SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTICANCER AGENTS

A thesis presented in part fulfilment of the requirement for the Degree of Doctor of Philosophy

by

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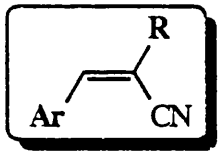
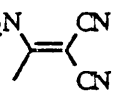
SUMMARY

Within the last ten years intense research has led to a greater understanding of the signalling pathways that control normal cell division and cancerous cell proliferation. Enzymes known as protein tyrosine kinases (PTKs) play a key rôle in such growth-related processes by catalysing the phosphorylation of tyrosine residues on intracellular protein substrates. This knowledge has led to the search for selective PTK inhibitors in the hope of generating chemotherapeutic agents of potential use in the treatment of cancer. Particular attention has focused on the signalling events that occur at a receptor tyrosine kinase (RTK) called the epidermal growth factor (EGF) receptor since a number of studies have implicated its involvement in many types of human cancer.

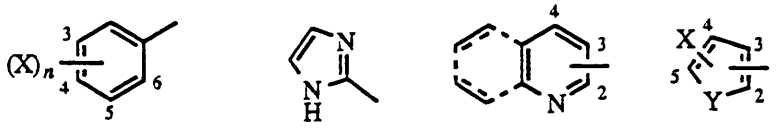
Compounds found to possess anticancer activity by acting as PTK inhibitors with a structural resemblance to tyrosine are known as the tyrphostins. In this project, an extensive array of 3-arylpropenonitriles (approximately 140 compounds) akin to the tyrphostins have been synthesised (Diagram) and biologically evaluated in cell assays designed to correlate PTK inhibition at the EGF receptor to antiproliferative activity. Efforts were directed at producing more stable tyrphostin analogues as well as those that incorporate a heterocyclic moiety.

Diagram: 3-Arylpropenonitriles (Tyrphostins)



R= CN, CO_2R', $CONH_2$, $CSNH_2$, 

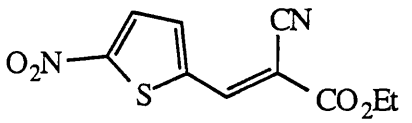
(R'= H or alkyl group)

Ar= 

X= H, O*R'*, NO_2, Cl and/or F *(n= 1, 2, or 3)* (Y= O or S)

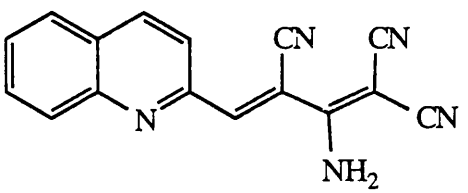
From initial studies, tyrphostins incorporating a nitrothiophene portion were shown to be highly potent antiproliferative agents and gave sub-nanomolar IC_{50} values against the breast adenocarcinoma MCF-7 cell line. Consequently, structurally isomeric tyrphostin sets were synthesised. More detailed biological analysis demonstrated that some of these compounds were acting in a non-selective cytotoxic fashion. Presently, the compound ethyl 2-cyano-3-

[5-(2-nitrothienyl)]propenoate (**A**) has been accepted by the National Cancer Institute to undergo an *in vivo* evaluation and represents an interesting new cytotoxic anticancer agent.



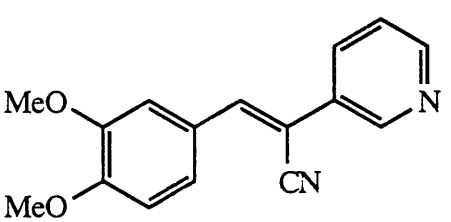
(**A**)

During a search for heterocyclic tyrphostins some quinoline derivatives were found to display encouraging antiproliferative activities against the MCF-7 cell line (IC_{50} values < 1 μM). The most potent compounds contained the 2-aminoethene-1,1-dicarbonitrile unit [Diagram: **R**= $C(NH_2)=C(CN)_2$] and, although they produced an additional cytotoxic action, they were found to inhibit selectively the phosphorylation of an unknown signalling protein that was found to be linked to EGF receptor activation. The 2-substituted quinoline (**B**) also displayed a certain degree of selectivity within a panel of 60 tumour cell lines (held at the US National Cancer Institute) and may act as an interesting lead for future studies.



(**B**)

In order to determine the stereochemical requirement preferred for biological activity, attempts were made to synthesise geometric isomers of some tyrphostins. In particular, the *cis*- and *trans*-forms of the known tyrphostin (**C**) were synthesised and characterised by detailed NMR spectroscopy and X-ray crystallography. Interestingly, the isomer (**C**) was about 3.5 times more potent than its geometric partner in an antiproliferative assay against a cell line (HN5) that overexpresses the EGF receptor. Also, synthesised were other 2,3-diarylpropenonitriles akin to compound (**C**) and some of these displayed equally effective IC_{50} values against the MCF-7 cell line.



(**C**)

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LIST OF ABBREVIATIONS

cAMP ..... cyclic adenosine monophosphate
AC ..... adenylyl cyclase
AP-1 ..... activating protein-1
ATP ..... adenosine triphosphate
CNS ..... central nervous system
DNA ..... deoxyribonucleic acid
D-*Ras* ..... GDP-bound *Ras* form
DSK ..... dual-specific kinase
DSP ..... dual-specific phosphatase
DAG ..... diacyl glycerol
EGF ..... epidermal growth factor
EGF-R ..... epidermal growth factor receptor
ER ..... endoplasmic reticulum
ERK ..... extracellular regulated kinase
GAP ..... GTPase activating protein
GDP ..... guanine diphosphate
cGMP ..... cyclic guanine monophosphate
GRP ..... guanine nucleotide releasing protein
GTP ..... guanine triphosphate
IR ..... insulin receptor
IP_3 ..... inositol-1,4,5-triphosphate
JAK ..... Janus kinase
LAK (cells) ..... lymphokine-activated killer (cells)
MAP ..... mitogen-activated protein
MAPK ..... MAP kinase
MAPKK ..... MAPK kinase (same as MEK)
MAPKKK ..... MAPKK kinase (same as MEKK)
MEK ..... MAP/ERK kinase
MEKK ..... MEK kinase
NO ..... nitric oxide

NOS ..... nitric oxide synthase

PDE ..... phosphodiesterase

PDGF ..... platelet-derived growth factor

PDGF-R ..... platelet-derived growth factor receptor

PH ..... pleckstrin homology

PG ..... prostaglandin

PIC ..... phosphatidylinositol-specific PLC or phosphinosidase C

PI-3K ..... phosphatidylinositol 3-kinase

PIP_2 ..... phosphatidylinositol-4,5-bisphosphate

PITP ..... phosphatidylinositol transfer protein

PKA ..... cAMP-dependent protein kinase

PKC ..... protein kinase C

c-PLA_2 ..... cytoplasmic phospholipase A_2

PLC ..... phospholipase C

PSK ..... protein serine/threonine kinase

PSP ..... protein serine/threonine phosphatase

PTB ..... phosphotyrosine binding

PTK ..... protein tyrosine kinase

RNA ..... ribonucleic acid (m= messenger; t= transfer; r= ribosomal)

RTK ..... receptor tyrosine kinase

SH2 or SH3 ..... *src*-homology 2 or 3

SIE ..... *sis* inducible element

SRE ..... serum response element

STAT ..... signal transducer and activation of transcription

TIL ..... tumour-infiltrative lymphocyte

TGF-α ..... transforming growth factor-α

◊ INTRODUCTION ◊

"The mission of pharmacology is to establish the active substances within natural drugs, to find chemical properties responsible for their action, and to prepare synthetically drugs that are more effective"; R. Buchheim, 1876.

PREFACE

One might define medicinal chemistry as Rudolf Bucheim had described pharmacology at the University of Giessen in 1876 (above quotation).[1] Indeed, this discovery/design/development description could equally apply to the objective of this research work which, over three years, has endeavoured to synthesise a more effective anticancer agent. As one might expect, this type of programme involves many disciplines and therefore it is necessary to equip the reader with some relevant background.

As an appropriate start, a brief general account of cancer is given followed by more in-depth sections into cell biology and such like. This should be suitable armoury for later sections which explain the biochemistry of a growth control pathway which is at the core of this Ph.D. project. As the reader will soon learn, a new era has begun, not only in cancer research but in the whole area of medicinal chemistry, namely: *de novo* design, *i.e.* the rational design of useful new drugs for a particular disease directly from knowledge of that disease.

CHAPTER «1» Cancer—An Overview

Cancer is a feared disease. Indeed, it is many diseases; that is one of the problems. Cancer arises from cells in the body that were once normal cells and these 'transformed' cells differ only subtly at the biochemical level. Indeed this feature has bedevilled the search for a 'cure' as there will always be an inherent lack of selectivity. In the words of Walt Kelly: "We have met the enemy and they are us." However, recent research is starting to decipher the intricate control mechanisms of cells and revealing potential strategies in this disease without affecting normal cells. In the clinic, the hope is no more side-effects.

This burgeoning knowledge has taken time to collate and has had to wait for advances in molecular biology and fundamental cell biology. Today these findings are being translated into clinically viable treatment strategies (see Section 4.3). Obviously it will take more time (years) before these are at a stage to be put to test in the clinic and although human society has waited for many centuries for a 'cancer cure' one should take heed of Alexander Fleming's statement on discovering penicillin: "The spores didn't just stand up on the agar and say, 'I produce an antibiotic, you know.'"

1.1 Cancer—A Brief History

Cancer is an old problem and has been with us throughout the history of the human race. Although, in saying this, it is not a disease which only afflicts humans and traces of tumours have been found in dinosaurs from the Cretaceous Age as well as in a Pleistocene cave bear. In human society it was well known to the ancient Egyptians and succeeding civilisations. Egyptian mummies from the third to fifth dynasties (*circa* 3000-2500 BC) displayed evidence of tumours and there are similar indications in Inca remains from about 500 BC. Two of the oldest documented accounts of cancer include the Edwin Smith Papyrus (*circa* 1660 BC) and the Ebers Papyrus, dating from about 1552 BC.[2]

The great medical authority, Hippocrates (460-370 BC) described many varieties of cancers (skin, breast, stomach, cervix uteri and rectum) and established an initial classification system.[3] Also his writers introduced a variety of terms– "phyma", "oidema", "karkinos" and "karkinoma"—to signify different types of chronic ulcerations, growths and swellings.[4] At least some of these descriptions appear to have been tumours in today's sense and the Greek term for cancer, "karkinos" meaning "crab", still aptly describes the crab-like appearance or texture of more advanced cases of this disease, *e.g.* breast cancer.

In 1800 and 1801, Marie François Xavier Bichat, a brilliant young French anatomist laid down the principle that all tissues of the body are fundamentally similar in structure and each consists of "cellular" units capable of reproducing themselves. Later that century Mathias Schleiden, Theodor Schwann and Johannes Mueller (1838) developed their cellular theory[5] and Wilhelm Waldeyer published two papers which outlined the fundamental principals on cancer.[6] From that time on, cell theory gave a solid conceptual basis for twentieth century cancer research.

1.2 Cancer of Today

Cancer holds a strange place in modern mythology. Although it is a common disease and it is true to say that one person in five will die of cancer, it is equally true to say that four out of five will die of some other disease. Heart disease, for example, a more common cause of death, does not carry with it the gloomy overtones, not always justifiable, of a diagnosis of cancer. Maybe the fear of cancer stems from the fact that one's own body has revolted against the whole, leading to destruction within. Indeed, cancer results from the abnormal growth of otherwise healthy cells and is a disease characterised by uncontrolled cell proliferation. Unless checked, the cancer cells invade surrounding normal tissues, enter the circulation, and spread throughout the body, and eventually interfere with the function of normal cells leading to death of an afflicted individual.

Ironically, it has been the triumph of medical science over the last century which has resulted in a shift from diseases which were prevalent in the past towards cancer and other disease types (Table 1). To put this in context, it is estimated that an adult of today will live to over 70 years of age whereas the average age of the onset of cancer is about 67 years.[7] This means that in say, the United States, one person dies from cancer every 62 seconds and 30 % of the living population will eventually be afflicted with the disease. On a more

Table 1 Status of Some Diseases

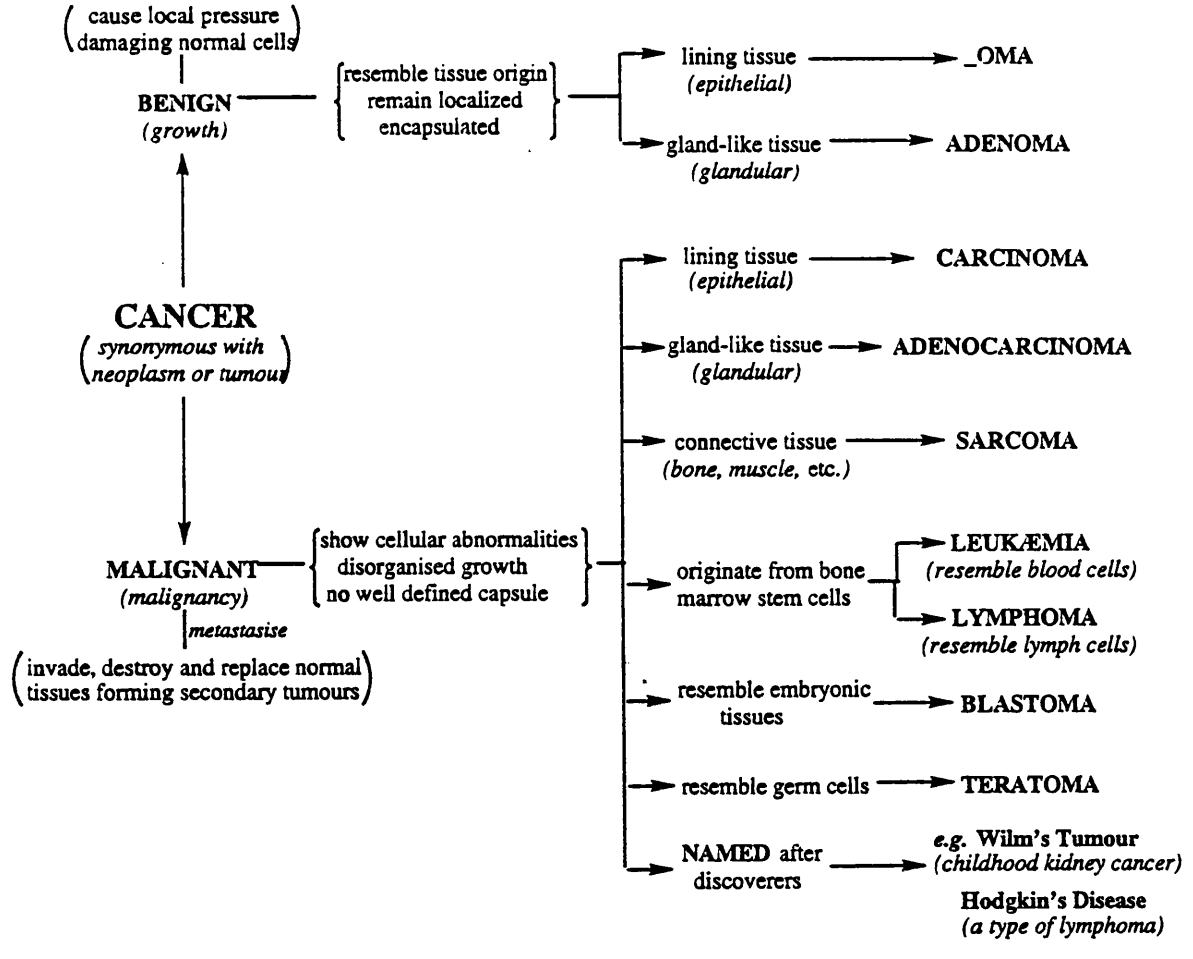
Diseases Largely Cured or Avoided	*Diseases Alleviated*
Cholera	Asthma
Diphtheria	Diabetes
Erysipelas	Heart Disease
Lobel pneumonia	Schizophrenia
Measles	Syphilis and other venereal diseases
Meningococcal meningitis	*Diseases Which Still Present Challenges*
Pertussis (whooping cough)	AIDS
Plague	Alzheimer's disease
Poliomyelitis	Arthritis
Rheumatic fever	Cancer
Scarlet fever	Cirrhosis
Smallpox	Common cold
Staphylococcal septicæmia	Genetically transmitted diseases
Subacute bacterial endocarditis	Genital herpes
Tuberculosis	Huntingdon's chorea
Typhoid fever	Influenza
Vitamin deficiency	Multiple sclerosis
	Parkinson's disease
	Pulmonary fibrosis
	Senility, geriatric problems

fundamental level, this reflects an important feature of cancer biology, namely: the cancerous transformation of a healthy body cell (carcinogenesis) is not a single step process and generally requires many years before enough abnormalities accumulate to the point of undermining the regulatory and safety mechanisms of normal cell growth.

1.3 The Development of Neoplasms—Carcinogenesis

Although over one hundred distinct forms of cancer have been identified, they can generally be classified by their tissue origin (Figure 1).[8] As indicated, the important distinction is that between a benign and a malignant neoplasm. Generally speaking, a neoplasm (literally, "new growth") is any abnormal growth of cells and the term tumour is usually used synonymously with neoplasm. In particular, a benign neoplasm is a growth that remains confined to its original location whereas a malignant neoplasm is capable of both invading adjacent tissues and of spreading (or disseminating) to other tissues of the body (to metastasise, the process of metastasis). Therefore, in contrast to malignancies, benign

Figure 1 Classification of Cancer by Tissue Origin



tumours are generally not life-threatening (except, for example, brain tumours) and can usually be completely removed by surgery (ablation).

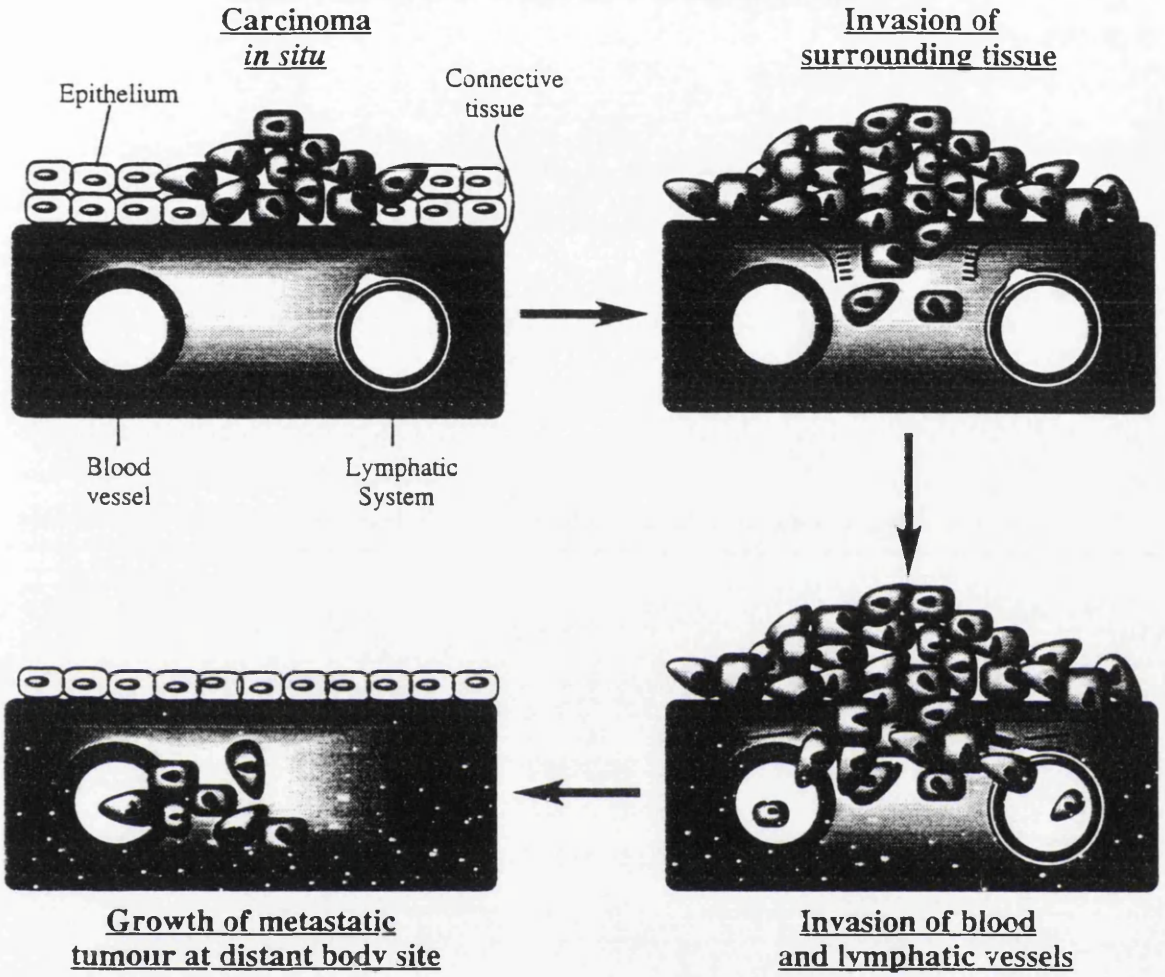
At the cellular level, the primary event (tumour initiation) in the development of a malignant neoplasm (carcinogenesis) most likely involves the damage or destruction (mutation) of the genetic material (DNA), probably in the stem cell population of the tissue involved.[9] These initiated cells remain latent until promoted into an increased state of proliferation (tumour promotion). At this stage additional mutations can occur and eventually one variant cell will have the effect of further increasing the growth potential of the neoplasm (tumour progression).[10] Due to this proliferative advantage, the descedants of a cell bearing such a mutation tend to outgrow the other cells in the tumour (clonal selection). This clonal selection process is repeated several times during tumour progression, resulting in the formation of increasingly hyperproliferative tumour cells.[11]

Moreover, there is a general movement towards a more aggressive behavioural pattern against surrounding tissues.[12] In the case of malignant neoplasms this is often manifested by the ability to invade and spread throughout the body (to metastasise) rather than remain confined (*in situ*) to their site of origin.[13] The first stage of this process involves the invasion and infiltration of surrounding normal tissue with the eventual penetration of small blood and lymphatic vessels (*i.e.* the capillaries). Once a passage to the circulatory system has been established there can be release of neoplastic cells, either as single entities or small clumps (emboli). At this stage the disseminated tumour cells must survive any natural defence mechanism (*e.g.* cells of the immune system) and be able to penetrate the junctions of the circulatory system before they can arrest in the capillary beds of distant organs and finally develop into a secondary tumour (see Figure 2). Luckily, the vast majority of tumour cells which enter the circulation are eliminated by such barriers and it has been estimated that less than one in ten thousand successfully establish a metastatic tumour.

1.4 Factors in Cancer Causation[14]

Substances that aggravate a cell into a neoplastic state are generally termed carcinogens.[15] Carcinogens which induce the critical mutation(s)[16] in the first stage of tumour formation are called initiating agents, whereas others which enhance carcinogenesis by stimulating cell proliferation[17] are called promoting agents[18] (see Figure 3 for chemical examples). The hormones provide examples which act solely as promotors—particularly oestrogen, a

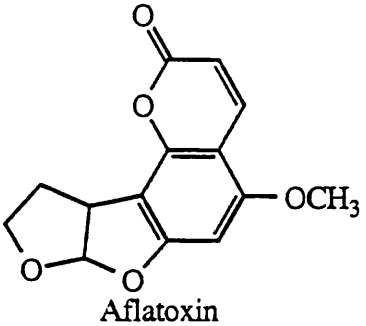
Figure 2 Invasion and the Metastasis Process



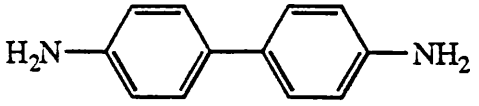
hormone which has been shown to be a major factor in the development of endometrial carcinoma.[19] Dietary fat is another example of a sole promoting agent and has been implicated in carcinomas of the breast, colon, prostate, ovary and uterus (endometrium).[20] However there are various dietary elements which studies suggest to be of benefit in *reducing* the risk of breast[21] and colorectal cancers:[22] *e.g.* high grain fibre,[23] fruits and vegetables,[24] and foods rich in vitamins A, C and E.[25]

"Lifestyle" has been attributed as one of the most common causes of cancer and, apart from stress and dietary factors, includes: smoking, alcohol use, reproductive history, sexual behaviour, and exposure to sunlight.[26] From this list, tobacco smoking is unquestionably the major identifiable cause of all cancers (30 %). Not only is it directly responsible for the vast majority (80–90 %) of lung cancers, but also for cancers of the mouth, pharynx, oesophagus, urinary bladder, pancreas, and kidney.[27] Heavy alcohol consumption,

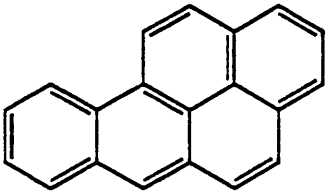
Figure 3 Structures of Some Representative Carcinogens



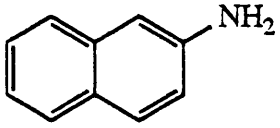
Aflatoxin



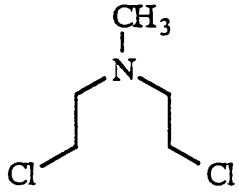
Benzidine



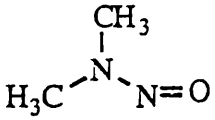
Benzo(a)pyrene



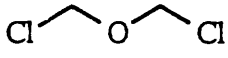
2-Naphthylamine



Nitrogen mustard(HN2)



Dimethylnitrosamine



Bis(chloromethyl)ether

Nickel carbonyl

particularly in combination with cigarette smoking, has also been linked with these cancers[28] and can result in chronic cell damage of the liver (cirrhosis), leading to excess cell proliferation, and hence liver cancer.

Genetic factors also play a part in cancer causation.[29] Although, inherited cancers represent a small fraction (perhaps 1-2 %) of total cancers,[30] dominant genetic inheritance accounts for about 40 % of retinoblastomas[31] and 20-40 % of Wilm's tumours (embryonal renal tumours) and neuroblastomas.[32] In these cases, a strong predisposition to cancer is transmitted directly from parent to child, and development of cancer is inherited like any other trait, such as hair or eye colour. Also the transmission of a single dominant gene can result in a cancer family syndrome,[33] for example, but luckily these are quite rare. Such disorders tend to result from defective maintenance of the genetic material, *i.e.* from their inherent genetic instability.[34]

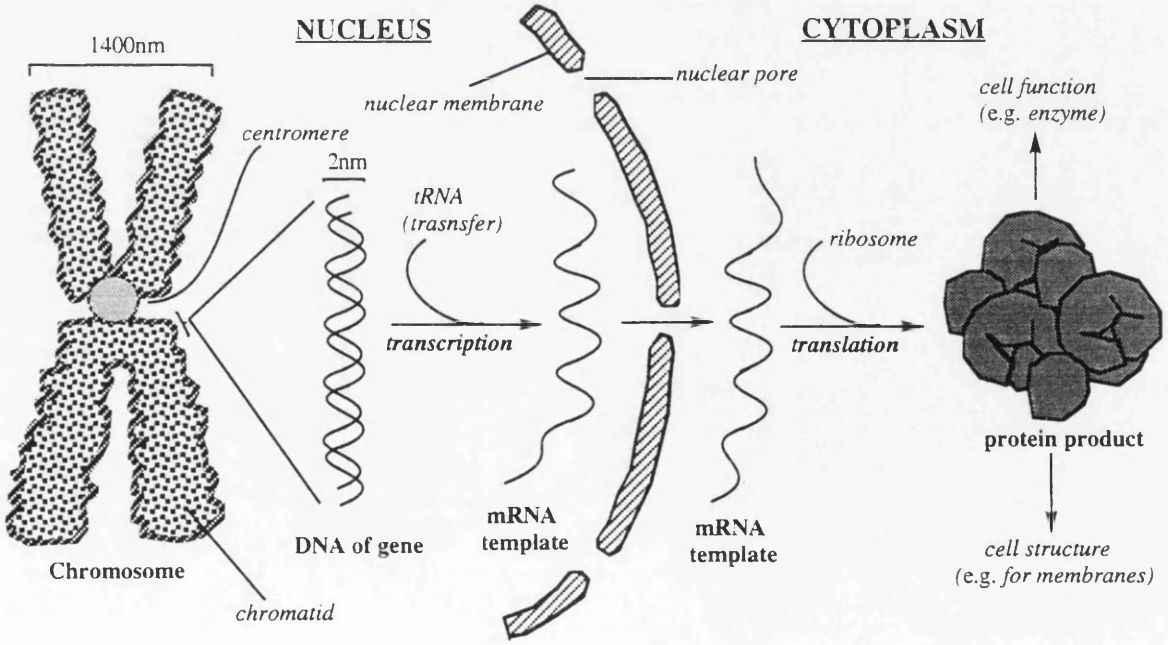
A further class of carcinogens would include chemicals or other agents that interfere with the normal function of the immune system.[35] This includes the genetic disorders from viral infection and inherited immunodeficiences[36]—as opposed to *acquired* immunodeficiences

which are mostly due to factors such as immunosuppressive agents or viruses, *e.g.* AIDS caused by viral (HIV) infection. Indeed viruses, and in particular oncogenic viruses are known to be associated with the generation of various human cancers.[37] These *non*cellular *intra*cellular parasites exhibit oncogenic activity by utilising the genetic material of host cells, thereby subverting normal cellular function.

1.5 Cancer Genetics

The control of gene expression is considered central to the understanding of cancer (see Figure 4). Specifically, it is abnormalities in the regulatory programme of a normal cell that transforms it into a neoplastic state. For this to occur, there must be specific alterations within critical regulatory genes, *viz.*: proto-oncogenes and tumour suppressor genes.[38] The proto-oncogenes are normal genes of the cell which play a major rôle in numerous aspects of cell growth and differentiation. These "pre-cancerous" genes require to be triggered into a state of abnormal function (a process called mutation) and thereby become fully-fledged oncogenes able to cause the pathological process of cancer. However, tumour suppressor genes have an indirect cause on cancer, whereby their function to inhibit tumour progression is switched-off or lost altogether.[39] Thus, the activation of oncogenes and inactivation of tumour suppressor genes represent complementary events in the carcinogenic process.

Figure 4 Gene Expression



The oncogenes found in tumour cells result from particular alterations in either the normal regulation, or structural (*i.e.* functional) integrity of the proto-oncogenes that are already native to the cell (so-called *cellular* proto-oncogenes).[40] These abnormal alterations are generally termed mutations (see Table 2) and may also elevate the levels of a product (*via* gene amplification)[41] to the point of subverting any normal cellular function. Interestingly, the oncogenes of retroviruses[42] are derived from such host-cell genes,[43] and this has led to the identification of various oncogenes associated with human tumours.

Table 2 Common Events in the Mutagenesis of DNA

Mutagenic Event	General Description
Point mutation	Genetic code becomes altered due to *substitution* of a nucleotide base pair (*i.e.* the basic unit, "alphabet", of the genetic language). Also can get "frame shift mutations" due to either a *deletion* or *insertion* of one or more base-pairs. So-called since the "words" of the genetic code become off-set and thus become garbled.
DNA rearrangement	This is where a proto-oncogene can be activated by recombination with other, previously unlinked, DNA sequences. Such a gene transfer usually occurs from viral transfection of cellular genomes and may disrupt regulatory sequences leading to abnormal gene expression. Alternatively, gene integration leads to recombinant fusion oncoproteins, often truncated, with aberrant functioning within a cell.
Chromosomal translocation[44]	A type of genetic rearrangement whereby regions of chromosomes become removed from their normal environment (*i.e. locus*) and become inserted into an abnormal position in a different chromosome. This *transposition* process results in oncogenes in a similar manner as described above but only involves cellular DNA (not viral).

Similarly, defective tumour suppressor genes[45] result from the mutagenic events described above but, unlike the oncogenes, can also result from inheritance of previously damaged forms. Noteworthy are the tumour supressor genes *p53* and *RB* which are frequently inactivated in most types of human cancers.[46, 47] Thus, in combination with the oncogenes, these "cancer-associated" genes contribute substantially to the pathogenesis of cancer (for a representative listing see Table 3).[48]

CHAPTER «2» Cell Communication—An Overview

The human body consists of about 5×10^{13} cells, of which there are approximately 100 different kinds, and a clear marvel of biology is in the proper functioning of such a complex system.[49] Ultimately this requires stringent control mechanisms so that not only proximal but remote cell types can communicate with each other. Thus, this section will concentrate on the signalling processes of cells, particularly in relation to cancer, and will lead on to a growth control pathway that forms the conceptual backbone of this research project.

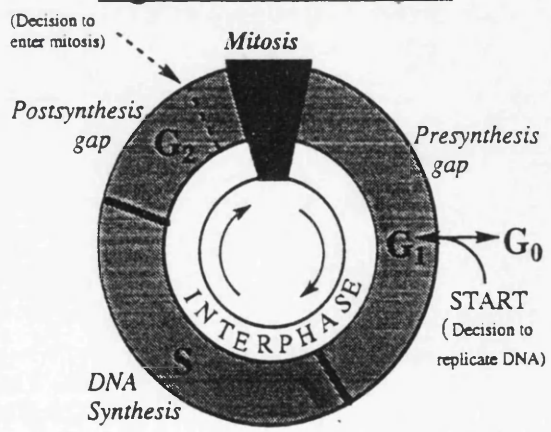
Table 3 Oncogenes &Tumour Suppressor Genes in Human Cancers

Tumour	Oncogenes	Tumour Suppressor Genes
Breast carcinoma[50]	c-*myc*, *erb*B-2	*RB*, *p53*, chromosomes 1, 3, 11, 18
Burkitt's lymphoma[51]	*BNLF*-1, c-*myc*	
Colon/rectum carcinoma[52]	*ras* K	*DCC*, *MCC*, *p53*, *chromosomes* 5, 18
Lung carcinoma[53]	*ras* K, c-*myc*, L-*myc*, N-*myc*	*RB*, *p53*, chromosome 3
Neuroblastoma[54]	N-*myc*	chromosome 1
Neurofibrosarcoma[55]		*NF* 1, *p53*

2.1 Factors of Cell Growth

Cell growth and replication (*i.e.* division)[56] is classically divided into four stages, collectively known as the cell cycle (Figure 5).[57] In situations where G_1 is very long, cells may be considered as being arrested and are said to be in a quiescent state (denoted by G_0). Connective tissue cells remain arrested in G_0 unless triggered by some "external factor". For skin fibroblasts this may be in response to damage made by a cut or injury. In this case, the "factor" involved is called platelet-derived growth factor (PDGF) and is released by blood cell fragments (*i.e.* platelets) during the blood clotting process.[58] It is this which stimulates proliferation of local skin cells. Such control substances are known as growth factors and they belong to diverse set of signalling molecules that also include the hormones.

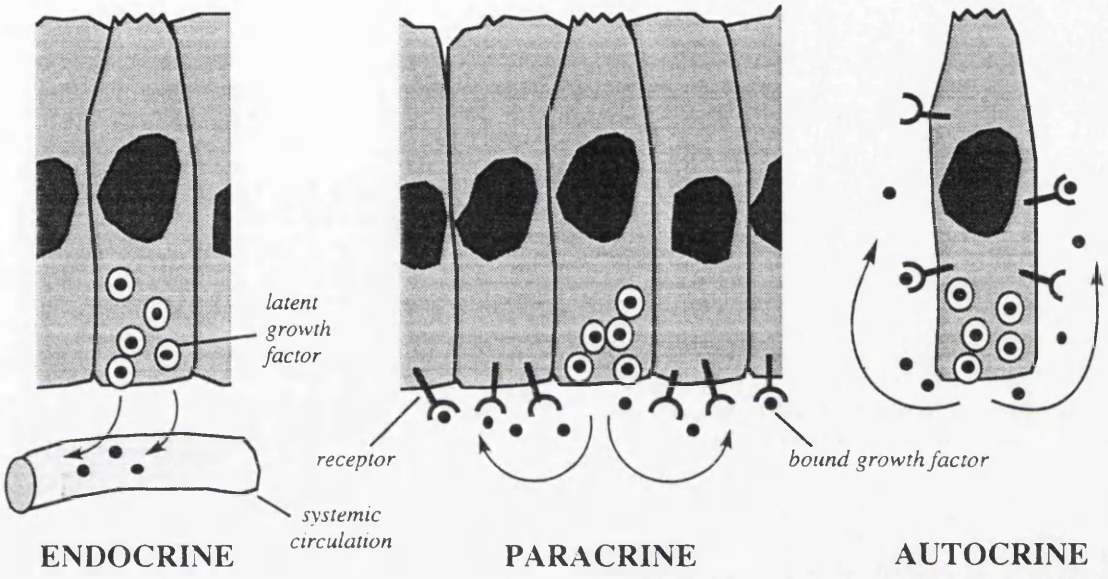
Figure 5 The Cell Cycle



An underlining theme in understanding the regulatory action of growth factors is exemplified in their endocrine, autocrine and paracrine modes of action (see Figure 6).[59] This understanding augments and extends the action given by the mobile endocrine (or

hormone) system. Thus, the global effect of the classical hormones often requires the local action of these polypeptide mediators.

Figure 6 Diagrammatic Representation of Secretion Modes[60]



2.2 Aspects of Growth Factors

Due to their function in influencing the cell to divide, *i.e.* to undergo mitosis, growth factors are often referred to as mitogens. However, although they do exert a mitogenic action in growth control processes, this is not their sole function. Indeed they are multifunctional in nature and may be regarded as simply the alphabet or symbols of a biological regulatory language (see Table 4).[61] Elucidation of the grammar and syntax of this language is one of the most challenging aspects of modern biology and an attempt to give a deciphered acount of this will be given in the next sections.

Often it is the case that growth factors (*e.g.* PDGF[62] and others[63]) regulate gene expression by controlling the synthesis and degradation of the extracellular matrix. The extracellular matrix is composed of collagens, glycoproteins and proteoglycans, and provides not only a physical support to the tissues but also maintains cellular viability, stability and homeostasis.[64] Thus, it comes as no surprise that abnormalities in growth factor action can lead to the development and progress of cancer.[65] In part, this is due to aberrant stimulation of a cell to produce proteolytic enzymes, *e.g.* plasminogen activator or collagenase.[66]

An important principle to put forward is that the type of cell that makes a growth factor does not normally respond to that factor. Rather, growth factors are produced in order to signal a

Table 4 Names and Abreviations of Growth Factors

Common Growth Factor Name	Alternative Functional Name(s)
Activins (A, AB, B)	FSH releasing protein (FRP)
Colony-stimulating factor 1 (CSF-1)	Macrophage colony-stimulating factor (M-CSF)
Epidermal growth factor (EGF)	Urogatrone
Erythropoietin (epo; EP)	Haemopoietin Erythrocyte stimulating factor (ESF)
Fibroblast growth factors (FGF)	Acidic fibroblast growth factor (aFGF) Basic fibroblast growth factor (bFGF) Brain-derived growth factor (BNDF; BDGF) Heparin binding growth factor (HBGF) Endothelial cell growth factor (ECGF) Retina-derived growth factor (RDGF) Eye-derived growth factor (EDGF) Kidney angiogenic factor (KAF) Adrenal growth factor (AGF) Corpus luteum angiogenic factor (CLAF) Ovarian growth factor (AGF) Placental angiogenic factor (PAG) Hepatocyte growth factor (HGF) Myogenic growth factor (MGF) Cartilage-derived growth factor (CDGF) Bone growth factor (BGF) Seminiferous growth factor (SGF) Prostatropin (PGF) Tumour-derived growth factor (TDGF) Hepatoma-derived growth factor (HDGF) Melanoma-derived growth factor (MDGF) Mammary tumour-derived growth factor (MTGF)
Gastrin-releasing peptide (GRP)	Mammalian bombesin
Granulocyte colony-stimulating factor (G-CSF)	Macrophage/granulocyte inducer type 1, granulocyte (MG-1G) Pluripotent colony-stimulating factor (pluripoetin) Granulocyte-macrophage colony-stimulating factor-β (GM-CSFβ)
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Macrophage-granulocyte inducer Colony-stimulating factor 1 (CSF-2)
Inhibin	
Insulin-like growth factor I (IGF-I)	Somatomedin A Somatomedin C Basic somatomedin
Insulin-like growth factor II (IGF-II)	Multiplication stimulating activity (MSA)
Interferon-α (IFN-α)	Leukocyte (Le) interferon Type I interferon
Interferon-β (IFN-β)	Fibroblast (F) interferon Type I interferon

Interferon-γ (IFN-γ)	Immune interferon T interferon Type II interferon
Interleukin-1 (IL-1)	Interleukin-1 a Interleukin-1 b Lymphocyte activating factor (LAF)
Interleukin-2 (IL-2)	T-cell growth factor (TCGF)
Interleukin-3 (IL-3)	Mast cell growth factor P-cell stimulating factor Multi-colony-stimulating factor Burst promoting activity WEHI-3 haematopoietic growth factor Thy-1 inducing factor Histamine cell-producing stimulating factor 20α-dehydrogenase-inducing factor
Interleukin-4 (IL-4)	B-cell stimulating factor 1 (BSF-1) T-cell growth factor (TCGF) B-cell growth factor 1 (BCGF-1) Mast-cell growth factor (MCGF)
Interleukin-5 (IL-5)	T-cell replacing factor (TRF-1) B-cell growth factor II (BCGF II) B-cell differentiation factor μ (BCDFμ) Eosinophil differentiation factor (EDF) IgA enhancing factor (IgA-EF) B-cell maturation factor (BMF) B-cell growth and differentiation factor (BGDF)
Interleukin-6 (IL-6)	B-cell stimulatory factor-2 (BSF-2) Interferon-β2 26-kDa protein Hepatocyte stimulatory factor Hybridoma/plasmacytoma growth factor Interleukin-HP1 Macrophage granulocyte inducer type 2
Lymphotoxin	Tumour necrosis factor-β (TNF-β)
Müllerian inhibiting substance (MIS)	Anti-Müllerian hormone (AMH) Müllerian inhibiting factor (MIF)
Nerve growth factor (NGF)	
Platelet-derived endothelial cell growth factor (PD-ECGF)	
Platelet-derived growth factor (PDGF)	(PDGF-AA, PDGF-AB, PDGF-BB)
Transforming growth factor-α (TGF-α)	Sarcoma growth factor (SGF)
Transforming growth factor-β (TGF-β)	Cartilage inducing factor-A (CIF-A) Cartilage inducing factor-B (CIF-B) BSC-1 growth inhibitor (BSC-1 GI) Differentiation inhibitor (DI) Polyergin
Tumour necrosis factor (TNF)	Cachectin Tumour necrosis factor-α (TNF-α)

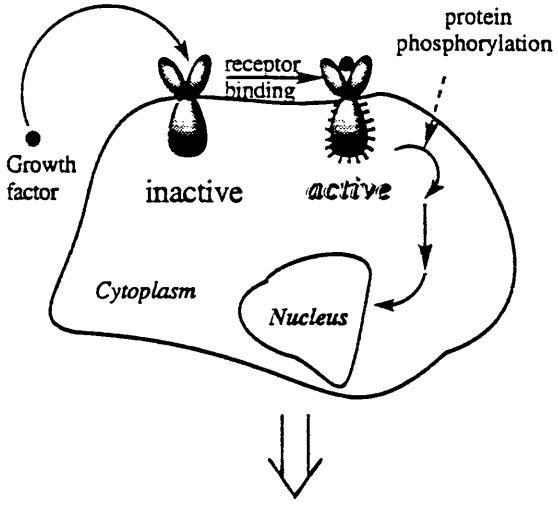
different cell type. In cancer, a cell can produce a growth factor that it also responds to (called autocrine growth stimulation) leading to continual cell proliferation.[67] Pertinent cases of this situation are given by: the viral oncogene v-*sis* and PDGF (causes sarcomas in monkeys),[68] the oncogenes *hst* and *int-2* (members of the FGF family which are sometimes amplified in human tumours),[69] and IL-3 (which is translocated in some human leukæmias).[70]

Another important concept to address is the contextual nature of growth factors, *i.e.* the cellular response will depend on other effectors and receptors which are present or have been set into action. For example TGF-β, in a particular fibroblastic cell line, will inhibit cell proliferation in the presence of EGF but stimulate proliferation in the presence of PDGF.[71] Also the action of growth factors depends on the state of development or differentiation of a target cell (see reference 72 for examples involving TGF-β action). Thus, peptide growth factors should be considered as a regulatory apparatus within a more complex array and serve simply as a means to convey information from one cell to another. It is the responsibility of the target cell from the whole context of these messengers to determine the actual cellular response.

2.3 Signal Transduction—An Overview

The biological action of growth factors are mediated by cell surface receptors, *i.e.* plasma membrane proteins. These serve as external sensors, acting to transmit information from outside the cell to its interior. Such a response is usually exhibited by the initiation of a cascade of enzymatic biochemical reactions that ultimately results in changes in gene expression and cell division (Figure 7). As indicated, activation of a receptor often leads to the phosphorylation of intracellular proteins. This is a common regulatory event within a cell[73] and not only serves to alter enzymatic action, as in this receptor-signalling system, but in the metabolic pathways of a cell[74] increases a substrates reactivity. In essence, phosphorylation "moves" a substrate from one state to another. Thus, an enzyme which catalyses the transfer of a phosphate groups from ATP to another molecule is called a kinase (from the Greek for motion or action, *kinēma*). If the recipient molecule is a protein and is phosphorylated on the amino acid tyrosine, then the enzyme is called a protein tyrosine kinase (PTK).

Figure 7 Schematic of Intracellular Signal Transduction



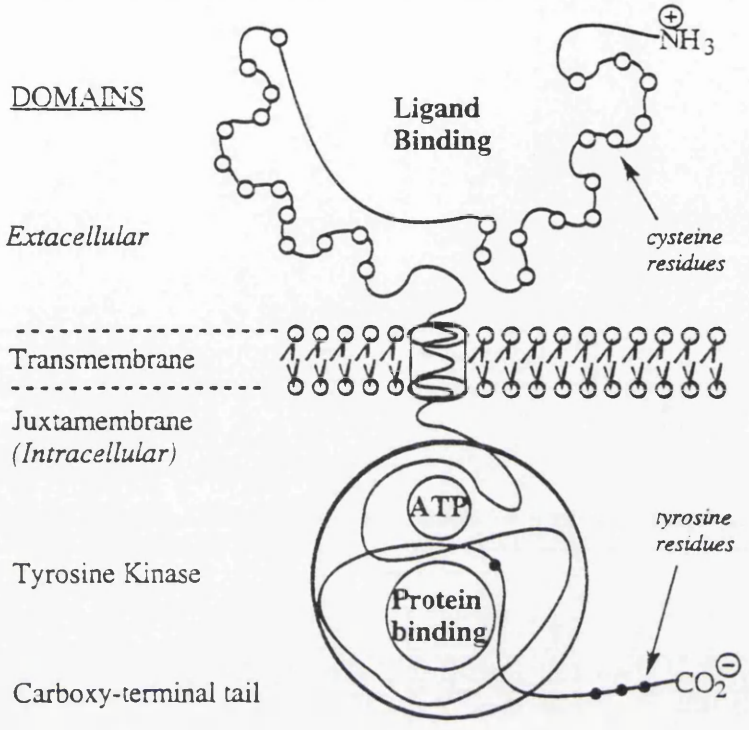
Cellular Response
(Gene Expression or Cell Division)

The PTKs can be divided into three categories, *viz.*: receptor-linked PTKs, or receptor tyrosine kinases (RTKs), which are found bound to the cell membrane; non-receptor linked, cytosolic PTKs, which are found in the more fluid portions of the cytoplasm; and non-receptor-linked PTKs, which are also found bound to the cell membane. A notable example of a RTK would include the epidermal growth factor receptor (EGF-R). This belongs to a family of growth factor receptors which possess intrinsic tyrosine kinase activity.[75] The structural organisation of this receptor[76], and family members[77], is composed of several distinct domains: an extracellular domain for ligand binding; a transmembrane α-helical domain; and a cytosolic PTK domain for ATP *and* substrate binding (Figure 8).

In cancer, such growth factor receptors have often been altered in their normal functioning. This could be due to an abnormally high expression of the cellular receptor itself, as in the case of the *erb*B-2 oncogene,[78] an EGF-R like product in breast and ovarian carcinomas. Alternatively, a number of receptors become oncogenic as a consequent of structural changes (or truncation) of their ligand-binding domains. These non-receptor-linked membrane-bound PTKs remain active even in the absence of growth factor stimulation and would include the oncogenes *ret*,[79] *trk*[80] and the *Src*-family[81] of PTKs.

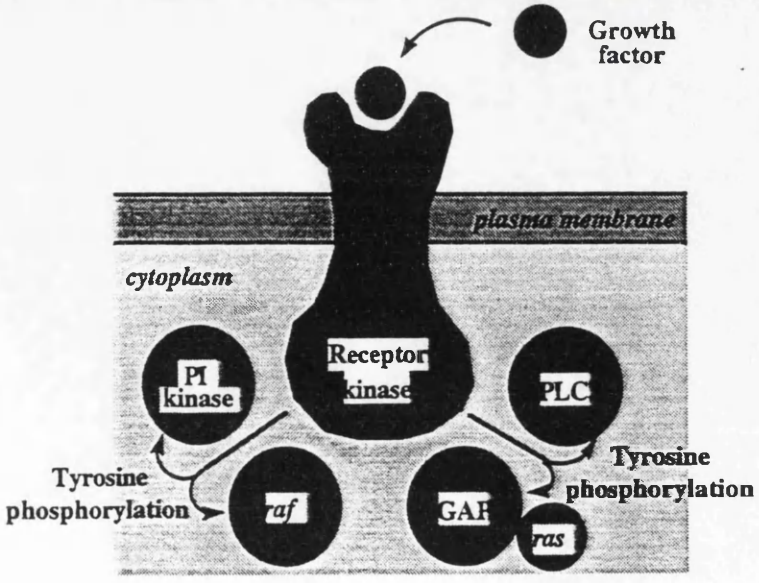
At least four distinct protein targets have been recognised to be initially phosphorylated by the RTKs in the signal transduction processes of the cell (Figure 9). These would include: phosphatidylinositol 3-kinase (PI 3-kinase) and phospholipase C (PLC), which regulate the

Figure 8 Essential Features of the EGF Receptor (EGF-R)



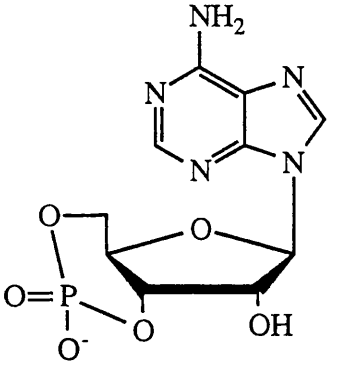
metabolism of inositol phospholipids; the *raf* proto-oncogene product; and GTPase activating protein (GAP) which functions to regulate the activity of *ras* proto-oncogenes. At this stage it is not necessary to deal with each of these in turn but it suffices to say that activation of these targets leads to a rapid change in the intracellular concentration of small

Figure 9 Targets of Growth Factor Receptors (RTKs)

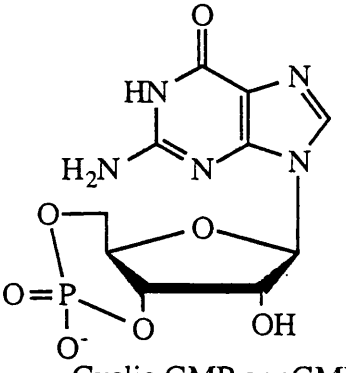


molecular weight compounds or ions. It is these so-called second messengers that activate a cascade of biochemical events which eventually leads to an end response. Figure 10 illustrates the most important types but excludes the calcium ion (Ca^{2+}) which is also a highly utilised second messenger in the signal transduction processes of the cell.

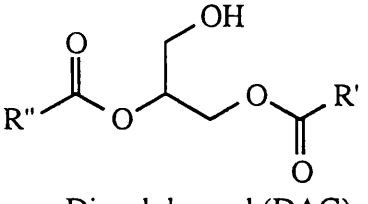
Figure 10 Important Second Messengers



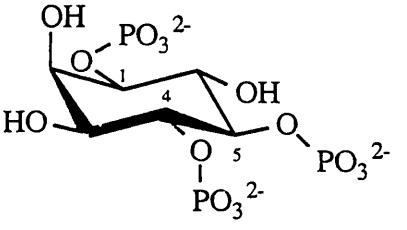
Cyclic AMP or cAMP
(adenosine 3',5'-cyclomonophosphate)



Cyclic GMP or cGMP
(guanosine 3',5'-cyclomonophosphate)



Diacylglycerol (DAG)
(R', R"= C_{20}-chains)



Inositol-1,4,5-triphosphate (IP_3)

As the phosphorylation of signalling proteins on tyrosine is essential for regulation of growth and differentiation, any excessive tyrosine phosphorylation would be expected to be detrimental to normal cellular activity. Indeed, the level of tyrosine phosphorylation in a cell is modulated by a family of enzymes called protein tyrosine phosphatases (PTPs) which specifically *de*phosphorylate tyrosine residues on their target proteins.[82] Furthermore, it is perhaps not surprising that PTPs are also involved in the pathogenesis of certain human diseases, including cancer.[83]

As for the PTKs, the PTPs can be grouped into discrete categories, namely those that are cytosolic or membrane-bound (non-receptor-like), and those that span the plasma membrane (receptor-like). But, despite the division, all PTPs share a common catalytic core of *ca.* 250 amino acids comprised of *ca.* 21 invariant residues, many of which appear to be essential for

phosphatase activity.[84] Unfortunately, this area of research is still in its infancy and little is known about the specific biological rôles of these proteins. However, the study of PTPs should provide important insights into the understanding of normal growth and development as well as the molecular basis of cancer. The beginings of this understanding is exemplified by the demonstration that certain PTPs (CD45 and Cdc25)[85] cause cells to enter mitosis as opposed to merely quenching signals that have been generated by the PTKs. Thus, PTPs have also been implicated as *positive* regulators of cellular processes.

Although phosphorylation and dephosphorylation events on tyrosine residues are an essential regulatory feature of many signal transduction pathways, it is not the sole amino acid to be utilised. Thus phosphate esters are also formed on serine and threonine residues which, as for tyrosine, are controlled by kinases (protein-serine/threonine kinases or PSKs) and phosphatases (protein serine/threonine phosphatases or PSPs).[86] In some cases the phosphoramidate counterparts of the basic amino acids histidine,[87] lysine,[88] and arginine[89] have been known to occur, and recently "dual specific" kinases (DSKs)[90] and phosphatases (DSPs)[91] have been described, so-named due to their ability to act both on tyrosine and serine/threonine residues.

Thus the enzymatic machinery of the signal transduction processes is complex and is finely tuned in order to compensate for each other's actions. Accordingly this is a complex area and it is beyond the scope of this thesis to give a detailed narrative on all aspects of cell communication. However an attempt has been made in Table 1.5 to summarise the (presently) known essential features of signal transduction and the reader should refer to the reviews for further information.

2.4 Signalling Guides—Modulators of Protein Interaction

The recent progress in deciphering the signalling pathways of a cell has highlighted key protein motifs that are intimately involved in the modulation of protein-protein interactions. These so-called protein modules are highly conserved sequences and examples, which include *Src* homology domains (SH2 and SH3) and pleckstrin homology domain (PH), are found in a wide variety of intracellular signalling proteins, either singly or in combination.[92] The SH2 domains function as motifs capable of binding to short protein sequences that bear phosphorylated tyrosine residues, whereas the SH3 domains prefer to bind to sequences with one or more proline residues (the PH domain is less understood). In any case the result

Table 5 Signal Transduction Processes—An Overview

Second Messengers in Signalling Pathways		
Second Messenger	**Main Substrates Affected**	**Reviews**
cyclic AMP (cAMP)	cAMP is generated by the adenylyl cyclase (AC) family of isozymes. Functions in muscle contractility, glycogenolysis, lipolysis, steroidogenesis, ion channel/pump modulation, and growth factor & inflammatory responses. Also cAMP controls a PSK family of cytosolic enzymes: called cAMP-dependent protein kinases (PKAs).	93 94
cyclic GMP (cGMP)	cGMP is formed by guanylyl cyclase: an enzyme which can be receptor-linked (controls water/Na^+ loss in kidney) or cytosolic (involved in muscle contractility) but is also activated by NO. Also cGMP controls a family of PSKs: cGMP-dependent protein kinases, types I and II; and directly controls retinal ion channels.	95 96
Phospholipid hydrolysis products (IP_3, DAG)	A phopholipase C (PLC) cleaves phosphatidylinositol-4,5-bisphosphate (PIP_2), a minor component of the cell membrane, to DAG and IP_3. DAG remains membrane-bound and with Ca^{2+} activates PSPs: *e.g.* protein kinase C (PKC) which, in turn, modulates AC, cAMP phosphodiesterases (PDEs), and ion channels/pumps. IP_3 migrates to, binds, and then activates receptors on the endoplasmic (or sarcoplasmic) reticulum stores thereby allowing rapid Ca^{2+} release.	97 98
Calcium ions (Ca^{2+})	Ca^{2+} concentrations play a fundamental rôle in muscle contraction, secretion, mitosis, chemotaxis, glycogenolysis, and steroidogenesis. Is mediated: extracellularly, *via* voltage-sensitive, receptor-operated, or second-messenger channels; intracellularly, *via* IP_3-receptors or ryanodine channels of endoplasmic reticulum Ca^{2+} stores. Important Ca^{2+} binding proteins include calmodulin and in muscle cells troponin C which bind and then modify the activity of other receptor proteins.	99
Nitric oxide (NO)	NO is a physiological mediator and is generated from arginine by Ca^{2+}-dependent nitric oxide synthases: iNOS (which is important in macrophage host-defences), and nNOS & eNOS (which are important in the regulation of blood pressure and CNS transmission).	100

is temporal localisation of a protein substrate to its host and enables the regulation of cellular events such as protein trafficking or relocalisation, and the enhancement of catalytic activity. Very recently an alternative phosphotyrosine-binding domain (designated PTB) has been identified but as yet its function and occurrence remains to be fully elucidated.[101]

2.5 Signalling and Transcription

To finalise fully a general discussion on signalling pathways, one should connect these intracellular pathways to the factors that directly influence gene expression, *i.e.* the transcription factors or gene promotor/enhancer proteins. These are factors which contain specific nucleotide regulatory sequences for a particular gene and are able to control DNA

Table 5 Signal Transduction Processes—An Overview (continued)

Generic Enzyme Signalling Pathways		
Enzyme Family	**Brief Description**	**Reviews**
Receptor-linked PTKs (RTKs)	RTKs such as EGF-R or PDGF-R are well recognised to participate in mediating cellular processes (proliferation, differentiation, division) by initiating a set of signalling molecules (PI3-kinase, S6 kinase, PIC or PLC-γ , GAP, Grb2, SHPTP-2 (a PTP), *Src* family) plus signalling cascades (*e.g. Ras*, MAP kinase & JAK-STAT)—see below.	102
G-protein coupled receptors	Hormones, neuropeptides/neurotransmitters and vasoactive peptides are known to transmit their signal *via* G-protein coupled receptors. These comprise a large superfamily of receptors which operate through guanine nucleotide binding (G) proteins (*e.g. Ras*) and are known to activate multiple effector systems (*e.g.* ion channels, AC; activation of PIC, PKC, and the MAP kinase cascade; plus possibly the JAK-STAT pathway–below entries). Structurally, they consist of a cluster of 7-transmembrane helical domains that describe a pocket for ligand binding-with intracellular loops involved in G-protein coupling	103
Kinases in phospholipid signalling	PIP_2 ($PtIns(4,5)P_2$) hydrolysis is a mitogenic signal implicated in both the normal and pathological control of cell growth. This is regulated by an inositol lipid-specific PLC called a phosphinositidase C (PIC) & generates IP_3 ($Ins(1,4,5)P_3$, which releases Ca^{2+}) plus DAG (which activates PKC). Many other phosphorylated forms of membrane-bound inositol lipids play several distinct (but still undefined) rôles in cell signalling. Phosphatidylinositol 3-kinase (PI 3-kinase)—a DSK activated by RTKs (*via* PLC_γ), G-protein coupled receptors (*via* PLC_β), and T-*Ras*—produces a set of 3-phosphorylated lipids from PIP_2 (PIP_3 or $PtdIns(3,4,5)P_3$; $PtdIns(3,4)P_2$; PIP or PtdIn(3)P) which have been correlated with cell proliferation and motility. One of these products PIP_3 is a specific activator of certain isozymes of PKC.	104 105 106

transcription by interaction with RNA polymerase III, a nuclear enzyme that synthesises RNA from a DNA template. Such an interaction may be considered analogous to the interactions of growth factors with their receptors and is exemplified by the c-*fos* gene product associating with the *jun* protein (p39) thereby forming an activating protein (AP-1) which can stimulate synthesis at its designated DNA binding site.[107]

Today a great deal of effort is being directed at ascertaining these transcriptional events and burgeoning evidence is giving a better insight into the contextual (or pleiotropic) nature of the external instigators of such events, *i.e.* the growth factors.[108] For example, signal convergence can occur from different growth factor receptors *via* the MAP kinase pathway and ends in the regulation of the activity of a transcriptional factor, serum response element (SRE), known to regulate activity at the c-*fos* gene.[109] In some cases, activation of a

Table 5 Signal Transduction Processes—An Overview (continued)

Generic Enzyme Signalling Pathways		
Enzyme Family	**Brief Description**	**Reviews**
MAP kinase cascade	Mitogen-activated protein (MAP) kinases and their cloned gene products (called extracellular regulated kinases, ERKs) are activated by a wide variety of external signals through many cell-surface receptors *via* c-*Raf*-1 (*e.g.* RTKs, serpentines, cytokines and others). They represent a ubiquitous unit of phosphorylation events that couple upstream input signals to a variety of outputs: *e.g.* negative growth control, differentiation, proliferation and stress responses; whereby each kinase is regulated by a partner kinase, *i.e.*: MAP kinase (MAPK) by MAPK kinase (MAPKK, or MEK for MAPK/ERK kinase); MAPKK by MAPKK kinase (MAPKKK, or MEKK).	110 111 112
Ras signalling network	*Ras*, a small G-protein, acts as a molecular switch which can interconvert between an active GTP-bound form (T-*Ras*) and an inactive GDP-bound form (D-*Ras*). This interconversion is stimulated by a few distinct GTPase activating proteins (GAPS) and is facilitated by GDP-releasing factors (GRFs). *Ras* may be activated to T-*Ras* by RTKs, cytokine receptor aggregates with cytosolic tyrosine kinases (*Lck*, *Lyn*), or by individual cytosolic tyrosine kinases (*Src*, *Abl*, *Neu*). T-*Ras* also activates c-*Raf*-1, and thus the MAP kinase cascade.	113 114 115
JAK-STAT pathways	This is a newly recognised pathway whereby the direct activation of transcriptional factors occurs *via* growth factor or cytokine receptor stimulation. Thus cytosolic STATs (signal transducers and activation of transcription) become transiently associated to activated receptors, whereby tyrosine phosphorylation induces STAT homo- & hetero-dimerisation and initiates dimer migration to the nucleus (by some unknown mechanism). Cytokine receptors lack intrinsic PTK activity & recruit members of the JAK (Janus kinase) family to act as adaptor proteins thereby forming aggregates able to phosphorylate the STATs.	116 117

transcription factor requires the dual or multiple action of different pathways of the cell, *e.g.* the full activation of the transcription factor AP-1 requires both the calcium and PKC dependent pathways in activated *T*-cells.[118] Futhermore, depending on the specific physical interactions among the STATs, JAKs, and receptors, a repertoire of differing STAT complexes may be formed. A few of these have been shown to elicit different DNA sequence specificities and thus illustrate a further mode of contextual regulation.[119]

CHAPTER «3» Signalling at the Epidermal Growth Factor–Receptor

Unequivocally, cancer is a genetic disease and disruption in the regulation of proto-oncogenes and tumour suppressor genes is the fundamental cause for the onset and progression of the neoplastic state. In general, most of these cancer-associated genes are integral participants in the signalling pathways of a cell (Table 6). In that sense, cancer may

Table 6 Functions of Oncogene & Tumour Suppressor Gene Products

Cellular Function (or Class)	Representative Gene (and/or Product)
Growth factors	*sis* (PDGF B-chain), *fgf*-5, *hst* & *int*-2 (all FGF-like) EGF, EPO, IL-2, IL-3, CSF-1, GM-CSF
Growth factor receptors	*erb*B (EGF-R), *erb*B-2 (heregulin-like receptor) *fms* (CSF-R), *kit* (SCF-R), *trk* (NGF-R) *met*, *ret*, *ros*
Intracellular transducers	*abl*, *lck*, *Src* family, & *yes* (all PTKs) *Raf* family, *pim*, & *mos* (all PSKs) *gsp* & *Ras* family (all G-proteins) *sos* (a GRP); *NF*-1 (a GAP-like suppressor gene) *grb*, *crk*, *nck*, & *shc* (all adaptor proteins)
Nuclear transcription factors	*fos/jun* (AP-1), *myc*, *myb*, *rel*, *E2A*, *RB*, *p53* &*WT*1 (all suppressor genes)
Steroids receptors (transcriptional regulation)	*erbA*; *RAR*
Cell adhesion/matrix control	*DCC*, *APC* & *NF*2 (all suppressor genes)
Cell survival	*bcl*-2 (inhibits apoptosis)

be considered a disease of intracellular signalling and that the overexpression, mutation, or inactivation of such gene products will subvert a normal cell into an unhealthy state.[120] For this reason, there has been a surge of activity in recent years to develop signal transduction inhibitors in order to circumvent or reverse such oncogenic processes.

3.1 Rationale for Targeting EGF-R

Within the plethora of signal transduction processes there are many conceivable areas of intervention. However, particular interest has centred on the signalling pathways associated with the epidermal growth factor receptor (EGF-R) since an abundance of experimental evidence has implicated its involvement in many human cancers.[121] Clinical evidence, amongst others, shows that high levels of EGF-R are often found in epithelial tumours of the breast, bladder, oesophagus, and lung, as well as gynecological malignancies of the ovary, cervix and vulva. Related studies have shown that the gene encoding EGF-R (c-*erb*B) is often amplified or overexpressed in human squamous cell carcinomas, mammary carcinomas and bladder carcinomas. Further genetic investigations have shown oncogene products to be homologous to EGF-R (*erb*B-2 or HER-2/*neu*, plus *erb*B-3 and *erb*B-4) and these oncoproteins are overexpressed in carcinomas of the breast, stomach, ovary, and

lung.[122] Moreover, the growth factors which activate EGF-R signalling (EGF and TGF-α) are commonly co-produced with EGF-R in either an autocrine or paracrine fashion (*cf.* Figure 1.6) to give continual growth stimulation of various human tumours including: glioblastomas, oesophageal squamous-cell carcinomas and adenocarcinomas of the lung, stomach, colon, pancreas, kidney, ovary and breast.[123] In addition, it has been observed that certain tumour cells are more dependent on the EGF-R signalling pathway for proliferation than their normal counterparts (*e.g.* breast carcinoma cells).[124]

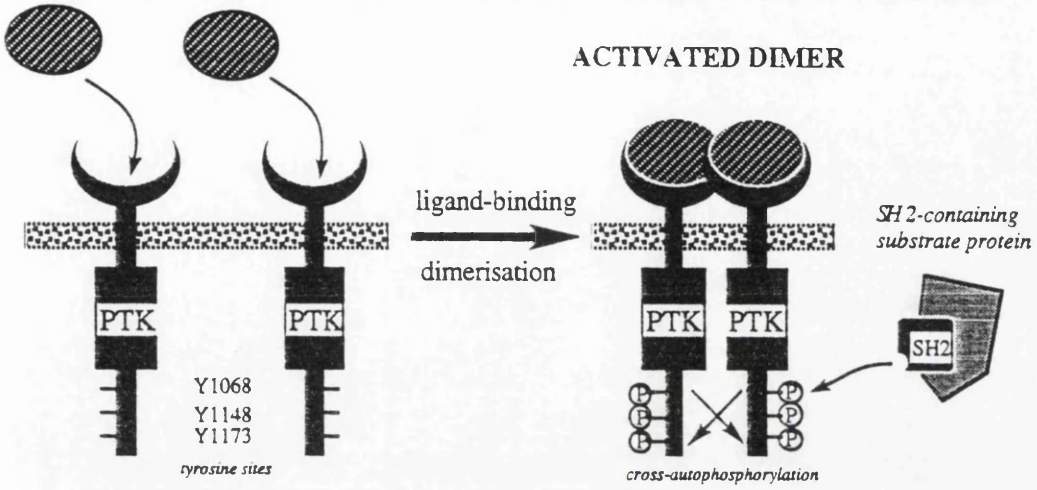
All this information has provided a strong impetus for the development of anticancer agents targeted at the EGF-R growth control pathway. However, there are many possible points of pharmacological intervention along this pathway, from inhibiting growth factor binding through interference with the phosphorylation of regulatory proteins and transcription factors *via* specific protein kinases and phosphatases. Therefore, in order to devise a precise target, this mitogenic signal transduction process should be studied in more detail.

3.2 EGF-R Kinase Activation

A variety of viral and cellular ligands are known to activate EGF-R but among these the most important include the mammalian polypeptide growth factors:[125] epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α), and amphiregulin (produced by human MCF-7 adenocarcinoma breast cells).[126] Ligand binding of these growth factors to the extracellular domain of EGF-R (see Figures 7 and 8) stimulates a conformational change that allows the transmembrane α-helical anchor to either rotate or slide within the cell membrane. In this model, this leads to adjacent receptors to cluster and is referred to as allosteric receptor oligomerisation (or dimerisation).[127] This then leads to the ability for intimate receptors to phosphorylate each other on tyrosine residues located within their adjacent carboxy-terminal segments[128] (a process called autophosphorylation or cross-phosphorylation; Figure 11).[129] These autophosphorylation sites serve the function of creating suitable docking regions for down-stream signal transduction proteins that contain SH2 domains. Importantly, this provides the necessary activation step required for substrate-binding to the intracellular PTK domain.

However, very recently, an alternative model for EGF-R activation has been proposed and suggests that dimerisation is not a prerequisite for receptor activation.[130] Moreover, the functional state of the EGF-R is a preformed but inactive dimer form that only becomes

Figure 11 Ligand-Induced Dimerisation and EGF-R Activation



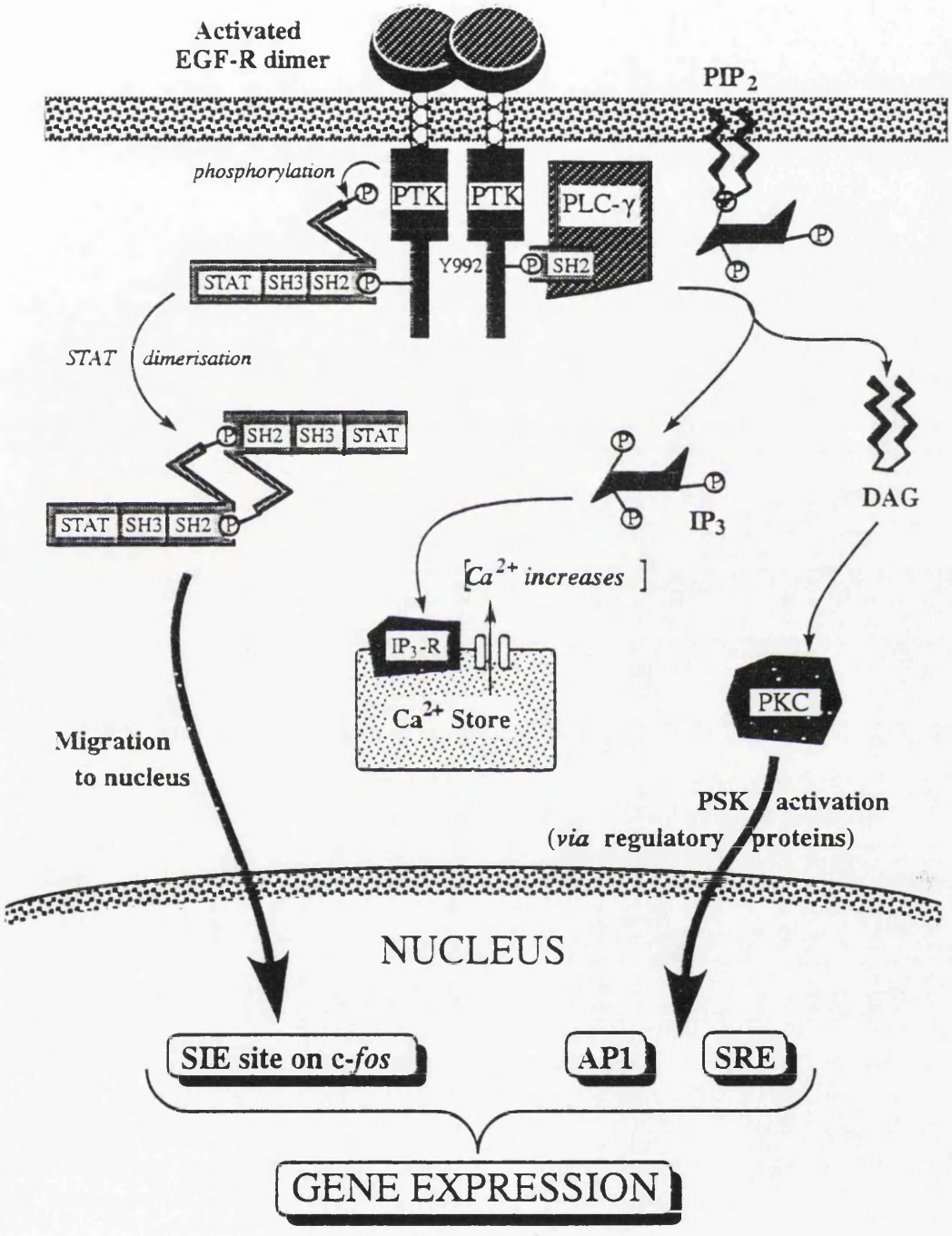
activated on ligand binding. Futhermore, it is postulated that the stereochemical requirements enforced by the closely bound ligands induce a mutual rotation (or twist) of the receptor monomers that enables an appropriate orientation for PTK activation to occur. In any case, the result is a fully functional cytosolic region of the EGF-R with intrinsic PTK activity and enables the subsequent tyrosine phosphorylation of many SH2-containing intracellular proteins (see Figure 9 for examples).

3.3 Signalling Cascades at EGF-R

The activation of EGF-R can initiate a variety of signalling cascades and, in general, the intervening regulatory steps still remain to be fully elucidated. However, recent progress has deciphered at least three main pathways that link the action of extracellular growth factors such as EGF and TGF-α to the transient induction of nuclear oncogenes that encode transcriptional regulatory proteins. Two of these signalling cascades have been illustrated in Figure 12 (see Table 5 and also "List of Abbreviations" if necessary). As indicated, the cytoplasmic STAT proteins become transiently associated with EGF-R during activation, and phosphorylation at their C-termini allows STAT dimerisation. Following this the STAT dimers translocate to the cell nucleus, by some unknown mechanism, whereby they bind directly to specific DNA sequences, *e.g.* the SIE (*sis* inducible element) site found on the c-*fos* gene (this is a base sequence previously recognised to induce *sis* gene expression).[131]

Alternatively, EGF-R activation triggers PLC-γ mediated PIP_2 hydrolysis to generate the second messengers DAG and IP_3. Recently it has been highlighted that this step requires a

Figure 12 Two Signalling Cascades at the EGF-R



further protein called phosphatidylinositol transfer protein (PITP).[132] In any case, the product DAG stimulates a PSK called protein kinase C (PKC) while IP_3 releases intracellular calcium from endoplasmic reticulum (ER) stores by acting on specific IP_3-receptors (IP_3-R).

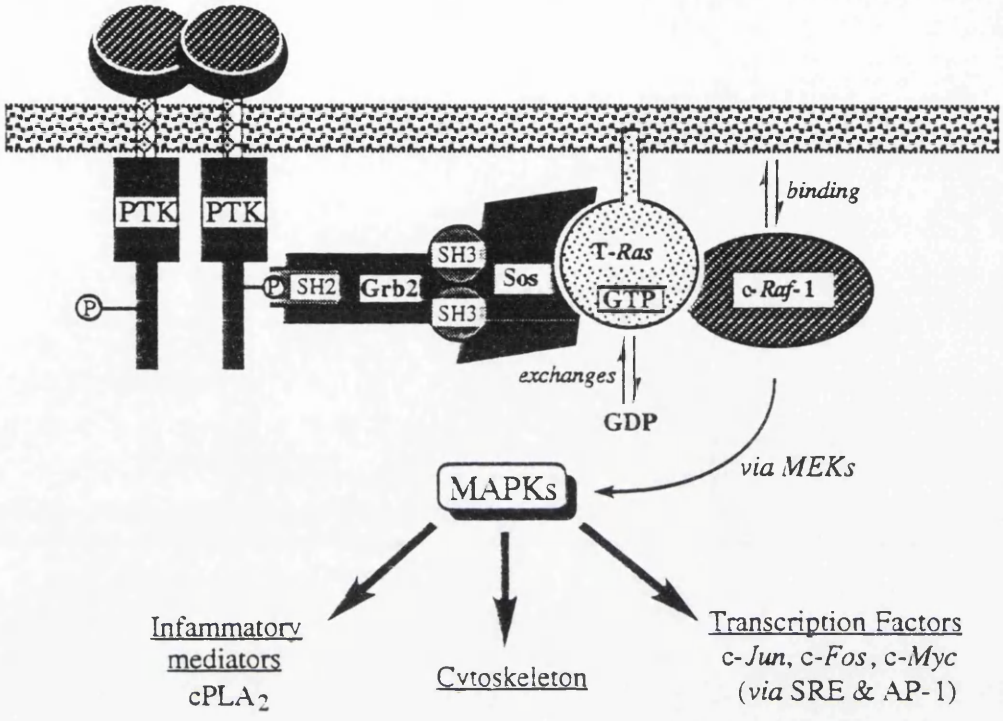
Through serine/threonine phosphorylation events, PKC leads to the activation of the transcription factors AP-1 and SRE which can then assist gene expression.

PKC is also mediated by calcium and initiates many, and as yet unmapped, kinase cascades. In addition, it has the function of down-regulating the EGF-R by phosphorylating a threonine residue (Thr^{654})—this is a common regulatory event to alter the signalling activity at this receptor.[133] Not shown in Figure 12 is the conversion of DAG into a C_{20}-fatty acid called arachidonate, a prostaglandin (PG) precursor, which can regulate cytosolic signalling or, as a PG, be transported out of the cell to act at specific adenylyl cyclase (AC) coupled receptors. Futhermore, changes in calcium levels modulate various calcium- and voltage-dependent ion channels for growth related processes and, while still poorly defined, this has recently been associated with the phenomenon of apoptosis.[134]

One of the most noteable achievements in recent years has been the connection of *Ras*, a small GDP/GTP-binding (G) protein, to the EGF-R or PDGF-R signal transduction pathways. The significance of this finding is that mutations in the *Ras* protein have long been associated with a large proportion (around 30 %) of all human carcinomas and, in particular, with 90 % of pancreas and 50 % of colon cancers.[135] This is yet more convincing evidence to concentrate on inhibiting EGF-R signailing pathways.

The sequence of events in this pathway is illustrated in Figure 13. Thus EGF-R activation promotes binding of a non-catalytic unit called growth factor binding protein (Grb2) *via* its SH2 domain. This so-called adaptor protein is constitutively bound to a GDP releasing factor (a GRP) called son-of-sevenless (*Sos*) *via* its two SH3 domains. This brings *Sos* to the membrane whereby it activates D-*Ras* into its active T-*Ras* state by facilitating the exchange of GDP for GTP (*cf.* "*Ras* signalling network" in Table 5). Not indicated, is the fact that the Grb2-*Sos* complex may also bind to EGF-R indirectly using an additional phosphorylated adaptor protein, *Shc*, which binds through either its SH2 domain or, more likely, through its PTB domain.[136] In any case, T-*Ras* associates with a MEKK called c-*Raf*-1 (a PSK) which, when bound to the cell membrane, becomes activated and thus able to initiate the MAP kinase cascade (see Table 5). At the end of this sequence, MAP kinases regulate the expression of various transcription factors, including SRE which regulates c-*fos* expression, as well as affecting other components of the cell, *e.g.* PSKs, the cytoskeleton, and inflammatory mediators such as cytoplasmic phospholipase A_2 ($cPLA_2$).

Figure 13 EGF-R Activation of MAP Kinase Cascade *via* Ras



CHAPTER «4» Cancer Treatment & Chemotherapy

If an individual has the unfortunate diagnosis of having cancer, a variety of possible treatment options may be considered (see Table 7). The choice of treatment is determined by the type of cancer and the extent to which the disease has progressed. Generally the limiting factor is metastasis and, more often than not, drug resistance results from a given chemotherapeutic drug. Above all this, the present day anticancer drugs are grossly unselective and interfere with rapidly dividing cells such as the hæmatopoeitic, intestinal epithelial, and hair follicle cells. These results have lead researchers to develop alternative treatment strategies (see later sections).

4.1 Cancer Treatment

Cancer treatments in the clinic are continually improving and often require the combination of surgeon, radiotherapist, clinician and oncologist. Together with the correct choice of chemotherapy a whole range of success stories have resulted. A list of these could include: acute lymphocytic leukæmia of childhood, Hodgkin's disease, Burkitt's lymphoma, Ewing's sarcoma (a form of bone cancer), Wilm's tumour (a kidney cancer in children), rhabdomyosarcoma (a cancer of the muscle tissue), choriocarcinoma (a malignancy of the

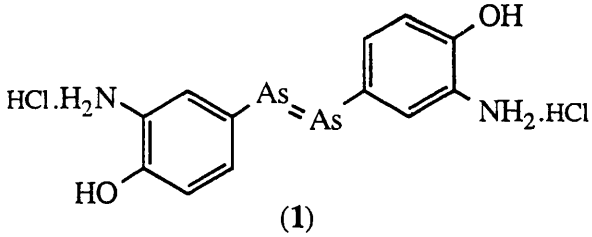
Table 7 Current Cancer Treatments

Treatment Method	Discussion
Surgery [137]	This is the first line of attack against most cancers. If operable (unlike brain tumours) removal of a benign tumour (or carcinoma *in situ* if diagnosed early) results in a complete cure. Thus early diagnosis is essential but unfortunately about 70% of cancers have already metastasised and therefore cannot be eliminated by surgery alone. Nevertheless, clinical staging of the primary tumour mass still forms an integral part of treatment, in combination with radiation and chemotherapy.
Radiotherapy [138]	This is primarily used for the treatment of localised cancers that have spread beyond the scope of surgical removal and is used in combination as such. For some cancers it is the preferred treatment due to their sensitivity to DNA damage (*via* radical formation). However, its effectiveness is limited by the fact that radiation is not selective for cancer cells and is often highly toxic to the patient leading to anaemia, nausea, vomiting, diarrhoea, skin damage, hair loss, and sterility.
Chemotherapy [139]	Surgery and radiotherapy are frequently limited by metastasis of cancer cells and often the adminstration of chemotherapeutic drugs is required. Unfortunately, most clinically used drugs act by damaging DNA or by interfering with DNA synthesis and, like radiation, unselectively kill rapidly dividing cells giving the same adverse side effects (see previous entry). The goal of the physician is to regulate doses such that cancer cell death is maximised while maintaining normal cell survival.
Drug Resistance [140] and Combination Chemotherapy	Often a drawback of chemotherapy is drug resistance whereby a small proportion of tumour cells remain and thus reform a tumour. In certain cases the genetic instability of cancer cells also renders them resistant to many drugs (multidrug resistance). This may be combatted by administering a spectrum of chemotherapeutic agents with alternative modes of action and toxicity patterns (combination chemotherapy). This has resulted in most of the progress in chemotherapy over the last few decades.
Bone marrow transplantation [141]	An approach to bypass the toxicity of cancer therapies to rapidly dividing haematopoeitic cells is to perform bone marrow transplantations thereby allowing higher drug or radiation doses. This is often employed for the treatment of leukæmias & lymphomas, but also for solid tumours. Unfortunately, during & after this operation the immune system is impaired & there is a high risk of infection.
Hormone therapy [142]	Some cancer cells still can respond to growth factors and manipulation of hormone levels can be an effective means of cancer treatment. For eample: breast cancers can be treated with tamoxifen (an antagonist at the oestrogen receptor); endometrial cancer is inhibited by progesterone (a natural inhibitor of proliferation in the menstrual cycle); prostate cancer can be suppressed by stopping androgen (*e.g.* testosterone) production at the testes; leukæmias and lymphomas may be controlled by glucocorticoids-usually prednisone, a natural antiproliferative of lymphoid cells.
Immunotherapy [143]	Immunotherapy is an attempt to stimulate the natural defence mechanisms of the body to eliminate cancer cells. In the treatment of human colon carcinomas the general immune stimulant, levamisole has been proved useful in combination with the chemotherapeutic agent, fluorouracil. More specific immunostimulants would include: tumour-infiltrating lymphocytes (possess antitumour activities) in combination with their cytokine growth factors, interleukin-2 (an interferon) or tumour necrosis factor; monoclonal antibodies, alone or for drug targeting.

placenta), testicular cancer, certain ovarian cancers, and oestrogenic sarcoma. Unfortunately, these have a relatively low incidence and the major types of cancers which occur in adults (breast, lung, colon, and prostate) remain largely incurable. Nevertheless, early diagnosis and prompt treatment by the forenamed arsenal of specialists, in combination with chemotherapy, still holds the best hope for a patient's long-term survival.

4.2 Classical Chemotherapeutic Agents

The beginings of chemotherapy can be traced back to the times of Paul Ehrlich (1854-1915)[144] who coined the term "chemotherapy" as the classical cure of infectious diseases (1891).[145] Although his seminal work (ending 1910) provided a "magic bullet" against syphilis (not cancer), the arsenobenzyl compound called salvarsan (**1**), was undoubtedly the first true chemotherapeutic agent. Importantly, this led to an organisation of pharmaceutical research which has not changed materially to the present day and is inextricably linked to cancer research. Since that time the emergence of various forms of chemotherapeutic agents targeted at cancer has resulted.



(**1**)

Today the choice of clinically proven chemotherapeutic agents is continually being reassessed as rapid developments in this field occur. The current status (from Goodman and Gilman)[146] of compounds worthy of being characterised as chemotherapeutic agents (with acceptable toxicity limits) has been classed and summarised in Table 8. These classes of drugs are still prodominantly used in cancer chemotherapy today and each class often reflects its mode of action. For an overall perspective on cancer chemotherapy and chemistry of anticancer agents the reader is refered to the literature.[147]

Ironically, the alkylating agents are very potent carcinogens and mutagens. As inferred by their name, they become covalently bound to a diverse set of cellular constituents, disrupt the normal function of the cell and therefore exhibit an overall cytotoxic effect. The nitrogen mustards act as DNA cross-linkers:[148] as do the aziridines, the alkane sulfonates, the nitrosoureas, and several of the platinum complexes. Repair mechanisms within the cell

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease

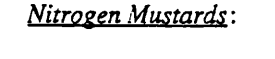
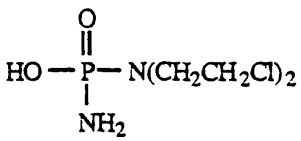
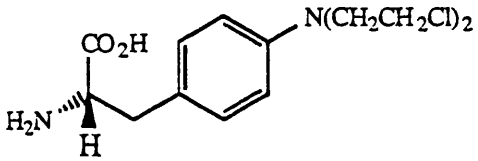
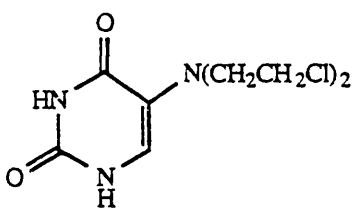
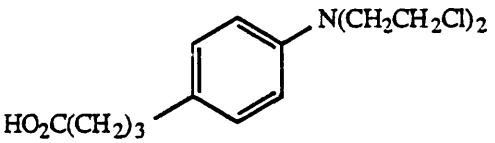
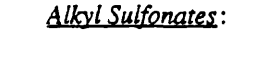
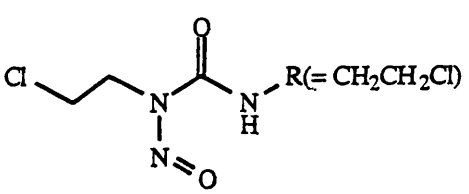
Name	Structure	Cancer Application
	Alkylating Agents	
Mechlorethamine (HN_2)	*Nitrogen Mustards*: 	Hodgkin's disease, non-Hodgkin's lymphomas
Cyclophosphamide (CP)		Acute and chronic lymphocytic leukæmias, Hodgkin's disease, non-Hodgkin's lymphomas, multiple myeloma, neuroblastoma, breast, ovary, lung, Wilm's tumour, rhabdomyocarcoma
Melphalan (L-sacolysin, L-PAM)		Multiple myeloma, breast, ovary
Uracil mustard (Dopan)		Chronic lymphocytic leukæmia, Hodgkin's disease, non-Hodgkin's lymphomas, ovary, primary thrombocytosis
Chlorambucil		Chronic lymphocytic leukæmia, Hodgkin's disease, non-Hodgkin's lymphomas
Myleran	*Alkyl Sulfonates*: 	Chronic myeloid leukæmia
Carmustine (BCNU)	*Nitrosoureas*: 	Hodgkin's disease, non-Hodgkin's lymphomas, primary brain tumours, multiple myeloma, malignant melanoma
Lomustine (CCNU)		Hodgkin's disease, non-Hodgkin's lymphomas, primary brain tumours, small-cell lung

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease (continued)

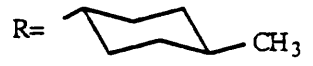
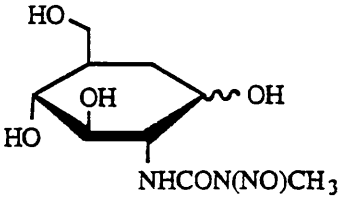
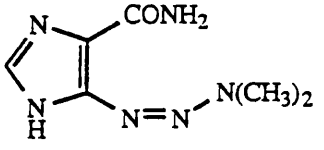
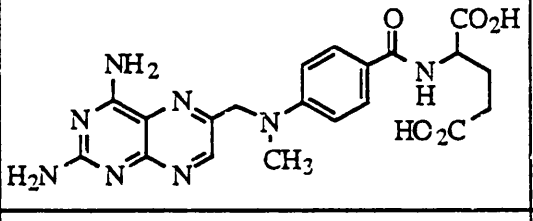
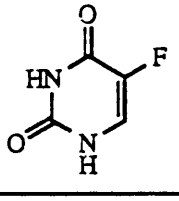
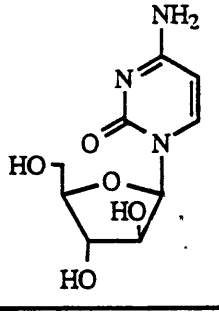
Semustine (MeCCNU)		Primary brain tumours, stomach, colon
Streptozocin (streptozotocin)		Malignant pancreatic insulinoma, malignant carcinoid
Dacarbazine (DTIC; dimethyltriazenoimidazole carboxamide)	*Triazenes*: 	Malignant melanoma, Hodgkin's disease, soft-tissue sarcomas
Antimetabolites		
Methotrexate (amethopterin)	*Folic Acid Analogues*: 	Acute lymphocytic leukæmia, choriocarcinoma, mycosis fungoides, breast, head and neck, lung, osteogenic sarcoma
Fluorouracil (5-fluorouracil; 5-HT)	*Pyrimidine Analogues*: 	Breast, colon, stomach, pancreas, ovary, head and neck, urinary bladder, premalignant skin lesions (topical)
Cytarabine [cytosine arabinoside; 1-(β-D-arabinofuranosyl)-cytosine, araC)]		Acute granulocytic and acute lymphocytic leukæmias

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease (continued)

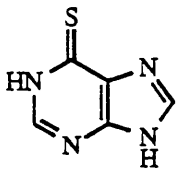
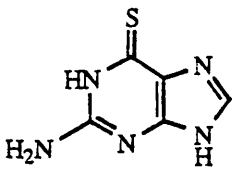
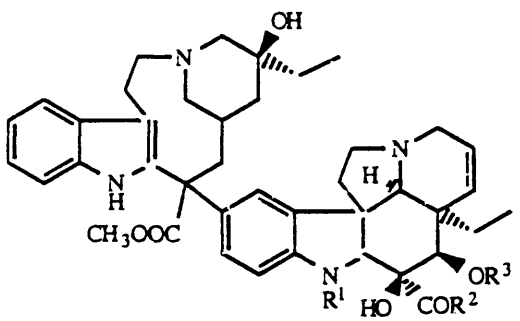
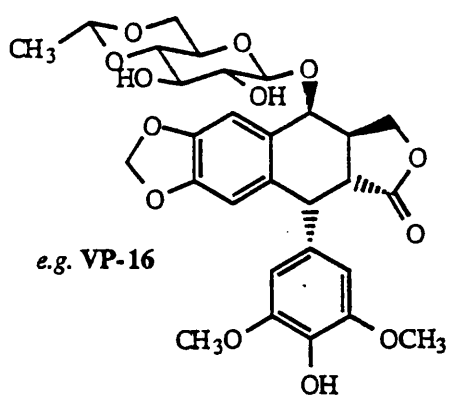
Mercaptopurine (6-mercaptopurine; 6-MP)	*Purine Analogues*: 	Acute lymphocytic, acute granulocytic, and chronic granulocytic leukæmias.
Thioguanine (6-thioguanine; TG)		Acute lymphocytic, acute granulocytic, and chronic granulocytic leukæmias.
Natural Products		
Vinblastine (VLB) [$R^1 = CH_3$, $R^2 = OCH_3$, $R^3 = COCH_3$]	*Vinca Alkaloids*: 	Hodgkin's disease, non-Hodgkin's lymphomas, breast, testis
Vincristine (VCR) [$R^1 = CHO$, $R^2 = OCH_3$, $R^3 = COCH_3$]		Acute lymphocytic leukæmia, neuroblastoma, Wilm's tumour, rhabdomyosarcoma, Hodgkin's disease, non-Hodgkin's lymphomas, small cell-lung
Vindesine (VDS) [$R^1 = CH_3$, $R^2 = NH_2$, $R^3 = H$]		Lymphomas, blastic crisis of chronic granulocytic leukæmia, systemic mastocytosis
Etoposide (VP-16) Teniposide (VM-26)	*Epipodophyllotoxins*:  *e.g.* **VP-16**	Testis, small-cell lung and other lung, breast, Hodgkin's disease, non-Hodgkin's lymphomas, acute granulocytic leukæmia, Kaposi's sarcoma

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease (continued)

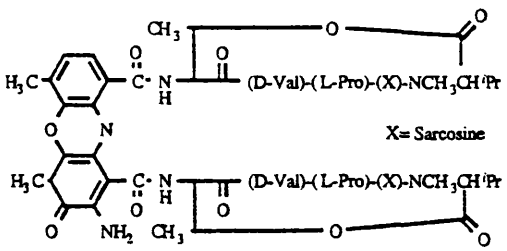
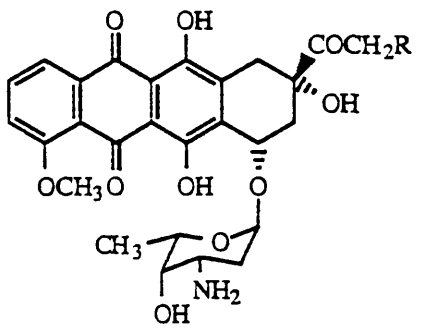
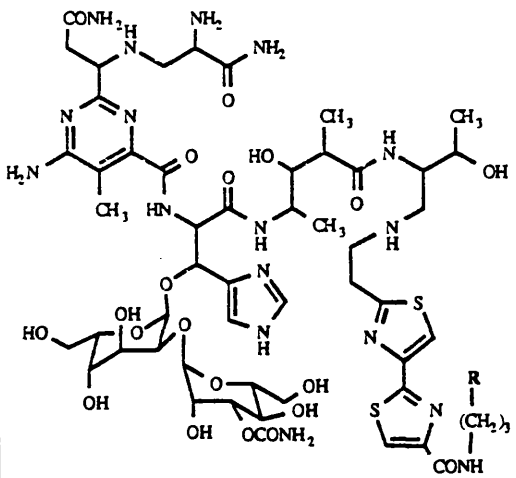
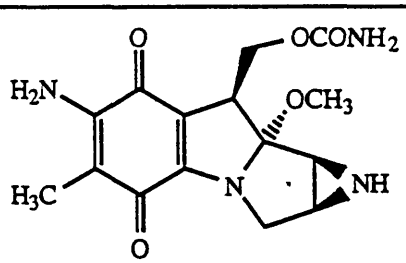
Dactinomycin (actinomycin D)	*Antibiotics*: 	Choriocarcinoma, Wilm's tumour, rhabdomyosarcoma, testis, Kaposi's sarcoma
Daunorubicin (R= H) (daunomycin; rubidomycin) Doxorubicin (R= OH) (adriamycin)		Acute granulocytic and acute lymphocytic leukæmias Soft-tissue, oesteogenic, and other sarcomas; Hodgkin's disease, non-Hodgkin's lymphomas, acute luekæmias, breast, genitourinary tract, thyroid, lung, stomach, neuroblastoma
Bleomycin (mixture of *ca.* 70% bleomycin A_2 and 30% bleomycin B_2)	 [A_2: R= $^+S(CH_3)_2$ & B_2: R= $CH_2NHC(=NH)NH_2$]	Testis, head and neck, skin, oesophagus, lung, and genitourinary tract; Hodgkin's disease, non-Hodgkin's lymphomas
Mitomycin (mitomycin C)		Stomach, cervix, colon, breast, pancreas, bladder, head and neck

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease (continued)

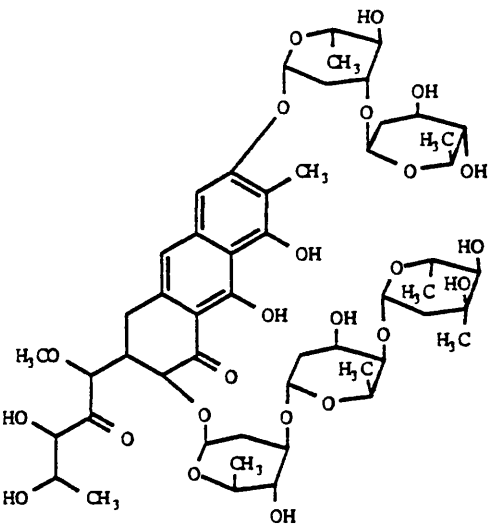
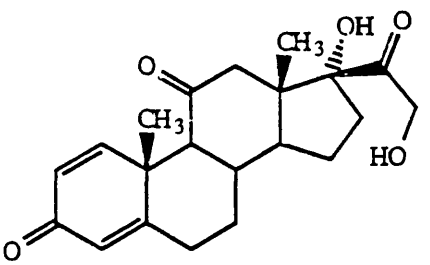
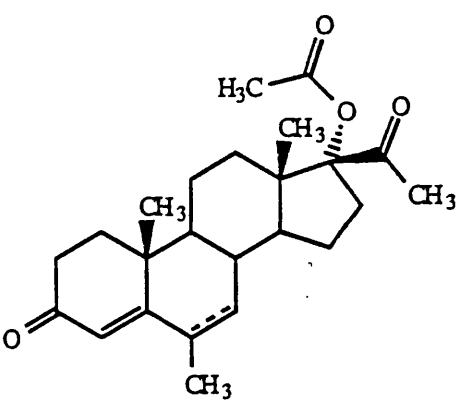
Plicamycin (mithramycin)		Testis, malignant hypercalcaemia
L-Asparaginase	*Enzymes*:	Acute lymphocytic leukæmia
	Hormones and antagonists	
Prednisone	*Adrenocorticosteroids*: 	Acute and chronic lymphocytic leukæmias, Hodgkin's disease, non-Hodgkin's lymphomas, breast
Medroxyprogesterone acetate (no double-bond) Megestrol acetate (contains double-bond, as shown)	*Progestins*: 	Endometrium, breast

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease (continued)

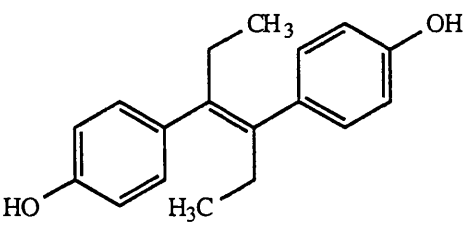
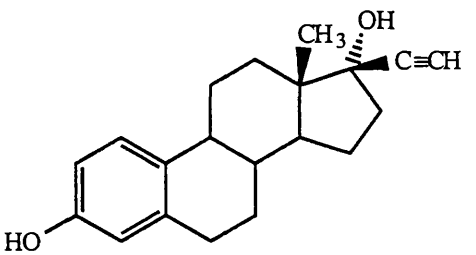
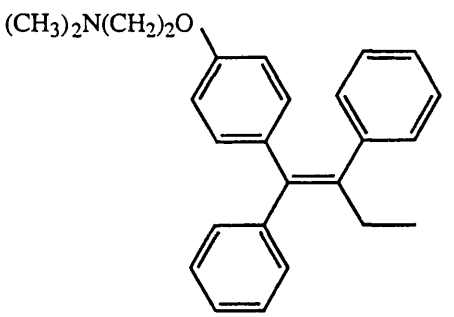
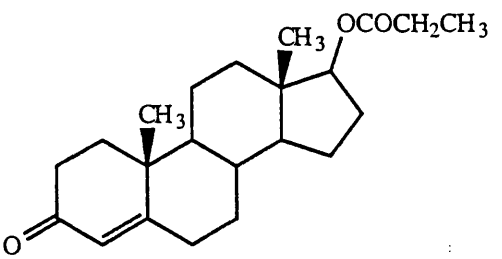
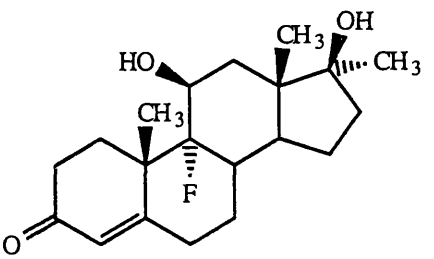
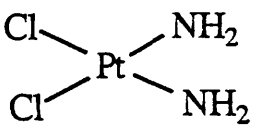
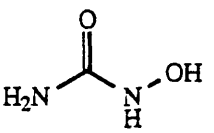
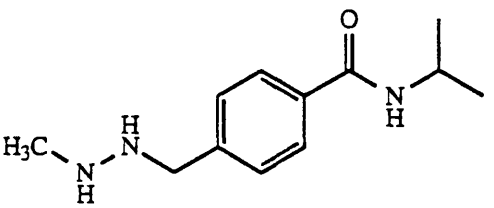
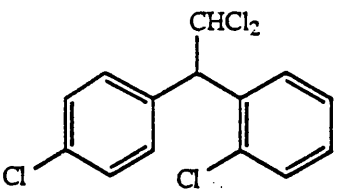
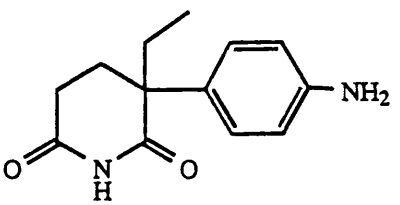
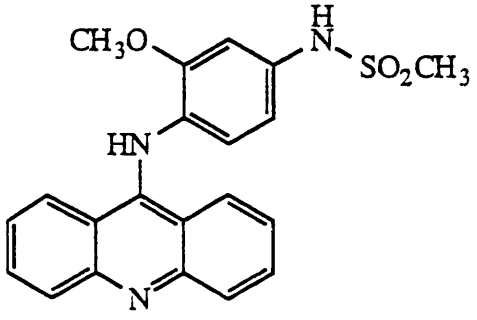
Diethylstilbestrol	*Oestrogens*: 	Breast, prostate
Ethinyl oestradiol		Breast, prostate
Tamoxifen	*Antioestrogens*: 	Breast
Testosterone propionate	*Androgens*: 	Breast
Fluoxymesterone		Breast

Table 8 Chemotherapeutic Agents Useful in Neoplastic Disease (continued)

Miscellaneous Agents		
Cisplatin (*cis*-DDP)	*Platinum Coordination Complexes*: 	Testis, ovary, bladder, head & neck, lung, thyroid, cervix, endometrium, neuroblastoma, osteogenic sarcoma
Hydroxyurea	*Substituted Urea*: 	Chronic granulocytic leukæmia, polycythemia vera, essential thrombocytosis, malignant melanoma
Procarbazine (*N*-methylhydrazine, MIH)	*Methylhydrazine Derivative*: 	Hodgkin's disease
Mitotane (*o,p'*-DDD)	*Adrenocorticol Suppressant*: 	Adrenal cortex
Aminoglutethimide		Breast

cannot cope with such adducts and often DNA strand scission occurs. Also, alkylation of DNA decreases its ability to act as a template for transcriptional processes.[149] Although alkylation can occur at any time, its biological consequences are more severe when it occurs during DNA synthesis[150] and alkylating agents are said to be proliferation dependent. Luckily, this has good implications for selecting out hyperproliferative cancer cells over their normal counterparts.

An alternative way of binding to DNA is in a noncovalent fashion and this is exhibited by the so-called intercalating agents. All such drugs possess planar regions capable of stacking between the paired bases in DNA. This forms a tight drug-DNA interaction that is critical to their cytoxic, mutagenic, and carcinogenic effects.[151] Examples of these would include the acridines, [*e.g.* amsacrine or *m*-AMSA; (2)], anthracyclines (daunorubicin and doxorubicin), dactinomycin, bleomycin and plicamycin (see Table 8).

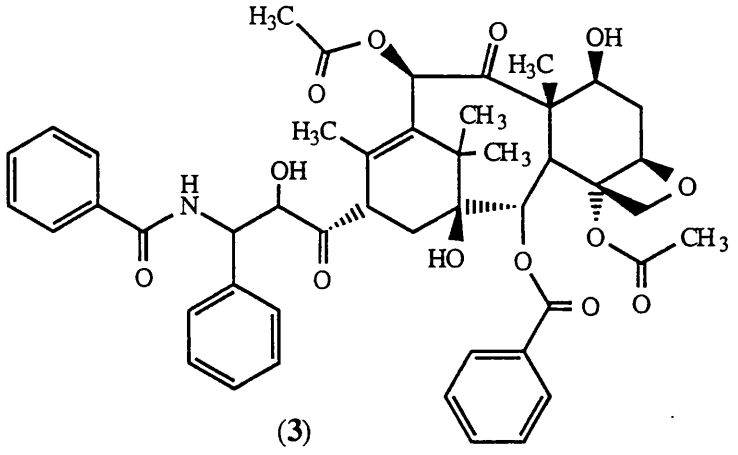


(2)

A further mechanism of action is given by the antimetabolites. These interfere with the production of nucleic acids by antagonising the synthesis of DNA precursors (*i.e.* the deoxyribo*nucleoside* triphosphates) and therefore inhibit DNA (*i.e nucleotide*) synthesis. Alternatively, or in addition, they may act as purine or pyrimidine base surrogates and, by deceiving polymerases, become incorporated into RNA or DNA, and thereby disrupt nucleic acid function. Both these mechanisms are demonstrated by 5-fluorouracil, the thiopurines (6-mercaptopurine and thioguanine) and also the modified nucleoside sugar, cytarabine (see Table 8).

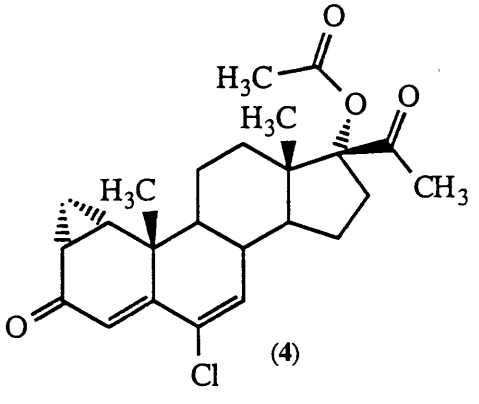
Other chemotherapeutic agents act by disrupting a stage in cell division whereby delicate chromosomal changes in conformation and position are occurring. Thus inhibitors of enzymes that unwind and expose regions of DNA for transcriptional processes (topoisomerase inhibitors) show anticancer properties. Notable examples would include the epipodophyllotoxins, etoposide and teniposide (see Table 8), and is the principle effect of amsacrine[152] (2) in creating DNA strand breaks and cross-links. The vinca alkaloids, vinblastine and vincristine, represent a class of microtubule inhibitors which disturb the functioning of protein polymers (composed of tubulin dimers)[153] that are involved in chromosomal segregation. These act by binding to free tubulin dimers and result in the net depolymerisation of microtubules, destruction of the mitotic spindle, and arrest of

metaphasic cells.[154] In contrast, a revived antitumour agent known as taxol [paclitaxel; (**3**)] acts by stablising the equilibrium in the direction of assembly,[155] rather than dissembly.[156]

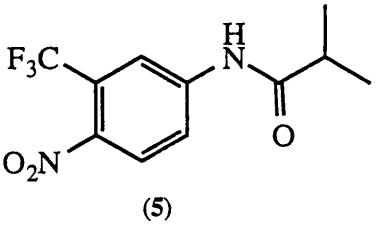


(**3**)

Tumour cells that arise from sexually differentiated tissues (breast, endometrium, prostate) normally contain hormone receptors and thus are able to respond to physiological hormones that naturally affect growth. This is the rationale behind the drugs that affect the endocrine system in steroid therapy. Examples include the glucocorticoids, oestrogens, progestins and androgens (see Table 8). On one hand it may be necessary to administer a hormone which inhibits growth and, on the other, to remove the effect of the growth stimulation made by endogenous hormones. This latter case may be acheived by organ removal (*e.g.* ovariectomy), prevention of hormone synthesis (*e.g.* aminoglutethimide is an aromatase inhibitor), or blocking hormone action [*e.g.* use of antioestrogens, such as tamoxifen; or antiandrogens, such as cyproterone acetate (**4**) or flutamide (**5**)].



(**4**)

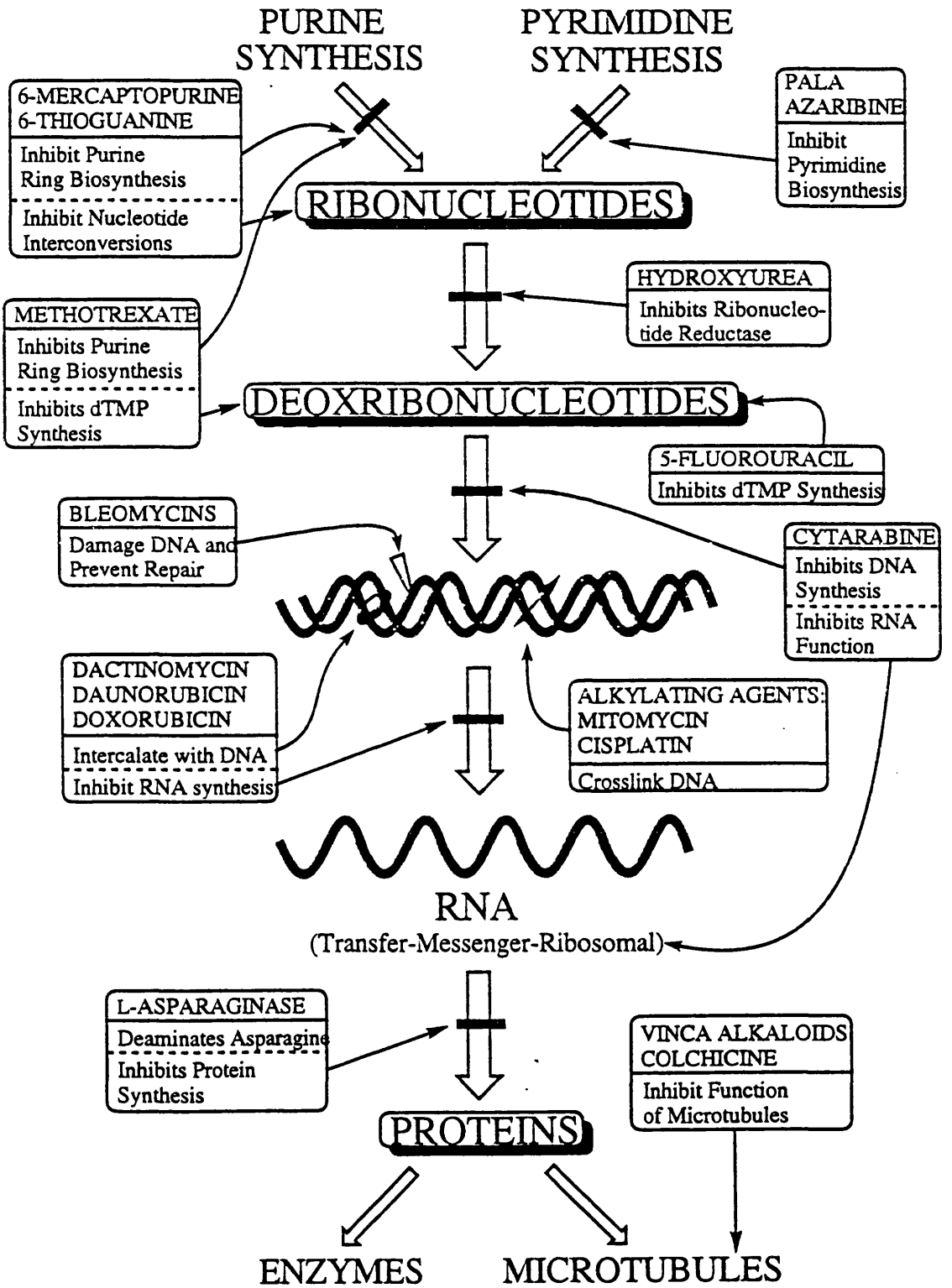


(**5**)

A representative summary of the previously described modes of action of the clinically useful chemotherapeutic agents has been given in Figure 14. As indicated these agents mostly intervene at the DNA level. However, this overall picture is changing and different

points of intervention are emerging as viable targets for the fight against neoplastic processes. This will be discussed in the next section.

Figure 14 Modes of Action of Representative Anticancer Agents



4.3 New Approaches to Cancer Chemotherapy

Most of the chemotherapeutic agents previously descibed have one major drawback—their toxicity to the patient. The root of the problem lies in the rather unselective manner in which they exert their anticancer activity. Obviously superior agents and new directions in drug discovery and treatment therapies must be developed. Potentially, novel anticancer agents may be obtained from a number of sources, as exemplified by the classical agents (Table 9).[157] But, ideally, future anticancer drug discovery should be based on more rational and mechanism-based approaches.

Table 9 Methods to the Discovery of Current Anticancer Agents

Targeted Synthesis	Screening of Natural Products	Screening of Chemicals	Analogue Synthesis	Serendipity & Rational Appl[n]
Antimetabolites: Methotrexate Thioguanine Mercaptopurine Fluorouracil Cytarabine PALA *Hormones*: Steroids Tamoxifen Flutamide Leuprolide Octreotide	Dactinomycin Vincristine Vinblastine Plicamycin Daunorubicin Doxoxrubicin Mitomycin C Bleomycin Streptozocin Taxol	Busulfan Dacarbazine Procarbazine Hydroxyurea Thiotepa Carmustine Lomustine Mitoxantrone Altretamine Pentostatin	Cyclophosphamide Chlorambucil Melphalan Ifosfamide Etoposide Teniposide Carboplatin	Mechlorethamine Asparaginase Mitotane Cisplatin Levamisole Interferons

In recent years, biological approaches to treating cancer have received a lot of attention (consult Table 10 for a referenced listing). This is an area where future breakthroughs will probably occur and so it is important to place these in context with other new therapeutic agents and régimes. Thus, using the technologies of recombinant DNA[158] and gene therapy,[159] one line of attack is to develop immunotherapy protocols[160] whereas others utilise the natural "magic bullets" of the body (*i.e.* the antibodies) to facilitate drug action (tumour-directed monoclonal antibodies, MoAbs). Today, radionuclide MoAb-conjugates are used to localise tumours diagnostically as well as for the purpose of radiotherapy.[161] Morever, in 1992, the first MoAb-based diagnostic agent for tumour γ-camera imaging (OncoScint CR/OV) received approval by the Federal of Drug Administration[162] and currently a number of antitumour MoAb-based therapeutics are in clinical trials.[163]

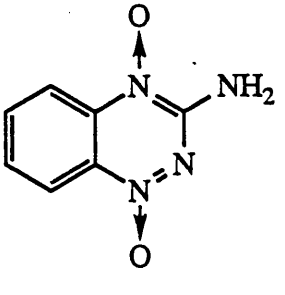
An important therapeutic strategy for the treatment of cancer involves the use of antisense or antigene oligonucleotides.[164] These are genetically engineered or chemically synthesised

Table 10 Biological Approaches to Cancer Treatment

Type of Treatment	Lit.	Type of Treatment	Lit.
Immunotherapies	165	*MoAb-Based Therapies*	168
Biomodulators	166	immunotoxin (IT) therapy	169
Interferon Therapy	167	ADEPT	170
Adoptive Immunotherapies (*interleukin therapies*)		*Gene Transfer Therapies*	
		V-DEPT	171
		G-DEPT	172
LAK-Cell Therapy	173	*Clinical approaches and drug delivery*	
		chemosensitivity testing	174
TIL-Therapy	175	liposome-encapsulation	176
CSF-Therapy	177	biodegradable microspheres	178
TNF-Therapy	179	implantable devices	180

to regulate gene expression selectively by acting as complementary sequences to either target mRNA (the antisense approach) or to target DNA (the antigene approach). The idea is to block an oncogene that is involved in cell immortalisation or cell transformation in the neoplastic process.

This is an area of great excitment and exemplifies the so-called mechanism-based approaches to the design of novel anticancer agents.[181] A further important example of this would include the exploitation of a characteristic of solid tumours, *i.e.* their low-oxygen (hypoxic) enviroment and enables the design of prodrugs which can become cytotoxic upon bioreduction, *e.g.* Tirapazamine™ or SR 4233 (**6**).[182] Moreover, this may lead the way to the treatment of the major types of solid cancers that have eluded the chemotherapist in the past.[183]



(6)

Thus, the search for new compounds for the treatment of cancer has been revolutionised by our burgeoning knowledge of the "workings" of a cell and how previously known anticancer agents exert their effects. Armed with such information, the drug discoverer has been able to "design" more effective analogues and many examples have been described in the recent literature.[184] However, such an exploitation of the classical chemotherapeutic agents will only partially circumvent the problems of cytotoxicity and drug resistance which are commonly encountered in the clinic today. Clearly, we must use the advancing knowledge of the regulatory mechanisms of a cell, together with any highlighted molecular (hence genetic) differences between normal and deranged cell types, to enable us to design *selectivity* into a potential anticancer agent.

4.4 Signalling & EGF-R Inhibition

As outlined previously, signalling at the EGF receptor represents a formidable target for drug intervention and many excellent reviews exist in this area.[185] Within the EGF-R signalling pathways (see Section 3.3) many specific pharmacological targets present themselves (see Table 11 and references therein). For the purpose of this research work, the focus of this discussion will be on the potential chemotherapeutic utility in developing signal inhibitors targeted at the EGF receptor. Theoretically, blocking signalling at or near the cell membrane should provide a more selective strategy than going further downstream. Indeed, signalling is better understood within this region. Unfortunately, there is the possibility that this will not affect a self-perpetuating oncogenic signal or one that is activated by other pathways. The mutated forms of the *Ras* family illustrate a point in this matter since these G-proteins are switched "on" irrespective of the activity at any given receptor. Thus, the inhibition of EGF-R would serve no function in these cases. However, it should be stressed that any excess activity of the normal forms of the *Ras* family will still contribute significantly to the condition of cancer (this is also true for c-*Src*, see reference in Table 11). Hence, these facts further implicate signal amplification at the EGF-R as being a significant factor towards the neoplastic state of a cancer cell (*cf.* Section 3.1).

In light of the above, the logical choice is to inhibit activity at the EGF-R. As indicated in Table 11, one line of attack is to develop growth factor antagonists or receptor-directed antibodies. Unlike the former case, the antibody based agents have shown some success in clinical trials and tend to induce internalisation of the receptor. This down-regulation

Table 11 Signal Transduction Targets of the EGF-R Pathways

Possible Target to Inhibit	Literature Examples	Ref.
Growth factor action at EGF-R	Growth factor antagonists (Suramin) EGF-R raised monoclonal antibodies (MoAb) Growth factor or MoAb toxin-conjugates	186 187 188
PTK activity at EGF-R	**SEE TEXT FOR DISCUSSION**	
Cytoplasmic PTKs	c-*Src* is a viable target	189
SH2/SH3 domains	None cited to date.	190
Ras activation (*via* Grb2/*Sos* & *Sos/Ras*)	Develop farnesylation inhibitors (as *Ras* is attached to membrane *via* a farnesyl group) "Grbstatins" & "Sostatins", which interfere with the complexes opposite, also suggested	191 192
c-*Raf*-1 and MAP kinases	None-cited to date	193
PKC	Suramin, phorbol esters, & bryostatins	194
PLC-γ	ether lipids	195
PI 3-K	*myo*-Inositol analogues & wortmannin	196
Transcription factors (SRE, AP-1)	None cited to date	197

of EGF-R signalling may also be achieved indirectly by stimulating or inhibiting the specific action of PSPs or PTPs.[198] For example, a cytosolic PTP isozyme denoted by PTP1C is known to form a high-affinity complex with EGF-R.[199] It is proposed that PTP1C dephosphorylates specific autophosphorylation sites and the augmentation of this process should provide an inhibitory effect on EGF-R signalling. Furthermore, agonist action on somatostatin receptors is known to stimulate tyrosine phosphatase activity and to inhibit the EGF-stimulated growth of pancreatic cancer.[200] Therefore, the development of somatostatin analogues is another indirect way of controlling signalling at the EGF receptor.

By far the most studied approach to the development of EGF-R signalling inhibitors is based on blocking the inherent PTK activity at the receptor itself. Conceivably such PTK inhibitors can exert their action in a number of ways, *viz.*: to prevent the dimerisation process; to inhibit the autophosphorylation process; to block the ATP-binding site within the receptor PTK domain; and/or to inhibit substrate-binding to the PTK domain (see Figures 8 and 11). Although all these are valid targets, it is debatable whether or not the dimerisation process is critical to receptor activation (see Section 3.2). Futhermore, the ATP-binding site is a highly conserved structural region in all protein kinases and an inhibitor designed to

only compete with ATP would be expected to exhibit low selectivity and could lead to undesirable side-effects, probably toxicity. However, the substrate-binding site is a variable region between PTKs and RTKs, and this should increase molecular selectivity and thus give a favourable toxicity profile for substrate-directed inhibitors *or* ATP/substrate mixed inhibitors. Preferably these PTK inhibitors should possess higher receptor-binding affinities *prior* to autophosphorylation in order to block this activation step and any subsequent phosphorylation of endogenous protein-substrates thereafter.

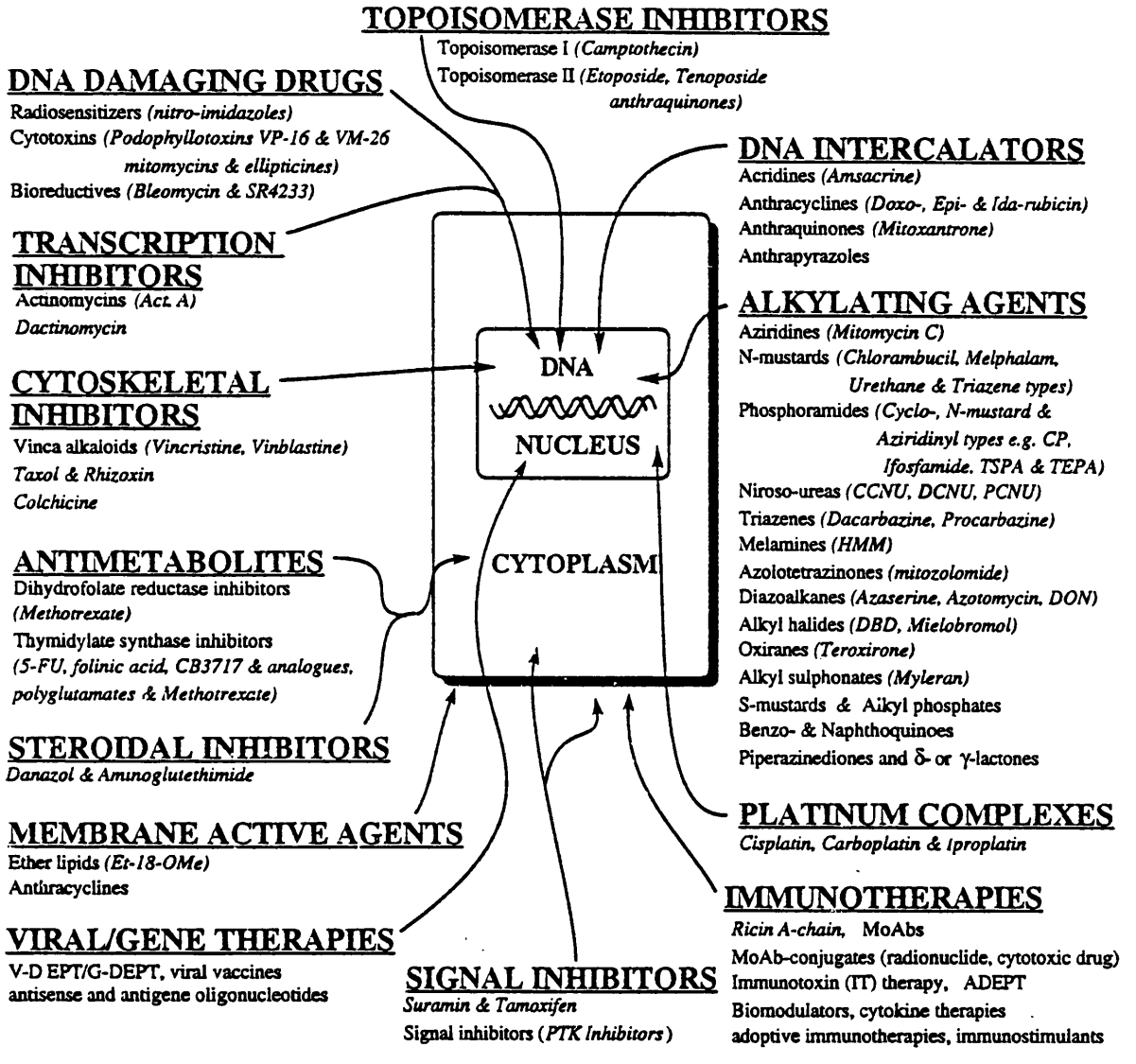
As chemotherapeutic agents, the PTK inhibitors should invoke a "calming" effect on the amplified signals within a cancer cell. In other words, PTK inhibitors should be able to "persuade" the cell to enter G_0 and block any internal signal that enforces a hyperproliferative state. Thus, unlike the cytotoxic chemotherapeutic agents of today, they should exert an overall cytostatic effect and provide a greater efficacy. In addition, this cytostatic effect should be able to cause tumour mass shrinkage since the "natural" loss of tumour cells by necrosis (or apoptosis) should outweigh any cell gain.[201]

Moreover, the strategy is to exploit the contextual and redundant nature of signalling processes (*cf.* Section 2.2) in order to acheive a therapeutic selectivity between tumour cells and normal cells. In addition, the strategy is to revert a tumour cell into a more differentiated form by blocking an overpowering oncogenic pathway to such a degree as to leave the intact normal cellular pathways to take over. In that sense, a PTK inhibitor will not merely be a new antiproliferative tool but an antisignalling agent with the capacity to control cellular processes such as differentiation, apoptosis, angiogenesis, and metastasis. Therefore, the PTK inhibitors should be an extremely valuable addition to the pharmacopœia of the clinician in his/her fight against the multiple forms and stages of the neoplastic state (see Figure 15).

CHAPTER «5» ProteinTyrosine Kinase (PTK) Inhibitors

On the whole, the search for PTK inhibitors has been semi-empirical in nature. Often this has involved the initial discovery of a "lead" compound from some natural source and/or correlating a design to one of several common motifs characteristic to PTKs or their protein substrates, and then manipulating its "chemical make-up" in order to obtain an attractive pharmacological profile. The reason for this is that prior to 1991, no X-ray model for a protein kinase was available to the scientific community. Nevertheless, there has been a

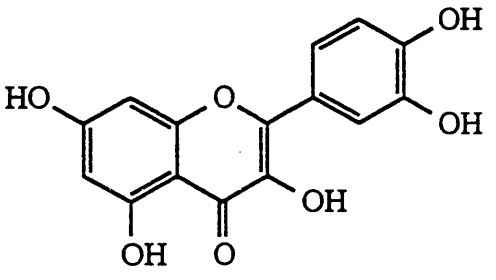
Figure 15 The Evolving Pharmacopœia of the Clinician



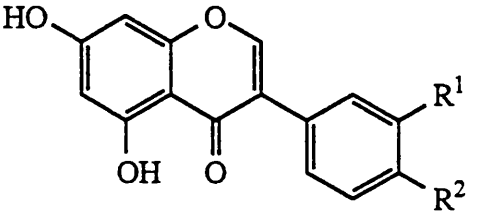
series of hopeful candidates in the last five or so years and these are outlined below—for further information and earlier references the reader should refer to review articles in this area.[202] Today two X-ray structures for a kinase exist. One constitutes the catalytic unit of a PSK and has been used to generate a homology model for the EGF-R kinase.[203] More recently, an X-ray of the PTK domain of the insulin receptor (IR) has been published.[204] These data have generated much excitement in cancer research since comparison of these two models should greatly facilitate the rational design of highly selective PTK inhibitors.

5.1 Flavonoids and Isoflavanoids

In 1975 a flavonol called quercetin (**7**) was found to inhibit malignant cells. Later this activity was shown to be due to the inhibition of tyrosine phosphorylation activity at the v-*src* gene product and lead to the first systematic search for PTK inhibitors based on similar plant products between 1985 and 1988. This screening programme identified a set of isoflavonoids including orobol (**8**) and its regioisomer genistein (**9**) plus a further set of flavonols, but these were not significantly more potent than quercetin (**7**).

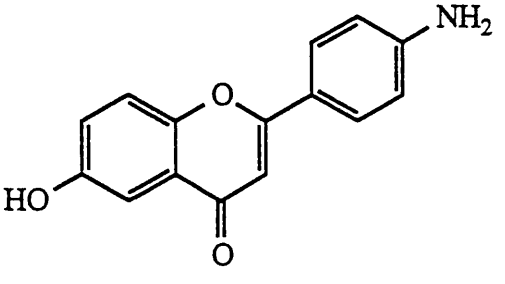


(**7**)

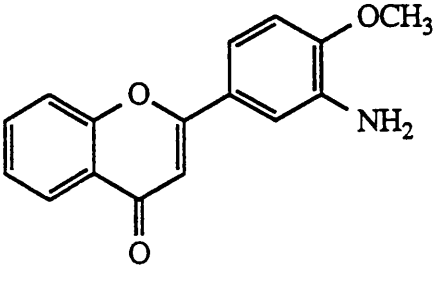


(**8**) R^1= OH, R^2= H
(**9**) R^1= H, R^2= OH

Quercetin is competitive against ATP both *in vitro* and *in vivo* (IC_{50}= 6 to 11 μM) but shows hardly any PTK selectivity and inhibits several other enzymes involved in cell signalling. On the other hand, genistein (**9**) is a competitive inhibitor with respect to ATP and can inhibit EGF-R at concentrations which do not effect PSKs. It has also exhibited cytostatic activity in experimental models but this may not be directly due to EGF-R inhibition. In spite of these ambiguities, these base structures have been used to develop other more selective inhibitors, *e.g.* from independent studies, the potent flavonoid derivatives (**10**) and (**11**) have been identified.[205] The compound (**11**) showed a 4-fold greater differential cytotoxic action against v-*Abl* transformed cells over their non-transformed counterparts and was a very poor ATP-inhibitor at the EGF-R kinase.

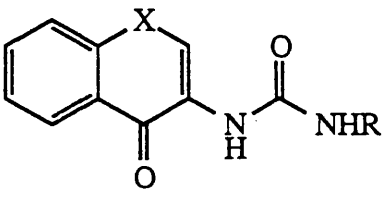


(**10**)

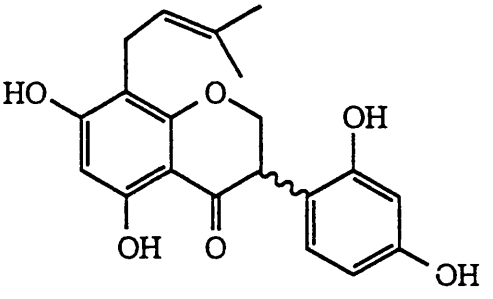


(**11**)

Another class of PTK-inhibitors structurally related to the isoflavones include the benzopyranones and benzothiopyranones, typified by derivatives (**12**). These provide the first examples to act as selective ATP-competitive inhibitors at the v-*Abl* oncoprotein (ID_{50}=1 to 7 μM), compared to EGF-R or PKC, and illustrate that PTK selectivity can be achieved in non-substrate-type competitors.[206] Recently, a naturally occurring isoflavonoid called kievitone (**13**) was identified to be a potent antiproliferative agent of breast cancer cell lines and has been shown to inhibit PTK activity at the EGF receptor. However, its mode of action remains to be determined as flavonoids in general inhibit numerous signalling molecules with varying degrees of selectivity.



(**12**) X= O or S

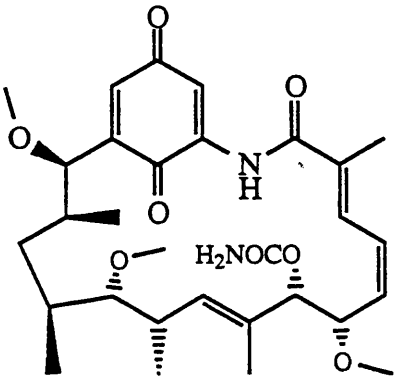


(**13**)

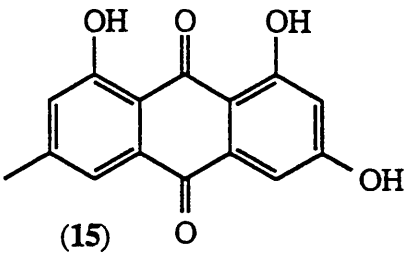
5.2 Quinones and Antibiotics

Several antibiotics of the benzoquinonoid ansamycin group exhibit moderate antitumour activity. The compound herbimycin A (**14**) has been studied the most and reverses malignant cell morphologies which have been induced by certain PTK oncogenes, including *yes*, *abl*, and *erb*B. An additional favourable characteristic is that the induction of differentiation was observed in K562 human leukæmic cells. Its activity is attributed to PTK inhibition of $p60^{v\text{-}src}$ and it has also been reported to inhibit EGF-R autophosphorylation. Herbimycin A (**14**) may be useful clinically as an adjuvant to other anticancer agents as it can reverse a malignant phenotype at higher concentrations, while displaying only partial cytotoxicity, and can establish a regular growth pattern on removal.

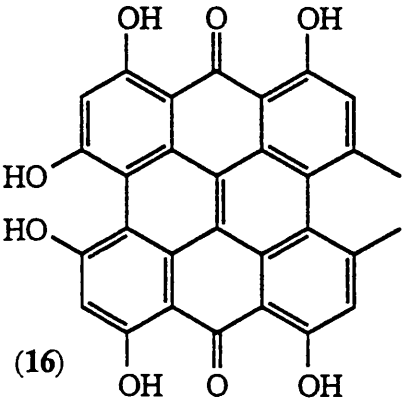
A large number of anthraquinones and naphthoquinones exhibit moderate to good PTK inhibition of $p56^{lck}$, *e.g.* emodin (**15**). Very recently a polycyclic bisanthraquinone called hypericin (**16**) has demonstrated potent EGF-R PTK inhibition and, although it is also a potent inhibitor of PKC, has been used as an adjuvant to treat brain tumours.[207] The known anthracycline antineoplastic antibiotic doxorubicin (see Table 1.8 under "Natural Products") has also been shown to inhibit a number of PTKs in a manner that is competitive to ATP.



(14)



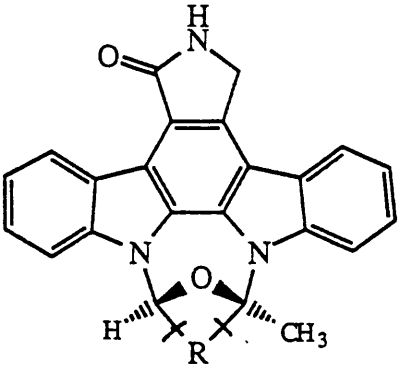
(15)

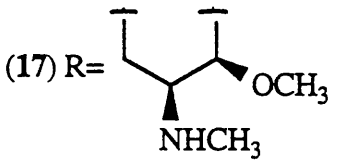


(16)

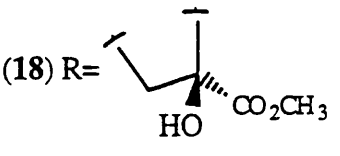
However, its value as a selective PTK inhibitor is limited due to its inhibition of PKC.

The indolecarbazole antibiotic staurosporine (**17**) has been shown to be a potent non-selective inhibitor of various kinases, including PTKs. However, the related analogue K252a (**18**) exhibits selectivity for the *Trk* family of oncogenes, having no inhibitory activity for EGF-R or *src*. Unfortunately, (**18**) is about ten-fold more efficient for the normal *Trk* proteins than their oncogenic forms. Also, *N*-benzoylstaurosporine (**19**) is known to inhibit PDGF-R autophosphorylation and c-*fos* gene expression in intact cells.[208]

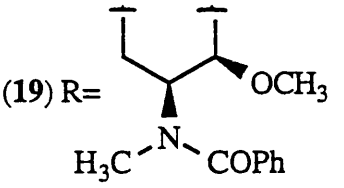




(17) R=

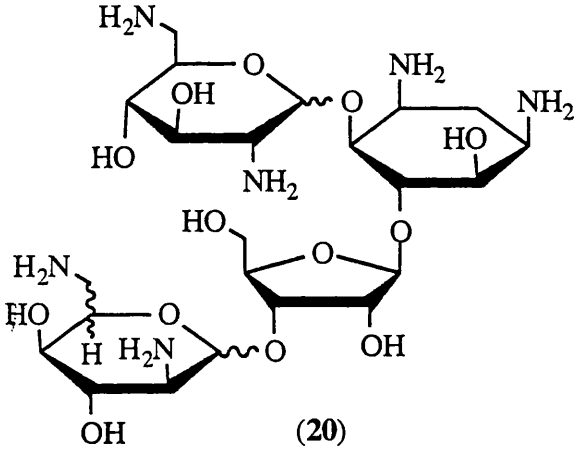


(18) R=

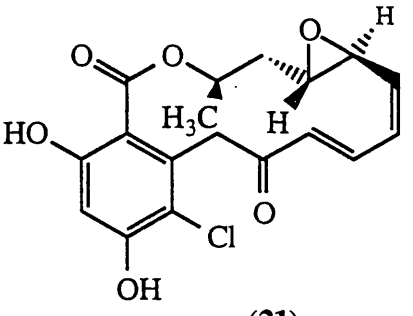


(19) R=

The aminoglycoside antibiotic neomycin (**20**) is known to act as a PTK inhibitor and prevents autophosphorylation of a PDGF class of receptor.[209] A notable example of an antibiotic which can act as a very potent PTK inhibitor to members of the *Src* family is the macrocycle radicicol (**21**), which shows inhibition of $p53/56^{lyn}$ with an IC_{50} value of 27 nM.[210] However, its selectivity to other PTKs and signalling enzymes is not fully established.



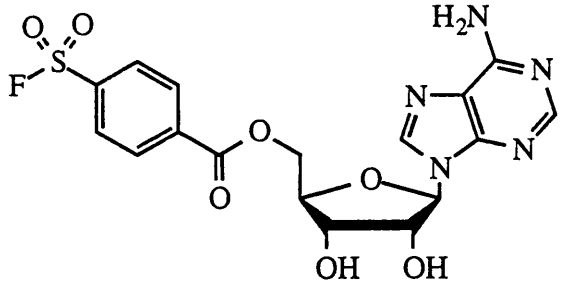
(**20**)



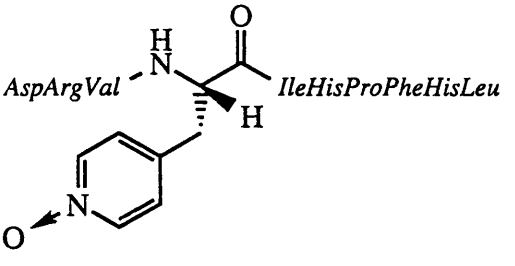
(**21**)

5.3 Nucleoside, Peptide and Amino Acid Analogues

Nucleoside-based analogues were among the first agents explored as potential inhibitors of ATP binding to PTKs. The known PSK inhibitor 5'-[4-(fluorosulfonyl)benzoyl]adenosine or 5'-FSBA (**22**) has been shown to form covalent bonds to lysine residues within the active site of PTKs.[211] However, these agents tend to lack selectivity and potency, possibly due to problems in traversing the cell membrane.



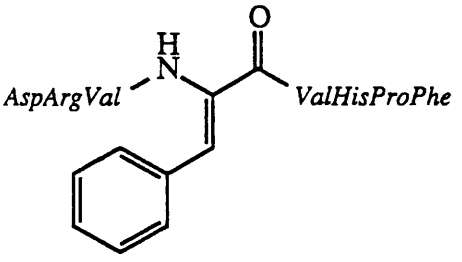
(**22**)



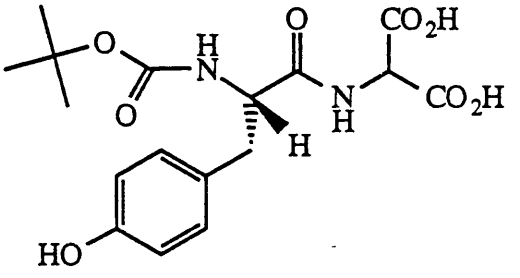
(**23**)

Apart from the many tyrosine-containing peptides modelled around PTK auto-phosphorylation sites, an interesting analogue (**23**) of angiotensin I has been designed whereby the 4-hyrdoxyphenyl group normally phosphorylated has been replaced by 4-pyridyl-1-oxide.[212] The rationale was that phosphorylation on the *N*-oxide would activate

the pyridine ring for nucleophilic attack by a lysine residue in the ATP-binding site (*cf.* 5'-FSBA) and thus act as a potential suicide inhibitor. Unfortunately, the peptide analogue (**23**) proved to be an extremely weak competitive inhibitor. However, a different type of isosteric replacement within angiotensin II gave a more successful PTK inhibitor, the dehydrophenylalanine derivative (**24**). Also there has been success among a series of amino acid-like derivatives, whereby ^{t}Boc-Tyr-aminomalonic acid (**25**) was found to be the most effective inhibitor of PTK activity at the insulin receptor.[213]



(**24**)

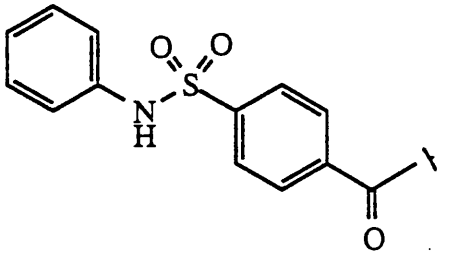


(**25**)

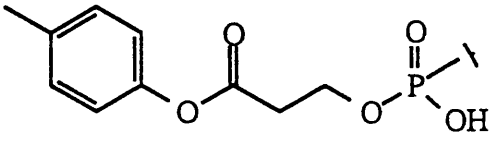
5.4 Bisubstrate Inhibitors

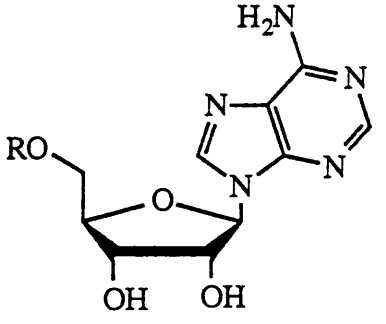
Compounds that are designed to incorporate structural aspects of both ATP and a tyrosyl-containing protein have been termed bisubstrate (or multisubstrate) inhibitors. In general, the most successful design strategy has emphasised the ATP moiety as a base structure to which a tyrosine-like portion is appended. The nucleoside analogues (**26a**) and (**26b**) illustrate this concept whereby the triphosphate chain of ATP is replaced in some isosteric manner or form. However, when tested in PTK assays, both these compounds are purely competitive with respect to ATP. Nevertheless, unlike the best 5'-FSBA sulfonamide analogue (**26a**), the compound (**26b**) still exhibits selectivity for PTKs over PSKs.

(**26a**) R=

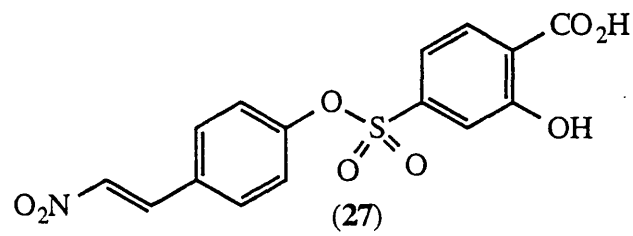


(**26b**) R=

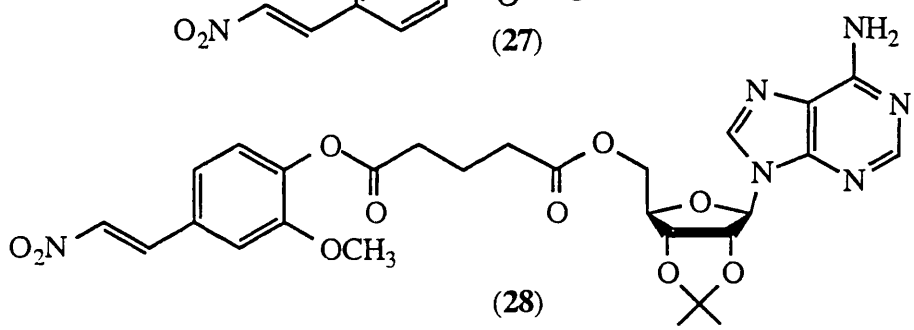




Bisubstrate inhibitors also have the potential to mimic the postulated transition state of the γ-phosphoryl transfer of the terminal phosphate group of MgATP to a tyrosine moiety and was the basis in the design of (**27**) and (**28**).[214] The compound (**27**) has been found to be a potent and selective inhibitor of EGF-R, and exhibits no inhibitory effect for $p60^{v\text{-}src}$ or the PSKs, PKC and PKA. The analogue (**28**), which utilises a glutarate group as a "triphosphate spacer", also gave a similar kinase activity, and it is believed that these types of PTK inhibitors are truly multisubstrate in nature and present an experimental model to design potentially superior agents.



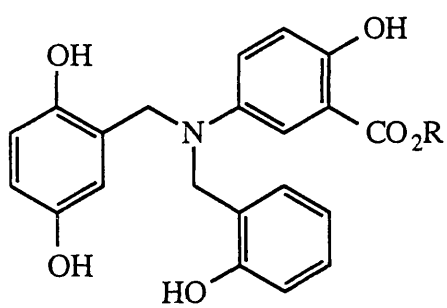
(**27**)



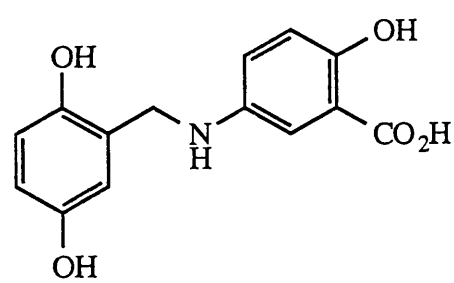
(**28**)

5.5 Miscellaneous PTK Inhibitors

Among the natural products with PTK inhibitory activity, lavendustin A (**29**) displays one of the most potent *in vitro* IC_{50} values of 12 nM against EGF-R and, at or near this concentration, does not inhibit PKA or PKC. The less potent methyl ester (**30**) is more easily taken up by the cell and inhibits EGF-R autophosphorylation and internalisation. The truncated version (**31**) also exhibits similar activity and, as for (**29**) and (**30**), is ATP competitive.

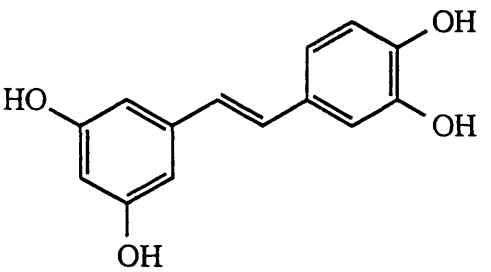


(**29**) R= H
(**30**) R= CH_3

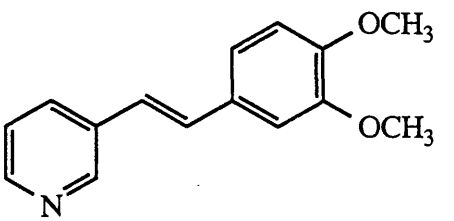


(**31**)

Another natural product, piceatannol (**32**), inhibits a variety of PTKs by competing for the peptide-substrate binding site and has no effect on PKAs. Initial attempts at improving its potency failed but substituting a 3-pyridyl ring and methoxylated phenyl ring generated the more successful analogue (**33**). Interestingly, although (**33**) has a similar structure and potency as (**32**) it differs in its mode of action and is competitive with respect to ATP.

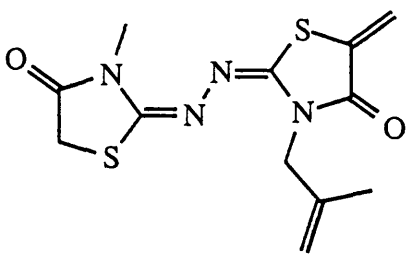


(**32**)

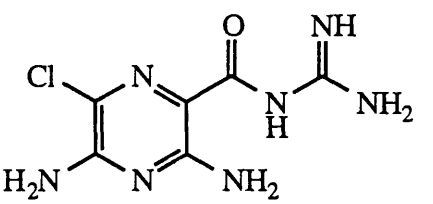


(**33**)

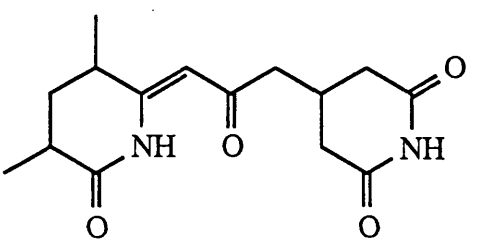
A novel class of PTK inhibitors, typified by (**34**), was shown to inhibit both EGF-R and c-*Src* kinase (without effecting v-*Abl*, PKA, or PKC) and further block EGF-R autophosphorylation and EGF-dependent proliferation in intact cells. Inhibition of PTKs by these thiazolidinediones is competitive with respect to ATP and the protein substrate. Apart from the ATP-competitive heterocycle amiloride (**35**), some other PTK inhibitors, although reasonably potent, lack selectivity and tend to elicit unknown modes of action. Examples of these would include: the glutarimide antibiotic, epiderstatin (**36**); a naturally occurring tyrosine metabolite, (+)-aeroplysinin-1 (**37**)—a potent inhibitor of EGF-R; an



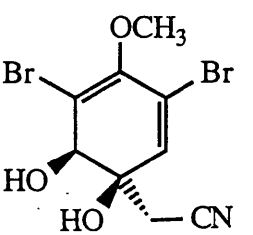
(**34**)



(**35**)

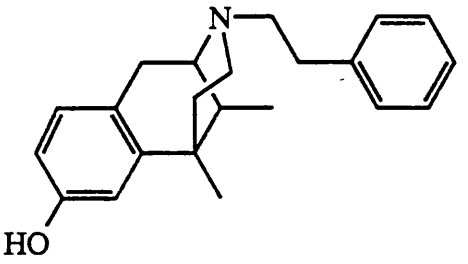


(**36**)

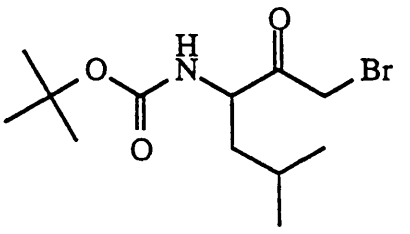


(**37**)

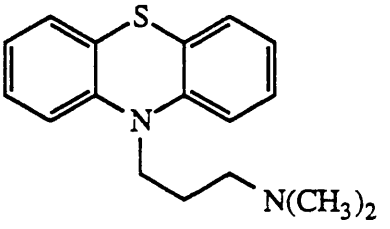
opioid tyrosine mimic, (±)-phenazocine (**38**)—a fairly potent EGF-R inhibitor; halomethyl ketones, *e.g.* ^{t}Boc-Leu-CH_2Br (**39**) inhibits EGF-R; and chlorpromazine (**40**), a lipid active inhibitor effective against c-*Src* kinase.



(**38**)



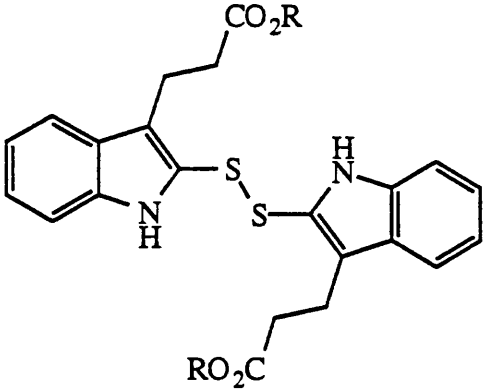
(**39**)



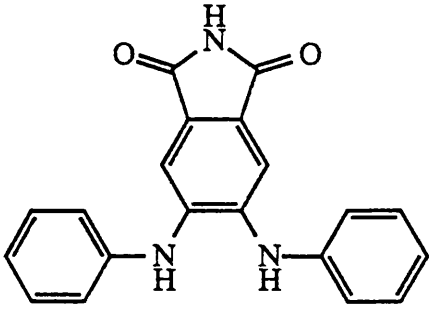
(**40**)

5.6 Recent Classes of PTK Inhibitors

A novel class of selective inhibitors of EGF-R kinase is based on 2,2'-dithiobis(1*H*-indole-3-propanoic acids), as typified by the most potent compound (**41**).[215] Generally, the esterified form (**42**) is considered less inhibitory to the isolated enzyme but shows better cellular growth inhibition, possibly due to more efficient membrane permeability. Based on this observation, a series of amide analogues was prepared.[216] The *N*-benzylamide derivative (**43**) was found to be the most potent (IC_{50}= 0.85 μM) in an *in vitro* assay against EGF-R kinase or pp60$^{v\text{-}src}$ but its inhibition of other kinases remains to be determined.



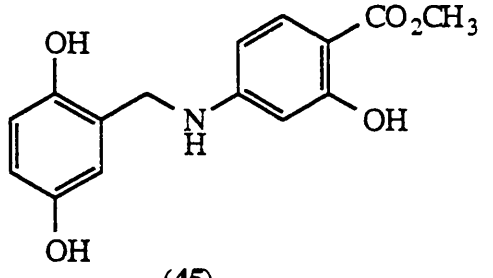
(**41**) R= H
(**42**) R= CH_3
(**43**) R= $NHCH_2Ph$



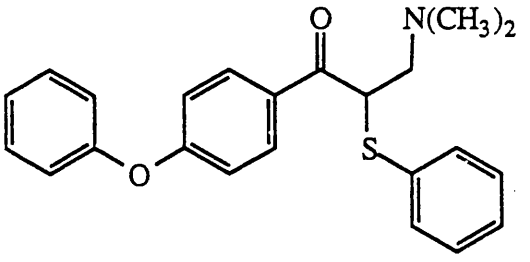
(**44**)

Based on the aglycone moiety of the non-selective PTK inhibitor staurosporine (**17**) a collection of ATP-competitive dianilinophthalimide derivatives was designed and found to be potent and selective inhibitors of EGF-R kinase in enzyme-free and intact-cell assays.[217] For example the compound (**44**) displayed a high selectivity for inhibiting EGF-R and *erb*B-2 autophosphorylation as well as c-*fos* expression (v-*abl*, c-*src*, and PDGF-R kinase activity were unaffected). Furthermore (**44**) demonstrated an *in vivo* antitumour activity in nude mice and offers a promising therapeutic lead.[218]

The truncated lavendustin A compound (**31**) has also proved to be a useful lead and has generated a series of amino derivatives, *e.g.* the methyl ester (**45**), which are non-competitive with respect to *both* ATP and the peptide-substrate at the EGF-R kinase.[219] This study provided information on the importance of a hydrophobic auxiliary binding group and demonstrated that (**45**) had a significantly lower inhibitory activity for PKC or PKA. Furthermore the study implies that the ester functionality may not be necessary for activity and a hydroiytically stable alternative should be sought. Based on the data given, probably an amide or ether linkage substituted with a phenyl or benzyl group would be advisable.



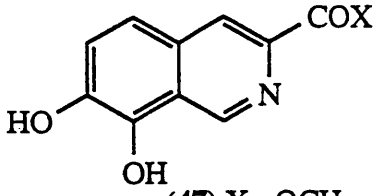
(**45**)



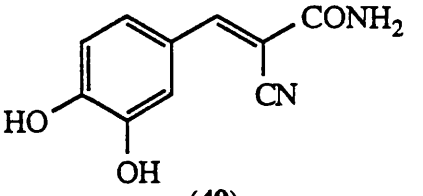
(**46**)

A novel class of potent and selective inhibitors of EGF-R kinase has recently been described.[220] Its most potent member, the acrylophenone (**46**), displays significant selectivity over other kinases such as v-*abl*, c-*src*, and PKC, as well as exhibiting potent antiproliferative properties towards intact cells and antitumour activity in nude mice. However the mechanism of action does not involve blocking autophosphorylation and needs to be investigated in more detail.

The most interesting set of compounds recently found to possess PTK inhibitory activity are based on bicyclic nitrogen heterocycles. The isoquinolines (**47**) and (**48**) were designed as conformationally locked mimetics of the known $p56^{lck}$ inhibitor (**49**) and represent potential new leads for this kinase.[221] An important lead compound for PTK inhibitors that act against



(47) X= OCH_3
(48) X= NH_2



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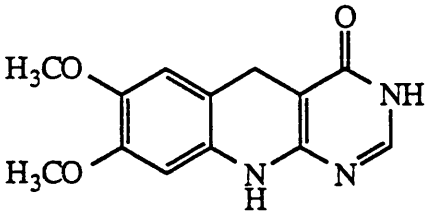
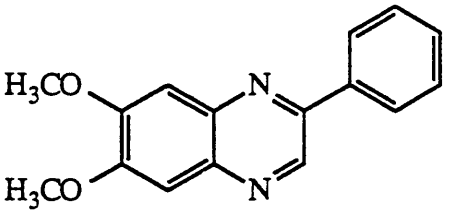


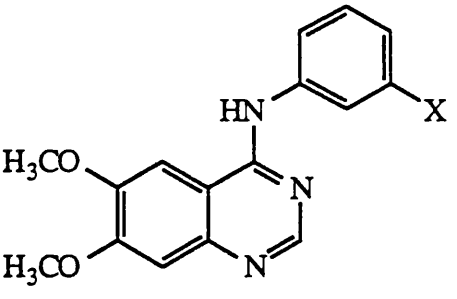
(50)

the $pp60^{c\text{-}src}$ kinase is the fused pyrimidinone (**50**) and acts in a mixed non-competitive, potent, and selective manner.

The quinoxaline (**51**) selectively inhibits PDGF-R kinase in intact cells and does not inhibit EGF-R or c-*src* kinases.[222] Undoubtedly the most potent and selective PTK inhibitors belong to the 4-anilinoquinazoline class. For example, the chloro derivative (**52**) has an IC_{50} value of 3 nM against the EGF-R kinase and is ATP-competitive.[223] The bromo derivative (**53**) has an IC_{50} in the low *picomolar* range against the EGF-R kinase and is clearly an exceptionally potent PTK inhibitor.[224]



(51)

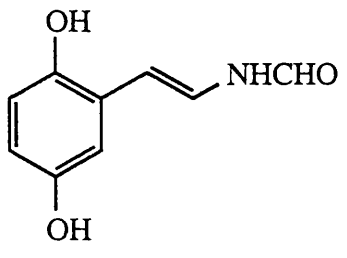


(52) X= Cl
(53) X= Br

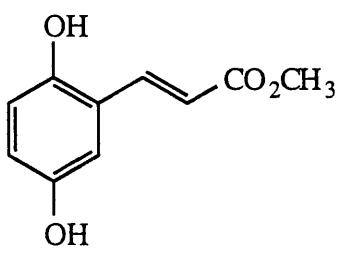
5.7 Erbstatin and the Tyrphostins

Among the PTK inhibitors discovered from natural product screening, erbstatin (**54a**) has been the most useful prototype used for drug design. It was isolated from an *Actinomycete* broth and has an *in vitro* IC_{50} value of 3 μM against EGF-R.[225] However, the action of erbstatin is ambiguous and probably effects other kinases apart from the PTKs. Also, its

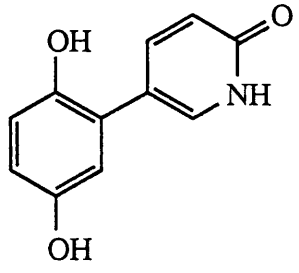
antiproliferative activity is partly due to inhibition of DNA topoisomerase I and II and its action *in vivo* requires Fe(III) chelators (*e.g.* feroxymithine).[226] Presumably, erbstatin (**54a**) is prone to oxidative degradation. Accordingly, much synthetic effort has been directed at developing stable analogues.



(**54a**)



(**54b**)

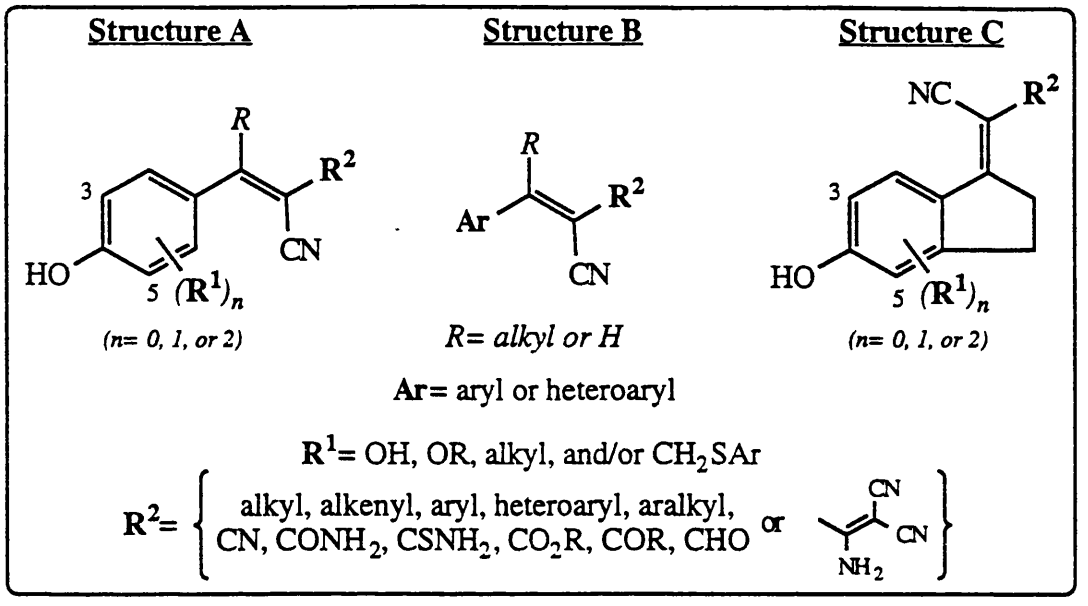


(**55**)

The methyl cinnamate (**54b**) was found to inhibit EGF-R kinase activity and EGF-R autophosphorylation in intact cells with equal potency to erbstatin, and was about four times more stable in aqueous solution.[227] The pyridone-based analogue (**55**) has also been designed and inhibits EGF-R non-competitively with respect to ATP and the protein-substrate.[228] In the same study, erbstatin (**54a**) was shown to inhibit the EGF-R kinase as a partial competitive inhibitor with respect to both ATP and the protein-substrate. This implies erbstatin binds at a site distinct from the ATP and protein-substrate binding sites of the enzyme, and thereby lowers the binding affinities of the enzyme for both substrates by some, still undefined, manner.

The most promising and well studied PTK inhibitors based on erbstatin (**54a**) are known as the "tyrphostins", for tyrosine phosphorylation inhibitors. Together with Shiraishi *et al.*,[229] this area has been pioneered by Levitzki and his co-workers.[230] Between these two groups, three main structural archetypes have been synthesised and each of these bear a diverse range of functionality (Figure 16). The key pharmacophoric feature appears to be a *p*-hydroxycinnamonitrile (Structure A) or, more generally, a 3-arylpropenonitrile (Structure B). This importantly includes the *cis* relative arrangement between the aryl and nitrile groups and is exemplified in the conformationally constrained indanones (Structure C)—the corresponding *trans*-forms of the tyrphostins are thought to be less active. On the whole, reducing out the double-bond, removing phenolic hydroxyl groups, or replacing the *p*-hydroxyphenyl ring with a heteroaryl ring, abolishes PTK inhibitory action.

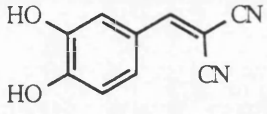
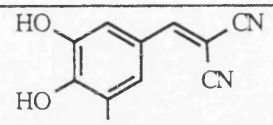
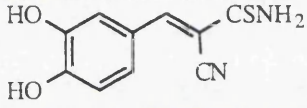
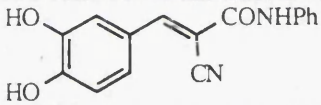
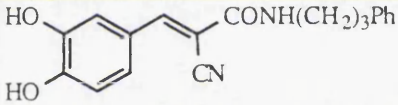
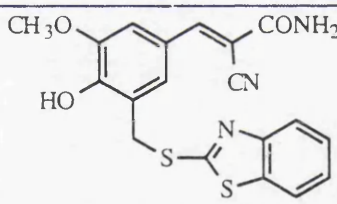
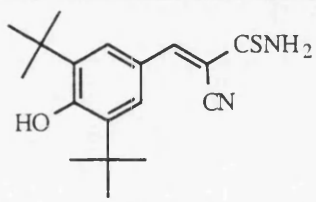
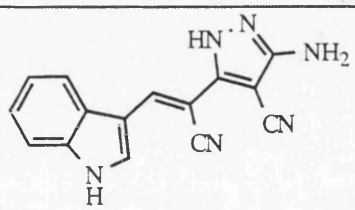
Figure 16 Tyrphostin Archetypes

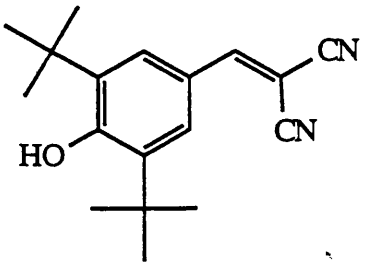


The tyrphostins are designed to be low molecular weight PTK inhibitors competitive for the peptide-binding site such that their selectivity and hence toxicity will be therapeutically favourable. However, in general, they exhibit a mixed-type competitive response against ATP and peptide binding (*i.e.* are bisubstrate inhibitors) but, fortunately, this does not affect their selectivity.[231] In fact, the tyrphostins are not only effective at distinguishing between PTKs and other signalling enzymes, but also among the PTKs themselves (see Table 12 for pertinent examples). Importantly, there is significant selectivity over the insulin-receptor (IR) kinase and this should circumvent the potential problem of signal inhibitors being toxic to cells of the pancreas.

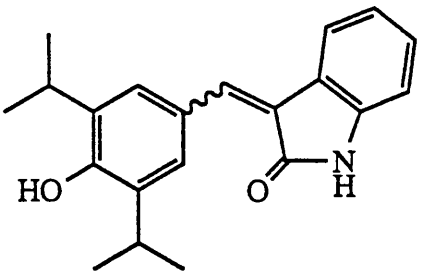
The biological activity of the tyrphostins generally correlates well to their PTK potency. This is exemplified by the tyrphostin (**58**) (Table 12) which inhibits EGF-dependent autophosphorylation at EGF-R kinase, EGF-stimulated DNA synthesis, and tyrosine phosphorylation of endogenous protein substrates. However, penetration into cells is often slow and experiments indicate that (**58**) is either unstable and/or is easily metabolised. An improvement on this is the analogue (**62**) (Table 12). This is related to the potent but non-selective inhibitor (**64**) and Shiraishi's compound (**65**),[229] a potent and selective PTK inhibitor of EGF-R. Thus, compound (**62**) preferentially inhibits *erb*B-2 kinase and *Trk* kinase over EGF-R and PDGF-R kinases. Clearly, while the *tert*-butyl groups add stability to (**62**), predicting the selectivity between related kinases remains a learning process.

Table 12 Selectivity of Some Tyrphostins

Tyrphostin	PTKs and IC_{50} values (μM)					
	EGF-R	*erb*B-2	PDGF-R	*Trk*	$p210^{Bcr\text{-}Abl}$	IR
 (**56**)	35		25	>100	75	4000
 (**57**)	3			>100	3.6	
 (**58**)	0.8		3	>100	6	640
 (**59**)	0.7	42	6		75	>100
 (**60**)	0.7	35				>100
 (**61**)	19	0.35	40		75	>100
 (**62**)	>500	1.0	>100	10		
 (**63**)	18.5				0.8	

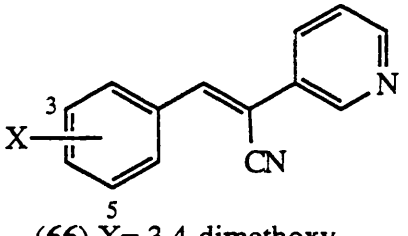


(64)

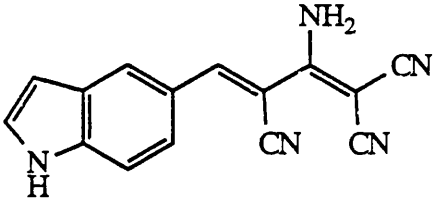


(65)

Pertaining to metabolic stability, it is preferable to design PTK inhibitors devoid of phenolic hydroxyl groups and the diaryl compounds (**66**) and (**67**) represent a significant development in this respect. These "second generation" tyrphostins have been used *in vivo* and inhibit EGF-dependent growth of EGF-R rich squamous cell carcinomas in nude mice.[232] This contradicts the notion that phenolic hydroxyl groups are essential for PTK inhibitory activity for a given tyrphostin (see above). Another contradiction is the existence of potent and selective *heteroaryl* tyrphostins, *e.g.* (**68**) selectively inhibits PDGF-induced mitogenesis and PTK activity in bone-marrow fibroblasts,[233] and a structurally related analogue (**63**) is highly selective for the PDGF-R kinase (Table 12). All these facts add another dimension to the design of potentially useful PTK inhibitors.



(**66**) X= 3,4-dimethoxy
(**67**) X= 3,5-dichloro



(**68**)

In general terms, the tyrphostins and related PTK inhibitors have a great therapeutic potential to control a whole range of proliferative diseases where PTKs are implicated, *e.g.* many human cancers, psoriasis and other skin conditions, atherosclerotic diseases, and inflammatory conditions (see Table 13).[234] However, before becoming clinically useful, the tyrphostins will have to display better pharmacological profiles such as bioavailability, cell permeability, metabolic stability, and non-toxicity. Furthermore, they need to overcome the significant problem of drug resistance, as this all too frequently occurs for the conventional anticancer drugs in the genetically unstable fertile environment of a tumour cell population.[235]

Table 13 Potential Therapeutic Application of PTK Inhibitors

Disease	PTK Implicated	Mechanism of PTK involvement	PTK Inhibitor
Certain cancers (squamous cell carcinoma)	EGF-R	Amplification of gene and overexpression of EGF-R	Compounds (**44**), (**52**), (**58**), (**60**), and (**66**)
Psoriasis	EGF-R	Overexpression of amphiregulin or TGF-α gene, leading to autocrine stimulation of EGF-R	Compounds (**58**) and (**60**)
Mammary and ovary carcinoma	*erb*B-2	Gene amplification and overexpression of erbB-2 kinase receptor	Compounds (**61**) and (**62**)
Atherosclerosis, restenosis, pulmonary fibrosis	PDGF-R	Amplification of PDGF-R by pathological release of PDGF (restenosis)	Compounds (**51**) and (**64**)
Gliomas, glioblastomas	PGDF-R	Amplification of PDGF-R; coexpression of PDGF and PDGFR in the tumour	Compounds (**51**) and (**64**)
Chronic myelogenous leukæmia (CML)	$p210^{Bcr\text{-}Abl}$ and $p185^{Bcr\text{-}Abl}$	Chromosome rearrangement that results in fusion of *Bcr* and $p140^{c\text{-}Abl}$, leading to enhanced kinase activity	Compound (**63**)

Nevertheless, the PTK inhibitors, and the tyrphostins in particular, are making an impressive impact on anticancer drug design. Not only can they serve as molecular tools to unravel the signalling processes of the cell, but can act as signal blockers capable of "sensitising" a tumour cell to the effects of traditional cytotoxic agents.[236] Indeed, they have already been shown to act as effective anticancer agents in their own right.[237] Moreover, once signalling "cross-talk" is better understood[238] and further X-ray structures for kinases become available, PTK inhibitors may be tailored more appropriately in order to exploit the contextual nature of signalling processes within tumour cells in a favourable manner over their normal cellular forms. In this way, the challenge is to design a predictive and efficacious signalling agent for the purpose of fighting against the age-old disease of cancer.

◊ RESULTS & DISCUSSION ◊

"Write the things which thou hast seen,
and the things which are,
and the things which shall be hereafter";
The Revelation of St. John the Divine, Chapter 1, Verse 19.

CHAPTER «6» Preliminary Tyrphostins Studied

At the onset of this project the most promising approach to the discovery of PTK inhibitors was that described for the so-called tyrphostins (see Section 5.7). As an appropriate start it was necessary to confirm and extend the work carried out by Levitzki and co-workers,[230f] and therefore synthesise hydroxylated aromatic tyrphostins (see Figure 16; Structure A). An additional purpose was to "design out" the undesirable feature of phenolic hydroxyl groups—a structural motif renowned for the instability and easy metabolism of compounds. Thus it was decided to explore the effects of replacing these hydroxyl groups with halogens or substituting in a whole new heteroaromatic moiety. Moreover, the impetus was to "discover" an interesting new "lead" and so a range of alternative aromatic moieties was investigated.

However, the purpose of this project was not only to synthesise potential PTK inhibitors but also to test them in an appropriate biological assay. Indeed, an additional aim was to develop structure-activity relationships for a tyrphostin's antiproliferative activity in a *tumour-derived* cell line—previously, Yaish *et al.*[230a] demonstrated that several of their compounds inhibited EGF-dependent proliferation of fibroblasts. For this purpose, the MCF-7 breast adenocarcinoma cell line was chosen as an apt model system. The reason for this was two fold: the growth of MCF-7 cells is stimulated by EGF in culture and is inhibited by EGF-R derived monoclonal antibodies;[239] and other mitogens such as oestrogen and the insulin-like growth factors (IGFs) are important for proliferation of MCF-7 cells.[240] Thus, signalling *via* the EGF-R kinase not only plays an important rôle in the proliferation of this tumour type but also depends on other growth factors. Therefore, it was important to determine the ability of these inhibitors to block growth of tumour cells where multiple

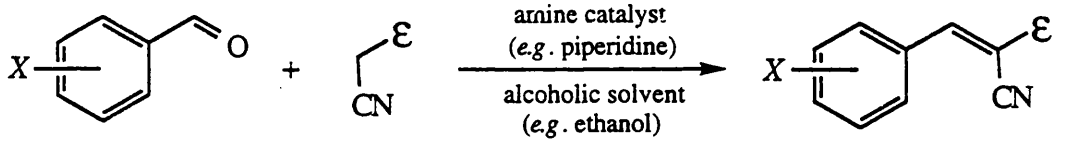
interactions control cell proliferation and to assess whether effects on MCF-7 cell growth could be attributed to inhibition of EGF-R kinase function. The synthesis and biological evaluation of these types of PTK inhibitors will be discussed here and some of this work has recently been published.[241]

The MCF-7 and some of the HER-14 cell-line assays were carried out by me at the CRC Medical Oncology unit, Beatson Institute, Glasgow University. Unless stated otherwise, the more detailed cell assays, including EGF-R kinase inhibition and cytotoxicity testing, were perfomed by Dr Valerie G. Brunton and co-workers. For the readers information, the protocols followed have been appended as an Experimental—Biology section.

6.1 Synthesis of Hydroxylated 3-Phenylpropenonitriles

The tyrphostin base structure is sometimes referred to as a benzylidenemalononitrile (or benzalmalononitrile, BMN) and, as inferred by its name, is normally made from the reaction between a benzaldehyde and an activated methylene derivative of malononitrile (Scheme 1). Classically this is called a Knoevenägel condensation[242] and an excellent general review on ylidenemalononitriles is given by Freeman.[243] The reaction belongs to the general class of base-catalysed aldol-type condensations and is usually performed in an alcohol and, for aromatic aldehydes, involves the use of a weak secondary amine or its equivalent salt.

Scheme 1 The Knoevenägel Condensation



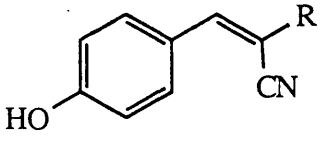
(X= OH, OR, halogen, etc.*)* *(X= OH, OR, halogen,* etc.*)*

Ɛ= electron withdrawing group, *e.g.* CN, $CONR_2$, CO_2R, COR, Ar, NO_2, $S(O)_nR$, *etc.*

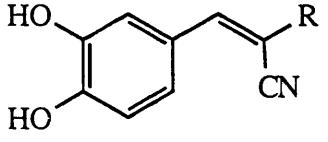
Depending on the starting materials, a judicious choice of base, solvent, reaction concentration, and temperature should be considered in order to obtain a clean conversion. With the correct choice of conditions the product will normally precipitate out or, better still, crystallise out of solution. In general, piperidine was used as the base, ethanol as the solvent, and yields ranged from 60 to 90 %. However, for some cases the reactions were extremely temperamental and gave complex mixtures, presumably *via* some kind of

Michael-type addition product (or *bis*-adduct) of the initially formed α,β-unsaturated compound.

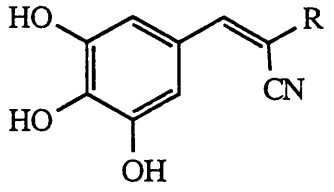
The first tyrphostins prepared contained the mono-, di-, and tri-hydroxylated phenyl portions. Thus, condensation of 4-hydroxybenzaldehyde with malononitrile, 1,1,3-tricyano-2-aminopropene (malononitrile dimer), cyanothioacetamide, ethyl cyanoacetate, and cyanoacetamide gave the 3-(4-hydroxyphenyl)propenonitrile series [(**69**)-(**73**)]—hereinafter called the *p*-hydroxyphenyl series. Similar reactions with 3,4-dihydroxybenzaldehyde led to the dihydroxyphenyl series [(**56**), (**58**), and (**74**)-(**76**)], and the trihydroxyphenyl series [(**57**) and (**77**)-(**80**)] were formed from 3,4,5-trihydroxybenzaldehyde. Apart from compounds (**71**), (**72**), (**75**), (**79**) and (**80**), these tyrphostins had previously been reported by Gazit *et al*.[230e]



(**69**) R= CN
(**70**) R= $C(NH_2)=C(CN)_2$
(**71**) R= $CSNH_2$
(**72**) R= CO_2Et
(**73**) R= $CONH_2$



(**56**) R= CN
(**74**) R= $C(NH_2)=C(CN)_2$
(**58**) R= $CSNH_2$
(**75**) R= CO_2Et
(**76**) R= $CONH_2$



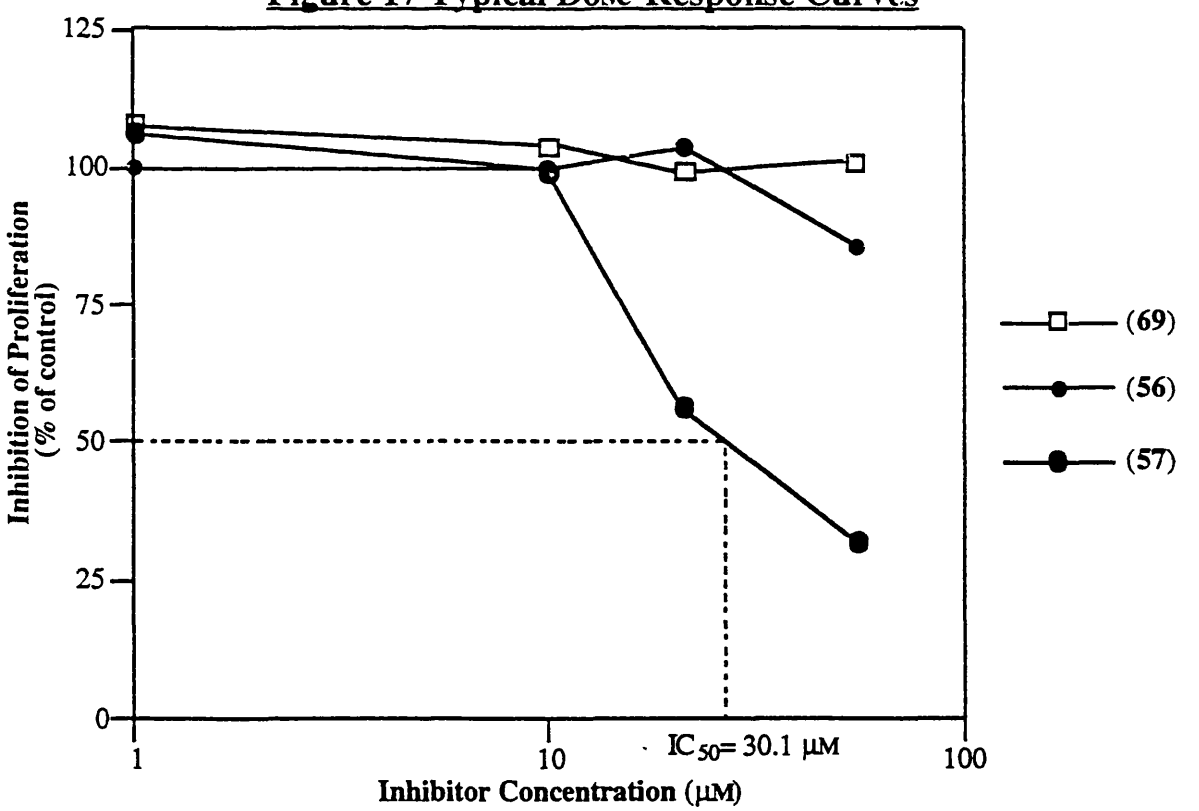
(**57**) R= CN
(**77**) R= $C(NH_2)=C(CN)_2$
(**78**) R= $CSNH_2$
(**79**) R= CO_2Et
(**80**) R= $CONH_2$

Synthesis of the *p*-hydroxyphenyl series was reasonably straightforward except for the malononitrile dimer product (**70**) and the thioamide (**71**) which tended to give low yields (below 30 %). This turned out to be a general trend and also occurred in the dihydroxyphenyl and trihydroxyphenyl series. In particular, synthesis of the known tyrphostin (**58**) proved problematic. The best procedure employed precluded light, used a minimum amount of piperidine, and isolation was performed *via* silica chromatography. Further purification by crystallisation was avoided due to the generation of impurities. Indeed, all the thioamide dervatives [(**71**), (**58**), and (**78**)] were light and air sensitive. Moreover, the dihydroxyphenyl and trihydroxyphenyl series all tended to go dark on standing at room temperature or at 4 °C. The best method of storage avoided light and air, since it was suspected that these hydroxylated series may readily oxidise to quinones. Clearly this instablity is not a desirable feature to have in a potential cytostatic agent since normal cell toxicity is likely to be increased.

6.2 Biological Evaluation of Hydroxylated 3-Phenylpropenonitriles

The antiproliferative activities of the compounds synthesised above were assessed in the breast adenocarcinoma cell line MCF-7 using [^{3}H]thymidine incorporation as a measure of DNA synthesis. In other words, the growth of MCF-7 cells in the presence of an inhibitor was compared to that in the absence of the inhibitor (a control system). Unhindered growth should allow a greater incorporation of the radioactive label into the DNA of new cells, than that of cells stunted by any inhibitor, and thus the quantity of radioactivity measured over a period of time should differ between the two test systems. The difference is best expressed as a percentage and gives a so-called typical dose-response curve, for example see compounds (**69**), (**56**), and (**57**) in Figure 17. The range of inhibitor concentrations used in these assays was: 1, 10, 20 and 50 μM and the concentration at which a 50 % inhibition of [^{3}H]thymidine incorporation occurs is defined as the IC_{50} value. This is indicated for compound (**57**). The other compounds have IC_{50} values greater than 50 μM.

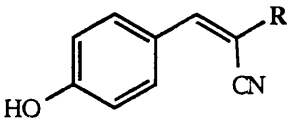
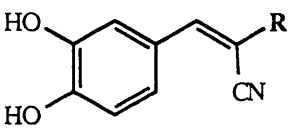
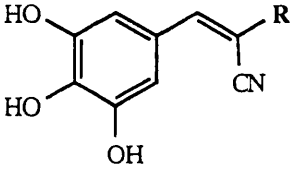
Figure 17 Typical Dose-Response Curves



Silmilar dose-response curves were obtained for the other compounds in the series and the data have been collated in Table 14. Also given are IC_{50} values for a cell free system whereby the only parameter to consider is enzyme inhibition of the EGF-R kinase (tyrosine

phosphorylation of a synthetic polypeptide is measured). This allows a direct comparison of the data between inhibiting signalling events at the EGF-R kinase with the observed antiproliferative activity against intact tumour cells.

Table 14 Biological Activities of Hydroxylated 3-Phenylpropenonitriles

Structure	No.	R	IC_{50} (μM)[a] MCF-7 cells	IC_{50} (μM)[b] EGF-R kinase
	(69)	CN	>50	560[c]
	(70)	$C(NH_2)=C(CN)_2$	>50	0.125[c]
	(71)	$CSNH_2$	>50	40
	(72)	CO_2Et	>50	>500
	(73)	$CONH_2$	>50	>800[c]
	(56)	CN	>50	35[c]
	(74)	$C(NH_2)=C(CN)_2$	>50	2.5[c]
	(58)	$CSNH_2$	31.3	2.4[c]
	(75)	CO_2Et	43.4	35
	(76)	$CONH_2$	40.9	10[c]
	(57)	CN	30.1	3[c]
	(77)	$C(NH_2)=C(CN)_2$	46.3	0.8[c]
	(78)	$CSNH_2$	32.6	1.7[d]
	(79)	CO_2Et	35.3	8
	(80)	$CONH_2$	>50	4.2

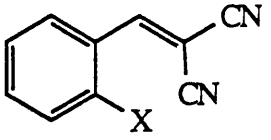
[a]Intact cell assay [b]Celi free assay
[c]Values taken from Gazit *et al.*[230b] [d]Value taken from Anafi *et al.*[244]

As can be observed in Table 14, the compounds exerted a range of antiproliferative activities against the MCF-7 cell line with IC_{50} values ranging from 30 to above 50 μM. From the collection of novel structures, compounds (**75**) and (**79**) displayed a reasonable correlation between EGF-R kinase inhibition and antiproliferative activity. However, the activity of compound (**80**) is disappointing in the MCF-7 assay. In general, the functional groups which contributed the most to potency tended to be the thioamide (**R**= $CSNH_2$) and malononitrile dimer (**R**= $C(NH_2)=C(CN)_2$) moieties.

From one series to the next, there is a significant enhancement of antiproliferative activity of MCF-7 cells over the *p*-hydroxyphenyl series, although this increase in activity is not so prominent when comparing the dihydroxyphenyl series to the trihydroxyphenyl series. In contrast, the inhibition of the EGF-R kinase shows a much more linear structure-activity relationship when the number of hydroxyl groups is increased on the phenyl group for a given analogue in a series. This agrees with the findings of Levitzki and co-workers.[230a]

However, not in agreement is the fact that there is no general correlation of antiproliferative activity of the MCF-7 cells with enzyme inhibiton of the EGF-R kinase. Previously, this correlation had been observed for the EGF-dependent proliferation of fibroblasts.

This result is not entirely surprising as other mitogens such as oestrogen and the IGFs are involved in MCF-7 cell growth, and signalling *via* the EGF-R kinase may not be the major pathway responsible for growth. Alternatively, the tyrphostins may act with low selectivity and so inhibit other growth pathways of the cell or, indeed, act in some unknown manner (*cf.* Faaland *et al.*[245]). Indeed, the tyrphostin 2-cyano-3-(4-hydroxyphenyl)propenonitrile (**69**) has been reported to prevent mitochondrial oxidative phosphorylation in animal and plant systems,[246] and this inhibition of a PTK-*independent* function has recently been confirmed for other tyrphostins.[247] Futhermore, the general toxicity of this class of BMNs is well known as numerous reports mention their associated cytotoxic and irritant properties,[248] and a detailed animal exposure of BMNs, including LD_{50} values,[249] has been reported by Jones.[250] Moreover, (2-chlorobenzylidene)malononitrile (**81**) and (2-nitrobenzylidene)-malononitrile (**82**) are severe irritants, and (**81**) has even found use as a riot control agent and in chemical warfare. In any case, this study has demonstrated that care must be taken in interpreting the mode of antiproliferative activity in tumour cells as the compounds may be altering the activity of multiple enzymes or pathways in a cell to exert their antiproliferative effects.



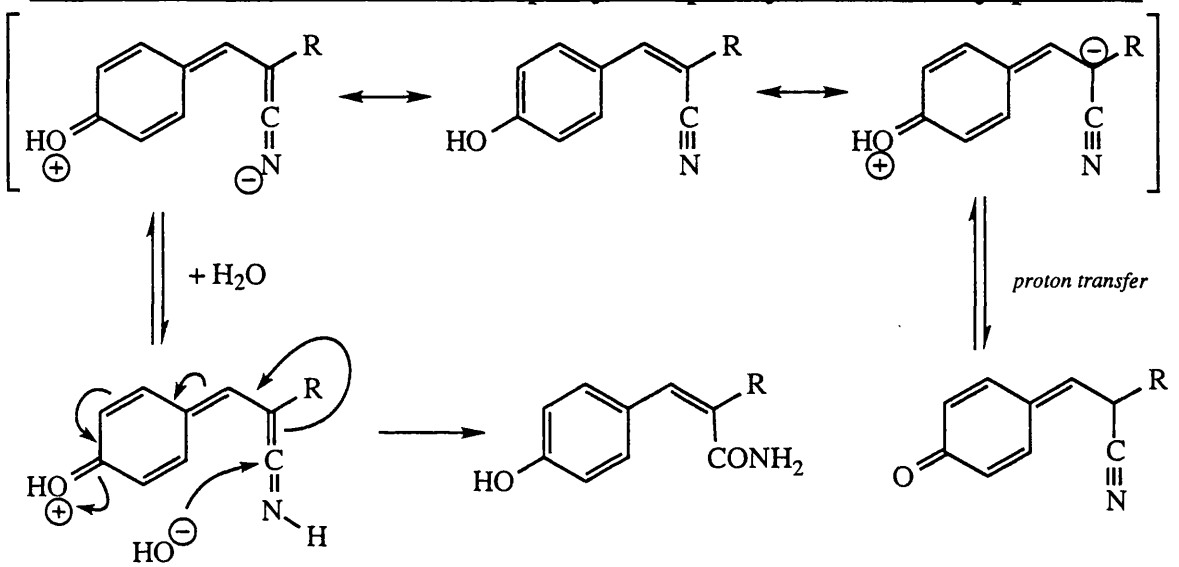
(**81**) X= Cl
(**82**) X= NO_2

6.3 Preliminary Tyrphostin Stability Study

As noted earlier and in Section 5.7 (Introduction) a major concern with the hydroxylated 3-phenylpropenonitriles is their biological and chemical instability. Even before reaching the metabolic processes of a cell, it is conceivable that compounds of this type may undergo hydrolysis of the nitrile group or undergo quinone formation.[251] Conceptually, the main reason for this is probably the powerful "push-pull" system of the 3-(4-hydroxyphenyl)propenonitrile generating a quinonoid-type structure able to facilitate protonation and subsequent nucleophilic attack (Scheme 2). This hydrolysis is particularly

pertinent for the thioamide group since this will generate hydrogen sulphide as a toxic side-product and may account for such compounds exhibiting higher IC_{50} values than their ester or amide counterparts (*cf.* Table 14).

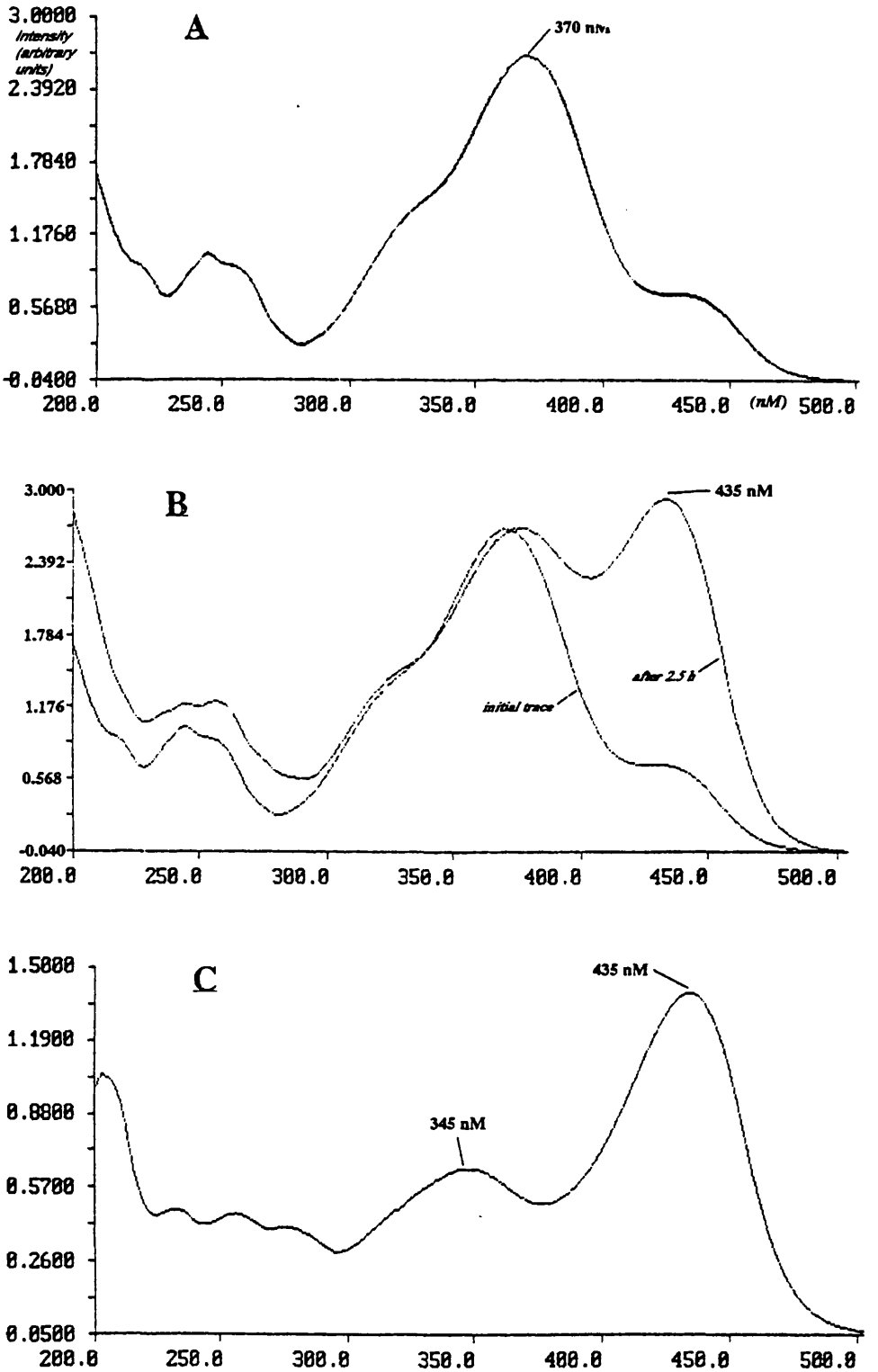
Scheme 2 Possible Reactions of *p*-Hydroxphenyl Series of Tyrphostins



Thus, it was important to ascertain the stability of a tyrphostin in aqueous solution. Consequently, 2-cyano-3-(3,4-dihydroxyphenyl)propenonitrile (**69**) was dissolved in distilled water (125 μg ml^{-1}) and monitored by UV/visible spectra (200-520 nm). The initial UV spectrum gave a main peak at 370 nm (Figure 18A) but during 2.5 h an additional peak formed and rose to a maximum near 435 nm (Figure 18B), after which the initial peak gradually diminished to a minimum near 345 nm over 24 h (Figure 18C). A similar result was obtained in the absence of light and clearly, the compound (**69**) is readily converted into one or more degradation products. Silica TLC (EtOAc/MeOH; various ratios) confirmed this observation and at least two products were formed which tended to be more polar than the parent compound. However, isolation of these proved to be extremely difficult and all attempts failed.

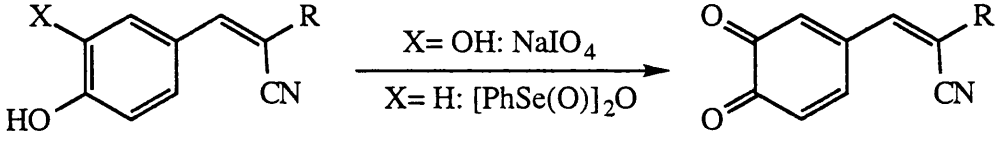
It was thought that at least one of these degradation products would be a quinone of some kind. Thus it was decided to attempt the synthesis of the *ortho*-quinones (**83**) and (**84**) in order to determine if these were responsible for the *in vitro* activity observed (see Scheme 3). Several oxidation methods were tried and included: sodium periodate[252] on compounds (**56**) or (**58**), and diphenylseleninic anhydride[253] on compound (**69**). Also, as

Figure 18 UV Spectra Illustrating the Degradation of 2-Cyano-3-(3,4-dihydroxyphenyl)propenonitrile (69)



the ferric ion is known to form the *para*-quinone of erbstatin (*cf.* Introduction), iron(III) choride catalyst plus (**56**) or (**58**) dispersed in dichloromethane or partitioned between an aqueous and organic layer were tried. However, all these attempts failed to produce the required *ortho*-quinones and so this work was abandoned.

Scheme 3 Attempted Formation of *Ortho*-quinones



(**56**) X= OH, R= CN
(**58**) X= OH, R= $CSNH_2$
(**69**) X= H, R= CN

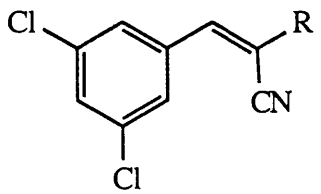
(**83**) R= CN
(**84**) R= $CSNH_2$

Very recently, the observation of a tyrphostin's instability in aqueous solution was reported to occur for the amide derivatives (**76**) and (**80**).[254] Both of these compounds gave a similar UV/visible profile as shown above for compound (**69**) and gave an initial main peak at 340 nm which shifted to 440 nm (*cf.* above). Moreover, the degree of inhibition of $pp60^{c\text{-}src}$ kinase and EGF-R kinase by these tyrphostins was shown to be linked to their instability and compound (**76**) displayed an enhanced inhibition in the presence of catalytic amounts of Mn^{2+}, Fe^{2+}, or Fe^{3+}. This implies that oxidation products (possibly quinones) may be formed and, although no characterisation data were given, these compounds were isolated and shown to be at least 5-fold more potent than the parent compounds.

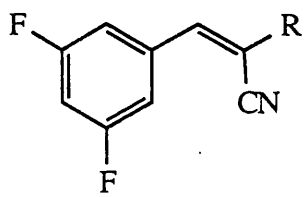
6.4 Synthesis and Biological Evaluation of 3-(3,5-Dihalophenyl)propenonitriles

From the information given above it was desirable to eliminate the presence of the phenolic hydroxyl groups altogether and replace these by an isostere which was not so activating. For this purpose a set of 3-(3,5-dihalophenyl)propenonitriles was synthesised. The substitution pattern on the phenyl ring was decided based on the data mentioned above for the hydroxyphenyl series. Thus, groups at the *ortho* and *para* positions relative to the attachment of the propenonitrile moiety were avoided due to the possible mesomeric effects that could aid nitrile hydrolysis (*cf.* Scheme 2), as well as the known toxicity of compound (**81**). Furthermore the *ortho* and *para* positions do not seem to contribute significantly to a compound's potency (*cf.* Table 14). Substitution at the *meta* positions seemed the more logical choice.

Thus, the 3-(3,5-dichlorophenyl)propenonitrile series—hereinafter called the dichlorophenyl series—was synthesised by reacting malononitrile, cyanothioacetamide, and ethyl cyanoacetate with 3,5-dichlorobenzaldehyde in the presence of catalytic amounts of piperidine to give (**85**), (**86**), and (**87**), respectively. All attempts at making the amide and malononitrile dimer adducts failed. Again formation and isolation of the thioamide derivative (**86**) proved problematic. However, after chromatography the product could be purified by a low temperature crystallisation, unlike the analogues (**58**) or (**78**).



(**85**) R= CN
(**86**) R= $CSNH_2$
(**87**) R= CO_2Et



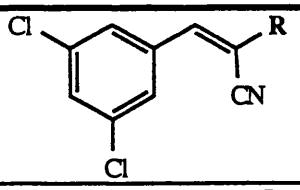
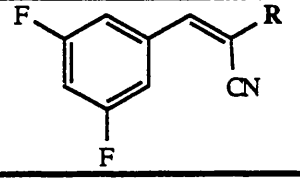
(**88**) R= CN
(**89**) R= $CONH_2$
(**90**) R= CO_2Et

The difluorophenyl series [(**88**), (**89**), and (**90**)] was similarly prepared by the condensation of 3,5-difluorobenzaldehyde with malononitrile, cyanoacetamide, and ethyl cyanoacetate. However, all attempts at obtaining analytically pure malononitrile dimer and cyanothioacetamide members of this series failed, and the impure samples isolated were considered unsuitable for testing. Futhermore, unlike the dichlorophenyl series, the compounds of the difluorophenyl series were best formed in isopropanol with the use of a very slight catalytic amount of piperidine. This was necessary to allow precipitation of the fluorinated compounds (which are too soluble in ethanol) and avoided the production of side products.

As for the hydroxylphenyl series, the biological activities of these compounds were assessed in MCF-7 intact-cell assays, but at concentrations of 0.1, 1.0, 10, 50, and 100 μM. The results are summarised in Table 15 and analogues which contain two cyano groups (**R**= CN) tended to be more potent than others in the series. In particular, compound (**85**) showed an encouraging IC_{50} (MCF-7) value of 11 μM and is at least 3-fold more potent than the corresponding analogues (**56**) and (**57**) in the hydroxyphenyl series. Moreover, this was in good correlation with the IC_{50} (EGF-R kinase) value of 0.1 μM.

Clearly compound (**85**) represents a promising successor to the tyrphostins (**56**) and (**57**). Indeed, a short term stability study in aqueous solution demonstrated that no untoward side

Table 15 Biological Activities of Halogenated 3-Phenylpropenonitriles

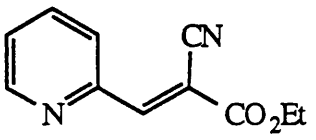
Structure	No.	R	IC_{50} (μM)[a] MCF-7 cells	IC_{50} (μM)[b] EGF-R kinase
	(85)	CN	11	0.1
	(86)	$CSNH_2$	37	n.d.
	(87)	CO_2Et	68	n.d.
	(88)	CN	35	n.d.
	(89)	$CONH_2$	86	n.d.
	(90)	CO_2Et	49	n.d.

[a]Intact cell assay. [b]Cell free assay (n.d.= not determined)

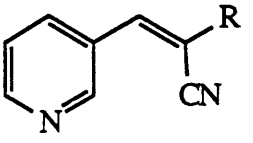
products are detectable by UV/visible or TLC analysis over a 24 h period. However, direct UV spectrophotometry is probably an inadequate technique for detailed analysis of the stability of this tyrphostin, since a degradation product may possess a chromophore similar to that of the parent compound—an HPLC technique would be preferable. Also, the activity of (**85**) in other kinase assays (*e.g.* IR, PDGF-R, $pp60^{c\text{-}src}$) should be studied, as well as its selectivity over PSKs such as PKC and PKA.

6.5 Synthesis and Biological Evaluation of 3-Heteroarylpropenonitriles

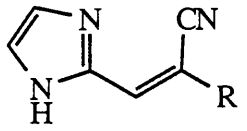
In order to develop tyrphostin analogues less prone to hydrolytic or metabolic processes, a series of 3-heteroarylpropenonitriles was synthesised. Gazit *et al.*[230e] had previously reported that heteroaromatic tyrphostins were inactive; however only a very small number of compounds were synthesised. Thus, a series of Knoevenägel condensations (Scheme 1) were carried out with 2-pyridinecarbaldehyde to give (**91**); 3-pyridinecarbaldehyde led to (**92**)-(**95**); 2-imidazolecarbaldehyde yielded (**96**)-(**100**); 2-thiophenecarbaldehyde generated (**101**)-(**105**); and 3-thiophenecarbaldehyde produced (**106**)-(**110**).



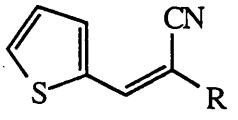
(**91**)



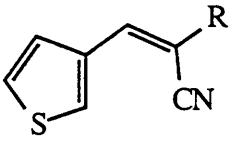
(**92**) R= CN
(**93**) R= $CSNH_2$
(**94**) R= CO_2Et
(**95**) R= $CONH_2$



(**96**) R= CN
(**97**) R= $C(NH_2)=C(CN)_2$
(**98**) R= $CSNH_2$
(**99**) R= CO_2Et
(**100**) R= $CONH_2$



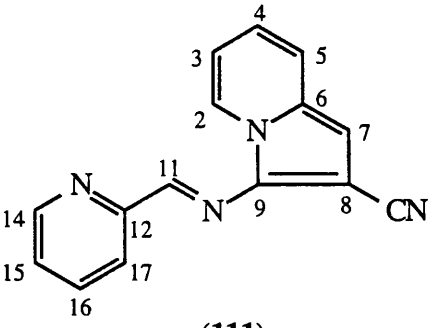
(**101**) R= CN
(**102**) R= $C(NH_2)=C(CN)_2$
(**103**) R= $CSNH_2$
(**104**) R= CO_2Et
(**105**) R= $CONH_2$



(**106**) R= CN
(**107**) R= $C(NH_2)=C(CN)_2$
(**108**) R= $CSNH_2$
(**109**) R= CO_2Et
(**110**) R= $CONH_2$

Formation of other members of the 2-pyridyl series, apart from compound (**91**), proved extremely problematic. The reactions of 2-pyridinecarbaldehyde with malononitrile, 1,1,3-tricyano-2-aminopropene (malononitrile dimer), cyanothioacetamide, and cyanoacetamide all gave black inseparable amorphous mixtures. Changing the reaction conditions (under nitrogen, absence of light, low temperature) seemed not to effect the outcome. The only success was acheived with the reaction between 2-pyridinecarbaldehyde and malononitrile in the presence of a non-nucleophilic amidine base, 1,8-diazabicylco[5.4.0]undec-7-ene (DBU). Although a compound was isolated, albeit in very low yield, and could be crystallised, it could not be assigned to the required structure.

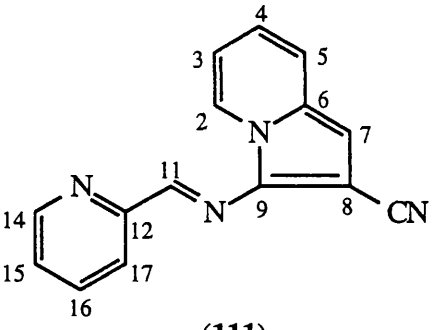
A plausible structure consistent with the data obtained is illustrated for the indolizine (**111**). The mass spectrum of (**111**) showed distinct ions at *m/z* (EI) 245 (assigned 100 % in intensity) and 246 (55 % relative intensity). For structure (**111**), the molecular ion for $C_{15}H_{10}N_4$ would be at 246. Moreover, this formula tallied well with the 200 MHz ^1H and ^{13}C NMR spectra which illustrated the presence of 10-aromatic methine hydrogens and carbons, plus at least four quaternary carbons (Figures 19 and 20).



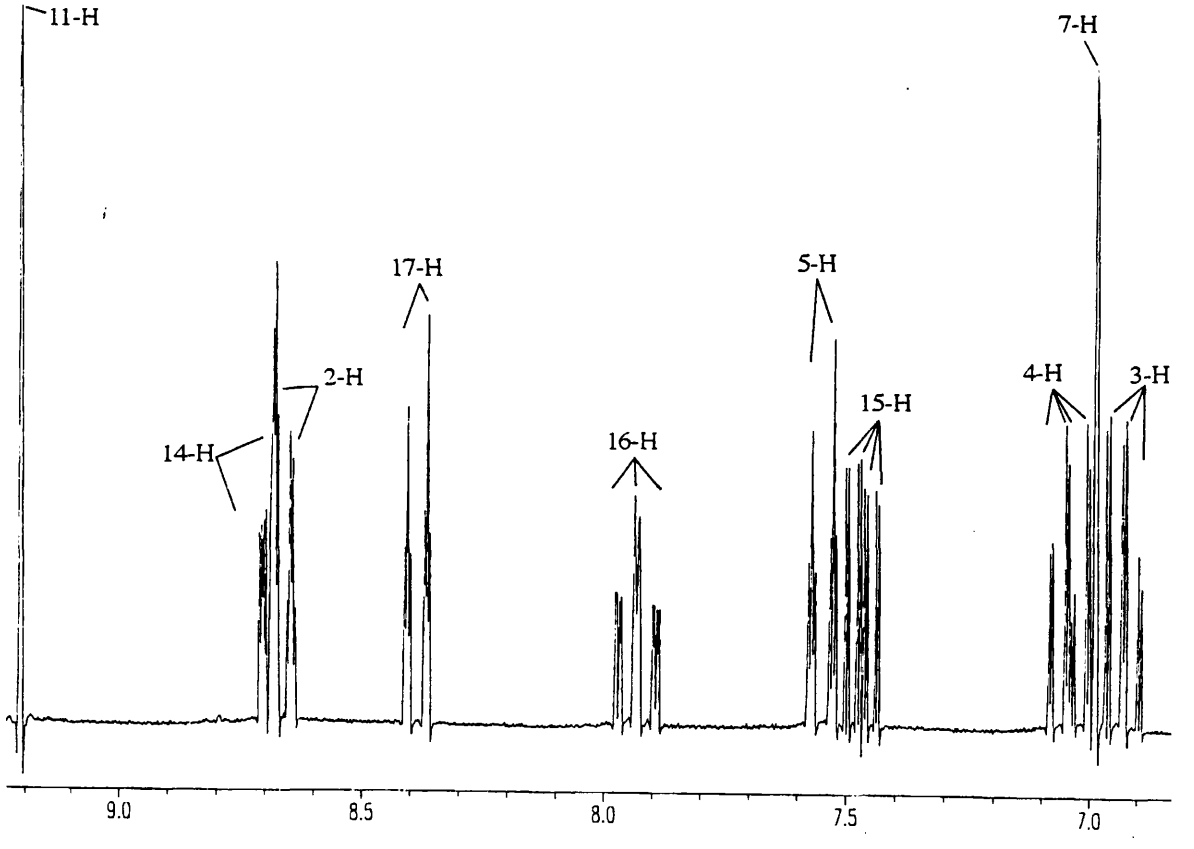
(**111**)

Importantly, the ^1H NMR spectrum showed the presence of two distinct aromatic coupling systems, each comprising of four contiguous protons, and it is likely that these signals are derived from two 2-substituted pyridine-like rings (see Figure 19). Consistent with the

Figure 19 ^{1}H-200 MHz Spectrum of Compound (111)



(111)

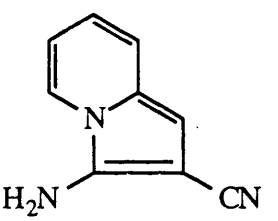


proposed structure was the fact that the protons 2- and 16-H displayed additional long-range couplings to their respective partners, 7- and 11-H. The coupling constant for the (2-H/7-H) and (16-H/11-H) proton pairs were ^{5}J 0.5 Hz and 0.7 Hz, respectively.

The full carbon spectrum of compound (**111**) is given in Figure 20A. Significantly, only one nitrile carbon is observed in the 110 to 120 ppm region. By close inspection of the DEPT ^{13}C NMR spectrum a further quaternary carbon signal seems to be apparent but is obscured

by a strong methine signal (Figure 20B). Futhermore, this signal is relatively broad and is likely to be experiencing the phenomenum of relaxation. Therefore, in structure (**111**) this signal may correspond to 9-C, a quaternary carbon flanked by two nitrogens but, for the full carbon assigments of compound (**111**) additional NMR data (*e.g.* 2D) is required.

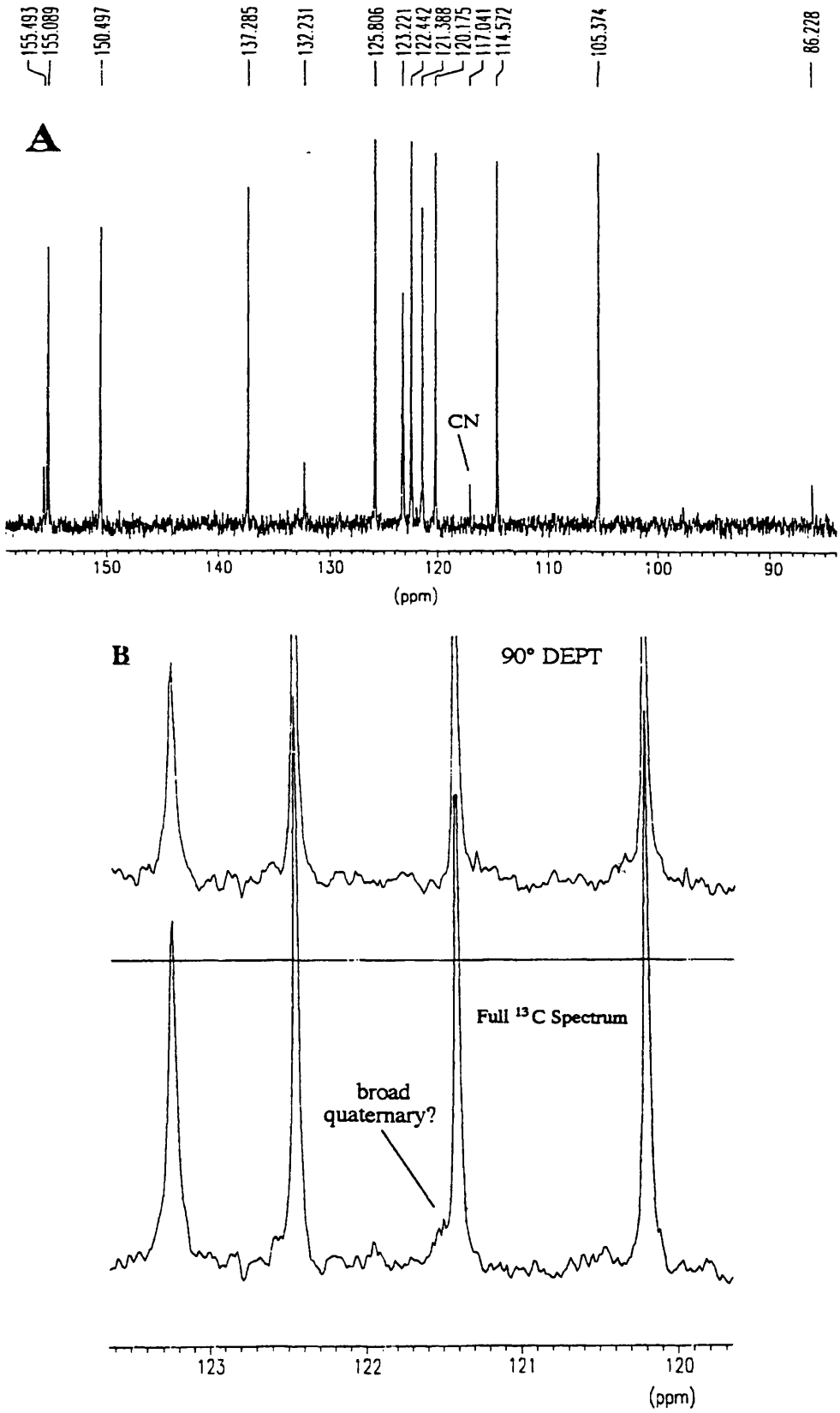
Clearly, formation of the imine product (**111**) involves the amine intermediate (**112**). However, the formation of (**112**) must require a reduction at some stage. This is inevitable for *any* product with the formula $C_9H_7N_3$ which is being generated from pyridine-2-carbaldehyde (C_6H_5NO) and malononitrile ($C_3H_2N_2$). This creates some difficulties at stipulating a likely mechanism since reductive reaction conditions were not employed. Nevertheless, if a hydride source is generated *in situ*, conceptually the overall reaction may be considered as a redox process. Indeed, this is the case for the Cannizzaro reaction[255] whereby aromatic aldehydes, under basic conditions, are converted to their correponding carbinols and acids. Thus, in this situation, the hydride source will likely be analogous to the hydride source in the Cannizzaro reaction[256] and, although alternative candidates may be envisaged, an example is presented in Scheme 4.



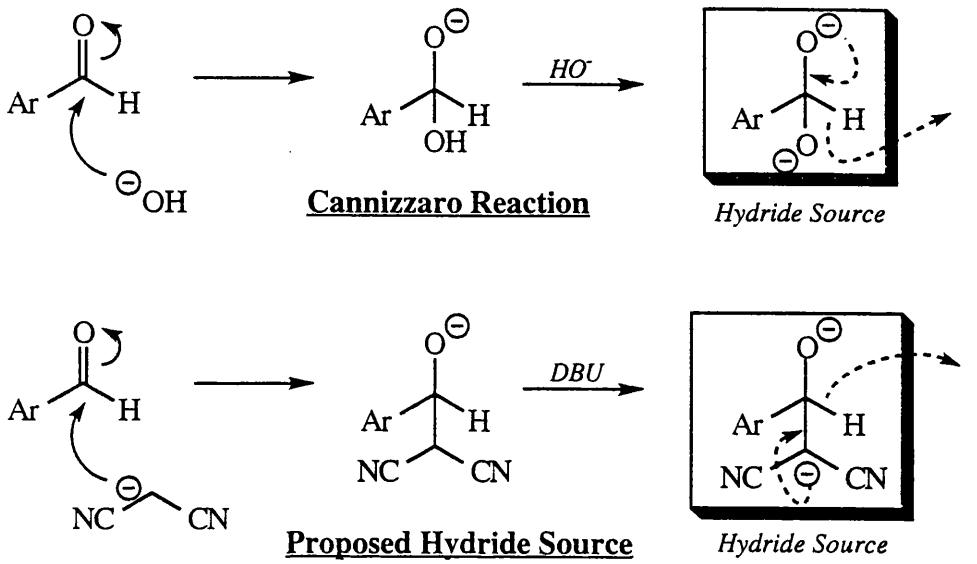
(**112**)

A postulated mechanism for the formation of the intermediate (**112**) is outlined in Figure 5. Initially, it is likely that a normal Knoevenägel condensation product (**A**) forms which would be expected to be a good hydride acceptor and thereby be reduced to its more saturated counterpart (**B**). Subsequent intramolecular attack of the pyridine nitrogen lone-pair on a nitrile grouping followed by a series of proton transfers will generate the ring closed structure (**112**). Although this unexpected side-reaction requires to be validated by experiment, this study provides an interesting basis for future investigation.

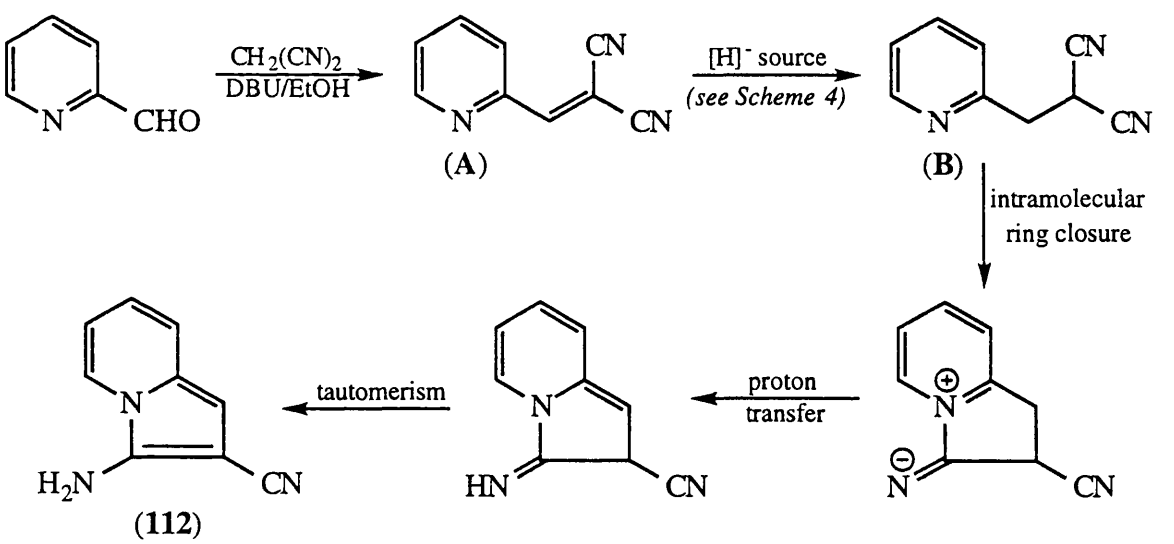
Figure 20 DEPT ^{13}C 200 MHz Spectra of Compound (111)



Scheme 4 Proposed Hydride Source in the Formation of Indolizine (112)

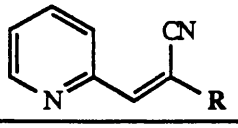
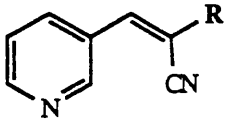
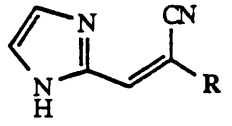
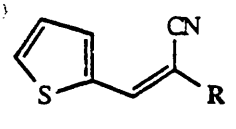
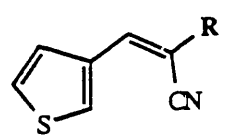


Scheme 5 Mechanistic Outline to the Formation of Indolizine (112)



In comparison to the discussion above, the synthesis of the 3-pyridyl series [(**92**)-(**95**)] was much more straightforward, although attempts at obtaining the 3-pyridinecarbaldehyde adduct with 1,1,3-tricyano-2-aminopropene failed. Also, the synthesis of the 2-imidazolyl series [(**96**)-(**100**)], 2-thienyl series [(**101**)-(**105**)], and 3-thienyl series [(**106**)-(**110**)], did not pose any serious problems. Consequently, together with the unknown (**111**), these were all biologically tested in the MCF-7 intact-cell assay, as well as the cell-free assay against the EGF-R kinase (Table 16).

Table 16 Biological Activities of Heteroarylpropenonitriles

Structure	No.	R	IC_{50} (μM)[a] MCF-7 cells	IC_{50} (μM)[b] EGF-R kinase
	(91)	CO_2Et	39.3	n.d.
See structure (111)			>50	n.d.
	(92)	CN	32.3	225[c]
	(93)	$CSNH_2$	>50	77
	(94)	CO_2Et	>50	>500
	(95)	$CONH_2$	>50	n.d.
	(96)	CN	26.3	532[c]
	(97)	$C(NH_2)=C(CN)_2$	>50	n.d.
	(98)	$CSNH_2$	29.4	56
	(99)	CO_2Et	>50	>100
	(100)	$CONH_2$	>50	n.d.
	(101)	CN	20.0	>100
	(102)	$C(NH_2)=C(CN)_2$	>50	>100
	(103)	$CSNH_2$	49.9	>100
	(104)	CO_2Et	>50	>100
	(105)	$CONH_2$	>50	n.d.
	(106)	CN	43.0	n.d.
	(107)	$C(NH_2)=C(CN)_2$	39.1	n.d.
	(108)	$CSNH_2$	22.7	250
	(109)	CO_2Et	40.9	>100
	(110)	$CONH_2$	>50	n.d.

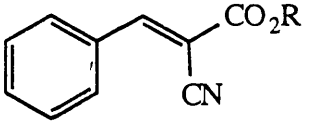
[a]Intact cell assay [b]Cell free assay (n.d.= not determined)
[c]Values taken from Gazit *et. al.*[230e]

On the whole the biological results were disappointing and there was no significant correlation between antiproliferative activity and EGF-R kinase inhibition. Futhermore none of these compounds seemed to possess the capacity to inhibit the enzymatic activity of the EGF-R kinase to the same extent as the dihydroxphenyl or trihydroxyphenyl series. For example the thioamide derivatives (**98**) and (**103**) showed reasonable IC_{50} values of 77 and 56 μM, respectively, against the EGF-R kinase, but this did not approach the 1-5 μM values exhibited by the best hydroxyphenyl analogues, *cf.* (**58**) and (**78**) in Table 14. It is clear, therefore, that the hydroxybenzylidene nucleus is needed for potent inhibitory action of the tyrphostins against the EGF-R tyrosine kinase. However, as noted previously, there is a major concern for this type of tyrphostin and probably potency and instability are inextricably linked with each other. Thus, it was decided to search for other heterocyclic

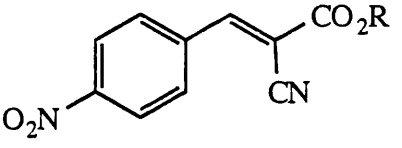
moieties that are able to exhibit low micromolar IC_{50} values. This will be discussed in Chapter 8.

6.6 Propenoic Acid and Other Tyrphostin Derivatives

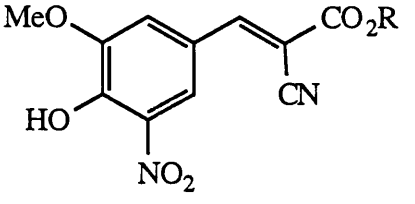
Previously, Levitzki and co-workers[230b] had synthesised acid and ester derivatives based on the *p*-hydroxyphenyl nucleus. Thus, it was planned to make a series of ester and acid derivatives based on alternative aryl groups. Particular interest was directed at exploring nitroaromatic tyrphostin analogues, as only a limited number of these had been reported. Initially, only methyl and ethyl cyanoacetate were available as starting materials. Thus, in order to determine appropriate conditions to form the corresponding acid derivatives, the cinnamic esters (**113**)-(**118**) were formed and used as model systems.



(**113**) R= Me
(**114**) R= Et



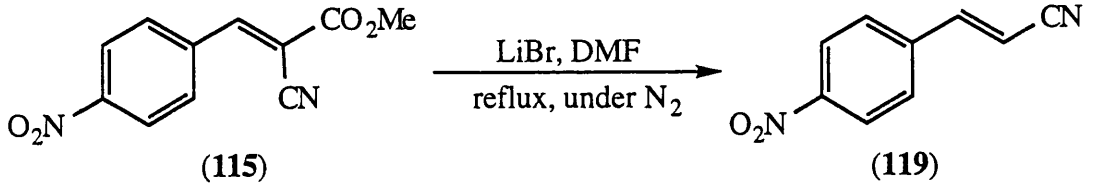
(**115**) R= Me
(**116**) R= Et



(**117**) R= Me
(**118**) R= Et

Due to the acid and base sensitivity of the nitrile group, a hydrolytic route to the acid derivatives of the above esters was avoided. Thus, it was required to use neutral conditions and the method initially chosen involved the S_N2 cleavage of methyl esters using anhydrous lithium iodide in refluxing DMF.[257] However, although a variety of conditions were used, this method gave complex mixtures with no identifiable product after extensive attempts at purification. Interestingly, the use of anhydrous lithium *bromide* on methyl 2-cyano-3-(4-nitrophenyl)propenonitrile (**115**) induced a demethoxycarbonylation and gave a low yield of the *trans*-cinnamonitrile (**119**)—as identified by 90 MHz ^{1}H NMR spectroscopy (see Scheme 6). This result is not surprising considering the powerful electron-withdrawing effect of the 3-(4-nitrophenyl)propenonitrile system since it will facilitate nucleophilic attack and subsequently stabilise any anionic intermediate. Furthermore, dipolar aprotic media are frequently used to effect this transformation.[258]

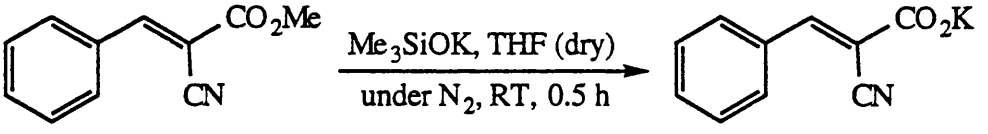
Scheme 6 Demethoxycarbonylation of Compound (115)



(**115**) (**119**)

Another S_N2 method employed the use of potassium trimethylsilanolate and was applied to the simplest tyrphostin model (**113**).[259] This method quickly and efficiently formed the potassium cinnamate salt (**120**) in 90 % yield (Scheme 7), but was found inappropriate for the nitroaromatics (**115**)-(**118**). This is probably due to nucleophilic attack of the powerful silanolate anion on the nitroaromatic rings.

Scheme 7 Demethylation of Compound (113)

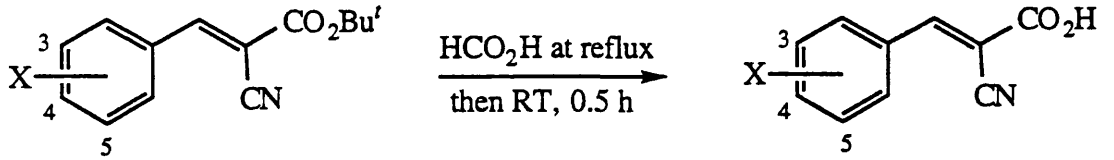


(**113**) (**120**)

After these disappointing results a milder non-nucleophilic method of cinnamic acid formation was sought and the use of trimethylsilyl iodide (TMS-I) made *in situ* from sodium iodide and trimethylsilylchloride was tried.[260] However, this method also met with failure and only starting material could be isolated, even when TMS-I was preformed from hexamethyldisilane and iodine.[261] Similar results were obtained with the use of bis(tributyltin)oxide—a mild procedure reported to cleave methyl esters selectively among co-existing functional groups of methyl penicillonates.[262] Clearly, an alternative mechanism of ester cleavage had to be investigated.

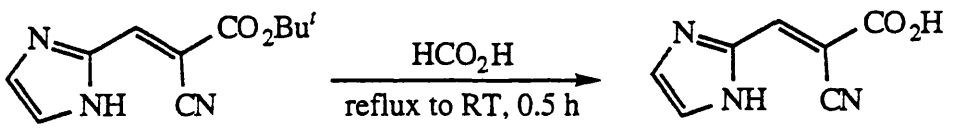
Originally, the idea had been to form *tert*-butyl ester derivatives of the tyrphostins by the Knoevenägel condensation and then generate the acid derivatives by a classical S_N1 fragmentation, expelling isobutylene, using excess trifluoroacetic acid in dichloromethane.[263] However, the methods described previously were adopted since, at that time, the *tert*-butyl cyanoacetate starting material was unavailable. When this material became available, a series of *tert*-butyl cinnamate esters were formed and consequently transformed into their acid counterparts (Scheme 8). Use of trifluoroacetic acid in dichloromethane was temperamental and required only a brief exposure to the solvent system. Trimethylsilyl triflate proved to be a milder method of fragmentation.[264] But the best protocol employed the use of formic acid[265] whereby the *tert*-butyl esters could be briefly heated at reflux in a minimum amount of this solvent and on cooling the acid derivatives tended to crystallise out.

Scheme 8 Formation of Propenoic Acid Tyrphostin Derivatives



(**121**) X= 3,4-dihydroxy
(**123**) X= 3,5-dichloro
(**125**) X= 3,5-difluoro
(**127**) X= 4-nitro
(**129**) X= 3,4-dimethoxy-6-nitro
(**131**) X= 3,4-methylenedioxy-6-nitro
(**133**) X= 3-methoxy-4-hydroxy-5-nitro

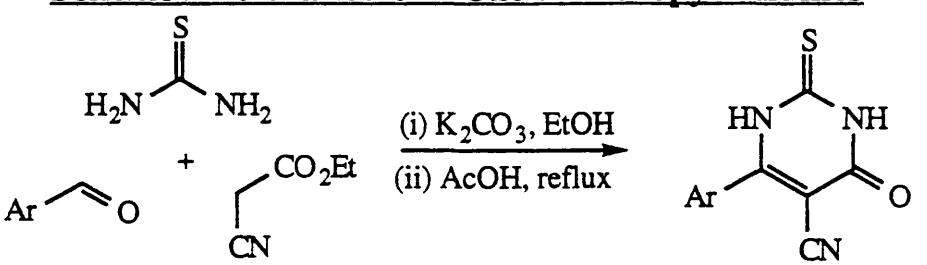
(**122**) X= 3,4-dihydroxy
(**124**) X= 3,5-dichloro
(**126**) X= 3,5-difluoro
(**128**) X= 4-nitro
(**130**) X= 3,4-dimethoxy-6-nitro
(**132**) X= 3,4-methylenedioxy-6-nitro



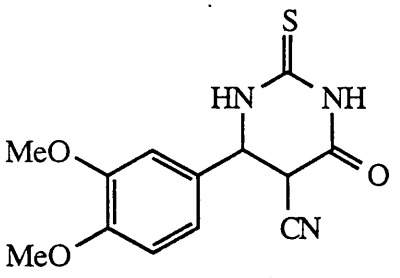
(**134**) (**135**)

After this work, other analogues based on the tyrphostins were sought. It was proposed to form a series of 4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidines (Scheme 9). The rationale behind this was two fold: firstly, to incorporate a less hydrolytically labile thioamide/thiourea functionality since, from previous work, the thioxo-group seemed to be important for potency, and secondly, to design a novel heterocyclic target that would enforce a *cis*-geometry about the aromatic and cyano portions (*cf.* Levitzki *et al.*[230f]).

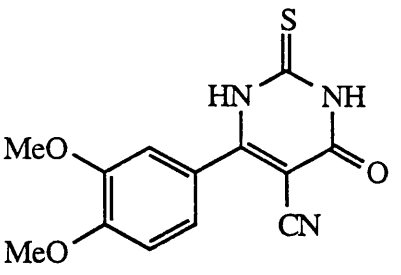
Scheme 9 Formation of 4-Oxo-2-thioxopyrimidines



Originally, it was planned to make a range of derivatives and incorporate the most active aromatic groups so far encountered in the project. However, this three component reaction could not be applied generally and only the reported synthesis of compound (**136**) could be successfully achieved, albeit in a low yield.[266] In particular, nitroaromatic systems proved to be problematic and reacting thiourea with the compounds (**115**), (**117**) or (**129**), or indeed the dichlorophenyl derivative (**87**), under a variety of conditions did not produce the desired product.



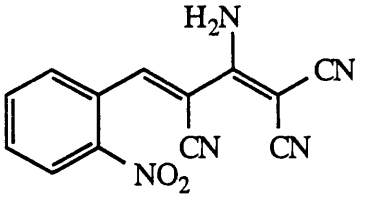
(**136**) K^+salt



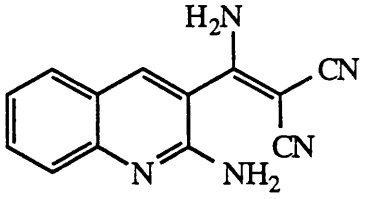
(**137**)

Consequently, this area of work was not investigated any further. However it should be noted that compound (**137**) was formed from the isolated potassium salt (**136**) by an oxidative process. Although 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) was initially used for this purpose, the best method employed vigorously heating the acidified product of (**136**) at reflux in glacial acetic acid in order to achieve an air-assisted mild oxidation.

Another area of interest was to develop other heterocyclic systems. For this purpose, the malononitrile dimer adduct of 2-nitrobenzaldehyde was formed (**138**). The rationale was to convert the nitro group into an amino group and then induce a cyclisation to the quinoline system (**139**). Such reductive cyclisations of benzylidene compounds with *ortho*-nitro groups have been documented before.[267] However all attempts based on catalytic hydrogenations (*e.g.* 5 % palladium on carbon)[268] were unsuccessful. Hydrolytic reduction methods (*e.g.* zinc or tin metal in mineral acid) were precluded due to the sensitivity of the nitrile grouping—although, in hindsight, the use of zinc or iron dust in acetic acid could have been tried.



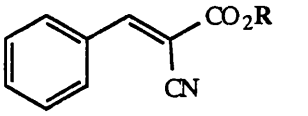
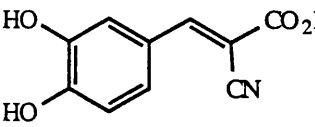
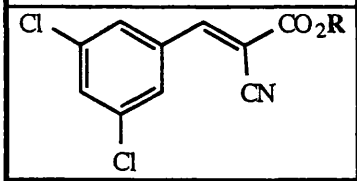
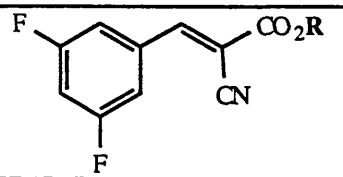
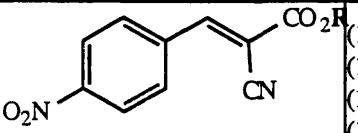
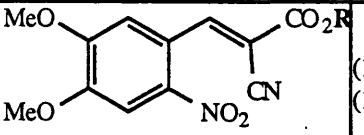
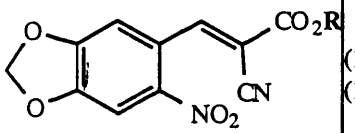
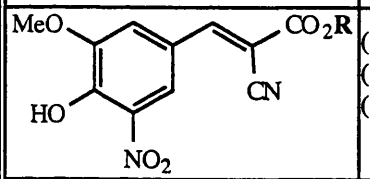
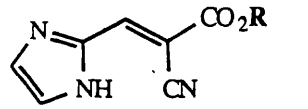
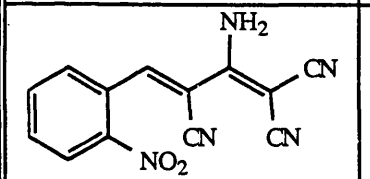
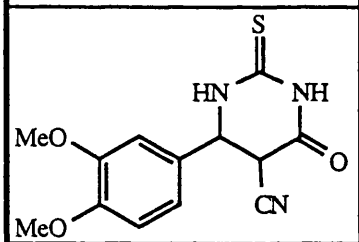
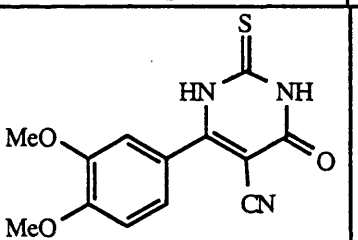
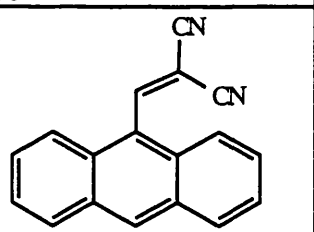
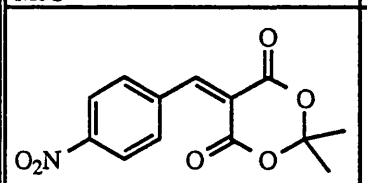
(**138**)



(**139**)

Other miscellaneous tyrphostin-based analogues were also made *via* the Knoevenägel condensation. These have been collated with the ester and acid tyrphostin derivatives in Table 17. Also displayed are the IC_{50} values measured for the compounds in the MCF-7 antiproliferative assay (no EGF-R kinase assays were performed in these cases).

Table 17 MCF-7 Data for Acid & Other Tyrphostin Derivatives

Structure	No.	R	IC_{50} (μM)	Structure	No.	R	IC_{50} (μM)
	(113) (114)	Me Et	30 3		(77) (121) (122)	Et ^{t}Bu H	43 31 33
	(87) (123) (124)	Et ^{t}Bu H	68 49 38		(90) (125) (126)	Et ^{t}Bu H	49 67 30
	(115) (116) (127) (128)	Me Et ^{t}Bu H	13 17 58 50		(129) (130)	^{t}Bu H	25 31
	(131) (132)	^{t}Bu H	30 46		(117) (118) (133)	Me Et ^{t}Bu	40 10 7
	(99) (134) (135)	Et ^{t}Bu H	>50 42 63		(138)		15
	(136) K^+ salt		33		(137)		12
	(139)		43		(140)		28

In some cases, the *tert*-butyl esters tended to be more active than their acid counterparts. This may be due to the more hydrophobic derivatives being more able to penetrate the cell membrane. However, there are a few exceptions to this statement. For example, in the 3,5-dihalophenyl series, there is a distinct advantage to possessing an acidic side group, *cf.* compounds (**87**), (**90**), and (**123**)-(**126**). Also, in the 4-nitrophenyl series [(**115**), (**116**), (**127**), and (**128**)], as the alkyl group (**R**) gets smaller the potency increases, although it is

it is not beneficial to have an acidic functionality. The imidazole derivatives [(**99**), (**134**), and (**135**)] all tend to lack potency. Similarly, both the anthracene (**139**) and the Meldrum's acid adduct (**140**) of 4-nitrobenzaldehyde, did not afford interesting results.

A strikingly clear trend in Table 17 is shown by the 5-nitrovanillin derivatives [(**117**), (**118**), and (**133**)] where as the size and hydrophobicity of the alkyl group (**R**) is increased so does the potency. For the the thioxopyrimidines, (**136**) and (**137**), the presence of a double bond seems to be important for potency and this, as a series, could show promise. However, in order to ascertain the likelihood that the observed antiproliferative activity is due to inhibiting the signalling function at the EGF receptor, the most potent members of Table 17, *i.e.* compounds (**114**)-(**116**), (**133**), (**137**), and (**138**), should be checked against the EGF-R kinase cell-free assay. Unfortunately, this was beyond my expertise and was not carried out. Nevertheless, for future reference, it would be advisable to develop the thioxopyrimidine series and incorporate alternative aromatic moieties into this base structure. Also, 5-nitro-vanillin derivatives, *cf.* (**133**), with an aralkyl hydrophobic side chain (**R**) should be made.

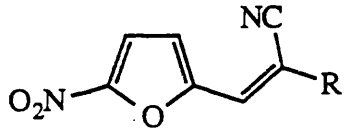
CHAPTER «7» Heteroaromatic Tyrphostin Derivatives

From the results given above, nitroaromatic tyrphostins generally exhibit a higher potency than the tyrphostins that possess a simple phenyl moiety (*cf.* Tables 14 to 17). This could mean that one should search for other nitroaromatic tyrphostins or an alternative electron-deficient system. For this latter case the most suitable system would be that of a nitrogen-heterocycle. However, as the formation of a 2-pyridine series of tyrphostins was unfruitful and the 3-pyridine series showed poor potency, it was decided to form a set of tyrphostins based on the quinoline nucleus. Futhermore, a set of nitro-substituted heterocycles was synthesised and tested for their biological activity. Thus, work relating to electron-deficient systems will be discussed in this chapter.

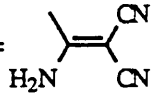
7.1 Nitrofuranylpropenonitrile Series

Earlier in the project various attempts at performing the Knoevenägel condensation (Scheme 1) on 2-nitrofuran-5-carbaldehyde were made. These were based around using neat piperidine and relatively dilute reaction concentrations at room temperature (Methods A and B). However these methods were generally unsuccessful or gave complex mixtures which were difficult to separate. So in order to ascertain an optimum procedure, a systematic investigation of reaction conditions was performed. This resulted in a new method based on

the observation that the product tended to be less soluble in the chosen alcohol solvent than the starting materials (Method C). This allowed the required product to precipitate or crystallise out so that it could not react any further. It was also important to minimise the reaction time as well as the quantity of piperidine catalyst. Indeed, this procedure is applicable to other activated nitroarylcarbaldehydes and, in this case, gave rise to the 3-(2-nitrofuran-5-yl)propenonitrile series (**141**)-(**148**). The acid derivative (**149**) was made from (**148**) using formic acid according to the conditions given in Scheme 8.



(**141**) R= CN

(**142**) R= 

(**143**) R= $CONH_2$

(**144**) R= $CSNH_2$

(**145**) R= CO_2Me

(**146**) R= CO_2Et

(**147**) R= $CO_2{}^nBu$

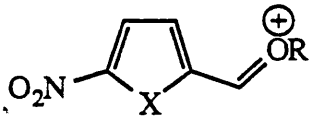
(**148**) R= $CO_2{}^tBu$

(**149**) R= CO_2H

In this series, the most difficult analogues to make were the products derived from malononitrile dimer (**142**), cyanoacetamide (**143**), and cyanothioacetamide (**144**). Both (**142**) and (**143**) required careful purification by silica chromatography, and it was found best not to use piperidine as a catalyst for the preparation of the thioamide (**142**). For this latter case it was surmised that there was enough basic character in the amino portion of the thioamide group to act as a self-catalyst for the condensation reation—a thioamide group is more like an α-thioxo-amine group due to poor orbital overlap. However, some other related reactions could be conducted without the presence of base catalyst. Thus an alternative reason may simply be the fact that 2-nitrofuran-5-carbaldehyde is a highly activated system for this type of reaction.

The observation that no base catalyst was required led to a brief ^1H NMR study of a related activated aldehyde (2-nitrothiophene-5-carbaldehyde) in deuteriated methanol. It was postulated that the aldehyde would be in an equilibrium between its free unsolvated and solvated form, *i.e.* as a dimethyl acetal. Indeed, this was observed and probably the condensation reactions as descibed above are facilitated by the alcohol solvent. The proposed rôle of piperidine as a catalyst is to form an iminium intermediate with an aldehyde and thereby make it more susceptible to nucleophilic attack. Thus, in this case, an

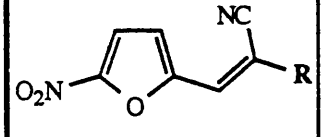
alkyl oxonium intermediate such as (**150**) could be involved in the reaction mechanism but clearly this is still open for investigation.



(**150**) X= O or S

Returning to the nitrofuranyl tyrphostin series, the condensation products (**141**)-(**149**) were subsequently tested in the MCF-7 intact-cell assay and the IC_{50} values obtained have been collated in Table 18. The amide (**143**) and *tert*-butyl ester (**148**) were the most potent compounds. However, unlike the other series so far encountered, the thioamide (**144**) and the 2-amino-1,1-dicyanopropene conjugate (**142**) were not the most potent members of this series. Also, a clear structure-activity relationship is presented for the ester derivatives (**145**)-(**148**), with the *tert*-butyl ester (**148**) being the most potent with a significantly low IC_{50} value of 4 μM. This same pattern was observed in the 5-nitrovanillin derivatives [(**117**), (**118**), and (**133**)] whereby an increase in hydrophobicity is correlated to an increase in potency—*cf.* Table 17. Again a loss in potency is observed for the acid derivative (**149**) and reflects the ease at which acids are able to traverse cellular membranes.

Table 18 MCF-7 Data of 3-(2-Nitrofuran-5-yl)propenonitriles

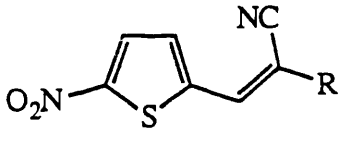
Structure	No.	R	IC_{50} (μM)
	(**141**)	CN	39
	(**142**)	$C(NH_2)=C(CN)_2$	41
	(**143**)	$CONH_2$	9
	(**144**)	$CSNH_2$	39
	(**145**)	CO_2Me	49
	(**146**)	CO_2Et	53
	(**147**)	$CO_2{}^nBu$	25
	(**148**)	$CO_2{}^tBu$	4
	(**149**)	CO_2H	30

This series was not investigated any further since more potent compounds were generated from 2-nitrothiophene-5-carbaldehyde. Also a promising series based on the quinoline nucleus had been discovered—see following sections. This aside, a clear recommendation for the nitrofuranyl tyrphostins would be to synthesise ester or amide derivatives with branched and unbranched alkyl chains. Also the incorporation of aralkyl groups would be advisable (particularly isosteres of tyrosine). Both these suggestions should not only aid the

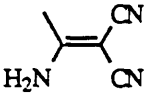
compound to traverse the cell membrane but the hydrophobic side chains should be able to act as auxiliary binding groups in their own right.

7.2 Synthesis of 3-(Nitrothienyl)propenonitriles

From initial undergraduate work carried out in our group it was apparent that certain tyrphostins derived from a nitrothiophene showed excellent biological activities (IC_{50} values less than 1 μM). Thus, it was of interest to synthesise a range of substituted nitrothiophenes in order to establish structure-activity relationships. Therefore, using readily available starting materials, a set of 5- and 4-substituted 2-nitrothiophenes were synthesised—hereinafter called the 2-nitrothien-5-yl series (**151**)-(**159**) and 2-nitrothien-4-yl series (**160**)-(**166**).



(**151**) R= CN

(**152**) R= 

(**153**) R= $CONH_2$

(**154**) R= $CSNH_2$

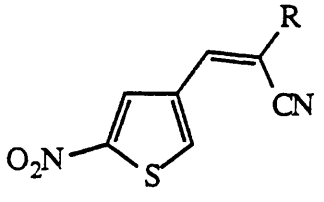
(**155**) R= CO_2Me

(**156**) R= CO_2Et

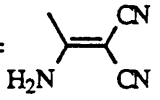
(**157**) R= $CO_2{}^nBu$

(**158**) R= $CO_2{}^tBu$

(**159**) R= CO_2H



(**160**) R= CN

(**161**) R= 

(**162**) R= $CONH_2$

(**163**) R= $CSNH_2$

(**164**) R= CO_2Me

(**165**) R= CO_2Et

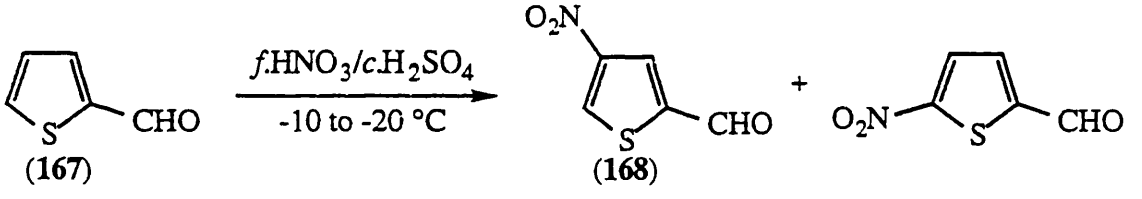
(**166**) R= $CO_2{}^nBu$

The method developed for the nitrofuranyl series was used to form these products—other methods tended to be less effective and more cumbersome. However, as encountered before, formation of the malononitrile dimer product (**152**) gave diffuculties. This was overcome by using THF as the solvent, instead of ethanol, which allowed the desired product to precipitate out as the reaction progressed. The esters (**155**)-(**158**) and (**164**)-(**166**) could all be crystallised directly from the reaction medium. The acid (**159**) was made from the

tert-butyl ester (**158**), in a similar manner as described before by using hot formic acid and could also be crystallised from this solvent. For crystallisation of other members in these series, generally isopropanol or ethyl acetate were used. However, the amide (**162**) had to be purified by column chromatography.

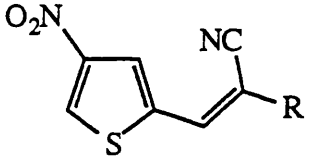
Having synthesised two isomeric series it was desirable to explore other substitution patterns. This was achieved after synthesising the appropriate nitrothiophene carbaldehydes. Thus, for one series, 4-nitrothiophene-2-carbaldehyde (**168**) was made by the β-nitration of thiophene-2-carbaldehyde (**167**) at -10 to 20 °C with a mixture of fuming nitric acid and concentrated sulfuric acid (Scheme 10).[269] An approximate 3:2 ratio of the β- and γ-nitrated products was obtained, as indicated by 90 MHz ^{1}H NMR spectroscopy. The desired isomer (**168**) was separated by column chromatography but needed a series of careful low temperature crystallisations in order to obtain an analytically pure product.

Scheme 10 β-Nitration of Thiophene-2-carbaldehyde

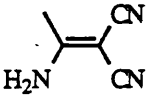


(**167**) (**168**)

In this reaction it was critical to maintain a temperature below -10 °C and to add the nitrating solution slowly with ample stirring, otherwise purification was problematic and the ratio of desired product to unwanted isomer plus overall yield diminished greatly. The best yield obtained was 33 %. Under the conditions of the Knoevenägel condensation (Method C), the aldehyde (**168**) was subsequently used to form a set of 2-substituted 4-nitrothiophenes, compounds (**169**)-(**175**)—hereinafter called the 4-nitrothien-2-yl series. Again the malononitrile dimer product (**170**) was formed in low yield and it was preferable not to use piperidine as catalyst in the formation of the thioamide (**172**).



(**169**) R= CN

(**170**) R= 

(**171**) R= $CONH_2$

(**172**) R= $CSNH_2$

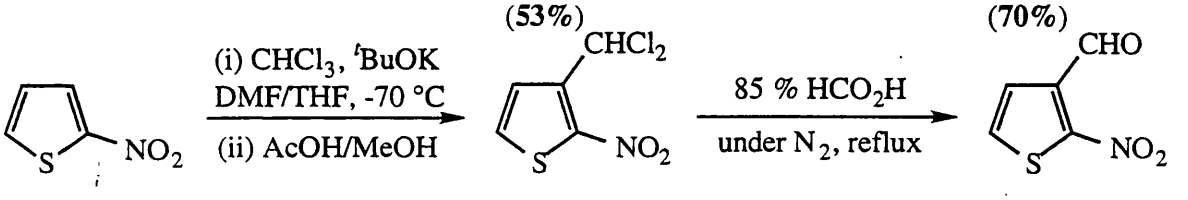
(**173**) R= CO_2Me

(**174**) R= CO_2Et

(**175**) R= $CO_2{}^nBu$

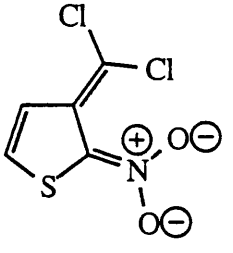
The synthesis of the other starting aldehyde (**178**) required a different strategy (Scheme 11).[270] This involved an initial *ortho*-dichloromethylation of 2-nitrothiophene (**176**) to give (**177**) followed by hydrolysis of the dichloromethyl moiety with 85 % formic acid. This last step required a little perseverance but the right reaction conditions were finally determined. Overall, this gave an *ortho*-formylation and is a good general method for introducing a carbaldehyde group to an electron-deficient aromatic system—as opposed to the Reimer-Tiemann reaction[271] which under different conditions works well on electron-rich systems such as phenols or pyrroles.

Scheme 11 *ortho*-Formylation of 2-Nitrothiophene



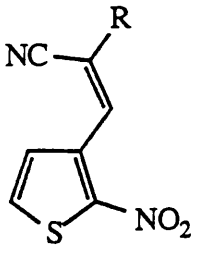
(**176**) (**177**) (**178**)

The initial reaction type has been termed by Mąkosza *et al.*[272] as a "vicarious nucleophilic substitution (VNS) of hydrogen". However, as it stands, this VNS description is probably confusing since hydrogen, as the hydride anion, does not actually act as the leaving group. Indeed, the reaction proceeds by an *ortho* or *para* attack on a nitro-aromatic by a carbanion which possesses a leaving group capable of a base induced β-elimination.[273] Under the anionic conditions, this generates an *ortho*-dichloromethylene *aci*-nitronate intermediate (**179**) that on acidification gives 3-dichloromethyl-2-nitrothiophene (**177**).

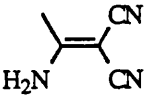


(**179**)

Subsequently, the novel aldehyde (**178**) was subjected to a series of Knoevenägel condensations to give the compounds (**180**)-(**185**)—hereinafter called the 2-nitrothien-3-yl series. Synthetically, this set of compounds did not pose too much problems and their biological evaluation, together with the isomeric series discussed above, will be presented in the next sections.



(**180**) R= CN

(**181**) R= 

(**182**) R= $CONH_2$

(**183**) R= $CSNH_2$

(**184**) R= CO_2Me

(**185**) R= CO_2Et

(**186**) R= $CO_2{}^nBu$

7.3 Biological Evaluation of 3-(Nitrothienyl)propenonitriles

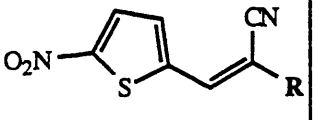
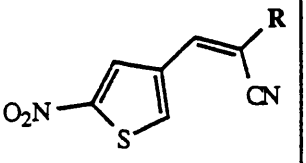
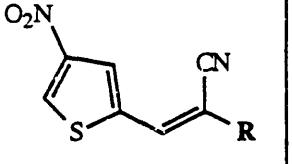
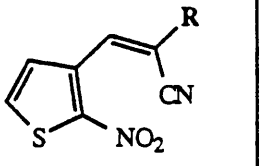
The biological activities of the various nitrothienyl series have been summarised in Table 19. Initially, the MCF-7 assay was conducted at inhibitor concentrations of 1 to 100 μM. However, as potencies of less than 1 μM were indicated, several assays were conducted at inhibitor concentrations down to 1 nM, and it was encouraging that some compounds even displayed inhibitory activities below this value.

As can be seen in Table 19 (MCF-7 column), most of the nitrothiophenes are highly potent antiproliferative agents. However, some compounds within a series displayed unexpectedly disappointing inhibitory actions, for example the methyl esters (**155**) and (**164**). The reason for this is not clear but it was found that methyl esters tended to be very insoluble in both organic and aqueous solvents. Thus, precipitation during an assay could account for these seemingly erroneous results.

For the EGF-R kinase assay, the IC_{50} values measured did not approach that observed for the MCF-7 cell assays. This indicates that the potent antiproliferative activity observed in the MCF-7 assay is not necessarily all due to inhibiting enzyme action at the EGF-R kinase. Nevertheless, a number compounds showed a reasonable correlation. For example, the nitrile (**R**= CN) derivatives [(**151**), (**160**), (**169**), (**180**)] consistently gave encouraging results in this respect. Also, the malononitrile dimer (**R**= $C(NH_2)=C(CN)_2$) products [(**152**), (**161**), (**181**)] and the thioamide (**R**= $CSNH_2$) derivatives [(**154**), (**163**), (**183**)] displayed a reasonable correlation.

By comparing Tables 17 to 19, an interesting general trend seems to be apparent for tyrphostin ester derivatives. Thus, for certain sets of tyrphostins such as the dihydroxyphenyl series [(**77**), (**121**), (**122**)], the nitrovanillin series [(**117**), (**118**), (**133**)], or the nitrofuranyl series [(**145**)-(**148**)], *increasing* the ester alkyl chain length tends to increase potency. However, for other sets of tyrphostins (particularly those with definite electron-

Table 19 Biological Activities of Nitrothienylpropenonitriles

Structure	No.	R	IC_{50} (μM)[a] MCF-7 cells	IC_{50} (μM)[b] EGF-R kinase
	(151)	CN	18.4	50
	(152)	$C(NH_2)=C(CN)_2$	12.0	12.25
	(153)	$CONH_2$	1.9	>500
	(154)	$CSNH_2$	<1	37.5
	(155)	CO_2Me	>100	>500
	(156)	CO_2Et	<1	34
	(157)	$CO_2{}^nBu$	17.8	>500
	(158)	$CO_2{}^tBu$	<1	75
	(159)	CO_2H	>50	150
	(160)	CN	<0.001	23
	(161)	$C(NH_2)=C(CN)_2$	<0.001	7
	(162)	$CONH_2$	0.9	>500
	(163)	$CSNH_2$	<0.001	10.5
	(164)	CO_2Me	>20	>500
	(165)	CO_2Et	<0.001	9
	(166)	$CO_2{}^nBu$	>20	>500
	(169)	CN	0.91	2
	(170)	$C(NH_2)=C(CN)_2$	<0.001	160
	(171)	$CONH_2$	<0.001	47
	(172)	$CSNH_2$	<0.001	>500
	(173)	CO_2Me	<0.001	18
	(174)	CO_2Et	<0.001	>500
	(175)	$CO_2{}^nBu$	<0.001	>500
	(180)	CN	<0.001	15.3
	(181)	$C(NH_2)=C(CN)_2$	0.025	14.6
	(182)	$CONH_2$	<0.001	400
	(183)	$CSNH_2$	0.8	22.7
	(184)	CO_2Me	6.0	50
	(185)	CO_2Et	<0.001	100
	(186)	$CO_2{}^nBu$	<0.001	>500

[a]Intact cell assay [b]Cell free assay

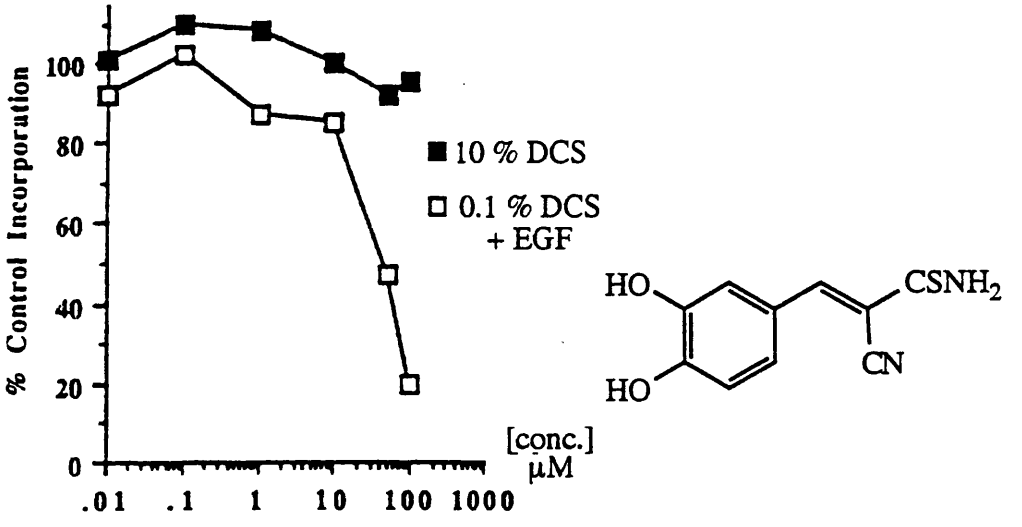
deficient character) such as the nitrothienyl series [above], 4-nitrophenyl series [(**115**), (**116**), (**127**), (**128**)], or difluorophenyl series [(**90**), (**125**), (**126**)], *decreasing* the chain length tends to increase potency. Although this contrasting structure-activity relationship is open to interpretation, it may be speculated that the site or, possibly, mode of action between these two classes of tyrphostins is different.

Due to this discrepancy and that the nitrothiophene set of tyrphostins exhibit highly potent antiproliferative properties, a more detailed biological analysis on their mode of action was

required. The data so far obtained indicates that some nitrothiophene compounds are able to block the phosphorylation of a synthetic polypeptide by inhibiting activity at the EGF-R kinase in a *cell-free* enzyme assay. It was therefore necessary to ascertain the inhibition of the EGF-R kinase in an *intact-cell* assay. For this purpose a similar assay to that performed for MCF-7 cells was carried out, except that fibroblast (HER-14) cells that have been transfected with the human EGF receptor were used. As before, the antiproliferative activity was assessed by using [^{3}H]thymidine incorporation as a measure of DNA synthesis and gave a similar dose-response curve as shown in Figure 17.

It is important to understand in these assays that HER-14 cells remain arrested in G_0, *i.e.* are quiescent, unless acted upon by two types of growth stimulators (*cf.* Section 2.1—Introduction). One of these is donor calf serum (DCS) and the other is EGF. DCS is ineffectual at a 0.1 % concentration but is growth stimulatory at 10 % and does not use EGF-R signalling to achieve this effect. Thus, parallel experiments were performed in which cells are grown in medium supplemented with 0.1 % DCS plus EGF[274], or just medium containing 10 % DCS. Ideally, selective EGF-R PTK inhibitors will only block EGF-stimulated growth and will have no effect on growth stimulated by 10 % DCS. This behaviour is exemplified in Figure 21 for the known tyrphostin, 2-cyano-3-(3,4-dihydroxyphenyl)propenothioamide (**58**)[275] which was used as a control in these assays.

Figure 21 Inhibition of EGF- & Serum- Dependent Proliferation of HER-14 Cells by 2-Cyano-3-(3,4-dihydroxyphenyl)propenothioamide (58)



The compounds (**152**), (**154**), (**156**), and (**160**) were tested in the HER-14 assay but, although significant antiproliferative activity was observed with IC_{50} values in the range of 20 to 80 μM, there was no observed selectivity (see Figures 22A to 22D). Unfortunately, these activities are not near the nanomolar values measured for the breast carcinoma MCF-7 cells. These results imply that another mode of action other than inhibition of EGF-R signalling is occurring.

Further investigations (carried out by Dr Valerie G. Brunton) assessed the ability of few of nitrothiophenes [(**152**), (**154**), (**156**), (**160**)] to inhibit EGF-R autophosphorylation in *intact* cells.[276] From this study, only the ethyl ester (**156**) was identified to inhibit this process but only at a concentration (100 μM) that was (probably) toxic to the cells and thus does not represent a specific effect on EGF-R function. As an unselective toxic action was suspected, Dr Brunton then went on to assess the ability of a selection of nitrothiophenes to inhibit EGF-R kinase activity in a cytotoxicity assay against a panel of four squamous cell carcinoma cell lines derived from tumours of the vulva, tongue, cervix and uterus (see Table 20). These cell lines were chosen as they are well characterised for their EGF-R status, with the A431 and HN5 cells having highly overexpressed levels of receptor and the CaSki and SiHa cell lines having levels around those found in normal cells.

Table 20 Cytotoxicity Data for Nitrothiophene Tyrphostins in a Panel of Squamous Cell Carcinoma Cell Lines

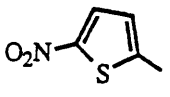
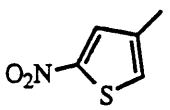
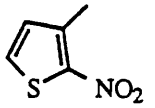
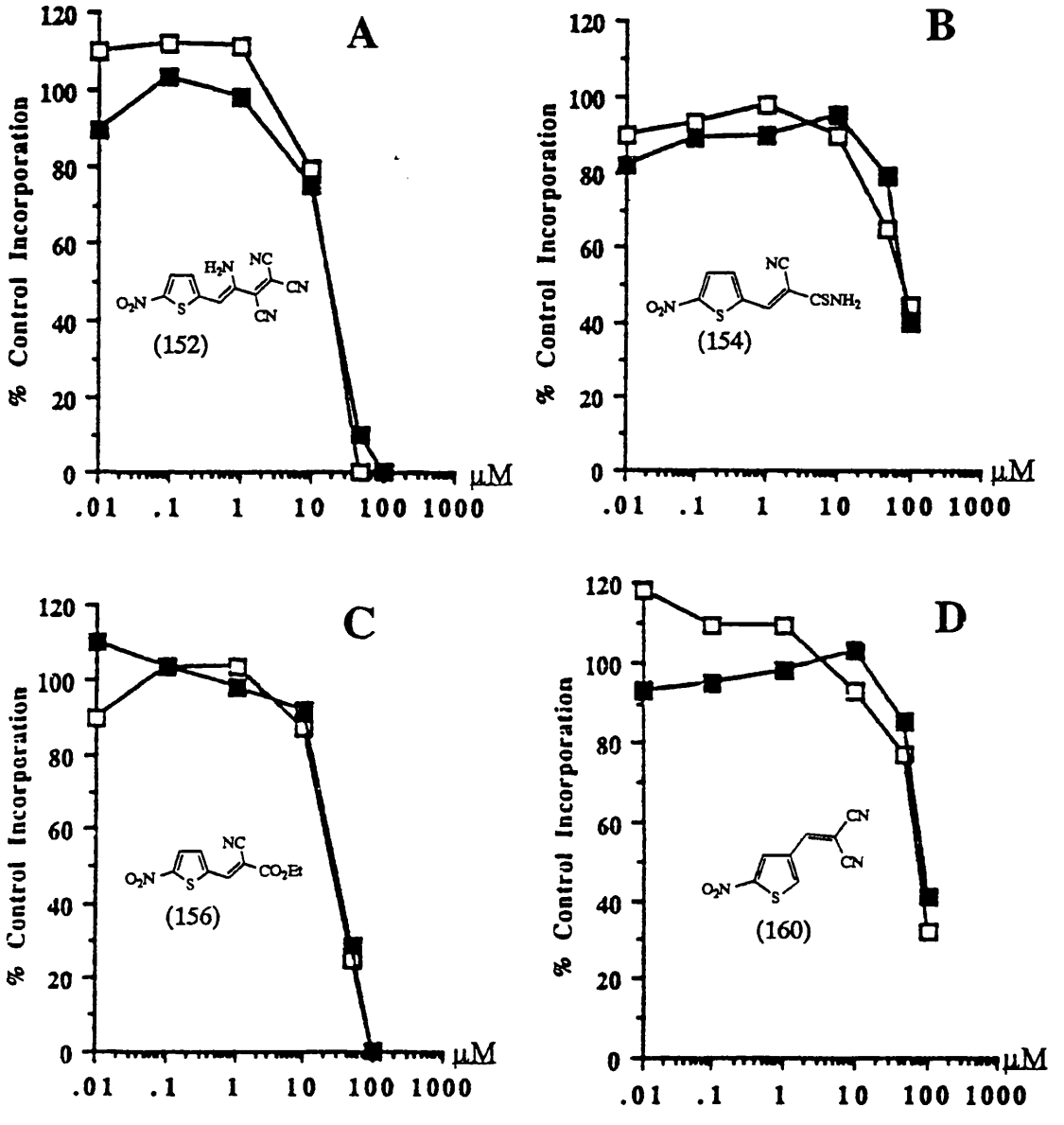
			IC_{50} values (μM) ± standard deviations			
Compound Structure and Number for: ArCH=C(CN)R			Values in brackets after each cell line represent the number of EGF receptors (× 10^6 per cell)			
Ar	**R**	**No.**	**HN5** (5.195)	**A431** (1.554)	**CaSki** (0.785)	**SiHa** (0.114)
	CN	(**151**)	20.7 ± 0.5	28.0 ± 1.4	25.8 ± 2.7	25.4 ± 3.8
	$C(NH_2)=C(CN)_2$	(**152**)	8.4 ± 1.1	6.1 ± 0.2	9.2 ± 0.2	13.4 ± 1.0
	$CSNH_2$	(**154**)	66.9 ± 9.7	71.6 ± 5.2	82.9 ± 8.3	82.1 ± 10.0
	CO_2Et	(**156**)	5.4 ± 0.3	4.6 ± 0.2	6.9 ± 2.8	4.6 ± 1.3
	CN	(**160**)	40.4 ± 5.8	28.4 ± 1.9	40.0 ± 6.4	44.6 ± 5.0
	$C(NH_2)=C(CN)_2$	(**161**)	>100	>100	>100	>100
	CO_2Et	(**165**)	>100	>100	>100	>100
	$C(NH_2)=C(CN)_2$	(**180**)	5.1 ± 2.4	4.7 ± 0.8	4.7 ± 1.2	7.8 ± 1.1
	$CSNH_2$	(**183**)	0.27 ± 0.08	0.11 ± 0.02	0.32 ± 0.02	0.35 ± 0.04

Figure 22 Inhibition of EGF- and Serum- Dependent Proliferation of HER-14 Cells by Nitrothiophenes (152), (154), (156), and (160)



KEY: ■ 10 % DCS □ 0.1 % DCS + EGF

In theory, cell lines that overexpress the EGF receptor will be more dependent on the EGF-R pathway for growth and thus will be more susceptible to an *EGF-R selective* inhibitor. Consequently, the potency of such an inhibitor should be greater in these cell lines as compared with ones that express lower numbers of EGF receptors, and should result in a gradual differential response from one cell line to another. However, although the compounds (**152**), (**156**), (**180**), and (**183**) were significantly potent in this assay, the results showed that nitrothiophenes were unselective (IC_{50} values remained constant).

7.4 Further Studies on Nitrothiophenes

The studies described above indicate that the nitrothiophenes are not displaying a selective inhibitory action on EGF-R kinase function. Nonetheless, these compounds exert an impressive cytotoxic effect against the breast carcinoma MCF-7 cells. Thus, although these compounds were originally designed to be cytostatic, it was of interest to investigate this mechanism of cell kill further.

From the information so far obtained it is more likely that the nitothiophenes exert their cytotoxic effect in a non-selective manner. With consideration to their structure, a plausible reason for this could be the presence of an aromatic nitro group, since it is known that nitro-reduction within a cell can generate DNA damaging species (usually radicals of some kind—see references on Tirapazamine™ (**6**) in Section 4.3). Futhermore, it has been reported that a number of nitrothiophenes[277] give initial one-electron reduction potentials in the order of -200 to -550 mV. This lies within the range for cellular reductions and so nitrothiophenes have the potential to act as bioreductively activated cytotoxins or radiosensitisers.

To test this suggestion, a selection of nitrothiophenes [(**152**), (**156**), (**161**), (**165**)] was evaluated for their ability to induce DNA strand breaks in A431 cells. This work was carried out by Dr Lloyd R. Kelland at the Institute of Cancer Research (Belmont, Surrey, SM2 5NG, UK) and used alkaline filter elution as described previously for *cis*-platin.[278] In summary, this study demonstrated that compounds (**152**) and (**156**) gave significant induction of DNA strand breaks at 100 μM, and compound (**152**) even partially displayed this activity at 50 μM. However, compounds (**161**) and (**165**) showed much less effecient activities. In contrast, the known typhostins (**58**) and (**66**) exhibited no induction of DNA strand breaks.

Having established that some nitrothiophenes can cause DNA damage, preliminary work carried out by a colleague[279] demonstrated that compounds belonging to the 2-nitrothien-5-yl and 2-nitrothien-3-yl series displayed one-electron reduction potentials[280] within the range likely to be reduced by cellular reducing agents, whereas compounds belonging to the 2-nitrothien-4-yl and 4-nitrothien-2-yl series did not. This result was not entirely surprising since, for electron withdrawing groups, an *ortho-* or *para-* substitution pattern is more able to accept and stabilise an additional electron than a *meta-* substitution pattern.

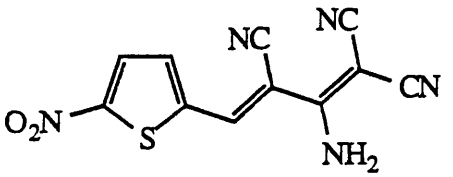
Interestingly, compounds (**152**) and (**156**) not only exhibited acceptable one-electron reduction potentials (-420 ± 20 mV and -330 ± 50 mV, respectively)[280] but also caused significant DNA damage as described above. Futhermore, it is notable that compounds (**161**) and (**165**) of the 2-nitrothien-4-yl series, which were inactive in the cytotoxicity panel at 100 μM (see Table 20) and did not induce DNA damage, gave reduction potentials outwith the range possible for cellular reduction (-1210 ± 30 mV and -720 ± 10 mV, respectively).[280] Such evidence agrees with the possibility that bioreduction contributes at least partially to the observed cytotoxicty of the compounds (**152**) and (**156**).

Due to the novelty and increasing interest in this class of compounds, a few promising candidates were selected to be screened against a panel of 60 human tumour cell lines held at the US National Cancer Institute.[281] Under a similar protocol to that performed for the MCF-7 cell line, tumour cells are subjected to a series of drug concentrations and the concentration which produces an apparent 50 % decrease in the number of tumour cells (relative to an untreated control) is defined as the GI_{50} value. The results for compounds (**152**), (**156**) and (**160**) are given in Figure 23. For each graph the vertical line represents the log of the mean GI_{50} value based on all the 60 cell lines. Thus, 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})].

As can be observed in Figures 23A and 23C, there was a very narrow range of GI_{50} values for compounds (**152**) and (**160**) in the different cell lines, indicating no preference for any particular tumour class. In contrast, compound (**156**) displayed a significant variation in sensitivity between the cell lines (Figure 23B). The leukæmia cell lines were particularly sensitive to the compound as were the majority of the colon cancer, melanoma and breast cancer cell lines, while the CNS derived lines and the non-small cell lung cancer lines were particularly resistant to compound (**156**). Furthermore, in order to search for similarities between drugs of known mechanism of action and the test compounds (**152**), (**156**), and (**160**), the data obtained was subjected to a COMPARE analysis.[282] This indicated that these compounds had unique profiles of sensitivity in the 60 cell lines as compared to a panel of anticancer agents with well defined modes of action.

Figure 23 Mean GI_{50} Values from US National Cancer Institute Panel of Human Cancer Cell Lines for Compounds (152), (156), and (160)

A: Compound (**152**)



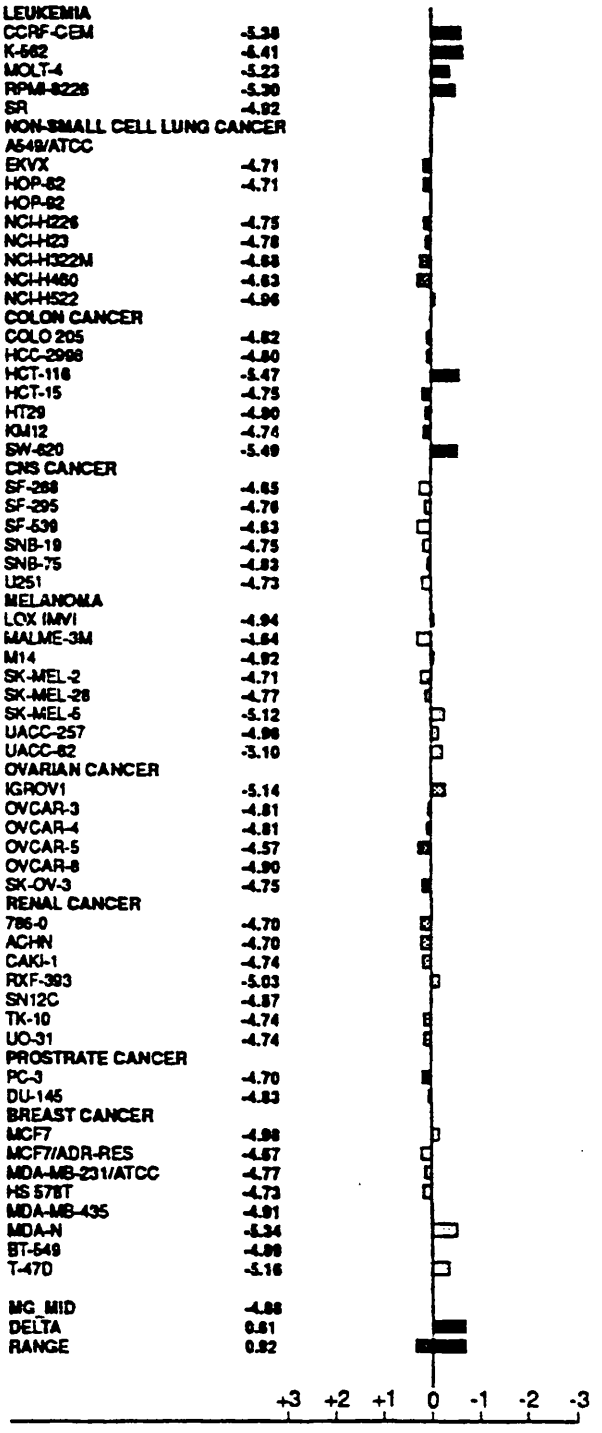
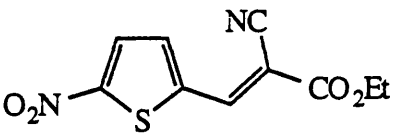


Figure 23 Mean GI_{50} Values from US National Cancer Institute Panel of Human Cancer Cell Lines for Compounds (152), (156), and (160)

B: Compound (**156**)



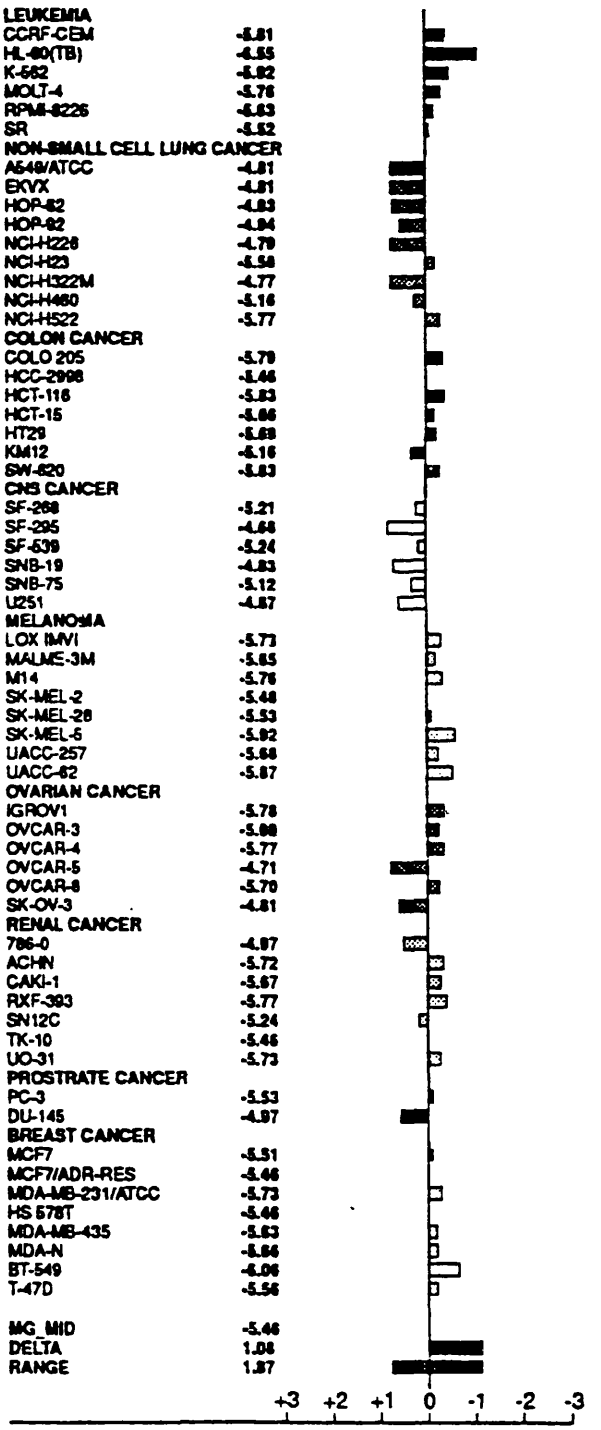
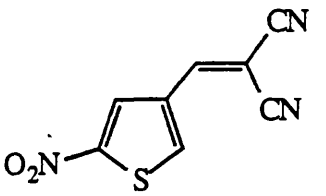
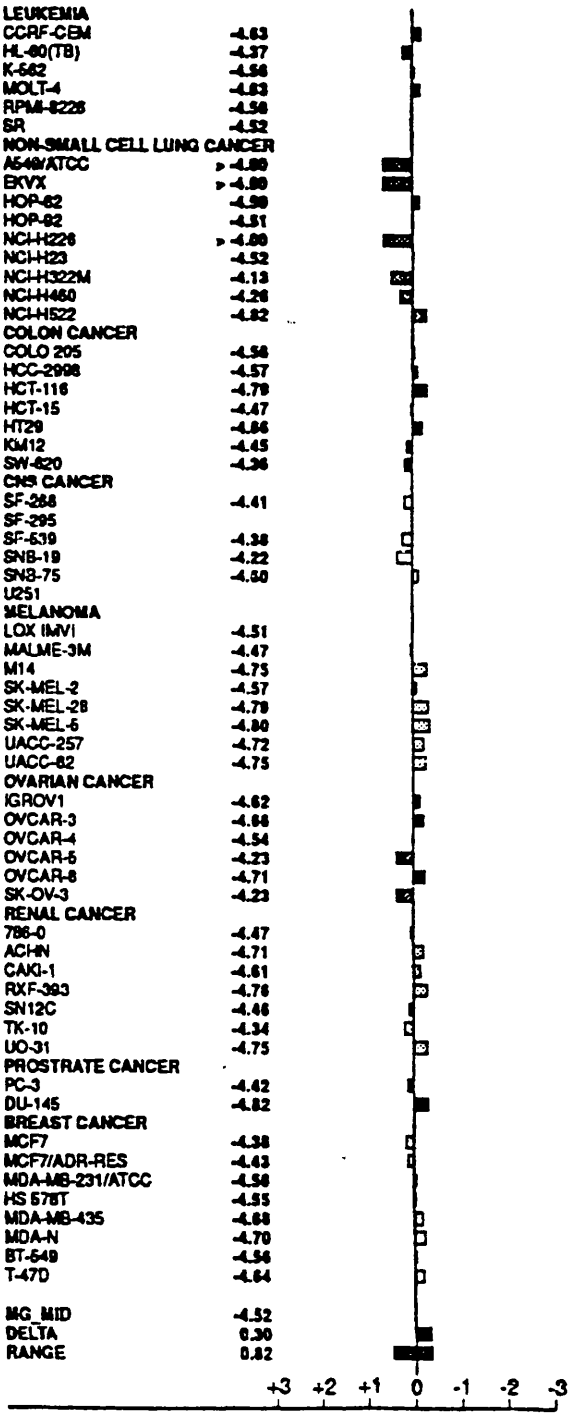


Figure 23 Mean GI_{50} Values from US National Cancer Institute Panel of Human Cancer Cell Lines for Compounds (152), (156), and (160)

C: Compound (**160**)



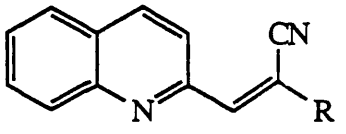


It is clear, therefore, that the nitrothiophenes represent an interesting (possibly novel) class of cytotoxic agent and may be promising candidates for future drug development. However, their exact mode(s) of action still remains to be determined and their overall selectivity does not look too encouraging. Nevertheless, from the study described above and in the previous section, particular attention should be directed at ethyl 2-cyano-3-[5-(2-nitrothienyl)]propenoate (**156**) due to its differential effects on various tumour cell lines. Currently this compound has been accepted by the NCI to undergo an *in vivo* evaluation and, if bioreduced, has the possibility of being solid tumour selective, although its sensitivity to rapidly dividing cells of the body still remains a concern. However, for the other nitrothiophenes [(**152**), (**160**), (**161**), (**163**), (**163**), (**165**), (**169**), (**173**), (**180**), (**181**), (**183**)] found signficantly active against the EGF-R kinase assay (see Table 19), the next step would be to test these against appropriate human tumour xenografts, first *in vitro* and then *in vivo*. Further recommendations would be to determine if the inhibition of DNA synthesis is reversible or not and to ascertain whether high dosing induces cell death (*e.g.* by lysis, apoptosis, necrosis, *etc*). This should allow the degree of cytotoxicity to be assessed in greater detail. It would also be advisable to test these compounds for damage against redox or metabolic (mitochondrial) processes of the cell because of their susceptibility to reduction (*cf.* closing discussion in Section 6.2). Indeed, they should be assayed against enzymes responsible for cellular reductions (*e.g.* DT diaphorase, P450 cytochromes, *etc*.). Clearly, this is an area open to further investigation and the results so far obtained have been submitted for publication.[283]

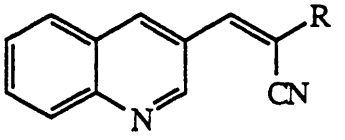
7.5 Synthesis of Quinolinylpropenonitrile Series

In Section 6.5, the synthesis and biological evaluation of heterocyclic tyrphostin derivatives was described. As an extension to this work and to explore alternative heteroaromatic systems, a series of quinoline derivatives was synthesised. Like the nitroaromatics described earlier in this chapter, these heterocycles are electron-deficient (but to a lesser degree). This may avoid the instability that is apparent in the hydroxylated phenyl series (*cf.* Section 6.3) and the cytotoxicity that is observed in the nitrothienyl series (to some extent). Although potency may diminish, it should be borne in mind that potency *per se* is less important than selectivity and the purpose of this project was not to develop yet another cytotoxic agent but to strive for a cytostatic antisignalling agent.

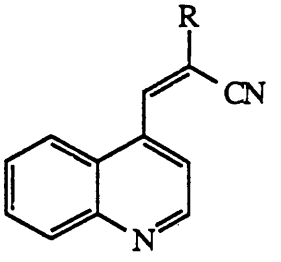
The synthesis of quinoline based tyrphostins was carried out under the conditions of the Knovenägel condensation (*cf.* Scheme 1). Thus, using a selection of activated methylene derivatives, the 2-quinolinyl series (**187**)-(**192**) was generated from 2-quinoline-carbaldehyde. Similarly, the 3-quinolinyl series (**193**)-(**197**) was produced from 3-quinolinecarbaldehyde; and the 4-quinolinyl series (**198**)-(**202**) was synthesised from 4-quinolinecarbaldehyde. The formation of the malononitrile products (**187**), (**193**), and (**198**) for use as charge transporters in electrophotographic photoconductors has recently been reported.[284] These compounds, together with the derivatives (**188**), (**194**), and (**199**), have also been prepared in order to study the mesomeric effects of an electron withdrawing sidearm on the ultraviolet spectrum of quinoline.[285]



(**187**) R= CN
(**188**) R= $C(NH_2)=C(CN)_2$
(**189**) R= $CONH_2$
(**190**) R= $CSNH_2$
(**191**) R= CO_2Et
(**192**) R= CO_2Bu^t



(**193**) R= CN
(**194**) R= $C(NH_2)=C(CN)_2$
(**195**) R= $CONH_2$
(**196**) R= $CSNH_2$
(**197**) R= CO_2Et



(**198**) R= CN
(**199**) R= $C(NH_2)=C(CN)_2$
(**200**) R= $CONH_2$
(**201**) R= $CSNH_2$
(**202**) R= CO_2Et

Out of these three series the formation of the 2-quinolinyl series posed the most problems (*cf.* 2-pyridyl series, Section 6.5). Although preparation of the compounds (**187**), (**191**), and (**192**) did not create too many difficulties, the malononitrile dimer (**188**) and amide (**189**) products were preferably made in THF while using DBU as the base catalyst. However, under reasonably concentrated conditions with a minimal amount of piperidine, the compound (**188**) could be made and precipitated from ethanol. This was not the case for the formation of the amide (**189**) which was extremely problematic and gave complex mixtures. The reason for this was not clear but careful elimination of water, using 4 Å molecular sieves, and an immediate purification by column chromatography, yielded the desired product, albeit in very low yield.

In contrast, although DBU was also used for some members of the 4-quinolinyl series, the amide (**200**) was produced in reasonable yield and could be crystallised. Apparently, a

heteroarylcarbaldehyde with an *ortho* azine-nitrogen is susceptible to further reaction under the conditions employed. One possibility is activation of the double-bond for *bis*-adduct formation, although other alternatives may be envisaged, *e.g.* nitrogen lone-pair attacking a carbonyl or nitrile carbon centre.

In the 3-quinolinyl series, the preparation of compounds (**193**) and (**195**)-(**197**) was reasonably straightforward. The malononitrile dimer product (**194**) was formed in very low yield but this was mainly due to the small scale of the reaction and product loss during crystallisation. The formation of the *tert*-butyl ester (**192**) in the 2-quinolinyl series, was carried out in order to obtain its acid counterpart. However, all attempts at treating (**192**) in formic acid or other media favourable for S_N1 reactions failed and gave intractable mixtures. In comparison, formation of the acid derivatives in the 3- and 4-quinolinyl series *is* possible (*via* the *tert*-butyl esters) and the formic acid reactions are reasonably facile and effective (see note[286]). The reason for this is again unclear but is probably connected to the problems encountered previously in the preparation of the 2-quinolinyl series.

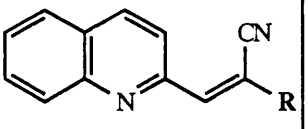
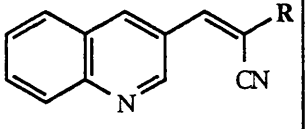
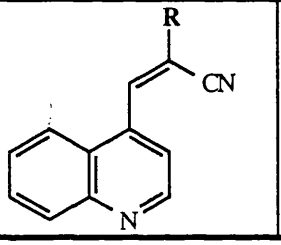
7.6 Biological Evaluation of Quinolinylpropenonitrile Series

Initially, a selection of tyrphostins incorporating a quinoline portion, as synthesised above, were evaluated in the MCF-7 antiproliferative assay (Table 21; "[^{3}H]Thymidine" column). This gave encouraging results since a number of substituted quinolines, notably (**187**), (**188**), and (**196**), displayed IC_{50} values less than 1 μM. Also, this set of compounds (as a whole) exhibited activities significantly higher than those encountered for the hydroxyphenyl series (*cf.* Section 6.2) or, indeed, the other heterocyclic series previously described (*cf.* Section 6.5).

Consequently, the compounds were tested for their ability to inhibit EGF-R kinase function directly (Table 21). This demonstrated that the most potent derivatives in each quinoline series contained the 2-aminoethene-1,1-dicarbonitrile moiety [**R**= $C(NH_2)=C(CN)_2$]. Thus, compounds (**188**), (**194**), and (**199**), gave respective IC_{50} values of 1.7, 27.0, and 4.7 μM. As can be seen, for each **R** group, the 2-substituted quinolines were generally more potent than their 3- or 4-substituted counterparts and the 3-substituted series were the least potent.

However, there did not seem to be a consistent correlation between antiproliferative activity and EGF-R kinase inhibition. This indicated that a non-selective mode of growth

Table 21 Biological Activities of Quinolinylpropenonitriles

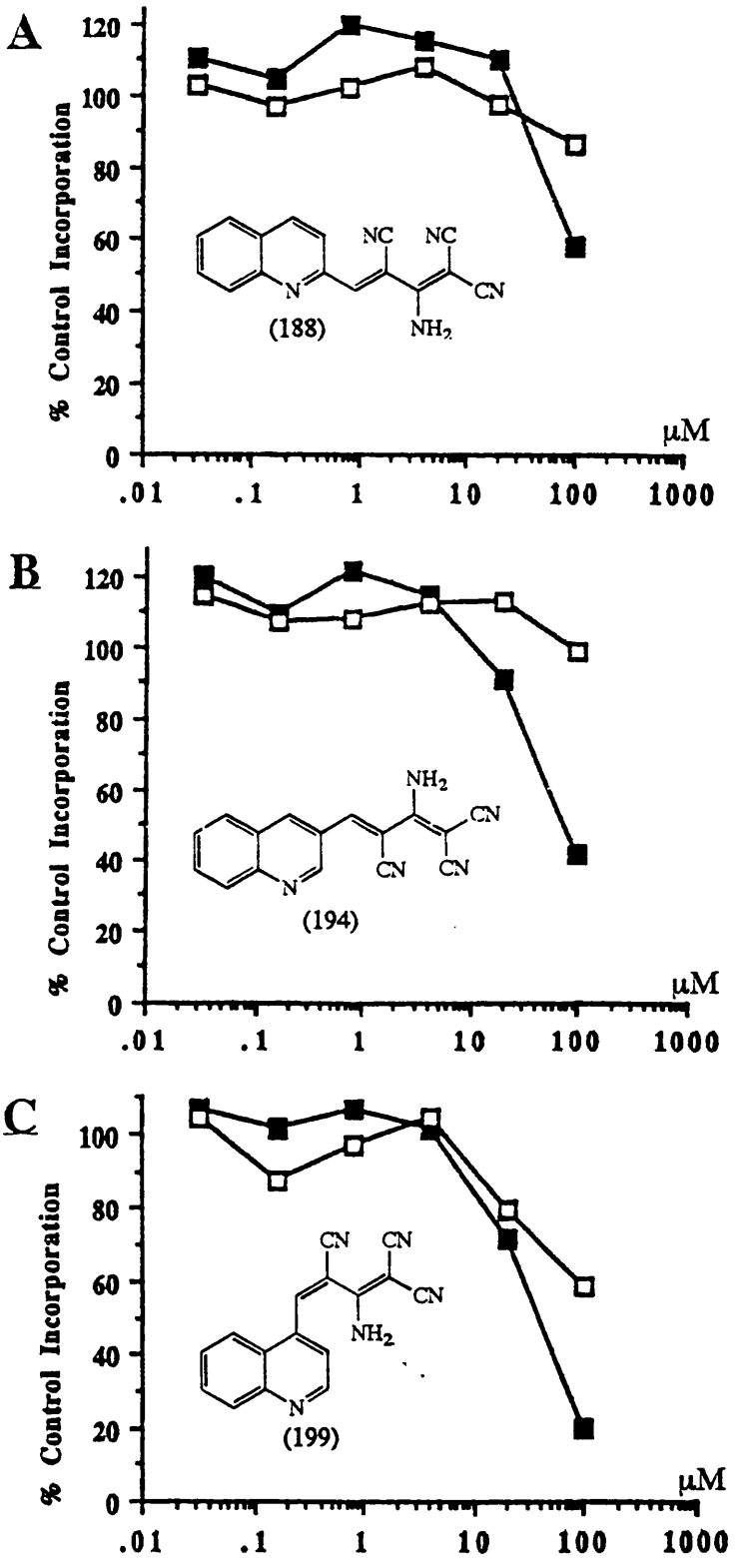
Compound			IC_{50} Values (μM)		
Structure	No.	R	[^{3}H]Thymidine[a]	MTT[b]	EGF-R kinase[c]
	(187)	CN	<1	>100	58
	(188)	$C(NH_2)=C(CN)_2$	<1	77	1.7
	(189)	$CONH_2$	49	34	79
	(191)	CO_2Et	n.d	17	240
	(192)	CO_2Bu^t	n.d	14	145
	(193)	CN	24.8	76	400
	(194)	$C(NH_2)=C(CN)_2$	n.d	52	27
	(195)	$CONH_2$	23.9	n.d	>500
	(196)	$CSNH_2$	<1	>100	300
	(197)	CO_2Et	>50	>100	>500
	(198)	CN	21.0	78	170
	(199)	$C(NH_2)=C(CN)_2$	n.d	55	4.7
	(200)	$CONH_2$	26.8	41	140
	(201)	$CSNH_2$	n.d	49	38
	(202)	CO_2Et	48.4	n.d	330

[a]MCF-7 antiproliferative assay [b]MCF-7 cytotoxicity assay
[c]Cell free assay on HN5 membrane fractions (n.d.= not determined)

inhibition was occurring, possibly toxicity, and the compounds required further investigation. For this purpose the compounds were assessed in a cytotoxicity assay against the MCF-7 cell line (see Table 21; "MTT" column). As indicated, there was a wide range of IC_{50} values (as low as to 14 μM and above 100 μM) but again no obvious correlation could be made between cytotoxicity and EGF-R kinase inhibition. Futhermore, although a reasonable correlation was apparent for the compounds (**188**), (**194**), and (**199**), there was no general pattern between potency and **R** group substituents or with the position of substitution on the quinoline ring system.

These findings raised the possibility that the observed antiproliferative effects of the quinoline-derived tyrphostins may be unrelated to EGF-R kinase inhibition. Thus, as performed for the nitrothiophenes, the most promising candidates [(**188**), (**194**), (**199**)] were assessed against transfected fibroblast (HER-14) cells (*cf.* Section 7.2). The dose-response curves obtained are given in Figure 24. Each graph displays parallel experiments that were carried out in which quiescent HER-14 fibroblasts were stimulated to undergo DNA synthesis in either 0.1 % DCS supplemented with EGF,[274] or just 10 % DCS over a

Figure 24 Inhibition of EGF- and Serum- Dependent Proliferation of HER-14 Cells by Quinolines (188), (194), and (199)



KEY: □ 10 % DCS ■ 0.1 % DCS + EGF

16 h period. Thus, all compounds were shown to preferentially inhibit EGF-dependent phosphorylation at 100 μM, although to a lesser degree as compared to the tyrphostin (**58**) (*cf.* Figure 21). The 3-substituted quinoline (**194**) even showed preferential inhibition at 20 μM (Figure 24B). Therefore, this study suggests that the mechanism of action of these compounds is at least partially linked to the EGF-R signalling pathway.

In an attempt to determine if there was a link between the molecular properties of this set of quinoline tyrphostins and PTK activity, detailed *ab initio* calculations were carried out by Dr R. Zwaans and Dr C. Thomson at the University of St. Andrews (North Haugh, St. Andrews, Fife, KY16 9ST).[287] Although sophisticated computer-aided techniques were used, conclusions were difficult to formulate and will not be discussed in detail here. However, it was suggested that three small negatively charged areas are required to attach compounds containing the 2-aminoethene-1,1-dicarbonitrile moiety [(**188**), (**194**), (**199**)] to the catalytic site of the enzyme and allow the quinoline portion to rotate freely in an appropriately orientated position.

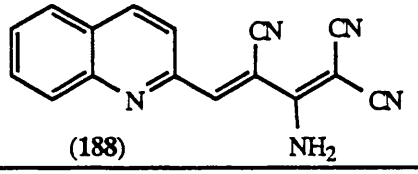
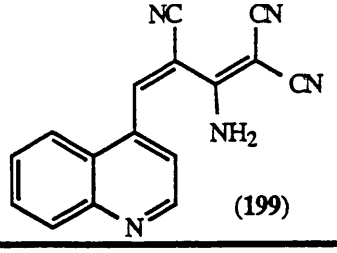
Further biological studies (carried out by Dr Valerie G. Brunton) investigated the effect that the compounds (**188**), (**194**), and (**199**) had on inhibiting the EGF-stimulated tyrosine phosphorylation of the EGF-R kinase as well as other cellular proteins in HER-14 cells. This showed that the 2- and 3- substituted quinolines [(**188**) and (**194**)] did not reduce EGF-R autophosphorylation at concentrations as high as 100 μM. In comparison, the 4-substituted quinoline (**199**) at 100 μM completely abolished EGF-induced tyrosine phosphorylation but this was probably due to a toxicity effect at this concentration (*cf.* Figure 24C). However, an interesting observation made from this study was that all three compounds were able to inhibit *selectively* the EGF-dependent phosphorylation of an unknown protein of around 50 kDa in mass—compounds (**188**) and (**194**) at 100 μM, and compound (**199**) at 50 μM.

These findings, therefore, imply that the selected quinoline compounds may not be acting on the EGF-R kinase itself but on at least one downstream protein involved in EGF-R signalling. However, the action of these compounds on EGF-R cannot be totally discounted since in *intact* cells they may be acting as inhibitors competitive for the ATP binding site and so would have to compete with very high intracellular levels of ATP (*cf.* Osherov *et al.*[288]). This would mask any effect the compounds had on receptor autophosphorylation.

However their action may still be sufficient to inhibit the association and/or phosphorylation of EGF-R kinase substrates that are involved in the mitogenic response to EGF. Such substrates may include the *Shc* family of adaptor proteins which are known to associate with and be phosphorylated by activated EGF receptors (see Section 3.3; Introduction). Unfortunately, although the *Shc* protein has a molecular weight of around 50 kDa, more detailed experiments showed that the EGF-dependent phosphorylation of *Shc* in HER-14 cells was unaltered by either compound (**188**), (**194**), or (**199**).[289]

The suggestion that the quinoline compounds (**188**) and (**199**) are affecting EGF-R kinase signalling was also indicated in the results obtained from their cytotoxicity profile against a panel of squamous cell carcinoma cell lines which express differing levels of the EGF receptor (Table 22). Thus, the high receptor-expressing cell lines were more sensitive to the quinolines than the cell lines expressing lower receptor levels (*cf.* Table 20 in Section 7.2). Although encouraging this outcome can not be used as direct evidence for inhibition of EGF-R kinase function either at the receptor itself or downstream in its signalling cascades.

Table 22 Cytotoxicity Data for Quinoline Tyrphostins (188) and (199) in a Panel of Squamous Cell Carcinoma Cell Lines

Compound Structure and Number	IC_{50} values (μM) ± standard deviations Values in brackets after each cell line represent the number of EGF receptors (× 10^6 per cell)			
	HN5 (5.195)	**A431** (1.554)	**CaSki** (0.785)	**SiHa** (0.114)
 (188)	38.3 ± 7.5	45.7 ± 7.5	69.0 ± 17.1	>100
 (199)	8.9 ± 2.4	6.3 ± 1.0	36.3 ± 3.8	>100

In each cytotoxicity assay, the 4-substituted quinoline (**199**) displayed greater potency than the 2-substituted quinoline (**188**) and so was selected to be evaluated for its potential to induce DNA strand breaks. This work was carried out by Dr Lloyd R. Kelland at the Institute of Cancer Research (Belmont, Surrey, SM2 5NG, UK) and showed that cells

treated with compound (**199**) displayed no evidence of DNA damage—*cf.* opening discussion in Section 7.3. Thus, the mechanism of cytoxicity remains unknown at present.

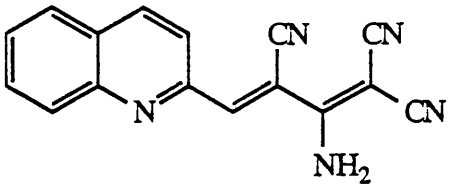
It is clear, therefore, that further work is required to elucidate the mechanism of action of this group of substituted quinoline tyrphostins and to establish whether this is related to inhibition of EGF-R kinase function or not. This is particularly pertinent for the most active compounds (**188**), (**194**), and (**199**) which, although possessing an additional cytotoxic action, selectively inhibit the EGF-dependent phosphorylation of a 50 kDa protein. An initial recommendation would be to determine if these three compounds are acting as substrate-based ATP inhibitors at the EGF-R kinase in *cell-free* assays. Also, their selectivity towards other signalling enzymes should be investigated as well as their effect on the mitochondrial processes of the cell.

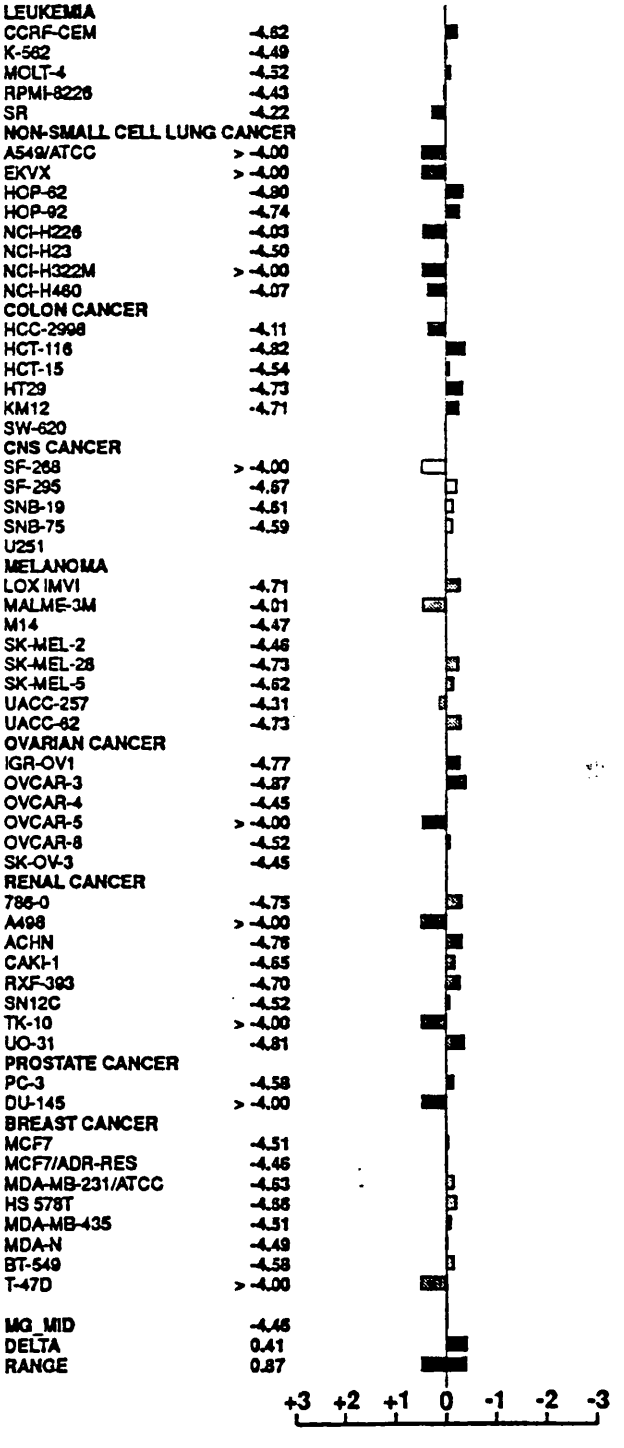
As suggested in the closing discussion for the nitrothiophenes (Section 7.3), the compounds (**188**), (**194**), and (**199**) should also be assessed in a panel of human xenograft models in order to establish sensitivity patterns. The 2-substituted quinoline (**188**) has already been screened by the US National Cancer Institute for its antiproliferative activity against a panel of 60 tumour cell lines. The mean GI_{50} graph (Figure 25) shows that the majority of non-small cell lung cancer tumours are resistant to compound (**188**), whereas the majority of the colon, CNS, melanoma and renal tumours are relatively sensitive.[290] Thus, this study has demonstrated that a certain degree of selectivity may be achieved with this type of antiproliferative agent and some of the results presented here have been submitted for publication.[291]

7.7 Attempted Synthesis of Polysubstituted Quinoline Derivatives

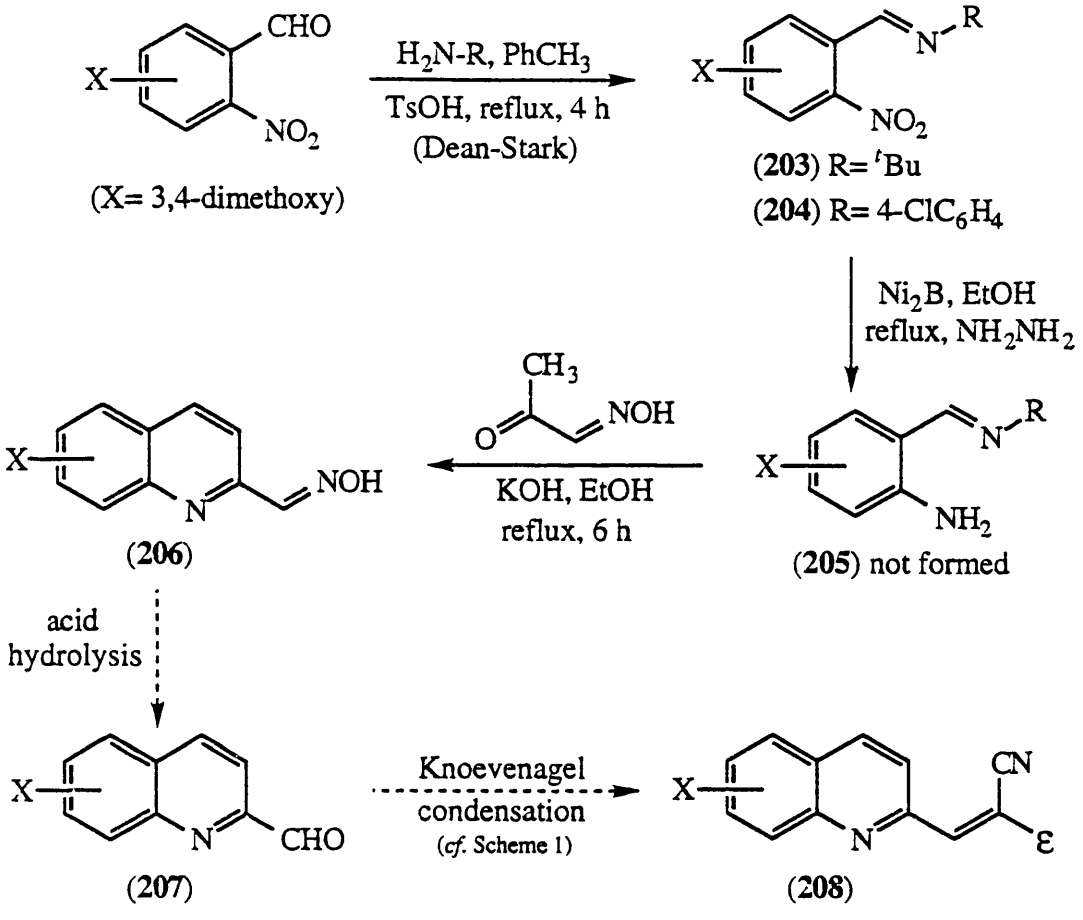
From the biological results obtained for the quinoline-based tyrphostins, the 2-substituted series gave the most potent compounds. Thus it seemed appropriate to develop a synthetic route to substituted 2-quinolinecarbaldehydes (**207**) in order to explore the effect substituents had on the biological activity of the tyrphostins (**208**) derived from these starting materials (Scheme 12). This work was initiated nearing the end of the PhD project and was not completed due to time restrictions but is included here due to its logical connection to the previous sections.

Figure 25 Mean GI_{50} Values from US National Cancer Institute Panel of Human Cancer Cell Lines for Compound (188)





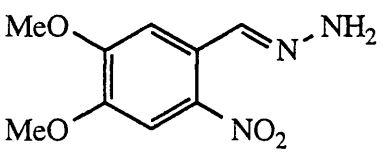
Scheme 12 Proposed Synthesis of Substituted 2-Quinolinecarbaldehydes



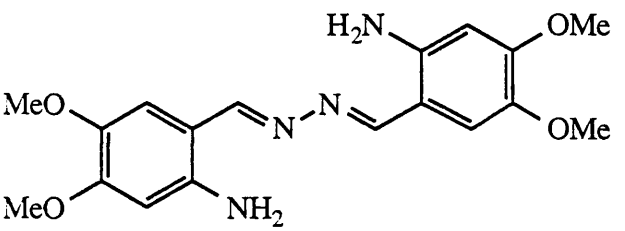
Scheme 12 outlines the Friedländer quinoline synthesis[292] and requires the initial imine protection of an *ortho*-nitrobenzaldehyde before reduction of the nitro group to form the *ortho*-aminobenzaldimine derivative (**205**). The reason for this is that unprotected *ortho*-aminobenzaldehydes are generally unstable and are proned to further reactions. Thus treatment of the intermediate (**205**) with the aldoxime derivative of glyoxal under basic conditions should generate the quinoline system (**206**). This step illustrates the Borsche modification[293] of the Friedländer quinoline synthesis and allows the incorporation of an oxime protected aldehyde functionality in the 2-position for further derivatisation.

As an appropriate starting material, 6-nitroveratraldehyde was chosen (X= 3,4-dimethoxy) and was converted under Dean-Stark conditions to the benzaldimines (**203**) and (**204**). The subsequent reduction of the nitro group to form the aniline (**205**) proved to be problematic. At the time, the method of choice was to use nickel boride in the presence of hydrazine since it has been reported to leave sensitive functionality such as amides, esters, nitriles, or

carbaldehydes, unreduced.[294] However, when the *N-tert*-butylimine (**203**) was added to a freshly prepared ethanolic suspension of nickel boride (from $Ni(OAc)_2$ and $NaBH_4$) followed by the addition of hydrazine, only the hydrazone derivative (**209**) could be isolated. This was also the case for the imine (**204**) and any alternative reaction condition which were employed.

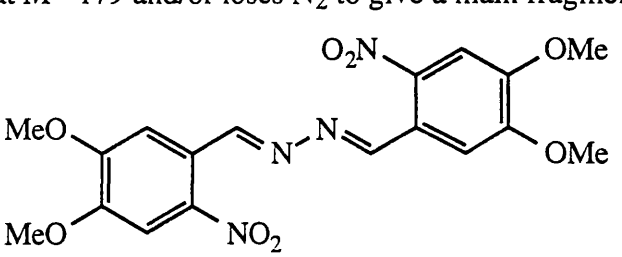


(**209**)



(**210**)

When the hydrazone (**209**) was treated with nickel boride in the presence of hydrazine only starting material could be isolated. Apparently this system is resistant to these reduction conditions. Interestingly, the only success in making a nitro-reduced product was achieved when *unprotected* 6-nitroveratraldehyde was treated with nickel boride in the presence of excess hydrazine. This reaction produced the hydrazone (**209**) in 17 % yield together with 3,4-dimethoxy-6-aminobenzaldazine (**210**) in 73 % yield, after silica chromatography.. Presumably, the nitrobenzaldazine (**211**) is formed as an intermediate and is more susceptibile to reduction than its hydrazone counterpart (**209**). The symmetrical azine (**210**) was consistent with NMR data and was identified *via* its mass spectra (M^+ 358)—cleaves into monomeric units at M^+ 179 and/or loses N_2 to give a main fragment at M^+ 165.



(**211**)

In retrospect the problems encountered are not entirely surprising and probably the use of sodium sulphide as a reductant, although unpleasant to work with, would have been advisable. Nevertheless, under the reaction conditions given in Scheme 12, a final attempt was made to form the quinoline system (**206**) (X= 3,4-dimethoxy) using the aminobenzaldazine (**210**). Unfortunately this met with failure and there was no more time to pursue this route any further.

CHAPTER «8» Further Tyrphostin Investigations

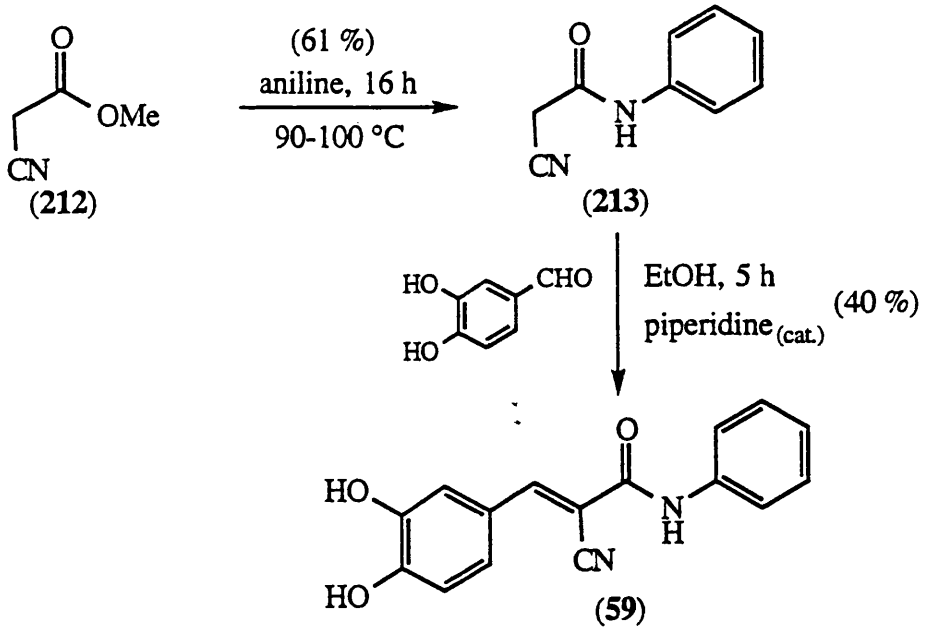
As mentioned in the Introduction (Section 5.7) tyrphostin derivatives devoid of phenolic functionality represent good candidates for lead development. Notable examples would include the 2,3-diarylpropenonitriles (**66**) and (**67**). Thus, for the purpose of reproducing the results of Levitzki *et al.*,[232] these and other therapeutically promising tyrphostin derivatives [(**59**) and (**61**)][230g] have been prepared. In addition, an aim of this project was to perform stereochemical studies and also to attempt the synthesis of geometric isomers of the tyrphostins in order to establish the stereochemical requirement preferred for biological activity. This and other related work will be described in this chapter.

8.1 Synthesis of the Tyrphostins (59) and (61)

As shown in Table 12, the tyrphostin (**59**) is about 50-60 times more potent towards inhibiting the EGF-R kinase over its highly homologous counterpart (*erb*B-2). The *reverse* situation is true for the tyrphostin (**61**). These two compounds, therefore, represent an interesting situation for further biological evaluation and, for this purpose, were requested by Dr J. Bartlett, Department of Surgery, Glasgow Royal Infirmary, Glasgow.

The preparation of the *N*-phenylamide tyrphostin derivative (**59**) initially involved the synthesis of *N*-phenylcyanoacetamide (**213**) (Scheme 13). Although amide coupling

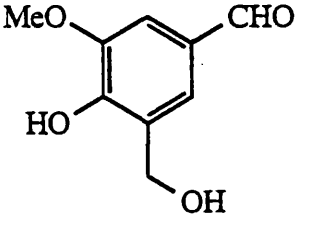
Scheme 13 Synthesis of *N*-Phenyl 2-Cyano-3-(3,4-dihydroxyphenyl)propenamide (59)



methodologies to cyanoacetic acid were envisaged, the procedure adopted involved heating neat methyl cyanoacetate (**212**) with aniline at *ca.* 100 °C in the absence of solvent.[230b] The evaporation of methanol drove the reaction forward and the product crystallised out on cooling making the procedure simple and effective. The subsequent Knoevenägel condensation between the amide (**213**) and 3,4-dihydroxybenzaldehyde afforded the desired target (**59**) in 40 % yield after crystallisation.

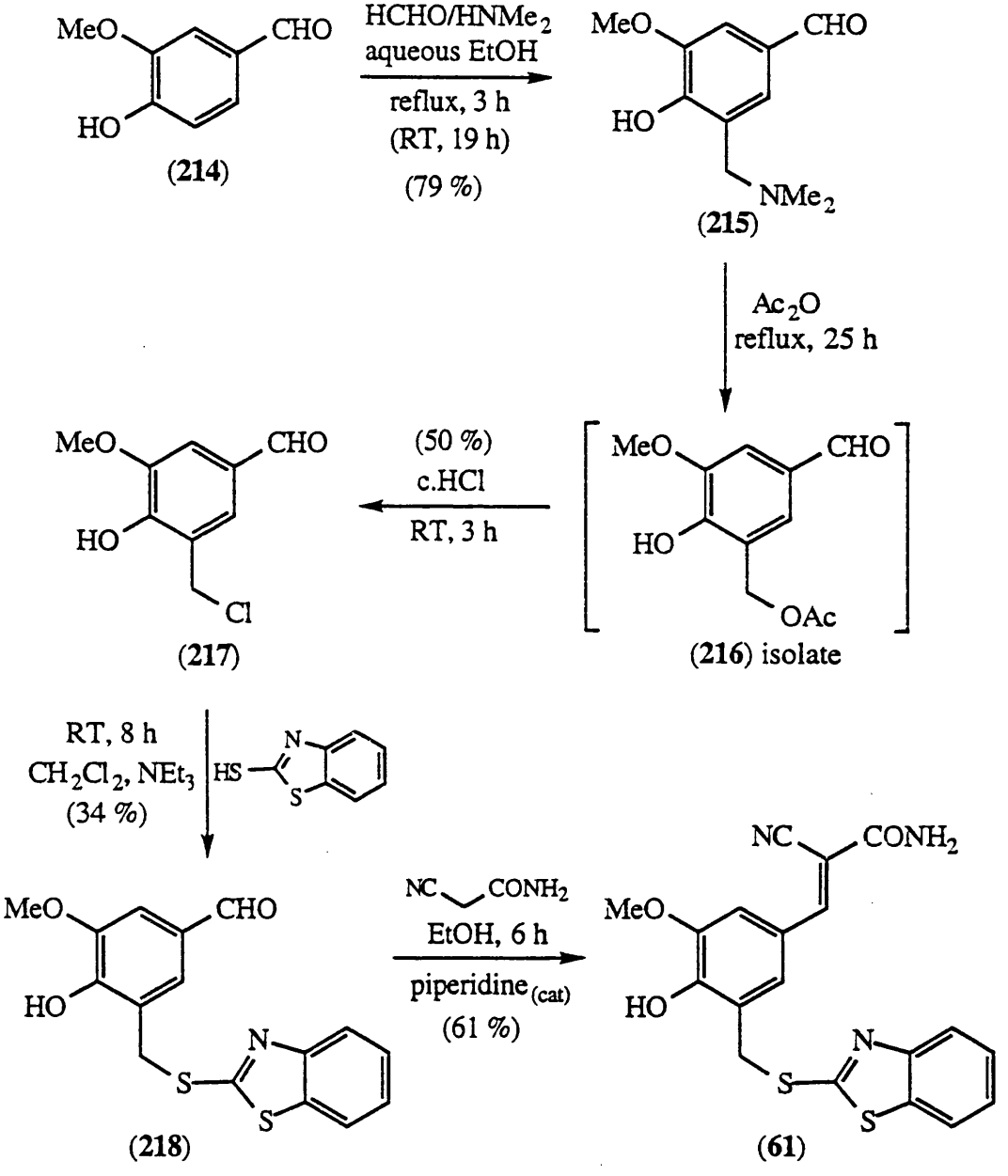
The synthesis of the tyrphostin (**61**) required a more complex route and is outlined in Scheme 14. Vanillin (**214**) was first converted into the 5-substituted compound (**215**) in a Mannich-type reaction whereby formaldehyde and dimethylamine form an iminium intermediate that acts as an electrophilic source of the (*N,N*-dimethylamino)methyl group.[295] The reaction works well on electron-rich systems and is probably facilitated by the mildly basic conditions generating a phenoxide intermediate.

Replacement of the dimethylamino group in compound (**215**) with a more appropriate leaving group was achieved by heating this compound in acetic anhydride at reflux. This gave the acetate intermediate (**216**) which was isolated directly from the reaction mixture by fractional distillation and then treated with concentrated hydrochloric acid to afford the chloromethyl derivative (**217**). The best yield obtained for these two steps was 50 %. It was important not to use aqueous base in the work-up procedure in isolating compound (**217**). When aqueous 0.1 M sodium hydroxide was used this generated, not surprisingly, the methyl alcohol (**219**), as identified by 90 MHz ^{1}H NMR spectroscopy. Also two other products were obtained after chromatography, although in impure form, with R_F values less than 0.2 (Silica/EtOH). Spectroscopy indicated that these contained many exchangeable protons and it is conceivable that at least one of these was the acid or carbinol product of a Cannizzaro reaction (*cf.* Section 6.5).[296]



(219)

Scheme 14 Synthesis of 2-Cyano-3-[4-hydroxy-3-methoxy-5-(2-benzothiazolylthiomethyl)phenyl]propenamide (61)



The next step in the synthesis of compound (**61**) required the displacement of the chloro group in compound (**217**) by 2-mercaptobenzothiazole to give the *S*-aryl thioether (**218**).[230g] This conversion was difficult to optimise but it was found best to slowly add the thiol and triethylamine together in dichloromethane. The highest yield obtained was 34 % after column chromatography. The synthesis of the target tyrphostin (**61**) was completed by perfoming a Knoevenägel condensation between the aldehyde (**218**) and cyanoacetamide in the usual manner.

8.2 Attempted & Proposed Synthesis of Tyrphostin Geometric Isomers

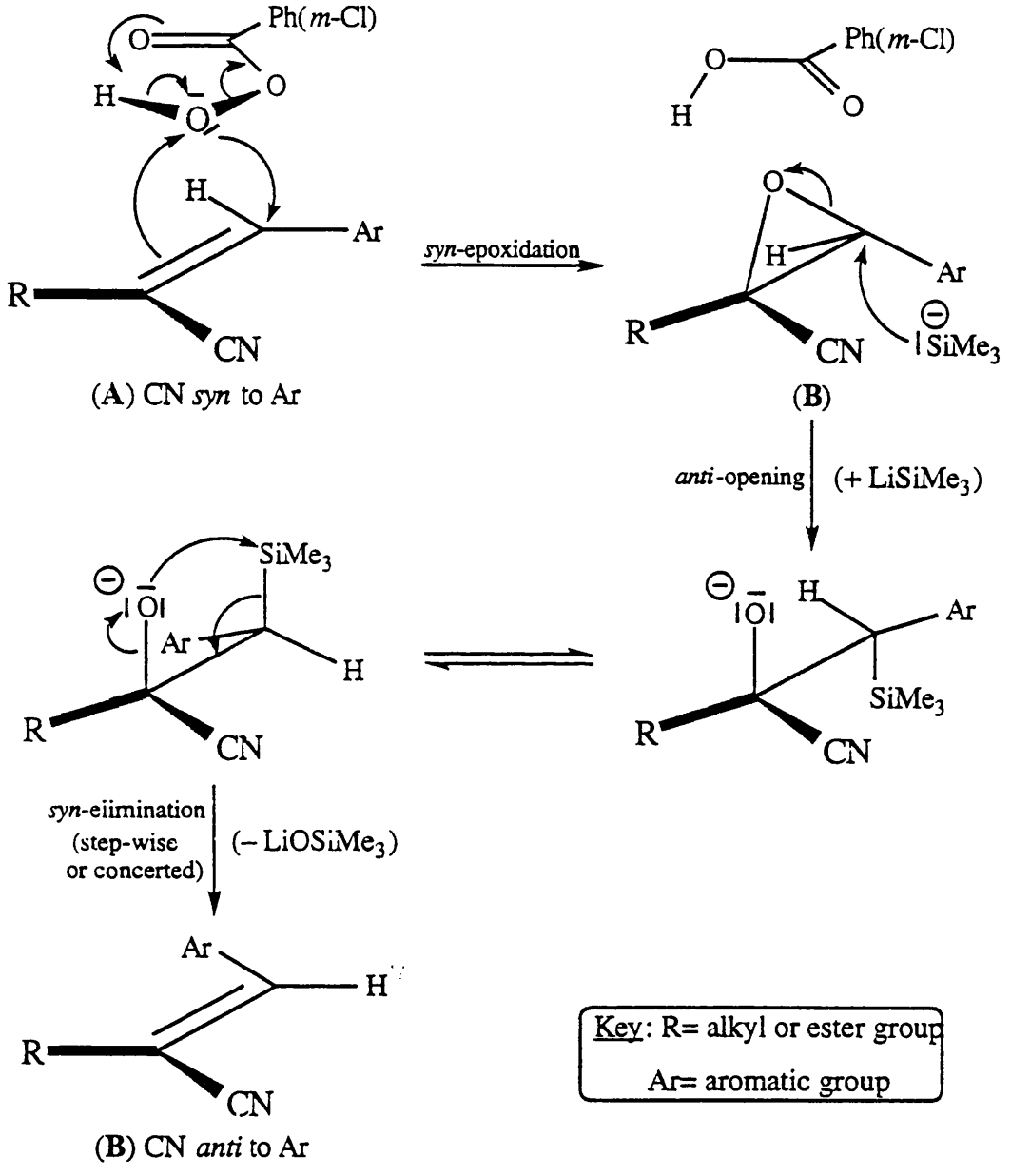
So far it has been assumed that the product which is formed in the Knoevenägel condensation is the one with the aromatic ring *syn* relative to the nitrile grouping (*cf.* Scheme 1). Indeed, mechanistic considerations[242] together with experimental data[297] agree with this assumption. It has also been presumed that the most active geometric form for a tyrphostin is that for this *syn*-arrangement. Although there is some evidence to substantiate this latter statement,[230f] it may be the case that small traces of the *anti*-isomer are contributing to the observed biological activity.

Thus, it was of interest to develop a general synthetic route to the *anti*-isomers of the tyrphostins. A strategy initially investigated has been generalised in Scheme 15 and involves the formation of an epoxide (**B**) from an alkene (**A**) in a stereoselective *syn*-manner by using *meta*-chloroperoxybenzoic acid (*m*cpba). Presuming only the *syn*-isomer originally existed this will lead to a racemate of the diastereoisomer (**B**).[298] In theory, *anti*-nucleophilic attack on the epoxide (**B**) with the anion of trimethylsilane, followed by *syn*-elimination of trimethylsilanolate,[299] will invert the relative stereochemistry about the epoxide (**B**) and therefore afford the desired isomer (**C**).[300]

However, when this strategy was conducted on the epoxide (**220**), prepared from compound (**116**) using *m*cpba in dichloromethane, a complex mixture of products was obtained (Scheme 16). As indicated trimethylsilyllithium was pre-formed by treatment of hexamethyldisilane with methyllithium and was then added to the epoxide in DMPU[301] dropwise at *ca.* 10 °C under an inert atmosphere. Although the reaction conditions could not be altered to improve the result, two products of the reaction after column chromatography were identified to be the α-keto ester (**221**) and the amide (**222**) (Scheme 16).

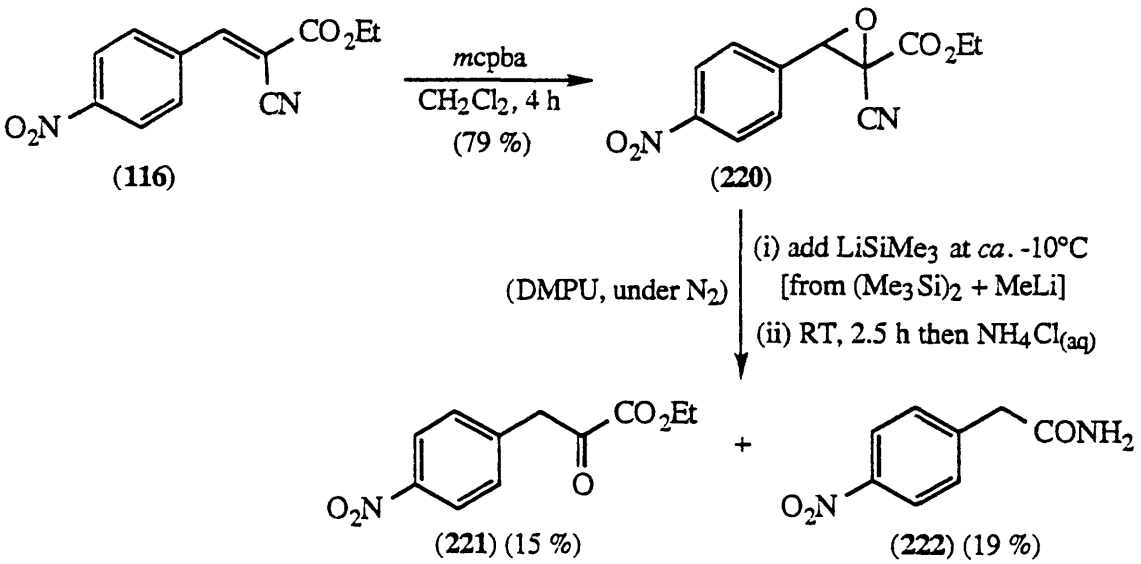
According to the ^{1}H NMR and infra-red spectra, the α-keto ester (**221**) exists in its enol form and displays rotameric behaviour. In retrospect, the production of this product is not entirely surprising and probably involves the mechanism as given in Scheme 17. However, the mechanism of formation of the amide (**222**) is not all that obvious. Nevertheless, an attempt has been made in Scheme 18. It is proposed that the source of the amide group is *via* hydrolysis of the nitrile group during the work-up stage of the reaction. The

Scheme 15 Proposed Method of Double-Bond Isomerism

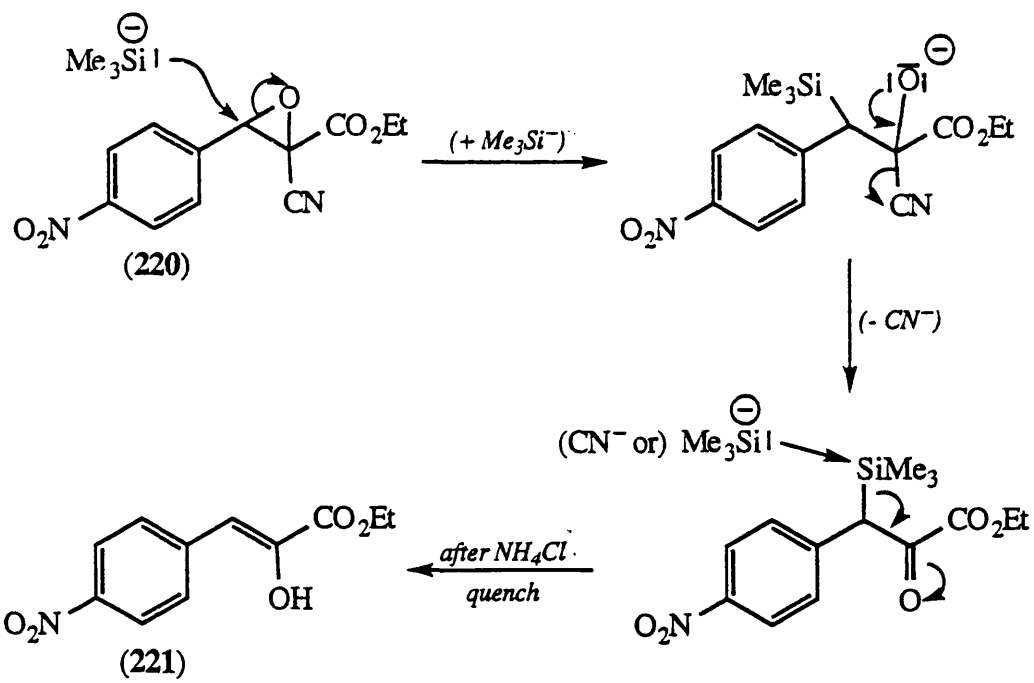


incorporation of the nitrile group at the benzylic position in compound (**224**) is possibly as a result of an *inter*molecular reaction on the epoxide (**220**) as it is conceivable that the cyanide nucleophile is readily generated under the reaction conditions employed (*cf.* Scheme 16). A plausible sequence of events (Scheme 18) would thus form the α-keto ester intermediate (**223**) which would then be able to fragment, under the influence of a nucleophile, to the stabilised acetonitrile anion (**224**). Hydrolytic work-up would therefore produce the amide (**222**).

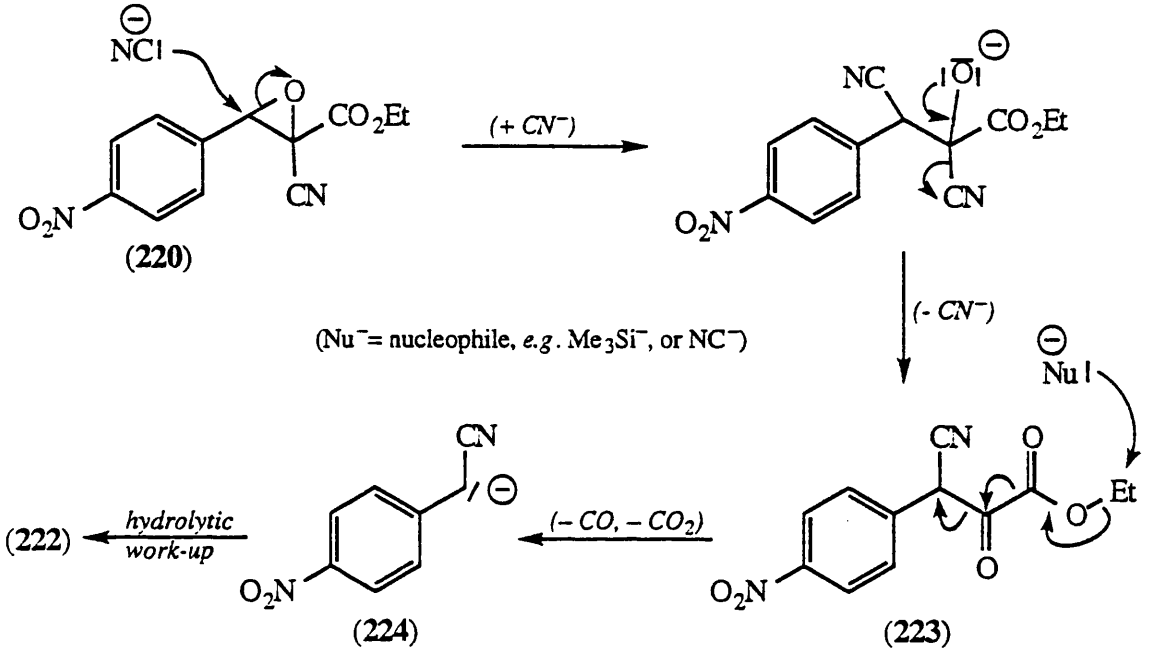
Scheme 16 Attempted Double-Bond Isomerism of Tyrphostin (116)



Scheme 17 Mechanism of Formation of the α-Keto ester (221)



Scheme 18 Proposed Mechanism of Formation of the Amide (222)



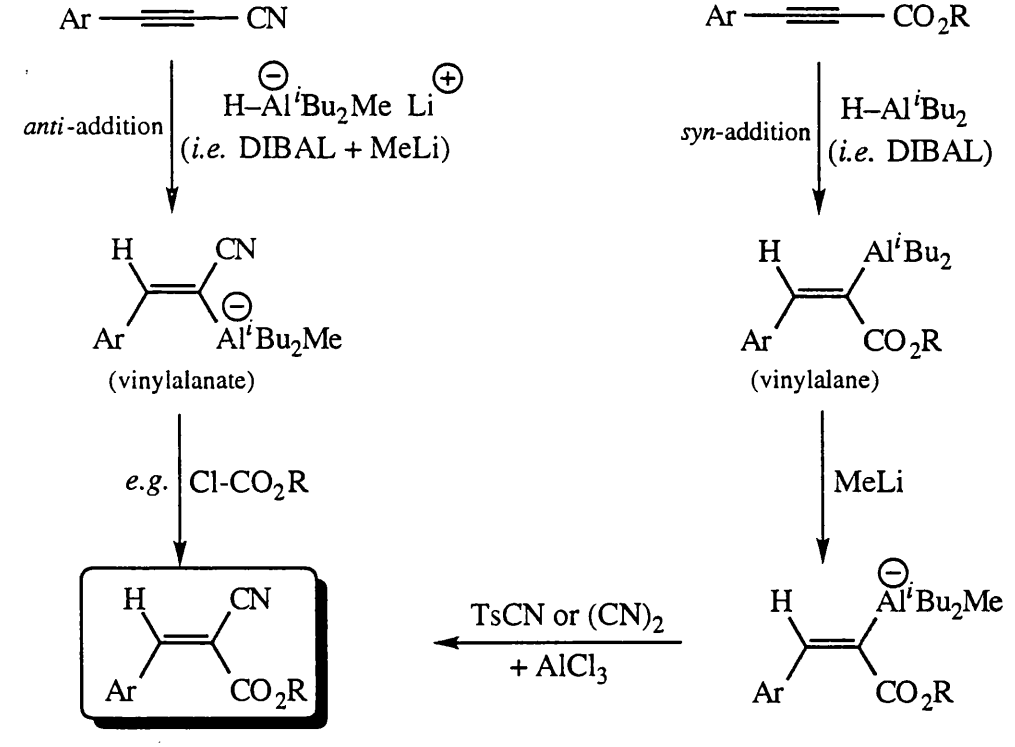
Although not carried out at the time, a test of this reaction mechanism would involve treatment of the epoxide (**220**) with a cyanide salt in order to establish if an acetonitrile product akin to the anion (**224**) could be generated. If so this could be an interesting method for the formation of arylacetonitriles.

However, due to the problems encountered, this method of inverting the stereochemistry about an olefin (Scheme 15) was abandoned and other alternative strategies were sought.[302] It was decided to avoid other protocols which utilised epoxides, such as those which involve intermediates found in a Horner-Wadsworth-Emmons[303] or Wittig-type olefination,[304] since these often involve strong anionic nucleophilic conditions and so are likely to be inappropriate for such functionalised compounds. The conclusion was that the desired geometric isomer of a tyrphostin probably had to be made in a stereocontrolled manner and two such approaches have been proposed in Schemes 19 and 20. Due to time limitations these routes were not initiated. Nonetheless, they should provide an interesting basis for future research and are included here due to their potential practical use.

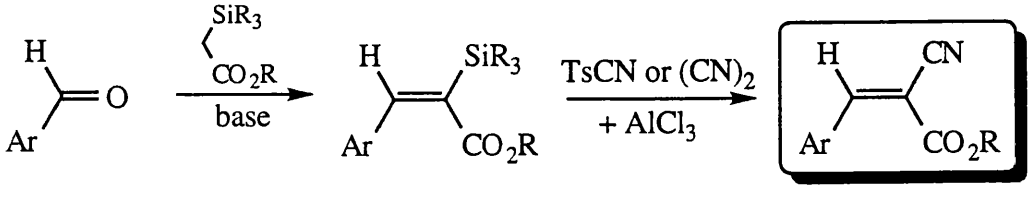
In summary, Scheme 19 illustrates a strategy which makes use of the stereoselective reductive addition of diisobutylaluminium hydride (DIBAL or $^{i}Bu_2Al$–H) or pre-formed alanate complex (DIBAL plus methyllithium) to alkynes followed by the *retentive* substitution of the metal centre by an electrophile.[305] From a literature survey, alternative

reagents and procedures could be envisaged to be used in a similar protocol and examples would include: use of zirconium complexes;[306] use of iron complexes;[307] and the technique of hydrocyanation.[308] However, DIBAL seemed more preferable due to its availability and ease of use. A different strategy involves the formation of *trans*-alkenylsilanes *via* an aldol-type condensation (Scheme 20). These isomers should predominantly form for large trisubstituted silyl groups and may be substituted with a nitrile group as shown. Under Friedel-Crafts conditions, such electrophilic desilylations[309] have been reported to occur with retention of configuration about the double-bond[310] and can be rationalised in a similar fashion to proto-desilyation.[311]

Scheme 19 Proposed Synthesis of Tyrphostin Geometric Isomers *via* Substituted Alkynes



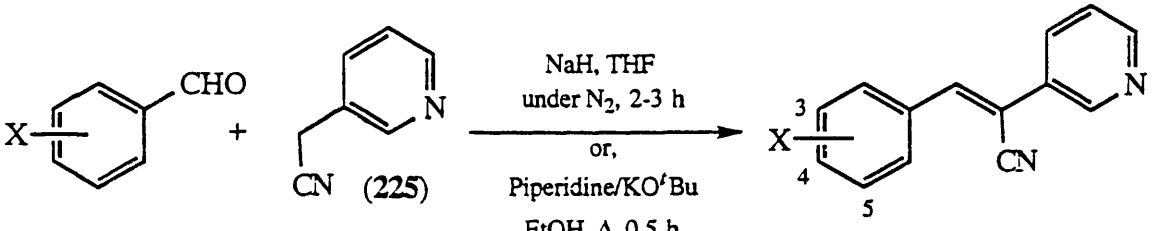
Scheme 20 Proposed Synthesis of Tyrphostin Geometric Isomers *via* trans-Alkenylsilanes



8.3 Synthesis and Biological Evaluation of 2,3-Diarylpropenonitriles

At the request of colleagues at the CRC Beatson Laboratories (Glasgow University) the so-called "second-generation" tyrphostins (**66**) and (**67**) were synthesised for the purpose of biological evaluation (Scheme 21). These 2,3-diarylpropenonitriles have previously been reported to be made by condensing the appropriate arylcarbaldehyde with 3-pyridylacetonitrile (**225**) using potassium carbonate as a base (yields not disclosed).[312] However, this method of preparation was found to be temperamental and often gave low yields of products. Thus, it was decided to obtain significant amounts of the compounds (**66**) and (**67**) by using sodium hydride as a base. In this case, the pre-formed sodium salt of 3-pyridylacetonitrile (**225**) was added to the appropriate arylcarbaldehyde and afforded the desired products (**66**) and (**67**) in respective yields of 54 % and 24 %, after crystallisation.

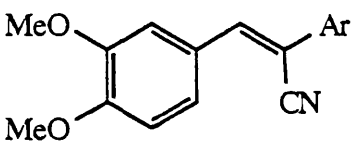
Scheme 21 Synthesis of 2,3-Diarylpropenonitriles



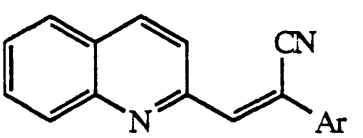
(**66**) X= 3,4-dimethoxy
(**67**) X= 3,5-dichloro
(**226**) X= H

Due to the precautions and lengthy procedure involved in the use of sodium hydride, together with the relatively low yields obtained, an alternative protocol was sought. Initially, piperidine was used as a base in a Knoevenägel condensation whereby the reactants under concentrated conditions were heated at reflux (Method C). However, this method failed and it was surmised that piperidine was not sufficiently basic to deprotonate 3-pyridylacetonitrile (**225**). Changing the base to potassium *tert*-butoxide, used in catalytic to stoichiometric amounts according to a similar procedure, consequently produced the desired products but the reaction was complicated by side-products and yields were generally low. Based on the idea that a secondary amine is important for activating arylcarbaldehydes, *via* an iminium intermediate, it was then decided to use piperidine and potassium *tert*-butoxide in combination. This led to a general procedure (Method D) that efficiently and quickly generated the desired compounds (**66**) and (**67**) in high yield—94 % and 80 %, respectively (see Scheme 21).

Having esablished that the method worked well on both the electron-rich and electron-deficient benzaldehyde precursors to compounds (**66**) and (**67**), the method was applied to forming the unsubstituted compound (**226**) (Scheme 21). Futhermore, the generality of this method was exemplified in the formation of the 2,3-diarylpropenonitriles (**227**)-(**232**). Thus, compounds (**227**)-(**229**) were made by reacting 3,4-dimethoxybenzaldehyde with either 2-pyridyl-, 2-thienyl-, or 3-thienylacetonitrile. For the preparation of the derivatives (**228**) and (**229**), it was found best to purify the thienylacetonitrile starting materials over activated charcoal prior to performing the condensation reaction. This was also found to be the case for the 3-thienyl derivative (**232**). Unfortunately, all attempts at completing the 2-quinolinyl series [(**230**)-(**232**)] by making a 2-thienyl analogue, failed.



(**227**) Ar= 2-pyridyl
(**228**) Ar= 2-thienyl
(**229**) Ar= 3-thienyl

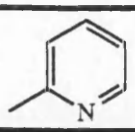
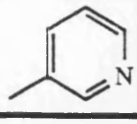
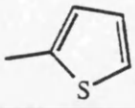
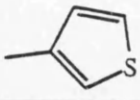
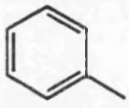
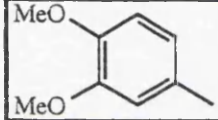
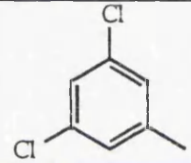
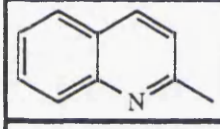


(**230**) Ar= 2-pyridyl
(**231**) Ar= 3-pyridyl
(**232**) Ar= 3-thienyl

An additional purpose to forming the compounds (**226**)-(**232**) was to explore alternative tyrphostin derivatives akin to (**66**) and (**67**), and thus evaluate their biological activities. The IC_{50} values obtained against the MCF-7 cell line in a growth inhibition assay using [^{3}H]thymidine as a measure of DNA synthesis have been collated in Table 23. As can be seen, the diaryl tryphostin derivatives are all reasonably potent antiproliferatives with many being comparable to the known tyrphostin (**66**). In particular the compounds (**227**) and (**229**)-(**231**) display significantly low IC_{50} values and are good examples for future investigation.

Although time limits prevented a complete study of these diaryl derivatives in the EGF-R kinase cell free assay, recent results (performed by Dr Valerie G. Brunton) indicated that the quinolines (**230**)-(**232**) exhibit IC_{50} values greater than 500 μM. These compounds are, therefore, not blocking phosphorylation events at the EGF receptor, implying that the observed growth inhibitory effects are due to some other mechanism. Nevertheless, this work has provided some interesting tyrphostin derivatives (2,3-diarylpropenonitriles) for future biological evalution and, in addition, a relatively facile method for their preparation.

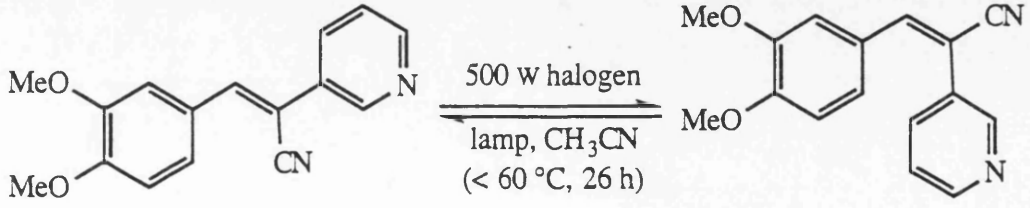
Table 23 MCF-7 Data for Some 2,3-Diarylpropenonitriles

IC_{50} (MCF-7) Values[a] for $Ar^1CH=CH(CN)Ar^2$				
Ar^1 \ Ar^2				
		(**226**) 36 μM		
	(**227**) 9 μM	(**66**) 11 μM	(**228**) 22 μM	(**229**) 13 μM
		(**67**) *(not determined)*		
	(**230**) 5 μM	(**231**) 8 μM		(**232**) 28 μM
[a]Growth Inhibition values are given below compound numbers				

8.4 Stereochemical, Crystallographic & Biological Studies

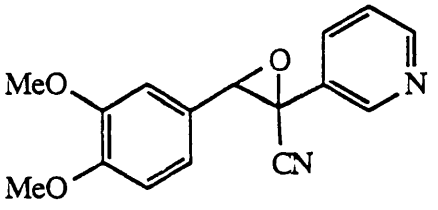
As mentioned in the Introduction (Section 5.7), the tyrphostin (**66**) has been shown to exhibit promising *in vivo* activity. In addition, it has been claimed to give a long lasting inhibitory effect in cell culture and prolongs the survival of tumour-bearing mice.[232] However, it has been briefly noted that tyrphostin (**66**) is able to undergo a light-induced *trans/cis* isomerism,[313] generating some speculation as to which isomer is actually attributable to the observed biological activity. For this reason, it was decided to investigate this isomerism process in more detail and therefore synthesise the individual geometric isomers. This was achieved by irradiating a dilute acetonitrile solution of the tyrphostin (**66**) with a 500 W halogen lamp for 26 h (Scheme 22).

Scheme 22 Light-induced *cis/trans* Interconversion of Tyrphostin (66)



(**66**) (**233**)

In this reaction, care must be taken not to allow the solvent to reach boiling point and it was found best to immerse a cold-finger condenser into a well stirred solution of compound (**66**). At equlibrium, an approximate 60:40 mixture of (**233**) to (**66**) was present (by 90 MHz ^{1}H NMR spectroscopy) and the desired isomer (**233**) was isolated in good yield (43 %) after column chromatography. Interestingly, this reaction also afforded the epoxide (**234**) in 8 % yield. Since the reaction was carried out in the presence of air, the most likely reason for this outcome was probably due to the generation and subsequent [2 + 2] cycloaddition of singlet oxygen to the double bond (Scheme 23).[314] In the presence of a proton source (*e.g.* from the acetonitrile solvent or, more likely, from any water present)[315] the oxygenated species (**235**) is likely to be protonated and then converted with the epoxide (**234**). This amounts to an overall reduction and would be concomitant with the oxidation of either acetonitrile to hydroxyacetonitrile, or water to hydrogen peroxide.[316]

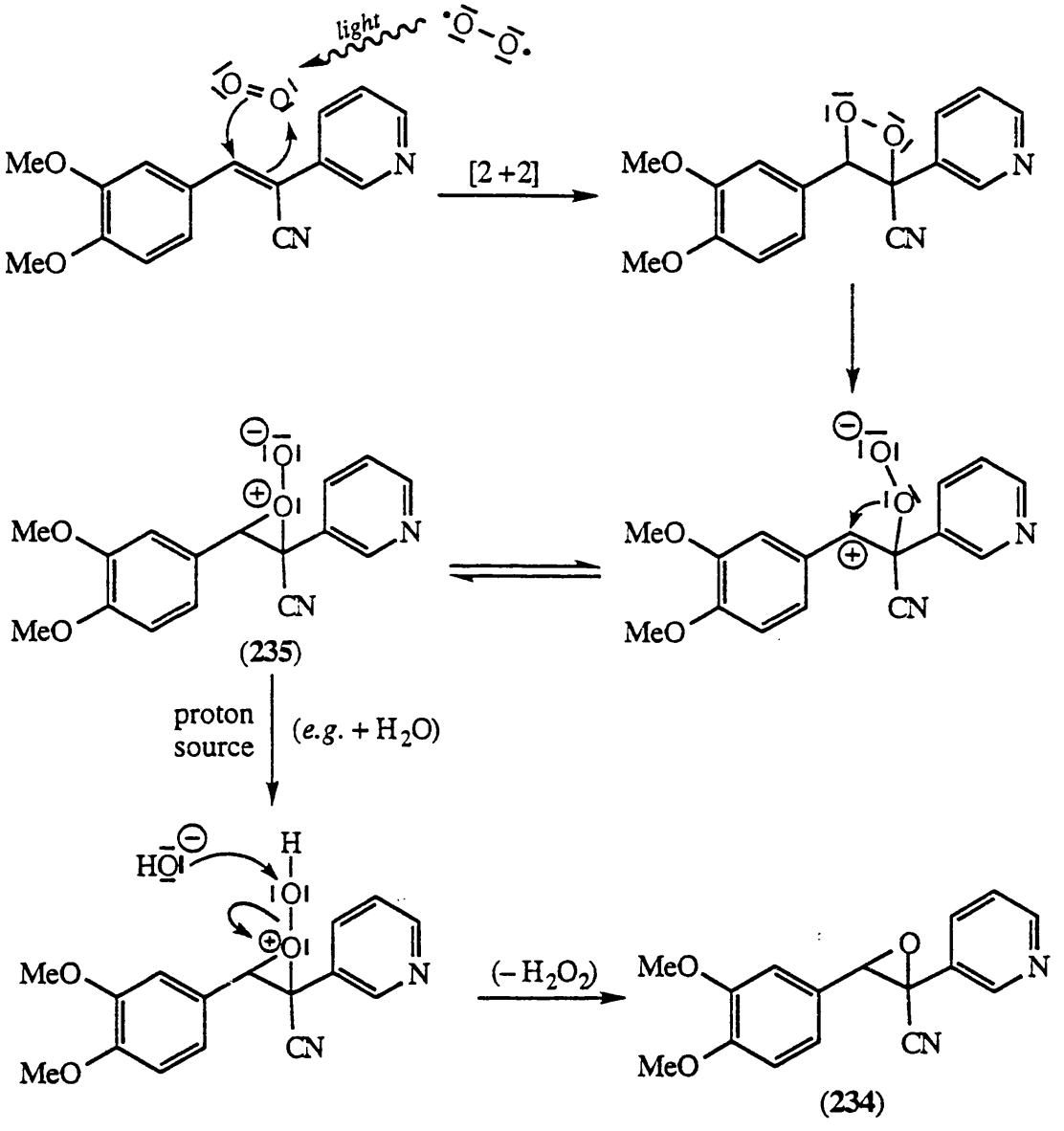


(234)

Having synthesised and isolated the compounds (**66**), (**233**), and (**234**) it was then necessary to determine their relative stereochemistry, unambiguously. Through the expertise of Dr David S. Rycroft (Glasgow University) this was achieved *via* detailed ^{1}H and ^{13}C NMR spectroscopic analysis of their ^{1}H/^{1}H and ^{1}H/^{13}C coupling constants, DEPT spectra, 2D δ_C/δ_H direct and long-range correlation experiments, as well as their NOE difference spectra. The ^{1}H and ^{13}C NMR data obtained (including NOE results) for $CDCl_3$ solutions of (*Z*)-(**66**), (*E*)-(**233**) and (*Z*)-(**234**) have been summarised in Tables 23 to 25.

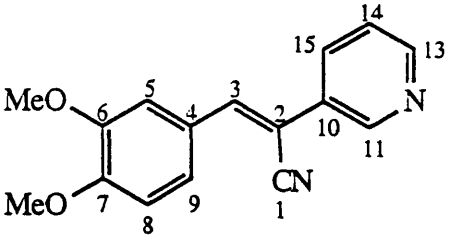
However, before discussing the results, it should be noted that it seems more sensible to ignore the Cahn-Ingold-Prelog sequencing rules for designating a (*Z*) or (*E*) assignment and focus only on the configurational relationship between the aromatic rings. Thus, the geometric isomers (*Z*)-(**66**) and (*E*)-(**233**) will hereinafter be called *trans* and *cis*, respectively, while the epoxide (*Z*)-(**234**) will be referred to as *trans*. This definition should eliminate any confusion that might occur later in the discussion when relating these structures to *trans*- and *cis*-stilbenes.

Scheme 23 Mechanism of Formation of the Epoxide (234)



As shown in Table 24, when 3-H was irradiated in compound (**66**) all four *ortho* protons of the aromatic rings (5-, 9-, 11-, and 15-H) displayed NOEs. The resulting spectrum of this NOE difference experiment is given in Figure 26 and indicates that compound (**66**) exists in a *trans*-configuration. Similar arguments identified the epoxide (**235**) to be *trans* (Table 26) and both cases were substantiated by the fact that NOEs between the two aromatic rings were not observed. This would be expected in a *cis*-arrangement and was seen in compound (**233**) between the aromatic protons (5-H, 9-H) and (11-H, 15-H). Importantly, the *cis*-assignment for compound (**233**) was reinforced by the fact that irradiation of 3-H caused NOEs at 5- and 9-H but not at 11- and 15-H (Table 25).

Table 24 NMR Data of *trans*-Isomer (66)—$CDCl_3$ solution



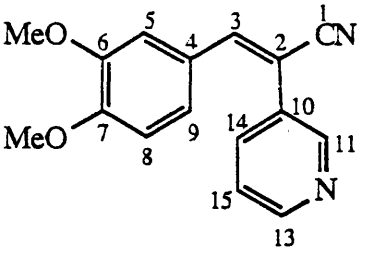
Position number	δ_C [a]	Protons showing long range ^{13}C correlations	Carbon multiplicity	$^1H/^{13}C$ couplings (Hz)				δ_H [a]	Proton multiplicity	Proton couplings (Hz)		
1	117.91	3	d	14.4								
2	105.03	15	dd	5	3							
3	143.56	5, 9	dt	154.6	4.9			7.49	s			
4	126.17	8	d	8.6								
5	110.63	3, 9	dt	157.8	4.9			7.72	d	2.2		
6	149.05	6-OMe, 5, 8	d quin	7.4	3.9							
7	151.63	7-OMe, 5, 8, 9	m	(obscured)								
8	110.91		dd	158.3	1.9			6.93	d	8.4		
9	124.92	3, 5	dt	160.1	6.3			7.38	ddd	8.4	2.2	0.5
10	130.82	3, 11, 14	qd	6.5	1.6							
11	146.82	13	ddd	178.8	11.5	5.5		8.90	dd	2.5	0.8	
13	149.59	11	dddd	180.3	11.0	6.9	3.0	8.60	dd	4.8	1.6	
14	123.55	13	ddd	164.4	8.6	0.9		7.37	ddd	8.1	4.8	0.8
15	133.07		dtd	161.6	6.0	1.0		7.93	ddd	8.1	2.5	1.6
6-OMe	55.96		q	144.8				3.97	s			
7-OMe	55.99		q	145.0				3.95	s			

Proton irradiated	% of Saturation	Observed NOEs Scaled for 100 % Saturation									
		3-H	5-H	8-H	9-H	11-H	13-H	14-H	15-H	6-OMe	7-OMe
3-H	69		7 %	-1 %	7 %	13 %			8 %		
5-H	64	5 %								4 %	
8-H	47				13 %						5 %
9-H	33	*		14 %							
11-H	71	6 %									
13-H	51				5 %						
14-H	29						7 %		15 %		
15-H	39	5 %						14 %			

[a]Relative to TMS at 0.00 ($CDCl_3$ appears at δ_C 77.15).

* The experimental integral was -2 %, but this result is dominated by the proximity of 3-H to the position of irradiation.

Table 25 NMR Data of *cis*-Isomer (233)—$CDCl_3$ solution

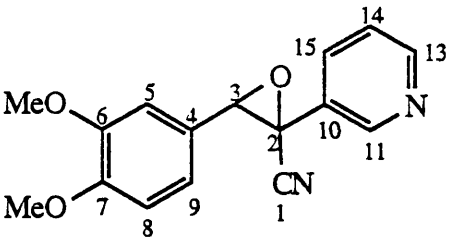


Position number	δ_C [a]	Protons showing long range ^{13}C correlations	Carbon multiplicity	$^1H/^{13}C$ couplings (Hz)				δ_H [a]	Proton multiplicity	Proton couplings (Hz)		
1	119.83	3	d	8.9								
2	107.86	3	dt	4	2							
3	146.06	5, 9	dt	157.6	4.5			7.40	s			
4	125.55	8	d	7								
5	111.66	3, 9	dt	157.6	6.2			6.55	d	1.9		
6	148.68	6-OMe, 5, 8	d quin	6.7	4.0							
7	151.07	7-OMe, 5, 8, 9	m	(obscured)								
8	110.95		d	160.1				6.77	d	8.4		
9	124.61	3, 5	dddd	162.3	6.6	5.8	0.9	6.84	ddd	8.4	1.9	0.3
10	129.59	3, 11, 14	dtd	9.3	7.1	1.7						
11	149.98	13	ddd	180	11	6		8.67	dd	2.3	0.9	
13	150.02	11	dddd	180	11	7	3	8.62	dd	4.9	1.7	
14	123.77	13	dm	165				7.37	ddd	7.9	4.9	0.9
15	136.52	11	dm	165				7.77	ddd	7.9	2.3	1.7
6-OMe	55.45		q	144.7				3.54	s			
7-OMe	55.93		q	145.0				3.87	s			

Proton irradiated	% of Saturation	Observed NOEs Scaled for 100 % Saturation									
		3-H	5-H	8-H	9-H	11-H	13-H	14-H	15-H	6-OMe	7-OMe
3-H	67		5 %	2 %	6 %						
5-H	56	6 %				2 %			1 %	4 %	
8-H	28										5 %
9-H	21	11 %				1 %			0.5 %		
11-H	34		1 %								
13-H	49							3 %			
14-H	23						6 %		11 %		
15-H	25		1 %					9 %			
6-OMe	67		22 %			0.3 %	1 %		0.3 %		
7-OMe	67			15 %	4 %						

[a]Relative to TMS at 0.00 ($CDCl_3$) appears at δ_C 77.18).

Table 26 NMR Data of *trans*-Epoxide (234)—$CDCl_3$ solution



Position number	δ_C [a]	DEPT ^{13}C multiplicity	Protons showing long range ^{13}C correlations[b]	δ_H [c]	Proton multiplicity	Proton couplings (Hz)		
1	120.82	s	3					
2	46.72	s	3					
3	54.81	d		5.21	s			
4	123.87	s	3, 8					
5	112.23	d	3	6.64	d	2.2		
6	148.91	s	6-OMe, 8					
7	149.77	s	7-OMe, 5, 9					
8	111.05	d		6.82	d	8.4		
9	122.64	d	3	6.93	dd	8.4	2.2	
10	130.83	s	3					
11	148.87	d		8.65	dd	2.6	0.7	
13	149.90	d		8.59	dd	4.8	1.5	
14	123.39	d		7.26	ddd	8.1	4.8	0.7
15	135.46	d		7.59	ddd	8.1	2.6	1.5
6-OMe	55.78	q		3.66	s			
7-OMe	55.78	q		3.87	s			

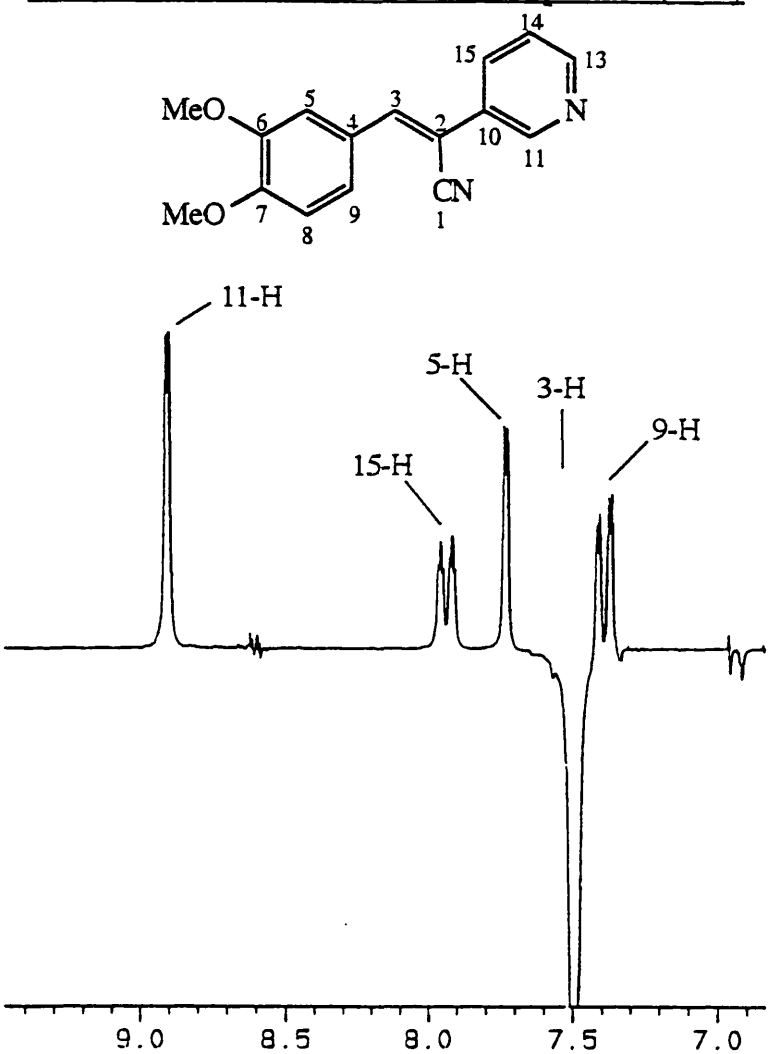
Proton irradiated	% of Saturation	Observed NOEs Scaled for 100 % Saturation									
		3-H	5-H	8-H	9-H	11-H	13-H	14-H	15-H	6-OMe	7-OMe
3-H	66		15 %		16 %	15 %		-1 %	15 %		
5-H	53	10 %								5 %	
8-H	41										6 %
9-H	33	13 %									
11-H	55	9 %									
13-H	43							13 %			
14-H	35						10 %		13 %		
15-H	36	7 %						11%			
6-OMe	67	-1 %	20 %			0.3 %	1 %		0.3 %		
7-OMe	67			20 %							

[a]Relative to $CDCl_3$ at δ_C 77.00.

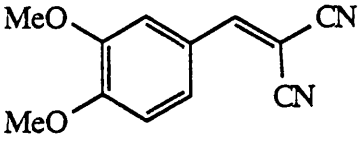
[b]Long-range (and direct) correlations were observed for (**234**) present as a 15 % impurity in a sample of (**66**).

[c]Relative to TMS at 0.00.

Figure 26 NOE Difference Spectrum of Tyrphostin (66) with Irradiation of double bond proton (3-H)



Additional evidence for the geometric assignments of the compounds (**66**) and (**233**) was derived from their proton-coupled ^{13}C NMR spectra (see Tables 24 and 25). In particular, the vicinal couplings ^{3}J(3-H...1-CN) of 14.4 Hz in structure (**66**) and 8.9 Hz in structure (**233**) were in respective agreement of a *trans-* and *cis-* mutual arrangement between the cyano group (1-CN) and olefinic proton (3-H) about the double bond. To act as a reference system, the dinitrile product (**236**) was synthesised from 3,4-dimethoxybenzaldehyde and malononitrile. In this case the corresponding *trans-* and *cis-* couplings were 14.1 Hz (δ_{CN} 113.6 ppm) and 8.4 Hz (δ_{CN} 114.5 ppm), respectively. In fact, $^{3}J(^{1}H...^{13}C)$ values are widely used to assign the stereochemistry of Knoevenägel condensation products[242] and, as expected, the magnitude of *trans*-couplings are generally greater than *cis*-couplings due to better orbital overlap.[317]



(136)

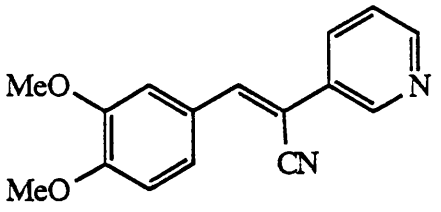
In collaboration with Dr Kenneth W. Muir and A. Ashgar Torabi (Glasgow University) the stereochemistry of the compounds (**66**) and (**233**) was confirmed by single crystal X-ray analysis.[318] The structures obtained are displayed in Figures 27 and 28 together with their crystallographic data—50 % probability ellipsoids are used to represent atomic positions, except for hydrogen atoms which are depicted by spheres of arbitrary radius. A selection of bond lengths/angles may be found in Table 27 (atom labels refer to Figures 27 and 28).[319]

Interestingly, the *cis*-isomer (**233**) exists in the solid state in two near identical forms (A and B; Table 27)[320] and, for both shown in Figure 28, the most striking feature is that the pyridyl ring is twisted out-of-plane to the rest of the molecule in a near perpendicular manner. In contrast, the *trans*-isomer (**66**) adopts a near flat conformation (Figure 27). However, a common feature for both isomers is that the (3,4-dimethoxyphenyl)-propenonitrile carbon skeleton [$(MeO)_2C_6H_3C{=}CCN$] remains close to planarity.

In order to assess how typical these *cis* and *trans* structures were, Dr Muir surveyed the Cambridge Structural Database for 1,2-diarylethenes [Ar–C=C–Ar] containing a central C=C double-bond in which both carbons carry a single six-membered aromatic ring (Ar).[321] In summary, this search identified 105 *trans* and 22 *cis* structures. Since it was of interest to determine the orientation of the aromatic rings (Ar) with respect to the double bond, each of these structures was defined by two torsion angles φ_1 and φ_2 (of the type $C{=}C\text{-}C_{Ar}\text{-}C_{Ar}$) and thus represented in a plot of φ_1 *versus* φ_2. Analysis of *trans*-Ar-C=C-Ar molecules (Figure 29) indicated that many are either centro-symmetric (with $\varphi_1 = -\varphi_2$) or close to C_2 point symmetry (with $\varphi_1 \approx \varphi_2$). For *cis*-Ar–C=C–Ar molecules (Figure 30) the data suggested that acceptable inter-ring distances occur when at least one aromatic ring (Ar) is twisted out of conjugation with the C=C double bond ($\varphi_2 > 45°$)—the other ring twists by variable amounts ($\varphi_2 = 15\text{-}90°$).[322]

Using these plots it may be concluded that the *trans*-isomer was not unusual (Figure 29). In comparison, the planarity (φ_1 close to zero) of the [$(MeO)_2C_6H_3C{=}CCN$] unit for the *cis*-molecules was somewhat unusual (Figure 30) and may reflect the resonance stabilisation

Figure 27 X-ray Crystal Structure and Data of *trans*-Isomer (66)



trans-$C_{16}H_{14}N_2O_2$, M_r= 266.30
orthorhombic, space group *Pbca*

crystallised from diisopropyl ether, 0 °C
crystal specimen: transparent needle
(0.80 × 0.25 × 0.15 mm)

a= 8.0279(8), b= 26.098(2), c= 13.3235(1) Å
β= 90°, V= 2791.4(5) Å^3, Z= 8
D_c= 1.267 g cm^{-3}, μ(Mo-*K*α)= 0.79 mm^{-1}

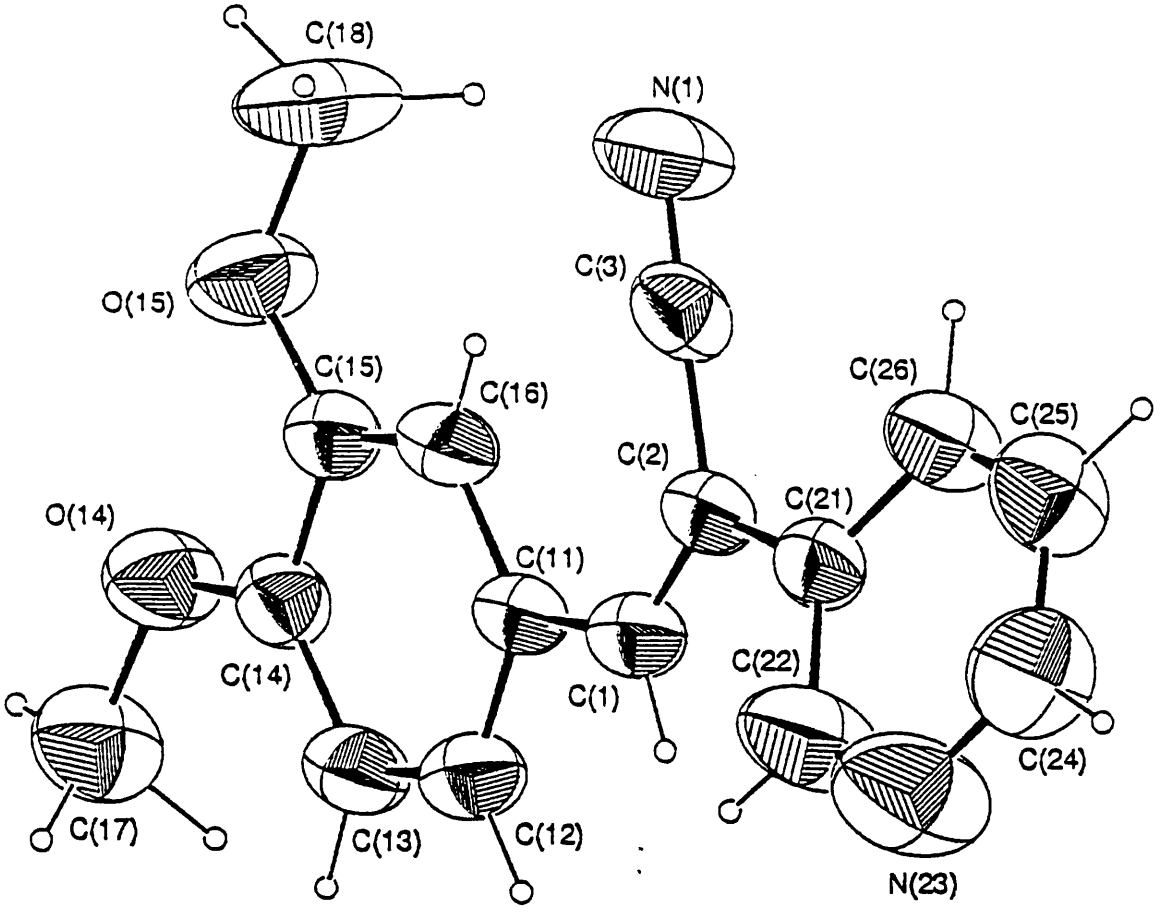
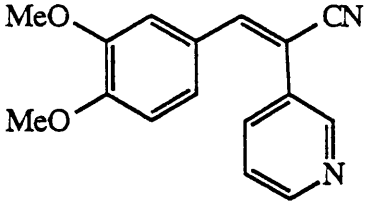


Figure 28 X-ray Crystal Structure and Data of *cis*-Isomer (233)

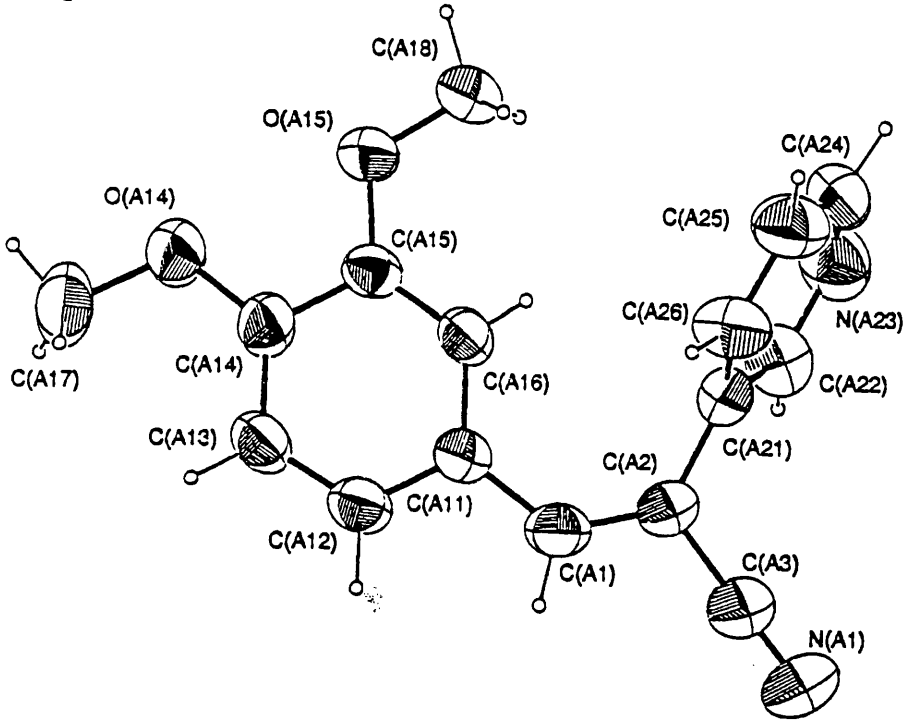


cis-$C_{16}H_{14}N_2O_2$, M_r= 266.30
monoclinic, space group $P2_1/c$

crystallised from diethyl ether, -10 °C
crystal specimen: transparent plate (0.48 × 0.42 × 0.30 mm)

a= 9.7755(5), b= 25.6816(9), c= 11.4330(6) Å
β= 93.625(4)°, V= 2864.5(2) Å^3, Z= 8
D_c= 1.235 g cm^{-3}, μ(Mo-*K*α)= 0.77 mm^{-1}

Molecule A (top view)



Molecule B (side view)

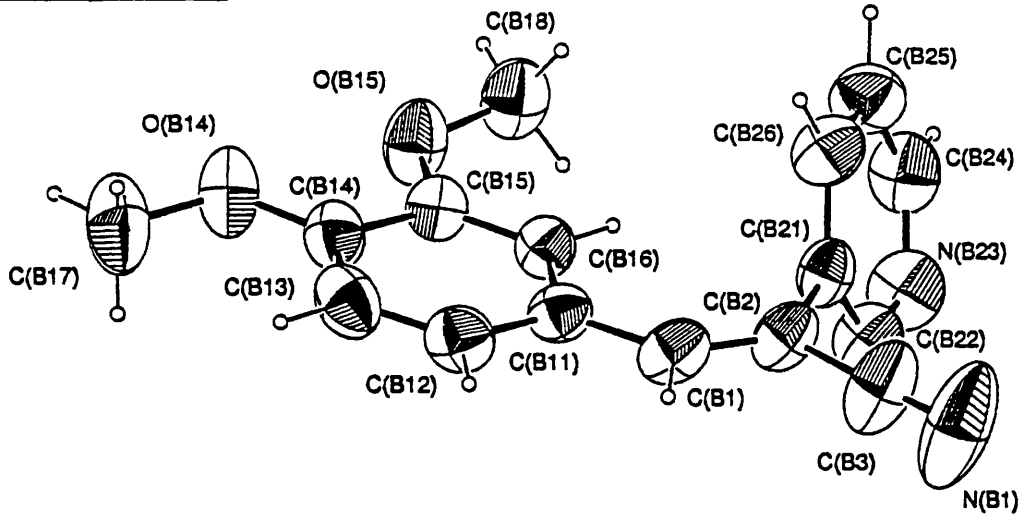


Table 27 Selected X-Ray Data of *trans*- and *cis*-isomers, (66) and (233)

Bond Angles[a]	*trans*	*cis*-A[c]	*cis*-B[c]	Bond Angles[b]	*trans*	*cis*-A[c]	*cis*-B[c]
				C(22)—N(23)—C(24)	117.8(5)	116.6(3)	116.6(2)
				C(14)—O(14)—C(17)	117.9(4)	117.1(2)	117.7(3)
				C(15)—O(15)—C(18)	117.0(4)	117.4(2)	117.0(2)
N(1)—C(3)	1.139(5)	1.141(3)	1.138(3)	C(2)—C(1)—C(11)	133.3(4)	132.0(2)	131.7(2)
N(23)—C(22)	1.329(6)	1.339(3)	1.332(3)	C(1)—C(2)—C(3)	120.4(4)	116.4(2)	117.6(2)
N(23)—C(24)	1.324(6)	1.332(3)	1.323(3)	C(1)—C(2)—C(21)	124.6(4)	128.7(2)	128.8(2)
O(14)—C(14)	1.351(4)	1.357(3)	1.366(3)	C(3)—C(2)—C(21)	115.0(3)	114.8(2)	113.5(2)
O(14)—C(17)	1.424(6)	1.437(3)	1.440(3)	N(1)—C(3)—C(2)	177.0(4)	178.4(3)	178.2(3)
O(15)—C(15)	1.363(4)	1.369(2)	1.364(3)	C(1)—C(11)—C(12)	118.1(4)	117.5(2)	118.2(2)
O(15)—C(18)	1.434(6)	1.425(3)	1.419(3)	C(1)—C(11)—C(16)	125.7(4)	124.9(2)	124.3(2)
C(1)—C(2)	1.338(5)	1.341(3)	1.338(3)	C(12)—C(11)—C(16)	116.2(4)	117.7(2)	117.5(2)
C(1)—C(11)	1.454(5)	1.454(3)	1.449(3)	C(11)—C(12)—C(13)	121.8(4)	122.0(2)	121.8(2)
C(2)—C(3)	1.461(6)	1.433(3)	1.432(3)	C(12)—C(13)—C(14)	120.9(4)	119.8(2)	120.1(2)
C(2)—C(21)	1.457(5)	1.497(3)	1.495(3)	O(14)—C(14)—C(13)	126.1(4)	125.4(2)	125.3(2)
C(11)—C(12)	1.392(5)	1.385(3)	1.390(3)	O(14)—C(14)—C(15)	115.4(4)	115.2(2)	115.3(2)
C(11)—C(16)	1.398(5)	1.406(3)	1.407(3)	C(13)—C(14)—C(15)	118.6(4)	119.4(2)	119.4(2)
C(12)—C(13)	1.368(6)	1.377(3)	1.378(3)	O(15)—C(15)—C(14)	114.5(4)	114.8(2)	115.2(2)
C(13)—C(14)	1.374(5)	1.381(3)	1.375(3)	O(15)—C(15)—C(16)	125.7(4)	124.8(2)	124.7(2)
C(14)—C(15)	1.403(5)	1.402(3)	1.404(3)	C(14)—C(15)—C(16)	119.8(4)	120.3(2)	120.1(2)
C(15)—C(16)	1.357(5)	1.368(3)	1.368(3)	C(11)—C(16)—C(15)	122.7(4)	120.8(2)	121.1(2)
C(21)—C(22)	1.382(5)	1.371(3)	1.387(3)	C(2)—C(21)—C(22)	122.8(4)	120.9(2)	119.5(2)
C(21)—C(26)	1.383(5)	1.373(3)	1.375(3)	C(2)—C(21)—C(26)	122.4(4)	121.5(2)	122.7(2)
C(24)—C(25)	1.365(7)	1.349(4)	1.372(3)	C(22)—C(21)—C(26)	114.8(4)	117.5(2)	117.7(2)
C(25)—C(26)	1.359(6)	1.377(3)	1.383(3)	N(23)—C(22)—C(21)	125.1(5)	123.9(3)	124.1(2)
				N(23)—C(24)—C(25)	122.0(5)	123.7(3)	124.2(2)
				C(24)—C(25)—C(26)	119.2(5)	118.9(3)	118.4(3)
				C(21)—C(26)—C(25)	121.1(5)	119.3(3)	119.0(2)

Torsion Angles[b]	*trans*	*cis*-A[c]	*cis*-B[c]
C(17)—O(14)—C(14)—C(13)	4.4(5)	1.4(3)	-4.3(3)
C(18)—O(15)—C(15)—C(16)	10.3(5)	-8.4(2)	-8.0(2)
C(11)—C(1)—C(2)—C(3)	-2.8(4)	-179.7(4)	179.6(4)
C(2)—C(1)—C(11)—C(12)	178.6(7)	-178.6(4)	-177.7(4)
C(2)—C(1)—C(11)—C(16)	-2.7(5)	2.0(3)	0.5(2)
C(11)—C(1)—C(2)—C(21)	178.1(7)	1.6(2)	-0.3(2)
C(1)—C(2)—C(21)—C(22)	-23.5(5)	-107.4(3)	-106.7(3)
C(1)—C(2)—C(21)—C(26)	157.5(6)	75.3(3)	75.0(3)
C(3)—C(2)—C(21)—C(22)	157.4(6)	73.9(3)	73.3(3)
C(3)—C(2)—C(21)—C(26)	-21.6(4)	-103.4(3)	-105.0(3)
O(14)—C(14)—C(15)—O(15)	0.4(3)	-0.5(2)	-0.4(2)

[a]In Angstroms (Å) [b]In degrees (°)
[a]Crystals of the *cis*-isomer contain two crystallographically independent molecules, A and B.

Figure 29 Plot of *trans*-1,2-Diarylethenes [Ar–C=C–Ar] Defined by Torsion Angles φ_1 and φ_2 (of the type C=C–C_{Ar}–C_{Ar})

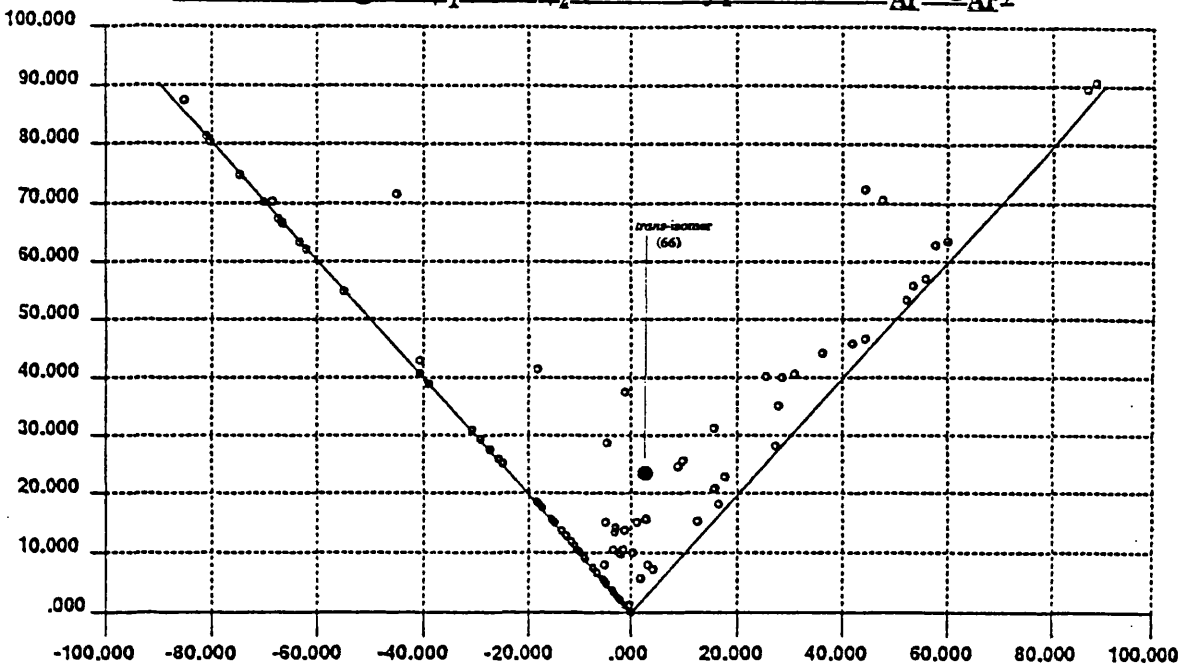
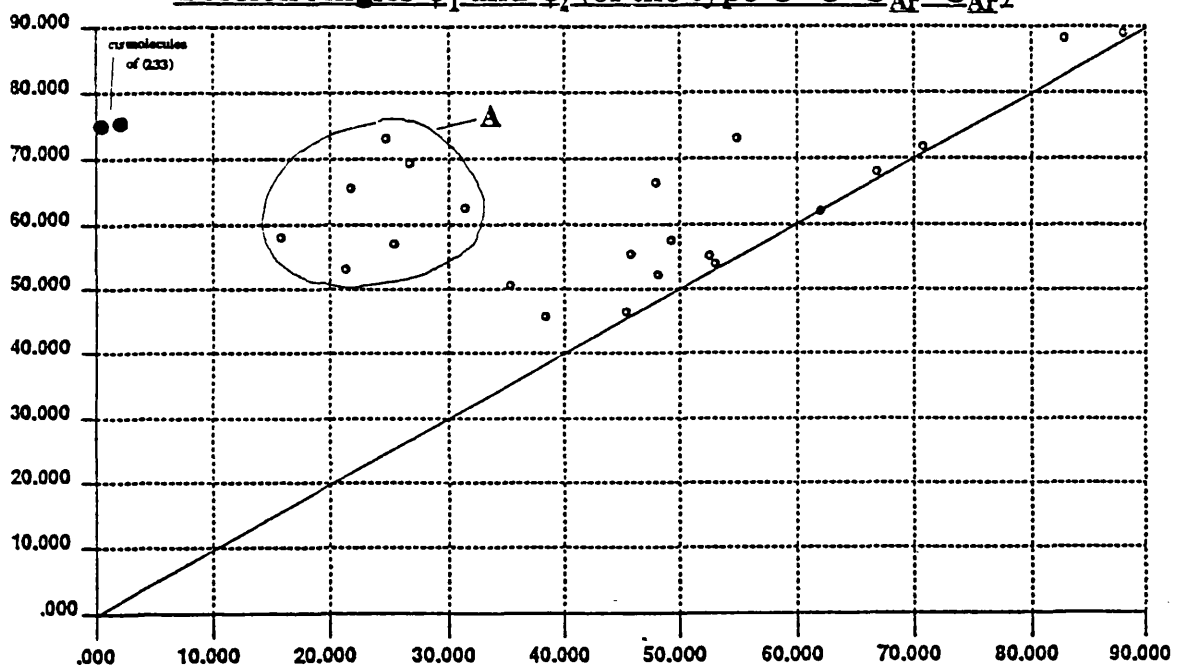
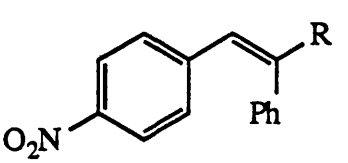


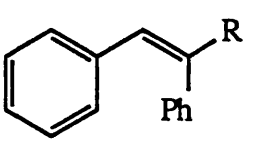
Figure 30 Plot of *cis*-1,2-Diarylethenes [Ar–C=C–Ar] Defined by Torsion Angles φ_1 and φ_2 (of the type C=C–C_{Ar}–C_{Ar})



Cluster A includes:
(other 15 data points are tetra-substituted 1,2-diarylethenes)



(R= H, CO_2Me, CN)



(R= N(O)=N^{n}Bu, CF_3)

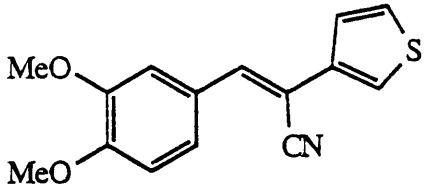
gained through an electron-donating group [$(MeO)_2C_6H_3$] being in conjugation with an electron-withdrawing group [CN]. However, only a small number of *cis*-compounds were found, with only a few being substituted similarly to compound (**233**)—see cluster marked A in Figure 29. Thus care must be taken in designating this *cis*-structure as unusual.

On the whole, the crystallographic data suggest (not unexpectedly) that the pyridyl ring is more prone to twist out of conjugation with the central double bond than the dimethoxyphenyl ring and this is more likely to occur for the *cis*-isomer (**233**) than the *trans*-isomer (**66**). However, the NOE results (Tables 23 and 24) demonstrate that the dimethoxyphenyl ring readily twists in and out of alignment with the double-bond (within the NMR timescale at ambient temperatures) since irradiation of 3-H causes NOEs at all four *ortho* protons in (**66**) [5-, 9-, 11-, and 15-H] and at both 5- and 9-H in (**233**).[323] Thus, given the present evidence it is difficult to draw detailed conclusions about the conformational preference for either isomer and thus shed any light on any plausible *bioactive* conformation.

When the *trans/cis* isomerism (Scheme 22) was repeated for the 3-thienyl compound (**229**), three distinct compounds were observed by TLC. Unfortunately, subsequent seperation by column chromatography and crystallisation of the presumed *cis*-isomer (aromatic groups mutually *cis*) only afforded the corresponding *trans*-isomer (**229**). All attempts failed thereafter and presumably the *cis*-isomer reverts back to its *trans* counterpart in the purification process. Nevertheless, a product akin to the epoxide (**234**), as identified by 90 MHz chromatography, could be isolated and the *trans*-isomer (**229**) was able to be crystallised in a form suitable for X-ray crystallography—see Figure 31 and Table 28.[319] As expected a near co-planar arrangement was found for the dimethoxyphenylpropenonitrile portion [$(MeO)_2C_6H_3C$=CCN] and the structure (**229**) adopts a very similar conformation to the *trans*-isomer (**66**)—compare torsional angles of aromatic groups relative to the double-bonds (*cf.* Tables 27 and 28).

Pertaining to the isomeric tyrphostins (**66**) and (**233**), it was of interest to study the effect daylight had on each compound. This was achieved by monitoring each isomer every 24 hours in d_6-DMSO by 200 MHz ^{1}H NMR spectroscopy (Figure 32). The composition of the mixture was determined by measuring the integrals of the methoxy signals at δ_H 3.89 and 3.86 for (**66**), and δ_H 3.79 for (**233**). In both cases, a near identical mixture was obtained

Figure 31 X-ray Crystal Structure and Data of *trans*-Isomer (229)



trans-$C_{15}H_{13}NO_2S$, M_r= 271.34

monoclinic, space group $P2_1/n$

crystallised from diethyl ether

crystal specimen: pale-yellow cuboid (0.55 × 0.38 × 0.20 mm)

a= 10.5062(5), b= 11.0185(7), c= 12.4749(8) Å

β= 107.36(4)°, V= 1378.3(1) Å^3, Z= 4

D_c= 1.308 g cm^{-3}, μ(Mo-*K*α)= 2.2 mm^{-1}

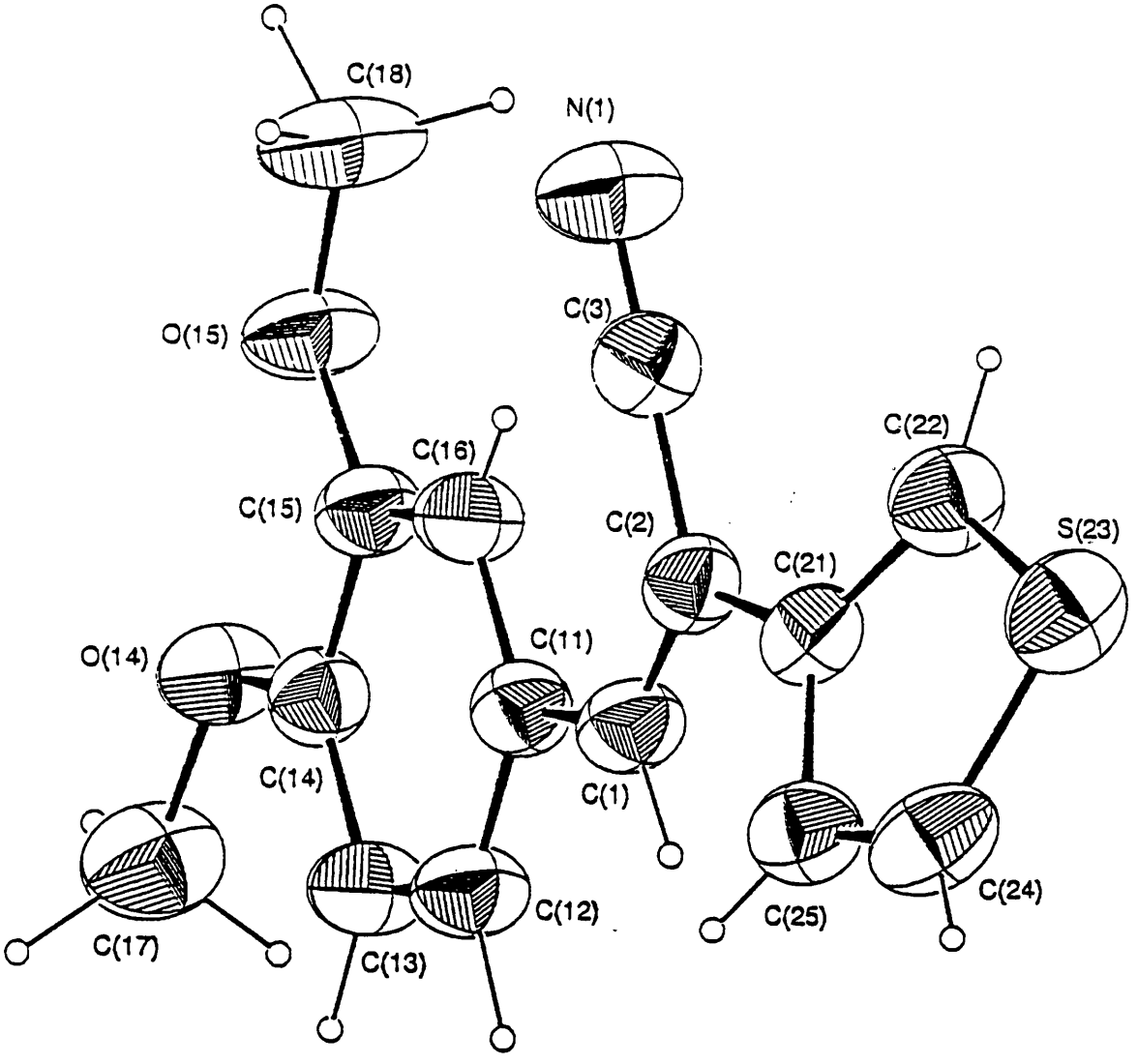
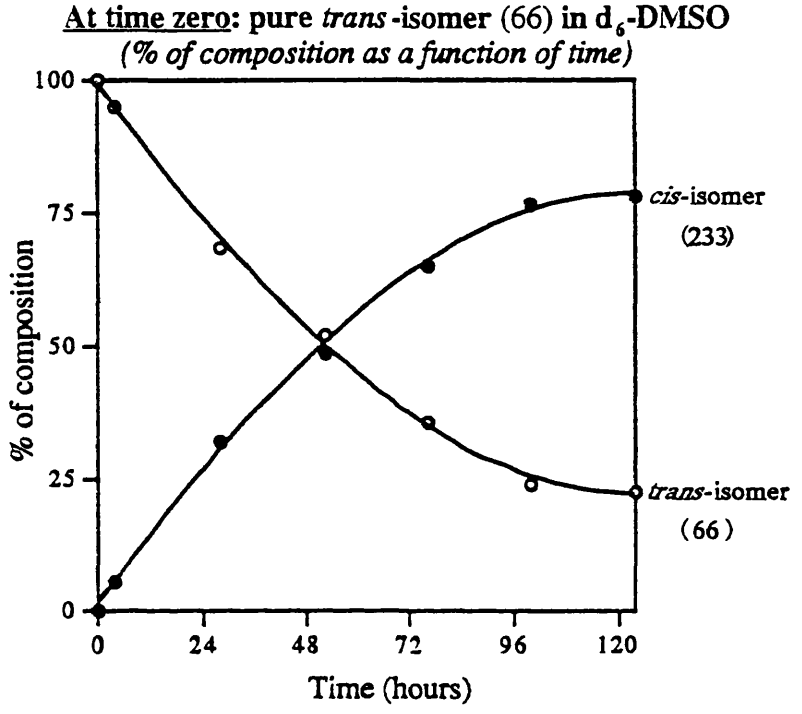
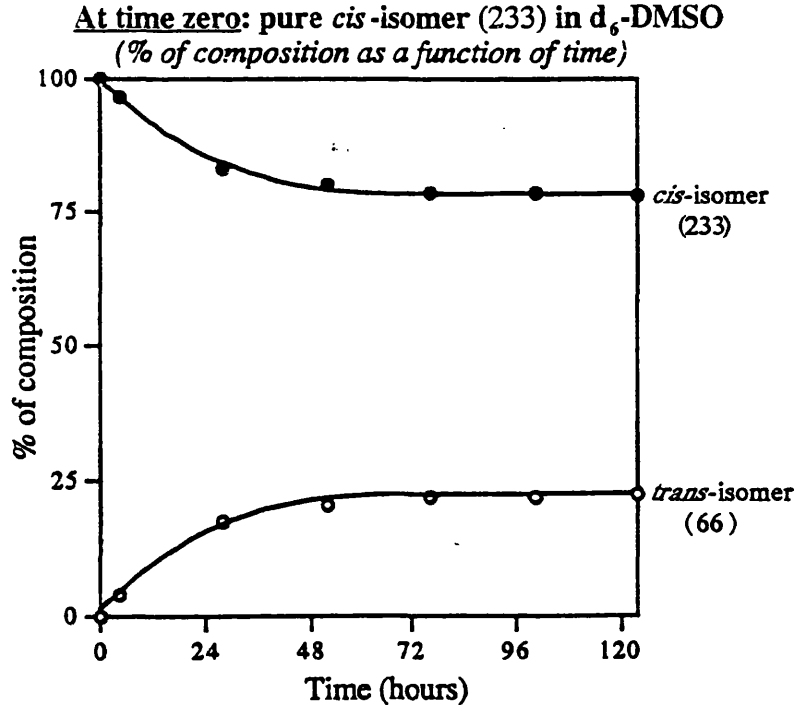


Table 28 Selected X-Ray Data of *trans*-isomer (229)

Bond Lengths (Å)			
S(23)—C(22)	1.705(2)	S(23)—C(24)	1.702(3)
N(1)—C(3)	1.137(3)	O(14)—C(3)	1.357(2)
O(14)—C(17)	1.428(3)	O(15)—C(15)	1.366(2)
O(15)—C(18)	1.419(3)	C(1)—C(2)	1.341(3)
C(1)—C(11)	1.456(3)	C(2)—C(3)	1.443(3)
C(2)—C(21)	1.477(3)	C(11)—C(12)	1.381(3)
C(11)—C(16)	1.415(3)	C(12)—C(13)	1.386(3)
C(13)—C(14)	1.380(3)	C(14)—C(15)	1.409(3)
C(15)—C(16)	1.371(3)	C(21)—C(22)	1.354(3)
C(21)—C(25)	1.437(3)	C(24)—C(25)	1.349(3)
Bond Angles (°)			
C(22)—S(23)—C(24)	91.5(1)	C(14)—O(14)—C(17)	117.9(2)
C(15)—O(15)—C(18)	117.2(2)	C(2)—C(1)—C(11)	132.2(2)
C(1)—C(2)—C(3)	122.2(2)	C(1)—C(2)—C(21)	123.2(2)
C(3)—C(2)—C(21)	114.2(2)	N(1)—C(3)—C(2)	177.3(3)
C(1)—C(11)—C(12)	118.3(2)	C(1)—C(11)—C(16)	123.8(2)
C(12)—C(11)—C(16)	117.8(2)	C(11)—C(12)—C(13)	121.8(2)
C(12)—C(13)—C(14)	120.1(2)	O(14)—C(14)—C(13)	125.6(2)
O(14)—C(14)—C(15)	115.3(2)	C(13)—C(14)—C(15)	119.2(2)
O(15)—C(15)—C(14)	115.1(2)	O(15)—C(15)—C(16)	124.6(2)
C(14)—C(15)—C(16)	120.2(2)	C(11)—C(16)—C(15)	120.9(2)
C(2)—C(21)—C(22)	124.3(2)	C(2)—C(21)—C(25)	124.1(2)
C(22)—C(21)—C(25)	111.6(2)	S(23)—C(22)—C(21)	112.4(2)
S(23)—C(24)—C(25)	112.5(2)	C(21)—C(25)—C(24)	112.0(2)
Torsion Angles (°)			
C(17)—O(14)—C(14)—C(13)		2.7(2)	
C(18)—O(15)—C(15)—C(16)		-4.8(2)	
C(11)—C(1)—C(2)—C(3)		2.5(2)	
C(2)—C(1)—C(11)—C(16)		5.0(2)	
C(11)—C(1)—C(2)—C(21)		176.9(4)	
C(1)—C(2)—C(21)—C(22)		152.1(3)	
C(1)—C(2)—C(21)—C(25)		-26.9(2)	
C(3)—C(2)—C(21)—C(22)		-27.3(2)	
C(3)—C(2)—C(21)—C(25)		153.6(3)	
O(14)—C(14)—C(15)—O(15)		0.5(2)	
C(25)—C(21)—C(22)—S(23)		0.8(2)	
C(22)—C(21)—C(25)—C(24)		-0.2(2)	
S(23)—C(24)—C(25)—C(21)		-0.4(2)	
C(24)—S(23)—C(22)—C(21)		-0.9(2)	
C(22)—S(23)—C(24)—C(25)		0.8(2)	

Figure 32 Light-induced *trans/cis* Interconversion of Tyrphostins (66) and (233).





within 5-6 days, with the *cis*-isomer (**233**) predominating by a ratio of 7:2, which showed that both forms can mutually equilibrate in the presence of light. By conducting a similar light study in d_8-THF, it was demonstrated that the equilibrium ratio was solvent dependent since (**66**) and (**233**) gave a 4:5 mixture, respectively, over *ca.* 8 days.

When each isomer was stored in the dark at ambient temperatures (over 17 hours) either as a d_6-DMSO solution or in the solid state, no *trans/cis* interconversion was observed. Also, no isomerised products or impurities were detected when (**66**) and (**233**) were exposed to light in the solid state (over 16 hours), and no discolouring of the transparent crystals of the *cis*-isomer (**233**) were observed for longer periods (two weeks in daylight hours). Thus, this study highlights the importance of *not* storing stilbene-like tyrphostins as stock solutions, especially in DMSO, and it would be advisable to reconstitute these drugs in the dark immediately *prior* to biological testing.

The stablity of the tyrphostin (**66**) in solution has previously been questioned by colleagues at the Beatson Institute (Glasgow University) and it was suggested that the parent drug seemed to be in equilibrium with a "main degradation product (mdp)" in DMSO.[324] Indeed, in later work, this "mdp" was isolated by reverse phase HPLC (55% H_2O/45% MeOH) and was identified by us to be the *cis*-isomer (**233**) using ^{1}H NMR spectroscopy. Futhermore, it was demonstrated by Dr Howard L. McLeod and colleagues (Beatson Institute, Glasgow University) that the *cis*-isomer was also formed in the plasma of nude mice, together with at least 6 other metabolites, after administration of the *trans*-compound (**66**). However, the results of Yoneda *et al.*[232] could not be reproduced in this study and the tyrphostin (**66**) did not show a promising *in vivo* profile against tumour-bearing nude mice.

Nevertheless, an interesting finding was that the isomeric tyrphostins (**66**) and (**233**) gave IC_{50} values of 11 and 38 μM, respectively, when tested in an antiproliferative assay against the HN5 cell line.[325] This finding supports Levitzki's premise[230f] that *trans*-forms of the tyrphostins (aromatic and nitrile groups mutually *cis*) are more potent than their *cis*-counterparts. However, the true reason for this result is not clear but, since the tyrphostin (**66**) is a mixed-type inhibitor[231] and therefore competes for the ATP-binding site, it may be speculated that a flat conformation analogous to the adenine portion of ATP is important to attain a high binding affinity (*cf.* Burke[202]). This situation is sterically dissallowed for the *cis*-isomer (**233**) and so would be expected to be less active. On the other hand, it may be

the directional nature of the nitrile group that is important for substrate binding interactions *via* hydrogen-bonding or by some electrostatic attraction, for example. Whatever the reason the present study has provided interesting data towards the interpretation of the biological data of the known tyrphostin (**66**) and is currently being compiled for publication both in a chemical and biological context.[326,327]

EXPERIMENTAL—CHEMISTRY

MATERIALS AND INSTRUMENTATION

All chemicals and solvents were purchased from Aldrich Chemical Company (Gillingham, Dorset, UK) except 2-nitrothiophene-4-carbaldehyde which was purchased from Lancaster Synthesis Limited (Morecambe, Lancashire, UK). Organic solutions were dried with anhydrous Na_2SO_4 or $MgSO_4$ and concentrated under 40 °C using a rotary evaporator. Unless otherwise stated, ether refers to diethyl ether and petroleum ether refers to the fraction which distills between 40-60 °C. R_F values were obtained by performing qualitative thin-layer chromatography (TLC) using Merck GF_{254} silica gel plates. Compounds were detected by viewing under UV and/or developing with iodine.

Mps are uncorrected and were determined on a Reichert-Kofler hot-stage apparatus. IR spectra were recorded as KBr discs on a Philips analytical PU9800 Fourier Transform IR spectrometer. IR frequencies, ν, are given in wave numbers (cm^{-1}) and the relative intensities (weak, medium and strong) are denoted by w, m, and s, respectively. Stated ^1H (200 Mz) and ^{13}C (50 Mz) NMR data were obtained from Brüker AM200SY or WP200SY instruments and the numbering systems refer to the appropriate structures in the Appendix. All chemical shifts are quoted in ppm on the δ scale using the residual protons from the deuteriated solvent as reference. Mass spectra (MS) were performed (as EI) on an AEI/Kratos MS 12 (low resolution) and an AEI/Kratos MS 902S (high resolution) instrument. Elemental compositions for C, H and N were determined on a Carlo Erba Strumentazione Elemental Analyzer-MOD 1106 with a Hewlett Packard 3394A Integrator. The presentation of the experimental data is according to the 1994 recomendations of the *Journal of the Chemical Society (Perkin Transaction 1)*.[328]

General Notes and Methods Used

Method A. A round-bottomed flask was charged with equimolar amounts of aryl carbaldehyde and malononitrile derivative (1-4 mmol) followed by absolute ethanol (3-15 ml) and a magnetic stirrer. After addition of neat piperidine (1-3 drops from a pasteur pipette) the reaction was stirred at room temperature until precipitation of the product had occurred (1-18 h). Failing this water and/or diethyl ether was added and the precipitate was collected and dried under suction. This procedure worked well with malononitrile and the alkyl cyanoacetate esters.

Method B. A 25 ml round-bottomed flask was charged with the appropriate aryl carbaldehyde (1.5 mmol) and malononitrile derivative (1.5 mmol) together with absolute ethanol (5 ml) and a magnetic stirrer. As a precaution the contents were occasionally heated and filtered to obtain a clear solution or else each reactant was separately dissolved in a minimum amount of ethanol, filtered and mixed later. The solution obtained was stirred at room temperature and 10% piperidine in ethanol (up to 1 ml) was added dropwise. The reaction was then stirred for up to 24 h or until TLC (silica gel/diethyl ether) showed completion. The precipitate was filtered, washed and dried under suction with diethyl ether. This procedure was preferred for the less soluble malononitrile derivatives, 1,1,3-tricyano-2-aminoacetate and cyanothioacetamide.

Method C. Equimolar amounts of an appropriate aryl carbaldehyde and malononitrile derivative (10-100 mmol) were dissolved in a minimum amount of ethanol (1-10 ml). After keeping the solution hot for *ca.* 5 min, piperidine in ethanol (5 to 50 %; 1-5 drops) was added, while stirring, and the mixture was left to cool to room temperature (unstirred). The crystals were filtered, washed (diethyl ether) and dried under suction. This procedure worked well with nitroaryl carbaldehydes *i.e.* activated systems.

Method D. Neat piperidine (1 drop per ml of solvent used) was added to a hot stirred solution of the appropriate aryl carbaldehyde (10-100 mmol) in a minimum amount of ethanol (1-10 ml). After keeping the solution hot for *ca.* 5 min and while stirring, an equimolar amount of the appropriate 3-substituted acetonitrile derivative was added followed by cautious addition of a catalytic amount of potassium *tert*-butoxide (*ca.* 10 mg). After the initial exotherm subsided, the solution was left to cool to room temperature (unstirred). The crystals were filtered, washed (diethyl ether) and dried under suction. This procedure worked well with less activated reaction systems and in particular for the 3-arylacetonitriles.

Experimental Procedures—Chapter 6

2-Cyano-3-(4-hydroxyphenyl)propenonitrile (**69**)—Using Method A, 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) and malononitrile (0.27 g, 4.1 mmol) reacted to give a yellow preciptate after 1 h: (0.68 g, 98 %), m.p. 155-163 °C (from EtOH) (lit.,[230c] 180 °C); R_F 0.69 (diethyl ether); (Found: M^+, 170.0487. $C_{10}H_6N_2O$ requires *M*, 170.0494); ν_{max}(KBr disc)/cm^{-1} 3400m (OH), 3025w (Ar–H), 2200s (CN), 1610m, 1580s, and 1560s

(Ar and C=C), 850m and 840m (Ar–H); δ_H(200 MHz; DMSO-d_6) 9.77 (1 H, br, OH), 8.09 (1 H, s, 3-H), 7.85 (2 H, d, ^3J 8.4 Hz, 5-H and 9-H), 6.30 (2 H, d, ^3J 8.4 Hz, 6-H and 8-H); δ_C(50 MHz; DMSO-d_6) 169.6 (7-C), 159.1 (3-CH), 134.5 (5- and 9-CH), 120.4 (4-C), 118.1 (6- and 8-CH), 116.2 and 115.5 (2 × CN), 69.8 (2-C); *m/z* 170 (M^+, 100%), 142 (M^+ – CO, 56), 119 (M^+ – CO, – CN, 60), 91 (M^+ – CO, – H-C≡C-CN, 46), 76 (16), 63 (32), 51 (25.5).

3-Amino-2,4-dicyano-5-(4-hydroxyphenyl)penta-2,4-dienonitrile (**70**)—Using Method A, 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) and 1,1,3-tricyano-2-aminopropene (0.54 g, 4.1 mmol) were reacted in ethanol (5 ml) with piperidine (1 drop) and after 4 h the product was precipitated with water and collected: (0.17 g, 18 %), m.p. 182 °C (from ethanol/water) (lit.,[230c] 225 °C); R_F 0.38 (diethylether); (Found: M^+, 236.0716. $C_{13}H_8N_3O$ requires *M*, 236.0734); ν_{max}(KBr disc)/cm^{-1} 3400s (OH), 3330s and 3240s (NH_2), 2200s and 2220s (CN), 1640s, 1605s and 1560s (C=C), 835m (Ar–H); δ_H(200 MHz; DMSO-d_6) 9.0 (3 H, v.br, OH and NH_2), 7.94 (1 H, s, 3-H), 7.93 (2 H, d, ^3J 8.6 Hz, 5-H and 9-H), 6.97 (2 H, d, ^3J 8.6 Hz, 6-H and 8-H); *m/z* 236 (M^+, 40 %), 210 (M^+ – CN, 68), 194 (M^+ – HC=CH–O, 3), 192 (M^+ – CN, – H_2O, 4), 181 (M^+ – H_2C=CH–C=O, 13), 116 (M^+ – CO, – $C(NH_2)$=$C(CN)_2$, 12.2), 165 (8), 152 (3.5), 143 (9), 142 (4.5), 139 (4), 107 (100), 89 (28), 84 (23), 77 (34), 63 (25), 56 (44), 51 (30.5).

2-Cyano-3-(4-hydroxyphenyl)propenothioamide (**71**)—Using Method A, 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) and cyanothioacetamide (0.41 g, 4.1 mmol) were reacted in ethanol (3 ml) with piperidine (1 drop) and after 2 h the product was precipitated with water: (0.31 g, 38 %), m.p. 189-193 °C; R_F 0.64 (diethyl ether); (Found: M^+, 204.0368. $C_{10}H_8N_2OS$ requires *M*, 204.0379); ν_{max}(KBr disc)/cm^{-1} 3360s (OH), 3290s and 3170s ($CSNH_2$), 2220 (CN), 1640 (C=S), 1605m, 1585s and 1565s (Ar and C=C), 845s and 830s (Ar–H); δ_H(200 MHz; DMSO-d_6) 10.70 (1 H, br, OH), 9.99 (1 H, br, NH_2), 9.45 (1 H, br, NH_2), 8.29 (1 H, s, 3-H), 7.93 (2 H, d, ^3J 8.6 Hz, 5-H and 9-H), 6.98 (2 H, d, ^3J 8.6 Hz, 6-H and 8-H); δ_C(50 MHz; DMSO-d_6) 192.9 (C=S), 162.0 (7-C), 147.9 (3-CH), 133.3 (5- and 9-CH), 122.8 (4-C), 116.5 (6- and 8-CH), 117.2 (CN), 107.8 (2-C); *m/z* 204 (M^+, 83%), 203 (M^+ – H, 100), 171 (M^+ – HS, 19), 116 (M^+ – CO, – $CSNH_2$, 16), 115 (11), 114 (15), 77 (8), 60 (34), 51 (22).

Ethyl 2-Cyano-3-(4-hydroxyphenyl)propenoate (**72**)—Using Method A, 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) and ethyl cyanoacetate (0.46 g, 4.1 mmol) were

reacted in ethanol (3 ml) with piperidine (1 drop) to give a white precipitate after 2 h: (0.85 g, 96 %), m.p. 169-170 °C (from EtOH); R_F 0.60 (diethyl ether); (Found: M^+, 217.0737. $C_{12}H_{11}NO_3$ requires *M*, 217.0736); ν_{max}(KBr disc)/cm^{-1} 3300s (OH), 2990w (Ar–H), 2230m (CN), 1730m and 1710m (C=O), 1585s (Ar and C=C), 835m (Ar–H); δ_H(200 MHz; DMSO-d_6) 10.68 (1 H, br, OH), 8.03 (1 H, s, 3-H), 7.80 (2 H, d, ^3J 8.4 Hz, 5-H and 9-H), 6.78 (2 H, d, ^3J 8.4 Hz, 6-H and 8-H), 4.10 (2 H, q, ^3J 6.8 Hz, CH_2), 1.13 (3 H, d, ^3J 6.8 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 163.0. (C=O), 162.7 (7-C), 154.7 (3-CH), 134..0 (5- and 9-CH), 122.6 (4-C), 116.5 (6- and 8-CH), 116.5 (CN), 97.1 (2-C), 62.0 (CH_2), 14.1 (CH_3); *m/z* 217 (M^+, 91%), 188 ($M^+ - CH_2CH_3$, 14), 173 ($M^+ - H_2O$, – CN, 5), 144 ($M^+ - CO_2CH_2CH_3$, 55), 118 ($M^+ - CO_2CH_2CH_3$, – CN, 26), 105 (2.5), 100 (20), 94 (9), 90 (22), 89 (93), 79 (2), 77 (3), 74 (6), 63 (23).

2-Cyano-3-(4-hydroxyphenyl)propenamide (**73**)—Using Method A, 4-hydroxybenzaldehyde (0.50 g, 4.1 mmol) and cyanoacetamide (0.34 g, 4.1 mmol) were reacted in ethanol (5 ml) with piperidine.(1 drop) After 5 h the product was obtained by precipitation on ice: (0.45 g, 58 %), m.p. 243 °C (from EtOH) (lit.,[230c] 250 °C); R_F 0.32 (diethyl ether); (Found: M^+, 188.0598. $C_{10}H_8N_2O_2$ requires *M*, 188.0610); ν_{max}(KBr disc)/cm^{-1} 3450s and 3370s (NH_2), 3190s (OH), 2230m (CN), 1655m (C=O), 1600s and 1570m (Ar and C=C), 840m (Ar–H); δ_H(200 MHz; DMSO-d_6) 10.62 (1 H, br, OH), 8.11 (1 H, s, 3-H), 7.81 (1 H, br, NH_2), 7.70 (1 H, br, NH_2), 7.92 (2 H, d, ^3J 8.3 Hz, 5-H and 9-H), 6.98 (2 H, d, ^3J 8.3 Hz, 6-H and 8-H); δ_C(50 MHz; DMSO-d_6) 163.5 (C=O), 161.9 (7-C), 150.7 (3-CH), 133.0. (5- and 9-CH), 123.0. (4-C), 117.5 (CN), 116.3 (6- and 8-CH), 101.5 (2-C); *m/z* 188 (M^+, 100%), 172 ($M^+ - NH_2$, 25), 144 ($M^+ - CONH_2$, 39), 118 ($M^+ - CONH_2$, – CN, 61), 114 (13), 104 (3.5), 93 (3), 91 (5.5), 89 (81), 65 (27), 64 (16), 63 (53), 51 (26).

2-Cyano-3-(3,4-dihydroxyphenyl)propenonitrile (**56**)—Using Method A, 3,4-dihydroxybenzaldehyde (1.10 g, 8.0 mmol) and malononitrile (0.55 g, 8.3 mmol) were reacted in ethanol (6 ml) under reflux with piperidine (1 drop) for 0.5 h. This gave a yellow precipitate which was crystallised from ethanol/water: (1.18 g, 80 %), m.p. 220 °C (decomp.) (from EtOH/H_2O) (lit.,[230c] 225 °C); R_F 0.72 (diethyl ether); (Found: M^+, 186.0415. $C_{10}H_6N_2O_2$ requires *M*, 186.0401); ν_{max}(KBr disc)/cm^{-1} 3460s and 3285s (2 × OH), 2220s and 2225s (2 × CN), 1610m, 1595m and 1570s (Ar and C=C), 905m and 825m

(Ar–H); δ_H(200 MHz; acetone-d_6) 9.00 (2 H, v.br, 2 × OH), 7.97 (1 H, s, 3-H), 7.65 (1 H, d, $^4J_{9\text{-}H}$ 2.25 Hz, 5-H), 7.47 (1 H, dd, $^4J_{5\text{-}H}$ 2.25 Hz, $^3J_{8\text{-}H}$ 8.4 Hz, 9-H), 7.00 (1 H, d, $^3J_{9\text{-}H}$ 8.4 Hz, 8-H); δ_C(50 MHz; acetone-d_6) 160.7 (3-CH), 153.2 (7-CH), 146.5 (6-CH), 128.1 (9-CH), 124.8 (4-C), 116.7 (5- or 8-CH), 116.5 (8- or 5-CH), 116.6 and 114.6 (2 × CN), 77.1 (2-C); *m/z* 186 (M^+, 100 %), 158 (M^+ – CO, 36), 130 (M^+ – 2 × CO, 52), 103 (M^+ – 2 × CO, – HCN, 39), 93 (2), 89 (4), 87 (18), 76 (52), 62 (30.5).

3-Amino-2,4-dicyano-5-(3,4-dihydroxyphenyl)penta-2,4-dienonitrile (**74**)—Using Method A, 3,4-dihydroxybenzaldehyde (0.50 g, 3.62 mmol) and 1,1,3-tricyano-2-aminopropene (0.47 g, 3.61 mmol) were reacted in ethanol (5 ml) with piperidine (1 drop) for 3 h. The product was precipitated on ice to give a yellow solid: (0.39 g, 43 %), m.p. 229 °C (from EtOH/H_2O) (lit.,[230c] 235 °C); R_F 0.26 (diethyl ether); (Found: M^+, 252.0640. $C_{13}H_8N_4O_2$ requires *M*, 252.0373); ν_{max}(KBr disc)/cm^{-1} 3380s (2 × OH), 3330s and 3220s (NH_2), 2220s and 2200s (CN), 1650s, 1610s and 1570s (C=C), 310w (Ar–H); δ_H(200 MHz; DMSO-d_6) 10.01 and 9.10 (4 H, br, 2 × OH and NH_2), 8.19 (1 H, s, 3-H), 7.95 (1 H, b r s, 5-H), 7.47 (1 H, br d, $^3J_{8\text{-}H}$ 8.5 Hz, 9-H), 7.07 (1 H, d, $^3J_{9\text{-}H}$ 8.5 Hz, 8-H); *m/z* 252 (M^+, 100%), 210 (M^+ – CN, 14), 198 (M^+ – CN, – CO, 11), 196 (M^+ – 2 × CO, 28), 195 (M^+ – CO, – NH_2, 31.5), 135 (M^+ – CN, – $C(NH_2)$=$C(CN)_2$, 3), 235 (25), 226 (47.5), 180 (32), 169 (27), 152 (20), 141 (23), 130 (14.5), 127 (6), 115 (10), 114 (34), 105 (11.5), 88 (36).

2-Cyano-3-(3,4-dihydroxyphenyl)propenothioamide (**58**)—Using Method B and in the absence of light, 3,4-dihydroxybenzaldehyde (2.085 g, 15.10 mmol) and cyanothioacetamide (1.510 g, 15.08 mmol) were reacted in ethanol (10 ml) for 5.5 h with 10% piperidine in ethanol (3 drops). The greenish-yellow precipitate was filtered, washed with isopropanol and dried under suction with diethyl ether. This was subsequently purified through a silica column with ethyl acetate/petroleum ether (bp 30-40 °C) elutions to give: (1.631 g, 49 %), m.p. 206-207 °C (from EtOAc/pet. ether; bp 30-40 °C) (lit.,[230c] 213 °C); R_F 0.58 (diethyl ether); (Found: M^+, 220.0365. $C_{10}H_8N_2O_2S$ requires *M*, 220.0373); ν_{max}(KBr disc)/cm^{-1} 3380s (2 × OH), 3290s and 3185s (NH_2), 2215m (CN), 1630m (C=S), 1610s and 1575s (Ar and C=C), 870m (Ar–H); δ_H (200 MHz; DMSO-d_6) 9.90 (2 H, br, 2 × OH), 9.92 (1 H, br s, NH_2), 9.40 (1 H, br s, NH_2), 8.01 (1 H, s, 3-H), 7.61 (1 H, d, $^4J_{9\text{-}H}$ 1.97 Hz, 5-H), 7.35 (1 H, dd, $^4J_{5\text{-}H}$ 1.97 Hz, $^3J_{8\text{-}H}$ 8.34 Hz, 9-H), 6.94 (1 H, d, $^3J_{9\text{-}H}$ 8.34 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 193.0 (C=S), 151.0 (7-C), 148.2 (3-CH), 145.9 (6-C), 125.9

(9-CH), 123.2 (CN), 117.2 (4-C), 116.5 (5- or 8-CH), 116.2 (8- or 5-CH), 107.4 (2-C); *m/z* 220 (M^+, 96%), 203 ($M^+ - CO, + H$, 31), 187 ($M^+ - HS$, 15), 132 ($M^+ - CO, - CSNH_2$, 4), 131 (9.5), 130 (40), 77 (14.5).

Ethyl 2-cyano-3-(3,4-dihydroxyphenyl)propenonitrile (**75**)—Using Method A, 3,4-hydroxybenzaldehyde (0.50 g, 3.62 mmol) and ethyl cyanoacetate (0.41 g, 3.62 mmol) were reacted in ethanol (3 ml) with piperidine (1 drop). After 1 h a precipitate was not observed and thus the reaction was heated for 2 h which yielded the title product (0.74 g, 87.4 %) on cooling to room temperature, m.p. 160-165 °C (from EtOH); R_F 0.67 (diethyl ether); (Found: M^+, 233.0684. $C_{12}H_{11}NO_4$ requires *M*, 233.0680); ν_{max}(KBr disc)/cm^{-1} 3470m and 3140m (2 × OH), 2205m (CN), 1700s (C=O), 1615w and 1580s (Ar), 860m and 745s (Ar–H); δ_H(200 MHz; DMSO-d_6) 10.85 (2 H, v.br, 2 × OH), 8.27 (1 H, s, 3-H), 7.83 (1 H, br s, 5-H), 7.54 (1 H, br d, $^3J_{8\text{-}H}$ 8.2 Hz, 9-H), 7.07 (1 H, d, $^3J_{9\text{-}H}$ 8.2 Hz, 8-H), 4.42 (2 H, q, ^3J 7.0 Hz, CH_2), 1.44 (3 H, t, ^3J 7.0 Hz, CH_3); *m/z* 233 (M^+, 48 %), 188 ($M^+ - CN$, $- CH_2CH_3$, 55), 160 ($M^+ - CO_2\ CH_2CH_3$, 54.5), 141 ($M^+ - 2 \times CO, - 2 \times H_2O$, 1), 114 ($M^+ - 2 \times CO, - H_2O, - OCH_2CH_3$, 100), 106 ($M^+ - CO_2CH_2CH_3, -$ HC=C–C=O, 5), 103 (34.5), 102 (16), 91 (2), 88 (7), 87 (6), 77 (13).

2-Cyano-3-(3,4-dihydroxyphenyl)propenamide (**76**)—Using Method A, 3,4-dihydroxybenzaldehyde (0.50 g, 3.62 mmol) and cyanoacetamide (0.30 g, 3.62 mmol) were reacted in ethanol (8 ml) with piperidine (2 drops) After 1.5 h, no precipitate formed and so the solution was heated at reflux for 16 h and precipitation induced by the addition of diethyl ether to give: (0.34 g, 46 %), m.p. 220-222 °C ($EtOH/Et_2O$) (lit.,[230c] 247 °C); R_F 0.22 (diethyl ether); (Found: M^+, 204.0527. $C_{10}H_8N_2O_3$ requires *M*, 204.0520); ν_{max}(KBr disc)/cm^{-1} 3460s, 3420s, 3320s and 3265s (OH, 2210m (CN), 1680m (C=O), 1605s and 1575m (C=C, Ar); δ_H(200 MHz; DMSO-d_6) 9.84 (2 H, v.br, 2 × OH), 8.11 (1 H, s, 3-H), 7.73 and 7.60 (2 × 1 H, 2 × br s, NH_2), 7.54 (1 H, d, $^4J_{9\text{-}H}$ 2.12 Hz, 5-H), 7.26 (1 H, dd, $^4J_{9\text{-}H}$ 2.12 Hz, $^3J_{8\text{-}H}$ 8.3 Hz, 9-H), 6.87 (1 H, d, $^3J_{9\text{-}H}$ 8.3 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 163.7 (C=O), 151.0 (3-CH), 150.9 (7-C), 145.8 (6-C), 125.5 (9-CH), 123.5 (CN), 117.5 (4-C), 116.3 (5- or 8-CH), 116.1 (8- or 5-CH), 101.2 (2-C); *m/z* 204 (M^+, 100 %), 185 ($M^+ - H_2O$, – H, 6.5), 176 ($M^+ - CO$, 6), 160 ($M^+ - CONH_2$, 11.5), 134 ($M^+ - CN, -$ CON H_2, 12), 132 ($M^+ - CO, - CONH_2$, 5), 114 ($M^+ - CO, - CONH_2, -$ OH, – H, 16), 104 ($M^+ - 2 \times CO, -$ $CONH_2$, 7), 103 (4), 102 (2.5), 88 (9), 87 (5), 78 (4), 77 (9), 76 (8.5).

2-Cyano-3-(3,4,5-trihydroxyphenyl)propenonitrile (**57**)—Using Method B, 3,4,5-dihydroxybenzaldehyde (0.109 g, 0.707 mmol) and ethanolic malononitrile (27.8 mg ml^{-1}, 0.715 mmol) (1.7 ml) were mixed and while stirring piperidine (1 drop) was added. After 3 h, the orange precipitate was collected: (61.3 mg, 43 %), m.p. 157-160 °C (from EtOH) (lit.,[230c] 245 °C); R_F 0.20 (diethyl ether); (Found: M^+, 202.0384. $C_{10}H_6N_2O_3$ requires *M*, 202.0390); ν_{max}(KBr disc)/cm^{-1} 3300m and 3170m (OH), 2955m (Ar–H), 2215s (CN), 1605m, 1570s and 1545s (Ar and C=C); δ_H(200 MHz; DMSO-d_6) 8.02 (3 H, br, 3 × OH), 7.26 (1 H, s, 3-H), 6.97 (2 H, br s, 5- and 9-H); *m/z* 202 (M^+, 20%), 174 (M^+ – CO, 3), 128 (M^+ – 2 × CO, – H_2O, 5), 118 (M^+ – 3 × CO, 100), 91 (M^+ – 3 × CO, – HCN, 21.5), 90 (16), 89 (4), 79 (1.5), 77 (3), 76 (16), 75 (11).

3-Amino-2,4-dicyano-5-(3,4,5-trihydroxyphenyl)penta-2,4-dienonitrile (**77**)—Using Method B, 3,4,5-dihydroxybenzaldehyde (0.10 g, 0.65 mmol) was added to 1,1,3-tricyano-2-aminopropene (0.12 g, 0.91 mmol) in ethanol (3 ml). While stirring 10% piperidine in ethanol (1 ml) was added and after 4 h the dark red solution was subjected to column chromatography (silica/diethyl ether) to give an orange solid: (0.10 g, 42 %), m.p. 180 °C (decomp.) (lit.,[230c] 275 °C); R_F 0.39 (diethyl ether); ν_{max}(KBr disc)/cm^{-1} 3315s and 3210s (NH_2 and OH), 2220m (CN), 1630s (C=S), 1600s, 1570s and 1525s (Ar and C=C), 875 and 800 (Ar-H); δ_H(200 MHz; acetone-d_6) 7.8 (5 H, v.br, NH_2 and 3 × OH), 7.65 (1 H, s, 3-H), 7.07 (2 H, br s, 5- and 9-H); *m/z* 268 (M^+, 0.5), 242 (M^+ – CN, 1), 224 (M^+ – CN, – H_2O, 0.4), 176 (M^+ – $C(NH_2)=C(CN)_2$, 2), 151 (M^+ – CN, $C(NH_2)=C(CN)_2$, 2), 125 (*Ar*, 37), 97 (*Ar* – CO, – H, 33.5), 79 (95), 66 (17).

2-Cyano-3-(3,4,5-trihydroxyphenyl)propenothioamide (**78**)—Using Method B and in the abscence of light, 3,4,5-dihydroxybenzaldehyde (0.20 g, 1.30 mmol) was added to cyanothioacetamide (0.18 g, 1.80 mmol) in degassed ethanol (2 ml), under a nitrogen atmosphere. While stirring, 10% piperidine in ethanol (1 ml) was added and after 4 h the dark red solution was subjected to column chromatography (silica/diethyl ether) to give an orange solid: (0.21 g, 50 %), m.p. 98 °C (decomp.); R_F 0.26 (diethyl ether); ν_{max}(KBr disc)/cm^{-1} 3315s and 3210s (NH_2 and OH), 2220m (CN), 1630s (C=S), 1600s, 1570s and 1525s (Ar and C=C), 875 and 800 (Ar–H); δ_H(200 MHz; DMSO-d_6) 9.42 and 8.81 (2 H, 2⊇× br s, NH_2), 8.2 (3 H, v.br, 3 × OH), 7.81 (1 H, s, 3-H), 6.90 (2 H, br s, 5- and 9-H); *m/z* 236 (M^+, 55), 219 (M^+ – NH_3, 25), 209 (M^+ – HCN, 2), 203 (M^+ – HS, 20), 176 (M^+ –

$CSNH_2$, 6.5), 148 ($M^+ - CSNH_2$, – CO, 8), 134 (M^+ – CN, – 2 × CO, – NH_2, 18.5), 118 (M^+ – $CSNH_2$, – CN, – CO, 65), 102 (36), 91 (35), 76 (69).

Ethyl 2-cyano-3-(3,4,5-trihydroxyphenyl)propenoate (**79**)—Using Method C, ethyl cyanoacetate (0.20 g, 1.8 mmol) was added to 3,4,5-dihydroxybenzaldehyde (0.20 g,1.30 mmol) in ethanol (1 ml) and were reacted with piperidine (1 drop) After 1.25 h, the reaction solution was directly applied onto a silica column and eluted with diethyl ether to give a yellow solid: (0.13 g, 40 %), m.p. 76 °C (decomp.); R_F 0.55 (diethyl ether); ν_{max}(KBr disc)/cm^{-1} 3365s (OH), 2985m (Ar–H), 2225m (CN), 1695s (C=O), 1655s (Ar and C=C), 635m (Ar–H); δ_H(200 MHz; DMSO-d_6) 9.0 (3 H, v.br, 3 × OH), 8.04 (1 H, s, 3-H), 7.17 (2 H, br s, 5- and 9-H), 4.31 (2 H, q, ^3J 7.08 Hz, CH_2), 1.32 (2 H, t, ^3J 7.08Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 163.0. (C=O), 155.3 (3-CH), 146.2 (6- and 8-C), 141.5 (7-C), 121.4 (4-C), 116.7 (CN), 111.2 (5- and 9-CH), 95.9 (2-C), 61.9 (CH_2), 14.2 (CH_3); *m/z* 249 (M^+, 39%), 221 ($M^+ - CH_2CH_3$, + H, 14), 204 ($M^+ - OCH_2CH_3$, 13.5), 193 (M^+ – CO, – CH_2CH_3, + H, 20), 165 (M^+ – CO, – CH_2CH_3, – HCN, 46), 148 (M^+ – CO, – $HOCH_2CH_3$, – HCN, 57), 119 (M^+ – 2 × CO, – CH_2CH_3, – HCN, 100), 102 (49), 91 (50), 76 (53), 65 (31).

2-Cyano-3-(3,4,5-trihydroxyphenyl)propenamide (**80**)—Using Method A, 3,4,5-dihydroxybenzaldehyde (0.20 g,1.30 mmol) and cyanoacetamide (0.15 g, 1.8 mmol) were reacted in ethanol (2 ml) and while stirring piperidine (1 drop) was added. After 3 h, precipitation was induced on ice to afford a yellow solid: (0.25 g, 71 %), m.p. 260 °C (decomp.); R_F 0.08 (diethyl ether); ν_{max}(KBr disc)/cm^{-1} 3410s ($CONH_2$ and OH), 2965m (Ar-H), 2215w (CN), 1680m (C=O), 1655s (Ar and C=C), 635m (Ar–H); δ_H(200 MHz; DMSO-d_6) 7.87 (1 H, s, 3-H), 7.75 and 7.62 (2 H, 2 × br s, NH_2), 7.05 (2 H, br s, 5- and 9-H), 5.8 (3 H, v.br, 3 × OH); δ_C(50 MHz; DMSO-d_6) 163.8 (C=O), 151.2 (3-CH), 146.1 (6- and 8-C), 139.1 (7-C), 122.2 (4-C), 117.4 (CN), 110.3 (5- and 9-CH), 101.1 (2-C); *m/z* 220 (M^+, 6.3%), 204 ($M^+ - NH_2$, 3), 176 ($M^+ - CONH_2$, 6), 165 (M^+ – CN, – CO, 10), 135 (M^+ – H, – $NC\text{-}CH_2CONH_2$, 4), 153 (84), 125 (54), 97 (23), 79 (67), 67 (2.5).

2-Cyano-3-(3,5-dichlorophenyl)propenonitrile (**85**)—Using Method C, 3,5-dichlorobenzaldehyde (1.040 g, 5.94 mmol) and malononitrile (0.393 g, 5.95 mmol) were reacted in absolute ethanol (5 ml) with the addition of 10 % piperidine in ethanol (10 drops). After 6.5 h the precipitate was collected (0.549 g, 41 %), m.p. 106-107 °C (from

EtOH); R_F 0.91 (diethyl ether); (Found: M^+, 221.9785 (for ^{35}Cl). $C_{10}H_4N_2Cl_2$ requires *M*, 221.9752 (for ^{35}Cl)); ν_{max}(KBr disc)/cm^{-1} 3080w, 3060m and 3035w (Ar–H), 2225m (CN), 1590m and 1555s (Ar), 1225m/s, 865m/s (=C–H deformation), 670m/s (=C–Cl); δ_H(200 MHz; acetone-d_6) 8.35 (1 H, s, 3-H), 7.97 (2 H, d, ^4J 1.54, 5- and 9-H), 7.79 (1 H, t, ^4J 1.62, 7-H); δ_C(50 MHz; acetone-d_6) 158.5 (3-CH), 136.5 (6- and 8-C), 135.1 (4-C), 133.7 (7-CH), 129.3 (5- and 9-CH), 114.0 and 113.0 (2 × CN), 86.8 (2-C); *m/z* 226 (($^{37}Cl)M^+$, 9%), 224 (($^{35}Cl/^{37}Cl)M^+$, 46), 222 (($^{35}Cl)M^+$, 72), 197 (($^{35}Cl/^{37}Cl)M^+$ – HCN, 33), 195 (($^{35}Cl)M^+$ – HCN, 20), 189 (($^{37}Cl)M^+$ – ^{37}Cl, 33), 187 (($^{35}Cl)M^+$ – ^{35}Cl and ($^{35}Cl/^{37}Cl)M^+$ – ^{37}Cl, 100), 175 (($^{37}Cl)M^+$ – 2 × CN, + H, 9), 173 (($^{35}Cl/^{37}Cl)M^+$ – 2 × CN, + H, 46), 171 (($^{35}Cl)M^+$ – 2 × CN, + H, 72), 160 (($^{35}Cl)M^+$ – ^{35}Cl or ($^{35}Cl/^{37}Cl)M^+$ – ^{37}Cl, – HCN, 8), 152 (M^+ – 2 × Cl, 16), 124 (M^+ – 2 × HCl, – CN, 18), 136 (M^+ – 2 × CN, – H^{35}Cl, 5), 111 (5), 99 (12).

2-Cyano-3-(3,5-dichlorophenyl)propenothioamide (**86**)—Using Method C, 3,5-dichlorobenzaldehyde (0.497 g, 2.82 mmol) and cyanothioacetamide (0.284 g, 2.84 mmol) were reacted in absolute ethanol (5 ml) with the addition of piperidine (3 drops). After 6 h the solution was concentrated *in vacuo* to an oil which was subjected to column chromatography through silica using a concentration gradient of 4:1 to 2:1 petroleum ether/ethyl acetate. The yellow main fraction obtained (0.352 g) was crystallised at *ca.* -15 °C in diisopropyl ether triturated with *n*-pentane (0.120 g, 17 %), m.p. 126-128 °C (from *i*-Pr_2O/*n*-pentane); R_F 0.81 (diethyl ether); (Found: M^+, 255.9661 (for ^{35}Cl). $C_{10}H_6N_2SCl_2$ requires *M*, 255.9629 (for ^{35}Cl)); ν_{max}(KBr disc)/cm^{-1} 3416m, 3357m and 3196m ($CSNH_2$), 3073m (=C–H), 2187m (CN), 1629m/s (C=C), 1569 s (Ar), 1434m/s, 808m (=C–H deformation), 685m (=C–Cl); δ_H(200 MHz; acetone-d_6) 9.07 and 8.16 (2 × 1 H, 2 × br s, NH_2), 7.48 (2 H, d, ^4J 1.81, 3-H), 7.36 (2 H, d, ^4J 1.87, 5- and 9-H), 7.33 and 7.27 (2 × 1 H, 2 × t, ^4J 1.89, 7-H); δ_C(50 MHz; acetone-d_6), 135.2 and 134.8 (6- and 8-C), 131.7 (4-C), 129.9 (3-CH), 129.0 (7-CH), 128.6 (5- and 9-CH), 117.7 (1-CN), 116.2 (2-C); *m/z* 260 (($^{37}Cl)M^+$, 6%), 259 (($^{37}Cl)M^+$ – H, 10), 258 (($^{35}Cl/^{37}Cl)M^+$, 31), 257 (($^{35}Cl/^{37}Cl)M^+$ – H, 41), 256 (($^{35}Cl)M^+$, 46), 255 (($^{35}Cl)M^+$ – H, 52), 230 (($^{35}Cl)M^+$ – CN, 5), 224 (($^{37}Cl)M^+$ – ^{37}Cl, + H, 47), 223 (($^{37}Cl)M^+$ – ^{37}Cl, 26), 222 (($^{35}Cl)M^+$ – ^{35}Cl and ($^{35}Cl/^{37}Cl)M^+$ – ^{37}Cl, + H, 72), 221 (($^{35}Cl)M^+$ – ^{35}Cl and ($^{35}Cl/^{37}Cl)M^+$ – ^{37}Cl, 31), 197 (($^{35}Cl/^{37}Cl)M^+$ – $CSNH_2$, 15), 195 (($^{35}Cl)M^+$ – $CSNH_2$, 23), 187 (M^+ – 2 × Cl, 100), 152 (M^+ – 2 × Cl, – H_2S, 18),

135 (M^+ – HCN, – $H^{35}Cl$, – $CSNH_2$, 6.5), 124 (M^+ – 2 × HCl, – $CSNH_2$, 24), 111 (12), 99 (19).

Ethyl 2-cyano-3-(3,5-dichlorophenyl)propenoate (**87**)—Using Method C, 3,5-dichlorobenzaldehyde (0.304 g, 1.74 mmol) and ethyl cyanoacetate (0.196 g, 1.73 mmol) were reacted in absolute ethanol (5 ml) with the addition of 10 % piperidine in ethanol (3 drops). After 0.5 h the white dense precipitate was collected (0.439 g, 93 %), m.p. 117.5-118.5 °C (from EtOH); R_F 0.91 (diethyl ether); (Found: M^+, 269.0010 (for ^{35}Cl). $C_{12}H_9NO_2Cl_2$ requires *M*, 269.0016 (for ^{35}Cl)); ν_{max}(KBr disc)/cm^{-1} 3065m/w (=C–H), 2905m/w and 2935m/w (CH_x), 2228m (CN), 1715s (C=O), 1610s and1560m/s (Ar), 1418m/s, 1278m/s and 1210m/s (C–O), 865m (=C–H deformation), 670m (=C–Cl); δ_H(200 MHz; DMSO-d_6; 333 K) 8.40 (1 H, s, 3-H), 8.06 (2 H, br s, 5- and 9-H), 7.84 (1 H, br s, 7-H), 4.39 (2 H, q, ^3J 6.98 Hz, CH_2), 1.38 (3 H, t, ^3J 7.00 Hz, CH_3); δ_C(50 MHz; DMSO-d_6; 333 K) 161.17 ($CO_2CH_2CH_3$), 151.86 (3-CH), 134.98 (6- and 8-C), 134.79 (4-C), 131.90 (7-CH), 128.79 (5- and 9-CH), 114.43 (1-CN), 106.18 (2-C), 62.77 (CH_2), 13.98 (CH_3); *m/z* 273 (($^{37}Cl)M^+$, 6%), 272 (($^{37}Cl)M^+$ – H, 5), 271 (($^{35}Cl/^{37}Cl)M^+$, 28), 270 (($^{35}Cl/^{37}Cl)M^+$ – H, 8), 269 (($^{35}Cl)M^+$, 42), 268 (($^{35}Cl)M^+$ – H, 3), 243 (($^{35}Cl)M^+$ – CN, 37), 242 (($^{35}Cl/^{37}Cl)M^+$ – C_2H_4, 20), 241 (($^{35}Cl)M^+$ – C_2H_4 and ($^{35}Cl/^{37}Cl)M^+$ – C_2H_5, 59), 240 (($^{35}Cl)M^+$ – C_2H_5, 22.5), 236 (($^{37}Cl)M^+$ – ^{37}Cl, 11), 234 (($^{35}Cl)M^+$ – ^{35}Cl and ($^{35}Cl/^{37}Cl)M^+$ – ^{37}Cl, 27), 226 (($^{35}Cl/^{37}C)M^+$ – OC_2H_5, 25), 224 (($^{35}Cl)M^+$ – OC_2H_5, 38), 199 (M^+ – 2 × Cl, 22.5), 198 (($^{35}Cl/^{37}Cl)M^+$ – $CO_2C_2H_5$, 30), 196 (($^{35}Cl)M^+$ – $CO_2C_2H_5$, 38), 135 (M^+ – HCN, – $H^{35}Cl$, –$CO_2C_2H_5$, 11), 124 (M^+ – 2 × HCl, – $CO_2C_2H_5$, 24), 109 (34), 99 (38).

2-Cyano-3-(3,5-difluorophenyl)propenonitrile (**88**)—Using Method C, 3,5-difluorobenzaldehyde (0.621 g, 4.37 mmol) and malononitrile (0.289 g, 4.37 mmol) were reacted in absolute ethanol (3 ml) with the addition of 10 % piperidine in ethanol (2 drops). After 5.5 h the precipitate was collected, washed with ether (some material dissolved) and dried with petroleum ether (30-40 °C) (0.150 g, 18 %), m.p. 82-83 °C (from EtOH); R_F 0.87 (diethyl ether); (Found: M^+, 190.0351. $C_{10}H_4N_2F_2$ requires *M*, 190.0346); ν_{max}(KBr disc)/cm^{-1} 3110w, 3090m/w and 3030w (Ar–H), 2230m (CN), 1615m (C=C), 1585s and 1430s (Ar), 1330s, 1130s and 990s (C–F), 865m/s (=C–H deformation), 670m/s (=C–F deformation); δ_H(200 MHz; acetone-d_6) 8.39 (1 H, s, 3-H), 7.97 (2 H, br d, ^3J_F

6.25 Hz, 5- and 9-H), 7.79 (1 H, tt, ^3J_F 8.93 Hz, $^4J_{5\text{-H}/9\text{-H}}$ 2.22 Hz, 7-H); δ_C(50 MHz; acetone-d_6) 164.0 (dd, ^1J_F 247.4 Hz, ^3J_F 19.4 Hz, 6- and 8-C), 159.0 (s, 3-CH), 135.0 (m, 4-C), 114.10 (d, ^2J_F 26.9 Hz, 5- and 9-CH), 114.0 (2 × CN), 109.8 (t, ^3J_F 24.3 Hz, 7-CH), 86.6 (s, 2-C); *m/z* 190 (M^+, 77%), 163 (M^+ – HCN, 100), 139 (M^+ – H-C≡C-CN, 32), 112 ($M^+ - H_2C{=}C(CN)_2$, 15), 99 (12), 88 (6.5), 75 (9).

2-Cyano-3-(3,5-difluorophenyl)propenamide (**89**)—Using Method C, 3,5-difluorobenzaldehyde (0.176 g, 1.24 mmol) and cyano acetamide (0.104 g, 1.24 mmol) were reacted in absolute ethanol (5 ml) with the addition of 3 drops of ethanolic piperidine (1:10 dilution). After 7.5 h no precipitate formed and thus the reaction was concentrated *in vacuo* and the gum obtained was crystallised from isopropanol to give the title compound: (0.043 g, 17 %), m.p. 134-136 °C (from *i*-PrOH); R_F 0.52 (diethyl ether); (Found: M^+, 208.0461; $C_{10}H_6N_2OF_2$ requires *M*, 208.0448); ν_{max}(KBr disc)/cm^{-1} 3440m, 3180m and 3410m ($CONH_2$), 3100m and 3020w (Ar–H), 2215m (CN), 1695s and 1675s (C=O), 1615m (C=C), 1600s, 1580s and 1435s (Ar), 1390s, 1340s, 1305s, 1130s and 990s (C–F), 870m/s and 850m (=C–H defomations), 675m and 670m (=C–F deformations); δ_H(200 MHz; acetone-d_6) ($CONH_2$ signals coincide with aromatic region), 7.27-7.06 (4 H, m, 3-H plus 5- and 9-H), 7.05-6.94 (2 H, m, *ca.* triplet, 7-H); δ_C(50 MHz; acetone-d_6) 163.9 (dd, ^1J_F 244.7 Hz, ^3J_F 19.1, 6- and 8-C), 117.4 (m, 4-C), 114.1 (m, 5- and 9-CH), (1-CN coincides with 5- and 9-CH), 103.7 (m, 7-CH), (2-C may overlap with 7-CH); *m/z* 208 (M^+, 92%), 207 (M^+ – H, 100), 192 ($M^+ - NH_2$, 19), 189 (M^+ – F, 14), 164 ($M^+ - CONH_2$, 53), 144 ($M^+ - CONH_2$, – HF, 48), 138 (M^+ – H-C≡C-H, – HCN, 32), 124 ($M^+ - CONH_2$, – 2 × HF, 17.5), 113 ($M^+ - HC{=}C(CN)_2$, 22.5), 99 (12), 88 (12), 75 (12).

Ethyl 2-cyano-3-(3,5-difluorophenyl)propenoate (**90**)—Using Method C, 3,5-difluorobenzaldehyde (0.540 g, 3.80 mmol) and ethyl cyanoacetate (0.430 g, 3.80 mmol) were reacted in isopropanol (5 ml) with the addition 10 % piperidine in isopropanol (4 drops). After 1 h the white precipitate was collected (0.660 g, 73 %), m.p. 97-98 °C (from EtOH); R_F 0.89 (diethyl ether); (Found: M^+, 237.0609. $C_{12}H_9NO_2F_2$ requires *M*, 237.0601); ν_{max}(KBr disc)/cm^{-1} 3077m and 3035w (Ar–H), 2228w (CN), 1724s (C=O), 1612m/s (C=C), 1590m/s and 1442m/s (Ar), 1368m, 1326m, 1263s, 1239m/s and 1000m (C–O and C–F), 875m/s and 861m (=C–H defomations), 672m (=C–F deformation); δ_H(200 MHz; acetone-d_6) 8.34 (1 H, s, 3-H), 7.70 (2 H, dm, ^3J_F 6.72 Hz, 5- and 9-H), 7.33

(1 H, tt, ^3J_F 8.96 Hz, $^4J_{5\text{-}H/9\text{-}H}$ 2.30 Hz, 7-H), 4.37 (2 H, q, ^3J 7.12 Hz, CH_2), 1.36 (3 H, t, ^3J 7.13 Hz, CH_3); δ_C(50 MHz; acetone-d_6) 163.8 (dd, ^1J_F 248.5 Hz, ^3J_F 13.0 Hz, 6- and 8-C), 162.1 (s, $CO_2CH_2CH_3$), 159.0 (t, ^4J_F 2.9 Hz, 3-CH), 135.7 (t, ^3J_F 10.1 Hz, 4-C), 115.4 (1-CN), 114.3 (d, ^2J_F 26.5 Hz, 5- and 9-CH), 108.8 (t, ^3J_F 26.0 Hz, 7-CH), 107.2 (s, 2-C), 63.5 (CH_2), 14.3 (CH_3); *m/z* 237 (M^+, 68%), 218 ($M^+ - F$, 18), 209 ($M^+ - CH_2CH_2$, 53), 192 ($M^+ - OCH_2CH_3$, 48), 190 (M^+ – HCN, – HF, 10), 171 (M^+ – HCN, – HF, – F, 10), 164 ($M^+ - CO_2CH_2CH_3$, 100), 144 ($M^+ - CO_2CH_2CH_3$, – HF, 73), 138 ($M^+ - CO_2CH_2CH_3$, – CN, 63), 124 ($M^+ - CO_2CH_2CH_3$, – 2 × HF, 16), 113 ($M^+ - CO_2CH_2CH_3$, H-C≡C-CN, 20), 99 (6), 87 (11), 75 (6).

Ethyl 2-cyano-3-(2-pyridyl)propenoate (**91**)—Using Method A, 2-pyridylcarbaldehyde (0.10 g, 0.93 mmol) and ethyl cyanoacetate (0.11 g, 0.94 mmol) were reacted in EtOH (1 ml) and while stirring piperidine (1 drop) was added. After 2 h, the yellow precipitate was collected: (0.12 g, 64 %), m.p. 81-87 °C (from EtOH); R_F 0.24 (diethyl ether); (Found: M^+, 203.0829. $C_{11}H_{11}N_2O_2$ requires *M*, 203.0821); ν_{max}(KBr disc)/cm^{-1} 3100m and 3030m (py–H), 2995w (C–H), 2220m (CN), 1720s (C=O), 1600m (C=C), 810m, 795m, 760m, 645s (2-py and =C–H); δ_H(200 MHz; DMSO-d_6) 8.34 (1 H, br d, $^3J_{7\text{-}H}$ 7.83 Hz, 6-H), 8.41 (1 H, s, 3-H), 8.03 (1 H, ddd, $^3J_{6\text{-}H}$ 7.83 Hz, $^3J_{8\text{-}H}$ 7.12 Hz, $^4J_{9\text{-}H}$ 1.17 Hz, 7-H), 8.01 (1 H, br d, $^3J_{8\text{-}H}$ 4.69 Hz, 9-H), 7.62 (1 H, ddd, $^3J_{7\text{-}H}$ 7.12 Hz, $^3J_{9\text{-}H}$ 4.69 Hz, $^4J_{6\text{-}H}$ 1.90 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 163.0 (C=O), 153.0 (3-CH), 150.3 (6-CH), 137.7 (8-CH), 128.6 (9-CH), 127.1 (7-CH), 114.8 (CN), 149.6 (4-C), 105.7 (2-C), 62.6 (CH_2), 14.1 (CH_3); *m/z* 203 (M^+, 1.2%), 158 ($M^+ - OCH_2CH_3$, 44), 130 ($M^+ - CO_2CH_2CH_3$, 100), 103 (M^+ – HCN, – $CO_2CH_2CH_3$, 5), 78 (*py*, 40), 51 (53).

2-Cyano-3-(3-pyridyl)propenonitrile (**92**)—Using Method B, 3-pyridinecarbaldehyde (5.690 g, 53.12 mmol) was added to malononitrile (2.709 g, 41.01 mmol) in EtOH (20 ml) followed by 10 % piperidine in ethanol (2 ml). After 0.75 h, the orange precipitate was collected:(5.593 g, 88 %), m.p. 77-78 °C (from EtOH); R_F 0.32 (diethyl ether); (Found: M^+, 155.0467. $C_9H_5N_3$ requires *M*, 155.0483); ν_{max}(KBr disc)/cm^{-1} 3030m and 3005m (py–H), 2200s (CN), 1595m, 1590s and 1580s (C=C and py), 1410s ($C=N_{py}$), 695s (2-py and =C–H); δ_H(200 MHz; $CDCl_3$) 8.85 (1 H, br d, $^4J_{7\text{-}H}$ 2.4 Hz, 5-H), 8.77 (1 H, dd, $^3J_{8\text{-}H}$ 4.83 Hz, $^4J_{9\text{-}H}$ 1.59 Hz, 7-H), 8.42 (1 H, dddd, $^3J_{8\text{-}H}$ 8.20 Hz, $^4J_{5\text{-}H}$ 2.30 Hz, $^4J_{7\text{-}H}$ 1.60 Hz, $^4J_{3\text{-}H}$ 0.56 Hz, 9-H), 7.83 (1 H, br d, $^4J_{9\text{-}H}$ 0.5 Hz, 3-H), 7.62 (1 H,

ddd, $^3J_{9\text{-}H}$ 8.20 Hz, $^3J_{7\text{-}H}$ 4.86 Hz, $^5J_{5\text{-}H}$ 0.6 Hz, 8-H); δ_C(50 MHz; $CDCl_3$) 156.6 (5-CH), 154.4 (7-CH), 152.1 (3-CH), 135.5 (9-CH), 126.8 (4-C), 124.1 (8-CH), 112.8 and 111.9 (2 × CN), 85.2 (2-C); *m/z* 155 (M^+, 100%), 128 (M^+ – HCN, 59), 104 (M^+ – H-C≡C-CN, 85), 101 (M^+ – 2 × HCN, 46), 77 (*py* – H, 8), 73 (2.5), 64 (6), 50 (74).

2-Cyano-3-(3-pyridyl)propenothioamide (**93**)—Using Method B, 3-pyridinecarbaldehyde (1.0 g, 9.34 mmol) and cyanothioacetamide (0.47 g, 4.67 mmol) were reacted in EtOH (3 ml) and while stirring piperidine (1 drop) was added. After 2 h, the dark red precipitate was collected: (0.54 g, 61 %), m.p. 170 °C (decomp.); R_F 0.21 (diethyl ether); (Found: M^+, 189.0379. $C_9H_7N_3S$ requires *M*, 189.0391); ν_{max}(KBr disc)/cm^{-1} 3310m and 3230m (NH_2), 3060m (py–H), 2210m (CN), 1655s (C=S), 1585m and 1580s (C=C and py), 1410s ($C{=}N_{py}$), 895s and 695s (2-py and =C–H); δ_H(200 MHz; DMSO-d_6) 10.30 and 9.80 (2 × 1 H, 2 × br s, NH_2), 9.01 (1 H, d, ^4J 1.7 Hz, 5-H), 8.68 (1 H, m, 9-H), 8.43 (1 H, br d, 7-H), 8.17 (1 H, s, 3-H), 7.64 (1 H, dd, $^3J_{9\text{-}H}$ 7.96 Hz, $^3J_{7\text{-}H}$ 4.79 Hz, 8-H); *m/z* 189 (M^+, 91.5%), 163 (M^+ – CN, 5.5), 155 (M^+ – H_2S, 8.5), 146 (M^+ – HCN, – NH_2, 5), 129 (M^+ – $CSNH_2$, 52), 122 (M^+ – NH_2, – H-C≡C-CN, 3), 102 (M^+ – HCN, – $CSNH_2$, 41.5), 90 (M^+ – NC-CH-$CSNH_2$, 3), 79 (16), 78 (12.5), 75 (23), 65 (5.5), 63 (22.5), 61 (10), 60 (94), 51 (71).

Ethyl 2-cyano-3-(3-pyridyl)propenoate (**94**)—Using Method A, 3-pyridinecarbaldehyde (0.50 g, 4.67 mmol) and ethyl cyanoacetate (0.53 g, 4.67 mmol) were reacted in EtOH (3 ml) and while stirring piperidine (1 drop) was added. After 0.5 h, the yellow precipitate was collected: (0.59 g, 62 %), m.p. 73-74 °C (from EtOH); R_F 0.45 (diethyl ether); (Found: M^+, 202.756. $C_{11}H_{10}N_2O_3$ requires *M*, 202.0737); ν_{max}(KBr disc)/cm^{-1} 3030m and 3005m (py–H), 2225s (CN), 1720 (C=O), 1610s, 1585s and 1565m (C=C and py), 1415s ($C{=}N_{py}$), 820s, 765m and 705s (2-py and =C–H); δ_H(200 MHz; DMSO-d_6) 9.13 (1 H, br s, 5-H), 8.79 (1 H, br d, $^3J_{7\text{-}H}$ 4.6 Hz, 7-H), 8.51 (2 H, br s, 3- and 9-H), 7.66 (1 H, br dd, $^3J_{9\text{-}H}$ 8.00 Hz, $^3J_{7\text{-}H}$ 4.84 Hz, 8-H), 4.36 (2 H, q, ^3J 7.08 Hz, CH_2), 1.35 (3 H, q, ^3J 7.12 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 161.4 (C=O), 153.3 (5-CH), 152.3 (7-CH), 152.2 (3-CH), 136.5 (9-CH), 127.7 (4-C), 124.3 (8-CH), 115.3 (CN), 105.0 (2-C), 62.7 (CH_2), 14.0. (CH_3); *m/z* 202 (M^+, 30%), 173 (M^+ – CH_2CH_3, 38), 157 (M^+ – OCH_2CH_3, 58), 147 (M^+ – CH_2CH_3, – CO, 2), 129 (M^+ – $CO_2CH_2CH_3$, 39), 103 (M^+ – CN, – $CO_2CH_2CH_3$, 20), 102 (22), 101 (7), 75 (46), 51 (52).

2-Cyano-3-(3-pyridyl)propenamide (**95**)—Using Method B, 3-pyridylcarbaldehyde (0.5 g, 4.67 mmol) was added to cyanoacetamide (0.4 g, 4.73 mmol) in EtOH (5 ml) followed by 10 % piperidine in ethanol (1 ml) After 0.5 h, the white precipitate was collected: (0.79 g, 98 %), m.p. 227-235 °C (from EtOH); R_F 0.14 (diethyl ether); (Found: M^+, 173.0616. $C_9H_7N_3O$ requires *M*, 173.0587); ν_{max}(KBr disc)/cm^{-1} 3340m, 3130 m/s and 3280w ($CONH_2$), 2120m (CN), 1720s ($CONH_2$), 1675s (C=C), 1595s and 1490m/w (Ar), 1415m, 1380m and 1225m (C–X), 695m/s and 635m (Ar–H deformations); δ_H(200 MHz; DMSO-d_6) 9.02 (1 H, d, ^4J 2.04 Hz, 5-H), 8.76 (1 H, dd, ^3J 4.79 Hz, ^4J 1.49 Hz, 7-H), 8.42 (1 H, dm, ^3J 8.14 Hz, ^4J 1.8 Hz, 9-H), 8.30 (1 H, s, 3-H), 8.07 and 7.94 (2 × 1 H, 2 × br s, CONH2), 7.65 (1 H, dd, ^3J 8.04 Hz, ^3J 4.42 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 162.3 ($CONH_2$), 152.5 (5-CH), 151.4 (7-CH), 147.9 (3-CH), 136.0 (9-CH), 128.2 (4-C), 124.3 (8-CH), 116.2 (1-CN), 109.0 (2-C); *m/z* 173 (M^+, 70%), 172 (M^+ – H, 100), 156 (M^+ – OH, 35), 147 (M^+ – CN, 14), 145 (M^+ – CO, 17), 129 (M^+ – $CONH_2$, 35.5), 118 (M^+ – CO, – HCN, 15), 103 (M^+ – $CONH_2$, – CN, 41), 91 (6), 76 (79), 73 (7.5), 63 (16.5).

2-Cyano-3-(2-imidazoyl)propenonitrile (**96**)—Using Method B, 2-imidazolecarbaldehyde (0.10 g, 1.04 mmol) and malononitrile (0.068 g, 1.05 mmol) were heated at 40 °C in EtOH (0.75) with 5 % piperidine in ethanol (1 ml). After 0.5 h the reaction was cooled and the addition of water gave a yellow precipitate: (0.081 g, 54 %), m.p. 165-169 °C (from EtOH); R_F 0.20 (diethyl ether); (Found: M^+, 144.0424. $C_7H_4N_4$ requires *M*, 144.0413); ν_{max}(KBr disc)/cm^{-1} 3145m and 3110m (HN_{im}), 3015m (im–H), 2230s (CN), 1610s (C=C), 790s and 775s (2-im and =C–H); δ_H(200 MHz; acetone-d_6) 13.23 (1 H, br s, 8-NH), 7.96 (1 H, s, 3-H), 7.57 (2 H, br s, 6- and 7-H); δ_C(50 MHz; acetone-d_6) 150.8 (3-C), 147.6 (4-C), 134.0 (6- and 7-CH (broad)), 119.7 and 118.0. (2 × CN), 83.7 (2-C); *m/z* 144 (M^+, 100%), 117 (M^+ – HCN, 33), 90 (M^+ –2 × HCN, 16), 77 (*HC=C(CN)*$_2$, 5), 68 (*im*, 41), 66 (*NC-CH*$_2$*-CN*, 13), 63 (17), 53 (1), 52 (10).

3-Amino-2,4-dicyano-5-(2-imidazoyl)penta-2,4-dienonitrile (**97**) — Using Method B, 2-imidazolecarbaldehyde (0.10 g, 1.04 mmol) and 1,1,3-tricyano-2-aminopropene (0.137 g, 1.05 mmol) were reacted in EtOH (0.75 ml) with 5 % piperidine in ethanol (1 ml). After 1 h, the yellow solid was collected: (0.125 g, 57 %), m.p. 173-177 °C (from EtOH); R_F 0.15 (diethyl ether); (Found: M^+, 210.0663. $C_{10}H_6N_6$ requires *M*,

210.0659); ν_{max}(KBr disc)/cm^{-1} 3395m (NH_2), 3330m and 3285m (HN_{im}), 3135m and 3055 (im–H), 2220s and 2200s (CN), 1660s and 1620m (C=C), 745m (im–H and =C–H); δ_H(200 MHz; DMSO-d_6) 13.17 (1 H, br s, 8-NH), 9.11 and 9.04 (2 × 1 H, 2 × br s, NH_2), 7.63 (1 H, s, 3-H), 7.38 (2 H, br s, 6- and 7-H); δ_C(50 MHz; acetone-d_6) 166.7 (3-C), 146.9 (6-C), 140.6 (4-C), 138.3 (5-CH), 134.0(9-C (broad), 122.5 (8-C (broad), 116.0 (115.2 and 115.0, 3 × CN), 100.2 (2-C); *m/z* 210 (M^+, 22.5%), 184 (M^+ – CN, 100), 145 (M^+ – NC-CH_2-CN,), 129 (M^+ – NC-CH_2-CN, NH_2, 10), 118 (M^+ – H_2N-C=C-$(CN)_2$, 9), 96 (15.5), 92 (10), 90 (4), 81 (*im*CH_2, 1), 77 (14), 68 (27), 52 (26).

2-Cyano-3-(2-imidazoyl)propenothioamide (**98**)—Using Method B, 2-imidazolecarbaldehyde (0.10 g, 1.04 mmol) and cyanothioacetamide (0.10 g, 1.05 mmol) were reacted in EtOH (3 ml) with 5 % piperidine in ethanol (1 ml) After 1 h, water was added and the red solid was collected: (0.088 g, 48 %), m.p. 183 °C (decomp.); R_F 0.72 (diethyl ether); (Found: M^+, 178.0401. $C_7H_6N_4S$ requires *M*, 178.0397); ν_{max}(KBr disc)/cm^{-1} 3320s (NH_2), 3180s (HN_{im}), 2210s (CN), 1615s (C=C), 800w and 710w (im–H and =C-H); δ_H(200 MHz; DMSO-d_6) 13.12 (1 H, br s, 8-NH), 10.15 and 9.43 (2 × 1 H, 2 × br s, NH_2), 8.09 (1 H, s, 3-H), 7.45 (2 H, m, 6- and 7-H); *m/z* 178 (M^+, 100%), 161 (M^+ – NH_2, 5), 150 (M^+ – CN, 14.5), 118 (M^+ – $CSNH_2$, 89), 92 (M^+ – CN, – $CSNH_2$, 54), 68 (*im*, 93), 77 (25), 68 (93), 52 (75).

Ethyl 2-cyano-3-(2-imidazoyl)propenoate (**99**)—Using Method B, 2-imidazolecarbaldehyde (0.10 g, 1.04 mmol) and ethyl cyanoacetate (0.12 g, 1.05 mmol) were reacted in EtOH (1 ml) with 5 % piperidine in ethanol (1 ml). After *ca.* 1 h, the white solid was collected: (0.186 g, 94 %), m.p. 195 °C (decomp.); R_F 0.24 (diethyl ether); (Found: M^+, 191.0712. $C_9H_9N_3O_2$ requires *M*, 191.0735); ν_{max}(KBr disc)/cm^{-1} 3145m and 3105m (HN_{im}), 3030m (im–H), 2920m (=C–H), 2220s (CN), 1720s (C=O), 1625s (C=C), 795m and 760m (=C–H); δ_H(200 MHz; acetone-d_6) 12.92 (1 H, br s, 8-NH), 8.09 (1 H, s, 3-H), 7.48 (2 H, br s, 6- and 7-H), 4.32 (2 H, q, CH_2), 1.32 (3 H, t, ^3J 7.83 Hz, CH_3); δ_C(50 MHz; acetone-d_6) 163.2 (C=O), 140.9 (4-C), 140.0 (3-C), 134.0. (7-CH (broad)), 123.3 (6-CH (broad)), 115.3 (CN), 100.0. (2-C); *m/z* 191 (M^+, 14%), 146 (M^+ – OCH_2CH_3, 36), 118 (M^+ – $CO_2CH_2CH_3$, 57), 92 (M^+ – CN, – $CO_2CH_2CH_3$, 30), 68 (*im*, 33), 64 (34), 52 (18).

2-Cyano-3-(2-imidazoyl)propenoamide (**100**)—Using Method B, 2-imidazolecarbaldehyde (0.10 g, 1.04 mmol) and cyanoacetamide (0.089 g, 1.03 mmol) were reacted in EtOH (1 ml) with 10 % piperidine in ethanol (1 ml) After 1.2 h, the white solid was collected: (0.059 g, 35 %), m.p. 178-180 °C (from EtOH); R_F 0.12 (diethyl ether); (Found: M^+, 162.0544. $C_7H_6N_4O$ requires *M*, 162.0522); ν_{max}(KBr disc)/cm^{-1} 3440m, 3330m and 3180m ($CONH_2$), 3070m (Ar–H), 2210m (CN), 1770s ($CONH_2$), 770m (Ar–H deformations); δ_H(200 MHz; DMSO-d_6) 8-NH (not observed in range), 9.69 (1 H, s), 7.94 (1 H, s), 7.79 (2 H, br s), 7.48 (3 H, d); δ_C(50 MHz; DMSO-d_6) 162.7 ($CONH_2$), 140.5 (4-C), 136.7 (3-CH), 127.4 (6- snd 7-CH (broad)), 116.2 (1-CN), 103.1 (2-C); *m/z* 162 (M^+, 13%), 145 ($M^+ - NH_3$, 10), 134 (M^+ – HCN, – H, 8), 118 ($M^+ - CONH_2$, 54), 107 (M^+ – CO, – HCN, 6), 96 ([H_2C=$C(CN)(CONH_2)$], 8), 92 (17), 79 ([$C_4H_3N_2$], 11), 68 ([$C_3H_4N_2$= *imidazyl*], 38).

2-Cyano-3-(2-thienyl)propenonitrile (**101**)—Using Method B, 2-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and malononitrile (0.29 g, 4.45 mmol) were reacted in EtOH (2.75) with 5% piperidine in ethanol (1 ml). After *ca.* 1 h, the pale orange solid was collected and crystallised from ethanol/water: (0.362 g, 51 %), m.p. 88-90 °C (from EtOH/H_2O); R_F 0.84 (diethyl ether); (Found: M^+, 160.0076. $C_8H_4N_2S$ requires *M*, 159.9978); ν_{max}(KBr disc)/cm^{-1} 3100m, 3090w and 3025m (th–H), 2230s (CN), 1570s (C=C), 1410s (C–S_{th}), 740s (=C–H); δ_H(200 MHz; $CDCl_3$) 7.91 (1 H, AB, $^3J_{6\text{-H, 8-H}}$ 0.7 Hz, 3-H), 7.90 (1 H, dAB, $^3J_{7\text{-H}}$ 5.22 Hz, $^4J_{6\text{-H}}$ 1.0 Hz, $^4J_{3\text{-H}}$ 0.5 Hz, 8-H,), 7.82 (1 H, ddd, $^3J_{7\text{-H}}$ 3.88 Hz, $^4J_{8\text{-H}}$ 1.08 Hz, $^5J_{3\text{-H}}$ 0.53 Hz, 6-H), 7.27 (1 H, dd, $^3J_{6\text{-H}}$ 3.91 Hz, $^3J_{8\text{-H}}$ 5.19 Hz, 7-H); δ_C(50 MHz; $CDCl_3$) 152.3 (3-CH), 136.9 (8-CH), 134.0 (4-C), 128.4 (6-CH), 126.7 (7-CH), 113.8 and 113.0 (2 × CN), 80.5 (2-C); *m/z* 160 (M^+, 100%), 134 (M^+ – CN, 5), 116 (M^+ – C≡S, 8), 109 (M^+ – H-C≡C-CN, 39), 100 (M^+ – CN, – H_2S, 2), 89 (M^+ – HCN, – C≡S, 4), 77 (*HC=C(CN)*$_2$, 3), 52 (*HC=CH-CN*, 1.5).

2,4-Cyano-3-amino-5-(2-thienyl)penta-2,4-dienonitrile (**102**)—Using Method A, 2-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and 1,1,3-tricyano-2-aminopropene (0.59 g, 4.45 mmol) were reacted in EtOH (3 ml) with piperidine (1 drop). After 1 h, the yellow precipitate (0.812 g, 81 %) was collected and crystallised from ethanol/water: (0.417 g, 42 %), m.p. 237-240 °C (from EtOH/H_2O); R_F 0.48 (diethyl ether); (Found: M^+, 226.0306. $C_{11}H_6N_4S$ requires *M*, 226.0319); ν_{max}(KBr disc)/cm^{-1} 3380s and 3340s (NH_2), 3225s and

disc)/cm^{-1} 3380s and 3340s (NH_2), 3225s and 3080m (th–H), 220s and 2210s (CN), 1665s and 1575m (C=C), 1410m (C–S_{th}), 735m (=C-H); δ_H(200 MHz; acetone-d_6) 8.6 and 8.4 (2 × 1 H, 2 × br s, NH_2), 8.26 (1 H, AB, $^3J_{6\text{-H},\ 8\text{-H}}$ 0.6 Hz, 3-H), 8.10 (1 H, dAB, $^3J_{7\text{-H}}$ 5.06 Hz, $^4J_{6\text{-H}}$ 1.16 Hz, $^4J_{3\text{-H}}$ 0.85 Hz, 8-H), 7.92 (1 H, ddd, $^3J_{7\text{-H}}$ 3.82 Hz, $^4J_{8\text{-H}}$ 1.22 Hz, $^5J_{3\text{-H}}$ 0.56 Hz, 6-H), 7.34 (1 H, dd, $^3J_{6\text{-H}}$ 3.81 Hz, $^3J_{8\text{-H}}$ 5.04 Hz, 7-H); δ_C(50 MHz; acetone-d_6) 166.2 (3-C), 146.5 (5-CH), 144.1 (4-C), 139.0. (10-CH), 136.5 (6-C), 136.3 (8-CH), 129.4 (9-CH), 115.6, 115.1 and 111.3 (3 × CN), 98.9 (2-C); *m/z* 226 (M^+, 100%), 200 (M^+ – CN, 56.5), 193 (M^+ – HS, 13), 184 (M^+ – CN, – NH_2, 2.5), 182 (M^+ – C≡S, 18), 172 (M^+ – 2 × HCN, 8.5), 168 (M^+ – CNS, 23.5), 161 (M^+ – $CH(CN)_2$, 9), 156 (M^+ – CH, – C≡S, 4), 134 (M^+ – H_2N-C=C$(CN)_2$, 13), 97 (*th*CH_2, 2), 92 (*H_2N-C=C(CN)*$_2$, 2.5), 66 (*CH_2(CN)*$_2$, 12), 57 (9), 45 (44).

2-Cyano-3-(2-thienyl)propenothioamide (**103**)—Using Method A, 2-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and cyanothioacetamide (0.45 g, 4.47 mmol) were reacted in EtOH (3 ml) with 10 % piperidine in ethanol (1.5 ml). After *ca.* 1 h, the dark red precipitate (0.651 g, 75%) was collected and crystallised from water/ethanol: (0.55 g, 64 %), m.p. 163-167 °C (from H_2O/EtOH); R_F 0.83 (diethyl ether); (Found: M^+, 193.9986. $C_8H_6N_2S_2$ requires *M*, 193.9978); ν_{max}(KBr disc)/cm^{-1} 3360s and 3260s (NH_2), 3135s (th-H), 2200m (CN), 1620s (C=S), 1575s (C=C), 1410m (C–S_{th}), 880s and 740s (=C–H), 535s; δ_H(200 MHz; acetone-d_6) 9.26 and 8.67 (2 × 1 H, 2 × br s, NH_2), 8.66 (1 H, AB, $^3J_{6\text{-H},\ 8\text{-H}}$ 0.7 Hz, 3-H), 8.05 (1 H, dAB, $^3J_{7\text{-H}}$ 5.07 Hz, $^4J_{6\text{-H}}$ 1.14 Hz, $^4J_{3\text{-H}}$ 0.90 Hz, 8-H,), 7.95 (1 H, ddd, $^3J_{7\text{-H}}$ 3.82 Hz, $^4J_{8\text{-H}}$ 1.18 Hz, $^5J_{3\text{-H}}$ 0.60 Hz, 6-H), 7.34 (1 H, dd, $^3J_{6\text{-H}}$ 3.82 Hz, $^3J_{8\text{-H}}$ 5.05 Hz, 7-H); δ_C(50 MHz; acetone-d_6) 193.2 (C=S), 145.4 (3-CH), 139.0 .(8-CH), 137.1 (4-C), 135.8 (6-CH), 129.4 (7-CH), 116.8 or 111.1 (CN), 107.8 (2-C); *m/z* 194 (M^+, 100%), 168 (M^+ – CN, 6.5), 149 (M^+ – H, – C≡S, 2), 134 (M^+ – $CSNH_2$, 30), 122 (M^+ – HCN, – C≡S, – H, 8.5), 106 (M^+ – HCN, – $CSNH_2$, 5), 94 (6), 90 (9), 76 (6), 60 (29), 52 (5.5), 45 (24).

Ethyl 2-cyano-3-(2-thienyl)propenoate (**104**)—Using Method A, 2-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and ethyl cyanoacetate (050 g, 4.46 mmol) were reacted in EtOH (3 ml) with piperidine (1 drop). After *ca.* 0.5 h, the white precipitate was collected: (0.78 g, 85 %), m.p. 95-96 °C (from EtOH) (lit.,[330] 95-96 °C); R_F 0.65 (diethyl ether); (Found: M^+, 207.0357. $C_{10}H_9NO_2S$ requires *M*, 207.0345); ν_{max}(KBr

disc)/cm^{-1} 3085s, 3070m and 3025m (th–H), 2985m and 2940m (CH_2 and CH_3), 2220m (CN), 1715s (C=O), 1600s (C=C), 1415m (C–S_{th}), 735s (=C–H); δ_H(200 MHz; acetone-d_6) 8.49 (1 H, br s, 3-H), 8.08 (1 H, dAB, $^3J_{7\text{-H}}$ 5.08 Hz, $^4J_{6\text{-H, 3-H}}$ 1.0 Hz, 8-H), 7.99 (1 H, dAX, $^3J_{7\text{-H}}$ 3.82 Hz, $^4J_{8\text{-H}}$ 1.2 Hz, $^5J_{3\text{-H}}$ 0.60 Hz, 6-H), 7.27 (1 H, dd, $^3J_{6\text{-H}}$ 3.87 Hz, $^3J_{8\text{-H}}$ 5.02 Hz, 7-H), 4.32 (2 H, q, ^3J 7.12 Hz, CH_2), 1.33 (3 H, t, ^3J 7.12 Hz, CH_3); δ_C(50 MHz; acetone-d_6) 163.0 (C=O), 147.6 (3-CH), 139.6 (8-CH), 136.8 (4-C), 136.7 (6-CH), 129.5 (7-CH), 116.2 (CN), 99.8 (2-C), 62.9 (CH_2), 14.4 (CH_3); *m/z* 207 (M^+, 100%), 178 ($M^+ - CH_2CH_3$, 5), 162 ($M^+ - OCH_2CH_3$, 93), 134 ($M^+ - CO_2CH_2CH_3$, 85), 108 ($M^+ - CN, - CO_2CH_2CH_3$, 35), 90 ($M^+ - NC\text{-}CH_2\text{-}CO_2CH_2CH_3$, 33), 84 (*th*, 9), 63 (26), 45 (46).

2-Cyano-3-(2-thienyl)propenamide (**105**)—Using Method A, 2-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and cyanoacetamide (0.45 g, 4.47 mmol) were reacted in EtOH (3 ml) with piperidine (2 drops). After 10 min, the orange precipitate was collected: (0.781 g, 99 %), m.p. 152-153 °C (from EtOH); R_F 0.35 (diethyl ether); (Found: M^+, 178.0202. $C_8H_6N_2OS$ requires *M*, 178.0190); ν_{max}(KBr disc)/cm^{-1} 3470s, 3360w, 3300w and 3235w (NH_2), 3140s (th–H), 2210s (CN), 1700s (C=O), 1580s (C=C), 1380s (C–S_{th}), 745s (=C–H), 460s; δ_H(200 MHz; DMSO-d_6) 7.89 and 7.78 (2 × 1 H, 2 × br s, NH_2), 8.45 (1 H, s, 3-H), 8.12 (1 H, d, $^3J_{7\text{-H}}$ 4.94 Hz, 8-H), 7.90 (1 H, d, $^3J_{7\text{-H}}$ 3.28 Hz, 6-H), 7.34 (1 H, dd, $^3J_{6\text{-H}, 8\text{-H}}$ 4.4 Hz, 7-H); δ_C(50 MHz; DMSO-d_6) 162.8 (C=O), 143.7 (3-CH), 137.9 (8-CH), 136.0 (4-C), 135.0 (6-CH), 128.7 (7-CH), 116.8 (CN), 102.1 (2-C); *m/z* 178 (M^+, 100%), 161 ($M^+ - NH_3$, 30), 145 (M^+ – HS, 8), 134 ($M^+ - CONH_2$, 36), 123 (M^+ – HCN, – CO, 2), 112 ($M^+ - NH_2$, – HO, – HS, 14), 108 (M^+ – CN, – $CONH_2$, 39), 90 (18), 75 (9), 63 (25.5), 50 (14).

2-Cyano-3-(3-thienyl)propenonitrile (**106**)—Using Method A, 3-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and malononitrile (0.29 g, 4.47 mmol) were reacted in EtOH (2.75 ml) 10 % piperidine in ethanol (1.5 ml). After *ca.* 1 h, the pale yellow solid was collected (0.70 g, 98 %) and crystallised from water: (0.084, 12 %), m.p. 84-85 °C (from H_2O); R_F 0.84 (diethyl ether); (Found: M^+, 160.0074. $C_8H_4N_2S$ requires *M*, 159.9978); ν_{max}(KBr disc)/cm^{-1} 3125m, 3100w and 3235w (NH_2), 3140s (th–H), 2210s (CN), 1700s (C=O), 1580s (C=C), 1380s (C–S_{th}), 745s (=C–H), 460s; δ_H(200 MHz; $CDCl_3$) 8.18 (1 H, ddd, $^4J_{8\text{-H}}$ 3.00 Hz, $^5J_{7\text{-H}}$ 1.31 Hz, $^5J_{3\text{-H}}$ 0.50 Hz, 5-H), 7.79 (1 H, dAX, $^4J_{5\text{-H}}$ 1.3 Hz, $^5J_{3\text{-H}}$ 0.3 Hz, 7-H), 7.78 (1 H, AB, $^5J_{5\text{-H}, 8\text{-H}}$ 0.4 Hz, 3-H), 7.52 (1 H, ddd, $^3J_{7\text{-H}}$ 5.25

Hz, $^4J_{5\text{-H}}$ 3.01 Hz, $^4J_{3\text{-H}}$ 0.49 Hz, 8-H); δ_C(50 MHz; $CDCl_3$) 152.3 (3-CH), 136.9 (8-CH), 134.0 (4-C), 128.4 (5-CH), 126.8 (7-CH), 113.8 and 113.0 (2 × CN), 80.5 (2-C); *m/z* 160 (M^+, 100%), 133 (M^+ – HCN, 47), 116 (M^+ – C≡S, 4), 109 (M^+ – H-C≡C-CN, 51), 89 (M^+ – HCN, – C≡S, 3), 84 (*th*, 2), 75 (7), 69 (16), 58 (19).

2-Amino-2,4-dicyano-3-(3-thienyl)penta-2,4-dienonitrile (**107**) — Using Method B, 3-thiophenelcarbaldehyde (0.50 g, 4.46 mmol) and 1,1,3-tricyano-2-aminopropene (0.59 g, 4.47 mmol) were reacted in EtOH (3 ml) with 10 % piperidine in ethanol (1 ml) After *ca.* 1 h, the yellow solid was collected (0.78, 79 %), m.p. 197-199 °C (from EtOH); R_F 0.45 (diethyl ether); (Found: M^+, 226.0315. $C_{11}H_6N_4S$ requires *M*, 226.0312); ν_{max}(KBr disc)/cm^{-1} 3340m and 3220m (NH_2), 3110w and 3090w (Ar–H), 2220s and 2210s (CN), 1665s ($(H_2N)C=C(CN)_2$), 1580s (broad Ar), 795m and 625m (Ar–H deformations); δ_H(200 MHz; acetone-d_6) 8.98 and 8.75 (2 × 1 H, 2 × br s, NH_2), 8.42 (1 H, dm, $^4J_{7\text{-H}}$ 1.31 Hz, $^4J_{8\text{-H}}$ 2.91 Hz, 5-H), 8.04 (1 H, s, 3-H), 7.87 (1 H, dt, $^4J_{5\text{-H}}$ 1.26 Hz, $^3J_{8\text{-H}}$ 5.18 Hz, 7-H), 7.77 (1 H, dd, $^4J_{5\text{-H}}$ 2.93 Hz, $^3J_{7\text{-H}}$ 5.18 Hz, 8-H); δ_C(50 MHz; acetone-d_6) 166.2 (H_2N-C=$C(CN)_2$), 147.0 (5-CH), 136.8 (3-CH), 135.0 (4-C), 129.2 (7-CH), 126.8 (8-CH), 115.8 and 115.2 (H_2N-C=C(C≡N)$_2$), 100.6 (2-C), 50.9 (H_2N-C=C$(CN)_2$); *m/z* 226 (M^+, 100%), 200 (M^+ – CN, 63), 199 (M^+ – HCN, 45), 193 (M^+ – HS, 14), 182 (M^+ – C≡S, 21), 166 (M^+ – HCN, – HS, 2), 161 (M^+ – $HC(CN)_2$, 7), 139 (M^+ – HCN, – C≡S, – NH_2, 2), 134 (M^+ – H_2N-C=$C(CN)_2$, 9), 84 ([C_4H_4S= *3-thienyl*], 3), 65 (7.5).

2-Cyano-3-(3-thienyl)propenothioamide (**108**)—Using Method A, 3-thiophenelcarbaldehyde (0.50 g, 4.46 mmol) and cyanothioacetamide (0.45 g, 4.47 mmol) were reacted in EtOH (2.75 ml) with 10 % piperidine in ethanol (1.5 ml). After *ca.* 1 h, the brown solid was collected (0.391 g, 45 %) and crystallised from ethanol/water: (0.226, 16 %), m.p. 150-151 °C (from EtOH/H_2O); R_F 0.84 (diethyl ether); (Found: M^+, 193.9979. $C_8H_6N_2S_2$ requires *M*, 193.9978); ν_{max}(KBr disc)/cm^{-1} 3360s and 3260m (NH_2), 3140s and 3055m (th–H), 2210s (CN), 1620s (C=S), 1590s (C=C), 1410m (C–S_{th}), 790s (=C–H), 535s; δ_H(200 MHz; acetone-d_6) 9.28 and 8.73 (2 × 1 H, 2 × br s, NH_2), 8.48 (1 H, AB, $^5J_{5\text{-H, 8-H}}$ 0.7 Hz, 3-H), 8.43 (1 H, ddd, $^4J_{8\text{-H}}$ 2.92 Hz, $^5J_{7\text{-H}}$ 1.31 Hz, $^4J_{3\text{-H}}$ 0.56 Hz, 5-H), 7.92 (1 H, ddd, $^3J_{8\text{-H}}$ 5.21 Hz, $^5J_{5\text{-H}}$ 1.33 Hz, $^4J_{3\text{-H}}$ 0.45 Hz, 7-H), 7.73 (1 H, ddd, $^3J_{7\text{-H}}$ 5.22 Hz, $^4J_{5\text{-H}}$ 2.95 Hz, $^4J_{3\text{-H}}$ 0.56 Hz, 8-H); δ_C(50 MHz; acetone-d_6) 193.7 (C=S), 145.5 (3-CH), 136.4 (8-CH), 135.6 (4-C), 128.9 (5-CH), 127.6 (7-CH), 117.1 (CN), 109.9 (2-C); *m/z* 194 (M^+,

100%), 178 ($M^+ - NH_2$, 4), 168 ($M^+ - CN$, 5.5), 161 ($M^+ - HS$, 33), 150 ($M^+ - C{\equiv}S$, 3), 140 ($M^+ - 2 \times HCN$, 3), 134 ($M^+ - CSNH_2$, 25.5), 126 ($M^+ - NH_2$, – H-C≡C-CN, 7), 107 ($M^+ -$ CN, $- CSNH_2$, + H, 7), 97 (4), 94 (3), 90 (14), 84 (*th*, 6.5), 76 (8), 60 (80), 52 (21), 45 (94).

Ethyl 2-cyano-3-(3-thienyl)propenoate (**109**)—Using Method A, 3-thiophenecarbaldehyde (0.50 g, 4.46 mmol) and ethyl cyanoacetate (0.50 g, 4.46 mmol) were reacted in EtOH (3 ml) with 1 piperidine (1 drop). After *ca.* 0.5 h, the white solid was collected: (0.782, 85 %), m.p. 66-67 °C (from EtOH); R_F 0.70 (diethyl ether); (Found: M^+, 207.0361. $C_{10}H_9NO_2S$ requires *M*, 207.0345); ν_{max}(KBr disc)/cm^{-1} 3090m and 3030m (th-H), 2985m, 2940w and 2920w (CH_2 and CH_3), 2215m (CN), 1720s (C=O), 1600s (C=C), 1360m (C-S_{th}), 810s, 795m and 760m (=C–H), 635s; δ_H(200 MHz; DMSO-d_6) 8.43 (1 H, br s, 3-H), 8.63 (1 H, dd, $^4J_{8\text{-H}}$ 2.66 Hz, $^5J_{7\text{-H}}$ 1.14 Hz, 5-H), 7.91 (1 H, dd, $^3J_{8\text{-H}}$ 5.17 Hz, $^5J_{5\text{-H}}$ 1.16 Hz, 7-H), 7.84 (1 H, dd, $^3J_{7\text{-H}}$ 5.13 Hz, $^4J_{5\text{-H}}$ 2.85 Hz, 8-H), 4.33 (2 H, q, ^3J 7.11 Hz, CH_2), 1.36 (3 H, t, ^3J 7.10 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 162.3 (C=O), 148.3 (3-CH), 138.4 (8-CH), 134.4 (4-C), 129.2 (5-CH), 126.4 (7-CH), 116.1 (CN), 100.0 (2-C), 62.3 (CH_2), 14.1 (CH_3); *m/z* 207 (M^+, 100%), 178 ($M^+ - CH_2CH_3$, 88), 161 ($M^+ -$ OCH_2CH_3, – H, 60), 151 ($M^+ - CH_2CH_3$, – CN, 7), 134 ($M^+ - CO_2CH_2CH_3$, 85), 110 ($M^+ -$ H-C≡C-CN, – OCH_2CH_3, – H, 7), 108 (M^+ – CN, – $CO_2CH_2CH_3$, 55), 90 ($M^+ -$ NC-CH_2-$CO_2CH_2CH_3$, 33.5), 84 (*th*, 10), 63 (33), 45 (51).

2-Cyano-3-(3-thienyl)propenamide (**110**)—Using Method A, 3-thiophenelcarbaldehyde (0.50 g, 4.46 mmol) and cyanoacetamide (0.38 g, 4.47 mmol) were reacted in EtOH (3 ml) with piperidine (2 drops). After 2 h, the reaction solution was poured onto ice and the white solid was collected: (0.559, 71 %), m.p. 104-106 °C (from EtOH/H_2O); R_F 0.49 (diethyl ether); (Found: M^+, 178.0199. $C_8H_6N_2OS$ requires *M*, 178.0190); ν_{max}(KBr disc)/cm^{-1} 3450s and 3330s (NH_2), 3190s (th–H), 2200 (CN), 1645s (C=O), 1585s (C=C), 745m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.42 (1 H, br d, 5-H), 8.25 (1 H, br d, 7-H), 7.83 (1 H, s, 3-H), 7.90 and 7.80 (2 × 1 H, 2 × br s, NH_2), 7.8 (1 H, br d, 8-H); δ_C(50 MHz; DMSO-d_6) 163.0 (C=O), 144.4 (3-CH), 135.6 (8-CH), 134.6 (4-C), 128.9 (5-CH), 126.4 (7-CH), 117.1 (CN), 104.1 (2-C); *m/z* 178 (M^+, 100%), 161 ($M^+ - NH_3$, 19), 136 (M^+ – CN, $- NH_2$, 6), 134 ($M^+ - CONH_2$, 43.5), 112 ($M^+ - CH_2(CN)_2$, 76), 108 ($M^+ -$ CN, $- CONH_2$, 52), 97 (*th*CH_2, M^+ – NC-CH-$CONH_2$, + H, 3), 90 ($M^+ - C{\equiv}S$, $- CONH_2$, 16.5), 84 (*th*, 5), 63 (14), 58 (14).

2-cyano-3-(2-pyridylimino)indolizine (**111**)—A 25 ml two-necked round bottomed flask, fitted with equilibrating dropping funnel (10 ml) and temperature probe, was charged with 2-pyridinecarbaldehyde (1.003 g, 9.36 mmol), a magnetic stirrer and of absolute ethanol (5 ml). The reaction was cooled to -6.0 °C on an ice/salt water bath and while stirring an ethanolic solution (5 ml) of malononitrile (0.617 g, 9.34 mmol), doped with DBU (3 drops), was added dropwise over 10 minutes. On addition the reaction turned distinctly black in colour and was stirred at -3.0 and -6.0 °C for 1h. After a further 1.5 h at room temperature the black solid which formed was collected (1.048 g, 72 %) and the mother liqours concentrated onto silica *in vacuo* for preparation for column chromatography. Using a concentration gradient from 1:1 ethyl acetate/petroleum ether to 100 % ethyl acetate a yellow product was isolated (0.154 g) and crystallised from isopropanol to give a product assigned to the title compound (44 mg), m.p. 167.5-168.0 °C (from *i*-PrOH); R_F 0.59 (diethylether); ν_{max}(KBr disc)/cm^{-1} 3107m, 3078m, 3062m and 2926m (=C–H), 2218s (CN), 1630m (broad C=C), 1575m and 1563m (*2-py*), 1408s and 1239s (C–$N_{2\text{-}py}$), 772s and 740s (=C–H); δ_H(200 MHz; acetone-d_6) 9.21 (1 H, s, 11-H), 8.69 (1 H, ddd, $^3J_{15\text{-}H}$ 4.6 Hz, $^4J_{16\text{-}H}$ 2.0 Hz, $^5J_{17\text{-}H}$ 1.0 Hz, 14-H), 8.66 (1 H, dq, $^3J_{3\text{-}H}$ 7.1 Hz, $^4J_{4\text{-}H}$ 1.1 Hz, 2-H), 8.38 (1 H, dt, $^3J_{16\text{-}H}$ 8.1 Hz, $^4J_{15\text{-}H}$ 1.1 Hz, 17-H), 7.93 (1 H, dddd, $^3J_{15\text{-}H}$ 7.8 Hz, $^3J_{16\text{-}H}$ 7.8 Hz, $^4J_{14\text{-}H}$1.8 Hz, $^5J_{11\text{-}H}$ 0.7 Hz, 16-H), 7.55 (1 H, dt, $^3J_{4\text{-}H}$ 8.8 Hz, $^4J_{3\text{-}H}$ 1.2 Hz, 5-H), 7.47 (1 H, ddd, $^3J_{16\text{-}H}$ 7.6 Hz, $^3J_{14\text{-}H}$ 4.9 Hz, $^4J_{17\text{-}H}$ 1.2 Hz, 15-H), 7.04 (1 H, ddd, $^3J_{5\text{-}H}$ 8.8, $^3J_{3\text{-}H}$ 6.6, $^4J_{2\text{-}H}$ 1.2 Hz, 4-H), 6.99 (1 H, d, $^5J_{2\text{-}H}$ 0.5 Hz, 7-H), 6.93 (1 H, td, $^3J_{2\text{-}H/4\text{-}H}$ 6.8 Hz, $^4J_{5\text{-}H}$ 1.5 Hz, 3-H); δ_C(50 MHz; acetone-d_6) 155.49 (s), 155.09 (d), 150.50 (d), 137.28 (d, 132.23 (6d), 125.80 (d), 123.22 (d), 122.44 (d), 121.39 (d), 120.17 (d), 117.03 (CN), 114.57 (d), 105.38 (d), 86.23 (s); *m/z* 246 (M^+, 55%), 245 (M^+ – H, 100), 220 (M^+ – CN, 8), 219 (M^+ – HCN, 4), 194 (M^+ – H-C≡C-CN, 1), 168 (M^+ – [*2-py*]$^+$ *i.e.* $[C_5H_4N]^+$, 15), 142 (M^+ – [*2-py*]$^+$, – CN, 9), 114 (M^+ – [*2-py*]-CH=NH, – CN, 13), 105 (3), 88 (4), 79 ([*2-py-H*]$^+$, 16.5), 51 (H-C≡C-CN, 10).

Methyl 2-cyano-3-phenylpropenoate (**113**)—Using Method D, benzaldehyde (8.12 g, 0.077 mol) and methyl cyanoacetate (7.67 g, 0.077 mmol) were reacted in methanol (25 ml) with the addition of piperidine (10 drops) and potassium *tert*-butoxide (*ca.* 20 mg). After 29 h the crystals were collected (8.34 g, 58 %), m.p. 86-87 °C (from MeOH) ; R_F 0.83 (diethyl ether); (Found: C, 73.12; H, 5.01; N, 7. 54; M^+, 187.0642. $C_{11}H_9NO_2$ requires C,

73.32; H, 5.03; N, 7.77%; *M*, 187.0633); ν_{max}(KBr disc)/cm^{-1} 3070w, 3033w and 3008w (=C–H), 2954w (CH_3), 2225w (CN), 1729s (CO_2Bu^t), 1607m/s and 1571m (Ar and C=C), 1267s, 1205s and 1190m/s (C–O), 768m/s and 684m (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.13 (1 H, s, 3-H), 7.86 (2 H, dd, $^3J_{6\text{-H}/8\text{-H}}$ 7.7 Hz, $^3J_{7\text{-H}}$ 1.6 Hz, 5- and 9-H), 7.41 (3 H, m, 6- and 7- and 8-H), 3.81 (3 H, s, CH_3); δ_C(50 MHz; $CDCl_3$) 162.8 ($\underline{C}O_2CH_3$), 155.2 (3-CH), 133.4 (7-CH), 131.3 (4-C), 131.0 (5- and 9-CH), 129.2 (6- and 8-CH), 115.4 (1-CN), 102.5 (2-C), 53.3 ($CO_2\underline{C}H_3$); *m/z* 187 (M^+, 100%), 172 ($M^+ - CH_3$, 8), 156 ($M^+ - OCH_3$, 98), 142 (M^+ – HCN, – H_2O, 8), 128 ($M^+ - CO_2CH_3$, 98), 121 ($M^+ - CH_3$, – H-C≡C-CN, 33), 101 ($M^+ - CO_2Me$, – HCN, 29), 77 ([*Ph*]$^+$, M^+ – H-C≡C-CN, – CO_2Me, 56).

Ethyl 2-cyano-3-phenylpropenoate (**114**)—Using Method D, benzaldehyde (6.48 g, 0.061 mol) and ethyl cyanoacetate (6.97 g, 0.062 mmol) were reacted in ethanol (20 ml) with the addition of piperidine (10 drops) and potassium *tert*-butoxide (20 mg). After 8 h the crystals were collected (6.54 g, 53 %), m.p. 63-65 °C (from EtOH) (lit.,[331] 63-65 °C); R_F 0.87 (diethyl ether); (Found: C, 71.47; H, 5.48; N, 6.88; M^+, 201.0803. $C_{12}H_{11}NO_2$ requires C, 71.63; H, 5.51; N, 6.96%; *M*, 201.0790); ν_{max}(KBr disc)/cm^{-1} 3030w and 3002w (=C–H), 2980w and 2940w (CH_x), 2224w (CN), 1727s (CO_2Bu^t), 1607m/s and 1574m (Ar and C=C), 1257s, 1202s, 1189m/s and 1011m/s (C–O), 768m/s (=C--H deformation); δ_H(200 MHz; $CDCl_3$) 8.50 (1 H, s, 3-H), 7.85 (2 H, dd, $^3J_{6\text{-H}/8\text{-H}}$ 7.8 Hz, $^3J_{7\text{-H}}$ 1.5 Hz, 5- and 9-H), 7.40 (3 H, m, 6- and 7- and 8-H), 4.23 (2 H, q, ^3J 7.14 Hz, $\underline{C}H_2CH_3$), 1.25 (3 H, t, ^3J 7.14 Hz, $CH_2\underline{C}H_3$); δ_C(50 MHz; $CDCl_3$) 162.4 ($\underline{C}O_2CH_2CH_3$), 155.0 (3-CH), 133.3 (7-CH), 131.4 (4-C), 131.0 (5- and 9-CH), 129.2 (6- and 8-CH), 115.5 (1-CN), 102.9 (2-C), 62.7 ($CO_2\underline{C}H_2CH_3$), 14.1 ($CO_2CH_2\underline{C}H_3$); *m/z* 201 (M^+, 89%), 172 ($M^+ - CH_2CH_3$, 86), 156 ($M^+ - OCH_2CH_3$, 92), 128 ($M^+ - CO_2CH_2CH_3$, 100), 102 ($M^+ - CO_2CH_2CH_3$, – CN, 72), 77 ([*Ph*]$^+$, M^+ – H-C≡C-CN, – $CO_2CH_2CH_3$, 85).

Methyl 2-cyano-3-(4-nitrophenyl)propenoate (**115**)—Using Method C, 4-nitrobenzaldehyde (1.453 g, 9.61 mmol) and methyl cyanoacetate (1.001 g, 10.10 mmol) were reacted in methanol (10 ml) with the addition of 5 % piperidine in methanol (5 drops). After 2 h the crystals were collected (1.541 g, 69 %), m.p. 173-174 °C (from EtOAc); R_F 0.88 (3:1 petroleum ether; bp 40-60 °C/diethyl ether); (Found: C, 56.65; H, 3.46; N, 11.83; M^+, 232.0485. $C_{11}H_8N_2O_4$ requires C, 56.90; H, 3.47; N, 12.06%; *M*, 232.0484); ν_{max}(KBr disc)/cm^{-1} 3103w, 3078w and 3034w (=C–H), 3004w, 2982w and 2940w (CH_3), 2250m

(CN), 1717s (CO_2Me), 1597m (Ar and C=C), 1529s (C–NO_2), 1346s and 1156s (C–O), 853m/s (=C–H deformation); δ_H(200 MHz; $CDCl_3$) 8.36 (2 H, d, $^3J_{5\text{-H}/9\text{-H}}$ 8.4 Hz, 6- and 8-H), 8.34 (1 H, s, 3-H), 8.14 (2 H, d, $^3J_{6\text{-H}/8\text{-H}}$ 8.4 Hz, 5- and 9-H), 3.98 (9 H, s, Me); δ_C(50 MHz; $CDCl_3$) 162.0 (CO_2Me), 152.1 (3-CH), 149.6 (7-C), 136.9 (4-C), 131.6 (6- and 8-CH), 124.4 (5- and 9-CH), 114.5 (1-CN), 107.0 (2-C), 53.8 (CH_3); *m/z* 232 (M^+, 14%), 202 (M^+ – N=O, 10), 186 (M^+ – NO_2, 100), 174 (MH^+ – CO_2Me, 63), 159 (M^+ – NO_2, – HCN, 25), 127 (M^+ – CO_2Me, – NO_2, 45), 100 (M^+ – CO_2Me, – NO_2, – HCN, 14), 89 (12), 76 (14).

Ethyl 2-cyano-3-(4-nitrophenyl)propenoate (**116**)—Using Method C, 4-nitrobenzaldehyde (7.210 g, 47.71 mmol) and ethyl cyanoacetate (5.407 g, 47.80 mmol) were reacted in ethanol (30 ml) with the addition of piperidine (2 drops). After 2 h the crystals were collected (8.741 g, 74 %), m.p. 165.5-166.5 °C (from EtOH); R_F 0.82 (diethyl ether); (Found: C, 58.34; H, 4.07; N, 11.12; M^+, 246.0645. $C_{12}H_{10}N_2O_4$ requires C, 58.54; H, 4.09; N, 11.38%; *M*, 246.0641); ν_{max}(KBr disc)/cm^{-1} 3096w, 3078w and 3038w (=C–H), 2992w, 2943w and 2905w (CH_3), 2226w (CN), 1721s (CO_2Bu^t), 1617m and 1594m (Ar and C=C), 1514s (C–NO_2), 1347s and 1267s (C–O), 860m/s, 766m and 687m (=C–H deformations); δ_H(200 MHz; acetone-d_6; 333 K) 8.48 (1 H, s, 3-H), 8.35 (2 H, dm, $^3J_{5\text{-H}/9\text{-H}}$ 8.95 Hz, 6- and 8-H), 8.20 (2 H, dm, $^3J_{6\text{-H}/8\text{-H}}$ 9.1 Hz, 5- and 9-H), 4.35 (2 H, q, ^3J 7.09 Hz, $\underline{CH}_2CH_3$), 1.33 (3 H, t, ^3J 7.09 Hz, $CH_2\underline{CH}_3$); δ_C(50 MHz; acetone-d_6; 333K) 160.8 ($\underline{CO}_2CH_2CH_3$), 152.3 (3-CH), 149.1 (7-C), 137.0 (4-C), 131.3 (6- and 8-CH), 123.8 (5- and 9-CH), 114.5 (1-CN), 106.6 (2-C), 62.5 ($\underline{CH}_2CH_3$), 13.6 ($CH_2\underline{CH}_3$); *m/z* 246 (M^+, 77%), 229 (M^+ – OH, 9), 218 (MH^+ – Et, 100), 216 (M^+ – N=O, 5), 201 (MH^+ – NO_2, 79), 199 (M^+ – HNO_2, 66), 188 (MH^+ – N=O, – Et, 26), 174 (MH^+ – CO_2Et, 20), 173 (M^+ – NO_2, – HCN, 26), 171 (M^+ – Et, – NO_2, 17), 155 (M^+ – OEt, – NO_2, 53), 127 (M^+ – CO_2Et, – NO_2, 57), 100 (M^+ – CO_2Et, – NO_2, – HCN, 35), 89 (34), 76 (39).

Methyl 2-cyano-3-(3-methoxy-4-hydroxy-5-nitrophenyl)propenoate (**117**)—Using Method B, 5-nitrovanillin (3-methoxy-4-hydroxy-5-nitrobenzaldehyde; 0.87 g, 4.41 mmol) and methyl cyanoacetate (0.453 g, 4.57 mmol) were reacted in methanol (15 ml) with the addition of piperidine (5 drops). After 29 h the reaction was concentrated to quarter volume which yielded the title compound (0.524 g, 36 %) on cooling to room temperature, m.p. 185.0-186.5 °C (from MeOH); R_F 0.69 (ethanol); (Found: C, 51.60; H, 3.45; N, 9.96;

M^+, 278.0547. $C_{12}H_{10}N_2O_6$ requires C, 51.81; H, 3.62; N, 10.07%; *M*, 278.0539); ν_{max}(KBr disc)/cm^{-1} 3430m (OH), 2954m and 2840w (CH_x), 2214w (CN), 1723s (CO_2Me), 1633m, 1604m and 1586m/s (Ar or C=C), 1545s (C–NO_2), 1435m, 1276m/s, 1236s, 1215s and 1158s (C–O), 760m (=C–H deformation); δ_H(200 MHz; DMSO-d_6) 8.51 (1 H, s, OH), 8.16 (1 H, d, $^4J_{5\text{-}H}$ 2.14 Hz, 9-H), 7.99 (1 H, s, 3-H), 7.55 (1 H, d, $^4J_{9\text{-}H}$ 2.17 Hz, 5-H), 3.80 (3 H, s, 6-OMe), 3.72 (3 H, s, CO_2Me); δ_C(50 MHz; DMSO-d_6) 164.9 and 164.3 (CO_2CH_3), 154.8 (8-C), 154.0 (3-CH), 130.64 (9-CH), 130.1 (6-C), 118.6 (4-C), 110.2 (1-CN), 108.1 (5-CH), 87.69 (2-C), 55.3 (OCH_3), 52.4 (CO_2CH_3); *m/z* 278 (M^+, 100%), 261 (M^+ – OH, 4), 247 (M^+ – H-N=O, 6), 230 (M^+ – H_2NO_2, 11), 215 (M^+ – HNO_2, – Me, 100), 204 (M^+ – HCN, – HNO_2, 3), 203 (MH^+ – CO_2Me, – OH, 3), 199 (M^+ – HNO_2, – MeOH, 10), 188 (M^+ – HNO_2, – Me, – HCN, 20), 173 (M^+ – NO_2, – MeOH, – HCN, 8), 158 (17), 144 (9), 130 (10), 116 (11), 101 (17).

Ethyl 2-cyano-3-(3-methoxy-4-hydroxy-5-nitrophenyl)propenoate (**118**)—Using Method B, 5-nitrovanillin (3-methoxy-4-hydroxy-5-nitrobenzaldehyde; 0.47 g, 2.38 mmol) and ethyl cyanoacetate (0.273 g, 2.41 mmol) were reacted in ethanol (10 ml) with the addition of piperidine (3 drops). After 5 h the reaction was concentrated to quarter volume which yielded the title compound (0.224 g, 34 %) on cooling to room temperature, m.p. 159-162 °C (from EtOH); R_F 0.72 (ethanol); (Found: C, 53.14; H, 4.13; N, 9.37; M^+, 292.0690. $C_{13}H_{12}N_2O_6$ requires C, 53.43; H, 4.14; N, 9.59%; *M*, 292.0695); ν_{max}(KBr disc)/cm^{-1} 3426m (OH), 2950m (CH_x), 2208w (CN), 1732s and 1708m/s (CO_2Et), 1604m (Ar or C=C), 1544s and 1527s (C–NO_2), 1288s, 1236s, 1210s, 1155m/s and 1094m/s (C-O), 760m (=C–H deformation); δ_H(200 MHz; DMSO-d_6) 8.47 (1 H, s, OH), 8.24 (1 H, d, $^4J_{5\text{-}H}$ 2.19 Hz, 9-H), 8.20 (1 H, s, 3-H), 7.79 (1 H, d, $^4J_{9\text{-}H}$ 2.20 Hz, 5-H), 4.30 (1 H, q, ^3J 7.09 Hz, $\underline{CH_2}CH_3$), 1.33 (1 H, t, ^3J 7.09 Hz, $CH_2\underline{CH_3}$), 3.84 (3 H, s, OMe); δ_C(50 MHz; DMSO-d_6) 163.2 ($CO_2CH_2CH_3$), 155.3 (8-C), 153.6 (3-CH), 152.2 (7-C), 137.28 (6-C), 125.6 (9-CH), 117.3 (4-C), 116.0 (1-CN), 112.4 (5-CH), 94.7 (2-C), 61.8 ($\underline{CH_2}CH_3$), 56.1 (OCH_3), 14.2 ($CH_2\underline{CH_3}$); *m/z* 292 (M^+, 63%), 278 (MH^+ – CH_3, 11), 264 (M^+ – CO, 8), 262 (M^+ – N=O, 4), 247 (MH^+ – NO_2, 11), 229 (M^+ – NO_2, – OH, 6), 216 (M^+ – NO_2, – Et, 18), 202 (M^+ – CO_2Me, – OH, 5), 197 (M^+ – NO_2, – OH, – MeOH, 66), 180 (35), 173 (M^+ – NO_2, – EtOH, – HCN, 7), 149 (18), 135 (23), 84 (100).

tert-*Butyl 2-cyano-3-(3,4-dihydroxyphenyl)propenoate* (**121**)—Using Method A, 3,4-dihydroxybenzaldehyde (1.238 g, 6.65 mmol) and *tert*-butyl cyanoacetate (0.942 g, 6.67 mmol) were reacted in isopropanol (8 ml) with the addition of piperidine (4 drops). After 3 h the precipitate was collected (1.241 g, 71 %), m.p. 201-203 °C (from *i*-PrOH); R_F 0.53 (diethyl ether); (Found: C, 64.17; H, 5.78; N, 5.11; M^+, 261.0991. $C_{14}H_{15}NO_4$ requires C, 64.36; H, 5.79; N, 5.36%; *M*, 261.0995); ν_{max}(KBr disc)/cm^{-1} 3245m/s (broad; 2 × OH), 2984m (CH_3), 2232m (CN), 1717s (C=O), 1580s and 1514s (Ar), 1372s, 1308s, 1291s, 1256s, 1157s, 1121m/s and 1098m/s (C–O), 841m and 810m (Ar–H deformations); δ_H(200 MHz; acetone-d_6) 9.0 (2 H, v.br, 2 × OH), 8.06 (1 H, s, 3-H), 7.74 (1 H, d, $^4J_{9\text{-H}}$ 2.19 Hz, 5-H), 7.43 (1 H, dd, $^4J_{5\text{-H}}$ 2.19 Hz, $^3J_{8\text{-H}}$ 8.36 Hz, 9-H), 7.07 (1 H, d, $^3J_{9\text{-H}}$ 8.32 Hz, 8-H), 1.55 (9 H, s, ^{t}Bu); δ_C(50 MHz; acetone-d_6) 162.6 (C=O), 154.6 (3-CH), 151.8 (7-C), 146.3 (6-C), 127.6 (9-CH), 124.8 (4-C), 117.0 (1-CN and 5- or 8-CH), 116.6 (8- or 5-CH), 100.5 (2-C), 83.3 ($\underline{C}(CH_3)_3$), 28.0 ($C(\underline{C}H_3)_3$); *m/z* 261 (M^+, 2 %), 205 ($M^+ - Bu^t$, + H, 21), 188 (M^+ – CN, – Bu^t, 20), 170 ($M^+ - 2 \times CO, - 2 \times H_2O$, + H, 1), 161 ($M^+ - CO_2Bu^t$, + H, 33), 133 ($M^+ - CO_2Bu^t$, – HCN, 30), 114 ($M^+ - 2 \times CO$, $-H_2O$, –O Bu^t, 61), 105 ($M^+ - CO_2Bu^t$, – HC=CH–C=O, 17), 104 (19), 103 (17), 88 (16), 77 (30), 57 ([Bu^t]$^+$, 79).

2-Cyano-3-(3,4-dihydroxyphenyl)propenoic acid (**122**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(3,4-dihydroxyphenyl)propenoate (0.456 g, 1.63 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 10 ml) by heating to reflux for 5 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the precipitate was filtered, washed in ethyl acetate and ether to give the title acid (0.301 g, 82 %), m.p. 207.0-207.5 °C (from HCO_2H) (lit.,[230c] 240 °C); (Found: C, 58.09; H, 3.34; N, 6.71; M^+, 205.0377. $C_{10}H_7NO_4$ requires C, 58.54; H, 3.44; N, 6.83%; *M*, 205.0375); ν_{max}(KBr disc)/cm^{-1} 3499m (sharp) and 3356m (broad; 2 × OH and CO_2H), 2230m (CN), 1671s (C=O), 1593s, 1578s and 1504s (Ar and C=C), 1296s and 1253s (C–O), 818m/w and 632m/w (Ar–H); δ_H(200 MHz; DMSO-d_6) 10.3 and 9.7 (2 H, v.br, 2 × OH), 8.11 (1 H, s, 3-H), 7.67 (1 H, d, $^4J_{9\text{-H}}$ 1.78 Hz, 5-H), 7.40 (1 H, dd, $^4J_{5\text{-H}}$ 1.78 Hz, $^3J_{8\text{-H}}$ 8.35 Hz, 9-H), 6.93 (1 H, d, $^3J_{9\text{-H}}$ 8.28 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 164.3 (C=O), 154.5 (3-CH), 151.8 (7-C), 145.9 (6-C), 126.7 (9-CH), 123.2 (4-C), 117.0 (1-CN), 116.5 (5- or 8-CH), 116.1 (8- or 5-CH), 98.0 (2-C); *m/z* 205 (M^+, 77%), 188 (M^+ – HCN,

13), 161 ($M^+ - CO_2$, 87), 133 ($M^+ - CO_2$, – CN, 73), 114 ($M^+ - CO_2H$, – CO, – H_2O, 62), 105 ($M^+ - CO_2H$, – HC=CH–C=O, 100), 88 (43), 77 (76).

tert-*Butyl 2-cyano-3-(3,5-dichlorophenyl)propenoate* (**123**)—Using Method B, 3,5-dichlorobenzaldehyde (0.234 g, 1.34 mmol) and *tert*-butyl cyanoacetate (0.197 g, 1.40 mmol) were reacted in absolute ethanol (5 ml) with the addition of 10 % piperidine in ethanol (10 drops). After 5 h the precipitate was collected (0.210 g, 53 %), m.p. 85.5-86.0 °C (from EtOH); R_F 0.95 (diethyl ether); (Found: C, 56.31; H, 4.38; N, 4.65; M^+, 297.0351 (for ^{35}Cl). $C_{14}H_{13}NO_2Cl_2$ requires C, 56.40; H, 4.39; N, 4.70%; *M*, 297.0323 (for ^{35}Cl)); ν_{max}(KBr disc)/cm^{-1} 3096w, 3076w and 3016w (=C–H), 2980m and 2939w (CH_3), 2222w (CN), 1722s (C=O), 1613s and1562m (Ar), 1420m/s, 1280m/s, 1256m/s, 1213m/s and 1152s (C–O), 865m and 816m (=C–H), 672m (=C–Cl); δ_H(200 MHz; $CDCl_3$) 8.00 (1 H, s, 3-H), 7.78 (2 H, d, ^4J 1.65 Hz, 5- and 9-H), 7.49 (1 H, t, ^4J 1.81 Hz, 7-H), 1.56 (9 H, s, ^{t}Bu); δ_C(50 MHz; $CDCl_3$) 160.2 (CO_2Bu^t), 150.5 (3-CH), 134.1 (6- and 8-C), (4-C not observed), 132.3 (7-CH), 128.5 (5- and 9-CH), 114.6 (1-CN), 107.9 (2-C), 27.8 (Bu^t); *m/z* 299 (($^{35}Cl/^{37}Cl$)M^+, 3), 297 ((^{35}Cl)M^+, 5), 255 (($^{35}Cl/^{37}C$)M^+ – O^{t}Bu, 1), 253 ((^{35}Cl)M^+ – O^{t}Bu, 1), 227 ($M^+ - 2 \times Cl$, 1), 226 (($^{35}Cl/^{37}Cl$)$M^+ - CO_2{}^t$Bu, 12), 224 ((^{35}Cl)$M^+ - CO_2{}^t$Bu, 38), 161 (M^+ – HCN, – H^{35}Cl, –$CO_2{}^t$Bu, 12), 124 ($M^+ - 2 \times$ HCl, – $CO_2{}^t$Bu, 3), 109 (3), 99 (3), 57 ([^{t}*Bu*]$^+$, 100).

2-Cyano-3-(3,5-dichlorophenyl)propenoic acid (**124**)—A 10 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(3,5-dichlorophenyl)propenoate (0.150 g, 0.503 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 2 ml) by heating to reflux for 10 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in ethyl acetate and ether to give the title acid (0.098 g, 80 %), m.p. 186-188 °C (from HCO_2H); (Found: C, 49.43; H, 2.05; N, 5.67; M^+, 240.9713. $C_{10}H_5NO_2Cl_2$ requires C, 49.62; H, 2.08; N, 5.79%; *M*, 240.9697); ν_{max}(KBr disc)/cm^{-1} 3422m (CO_2H), 3072m (=C–H), 2226m (CN), 1703s (C=O), 1610s and 1563s (Ar), 1420m, 1284m, 1256m, 1215m (C–O), 862m/s and 787m (=C–H deformation), 671m/s (=C–Cl); δ_H(200 MHz; DMSO-d_6) (acid proton not observed), 8.10 (1 H, s, 3-H), 8.00 (2 H, s, 5- and 9-H), 7.81 (1 H, br s, 7-H); δ_C(50 MHz; DMSO-d_6) 162.4 (CO_2H), 148.8 (3-CH), 134.7 (6- and 8-C), 128.0 (5- and 9-CH), 130.4 or 126.42 (7-CH); *m/z* 245 ((^{37}Cl)M^+, 9%), 244 ((^{37}Cl)M^+ – H, 9), 243 (($^{35}Cl/^{37}Cl$)M^+, 50), 242 (($^{35}Cl/^{37}Cl$)M^+ – H,

30), 241 ((^{35}Cl)M^+, 77), 240 ((^{35}Cl)M^+ – H, 32), 214 ((^{35}Cl)M^+ – HCN, 5), 208 ((^{37}Cl)M^+ – ^{37}Cl, 18), 206 ((^{35}Cl)M^+ – ^{35}Cl and (^{35}Cl/^{37}Cl)M^+ – ^{37}Cl, 51), 199 ((^{35}Cl/^{37}C)M^+ – CO_2, 62), 197 ((^{35}Cl)M^+ – CO_2, 98), 170 (M^+ – 2 × Cl, – H, 28), 164 ((^{35}Cl/^{37}C)M^+ – CO_2, – ^{35}Cl, 33), 162 ((^{35}Cl)M^+ – CO_2, – ^{35}Cl, 100), 127 (M^+ – CO_2, – 2 × Cl, 100), 109 (24), 99 (44).

tert-*Butyl 2-cyano-3-(3,5-difluorophenyl)propenoate* (**125**)—Using Method B, 3,5-difluorobenzaldehyde (0.531 g, 3.03 mmol) and *tert*-butyl cyanoacetate (0.450 g, 3.19 mmol) were reacted in absolute ethanol (6 ml) with the addition of 10 % piperidine in ethanol (5 drops). After 4 h the precipitate was collected (0.623 g, 78 %), m.p. 103-104 °C (from EtOH); R_F 0.75 (diethyl ether); (Found: C, 63.12; H, 4.92; N, 5.05; M^+, 265.0896. $C_{14}H_{13}NO_2F_2$ requires C, 63.40; H, 4.94; N, 5.28%; *M*, 265.0914); ν_{max}(KBr disc)/cm^{-1} 3110w, 3088m/w, 3063w and 3028w (Ar–H), 2989m/w and 2945w (^{t}Bu), 2226w (CN), 1714s (C=O), 1620m and 1612m (C=C), 1592m/s, 1456m and 1438m (Ar), 1371m, 1315m, 1281s, 1260m/s, 1150m/s and 996m (C–O and C–F), 862m/s and 834m (=C–H deformations), 674m (=C–F deformation); δ_H(200 MHz; $CDCl_3$) 7.89 (1 H, s, 3-H), 7.31 (2 H, dm, ^3J_F 6.02 Hz, 5- and 9-H), 6.82 (1 H, tt, ^3J_F 8.52 Hz, $^4J_{5\text{-H/9-H}}$ 2.21 Hz, 7-H), 1.41 (9 H, s, ^{t}Bu); δ_C(50 MHz; $CDCl_3$) 162.8 (dd, unresolved, 6- and 8-C), 160.3 (CO_2Bu^t), 151.0 (t, unresolved, 3-CH), 134.2 (s, 4-C), (1-CN weak signal), 113.3 (d, ^2J_F 25.06 Hz, 5- and 9-CH), 108.0 (t, ^3J_F 25.19 Hz, 7-CH), 84.5 (s, 2-C), 27.8 (Bu^t); *m/z* 265 (M^+, 5%), 250 (M^+ – CH_3, 1), 221 (M^+ – CN, – F, + H, 3), 209 (M^+ – Bu^t, + H, 3), 192 (M^+ – OBu^t, 34), 164 (M^+ – CO_2Bu^t, 14), 144 (M^+ – CO_2Bu^t, – HF, 12.5), 138 (M^+ – CO_2Bu^t, – CN, 4), 124 (M^+ – CO_2Bu^t, – 2 × HF, 3), 113 (M^+ – CO_2Bu^t, H-C≡C-CN, 3), 87 (1.5), 57 ([*Bu*^{t}]$^+$, 100).

2-Cyano-3-(3,5-difluorophenyl)propenoic acid (**126**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(3,5-difluorophenyl)propenoate (0.550 g, 2.07 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 5 ml) by heating to reflux for 15 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in ethyl acetate and ether to give the title acid (0.348 g, 80 %), m.p. 177-178 °C (from HCO_2H); (Found: C, 57.11; H, 2.39; N, 6.49; M^+, 209.0291. $C_{10}H_5NO_2F_2$ requires C, 57.43; H, 2.41; N, 6.70%; *M*, 209.0288); ν_{max}(KBr disc)/cm^{-1} 3432m (CO_2H), 3097m and 3039m (Ar–H), 2230m/w (CN), 1697s (C=O), 1608s (C=C), 1585s, 1428s and 1438s (Ar), 1325m/s, 1273s, 1233m, 1131m/s and 992m/s (C–O

and C–F), 871m and 859m (=C–H deformations), 672m (=C–F deformations; δ_H(200 MHz; DMSO-d_6) (acid proton not observed), 8.36 (1 H, s, 3-H), 7.75 (2 H, dm, ^3J_F 6.65 Hz, 5- and 9-H), 7.57 (1 H, tt, ^3J_F 8.06 Hz, $^4J_{5\text{-H/9-H}}$ 2.17 Hz, 7-H); δ_C(50 MHz; DMSO-d_6) 162.7 (s, CO_2H), 162.4 (dd, ^1J_F 247.4 Hz, ^3J_F 13.3 Hz, 6- and 8-C), 151.6 (t, unresolved, 3-CH), 134.8 (t, ^3J_F 10.2 Hz, 4-C), 115.5 (1-CN), 113.5 (d, ^2J_F 26.4 Hz, 5- and 9-CH), 108.2 (t, ^3J_F 26.1 Hz, 7-CH), 107.0 (s, 2-C); *m/z* 209 (M^+, 76%), 190 ($M^+ - F$, 9), 165 ($M^+ - CO_2$, 100), 144 ($M^+ - CO_2H$, – HF, 42), 138 ($M^+ - CO_2H$, – CN, 77), 124 ($M^+ - CO_2H$, – 2 × HF, 12), 114 ($M^+ - CO_2$, H-C≡C-CN, 3), 99 (9), 75 (12).

tert-*Butyl 2-cyano-3-(4-nitrophenyl)propenoate* (**127**)—Using Method B, 4-nitrobenzaldehyde (2.183 g, 14.44 mmol) and *tert*-butyl cyanoacetate (2.103 g, 14.90 mmol) were reacted in ethanol (10 ml) with the addition of piperidine (5 drops). After 8 h the crystals were collected (1.541 g, 39 %), m.p. 148.0-148.8 °C (from EtOH); R_F 0.88 (diethyl ether); (Found: C, 61.05; H, 5.11; N, 10.26; M^+, 274.0951. $C_{14}H_{14}N_2O_4$ requires C, 61.31; H, 5.14; N, 10.21%; *M*, 274.0954); ν_{max}(KBr disc)/cm^{-1} 3103w, 3078w and 3034w (=C–H), 3004w, 2982w and 2940w (CH_3), 2230w (CN), 1717s (CO_2Bu^t), 1686m, 1597m and 1620m (Ar and C=C), 1522s (C–NO_2), 1345s, 1300s, 1291s and 1156s (C–O), 853m/s (=C–H deformation); δ_H(200 MHz; $CDCl_3$) 8.30 (2 H, dm, $^3J_{5\text{-H/9-H}}$ 8.89 Hz, 6- and 8-H), 8.19 (1 H, s, 3-H), 8.08 (2 H, dm, $^3J_{6\text{-H/8-H}}$ 8.80 Hz, 5- and 9-H), 1.56 (9 H, s, ^{t}Bu); δ_C(50 MHz; $CDCl_3$) 160.1 ($CO_2C(CH_3)_3$), 150.8 (3-CH), 149.4 (7-C), 137.1 (4-C), 131.3 (6- and 8-CH), 124.2 (5- and 9-CH), 114.8 (1-CN), 108.9 (2-C), 84.7 ($\underline{C}(CH_3)_3$), 27.8 ($C(\underline{C}H_3)_3$); *m/z* 274 (M^+, 4%), 259 ($M^+ - O$, + H, 1), 244 ($M^+ - N{=}O$, 0.3), 230 ($MH_2^+ - NO_2$, 2), 218 ($MH^+ - Bu^t$, 1), 201 ($M^+ - NO_2$, – HCN, 15), 188 ($MH^+ - N{=}O$, – Bu^t, 3), 174 ($MH^+ - CO_2Bu^t$, 2), 171 ($M^+ - Bu^t$, – NO_2, 1), 155 ($M^+ - OBu^t$, – NO_2, 7), 127 ($M^+ - CO_2Bu^t$, – NO_2, 5), 100 ($M^+ - CO_2Bu^t$, – NO_2, – HCN, 4), 89 (2), 76 (4), 57 ([Bu^t]$^+$, 100).

2-Cyano-3-(4-nitrophenyl)propenoic acid (**128**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(4-nitrophenyl)propenoate (1.034 g, 3.77 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 10 ml) by heating to reflux for 5 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in ethyl acetate and ether to give the title acid (0.745 g, 91 %), m.p. > 300 °C (from HCO_2H) (lit.,[230c] 205 °C); (Found: C, 54.87; H, 2.73; N, 12.61; M^+, 218.0334. $C_{10}H_6N_2O_4$ requires C, 55.05; H, 2.77; N, 12.84%; *M*, 218.0328); ν_{max}(KBr

disc)/cm^{-1} 3468m (broad CO_2H), 3113w, 3094w and 3032w (=C–H), 2218w (CN), 1686s (CO_2H), 1639m and 1593m (Ar and C=C), 1522s (C–NO_2), 1350s, 1298s and 1277s (C–O), 853m/s (=C–H deformation); δ_H(200 MHz; DMSO-d_6) 8.35 (2 H, br d, $^3J_{5\text{-H}/9\text{-H}}$ 8.2 Hz, 6- and 8-H), 8.09 (1 H, s, 3-H), 7.96 (2 H, br d, $^3J_{6\text{-H}/8\text{-H}}$ 8.7 Hz, 5- and 9-H); δ_C(50 MHz; DMSO-d_6) 162.4 (CO_2H), 148.1 (7-C), 144.9 (3-CH), 139.9 (4-C), 130.4 (6- and 8-CH), 124.1 (5- and 9-CH), 113.6 (1-CN), 109.3 (2-C); *m/z* 218 (M^+, 37%), 201 (M^+ – OH, 14), 188 (M^+ – N=O, 16), 174 (M^+ – CO_2, 35), 172 (M^+, – NO_2, 8), 144 (M^+ – HNO_2, – HCN, 55), 128 (M^+ – CO_2, – NO_2, 48), 116 (M^+ – CO_2, – NO, – CO, 100), 101 (M^+ – CO_2, – NO_2, – HCN, 78), 89 (95), 75 (84).

tert-*Butyl 2-cyano-3-(3,4-dimethoxy-6-nitrophenyl)propenoate* (**129**)—Using Method B, 6-nitroveratraldehyde (3,4-dimethoxy-6-nitrobenzaldehyde; 2.214 g, 10.48 mmol) and *tert*-butyl cyanoacetate (1.485 g, 10.52 mmol) was reacted in ethanol (40 ml) with the addition of piperidine (10 drops). After 19 h a precipitate was not observed and thus the reaction was refluxed for 0.5 h and concentrated to half volume which yielded the title acid (2.287 g, 65 %) on cooling to room temperature, m.p. 228-229 °C (from EtOH); R_F 0.79 (diethyl ether); (Found: C, 57.13; H, 5.41; N, 8.62; M^+, 334.1168. $C_{16}H_{18}N_2O_6$ requires C, 57.48; H, 5.43; N, 8.80%; *M*, 334.1165); ν_{max}(KBr disc)/cm^{-1} 3090w (=C–H), 2979w and 2940w (CH_x), 2232w (CN), 1715s (CO_2Bu^t), 1611m and 1567m/s (Ar or C=C), 1524s (C–NO_2), 1330m/s, 1285s, 1226s and 1160s (C–O), 883m, 796m/w and 748m/w (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.58 (1 H, s, 3-H), 7.69 (1 H, s, 8-H), 7.29 (1 H, s, 5-H), 3.96 and 3.94 (6 H, 2 × s, 2 × OMe), 1.52 (9 H, s, Bu^t); δ_C(50 MHz; $CDCl_3$) 160.1 ($CO_2C(CH_3)_3$), 153.2 (9-C), 152.0 (3-CH), 151.0 (7-C), 141.0 (6-C), 121.8 (4-C), 114.9 (1-CN), 111.4 (8-CH), 108.6 (2-C), 108.1 (5-CH), 84.3 ($\underline{C}(CH_3)_3$), 56.8 and 56.6 (2 × OCH_3), 27.8 ($C(\underline{C}H_3)_3$); *m/z* 334 (M^+, 6%), 278 (MH^+ – Bu^t, 1), 261 (M^+ – HCN, – NO_2, (or – OBu^t), 5), 232 (M^+ – NO_2, – Bu^t, 100), 215 (M^+ – OBu^t, – NO_2, 11), 164 (9), 136 (9), 57 ($[Bu^t]^+$, 37).

2-cyano-3-(3,4-dimethoxy-6-nitrophenyl)propenoic acid (**130**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(3,4-dimethoxy-6-nitrophenyl)propenoate (1.437 g, 4.30 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 10 ml) by heating to reflux for 5 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in

ethyl acetate and ether to give the title acid (0.890 g, 74 %), m.p. 226-228 °C (from HCO_2H); (Found: C, 51.72; H, 3.67; N, 9.87; M^+, 278.0531. $C_{12}H_{10}N_2O_6$ requires C, 51.81; H, 3.62; N, 10.07%; *M*, 278.0539); ν_{max}(KBr disc)/cm^{-1} 3328m/s (CO_2H), 3088m/w and 2946m/w (=C–H), 2230w (CN), 1735s and 1702m/s (CO_2H), 1654m/w, 1607m and 1570m/s (Ar or C=C), 1527s (C–NO_2), 1333m/s, 1292s and 1230s (C–O), 884m, 794m and 771m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 8.69 (1 H, s, 3-H), 7.83 (1 H, s, 8-H), 7.57 (1 H, s, 5-H), 3.98 and 3.97 (6 H, 2 × s, 2 × OMe); δ_C(50 MHz; DMSO-d_6) 162.8 ($CO_2C(CH_3)_3$), 153.7 (3-CH), 152.9 (9-C), 150.5 (7-C), 140.8 (6-C), 122.0 (4-C), 115.3 (1-CN), 112.3 (8-CH), 108.3 (5-CH), 107.9 (2-C), 56.8 and 56.5 (2 × OCH_3); *m/z* 278 (M^+, 14%), 234 ($M^+ - CO_2$, 10), 232 ($M^+ - NO_2$, 100), 217 ($M^+ - OCH_3$, – N=O, 7), 205 ($M^+ - NO_2$, – HCN, 10), 188 ($M^+ - NO_2$, – CO_2, 100), 164 (21), 136 (23), 102 (12).

tert-*Butyl 2-cyano-3-(3,4-methylenedioxy-6-nitrophenyl)propenoate* (**131**)—Using Method B, 6-nitropiperonal (3,4-methylenedioxy-6-nitrobenzaldehyde; 1.460 g, 7.48 mmol) and *tert*-butyl cyanoacetate (1.060 g, 7.51 mmol) were reacted in ethanol (25 ml) with the addition of piperidine (10 drops). After 18 h a precipitate was not observed and thus the reaction was concentrated to quarter volume and left to crystallise. This gave the title compound on cooling to room temperature (0.845 g, 35 %), m.p. 116-117 °C (from EtOH); R_F 0.83 (diethyl ether); (Found: C, 56.35; H, 4.40; N, 8.68; M^+, 318.0856. $C_{15}H_{14}N_2O_6$ requires C, 56.60; H, 4.43; N, 8.80%; *M*, 318.0852); ν_{max}(KBr disc)/cm^{-1} 3113w and 3059w (=C–H), 2984w and 2908w (CH_x), 2233w (CN), 1715s (CO_2Bu^t), 1602m (Ar or C=C), 1522s (C–NO_2), 1329s, 1286s and 1268s (C–O), 882m (=C–H deformation); δ_H(200 MHz; $CDCl_3$) 8.51 (1 H, d, $^4J_{5\text{-}H}$ 0.5 Hz, 3-H), 7.68 (1 H, s, 8-H), 7.07 (1 H, d, $^4J_{3\text{-}H}$ 0.4 Hz, 5-H), 6.21 (2 H, s, OCH_2O), 1.56 (9 H, s, Bu^t); δ_C(50 MHz; $CDCl_3$) 159.9 ($CO_2C(CH_3)_3$), 155.0 (3-CH), 152.5 (9-C), 150.3 (7-C), 142.6 (6-C), 124.4 (4-C), 114.3 (1-CN), 109.1 (2-C), 108.9 (8-CH), 106.0 (5-CH), 104.0 (OCH_2O), 84.4 ($\underline{C}(CH_3)_3$), 27.9 ($C(\underline{C}H_3)_3$); *m/z* 318 (M^+, 5%), 262 ($MH^+ - Bu^t$, 0.5), 245 (M^+ – HCN, – NO_2, (or – OBu^t), 11), 216 ($M^+ - NO_2$, – Bu^t, 100), 199 ($M^+ - OBu^t$, – NO_2, 21), 163 (7), 135 (10), 114 (5).

2-Cyano-3-(3,4-methylenedioxy-6-nitrophenyl)propenoic acid (**132**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(3,4-methylenedioxy-6-nitrophenyl)propenoate (0.636 g, 2.00 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 7 ml) by heating to reflux for 5 min. After this time the reaction was

allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in ethyl acetate and ether to give the title acid (0.412 g, 79 %), m.p. 142-144 °C (from EtOH); (Found: C, 50.16; H, 2.30; N, 10.42; M^+, 262.0222. $C_{11}H_6N_2O_6$ requires C, 50.39; H, 2.31; N, 10.68%; *M*, 262.0226); ν_{max}(KBr disc)/cm^{-1} 3424w (CO_2H), 3069w (=C–H), 2233w (CN), 1718s (CO_2H), 1599m (Ar or C=C), 1508s (C–NO_2), 1329s and1276s (C–O), 883m and 818m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) (CO_2H not observed below 9 ppm), 8.63 (1 H, s, 3-H), 7.90 (1 H, s, 8-H), 7.50 (1 H, s, 5-H), 6.39 (2 H, s, OCH_2O); δ_C(50 MHz; DMSO-d_6) 162.7 ($CO_2C(CH_3)_3$), 154.0 (3-CH), 152.4 (9-C), 150.0 (7-C), 142.4 (6-C), 124.7 (4-C), 108.9 (8-CH), 115.0 (1-CN), 108.0 (2-C), 105.8 (5-CH), 104.6 (OCH_2O); *m/z* 262 (M^+, 16%), 232 (M^+ – N=O, 3), 218 (M^+ – CO_2, 26), 216 (M^+ – NO_2, 100), 201 (M^+ – OCH_3, – N=O, 11), 189 (M^+ – HCN, – NO_2, (or – OBu^t), 13), 163 (29), 158 (32), 135 (32), 114 (50).

tert-Butyl 2-cyano-3-(3-methoxy-4-hydroxy-5-nitrophenyl)propenoate (**133**)—Using Method B, 5-nitrovanillin (3-methoxy-4-hydroxy-5-nitrobenzaldehyde; 1.456 g, 4.55 mmol) and *tert*-butyl cyanoacetate (1.043 g, 7.39 mmol) were reacted in ethanol (40 ml) with the addition of piperidine (10 drops). After 19 h the reaction was concentrated to quarter volume which yielded the title acid (0.524 g, 36 %) on cooling to room temperature, m.p. 166.5-167.5 °C (from EtOH); R_F 0.77 (diethyl ether); (Found: C, 56.00; H, 5.00; N, 8.63; M^+, 320.0997. $C_{15}H_{16}N_2O_6$ requires C, 56.25; H, 5.04; N, 8.75%; *M*, 320.1008); ν_{max}(KBr disc)/cm^{-1} 3080w (=C–H), 2981w and 2942w (CH_x), 2234w (CN), 1715s and 1685m/s (CO_2Bu^t), 1613m and 1578m (Ar or C=C), 1548s (C–NO_2), 1336m/s, 1268s, 1233s and 1105s (C–O), 919m, 875m, 769m and 626m (=C–H deformations); δ_H(200 MHz; acetone-d_6) 9.94 (1 H, s, OH), 8.32 (1 H, s, 3-H), 8.22 (1 H, d, $^4J_{5\text{-}H}$ 1.80 Hz, 9-H), 7.69 (1 H, d, $^4J_{9\text{-}H}$ 1.77 Hz, 5-H), 4.03 (3 H, s, OMe), 1.51 (9 H, s, Bu^t); δ_C(50 MHz; acetone-d_6) 163.4 ($CO_2C(CH_3)_3$), 153.4 (3-CH), 151.5 (8-C), 148.9 (7-C), 128.7 (6-C), 123.5 (4-C), 121.4 (9-CH), 116.55 (1-CN), 113.9 (5-CH), 103.6 (2-C), 84.2 ($\underline{C}(CH_3)_3$), 57.2 (OCH_3), 27.7 ($C(\underline{C}H_3)_3$); *m/z* 320 (M^+, 12%), 290 (M^+ – N=O, 1), 264 (MH^+ – MeO, – CN, (or – Bu^t), 100), 247 (M^+ – HCN, – NO_2, (or – OBu^t), 20), 216 (M^+ – HNO_2, – HBu^t, 100), 197 (M^+ – OBu^t, – NO_2, 11), 180 (23), 149 (13), 135 (17), 57 ([Bu^t]$^+$, 56).

tert-*Butyl 2-cyano-3-(2-imidazolyl)propenoate* (**134**)—Using Method B, 2-imidazolylcarbaldehyde (0.121 g, 1.26 mmol) and *tert*-butyl cyanoacetate (0.184 g,

1.30 mmol) were reacted in isopropanol (2 ml) with 5 % piperidine in isopropanol (3 drops). After *ca.* 1 h, the white solid which formed was collected (0.146 g, 53 %), m.p. 164.5-165.0 °C (from *i*-PrOH); R_F 0.43 (diethyl ether); (Found: C, 59.98; H, 5.91; N, 18.99; M^+, 219.1005. $C_{11}H_{13}N_3O_2$ requires C, 60.26; H, 5.98; N, 19.17%; *M*, 219.1008); ν_{max}(KBr disc)/cm^{-1} 3134m/s, 3109m/s and 3031m (HN_{im} and *im*–H), 2990m and 2911m (=C–H), 2228m/s (CN), 1719s (C=O), 1622s (C=C), 1442s, 1294s, 1244s and 1111s (C–N_{im} and C-O), 797m/s and 760m/s (=C–H deformations); δ_H(200 MHz; acetone-d_6/$CDCl_3$) (8-NH not below 12 ppm), 7.82 (1 H, s, 3-H), 7.23 (2 H, br s, 6- and 7-H), 1.33 (9 H, s, Bu^t); δ_C(50 MHz; acetone-d_6/$CDCl_3$) 161.3 (C=O), 140.5 (4-C), 139.7 (3-C), 133.5 (7-CH (broad)), 122.4 (6-CH (broad)), 115.6 (CN), 102.3 (2-C), 83.3 ($\underline{C}(CH_3)_3$), 27.8 ($C(\underline{C}H_3)_3$); *m/z* 219 (M^+, 4%), 163 ($M^+ - Bu^t$, + H, 11), 146 ($M^+ - OBu^t$, 24), 119 ($M^+ - CO_2Bu^t$, + H, 100), 92 (M^+ – CN, – CO_2Bu^t, 17), 68 (*im*, 13), 64 (25), 57 ([Bu^t]$^+$, 18).

2-Cyano-3-(2-imidazolyl)propenoic acid (**135**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-(2-imidazolyl)propenoate (0.096 g, 0.438 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 2 ml) by heating to reflux for 5 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the precipitate was filtered, washed in ethyl acetate and ether to give the title acid (0.056 g, 78 %), m.p. 189.5-190.5 °C (from HCO_2H); (Found: C, 51.32; H, 3.07; N, 25.64; M^+, 163.0379. $C_7H_5N_3O_2$ requires C, 51.54; H, 3.09; N, 25.76%; *M*, 163.0382); ν_{max}(KBr disc)/cm^{-1} 3428m/w (CO_2H), 3173m/s and 3125m (HN_{im}), 3056m/w and 3015m/w (im–H), 2926m/w and 2855m/w (=C–H), 2222m/w (CN), 1641m/s (C=O), 1609m/s (C=C), 1392s, 1342s and 1304s (C–X), 799s and 747m (=C–H); δ_H(200 MHz; acetone-d_6) (8-NH not observed below 12 ppm), 7.87 (1 H, s, 3-H), 7.25 (2 H, br s, 6- and 7-H); δ_C(50 MHz; acetone-d_6) 163.7 (C=O), 141.1 (4-C), 139.9 (3-CH), 133.6 (7-CH (broad)), 122.7 (6-CH (broad)), 114.4 (CN), 102.8 (2-C); *m/z* 163 (M^+, 2.5%), 119 ($M^+ - CO_2$, 100), 92 (M^+ – CN, – CO_2H, 41), 68 (*im*, 65), 64 (34), 52 (28).

5-Cyano-6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4,5,6-hexahydro pyrimidine, potassium salt (**136**)[266]— To a heated solution at reflux of 6-nitroveratraldehyde (3, 4-dimethoxy-6-nitrobenzaldehyde; 2.230 g, 10.56 mmol), ethyl cyanoacetate (1.194 g, 10.56), thiourea (0.804 g, 10.56 mmol) in absolute ethanol (20 ml) was added anhydrous sodium carbonate (1.460 g, 10.56 mmol) over 15 min. After 5.25 h the precipitate was

filtered, washed in ethanol and tetrahydrofuran, and air dried to give the potassium salt of 5-cyano-6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimidine (3.19 g, 80 %), m.p. 238-241 °C (from EtOH); ν_{max}(KBr disc)/cm^{-1} 3322m/s and 3184m (HNC(S)NH), 2155m/s (CN), 1616s (broad; C=O of amide and C=S of thiourea), 1570m/s (thiourea stretch), 1620m and 1512m (C=C and Ar), 1423s, 1298m, 1280m/s, 1152m and 1131m/s (C–N and C–O stretches), 780m and 686m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 8.93 (2 H, br s, $(NH)_2C$=S), 7.07 (1 H, d, $^3J_{8\text{-}H}$ 8.3 Hz, 9-H), 7.05 (1 H, s, 5-H), 6.83 (1 H, d, $^3J_{9\text{-}H}$ 8.20 Hz, $^4J_{5\text{-}H}$ 2.20 Hz, 8-H), 3.77 (6 H, s, 6- and 7-OMe); δ_C(50 MHz; DMSO-d_6) 175.8 (C=S), 160.6 (C=O), 148.6 and 148.0 (6- and 7-C), 138.5 (3-C), 126.4 (4-C), 118.3 (9-CH), (1-CN not observed), 111.7 (5-CH), 110.6 (8-CH), 49.3 (2-C), 56.6 (3-C), 55.7 and 55.6 (6- and 7-OCH_3); *m/z* 307 (M^+ – 22, $[M_1]^+$, 26%), 292 ($[M_1]^+ - CH_3$, 10), 276 ($[M_1]^+ - OCH_3$, 16), 234 ($[M_1H]^+ - (HN)_2C$=S, 10), 232 ($[M_1]^+ - (HN)_2C$=S, – H, 10), 216 ($[M_1]^+$ – HN-C=S, – CO, 10), 151 (M^+ – HN-C=S, – NC-C-CONH, 10), 137 (M^+ – HN-C=S, – H-C=C(CN)CONH, 10), 107 (12), 94 (20), 79 (15).

5-Cyano-6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro pyrimidine (**137**)—The potassium salt of 5-cyano-6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimidine (2.04 g, 5.43 mmol) was dissolved in distilled water (100 ml) and filtered prior to acidification with glacial acetic acid (5 ml). The solid formed was filtered, washed with water (2 × 20 ml) and ethyl acetate (2 × 10 ml), and dried over a sinter with diethyl ether. This solid (1.23 g) was dissolved in acetic acid (100 ml) and vigorously stirred at reflux (in the presence of air) for 2 h. On cooling the precipitate was filtered, washed in ethyl acetate (2 × 20 ml) and diethyl ether (2 × 20 ml) to give the title compound as a yellow powder (0.673 g, 37 %), m.p. 279-280 °C (from AcOH); R_F 0.06 (diethyl ether); (Found: C, 53.86; H, 3.84; N, 14.59; M^+, 289.0532. $C_{13}H_{11}N_3O_3S$ requires C, 53.97; H, 3.83; N, 14.52%; *M*, 289.0521); ν_{max}(KBr disc)/cm^{-1} 3440m and 3273m (HNC(S)NH), 2937m (CH_3), 2224m/s (CN), 1678s (broad; C=O of amide and C=S of thiourea), 1575m/s (thiourea stretch), 1598s, 1548s and 1517s (Ar and C=C), 1439m/s, 1273m/s, 1213m/s and 1016m (C–N and C–O stretches), 764m (=C–H deformation); δ_H(200 MHz; DMSO-d_6) 13.17 (2 H, br s, $(NH)_2C$=S), 7.37 (1 H, d, $^3J_{8\text{-}H}$ 8.6 Hz, 9-H), 7.34 (1 H, s, 5-H), 7.55 (1 H, d, $^3J_{9\text{-}H}$ 8.3 Hz, 8-H), 3.89 and 3.87 (6 H, 2 × s, 6- and 7-OMe); δ_C(50 MHz; DMSO-d_6) 176.2 (C=S), 160.4 (C=O), 158.8 (3-C), 152.2 and 148.1 (6- and

7-C), 122.8 (9-CH), 120.9 (4-C), 115.3 (1-CN), 112.4 (5-CH), 111.3 (8-CH), 89.8 (2-C), 55.9 and 55.8 (6- and 7-OCH_3); *m/z* 289 (M^+, 100%), 274 ($M^+ - CH_3$, 9), 258 ($M^+ - OCH_3$, 8), 246 ($M^+ - CH_3$, – CO, 6), 231 ($M^+ - OCH_3$, – HCN, 11), 215 ($M^+ - (HN)_2C{=}S$, 10), 201 ($M^+ - 2 \times OCH_3$, 8), 187 (M^+ – HN-C=S, – CO, 10), 164 (M^+ – HN-C=S, – CN, – C≡C-H, 18), 144 (M^+ – HN-C=S, – 2 × CO, – CH_3, 10), 115 (12), 89 (7), 77 (7).

3-Amino-2,4-dicyano-5-(2-nitrophenyl)penta-1,3-dienonitrile (**138**)—Using Method C, 2-nitrobenzaldehyde (1.563 g, 10.34 mmol) and 1,1,3-tricyano-2-aminopropene (1.345 g, 10.18 mmol) were reacted in absolute ethanol (10 ml) with the addition of 10 % piperidine in ethanol (5 drops). After 4 h the crystals were collected (1.789 g, 66 %), m.p. 200-202 °C (from EtOH); R_F 0.40 (diethyl ether); (Found: C, 58.78; H, 2.67; N, 26.32; M^+, 265.0612. $C_{13}H_7N_5O_2$ requires C, 58.87; H, 2.66; N, 26.40%; *M*, 265.0600); ν_{max}(KBr disc)/cm^{-1} 3373m, 3333m and 3213m (NH_2), 2223m/s and 2209m/s (H_2N-C=C($\underline{CN}$)$_2$), 1657s (H_2N-C=C(CN)$_2$), 1622m and 1604m (C=C), 1543s and 1523s (C–NO_2), 1343s (C-N), 795m and 652m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 9.30 and 9.23 (2 × 1 H, 2 × br s, NH_2), 8.49 (1 H, s, 3-H), 8.35 (1 H, d, ^3J 8.21 Hz, 6-H), 8.35 (1 H, m, *ca.* dt, ^3J 7.6 Hz, 8-H), 7.62 (2 H, m (*ca.* dt), ^3J 7.6 Hz, 7- and 9-H); δ_C(50 MHz; DMSO-d_6) 164.0 (H_2N-C=$\underline{C}$(CN)$_2$), 152.6 (3-CH), 147.1 (5-C), 135.0 (6-CH), 132.6 (8-CH), 130.6 (9-CH), 128.1 (4-C), 115.1 (1-CN), 114.4 and 113.8 (H_2N-C=C($\underline{CN}$)$_2$), 107.0 (2-C), 50.7 (H_2N-C=C($\underline{CN}$)$_2$); *m/z* 265 (M^+, 12%), 235 (M^+ – N=O, 11), 219 ($M^+ - NO_2$, 100), 192 ($M^+ - NO_2$, – HCN, 35), 165 ($M^+ - NO_2$, – 2 × HCN, 38), 153 ($M^+ - HNO_2$, –$HC(CN)_2$, 38), 138 ($M^+ - NO_2$, – 3 × HCN, 12), 126 (14), 104 (21), 92 (43), 76 (26).

2-Cyano-3-(9-anthryl)propenonitrile (**139**)—Using Method B, 9-anthracene carbaldehyde (2.289 g, 11.10 mmol) and malononitrile (0.715 g, 10.82 mmol) were reacted with piperidine (4 drops) in chloroform (30 ml). After *ca.* 10 min a fine orange crystalline mesh formed which was collected (1.593 g, 58 %), m.p. 203-204 °C (from $CHCl_3$); R_F 0.82 (diethyl ether); (Found: C, 85.24; H, 3.98; N, 10.89; M^+, 254.0863. $C_{18}H_{10}N_2$ requires C, 85.02; H, 3.96; N, 11.02%; *M*, 254.0844); ν_{max}(KBr disc)/cm^{-1} 3053w and 3016w (Ar), 2229m/s (CN), 1621m (C=C), 1575m/s, 1552m/s and 1519m (Ar), 734s (=C–H deformation); δ_H(200 MHz; DMSO-d_6;338 K) 9.30 (1 H, s, 3-CH), 8.67 (1 H, s, 5-CH), 8.22 (4 H, 2 × d, ^3J 8.54 Hz, ^3J 8.09 Hz, 7- and 10-H), 7.62 (4 H, m, *ca.* two overlapping triplets, 8- and 9-H); δ_C(50 MHz; DMSO-d_6;338 K) 161.7 (3-CH), 131.5 (5-CH), 130.6 (11-C),

129.0 and 127.7 (6- and 9-CH), 128.7 (6-C), 125.7 and 124.2 (7- and 8-CH), 124.0 and112.8 (2 × CN), 91.9 (2-C); *m/z* 254 (M^+, 86%), 227 (M^+ – HCN, 100), 200 (M^+ – 2 × HCN, 15), 187 (6), 174 (6), 150 (6), 113 (24), 100 (26), 87 (11).

5-(4-Nitrophenylene)-2-dimethyl-1,3-dioxane-4,6-dione (**140**)—Using Method C, 4-nitrobenzaldehyde (1.231 g, 8.15 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid; 1.124 g, 7.81 mmol) were reacted in ethanol (20 ml) with the addition of 10 % piperidine in methanol (5 drops). After 2 h the crystals were collected (1.633 g, 75 %), m.p. 201-203 °C (from EtOH); R_F 0.80 (diethyl ether); (Found: C, 56.13; H, 3.96; N, 4.83; M^+, 277.0597. $C_{13}H_{11}NO_6$ requires C, 56.32; H, 4.00; N, 5.05%; *M*, 277.0586); ν_{max}(KBr disc)/cm^{-1} 3119w and 3014w (=C–H), 2989w and 2941w (CH_3), 1760m and 1729s (C=O), 1630m and 1601m (Ar and C=C), 1528s (C–NO_2), 1350s, 1306s, 1293s and 1198s (C–O), 930m, 853m, 832m and 796m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 8.54 (1 H, s, 2-H), 8.36 (2 H, d, $^3J_{5\text{-H}/7\text{-H}}$ 8.77 Hz, 4- and 8-H), 8.14 (2 H, d, $^3J_{4\text{-H}/8\text{-H}}$ 8.67 Hz, 5- and 7-H), 1.83 (6 H, s, $OC(CH_3)_2O$); δ_C(50 MHz; DMSO-d_6) 154.0 (2-CH), 150 (6-C), 132.6 (5- and 7-CH), 123.2 (4- and 8-CH), 107.0 (1-C), 71.6 ($O\underline{C}(CH_3)_2O$), 27.4 ($OC(\underline{CH_3})_2O$); *m/z* 277 (M^+, 0.4%), 262 (M^+ –CH_3, 3), 219 (M^+ – $(CH_3)_2C{=}O$, 100), 202 (M^+ – $(CH_3)_2C(OH)O$, 80), 191 (M^+ – CO_2, – $(CH_3)_2C$, 8), 175 (M^+ – CO_2, – $(CH_3)_2C{=}O$, 8), 172 (M^+ – HNO_2, – $(CH_3)_2C{=}O$, 37), 146 (M^+ – 2 × CO_2, – $(CH_3)_2CH$, 7), 129 (M^+ – CO_2, – NO_2, – $(CH_3)_2C{=}O$, 20), 117 (M^+ – 2 × CO_2, – N=O, – $(CH_3)_2C$, 14), 101 (51), 89 (22), 75 (39).

Experimental Procedures—Chapter 7

2-Cyano-3-[5-(2-nitrofuranyl)]propenonitrile (**141**)—Using Method C, a 100 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (1.020 g, 7.23 mmol), absolute ethanol (7 ml), malononitrile (0.478 g, 7.24 mmol) and ethanolic piperidine (1:1, 1 drop). After 1.25 h the orange precipitate was filtered (1.187 g, 87 %) and a portion was crystallised from ethyl acetate: m.p. 173-174 °C (from EtOAc); R_F 0.43 (diethyl ether); (Found: C, 50.78; H, 1.51; N, 22.35; M^+, 189.0378. $C_8H_3N_3O_3$ requires C, 50.81; H, 1.60; N, 22.22%; *M*, 189.0359); ν_{max}(KBr disc)/cm^{-1} 3128m and 3071m (=C-H), 2232m (3 × CN), 1614m (Ar-C=C), 1558s (Ar), 1517s (NO_2), 1470s and 1344s (C-O_{furan}), 842m, 814m, 789m and 742m/w (=C–H); δ_H(200 MHz; acetone-d_6) 8.29 (1 H, s, 3-H), 7.74 (1 H, d, $^3J_{5\text{-H}}$ 4.03 Hz, 6-H), 7.68 (1 H, d, $^3J_{6\text{-H}}$ 4.03 Hz, 5-H); δ_C(50 MHz; acetone-d_6) 153.1 (7-C), 149.0

(4-C), 144.2 (3-CH), 124.7 (6-CH), 115.4 (1-CN), 114.0 (5-CH), 112.7 (1-CN), 84.2 (2-C), (other CN visible at 113.96 in $^{1}H...^{13}C$ coupled spectrum); *m/z* 189 (M^+, 71%), 173 (M^+ – O, 7), 160 (M^+ – HCN, 8), 159 (M^+ – N=O, 83), 131 (M^+ –CN, – N=O, 31), 115 (M^+ –CN, – NO_2, 51), 105 (100), 88 (65), 77 (27), 64 (47).

3-Amino-2,4-dicyano-5-[5-(2-nitrofuranyl)]penta-2,4-dienonitrile (**142**)—Using Method C, a 100 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (1.203 g, 8.53 mmol) absolute ethanol (10 ml), 1.1.3-tricyano-2-amino-propene (1.127 g, 8.53 mmol) and ethanolic piperidine (1:1,.2 drops). After an initial heating to near reflux and 0.5 h at room temperature, the green precipitate was collected (1.714 g, 79 %), dissolved in THF and filtered. The liquors were subjected to column chromatography through a plug of silica using ethyl acetate/petroleum ether (1:1) and the main fraction was crystallised from acetone/ethyl acetate: m.p. 137-138 °C (from acetone/EtOAc); R_F 0.09 (diethyl ether); (Found: C, 51.79; H, 1.97; N, 27.57; M^+, 255.0285. $C_{11}H_5N_5O_3$ requires C, 51.71; H, 1.98; N, 27.44%; *M*, 255.0253); ν_{max}(KBr disc)/cm^{-1} 3372m, 3325m and 3217m (NH_2), 3154m, 3130m and 3068m (=C–H), 2223m/s and 2206m/s (3 × CN), 1650m/s (H_2N-C=C$(CN)_2$), 1609m (Ar-C=C), 1558s (Ar), 1514s (NO_2), 1350s (C–O_{furan}), 821m/w, 812m and 738m/w (=C–H); δ_H(200 MHz; DMSO-d_6) 9.23 (2 H, br s, NH_2), 8.11 (1 H, s, 3-H), 7.87 (1 H, d, $^{3}J_{5\text{-}H}$ 4.01 Hz, 6-H), 7.59 (1 H, d, $^{3}J_{6\text{-}H}$ 4.03 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 164.1 (H_2N-C=C$(CN)_2$), 153.1 (7-C), 148.4 (4-C), 137.3 (3-CH), 122.8 (6-CH), 115.4 (1-CN), 114.0 (5-CH), 114.5 and 113.8 (H_2N-C=C(CN)$_2$), 103.4 (2-C), 50.4 (H_2N-C=C(CN)$_2$); *m/z* 255 (M^+, 36.5%), 225 (M^+ – N=O, 2), 209 (M^+ – NO_2, 100), 181 (M^+ – NO_2, – CN, 53), 154 (MH^+ – NO_2, – 2 × CN, 60), 127 ([HC=C(CN)-C=C$(CN)_2$]$^+$, 29), 100 (14), 89 (14).

2-Cyano-3-[5-(2-nitrofuranyl)]propenamide (**143**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (1.155 g, 8.19 mmol), absolute ethanol (2 ml), cyanoacetamide (0.688 g, 8.18 mmol) in hot ethanol (8 ml) and ethanolic piperidine (1:1, 2 drops). After 1 h the black precipitate was filtered, subjected to column chromatography on silica with ethyl acetate/petroleum ether (30-40°) dilutions and gave: (0.327 g, 19 %), m.p. 190-193 °C (from EtOAc/Petroleum ether; bp 30-40 °C); R_F 0.21 (diethylether); (Found: C, 46.23; H, 2.56; N, 20.27; M^+, 207.0492. $C_8H_5N_3O_4$ requires C, 46.39; H, 2.43; N, 20.28%; *M*, 207.0475); ν_{max}(KBr disc)/cm^{-1} 3432m, 3379m

and 3317m (NH_2), 3180m, 3142m and 3058m (=C–H), 2221w (CN), 1687s (C=O), 1614s and1563s (Ar), 1535s (NO_2), 1474m/s (C–NH_2), 1349s (C–O_{furan}), 836m, 812m, 804m and 738m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.11 (1 H, s, 3-H), 8.04 and 7.96 (2 H, 2 × br s, NH_2), 7.86 (1 H, d, $^3J_{5\text{-}H}$ 3.66 Hz, 6-H), 7.58 (1 H, d, $^3J_{6\text{-}H}$ 3.62 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 161.6 (C=O), 152.7 (7-C), 149.4 (4-C), 134.8 (3-CH), 121.6 (6-CH), 115.2 (1-CN), 114.2 (5-CH), 108.0 (2-C); *m/z* 207 (M^+, 37%), 191 ($M^+ - NH_2$, 1), 162 ($M^+ - CO$, $-NH_3$, 13), 161 ($M^+ - NO_2$, 100), 133 ($M^+ - NO_2$, – CN, 26), 118 (7.5), 105 (28), 89 (20), 78 (15).

2-Cyano-3-[5-(2-nitrofuranyl)]propenothioamide (**144**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (2.040 g, 14.46 mmol), absolute ethanol (5 ml) and cyanothioacetamide (1.448 g, 14.46 mmol) dissolved in ethanol (10 ml). After the reaction reached ambient temperature the precipitate was filtered, washed with ethyl acetate, and dried with ether washings to give (2.550 g, 79 %), m.p. 189 °C (decomp.) (from EtOH); R_F 0.63 (diethyl ether); (Found: C, 43.05; H, 2.46; N, 18.72; M^+, 223.0180. $C_8H_5N_3O_3S$ requires C, 43.05; H, 2.26; N, 18.82%; *M*, 223.0137); ν_{max}(KBr disc)/cm^{-1} 3373m and 3275m (NH_2), 3171m, 3142m and 3027m/w (=C–H), 2219w (CN), 1625s (C=S), 1560s (Ar), 1537s (NO_2), 1472m/s (C–NH_2), 1346s (C–O_{furan}), 878s, 831m/s, 810s and 738m/s (=C–H); δ_H(200 MHz; DMSO-d_5) 10.30 and 9.73 (2 H, 2 × br s, NH_2), 8.04 (1 H, s, 3-H), 7.86 (1 H, d, $^3J_{5\text{-}H}$ 4.01 Hz, 6-H), 7.62 (1 H, d, $^3J_{6\text{-}H}$ 4.03 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) (C=S not within scale), 152.7 (7-C), 149.4 (4-C), 131.1 (3-CH), 121.3 (6-CH), 115.1 (1-CN), 114.4 (5-CH), 113.0 (2-C); *m/z* 223 (M^+, 25%), 193 (M^+ – N=O, 3), 189 ($M^+ - H_2S$, 8), 177 ($M^+ - NO_2$, 100), 159 ($M^+ - H_2S$, – HCN, – H, 21.5), 149 ($M^+ - NO_2$, – CN, 49), 122 (24), 105 (55), 88 (21).

Methyl 2-cyano-3-[5-(2-nitrofuranyl)]propenoate (**145**)—Using Method C, a 100 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (1.125 g, 7.97 mmol), methanol (4 ml) and methyl cyanoacetate (0.790 g, 7.97 mmol) diluted in methanol (5 ml). The reaction was heated with methanolic piperidine (5 % piperidine, 1 drop) and after 35 min the light orange precipitate was collected (1.464 g, 83 %). A portion of this was recrystallised from acetone to give as yellow flakey crystals, m.p. 211-212 °C; R_F 0.65 (ethyl acetate); (Found: C, 48.41; H, 2.62; N, 12.42; M^+, 222.0912. $C_9H_6N_2O_5$ requires C, 48.66; H, 2.72; N, 12.61%; *M*, 222.0953); ν_{max}(KBr disc)/cm^{-1}

3145m and 3059m (=C–H), 2984m/w, 2960w (CH_3), 2235m/w (CN), 1715s (C=O), 1622m (C=C), 1562m/s (Ar), 1532s (NO_2), 1473m/s and 1343s (C–O_{furan}), 844m, 812m, 767m/w and 741m/w (=C–H); δ_H(200 MHz; DMSO-d_6) 8.27 (1 H, s, 3-H), 7.83 (1 H, d, $^3J_{5\text{-}H}$ 3.98 Hz, 6-H), 7.68 (1 H, d, $^3J_{6\text{-}H}$ 3.99 Hz, 5-H), 3.93 (3 H, s, CH_3); δ_C(50 MHz; DMSO-d_6) 161.7 (C=O), 153 (7-C; weak signal), 148.8 (4-C), 138.3 (3-CH), 123.8 (6-CH), 113.9 (5-CH), (1-CN masked), 103.8 (2-C), 53.7 (CH_3); *m/z* 222 (M^+, 25%), 206 ($M^+ - CH_4$, 2), 191 ($M^+ - OCH_3$, 11), 176 ($M^+ - NO_2$, 100), 164 ($MH^+ - CO_2CH_3$, 3), 161 ($MH^+ - NO_2$, – O, 22), 148 ($M^+ - NO_2$, – CN, 17), 117 ($M^+ - NO_2$, – CO_2CH_3, 8), 133 (19), 105 (21), 88 (24).

Ethyl 2-cyano-3-[5-(2-nitrofuranyl)]propenoate (**146**)—Using Method B, a 100 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (1.000 g, 7.09 mmol), absolute ethanol (5 ml) and ethyl cyanoacetate (0.802 g, 7.09 mmol) diluted in ethanol (5 ml). The reaction was heated with ethanolic piperidine (1:1, 2 drops) and on cooling (after 20 min) the crystals were collected (1.292 g, 77 %). A portion was recrystallised from ethyl acetate and gave: m.p. 146-147 °C (from EtOAc); R_F 0.73 (diethyl ether); (Found: C, 50.93; H, 3.54; N, 11.76; M^+, 236.0902. $C_{10}H_8N_2O_5$ requires C, 50.86; H, 3.41; N, 11.86%; *M*. 236.0852); ν_{max}(KBr disc)/cm^{-1} 3160m/w, 3138m/w and 3045m (=C–H), 2984m/w, 2938m/w and 2871w (CH_x), 2232m/w (CN), 1738s (C=O), 1625m (C=C), 1561s (Ar), 1526s (NO_2), 1474m/s and 1348s (C–O_{furan}), 823m, 812m, 762m and 739m/s (=C–H); δ_H(200 MHz; acetone-d_6) 8.20 (1 H, s, 3-H), 7.72 (1 H, d, $^3J_{5\text{-}H}$ 4.01 Hz, 6-H), 7.69 (1 H, d, $^3J_{6\text{-}H}$ 4.01 Hz, 5-H), 4.37 (2 H, q, ^3J 7.12 Hz, CH_2), 1.36 (3 H, t, ^3J 7.11 Hz, CH_3); δ_C(50 MHz; acetone-d_6) 161.9 (C=O), 154 0(7-C (?), 149.9 (4-C), 138.6 (3-CH), 123.1 (6-CH), 114.7 (1-CN), 114.0 (5-CH), 105.8 (2-C), 63.6 (CH_2), 14.2 (CH_3); *m/z* 236 (M^+, 20%), 220 ($M^+ - CH_4$, 1), 208 ($MH^+ - CH_2CH_3$, 2), 191 ($M^+ - OCH_2CH_3$, 17), 190 ($M^+ - NO_2$, 18), 178 ($M^+ - NO$, – CN, 7), 162 ($M^+ - HCO_2$, – CH_2CH_3, 100), 134 ($M^+ - HNO_2$, – CO_2, – CH_2CH_3, 49), 106 (14), 89 (26.5).

n-*Butyl 2-cyano-3-[5-(2-nitrofuranyl)]propenoate* (**147**)—Using Method C, a 100 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (1.197 g, 8.48 mmol), *n*-butanol (5 ml) and *n*-butyl cyanoacetate (1.198 g, 8.49 mmol) dissolved in *n*-butanol (5 ml). The reaction was heated with 5% piperidine in ethanol (1 drop) and on cooling the cream crystalline precipitate was collected over a sinter, washed with ethyl

acetate and dried with *n*-pentane/ether washings under suction: (1.753 g, 51 %), m.p. 125.0-125.3 °C (from *n*-butanol); R_F 0.82 (diethyl ether); (Found: C, 54.83; H, 4.38; N, 10.62; M^+, 264.0489. $C_{12}H_{12}N_2O_5$ requires C, 54.55; H, 4.58; N, 10.60%; *M*, 264.0427); ν_{max}(KBr disc)/cm^{-1} 3157w, 3137m/w and 3060m (=C–H), 2965m, 2933m/w, 2899m/w and 2875m (CH_x), 2236w (CN), 1720s (C=O), 1621m (C=C), 1562s (Ar), 1530s (NO_2), 1475m/s and 1344s (C–O_{furan}), 829m/s, 813m/s and 738m/s (=C–H); δ_H(200 MHz; DMSO-d_6) 8.28 (1 H, s, 3-H), 7.87 (1 H, d, $^3J_{5\text{-}H}$ 3.99 Hz, 6-H), 7.69 (1 H, d, $^3J_{6\text{-}H}$ 4.00 Hz, 5-H), 4.32 (2 H, t, ^3J 6.47 Hz, $\underline{CH_2}CH_2CH_2CH_3$), 1.72 (2 H, q t, ^3J 6.8 Hz, ^3J 6.45 Hz, $CH_2CH_2\underline{CH_2}CH_3$), 1.43 (2 H, tt, ^3J 7.23 Hz, ^3J 7.0 Hz, $CH_2\underline{CH_2}CH_2CH_3$), 0.97 (3 H, t, ^3J 7.26 Hz, $CH_2CH_2CH_2\underline{CH_3}$); δ_C(50 MHz; DMSO-d_6) 161.2 (C=O), 153.2 (7-C), 148.8 (4-C), 138.2 (3-CH), 124.3 (6-CH), 114.2 (1-CN), 114.0 (5-CH), 103.6 (2-C), 66.4 ($\underline{CH_2}CH_2CH_2CH_3$), 30.0 ($CH_2\,\underline{CH_2}CH_2CH_3$), 18.6 ($CH_2\,CH_2\underline{CH_2}CH_3$), 13.6 ($CH_2CH_2CH_2\underline{CH_3}$); *m/z* 264 ($M^+$, 21%), 234 ($M^+$ – N=O, 2), 218 (M^+ – NO_2, 17), 209 (M^+ – $CH_2CH_2CH_2CH_3$, 21), 191 (MH^+ – NO_2, – CN, 46), 162 (MH^+ – $CO_2CH_2CH_2CH_2CH_3$, 91), 134 (MH^+ – $CO_2CH_2CH_2CH_2CH_3$, – CN, 39), 117 (M^+ – NO_2, – $CO_2CH_2CH_2CH_2CH_3$, 17), 105 (16), 89 (38), 77 (13), 62 (24), 56 ([$CH_3CH{=}CHCH_3$]$^+$, 100).

tert-*Butyl 2-cyano-3-[5-(2-nitrofuranyl)]propenoate* (**148**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrofuran-5-carbaldehyde (0.985 g, 6.98 mmol), isopropanol (5 ml) and *tert*-butyl cyanoacetate (1.050 g, 7.44 mmol). The reaction was heated with 5 % piperidine in isopropanol (2 drops) and after 1 h the precipitate was collected (1.214 g, 66 %), m.p. 157-158 °C (from *i*-PrOH); R_F 0.83 (diethyl ether); (Found: C, 54.43; H, 4.71; N, 10.45; M^+, 264.0749. $C_{12}H_{12}N_2O_5$ requires C, 54.55; H, 4.58; N, 10.60%; *M*, 264.0746); ν_{max}(KBr disc)/cm^{-1} 3132m and 3059m (=C–H), 2996m, 2940w (CH_3), 2232m/w (CN), 1718s (C=O), 1622m (C=C), 1561m/s (Ar), 1527s (NO_2), 1473m/s and 1344s (C–O_{furan}), 823m/s, 812m/s, 766m/w and 738m/w (=C–H); δ_H(200 MHz; DMSO-d_6) 8.17 (1 H, s, 3-H), 7.83 (1 H, d, $^3J_{5\text{-}H}$ 3.99 Hz, 6-H), 7.66 (1 H, d, $^3J_{6\text{-}H}$ 3.96 Hz, 5-H), 1.59 (3 H, s, Bu^t); δ_C(50 MHz; DMSO-d_6) 160.0 (C=O), 149.0 (7-C), 137.6 (3-CH), 123.2 (6-CH), 114.3 (1-CN), 114.0 (5-CH), 105.7 (4-C), 84.2 (2-C), 27.7 (^tBu); *m/z* 264 (M^+, 25%), 249 (M^+ – CH_3, 2), 220 (M^+ – NO_2, 1), 208 (M^+ – Bu^t, + H, 6), 191 (M^+ –

OBu^t, 27), 162 ($M^+ - CO_2Bu^t$, – H, 3), 148 ($M^+ - NO_2$, – CN, 17), 134 ($M^+ - CO_2$, – HBu^t, – HCN, 3), 117 ($M^+ - NO_2$, – CO_2Bu^t, 7), 105 (3), 89 (10), 57 ($[^tBu]^+$, 100)

2-Cyano-3-[5-(2-nitrofuranyl)]propenoic acid (**149**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-[5-(2-nitrofuranyl)]propenoate (0.763 g, 2.89 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 10 ml) by heating to reflux for 15 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in ethyl acetate and ether to give the title acid (0.412 g, 68 %), m.p. 242 °C (decomp.) (from HCO_2H); (Found: C, 46.02; H, 1.89; N, 13.42; M^+, 208.0123. $C_8H_4N_2O_5$ requires C, 46.17; H, 1.94; N, 13.46%; *M*, 208.0120); ν_{max}(KBr disc)/cm^{-1} 3436m/w (broad CO_2H), 3141m and 3054m (=C–H deformations), 2235m/w (CN), 1688s (C=O), 1616s (C=C), 1561m/s (Ar), 1529s (NO_2), 1474s and 1344s ($C–O_{furan}$), 842m and 809m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.21 (1 H, s, 3-H), 7.86 (1 H, d, $^3J_{5\text{-}H}$ 3.97 Hz, 6-H), 7.64 (1 H, d, $^3J_{6\text{-}H}$ 3.99 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 162.6 (C=O), 153.4 (7-C), 149.0 (4-C), 137.7 (3-CH), 123.6 (6-CH), 114.8 (1-CN), 114.1 (5-CH), 105.1 (2-C); *m/z* 208 (M^+, 42%), 192 (M^+ – O, 4), 178 (M^+ – N=O, 32), 164 ($M^+ - CO_2$, 31), 162 ($M^+ - NO_2$, 58), 148 ($M^+ - NO_2$, – CN, 3.5), 134 ($M^+ - CO_2H$, – HCN, 100), 124 (M^+ – NC-C-CO_2, 21), 117 ($M^+ - NO_2$, – CO_2H, 4), 106 (43), 90 (32).

2-Cyano-3-[5-(2-nitrothienyl)]propenonitrile (**151**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (1.045 g, 6.65 mmol), absolute ethanol (5 ml), malononitrile (0.439 g, 6.65 mmol) and 50 % ethanolic piperidine (1 drop). After 30 min the precipitate was filtered: (1.121 g, 82 %), m.p. 147-149 °C (from EtOH); R_F 0.55 (ethyl acetate/petroleum ether 1:1); (Found: C, 46.44; H, 1.53; N, 20.75; M^+, 204.9949. $C_8H_3N_3O_2S$ requires C, 46.83; H, 1.47; N, 20.47%; *M*, 204.9971); ν_{max}(KBr disc)/cm^{-1} 3050s (=C–H), 2240s (CN), 1590s and 1545m (C=C), 1485s (NO_2), 815w (=C–H); δ_H(200 MHz; DMSO-d_6) 8.79 (1 H, s, 3-H), 8.23 (1 H, d, $^3J_{5\text{-}H}$ 4.4 Hz, 6-H), 7.89 (1 H, d, $^3J_{6\text{-}H}$ 4.4 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 155.9 (7-C), 152.5 (3-CH), 139.2 (4-C), 137.9 (6-CH), 129.6 (5-CH), *ca.* 113 (2 × CN, broad signals), 82.7 (2-C); *m/z* 205 (M^+, 61%), 175 (M^+ – N=O, 14), 159 ($M^+ - NO_2$, 31), 147 (M^+ – HCN, – HS, 9), 132 ($M^+ - NO_2$, – HCN, 21), 115 ($M^+ - NO_2$, – C≡S, 52), 94 (13), 88 (38), 82 (17).

3-Amino-2,4-dicyano-5-[5-(2-nitrothienyl)]penta-2,4-dienonitrile (**152**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde

(2.875 g, 18.29 mmol), THF (10 ml), 1.1.3-tricyano-2-amino-propene (2.415 g, 18.28 mmol) and 50 % piperidine in ethanol (5 drops). After 6 h the orange precipitate was filtered and crystallised from ethyl acetate to give dark red crystals: (1.501 g, 30 %), m.p. 214-215 °C (from EtOAc); R_F 0.51 (ethyl acetate); (Found: C, 48.51; H, 1.90; N, 25.93; M^+, 271.0134. $C_{11}H_5N_5O_2S$ requires C, 48.71; H, 1.86; N, 25.82%; *M*, 271.0172); ν_{max}(KBr disc)/cm^{-1} 3330m (NH_2), 3090w (=C–H), 2250s and 2220s (3 × CN), 1640s (H_2N-C=C$(CN)_2$), 1585w and 1540w (Ar), 1520s (NO_2), 815ws (=C–H); δ_H(200 MHz; DMSO-d_6) 9.17 (2 H, br s, NH_2), 8.41 (1 H, d, $^4J_{5\text{-}H}$ 0.4 Hz, 3-H), 8.23 (1 H, d, $^3J_{5\text{-}H}$ 4.4 Hz, 6-H), 7.88 (1 H, dd, $^3J_{6\text{-}H}$ 4.4 Hz, $^4J_{3\text{-}H}$ 0.4 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 163.8 (H_2N-C=C$(CN)_2$), 154.9 (7-C), 145.3 (3-CH), 139.9 (4-C), 136.8 (6-CH), 129.7 (5-CH), 115.3 and 114.4 (1- CN and H_2N-C=C(CN)$_2$), 103.0 (2-C), 50.4 (H_2N-C=C(CN)$_2$); *m/z* 271 (M^+, 34%), 241 (M^+ – N=O, 7), 225 (M^+ – NO_2, 13), 198 (MH^+ – NO_2, – CN, 54), 154 (M^+ – CN, – H_2N-C=C$(CN)_2$, + H, 23), 127 (9), 88 (11).

2-Cyano-3-[5-(2-nitrothienyl)]propenamide (**153**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (1.283 g, 8.16 mmol), absolute ethanol (4 ml), cyanoacetamide (0.686 g, 8.16 mmol) in hot ethanol (11 ml) and 50 % piperidine in ethanol (2 drops). After 1 h the precipitate was filtered and gave: (1.203 g, 66 %), m.p. 231-233 °C (from EtOH); R_F 0.58 (ethyl acetate); (Found: C, 43.68; H, 2.17; N, 18.59; M^+, 223.0956. $C_8H_5N_3O_3S$ requires C, 43.05; H, 2.26; N, 18.83%; *M*, 223.0998); ν_{max}(KBr disc)/cm^{-1} 3350m and 3250m (NH_2), 3005w (=C–H), 2200w (CN), 1690s (C=O), 1599s and 1542m (Ar, C=C), 1500s (NO_2), 820m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.46 (1 H, s, 3-H), 8.24 (1 H, d, $^3J_{5\text{-}H}$ 4.4 Hz, 6-H), 8.03 and 7.96 (2 H, 2 × br s, NH_2), 7.90 (1 H, d, $^3J_{6\text{-}H}$ 4.4 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 161.4 (C=O), 154.1 (7-C), 142.6 (3-CH), 141.1 (4-C), 136.2 (6-CH), 129.7 (5-CH), 115.8 (1-CN), 107.4 (2-C); *m/z* 223 (M^+, 37%), 193 (M^+ – N=O, 7), 179 (M^+ – $CONH_2$, 48), 177 (M^+ – NO_2, 100), 127 (9), 107 (6), 92 (5).

2-Cyano-3-[5-(2-nitrothienyl)]propenothioamide (**154**)—Using Method C, a 100 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (2.044 g, 13.01 mmol), absolute ethanol (10 ml) and cyanothioacetamide (1.303 g, 13.01 mmol). After the reaction reached ambient temperature the precipitate was filtered and gave: (2.503 g, 80 %), m.p. 137-139 °C (from EtOH); R_F 0.46 (ethyl acetate); (Found: C, 39.89;

H, 2.07; N, 17.22; M^+, 238.9940. $C_8H_5N_3O_2S_2$ requires C, 40.16; H, 2.11; N, 17.56%; *M*, 238.9958); ν_{max}(KBr disc)/cm^{-1} 3360m and 3150s (NH_2), 3060w (=C–H), 2190w (CN), 1620s (C=S), 1500s (NO_2), 1460s (C-N), 820m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.46 (1 H, s, 3-H), 8.22 (1 H, d, $^3J_{5\text{-H}}$ 4.4 Hz, 6-H), 7.98 and 7.95 (2 H, 2 × br s, NH_2), 7.90 (1 H, d, $^3J_{6\text{-H}}$ 4.4 Hz, 5-H); *m/z* 239 (M^+, 37%), 206 ($M^+ - H_2S$, 11), 193 ($M^+ - NO_2$, 68), 179 ($M^+ - CSNH_2$, 37), 88 (8).

Methyl 2-cyano-3-[5-(2-nitrothienyl)]propenoate (**155**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (2.417 g, 15.38 mmol), methanol (10 ml) and methyl cyanoacetate (1.354 g, 13.66 mmol). The reaction was heated and on cooling the crystals were collected: (2.948 g, 91 %), m.p. 172-173 °C (from MeOH); R_F 0.62 (ethyl acetate); (Found: C, 45.10; H, 2.49; N, 11.61; M^+, 238.0052. $C_9H_6N_2O_4S$ requires C, 45.38; H, 2.54; N, 11.76%; *M*, 238.0080); ν_{max}(KBr disc)/cm^{-1} 3100m (=C–H), 2950m (CH_x), 2220w (CN), 1720s (C=O), 1500s (NO_2), 1340s (C–O), 835m and 815m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.67 (1 H, s, 3-H), 8.22 (1 H, d, $^3J_{5\text{-H}}$ 4.4 Hz, 6-H), 8.02 (1 H, d, $^3J_{6\text{-H}}$ 4.4 Hz, 5-H), 3.85 (3 H, s, CH_3); *m/z* 238 (M^+, 40%), 207 ($M^+ - OCH_3$, 15), 192 ($M^+ - NO_2$, 100), 179 ($M^+ - CO_2CH_3$, 18), 133 ($M^+ - NO_2$, – CO_2CH_3, 34), 127 (7), 88 (9).

Ethyl 2-cyano-3-[5-(2-nitrothienyl)]propenoate (**156**)—Using Method C, a 100 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (2.001 g, 12.73 mmol), absolute ethanol (20 ml), ethyl cyanoacetate (1.440 g, 12.73 mmol) and 50 % piperidine in ethanol (4 drops). The reaction was heated and on cooling (after 20 minutes) the crystals were collected: (1.725 g, 54 %), m.p. 126-127 °C (from EtOH); R_F 0.54 (ethyl acetate); (Found: C, 47.37; H, 3.14; N, 11.00; M^+, 252.0221. $C_{10}H_8N_2O_4S$ requires C, 47.62; H, 3.20; N, 11.10%; *M*, 252.0201); ν_{max}(KBr disc)/cm^{-1} 3132m, 3102m and 3026w (=C–H), 2991w and 2942m (CH_2CH_3), 2225m (CN), 1718s (C=O), 1607s (C=C), 1539s (NO_2), 1259s (C–O), 879m, 782m, 762m and 736m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.64 (1 H, s, 3-H), 8.22 (1 H, d, $^3J_{5\text{-H}}$ 4.3 Hz, 6-H), 8.03 (1 H, d, $^3J_{6\text{-H}}$ 4.3 Hz, 5-H), 4.35 (2 H, q, ^3J 7.1 Hz, CH_2), 1.33 (3 H, t, ^3J 7.1 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 161.0 (C=O), 155.1 (7-C), 146.4 (3-CH), 140.3 (4-C), 138.0 (6-CH), 129.6 (5-CH), 115.2 (1-CN), 103.6 (2-C), 62.8 (CH_2), 14.0 (CH_3); *m/z* 252 (M^+, 74%), 236 ($M^+ - CH_4$, 3), 224 ($M^+ - CH_2CH_3$, + H, 29), 207 ($M^+ - OCH_2CH_3$, 69), 179 ($M^+ - CO_2CH_2CH_3$, 25), 174 ($M^+ - NO_2$, – S, 91), 161

(M^+ – NO_2, – H-CS, 21), 146 (M^+ – $CO_2CH_2CH_3$, – HS, 100), 133 (M^+ – NO_2, – $CO_2CH_2CH_3$, 45), 122 (21), 107 (16), 94 (14), 82 (29).

n-*Butyl 2-cyano-3-[5-(2-nitrothienyl)]propenoate* (**157**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (1.475 g, 9.39 mmol), isopropanol (20 ml) and *n*-butyl cyanoacetate (1.325 g, 9.39 mmol). The reaction was heated and on cooling the crystals were collected over a sinter, washed with *iso*propanol and dried with *n*-pentane washings under suction: (1.753 g, 71 %), m.p. 112-113 °C (from *i*-PrOH); R_F 0.73 (ethyl acetate); (Found: C, 51.07; H, 4.21; N, 10.13; M^+, 280.0489. $C_{12}H_{12}N_2O_4S$ requires C, 51.42; H, 4.32; N, 9.99%; *M*, 280.0520); ν_{max}(KBr disc)/cm^{-1} 3100m (=C–H), 2950m (CH_x), 2225w (CN), 1720s (C=O), 1605w (C=C), 1500s (NO_2), 1340s (C–O), 840m and 825m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.65 (1 H, s, 3-H), 8.22 (1 H, d, $^3J_{5\text{-H}}$ 4.5 Hz, 6-H), 8.03 (1 H, d, $^3J_{6\text{-H}}$ 4.5 Hz, 5-H), 4.26 (2 H, t, ^3J 6.4 Hz, $\underline{CH}_2CH_2CH_2CH_3$), 1.65 (2 H, q t, ^3J 7.2 Hz, ^3J 6.4 Hz, $CH_2CH_2\underline{CH}_2CH_3$), 1.33 (2 H, tt, ^3J 7.2 Hz, ^3J 7.2 Hz, $CH_2\underline{CH}_2CH_2CH_3$), 0.91 (3 H, t, ^3J 7.2 Hz, $CH_2CH_2CH_2\underline{CH}_3$); *m/z* 280 ($M^+$, 22%), 234 ($M^+$ – NO_2, 7), 224 (M^+ – CH_2=$CHCH_2CH_3$, 41), 180 (MH^+ – $CO_2CH_2CH_2CH_2CH_3$, 18), 133 (M^+ – NO_2, – $CO_2CH_2CH_2CH_2CH_3$, 17), 56 ([CH_3CH=$CHCH_3$], 100).

tert-*Butyl 2-cyano-3-[5-(2-nitrothienyl)]propenoate* (**158**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-5-carbaldehyde (1.245 g, 7.92 mmol), isopropanol (10 ml) and *tert*-butyl cyanoacetate (1.302 g, 9.22 mmol). The reaction was heated and on cooling the crystals were collected (0.948 g, 45 %), m.p. 170-171 °C (from EtOH); R_F 0.73 (1:1 ethyl acetate/petroleum ether); (Found: C, 51.76; H, 4.29; N, 9.87; M^+, 280.0522. $C_{12}H_{12}N_2O_4S$ requires C, 51.42; H, 4.32; N, 9.99%; *M*, 280.0538); ν_{max}(KBr disc)/cm^{-1} 3106m, 3079m and 3032m (=C–H), 2986m, 2955m and 2937m ($C(CH_3)_3$), 2224m (CN), 1728s (C=O), 1604s (C=C), 1531s (NO_2), 1508s (Ar), 1343s, 1275s and 1163 (C–O), 839m/s and 824m/s (=C–H deformations); δ_H(200 MHz; DMSO-d_6; 333 K) 8.57 (1 H, s, 3-H), 8.24 (1 H, d, $^3J_{5\text{-H}}$ 4.31 Hz, 6-H), 8.04 (1 H, d, $^3J_{6\text{-H}}$ 4.33 Hz, 5-H), 1.58 (9 H, s, Bu^t); δ_C(50 MHz; DMSO-d_6; 333 K) 159.6 (CO_2Bu^t), 154.7 (7-C), 145.4 (3-CH), 140.2 (4-C), 137.1 (6-CH), 129.4 (5-CH), 115.0 (1-CN), 105.0 (2-C), 52.2 ($\underline{C}(CH_3)_3$), 27.4 ($C(\underline{C}H_3)_3$); *m/z* 280 (M^+, 2%), 265 ($M^+ - CH_3$, 0.2), 234 ($M^+ - NO_2$,

0.5), 207 ($M^+ - OBu^t$, 12), 178 ($M^+ - CO_2$, $- HBu^t$, 10), 133 ($M^+ - NO_2$, $- CO_2Bu^t$, 11), 107 (4), 88 (4), 82 (11), 69 (10), 57 ($[Bu^t]^+$, 100).

2-Cyano-3-[5-(2-nitrothienyl)]propenoic acid (**159**)—A 25 ml round-bottomed flask was charged with *tert*-butyl 2-cyano-3-[5-(2-nitrothienyl)]propenoate (0.456 g, 1.63 mmol) and dissolved in a minimum amount of 98 % formic acid (*ca.* 3 ml) by heating to reflux for 5 min. After this time the reaction was allowed to cool to room temperature (unstirred) and the crystals were filtered, washed in ethyl acetate and ether to give the title acid (0.301 g, 82 %), m.p. 192-194 °C (from HCO_2H); (Found: C, 42.65; H, 1.87; N, 12.24; M^+, 223.9903. $C_8H_4N_2O_4S$ requires C, 42.86; H, 1.78; N, 12.50%; *M*, 223.9892); ν_{max}(KBr disc)/cm^{-1} 3428m (broad CO_2H), 3100m and 3047m (=C–H), 2243w (CN), 1694s (C=O), 1599s (C=C), 1527s (NO_2), 1501s (Ar), 1339s, 1279m/s and 1210m/s (C–O), 818m/s and 731m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.62 (1 H, s, 3-H), 8.27 (1 H, d, $^3J_{5\text{-H}}$ 4.26 Hz, 6-H), 8.04 (1 H, d, $^3J_{6\text{-H}}$ 4.34 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 162.4 (CO_2H), 154.8 (7-C), 145.5 (3-CH), 140.8 (4-C), 137.3 (6-CH), 129.7 (5-CH), 113.8 (1-CN), 105.5 (2-C); *m/z* 224 (M^+, 19%), 194 ($M^+ - NO$, 3), 180 ($M^+ - CO_2$, 50), 178 ($M^+ - NO_2$, 38), 150 ($M^+ - HNO_2$, – HCN, 88), 134 ($M^+ - NO_2$, $- CO_2$, 15), 122 (78), 107 (18), 88 (16.5).

2-Cyano-3-[4-(2-nitrothienyl)]propenonitrile (**160**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (0.540 g, 3.44 mmol), absolute ethanol (5 ml), malononitrile (0.227 g, 3.44 mmol) and 50 % piperidine in ethanol (2 drops). After 5 h the precipitate was filtered and crystallised from isopropanol to give: (0.195 g, 28 %), m.p. 106-107 °C (from *i*-PrOH); R_F 0.60 (ethyl acetate); (Found: C, 46.74; H, 1.53; N, 20.55; M^+, 204.9941. $C_8H_3N_3O_2S$ requires C, 46.83; H, 1.47; N, 20.47%; *M*, 204.9989); ν_{max}(KBr disc)/cm^{-1} 3103s (=C–H), 2233s (CN), 1597s, 1545m and 1530s (Ar), 1508s (NO_2), 819s (=C–H); δ_H(200 MHz; DMSO-d_6) 8.75 (1 H, dd, $^4J_{5\text{-H}}$1.93 Hz, $^4J_{3\text{-H}}$0.48 Hz, 8-H), 8.48 (1 H, dd, $^4J_{5\text{-H}}$0.95 Hz, $^4J_{8\text{-H}}$0.48 Hz, 3-H), 8.45 (1 H, dd, $^4J_{8\text{-H}}$1.93 Hz, $^4J_{3\text{-H}}$0.95 Hz, 5-H); δ_C(50 MHz; DMSO-d_6) 153.4 (3-CH), 152.8 (7-C), 143.5 (8-CH), 132.0 (4-C), 127.5 (5-CH), 113.7 and 113.0 (2 × CN), 82.1 (2-C); *m/z* 205 (M^+, 67%), 175 ($M^+ - N{=}O$, 23), 159 ($M^+ - NO_2$, 13), 147 ($M^+ - HCN$, – HS, 88), 132 ($M^+ - NO_2$, – HCN, 18), 115 ($M^+ - NO_2$, $- C{\equiv}S$, 31), 88 (33), 82 ($[th]^+$, 10), 69 (23), 45 (100).

3-Amino-2,4-dicyano-5-[4-(2-nitrothienyl)]penta-2,4-dienonitrile (**161**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (1.036 g, 6.59 mmol), tetrahydrofuran (10 ml), 1.1.3-tricyano-2-amino-propene (0.871 g, 6.59 mmol) and 50 % piperidine in ethanol (5 drops). After 4.5 h the precipitate was filtered and subjected to chromatography through silica using ethyl acetate:petroleum ether (1:1) elutions. The main fraction obtained (0.74 g, 41 %) was then crystallised from isopropanol to give: (0.350 g, 20 %), m.p. 185-186 °C (from *i*-PrOH); R_F 0.52 (ethyl acetate/petroleum ether 1:1); (Found: C, 48.99; H, 1.97; N, 26.08; M^+, 271.0150. $C_{11}H_5N_5O_2S$ requires C, 48.71; H, 1.86; N, 25.82%; *M*, 271.0115); ν_{max}(KBr disc)/cm^{-1} 3330m (NH_2), 3038w (=C-H), 2231s and 2229s (CN), 1636s (C=C$(CN)_2$), 1585s and 1540s (Ar), 1522s (NO_2), 870s and 834s (=C–H); δ_H(200 MHz; DMSO-d_6) 9.10 (2 H, br s, NH_2), 8.70 (1 H, d, $^4J_{5\text{-}H}$ 2.3 Hz, 8-H), 8.58 (1 H, d, $^4J_{8\text{-}H}$ 2.3 Hz, 5-H), 8.15 (1 H, s, 3-H); δ_C(50 MHz; DMSO-d_6) 164.6 (H_2N-C=C$(CN)_2$), 152.9 (7-C), 145.8 (3-CH), 141.6 (8-CH), 132.4 (4-C), 127.6 (5-CH), 115.4 (1-CN), 114.7 and 114.6 (H_2N-C=C$(CN)_2$), 102.6 (2-C), 50.1 (H_2N-C=C$(CN)_2$); *m/z* 271 (M^+, 65%), 241 (M^+ – N=O, 23), 225 (M^+ – NO_2, 100), 198 (M^+ – NO_2, – HCN, 43), 181 (M^+ – NO_2, – C≡S, 68), 154 (M^+ – CN, – H_2N-C=C$(CN)_2$, + H, 18), 142 (M^+ – NC-C(H_2N)=C$(CN)_2$, + H, 12), 92 ([H_2N-C=C$(CN)_2$]$^+$, 13).

2-Cyano-3-[4-(2-nitrothienyl)]propenamide (**162**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (0.517 g, 3.29 mmol), absolute ethanol (5 ml), cyanoacetamide (0.277 g, 3.29 mmol) and 50 % piperidine in ethanol (3 drops). After 5 h the precipitate was filtered, subjected to column chromatography on silica with ethyl acetate/petroleum ether (bp 30-40 °C) dilutions and gave: (0.229 g, 31 %), m.p. 217-219 °C (from EtOH); R_F 0.56 (ethyl acetate:petroleum/ether,1:1); (Found: C, 43.02; H, 2.22; N, 18.90; M^+, 223.0897. $C_8H_5N_3O_3S$ requires C, 43.05; H, 2.26; N, 18.83%; *M*, 223.0973); ν_{max}(KBr disc)/cm^{-1} 3422m, 3335m and 3289m (NH_2), 3125m (=C–H), 2849w (C–NH_2), 2200w (CN), 1686s (C=O), 1597s and 1491s (C=C, Ar), 1526s (NO_2), 840m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.63 (1 H, d, $^4J_{5\text{-}H}$ 1.7 Hz, 8-H), 8.50 (1 H, d, $^4J_{8\text{-}H}$ 1.7 Hz, 5-H), 8.13 (1 H, s, 3-H), 7.84 and 7.80 (2 × 1 H, 2 × br s, NH_2); δ_C(50 MHz; $CDCl_3$) 162.0 (C=O), 152.4 (7-C), 143.2 (3-CH), 140.8 (8-CH), 133.1 (4-C), 127.9 (5-CH), 116.3 (1-CN), 106.8 (2-C); *m/z* 223 (M^+, 12%),

207 ($M^+ - NH_2$, 2), 179 ($M^+ - CONH_2$, 8), 177 ($M^+ - NO_2$, 100), 149 ($M^+ - HNO_2$, – HCN, 18), 133 ($M^+ - NO_2$, – $CONH_2$, 12), 122 (10), 105 (17), 82 ([*th*]$^+$, 8).

2-Cyano-3-[4-(2-nitrothienyl)]propenothioamide (**163**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (0.536 g, 3.41 mmol), absolute ethanol (5 ml) and cyanothioacetamide (0.342 g, 3.42 mmol). After the reaction reached ambient temperature the precipitate was filtered and gave: (0.573 g, 70 %), m.p. 199-201 °C (from EtOH); R_F 0.51 (ethyl acetate/petroleum ether, 1:1); (Found: C, 40.12; H, 2.08; N, 17.32; M^+, 238.9910. $C_8H_5N_3O_2S_2$ requires C, 40.16; H, 2.11; N, 17.56%; *M*, 238.9956); ν_{max}(KBr disc)/cm^{-1} 3135s (NH_2), 3080m (=C–H), 2215m (CN), 1480s (NO_2), 1470s (S=C–NH_2), 840w (=C–H); δ_H(200 MHz; DMSO-d_6) 8.69 and 8.61 (2 × 1 H, 2 × br s, NH_2), 8.17 (1 H, d, $^4J_{5\text{-}H}$ 1.9 Hz, 8-H), 8.10 (1 H, d, $^4J_{8\text{-}H}$ 1.9 Hz, 5-H), 8.02 (1 H, s, 3-H); δ_C(50 MHz; DMSO-d_6) (C=S not observed), 152.4 (7-C), 140.8 (8-CH), 139.3 (3-CH), 132.9 (4-C), 128.0 (5-CH), 116.1 (1-CN), 112.6 (2-C); *m/z* 239 (M^+, 22%), 205 ($M^+ - H_2S$, – H, 2), 193 ($M^+ - NO_2$, 100), 166 ($M^+ - NO_2$, – HCN, 21), 161 ($M^+ - NO_2$, – S, 9), 149 ($M^+ - NO_2$, – C≡S, 32), 122 ($M^+ - NO_2$, – C≡S, – HCN, 13), 105 (9), 90 (11), 82 ([*th*]$^+$, 9), 69 (16.5), 60 ([*CSNH*$_2$]$^+$, 40).

Methyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate (**164**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (0.504 g, 3.21 mmol), methanol (5 ml), methyl cyanoacetate (0.318 g, 3.21 mmol) and 50 % piperidine in methanol (1 drop). The reaction was heated and on cooling the crystalline precipitate was collected: (0.303 g, 68 %), m.p. 89-90 °C (from MeOH); R_F 0.50 (ethyl acetate/petroleum ether, 1:1); (Found: C, 45.04; H, 2.41; N, 11.50; M^+, 238.0035. $C_9H_6N_2O_4S$ requires C, 45.38; H, 2.54; N, 11.76%; *M*, 238.0051); ν_{max}(Nujol)/cm^{-1} 2200w (CN), 1720m (C=O), 1618s (C=C), 1498s (NO_2), 1265s (C–O); δ_H(200 MHz; DMSO-d_6) 8.86 (1 H, d, $^4J_{5\text{-}H}$0.5 Hz, 8-H), 8.62 (1 H, d, $^4J_{8\text{-}H}$0.5 Hz, 5-H), 8.45 (1 H, s, 3-H), 3.85 (3 H, s, CH_3); δ_C(50 MHz; DMSO-d_6) 162.0 (C=O), 153.0 (7-C), 147.3 (3-CH), 142.9 (8-CH), 132.8 (4-C), 128.3 (5-CH), 115.5 (1-CN), 102.8 (2-C), 53.5 (CH_3); *m/z* 238 (M^+, 21%), 207 (M^+ –OCH_3, 13), 192 ($M^+ - NO_2$, 100), 179 ($M^+ - CO_2CH_3$, 7), 177 ($M^+ - NO_2$, – CH_3, 19), 161 ($MH^+ - NO_2$, – S, 6.5), 133 ($M^+ - NO_2$, – CO_2CH_3, 20.5), 121 (10), 106 (7), 82 ([*th*]$^+$, 20), 69 (15), 59 ([CO_2CH_3]$^+$, 18).

Ethyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate (**165**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (0.535 g, 3.40 mmol), absolute ethanol (5 ml), ethyl cyanoacetate (0.385 g, 3.40 mmol) and 50 % piperidine in ethanol (2 drops). The reaction was heated and on cooling the precipitate was collected and crystallised from isopropanol: (0.351 g, 76 %), m.p. 108-109 °C (from *i*-PrOH); R_F 0.45 (ethyl acetate/petroleum ether, 1:1); (Found: C, 47.49; H, 3.09; N, 10.95; M^+, 252.0224. $C_{10}H_8N_2O_4S$ requires C, 47.62; H, 3.20; N, 11.10%; *M*, 252.0199); ν_{max}(KBr disc)/cm^{-1} 3100m (=C–H), 2990w (CH_x), 2240m (CN), 1720m (C=O), 1450s (NO_2), 1300s (C–O), 825m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.86 (1 H, d, $^4J_{5\text{-}H}$ 1.96 Hz, 8-H), 8.63 (1 H, d, $^4J_{8\text{-}H}$ 1.96 Hz, 5-H), 8.42 (1 H, s, 3-H), 4.30 (2 H, q, ^3J 7.1 Hz, CH_2), 1.29 (3 H, q, ^3J 7.1 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 161.5 (C=O), 152.5 (7-C), 147.1 (3-CH), 142.8 (8-CH), 132.6 (4-C), 128.3 (5-CH), 115.5 (1-CN), 103.0 (2-C), 62.6 (CH_2), 14.0 (CH_3); *m/z* 252 (M^+, 19%), 224 ($MH^+ - CH_2CH_3$, 2), 206 ($M^+ - HOCH_2CH_3$, 53), 178 ($M^+ - CO_2CH_2CH_3$, – H, 100), 161 ($M^+ - NO_2$, – H-CS, 7), 150 (18), 133 ($M^+ - NO_2$, – $CO_2CH_2CH_3$, 25), 122 (19), 106 (9), 82 ([*th*]$^+$, 26).

n-*Butyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate* (**166**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-4-carbaldehyde (0.601 g, 3.88 mmol), *n*-butanol (6 ml) and *n*-butyl cyanoacetate (0.540 g, 3.82 mmol). The reaction was heated and on cooling the crystals which formed were collected: (0.420 g, 39 %), m.p. 144-145 °C (from *i*-PrOH); R_F 0.71 (ethyl acetate/petroleum ether, 1:1); (Found: C, 51.37; H, 4.08; N, 10.10; M^+, 280.0527. $C_{12}H_{12}N_2O_4S$ requires C, 51.42; H, 4.32; N, 9.99%; *M*, 280.0521); ν_{max}(KBr disc)/cm^{-1} 3100m (=C–H), 2940m (CH_x), 2200w (CN), 1718m (C=O), 1610m (C=C), 1491s (NO_2), 1280s (C–O), 817w (=C–H); δ_H(200 MHz; DMSO-d_6) 8.86 (1 H, m, 8-H), 8.64 (1 H, m, 5-H), 8.42 (1 H, m, 3-H), 4.26 (2 H, t, ^3J 6.5 Hz, $\underline{CH_2}CH_2CH_2CH_3$), 1.63 (2 H, tq, ^3J 7.2 Hz, ^3J 6.4 Hz, $CH_2CH_2\underline{CH_2}CH_3$), 1.39 (2 H, tt, ^3J 6.4 Hz, ^3J 6.5 Hz, $CH_2\underline{CH_2}CH_2CH_3$), 0.90 (3 H, t, ^3J 7.2 Hz, $CH_2CH_2CH_2\underline{CH_3}$); *m/z* 280 ($M^+$, 21%), 234 ($M^+ - NO_2$, 100), 223 ($M^+ - CH_2CH_2CH_2CH_3$, 31), 207 ($M^+ - OCH_2CH_2CH_2CH_3$, 32), 179 ($M^+ - CO_2CH_2CH_2CH_2CH_3$, 43), 133 ($M^+ - NO_2$, – $CO_2CH_2CH_2CH_2CH_3$, 27), 82 ([*th*]$^+$, 14), 69 (25), 57 ([$CH_2CH_2CH_2CH_3$]$^+$, 87).

4-Nitro-2-thiophenecarbaldehyde (**168**)—While stirring at ambient temperatures with an overhead mechanical stirrer, a 50 ml beaker was charged with concentrated sulfuric

concentrated sulfuric acid (98 % (w/v), 21.20 g, 0.22 mol) and thiophene-2-carbaldehyde (8.71 g, 0.078 mol). This solution was cooled to *ca.* -10 °C in an ice/salt water bath while a nitrating solution of concentrated sulfuric acid (16.3 g, 0.17 mol) plus *fuming* nitric acid (18.9 g, 0.30 mol) was prepared and similarly cooled to *ca.* -10 °C. The nitrating solution was slowly added to the reaction solution (1.5 h) while keeping the temperature between -10 and -20 °C by the addition of freshly crushed carbon dioxide snow. The reaction mixture was allowed to reach room temperature over 1.5 h and was poured onto ice (300 g) in a 1 l wide-necked conical flask. The aqueous solution was extracted with diethyl ether (3 × 150 ml), and the ether extracts were dried over anhydrous sodium sulphate and concentrated *in vacuo*. By 90 MHz ^{1}H NMR spectroscopy an *ca.* 3:2 mixture of the 4- and 5-nitrothiophene-2-carbaldehyde products were obtained. This was subjected to column chromatography on silica with petroleum ether (bp 30-40 °C)/ethyl acetate (4:1) and the oily main fraction was subjected to a series of crystallisations at *ca.* O °C and -15 °C in isopropanol with added *n*-pentane or petroleum ether (bp 30-40 °C). This gave 4-nitrothiophene-2-carbaldehyde as flakey brown crystals (4.07 g, 33 %), m.p. 47.4 °C (*i*-PrOH/*n*-pentane) (lit.,[269] 35-37 °C); R_F 0.84 (diethylether); (Found: C, 37.70; H, 1.80; N, 9.22; M^+, 157.1483. $C_5H_3NO_3S$ requires C, 38.22; H, 1.92; N, 8.91%; *M*, 157.1419); ν_{max}(KBr disc)/cm^{-1} 3118m and 3088m (=C–H), 2835w (CHO), 1683s (C=O), 1540s (C-NO_2), 1513s (Ar), 881s, 784s, 738s, 710s and 662s (=C–H); δ_H(200 MHz; $CDCl_3$) 9.93 (1 H, d, $^4J_{3\text{-H}}$ 1.38 Hz, 1-HC=O), 8.63 (1 H, dd, $^4J_{1\text{-H/5-H}}$ 1.43 Hz, 3-H), 8.26 (1 H, d, $^4J_{3\text{-H}}$ 1.48 Hz, 5-H); δ_C(50 MHz; $CDCl_3$) 182.0 (CHO), 148.7 (4-C), 134.4 (3-CH), 131.2 (2-C), 129.4 (5-CH); *m/z* 157 (M^+, 100%), 127 (M^+ – NO, 15), 110 (M^+ – NO_2, – H, 16), 82 ([*th*]$^+$, M^+ – NO_2, – CHO, 46).

2-Cyano-3-[2-(4-nitrothienyl)]propenonitrile (**169**)—Using Method C, a 50 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.232 g, 1.48 mmol), absolute ethanol (3 ml), malononitrile (0.098 g, 1.48 mmol) and ethanolic piperidine (1:1, 1 drop). After 10 min the precipite was filtered and gave: (0.212 g, 70 %), m.p. 169-171 °C (from EtOH); R_F 0.81 (diethyl ether); (Found: C, 46.44; H, 1.53; N, 20.75; M^+, 204.9938. $C_8H_3N_3O_2S$ requires C, 46.83; H, 1.47; N, 20.47%; *M*, 204.9983); ν_{max}(KBr disc)/cm^{-1} 3092s, 3073s and 3039s (=C–H), 2232s (CN), 1685w (C=C), 1592s, 1542m and 1531s (Ar), 1531s (NO_2), 883m, 782s and 746s (=C–H); δ_H(200 MHz; acetone-d_6) 9.11 (1 H, q, $^3J_{7\text{-H}}$ 1.52 Hz, *J* 0.56 Hz, 5-H), 8.52 (1 H, s, 3-H), 8.46 (1 H, dd, $^4J_{5\text{-H}}$ 1.60 Hz, $^4J_{3\text{-H}}$

0.44 Hz 7-H); δ_C(50 MHz; acetone-d_6) 151.9 (3-CH), 149.8 (6-C), 136.1 (5-CH), 132.2 (7-CH), 117.3 (4-C), 112.6 and 113.1 (2 × CN), 84.3 (2-C); *m/z* 205 (M^+, 61%), 204 (M^+ – H, 100), 175 (M^+ – N=O, 14), 159 (M^+ – NO_2, 31), 147 (M^+ – HCN, – HS, 9), 132 (M^+ – NO_2, – HCN, 21), 115 (M^+ – NO_2, – C≡S, 52), 94 (13), 88 (38), 82 (17), 69 (22).

3-Amino-2,4-dicyano-5-[2-(4-nitrothienyl)]penta-2,4-dienonitrile (**170**)—Using Method C, a 100 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.550 g, 3.50 mmol), absolute ethanol (10 ml), 1.1.3-tricyano-2-amino-propene (0.461 g, 3.49 mmol) and ethanolic piperidine (1:1, 1 drop). After 1 h the yellow precipitate was filtered and gave: (0.526 g, 56 %), m.p. 270-273 °C (from EtOH); R_F 0.16 (diethyl ether); (Found: C, 48.47; H, 1.73; N, 25.88; M^+, 271.0169. $C_{11}H_5N_5O_2S$ requires C, 48.71; H, 1.86; N, 25.82%; *M*, 271.0112); ν_{max}(KBr disc)/cm^{-1} 3373m, 3330m and 3216m (NH_2), 3094s and 3005m (=C–H), 2227s and 2215s (CN), 1654s (C=C), 1587s and 1540s (Ar), 1539s (NO_2), 780m, 733s and 618s (=C–H); δ_H(200 MHz; DMSO-d_6) 9.19 and 9.17 (2 H, 2 × br s, NH_2), 9.18 (1 H, s, 5-H), 8.42 (2 H, s, 3- and 5-H); δ_C(50 MHz; DMSO-d_6) 164.3 (H_2N-C=C$(CN)_2$), 147.9 (6-C), 145.1 (3-CH), 136.8 (5-CH), 130.8 (7-CH), 130.7 (4-C), 115.4 (1-CN), 114.6 and 114.6 (2 × CN), 101.2 (2-C), 50.2 (H_2N-C=C$(CN)_2$); *m/z* 271 (M^+, 39%), 254 (M^+ –NH_3, 3), 241 (M^+ – N=O, 14), 224 (M^+ – H, – NO_2, 13), 193 (M^+ – NO_2, – HS, 100), 154 (M^+ – CN, – H_2N-C=C$(CN)_2$, + H, 10), 139 (7), 92 ([H_2N-C=C$(CN)_2$]$^+$, 9.

2-Cyano-3-[2-(4-nitrothienyl)]propenamide (**171**)—Using Method C, a 50 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.397 g, 2.53 mmol), absolute ethanol (4 ml), cyanoacetamide (0.212 g, 2.52 mmol) and ethanolic piperidine (1:1, 1 drop). After 3.25 h the precipitate was filtered, subjected to chromatography on silica with ethylacetate/petroleum ether (bp 30-40 °C) dilutions and gave: (0.103 g, 18 %), m.p. 202-204 °C (from EtOH); R_F 0.34 (diethyl ether); (Found: C, 43.98; H, 2.08; N, 18.59; M^+, 223.0896. $C_8H_5N_3O_3S$ requires C, 43.05; H, 2.26; N, 18.83%; *M*, 223.0968); ν_{max}(KBr disc)/cm^{-1} 3423m and 3181m (NH_2), 3089s and 3048m (=C–H), 2216s (CN), 1690s (C=O), 1599s and 1542m (Ar), 1541s (NO_2), 865m, 781m and 738m (=C–H); δ_H(200 MHz; DMSO-d_6) 9.16 (1 H, br s, 5-H), 8.62 (1 H, s, 3-H), 8.41 (1 H, br s, 7-H), 7.92 (2 H, v.br s, NH_2); δ_C(50 MHz; DMSO-d_6) 161.8 (C=O), 147.8 (6-C), 142.6 (3-CH), 136.6 (4-C), 134.8 (5-CH), 130.0 (7-CH), 116.2 (1-CN), 105.6 (2-C); *m/z* 223 (M^+,

13%), 207 ($M^+ - NH_2$, 4), 193 ($M^+ - N{=}O$, 5), 179 ($M^+ - CONH_2$, 3), 176 ($M^+ - NO_2, - H$, 8), 145 ($M^+ - NO_2, - S$, 100), 136 (22), 133 (12), 107 (6), 90 (5), 82 (8), 69 (11), 63 (14.5).

2-Cyano-3-[2-(4-nitrothienyl)]propenothioamide (**172**)—Using Method C, a 50 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.668 g, 4.25 mmol), absolute ethanol (5 ml) and cyanothioacetamide (0.426 g, 4.25 mmol). After the reaction reached ambient temperature the precipitate was filtered and gave: (0.535 g, 53 %), m.p. 212 °C (decomp.) (from EtOH); R_F 0.62 (diethyl ether); (Found: C, 39.77; H, 2.09; N, 17.01; M^+, 238.9910. $C_8H_5N_3O_2S_2$ requires C, 40.16; H, 2.11; N, 17.56%; *M*, 238.9956); ν_{max}(KBr disc)/cm^{-1} 3367m, 3277m and 3170m (NH_2), 3098s, 3084s and 3012m (=C–H), 2209m (CN), 1628s (C=S), 1596s and 1541m (Ar), 1540s (NO_2), 877s, 865m, 780m and 732m (=C–H); δ_H(200 MHz; DMSO-d_6) 10.22 and 9.66 (2 × 1 H, 2 × br s, NH_2), 9.15 (1 H, d, $^4J_{7\text{-H}}$ 1.17 Hz, 5-H), 8.40 (1 H, d, $^4J_{7\text{-H}}$ 1.17 Hz, 7-H), 8.36 (1 H, s, 3-H); δ_C(50 MHz; DMSO-d_6) 191.1 (C=S), 147.9 (6-C), 148.8 (3-CH), 136.5 (4-C), 135.0 (5-CH), 130.0 (7-CH), 116.1 (1-CN), 111.3 (2-C); *m/z* 239 (M^+, 17%), 205 ($M^+ - H_2S, - H$, 11), 192 ($M^+ - NO_2, - H$, 8), 161 ($M^+ - NO_2, - S$, 100), 148 ($M^+ - NO_2, - C{\equiv}S, - H$, 8), 133 (5), 115 (6), 88 (7.5), 82 (6), 69 (12), 60 (21.5).

Methyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate (**173**)—Using Method C, a 50 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.370 g, 2.35 mmol), methanol (3 ml) and methyl cyanoacetate (0.234 g, 2.36 mmol). The reaction was heated and on cooling the crystals were collected: (0.303 g, 54 %), m.p. 173-174 °C (from EtOH); R_F 0.81 (diethyl ether); (Found: C, 45.15; H, 2.47; N, 11.90; M^+, 238.0031. $C_9H_6N_2O_4S$ requires C, 45.38; H, 2.54; N, 11.76%; *M*, 238.0052); ν_{max}(KBr disc)/cm^{-1} 3114m, 3102m and 3025m (=C–H), 2958w and 2854m (CH_3), 2225m (CN), 1716s (C=O), 1604s (C=C), 1533s (NO_2), 1266s (C–O), 875m, 782m, 762m and 742m (=C–H); δ_H(200 MHz; DMSO-d_6) 9.18 (1 H, d, $^4J_{7\text{-H}}$ 1.37 Hz, 5-H), 8.69 (1 H, s, 3-H), 8.59 (1 H, d, $^4J_{7\text{-H}}$ 1.36 Hz, 7-H), 3.90 (3 H, s, CH_3); δ_C(50 MHz; DMSO-d_6) 161.9 (C=O), 147.8 (6-C), 146.5 (3-CH), 136.4 (5-CH), 136.0 (4-C), 132.3 (7-CH), 115.3 (1-CN), 101.4 (2-C), 53.6 (CH_3); *m/z* 238 (M^+, 43%), 222 ($M^+ - CH_4$, 2), 207 ($M^+ - OCH_3$, 33), 191 ($M^+ - NO_2, - H$, 9), 179 ($M^+ - CO_2CH_3$, 9), 160 ($M^+ - NO_2, - S$, 100), 133 ($M^+ - NO_2, - CO_2CH_3$, 17), 121 (7), 107 (5.5), 88 (9).

Ethyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate (**174**)—Using Method C, a 50 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.380 g, 2.42 mmol), absolute ethanol (3 ml) and ethyl cyanoacetate (0.274 g, 2.42 mmol). The reaction was heated and on cooling the crystals were collected: (0.351 g, 57.5 %), m.p. 170-171 °C (from EtOH); R_F 0.84 (diethyl ether); (Found: C, 47.38; H, 3.20; N, 11.28; M^+, 252.0221. $C_{10}H_8N_2O_4S$ requires C, 47.62; H, 3.20; N, 11.10%; *M*, 252.0201); ν_{max}(KBr disc)/cm^{-1} 3132m, 3102m and 3026w (=C–H), 2991w and 2942m (CH_2CH_3), 2225m (CN), 1718s (C=O), 1607s (C=C), 1539s (NO_2), 1259s (C–O), 879m, 782m, 762m and 736m (=C–H); δ_H(200 MHz; DMSO-d_6) 9.16 (1 H, d, $^4J_{7\text{-H}}$ 0.80 Hz, 5-H), 8.69 (1 H, s, 3-H), 8.61 (1 H, d, $^4J_{5\text{-H}}$ 0.93 Hz, 7-H), 4.39 (2 H, q, ^3J 7.11 Hz, CH_2), 1.38 (3 H, t, ^3J 7.11 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 161.2 (C=O), 148.0 (6-C), 145.8 (3-CH), 136.1 (4-C), 135.3 (5-CH), 131.5 (7-CH), 114.8 (1-CN), 102.0 (2-C), 62.4 (CH_2), 13.4 (CH_3); *m/z* 252 (M^+, 74%), 236 ($M^+ - CH_4$, 3), 224 ($M^+ - CH_2CH_3$, + H, 29), 207 ($M^+ - OCH_2CH_3$, 69), 179 ($M^+ - CO_2CH_2CH_3$, 25), 174 ($M^+ - NO_2$, – S, 91), 161 ($M^+ - NO_2$, – H-CS, 21), 146 ($M^+ - CO_2CH_2CH_3$, – HS, 100), 133 ($M^+ - NO_2$, – $CO_2CH_2CH_3$, 45), 122 (21), 107 (16), 94 (14), 82 (29).

n-*Butyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate* (**175**)—Using Method C, a 50 ml round-bottomed flask was charged with 4-nitrothiophene-2-carbaldehyde (0.389 g, 2.48 mmol), isopropanol (3 ml) and *n*-butyl cyanoacetate (0.349 g, 2.47 mmol). The reaction was heated and on cooling the crystals were collected: (0.283 g, 41 %), m.p. 150-151 °C (from *i*-PrOH); R_F 0.89 (diethyl ether); (Found: C, 51.36; H, 4.10; N, 10.15; M^+, 280.0519. $C_{12}H_{12}N_2O_4S$ requires C, 51.42; H, 4.32; N, 9.99%; *M*, 280.0518); ν_{max}(KBr disc)/cm^{-1} 3123m, 3097m and 3024w (=C–H), 2964m, 2937m and 2875w (CH_2CH_3), 2223w (CN), 1713s (C=O), 1608s (C=C), 1540s (NO_2), 1278s (C–O), 878m, 782m, 761m and 736s (=C–H); δ_H(200 MHz; DMSO-d_6) 9.20 (1 H, q, $^3J_{7\text{-H}}$ 1.66 Hz, *J* 0.70 Hz, 5-H), 8.71 (1 H, s, 3-H), 8.63 (1 H, dd, $^4J_{5\text{-H}}$ 1.66 Hz, $^4J_{3\text{-H}}$ 0.42 Hz 7-H), 4.31 (2 H, t, ^3J 6.44 Hz, $\underline{CH_2}CH_2CH_2CH_3$), 1.71 (2 H, tt, ^3J 6.42 Hz, ^3J 6.84 Hz, $CH_2\underline{CH_2}CH_2CH_3$), 1.44 (2 H, q t, *J* 7.3 Hz, $CH_2CH_2\underline{CH_2}CH_3$), 0.97 (3 H, t, ^3J 7.27 Hz, $CH_2CH_2CH_2\underline{CH_3}$); δ_C(50 MHz; DMSO-d_6) 161.4 (C=O), 147.9 (6-C), 146.4 (3-CH), 136.4 (5-CH), 136.1 (4-C), 132.3 (7-CH), 115.4 (1-CN), 101.7 (2-C), 66.2 ($\underline{C}H_2CH_2CH_2CH_3$), 30.1 ($CH_2\underline{CH_2}CH_2CH_3$), 18.6 ($CH_2CH_2\underline{CH_2}CH_3$), 13.7 ($CH_2CH_2CH_2\underline{CH_3}$); *m/z* 280 ($M^+$, 17%), 224 ($M^+$ –

$CH_2CH_2CH_2CH_3$, + H, 36), 207 ($M^+ - OCH_2CH_2CH_2CH_3$, 41), 202 ($M^+ - NO_2$, – S, 39), 180 ($M^+ - CO_2$ $CH_2CH_2CH_2CH_3$, + H, 13), 146 ($M^+ - CO_2CH_2CH_2CH_2CH_3$, – HS, 23), 133 ($M^+ - NO_2$, – $CO_2CH_2CH_2CH_2CH_3$, 23), 82 (14), 69 (14), 52 ([$CH_3CH{=}CHCH_3$], 100).

2-Nitro-3-(dichloromethyl)thiophene (**177**)—Using oven dried glassware, a two-necked 250 ml round-bottomed flask equipped with a nitrogen bubbler and equilibrating dropping funnel was charged with a magnetic stirrer and potassium *tert*-butoxide (11.48 g, 0.102 mol, 3 equivalents). Under a nitrogen atmosphere, dry DMF (25 ml) and THF (25 ml) (recently distilled over sodium/benzophenone) were added and the dispersion formed was cooled below -70 °C. While maintaining the temperature below -65 °C, a solution of 2-nitrothiophene (4.326 g, 0.0335 mol) and dry chloroform (4.3 ml, 0.0345 mol) in DMF (6 ml), was added dropwise. After the addition was complete the dark coloured solution was immediately quenched over 10 min with acetic acid/methanol (1:1, 12 ml, 3 equivalents) and then further acidified with another 12 ml. The reaction was allowed to reach ambient temperature, transferred to a separating funnel and partitioned between distilled water (150 ml) and diethyl ether (3 × 150 ml). The combined ether layers were washed with saturated sodium hydrogencarbonate (100 ml), brine (100 ml), dried over sodium sulphate and concentrated *in vacuo* to give a dark brown oil. This was subjected to column chromatography through a plug of silica using *c*-hexane and gave the title compound as a yellow crystalline paste (3.782 g, 53 %), δ_H(200 MHz; acetone-d_6) 8.01 (1 H, d, $^3J_{4\text{-}H}$ 5.69 Hz, 5-H), 7.75 (1 H, s, $CHCl_2$), 7.66 (1 H, d, $^3J_{5\text{-}H}$ 5.70 Hz, 4-H); δ_C(50 MHz; acetone-d_6) 140.9 (2-C), 133.3 (5-CH), 133.1 (3-C), 128.9 (4-CH), 63.9 ($CHCl_2$).

2-Nitrothiophene-3-carbaldehyde (**178**)[270]—Under a nitrogen atmosphere, a 250 ml round-bottomed flask equipped with a reflux condenser and nitrogen bubbler was charged with 2-nitro-3-(dichloromethyl)thiophene (3.529 g, 0.0166 mol), a magnetic stirrer and 85 % formic acid (100 ml: prepared from 98-100 % formic acid and distilled water) was degassed under vacuum and put through a nitrogen atmosphere. The reaction was heated at reflux for 18 h, left to reach room temperature for 2 h, concentrated *in vacuo* and partitioned between water (200 ml) and diethyl ether (3 × 150 ml). The combined ether extracts were washed with water (2 × 150 ml), then re-washed with water (150 ml; which was continually

basified with sodium hydrogencarbonate until pH remained at 8), dried over sodium sulphate and concentrated *in vacuo* to give a light brown oil which crystallised on standing (1.823 g, 70 %). This was dissolved in isopropanol/petroleum ether (3:5, 30 ml), filtered through a pad of Celite and crystallised at *ca.* -15 °C to give the title compound as yellow flakey crystals (1.251 g, 48 %), m.p. 60-61 °C (from *i*-PrOH/petroleum ether) (lit.,[270] 54 °C); R_F 0.38 (9:4 *n*-hexane/diethylether); (Found: C, 38.11; H, 1.87; N, 8.85; M^+, 156.9913. $C_5H_3NO_3S$ requires C, 38.22; H, 1.92; N, 8.91%; *M*, 156.9925); ν_{max}(KBr disc)/cm^{-1} 3110m, 3096m and 3080m (C=C–H), 2910w (O=C–H), 1680s (ArCHO carbonyl), 1534s (C–NO_2), 1510s (Ar), 1387s, 1332s and 1228s (C–S_{th}), 820s, 743m/s and 774m/s (=C–H); δ_H(200 MHz; DMSO-d_6) 10.55 (1 H, s, CHO), 7.76 (1 H, dd, $^3J_{4\text{-H}}$ 5.56 Hz, $^4J_{CHO}$ 0.70 Hz, 5-H), 7.51 (1 H, d, $^3J_{5\text{-H}}$ 5.57 Hz, 4-H); δ_C(50 MHz; DMSO-d_6) 183.7 (CHO), (2-C not observed), 137.2 (3-C), 131.1 (5-CH), 126.7 (4-CH); *m/z* 157 (M^+, 1.4%), 140 (M^+ – OH, 7), 127 (M^+ – CHO, – H, 9), 113 (M^+ – C≡S, 42), 110 (M^+ – HNO_2, 48.5), 99 (M^+ – H-C≡C-SH, 11), 82 ([*th*]$^+$, M^+ – CHO, – NO_2, 48), 71 ([C_3H_3S]$^+$ *e.g* [H_2C=CH–C≡S]$^+$, 18), 55 ([C_3H_3O]$^+$ *e.g.* [H_2C=CH–C≡O]$^+$, 34), 39 (86), 28 (100).

2-Cyano-3-[3-(2-nitrothienyl)]propenonitrile (**180**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.162 g, 1.03 mmol), absolute ethanol (2 ml), malononitrile (0.068 g, 1.03 mmol) and ethanolic piperidine (1:1 ,1 drop). After 1 h the precipitate was filtered to give (0.142 g, 67 %), m.p. 194-195 °C (from EtOH); R_F 0.67 (ethyl acetate/petroleum ether, 1:1); (Found: C, 46.47; H, 1.51; N, 20.72; M^+, 205.0003. $C_8H_3N_3O_2S$ requires C, 46.83; H, 1.47; N, 20.47%; *M*, 204.9987); ν_{max}(KBr disc)/cm^{-1} 3038s (=C–H), 2230w (CN), 1636m (C=C), 1580s and1545m (Ar), 1522s (C–NO_2), 870w and 834w (=C–H); δ_H(200 MHz; DMSO-d_6) 8.72 (1 H, s, 3-H), 8.22 (1 H, d, $^3J_{8\text{-H}}$ 5.7 Hz, 7-H), 7.76 (1 H, d, $^3J_{7\text{-H}}$ 5.7 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) (5-C maybe masked by 3-CH), 152.2 (3-CH), 134.3 (7-CH), 131.6 (4-C), 127.3 (8-CH), 113.3 and 112.2 (2 × CN), 88.3 (2-C); *m/z* 205 (M^+, 46%), 188 (M^+ – OH, 15), 175 (M^+ – N=O, 4.5), 159 (M^+ – NO_2, 20), 147 (M^+ – HCN, – HS, 8), 132 (M^+ – NO_2, – HCN, 17), 115 (M^+ – NO_2, – C≡S, 18), 97 (51), 88 (49), 82 ([*th*]$^+$, 17), 70 (44).

3-Amino-2,4-dicyano-5-[3-(2-nitrothienyl)]penta-2,4-dienonitrile (**181**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.176 g, 1.12 mmol), absolute ethanol (10 ml), 1.1.3-tricyano-2-amino-propene (0.149 g,

1.13 mmol) and ethanolic piperidine (1:1, 1 drop). After 1 h the yellow precipitate was filtered to give (0.185 g, 61 %), m.p. 210-212 °C (from EtOH); R_F 0.58 (ethyl acetate/petroleum ether, 1:1); (Found: C, 48.58; H, 1.70; N, 25.71; M^+, 271.0027. $C_{11}H_5N_5O_2S$ requires C, 48.71; H, 1.86; N, 25.82%; *M*, 271.0112); ν_{max}(KBr disc)/cm^{-1} 3052m (=C–H), 2726m (C–NH_2), 2223m and 2209m (CN), 1647s (C=C), 1607s and 1540s (Ar), 1489s (C–NO_2), 820w (=C–H); δ_H(200 MHz; DMSO-d_6) 9.27 (2 H, br s, NH_2), 8.40 (1 H, s, 3-H), 8.23 (1 H, d, $^3J_{8\text{-}H}$ 5.6 Hz, 7-H), 7.78 (1 H, d, $^3J_{7\text{-}H}$ 5.6 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 164.0 (H_2N-C=C(CN)$_2$), 151.2 (5-C), 143.8 (3-CH), 134.3 (7-CH), 132.2 (4-C), 127.2 (8-CH), 115.1 (1-CN), 114.4 and 114.0 (H_2N-C=C(CN)$_2$), 108.2 (2-C), 50.7 (H_2N-C=C(CN)$_2$); *m/z* 271 (M^+, 5%), 240 (M^+ – H-N=O, 12), 224 (M^+ – H, – NO_2, 100), 197 (M^+ – HNO_2, – HCN, 56), 180 (M^+ – HN-C=C(CN)$_2$, 30), 170 (M^+ – HNO_2, – 2 × CN, 29), 158 (M^+ – NO_2, – HC(CN)$_2$, 17), 92 ([H_2N-C=C(CN)$_2$]$^+$, 15).

2-Cyano-3-[3-(2-nitrothienyl)]propenamide (**182**)—Using Method C, a 50 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.156 g, 0.99 mmol), absolute ethanol (2 ml), cyanoacetamide (0.091 g, 1.08 mmol) and ethanolic piperidine (1:1, 1 drop). After 3 h the precipitate was filtered to give (0.064 g, 29 %), m.p. 217-219 °C (from EtOH); R_F 0.57 (ethyl acetate/petroleum ether, 1:1); (Found: C, 43.76; H, 2.07; N, 18.47; M^+, 223.0876. $C_8H_5N_3O_3S$ requires C, 43.05; H, 2.26; N, 18.83%; *M*, 223.0959); ν_{max}(KBr disc)/cm^{-1} 3455m and 3380m (NH_2), 3077s (=C–H), 2200m (CN), 1618s (C=O), 1599m (Ar), 1495m (NO_2), 824m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.21 (1 H, s, 3-H), 8.19 (1 H, d, $^3J_{8\text{-}H}$ 5.6 Hz, 7-H), 8.08 (2 H, br s, NH_2), 7.79 (1 H, d, $^3J_{7\text{-}H}$ 5.6 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 161.3 (C=O), 150.5 (5-C), 141.9 (3-CH), 133.8 (7-CH), 133.4 (4-C), 127.7 (8-CH), 115.3 (1-CN), 112.3 (2-C); *m/z* 223 (M^+, 5%), 206 (M^+ – NH_3, 2), 193 (M^+ – N=O, 5), 179 (M^+ – $CONH_2$, 11), 177 (M^+ – NO_2, 100), 163 (13.5), 153 (M^+ – $CONH_2$, – CN, 17), 136 (19), 133 (M^+ – NO_2, – $CONH_2$, 17), 110 (14), 107 (12), 97 (15), 95 (23.5), 88 (18), 82 ([*th*]$^+$, 14), 69 (24).

2-Cyano-3-[3-(2-nitrothienyl)]propenothioamide (**183**)—Using Method C, in the absence of light and under a nitrogen atmosphere, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.152 g, 0.97 mmol), absolute ethanol (2 ml) and cyanothioacetamide (0.098 g, 0.98 mmol). After the reaction reached ambient temperature no precipitation occurred. The reaction was re-heated to near boiling and

petroleum ether was added. On cooling the precipitate was filtered off to give (0.155 g, 66 %), m.p. 90-93 °C (from EtOH/Petroleum ether); R_F 0.55 (ethyl acetate/petroleum ether, 1:1); (Found: C, 39.81; H, 2.06; N, 17.13; M^+, 239.0001. $C_8H_5N_3O_2S_2$ requires C, 40.16; H, 2.11; N, 17.56%; *M*, 238.9941); ν_{max}(KBr disc)/cm^{-1} 3330m (NH_2), 3096s (=C–H), 2188m (CN), 1625s (C=S), 1598m and 1545m/w (Ar), 1507s (NO_2), 818w (=C–H); δ_H(200 MHz; DMSO-d_6) 9.84 and 9.55 (2 × 1 H, 2 × br s, NH_2), 8.05 (1 H, d, $^3J_{8\text{-}H}$ 5.6 Hz, 7-H), 7.73 (1 H, s, 3-H), 7.62 (1 H, d, $^3J_{7\text{-}H}$ 5.6 Hz, 8-H); *m/z* 239 (M^+, 7%), 193 ($M^+ - NO_2$, 100), 160 ($M^+ - NO_2$, – HS, 10), 149 ($M^+ - NO_2$, – C≡S, 8), 133 ($M^+ - NO_2$, – $CSNH_2$, 17), 95 (23), 88 (14), 82 ([*th*]$^+$, 16), 69 (12).

Methyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate (**184**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.130 g, 0.83 mmol), methanol (1.5 ml) and methyl cyanoacetate (0.103 g, 1.04 mmol). The reaction was heated with 5% piperidine in methanol (1 drop) and on cooling the crystals were collected to give (0.085 g, 43 %), m.p. 120-121 °C (from MeOH); R_F 0.46 (ethyl acetate/petroleum ether, 1:1); (Found: C, 45.27; H, 2.48; N, 11.63; M^+, 238.0036. $C_9H_6N_2O_4S$ requires C, 45.38; H, 2.54; N, 11.76%; *M*, 238.0057); ν_{max}(KBr disc)/cm^{-1} 3125m (=C–H), 2920w (CH_3), 2250w (CN), 1717s (C=O), 1530m/s (C–NO_2), 1270s (C-O), 820m (=C–H); δ_H(200 MHz; DMSO-d_6) 9.00 (1 H, s, 3-H), 8.28 (1 H, d, $^3J_{8\text{-}H}$ 5.7 Hz, 7-H), 7.83 (1 H, d, $^3J_{7\text{-}H}$ 5.7 Hz, 8-H), 3.86 (3 H, s, CH_3); δ_C(50 MHz; DMSO-d_6) 161.6 (C=O), 151.8 (5-C), 145.4 (3-CH), 133.9 (7-CH), 132.3 (4-C), 127.3 (8-CH), 114.6 (1-CN), 108.1 (2-C), 53.8 (CH_3); *m/z* 238 (M^+, 9%), 207 (M^+ –OCH_3, 2.5), 192 ($M^+ - NO_2$, 100), 179 ($M^+ - CO_2CH_3$, 5), 177 (12), 161 ($MH^+ - NO_2$, – S, 19), 133 ($M^+ - NO_2$, – CO_2CH_3, 18), 121 (12), 111 (16), 107 (11), 97 (16), 88 (18), 59 ([CO_2Me]$^+$, 33.5).

Ethyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate (**185**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.127 g, 0.81 mmol), absolute ethanol (1.5 ml)and ethyl cyanoacetate (0.118 g, 1.04 mmol). The reaction was heated with 5% piperidine in ethanol (1 drop) and on cooling the crystals were collected: (0.096 g, 47 %), m.p. 90-91 °C (from EtOH); R_F 0.63 (ethyl acetate/petroleum ether, 1:1); (Found: C, 47.35; H, 3.23; N, 11.04; M^+, 252.0183. $C_{10}H_8N_2O_4S$ requires C, 47.62; H, 3.20; N, 11.10%; *M*, 252.0199); ν_{max}(KBr disc)/cm^{-1} 3115m (=C–H), 2993w and 2942m (CH_x), 2240w (CN), 1725s (C=O), 1607s and 1501s (Ar and C=C), 1532s (C–NO_2),

1265s (C–O), 819m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.72 (1 H, s, 3-H), 8.20 (1 H, d, $^3J_{8\text{-}H}$ 5.6 Hz, 7-H), 7.83 (1 H, d, $^3J_{7\text{-}H}$ 5.6 Hz, 8-H), 4.36 (2 H, q, ^3J 7.1 Hz, CH_2), 1.36 (3 H, t, ^3J 7.1 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 161.2 (C=O), 151.8 (5-C), 145.3 (3-CH), 133.9 (7-CH), 132.4 (4-C), 127.4 (8-CH), 114.6 (1-CN), 108.4 (2-C), 63.0 (CH_2), 14.0 (CH_3); *m/z* 252 (M^+, 7%), 222 ($M^+ - CH_2CH_3$, – H, 2), 206 ($M^+ - HOCH_2CH_3$, 20), 178 ($M^+ - CO_2CH_2CH_3$, – H, 93), 161 ($M^+ - NO_2$, – H-C=S, 25), 150 ($M^+ - NO_2$, – C=C=S, 36.5), 147 ($M^+ - CO_2CH_2CH_3$, – S, 4), 133 ($M^+ - NO_2$, – $CO_2CH_2CH_3$, 19), 122 (14), 107 (9), 97 (22), 82 ([*th*]$^+$, 29).

n-*Butyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate* (**186**)—Using Method C, a 25 ml round-bottomed flask was charged with 2-nitrothiophene-3-carbaldehyde (0.090 g, 0.57 mmol), isopropanol (1 ml) and *n*-butyl cyanoacetate (0.146 g, 1.03 mmol). The reaction was heated with 5% piperidine in ethanol (1 drop) and on cooling the crystals were collected. These were washed briefly with *iso*propanol/*n*-pentane (1:2, 2 × 3 ml) and then *n*-pentane (3 × 5 ml) to give (0.078 g, 49 %), m.p. 69-70 °C (from *i*-PrOH/*n*-pentane); R_F 0.60 (ethyl acetate/petroleum ether, 1:1); (Found: C, 51.34; H, 4.28; N, 9.72; M^+, 280.0525. $C_{12}H_{12}N_2O_4S$ requires C, 51.42; H, 4.32; N, 9.99%; *M*, 280.0517); ν_{max}(KBr disc)/cm^{-1} 3120w (=C–H), 2950w (CH_x), 2250w (CN), 1725s (C=O), 1608s and 1522s (Ar and C=C), 1530s (NO_2), 1275s (C–O), 822s (=C–H); δ_H(200 MHz; DMSO-d_6) 8.82 (1 H, s, 3-H), 8.20 (1 H, d, $^3J_{8\text{-}H}$ 5.6 Hz, 7-H), 7.86 (1 H, d, $^3J_{7\text{-}H}$ 5.6 Hz, 8-H), 4.35 (2 H, t, ^3J 6.4 Hz, $\underline{CH}_2CH_2CH_2CH_3$), 1.73 (2 H, tt, ^3J 7.8 Hz, ^3J 6.4 Hz, $CH_2\underline{CH}_2CH_2CH_3$), 1.41 (2 H, q t, ^3J 7.8 Hz, ^3J 7.1 Hz, $CH_2CH_2\underline{CH}_2CH_3$), 0.93 (3 H, t, ^3J 7.1 Hz, $CH_2CH_2CH_2\underline{CH}_3$); *m/z* 280 ($M^+$, 11%), 234 ($M^+ - NO_2$, 39), 223 ($M^+ - CH_2CH_2CH_2CH_3$, 16), 207 ($M^+ - OCH_2CH_2CH_2CH_3$, 21), 179 ($M^+ - CO_2\ CH_2CH_2CH_2CH_3$, + H, 81), 147 ($M^+ - CO_2CH_2CH_2CH_2CH_3$, – S, 9), 133 ($M^+ - NO_2$, – $CO_2CH_2CH_2CH_2CH_3$, 13), 107 (13), 97 (23), 82 ([*th*]$^+$, 11), 69 (14), 57 ([$CH_2CH_2CH_2CH_3$], 26).

2-Cyano-3-(2-quinolinyl)propenonitrile (**187**)—Using Method B, 2-quinolinecarbaldehyde (1.270 g, 8.08 mmol) and malononitrile (0.536 g, 8.11 mmol) were reacted in ethanol (10 ml) with piperidine (2 drops). After 1.5 h the orange precipitate was collected (1.491 g, 90 %) and crystallised from isopropanol: (0.567 g, 35 %), m.p. 168-170 °C (from *i*-PrOH); R_F 0.67 (diethyl ether); (Found: C, 76.00; H, 3.47; N, 20.42; M^+, 205.0636. $C_{13}H_7N_3$ requires C, 76.10; H, 3.41; N, 20.49%; *M*, 205.0632); ν_{max}(KBr

disc)/cm^{-1} 3045m (Ar–H), 2230s (CN), 1615w (C=C), 1600m, 1585m and 1500m (Ar), 825s and 765s (=C–H); δ_H(200 MHz; DMSO-d_6) 8.63 (1 H, s, 3-H), 8.52 (1 H, d, ^3J 8.46 Hz, 7-H), 8.00 (2 H, 2 × br d (eclipsing), ^3J 7.84 Hz, ^3J 8.44 Hz, 12- and 13-H), 7.85 (1 H, m, ^3J 6.75Hz, 8-H), 7.76 (1 H, d, 10-H), 7.71 (1 H, m, ^3J 6.73Hz, 9-H); δ_C(50 MHz; DMSO-d_6) 158.5 (3-CH), 148.8 (4-C), 146.9 (6-C), 137.9 (12-CH), 131.2 (7-CH), 129.4 and 129.2 (8- and 9-CH), 128.9 (11-C), 128.1 (10-CH), 124.1 (13-CH), 114.4 and 112.9 (2 × CN), 85.7 (2-C); *m/z* 205 (M^+, 100%), 179 (M^+ – CN, 12), 154 (35), 129 (M^+ – $HC{=}C(CN)_2$, + H, 25), 102 (M^+ – $HC{=}C(CN)_2$, – H-C≡C-H, 22.5), 75 (52), 63 (33), 51 (49).

3-Amino-2,4-dicyano-5-(2-quinolinyl)penta-2,4-dienonitrile (**188**) — Using Method B, 2-quinolinecarbaldehyde (1.053 g, 6.70 mmol) and 1,1,3-tricyano-2-aminopropene (0.887 g, 6.71 mmol) were reacted in THF (10 ml) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3 drops) and after 8.5 h the yellow precipitate was collected: (0.844 g, 46 %), m.p. 190 °C (decomp.) (from THF); R_F 0.23 (diethyl ether); (Found: C, 70.84; H, 3.34; N, 25.82; M^+, 271.0854. $C_{16}H_9N_5$ requires C, 70.61; H, 3.20; N, 25.63%; *M*, 271.0851); ν_{max}(KBr disc)/cm^{-1} 3325s and 3180s (NH_2), 3045w (Ar-H), 2210s and 2020s (CN), 1650s and 1620m (C=C), 1605m and 1545s (Ar), 830m and 765m (Ar-H); δ_H(200 MHz; DMSO-d_6) 9.39 and 9.31 (2 × 1 H, 2 × br s, NH_2), 8.62 (1 H, d, $^3J_{13\text{-H}}$ 8.43 Hz, 12-H), 8.30 (1 H, s, 3-H), 8.10 (2 H, 2 × d (overlapping), *J* 6.3 Hz, 7- and 10-H), 7.92 (2 H, t and d (overlapping), $^3J_{12\text{-H}}$ 8.47 Hz, 9- and 13-H), 7.78 (1 H, dt, *J* 7.6 Hz, *J* 1.1 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 165.7 (H_2N-C=$C(CN)_2$), 150.4 (3-CH), 149.5 (4-C), 147.1 (6-C), 137.8 (12-CH), 131.0 (7-CH), 129.1 (8-CH), 128.8 (10-CH), 128.4 (11-C), 128.1 (9-CH), 123.6 (13-CH), 115.6 (1-CN), 114.5 (H_2N-C=C($CN)_2$), 106.6 (2-C); *m/z* 271 (M^+, 89%), 245 (M^+– CN, 88), 217 (M^+– 2 × HCN, 17), 206 (M^+– $CH(CN)_2$, 61), 179 (M^+– H_2N-C=$C(CN)_2$, 39), 129 ($[Ar]^+$, M^+ – H_2N-C=$C(CN)_2$, – C≡C-CN, 100), 101 (38), 77 (38), 63 (23).

2-Cyano-3-(2-quinolinyl)propenamide (**189**)—Using Method B and oven dried equipment, under a nitrogen atmosphere, 2-quinolinecarbaldehyde (1.038 g, 6.60 mmol) and cyanoacetamide (0.555 g, 6.60 mmol) were reacted in freshly distilled THF (10 ml) containing 4 Å molecular sieves (*ca* .1.5.g) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 3 drops). After 4.5 h the reaction solution was concentrated under vacuum with some silica

gel in preparation for column chromatography. After eluting with ethyl acetate and petroleum ether; bp 30-40 °C (1:1 to 8:5 mixtures) the main fraction (0.485 g, 32 %) was triturated in ethylacetate to give the title compound (0.111 g, 7.2 %), m.p. 245 °C (decomp.) (from EtOAc); R_F 0.10 (diethyl ether); (Found: C, 69.49; H, 3.98; N, 18.67; M^+, 223.0733. $C_{13}H_9N_3O$ requires C, 69.95; H, 4.06; N, 18.82%; *M*, 223.0721); ν_{max}(KBr disc)/cm^{-1} 3200m (NH_2), 2210w (CN), 1710s (C=O), 1645w (C=C), 1590s and 1495m (Ar), 1380s, 1175m and 1130m (C–O), 880m, 855m/s and 680m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.57 (1 H, d, $^3J_{13\text{-}H}$ 8.5, 12-H), 8.31 (1 H, s, 3-H), 8.08 (2 H, t & d (overlapping), ^3J 8.4 Hz, 9- and 13-H), 7.98 (2 H, 2 × d (overlapping), 7- and10-H), 7.73 (1 H, m, 8-H); δ_C(50 MHz; DMSO-d_6) 163.0 (C=O), 151.5 (3-CH), 148.0 (6-C), 147.3 (4-C), 136.0 (12-CH), 130.1 (8-CH), 130.1 (7-CH), 129.8 (10-CH), 128.7 (11-C), 127.6 (9-CH), 122.7 (13-CH), 115.5 (CN), 111.8 (2-C); *m/z* 223 ($[M]^+$, 61%), 206 ($[M]^+ - NH_3$, 9), 194 ($[M]^+$ – HCN, 24), 179 ($[M]^+ - NH_3$, – CO, 31), 152 ($[M]^+$ – HCN, – $CONH_2$, 36), 128 ($[M]^+ - CONH_2$, – H-C≡C-CN, 53), 101 (22), 75 (35).

2-Cyano-3-(2-quinolinyl)propenothioamide (**190**)—Using Method B, 2-quinolinecarbaldehyde (0.207 g, 1.32 mmol) and cyanothioacetamide (0.132 g, 1.32 mmol) were reacted in ethanol (4 ml) with piperidine (2 drops) and after 1.5 h the solid was collected: (0.104 g, 33 %), m.p. 210 °C (decomp.) (from EtOH); R_F 0.23 (diethyl ether); (Found: C, 64.87; H, 3.57; N, 17.16; M^+, 239.0524. $C_{13}H_9N_3S$ requires C, 65.25; H, 3.79; N, 17.56%; *M*, 239.0518); ν_{max}(KBr disc)/cm^{-1} 3335s ($CSNH_2$), 3020s and 3005s (Ar–H), 2215s (CN), 1660s (C=S), 1600s, 1570s and 1490s (Ar–H), 885s, 785s, 745s and 620s (=C-H); δ_H(200 MHz; DMSO-d_6) 10.1 (2 × 1 H, 2 × br s, NH_2), 8.65 (1 H, d, $^3J_{13\text{-}H}$ 8.4 Hz, 12-H), 8.17 (1 H, s, 3-H), 8.12 (1 H, br d, $^3J_{13\text{-}H}$8.6 Hz, 12-H), 8.10 (2 H, t & d (overlapping), $^3J_{12\text{-}H}$ 8.4 Hz, 9- and 13-H), 8.02 (2 H, 2 × d (overlapping), ^3J 7.8 Hz, 7- and 10-H), 7.78 (1 H, dt, *J* 7.0 Hz, *J* 1.2 Hz, 8-H); δ_C(50 MHz; DMSO-d_6) 192.7 (C=S), 152.2 (3-CH), 147.2 (6-C), 143.3 (4-C), 137.0 (12-CH), 130.2 (7-CH), 130.1 (8-CH), 129.0 (10-CH), 128.6 (11-C), 127.7 (9-CH), 122.7 (13-CH), 117.8 (2-C), 115.5 (CN); *m/z* 239 (M^+, 91%), 206 (M^+ – HS, 79), 179 ($M^+ - CSNH_2$, 41), 152 ($M^+ - CSNH_2$, – HCN, 32), 142 (M^+ – NC-CH-$CSNH_2$, 33), 128 ($M^+ - CSNH_2$, – H-C≡C-CN, 24), 115 (16), 101 (26), 87 (18), 75 (36).

Ethyl 2-cyano-3-(2-quinolinyl)propenoate (**191**)—Using Method B, 2-quinolinecarbaldehyde (0.515 g, 3.28 mmol) and ethyl cyanoacetate (0.371 g, 3.28 mmol) were reacted in ethanol (5 ml) with piperidine (4 drops) and after 2.5 h the precipitate was collected: (0.490 g, 59 %), m.p. 108-109 °C (from EtOH); R_F 0.74 (diethyl ether); (Found: C, 71.24; H, 4.68; N, 11.04; M^+, 252.0902. $C_{15}H_{12}N_2O_2$ requires C, 71.42; H, 4.79; N, 11.10%; *M*, 252.0890); ν_{max}(KBr disc)/cm^{-1} 3055m (Ar-H), 2985m and 2960m (CH_x), 2220m (CN), 1730s (C=O), 1620m (C=C), 1600s (Ar), 1250s, 1230s and 1205s (C-O), 825s and 755s (Ar-H deformations); δ_H(200 MHz; $CDCl_3$) 8.48 (1 H, s, 3-H), 8.29 (1 H, br d, $^3J_{13\text{-H}}$ 8.60 Hz, 12-H), 8.21 (1 H, dm, *J* 8.4 Hz, 10-H), 8.05 (1 H, d, $^3J_{12\text{-H}}$ 8.54 Hz, 13-H), 7.95 (1 H, dd, $^3J_{8\text{-H}}$ 8.12 Hz, $^4J_{9\text{-H}}$ 1.2 Hz, 7-H), 7.80 (1 H, ddd, $^3J_{10\text{-H}}$ 8.48 Hz, $^3J_{8\text{-H}}$ 6.90 Hz, $^4J_{7\text{-H}}$ 1.60 Hz, 9-H), 7.65 (1 H, ddd, $^3J_{7\text{-H}}$ 8.08 Hz, $^3J_{9\text{-H}}$ 6.88 Hz, $^4J_{10\text{-H}}$ 1.25 Hz, 8-H), 4.43 (2 H, q, ^3J 7.14 Hz, CH_2), 1.43 (3 H, t, ^3J 7.13 Hz, CH_3); δ_C(50 MHz; $CDCl_3$) 162.0 (C=O), 153.8 (3-CH), 149.9 (4-C), 148.2 (6-C), 137.2 (12-CH), 130.6 (7-CH), 130.3 (8-CH), 129.0 (10-CH), 128.6 (11-C), 127.6 (9-CH), 121.9 (13-CH), 114.8 (1-CN), 107.8 (2-C), 63.0 (CH_2), 14.2 (CH_3); *m/z* 252 (M^+, 3%), 223 ($M^+ - C_2H_5$, 2), 208 ($M^+ - OC_2H_5$, + H, 59), 180 ($M^+ - CO_2C_2H_5$, + H, 100), 153 ($M^+ - CO_2C_2H_5$, – CN, 6), 128 ([C_9H_5N, *Ar*]$^+$, $-CO_2C_2H_5$, – H-C≡C-CN, 43), 101 ([C_8H_5]$^+$, [*Ar*]$^+$ – CN, 16), 77 (10).

tert-*Butyl 2-cyano-3-(2-quinolinyl)propenoate* (**192**)—Using Method B, 2-quinolylcarbaldehyde (0.515 g, 3.28 mmol) and *tert*-butyl cyanoacetate (0.371 g, 3.28 mmol) were reacted in absolute ethanol (5 ml) with the addition of piperidine (4 drops) and after 2.5 h the precipitate was collected (0.490 g, 59 %), m.p. 73-74 °C (from *i*-PrOH); R_F 0.82 (diethyl ether); (Found: C, 72.86; H, 5.81; N, 10.01; M^+, 280.1213. $C_{17}H_{16}N_2O_2$ requires C, 72.84; H, 5.75; N, 9.99%; *M*, 280.1211); ν_{max}(KBr disc)/cm^{-1} 3074w and 3010w (Ar-H), 2976m and 2930w (CH_3), 2224w (CN), 1720s (C=O), 1626m (C=C), 1592m/w and 1504m/w (Ar), 1284s, 1256m/s, 1248m and 1156s (C–O), 834m/s and 752m/s (Ar-H deformations); δ_H(200 MHz; $CDCl_3$) 8.39 (1 H, s, 3-H), 8.29 (1 H, br d, $^3J_{13\text{-H}}$ 8.52 Hz, 12-H), 8.21 (1 H, dm, *J* 8.3 Hz, 10-H), 8.07 (1 H, d, $^3J_{12\text{-H}}$ 8.55 Hz, 13-H), 7.87 (1 H, br d, $^3J_{8\text{-H}}$ 8.10 Hz, $^4J_{9\text{-H}}$ (unresolved), 7-H), 7.80 (1 H, ddd, $^3J_{10\text{-H}}$ 8.46 Hz, $^3J_{8\text{-H}}$ 6.96 Hz, $^4J_{7\text{-H}}$ 1.54 Hz, 9-H), 7.64 (1 H, ddd, $^3J_{7\text{-H}}$ 8.07 Hz, $^3J_{9\text{-H}}$ 6.85 Hz, $^4J_{10\text{-H}}$ 1.22 Hz, 8-H), 1.62 (9 H, s, ^{t}Bu); δ_C(50 MHz; $CDCl_3$) 160.7 (C=O), 153.0 (3-CH), 150.2 (6-C), 137.1 (12-CH), 130.5 (7-CH), 130.2 (8-CH), 128.8 (10-CH), 128.4 (11-C), 127.5 (9-CH), 121.9 (13-CH), 115.0

(1-CN), 109.5 (4-C), 84.2 (2-C), 27.9 (Bu^t); *m/z* 280 (M^+, 17%), 224 ($M^+ - {}^tBu, + H$, 5), 207 ($M^+ - O^tBu$, 27), 180 ($M^+ - CO_2{}^tBu, + H$, 75), 152 ($M^+ - CO_2{}^tBu, - HCN$, 5), 128 ($[C_9H_5N, Ar]^+, - CO_2{}^tBu, - H\text{-}C{\equiv}C\text{-}CN$, 39), 101 ($[C_8H_5]^+$, $[Ar]^+ - CN$, 19), 77 (13), 57 ($[^tBu]^+$, 100).

2-Cyano-3-(3-quinolinyl)propenonitrile (**193**)—Using Method B, 3-quinolinecarbaldehyde (0.147 g, 0.935 mmol) and malononitrile (0.063 g, 0.960 mmol) were reacted in ethanol (2.5 ml) with piperidine (1 drop) and after 2.5 h the precipitate was collected: (0.122 g, 64 %), m.p. 171-172 °C (from EtOH); R_F 0.68 (diethyl ether); (Found: C, 76.03; H, 3.19; N, 20.43; M^+, 205.0639. $C_{13}H_7N_3$ requires C, 76.09; H, 3.44; N, 20.48%; *M*, 205.0640); ν_{max}(KBr disc)/cm^{-1} 3040m (Ar–H), 2230s (CN), 1615m (C=C), 1595s, 1565m and 1490m (Ar), 790s and 765m (=C–H); δ_H(200 MHz; DMSO-d_6) 9.25 (1 H, s, 3-H), 8.86 (1 H, s, 5-H), 8.72 (1 H, s, 13-H), 8.09 (2 H, 2 × d (coinciding), *J* 7.0 Hz, 8- and 11-H), 7.94 (1 H, AA' (= t), $^3J_{10\text{-}H}$ 7.68 Hz, 9-H), 7.71 (1 H, AA' (= t), $^3J_{9\text{-}H}$ 7.41 Hz, 10-H); δ_C(50 MHz; DMSO-d_6) 158.8 (3-CH), 150.0 (5-CH), 148.8 (7-C), 138.8 (13-CH), 133.1 (9-CH), 129.8 (8-CH), 128.9 and 128.2 (10- and 11-CH), 126.3 (12-C), 124.8 (4-C), 113.9 and113.1 (2 × CN), 83.4 (2-C); *m/z* 205 (M^+, 100%), 178 ($M^+ - HCN$, 35), 154 ($M^+ - H\text{-}C{\equiv}C\text{-}CN$, 46), 151 ($M^+ - 2 \times HCN$, 28), 140 ($M^+ - CH(CN)_2$, 1.5), 129 ($M^+ - HC{=}C(CN)_2, + H$, 23), 124 (11), 100 (14), 75 (23), 63 (18).

2-Amino-2,4-dicyano-3-(3-quinolinyl)penta-2,4-dienonitrile (**194**)—Using Method B, 3-quinolinecarbaldehyde (0.108 g, 0.687 mmol) and 1,1,3-tricyano-2-aminopropene (0.093 g, 0.706 mmol) were reacted in methanol (2.5 ml) with piperidine (2 drops). After 1.5 h the reaction was heated for 0.5 h and methanol was evaporated off. Crystallisation from ethyl acetate/ethanol gave (16 mg, 0.84 %), m.p. 200 °C (decomp.) (from EtOH/EtOAc); R_F 0.09 (diethyl ether); (Found: C, 70.71; H, 3.18; N, 25.62; M^+, 271.0847. $C_{16}H_9N_5$ requires C, 70.84; H, 3.34; N, 25.82%; *M*, 271.0858); ν_{max}(KBr disc)/cm^{-1} 3415m, 3325m and 3230s (NH_2), 2215s and 2205m (CN), 1645s and 1620w (C=C), 1600m, 1581m and 1525m (Ar), 790w and 760w (Ar-H); δ_H(200 MHz; DMSO-d_6) 9.31 (1 H, d, $^4J_{13\text{-}H}$ 2.2 Hz, 5-H), 9.31 and 9.26 (2 × 1 H, 2 × br s, NH_2), 8.99 (1 H, d, $^4J_{5\text{-}H}$ 2.0 Hz, 13-H), 8.40 (1 H, s, 3-H), 8.18 (2 H, 2 × d (overlapping), 8- and 11-H), 7.99 (1 H, ddd, $^3J_{8/10\text{-}H}$ 6.9 Hz, $^4J_{11\text{-}H}$ 1.34 Hz, 9-H), 7.78 (1 H, ddd, $^3J_{9/11\text{-}H}$ 7.5 Hz, $^4J_{8\text{-}H}$ 1.03 Hz, 10-H); δ_C(50 MHz; DMSO-d_6) 165.0 ($H_2N\text{-}\underline{C}{=}C(CN)_2$), 150.8 (3-CH), 150.2 (5-CH), 148.6

(7-C), 137.8 (13-CH), 132.6 (8-CH), 129.6 (9-CH), 129.4 (11-CH), 128.2 (10-CH), 126.7 (12-C), 115.5 (1-CN), 114.8 and 114.7 (H_2N-C=C(CN)$_2$), 104.3 (2-C); *m/z* 271 (M^+, 79%), 245 (M^+– CN, 45), 217 (M^+– 2 × HCN, 17), 206 (M^+– $CH(CN)_2$, 72), 179 (M^+– H_2N-C=$C(CN)_2$, 11), 142 ([$C_{10}H_8N$, $ArCH_2$]$^+$, M^+ – NC-CH-C(NH_2)=$C(CN)_2$, 96), 101 (11), 76 (16).

2-Cyano-3-(3-quinolinyl)propenamide (**195**)—Using Method B, 3-quinolinecarbaldehyde (0.110 g, 0.700 mmol) and cyanoacetamide (0.060 g, 0.714 mmol) were reacted in ethanol (2.5 ml) with piperidine (2 drops) and after 2 h the precipitate was collected (0.119 g, 76 %), m.p. 240 °C (decomp.) (from EtOH); R_F 0.10 (diethyl ether); (Found: C, 69.87; H, 3.91; N, 18.77; M^+, 223.0732. $C_{13}H_9N_3O$ requires C, 69.95; H, 4.06; N, 18.82%; *M*, 223.0719); ν_{max}(KBr disc)/cm^{-1} 3230m and 3100m (NH_2), 2200w (CN), 1710s (C=O), 1645w (C=C), 1590s and 1495m (Ar), 1380s, 1175m and 1130m (C–X), 880m, 855m/s and 680m (Ar–H); δ_H(200 MHz; DMSO-d_6) 9.26 (1 H, d, $^4J_{13\text{-}H}$ 2.27, 5-H), 8.88 (1 H, d, $^4J_{5\text{-}H}$ 2.21, 13-H), 8.39 (1 H, s, 3-H), 8.06 (3 H, d, 8-H/11-H and $^1/_2$ NH_2), 7.88 (2 H, ddd, ^4J 1.4, $^3J_{10\text{-}H}$ 6.79, 9-H and $^1/_2$ NH_2), 7.68 (1 H, ddd, ^4J 1.3, $^3J_{9\text{-}H}$ 6.82, 10-H); δ_C(50 MHz; DMSO-d_6) 162.3 (C=O), 150.5 (3-CH), 148.3 (7-C), 147.8 (5-CH), 137.5 (13-CH), 132.0 (9-CH), 129.3 (8-CH). 128.9 (11-CH), 127.8 (10-CH), 126.7 (12-C), 125.5 (4-C), 116.3 (CN), 108.6 (2-C); *m/z* 223 ([M]$^+$, 75%), 206 ([M]$^+$ – NH_3, 53), 194 ([M]$^+$ – HCN, 17), 179 ([M]$^+$ – NH_3, – CO, 30), 152 ([M]$^+$ – HCN, – $CONH_2$, 46), 128 ([M]$^+$ – $CONH_2$, – H-C≡C-CN, 13), 125 (24), 101 (22), 75 (35).

2-Cyano-3-(3-quinolinyl)propenothioamide (**196**)—Using Method B, 3-quinolinecarbaldehyde (0.102 g, 0.649 mmol) and cyanothioacetamide (0.069 g, 0.692 mmol) were reacted in ethanol (3 ml) with piperidine (1 drop) and after 2.75 h the precipitate was collected: (0.113 g, 73 %), m.p. 235 °C (decomp.) (from EtOH); R_F 0.27 (diethyl ether); (Found: C, 65.07; H, 3.51; N, 17.36; M^+, 239.0512. $C_{13}H_9N_3S$ requires C, 65.25; H, 3.79; N, 17.56%; *M*, 239.0517); ν_{max}(KBr disc)/cm^{-1} 3365s ($CSNH_2$), 3020s and 3005s (Ar–H), 2210s (CN), 1655s (C=S), 1600s, 1570s and 1490s (Ar–H), 885s, 785s, 745s and 620s (=C–H); δ_H(200 MHz; DMSO-d_6) 10.27 and 9.77 (2 × 1 H, 2 × br s, NH_2), 9.24 (1 H, d, $^4J_{13\text{-}H}$ 2.3 Hz, 5-H), 8.93 (1 H, d, $^4J_{5\text{-}H}$ 2.2 Hz, 13-H), 8.28 (1 H, s, 3-H), 8.10 and 8.08 (2 H, 2 × dd (overlapping), ^3J 7.89 Hz, ^3J 7.91 Hz, 8- and 11-H), 7.90 (1 H, td, $^3J_{8\text{-}H/10\text{-}H}$ 6.91 Hz, $^4J_{11\text{-}H}$ 1.5 Hz, 9-H), 7.70 (1 H, td, $^3J_{9\text{-}H/11\text{-}H}$ 6.88 Hz, $^4J_{8\text{-}H}$ 1.3 Hz, 10-H);

δ_C(50 MHz; DMSO-d_6) 191.8 (C=S), 150.7 (3-CH), 148.2 (7-C), 143.7 (5-CH), 137.5 (13-CH), 132.1 (9-CH), 129.3 (8-CH), 128.9 (11-CH), 127.9 10-CH, 126.8 (12-C), 125.5 (4-C), 116.2 (CN), 114.4 (2-C); *m/z* 239 (M^+, 95%), 206 (M^+ – HS, 78), 179 (M^+ – $CSNH_2$, 38), 152 (M^+ – $CSNH_2$, – HCN, 38), 142 (M^+ – NC-CH-$CSNH_2$, 31), 128 (M^+ – $CSNH_2$, – H-C≡C-CN, 17), 115 (16), 101 (26), 87 (15), 75 (46), 60 (47).

Ethyl 2-cyano-3-(3-quinolinyl)propenoate (**197**)—Using Method B, 3-quinolinecarbaldehyde (0.105 g, 0.668 mmol) and ethyl cyanoacetate (0.078 g, 0.687 mmol) were reacted in ethanol (2 ml) with piperidine (2 drops) and after 0.5 h the precipitate was collected:(0.129 g, 77 %), m.p. 174-175 °C (from EtOH); R_F 0.55 (diethyl ether); (Found: C, 71.33; H, 6.64; N, 11.00; M^+, 252.0888. $C_{15}H_{12}N_2O_2$ requires C, 71.42; H, 4.79; N, 11.10%; *M*, 252.0899); ν_{max}(KBr disc)/cm^{-1} 3030w (Ar–H), 2985m and 2940w (CH_2CH_3), 2210w (CN), 1720s (C=O), 1605s, 1590s and 1485m (C=C, 1280s and 1250s (C–O), 760s (=C–H); δ_H(200 MHz; DMSO-d_6) 9.36 (1 H, d, $^4J_{13\text{-}H}$ 2.25 Hz, 5-H), 8.90 (1 H, d, $^4J_{5\text{-}H}$ 2.21 Hz, 13-H), 8.58 (1 H, s, 3-H), 8.08 (2 H, br d, ^3J 8.4 Hz, 8- and 11-H), 7.92 (1 H, td, 9-H), 7.72 (1 H, td, 10-H), 4.36 (2 H, q, ^3J 7.10 Hz, CH_2), 1.33 (3 H, t, ^3J 7.09 Hz, CH_3); δ_C(50 MHz; DMSO-d_6) 161.3 (C=O), 152.0 (3-CH), 150.4 (5-CH), 148.5 (7-C), 138.6 (13-CH), 132.3 (9-CH), 129.2 (8-CH), 128.7 (11-CH), 127.8 (10-CH), 126.4 (12-C), 124.8 (4-C), 115.3 (CN), 104.5 (2-C), 62.4 (CH_2), 13.8 (CH_3); *m/z* 252 (M^+, 65%), 223 (M^+ – C_2H_5, 30), 208 (M^+ – OC_2H_5, + H, 100), 179 (M^+ – $CO_2C_2H_5$, 37), 152 (M^+ – $CO_2C_2H_5$, – HCN, 49), 128 ([C_9H_5N, *Ar*]$^+$, – $CO_2C_2H_5$, – H-C≡C-CN, 13), 101 ([C_8H_5]$^+$, [*Ar*]$^+$ – CN, 22), 77 (10).

2-Cyano-3-(4-quinolinyl)propenonitrile (**198**)—Using Method B, 4-quinolinecarbaldehyde (0.508 g, 3.23 mmol) and malononitrile (0.214 g, 3.24 mmol) were reacted in ethanol (5 ml) with piperidine (3 drops) and after 1 h the precipitate was collected (0.330 g, 50 %) and crystallised from isopropanol to give the title compound as yellow needles (0.165 g, 25 %), m.p. 170-173 °C (from *i*-PrOH) (lit.,[332] 146-147 °C); R_F 0.62 (diethyl ether); (Found: C, 75.83; H, 3.46; N, 20.42; M^+, 205.0630. $C_{13}H_7N_3$ requires C, 76.10; H, 3.41; N, 20.49%; *M*, 205.0589); ν_{max}(KBr disc)/cm^{-1} 3065w, 3045w and 3030 (Ar–H), 2220m/s (CN), 1615w (C=C), 1595s, 1565m and 1505m (Ar), 1245m and 1145m (C–X), 920m/s, 785s and 765m (=C–H); δ_H(200 MHz; DMSO-d_6) 9.40 (1 H, s, 3-H), 9.11 (1 H, d, $^3J_{5\text{-}H}$ 4.5 Hz, 6-H), 8.22 (1 H, d, $^3J_{11\text{-}H}$ 8.4 Hz, 12–H), 8.14 (1 H, d, $^3J_{10\text{-}H}$ 8.2 Hz,

9–H), 7.93 (1 H, d, $^3J_{6\text{-H}}$ 4.7 Hz, 5-H), 7.89 (1 H, dt, 11-H), 7.75 (1 H, dt, 10-H); δ_C(50 MHz; DMSO-d_6) 158.9 (3-CH), 150.4 (6-CH), 147.9 (8-C), 136.3 (4-C), 131.2 (9-CH), 130.6 (5-CH), 129.7 (10-CH), 129.2 (11-CH), 124.4 (12-CH), 124.2 (13-C), 120.2 (9-CH), 113.2 and 112.2 (2 × CN), 89.8 (2-C); *m/z* 205 (M^+, 92%), 178 (M^+ – HCN, 100), 151 (M^+ – 2 × HCN, 32), 127 ($M^+ - H_2C{=}C(CN)_2$, 14), 100 ($M^+ - H_2C{=}C(CN)_2$, $-H_2C{=}CH$, 21), 75 (34), 63 (21).

2-Amino-2,4-dicyano-3-(4-quinolinyl)penta-2,4-dienonitrile (**199**) — Using Method B, 4-quinolinecarbaldehyde (0.102 g, 0.649 mmol) and 1,1,3-tricyano-2-aminopropene (0.087 g, 0.658 mmol) were reacted in methanol (3 ml) with DBU (1 drop). After 2 h the reaction was left at *ca.* 0 °C overnight (17 h), the solvent was removed under vacuum and crystallisation of the gum formed was attempted from isopropanol at 40 °C. However only a solid formed (0.127 g, 72 %), m.p. 190 °C (decomp.) (from *i*-PrOH); R_F 0.07 (diethyl ether); (Found: C, 70.84; H, 3.34; N, 25.82; M^+, 271.0856. $C_{16}H_9N_5$ requires C, 70.51; H, 3.17; N, 25.73%; *M*, 271.0854); ν_{max}(KBr disc)/cm^{-1} 3445s, 3335s and 3175s (NH_2), 3030m (Ar-H), 2230s and 2220s (CN), 1685m and 1660s (C=C), 1605w, 1545s and 1535s (Ar), 800w and 780w (Ar-H); δ_H(200 MHz; DMSO-d_6) 9.47 and 9.36 (2 × 1 H, 2 × br s, NH_2), 9.16 (1 H, d, $^3J_{5\text{-H}}$ 4.46 Hz, 6-H), 8.95 (1 H, s, 3-H), 8.29 (1 H, d, $^3J_{11\text{-H}}$ 8.10 Hz, 12-H), 8.20 (1 H, d, $^3J_{10\text{-H}}$ 8.25 Hz, 9-H), 7.93 (1 H, t, $^3J_{9\text{-H/11-H}}$ 7.3 Hz, 10-H), 7.83 (2 H, d & t (overlapping), 5- and 11-H); δ_C(50 MHz; DMSO-d_6) 164.2 (H_2N-$\underline{C}$=$C(CN)_2$), 150.9 and 150.6 (3-CH and 6-CH), 147.9 (8-C), 137.6 (4-C), 130.7 (9-CH), 129.8 (10-CH), 128.0 (12-CH), 124.9 (13-C), 124.7 (11-CH), 120.2 (5-CH), 115.8 (1-CN), 113.9 and 113.7 (H_2N-C=$C(\underline{C}N)_2$), 109.9 (2-C), 50.7 (H_2N-C=$\underline{C}(CN)_2$); *m/z* 271 (M^+, 100%), 245 (M^+– CN, 85), 217 (M^+– 2 × HCN, 23), 206 (M^+– $CH(CN)_2$, 53), 179 ($M^+ - H_2N$-C=$C(CN)_2$, 23), 152 ($M^+ - H_2N$-C=$C(CN)_2$, – CN, 23), 101 (19), 75 (34).

2-Cyano-3-(4-quinolinyl)propenamide (**200**)—Using Method B, 4-quinolinecarbaldehyde (0.241 g, 1.53 mmol) and cyanoacetamide (0.129 g, 1.53 mmol) were reacted in ethanol (3 ml) with piperidine (2 drops). After 2 h the precipitate (0.286 g, 80 %) was dissolved (reluctantly) in methanol (50 ml), plus DMSO (*ca*, 5 ml) and crystallisation at *ca.* 0 °C gave (0.095 g, 27%), m.p. 200 °C (decomp.) (from MeOH/DMSO) (lit.,[332] 253 °C); R_F 0.02 (diethyl ether); (Found: C, 69.87; H, 3.96; N, 18.90; M^+, 223.0739. $C_{13}H_9N_3O$ requires C, 69.95; H, 4.06; N, 18.82%; *M*, 223.0732); ν_{max}(KBr

disc)/cm^{-1} 3440s and 3430sh (NH_2), 2200w (CN), 1700s (C=O), 1608m, 1585m and 1505m (C=C, 1395s, 1385s and1240m (C–X), 795w, 760w and 750w (Ar–H); δ_H(200 MHz; DMSO-d_6) 9.05 (1 H, d, $^3J_{5\text{-H}}$ 4.47 Hz, 6-H), 8.83 (1 H, s, 3-H), 8.34 and 8.01 (2 × 1 H, 2 × br s, NH_2), 8.15 (2 H, 2 × d (overlapping= t), ^3J 9 Hz, 9- and 12--H), 7.87 (2 H, m (= overlapping t & d), 10- and 5--H), 7.72 (1 H, ddd, $^4J_{9\text{-H}}$ 1.38 Hz, $^3J_{12\text{-H}}$ 8.30 Hz, $^3J_{10\text{-H}}$ 6.92 Hz, 11-H); δ_C(50 MHz; DMSO-d_6) 162.0 ($CONH_2$), 150.4 (3-CH), 147.8 (8-C), 146.7 (6-CH), 138.0 (4-C), 130.3 (9-CH), 129.8 (5-CH), 127.8 (12-CH), 125.0 (13-C), 124.4 (10-CH), 120.3 (11-CH), 115.2 (1-CN), 114.8 (2-C); *m/z* 223 ($[M]^+$, 99.9%), 206 ($[M]^+ - NH_3$, 22.5), 194 ($[M]^+$ – HCN, 15), 179 ($[M]^+ - NH_3$, – CO, 100), 152 ($[M]^+$ – HCN, – $CONH_2$, 50), 128 ($[M]^+ - CONH_2$, – H-C≡C-CN, 13), 125 (21), 101 (25), 75 (49).

2-Cyano-3-(4-quinolinyl)propenothioamide (**201**)—Using Method B, 4-quinolinecarbaldehyde (0.132 g, 0.840 mmol) and cyanothioacetamide (0.085 g, 0.849 mmol) were reacted in ethanol (3 ml) with DBU (2 drops) and after 3.3 h the product was precipitated with diethyl ether: (0.139 g, 69 %), m.p. 180 °C (decomp.) (from EtOH); R_F 0.25 (diethyl ether); (Found: C, 65.07; H, 3.51; N, 17.36; M^+, 239.0523. $C_{13}H_9N_3S$ requires C, 64.81; H, 3.39; N, 17.28%; *M*, 239.0519); ν_{max}(KBr disc)/cm^{-1} 3365s ($CSNH_2$), 3015s (Ar–H), 2210s (CN), 1650s (C=S), 1600s, 1570s and 1490s (Ar–H), 780m and 760m (=C–H); δ_H(200 MHz; DMSO-d_6) 8.9 (1 H, d, $^3J_{5\text{-H}}$ 4.5 Hz, 6-H), 8.5 (1 H, s, 3-H), 8.3 (2 H, 2 × d, *J* 8.2 Hz, 12-H and 9-H), 7.8 (3 H, m, 5-H and 10-H/11-H); *m/z* 239 (M^+, 84%), 206 (M^+ – HS, 67), 179 ($M^+ - CSNH_2$, 27), 152 ($M^+ - CSNH_2$, – HCN, 29), 142 (M^+ – NC-CH-$CSNH_2$, 20), 128 ($M^+ - CSNH_2$, – H-C≡C-CN, 7), 115 (18), 101 (27), 87 (13), 75 (43).

Ethyl 2-cyano-3-(4-quinolinyl)propenoate (**202**)—Using Method B, 4-quinolinecarbaldehyde (0.349 g, 2.22 mmol) and ethyl cyanoacetate (0.251 g, 2.22 mmol) were reacted in ethanol (3 ml) with piperidine (3 drops) and after 2.5 h the solvent was removed under vacuum. The crude product was dissolved in ethyl acetate and put through a plug of silca gel using petroleum ether; bp 30-40 °C/ethyl acetate mixtures. The main fraction was then crystallised from ethyl acetate (0.120 g, 21 %), m.p. 98-99 °C (from EtOAc) (lit.,[332] 98-99 °C); R_F 0.62 (diethyl ether); (Found: C, 71.54; H, 4.78; N, 11.14; M^+, 252.0903. $C_{15}H_{12}N_2O_2$ requires C, 71.42; H, 4.79; N, 11.10%; *M*, 252.0891); ν_{max}(KBr disc)/cm^{-1} 3020w (Ar-H), 2995w (CH_x), 2230w (CN), 1725s (C=O), 1610m (C=C), 1280s,

1270s and 1235s (C–O), 850m, 775m, 755m/s and 745m (Ar-H deformations); δ_H(200 MHz; $CDCl_3$) 9.07 (1 H, d, $^3J_{5\text{-H}}$ 4.52 Hz, 6-H), 9.00 (1 H, s, 3-H), 8.22 (1 H, d, $^3J_{11\text{-H}}$ 8.47 Hz, 12–H), 8.14 (2 H, 2 × d, overlapping, 9–H and 5–H), 7.83 (1 H, ddd, $^4J_{9\text{-H}}$ 1.47 Hz, $^3J_{12\text{-H}}$ 8.40 Hz, $^3J_{10\text{-H}}$ 6.93 Hz, 11-H), 7.83 (1 H, ddd, $^3J_{9\text{-H}}$ 8.29 Hz, $^3J_{11\text{-H}}$ 6.94 Hz, $^4J_{12\text{-H}}$ 1.35 Hz, 10-H), 4.47 (2 H, q, ^3J 7.14 Hz, CH_2), 1.45 (3 H, t, ^3J 7.15 Hz, CH_3); *m/z* 252 (M^+, 52%), 224 ($M^+ - C_2H_6$, 6), 207 ($M^+ - OC_2H_5$, 17), 179 ($M^+ - CO_2C_2H_5$, 100), 153 ($M^+ - CO_2C_2H_5$, – CN, 37), 125 (15), 101 ($[C_8H_5]^+$, $[Ar]^+$ – CN, 11), 75 (17).

N-tert-*Butyl-3,4-dimethoxy-6-nitrobenzaldimine* (**203**)—A solution of 3,4-dimethoxy-6-nitrobenzaldehyde (6-nitroveratraldehyde; 5.43 g, 0.0257 mol), *tert*-butylamine (1.95 g, 0.0267 mol) and *p*-toluenesulphonic acid (15 mg) in toluene (200 ml) was azeotropically heated at reflux (Dean-Stark apparatus) for 4 h. The solvent was removed *in vacuo* and the solid washed and dried with diethyl ether under suction to give the title compound (6.21 g, 91 %), m.p. 110-111 °C; R_F 0.72 (diethyl ether); (Found: C, 58.72; H, 6.86; N, 10.61; M^+, 266.1276. $C_{13}H_{18}N_2O_4$ requires C, 58.64; H, 6.81; N, 10.52%; *M*, 266.1267); ν_{max}(KBr disc)/cm^{-1} 3096w and 3001w (=C–H of aromatic), 2936m and 2850m/w (CH_3), 1638m/s (C=N), 1596m/s and 1514s (=C–H of aromatic), 1285s and 1117s (C–O), 789m (=C–H deformation); δ_H(200 MHz; acetone-d_6) 8.52 (1 H, s, 1-H), 7.34 (2 H, br s, 3- and 6-H), 3.83 and 3.82 (6 H, s, 4- and 5-OMe), 1.15 (6 H, s, $C(CH_3)_3$); δ_C(50 MHz; acetone-d_6) 154.0 (4-C), 152.2 (1-CH), 151.0 (5-C), 143.1 (7-C), 127.2 (2-C), 110.0 (6-CH), 107.6 (3-CH), 57.2 ($\underline{C}(CH_3)_3$), 56.4 and 56.2 (4- and 5-OCH_3), 29.4 ($C(\underline{CH}_3)_3$); *m/z* 266 (M^+, 3%), 251 ($M^+ - CH_3$, 11), 236 (M^+ – N=O, 2), 210 ($M^+ - H_2C{=}C(CH_3)_2$, 9), 193 ($M^+ - OC(CH_3)_3$, 12), 180 ($M^+ - H_2C{=}C(CH_3)_2$, – N=O, 9), 163 ($M^+ - C(CH_3)_3$, – NO_2, 23), 150 ($M^+ - C(CH_3)_3$, – CO, – N=O, 9), 136 (M^+ –C=N-$C(CH_3)_3$, – NO_2, [*dimethoxybenzyne*]$^+$, 36), 121 ([*dimethoxybenzyne*]$^+$ – CH_3, 13), 104 ([*tropylium-O*]$^+$, 16), 93 ([*phenoxy*]$^+$, 29), 77 ([*phenyl*]$^+$, 24), 63 (18), 57 ([$C(CH_3)_3$]$^+$, 92), 41 (100).

N-*4-Chlorophenyl-3,4-dimethoxy-6-nitrobenzaldimine* (**204**)—A solution of 3,4-dimethoxy-6-nitrobenzaldehyde (6-nitroveratraldehyde; 5.03 g, 0.0238 mol), 4-chloroaniline (3.12 g, 0.0244 mol) and *p*-toluenesulphonic acid (15 mg) in toluene (150 ml) was azeotropically heated at reflux (Dean-Stark apparatus) for 4 h. The solvent was removed *in vacuo* and the solid was washed and dried with diethyl ether under suction and

crystallised from ethyl acetate to give the title compound (5.08 g, 65 %), m.p. 130-131 °C; R_F 0.81 (diethyl ether); (Found: C, 56.32; H, 4.11; N, 8.88; M^+, 320.0550. $C_{13}H_{18}N_2O_4{}^{35}Cl$ requires C, 56.17; H, 4.09; N, 8.73%; *M*, 320.0564); ν_{max}(KBr disc)/cm^{-1} 3098w (=C–H of aromatic), 2928m and 2850m/w (CH_3), 1608m/w (C=N-Ar), 1566m/s and 1516s (=C–H of aromatic), 1224s and 1064m/s (C–O), 882m/w, 830m/w, 798m and 754m/w (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.95 (1 H, s, 1-H), 7.70 (1 H, s, 6-H), 7.60 (1 H, s, 3-H), 7.33 (2 H, dm, $^3J_{3'\text{-H}/5'\text{-H}}$ 8.71 Hz, 2'- and 6'-H), 7.17 (2 H, dm, $^3J_{2'\text{-H}/6'\text{-H}}$ 8.71 Hz, 3'- and 5'-H), 4.04 (3 H, s, 4- or 5-OMe), 3.97 (3 H, s, 5- or 4-OMe); δ_C(50 MHz; $CDCl_3$) 156.4 (1-CH), 153.1 (4'-C), 150.7 (7-C), 149.6 (4-C), 142.6 (5-C), 132.2 (1'-C), 129.3 (2'- and 6'-CH), 125.4 (2-C), 122.5 (3'- and 5'-CH), 109.7 (6-CH), 107.3 (3-CH), 56.6 (4- or 5-OMe), 56.5 (5- or 4-OMe); *m/z* 322 ((^{37}Cl)M^+, 8%), 320 ((^{35}Cl)M^+, 23%), 305 ((^{37}Cl)M^+ – OH, 33), 303 ((^{35}Cl)M^+ – OH, 100), 290 ((^{35}Cl)M^+ – N=O, 11), 275 ((^{35}Cl)M^+ – N=O, – CH_3, 9), 260 ((^{35}Cl)M^+ – N=O, – 2 × CH_3, 9), 244 ((^{35}Cl)M^+ – NO_2, – 2 × CH_3, 20), 225 (26), 197 (24), 164 (M^+ – PhCl, – NO_2, 36), 150 (M^+ – NPhCl, – NO_2, 9), 136 (M^+ – C=N-PhCl, – NO_2, [*dimethoxybenzyne*]$^+$, 23), 113 ([*chloro(37)phenyl*]$^+$, 12), 111 ([*chloro(35)phenyl*]$^+$, 36), 93 ([*phenoxy*]$^+$, 14), 75 (26).

Hydrazone of 3,4-dimethoxy-6-nitrobenzaldehyde (**209**)—A fresh sample of nickel boride was prepared by adding an aqueous solution of 1.0 M sodium borohydride in 0.1 M sodium hydroxide (6.0 ml, 6.0 mmol) to a stirred aqueous solution of 0.1 M nickel(II) acetate (60 ml, 6.0 mmol). After the liberation of hydrogen gas subsided the black voluminous precipitate was allowed to settle, the supernatant was decanted, the solid was washed with absolute ethanol (4 × 30 ml) and then immersed in ethanol (40 ml). To this was added *N-tert*-butyl-3,4-dimethoxy-6-nitrobenzaldimine (1.31 g, 4.92 mmol) and at reflux temperature a solution of hydrazine monohydrate (0.53 g, 10.58 mmol) in absolute ethanol (20 ml) was added dropwise over 20 min. After refluxing for 2 h the reaction was filtered through a pad of celite, the solvent was removed *in vacuo* and the residue was subjected to silica column chromatography using hexane/ethyl acetate elutions to give predominantly the undesired hydrazone (**209**) of 3,4-dimethoxy-6-nitrobenzaldehyde (0.81 g, 73 %), m.p. 176.0-177.5 °C; R_F 0.54 (diethyl ether); (Found: C, 48.11; H, 4.95; N, 18.71; M^+, 225.0744. $C_9H_{11}N_3O_4$ requires C, 48.00; H, 4.92; N, 18.66%; *M*, 225.0750); ν_{max}(KBr disc)/cm^{-1} 3424m and 3275w (NH_2), (=C–H of aromatic obscured), 2932m/w and 2830w (CH_3),

1623m/s (C=N-NH_2), 1554m/s and 1509s (=C–H of aromatic), 1276m/s, 1239s and 1142s (C–X), (=C–H deformations weak); δ_H(200 MHz; acetone-d_6) 8.52 (1 H, s, 1-H), 7.34 (2 H, br s, 3- and 6-H), 3.83 and 3.82 (6 H, s, 4- and 5-OMe), 1.15 (6 H, s, $C(CH_3)_3$); δ_C(50 MHz; acetone-d_6) 153.1 (4-C), 158.7 (5-C), 139.8 (7-C), 138.2 (1-CH), 125.5 (2-C), 108.2 (6-CH), 107.2 (3-CH), 56.4 and 56.3 (4- and 5-OCH_3); *m/z* 225 (M^+, 5%), 196 ($M^+ - N_2$, – H, 19), 180 ($M^+ - HN_2O$, 9), 168 (M^+ – HC=N-N=O, 100), 164 (M^+ –CH_3, – NH_2, – N=O, 12), 152 ($M^+ - NO_2$, – HCN, 9), 136 (M^+ – HC=N-NH_2, – NO_2, [*dimethoxybenzyne*]$^+$, 74), 121 ([*dimethoxybenzyne*]$^+ - CH_3$, 24), 108 ([*phenyl-N=OH*]$^+$, 56), 104 ([*tropylium-O*]$^+$, 18), 94 ([*phenoxy-H*]$^+$, 52), 77 ([*phenyl*]$^+$, 49), 63 (18).

3,4-Dimethoxy-6-aminobenzaldazine (**210**)—A fresh sample of nickel boride was prepared by adding an aqueous solution of 1.0 M sodium borohydride in 0.1 M sodium hydroxide (35.0 ml, 0.035 mol) to a stirred aqueous solution of 0.1 M nickel(II) acetate (350 ml, 0.035 mol). After the liberation of hydrogen gas subsided the black voluminous precipitate was allowed to settle, the supernatant was decanted, the solid was washed with absolute ethanol (4 × 50 ml) and then immersed in ethanol (150 ml). To this was added 3,4-dimethoxy-6-nitrobenzaldehyde (7.02 g, 0.0332 mol) and at reflux temperature a solution of hydrazine monohydrate (5.12 g, 0.102 mol) in absolute ethanol (50 ml) was added dropwise over 30 min. After heating at reflux for 4.5 h the reaction was filtered through a pad of celite, the solvent was removed *in vacuo* and the residue was subjected to column chromatography using hexane/ethyl acetate elutions to give the hydrazone of 3,4-dimethoxy-6-nitrobenzaldehyde (1.28 g, 17 %) and the undesired title compound (8.74 g, 73 %) which was crystallised from ethyl acetate (3.210 g, 27 %), m.p. 246-248 °C; R_F 0.24 (diethyl ether); (Found: C, 60.11; H, 6.24; N, 15.82; M^+, 358.1647. $C_{18}H_{22}N_4O_4$ requires C, 60.32; H, 6.19; N, 15.63%; *M*, 358.1641); ν_{max}(KBr disc)/cm^{-1} 3440m (with shoulders - NH_2), 2933m/w and 2830w (CH_3), 2361w and 2343w (cummulative double bonds), 1628s and 1606m/s (C=N–N=C), 1560m/s and 1509s (=C–H of aromatic), 1274m/s, 1240s and 1144s (C–X), 812 m (=C–H deformation); δ_H(200 MHz; DMSO-d_6/acetone-d_6) 8.69 (1 H, s, 1-H), 6.94 (1 H, s, 3- H), 6.87 (2 H, br s, 7-NH_2), 6.48 (1 H, s, 6- H), 3.83 (3 H, s, 4- or 5-OMe), 3.76 (3 H, s, 5- or 4-OMe); δ_C(50 MHz; DMSO-d_6/acetone-d_6) 161.7 (1-CH), 152.9 (7-C), 145.4 (5-C), 140.1 (4-C), 116.6 (3-CH), 106.8 (2-C), 98.5 (6-CH), 56.0 (4- or 5-OCH_3), 54.7 (5- or 4-OCH_3); *m/z* 358 (M^+, 100%), 342 ($M^+ - NH_2$, 45), 327 (M^+ –

OCH_3, 31), 311 (M^+ – OCH_3, – NH_2, 9), 296 (M^+ – 2 × OCH_3, 38), 179 ([*$(CH_3O)_2(NH_2)Ph$-CH=N*]$^+$, 46), 165 ([*$(CH_3O)_2(NH_2)Ph$-CH*]$^+$, 65), 150 ([*$(CH_3O)_2PhCH$*]$^+$, 53), 135 ([*dimethoxybenzyne – H*]$^+$, 29), 120 ([*dimethoxybenzyne*]$^+$ – CH_4, 19), 105 ([*tropylium-OH*]$^+$, 11), 94 ([*phenoxy-H*]$^+$, 35).

Experimental Procedures—Chapter 8

N-*Phenylcyanoethanamide* (**213**)[230e]—A stirred mixture of methyl cyanoacetate (15 ml) and aniline (4.56 g, 0.049 mol) was heated at 90-100 °C for 16 h. After cooling the crystals were collected, washed with ethyl acetate and dried under suction using diethyl ether to give the title compound as off-white crystalline flakes (4.78 g, 61 %), m.p. 194.5-195.5 °C (lit.,[230c] 192 °C); R_F 0.32 (diethyl ether); (Found: C, 67.53; H, 5.04; N, 17.55; M^+, 160.0641. $C_9H_8N_2O$ requires C, 67.49; H, 5.03; N, 17.49%; *M*, 160.0637); ν_{max}(KBr disc)/cm^{-1} 3271m and 3209m (CONHPh), 3146m, 3105m, 3054m/w and 3031w (Ph), 2955w and 2916w (CH_2), 2261w (CN), 1670s (CONHPh), 1622m/s and 1611m/s (double bond character), 1601m/s, 1561 m/s and 1447m/s (Ph), 1350m/s and 1300m/s (C–O), 948m, 754s and 698m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 10.34 (1 H, br s, 4-NH), 7.60 (2 H, dm, ^3J 8.1 Hz, 6- and 10-H), 7.38 (2 H, tm, ^3J 7.85 Hz, 7- and 9-H), 7.13 (1 H, tm, $^3J_{7\text{-H}/9\text{-H}}$ 7.34 Hz, 8-H), 3.95 (2 H, s, 2-CH_2); δ_C(50 MHz; DMSO-d_6) 161.1 (3-C=O), 138.5 (5-C), 129.0 (7- and 9-CH), 124.0 (8-CH), 119.4 (6- and 10-CH), 116.1 (1-CN), 26.9 (2-CH_2); *m/z* 160 (M^+, 72), 120 (M^+ – CH_2CN, 32), 93 (MH^+ – C≡O, – CH_2CN, [*$PhNH_2$*]$^+$, 100), 77 ([*$PhNH_2$*]$^+$ – NH_2, [*Ph*]$^+$, 31), 65 ([*$PhNH_2$*]$^+$ – C=NH_2, [C_5H_5]$^+$, 30), 51 ([*Ph*]$^+$ – H-C≡C-H, [C_4H_3]$^+$, 13), 39 ([C_3H_3]$^+$, 22).

N-*Phenyl-2-cyano-3-(3,4-dihydroxyphenyl)propenamide* (**59**)—Using Method B, 3,4-dihydroxybenzaldehyde (1.034 g, 7.49 mol) and *N*-phenylcyanoethanamide (1.254 g, 7.83 mol) were reacted in absolute ethanol (15 ml) with 10 % ethanolic piperidine (5 drops). After 5 h the precipitate was crystallised from ethyl acetate to give the title compound as fine yellow crystalline needles (0.845 g, 40 %), m.p. 249.5-250.5 °C (lit.,[230c] 258 °C); R_F 0.38 (diethyl ether); (Found: C, 68.64; H, 4.33; N, 10.16; M^+, 280.0851. $C_{16}H_{12}N_2O_3$ requires C, 68.57; H, 4.32; N, 9.99%; *M*, 280.0848); ν_{max}(KBr disc)/cm^{-1} 3410m/s (broad OH stretches and CONHPh), (C–H stretches obscured), 2361w and 2342w (cummulative double-bonds), 2210w (CN), 1664s (CONHPh), 1603m/s (C=C), 1577s, 1498s and1445s (Ph), 1249m/s, 1195m/s and 1175m/s (C–O), 948m, (=C–H deformations

weak); δ_H(200 MHz; DMSO-d_6/acetone-d_6) 10.02 (1 H, br s, 4-NH), (OH broad signal observed along baseline from 10.5 to 8.5), 8.12 (1 H, s, 3-H), 7.79 (2 H, dm, ^3J 8.0 Hz, 3'- and 5'-H), 7.73 (1 H, d, $^4J_{9\text{-H}}$ 2.18 Hz, 5-H), 7.43 (*ca.* 1 H, dd, $^3J_{9\text{-H}}$ 8.18 Hz, $^4J_{5\text{-H}}$ 2.15 Hz, 9-H), 7.39 (*ca.* 2 H, tm, ^3J 7.82 Hz, 2'- and 6'-H, overlaps with 9-H), 7.15 (1 H, tt, $^3J_{3'\text{-H}/5'\text{-H}}$ 7.36 Hz, $^4J_{2'\text{-H}/6'\text{-H}}$ 1.11 Hz, 4'-H), 7.00 (1 H, d, $^3J_{9\text{-H}}$ 8.29 Hz, 8-H); δ_C(50 MHz; DMSO-d_6/acetone-d_6) 161.0 (CONHPh), 151.0 (7-C), 150.9 (3-CH), 145.9 (6-C), 138.7 (1'-C), 128.4 (3'- and 5'-CH), 125.1 (9-CH), 123.8 (4'-CH), 123.5 (4-C), 120.4 (2'- and 6'-CH), 116.8 (1-CN), 116.3 (5'-CH), 115.8 (8'-CH), 101.8 (2-C); *m/z* 280 (M^+, 39), 188 (M^+ – PhNH, 30), 170 (M^+ – $PhNH_2$, – HO, 30), 160 (M^+ – PhNHCO, 8), 142 (M^+ – PhNHCO, – CO, 8), 114 (M^+ – PhNHCO, – 2 × CO, 17), 93 ($[PhNH_2]^+$, 100), 77 ($[PhNH_2]^+$ – NH_2, $[Ph]^+$, 10), 65 ($[PhNH_2]^+$ – C=NH_2, $[C_5H_5]^+$, 7), 51 ($[Ph]^+$ – H-C≡C-H, $[C_4H_3]^+$, 6), 39 ($[C_3H_3]^+$, 6).

*4-Hydroxy-3-methoxy-5-(*N,N*-dimethylamino)methylbenzaldehyde* (**215**)[295]—To a stirred solution of aqueous formaldehyde (*ca.* 37 % *w/v*; 30.1 g, 0.37 mol) and aqueous dimethylamine (*ca.* 40 % *w/v*; 45.2 g, 0.40 mol) in absolute ethanol (250 ml) was added vanillin (3-methoxy-4-hydroxybenzaldehyde; 38.0 g, 0.25 mmol). The mixture was refluxed for 3 h and then stirred at room temperature for 19 h. The solid obtained was collected by filtration, washed with acetone (30 ml at *ca.* 0 °C) and dried under suction (28.24 g, 54 %). The liquors were concentrated to *ca.* two-thirds volume and crystallised at *ca.* O °C to give the title compound as large yellow crystals (13.16 g, 25 %), m.p. 135.0-136.5 °C (from EtOH/H_2O) (lit.,[295] 139-141 °C); R_F 0.10 (ethanol); (Found: C, 62.98; H, 7.21; N, 6.92; M^+, 209.1057. $C_{11}H_{15}NO_3$ requires C, 63.15; H, 7.23; N, 6.70%; *M*, 209.1052); ν_{max}(KBr disc)/cm^{-1} 3426w (OH), 3002w (=C–H of aromatic), 2952w, 2920w and 2834m/w (CH_x), 2807m/w and 2751w (CHO), 1649s (H-C=O), 1594s, 1469s, 1448m/s and 1435s (=C–H of aromatic), 1269s and 1148s (C–O), 710m (=C–H deformation); δ_H(200 MHz; DMSO-d_6) 9.74 (1 H, s, 1-CHO), 8.30 (1 H, v.br s, 5-OH), 7.34 (2 H, br s, 3- and 7-H), 3.85 (3 H, s, 4-OMe), 3.76 (2 H, s, 6-CH_2), 2.36 (6 H, s, $N(CH_3)_2$); δ_C(50 MHz; DMSO-d_6) 190.6 (1-CHO), 155.2 (4-C), 148.3 (5-C), 126.4 (2-C), 126.0 (7-CH), 122.1 (6-C), 109.6 (3-CH), 59.8 ($\underline{CH_2}N(CH_3)_2$), 55.5 ($OCH_3$), 43.7 ($CH_2N(\underline{CH_3})_2$); *m/z* 209 ($M^+$, 81%), 192 ($M^+$ – OH, 5), 178 (M^+ – OMe, 4), 166 (M^+ – Me, – CO, 12), 165 (M^+ – NMe_2, 21), 164 (M^+ – $HNMe_2$, 31), 163 (M^+ – $HNMe_2$, – H, 34), 150 (M^+ – H_2C=NMe_2, 11), 134 (M^+ – $HNMe_2$,

– H, – CHO, 18), 121 ([*MeO-phenylCH*$_2$], 8), 107 ([*MeO-phenyl*]$^+$, 13), 93 ([*phenoxy*]$^+$, 7), 77 ([*phenyl*]$^+$, 12), 65 (18), 58 ([H_2C=NMe_2]$^+$, 80), 44 ([NMe_2]$^+$, 100).

5-Chloromethyl-4-hydroxy-3-methoxybenzaldehyde (**217**)[295]—A solution of 4-hydroxy-3-methoxy-5-(*N*,*N*-dimethylamino)methylbenzaldehyde (15.5 g, 0.074 mol) in acetic anhydride (60 ml) was refluxed for 25 h and then subjected to fractional distillation. To the main fraction (bp 40-65 °C, at *ca.* 0.1 mbar/140 °C) was added 37 % hydrochloric acid (50 ml) and the mixture was stirred at room temperature for 3 h. The precipitate was filtered, washed with copius amounts of water and air dried to give the title compound as a discoloured white powder (7.5 g, 50 %), m.p. 103.5-105.0 °C; R_F 0.22 (diethyl ether); (Found: C, 53.54; H, 4.39; M$^+$, 200.0247 (for ^{35}Cl). $C_9H_9O_3Cl$ requires C, 53.88; H, 4.52%; *M*, 200.0240 (for ^{35}Cl)); ν_{max}(KBr disc)/cm^{-1} 3327m (OH), 2964w, 2936w and 2866m/w (CH_x), 1666s (H-C=O), 1594s, 1501s, 1468m/s and 1431s (=C–H of aromatic), 1295s, 1267s and 1145s (C–O), 697m (=C–H deformation); δ_H(200 MHz; $CDCl_3$) 9.81 (1 H, s, 1-CHO), 7.51 (1 H, d, $^4J_{3\text{-}H}$ 1.70 Hz, 7-H), 7.38 (1 H, d, $^4J_{7\text{-}H}$ 1.72 Hz, 3-H), 6.65 (1 H, br s, 5-OH), 4.70 (2 H, s, 6-CH_2), 3.95 (3 H, s, 4-OMe); δ_C(50 MHz; $CDCl_3$) 190.7 (1-CHO), 149.6 (4-C), 147.1 (5-C), 129.2 (2-C), 128.1 (7-CH), 123.4 (6-C), 108.6 (3-CH), 56.4 (OCH_3), 40.0 (CH_2Cl); *m/z* 202 ((^{37}Cl)M$^+$, 12%), 202 ((^{35}Cl)M$^+$, 35%), 182 ((^{35}Cl)M$^+$ – H_2O, 4), 165 (M$^+$ – Cl, + H, 21), 164 (M$^+$ – HCl, 31), 163 (M$^+$ – HCl, – H, 34), 150 (M$^+$ – [H_2C–Cl]$^+$, 8), 134 (M$^+$ – HCl, – H, – CHO, 38), 121 ([*MeO-phenylCH*$_2$], 27), 107 ([*MeO-phenyl*]$^+$, 34), 93 ([*phenoxyl*]$^+$, 25), 77 ([*phenyl*]$^+$, 34), 65 (100).

4-Hydroxy-3-methoxy-5-(2-benzothiazolylthiomethyl)benzaldehyde (**218**)[230g]—To a stirred solution of 5-chloromethyl-4-hydroxy-3-methoxybenzaldehyde (3.07 g, 0.0153 mol) in dichloromethane (10 ml) was added dropwise a solution of 2-mercaptobenzothiazole (2.52 g, 0.0151 mol) and triethylamine (1.67 g, 0.0165 mol) in dichloromethane (10 ml) over 10 min. After stirring for 8 h at room temperature the solvent was removed *in vacuo* and the residue subjected to silica column chromatography using ethyl acetate/hexane elutions to give the title compound as a white amorphous solid (1.64 g, 34 %), m.p. 108.6-109.3 °C (lit.,[230g] 116 °C); R_F 0.57 (diethyl ether); (Found: C, 58.17; H, 3.98; N, 4.51; M$^+$, 331.0339. $C_{16}H_{15}NO_3S_2$ requires C, 57.99; H, 3.95; N, 4.23%; *M*, 331.0337); ν_{max}(KBr disc)/cm^{-1} 3406m and 3171m (OH), 2964m/w, 2934w and 2848m/w (CH_x), 1665s (H-C=O), 1593s, 1499s, 1464s and 1428s (=C–H of aromatic), 1308s, 1288s, 1263s and 1146s

(C–X), 752m, 746m and 718m (=C–H deformations); δ_H(200 MHz; $CDCl_3$) (5-OH not observed), 9.65 (1 H, s, 1-CHO), 7.80 (1 H, dm, $^3J_{7'\text{-H}}$ 8.03 Hz, 8'-H), 7.57 (1 H, dm, $^3J_{6'\text{-H}}$ 7.33 Hz, 5'-H), 7.36 (1 H, d, $^4J_{3\text{-H}}$ 1.65 Hz, 7-H), 7.28 (1 H, dt, $^3J_{8'\text{-H}}$ 8.02 Hz, $^3J_{5'\text{-H}}$ 1.30 Hz, 7'-H), 7.18 (1 H, d, $^4J_{7\text{-H}}$ 1.56 Hz, 3-H), 7.15 (1 H, dt, $^3J_{7'\text{-H}}$ 7.32 Hz, $^3J_{5'\text{-H}}$ 1.25 Hz, 6'-H, overlaps with 3-H and residual $CHCl_3$), 4.49 (2 H, s, 6-CH_2), 3.74 (3 H, s, 4-OMe); δ_C(50 MHz; $CDCl_3$) 190.8 (1-CHO), 168.3 (2'-C), 151.9 (4-C), 150.8 (9'-C), 148.9 (5-C), 134.9 (4'-C), 129.0 (2-C), 128.3 (7-CH), 126.4 (8'-CH), 124.7 (5'-CH), 123.9 (6-C), 121.1 and 121.0 (6'- and 7'-CH), 108.8 (3-CH), 56.1 (OCH_3), 32.3 (CH_2SAr); *m/z* 331 (M^+, 22%), 298 (M^+ – MeOH, – H, 5), 167 ([*2-mercaptobenzothiazole*]$^+$, 100), 165 (M^+ – [*2-dehydromercaptobenzothiazole*]$^+$, 16), 150 (M^+ – [*2-methylenemercaptobenzothiazole*]$^+$, 2.5), 135 (M^+ – [*2-mercaptobenzothiazole*]$^+$, – CHO, or [*benzothiazole*]$^+$, 18), 123 ([*2-mercaptobenzothiazole*]$^+$ – C≡S, 8), 121 ([*MeO-phenylCH*$_2$], 5), 109 ([*2-mercaptobenzothiazole*]$^+$ – S-C≡N, 11), 107 ([*MeO-phenyl*]$^+$, 4), 93 ([*phenoxy*]$^+$, 3), 91 ([*2-mercaptobenzothiazole*]$^+$ – CS_2, 9), 77 ([*phenyl*]$^+$, 6), 65 (14).

2-Cyano-3-[4-hydroxy-3-methoxy-5-(2-benzothiazolylthiomethyl)phenyl] propenamide (**61**)[230g]—To a stirred solution of 4-hydroxy-3-methoxy-5-(2-benzothiazolyl thiomethyl)benzaldehyde (0.590 g, 1.78 mmol) and cyanoacetamide (0.179 g, 2.12 mmol) in absolute ethanol (5 ml) was added piperidine (3 drops). After stirring for 6 h at room temperature the precipitate was collected and crystallised from ethyl acetate to give the title compound as a fine yellow mesh (0.121 g, 17 %). The combined liquors were concentrated *in vacuo* and the residue subjected to silica column chromatography using ethyl acetate/hexane elutions (0.314 g, 44 %), m.p. 254-256 °C (from EtOAc) (lit.,[230g] 268 °C); R_F 0.29 (diethyl ether); (Found: C, 57.57; H, 3.82; N, 10.51; M^+, 397.0559. $C_{19}H_{15}N_3O_3S_2$ requires C, 57.41; H, 3.80; N, 10.57%; *M*, 397.0555); ν_{max}(KBr disc)/cm^{-1} 3302m, 3215m and 3152m (OH and $CONH_2$), 2962m/w, 2936w and 2851m/w (CH_x), 1695s ($CONH_2$), 1592s (C=C), 1502s, 1463s and 1427s (=C–H of aromatic), 1308s, 1285s, 1260s and 1149s (C–X), 758m, 745m and 716m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 8.07 (1 H, s, 3-H), 8.03 (1 H, dm, $^3J_{7'\text{-H}}$ 7.83 Hz, 8'-H), 7.96 (1 H, dm, $^3J_{6'\text{-H}}$ 7.85 Hz, 5'-H), 7.80 (1 H, d, $^4J_{5\text{-H}}$ 1.92 Hz, 9-H), 7.68 (1 H, d, $^4J_{9\text{-H}}$ 1.99 Hz, 5-H), ($CONH_2$ singlets coincide with 5- and 9-H doublets), 7.52 (1 H, dt, $^3J_{8'\text{-H}}$ 7.7 Hz, $^3J_{5'\text{-H}}$ 1.35 Hz, 7'-H), 7.40 (1 H, dt, $^3J_{7'\text{-H}}$ 7.6 Hz, $^3J_{5'\text{-H}}$ 1.30 Hz, 6'-H), 4.66 (2 H, s, 8-CH_2), 3.91 (3 H, s, 6-OMe); δ_C(50 MHz;

DMSO-d_6) 166.5 (2'-C), 163.3 ($CONH_2$), 152.7 (6-C), 150.5 (3-CH), 149.5 (9'-C), 147.7 (7-C), 134.8 (4'-C), 126.6 (9-CH), 126.5 (8'-CH), 124.6 (5'-CH), 123.8 (8-C), 122.7 (4-C), 121.9 and 121.3 (6'- and 7'-CH), 117.4 (1-CN), 112.7 (5-CH), 102.2 (2-C), 56.1 (OCH_3), 31.8 (CH_2SAr); *m/z* 397 (M^+, 26%), 380 ($M^+ - NH_3$, 4), 327 ($M^+ - CN, - CONH_2$, 13), 230 ($M^+ - [2\text{-}dehydromercaptobenzothiazole]^+$, 17), 167 ($[2\text{-}mercaptobenzothiazole]^+$, 100), 150 ($M^+ - [2\text{-}methylenemercaptobenzothiazole]^+$, 3), 135 ($M^+ - [2\text{-}mercaptobenzothiazole]^+$, – CHO, or $[benzothiazole]^+$, 15), 123 ($[2\text{-}mercaptobenzothiazole]^+$ – C≡S, 9), 121 ($[MeO\text{-}phenylCH_2]$, 7), 109 ($[2\text{-}mercaptobenzothiazole]^+$ – S-C≡N, 10), 107 ($[MeO\text{-}phenyl]^+$, 5), 93 ($[phenoxy]^+$, 4), 91 ($[2\text{-}mercaptobenzothiazole]^+ - CS_2$, 7), 77 ($[phenyl]^+$, 6), 65 (18).

Ethyl 2-cyano-2,3-epoxy-3-(4-nitrophenyl)propanonitrile (**220**)—To a stirred solution of ethyl 2-cyano-3-(4-nitrophenyl)propenonitrile (4.948 g, 20.10 mmol) in dry dichloromethane (250 ml) was added *ca.* 80 % *meta*-chloroperoxybenzoic acid (5.015 g, 44.11 mmol) over 15 min. After 4 h, TLC indicated that the reaction was incomplete. Thus *ca.* 80 % *meta*-chloro*per*benzoic acid (5.015 g, 44.11 mmol) was added and the reaction was stirred at room temperature for a further 27 h. After this time saturated sodium carbonate (150 ml) was added, the dichloromethane layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 100 ml). The organic layers were dried over sodium sulphate (anhydrous), combined, concentrated *in vacuo* and the solid obtained (6.437 g) was crystallised from dichloromethane to give the title epoxide (4.142 g, 79 %), m.p. 116-117 °C (from EtOAc); R_F 0.72 (diethyl ether); (Found: C, 54.63; H, 3.82; N, 10.45; M^+, 274.0951. $C_{14}H_{14}N_2O_4$ requires C, 54.96; H, 3.84; N, 10.68%; *M*, 274.0954); ν_{max}(KBr disc)/cm^{-1} 3125w and 3093w (=C–H), 2992w and 2948w (CH_3), 2361w (CN), 1760s (CO_2Et), 1611m (Ar), 1519s (C–NO_2), 1348s, 1300s, 1290s and 1011m (C–O), 860m, 828m, 730m/s and 696m (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.28 (2 H, d, $^3J_{5\text{-}H/9\text{-}H}$ 8.80 Hz, 6- and 8-H), 7.62 (2 H, d, $^3J_{6\text{-}H/8\text{-}H}$ 8.72 Hz, 5- and 9-H), 4.64 (1 H, s, 3-H), 4.39 (2 H, dq, ^3J 7.14 Hz, *J* 1.55 Hz, $\underline{CH_2}CH_3$), 1.37 (3 H, t, ^3J 7.14 Hz, $CH_2\underline{CH_3}$); δ_C(50 MHz; $CDCl_3$) 161.9 ($CO_2CH_2CH_3$), 149.0 (7-C), 136.8 (4-C), 127.9 (6- and 8-CH), 123.9 (5- and 9-CH), 112.3 (1-CN), 64.5 ($\underline{CH_2}CH_3$), 62.9 (3-CH), 53.1 (2-C), 13.9 ($CH_2\underline{CH_3}$); *m/z* 262 (M^+, 4%), 246 (M^+ – O, 5), 234 (M^+ – HCN, – H, 18), 218 ($MH_2^+ - NO_2$, 8), 218 (MH^+ – Et, 8), 218 (M^+ – OH, – HCN, 8), 188 ($M^+ - NO_2$, – CN, 3), 188 (MH^+ – N=O, – Et, 3), 180 (MH^+ – OEt, –

CCN, 12), 172 ($M^+ - CO_2Et$, – H, 3), 152 (MH^+ – NC-C-CO_2Et, 100), 135 (M^+ – NC-C-CO_2Et, –OH, 5), 122 (6), 115 (6), 105 (7), 89 (23), 83 (24), 77 (13).

Attempted formation of the trans*–form of Ethyl 2-cyano-3-(4-nitrophenyl) propenonitrile* (**116**)*: Ethyl 2-oxo-3-(4-nitrophenyl)propanoate* (**221**) *isolated.*—Using oven-dried glassware, a 50 ml three-necked round-bottomed flask (equipped with an equilibrating dropping funnel capped with a rubber septum, digital temperature probe and nitrogen bubbler) was charged with ethyl 2-cyano-2,3-epoxy-(4-nitrophenyl)propenonitrile (**220**) (0.868 g, 3.31 mmol), 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (*N*,*N*'-dimethyl-*N*,*N*'-propylene urea or DMPU; 4 ml) and a magnetic stirrer bar. The reaction system was thoroughly evacuated and then purged with dry nitrogen. To the dropping funnel (closed) was added, *via* septum, a solution of hexamethyldisilane (0.776 g, 5.32 mmol) in DMPU (4 ml) followed by the slow administration of *ca.* 1.4 M methyllithium (in diethyl ether) over 20 min. The reaction mixture was then cooled to *ca.* -10 °C on an ice/salt water bath and the preformed solution of trimethylsilyllithium was added dropwise over 20 min. After 30 min at -10 °C and 2.5 h at room temperature the reaction was quenched with saturated ammonium chloride solution (10 ml) and partioned with toluene (3 × 30 ml). The combined organic layers were washed with brine (2 × 20 ml), dried (anhydrous Na_2SO_4) and concentrated *in vacuo*. The brown oil was subjected to column chromatography (silica) using ethyl acetate/petroleum ether (bp 40-60 °C) elutions (20 % to 100 %). The required *trans*-isomer of ethyl 2-cyano-3-(4-nitrophenyl)-propenonitrile was not formed but from the complex mixture the title β-keto ester was identified (0.120 g, 15 %), m.p. 161-163 °C (from EtOAc); R_F 0.11 (ethyl acetate); (Found: C, 55.34; H, 4.39; N, 6.12; M^+, 237.0644. $C_{11}H_{11}NO_5$ requires C, 55.70; H, 4.67; N, 5.90%; *M*, 237.0637); ν_{max}(KBr disc)/cm^{-1} 3103w, 3078w and 3034w (=C–H), 2982w and 2940w (CH_x), 1740s (CO_2Et), 1686m/s, 1597m and 1625m/s (Ar and C=C–OH), 1522s (C–NO_2), 1345s, 1300s, 1291s and 1156s (C–O), 853m/s (=C–H deformation); δ_H(90 MHz; $CDCl_3$) 8.2 (2 H, d, $^3J_{3\text{-H}/7\text{-H}}$ 8.7 Hz, 4- and 6-H), 8.1 (2 H, d, $^3J_{4\text{-H}/6\text{-H}}$ 8.8 Hz, 3- and 7-H), 6.5 (1 H, s, 3-H), 4.45 (major rotamer) and 4.2 (minor rotamer) (2 H, 2 × q, ^3J 7.1 Hz, $\underline{CH_2}CH_3$), 1.55 (major rotamer) and 1.35 (minor rotamer) (3 H, 2 × t, ^3J 7.1 Hz, $CH_2\underline{CH_3}$); *m/z* 237 (M^+, 24%), 207 (M^+ – N=O, 0.7), 192 ($MH^+ - NO_2$, 0.9), 163 ($M^+ - CO_2Et$, – H, 100), 147 ($M^+ - CO_2Et$, – O, 9), 136 ($M^+ - CO_2Et$, – CO, 87), 133 ($M^+ - CO_2Et$, – NO, 45), 117 (M^+ –

CO_2Et, $-NO_2$, 11), 106 ($M^+ - CO_2Et$, $-CO$, $-NO$, 25), 89 ($M^+ - CO_2Et$, $-CO$, $-HNO_2$, 81), 78 (44).

4-Nitrophenylethanamide (**222**)— After performing chromatography as described for the α-keto ester (**221**) the title acetamide (**222**) was also isolated (0.113 g, 19 %), m.p. 182-184 °C (from EtOAc); R_F 0.19 (ethyl acetate); (Found: C, 53.01; H, 4.42; N, 15.10; M^+, 180.0545. $C_8H_8N_2O_3$ requires C, 53.33; H, 4.48; N, 15.55%; *M*, 180.0535); ν_{max}(KBr disc)/cm^{-1} 3414m/s and 3335m (NH_2), 1655s ($CONH_2$), 1686m, 1624m and 1613m (Ar and C=C), 1539s (C–NO_2), 1395m/s and1352s (C–O), 771m/s and 706m (=C–H deformations); δ_H(200 MHz; DMSO-d_6) 8.22 (2 H, d, $^3J_{4\text{-H}/8\text{-H}}$ 8.78 Hz, 5- and 7-H), 7.61 and 7.07 (2 × 1 H, 2 × br s, $CONH_2$), 7.58 (2 H, d, $^3J_{5\text{-H}/7\text{-H}}$ 8.75 Hz, 4- and 8-H), 3.60 (2 H, s, 2-CH_2); δ_C(50 MHz; DMSO-d_6) 171.1 ($CONH_2$), 146.3 (6-C), 144.7 (3-C), 130.6 (5- and 7-CH), 123.4 (4- and 8-CH), 41.9 (2-CH_2); *m/z* 180 (M^+, 4%), 162 ($M^+ - H_2O$, 1), 150 (M^+ – N=O, 0.6), 137 ($MH^+ - CONH_2$, 100), 120 ($M^+ - CONH_2$, – O, 14), 107 ($MH^+ - CONH_2$, – N=O, 50), 90 ($M^+ - CONH_2$, $-NO_2$, 62), 89 ($M^+ - CONH_2$, $-HNO_2$, 54), 78 (21).

(Z)-2-(3-Pyridyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**66**)—An oven dried two-necked round bottom flask (50 ml), equipped with a rubber septum and nitrogen inlet, was charged with 3-pyridylacetonitrile (1.507 g, 0.0127 mol) and dry tetrahydrofuran (20 ml). The solution was cooled on an ice/salt water bath and 95% NaH (0.324 g, 0.0135 mol) was cautiously added. This was stirred at room temperature for 2 h and carefully added to a solution of 3,4-dimethoxybenzaldehyde (1.997 g, 0.120 mol) in tetrahydrofuran (10 ml). The reaction mixture was stirred for a further 2 h, quenched with ethyl acetate (50 ml) and the tetrahydrofuran removed under reduced pressure. The residue was partitioned between water (50 ml) and ethyl acetate (3 × 50 ml) and the ethyl acetate extracts dried (Na_2SO_4) filtered and concentrated to give 3.0 g of crude product which was crystallised from isopropanol to yield the title compound (1.73 g, 54 %).

Alternatively, using Method D, piperidine (10 drops) was added to a hot stirred solution of 3,4-dimethoxybenzaldehyde (2.08 g, 0.0125 mol) in absolute ethanol (5 ml). After 5 min, 3-pyridylacetonitrile (1.46 g, 0.0124 mol) was added followed by cautious addition of a catalytic amount of potassium *tert*-butoxide (*ca.* 10 mg) and the yellow crystals obtained were filtered, washed (diethyl ether) and dried to afford the title compound (3.13 g, 94 %). Recrystallisation from ethyl acetate gave yellow flakes (2.83 g, 85 %) and, for

crystallographic purposes, *ca.* 0.5 g of this was further recrystallised from diisopropyl ether at 0 °C to give pale yellow rhombic crystals, m.p. 117.7 °C; R_F 0.42 (1:2 ethyl acetate/petroleum ether, bp 30-40°C); (Found: C, 72.18; H, 5.35; N, 10.69; M^+, 266.1051. $C_{16}H_{14}N_2O_2$ requires C, 72.17; H, 5.30; N, 10.51%; *M*, 266.1055); ν_{max}(KBr disc)/cm^{-1} 3050w and 3015w (=C–H), 2969w and 2938w (CH_3), 2843w (CH_3OAr), 2361w and 2344w (cumulative double-bonds), 2207m (CN), 1593s (C=C), 1578m, 1570m and 1516s (Ar), 1447m and 1424m (CH_3 deformations), 1275s, 1147s and 1020s (C–O), 808m and 708m (=C–H deformations); δ_H(200 MHz; acetone-d_6) 8.94 (dd, 1 H, $^4J_{15\text{-}H}$ 2.52 Hz, $^4J_{14\text{-}H}$ 0.87 Hz, 11-H), 8.60 (dd, 1 H, $^3J_{14\text{-}H}$ 4.71 Hz, $^4J_{15\text{-}H}$ 1.56 Hz, 13-H), 8.08 (ddd, 1 H, $^3J_{14\text{-}H}$ 8.11 Hz, $^4J_{11\text{-}H}$ 2.54 Hz, $^4J_{13\text{-}H}$ 1.57 Hz, 15-H), 7.92 (1 H, br s, 3-H), 7.77 (d, 1 H, $^4J_{9\text{-}H}$ 2.16 Hz, 5-H), 7.58 (ddd, 1 H, $^3J_{8\text{-}H}$ 8.45 Hz, $^4J_{5\text{-}H}$ 2.17 Hz, $^4J_{3\text{-}H}$ 0.60 Hz, 9-H), 7.48 (ddd, 1 H, $^3J_{15\text{-}H}$ 8.08 Hz, $^4J_{13\text{-}H}$ 4.75 Hz, $^4J_{11\text{-}H}$ 0.86 Hz, 14-H), 7.11 (d, 1 H, $^3J_{9\text{-}H}$ 8.46 Hz, 8-H), 3.91 (s, 3 H, 7-OMe), 3.89 (s, 3 H, 6-OMe); δ_H(200 MHz; $CDCl_3$) 8.90 (dd, 1 H, $^4J_{15\text{-}H}$ 2.50 Hz, $^4J_{14\text{-}H}$ 0.84 Hz, 11-H), 8.59 (dd, 1 H, $^3J_{14\text{-}H}$ 4.78 Hz, $^4J_{15\text{-}H}$ 1.56 Hz, 13-H), 7.93 (ddd, 1 H, $^3J_{14\text{-}H}$ 8.05 Hz, $^4J_{11\text{-}H}$ 2.50 Hz, $^4J_{13\text{-}H}$ 1.57 Hz, 15-H), 7.72 (d, 1 H, $^4J_{9\text{-}H}$ 2.14 Hz, 5-H), 7.49 (1 H, br s, 3-H), 7.38 (ddd, 1 H, $^3J_{8\text{-}H}$ 8.3 Hz, $^4J_{5\text{-}H}$ 2.2 Hz, $^4J_{3\text{-}H}$ 0.6 Hz, 9-H), 7.36 (ddd, 1 H, $^3J_{15\text{-}H}$ 8.1 Hz, $^4J_{13\text{-}H}$ 4.85 Hz, $^4J_{11\text{-}H}$ 0.85 Hz, 14-H, overlaps with 9-H), 6.93 (d, 1 H, $^3J_{9\text{-}H}$ 8.46 Hz, 8-H), 3.97 (s, 3 H, 6-OMe), 3.95 (s, 3 H, 7-OMe); δ_H(200 MHz; THF-d_8 at 3.584 and 1.731 ppm) 8.92 (dd, 1 H, $^4J_{15\text{-}H}$ 2.50 Hz, $^4J_{14\text{-}H}$ 0.76 Hz, 11-H), 8.53 (dd, 1 H, $^3J_{14\text{-}H}$ 4.73 Hz, $^4J_{15\text{-}H}$ 1.55 Hz, 13-H), 8.01 (ddd, 1 H, $^3J_{14\text{-}H}$ 8.08 Hz, $^4J_{11\text{-}H}$ 2.53 Hz, $^4J_{13\text{-}H}$ 1.57 Hz, 15-H), 7.78 (d, 1 H, $^4J_{9\text{-}H}$ 2.18 Hz, 5-H), 7.77 (1 H, s, 3-H, overlaps with 5-H), 7.48 (dd, 1 H, $^3J_{8\text{-}H}$ 8.41 Hz, $^4J_{5\text{-}H}$ 2.0 Hz, 9-H), 7.38 (ddd, 1 H, $^3J_{15\text{-}H}$ 8.07 Hz, $^4J_{13\text{-}H}$ 4.75 Hz, $^4J_{11\text{-}H}$ 0.85 Hz, 14-H), 7.03 (d, 1 H, $^3J_{9\text{-}H}$ 8.44 Hz, 8-H), 3.87 (br s, 6 H, 7- and 6-OMe); δ_C(50 MHz; acetone-d_6) 152.9 (7-C), 150.4 (11-CH), 150.1 (6-C), 147.7 (13-CH), 144.7 (3-CH), 133.5 (15-CH), 131.7 (10-C), 127.3 (4-C), 125.3 (9-CH), 124.5 (14-CH), 118.7 (1-CN), 112.5 (5-CH), 112.3 (8-CH), 105.3 (2-C), 56.1 (6-OCH_3), 56.0 (7-OCH_3); δ_C(50 MHz; $CDCl_3$) 151.6 (7-C), 149.5 (11-CH), 149.0 (6-C), 146.8 (13-CH), 143.5 (3-CH), 133.0 (15-CH), 130.8 (10-C), 126.1 (4-C), 124.9 (9-CH), 123.4 (14-C), 117.8 (1-C), 110.9 (5-CH), 110.5 (8-CH), 104.9 (2-C), 55.9 (6- and 7-OCH_3); *m/z* 266 (M^+, 100%), 251 ($M^+ - CH_3$, 18), 223 ($M^+ - CH_3$, – CO, 22), 220 ($M^+ - CH_3$, – CH_3O, 6), 208 ($M^+ - 2 \times CH_3$, – CO, 21), 195 ($M^+ - CH_3$, – H-C=O, – HCN, 22), 192 ($M^+ - CH_3$, – CH_3O, – CO, 32), 191 ($M^+ - CH_3$, – CH_3OH, – CO, 34), 180 ($M^+ - 2 \times CH_3$, – 2 × CO, 32),

179 ($M^+ - 2 \times CH_3$, $- 2 \times CO$, $- H$, 66), 154 ([*H-C≡C-CH=C(CN)-Py*]$^+$, $M^+ - H_2C{=}C(OCH_3)\text{-}C(OCH_3){=}CH_2$, 20), 167 (12), 164 (15), 138 ([$(CH_3O)_2Ph$]$^+$, 6), 114 (7), 100 (12), 87 (10), 77 ([*Py*]$^+ - H$, 10), 63 (21), 51 (21).

2-(3-Pyridyl)-3-(3,5-dichlorophenyl)propenonitrile (**67**)—An oven dried two-necked round bottom flask (50 ml), equipped with a rubber septum and nitrogen inlet, was charged with 3-pyridylacetonitrile (1.03 g, 8.68 mmol) and dry tetrahydrofuran (20 ml). The solution was cooled on an ice/salt water bath and 95% NaH (0.26 g, 0.011 mol) was cautiously added. This was stirred at room temperature for 2 h and carefully added to a solution of 3,5-dichlorobenzaldehyde (1.50 g, 8.57 mmol) in tetrahydrofuran (10 ml). The reaction mixture was stirred for a further 2.5 h. After quenching with ethyl acetate (50 ml) and removing tetrahydrofuran under reduced pressure, the residue was partitioned between water (50 ml) and ethyl acetate (3 × 50 ml). The ethyl acetate extracts were dried, filtered and concentrated to give 1.0 g of an orange crude viscous oil which was crystallised from ethyl acetate to yield the title compound (0.56 g, 24 %).

Alternatively, using Method D, piperidine (8 drops) was added to a hot stirred solution of 3,5-dichlorobenzaldehyde (1.78 g, 0.0102 mol) in absolute ethanol (5 ml). After 5 min, 3-pyridylacetonitrile (1.18 g, 0.0100 mol) was added followed by cautious addition of a catalytic amount of potassium *tert*-butoxide (*ca.* 10 mg) and the yellow crystalline mesh obtained was filtered, washed (diethyl ether) and dried to afford the title compound (2.19 g, 80 %). Recrystallisation from ethyl acetate gave light-yellow fine needles (1.34 g, 49 %) m.p. 148.1-148.5 °C (EtOAc); R_F 0.42 (diethyl ether); (Found: C, 61.02; H, 3.07; N, 10.16; M^+, 274.0061 (for ^{35}Cl). $C_{14}H_8N_2Cl_2$ requires C, 61.12; H, 2.93; N, 10.18%; *M*, 274.0065 (for ^{35}Cl)); ν_{max}(KBr disc)/cm^{-1} 3098m, 3020m and 3018m (=C–H), 2217m (CN), 1579m/s, 1560s, 1478m/s, 1412m/s and 1354m/s (C=C and Ar), 1256m/s, 1213m and 1008s (C-N), 855s, 807s, 704s and 670s (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.92 (dd, 1 H, $^4J_{15\text{-}H}$ 2.50 Hz, $^5J_{14\text{-}H}$ 0.81 Hz, 11-H), 8.67 (dd, 1 H, $^4J_{14\text{-}H}$ 4.80 Hz, $^5J_{15\text{-}H}$ 1.56 Hz, 13-H), 7.96 (ddd, 1 H, $^3J_{14\text{-}H}$ 8.07 Hz, $^4J_{11\text{-}H}$ 2.50 Hz, $^4J_{13\text{-}H}$ 1.60 Hz, 15-H), 7.75 (dd, 2 H, $^4J_{7\text{-}H}$ 1.8 Hz, $^5J_{3\text{-}H}$ 0.6 Hz, 5- and 9-H), 7.46 (br s, 1 H, 3-H), 7.43 (ddd, 1 H, $^3J_{15\text{-}H}$ 8.05 Hz, $^3J_{13\text{-}H}$ 4.82 Hz, $^5J_{11\text{-}H}$ 0.85 Hz, 14-H), 7.42 (t, 1 H, $^4J_{7\text{-}H}$ 1.9 Hz, 7-H); δ_C(200 MHz; $CDCl_3$) 150.7 (11-CH), 147.1 (13-CH), 140.1 (3-CH), 135.7(5) and 135.7(0) (6- and 8-C), 133.5 (15-CH), 130.6 (14-CH), 129.6 (4-C), 127.3 (5- and 9-CH), 123.7 (7-CH), 116.2 (1-CN), 111.5 (2-C);

m/z 278 (($^{37}Cl)M^+$, 10%), 276 (($^{35}Cl/^{37}Cl)M^+$, 60), 274 (($^{35}Cl)M^+$, 100), 247 (($^{35}Cl)M^+$ – HCN, 6.5), 241 (($^{37}Cl)M^+ - {}^{37}Cl$, 16), 239 (($^{35}Cl)M^+ - {}^{35}Cl$ and $(^{35}Cl/^{37}Cl)M^+ - {}^{37}Cl$, 51), 213 (($^{35}Cl)M^+ - {}^{35}Cl$ or $(^{35}Cl/^{37}Cl)M^+ - {}^{37}Cl$, – CN, 11.5), 211 (($^{35}Cl)M^+ - H^{35}Cl$ or $(^{35}Cl/^{37}Cl)M^+ - H^{37}Cl$, – HCN, 10), 203 ($M^+ - 2 \times Cl$, – H, 34), 177 ($M^+ - 2 \times Cl$, – HCN, 29), 150 (14), 101 (13), 88 (11), 75 (12).

2-(3-Pyridyl)-3-phenylpropenonitrile (**226**)—Using Method D, benzaldehyde (2.46 g, 0.027 mol) and 3-pyridylacetonitrile (3.22 g, 0.027 mol) were reacted in absolute ethanol (*ca.* 5 ml) with the addition of piperidine (10 drops) and potassium *tert*-butoxide (*ca.* 15 mg) to give the title compound as light-brown crystals (2.67 g, 48 %), m.p. 96.0-96.5 °C; R_F 0.44 (diethyl ether); (Found: C, 81.35; H, 4.85; N, 13.44; M^+, 206.0849. $C_{14}H_{10}N_2$ requires C, 81.53; H, 4.89; N, 13.58%; *M*, 206.0844); ν_{max}(KBr disc)/cm^{-1} 3092m, 3038m and 3018m (=C–H), 2213m (CN), 1604m, 1598m, 1572m, 1566m, 1496m, 1483m, 1449m, 1427m/s and 1362m/s (C=C and Ar), 1024m/s and 990m/s (C-N), 807s, 701s and 686s (=C-H deformations); δ_H(200 MHz; $CDCl_3$) 8.87 (dd, 1 H, $^4J_{15\text{-}H}$ 2.44 Hz, $^5J_{14\text{-}H}$ 0.69 Hz, 11-H), 8.57 (dd, 1 H, $^4J_{14\text{-}H}$ 4.81 Hz, $^5J_{15\text{-}H}$ 1.52 Hz, 13-H), 7.92 (ddd, 1 H, $^3J_{14\text{-}H}$ 8.07 Hz, $^4J_{11\text{-}H}$ 2.47 Hz, $^4J_{13\text{-}H}$ 1.57 Hz, 15-H), 7.8-7.9 (m, 2 H, 5- and 9-H), 7.53 (br s, 1 H, 3-H), 7.43 (m, 3 H, 6- and 7- and 8-H), 7.33 (ddd, 1 H, $^3J_{15\text{-}H}$ 8.10 Hz, $^3J_{13\text{-}H}$ 4.81 Hz, $^5J_{11\text{-}H}$ 0.80 Hz, 14-H); δ_C(200 MHz; $CDCl_3$) 149.9 (3-CH), 146.9 (11-CH), 143.8 (13-CH), 133.4 (15-CH), 133.1 (10-C), 131.1 (14-CH), 130.5 (4-C), 129.4 (5- and 9-CH), 129.0 (6- and 8-CH), 123.6 (7-CH), 117.1 (1-CN), 108.3 (2-C); *m/z* 206 (M^+, 44%), 205 (M^+ – H, 100), 179 (M^+ – HCN, 5), 177 (9), 151 (15), 126 (6), 102 (6), 84 (6), 76 (12).

2-(2-Pyridyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**227**)—Using Method D, 3,4-dimethoxybenzaldehyde (1.93 g, 11.61 mmol) and 2-pyridylacetonitrile (1.44 g, 12.19 mmol) were reacted in absolute ethanol (8 ml) with the addition of piperidine (8 drops) and potassium *tert*-butoxide (15 mg). The crystals formed (2.87 g, 93 %) were recrystallised from ethyl acetate to give the title compound as a light yellow microcrystalline powder (2.28 g, 74 %), m.p. 108.5-109.0 °C; R_F 0.65 (diethyl ether); (Found: C, 72.01; H, 5.32; N, 10.46; M^+, 266.1057. $C_{16}H_{14}N_2O_2$ requires C, 72.17; H, 5.30; N, 10.51%; *M*, 266.1055); ν_{max}(KBr disc)/cm^{-1} 3001w (=C–H), 2963w and 2936w (CH_3), 2211m (CN), 1595m/s (C=C), 1585s and 1516s (Ar), 1470m (CH_3 deformation), 1280s, 1269s, 1148m and 1024m (C–O), 793m and 775m (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.59 (ddd,

1 H, $^3J_{13\text{-H}}$ 4.73 Hz, $^4J_{14\text{-H}}$ 1.70 Hz, $^5J_{15\text{-H}}$ 0.70 Hz, 12-H), 7.36 (1 H, br s, 3-H), 7.79-7.66 (3 H, m, 5- and 14- and 15-H), 7.47 (dd, 1 H, $^3J_{8\text{-H}}$ 8.55 Hz, $^4J_{5\text{-H}}$ 2.17 Hz, 9-H), 7.48 (ddd, 1 H, $^3J_{14\text{-H}}$ 6.65 Hz, $^3J_{12\text{-H}}$ 4.72 Hz, $^4J_{15\text{-H}}$ 1.95 Hz, 13-H, overlaps with $CHCl_3$ signal), 6.91 (d, 1 H, $^3J_{9\text{-H}}$ 8.44 Hz, 8-H), 3.94 (s, 3 H, 7-OMe), 3.92 (s, 3 H, 6-OMe); δ_C(50 MHz; $CDCl_3$) 151.6 and 151.4 (7- and 6-C), 149.5 (12-CH), 148.9 (10-C), 144.9 (3-CH), 137.3 (14-CH), 126.3 (4-C), 125.5 (9-CH), 123.0 (15-CH), 120.9 (13-CH), 118.5 (1-CN), 111.2 (5-CH), 110.9 (8-CH), 106.9 (2-C), 55.9 (6- and 7-OCH_3); *m/z* 266 (M^+, 56%), 265 (M^+ – H, 100), 251 (M^+ – CH_3, 18), 223 (M^+ – CH_3, – CO, 19), 220 (M^+ – CH_3, – CH_3O, 9), 208 (M^+ – 2 x CH_3, – CO, 34), 192 (M^+ – CH_3, – CH_3O, – CO, 46), 179 (M^+ – 2 x CH_3, – 2 x CO, – H, 95), 154 ([*H-C≡C-CH=C(CN)-Py*]$^+$, M^+ – H_2C=C(OCH_3)-C(OCH_3)=CH_2, 36), 166 (11), 139 ([*(CH$_3$O)$_2$PhH*]$^+$, 7), 127 ([*H-C≡C-C≡C-Py*]$^+$, M^+ – H_2C=C(OCH_3)-C(OCH_3)=CH_2, – HCN, 18), 114 (7), 100 (12), 87 (11), 78 ([*Py*]$^+$, 38).

2-(2-Thienyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**228**)—Using Method D, 3,4-dimethoxybenzaldehyde (2.38 g, 14.32 mmol) and 2-thienylacetonitrile (previously purified over activated charcoal; 1.81 g, 14.69 mmol) were reacted in absolute ethanol (4 ml) with the addition of piperidine (5 drops) and potassium *tert*-butoxide (13 mg). The crystals formed (3.68 g, 95 %) were recrystallised from ethyl acetate to give the title compound (3.15 g, 81 %), m.p. 80-81 °C; R_F 0.79 (diethyl ether); (Found: C, 66.41; H, 4.87; N, 5.14; M^+, 271.0649. $C_{15}H_{13}NO_2S$ requires C, 66.42; H, 4.74; N, 5.16%; *M*, 271.0667); ν_{max}(KBr disc)/cm^{-1} 3102w and 3097w (=C–H of *thienyl*), 3043w (=C–H of *phenyl*), 2969w and 2938w (CH_3), 2843w (CH_3OPh), 2363w and 2347w (cumulative double-bonds), 2212m (CN), 1602m/w and 1595m/w (C=C), 1574m/w, 1540m and 1512m/s (Ar), 1275s, 1147m and 1020s (C–O), 738m (=C–H deformation); δ_H(200 MHz; $CDCl_3$) 7.60 (d, 1 H, $^4J_{9\text{-H}}$ 2.03 Hz, 5-H), 7.31-7.22 (m, 4 H, 3- and 9- and 12- and 14-H), 7.02 (dd, 1 H, $^3J_{14\text{-H}}$ 5.11 Hz, $^3J_{12\text{-H}}$ 3.67 Hz, 13-H), 6.87 (d, 1 H, $^3J_{9\text{-H}}$ 8.40 Hz, 8-H), 3.93 and 3.90 (2 × s, 6 H, 6- and 7-OMe); δ_C(50 MHz; $CDCl_3$) 151.1 (7-C), 149.0 (6-C), 139.6 (3-CH), 139.5 (10-C), 128.0 (12-CH), 126.4 (14-CH), 126.3 (4-C), 125.6 (13-CH), 124.2 (9-CH), 117.4 (1-CN), 110.9 (5-CH), 110.4 (8-CH), 103.2 (2-C), 55.9 (6- and 7-OCH_3); *m/z* 271 (M^+, 67%), 256 (M^+ – CH_3, 19), 228 (M^+ – CH_3, – CO, 27), 224 (M^+ – CH_3, – CH_3OH, 11), 212 (M^+ – 2 x CH_3, – CO, – H, 12), 200 (M^+ – CH_3, – H-C=O, – HCN, 22), 196 (M^+ – CH_3, – CH_3OH, – CO, 100), 185 (M^+ – 2 x CH_3, – 2 x CO, 45), 184 (M^+ – 2 x CH_3, – 2 x CO, – H,

32), 171 ($M^+ - CH_3, - CH_3O, - CO, - CN$, 100), 159 ([*H-C≡C-CH=C(CN)-thienyl*]$^+$, $M^+ - H_2C{=}C(OCH_3){-}C(OCH_3){=}CH_2$, 20), 143 ([$(CH_3O)_2Ph$]$^+$, 6), 127 (17), 114 (23), 82 ([*thienyl*]$^+ - H$, 8).

(Z)-2-(3-Thienyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**229**)—Using Method D, 3,4-dimethoxybenzaldehyde (2.78 g, 16.73 mmol) and 3-thienylacetonitrile (previously purified over activated charcoal; 2.06 g, 16.72 mmol) were reacted in absolute ethanol (5 ml) with the addition of piperidine (5 drops) and potassium *tert*-butoxide (15 mg). The crystals formed (3.97 g, 88 %) were recrystallised from ethyl acetate and then diethyl ether to give the title compound as yellow rhombic crystals suitable for X-ray crystallography (3.28 g, 72 %), m.p. 95-96 °C (from EtOAc); R_F 0.74 (diethyl ether); (Found: C, 66.37; H, 4.72; N, 5.16; M^+, 271.0667. $C_{15}H_{13}NO_2S$ requires C, 66.42; H, 4.74; N, 5.16%; *M*, 271.0667); ν_{max}(KBr disc)/cm^{-1} 3110w, 3098w and 3084w (=C–H of *thienyl*), 3026w and 3011w (=C–H of *phenyl*), 2951w and 2932w (CH_3), 2840w and 2830w (CH_3OPh), 2205w (CN), 1593m (C=C), 1574m/w, 1522m and 1506m/s (Ar), 1269s, 1145m and 1020m (C–O), 795m and 629m (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 7.63 (d, 1 H, $^4J_{9\text{-}H}$ 2.07 Hz, 5-H), 7.51 (dd, 1 H, $^4J_{14\text{-}H}$ 2.84 Hz, $^4J_{13\text{-}H}$ 1.45 Hz, 11-H), 7.38-7.27 (m, 4 H, 9- and 13- and 14-H), 7.34 (s, 3-H, overlaps with multiplet signals), 6.89 (d, 1 H, $^3J_{9\text{-}H}$ 8.41 Hz, 8-H), 3.94 and 3.91 (2 × s, 6 H, 6- and 7-OMe); δ_C(50 MHz; $CDCl_3$) 150.9 (7-C), 149.0 (6-C), 140.3 (3-CH), 136.7 (10-C), 127.2 (11-CH), 126.5 (4-C), 124.0 (14- and 13-CH), 122.8 (9-CH), 118.5 (1-CN), 110.9 (5-CH), 110.5 (8-CH), 103.9 (2-C), 55.9 (6- and 7-OCH_3); *m/z* 271 (M^+, 83%), 256 ($M^+ - CH_3$, 11), 228 ($M^+ - CH_3, - CO$, 31), 225 ($M^+ - CH_3, - CH_3O$, 17), 213 ($M^+ - 2$ x $CH_3, - CO$, 16), 200 ($M^+ - CH_3, -$ H-C=O, – HCN, 12), 196 ($M^+ - CH_3, - CH_3OH, - CO$, 100), 185 ($M^+ - 2$ x $CH_3, - 2$ x CO, 45), 184 ($M^+ - 2$ x $CH_3, - 2$ x CO, – H, 33), 171 ($M^+ - CH_3, - CH_3O, - CO, - CN$, 100), 159 ([*H-C≡C-CH=C(CN)-thienyl*]$^+$, $M^+ - H_2C{=}C(OCH_3){-}C(OCH_3){=}CH_2$, 25), 127 (15), 114 (22), 82 ([*thienyl*]$^+ - H$, 7).

2-(2-Pyridyl)-3-(2-quinolinyl)propenonitrile (**230**)—Using Method D, 2-quinolylcarbaldehyde (0.524 g, 3.33 mmol) and 2-pyridylacetonitrile (0.394 g, 3.34 mmol) were reacted in absolute ethanol (*ca.* 2 ml) with the addition of piperidine (5 drops) and potassium *tert*-butoxide (*ca.* 10 mg) to give the title compound as yellow crystals (0.612 g, 71 %), m.p. 124.5-125.1 °C (EtOH); R_F 0.59 (diethyl ether); (Found: C, 79.12; H, 4.14; N, 16.19; M^+, 257.0939. $C_{17}H_{11}N_3$ requires C, 79.36; H, 4.31; N, 16.33%;

M, 257.0952); ν_{max}(KBr disc)/cm^{-1} 3062w, 3046w and 2998w (=C–H), 2218m (CN), 1590s, 1582s, 1566m/s, 1498s, 1468s, 1434s and 1422s (aromatic stretches), 822s, 782s, 772s and 760s (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.64 (ddd, 1 H, $^3J_{17\text{-H}}$ 4.71 Hz, $^4J_{18\text{-H}}$ 1.77 Hz, $^5J_{19\text{-H}}$ 0.99 Hz, 16-H), 8.62 (1 H, s, 3-H), 8.62 (2 H, d, $^3J_{13\text{-H}}$ 8.50 Hz, 12-H, multiplet from 19-H coincides), 7.92-7.66 (5 H, multiplet signals), 7.92 (1 H, d, $^3J_{12\text{-H}}$ 8.50 Hz, 13-H), 7.87 (dt, 1 H, *J* 6.73 Hz, $^3J_{16\text{-H}}$ 1.18 Hz, 7-H), 7.80 (m, 1 H, $^4J_{16\text{-H}}$ 1.80 Hz, 18-H), 7.73 (2 H, 2 × m (overlapping), *J* 6.89 Hz, *J* 1.57 Hz, 8- and 10-H), 7.55 (ddd, 1 H, $^3J_{10\text{-H}}$ 8.10 Hz, $^3J_{8\text{-H}}$ 6.89 Hz, $^4J_{7\text{-H}}$ 1.21 Hz, 9-H), 7.28 (ddd, 1 H, $^3J_{18\text{-H}}$ 7.31 Hz, $^3J_{16\text{-H}}$ 4.73 Hz, $^4J_{19\text{-H}}$ 1.34 Hz, 17-H); δ_C(50 MHz; $CDCl_3$) 151.6 (4-C), 150.8 (14-C), 149.7 (16-CH), 143.6 (3-CH), 137.4 (12-CH), 136.8 (18-CH), 130.2 (7-CH), 129.9 (8-CH), 128.0 (11-C), 127.9 (9-CH), 127.5 (10-CH), 124.1 (19-CH), 122.0 (17-CH), 121.8 (13-CH), 117.2 (1-CN), 114.7 (2-C); *m/z* 257 (M^+, 95%), 256 (M^+ – H, 100), 229 (M^+ – H, – HCN, 15), 203 (M^+ – 2 × HCN, 4), 179 (M^+ – $[C_4H_4N]^+$ *i.e.* $[pyridyl]^+$, 13), 152 (M^+ – $[C_4H_4N]^+$ *i.e.* $[pyridyl]^+$, – CN, 5), 128 ($[C_9H_6N]^+$ *i.e.* $[quinolinyl]^+$, M^+ – $[pyridyl]^+$, – C≡C-CN, 30), 115 ($[quinolinyl\text{-}H]^+$ – N, 38), 101 ($[quinolinyl\text{-}H]^+$ – CN, 38), 78 (16), 63 (7), 51 (21).

2-(3-Pyridyl)-3-(2-quinolinyl)propenonitrile (**231**)—Using Method D, 2-quinolylcarbaldehyde (0.352 g, 2.24 mmol) and 3-pyridylacetonitrile (0.265 g, 2.24 mmol) were reacted in absolute ethanol (*ca.* 1.5 ml) with the addition of piperidine (3 drops) and potassium *tert*-butoxide (*ca.* 7 mg) to give the title compound as yellow crystals (0.402 g, 70 %), m.p. 134.9-135.2 °C (EtOH); R_F 0.15 (diethyl ether); (Found: C, 78.94; H, 4.07; N, 16.27; M^+, 257.0941. $C_{17}H_{11}N_3$ requires C, 79.36; H, 4.31; N, 16.33%; *M*, 257.0953); ν_{max}(KBr disc)/cm^{-1} 3070w, 3034w and 3000w (=C–H), 2214m/w (CN), 1592s, 1502s, 1478m/s and 1426m/s (aromatic stretches), 912m/s, 842m/s, 830m/s, 748s and 698s (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 9.00 (dd, 1 H, $^4J_{19\text{-H}}$ 2.50 Hz, $^5J_{18\text{-H}}$ 0.85 Hz, 15-H), 8.63 (dd, 1 H, $^3J_{18\text{-H}}$ 4.81 Hz, $^4J_{19\text{-H}}$ 1.57 Hz, 17-H), 8.21 (dd, 2 H, $^3J_{13\text{-H}}$ 8.59 Hz, $^4J_{10\text{-H}}$ 0.65 Hz, 12-H), 8.17 (dm, unresolved, 10-H, coincides with 12-H), 8.03 (ddd, 1 H, $^3J_{18\text{-H}}$ 8.08 Hz, $^4J_{15\text{-H}}$ 2.50 Hz, $^4J_{17\text{-H}}$ 1.58 Hz, 19-H), 8.62 (1 H, d, $^3J_{12\text{-H}}$ 8.57 Hz, 13-H), 8.62 (2 H, s, 3-H), 7.80 (dm, $^3J_{8\text{-H}}$ 8.5 Hz, $^4J_{9\text{-H}}$ 1.2 Hz, 7-H, coincides with 3-H), 7.72 (ddd, 1 H, $^3J_{7\text{-H}}$ 8.49 Hz, $^3J_{9\text{-H}}$ 6.91 Hz, $^4J_{10\text{-H}}$ 1.58 Hz, 8-H), 7.56 (ddd, 1 H, $^3J_{10\text{-H}}$ 8.13 Hz, $^3J_{8\text{-H}}$ 6.90 Hz, $^4J_{7\text{-H}}$ 1.23 Hz, 9-H), 7.37 (ddd, 1 H, $^3J_{19\text{-H}}$ 8.13 Hz, $^3J_{17\text{-H}}$ 4.82 Hz, $^5J_{15\text{-H}}$ 0.89 Hz, 18-H); δ_C(50 MHz; $CDCl_3$) 151.3 (4-C), 150.6 (15-CH), 148.0

(6-C), 147.4 (17-CH), 142.5 (19-CH), 137.0 (3-CH), 133.8 (12-CH), 130.4 (7-CH), 130.1 (11-C), 129.7 (8-CH), 128.0(5) (10-CH), 127.9(6) (14-C), 123.7 (18-CH), 121.0 (13-CH), 116.6 (1-CN), 112.8 (2-C); *m/z* 257 (M^+, 100%), 229 (M^+ – H, – HCN, 22), 203 (M^+ – 2 × HCN, 14), 179 (M^+ – $[C_4H_4N]^+$ *i.e.* [*pyridyl*]$^+$, 3), 128 ($[C_9H_6N]^+$ *i.e.* [*quinolyl*]$^+$, M^+ – [*pyridyl*]$^+$, – C≡C-CN, 19), 114 ([*quinolyl*]$^+$ – N, 38), 101 ([*quinolyl-H*]$^+$ – CN, 25), 88 (7), 75 (18), 63 (10), 51 (18).

2-(3-Thienyl)-3-(2-quinolinyl)propenonitrile (**232**)—Using Method D, 2-quinolylcarbaldehyde (0.336 g, 2.14 mmol) and 3-thienylacetonitrile (previously purified with activated carbon; 0.263 g, 2.14 mmol) were reacted in absolute ethanol (*ca.* 2 ml) with the addition of piperidine (5 drops) and potassium *tert*-butoxide (*ca.* 10 mg) to give the title compound as yellow crystals (0.312 g, 56 %), m.p. 135.3-135.6 °C (EtOH); R_F 0.75 (diethyl ether); (Found: C, 72.84; H, 3.62; N, 10.38; M^+, 262.0546. $C_{16}H_{10}N_2S$ requires C, 73.26; H, 3.84; N, 10.68%; *M*, 262.0531); ν_{max}(KBr disc)/cm^{-1} 3098w, 3074w and 3056w (=C–H), 2226w (CN), 1594s, 1550m/s, 1566m/s and 1500s (aromatic stretches), 922m/s, 838s, 784s and 760s (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 8.1 (2 H, br s), 7.9 (1 H, br d, *J* 9.3 Hz), 7.6(5) (4 H, br d, *J* 18.2 Hz, includes 3-H), 7.6 (0.5 H, br d, *J* 7.1 Hz), 7.4 (2.5 H, br s); δ_C(50 MHz; $CDCl_3$) 152.0 (4-C), 148.0 (6-C), 139.1 (15-CH), 136.8 (3-CH), 136.3 (11-C), 130.2 (12-CH), 129.6 (7-CH), 127.6 (8- and 9- and 10-CH), 125.5 (17-CH), 124.2 (18-CH), 120.7 (13-CH), 117.3 (1-CN), 110.8 (2-C); *m/z* 262 (M^+, 75%), 261 (M^+ – H, 100), 236 (M^+ – CN, 21), 229 (M^+ – HS, 50), 216 (M^+ – H-C≡S, – H, 15), 203 (M^+ – H-C≡C-H, – HS, 6), 190 (M^+ – H-C≡S, – HCN, 8), 128 ($[C_9H_6N]^+$ *i.e.* [*quinolyl*]$^+$, M^+ – [*thienyl*]$^+$, – C≡C-CN, 12), 114 ([*quinolyl*]$^+$ – N, 4), 101 ([*quinolyl-H*]$^+$ – CN, 15), 77 (10), 63 (13), 51 (15).

(E)-2-(3-Pyridyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**233**)—Using a reflux condenser and cold-finger to prevent solvent loss, a solution of (*Z*)-2-(3-pyridyl)-3-(3,4-dimethoxyphenyl)propenonitrile (1.175 g) in acetonitrile (180 ml) was stirred under irradiation from a 500 W halogen lamp for 26 h. The temperature did not exceed 60 °C throughout the experiment. After additional stirring at room temperature for 2 h, some silica was added and the solvent was removed in preparation for column chromatography. The two isomers were separated (*ca.* 60:40 (*E*) to (*Z*)-forms by 90 MHz ^{1}H NMR spectroscopy) through silica using cyclohexane/diethyl ether combinations followed by pure diethyl ether and ethyl acetate elutions. This gave the (*Z*)-isomer (0.48 g, 41 %) and the desired

(*E*)-isomer (0.51 g, 43 %) which was crystallised from diethyl ether at 0 °C to afford white needles (0.311 g, 26.5 %). For crystallographic purposes, *ca.* 0.1 g of this was further crystallised from diethyl ether at *ca.* -10°C to give white rod-like crystals, m.p. 86.8 °C; R_F 0.34 (1:2 ethyl acetate/petroleum ether, bp 30-40°C); (Found: C, 72.10; H, 5.36; N, 10.51; M^+, 266.1062. $C_{16}H_{14}N_2O_2$ requires C, 72.17; H, 5.30; N, 10.51%; *M*, 266.1055); ν_{max}(KBr disc)/cm^{-1} 3056w and 3004w (=C–H), 2967w, 2938w and 2917w (CH_3), 2842w (CH_3OAr), 2203m (CN), 1597s (C=C), 1576w, 1566w and1512s (Ar), 1426m and 1412m (CH_3 deformations), 1273s, 1246m and 1019s (C–O), 814m, 770m and 718m (=C–H deformations); δ_H(200 MHz; acetone-d_6) 8.64 (br dd, 2 H, $^3J_{14\text{-H}}$ 4.85 Hz, $^4J_{15\text{-H}}$ 1.64 Hz, 13-H with 11-H overlapping), 7.88 (ddd, 1 H, $^3J_{14\text{-H}}$ 7.92 Hz, $^4J_{11\text{-H}}$ 2.28 Hz, $^4J_{13\text{-H}}$ 1.70 Hz, 15-H), 7.57 (1 H, br s, 3-H), 7.51 (ddd, 1 H, $^3J_{15\text{-H}}$ 7.93 Hz, $^4J_{13\text{-H}}$ 4.81 Hz, $^4J_{11\text{-H}}$ 0.91 Hz, 14-H), 6.91 (d, 2 H, $^4J_{9\text{-H}}$ 1.15 Hz, 8- and 9-H coincide), 6.68 (br t, 1 H, unresolved, 5-H), 3.82 (s, 3 H, 6-OMe), 3.48 (s, 3 H, 7-OMe); δ_H(200 MHz; $CDCl_3$) 8.67 (dd, 1 H, $^4J_{15\text{-H}}$ 2.33 Hz, $^5J_{14\text{-H}}$ 0.88 Hz, 11-H), 8.62 (dd, 1 H, $^3J_{14\text{-H}}$ 4.86 Hz, $^4J_{15\text{-H}}$ 1.67 Hz, 13-H), 7.77 (ddd, 1 H, $^3J_{14\text{-H}}$ 7.91 Hz, $^4J_{11\text{-H}}$ 2.22 Hz, $^4J_{13\text{-H}}$ 1.76 Hz, 15-H), 7.10 (1 H, br s, 3-H), 7.51 (ddd, 1 H, $^3J_{15\text{-H}}$ 7.93 Hz, $^4J_{13\text{-H}}$ 4.85 Hz, $^4J_{11\text{-H}}$ 0.89 Hz, 14-H), 6.83 (ddd, 1 H, $^3J_{8\text{-H}}$ 8.37 Hz, $^4J_{5\text{-H}}$ 1.94 Hz, $^4J_{3\text{-H}}$ 0.32 Hz, 9-H), 6.77 (d, 1 H, $^3J_{9\text{-H}}$ 8.39 Hz, 8-H), 6.68 (br d, 1 H, $^4J_{9\text{-H}}$ 1.93 Hz, 5-H), 3.87 (s, 3 H, 6-OMe), 3.54 (s, 3 H, 7--OMe); δ_H(200 MHz; THF-d_8 at 3.584 and 1.731 ppm) 8.60 (dd, 1 H, $^4J_{15\text{-H}}$ 2.3 Hz, $^5J_{14\text{-H}}$ 0.8 Hz, 11-H), 8.56 (dd, 1 H, $^3J_{14\text{-H}}$ 4.81 Hz, $^4J_{15\text{-H}}$ 1.66 Hz, 13-H), 7.78 (ddd, 1 H, $^3J_{14\text{-H}}$ 7.92 Hz, $^4J_{11\text{-H}}$ 2.25 Hz, $^4J_{13\text{-H}}$ 1.76 Hz, 15-H), 7.49 (1 H, s, 3-H), 7.39 (ddd, 1 H, $^3J_{15\text{-H}}$ 7.88 Hz, $^4J_{13\text{-H}}$ 4.81 Hz, $^4J_{11\text{-H}}$ 0.87 Hz, 14-H), 6.83 (d, 2 H, *J* 1.04 Hz, 8- and 9-H), 6.64 (br t, 1 H, unresolved, 5-H), 3.77 (s, 3 H, 6-OMe), 3.46 (s, 3 H, 7-OMe); δ_C(50 MHz; acetone-d_6) 152.3 (7-C), 150.9 (11-CH), 150.6 (13-CH), 149.8 (6-C), 146.9 (3-CH), 137.3 (15-CH), 130.9 (10-C), 126.7 (4-C), 125.5 (9-CH), 124.9 (14-CH), 120.6 (1-CN), 113.2 (5-CH), 112.3 (8-CH), 108.4 (2-C), 56.0 (6-OCH_3), 55.5 (7-OCH_3); δ_C(50 MHz; $CDCl_3$) 151.1 (7-C), 150.0 (2) and 149.9 (8) (11- and 13-CH), 148.7 (6-C), 146.0 (3-CH), 136.5 (15-CH), 129.6 (10-C), 125.6 (4-C), 124.6 (9-CH), 123.8 (14-CH), 119.8 (1-CN), 111.7 (5-CH), 110.9 (8-CH), 107.9 (2-C), 55.9 (6-OCH_3), 55.4 (7-OCH_3); *m/z* 266 (M^+, 100%), 251 ($M^+ - CH_3$, 16), 223 ($M^+ - CH_3$, – CO, 28), 220 ($M^+ - CH_3$, – CH_3O, 8), 208 ($M^+ - 2 \times CH_3$, – CO, 29), 195 ($M^+ - CH_3$, – H-C=O, – HCN, 30), 192 ($M^+ - CH_3$, – CH_3O, – CO, 46), 191 ($M^+ - CH_3$, – CH_3OH, – CO, 46), 180 ($M^+ - 2 \times CH_3$, – 2 × CO, 49), 179 ($M^+ - 2 \times CH_3$, –

2 × CO, – H, 96), 154 ([*H-C≡C-CH=C(CN)-Py*]$^+$, M^+ – $H_2C{=}C(OCH_3){-}C(OCH_3){=}CH_2$, 33), 167 (17), 164 (22), 139 ([*$(CH_3O)_2PhH$*]$^+$, 13), 127 ([*H-C≡C-C≡C-Py*]$^+$, M^+ – $H_2C{=}C(OCH_3){-}C(OCH_3){=}CH_2$, – HCN, 33), 114 (11), 100 (21), 87 (18), 77 ([*Py*]$^+$ – H, 17), 63 (39), 51 (42).

(Z)-2,3-Epoxy-2-(3-pyridyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**2 3 4**) — After performing chromatography as described for (*E*)-2-(3-pyridyl)-3-(3,4-dimethoxyphenyl)propenonitrile (**233**) the (*Z*)-epoxide (0.105 g, 8.4 %) was isolated, extracted into diethyl ether, to remove insoluble material, and crystallised from acetone, m.p. 108 °C; R_F 0.12 (1:2 ethyl acetate/petroleum ether, bp 30-40 °C); (Found: C, 72.18; H, 5.35; N, 10.69; M^+, 282.1011. $C_{16}H_{14}N_2O_3$ requires C, 72.17; H, 5.30; N, 10.51%; *M*, 282.1004); ν_{max}(KBr disc)/cm^{-1} 3047w and 3011w (=C–H), 2968w and 2940w (CH_3), 2844w (CH_3OAr), 2243w (CN), 1596s and 1518s (Ar), 1447m and 1424m (CH_3 deformations), 1276s, 1149s and 1019s (C–O), 808m and 712m (=C–H deformations); δ_H(200 MHz; acetone-d_6) 8.64 (dd, 1 H, $^4J_{15\text{-}H}$ 2.65 Hz, $^4J_{14\text{-}H}$ 0.86 Hz, 11-H), 8.63 (dd, 1 H, $^3J_{14\text{-}H}$ 4.77 Hz, $^4J_{15\text{-}H}$ 1.53 Hz, 13-H), 7.89 (ddd, 1 H, $^3J_{14\text{-}H}$ 8.15 Hz, $^4J_{11\text{-}H}$ 2.65 Hz, $^4J_{13\text{-}H}$ 1.55 Hz, 15-H), 7.41 (ddd, 1 H, $^3J_{15\text{-}H}$ 8.11 Hz, $^4J_{13\text{-}H}$ 4.77 Hz, $^4J_{11\text{-}H}$ 0.83 Hz, 14-H), 7.23 (dd, 1 H, $^3J_{8\text{-}H}$ 8.36 Hz, $^4J_{5\text{-}H}$ 2.23 Hz, 9-H), 6.98 (d, 1 H, $^4J_{9\text{-}H}$ 2.21 Hz, 5-H), 6.92 (d, 1 H, $^3J_{9\text{-}H}$ 8.37 Hz, 8-H), 5.77 (1 H, s, 3-H), 3.80 (s, 3 H, 7-OMe), 3.60 (s, 3 H, 6-OMe); δ_C(50 MHz; acetone-d_6) 150.9 (7-C), 150.4 (11-CH), 150.3 (13-CH), 149.8 (6-C), 136.5 (15-CH), 132.2 (10-C), 125.9 (4-C), 124.3 (9-CH), 124.2 (14-C), 121.9 (1-CN), 114.9 (5-CH), 112.1 (8-CH), 56.0 (6-OCH_3), 55.5 (7-OCH_3), 54.4 (3-CH), 48.0 (2-C); *m/z* 282 (M^+, 15%), 267 ($M^+ - CH_3$, 14), 266 (M^+ – O, 6), 256 (M^+ – CN, 4), 239 ($M^+ - CH_3$, – CO, 26), 236 ($M^+ - CH_3$, – CH_3O, 8), 224 ($M^+ - 2 \times CH_3$, – CO, 23), 211 ($M^+ - CH_3$, – H-C=O, – HCN, 27), 208 ($M^+ - CH_3$, – CH_3O, – CO, 34), 2071 ($M^+ - CH_3$, – CH_3OH, – CO, 35), 196 ($M^+ - 2 \times CH_3$, – 2 × CO, 34), 195 ($M^+ - 2 \times CH_3$, – 2 × CO, – H, 54), 148 ([*H-C=C=CH-C(O)-Py*]$^+$, M^+ – $H_2C{=}C(OCH_3){-}C(OCH_3){=}CH_2$, – CN, 10), 167 (15), 164 (17), 138 ([*$(CH_3O)_2Ph$*]$^+$, 7), 100 (12), 87 (13), 77 ([*Py*]$^+$ – H, 12).

2-Cyano-3-(3,4-dimethoxyphenyl)propenonitrile (**236**)—Using Method C, 3,4-dimethoxybenzaldehyde (6.996 g, 0.0421 mol) and malononitrile (2.781 g, 0.0421 mol) were reacted in absolute ethanol (15 ml) with the addition of piperidine (5 drops) and after 2 h the crystals were collected (7.683 g, 85 %), m.p. 144.3-145.4 °C (from EtOH) (lit.,[333]

144 °C); R_F 0.93 (diethyl ether); (Found: C, 67.12; H, 4.52; N, 13.01; M^+, 214.0745. $C_{12}H_{10}N_2O_2$ requires C, 67.29; H, 4.67; N, 13.08%; *M*, 214.0742); ν_{max}(KBr disc)/cm^{-1} 3105w, 3026w and 3009w (=C–H), 2965w and 2936w (CH_3), 2849w and 2834w (CH_3OPh), 2224m/s (CN), 1601m/w (C=C), 1581m, 1568m and 1508s (Ar), 1273s, 1144m/s and 1016m (C–O), 850m/w, 822m/w and 629m/w (=C–H deformations); δ_H(200 MHz; $CDCl_3$) 7.68 (d, 1 H, $^4J_{9\text{-}H}$ 2.2 Hz, 5-H), 7.67 (s, 1 H, 3-H, overlaps with 5-H), 7.51 (dd, 1 H, $^3J_{9\text{-}H}$ 8.46 Hz, $^4J_{5\text{-}H}$ 2.12 Hz, 9-H), 6.98 (d, 1 H, $^3J_{9\text{-}H}$ 8.48 Hz, 8-H), 4.00 and 3.94 (2 × s, 6 H, 6- and 7-OMe); δ_C(50 MHz; $CDCl_3$) 159.5 (3-CH), 154.6 (7-C), 149.4 (6-C), 128.2 (9-CH), 124.2 (4-C), 114.4 and 113.6 (2 × CN), 111.1 (5-CH), 110.7 (8-CH), 78.2 (2-C), 56.3 and 56.0 (6- and 7-OCH_3); *m/z* 214 (M^+, 45%), 199 ($M^+ - CH_3$, 26), 171 ($M^+ - CH_3$, – CO, 87), 168 ($M^+ - CH_3$, – CH_3O, 3), 156 ($M^+ - 2 \times CH_3$, – CO, 3), 143 ($M^+ - CH_3$, – H-C=O, – HCN, 83), 128 ($M^+ - 2 \times CH_3$, – 2 × CO, 49), 116 ($M^+ - CH_3$, – CH_3O, – 2 × CN, 100), 114 ($M^+ - CH_3$, – CH_3O, – CO, – CN, 44), 101 ([H_2C=C-CH=C(CN)$_2$]$^+$, M^+ – HC=C(OCH_3)-C(OCH_3)=CH_2, 87), 89 (39), 75 (46), 63 (37), 50 (52), 39 (28).

EXPERIMENTAL—BIOLOGY

MATERIALS AND INSTRUMENTATION

Glutamine, RPMI 1640, Dubeco's Modified Eagle's Medium (DMEM), foetal calf serum (FCS), and donor calf serum (DCS) were all obtained from Gibco BRL (Paisley, UK). [^{3}H]Thymidine and [γ-^{32}P]ATP were obtained from Amersham Ltd (Amersham, UK). Ecoscint A was obtained from National Diagnostics.

MCF-7 breast adenocarcinoma cells were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in RPMI 1640 supplemented with 2 mM glutamine and 10 % (v/v) FCS. Fibroblasts transfected with the human EGF receptor (HER 14)[334] were a kind gift from Dr J. Schlessinger (New York University School of Medicine) and were maintained in DMEM supplemented with 2 mM glutamine and 10 % DCS. The HN5 cell line derived from a human squamous cell carcinoma of the tongue,[335] was a kind gift from Dr B. Ozanne (Beatson Institute, Glasgow). The cell lines A431[336], CaSki[337] and SiHa[338] were all obtained from the ATCC and maintained in Ham's F10:DMEM (50:50) supplemented with 2 mM glutamine and 10 % (v/v) FCS.

Cell harvesting was performed on an LKB Cell Harvester and radioactivity was determined with a Packard Liquid Scintillation Analyser.

Experimental Procedures

All of the biochemical methods were performed at the Cancer Research Campaign (CRC) Department of Medical Oncology, CRC Beatson Laboratories, University of Glasgow, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD, UK.

MCF-7 Antiproliferative Assay

MCF-7 cells were plated at a concentration of 1×10^3 cells/well in 96 well plates and grown in a humidified atmosphere of 5 % (v/v) carbon dioxide:air at 37 °C for 72 h. For each plate, a range of inhibitor concentrations in 10 % (v/v) FCS was then added (in triplicate) and 12 wells were left untreated to act as a control. After 24 h, [^{3}H]thymidine (0.25 μCi/well) was added to each well for a 1 h pulse. Following removal of the medium, the cell sheets were washed twice with ice-cold phosphate-buffered saline, subjected to trypsin/EDTA (0.25 %/1 mM) for a 20 min period and then harvested onto filter mats. These were washed

three times with distilled water, allowed to dry, covered with Ecoscint A (10 ml) and their radioactivity measured in counts-per-minute (cpm). The amount of incorporation of [^{3}H]thymidine (in cpm) was then expressed as a percentage of the uninhibited growth of the control. Log-concentration response curves were generated from which IC_{50} values were determined as the concentration of drug required to inhibit growth of the control by 50 %.

The inhibitor concentrations were prepared by forming *fresh* 10 mM solutions of the inhibitors in DMSO and then diluting with 10 % (v/v) FCS to the desired concentration. Depending on the inhibitor, the concentration range was generally [1.0 10.0, 20.0, 50.0, and 100.0 μM] or [0.1, 1.0, 10.0, 20.0, and 50.0 μM] or [0.001, 0.01, 0.1, 1.0, and 20.0 μM]. For most experiments, use of *stock* 10 mM solutions stored at -20 °C was avoided due to suspected inhibitor instability.

Initially for the MCF-7 antiproliferatve assay a parallel experiment using charcoal stripped serum (CSS) was performed. Compared to FCS the results obtained were near identical and the use of CSS, which lacks the growth factor oestrogen, was no longer necessary.

HER-14 Antiproliferative Assay

For the HER-14 cell line the procedure was as for MCF-7 except: a 2 % (v/v) carbon dioxide:air atmosphere was generally used to confluence; inhibitors were added in 10 % (v/v) DCS and 0.1% (v/v) DCS plus 10 ng/ml EGF; a 48 h inhibitor exposure time was adopted and [^{3}H]thymidine incorporation was measured over 16 h.

Cytotoxicity Assay

Compounds against an appropriate carcinoma cell line, *e.g.* MCF-7, HN5, A431, CaSki or SiHa, were assayed for their cytotoxicity as given below. This work was carried out by Dr Valerie G. Brunton. For a comparative procedure see reference 339.

Carcinoma cells were seeded at the appropriate cell density in 96-well plates and grown for 72 h in a humidified atmosphere of 2 % (v/v) CO_2:air at 37 °C before addition of a range of inhibitor concentrations in 200 μl of medium. Four replicate wells were used for each drug concentration. Cells alone were used as a control and medium alone was used as a blank. After 24 h the inhibitors were removed and fresh medium added. The medium was replaced every 24 h for a further 3 days allowing the cells to pass through 2 to 3 doublings. This allowed the assay to be used as a measure of cell kill. On the third day 3-(4,5-dimethylthiaz-

2-yl)-2,5-diphenyltetrazolium (MTT) (5 mg/ml) was added to each well. The plates were then incubated in the dark at 37 °C for 4 h. Medium and MTT were then removed and the formazan crystals (formed following redution of MTT by live cells) were dissolved in DMSO (200 µl) and Sorensen's glycine buffer (25 µl) (0.1 M glycine plus 0.1 M NaCl adjusted to pH 10.5 with 0.1 M NaOH) then added. The absorbance was read at 570 nm in an enzyme-linked immunosorbent assay plate reader (Model 2550; Bio-Rad Laboratories). Log-concentration response curves were generated from which IC_{50} values were determined as the dose to inhibit MTT absorbance by 50 %.

Measurement of EGF-R Kinase Activity

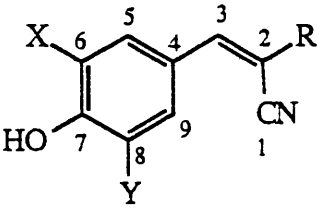
EGF receptor tyrosine kinase activity was measured in membrane fractions prepared from the HN5 cell line by measuring the EGF-dependent phosphorylation of a synthetic substrate, poly(Glu_6, Ala_3, Tyr_1). This work was performed by Dr Valerie G. Brunton.

The membrane fractions (0.5 µg protein) were incubated with kinase buffer (45 mM HEPES, pH 7.4, 100 µM sodium orthovanadate, 0.05 % Triton X-100, 2 % glycerol), 25 mM $MgCl_2$, 2 mg/ml poly(Glu_6, Ala_3, Tyr_1) and inhibitors at a range of concentrations. Parallel incubations were carried out in either the presence or absence of 0.25 µM EGF. The inhibitors were prepared as stock solutions in dimethylsulphoxide (DMSO) and the final concentration of DMSO in the incubations (1 %) had no effect on the reaction rate. The reaction was initiated by addition of [γ-^{32}P]ATP (0.5 µCi, 20 µM, *sp. ac.* 3000 Ci/mmol) and after 20 min at 30 °C the reaction was terminated by the addition of 1 mM ATP on ice. The products were spotted onto Whatman 3MM paper and the peptide was precipitated with 10 % trichloroacetic acid containing 0.01 M sodium pyrophosphate. The filters were then washed extensively with the trichloroacetic acid solution, allowed to dry, transferred to scintillation vials, Ecoscint A (10 ml) was added and the radioactivity was measured.

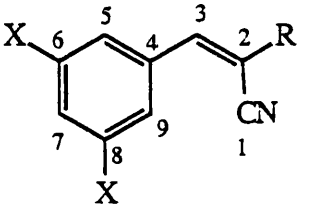
◊ APPENDIX ◊

Listed below are the numbering systems used for the compounds stated in the experimental. An arbitrary system is adopted which emphasises the propenonitrile moiety and the (R) label refers to anyone of the following functional groups: CN, $CONH_2$, $CSNH_2$, CO_2Me, CO_2Et, $CO_2{}^nBu$, $CO_2{}^tBu$, or $C(NH_2)=C(CN)_2$. Each group is clearly assigned in the experimental data for a given compound.

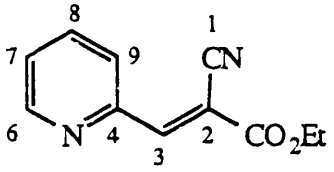
Chapter 6



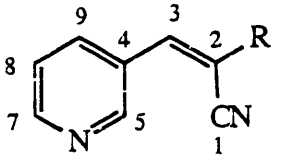
(**56**)-(**58**) and (**69**)-(**80**)
(X, Y= H and/or OH)



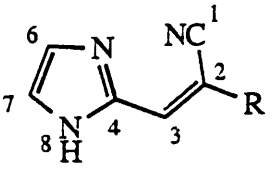
(**85**)-(**90**)
(X= Cl or F)



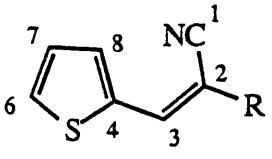
(**91**)



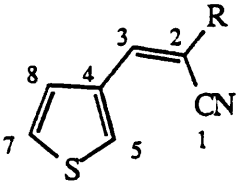
(**92**)-(**95**)



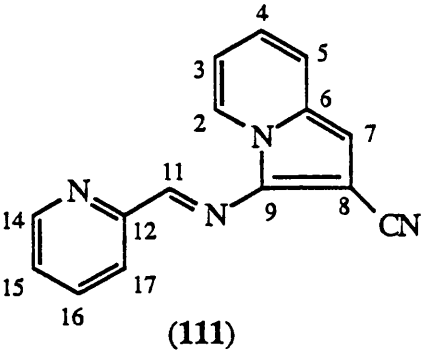
(**96**)-(**100**)



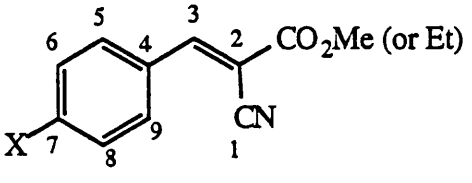
(**101**)-(**105**)



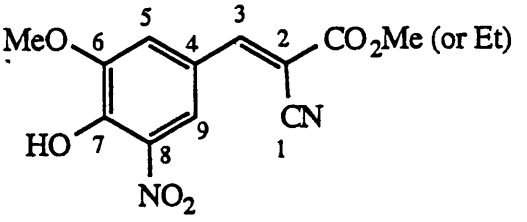
(**106**)-(**110**)



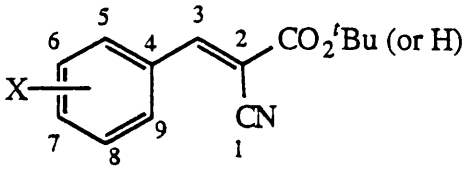
(**111**)



(**113**)-(**116**)
(X= H or NO_2)

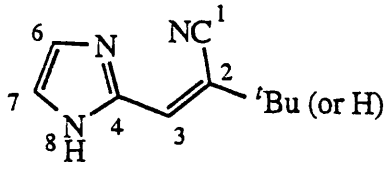


(**117**)-(**118**)

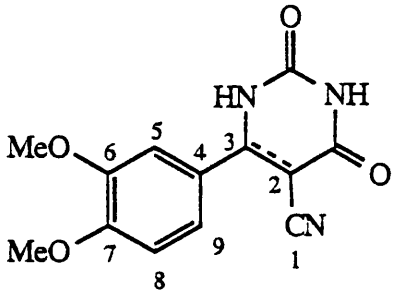


(**121**)-(**133**)

(X= H, F, Cl, NO_2,OH, OMe, and/or OCH_2O)

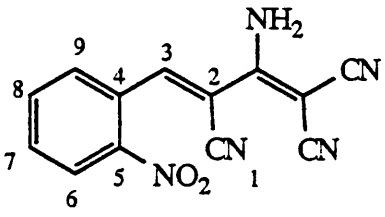


(**134**)-(**135**)



(**136**) K^+ salt (no double-bond)

(**137**) includes double bond



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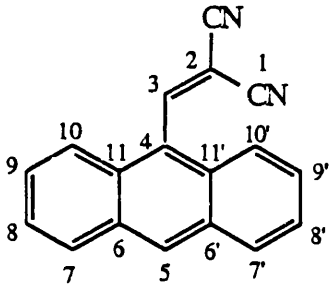

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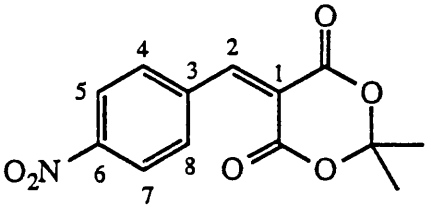
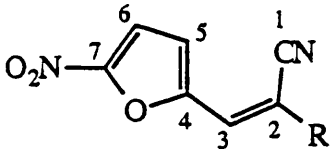


(**140**)

Chapter 7



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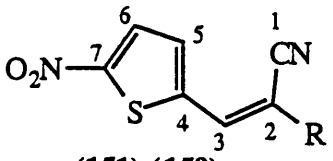

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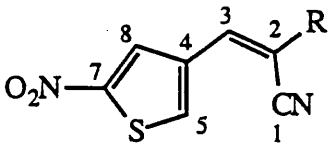

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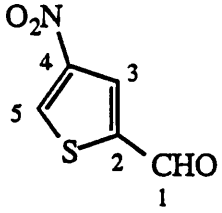

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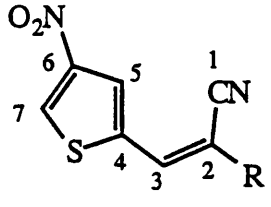
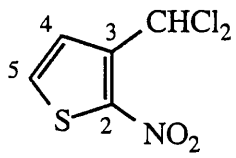


(**169**)-(**175**)



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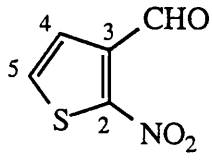

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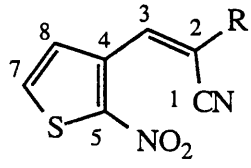
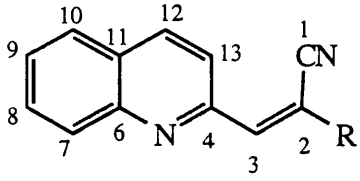


(180)-(186)



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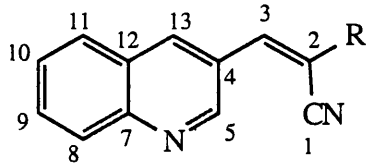

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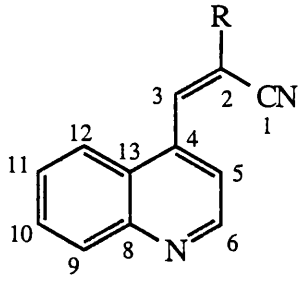
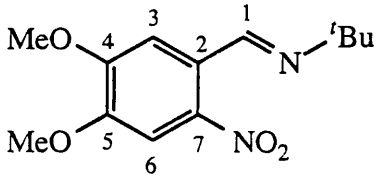


(198)-(202)



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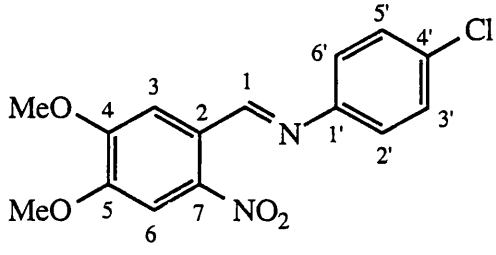

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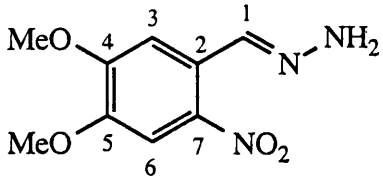

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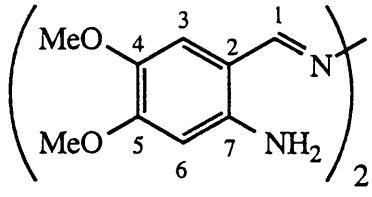

(210)

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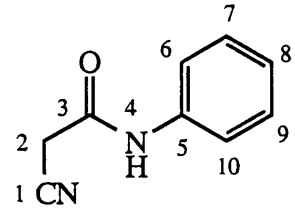

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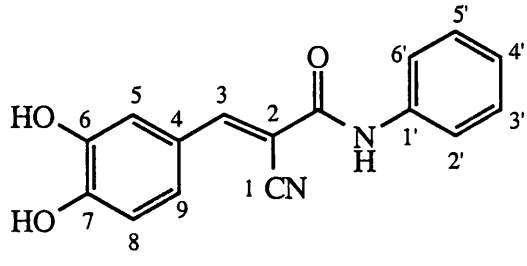

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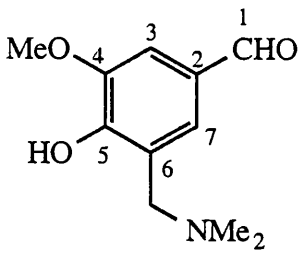

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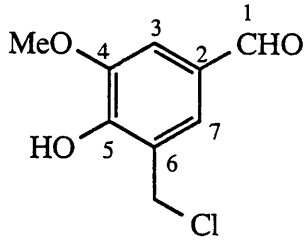

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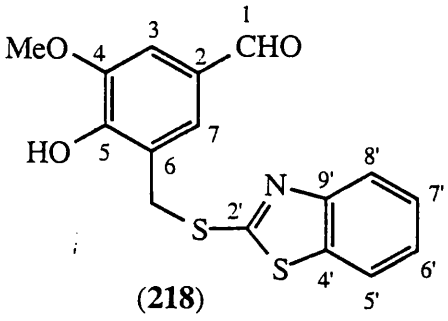

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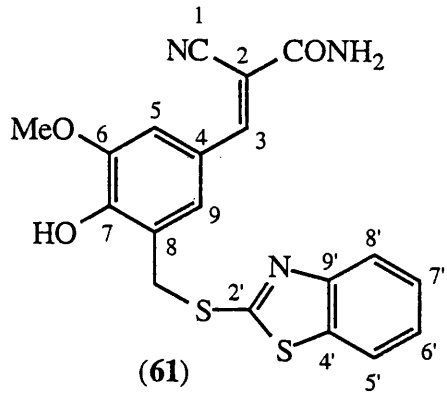

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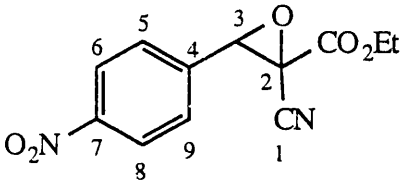

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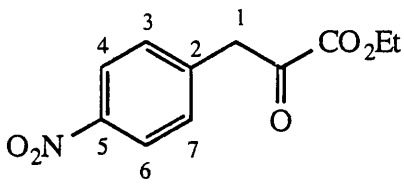

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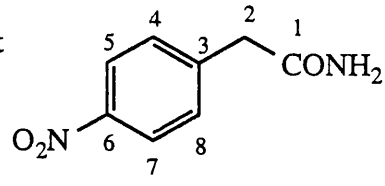

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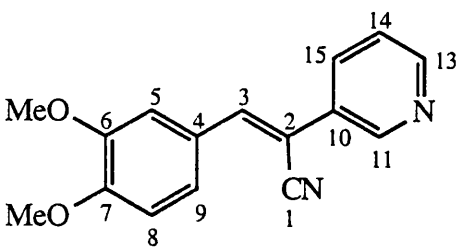

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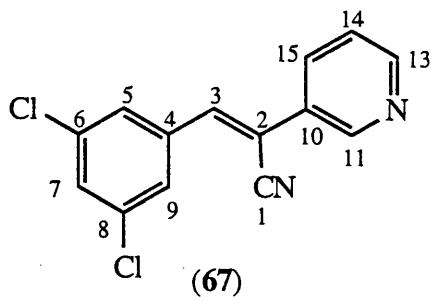

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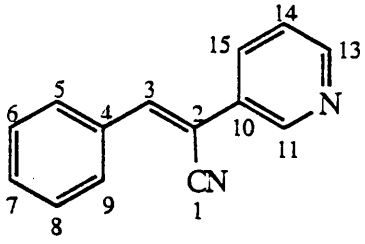

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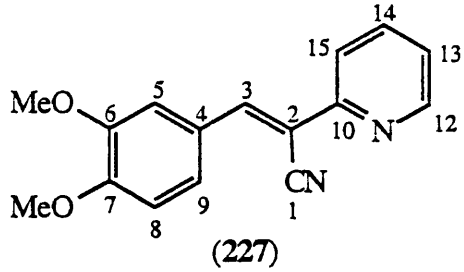

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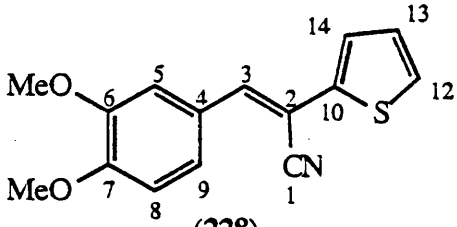

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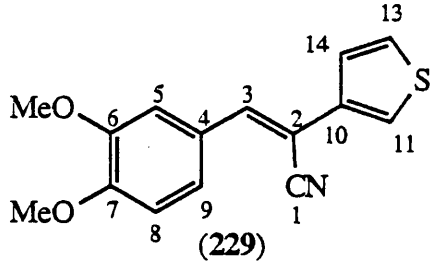

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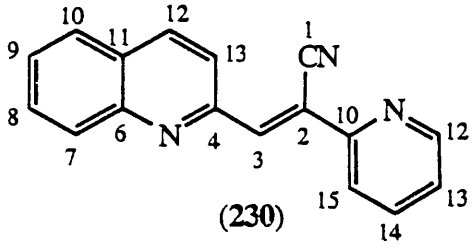

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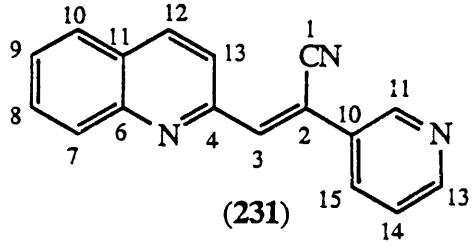

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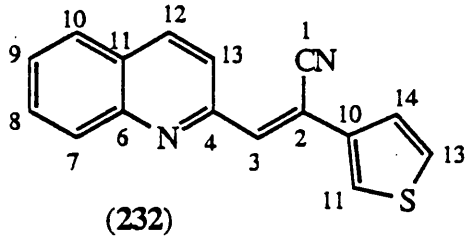

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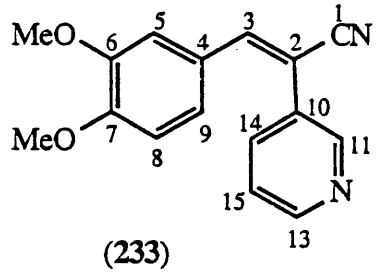

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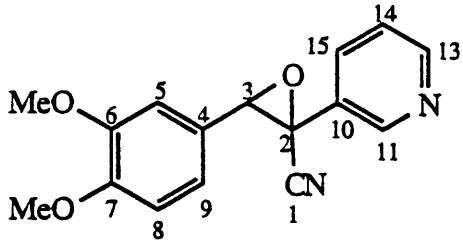

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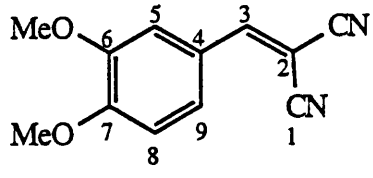

(236)

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