 8.07 (1 H, d, $^3J$= 8.5, 5-H), 7.87 (1 H, d, $^3J$= 8.1, 8-H), 7.80 (1 H, m, $^4J$= 1.6, 10-H), 7.64 (1 H, m, $^4J$= 1.2, 9-H) and 1.62 (9 H, s, C(C*H*$_3$)$_3$); $\delta_C$ ($CDCl_3$) 160.7

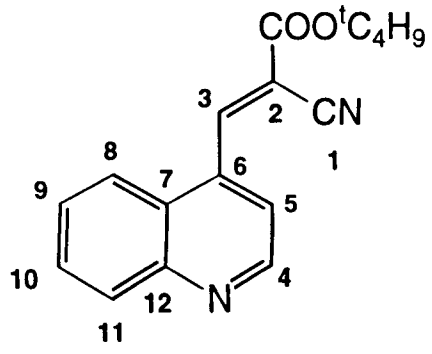
(*C*OOC($CH_3$)$_3$), 153.0 (C-3), 150.1 (C-4), 148.2 (C-12), 137.1 (C-6), 130.5 (C-10), 130.2 (C-11), 128.7 (C-9), 128.4 (C-7), 127.5 (C-8), 121.7 (C-5), 115.0 (C-1), 109.5 (C-2), 84.2 (COO*C*($CH_3$)$_3$) and 27.9 (COOC(*C*$H_3$)$_3$); m/z 280 ($M^+$, 5.0), 236 (9.6), 208 (38.5), 180 (100) and 128 (78.2%) (Found: $M^+$, 280.1213; C, 72.87; H, 5.84; N, 10.03%. $C_{17}H_{16}N_2O_2$ requires *M*, 280.1208; C, 72.82; H, 5.75; N, 10.00%).

**$^t$Butyl *(E)*-2-cyano-3-(3-quinolinyl)propenoate (59).**



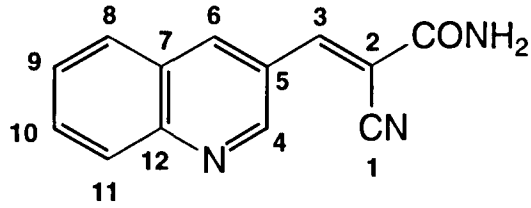

Method A gave ester **59** as a cream precipitate which was recrystallised, giving large rhombic crystals (1.104 g, 78%), m.p. 125-126 °C (from EtOAc); $R_f$ 0.67; $\nu_{max}$ (KBr) 3051w (unsaturated C-H), 2982m (aliphatic C-H), 2228m (CN), 1709s (ester C=O) and 1608m (aromatic C=C) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 9.13 (1 H, d, $^4J$= 2.3, 4-H), 8.96 (1 H, d, $^4J$= 2.3, 6-H), 8.28 (1 H, s, 3-H), 8.09 (1 H, d, $^3J$= 8.5, 11-H), 7.92 (1 H, d, $^3J$= 8.2, 8-H), 7.81 (1 H, ddd, $^4J$= 1.5, 10-H), 7.60 (1 H, ddd, $^4J$= 1.1, 9-H) and 1.58 (9 H, s, C(C$H_3$)$_3$); $\delta_C$ ($CDCl_3$) 160.6 (*C*OOC($CH_3$)$_3$), 151.5 (C-4), 150.2 (C-3), 149.3 (C-12), 137.7 (C-6), 132.3 (C-10), 129.4 (C-11), 129.3 (C-8), 127.8 (C-9), 127.0 (C-7), 124.6 (C-5), 115.4 (C-1), 106.6 (C-2), 84.2 (COO*C*($CH_3$)$_3$) and 27.9 (COOC(*C*$H_3$)$_3$); m/z 280 ($M^+$, 7.1), 224 (39.2), 207 (38.3), 180 (60.0) and 152 (56.6%) (Found: $M^+$, 280.1218; C, 73.01; H, 5.79; N, 10.04%. $C_{17}H_{16}N_2O_2$ requires *M*, 280.1208; C, 72.82; H, 5.75; N, 10.00%).

**$^t$Butyl *(E)*-2-cyano-3-(4-quinolinyl)propenoate (60).**



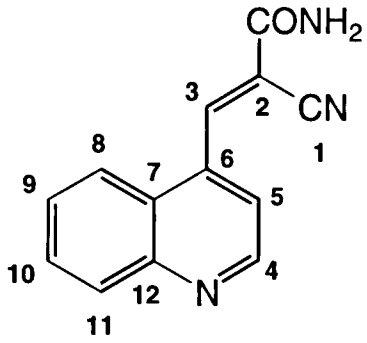
Method A afforded ester **60** as a cream precipitate which was recrystallised, giving large rhombic crystals (1.188 g, 85%), m.p. 110-111 °C (from EtOAc); $R_f$ 0.61; $\nu_{max}$ (KBr disc) 3065w (unsaturated C-H), 2991m (aliphatic C-H), 2229m (CN), 1714s (ester C=O) and 1618m (alkene C=C) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 9.03 (1 H, d, $^3J$= 4.5, 4-H), 8.87 (1 H, s, 3-H), 8.17 (1 H, d, $^3J$= 8.3, 11-H), 7.97-7.89 (2 H, m, 5-H & 8-H), 7.78 (1 H, m, 10-H), 7.63 (1 H, m, 9-H) and 1.61 (9 H, s, $C(CH_3)_3$); $\delta_C$ ($CDCl_3$) 159.9 (C=O), 150.1 (C-4), 149.7 (C-3), 148.5 (C-12), 136.4 (C-6), 130.6 (quin C-H), 130.2 (quin C-H), 128.0 (quin C-H), 125.4 (C-7), 122.9 (quin C-H), 120.0 (C-5), 114.2 (C-1), 111.8 (C-2), 84.8 ($C(CH_3)_3$) and 27.9 ($C(CH_3)_3$); m/z 280 ($M^+$, 11.0), 224 (21.8), 207 (35.1), 179 (69.9) and 152 (50.3%) (Found: $M^+$, 280.1217; C, 73.00; H, 5.81; N, 9.98%. $C_{17}H_{16}N_2O_2$ requires *M*, 280.1208; C, 72.82; H, 5.75; N, 10.00%).

***(E)*-2-cyano-3-(3-quinolinyl)propenamide (62).**



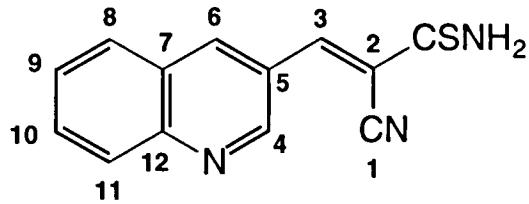

Method A afforded amide **62** as a white solid (0.273, 81%), m.p. 245 °C (d.); $R_f$ 0.46 (EtOH); $\nu_{max}$ (KBr) 3100m ($NH_2$), 2225m (CN), 1710s (amide I), 1640s (amide II), 1585s and 1500m (aromatic C=C) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.32 (1 H, d, $^4J$= 2.2, 4-H), 8.95 (1 H, d, $^4J$= 2.0, 6-H), 8.45 (1 H, s, 3-H), 8.16-8.11 (2 H, m, 8-H & 11-H), 8.10-7.90 (3 H, m, 10-H & $NH_2$) and 7.57 (1 H, m, $^4J$= 1.1, 9-H); $\delta_C$ ($D_6$-DMSO) 162.3 ($CONH_2$), 150.5 (C-4), 148.3 (C-12), 147.8 (C-3), 137.5 (C-6), 132.0 (quin C-H), 129.3 (quin C-H), 128.9 (quin C-H), 127.8 (quin C-H), 126.7 (C-7), 125.5 (C-5), 116.3 (C-1) and 108.7 (C-2); m/z 223 ($M^+$, 76.6), 222 (100), 206 (60.0), 179 (27.9) and 152 (35.8%) (Found: $M^+$, 223.0731; C, 69.92; H, 3.84; N, 18.77%. $C_{13}H_9N_3O$ requires *M*, 223.0744; C, 69.93; H, 4.07; N, 18.83%).

***(E)*-2-Cyano-3-(4-quinolinyl)propenamide (63).**




Method A yielded amide **63** as a white solid (0.354 g, 79%), m.p. 195 °C (d.); $R_f$ 0.47 (EtOAc); $\nu_{max}$ (KBr) 3439s, 3400m ($NH_2$), 2218m (CN), 1701s (amide I), 1608m, 1583m and 1504s (aromatic C=C) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.11 (1 H, d, $^3J$= 4.4, 4-H), 8.87 (1 H, s, 3-H), 8.36 (1 H, br s, $NH_2$), 8.20 (2 H, m, 8-H & 11-H), 8.04 (1 H, br s, $NH_2$), 7.92 (2 H, m, 5-H & 10-H) and 7.72 (1 H, m, 9-H); $\delta_C$ ($D_6$-DMSO) 162.1 (*C*$ONH_2$), 150.5 (C-4), 147.9 (C-12), 146.8 (C-3), 138.0 (C-6), 130.4 (quin C-H), 129.9 (quin C-H), 127.9 (quin C-H), 125.0 (C-7), 124.4 (quin C-H), 120.4 (C-5), 115.3 (C-1) and 114.9 (C-2); m/z 223 ($M^+$, 100), 222 (41.8), 206 (18.1), 194 (9.4), 179 (58.9) and 153 (37.1%) (Found: $M^+$, 223.0741; C, 69.86; H, 3.99; N, 18.95%. $C_{13}H_9N_3O$ requires *M*, 223.0744; C, 69.93; H, 4.07; N, 18.83%).

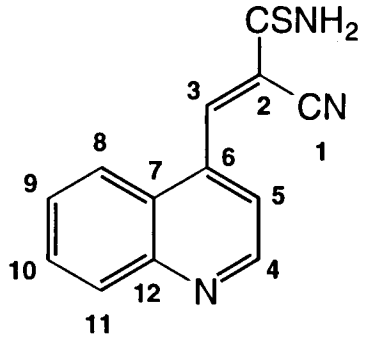
***(E)*-2-Cyano-3-(3-quinolinyl)propenethioamide (65).**




Method A afforded thioamide **65** as an orange solid which recrystallised to give needles (0.311 g, 65%), m.p. 179 °C (from EtOAc, d.); $R_f$ 0.25; $\nu_{max}$ (KBr) 3312s ($CSNH_2$), 3022w (unsaturated C-H), 2210m (CN), 1616 (amide II), 1491 (aromatic C=C) and 1269 (amide I) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 10.31 and 9.99 (2 H, 2 br s, $CSNH_2$), 9.32 (1 H, d, $^4J$= 2.2, 4-H), 9.01 (1 H, d, $^4J$= 2.2, 6-H), 8.44 (1 H, s, 3-H), 8.15-8.11 (2 H, m, 8-H & 11-H), 7.94 (1 H, ddd, 10-H) and 7.74 (1 H, ddd, 9-H); $\delta_C$ ($D_6$-DMSO) 194.4, (C=S), 150.7 (C-4), 148.1 (C-12), 143.2 (C-3), 137.4 (C-6), 131.9 (quin C-H), 129.2 (quin C-H), 128.9 (quin C-H), 127.8 (quin C-H), 126.7 (C-7), 125.5 (C-5), 116.2 (C-1) and 114.3 (C-2); m/z 239 ($M^+$, 100), 206 (63.6), 179 (30.1), 152 (37.5) and 75

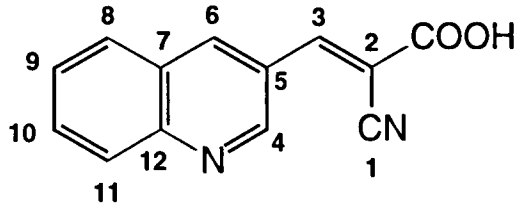
(54.8%) (Found: $M^+$, 239.0514; C, 65.02; H, 3.98; N, 17.34%. $C_{13}H_9N_3S$ requires $M$, 239.0516; C, 65.25; H, 3.79; N, 17.57%).

**(*E*)-2-Cyano-3-(4-quinolinyl)propenethioamide (66).**




Method A afforded thioamide **66** as an orange powder (0.334 g, 69%), m.p. 189 °C (d.); $R_f$ 0.26; $\lambda_{max}$ (EtOH)/nm 212 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 81600), 251 (32700) and 327 (16700); $\nu_{max}$ (KBr) 3355s ($CSNH_2$), 3017w (unsaturated C-H), 2213m (CN), 1650 (amide II), 1621 (alkene C=C), 1503 (aromatic C=C), 1263 (amide I) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 10.59 and 10.44 (2 H, 2 br s, $CSNH_2$), 9.10 (1 H, d, $^3J$= 4.2, 4-H), 8.70 (1 H, s, 3-H), 8.28 (1 H, d, $^3J$= 8.2, 11-H), 8.17 (1 H, d, $^3J$= 8.2, 8-H), 7.94-7.87 (2 H, m, 5-H & 10-H) and 7.76 (1 H, ddd, 9-H); $\delta_C$ ($D_6$-DMSO) 192.5 (C=S), 150.5 (C-4), 147.9 (C-12), 142.0 (C-3), 138.2 (C-6), 130.4 (C-H), 129.9 (C-H), 127.8 (C-H), 125.3 (C-7), 124.7 (C-H), 120.4 (C-5), 119.5 (C-2) and 115.3 (C-1); m/z 239 ($M^+$, 100.0), 206 (29.6), 179 (27.6) and 152 (22.3%) (Found: $M^+$, 239.0508; C, 65.32; H, 3.91; N, 17.43%. $C_{13}H_9N_3S$ requires $M$, 239.0516 C, 65.27; H, 3.77; N, 17.57%).

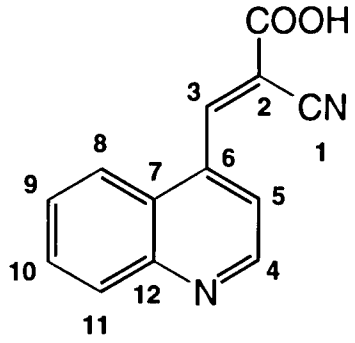
**(*E*)-2-Cyano-3-(3-quinolinyl)propenoic acid (69).**




Using ester **59** as the starting material, Method B afforded acid **69** as a white powder (0.524, 83%), m.p. 198 °C (d.); $R_f$ 0.70 (EtOH); $\nu_{max}$ (KBr) 3400-3200s (COOH), 3030w (unsaturated C-H), 2224m (CN), 1707s (acid C=O), 1610s and 1496s (aromatic C=C) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.41 (1 H, d, $^4J$= 2.2, 4-H), 9.05 (1 H, d, $^4J$= 2.2, 6-H), 8.60 (1 H, s, 3-H), 8.18-8.16 (2 H, m, 8-H &

11-H), 7.97 (1 H, m, 10-H) and 7.76 (1 H, m, 9-H); $\delta_C$ ($D_6$-DMSO) 163.0 (C=O), 151.6 (C-3), 150.7 (C-4), 148.3 (C-12), 138.8 (C-6), 132.7 (quin C-H) 129.6 (quin C-H), 128.8 (quin C-H), 128.2 (quin C-H), 126.8 (C-7), 125.2 (C-5), 116.1 (C-1) and 106.0 (C-2); m/z 224 ($M^+$, 33.2),180 (100.0), 153 (50.7), 129 (32.9) and 75 (28.6%) (Found: $M^+$, 224.0579. $C_{13}H_8N_2O_2$ requires *M*, 224.0584).

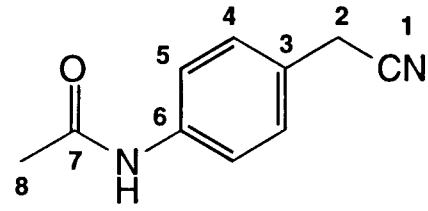
***(E)*-2-Cyano-3-(4-quinolinyl)propenoic acid (70).**




Using ester **60** as the starting material, Method B afforded acid **70** as a white powder (0.374 g, 75%), m.p. 200 °C (d.); $R_f$ 0.70 (EtOH); $\nu_{max}$ (KBr) 3400-3300s (COOH), 3038w (unsaturated C-H), 2224m (CN), 1701s (acid C=O), 1610s and 1499m (aromatic C=C) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.05 (1 H, d, 4-H), 9.01 (1 H, s, 3-H), 8.16 (1 H, d, $^3J$= 8.3, 11-H), 8.08 (1 H, d, $^3J$= 8.2, 8-H), 7.93-7.86 (2 H, m, 5-H & 10-H) and 7.74 (1 H, m, 9-H); $\delta_C$ ($D_6$-DMSO) 162.3 (C=O), 151.1 (C-3), 150.4 (C-4), 147.8 (C-12), 137.7 (C-6), 130.5 (quin C-H), 129.8 (quin C-H), 128.0 (quin C-H), 124.8 (C-7), 124.3 (quin C-H), 120.4 (C-5), 115.0 (C-1) and 112.3 (C-2); m/z 224 ($M^+$, 7.8), 180 (58.6), 179 (100), 153 (33.4) and 75 (40.1%) (Found: $M^+$, 224.0592. $C_{13}H_8N_2O_2$ requires 224.0584).

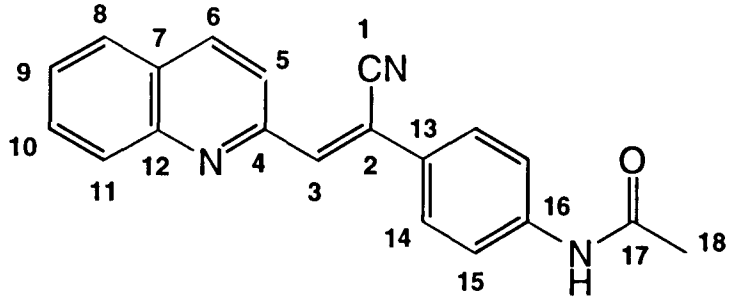
## 7.3 Experimental to Chapter 4

### *N*-[4-(cyanomethyl)phenyl]acetamide (73).



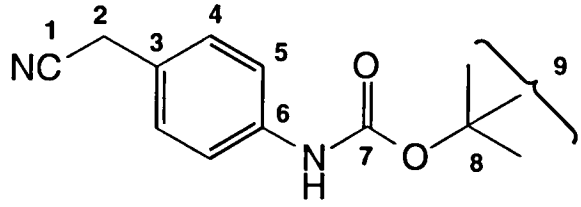

The title compound was prepared using conditions described for the acetylation of aniline.[92] 4-Aminobenzyl cyanide (0.733 g, 5.5 mmol), water (12.5 ml) and conc. HCl (35%, 0.45 ml) were stirred together until the solid dissolved. Acetic anhydride (0.639 g, 6.3 mmol) was added with stirring until a heterogeneous mixture was obtained. Immediately, a solution of sodium acetate (0.825 g, 12 mmol) in water (2.5 ml) was added. The mixture was stirred vigorously and then cooled in ice. A cream precipitate formed which was filtered off, washed with petroleum ether and dried under suction giving amide **73** (0.910 g, 95%), m.p. 84-85 °C (lit.,[93] 85-87 °C), $R_f$ 0.58 (EtOAc); $\nu_{max}$ (KBr) 3345, 3318 (NH), 3055, 2960, 2247 (unconjugated CN), 1669 (amide I), 1600 (aromatic C=C), 1523 (amide II) and 814 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-acetone) 9.37 (1 H, br s, N-H), 7.68 (2 H, AA'BB', 5-H), 7.29 (2 H, AA'BB', 4-H), 3.88 (2 H, s, 2-H) and 2.08 (3 H, s, 8-H); $\delta_C$ ($D_6$-acetone) 168.5 (C=O), 138.9 (C-6), 128.6 (C-4), 125.6 (C-3), 119.5 (C-5), 114.7 (CN), 24.1 (C-8) and 21.9 (C-2); *m/z* 174 ($M^+$, 26.3), 132 (100), 104 (11.6) and 77 (11.1%) (Found: $M^+$, 174.0794. $C_{10}H_{10}N_2O$ requires *M*, 174.0791).

### Condensation product (74) of 2-quinolinecarbaldehyde with compound 73.



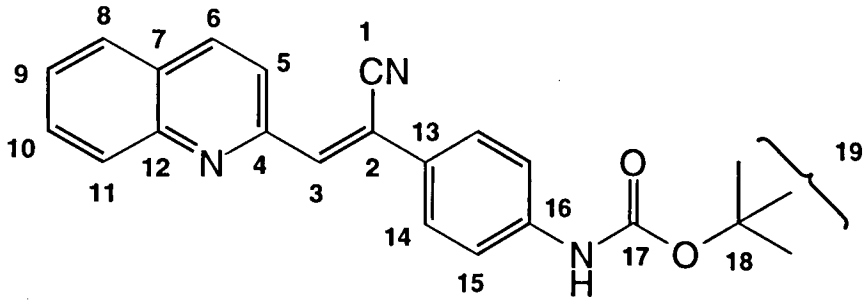
Compound **73** (0.255 g, 1.46 mmol) was dissolved in EtOH (2 ml) and added to a stirred solution of 2-quinolinecarbaldehyde (0.236 g, 1.5 mmol) in EtOH (2 ml). After 30 min the cream precipitate was filtered off, washed with petroleum ether and dried under suction. Amide **74** (0.338 mg, 74%) was obtained as a white powder, m.p. 193-195 °C; $R_f$ 0.49 (EtOAc); $\nu_{max}$ (KBr) 3056, 2925, 1667 (amide I), 1588 (C=C), 1514 (amide II), 836 (*para*-disubstituted benzene) and 755 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 10.26 (1 H, br s, N-H), 8.45 (1 H, d, $^3J$= 8.5, 6-H), 8.08-7.96 (3 H, m), 7.87-7.72 (6 H, m), 7.63 (1 H, ddd, 9-H) and 2.08 (3 H, s, 18-H); $\delta_C$ ($D_6$-DMSO) 168.7 (C-17), 152.0 (C-4), 147.1 (C-12), 140.9 (C-16), 138.7 (C-3), 137.0 (C-6), 130.4 (quin C-H), 128.8 (quin C-H), 128.0 (C-7), 127.8 (quin C-H), 127.5 (C-13), 127.5 (quin C-H), 126.9 (C-14), 122.6 (C-5), 119.1 (C-15), 117.3 (C-2), 114.2 (CN) and 24.1 (C-18); *m/z* 313 (*M*+, 93.9), 270 (100), 255 (18.3) and 243 (11.2%) (Found: *M*+, 313.1185. $C_{20}H_{15}N_3O$ requires *M*, 313.1212).

***N*-$^t$Butoxycarbonyl-4-cyanomethylaniline (75).**



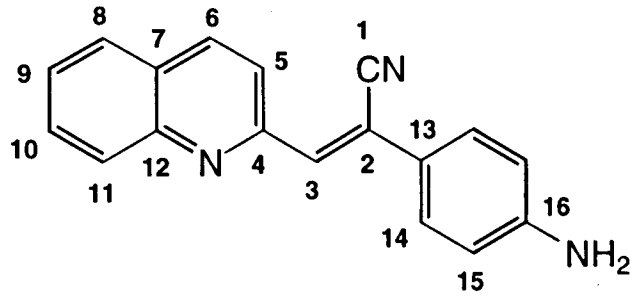

4-Aminobenzyl cyanide (0.66 g, 5 mmol) and $(BOC)_2O$ (1.2 g, 5.5 mmol) were stirred together in dioxane-water (1:1, 6 ml) for 18 h. Ethanol (10 ml) was added and the solvents removed under reduced pressure to give carbamate **75** (1.160 g, 99%), m.p. 117.5-118.5 °C; $R_f$ 0.46; $\nu_{max}$ (KBr) 3388 (N-H), 2983, 2248 (unconjugated CN), 1700 (carbamate C=O), 1597 (C=C), 1238 (carbamate C-O) and 820 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.44 (1 H, br s, N-H), 7.50 (2 H, AA'BB', $^3J$= 8.6, 5-H) 7.25 (2 H, AA'BB', $^3J$= 8.6, 4-H), 3.96 (2 H, s, 2-H) and 1.51 (9 H, s, 9-H); $\delta_C$ ($D_6$-DMSO) 152.7 (C-7), 139.1 (C-6), 128.5 (C-4), 124.5 (C-3), 118.5 (C-5), 114.9 (C-1), 79.2 (C-8), 28.2 (C-9) and 21.8 (C-2); *m/z* 232 (*M*+, 6.0), 176 (30.7), 132 (34.7) and 104 (6.7%) (Found: *M*+, 232.1217; C, 67.17; H, 7.09; N, 12.13%. $C_{13}H_{16}N_2O_2$ requires *M*, 232.1208; C, 67.20; H, 6.95; N, 12.07%).

**Condensation product (76) of 2-quinolinecarbaldehyde with compound 75.**




Carbamate **75** (0.464 g, 2 mmol) was dissolved in EtOH (2 ml) and added to a stirred solution of 2-quinolinecarbaldehyde (0.314 g, 2 mmol) in EtOH (2 ml). KO$^t$Bu (0.224 g, 2 mmol) was added and after 5 min a light brown precipitate formed. This was filtered off, washed with petroleum ether and dried under suction to give carbamate **76** (0.527 g, 71%) as a beige powder, m.p. 180-182 °C; $R_f$ 0.85 (EtOAc); $\nu_{max}$ (KBr) 3058, 2975, 1715 (carbamate C=O), 1588 (C=C), 1522, 1231 (carbamate C-O), 1158, 832 (*para*-disubstituted benzene) and 756 (*ortho*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.77 (1 H, br s, N-H), 8.50 (1 H, d, $^3J$= 8.5, 6-H), 8.12 (1 H, s, 3-H), 8.10-8.00 (2 H, m), 7.93-7.82 (4 H, m), 7.73-7.65 (3 H, m) and 1.53 (9 H, s, 19-H); $\delta_C$ ($D_6$-DMSO) 152.7 (C-17), 152.2 (C-4), 147.3 (C-12), 141.5 (C-16), 138.4 (C-3), 137.1 (C-6), 130.5 (quin C-H), 128.9 (quin C-H), 128.0 (quin C-H), 127.7 (quin C-H), 127.6 (C-7 or C-13), 127.3 (C-13 or C-7), 127.1 (C-14), 122.7 (C-5), 118.3 (C-15), 117.5 (C-2), 114.4 (CN), 79.7 (C-18) and 28.2 (C-19); *m/z* 371 ($M^+$, 4.5), 315 (23.1), 297 (36.6), 270 (100) and 242 (15.7%) (Found: $M^+$, 371.1598; $C_{23}H_{21}N_3O_2$ requires *M*, 371.1629).

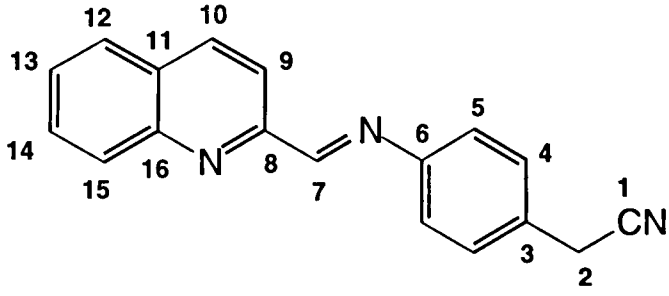
**2-(4-Aminophenyl)-3-(2-quinolinyl)propenenitrile (77).**




Carbamate **76** (0.216 g, 0.6 mmol) was dissolved in EtOAc (10 ml) and HCl gas was bubbled through the solution for 30 min. The orange precipitate

was filtered off, dissolved in water and treated with NaOH solution (1M) to pH 8. The aqueous solution was extracted with EtOAc (3 x 25 ml), dried and the organic solvents were removed under reduced pressure to give crude compound **77** (0.142 g, 90%). The $^1$H NMR showed the presence of a small amount (<5%) of **76** in this crude mixture. This compound was used in a further experiment without further characterisation. The NMR spectra are quoted for the deprotected amine **77**; m.p (crude, 174-175 °C), $R_f$ 0.46 (EtOAc); $\delta_H$ ($D_6$-acetone) 8.25 (1H, d, $^3J$= 8.6, 6-H), 7.96 (1 H, dd, 11-H), 7.83 (1 H, dd, 8-H), 7.79 (1 H, d, $^3J$= 8.6, 5-H), 7.65 (2H, m, 3-H & 10-H), 7.49 (3H, m, 14-H & 9-H), 6.67 (2H, d, $^3J$= 8.7, 15-H) and 5.16 (2 H, br s, $NH_2$); $\delta_C$ ($D_6$-acetone) 153.9 (C-16), 151.6 (C-4), 148.6 (C-12), 137.4 (C-3 or C-6), 135.5 (C-6 or C-3), 131.1 (C-7), 130.8 (quin C-H), 130.1 (quin C-H), 128.6 (quin C-H), 128.5 (C-14), 128.0 (quin C-H), 123.0 (C-13), 122.7 (C-5), 116.6 (C-2), 115.1 (C-15) and 114.7 (CN).

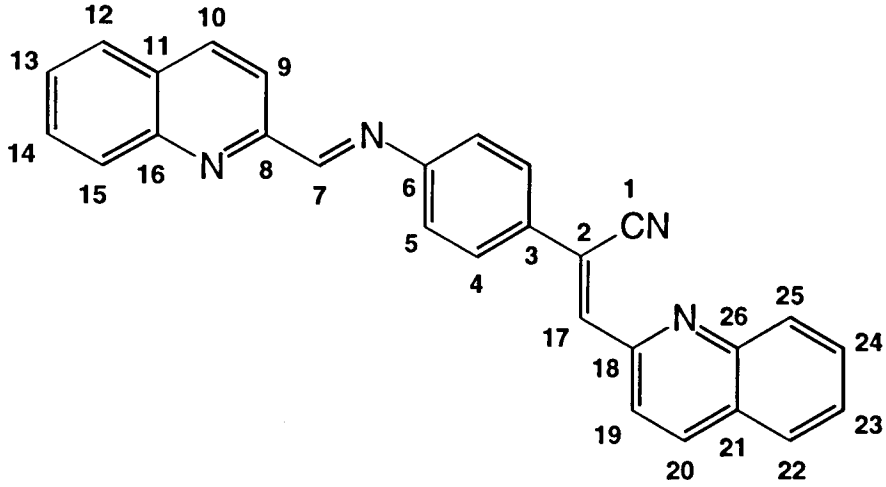
***N*-[(4-cyanomethyl)phenyl]-2-quinolinealdimine (78).**




2-Quinolinecarbaldehyde (0.470 g, 3 mmol) and 4-aminobenzyl cyanide (0.396 g, 3 mmol) were dissolved in EtOH (3 ml) with stirring. Within 5 minutes a brown precipitate formed which was recovered and recrystallised from EtOAc to afford nitrile **78** (0.523 g, 65%) as brown plates, m.p. 131-132 °C; $R_f$ 0.58 (EtOAc); $\nu_{max}$ (KBr) 3063 (aromatic C-H), 2943 (aliphatic C-H), 2246 (unconjugated CN), 1624 (C=N), 1593, 1501 (aromatic C=C), 1417 ($CH_2$ deformation), 834 (*para*-disubstituted benzene) and 759 (*ortho*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 8.75 (1 H, s, 7-H), 8.32 (1 H, d, $^3J$= 8.5, 10-H), 8.20 (1 H, d, $^3J$= 8.6, 9-H), 8.17 (1 H, dd, $^3J$= 8.5, 15-H), 7.82 (1 H, dd, 12-H), 7.71 (1 H, ddd, 14-H), 7.57 (1 H, ddd, $^4J$= 1.1, 13-H), 7.34 (4 H, m, 4-H & 5-H) and 3.75 (2 H, s, 2-H); $\delta_C$ ($CDCl_3$) 161.5 (C-7), 154.5 (C-6), 150.7 (C-8), 147.9 (C-16), 136.7 (C-10), 130.0 (quin C-H), 129.8 (quin C-H), 128.9 (C-4), 128.3 (C-11), 127.9 (quin C-H), 127.8 (quin C-H), 121.9 (C-5), 118.6 (C-9), 117.8 (C-3) 115.2 (CN) and 23.2 (C-2); *m/z* 271 ($M^+$, 97.6), 243 (100), 204 (23.7), 155 (25.7) and 129 (74.0%) (Found: $M^+$, 271.1097; C, 79.54; H,

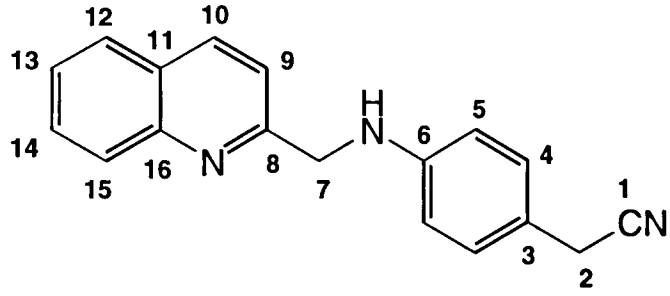
4.92; N, 15.53%. $C_{18}H_{13}N_3$ requires *M*, 271.1107; C, 79.67; H, 4.83; N, 15.50%).

**Condensation product (79) of 2-quinolinecarbaldehyde with 78.**



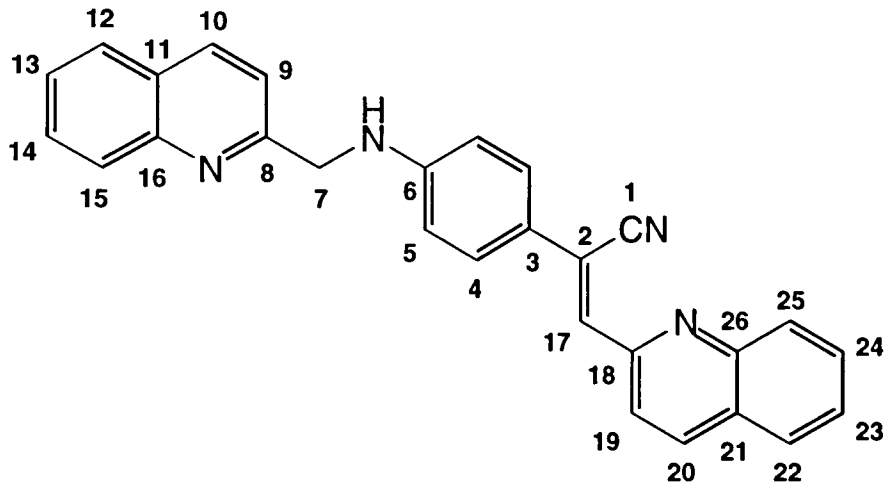

2-Quinolinecarbaldehyde (0.118 g, 0.75 mmol) was dissolved in EtOH (2 ml) with stirring. A solution of nitrile **78** (0.203 g, 0.75 mmol) in EtOH (2 ml) was added, followed by an equivalent of $^t$BuOK (84 g, 0.75 mmol) in EtOH (2 ml). After stirring for 30 min a solid had precipitated which was filtered, washed and dried to afford nitrile **79** (0.217 g, 71%) as a yellow powder, m.p. 145 °C (d.); $R_f$ 0.50 (EtOAc); $\lambda_{max}$ (EtOH)/nm 205 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 85300), 233 (66400) and 370 (41000); $\nu_{max}$ (KBr) 3042 (unsaturated C-H), 2214 (*α,β*-unsaturated CN), 1616 (C=N), 1504 (C=C), 834 (*para*-disubstituted benzene) and 752 (*ortho*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 8.70 (1 H, s, 7-H), 8.36 (2 H, 2 d, 10-H & 20-H), 8.23 (1 H, d, $^3J$= 8.6, 9-H), 8.04 (2 H, 2 dd, 15-H & 25-H), 8.01 (1 H, s, 17-H), 7.95-7.85 (2 H, m, 12-H & 22-H), 7.92 (2 H, AA'BB', $^3J$= 8.8, 4-H), 7.85 (1 H, d, $^3J$= 8.6, 19-H), 7.76-7.66 (2 H, 2 ddd, 14-H & 24-H), 7.62-7.50 (2 H, 2 ddd, 13-H & 23-H) and 7.45 (2 H, AA'BB', $^3J$= 8.8, 5-H); $\delta_C$ ($CDCl_3$) 161.9 (C-7), 154.2 (C-6), 151.6 (C-8 or C-18), 151.3 (C-18 or C-8), 147.3 (C-16 or C-20), 147.2 (C-20 or C-16), 140.3 (C-17), 137.3 (C-10 or C-20), 135.3 (C-20 or C-10), 130.5, 129.4, 129.0 (all C-H), 128.7 (C-11), 128.3, 128.1, 127.8, 127.6, 126.1, 123.1, 122.4, 121.8, 118.4 (all C-H) 117.2 (C-2) and 113.9 (CN); *m/z* 410 (*M*$^+$, 21.8), 270 (100), 253 (18.3), 242 (10.4) and 135 (16.8%) (Found: *M*$^+$, 410.1525. $C_{28}H_{18}N_4$ requires *M*, 410.1528)

***N*-[(4-cyanomethyl)phenyl]-2-quinolinemethylamine (80).**



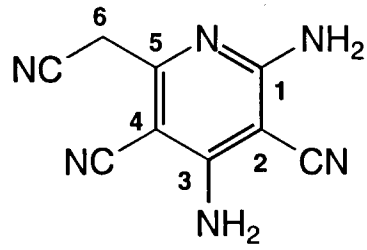

The conditions for the borohydride reduction were the same as those employed by Moriarty *et al.*[95] Nitrile **78** (0.540 g, 2 mmol) was dissolved in dry MeOH (5 ml) and the solution stirred at 0 °C under $N_2$. $NaBH_4$ (0.756 g, 20 mmol) was added in small quantities over a period of 10 min and the reaction was allowed to stir for a further 2 h. After this time the mixture was diluted with brine (15 ml) and extracted with $CHCl_3$ (3 x 10 ml). After drying, the solvent was removed under reduced pressure to afford nitrile **80** (0.432 g, 79%) as a fawn coloured solid, m.p. 79-81 °C; $R_f$ 0.88 (EtOAc); $\nu_{max}$ (KBr) 3348 (N-H), 3054 (aromatic C-H), 2912 (aliphatic C-H), 2244 (unconjugated CN), 1614, 1522 (aromatic C=C), 824 (*para*-disubstituted benzene) and 748 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.33 (1 H, d, $^3J$= 8.5, 10-H), 8.04 (1 H, dd, $^3J$= 8.4, 15-H), 7.97 (1 H, dd, $^3J$= 8.1, 12-H), 7.79 (1 H, ddd, 14-H), 7.59 (1 H, ddd, 13-H), 7.56 (1 H, d, $^3J$= 8.5, 9-H), 7.05 (2 H, dd, AA'BB', 4-H), 6.67 (3 H, m, N-H & 5-H), 4.57 (2 H, d, $^3J$= 6.1, 7-H) and 3.80 (2 H, s, 2-H); $\delta_C$ ($D_6$-DMSO) 160.7 (C-6), 147.9 (C-8), 147.1 (C-16), 136.7 (C-10), 129.6 (quin C-H), 128.9 (C-4), 128.4 (quin C-H), 127.9 (quin C-H), 127.0 (C-11), 126.1 (quin C-H), 119.6 (C-9), 117.9 (CN), 112.6 (C-5), 49.1 (C-7) and 21.5 (C-2); *m/z* 273 ($M^+$, 40.8), 157 (40.3), 145 (74.3), 129 (84.7) and 116 (97.7%) (Found: $M^+$, 273.1271. $C_{18}H_{15}N_3$ requires *M*, 273.1263).

**Condensation product (81) of 2-quinolinecarbaldehyde with 80.**



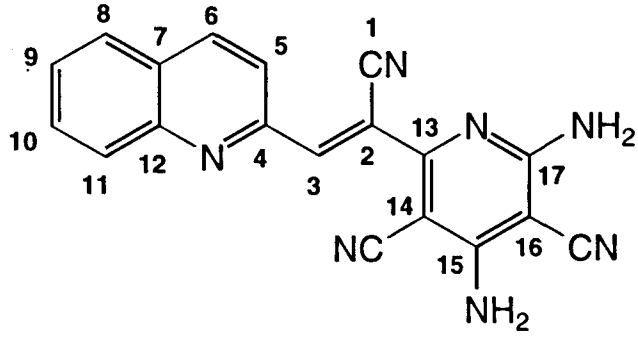

Nitrile **80** (0.165 g, 0.6 mmol) was dissolved in EtOH (2 ml) with stirring. To this solution was added $^{t}$BuOK (67 mg, 0.6 mmol) in EtOH (2 ml) and, after stirring for 1 min, 2-quinolinecarbaldehyde (94 mg, 0.6 mmol) in EtOH (1 ml). After 2 h, TLC indicated formation of a new product. The reaction solution was adsorbed onto silica and was purified by column chromatography with petroleum ether-EtOAc (2:1) as eluent to give nitrile **81** (83 mg, 34%) as a yellow solid, m.p. 214 °C (d.); $R_f$ 0.86 (EtOAc); $\nu_{max}$ (KBr) 3387 (N-H), 3054 unsaturated C-H), 2961 (aliphatic C-H), 2213 (conjugated CN), 1608, 1522 (C=C), 823 (*para*-disubstituted benzene) and 755 (*ortho*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.35 (1 H, d, $^3J$= 8.5, 10-H or 20-H), 8.25 (1 H, d, $^3J$= 8.5, 20-H or 10-H), 8.00-7.86 (4 H, m), 7.79 (1 H, s, 17-H), 7.75-7.64 (3 H, m), 7.58-7.45 (5 H, m), 6.70 (2 H, AA'BB', $^3J$= 8.6, 5-H) and (2 H, d, 7-H); $\delta_C$ ($D_6$-DMSO) 160.2 (C-6), 152.7 (C-18 or C-8), 150.3 (C-8 or C-18), 147.4 (C-16 or C-26), 147.1 (C-26 or C-16), 136.9 (C-10 or C-20), 134.5 (C-20 or C-10), 130.3, 129.8, 128.8, 128.5 (all C-H), 128.0 (C-4), 127.7, 127.4 (both C-H), 126.8 (C-11 & C-21), 126.3 (C-H), 122.5 (C-19), 121.4 (C-3), 119.7 (C-9), 117.5 (C-2), 115.0 (CN), 112.6 (C-5) and 48.9 (C-7); *m/z* 412 ($M^+$, 1.8), 270 (100), 245 (10.2) and 135 (17.0%) (Found: $M^+$, 412.1703. $C_{28}H_{20}N_4$ requires *M*, 412.1684).

**2,4-Diamino-6-cyanomethyl-3,5-pyridinedicarbonitrile (82).**




Trinitrile **82** was prepared as previously described in the literature.[96] A mixture of malononitrile (3.0 g, 45 mmol) and anhydrous $ZnCl_2$ (1 g, 7.4 mmol) in DMF (5 ml) was heated with stirring for 15 min at 95 °C and for 15 min at RT. The yellowish solution was diluted with MeOH to give a pale yellow precipitate. This was filtered off and recrystallised from dioxane to give compound **82** (1.148 g, 38%) m.p. 329-331 °C (lit.,[96] 330-332 °C); $R_f$ 0.61; $\nu_{max}$ (KBr) 3411, 3335 ($NH_2$), 2262 (unconjugated CN), 2214 (conjugated CN), 1649 (C=N), 1573 and 1553 $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 7.51 and 7.34 (4 H, 2 br s, 2 x $NH_2$) and 4.14 (2 H, s, 6-H); $\delta_C$ ($D_6$-DMSO) 161.5 (C-5), 158.9 (C-1), 158.2 (C-3), 116.5, 115.2, 114.7 (all CN), 81.4 (C-4), 70.9 (C-2) and 25.8 (C-6); *m/z* 198 ($M^+$, 100), 171 (11.0), 144 (17.9) and 67 (18.0%) (Found: $M^+$, 198.0655. $C_9H_6N_6$ requires *M*, 198.0654).

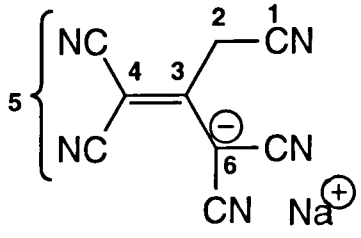
**2-[6-(2,4-Diamino-3,5-dicyanopyridyl)]-3-(2-quinolinyl) propenenitrile (83).**




To a solution of 2-quinolinecarbaldehyde (0.314 g, 2 mmol) in DMF (2 ml) was added a catalytic amount of piperidine and a solution of trinitrile **82** (0.396 g, 2 mmol) in DMF (2 ml). A small quantity of $^t$BuOK was introduced and the reaction was stirred at RT for 4 h. The reaction mixture was diluted with EtOAc and washed with water (2 x 10 ml), brine (2 x 10 ml) and water (2 x 10 ml). After drying, most of the EtOAc was removed under reduced

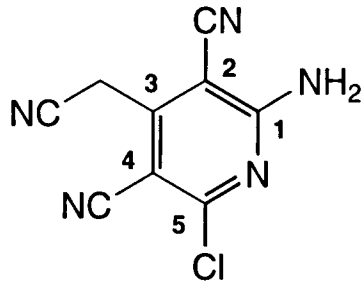
pressure, leaving a slurry of the precipitated product. This slurry was filtered, washed with petroleum ether and dried under suction to afford nitrile **83** (0.323 g, 48%) as a fawn coloured solid, m.p. 230 °C (d.); $R_f$ 0.34 (EtOAc); $\nu_{max}$ (KBr) 3642, 3367 ($NH_2$), 2208 (CN), 1662 (alkene C=C), 1566, 1543 (aromatic C=C & C=N) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.60 (1 H, d, $^3J$= 8.5, 6-H), 8.23 (1 H, s, 3-H), 8.17-8.06 (2 H, m, 8-H & 11-H), 7.99-7.85 (2 H, m, 5-H & 10-H) and 7.83-7.47 (5 H, m, 9-H & 2 x $NH_2$); $\delta_C$ ($D_6$-DMSO) 161.5 (C-17), 159.3 (C-13), 158.9 (C-15), 150.7 (C-4), 148.4 (C-3), 147.3 (C-12), 137.7 (C-6), 130.9, 129.3, 128.5, 128.2 (all quin C-H), 128.1 (C-7), 122.9 (C-5), 115.9, 115.5, 114.7 (all CN), 112.7 (C-2), 81.8 (C-16) and 71.9 (C-14); *m/z* 337 ($M^+$, 100), 311 (86.5), 198 (19.9), 179 (30.1), 128 (45.8) and 101 (21.3%). (Found: $M^+$, 337.1093. $C_{19}H_{11}N_7$ requires *M*, 337.1075).

**2-Cyanomethyl-propene-1,1,3,3-tetranitrile, sodium salt (84).**



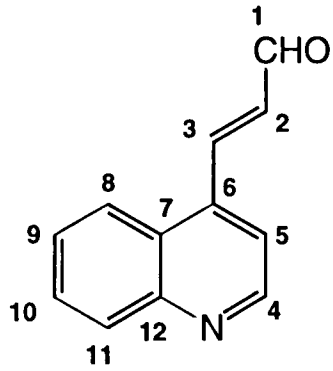

A solution of malononitrile (1.98 g, 30 mmol) in $Na_2CO_3$ solution (1M, 20 ml) was stirred for 17 h at room temperature. The solution was acidified to pH3 with concentrated HCl and extracted with EtOAc (4 x 50 ml). Anhydrous $NaHCO_3$ (2.5 g) was added to the combined extracts and the slurry was stirred for 2 h. The precipitate was recovered by filtration and washed with EtOAc-$CHCl_3$ (3:2, 2 x 50 ml). The filtrate and washings were combined and diluted with $CHCl_3$ to precipitate an oil which crystallised giving nitrile **84**, (53%) m.p. 249-250 °C (lit.,[99] 248-250 °C); $R_f$ 0.23 (EtOAc); $\nu_{max}$ (KBr) 2200 (conjugated CN), 1648 (aliphatic C=C) and 1492 $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 3.64 (2 H, s, 2-H); $\delta_C$ ($D_6$-DMSO) 156.6 (C-3), 117.6 (C-1), 115.6 (CN), 115.2 (CN), 53.7 (C-4 & C-6) and 23.0 (C-2). This compound was used in subsequent reaction without further characterisation.

**2-Amino-6-chloro-4-cyanomethyl-3,5-pyridinedicarbonitrile (85).**




Nitrile **84** (0.880 g, 4.33 mmol) was dissolved in acetone (15 ml), concentrated HCl (0.5 ml) was added and the reaction was heated at 60 °C for 20 min. The reaction mixture was concentrated under reduced pressure and dissolved in water (20 ml). NaOH (1M) was added to pH 8. The organic product was extracted with EtOAc and the solution was dried before removing the organic solvents under reduced pressure. The crude product was purified by column chromatography to afford nitrile **85** (0.131 g, 14%), $R_f$ 0.42; $\delta_H$ ($D_6$-DMSO) 8.85 and 8.50 (2 H, 2 br s, $NH_2$) and 4.27 (2 H, s, $CH_2CN$); *m/z* 217 ($M^+$, 100), 181 (24.5), 155 (23.9), 128 (18.4) and 101 (8.2%). This product was used in a subsequent reaction without further characterisation.

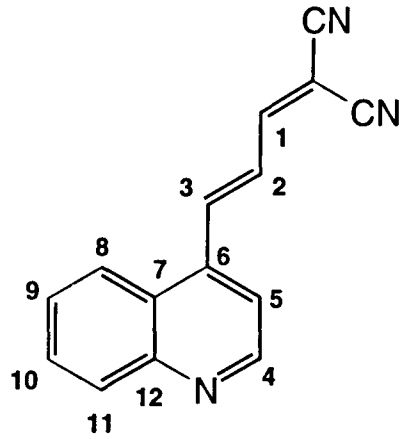
**3-(4-Quinolinyl)-2-propenal (87).**




Compound **87** was prepared as described previously in the literature.[100] To a suspension of triphenylphosphoranylideneacetaldehyde (0.608 g, 2 mmol) in nitrobenzene (2 ml) under $N_2$ in dry conditions was added 4-quinolinecarbaldehyde (0.314 g, 2 mmol). After the initial exotherm had subsided, the mixture was stirred, excluding light and air, for 24 h at RT. HCl (2 M, 5 x 2 ml) was added and the aqueous phase separated and washed with ether (3 x 20 ml) and pentane (3 x 20 ml) until the smell of nitrobenzene

had disappeared. The acid solution was carefully treated with $Na_2CO_3$ until a precipitate formed and the pH became alkaline. The crude product was filtered off, dried and purified by flash column chromatography to yield **87** (0.335 g, 54%) as a mixture of *E*- and *Z*- isomers (92:8 by $^1H$ NMR spectroscopy), m.p. 68.5-70 °C (lit.,[100] 91-92 °C for *E*- isomer); $R_f$ 0.31 (EtOAc); $\nu_{max}$ (KBr) 3052, 1682 ($\alpha,\beta$–unsaturated aldehyde C=O), 1612 ($\alpha,\beta$– unsaturated C=C), 1506 (aromatic C=C) and 760 (*ortho*-disubstituted benzene) $cm^{-1}$; NMR data quoted for *E*-isomer; $\delta_H$ ($D_6$-acetone) 9.94 (1 H, d, $^3J_{12}$=7.6, 1-H), 8.96 (1 H, d, $^3J$= 4.5, 4-H), 8.56 (1 H, d, $^3J_{23}$=15.9, 3-H), 8.35 (1 H, dd, $^3J$= 8.0, 11-H), 8.12 (1 H, dd, $^3J$= 7.8, 8-H), 7.80 (2 H, m, 5-H & 10-H), 7.67 (1 H, ddd, $^4J$= 1.3, 9-H) and 6.98 (1 H, dd, $^3J_{12}$=7.6, $^3J_{23}$=15.9, 2-H); $\delta_C$ ($D_6$-acetone) 194.2 (C-1), 151.1 (C-4), 149.7 (C-12), 146.9 (C-3), 139.8 (C-6), 134.6 (C-2), 131.0 (quin C-H), 130.4 (quin C-H), 128.2 (quin C-H), 126.4 (C-7), 124.1 (quin C-H) and 119.1 (C-5); *m/z* 183 ($M^+$, 56.2) 154 (100), 127 (34.3) and 75 (16.9%) (Found: $M^+$, 183.0679. $C_{12}H_9NO$ requires 183.0682).

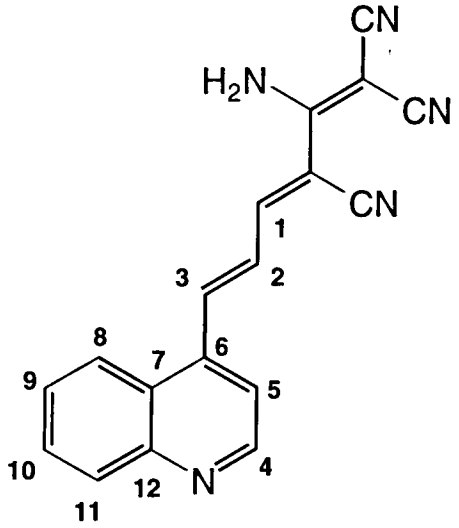
**4-(4-quinolinyl)-1,3-butadiene-1,1-dicarbonitrile (88).**




Malononitrile (36.1 mg, 0.55 mmol) was dissolved in EtOH (2 ml) and added to a solution of aldehyde **87** (100 mg, 0.55 mmol) in EtOH (2 ml). EtOH-piperidine solution (10:1, 2 drops) was added and the reaction was stirred for 48 h. The solvents were removed under reduced pressure and the crude product was subjected to column chromatography in pet. ether-EtOAc (1:1), affording nitrile **88** (83 mg, 66%), $R_f$ 0.55; $\nu_{max}$ 2963, 2221 (CN), 1603, 1563 (C=C and C=N) and 1504; $\delta_H$ ($D_6$-DMSO) 9.04 (1 H, d, $^3J$= 4.61, 4-H), 8.59 (1 H, d, $^3J$= 15.0, 3-H), 8.46 (1 H, d, $^3J$= 11.4, 1-H), 8.36 (1 H, dd, $^3J$= 8.2, 11-H), 8.19-8.07 (2 H, m, 8-H & 5-H), 7.95 - 7.75 (2 H, m, 9-H & 10-H)

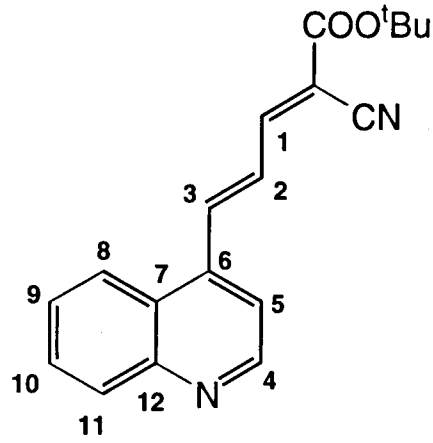
and 760 (1 H, dd, $^3J$= 15.0, 3J=11.3, 2-H); *m/z* 270 (*M*$^+$, 3.3), 257 (5.2), 231 (100), 204 (43.2) and 166 (91.2%).

**2-Amino-6-(4-quinolinyl)-1,3,5-hexatriene-1,1,3-tricarbonitrile (89).**



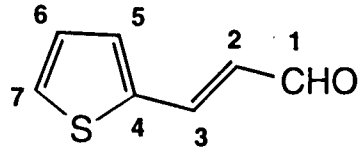

Malononitrile dimer (72 mg, 0.55 mmol) was dissolved in EtOH (2 ml) and added to a stirred solution of aldehyde **87** (0.100 g, 0.55 mmol) in EtOH (2 ml). After 1 h a yellow precipitate had formed which was filtered off and purified by flash column chromatography to give nitrile **89** (101 mg, 62%) (>90% of one isomer by $^1$H NMR spectroscopy), m.p. 140 °C (d.); $R_f$ 0.08 (EtOAc); $\nu_{max}$ (KBr) 3324, 3210 ($NH_2$), 2960, 2215 (conjugated CN), 1647 (alkene C=C), 1607, 1575 (aromatic C=C & C=N) and 761 (*ortho*-disubstituted benzene) cm$^{-1}$; NMR data quoted for major isomer (*E,Z*- shown above); $\delta_H$ ($D_6$-DMSO) 9.15 (2 H, br s, $NH_2$), 9.02 (1 H, d, 4-H), 8.56 (1 H, d, $^3J_{23}$= 15.0, 3-H), 8.39 (1 H, d, $^3J$= 8.5, 11-H), 8.20-8.05 (3 H, m, 1-H, 8-H & 5-H), 7.95-7.75 (2 H, m, 10-H & 9-H) and 7.57 (1 H, dd, $^3J_{23}$= 15.0, $^3J_{12}$=11.2, 2-H); $\delta_C$ ($D_6$-DMSO) 164.3 (*C*($NH_2$)), 153.7 (C-1), 150.6 (C-4), 148.5 (C-12), 142.2 (C-3), 139.2 (C-6), 130.0 (quin C-H), 129.9 (quin C-H), 128.2 (quin C-H), 127.7 (C-2), 125.4 (C-7), 123.3 (quin C-H), 118.4 (C-5), 115.5, 114.8, 113.6 (all CN), 105.6 (=*C*(CN)-C($NH_2$)=C($CN)_2$), 49.8 (*C*($CN)_2$); *m/z* 297 (*M*$^+$, 100), 269 (26.2), 205 (27.9) and 166 (47.9%) (Found: *M*$^+$, 297.1010. $C_{18}H_{11}N_5$ requires *M*, 297.1013).

**$^t$Butyl-2-Cyano-5-(4-quinolinyl)-2,4-pentadieneoate (90).**




$^t$Butyl cyanoacetate (66 mg, 0.47 mmol) was added to a stirred solution of aldehyde **87** (86 mg, 0.47 mmol) in EtOH (2 ml). After 18 h a yellow precipitate had formed which was filtered off and purified by flash column chromatography to give ester **90** (79 mg, 57%), (>90% one isomer by $^1$H NMR spectrospcopy), m.p. 131-133 °C; $R_f$ 0.53 (EtOAc); $\nu_{max}$ (KBr) 2978, 1724 (ester C=O), 1654 (alkene C=C), 1586 (aromatic C=C) and 1260 (ester C-O) cm$^{-1}$; NMR quoted for major isomer (*E,E*- shown above); $\delta_H$ ($D_6$-DMSO) 8.94 (1 H, d, $^3J$= 4.6, 4-H), 8.58 (1 H, d, $^3J_{23}$=15.1, 3-H), 8.38 (1 H, dd, $^3J$= 8.4, 11-H), 8.28 (1 H, d, $^3J_{12}$=11.5, 1-H), 8.07 (1 H, dd, $^3J$= 8.3, 8-H), 7.95 (1 H, d, $^3J$= 4.6, 5-H), 7.82 (1 H, ddd, 10-H), 7.70 (1 H, ddd, 9-H), 7.45 (1 H, dd, $^3J_{12}$=11.5, $^3J_{23}$=15.1, 2-H) and 1.57 (9 H, s, $CH_3)_3$); $\delta_C$ ($D_6$-DMSO) 160.6 (C=O), 154.4 (C-1), 150.6 (C-4), 148.5 (C-12), 142.9 (C-3), 139.2 (C-6), 130.0 (quin C-H), 129.9 (quin C-H), 128.2 (quin C-H), 127.5 (C-2), 125.5 (C-7), 123.6 (quin C-H), 118.2 (C-5), 114.4 (CN), 107.6 (=*C*(CN)), 83.8 (*C*$(CH_3)_3$) and 27.6 ($CH_3$); *m/z* 306 ($M^+$, 12.5), 250 (22.5), 233 (11.9), 205 (90.1) and 166 (37.3%) (Found: $M^+$, 306.1354. $C_{19}H_{18}N_2O_2$ requires *M*, 306.1364).

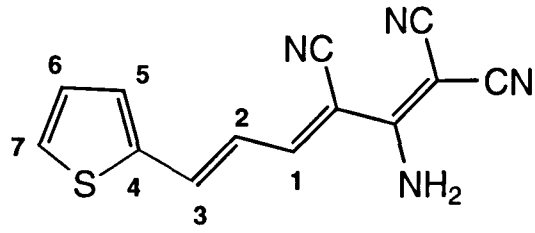
**3-(2-Thienyl)propenal (91).**




Aldehyde **91** was prepared as previously described in the literature.[102] NaOH (1 g, 25 mmol) was added to a solution of EtOH-water (1:2, 18 ml) and over a period of 15 min, freshly distilled 2-thienaldehyde (4.5 g, 40 mmol)

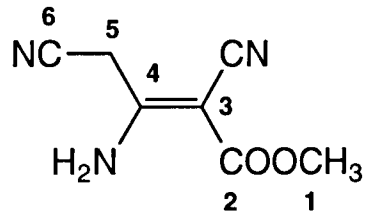
was added dropwise. To this mixture a solution of acetaldehyde in water (40% v/v, 10 ml) was added at 0 °C over 3.5 h with vigorous stirring. Following neutralisation with ice-cold acetic acid the mixture was extracted with benzene, washed with water and dried. Distillation afforded aldehyde **91** (1.158g, 34%) as a colourless liquid, b.p. 131-135 °C (lit.,[102] 105-108 °C (0.4 atm.)); $R_f$ 0.79 (EtOAc); $\nu_{max}$ (KBr) 3040 (aromatic C-H), 1672 ($\alpha,\beta$--unsaturated aldehyde C=O), 1612, 1421, 1226 (C=C in-plane vibration), 1118 and 711 (monosubstituted thiophene) $cm^{-1}$; $\delta_H$ (neat) 9.46 (1 H, d, $^3J_{12}$=7.7, 1-H), 7.47 (1 H, d, $^3J_{23}$=15.6, 3-H), 7.37 (1 H, d, $^3J$= 5.0, 7-H), 7.24 (1 H, d, $^3J$= 3.4, 5-H), 6.95 (1 H, m, 6-H) and 6.35 (1 H, dd, $^3J_{12}$=7.7, $^3J_{23}$=15.6, 2-H); $\delta_C$ (neat) 192.9 (C-1), 144.5 (C-3), 139.1 (C-4), 132.2 (C-5), 130.5 (C-7), 128.5 (C-6) and 127.0 (C-2); *m/z* 138 ($M^+$, 86.3), 109 (66.8), 84 (70.0) and 65 (66.8%).

**2-Amino-6-(2-thienyl)-1,3,5-hexatriene-1,1,3-tricarbonitrile (92).**



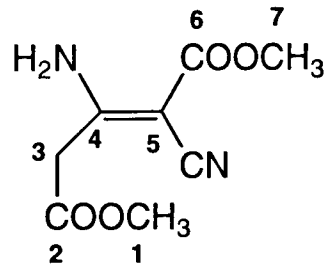

Aldehyde **91** (124 mg, 0.89 mmol) was added to a stirred solution of malononitrile dimer (118 mg, 0.89 mmol) in EtOH (5 ml). After 1 h the EtOH was removed under reduced pressure and the solid product was purified by column chromatography twice on silica with petroleum ether-EtOAc (1:1) and diethyl ether as eluents. This afforded compound **92** (0.153 g, 68%) as a yellow solid, m.p. 212 °C (d.); $R_f$ 0.88 (EtOAc); $\nu_{max}$ (KBr) 3334, 3217 ($NH_2$), 2212 (CN), 1658 (alkene C=C), 1599, 1575 and 1533 $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.83 (2 H, br s, $NH_2$), 7.82 (1 H, d, $^3J_{12}$=11.2, 1-H), 7.80 (1 H, d, $^3J$= 5.0, 7-H), 7.68 (1 H, d, $^3J_{23}$=14.9, 3-H), 7.56 (1 H, d, $^3J$= 3.4, 5-H), 7.16 (1 H, dd, 6-H) and 6.81 (1 H, dd, $^3J_{23}$=14.9, $^3J_{12}$=11.3, 2-H); $\delta_C$ ($D_6$-DMSO) 165.0 (-*C*($NH_2$)=C($CN)_2$), 154.0 (C-1), 141.3 (C-3), 139.9 (C-4), 133.4 (C-5), 132.0 (C-7), 129.4 (C-6), 121.3 (C-2), 115.8, 115.7, 115.1 (all CN), 101.5 (-*C*($NH_2$)=C($CN)_2$) and 52.7 (-C($NH_2$)=*C*($CN)_2$); *m/z* 252 ($M^+$, 100), 226 (24.8), 197 (10.5) 160 (30.3) and 122 (30.7%) (Found: $M^+$, 252.0473. $C_{13}H_8N_4S$ requires *M*, 252.0469).

**Methyl *(Z)*-3-amino-2,4-dicyano-2-butenoate (93).**




Sodium methoxide (3.51 g, 65 mmol) was suspended in dry toluene (30 ml) and heated to reflux. A solution of methylcyanoacetate (6.44 g, 65 mmol) and malononitrile (4.292 g, 65 mmol) was added dropwise over 10 min. The solution was refluxed for a further 50 min. The coloured sodium salt was filtered off, dissolved in water (30 ml) and the pH adjusted to 5 with concentrated HCl. After cooling overnight a solid had precipitated which consisted of malononitrile dimer and the desired product. The solid was filtered off and purified by column chromatography, yielding **93** (0.072 g, <1%), m.p. 95-96 °C (lit.,[103] 95-96 °C); $R_f$ 0.27; $\delta_H$ ($D_6$-DMSO) 9.12 (2 H, br s, $NH_2$), 3.87 (2 H, s, 5-H) and 3.72 (3 H, s, 1-H); *m/z* 165 (75.1), 138 (54.3), 133 (100), 107 (25.9) and 68 (95.6%).

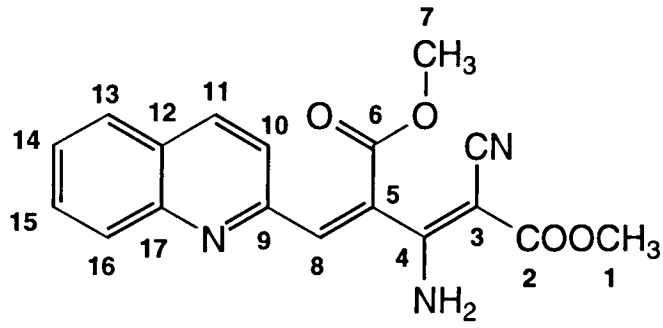
**Dimethyl-3-amino-2-cyano-pent-2-enedioate (94).**




To a solution of sodium (1.15 g, 50 mmol) in dry MeOH (22 ml) was added methyl cyanoacetate (10.7 g, 100 mmol) and the solution was heated at reflux for 1 h. Upon cooling to RT, a colourless precipitate formed which was recovered and stirred in ice cooled HCl (0.5 M, 50 ml). The crystals were filtered off, washed with water and recrystallised from MeOH-water giving diester **94** (6.403 g, 61%) as white crystals m.p. 132-133 °C (lit.,[104] 131 °C); $R_f$ 0.31 (EtOAc); $\nu_{max}$ (KBr) 3402, 3320 ($NH_2$), 2206 (CN), 1708 (ester C=O), 1636 (alkene C=C) and 1280 (ester C-O) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.13 and 8.94 (2 H, 2 br s, $NH_2$), 3.71 (3 H, s, 1-H or 7-H), 3.70 (3 H, s, 7-H or 1-H) and 3.63 (2 H, s, 3-H); $\delta_C$ ($D_6$-DMSO) 167.8 (C-6 or C-2), 167.2 (C-2 or C-6), 165.8 (C-4), 118.3 (CN), 70.5 (C-5), 52.5 (C-7 or C-1), 51.2 (C-1 or C-7) and 39.8 (C-

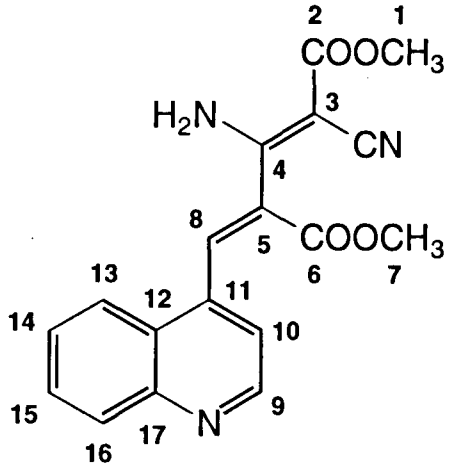
3); *m/z* 198 ($M^+$, 29.7), 166 (39.8), 138 (100), 123 (18.0) and 79 (21.1%) (Found: $M^+$, 198.0628; C, 48.60; H, 5.09; N, 14.39%. $C_8H_{10}N_2O_4$ requires *M*, 198.0638; C, 48.47; H, 5.09; N, 14.14%).

**Condensation product (95) of 2-quinolinecarbaldehyde with diester 94.**



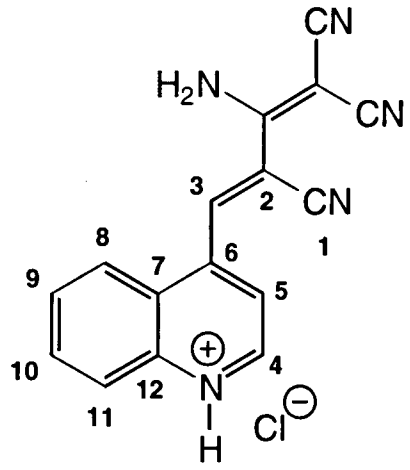

To a solution of 2-quinolinecarbaldehyde (0.314 g, 2 mmol) in DMF (2 ml) was added a catalytic amount of piperidine and a solution of diester **94** (0.396 g, 2 mmol) in DMF (2 ml). A small quantity of $^t$BuOK was introduced and the reaction was stirred at 90 °C for 16 h. The reaction mixture was cooled, diluted with EtOAc and washed with water (2 x 10 ml), brine (2 x 10 ml) and water (2 x 10 ml). After drying, most of the EtOAc was removed under reduced pressure, leaving a slurry of the precipitated product. This slurry was filtered, washed with petroleum ether and dried under suction to afford **95** (0.323 g, 46%) as a fawn-coloured solid, m.p. 185 °C (d.); $R_f$ 0.86 (EtOAc); $\nu_{max}$ (KBr) 3422 ($NH_2$), 2208 (CN), 1726 (ester C=O), 1628 (alkene C=C), 1522 (aromatic C=C) and 1266 (ester C-O) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.19 and 9.14 (2 H, 2 br s, $NH_2$), 8.45 (1 H, d, $^3J$= 8.5, 11-H), 8.32 (1 H, s, 8-H), 8.01 (2 H, m), 7.82 (2 H, m), 7.65 (1 H, m, 14-H), 3.89 (3 H, s, 7-H or 1-H) and 3.81 (3 H, s, 1-H or 7-H); $\delta_C$ ($D_6$-DMSO) 167.4 (C-2 or C-6), 167.3 (C-6 or C-2), 164.2 (C-4), 151.2 (C-9), 147.5 (C-17), 142.0 (C-8), 137.2 (C-11), 130.6 (quin C-H), 129.4 (quin C-H), 129.2 (C-5), 128.2 (quin C-H), 128.0 (quin C-H), 127.5 (C-12), 123.2 (C-10), 118.9 (CN), 70.7 (C-3), 53.0 (C-7 or C-1) and 51.2 (C-1 or C-7); *m/z* 337 ($M^+$, 1.5), 278 (100), 246 (10.7), 203 (10.9) and 128 (20.7%) (Found: $M^+$, 337.1048. $C_{18}H_{15}N_3O_4$ requires *M*, 337.1059).

**Condensation product (96) of 4-quinolinecarbaldehyde with diester 94.**



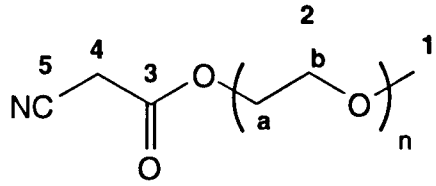

Diester **96** was prepared using the method described for the preparation of compound **95**. Recrystallisation from EtOAc afforded compound **96** (0.108 g, 16%) as a fawn-coloured solid, m.p. 179 °C (d.); $R_f$ 0.57 (EtOAc); $\nu_{max}$ (KBr) 3406, 3308 ($NH_2$), 2214 (CN), 1720 (ester C=O), 1664 (alkene C=C), 1620, 1528 (aromatic C=C & C=N), 1240 (ester C-O) and 754 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.11 and 9.04 (2 H, 2 br s, $NH_2$), 8.87 (1 H, d, $^3J$= 4.4, 9-H), 8.46 (1 H, s, 8-H), 7.99 ( 2 H, m, 13-H & 16-H), 7.75 (1 H, ddd, 15-H), 7.62 (1 H, ddd, 14-H), 7.40 (1 H, d, $^3J$= 4.3, 10-H), 3.81 (3 H, s, 7-H) and 3.52 (3 H, s, 1-H); $\delta_C$ ($D_6$-DMSO) 166.6 (C-2 or C-6), 164.7 (C-6 or C-2), 163.5 (C-4), 150.3 (C-9), 147.8 (C-17), 140.5 (C-8), 138.7 (C-11), 131.3 (C-5), 130.2 (quin C-H), 129.6 (quin C-H), 127.4 (quin C-H), 125.4 (C-12), 125.1 (quin C-H), 119.5 (C-5), 118.0 (CN), 71.7 (C-3), 53.2 (C-7) and 51.3 (C-1); *m/z* 337 ($M^+$, 5.9), 278 (56.7), 246 (100), 191 (15.1) and 128 (50.5%) (Found: $M^+$, 337.1079. $C_{18}H_{15}N_3O_4$ requires *M*, 337.1059).

**(*Z*)-2-Amino-4-(4-quinolinyl)-1,3-butadiene-1,1,3-tricarbonitrile, hydrochloride salt (97).**




HCl gas was bubbled through a solution of compound **48** (0.1 g, 0.37 mmol) in EtOH (5 ml). A yellow precipitate formed instantly and it was filtered off and dried under suction giving compound **97** (110 mg, 97%), m.p. 231 °C (d.); $R_f$ 0.77 (EtOH-acetic acid, 1:1); $\nu_{max}$ (KBr) 3426, 3344 ($NH_2$), 2430 (+N-H), 2208 (CN), 1650 (alkene C=C) and 1596 (aromatic C=C) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 10.49 (1 H, br s, +N-H), 9.81 and 9.35 (2 H, 2 br s, $NH_2$), 9.27 (1 H, d, $^3J_{45}$=5.1, 4-H), 8.91 (1 H, s, 3-H), 8.34 (2 H, m, 8-H & 11-H), 8.03 (2 H, m, $^3J_{45}$=5.1, 5-H &10-H) and 7.84 (1 H, ddd, 9-H); $\delta_C$ ($D_6$-DMSO) 163.5 (-*C*($NH_2$)=C($CN)_2$), 149.2 (C-4), 147.0 (C-3), 143.4 (C-12), 141.5 (C-6), 133.2 (quin C-H), 129.4 (quin C-H), 125.7 (C-7), 125.3 (quin C-H), 124.5 (quin C-H), 120.9 (C-5), 115.5, 114.1, 113.2 (all CN), 111.5 (C-2) and 50.3 (-C($NH_2$)=*C*($CN)_2$); *m/z* 271 (*M*-36$^+$, 69.0), 245 (54.8), 217 (16.3), 206 (36.0) and 142 (22.1%) (Found C, 62.62; H, 3.35; N, 22.57%. $C_{16}H_{10}ClN_5$ requires C, 62.53; H, 3.28; N, 22.80%).

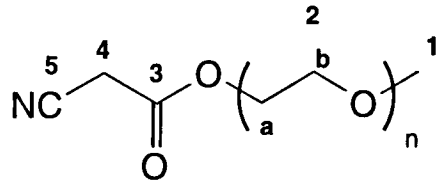
**(Trimethyleneglycol methyl ether) cyanoacetate (99).**




Cyanoacetic acid (4.155 g, 49 mmol) and triethyleneglycol methyl ether (7.990 g, 49 mmol) were heated at reflux in toluene (100 ml) for 24 h under Dean-Stark conditions. After removal of the toluene under reduced pressure

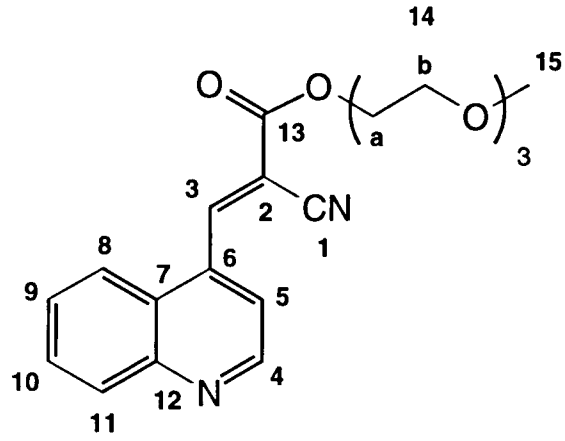
the clear liquid product was subjected to flash column chromatography with EtOAc as eluent. This afforded **99** (7.523 g, 66%) as a viscous, clear oil, $R_f$ 0.14 (EtOAc); $\nu_{max}$ (KBr) 2891s (aliphatic C-H), 2261 (unconjugated CN), 1749 (ester C=O), 1257 (ester C-O) and 1104 (ether C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 4.17 (2 H, m, ester terminal ethylene glycol unit, 2a-H), 3.56-3.34 (12 H, m) and 3.20 (3 H, s, 1-H); $\delta_C$ ($CDCl_3$) 161.9 (C-3), 112.0 (CN), 69.9, 68.6, 68.5, 66.6, 63.7 (all $CH_2$), 56.9 (C-1) and 22.7 (C-4); *m/z* 232 (*M*+1, 0.2), 186 (3.0), 156 (4.1), 112 (92.8) and 68 (55.9%) (Found: $M^+$, 232.1209. $C_{10}H_{17}NO_5$ requires *M*, 231.1102).

**(Polyethyleneglycol methyl ether) cyanoacetate (101).**



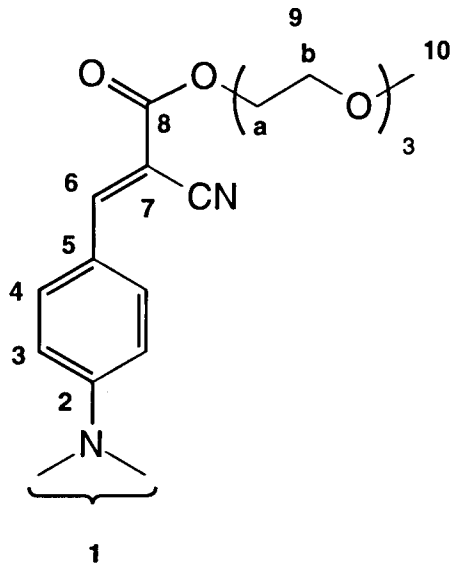

Cyanoacetic acid (0.637 g, 7.4 mmol) and polyethyleneglycol methyl ether (15 g, 7.5 mmol) were heated at reflux in toluene (100 ml) with a catalytic amount of PTSA for 24 h under Dean-Stark conditions. After removal of the toluene under reduced pressure the white solid was taken up in acetone and precipitated by addition to ice-cooled diethyl ether. The precipitate was recovered by filtration and purified from residual cyanoacetic acid by filtration through Sephadex LH-20 beads (50 g) with water as eluent giving ester **101** (10.994 g, 71%), m.p. 48-50 °C; $R_f$ 0.05 (EtOH); $\delta_H$ ($CDCl_3$) 4.26 (2 H, m, ester terminal polyethylene glycol unit, 2a-H), 3.90 (2 H, m, ester terminal polyethyleneglycol unit, 2b-H), 3.56 (178 H, m, 2-H and 4-H) and 3.23 (3 H, s, 1-H); $\delta_C$ ($CDCl_3$) 165.3 (C-3), 115.3 (CN), 83.0, 77.1, 72.7, 71.2 (strong), 69.3, 66.2, 65.3, 59.4 (C-1) and 24.9 (C-4)

**(Trimethyleneglycol methyl ether)-2-cyano-3-(4-quinolinyl) propenoate (102).**



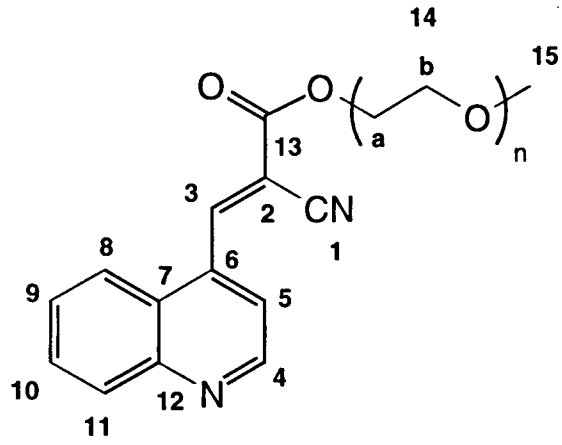

4-Quinolinecarbaldehyde (0.471 g, 3 mmol) was dissolved in diethyl ether (5 ml) and a catalytic amount of EtOH-piperidine solution (10:1) was added. Compound **99** (0.693 g, 2.9 mmol) was introduced and the reaction was stirred for 18 h. After this time TLC indicated formation of a new product. After removing the solvent under reduced pressure, the crude oil was twice purified by column chromatography on silica with EtOAc as eluent to afford ester **102** (0.318 g, 29%), $R_f$ 0.24 (EtOAc); $\nu_{max}$ (KBr) 2878s (aliphatic C-H), 2230 (CN), 1734 (ester C=O), 1272 (ester C-O), 1103 (ether C-O), 754 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-acetone) 8.91 (1 H, d, $^3J$= 4.5, 4-H), 8.86 (1 H, s, 3-H), 7.97 (1 H, d, $^3J$= 8.5, 11-H), 7.85 (1 H, d, $^3J$= 8.3, 8-H), 7.76 (1 H, d, $^3J$= 4.5, 5-H), 7.66 (1 H, ddd, $^4J$= 1.4, 10-H), 7.50 (1 H, ddd, $^4J$= 1.3, 9-H), 4.36 (2 H, m, terminal ethylene glycol unit, 14a-H), 3.71-3.22 (10 H, m) and 3.06 (3 H, s, 15-H); $\delta_C$ ($D_6$-acetone) 161.9 (C-13), 152.1 (C-3), 151.2 (C-4), 149.3 (C-12), 137.7 (C-6), 131.2 (quin C-H), 131.0 (quin C-H), 128.8 (quin C-H), 125.9 (C-7), 124.5 (quin C-H), 121.1 (C-5), 114.9 (CN), 111.6 (C-2), 72.6, 71.4, 71.3, 71.1, 69.3, 67.0 (all ethylene glycol $CH_2$) and 58.9 (C-15); *m/z* 371 ($M^+$, 0.3), 294 (1.2), 251 (8.4), 207 (25.3) and 152 (25.0%) (Found: $M^+$, 370.1510; C, 64.54; H, 6.19; N, 7.59%. $C_{20}H_{22}N_2O_5$ requires *M*, 370.1523; C, 64.83; H, 5.99; N, 7.57%).

**(Trimethyleneglycol methyl ether)-2-cyano-3-(4-dimethylamino phenyl)-2-propenoate (103).**



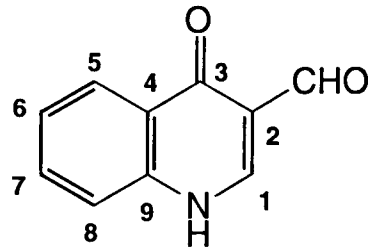

4-Dimethylaminobenzaldehyde (0.298 g, 2 mmol) was dissolved in EtOAc (5 ml) and a catalytic amount of neat piperidine was added. Compound **99** (0.462 g, 2 mmol) was introduced, giving a rapid colour change to orange. After stirring for 1 h, TLC indicated the formation of a new product. The solvent was removed under reduced pressure and the crude oil was subjected to column chromatography with EtOAc as eluent to afford **103** (0.52 g, 72%) as an orange oil, $R_f$ 0.55 (EtOAc); $\nu_{max}$ (KBr) 2878s (aliphatic C-H), 2212 (CN), 1714 (ester C=O), 1614 (C=C), 1574, 1520 (aromatic C=C), 1278 (ester C-O) and 1188s (ether C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.87 (1 H, s, 6-H), 7.74 (2 H, d, $^3J$= 9.0, 4-H), 6.52 (2 H, d, AA'BB', $^3J$= 9.1, 3-H), 4.30 (2 H, ester terminal ethylene glycol unit, 9a-H), 3.73-3.39 (10 H, m), 3.22 (3 H, s, 10-H) and 2.95 (6 H, s, 1-H); $\delta_C$ ($CDCl_3$) 163.9 (C-8), 154.3 (C-6), 153.4 (C-2), 133.9 (C-4), 118.8 (C-5), 117.2 (CN), 111.3 (C-3), 92.8 (C-7), 71.7, 70.5, 70.4, 70.3, 68.6, 64.7 (all ethylene glycol $CH_2$), 58.7 (C-10) and 40.1 (C-1); *m/z* 362 ($M^+$, 77.5), 304 (13.9), 260 (17.3), 216 (67.9), 199 (100%) and 171 (56.7%) (Found: $M^+$, 362.1835; $C_{20}H_{26}N_2O_5$ requires *M*, 362.1835).

**(Polyethyleneglycol methyl ether)-2-cyano-3-(4-quinolinyl)-2-propenoate (104).**



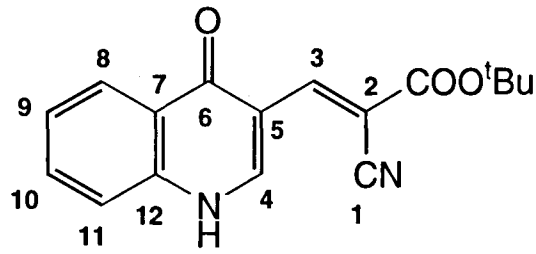

To a solution of 4-quinolinecarbaldehyde (0.079 g, 0.5 mmol) in EtOH (2 ml) was added a solution of compound **101** (1.034 g, 0.5 mmol) in EtOH (5 ml). After 18 h, the solvent was removed under reduced pressure and the solid product was taken up in acetone and precipitated with by addition to ice-cooled diethyl ether. The cream-coloured product was filtered off and dried under suction. TLC indicated no residual aldehyde but the presence of ~6% unreacted **101**. Compound **104** (0.583 g, 56%), m.p. 49-50 °C; $R_f$ 0.05 (EtOH); $\nu_{max}$ (KBr) 2887 (aliphatic C-H), 2214 (CN), 1738 (ester C=O), 1562 (aromatic C=C), 1241 (ester C-O) and 1112 (ether C-O) $cm^{-1}$; NMR data quoted for the major product; $\delta_H$ ($CDCl_3$) 9.12 (0.96 H, d, 4-H), 9.11 (0.96 H, s, 3-H), 8.15 (1.92 H, m, 8-H & 11-H), 7.95 (0.96 H, d, 5-H), 7.91 (0.96 H, ddd, 10-H), 7.75 (0.96 H, ddd, 9-H), 4.52 (2 H, m, PEG ester terminal unit 14a-H) and 3.95-3.20 (181 H, m, PEG $CH_2$ & 15-H); $\delta_C$ ($CDCl_3$) 161.9 (C-13), 152.4 (C-3), 151.3 (C-4), 149.4 (C-12), 137.9 (C-6), 131.2 (quin C-H), 131.1 (quin C-H), 128.8 (C-7), 126.0, 124.7, 111.8 (CN), 100.8 (C-2), 84.5, 77.1, 72.6, 71.2 (strong), 69.2, 66.9, 65.3 and 58.8 (C-15); ESMS showed a broad molecular weight distribution centred on approximately 2200 (Chapter 4, Section 4.5).

**4-Hydroxy-3-quinolinecarbaldehyde (105).**



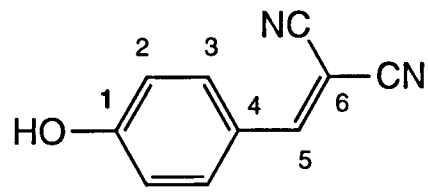

Compound **105** was prepared as described previously in the literature.[106] Quinolinol (2.0 g, 13.4 mmol) and powdered NaOH (2.0 g, 50 mmol) were mixed and $CHCl_3$ (4 ml) was added. While heating under reflux and shaking vigorously, water (4 ml) was added in four equal portions, the reaction being allowed to subside after each addition. The mixture was heated under reflux and from time to time was shaken vigorously during 1 h when further $CHCl_3$ (2 ml) and water (1 ml) were added. The mixture was then heated under reflux for 5 h. The solvent was removed under reduced pressure and the remaining aqueous solution and insoluble gum were cooled. The aqueous solution was separated and filtered and the residual gum was extracted twice by boiling with aqueous NaOH solution (2 M, 5 ml), followed by cooling and filtering. The combined aqueous extract was acidified to pH 6 with glacial acetic acid and cooled in ice. After several hours the precipitated product was collected, washed with cold water and dried. Recrystallisation from EtOH gave colourless crystals of aldehyde **105** (1.342 g, 58%), m.p. 271-273 °C (lit.,[106] 272-275 °C); $R_f$ 0.38 (EtOAc); $\nu_{max}$ (KBr) 3444, 3078, 3050, 2992, 2946, 1704 ($\alpha,\beta$-unsaturated aldehyde), 1682, 1616 (amide I), 1586, 1554 (amide II) and 774 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 10.22 (1 H, s, CHO) and 8.50-7.45 (6-H, m); $\delta_C$ ($D_6$-DMSO) 188.8 (C=O), 176.3 (C-3), 143.4 (C-1), 139.4 (C-9), 133.1 (C-7), 127.8 (C-4), 125.4 (C-5 & C-6), 119.5 (C-8) and 116.4 (C-2); *m/z* 173 ($M^+$, 100), 144 (13.2), 127 (13.0), 116 (25.8) and 104 (35.1%) (Found: $M^+$, 173.0472; C, 69.02; H, 3.96; N, 7.94%. $C_{10}H_7NO_2$ requires *M*, 173.0475; C, 69.36; H, 4.05; N, 8.09%).

**Condensation product of 105 with $^t$butyl cyanoacetate (107).**




To a stirred solution of compound **105** (0.198 g, 1.1 mmol) in DMF (2 ml) was added $^t$butylcyanoacetate (0.161 g, 1.1 mmol). After 16 h the reaction mixture was diluted with EtOAc (20 ml) and washed with water (2 x 10 ml), brine (2 x 10 ml) and water (2 x 10 ml). After drying, most of the EtOAc was removed under reduced pressure, leaving a slurry of the precipitated product. This slurry was filtered, washed with petroleum ether and dried under suction. The crude product was purified by column chromatography on silica with EtOAc as the eluent, affording amide **107** (46 mg, 14%) as a cream powder, m.p. 260-262 °C (d.); $R_f$ 0.84 (EtOAc); $\nu_{max}$ (KBr) 3426 (N-H), 3063, 2930, 2216 (CN), 1719 (ester C=O), 1621 (amide I), 1553 (amide II) and 1253 (ester C-O) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.02 (1 H, s, 4-H), 8.63 (1 H, s, 3-H), 8.23 (1 H, dd, $^3J$= 8.1, 8-H), 7.81 (1 H, ddd, $^4J$= 1.5, 10-H), 7.70 (1 H, d, 11-H), 7.54 (1 H, ddd, $^4J$= 1.2, 9-H) and 1.58 (9 H, s, $(CH_3)_3$); $\delta_C$ ($D_6$-DMSO) 174.9 (C-6), 161.9 (ester C=O), 148.2 (C-4), 142.1 (C-3), 138.4 (C-12), 133.3 (C-10), 125.9 (C-8 or C-9), 125.8 (C-9 or C-8), 125.6 (C-7), 119.6 (C-11), 117.0 (CN), 112.0 (C-5), 97.1 (C-2), 82.6 ($C(CH_3)_3$) and 27.8 ($C(CH_3)_3$); *m/z* 296 ($M^+$, 2.8), 195 (100), 140 (16.6) and 113 (4.7%) (Found: $M^+$, 296.1146. $C_{17}H_{16}N_2O_3$ requires *M*, 296.1157).

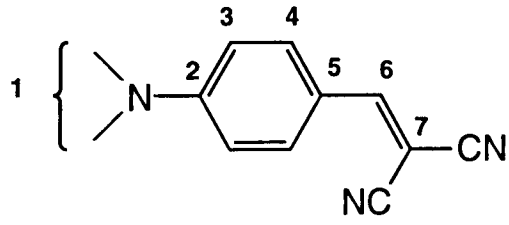
**2-Cyano-3-(4-hydroxyphenyl)propenenitrile (108).**[80]




To a solution of 4-hydroxybenzaldehyde (0.366 g, 3 mmol) in EtOH (2 ml) was added a solution of malononitrile (0.198 g, 3 mmol) in EtOH (2 ml). A catalytic amount of piperidine was added and the reaction was stirred for 16 h. The product, a yellow precipitate, was recovered by filtration, then washed

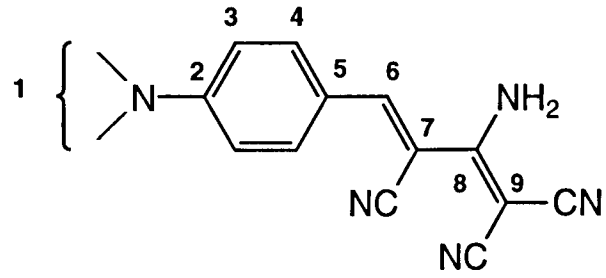
and dried under suction giving nitrile **108** (0.360 g, 71%), m.p. 175-177 °C; $R_f$ 0.89 (EtOAc); $\nu_{max}$ (KBr) 3350 (OH), 2228 (CN), 1610, 1580, 1564 (aromatic C=C & C=N) and 1298 (C-O) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.36 (1 H, s, O-H), 8.30 (1 H, s, 5-H), 7.92 (2 H, AA'BB', $^3J$= 8.7, 3-H) and 7.01 (2 H, AA'BB', 2-H, $^3J$= 8.7, 2-H); $\delta_C$ ($D_6$-DMSO) 164.6 (C-1), 160.5 (C-5), 134.0 (C-3), 122.6 (C-4), 116.9 (C-2), 115.3 and 114.4 (both CN), 74.7 (C-6); *m/z* 170 ($M^+$, 100), 142 (40.7), 119 (43.3) and 91 (25.5%) (Found: $M^+$, 170.0474; C, 70.39; H, 3.64; N, 16.42%. $C_{10}H_6N_2O$ requires *M*, 170.0479; C, 70.57; H, 3.56; N, 16.47%).

**2-Cyano-3-(4-dimethylaminophenyl)propenenitrile (109).**



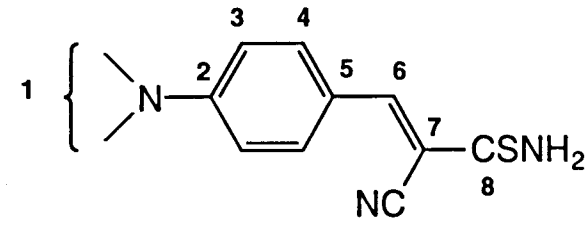

Malononitrile (0.198 g, 3 mmol) was dissolved in EtOH (2 ml) and added to a stirred solution of 4-dimethylaminobenzaldehyde (0.447 g, 2 mmol) in EtOH (4 ml). A catalytic amount of EtOH-piperidine solution (10:1) was added and after 2 h an orange precipitate had formed. The crude product was filtered, washed and dried to yield nitrile **109** (0.346 g, 58%), m.p. 175-177 °C ; $R_f$ 0.55 (EtOAc); $\nu_{max}$ (KBr) 2208 (CN), 1612, 1522 (aromatic C=C), 1388 ($CH_3$ symmetrical deformation) and 816 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.06 (1 H, s, 6-H), 7.86 (2 H, AA'BB', $^3J$= 9.2, 4-H), 6.88 (2 H, AA'BB', $^3J$= 9.2, 3-H) and 3.14 (6 H, s, 1-H); $\delta_C$ ($D_6$-DMSO) 158.9 (C-6), 154.4 (C-2), 133.7 (C-4), 118.8 (C-5), 115.7 (2 x CN), 111.9 (C-3), 68.9 (C-7) and 39.6 (C-1); *m/z* 197 ($M^+$, 83.9), 196 (100), 180 (13.7) and 126 (13.5%) (Found: $M^+$, 197.0945; C, 73.02; H, 5.68; N, 21.44%. $C_{12}H_{11}N_3$ requires *M*, 197.0951; C, 73.06; H, 5.62; N, 21.31%).

**2-Amino-4-(4-dimethylaminophenyl)-1,3-butadiene-1,1,3-tricarbonitrile (110).**




Malononitrile dimer (0.264 g, 2 mmol) was dissolved in EtOH (2 ml) and added to a stirred solution of 4-dimethylaminobenzaldehyde (0.298 g, 2 mmol) in EtOH (2 ml). A catalytic amount of EtOH-piperidine solution (10:1) was added and after 4 h an orange precipitate formed. The crude product was filtered off and purified by column chromatography on basic alumina to yield nitrile **110** (68 mg, 13%), m.p. 190 °C (d.); $R_f$ 0.26 (EtOAc); $\lambda_{max}$ (EtOH)/nm 202 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 16800), 251 (10500) and 432 (22300); $\nu_{max}$ (KBr) 3339, 3207 ($NH_2$), 2225, 2221 (CN), 1679 (alkene C=C), 1614, 1516 (aromatic C=C) and 810 (*para*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.71 (2 H, br s, $NH_2$), 7.83 (2 H, d, AA'BB', $^3J$= 9.1, 4-H), 7.70 (1 H, s, 6-H), 6.78 (2 H, d, AA'BB', $^3J$= 9.1, 3-H) and 3.02 (6 H, s, $CH_3$); $\delta_C$ ($D_6$-DMSO) 167.0 (C-8), 153.6 (C-2), 152.9 (C-6), 133.3 (C-4), 118.6 (C-5), 116.7 (CN), 116.0 (CN), 115.6 (CN), 111.6 (C-3), 92.3 (C-7), 48.4 (C-9) and 39.6 (C-1); *m/z* 263 ($M^+$, 100), 237 (61.4), 219 (21.4), 196 (32.1) and 134 (80.2) (Found: $M^+$, 263.1162. $C_{15}H_{13}N_5$ requires *M*, 263.1169).

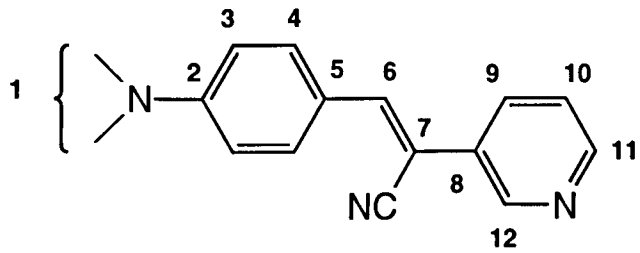
**2-Cyano-3-(4-dimethylaminophenyl)propenethioamide (111).**




Cyanothioacetamide (0.300 g, 3 mmol) was dissolved in EtOH (3 ml) and added to a stirred solution of 4-dimethylaminobenzaldehyde (0.447 g, 3 mmol) in EtOH (4 ml). A catalytic amount of EtOH-piperidine solution (10:1) was added and after 3.5 h a dark red precipitate had formed. The crude product was filtered off, washed with petroleum ether and dried to yield

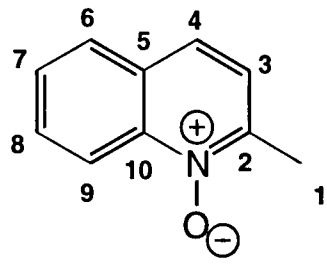
thioamide **111** (0.294 g, 33%), m.p.160 °C (d.); $R_f$ 0.56 (EtOAc); $\lambda_{max}$ (EtOH)/nm 202 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$, 32300), 238 (16200), 261 (15200) and 439 (45300); $\nu_{max}$ (KBr) 3331, 3285 ($NH_2$), 2215 (CN), 1608, 1563, 1519 (C=C), 1370 ($CH_3$ symm. deformation) and 810 (*para*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.74 and 9.13 (2 H, 2 br s, $NH_2$), 8.10 (1 H, s, 6-H), 7.13 (2 H, AA'BB', $^3J$= 9.0, 4-H), 6.85 (2 H, AA'BB', $^3J$= 9.0, 3-H) and 3.10 (6 H, s, 1-H); $\delta_C$ ($D_6$-DMSO), 193.1 (C-8), 153.2 (C-2), 148.9 (C-6), 133.4 (C-4), 118.4 (C-5), 118.1 (CN), 111.9 (C-3), 103.9 (C-7) and 39.7 (C-1) ; *m/z* 231 (*M*$^+$, 100), 198 (67.2), 187 (27.1), 155 (39.5) and 128 (20.3%) (Found: *M*$^+$, 231.0826; C, 62.46; H, 5.56; N, 18.01%. $C_{12}H_{13}N_3S$ requires *M*, 231.0828; C, 62.31; H, 5.67; N, 18.18%).

**2-(3-pyridyl)-3-(4-dimethylaminophenyl)propenenitrile (112).**



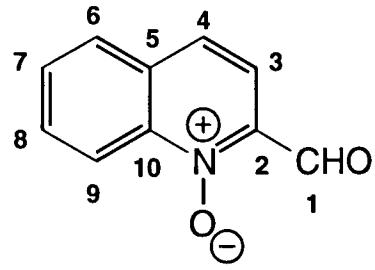

3-Pyridylacetonitrile (0.295 g, 2.5 mmol) was added to a stirred solution of 4-dimethylaminobenzaldehyde (0.372 g, 2.5 mmol) in EtOH (4 ml). A catalytic amount of KO$^t$Bu was added and after 10 min a yellow precipitate had formed. The crude product was filtered off, washed with petroleum ether and dried to yield nitrile **112** (0.347 g, 55%), m.p. 129-130 °C; $R_f$ 0.31 (EtOAc); $\lambda_{max}$ (EtOH)/nm 202 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$, 28000), 260 (9960) and 408 (38000); $\nu_{max}$ (KBr) 3022, 2199 (CN), 1613, 1586, 1529 (aromatic C=C & C=N), 1378 ($CH_3$ symm. deformation) and 811 (*para*-disubstituted benzene) cm$^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.91 (1 H, d, $^4J$= 2.4, 12-H), 8.57 (1 H, dd, $^3J$= 4.7, 11-H), 8.06 (1 H, m, 9-H), 7.92 (2 H, d, AA'BB', $^3J$= 8.9, 4-H), 7.92 (1 H, s, 6-H), 7.50 (1 H, dd, $^3J$= 4.8, 10-H), 6.82 (2 H, d, AA'BB', $^3J$= 9.0, 3-H) and 3.04 (6 H, s, 1-H); $\delta_C$ ($D_6$-DMSO) 152.0 (C-2), 148.7 (C-12), 146.2 (C-11), 144.4 (C-6), 132.4 (C-9), 131.5 (C-4), 131.0 (C-8), 123.9 (C-10), 120.7 (C-5), 118.8 (CN), 111.6 (C-3), 98.9 (C-7) and 39.6 (C-1); *m/z* 249 (*M*$^+$, 100), 234 (55.2), 220 (28.2), 206 (52.5) and 177 (16.8%) (Found: *M*$^+$, 249.1260; C, 77.33; H, 6.03; N, 16.61%. $C_{16}H_{15}N_3$ requires *M*, 249.1263; C, 77.07; H, 6.07; N, 16.86%).

**Quinaldine *N*-oxide monohydrate (113).**



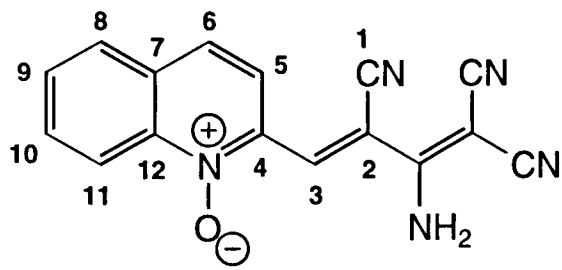

Glacial acetic acid (75 ml), quinaldine (31.74 g, 0.222 mol) and hydrogen peroxide solution (27.5%, 23 ml, 0.186 mol) were heated at 70 °C for 30 min. More peroxide solution (20 ml) was introduced and the mixture was heated at reflux for a further 3 h. The excess acid was removed under reduced pressure and the remaining solution was neutralised with saturated $Na_2CO_3$ solution. The mixture was filtered and the filtrate extracted with $CHCl_3$ (2 x 100 ml). The filtered solid was also extracted with hot $CHCl_3$ (2 x 50 ml). After combining these extracts and removing the solvent under reduced pressure the dark brown liquid was diluted with DCM and pre-adsorbed onto silica. Flash column chromatography with EtOAc removed the residual quinaldine and the desired quinaldine N-oxide was eluted with MeOH. Removal of the solvent under reduced pressure and recrystallisation from MeOH afforded compound **113** (12.86 g, 36%) as fawn-coloured needles, m.p. 76-78 °C (lit.,[109] 77-78 °C); $R_f$ 0.56 (EtOH); $\nu_{max}$ (KBr) 3416 (water of crystallisation), 1566, 1516 (C=C & C=N), 1242s (N-O) and 734 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 8.55 (1 H, d, $^3J$= 8.7, 4-H), 7.97 (1 H, dd, $^4J$= 1.0, $^3J$= 8.0, 9-H), 7.74 (2 H, m, 6-H & 8-H), 7.62 (1 H, ddd, $^4J$= 1.0, 7-H), 7.48 (1 H, d, $^3J$= 8.6, 3-H) and 2.56 (3 H, s, 1-H); $\delta_C$ ($D_6$-DMSO) 145.2 (C-2), 140.7 (C-10), 130.2 (C-8), 129.0 (C-5), 128.5 (C-7), 127.7 (C-6), 124.5 (C-4), 123.4 (C-3), 118.7 (C-9) and 18.32 (C-1); *m/z* 159 ($M^+$, 52.2), 143 (60.3) and 115 (53.0%) (Found: $M^+$, 159.0688; $C_{10}H_9NO$ requires *M*, 159.0684).

**2-Quinolinecarbaldehyde *N*-oxide (114).**




To a solution of $SeO_2$ (1.109 g, 10 mmol) in dioxane (10 ml) at 70-80 °C, a solution of compound **113** (1.305 g, 9.0 mmol) in dioxane (1.5 ml) was added dropwise. The mixture was heated at reflux for 1 h to deposit selenium metal which was filtered off. Upon addition of MeOH a white solid precipitated which was also removed by filtration. Chromatography of the residue afforded aldehyde **114** (0.851 g, 55%) as a yellow solid, m.p. 98-100 °C (lit.,[110] 95-96 °C); $R_f$ 0.41 (EtOAc); $\nu_{max}$ (KBr) 3058 (aromatic C-H), 1694 (aldehyde C=O) and 1206 (N-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 10.82 (1 H, s, 1-H), 8.75 (1 H, d, 4-H) and 7.90-7.69 (5 H, m); $\delta_C$ ($CDCl_3$) 186.5 (C-1), 142.0 (C-2), 140.0 (C-10), 131.9 (C-5), 130.9 (C-8 & C-7), 128.4 (C-6), 124.7 (C-4), 119.8 (C-3 or C-9) and 118.9 (C-9 or C-3); *m/z* 173 ($M^+$, 7.0), 156 (13.4), 128 (100) and 101 (26.9%) (Found: $M^+$, 173.0469; $C_{10}H_7NO_2$ requires *M*, 173.0476).

***(Z)*-2-Amino-4-(2-quinolinyl)-1,3-butadiene-1,1,3-tricarbonitrile, *N*-Oxide (115).**




Compound **114** (0.140g, 8.1 mmol) and malononitrile dimer (0.106 g, 8.0 mmol) were dissolved in EtOH (2 ml). While stirring for 2 h, a yellow precipitate formed which was filtered off and purified by column chromatography to give compound **115** as a yellow solid (0.089 g, 39%), m.p. 140 °C (d.); $R_f$ 0.1 (EtOAc); $\nu_{max}$ (KBr) 3204, 3070 (unsaturated C-H), 2962 (aliphatic C-H), 2216 (CN), 1666, 1620 (alkene C=C), 1564, 1540 (aromatic C=C & C=N) and 1262 (N-O) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.35 and 9.30 (2

H, 2 br s, $NH_2$), 8.60 (1 H, d, 6-H), 8.43 (1 H, s, 3-H), 8.18-8.13 (3 H, m) and 7.95-7.91 (2 H, m); $\delta_C$ ($D_6$-DMSO) 164.3 (-*C* ($NH_2$)=C($CN)_2$), 143.3 (C-3), 141.1 (C-4), 138.7 (C-12), 131.7 (C-10), 130.8 (C-8 or C-9), 130.7 (C-7), 129.1 (C-8 or C-9), 125.2 (C-6), 120.7 (C-5), 119.3 (C-11), 115.3, 114.5, 113.9 (all CN) 107.8 (C-2) and 50.6 (-C ($NH_2$)=*C*($CN)_2$); *m/z* 287 ($M^+$, 16.5), 273 (23.2), 271 (57.0), 245 (73.3) and 207 (100%) (Found: $M^+$, 287.0823; $C_{16}H_9N_5O$ requires *M*, 287.0806)

## 7.4 Experimental to Chapter 6

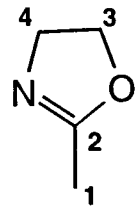
### 7.4.1 General Procedures:

***Method A-Preparation of oxazolines from nitriles:***[153] The nitrile (1 mol) was heated at reflux with the appropriate amino alcohol (1-1.5 mol) and cadmium acetate (0.025 mol) for 25 h. Ammonia was evolved during the course of the reaction and progress was monitored by taking the pH of the exit gases from the condenser. The oxazolines were recovered by distillation of the reaction mixture at atmospheric pressure.

***Method B(i)-Preparation of 2-hydroxyamides:***[156,166] The acid chloride (50 mmol) was dissolved in DCM (25 ml) and added dropwise with stirring to an ice-cooled solution of the appropriate amino alcohol (0.1 mol) in DCM (25 ml). After stirring at RT for 4 h, the reaction mixture was filtered and the solvent removed under reduced pressure to give the amide in good to excellent yields.

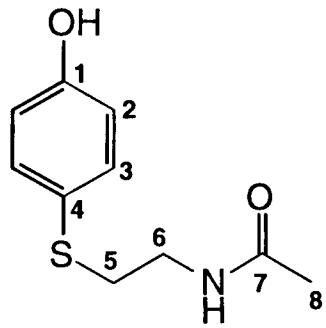
***Method B(ii)-Preparation of oxazolines by cyclisation of 2-hydroxyamides:***[156,166] Thionyl chloride (60 mmol) was added dropwise with stirring to an ice-cooled solution of the 2-hydroxyamide (20 mmol) in DCM (20ml). After 2 h reaction time, during which HCl was evolved, the solvent was removed under reduced pressure leaving the oxazoline as its hydrochloride salt. The oily solid was dissolved in water (5 ml), neutralised with NaOH (1M) and extracted with EtOAc (3 x 10 ml). The organic extracts were dried and concentrated under reduced pressure to yield the desired oxazoline as a yellow oil.

**2-Methyl-2-oxazoline (145).**[154]




Acetonitrile (20.5 g, 0.5 mol), ethanolamine (39.7 g, 0.65 mol) and cadmium acetate (3.33 g, 12 mmol) were reacted according to method A. Distillation of the reaction mixture at atmospheric pressure yielded oxazoline **145** (22.720 g, 54%) as a colourless liquid, b.p. 108-110 °C (lit.,[154] 109.5-110.5 °C); $R_f$ 0.32 (EtOAc); $\nu_{max}$ (KBr) 2976, 2938 (aliphatic C-H), 1674 (C=N) and 1228 (imidic ester C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 3.48 (2 H, t, $^3J$= 9.5, 3-H), 3.05 (2 H, t, $^3J$= 9.4, 4-H) and 1.21 (3 H, s, 1-H); $\delta_C$ ($CDCl_3$) 164.4 (C-2), 66.5 (C-3), 53.8 (C-4) and 12.8 (C-1); *m/z* 85 ($M^+$, 40.7), 55 (100) and 43 (20.4%) (Found: $M^+$, 85.0528. $C_4H_7NO$ requires *M*, 85.0532).

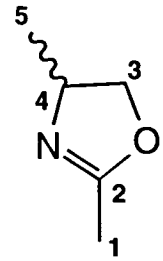
***N*-{2-[(4-Hydroxyphenyl)thio]ethyl}acetamide (142).**




The title compound was synthesised as described previously in the literature.[144] 4-Hydroxythiophenol (3.402 g, 27 mmol) and oxazoline **145** (2.55 g, 30 mmol) were heated at reflux under $N_2$ for 2 h at 130 °C. Upon cooling, the viscous reaction mixture was dissolved in hot EtOH (20 ml) and diluted with water (50 ml) to precipitate a white solid which was filtered off and recrystallised to yield white crystals of amide **142** (5.24 g, 92%), m.p. 124-125 °C (from EtOAc, lit.,[144] 123-125 °C); $R_f$ 0.2 (EtOAc); $\nu_{max}$ (KBr) 3418m (N-H), 3038 (aromatic C-H), 2960 (aliphatic C-H), 1636s (amide I), 1580 (C=C), 1522 (amide II) and 834 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-acetone) 7.34 (2 H, AA'BB', $^3J$= 8.7, 3-H), 6.83 (2 H, AA'BB', $^3J$= 8.7, 2-H), 3.34 (2 H, t, $^3J$= 6.6, 6-H), 2.90 (2 H, t, $^3J$= 6.5, 5-H) and 1.89 (3 H, s, 8-H); $\delta_C$ ($D_6$-acetone) 170.8 (C-7), 157.9 (C-1), 134.5 (C-3), 124.6 (C-4), 116.8 (C-2), 39.5 (C-6), 35.53 (C-5) and 22.7 (C-8); *m/z* 211 ($M^+$, 19.5), 152 (90.6), 139 (16.3), 125 (23.1) and 107 (16.7%) (Found: $M^+$, 211.0666; C, 56.87; H,

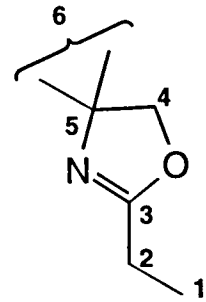
6.17; N, 6.62%. $C_{10}H_{13}NO_2S$ requires *M*, 211.0666; C, 56.85; H, 6.21; N, 6.63%).

**(*R,S*)-2,4-Dimethyl-2-oxazoline (146).**[158]



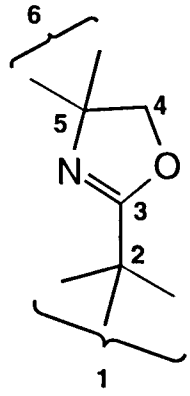

Acetonitrile (1.845 g, 45 mmol), 2-amino-propan-1-ol (4.506 g, 60 mmol) and cadmium acetate (0.300 g, 1.1 mmol) were reacted according to method A to yield oxazoline **146** (2.182 g, 49%) as a colourless oil, b.p. 115-117 °C (lit.,[158] 116-117 °C); $R_f$ 0.38 (EtOAc); $\nu_{max}$ (KBr) 2970, 2930 (aliphatic C-H), 1674 (C=N) and 1234 (imidic ester C-O) $cm^{-1}$; $\delta_H$ (neat) 4.41-3.62 (3 H, m, 4-H and 3-H), 1.86 (3 H, s, 1-H) and 1.16 (3 H, d, $^3J$= 6.0, 5-H); *m/z* 99 (*M*$^+$, 0.6), 86 (22.8), 60 (10.0) and 44 (100%).

**2-Ethyl-4,4-dimethyl-2-oxazoline (147).**



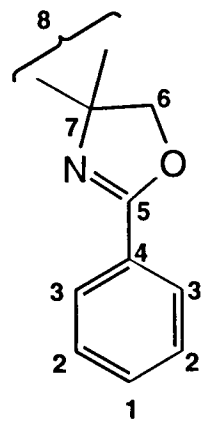

Propanonitrile (11.016 g, 0.2 mol), 2-amino-2-methyl-1-propanol (18.00 g, 0.2 mol) and cadmium acetate (1.332 g, 5 mmol) were reacted together according to method A. The reaction mixture was distilled at atmospheric pressure to yield oxazoline **147** (17.36 g, 68%) as a colourless liquid, b.p. 125-127 °C (lit.,[159] 129-130 °C); $R_f$ 0.56 (EtOAc); $\nu_{max}$ (KBr) 2971 (aliphatic C-H), 1667 (C=N) and 1195 (imidic ester C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 3.60 (2 H, s, 4-H), 1.94 (2 H, q, $^3J$= 7.6, 2-H), 0.96 (6 H, s, 6-H) and 0.87 (3 H, t, $^3J$= 7.6, 1-H); $\delta_C$ ($CDCl_3$) 166.6 (C-3), 78.5 (C-4), 66.3 (C-5), 27.9 (C-6), 21.1 (C-2) and 9.9 (C-1); *m/z* 127 (*M*$^+$, 12.5), 112 (72.4), 97 (44.4) and 84 (23.9%) (Found: *M*$^+$, 127.1004; $C_7H_{13}NO$ requires *M*, 127.0997).

**2-$^{t}$Butyl-4,4-dimethyl-2-oxazoline (148).**




Pivaloyl chloride (6.029 g, 50 mmol) and 2-amino-2-methyl-1-propanol (8.903 g, 0.1 mol) were reacted according to method B(i) to give a quantitative yield of *N*-(1,1-dimethyl-2-hydroxyethyl)-2,2-dimethyl propanamide. This amide (3.0 g, 17.3 mmol) was cyclised according to method B(ii) by reaction with thionyl chloride (6.54 g, 55 mmol) yielding oxazoline **148** (1.805 g, 69%) as a yellow oil, b.p. 131-135 °C (lit.,[160] 135 °C); $R_f$ 0.65 (EtOAc); $\nu_{max}$ (KBr) 2970 (aliphatic C-H), 1658 (C=N) and 1122 (imidic ester C-O) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 3.45 (2 H, s, 4-H), 0.83 (6 H, s, 6-H) and 0.78 (9 H, s, 1-H); $\delta_C$ ($CDCl_3$) 171.7 (C-3), 78.5 (C-4), 66.3 (C-5), 59.8 (C-2), 27.8 (C-6) and 27.3 (C-1); *m/z* 155 ($M^+$, 17.8), 140 (100), 125 (16), 110 (12.5) and 84 (57.6%).

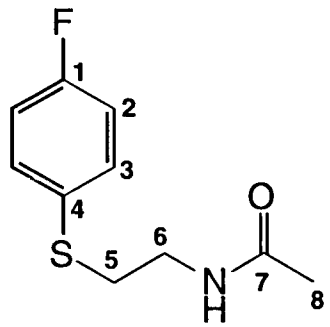
**4,4-Dimethyl-2-phenyl-2-oxazoline (149).**[160]




Benzoyl chloride (7.025 g, 50 mmol) and 2-amino-2-methyl-1-propanol (8.90 g, 0.1 mol) were reacted according to method B(i) to give a quantitative yield of *N*-(1,1-dimethyl-2-hydroxyethyl)-benzamide. This amide (5 g, 26 mmol) was cyclised according to method B(ii) by reaction with thionyl chloride (9.282 g, 78 mmol) yielding oxazoline **149** (3.18 g, 70%) as a pleasant

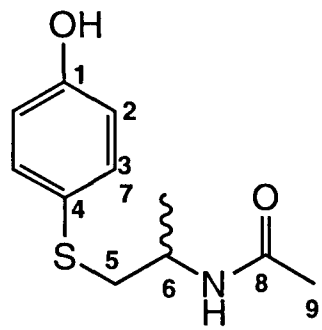
smelling orange oil; $R_f$ 0.59 (EtOAc); $\nu_{max}$ (KBr) 3032 (aromatic C-H), 2968 (aliphatic C-H), 1650 (C=N) and 696 (monosub. benzene) and 1062 (C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.83 (2 H, dd, 3-H), 7.33-7.19 (3 H, m, 2-H and 1-H), 3.93 (2 H, s, 6-H) and 1.24 (6 H, s, 8-H); $\delta_C$ ($CDCl_3$) 161.9 (C-5), 131.1 (C-1), 128.3 (C-2 & C-3), 127.9 (C-4), 78.9 (C-6), 67.3 (C-7) and 28.3 (C-8); *m/z* 175 ($M^+$, 2.0), 162 (30.5), 122 (5.8) and 105 (100%).

**<u>*N*-{2-[(4-Fluorophenyl)thio]ethyl}acetamide (150).</u>**



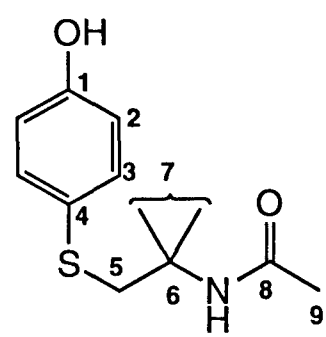

4-Fluorothiophenol (1.154 g, 9.2 mmol) and oxazoline **145** (1.507 g, 17.7 mmol) were heated at reflux under $N_2$ for 2 h at 120 °C. Upon cooling, an orange solid precipitated which was recrystallised twice to yield large white crystals of amide **150** (1.526 g, 70%), m.p. 96-97.5 °C (from EtOAc); $R_f$ 0.23 (EtOAc); $\nu_{max}$ (KBr) 3287 (N-H), 2980 (aliphatic C-H), 1637 (amide I), 1560 (amide II), 1494 (C=C), 1242 (C-F) and 822 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.38 (2 H, m, 3-H ) 7.00 (2 H, m, 2-H ) 6.53 (1 H, br s, N-H), 3.39 (2 H, m, 6-H), 3.00 (2 H, t, $^3J$= 6.6, 5-H) and 1.94 (3 H, s, 8-H); $\delta_C$ ($CDCl_3$) 170.4 (C-7), 164.3 and 159.4 (d, $J_{CF}$=245, C-1), 132.6 and 132.5 (d, $^4J_{CF}$=8, C-3), 129.9 (C-4), 116.3 and 115.9 (d, $^3J_{CF}$=22, C-2) 38.6 (C-6), 34.4 (C-5) and 23.0 (C-8); *m/z* 213 ($M^+$, 11.4), 154 (100), 141 (12.6), 127 (19.6) and 109 (17.8%) (Found: $M^+$, 213.0607; C, 56.21; H, 5.68; N, 6.59%. $C_{10}H_{12}FNOS$ requires *M*, 213.0623; C, 56.32; H, 5.67; N, 6.57%).

**(R,S)-*N*-{[(2-(4-Hydroxyphenyl)thio)-1-methyl]ethyl}acetamide (151).**




The title compound has been prepared previously[144] but has not been fully characterised. 4-Hydroxythiophenol (1.134 g, 9 mmol) and oxazoline **146** (0.99 g, 10 mmol) were heated at reflux under $N_2$ for 2 h at 130 °C. After cooling, the crude product was precipitated by addition of DCM (10 ml), filtered and dried under suction. Recrystallisation yielded amide **151** (1.375 g, 61%) as white crystals, m.p. 118-119 °C (from EtOAc, lit.,[144] 120-122 °C); $R_f$ 0.21 (EtOAc); $\nu_{max}$ (KBr) 3311 (N-H), 2976 (aliphatic C-H), 1632 (amide I), 1598, 1581 and 1494 (C=C), 1548 (amide II) and 833 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-acetone) 8.91 (1 H, s, O-H), 7.28 (2 H, AA'BB', $^3J$= 8.8, 3-H), 7.18 (1 H, s, N-H), 6.76 (2 H, AA'BB', $^3J$= 8.8, 2-H), 4.01 (1 H, m, 6-H), 2.86 (2 H, ABX, $^2J$= 13.4, $^3J$= 6.2 and 6.7, 5-H), 1.85 (3 H, s, 9-H) and 1.15 (3 H, d, $^3J$= 6.7, 7-H) ; $\delta_C$ ($D_6$-acetone) 170.3 (C=O), 157.6 (C-1), 133.9 (C-3), 124.9 (C-4), 116.5 (C-2), 45.3 (C-6), 41.9 (C-5), 22.8 (C-9) and 19.46 (C-7); *m/z* 225 ($M^+$, 28.2), 166 (100), 140 (19.5), 125 (25.3) and 100 (34.6%). (Found: $M^+$, 225.0824; C, 58.52; H, 6.42; N, 6.13%. $C_{11}H_{15}NO_2S$ requires *M*, 225.0823; C, 58.64; H, 6.72; N, 6.22%).

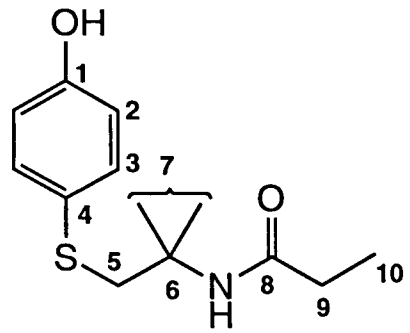
***N*-{[(2-(4-Hydroxyphenyl)thio)-1,1-dimethyl]ethyl}acetamide (152).**




4-Hydroxythiophenol (2.268 g, 18 mmol) and 2,4,4-trimethyloxazoline(2.260 g, 20 mmol) were heated at reflux under $N_2$ for 5 h at 120 °C. After cooling,

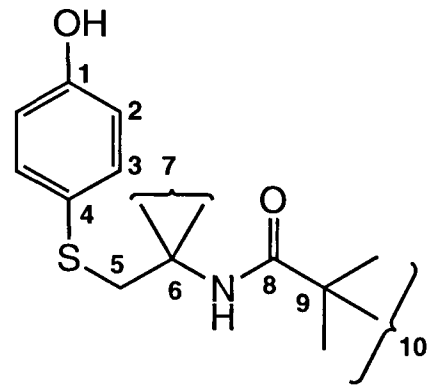
the reaction mixture was dissolved in EtOH (10 ml) and diluted with water (20 ml) to give a white precipitate. This was filtered off, dissolved in acetone and pre-adsorbed onto silica. Flash column chromatography on silica with EtOAc as eluent, followed by recrystallisation afforded white crystals of compound **152** (2.249 g, 52%), m.p. 108-110 °C (from EtOAc); $R_f$ 0.40 (EtOAc); $\nu_{max}$ (KBr) 3307 (N-H), 2966 (aliphatic C-H), 1636 (amide I), 1601, 1583, 1559 (amide II), 1494 and 823 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-acetone) 9.04 (1 H, br s, N-H), 7.12 (2 H, AA'BB', d, $^3J$= 8.6, 3-H), 7.02 (1 H, br s, 0-H), 6.59 (2 H, AA'BB', $^3J$= 8.6, 2-H), 3.19 (2 H, s, 5-H), 1.59 (3 H, s, 9-H) and 1.18 (6-H, s, 7-H); $\delta_C$ ($D_6$-acetone)171.6 (C=O), 157.8 (C-1), 134.3 (C-3), 126.5 (C-4), 116.9 (C-2), 55.0 (C-6), 45.8 (C-5), 27.1 (C-7) and 23.7 (C-9); *m/z* 239 ($M^+$, 3.8), 180 (29.5), 140 (4.0), 125 (8.9) and 100 (10.9%) (Found: $M^+$, 239.0984; $C_{12}H_{17}NO_2S$ requires *M*, 239.0979).

**<u>*N*-{[(2-(4-Hydroxyphenyl)thio)-1,1-dimethyl]ethyl}propanamide (153).</u>**



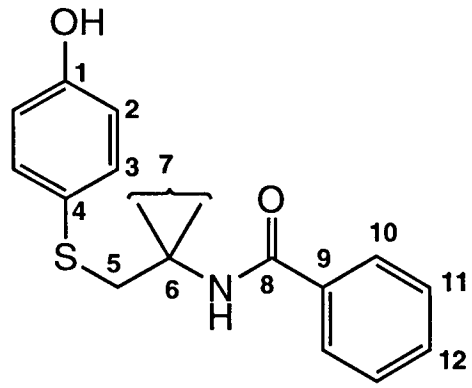

4-Hydroxyphenol (1.80 g, 14 mmol) and 4,4-dimethyl-2-ethyl oxazoline (2.00 g, 15 mmol) were heated at reflux under $N_2$ for 4 h at 120 °C. Upon cooling, the reaction mixture was dissolved in EtOH (10 ml) and diluted with water (20 ml) to give a white precipitate of the crude product. This was filtered off and recrystallised to give white crystals of amide **153** (1.788 g, 52%), m.p. 112-113 °C (from EtOAc); $R_f$ 0.37 (EtOAc); $\nu_{max}$ (KBr) 3321 (N-H), 1636 (amide I), 1598, 1579, 1558 (amide II), 1494 (C=C) and 822 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 9.01 (1 H, br s, O-H) 7.20 (2 H, AA'BB', $^3J$= 8.5, 3-H) 6.70 (2 H, AA'BB', $^3J$= 8.5, 2-H) 5.61 (1 H, br s, N-H) 3.25 (2 H, s, 5-H) 1.93 (2 H, q, $^3J$= 7.6, 9-H), 1.31 (6 H, s, 7-H) and 0.93 (3 H, t, $^3J$= 7.5, 10-H); $\delta_C$ ($CDCl_3$) 174.6 (C=O), 156.5 (C-1), 133.4 (C-3), 125.1 (C-4), 116.2 (C-2), 54.5 (C-6), 45.5 (C-5), 30.2 (C-9), 26.8 (C-7) and 9.8 (C-10); *m/z* (Found: $M^+$, 253.1158; C, 61.47; H, 7.58; N, 5.49%. $C_{13}H_{19}NO_2S$ requires *M*, 253.1132; C, 61.63; H, 7.57; N, 5.53%).

***N*-{[(2-(4-Hydroxyphenyl)thio)-1,1-dimethyl]ethyl}2,2-dimethyl propanamide (154).**



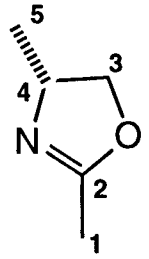

4-Hydroxythiophenol (0.731 g, 5.8 mmol) was heated at reflux with oxazoline **148** (1.00 g, 6.4 mmol) under $N_2$ for 2 h at 130 °C. TLC indicated residual thiol, so the reaction mixture was dissolved in EtOAc (10 ml) and shaken twice with $Na_2CO_3$ solution (1 M, 2 x 10 ml). After drying, the organic extract was concentrated yielding the crude product (1.145 g, 70%). Recrystallisation gave colourless rhombic crystals of compound **154** (0.771 g, 47%), m.p. 170-172 °C (from EtOH); $R_f$ 0.69 (EtOAc); $\nu_{max}$ (KBr) 3397m (N-H), 2970 (aliphatic C-H), 1620 (amide I), 1601, 1581 and 1496 (C=C), 1525 (amide II) and 827 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.53 (1 H, br s, O-H), 7.20 (2 H, AA'BB', $^3J$= 8.6, 3-H), 6.70 (2 H, AA'BB', $^3J$= 8.6, 2-H), 6.58 (1 H, br s, N-H), 3.29 (2 H, s, 5-H), 1.28 (6 H, s, 7-H) and 1.06 (9 H, s, 10-H); $\delta_C$ ($D_6$-DMSO) 177.3 (C-8), 156.5 (C-1), 132.4 (C-3), 125.3 (C-4), 116.1 (C-2), 53.5 (C-6), 45.0 (C-5), 38.6 (C-9), 27.5 (C-10) and 26.6 (C-7); *m/z* 281 ($M^+$, 2.2), 180 (49.6), 142 (11.4) and 125 (27.3%) (Found: $M^+$, 281.1445; C, 64.14; H, 8.26; N, 4.87; S, 11.55%. $C_{15}H_{23}NO_2S$ requires *M*, 281.1449; C, 64.02; H, 8.24; N, 4.98; S, 11.37%).

***N*-{[(2-(4-Hydroxyphenyl)thio)-1,1-dimethyl]ethyl}benzamide (155).**




4-Hydroxythiophenol (0.454 g, 3.6 mmol) was heated at reflux with oxazoline **149** (0.700g, 4 mmol) in DMF (5 ml) for 24 h at 120 °C. Upon cooling, the reaction mixture was dissolved in EtOAc (25 ml) and washed with brine (2 x 25 ml) and water (2 x 25 ml). After drying, the organic layer was concentrated and the residual solid was dissolved in EtOH (10 ml). Upon addition of water (20 ml) an oily solid precipitated which crystallised upon vigorous scratching. This solid was recovered and recrystallised to yield benzamide **155** (0.225 g, 21%) as white crystals, m.p. 117-119 °C (from EtOAc); $R_f$ 0.79 (EtOAc); $\nu_{max}$ (KBr) 3380 (N-H), 3109 br (Ar-H), 1633 (amide I),1602, 1575, 1533 (amide II), 1493, 817 (*para*-disubstituted benzene) and 715 (monosub. benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.22 (1 H, br s, O-H), 7.54 (2 H, dd, 10-H), 7.48-7.35 (3 H, m, 11-H &12-H), 7.26 (2 H, AA'BB', 3-H), 6.77 (2 H, AA'BB', 2-H), 6.30 (1 H, br s, N-H), 3.39 (2 H, s, 5-H) and 1.54 (6 H, s, 7-H); $\delta_C$ ($CDCl_3$) 168.2 (C-8), 156.3 (C-1), 134.8 (C-9), 133.5 (C-3), 131.5 (C-12), 128.5 (C-10), 126.7 (C-11), 125.2 (C-4), 116.4 (C-2), 55.2 (C-6), 46.7 (C-5), 26.7 (C-7); *m/z* 301 ($M^+$, 1.6), 180 (35.0), 162 (8.9), 125 (11.1) and 105 (100%) (Found: $M^+$, 301.1125; C, 67.49; H, 6.18; N, 4.59%. $C_{17}H_{19}NO_2S$ requires *M*, 301.1135; C, 67.75; H, 6.36; N, 4.65%).

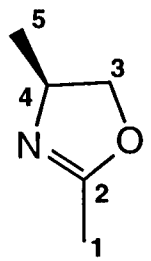
***(R)*-2,4-Dimethyl-2-oxazoline (157a).**




This compound has previously been synthesised[161] but has not been fully characterised. Acetonitrile (0.652 g, 16 mmol), *(R)*-2-amino-1-propanol

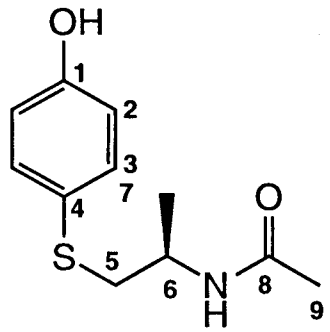
(1.196 g, 16 mmol) and cadmium acetate (0.106 g, 0.4 mmol) were reacted according to method A. Distillation of the reaction mixture at atmospheric pressure yielded oxazoline **157a** (0.538 g, 34%) as a colourless liquid, b.p. 65-70 °C; $[\alpha]_D^{22}$ +80.7 ° (*c* = 3.28 in MeOH); $R_f$ 0.39 (EtOAc); $\nu_{max}$ (KBr) 2972 (aliphatic C-H), 1674 (C=N) and 1236 (imidic ester C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 4.28-3.62 (2 H, m, ABX, 3-H), 4.02 (1 H, m, 4-H), 1.87 (3 H, s, 1-H) and 1.15 (3 H, d, $^3J$= 6.5, 5-H); $\delta_C$ ($CDCl_3$) 164.2 (C-2), 73.7 (C-3), 61.2 (C-4), 21.2 (C-5) and 13.6 (C-1); *m/z* 99 ($M^+$, 33.7), 84 (57.2), 69 (68.9) and 56 (42.7%) (Found: $M^+$, 99.0680; $C_5H_9NO$ requires *M*, 99.0682).

***(S)*-2,4-Dimethyl-2-oxazoline (157b).**



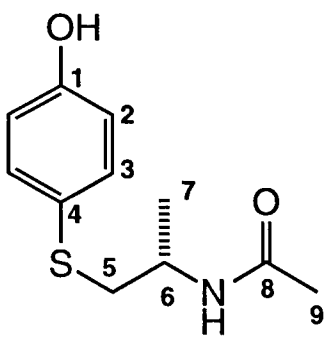

This compound has previously been synthesised[161,162] but has not been fully characterised. Acetonitrile (1.165 g, 27 mmol), *(S)*-2-amino-1-propanol (2.136 g, 28 mmol) and cadmium acetate (1.866 g, 7 mmol) were reacted together according to method A. Distillation of the reaction mixture at atmospheric pressure yielded **157b** (0.327 g, 12%) as a colourless liquid, b.p. 65-70 °C; $[\alpha]_D^{20}$ = -80.2 ° (*c*= 2.43, MeOH); $R_f$ 0.39 (EtOAc); $\nu_{max}$ (KBr) 2972, 2930 (aliphatic C-H), 1672 (C=N) and 1234 (imidic ester C-O) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 4.30-3.64 (2 H, m, ABX, 3-H), 4.05 (1 H, m, 4-H), 1.89 (3 H, s, 1-H) and 1.17 (3 H, d, $^3J$= 6.5, 5-H); $\delta_C$ ($CDCl_3$) 164.5 (C-2), 73.9 (C-3), 61.3 (C-4), 21.3 (C-5) and 13.8 (C-1); *m/z* 99 ($M^+$, 30.2), 84 (50.8), 69 (65.3) and 56 (38.6%) (Found: $M^+$, 99.0687; $C_5H_9NO$ requires *M*, 99.0682).

***(R)-N*-{[(2-(4-Hydroxyphenyl)thio)-1-methyl]ethyl}acetamide (159a).**



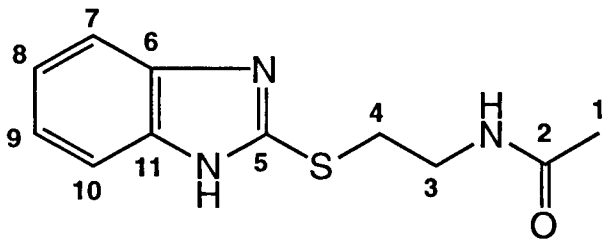

This compound has previously been prepared[161] but has not been fully characterised. 4-Hydroxythiophenol (0.567 g, 4.5 mmol) and oxazoline **157a** (0.445 g, 4.5 mmol) were heated at reflux under $N_2$ at 120 °C for 24 h. The reaction mixture was dissolved in acetone (50 ml), pre-adsorbed onto silica and subjected to flash column chromatography on silica with EtOAc as eluent. The oily product was triturated in hexane for 72 h yielding white crystals of amide **159a** (0.583 g, 58%), m.p. 60-62 °C; $[\alpha]_D^{22}$= -30.0 ° (*c* = 0.97 in MeOH); $R_f$ 0.2 (EtOAc); $\nu_{max}$ (KBr) 3401 and 3349 (N-H), 1636 (amide I), 1578, 1494 (C=C), 1545 (amide II) and 825 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.71 (1 H, br s, O-H), 7.27 (2 H, AA'BB', $^3J$= 8.6, 3-H), 6.75 (2 H, AA'BB', $^3J$= 8.6, 2-H), 5.62(1 H, br d, N-H), 4.05 (1 H, m, 6-H), 2.82 (2 H, d, $^3J$= 6.0, 5-H), 1.82 (3 H, s, 9-H) and 1.16 (3 H, d, $^3J$= 6.6, 7-H); $\delta_C$ 170.9 (C=O), 156.9 (C-1), 133.8 (C-3), 123.8 (C-4), 116.3 (C-2), 45.2 (C-6), 41.5 (C-5), 23.0 (C-9) and 19.4 (C-7); *m/z* 225 ($M^+$, 12.5), 166 (77.1), 151 (8.2), 140 (9.2), 125 (13.3) and 100 (16.8%) (Found: $M^+$, 225.0829; C, 58.76; H, 6.46; N, 6.12%. $C_{11}H_{15}NO_2S$ requires *M*, 225.0823; C, 58.64; H, 6.72; N, 6.22%).

***(S)-N*-{[(2-(4-Hydroxyphenyl)thio)-1-methyl]ethyl}acetamide (159b).**



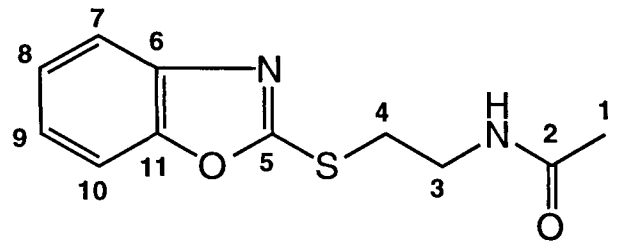
This compound has previously been prepared[161] but has not been fully characterised. 4-Hydroxythiophenol (0.176 g, 1.4 mmol) and oxazoline **157b** (0.140 g, 1.4 mmol) were heated at reflux under $N_2$ for 2 h at 120 °C. The reaction mixture was dissolved in acetone (50 ml) and pre-adsorbed onto silica. Column chromatography on silica with EtOAc-petroleum ether (1:1) as the eluent afforded **159b** as a yellow oil. Trituration in hexane for 48 h gave white crystals of amide **159b** (0.18 g, 57%), m.p. 60-62 °C; $[\alpha]_D^{22}$ +30.9 ° (*c* = 0.53 in MeOH); $R_f$ 0.2 (EtOAc); $\nu_{max}$ (KBr) 3401 and 3348 (N-H), 1636 (amide I), 1595, 1578 and 1494 (C=C), 1545 (amide II) and 825 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 8.80 (1 H, br s, O-H), 7.26 (2 H, AA'BB', $^3J$= 8.6, 3-H), 6.73 (2 H, AA'BB', $^3J$= 8.6, 2-H), 5.66 (1 H, br d, N-H), 4.04 (1 H, m, 6-H), 2.82 (2 H, d, $^3J$= 6.0, 5-H), 1.82 (3 H, s, 9-H) and 1.16 (3 H, d, $^3J$= 6.6, 7-H); $\delta_C$ 170.8 (C=O), 156.9 (C-1), 133.7 (C-3), 123.8 (C-4), 116.3 (C-2), 45.2 (C-6), 41.5 (C-5), 23.0 (C-9) and 19.4 (C-7); *m/z* 225 ($M^+$, 29.3), 166 (100), 151 (16.3), 140 (21.7), 125 (32.0) and 100 (30.9%) (Found: $M^+$, 225.0818; $C_{11}H_{15}NO_2S$ requires *M*, 225.0823).

***N*-{2-[(2-Benzimidazolyl)thio]ethyl}acetamide (161).**



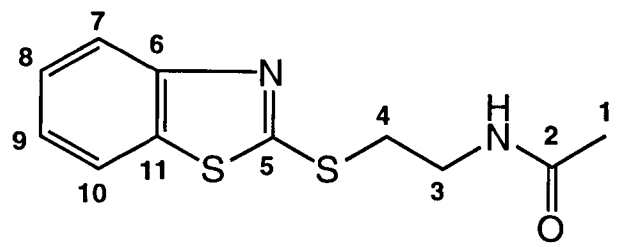

2-Mercaptobenzimidazole (1.00 g, 6.6 mmol) was heated at reflux with oxazoline **145** (4 ml) under $N_2$ for 4 h between 90-100 °C. Upon cooling, needle like crystals of amide **161** formed (0.150 g, 10%). The reaction mixture was concentrated and the residual solid was recrystallised to yield a second crop of **161** (0.295 g, 19%), m.p. 247-249 °C (EtOAc); $R_f$ 0.77 (EtOAc); $\nu_{max}$ (KBr) 3155 (N-H, H-bonded), 1666 (amide I), 1514, 1468 (C=C), 1238 (C-S) and 742 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_4$-methanol) 7.23-7.12 (4 H, m, phenyl), 5.02 (2 H, br s, 2 x N-H), 4.28 (2 H, t, $^3J$= 9.6, 3-H), 3.76 (2 H, t, $^3J$= 9.5, 4-H) and 1.94 (3 H, s, 1-H); $\delta_C$ ($D_6$-DMSO) 168.2 (C-2), 163.9 (C-5), 132.4 (C-6 & C-11), 122.4 (C-8 & C-9), 109.6 (C-7 & C-10), 66.9 (C-3), 54.1 (C-4) and 13.5 (C-1); *m/z* 235 ($M^+$, 0.3), 150 (100), 118 (22.4) and 106 (28.7%) (Found: $M^+$, 235.0789; C, 55.98; H, 5.31; N, 17.88%. $C_{11}H_{13}N_3OS$ requires *M*, 235.0779; C, 56.15; H, 5.57; N, 17.87%).

***N*-{2-[(2-benzoxazolyl)thio]ethyl}acetamide (162).**




2-Mercaptobenzoxazole (1.00 g, 6.6 mmol) was heated at reflux with oxazoline **145** (4 ml) under $N_2$ for 4 h between 90-100 °C. The reaction mixture was concentrated and the solid product was recrystallised to yield crude **162** (1.469 g, 94%) as cream coloured crystals, m.p. 91-92 °C (from EtOAc); $R_f$ 0.16 (EtOAc); $\nu_{max}$ (KBr) 3308 (N-H), 3024 (aromatic C-H), 2963 (aliphatic C-H), 1649 (amide I), 1556 (amide II), 1506 (C=C), 1236 (C-S) and 744 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.59 (1 H, m, dd, 7-H or 10-H), 7.45 (1 H, m, dd, 10-H or 7-H), 7.27 (2 H, m, 8-H & 9-H), 6.74 (1 H, br s, N-H), 3.71 (2 H, m, 3-H), 3.45 (2 H, t, 4-H) and 1.97 (3 H, s, 3-H); $\delta_C$ ($CDCl_3$) 170.6 (C=O), 164.8 (C-5), 151.9 (C-11), 141.5 (C-6), 124.5 (C-8), 124.2 (C-9), 118.3 (C-7), 110.0 (C-10), 39.7 (C-3), 31.7 (C-4) and 23.1 (C-1); *m/z* 236 ( $M^+$, 1.4), 177 (17.0), 151 (13.0) and 122 (27.4%) (Found: $M^+$, 236.0616; C, 55.82; H, 4.98; N, 11.84%. $C_{11}H_{12}N_2O_2S$ requires *M*, 236.0619; C, 55.91; H, 5.12; N, 11.86%).

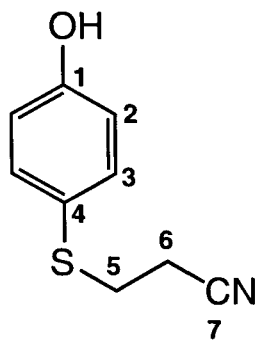
***N*-{2-[(2-benzothiazolyl)thio]ethyl}acetamide (163).**




2-Mercaptobenzothiazole (1.00 g, 5.98 mmol) was heated at reflux with oxazoline **145** (4 ml) under $N_2$ for 4 h between 90-100 °C. The reaction mixture was concentrated and the solid product was recrystallised to yield amide **163** (0.593g, 40%) as white needles, m.p. 88-89 °C (EtOAc); $R_f$ 0.19 (EtOAc); $\nu_{max}$ (KBr) 3298 (N-H), 2984 (aliphatic C-H), 1642 (amide I), 1544 (amide II), 1236 (C-S) and 756 (*ortho*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.80 (1 H, dd, $^3J$= 8.1, 7-H), 7.72 (1 H, dd, $^3J$= 8.0, 10-H), 7.39 (1 H, ddd, 8-H), 7.20 (1 H, ddd, 9-H), 7.03 (1 H, br s, N-H), 3.64 (2 H, m, 3-H), 3.45, (2 H, t, 4-H), 1.94 (3 H, s, 1-H); $\delta_C$ ($CDCl_3$) 170.6 (C=O), 166.8 (C-5), 152.7 (C-11), 135.3 (C-6), 126.2 (C-8), 124.5 (C-9), 121.2 (C-10 or C-7), 121.1 (C-

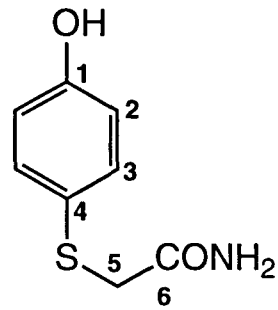
7 or C-10), 40.0 (C-3), 32.9 (C-4) and 23.1 (C-1); *m/z* 252 ($M^+$, 1.0), 192 (11.1), 180 (8.4), 167 (14.7), 136 (16.0) and 108 (29.2%) (Found: $M^+$, 252.0392; C, 52.24; H, 4.77; N, 11.07%. $C_{11}H_{12}N_2OS_2$ requires *M*, 252.0391; C, 52.37; H, 4.79; N, 11.11%).

**3-[(4-Hydroxyphenyl)thio]propanonitrile (165).**



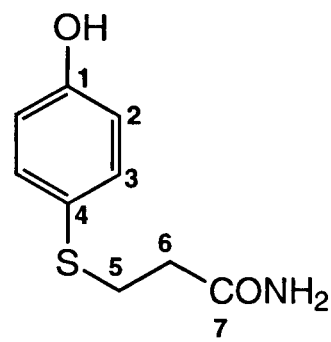

4-Hydroxythiophenol (0.609 g, 4.8 mmol) was dissolved in acetonitrile (5 ml) and stirred with NaOH (2M, 2.5 ml) for 10 min. Bromopropionitrile (0.709 g, 5.3 mmol) was introduced and the reaction mixture was heated at reflux for 3 h. After cooling, the reaction mixture was acidified with HCl (1 M) to pH 3 and extracted with $CHCl_3$ (3 x 20 ml). After drying, the organic extracts were concentrated under reduced pressure and the crude product pre-adsorbed onto silica. Chromatography (silica) with petroleum ether-EtOAc (1:1) as eluent, followed by recrystallisation, afforded nitrile **165** (0.496 g, 58%), m.p. 69-70 °C (from benzene, lit.,[163] 72-73 °C); $R_f$ 0.84 (EtOAc); $\nu_{max}$ (KBr) 3325br (O-H), 3007w (aromatic C-H), 2928w (aliphatic C-H), 2266m (CN), 1599, 1579, 1493 (C=C) and 829 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($CDCl_3$) 7.27 (2 H, AA'BB', $^3J$= 8.7, 3-H), 6.76 (2 H, AA'BB', $^3J$= 8.7, 2-H), 6.04 (1 H, br s, O-H), 2.91 (2 H, t, $^3J$= 7.2, 5-H) and 2.45 (2 H, t, $^3J$= 7.1, 6-H) ; $\delta_C$ ($CDCl_3$) 156.5 (C-1), 135.4 (C-3), 122.8 (C-4), 118.2 (C-7), 116.5 (C-2), 31.8 (C-5), 16.2 (C-6); *m/z* 179 ($M^+$, 56.7), 139 (86.6), 125 (59.5) and 97 (61.0%) (Found: $M^+$, 179.0381; C, 60.47; H, 5.24; N, 7.98%. $C_9H_9NOS$ requires *M*, 179.0404; C, 60.32; H, 5.03; N, 7.82%).

**2-[(4-Hydroxyphenyl)thio]acetamide (166).**




Amide **166** was prepared by partial hydrolysis of nitrile **165** according to established methodology.[164] The nitrile (0.490 g, 3.0 mmol) was added with vigorous stirring to HCl (35%, 5 ml) maintained at a temperature of 40 °C. After 40 min, the flask was cooled to 15-20 °C and cold distilled water (5 ml) was added . The reaction mixture was extracted with EtOAc (2 x 20 ml) and washed with water (2 x 50 ml). After drying, the organic extracts were concentrated yielding a white solid which was recrystallised to give **166** (0.417 g, 76%) as white needles, m.p. 156-157 °C; $R_f$ 0.21 (EtOAc); $\nu_{max}$ (KBr) 3429 and 3179 ($NH_2$), 1670br (amide I & II), 1498 (C=C) and 825 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 9.73 (1 H, br s, O-H), 7.50 and 7.14 (2 H, 2 br s, $NH_2$), 7.30 (2 H, AA'BB', $^3J$= 8.6, 3-H), 6.77 (2 H, AA'BB', $^3J$= 8.6, 2-H) and 3.47 (2 H, s, 5-H); $\delta_C$ ($D_6$-DMSO) 170.7 (C-6), 157.3 (C-1), 132.8 (C-3), 124.0 (C-4), 116.4 (C-2) and 39.2 (C-5); *m/z* 183 ($M^+$, 47.8), 139 (47.7), 125 (25.9) and 95 (21.9%) (Found: $M^+$, 183.0362; C, 52.69; H, 4.81; N, 7.57%. $C_8H_9NO_2S$ requires *M*, 183.0354; C, 52.44; H, 4.95; N, 7.65%).

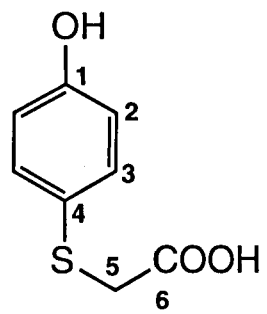
**3-[(4-Hydroxyphenyl)thio]propanamide (167).**




Compound **167** was prepared using the same method as described for compound **166**, affording a white solid which was recrystallised to give amide **167** (0.275 g, 56%) as white needles, m.p. 110-111 °C; $R_f$ 0.16 (EtOAc); $\nu_{max}$ (KBr) 3379 and 3188 ($NH_2$), 1657br (amide I & II), 1495 (C=C) and 821 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-acetone) 8.64 (1 H, br s,

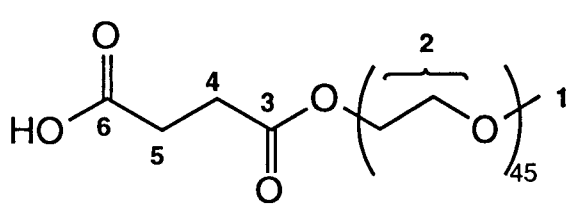
O-H), 7.28 (2 H, AA'BB', $^3J$= 8.7, 3-H), 6.96 and 6.45 (2 H, 2 br s, $NH_2$), 6.80 (2 H, AA'BB', $^3J$= 8.7, 2-H), 3.01 (2 H, t, $^3J$= 7.3, 5-H) and 2.43 (2 H, t, $^3J$= 7.4, 6-H); $\delta_C$ ($D_6$-acetone) 173.6 (C-7), 157.9 (C-1), 134.5 (C-3), 125.1 (C-4), 116.9 (C-2), 36.0 (C-5) and 31.9 (C-6); *m/z* 197 ($M^+$, 50.1), 152 (11.2), 139 (12.9), 125 (54.4) and 97 (51.3%) (Found: $M^+$, 197.0509; C, 54.64; H, 5.58; N, 7.07%. $C_9H_{11}NO_2S$ requires *M*, 197.0510; C, 54.81; H, 5.62; N, 7.10%).

**2-[(4-Hydroxyphenyl)thio]acetic acid (168).**




The title compound was prepared by hydrolysis of nitrile **164** according to established methodology.[165] The nitrile (0.500 g, 3.0 mmol) was heated at reflux with NaOH (30%, 5 ml) for 3 h. Upon cooling, the reaction mixture was diluted with water (5 ml) and $H_2SO_4$ (50%, 7 ml) and extracted with EtOAc (2 x 20 ml). After drying, the organic solvents were removed *in vacuo* and the white powder obtained was recrystallised to yield acid **164** (0.279 g, 50%) as rhombic crystals, m.p. 145-146 °C (from EtOAc/hexane); $R_f$ 0.1 (EtOAc); $\nu_{max}$ (KBr) 3160br (COOH), 1693 (C=O), 1586 and 1494 (C=C), 835 (*para*-disubstituted benzene) $cm^{-1}$; $\delta_H$ ($D_6$-DMSO) 12.56 (1 H, br s, COOH), 9.69 (1 H, br s, O-H), 7.29 (2 H, AA'BB', $^3J$= 8.6, 3-H), 6.77 (2 H, AA'BB', $^3J$= 8.6, 2-H) and 3.60 (2 H, s, 5-H); $\delta_C$ ($D_6$-DMSO) 171.0 (C-6), 157.2 (C-1), 133.0 (C-3), 123.2 (C-4), 116.2 (C-2) and 37.8 (C-5); *m/z* 184 ($M^+$, 75.6), 139 (70.6), 125 (48.7) and 95 (28.9%) (Found: $M^+$, 184.0196; C, 52.06; H, 4.57%. $C_8H_8O_3S$ requires *M*, 184.0194; C, 52.17; H, 4.38%).

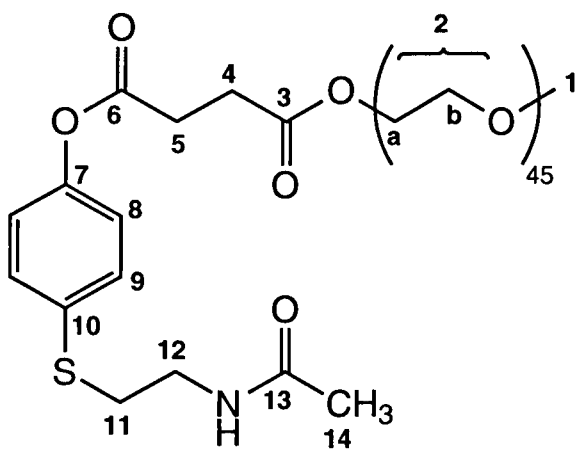
**Methoxypolyethyleneglycol (2000) succinate (MPEG-2000 succinate) (169).**




The title compound was prepared using established methodology.[105] MPEG-2000 (10 g, 5 mmol) was dissolved in toluene (50 ml) and dried by distilling

off most of the toluene. Succinic anhydride (20 g, 20 mmol) was added and the mixture was stirred for 5 h on an oil bath at 150 °C. The mixture was cooled, concentrated and purified from excess succinic anhydride by repeated recrystallisation from toluene. The crude product was subjected to filtration through Sephadex LH-20 (10 g) with water as eluent, affording succinate **169** as a white solid (10.01 g, 95%). The $^1$H NMR spectrum of **169** indicated the presence of unreacted MPEG-2000 (16%) in the crude product, as did titration with standard NaOH solution (See Chapter 6, Section 6.7). Overall yield of MPEG succinate was therefore 80%. The crude material was used in successive experiments without further purification. $R_f$ 0.23 (acetic acid-MeOH (1:1)); $\nu_{max}$ (KBr) 2889s (aliphatic C-H), 1736 (ester C=O) and 1114 (ester C-O) cm$^{-1}$; $\delta_H$ ($CDCl_3$) 3.57 (180 H, s, 2-H), 3.31 (3 H, s, 1-H) and 2.56 (3.4 H, s, 4-H & 5-H); $\delta_C$ ($CDCl_3$) 173.7 (C-6), 172.5 (C-3), 70.29 (C-2), 63.5 (C-1), 29.1 and 28.7 (C-4 & C-5); ESMS showed a broad molecular weight distribution centred at approximately 2100.

**Methoxypolyethyleneglycol (2000) succinate derivative with 142 (170).**




The title compound was prepared using established methodology.[105] Crude MPEG-2000 succinate (2.100 g, 0.84 mmol), *N*-{2-[(4-Hydroxyphenyl)thio]ethyl} acetamide (0.232 g, 1.1 mmol) and DMAP (30 mg, 0.25 mmol) were dissolved in dry DCM (25 ml) in a flame dried apparatus under $N_2$. A solution of DCC (300 mg, 1.2 mmol) in DCM (5 ml) was added and the mixture was stirred for 16 h. The white precipitate of DCU was filtered off and the organic filtrate was concentrated, taken up in acetone and the product precipitated by pouring into ice-cold diethyl ether. Filtration and drying under suction yielded crude **170** (0.914 g, 49%). The $^1$H NMR spectra indicated that 75% of this crude product was the title compound. The remainder was unreacted MPEG-2000 or MPEG-2000 succinate The

integrals for the polymer protons in the $^1H$ NMR spectrum include these impurities, giving fractional integrals; $R_f$ 0.31 (acetic acid-MeOH (1:1)); $\nu_{max}$ (KBr) 2921 (aliphatic C-H), 1730 (ester C=O), 1660 (amide C=O), 1463, 1376 and 1120 $cm^{-1}$; $\delta_H$ ($CDCl_3$); 7.52 (2 H, AA'BB', $^3J$= 8.7, 9-H), 7.16 (2 H, AA'BB', $^3J$= 8.6, 8-H), 6.89 (1 H, br s, N-H), 4.34 (2.6 H, m, terminal PEG unit, $CH_2$ adjacent to ester, 2a-H), 4.03 (2.6 H, m, terminal PEG unit, penultimate $CH_2$, 2b-H), 3.56 (241 H, br m, 2-H, 1-H & 12-H), 2.95 (2 H, m, 11-H) and 1.97 (3 H, s, 14-H); $\delta_C$ ($CDCl_3$) 173.2 (C-6), 172.0 (C-3), 171.5 (C-13), 131.3 (C-9), 122.5 (C-8), 70.4 (C-2), 63.7 (C-1), 39.0 (C-12), 34.0 (C-11), 29.5 (C-4 & C-5) and 23.3 (C-14); ESMS showed a broad molecular weight distribution centred at approximately 2300.

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