Synthesis of Inhibitors of Histone Deacet

A thesis submitted by

-

Jonathon Dines

In partial fulfilment of the requirements for the degree o:

DOCTOR OF PHILOSOPHY

Dept. of Chemistry
Christopher Ingold Building
20 Gordon Street
University College London
London
WC1E 6BT

June

UMI Number: U592546

UMI

Dissertation Publishing

ProQuest

Abstract

It has been known for some years now that histone deacetylase (HDAC) inhibition exerts a variety of effects on cellular proliferation and apoptosis. The potential for use of inhibitors of HDAC in the field of cancer therapy has now been realised; the first drug of this class recently being approved for use.[1] Structurally, a great variety of molecules have been shown to be capable of producing an inhibitory effect, through mechanisms elucidated by crystallographic studies of the inhibitor-bound enzyme. Despite the diversity of these compounds, several structural features common to most of the known inhibitors can be identified, including a moiety capable of binding a zinc ion present at the enzymes active site. A great number of the known inhibitors bear a hydroxamic acid group for this reason, despite the reportedly unfavourable characteristics of this functionality *in vivo*.[2] This thesis focuses on the synthesis of novel inhibitors of HDAC, attempts to develop non-hydroxamate inhibitors and biological evaluation of the HDAC inhibitory capability of compounds synthesised within this group.

A series of compounds bearing aryloxyalkanoic acid hydroxyamide motifs were synthesised following the identification of this class of compounds as potent HDAC inhibitors. Systematic variation of these molecules allowed optimisation of their activity in isolated enzyme inhibition assays and in human cancer cell line cytotoxicity assays.

Attempts to identify novel zinc-binding groups capable of replacing the hydroxamic acid motif were possible through use of the structure of a known potent inhibitor and substitution of the motif with a variety of structures proposed to provide the same role. A further group of novel hydroxamate inhibitors were synthesised based on the simple structure of a known inhibitor. Diversification of the structure and substitution of bio-isosteres led to enhancement of inhibitory activity. Finally, a selection of novel compounds synthesised within this group were evaluated for their effect on cell cycle, cell protein expression and isolated HDAC inhibition.

Acknowledgements

The work for this thesis would not have been possible without the assistance of a great number of people. I would like to thank Prof. Charles Marson for his supervision and guidance throughout the project and the Mandeville Trust for funding this research. The biological work I was able to carry out at King's College is thanks to Dr Shaun Thomas, with patient help from Steve Orr. Back at UCL, I am forever grateful for the contributions of the group, particularly for early technical help from Stephane Sengmany and Alf Rioja. I owe thanks to many others; Richard Fitzmaurice, Jon Wilden, Alex Lewis and Jamie Baker for education; all the good people of the fourth floor for coffee, the Crunchy Nuts and Tom for enlightenment, and finally, Elodie, for everything.

Table of Contents

Abbreviations and Acronyms

Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOP	benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
Bz	benzoyl
CBHA	*m*-carboxycinnamic acid bis hydroxamide
DCC	*N,N*-dicyclohexycarbodiimide
DCM	dichloromethane
DHAD	dihydroxyacetone dimer
DIPEA	ethyl diisopropylamine
DMAP	*N,N*-dimethyl-4-aminopyridine
DMEM	Dulbecco's minimal essential medium
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
FITC	fluorescein isothiocyanate
GF	growth factor
HAT	histone acetyl transferase
HBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HDAC	histone deacetylase
HMT	histone methyl transferase
HOBT	1-hydroxybenzotriazole
HRMS	high-resolution mass spectrometry
HRP	horse-raddish peroxidase
Hz	Hertz
IR	infra-red
LHMDS	lithium hexamethyldisilazide
LRMS	low-resoluition mass spectrometry
MAPK	mitogen-activated protein kinase
MeOH	methanol
mp	melting point
NAD^+	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
NBS	*N*-bromosuccinnimide
NFF	neonatal forskin fibroblast
NLS	nuclear-locating signal
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NO	nitric oxide
PARP	poly(ADP-ribose) polymerase
PBS	phosphate buffered solution

PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PI	propidium iodide
^{i}PrOH	2-propanol
Rb	retinoblastoma
SAHA	suberoylanilide hydroxamic acid
SAR	structure-activity relationship
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SRB	sulphorhodamine B
TCCA	trichloroisocyanuric acid
TCP	2,4,6-trichlorophenol
TEMPO	2,2,6,6,-tetramethylpiperidin-1-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TNF	tumour necrosis factor
Trt	trityl
TSA	trichostatin A

1 Introduction

1.1 Objectives

This thesis focuses on inhibitors of histone deacetylase enzymes, and their potential for use as anti-cancer drugs. Observed effects of compounds of this class on cellular proliferation and apoptosis have led to their identification as a promising new target for cancer therapy. The field is already bearing fruit, with the first histone deacetylase inhibitor recently having successfully completed clinical trials and being approved for use in the United States.[1] This chapter commences with an overview of cancer and the role of the enzymes in question, and proceeds to a review of currently known inhibitors.

1.2 Cancer: The Disease

Cancer is the broad term used to describe the diseases that result from unregulated cellular proliferation. When cells cease to be controlled by the regulatory circuits that bring order to 'normal' cell growth they can acquire the ability to form neoplastic tissue masses, referred to as tumours. If further loss of regulation occurs, tumour cells may also attain the ability to invade remote sites in the body, to metastasise, forming secondary tumours where the cells are deposited by the vascular and or endocrine systems. Not all neoplastic tissue is cancerous; tumours may be benign or malignant. Whilst both arise from a loss of control of cellular proliferation, benign tumours, such as warts, retain control of differentiation and may not transfer to different parts of the body.

Malignant neoplastic tissue will commonly display five key characteristics: increased rate of proliferation, development of pleomorphisms (mixtures of differently differentiated cell types), disturbance of cellular arrangement, invasion of adjacent tissues and formation of secondary tumours or metastases. In some instances 'normal' cells display all of these characteristics, such as the implantation of a fertilised egg in the uterine wall, however these

events are tightly controlled. Although the development of cancers may appear rapid due to the exponential growth of tumours, formation of a tumour from a single cell may take decades.

The original tissue type in which a cancer develops gives the disease its name, for example, carcinoma for epithelial tissue, sarcoma for connective tissue or lymphoma for lymphatic cells. It has been suggested that in order to generate the common characteristics of all cancers there are six essential alterations in cell physiology that must occur.[2] These combined alterations, irrespective of how they arise, are necessary and sufficient to dictate malignant growth. These changes that must occur for a cell to become transformed are: self sufficiency from growth signals, resistance to growth-inhibition signals, evasion of programmed cell death (apoptosis), unlimited replication potential, sustained angiogenesis and tissue invasion and metastasis.[2]

1.3 Requirements for Cancer

We shall now look in more detail at the mechanisms by which the six key alterations mentioned above may be effected.

1.3.1 Self Sufficiency in Growth Signals

It is possible for a cell to become self sufficient in growth signals in three ways: (i) excess production of growth factors (GFs), (ii) aberrant activity of the cell surface receptors for these signals or (iii) of the complex signal transduction pathways for these stimuli.[2]

The effect of most GFs is regulated under normal conditions through their expression by one tissue type to act on another. If a cell is able to produce a GF that stimulates itself, it may become independent of the supply from other tissues. An alternative to self-expression of GFs could be over expression of the cell surface receptor for that GF, allowing the cell to become hyper sensitive to normal GF concentrations. Finally, over expression or activity of any of the proteins in the pathway that transmits the extracellular signal may lead to the same effect.

1.3.2 Resistance to Growth-Inhibition Signals

Normal inhibition of growth can be mediated through proteins that block entry into new phases of the cell cycle. Two such proteins, the products of the retinoblastoma (Rb) and TP53 tumour-suppressor genes, are the proteins pRb and p53. Until the correct level growth signals have been reached, pRb will prevent progression of the cell cycle. Activation of p53 by cellular stress (such as DNA damage) also acts to prevent potential replication of damaged cells. Mutations or deletions of the TP53 gene occur in 50% of all cancers.[3] It is not surprising, with these crucial roles, that loss of activity of either of these proteins can have dramatic effects on the control of proliferation.

1.3.3 Evasion of Apoptosis

It is a crucial ability of most, if not all animal cells, to be able to undergo a process of controlled suicide - apoptosis. Apoptosis can be triggered as a result of cellular stress such as DNA damage (in this case mediated through the activity of p53). Through this process a single cell could be sacrificed on detection of DNA damage as a means of preventing propagation of any potential mutations. In contrast to necrotic cell death, apoptosis is a highly ordered process. Once initiated, the cell begins to shrink as the cytoskeleton and organelles are degraded. The nucleus fragments as chromatin is broken down and eventually the cell breaks into separate vesicles which are phagocytosed by neighbouring cells.

Upregulation of anti-apoptotic genes and loss of activity of pro-apoptotic factors (including p53) have been identified in several cancers as a way of avoiding this barrier to tumour formation.

1.3.4 Immortalisation

In the event of a cell escaping the normal proliferation control and avoiding apoptosis, its potential for tumour formation is still limited. Under normal circumstances, cells are not able to continue dividing indefinitely: observation of human cells in culture has shown that typically a cell is capable of only 60 to 70 divisions. The method by which a cell ages, or senesces, has been identified as shortening of chromosome ends with each round of cell division. The end of a normal chromosome consists of a six base sequence, TTAGG,

repeated several thousand times forming a structure known as a telomere, protecting against unwanted fusion of chromosome ends. Under normal circumstances the shortening telomeres of an ageing cell are interpreted by p53 as DNA damage, leading to apoptosis. Almost all tumours avoid this process and achieve an unlimited replicative potential by actively maintaining telomeres through the aberrant activity of telomerase – an enzyme capable of extending the hexanucleotide repeats.

1.3.5 Sustained Angiogenesis

In order for a small collection of cancer cells to develop into a tumour greater than one or two millimetres in diameter the cells must increase the supply of oxygen and nutrients. This is achieved through vascularisation of the tumour by invasion of new endothelial cells towards the tumour to produce new capillaries. Angiogenesis – the process of new blood vessel growth – is tightly controlled, and this restriction must be bypassed if the tumour is to continue to expand. The process can be viewed as having four stages:

(i) Initiation – "angiogenic switch"[2] triggered by action of pro-angiogenic factors. This can be either by tumour cells directly, or by surrounding cells as stimulated by the anaerobic environment created by the tumour.

(ii) Progression – degradation and remodelling of the extracellular matrix to promote survival.

(iii) Differentiation – attraction of endothelial cells and re-establishment of cell-cell contacts to form the luminal structure.

(iv) Maturation into functional blood vessels.

In addition to providing the necessary oxygen and nutrient supply, vascularisation of a tumour allows release of cancer cells into the circulatory system, providing a possible route to metastasis.

1.3.6 Tissue Invasion and Metastasis

The formation of secondary tumours is a part of the development of most cancers, and it is these metastases that are responsible for the vast majority of human cancer deaths. Migration of tumour cells into body cavity fluid, the vascular or the lymphatic system provides a mechanism of colonising distant sites in the body. The frequency of metastasis is

very low – invading tumour cells must contend with the normal immune response and the process itself is extremely inefficient. For metastatic tumour to develop, ten key steps must be achieved: progressive growth of the primary tumour, penetration of the basement membrane, vascularisation, entry of tumour cells into vasculature, migration and survival in lymph or blood, arrest in distant capillary bed, exit of vasculature and disruption by mechanical expansion, migration to parenchyma of new organ and progressive growth from one cell to over 1×10^9 cells.

These six capabilities provide the basic requirements for a tumour cell to transform and fully develop the disease. Several genes have been mentioned which are commonly found to be abnormally overactive or inactive (oncogenes and tumour-suppressor genes respectively) and these genes have traditionally been the focus of studies on genetic mutations that produce cancer. It is now known, however, that 50% of the mutated genes that cause inherited forms of cancer can be silenced by epigenetic mechanisms.[4] An explanation of epigenetic control of gene expression and its implications shall follow.
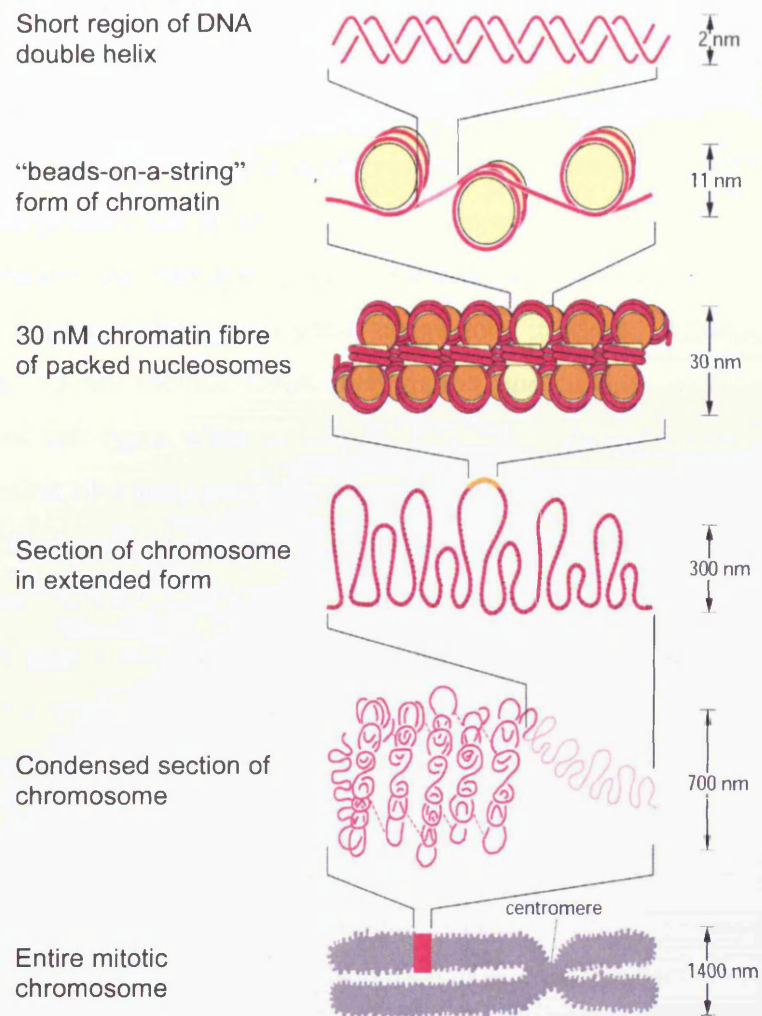
1.4 Histones, Chromatin and the Availability of Genes

It is hardly necessary to state that an enormous amount of information is required in order to produce a living human entity. The human genome is currently thought to contain 20-25,000 protein coding genes, encoded by 2.85 billion nucleotides.[5] This information in its entirety is stored within the nucleus of virtually every cell of the organism. It is not surprising, therefore, that an astoundingly complex system exists for not only storage of this information, but for retrieval of the coding necessary at any given time and place.

1.4.1 The Role of the Histone

Fully extended, the longest human DNA molecules would reach over 8 cm in length – a fact that demonstrates the achievement of normal DNA condensation. Nuclear DNA in its chromosomal conformation, termed chromatin, exists in a protein-DNA complex of approximately equal proportions by weight. Of these proteins, histones constitute the vast majority and are involved at the first level of DNA organisation. Histones are small, basic

proteins consisting of ~25% lysine and arginine residues which, through their protonated side-chains, provide a means of interaction with the negatively charged phosphate backbone of the DNA double helix.[6] Of the five main types of histones; H1, H2A, H2B, H3 and H4, all but H1 are required for nucleosome formation. The nucleosome is the basic repeating unit of chromatin and consists of a core histone-octamer, consisting of two copies each of histones H2A, H2B, H3 and H4, which provide a spool for DNA to coil around. The DNA passes around this core 1.65 times,[7] with linker regions between nucleosomes producing a 'beads-on-a-string' effect (figure 1.1).[8] With the aid of histone H1 (an example of a 'linker histone', so called because of its interaction with the linking DNA regions), this structure coils on itself to form a 30 nm fibre. The fibre is then arranged in loops radiating from a central protein scaffold, to produce the recognisable chromosome structure. The extent of chromosomal condensation is not fixed, and the maximum density occurs during the metaphase of a cell's mitotic division, when the largest chromosome is reduced to ~10 μm in length. In this extreme conformation there is no access for the transcriptional apparatus to DNA, and so this ultra efficient form of packaging is not adopted during interphase.

Short region of DNA
double helix

2 nm

"beads-on-a-string"
form of chromatin

11 nm

30 nM chromatin fibre
of packed nucleosomes

30 nm

Section of chromosome
in extended form

300 nm

Condensed section of
chromosome

700 nm

centromere

Entire mitotic
chromosome

1400 nm

Net result: each DNA molecule has been packaged into a mitotic
chromosome that is 50,000x shorter than its extended length

Figure 1.1. DNA Condensation.[8]

Although it is not possible for chromosomes to be fully condensed all of the time, it is also not necessary for an entire chromosome to be accessible for transcription, even during interphase. Every human cell (excluding erythrocytes) contains at least one copy of the genome in its entirety, however, not all genes are required for expression in any single cell. A cell's identity, and therefore its role, is defined by expression of a specific combination of genes, and so not only is it unnecessary to have transcriptional access to every gene, it is potentially dangerous. This is highlighted by the effects of aberrant expression of oncogenes,

the products of which are often required only in embryonic development, or, conversely, the repression of tumour suppressor genes such as p53 and pRb (discussed in the previous section). It is becoming increasingly clear that post-translational modification of histone proteins has profound effects on the conformation of chromosomal DNA, and therefore, a cell's expression profile. On a large scale, regions of the chromosome may be rendered accessible or inaccessible through epigenetic events. Epigenetic events can be defined as 'reversible but heritable changes in gene expression, produced without alteration of the DNA sequence.'[9] They include DNA methylation and histone modification, and allow differentiation of cell types when essentially all cells contain the same information - the genome. Formation of transcriptionally unavailable heterochromatin is a complex process, requiring the activity of histone-modifying enzymes, chromatin-remodeling proteins, DNA methyltransferases and RNA-directed silencing complexes in addition to other architectural proteins.[10] With such a broad range of control mechanisms, it is sadly not within the scope of this thesis to consider them all, and it is the post-translational modification of histones that will provide the focus from here on.

1.4.2 Histone Post-Translational Modifications

The high proportion of lysine and arginine residues in these proteins render histones particularly susceptible to a variety of post-translational modifications. The extent of these modifications vary, but include acetylation of lysine residues by histone acetyltransferases (HATs), methylation of lysine and arginine residues by histone methyltransferases (HMTs), serine phosphorylation by serine kinases, ubiquitination by ubiquitin ligases, poly ADP-ribosylation by poly ADP-ribose polymerase and sumoylation of lysine by the small ubiquitin-like modifier (SUMO). It should be noted that all known covalent histone modifications are reversible, and the enzymes responsible for attachment and removal of the modifying groups are often present at the same time.[7] These competing reactions are achieved through the action of enzymes such as histone deacetylases (HDACs) (scheme 1.1), methylases, phosphatases and proteases.
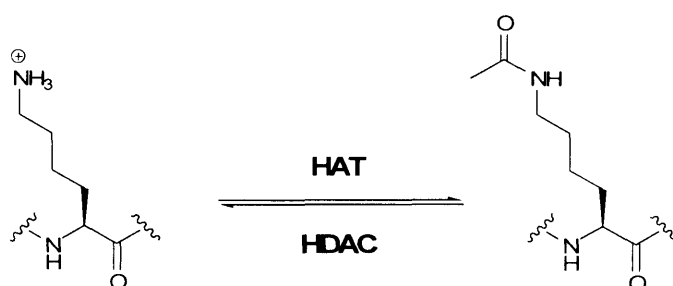
Scheme 1.1. Opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) on lysine acetylation.

There are three key ways in which histone modifications are thought to affect chromatin structure; first, modifications such as lysine acetylation and methylation alter the charge of the amino acid residue (scheme 1.1), thereby altering its electrostatic interaction with the negatively charged phosphate backbone of the DNA. This original hypothesis would suggest that the degree of acetylation of lysine residues would produce a cumulative effect to bring about large conformational changes. Second, modification can affect the recruitment of DNA binding factors, thereby indirectly eliciting an effect. This is analogous to the effect of phosphorylation on many protein-protein interactions and implies, for example, that acetylation of a single specific residue could create a new binding surface sufficient to recruit another protein.[11] One protein structure capable of responding to this is the bromo domain, which recognises acetylated lysine residues. This domain has been identified through sequence analysis as being potentially present in more than 72 human proteins.[12] Consideration of this mode of action has led to the hypothesis of an epigenetic code,[13] whereby individual modifications or combinations thereof (in conjunction with DNA methylation) mediate a variety of nuclear functions through their sequence-specific recognition by specific domains contained in effector proteins.[14] The third possible mechanism is through modification affecting nucleosome-nucleosome interactions, and therefore, higher order chromatin structure.[7]

Of the modifications described above, acetylation is the best characterised – several families of proteins have been identified as histone acetyltransferases (HATs) each displaying different specificities.[14] Many proteins identified as possessing HAT activity had been

previously known as transcriptional regulators, with diverse functions such as control of cell cycle, differentiation and apoptosis.[12] A characteristic of this group of enzymes is that they function as transcriptional co-activators, binding to DNA-binding proteins (as opposed to directly binding DNA itself). On recruitment to promoter regions by these DNA-binding proteins, HATs are then able to exert their catalytic effects as components of large, multi-subunit complexes.[15] The enzymes catalyse the acetyl coenzyme A-dependent acetylation of the ε-amino groups of lysine residues (scheme 1.1). Unsurprisingly, considering their key roles in cellular processes, aberrant activity of HATs has been observed in cancer.[12] Traditionally, the stable hyperacetylation of histones has been considered a characteristic of transcriptionally active genes, however it is now though possible that rapid turnover of acetylation/deacetylation could be more important for transcription.[11] Histones are not the only substrates of these enzymes, and other targets include general transcription factors and nuclear proteins,[16] further complicating a precise description of their role.

1.5 The Histone Deacetylases

The four classes of mammalian proteins that have so far been identified as possessing HDAC activity are distinguished by their structural homology to yeast HDACs.[17] Class I are most closely related to the *S. cerivisiae* transcriptional regulator Rpd3 (reduced potassium dependency) and includes HDACs 1, 2, 3 and 8. Members of class II, including HDACs 4, 5, 6, 7, 9 and 10, are homologous to yeast deacetylase Hda1. The sole member of class IV is HDAC11, which does not have sufficient sequence similarity to be considered a member of class I or II. All of these enzymes contain a highly conserved catalytic domain, however the members of class II are typically two to three times larger in size than the class I proteins.[18] The third class of HDACs, the sirtuins (related to yeast transcriptional repressor Sir2) are evolutionarily distinct, requiring the presence of NAD^+ as a cofactor for enzymatic activity. Class III HDAC's alternative mechanism of action allows them to remain unaffected by the majority of HDAC inhibitors currently under development, and so this class will not be included in the following discussion.

HDACs function almost exclusively in multi-protein complexes; most recombinantly expressed HDACs (with the exception of HDAC8) will not function in the absence of other cofactors.[19] The complexes generally include proteins which influence recruitment, DNA-binding and chromatin re-modelling. Further cellular regulation of HDAC activity exists at the levels of gene expression, proteolytic processing and post-translational modification.

Active HDACs 1 and 2 are localised exclusively to the nucleus and have been identified in three different protein complexes: Sin3, NuRD (nucleosome remodelling and deacetylating, also known as Mi2) and CoREST.[18] Recombinantly expressed versions of these two enzymes are inactive alone and require the other members of the complex for DNA-binding, recruitment and modulation of deacetylase activity. Both HDACs 1 and 2 interact with the previously mentioned tumour-suppressor protein pRb.[20] HDAC3 is capable of leaving the nucleus owing to its possession of both nuclear localisation signal (NLS) and nuclear export signal (NES) sequences. HDAC8 is present in the nucleus, however only in very low abundance.

Class II HDACs are more able to shuttle between the nucleus and cytoplasm; HDACs 4, 5 and 7 are exported from the nucleus on phosphorylation.[19] HDAC6 is found predominantly in the cytoplasm and is thought to play a role in cell motility owing to its activity as a tubulin deacetylase.

The first structural information on histone deacetylases came from crystallographic studies of a HDAC homologue, HDLP, from the hyperthermophilic bacterium *Aquifex aeolicus*.[21] The enzyme shares 35.2% homology with human HDAC1, and in addition to the pure enzyme, crystal structures of HDLP complexed with the HDAC inhibitors trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) (figure 1.3) were elucidated, suggesting mechanistic information on the enzyme-catalysed reaction (figure 1.2), which shows characteristic elements of both metallo- and serine-proteases. The crystal structure showed a tubular pocket approximately 11 Å deep and ~4.5 Å across at its narrowest point, where the aromatic rings of two phenylalanine residues face each other across the tunnel.
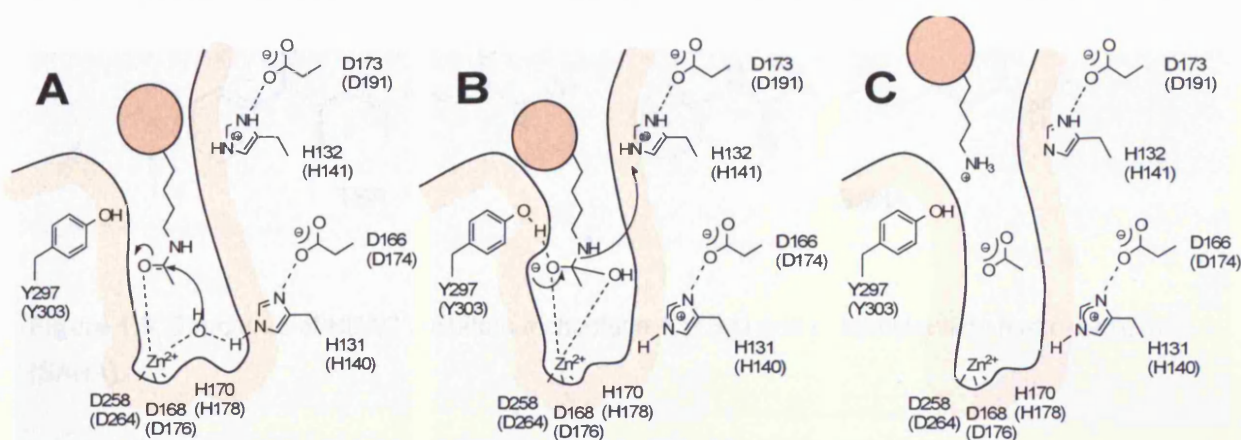
Figure 1.2. Proposed mechanism for deacetylation of acetylated Lys by HDLP. Active site residues (and HDAC1 counterparts) are labelled.[21]

The pocket is lined with hydrophobic and aromatic residues, with a zinc ion and water molecule present near the base of the cavity. No deacetylase activity was seen without incubation of the purified enzyme with zinc chloride (or a cobalt substitute) showing the ion is a necessary component of the catalytic site. A charge-relay system that is characteristic of serine proteases is present in the pocket: two histidines (H132 and H131, figure 1.2) each hydrogen-bond with an aspartic acid residue (D173 and D166 respectively), thereby polarising the imidazole ring and increasing its basicity. The lower of these two histidine residues, H131, hydrogen-bonds with the water molecule, which is also bonding with the zinc ion. Another structural feature of the enzyme is a second cavity adjacent to the pocket. The cavity is composed of hydrophobic residues and its function is not clear.

The crystal structure of the enzyme bound to the highly potent hydroxamic acid inhibitor TSA (figure 1.3) shows the aliphatic chain penetrating the pocket, projecting the hydroxamic acid moiety into the proximity of the zinc ion and making numerous contacts with the hydrophobic residues lining the tube. The length of the chain allows coordination of the hydroxamic acid with the zinc ion and also interaction of the aromatic ring with residues at the pocket entrance, as shown schematically in figure 1.4.
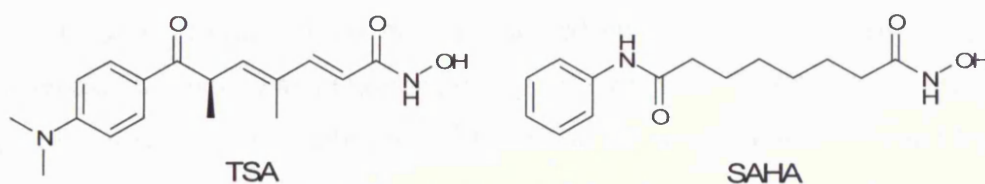
Figure 1.3. Structures of HDAC inhibitors trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA).
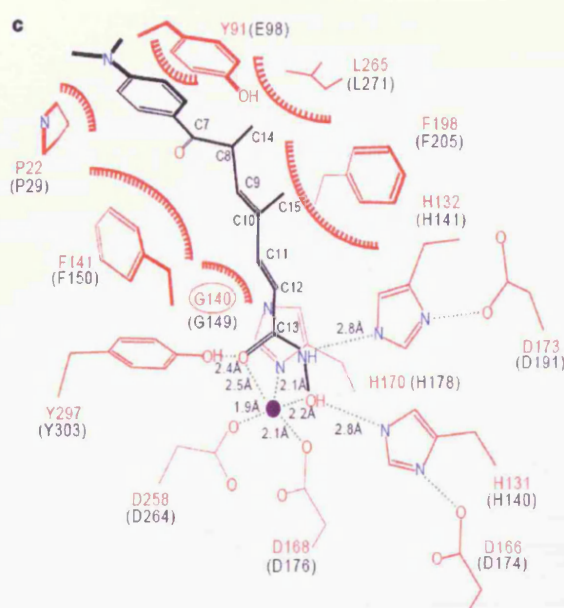

Figure 1.4. Schematic representation of HDLP-TSA interactions (HDLP residues are labelled in red with HDAC1 counterparts indicated in black).[21]

Coordination of the carbonyl and hydroxyl oxygens of the hydroxamic acid result in a pentavalent zinc ion, showing similarities with the binding of hydroxamic acids to zinc metalloproteases such as MMP-1.[22] Binding of SAHA is in a similar fashion, however, several structural differences may help to explain SAHA's significantly lower inhibitory capacity. The unbranched aliphatic chain of SAHA fits the pocket less well and is more flexible than the TSA chain, resulting in fewer van der Waals interactions with residues lining the pocket. Also, the phenyl group of SAHA has a lower electron density, suggesting it packs less well at the cap region.

Further structural information has been elucidated on human HDACs by the publication (simultaneously by two separate groups) of crystal structures of HDAC8 complexed with a variety of hydroxamic acid inhibitors.[23,24] Based on the structure of the bound hydroxamic acid, Somoza et al. propose a mechanism of acetylated lysine binding (figure 1.5) that is similar to that proposed by Finnin et al. given in figure 1.2.
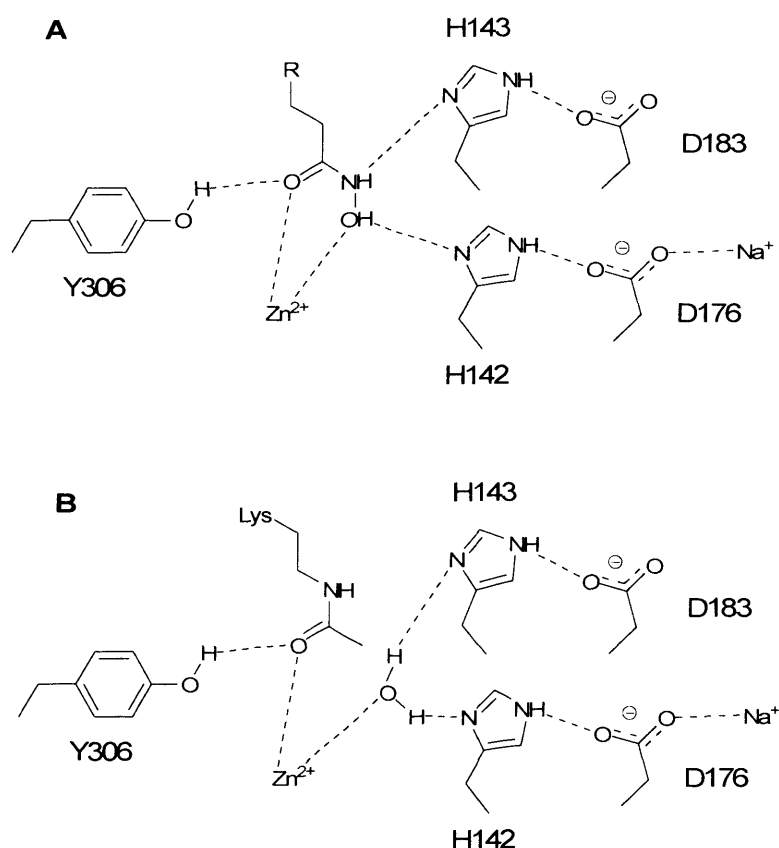

Figure 1.5. A. Schematic representation of binding of a hydroxamic acid with human HDAC8. **B.** Proposed binding of acetyl Lys and water.[24]

With the current understanding of the structure and mechanism of histone deacetylases thus summarised, it is useful to take a step back and review the basic principles of enzyme kinetics before moving on to discussion of the wide variety of HDAC inhibitors under development.

1.6 Enzyme Inhibition

At the most fundamental level, enzymes reduce the free energy (ΔG) required for chemical reaction by providing an alternative pathway to the uncatalysed reaction with a lower energy transition state (figure 1.6). The Gibbs free energy of activation, denoted as $\Delta G^{\ddagger}$, is the increase in energy required to convert the substrate to the high energy transition state.
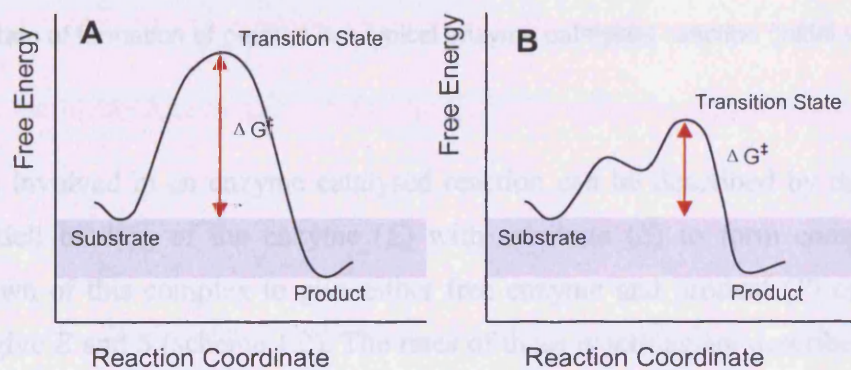

Figure 1.6. Free energy profiles of **A**: an uncatalysed reaction and **B**: a catalysed reaction.[25]

The overall rate of an enzyme catalysed reaction is described as its velocity, and the initial velocity, V_0, produced before consumption of 10% of the substrate (S) is the maximum value (figure 1.7). The velocity then begins to decrease as substrate concentration ([S]) decreases and product concentration ([P]) increases. The rate is typically dependent on three key factors: [S], temperature and pH. Increasing [S] will increase reaction rate until [S] reaches the enzymes saturation point, at which point increasing [S] will no longer increase the rate, and the reaction becomes dependent on enzyme concentration [E]. The enzyme will operate at an optimum temperature, typically close to 37 °C. Reaction rate will tend to increase fairly steadily until the optimum temperature is reached then drop rapidly when exceeded owing to denaturating of the protein. Similarly, an enzyme will have an optimum pH range.
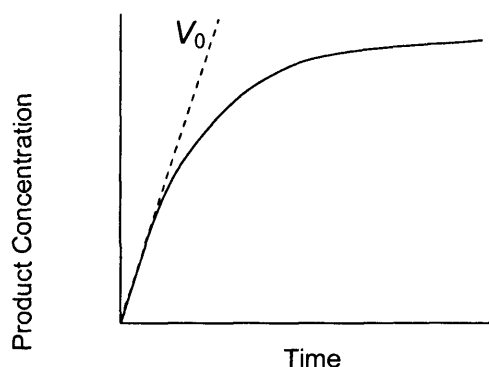
Figure 1.7. Rate of formation of product in a typical enzyme catalysed reaction (initial velocity, V_0, indicated).

The process involved in an enzyme catalysed reaction can be described by the Michaelis-Menten model: binding of the enzyme (E) with substrate (S) to form complex ES and breaking down of this complex to give either free enzyme and product (P) or the reverse reaction to give E and S (scheme 1.2). The rates of these reactions are described as k_{+1}, k_{+2}, and k_{-1} respectively and the overall reaction is considered irreversible as conversion of P to back to ES is assumed to be at an insignificant rate.

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \overset{k_{+2}}{\longrightarrow} E + P$$
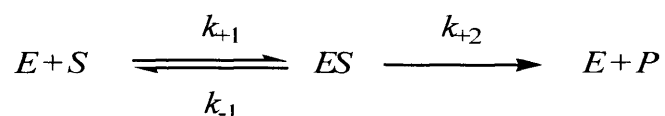
Scheme 1.2. Stages of enzyme catalysed reactions.

There are two stages at which the kinetics of a reaction can be studied: (i) the pre-steady state, which concerns the very first round of reaction on contact of E with S and (ii) the steady-state. The steady-state concerns turnover of multiple rounds of reaction when S in present in excess, and so this is the case over the majority of the course of a reaction provided it is not inhibited by formation of P. Under these conditions the concentration of ES ($[ES]$) remains constant and it is possible to describe the relationship between $[S]$, V_0, maximum velocity (V_{max}) and a constant, K_m, derived from the rate constants shown in scheme 1.2.

Calculation of V_0 at a range of values for $[S]$, as in figure 1.8, shows that V_0 is directly proportional to $[S]$ at low concentrations, whereas at high $[S]$, it tends towards a maximum value (V_{max}).
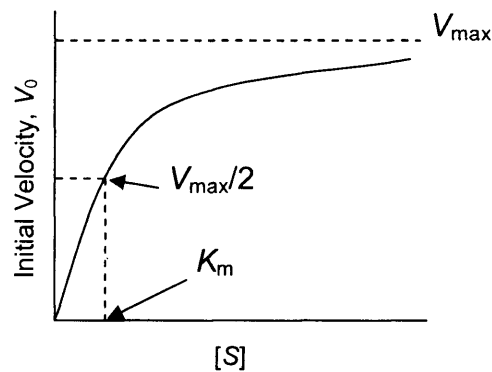

Figure 1.8. Relationship between $[S]$ and V_0.

Finally, these terms are combined in the Michaelis-Menten equation:

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

Equation 1.1. Michaelis-Menten

As V_{max} is only achieved at an infinite substrate concentration it is not possible to determine this factor from the hyperbolic plot of $[S]$ against V_0 (figure 1.8). Manipulation of the data to produce a double reciprocal plot, known as the Lineweaver-Burke plot (equation 1.2, figure 1.9), allows calculation of V_{max} from experimental values.

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

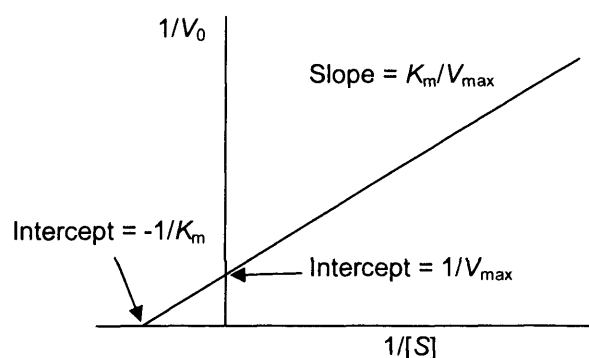**Equation 1.2.** Double reciprocal of the Michaelis-Menten equation.

Figure 1.9. Lineweaver-Burk Plot allowing calculation of V_{max} and K_m.

Information about the mechanism of an enzyme inhibitor may be obtained by study of enzyme kinetics in this way. Any compound that reduces the rate of an enzyme catalysed reaction can be called an inhibitor, and these compounds can be divided into two main groups; reversible and irreversible inhibitors. Irreversible inhibitors cause permanent loss of activity, generally by covalently bonding to the enzymes active site. Reversible inhibitors have traditionally been divided into three sub-classes: (i) non-competitive, when the degree of inhibition is unaffected by change in [S], (ii) competitive, when inhibition is reduced by increasing [S] and (iii) un-competitive, where the reverse is true, and inhibition increases with increasing [S].[26] Lineweaver-Burk plots of reactions in the presence of these types of inhibitors show characteristic differences (figure 1.10).
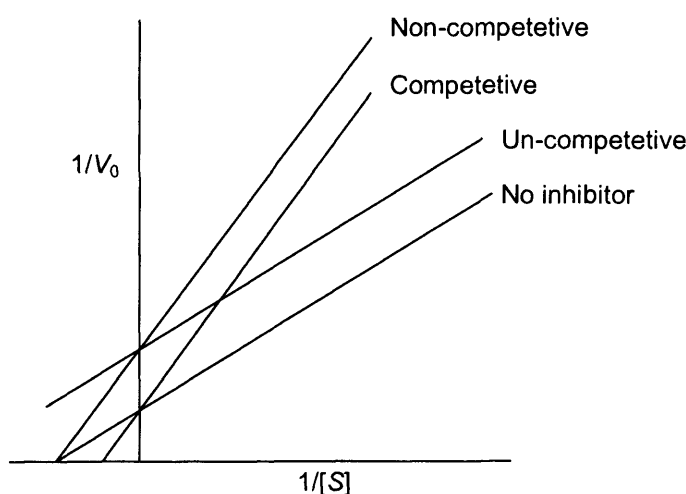
Figure 1.10. Comparison of Lineweaver-Burk plots in the presence of classical non-competitive, competitive and un-competitive inhibitors.

With the background topics including the molecular basis of cancer, histone acetylation/deacetylation, HDAC structure and enzyme kinetics now reviewed, the focus of this introduction now comes to the use of HDAC inhibitors as therapeutic agents. Before considering in detail the wide ranging structural characteristics of current HDAC inhibitors an overview of the biochemical effects of this class of compounds shall be given.

1.7 Cellular Effects of HDAC Inhibition

By considering the six characteristic requirements for development of cancer (self sufficiency in growth signals, resistance to growth inhibition signals, evasion of apoptosis, immortalisation, sustained angiogenesis and invasion and metastasis, discussed in chapter 1.3) the complex anti-tumour action of HDAC inhibitors can be begun to be understood. As has previously been mentioned, the balanced actions of HATs and HDACs in their respective acetylation and deacetylation of various proteins plays a key role in regulation of transcription. Gene profiling studies have shown that treatment of cells with HDAC inhibitors leads to alteration in expression of 2-5% of expressed genes in malignant cells,[27] with similar proportions of these genes being activated and repressed. It is important to note

that among this small percentage of genes are key regulators of all six properties required for cancer, some of which are listed in table 1.1.[28]

Oncogenes Down-Regulated by HDAC Inhibitors

TGFβ	Regulator of cell proliferation and differentiation
Thioredoxin	Cytokine capable of inhibiting apoptosis
Telomerase	Prevents telomer erosion
VEGF	Angiogenic factor
cyclins A, B and D	Cell cycle regulators

Tumour-Suppressor Genes Activated by HDAC Inhibitors

p53	Pro-apoptotic
Fas/FasL	Pro-apoptotic
p21$^{WAF1/Cip1}$	Cell cycle regulator
p27^{Kip1}	Cell cycle regulator

Table 1.1. Some tumour-associated proteins transcriptionally affected by HDAC inhibitors.[28]

It has been suggested that the reason for only a relatively small subset of genes to be affected by the induced global increase in histone acetylation is due to the effect this change has on histone and DNA methylation, which exerts a dominant effect over histone acetylation.[29] A summary of the currently understood functions of HDAC inhibitors in cancer therapy is given in table 1.2.

Inhibition of Cell Proliferation

Augmentation of nuclear receptor response driving terminal cell differentiation

Reversal of repression by fusion transcription factors or over-expressed repressors

Induction of p21, G1 arrest and cellular differentiation

Re-activation of silenced tumour suppressor genes in combination with DNA methyltransferase inhibitors

Suppression of telomerase gene expression

Induction of Apoptosis

Activation of mitochondria-dependent apoptosis

Activation and/or sensitization of death-receptor mediated killing

Mitotic dysfunction, aberrant chromosomal segregation and DNA damage

Induction of topoisomerase II may alter sensitivity to DNA-damaging agents

Inhibition of Cancer Cell Migration, Invasion and Metastasis

Activation of suppressors of invasion and metastasis including TIMP1, KAI1 and RhoB

Suppression of promoters of migration, invasion and metastasis including TGFβ1, MTA1 and MMP2

Inhibition of Tumour-Driven Angiogenesis

Activation of suppressors including p53

Suppression of promoters including VEGF, VEGF receptor 1 and 2 and MMP2

Other Mechanisms

Alteration of microtubule function

Induction of MHC antigens on cell surface to augment immune response

Suppression of IL-2-mediated gene expression

Table 1.2. Summary of HDAC inhibitors' effects in cancer therapy.[29,27]

1.8 Known Inhibitors of Histone Deacetylase

Having now discussed the biological effects of HDAC inhibitors, the attention shall now turn to a review and classification of current molecules known to inhibit the enzyme. Many apparently structurally-diverse compounds fall within this field, however it is almost always possible to describe inhibitors with respect to a common pharmacophore model based on the

crystallographic studies of Finnin et al.[21] (discussed in chapter 1.5). The model divides inhibitors into three sections: a zinc-binding domain, a spacer domain and a capping domain (figure 1.11)
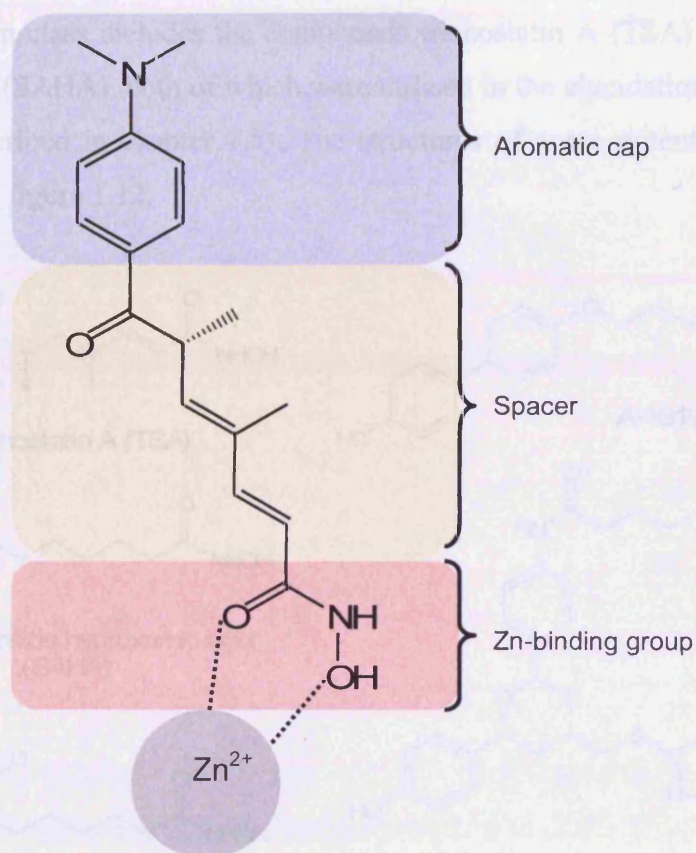

Figure 1.11. Pharmacophore model for HDAC inhibitors using TSA as an example.

It has been established that many chemical moieties are capable of functioning as the zinc-binding group, the most commonly used being a hydroxamic acid. The other known categories of inhibitors, including benzamides, carboxylates and macrocycles, will be discussed presently. The spacer region may take the form of saturated or unsaturated alkyl chains, or cyclic linkers and the cap regions are commonly hydrophobic, aromatic groups, however another major class of inhibitors contain macrocyclic domains. Much of the work done in establishing this pharmacophore is summarised in the excellent review by Miller et

al.[30] We shall commence the review with the hydroxamate inhibitors as this class provides the greatest number of examples.

1.8.1 Straight-Chain Hydroxamic Acids

The straight-chain class includes the compounds trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), both of which were utilised in the elucidation of HDAC catalytic mechanism (described in chapter 1.5). The structures of some potent compounds of this class are given in figure 1.12.
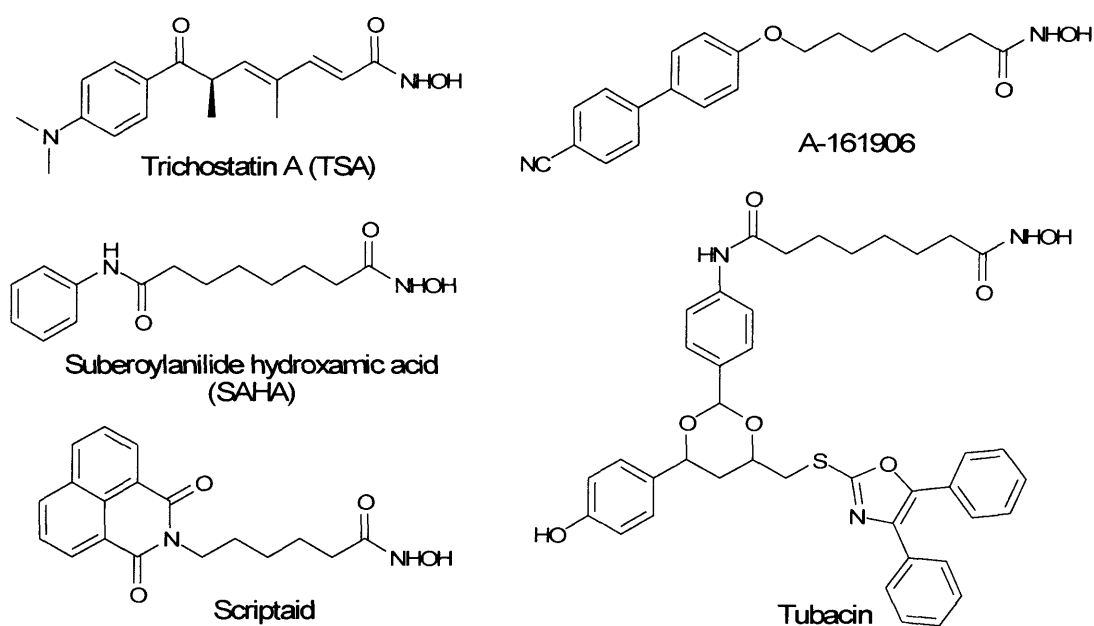

Figure 1.12 Examples of straight chain hydroxamate inhibitors.

The potent natural product TSA, isolated from *Streptomyces hygroscopicus*, was first identified as a non-competitive HDAC inhibitor by Yoshida and co-workers in 1990, with Ki = 3.4 nM.[31] As has previously been mentioned, the structurally related, unsaturated SAHA has a significantly lower inhibitory capacity, with activity at low micromolar concentrations.[32] Extensive work has been carried out to identify the structural requirements for TSA's potency. Synthesis of the un-natural (*S*)-TSA showed this enantiomer to be inactive.[33] Hybrid structures blending elements of these two compounds in the form of

amide TSA-analogues show the importance of TSA's unique chain (figure 1.13).[34] The fully saturated analogue **1a** shows an IC_{50} one tenth of the parent compound, methyl-substitution on the chain of **1b** reduces activity by a further half and unsaturation (**1c**) effectively destroys the inhibitory activity.
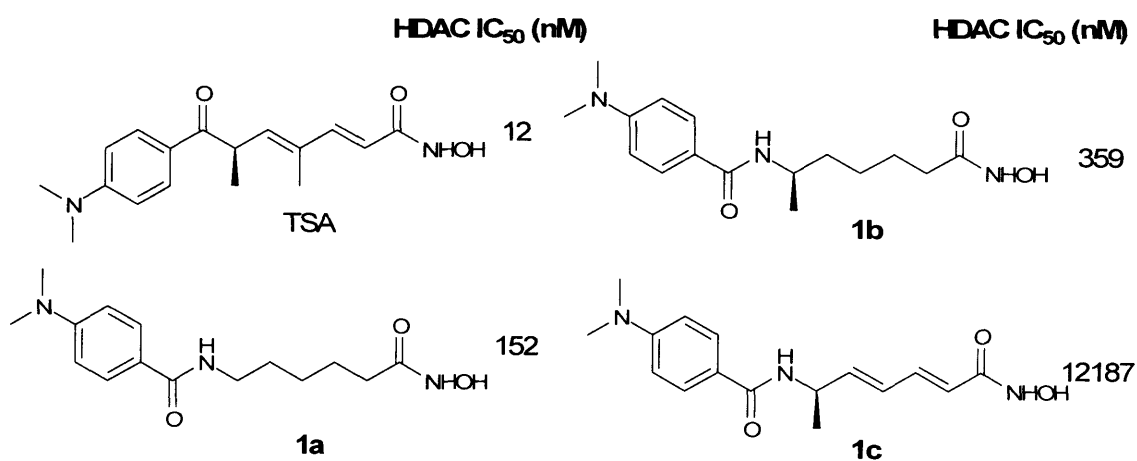

Figure 1.13. Amide analogues of TSA.[34]

Compound **1a** also featured in a structure-activity relationship (SAR) study of cap groups on the unsaturated amide analogue by Remiszewski et al. in 2002 (figure 1.14).[35]
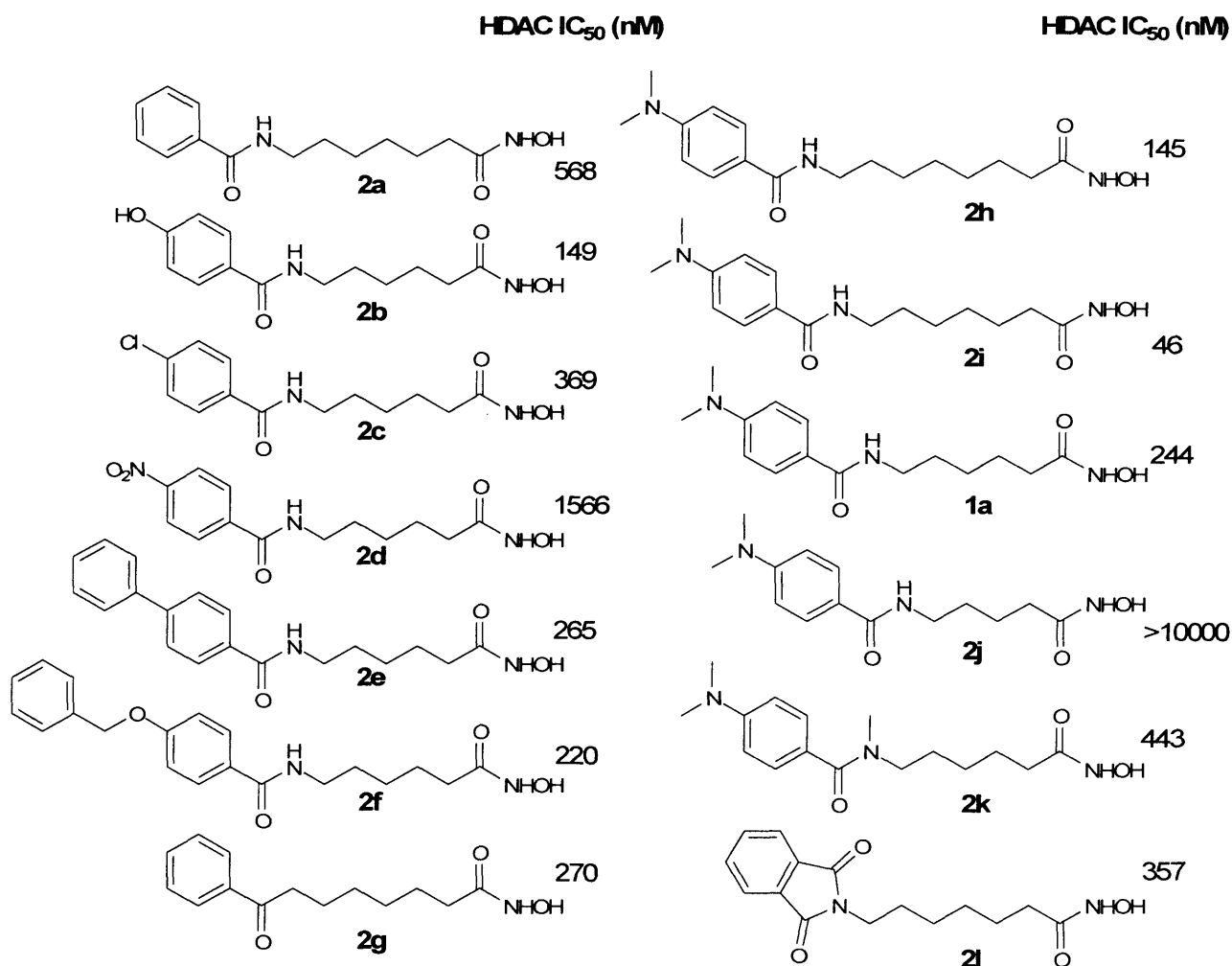
Figure 1.14. Further amide analogues of TSA.[35]

This study identified the longer chain length of six methylene units as being the optimal length in this case, the potency being markedly increased by inclusion of the 4-N,N-dimethyl cap (**2i**). Non-amide compound **2g**, with a longer chain than that of TSA, displayed a 20-fold reduction in potency. N-Methylation of the amide group (**2k**) reduced activity, however, annulation in the form of phthalimide **2l** produced an improvement compared with **2a**.

Another study centering on this class of inverted-amide SAHA analogues investigated the effect of heterocycles in the cap region, with a particular emphasis on indoles.[36] The

inclusion of electron-rich aromatic groups including indoles and a benzofuran showed remarkable effects on activity.
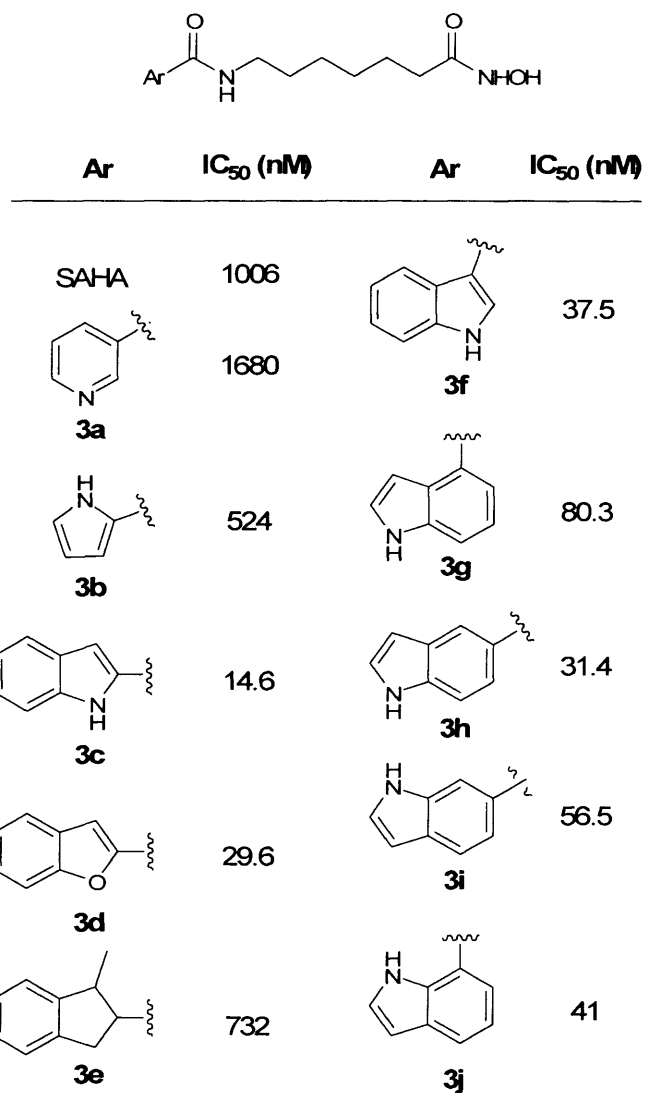

Ar	IC_{50} (nM)	Ar	IC_{50} (nM)
SAHA	1006	3f	37.5
3a	1680		
3b	524	3g	80.3
3c	14.6	3h	31.4
3d	29.6	3i	56.5
3e	732	3j	41

Figure 1.15. Development of indole amide SAHA analogues.[36]

Substitution of a pyrrole group for the phenyl group of SAHA in **3b** (figure 1.15) was found to be favourable and extension of this moiety into an indole functionality produced a ten-fold increase in activity. Attachment in the 2-position of **3c** led to an IC_{50} approaching the single-figure nanomolar range.

Departing from the amide-linked cap regions, inclusion of ketone **2g** in Remiszewski's series (figure 1.14) leads conveniently to further work around this unsaturated, unsubstituted analogue of TSA. The following series by Woo and co-workers (published shortly after Remiszewski's series of figure 1.14), given in figure 1.16, includes the structure **2g** and provides an extensive study on the chain length and cap region of this compound.[37] The study identifies the six-methylene linker to be the optimal length, with the most potent compounds possessing highly hydrophobic naphthyl or biphenyl cap groups. The methoxy-substituted phenyl cap groups of compounds **4h** and **4i** indicate that substitution in the 2-position of the former is detrimental, whereas the 4-position produces a favourable effect. Replacement of the ketone with an oxime appears to be well tolerated (**4n** and **4o**), as is methylene **4p**. Removal of the group altogether does not significantly affect potency, implying a degree of freedom in the pharmacophore at this point.

Substitution adjacent to the hydroxamic acid, as with the methyl group of **4u**, dramatically reduces the inhibitory capacity – a point seemingly confirmed in a recent study on the effect of substitution of SAHA in this position. The study showed inclusion of any group, ranging in bulk from methyl to benzyl, in this position significantly reduced activity.[38]
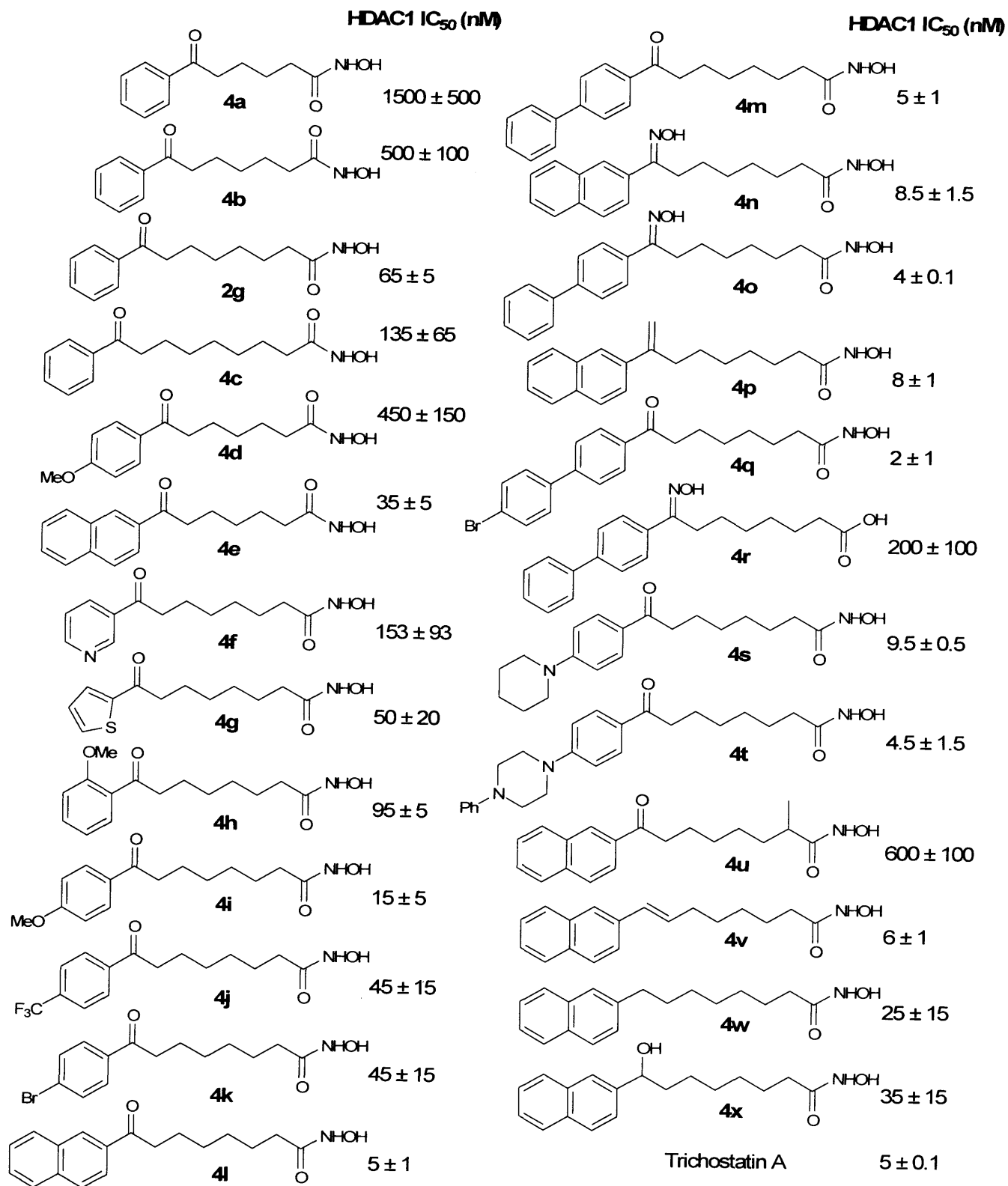
HDAC1 IC$_{50}$ (nM)   HDAC1 IC$_{50}$ (nM)

4a   1500 ± 500   **4m**   5 ± 1

4b   500 ± 100   **4n**   8.5 ± 1.5

2g   65 ± 5   **4o**   4 ± 0.1

4c   135 ± 65   **4p**   8 ± 1

4d   450 ± 150   **4q**   2 ± 1

4e   35 ± 5   **4r**   200 ± 100

4f   153 ± 93   **4s**   9.5 ± 0.5

4g   50 ± 20   **4t**   4.5 ± 1.5

4h   95 ± 5   **4u**   600 ± 100

4i   15 ± 5   **4v**   6 ± 1

4j   45 ± 15   **4w**   25 ± 15

4k   45 ± 15   **4x**   35 ± 15

4l   5 ± 1   Trichostatin A   5 ± 0.1

Figure 1.16. SAR of straight-chain analogues of TSA.[37]

A series of straight chain, polyunsaturated analogues of TSA synthesised by the Marson group[39] showed that good potency could be achieved with a shorter chain possessing no heteroatom at the join between chain and cap, as with the highly potent **4v** and **4w** above (figure 1.16). The examples given in figure 1.17 indicate that with the unsaturated, carbon-only linker a much shorter chain is tolerated than in the previous series (figure 1.16). The unsaturated chain of **5d** reduces activity by a factor of ten, and whilst *para*-chloro substitution is tolerated in **5b**, the *para*-dimethylamino group of **5c** greatly reduces activity. It may be surmised that the shorter chain of these compounds does not allow the amine to sit in the same position at the top of the enzymes active site as is occupied by the corresponding group in TSA. Additional bulk in the chain has little effect in the chloro example **5e**, but greatly improves activity with amine **5f** (although still considerably less active than **5e**).

HDAC IC$_{50}$ (nM)   HDAC IC$_{50}$ (nM)
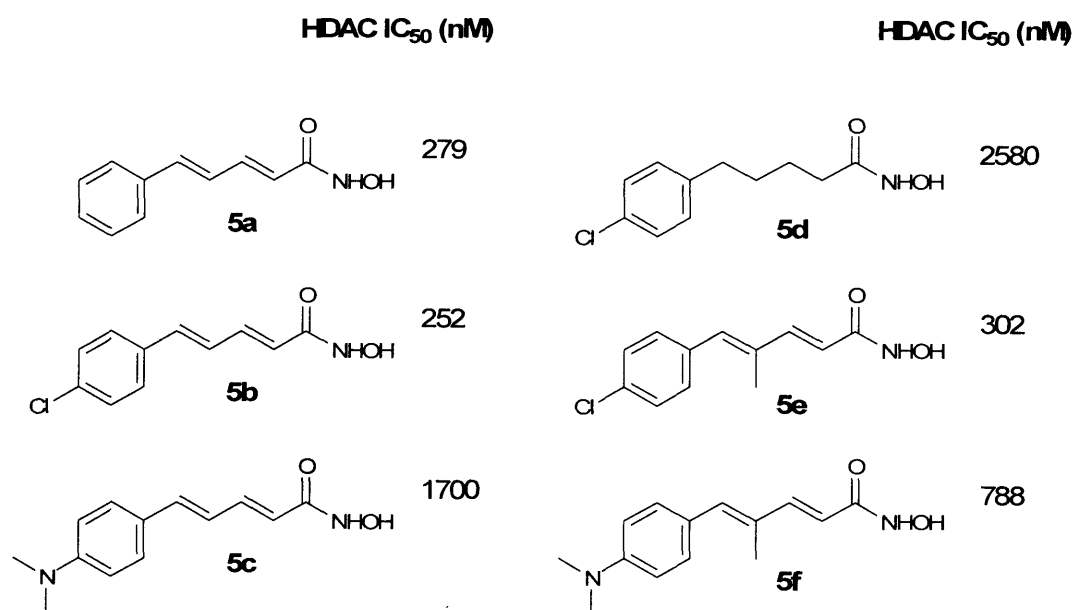
Figure 1.17. Polyunsaturated straight chain analogues of TSA.[39]

Conformationally constrained analogues of TSA were recently reported by Charrier and co-workers as potent inhibitors.[40] The tetralone, **6a**, and three indanone analogues **6b-d** depicted in figure 1.18 all retained moderate to good inhibitory activity, the indanone proving superior in the unsubstituted examples **6a** and **6b**. Substitution on the phenyl ring

increased activity, with the direct TSA analogue, bearing a dimethylamino moiety, **6d**, showing the greatest activity with a sub-micromolar IC_{50} in H661 cell assays.
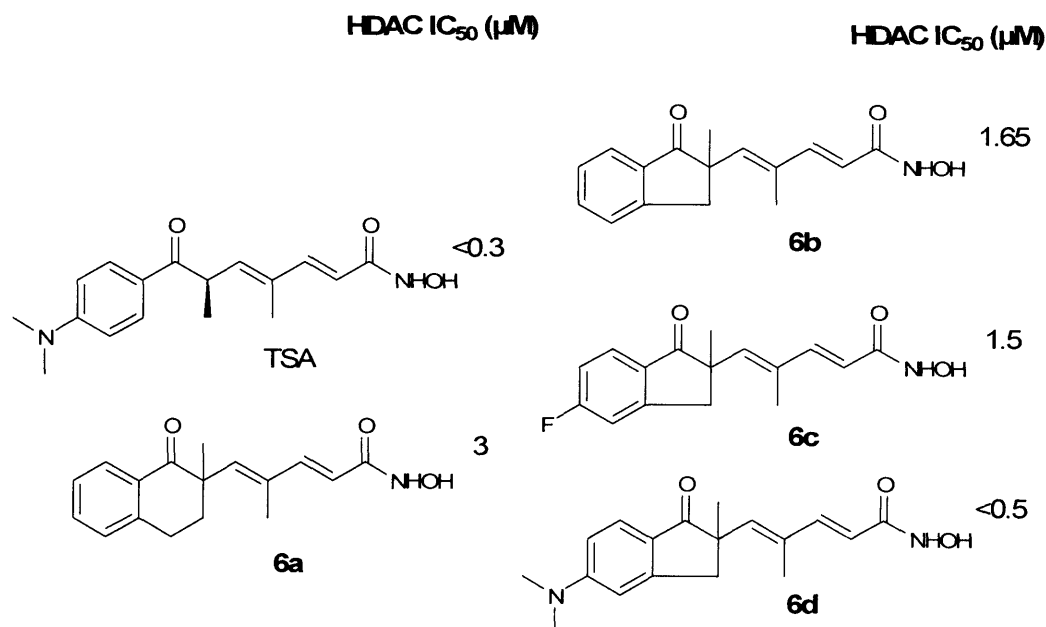
Figure 1.18. Rigid analogues of TSA.[40]

It should be noted that although the imposed rigidity at the joining region between cap and chain is tolerated in this case, albeit at some reduction in potency, the chain of TSA is already considerably restricted in its conformations owing to its unsaturation (despite potential rotation arising from 1,3-allyl steric clash0. If the rigid chain of TSA does confer favourable attributes when the molecule is docking in the pocket of the enzymes active site, it might be suggested that SAHA, with its fully saturated chain, would be an appropriate target for this type of conformational analysis. It may be the case that increasing the rigidity of the chain of SAHA in this way would produce more favourable effects.

The capacity of the attachment point of cap and chain to tolerate diverse chemical entities is further confirmed by a series of potent aromatic sulfur-linked compounds (figure 1.19), synthesised by the Marson group.[41]
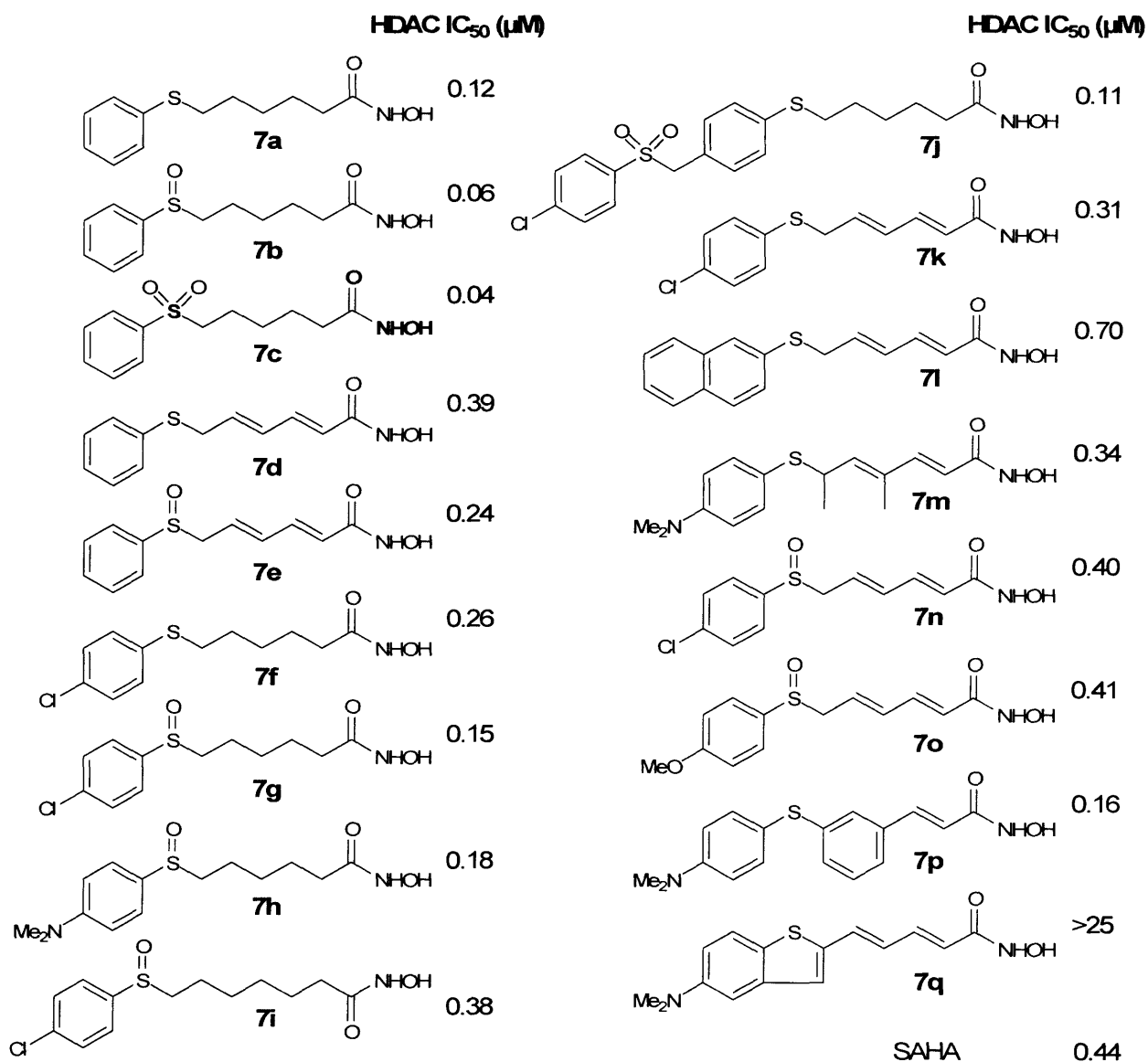
Figure 1.19. Aromatic sulfide analogues of TSA and SAHA.[41]

Almost all of the aromatic sulfur-linked inhibitors depicted in figure 1.19 showed lower IC_{50}s than SAHA, compounds **7b** and **7c** indicated that increase in oxidation state of the sulfur to sulfoxide and sulfone respectively increased potency. This was not the case with unsaturated compounds **7k** and **7n**, which retained approximately the same activity.

An aromatic thiol structurally related to the above series (figure 1.19) was recently synthesised by Kim et al.[42] The 2-naphthylthiol analogue of SAHA, **8**, (figure 1.20) proved to be a potent inhibitor *in vitro*. Additionally, sulfoxide **7g** was effective *in vivo*, exhibiting stronger potency than SAHA in xenograft mice tumour models.

8
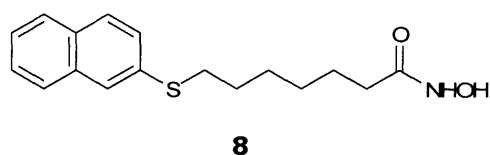
Figure 1.20. 2-Naphthylthiol SAHA analogue.[42]

Uracil-based thiols have been synthesised by Mai and co-workers.[43] The unsubstituted uracil cap of compound **9a** (figure 1.21) showed a modest inhibitory capacity and the cap region was optimised, producing low nanomolar inhibition, by inclusion of benzyl or phenyl groups in **9d** and **9e** respectively.

	HD2 IC$_{50}$ (nM)
9a	213
9b	110
9c	135
SAHA	50

	HD2 IC$_{50}$ (nM)
9d	18
9e	12
9f	35
TSA	7
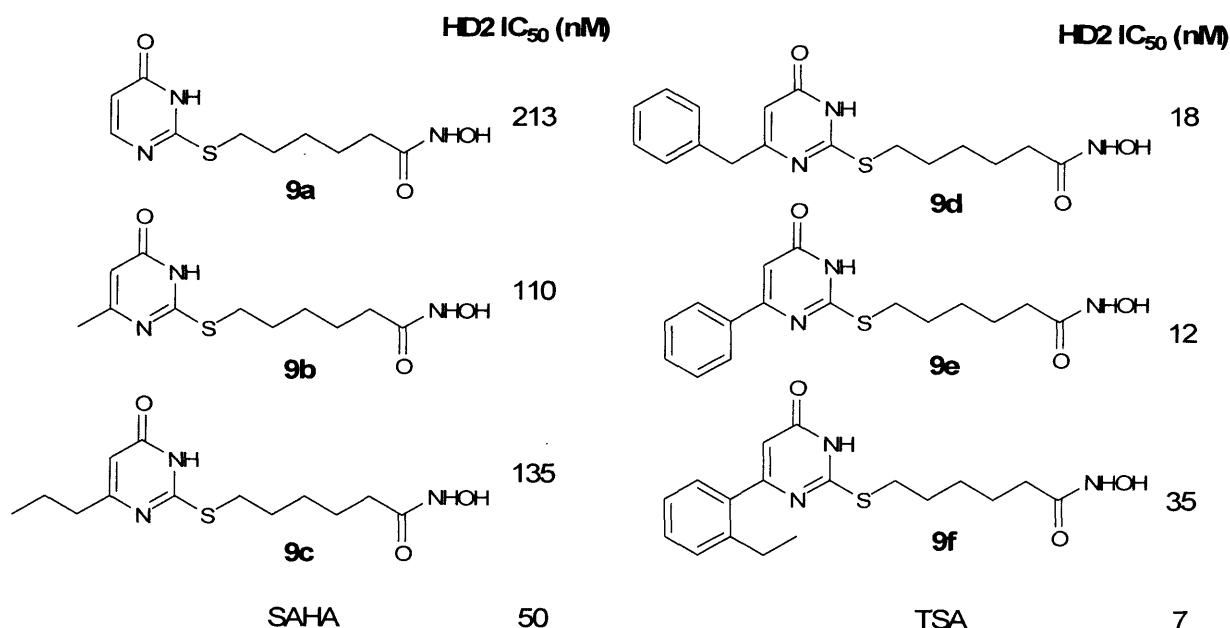
Figure 1.21. Uracil-based hydroxamates.[43]

A solitary example of an aryl ether linked straight chain hydroxamic acid inhibitor was reported by Dai and co-workers (10, figure 1.22), this simple example showed modest activity in isolated HDAC assays.[44] The biaryl analogue 11, reported the same year by Wada and co-workers, showed greatly improved activity.
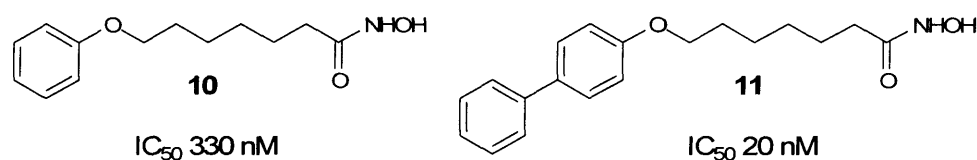
Figure 1.22. Aryl ether straight chain hydroxamates.[44,45]

Previous work within the Marson group greatly enhanced the potential of this motif by introducing p-substitution. A lead compound in bearing a 4-amino group substituted with pyridyl and methyl moieties (12, figure 1.23) showed an IC_{50} of 15 nM and the simple naphthyl group of 13 produced exceptional potency.[46]
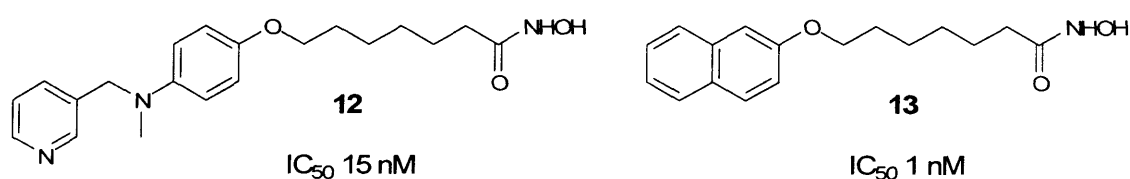
Figure 1.23. Additional aryl ethers.[46]

In addition to his work on indole amide hydroxamic acids (figure 1.15), Dai et al. have demonstrated it to be possible to include an aromatic connecting unit in place of the linear amide, ketone or sulfur examples reviewed thus far. Exchange of SAHA's amide linker for oxazole or thiazole moieties (figure 1.24) significantly raised the activity of the inhibitors.[44] Potency in this series was further enhanced with substitution on the phenyl ring, p-methoxy and p-bromo analogues of 14a lowering the IC_{50}s to 2 nM and 3 nM respectively.
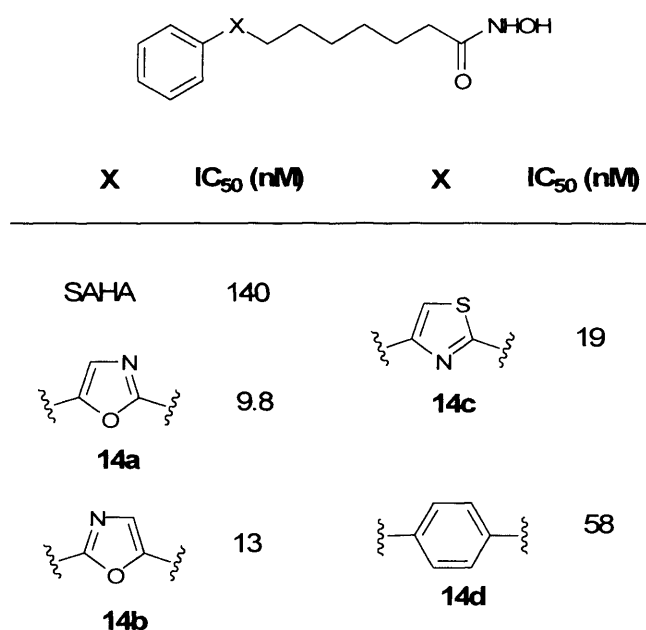
Figure 1.24. HDAC inhibitors incorporating aromatic connection units. [44]

A one-off, related compound, containing a thiazole ring in the cap region (this time retaining the amide linker) was synthesised and tested by Glaser and co-workers as part of a structurally diverse series of inhibitors.[47] Retention of the amide connecting unit in **15** (figure 1.25) resulted in exceptional potency in *in vitro* assays.
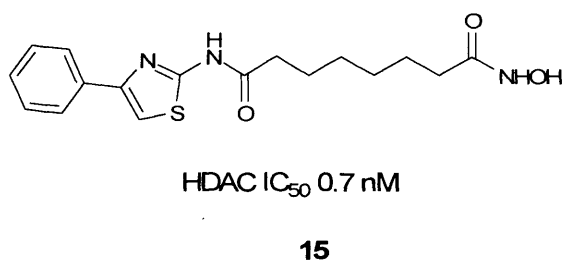

HDAC IC_{50} 0.7 nM

15

Figure 1.25. A thiazole-containing inhibitor.[47]

The potential of the framework of **15** to form the basis of a drug-like molecule has been explored in greater detail by Suzuki et al. whose analogous compound (discussed later) replaces the hydroxamic acid moiety with, initially, a simple thiol.[48]

The somewhat unique structure of the previously depicted tubacin (figure 1.12) was identified from a mass screen of a diversity-oriented synthesis library by Schreiber and co-workers.[49] This vast study also identified a total of 617 small molecule inhibitors of HDAC.

As shall been seen to a greater extent later with macrocyclic inhibitors, the cap region of these compounds is not restricted to linearly linked aromatic groups. A series of phenylalanine-based SAHA analogues produced by Jung and co-workers demonstrated the potency of bifurcated caps. Figure 1.26 gives a selection of compounds identified as most potent in cell-based assays. As has been demonstrated in other systems, the biphenyl motif of **16d** provided good activity, with the aliphatic valine derivative, naphthyl and thienyl compounds **16e**, **16g** and **16h** all displaying low micromolar values.

X	IC$_{50}$ (µM) Friend leukaemic cells	X	IC$_{50}$ (µM) Friend leukaemic cells
TSA	0.04	16f	22
SAHA	0.99	16g	5.2
16a	15	16h	5.9
16b	15	16i	13
16c	11	16j	15
16d	0.84		
16e	6.2		
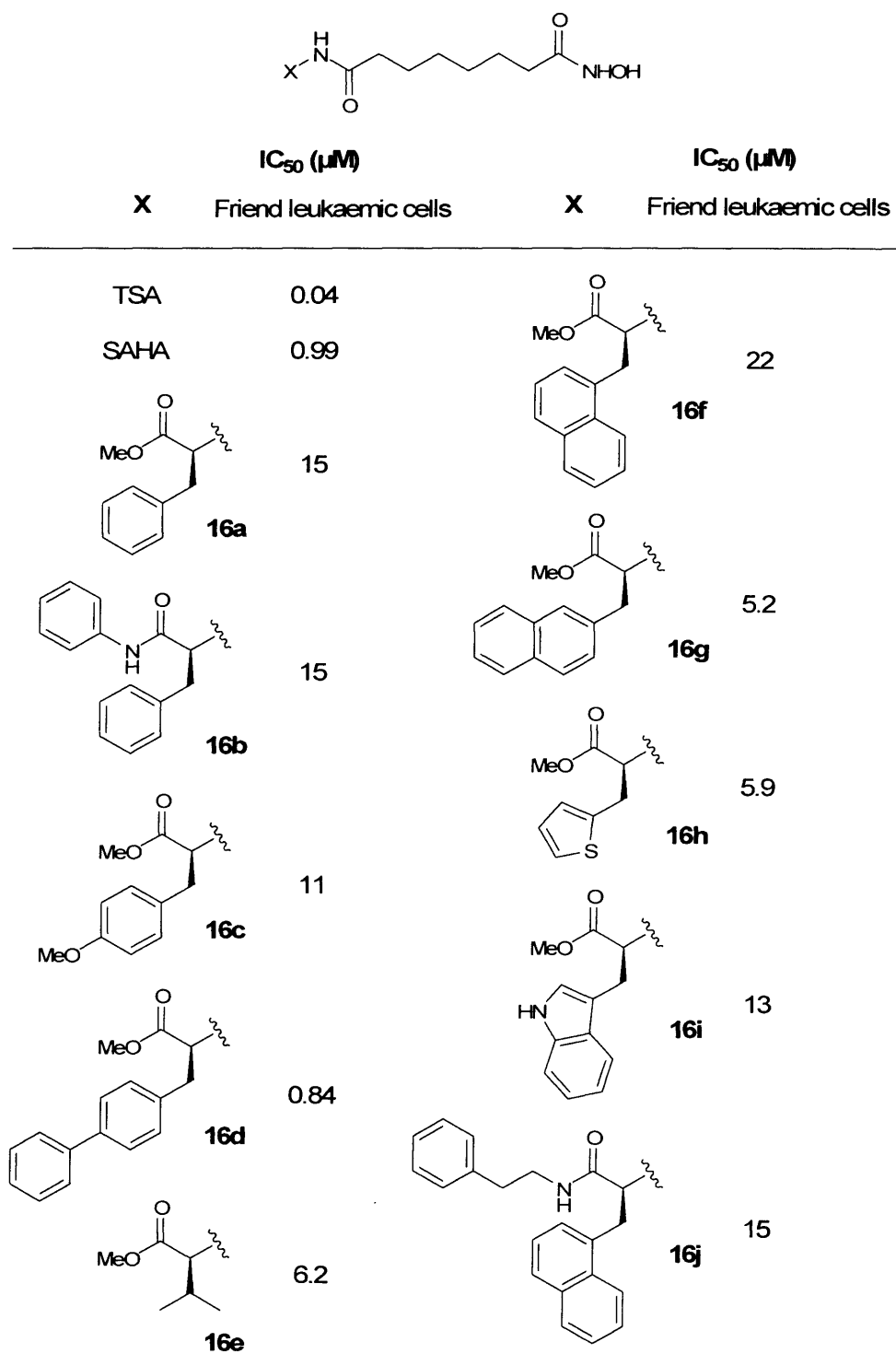
Figure 1.26. Phenylalanine-based SAHA analogues

In an extensive study also involving compounds possessing the bifurcated cap region, this time with derivatives of aminosuberic acid, Kahnberg and co-workers synthesised and evaluated over 50 novel inhibitors.[50] This study not only identified many potently cytotoxic compounds in assays with MM96L human melanoma cells, but it also sought to identify compounds that showed selectivity for toxicity to cancer cells over untransformed neonatal foreskin fibroblasts (NFF) cells. Comparison of the IC_{50} values from assays with each of these cell types individually were used to give a qualitative selectivity index (SI). The most cytotoxic and selective structures from this study are given in figure 1.27. Six compounds were found to have IC_{50} 20-80 nM, activity comparable to that of TSA. The series focuses heavily on aromatic substituents of the parent amino acid, with minimal flexibility linking at positions R_1 and R_2. Compounds bearing linkers of two or more carbon atoms between the carbonyl and aromatic group (not featured here) showed reduced potency. The most potent compounds, 17k-m, contained the rigid and bulky 8-aminoquinoline substituent in the R_2 position, and exchange of this group for a benzyl moiety resulted in a 2-80 fold loss of potency. It was noted that the ten-fold increase in potency of the (R)-enantiomer 17b over (S)-17a was consistent with the predicted molecular modelling study which indicated greater H-bonding and van der Waals interactions with the enzyme for this enantiomer. It is therefore suggested from this that the unnatural D-amino acids may prove to be more potent than the L-forms.

	R^1	R^2	*	IC$_{50}$ (μM)		
				NFF	MM96L	SI[a]
TSA				0.20	0.03	6.7
17a	NMe$_2$		(S)	12	1.6	7.8
17b	NMe$_2$		(R)	1	0.16	6.0
17c	(furan)		rac	4.6	0.2	23
17d	(styryl)		(S)	4.01	0.46	8.7
17e	(indole) HN		(S)	1.26	0.25	5.0
17f	(indole) NH		(S)	>2.2	0.33	>7
17g	(hydroxyindole) HN OH		(S)	2.21	0.33	6.7
17h	CF$_3$	Me$_2$N	(S)	0.40	0.22	1.8
17i	O	(biphenyl)	(S)	2.2	0.33	6.7
17j	Br	(biphenyl)	(S)	0.19	0.07	2.5
17k	NMe$_2$	(quinoline) N	(S)	0.57	0.02	28
17l	HN (indole)	(quinoline) N	(S)	0.34	0.02	16
17m	(styryl)	(quinoline) N	(S)	1.24	0.02	59

Figure 1.27. Novel aminosuberic acid-based inhibitors compared in assays against normal NNF cells versus MM96L melanoma cells. [a] Selectivity index.[50]

A recent communication reporting a similar series of aromatic derivatives of aminosuberic acid, which included other similar structures to those in the series described above, also identified this framework as being potent in HDAC1 assays and SC9 murine erythroleukaemia cell cytotoxicity assays.[51] This series reduced flexibility in the cap group still further by including aniline substituents where benzylamine had been used above. The most potent cell kill achieved from the compounds of figure 1.28 was of low nanomolar potency, by compounds bearing a combination of phenyl and quinolyl groups (**18c** and **18e**).

R¹	R²			
	⟨phenyl⟩	⟨pyridyl⟩	⟨quinolyl⟩	⟨benzyloxy⟩
⟨benzyl⟩	**18a** 12.1 / 73	**18b** 11.1 / 250	**18c** 10 / 33	**18d** 18 / 102
⟨isoquinolinyl⟩	**18e** 9.4 / 43	**18f** 4 / 367	**18g** 42 / 165	**18h** 14.5 /39
⟨quinolinyl⟩	**18i** 3.5 / 90	**18j** 1.3 / 756	**18k** 54 / 107	**18l** 7.7 / 110
⟨phenethyl⟩	**18m** 93 / 1039	**18n** 120 / 3423	**18o** 51.3 / 374	**18p** 48 / 412

Values shown for IC$_{50}$ HDAC1 / IC$_{50}$ SC9 (nM)
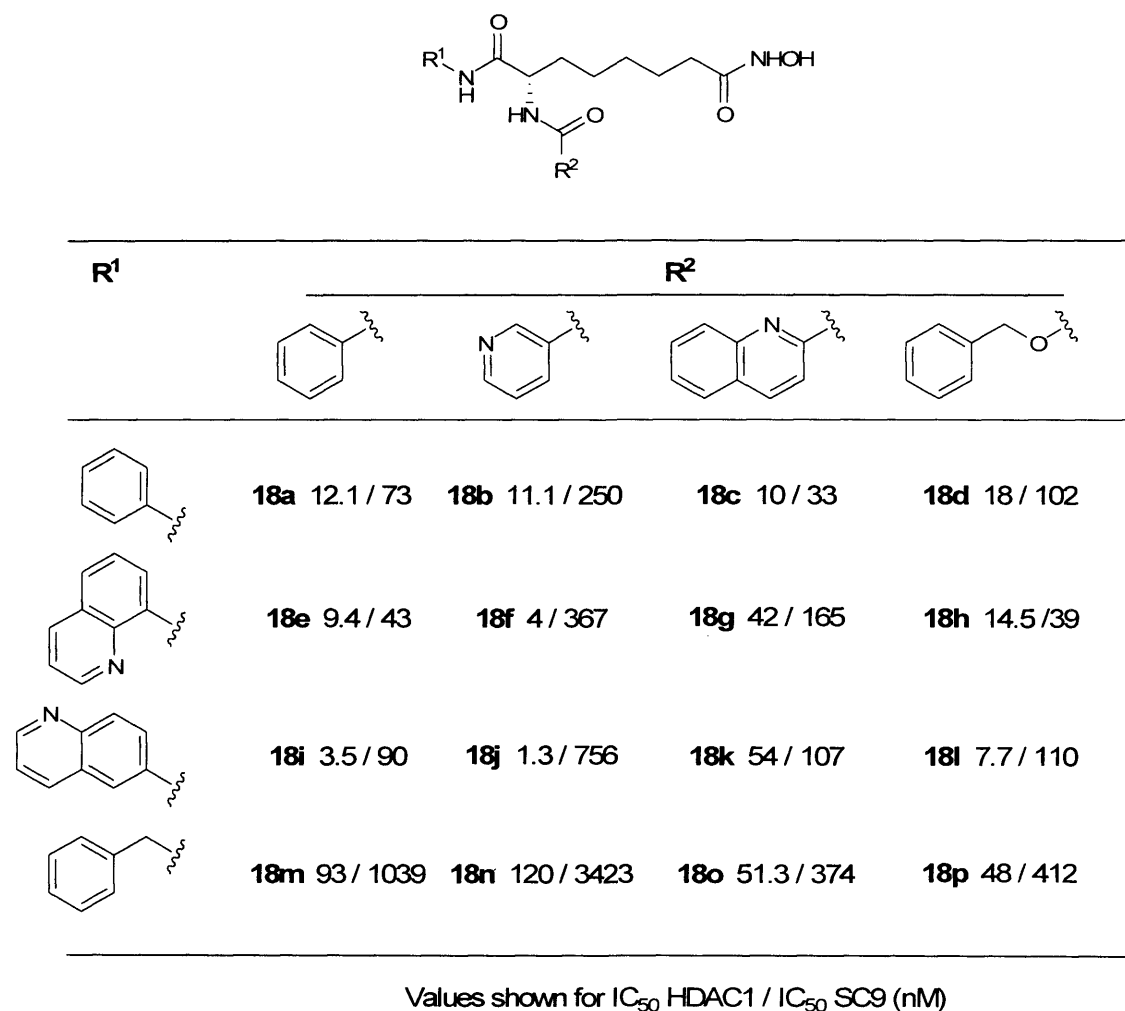
Figure 1.28. Aminosuberic acid derivatives.

Additional flexibility of the R^1 group was seen to dramatically reduce potency (**18m**), however inclusion of a benzyl ester at R^2 did not reduce the activity to such a great extent in most cases (**18d, 18h** and **18l**).

Other inhibitors bearing bifurcated cap regions related to the class of compounds described above, but based on malonamide branch points, have also been investigated. As the previously reported structures are not straight-chain, hydroxamic acid inhibitors they shall be discussed in later sections. However, additional compounds that do fall into this category have been synthesised as part of this thesis and will be discussed in the appropriate chapter.

1.8.2 Hydroxamic Acids Bearing Cyclic Linker Domains

The discovery that cyclic groups could be tolerated in the linker regions of inhibitors came with development of the so-called second generation of hybrid polar compounds.[52] Included in this class was the previously discussed SAHA and *m*-carboxycinnamic acid bis hydroxamide (CBHA). Although it may seem surprising that a bulky benzene ring may provide a suitable replacement for an alkyl chain, comparison with the linker of TSA shows good overlap with this highly effective scaffold (figure 1.29).
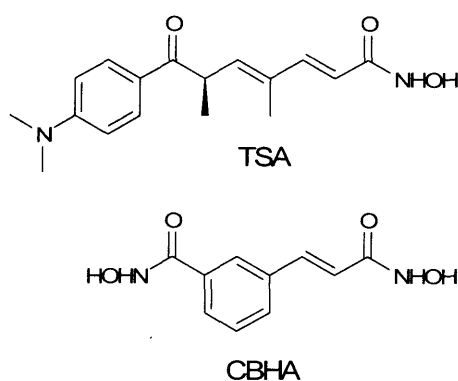
Figure 1.29. Similarity of TSA and CBHA linkers.[51]

An early example of cyclic-linker hydroxamic acid inhibitors came from Jung in 1999.[53] Comparison of the activity of the two compounds **19** and **20**, figure 1.30, indicates a

dramatic influence on activity produced by the positioning of the cyclic group in the chain. This dramatic difference may indicate that different conformations are being adopted by these two molecules, which prevent **20** from binding well to the active site.

HD2 IC_{50} (nM)

180

>40,000
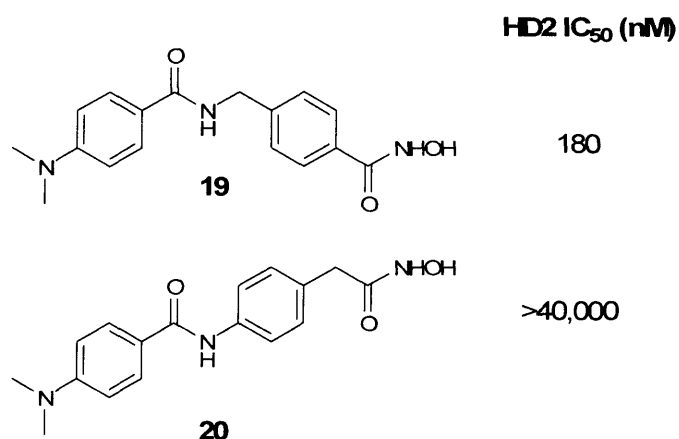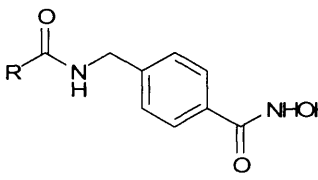
Figure 1.30. Amide analogues of TSA.[53]

The potent benzylic hydroxamic acid **19** is the same overall length as its analogue **20**, which should theoretically allow the same contacts of the cap groups with the top of the enzymes active site, however, placing the methylene unit of the chain before the phenyl ring (**20**) instead of after results in what is effectively a total loss of activity. These two compounds were the only cyclic-linker inhibitors evaluated in this study but further work making use of the linker unit of **19** has been carried out by Maeda and co-workers. They produced a short series of benzylic hydroxamic acids with varied aromatic capping groups that showed good activity in isolated enzyme assays and were able to optimise the cap to give low single figure micromolar inhibition in HCT116 cell assays (figure 1.31).[54]
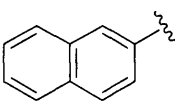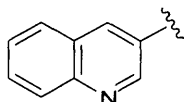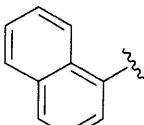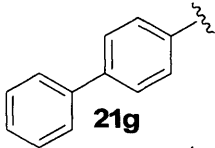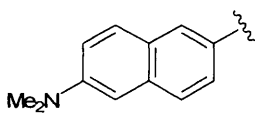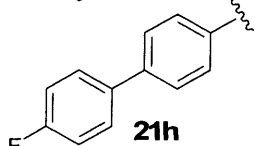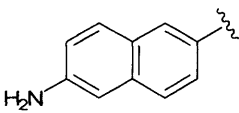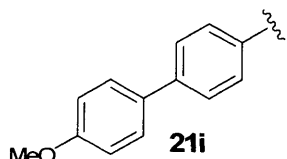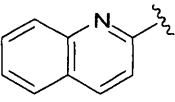
| | IC_{50} | | | IC_{50} | |
R	HDAC (nM)	HCT116 (µM)	R	HDAC (nM)	HCT116 (µM)
21a	44	0.7	21f	43	3.6
21b	830	9.7	21g	240	3.2
21c	>10,000	80.4	21h	830	8.2
21d	39	3.9	21i	>10,000	36.1
21e	26	3.8	TSA	3	0.5
			SAHA	263	6.3

Figure 1.31. N-Hydroxybenzamide HDAC inhibitors.[54]

Although the most potent compound in this series was naphthyl analogue **21a**, it was the more water-soluble amine **21d** which was selected for further evaluation in the form of a murine tumour model, showing promising results.

Benzylic hydroxamic acids bearing shorter linker units than those above have also been shown to be effective by Chen and co-workers.[55] The phenylbutyrate based compound

HTBP (figure 1.32) was identified in a screen of short chain fatty acids tethered with Zn-chelating motifs.
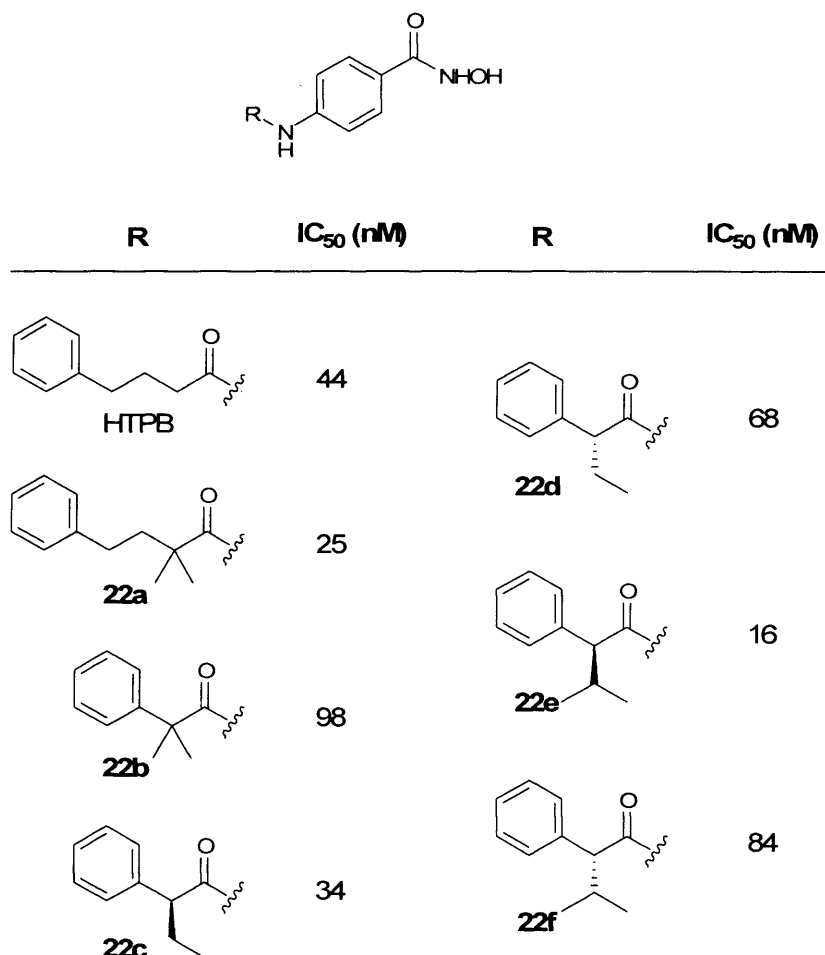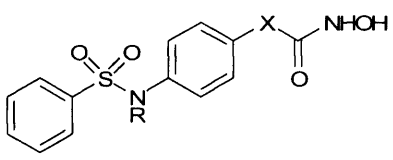
Figure 1.32. Phenybutyrate-derived HDAC inhibitors.[55]

The phenylbutyrate moiety was further optimised on the basis of computer docking models to shorter, bulkier 3-methyl-2-phenylbutanoyl **22e**. The (R)-enantiomer **22f** showed a significantly reduced activity, as was the case with 2-phenylbutanoyl compounds **22c** and **22d**. This property was consistent with the computer predicted binding energies for the different enantiomers.

Work on optimisation of the cyclic linker of a class of sulfonamide-containing hydroxamic acids was carried out by Lavoie and co-workers (figure 1.33).[56]
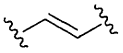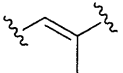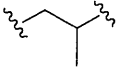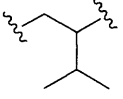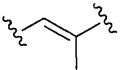

	X	R	HDAC1	HCT116
			IC$_{50}$ (µM)	
	TSA		0.005	1
23a	(-)	H	0.9	2
23b	CH$_2$	H	1	22
23c	(CH$_2$)$_2$	H	0.1	2
23d	(CH$_2$)$_3$	H	1	8
23e		H	0.2	3
23f		H	2	15
23g		H	17	>50
23h		H	>25	30
23i		CH$_3$	0.6	4

Figure 1.33. Sulfonamide-containing compounds.

The optimum linker between aryl group and hydroxamic acid was identified as two carbon units, with little difference on this scale between the saturated dihydrocinnamic acid-type **23c** and unsaturated cinnamic acid-type **23e**. Reduction or increase of this separation in **23a**, **23b** and **23d** reduced activity significantly in this series. Introduction of alkyl substituents to

the chain was also not favourable for activity, it was, however, better tolerated in the *N*-methyl analogue **23i**. As has been shown by comparison of TSA with CBHA, the cinnamic acid group provides excellent analogy with the substituted, unsaturated chain of TSA, and this group has been shown repeatedly to be an excellent linker. This study further optimised the unsaturated chain analogue **23e** by substitution in the aryl cap group, identifying the 4-chloro and 4-methoxy analogues as substantially more active inhibitors.

This optimisation of the aromatic cap was continued in later work to include polycyclic moieties.[57] As is consistent with previously described work, the biphenyl (**24e**) and naphthyl (**24f**) groups proved suitable units for capping the structure (figure 1.34). Analogues in this series bearing the reverse order of sulfonamide units (NHSO$_2$) were also evaluated and found to give comparable activity to those shown.
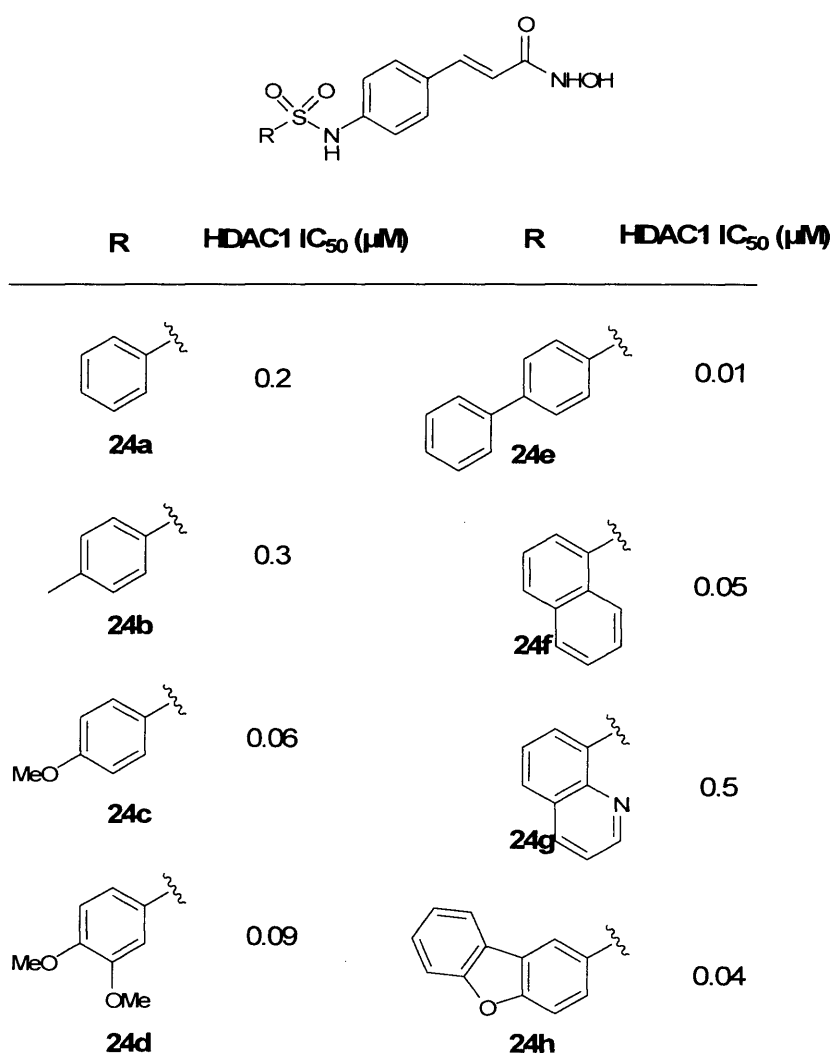
R | HDAC1 IC$_{50}$ (μM) | R | HDAC1 IC$_{50}$ (μM)

24a — 0.2

24b — 0.3

24c — 0.06

24d — 0.09

24e — 0.01

24f — 0.05

24g — 0.5

24h — 0.04

Figure 1.34. Further cinnamyl-based sulfonamides.[57]

Significant attention has been given to cyclic linker units containing pyrrole functions, with examples being reported by Massa in 1990.[58] Massa and Mai have continued work on these compounds, reporting a series of pyrrole-containing inhibitors of moderate activity in 2001.[59] Later work by the same groups was able to improve activity of this class of compounds by increasing separation of the aryl cap from the pyrrole by inclusion of alkyl chains of various lengths.[60] Building on the information gained in this study on the benefit of a separating group between pyrrole and cap, work was continued with an unsaturated

linker.[61] These compounds proved to be highly selective inhibitors for class II HDACs (figure 1.35).

R¹	R²	HD2 IC₅₀ (µM)	R¹	R²	HD2 IC₅₀ (µM)
25a	H	0.28	25f (MeO)	H	0.57
25b (Br)	H	0.36	25g	H	0.20
25c (Me)	H	0.27	25h	Me	0.68
25d (OMe)	H	0.25	25i	Et	0.42
25e (OMe)	H	0.54	TSA		0.007
			SAHA		0.05

Figure 1.35. Unsaturated linker pyrrole inhibitors.[61]

The potent inhibitor NVP-LAQ824 (now in phase II clinical trials) (figure 1.36) was the optimised product of an SAR study stemming from the identification of compound **26** in a high-throughput screen of the Novartis compound archive.[62]
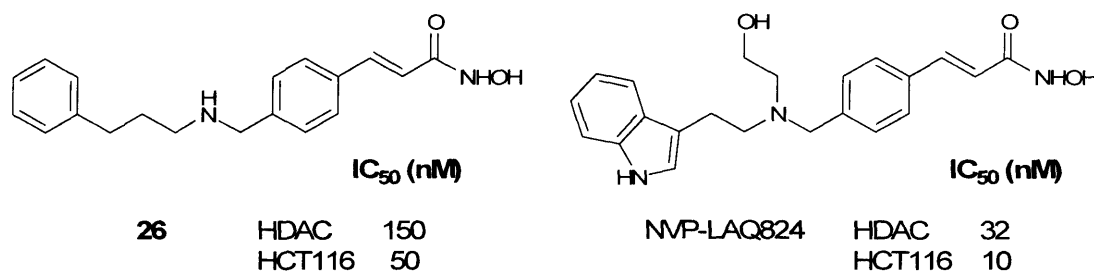
Figure 1.36. Optimisation of hit compound **26** to NVP-LAQ824.[62]

A series of benzyl hydroxamate analogues of NVP-LAQ824, with variation of the heterocycle in the cap region was produced by Nagaoka and co-workers, however, all examples showed substantially lower activities than the cinnamoyl NVP-LAQ824.[63] The direct benzyl analogue (**27**) of NVP-LAQ824 was reported as having an isolated enzyme IC_{50} with more than a ten-fold reduction in potency, and with 30,000 times lower activity in the HCT116 assay (figure 1.37). It may be suggested from these observed effects that the shorter chain of **27** does not allow the same contacts to be made by the cap group with the opening of the enzyme active site.
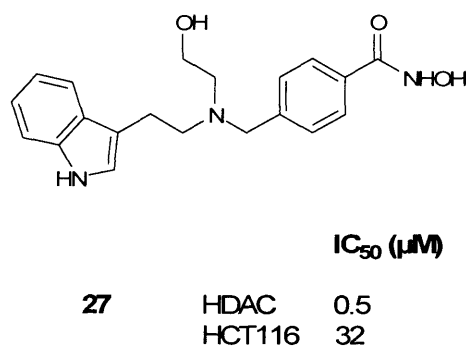
Figure 1.37. Benzyl hydroxamate analogue of NVP-LAQ824.[63]

It was shown by Shinji and co-workers in 2005 that a phthaloyl group is also capable of being tolerated in linker region.[64] The unsaturated general structure shown in figure 1.38 was determined as being preferable to an unsaturated linker or direct attachment of the hydroxamic acid to the aromatic ring. It was determined that an additional unsaturated

linker attaching an aryl group to the phthaloyl nitrogen was optimal, the unsubstituted two-carbon linker of **28b** showing greatest potency in this series.
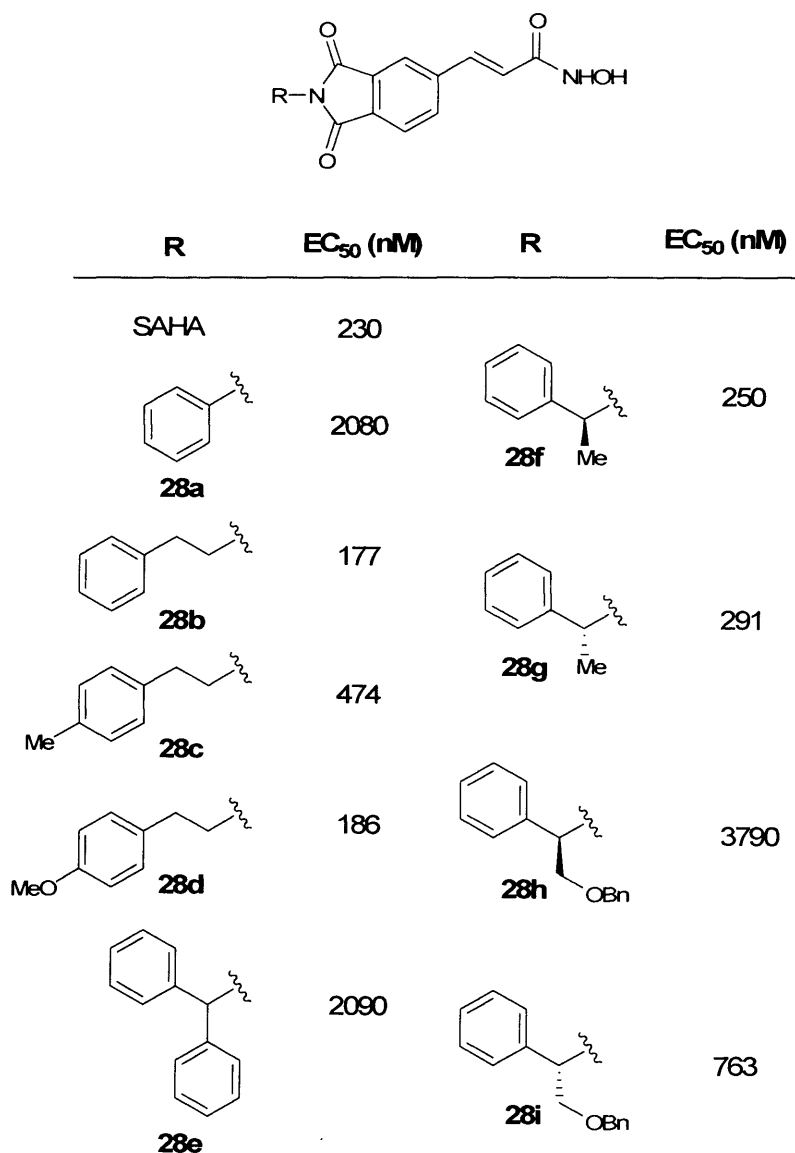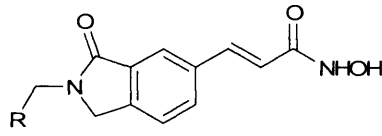

R	EC$_{50}$ (nM)	R	EC$_{50}$ (nM)
SAHA	230		
28a	2080	**28f** Me	250
28b	177	**28g** Me	291
Me **28c**	474		
MeO **28d**	186	**28h** OBn	3790
28e	2090	**28i** OBn	763

Figure 1.38. Phthalimide based inhibitors.[64]

In a similar vein to the phthalimide series above, an isoindolinone linker was identified by Miyachi and co-workers.[65] The *N*-benzyl analogue **29a** (figure 1.39) showed moderate activity against HDAC1 and 6, with ten-fold selectivity for HDAC4.
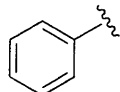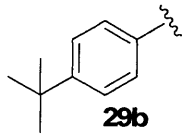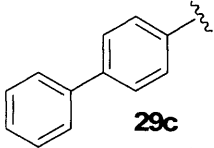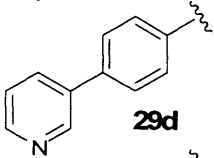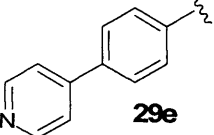
| R	IC$_{50}$ (nM)		
	HDAC1	HDAC4	HDAC6
TSA	16	29	53
29a	250	20	230
29b	230	180	990
29c	220	98	750
29d	92	56	280
29e	3300	2100	15,000

Figure 1.39. Isoindolinone inhibitors.[65]

The first instance of benzo[b]thiophene-linked HDAC inhibitors was recently reported, with compounds in this series displaying sub-micromolar activity against SC9 murine erythroleukaemia cells (figure 1.40).[66] Of the chiral amide examples **30f** and **30g**, the *S*-enantiomer **30f** showed approximately a three-fold greater activity.

	IC_{50}			IC_{50}	
R	HDAC1 (nM)	SC9 (μM)	R	HDAC1 (nM)	SC9 (μM)
30a	55	1.11	30f	20	0.14
30b	60	2.08	30g	60	0.53
30c	10	0.22	30h	50	0.16
30d	20	0.23	30i	128	0.46
30e	20	0.21	30j	10	0.19

Figure 1.40. Benzo[b]thiophene inhibitors.[66]

Insertion of a δ-lactam into the otherwise straight, aliphatic chains of compounds **31a-d**, figure 1.41, resulted in inhibitors approaching the potency of SAHA.[67] It was also noted that, in addition to HDAC inhibition, these compounds inhibited production of pro-inflammatory agents tumour necrosis factor α (TNFα) and nitric oxide (NO), although again, to a lesser extent than SAHA.
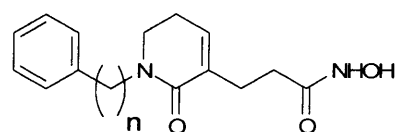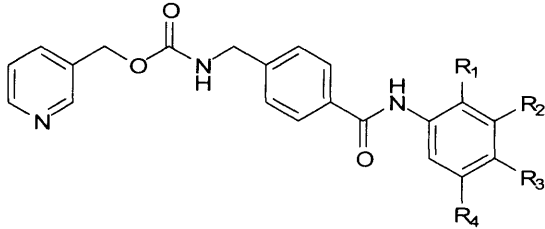
	n	HDAC IC$_{50}$ (μM)
31a	1	0.67
31b	2	0.35
31c	3	0.27
31d	4	0.13
SAHA		0.11

Figure 1.41. δ-Lactam-based HDAC inhibitors.[67]

1.8.3 Benzamides

Having covered the broad category of hydroxamate inhibitors we shall now review the known inhibitors in the benzamide class. The class typically displays lower potency than the corresponding hydroxamic acids. It is also a common trait of this class to display relatively poor potency in isolated enzyme assays whilst maintaining good potency in anti-proliferation assays. For example, many benzamides are only active at micromolar concentrations in isolated enzyme assays and have anti-proliferation activity at similar concentrations. This low specific activity may suggest that HDAC inhibition is not the only mode of action of benzamides in effecting their cytotoxic response, or that hydroxamates suffer from a reduction in expected potency on moving from isolated enzyme to cell-based assays.

An early study which established some of the key structural necessities for this class identified the 2-aminobenzanilide MS-275 (figure 1.42).[68] The study clearly demonstrated the requirement of a coordinating heteroatom (in this case $-NH_2$ or -OH) in the 2-position, with re-location to the 3- or 4-positions eliminating activity. Additional bulk on the ring in the form of methyl, methoxy or chloro groups is not tolerated in the 3- or 4- positions (**32f-i**), but seems to be allowed in the 5-position (**32j-m**).

	R_1	R_2	R_3	R_4	HDAC IC$_{50}$ (µM)
MS-275	NH$_2$	H	H	H	4.8
32a	H	H	H	H	>100
32b	H	NH$_2$	H	H	>100
32c	H	H	NH$_2$	H	>100
32d	NHAc	H	H	H	>100
32e	OH	H	H	H	2.2
32f	NH$_2$	CH$_3$	H	H	>100
32g	NH$_2$	H	CH$_3$	H	>100
32h	NH$_2$	H	OCH$_3$	H	44
32i	NH$_2$	H	Cl	H	40
32j	NH$_2$	H	H	CH$_3$	2.8
32k	NH$_2$	H	H	OCH$_3$	4.6
32l	NH$_2$	H	H	Cl	7.7
32m	NH$_2$	H	H	F	6.0
TSA					0.0046

Figure 1.42. Identification of the 2-aminobenzamide moiety in development of MS-275.[68]

With the 2-aminobenzanilide group generally adopted as the Zn-binding group of choice in this class, further experimentation has focused on the linking and capping regions. The linker groups tend to contain cyclic groups as opposed to straight aliphatic chains. The direct analogue of SAHA being 50 times less active in isolated enzyme assays (discussed later).[69] A series of cinnamoyl-type benzamide containing a sulfonamide group was synthesised as part of a previously discussed hydroxamic acid series (figure 1.34).[57]

The compounds of figure 1.43 are the most potent of the series. Dimethoxy analogue **33b** showed only modest HDAC1 inhibition, but inhibited HCT116 proliferation at 1 μM. Again, compounds bearing a biphenyl cap group displayed the lowest enzyme IC_{50}s, the order of the sulfonamide bearing little influence.

	Ar	X-Y	IC_{50} (μM)			
			HDAC1	H4-Ac	HCT116	T24
33a	Me	SO_2NH	3	5	4	8
33b	MeO, MeO	SO_2NH	4	10	1	6
33c	Ph	SO_2NH	1	15	15	>36
33d	Me	$NHSO_2$	2	2	3.5	6.5
33e	MeO, MeO	$NHSO_2$	3	3	4	7.5
33f	Ph	$NHSO_2$	1	5	N.T.	N.T.

Figure 1.43. Sulfonamide-containing benzamides.[57]

The promising drug-candidate MGCD0103 (figure 1.44) was discovered by Methylgene to bear more favourable biological properties than MS-275. By directly replacing the carbamate of MS-275 with a more stable aminopyrimidine ring, the two oxygen atoms are exchanged for nitrogen but their positions remain the same.
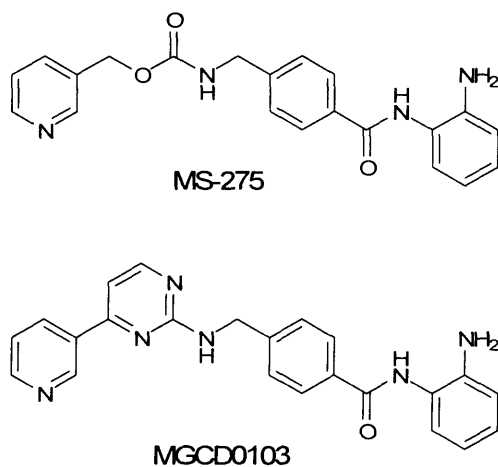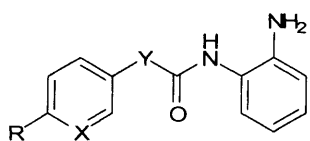
Figure 1.44. MGCD0103: Improved analogue of MS-275.

A later study by Methylgene, referred to in figure 1.45, identifies several benzamides active at sub-micromolar concentrations. The cinnamyl compounds **34a-e** were found to be less potent than the benzyl variants **34f-j**. The appreciable activity of *p*-methoxy analogue **34f** was not improved by increased flexibility of the cap in **34g**. The same appeared true for translocation of the cap amine group (cf. **34i** and **34j**) with cellular assays.
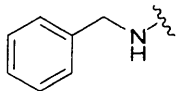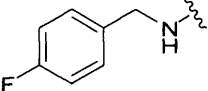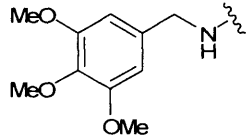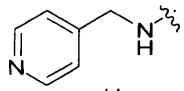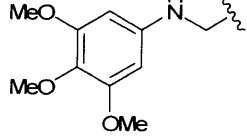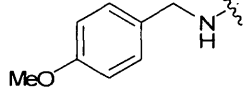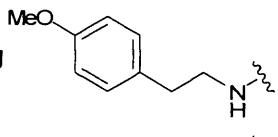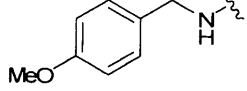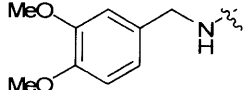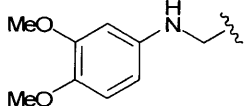
	R	X	Y	IC$_{50}$ (µM)	
				HDAC1	HCT116
34a	(benzyl-NH)	N	CHCH	3	1
34b	(4-F-benzyl-NH)	N	CHCH	5	2
34c	(3,4,5-triMeO-benzyl-NH)	N	CHCH	4	2
34d	(pyridin-4-ylmethyl-NH)	CH	CHCH	10	2
34e	(3,4,5-triMeO-phenyl-NH-CH$_2$)	CH	CHCH	2	2
34f	(4-MeO-benzyl-NH)	N	(-)	4	0.5
34g	(4-MeO-phenethyl-NH)	N	(-)	3	0.8
34h	(4-MeO-benzyl-NH)	CH	(-)	2	1
34i	(3,4-diMeO-benzyl-NH)	CH	(-)	4	0.6
34j	(3,4-diMeO-phenyl-NH-CH$_2$)	CH	(-)	2	0.4

Figure 1.45. Amine-linked benzamides.[70]

Analogues of MS-275 bearing short-chain fatty acid cap groups have also been synthesised, demonstrating comparable activities to the parent compound in enzyme assays (figure 1.46).[71] The highly flexible aromatic cap of **35c** provided best potency from these compounds.
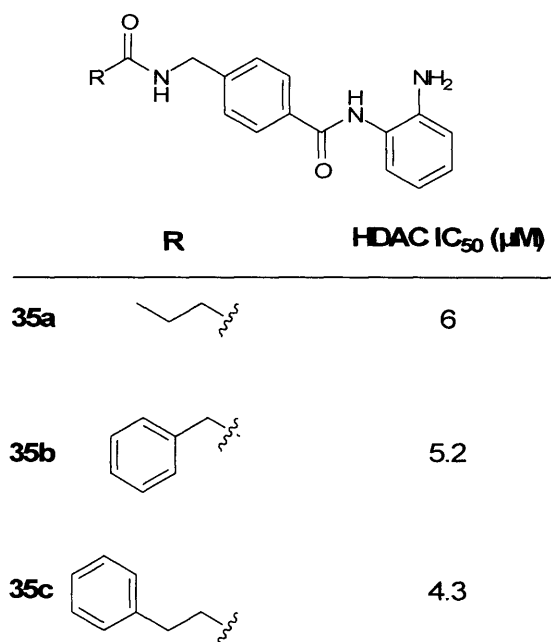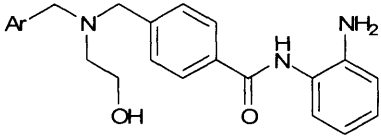

R	HDAC IC_{50} (µM)
35a	6
35b	5.2
35c	4.3

Figure 1.46. Short-chain fatty acids.[71]

Hydroxyethyl compounds bearing structural resemblance to NVP-LAQ824 (figure 1.36) have shown moderate activity.[63] Variation of the aromatic cap groups in figure 1.47 had little effect on potency with these compounds unlike in the case of hydroxamic acid NVQ-LAQ824, which required the presence of an indole cap group for cellular activity.
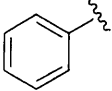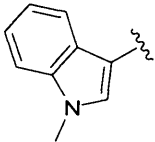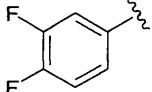
Ar	IC$_{50}$ (μM)	
	HDAC	HCT116
36a	3.7	4.7
36b	2.4	4.5
36c	3.1	4.6

Figure 1.47. Hydroxyethyl amines.[63]

1.8.4. Carboxylates

A small number of short-chain fatty acids are known as relatively feeble inhibitors of HDACs, with activity typically in the millimolar range (figure1.48).[28]

Figure 1.48. Carboxylate inhibitors.

Despite the low activity of this class of inhibitor some examples are still being clinically investigated, partly owing to the fact that they have already passed safety testing for other clinical uses.

1.8.5. Macrocyclic Inhibitors

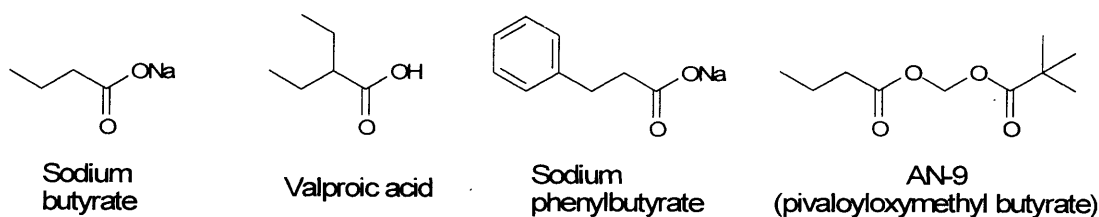This heading shall include both cyclic peptides and related hybrid compounds. Compounds of this class generally demonstrate very good activity, however, the disulfide-bridged compound FK228 (figure 1.49) is the only member of this group currently in clinical trials. The bridged 16-membered ring structure of this compound does not immediately resemble the generalised formula followed by the previously discussed inhibitors. Work by Furumai[72] elucidating the mode of action of FK228 demonstrated that the native compound in fact acts as a prodrug, and the active structure is the reduced form, **37**, which provides a free thiol moiety which could be capable of co-ordinating to the enzyme's active site.
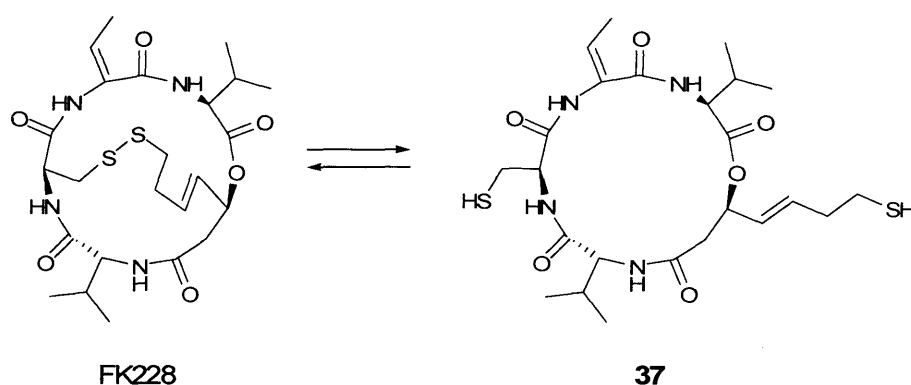

Figure 1.49. FK228 prodrug and its active form.[72]

Depicted in figure 1.50 are the structures of some analogous cyclic tetrapeptide inhibitors. All show similarly substituted peptidic cap regions, with saturated straight-chain linkers. Trapoxin B is an example of an irreversible HDAC inhibitor; reaction of the terminal epoxide with the enzymes active site covalently binds the inhibitor (possibly the nucleophilic histidine residue on Lewis acid activation of the epoxide by zinc), permanently blocking activity. CHAP31 is the reversible analogue of this compound, bearing the common hydroxamic acid Zn-coordinating group. The naturally occurring apicidin demonstrates the potency conferred by the cyclic peptide cap group. The compound is active at low nanomolar concentrations even though it does not bear a group capable of strong coordination with the enzymes Zn ion. The recently reported compound **38** bears a trifluoromethylketone moiety,[73] a group known to act as a coordinating group through the

carbonyl. The trifluoromethylketone functionality, although capable of conferring good activity, has been deemed to be unsuitable for *in vivo* use owing to its ready metabolism to the inactive alcohol.
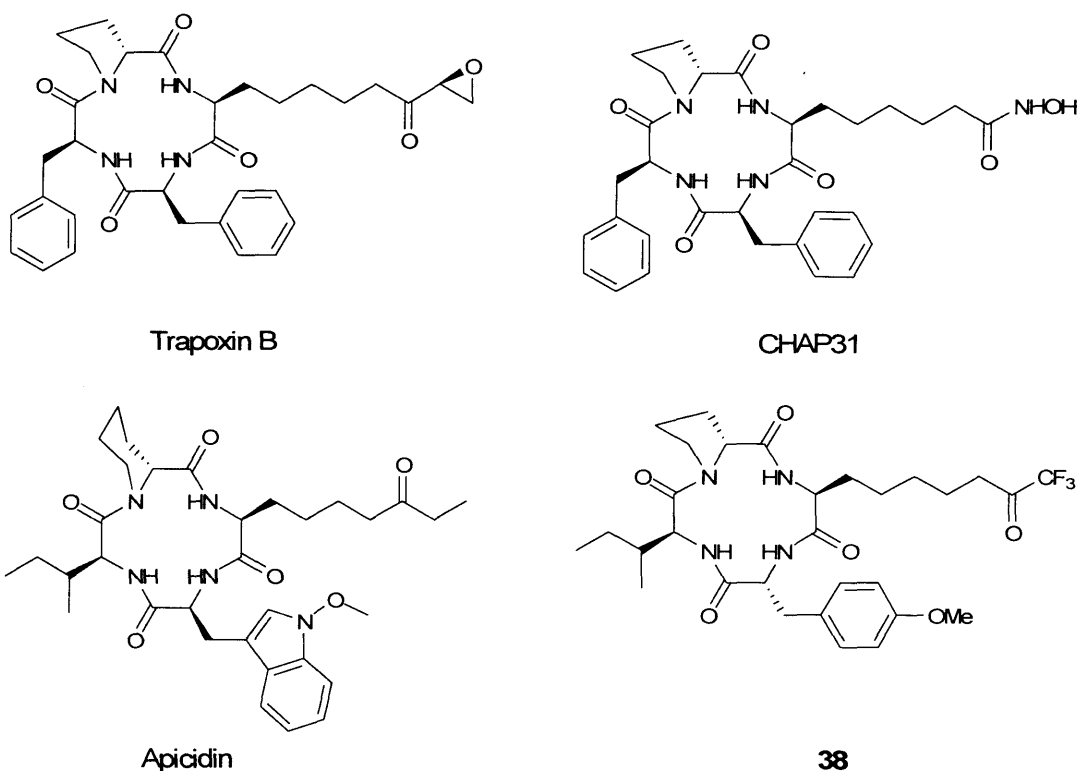
Figure 1.50. Cyclic tetrapeptides.

The compounds **39** and **40** (figure 1.51) could have been included with other straight-chain hydroxamic acid inhibitors, however, they have been included here as hybrids, blurring the boundaries between the classifications. Peptide-mimic **39** showed low nanomolar inhibition for mixed HDACs and approximately a ten-fold selectivity for HDAC1 over HDAC8.[74] The benzodiazepine **40** is an interesting example of a peptide-mimetic, but gave more modest potency, displaying low micromolar activity in H661 cell assays.[75]
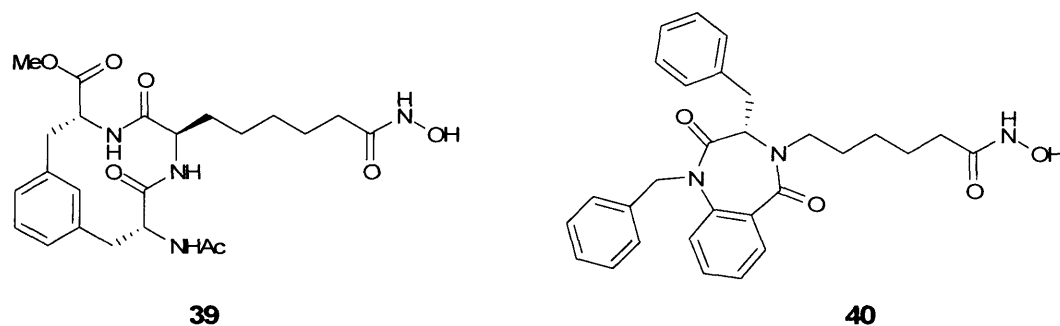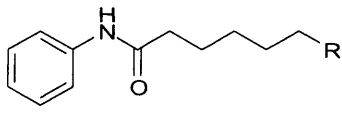
Figure 1.51. Cyclic peptide mimics.[74,75]

1.8.6. Miscellaneous Zinc-Binding Groups

Having discussed examples of the most commonly used zinc-binding groups present in HDAC inhibitors some attention must now fall on work that has sought to identify new alternatives to these groups. As has been mentioned before, the hydroxamic acid group generally provides excellent activity but suffers *in vivo* from rapid metabolism and poor bioavailability. The benzamide functionality does not suffer these drawbacks, but generally confers considerably lower activity. Figure 1.52 summarises the examples of new Zn-binding groups that can be readily compared in efficacy owing to their possession of the same linking and cap groups – in this case, the unsaturated chain and anilide groups of SAHA.
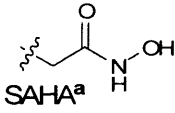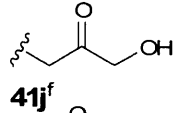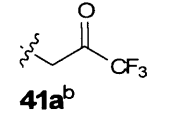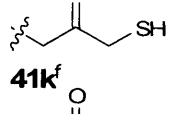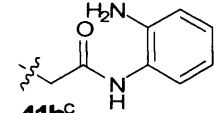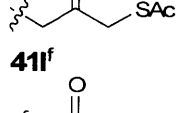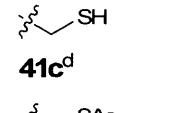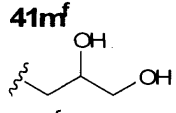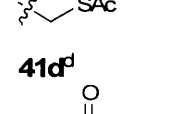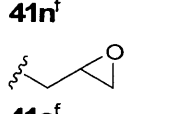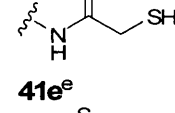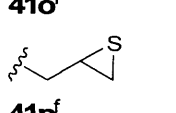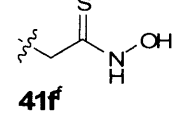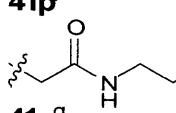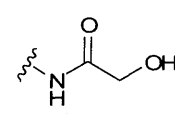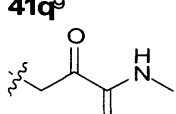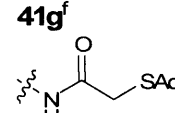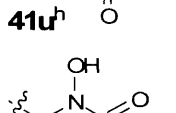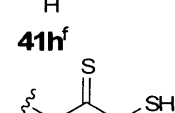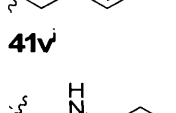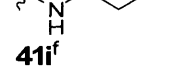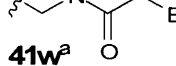
R	IC$_{50}$ (µM)	R	IC$_{50}$ (µM)
SAHA[a]	0.28	41j[f]	>40
41a[b]	6.7	41k[f]	0.15
41b[c]	120	41l[f]	0.08
41c[d]	0.21	41m[f]	>40
41d[d]	7.1	41n[f]	>40
41e[e]	0.39	41o[f]	>40
41f[f]	>10	41p[f]	>40
41g[f]	>40	41q[g]	2.35
41h[f]	20.1	41u[h]	0.34
41i[f]	13.3	41v[i]	2.8
		41w[a]	14

Figure 1.52. SAHA analogues with Zn-binding groups.

[a] Ref[76]; [b] ref[77]; [c] ref[69]; [d] ref[48]; [e] ref[78]; [f] ref[79]; [g] ref[80]; [h] ref[45]; [i] ref[81].

It is noted that, as previously mentioned, the benzamide analogue **41b** shows essentially no activity compared with its hydroxamate analogue SAHA.[69] The most promising alternative groups appear to be those containing a sulfur atom for coordination. The simple thiol **41c** appears to be an improvement on the hydroxamate, with acetylation (**41d**) greatly attenuating activity.[48] The sulfur atom in potent mercaptoamide **41e** cannot be replaced with oxygen (**41g**),[78,79] but removal of the nitrogen to give mercaptoketone **41k** improves activity further.[79] In this case, acetylation of the sulfur, in **41l**, is not only tolerated but produces a favourable effect. The only other example of sub-micromolar activity is from α-ketoamide **41u**, however it was determined that this functionality is also readily metabolised to the alcohol *in vivo*.[45] Substitution of SAHA's carbonyl oxygen for sulfur in **41f** and the subsequent loss of activity may indicate that the acidity of the hydroxamic acid group is necessary for its high activity.

The direct thiol analogue of SAHA (**41c**) has been the subject of considerable study by Suzuki and co-workers. First identified in 2004, it was optimised in the cap region by substitution of various aromatic groups (figure 1.53).[48]
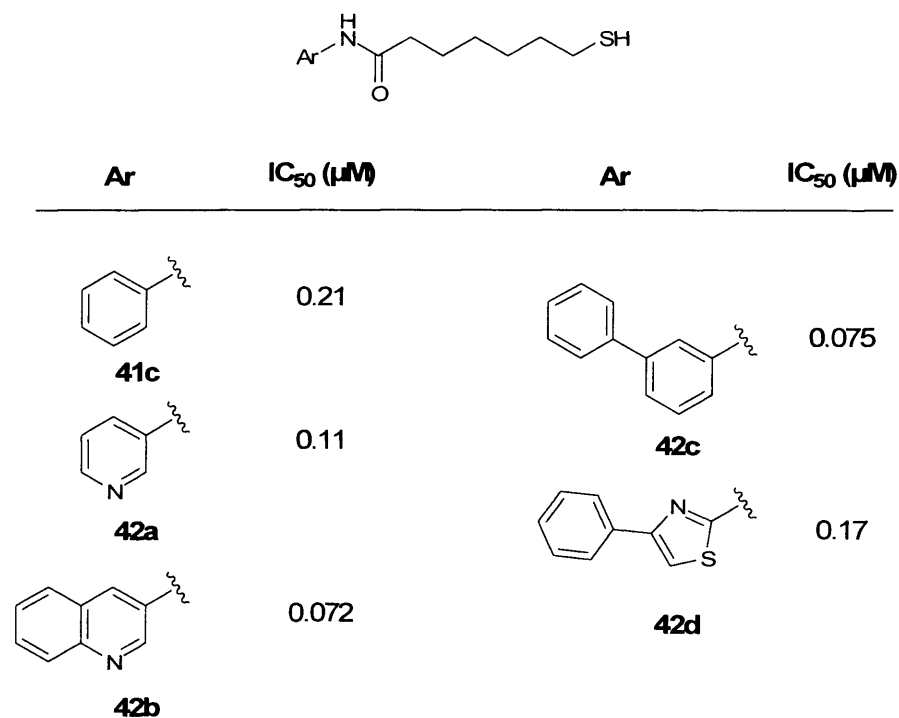
Figure **1.53**. Optimisation of thiol SAHA analogue.[48]

The cap group of **42d** (the hydroxamic acid analogue of which, **15**, (figure 1.25) was described by Glaser) was selected for further study, resulting in the pro-drug form **43** (figure 1.59), which performed at low micromolar concentrations against a panel of cancer cell lines.[82]
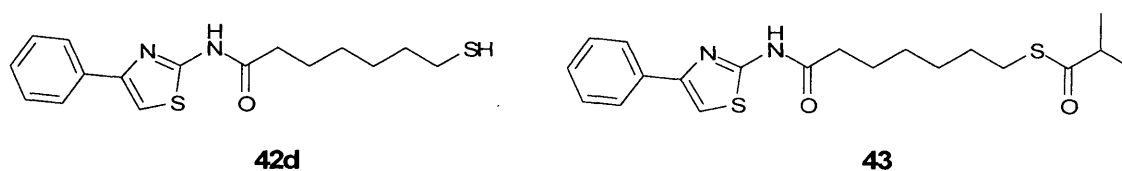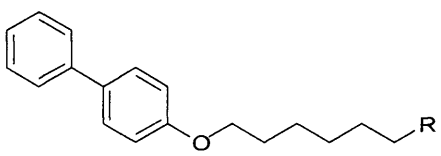
Figure **1.54**. Thiol inhibitor and enhanced performance pro-drug.[82]

As part of the study that discovered α-ketoamide **41u** (figure 1.52), biphenyl compounds **44a-f** were also synthesised (figure 1.55). The α-ketoester **44d** also showed very promising

activity but proved unstable. Using the same general structure, Vasudevan and co-workers synthesised a series of heterocyclic ketones.[83] The most active of these compounds (**44g-i**) showed low micromolar potency.
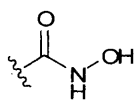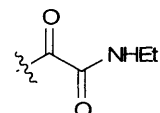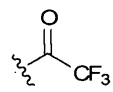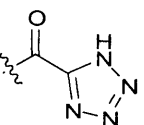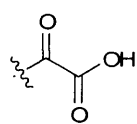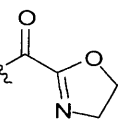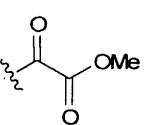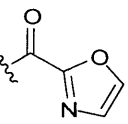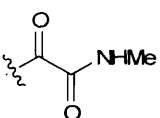
R	HDAC IC$_{50}$ (µM)	R	HDAC IC$_{50}$ (µM)
44a[a]	0.02	**44f**[a]	4.9
44b[a]	2.9	**44g**[b]	2.0
44c[a]	3.7	**44h**[b]	1.8
44d[a]	0.062	**44i**[b]	1.1
44e[a]	0.11		

Figure 1.55. Further Zn-binding groups.
[a] Ref[45]; [b] ref[83].

1.9 References

1. Grant, S.; Easley, C.; Kirkpatrick, P. Vorinostat. *Nature Reviews Drug Discovery* **2007**, *6*, (1), 21-22.

2. Hanahan, D.; Weinberg, R. A. The Hallmarks of Cancer. *Cell* **2000**, *100*, (1), 57-70.

3. Carson, D. A.; Lois, A. Cancer progression and p53. *The Lancet* **1995**, *346*, (8981), 1009-1011.

4. Konstantinopoulos, P. A.; Papavassiliou, A. G. Chromatin-modulating agents as epigenetic anticancer drugs - 'the die is cast'. *Drug Discovery Today* **2006**, *11*, (3-4), 91-93.

5. Finishing the euchromatic sequence of the human genome. *Nature* **2004**, *431*, (7011), 931-945.

6. Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **1997**, *389*, (6648), 251-260.

7. Luger, K. Dynamic nucleosomes. *Chromosome Research* **2006**, *14*, (1), 5-16.

8. Alberts; Bray; Johnson; Lewis; Raff; Roberts; Walter. http://www.essentialcellbiology.com. *http://www. essentialcellbiology. com* **1998**,

9. Galm, O.; Herman, J. G.; Baylin, S. B. The fundamental role of epigenetics in hematopoietic malignancies. *Blood Reviews* **2006**, *20*, (1), 1-13.

10. Tremethick, D. Chromatin: the dynamic link between structure and function. *Chromosome Research* **2006**, *14*, (1), 1-4.

11. Clayton, A. L.; Hazzalin, C. A.; Mahadevan, L. C. Enhanced Histone Acetylation and Transcription: A Dynamic Perspective. *Molecular Cell* **2006**, *23*, (3), 289-296.

12. Inche, A. G.; La Thangue, N. B. Keynote review: Chromatin control and cancer-drug discovery: realizing the promise. *Drug Discovery Today* **2006**, *11*, (3-4), 97-109.

13. Strahl, B. D.; Allis, C. D. The language of covalent histone modifications. *Nature* **2000**, *403*, (6765), 41-45.

14. Couture, J. F.; Trievel, R. C. Histone-modifying enzymes: encrypting an enigmatic epigenetic code. *Current Opinion in Structural Biology* **2006**, *16*, (6), 753-760.

15. Berger, S. L. Histone modifications in transcriptional regulation. *Current Opinion in Genetics & Development* **2002**, *12*, (2), 142-148.

16. Imhof, A.; Yang, X. J.; Ogryzko, V. V.; Nakatani, Y.; Wolffe, A. P.; Ge, H. Acetylation of general transcription factors by histone acetyltransferases. *Current Biology* **1997**, *7*, (9), 689-692.

17. de Ruijter, A. J.; van Gennip, A. H.; Caron, H. N.; Kemp, S.; van Kuilenburg, A. B. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* **2003**, *370*, (Pt 3), 737-749.

18. Grozinger, C. M.; Schreiber, S. L. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. *Chemistry & Biology* **2002**, *9*, (1), 3-16.

19. Sengupta, N.; Seto, E. Regulation of histone deacetylase activities. *Journal of Cellular Biochemistry* **2004**, *93*, (1), 57-67.

20. Cress, W. D.; Seto, E. Histone deacetylases, transcriptional control, and cancer. *Journal of Cellular Physiology* **2000**, *184*, (1), 1-16.

21. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **1999**, *401*, (6749), 188-193.

22. Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, R. T.; Campbell, J. A.; Walker, K. A.; Martin, R.; Van Wart, H.; Browner, M. F. Crystal structures of MMP-1 and -13 reveal the structural basis for selectivity of collagenase inhibitors. *Nature Structural Biology* **1999**, *6*, (3), 217-221.

23. Vannini, A.; Volpari, C.; Filocamo, G.; Casavola, E. C.; Brunetti, M.; Renzoni, D.; Chakravarty, P.; Paolini, C.; De Francesco, R.; Gallinari, P.; Steinkuhler, C.; Di Marco, S. Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. *Proceedings of the National Academy of Sciences of the United States of America* **2004**, *101*, (42), 15064-15069.

24. Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B. C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. *Structure. (Camb.)* **2004**, *12*, (7), 1325-1334.

25. Stryer, L. *Biochemistry, 4th ed.,* **1999**, Freeman.

26. Stojan, J. *Enzyme Inhibitors,* Chapter 4, *Enzymes and Their Inhibition Drug Development,* **2005**, CRC Press.

27. Liu, T.; Kuljaca, S.; Tee, A.; Marshall, G. M. Histone deacetylase inhibitors: Multifunctional anticancer agents. *Cancer Treatment Reviews* **2006**, *32*, (3), 157-165.

28. Drummond, D. C.; Noble, C. O.; Kirpotin, D. B.; Guo, Z.; Scott, G. K.; Benz, C. C. Clinical development of histone deacetylase inhibitors as anticancer agents. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 495-528.

29. Johnstone, R. W.; Licht, J. D. Histone deacetylase inhibitors in cancer therapy: Is transcription the primary target? *Cancer Cell* **2003**, *4*, (1), 13-18.

30. Miller, T. A.; Witter, D. J.; Belvedere, S. Histone deacetylase inhibitors. *Journal of Medicinal Chemistry* **2003**, *46*, (24), 5097-5116.

31. Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* **1990**, *265*, (28), 17174-17179.

32. Marks, P. A. Discovery and development of SAHA as an anticancer agent. *Oncogene* **2007**, *26*, (9), 1351-1356.

33. Mori, K.; Koseki, K. Synthesis of trichostatin a, a potent differentiation inducer of friend leukemic cells, and its antipode. *Tetrahedron* **1988**, *44*, (19), 6013-6020.

34. Van Ommeslaeghe, K.; Elaut, G.; Brecx, V.; Papeleu, P.; Iterbeke, K.; Geerlings, P.; Tourwe, D.; Rogiers, V. Amide analogues of TSA: synthesis, binding mode analysis and HDAC inhibition. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (11), 1861-1864.

35. Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Cornell, W. D.; Green, M. A.; Howell, K. L.; Jung, M.; Kwon, P.; Trogani, N.; Walker, H. Inhibitors of human histone deacetylase: synthesis and enzyme and cellular activity of straight chain hydroxamates. *Journal of Medicinal Chemistry* **2002**, *45*, (4), 753-757.

36. Dai, Y.; Guo, Y.; Guo, J.; Pease, L. J.; Li, J.; Marcotte, P. A.; Glaser, K. B.; Tapang, P.; Albert, D. H.; Richardson, P. L.; Davidsen, S. K.; Michaelides, M. R. Indole amide hydroxamic acids as potent inhibitors of histone deacetylases. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (11), 1897-1901.

37. Woo, S. H.; Frechette, S.; Khalil, E. A.; Bouchain, G.; Vaisburg, A.; Bernstein, N.; Moradei, O.; Leit, S.; Allan, M.; Fournel, M.; Trachy-Bourget, M. C.; Li, Z.; Besterman, J. M.; Delorme, D. Structurally Simple Trichostatin A-Like Straight Chain Hydroxamates as Potent Histone Deacetylase Inhibitors. *Journal of Medicinal Chemistry* **2002**, *45*, (13), 2877-2885.

38. Bieliauskas, A. V.; Weerasinghe, S. V. W.; Pflum, M. K. Structural requirements of HDAC inhibitors: SAHA analogs functionalized adjacent to the hydroxamic acid. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (8), 2216-2219.

39. Marson, C. M.; Serradji, N.; Rioja, A. S.; Gastaud, S. P.; Alao, J. P.; Coombes, R. C.; Vigushin, D. M. Stereodefined and polyunsaturated inhibitors of histone deacetylase based on (2E,4E)-5-arylpenta-2,4-dienoic acid hydroxyamides. *Bioorganic & Medicinal Chemistry Letters* **2004**, *14*, (10), 2477-2481.

40. Charrier, C.; Bertrand, P.; Gesson, J. P.; Roche, J. Synthesis of rigid trichostatin A analogs as HDAC inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, (20), 5339-5344.

41. Marson, C. M.; Savy, P.; Rioja, A. S.; Mahadevan, T.; Mikol, C.; Veerupillai, A.; Nsubuga, E.; Chahwan, A.; Joel, S. P. Aromatic sulfide inhibitors of histone deacetylase based on arylsulfinyl-2,4-hexadienoic acid hydroxyamides. *Journal of Medicinal Chemistry* **2006**, *49*, (2), 800-805.

42. Kim, D. H.; Lee, J.; Kim, K. N.; Kim, H. J.; Jeung, H. C.; Chung, H. C.; Kwon, H. J. Anti-tumor activity of N-hydroxy-7-(2-naphthylthio) heptanomide, a novel histone deacetylase inhibitor. *Biochemical and Biophysical Research Communications* **2007**, *356*, (1), 233-238.

43. Mai, A.; Massa, S.; Rotili, D.; Pezzi, R.; Bottoni, P.; Scatena, R.; Meraner, J.; Brosch, G. Exploring the connection unit in the HDAC inhibitor pharmacophore model: Novel uracil-based hydroxamates. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, (21), 4656-4661.

44. Dai, Y.; Guo, Y.; Curtin, M. L.; Li, J.; Pease, L. J.; Guo, J.; Marcotte, P. A.; Glaser, K. B.; Davidsen, S. K.; Michaelides, M. R. A novel series of histone deacetylase inhibitors

incorporating hetero aromatic ring systems as connection units. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (21), 3817-3820.

45. Wada, C. K.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Garland, R. B.; Holms, J. H.; Li, J.; Pease, L. J.; Guo, J.; Glaser, K. B.; Marcotte, P. A.; Richardson, P. L.; Murphy, S. S.; Bouska, J. J.; Tapang, P.; Magoc, T. J.; Albert, D. H.; Davidsen, S. K.; Michaelides, M. R. Alpha-keto amides as inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (19), 3331-3335.

46. Marson, C. M.; Mahadevan, T.; Dines, J.; Sengmany, S.; Morrell, J. M.; Alao, J. P.; Joel, S. P.; Vigushin, D. M.; Charles Coombes, R. Structure-activity relationships of aryloxyalkanoic acid hydroxyamides as potent inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (1), 136-141.

47. Glaser, K. B.; Li, J.; Pease, L. J.; Staver, M. J.; Marcotte, P. A.; Guo, J.; Frey, R. R.; Garland, R. B.; Heyman, H. R.; Wada, C. K.; Vasudevan, A.; Michaelides, M. R.; Davidsen, S. K.; Curtin, M. L. Differential protein acetylation induced by novel histone deacetylase inhibitors. *Biochemical and Biophysical Research Communications* **2004**, *325*, (3), 683-690.

48. Suzuki, T.; Kouketsu, A.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. Thiol-based SAHA analogues as potent histone deacetylase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2004**, *14*, (12), 3313-3317.

49. Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Butcher, R. A.; Schreiber, S. L. Multidimensional Chemical Genetic Analysis of Diversity-Oriented Synthesis-Derived Deacetylase Inhibitors Using Cell-Based Assays. *Chemistry & Biology* **2003**, *10*, (5), 383-396.

50. Kahnberg, P.; Lucke, A. J.; Glenn, M. P.; Boyle, G. M.; Tyndall, J. D. A.; Parsons, P. G.; Fairlie, D. P. Design, Synthesis, Potency, and Cytoselectivity of Anticancer Agents Derived by Parallel Synthesis from α-Aminosuberic Acid. *Journal of Medicinal Chemistry* **2006**, *49*, (26), 7611-7622.

51. Belvedere, S.; Witter, D. J.; Yan, J.; Secrist, J. P.; Richon, V.; Miller, T. A. Aminosuberoyl hydroxamic acids (ASHAs): A potent new class of HDAC inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (14), 3969-3971.

52. Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; Ngo, L.; Civoli, F.; Breslow, R.; Rifkind, R. A.; Marks, P. A. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *Proceedings of the National Academy of Sciences of the United States of America* **1996**, *93*, (12), 5705-5708.

53. Jung, M.; Brosch, G.; Kolle, D.; Scherf, H.; Gerhauser, C.; Loidl, P. Amide Analogues of Trichostatin A as Inhibitors of Histone Deacetylase and Inducers of Terminal Cell Differentiation. *Journal of Medicinal Chemistry* **1999**, *42*, (22), 4669-4679.

54. Maeda, T.; Nagaoka, Y.; Kuwajima, H.; Seno, C.; Maruyama, S.; Kurotaki, M.; Uesato, S. Potent histone deacetylase inhibitors: N-hydroxybenzamides with antitumor activities. *Bioorganic & Medicinal Chemistry* **2004**, *12*, (16), 4351-4360.

55. Lu, Q.; Wang, D. S.; Chen, C. S.; Hu, Y. D.; Chen, C. S. Structure-Based Optimization of Phenylbutyrate-Derived Histone Deacetylase Inhibitors. *Journal of Medicinal Chemistry* **2005**, *48*, (17), 5530-5535.

56. Lavoie, R.; Bouchain, G.; Frechette, S.; Woo, S. H.; Khalil, E. A.; Leit, S.; Fournel, M.; Yan, P. T.; Trachy-Bourget, M. C.; Beaulieu, C. Design and synthesis of a novel class of histone deacetylase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2001**, *11*, (21), 2847-2850.

57. Bouchain, G.; Leit, S.; Frechette, S.; Khalil, E. A.; Lavoie, R.; Moradei, O.; Woo, S. H.; Fournel, M.; Yan, P. T.; Kalita, A.; Trachy-Bourget, M. C.; Beaulieu, C.; Li, Z.; Robert, M. F.; MacLeod, A. R.; Besterman, J. M.; Delorme, D. Development of Potential Antitumor Agents. Synthesis and Biological Evaluation of a New Set of Sulfonamide Derivatives as Histone Deacetylase Inhibitors. *Journal of Medicinal Chemistry* **2003**, *46*, (5), 820-830.

58. Massa, S.; Artico, M.; Corelli, F.; Mai, A.; Di Santo, R.; Cortes, S.; Marongiu, M. E.; Pani, A.; La Colla, P. Synthesis and antimicrobial and cytotoxic activities of pyrrole-containing analogues of trichostatin A. *Journal of Medicinal Chemistry* **1990**, *33*, (10), 2845-2849.

59. Massa, S.; Mai, A.; Sbardella, G.; Esposito, M.; Ragno, R.; Loidl, P.; Brosch, G. 3-(4-aroyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides, a new class of synthetic histone deacetylase inhibitors. *Journal of Medicinal Chemistry* **2001**, *44*, (13), 2069-2072.

60. Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Ragno, R.; Bottoni, P.; Scatena, R.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1-methyl-1*H*-2-pyrrolyl)-*N*-hydroxy-2-propenamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 2. Effect of Pyrrole-C_2 and/or -C_4 Substitutions on Biological Activity. *Journal of Medicinal Chemistry* **2004**, *47*, (5), 1098-1109.

61. Mai, A.; Massa, S.; Pezzi, R.; Simeoni, S.; Rotili, D.; Nebbioso, A.; Scognamiglio, A.; Altucci, L.; Loidl, P.; Brosch, G. Class II (IIa)-selective histone deacetylase inhibitors. 1. Synthesis and biological evaluation of novel (aryloxopropenyl)pyrrolyl hydroxyamides. *Journal of Medicinal Chemistry* **2005**, *48*, (9), 3344-3353.

62. Remiszewski, S. W.; Sambucetti, L. C.; Bair, K. W.; Bontempo, J.; Cesarz, D.; Chandramouli, N.; Chen, R.; Cheung, M.; Cornell-Kennon, S.; Dean, K.; Diamantidis, G.; France, D.; Green, M. A.; Howell, K. L.; Kashi, R.; Kwon, P.; Lassota, P.; Martin, M. S.; Mou, Y.; Perez, L. B.; Sharma, S.; Smith, T.; Sorensen, E.; Taplin, F.; Trogani, N.; Versace, R.; Walker, H.; Weltchek-Engler, S.; Wood, A.; Wu, A.; Atadja, P. N-hydroxy-3-phenyl-2-propenamides as novel inhibitors of human histone deacetylase with in vivo antitumor activity: discovery of (2E)-N-hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (NVP-LAQ824). *Journal of Medicinal Chemistry* **2003**, *46*, (21), 4609-4624.

63. Nagaoka, Y.; Maeda, T.; Kawai, Y.; Nakashima, D.; Oikawa, T.; Shimoke, K.; Ikeuchi, T.; Kuwajima, H.; Uesato, S. Synthesis and cancer antiproliferative activity of new histone deacetylase inhibitors: hydrophilic hydroxamates and 2-aminobenzamide-containing derivatives. *European Journal of Medicinal Chemistry* **2006**, *41*, (6), 697-708.

64. Shinji, C.; Nakamura, T.; Maeda, S.; Yoshida, M.; Hashimoto, Y.; Miyachi, H. Design and synthesis of phthalimide-type histone deacetylase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, (20), 4427-4431.

65. Lee, S.; Shinji, C.; Ogura, K.; Shimizu, M.; Maeda, S.; Sato, M.; Yoshida, M.; Hashimoto, Y.; Miyachi, H. Design, synthesis, and evaluation of isoindolinone-hydroxamic acid

derivatives as histone deacetylase (HDAC) inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (17), 4895-4900.

66. Witter, D. J.; Belvedere, S.; Chen, L.; Secrist, J. P.; Mosley, R. T.; Miller, T. A. Benzo[b]thiophene-based histone deacetylase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (16), 4562-4567.

67. Kim, H. M.; Ryu, D. K.; Choi, Y.; Park, B. W.; Lee, K.; Han, S. B.; Lee, C. W.; Kang, M. R.; Kang, J. S.; Boovanahalli, S. K.; Park, S. K.; Han, J. W.; Chun, T. G.; Lee, H. Y.; Nam, K. Y.; Choi, E. H.; Han, G. Structure-Activity Relationship Studies of a Series of Novel δ-Lactam-Based Histone Deacetylase Inhibitors. *Journal of Medicinal Chemistry* **2007**, *50*, (11), 2737-2741.

68. Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N.; Saito, A.; Mariko, Y.; Yamashita, T.; Nakanishi, O. Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives. *Journal of Medicinal Chemistry* **1999**, *42*, (15), 3001-3003.

69. Wong, J. C.; Hong, R.; Schreiber, S. L. Structural Biasing Elements for In-Cell Histone Deacetylase Paralog Selectivity. *Journal of the American Chemical Society* **2003**, *125*, (19), 5586-5587.

70. Moradei, O.; Leit, S.; Zhou, N.; Frechette, S.; Paquin, I.; Raeppel, S.; Gaudette, F.; Bouchain, G.; Woo, S. H.; Vaisburg, A. Substituted N-(2-aminophenyl)-benzamides, (E)-N-(2-aminophenyl)-acrylamides and their analogues: Novel classes of histone deacetylase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, (15), 4048-4052.

71. Lu, Q.; Yang, Y. T.; Chen, C. S.; Davis, M.; Byrd, J. C.; Etherton, M. R.; Umar, A.; Chen, C. S. Zn^{2+}-Chelating, Motif-Tethered, Short-Chain Fatty Acids as a Novel Class of Histone Deacetylase Inhibitors. *Journal of Medicinal Chemistry* **2004**, *47*, (2), 467-474.

72. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Research* **2002**, *62*, (17), 4916-4921.

73. Jose, B.; Oniki, Y.; Kato, T.; Nishino, N.; Sumida, Y.; Yoshida, M. Novel histone deacetylase inhibitors: cyclic tetrapeptide with trifluoromethyl and pentafluoroethyl ketones. *Bioorganic & Medicinal Chemistry Letters* **2004**, *14*, (21), 5343-5346.

74. Liu, T.; Kapustin, G.; Etzkorn, F. A. Design and Synthesis of a Potent Histone Deacetylase Inhibitor. *Journal of Medicinal Chemistry* **2007**, *50*, (9), 2003-2006.

75. Loudni, L.; Roche, J.; Potiron, V.; Clarhaut, J.; Bachmann, C.; Gesson, J. P.; Tranoy-Opalinski, I. Design, synthesis and biological evaluation of 1,4-benzodiazepine-2,5-dione-based HDAC inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (17), 4819-4823.

76. Suzuki, T.; Nagano, Y.; Matsuura, A.; Kohara, A.; Ninomiya, S. i.; Kohda, K.; Miyata, N. Novel histone deacetylase inhibitors: design, synthesis, enzyme inhibition, and binding mode study of SAHA-Based non-hydroxamates. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (24), 4321-4326.

77. Frey, R. R.; Wada, C. K.; Garland, R. B.; Curtin, M. L.; Michaelides, M. R.; Li, J.; Pease, L. J.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J. Trifluoromethyl ketones as inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2002**, *12*, (23), 3443-3447.

78. Suzuki, T.; Matsuura, A.; Kouketsu, A.; Nakagawa, H.; Miyata, N. Identification of a potent non-hydroxamate histone deacetylase inhibitor by mechanism-based drug design. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, (2), 331-335.

79. Gu, W.; Nusinzon, I.; Smith, J.; Horvath, C. M.; Silverman, R. B. Carbonyl- and sulfur-containing analogs of suberoylanilide hydroxamic acid: Potent inhibition of histone deacetylases. *Bioorganic & Medicinal Chemistry* **2006**, *14*, (10), 3320-3329.

80. Anandan, S. K.; Ward, J. S.; Brokx, R. D.; Bray, M. R.; Patel, D. V.; Xiao, X. X. Mercaptoamide-based non-hydroxamic acid type histone deacetylase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, (8), 1969-1972.

81. Wu, T. Y. H.; Hassig, C.; Wu, Y.; Ding, S.; Schultz, P. G. Design, synthesis, and activity of HDAC inhibitors with a N-formyl hydroxylamine head group. *Bioorganic & Medicinal Chemistry Letters* **2004**, *14*, (2), 449-453.

82. Suzuki, T.; Hisakawa, S.; Itoh, Y.; Maruyama, S.; Kurotaki, M.; Nakagawa, H.; Miyata, N. Identification of a potent and stable antiproliferative agent by the prodrug formation of a thiolate histone deacetylase inhibitor. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (6), 1558-1561.

83. Vasudevan, A.; Ji, Z.; Frey, R. R.; Wada, C. K.; Steinman, D.; Heyman, H. R.; Guo, Y.; Curtin, M. L.; Guo, J.; Li, J.; Pease, L.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J.; Davidsen, S. K.; Michaelides, M. R. Heterocyclic ketones as inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (22), 3909-3913.

2 The Oxygen Series

The initial study of this project consisted of the synthesis of a series of potential HDAC inhibitors containing a common aryloxyalkanoic acid hydroxyamide motif[1] – more easily referred to as the oxygen series.

2.1 Synthesis

The general form of this series was selected by beginning from the structure of trichostatin A (TSA) and the frequently observed mechanical formula of Zn-binding group – linker – capping group (discussed previously). When considering the mode of attachment of the alkyl chain to the aromatic cap, it was noted that a benzyl ketone is present in TSA – previous work has shown that this may be replaced with a variety of functional groups, including a sulfoxide, thioether or aromatic ether without harming inhibitory activity. The structurally most simple example phenyl ether 10 showed some promise,[2] with moderate inhibitory activity. Biphenyl analogue 11 showed a greater than ten-fold increase in activity (figure 2.1).[3]
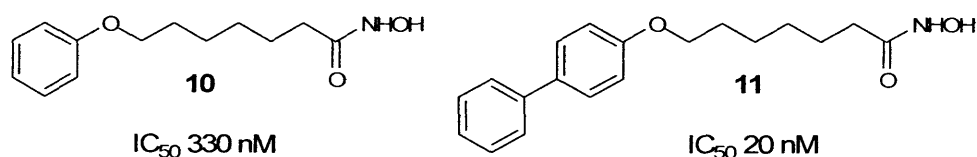
Figure 2.1. Known arylether hydroxamates.

Further work within this group focusing on development of this framework had led to the identification of compounds **12** and **13** (figure 2.2) as extremely potent in isolated enzyme assays.
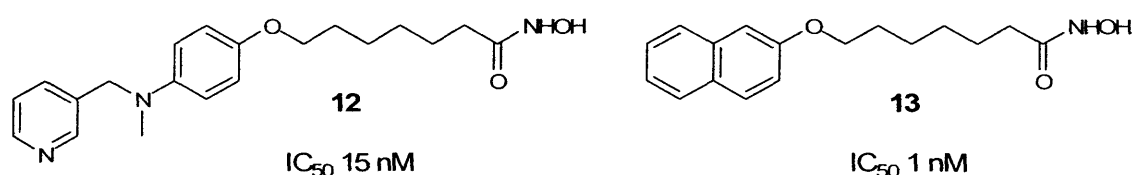
Figure 2.2. Previously synthesised oxygen series compounds.

Despite the excellent activity of **12** and **13**, performance in cellular assays remained modest. It was from this starting point that the oxygen series study began, with the aim of developing the identified active framework to improve cellular activity of this class of compounds. It was decided that the series designed to explore the potential of this template should contain a terminal *N*-hydroxyamide, a saturated alkyl chain linking to an aromatic group capable of expedient diversification. With this decided, it was noted that the *p*-amino group of **12** would retain the structural analogy to TSA and provide a point for variation from its dimethylamine (figure 2.3).
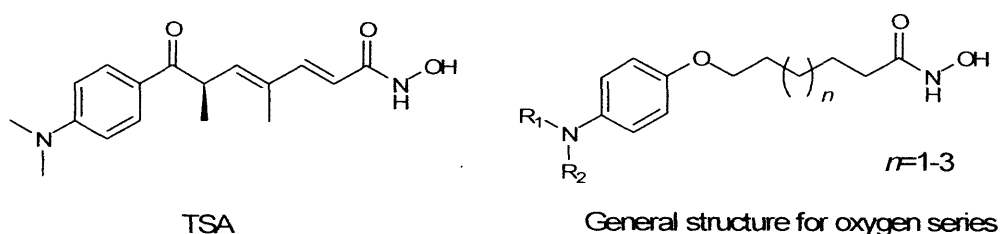
Figure 2.3. Structural basis of the oxygen series.

For this arrangement the key intermediate amines **51-53** could be readily reached from the commercially available 4-nitrophenol and alkyl bromides **(45-47)**. A Williamson ether synthesis, with caesium carbonate and refluxing in DMF, gave the various ethers **(48-50)** (scheme 2.1). The chain length of the initial alkyl bromide was the first point of variation for the structure-activity relationship study, but the chain always contained a terminal ester ready for later conversion to the hydroxamic acid. TSA possesses a linker seven carbons in length, so oxygen series variants with six, seven and eight carbon linkers were synthesised to determine the optimum length. Both the ethyl-6-bromohexanoate **(45)** and ethyl-7-bromoheptanoate **(46)** starting materials (scheme 2.1) were commercially available. The known ester methyl-8-bromooctanoate **(47)**[4] was easily obtained in from the commercially available acid **(54)** by refluxing in 4% conc. H_2SO_4 in methanol (scheme 2.2), basic work-up providing the pure compound.

With the ether link installed in excellent yield, reduction of the nitro group by catalytic hydrogenolysis using 5% palladium on carbon provided the aromatic amine in good to excellent yield and requiring no further purification after removal of catalyst by filtration.

	n	R	
45	1	Et	
46	2	Et	
47	3	Me	

	n	R	
48	1	Et	85 %
49	2	Et	90 %
50	3	Me	97 %

	n	R	
51	1	Et	83 %
52	2	Et	62 %
53	3	Me	95 %
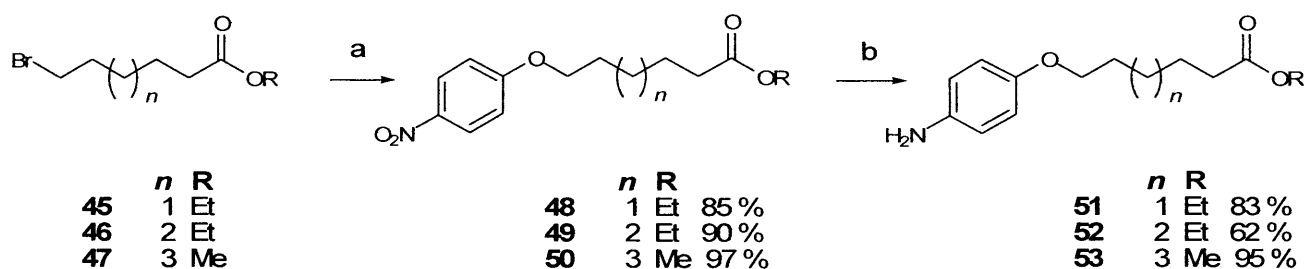
(a) 4-Nitrophenol, Cs_2CO_3, DMF; reflux; (b) H_2, 5 % Pd/C, EtOH

Scheme 2.1. Synthesis of intermediate amines **51-53**.
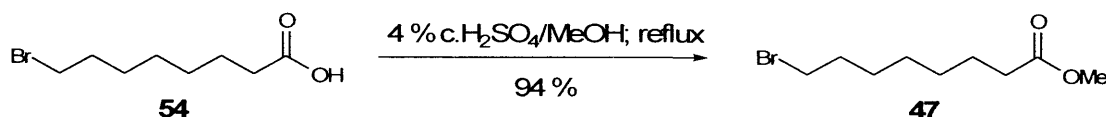
Scheme 2.2. Synthesis of methyl-8-bromooctanoate.

The direct analogue of TSA, possessing the dimethylamino cap ($R^1=R^2=Me$), was a logical compound to begin the series. The intermediate amine (52) provided a straightforward point for divergence of structure at the cap region through alkylation or acylation, the dimethylamino compound being available through double reductive alkylation of amine 52 (scheme 2.3). A solution of aqueous formaldehyde, sodium cyanoborohydride and catalytic acetic acid gave the ester 55 in good yield.
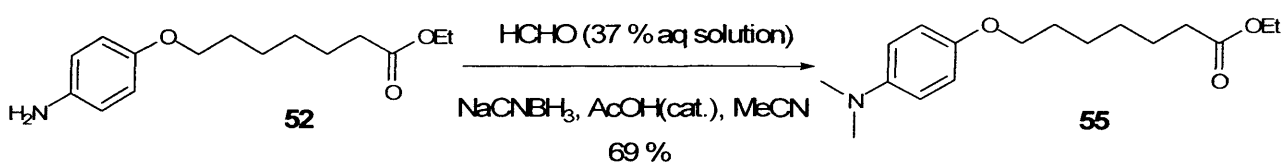
Scheme 2.3. Dimethylamine 55.

A standard procedure was employed with all compounds of this series for the conversion of the ethyl esters (or methyl ester, in the case of 47) into the corresponding hydroxamic acids. Moderate to poor yields of the hydroxamic acids were obtained from the addition of methanolic solutions of hydroxylamine and potassium hydroxide to solutions of the esters in THF with stirring typically for at least one day. Purification of these highly polar compounds was initially problematic. Extremely poor recovery was experienced with column chromatography, and poor solubility in organic solvents eliminated the possibility of recrystallisation in most cases. The insoluble nature of the compounds was eventually utilised through the repeated trituration of the hydroxamic acids in various solvents to wash out any impurities and provide analytically pure material.

The amines 51-53 provided very few restrictions on possible functionalisation of our cap group, leaving us with a wide range of possibilities. Investigation into the SAR of another HDAC inhibitor, NVP-LAQ824 had shown inclusion of an indole group to be necessary for cellular activity (figure 2.4, discussed in the introduction),[5] and although there is little

structural similarity between NVP-LAQ824 and our series, it seemed an interesting point to investigate.
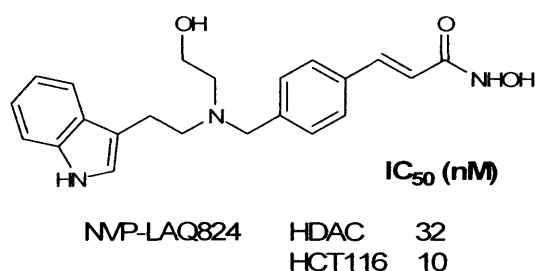
Figure 2.4. Indole-containing compound NVP-LAQ824.[5]

Acylation of amine **52** with indole-3-acetic acid using the coupling system of EDCI, HOBT and NMM as base in DMF proceeded in good yield, as did reaction with indole-3-propionic acid under the same conditions to produce the more flexibly linked analogue (**57** and **58** respectively, scheme 2.4). The six-carbon and eight-carbon chain analogues were acylated under the same conditions in excellent yield (**56** and **59** respectively). These esters, after recrystallisation from ethanol were converted to the hydroxamic acids in the standard manner.
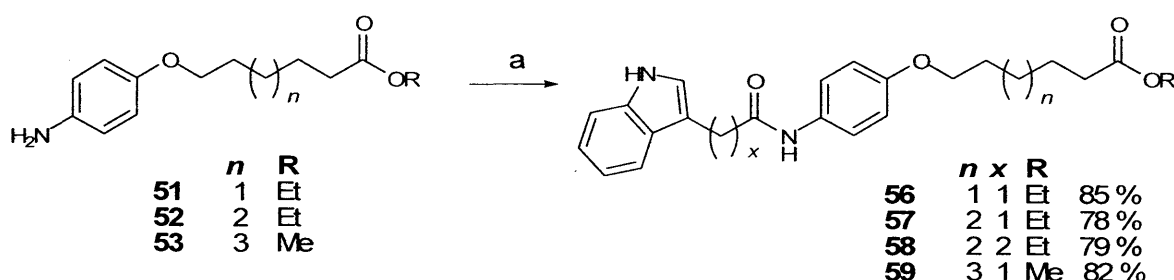
(a) **56, 57, 58**; x=1: Indole-3-acetic acid, EDC.HCl, HOBT, NMM, DMF; **58**; x=2: indole-3-propionic acid, EDC.HCl, HOBT, NMM, DMF.

Scheme 2.4. Acylation of amines **51-53**.

Compound **60**, the amine analogue of amide **57**, was not available from the reduction of **57** with borane-THF complex or sodium borohydride under standard conditions. Reduction of a similar amide using sodium borohydride in the presence of acetic acid had been reported,[5] and this approach proved successful with warming to 45 °C and stirring for 17 h (scheme 2.5). The modest yield in this reaction appeared to be due, at least in part, to the unwanted reduction of the ester to a small extent.

(a)(i) AcOH, NaBH₄, THF; (ii) **57**, THF, 45 °C.
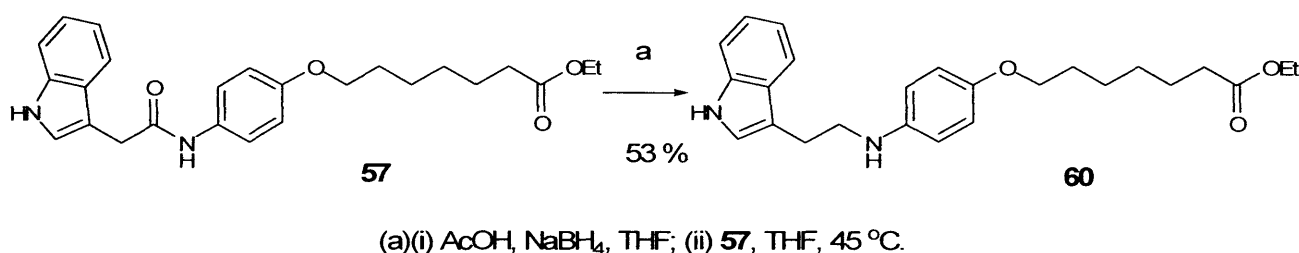
Scheme 2.5. Reduction of amide **57**.

Derivatization of the series continued with attachment of a pyridine moiety to the cap region, the lead compound **12** warranting further investigation of this system. The secondary amine **61** (scheme 2.6) was the product of a reductive alkylation of primary amine **52** with 4-pyridinecarboxaldehyde. The imine formed from mixing the aldehyde and amine in methanol was reduced by addition of sodium cyanoborohydride and zinc chloride to give **61** in good yield.

An indole was incorporated by acylation with indole-3-acetic acid or indole-3-propionic acid to give compounds **62** and **63** respectively. Initial attempts to carry out this acylation under the conditions used for **56-59**, using EDCI.HCl/HOBT, were unsuccessful - as were attempts using BOP. An excellent yield, however, resulted from the use of isobutyl chloroformate to activate the acid, with *N*-methylmorpholine in THF. The more flexibly-linked compound **63** was converted to the hydroxamic acid in the usual way, however, the shorter **62** proved problematic. The reaction in this case proceeded as expected, but the crude product after work-up was a viscous oil. Purification by column chromatography was

unsuccessful owing to the extremely polar and insoluble nature of the compound, and as recrystallisation or washing of the product was not an option this compound remained unpurified and so is not included in this series.

(a) (i) 4-Pyridinecarboxaldehyde, MeOH; (ii) NaCNBH$_3$, ZnCl$_2$; (b) n=1: 3-indoleacetic acid, NMM, isobutyl chloroformate, THF; n=2: 3-indolepropionic acid, NMM, isobutyl chloroformate, THF.
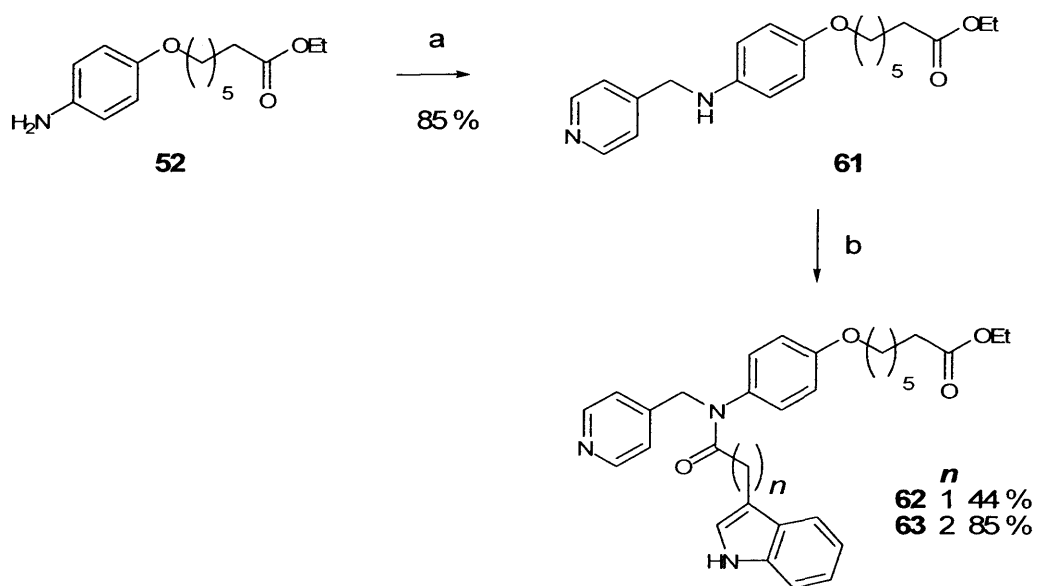
Scheme 2.6. Reductive alkylation and acylation of amine **52**.

The amide **63** was seen as a useful potential stepping stone to a tertiary amine analogue. It was hoped that selective reduction of the amide carbonyl would provide the amine analogue **64**, ready for conversion to the hydroxamic acid (scheme 2.7). Unfortunately, attempts at reduction with BH$_3$.THF or the previously successful use of sodium borohydride in the presence of acetic acid proved fruitless.
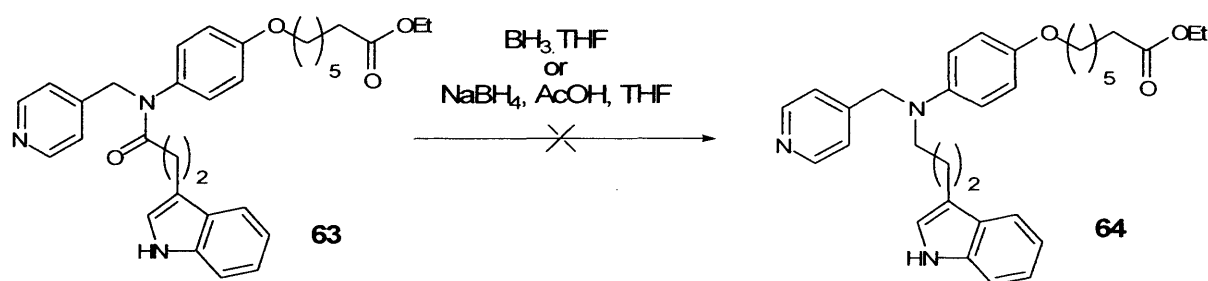
Scheme 2.7. Attempted selective reduction of amide.

It was decided, therefore, that the more vigorous reduction with lithium aluminium hydride would be required, followed by re-oxidation of the resultant alcohol **65** (scheme 2.8).
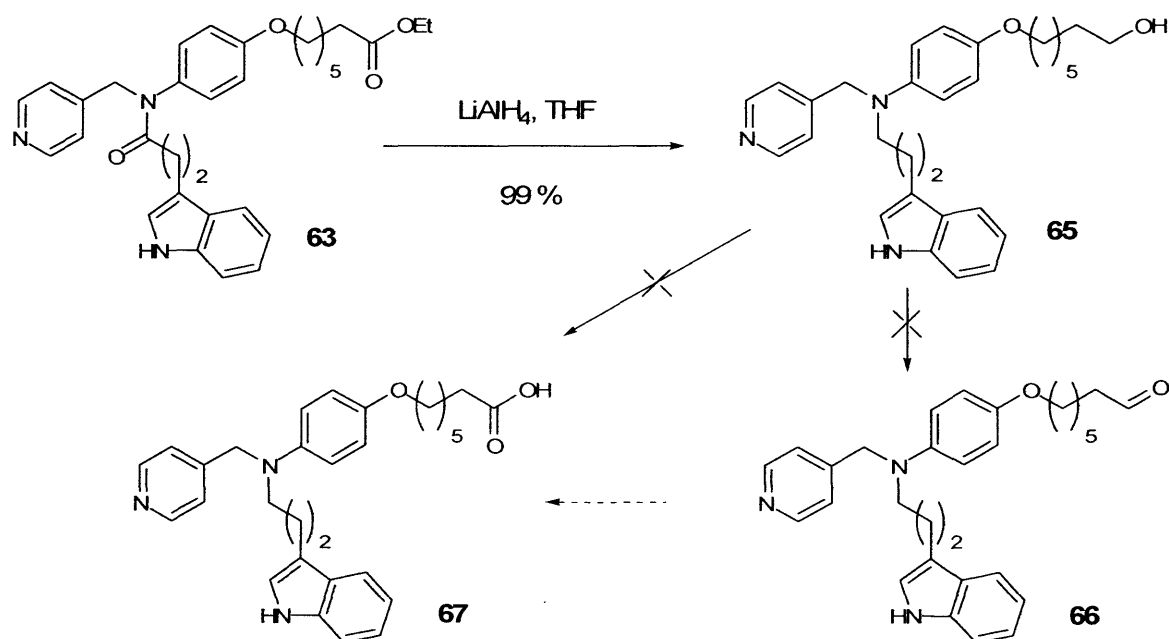
Scheme 2.8. Reduction and attempted re-oxidation.

Whilst the aggressive reduction proceeded in excellent yield, oxidation was less successful. Attempts were made to reach the acid directly using ruthenium (III) chloride, PDC in DMF

and TEMPO, all without success. Further unsuccessful attempts to reach the aldehyde as an intermediate were made using PDC in dichloromethane or PCC in dichloromethane.

Returning to the intermediate primary amine **52**, an alternative attachment of an indole group was possible from reductive amination of indole-5-carboxaldehyde to give **68** (scheme 2.9). The highly functionalised analogue **69** was selected with the aim of improving water solubility, as an analogue that retained the pyridyl group. In these cases, substitution of sodium borohydride for the cyanoborohydride – zinc chloride system was found to be preferential. Both esters were then converted to the hydroxamic acids.

(a)(i) Pyridoxal hydrochloride, Et₃N, MeOH; (ii) NaBH₄;
(b)(i) Indole-5-carboxaldehyde, MeOH; (ii) NaBH₄.
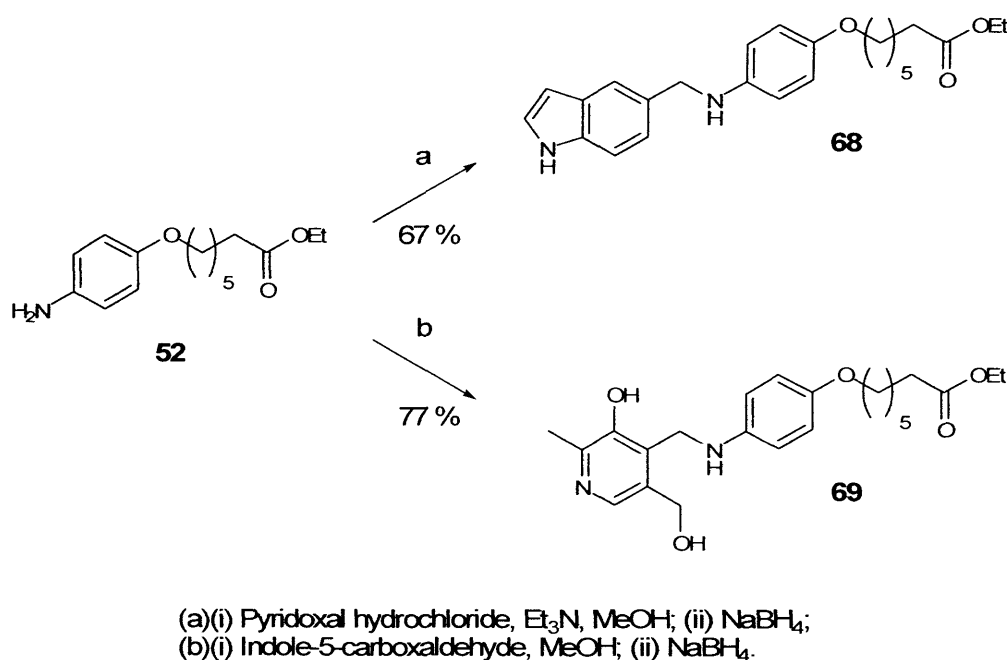
Scheme 2.9. Additional reductive alkylations.

Another interesting compound (**71**) was available through *O*-alkylation of 5-hydroxyindole (**70**) in the place of 4-nitrophenol (scheme 2.9). It was observed that inclusion of the potentially desirable indole moiety in this fashion provided an annulated analogue which fitted perfectly with the general structure planned for this series as it retained the 4-aminophenyl structure. Only one step was required to reach the target ester, ready for

hydroxamation – the Williamson ether synthesis with 5-hydroxyindole and ethyl-7-bromoheptanoate providing an acceptable yield of the ester under standard conditions (scheme 2.10).
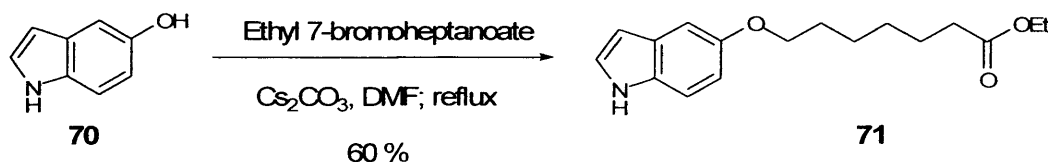
Scheme 2.10. Alkylation of 5-hydroxyindole.

With a range of groups now installed in the cap region, a final study into any effects of electron-withdrawing substituents on the benzene ring was desired. Fluorine atoms present at the 2- or 3-position on the ring will exert an influence on the electron densities of the aromatic amine and ether linkages, and so may influence the biological stability of the compound. With the commercial availability of both 2- and 3-fluoro-4-nitrophenol no change in synthetic route was necessary. Both compounds successfully underwent the Williamson ether synthesis and catalytic hydrogenolysis in high to excellent yields (scheme 2.11).

	R_1	R_2	
72	F	H	79 %
73	H	F	74 %

	R_1	R_2	
74	F	H	89 %
75	H	F	95 %
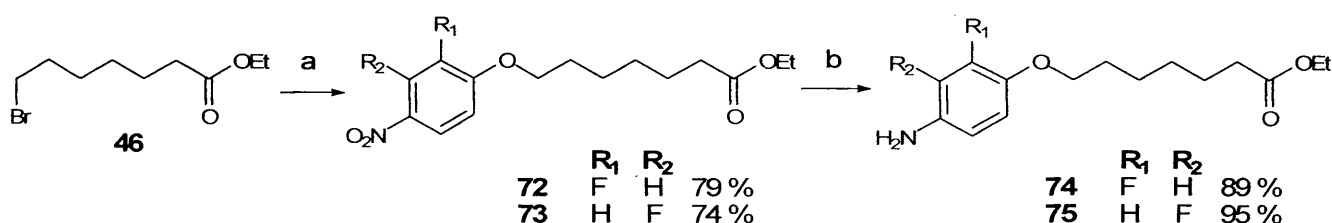
(a) R_1=F: 2-Fluoro-4-nitrophenol, Cs_2CO_3, DMF; reflux; R_2=F: 3-fluoro-4-nitrophenol, Cs_2CO_3, DMF; reflux;
(b) H_2, 5 % Pd/C, EtOH

Scheme 2.11. Incorporation of electron-withdrawing substituents on the benzene ring.

The amines **74** and **75** underwent acylation under the conditions previously used to provide

the analogues **76** and **77** in excellent yield (scheme 2.12).
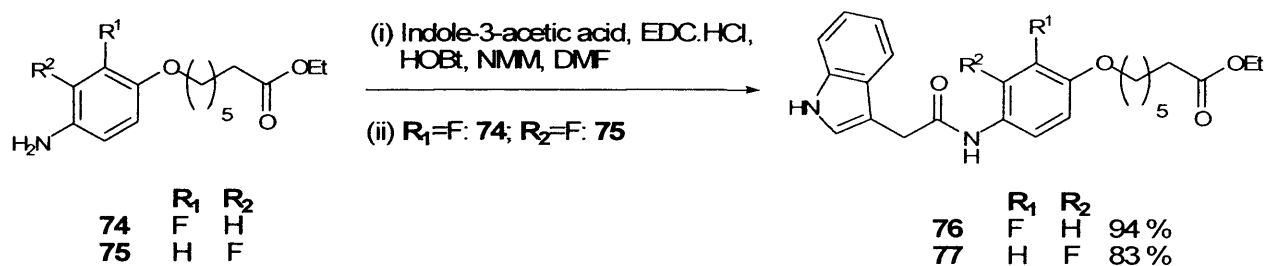
Scheme 2.12. Acylation of fluoro analogues.

2.2 Results

Conversion of all esters to the hydroxamic acids under the conditions previously described

provided a systematically varied range of compounds with which to begin our analysis of

histone deacetylase inhibition. Un-optimised yields for the final conversion of esters to

hydroxamic acids along with results from the isolated enzyme assay are given in table 2.1.

R—O⫯⫯⫯ⁿ—C(=O)—NH-OH
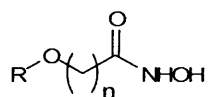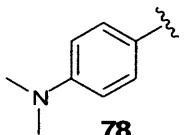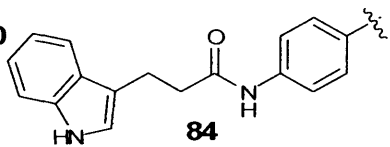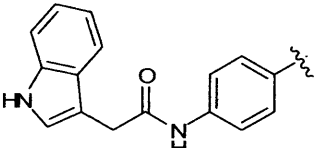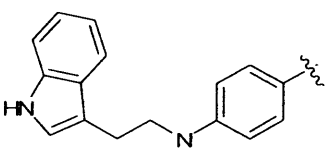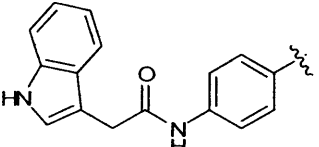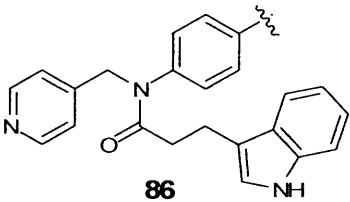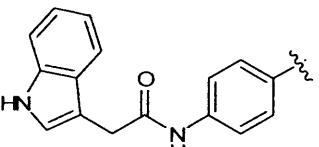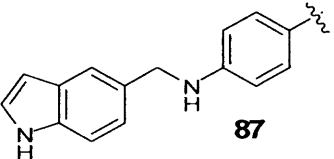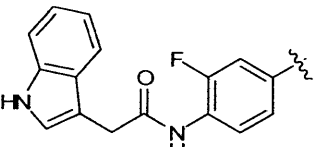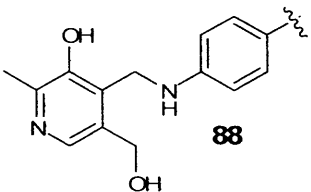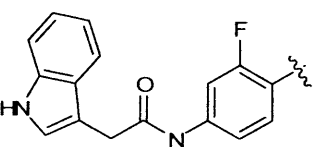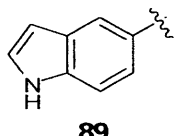
R	n	Yield	IC$_{50}$ (nM)	R	n	Yield	IC$_{50}$ (nM)
78	6	38 %	18.8 ± 6.0	**84**	6	39 %	16.0 ± 5.0
79	5	59 %	50 ± 2.1	**85**	6	69 %	6.2 ± 3.5
80	6	36 %	12.8 ± 5.2	**86**	6	81 %	16.4 ± 8.0
81	7	42 %	22.4 ± 9.0	**87**	6	59 %	14.3 ± 5.7
82	6	28 %	13.1 ± 5.0	**88**	6	59 %	22.0 ± 12.0
83	6	77 %	23.5 ± 9.0	**89**	6	66 %	3.6 ± 1.8

Table 2.1. Hydroxamic acids of the oxygen series with percentage yields and HDAC IC$_{50}$ values.

Biological evaluation of most of these compounds was carried out by Alao and Vigushin at Imperial College and some others by Joel at St. Bartholomew's Hospital, London. All compounds of the oxygen series proved to be potent, low nanomolar inhibitors of histone deacetylase in isolated enzyme assays.

The 4-dimethylamino cap group of **78** (analogous to that of TSA) showed excellent activity, being dramatically more active than the known unsubstituted analogue **10** (figure 2.1). Comparison of the three indolylacetamide compounds **79-81** showed that the seven-carbon chain length provided optimal activity in this case, although six and eight-carbon chains were tolerated reasonably well. Fluorination of the aryl ring in the 3-position **(82)** showed no change in activity, however, in the 2-position **(83)** a moderate reduction in potency was observed. Increasing flexibility of the attached indole group with **84** did not appear to affect activity, and somewhat surprisingly, increasing the aromatic character of the cap by inclusion of the pyridyl group of **86** did not improve activity.

Reduction of the amide to secondary amine **85** would also allow greater flexibility in addition to changing potential interactions at the cap region, this compound showing greater potency than the corresponding amide **80**. The alternative attachment of the indole group in amine **87**, with reduced flexibility and changed position of the indole-amine still showed excellent activity. The more polar cap group of **88** was tolerated, but did not increase activity. It was the simple indole **89**, which retains the 4-amino function (as with TSA analogue **78**) but with the addition of a fused ring, that showed the greatest potency of this series.

Antiproliferation assays were also carried out on compounds **85** and **89** with two cancer cell lines. With HeLa cells, both compounds gave an IC_{50} of 7 μM, and with MCF-7 cells compound **85** again showed an IC_{50} of 7 μM and **89** 5 μM.

Work in this series produced some very promising results in the form of excellent activity in the isolated enzyme assay. The general structure was clearly a suitable basis for development of compounds with high affinity for the enzyme, and it was indeed possible to

identify one compound in particular which displayed not only excellent activity, but was synthetically simple to make and structurally very efficient. The indole **89** appeared to be an ideal candidate for further development, but the trend that was observed with the series with respect to cellular activity was unsatisfactory. TSA gives nanomolar activity in the isolated cell assay and this transfers to still nanomolar activity in cell-based assays. The oxygen series developed compounds with activities in the same range as TSA, but this did not track in results from cellular assays. The trend observed was of reduction in potency to micromolar figures, the best activity achieved being 5µM in one cell line. With this trend still being observed despite our efforts to optimise the series, it was felt that a problem was likely to lie with the general aryl-ether structure when it came to cellular penetration. No further development of this series was carried out, but it is possible that were the cap-group development undertaken here to be transferred to a different linker-structure, improvements in cellular activity may be achieved.

2.3 References

1. Marson, C. M.; Mahadevan, T.; Dines, J.; Sengmany, S.; Morrell, J. M.; Alao, J. P.; Joel, S. P.; Vigushin, D. M.; Charles Coombes, R. Structure-activity relationships of aryloxyalkanoic acid hydroxyamides as potent inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (1), 136-141.

2. Dai, Y.; Guo, Y.; Curtin, M. L.; Li, J.; Pease, L. J.; Guo, J.; Marcotte, P. A.; Glaser, K. B.; Davidsen, S. K.; Michaelides, M. R. A novel series of histone deacetylase inhibitors incorporating hetero aromatic ring systems as connection units. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (21), 3817-3820.

3. Wada, C. K.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Garland, R. B.; Holms, J. H.; Li, J.; Pease, L. J.; Guo, J.; Glaser, K. B.; Marcotte, P. A.; Richardson, P. L.; Murphy, S. S.; Bouska, J. J.; Tapang, P.; Magoc, T. J.; Albert, D. H.; Davidsen, S. K.; Michaelides, M. R. Alpha-keto amides as inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (19), 3331-3335.

4. Takahashi, T.; Mizuno, T.; Ido, T.; Iwata, R.; Watanabe, K. i. Improved synthesis of pure [18F]fluoro-compounds for PET studies from bromo-compounds. *Applied Radiation and Isotopes* **2003**, *58*, (5), 557-566.

5. Remiszewski, S. W.; Sambucetti, L. C.; Bair, K. W.; Bontempo, J.; Cesarz, D.; Chandramouli, N.; Chen, R.; Cheung, M.; Cornell-Kennon, S.; Dean, K.; Diamantidis, G.; France, D.; Green, M. A.; Howell, K. L.; Kashi, R.; Kwon, P.; Lassota, P.; Martin, M. S.; Mou, Y.; Perez, L. B.; Sharma, S.; Smith, T.; Sorensen, E.; Taplin, F.; Trogani, N.; Versace, R.; Walker, H.; Weltchek-Engler, S.; Wood, A.; Wu, A.; Atadja, P. N-hydroxy-3-phenyl-2-propenamides as novel inhibitors of human histone deacetylase with in vivo antitumor activity: discovery of (2E)-N-hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (NVP-LAQ824). *Journal of Medicinal Chemistry* **2003**, *46*, (21), 4609-4624.

3 Cyclic-Linker Non-Hydroxamates

Work in this chapter focuses on the exploration of new zinc-binding moieties. The development initially began utilising the frame of the known potent inhibitor MGCD0103 (figure 3.1).

R=

MGCD0103   90   91   92   93   94   95
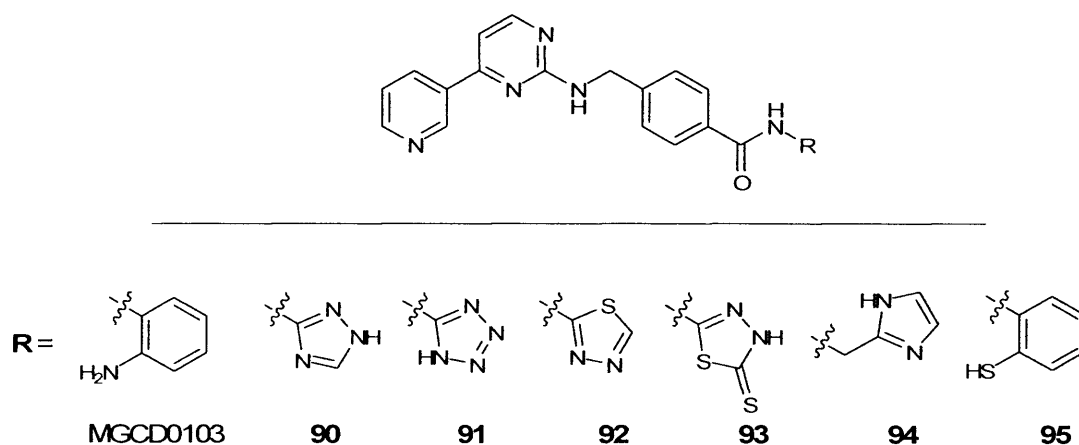
Figure 3.1. Exploration of new potential Zn-binding groups.

Whilst the hydroxamic acid moiety present in many HDAC inhibitors provides an excellent zinc-binding functionality, its presence in a drug can lead to undesired characteristics such as low oral bio-availability and short half-life *in vivo*.[1] With this in mind, there is a clear need to discover alternative groups capable of chelating the Zn^{2+} ion with high affinity and which do not produce the undesired physiological affects of hydroxamic acids. By considering the mode in which the heteroatoms coordinate the metal ion, it seems plausible that amino heterocycles (**90-95**) could provide suitable alternatives (figure 3.2).

Figure 3.2. Suggested potential mode of binding of new end groups.

The imidazole group has been shown to function as a zinc-binding group when incorporated in inhibitors of the enzyme farnesyl transferase, which also utilises a zinc ion at the active site.[2] With this in mind, an additional, structurally simple, example of an imidazole-containing compound, **96**, was synthesised (figure 3.3).
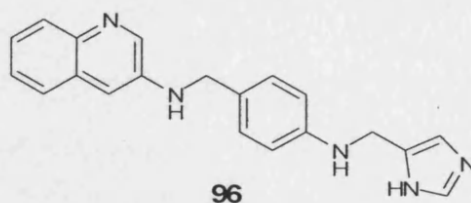
Figure 3.3. Additional imidazole-containing target compound.

Additional targets **97** and **98** were also desired for evaluation of the novel end groups, however, despite synthetic effort towards these compounds the final products were unfortunately not reached (figure 3.4).
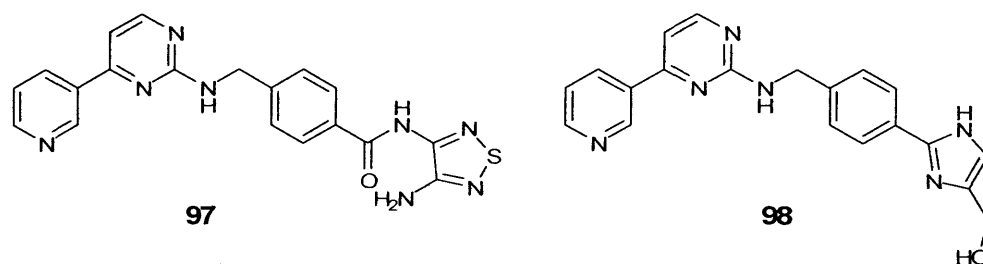
Figure 3.4. Additional planned targets.

Although synthesis of hydroxymethylimidazole **98** was not completed, synthesis of interesting intermediate compounds suggested another possible zinc-binding group. It seemed surprising that no examples of compounds bearing the amidoxime functionality of **99** (figure 3.5) had been previously reported when considering the similarity to a hydroxamic acid. This compound was duly synthesised and evaluated. In addition to the MGCD0103 target a small number of synthetically simple amidoximes were also synthesised.
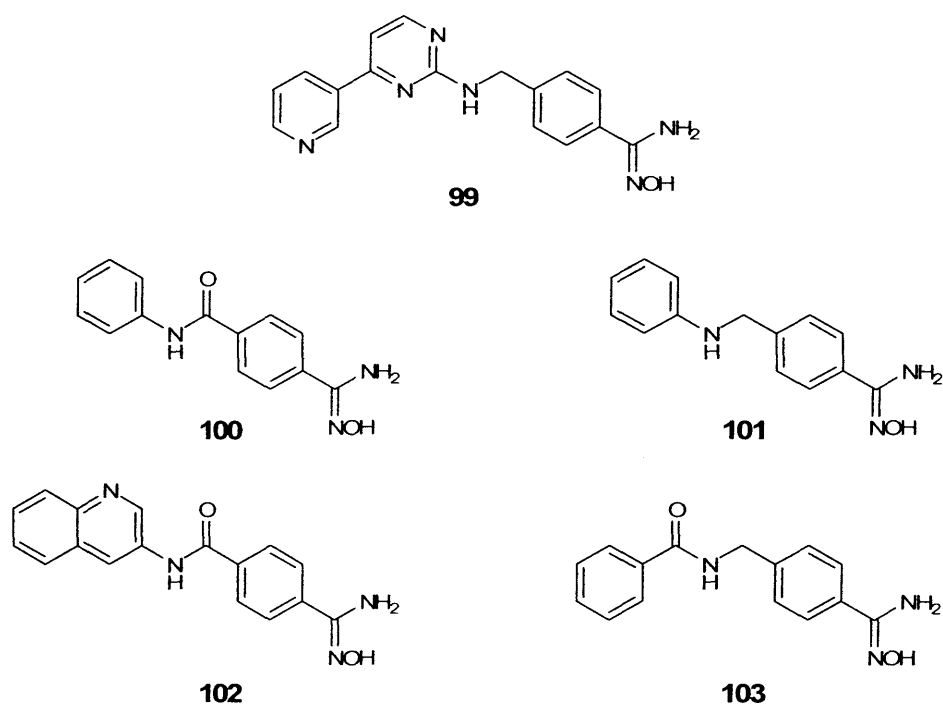
Figure 3.5. Amidoxime compounds.

A final example of this class was synthesised in an attempt to directly compare the activity of the amidoxime group with that of a hydroxamic acid. The direct amidoxime analogue of SAHA (**104**, figure 3.6) was selected for this purpose and shall be included here for the purpose of comparison with other amidoximes despite it not containing a cyclic linker.
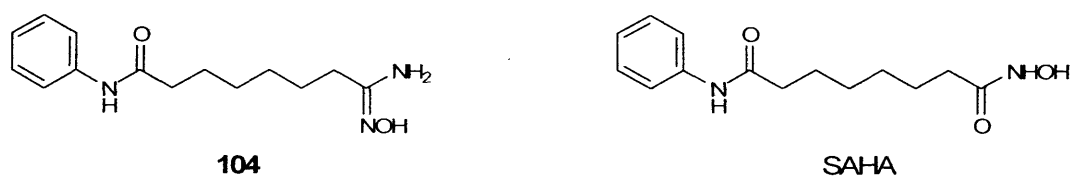
Figure 3.6. Amidoxime analogue of SAHA.

3.1 Synthesis

As the many of the compounds in this chapter are structurally based on MGCD0103, it was necessary to have a sample of the original compound for comparison of biological properties. The patented route[3] of Methylgene to this compound was followed without difficulty (scheme 3.1).
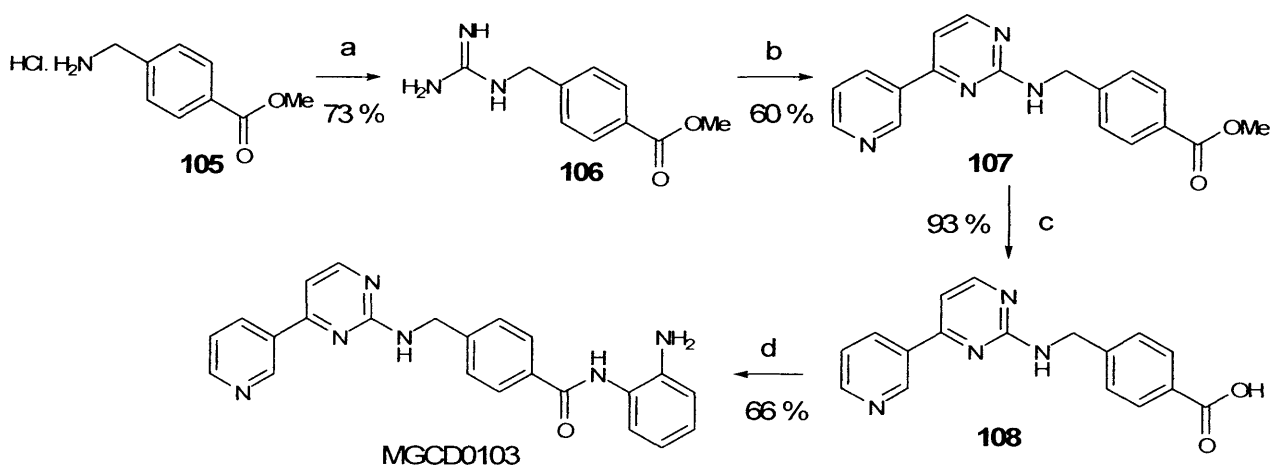
(a) Pyrazole carboxamidine.HCl, DIPEA, DMF; 5 h, r.t.; (b), **110**, ¹PrOH; 20 h, reflux; (c) LiOH, THF, MeOH, H₂O; 1 d, r.t.; (d) 1, 2-Phenylenediamine, BOP, Et₃N, DMF; 1 d, r.t.

Scheme 3.1. Synthesis of MGCD0103.

Guanidine **106** was the product of stirring a solution of commercially available amine **105** and pyrazole carboxamidine hydrochloride with an amine base in DMF at room temperature. Condensation of **106** with **110** (produced in one step from 3-acetylpyridine (**109**) by refluxing in dimethylformamide dimethylacetal, scheme 3.2) gave the aminopyrimidine **107**, requiring only trituration with ethyl acetate for purification.
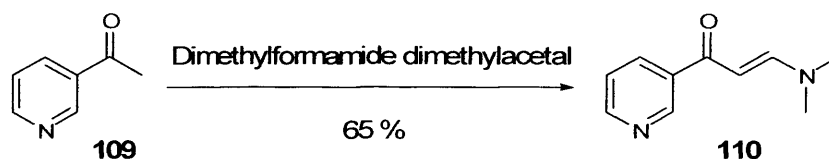
Scheme 3.2. MGCD0103 intermediate.

A likely mechanism for the formation of the aminopyrimidine ring involving the early irreversible loss of volatile methylamine is given in scheme 3.3.
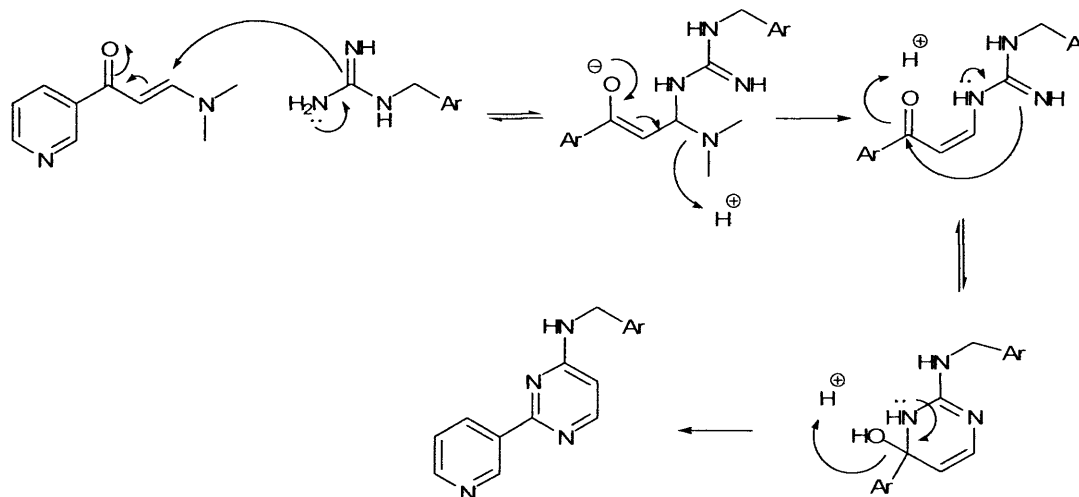
Scheme 3.3. Proposed mechanism of ring formation for **107**

The final compound was obtained by hydrolysis of methyl ester **107** under standard conditions and acylation of 1,2-phenylenediamine with the resultant acid **108**, using BOP as an activating agent. With a control sample of MGCD0103 now in hand, it was possible to utilise intermediate acid **108** for acylation of the amines required for the novel groups of targets **90-95**.

3.1.1 Preparation of Amines

The first target was the direct sulfur analogue, **95**, of the parent compound. It again appeared surprising that this group had not been evaluated before, especially when the excellent activity of other thiol-based inhibitors is considered.[4] The 2-amino substituted group, used in so many inhibitors, was identified during the development of inhibitor MS-275, and during this development the 2-hydroxy analogue was also identified as having excellent activity (discussed in the introduction).[5]

Direct acylation of 2-aminothiophenol (**111**) with **108** was not attempted owing to the probability of forming the benzothiazole (scheme 3.4).[6]

111
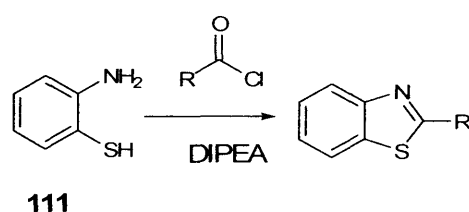
Scheme 3.4. Likely formation of benzothiazoles.[6]

It was therefore deemed necessary to protect the thiol before coupling the amine. The method of Noveron et al.[7] was followed, trityl protection occurring on sulfur under highly acidic conditions with triphenylmethanol (scheme 3.5).

111   **112**
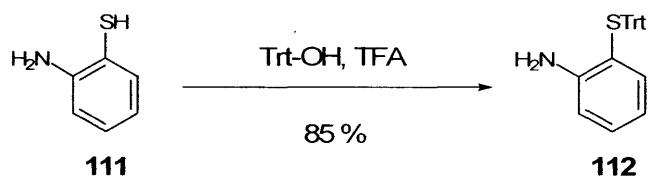
Scheme 3.5. Trityl protection of aminothiophenol.
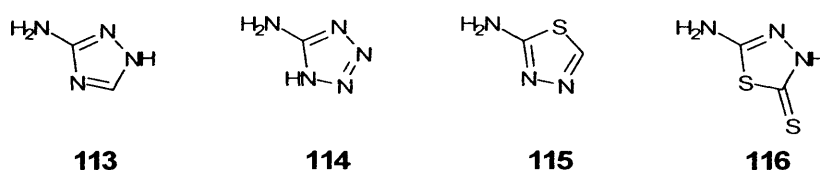
Figure 3.7. Commercially available amino heterocycles.

The heterocyclic amines required for targets **90-93** are all commercially available (figure 3.7). Aminomethylimidazole **119** required for analogue **94** was not commercially available and was prepared as the dihydrochloride salt in two steps from imidazole according to the procedure of Bastiaansen et al. (scheme 3.6).[8]
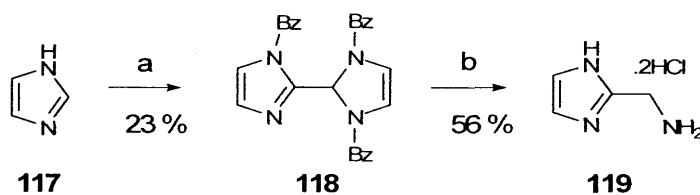
(a) Benzoyl chloride, Et$_3$N; 2 h, 0°C-r.t.; (b) MeOH, ^{i}PrOH (sat^d with HCl); 4 h, reflux.

Scheme 3.6. Synthesis of aminomethylimidazole intermediate.

Proposed mechanisms for the formation of **118** and **119** are given in scheme 3.7.
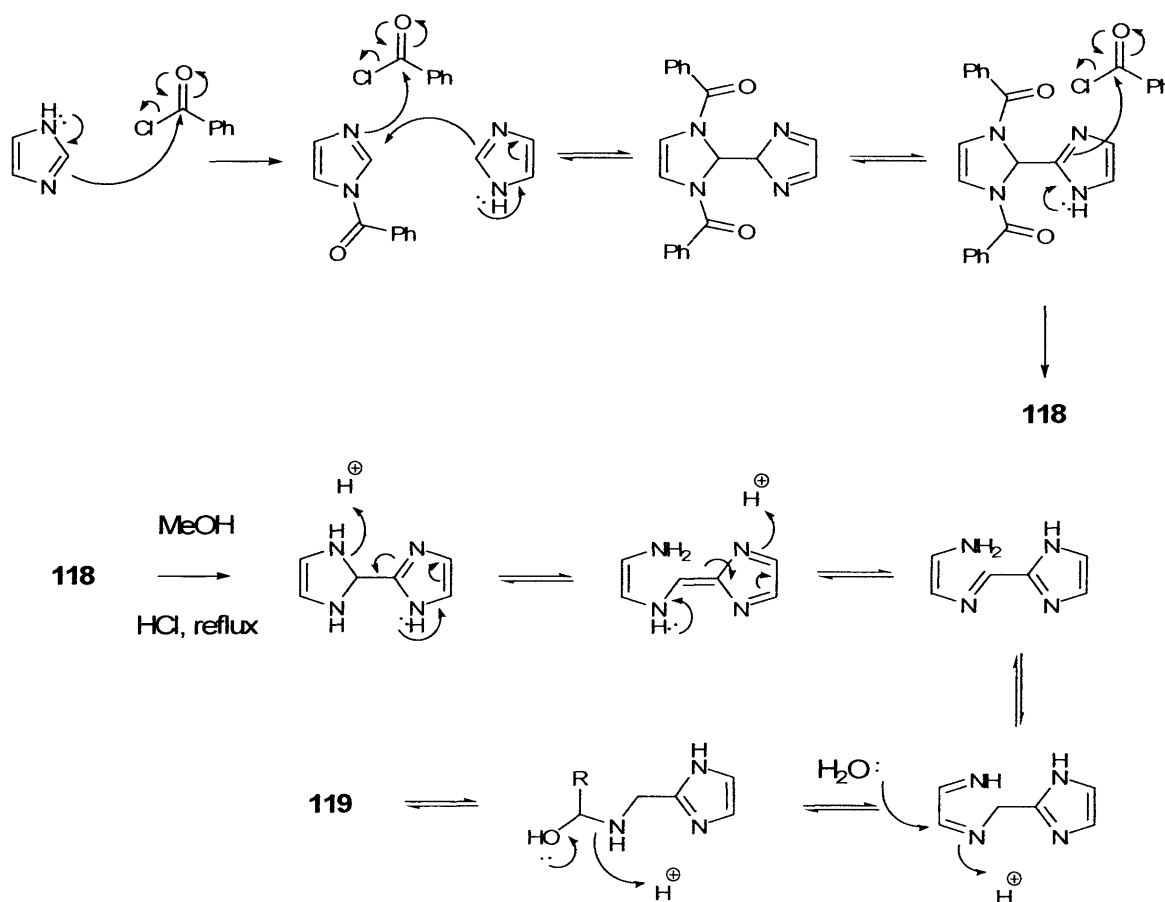
Scheme 3.7. Proposed mechanisms for formation of **118** and **119**.

The heterocyclic amine required for target **97** was the 1,2,5-thiadiazole-3,4-diamine **123** (scheme 3.8). Synthesis of the diamine has been reported via the bicyclic compound **122**. This is formed from reaction of diamidoxime **121** with freshly prepared sulfur dichloride. This precursor was, in this case, prepared from aqueous glyoxal (**120**) and hydroxylamine with aqueous sodium hydroxide (scheme 3.8).
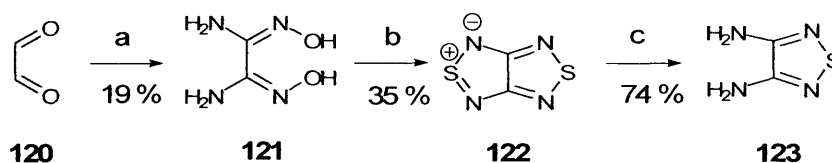
(a) NH$_2$OH.HCl, NaOH, H$_2$O; 5h, 0 to 95 °C; (b) SCl$_2$, DMF; 0 to 55 °C, 14 h;
(c) 3 M NH$_4$OH; 100 °C, 10 min.

Scheme 3.8. Preparation of 1,2,5-thiadiazole-3,4-diamine.

Whilst the yield of the initial step[9] in this sequence is poor, starting materials are cheap and easily handled; this is as opposed to alternate routes utilising the gaseous and highly toxic cyanogen in place of glyoxal, which itself must be prepared from a cyanide metal salt. Conversion of **121** into heterocycle **122** is also a less than ideal reaction – sulfur dichloride is not commercially available on less than an industrial scale, and so must be generated from the more stable sulfur monochloride and chlorine gas. The low boiling fraction of SCl$_2$ is then distilled as a cherry red, noxious liquid and used immediately in reaction with **121** according to the procedure of Komin et al.[10] Thorough studies on compound **122** in the same paper show that efficient hydrolysis to target diamine **123** is possible by heating in the presence of ammonium hydroxide, without which, any sulfur dioxide generated in the reaction leads to over hydrolysis of **122** to glyoxime and elemental sulfur.

Having prepared the required amines, coupling with the acid **108** was carried out. Examples **90-94** utilised EDCI.HCl/HOBT coupling agents in place of BOP in order to ease isolation. Purification of MGCD0103 involved removal of solvent from the reaction and column chromatography of the crude mixture. The targets synthesised here proved to be extremely insoluble, rendering column chromatography out of the question. With the water soluble by-products of the EDCI/HOBT system, the products could be precipitated from the reaction mixture by addition of copious amounts of water. This method afforded good yields for the above examples (scheme 3.9).
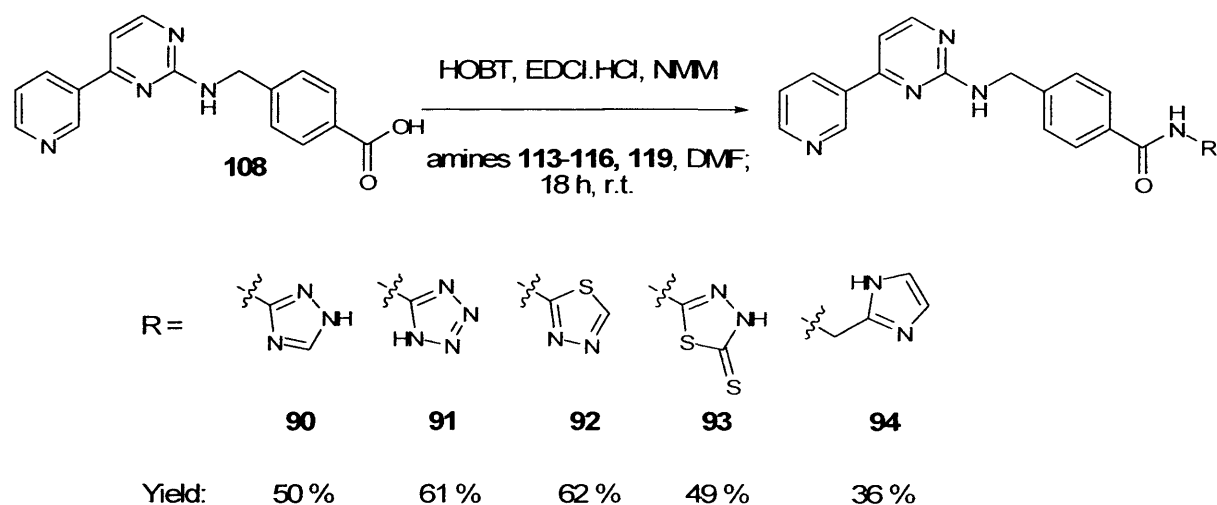
Scheme 3.9. Coupling of amino heterocycles.

The trityl protected 2-aminothiophenol (112) failed to react under these conditions, possibly due to the steric bulk of the trityl group. In light of this, the more reactive acid chloride was employed. Initial attempts at the new coupling involved formation of the acid chloride through refluxing in thionyl chloride and were not successful. As it was suspected that the conditions for acyl chloride formation were perhaps unnecessarily harsh, the reaction was tried again using oxalyl chloride with catalytic DMF at room temperature. Despite a reaction time of 2 d for the coupling, this method did furnish the protected intermediate 124, which was readily deprotected with trifluoroacetic acid and triethylsilane to give the target thiol 95 (scheme 3.10). Whilst microanalytical evidence identified the product of this reaction as free thiol 95, later analysis by mass spectrometry and repeat of microanalysis demonstrated that decomposition to the benzothiazole 125 had occurred.
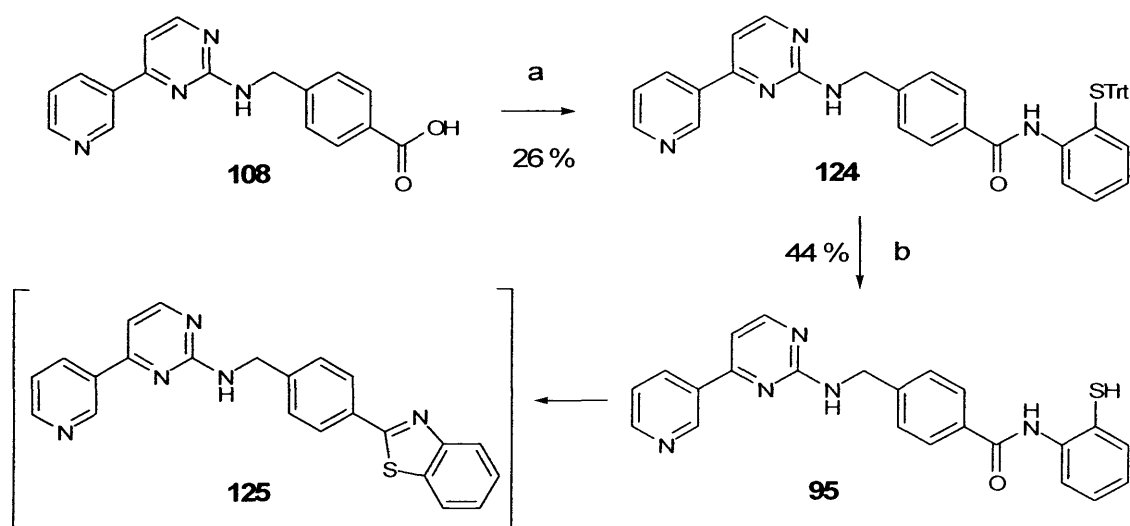
(a)(i) Oxalyl chloride, cat. DMF, DCM; 3 h, r.t.; (ii) trityl 2-aminothiophenol, CHCl$_3$; 2 d, r.t.;
(b) triethylsilane, TFA, DCM; 10 min, r.t.

Scheme 3.10. Synthesis of 2-aminothiophenol analogue.

Unfortunately, coupling of the 1,2,5-thiadiazole-3,4-diamine **123** was unsuccessful under all the conditions described above, in each case no reaction occurring.

In addition to assessing imidazole **94**, it was desired that an additional example of an aminomethylimidazole-containing compound be synthesised. This particular group has been utilised as a zinc-binding group in inhibitors of another zinc-containing enzyme, farnesyl transferase.[2]

The benzylamine-linked compound **96** was available in three simple steps from 4-nitrobenzaldehyde, **126** (scheme 3.11). Reductive amination with 3-aminoquinoline proceeded in excellent yield, requiring only hydrogenolysis of the nitro group (**127**), then another reductive amination, this time with 4(5)-imidazole carboxaldehyde.
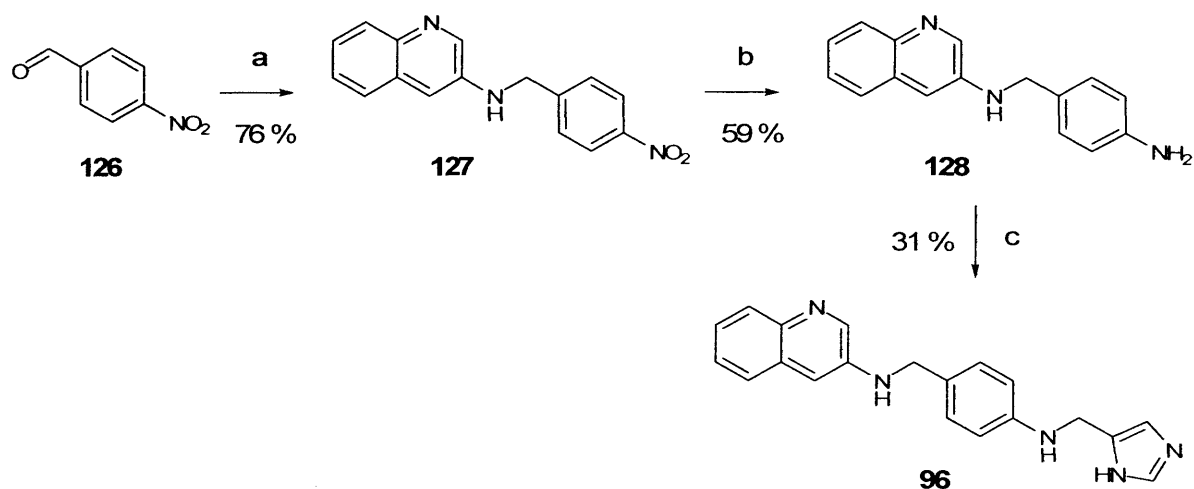
(a)(i) 3-Aminoquinoline, cat. AcOH, MeOH; 18 h, r.t.; (ii) NaBH₄; 1h, r.t.; (b) H₂, 5% Pd/C, EtOH; 18 h, r.t.; (c)(i) 4(5)-imidazole carboxaldehyde, cat. AcOH, MeOH; 1 h, r.t.; (ii) NaBH₄; 3 d, r.t.

Scheme 3.11. Additional imidazole Zn-binding group.

Despite the structural diversity present in the above compound's proposed zinc-binding moieties all compounds failed to produce any significant effect in an *in vitro* HDAC inhibition screening assay carried out at standard 5 μM concentrations.

3.1.2 Hydroxymethylimidazole Cap Group

Efforts towards synthesis of target **98** (figure 3.4) were reliant on the possibility of converting an amidine group into the desired imidazole. An example of this reaction, utilising a procedure of Thurkauf and co-workers[11], was carried out making use of dihydroxyacetone dimer (DHAD) and ammonium chloride in ammonium hydroxide to convert benzamidine **129** to the bicyclic **130**, albeit in poor yield (scheme 3.12).
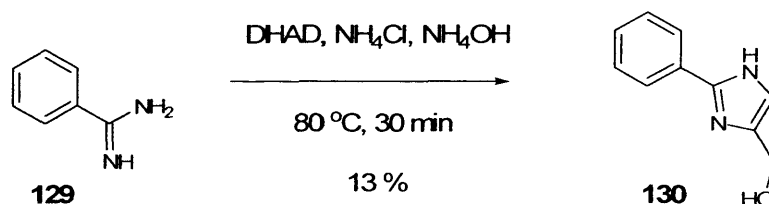
Scheme 3.12. Trial formation of hydroxymethylbenzimidazole.

A proposed mechanism for the formation of **130** is given in scheme 3.13.
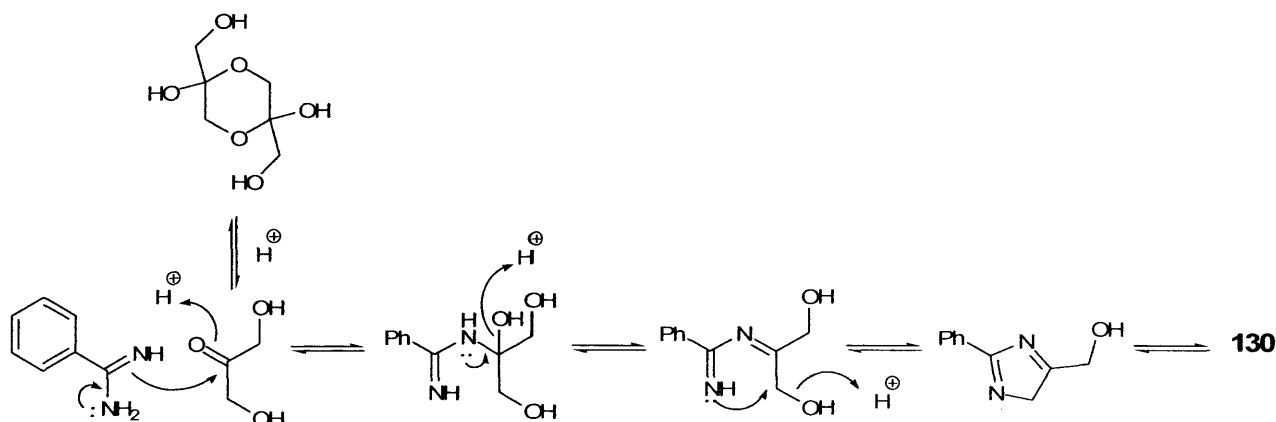
Scheme 3.13. Proposed mechanism for formation of **130**.

In the literature example the benzamidine was formed from a nitrile by reaction with lithium hexamethyldisilazide (LHMDS), therefore it was proposed that our target might be reached from a benzonitrile precursor analogous to acid **108** above.

With the reaction known to work in this simple context, work began on production of the necessary nitrile precursor **133** (scheme 3.14). It was found that the same experimental procedures as for the original MGCD0103 could be used, merely substituting the methyl-4-(aminomethyl)benzoate for commercially available 4-(aminomethyl)benzonitrile (**131**).
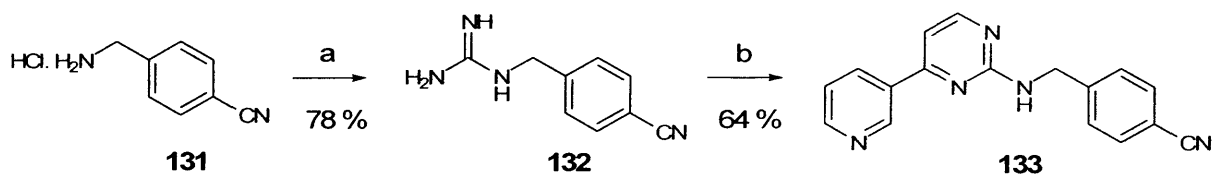
(a) Pyrazole carboxamidine.HCl, DIPEA, DMF; 7 h, r.t.; (b), **110**, ⁱPrOH; 18 h, reflux.

Scheme 3.14. Synthesis of intermediate nitrile.

The nitrile was now ready for conversion to the amidine, and hence heterocycle formation. The first method attempted was similar to that of Eisen and Kapon, which, as described above, involved formation of a complex with LHMDS followed by acid hydrolysis.[12] Unfortunately this reaction proved problematic, and reaction of either the free amine **133** or the Boc protected analogue **135** did not result in an isolable, pure product (scheme 3.15). The original procedure utilised aqueous HCl for hydrolysis, but this was not successful in the present case. As the product of hydrolysis appeared to be extremely hygroscopic, attempts were made using ethanolic HCl or TFA, both of which gave similarly unsuccessful results.
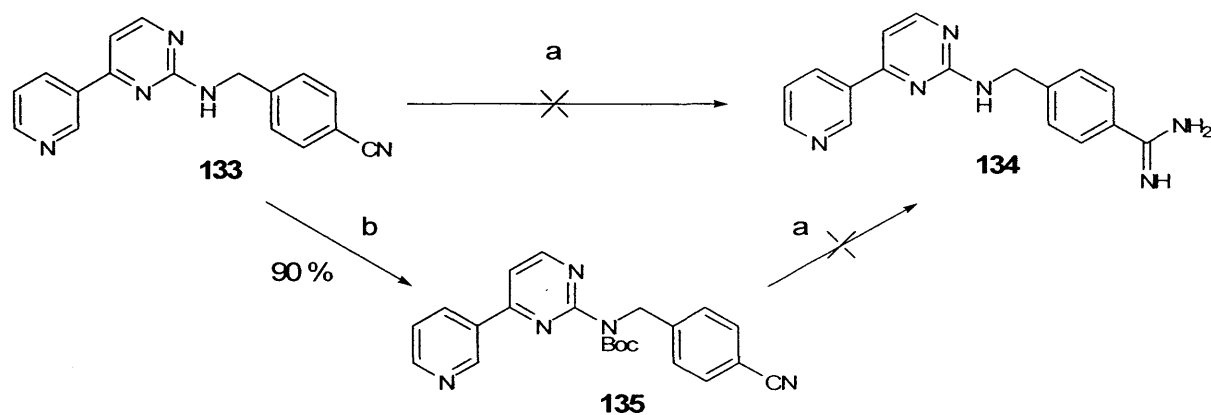
(a)(i) LHMDS, THF; (ii) acid; (b) Boc₂O, DMAP (cat), pyridine, 4 d, r.t.

Scheme 3.15. Attempted formation of benzamidine.

3.1.3 Amidoximes

During the attempted synthesis of the hydroxymethylimidazole **98**, it was noted that the reaction of a nitrile group with hydroxylamine would result in formation of an amidoxime, a structure closely related to that of hydroxamic acids. It is known that the (Z)-amidoxime is the favoured isomer in cases without N-substitution,[13] and their coordination with metal ions has been studied.[14] Amidoximes have also been used as metal coordinators in quantitative analysis.[15] The similarity of this group to a hydroxamic acid, combined with the fact that no example of this class of compound had been reported in the field of HDAC inhibitors suggested that it might be an interesting group to assess.

It was found that the direct amidoxime-analogue of MGCD0103, **99**, was available in excellent yield with easy purification by recrystallisation by reaction of hydroxylamine with intermediate benzonitrile **133** (scheme 3.16).
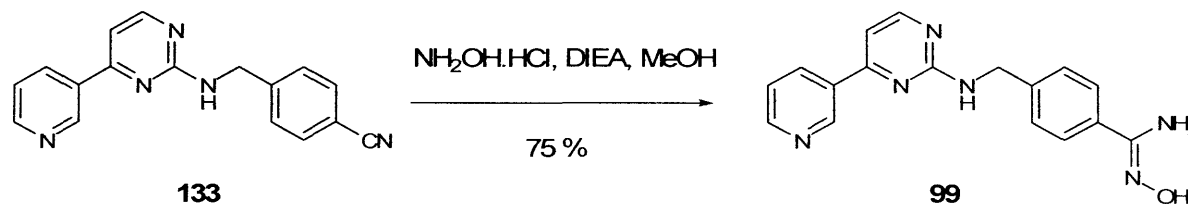
Scheme 3.16. Amidoxime analogue of MGCD0103.

Further examples of amidoximes were desired to rapidly assess the suitability of this group as a hydroxamic acid-substitute. The four examples **100-103** (figure 3.5) were selected as a sub-series of simple compounds possessing structures that would be likely to be potent in the case of hydroxamic acid inhibitors.

Amides **137** and **138** were synthesised in three steps from 4-cyanobenzoic acid (**136**) by coupling with aniline and 3-aminoquinoline respectively (via the acid chloride), followed by conversion of the nitrile into amidoxime under the same conditions as above (scheme 3.17).
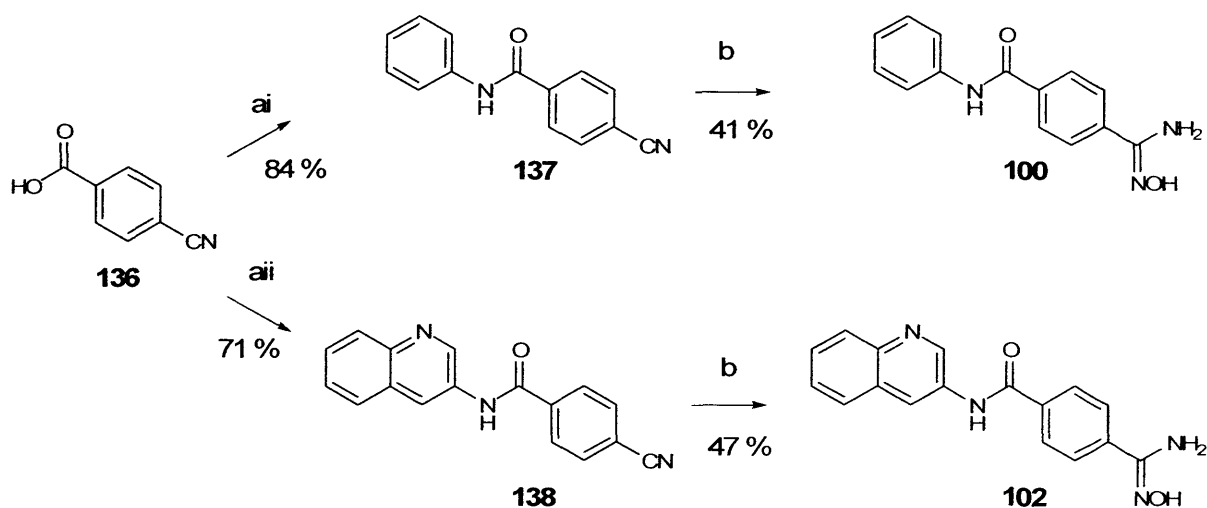
(ai) (i) SOCl₂; 1 h, 90 °C; (ii) aniline, pyridine, DCM; 1 h, 0 °C; (aii) (i) SOCl₂; 1 h, 90 °C;
(ii) 3-aminoquinoline, pyridine, DCM; 2 h, 0 °C; (b) NH₂OH.HCl, DIEA, MeOH; 18 h, r.t.

Scheme 3.17. Synthesis of amide-linked amidoximes.

The more flexibly-linked amine **101** was the product of the reductive amination of 4-cyanobenzaldehyde (**139**) and conversion of the nitrile **140** into the amidoxime. Formation of the un-isolated imine by mixing the aldehyde and aniline in methanol was assisted by addition of catalytic acetic acid, and was reduced with sodium borohydride (scheme 3.18).
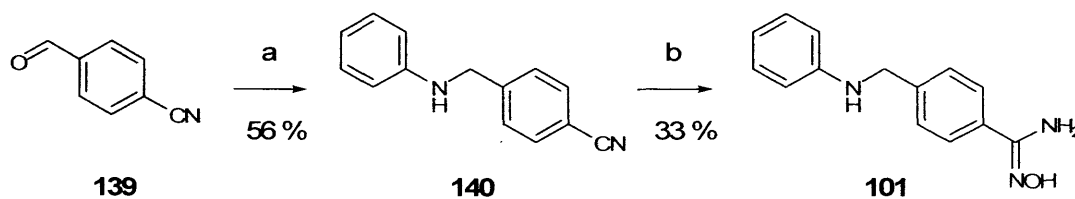
(a)(i) Aniline, MeOH, cat. AcOH; 24 h, r.t.; (ii) NaBH₄; 18 h, r.t.;
(b) NH₂OH.HCl, DIEA, MeOH; 3 d, r.t.

Scheme 3.18. Amine-linked amidoxime.

Finally, reaction of benzoyl chloride with 4-(aminomethyl)benzonitrile (**131**) provided the more flexible amide **141** in excellent yield, amidoxime formation proceeding under the standard conditions (scheme 3.19).
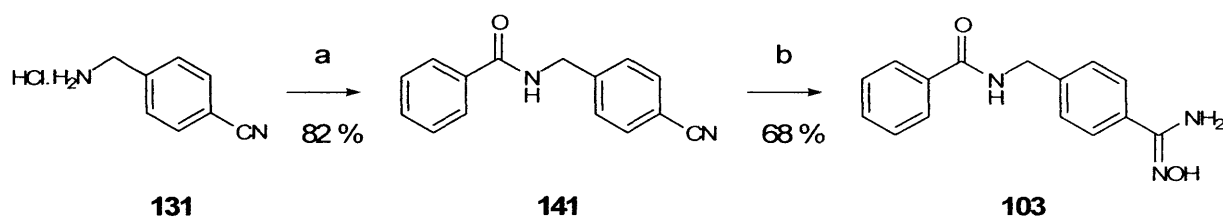
(a) Benzoyl chloride, Et$_3$N, DCM; 3 h, 0 °C; (b) NH$_2$OH.HCl, DIEA, MeOH; 20 h, r.t.

Scheme 3.19. Extended amide-linked amidoxime.

The amidoximes thus far synthesized were subjected to the standard *in vitro* HDAC inhibition screening. Unfortunately, none of the above examples displayed any significant activity at concentrations of 5 μM. Despite these results, it was felt that one final example of this class should be synthesized in order to compare directly with a known straight-chain hydroxamic acid.

3.1.4 Amidoxime SAHA analogue

The route initially selected for synthesis of amidoxime analogue of SAHA **104** was based on the oxidation of amine **142** to nitrile **143** (scheme 3.20). In a procedure reported by Chen et al.[16] a variety of amines including 7-aminoheptanoic acid (which was thought to provide good analogy for 8-aminooctanoic acid required in this case) were converted to the nitrile in 80 % yield under very mild conditions, using trichloroisocyanuric acid (TCCA) and catalytic TEMPO. Unfortunately, treatment of amine **142** with these reagents under a variety of conditions, including adjusting the temperature to 0, 5, 10 or 20 °C, increasing the catalyst from 1 mol% to 10 mol% and even addition of base to deprotonate the acid, all resulted in rapid formation of an unwanted product in quantitative yield.

(a) TCCA, cat. TEMPO, DCM; 2-7h, 0-10 °C
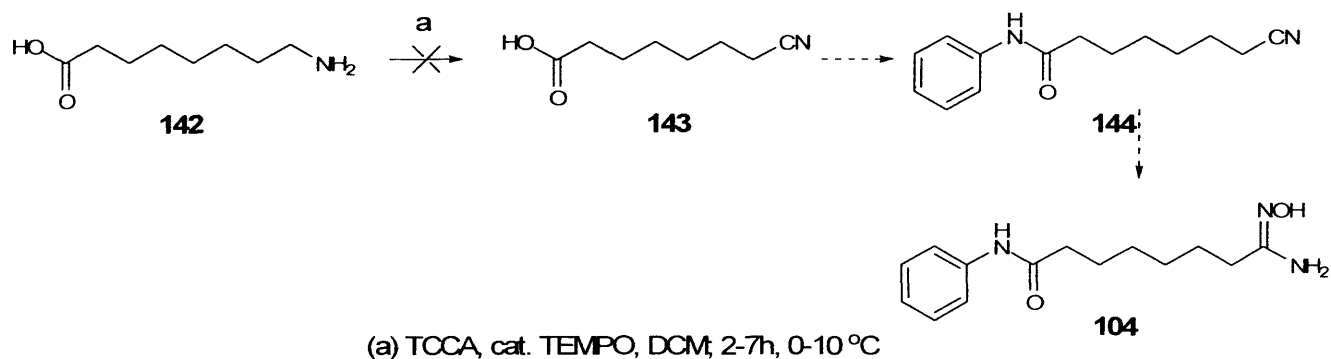
Scheme 3.20. Proposed route to amidoxime SAHA analogue.

With convenient oxidation of the amine ruled out as a route to the nitrile, the alternative alkylation of an alkyl bromide with a cyanide salt was investigated (scheme 3.21).

(a) 2M LiOH, MeOH; 18h, r.t.; (b) NaCN, K$_2$CO$_3$, H$_2$O; 18h, reflux.
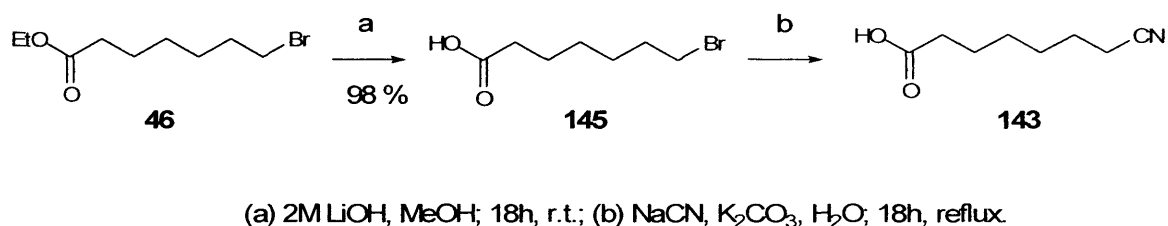
Figure 3.21. Alternative route to nitrile **143**.

Bromoacid **145** was not commercially available and so was given by the hydrolysis of the ethyl ester according to the procedure of Suzuki et al.,[17] and although this extra step is undesirable, it proceeds in excellent yield. The alkylation of 6-bromohexanoic acid is reported by Schultz[18] in good yield on the scale of 100 g, however, a small scale run of this reaction resulted in poor yield (by NMR) of **143** and so it was thought that this would be improved by derivatization of the carboxylic acid before alkylation (scheme 3.22)
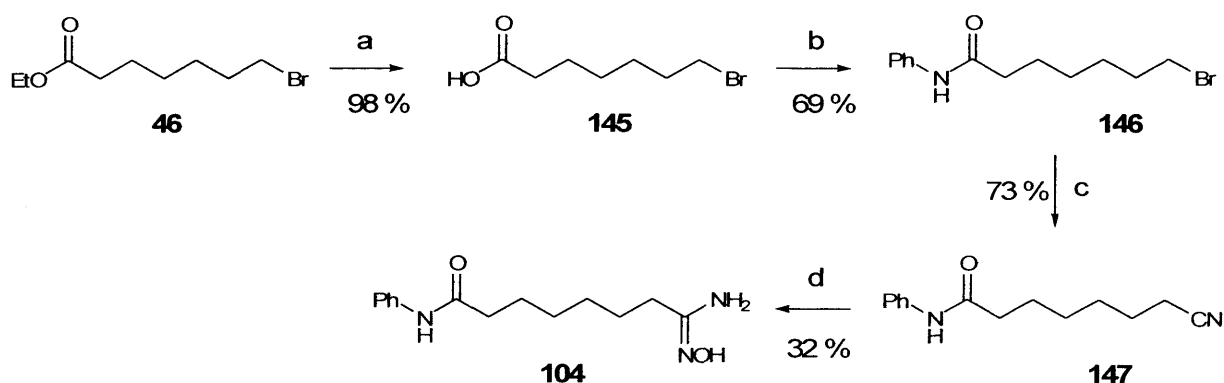
(a) 2M LiOH, MeOH, 18h r.t.; (b)(i) SOCl$_2$; 1h, 70 °C; (ii) aniline, pyridine, DCM, 2h, 0 °C;
(c) KCN, EtOH, 18h, 75 °C; (d) 1M NH$_2$OH, MeOH, 4 d, r.t.

Scheme 3.22. Final route to amidoxime **104**.

Coupling of acid **145** with aniline proceeded in good yield via the acid chloride, and it was seen that amide **146** did indeed undergo the alkylation step in excellent yield. Whilst this sequence is not as convenient as that depicted in scheme 3.21 with respect to ease of derivatization of the amide group, it was deemed preferable under these circumstances to the use of excessive amounts of cyanide salts in a low yielding step.

Conversion of the alkyl nitrile group of **147** into the amidoxime occurred far less readily than with the previous aromatic examples. Under all conditions trialled the reaction proceeded slowly and did not run to completion. Efforts to increase the rate of reaction attempted to adapt the procedure of Gissot et al. for formation of O-benzyl hydroxamates from esters.[19] In this case, the O-protected hydroxylamine is deprotonated with lithium hexamethyldisilazide then the ester is added, giving the protected hydroxamate in excellent yield within ten minutes. This procedure was unsuccessful when applied to reaction with the nitrile (to give the O-benzyl amidoxime) in place of the ester, and it has been reported that O-alkyl amidoximes are in fact known to be unstable.[15] Other conditions experimented with to optimise this reaction included use of free hydroxylamine or the hydrochloride salt with various bases (DIPEA, Na$_2$CO$_3$, KOH, or no base) in water, methanol, ethanol or dimethylformamide. The best yields were obtained by stirring the nitrile in a 1 M

methanolic solution of hydroxylamine for 4 d. Although this reaction did not go to completion, stirring for longer periods led to decomposition of the product, and after 4 d any remaining unreacted starting material was recovered by column chromatography.

The final compound was subjected to the standard screening assay at 5 μM concentration and was found to be inactive. It was concluded that, after this result combined with the negative results from the previous amidoximes, this group was unfortunately not a suitable substitute for a hydroxamic acid zinc-binding group, and our investigation in this area was closed.

Efforts in this chapter to develop potent new non-hydroxamate HDAC inhibitors as potential anti-cancer drugs were deemed unsuccessful on the basis of poor activity in cell-based assay screens. This lack of activity was un-surprising in some cases, with poor solubilities being noted during synthesis. It was, however, disappointing to not see some significant activity from the range of new groups tested, given the wide range of groups known to act as substitutes for hydroxamic acids (discussed in the introduction). It should also be noted that, because of funding constraints on the screening process, compounds were required to have shown an appreciable activity in cell-based assays before any further analysis could be carried out. This single concentration-point assay provided little information for direction of further development. It is possible that if the compounds of this chapter were subjected to an isolated enzyme assay, some information may be obtained that could allow further development of this work. The isolated enzyme assay would also be of particular interest in the case of the amidoxime compounds in determining the requirements for replacement hydroxamates. The similarity of amidoximes to hydroxamic acids could prove a useful tool in elucidating the binding requirements of inhibitors if it were possible to compare directly the isolated enzyme activity of these analogues. As it remains we are unable to say whether the observed poor activity is due to poor HDAC-binding or just a result of poor cellular penetration.

3.2 References

1. Frey, R. R.; Wada, C. K.; Garland, R. B.; Curtin, M. L.; Michaelides, M. R.; Li, J.; Pease, L. J.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J. Trifluoromethyl ketones as inhibitors of histone deacetylase. *Bioorganic & Medicinal Chemistry Letters* **2002**, *12*, (23), 3443-3447.

2. Ohkanda, J.; Lockman, J. W.; Kothare, M. A.; Qian, Y.; Blaskovich, M. A.; Sebti, S. M.; Hamilton, A. D. Design and synthesis of potent nonpeptidic farnesyltransferase inhibitors based on a terphenyl scaffold. *Journal of Medicinal Chemistry* **2002**, *45*, (1), 177-188.

3. Zhou, Z.; Delorme, D.; et al. Inhibitors of Histone Deacetylase. **2004**, (WO2004/069823),

4. Suzuki, T.; Hisakawa, S.; Itoh, Y.; Maruyama, S.; Kurotaki, M.; Nakagawa, H.; Miyata, N. Identification of a potent and stable antiproliferative agent by the prodrug formation of a thiolate histone deacetylase inhibitor. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, (6), 1558-1561.

5. Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. *Proceedings of the National Academy of Sciences of the United States of America* **1999**, *96*, (8), 4592-4597.

6. Mourtas, S.; Gatos, D.; Barlos, K. Solid phase synthesis of benzothiazolyl compounds. *Tetrahedron Letters* **2001**, *42*, (11), 2201-2204.

7. Noveron, J. C.; Olmstead, M. M.; Mascharak, P. K. A Synthetic Analogue of the Active Site of Fe-Containing Nitrile Hydratase with Carboxamido N and Thiolato S as Donors: Synthesis, Structure, and Reactivities. *Journal of the American Chemical Society* **2001**, *123*, (14), 3247-3259.

8. Bastiaansen, L. A. M.; Godefroi, E. F. 2-Aminomethylimidazole and imidazole-2-carboxaldehyde: two facile syntheses. *Journal of Organic Chemistry* **1978**, *43*, (8), 1603-1604.

9. Zelenin, A. K.; Trudell, M. L. A Two-Step Synthesis of Diaminofurazan and Synthesis of N-Monoarylmethyl and N, N'-Diarylmethyl Derivatives. *Journal of Heterocyclic Chemistry* **1997**, *34*, 1057-1060.

10. Komin, A. P.; Street, R. W.; Carmack, M. Chemistry of 1,2,5-thiadiazoles. III. [1,2,5]thiadiazolo[3,4-c][1,2,5]thiadiazole. *Journal of Organic Chemistry* **1975**, *40*, (19), 2749-2752.

11. Thurkauf, A.; Hutchison, A.; Peterson, J.; Cornfield, L.; Meade, R.; Huston, K.; Harris, K.; Ross, P. C.; Gerber, K.; Ramabhadran, T. V. 2-Phenyl-4-(aminomethyl)imidazoles as potential antipsychotic agents. Synthesis and dopamine D2 receptor binding. *Journal of Medicinal Chemistry* **1995**, *38*, (12), 2251-2255.

12. Eisen, M. S.; Kapon, M. Synthesis, Molecular Structure and Solution Dynamics of Dimeric Benzamidinates containing a Double Diazaallyl Lithium Bridge. A Rapid Interconversion of sigma and pi Bonds. *Journal of the Chemical Society, Dalton Transactions* **1994**, 3507-3510.

13. Andrianov, V. G.; Eremeev, A. V. Influence of substituents at the nitrogen atom on the structure of amidoximes. *Journal of Structural Chemistry* **1992**, *32*, (4), 477-482.

14. Pearse, G. A.; Pflaum, R. T. Interaction of Metal Ions with Amidoximes. *Journal of the American Chemical Society* **1959**, *81*, 6505-6508.

15. Eloy, F.; Lenaers, R. The Chemistry of Amidoximes and Related Compounds. *Chemical Reviews* **1962**, *62*, (2), 155-183.

16. Chen, F. E.; Kuang, Y. Y.; Dai, H. F.; Lu, L.; Huo, M. A Selective and Mild Oxidation of Primary Amines to Nitriles with Trichloroisocyanuric Acid. *Synthesis* **2003**, *17*, 2629-2631.

17. Suzuki, T.; Matsuura, A.; Kouketsu, A.; Hisakawa, S.; Nakagawa, H.; Miyata, N. Design and synthesis of non-hydroxamate histone deacetylase inhibitors: identification of a selective histone acetylating agent. *Bioorganic & Medicinal Chemistry* **2005**, *13*, (13), 4332-4342.

18. Schultz, E. M. The Reaction of Aminoesters with Ethyl Isocyanate: Open Chain Models of Desthiobiotin. *Journal of the American Chemical Society* **1947**, *69*, (5), 1056-1057.

19. Gissot, A.; Volonterio, A.; Zanda, M. One-Step Synthesis of *O*-Benzyl Hydroxamates from Unactivated Aliphatic and Aromatic Esters. *Journal of Organic Chemistry* **2005**, *70*, (17), 6925-6928.

4 Analogues of SAHA

The work described in this chapter covers the synthesis of various novel hydroxamic acids bearing structural relationships to suberoylanilide hydroxamic acid (SAHA) (figure 4.1). The well established hydroxamic acid zinc-binding group was utilised in each of the following examples, with efforts to improve inhibitory activity focusing on developing the structurally simple linker (148) and cap region (149-155) of SAHA.

The piperidyl linker of compound 148 was proposed as a potential improvement on a saturated alkyl chain as it would provide more bulk to fill the pocket of the enzyme's active site. Inclusion of an amine in the linker would improve solubility of the compound and simplify synthesis. Carboxylic acid 149 and alcohol 150 provide increased bulk in the cap region, whilst also potentially greatly improving water solubility. Benzimidazole 151 was synthesised to asses the suitability of the fused system as a substitute for the anilide moiety of SAHA, and addition of the group in 152 greatly increases aromatic bulk in the cap. Inclusion of an ester with the benzimidazole of 153 was also carried out to increase bulk and potentially behave as a pro-drug by hydrolysis to the acid. Finally, piperidinones 154 and 155 retain good structural analogy to SAHA whilst increasing the area covered by the cap.

In order to compare the biological activity of these compounds a sample of the parent compound was also required, and so laboratory work in this series began with a literature synthesis of SAHA.
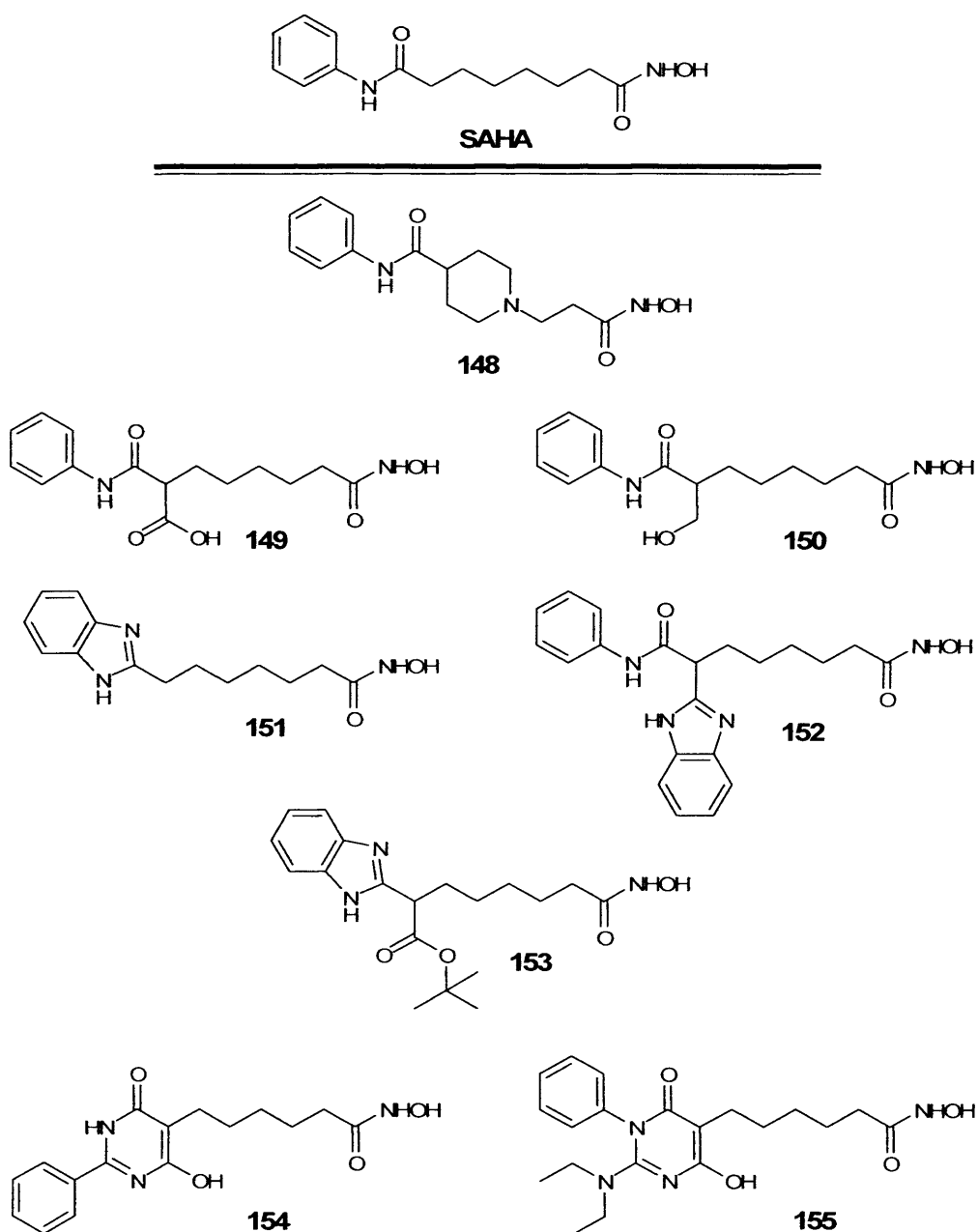
Figure 4.1. Novel hydroxamic acids based on SAHA.

4.1 Suberoylanilide Hydroxamic Acid

Several syntheses of SAHA have been reported and the procedure of Mai et al.[1] was selected in this case (scheme 4.1).
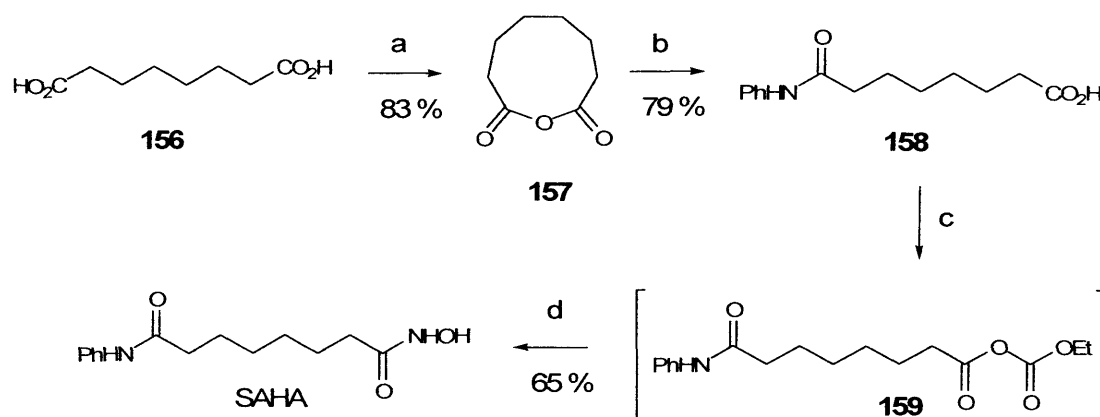
(a) Ac_2O, 1h, reflux; (b) aniline, THF, 45 min, r.t.; (c) ethyl chloroformate, THF, 20 min, 0 °C; (d) NH_2OH, MeOH, 20 min, r.t.

Scheme 4.1. Synthesis of SAHA.[1]

Suberic acid was converted to its anhydride by refluxing in acetic anhydride for 1 h, this transformation allowing selective reaction of one acid group simply by opening of the anhydride **157** with aniline in THF. The acid **158** is itself not active to attack of hydroxylamine, therefore prior conversion to the mixed anhydride by reaction with ethyl chloroformate is necessary, this is however generated *in situ*, without requiring isolation.

Whilst this route provides a quick and high-yielding synthesis of SAHA, it should be noted that difficulties were encountered, particularly in the separation of the final product from un-reacted acid **158** by recrystallisation.

4.2 Piperidyl SAHA Analogue

A reason that is commonly proposed for the lower activity of SAHA when compared with TSA is the high flexibility of the unsaturated chain of SAHA. The more rigid, unsaturated chain of TSA, along with its bulkier protruding methyl groups provide a more snug fit in this space.[2] This observation led to the proposal of the piperidyl compound **148** (figure 4.1) as a potential improvement on the simple alkyl chain by providing more conformational constraint in addition to improved solubility.

An analogue of SAHA (**160**) previously synthesised by this group had demonstrated the benefit to activity of bifurcation of the cap region in the form of a second anilide group (un-published results) (figure 4.2). With this knowledge, an additional piperidyl target also bearing the di-anilide cap (**161**) was proposed.
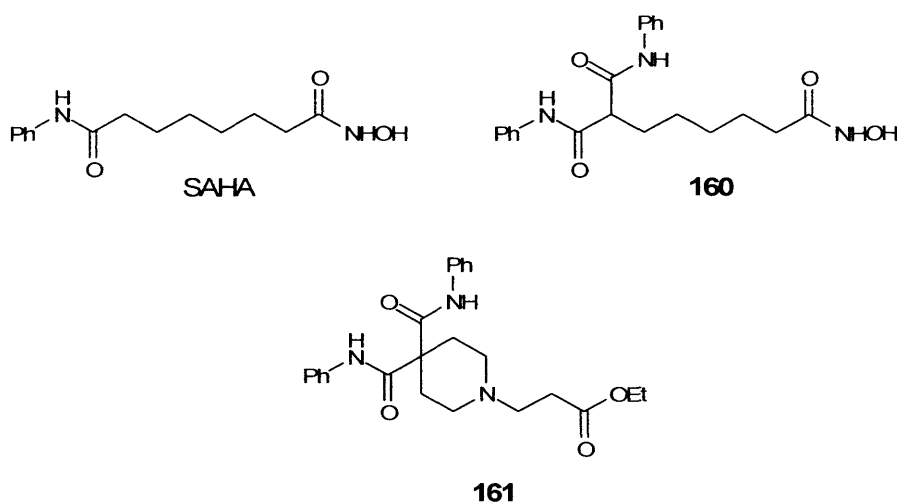
Figure 4.2. SAHA, previously synthesised bifurcated analogue and proposed piperidyl target.

4.2.1 Mono-anilide Analogue

The direct route to mono-anilide **148**, given in scheme 4.2, began from commercially available isonipecotic acid (**162**). Protection of the amine with a Boc group allowed trouble-

free coupling of the acid **163** with aniline using EDCI/HOBt. Monitoring the deprotection of the amine with TFA in dichloromethane via TLC indicated that the reaction proceeded rapidly as might be expected, however, the uninspiring yield of 68% is likely to be due to the reluctance of amine **165** to be extracted from the aqueous phase in the work-up. Extraction with ethyl acetate showed small amounts of amine by TLC, and although it is likely that the yield could be improved with further extraction, a compromise was met after 10 washes.
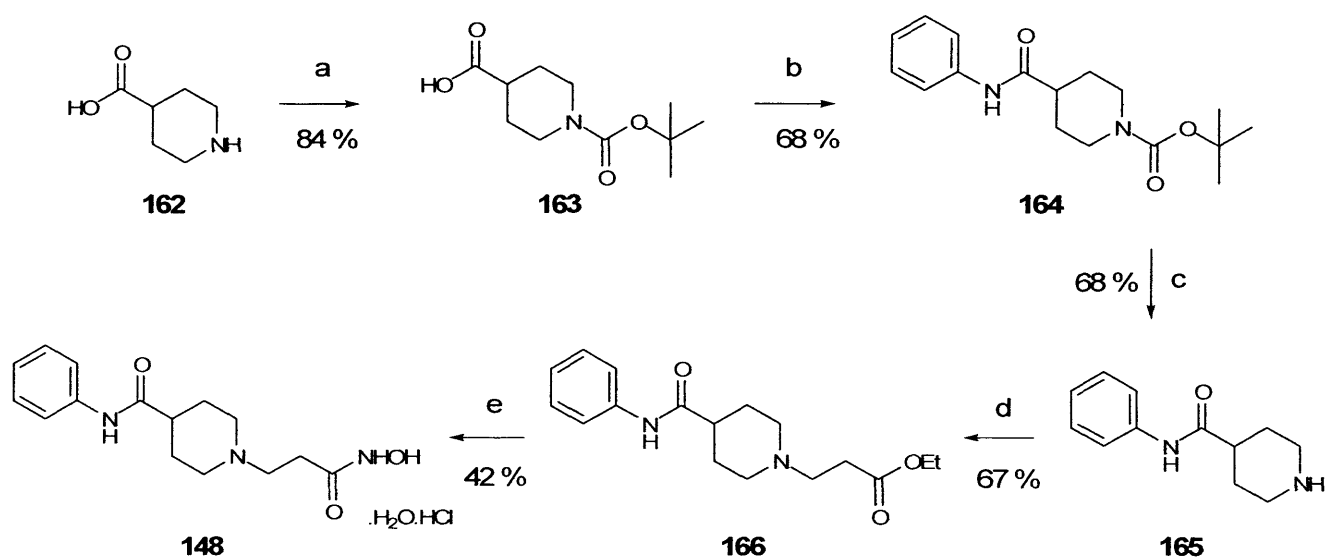
(a) K_2CO_3, Boc_2O, water, THF; 4 h, r.t.; (b) HOBT, EDCI.HCl, NMM, aniline, DMF; 18 h, r.t.; (c) DCM-TFA, 1:1; 17 h, r.t.; (d) ethyl acrylate, EtOH; 4 d, r.t.; (e) NH_2OH, KOH, THF, MeOH, 18 h, r.t.

Scheme 4.2. Synthesis of mono-anilide piperidyl analogue.

The spacer region was completed by conjugate addition of piperidine **165** to ethyl acrylate. The reaction proceed slowly at room temperature in ethanol providing an acceptable yield of ester **166** after four days, however, it may be possible to improve this by extending the reaction time or heating. Separation of the product by column chromatography was easily achieved, providing the crystalline ester. Conversion to the hydroxamic acid was achieved by treatment with methanolic hydroxylamine and potassium hydroxide in THF at room

temperature. After neutralisation with HCl the crude product was recrystallised from 5% methanol-acetonitrile to afford the product as the hydrochloride salt monohydrate **148**.

4.2.2 Di-anilide Analogue

The synthetic route proposed to the di-anilide **161** could potentially also provide an alternative route to the mono-anilide **148**. This approach involves formation of the piperidine ring in the third step by dialkylation of di-*tert*-butyl malonate (scheme 4.3).
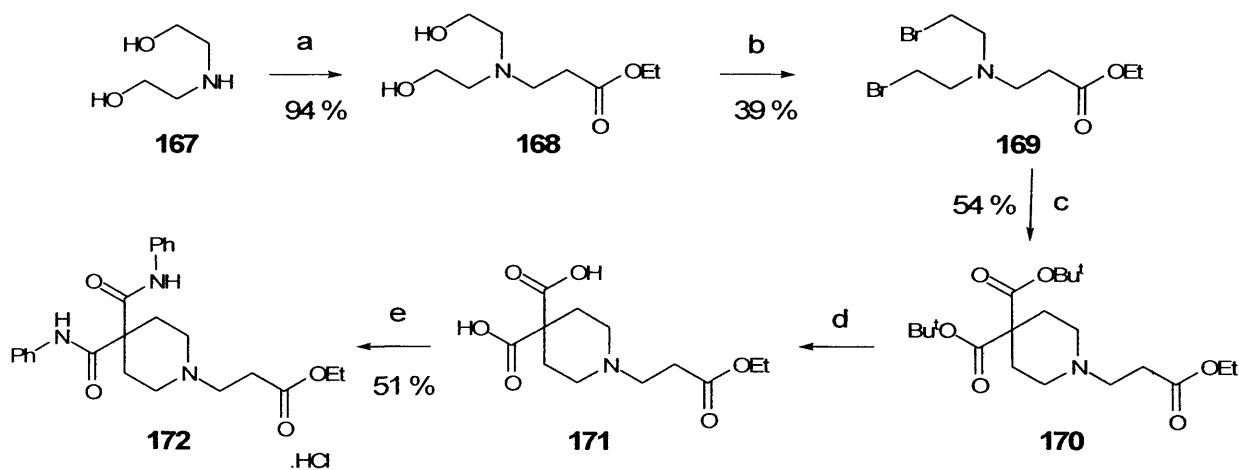
(a) Ethyl acrylate, EtOH, 7 d, r.t.; (b) NBS, PPh₃, DCM; (c) (i) di-*t*-butyl malonate, NaH (1.5 eq), DMF; (ii) **169**; (iii) NaH (1.5 eq); 70 °C, 18 h; (d) TFA, DCM, 20 h, r.t.; (e)(i) SOCl₂; (ii) PhNH₂, pyridine, DCM, 3 h, r.t.

Scheme 4.3. Route to di-anilide.

Selective hydrolysis of the *tert*-butyl esters of **170** would then provide an interesting diacid (**171**) which could potentially be decarboxylated (**173**), or coupled with one equivalent of aniline leaving a free carboxylic acid for further derivatization (**174**, scheme 4.6).
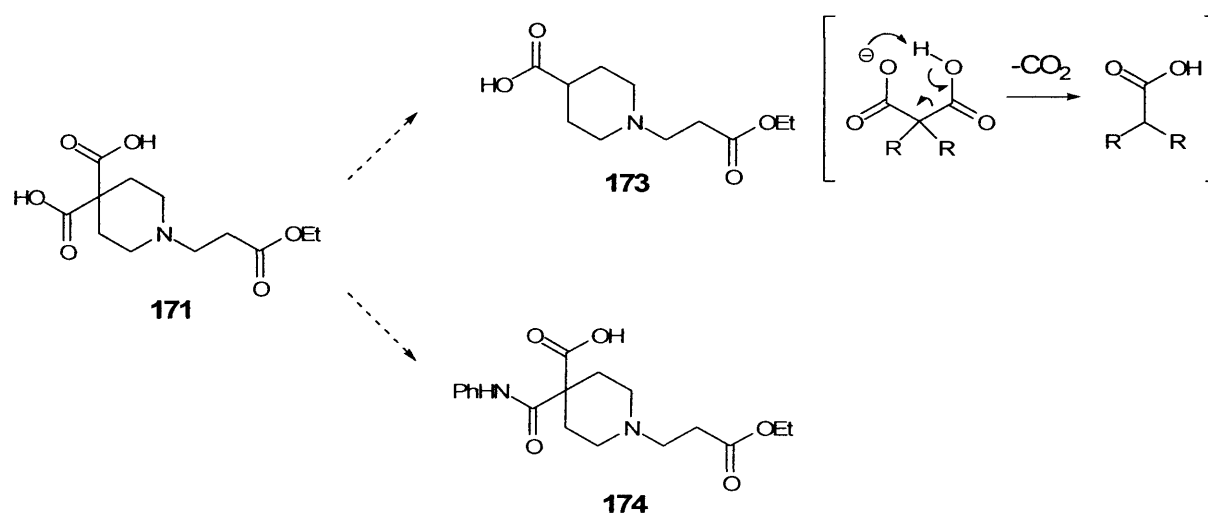
Scheme 4.4. Potential derivatives available from di-acid **171**.

Initially, the Michael addition of ethanolamine (**167**) to ethyl acrylate was carried out under the same conditions as for addition of piperidine **165** (scheme 4.3), viz, in ethanolic solution at room temperature. Whilst this did furnish the addition product **168** in excellent yield, reaction was sluggish, requiring seven days. The reaction time was subsequently reduced to 5 h by heating to 120 °C whilst retaining an excellent yield. Isolation of di-bromide **169** after conversion with triphenylphosphine and *N*-bromosuccinimide was hampered by the presence of two equivalents of the unwanted triphenylphosphine oxide side-product, possibly contributing to the modest yield. Formation of the piperidine ring of **170** was then carried out in two stages by deprotonation of di-*tert*-butyl malonate with two successive portions of sodium hydride with cautious heating in DMF. Attempts to improve the yield for the alkylation by using the potentially more reactive di-mesylate in place of di-bromide **169** was unsuccessful as the only product isolated from the reaction of di-alcohol **168** with methanesulfonyl chloride appeared to be the self-reacted morpholine.

From ester **170** was provided di-acid **171** by cleavage in TFA-DCM. Preparation of the di-amide derivative **172** proceeded well through formation of the di-acyl chloride with thionyl chloride, then coupling with two equivalents of aniline. This method of coupling was found

to be preferable to the use of EDCI/HOBt, which provided only a poor yield with longer reaction time.

Unfortunately, the synthesis of this di-anilide analogue was not continued beyond this point as results from biological testing of initial target **148** were received, showing no significant activity in cell viability assays. This information led us to believe that the piperidyl spacer is not a tolerable alternative to the alkyl chain of SAHA, and this line of investigation was therefore discontinued.

4.3 Malonamide-Based Compounds

This section incorporates the unsymmetrical, malonamide-derived compounds **149**, **150**, **152** and **153**, and also, owing to its role in the development of the series, symmetrical benzimidazole **151**. Synthesis of this compound was based on that of SAHA, utilising the suberic anhydride intermediate **157** (scheme 4.5).
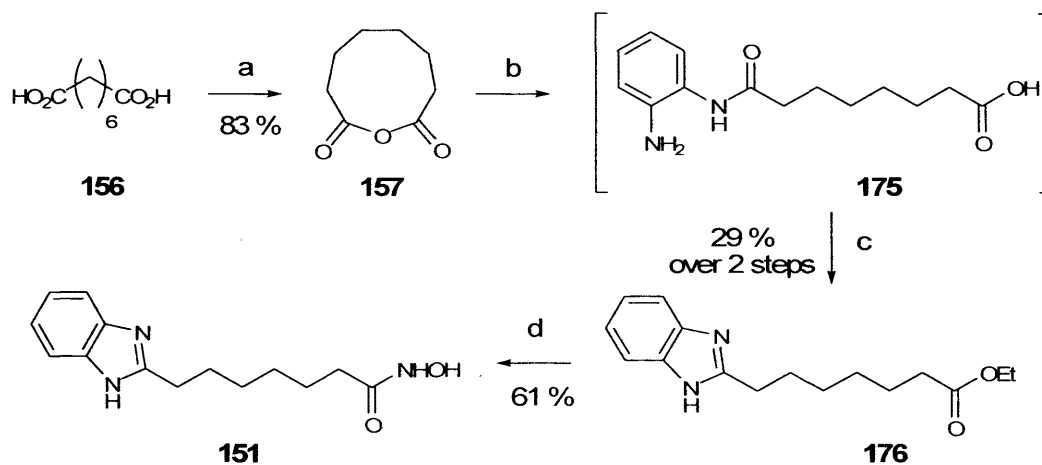
(a) Ac$_2$O; 2h, reflux; (b) 1,2-phenylenediamine, THF; 20 min, r.t.; (c) 4% conc.H$_2$SO$_4$/EtOH; 16 h, reflux; (e) NH$_2$OH(aq), 1M KOH/MeOH, THF; 6 h, r.t.

Scheme 4.5. Synthesis of benzimidazole SAHA analogue.

The amino-acid intermediate **175** was not isolated, as dehydration to form the benzimidazole and esterification was possible in one step. Reflux in 4% concentrated sulfuric acid in ethanol gave the more easily purified ester **176**, which was converted to the corresponding hydroxamic acid with aqueous hydroxylamine and methanolic potassium hydroxide. It should be noted that, whilst the formation of the benzimidazole under these harsh conditions was not a significant problem in this case, much milder methods are possible from reaction of phenylenediamine with aldehydes in the presence of oxidising agents such as hypervalent iodine.[3]

The three anilide-containing compounds **149**, **150** and **152** were all derived from malonic acid via the intermediate amide-ester **179** (scheme 4.6). Mono-esterification was carried out in excellent yield with *tert*-butyl alcohol according to the procedure of Melman et al., using DCC as a coupling agent.[4] Acylation of aniline with the resultant acid **178** was carried out with three different methods; initially, EDCI.HCl and HOBt coupling reagents were used, giving the product in 63% yield and requiring no purification after aqueous work-up. On repetition of the synthesis, DCC was substituted for the EDCI.HCl/HOBt system to provide a more economically viable route for use on larger scale. The reaction proceeded well, improving the yield to 77%, however, as the by-product of this reaction is not water soluble, purification by column chromatography was required. Finally, activation of the acid in the form of the acid chloride provided an excellent yield and easy purification, capable of use on a large scale.
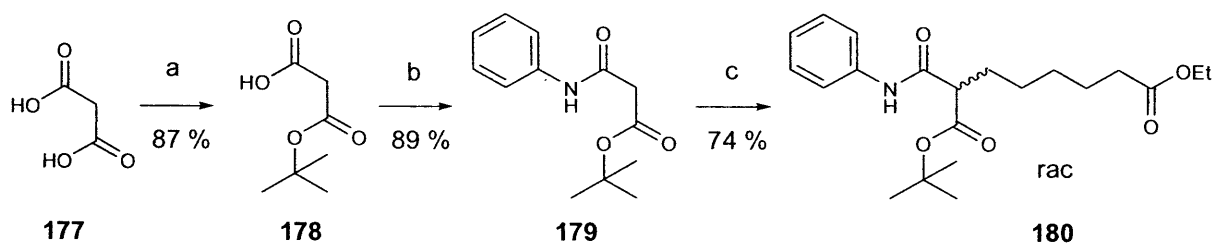
(a) Bu^{t}OH, DCC, MeCN; 2 h, r.t.; (b)(i) oxalyl chloride, DMF (cat), DCM; 2 h, 40 °C; (ii) PhNH$_2$, DMAP, DCM; 2 h, r.t.; (c)(i) NaH, THF, 20 min, r.t.; (ii) ethyl 6-bromohexanoate; 18 h, 70 °C.

Scheme 4.6. Synthesis of alkylmalonamide ester intermediate.

It proved possible to alkylate the intermediate malonamide ester **179** under standard conditions despite the reduced acidity of the methylene group compared with that of a malonate ester. Deprotonation with sodium hydride in tetrahydrofuran was followed by alkylation with ethyl 6-bromohexanoate at reflux in excellent yield. It was envisaged that the synthesis of these compounds would initially aim at producing racemic material. Any racemic compounds that showed promising activity would then be targeted for asymmetric synthesis or chiral resolution in order to assess any difference in activity between enantiomers. Separation of the product from unreacted starting material was difficult to achieve completely by column chromatography, however, pure material could be obtained with subsequent recrystallisation from diethyl ether-petroleum ether.

Synthesis of the acid-containing compound **149** was first attempted by selective hydrolysis of the *tert*-butyl ester with trifluoroacetic acid (intermediate **182**, scheme 4.8), then conversion of the ethyl ester to hydroxamic acid. Whilst this route did provide the target compound, it proved extremely difficult to isolate the pure product from the highly polar starting material. It was therefore decided to attempt conversion of the ethyl ester of **180** to the hydroxamic acid in the presence of the *tert*-butyl ester (scheme 4.7). Reaction proceeded rapidly to give the hydroxamic acid precursor **181**, which was purified by column chromatography before hydrolysis of the *tert*-butyl ester to give the final compound.
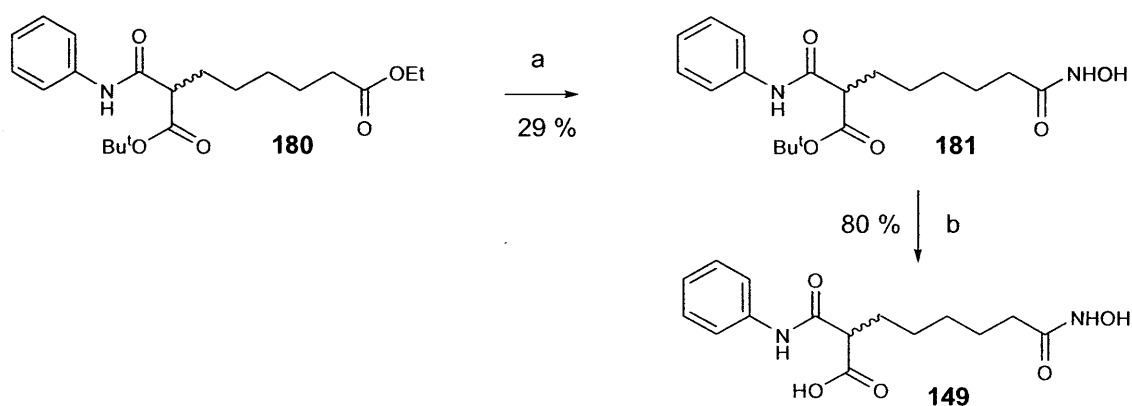
(a) NH$_2$OH(aq), 1 M KOH/MeOH, THF; 45 min, r.t.; (b) TFA, DCM; 1h, r.t.

Scheme 4.7. Synthesis of acid **149**.

The hydroxymethyl analogue **150** was seen to be available through hydrolysis of the *tert*-butyl ester, selective reduction of the resultant acid **182** and conversion to the hydroxamic acid (scheme 4.8). Hydrolysis proceeded in excellent yield, however, reduction of the acid proved more troublesome. Initially it was envisaged that reduction with borane would be suitably selective for the carboxylic acid; reaction with up to two equivalents of BH₃.THF produced no reaction, but increasing to five equivalents resulted in additional reduction of the amide. It was possible to reduce the acid in the presence of the amide and ester by formation of the mixed anhydride with ethyl chloroformate, followed by reduction with sodium borohydride, adapting the procedure of Polla et al.[5]
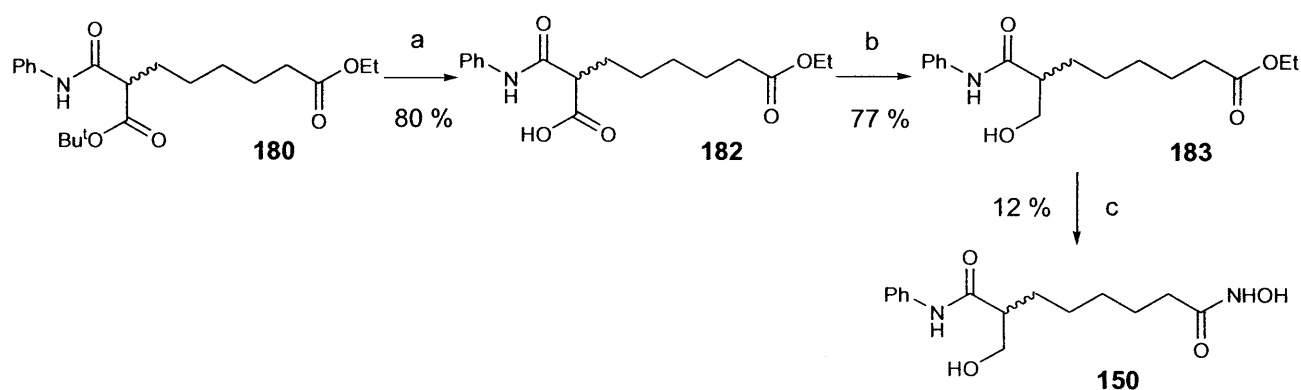
(a) TFA, DCM; 6 h, r.t.; (b)(i) ethyl chloroformate, Et₃N, THF; 30 min, 0 °C; (ii) NaBH₄, MeOH; 1.5 h, 10 °C; (c) NH₂OH(aq), 1 M KOH/MeOH, THF;

Scheme 4.8. Hydroxymethyl analogue.

The intermediate acid **182** provided a route to many potential targets bearing two different aromatic groups in the cap region. A compound of particular interest was the hybrid combination of SAHA with our benzimidazole analogue **151**, in the form of benzimidazole-containing anilide **152**. The synthesis involved coupling of the acid with 1,2-phenylenediamine followed by dehydrative cyclization of the free amino group on the amide carbonyl under acidic conditions (in the same manner as with benzimidazole **176**,

scheme 4.5). In practice, it was found that yields in the coupling step were improved by use of a mono-protected 1,2-diamine **185** (scheme 4.9).
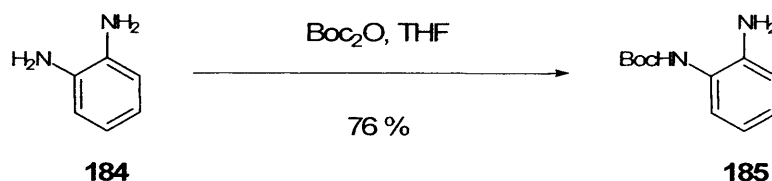
Scheme 4.9. Mono-protection of 1,2-phenylenediamine.

Acylation of the free amino group of **185** with acid **182** using EDCI.HCl and HOBt coupling gave amide **186** in good yield (scheme 4.10). It was then possible to deprotect the amine and form the benzimidazole in one step by warming in trifluoroacetic acid. Attempts to convert the ester of **187** directly to the hydroxamic acid resulted in difficulties in purification of the product. Column chromatography and preparative TLC proved ineffective owing to strong adherence of the compound to silica gel, and recrystallisation from various solvents also failed to remove by-products. It was therefore decided to protect the NH group of the benzimidazole in an effort to reduce the polarity of the product hydroxamate, potentially allowing separation by column chromatography. Boc-protection to give **188** proceeded very slowly, after 4 d only yielding 35% of the protected product. It was, however, possible to recover 45% of the starting material from this reaction. Subjection of the product to the standard hydroxamation conditions of aqueous hydroxylamine and methanolic potassium hydroxide resulted in spontaneous deprotection of the amine. This effect was observed by TLC comparison and is presumably due to the high concentration of hydroxylamine (10 equivalents) in the reaction. Despite this deprotection, the reaction proceeded cleanly and the final product (**152**) purified by recrystallisation from ethanol.
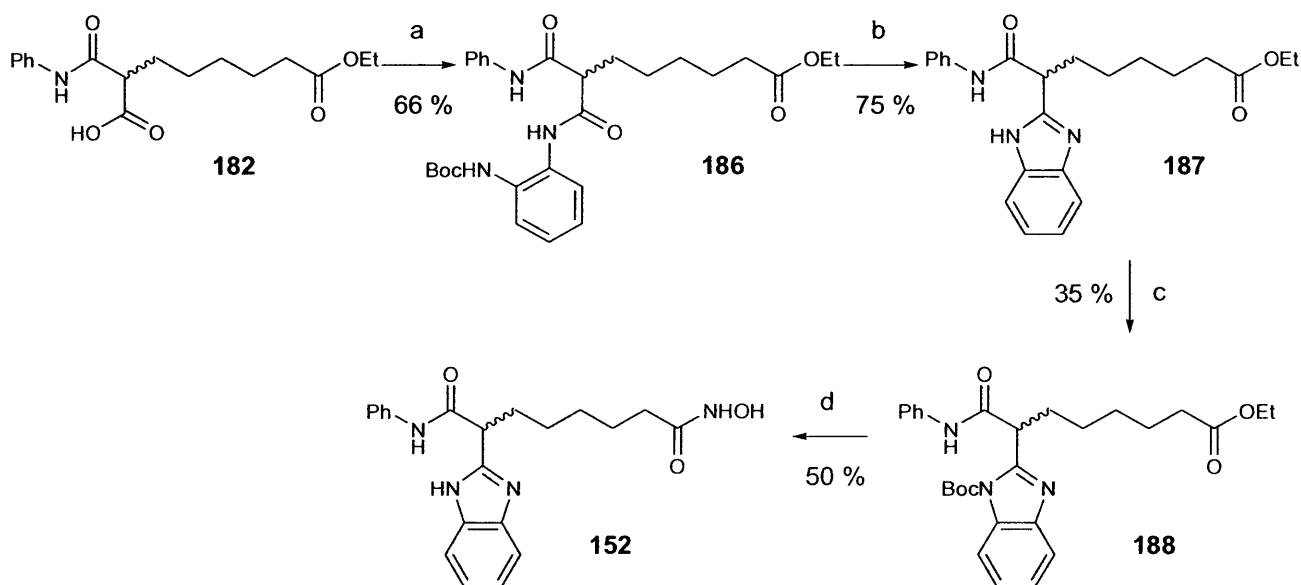
(a) **185**, EDC.HCl, HOBT, Et₃N, DMF; 18 h, r.t.; (b) TFA, DCM; 2 h, reflux;
(c) Boc₂O, THF; 4 d, r.t.; (d) NH₂OH(aq) 1M KOH/MeOH, THF; 6 h, r.t.

Scheme 4.10. Route to benzimidazole analogue.

The hybrid, asymmetric benzimidazole **153** was available using the same methodologies developed with the previous compounds of this class. Acylation of 1,2-phenylenediamine with the *tert*-butyl malonate **178** using DCC coupling agent proceeded rapidly in moderate yield (scheme 4.11). Subsequent dehydration to form the benzimidazole ring occurred on heating **189** in glacial acetic acid in quantitative yield. An initial attempt at alkylation of this compound under the same conditions used for previous malonamide and malonate examples resulted in *N*-alkylation only. The NH group was therefore protected, allowing alkylation on carbon to give **192** in modest yield. Conversion to the hydroxamic acid also resulted in deprotection of the amine, as seen with previously (**152**, scheme 4.10).
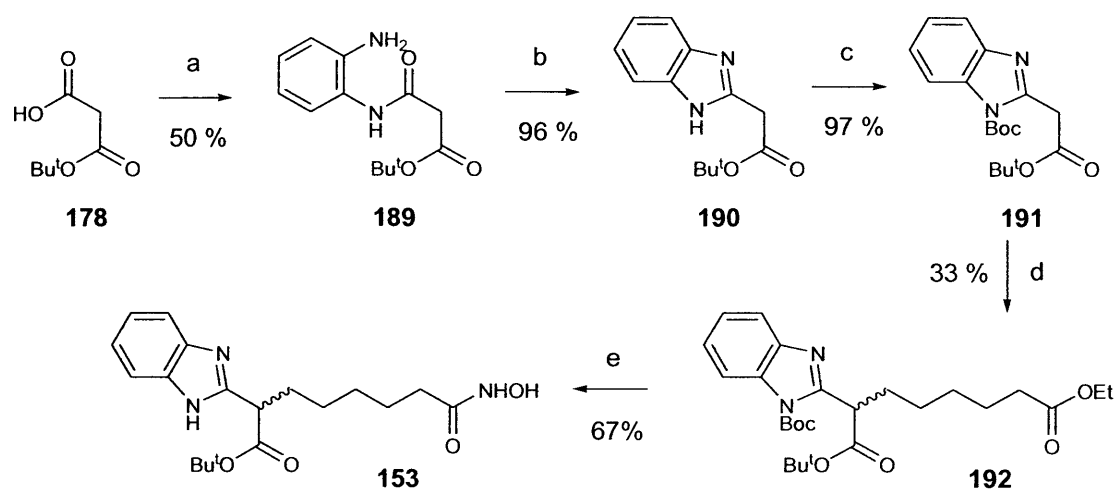
(a) 1,2-Phenylenediamine, DCC, MeCN; 20 min, r.t.; (b) AcOH; 1 h, 90 °C; (c) Boc$_2$O, THF; 3 d, r.t.;
(d)(i) NaH, THF; 30 min, r.t.; (ii) ethyl 6-bromohexanoate; 18 h, 60 °C; (e)NH$_2$OH(aq) 1M KOH/MeOH,
THF; 30 min, r.t.

Scheme 4.11. Functionalised benzimidazole analogue.

4.4 Piperidinone Analogues

This class of compounds retains the general structure of SAHA, including the alkyl spacer,
but introduces some rigidity in the cap region. Formation of piperidinone rings is commonly
carried out with malonic esters under harsh, basic conditions. This would not be possible in
our case as the compound would be required to contain an alkyl ester for subsequent
conversion to the hydroxamic acid. After attempts involving several different methods of
activating the malonyl carboxylic acids to nucleophilic attack, an acceptable method for
formation of the ring was found, and the synthesis is given in scheme 4.12.
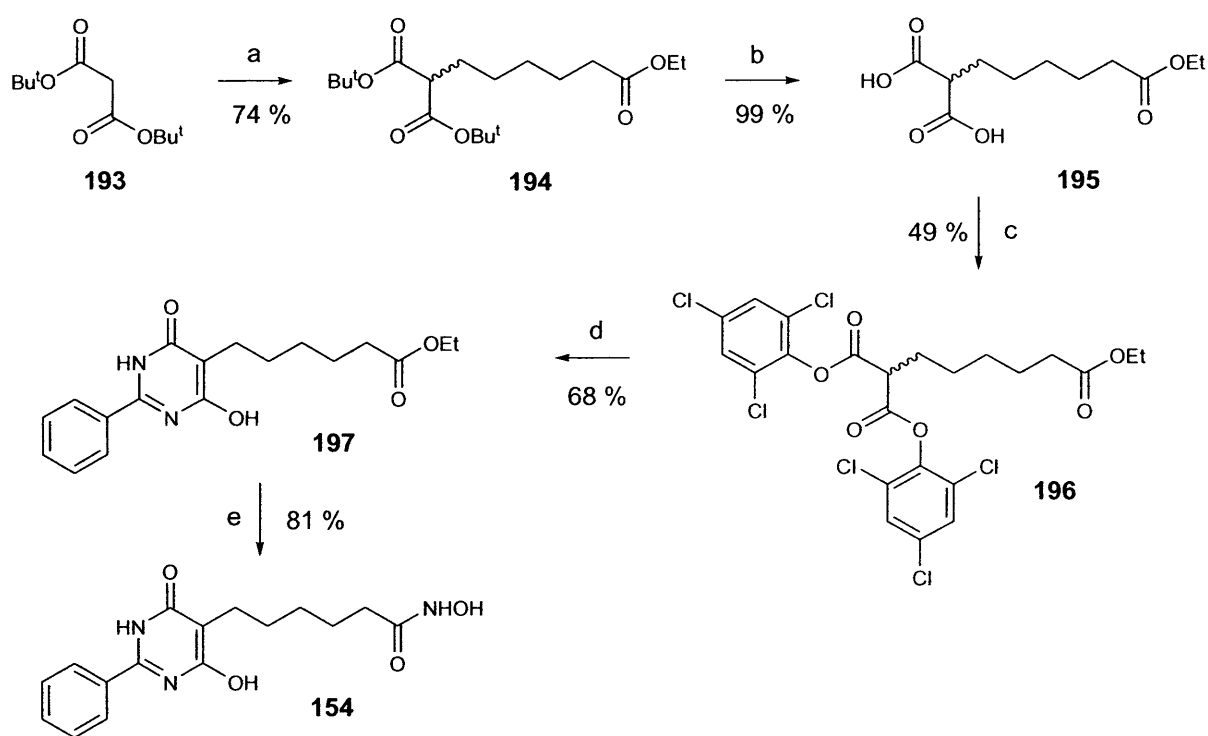
(a)(i) NaH, THF; 30 min, r.t.; (ii) ethyl 6-bromohexanoate; 16 h, 85 °C; (b) TFA, DCM; 24 h, r.t.;
(c) 2,4,6-trichlorophenol, POCl₃; 5 h, 100 °C; (d) benzamidine, DCM; 1 h, 40 °C;
(e) NH₂OH(aq), 1M KOH/MeOH, THF; 2h, r.t.

Scheme 4.12. Synthesis of piperidinone **154**.

Alkylation of di-*tert*-butyl malonate was carried out under established conditions, with sodium hydride in tetrahydrofuran. The two *tert*-butyl esters of **194** were selectively hydrolysed with trifluoroacetic acid to give di-acid **195** in excellent yield. Attempts at formation of the piperidinone via formation of the di-acyl chloride or activation of the carboxylic acids via formation of the mixed anhydride with ethyl chloroformate both failed to give the desired product on treatment with benzamidine. The transformation was eventually completed through the di-2,4,6-trichlorophenol ester **196**, formed by refluxing the acid **195** with 2,4,6-trichlorophenol in phosphorus oxychloride,[6] which reacted rapidly and cleanly with the amidine, the products poor solubility allowing purification by washing with ethanol. Presumably the trichlorophenol ester provided the correct level of activity to

allow reaction with the amidine but avoid formation of unwanted side-products. The ethyl ester of **197** was then converted to the hydroxamic acid **154** with aqueous hydroxylamine and methanolic potassium hydroxide solution.

It was thought possible that it may be able to reduce the number of steps to this ester intermediate (**196**) by directly alkylating the bis-trichlorophenyl malonate **198**. This ester was available from reaction of malonic acid with the phenol under the same conditions as above,[6] however, attempts to alkylate were unsuccessful (scheme 4.13). In light of the undesired result of this reaction, it is noted that deprotonation of **198** could readily result in elimination of a TCP group to form a highly reactive ketene intermediate.
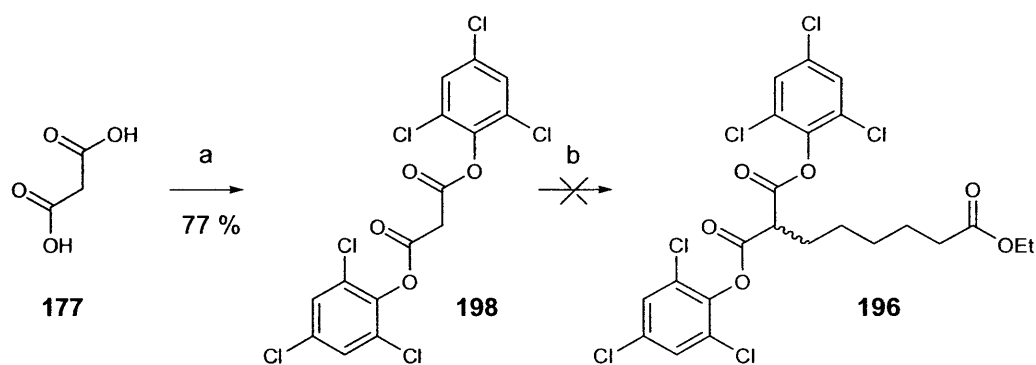
(a) 2,4,6-Trichlorophenol, POCl$_3$; 2.5h, 100 °C; (b)(i) NaH, THF; 30 min, r.t.;
(ii) ethyl 6-bromohexanoate; 18h, 75 °C

Scheme 4.13. Attempted alkylation of malonate ester.

The second piperidinone compound (**155**) required synthesis of the guanidine **201** before formation of the ring. The third nitrogen of the group was installed by addition of aniline to nitrile **200**, requiring an extended period of heating in toluene and still only proceeding in very modest yield (scheme 4.14). Diethyl cyanamide (**200**) was prepared by the procedure

of Keil and Hartmann through careful addition of diethylamine to cyanogen bromide on ice.[7]
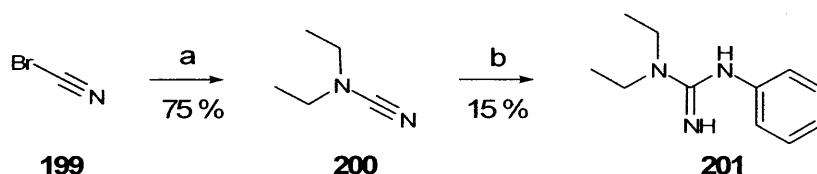
(a) Diethylamine, Et_2O, 30 min, $0^{\circ}C$; (b) aniline.HCl, PhMe; 3d, reflux.

Scheme 4.14. Synthesis of guanidine precursor.

With guanidine **201** now in hand, the pyrimidinone of **202** was formed from the previously synthesised TCP-precursor **196** (scheme 4.15). The reaction did not proceed as readily as with benzamidine, but a good yield was achieved by heating the two compounds neat for 5 min at 150 °C, according to the procedure of Gotthard et al.[8] Hydroxamation followed under standard conditions to give the target compound **155**. Unfortunately, the physical properties of the hydroxamic acid appear to be unsuitable for a drug molecule – no solid was obtained, it being unclear whether the product is an oil, or, as may be more likely, an extremely hygroscopic solid.
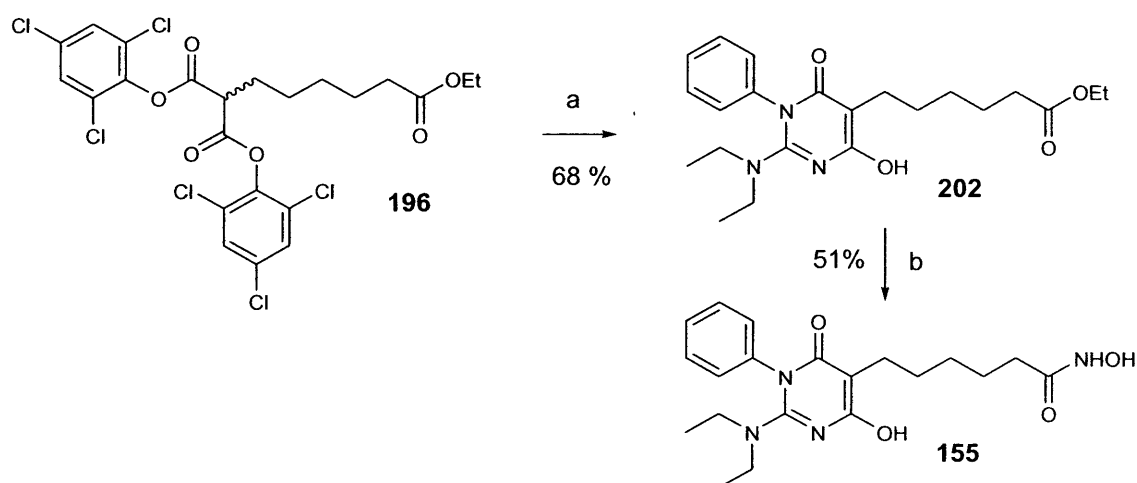
(a) **201**; 5 min, 150 °C; (b) NH₂OH(aq), 1 M KOH/MeOH, THF; 2 h, r.t.

Scheme 4.15. Synthesis of functionalised piperidinone.

The compounds synthesised in this series were subjected to a routine screen to biological activity. Screening was in the form of a cellular proliferation assay carried out at a standard 5 µM concentration and was performed by Alexandra V. Stavropoulou at Imperial College London. The majority of compounds described here unfortunately did not show significant activity, however, the benzimidazole SAHA analogue **151** and hydroxymethyl analogue **150** (figure 4.1) did show promising results. Further analysis was carried out on these compounds, respective IC₅₀ values of 2.4 µM and 4.4 µM being determined. Full details of the analysis of these compounds is given in chapter 5, where explanations of methods used are also included.

The development of the series described here is in its early stages, but looks promising for further work. The versatility of the malonate-based compounds for development of further analogues is excellent, with alternative amides, amines, ethers, esters or heterocycles readily accessible from this structure (particularly indoles, bearing in mind the work of chapter 2). In addition to production of further analogues in this series, it would be necessary to consider evaluation of enantiomerically pure compounds. Having observed the activity of

racemic compounds such as the promising alcohol **150**, the analysis of activity of the pure enantiomers is now highly desirable. Work to consider in the immediate future could focus on racemic alcohol **150**, which could be suitable for chiral resolution by formation of an ester with a chiral acid. The proximity of the alcohol to the chiral centre would potentially mean this is a viable method for separating the resultant diastereomers. An alternative would be an asymmetric synthesis, possibly involving an asymmetric aldol reaction.

4.5 References

1. Mai, A.; Esposito, M.; Sbardella, G.; Massa, S. *Organic Preparations and Procedures International* **2001**, *33*, (4), 391-394.

2. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **1999**, *401*, (6749), 188-193.

3. Du, L.; Wang, Y. A Rapid and Efficient Synthesis of Benzimidazoles Using Hypervalent Iodine as Oxidant. *Synthesis* **2007**, *5*, 675-678.

4. Shelkov, R.; Nahmany, M.; Melman, A. Acylation through Ketene Intermediates. *Journal of Organic Chemistry* **2002**, *67*, (25), 8975-8982.

5. Polla, M. O.; Tottie, L.; Norden, C.; Linschoten, M.; Musil, D.; Trumpp-Kallmeyer, S.; Aukrust, I. R.; Ringom, R.; Holm, K. H.; Neset, S. M.; Sandberg, M.; Thurmond, J.; Yu, P.; Hategan, G.; Anderson, H. Design and synthesis of potent, orally active, inhibitors of carboxypeptidase U (TAFIa). *Bioorganic & Medicinal Chemistry* **2004**, *12*, (5), 1151-1175.

6. Varga, M.; Kapui, Z.; Batori, S.; Nagy, L. T.; Vasvari-Debreczy, L.; Mikus, E.; Urban-Szabo, K.; Aranyi, P. A novel orally active inhibitor of HLE. *European Journal of Medicinal Chemistry* **2003**, *38*, (4), 421-425.

7. Keil, D.; Hartmann, H. Preparation and Characterisation of *N,N*-disubstituted 2-amino-selenazoles. *Phosphorus, Sulfur and Silicon* **1999**, *152*, 169-184.

8. Gotthardt, H.; Blum, J.; Schenk, K. H. Synthesis of 2,6-diazabicyclo[2.2.2]oct-7-ene-3,5-diones and 2(1*H*)-pyridinones - A Contribution to the Mechanism and to the Regiochemistry of 1,4-Dipolar Cycloaddition Reactions of New and Known 3,6-Dihydro-6-oxo-1-pyrimidinium-4-olates to Electron-Rich and Electron-Poor Alkynes. *Chemische Berichte-Recueil* **1986**, *119*, 1315-1330.

5 Biological Analysis

In addition to the biological evaluation of compounds synthesised for this thesis, which was carried out by collaborating groups, compounds previously synthesised (unpublished work) within this group were tested for biological effect. In these cases, evaluation took the form of cell protein and cell cycle analysis. Work was carried out at the Rayne Institute, King's College London under the supervision of Dr N. S. B. Thomas. Protein analysis was carried out by SDS-PAGE and western blot and cell cycle analysis by flow cytometry on two human cancer cell lines, HCT116 (colon) and HeLa (cervical). An *in vitro* assay for histone deacetylase activity was also carried out.

Also discussed in this chapter are the results of the biological analysis of SAHA analogues **150** and **151**, as carried out by Alexandra V. Stavropoulou. The synthesis of these compounds was discussed in chapter 4.

5.1 SST4-4 and SST4-5

Novel histone deacetylase (HDAC) inhibitors SST4-4 and SST4-5 were assessed for biological function by whole cell protein analysis and cell cycle analysis. The pair of compounds are complex macrocycles, structurally analogous to the known inhibitor depsipeptide (also known as FK228). Although biological activity of these compounds was lower than expected on the basis of structural analogy with depsipeptide, some differences in biological effect were observed between the compounds and between the cell lines.
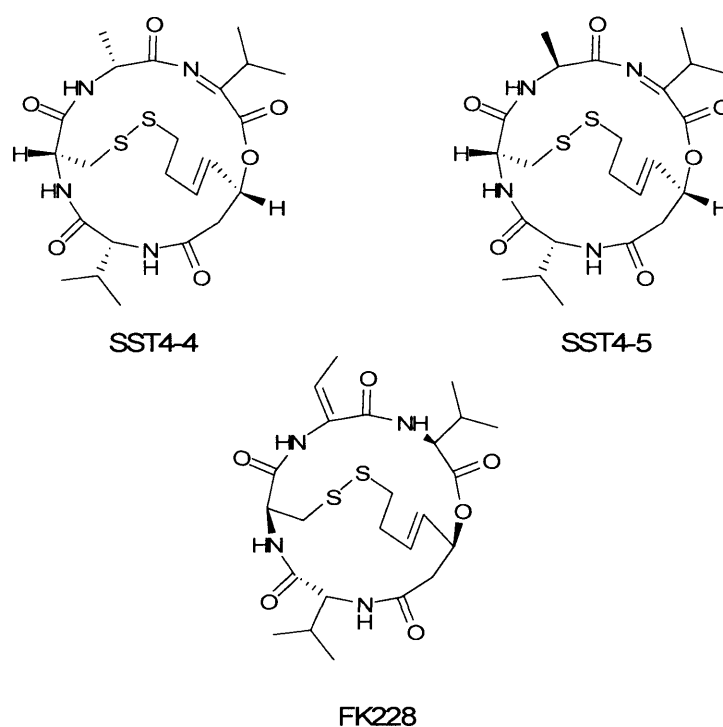
Figure 5.1 Structures of SST4-4, SST4-5 and FK228

5.1.1 Protein Analysis

Typical experiments for analysis of protein by western blot involved the incubation of cells for 24 h in the presence of a range of concentrations of inhibitors. Controls and standards in the form of known inhibitors trichostatin A (TSA) and apicidin were always included in the assay. On completion of incubation, cells were harvested, samples fixed in SDS-lysis buffer and heated to 100 °C for 10 min before storage at -20 °C.

After SDS-PAGE and western blot, membranes were probed with antibodies to assess deacetylase activity and potential apoptosis. Anti hyperacetylated histone H4 is a marker for inhibition of class I HDACs which are most commonly localised to the nucleus. Class II HDACs are generally able to shuttle between the nucleus and cytoplasm, allowing detection of cytoplasmic substrates to provide an indication of class II inhibition. For this, anti acetylated α-tubulin was used as the marker. Anti PARP was employed as a marker for caspase-

dependent apoptosis. Anti MAPK was used as an indication of protein loading for the electrophoresis.

Preliminary experiments at low nanomolar inhibitor concentrations showed little effect on levels of the probed proteins, therefore concentrations of SST4-4 and SST4-5 were increased to a maximum of 10 µM.

5.1.2 Results

Qualitative protein analysis after culturing HeLa cells with SST4-4 and SST4-5 at concentrations from 50 nM to 10 µM are shown in figure 5.2. Trichostatin A (TSA) and apicidin were included at concentrations of 1 µM and 100 nM respectively as controls.
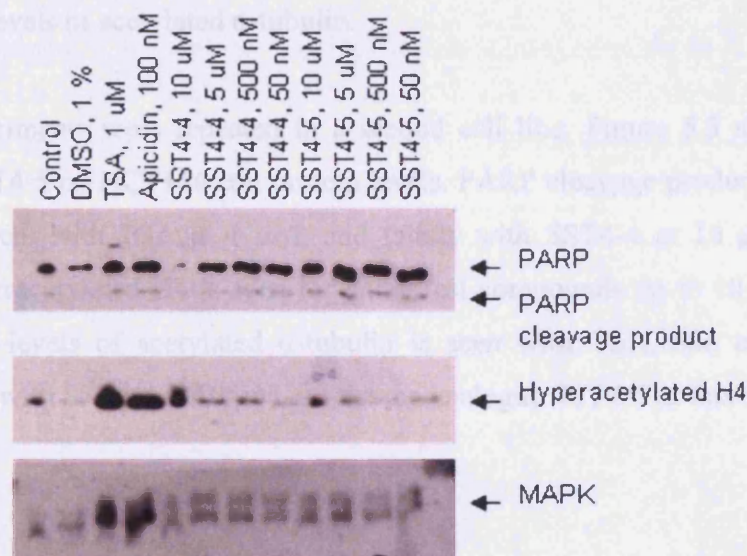
Figure 5.2. Accumulation of hyperacetylated H4 in HeLa cells on 24 h incubation with HDAC inhibitors as detected by western blot. Test compounds SST4-4 and SST4-5 were incubated at concentrations ranging from 50 nM to 10 µM. Detection of PARP and its respective cleavage product was used as an indication of apoptosis and MAPK expression was used as a loading control. Representative of n=2 experiments.

Faint PARP cleavage products are seen in most lanes, however, no full-length PARP is present with SST4-4 at 10 µM (**A,** figure 5.2). This total loss of PARP at high concentrations of SST4-

4 is consistent with high levels of apoptosis identified by flow cytometry (discussed later). In comparison with the DMSO control, distinct increases in levels of hyperacetylated H4 (**B**) are seen with apicidin, TSA and SST4-4 at 10 μM. A slight increase is also seen with SST4-5 at 10 μM. Detection of MAPK as a protein-loading control (**C**) indicated that overloading may have occurred in the TSA and apicidin lanes, but all other cases appear approximately equal. Probing with anti acetylated α-tubulin showed no detectable levels of protein under all conditions (results not shown).

The results obtained from the protein analysis in HeLa cells indicate that SST4-4 is capable of increasing accumulation of hyperacetylated H4 when present in high concentrations. As indicated by the known inhibitors TSA and apicidin, this is the predicted result of HDAC inhibition. No inference can be made with respect to HDAC class specificity owing to extremely low levels of acetylated α-tubulin.

The same experiments were repeated in a second cell line. Figure 5.3 shows the effect of SST4-4 and SST4-5 on HCT116 cell protein levels. PARP cleavage products can be seen as a result of treatment with TSA at 1 μM, and faintly with SST4-4 at 10 μM. No significant increase in hyperacetylated H4 is seen for either test compounds up to 10 μM concentration. An increase in levels of acetylated α-tubulin is seen with TSA, and crucially, a distinct increase is seen with SST4-4 at 10 μM but not the analogue SST4-5 at this concentration.
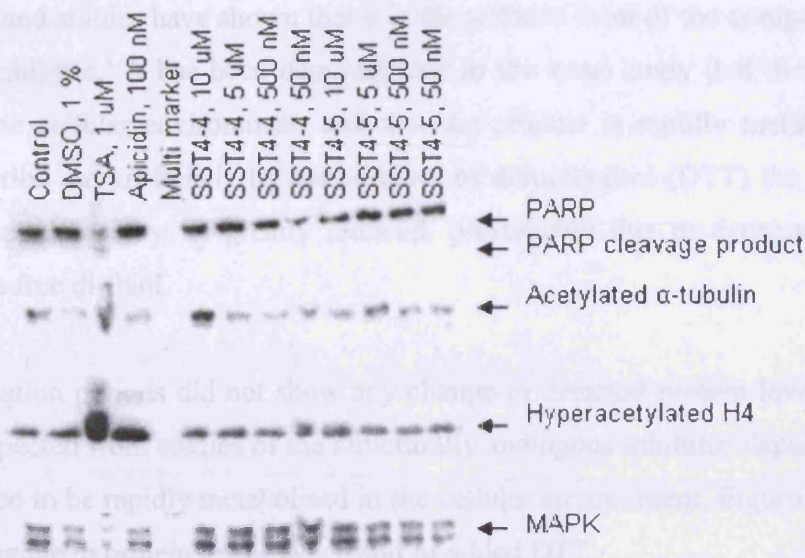
Figure 5.3. Accumulation of hyperacetylated H4 and acetylated α-tubulin in HCT116 cells on 24 h incubation with HDAC inhibitors as detected by western blot. Test compounds SST4-4 and SST4-5 were incubated at concentrations ranging from 50 nM to 10 µM. Detection of PARP and its respective cleavage product was used as an indication of apoptosis and MAPK expression was used as a loading control. Representative of n=2 experiments.

These results indicate that SST4-4 is a weak inhibitor of class II HDACs in HCT116. It is not possible to say from these results whether this inhibition is specific to class II over class I from the comparison with hyperacetylated H4 accumulation. This is owing to high naturally occurring background levels of the protein in this cell line, making subtle changes in level hard to detect. There is no obvious effect on the protein levels assessed from treatment with the analogue SST4-5.

Further experiments were carried out with increased incubation time using HCT116 cells. In addition to 24 h, samples were taken at 48 h and 72 h to determine any possible delayed effects of treatment, and a final experiment to determine the effect of reduction of the internal disulfide bridge present in SST4-4 and SST4-5 was included. The functionality is also present

in depsipeptide and studies have shown that it is the reduced form of the compound that is the active HDAC inhibitor.[1] It has been demonstrated in the same study that the reduction will take place in the cellular environment, and that the product is rapidly metabolised. If this reduction is carried out artificially by the addition of dithiothreitol (DTT) the potency of the compound in cellular assays is greatly reduced, presumably due to decreased uptake and instability of the free di-thiol.

Increased incubation periods did not show any change in detected protein levels (figure 5.4). This may be expected from studies of the structurally analogous inhibitor depsipeptide, which has been reported to be rapidly metabolised in the cellular environment. Figure 5.4 also shows no detectable change in protein levels as a result of added DTT.

1. 24 h

Control
DMSO, 1 %
TSA, 0.3 uM
Apicidin, 0.1 µM
SST4-4, 10 uM
SST4-5, 10 uM

← PARP
← PARP cleavage product

← Acetylated α-tubulin

← Hyperacetylated H4

← MAPK

2. 24 h + DTT

Control
DMSO, 1 %
TSA, 0.3 uM
Apicidin, 0.1 µM
SST4-4, 10 uM
SST4-5, 10 uM

← PARP
← PARP cleavage product

← Acetylated α-tubulin

← Hyperacetylated H4

← MAPK

3. 48 h

Control
DMSO, 1 %
TSA, 0.3 uM
Apicidin, 0.1 µM
SST4-4, 10 uM
SST4-5, 10 uM

← PARP
← PARP cleavage product

← Acetylated α-tubulin

← Hyperacetylated H4

← MAPK

4. 72 h

Control
DMSO, 1 %
TSA, 0.3 uM
Apicidin, 0.1 µM
SST4-4, 10 uM
SST4-5, 10 uM

← PARP
← PARP cleavage product

← Acetylated α-tubulin

← Hyperacetylated H4

← MAPK

Figure 5.4. Accumulation of hyperacetylated H4 on incubation of HCT116 cells for extended time periods (24, 48 and 72 h) in the presence of SST4-4 or SST4-5 at 10 µM. Incubation (24 h) in the presence of DTT showed no significant effect.

5.1.3 Cell Cycle Analysis

Cell cycle analysis was carried out by flow cytometry. The technique allows identification of the cell cycle stage of individual cells by detection of protein and DNA levels. Treatment of cells to be sampled with a fluorescent dye that bind non-specifically to protein, and another for DNA allows quantification by detection of fluorescence from the respective tags on laser excitation. Detection is possible by passing a single-file stream of the labelled cells in front of the laser and as each cell passes, the resultant scattered light is recorded as an 'event'. For each event, the relative level of both cell protein and DNA content is determined from light emitted by the respective tags, and the data plotted. After calibration of the cell cycle profile of the desired cell type, the populations of cells in each stage of the cell cycle can be determined from the characteristic proportions of DNA-to-protein levels. For example, from a sample of 'normally' dividing cells, a band will be seen containing 2n equivalents of DNA – these cells can be identified as residing in G0/G1 phase. Cells in G2/M, which are preparing to divide, contain 4n equivalents of DNA and higher total protein levels. Those cells possessing DNA and protein levels between these groups are assigned to S phase, and those with levels below that of G0/G1 are designated to be undergoing apoptosis (figure 5.5).
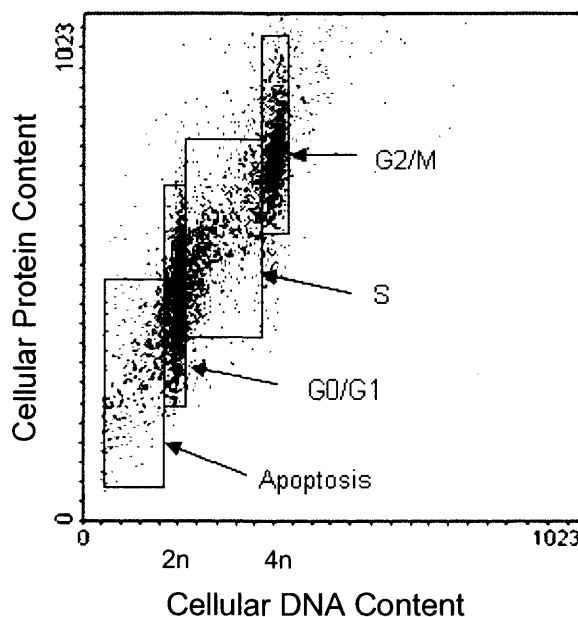
Figure 5.5. Example of cell cycle data obtained from flow cytometry of normal HCT116 cells.

Results from flow cytometry of HeLa cells incubated for 24 h with SST4-4 are shown in figure 5.6. Figures are given for the percentage of cells in the samples taken at each stage of the cell cycle (G0/G1, S or G2/M) and those undergoing apoptosis. Displayed here are the results for the control sample, a DMSO control, TSA and apicidin standards and SST4-4 at 50 nM, 500 nM, 5 µM and 10 µM.

SST4-4 HeLa
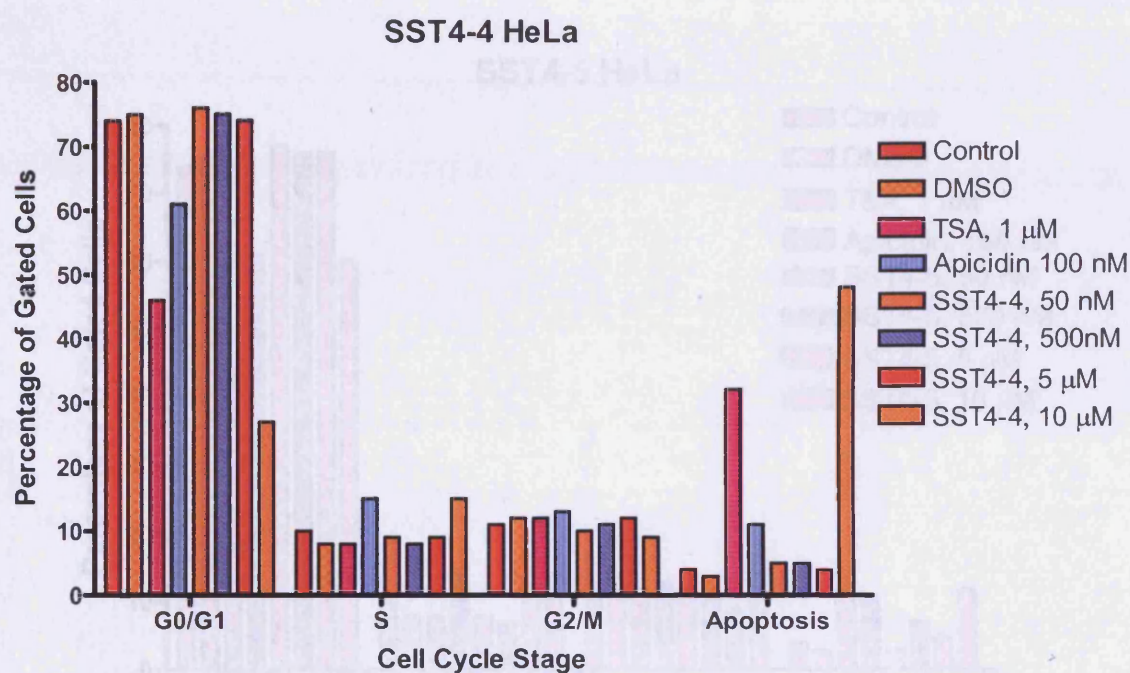
Figure 5.6. Apoptosis in HeLa cells induced by SST4-4. Cell cycle analysis by flow cytometry of HeLa cells cultured for 24 h with SST4-4 at concentrations up to 10 µM.

The effect of TSA on the cell cycle can be clearly seen with a reduction of cells in G0/G1 and a dramatic increase of those undergoing apoptosis. Concentrations of SST4-4 up to 5 µM show no significant difference in cell cycle profile from the controls, however at 10 µM there is a very pronounced drop in cells in G0/G1 accompanied by a massive increase in apoptosis. This observed high level of apoptosis is consistent with the loss of PARP (identified by

western blot, figure 5.1) from cells cultured in this concentration of SST4-4. The result also highlights the clear difference between the effects of SST4-4 and SST4-5 on HeLa cells (figure 5.7). The cell cycle profile of HeLa cells treated with the same concentration range of SST4-5 is shown in figure 5.7. The internal standards TSA and apicidin can again be seen to exert a consistent effect on the profile. As with SST4-4, SST4-5 shows no significant change in cell cycle profile up to 5 μM, however, unlike SST4-4, at 10 μM there is only a very modest increase in apoptosis.
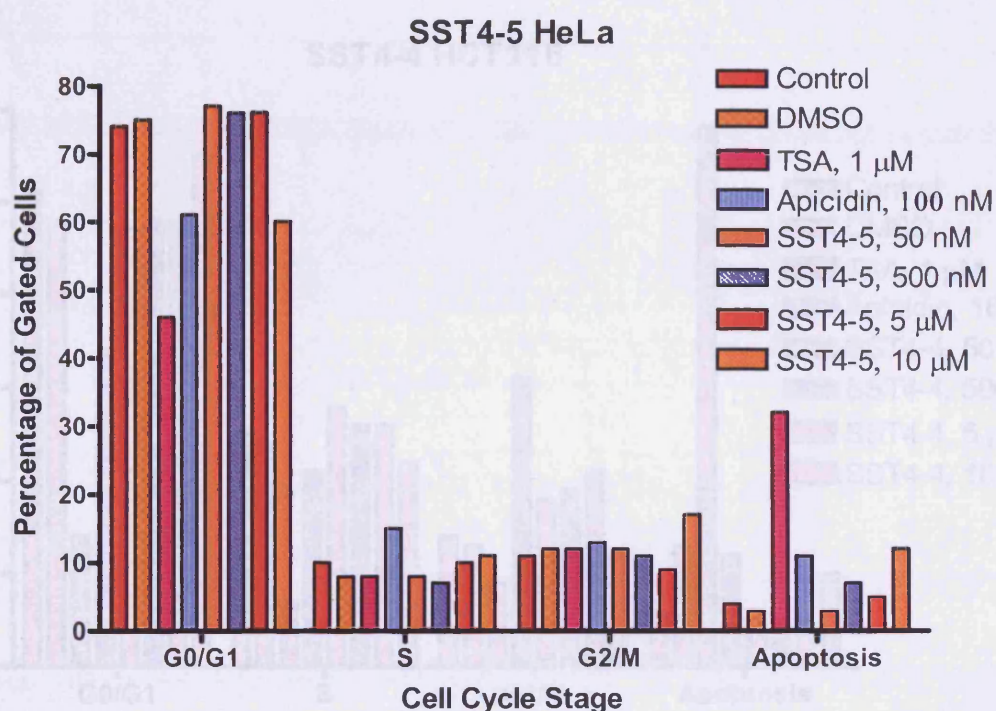
Figure 5.7. No significant change in cell cycle profile of HeLa cells cultured for 24 h with SST4-5 at concentrations up to 10 μM.

These results, in addition to results of protein analysis, show a clear difference in activity between analogues SST4-4 and SST4-5, with SST4-4 producing accumulation of hyperacetylated H4 and high levels of apoptosis at 10 μM but little or no effect by SST4-5 at the same concentration.

As may have been expected from the limited effects of both compounds on HCT116 cells, no dramatic changes in cell cycle profile were observed on culturing the cells for 24 h with concentrations up to 10 μM. Figure 5.8 demonstrates the pronounced effect of TSA on the cells, producing a massive decrease in cells in G0/G1 along with increase in apoptosis at 1 μM concentration. Apicidin shows no great increase in apoptosis, but does produce an accumulation of cells in the G2/M stage. SST4-4 does not show any significant effect on cell cycle at any concentration.
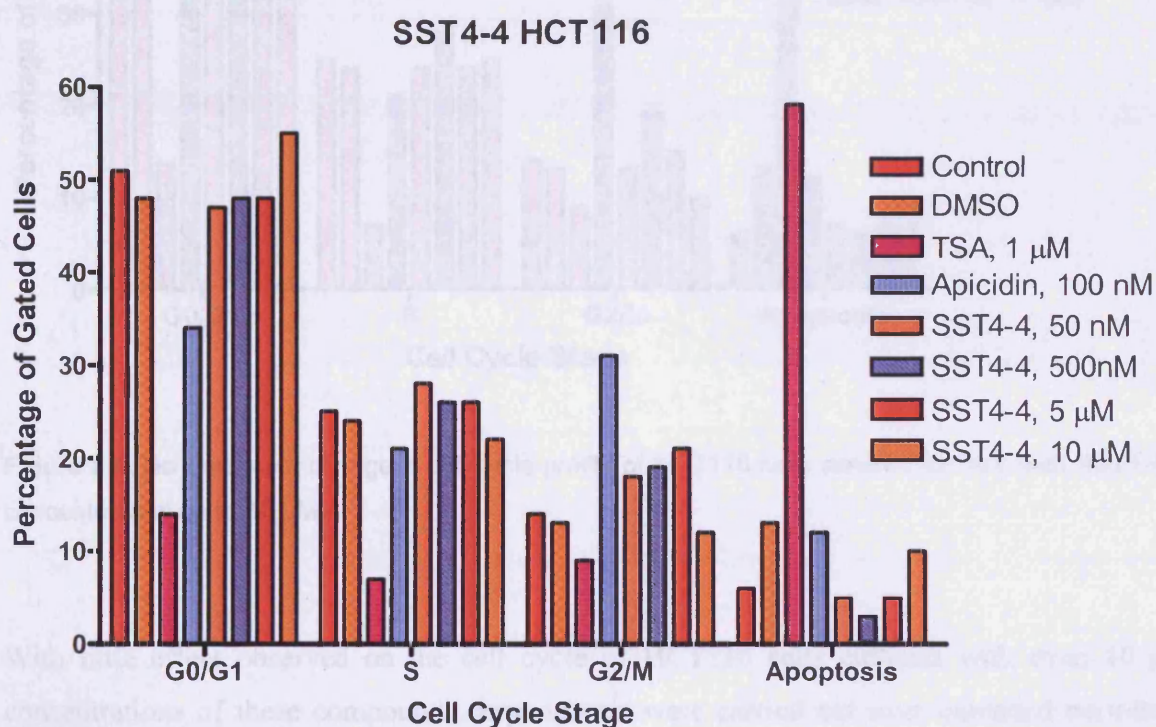
Figure 5.8. No significant change in cell cycle profile of HCT116 cells cultured for 24 h with SST4-4 at concentrations up to 10 μM.

Similar profiles are obtained from 24 h incubation of HCT116 cells with SST4-5 (figure 5.9).
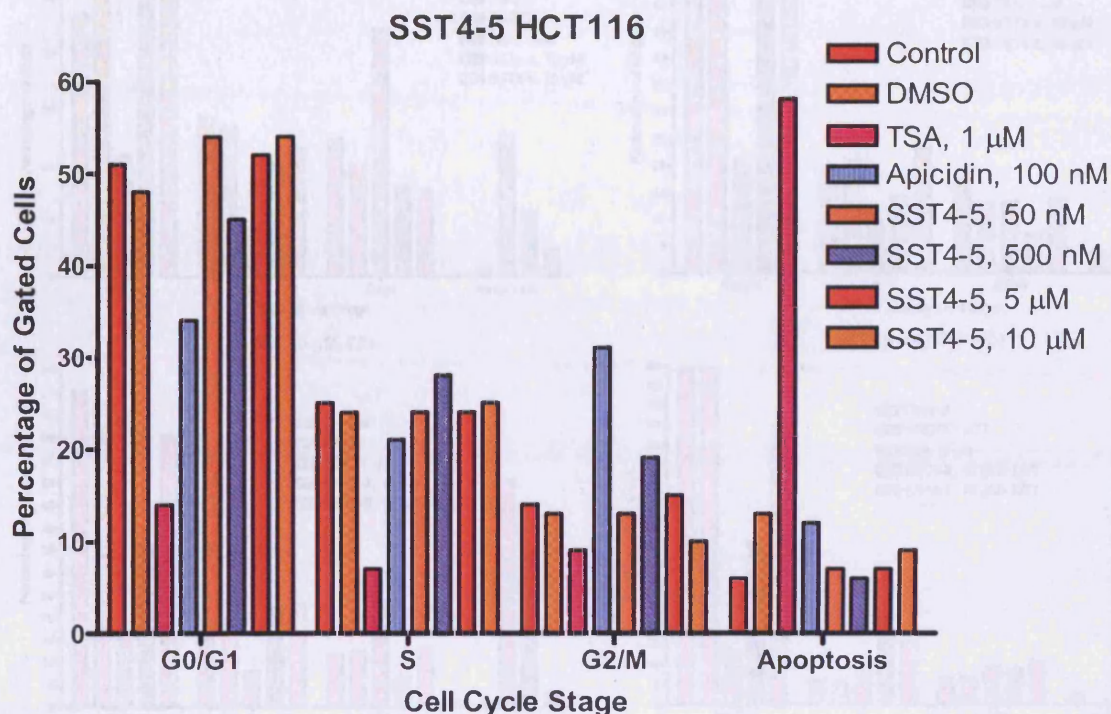
Figure 5.9. No significant change in cell cycle profile of HCT116 cells cultured for 24 h with SST4-4 at concentrations up to 10 μM.

With little effect observed on the cell cycle of HCT116 cells cultured with even 10 μM concentrations of these compounds, experiments were carried out over extended periods to determine any delayed effects. Figure 5.10 shows the effect of single concentration 10 μM doses over 24 h, 48 h and 72 h. In addition to the extended time periods, the influence of the reducing agent DTT with 24 h culture was determined.
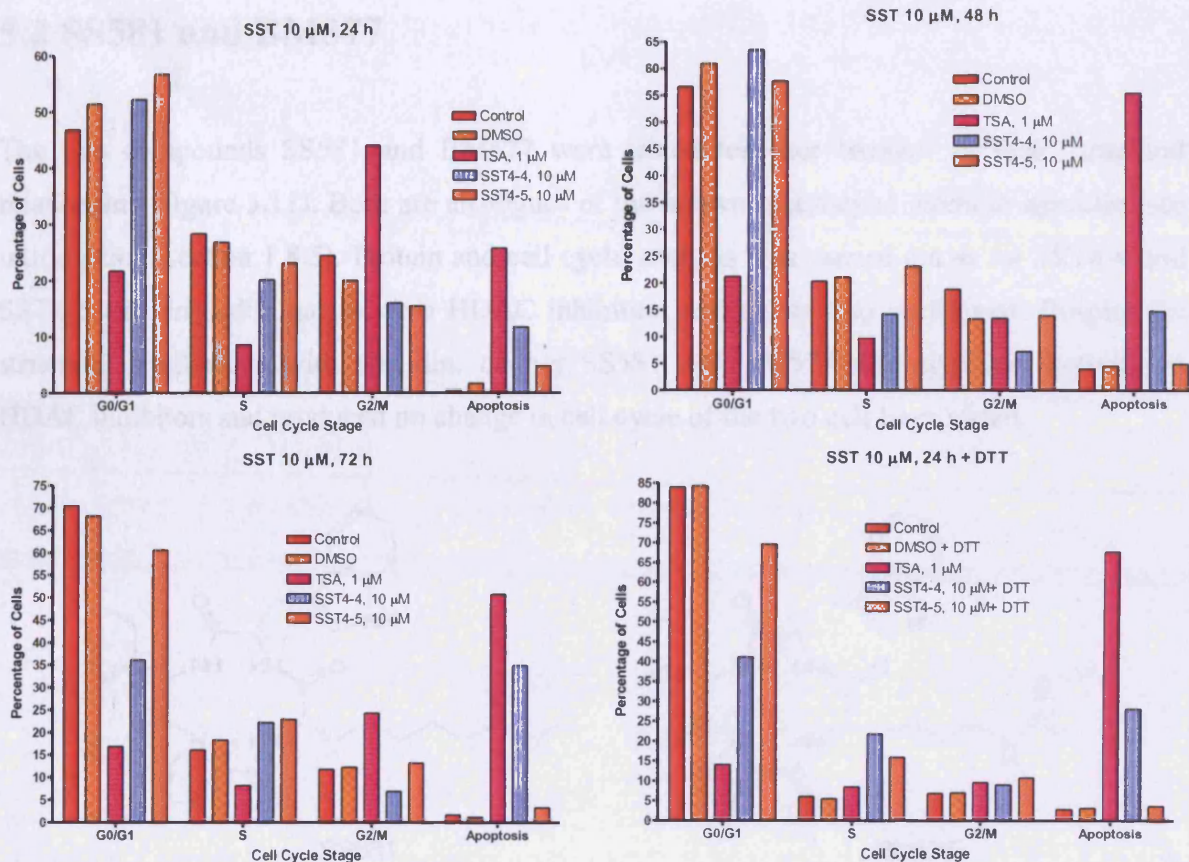
Figure 5.10. Increase in induction of apoptosis in HCT116 cells on extended incubation with SST4-4 but not SST4-5 at 10 µM. Samples were taken for flow cytometry at 24, 48 and 72 h. Some increased levels of apoptosis are also seen on incubation of SST4-4 in the presence of DTT for 24 h.

As was expected, neither SST4-4 or SST4-5 produced a pronounced effect over 24 h, however, prolonged exposure to SST4-4 did increase levels of apoptosis. Incubation with added DTT did appear to significantly decrease the population of cells residing in G0/G1, with a slight increase in apoptotic cells over 24 h incubation in the absence of DTT.

5.2 SS581 and BM577

The two compounds SS581 and BM577 were tested together because of their structural relationship (figure 5.11). Both are analogues of the known macrocylic inhibitor apicidin (see introduction, section 1.8.5). Protein and cell cycle analysis was carried out as for SST4-4 and SST4-5 and an additional *in vitro* HDAC inhibition assay was also performed. Despite the structural similarities with apicidin, neither SS581 nor BM577 displayed any activity as HDAC inhibitors and produced no change in cell cycle of the two cell lines tested.

SS581   BM577
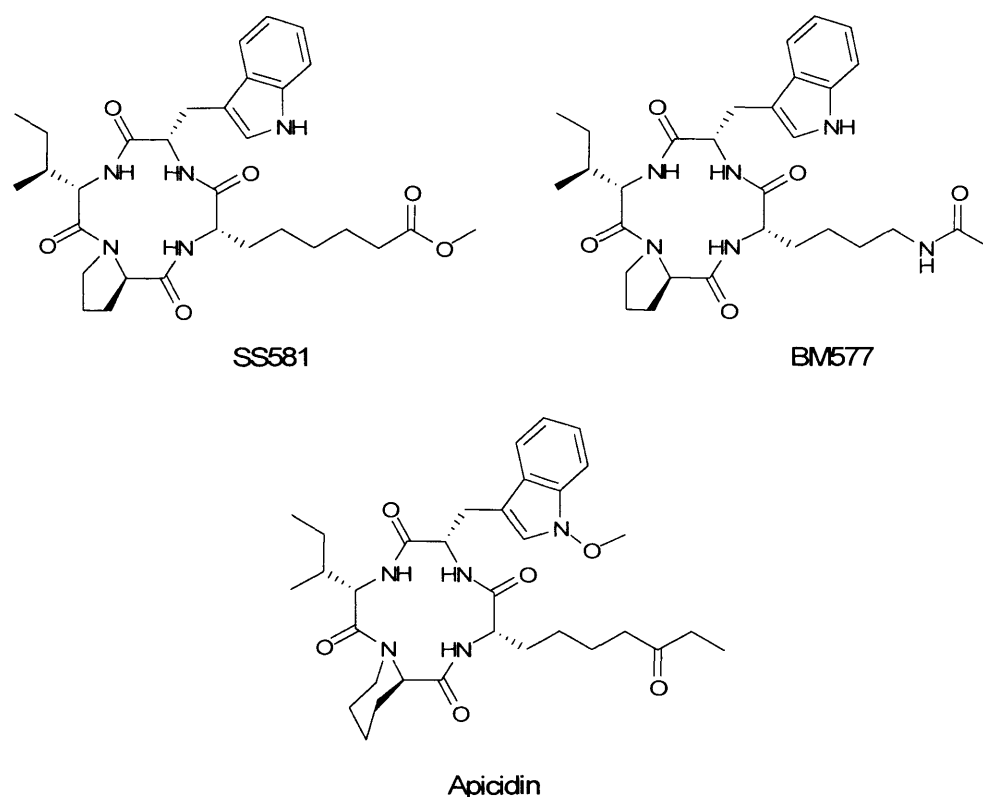
Apicidin

Figure 5.11. Structures of SS581 and BM577 compared with apicidin.

Figure 5.12 shows that cellular levels of hyperacetylated H4 were not increased by either compound up to concentrations of 100 μM. The failure to detect any PARP cleavage product

indicated that apoptosis was not being induced – this was confirmed by the results of cell cycle analysis.

HCT116   **HeLa**

Control
DMSO
TSA, 500 nM
Apicidin, 250 nM
SS581, 10 µM
SS581, 50 µM
SS581, 100 µM
BM577, 10 µM
BM577, 50 µM
BM577, 100 µM

← PARP
← PARP cleavage product

← Hyperacetylated H4

← MAPK

Figure 5.12. Accumulation of hypercetylated H4 in HCT116 and HeLa cells on 24 h incubation with HDAC inhibitors as detected by western blot. Test compounds SS581 and BM577 were incubated at concentrations ranging from 10 µM to 100 µM. Detection of PARP and its respective cleavage product was used as an indication of apoptosis and MAPK expression was used as a loading control. Representative of n=2 experiments.

Results of cell cycle analysis on incubation of both cell lines with each compound up to concentrations of 100 µM for 24 h indicated no effect (figures 5.13 and 5.14).

Figure 5.13. Influence of SS581 and BM577 on HCT116 cells after 24 h incubation. No discernable effect is seen on incubation with test compounds up to 100 μM concentration.

SS581, BM577 HeLa
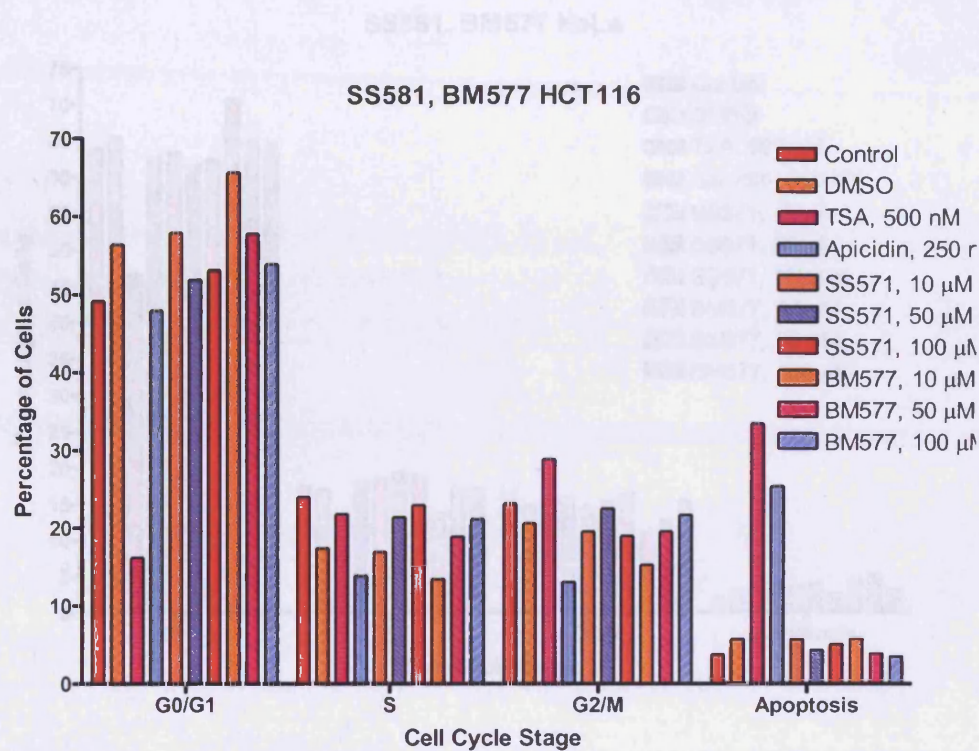
Figure 5.14. Influence of SS571 and BM577 on HeLa cells after 24 h incubation. No discernable effect is seen on incubation with test compounds up to 100 μM concentration.

A final test of these compounds was carried out in the form of an isolated HDAC-inhibition assay. This would allow detection of any capability to act as HDAC inhibitors that may not have been observable in the cellular assays owing to reasons such as metabolism to an inactive form. The assay selected was the commercially available *Fluor de Lys*™ (AK500, BIOMOL) system (figure 5.15).
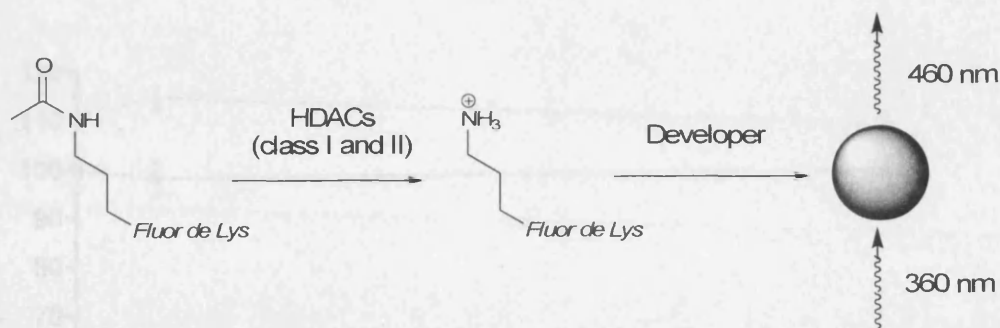
Figure 5.15. Schematic representation of *Fluor de Lys* assay. Deacetylation of the substrate sensitizes it to the developer, which then generates a fluorophore. The fluorophore is excited with 360 nm light and the emitted 460 nm light is detected on a fluorometric plate reader.

An initial experiment was carried with concentrations of SS581 and BM577 up to 500 nM, which displayed no inhibitory activity. The experiment was repeated, increasing concentrations to a maximum of 50 μM (figure 5.16). A control was included in the form of TSA (10 nM), which reduced HDAC activity to below 40 %. The two test compounds still failed to produce any inhibitory effect.

The reasons for the inactivity of this pair of compounds can not be known for certain, but it may be suggested that, although the cyclic tetra-peptide cap regions bear similarity to that of apicidin, the methyl ester end-group is not present in apicidin. It should also be noted that the aliphatic chain of SS581 is one unit longer than that of apicidin. It may be the case that despite the strong structural homology to the cap of the potent inhibitor, this new end-group does not function in a zinc-binding capacity and or, that the linker region of the compounds are not suitable for effective enzyme binding.
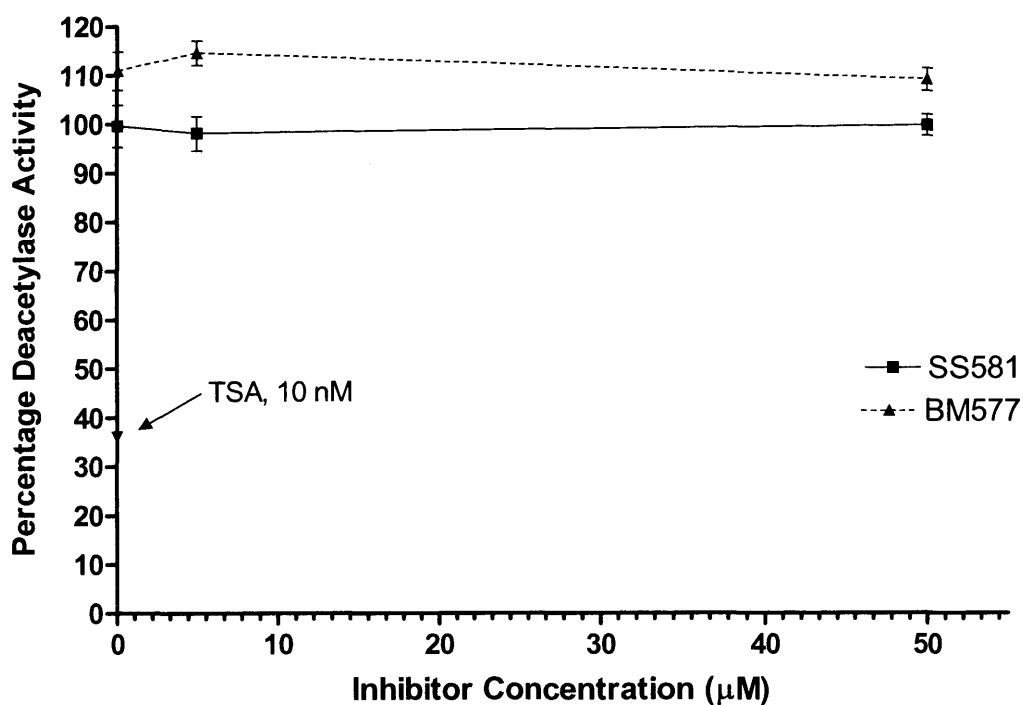
Figure 5.16. Isolated HDAC inhibition assay. Assays were carried out using an HDAC fluorescent activity kit (AK500, BIOMOL). Results are typical of n=2 experiments.

5.3 SAHA Analogues 150 and 151

The synthesis of the two SAHA analogues **150** and **151** (figure 5.17) was discussed in chapter 4. As was described, the compounds were selected for further analysis after displaying promising activity in the compound screen. Analysis of these compounds was carried out by Alexandra V. Stavropoulou.
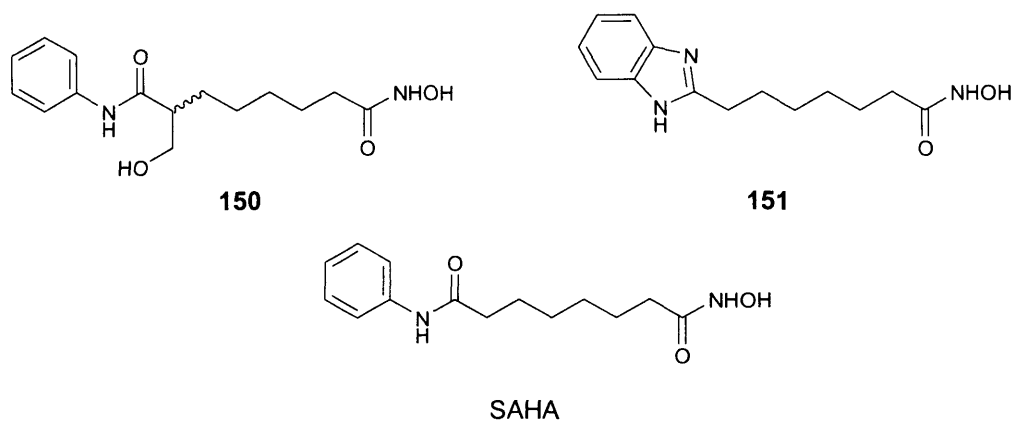
Figure 5.17. Structures of novel HDAC inhibitors **150** and **151** compared with SAHA.

The employed method for determination of inhibitor effects on cell proliferation was the SRB (sulphorhodamine B) assay. The assay involves incubation of a human cancer cell line (in this case MCF-7) with the test compounds in the presence of the aminoxanthine dye (SRB), which is taken up by the cells. Cell numbers may then be determined after the incubation period by colourimetric methods, intensity of colour being correlated to cell number after residual medium is washed away and remaining cells are lysed to release the absorbed dye. The results of the initial screening assay of the above compounds are shown in figure 5.18, along with TSA and a control. It can be seen that, at the same concentration as SAHA, both compounds appear to result in a greater reduction in cell proliferation at time periods over 24 h.
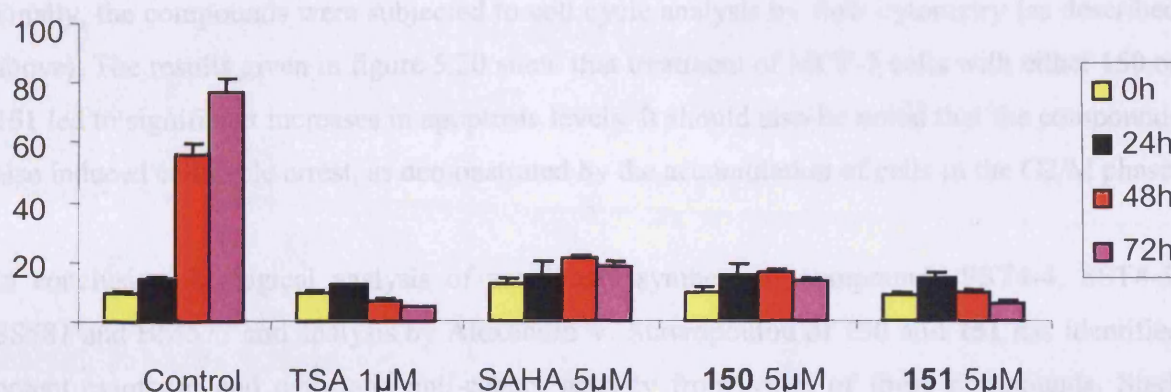
Figure 5.18. SRB anti-proliferation assay of compounds **150** and **151**. Compounds screened at 5 µM showed comparable activity to SAHA at the same concentration and to TSA at 1 µM. Proliferation determined at 0, 24, 48 and 72 h.

With the identification of **150** and **151** in the above screen, a follow-up proliferation assay was carried out at a range of concentrations to determine the IC_{50} values (figure 5.19). Results of this assay were used to calculate respective IC_{50} values of 4.4 µM and 2.4 µM for **150** and **151**.
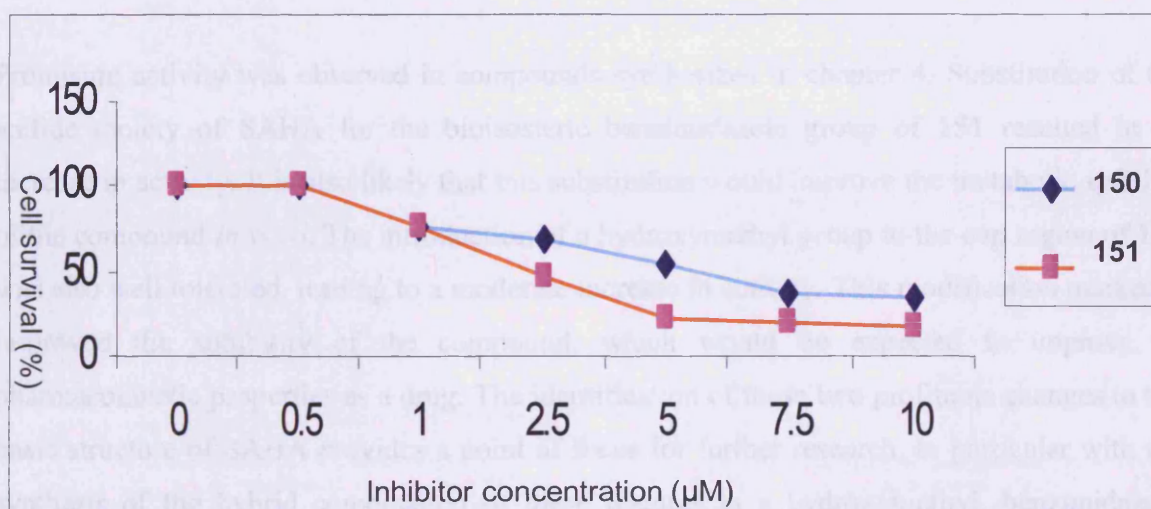
Figure 5.19. Effect of increasing concentration of **150** and **151** on MCF-7 proliferation (SRB assay).

Finally, the compounds were subjected to cell cycle analysis by flow cytometry (as described above). The results given in figure 5.20 show that treatment of MCF-7 cells with either **150** or **151** led to significant increases in apoptosis levels. It should also be noted that the compounds also induced cell cycle arrest, as demonstrated by the accumulation of cells in the G2/M phase.

In conclusion, biological analysis of previously synthesized compounds SST4-4, SST4-5, SS581 and BM577 and analysis by Alexandra V. Stavropoulou of **150** and **151** has identified potent cytotoxic and cytostatic anti-cancer activity from some of these compounds. Stark differences in biological activity were observed between epimers SST4-4 and SST4-5, apparently arising from inversion of a single chiral centre (possibly resulting in poor binding interaction of the non-natural isomer with the enzyme's surface). Of the two cell-lines tested, a more significant effect was noted in HeLa cells over HCT116, with increased levels of hyperacetylated H4 and apoptosis being observed.

Novel cyclic tetrapeptides SS581 and BM577 failed to display any significant activity as inhibitors of HDAC or on cell cycle despite their structural similarity to known inhibitors. This lack of activity could be due to a number of factors, possibly including non-optimum chain length of the linker regions and or poor binding capabilities of the end groups.

Promising activity was observed in compounds synthesized in chapter 4. Substitution of the anilide moiety of SAHA for the bioisosteric benzimidazole group of **151** resulted in an increase in activity. It is also likely that this substitution would improve the metabolic stability of the compound *in vitro*. The introduction of a hydroxymethyl group to the cap region of **150** was also well tolerated, leading to a moderate increase in activity. This modification markedly improved the solubility of the compound, which would be expected to improve its pharmacokinetic properties as a drug. The identification of these two profitable changes to the basic structure of SAHA provides a point of focus for further research, in particular with the synthesis of the hybrid combination of these features in a hydroxymethyl, benzimidazole analogue.
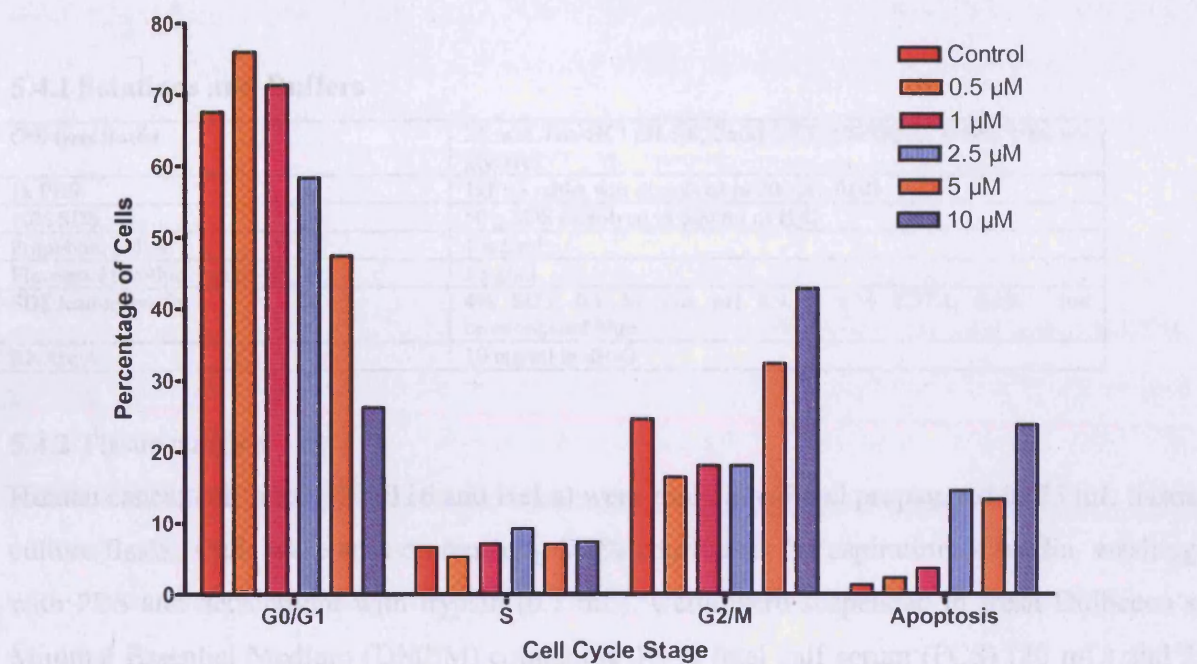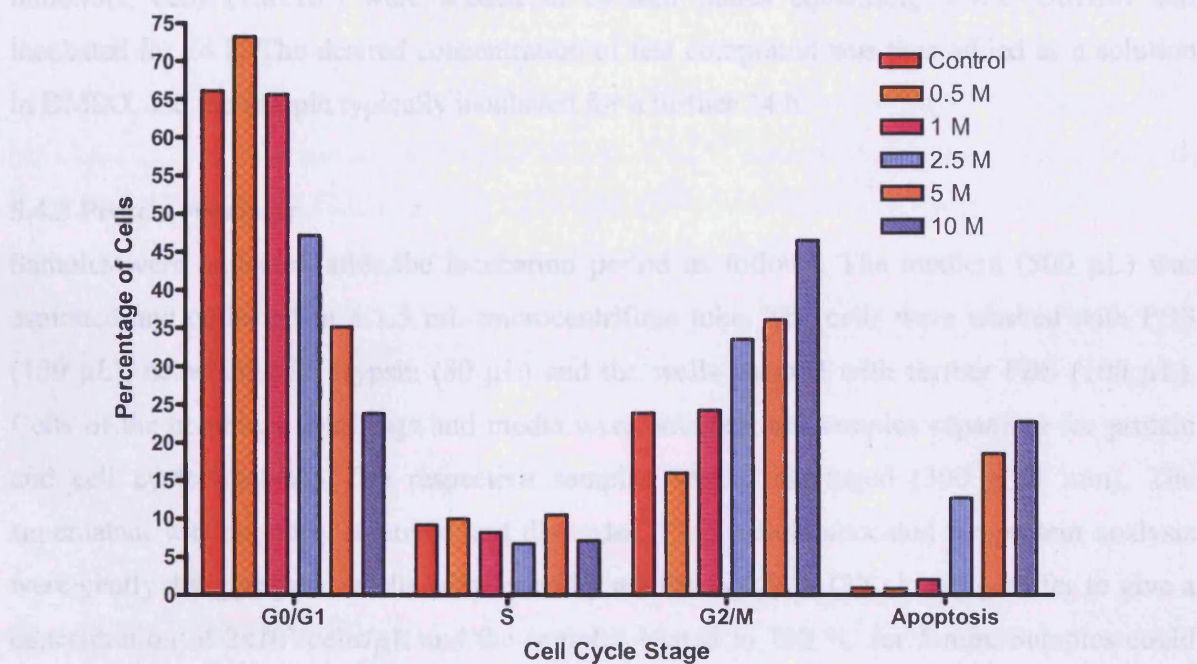
Figure 5.20. Cell cycle analysis of MCF-7 cells by flow cytometry after incubation with test compounds **150** and **151** at various concentrations for 48 h.

5.4 Experimental

5.4.1 Solutions and Buffers

Cell lysis Buffer	25 mM Tris-HCl pH 7.8, 2mM DTT, 1% Triton X-100, 10% v/v glycerol
1x PBS	1xPBS tablet was dissolved in 200 ml dH$_2$O
10% SDS	50 g SDS dissolved in 500 ml of H$_2$O
Propidium Iodide	1 mg/ml
Fluorescein Isothiocyanate (FITC)	1 μg/ml
SDS loading buffer	4% SDS, 0.1 M Tris pH 8.9. 2 mM EDTA. 0.1% and bromophenol blue
RNAse A	10 mg/ml in dH$_2$O

5.4.2 Tissue culture

Human cancer cell lines (HCT116 and HeLa) were maintained and propagated in 75 mL tissue culture flasks. Cells were split on reaching 80 % confluency by aspiration of media, washing with PBS and detachment with trypsin (0.1 mL). Cells were suspended in fresh Dulbecco's Minimal Essential Medium (DMEM) containing 10 % fetal calf serum (FCS) (20 mL) and 5 mL aliquots added to fresh tissue culture flasks containing 50 mL DMEM. For incubation with inhibitors, cells (1.8×10^5) were seeded in 24-well plates containing 5 mL DMEM and incubated for 24 h. The desired concentration of test compound was then added as a solution in DMSO, and the sample typically incubated for a further 24 h.

5.4.3 Protein Analysis

Samples were collected after the incubation period as follows. The medium (500 μL) was aspirated and collected in a 1.5 mL microcentrifuge tube. The cells were washed with PBS (100 μL), detached with trypsin (80 μL) and the wells washed with further PBS (100 μL). Cells of the combined washings and media were counted and samples separated for protein and cell cycle analysis. The respective samples were centrifuged (300 g, 8 min), The supernatant was carefully removed and discarded. Cell pellets allocated for protein analysis were gently disaggregated. Cells were lysed by addition of SDS (2X) loading buffer to give a concentration of 2×10^4 cells/μL and the samples heated to 100 °C for 5 min. Samples could then be stored indefinitely at -20 °C. Before separation by SDS-polyacrylamide gel electrophoresis (PAGE), samples were re-heated to 100 °C to fully denature proteins, centrifuged to collect any evaporated water, re-mixed and centrifuged briefly.

For SDS-PAGE analysis, 4-12% NuPage Bis-Tris precast gels were used. Sample volumes of 10 µl were loaded into each well and a potential of 160 volts was applied to the apparatus for 1 hour. As an indication of molecular weight, Novagen chromophore-conjugated protein markers were separated on the same gel.

Western blotting was carried out after separation by SDS-PAGE. The gel was placed on a nitrocellulose membrane (Hybond-C), pieces of 3mm paper placed over the reverse side of the gel and over the membrane and the combined layers placed in a cassette between cotton pads saturated with 20 % methanol-Tris-glycine. Proteins were transferred from the gel to the membrane by application of an electric current (160 mA, 25 V) across the cassette for 90 min. The membrane was then carefully removed from the cassette and blocked for 1 h in a solution of dried, skimmed milk (Marvel) in PBS-0.05 % Tween20, then rinsed in PBS-Tween.

For the detection of acetylated α-tubulin, membranes were incubated overnight at 4 °C in a 1:4000 dilution of an anti-acetylated α-tubulin monoclonal antibody in 1 % dried, skimmed milk in PBS 0.05 %-Tween. Anti-hyperacetylated histone H4 rabbit (1:10,000 dilution), anti-PARP mouse (1:1000) and anti-MAPK rabbit (1:1000) antibodies were used in the same way. Membranes were subsequently washed with PBS-0.05 % Tween (3 x 5 min) Incubation with the secondary antibody followed for 1 hour at a 1:2000 dilution of either anti-rabbit or anti-mouse Horse Radish Peroxidase (HRP) conjugated antibody in 1% (w/v) dried skimmed milk diluted in PBS-0.05% Tween. Membranes were again washed in PBS-0.05 % Tween (3 x 5 min) and bound antibodies detected with a chemiluminescence detection product (ECL-plus). After incubation for 5 minutes at RT with ECL-plus the membranes were wrapped in clear plastic membrane (Saran wrap) and exposed to photosensitive film.

5.4.4 Cell Cycle Analysis

Samples of pelleted cells collected as described in section 5.4.3. The pellets were gently disaggregated then fixed by addition of 70 % ice-cold ethanol with vortexing. Samples could then be stored indefinitely at -20 °C.

For analysis, fixed sample were centrifuged (400 g, 10 min) and the supernatant (70 % ethanol) removed by inverting the tubes. Cells were then re-suspended in a staining solution (500 μL) consisting of propidium iodide (PI, for DNA staining) (400 μL), fluorescein isothiocyanate (FITC, for protein staining) (50 μL) and RNAase A (500 μL) made up to 10 mL in ddH$_2$O.

The stained cells were analysed by flow cytometry (Beckton-Dickinson, FACScan) with FITC (total cell protein content, proportional to cell size) on FL-1 and PI (DNA content) on FL-2. Cell cycle stages were then assigned according to cell size and DNA content.

5.4.5 Histone Deacetylase Inhibition Assay

The HDAC inhibition assay was carried out in a 96-well plate with a fluorescence activity drug discovery kit (AK500, BIOMOL). The sample compounds at various concentrations were diluted in the assay buffer (10 μL), then a source of HDAC enzymes from HeLa nuclear extract (15 μL) added. The solutions were allowed to equilibrate at 25 °C for 15 min, then the *Fluor de Lys* substrate (25 μL) added and the plate incubated at 25 °C for 10 min. The reaction was stopped after this time by addition of *Fluor de Lys* developer (50 μL) including TSA at a final concentration of 1 μM. After developing for 15 min at 25 °C the fluorescence of the wells was measured on a fluorometric plate reader with excitation at 360 nM and detection at 460 nM. The percentage of deacetylase activity was calculated from the readings corrected to the control wells containing no inhibitor (100 % activity) and no enzyme (0 % activity).

5.5 References

1. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.;
    Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. FK228 (depsipeptide) as a natural
    prodrug that inhibits class I histone deacetylases. *Cancer Research* **2002**, *62*, (17),
    4916-4921.

6 Experimental

Melting points were determined on a microscope hot-stage Electrothermal 9100 apparatus, and are uncorrected. Infra-red (IR) spectra were recorded on a Perkin-Elmer PE-983 spectrophotometer; absorptions are quoted in wavenumbers (v_{max} in cm^{-1}). ^{1}H NMR spectra were recorded on a Bruker AC300 (300 MHz) spectrometer; data reported in parts per million (δ). Coupling constants (J) are given in Hertz (Hz). The following abbreviations are used in signal assignments: singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q), and multiplet (m). ^{13}C NMR spectra were obtained using Bruker AMX300 (75 MHz), Bruker AMX 400 (100 MHz) and Bruker AMX 500 (125 MHz) spectrometers and are recorded in parts per million (δ) with $CHCl_3$ as an internal standard. Mass spectra were recorded on a VG7070H mass spectrometer with Finigan Incos II data system at University College London. Thin-layer chromatography was performed on Merck 0.2 mm aluminium-backed silica gel 60 F_{254} plates and visualised by ultra violet light or an alkaline potassium permanganate spray with subsequent heating. Evaporation refers to the removal of solvent under reduced pressure. Glassware, syringes and needles for moisture-sensitive reactions were pre-dried in an oven (130 °C). Temperatures below 0 °C were obtained from various mixtures of water, freezing salt and ice, acetone and ice, acetone and dry ice or liquid nitrogen.

6.1 The Oxygen Series

Methyl 8-bromooctanoate (47)[1]
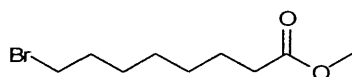
A solution of 8-bromooctanoic acid (1.50 g, 6.72 mmol) in 4 % cH_2SO_4-methanol (65 mL) was heated to 50 °C for 3 h. On cooling to room temperature the solvent was removed *in vacuo* and the colourless oil dissolved in ethyl acetate (30 mL). The solution was washed with saturated aqueous sodium hydrogen carbonate solution (2 x 30 mL), water (20 mL) and brine (20 mL) then dried over $MgSO_4$. The solvent was evaporated to leave a pale yellow oil (1.50 g, 94 %) requiring no purification; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 3.55 (3H, s, CH$_3$), 3.30 (2H, m, BrCH_2), 2.19 (2H, t, J=7.4 Hz, CH_2COO), 1.73 (2H, m, BrCH$_2$CH_2), 1.53 (2H, m, CH_2CH$_2$COO), 1.34-1.21 (6H, m, BrCH$_2$CH$_2$CH_2, BrCH$_2$CH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.87 (COO), 51.30 (CH$_3$), 33.85 (CH$_2$COO), 28.82 (CH$_2$CH$_2$CH$_2$COO), 28.31 BrCH$_2$CH$_2$CH$_2$CH$_2$), 27.87 (BrCH$_2$CH$_2$CH$_2$), 24.71 (CH$_2$CH$_2$COO).

Ethyl 6-(4-nitrophenoxy)hexanoate (48)
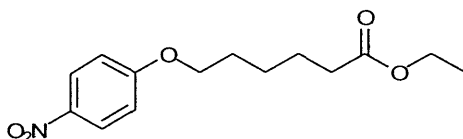
A mixture of 4-nitrophenol (935 mg, 6.72 mmol), ethyl 6-bromohexanoate (1.80 g, 8.07 mmol) and caesium carbonate (2.63 g, 8.07 mmol) in dimethylformamide (35 mL) was refluxed at 150 °C for 20 h. On cooling to room temperature water (50 mL) was added and the aqueous solution extracted with ethyl acetate (3 x 20 mL) and the combined organics washed with 1 M sodium hydroxide (20 mL), water (2 x 20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$ and evaporated to leave a pale yellow solid which was recrystallised from diethyl ether-petroleum ether (40 - 60) to give the product (1.83 g, 97 %) as pale yellow crystals, mp 68-70 °C; IR (KBr, thin film) (cm^{-1}) 2949, 1728, 1498, 1267. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.13 (2H, d, J=9.3 Hz, 3, 5-aryl), 6.89 (2H, d, J=9.3 Hz, 2, 6-aryl), 4.09 (2H, q, J=7.1 Hz, OCH_2CH$_3$), 4.01 (2H, t, J=6.4 Hz, OCH_2CH$_2$), 2.30 (2H, t,

J=7.3 Hz, CH₂COO), 1.81 (2H, m, OCH₂CH_2), 1.66 (2H, m, CH_2CH₂COO), 1.53 (2H, m, OCH₂CH₂CH_2), 1.21 (3H, t, J=7.1 Hz, CH₂CH_3); ¹³C-NMR (CDCl₃, 75 MHz) δ 173.47 (COO), 164.13 (1-aryl), 141.30 (4-aryl), 125.85 (3, 5-aryl), 114.38 (2, 6-aryl), 68.52 (OCH_2CH₂), 60.26 (OCH_2CH₃), 34.11 (CH_2COO), 28.65 (OCH₂CH_2), 25.48 (OCH₂CH₂CH_2), 24.58 (CH_2CH₂COO), 14.23 (CH₂CH_3). Anal. Calcd for C₁₄H₁₉NO₅ C, 59.78; H, 8.81; N, 4.98. Found: C, 59.42; H, 6.80; N, 4.90.

Ethyl 7-(4-nitrophenoxy)heptanoate (49)
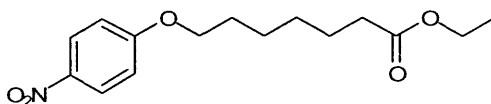
To a stirred solution of 4-nitrophenol (2.97 g, 21.4 mmol) in dimethylformamide (12 mL) was added cesium carbonate (8.34 g, 25.6 mmol) followed by ethyl-7-bromoheptanoate (6.08g, 25.6 mmol). The mixture was heated to reflux for 18 h then allowed to cool to room temperature. Water (50 mL) was added and the aqueous solution extracted with ethyl acetate (2 x 40 mL). The yellow organic phase was washed with 1 M sodium hydroxide solution (50 mL) and water (2 x 50 mL) before drying (MgSO₄). The solvent was removed *in vacuo* to yield a low melting solid which was recrystallised from ethanol to give the title compound (5.33 g, 85 %) as white needles, mp 31-32 °C; ¹H-NMR (CDCl₃, 300 MHz) δ 8.15 (2H, d, J=9.2 Hz, 3, 5-aryl), 6.90 (2H, d, J=9.2 Hz, 2, 6-aryl), 4.09 (2H, q, J=7.1 Hz, OCH_2CH₃), 4.01 (2H, t, J=6.4 Hz, OCH_2CH₂), 2.28 (2H, t, J=7.4 Hz, CH₂COO), 1.80 (2H, m, OCH₂CH_2), 1.63 (2H, m, CH_2CH₂COO), 1.49–1.34 (4H, m, OCH₂CH₂CH_2, CH_2CH₂CH₂COO), 1.22 (3H, t, J=7.1 Hz, CH₂CH_3); ¹³C-NMR (CDCl₃, 75 MHz) δ 173.67 (COO), 164.18 (1-aryl), 141.33 (4-aryl), 125.90 (3, 5-aryl), 114.39 (2, 6-aryl), 68.70 (OCH_2CH₂), 60.24 (OCH_2CH₃), 34.19 (CH_2COO), 28.77 (CH_2CH₂CH₂COO, OCH₂CH_2), 25.62 (OCH₂CH₂CH_2), 24.79 (CH_2CH₂COO), 14.26 (CH₂CH_3).

Methyl 8-(4-nitrophenoxy)octanoate (50)
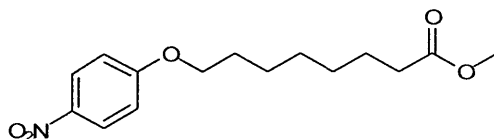
A mixture of 4-nitrophenol (694 mg, 4.99 mmol), methyl 8-bromooctanoate (1.42 g, 5.99 mmol) and caesium carbonate (1.95 g, 5.99 mmol) in dimethylformamide (30 mL) was refluxed at 150 °C for 20 h. On cooling to room temperature, water (50 mL) was added, and the aqueous solution extracted with ethyl acetate (3 x 20 mL) and the combined organics washed with 1 M sodium hydroxide (20 mL), water (2 x 20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$ and evaporated to leave a pale yellow solid which was recrystallised from diethyl ether-petroleum ether (40 - 60) to give the product (1.33 g, 90 %) as yellow crystals, mp 71-72 °C; IR (KBr, thin film) (cm^{-1}) 2930, 1732, 1499, 1337, 1265. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.15 (2H, m, 3, 5-aryl), 6.91 (2H, m, 2, 6-aryl), 4.01 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 3.63, (3H, s, CH$_3$), 2.28 (2H, t, J=7.4 Hz, CH$_2$COO), 1.77 (2H, m, OCH$_2$CH_2), 1.61 (2H, m, CH_2CH$_2$COO), 1.47–1.32 (6H, m, OCH$_2$CH$_2$CH_2, OCH$_2$CH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.16 (COO), 164.21 (1-aryl), 141.27 (4-aryl), 125.87 (3, 5-aryl), 114.38 (2, 6-aryl), 68.78 (OCH$_2$CH$_2$), 51.45 (CH$_3$), 33.97 (CH$_2$COO), 28.97 (OCH$_2$CH$_2$), 28.91 (CH$_2$CH$_2$CH$_2$CH$_2$COO), 28.87 (CH$_2$CH$_2$CH$_2$COO), 25.71 (OCH$_2$CH$_2$CH$_2$), 24.80 (CH$_2$CH$_2$COO). Anal. Calcd for C$_{15}$H$_{21}$NO$_5$ C, 61.00; H, 7.17; N, 4.74. Found: C, 60.96; H, 7.22; N, 4.71.

Ethyl 6-(4-aminophenoxy)hexanoate (51)
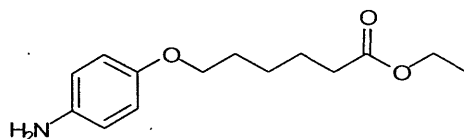
To a stirred solution of ethyl 6-(4-nitrophenoxy)hexanoate (48) (1.67 g, 5.94 mmol) in ethanol (30 mL), was added 5 % palladium on carbon (20 mg) under nitrogen. The system was evacuated and replaced with hydrogen at atmospheric pressure. After stirring for 36 h the catalyst was removed and the solvent removed in vacuo to yield a red oil (1.42 g, 95 %) which required no further purification; IR (KBr, thin film) (cm^{-1}) 3362, 2937, 1732, 1512, 1236. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 6.72 (2H, m, 2, 6-aryl), 6.59 (2H, m, 3, 5-aryl), 4.10

(2H, q, J=7.1 Hz, OCH_2CH$_3$), 3.85 (2H, t, J=6.6 Hz, OCH_2CH$_2$), 2.30 (2H, t, J=7.2 Hz, CH$_2$COO), 1.75-1.61 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.46 (2H, m, OCH$_2$CH$_2$CH_2), 1.23 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.67 (COO), 152.09 (1-aryl), 140.09 (4-aryl), 116.34 (3, 5-aryl), 115.62 (2, 6-aryl), 68.31 (OCH_2CH$_2$), 60.23 (OCH_2CH$_3$), 34.26 (CH_2COO), 29.10 (OCH$_2$CH_2), 25.67 (OCH$_2$CH$_2$CH_2), 24.75 (CH_2CH$_2$COO), 14.26 (CH$_2$CH_3). LRMS (EI) m/z 251 (M$^+$, 35 %), 206 (13 %), 143 (32 %), 109 (100 %). HRMS calcd for C$_{14}$H$_{21}$NO$_3$ 251.15214. Found: 251.15197.

Ethyl 7-(4-aminophenoxy)heptanoate (52)
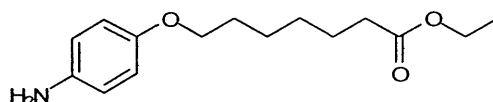
To a stirred solution of ethyl 7-(4-nitrophenoxy)heptanoate (49) (1.80 g, 6.1 mmol) in ethanol (20 mL), was added 5 % palladium on carbon (15 mg) under nitrogen. The system was evacuated and replaced with hydrogen at atmospheric pressure. After stirring for 4 h, the catalyst was removed and the solvent removed in vacuo to yield a pale brown solid which was recrystallised from ethanol to give the title compound (1.34 g, 83 %) as white flakes, mp 47-48 °C; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 6.67 (4H, m, aryl), 4.12 (2H, q, J=7.1 Hz, OCH_2CH$_3$), 3.85 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 3.26 (2H, s, NH$_2$), 2.29 (2H, t, J=7.5 Hz, CH$_2$COO), 1.75-1.61 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.48-1.36 (4H, m, OCH$_2$CH$_2$CH_2, OCH$_2$CH$_2$CH$_2$CH_2), 1.24 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.82 (COO), 152.29 (1-aryl), 139.82 (4-aryl), 116.44 (3, 5-aryl), 115.65 (2, 6-aryl), 68.51 (OCH_2CH$_2$), 60.21 (OCH_2CH$_3$), 34.30 (CH_2COO), 29.25 (OCH$_2$CH_2), 28.91 (OCH$_2$CH$_2$CH$_2$CH_2), 25.77 (OCH$_2$CH$_2$CH_2), 24.91 (CH_2CH$_2$COO), 14.27 (CH$_2$CH_3). Anal. Calcd for C$_{15}$H$_{23}$NO$_3$ C, 67.90; H, 8.74; N, 5.28. Found: C, 67.81; H, 8.79; N, 5.30.

Methyl 8-(4-aminophenoxy)octanoate (53)
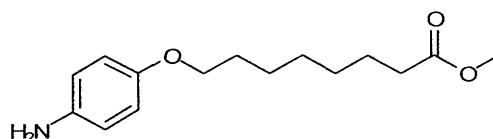
To a stirred solution of methyl 8-(4-nitrophenoxy)octanoate (50) (1.24 g, 4.20 mmol) in ethanol (30 mL), was added 5 % palladium on carbon (15 mg) under nitrogen. The system

was evacuated and replaced with hydrogen at atmospheric pressure. After stirring for 36 h, the catalyst was removed and the solvent evaporated to yield a red oil (0.69 g, 62 %); ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 6.73 (2H, m, 2, 6-aryl), 6.59 (2H, m, 3, 5-aryl), 3.85 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 3.65, (3H, s, CH$_3$), 3.32 (2H, brs, NH$_2$), 2.30 (2H, t, J=7.4 Hz, CH$_2$COO), 1.74-1.60 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.45-1.31 (6H, m, OCH$_2$CH$_2$CH_2, OCH$_2$CH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.28 (COO), 152.24 (1-aryl), 139.93 (4-aryl), 116.39 (3, 5-aryl), 115.64 (2, 6-aryl), 68.58 (OCH$_2$CH$_2$), 51.47 (CH$_3$), 34.07 (CH$_2$COO), 29.36 (OCH$_2$CH$_2$), 29.07 (CH$_2$CH$_2$CH$_2$CH$_2$COO, CH$_2$CH$_2$CH$_2$COO), 25.91 (OCH$_2$CH$_2$CH$_2$), 24.89 (CH$_2$CH$_2$COO). LRMS (EI) m/z 265 (M$^+$, 66 %), 235 (8 %), 109 (100 %). HRMS calcd for C$_{15}$H$_{23}$NO$_3$ 265.16778. Found: 265.16738.

Ethyl 7-(4-(dimethylamino)phenoxy)heptanoate (55)
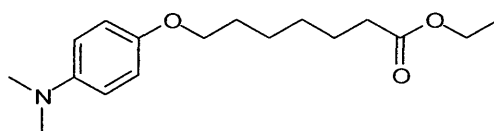
To a solution of ethyl 7-(4-aminophenoxy)heptanoate (52) (300 mg, 1.13 mmol), formaldehyde (0.55 mL of a 37 % aqueous solution, 6.78 mmol) and sodium cyanoborohydride (106 mg, 1.70 mmol) in acetonitrile (10 mL) was added acetic acid (34 mg, 0.57 mmol) and the solution stirred for 18 h. A further aliquot of acetic acid (34 mg, 0.57 mmol) was then added and stirring continued for 1 d. The reaction was quenched with water (10 mL) and the solution extracted with ethyl acetate (10 mL). The organic phase was washed with saturated aqueous sodium hydrogen carbonate solution (10 mL) and brine (10 mL) before drying over MgSO$_4$. The solvent was removed *in vacuo* and the crude yellow oil purified by column chromatography (hexane-ethyl acetate, 4:1) to give the title compound (230 mg, 69 %) as a pale yellow oil; IR (KBr, thin film) (cm^{-1}) 2937, 1734, 1514, 1244. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 6.88 (2H, m, 2, 6-aryl), 6.73 (2H, m, 3, 5-aryl), 4.13 (2H, q, J=7.2 Hz, OCH_2CH$_3$), 3.89 (2H, t, J=6.5 Hz, OCH$_2$), 2.85 (6H, s, N(CH$_3$)$_2$), 2.30 (2H, t, J=7.4 Hz, CH$_2$COO), 1.78-1.63 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.48-1.38 (4H, m, OCH$_2$CH$_2$CH_2, OCH$_2$CH$_2$CH$_2$CH_2), 1.25 (3H, t, J=7.2 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.25 (COO), 151.45 (1-aryl), 145.73 (4-aryl), 115.47 (2, 6-aryl), 114.87 (3, 5-aryl), 68.48 (OCH$_2$CH$_2$), 60.17 (OCH$_2$CH$_3$), 41.81 (N(CH$_3$)$_2$), 34.29 (CH$_2$COO), 29.30

(OCH$_2$CH$_2$), 28.93 (CH$_2$CH$_2$CH$_2$COO), 25.80 (OCH$_2$CH$_2$CH$_2$), 24.93 (CH$_2$CH$_2$COO), 14.28 (CH$_2$CH$_3$). LRMS (EI) m/z 293 (M$^+$, 100 %), 248 (14 %), 136 (95 %), 108 (38 %). HRMS calcd for C$_{17}$H$_{27}$NO$_3$ 293.19908. Found: 293.19859.

Ethyl 6-(4-(2-(1H-indol-3-yl)acetamido)phenoxy)hexanoate (56)
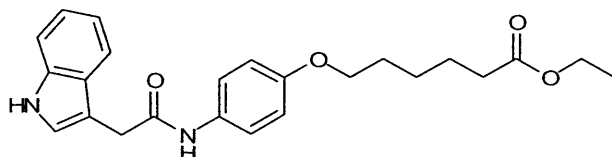
3-Indole acetic acid (0.99 g, 5.64 mmol) was dissolved in dimethyl formamide (55 mL) and 1-hydroxybenzotriazole (0.94 g, 6.98 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (1.18, 6.18 mmol) and N-methyl morpholine (1.25 g, 12.35 mmol) added successively and the solution stirred at room temperature for 30 min. To the solution was added ethyl 6-(4-aminophenoxy)hexanoate (51) (1.35 g, 5.37 mmol) in dimethyl formamide (4 mL) and the solution stirred for 21 h. The reaction was quenched by the addition of 1 M HCl and the resultant suspension extracted with ethyl acetate (4 x 20 mL). The combined organics were washed with saturated aqueous sodium hydrogen carbonate solution (40 mL), water (2 x 40 mL) and brine (40 mL). The solvent was removed *in vacuo* to yield an off-white solid which was recrystallised from ethanol to give the title compound (1.86 g, 85 %) as white crystals, 114-116 °C; IR (KBr, thin film) (cm^{-1}) 3412, 2939, 1732, 1649, 1510. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.75 (1H, s, NH), 7.61 (1H, d, J=7.8 Hz, 5-indolyl), 7.47 (1H, s, NH), 7.40 (1H, d, J=8.0 Hz, 8-indolyl), 7.27-7.13 (5H, m, 7-indolyl, 3, 5-aryl, 6, 2-indolyl), 6.75 (2H, d, J=9.0 Hz, 2, 6-aryl), 4.12 (2H, q, J=7.1 Hz, OCH$_2$CH$_3$), 3.86 (4H, m, OCH$_2$CH$_2$, CH$_2$CONH), 2.32 (2H, t, J=7.4 Hz, CH$_2$COO), 1.77-1.65 (4H, m, OCH$_2$CH$_2$, CH$_2$CH$_2$COO), 1.48 (2H, m, CH$_2$CH$_2$CH$_2$COO), 1.25 (3H, t, J=7.2 Hz, CH$_2$CH$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.79 (COO), 169.90 (CONH), 155.97 (1-aryl), 136.56 (9-indolyl), 130.61 (4-aryl), 126.95 (4-indolyl), 124.16 (2-indolyl), 122.66 (6-indolyl), 122.09 (3, 5-aryl), 120.20 (7-indolyl), 118.62 (5-indolyl), 114.66 (2, 6-aryl), 111.66 (8-indolyl), 108.45, (3-indolyl), 67.90 (OCH$_2$CH$_2$), 60.32 (OCH$_2$CH$_3$), 34.28 (OOCCH$_2$, CH$_2$CONH), 28.93 (OCH$_2$CH$_2$), 25.63 (OCH$_2$CH$_2$CH$_2$), 24.73 (CH$_2$CH$_2$COO), 14.28 (CH$_2$CH$_3$). Anal. Calcd for C$_{24}$H$_{28}$N$_2$O$_4$ C, 71.57; H, 6.91; N, 6.86. Found: C, 70.42; H, 6.80; N, 6.86.

Ethyl 7-(4-(2-(1H-indol-3-yl)acetamido)phenoxy)heptanoate (57)
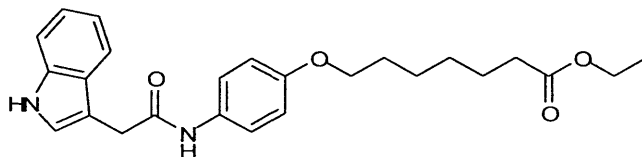
3-Indole acetic acid (0.52 g, 2.95 mmol) was dissolved in dimethyl formamide (8 mL) and 1-hydroxybenzotriazole (0.49 g, 3.65 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (0.62, 3.23 mmol) and N-methyl morpholine (0.72 mL, 6.46 mmol) added successively at 0 °C. To the solution was added ethyl 7-(4-aminophenoxy)heptanoate (52) (1.0 g, 3.77 mmol) in dimethyl formamide (4 mL) and the solution stirred for 16 h. The reaction was quenched by the addition of 1 M HCl (15 mL) and the resultant solution extracted with ethyl acetate (2 x 20 mL). The combined organics were washed with 5 % aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL) before drying (MgSO$_4$). The solvent was removed *in vacuo* to yield an off-white solid which was recrystallised from ethanol to give the title compound (0.95 g, 78 %) as white crystals, mp 107-108 °C; IR (KBr, thin film) (cm^{-1}) 3409, 2939, 1720, 1649, 1510. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.58 (1H, s, NH), 7.61 (1H, d, J=7.8 Hz, 5-indolyl), 7.41 (2H, d, J=8.0 Hz, Ar), 7.21 (4H, m, 8, 2, 7, 6-indolyl), 6.75 (2H, d, J=8.8 Hz, 2, 6-aryl), 4.12 (2H, q, J=7.1 Hz, OCH_2CH$_3$), 3.87 (4H, m, OCH_2CH$_2$, CH_2CONH), 2.30 (2H, t, J=7.5 Hz, CH$_2$COO), 1.87 (2H, m, OCH$_2$CH_2), 1.68 (2H, m, CH_2CH$_2$COO), 1.40 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.24 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.87 (COO), 169.76 (CONH), 156.02 (1-aryl), 136.52 (9-indolyl), 130.54 (4-aryl), 126.53 (4-indolyl), 124.10 (2-indolyl), 122.75 (6-indolyl), 122.03 (3, 5-aryl), 120.29 (7-indolyl), 118.66 (5-indolyl), 114.66 (2, 6-aryl), 111.61 (8-indolyl), 108.58, (3-indolyl), 68.08 (OCH_2CH$_2$), 60.27 (OCH_2CH$_3$), 34.29 (OOCCH_2CH$_2$, CH_2CONH), 29.07 (OCH$_2$CH_2), 28.87 (OCH$_2$CH$_2$CH$_2$CH_2), 25.72 (OCH$_2$CH$_2$CH_2), 24.88 (CH_2CH$_2$COO), 14.28 (CH$_2$CH_3). Anal. Calcd for C$_{25}$H$_{30}$N$_2$O$_4$ C, 71.07; H, 7.16; N, 6.63. Found: C, 70.93; H, 7.22; N, 6.56.
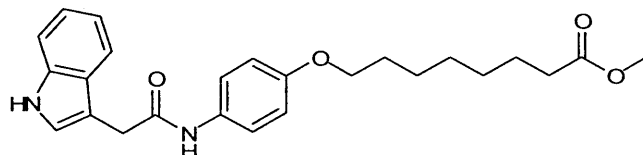
**Methyl 8-(4-(2-(1H-indol-3-yl)acetamido)phenoxy)octanoate (59)**

3-Indole acetic acid (0.44 g, 2.95 mmol) was dissolved in dimethyl formamide (25 mL) and 1-hydroxybenzotriazole (0.42 g, 3.08 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (0.52, 2.73 mmol) and N-methyl morpholine (0.55 g, 5.45 mmol) added successively and the resultant solution stirred at room temperature for 30 min. To the solution was added methyl 8-(4-aminophenoxy)octanoate (53) (0.63 g, 2.37 mmol) in dimethyl formamide (4 mL) and the solution stirred for 21 h. The reaction was quenched by the addition of 1 M HCl and the resultant suspension extracted with ethyl acetate (1 x 20 mL, 3 x 10 mL). The combined organics were washed with saturated aqueous sodium hydrogen carbonate solution (25 mL), water (2 x 25 mL) and brine (20 mL). The solvent was removed *in vacuo* to yield a pale pink solid which was recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (0.82 g, 82 %) as white crystals, mp 133-134 °C; IR (KBr, thin film) (cm^{-1}) 3416, 2941, 1738, 1651. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.61 (1H, brs, NH), 7.62 (1H, d, J=7.8 Hz, 5-indolyl), 7.41 (2H, m, 8-indolyl, NH), 7.20 (5H, m, 7-indolyl, 3, 5-aryl, 6, 2-indolyl), 6.75 (2H, d, J=9.0 Hz, 2, 6-aryl), 3.86 (4H, m, OCH$_2$CH$_2$, CH$_2$CONH), 3.66 (3H, s, CH$_3$), 2.30 (2H, t, J=7.4 Hz, CH$_2$COO), 1.77-1.60 (4H, m, OCH$_2$CH$_2$, CH$_2$CH$_2$COO), 1.44-1.32 (6H, m, OCH$_2$CH$_2$CH$_2$, OCH$_2$CH$_2$CH$_2$CH$_2$, CH$_2$CH$_2$CH$_2$COO); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.35 (COO), 169.74 (CONH), 156.03 (1-aryl), 136.53 (9-indolyl), 130.55 (4-aryl), 126.94 (4-indolyl), 124.08 (2-indolyl), 122.73 (6-indolyl), 122.03 (3, 5-aryl), 120.27 (7-indolyl), 118.65 (5-indolyl), 114.67 (2, 6-aryl), 111.61 (8-indolyl), 108.59, (3-indolyl), 68.16 (OCH$_2$CH$_2$), 51.51 (CH$_3$), 34.32 (CH$_2$CONH), 34.08 (OOCCH$_2$CH$_2$), 29.17 (OCH$_2$CH$_2$), 29.04 (CH$_2$CH$_2$CH$_2$CH$_2$COO, CH$_2$CH$_2$CH$_2$COO), 25.84 (OCH$_2$CH$_2$CH$_2$), 24.87 (CH$_2$CH$_2$COO). Anal. Calcd for C$_{25}$H$_{30}$N$_2$O$_4$ C, 71.07; H, 7.16; N, 6.63. Found: C, 70.88; H, 7.21; N, 6.58.

## Ethyl 7-(4-(3-(1*H*-indol-3-yl)propanamido)phenoxy)heptanoate (58)
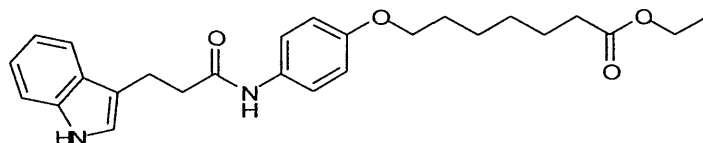
3-Indolepropionic acid (449 mg, 2.37 mmol) was dissolved in dimethyl formamide (9 mL) and 1-hydroxybenzotriazole (397 mg, 2.94 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (498 mg, 2.60 mmol) and *N*-methyl morpholine (526 mg, 5.20 mmol) added successively at 0 °C. To the solution was added ethyl 7-(4-aminophenoxy)heptanoate (52) (600 mg, 2.26 mmol) in dimethyl formamide (5 mL) and the solution stirred for 16 h. The reaction was quenched by the addition of 1 M HCl (8 mL) and the resultant solution extracted with ethyl acetate (2 x 20 mL). The combined organics were washed with 5 % aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL) before drying (MgSO$_4$). The solvent was removed *in vacuo* to yield a cream coloured solid which was recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (0.78 g, 79 %) as white crystals, mp 110-111 °C; IR (KBr, thin film) (cm^{-1}) 3408, 3304, 2934, 1732, 1655, 1510. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.31 (1H, brs, NH) 7.58 (1H, d, *J*=7.7 Hz, 5-indolyl), 7.51 (1H, s, NH), 7.28 (3H, m, 3, 5-aryl, 8-indolyl), 7.18-7.09 (2H, m, 7, 6-indolyl), 6.86 (1H, d, *J*=0.7 Hz, 2-indolyl), 6.76 (2H, d, *J*=8.9 Hz, 2, 6-aryl), 4.13 (2H, q, *J*=7.1 Hz, OC*H$_2$*CH$_3$), 3.85 (2H, t, *J*=6.5 Hz, OC*H$_2$*CH$_2$), 3.14 (2H, t, *J*=7.4 Hz, C*H$_2$*CH$_2$CONH), 2.66 (2H, t, *J*=7.4 Hz, C*H$_2$*CONH), 2.31 (2H, t, *J*=7.4 Hz, CH$_2$COO), 1.76-1.62 (4H, m, OCH$_2$C*H$_2$*, C*H$_2$*CH$_2$COO), 1.40 (4H, m, OCH$_2$CH$_2$C*H$_2$*, C*H$_2$*CH$_2$CH$_2$COO), 1.26 (3H, t, *J*=7.1 Hz, CH$_2$C*H$_3$*); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.88 (COO), 171.02 (CONH), 155.87 (1-aryl), 136.38 (9-indolyl), 130.80 (4-aryl), 127.05 (4-indolyl), 122.07 (2-indolyl), 122.01 (3, 5-aryl), 121.88 (6-indolyl), 119.39 (7-indolyl), 118.65 (5-indolyl), 114.70 (2, 6-aryl, 8-indolyl), 111.34 (3-indolyl), 68.10 (OCH$_2$CH$_2$), 60.28 (OCH$_2$CH$_3$), 38.13 (CH$_2$CONH), 34.30 (CH$_2$COO), 29.08 (OCH$_2$CH$_2$), 28.87 (CH$_2$CH$_2$CH$_2$COO), 25.73 (OCH$_2$CH$_2$CH$_2$), 24.89 (CH$_2$CH$_2$COO), 21.31 (CH$_2$CH$_2$CONH)), 14.28 (CH$_2$CH$_3$). Anal. Calcd for C$_{26}$H$_{32}$N$_2$O$_4$ C, 71.53; H, 7.39; N, 6.42. Found: C, 71.53; H, 7.39; N, 6.42.

Ethyl 7-(4-(2-(1*H*-indol-3-yl)ethylamino)phenoxy)heptanoate (60)
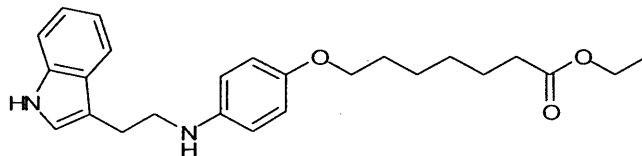
A solution of acetic acid (1.99 g, 33.1 mmol) in tetrahydrofuran (5 mL) was added dropwise to a stirred suspension of sodium borohydride (1.25 g, 33.1 mmol) in tetrahydrofuran (30 mL). After stirring for 2 h, a solution of ethyl 7-(4-(2-(1*H*-indol-3-yl)acetamido)phenoxy)heptanoate (57) (1.40 g, 3.31 mmol) in tetrahydrofuran (10 mL) was added dropwise, and the suspension warmed to 45 °C and stirred for 17 h. On cooling to room temperature, the reaction was quenched with brine (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organics were washed with water (2 x 20 mL) and brine (20 mL), then dried (MgSO$_4$). The solvent was removed *in vacuo* and the colourless oil purified by column chromatography (ethyl acetate-petroleum ether (40 - 60), 1:1) to give the product (0.72 g, 53 %) as a white crystalline solid, mp 76-77 °C; IR (KBr, thin film) (cm^{-1}) 3393, 2937, 1730, 1512, 1231. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.06 (1H, brs, NH), 7.63 (1H, d, *J*=7.9 Hz, 5-indolyl), 7.37 (1H, d, *J*=8.1 Hz, 8-indolyl), 7.21 (1H, ddd, *J*=1.1, 7.1, 8.1 Hz, 7-indolyl), 7.13 (1H, ddd, *J*=1.1, 7.1, 8.1 Hz, 6-indolyl), 7.04 (1H, d, *J*= 2.3 Hz, 2-indolyl), 6.77 (2H, d, *J*=8.9 Hz, 3, 5-aryl) 6.58 (2H, d, *J*=8.9 Hz, 2, 6-aryl), 4.13 (2H, q, *J*=7.1 Hz, OC*H*$_2$CH$_3$), 3.88 (2H, t, *J*=6.5 Hz, OC*H*$_2$CH$_2$), 3.43 (2H, t, *J*=6.8 Hz, C*H*$_2$NH), 3.08 (2H, t, *J*=6.8 Hz, C*H*$_2$CH$_2$NH), 2.31 (2H, t, *J*=7.5 Hz, CH$_2$COO), 1.69 (4H, m, OCH$_2$C*H*$_2$, C*H*$_2$CH$_2$COO), 1.41 (4H, m, OCH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$COO), 1.26 (3H, t, *J*=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.85 (COO), 151.57 (1-aryl), 142.47 (4-aryl), 136.42 (9-indolyl), 127.47 (4-indolyl), 122.17 (2-indolyl), 122.05 (6-indolyl), 119.43 (7-indolyl), 118.85 (5-indolyl), 115.82 (3, 5-aryl), 114.40 (2, 6-aryl), 113.53 (8-indolyl), 111.21 (3-indolyl), 68.65 (OCH$_2$CH$_2$), 60.22 (OC*H*$_2$CH$_3$), 44.98 (CH$_2$NH), 34.33 (*C*H$_2$COO), 29.30 (OCH$_2$*C*H$_2$), 28.93 (*C*H$_2$CH$_2$CH$_2$COO), 25.79 (*C*H$_2$CH$_2$NH), 25.20 (*C*H$_2$CH$_2$COO), 24.93 (OCH$_2$CH$_2$*C*H$_2$), 14.28 (CH$_2$*C*H$_3$). Anal. Calcd for C$_{25}$H$_{32}$N$_2$O$_3$ C, 73.50; H, 7.90; N, 6.86. Found: C, 73.25; H, 7.93; N, 6.88.

Ethyl 7-(4-(pyridin-4-ylmethylamino)phenoxy)heptanoate (61)
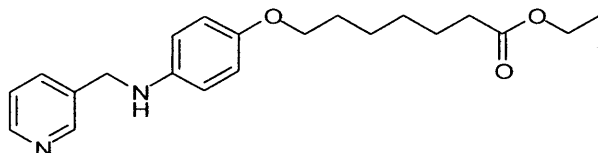
To a solution of ethyl 7-(4-aminophenoxy)heptanoate (**52**) (1.17 g, 4.43 mmol), in methanol (80 mL) was added 4-pyridine carboxaldehyde (0.56 mL, 4.43 mmol) and the solution stirred at room temperature for 2 h, during which time a yellow precipitate formed. The suspension was cooled to 0 °C and sodium cyanoborohydride (0.56 g, 8.86 mmol) and zinc chloride (0.6 g, 4.43 mmol) added. The reaction was allowed to warm to room temperature and was stirred for a further 2 h. Water (5 mL) was added and the solvent removed *in vacuo*. The residue was dissolved in ethyl acetate and was washed with saturated aqueous sodium hydrogen carbonate solution (2 x 20 mL) and brine (20 mL) then dried (MgSO$_4$). The solvent was removed *in vacuo* to yield a pale orange solid which was recrystallised from isopropanol to give the title compound (1.14 g, 72 %) as pale yellow crystals, mp 98-99 °C; IR (KBr, thin film) (cm^{-1}) 3371, 2935, 1714, 1518, 1231. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.51 (2H, m, 2, 6-pyridyl), 7.27 (2H, m, 3, 5-pyridyl), 6.73 (2H, d, *J*=8.8 Hz, 2, 6-aryl), 6.50 (2H, d, *J*=8.8 Hz, 3, 5-aryl), 4.30 (2H, s, C*H*$_2$NH), 4.10 (2H, q, *J*=7.1 Hz, C*H*$_2$CH$_3$), 3.84 (2H, t, *J*=6.4 Hz, OC*H*$_2$CH$_2$), 2.28 (2H, t, *J*=7.5 Hz, C*H*$_2$COO), 1.74-1.60 (4H, m, OCH$_2$C*H*$_2$, C*H*$_2$CH$_2$COO), 1.44-1.33 (4H, m, OCH$_2$CH$_2$C*H*$_2$, OCH$_2$CH$_2$CH$_2$C*H*$_2$), 1.23 (3H, t, *J*=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.80 (COO), 151.88 (1-aryl), 149.91 (2, 6-pyridyl), 149.42 (4-pyridyl), 141.57 (4-aryl), 122.19 (3, 5-pyridyl), 115.75 (3, 5-aryl), 114.08 (2, 6-aryl), 68.50 (OCH$_2$CH$_2$), 60.21 (OCH$_2$CH$_3$), 47.91 (CH$_2$NH), 34.28 (CH$_2$COO), 29.24 (OCH$_2$CH$_2$), 28.90 (OCH$_2$CH$_2$CH$_2$CH$_2$), 25.76 (OCH$_2$CH$_2$CH$_2$), 24.90 (CH$_2$CH$_2$COO), 14.27 (CH$_2$CH$_3$). Anal. Calcd for C$_{21}$H$_{28}$N$_2$O$_3$ C, 70.76; H, 7.92; N, 7.86. Found: C, 70.50; H, 7.94; N, 7.84.

7-{4-[(2-1*H*-Indol-3-yl-acetyl)-pyridin-4-ylmethyl-amino]-phenoxy}-heptanoic acid ethyl ester (63)
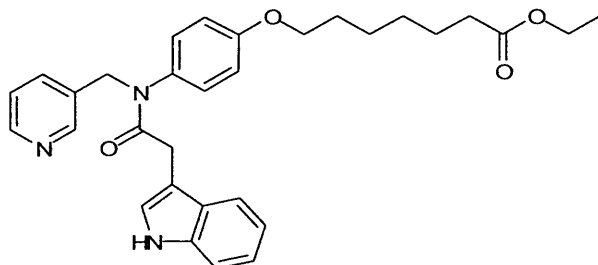
3-Indoleacetic acid (100 mg, 0.56 mmol) was dissolved in dry dichloromethane (12 mL) and to the solution was added triethylamine (135 mg 1.34 mmol). The solution was cooled to -20 °C and isobutyl chloroformate (110 mg, 0.84 mmol) added dropwise. After stirring for 15 min at -20 °C, 7-{4-[(pyridin-4-ylmethyl)-amino]-phenoxy}-heptanoic acid ethyl ester (61) (200 mg, 0.56 mmol) was added and the reaction allowed to warm to room temperature for 3 h. The solvent was removed *in vacuo* and the residue dissolved in ethyl acetate (10 mL) then washed with 10 % aqueous citric acid (2 x 10 mL), saturated aqueous sodium hydrogen carbonate solution (2 x 10 mL) and brine (10 mL). The organic phase was dried (MgSO₄) and the solvent removed *in vacuo* to give the crude product as a brown oil which was purified by column chromatography (ethyl acetate-1 % acetic acid) to yield the title compound (127 mg, 44 %) as a pale yellow oil; ^{1}H-NMR (CDCl₃, 300 MHz) δ 8.85 (1H, s, NH), 8.47 (2H, brs, Ar), 7.39 – 6.57 (11H, m, Ar), 4.84 (2H, s, CH₂N), 4.11 (2H, q, *J*=7.1 Hz, OC*H*₂CH₃) 3.90 (2H, t, *J*=6.4 Hz, OC*H*₂CH₂), 3.64 (2H, s, NCOCH₂), 2.31 (2H, t, *J*=7.4 Hz, CH₂COO), 1.77 (2H, m, OCH₂C*H*₂), 1.66 (2H, m, C*H*₂CH₂COO) 1.43 (4H, m, OCH₂CH₂C*H*₂, C*H*₂CH₂CH₂COO) 1.24 (3H, t, *J*=7.1 Hz, CH₂C*H*₃); ^{13}C-NMR (CDCl₃, 75 MHz) δ 173.84 (COO), 172.28 (CON), 158.70 (1-aryl), 149.66 (Ar), 146.79 (Ar), 136.14 (Ar), 134.85 (Ar), 129.20 (Ar), 127.18 (Ar), 123.31 (Ar), 121.80 (Ar), 119.22 (5-indolyl), 118.81 (7-indolyl), 115.26 (2, 6-aryl), 111.23 (3-indolyl), 108.88 (8-indolyl), 68.09 (OC*H*₂CH₂), 60.29 (OCH₂CH₃), 52.63 (CH₂N) 34.25 (OOCC*H*₂CH₂), 31.16 (CH₂CON), 28.97 (OCH₂CH₂), 28.83 (OCH₂CH₂CH₂C*H*₂), 25.73 (OCH₂CH₂C*H*₂), 24.86 (C*H*₂CH₂COO), 14.28 (CH₂C*H*₃). LRMS (CI) *m/z* 514 (M+H, 50 %), 457 (29 %), 306 (23 %), 154 (100 %). HRMS (M+H) calcd for C₃₁H₃₅N₃O₄ 514.27057. Found: 514.26977.

Ethyl 7-(4-(3-(1*H*-indol-3-yl)-*N*-(pyridin-4-

ylmethyl)propanamido)phenoxy)heptanoate (63)
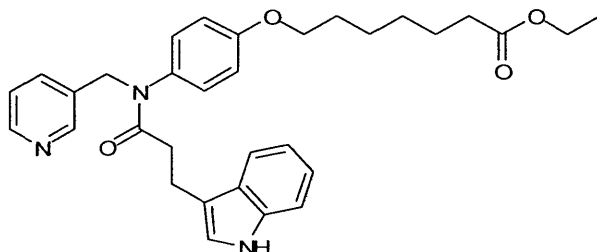
3-Indolepropionic acid (441 mg, 2.33 mmol) was dissolved in THF (23 mL) at 0 °C and to the solution was added *N*-methyl morpholine (236 mg, 2.33 mmol) and isobutyl chloroformate (318 mg, 2.33 mmol). After stirring for 30 min at room temperature, ethyl 7-(4-(pyridin-4-ylmethylamino)phenoxy)heptanoate (**61**) (830 mg, 2.33 mmol) was added and stirring continued for 16 h. Water (15 mL) was added and the aqueous phase extracted with ethyl acetate (2 x 10 mL). The combined organics were washed with 10 % aqueous citric acid (20 mL), saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL). The organic phase was dried (MgSO$_4$) and the solvent removed *in vacuo* to give the crude product as a yellow oil which was purified by column chromatography (20 % petroleum ether (40 - 60)-ethyl acetate increasing to 5 % methanol-ethyl acetate) to yield the title compound (1.05 g, 85 %) as a yellow oil; IR (KBr, thin film) (cm^{-1}) 3306, 2936, 1732, 1651, 1510, 1248. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 9.15 (1H, s, NH), 8.45 (2H, d, *J*=5.2 Hz, 2, 6-pyridyl), 7.33 (2H, m, 5, 8-indolyl), 7.13 (1H, m, indolyl), 7.01 (3H, m, 3, 5-pyridyl, indolyl), 6.92 (1H, d, *J*=2.0 Hz, 2-indolyl), 6.62 (4H, m, aryl), 4.79 (2H, s, CH$_2$N), 4.12 (2H, q, *J*=7.2 Hz, OC*H*$_2$CH$_3$), 3.84 (2H, t, *J*=6.3 Hz, OC*H*$_2$CH$_2$), 3.11 (2H, t, *J*=7.3 Hz, NCOCH$_2$C*H*$_2$), 2.53 (2H, t, *J*=7.4 Hz, NCOCH$_2$), 2.30 (2H, t, *J*=7.4 Hz, CH$_2$COO), 1.76-1.62 (4H, m, OCH$_2$C*H*$_2$,C*H*$_2$CH$_2$COO), 1.41 (4H, m, OCH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$COO), 1.23 (3H, t, *J*=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.83 (COO), 173.58 (CON), 158.57 (1-aryl), 149.64 (2, 6-pyridyl), 146.78 (4-pyridyl), 136.44 (9-indolyl), 134.58 (4-aryl), 129.05 (2, 6-aryl), 127.25 (4-indolyl), 123.37 (3, 5-pyridyl), 122.25 (2-indolyl), 121.71 (6-indolyl), 118.98 (5-indolyl), 118.84 (7-indolyl), 115.21 (3, 5-aryl), 114.60 (3-indolyl), 111.27 (8-indolyl), 68.01 (OC*H*$_2$CH$_2$), 60.28 (OC*H*$_2$CH$_3$), 52.36 (CH$_2$N), 34.81 (*C*H$_2$CON), 34.26 (*C*H$_2$COO), 28.97 (OCH$_2$*C*H$_2$), 28.83 (OCH$_2$CH$_2$CH$_2$*C*H$_2$), 25.73 (OCH$_2$CH$_2$*C*H$_2$), 24.86 (*C*H$_2$CH$_2$COO), 21.55 (NCOCH$_2$*C*H$_2$), 14.28 (CH$_2$*C*H$_3$).

LRMS (CI) m/z 528 (M+H, 54 %), 419 (25 %), 287 (32 %), 154 (100 %). HRMS (M+H) calcd for $C_{32}H_{37}N_3O_4$ 528.28622. Found: 528.28636.

7-(4-{[3-(Indol-3-yl)-propyl]-pyridin-4-ylmethyl-amino}-phenoxy)-heptan-1-ol (65)
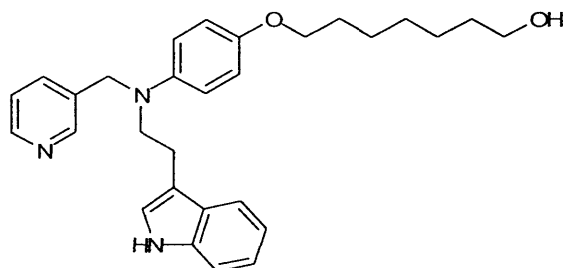
To a suspension of lithium aluminium hydride (0.66 g, 17.4 mmol) in dry tetrahydrofuran (40 mL) at 0 °C was added a solution of 7-{4-[(3-1H-indol-3-yl-propionyl)-pyridin-4-ylmethyl-amino]-phenoxy}-heptanoic acid ethyl ester (63) (2.30 g, 4.36 mmol) in dry tetrahydrofuran (5 mL) dropwise. The solution was allowed to warm to room temperature and stirred for 4 h then quenched with water (0.7 mL), 15 % aqueous sodium hydroxide solution (0.7 mL) and water (2.1 mL). The suspension was filtered through celite and the precipitate washed with ethyl acetate. The solvent was removed *in vacuo* and the crude oil purified by column chromatography (3 % methanol, 1 % triethylamine, 96 % ethyl acetate) to give the title compound (2.05 g, 99 %) as a colourless oil; IR (KBr, thin film) (cm^{-1}) 3261, 2934, 1512, 1240, 1217. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.79 (1H, s, NH), 8.46 (2H, dd, J=1.5 Hz, J=4.6 Hz, 2, 6-pyridyl), 7.56 (1H, d, J=7.7 Hz, indolyl), 7.32 (1H, d, J=8.0 Hz, indolyl), 7.14 (4H, m, Ar), 6.91 (1H, d, J=2.1 Hz, 2-indolyl), 6.75 (2H, d, J=9.1 Hz, 2, 6-aryl), 6.57 (2H, d, J=9.1 Hz, 3, 5-aryl), 4.38 (2H, s, CH$_2$N), 3.86 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 3.63 (2H, t, J=6.6 Hz, CH_2OH), 3.38 (2H, t, J=7.1 Hz, NCH_2CH$_2$), 2.95 (1H, br s, OH), 2.80 (2H, t, J=7.3 Hz, NCH$_2$CH$_2$CH_2), 2.06 (2H, m, NCH$_2$CH_2CH$_2$) 1.73 (2H, m, OCH$_2$CH_2), 1.58 (2H, m, CH_2CH$_2$OH) 1.43 (6H, m, CH_2CH_2CH_2CH$_2$CH$_2$OH); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 151.35 (1-aryl), 149.59 (2, 6-pyridyl), 142.73 (4-pyridyl), 136.57 (4-aryl), 127.41 (4-indolyl), 122.28 (3, 5-pyridyl), 121.81 (indolyl), 121.52 (indolyl), 119.03 (indolyl), 118.78 (indolyl), 115.67 (Ar), 115.37 (indolyl), 114.78 (3, 5-aryl), 111.32 (8-indolyl), 68.60 (OCH$_2$), 62.69 CH$_2$OH), 55.03 (pyridine-CH_2N), 52.16 (CH$_2$CH_2N), 32.79 (CH_2CH$_2$OH), 29.41(OCH$_2$CH_2), 29.26 (CH_2CH$_2$CH$_2$CH$_2$OH), 27.50 (CH_2CH$_2$N), 26.11 (CH_2CH$_2$CH$_2$OH), 25.82 (OCH$_2$CH$_2$CH_2), 22.70 (indole-CH$_2$). LRMS (CI) m/z 471 (M+,

100 %), 380 (22 %), 327 (46 %), 250 (19 %). HRMS (M+H) calcd for $C_{29}H_{35}N_3O_2$ 472.29639. Found: 472.29496.

Ethyl 7-(4-((1*H*-indol-5-yl)methylamino)phenoxy)heptanoate (68)
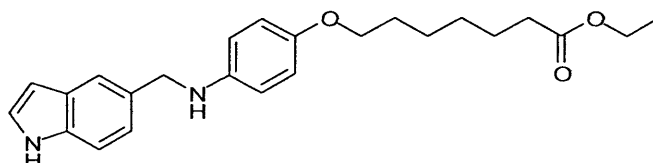
A solution of ethyl 7-(4-aminophenoxy)heptanoate (**52**) (683 mg, 2.57 mmol) and indole-5-carboxaldehyde (374 mg, 2.57 mmol) in methanol (35 mL) was stirred for 30 min. The orange solution was then cooled to 0 °C and sodium borohydride (243 mg, 6.43 mmol) added portion-wise. After stirring for a further 1 h the solvent was removed in vacuo and replaced with ethyl acetate (20 mL) then the solution washed with water (2 x 20 mL) and brine (20 mL). The organic phase was dried (MgSO4) and evaporated to leave an orange/brown solid which was recrystallised from ethanol to give the product (675 mg, 67 %) as orange crystals, mp 87-89 °C; IR (KBr, thin film) (cm^{-1}) 3408, 2936, 1720, 1510, 1232. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.49 (1H, s, NH), 7.66 (1H, s, 5-indolyl), 7.32 (1H, d, J=8.3 Hz, 8-indolyl), 7.24 (1H, dd, J=1.3, 8.4 Hz, 7-indolyl), 7.15 (1H, t, J=2.7 Hz, 2-indolyl), 6.79 (2H, d, J=8.8 Hz, 2, 6-aryl), 6.67 (2H, d, J=8.9 Hz, 3, 5-aryl), 6.53 (1H, m, 3-indolyl), 4.36 (2H, s, C*H*$_2$NH), 4.19 (2H, q, J=7.1 Hz, OC*H*$_2$CH$_3$), 3.91 (2H, t, J=6.5 Hz, OC*H*$_2$CH$_2$), 2.35 (2H, t, J=7.4 Hz, CH$_2$COO), 1.82-1.67 (4H, m, OCH$_2$C*H*$_2$, C*H*$_2$CH$_2$COO), 1.47 (4H, m, OCH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$COO), 1.30 (3H, t, J=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.03 (COO), 151.51 (1-aryl), 142.91 (4-aryl), 135.27 (9-indolyl), 130.84 (6-indolyl), 128.09 (4-indolyl), 124.89 (2-indolyl), 122.15 (7-indolyl), 119.81 (5-indolyl), 115.89 (2, 6-aryl), 114.22 (3, 5-aryl), 111.30 (8-indolyl), 102.36 (3-indolyl), 68.72 (OC*H*$_2$CH$_2$), 60.34 (C*H*$_2$CH$_3$), 49.92 (CH$_2$NH), 34.38 (C*H*$_2$COO), 29.35 (OCH$_2$C*H*$_2$), 28.97 (C*H*$_2$CH$_2$CH$_2$COO), 25.83 (OCH$_2$CH$_2$C*H*$_2$), 24.99 (C*H*$_2$CH$_2$COO), 14.34 (CH$_2$C*H*$_3$). Anal. Calcd for $C_{24}H_{30}N_2O_3$ C, 73.07; H, 7.66; N, 7.10. Found: C, 72.67; H, 7.71; N, 6.98.

Ethyl 7-(4-((3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylamino)phenoxy)heptanoate (69)
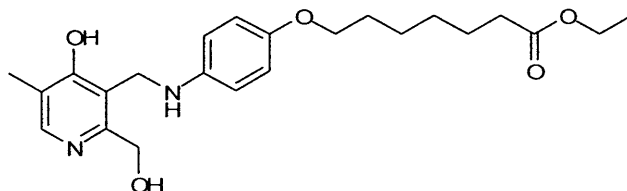
To a stirred solution of ethyl 7-(4-aminophenoxy)heptanoate (52) (300 mg, 1.13 mmol) in methanol (12 mL) was added pyridoxal hydrochloride (230 mg, 1.13 mmol) and triethylamine (114 mg, 1.13 mmol). After stirring for 30 min sodium borohydride (107 mg, 2.83 mmol) was added portionwise to the yellow suspension and the resultant solution stirred for a further 20 min. The solvent was removed in vacuo to leave a light-brown solid which was recrystallised from ethyl acetate-petroleum ether (40 - 60) to yield the title compound (362 mg, 77 %) as white crystals, mp 134-135 °C; IR (KBr, thin film) (cm^{-1}) 3277, 2860, 1736, 1514, 1242. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.69 (1H, s, 6-pyridyl), 6.77 (4H, m, 2, 3, 5, 6-aryl), 4.58 (2H, s, CH_2OH), 4.49 (2H, s, CH_2NH), 4.11 (2H, q, J=7.1 Hz, OCH_2CH$_3$), 3.86 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 2.39 (3H, s, Ar-CH$_3$), 2.29 (2H, t, J=7.4 Hz, CH$_2$COO), 1.76-1.61 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.41 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.24 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.83 (COO), 154.55 (3-pyridyl), 152.31 (2-pyridyl), 147.85 (1-aryl), 139.74 (6-pyridyl), 139.31 (4-pyridyl), 131.65 (4-aryl), 128.63 (5-pyridyl), 118.15 (3, 5-aryl), 115.53 (2, 6-aryl), 68.31 (OCH_2CH$_2$), 60.72 (CH$_2$OH), 60.24 (OCH_2CH$_3$), 45.88 (CH$_2$NH), 34.28 (CH_2COO), 29.12 (OCH$_2$CH_2), 28.87 (CH_2CH$_2$CH$_2$COO), 25.73 (OCH$_2$CH$_2$CH_2), 24.87 (CH_2CH$_2$COO), 18.67 (Ar-CH$_3$), 14.26 (CH$_2$CH_3). Anal. Calcd for C$_{23}$H$_{32}$N$_2$O$_5$ C, 66.32; H, 7.74; N, 6.73. Found: C, 65.96; H, 7.77; N, 6.84.

Ethyl 7-(1H-indol-5-yloxy)heptanoate (71)
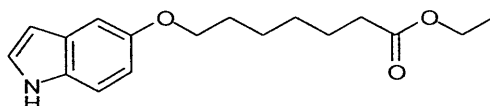
To a solution of 5-hydroxyindole (1.47 g, 11.04 mmol) in dimethylformamide (40 mL) was added caesium carbonate (4.32 mg, 13.25 mmol) and ethyl-7-bromoheptanoate (3.14 g, 13.25 mmol) and the resultant mixture heated to 120 °C for 18 h. Water (35 mL) was added

and the solution extracted with ethyl acetate (1 x 40 mL, 3 x 20 mL). The combined extracts were washed with water (2 x 30 mL) and brine (30 mL) then dried over $MgSO_4$ and evaporated. The brown oil was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 3:1) to give a pale yellow solid which was recrystallised from diethyl ether-petroleum ether (40 - 60) to give the product (1.90 g, 60 %) as a white, crystalline solid, mp 64 °C; IR (KBr, thin film) (cm^{-1}) 3377, 2937, 1717, 1456, 1225. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.28 (1H, s, NH), 7.25 (1H, d, J=8.6 Hz, 8-indolyl), 7.14 (2H, m, 2, 5-indolyl), 6.88 (1H, dd, J=2.4, 8.8 Hz, 7-indolyl), 6.48 (1H, ddd, J=0.9, 2.0, 3.0 Hz, 3-indolyl), 4.19 (2H, q, J=7.0 Hz, OCH_2CH$_3$), 4.01 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 2.35 (2H, t, J=7.4 Hz, CH$_2$COO), 1.83 (2H, m, OCH$_2$CH_2), 1.70 (2H, m, CH_2CH$_2$COO), 1.55–1.42 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.28 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.03 (COO), 153.54 (6-indolyl), 131.09 (4-indolyl), 128.33 (9-indolyl), 124.98 (2-indolyl), 112.85 (8-indolyl), 111.76 (7-indolyl), 103.52 (3-indolyl), 102.18 (5-indolyl), 62.72 (OCH_2CH$_2$), 60.32 (CH_2CH$_3$), 34.38 (CH_2COO), 29.36 (OCH$_2$CH_2), 28.98 (CH_2CH$_2$CH$_2$COO), 25.89 (OCH$_2$CH$_2$CH_2), 24.99 (CH_2CH$_2$COO), 14.31 (CH$_2$CH_3). Anal.Calcd for C$_{17}$H$_{23}$NO$_3$ C, 70.56; H, 8.01; N, 4.84. Found: C, 70.37; H, 8.08; N, 4.77.

Ethyl 7-(2-fluoro-4-nitrophenoxy)heptanoate (72)
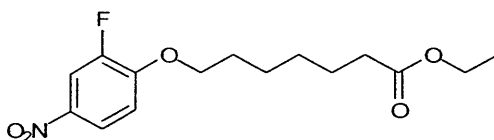
To a stirred solution of 2-fluoro-4-nitrophenol (500 mg, 3.18 mmol) in dimethylformamide (30 mL) was added cesium carbonate (1.24 g, 3.82 mmol) followed by ethyl-7-bromoheptanoate (0.90 g, 3.82 mmol). The mixture was heated to 130 °C for 20 h then allowed to cool to room temperature. Water (40 mL) was added and the aqueous solution extracted with ethyl acetate (2 x 30 mL, 2 x 15 mL). The combined yellow organics were washed with 1 M sodium hydroxide solution (30 mL), water (2 x 30 mL) and brine before drying (MgSO$_4$). The solvent was removed *in vacuo* to yield a yellow oil which was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 4:1) to give a pale yellow solid. The solid was recrystallised from diethyl ether-petroleum ether (40 - 60) to yield the title compound (0.79 g, 79 %) as pale yellow crystals, mp 40-42 °C; IR (KBr,

thin film) (cm^{-1}) 2941, 1732, 1524, 1346, 1286. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.99 (1H, ddd, J=1.5, 2.7, 9.0 Hz, 5-aryl), 7.92 (1H, dd, J=2.7, 10.7 Hz, 3-aryl), 6.99 (1H, dd, J=8.1, 9.0 Hz, 6-aryl), 4.08 (4H, m, OCH_2CH$_3$, OCH_2CH$_2$), 2.27 (2H, t, J=7.4 Hz, CH$_2$COO), 1.84 (2H, m, OCH$_2$CH_2), 1.63 (2H, m, CH_2CH$_2$COO), 1.51–1.37 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.21 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.62 (COO), 152.99 (d, J_{CF}=10.6 Hz, 1-aryl), 151.19 (d, J_{CF}=251.1 Hz, 2-aryl), 140.57 (d, J_{CF}=7.3 Hz, 4-aryl), 120.91 (d, J_{CF}=3.1 Hz, 5-aryl), 112.85 (6-aryl), 112.17 (d, J_{CF}=22.9 Hz, 3-aryl), 69.70 (OCH$_2$CH$_2$), 60.20 (OCH$_2$CH$_3$), 34.13 (CH$_2$COO), 28.69 (OCH$_2$CH$_2$), 28.65 (CH$_2$CH$_2$CH$_2$COO), 25.49 (OCH$_2$CH$_2$CH$_2$), 24.74 (CH$_2$CH$_2$COO), 14.21 (CH$_2$CH$_3$). Anal. Calcd for C$_{15}$H$_{20}$FNO$_5$ C, 57.50; H, 6.43; N, 4.47. Found: C, 57.47; H, 6.47; N, 4.32.
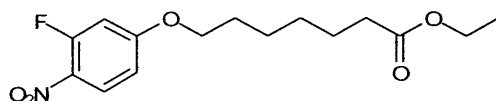

Ethyl 7-(3-fluoro-4-nitrophenoxy)heptanoate (73)

To a stirred solution of 3-fluoro-4-nitrophenol (400 mg, 2.55 mmol) in dimethylformamide (25 mL) was added cesium carbonate (980 mg, 3.06 mmol) followed by ethyl-7-bromoheptanoate (720 mg, 3.06 mmol). The mixture was heated to 120 °C for 5 h then allowed to cool to room temperature. Water (40 mL) was added and the aqueous solution extracted with ethyl acetate (3 x 20 mL). The combined yellow organics were washed with 1 M sodium hydroxide solution (30 mL), water (2 x 30 mL) and brine before drying (MgSO$_4$). The solvent was removed *in vacuo* to yield a yellow oil which was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 4:1) to give the product (594 mg, 74 %) as a pale yellow oil; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.02 (1H, m, 5-aryl), 6.69 (2H, m, 2, 6-aryl), 4.05 (4H, m, OCH_2CH$_3$, OCH_2CH$_2$), 2.27 (2H, t, J=7.4 Hz, CH$_2$COO), 1.79 (2H, m, OCH$_2$CH_2), 1.62 (2H, m, CH_2CH$_2$COO), 1.42 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.21 (3H, t, J=7.2 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.62 (COO), 164.90 (d, J_{CF}=11.3 Hz, 4-aryl), 157.47 (d, J_{CF}=263.3 Hz, 3-aryl), 130.46 (1-aryl), 127.84 (5-aryl), 110.75 (6-aryl), 103.48 (d, J_{CF}=24.0 Hz, 2-aryl), 69.22 (OCH$_2$CH$_2$), 60.21 (OCH$_2$CH$_3$), 34.13 (CH$_2$COO), 28.69 (OCH$_2$CH$_2$), 28.58 (CH$_2$CH$_2$CH$_2$COO), 25.52 (OCH$_2$CH$_2$CH$_2$), 24.74 (CH$_2$CH$_2$COO), 14.22 (CH$_2$CH$_3$). LRMS

(CI) m/z 314 (M+H, 82 %), 284 (28 %), 268 (100 %), 238 (28 %), 158 (39 %). HRMS calcd for $C_{15}H_{20}FNO_5$ (M+H) 314.14037. Found: 314.13936.

Ethyl 7-(4-amino-2-fluorophenoxy)heptanoate (74)
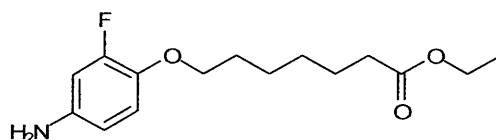
To a stirred solution of ethyl 7-(2-fluoro-4-nitrophenoxy)heptanoate (72) (500 mg, 1.60 mmol) in ethanol (16 mL), was added 5 % palladium on carbon (15 mg) under nitrogen. The system was evacuated and replaced with hydrogen at atmospheric pressure. After stirring for 4 h, the catalyst was removed and the solvent removed in vacuo to yield a red/brown oil which was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 4:1 increasing to 2:1) to give the product (403 mg, 89 %) as a light brown oil; IR (KBr, thin film) (cm^{-1}) 3371, 2937, 1724, 1518, 1263. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 6.71 (1H, t, J=8.9 Hz, 6-aryl), 6.38 (1H, d, J=12.8 Hz, 5-aryl), 6.29 (1H, d, J=8.5 Hz, 3-aryl), 4.06 (2H, q, J=7.1 Hz, OCH_2CH$_3$), 3.85 (2H, t, J=6.4 Hz, OCH_2CH$_2$), 2.24 (2H, t, J=7.4 Hz, CH$_2$COO), 1.73-1.54 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.43–1.31 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.19 (3H, t, J=7.1 Hz, CH$_2$CH_3). ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.62 (COO), 153.75 (d, J_{CF}=244.7 Hz, 2-aryl), 141.50 (d, J_{CF}=9.4 Hz, 4-aryl), 139.19 (d, J_{CF}=11.3 Hz, 1-aryl), 117.87 (d, J_{CF}=2.7 Hz, 6-aryl), 110.25 (d, J_{CF}=3.0 Hz, 5-aryl), 103.95 (d, J_{CF}=21.6 Hz, 3-aryl), 70.80 (OCH$_2$CH$_2$), 60.14 (OCH$_2$CH$_3$), 34.19 (CH$_2$COO), 29.23 (OCH$_2$CH$_2$), 28.80 (CH$_2$CH$_2$CH$_2$COO), 25.57 (OCH$_2$CH$_2$CH$_2$), 24.83 (CH$_2$CH$_2$COO), 14.13 (CH$_2$CH$_3$). Anal. Calcd for $C_{15}H_{22}FNO_3$ C, 63.58; H, 7.83; N, 4.94. Found: C, 63.70; H, 7.89; N, 4.93.

Ethyl 7-(4-amino-3-fluorophenoxy)heptanoate (75)
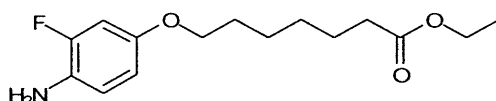
To a stirred solution of ethyl 7-(3-fluoro-4-nitrophenoxy)heptanoate (73) (595 mg, 1.90 mmol) in ethanol (19 mL), was added 5 % palladium on carbon (20 mg) under nitrogen. The system was evacuated and replaced with hydrogen at atmospheric pressure. After stirring

for 4 h, the catalyst was removed and the solvent removed in vacuo to yield a red/brown oil which was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 4:1 increasing to 2:1) to give the product (511 mg, 95 %) as a red oil; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 6.55 (3H, m, 2, 5, 6-aryl), 4.07 (2H, q, J=7.1 Hz, OCH_2CH$_3$), 3.78 (2H, t, J=6.5 Hz, OCH_2CH$_2$), 3.44 (2H, s, NH$_2$), 2.25 (2H, t, J=7.4 Hz, CH$_2$COO), 1.72-1.57 (4H, m, OCH$_2$CH_2, CH_2CH$_2$COO), 1.36 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO), 1.20 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.69 (COO), 151.91 (d, J_{CF}=238.9 Hz, 3-aryl), 152.09 (d, J_{CF}=9.4 Hz, 1-aryl), 127.80 (d, J_{CF}=13.6 Hz, 4-aryl), 117.53 (d, J_{CF}=4.8 Hz, 5-aryl), 110.48 (d, J_{CF}=2.8 Hz, 6-aryl), 102.90 (d, J_{CF}=22.1 Hz, 2-aryl), 68.53 (OCH$_2$CH$_2$), 60.15 (OCH_2CH$_3$), 34.19 (CH_2COO), 29.06 (OCH$_2$CH_2), 28.82 (CH_2CH$_2$CH$_2$COO), 25.68 (OCH$_2$CH$_2$CH_2), 24.84 (CH_2CH$_2$COO), 14.21 (CH$_2$CH_3). LRMS (CI) m/z 284 (M+H, 63 %), 283 (100 %), 157 (12 %), 127 (37 %). HRMS calcd for C$_{15}$H$_{22}$FNO$_3$ 284.16565. Found: 284.16688.

Ethyl 7-(4-(2-(1H-indol-3-yl)acetamido)-2-fluorophenoxy)heptanoate (76)
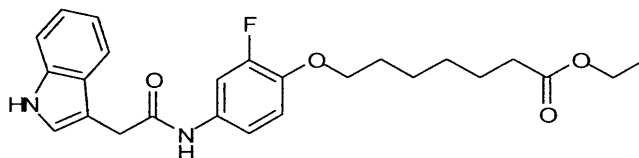
3-Indole acetic acid (449 mg, 2.56 mmol) was dissolved in dimethyl formamide (24 mL) and 1-hydroxybenzotriazole (429 g, 3.17 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (538 mg, 2.81 mmol) and N-methyl morpholine (568 mg, 5.61 mmol) added successively and the solution stirred at room temperature for 20 min. To the solution was added ethyl 7-(4-amino-2-fluorophenoxy)heptanoate (74) (691 mg, 2.44 mmol) in dimethyl formamide (4 mL) and the solution stirred for 16 h. The reaction was quenched by the addition of 1 M HCl and the resultant suspension extracted with ethyl acetate (3 x 20 mL). The combined organics were washed with saturated aqueous sodium hydrogen carbonate solution (30 mL), water (2 x 20 mL) and brine (30 mL) before drying (MgSO$_4$). The solvent was removed in $vacuo$ to yield a brown solid which was recrystallised from ethanol to give the title compound (1.02 g, 94 %), mp 107-109 °C; IR (KBr, thin film) (cm^{-1}) 3319, 2936, 1738, 1661, 1375, 1269. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.87 (1H, s, NH), 7.76 (1H, s, NH), 7.58 (1H, d, J=7.8 Hz, 5-indolyl), 7.37 (1H, d, J=8.1

Hz, 8-indolyl), 7.31 (1H, m, 3-aryl), 7.22 (1H, t, *J*=7.3 Hz, 6-indolyl), 7.13 (2H, m, 2-indolyl, 5-aryl), 6.92 (1H, d, *J*=8.8 Hz, 6-aryl), 6.76 (1H, m, 7-indolyl), 4.13 (2H, q, *J*=7.1 Hz, OC*H₂*CH₃), 3.91 (2H, t, *J*=6.5 Hz OC*H₂*CH₂), 3.84 (2H, s, C*H₂*CONH), 2.30 (2H, t, *J*=7.4 Hz, CH₂COO), 1.78-1.64 (4H, m, OCH₂C*H₂*, C*H₂*CH₂COO), 1.41 (4H, m, OCH₂CH₂C*H₂*, C*H₂*CH₂CH₂COO), 1.25 (3H, t, *J*=7.1 Hz, CH₂C*H₃*); ¹³C-NMR (CDCl₃, 75 MHz) δ 174.04 (COO), 170.29 (CONH), 152.31 (d, *J*cf=243.8 Hz, 1-aryl), 136.52 (9-indolyl), 131.17 (d, *J*cf=11.3 Hz, 4-aryl), 126.92 (4-indolyl), 124.18 (d, *J*cf=12.0 Hz, 5-aryl), 122.53 (6-indolyl), 120.06 (2-indolyl), 118.47 (5-indolyl), 116.50 (6-aryl), 115.18 (7-indolyl), 111.74 (8-indolyl), 109.48 (d, *J*cf=22.5 Hz, 3-aryl), 108.02 (3-indolyl), 69.74 (OC*H₂*CH₂), 60.35 (OC*H₂*CH₃), 34.30 (OOCC*H₂*CH₂, C*H₂*CONH), 29.03 (OCH₂C*H₂*), 28.82 (OCH₂CH₂CH₂C*H₂*), 25.59 (OCH₂CH₂C*H₂*), 24.87 (C*H₂*CH₂COO), 14.27 (CH₂C*H₃*). Anal. Calcd for C₂₅H₂₉FN₂O₄ C, 68.16; H, 6.64; N, 6.36. Found: C, 68.06; H, 6.73; N, 6.32.

Ethyl 7-(4-(2-(1H-indol-3-yl)acetamido)-3-fluorophenoxy)heptanoate (77)
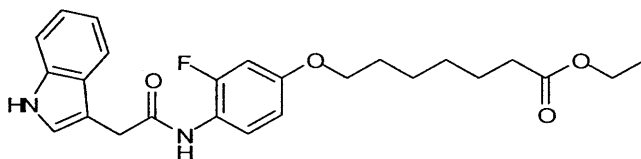
3-Indole acetic acid (0.71 g, 4.08 mmol) was dissolved in dimethyl formamide (38 mL) and 1-hydroxybenzotriazole (0.68 g, 5.04 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (0.85 g, 4.46 mmol) and *N*-methyl morpholine (0.90 g, 8.92 mmol) added successively and the solution stirred at room temperature for 20 min. To the solution was added ethyl 7-(4-amino-3-fluorophenoxy)heptanoate (75) (1.10 g, 3.88 mmol) and the solution stirred for 24 h. The reaction was quenched by the addition of 1 M HCl and the resultant suspension extracted with ethyl acetate (4 x 20 mL). The combined organics were washed with saturated aqueous sodium hydrogen carbonate solution (30 mL), water (2 x 20 mL) and brine (30 mL) before drying (MgSO₄). The solvent was removed *in vacuo* to yield a red oil which solidified on cooling and was recrystallised from ethanol to give the title compound (1.42 g, 83 %), mp 94-96 °C; IR (KBr) (cm⁻¹) 3053, 2988, 1421, 1258, 898. ¹H-NMR (CDCl₃, 300 MHz) δ 8.96 (1H, s, NH), 7.92 (1H, t, *J*=9.0 Hz, 5-aryl), 7.61 (2H, m, 5-indolyl, NH), 7.37 (1H, d, *J*=8.0 Hz, 8-indolyl), 7.19 (3H, m, 6, 7, 2-indolyl), 6.56 (2H, m, 2, 6-aryl), 4.15 (2H, q, *J*=7.2 Hz, OC*H₂*CH₃), 3.90 (2H, s, C*H₂*CONH), 3.80 (2H, t,

J=6.4 Hz OC*H₂*CH₂), 2.31 (2H, t, *J*=7.4 Hz, CH₂COO), 1.68 (4H, m, OCH₂C*H₂*, C*H₂*CH₂COO), 1.42 (4H, m, OCH₂CH₂C*H₂*, C*H₂*CH₂CH₂COO), 1.26 (3H, t, *J*=7.1 Hz, CH₂C*H₃*); ¹³C-NMR (CDCl₃, 75 MHz) δ 173.95 (COO), 170.26 (CONH), 156.19 (d, *J*_{CF}=10.4 Hz, 1-aryl), 154.06 (d, *J*_{CF}=244.9 Hz, 3-aryl), 136.90 (9-indolyl), 126.94 (4-indolyl), 124.20 (2-indolyl), 123.91 (5-aryl), 122.52 (6-indolyl), 120.01 (7-indolyl), 118.64 (4-aryl), 118.56 (5-indolyl), 111.68 (8-indolyl), 109.91 (d, *J*_{CF}=2.1 Hz, 6-aryl), 108.08 (3-indolyl), 102.12 (d, *J*_{CF}=22.7 Hz, 2-aryl), 68.32 (OCH₂CH₂), 60.33 (OCH₂CH₃), 34.28 (OOCCH₂CH₂, CH₂CONH), 28.90 (OCH₂CH₂), 28.83 (OCH₂CH₂CH₂CH₂), 25.66 (OCH₂CH₂CH₂), 24.87 (CH₂CH₂COO), 14.28 (CH₂CH₃). Anal. Calcd for C₂₅H₂₉FN₂O₄ C, 68.16; H, 6.64; N, 6.36. Found: C, 68.23; H, 6.66; N, 6.40.

7-(4-(Dimethylamino)phenoxy)-*N*-hydroxyheptanamide (78)
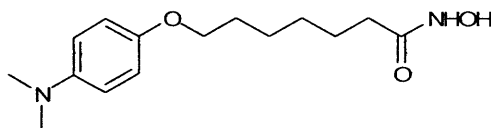
To a solution of ethyl 7-(4-(dimethylamino)phenoxy)heptanoate (52) (1.04 g, 3.54 mmol) in tetrahydrofuran (10 mL) at 0 °C was added a 1M solution of hydroxylamine in methanol (35.4 mL, 35.4 mmol) dropwise, followed by a 1M solution of potassium hydroxide in methanol (7.09 mL, 7.09 mmol) dropwise. The reaction was allowed to warm to room temperature and stirred for 2 d. After cooling to 0 °C, water (20 mL) was added and the acidity of the solution adjusted to pH6 by addition of 1M HCl. The solution was extracted ethyl acetate (3 x 30 mL) and 10% methanol-ethyl acetate (2 x 20 mL). The combined organics were dried (MgSO₄) and evaporated to leave an off-white solid which was recrystallised from methanol to give the product (377 mg, 38 %) as off-white crystals, mp 105-107 °C; IR (KBr) (cm⁻¹) 3188, 2934, 1624, 1514, 1259. ¹H-NMR ((CD₃)₂SO, 300 MHz) δ 8.69 (1H, brs, NH), 6.77 (2H, m, 2, 6-aryl), 6.68 (2H, m, 3, 5-aryl), 3.82 (2H, t, *J*=6.2, OCH₂), 2.76 (6H, s, N(CH₃)₂), 1.94 (2H, t, *J*=7.2, CH₂CO), 1.62 (2H, m, OCH₂C*H₂*), 1.51-1.23 (6H, m, C*H₂*CH₂CO, OCH₂CH₂C*H₂*, C*H₂*CH₂CH₂CO); ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 169.10 (CO), 150.59 (1-aryl), 145.26 (4-aryl), 115.12 (2, 6-aryl), 114.28 (3, 5-aryl), 67.69 (OCH₂), 41.12 (N(CH₃)₂), 32.15 (CH₂CO), 28.69 (OCH₂CH₂), 28.30

($CH_2CH_2CH_2CO$), 25.23 (CH_2CH_2CO), 25.02 ($OCH_2CH_2CH_2$). Anal.Calcd for $C_{15}H_{24}N_2O_3$ C, 64.26; H, 8.63; N, 9.99. Found: C, 64.34; H, 8.84; N, 9.95.

6-(4-(2-(1H-Indol-3-yl)acetamido)phenoxy)-N-hydroxyhexanamide (79)
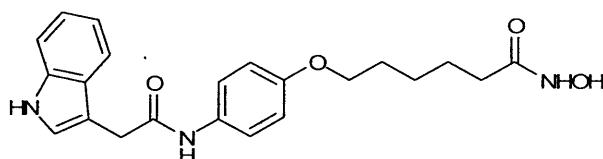
To a solution of ethyl 6-(4-(2-(1H-indol-3-yl)acetamido)phenoxy)hexanoate (56) (900 mg, 2.20 mmol) in tetrahydrofuran (6 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol (22 mL) and a 1M solution of KOH in methanol (4.4 mL) dropwise. The white suspension was stirred for 3 d at room temperature then water (20 mL) added. The white precipitate was collected by filtration and washed with cold methanol to give the product (383 mg, 59 %) as a white powder, mp 204 °C dec.; IR (KBr) (cm^{-1}) 3385, 2937, 1661, 1512, 1244, 723. ^{1}H-NMR ((CD_3)$_2$SO, 300 MHz) δ 7.61 (1H, d, J=7.8 Hz, 5-indolyl), 7.51 (2H, d, J=9.1 Hz, 3, 5-aryl), 7.35 (1H, d, J=8.0 Hz, 8-indolyl), 7.24 (1H, s, 2-indolyl), 7.06 (1H, ddd, J=1.2, 7.1, 8.1 Hz, 7-indolyl), 6.96 (1H, ddd, J=1.1, 7.1, 7.9 Hz, 6-indolyl), 6.82 (2H, d, J=9.1 Hz, 2, 6-aryl), 3.83 (2H, t, J=6.4 Hz, OCH_2CH$_2$), 3.69 (2H, s, CH_2CONH), 1.91 (2H, t, J=7.2 Hz, CH_2CONHOH), 1.67-1.46 (4H, m, OCH$_2$CH_2, CH_2CH$_2$CONHOH), 1.33 (2H, m, CH_2CH$_2$CH$_2$CONHOH); ^{13}C-NMR ((CD_3)$_2$SO, 75 MHz) δ 169.19 (CONH), 167.97 (CONHOH), 154.39 (1-aryl), 136.09 (9-indolyl), 132.49 (4-aryl), 127.20 (4-indolyl), 123.80 (2-indolyl), 120.82 (6-indolyl), 120.53 (3, 5-aryl), 118.66 (7-indolyl), 118.23 (5-indolyl), 114.25 (2, 6-aryl), 111.30 (8-indolyl), 108.67, (3-indolyl), 67.44 (OCH$_2$CH$_2$), 33.60 (OOCCH_2CH$_2$, CH_2CONH), 28.56 (OCH$_2$CH_2), 25.47 (CH_2CH$_2$CONHOH), 25.34 (OCH$_2$CH$_2$CH_2). Anal. Calcd for $C_{22}H_{25}N_3O_4$ C, 66.82; H, 6.37; N, 10.73. Found: C, 66.63; H, 6.34; N, 10.52.

7-(4-(2-(1H-Indol-3-yl)acetamido)phenoxy)-N-hydroxyheptanamide (80)
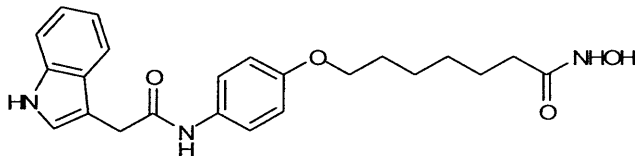
To a solution of 7 ethyl 7-(4-(2-(1H-indol-3-yl)acetamido)phenoxy)heptanoate (57) (1.00 g, 2.37 mmol) in tetrahydrofuran (8 mL) at 0 °C was added a 1M solution of hydroxylamine in methanol (23.7 mL, 23.7 mmol) dropwise, followed by a 1M solution of potassium hydroxide in methanol (4.7 mL, 4.7 mmol) dropwise. The reaction was allowed to warm to room temperature and stirred for 2 d. After cooling to 0 °C, water (30 mL) was added to the white suspension and the acidity adjusted to pH6 by addition of 1M HCl. The solution was extracted ethyl acetate (1 x 50 mL, 2 x 30 mL) and 10% methanol-ethyl acetate (2 x 30 mL). The combined organics were evaporated to leave an off-white solid which was repeatedly triturated (petroleum ether (40 - 60)-ethyl acetate, 4:1) to give the product (349 mg, 36 %) as an off-white powder, mp 155-158 °C; IR (KBr) (cm^{-1}) 3378, 2935, 1663, 1512, 1244, 741. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.89 (1H, brs, NH), 10.32 (1H, brs, OH), 9.92 (1H, brs, NH), 8.65 (1H, brs, NH), 7.59 (1H, d, J=7.8 Hz, 5-indolyl), 7.48 (2H, d, J=9.1 Hz, 3, 5-aryl), 7.34 (1H, d, J=8.0 Hz, 8-indolyl), 7.23 (1H, d, J=2.2 Hz, 2-indolyl), 7.06 (1H, ddd, J=1.2, 7.1, 8.1, 7-indolyl), 6.97 (1H, ddd, J=1.1, 7.1, 7.9 Hz, 5-indolyl), 6.84 (2H, d, J=9.1 Hz, 2, 6-aryl), 3.88 (2H, t, J=6.4 Hz, OCH$_2$), 3.67 (2H, s, CH_2C=ONH), 1.93 (2H, t, J=7.3 Hz, CH_2CONHOH), 1.65 (2H, m, OCH$_2$CH_2), 1.49 (2H, m, CH_2CH$_2$CONHOH), 1.40-1.23 (4H, m, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$CONHOH); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 169.13 (CONHOH), 169.06 (CONH), 154.42 (1-aryl), 136.05 (9-indolyl), 132.41 (4-aryl), 127.17 (4-indolyl), 123.76 (2-indolyl), 120.90 (6-indolyl), 120.54 (3, 5-aryl), 118.63 (7-indolyl), 118.29 (5-indolyl), 114.32 (2, 6-aryl), 111.29 (8-indolyl), 108.67 (3-indolyl), 67.41 (OCH$_2$), 33.62 (CH$_2$CONH), 32.15 (CH$_2$CONHOH), 28.55 (OCH$_2$CH$_2$), 28.28 (CH$_2$CH$_2$CH$_2$CONHOH), 25.19 (CH$_2$CH$_2$CONHOH), 25.01 (OCH$_2$CH$_2$CH$_2$). Anal.Calcd for C$_{23}$H$_{27}$N$_3$O$_4$ C, 67.46; H, 6.65; N, 10.26. Found: C, 67.24; H, 6.62; N, 9.99.
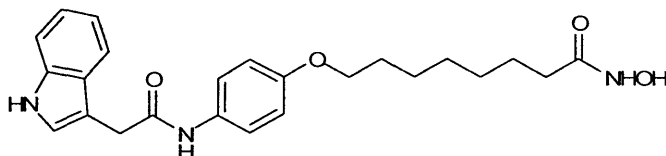
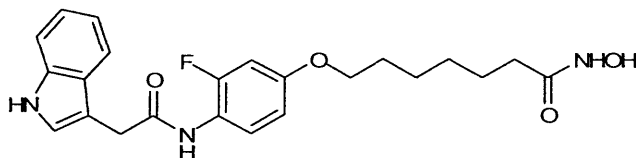## 8-(4-(2-(1*H*-Indol-3-yl)acetamido)phenoxy)-*N*-hydroxyoctanamide (81)

To a suspension of methyl 8-(4-(2-(1*H*-indol-3-yl)acetamido)phenoxy)octanoate (59) (750 mg, 1.78 mmol) in tetrahydrofuran (5 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol (17.8 mL) and a 1M solution of KOH in methanol (3.6 mL) dropwise. The white suspension was stirred for 3 d at room temperature then water (50 mL) added and the suspension neutralised by the addition of 2M HCl. The aqueous phase was extracted with ethyl acetate (1 x 90 mL, 3 x 15 mL) and the combined extracts washed with brine then the solvent removed *in vacuo*. The crude product was triturated with 1:1 ethyl acetate-petroleum ether (40 - 60) and washed with cold methanol to give the title compound (316 mg, 42 %) as an off-white powder, mp 164-165 °C; IR (KBr) (cm^{-1}) 3381, 3308, 2936, 1661, 1514, 1244. ^{1}H-NMR ((CD_3)$_2$SO, 300 MHz) δ 8.74 (1H, s, NH), 7.64 (1H, d, *J*=7.8 Hz, 5-indolyl), 7.55 (2H, d, *J*=9.0 Hz, 3, 5-aryl), 7.35 (1H, d, *J*=8.0 Hz, 8-indolyl), 7.27 (1H, d, *J*=2.0 Hz, 2-indolyl), 7.05 (1H, m, 7-indolyl), 6.96 (1H, m, 6-indolyl), 6.83 (2H, d, *J*=9.0 Hz, 2, 6-aryl), 3.87 (2H, t, *J*=6.3 Hz, OC*H*$_2$CH$_2$), 3.43 (2H, s, C*H*$_2$CONH), 1.95 (2H, t, *J*=7.2 Hz, C*H*$_2$CONHOH), 1.64 (2H, m, OCH$_2$C*H*$_2$), 1.50 (2H, m, C*H*$_2$CH$_2$CONHOH), 1.35-1.26 (6H, m, OCH$_2$CH$_2$C*H*$_2$, OCH$_2$CH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$CONHOH); ^{13}C-NMR ((CD_3)$_2$SO, 75 MHz) δ 169.22 (CONH), 169.14 (CONHOH), 154.35 (1-aryl), 136.03 (9-indolyl), 132.56 (4-aryl), 127.20 (4-indolyl), 123.80 (2-indolyl), 120.81 (6-indolyl), 120.50 (3, 5-aryl), 118.73 (7-indolyl), 118.21 (5-indolyl), 114.24 (2, 6-aryl), 111.27 (8-indolyl), 108.73, (3-indolyl), 67.43 (OCH$_2$CH$_2$), 33.58 (*C*H$_2$CONH), 32.16 (*C*H$_2$CONHOH), 28.62 (OCH$_2$CH$_2$), 28.42 (OCH$_2$CH$_2$CH$_2$*C*H$_2$, OCH$_2$CH$_2$CH$_2$CH$_2$*C*H$_2$), 25.36 (OCH$_2$CH$_2$*C*H$_2$), 25.00 (*C*H$_2$CH$_2$CONHOH). Anal. Calcd for $C_{24}H_{29}N_3O_4$ C, 68.06; H, 6.90; N, 9.92. Found: C, 67.94; H, 6.93; N, 9.80.

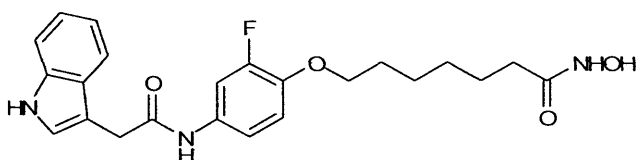### 7-(4-(2-(1*H*-Indol-3-yl)acetamido)-3-fluorophenoxy)-*N*-hydroxyheptanamide (82)

To a solution of ethyl 7-(4-(2-(1*H*-indol-3-yl)acetamido)-3-fluorophenoxy)heptanoate (77) (1.05 g, 2.38 mmol) in tetrahydrofuran (5 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol (23.8 mL) and a 1M solution of KOH in methanol (4.8 mL) dropwise. After stirring for 2 d at room temperature water (50 mL) was added and the white precipitate collected by filtration. The precipitate was washed with cold methanol and dried *in vacuo* to give the product (0.78 g, 77 %) as a pale pink solid, mp 138-141 °C; IR (KBr) (cm^{-1}) 3381, 3195, 2934, 1672, 1533, 1117. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.64 (1H, brs, NH), 7.59 (2H, m, 5-indolyl, 5-aryl), 7.36 (1H, d, *J*=8.0 Hz, 8-indolyl), 7.26 (1H, s, 2-indolyl), 7.07 (1H, m, 7-indolyl), 7.01 (1H, m, 6-indolyl), 6.87 (1H, dd, *J*=2.6, 12.6 Hz, 6-aryl), 6.71 (1H, dd, *J*=1.8, 8.8 Hz, 2-aryl), 3.91 (2H, t, *J*=6.4 Hz OC*H*$_2$CH$_2$), 3.76 (2H, s, C*H*$_2$CONH), 1.95 (2H, t, *J*=7.2 Hz, C*H*$_2$CONHOH), 1.66 (2H, m, OCH$_2$C*H*$_2$), 1.50 (2H, m, C*H*$_2$CH$_2$CONHOH), 1.42-1.27 (4H, m, OCH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$CONHOH); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 169.84 (CONH), 169.06 (CONHOH), 156.31 (d, *J*$_{CF}$=10.2 Hz, 1-aryl), 154.91 (d, *J*$_{CF}$=245.0 Hz, 3-aryl), 136.07 (9-indolyl), 127.17 (4-indolyl), 125.86 (2-indolyl), 123.85 (5-aryl, 6-indolyl), 120.93 (7-indolyl), 118.69 (4-aryl), 118.31 (5-indolyl), 111.30 (8-indolyl), 110.01 (d, *J*$_{CF}$=2.2 Hz, 6-aryl), 108.50 (3-indolyl), 102.13 (d, *J*$_{CF}$=23.1 Hz, 2-aryl), 67.90 (OC*H*$_2$CH$_2$), 32.89 (OOCC*H*$_2$CH$_2$) 32.15 (C*H*$_2$CONH), 28.36 (OCH$_2$C*H*$_2$), 28.23 (OCH$_2$CH$_2$CH$_2$C*H*$_2$), 25.12 (C*H*$_2$CH$_2$CONHOH), 25.00 (OCH$_2$CH$_2$C*H*$_2$). Anal. Calcd for C$_{23}$H$_{26}$FN$_3$O$_4$ C, 64.62; H, 6.13; N, 9.83. Found: C, 64.40; H, 6.14; N, 9.80.

7-(4-(2-(1*H*-Indol-3-yl)acetamido)-2-fluorophenoxy)-*N*-hydroxyheptanamide (83)

To a solution of ethyl 7-(4-(2-(1*H*-indol-3-yl)acetamido)-2-fluorophenoxy)heptanoate (**76**) (0.53 g, 1.20 mmol) in tetrahydrofuran (3 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol (12.0 mL) and a 1M solution of KOH in methanol (2.4 mL) dropwise. After stirring for 2 d at room temperature water (50 mL) was added and the white precipitate collected by filtration. The white solid was washed with cold methanol and dried *in vacuo* to give the product (0.14 g, 28 %) as a white powder, mp 179-180 °C dec.; IR (KBr) (cm^{-1}) 3377, 3307, 2937, 1661, 1518, 1223. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.61 (2H, m, Ar), 7.29 (3H, m, Ar), 7.00 (3H, m, Ar), 3.92 (2H, t, *J*=6.5 Hz OC*H$_2$*CH$_2$), 3.70 (2H, s, C*H$_2$*CONH), 1.87 (2H, t, *J*=7.2 Hz, CH$_2$CONHOH), 1.64 (2H, m, OCH$_2$C*H$_2$*), 1.51-1.24 (6H, m, C*H$_2$*CH$_2$CONHOH, OCH$_2$CH$_2$C*H$_2$*, C*H$_2$*CH$_2$CH$_2$CONHOH); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 169.62 (CONH), 167.94 (CONHOH), 151.10 (d, *J*$_{CF}$=241.6 Hz, 2-aryl), 141.87 (d, *J*$_{CF}$=11.0 Hz, 1-aryl), 136.09 (9-indolyl), 133.12 (d, *J*$_{CF}$=9.6 Hz Hz, 4-aryl), 127.18 (4-indolyl), 123.89 (5-aryl), 120.81 (6-indolyl), 118.64 (2-indolyl), 118.23 (5-indolyl), 115.25 (6-aryl), 114.90 (7-indolyl), 111.31 (8-indolyl), 108.42 (3-indolyl), 107.47 (d, *J*$_{CF}$=22.8 Hz, 3-aryl), 68.91 (OCH$_2$CH$_2$), 33.59 (OOCC*H$_2$*CH$_2$) 32.89 (*C*H$_2$CONH), 28.56 (OCH$_2$C*H$_2$*, OCH$_2$CH$_2$CH$_2$C*H$_2$*), 25.70 (*C*H$_2$CH$_2$CONHOH), 25.18 (OCH$_2$CH$_2$C*H$_2$*). Anal. Calcd for C$_{23}$H$_{26}$FN$_3$O$_4$ C, 64.62; H, 6.13; N, 9.83. Found: C, 64.53; H, 6.06; N, 9.69.

7-(4-(3-(1*H*-Indol-3-yl)propanamido)phenoxy)-*N*-hydroxyheptanamide (84)
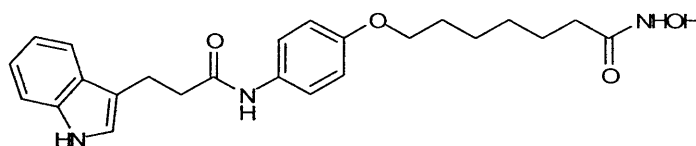
To a solution of ethyl 7-(4-(3-(1*H*-indol-3-yl)propanamido)phenoxy)heptanoate (**58**) (1.0 g, 2.29 mmol) in tetrahydrofuran (7 mL) at 0 °C was added a 1M solution of hydroxylamine in methanol (22.9 mL, 22.9 mmol) dropwise, followed by a 1M solution of potassium hydroxide in methanol (4.6 mL, 4.6 mmol) dropwise. The reaction was allowed to warm to room temperature and stirred for 2 d. After cooling to 0 °C, water (30 mL) was added to the white suspension and the acidity adjusted to pH6 by addition of 1M HCl. The solution was extracted ethyl acetate (1 x 50 mL, 2 x 30 mL) and 10% methanol-ethyl acetate (2 x 30 mL). The combined organics were evaporated to leave an off-white solid which was triturated (petroleum ether (40 - 60)-ethyl acetate, 4:1) to give the product (378 mg, 39 %) as an off-

white powder, mp 149-150 °C; IR (KBr) (cm⁻¹) 3418, 3285, 2928, 1653, 1510, 1244. ^{1}H-NMR ((CD₃)₂SO, 300 MHz) δ 10.76 (1H, s, NH), 10.33 (1H, s, OH), 9.75 (1H, s, NH), 8.67 (1H, brs, NH), 7.56 (1H, d, J=7.8 Hz, 5-indolyl), 7.48 (2H, d, J=9.1, 3, 5-aryl), 7.32 (1H, d, J=8.0 Hz, 8-indolyl), 7.12 (1H, d, J=0.7 Hz, 2-indolyl), 7.06 (1H, m, 7-indolyl), 7.02 (1H, m, 6-indolyl), 6.84 (2H, d, J= 9.1 Hz, 2, 6-aryl), 3.88 (2H, t, J=6.4 Hz, OCH_2CH₂), 3.00 (2H, t, J=7.2 Hz, CH_2CONH), 2.62 (2H, t, J=7.2 Hz, CH_2CH₂CONH), 1.94 (2H, t, J=7.2 Hz, CH₂CONHOH), 1.66 (2H, m, OCH₂CH_2), 1.50 (2H, m, CH_2CH₂CONHOH), 1.40-1.27 (4H, m, OCH₂CH₂CH_2, CH_2CH₂CH₂CONHOH); ^{13}C-NMR ((CD₃)₂SO, 75 MHz) δ 170.38 (CONH), 169.09 (CONHOH), 154.38 (1-aryl), 136.19 (9-indolyl), 132.37 (4-aryl), 126.99 (4-indolyl), 122.08 (2-indolyl), 120.86 (6-indolyl), 120.55 (3, 5-aryl), 118.31 (7-indolyl), 118.10 (5-indolyl), 114.30 (2, 6-aryl), 113.70 (8-indolyl), 111.27 (3-indolyl), 67.42 (OCH₂CH₂), 37.11 (CH₂CONH), 32.16 (CH₂CONHOH), 28.57 (OCH₂CH₂), 28.29 (CH₂CH₂CH₂CONHOH), 25.21 (CH₂CH₂CONHOH), 25.03 (OCH₂CH₂CH₂), 20.84 (CH₂CH₂CONH). Anal. Calcd for C₂₄H₂₉N₃O₄: C, 68.06; H, 6.90; N, 9.92. Found: C, 67.79; H, 6.91; N, 9.74.

7-(4-(2-(1H-Indol-3-yl)ethylamino)phenoxy)-N-hydroxyheptanamide (85)
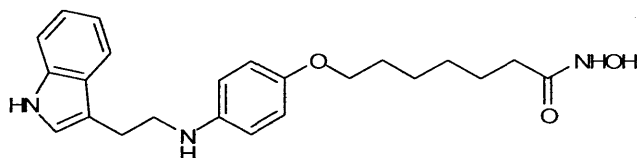
To a solution of ethyl 7-(4-(2-(1H-indol-3-yl)ethylamino)phenoxy)heptanoate (57) (700 mg, 1.71 mmol) in tetrahydrofuran (5 mL) at 0 °C was added a 1M solution of hydroxylamine in methanol (17.1 mL, 17.1 mmol) and a 1M solution of KOH in methanol (3.43 mL, 3.43 mmol) and the solution stirred for 1 d. Diethyl ether (20 mL) was added to the reaction and the white precipitate collected by filtration. The crude product was washed with water and 10 % methanol-ethyl acetate to give a white powder (468 mg, 69 %), mp 104-106 °C; IR (KBr) (cm⁻¹) 3391, 3223, 2934, 1616, 1514, 1244. ^{1}H-NMR ((CD₃)₂SO, 300 MHz) δ 10.94 (1H, brs, NH), 7.54 (1H, d, J=7.6 Hz, 5-indolyl), 7.38 (1H, d, J=7.8 Hz, 8-indolyl), 7.21 (1H, s, 2-indolyl), 7.03 (2H, m, 6, 7-indolyl), 6.71 (2H, d, J=8.3 Hz, 2, 6-aryl), 6.56 (2H, d, J=8.3 Hz, 3, 5-aryl), 5.10 (1H, s, NH), 3.78 (2H, s, OCH_2CH₂), 3.26 (2H, s, CH_2NH), 2.96 (2H, m, CH_2CH₂NH), 1.98 (2H, t, J=6.7 Hz, CH₂COO), 1.62-1.30 (8H, m, OCH₂CH_2,

CH_2CH$_2$COO, OCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO). ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 168.93 (CONHOH), 149.93 (1-aryl), 143.07 (4-aryl), 136.25 (9-indolyl), 127.23 (4-indolyl), 122.71 (2-indolyl), 120.81 (7-indolyl), 118.17 (8, 6-indolyl), 115.45 (2, 6-aryl), 113.05 (3, 5-aryl), 112.13 (3-indolyl), 111.37 (5-indolyl), 67.83 (OCH_2CH$_2$), 44.67 (CH$_2$NH), 32.32 (CH_2CONHOH), 28.82 (OCH$_2$CH_2), 28.41 (CH_2CH$_2$CH$_2$COO), 25.32 (OCH$_2$CH$_2$CH_2), 25.19 (CH_2CH$_2$CONHOH), 24.86 (indole-CH$_2$). Anal. Calcd for C$_{23}$H$_{29}$N$_3$O$_3$ + ⅓H$_2$O C, 68.81; H, 7.45; N, 10.47. Found: C, 68.71; H, 7.37; N, 10.26.

7-(4-(3-(1H-Indol-3-yl)-N-(pyridin-4-ylmethyl)propanamido)phenoxy)-N-hydroxyheptanamide (86)
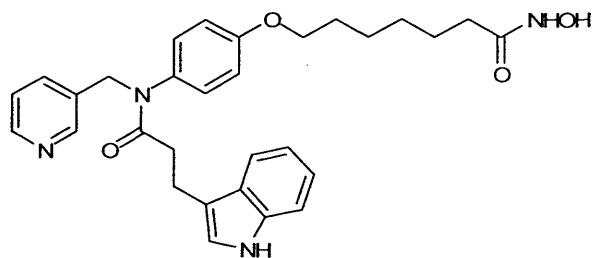
To a stirred solution of ethyl 7-(4-(2-(1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamido)phenoxy)heptanoate (63) (0.99 g, 1.88 mmol) in tetrahydrofuran (3 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol (18.8 mL) and a 1M solution of KOH in methanol (3.8 mL) dropwise. After stirring for 3 d at room temperature water (30 mL) was added and the white precipitate collected by filtration and recrystallised from methanol to give the product (783 mg, 81 %) as white crystals, mp 163-170 °C dec.; IR (KBr) (cm^{-1}) 3215, 2936, 1665, 1512, 1256. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 8.86 (1H, s, NH), 8.44 (2H, d, J=5.4 Hz, 2, 6-pyridyl), 7.36 (1H, d, J=8.1 Hz, 8-indolyl), 7.29 (1H, d, J=7.8 Hz, 5-indolyl), 7.08 (4H, m, 3, 5-pyridyl, 7, 2-indolyl), 6.91 (3H, m, 3, 5-aryl, 6-indolyl), 6.77 (2H, d, J=8.7 Hz, 2, 6-aryl), 4.82 (2H, s, CH$_2$N), 3.83 (2H, t, J=5.7 Hz, OCH_2CH$_2$), 2.97 (2H, t, J=7.2 Hz, NCOCH$_2$CH_2), 2.46 (2H, t, J=7.7 Hz, NCOCH$_2$), 1.97 (2H, t, J=7.1Hz, CH_2CONH), 1.62-1.49 (4H, m, OCH$_2$CH_2, CH_2CH$_2$CONH), 1.30 (4H, m, CH_2CH$_2$CH$_2$CO, OCH$_2$CH$_2$CH_2); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 172.28 (CON), 169.15 (CONH), 157.76 (1-aryl), 149.42 (2, 6-pyridyl), 146.69 (4-pyridyl), 136.18 (9-indolyl), 134.56 (4-aryl), 129.02 (3, 5-aryl), 126.85 (4-indolyl), 122.65 (3, 5-pyridyl), 122.38 (6-indolyl), 120.85 (2-indolyl), 118.21 (7-indolyl), 118.08 (5-indolyl), 114.88 (2, 6-aryl),

113.42 (3-indolyl), 111.31 (8-indolyl), 67.46 (OCH_2CH_2), 51.37 (CH_2N), 34.56 (CH_2CONH), 32.18 (CH_2CONH), 28.44 (OCH_2CH_2), 28.26 ($OCH_2CH_2CH_2CH_2$), 25.18 ($OCH_2CH_2CH_2$), 25.03 (CH_2CH_2CONH), 20.96 ($NCOCH_2CH_2$). Anal. Calcd for $C_{30}H_{34}N_4O_4 + \frac{1}{4}H_2O$ C, 69.41; H, 6.70; N, 10.79. Found: C, 69.31; H, 6.77; N, 10.57.

7-(4-((1*H*-Indol-5-yl)methylamino)phenoxy)-*N*-hydroxyheptanamide (87)
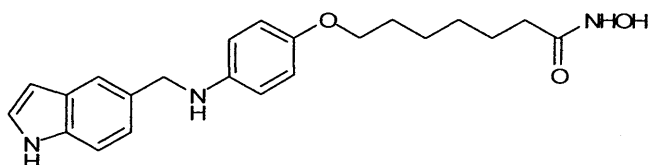
To a solution of ethyl 7-(4-((1*H*-indol-5-yl)methylamino)phenoxy)heptanoate (**68**) (550 mg, 1.39 mmol) in tetrahydrofuran (4 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol (13.9 mL) and a 1M solution of KOH in methanol (2.8 mL) dropwise. The reaction was stirred for 7 d at room temperature then water (40 mL) added and the aqueous solution extracted with ethyl acetate (5 x 20 mL). The combined organics were washed with brine (30 mL) and dried ($MgSO_4$) then the solvent removed *in vacuo*. The crude product was purified by column chromatography (30 % ethyl acetate-petroleum ether (40 - 60) increasing to 5 % methanol-ethyl acetate) to give an orange oil which was triturated with diethyl ether to give the product (164 mg, 31 %) as a pale yellow powder, mp 99-102 °C; IR (KBr) (cm^{-1}) 3416, 3261, 2930, 1663, 1514, 1242. ^{1}H-NMR ((CD_3)$_2$SO, 300 MHz) δ 10.32 (1H, brs, NH), 8.66 (1H, s, NH), 7.49 (1H, s, 5-indolyl), 7.30 (2H, m, 8, 2-indolyl), 7.08 (1H, d, *J*=8.2 Hz, 7-indolyl), 6.64 (2H, d, *J*=8.5 Hz, 2, 6-aryl), 6.52 (2H, d, *J*=8.7 Hz, 3, 5-aryl), 6.34 (1H, s, 3-indolyl), 5.68 (1H, s, NH), 4.22 (2H, s, C*H*$_2$NH), 3.76 (2H, t, *J*=6.2 Hz, OC*H*$_2$CH$_2$), 1.92 (2H, t, *J*=7.0 Hz, CH$_2$CONH), 1.62-1.24 (8H, m, OCH$_2$C*H*$_2$, C*H*$_2$CH$_2$COO, OCH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$COO); ^{13}C-NMR ((CD_3)$_2$SO, 75 MHz) δ 169.08 (COO), 149.82 (1-aryl), 143.12 (4-aryl), 134.94 (9-indolyl), 130.43 (6-indolyl), 127.55 (4-indolyl), 125.30 (2-indolyl), 120.91 (7-indolyl), 118.47 (5-indolyl), 115.26 (2, 6-aryl), 113.24 (3, 5-aryl), 111.07 (8-indolyl), 100.76 (3-indolyl), 67.77 (OCH$_2$CH$_2$), 47.89 (CH$_2$NH), 32.16 (CH$_2$CON), 28.75 (OCH$_2$CH$_2$), 28..31 (CH$_2$CH$_2$CH$_2$CON), 25..25 (OCH$_2$CH$_2$CH$_2$), 25.03 (CH$_2$CH$_2$CON). Anal. Calcd for $C_{22}H_{27}N_3O_3 + \frac{1}{4}H_2O$ C, 68.46; H, 7.18; N, 10.89. Found: C, 68.48; H, 7.03; N, 10.62.

N-Hydroxy-7-(4-((3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylamino)phenoxy)heptanamide (88)
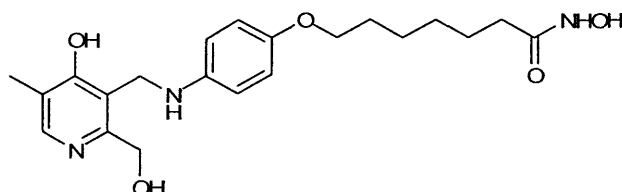
To a solution of ethyl 7-(4-((3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylamino)phenoxy)heptanoate (**69**) (300 mg, 0.72 mmol) in tetrahydrofuran (8 mL) was added hydroxylamine (0.40 mL of a 50 % aqueous solution, 6.12 mmol) and 1M KOH in methanol (1.22 mL) at 0 °C. The solution was stirred for 3 h, then water (8 mL) followed by acidification with 1M HCl. The suspension was extracted with a 2:1 solution of chloroform-ethanol (2 x 10 mL) and the combined organics dried (MgSO₄). The solvent was removed *in vacuo* and the residue dried under high vacuum. The crude product was purified by column chromatography (5 % water-acetonitrile) followed by recrystallisation (acetonitrile-water) to yield the title compound (171 mg, 59 %) as white crystals, mp 159-161 °C; IR (KBr) (cm^{-1}) 3252, 2943, 1670, 1514, 1244. ^{1}H-NMR ((CD₃)₂SO, 300 MHz) δ 7.88 (1H, s, 6-pyridyl), 6.73 (2H, d, *J*= 9.1 Hz, 2, 6-aryl), 6.66 (2H, d, *J*=9.1 Hz, 3, 5-aryl), 4.51 (2H, s, C*H₂*OH), 4.28 (2H, s, C*H₂*NH), 3.81 (2H, t, *J*=6.4 Hz, OC*H₂*CH₃), 2.31 (3H, s, CH3), 1.93 (2H, t, *J*=7.3 Hz, C*H₂*CONHOH), 1.61 (2H, m, OCH₂C*H₂*), 1.33 (6H, m, C*H₂*CH₂CONH, OCH₂CH₂C*H₂*, C*H₂*CH₂CH₂CONH); ^{13}C-NMR ((CD₃)₂SO, 75 MHz) δ 169.21 (CONH), 151.61 (3-pyridyl), 145.56 (1-aryl), 141.89 (2-pyridyl), 139.03 (Ar), 129.98 (Ar), 115.21 (Ar), 67.69 (OC*H₂*CH₂), 58.90 (CH₂OH), 41.34 (CH₂NH), 32.12 (C*H₂*CONH), 28.61 (OCH₂C*H₂*), 28.24 (C*H₂*CH₂CH₂CONH), 25.18 (OCH₂CH₂C*H₂*), 24.99 (C*H₂*CH₂CONH), 19.14 (Me). Anal. Calcd for C₂₁H₂₉N₃O₅ C, 62.51; H, 7.24; N, 10.41. Found: C, 62.50; H, 7.44; N, 10.42.

7-(1*H*-Indol-5-yloxy)-*N*-hydroxyheptanamide (89)
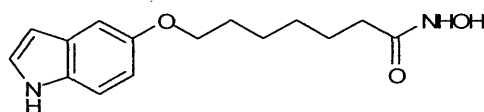
To a solution of ethyl 7-(1*H*-indol-5-yloxy)heptanoate (**71**) (328 mg, 1.13 mmol) in tetrahydrofuran (3 mL) at 0 °C were added a 1M solution of hydroxylamine in methanol

(11.3 mL) and a 1M solution of KOH in methanol (2.3 mL) dropwise. The solution was stirred for 3 d at room temperature then water (10 mL) added. The aqueous solution was extracted with ethyl acetate (5 x 15 mL) and the combined organics washed with brine (20 mL) and dried (MgSO$_4$). The solvent was removed *in vacuo* to leave a viscous, purple oil which was purified by column chromatography (20 % ethyl acetate-petroleum ether (40 - 60) increasing to 100 % ethyl acetate) to give the product (206 mg, 66 %) as an off-white crystalline solid, mp 119-122 °C; IR (KBr) (cm^{-1}) 3400, 3184, 2939, 1666, 1585, 1225, 1155. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.63 (2H, m, 2 NH), 9.02 (1H, brs, OH), 7.24 (2H, m, 8, 2-indolyl), 6.96 (1H, s, 5-indolyl), 6.67 (1H, m, 7-indolyl), 6.30 (1H, s, 3-indolyl), 3.82 (2H, t, *J*=6.3 Hz, OC*H*$_2$CH$_2$), 1.95 (2H, t, *J*=6.9 Hz, CH$_2$CONH), 1.60 (2H, m, OCH$_2$C*H*$_2$), 1.46 (2H, m, C*H*$_2$CH$_2$CONH), 1.26 (4H, m, OCH$_2$CH$_2$C*H*$_2$, C*H*$_2$CH$_2$CH$_2$CONH); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 170.31 (COO), 152.31 (6-indolyl), 130.88 (4-indolyl), 127.88 (9-indolyl), 125.62 (2-indolyl), 111.93 (8-indolyl), 111.59 (7-indolyl), 102.83 (3-indolyl), 100.90 (5-indolyl), 67.89 (OCH$_2$CH$_2$), 32.06 (*C*H$_2$CONH), 28.53 (OCH$_2$*C*H$_2$), 28.06 (*C*H$_2$CH$_2$CH$_2$CONH), 25.11 (OCH$_2$CH$_2$*C*H$_2$), 24.91 (*C*H$_2$CH$_2$CONH). Anal.Calcd for C$_{15}$H$_{20}$N$_2$O$_3$ C, 65.20; H, 7.30; N, 10.14. Found: C, 65.30; H, 7.37; N, 9.97.

6.2 Cyclic-Linker Non-Hydroxamates

Methyl 4-(guanidinomethyl)benzoate (106)
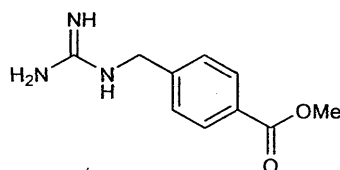
To a suspension of methyl-4-(aminomethyl)benzoate hydrochloride (5.10 g, 25.29 mmol) in dimethylformamide (125 mL) was added 1*H*-pyrazole-1-carboxamidine hydrochloride (4.08 g, 27.28 mmol) and diispopropylethylamine (3.92 g, 30.35 mmol) in dimethylformamide (15 mL). The white suspension was stirred at room temperature for 5 h to give a clear solution. The solvent was removed *in vacuo* and the remaining slurry treated with 5 % aqueous sodium hydrogen carbonate solution (15 mL) to give a white suspension. The white

precipitate was collected by filtration and washed with water and diethyl ether to give the product (3.82 g, 73 %) as a fine white powder, mp 158-160 °C; IR (cm⁻¹) 3410, 2951, 1684, 1274, 1109, 745. ^{1}H-NMR ((CD₃)₂SO, 300 MHz) δ 8.32 (4H, brs, NH, NH, NH₂), 7.88 (2H, d, J=8.0 Hz, 2, 6-aryl), 7.37 (2H, d, J=8.0 Hz, 3, 5-aryl), 4.32 (2H, s, CH₂), 3.83 (3H, s, CH₃); ^{13}C-NMR ((CD₃)₂SO, 75 MHz) δ 165.96 (CO), 157.69 (C(NH)NH₂), 143.95 (4-aryl), 129.18 (2, 6-aryl), 128.29 (1-aryl), 127.02 (3, 5-aryl), 51.98 (CH₃), 43.26 (CH₂). LRMS (CI) m/z 208 (M+H, 100 %), 194 (37 %), 166 (79 %), 151 (85 %).

(E)-3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (110)
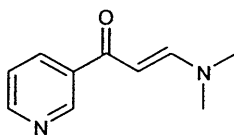
3-Acetylpyridine (1 g, 8.25 mmol) was added to dimethylformamide dimethylacetal (2.89 g, 16.50 mmol) and the solution refluxed for 5 h. The reaction was then evaporated to dryness and the brown solid triturated with diethyl ether and filtered to give the product (0.95 g, 65 %) as a yellow solid; IR (cm⁻¹) 3055, 1637, 1575, 1065, 902. ^{1}H-NMR (CDCl₃, 300 MHz) δ 8.98 (1H, d, J=1.9 Hz, 2-pyridyl), 8.55 (1H, dd, J=1.3, 4.7 Hz, 6-pyridyl), 8.07 (1H, td, J=1.5, 7.8 Hz, 4-pyridyl), 7.73 (1H, d, J=12.3 Hz, CHNMe₂), 7.24 (1H, dd, J=4.8, 7.9 Hz, 5-pyridyl), 5.57 (1H, d, J=12.3 Hz, COCH), 3.06 (3H, s, CH₃), 2.83 (3H, s, CH₃). ^{13}C-NMR (CDCl₃, 75 MHz) δ 186.22 (CO), 154.60 (6-pyridyl), 151.39 (2-pyridyl), 148.87 (CHN), 135.58 (3-pyridiyl), 134.93 (4-pyridyl), 123.20 (5-pyridyl), 91.74 (COCH), 45.15 (CH₃), 37.32 (CH₃). LRMS (CI) m/z 177 (M+H, 100 %), 159 (4 %), 134 (8 %), 98 (6 %).

Methyl 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoate (107)
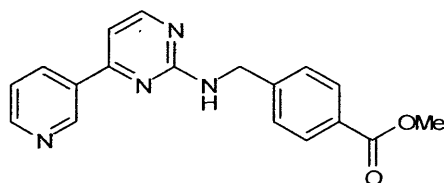
A suspension of (E)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (110) (750 mg, 3.62 mmol) and methyl 4-(guanidinomethyl)benzoate (106) (532 mg, 3.02 mmol) in propan-2-ol (9 mL) was refluxed for 20 h. The orange solution was evaporated to dryness and the

yellow residue triturated with cold ethyl acetate then filtered to give the product (578 mg, 60 %) as a tan solid, mp 151-153 °C; IR (cm⁻¹) 3238, 1713, 1587, 1549, 1273, 1105, 707. ¹H-NMR ((CD₃)₂SO, 300 MHz) δ 9.18 (1H, d, J=1.4 Hz, 2-pyridyl), 8.66 (1H, dd, J=1.5, 4.8 Hz, 6-pyridyl), 8.33 (1H, d, J=4.9 Hz, 4-pyrimidine), 8.24 (1H, d, J=8.0 Hz, 4-pyridyl), 7.99 (2H, d, J=8.3 Hz, 2, 6-aryl), 7.44 (2H, d, J=8.1 Hz, 3, 5-aryl), 7.36 (1H, dd, J=4.8. 7.0 Hz, 5-pyridyl), 7.00 (1H, d, J=5.2 Hz, 5-pyrimidine), 6.05 (1H, brs, NH), 4.77 (2H, d, J=6.1 Hz, CH₂), 3.88 (3H, s, CH₃). ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 166.91 (COOMe), 162.51 (pyrimidine), 159.05 (4-pyrimidine), 151.32 (2-pyridyl), 148.50 (6-pyridyl), 144.73 (4-aryl), 134.36 (4-pyridyl), 132.85 (3-pyridyl), 129.90 (2, 6-aryl), 129.06 (1-aryl), 127.01 (3, 5-aryl), 123.53 (4-pyridyl), 106.90 (5-pyrimidine), 52.57 (CH₃), 45.21 (CH₂). LRMS (CI) m/z 321 (M+H, 100 %), 201 (19 %), 177 (47 %), 151 (13 %).

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (108)
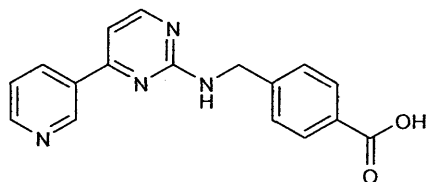
A suspension of methyl 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoate (107) (140 mg, 0.44 mmol) and lithium hydroxide monohydrate (22 mg, 0.53 mmol) in tetrahydrofuran (0.9 mL), water (0.45 mL) and methanol (0.9 mL) was stirred at room temperature for 1 d. The white suspension was acidified to pH 3 by addition of 2 M HCl to give a pale yellow solution. The solvent was removed *in vacuo* and the pale yellow solid suspended in cold water (3 mL) then filtered and washed with cold water to give the product (126 mg, 93 %) as pale yellow solid; IR (cm⁻¹) 3352, 3151, 3029, 1612, 1450, 1184, 796. ¹H-NMR ((CD₃)₂SO, 300 MHz) δ 9.28 (1H, s, 2-pyridyl), 8.77 (1H, d, J=4.1 Hz, 6-pyridyl), 8.60 (1H, J=7.9 Hz, 4-pyridyl), 8.44 (1H, d, J=5.0 Hz, 4-pyrimidyl), 8.11 (1H, brs, NH), 7.88 (2H, d, J=8.2 Hz, 2, 6-aryl), 7.70 (1H, dd, J=5.1, 7.6 Hz, 5-pyridyl), 7.47 (2H, d, J=6.9 Hz, 3, 5-aryl), 7.32 (1H, d, J=5.2 Hz, 5-pyrimidyl), 4.65 (2H, s, CH₂); ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 167.14 (CO), 161.49 (2-pyrimidyl), 160.54 (6-pyrimidyl), 159.05 (4-pyrimidyl), 148.55 (2-pyridyl), 145.47 (6-pyridyl, 4-aryl), 137.08 (4-pyridyl), 133.28 (3-pyridyl), 129.29 (2, 6-aryl), 129.09 (1-aryl), 127.07 (3, 5-aryl), 124.91 (5-pyridyl), 106.27 (5-

pyrimidyl), 43.96 (CH$_2$). LRMS (CI) m/z 307 (M+H, 46 %), 263 (8 %), 173 (19 %), 82 (100 %).

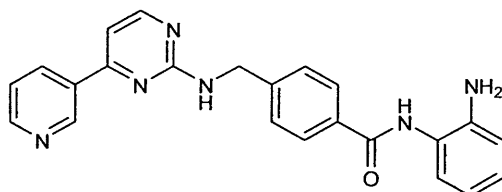### N-(2-Aminophenyl)-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzamides (MGCD0103)

A solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (**108**) (300 mg, 0.98 mmol), (benzotriazol-1-yloxy)tris(dimthylamino)phosphonium hexafluorophosphate (498 mg, 1.13 mmol), 1,2-phenylenediamine (212 mg, 1.96 mmol) and triethylamine (198 mg, 1.96 mmol) in dimethylformamide (10 mL) was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the black residue purified by column chromatography (ethyl acetate, increasing to 5 % methanol-ethyl acetate) to give the product (256 mg, 66 %) as a tan solid, mp 183-185 °C; IR (cm^{-1}) 3410, 3246, 1621, 1586, 795. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.59 (1H, s, CONH), 9.24 (1H, s, 2-pyridyl), 8.67 (1H, d, J=3.7 Hz, 6-pyridyl), 8.40 (2H, m, 4-pyrimidyl, 4-pyridyl), 8.01 (1H, t, J=6.2 Hz, NH), 7.92 (2H, d, J=8.1 Hz, 2, 6-aryl), 7.48 (3H, m, 3, 5-aryl, 5-pyridyl), 7.25 (1H, d, J=5.1 Hz, 5-pyrimidyl), 7.14 (1H, d, J=7.6 Hz, 6-aniliyl), 6.95 (1H, t, J=7.0 Hz, 4-anilyl), 6.76 (1H, d, J=7.0 Hz, 3-anilyl), 6.57 (1H, t, J=7.1 Hz, 5-anilyl), 4.87 (2H, s, NH$_2$), 4.64 (2, d, J=5.9 Hz, CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 165.20 (CO), 162.54 (2-pyrimidyl), 159.33 (4, 6-pyrimidyl), 151.19 (6-pyridyl), 147.95 (2-pyridyl), 144.11 (4-aryl), 143.02 (2-anilyl), 134.13 (4-pyridyl), 132.89 (1-aryl), 132.31 (3-pyridyl), 127.71 (2, 6-aryl), 126.84 (3, 5-aryl), 126.59 (4-anilyl), 126.39 (1-anilyl), 123.74 (5-pyridyl), 123.34 (6-anilyl), 116.26 (5-anilyl), 116.10 (3-anilyl), 106.11 (4-pyrimidyl), 43.93 (CH$_2$). LRMS (CI) m/z 419 (M+Na, 31 %), 397 (10 %), 329 (10 %), 289 (19 %), 176 (100 %). Anal.Calcd for C$_{23}$H$_{20}$N$_6$O C, 69.68; H, 5.08; N, 21.20. Found: C, 69.40; H, 5.10; N, 20.97.

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)-*N*-(1*H*-1,2,4-triazol-3-yl)benzamides (90)
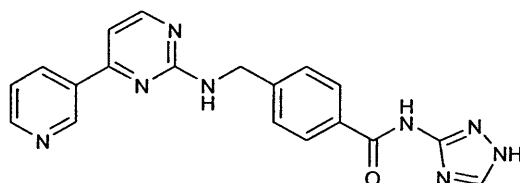
A solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (108) (300 mg, 0.98 mmol), 1-hydroxybenzotriazole (159 mg, 1.18 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (226 mg, 1.18 mmol), 3-amino-1,2,4-triazole (82 mg, 0.98 mmol) and *N*-methylmorpholine (218 mg, 2.16 mmol) in dimethylformamide (10 mL) was stirred at room temperature for 18 h. Water (20 mL) was added to the pale yellow solution and the white precipitate collected by filtration. The product was washed with water and methanol to give the title compound (181 mg, 50 %) as a white solid, mp 186 °C; IR (cm^{-1}) 3396, 3312, 3134, 1703, 1579, 931. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.22 (1H, brs, 2-pyridyl), 8.67 (1H, brs, 6-pyridyl), 8.40 (2H, m, 4-pyrimidyl, 4-pyridyl), 8.01 (3H, m, 2, 6-aryl, NH), 7.68 (2H, s, NH, NH), 7.60 (1H, s, triazolyl), 7.50 (3H, m, 3, 5-aryl, 5-pyridyl), 7.26 (1H, d, *J*=7.1 Hz, 5-pyrimidyl), 4.66 (2H, d, *J*=5.4 Hz, CH$_2$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 167.54 (CO), 162.30 (2-pyrimidyl), 159.40 (4, 6-pyrimidyl), 158.24 (3, 5-triazolyl), 151.21 (6-pyridyl), 147.93 (2-pyridyl), 146.08 (4-aryl), 134.13 (4-pyridyl), 132.38 (3-pyridyl), 130.78 (1-aryl), 130.10 (2, 6-aryl), 126.45 (3, 5-aryl), 123.73 (5-pyridyl), 106.20 (4-pyrimidyl), 43.96 (CH$_2$). Anal.Calcd for C$_{19}$H$_{16}$N$_8$O + ½H$_2$O C, 59.83; H, 4.49; N, 29.30. Found: C, 59.44; H, 4.16; N, 29.48.

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)-*N*-(1*H*-tetrazol-5-yl)benzamides (91)
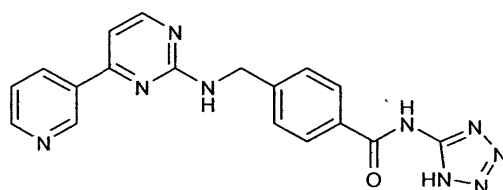
A solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (108) (500 mg, 1.63 mmol), 1-hydroxybenzotriazole (330 mg, 2.45 mmol), 1-(3-dimethylaminopropyl)-3-

ethyl carbodiimide (345 mg, 1.80 mmol), 5-aminotetrazole monohydrate (168 mg, 1.63 mmol) and N-methylmorpholine (363 mg, 3.59 mmol) in dimethylformamide (16 mL) was stirred at room temperature for 18 h. Water (15 mL) was added and the white precipitate collected by filtration. The crude product was washed with ethyl acetate then recrystallised from dimethylformamide-water. The white solid was triturated with diethyl ether to give the title compound (371 mg, 61 %) as a white solid, mp 270 °C dec.; IR (cm^{-1}) 3377, 2844, 1672, 1586, 1023. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 12.34 (1H, brs, NH), 9.22 (1H, s,), 8.67 (1H, d, J=3.2 Hz,), 8.40 (2H, m,), 8.02 (3H, m,), 7.53 (3H, m,), 7.26 (1H, d, J=5.2 Hz,), 4.66 (2H, d, J=5.7 Hz, CH$_2$). Anal.Calcd for C$_{18}$H$_{15}$N$_9$O + ½H$_2$O C, 57.14; H, 4.28; N, 32.42. Found: C, 57.04; H, 3.99; N, 32.63.

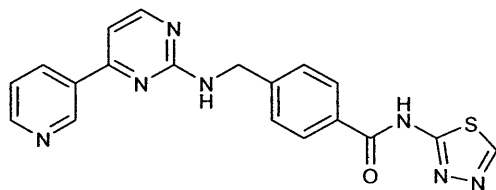## 4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)-N-(1,3,4-thiadiazol-2-yl)benzamides (92)

A solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (108) (500 mg, 1.63 mmol), 1-hydroxybenzotriazole (330 mg, 2.45 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (345 mg, 1.80 mmol), 2-amino-1,3,4-thiadiazole (165 mg, 1.63 mmol) and N-methylmorpholine (363 mg, 3.59 mmol) in dimethylformamide (16 mL) was stirred at room temperature for 18 h. Water (20 mL) was added and the solution cooled to 5 °C. The white precipitate was collected by filtration and recrystallised from dimethylformamide-water. The white solid was washed with methanol to give the title compound (391 mg, 62 %) as a white solid, mp 258-9 °C; IR (cm^{-1}) 3240, 3047, 1663, 1527, 1296. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 13.01 (1H, brs, CONH), 9.21 (2H, m, 4-pyridyl, 2-thiadiazolyl), 8.67 (1H, m, 6-pyridyl), 8.40 (2H, m, 4-pyrimidyl, 4-pyridyl), 8.04 (3H, m, 2, 6-aryl, NH), 7.52 (3H, m, 3, 5-aryl, 5-pyridyl), 7.26 (1H, d, J=5.1 Hz, 5-pyrimidyl), 4.66 (2H, d, J=5.5 Hz, CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 165.02 (CO), 162.03 (2-pyrimidyl), 159.46 (4, 6-pyrimidyl), 151.21 (6-pyridyl), 148.88 (2, 5-thiadiazolyl), 147.97 (2-pyridyl), 145.97 (4-aryl), 134.12 (4-pyridyl), 132.27 (3-pyridyl), 129.83 (1-aryl), 128.38

(2, 6-aryl), 127.09 (3, 5-aryl), 123.72 (5-pyridyl), 106.21 (4-pyrimidyl), 43.93 (CH_2). Anal. Calcd for $C_{19}H_{15}N_7OS$ C, 58.60; H, 3.88; N, 25.18. Found: C, 58.45; H, 3.85; N, 25.53.

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)-*N*-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)benzamides (93)
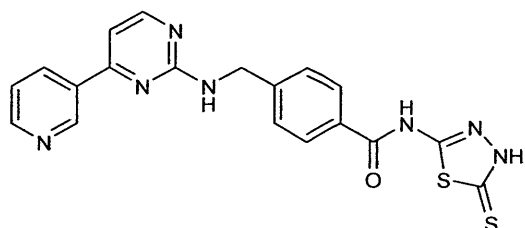
A solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (**108**) (500 mg, 1.63 mmol), 1-hydroxybenzotriazole (330 mg, 2.45 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (345 mg, 1.80 mmol), 5-amino-1,3,4-thiadiazole-2-thiol (217 mg, 1.63 mmol) and *N*-methylmorpholine (363 mg, 3.59 mmol) in dimethylformamide (16 mL) was stirred at room temperature for 18 h. Water (20 mL) was added to the pale yellow solution and the pale yellow precipitate collected by filtration. The product was washed with water, methanol and diethyl ether to give the title compound (339 g, 49 %) as a yellow solid, mp 175 °C dec.; IR (cm^{-1}) 3299, 2905, 1655, 1563, 1527, 1061. ^{1}H-NMR ((CD_3)$_2$SO, 300 MHz) δ 13.26 (1H, brs, CONH), 9.21 (1H, brs, 2-pyridyl), 8.66 (1H, d, *J*=3.1 Hz, 6-pyridyl), 8.38 (2H, m, 4-pyridyl , 4-pyrimidyl), 8.01 (3H, m, 2, 6-aryl, NH), 7.52 (3H, m, 3, 5-aryl, 5-pyridyl), 7.24 (1H, d, *J*=5.1 Hz, 4-pyrimidyl), 4.66 (2H, d, *J*=5.7 Hz, CH_2); Anal. Calcd for $C_{19}H_{15}N_7OS_2$ + ½H_2O C, 53.01; H, 3.75; N, 22.77. Found: C, 52.71; H, 3.59; N, 22.45.

(2-(1-Benzoyl-1*H*-imidazol-2-yl)-2*H*-imidazole-1,3-diyl)bis(phenylmethanone) (118)[2]
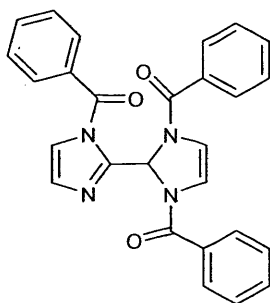
Benzoyl chloride (20.65 g, 146.89 mmol) was added slowly to a suspension of imidazole (5.0 g, 73.44 mmol) and triethylamine (14.85 g, 146.89 mmol) in acetonitrile (75 mL) at 0 °C. The ensuing white suspension was stirred for a further 2 h at room temperature then water (300 mL) and diethyl ether (75 mL) added. The mixture was shaken and the white precipitate collected by filtration and washed with diethyl ether to give the title compound (14.30 g, 43 %) as a white solid, mp 191-2 °C; IR (cm^{-1}) 3149, 1720, 1390, 1280, 1156, 867. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.75 (4H, m, Ar), 7.57 (11H, m, Ar), 7.30 (1H, brs, Ar), 7.03 (1H, s, Ar), 6.50 (2H, brs, Ar); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 167.60 (CO), 164.68 (CO), 146.83 (2-imidazolyl), 133.71 (Ar), 133.37 (Ar), 132.98 (Ar), 131.11 (Ar), 130.67 (Ar), 128.57 (3, 5-aryl), 127.86 (2, 6-aryl), 120.80 (Ar), 114.64 (3, 4-imidazolyl).LRMS (CI) m/z 449 (M+H, 11 %), 343 (29 %), 277 (100 %), 105 (99 %).

(1H-Imidazol-2-yl)methanamine dihydrochloride (119)^3
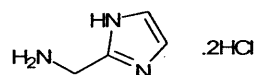
Propan-2-ol saturated with HCl (25 mL) was added to a solution of (2-(1-benzoyl-1H-imidazol-2-yl)-2H-imidazole-1,3-diyl)bis(phenylmethanone) (**118**) (10.0 g, 22.30 mmol) in methanol (100 mL) and the yellow solution refluxed for 4 h. The solvent was removed *in vacuo* and the residue triturated with cold acetone to give the title compound (1.42 g, 37 %) as an off-white solid, mp 237-8 °C (lit.^{3} 240-42 °C); IR (cm^{-1}) 3108, 2957, 2585, 1604, 1389, 1058. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.48 (2H, brs, NH$_2$), 7.71 (2H, s, Ar), 7.61 (1H, s, NH), 7.44 (1H, s, NH), 7.27 (1H, s, NH), 4.40 (2H, s, CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 139.65 (NCNH), 119.68 (C=C), 32.91 (CH$_2$). LRMS (CI) m/z 98 (M+H, 46 %), 81 (100 %), 69 (25 %).

**N-((1H-Imidazol-2-yl)methyl)-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzamides (94)**
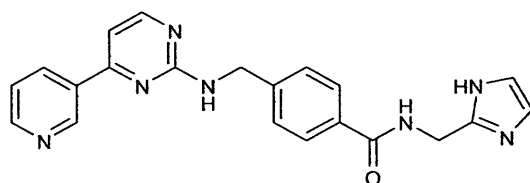
A solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (**108**) (400 mg, 1.31 mmol), 1-hydroxybenzotriazole (264 mg, 1.96 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (276 mg, 1.44 mmol), (1*H*-imidazol-2-yl)methanamine dihydrochloride (444 mg, 2.61 mmol) and *N*-methylmorpholine (726 mg, 7.18 mmol) in dimethylformamide (13 mL) was stirred at room temperature for 18 h. The solvent was removed *in vacuo* and replaced with water (10 mL). Saturated aqueous sodium hydrogen carbonate solution (5 mL) was added and the white precipitate collected by filtration. The crude product was recrystallised from ethanol to give the title compound (180 mg, 36 %) as a white crystalline solid, mp 236-9 °C dec.; IR (cm^{-1}) 3253, 3054, 1636, 1563, 1403, 1108. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 11.77 (1H, s, NH imidazolyl), 9.23 (1H, s, 4-pyrimidyl), 8.90 (1H, t, *J*=5.3 Hz, NH), 8.67 (1H, d, *J*=3.7 Hz, 2-pyridyl), 8.39 (2H, m, 4, 6-pyridyl), 7.98 (1H, t, *J*=6.0 Hz, NH), 7.85 (2H, d, *J*=8.1 Hz, 2, 6-aryl), 7.48 (3H, m, 3, 5-aryl, 5-pyridyl), 7.24 (1H, d, *J*=5.1 Hz, 5-pyrimidyl), 6.98 (1H, s, 4-imidazolyl), 6.80 (1H, s, 5-imidazolyl), 4.63 (2H, d, *J*=5.8 Hz, CH$_2$-Ph), 4.47 (2H, d, *J*=5.5 Hz, CONHC*H$_2$*); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 166.12 (CO), 162.33 (2-pyrimidyl), 159.41 (6-pyridyl), 151.20 (2-pyridyl), 147.96 (4-pyrimidyl), 144.97 (Ar), 143.92 (Ar), 134. 10 (4-pyridyl), 127.33 (2, 6-aryl), 126.76 (3, 5-aryl), 123.71 (5-pyridyl), 116.19 (Ar), 106.08 (5-pyrimidyl), 43.89 (CH$_2$-Ph), 37.01 (CONHCH$_2$). LRMS (CI) *m/z* 386 (M+H, 100 %). Anal. Calcd for C$_{21}$H$_{19}$N$_7$O C, 65.44; H, 4.97; N, 25.44. Found: C, 65.17; H, 4.97; N, 25.31.

2-(Tritylthio)benzenamine (112)[4]
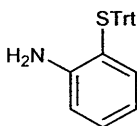
To a solution of triphenylmethanol (2.08 g, 7.99 mmol) in TFA (14 mL) was added a solution of 2-aminothiophenol (1.0 g, 7.99 mmol) in dichloromethane (1.5 mL) and the reaction stirred for 3 h. The solvent was removed in vacuo and the residue stirred in saturated aqueous sodium hydrogen carbonate solution for 2 h to give an off-white, powdery suspension. The solid was collect by filtration an recrystallised from a large volume of methanol to give the title compound (2.50 g, 85 %) as a pale yellow crystalline solid, mp 143-144 °C; IR (cm^{-1}) 3463, 3369, 3055, 1605, 1478, 1444, 743. ^{1}H-NMR (CDCl$_3$, 300

MHz) δ 7.44 (5H, m, Ar), 7.27 (10H, m, Ar), 7.06 (2H, m, Ar), 6.47 (2H, m, Ar), 3.58 (2H, brs, NH$_2$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 151.42 (2-thiophenyl), 144.54 (1-aryl), 137.91 (6-thiophenyl), 130.81 (4-thiophenyl), 130.02 (3, 5-aryl), 127.67 (2, 6-aryl), 126.80 (4-aryl), 117.98 (5-thiophenyl), 116.29 (1-thiophenyl), 115.19 (3-thiophenyl), 70.93 (C(Ph)$_3$). LRMS (CI) m/z 390 (M+Na, 4 %), 329 (34 %), 286 (32 %), 243 (75 %), 176 (100 %).

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)-N-(2-(tritylthio)phenyl)benzamides (124)
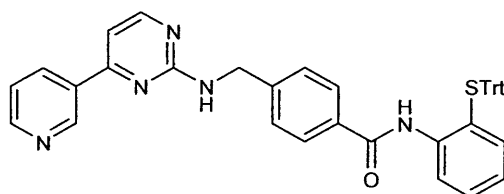
To a suspension of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzoic acid (108) (300 mg, 0.98 mmol) in dichloromethane (10 mL) was added oxalyl chloride (497 mg, 3.92 mmol) and dry dimethylformamide (3 drops) and the resultant suspension stirred for 3 h at room temperature. The solvent was evaporated and chloroform (10 mL) added, followed by N,N-dimethylaminopyridine (132 mg, 1.08 mmol) and 2-(tritylthio)benzenamine (360 mg, 0.98 mmol). The solution was stirred for 2 d at room temperature then the solvent removed in vacuo. The crude product was purified by column chromatography (ethyl acetate, increasing to 10 % methanol-ethyl acetate) to give the title compound (167 mg, 26 %) as a yellow oil; IR (cm^{-1}) 3343, 3054, 1674, 1575, 1501, 1430, 1300, 733. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 9.23 (1H, d, J=1.3 Hz, 2-pyridyl), 8.82 (1H, s, NH), 8.63 (1H, dd, J=1.2, 4.7 Hz, 6-pyridyl), 8.46 (1H, dd, J=1.2, 8.3 Hz, 3-thiophenyl), 8.35 (1H, d, J=5.1 Hz, 4-pyrimidyl), 8.28 (1H, td, J=1.9, 8.0 Hz, 4-pyridyl), 7.54 (2H, d, J=8.3 Hz, 2, 6-aryl), 7.42 (2H, d, J=8.3 Hz, 3, 5-aryl), 7.35 (2H, m, 4, 6-thiophenyl), 7.27 (7H, m, 3, 5-trityl, 5-pyridyl), 7.14 (9H, m, 2, 6-trityl, 4-trityl), 7.02 (1H, d, J=5.2 Hz, 5-pyrimidyl), 6.81 (1H, dt, J=1.3, 7.6 Hz, 5-thiophenyl), 6.10 (1H, brs, NH), 4.79 (2H, d, J=6.1 Hz, CH$_2$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 164.28 (CO), 162.55 (2-pyrimidyl), 159.07 (4-pyrimidyl), 151.36 (6-pyridyl), 148.52 (2-pyridyl), 1433.67 (6-pyrimidyl), 143.29 (1-trityl, 4-aryl), 142.42 (2-thiophenyl), 137.54 (6-thiophenyl), 134.39 (4-pyridyl), 133.81 (3-pyridyl), 132.87 (1-aryl), 131.29 (5-pyridyl), 129.64 (3, 5-trityl), 127.83 (2, 6-trityl), 127.47 (2, 6-aryl), 127.36 (3, 5-aryl), 127.20 (4-

trityl), 123.57 (4-thiophenyl), 123.30 (5-thiophenyl), 120.68 (1-thiophenyl), 119.57 (3-thiophenyl), 106.92 (5-pyrimidyl), 71.80 (C(Ph)$_3$), 45.17 (CH$_2$). LRMS (CI), m/z 678 (M+Na, 77 %), 656 (M+H, 42 %), 299 (35 %), 243 (100 %). HRMS (M+H) calcd for C$_{42}$H$_{33}$N$_5$OS 656.24786. Found: 656.24612.

N-(2-Mercaptophenyl)-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzamides (95)
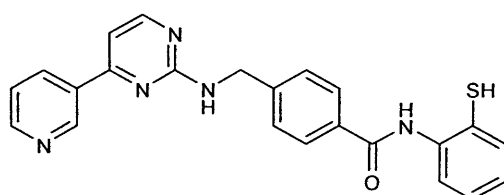
Trifluoroacetic acid (0.5 mL) was added to a solution of 4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)-N-(2-(tritylthio)phenyl)benzamide (124) (160 mg, 0.24 mmol) in dichloromethane (0.25 mL), developing a bright red colour. Triethylsilane (112 mg, 0.96 mmol) was then added to the reaction and stirring continued for 10 min at room temperature. The solvent was evaporated and the residue subjected to column chromatography (5 % methanol-ethyl acetate). A white precipitate formed in the eluted solvent which would not re-dissolve. The white solid was filtered and washed with methanol and dichloromethane to give the pure title compound (44 mg, 44 %) as a crystalline white solid, mp 206-209 °C dec.; ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.23 (1H, s, 2-pyridyl), 8.67 (1H, d, J=4.2 Hz, 6-pyridyl), 8.40 (2H, m, 4-pyridyl, 4-pyrimidyl), 8.12 (1H, d, J=7.8 Hz, 3-thiophenyl), 8.04 (4H, m, 2, 6-aryl, 6-thiophenyl, NH), 7.51 (5H, m, 3, 5-aryl, 4, 6, 5-thiophenyl), 7.26 (1H, d, J=5.1 Hz, 5-pyrimidyl), 4.67 (2H, d, J=5.3 Hz, CH$_2$). Anal.Calcd for C$_{23}$H$_{19}$N$_5$OS C, 66.81; H, 4.63; N, 16.94. Found: C, 66.81; H, 4.43; N, 16.98.

(1E,2E)-N1,N2-Dihydroxyoxalimidamide (121)[5]
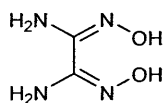
To a cooled solution of sodium hydroxide (25.3 g, 633.2 mmol) in water (72 mL) was added hydroxylamine hydrochloride (40.0 g, 575.6 mmol) portionwise and the solution stirred for

10 min at 0 °C. Glyoxal (20.9 mL of a 40 % aqueous solution, 143.9 mmol) was added and stirring continued at 0 °C for another 10 min before heating to 95 °C for 5 h. The volume of the red/brown solution was reduced by approximately 1/3 *in vacuo* and cooled to 5 °C over night. The product (3.26 g, 19 %) was collected by filtration as brown needles, mp 203-204 °C (lit.[5] 203 °C); IR (cm^{-1}) 3420, 3333, 3208, 2828, 1639, 1576, 1431, 933. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.76 (2H, brs, OH), 5.17 (4H, brs, NH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 145.26 (CN). LRMS (CI) *m/z* 199 (M+H, 100 %), 86 (27 %), 59 (24 %).

[1,2,5]Thiadiazolo[3,4-*c*][1,2,5]thiadiazole (122)[6]
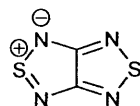
(1E,2E)-*N*1,*N*2-Dihydroxyoxalimidamide (121) (2.50 g, 21.17 mmol) in dry DMF (30 mL) was added dropwise to a stirred solution of SCl$_2$ (13.08 g, 127.02 mmol) in dry DMF (50 mL) at 0 °C. After the mixture had been heated to 55 °C for 14 h, it was cooled to 5 °C and poured onto ice (80 g) to give a yellow suspension. The sludgy, aqueous suspension was extracted with diethyl ether (10 x 30 mL) and the combined pale yellow extracts washed with water (3 x 25 mL) and brine (25 mL). The organic phase was dried over Na$_2$SO$_4$ and evaporated to leave a yellow solid which was recrystallised from methanol to give pale yellow platelets (1.05 g, 35 %), mp 113 °C (lit.[6] 115-116 °C); IR (cm^{-1}) 916, 806, 686. ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 169.35 (CN). LRMS (EI) *m/z* 144 (M$^+$, 100 %), 128 (48 %), 96 (36 %), 78 (35 %).

1,2,5-Thiadiazole-3,4-diamine (123)[6]
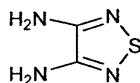
A suspension of [1,2,5]thiadiazolo[3,4-*c*][1,2,5]thiadiazole (122) (800 mg, 5.55 mmol) in 3 M NH$_4$OH (16 mL) was heated to 100 °C for 10 min to give a pale yellow solution. The solution was cooled to room temperature and approximately half the solvent removed *in vacuo*, the remaining suspension was heated to dissolve the precipitate and allowed to recrystallise to give the product (477 mg, 74 %) as pale yellow prisms, mp 178-179 °C (lit.

181-182 °C); IR (cm⁻¹) 3277, 3134, 2807, 1676, 1614, 1027. ¹H-NMR ((CD₃)₂SO, 300 MHz) δ 6.28 (4H, s, NH₂); ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 148.97. LRMS (CI) *m/z* 117 (M+H, 100 %), 90 (6 %), 74 (15 %).

N-(4-Nitrobenzylidene)quinolin-3-amine
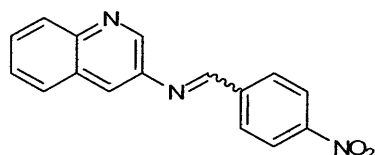
Acetic acid (99 mg, 1.65 mmol) was added to a solution of 3-aminoquinoline (2.39 g, 16.54 mmol) and 4-nitrobenzaldehyde (2.50 g, 16.54 mmol) in methanol (180 mL) and the colourless solution stirred for 18 h at room temperature. The solvent was reduced in volume and the yellow crystalline product (3.68 g, 80 %) collected by filtration, washed with cold methanol and used directly in the next step.

N-(4-Nitrobenzyl)quinolin-3-amine (127)
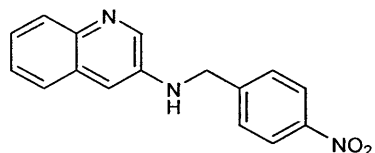
Sodium borohydride (312 mg, 8.26 mmol) was added to a yellow suspension of *N*-(4-nitrobenzylidene)quinolin-3-amine (2.29 g, 8.26 mmol) in methanol (70 mL). After stirring for 1 h at room temperature, the reaction was warmed to dissolve the orange suspension and then cooled on ice. The suspension was filtered and the solid washed with water to give the product (2.19 g, 95 %) as a yellow crystalline solid, mp 151-152 °C; IR (nujol) (cm⁻¹) 3246, 1610, 1515, 1344, 1224. ¹H-NMR ((CD₃)₂SO, 300 MHz) δ 8.58 (1H, d, *J*=2.8 Hz, 2-quinolyl), 8.19 (2H, d, *J*=8.8 Hz, 2, 6-aryl), 7.78 (1H, d, *J*=7.1 Hz, 9-quinolyl), 7.66 (2H, d, *J*=8.8 Hz, 3, 5-aryl), 7.54 (1H, d, *J*=7.4 Hz, 6-quinolyl), 7.32 (2H, m, 7, 8-quinolyl), 7.11 (1H, d, *J*=6.1 Hz, NH), 6.94 (1H, d, *J*=2.5 Hz, 4-quinolyl), 4.56 (2H, d, *J*=6.0 Hz, CH₂); ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 147.68 (4-aryl), 146.48 (1-aryl), 143.52 (2-quinolyl), 141.81 (10-quinolyl), 141.05 (3-quinolyl), 129.21 (5-quinolyl), 128.44 (9-quinolyl), 128.16 (3, 5-aryl), 126.55 (7-quinolyl), 125.71 (6-quinolyl), 124.09 (8-quinolyl), 123.51 (2, 6-aryl),

108.38 (4-quinolyl), 45.45 (CH$_2$). LRMS (CI) m/z 280 (M+H, 100 %), 234 (9 %).
Anal.Calcd for C$_{16}$H$_{15}$N$_3$ + ¼H$_2$O C, 67.71; H, 4.79; N, 14.81. Found: C, 67.88; H, 4.73; N, 14.75.

N-(4-Aminobenzyl)quinolin-3-amine (128)
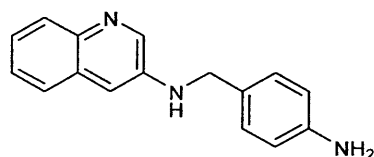
To a stirred suspension of ethyl N-(4-nitrobenzyl)quinolin-3-amine (127) (2.04 g, 7.30 mmol) in ethanol (70 mL), was added 5 % palladium on carbon (30 mg) under nitrogen. The system was evacuated and replaced with hydrogen at atmospheric pressure. After stirring for 18 h, the catalyst was removed by filtration and the solvent evaporated to yield a pale brown solid which was recrystallised from ethanol-water to give the title compound (1.08 g, 59 %) as off-white crystals, mp 150-152 °C; IR (nujol) (cm^{-1}) 3402, 3300, 3179, 1612, 1560. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 8.56 (1H, d, J=2.7 Hz, 2-quinolyl), 7.78 (1H, d, J=8.0 Hz, 9-quinolyl), 7.59 (1H, dd, J=1.3, 8.0 Hz, 6-quinolyl), 7.39-7.25 (2H, m, 7, 8-quinolyl), 7.09 (2H, d, J=8.3 Hz, 2, 6-aryl), 6.66 (1H, t, J=5.5 Hz, NH), 6.57 (2H, d, J=8.4 Hz, 3, 5-aryl), 5.00 (2H, s, NH$_2$), 4.17 (2H, d, J=5.5 Hz, CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 147.61 (1-aryl), 143.82 (2-quinolyl), 142.47 (10-quinolyl), 140.78 (3-quinolyl), 129.49 (Ar), 128.41 (3, 5-aryl, 9-quinolyl), 126.43 (7-quinolyl), 125.66 (6-quinolyl), 125.58 (Ar), 123.66 (8-quinolyl), 113.83 (2, 6-aryl), 107.80 (4-quinolyl), 46.09 (CH$_2$). LRMS (CI) m/z 250 (M+H, 68 %), 173 (27 %), 145 (100 %), 106 (67 %). Anal.Calcd for C$_{16}$H$_{15}$N$_3$ C, 77.08; H, 6.06; N, 16.85. Found: C, 77.11; H, 6.07; N, 16.65.

N-(4-((1H-Imidazol-5-yl)methylamino)benzyl)quinolin-3-amine (96)
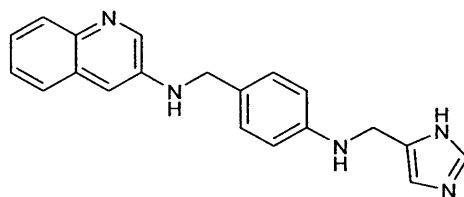
A solution of N-(4-aminobenzyl)quinolin-3-amine (128) (200 mg, 0.80 mmol), 4(5)-imidazole carboxaldehyde (77 mg, 0.80 mmol) and acetic acid (5 mg, 80 μmol) in methanol

(8 mL) was stirred for 1 h at room temperature. Sodium borohydride (30 mg, 0.80 mmol) was then added and stirring continued for 3 d, then the solvent removed in vacuo. The residue was suspended in saturated aqueous sodium hydrogen carbonate solution (15 mL) and the aqueous phase extracted with dichloromethane (3 x 15 mL). The combined organics were washed with brine then dried (Na_2SO_4) and the solvent removed *in vacuo*. The orange oil was purified by column chromatography ($CHCl_3$:acetone:ethanol, 100:40:8) to give the title compound (81 mg, 31 %) as off-white crystals, mp 190-192 °C; IR (nujol) (cm^{-1}) 3377, 1612, 1517, 1279, 1126. ^{1}H-NMR (($CD_3)_2SO$, 300 MHz) δ 11.92 (1H, brs, NH), 8.54 (1H, d, *J*=2.6 Hz, 2-quinolyl), 7.77 (1H, d, *J*=7.9 Hz, 9-quinolyl), 7.59 (2H, m, 6-quinolyl, 3-imidazolyl), 7.32 (2H, m, 7, 8-quinolyl), 7.14 (2H, d, *J*=8.3 Hz, 3, 5-aryl), 7.00 (1H, d, *J*=2.4 Hz, 5-imidazolyl), 6.93 (1H, s, NH), 6.65 (3H, d, *J*=8.4 Hz, 3, 5-aryl, 4-quinolyl), 5.76 (1H, s, NH), 4.17 (2H, d, *J*=5.4 Hz, quinoline-NHCH$_2$), 4.12 (2H, d, *J*=4.7 Hz, aniline-CH$_2$); ^{13}C-NMR (($CD_3)_2SO$, 75 MHz) δ 147.86 (1-aryl), 143.80 (2-quinolyl), 142.46 (10-quinolyl), 140.77 (3-quinolyl), 134.75 (3-imidazolyl), 129.48 (Ar), 128.42 (9-quinolyl), 128.34 (3, 5-aryl), 126.42 (7-quinolyl), 125.65 (6-quinolyl, Ar), 123.64 (8-quinolyl), 112.27 (2, 6-aryl), 107.78 (4-quinolyl), 46.05 (quinoline-NHCH$_2$), 40.32 (aniline-CH$_2$). LRMS (CI) *m/z* 252 (M+Na, 9 %), 329 (82 %), 199 (22 %), 176 (100 %), 154 (79 %). Anal. Calcd for $C_{20}H_{19}N_5$ C, 72.93; H, 5.81; N, 21.26. Found: C, 72.84; H, 5.78; N, 21.12.

(2-Phenyl-1*H*-imidazol-5-yl)methanol (130)
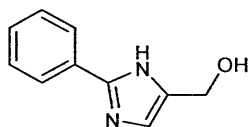
A mixture of benzamidine (767 mg, 6.39 mmol), dihydroxyacetone dimmer (1.21 g, 6.71 mmol) and ammonium chloride (1.37 g, 25.56 mmol) in ammonium hydroxide (10 mL) was heated to 80 °C for 40 min. The orange suspension was allowed to cool to room temperature then extracted with ethyl acetate (8 x 10 mL), the combined extracts dried ($MgSO_4$) and evaporated to leave an orange oil. The crude product was purified by column chromatography (5 % methanol-ethyl acetate) to give a yellow solid which was recrystallised from ethanol-diethyl ether, providing the title compound (150 mg, 13 %) as a crystalline white solid, mp 146-7 °C; IR (cm^{-1}) 3042, 2929, 2723, 2614, 1574, 1455, 1353,

1137, 1026, 988, 772. LRMS (CI) m/z 175 (M+H, 52 %), 157 (100 %), 145 (13 %), 104 (16 %). Anal.Calcd for $C_{10}H_{10}N_2O$ C, 68.95; H, 5.79; N, 16.08. Found: C, 68.94; H, 5.81; N, 16.00.

1-(4-Cyanobenzyl)guanidine (132)
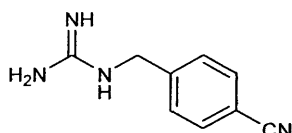
A solution of 4-(aminomethly)benzonitrile hydrochloride (2.0 g, 11.86 mmol), pyrazole-1-carboxamidine hydrochloride (1.91 g, 13.05 mmol) and diisopropylethylamine (3.37 g, 26.09 mmol) in dimethylformamide (60 mL) was stirred for 7 h at room temperature. The solvent was removed *in vacuo* and saturated aqueous sodium hydrogen carbonate solution (20 mL) added. The white suspension was filtered and washed with water and diethyl ether to give the product (1.61 g, 78 %) as a white solid; IR (cm-1) 3416, 3343, 2890, 2234, 1694, 1608, 1359, 830. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 8.67 (4H, brs, NH), 7.80 (2H, d, J=8.3 Hz, 2, 6-aryl), 7.44 (2H, d, J=8.2 Hz, 3, 5-aryl), 4.41 (CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 160.54 (CNH$_2$), 143.79 (4-aryl), 132.29 (3, 5-aryl), 127.73 (2, 6-aryl), 118.71 (CN), 109.88 (1-aryl), 43.11 (CH$_2$).

4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzonitrile (133)
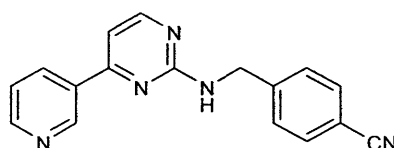
A suspension of 1-(4-cyanobenzyl)guanidine (132) (2.65 g, 15.21 mmol) and (E)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (2.68 g, 15.21 mmol) in propan-2-ol (40 mL) was refluxed for 18 h. The orange solution was evaporated to dryness and the yellow solid triturated with cold ethyl acetate to give the product (2.79 g, 64 %) as an off-white crystalline solid, mp 169-170 °C; IR (cm^{-1}) 3261, 2228, 1584, 1407, 799. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.20 (1H, s, 6-pyridyl), 8.66 (1H, d, J=3.6 Hz, 2-pyridyl), 8.39 (2H, m, 4-pyrimidyl, 4-pyridyl), 8.02 (1H, t, J=6.2 Hz, NH), 7.76 (2H, d, J=8.2 Hz, 2, 6-aryl), 7.54 (3H, m, 3, 5-aryl, 5-pyridyl), 7.25 (1H, d, J=5.1 Hz, 5-pyrimidyl), 4.64 (2H, d, J=5.5

Hz, CH₂); ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 162.24 (Ar), 159.38 (4-pyrimidyl), 151.22 (2-pyridyl), 147.95 (6-pyridyl), 146.56 (4-aryl), 134.10 (4-pyridyl), 132.22 (3-pyridyl), 132.12 (2, 6-aryl), 127.84 (3, 5-aryl), 123.71 (5-pyridyl), 118.89 (CN), 109.22 (1-aryl), 106.30 (5-pyrimidyl), 43.93 (CH₂). Anal.Calcd for C₁₇H₁₃N₅ C, 71.06; H, 4.56; N, 24.37. Found: C, 70.66; H, 4.49; N, 24.19.

N'-Hydroxy-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzimidamide (99)
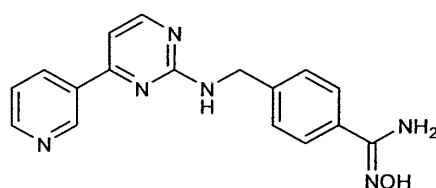
Hydroxylamine hydrochloride (726 mg, 10.45 mmol) was dissolved in a solution of DIEA (1.62 g, 12.54 mmol) in methanol (10 mL)and the colourless solution stirred for 30 min. 4-((4-(Pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzonitrile (133) (600 mg, 2.09 mmol) was added and the white suspension stirred for 3 d at room temperature. The solvent was removed *in vacuo* and replaced with water (200 mL). The white precipitate was filtered and recrystallised from 10 % methanol-ethyl acetate to give the title compound (499 mg, 75 %) as white crystals, mp 200-1 °C; IR (cm⁻¹) 3421, 3318, 1636, 1579, 1408, 933, 798. ¹H-NMR ((CD₃)₂SO, 300 MHz) δ 9.56 (1H, s, OH), 9.23 (1H, s, 6-pyridyl), 8.67 (1H, d, J=3.7 Hz, 2-pyridyl), 8.39 (2H, m, 4-pyrimidyl, 4-pyridyl), 7.92 (1H, t, J=6.1 Hz, NH), 7.60 (2H, d, J=8.2 Hz, 2, 6-aryl), 7.51 (1H, dd, J=4.9, 7.6 Hz, 5-pyridyl), 7.35 (2H, d, J=7.6 Hz, 3, 5-aryl), 7.23 (1H, d, J=5.1 Hz, 5-pyrimidyl), 5.74 (2H, s, NH₂), 4.58 (2H, d, J=5.9 Hz, CH₂); ¹³C-NMR ((CD₃)₂SO, 75 MHz) δ 162.24 (2-pyrimidyl), 159.34 (4-pyrimidyl), 151.89 (2-pyridyl), 150.68 (C(NH₂)NOH), 147.96 (6-pyridyl), 141.20 (4-aryl), 134.10 (4-pyridyl), 132.33 (3-pyridyl), 131.66 (1-aryl), 126.77 (3, 5-aryl), 125.22 (2, 6-aryl), 123.72 (5-pyridyl), 105.98 (5-pyrimidinyl), 43.83 (CH₂). LRMS (CI) m/z 343 (M+Na, 100 %), 321 (84 %), 303 (26 %), 286 (22 %), 185 (14 %), 173 (18 %). Anal.Calcd for C₁₇H₁₆N₆O C, 63.74; H, 5.03; N, 26.23. Found: C, 63.78; H, 5.06; N, 26.32.

4-Cyano-*N*-phenylbenzamide (137)
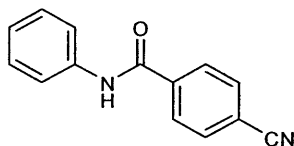
4-Cyanobenzoic acid (200 mg, 1.36 mmol) was dissolved in thionyl chloride (4 mL) and the solution heated to 90 °C for 1 h. The solution was then evaporated and the pale yellow solid dissolved in dichloromethane (15 mL) and cooled to 0 °C. Aniline (139 mg, 1.50 mmol) was added slowly to the cooled solution, followed by pyridine (119 mg, 1.50 mmol). The reaction was stirred at 0 °C for 1 h then washed with 2 M HCl (15 mL) and saturated aqueous sodium hydrogen carbonate solution (15 mL) before drying over MgSO$_4$. The solvent was evaporated to leave a white solid which was recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (254 mg, 84 %) as white crystals, mp 176-178 °C; IR (cm^{-1}) 3354, 2232, 1655, 1600, 1531, 1440, 854. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.48 (1H, s, NH), 8.10 (2H, d, *J*=8.5 Hz, Ar(*o*-CN)), 8.02 (2H, d, *J*=8.6 Hz, Ar(*m*-CN)), 7.76 (2H, d, *J*=7.7 Hz, Ar(*o*-NH)), 7.36 (2H, t, *J*=7.9 Hz, Ar(*m*-NH)), 7.12 (1H, t, *J*=7.4 Hz, Ar(*p*-NH)); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 164.08 (CO), 138.92 (Ar), 138.67 (Ar), 132.40 (Ar(*m*-CN)), 128.63 (Ar), 128.47 (Ar), 124.04 (Ar(*p*-NH)), 120.38 (Ar(*o*-NH)), 118.26 (Ar(*p*-NH)), 113.76 (CN). LRMS (CI) *m*/*z* 223 (M+H, 100 %), 130 (6 %), 104 (3 %).

4-(*N'*-Hydroxycarbamimidoyl)-*N*-phenylbenzamide (100)
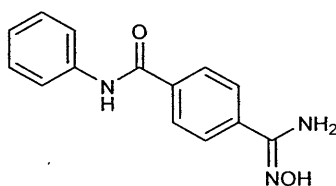
Hydroxylamine hydrochloride (312 mg, 4.50 mmol) was dissolved in a solution of methanol (4.5 mL) and DIEA (605 mg, 4.68 mmol) and the colourless solution stirred for 30 min. 4-Cyano-*N*-phenylbenzamide (137) (200 mg, 0.90 mmol) was added and stirring continued for 20 h at room temperature. The white suspension was filtered washed with a small amount of ethyl acetate to give the title compound (94 mg, 41 %), requiring no further purification, as a crystalline white solid, mp 273 °C; IR (cm^{-1}) 3452, 3359, 3270, 1648, 927.

^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.25 (1H, s, OH), 9.84 (1H, s, NH), 7.91 (2H, d, J=8.3 Hz, Ar(o-CNOH)), 7.80 (4H, m, Ar(m-CNOH), Ar(o-NH)), 7.34 (2H, t, J=7.8 Hz, Ar(m-NH)), 7.09 (1H, t, J=7.3 Hz, Ar(p-NH)), 5.94 (2H, s, NH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 164.99 (CO), 150.06 (CNOH), 138.67 (Ar(i-NH)), 136.09 (Ar(p-CNOH)), 134.92 (Ar(i-CNOH)), 128.55 (Ar(m-NH)), 127.45 (Ar(m-CNOH)), 125.11 (Ar(o-CNOH)), 123.63 (Ar(p-NH)), 120.29 (Ar(o-NH)). LRMS (CI) m/z 256 (M+H, 100 %). Anal.Calcd for C$_{14}$H$_{13}$N$_3$O$_2$ C, 65.87; H, 5.13; N, 16.46. Found: C, 65.46; H, 5.12; N, 16.25.

4-((Phenylamino)methyl)benzonitrile (140)
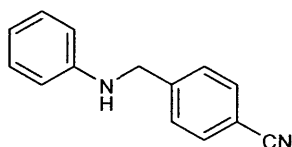
Aniline (448 mg, 4.81 mmol) was added to a solution of 4-cyanobenzaldehyde (631 mg, 4.81 mmol) in methanol (45 mL) and the pale yellow solution stirred at room temperature for 24 h. Sodium borohydride (260 mg, 6.87 mmol) was then added and the solution stirred for a further 18 h. The solvent was evaporated and replaced with ethyl acetate (20 mL) and the suspension washed with water (2 x 20 mL). The organic phase was dried over MgSO$_4$ and evaporated to leave a yellow oil which solidified on standing. The crude product was recrystallised from diethyl ether to give the title compound (557 mg, 56 %) as a crystalline pale yellow solid, mp 87-88 °C; IR (cm^{-1}) 3426, 2219, 1600, 1507, 1266. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.61 (2H, d, J=8.3 Hz, Ar(o-CN)), 7.48 (2H, d, J=8.3 Hz, Ar(m-CN)), 7.20 (2H, dd, J=7.4, 8.5 Hz, Ar(m-NH)), 6.77 (1H, t, J=7.3 Hz, Ar(p-NH)), 6.61 (2H, dd, J=0.9, 8.5 Hz, Ar(o-NH)), 4.43 (2H, s, CH$_2$), 4.30 (1H, brs, NH); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 147.54 (Ar(i-NH)), 145.59 (Ar(p-CN)), 132.47 (Ar(o-CN)), 129.43 (Ar(m-NH)), 127.78 (Ar(m-CN)), 119.04 (CN), 118.07 (Ar(p-NH)), 112.95 (Ar(o-NH)), 110.82 (Ar(i-CN)), 47.76 (CH$_2$). LRMS (CI) m/z 209 (M+H, 100 %), 118 (26 %), 94 (15 %). Anal.Calcd for C$_{14}$H$_{12}$N$_2$ + $^1/_8$H$_2$O C, 79.88; H, 5.87; N, 13.31. Found: C, 80.20; H, 5.77; N, 13.26.

N'-Hydroxy-4-((phenylamino)methyl)benzimidamide (101)
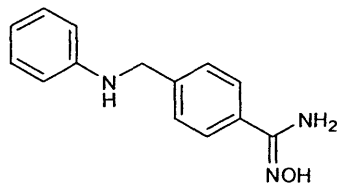
4-((Phenylamino)methyl)benzonitrile (140) (331 mg, 1.59 mmol) was dissolved in a 1M methanolic solution of hydroxylamine (7.95 mL, 7.95 mmol) and the colourless solution stirred for 3 d at room temperature. The solvent was evaporated, water (10 mL) added and the aqueous suspension extracted with ethyl acetate (3 x 10 mL). The combined extracts were dried (MgSO$_4$) and evaporated to leave a yellow oil which solidified on standing. The crude product was purified by column chromatography (65 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (177 mg, 46 %) as a white solid, mp 132-133 °C; IR (cm^{-1}) 3469, 3351, 2831, 1672, 1601, 1502, 939. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.59 (1H, s, OH), 7.62 (2H, d, J=8.3 Hz, Ar(o-CNOH)), 7.34 (2H, d, J=8.3 Hz, Ar(m-CNOH)), 7.03 (2H, m, Ar(m-NH)), 6.52 (3H, m, Ar(o-NH), Ar(p-NH)), 6.24 (1H, t, J=6.1 Hz, NH), 5.77 (2H, s, NH$_2$), 4.27 (2H, d, J=6.1 Hz, CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 150.73 (CNOH), 148.50 (Ar(i-NH)), 141.12 (Ar(p-CNOH)), 131.72 (Ar(i-CNOH)), 128.76 (Ar(m-NH)), 126.77 (Ar(m-CNOH)), 125.30 (Ar(o-CNOH)), 115.73 (Ar(p-NH)), 112.25 (Ar(o-NH)), 46.10 (CH$_2$). LRMS (CI) m/z 242 (M+H, 100 %). Anal.Calcd for C$_{14}$H$_{15}$N$_3$O + $^1/_{10}$H$_2$O C, 69.17; H, 6.30; N, 17.29. Found: C, 69.28; H, 6.27; N, 17.18.

4-Cyano-N-(quinolin-3-yl)benzamide (138)
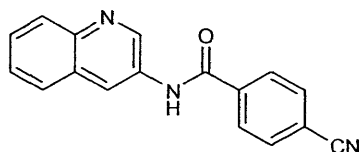
4-Cyanobenzoic acid (200 mg, 1.36 mmol) was dissolved in SOCl$_2$ (4 mL) and the solution heated to 90 °C for 1 h. The solution was then evaporated and the pale yellow solid dissolved in dichloromethane (15 mL) and cooled to 0 °C. 3-Aminoquinoline (287 mg, 1.99 mmol) was added to the solution to give a yellow precipitate. Pyridine (157 mg, 1.99 mmol) was added slowly and the reaction was stirred at 0 °C for 2 h. The solvent was evaporated

and the residue washed with 2 M HCl, water and methanol to leave the title compound (286 mg, 77 %) as a yellow solid which was used without further purification.

## 4-(*N'*-Hydroxycarbamimidoyl)-*N*-(quinolin-3-yl)benzamide (102)
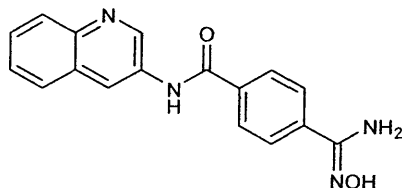
Hydroxylamine hydrochloride (381 mg, 5.49 mmol) was dissolved in a solution of methanol (5.5 mL) and DIEA (738 mg, 5.71 mmol) and the colourless solution stirred for 30 min. 4-Cyano-*N*-(quinolin-3-yl)benzamide (**138**) (300 mg, 1.10 mmol) was added and stirring continued for 18 h at room temperature. The white suspension was filtered washed with a small amount of methanol to give the title compound (210 mg, 62 %) requiring no further purification as a white solid, mp 262-263 °C dec; IR (cm^{-1}) 3404, 3300, 2820, 1640, 1530, 1490, 1369, 1277, 752. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.71 (1H, s, OH), 9.88 (1H, s, NH), 9.16 (1H, d, *J*=2.4 Hz, 2-quinolyl), 8.86 (1H, d, *J*=2.1 Hz, 4-quinolyl), 8.05 (2H, d, *J*=8.4 Hz, 2, 6-aryl), 7.97 (2H, t, *J*=7.3 Hz, 5, 8-quinolyl), 7.88 (2H, d, *J*=8.4 Hz, 3, 5-aryl), 7.66 (1H, m, 7-quinolyl), 7.58 (1H, m, 6-quinolyl), 5.97 (2H, s, NH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 165.65 (CO), 150.06 (C(NH$_2$)NOH), 145.43 (2-quinolyl), 144.32 (9-quinolyl), 136.50 (3-quinolyl), 134.27 (1-aryl), 132.83 (4-aryl), 128.51 (8-quinolyl), 127.98 (6-quinolyl), 127.75 (7-quinolyl), 127.70 (5-quinolyl), 127.60 (2, 6-aryl), 126.99 (6-quinolyl), 125.25 (3, 5-aryl), 123.34 (4-quinolyl). LRMS (CI) *m/z* 307 (M+H, 100 %). Anal.Calcd for C$_{17}$H$_{14}$N$_4$O$_2$ C, 66.66; H, 4.61; N, 18.29. Found: C, 66.47; H, 4.61; N, 18.00.

N-(4-Cyanobenzyl)benzamide (141)
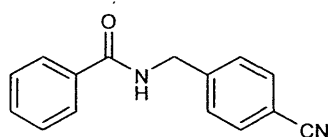
Benzoyl chloride (366 mg, 2.61 mmol) was added slowly to a solution of 4-(aminomethyl)benzonitrile hydrochloride (400 mg, 2.37 mmol) and triethylamine (503 mg, 4.98 mmol) in dichloromethane (20 mL) at 0 °C. The solution was stirred at 0 °C for 3 h

then washed with 2 M HCl (10 mL) and saturated aqueous sodium hydrogen carbonate solution (10 mL) before drying over $MgSO_4$. The solvent was evaporated to leave a white solid which was recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (459 mg, 82 %) as a white crystalline solid, mp 157-158 °C; IR (cm^{-1}) 3249, 3072, 2229, 1634, 1546, 794. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.80 (2H, dt, J=1.8, 7.0, Hz, Ar(o-CO)), 7.60 (2H, d, J=8.4 Hz, Ar(m-CO)), 7.53 (1H, tt, J=1.4, 7.3, Hz, Ar(p-CO)), 7.43 (4H, m, Ar), 6.75 (1H, brs, NH), 4.68 (2H, d, J=6.1 Hz, CH$_2$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 167.64 (CO), 143.95 (Ar(p-CN)), 133.80 (Ar(i-CO)), 132.49 (Ar(o-CN)), 131.94 (Ar(p-CO)), 128.72 (Ar), 128.22 (Ar), 127.02 (Ar), 118.72 (CN), 111.27 (Ar(i-CN)), 43.51 (CH$_2$). LRMS (CI) m/z 237 (M+H, 100 %), 122 (44 %), 104 (13 %). Anal.Calcd for C$_{15}$H$_{12}$N$_2$O C, 76.25; H, 5.12; N, 11.86. Found: C, 75.87; H, 5.13; N, 11.77.

N-(4-(N'-Hydroxycarbamimidoyl)benzyl)benzamide (103)
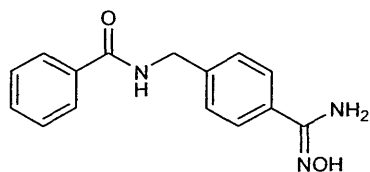
Hydroxylamine hydrochloride (397 mg, 5.71 mmol) was dissolved in a solution of methanol (5.7 mL) and DIEA (766 mg, 5.93 mmol) and the colourless solution stirred for 30 min. N-(4-Cyanobenzyl)benzamide (141) (270 mg, 1.14 mmol) was added and stirring continued for 20 h at room temperature. The solution was evaporated and water (10 mL) added to the colourless oil. The aqueous solution was extracted with ethyl acetate (3 x 10 mL) and the combined organics dried (MgSO$_4$) and evaporated. The white solid was recrystallised from methanol to give the title compound (210 mg, 68 %) as a crystalline white solid, mp 188-190 °C; IR (cm^{-1}) 3452, 3347, 3309, 3047, 1630, 1531, 1313, 942. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.60 (1H, s, OH), 9.07 (1H, t, J=5.9Hz, NH), 7.89 (2H, m, Ar(o-CO)), 7.62 (2H, d, J=8.3 Hz, Ar(o-CNOH)), 7.49 (3H, m, Ar(m,p-CO)), 7.30 (2H, d, J=8.3 Hz, Ar(m-CNOH)), 5.80 (2H, s, NH$_2$), 4.89 (2H, d, J=5.9 Hz, CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 166.16 (CO), 150.67 (CNOH), 140.39 (Ar(p-CNOH)), 134.22 (Ar(i-CO)), 131.80 (Ar), 131.20 (Ar), 128.27 (Ar), 127.18 (Ar), 126.84 (Ar), 125.30 (Ar), 42.33 (CH$_2$). LRMS (CI) m/z 270 (M+H, 100 %). Anal.Calcd for C$_{15}$H$_{15}$N$_3$O$_2$ C, 66.90; H, 5.61; N, 15.60. Found: C, 66.83; H, 5.63; N, 15.48.

7-Bromoheptanoic acid (145)[7]

$$\text{HO} \overset{O}{\underset{}{\parallel}} \text{C} \diagup\diagdown\diagup\diagdown\diagup\text{Br}$$
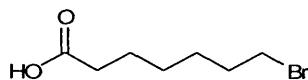
To a solution of ethyl 7-bromoheptanoate (1.0 g, 4.22 mmol) in methanol (30 mL) was added 2 M aqueous LiOH (5.06 mL) and the solution stirred for 18 h at room temperature. The methanol was removed *in vacuo* to leave a white slurry and ethyl acetate (30 mL) was added. The mixture was washed with 2 M HCl (2 x 15 mL) and brine (15 mL) before drying over MgSO$_4$. The colourless solution was evaporated to leave a colourless oil which solidified on standing to give the product (861 mg, 98 %) as a white solid which was spectroscopically identical to the literature; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 11.33 (1H, brs, OH), 3.38 (2H, t, J=6.8 Hz, CH$_2$Br), 2.34 (2H, t, J=7.4 Hz, COCH$_2$), 1.84 (2H, m, CH_2CH$_2$Br), 1.62 (2H, m, COCH$_2$CH_2), 1.40 (4H, m, COCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$Br); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 180.32 (COOH), 33.95 (COOHCH$_2$), 33.78 (CH$_2$Br), 32.50 (CH$_2$CH$_2$Br), 28.14 (CH$_2$CH$_2$CH$_2$Br), 27.76 (COCH$_2$CH$_2$$CH_2$), 24.41 (COCH$_2$$CH_2$).

7-Bromo-*N*-phenylheptanamide (146)[7,8]

$$\text{(phenyl)}\underset{H}{N} \overset{O}{\underset{}{\parallel}} \text{C} \diagup\diagdown\diagup\diagdown\diagup\text{Br}$$
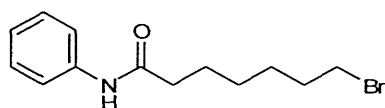
7-Bromoheptanoic acid (145) (840 mg, 4.02 mmol) in thionyl chloride (10 mL) was heated to 70 °C for 1 h. The solution was evaporated to leave a pale yellow oil which was dissolved in dichloromethane (40 mL) and cooled to 0 °C. Aniline (412 mg, 4.42 mmol) was added dropwise followed by pyridine (350 mg, 4.42 mmol) also dropwise and stirring continued for 3 h at 0 °C. The suspension was washed with 2 M HCl (20 mL) and saturated aqueous sodium hydrogen carbonate solution (20 mL) then dried (MgSO$_4$). The solvent was removed *in vacuo* to leave a peach coloured solid which was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 2:1) to give the title compound (790 mg, 69 %) as white needles, mp 98-99 °C, which were spectroscopically identical to the literature; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.51 (2H, d, J=7.7 Hz, 2, 6-aryl), 7.30 (2H, t, J=7.8 Hz, 3, 5-aryl), 7.09 (1H, t, J=7.3 Hz, 4-aryl), 3.39 (2H, t, J=6.7 Hz, CH$_2$Br), 2.35 (2H, t, J=7.4 Hz, COCH$_2$), 1.89-1.68 (4H, m, COCH$_2$CH_2, CH_2CH$_2$Br), 1.44 (4H, m, COCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$Br); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 171.34 (CO), 137.96 (1-

aryl), 128.98 (3, 5-aryl), 124.24 (4-aryl), 119.89 (2, 6-aryl), 37.51 (COCH_2), 33.89 (CH$_2$Br), 32.51 (CH_2CH$_2$Br), 28.31 (COCH$_2$CH$_2$CH_2), 27.84 (CH_2CH$_2$CH$_2$Br), 25.36 (COCH$_2$CH_2). LRMS (CI) m/z 284 (M+H, 85 %), 204 (100 %), 93 (37 %). Anal.Calcd for C$_{13}$H$_{18}$NOBr C, 54.94; H, 6.38; N, 4.93. Found: C, 55.30; H, 6.43; N, 4.81.

7-Cyano-*N*-phenylheptanamide (147)
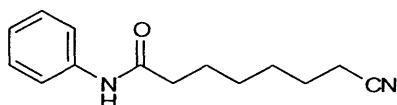
7-Bromo-*N*-phenylheptanamide (**146**) (770 mg, 2.71 mmol) was added to a solution of KCN (212 mg, 3.25 mmol) in dry ethanol (13 mL) and the solution heated to 75 °C for 19 h. The reaction was quenched by addition of 2 M HCl (5 mL) (caution: HCN evolved). The inorganic precipitates were removed by filtration and the filtrate evaporated to give a pale yellow solid. The solid was re-dissolved in ethanol and filtered to remove remaining inorganics, then evaporated. The crude solid was purified by column chromatography (petroleum ether (40 - 60)-ethyl acetate, 1:1) to give the title compound (458 mg, 73 %) as a white crystalline solid, mp 99-101 °C; IR (cm^{-1}) 3300, 3260, 2944, 2247, 1664, 1599. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.64 (1H, s, NH), 7.52 (2H, d, J=7.8 Hz, 2, 6-aryl), 7.29 (2H, t, J=7.9 Hz, 3, 5-aryl), 7.08 (1H, t, J=7.4 Hz, 4-aryl), 2.33 (4H, t, J=7.2 Hz, , CH$_2$CN), 1.77-1.58 (4H, m, COCH$_2$CH_2, CH_2CH$_2$CN), 1.41 (4H, m, COCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$CN); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 171.32, (CO), 138.05 (1-aryl), 128.97 (3, 5-aryl), 124.20 (4-aryl), 119.86 (2, 6-aryl, CN), 37.29 (COCH_2), 28.33 (COCH$_2$CH$_2$CH_2), 28.28 (CH_2CH$_2$CH$_2$CN), 25.13 (COCH$_2$CH_2), 25.09 (CH_2CH$_2$CN), 17.07 (CH_2CN). LRMS (CI) m/z 231 (M+H, 100 %), 138 (26 %), 94 (37 %). Anal. Calcd for C$_{14}$H$_{18}$N$_2$O C, 73.01; H, 7.88; N, 12.16. Found: C, 73.01; H, 7.98; N, 11.87.

8-Amino-8-(hydroxyimino)-*N*-phenyloctanamide (104)
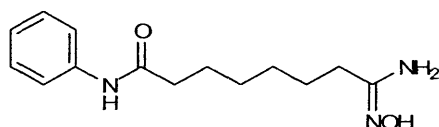
To a solution of 7-cyano-*N*-phenylheptanamide (**147**) (1.50 g, 6.51 mmol) was added a 1 M methanolic solution of hydroxylamine (32.46 mL, 32.56 mmol) and the resultant solution

stirred for 2 d at room temperature. The solvent was evaporated to leave a white solid which was purified by column chromatography (50 % ethyl acetate-petroleum ether (40 - 60) increasing to 100 % ethyl acetate) and recrystallisation from ethyl acetate-petroleum ether (40 - 60) to give the starting material (800 mg, 53 %) and the title compound (233 mg, 14 %) as a pale-grey crystalline solid, mp 127 °C; IR (cm^{-1}) 3290, 2940, 1662, 1601, 1544, 1444. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.84 (1H, s, OH), 9.30 (1H, s, NH), 8.22 (1H, s, NH), 7.88 (1H, s, NH), 7.57 (2H, d, J=7.7 Hz, 2, 6-aryl), 7.26 (2H, t, J=7.9 Hz, 3, 5-aryl), 7.00 (1H, t, J=7.4 Hz, 4-aryl), 2.28 (2H, t, J=7.4 Hz, , COCH$_2$), 2.16 (2H, t, J=7.5 Hz, CH$_2$C(NH$_2$)NOH), 1.62-1.46 (4H, m, COCH$_2$CH_2, CH_2CH$_2$CNH$_2$), 1.29 (4H, m, COCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$CNH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 171.19, (CO), 157.26 (C(NH$_2$)NOH), 139.28 (1-aryl), 128.56 (3, 5-aryl), 122.84 (4-aryl), 118.96 (2, 6-aryl), 36.35 (COCH$_2$), 28.53 (CH$_2$CH$_2$CH$_2$CNH$_2$), 28.42 (COCH$_2$CH$_2$CH$_2$), 27.69 (CH$_2$CNH$_2$), 25.80 (COCH$_2$CH$_2$), 25.03 (CH$_2$CH$_2$CNH$_2$), 17.07 (CH$_2$CN). LRMS (EI) m/z 263 (M$^+$, 7 %), 249 (12 %), 231 (13 %), 190 (24 %), 135 (27 %), 93 (100 %). HRMS calcd for C$_{14}$H$_{21}$N$_3$O$_2$ 263.16283. Found: 263.16266.

6.3 Analogues of SAHA

Oxonane-2,9-dione (157)^9
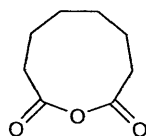
Suberic acid (5.0 g, 28.7 mmol) was refluxed at 150 °C in acetic anhydride (10 mL) for 1 h. The solution was then evaporated to leave a white solid which was recrystallised from ethyl acetate-petroleum ether (40 - 60) to give a crystalline white solid (5.40 g, 83 %), mp 50-52 °C (lit.^{9} 51-52 °); IR (cm^{-1}) 1799, 1740, 1473, 1415, 1077, 910. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 2.43 (4H, t, J=7.3 Hz, CH$_2$CO), 1.64 (4H, m, CH_2CH$_2$CO), 1.36 (4H, m, CH_2CH$_2$CH$_2$CO); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 169.41 (CO), 35.07 (CH$_2$CO), 28.38 (CH$_2$CH$_2$CO), 23.89 (CH$_2$CH$_2$CH$_2$CO). LRMS (CI) m/z 157 (M+H, 100%), 111 (12 %).

8-Oxo-8-(phenylamino)octanoic acid (158)[9]
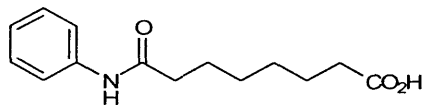
Aniline (1.48 g, 15.88 mmol) was added to a white suspension of oxonane-2,9-dione (157) (2.48 g, 15.88 mmol) in dry tetrahydrofuran (25 mL) and the resultant yellow solution stirred for 45 min at room temperature. The white suspension was then poured onto water (200 mL) and the precipitate filtered then recrystallised form methanol-water to give the product (3.12 g, 79 %) as a white crystalline solid, mp 123-124 °C (lit.[9] 122-123 °C); IR (cm^{-1}) 3304, 2936, 1690, 1659, 1598, 1442, 1250, 1178. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 12.01 (1H, brs, OH), 9.84 (1H, s, NH), 7.58 (2H, d, J=7.8 Hz, 2, 6-aryl), 7.26 (2H, d, J=7.8 Hz, 3, 5-aryl), 7.00 (1H, t, J=7.3 Hz, 4-aryl), 2.28 (2H, t, J=7.4 Hz, NHCOCH_2), 2.18 (2H, t, J=7.3 Hz, CH$_2$COO), 1.53 (4H, m, NHCOCH$_2$CH_2, CH_2CH$_2$COO), 1.28 (4H, m, CH_2CH_2CH$_2$CH$_2$COO); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 174.43 (COOH), 171.17 (CONH), 139.26 (1-aryl), 128.54 (3, 5-aryl), 122.84 (4-aryl), 118.97 (2, 6-aryl), 36.29 (NHCOCH_2), 33.55 (CH_2COO), 28.33 (CH_2CH$_2$CH$_2$COO), 28.26 (NHCOCH$_2$CH$_2$CH_2), 24.92 (NHCOCH$_2$CH_2), 24.32 (CH_2CH$_2$COO). LRMS (CI) m/z 250 (M+H, 82 %), 232 (100 %), 157 (16 %), 93 (53 %).

N^1-hydroxy-N^8-phenyloctanediamide (SAHA)[9]
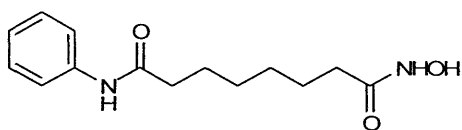
Ethyl chloroformate (5.69 mg, 5.24 mmol) and triethylamine (611 mg, 6.04 mmol) were added to a solution of 8-oxo-8-(phenylamino)octanoic acid (158) (1.0 g, 4.03 mmol) in dry tetrahydrofuran at 0 °C. The mixture was stirred for 20 min then the solid filtered and the filtrate added to a methanolic solution of hydroxylamine (prepared by addition of a solution of hydroxylamine hydrochloride (476 mg, 6.85 mmol) in methanol (10 mL) to a solution of KOH (384 mg, 6.85 mmol) in methanol (10 mL) at 0 °C, stirring for 15 min then filtration of the precipitate). The solution was stirred for 20 min then evaporated to leave a white solid which was recrystallised from 1 % methanol-acetonitrile to give the title compound (685 mg, 65 %) as a white crystalline solid, mp 159-160 °C (lit.[9] 159.5-160 °C); IR (cm^{-1})

3193, 2935, 1655, 1598, 1527, 1443, 1317, 1248. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.34 (1H, brs, NH), 9.86 (1H, s, NH), 8.66 (1H, s, OH), 7.57 (2H, d, J=8.4 Hz, 2, 6-aryl), 7.26 (2H, t, J=7.8 Hz, 3, 5-aryl), 7.00 (1H, t, J=7.3 Hz, 4-aryl), 2.27 (2H, t, J=7.4 Hz, PhNHCOCH_2), 1.92 (2H, t, J=7.3 Hz, CH_2CONHOH), 1.52 (4H, m, PhNHCOCH$_2$CH_2, CH_2CH$_2$CONHOH), 1.26 (4H, m, CH_2CH$_2$CH$_2$CH$_2$CONHOH, CH_2CH$_2$CH$_2$CONHOH); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 171.16 (PhNHCO), 169.04 (CONHOH), 139.28 (1-aryl), 128.55 (3, 5-aryl), 122.83 (4-aryl), 118.95 (2, 6-aryl), 36.30 (PhNHCOCH_2), 32.18 (CH_2CONHOH), 28.34 (CH$_2$CH$_2$CH$_2$CONHOH, PhNHCOCH$_2$CH$_2$CH$_2$), 24.96 (PhNHCOCH$_2$CH_2, CH_2CH$_2$CONHOH). LRMS (CI) m/z 287 (M+Na, 78 %), 265 (M+H, 100 %), 232 (8 %).

1-(*tert*-Butoxycarbonyl)piperidine-4-carboxylic acid (163)[10]
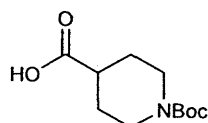
Isonipecotic acid (5.0 g, 38.71 mmol) was added to a solution of potassium carbonate (10.70 g, 77.42 mmol) in water (80 mL) at 0 °C. To the resultant solution was added a solution of di-*tert*-butyl dicarbonate (16.90 g, 77.42 mmol) in tetrahydrofuran (80 mL) dropwise at 0 °C. After stirring for 4 h at room temperature the tetrahydrofuran was evaporated and the aqueous solution washed with diethyl ether (2 x 30 mL). The aqueous phase was then acidified with 1 M HCl and extracted with ethyl acetate (4 x 20 mL). The combined extracts were washed with brine (20 mL) and dried (MgSO$_4$). The solvent was removed *in vacuo* to give the product (7.45 g, 84 %) as a white crystalline solid, mp 148-150 °C; IR (cm^{-1}) 3217, 2974, 1732, 1651, 1431, 1286. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 11.21 (1H, s, COOH), 3.99 (2H, d, J=11.8 Hz, 2-piperidyl), 2.83 (2H, t, J=11.4 Hz, 2-piperidyl), 2.45 (1H, tt, J=3.8, 10.8 Hz, 4-piperidyl), 1.87 (2H, dd, J=2.5, 13.1, Hz, 3-piperidyl), 1.61 (2H, m, 3-piperidyl), 1.42 (9H, s, C(CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 180.73 (COOH), 154.82 (NCOO), 79.86 (*C*(CH$_3$)$_3$), 42.99 (4-piperidyl), 40.81 (2, 6-piperidyl), 28.40 (C(*C*H$_3$)$_3$), 27.71 (3, 5-piperidyl). LRMS (CI) m/z 230 (M+H, 36 %), 202 (48 %), 174 (100 %), 156 (97 %), 139 (79 %), 112 (82 %).

tert-Butyl 4-(phenylcarbamoyl)piperidine-1-carboxylate (164)[11]
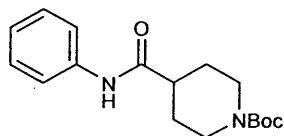
A solution of 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (**163**) (4.63 g, 20.19 mmol), 1-hydroxybenzotriazole (4.09 g, 30.29 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (4.26 g, 22.21 mmol), aniline (1.88 g, 20.19 mmol) and *N*-methylmorpholine (4.49 g, 44.42 mmol) in dimethylformamide (100 mL) was stirred at room temperature for 18 h. The reaction was acidified with 0.5 M HCl (90 mL) and extracted with ethyl acetate (4 x 20 mL). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL) before drying (MgSO$_4$). The solvent was removed *in vacuo* to leave a brown oil which was triturated with diethyl ether to give a white solid. The crude product was recrystallised from ethanol-water to give the title compound (4.20 g, 68 %) as a white crystalline solid, mp 154-6 °C; IR (cm^{-1}) 3316, 2848, 1698, 1663, 1603, 1546, 760. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.51 (2H, d, J=7.2 Hz, 2, 6-aryl), 7.29 (2H, m, 3, 5-aryl), 7.10 (1H, t, J=7.0 Hz, 4-aryl), 4.17 (2H, d, J=12.3 Hz, 2, 6eq-piperidyl), 2.75 (2H, t, J=12.3 Hz, 2, 6ax-piperidyl), 2.38 (1H, m, 4-piperidyl), 1.87 (2H, d, J=12.3 Hz, 3, 5eq-piperidyl), 1.74 (2H, m, 3, 5ax-piperidyl), 1.46 (9H, s, C(CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 172.78 (NHCO), 154.71 (NCOO), 137.84 (1-aryl), 129.02 (3, 5-aryl), 124.40 (4-aryl), 119.95 (2, 6-aryl), 79.78 (*C*(CH$_3$)$_3$), 44.26 (2, 6-piperidyl), 43.26 (4-piperidyl), 28.61 (3, 5-piperidyl), 28.45 (C(*C*H$_3$)$_3$). LRMS (CI) *m/z* 327 (M+Na, 100 %), 227 (63 %). Anal.Calcd for C$_{17}$H$_{24}$N$_2$O$_3$ C, 67.08; H, 7.95; N, 9.20. Found: C, 67.04; H, 7.93; N, 9.00.

N-Phenylpiperidine-4-carboxamide (165)[12]
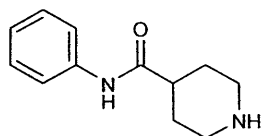
tert-Butyl 4-(phenylcarbamoyl)piperidine-1-carboxylate (**164**) (3.0 g, 9.86 mmol) was dissolved in a 1:1 solution of dichloromethane-trifluoroacetic acid and the resultant solution stirred for 17 h. The reaction was quenched with saturated aqueous sodium hydrogen

carbonate solution and the organic layer separated. The aqueous phase was further extracted with ethyl acetate (10 x 50 mL) and the combined organics dried with MgSO₄. The solvent was removed *in vacuo* to give the product (1.37, 68 %) without further purification as a viscous white oil; IR (cm⁻¹) 3268, 2940, 1645, 1527, 1440, 731. ¹H-NMR (CD₃OD, 300 MHz) δ 7.55 (2H, d, *J*=7.6 Hz, 2, 6-aryl), 7.27 (2H, t, *J*=7.9 Hz, 3, 5-aryl), 7.05 (1H, t, *J*=7.4 Hz, 4-aryl), 3.11 (2H, d, *J*=12.5 Hz, 2, 6eq-piperidyl), 2.67 (2H, dt, *J*=3.0, 12.3 Hz, 2, 6ax-piperidyl), 2.53 (1H, m, 4-piperidyl), 1.76 (4H, d, *J*=12.3 Hz, 3, 5-piperidyl); ¹³C-NMR (CD₃OD, 75 MHz) δ 175.83 (CO), 139.96 (1-aryl), 129.89 (3, 5-aryl), 125.56 (4-aryl), 121.42 (2, 6-aryl), 45.86 (2, 6-piperidyl), 44.25 (4-piperidyl), 29.46 (3, 5-piperidyl). LRMS (EI) *m/z* 204 (M⁺, 54 %), 148 (100 %), 92 (93 %). HRMS calcd for C₁₂H₁₆N₂O 204.12626. Found: 204.12588.

Ethyl 3-(4-(phenylcarbamoyl)piperidin-1-yl)propanoate (166)
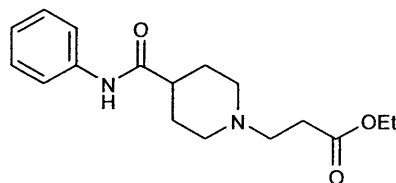
Ethyl acrylate (0.66 g, 6.56 mmol) was added to a solution of *N*-phenylpiperidine-4-carboxamide (165) (1.34 g, 6.56 mmol) in ethanol (30 mL) and the solution stirred for 4 d. The solvent was removed *in vacuo* to leave a white solid which was purified by column chromatography (ethyl acetate) and recrystallisation from ethyl acetate-petroleum ether (40-60) to give a white crystalline solid (1.34 g, 67 %), mp 115-116 °C; IR (cm⁻¹) 3281, 2962, 1730, 1653, 1525, 1249, 730. ¹H-NMR (CDCl₃, 300 MHz) δ 7.51 (2H, d, *J*=7.8 Hz, 2, 6-aryl), 7.30 (2H, t, *J*=7.9 Hz, 3, 5-aryl), 7.08 (1H, t, *J*=7.4 Hz, 4-aryl), 4.14 (2H, q, *J*=7.1 Hz, C*H*₂CH₃), 3.02 (2H, d, *J*=11.5 Hz, 2, 6eq-piperidyl), 2.75 (2H, t, *J*=7.3 Hz, C*H*₂CH₂COO), 2.53 (2H, t, *J*=7.3 Hz, CH₂COO), 2.30-2.10 (3H, m, 4-piperidyl, 2, 6ax-piperidyl), 1.97-1.85 (4H, m, 3, 5-piperidyl), 1.25 (3H, s, CH₃); ¹³C-NMR (CDCl₃, 75 MHz) δ 173.05 (COO), 172.30 (NHCO), 137.91 (1-aryl), 129.00 (3, 5-aryl), 124.29 (4-aryl), 119.90 (2, 6-aryl), 60.60 (*C*H₂CH₃), 53.56 (*C*H₂CH₂COO), 52.65 (2, 6-piperidyl), 43.73 (4-piperidyl), 32.10 (*C*H₂COO), 28.50 (3, 5-piperidyl), 14.22 (CH₃). LRMS (CI) *m/z* 327 (M+Na, 100 %),

305 (63 %), 217 (46 %), 212 (27 %). Anal.Calcd for $C_{17}H_{24}N_2O_3.\frac{1}{2}H_2O$ C, 65.15; H, 8.04; N, 8.94. Found: C, 65.59; H, 7.73; N, 8.71.

1-(3-(Hydroxyamino)-3-oxopropyl)-N-phenylpiperidine-4-carboxamide (148)
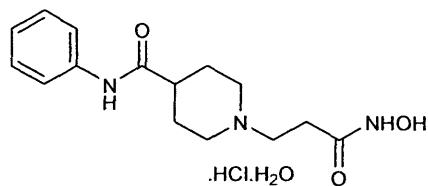
Ethyl 3-(4-(phenylcarbamoyl)piperidin-1-yl)propanoate (166) (513 mg, 1.69 mmol) in tetrahydrofuran (3 mL) was added to a 1M solution of hydroxylamine in methanol (16.9 mL, 16.9 mmol) and to the solution was added a 1M solution of KOH (3.37 mL, 3.37 mmol). After stirring over night at room temperature, the orange suspension was neutralised with 2 M HCl and evaporated. The pale orange solid dissolved in 10 % methanol-ethyl acetate and an insoluble white solid removed by filtration. The filtrate was evaporated to leave a pale orange solid which was recrystallised from 5 % methanol-acetonitrile to give the title compound (247 mg, 42 %) as white crystals, mp 118-120 °C; IR (cm^{-1}) 3463, 3232, 2949, 2714, 1657, 1446, 947. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.79 (1H, s, NH), 10.52 (1H, brs, OH), 10.27 (1H, s, NH), 8.93 (1H, s, NH), 7.62 (2H, d, J=7.8 Hz, 2, 6-aryl), 7.27 (2H, t, J=7.7 Hz, 3, 5-aryl), 7.01 (1H, t, J=7.2 Hz, 4-aryl), 3.48 (2H, d, J=9.6 Hz, 2, 6eq-piperidyl), 3.27 (2H, m, NCH$_2$), 2.91 (2H, m, 2, 6ax-piperidyl), 2.59 (3H, m, CH$_2$CO, 4-piperidyl), 1.98 (4H, m, 3, 5-piperidyl); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 171.78 (PhNHCO), 165.70 (CONHOH), 139.07 (1-aryl), 128.56 (3, 5-aryl), 123.13 (4-aryl), 119.16 (2, 6-aryl), 51.92 (NCH$_2$), 50.89 (2, 6-piperidyl), 39.89 (4-piperidyl), 26.80 (CH$_2$CO), 25.62 (3, 5-piperidyl). IR (nujol) (cm^{-1}) 3238, 2588, 1654, 752. LRMS (CI) m/z 292 (M+H, 100 %), 217 (19 %), 154 (74 %). Anal.Calcd for $C_{15}H_{21}N_3O_3.H_2O.HCl$ C, 52.10; H, 7.00; N, 12.15. Found: C, 52.15; H, 7.10; N, 12.09.

Ethyl 3-(bis(2-hydroxyethyl)amino)propanoate (168)

HO⌐
     \
HO⌐_N_⌐_OEt
          ‖
          O
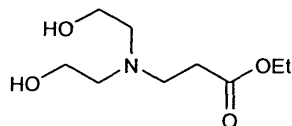
A solution of diethanolamine (10.42 g, 99.11 mmol) and ethyl acrylate (9.92 g, 99.11 mmol) in ethanol (250 mL) was refluxed at 120 °C for 5 h. The solvent was removed *in vacuo* and the colourless oil purified by column chromatography (10 % methanol-ethyl acetate) to give the product (18.14 g, 90 %) as a colourless oil; IR (cm^{-1}) 3371, 2967, 1728, 1187, 1042. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 4.10 (2H, q, *J*=7.1 Hz, CH_2CH$_3$), 3.55 (4H, t, *J*=5.2 Hz, CH_2OH), 3.25 (2H, brs, OH), 2.80 (2H, t, *J*=6.5 Hz, CH$_2$COO), 2.59 (4H, t, *J*=5.1 Hz, NCH_2CH$_2$OH), 2.43 (2H, t, *J*=6.5 Hz, NCH_2CH$_2$COO), 1.21 (3H, t, *J*=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.25 (COO), 60.67 (CH$_2$CH$_3$), 59.49 (CH$_2$OH), 56.06 (NCH$_2$CH$_2$OH), 49.66 (NCH$_2$CH$_2$COO), 32.65 (CH$_2$COO), 14.10 (CH$_2$CH$_3$). LRMS (CI) *m/z* 228 (M+Na, 74 %), 206 (M+H, 43 %), 188 (100 %).

Ethyl 3-(bis(2-bromoethyl)amino)propanoate (169)

Br⌐
     \
Br⌐_N_⌐_OEt
          ‖
          O
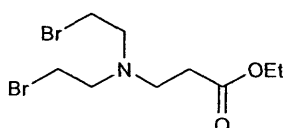
To a solution of ethyl 3-(bis(2-hydroxyethyl)amino)propanoate (168) (3.0 g, 14.62 mmol) in dichloromethane (150 mL) was added triphenylphosphine (8.05 g, 30.70 mmol) followed by *N*-bromosuccinamide (5.46 g, 30.70 mmol) slowly and the pale yellow solution stirred for 6 h at room temperature. The solvent was removed *in vacuo* to leave an off-white solid which was recrystallised from acetone-petroleum ether (40 - 60). The white, crystalline triphenylphosphine oxide was filtered off and washed with 10 % ethyl acetate-petroleum ether (40 - 60). The filtrate was then evaporated to give a pale yellow solid which was purified by column chromatography (10 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (2.97 g, 61 %) as a colourless oil; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 4.12 (2H, q, *J*=7.1 Hz, CH_2CH$_3$), 3.32 (4H, t, *J*=7.4 Hz, CH_2Br), 2.89 (6H, m, (CH$_2$)$_3$N), 2.42 (2H, t, *J*=6.9 Hz, CH$_2$COO), 1.25 (3H, t, *J*=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 172.01 (COO), 60.48 (CH$_2$CH$_3$), 56.27 (CH$_2$CH$_2$Br), 49.95 (NCH$_2$CH$_2$COO), 33.56 (CH$_2$COO), 30.13 (CH$_2$Br), 14.26 (CH$_2$CH$_3$). LRMS (CI) *m/z* 330 (M+H, 39 %), 282 (77

%), 252 (53 %), 236 (70 %), 202 (100 %). HRMS (M+H) calcd for $C_9H_{17}NO_2Br_2$ 329.97042. Found: 329.96970.

Di-tert-butyl 1-(3-ethoxy-3-oxopropyl)piperidine-4,4-dicarboxylate (170)
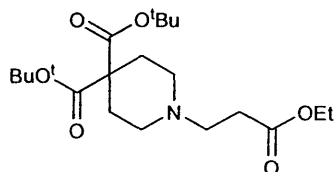
A suspension of di-*tert*-butyl malonate (2.61 g, 12.09 mmol) and sodium hydride (725 mg of a 60 % dispersion in mineral oil, 18.14 mmol) in dry dimethylformamide was stirred at room temperature for 30 min before addition of ethyl 3-(bis(2-bromoethyl)amino)propanoate (**169**) (4.00 g, 12.09 mmol). The reaction was stirred at 70 °C for 18 h then further sodium hydride (725 mg of a 60 % dispersion in mineral oil, 18.14 mmol) added and stirring continued for 24 h at 70 °C. Water (200 mL) was added slowly and the solution extracted with ethyl acetate (60 mL then 3 x 30 mL). The combined extracts were washed with brine and dried with $MgSO_4$ before evaporation to leave an orange oil. The crude material was purified by column chromatography (30 % ethyl acetate-petroleum ether (40 - 60) increasing to 100 % ethyl acetate) to give a pale yellow oil (2.51 g, 54 %); IR (cm^{-1}) 2978, 1730, 1257, 1168, 850. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 4.09 (2H, q, J=7.1 Hz, CH_2CH$_3$), 2.61 (2H, t, J=7.4 Hz, CH_2CH$_2$COO), 2.42 (6H, m, 3, 5-piperidyl, CH$_2$COO), 1.99 (4H, 2, 6-piperidyl), 1.40 (18H, s, C(CH$_3$)$_3$), 1.21 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 172.50 (COOEt), 170.40 (COO^{t}Bu), 81.12 (C(CH$_3$)$_3$), 60.33 (CH$_2$CH$_3$), 53.81 (4-piperidyl), 53.66 (CH$_2$CH$_2$COOEt), 50.20 (2, 6-piperidyl), 32.44 (CH$_2$COO), 30.71 (3, 5-piperidyl), 14.20 (CH$_2$CH$_3$). LRMS (EI) m/z 385 (M$^+$, 13 %), 298 (93 %), 228 (86 %), 186 (68 %), 96 (100 %). HRMS calcd for $C_{20}H_{35}NO_6$ 385.24643. Found: 385.24581.

1-(3-Ethoxy-3-oxopropyl)piperidine-4,4-dicarboxylic acid (171)
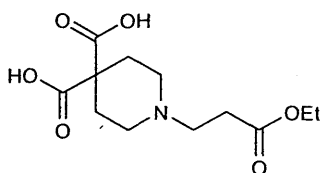
Trifluoroacetic acid (5 mL) was added dropwise to a solution of di-*tert*-butyl 1-(3-ethoxy-3-oxopropyl)piperidine-4,4-dicarboxylate (**170**) (2.23 g, 5.79 mmol) in dichloromethane (5 mL) and the brown solution stirred for 3 d. The solvent was evaporated to leave a brown oil which was triturated with diethyl ether to give a white powder which was recrystallised from ethanol to give the product (1.57 g, 99 %) as colourless crystals which were used without further purification.

Ethyl 3-(4,4-bis(phenylcarbamoyl)piperidin-1-yl)propanoate hydrochloride (172)
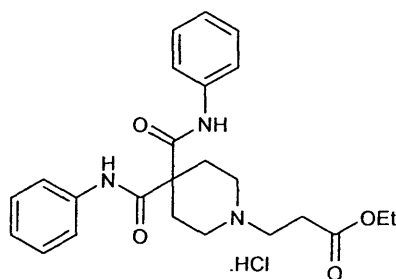
1-(3-Ethoxy-3-oxopropyl)piperidine-4,4-dicarboxylic acid (**171**) (750 mg, 2.74 mmol) in thionyl chloride (9 mL) was heated to 80 °C for 2 h then evaporated to give a light brown solid. The crude acyl chloride was dissolved in dichloromethane and cooled to 0 °C before dropwise addition of aniline (537 mg, 5.76 mmol). The orange suspension was stirred for 5 min at 0 °C then pyridine (477 mg, 6.03 mmol) added dropwise. Stirring was continued at 0 °C for 3 h then the solvent removed *in vacuo*. The orange solid was washed with 0.5 M H_2SO_4 (20 mL) and water (10 mL) then dried. The pale orange solid was recrystallised from ethanol to give the product (637 mg, 51 %) as white crystals, mp 231-233 °C; IR (cm^{-1}) 3224, 2549, 1736, 1668, 1528, 1191. ^{1}H-NMR ((CD$_3$)$_2$SO, 500 MHz) δ 10.73 (1H, brs, NH), 10.03 (1H, s, NH), 9.72 (1H, s, NH), 7.69 (4H, brs, 2, 6-aryl), 7.29 (4H, t, *J*=7.5 Hz, 3, 5-aryl), 7.08 (2H, t, *J*=7.1 Hz, 4-aryl), 4.09 (2H, q, *J*=7.1 Hz, C*H*$_2$CH$_3$), 3.55 (2H, d, *J*=7.3 Hz, 2, 6ax-piperidyl), 3.31 (2H, t, *J*=7.5 Hz, NC*H*$_2$CH$_2$COO), 3.03 (4H, m, 3, 5ax-piperidyl, 2, 6eq-piperidyl), 2.92 (2H, t, *J*=7.7 Hz, CH$_2$COO), 2.25 (2H, brs, 3, 5eq-piperidyl), 1.19 (3H, t, *J*=7.1 Hz, CH$_3$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 169.95 (COO), 168.04 (CONH), 167.03 (CONH), 138.40 (1-aryl), 128.38 (3, 5-aryl), 124.08 (4-aryl), 121.06 (2, 6-aryl), 120.78 (2, 6-aryl), 60.54 (C*H*$_2$CH$_3$), 52.76 (4-piperidyl), 50.56 (NCH$_2$), 49.17 (2, 6-piperidyl), 28.49 (CH$_2$COO), 27.61 (3, 5-piperidyl), 13.93 (CH$_3$). LRMS (CI) *m/z* 424

(M+H, 100 %), 287 (6 %). Anal.Calcd for $C_{24}H_{29}N_3O_4$.HCl C, 62.67; H, 6.57; N, 9.14. Found: C, 62.49; H, 6.52; N, 8.87.

Ethyl 7-(1H-benzo[d]imidazol-2-yl)heptanoate (176)
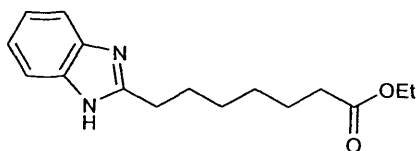
A solution of oxonane-2,9-dione (**157**) (3.0 g, 19.21 mmol) in tetrahydrofuran (15 mL) was added dropwise to a solution of 1,2-phenylenediamine (2.08 g, 19.21 mmol) in tetrahydrofuran (30 mL) and the brown solution stirred for 45 min. The solvent was removed *in vacuo* and the crude product dissolved in a 4 % solution of concentrated sulfuric acid in ethanol (190 mL). The orange solution was heated to 90 °C for 16 h then quenched with saturated aqueous sodium hydrogen carbonate solution and evaporated. Water (20 mL) was added and the mixture extracted with ethyl acetate (3 x 20 mL). The combined extracts were dried ($MgSO_4$) and evaporated to leave a brown oil which was purified by column chromatography (20 % ethyl acetate-petroleum ether (40 - 60)) to give an off-white solid. Recrystallisation from diethyl ether gave the title compound (1.52 g, 29 %) as white needles, mp 95-97 °C; IR (cm^{-1}) 2934, 1720, 1175, 1026, 747. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 10.78 (1H, brs, NH), 7.54 (2H, dd, J=3.2, 6.0 Hz, 4, 7-benzimidazolyl), 7.20 (2H, dd, J=3.2, 6.0 Hz, 5, 6-benzimidazolyl), 4.10 (2H, q, J=7.1 Hz, CH_2CH$_3$), 2.93 (2H, t, J=7.6 Hz, NCCH_2), 2.21 (2H, t, J=7.4 Hz, CH$_2$COO), 1.84 (2H, m, CH_2CH$_2$COO), 1.54 (2H, m, NCCH$_2$CH_2), 1.39-1.19 (7H, m, NCCH$_2$CH$_2$CH_2, CH_2CH$_2$CH$_2$COO, CH$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.96 (COO), 155.49 (2-benzimidazolyl), 138.64 (4, 9-benzimidazolyl), 122.03 (6, 7-benzimidazolyl), 114.60 (5, 8-benzimidazolyl), 60.31 (CH_2CH$_3$), 34.17 (CH_2COO), 29.18 (NCCH_2), 28.84 (NCCH$_2$CH_2), 28.63 (CH_2CH$_2$CH$_2$COO), 28.13 (NCCH$_2$CH$_2$CH_2); 24.70 (CH_2CH$_2$COO), 14.23 (CH$_3$). LRMS (CI) m/z 275 (M+H, 100 %), 229 (6 %), 187 (4 %). Anal. Calcd for $C_{16}H_{22}N_2O_2$ C, 70.04; H, 8.08; N, 10.21. Found C, 69.87; H, 8.07; N, 10.12.

## 7-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*-hydroxyheptanamide (151)
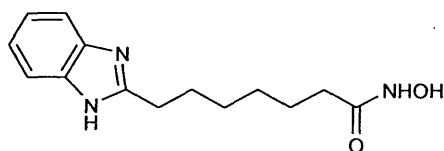
To a solution of ethyl 7-(1*H*-benzo[*d*]imidazol-2-yl)heptanoate (**176**) (1.20 g, 4.37 mmol) in tetrahydrofuran (40 mL) was added hydroxylamine (2.89 mL of a 50 % aqueous solution, 43.74 mmol) followed by slow addition of a 1 M methanolic solution of potassium hydroxide (6.56 mL). The solution was stirred for 2 h at room temperature then evaporated to leave a yellow residue. Water (60 mL) was added and the solution acidified with 2 M HCl to give a white precipitate which was collected by filtration. The product was washed with water, methanol and diethyl ether to give the title compound (696 mg, 61 %) as a white solid, mp 226-227 °C; IR (cm^{-1}) 3284, 2930, 2324, 1642, 1443, 1275, 727. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.33 (1H, s, NH), 8.67 (1H, s, NH), 7.34 (2H, m, 8, 5-benzimidazolyl), 7.09 (2H, m, 6, 7-benzimidazolyl), 2.77 (2H, t, *J*=7.5 Hz, NNCC*H*$_2$), 1.92 (2H, t, *J*=7.3 Hz, C*H*$_2$CO), 1.74 (2H, m, NNCCH$_2$C*H*$_2$), 1.48 (2H, m, C*H*$_2$CH$_2$CO), 1.29 (4H, m, C*H*$_2$CH$_2$CH$_2$CH$_2$CO, C*H*$_2$CH$_2$CH$_2$CO); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 174.24 (CO), 160.23 (2-benzimidazolyl), 148.39 (9-benzimidazolyl), 139.37 (4-benzimidazolyl), 126.42 (7-benzimidazolyl), 125.86 (6-benzimidazolyl), 123.11 (8-benzimidazolyl), 115.77 (5-benzimidazolyl), 37.33 (NNCC*H*$_2$), 33.55 (C*H*$_2$CO), 33.47 (NNCCH$_2$C*H*$_2$), 33.37 (C*H*$_2$CH$_2$CH$_2$CO), 32.54 (C*H*$_2$CH$_2$CH$_2$CH$_2$CO), 30.10 (C*H*$_2$CH$_2$CO). LRMS (CI) *m/z* 262 (M+H, 100 %), 242 (57 %), 201 (9 %). Anal. Calcd for C$_{14}$H$_{19}$N$_3$O$_2$ C, 64.35; H, 7.33; N, 16.08. Found C, 64.36; H, 7.51; N, 15.89.

3-*tert*-Butoxy-3-oxopropanoic acid (178)[13]
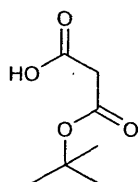
A solution of dicyclohexycarbodiimide (32.72 g, 158.6 mmol) in acetonitrile (150 mL) was added to a solution of malonic acid (15.0 g, 144.2 mmol) and *tert*-butyl alcohol (21.37 g, 288.3 mmol) in acetonitrile (380 mL). The resultant white suspension was stirred for 2 h at

room temperature then filtered and evaporated. The colourless oil was dissolved in diethyl ether (75 mL) and extracted with saturated aqueous sodium hydrogen carbonate solution (4 x 50 mL). The combined aqueous phases were acidified with 2 M HCl and extracted with ethyl acetate (4 x 50 mL). The combined organics were dried (MgSO$_4$) and evaporated to give the pure title compound (20.05 g, 87 %) as a colourless oil; IR (cm^{-1}) 2937, 1732, 1371, 1150, 837. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 11.02 (1H, brs, COOH), 3.31 (2H, s, CH$_2$), 1.44 (9H, s, (CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 172.02 (COOH), 166.22 (COOBu^t), 82.88 (C(CH$_3$)$_3$), 42.01 (CH$_2$), 27.84 ((CH$_3$)$_3$). LRMS (CI) m/z 161 (M+H, 47 %), 145 (14 %), 133 (46 %), 115 (33 %), 105 (100 %).

tert-Butyl 3-oxo-3-(phenylamino)propanoate (179)
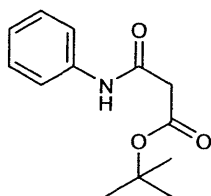
Method 1

A solution of 3-tert-butoxy-3-oxopropanoic acid (178) (2.05 g, 12.80 mmol), EDCI.HCl (2.70 g, 14.08 mmol), HOBt (1.90 g, 14.08 mmol), triethylamine (2.85 g, 28.16 mmol) and aniline (1.19 g, 12.80 mmol) in DMF (60 mL) was stirred for 3 h at room temperature. The yellow solution was evaporated and the residue suspended in 2 M HCl (50 mL) and extracted with ethyl acetate (3 x 15 mL). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate solution (20 mL) and brine (20 mL) before drying over MgSO$_4$. The solvent was removed in vacuo to leave the pure title compound (2.03 g, 63 %) as a peach-coloured solid.

Method 2

A solution of DCC (25.17 g, 122 mmol) in MeCN (180 mL) was added to a solution of 3-tert-butoxy-3-oxopropanoic acid (178) (17.86 g, 111 mmol) and aniline (11.40 g, 122 mmol) in MeCN (450 mL). The solution was stirred for 3 h then filtered and the filtrate evaporated. The pale pink oil was dissolved in ethyl acetate (100 mL) and washed with 2 M HCl (2 x 30 mL) and saturated aqueous sodium hydrogen carbonate solution (30 mL) and

brine (30 mL). The organic phase was dried (MgSO$_4$) and evaporated to leave a pale peach-coloured solid which was purified by column chromatography (30 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (21.51 g, 77 %) as a white, crystalline solid.

Method 3

Oxalyl chloride (11.72 g, 92.34 mmol) was added slowly to a solution of 3-*tert*-butoxy-3-oxopropanoic acid (**178**) (4.93 g, 30.78 mmol) in DCM (300 mL) followed by DMF (10 drops). The reaction was warmed to 40 °C for 2 h then evaporated. The red oil was dissolved in DCM (300 mL) and aniline (3.09 g, 33.86 mmol) added to give a precipitate. DMAP (4.14 g, 33.86 mmol) was added and the resultant solution stirred for 2 h at room temperature. The solvent was evaporated and the residue dissolved in ethyl acetate (50 mL). The organic phase was washed with 2 M HCl (2 x 30 mL) and saturated aqueous sodium hydrogen carbonate solution (30 mL), then dried (MgSO$_4$). The solvent was evaporated to leave a red oil which was purified by column chromatography (30 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (6.92 g, 89 %) as a white, crystalline solid, mp 72-74 °C; IR (cm^{-1}) 3308, 3267, 1721, 1556, 1146, 860. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 9.35 (1H, s, NH), 7.55 (2H, dd, J=0.9, 8.5 Hz, 2, 6-aryl), 7.29 (2H, t, J=7.9 Hz, 3, 5-aryl), 7.08 (1H, t, J=7.4 Hz, 4-aryl), 3.36 (2H, s, CH$_2$), 1.48 (9H, s, C(CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 168.89 (*C*OOBu^t), 163.83 (CONH), 137.70 (1-aryl), 128.93 (3, 5-aryl), 124.42 (4-aryl), 120.12 (2, 6-aryl), 82.90 (*C*(CH$_3$)$_3$), 42.87 (CH$_2$), 28.00 ((CH$_3$)$_3$). LRMS (EI) *m/z* 235 (M$^+$, 41 %), 179 (81 %), 162 (17 %), 119 (19 %), 93 (100 %). Anal. Calcd for C$_{13}$H$_{17}$NO$_3$ C, 66.36; H, 7.28; N, 5.95. Found C, 66.35; H, 7.34; N, 5.98.
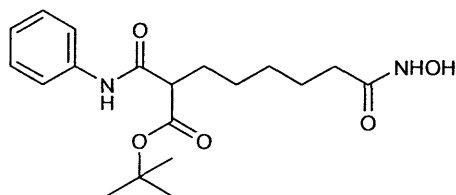
1-*tert*-Butyl 8-ethyl 2-(phenylcarbamoyl)octanedioate (180)
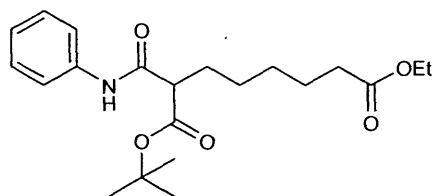
Sodium hydride (773 mg of a 60 % dispersion in oil, 19.33 mmol) was added slowly to a solution of *tert*-butyl 3-oxo-3-(phenylamino)propanoate (**179**) (4.45 g, 17.57 mmol) in dry

tetrahydrofuran (175 mL) at 0 °C. After stirring for 20 min at room temperature, ethyl 6-bromohexanoate (3.92 g, 17.57 mmol) was added and the reaction heated to 70 °C for 18 h. The white suspension was allowed to cool to room temperature and the solvent removed *in vacuo*. The residue was taken up in ethyl acetate (50 mL) and washed with 2 M HCl (2 x 20 mL) and brine (20 mL) before drying over MgSO$_4$. The crude product was purified by column chromatography (10 % ethyl acetate-petroleum ether (40 – 60)) to give the title compound (4.94 g, 74 %) as a crystalline, pale yellow solid, mp 65-66 °C; IR (cm^{-1}) 3307, 2944, 1726, 1656, 1554, 1154, 758. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.75 (1H, s, NH), 7.54 (2H, d, J=8.3 Hz, 2, 6-aryl), 7.32 (2H, t, J=7.9 Hz, 3, 5-aryl), 7.10 (1H, t, J=7.4 Hz, 4-aryl), 4.11 (2H, q, J=7.1 Hz, CH_2CH$_3$), 3.24 (1H, t, J=7.3 Hz, CH), 2.28 (2H, t, J=7.4 Hz, CH$_2$COO), 1.95 (2H, m, CHCH_2), 1.62 (2H, m, CH_2CH$_2$COO), 1.49 (9H, s, (CH$_3$)$_3$), 1.38 (4H, m, CH_2CH$_2$CH$_2$COO, CHCH$_2$CH_2), 1.24 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.62 (COOEt), 171.95 (CONH), 166.96 (COOBu^t), 137.76 (1-aryl). 128.96 (3, 5-aryl), 124.33 (4-aryl), 119.89 (2, 6-aryl), 82.68 (C(CH$_3$)$_3$), 60.22 (CH$_2$CH$_3$), 54.40 (CH), 34.16 (CH$_2$COO), 31.45 (CHCH$_2$), 28.64 (CH$_2$CH$_2$CH$_2$CHOO), 27.99 (CH$_3$)$_3$), 26.86 (CHCH$_2$$CH_2$), 24.63 ($CH_2CH_2$COO), 14.24 (CH$_2$$CH_3$). LRMS (EI) *m/z* 377 (M$^+$, 13 %), 322 (12 %), 258 (11 %), 179 (53 %), 161 (37 %), 93 (100 %). Anal. Calcd for C$_{21}$H$_{31}$NO$_5$ C, 66.82; H, 8.28; N, 3.71. Found C, 66.72; H, 8.32; N, 3.69.

tert-Butyl 8-(hydroxyamino)-8-oxo-2-(phenylcarbamoyl)octanoate (181)

To a solution of 1-*tert*-butyl 8-ethyl 2-(phenylcarbamoyl)octanedioate (180) (300 mg, 0.79 mmol) in tetrahydrofuran (8 mL) was added hydroxylamine (0.53 mL of a 50 % aqueous solution, 7.95 mmol) and 1 M KOH/methanol (2.37 mL, 2.37 mmol) dropwise. The solution was stirred for 1 h at room temperature then acidified with 2 M HCl. The solvent was evaporated to approximately 5 mL in volume and water (20 mL) added. The aqueous solution was extracted with ethyl acetate (4 x 15 mL) and the combined yellow extracts dried over Na$_2$SO$_4$. The solvent was removed *in vacuo* to leave a yellow oil which was

purified by column chromatography (ethyl acetate) to give the title compound (84 mg, 29 %) as a yellow oil; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 9.02 (1H, s, NH), 7.52 (2H, d, J=7.7 Hz, 2, 6-aryl), 7.26 (2H, t, J=7.7 Hz, 3, 5-aryl), 7.06 (1H, t, J=7.3 Hz, 4-aryl), 3.27 (t, J=7.0 Hz, CH), 2.08 (2H, m, CH_2CONHOH), 1.86 (2H, m, CHCH_2), 1.54 (CH_2CH$_2$CONHOH), 1.44 (9H, s, C(CH$_3$)$_3$), 1.28 (4H, m, CHCH$_2$CH_2, CHCH$_2$CH$_2$CH_2); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 171.65 (COOC(CH$_3$)$_3$), 171.32 (CONHOH), 167.82 (CONHPh), 137.67 (1-aryl), 128.94 (3, 5-aryl), 124.57 (4-aryl), 120.28 (2, 6-aryl), 82.68 (C(CH$_3$)$_3$), 54.20 (CH), 32.52 (CH$_2$CONHOH), 30.51 (CHCH$_2$), 28.27 (CHCH$_2$CH$_2$$CH_2$), 27.97 (C($CH_3$)$_3$), 26.59 ($CH_2CH_2$CONHOH), 25.02 (CHCH$_2$$CH_2$). LRMS (CI) m/z 387 (M+Na, 100 %), 365 (M+H, 45 %), 328 (22 %), 309 (35 %). HRMS calcd for C$_{19}$H$_{28}$N$_2$O$_5$ (M+H) 365.20710. Found: 365.20792.

8-(Hydroxyamino)-8-oxo-2-(phenylcarbamoyl)octanoic acid (149)
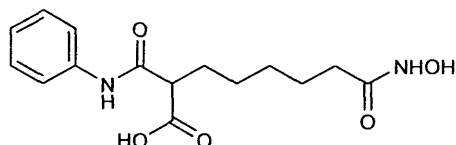
To a solution of *tert*-butyl 8-(hydroxyamino)-8-oxo-2-(phenylcarbamoyl)octanoate (181) (170 mg, 0.47 mmol) in DCM (2 mL) was added trifluoroacetic acid (2 mL) and the resultant solution stirred for 30 min. The solvent was evaporated to leave a pale yellow solid which was washed with cold diethyl ether and DCM to leave the pure title compound (116 mg, 80 %) as a white solid, mp 150-152 °C; IR (cm^{-1}) 2931, 2856, 1733, 1597, 1547, 1172. ^{1}H-NMR (CD$_3$OD, 300 MHz) δ 7.55 (2H, d, J=7.6 Hz, 2, 6-aryl), 7.30 (2H, t, J=7.3 Hz, 3, 5-aryl), 7.09 (1H, t, J=7.1 Hz, 4-aryl), 3.44 (1H, t, J=7.1' Hz, CH), 2.07 (2H, t, J=6.8 Hz, CH_2CONH), 1.92 (2H, m, CHCH_2), 1.61 (2H, m, CH_2CH$_2$CONH), 1.38 (4H, m, CHCH$_2$CH_2, CHCH$_2$CH$_2$CH_2); ^{13}C-NMR (CD$_3$OD, 75 MHz) δ 173.39 (COOH), 172.95 (CONHPh), 170.37 (CONHOH), 139.58 (1-aryl), 129.85 (3, 5-aryl), 125.53 (4-aryl), 121.40 (2, 6-aryl), 54.43 (CH), 33.66 (CH$_2$CONH), 30.17 (CHCH$_2$), 29.88 (CHCH$_2$CH$_2$$CH_2$), 28.11 ($CH_2CH_2$CONH), 26.53 (CHCH$_2$$CH_2$). LRMS (CI) m/z 331 (M+Na, 100 %), 309 (M+H, 83 %). Anal. Calcd for C$_{15}$H$_{20}$N$_2$O$_5$ C, 58.43; H, 6.54; N, 9.09. Found C, 58.16; H, 6.51; N, 8.75.

## 8-Ethoxy-8-oxo-2-(phenylcarbamoyl)octanoic acid (182)
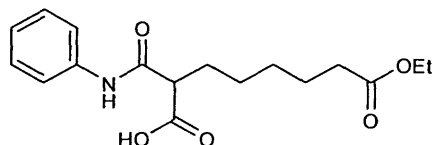
Trifluoroacetic acid (9 mL) was added to a solution of 1-*tert*-butyl-8-ethyl-2-(phenylcarbamoyl)octanedioate (**180**) (1.33 g, 3.52 mmol) in DCM (9 mL) and the solution stirred for 6 h. The solvent was removed in vacuo and the residue washed with a 1:1 solution of petroleum ether (40 - 60)-diethyl ether to give the title compound (0.91 g, 80 %) as a white powder, mp 108-110 °C; IR (cm^{-1}) 3426, 3340, 2933, 1730, 1600, 1174. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 10.17 (1H, brs, OH), 9.05 (1H, s, NH), 7.50 (2H, d, J=7.8 Hz, 2, 6-aryl), 7.30 (2H, t, J=7.7 Hz, 3, 5-aryl), 7.14 (1H, t, J=7.3 Hz, 4-aryl), 4.12 (2H, q, J=7.1 Hz, CH_2CH$_3$), 3.47 (1H, t, J=6.2 Hz, CH), 2.29 (2H, t, J=7.3 Hz, CH$_2$COO), 1.99 (2H, m, CHCH_2), 1.59 (2H, m, CH_2CH$_2$COO), 1.36 (4H, m, CH_2CH$_2$CH$_2$COO, CHCH$_2$CH_2), 1.24 (3H, t, J=7.1 Hz, CH$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 175.11 (COOH), 174.56 (COOEt), 169.13 (CONH), 136.72 (1-aryl), 129.07 (3, 5-aryl), 125.47 (4-aryl), 120.84 (2, 6-aryl), 60.94 (CH$_2$CH$_3$), 52.18 (CH), 34.14 (CH$_2$COO), 31.06 (CHCH$_2$), 28.32 (CH$_2$CH$_2$CH$_2$COO), 26.50 (CHCH$_2$CH$_2$), 24.37 (CH$_2$CH$_2$COO), 14.08 (CH$_3$). LRMS (EI) m/z 321 (M^{+}, 8 %), 277 (43 %), 232 (54 %), 179 (36 %), 135 (97 %), 93 (100 %). Anal. Calcd for C$_{17}$H$_{23}$NO$_5$ + ^{1}/$_5$H$_2$O C, 62.83; H, 7.26; N, 4.31. Found C, 63.08; H, 7.18; N, 3.92.

Ethyl 7-(hydroxymethyl)-8-oxo-8-(phenylamino)octanoate (183)
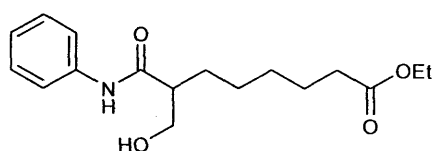
To a solution of 8-ethoxy-8-oxo-2-(phenylcarbamoyl)octanoic acid (**182**) (300 mg, 0.94 mmol) in dry tetrahydrofuran (3 mL) at 0 °C was added triethylamine (114 mg, 1.13 mmol) and ethyl chloroformate (152 mg, 1.40 mmol). The reaction was stirred at 0 °C for 30 min then filtered. The solid was washed with tetrahydrofuran (2 x 3 mL) and the combined filtrate cooled to 10 °C. Sodium borohydride (107 mg, 2.82 mmol) was added in one portion, followed by dropwise addition of methanol (0.6 mL) over a period of 1 h. After stirring for a further 30 min the reaction was quenched by dropwise addition of 2 M HCl

and extracted with DCM (10 mL). The organics were dried (MgSO$_4$) and evaporated, the crude product being purified by column chromatography (60 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (222 mg, 77 %) as a crystalline white solid, mp 132-134 °C; IR (cm^{-1}) 2935, 2669, 1684, 1408, 1250, 1188, 923. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.73 (1H, s, NH), 7.53 (2H, d, J=7.7 Hz, 2, 6-aryl), 7.23 (2H, t, J=7.9 Hz, 3, 5-aryl), 7.04 (1H, t, J= 7.4 Hz, 4-aryl), 4.08 (2H, q, J=7.1 Hz, CH$_2$CH$_3$), 3.95 (1H, brs, OH), 3.73 (1H, t, J=9.0 Hz, CH$_2$OH), 3.62 (1H, m, CH$_2$OH), 2.45 (1H, m, CH), 2.22 (2H, t, J=7.4 Hz, CH$_2$COO), 1.56 (3H, CHCHH, CH$_2$CH$_2$COO), 1.30 (8H, m, CHCHH, CH$_2$CH$_2$CH$_2$COO, CHCH$_2$CH$_2$, CH$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.13 (COOEt), 174.05 (CONH), 138.09 (1-aryl), 128.84 (3, 5-aryl), 124.24 (4-aryl), 120.20 (2, 6-aryl), 63.51 (CH$_2$OH), 60.35 (CH$_2$CH$_3$), 49.71 (CH), 34.18 (CH$_2$COO), 28.95 (CH$_2$CH$_2$CH$_2$COO), 28.74 (CHCH$_2$), 26.88 (CHCH$_2$CH$_2$), 24.61 (CH$_2$CH$_2$COO), 14.21 (CH$_3$). LRMS (CI) m/z 308 (M+H, 100 %), 262 (53 %), 197 (63 %), 169 (51 %). Anal. Calcd for C$_{17}$H$_{25}$NO$_4$ C, 66.43; H, 8.20; N, 4.56. Found C, 66.49; H, 8.34; N, 4.65.

N^8-hydroxy-2-(hydroxymethyl)-N^1-phenyloctanediamide (150)
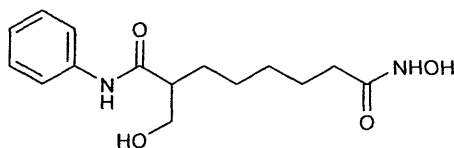
To a solution of ethyl 7-(hydroxymethyl)-8-oxo-8-(phenylamino)octanoate (183) (600 mg, 1.95 mmol) in tetrahydrofuran (20 mL) was added hydroxylamine (0.64 mL of a 50 % aqueous solution, 9.76 mmol) and 1 M methanolic potassium hydroxide solution (3.9 mL, 3.9 mmol) and the resultant solution stirred for 1.5 h. The yellow solution was evaporated and 0.5 M HCl (10 mL) added. The title compound (70 mg, 12 %) crystallised from the aqueous solution as an off-white solid, mp 150-151 °C; IR (cm^{-1}) 3398, 3291, 3185, 2920, 1626, 1543, 1446, 968. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.30 (1H, s, NH), 9.83 (1H, s, NHOH), 8.62 (NH), 7.62 (2H, d, J=7.8 Hz, 2, 6-aryl), 7.27 (2H, t, J=7.9 Hz, 3, 5-aryl), 7.00 (1H, t, J=7.4 Hz, 4-aryl), 4.71 (1H, t, J=5.0 Hz, OH), 3.58 (1H, m, CHHOH), 3.45 (1H, td, J=5.1, 10.2 Hz, CHHOH), 2.50 (1H, m, CH), 1.90 (2H, t, J=7.3 Hz, CH$_2$CONH), 1.42 (4H, m, CH$_2$CH$_2$CONH, CHCH$_2$), 1.24 (4H, m, CHCH$_2$CH$_2$CH$_2$, CHCH$_2$CH$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 173.02 (PhNHCO), 169.03 (CONHOH), 139.29 (1-aryl), 128.50 (3,

5-aryl), 122.87 (4-aryl), 119.09 (2, 6-aryl), 62.89 (CH_2OH), 49.75 (CH), 32.18 (CH_2CONH), 28.67 ($CHCH_2CH_2CH_2$), 28.57 ($CHCH_2$), 26.60 ($CHCH_2CH_2$), 24.96 (CH_2CH_2CONH). LRMS (CI) m/z 317 (M+Na, 100 %), 285 (17 %), 276 (15 %), 242 (13 %), 217 (13 %). Anal.Calcd for $C_{15}H_{22}N_2O_4 + \frac{1}{5}H_2O$ C, 60.47; H, 7.46; N, 9.40. Found: C, 60.71; H, 7.49; N, 9.39.

tert-Butyl 2-aminophenylcarbamate (185)
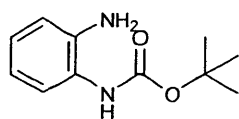
A solution of di-*tert*-butyl dicarbonate (10.09 g, 46.24 mol) in tetrahydrofuran (20 mL) was added to a solution of 1,2-phenylenediamine (5.0 g, 46.24 mmol) in tetrahydrofuran (80 mL) and the solution stirred for 2 h. The solvent was evaporated and the crude solid purified by column chromatography (20 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (7.30 g, 76 %) as a white, crystalline solid, mp 112-116 °C; ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.26 (1H, d, J=7.6 Hz, 6-aryl), 6.99 (1H, dt, J=1.4, 7.7 Hz, 4-aryl), 6.76 (2H, m, 3, 5-aryl), 6.36 (1H, brs, NH), 3.74 (2H, brs, NH$_2$), 1.51 (9H, s, C(CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 153.93 (CO), 140.01 (2-aryl), 126.13 (4, 1-aryl), 124.79 (6-aryl), 119.56 (5-aryl), 117.57 (3-aryl), 80.50 (C(CH$_3$)$_3$), 28.37 (C(CH$_3$)$_3$). LRMS (EI) m/z 208 (M+, 11 %), 152 (28 %), 108 (100 %).

Ethyl 8-(2-(tert-butoxycarbonylamino)phenylamino)-8-oxo-7-(phenylcarbamoyl)octanoate (186)
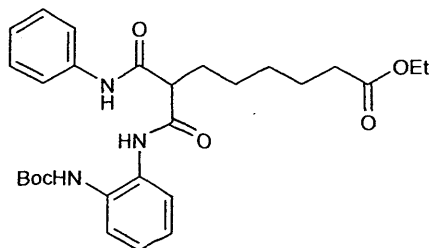
To a solution of 8-ethoxy-8-oxo-2-(phenylcarbamoyl)octanoic acid (182) (1.0 g, 3.11 mmol) in DMF (30 mL) was added *tert*-butyl 2-aminophenylcarbamate (648 mg, 3.11

mmol), triethylamine (692 mg, 6.84 mmol), EDC.HCl (657 mg, 3.42 mmol) and HOBt (462 mg, 3.42 mmol). The solution was stirred for 18 h then the solvent evaporated and water (50 mL) added. The solution was extracted with ethyl acetate (2 x 25 mL) and the combined extracts washed with water (15 mL), saturated ammonium chloride solution (15 mL) and saturated aqueous sodium hydrogen carbonate solution (15 mL). The organic phase was dried over MgSO₄ and evaporated to leave a yellow oil which was purified by column chromatography (33 % petroleum ether (40 - 60)-ethyl acetate) to give the title compound (1.05 g, 66 %) as a white solid, used without further purification.

Ethyl 7-(1*H*-benzo[*d*]imidazol-2-yl)-8-oxo-8-(phenylamino)octanoate (187)
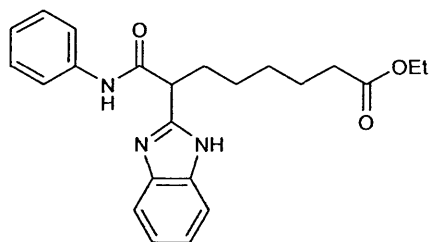
From ethyl 8-(2-aminophenylamino)-8-oxo-7-(phenylcarbamoyl)octanoate:

A solution of ethyl 8-(2-aminophenylamino)-8-oxo-7-(phenylcarbamoyl)octanoate (620 mg, 1.36 mmol) in glacial acetic acid (6.8 mL) was heated to 100 °C for 1 h. The solvent was evaporated and the crude product recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (410 mg, 77 %) as a crystalline white solid, mp 211-212 °C; IR (cm⁻¹) 3284, 2926, 1734, 1666, 1430, 1186, 730. ¹H-NMR (CDCl₃, 300 MHz) δ 11.95 (1H, s, NH), 11.47 (1H, s, NH), 7.66 (2H, d, m, 2, 6-aryl, 7-benzimidazolyl), 7.48 (1H, m, 6-benzimidazolyl), 7.25 (4H, m, 3, 5-aryl, 5, 8-benzimidazolyl), 7.10 (1H, t, *J*=7.4 Hz, 4-aryl), 4.65 (1H, t, *J*=7.7 Hz, CH), 4.02 (2H, q, *J*=7.1 Hz, CH₂CH₃), 2.32 (1H, m, CHC*H*H), 2.15 (1H, m, CHCH*H*), 1.99 (2H, t, *J*=7.5 Hz, CH₂COO), 1.36 (4H, m, CHCH₂C*H*₂, C*H*₂CH₂COO), 1.19 (5H, m, C*H*₂CH₂CH₂COO, CH₂C*H*₃); ¹³C-NMR (CDCl₃, 75 MHz) δ 173.52 (COO), 170.84 (NHCO), 153.32 (2-benzimidazolyl), 142.08 (9-benzimidazolyl), 138.16 (1-aryl), 134.20 (4-benzimidazolyl), 128.99 (3, 5-aryl), 124.70 (4-aryl), 123.15 (8-benzimidazolyl), 122.43 (5-benzimidazolyl), 120.41 (2, 6-aryl), 118.34 (7-benzimidazolyl), 111.69 (6-benzimidazolyl), 60.12 (CH₂CH₃), 48.26 (CH), 33.95 (CH₂COO), 33.60 (CHCH₂), 28.52 (CH₂CH₂CH₂COO), 27.21 (CHCH₂CH₂), 24.54 (CH₂CH₂COO), 14.21

(CH$_2$CH$_3$). LRMS (CI) m/z 394 (M+H, 100 %), 293 (58 %), 274 (23 %). Anal. Calcd for C$_{23}$H$_{27}$N$_3$O$_3$ C, 70.21; H, 6.92; N, 10.68. Found C, 70.17; H, 7.01; N, 10.79.

From ethyl 8-(2-(tert-butoxycarbonylamino)phenylamino)-8-oxo-7-(phenylcarbamoyl)octanoate (186):

To a solution of ethyl 8-(2-(tert-butoxycarbonylamino)phenylamino)-8-oxo-7-(phenylcarbamoyl)octanoate (186) (0.88 g, 1.72 mmol) in DCM (9 mL) was added trifluoroacetic acid (9 mL) and the solution refluxed for 2 h. The solvent was evaporated and the residue recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (505 mg, 75 %).

tert-Butyl 2-(8-ethoxy-1,8-dioxo-1-(phenylamino)octan-2-yl)-1*H*-benzo[*d*]imidazole-1-carboxylate (188)
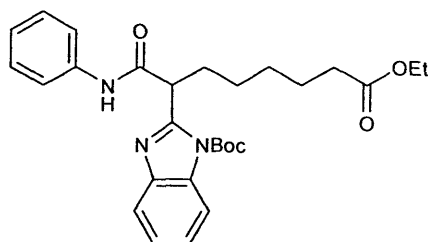
Di-*tert*-butyl dicarbonate (403 mg, 1.85 mmol) was added to a solution of ethyl 7-(1*H*-benzo[*d*]imidazol-2-yl)-8-oxo-8-(phenylamino)octanoate (187) (484 mg, 1.23 mmol) in tetrahydrofuran (12 mL) and the colourless solution stirred for 7 d at room temperature. The solvent was evaporated and the product purified by column chromatography (20 % ethyl acetate-petroleum ether (40 - 60)) to give the starting material (220 mg, 45 %) and the title compound (210 mg, 35 %) as a colourless oil; IR (cm^{-1}) 3319, 2936, 1732, 1600, 1152, 744. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 9.46 (1H, s, NH), 7.89 (1H, m, 8-benzimidazolyl), 7.77 (1H, m, 5-benzimidazolyl), 7.57 (2H, d, J=8.5 Hz, 2, 6-aryl), 7.31 (4H, m, 6, 7-benzimidazolyl, 3, 5-aryl), 7.04 (1H, t, J=7.4 Hz, 4-aryl), 4.82 (1H, t, J=7.2 Hz, CH), 4.07 (2H, q, J=7.1 Hz, C*H*$_2$CH$_3$), 2.28 (4H, m, CHC*H*$_2$, CH$_2$COO), 1.72 (9H, s, C(CH$_3$)$_3$), 1.61 (2H, m, C*H*$_2$CH$_2$COO), 1.43 (4H, m, CHCH$_2$C*H*$_2$, CHCH$_2$CH$_2$C*H*$_2$), 1.21 (3H, t, J=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.65 (COOEt), 168.09 (CONH), 154.12 (COO^{t}Bu), 149.18 (2-benzimidazolyl), 141.71 (9-benzimidazolyl), 138.19 (1-aryl), 132.63

(4-benzimidazolyl), 128.89 (3, 5-aryl), 124.99 (7-benzimidazolyl), 124.52 (6-benzimidazolyl), 124.06 (4-aryl), 119 93 (8-benzimidazolyl), 119.75 (2, 6-aryl), 115.18 (5-benzimidazolyl), 86.71 (C(CH3)3), 60.15 (CH_2CH_3), 48.13 (CH), 34.21 (CH_2COO), 33.48 ($CHCH_2$), 28.86 (CHCH$_2$CH$_2$CH$_2$), 28.08 (C(CH_3)$_3$), 26.99 (CHCH$_2CH_2$), 24.69 (CH_2CH$_2$COO), 14.25 (CH$_2$$CH_3$). LRMS (CI) m/z 516 (M+Na, 49 %), 494 (M+H, 92 %), 394 (28 %), 335 (100 %), 276 (19 %). HRMS (M+H) calcd for $C_{28}H_{35}N_3O_5$ 494.26550. Found: 494.26504.

## 2-(1H-Benzo[d]imidazol-2-yl)-N^8-hydroxy-N^1-phenyloctanediamide (152)
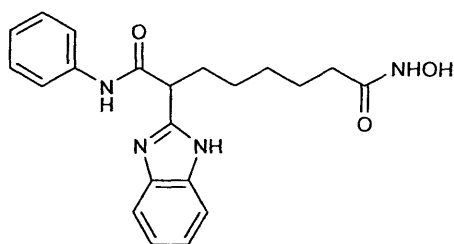
To a solution of $tert$-butyl 2-(8-ethoxy-1,8-dioxo-1-(phenylamino)octan-2-yl)-1H-benzo[d]imidazole-1-carboxylate (188) (210 mg, 0.43 mmol) in tetrahydrofuran (4 mL) was added hydroxylamine (0.28 mL of a 50 % aqueous solution, 4.25 mmol) followed by dropwise addition of 1 M methanolic KOH (1.29 mL). The pale yellow solution was stirred for 6 h the evaporated. Water (10 mL) was added followed by saturated aqueous ammonium chloride solution (10 mL) and the white precipitate collected by filtration. The crude product was recrystallised from ethanol to give the title compound (81 mg, 50 %) as a crystalline white solid, mp 184-185 °C; IR (cm^{-1}) 3261, 3093, 2924, 1637, 1437, 1133. ^{1}H-NMR (CD$_3$OD, 300 MHz) δ 7.58 (4H, m, 2, 6-aryl, 5, 8-benzimidazolyl), 7.27 (2H, t, J=7.7 Hz, 3, 5-aryl), 7.19 (2H, m, 6, 7-benzimidazolyl), 7.06 (1H, t, J=7.3 Hz, 4-aryl), 4.09 (1H, m, CH), 2.13 (4H, m, CHCH_2, CH_2CONH), 1.58 (2H, m, CH_2CH$_2$CONH), 1.37 (4H, m, CHCH$_2$CH_2, CHCH$_2$CH$_2$CH_2); ^{13}C-NMR ((CD$_3$)$_2$SO, 125 MHz) δ 169.29 (PhNHCO), 169.25 (CONHOH), 152.66 (2-benzimidazolyl), 142.65 (benzimidazolyl), 138.92 (1-aryl), 134.60 (benzimidazolyl), 128.74 (3, 5-aryl), 123.51 (4-aryl), 121.80 (benzimidazolyl), 121.01 (benzimidazolyl), 119.28 (benzimidazolyl), 118.33 (benzimidazolyl), 111.25 (benzimidazolyl), 47.79 (CH), 32.22 (CH_2CONHOH), 31.14 (CHCH_2), 28.36

(CHCH$_2$CH$_2$CH$_2$), 26.79 (CHCH$_2$CH$_2$), 24.95 (CH$_2$CH$_2$CONHOH). Anal. Calcd for C$_{21}$H$_{24}$N$_4$O$_3$ + ^{1}/$_3$H$_2$O C, 65.27; H, 6.43; N, 14.50. Found C, 65.18; H, 6.36; N, 14.13.

tert-Butyl 3-(2-aminophenylamino)-3-oxopropanoate (189)
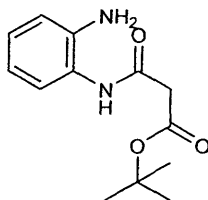
To a solution of 3-*tert*-butoxy-3-oxopropanoic acid (178) (200 mg, 1.25 mmol) and 1,2-phenylenediamine (135 mg, 1.25 mmol) in acetonitrile (5 mL) was added a solution of DCC (283 mg, 1.37 mmol) in acetonitrile (2 mL) and the reaction stirred for 20 min. The white suspension was filtered and evaporated to leave a pale yellow/brown oil which was purified by column chromatography (ethyl acetate-petroleum ether (40 - 60), 1:1) followed by recrystallisation from ethyl acetate-petroleum ether (40 - 60) to give the title compound (155 mg, 50 %) as a white, crystalline solid, mp 122-123 °C; IR (cm^{-1}) 3428, 3318, 1718, 1655, 1353, 750. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 8.89 (1H, s, NH), 7.26 (1H, m, 3-aryl), 7.05 (1H, m, 5-aryl), 6.78 (2H, m, 4, 6-aryl), 3.59 (2H, brs, NH$_2$), 3.40 (2H, s, CH$_2$), 1.50 (9H, s, (CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 169.08 (COOBu^t), 164.18 (CONH), 140.59 (2-aryl), 127.17 (4-aryl), 125.26 (6-aryl), 123.75 (1-aryl), 119.26 (5-aryl), 117.69 (3-aryl), 83.07 (C(CH$_3$)$_3$), 42.40 (CH$_2$), 28.03 ((CH$_3$)$_3$). Anal. Calcd for C$_{13}$H$_{18}$N$_2$O$_3$ C, 62.38; H, 7.25; N, 11.19. Found C, 62.29; H, 7.28; N, 11.13.
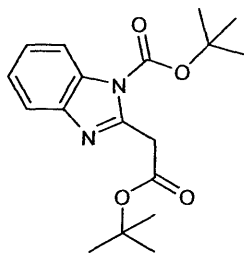
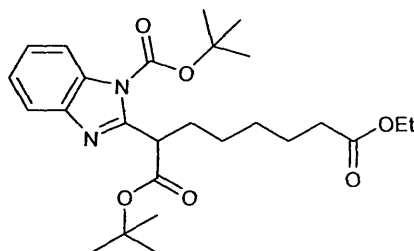### *tert*-Butyl 2-(1*H*-benzo[*d*]imidazol-2-yl)acetate (190)
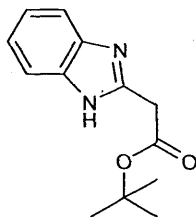
tert-Butyl 3-(2-aminophenylamino)-3-oxopropanoate (189) (70 mg, 0.28 mmol) was dissolved in acetic acid (0.15 mL) and the solution heated to 90 °C for 1 h. The solvent was removed in vacuo and the crude product recrystallised from ethyl acetate-petroleum ether

(40 - 60) to give the title compound (62 mg, 96 %) as a white, crystalline solid, mp 158-161 °C; IR (cm^{-1}) 2984, 1722, 1435, 1270, 1156, 755. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 10.01 (1H, brs, NH), 7.56 (2H, dd, J=3.2, 6.0 Hz, 4, 7-benzimidazolyl), 7.22 (2H, dd, J=3.2, 6.0 Hz, 5, 6-benzimidazolyl), 3.99 (2H, s, CH$_2$), 1.46 (9H, s, (CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 168.99 (COOBu^t), 147.79 (2-benzimidazolyl), 138.27 (4, 9-benzimidazolyl), 122.45 (6, 7-benzimidazolyl), 114.98 (5, 8-benzimidazolyl), 82.69 (C(CH$_3$)$_3$), 35.65 (CH$_2$), 28.01 ((CH$_3$)$_3$). Anal. Calcd for C$_{13}$H$_{16}$N$_2$O$_2$ C, 67.22; H, 6.49; N, 12.06. Found C, 67.10; H, 6.97; N, 11.97.
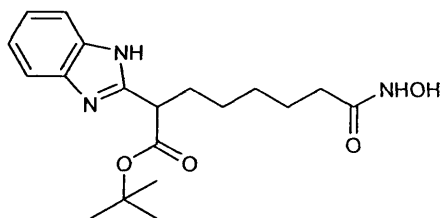
tert-Butyl 2-(2-tert-butoxy-2-oxoethyl)-1H-benzo[d]imidazole-1-carboxylate (191)

To a solution of *tert*-butyl 2-(1H-benzo[d]imidazol-2-yl)acetate (190) (3.37 g, 14.52 mmol) in tetrahydrofuran (33 mL) was added di-*tert*-butyl dicarbonate (3.80 g, 17.43 mmol) and the solution stirred for 3 d at room temperature. The solvent was evaporated and the product subjected to column chromatography (20 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (4.68 g, 97 %) as a viscous, colourless oil which solidified on standing to a white solid, mp 82-83 °C; IR (cm^{-1}) 2979, 1750, 1729, 1361, 1258, 1127. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.87 (1H, m, 7-benzimidazolyl), 7.67 (1H, m, 4-benzimidazolyl), 7.30 (2H, m, 5, 6-benzimidazolyl), 4.19 (2H, s, CH$_2$), 1.68 (9H, s, (CH$_3$)$_3$), 1.43 (9H, s, (CH$_3$)$_3$); ^{13}C-NMR (CDCl$_3$, 300 MHz) δ 167.87 (NCOO), 149.67 (CH$_2$COO), 148.91 (2-benzimidazolyl), 142.07 (4-benzimidazolyl), 132.90 (9-benzimidazolyl), 124.63 (6-benzimidazolyl), 124.12 (7-benzimidazolyl), 119.84 (5-benzimidazolyl), 114.93 (8-benzimidazolyl), 85.66 (C(CH$_3$)$_3$), 81.72 (C(CH$_3$)$_3$), 39.10 (CH$_2$), 28.02 ((CH$_3$)$_3$), 27.99 ((CH$_3$)$_3$). Anal. Calcd for C$_{18}$H$_{24}$N$_2$O$_4$ C, 65.04; H, 7.28; N, 8.43. Found C, 65.02; H, 7.26; N, 8.30.

1-*tert*-butyl 8-ethyl 2-(1-(tert-butoxycarbonyl)-1*H*-benzo[*d*]imidazol-2-yl)octanedioate (192)

Sodium hydride (337 mg of a 60 % dispersion in mineral oil, 14.03 mmol) was added to a solution of *tert*-butyl 2-(2-tert-butoxy-2-oxoethyl)-1*H*-benzo[*d*]imidazole-1-carboxylate (191) (4.24 g, 12.76 mmol) in dry tetrahydrofuran (130 mL). The suspension was stirred for 30 min to give a pale yellow, clear solution, then ethyl 6-bromohexanoate (3.13 g, 14.03 mmol) added and the solution stirred at 60 °C for 18 h. A further portion of sodium hydride (337 mg, 14.03 mmol) was added to the reaction (with effervescence) and stirring continued at 60 °C for 4 h. The solvent was evaporated and replaced with ethyl acetate (80 mL). The suspension was washed with saturated aqueous ammonium chloride solution (40 mL), dried over $MgSO_4$ and evaporated to leave a brown oil. The crude product was purified by column chromatography (15 % ethyl acetate-petroleum ether (40 - 60)) to give the title compound (2.01 g, 33 %) as a yellow oil; IR (cm^{-1}) 2978, 1731, 1453, 1333, 1152, 745. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.83 (1H, m, 7-benzimidazolyl), 7.69 (1H, m, 4-benzimidazolyl), 7.26 (2H, m, 5, 6-benzimidazolyl), 4.39 (1H, dd, *J*=6.1, 8.3 Hz, CH), 4.05 (2H, q, *J*=7.1 Hz, C*H*$_2$CH$_3$), 2.36-2.09 (4H, m, CHC*H*$_2$, CH$_2$COO), 1.61 (11H, m, (CH$_3$)$_3$, C*H*$_2$CH$_2$COO), 1.44 (13H, m, CHCH$_2$C*H*$_2$, CHCH$_2$CH$_2$C*H*$_2$, (CH$_3$)$_3$), 1.18 (3H, t, *J*=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.64 (COOEt), 170.50 (CHCOOBu^t), 153.50 (NCOO), 149.07 (2-benzimidazolyl), 142.08 (4-benzimidazolyl), 132.83 (9-benzimidazolyl), 124.44 (6-benzimidazolyl), 123.98 (7-benzimidazolyl), 120.00 (5-benzimidazolyl), 114.95 (8-benzimidazolyl), 85.53 (*C*(CH$_3$)$_3$), 81.19 (*C*(CH$_3$)$_3$), 60.05 (*C*H$_2$CH$_3$), 48.23 (CH), 34.21, (*C*H$_2$COO), 29.82 (CH*C*H$_2$), 28.89 (CHCH$_2$CH$_2$CH$_2$), 28.05 ((CH$_3$)$_3$), 27.83 ((CH$_3$)$_3$), 27.45 (CHCH$_2$*C*H$_2$), 24.72 (*C*H$_2$CH$_2$COO), 14.22 (CH$_2$*C*H$_3$). LRMS (EI) *m/z* 497 (M+H, 34 %), 441 (33 %), 397 (57 %), 297 (100 %), 251 (86 %). HRMS (M+H) calcd for C$_{26}$H$_{38}$N$_2$O$_6$ 497.26276. Found: 497.26199.

tert-Butyl 2-(1*H*-benzo[*d*]imidazol-2-yl)-8-(hydroxyamino)-8-oxooctanoate (153)

To a solution of 1-*tert*-butyl 8-ethyl 2-(1-(*tert*-butoxycarbonyl)-1*H*-benzo[*d*]imidazol-2-yl)octanedioate (**192**) (1.0 g, 2.11 mmol) in tetrahydrofuran (20 mL) was added hydroxylamine (1.39 mL of a 50 % aqueous solution, 21.07 mmol) followed by dropwise addition of 1 M methanolic potassium hydroxide (6.33 mL). The solution was stirred for 30 min and the solvent evaporated to leave a yellow oil. Water (15 mL) was added and the solution neutralised with saturated aqueous ammonium hydroxide solution. On cooling, the product precipitated from the solution and was collected by filtration. The crude product was recrystallised from ethanol-water to give the title compound (508 mg, 67 %) as a white crystalline solid, mp 110-115 °C; IR (cm^{-1}) 3341, 2934, 1704, 1650, 1438, 1274, 1151. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 12.31 (1H, brs, OH), 10.32 (1H, brs, NH), 8.65 (1H, brs, NH), 7.54 (1H, d, *J*=7.6 Hz, 5-benzimidazolyl), 7.44 (1H, d, *J*=7.0 Hz, 8-benzimidazolyl) 7.12 (2H, m, 6, 7-benzimidazolyl), 3.80 (1H, t, *J*=7.7 Hz, CH), 1.98 (2H, m, CHC*H*$_2$), 1.90 (2H, t, *J*=7.3 Hz, C*H*$_2$CONH), 1.45 (2H, m, C*H*$_2$CH$_2$CONH), 1.37 (9H, s, C(CH$_3$)$_3$), 1.24 (4H, m, CHCH$_2$C*H*$_2$, CHCH$_2$CH$_2$C*H*$_2$); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 170.18 (COO), 168.97 (CONH), 151.73 (2-benzimidazolyl), 142.80 (4-benzimidazolyl), 134.35 (9-benzimidazolyl), 121.78 (7-benzimidazolyl), 120.93 (6-benzimidazolyl), 118.44 (5-benzimidazolyl), 111.08 (8-benzimidazolyl), 80.81 (*C*(CH$_3$)$_3$), 46.79 (CH), 32.13 (*C*H$_2$CONH), 30.46 (CHCH$_2$), 28.19 (CHCH$_2$CH$_2$C*H*$_2$), 27.56 (C(*C*H$_3$)$_3$), 26.52 (*C*H$_2$CH$_2$CONH), 24.86 (CHCH$_2$C*H*$_2$). LRMS (EI) *m/z* 384 (M+Na, 100 %), 362 (54 %), 329 (79 %), 306 (36 %), 176 (99 %), 154 (99 %). HRMS (M+Na) calcd for C$_{19}$H$_{27}$N$_3$O$_4$ 384.18992. Found: 384.19056.

6-Ethyl 1,1-bis(*tert*-butyl) hexane-1,1,6-tricarboxylate (194)
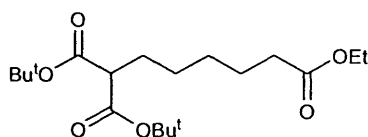
To a solution of di-*tert*-butyl malonate (5.0 g, 23.13 mmol) in dry tetrahydrofuran 230 mL) was added sodium hydride (1.02 g of a 60 % dispersion in mineral oil, 25.44 mmol). After stirring for 20 min at room temperature, ethyl-6-bromohexanoate (5.16 g, 23.13 mmol) was added and the reaction refluxed at 85 °C for 16 h. The solvent was then evaporated and water (50 mL) added. The aqueous phase was extracted with diethyl ether (2 x 30 mL) and the combined organics dried (MgSO$_4$) and the solvent removed *in vacuo* leaving a pale yellow oil. The crude product was purified by column chromatography (10 % ethyl acetate-petroleum ether (40 - 60)) to give the product (6.11 g, 74 %) as a colourless oil; IR (cm^{-1}) 2935, 1732, 1369, 1138, 847. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 4.04 (2H, q, *J*=7.1 Hz, CH$_2$O), 3.02 (2H, t, *J*=7.6 Hz, CH$_2$COOEt), 2.20 (2H, t, *J*=7.49 Hz, CHC*H*$_2$), 1.71 (2H, m, C*H*$_2$CH$_2$COOEt, 1.55 (2H, m, CHCH$_2$CH$_2$C*H*$_2$), 1.38 (18H, s, C(CH$_3$)$_3$), 1.26 (4H, m, CHC*H*$_2$, CHCH$_2$C*H*$_2$), 1.17 (3H, t, *J*=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 173.51 (*C*OOEt), 168.80 (*C*OOC(CH$_3$)$_3$), 81.09 (*C*(CH$_3$)$_3$), 60.06 (*C*H$_2$CH$_3$), 53.80 (CH), 34.12 (*C*H$_2$COOEt), 28.70 (CH*C*H$_2$), 28.30 (*C*H$_2$CH$_2$CH$_2$COOEt), 27.84 (C(*C*H$_3$)$_3$), 26.78 (CHCH$_2$*C*H$_2$), 24.62 (*C*H$_2$CH$_2$COOEt), 14.16 (*C*H$_3$CH$_2$). LRMS (CI) *m/z* 381 (M+H, 100 %), 269 (25 %), 247 (75 %), 201 (13 %). HRMS (M+Na) calcd for C$_{11}$H$_{18}$O$_6$ 381.22530. Found: 381.22599.

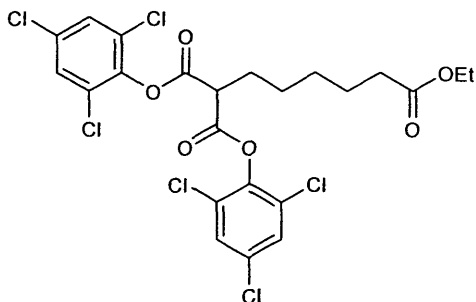## 2-Carboxy-octanedioic acid 8-ethyl ester (195)
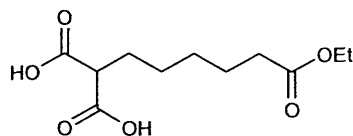
To a solution of 6-ethyl 1,1-bis(*tert*-butyl) hexane-1,1,6-tricarboxylate (194) (6.09 g, 16.99 mmol) in DCM (170 mL) was added trifluoroacetic acid (11.62 g, 101.9 mmol) and the solution stirred for 1 d. The volatile material was then removed in vacuo, leaving the acid (4.14 g, 99 %) as a white, crystalline solid without further purification, mp 71-73 °C; IR (cm^{-1}) 2939, 2613, 1705, 1183, 916. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 4.12 (2H, q, *J*=7.1 Hz, C*H*$_2$CH$_3$), 3.42 (1H, t, *J*=7.4 Hz, CH), 2.30 (2H, t, *J*=7.4 Hz, C*H*$_2$COOEt), 1.93 (2H, m, C*H*$_2$CH$_2$COOEt), 1.61 (2H, m, CHC*H*$_2$), 1.37 (4H, m, CHCH$_2$C*H*$_2$, CHCH$_2$CH$_2$C*H*$_2$), 1.24 (3H, t, *J*=7.1 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.62 (COOH), 174.55 (COOEt), 60.69 (*C*H$_2$CH$_3$), 51.46 (CH), 34.21 (*C*H$_2$COOEt), 28.59 (CH*C*H$_2$CH$_2$CH$_2$),

28.46 (CHCH₂), 26.82 (CHCH₂CH₂), 24.53 (CH₂CH₂COOEt), 14.16 (CH₂CH₃). LRMS (CI) m/z 247 (M+H, 31 %), 201 (21 %), 185 (100 %), 157 (27 %). Anal.Calcd for $C_{11}H_{18}O_6$ C, 53.65; H, 7.37; N, 0.00. Found: C, 53.48; H, 7.41; N, 0.00.
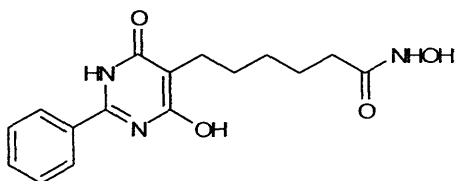
6-Ethyl 1,1-bis(2,4,6-trichlorophenyl) hexane-1,1,6-tricarboxylate (196)

A solution of 2-carboxy-octanedioic acid 8-ethyl ester (195) (1.34 g, 5.44 mmol) and trichlorophenol (2.15 g, 10.88 mmol) in phosphorusoxychloride (2.17 g, 14.4 mmol) was heated to 100 °C for 5 h, then water (10 mL) added. The aqueous solution was extracted with ethyl acetate (2 x 10 mL) and the combined extracts washed with saturated aqueous sodium hydrogen carbonate solution (10 mL) and brine (10 mL). The solution was dried (MgSO₄) and the solvent removed *in vacuo* leaving a brown oil which was purified by column chromatography (5 % ethyl acetate-petroleum ether (40 - 60)) to yield a colourless oil (1.61 g, 49 %); IR (cm⁻¹) 2937, 1769, 1731, 1566, 1446, 1231. ¹H-NMR (CDCl₃, 300 MHz) δ 7.38 (4H, m, 3, 5-aryl), 4.13 (2H, q, J=7.1 Hz, CH₂CH₃), 4.05 (1H, t, J=7.4 Hz, COCHCO), 2.29 (4H, m, CHCH₂, CH₂CO₂Et), 1.65 (4H, m, CH₂CH₂CO₂Et, CHCH₂CH₂), 1.47 (2H, m, CHCH₂CH₂CH₂), 1.25 (3H, t, J=7.1 Hz, CH₂CH₃). ¹³C-NMR (CDCl₃, 75 MHz) δ 173.52 (COOEt), 164.61 (COOAr), 142.44 (1-Ar), 132.56 (4-Ar), 129.53 (Ar) 128.75 (Ar), 60.30 (CH₂CH₃), 50.72 (CH), 34.12 (CH₂COOEt), 29.24 (CHCH2), 28.62 (CH₂CH₂CH₂COOEt), 26.97 (CHCH₂CH₂), 24.60 (CH₂CH₂COOEt), 14.12 (CH₃CH₂). LRMS (CI) m/z 626 (18 %), 211 (26 %), 176 (100 %). HRMS calcd for $C_{23}H_{20}Cl_6O_6$ (M+Na) 624.92887, found 624.92751.

Ethyl 6-(4-hydroxy-6-oxo-2-phenyl-1,6-dihydropyrimidin-5-yl)hexanoate (197)
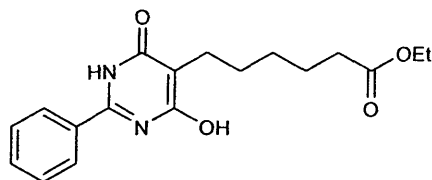

Benzamidine (361 mg, 3.01 mmol) was added to a solution of 6-ethyl 1,1-bis(2,4,6-trichlorophenyl) hexane-1,1,6-tricarboxylate (**196**) (1.82 g, 3.01 mmol) in DCM (10 mL) and the resultant solution stirred for 30 min at 40 °C. The solvent was evaporated, the residue washed with diethyl ether (30 mL) and filtered to give the title compound (805 mg, 81 %) as a yellow solid, mp 272-273 °C; IR (cm^{-1}) 2921, 2640, 1738, 1611, 1500, 1185. ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 11.71 (1H, brs, OH), 8.05 (2H, m, 2, 6-aryl), 7.51 (3H, m, 3, 4, 5-aryl), 4.02 (2H, q, J=7.1 Hz, CH_2CH$_3$), 2.29 (4H, m, CCH$_2$, CH$_2$COO), 1.59-1.36 (4H, m, CCH$_2$CH_2, CH_2CH$_2$COO), 1.28 (2H, m, CCH$_2$CH$_2$CH_2), 1.15 (3H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR ((CD$_3$)$_2$SO, 75 MHz) δ 172.87 (COO), 165.13 (4, 6-pyrimidyl), 153.94 (2-pyrimidyl), 132.11 (1-aryl), 131.33 (4-aryl), 128.49 (3, 5-aryl), 127.41 (2, 6-aryl), 101.00 (5-pyrimidyl), 59.55 (CH$_2$CH$_3$), 33.43 (CH$_2$COO), 28.32 (CCH$_2$CH$_2$CH$_2$), 27.20 (CCH$_2$CH$_2$), 24.34 (CH$_2$CH$_2$COO), 21.96 (CCH$_2$), 14.02 (CH$_2$CH$_3$). LRMS (CI) m/z 411 (M+Na, 100 %), 389 (M+H, 64 %), 321 (17 %), 192 (70 %). Anal.Calcd for C$_{18}$H$_{22}$N$_2$O$_4$ + ¼H$_2$0 C, 64.56; H, 6.77; N, 8.37. Found: C, 64.83; H, 6.60; N, 8.24.

N-Hydroxy-6-(4-hydroxy-6-oxo-2-phenyl-1,6-dihydropyrimidin-5-yl)hexanamide (154)

Benzamidine (361 mg, 3.01 mmol) was added to a solution of 6-ethyl 1,1-bis(2,4,6-trichlorophenyl) hexane-1,1,6-tricarboxylate (**197**) (1.82 g, 3.01 mmol) in DCM (10 mL) and the resultant solution stirred for 30 min at 40 °C. The solvent was evaporated, the residue washed with diethyl ether (30 mL) and filtered to give the title compound (805 mg, 81 %) as a yellow solid, mp 272-273 °C; ^{1}H-NMR ((CD$_3$)$_2$SO, 300 MHz) δ 11.71 (1H, brs, OH), 8.05 (2H, m, 2, 6-aryl), 7.51 (3H, m, 3, 4, 5-aryl), 4.02 (2H, q, J=7.1 Hz, CH_2CH$_3$), 2.29 (4H, m, CCH$_2$, CH$_2$COO), 1.59-1.36 (4H, m, CCH$_2$CH_2, CH_2CH$_2$COO), 1.28 (2H, m,

$CCH_2CH_2CH_2$), 1.15 (3H, t, J=7.1 Hz, CH_2CH_3); ^{13}C-NMR (($CD_3)_2SO$, 75 MHz) δ 172.87 (COO), 165.13 (4, 6-pyrimidyl), 153.94 (2-pyrimidyl), 132.11 (1-aryl), 131.33 (4-aryl), 128.49 (3, 5-aryl), 127.41 (2, 6-aryl), 101.00 (5-pyrimidyl), 59.55 (CH_2CH_3), 33.43 (CH_2COO), 28.32 ($CCH_2CH_2CH_2$), 27.20 (CCH_2CH_2), 24.34 (CH_2CH_2COO), 21.96 (CCH_2), 14.02 (CH_2CH_3). LRMS (CI) m/z 411 (M+Na, 100 %), 389 (M+H, 64 %), 321 (17 %), 192 (70 %). Anal.Calcd for $C_{18}H_{22}N_2O_4$ + ¼H_2O C, 64.56; H, 6.77; N, 8.37. Found: C, 64.83; H, 6.60; N, 8.24.

Diethyl-cyanamide (200)[14]
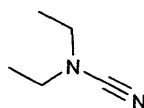
A solution of cyanogen bromide (2.0 g, 18.9mmol) in dry diethyl ether (9 mL) was added drop-wise to a solution of diethylamine (2.76 g, 37.8 mmol) in dry diethyl ether (7.5 mL) at 0 °C. The reaction was stirred for a further 30 min at 0 °C then the white precipitate removed by filtration and washed with diethyl ether. The combined organic phases were dried ($MgSO_4$) and the solvent removed *in vacuo* to leave the product (1.40 g, 75 %) as a pale yellow oil requiring no further purification; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.89 (4H, q, J=7.2 Hz, CH_2), 1.11 (6H, t, J=7.3 Hz, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 117.16 (NCN), 45.77 (CH_2), 12.72 (CH_3). LRMS (CI) m/z 99 (M+H, 100 %).
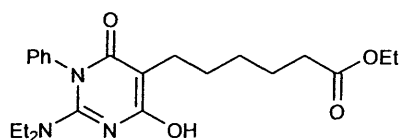
***N,N*-Diethyl-*N'*-phenyl-guanidine (201)**
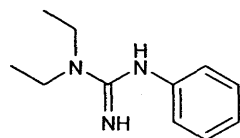
A solution of diethyl cyanamide (200) (3.37 g, 34.3 mmol) and aniline hydrochloride (4.45 g, 34.3 mmol) in dry toluene (50 mL) was refluxed for 3 d. The solution was allowed to cool to room temperature and water (20 mL) added. The organic layer was separated and the aqueous phase extracted with diethyl ether (20 mL) before being made basic by the addition of sodium hydroxide. The basic aqueous solution was extracted with diethyl ether (2 x 20 mL). The combined organics were dried ($MgSO_4$) and the solvent removed *in vacuo* leaving a brown oil which was purified by column chromatography (10 % methanol-ethyl acetate, 1

% triethylamine) to give the product as a brown oil (0.94 g, 15 %); ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.23 (2H, t, J=7.1 Hz, 2, 6-aryl), 6.89 (3H, m, 3, 4, 5-aryl), 3.89 (1H, brs, NH), 3.35 (4H, q, J=7.1 Hz, CH_2CH$_3$), 1.18 (6H, t, J=7.1 Hz, CH$_2$CH_3); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 151.03 (CNH), 150.89 (1-aryl), 129.22 (3, 5-aryl), 123.52 (2, 6-aryl), 121.35 (4-aryl), 41.82 (CH$_2$), 13.71 (CH$_3$). LRMS (CI) m/z 192 (M+H, 100 %), 175 (19 %), 119 (21 %).

Ethyl 6-(2-(diethylamino)-4-hydroxy-6-oxo-1-phenyl-1,6-dihydropyrimidin-5-yl)hexanoate (202)

A mixture of 6-ethyl 1,1-bis(2,4,6-trichlorophenyl) hexane-1,1,6-tricarboxylate (**196**) (1.40 g, 2.31 mmol) and N,N-diethyl-N'-phenyl-guanidine (443 mg, 2.31 mmol) was heated to 150 °C with stirring for 5 min. On cooling to room temperature, the viscous brown oil was purified by column chromatography (50 % ethyl acetate-hexane) to give the product (635 mg, 68 %) as a pale yellow oil; IR (cm^{-1}) 2935, 1730, 1595, 1524, 1243, 752. ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.36 (2H, t, J=7.4 Hz, 3, 5-aryl), 7.28 (1H, t, J=7.3 Hz, 4-aryl), 7.19 (2H, d, J=7.1 Hz, 2, 6-aryl), 4.02 (2H, q, J=7.1 Hz, CH_2CH$_3$), 2.97 (4H, q, J=6.9 Hz, N(CH_2CH$_3$)$_2$), 2.33 (2H, t, J=7.5 Hz, CCH_2), 2.20 (2H, t, J=7.6 Hz, CH_2COOEt), 1.56 (2H, m, CH_2CH$_2$COOEt), 1.44 (2H, m, CCH$_2$CH_2), 1.29 (2H, m, CCH$_2$CH$_2$CH_2), 1.15 (3H, t, J=7.1 Hz, CH$_2$CH_3), 0.75 (6H, t, J=7.0 Hz, N(CH$_2$CH_3)$_2$). ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 174.05 (COOEt), 165.08 (4-pyrimidyl), 163.69 (6-pyrimidyl), 155.45 (2-pyrimidyl), 138.07 (1-aryl), 128.91 (3, 5-aryl), 128.80 (2, 6-aryl), 128.01 (4-aryl), 95.11 (5-pyrimidyl), 60.06 (CH_2CH$_3$), 44.60 (N(CH_2CH$_3$)$_2$), 34.38 (CH_2COOEt), 29.14 (CCH$_2$CH$_2$CH_2), 27.89 (CHCH$_2$CH_2), 24.89 (CH_2CH$_2$COOEt), 23.05 (CCH_2), 14.19 (CH$_2$CH_3), 12.37 (N(CH$_2$CH_3)$_2$). LRMS (EI) m/z 401 (M$^+$, 2 %), 291 (27 %), 171 (59 %), 125 (100 %). HRMS calcd for C$_{22}$H$_{31}$N$_3$O$_4$ 401.23144. Found: 401.23108.

6-(2-(Diethylamino)-4-hydroxy-6-oxo-1-phenyl-1,6-dihydropyrimidin-5-yl)-*N*-hydroxyhexanamide (155)
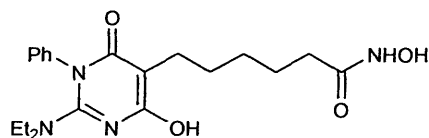
To a solution of ethyl 6-(2-(diethylamino)-4-hydroxy-6-oxo-1-phenyl-1,6-dihydropyrimidin-5-yl)hexanoate (202) (250 mg, 0.62 mmol) in tetrahydrofuran (6 mL) was added hydroxylamine (0.41 mL of a 50 % aqueous solution, 6.23 mmol) followed by dropwise addition of 1 M methanolic KOH (1.87 mL). The yellow solution was stirred for 5 min then evaporated and water (20 mL) added. The solution was neutralised with 1 M HCl and the aqueous phase extracted with ethyl acetate (4 x 20 mL). The combined organics were dried (Na$_2$SO$_4$) and evaporated to leave an orange oil. The crude product was purified by column chromatography (ethyl acetate) to give the title compound (124 mg, 51 %) as an orange oil; IR (cm^{-1}) 3192, 2932, 1616, 1522, 1202, 708. ^{1}H-NMR (CD$_3$OD, 300 MHz) δ 7.46 (3H, m, 3, 5-aryl, 4-aryl), 7.27 (2H, d, *J*=7.1 Hz, 2, 6-aryl), 3.08 (4H, q, *J*=7.0 Hz, N(C*H*$_2$CH$_3$)$_2$), 2.36 (2H, t, *J*=7.5 Hz, CH$_2$CO), 2.07 (2H, t, *J*=7.4 Hz, CCH$_2$), 1.62 (2H, td, *J*=7.5, 15.0 Hz, C*H*$_2$CH$_2$CO), 1.40 (4H, m, C*H*$_2$CH$_2$CH$_2$CH$_2$CO, C*H*$_2$CH$_2$CH$_2$CO), 0.80 (6H, t, *J*=7.0 Hz, N(CH$_2$C*H*$_3$)$_2$); ^{13}C-NMR (CD$_3$OD, 75 MHz) δ 173.15 (4-pyrimidyl), 167.68 (CONHOH), 167.22 (6-pyrimidyl), 160.00 (2-pyrimidyl), 139.88 (1-aryl), 130.28 (3, 5-aryl), 130.21 (2, 6-aryl), 129.33 (4-aryl), 95.84 (5-pyrimidyl), 45.96 (N(*C*H$_2$CH$_3$)$_2$), 33.74 (*C*H$_2$CO), 30.00 (*C*H$_2$CH$_2$CH$_2$CO), 29.03 (*C*H$_2$CH$_2$CH$_2$CH$_2$CO), 26.65 (*C*H$_2$CH$_2$CO), 23.94 (C*C*H$_2$), 12.88 (N(CH$_2$*C*H$_3$)$_2$). LRMS (CI) *m/z* 411 (M+Na, 100 %), 389 (M+H, 65 %), 321 (17 %), 192 (70 %). HRMS calcd for C$_{20}$H$_{28}$N$_4$O$_4$ (M+H) 389.21833, found 389.21984.

Bis(2,4,6-trichlorophenyl) malonate (198)[15]
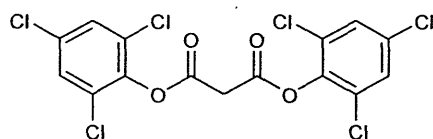
A mixture of malonic acid (2.0 g, 19.22 mmol) and 2,4,6-trichlorophenol (7.59 g, 38.44 mmol) in phosphorus oxychloride (7.66 g, 49.97 mmol) was heated to 100 °C for 2.5 h. The

reaction was then allowed to cool to room temperature and water (50 mL) added slowly. The off-white precipitate was filtered and suspended in a saturated aqueous sodium hydrogen carbonate solution (20 mL). After stirring for 1 h, the solid was collected by filtration, washed with water (3 x 20 mL), dried and recrystallised from ethyl acetate-petroleum ether (40 - 60) to give the title compound (6.81 g, 77 %), mp 152-155 °C (lit.[15] 154-156 °C); ^{1}H-NMR (CDCl$_3$, 300 MHz) δ 7.40 (4H, s, Ar), 4.06 (2H, s, CH$_2$); ^{13}C-NMR (CDCl$_3$, 75 MHz) δ 161.48 (COO), 142.38 (1-aryl), 132.72 (4-aryl), 129.50 (2, 6-aryl), 128.76 (3, 5-aryl), 39.72 (CH$_2$). LRMS (CI) m/z 463 (46 %), 265 (98 %), 197 (100 %).

6.4 References

1. Takahashi, T.; Mizuno, T.; Ido, T.; Iwata, R.; Watanabe, K. i. Improved synthesis of pure [18F]fluoro-compounds for PET studies from bromo-compounds. *Applied Radiation and Isotopes* **2003**, *58*, (5), 557-566.

2. Regel, E. *Justus Liebigs Annalen der Chemie* **1977**, 159-

3. Bastiaansen, L. A. M.; Godefroi, E. F. 2-Aminomethylimidazole and imidazole-2-carboxaldehyde: two facile syntheses. *Journal of Organic Chemistry* **1978**, *48*, (8), 1603-1604.

4. Noveron, J. C.; Olmstead, M. M.; Mascharak, P. K. A Synthetic Analogue of the Active Site of Fe-Containing Nitrile Hydratase with Carboxamido N and Thiolato S as Donors: Synthesis, Structure, and Reactivities. *Journal of the American Chemical Society* **2001**, *123*, (14), 3247-3259.

5. Zelenin, A. K.; Trudell, M. L. A Two-Step Synthesis of Diaminofurazan and Synthesis of N-Monoarylmethyl and N, N'-Diarylmethyl Derivatives. *Journal of Heterocyclic Chemistry* **1997**, *34*, 1057-1060.

6. Komin, A. P.; Street, R. W.; Carmack, M. Chemistry of 1,2,5-thiadiazoles. III. [1,2,5]thiadiazolo[3,4-c][1,2,5]thiadiazole. *Journal of Organic Chemistry* **1975**, *40*, (19), 2749-2752.

7. Suzuki, T.; Matsuura, A.; Kouketsu, A.; Hisakawa, S.; Nakagawa, H.; Miyata, N. Design and synthesis of non-hydroxamate histone deacetylase inhibitors: identification of a selective histone acetylating agent. *Bioorganic & Medicinal Chemistry* **2005**, *13*, (13), 4332-4342.

8. Suzuki, T.; Nagano, Y.; Matsuura, A.; Kohara, A.; Ninomiya, S. i.; Kohda, K.; Miyata, N. Novel histone deacetylase inhibitors: design, synthesis, enzyme inhibition, and binding mode study of SAHA-Based non-hydroxamates. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, (24), 4321-4326.

9. Mai, A.; Esposito, M.; Sbardella, G.; Massa, S. *Organic Preparations and Procedures International* **2001**, *33*, (4), 391-394.

10. Klein, S. I.; Molino, B. F.; Czekaj, M.; Gardner, C. J.; Chu, V.; Brown, K.; Sabatino, R. D.; Bostwick, J. S.; Kasiewski, C.; Bentley, R.; Windisch, V.; Perrone, M.; Dunwiddie, C. T.; Leadley, R. J. Design of a new class of orally active fibrinogen receptor antagonists. *Journal of Medicinal Chemistry* **1998**, *41*, (14), 2492-2502.

11. Elliott, J. M.; Broughton, H.; Cascieri, M. A.; Chicchi, G.; Huscroft, I. T.; Kurtz, M.; MacLeod, A. M.; Sadowski, S.; Stevenson, G. I. Serine derived NK1

antagonists 2: a pharmacophore model for arylsulfonamide binding. *Bioorganic & Medicinal Chemistry Letters* **1998**, *8*, (14), 1851-1856.

12. Ismaiel, A. M.; Los Angeles, J.; Teitler, M.; Ingher, S.; Glennon, R. A. Antagonism of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane stimulus with a newly identified 5-HT2- versus 5-HT1C-selective antagonist. *Journal of Medicinal Chemistry* **1993**, *36*, (17), 2519-2525.

13. Shelkov, R.; Nahmany, M.; Melman, A. Acylation through Ketene Intermediates. *Journal of Organic Chemistry* **2002**, *67*, (25), 8975-8982.

14. Keil, D.; Hartmann, H. *Phosphorus, Sulfur and Silicon* **2007**, *152*, 169-184.

15. Varga, M.; Kapui, Z.; Batori, S.; Nagy, L. T.; Vasvari-Debreczy, L.; Mikus, E.; Urban-Szabo, K.; Aranyi, P. A novel orally active inhibitor of HLE. *European Journal of Medicinal Chemistry* **2003**, *38*, (4), 421-425.