The Design and Synthesis of Non-Peptide Bradykinin B2 Receptor Antagonists

A thesis presented to the University of London in partial fulfilment of the requirements for the degree of Doctor of Philosophy

by

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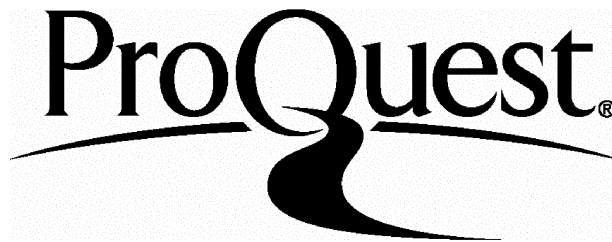London WC1H 0AJ

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Abstract

Bradykinin (BK; Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is a nonapeptide with a number of important biological actions. BK has been shown to act as a pathological mediator of pain and inflammation and thus drugs which can block this action of BK have a potential role as novel analgesics.

HOE140 (Icatibant; D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-D-Tic^7-Oic^8-Arg^9) is one of the most potent BK B2 receptor antagonists to have been synthesised. The unnatural amino acids at positions 7 and 8, D-Tic and Oic, are believed to induce a C-terminal β–turn which is thought to be responsible for its high binding affinity. A non-peptide antagonist of BK would offer a number of advantages over HOE140, including better oral bioavailability and increased metabolic stability. Our attempts at synthesising a non-peptide BK antagonist modelled on the C-terminal β-turn present in HOE140 are described.

Two recent literature reports on the use of the 1,4-benzodiazepin-2-one (BZD) scaffold to mimic a peptide reverse turn are outlined. These reports have been used to formulate a hypothesis that the C-terminal β-turn in HOE140 can be replaced by the BZD scaffold to form a non-peptide antagonist of BK. Two overlay models of the BZD scaffold on the C-terminal β-turn in HOE140 are provided and the synthesis and biological evaluation of over forty BZD ligands based on these models are described. Moderate biological activity was achieved with only the third compound tested, with Compound **61** exhibiting a K_i of 9.2 μM at the human BK B2 receptor. Analogues of this compound with a variety of C3 and N1 side chains on the BZD system are reported, as are compounds varying at the C5 position. These analogues were synthesised by changing either the amino acid or 2-aminobenzophenone portion of the BZD or by alkylating the BZD with a different electrophile. These studies have resulted in the synthesis of a more potent analogue, **121**, with a sub-micromolar binding affinity; compound **121** has a K_i of 0.95 μM at the human BK B2 receptor. The structure activity relationships of the compounds tested are discussed and the attempts to optimise the biological activity are summarised. Preliminary studies on the alkylation of the C3 position are also documented.

In describing the direct conversion of a second generation peptide antagonist into a non-peptide lead, this work has provided another example of a "privileged structure" furnishing useful leads in the search for new receptor-binding ligands.

Acknowledgements

Glossary

BSA	bovine serum albumin
BK	bradykinin
Boc	butoxycarbonyl
BZD(s)	benzodiazepine(s)
$CDCl_3$	deuterated chloroform
CD	circular dichroism
CHO	Chinese hamster ovary
DIPC	N,N'-diisopropylcarbodiimide
DCM	dichloromethane
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol-bis[β-aminoethylether]-N,N,N'N'-tetraacetic acid
EI	electron Impact
FAB	fast atom bombardment
HEPES	N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
HHBSA	HEPES-Hanks bovine serum albumin
HOBT	1-hydroxybenzotriazole
KO^tBu/THF	1M potassium tertiary butoxide solution in THF
PBS	phosphate buffered saline

TE	Tris-EDTA buffer
TES	N-*tris*(hydroxymethyl)methyl-2-aminoethanesulfonic acid; 2-([2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)-ethanesulfonic acid
TFA	trifluoroacetic acid
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
Tris-HCl	2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride
Z	benzyloxycarbonyl
1X	single strength

Contents

9

1. INTRODUCTION

1.1. The Problem of Pain

The alleviation of pain or, at the very least, its effective management is one of the most formidable problems in medicine.

Pain is such a common experience that we rarely pause to define it in everyday conversation. And yet how we express pain, and how we cope with it, varies enormously and is linked to psychological, social, and even cultural factors.

Pain influences physiology and psychology, the latter component complicating a strictly scientific approach to treatment. It is usually caused by intense, noxious stimulation, yet it sometimes occurs spontaneously without apparent cause. It normally signals physical injury, but it sometimes fails to occur even when extensive areas of the body have been seriously injured; at other times it persists after all the injured tissue has healed and becomes a crippling problem that may require urgent radical treatment. As the pain's severity and duration increases, the person's physical and mental response is increasingly dominated by the condition.

At first glance there would appear to be many products available for relieving pain. But ninety-nine percent of drug prescriptions for pain relief come from only two families of compounds, the non-steroidal anti-inflammatory group (aspirin, paracetamol, ibuprofen, *etc.*) and the opiates. No new class of drug for pain relief has been developed this century. That a search for novel analgesics is worthwhile is underscored by the realisation that, in an era of cost cutting, better treatment is not only humane, but also speeds recovery and saves money.

1.1.1. The Use of Aspirin in Pain Relief

Although extracts of the willow *Salix alba* have been in use in herbal medicines for centuries to relieve pain and inflammation, it was only in 1827 that Leroux isolated the active compound and named it salicin. Salicylic acid, as it was later known, had an unpleasant taste and irritated the membranes lining the mouth, oesophagus and stomach when swallowed. These unpleasant side-effects limited the use of salicylic acid to patients whose rheumatism or arthritis had

become intolerably painful. One such sufferer had a son who worked at the Bayer laboratories in Germany. Felix Hoffmann would return home after work and find his father racked with pain. Felix's father had a sensitive stomach which meant that the pain from his abdomen from salicylic acid was as bad as the pain of his arthritis. Felix and a colleague Heinrich Dreser decided to start a research program with the aim of making a variant of salicylic acid without its harmful effects. In 1899 Dreser produced acetyl salicylic acid and this was marketed by Bayer under the trade name aspirin[1]. This compound has fewer side-effects than salicylic acid and many variants have been produced. One study suggested that 40 000 tons of aspirin are consumed in the world each year[2].

Figure 1. Conversion of arachidonic acid to PGG_2

Prostacyclins, Prostaglandins and Thromboxanes
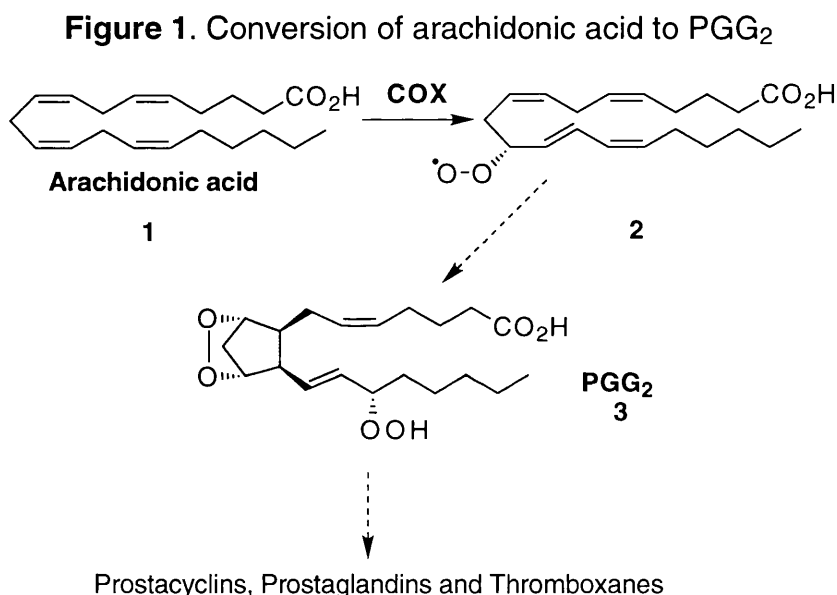
It was not until 1971[3] that the mode of action of aspirin-like compounds was determined. Sudden injury sets off a chain of reactions culminating in the perception of pain. First small molecules such as histamine and serotonin are released. Then larger peptides such as bradykinin appear and finally prostaglandins are synthesised and released. Aspirin blocks the synthesis of prostaglandins by inhibiting the enzyme cyclooxygenase (COX, see Figure 1). COX catalyses the oxygenation of arachidonic acid (**1**) to the cyclic endoperoxide PGG_2 (**3**). Drugs which prevent the synthesis of prostaglandins are effective against slow, prolonged tissue damage and the pain it causes. A broken leg and an arthritic joint have all these reactions and therefore the same sensitivity to these drugs. Importantly, pain triggered by events that do not produce inflammatory reactions do not respond to these drugs.

It has recently been established that there are two forms of the enzyme cyclooxygenase, COX-1 and COX-2[4]. The constitutive form of the enzyme, COX-1, is found in the gastric mucosal layer and in other tissues. In the gastric mucosa, COX-1 is used to form prostacyclin which protects the stomach wall from the effects of gastric juices. The other form of the enzyme, COX-2, has been shown to be present in inflammatory conditions. Pro-inflammatory cytokines which are produced at the site of tissue damage induce the synthesis of COX-2. This induction results in the production of prostaglandins, and thus to pain and inflammation. Aspirin blocks the actions of both COX-1 and COX-2, but is more potent against COX-1[4]. The effect of aspirin on COX-1 leads to the well-known side effects, gastric irritation and bleeding. There has been an intensive effort to synthesise selective COX-2 inhibitors[4]. A number of such compounds are currently undergoing clinical trials. The therapeutic benefits of these compounds, however, have yet to be established.

1.1.2. The Use of Opiates in Pain Relief

The power of opium in pain relief was known to the Sumerians in 4000 BC. The first medical document, the Eber's papyrus of 1530 BC, recommended its use for crying children[1].

In 1803 morphine was isolated by Sertürner[1] from opium. Opium contains about 10% morphine and smaller amounts of many other alkaloids, two of which, codeine and thebaine, are related to morphine. From these three molecules a large number of synthetic compounds have been generated, including heroin and pethidine.

The individual members of this group differ widely in their potency, duration of action, penetration and side effects. They are, however, fundamentally similar in their mode of action. All produce analgesia and, as the dose rises, varying degrees of drowsiness, change of mood, mental clouding, depression of respiration, and constipation.

The site of action of opiates is in the central nervous system. These compounds act by imitating the endorphins and the enkephalins that are produced in the brain and spinal cord[5].

1.2. Bradykinin as a Mediator of Pain and Inflammation

1.2.1. The Discovery of the Kinin System

The first evidence for the existence of the kinin system came from Frey[6] in the 1920s. Frey was investigating the fall of blood pressure caused by intravenous injection of urine. He found that urine contained an enzyme which acted on plasma to produce a substance which caused a fall in blood pressure. He tested several glands and tissues for the presence of this enzyme and found the richest source to be the pancreas. He named this enzyme kallikrein after the Greek name for the pancreas, *kallikreas*. Frey then named the vasopressor substance formed by this enzyme 'kallidin'. In 1949 Rocha e Silva[7] found that the addition of the venom from the Brazilian snake *Bothrops jararaca* to plasma produced a substance which causes a distinctive slow contraction of isolated guinea pig ileum. Because of this slow movement he christened this new substance 'bradykinin' (**4**, BK). He inferred that BK was a peptide because it was degraded by the enzyme chymotrypsin and was also aware that it had similar biological properties to kallidin.

Figure 2. Amino acid sequence and structure of BK

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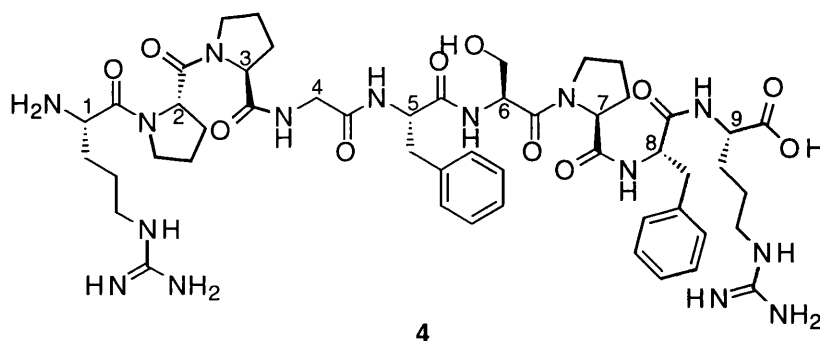
Bradykinin: Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9

The structural resemblance between kallidin and bradykinin was not established until 1960. Elliot *et al.*[8] determined the amino acid sequence of BK, which was then synthesised by Boisonnas *et al.*[9]. The amino acid sequence of kallidin was determined by Pierce *et al.*[10] and it was then synthesised by Nicolaides *et al.*[11] (see Figure 3).

There are two inactive protein precursors to the kinins termed 'high molecular weight kininogen' (HMWK) and 'low molecular weight kininogen' (LMWK)[12]. They are present in all tissues and are only activated when they come in

contact with either the enzymes plasma kallikrein (PK) or tissue kallikrein (TK) (see Figure 3). When the PK enzyme is activated, it binds to HMWK and cleaves the Lys^0-Arg^1 bond at the N-terminus and the Arg^9-Ser^{10} bond at the C-terminus to form BK. TK acts differently in that it recognises LMWK and cleaves the $Met^{-1}Lys^0$ bond at the N-terminal and the Arg^9-Ser^{10} bond to form Kallidin (see Figure 3). Met-Lys-BK is formed by the action of a different enzyme, neutrophil elastase. This enzyme is released from leukocytes activated by the immune system and cleaves the Leu^{-2}-Met^{-1} bond at the N-terminal and the Arg^9-Ser^{10} at the C-terminal to form Met-Lys-BK^{13}(see Figure 3).

Figure 3. Enzymatic synthesis of kinin peptides from the kininogens

HM or LM Kininogen: R-Ser^{-3}-Leu^{-2}-Met^{-1}-Lys^0-$Bradykinin$-Ser^{10}-R

Met-Lys-BK: Met-Lys^0-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9

Kallidin: Lys^0-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9

BK: Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9

Enzymatic Cleavage Sites:
A + D = Neutrophil Elastase
B + D = TK
C + D = PK
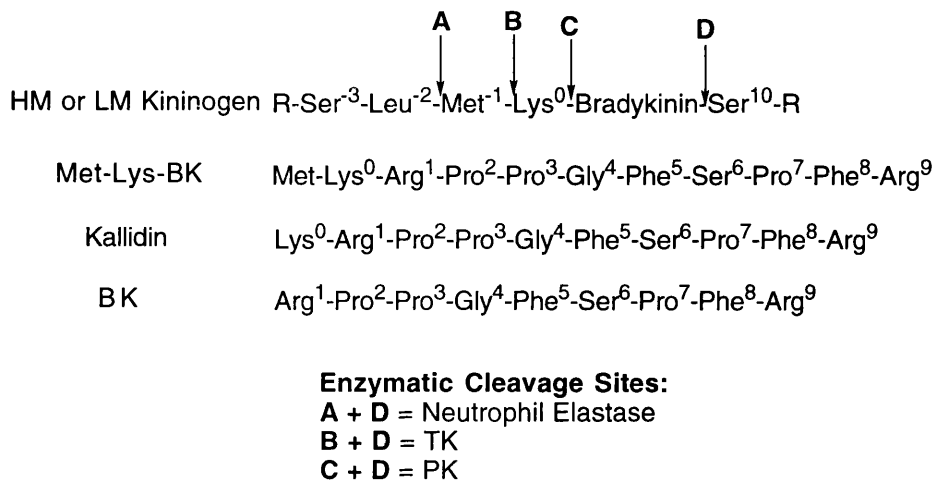
1.2.2. The BK Receptors

There are two classes of bradykinin receptors, B1 and B2. Most physiological processes are mediated by the B2 class[14,15,16]. B1 receptors appear to be absent *in vivo* under normal conditions and only appear in tissues after prolonged exposure to noxious stimuli. The B1 receptors selectively bind BK(1-8) and are believed to mediate certain chronic inflammatory processes[17,18]. Both BK receptors belong to the G-protein coupled receptor class (see Figure 4).

Figure 4. Model of the Rat BK B2 receptor.
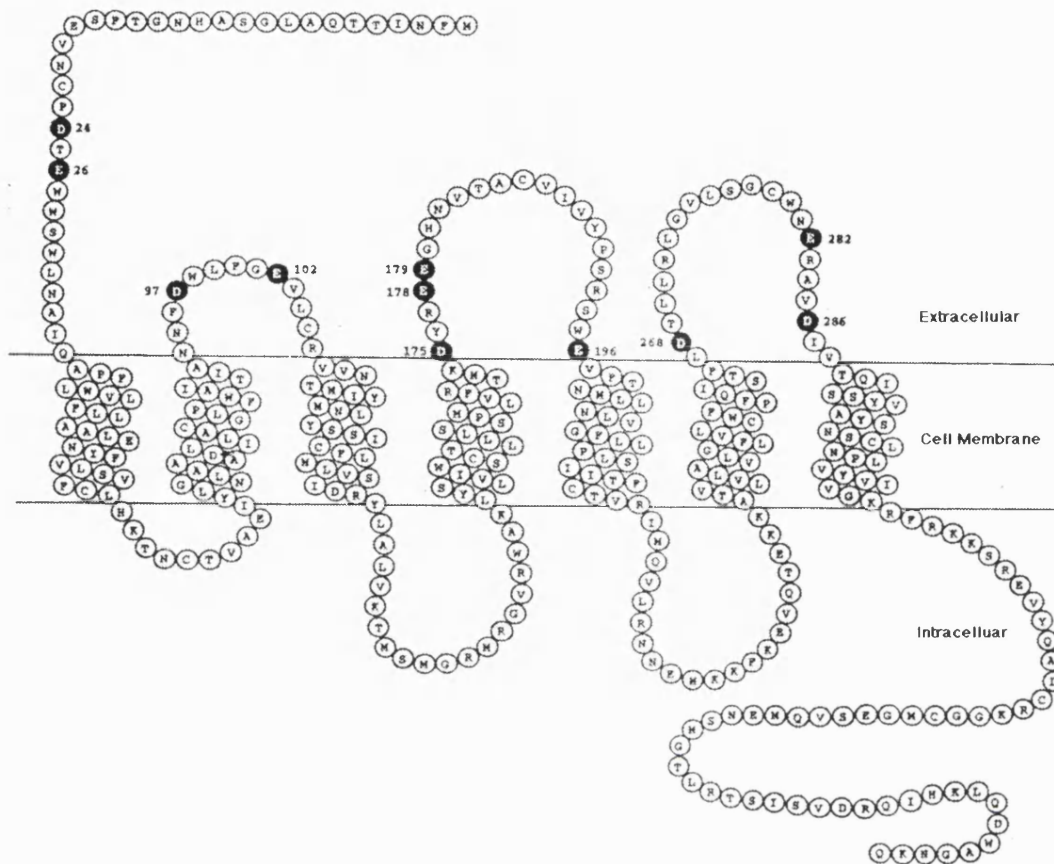
All the major second messenger systems have been shown to be linked, *via* G-proteins, to BK receptors[13]. BK receptors in endothelial cells are linked to NO synthase[19]. In these cells NO diffuses and activates cyclic GMP, which causes smooth muscle relaxation. In inflammation[20], activation of phospholipase A_2 (PLA_2) and phospholipase C is widespread.

BK is destroyed *in vivo* by the enzymes angiotensin converting enzyme (ACE), carboxypeptidase N (CPN)[13], plasma aminopeptidase IV (PAIV)[21] and metalloendopeptidase 3.4.24.11 (MEP)[22] (see Figure 5). ACE removes successively the two C-terminal amino acids from BK to form an inactive peptide[23]. CPN, a soluble enzyme found in plasma, removes the C-terminal arginine residue from BK. The remaining peptide BK(1-8) has less than 1% of the potency of BK at the B2 receptor but is a full agonist at the B1 receptor. PAIV completely inactivates BK by removing the N-terminal arginine residue. MEP cleaves BK internally at Phe[5] to form an inactive substrate.

Figure 5. Enzymatic degradation of kinin peptides

$$\begin{array}{cccc} \text{PAIV} & \text{MEP} & \text{ACE} & \text{CPN} \\ \downarrow & \downarrow & \downarrow & \downarrow \end{array}$$

BK $\qquad$ $Arg^1\text{-}Pro^2\text{-}Pro^3\text{-}Gly^4\text{-}Phe^5\text{-}Ser^6\text{-}Pro^7\text{-}Phe^8\text{-}Arg^9$

Kallidin $\qquad$ $Lys^0\text{-}Arg^1\text{-}Pro^2\text{-}Pro^3\text{-}Gly^4\text{-}Phe^5\text{-}Ser^6\text{-}Pro^7\text{-}Phe^8\text{-}Arg^9$
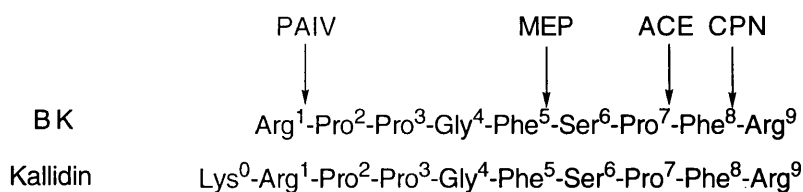
1.2.3. Functions of Kinins in Physiological Regulation.

Kinins perform many important physiological roles. In the digestive system BK is a major agent of intestinal motility[13]. It is also a regulator of chloride ion transport in the intestine and, as such, is important in fluid transport[24]. In the airways, pulmonary transmembrane fluid transport is mediated by bradykinin and since airways contract in the presence of BK, its presence is a regulatory factor in airway muscle tone[25]. In the kidney, BK has been shown to be a local mediator in the action of atrial natriuretic peptides, which regulate sodium excretion[26]. BK can act as a regulator in blood pressure. In the renin-angiotensin system, ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor. ACE also deactivates BK as a vasodilator by removing the two C-terminal amino acids. In experiments with BK antagonists, over half the actions of ACE inhibitors have been shown to be due to their prevention of BK degradation[27,28]. In ovulation, which has been described as a type of inflammation, BK has been shown to be involved in egg release[29]. In the nervous system, peripheral BK is very important in the initiation of pain signals[30]. In the CNS, BK receptors have been found but the precise roles for these neurones are unclear. It has been speculated that they are involved in blood pressure[13].

1.2.4. The Kinin System in Inflammation

All the classical signs of inflammation, *dolor* (pain), *rubor* (redness; erythema), *tumor* (edema) and *calor* (hyperthermia), can be invoked by BK. Kinin specific 'nociceptors' on sensory pain transmitting A-δ and C fibres bind kinins produced by contact activation, occlusion, bacterial enzymes or immune stimulation. These neurones transmit pain signals centrally using substance P as the neurotransmitter. Activation of phospholipase C by BK receptors generates inositol triphosphate (IP_3)[31] which causes an increase in intracellular calcium. In afferent sensory fibres this increase leads to the release of substance P, which propagates a pain signal[30,32]. Activation of PLA_2 by BK receptors leads to the production of prostaglandins and thromboxanes which are important mediators

of pain and edema. These additional substances work together with BK to stimulate the release of histamine and thus increase the inflammation and pain. Indeed, bradykinin has been described as the most potent endogenous algogenic substance known[33]. Erythema caused by BK can be illustrated by the example of sitting with crossed legs; the ischemia causes acidification and hence kallikrein activation. The released BK leads to vasodilatation and erythema when the pressure is reduced. BK has been shown to cause attraction and extravasation of leukocytes into areas of infection, injury and inflammation[34]. BK release leads to the relaxation of arteriolar pressure and constriction of venules. These events lead to a local increase in the temperature of the affected area and cause the capillary bed to extrude fluids. This leads to the movement of cells and flow of fluids from the circulation to the affected area.

1.2.5. The Search for Bradykinin Antagonists

It is clear from the discussion above that a potent, long-lasting, antagonist of bradykinin would have an important clinical role. The first bradykinin antagonist was discovered by Vavrek and Stewart[35] who replaced the proline residue at position 7 with D-phenylalanine. It was then found that replacing Pro[7] with any other D-aromatic amino acid confers antagonistic activity to the peptide[36]. Other workers attempted to synthesise analogues which enhanced receptor affinity and prevented enzymatic degradation. It has been shown that addition of basic amino acids to the N-terminus greatly decreases affinity of the peptide to the degradation enzymes. Most peptide antagonists synthesised have a D-Arg or a Lys-Lys- sequence at the N-terminus. These 'first generation' antagonists had low affinities for the BK receptors compared to BK and had a very limited *in vivo* lifetime. Despite their different amino acid sequence, the peptides were still substrates for CPN[37], although the D-residue at position 7 blocked the action of ACE[38].

The 'second generation' BK antagonists, of which HOE140 (**5**) is a member,[39] are resistant to CPN and have effective *in vivo* lifespans. At Cortech[40] two first generation cysteine containing BK antagonists were linked by hexamethylene bis-maleimide (CP-0127), which greatly increases the *in vivo* half life and potency. Both HOE-140 and CP-0127 antagonise BK at the B2 receptor. A 'dual' B1 and B2 antagonist, CP-0364, was developed by Cortech by joining a B1 antagonist and B2 antagonist through a hexamethylene bis-maleimide linker[41]. There is evidence that in solution most potent B2 receptor antagonists

adopt a C-terminal β-bend conformation, although it is not clear that this conformation is maintained by the antagonists when they are bound to the BK B2 receptor.

Figure 6. Structures of some peptidal BK antagonists
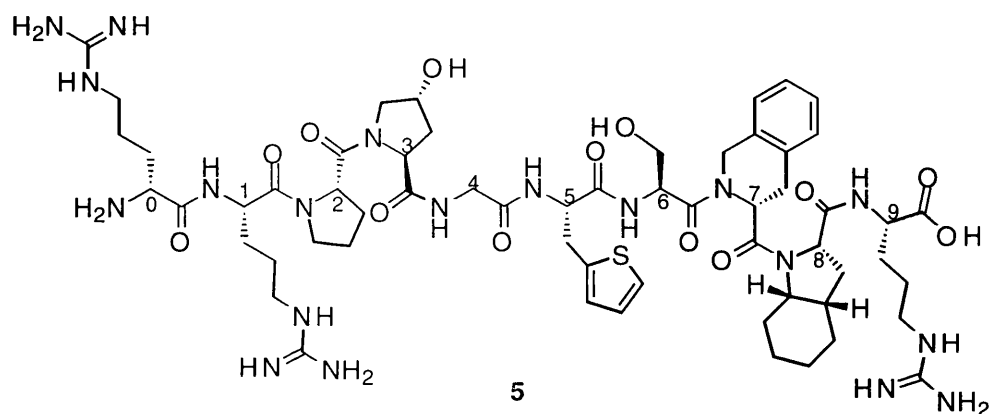
HOE140 H-(D)-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-DTic^7-Oic^8-Arg^9

D-Arg-Arg-Pro-Hyp-Gly-Thi-Cys-DPhe-Leu-Arg
                        |
                        S
                        |
                      Linker
                        |
                        S
                        |
D-Arg-Arg-Pro-Hyp-Gly-Thi-Cys-DPhe-Leu-Arg

CP-0127

D-Arg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Leu-Arg
                        |
    S —— Linker —— S   (B2 antagonist)
    |
D-Arg-Cys-Pro-Hyp-Gly-Phe-Ser-Pro-Leu
                        (B1 antagonist)

CP-0364
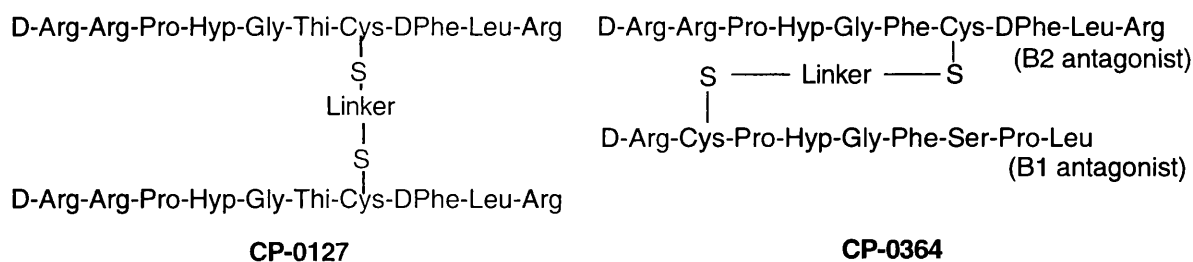
1.2.6. The Clinical Potential of BK Antagonists

Clinical trials undertaken by Nova Pharmaceutical Corporation with a first generation antagonist in the treatment of rhinitis and burn pain were unsuccessful. This was probably due to the low potency and short half-life *in vivo*[42]. In animal models of allergic rhinitis, asthma, septic shock, and head trauma the second generation BK antagonists were shown to be effective[43,44]. Currently CP-0127 is in phase II clinical trials for head trauma. In the human septic shock clinical trial, CP-0127 was found to be ineffective. In this lethal condition, ACE disappears from the pulmonary circulation. This causes the circulating BK to be degraded by CPN to yield BK(1-8), an agonist at the B1 receptor. The vasodilatory properties of this molecule cause the collapse of blood pressure and hence shock[45,46]. The other kininases act poorly on BK(1-

8) and hence the actions of this molecule cannot be readily terminated. The 'dual' antagonist, CP-0364, may have more success in this condition since it can block the action of BK at both the B1 and B2 receptors. Another possible use of BK antagonists is the area of analgesia. Burn centre physicians report that there are no available analgesics which afford adequate comfort to patients during debridement of large area burns. Chronic long term inflammatory diseases may also be a target for BK antagonists[47]. These conditions include arthritis, inflammatory bowel disease, asthma, and rhinitis. It is anticipated that BK antagonists will have an important place in anti-inflammatory medicine. However, the problems of the current peptide drugs (see Section **1.3.1.**) are likely to prevent their widespread use and a non-peptide antagonist of BK will thus have an obvious market.

1.3. Peptidomimetics

1.3.1. The Problems of Peptides as Drugs

There are a number of problems with the idea of formulating peptides as drugs.

(a) Their low metabolic stability towards proteases. This means that their *in vivo* half-lives are very short.

(b) Their poor absorption after oral ingestion. This is due to the combination of the presence of digestive enzymes in the stomach and the large size of the peptide molecule.

(c) Their rapid excretion through the kidney and liver.

(d) Their undesired interaction with other receptors (due to their conformational flexibility).

Much work has been undertaken in the search for peptidomimetics[48,49,50,51]. Peptidomimetics can be defined as "structures which serve as appropriate substitutes for peptides in interactions with receptors and enzymes"[50]. These peptidomimetics offer a number of advantages over native peptides, including resistance to proteases, good oral bioavailability, high receptor affinity and selectivity, and minimal side effects.

1.3.2. Peptidal Peptidomimetics

1.3.2.1. Amino Acid Manipulations

One of the techniques used to synthesise peptide antagonists is to change the amino acid sequence of the parent peptide[51]. When the smallest active fragment of a peptide has been determined, the role of each amino acid in the peptide is evaluated. This can be achieved by systematically replacing the side chains with methyl groups; that is, exchange one amino acid at a time by alanine. This analysis enables the identification of side chains of importance either for receptor interaction or for folding of the peptide into its bioactive conformation. A systematic replacement of L-amino acids with D-amino acids may also be informative in the initial exploration of structural requirements for receptor recognition and binding. Another strategy involves the systematic introduction of conformational constraints into the peptide fragment[50]. This may take the form of methylation at either nitrogen or the α-carbon, or the incorporation of an unnatural amino acid into the peptide. There are a large number of these unnatural amino acids and some of them offer the advantage of having a favoured conformational preference. When such conformationally constricted amino acids are introduced into the peptide then there is a possibility that the peptide will have a single, preferred low energy conformation. Ideally, this conformation is identical to its biologically active conformation. Listed below are some of these unnatural conformationally restricted amino acids and their natural surrogates.

Figure 7. Conformationally restricted amino acids

Amino acids   Conformationally restricted analogues

Phe   DTic

Leu

Trp   Tpi
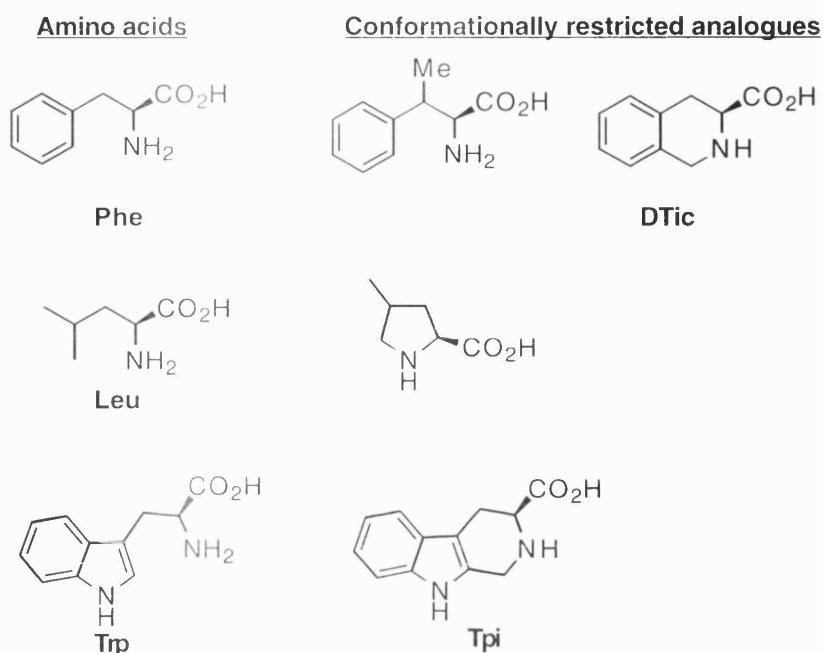
1.3.2.2. Amide Bond Modifications

In peptides the amide bond framework serves as a structural matrix so that side chains are projected in defined spatial positions. A combination of the appropriate side chain and projection then provides optimal interactions with the enzyme or receptor protein. In some cases the amide bonds themselves may also be of importance for binding to receptors or enzymes. For this reason several amide bond isosteres have been designed, synthesised and introduced into peptide analogues[48,51,52]. These isosteres have been used to enhance the stability towards protease degradation of the peptide but have similar geometrical, conformational, electrostatic and hydrogen bonding properties to the amide bond itself. Although a large number of amide isosteres are known, according to Giannis[48] "a convincing imitation of the amide bond in the ground state has not been achieved". A few examples of these isosteres are shown in Figure 8.

Figure 8. Some amide bond isosteres

Amide

N-methyl amide   hydroxymethylene   thioamide   thioester   retro-inverso amide

methyleneamino   ketomethylene   fluorovinyl   (*E*)-vinyl   phosphonate
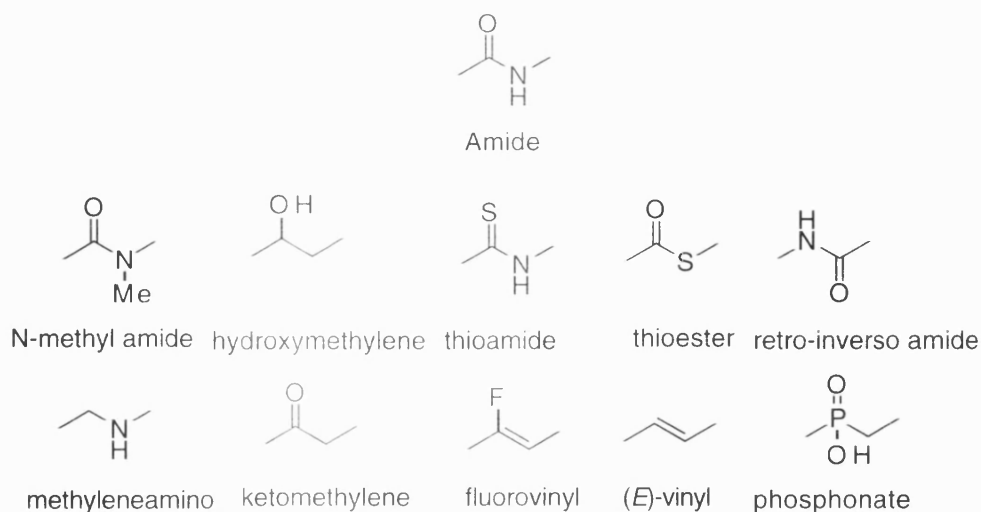
1.3.2.3. Dipeptide Analogues

Another approach to the design of peptidomimetics is the systematic replacement of dipeptide units with lactams[53], **6** and **7**, or azoles[54], **8**. These non-peptidic structures offer a number of advantages, including resistance to proteases and conformational stability. Examples are shown in Figure 9.

Figure 9. Dipeptide analogues

6   7   8
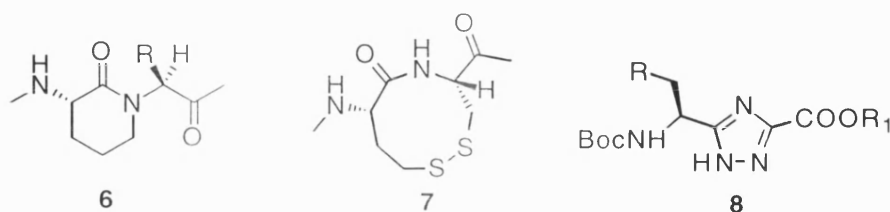
1.3.2.4. Conformational Constraints and Turn Mimetics

The flexible nature of a peptide means that its bioactive conformation may be poorly populated in the absence of the receptor. The conformations observed from ^{1}H-nmr spectroscopy or x-ray crystallography may be quite different from the receptor bound conformation[55]. Perhaps the simplest way to determine the bioactive conformation is to synthesise conformationally restricted peptidomimetics. This idea has been applied to the synthesis of turn mimetics. Turns and loops are the conformational preferences for peptides and proteins. A β–turn is formed from four amino acids and is stabilised by an intramolecular hydrogen bond. A γ–turn is formed from three amino acids. A large number of

turn mimetics have been synthesised and have been incorporated into peptides (see Figure 10).

Figure 10. β- and γ-turn mimetics

β-Turn

β-Turn mimetics

γ-Turn

γ-Turn Mimetics
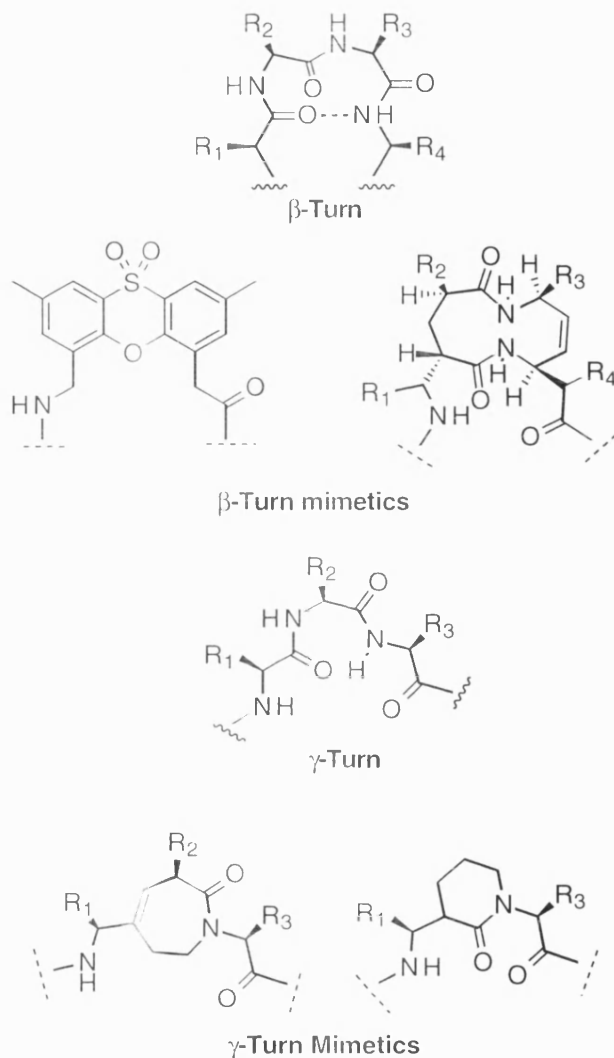
1.3.2.5. Scaffold Mimetics

Another approach used in the design of peptide mimetics utilises a non-peptidic scaffold to project binding determinants (amino acid side-chains) in the same directions as those in the bioactive peptide.

It has been shown that the tripeptide sequence Arg-Gly-Asp (RGD, **9**) antagonised the binding of fibrinogen to the platelet receptor, glycoprotein IIb-IIIa (GPIIbIIIa)[56]. This action prevents thrombus growth and hence fibrinogen antagonists have been targeted as anti-thrombotic agents. Hirschmann *et al.*[57] used a steroid molecular template to synthesise an RGD antagonist **10** (see Figure 11).

Figure 11. Steroidal scaffold mimetic

-Arg-Gly-Asp- (RGD)

9

GPIIbIIIa antagonist

10
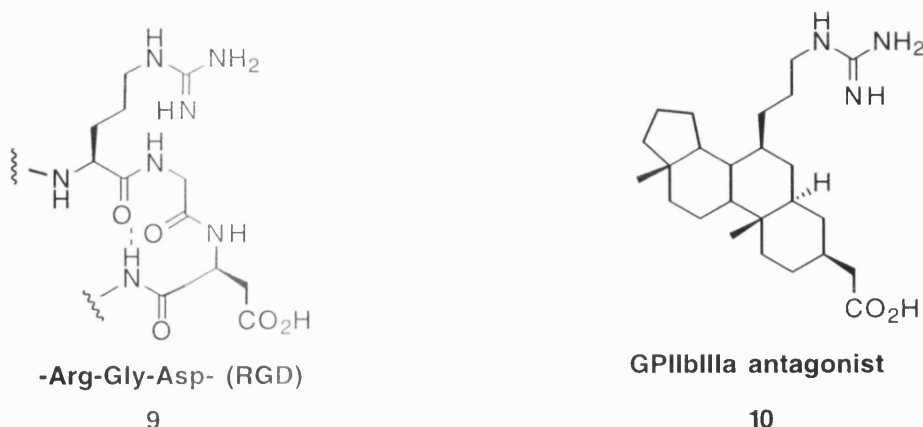
The peptide hormone somatostatin (SRIF, **11**), a cyclic tetradecapeptide which inhibits the release of several hormones including growth hormone (GH), has attracted attention as a potential therapeutic agent. Its very short half-life led chemists to the design and synthesis of longer acting peptidal peptidomimetics. SRIF contains a β-turn composed of the tetrapeptide sequence Phe^1-Trp^2-Lys^3-Thr^4. This tetrapeptide sequence retains the ability to elicit SRIF-like effects as long as the side chains of the four amino acids are constrained in nearly the same orientation as the bioactive conformation of SRIF. The cyclic hexapeptide **12**, which contains the four amino acids with the desired side chain conformations, is a potent agonist. ^{1}H-nmr studies on the peptide indicated the presence of a type II' β-turn in solution and suggested that the Phe-Pro amide bond exists in a *cis* arrangement, holding the peptide in the desired conformation. The properties of the individual amino acids in the β-turn have been evaluated and only the replacement of Thr by Ala resulted in no loss of activity (Figure 12).

Hirschmann *et al.*[58] observed that D-glucose appeared to be an attractive non-peptide scaffold in the design of a SRIF mimic. The pyranose ring of glucose provided several advantages, including its well-defined conformation, the ability to position the required side chains in an equatorial disposition, the enantiomeric purity of the starting material, and the vast array of synthetic knowledge. Molecular modelling studies suggested that the glycoside **13**, with substituents at C-2, C-1 and C-6, promised to provide appropriately positioned replacements for the crucial Phe^1-Trp^2-Lys^3 side chains (see Figure 12). The hydrophobic group at position C-4 could also mimic the Phe-Pro sub-unit in the cyclic peptide. The biological results from the peptide-mimetic showed

moderate activity. Compound **13** bound in a dose dependent manner to the SRIF receptor (IC_{50}: 15 µM, ^{125}I-Tyr-SRIF ligand).

Figure 12. Scaffold mimetic derived from β-D-glucopyranose

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp
| |
HO-Cys-Ser-Thr-Phe-Thr-Lys

Somatostatin
11

c-(Phe^1-D-Trp^2-Lys^3-Thr^4-Phe^5-Pro^6)

12

13
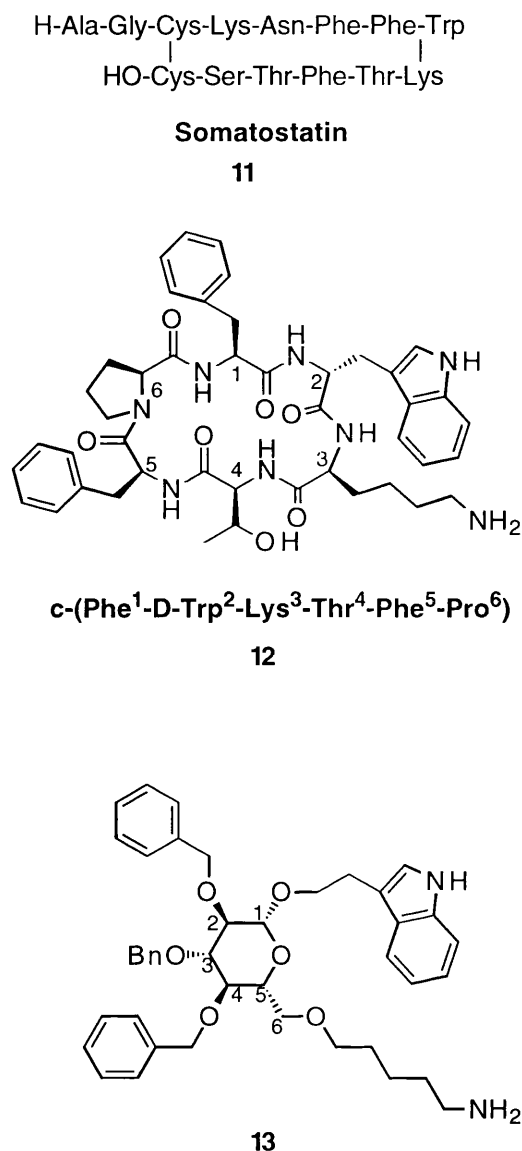
1.3.3. Non-Peptidal Peptidomimetics

1.3.3.1. The Message and Address Concept

In many peptides only a small number of amino acids (4-8) are responsible for the recognition and activation of the receptor (the "message" part)[59,60]. The important amino acids can be identified by amino acid substitutions, which should lead to pronounced changes in the biological response. The part of the peptide not directly involved in the binding of the ligand to the receptor (the "address" part) serves to fix the important amino acids in a proper spatial

arrangement and confers additional affinity and selectivity for the receptor. This concept, developed by researchers working on adrenocorticotropic hormone (ACTH)[61], can be applied in an analysis of the enkephalins. Morphine **(14)** acts as an agonist at the opiate receptor, whose normal ligands are the endogenous opiate peptides. These peptides, enkephalin, endorphin, and dynorphin act at the μ, κ, and δ receptors on sensory neurones[62]. The N-terminals of these peptides contain the common amino acid sequence of Tyr-Gly-Gly-Phe and this sequence is vital for receptor recognition. The tyrosine side chain acts as the message and the two glycine residues as the spacer linkage. Morphine binds to all three receptors. However, incorporating a functionality at the nitrogen of the morphine analogue **(16)** confers specificity[52] (see Figure 13). This example illustrates that not only can small molecules bind specifically at peptide receptors, but they can have enough functionality to act as agonists.

Figure 13. The message and address concept applied to the enkephalins

Dynorphin:
Tyr-Gly-Gly-Phe-Leu-Arg-Arg-
Ile-Arg-Pro-Lys-Leu-Lys
Enkephalins:
Tyr-Gly-Gly-Phe-Met
Tyr-Gly-Gly-Phe-Leu

Morphine
14

message   spacer   address
15

message   spacer   address
R = Phe-Leu (δ receptor selective)
R = Phe-Leu-Arg-Arg-Ile-OMe
(κ receptor selective)
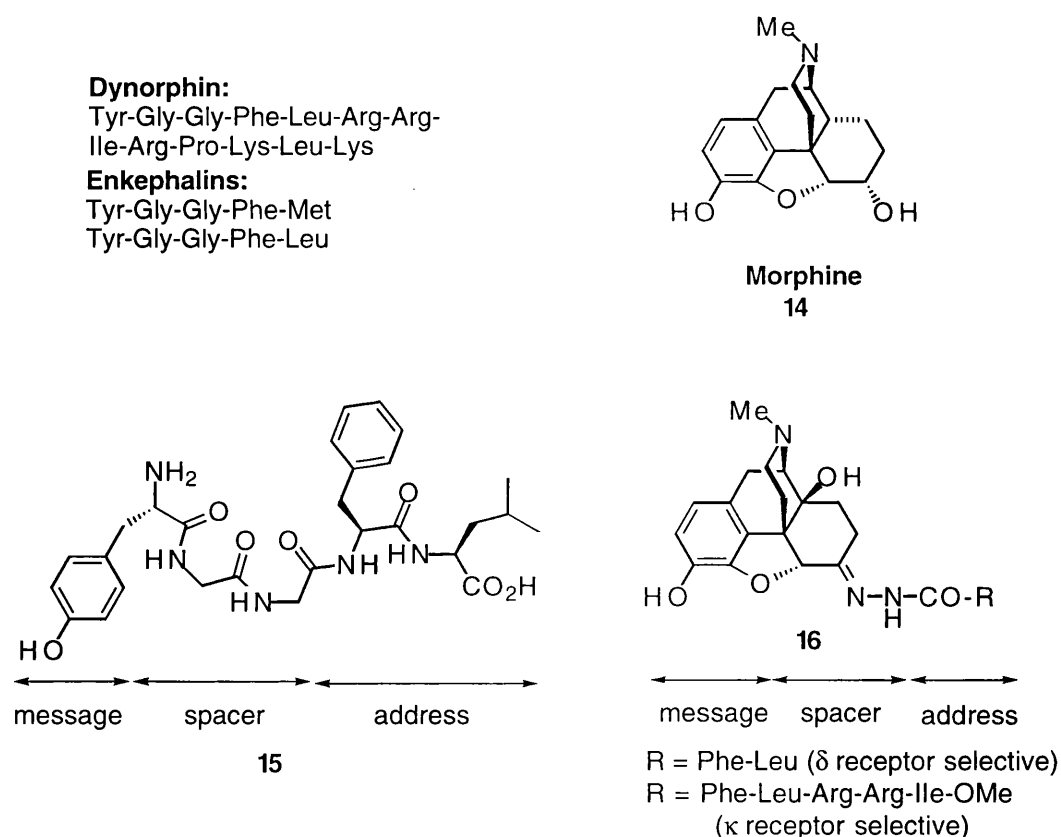
Adapted from Luthman[52]

There is no accepted strategy which guarantees the discovery of non-peptide ligands with high affinity, efficacy and specificity. In general, the discovery of novel ligands for peptide receptors has not been based on a thorough

understanding of the key-intermolecular interactions. Instead, receptor-based random screening and lead optimisation have been the preferred methods.

1.3.3.2. Development of Peptide Mimetics by Design: The Farmer Rules

The one dimensional genetic code contains all the information required to synthesise large, complicated, three dimensional structures. The predominance of peptides and proteins in nature does not derive from the unique receptor binding properties of these molecules, but from the fact that the structural information of these molecules can be contained within a one dimensional code[63]. Chemists are not constrained to this code and in the design of peptide mimetics researchers have tried to develop some general rules. Farmer[64] reviewed the general strategy for the rational design of non-peptide peptidomimetics. He suggests four "tentative rules" to follow.

(a) "The design of non-peptidic analogues of a bioactive peptide should start with the simplest conceivable structure that might possibly have specific peptidomimetic activity." Large parts of the peptide can be discarded without loss of activity. The determination of the smallest active fragment should then be performed by the stepwise cleavage of amino acids from both the N- and C-terminal ends of the natural ligand.

(b) "A non-peptidic analogue of a bioactive peptide should not occupy space outside that believed to be occupied by the peptide itself."

(c) "Conformational flexibility should be maximised until lead activity is discovered in a non-peptidic structure." At least some conformational flexibility should be retained in the first set of mimetic compounds. Potency and selectivity may then be improved by the introduction of conformational constraints.

(d) "The design of non-peptidic peptidomimetics should not rely heavily on mimicking the topology of backbone peptide bonds, especially where there are regular secondary structures". The common occurrences of α–helices and β–sheets mean that analogues of these secondary structures are unlikely to exhibit selectivity. Analogues mimicking tertiary structures will be more selective, although these mimics will be more difficult to design and synthesise.

In addition, Ariens[65] has studied the interaction between small molecules and peptide receptors and formulated a three point binding model. Two of these binding sites are believed to be an ion pair interaction site and an accessory binding site. The accessory binding site has been proposed[55,65] as recognising the "double-ring" motif, exemplified by the benzhydryl group (see below).

## 1.3.3.3. General Characteristics of Peptide Mimetics

Rich reviewed the general properties of a number of published peptide antagonists[55]. He concluded that all the compounds show a common chemical property: the inclusion of hydrophobic groups that are conformationally restricted. These groups prevent intramolecular hydrophobic interactions that would distort the ligand shape in an aqueous environment.

When large organic molecules dissolve in water they adopt conformations that minimise interactions between the hydrophobic groups and water. This is due to the powerful attraction of water molecules for each other. This effect, called the hydrophobic effect by Tanford[66], is central to all biological processes. Enzymes and receptors have evolved structures that generate stable hydrophobic pockets at their binding site. These hydrophobic pockets are stable in the presence of water and the absence of the ligand.

The peptide ligands for their part have evolved such that their hydrophobic groups are projected to interact appropriately with the enzyme or receptor for which they have been selected. Flexible peptides do not possess the rigidity required to prevent their hydrophobic side chains from interacting intramolecularly in aqueous media. The conformation therefore collapses to a hydrophobically stabilised conformation.

In the design of small molecule peptidomimetics, the template used should be resistant to hydrophobic collapse. It is important not to synthesise inactive collapsed conformations of the target molecule. Rich suggests that peptidomimetics will be discovered more readily in templates or scaffolds that conformationally restrict hydrophobic groups so that they cannot stack intramolecularly in water. Interestingly, most examples are found to contain the diphenylmethyl pharmacophoric group (see Figure 14).

28

Figure 14. Structural units which do not stack hydrophobic groups intramolecularly in aqueous media.
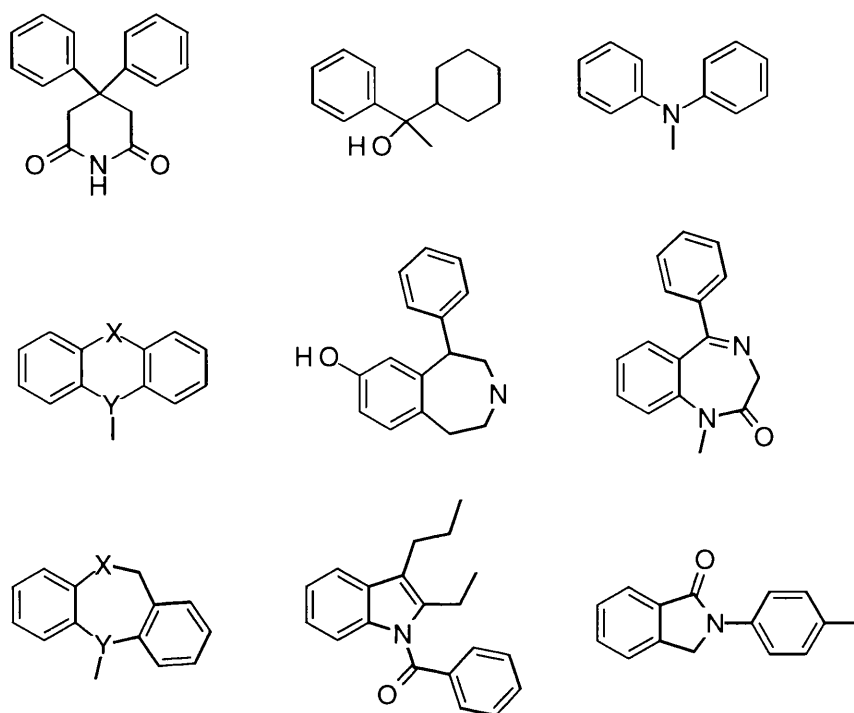
Adapted from Rich[55]

Rich then proposes that one should focus the design of peptide mimetics on those scaffolds which retain their ideal conformations in aqueous solution or are forced into hydrophobically collapsed bioactive conformations. If another type of scaffold is to be designed, he suggested that an energy minimised conformation in aqueous solution should be determined from molecular modelling.
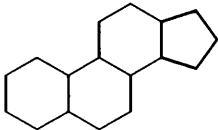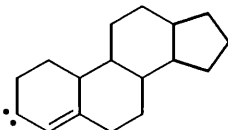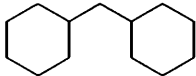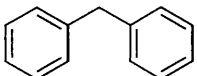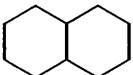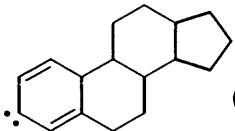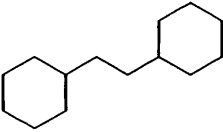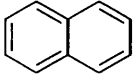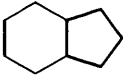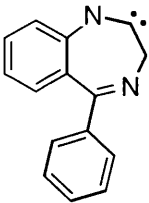
Why is the diphenylmethyl group recognised by a number of receptors? Rich speculated that, because of its affinity for a broad range of receptor systems, there is some structural element complementary to the diphenylmethyl moiety present in receptors for peptides. Nearly all seven-transmembrane superfamily of peptide receptors are G-protein linked systems[67]. The ligand binding domains in these receptor systems are in the hydrophobic core of the protein. Rich suggests that a hydrophobic pocket, complementary to different conformations of the diphenylmethyl unit, is formed by hydrophobic side chains from two or more α–helices buried within the membrane. As evidence Rich cited the work of Tota *et al.*[68] who used site directed mutagenesis of the β-adrenergic receptor to characterise the interactions between agonists and antagonists in three α–helices. Tota's work suggests that a series of aromatic

amino acid side chains in transmembrane helix 6 might be a candidate for this hydrophobic binding pocket. Such deletion mutation experiments could be used to characterise peptide receptor systems in the future and show which binding groups interact with the peptide and hence facilitate peptidomimetic design.

In a more general study of the properties of currently used drugs, Bemis and Murcko analysed a database of commercially available drugs[69]. The Comprehensive Medicinal Chemistry (CMC) database contains two and three dimensional information on the structures of known drugs. Of the 6700 compounds listed, Bemis and Murcko eliminated compounds which had no therapeutic activity class given. These included insecticides, laxatives and many miscellaneous examples. In the first analysis Bemis and Murcko were interested in the shape or 'framework' of the molecule. They defined the 'framework' as "the union of ring systems and linkers in a molecule" and excluded side chains and substituents from the study. In the first analysis Bemis and Murcko found that there were 1179 different frameworks among the 5120 compounds analysed. Of these frameworks, 783 (66%) were unique. They also found that 32 frameworks accounted for 50% of the total number of drug molecules (Table 1). The six-membered ring is the most commonly used framework for the drug molecules and acyclic compounds only account for 6% of the drugs examined.

In a second analysis, which took into account atom type and hybridisation, the number of unique frameworks was larger (76%), but a small set of 41 frameworks again account for a considerable proportion of compounds (24%).

Table 1. The seven most frequent frameworks in the CMC database from analysis 1 and analysis 2.

ANALYSIS 1	ANALYSIS 2

: = sp^2 carbon attached to side chain
(figure in parenthesis are the number of hits)
Adapted from Bemis[69]

The authors do not suggest that the database contains all the shapes a drug can take. Their results may reflect a me-too approach to drug design or perhaps synthetic limitations. However, they stress that half of the known drugs fall into only 32 shape categories and that the drugs have a similar topological shape.

1.3.4. Benzodiazepines as Non-Peptidal Peptide Mimetics

Benzodiazepines (BZDs) are one of the most important classes of biologically active molecules and have been used as templates for the construction of peptidomimetics. BZDs have other advantages which are relevant in drug

design. They are relatively compact structures, easily synthesised and show excellent oral bioavailability and safety profiles.

1.3.4.1. The Discovery of BZDs

BZDs were discovered serendipitously in the 1950s by Sternbach[70] at the Roche laboratories in Nutley, New Jersey. At that time Roche wanted to develop a new tranquilliser because the current drugs had significant side-effects. Drawing on post-doctoral experience with a class of compound known as the benzheptoxadiazines, Sternbach planned to synthesise a number of 2-aminophenones (**17**) bearing different substituents in the benzene ring and then effect intramolecular ring closure to afford 2,6-diaminooxepines **19**. By acylating a variety of 2-aminophenones, a large number of new compounds of type **19** could be synthesised quite readily. Sternbach then wanted to incorporate a basic functionality into the molecule as in **21**, since he thought this might impart biological activity.
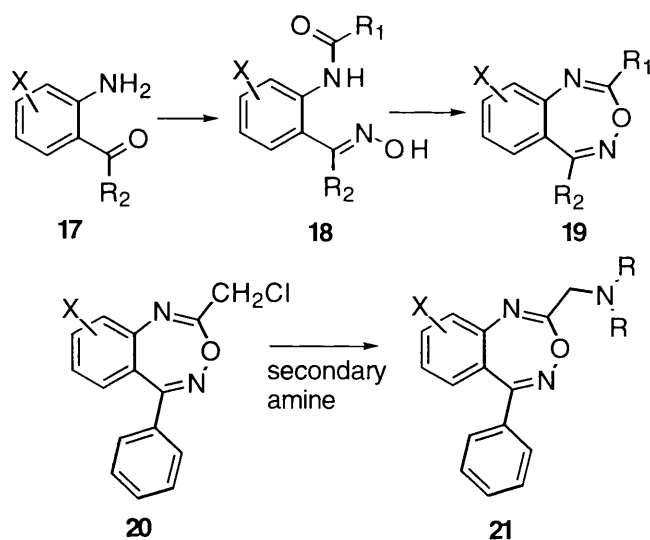
Scheme 1

After their apparent synthesis by this route, doubts about the structure of the compounds **20** and **21** arose. When compounds **20** and **21** were hydrogenated, the oxygen was removed with great ease and the products were found to be quinazolines. Further chemical studies proved conclusively that the compounds originally formed were not the 7-membered ring compounds **20, 21** but quinazoline 3-oxides **22** and **23**.
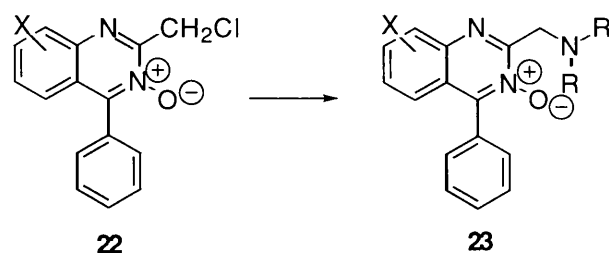
Scheme 2

Unfortunately, although crystalline and formed in good yield, these compounds did not elicit any interesting biological properties. After two years working on a new project, one of Sternbach's co-workers noticed that a crystalline product had formed from the treatment of **22** with methylamine. The compound itself was not tested because of an oversight, but the water soluble hydrochloride was submitted for pharmacological evaluation. It transpired that the compound synthesised showed significant muscle relaxation, sedation and anti-convulsant activity in a number of animal models. Concerns about the structure prompted further studies which finally unambiguously assigned the diazepine structure **26** to the compound.
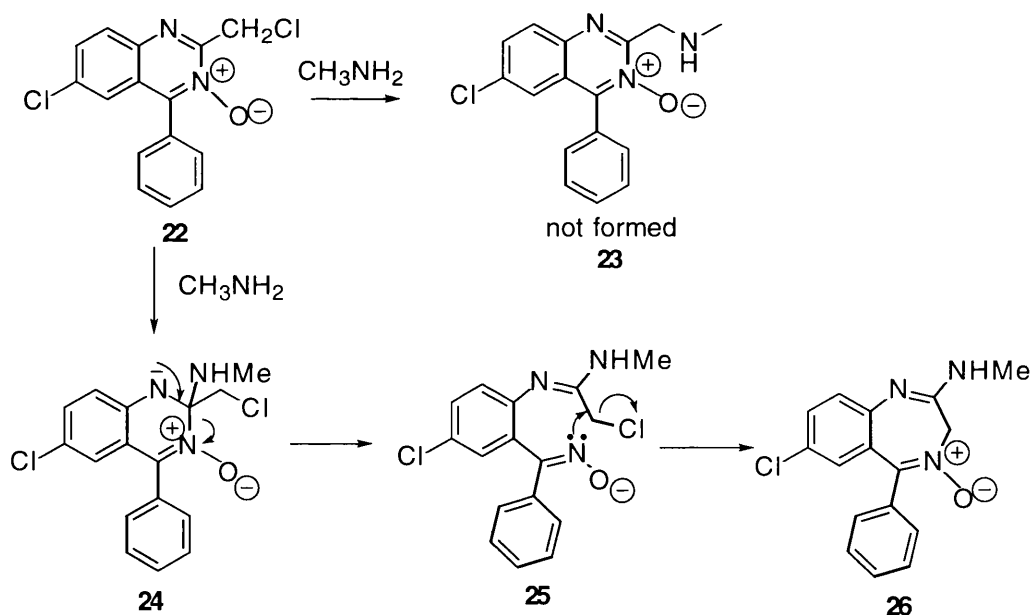
Scheme 3

The group then investigated the unusual transformation of a quinazoline 3-oxide **22** into a benzodiazepine 4-oxide **26**. The methylamine attacks the quinazoline N-oxide at the 2 position rather than replacing the reactive chlorine atom as might be expected. This causes the formation of an intermediate of type **24** which is probably transformed *via* **25** into the 1,4-benzodiazepine derivative **26**.

After completing toxicological studies, compound **26** was marketed by Roche under the name Librium. During studies to synthesise a derivative which did not have such a bitter taste and was less hygroscopic, compound **27** was found to be formed from **26** in the presence of aqueous acid. In another stroke of luck it emerged that when **27** was treated with PCl_3 the derivative **28** formed was not bitter or hygroscopic and was more potent. Subsequently over fifteen 1,4-benzodiazepines have found widespread use in a number of clinical conditions[71].
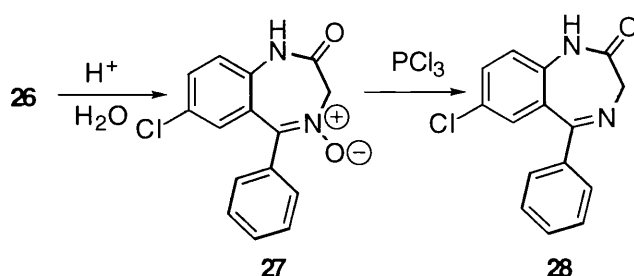
Scheme 4

The BZDs exert their biological effects at the GABA receptor by facilitating chloride flux. $GABA_A$ receptors in the CNS contain a chloride ion channel and have an independent benzodiazepine binding site. BZDs do not open the ion channel directly but increase the affinity of the receptor for GABA and potentiate its effects. The identification of the BZD receptor has led to speculation as to the identity of the natural ligand. One candidate, a 10 kDa peptide diazepam-binding inhibitor, has been isolated from rat brain and has an anxiogenic and pro-convulsant effect. Other evidence suggests that BZDs themselves may occur naturally in the brain. A recent autopsy of a human who died 57 years ago detected the presence of BZD **28**, at a time long before it was available as a synthetic compound[72,73].

1.3.4.2. The Development of Benzodiazepine CCK Antagonists.

BZDs have been used by workers from Merck, Sharpe and Dohme[74,75] as a template in the design of novel non-peptide cholecystokinin (CCK) antagonists. From random screening, a lead compound was discovered, the fungal metabolite asperlicin (**29**), which was found to exhibit an IC_{50} 1 μM as a CCK antagonist. A synthetic analogue program was initiated to prepare a compound with increased potency and oral bioavailability. This unfortunately failed and the workers were left to decide upon a new approach to the program. They decided that common structural and conformational elements shared by peptides might also be structural requirements for their non-peptidal surrogates. They were

aware that tryptophan was a key amino acid in the sequence of CCK. They discovered that tryptophan was a requirement for the biosynthesis of asperlicin. They then made the astute observation that, contained within the numerous substructures of asperlicin, was a BZD framework. This enabled them to simplify their lead to compound **30**. They then focused on C-3 substitutions which allowed them to synthesise extremely potent and specific antagonists at the CCK_A and CCK_B receptors.

Figure 15. The design of CCK antagonists.

29, Asperlicin (IC_{50} = 1 μM)

30, IC_{50} = 3.4μM

CCK_A MK-329 (IC_{50} = 0.8 nM)

CCK_B L-365,260 (IC_{50} = 2nM)
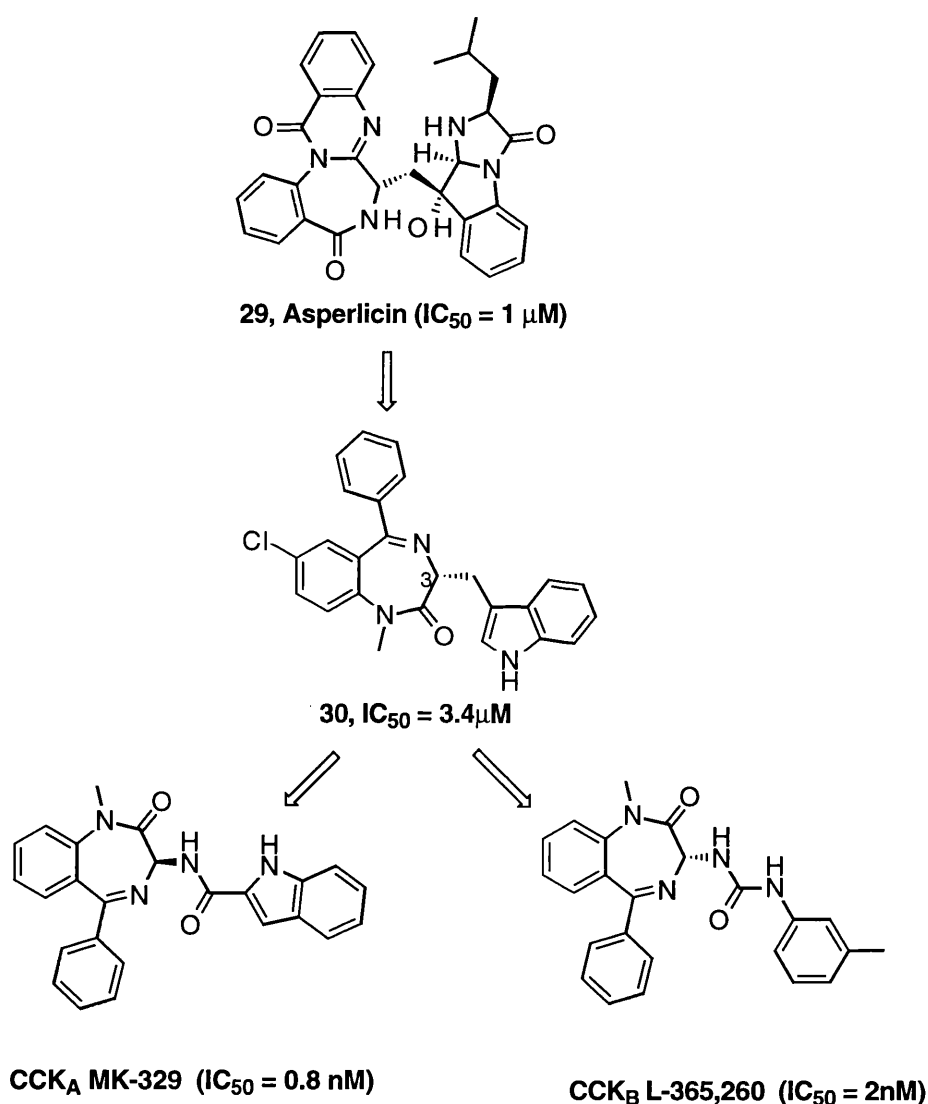
1.3.4.3. The Design of Benzodiazepine RGD Antagonists.

Workers at SmithKline Beecham were interested in synthesising non-peptide RGD analogues for the reasons described in Section **1.3.2.5**.[76]. They initially synthesised peptide **32** which displayed a high affinity for the fibrinogen receptor and considerable potency in inhibiting platelet aggregation. The

conformational rigidity of the small peptide allowed the use of ^{1}H-nmr spectroscopy to study its conformational preferences. Complete analysis of the ^{1}H nmr data for **32** revealed the RGD sequence to be characterised by a γ-turn. A x-ray structure was also determined for **32**, which strongly confirmed the turn-extended-turn conformation of RGD.

Since the conformational preference of the molecule was established, this provided them with the information to design a non-peptide analogue. In linear RGD peptides, the Asp cannot be replaced by either a D-Asp or Glu without complete loss of activity, suggesting that it is a critical recognition element. A number of diverse linkers have been shown to correctly deliver the guanidine moiety of arginine. In addition, a lipophilic group following Asp appeared to increase activity. Two particular conformational features of the peptide backbone, the turn at Asp and the extended Gly residue, were critical to the 3-dimensional placement of the Arg and Asp side chains (Figure 16).

Figure 16. The design of a non-peptide RGD antagonist

Ac-Arg-Gly-Asp-Ser-NH$_2$

31

SK&F 107260

32

SB 207448, R = H

human GPIIbIIIa receptor:
K$_i$ = 1.5 nM

33
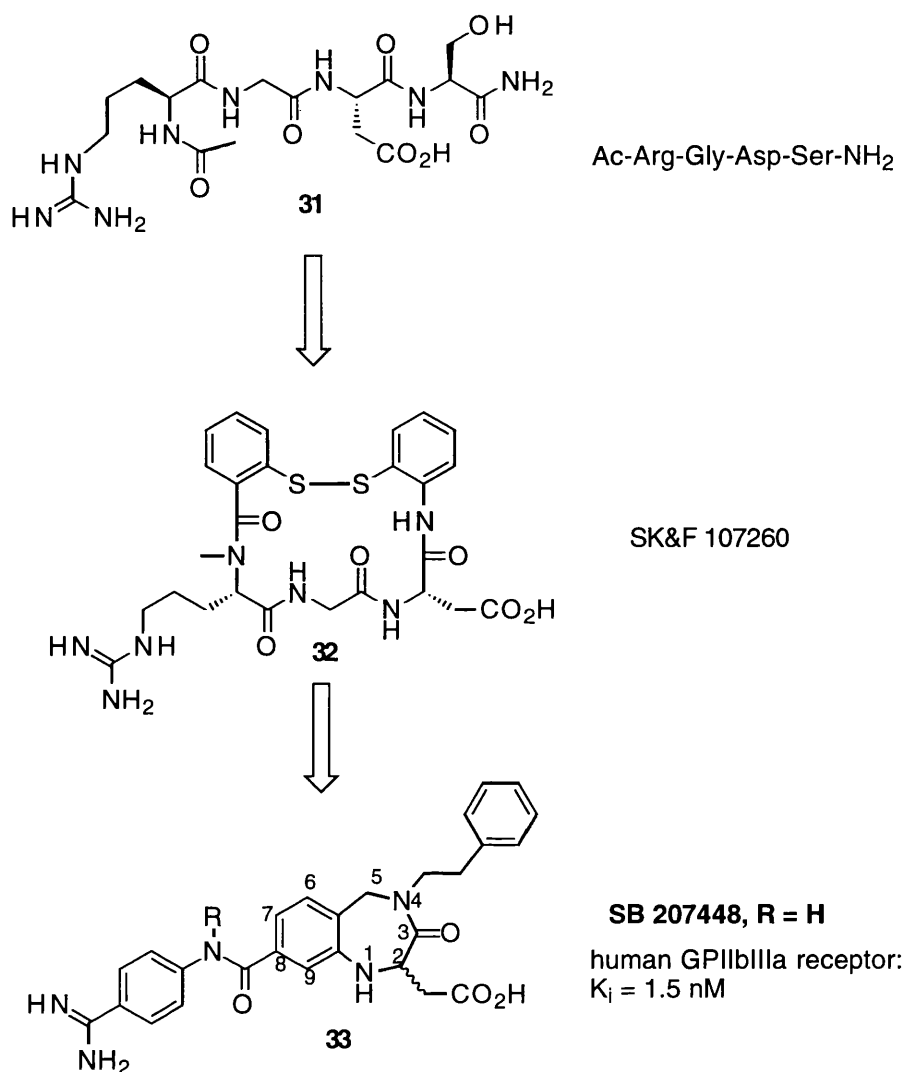
These were the features for the non-peptide to mimic. The 1,4-benzodiazepine nucleus offered the potential to mimic both the turn and the extended Gly in a manner that restricted conformational flexibility. Molecular modelling indicated that the 2 position of the BZD is equivalent to the Asp Cα. Attachment of an acetic acid moiety to this position was likely to maintain the Arg to Asp spatial relationship as it is found in the cyclic peptide. An amide group was added at position 8 of BZD to provide a mimic of the corresponding Arg carbonyl in the peptide. Taken together, the diazepine, the aromatic ring, and the amide functions of **33** represent a small molecular template that mimics the Arg-Gly-Asp backbone region of the peptide. Appropriate placement of the amidinophenyl group (as an Arg side-chain equivalent), the acetic acid moiety, and the lipophilic 2-phenylethyl group completed the design.

Genentech scientists also used the same approach to design a non-peptide RGD mimetic[77,78]. They studied the [1]H-nmr solution conformation of a rigid and potent peptide antagonist (G4120) and, rather than use sequential modifications to evolve a non-peptide structure from that of a peptide, they elected to design a non-peptidal scaffold to fit the contour and volume of the peptide backbone of G4120 (Farmer rule (b), Section **1.3.3.2.**, Figure 17). The group were confident that the "cupped shape" of the solution structure of the peptide related to its bound state conformation. The framework selected had to be flexible to allow systematic variations of the side chain. They chose a benzodiazepinedione scaffold after consulting crystallographic and modelling data, since the BZD is quite rigid with six of the atoms in the seven membered ring participating in conjugated systems and enforcing the "cupped shape". The aromatic ring allows the Arg side chain to be mimicked, to be directed in a number of positions and incorporation of a third ring allows the precise control of the Asp side chain (see Figure 17).

Figure 17. The design of RGD antagonist **35** from **G4120**
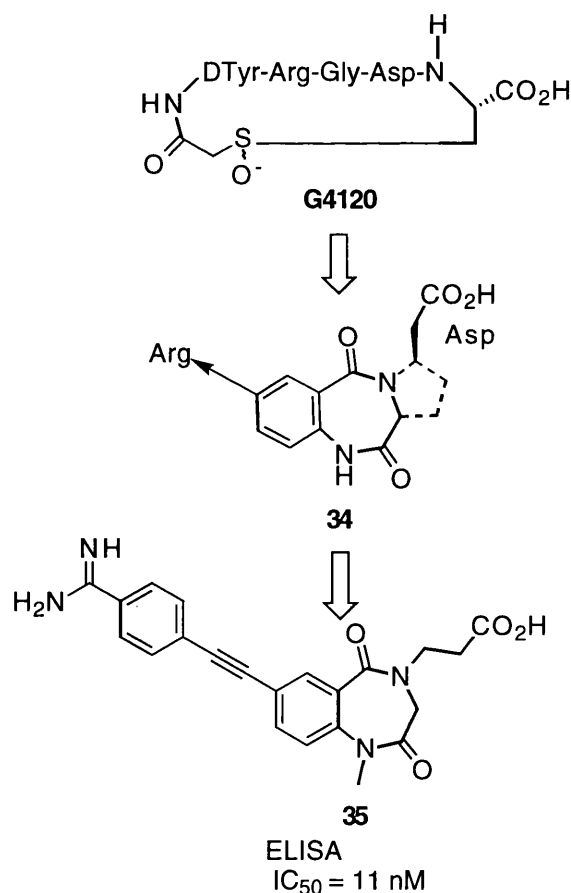
The original tricyclic analogues synthesised were inactive, but the bicyclic analogues possessed moderate activity. The biological activity was optimised

by N-methylation and attachment of the benzamidine moiety to an acetylene linker to give a potent RGD antagonist.

1.3.4.4. Structures of Benzodiazepine Peptidomimetics

BZDs have been recognised by a wide variety of peptide receptors[48,74]. Figure 18 shows various BZDs that either mimic or otherwise interact with peptides: (36)[79], (37)[80], (38)[81], (39)[82].

Figure 18. The variation in structure of some benzodiazepine ligands
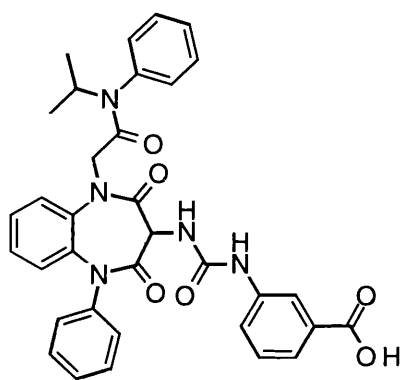
36
Glaxo (CCK$_A$ agonist)
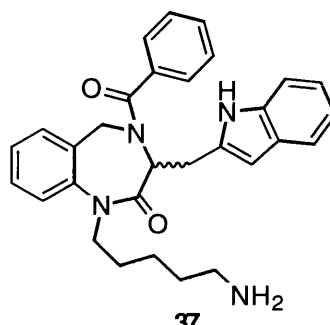
37
Novartis (Somatostatin agonist)
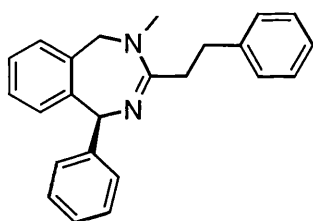
38
Sterling Research group
(Antiarrhythmic agent)
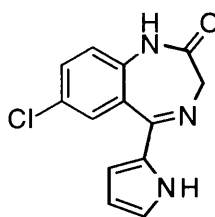
39
Roche (HIV-1 Tat inhibitor)

The BZDs have been reported as ligands for a surprisingly diverse collection of receptors. The natural ligands of these receptors bear little resemblance to one another or to the BZD analogues. The only similarities are among the BZDs themselves. BZDs may contain common features which facilitate binding to various protein surfaces, perhaps different from those of the natural ligand.

The observation that certain compounds are able to provide high affinity ligands for more than one receptor contradicts the concept of individuality and selectivity of receptors predominant in most models of receptor function. However, the family of G-protein coupled receptors is thought to have evolved

from a single ancestor. The ligand binding domain of this family of receptors is buried within the membrane on one or more of the α-helical segments. This section of the receptor is one of the most conserved amongst this class and, as Rich suggests[55], compounds which contain an embedded diphenylmethyl motif may bind to a complementary hydrophobic binding pocket in that area.

The BK receptors belong to this superfamily of G-protein coupled receptors and a non-peptide antagonist of BK would have significant therapeutic potential. As an approach to the design of antagonists, Evans[75] suggests: "What is clear is that certain 'privileged structures' are capable of providing useful ligands for more than one receptor and that judicious modifications of such structures could be a viable alternative in the search for new receptor agonists and antagonists."

The benzodiazepines are one class of compound which can incorporate a diphenylmethyl motif. The BZDs have also been described as one of these 'privileged structures'[48,52] and, as such, modification of their structure may furnish a novel class of BK antagonist.

2. RESULTS AND DISCUSSION

2.1 Models for the Design of BZD-Derived Peptidomimetics

Our approach to the design of non-peptidal BK antagonists was based on the ideas contained in two literature reports. These papers are examined briefly before our application of these concepts are discussed in Section **2.1.2.**

2.1.1 Examples of Benzodiazepines as β–Turn Mimetics in the Literature.

An important structural feature of many peptides and proteins is the β-turn motif. β-Turns consist of a segment of four amino acids (residues *i* to *i* + 3) and occur where the peptide chain reverses direction, due to the tendency of the backbone amide bonds to form intramolecular hydrogen bonds[83]. These turns are often situated on the protein surface and offer the opportunity for intermolecular interactions with other protein surfaces and hence provide sites for intermolecular recognition[84].

In biological systems β-turns in peptides and proteins may well be crucial in receptor interactions that ultimately lead to biological activity[85,86]. In recognition of this there have been several attempts to synthesise organic molecules that might mimic a specific β-turn in a peptide of interest[87,88].

In an attempt to synthesise a general non-peptide β–turn mimetic, one applicable to a variety of β–turn types, workers from Du Pont Merck[89] sought a rigid molecule that possesses the proper turn geometry and correct side chain orientation. A Type I bend was initially modified, the hydrogen bond between residues (*i*) and (*i* + 3) being replaced with two methylene units and the backbone amide bonds with three double bonds to form compound **41** (See Figure 19). In a second case, the alkene bond between R$_2$ and R$_3$ was replaced with an amide linkage to give **42**.

Figure 19. The design of non-peptide β-turn mimetics
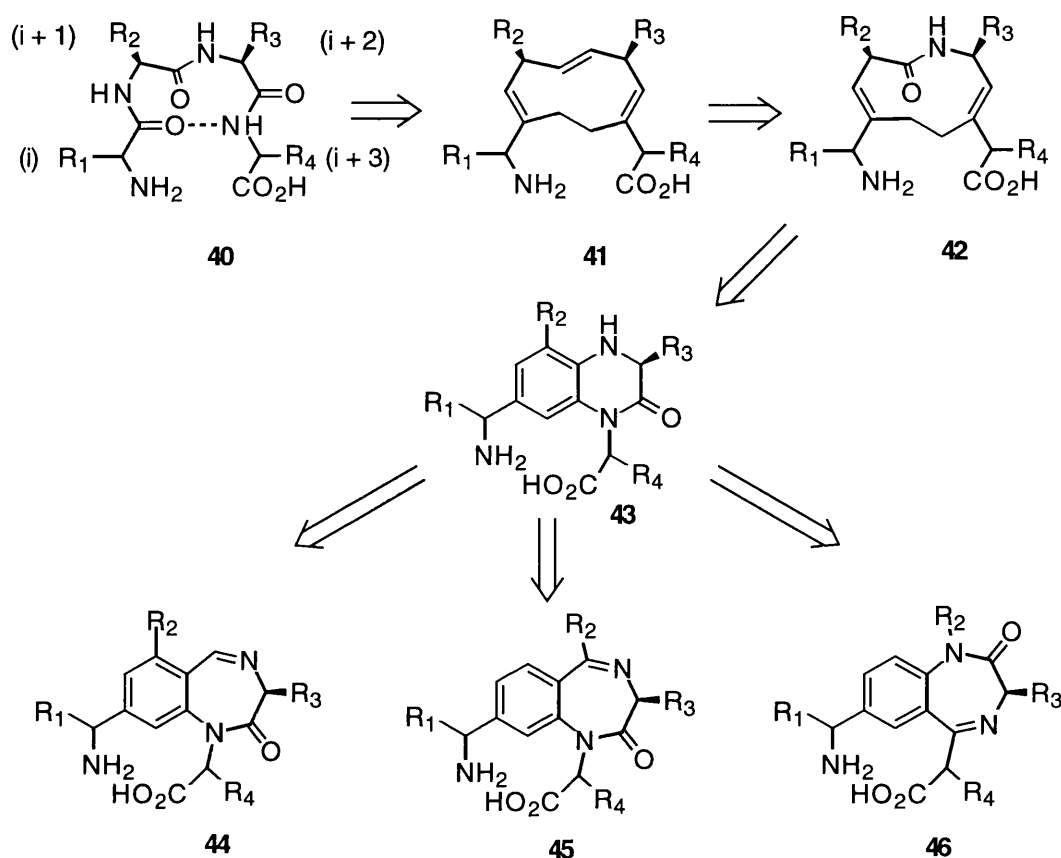
The energy minimised conformations of the macrocycles **41** and **42** were superimposed over the original β–turn **40**. The conformations were found to be a close match. The challenges envisaged in the syntheses of these macrocycles, however, led them to look for alternative systems. The initial alternative structure was the bicyclic system **43** but the perceived potential lack of stability and problems of synthesis led to the design of structures with a larger heterocyclic ring. The resulting benzodiazepine derivatives **44, 45, 46** show a good fit to the most common types of β–turn. However, only in **44** and **45** can the side chains R_1 to R_4 adopt similar relative positions to those present in the peptides.

One of the amide bonds between R_3 and R_4 is preserved in **45**. This means that the absolute stereochemistry of R_3 can easily be set by the use of an appropriate amino acid. The side chain R_2 can be in one of two positions (**44** or **45**) and still reach similar positions in space. Since proline is found 30% of the time at position 2 of β–turns (*i* + *1*), this suggests that in most cases either no substituent or a hydrophobic group (*e.g.* phenyl) might be acceptable at this position[89].

In order to support their hypothesis that the BZD nucleus can act as a non-peptide β–turn mimic, the Du Pont Merck group examined the conformation of the BZD β–turn mimetic in two situations.

In the first case, the conformation of the BZD β–turn mimetic was studied by 1H-nmr spectroscopy. Kopple[90] has shown by [1]H-nmr spectroscopy and x-ray analysis that the cyclic octapeptide c(Gly-Pro-DPhe-DAla)$_2$ **47** exists in a C$_2$ symmetric, stable double β–turn conformation. Substitution of one of the β–turns with a different four amino acid sequence destabilises the structure of the resulting cyclic octapeptide. The Du Pont Merck group substituted one of the β–turns with a BZD scaffold to form compound **48** (Figure 20).

Figure 20. The benzodiazepine scaffold as a β-turn mimetic
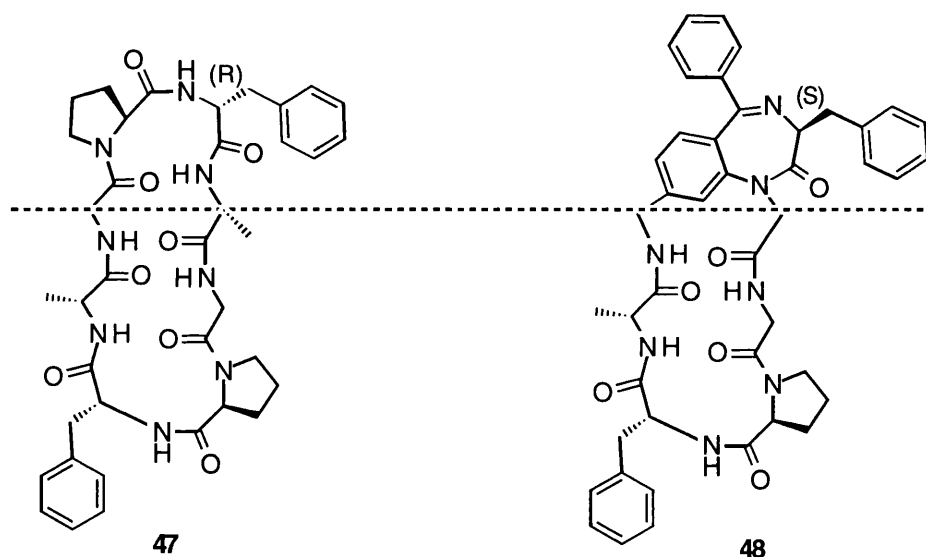
The 2D [1]H-nmr spectrum of compound **48** was obtained and compared to that of the cyclic octapeptide. The spectrum showed that the backbone conformation of the β–turn mimetic **48** and the peptide **47** were similar[89]. This suggests that the BZD is stabilising the β–turn present in the peptide portion of **48**.

The Du Pont group then decided to investigate whether the benzodiazepine could adopt the correct conformation to act as a non-peptide β–turn mimetic in a biological assay. Gramicidin S (GS, **49**) has been examined by ^{1}H nmr spectroscopy, circular dichroism, and x-ray analysis and shown to exist in a C$_2$ symmetric, stable, double β–turn conformation with a β–sheet connecting the two turns[91]. GS is a biologically active (anti-bacterial) peptide and its biological activity has been shown to be related to its conformation.

Figure 21. The benzodiazepine scaffold as a β-turn mimetic
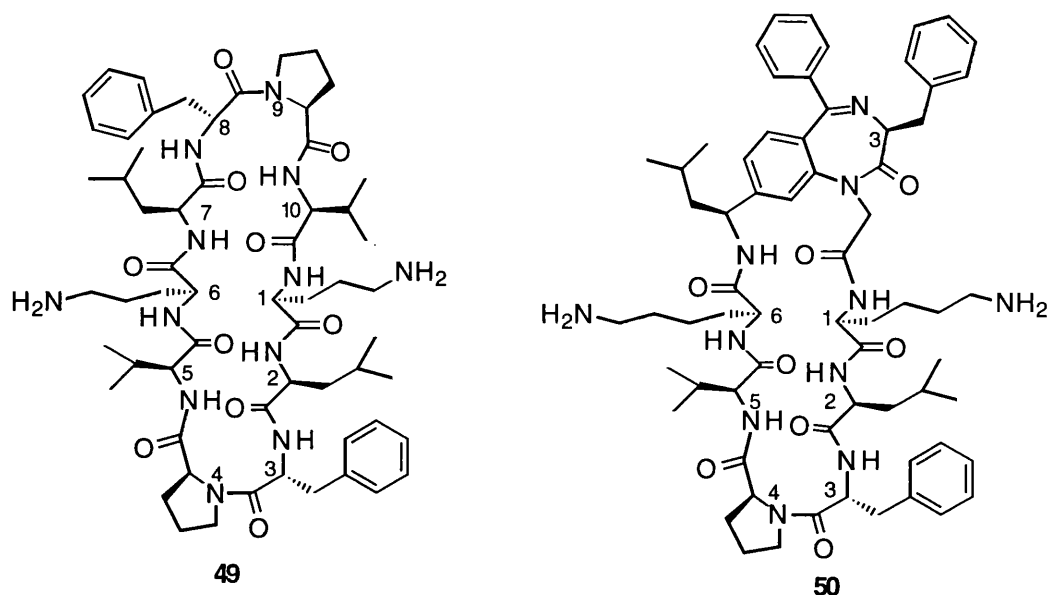
In order to synthesise the BZD peptide mimetic **50**, the group first had to simplify the molecule. They replaced the lysine residues (1, 6) by ornithine, this replacement having been shown not to affect the conformation of the molecule but to decrease biological activity by 50%[91]. The group incorporated the phenylalanine side chain at C-3 in the BZD to mimic the proline present at the (*i* + 2) position of the β–turn of **49**.

The group then studied the 2D ^{1}H nmr spectrum of the molecule and found that the four amino acids Leu^2-DPhe^3-Pro^4-Val^5 adopt a β–turn conformation, strongly suggesting that the BZD is topologically mimicking a β–turn and maintaining the overall native conformation of this cyclic peptide. When the antibacterial activity of compound **50** was compared to that of the original peptide against *Staphylococcus aureus*, **49** had a minimum inhibitory concentration (M.I.C.) of 4 μgml^{-1} compared to that of the BZD peptide mimetic **50** of 8 μgml^{-1}. Since the ornithine for lysine substitution is known to halve the activity of **49**, it is seen that compound **50** is an excellent non-peptide mimic of GS[92].

Another example of the use of benzodiazepines in the area of turn mimicry is found in the work of James *et al.*[93]. Most current cancer chemotherapeutic regimes target the cellular DNA. This chemical interference causes damage to the genetic material which stops cellular growth and hence uncontrolled cell division. These drugs are, however, indiscriminate in their actions and target all dividing cells, which include hair follicles, bone marrow cells and gastric

mucosal cells, causing the well-documented side-effects of cancer chemotherapy. These problems have led to the search for more specific drugs which act at a different target.

The development of cancer is a consequence of abnormal expression or function of specific cellular genes, called cellular oncogenes. The Ras oncogenes encode plasma membrane proteins that form part of the intracellular network responsible for the control of cell growth and differentiation[94,95]. Ras is a guanosine triphosphate (GTP) binding protein and when GTP is bound to Ras, cell division is triggered. Normal Ras possesses GTPase activity, leading eventually to the hydrolysis of the bound GTP to GDP, so that the mitogenic signal is terminated. The mutant forms of Ras have an impaired GTPase activity[94], and the consequence of this is that the mutant Ras protein remains permanently bound to GTP, leading to unregulated cell proliferation. Mutation of Ras genes is one of the most common genetic abnormalities in human malignancy, occurring in 90% of pancreatic carcinomas, 50% of colon carcinomas and 20-30% of acute leukaemias[96]. The transforming activity of Ras is induced by a single point mutation[97].

Farnesylation of both normal and oncogenic Ras proteins is necessary for attachment to the cell membrane and the resultant biological effects[98] (see Figure 22). This process is catalysed by the enzyme farnesyltransferase (FTase), a member of the recently discovered family of Zn^{2+} dependent prenyl transferase enzymes which catalyse the attachment of lipophilic moieties to the carboxyl terminal domains of certain intracellular proteins[99,100,101].

Figure 22. Membrane localisation of Ras
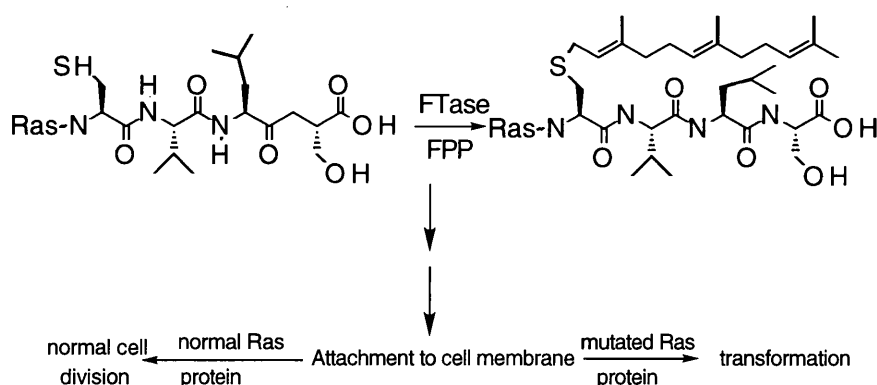
The enzyme FTase can be inhibited by a tetra-peptide sequence CAAX, in which C is cysteine, A is an aliphatic amino acid, and X is a serine or methionine[99,102,103]. CAAX tetra-peptides have no effect on Ras processing

when added to intact cells because they are either degraded by peptidases or are not able to penetrate the cell membrane. Because of the absolute requirement of the cysteine and methionine/serine residues at the N- and C-termini of the peptide, work concentrated on modification of the central hydrophobic residues. It is proposed that the zinc atom within the enzyme is simultaneously co-ordinated by the sulfur of the cysteine and the carboxyl group of the methionine/serine residue with the other amino acids forming a reverse turn[93]. The two aliphatic amino acids at positions (i + 1) and (i + 2) of the turn were replaced by the BZD scaffold (Figure 23). Molecular modelling studies showed that the BZD favourably presents the cysteine residue and the C-terminal methionine residue in the correct orientations to form potent inhibitors of FTase (Figure 24). This result again demonstrates the flexibility of the benzodiazepine framework in the area of turn mimetics.

Figure 23. Benzodiazepine turn mimetic
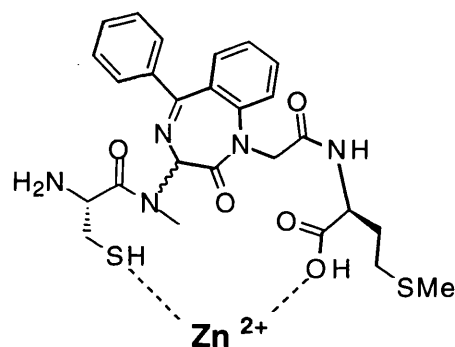
Figure 24. Ras farnesylation inhibitors
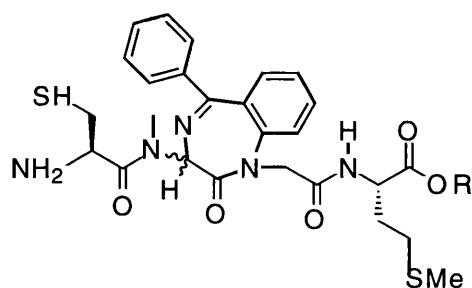
BZD A : R=H IC_{50}= 0.85nM
BZD B : R=Me IC_{50}=32-50nM

BZDs A and B act as peptide mimetics
and are potent inhibitors of Ras farnesylation

2.1.2. Can the BZD Scaffold Mimic the β–Turn in HOE140?

HOE140 (D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Thi[5]-Ser[6]-D-Tiq[7]-Oic[8]-Arg[9])[39,104] is a potent long-lasting BK antagonist, whose therapeutic potential has already

been mentioned (Section **1.2.6.**). A β-turn is believed to be induced by the unusual amino acids at positions 7 and 8 (see Figure 25) and, given the examples in the previous section, it might be possible to construct a BZD framework at this location. Selection of an appropriate BZD scaffold would also need to take into account those observations described in Sections **1.3.3.2.** and **1.3.3.3.**

2.1.2.1 Benzodiazepine Overlay Model I

There are a number of ways of overlaying a generalised BZD core on the β–turn in HOE140*. Ripka and co-workers have overlaid a BZD at the (*i* + 1) corner of the β–turn in the model cyclic peptide **47** and the cyclic antibiotic **49** as previously described in **2.1.1.** In this overlay model, the BZD acts as an internal β–turn mimetic. An internal β–turn mimetic is a molecule in which the mimetic skeleton approximately replaces the space that was occupied by the peptide backbone in the natural β–turn[105]. The substituents on the BZD then radiate along the corresponding peptide side chain trajectories, with the lactam moiety more or less superimposed onto the peptide bond between amino acid residues (*i* + 2) and (*i* + 3). Figure 25 illustrates the overlay with respect to the peptide template, HOE140, and highlights the amino acid residues chosen to be mimicked, assuming that this conformation is maintained in the receptor bound state.

By utilising the phenyl substituent at C-5 of the BZD framework as the (*i* + 1) binding determinant (retaining the "double ring" motif), the subsequent introduction of additional functionality would be immediately facilitated. A C-3 hydrophobic mimic of the (*i* + 2) residue would easily be incorporated by use of an appropriate amino acid, while a mimic of the important Arg[9] side chain could conceivably be elaborated from N-1 by alkylation with a suitable electrophile. Alternatively, the (*i* + 3) position could comprise of a carboxylic acid function in accordance with peptide structure activity requirements[106].

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* There are a number of benzodiazepine scaffolds which could have been chosen as the design core (see Section 1.3.4.4.). The 1,4-benzodiazepin-2-one depicted was chosen on the strength of the literature observations detailed in the previous section (2.1.1.).

Figure 25. Overlay model I design rationale

HOE140 C-terminus β-turn

Projects towards D-Tic[7]
in (R)-enantiomer

BASIC
FUNCTION — LINK

HYDROPHOBIC
SIDE CHAIN

Mimics Arg[9]

Mimics Oic[8]
(pseudo-equatorial)

51
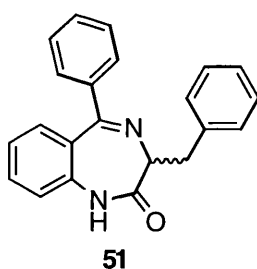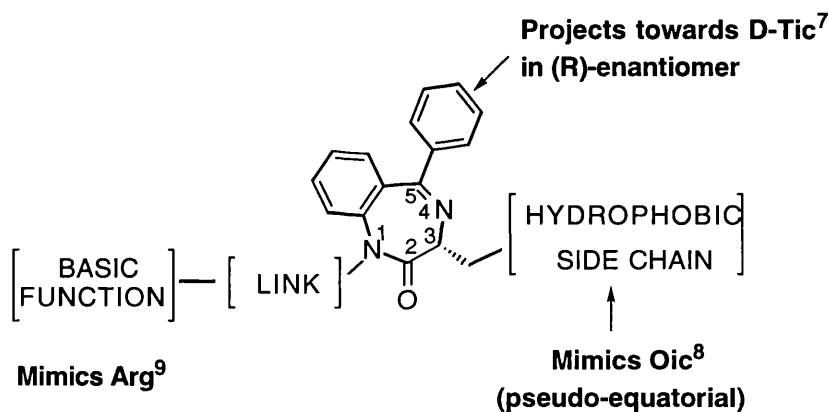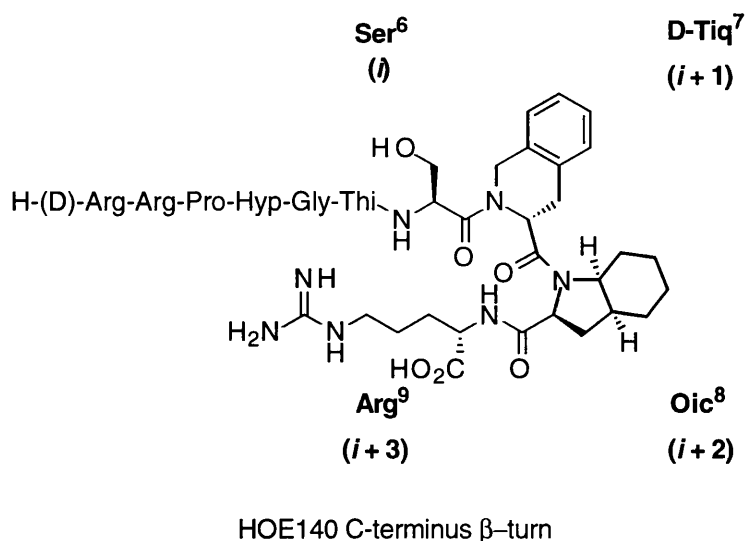
The seven membered heterocyclic ring of the unsubstituted BZD can flip between two *pseudo*-chair conformations of equal energy. In compound **51**, the group at C-3 can orientate itself either in a *pseudo*-axial or a *pseudo*-equatorial position. Ripka[92] states that, when there is a substituent in the C-3 position, the chair form with the substituent in a *pseudo*-equatorial position is thermodynamically favoured. Although there are a number of literature precedents for this suggestion[71], an independent determination of the

conformation was made, as this has implications in the correct positioning of the C-5 phenyl substituent.

An X-ray crystal structural analysis of BZD **51** was obtained, showing that the benzyl group orientates itself in an *pseudo*-equatorial position. The crystal structure, obtained on the racemic mixture, shows that the unit cell exist as a dimer, the components of which have similar but not identical conformations (see Figure 26 and appendix).

Figure 26. X-ray crystallographic structure of **51**
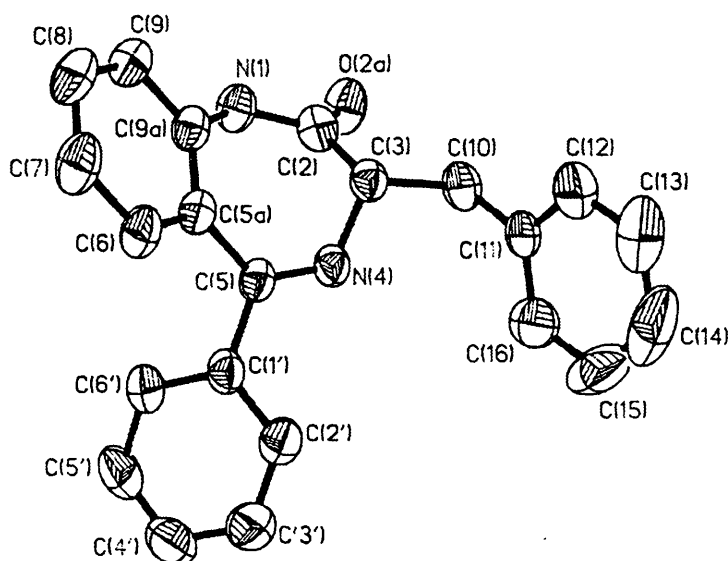
The first overlay model of the BZD core **51** on HOE140 requires the benzyl group to project in the general area previously occupied by the amino acid residue Oic and the phenyl group to project itself in the region previously occupied by the amino acid D-Tiq. In order to accommodate the C-3 substituent in a *pseudo*-equatorial position, the seven-membered ring is puckered in such a way that the C-5 substituent is projected on one side of the lactam plane in the (S)-enantiomer and on the other side in the antipode. Molecular modelling reveals that only the (R)-BZD enantiomer is capable of maintaining all three binding determinants in the appropriate positions*. Despite the stereochemical

---

* Note also that in the model system **48** (Figure 20, p 43) a BZD of (S) stereochemistry is employed to mimic an (R) amino acid at the (i + 2) position in **47**. This supports our preference for a BZD of (R) stereochemistry to mimic the (S)- phenylalanine residue at (i + 2) in the C-terminal β-turn of BK, or the (2S)-Oic residue at the same position in HOE140.

bias in this paradigm, it was prudent and expedient to evaluate racemic mixtures at the onset of the lead finding process. In this way the (S)-enantiomers would not be disqualified from revealing an antagonistic activity arising from an alternative mode of binding.

2.1.2.2. Benzodiazepine Overlay Model II

In the overlay model of James *et al.*[93] for Ras farnesyltransferase inhibitors, the benzodiazepine forms an external β–turn mimetic core. When this model is applied to HOE140 the C-3 substituent mimics the hydrophobic amino acid D-Tiq and the benzo-fused ring in turn mimics the amino acid Oic (Figure 27). The C-5 phenyl substituent has no obvious role in this model but was retained because its presence was shown previously not to be a disadvantage. Because the BZD is located at a slightly greater distance from the basic function that mimics Arg[9], an extra acetamido function needs to be inserted in the side chain.

Figure 27. Overlay model II design rationale

HOE-140 C-terminus β–turn
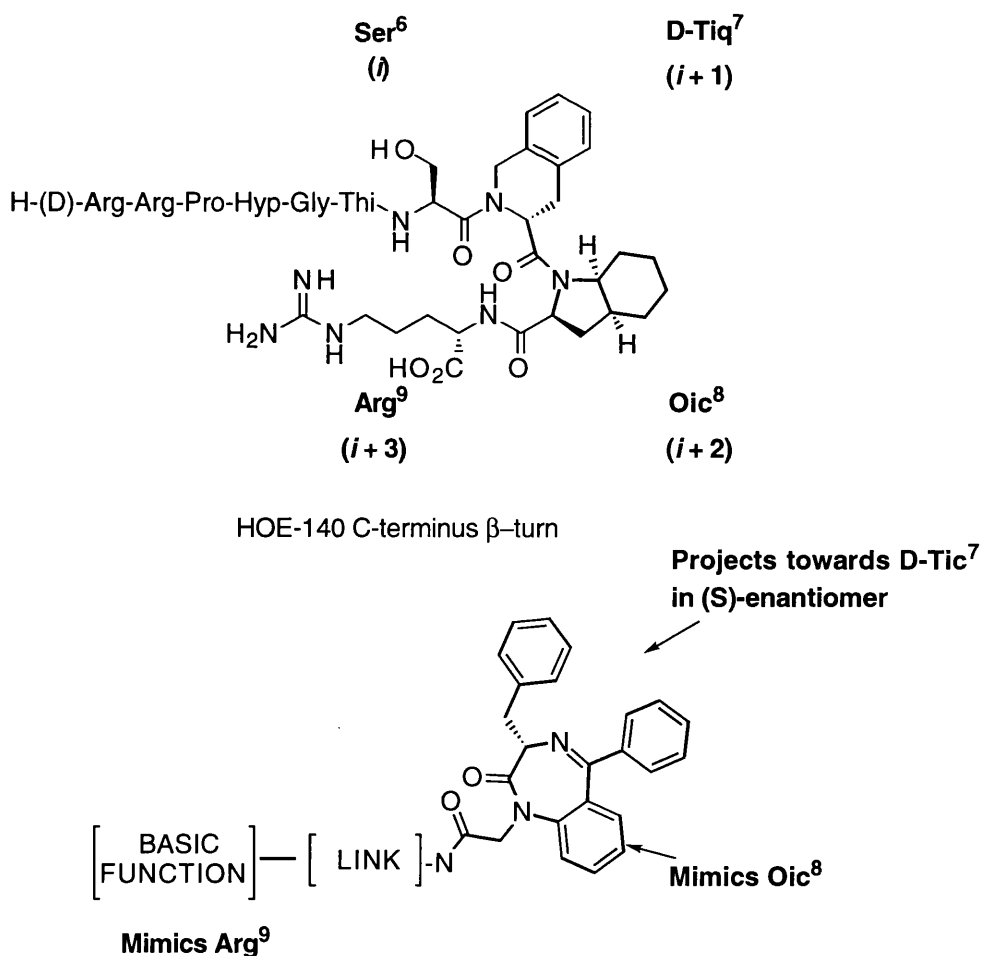
2.2. Chemistry

2.2.1. Variation in 1,4-Benzodiazepin-2-one Side Chain - Overlay Model I

The BZD **51** was synthesised in two steps from N-Boc phenylalanine (**52**) and 2-aminobenzophenone[89] (**53**, see Scheme 5).
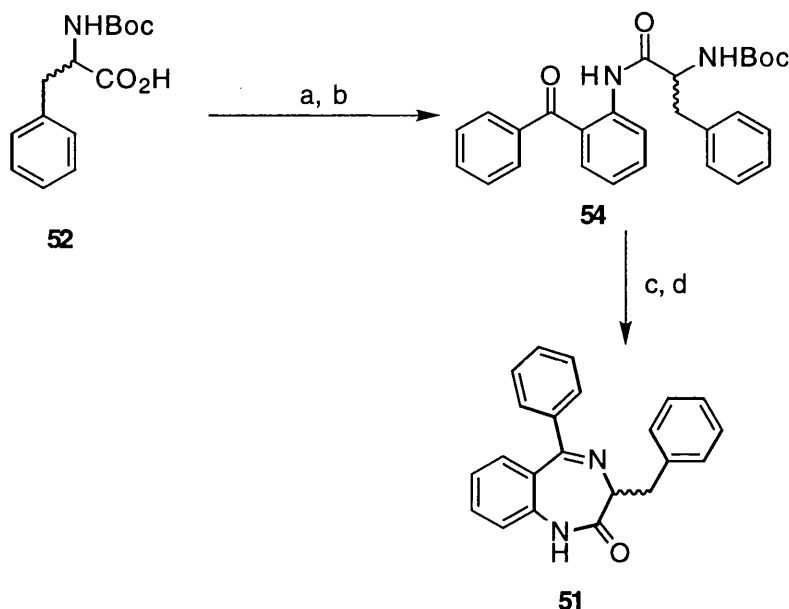
Reagents: (a) Et₃N, EtOCOCl (b) 2-aminobenzophenone (**53**)
(c) HCl (g) (d) NaOH

Scheme 5

A number of side chains were then incorporated into the phenylalanine-derived BZD scaffold **51**. In order to mimic the Arg[9] basic side chain in HOE140, a four carbon chain equivalent was introduced between the BZD lactam nitrogen and the basic terminal group. Compound **55** was synthesised from **51**, 1-iodo-4-nitrobenzene, NaOAc and Cu powder *via* a modified Ullman reaction[107]. It was then planned to reduce the nitro group in **55** to an amine, but problems occurred. Treatment with 10% palladium on charcoal resulted in reduction of the imine to form **56** as a mixture of diastereoisomers (see Scheme 6). When the nitro group was reduced with SnCl₂[108], however, no reduction of the imine functional group occurred and the desired compound (**57**) was obtained.

51 →(a)→ 55 →(b)→ 56

55 →(c)→ 57
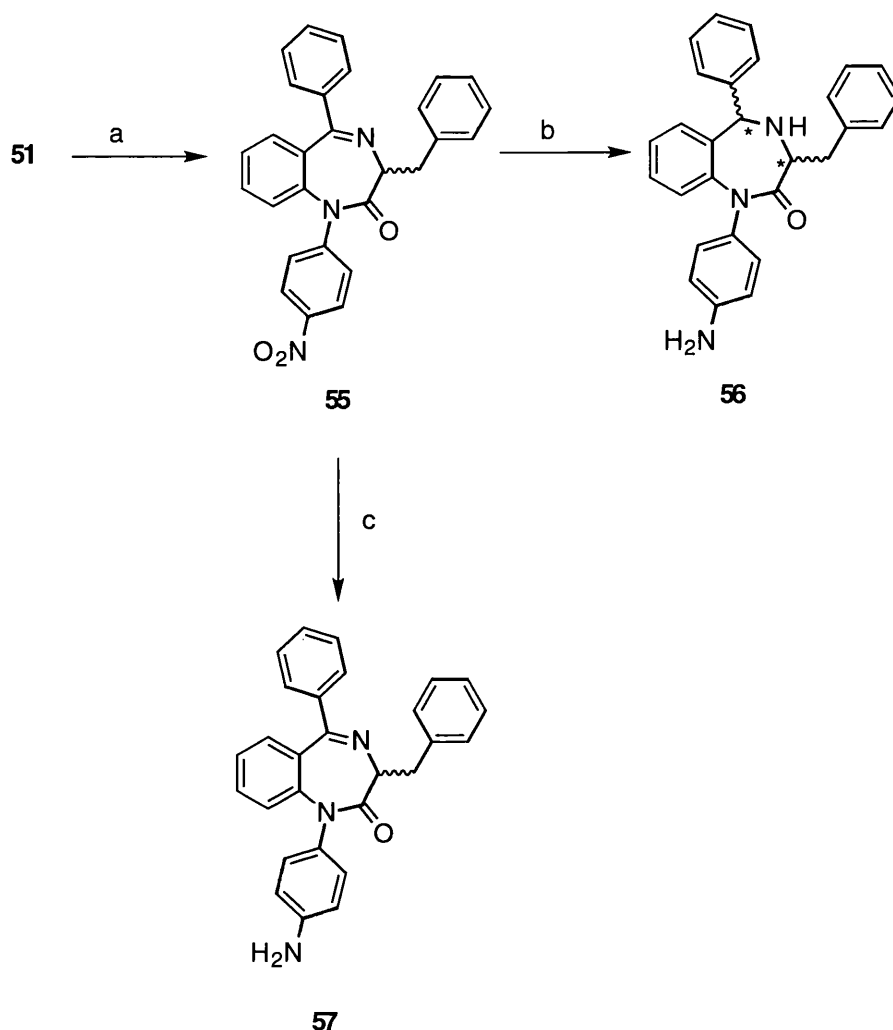
Reagents: (a) NaOAc, Cu, 1-iodo-4-nitrobenzene, DMF, reflux
(b) H$_2$ / 10% Pd-C (c) SnCl$_2$, EtOAc

Scheme 6

Incorporation of additional basicity into the molecule required the guanylation of the amino group of the aniline **57**. Although there are many standard methods for producing aliphatic guanidines, forming aromatic guanidines is more difficult since the deactivating effect of the phenyl ring reduces the nucleophilicity of the amine nitrogen. Compound **57** was treated with aminoiminomethansulfonic acid[109] (AIMSA; **58**, Scheme 7) but the only component isolated from the reaction mixture was starting material.

57 + H$_2$N–C(=NH)–SO$_3$H —MeOH, 3 h→ 57

58
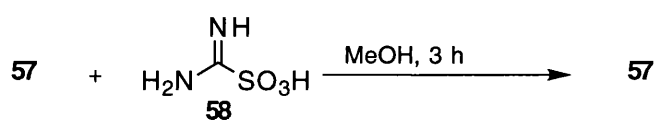
Scheme 7

A recent method for guanylation[110] uses N,N'-bis-*tert*-butoxycarbonylthiourea (59) and mercury chloride (see Scheme 8). Compound 57 was guanylated using this procedure and the resulting bis-Boc guanidine 60 was treated with trifluoroacetic acid (TFA) to give 61*. Compound 61 was purified by preparative HPLC to give the TFA salt and this was tested for biological activity.
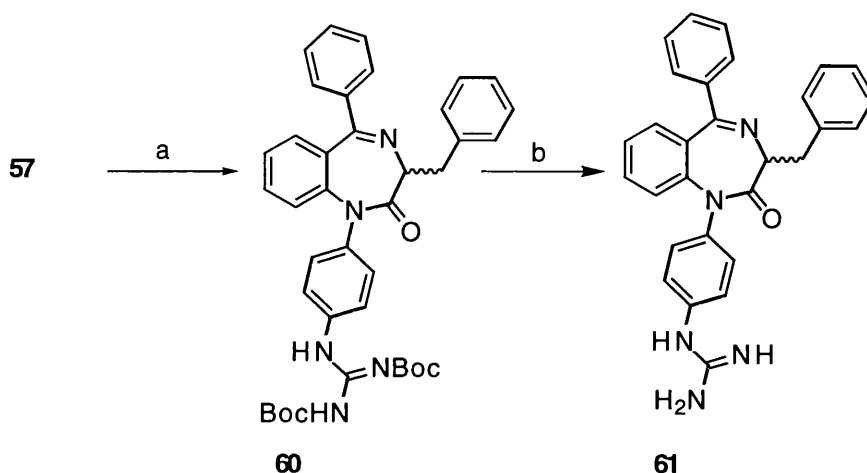
Reagents: (a) **59**, HgCl$_2$, DMF (b) TFA , DCM

Scheme 8

In order to introduce more conformational freedom between the lactam nitrogen and the basic terminal group, it was decided to prepare **64** (Scheme 9). BZD **51** was treated with KO^{t}Bu and 3-nitrobenzyl bromide. The resulting nitrophenyl derivative **62** was then reduced with SnCl$_2$ and guanylated to produce compound **63**. Compound **63** was then treated with TFA to form compound **64** as the TFA salt.

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* **NOTE: compounds are shown as the neutral BZD rather than as the corresponding charged TFA salt in which form all of them were isolated.**
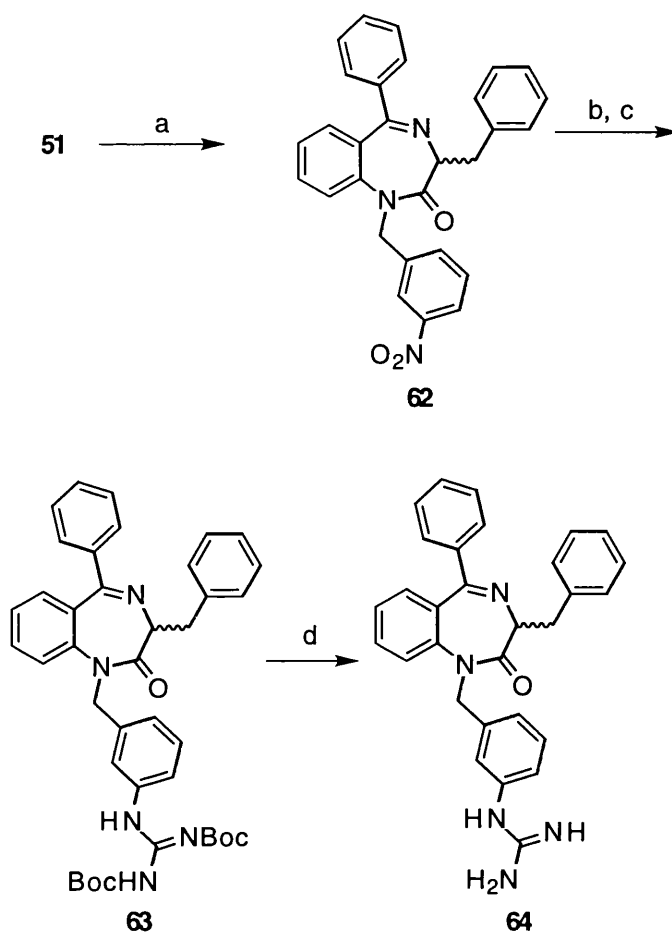
Reagents: (a) KO^{t}Bu/THF, 3-nitrobenzyl bromide, (b) SnCl$_2$.2H$_2$O, EtOAc, 70 °C, (c) **59**, HgCl$_2$, DMF (d) TFA , DCM

Scheme 9

Compound **64** was initially synthesised as the racemate, and encouraging biological results were obtained which suggested that the individual enantiomers should also be examined. The separate enantiomers were then synthesised from (S)- and (R)-phenylalanine (**70** and **67**) using the same series of reactions (Scheme 10).
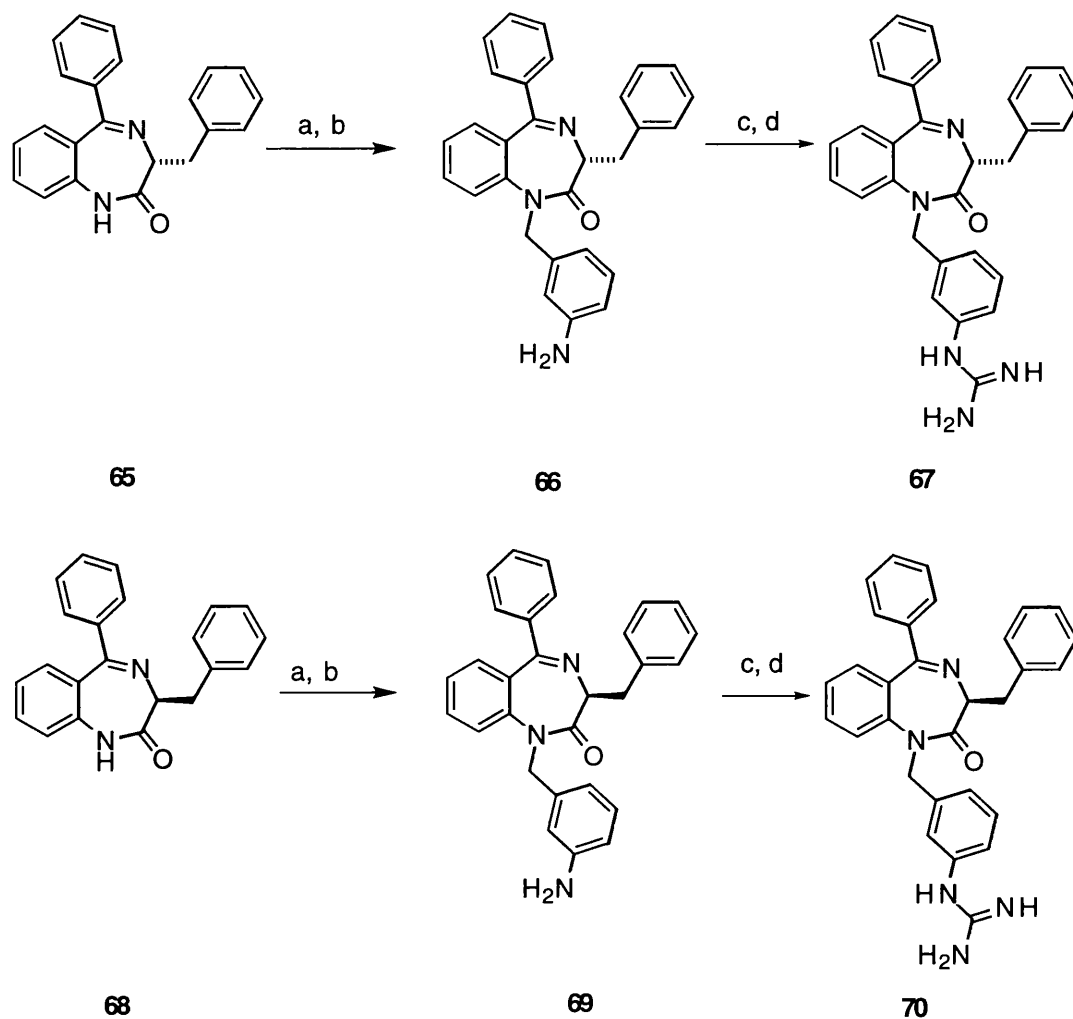
Reagents: (a) KO^{t}Bu/THF, 3-nitrobenzyl bromide, (b) SnCl$_2$.2H$_2$O, EtOAc, 70 °C, (c) **59**, HgCl$_2$, DMF (d) TFA , DCM

Scheme 10

The optical purity of the enantiomers was then investigated. As isolated, the R isomer (**67**), had a rotation of +8.9 ° (c = 1.007) and the S isomer (**70**), had a rotation of -8.7 ° (c = 1.01) in methanol at room temperature. Equal and opposite optical rotations values were a necessary though insufficient marker of optical purity. Chiral HPLC was therefore used in an attempt to establish the optical purity. Separation was attempted using normal phase conditions on a number of different column types, including a β-cyclodextrin, a chiracell OD and a Hichrom chi-L-leucine column. No separation was observed under these conditions. Under reverse phase conditions, involving a mixture of acetonitrile and water, two peaks were observed for the racemate **64** using the chi-L-leucine column. Unfortunately, the column degraded under these harsh conditions, and the results could not be repeated on the enantiomers. It was

then decided to investigate the optical purity of the BZD core. Chiral HPLC analysis of BZD **51** under normal phase conditions led to excellent separation of the enantiomers. The R and S enantiomers **65** and **68** were then separately analysed. The S isomer had a retention time of 8.5 minutes while the R isomer had a retention time of 13 minutes. In each of the samples there was a negligible amount of the other isomer. Mulzer *et al.*[111] have suggested that deprotonation at the C-3 position is difficult in this kind of ring system because of the planar structure the resulting 'cycloheptatriene' ring must adopt upon enolisation. If this is correct then it appears unlikely that significant racemisation would occur as the planar system would be antiaromatic ($4n$ electron system; n = 2). The CD spectra of the two enantiomers **67** and **70** were obtained and these showed equal and opposite rotations over a range of wavelengths (Figure 28).

Figure 28. CD spectra of the enantiomers **67** and **70**
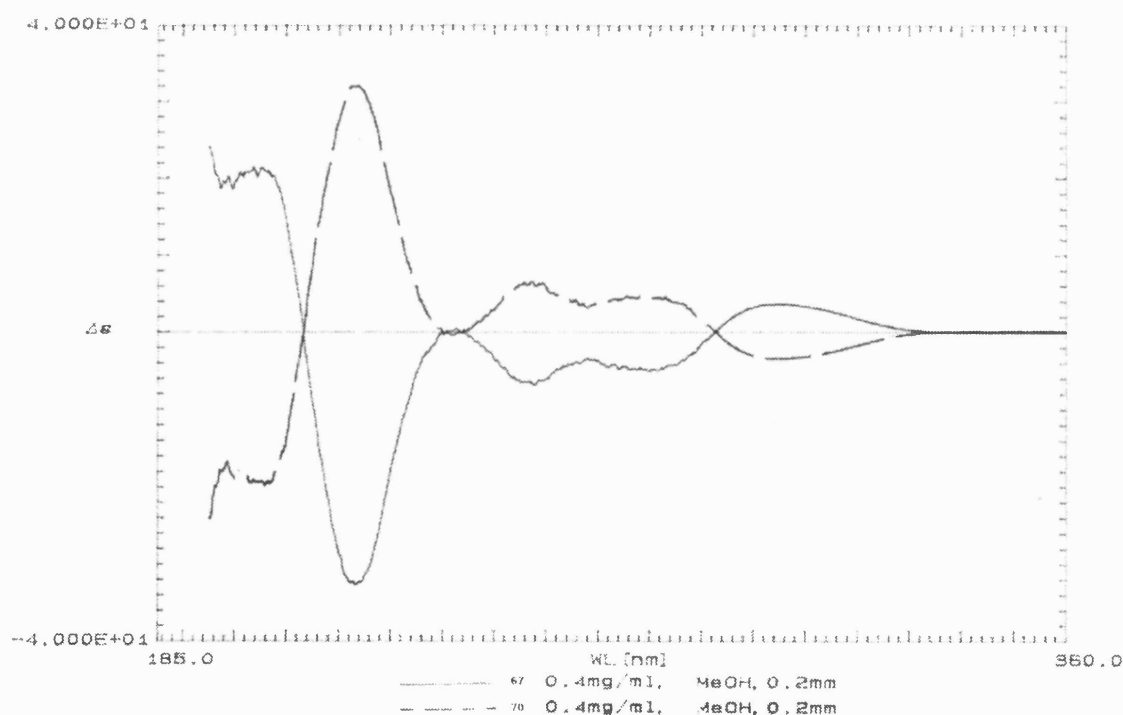
In order to complete the set of side-chain regioisomers, the amide anion of compound **51** was treated with both 4-nitrobenzyl bromide and 2-nitrobenzyl bromide to form compounds **71** and **72** (Scheme 11). These were converted to the target molecules **73** and **74** using the standard procedures described previously.

51 → (a) →

71 : R$_1$ = H, R$_2$ = NO$_2$
72 : R$_1$ = NO$_2$, R$_2$ = H

(b, c, d) → **73**

72 → (b, c, d) → **74**
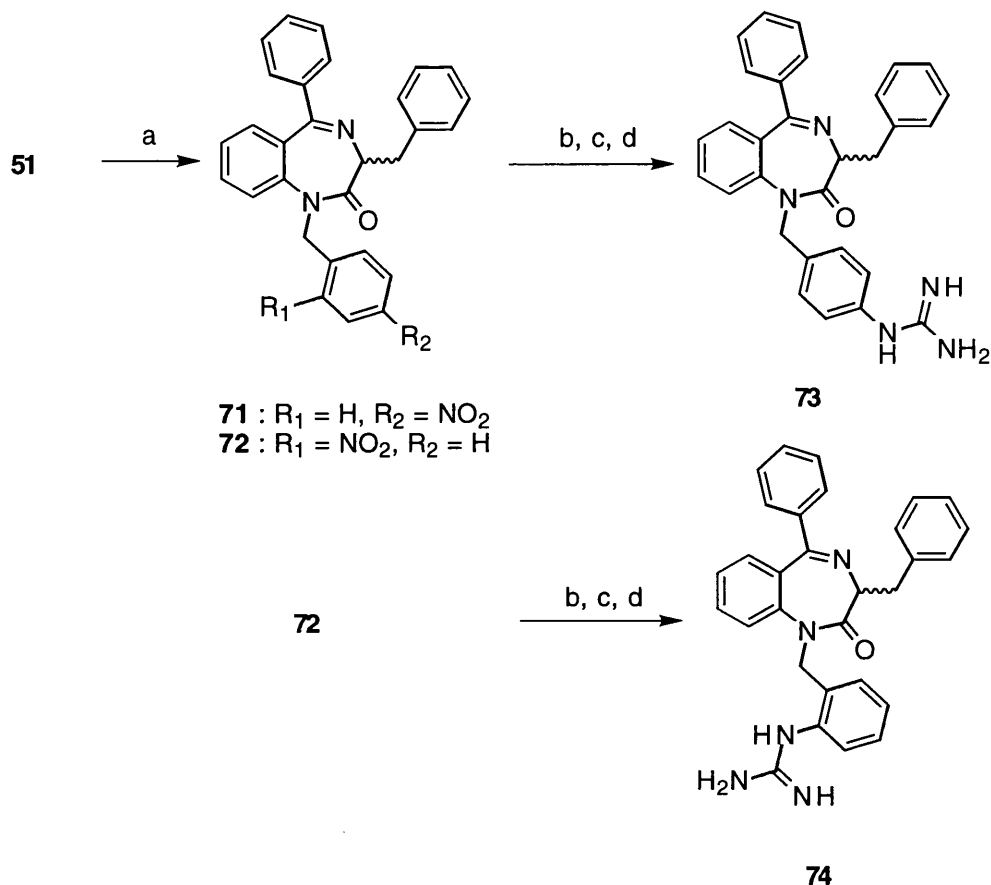
Reagents: (a) KO^{t}Bu/THF, 2- or 4-nitrobenzyl bromide,
(b) SnCl$_2$.2H$_2$O, EtOAc, 70 °C,(c)**59**, HgCl$_2$, DMF (d) TFA , DCM

Scheme 11

More conformational flexibility in the side chain could be provided by an aliphatic chain. The synthesis of the aliphatic guanidines, however, took some time to optimise. The synthetic scheme outlined in Scheme 12 involves alkylation with ethyl 5-bromovalerate, saponification and synthesis of the acyl azide with diphenyl phosphonic azide[112] (DPPA). Under these reaction conditions the acyl azide rearranges to the isocyanate which is trapped by a nucleophile. The reaction was successful with a number of nucleophiles, for example benzyl alcohol, 9-fluorenemethanol and t-butanol.
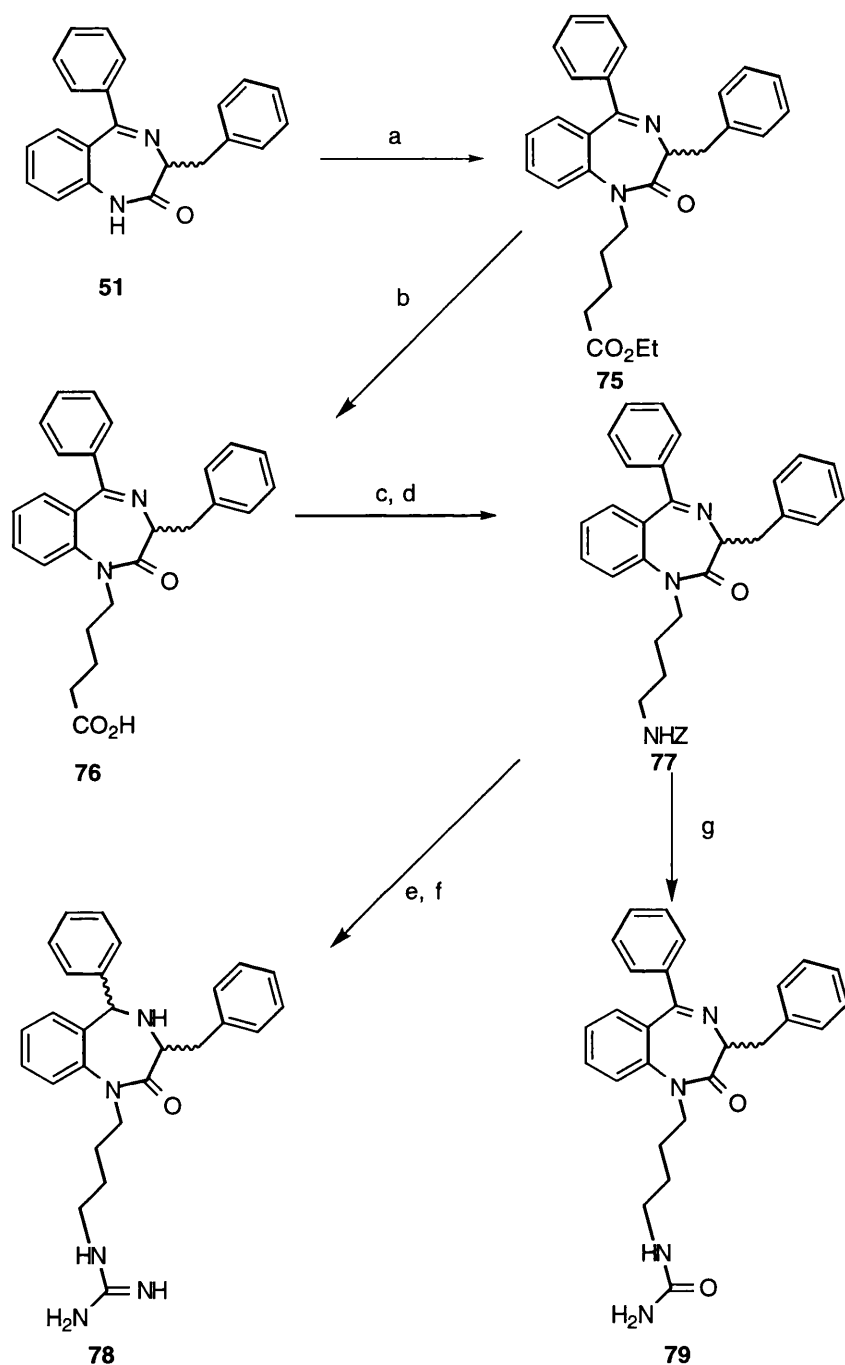
Scheme 12

Reagents: (a) (i) KOtBu / THF, -15 $^\circ$ C, (ii) 5-bromoethyl valerate (b) KOH, EtOH,
(c) DPPA, Et$_3$N, C$_6$H$_6$, 80 $^\circ$ C 4h (d) PhCH$_2$OH, 80 $^\circ$ C, 24 h (e) H$_2$, Pd/C (f) guanylation
(g) HCO$_2$$^-NH_4$$^+$, 10% Pd/C

The Z-group deprotection proved a problem. When the standard deprotection method, hydrogenation, was used, the imine functional group was also reduced. The mixture was analysed by HPLC and was found to be too complex to separate. Hydrogen transfer conditions were tried but these led to the urea **79** and not to the desired amine.

Acidic hydrolysis of the Z-group with 33% HBr/AcOH was then attempted. When the reaction was performed on a small scale the reaction proceeded successfully but when the reaction was scaled up a number of uncharacterised by-products were also obtained. The reaction mixture could not be separated by preparative HPLC.

An alternative synthetic route to **83** was then explored. Alkylation of **51** with N-(4-bromobutyl) phthalimide formed compound **81**. Treating this compound with hydrazine hydrate afforded the amine **83** which was guanylated in two steps to afford the target guanidine **85** (see Scheme 13).

$$n = 2 = 80$$
$$n = 3 = 81$$

| n = 2 = 82 | n = 2 = 84 |
| n = 3 = 83 | n = 3 = 85 |
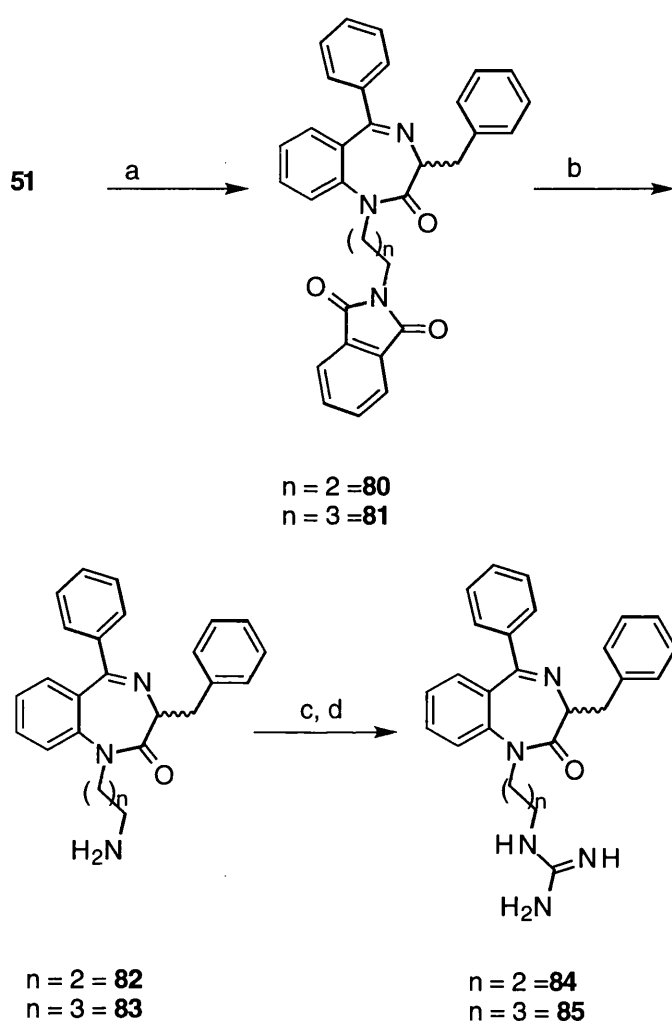
Reagents: (a) KO^{t}Bu/THF,N-(4-bromobutyl or 3-propyl) phthalimide, (b) N$_2$H$_4$, EtOH (c)**59**, HgCl$_2$, DMF (d) TFA , DCM

Scheme 13

The length of the linker chain between the basic terminal group and the lactam nitrogen of the BZD was then changed. Compound **51** was alkylated with N-(3-bromopropyl) phthalimide to give **80** which was then treated with hydrazine

hydrate to yield the amine **82** (Scheme 13). The amine **82** was then guanylated in two steps to afford the guanidine **84**.

The basic terminal group was then changed in compounds **88** and **89**. It has been demonstrated that benzamidines act as excellent arginine mimics in a number of peptide mimetics[54]. Compound **51** was treated with KO^{t}Bu and 3-bromomethyl benzonitrile to afford **86**. The nitrile **86** was then treated with HCl gas followed by ammonia to give the benzamidine **88**. The isomeric benzamidine **89** was synthesised by the same series of reactions from 4-bromomethyl benzonitrile (see Scheme 14).

86 : R$_1$ = CN, R$_2$ = H
87 : R$_1$ = H, R$_2$ = CN
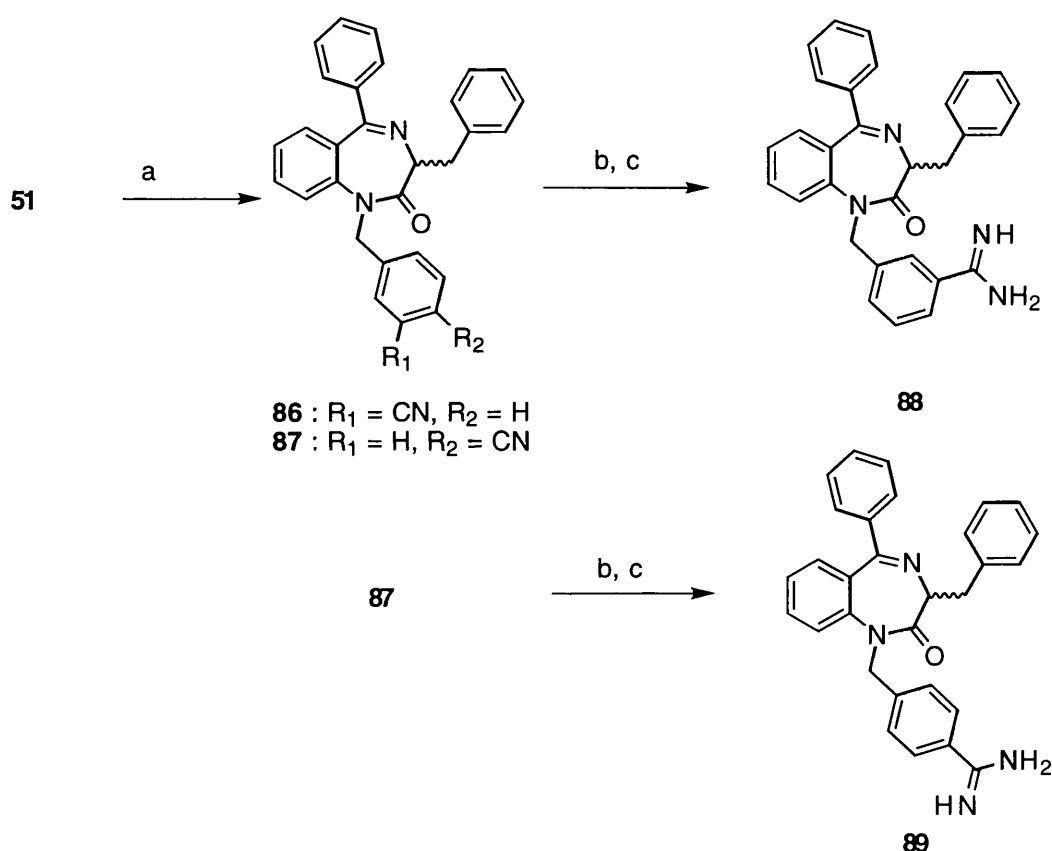
Reagents: (a) KO^{t}Bu/THF, 3 or 4-bromomethyl benzonitrile,
(b) HCl (g), EtOH, (c) NH$_3$, MeOH

Scheme 14

2.2.2. Variation in 1,4-Benzodiazepin-2-one Core Structure - Overlay Model I

Attention was next directed at the BZD core structure. The principal structural modifications were the alteration of the amino acid side chain and substitution of the aromatic rings with different functional groups. Compound **90** was synthesised from (RS) N-Boc alanine and 2-aminobenzophenone using the

standard literature procedure (see Scheme 15). Compound **90** was then treated with KO^{t}Bu and 3-nitrobenzyl bromide to afford the BZD **91** which was then reduced, guanylated, and treated with TFA to yield the target compound **92** (Scheme 15).
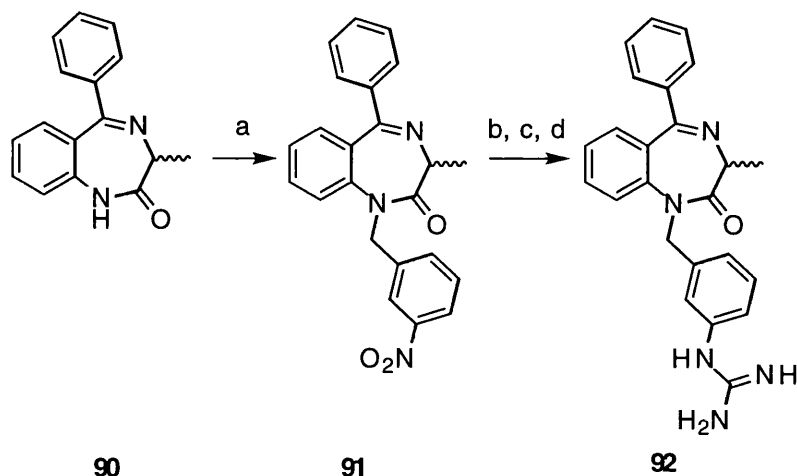
90   **91**   **92**

Reagents: (a) KO^{t}Bu/THF, 3-nitrobenzyl bromide, (b) SnCl$_2$.2H$_2$O, EtOAc, 70 °C, (c)59, HgCl$_2$, DMF (d) TFA , DCM

Scheme 15

In order to assess the importance of the embedded diphenylmethyl motif, it was decided to interchange the methyl group at C-3 with a phenyl group at C-5. Compound **93** was synthesised from 2-aminoacetophenone and N-Boc phenylalanine. BZD **93** was then alkylated with 3-nitrobenzyl bromide to form compound **94** which was reduced, guanylated and treated with TFA to form the desired guanidine **95** (Scheme 16).
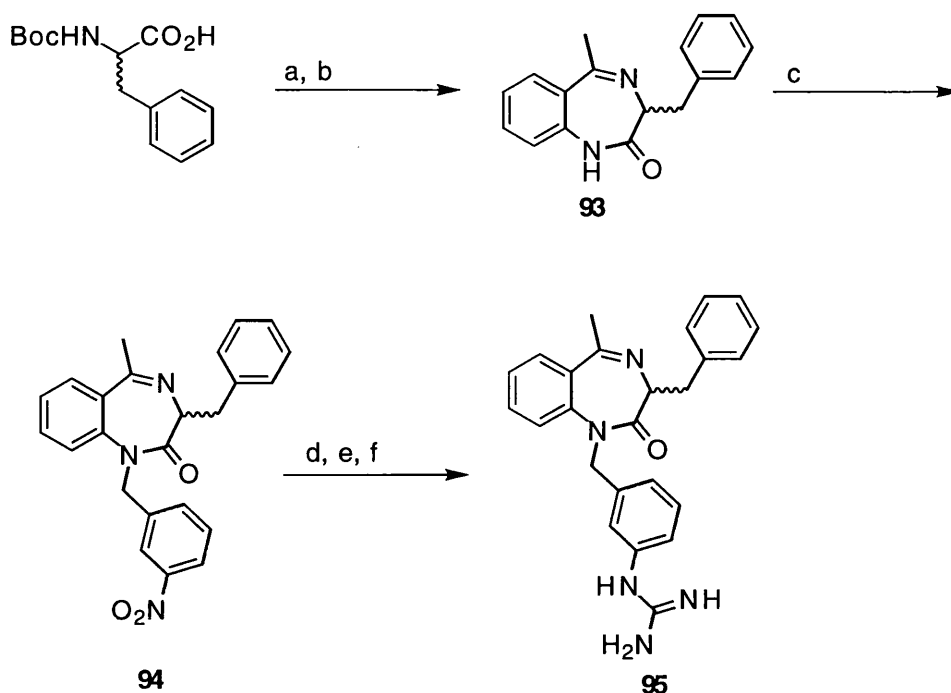
Reagents: (a) (i)EtOCOCl, Et$_3$N, (ii) 2-aminoacetophenone, (b) (i) HCl (g), (ii) NaOH
(c) KO^{t}Bu/THF, 3-nitrobenzyl bromide, (d) SnCl$_2$.2H$_2$O, EtOAc, 70 °C,
(e)59, HgCl$_2$, DMF (f) TFA , DCM

Scheme 16

In order to prepare other substituted BZDs, 2-aminobenzophenones which were not commercially available were synthesised by literature procedures[82,113,114]. These methods use 2-phenylbenzoxazinone (99), which can be prepared under mild conditions from anthranilic acid 96 and two equivalents of benzoyl chloride 97 in pyridine (see Scheme 17).
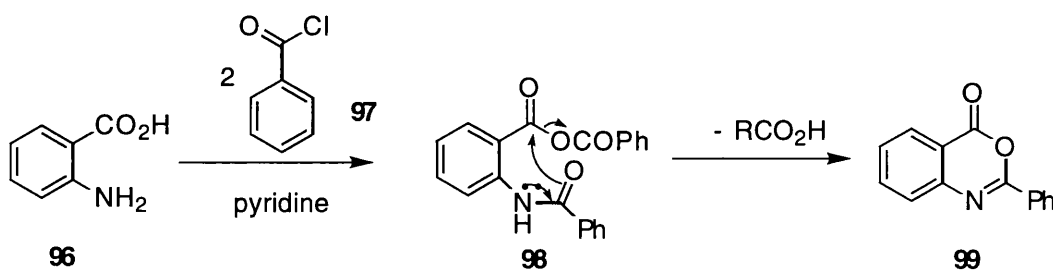
Scheme 17

Compound 102 was prepared in two steps from 2-bromonaphthalene and 2-phenyl-4H-3,1-benzoxazin-4-one (99, Scheme 18)[115]. When 99 was treated with 2-naphthylmagnesium bromide (100) at -20 °C, a significant amount of both the bis-arylation product (101) and the starting material was observed together with the desired product 102. The conditions were modified from the literature procedure by performing the reaction at a lower temperature (-40 °C), but again significant amounts of starting material and bis-arylation product were

observed. The reaction was then repeated on a large scale and the crude product was saponified and the desired compound **102** was isolated, albeit in low yield (11%).

Scheme 18
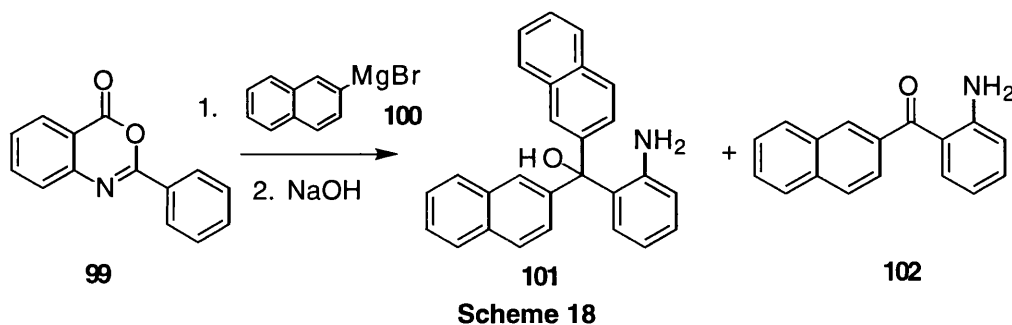
The same procedure was used to synthesise a number of other 2-aminobenzophenones. 2-Phenyl-4H-3,1-benzoxazin-4-one (**99**) was treated with the Grignard reagents formed from 4-bromoanisole, 4-bromotoluene, 4-bromochlorobenzene, 2-bromochlorobenzene to give the respective benzamides. The benzamides were then saponified to form the 2-aminobenzophenones shown in Scheme 19.

103, R_1 = Me, R_2 = H
104, R_1 = OMe, R_2 = H
105, R_1 = Cl, R_2 = H
106, R_1 = H, R_2 = Cl
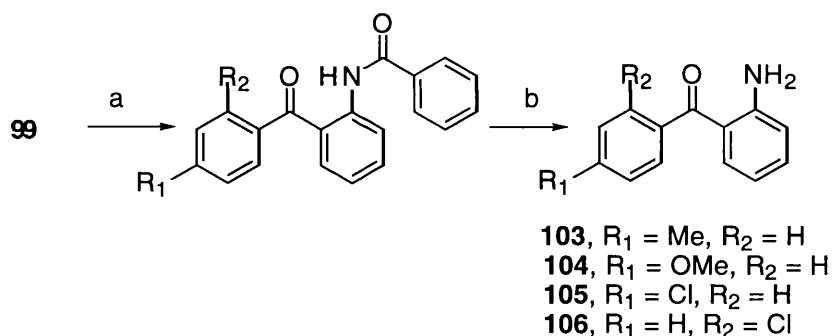
Reagents: (a) bromobenzene derivative, Mg, -40 °C
(b) 10 M NaOH, MeOH, reflux

Scheme 19

The next series of compounds prepared involved changing the phenylalanine moiety in the core compound **51**. A variety of amino acids were used as indicated by the formation of compounds **90, 112, 113, 114** and **115** in Scheme 20. These compounds were transformed in moderate yield to the target compounds **121, 122, 123, 124** and **125**.
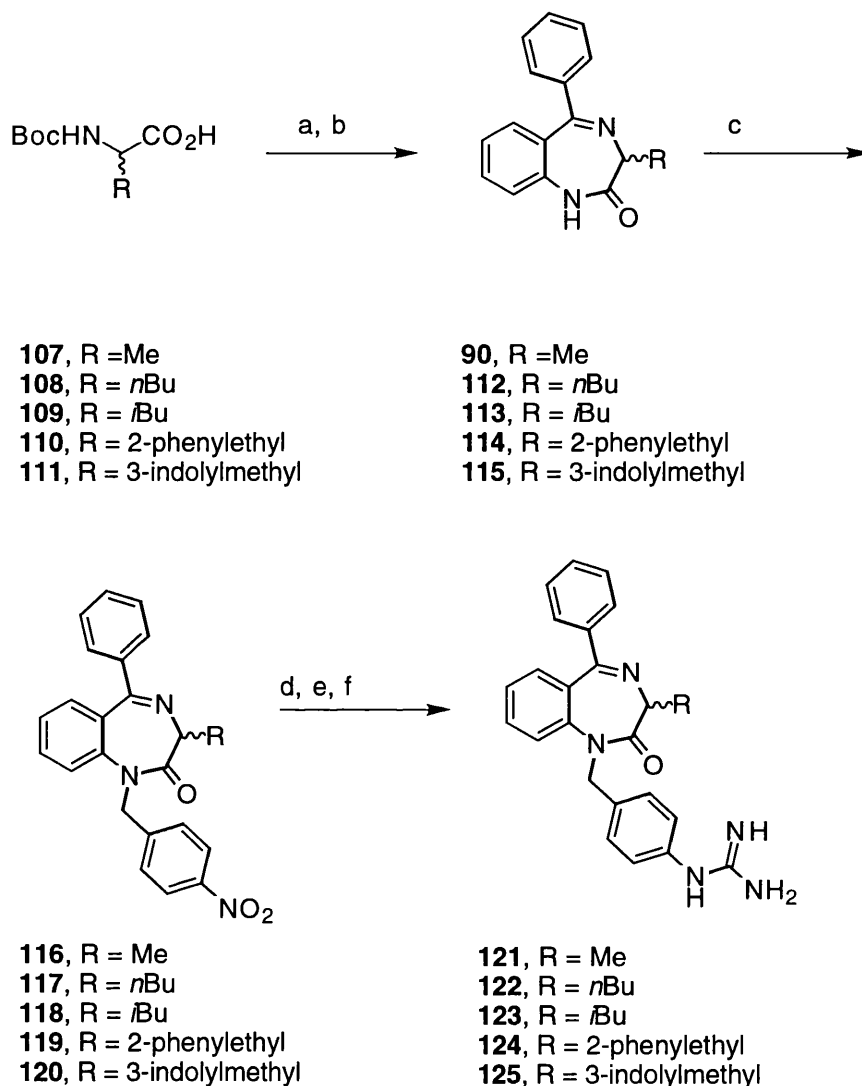
107, R =Me	**90**, R =Me
108, R = *n*Bu	**112**, R = *n*Bu
109, R = *i*Bu	**113**, R = *i*Bu
110, R = 2-phenylethyl	**114**, R = 2-phenylethyl
111, R = 3-indolylmethyl	**115**, R = 3-indolylmethyl

116, R = Me	**121**, R = Me
117, R = *n*Bu	**122**, R = *n*Bu
118, R = *i*Bu	**123**, R = *i*Bu
119, R = 2-phenylethyl	**124**, R = 2-phenylethyl
120, R = 3-indolylmethyl	**125**, R = 3-indolylmethyl

Reagents: (a) (i)EtOCOCl, Et$_3$N, (ii) 2-aminobenzophenone, (b) (i) HCl (g), (ii) NaOH (c) KO^{t}Bu/THF, 4-nitrobenzyl bromide, (d) SnCl$_2$.2H$_2$O, EtOAc, 70 °C, (e) **59**, HgCl$_2$, DMF (f) TFA , DCM

Scheme 20

The 2-aminobenzophenones that had been prepared were used for the next series of compounds. Compound **126** was synthesised from **102** and N-Boc phenylalanine using the standard literature procedure[89]. Compound **126** was then alkylated with 4-nitrobenzyl bromide to afford the BZD **127** which was then reduced, guanylated and treated with TFA to form compound **128** (Scheme 21).
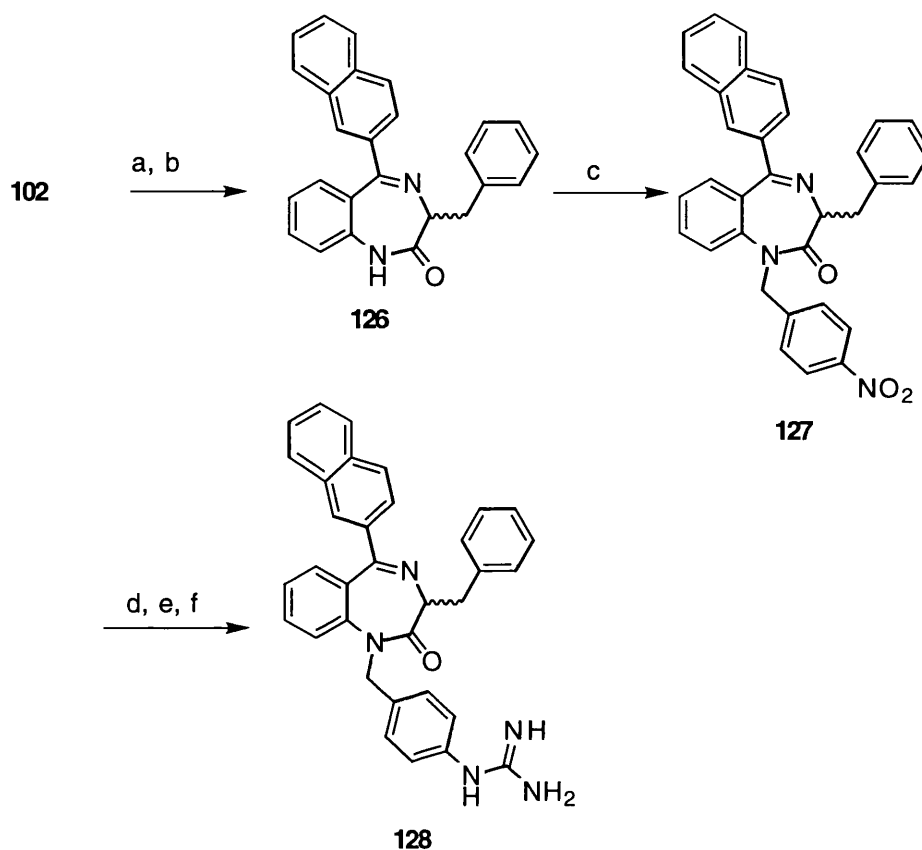
Reagents: (a) EtOCOCl, Et₃N, N-Boc Phe (b) (i) HCl (g), (ii) NaOH
(c) KO^tBu/THF, 4-nitrobenzyl bromide, (d) SnCl₂.2H₂O, EtOAc, 70 °C,
(e) **59**, HgCl₂, DMF (f) TFA , DCM

Scheme 21

Compound **103** was synthesised in two steps from 4-bromotoluene and **99**. Compound **103** was treated with the appropriate N-Boc amino acid to form the BZDs **129**, **130** and **131**. These were transformed to the target molecules **135**, **136** and **137** in moderate yield by the reactions shown in Scheme 22.

99

103

129, R = Me
130, R = nBu
131, R = Bn

132, R = Me
133, R = nBu
134, R = Bn

135, R = Me
136, R = nBu
137, R = Bn

Reagents: (a) (i) 4-bromotoluene, Mg, -78 °C, Et_2O, (ii) 10 M NaOH, MeOH, 80 °C
(b) (i) N-Boc Ala, Phe or nLeu, EtOCOCl, Et_3N, (ii) HCl (g), (iii) NaOH (c) KO^tBu/THF,
4-nitrobenzyl bromide, (d) $SnCl_2.2H_2O$, EtOAc, 70 °C, (e) **59**, $HgCl_2$, DMF
(f) TFA , DCM

Scheme 22

Compound **104** was synthesised in two steps from 4-bromoanisole and **99**. Compound **104** was reacted with either N-Boc phenylalanine or N-Boc norleucine to form the BZDs **138** and **139**. Compounds **139** and **138** were then converted into the target molecules **142** and **143** in moderate yield using the reactions shown in Scheme 23.

Reagents: (a) (i) 4-bromoanisole, Mg, -78 °C, Et₂O, (ii) 10 M NaOH, MeOH, 80 °C
(b) (i) N-Boc nLeu or Phe, EtOCOCl, Et₃N, (ii) HCl (g), (iii) NaOH (c) KO^{t}Bu/THF,
4-nitrobenzyl bromide, (d) SnCl₂.2H₂O, EtOAc, 70 °C, (e) **59**, HgCl₂, DMF
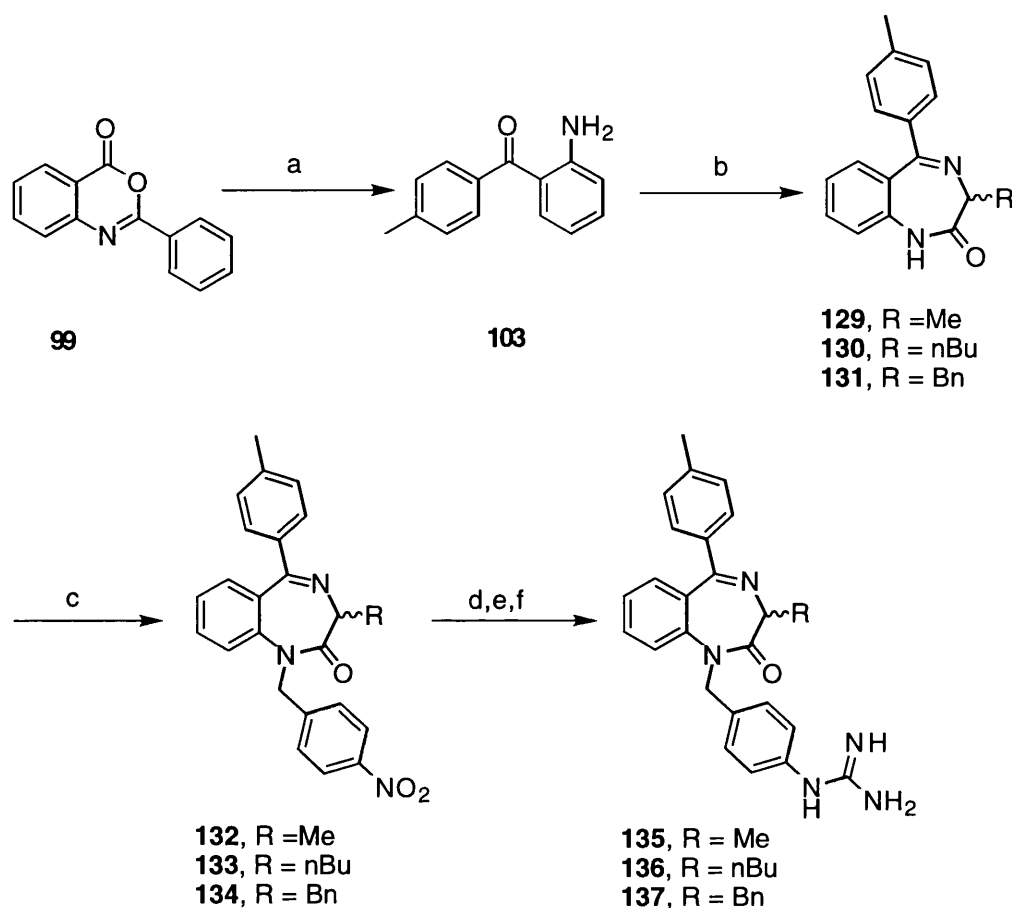(f) TFA , DCM

Scheme 23

Compound **105** was synthesised in two steps from 4-bromochlorobenzene and **99**. Compound **105** was treated with either N-Boc alanine or N-Boc phenylalanine to form compounds **144** and **145**. These compounds were transformed in moderate yield to the targets **148** and **149** (Scheme 24).
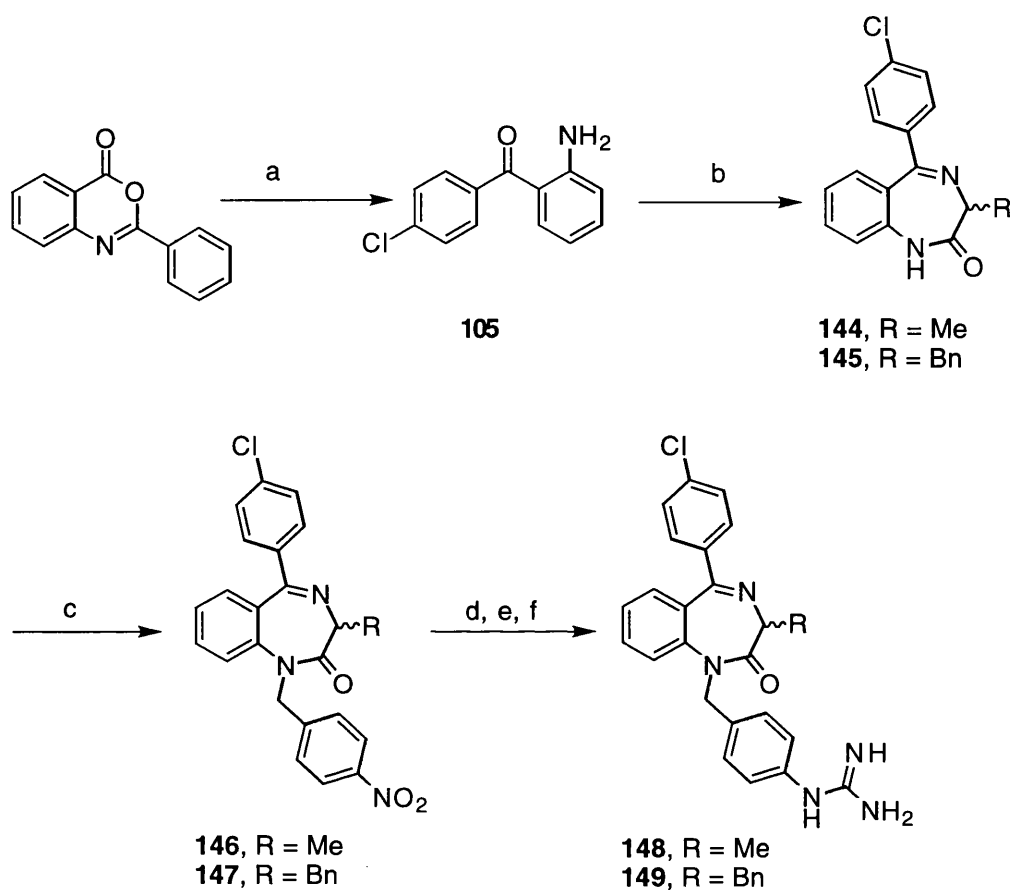
Reagents: (a) (i) 4-bromochlorobenzene, Mg, -78 °C, Et$_2$O, (ii) 10 M NaOH, MeOH, 80 °C (b) (i) N-Boc Ala or Phe, EtOCOCl, Et$_3$N, (ii) HCl (g), (iii) NaOH (c) KO^{t}Bu/THF, 4-nitrobenzyl bromide, (d) SnCl$_2$.2H$_2$O, EtOAc, 70 °C, (e) **59**, HgCl$_2$, DMF (f) TFA , DCM

Scheme 24

Compound **106** was synthesised in two steps from 2-bromochlorobenzene and **99** and was then treated with N-Boc alanine to form compound **150**. Compound **150** was then transformed to the target **152** as shown in Scheme 25.
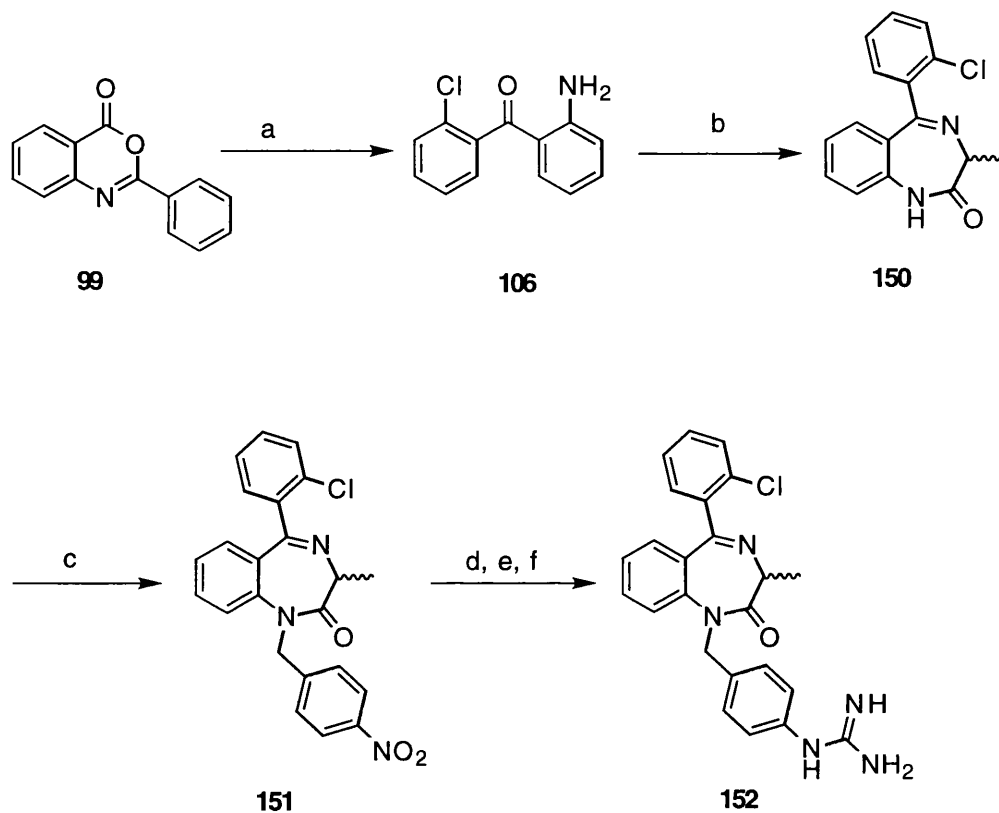
Reagents: (a) (i) 2-bromochlorobenzene, Mg, -78 °C, Et$_2$O, (ii) 10 M NaOH, MeOH, 80 °C (b) (i) N-Boc Ala, EtOCOCl, Et$_3$N, (ii) HCl (g), (iii) NaOH (c) KO^{t}Bu/THF, 4-nitrobenzyl bromide, (d) SnCl$_2$.2H$_2$O, EtOAc, 70 °C, (e) **59**, HgCl$_2$, DMF (f) TFA , DCM
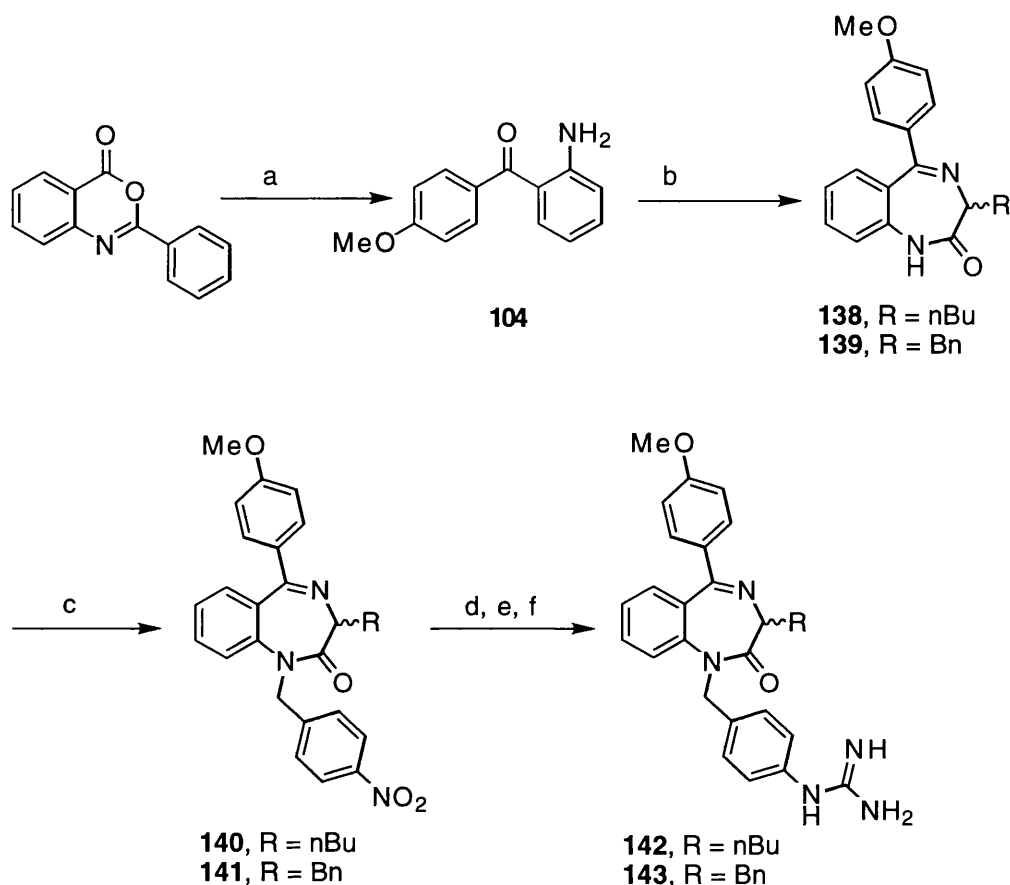
Scheme 25

Compounds **154**, **155**, **156** were synthesised from the appropriate N-Boc amino acid and commercially available 2-amino-5-chlorobenzophenone, using the standard procedures. These compounds were then transformed in moderate yield to the target compounds **162**, **163** and **164**. (Scheme 26).

154, R_1 = H, R_2 = Bn
155, R_1 = H, R_2 =Me
156, R_1 = H, R_2 = nBu
157, R_1 = Cl, R_2 =Me

153

158, R_1 = H, R_2 = Bn
159, R_1 = H, R_2 = Me
160, R_1 = H, R_2 = nBu
161, R_1 = Cl, R_2 =Me

162, R_1 = H, R_2 =Bn
163, R_1 = H, R_2 =Me
164, R_1 = H, R_2 = nBu
165, R_1 = Cl, R_2 =Me
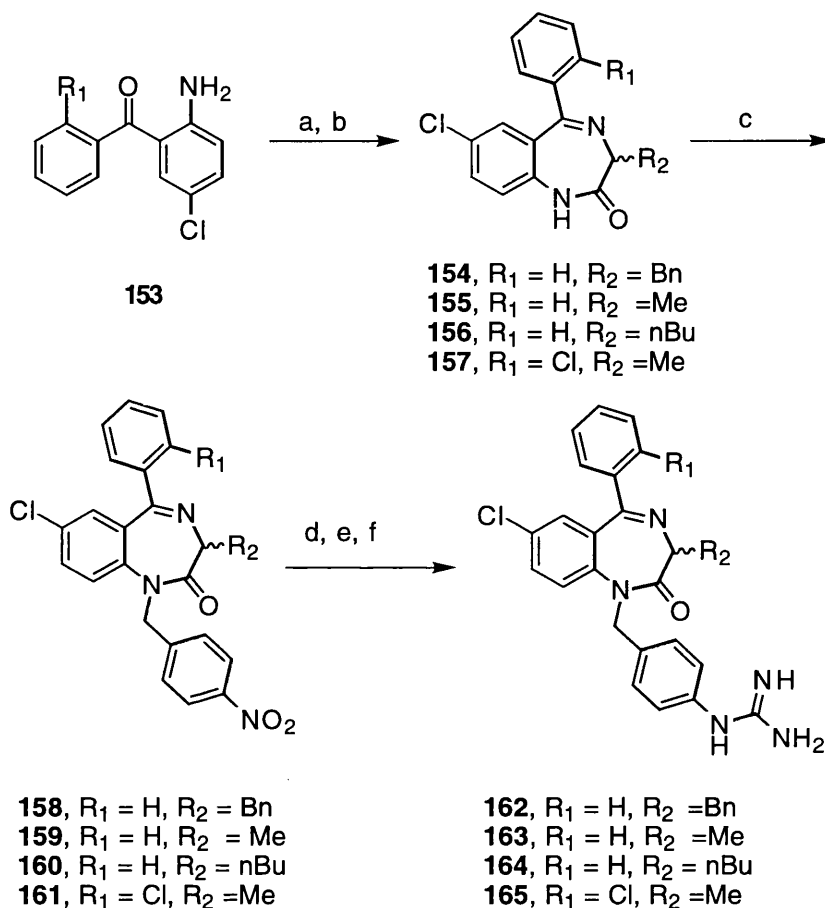
Reagents: (a) N-Boc Ala, Phe, nLeu, EtOCOCl, Et_3N, (b) HCl (g), (iii) NaOH
(c) KO^tBu/THF, 4-nitrobenzyl bromide, (d) $SnCl_2.2.H_2O$, EtOAc, 70 °C,
(e) **59**, $HgCl_2$, DMF (f) TFA , DCM

Scheme 26

Compound **161** was synthesised from (RS)-N-Boc alanine and the commercially available 2-amino-2',5-dichlorobenzophenone, using the standard procedures. Compound **161** was then transformed into the target **165** using the procedures described earlier (Scheme 26).

53, R₁ = R₂ = H
166, R₁ = R₂ = Cl
167, R₁ = Cl, R₂ = H

169, R₁ = R₂ = H
170, R₁ = R₂ = Cl
171, R₁ = Cl, R₂ = H
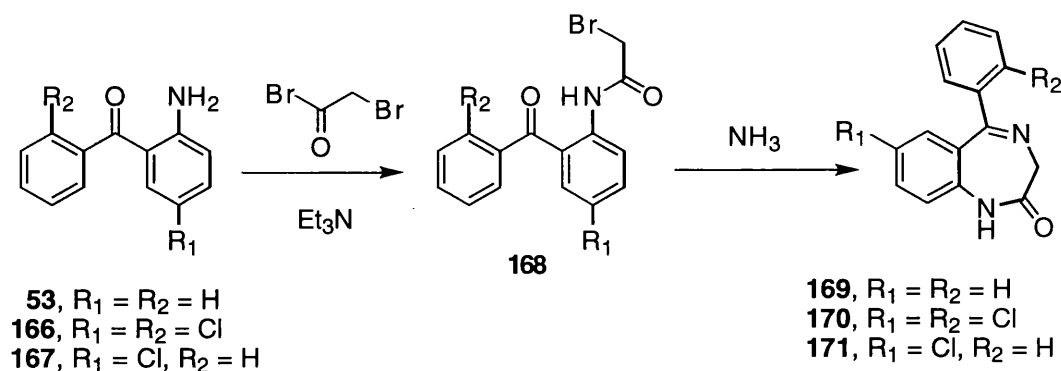
Scheme 27

BZDs formally derived from glycine were synthesised using an alternative procedure[116]. Compounds **169**, **170** and **171** were synthesised from the 2-aminobenzophenone derivatives shown in Scheme 27 and bromoacetyl bromide. The acetyl bromide intermediates (**168**) were treated with ammonia and heated at reflux for 10 h to yield the desired compounds in moderate yield.

169, R₁ = R₂ = H
170, R₁ = R₂ = Cl
171, R₁ = Cl, R₂ = H

172, R₁ = R₂ = H
173, R₁ = R₂ = Cl
174, R₁ = Cl, R₂ = H

175, R₁ = R₂ = H
176, R₁ = R₂ = Cl
177, R₁ = Cl, R₂ = H

Reagents: (a) KOᵗBu/THF, 4-nitrobenzyl bromide, (b) SnCl₂.2H₂O, EtOAc, 70 °C, (c) **59**, HgCl₂, DMF (d) TFA , DCM

Scheme 28

Compounds **169, 170** and **171** were then transformed into the target compounds **175, 176** and **177** using the standard conditions shown in Scheme 28.

Compound **179** was synthesised from (2S)-5-benzyloxycarbonylamino-2-*tert*-butoxycarbonylaminopentanoic acid (**178**) and 2-aminobenzophenone (**53**) using the standard literature procedure[89] (Scheme 29). Compound **179** was then treated with KOᵗBu and 4-nitrobenzyl bromide and the product then reduced and guanylated to yield **180**. Compound **180** was then treated with

TFA to yield the target **181**. Compound **181** represents an example of a compound with a large substituent at C-3 which will test the steric requirement of the receptor at this position. Removal of the Z- protecting group would reveal a second basic centre and when **179** was treated with 10% Pd/C, the desired aliphatic amine was obtained without the presence of diastereoisomers. However, this same transformation could not be achieved with **180**. The reaction, which was more sluggish, resulted in a mixture of uncharacterised products and starting materials.
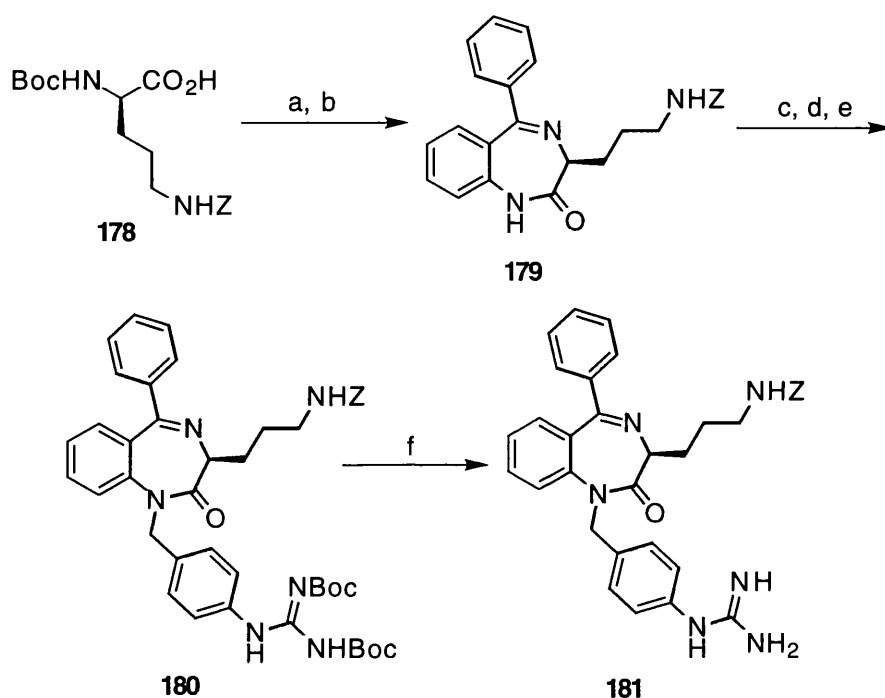
Reagents: (a) (i)EtOCOCl, Et₃N, (ii) 2-aminobenzophenone, (b) (i) HCl (g), (ii) NaOH
(c) KO^tBu/THF, 4-nitrobenzyl bromide, (d) SnCl₂.2H₂O, EtOAc, 70 °C,
(e) **59**, HgCl₂, DMF (f) TFA , DCM

Scheme 29
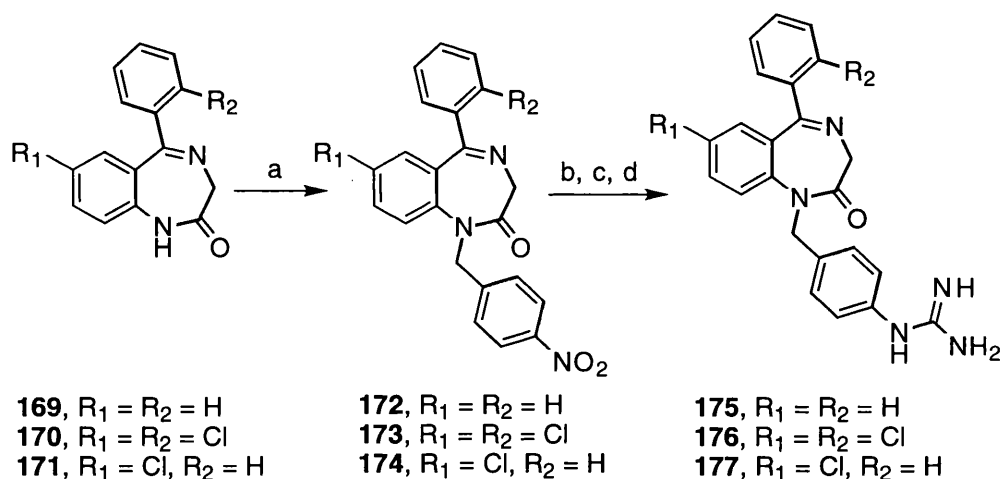
A derivative with a hydrophobic substituent at N-1 and a basic substituent at C-3 was required to compare its biological activity to **73**. Compound **183** was therefore synthesised from (RS)-N-Boc-4-nitrophenylalanine (**182**) and 2-aminobenzophenone (**53**) using the standard literature procedure[89]. Compound **183** was then treated with KO^tBu and benzyl bromide to yield compound **184** which was then transformed to the target compound **186** using the standard conditions described in Scheme 30.

Attention was then turned to the synthesis of the bis-guanidine **187**, since Salvino[117] had recently published the structure of a non-peptide BK B2 receptor antagonist which contained two basic groups separated by a spacer unit.

Compound **183** was treated with KO^{t}Bu and 4-nitrobenzyl bromide to form compound **185**. This was then transformed into the target compound **187** using the standard conditions shown in Scheme 30.

184, R$_1$ = H
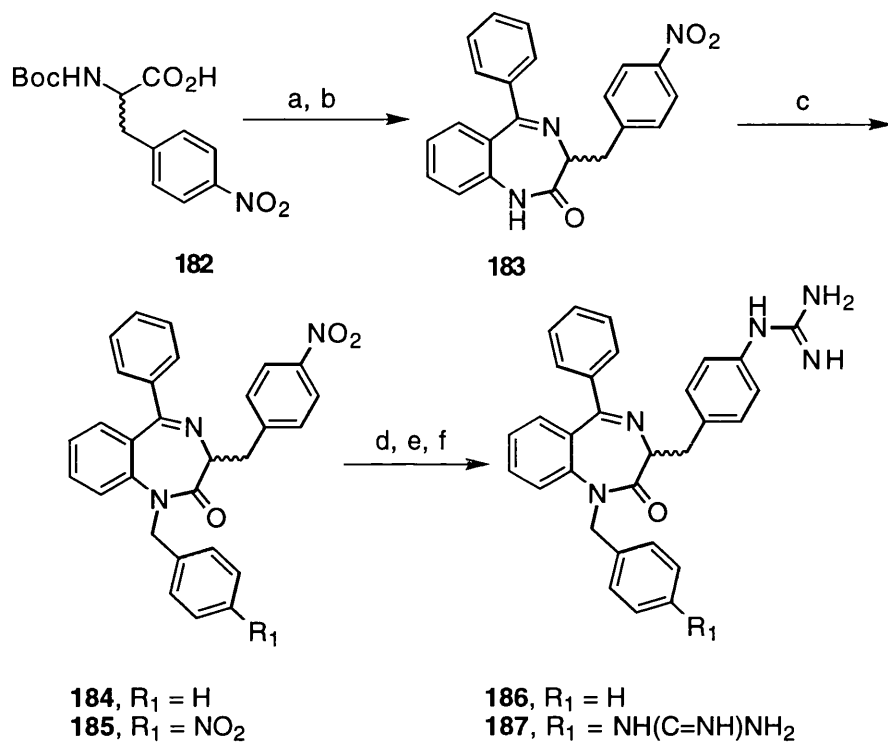**185**, R$_1$ = NO$_2$

186, R$_1$ = H
187, R$_1$ = NH(C=NH)NH$_2$

Reagents: (a) (i)EtOCOCl, Et$_3$N, (ii) 2-aminobenzophenone, (b) (i) HCl (g), (ii) NaOH
(c) KO^{t}Bu/THF, 4-nitrobenzyl bromide or benzyl bromide, (d) SnCl$_2$.2H$_2$O, EtOAc,
70 °C, (e) **59**, HgCl$_2$, DMF (f) TFA , DCM

Scheme 30

2.2.3. Variation in 1,4-Benzodiazepin-2-one Side Chain - Overlay Model II

There are two literature examples describing the application of BZDs as β-turn mimetics. The compounds described in Sections **2.2.1.** and **2.2.2.** belong to the overlay model of Ripka *et al.*[89] discussed in Section **2.1.2.1.** The compounds described in this Section are from the overlay model described by James *et al.*[93] in Section **2.1.2.2.**

Compound **189** was synthesised from **188**, N-Boc-1,4-diaminobutane, HOBT and DIPC[118] and was then treated with TFA to form compound **190**. The amine was then guanylated to form **191** which was treated with TFA to form compound **192** (Scheme 31).
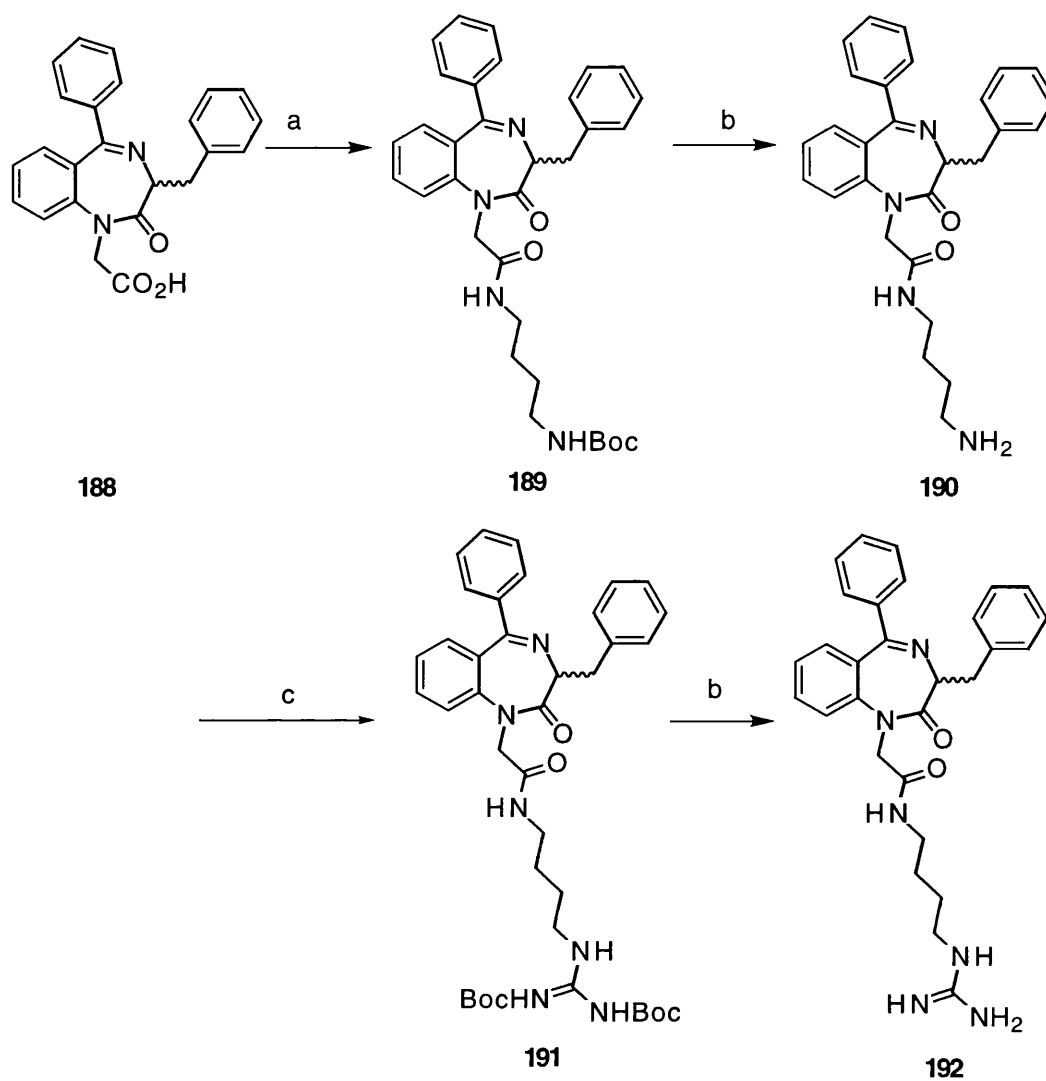
Reagents: (a) DIPC, HOBT, N-Boc-1,4-diaminobutane,
(b) TFA, DCM (c) **59**, HgCl₂, DMF

Scheme 31

Compound **193** was synthesised from **188**, *tert*-butyl-4-aminophenylcarbamate, HOBT and DIPC and was then treated with TFA to form the amine **194**. The amine was then guanylated to form **195** and this was treated with TFA to form compound **196**, a conformationally constrained analogue of **192** (Scheme 32).

188 →(a)→ **193** →(b)→ **194**

→(c)→ **195** →(b)→ **196**
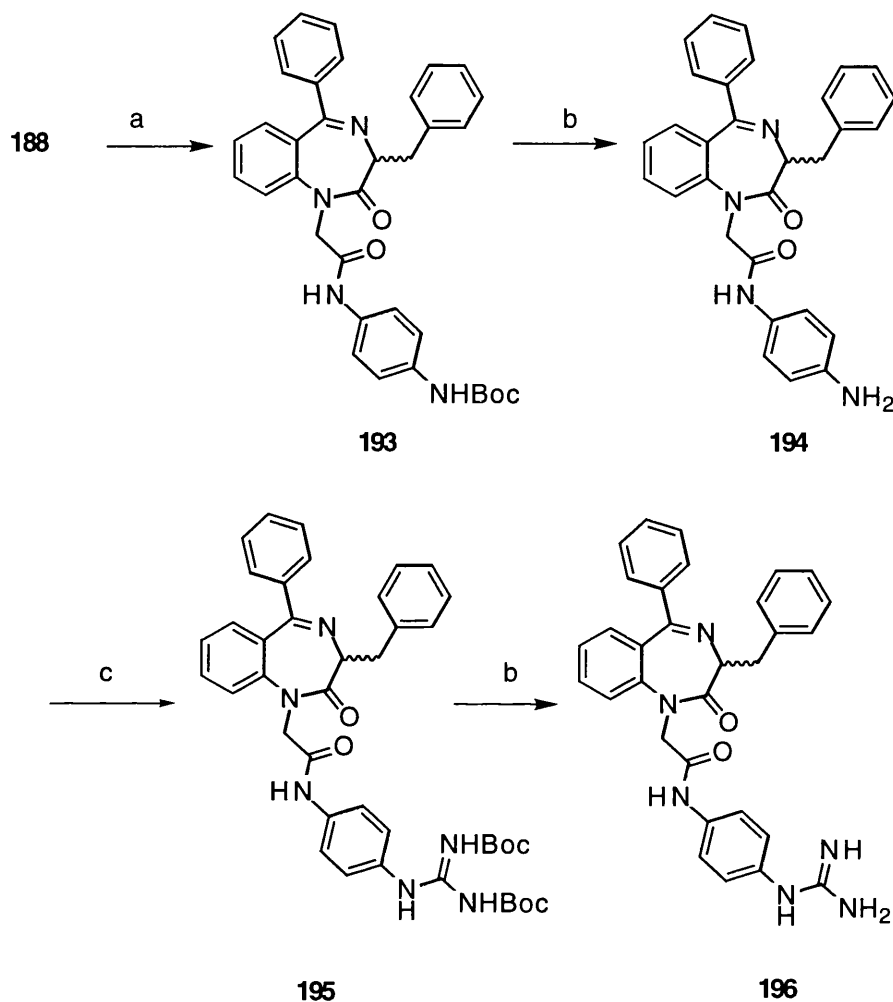
Reagents: (a) DIPC, HOBT,*tert*-butyl-4-aminophenylcarbamate,
(b) TFA, DCM (c) **59**, HgCl₂, DMF

Scheme 32

Compound **197** was synthesised in one step from **188**, 3,3'-iminobis (*N,N*-dimethyl-propylamine) (**198**), HOBT and DIPC (Scheme 33).
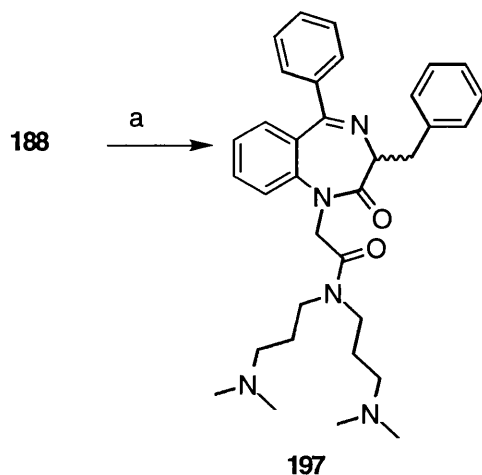
Reagents: (a) DIPC, HOBT,
3,3'-iminobis(N,N-dimethyl-propylamine) (**198**)

Scheme 33

2.2.4. Miscellaneous Compounds

Two compounds not related to either of the overlay models were also prepared. Compound **199** was synthesised from 2-aminobenzophenone (**53**), 4-nitrobenzyl bromide and calcium carbonate. The crude product was reduced, guanylated and treated with TFA to form the target guanidine **200** (Scheme 34).
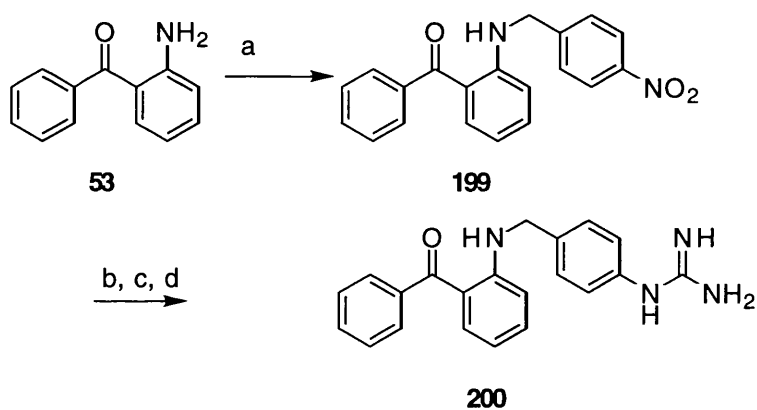
Reagents: (a) 4-nitrobenzyl bromide, CaCO₃, dioxane (b) SnCl₂.2H₂O, EtOAc, 70 °C, (c) **59**, HgCl₂, DMF (d) TFA , DCM

Scheme 34

Compound **183** was reduced, guanylated and treated with TFA to form the target guanidine **201**.
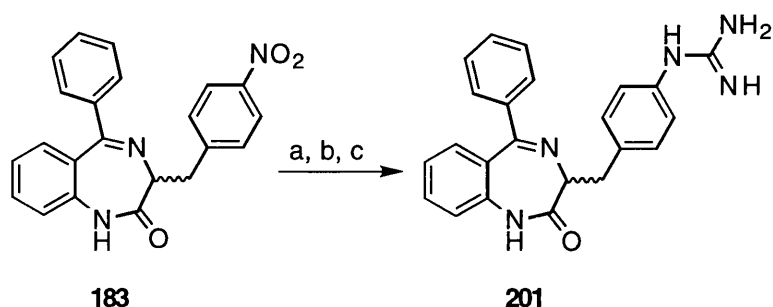
Reagents: (a) $SnCl_2.2H_2O$, EtOAc, 70 °C, (e) **59**, $HgCl_2$, DMF
(f) TFA , DCM

Scheme 35

2.2.5. Alkylation Studies on a Glycine-Derived BZD Intermediate.

The synthetic route we have described in the previous sections requires the synthesis of a distinct BZD core from the appropriate amino acid and the correct aminobenzophenone. A disadvantage with this route is that the number of commercially available amino acids is limited. If a synthetic route could be developed to allow for the inclusion of natural and unnatural amino acid side chains, this would greatly expand the availability of receptor-probing functionality at C-3.

One possible method would be to synthesise a BZD which is unfunctionalised at C-3 (formally derived from glycine) and introduce functionality by alkylation, as the numbers of commercially available alkylating agents is large. There are a number of examples in the literature where alkylation was used to synthesise a variety of C-3 functionalised BZDs[119]. To investigate this route, compound **173** was synthesised in moderate yield by the standard procedures described earlier and treated at low temperature with KO^{t}Bu. Addition of methyl iodide as a test electrophilic agent resulted in the formation of the alkylated product (**161**) in good yield (Scheme 36).

78
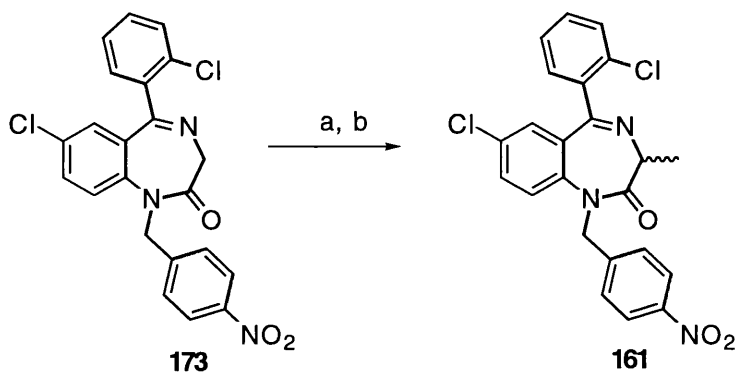
Reagents: (a) KO^{t}Bu/THF, -15 °C (b) MeI, -70 °C

Scheme 36

In order to begin investigating the scope of the reaction, compound **172** was treated with KO^{t}Bu and quenched separately with ethyl iodide and propyl iodide. When compound **172** was treated with ethyl iodide in this way, it was noticeable that a gas was evolved upon addition. The identity of this gas, although not determined, was suspected to be ethene, formed from the E2 elimination of iodide. When the reaction was worked up, a small amount of the desired product (**202**) was formed together with a sizeable amount of starting material and uncharacterised by-products. It quickly became apparent that more time than was available at this point in the project was required to optimise the synthetic route.
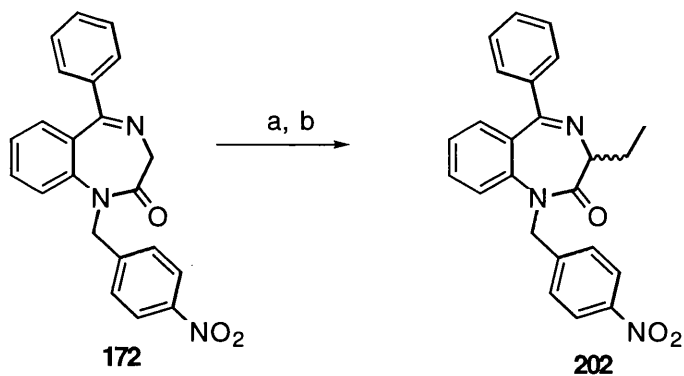
Reagents: (a) KO^{t}Bu/THF, -15 °C (b) CH$_3$CH$_2$I, -70 °C

Scheme 37

2.3. Biological Results

The experimental work described in this section was carried out by Dr Michael Brown and colleagues at the Novartis Institute for Medical Sciences, Gower Place, London.

2.3.1. Binding Affinities of Benzodiazepine Analogues.

The binding affinities are reported as the inhibition constant (K_i) and indicate the ability of each BZD analogue to displace 50% of the bound ligand [^{3}H]-BK from human BK B2 receptors transiently transfected into Cos-7 cells (or permanently transfected into CHO cells).

2.3.2. Transient Expression of DNA and Harvesting of Cells.

2.3.2.1. Cell Culture

Cos-7 cells were grown in Dulbecco's Modified Eagle medium with sodium pyruvate and glucose (1000 mg litre^{-1}) supplemented with penicillin (100 IU ml^{-1}), streptomycin (100 μg ml^{-1}), L-glutamine (2 mM) and 10% foetal bovine serum. The cells were sub-cultured as necessary using trypsin-EDTA solution (0.5 g litre^{-1} trypsin, 0.2 g litre^{-1} EDTA in Modified Puck's saline).

2.3.2.2. Preparation of Recombinant Vector DNA.

Plasmid DNA was isolated from 1 litre to 3 litres of overnight bacterial cultures using Promega Magic Megaprep DNA isolation kits according to the manufacturers instructions. Plasmid DNA was sterilised by precipitation with sodium acetate (0.3 M) and 2.5 volumes of absolute ethanol at -20 °C. The precipitate was spun down and rinsed once with 70% ethanol. The pellet was then resuspended in Tris (0.1 mM), EDTA (0.01 mM), pH 7.5 (0.1X TE) to a concentration of 120 μg ml^{-1}.

2.3.2.3. Transient Transfection of Cos-7 Cells.

Cos-7 cells were grown in 175 cm^2 surface area flasks and were split the day before transfection such that the cells would be about 75% confluent at the time of transfection. The DNA-calcium phosphate co-precipitate was prepared by mixing the following sterile solutions: 440 μl of the DNA solution (40 μg ml^{-1} in 0.1X TE) with 500 μl of (280 mM NaCl, 10 mM KCl, 1.5 mM Na$_2$HPO$_4$.2H$_2$O, 12 mM dextrose, 50 mM HEPES, pH 7.05). To this solution 62 μl of CaCl$_2$ (2.5 M) was added in a dropwise manner with gentle mixing. The solution was left for

about 20 min to allow for the precipitate to form. Old growth medium was removed from the cells and 20 ml of fresh medium was added. DNA-calcium phosphate co-precipitate solution (1.0 ml) was added to the flask. The cells were then incubated in the presence of the precipitate overnight at 37 °C, 5% CO_2. The following day the growth medium was removed. The cells were washed once with sterile PBS and fresh growth medium was added. The cells were left to grow for a further 24 h before harvesting.

2.3.2.4. Harvesting of Cells

The cells were rinsed once with 10 mM HEPES, 1X Hanks (minus calcium and magnesium), pH 7.4, 1 mM EDTA. The cells were then incubated at room temperature in the same medium for several minutes until the cells begin to detach. HEPES (10 mM), 1X Hanks (minus calcium and magnesium), pH 7.4, 0.2% BSA (fraction V, Sigma) (HHBSA) was added and the cell suspensions were pooled in centrifuge bottles. The cells were spun down at 500g, 5 min. The pellet was resuspended in HHBSA and the washing repeated twice or more as above. A cell suspension was prepared by diluting the pellet in 10% glycerol and stored by freezing at -70 °C.

2.3.3. Preparation of Membranes Containing Human BK Receptors.

The cells were thawed and homogenised at 10 000 rpm for 30 seconds using a Kinematica homogeniser. The resultant suspension was centrifuged for 30 min at 38720 x g_{max} (18 000 rpm) The pellet was washed a further two times by re-suspension in Tris-HCl (50 mM, pH 7.4) and re-centrifugation. The final pellet was resuspended at 2-8 mg protein/ml in Tris-HCl (50 mM, pH 7.4) in 500 µl aliquots with an 8.8 % glycerol solution then frozen and used when required.

2.3.4. Binding Assays: Saturation and Competition Studies.

Binding assays were performed in 1.2 ml micronic polypropylene tubes containing a final volume of 1 ml. The membranes were thawed, homogenised and diluted with binding buffer (composition: pH 7.4, TES (10 mM), EGTA (1 mM), bacitracin (0.1 mM), chymostatin (54 µg ml^{-1}) and 0.2 % BSA). The assay mixture comprised of [^{3}H]-BK (100 µl), 50 µl of either the test compound, DMSO (for total binding measurements), or unlabelled BK (20 µM, for estimating non-specific binding), and binding buffer (100 µl). The assay was induced by addition of membrane suspension (750 µl). Samples were incubated for 60 min at 4 °C, then filtered through GF/B filterplates pre-soaked

in polyethylenimine (6 g per litre) for 2-3 hours at room temperature. Microscint-40 scintillation cocktail (50 μl) was added to each filter sample and subsequently counted in a Canberra Packard TopCount scintillation counter. Binding parameters were calculated by using LIGAND[120].

2.3.5. Kinetic Experiments

Kinetic experiments were performed under the same conditions as the competition and saturation studies. For association kinetics the specific binding was measured at various times (5, 10, 15, 20, 40, 50, 60, 90 minutes). The dissociation was initiated after 60 minutes of incubation (at which time steady state would be reached) by the addition of 10 μM BK. Binding parameters were calculated by the method of McPherson using KINETIC[121].

2.3.6. Data Analysis

The K_i (inhibition constant) values were calculated for each compound from the IC_{50} values using the Cheng-Prusoff equation:

$$K_i = IC_{50}/(1+[ligand]/K_D)$$

K_i = the dissociation constant for the inhibitor-receptor complex.

IC_{50} = concentration of competing drug that reduces specific binding by 50%.

[ligand] = the concentration of [^{3}H]-BK introduced into each assay tube (normally 0.5 nM for calculations).

K_D = dissociation constant of the bradykinin-receptor complex (0.08 nM).

2.3.7. Biological Results of the Compounds From Overlay Model I

In the first overlay model, two hydrophobic groups are needed to mimic the two hydrophobic amino acids D-Tic and Oic at the presumed C-terminal β–turn in HOE140. Intermediate **51** was tested in the binding assay and was found to be inactive, exhibiting an IC_{50} value of >100 μM, suggesting that insufficient functionality is present in the molecule to afford biological activity.

A ligand needs to have a minimum of three receptor binding groups for it to bind tightly to a receptor[65]. Although HOE140 may have more binding determinants, a non-peptide analogue with only three binding sites could have an equally high potency and selectivity. As a general rule, biologically active peptides have binding affinities (K_D) at their receptors of between 10^{-9}-10^{-12} M. Introducing

these values for K_D into the Gibbs-Helmholtz equation (1) gives a value for $\Delta G°$, the free energy change, of -12.75 to -17 kcal mol^{-1} at 310 K.

$$\Delta G° = -RT \ln K_D \qquad \text{(equation 1)}$$

Where $G°$ represents the standard free energy; R = the gas constant (1.987 cal deg^{-1} mol^{-1}); and T, the absolute temperature.

A measured K_i can also be converted into a binding free energy:

$$\Delta G = RT \ln (K_i) \qquad \text{(equation 2)}$$

$$= 2.303 \, RT \log (K_i) \qquad \text{(equation 3)}$$

The factor 2.303 RT is approximately 1.4 kcal mol^{-1} at 25 °C. This gives a useful rule of thumb for converting a K_i to binding free energy:

e.g., if a $K_i = 10^{-8}$ M (10 nM), then $\Delta G = -8$ (1.4) = -11 kcal mol^{-1}.
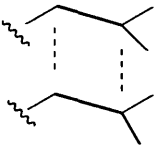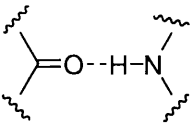
If we know the K_i for two molecules, X and Y, then the difference in free energy of binding between these is given by:

$$\Delta\Delta G = 1.4 \, [\log K_i \, (X) - \log K_i \, (Y)] \qquad \text{(equation 4)}$$

By comparing binding affinity differences between pairs of molecules, in this way, we can estimate functional group contributions to the free energy of binding.

The interaction between a ligand and receptor has been studied by a number of groups and three main modes of binding have been established: ionic, hydrophobic, and hydrogen bonding[64]. Numerous sub-groups are contained within these three classes. Amongst the naturally occurring amino acids, at least fifteen different potential receptor binding modes distinguishable on the basis of size and charge distribution have been described[64]. A few examples are shown in Table 2:

Table 2. Types of ligand binding interactions and their energies

Bond type	Example	Bond radius (nm)	Binding energy (kcal mol⁻¹)
Hydrophobic		0.2-0.4	-1(per methylene)
Hydrogen Bond		0.2-0.4	-(1-7)
Ionic		0.5-1.0	-5

Since compound **51** did not bind to the receptor with any great affinity, an additional binding determinant was then sought. Based on the knowledge of the structure of HOE140, the first overlay model suggests this would be a basic binding group.

Figure 29. Biological affinities of BZD BK analogues

203	**61**	**64**
$IC_{50} > 100\ \mu M$	$K_i = 9.2 \pm 5.4\mu M$	$K_i = 8.9 \pm 4.3\ \mu M$
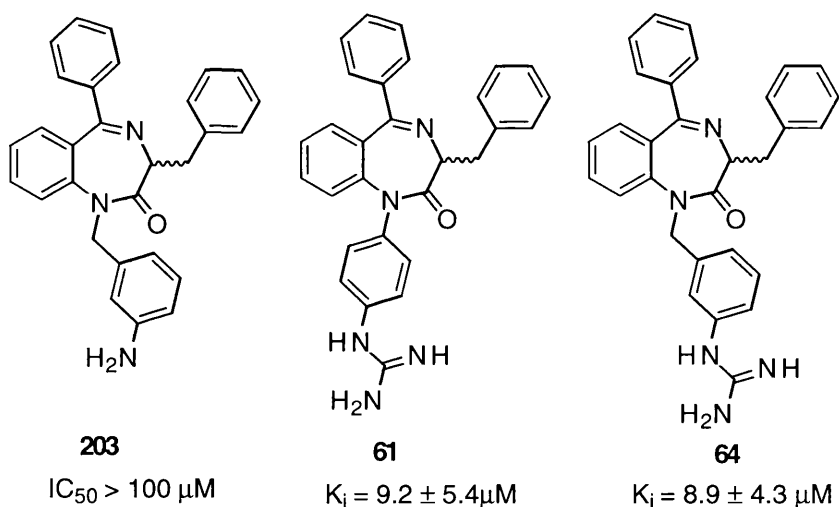
From our first overlay model (Section **2.1.2.1.**) a distance equivalent to four carbon atoms is required between the lactam nitrogen of the BZD to the basic binding determinant. Compound **203** was prepared from **51** and tested for biological activity. Its lack of affinity, $IC_{50} > 100\ \mu M$, suggested that the interaction between the aniline amine and the receptor is not sufficient for biological activity. This lack of activity is probably due to the aniline's lack of

basicity ($pK_a \approx 2.2$). It was then decided to introduce the guanidine functional group into the molecule ($pK_a \approx 10.7$). Compound **61** was prepared and its moderate biological activity (9.6 µM) was pleasing as it was only the third compound submitted for testing (Figure 29). A more flexible analogue **64** was then prepared, and it exhibited a similar biological activity (K_i = 8.9 µM). This result indicates that the guanidine functional group can adopt a similar orientation and position in both molecules.

By substituting the values of K_D, IC_{50} and ligand concentration for compound **203** into the Cheng-Prussof equation, we can calculate the approximate binding energy contribution for the guanidine functional group in **64** by using equation 4. If we assume that the K_i obtained is 100 µM:

$$\Delta\Delta G = 1.4 \left[\log K_i\,(\mathbf{203}) - \log K_i\,(\mathbf{64}) \right]$$

$$\Delta\Delta G = 1.4 \left[\log K_i\,(100000) - \log K_i\,(8900) \right]$$

$$\Delta\Delta G = 1.47 \text{ kcal mol}^{-1}$$

The energy difference between the two molecules, -1.47 kcal mol^{-1} at 25 °C, possibly reflects the better ionic bonding capabilities of the guanidine functional group. Since at pH 7.4, the guanidine functional group is charged whereas the aniline functional group largely exists in the unprotonated form.

Figure 30. Biological affinities of BZD BK analogues

70	**67**
K_i = 15.8 ± 7.6 µM	K_i = 11.9 ± 3.8 µM
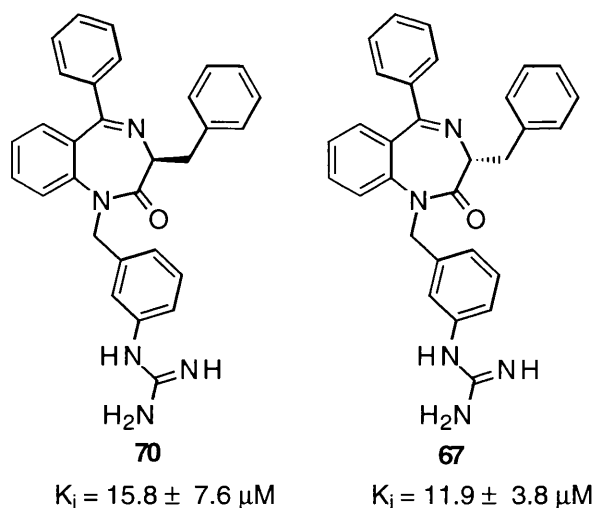
Although exhibiting a modest potency, we decided to synthesise the separate enantiomers of **64** to determine whether a stereochemical bias existed in the binding of our ligand to the receptor. There are many examples in the literature

where enantiomers display different activity. Our overlay model suggests that the R isomer (**67**) would bind in a manner preferential to the corresponding S-isomer (**70**). When tested, however, both compounds (**70** and **67**), exhibited similar binding affinities, within the range of the initial lead **64**. These results suggest that our binding model may need refining as the stereogenic centres of the enantiomers are obviously not being discriminated by the receptor. It was decided to continue to test racemic mixtures of compounds until the potencies were improved. When the affinities were sufficiently high it would become necessary to test the enantiomers separately in order to determine whether our overlay model is valid.

Figure 31. Biological affinities of BZD BK analogues

73	**74**
K_i 6.4 ±1.5 µM	<10 % inhibition at 10µM
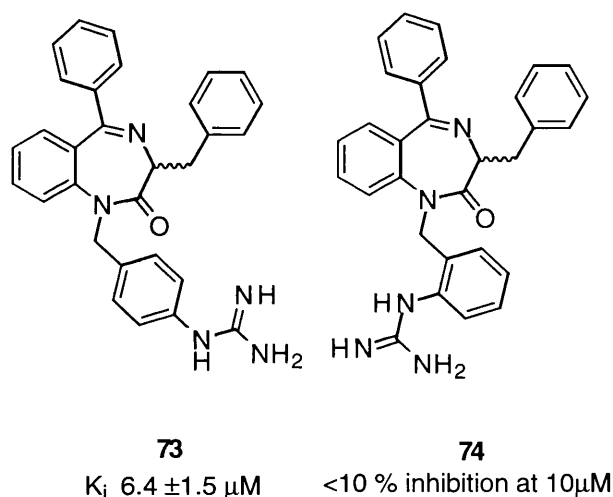
The regioisomers of **64** with the guanidine moiety at the 2 and 4 positions, **74** and **73**, were then tested. Compound **73** had a slightly improved biological affinity of 6.4 µM while **74** had a very poor affinity (Figure 31), suggesting at this early stage that chain length was obviously going to be important in the design of these compounds.

Figure 32. Biological affinities of BZD BK analogues

82

$IC_{50} > 100\mu M$

83

$26 \pm 5\%$ inhibition at 10 µM

84

<10 % inhibition at 10 µM

85

$K_i = 21.3 \pm 4.0\mu M$
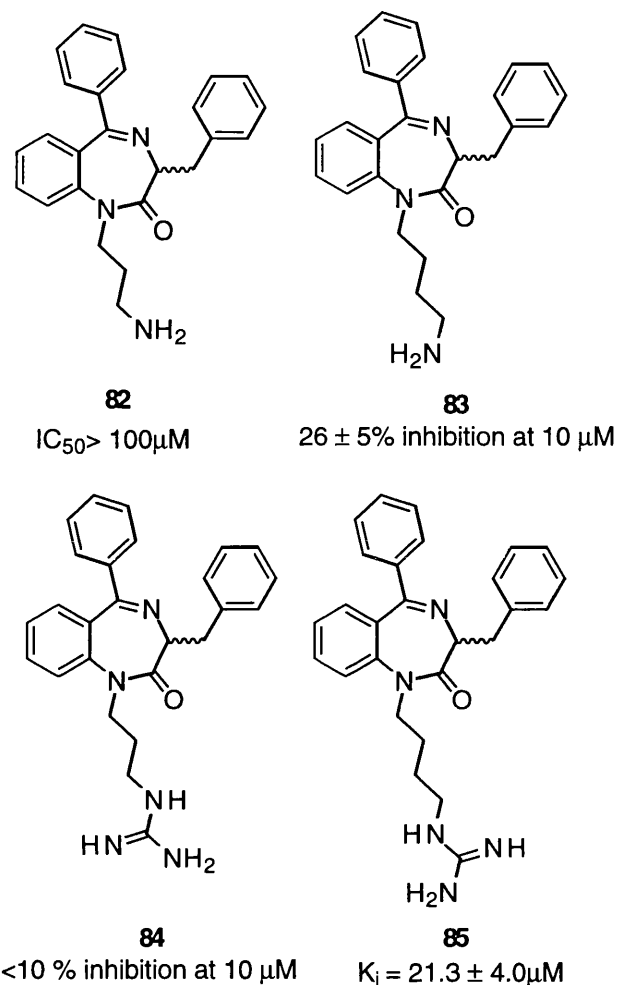
In the active compounds tested so far, the guanidine moiety is attached directly to a benzyl or phenyl 'linker' group. These linker groups have limited flexibility and this restriction may decrease biological activity if functionality is thereby projected into non-complementary areas of the receptor. The aromatic rings may also have an electronic influence on the molecules' binding properties. We were also aware of one of the 'rules' in peptide-mimetic design which states that "flexibility in a ligand should be retained until lead activity is discovered"[64]. Although a novel non-peptide lead had already been generated, it was still desirable to examine a flexible 'linker' between the lactam nitrogen and the basic moiety. The aliphatic amino derivatives, **82** and **83**, were tested for biological activity (Figure 32). The propyl amine derivative **82** was found to be inactive and the butyl amine derivative **91** to be weakly active. Like the results for compound **203**, these results suggest that an amine moiety is sub-optimal for biological activity. However, the basicities of the aliphatic amine and the aromatic guanidine are similar: both phenylguanidine and *n*-butylamine have a

pK_a value of 10.7. The amines were then transformed into the guanidines **84** and **85**. Aliphatic guanidines have higher pK_a values than aromatic guanidines ($pK_a \approx$ 13.2 compared to 10.7). With this additional basicity, we expected the aliphatic guanidines to bind with a greater affinity to the BK receptor than the aromatic guanidines. However, the butyl guanidine derivative **85** was found to exhibit a biological activity of 21μM, which when compared to the constrained analogue **64** potency of 9 μM, indicates that the greater flexibility imposes an entropic penalty. The propyl guanidine derivative was weakly active, with less than 10% of the ligand bound at the receptor at 10 μM concentration. This binding affinity, identical to that of compound **74**, suggests that a 'minimum distance' of four atoms between the lactam nitrogen of the BZD and the basic guanidine is required for the guanidine group to be in the appropriate position for biological activity. In addition, these results suggest that some conformational restriction between the basic group and the BZD core appears to be a requirement for high biological activity. Although the compounds **84** and **85** are more basic than their constrained aromatic counterparts, **64** and **74**, their increased conformational flexibility appears to be disadvantageous and thus bind to the receptor with lower affinities.

Figure 33. Three common modes of guanidine-receptor interactions and their associated binding energy (from Zablocki[122])

Hydrogen Bond Donor	Ionic	Reinforced Ionic
-1-7 Kcal mol^{-1}	-5 Kcal mol^{-1}	-10 Kcal mol^{-1}
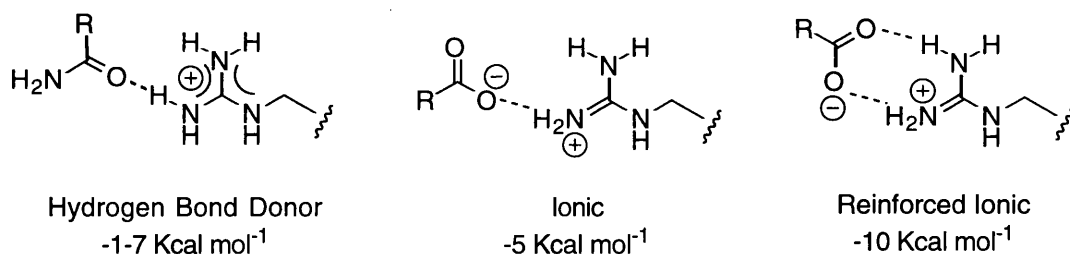
The nature of the interaction between our active compounds and the receptor was then investigated. Guanidines have been shown in the literature to interact with the receptor in three binding modes (see Figure 33). In order to test whether the guanidine moiety exerts it biological effect by hydrogen bonding or by an ionic interaction, compound **79**, a urea, was synthesised (Figure 34). The compound was found to be inactive with an $IC_{50} >$ 100 μM, suggesting again that an ionic or reinforced ionic interaction is required for biological activity rather than hydrogen bonding.

Figure 34. Biological affinity of BZD BK analogue **79**

79
$IC_{50} > 100 \ \mu M$
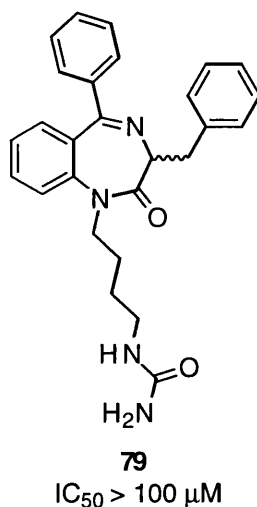
In a literature report on the synthesis of RGD mimetics[122], the authors studied the interaction between the guanidine moiety and a negatively charged receptor component and compared this interaction with that of benzamidine and the negatively charged component. They suggested that benzamidines, which have their positive charge localised on two nitrogen atoms as opposed to three for the guanidines, form favourable electrostatic interactions with a negatively charged receptor site (see Figure 35).

Figure 35. The interaction between the benzamidine and guanidine functional group with carboxylic acids
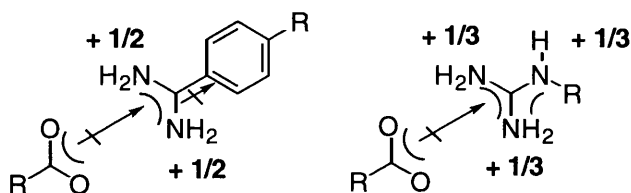
The reinforced ionic interaction is enhanced with
the benzamidine (from Zablocki)[122]

They compared the dipole moments between the two groups and found that in the reinforced ionic interaction (see Figure 33) the benzamidine provided a more favourable alignment of dipole moments (7.1 Debye) as compared to the guanidine (3.4 Debye). With this observation in mind, the 3- and 4-amidinobenzyl BZDs were tested for biological activity. Compounds **88** and **89** were less potent than their guanidine counterparts **64** and **73**, exhibiting a K_i of 14.5 and 13.5 μM respectively (Figure 36). Perhaps a larger alkyl chain is required between the lactam nitrogen and the benzamidine terminus. This

result also illustrates the difficulty in designing these types of antagonists, as the orientation of the guanidine or amidine function as well as its distance from the BZD core has to be taken into account.

Figure 36. Biological affinities of BZD BK analogues

88
$K_i = 14.5 \pm 6.3 \, \mu M$

89
$K_i = 13.5 \pm 3.2 \mu M$
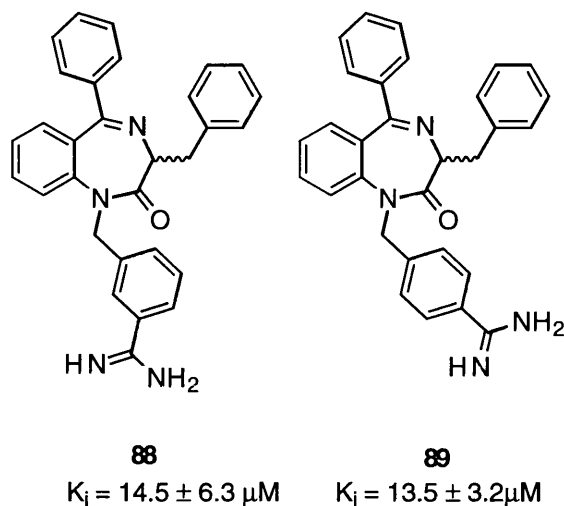
In compound **51** the hydrophobic core did not bind to the receptor with any great affinity. However, when a basic moiety was appended to N-1, moderate activity was achieved. We next wanted to determine which substituents in the hydrophobic core were important for biological activity.

Figure 37. Biological affinities of BZD BK analogues

95
$IC_{50} > 100 \, \mu M$

128
$K_i = 2.42 \pm 1.16 \mu M$
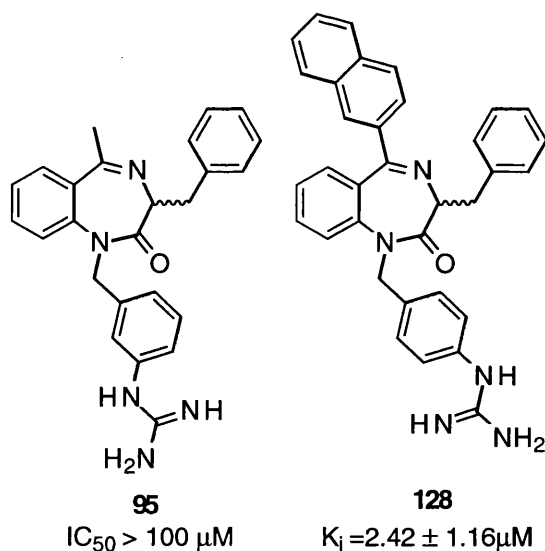
In the first study, the guanidinobenzyl moiety was retained as it had been shown to give maximal biological activity and the C-5 phenyl substituent was replaced with a methyl group as in **95** (Figure 37). The replacement of the C-5

phenyl by methyl leads to a great decrease in affinity, (IC_{50} > 100 μM compared to K_i = 8.9 μM), suggesting that the phenyl ring must be a major binding component. This result is in accord with the literature observation that the diphenylmethyl motif is a recognition element in binding of non-peptidal peptidomimetics to G-protein coupled receptors[55].

It was next decided to determine what effect a large hydrophobic group at C-5 would have on the biological activity of the molecule. Compound **128**, with a naphthyl group at C-5, exhibited a potency of 2.4 μM, an improv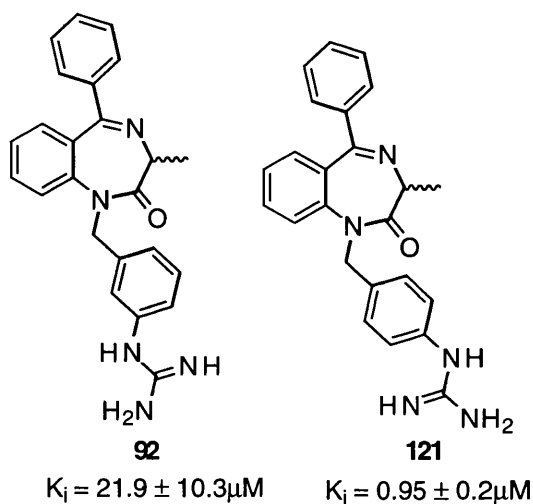ement when compared to **73** (K_i = 6.4 μM, Figure 37). This result suggests that there is a large amount of space at the receptor corresponding to the C-5 position.

Figure 38. Biological affinities of BZD BK analogues

92

K_i = 21.9 ± 10.3μM

121

K_i = 0.95 ± 0.2μM

The C-3 benzyl substituent was then replaced with the methyl group in compounds **92** and **121** (Figure 38). Unlike the C-5 phenyl ring, replacement of the phenyl ring at C-3 did not lead to a total loss of affinity. Compound **92** had an affinity of 21 μM which compared unfavourably to the biological activity of the benzyl substituted **64**, which had a K_i of 8.9 μM. However, the 4-guanidinobenzyl derivative **121** had a K_i of 0.95 μM, which shows an improvement in binding affinity over the benzyl substituted **73** (K_i = 6.4 μM). This may indicate that the presence of the benzyl group at C-3 results in a less precise orientation between the diphenylmethyl binding region and the guanidine binding site.

Figure 39. Biological affinities of BZD BK analogues

124	125	181
$K_i = 4.5 \pm 0.3\ \mu M$	$IC_{50} = 10\text{-}100\mu M$	$K_i = 2.5 \pm 0.31\mu M$
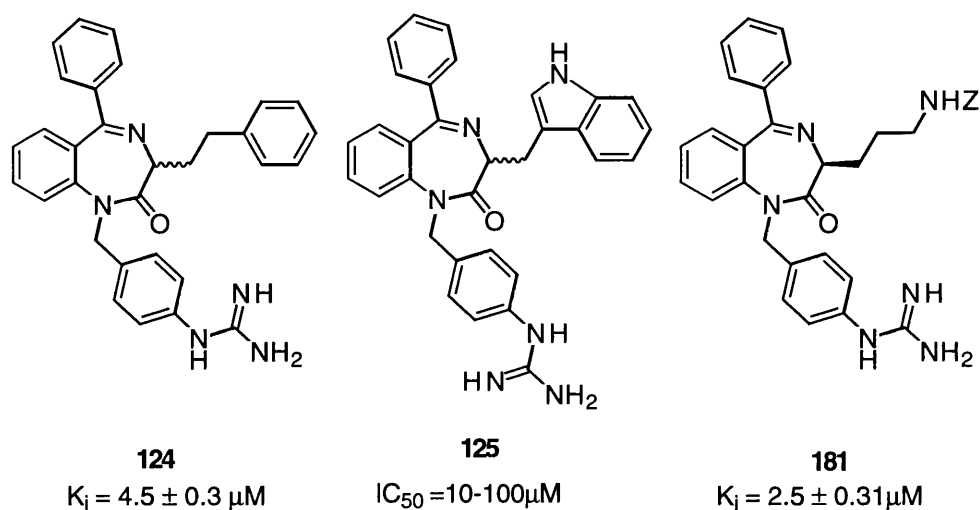
The homologue of **73** with a 2-phenylethyl side chain, **124**, was then tested This compound exhibited a slightly enhanced potency of 4.5 μM, compared to **73**, suggesting that the side chain was not adequately filling the receptor pocket at this position in **73**. A constrained analogue of **124** containing a 3-indolylmethyl side chain, **125**, had some biological activity; however, an insufficient amount was prepared to obtain a K_i and only an activity range of between 10-100 μM could be determined. A BZD with a larger C-3 substituent, prepared from N-protected ornithine, **181**, exhibited a potency of 2.5 μM which, when combined with the results for **125** and **124**, suggests that there is space at the receptor which the C-3 substituents are not accessing. This may explain the results with the enantiomers of **64**, and the potency of **121**. Indeed, if the amino acid component of the BZD ring is omitted, as in **200**, binding affinity is more or less preserved ($K_i = 16.3$ μM, see Figure 40). The greater affinity of compound **121** may reflect the importance of restricting the rotation of the C-5 phenyl group, compared to **200**, although this may be partly provided by intramolecular hydrogen bonding in the latter compound. We elected to continue with BZD-derived compounds rather than those based on **200** in order not to lose the source of a potential third binding determinant. We thus turned our attention briefly to aliphatic, hydrophobic side chains.

Figure 40. Biological affinities of BZD BK analogues

200
$K_i = 16.3 \pm 5.6\ \mu M$

122
$K_i = 15.7 \pm 5.7\ \mu M$

123
$K_i = 3.26 \pm 0.54\ \mu M$
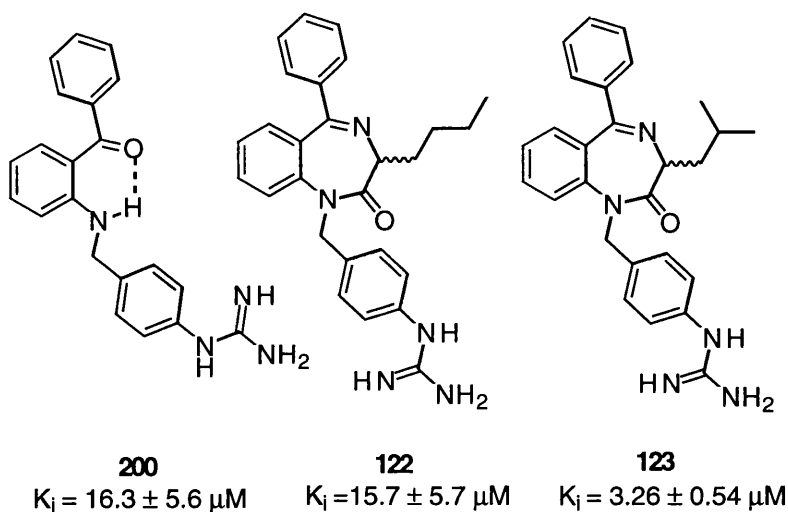
The BZD formed from norleucine, **122**, has a hydrophobic, flexible *n*-butyl side chain and exhibited a disappointing K_i of 15.7 μM, worse than both the BZDs derived from phenylalanine and alanine. It has been suggested by Nishio *et al.*[123] that amino acids with straight aliphatic side chains form less favourable hydrophobic interactions than amino acids that contain branched aliphatic side chains. Neither norvaline or norleucine are found in proteins in nature, but their branched counterparts are. The BZD prepared from leucine, compound **123**, was encouragingly found to have an enhanced biological affinity of 3.26 μM in comparison. However, this was still only comparable to the binding value of **73**, the phenylalanine-derived BZD.

Figure 41. Biological affinities of BZD BK analogue **175**

175
$IC_{50} > 100\ \mu M$
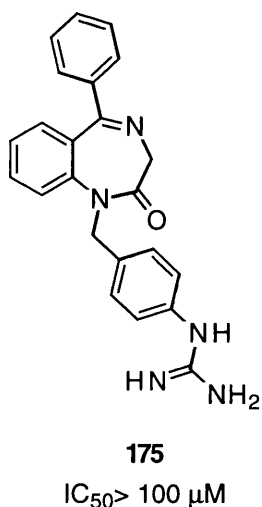
From the results discussed so far, it is noticeable that compounds with a smaller size substituent at C-3, tend to be more potent. Thus **73** (R = benzyl) is

93

less potent than **123** (R = *i*-butyl) which is less potent than **121** (R = Me). This relationship in the substituent size at C-3 led us to synthesise the glycine derived BZD **175** (Figure 41). When tested the BZD was found to be inactive which was most surprising given the result with **200**.

Figure 42. Biological affinities of BZD BK analogues

187	**186**	**73**
IC_{50} =10-100 μM	K_i = 6.1 ±1.8 μM	K_i = 6.4 ±1.5 μM
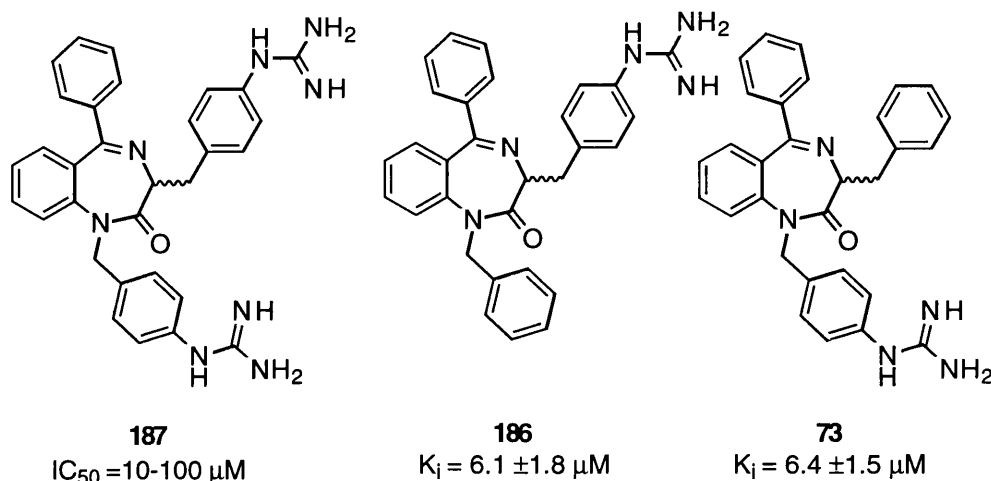
In order to further explore the configurable requirement of the binding pocket, a derivative with a hydrophobic substituent at N-1 and a basic substituent at C-3 (**186**) was prepared and tested. Compound **186** was found to exhibit a K_i value of 6.1 μM which is almost the same value as compound **73**. Compound **73** has a basic group at N-1 and a hydrophobic group at C-3 and has a binding affinity of 6.4 μM. This result suggests that although the binding sites are on different parts of the BZD scaffold, both the BZDs are able to access the same binding pockets.

The bis-guanidine **187** was then tested. The amount of compound synthesised was insufficient for a K_i determination to be made and only a binding affinity range of between 10-100 μM could be obtained.

Figure 43. Biological affinities of BZD BK analogues

201
$IC_{50} > 100 \mu M$

175
$IC_{50} > 100 \mu M$
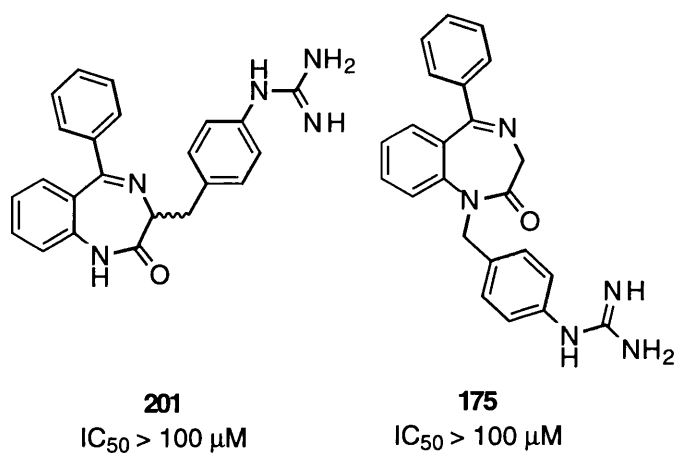
Compound **201**, which has a basic group at C-3 and no substituent at N-1 was found to have an IC_{50} value of $> 100 \mu M$ (Figure 43). Compound **175** with a basic group at N-1 and no substituent at C-3 was also found to exhibit an IC_{50} $>100 \mu M$.

Figure 44. Biological affinities of chlorinated BZD BK analogues

73, R = H,
$K_i = 6.4 \pm 1.5 \mu M$
162, R = Cl,
$K_i = 3.4 \pm 0.98 \mu M$

122, R = H,
$K_i = 15.7 \pm 5.7 \mu M$
164, R = Cl,
$K_i = 6.9 \pm 0.5 \mu M$

121, R = H,
$K_i = 0.95 \pm 0.2 \mu M$
163, R = Cl,
$IC_{50} > 100 \ \mu M$

175, R = H,
$IC_{50} > 100 \ \mu M$
177, R = Cl,
$K_i = 23.8 \pm 3.4 \mu M$

176
$K_i = 11.1 \pm 0.92 \ \mu M$
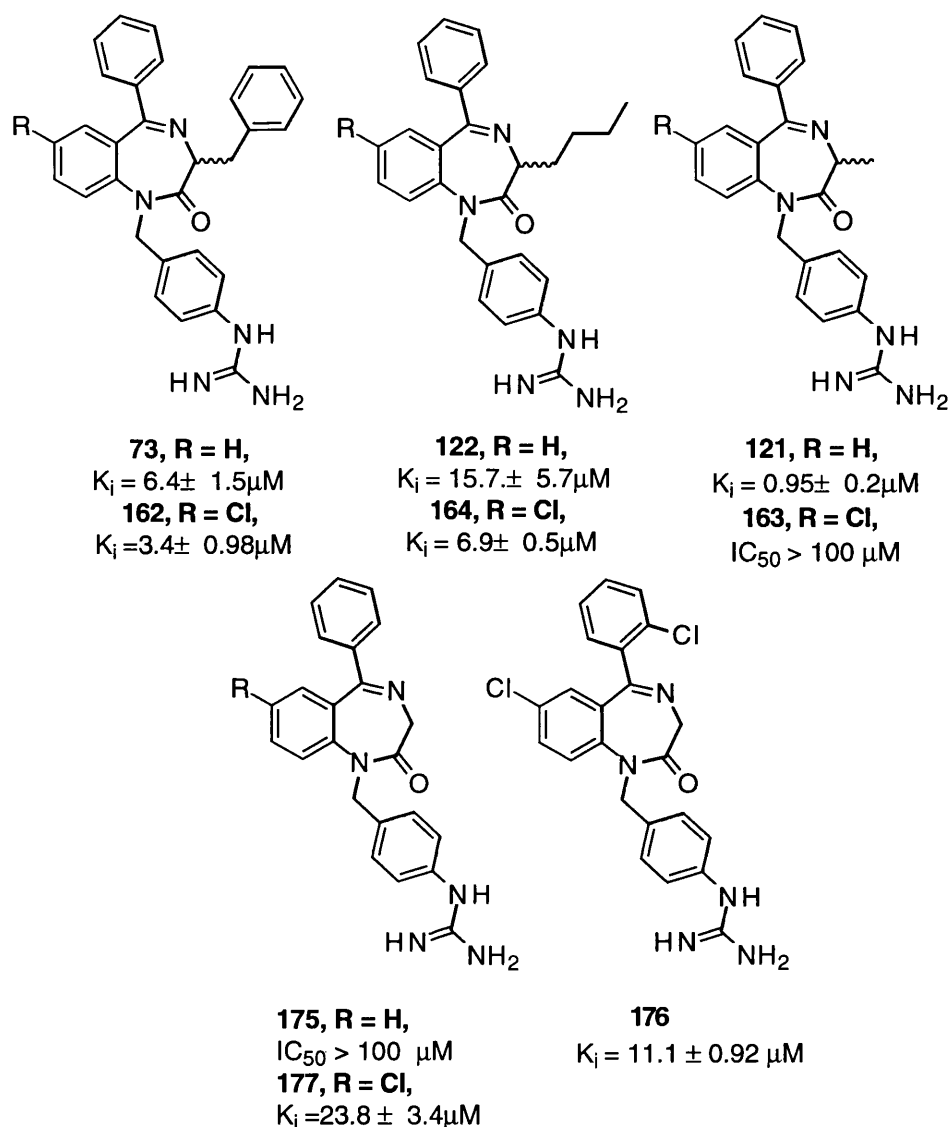
The next stage of the project was to try and improve potency by changing the electronic properties of the fused aromatic ring. Chlorine was introduced into the aromatic ring at position 7 by using 2-amino-5-chlorobenzophenone as the BZD precursor. Analogues of **73**, **121**, **122** and **175** were tested (Figure 44). The chlorine analogue of the BZD derived from phenylalanine, **162**, exhibited almost a doubling in biological activity to 3.4 μM. The chlorine analogue of the BZD derived from norleucine, **164**, again showed a doubling of binding affinity, with a K_i value of 6.9 μM. The chlorine analogue of the BZD derived from alanine, **163**, was then prepared in expectation that the activity would be well below micro molar; however, the compound was found to exhibit a $IC_{50} > 100$ μM. This result strongly suggests that the alanine derived BZDs may bind to a different region of the receptor than the phenylalanine and norleucine derived BZDs. The biological activity trend of the alanine series is in contrast to the

glycine series, whose potency returns with the introduction of a chlorine atom at C-7; compound **177** having a K_i of 23.8 μM. It seems unlikely that the chlorine atom alters the ring conformation greatly and suggests that the biological result of **175** is of doubtful value. Introduction of a second chlorine at C-2' in the C-5 attached phenyl ring produced a further increase in potency in compound **176** (11.1 μM).

2.3.7.1. Topliss Analysis of The BZDs

Attention was next directed at the C-5 phenyl group of the BZD. This portion of the molecule has been shown to be a crucial recognition element. We wanted to improve the potencies of our compounds by substituting different functional groups into this ring.

The ability to predict the change in biological activity of a molecule after changing a single substituent has long been a goal in medicinal chemistry. Drug-receptor interactions are dependent on the physicochemical and structural parameters of the drug and changes in these parameters can lead to pronounced changes in biological activity. Although there are an enormous number of physical parameters in even the simplest drug molecule, substituents which can alter three key parameters have been shown to influence biological activity.

The substituent hydrophobic constant, π_x, compares the lipophilicity of the substituent to hydrogen ($\pi_H = 0$). The more positive the value of π, the more lipophilic the substituent. Thus the hydrophobic CH_3 group has a π value of 0.56 and the hydrophilic CH_2OH substituent has a value of -1.03.

The Hammet substituent constant σ_x, reflects both the inductive and resonance effects of the substituent compared to hydrogen ($\sigma_H = 0$). A positive value indicates the substituent is an electron withdrawing group, *e.g.* NO_2, Cl, and a negative value indicate the substituent is electron donating, *e.g.* OMe, CH_3.

The third constant is the Taft steric parameter E_s This constant determines the steric bulk of the substituent compared to hydrogen. These constants have been incorporated by Hansch into equation 5.

$$\text{Log } 1/C = a\pi + bE_s + c\sigma + d \qquad (5)$$

In the Hansch equation C is the drug concentration for a chosen standard biological effect, and a, b, c, and d are regression coefficients.

Topliss has incorporated these concepts in his "decision tree" approach to lead optimisation (Figure 45). The scheme is a non-mathematical, non-statistical and non-computerised guide to the use of basic Hansch principles. The scheme considers the electronic, lipophilic and steric properties of the substituent and allows for the optimisation of activity of a lead structure containing benzene rings.

The Topliss analysis first requires the substitution of hydrogen by an electron withdrawing and hydrophobic 4-chloro substituent (see Figure 45). The biological activity of this compound is obtained and is compared to the biological activity of the parent compound. If the compound formed is more potent, then a positive π (hydrophobic) or σ (electron withdrawing) correlation with activity is suggested. The next compound synthesised should be the 3,4-dichloro substituted compound (see Figure 45). If the 4-chloro substituted compound is less potent, this suggests that an electron donating 4-MeO substituent should be incorporated into the molecule. Depending on that compound's biological activity a new substituent with the appropriate characteristics would then be incorporated into the molecule, and so on.

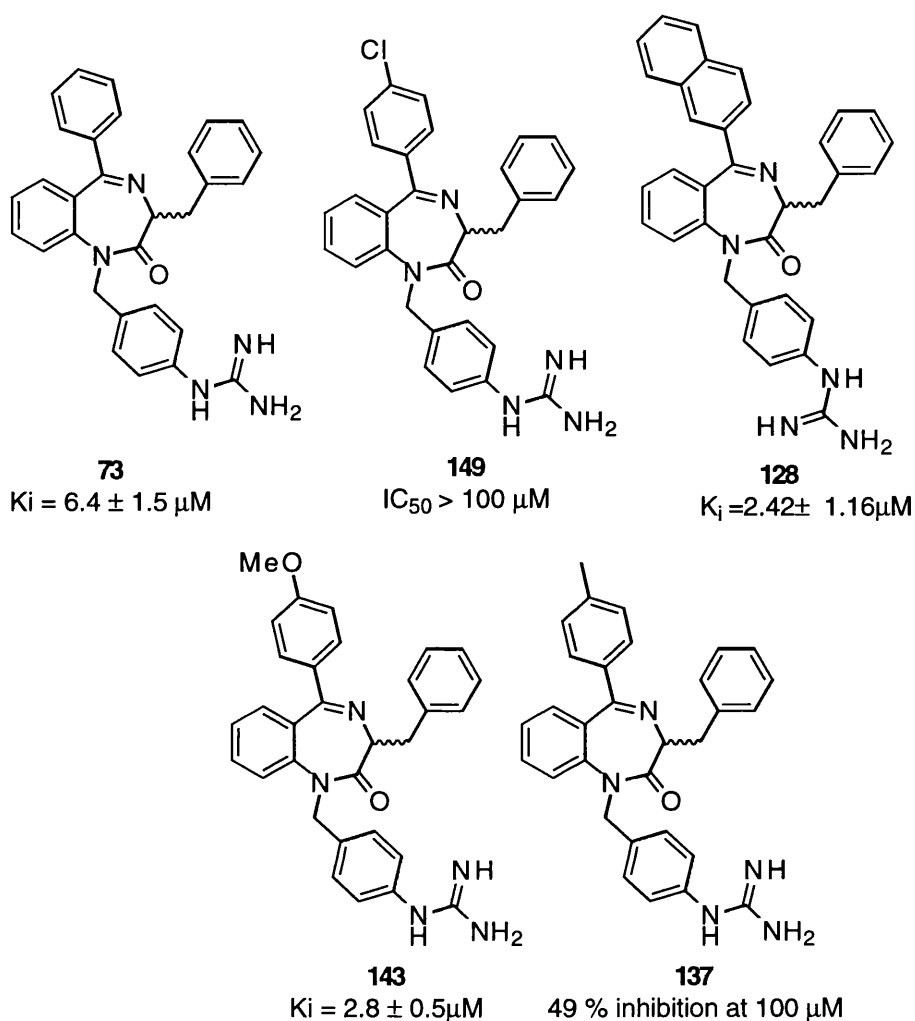**Figure 45.** Topliss decision tree for aromatic compounds.
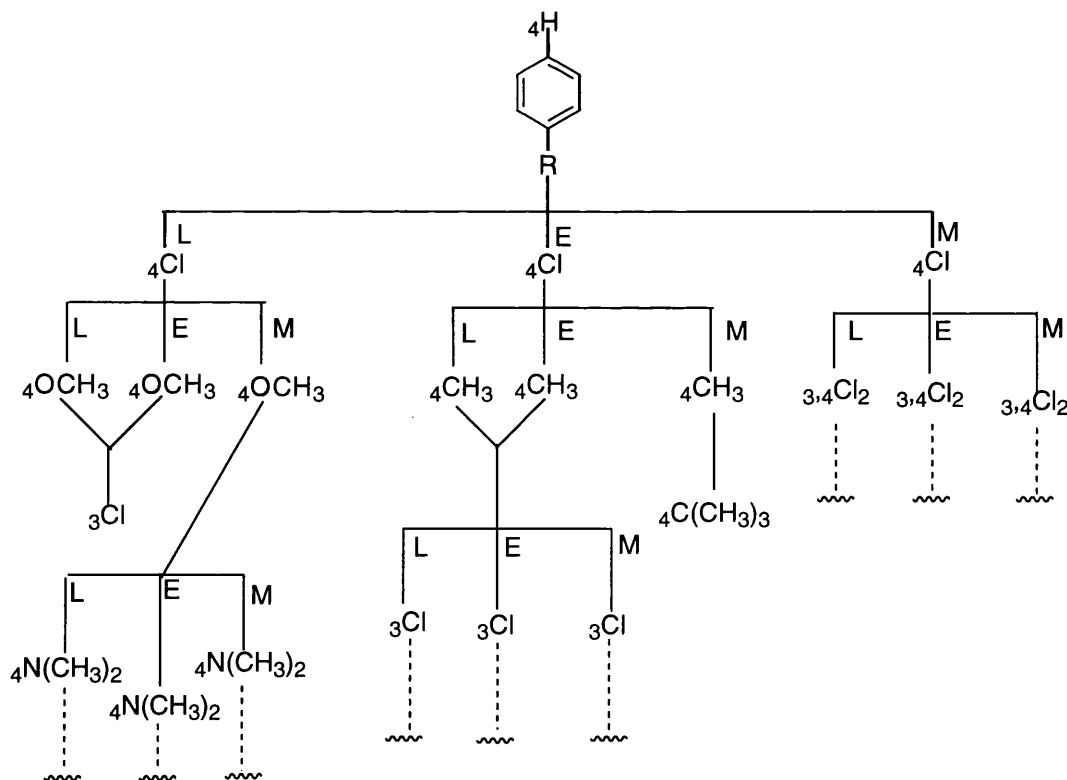
Descending lines indicate sequence of compounds to be prepared.
Dashed lines lead to different substituents (not shown). L: less active;
E: equiactive; M: more active

98

This approach has been used successfully in a number of different situations. In a recent example, Doherty *et al.*[124] used this approach to optimise a lead endothelin ET_A antagonist (**204**, see Figure 46). Compound **204** had an IC_{50} of 600 nM and after a Topliss analysis of one of the benzene rings a target compound with a predicted increased activity, **205**, was synthesised. This compound had an IC_{50} of 7.4 nM (Figure 46) and a further improvement in potency was obtained by performing a separate Topliss analysis on the second aromatic ring. This suggested the introduction of a *p*-OMe group and when this was carried out the new compound, **206**, exhibited an IC_{50} of 1.8 nM.

Figure 46. An application of the Topliss analysis
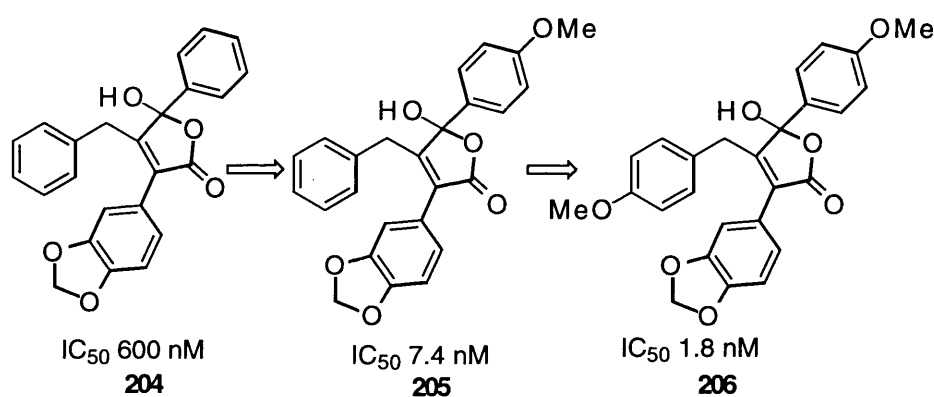
The Topliss analysis was performed on the C-5 phenyl group on the BZDs derived from norleucine (**122**), phenylalanine, (**73**) and alanine (**121**). The lag time between the synthesis of the target compounds and the biological results was up to two months which meant that we could not wait for the biological result to determine the next compound to be synthesised. We therefore decided to synthesise three sets of compounds. The 4-Cl, 4-Me, 4-MeO substitutions were made to the C-5 phenyl group on the BZDs **122**, **73** and **121**. The 4-MeO substituent has a -σ (electron donating) and -π effect (hydrophilic) on the phenyl ring. The Me substituent has a -σ and +π effect (hydrophobic) effect and the Cl substituent has a +σ (electron withdrawing) and +π effect on the C-5 phenyl ring. The complete set of nine compounds was not synthesised due to lack of time. However, enough compounds were obtained to generate a structure activity profile.

Figure 47. Topliss analysis of **73**

73
Ki = 6.4 ± 1.5 μM

149
IC$_{50}$ > 100 μM

128
K$_i$ =2.42± 1.16μM

143
Ki = 2.8 ± 0.5μM

137
49 % inhibition at 100 μM

In the BZD derived from phenylalanine (**73**), the 4-chloro substitution (**149**) was found to abolish activity completely (K$_i$ > 100 μM). Although the chlorine atom has lone pairs of electrons that conjugate with the aromatic ring, it is an electron withdrawing substituent with a positive σ value. This property is believed to hamper its binding affinity. It is unlikely to be a steric effect since compound **128** is active. Compound **143**, with a 4-OMe group, showed an improved biological activity with a K$_i$ of 2.8 μM. The methoxy substituent is electron donating with a negative σ value. Compound **137**, with a 4-Me group, was found to be less active than **73** with a 49 % inhibition of the receptor at 100 μM. To follow the Topliss analysis, the increase in affinity by the 4-MeO substitution now requires the synthesis of a N(CH$_3$)$_2$ substituent, but the biological results did not arrive in time for us to do this.

Figure 48. Topliss analysis of **122**

MeO

| **122**	**142**	**164**
Ki = 15.7± 5.7 µM	Ki = 4.6± 0.7 µM	IC$_{50}$ =10-100 µM
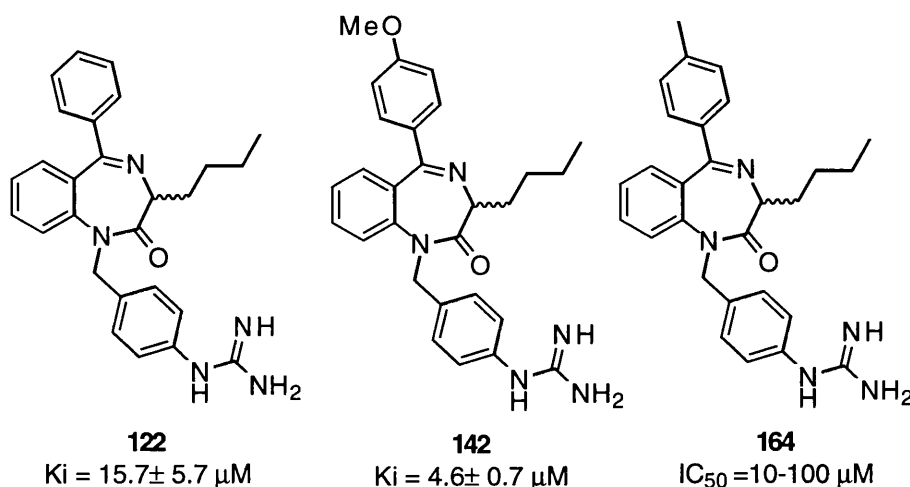
Both of the analogues with a 4-OMe group, **142**, and a 4-Me group, **164**, were prepared from the BZD derived from norleucine. An enhancement in biological activity was observed with compound **142**, which has a K$_i$ of 4.6 µM. This result is similar to that for the BZD derived from phenylalanine and confirms earlier observations which suggest that both BZDs bind to the same binding pocket. Compound **164** is expected to be less active than **122**, but an insufficient amount was prepared for the tests necessary to calculate a K$_i$, and only a binding affinity range of between 10-100 µM could be obtained.

Figure 49. Topliss analysis of **121**

Cl

121	**148**	**135**
Ki = 0.95 ±0.2 µM	Ki = 9.6 ± 2.1 µM	IC$_{50}$> 100 µM
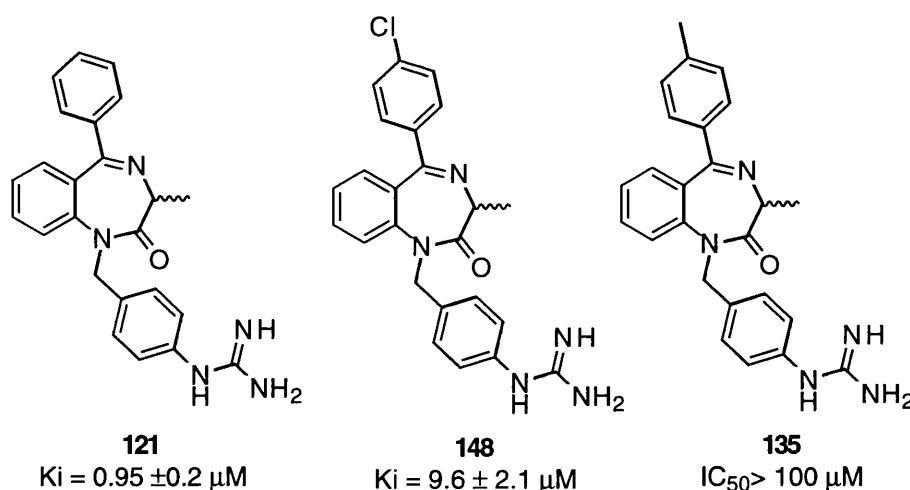
In the BZD derived from alanine the 4-Cl substituent, **148**, was found to be an order of magnitude less active than the original lead compound **121**. The methyl substituted BZD, **135**, was inactive with an IC$_{50}$ > 100 µM. Both the methyl and the chlorine functional groups are hydrophobic. However, the chlorine atom is electron withdrawing with a positive σ value and this may not be as

disadvantageous as a negative σ value. The 4-methoxy derivative would be required to determine whether the emerging trend was supported.

Figure 50. Biological affinities of BZD BK analogues

163	165	152
$IC_{50} > 100\mu M$	$IC_{50} = 10\text{-}100\ \mu M$	$IC_{50} > 100\mu M$
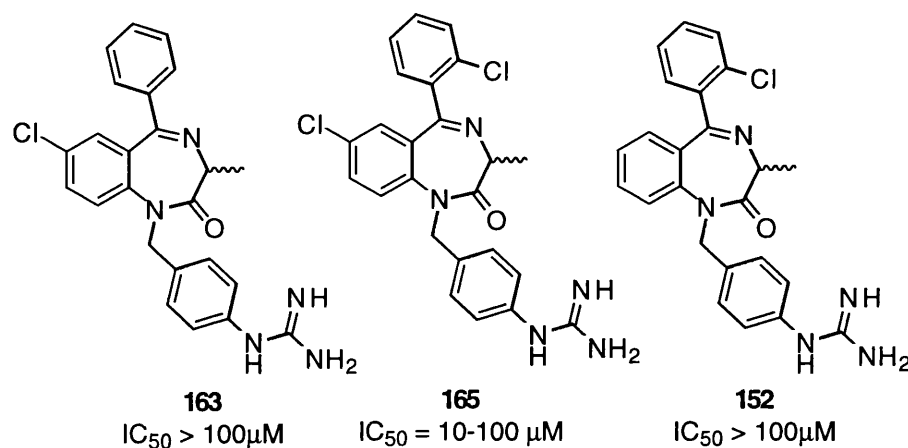
The dichloro derivative **165** exhibited a binding affinity of between 10-100 μM. This was encouraging since compound **163** exhibited an $IC_{50} > 100\ \mu M$. The only difference between the two molecules is the additional chlorine in the 2-position of the C-5 phenyl group. The substituent is believed to confer an additional affinity for the receptor despite the presence of the disadvantageous 7-chloro substituent. Compound **152** was prepared in expectation of it exhibiting an increased potency. However, when it was tested it was found to exhibit an $IC_{50} > 100\ \mu M$. This result suggests that the biological activity of **165** was possibly in the upper reaches of the binding affinity range. If this is so, it highlights another difference with the glycine series, that is, **165** versus **176**.

The Topliss analysis did not produce the desired increase in biological activity. This lack of success was mainly due to the lag time between the synthesis of the compounds and the receipt of biological results. This delay did not allow a focused approach to lead optimisation, as in the example of Doherty *et al.*, but resulted in only a preliminary examination. However, the results obtained from the BZDs derived from phenylalanine and norleucine suggest that a strongly electron donating substituent, such as a $-N(CH_3)_2$ group, could further increase the biological activity.

Most of the structure activity relationships (SAR) discussed earlier can be readily understood and explained. However, some of the results obtained from the alanine- and glycine-derived BZDs are puzzling. The modest activities of the BZDs synthesised are such that a substituent that decreases the biological activity by an order of magnitude can lead to an $IC_{50} > 100\mu M$. In this situation,

it is difficult to predict and observe subtle trends in SAR: a compound becomes either active or inactive. This may account, at least in part, for the apparently anomalous biological results of some members of the data set.

It has been suggested earlier that the alanine series of BZDs binds to a different portion of the receptor than the BZDs derived from norleucine and phenylalanine. Energy minimised conformations of the alanine-derived BZD, **121**, suggest that the C-5 phenyl group adopts a different conformation than that in the phenylalanine-derived BZD **73**. In **73** the phenyl group stacks with the benzyl side chain, while in **121** this phenomenon cannot take place. [see Figures 56 and 57, Appendix **2.** p 284] This different orientation may explain the difference in biological activities between the two compounds, assuming that the orientations are maintained within the receptor.

Within the alanine series of BZDs, some unusual results have been obtained. The introduction of the 4-Cl substituent in the C-5 ring, as in **148**, reduces the affinity of the compound compared to **121** by a factor of 10. However, in **152** which has a chlorine atom at the 2 position of the C-5 phenyl ring, the activity of the compound is abolished ($IC_{50} > 100$ μM). When an additional chlorine atom is added to the 7 position, as in **165**, the biological activity of the series is restored with an activity in the range of between 10-100 μM. However, when there is only one chlorine atom in position 7, as in **163**, the activity of the series is again abolished. A logical explanation of these results has so far eluded us but, as mentioned above, some of the confusion may result from the generally low binding affinities of the compounds.

The results for the glycine-derived BZDs are again very unusual. A trend of activity within that series of compounds has been established. This trend suggests that the activity of the compounds increases with the number of chlorine atoms in the molecule. The difference between **175** and **177** is a single chlorine atom at position 7. Molecular modelling calculations suggest that the energy minimised conformations of the two molecules are very similar [see Figures 58 and 59, Appendix **2.** p 286]. Again the possibility arises that the biological results are insufficiently precise because of the low binding affinities for a correct evaluation of the situation. A further improvement in biological activity is seen with compound **176**. Compound **176** has an additional chlorine atom in the C-5 phenyl ring and this could possibly alter the orientation of that ring, such that it can bind in a more preferential manner.

In order to attempt to disentangle some of these problems, the compounds in the glycine series are currently being re-tested simultaneously to determine whether some of the problems in the biological results may be resolved.

The next series of compounds were based on the overlay model described by James *et al.* in Section **2.1.2.2.**

### 2.3.8. Biological Results of the Compounds from Overlay Model II

Figure 51. Biological results of BZD BK analogues

188	190
$IC_{50} > 100$ μM	$K_i = 11.4 \pm 3.0$ μM
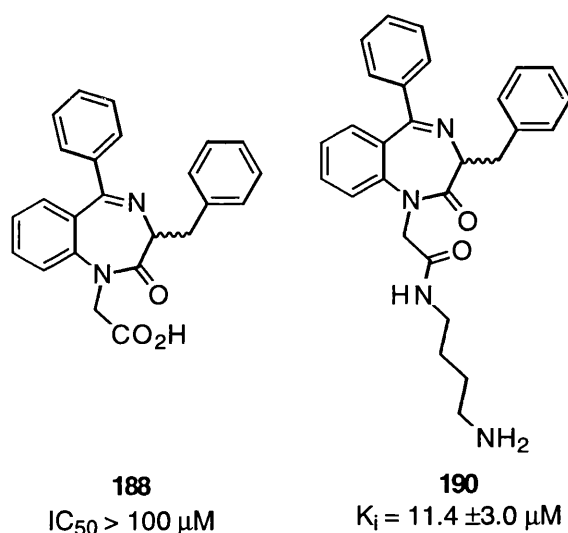
The presence of a C-terminus carboxylic acid has been demonstrated in BK to be essential for biological activity[106]. From overlay model II (Section **2.1.2.2.**) a one carbon linker group is required between the lactam nitrogen of the BZD and the carboxyl functional group. The carboxylic acid containing BZD **188** was tested for biological activity and found to exhibit a $IC_{50} > 100$ μM. This was the only carboxylic acid derivative tested. BZDs containing longer linker groups should be synthesised before it is concluded absolutely that the carboxylic acid functional group is inappropriate. In the next set of compounds synthesised, a basic group was incorporated into the molecule. In the first example, a flexible 4-aminobutyl chain was attached to the carboxylic acid to form **190**. Compound **190** was found to exhibit a moderate affinity with a K_i of 11.4 μM.

Figure 52. Biological results of BZD BK analogues **192** and **196**

192
$K_i = 15.6 \pm 5.6\ \mu M$

196
$K_i = 5.4 \pm 0.42\ \mu M$
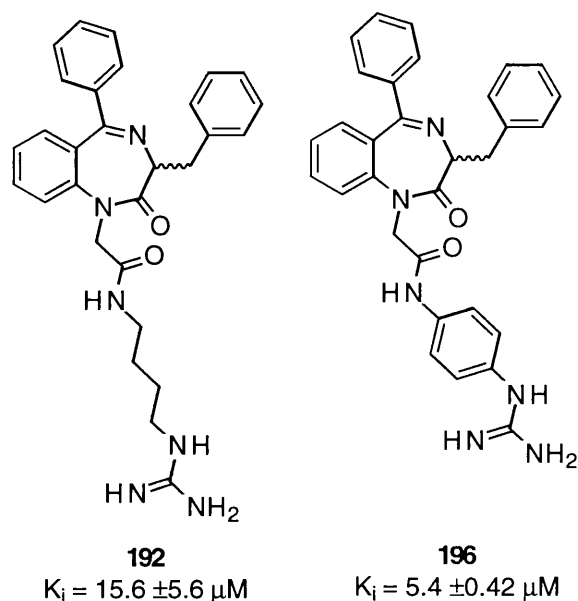
A more basic guanidine moiety was then incorporated into the molecule to form **192**. This compound, which was expected to have a greater binding affinity, was found to be slightly less potent than the amine containing analogue ($K_i = 15.6\ \mu M$). A rigid phenyl 'linker' was then introduced between the amide bond and the basic group to form **196**. As in the previous series of compounds, an improvement in affinity was observed with a K_i value of $5.4\ \mu M$, perhaps reflecting the decrease in entropy.

Figure 53. Biological results of BZD BK analogue **197**

197
$K_i = 29.1 \pm 7.6\ \mu M$
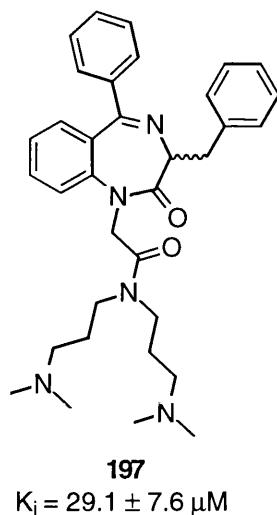
The final compound in this series, **197** has two basic groups separated from the amide bond by a flexible propyl 'linker'. These basic groups are potentially able to bind to more than one acidic amino acid side-chain at the receptor site and

this could increase potency. Indeed, it is known from mutagenesis experiments that aspartic acids at positions 268 and 286 (helices 6 and 7 respectively) are important for binding BK to the human B2 receptor[125]. Unfortunately compound **197** had the worst affinity in this limited series with a binding value of 29 μM.

The small set of compounds synthesised in this section limits the scope of the discussion. The overlay model of James *et al.* has been applied successfully to BK, reinforcing their conclusion that BZDs can act as turn mimetics* . The structure of the most active compound in this series, **196**, would form the basis of any further lead optimisation program.

2.3.9. Conclusion

In a recent publication by Jarnagin *et al*[126], site directed mutagenesis experiments were used to investigate the ligand binding domain of the BK receptor. The authors found that the affinity of BK at the human B2 BK receptor was greatly reduced by replacing three key amino acids by alanine. These three key amino acids are Phe[261], Thr[265], and Asp[286]. Replacement by alanine gives reduction in BK affinity of 2200 fold, 490 fold, and 60 fold respectively.

These biological results and [1]H-nmr spectroscopy studies on the conformation of BK were used by the authors to formulate a model for the binding of BK to the human BK B2 receptor. This model suggests that a C-terminal β-turn, featuring residues 6 to 9, binds within the interhelical bundle cavity. A salt bridge exists between Asp[286] and Arg[9] of BK and a hydrophobic pocket, composed of Tyr[117], Trp[157], Phe[261], and Trp[258], surrounds Phe[8] of BK. The amino terminus of BK rises out of the receptor and interacts weakly with Asp[268] (see Figure 54).

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* **Although based on the overlay model of James *et al.*, the receptor affinity of BZD 196 may simply be an extension of the Overlay Model I side chain investigation.**

Figure 54. Proposed binding model of BK to the B2 receptor
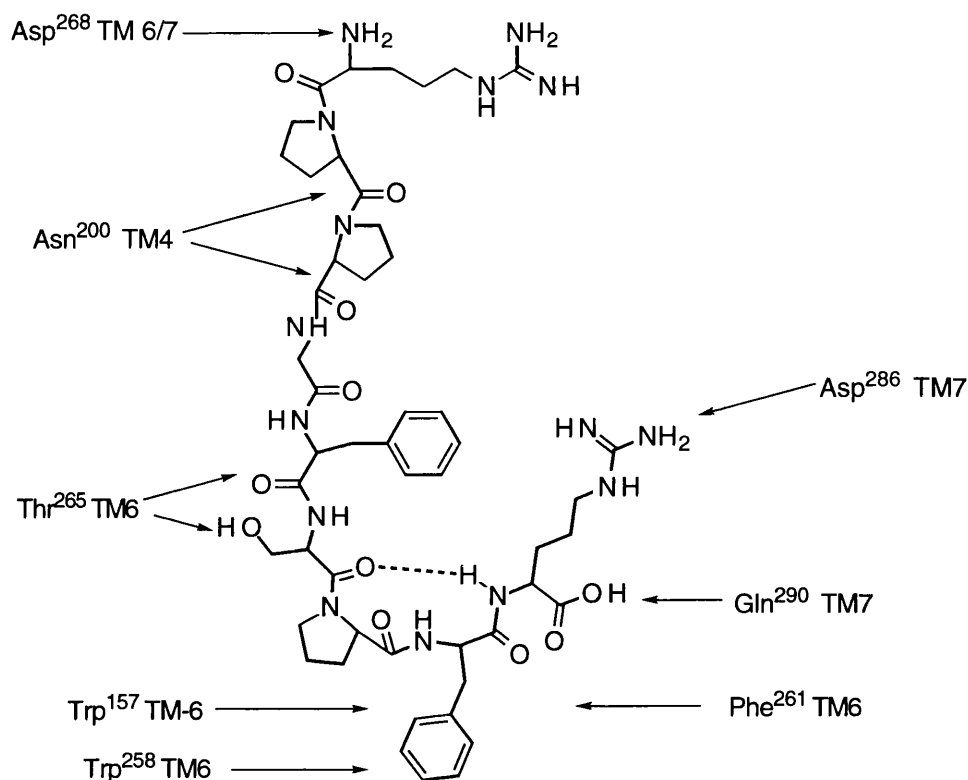
Despite the structural similarities between HOE140 and BK, the same mutations in the human BK B2 receptor reduced HOE140 affinity by much smaller amounts. The replacement of Phe[261] by Ala[261] led to a reduction of affinity by 2.2 fold. Similarly, the replacement of Thr[265] and Asp[286] by alanine resulted in the reduction of affinity by only 1.2 and 2.2 fold, respectively. A number of other alanine substitutions were found to affect the binding of HOE140 to a small extent, but these same mutations were not found to affect the binding to the receptor of BK. The authors concluded that these results suggest that the compounds bind to different regions of the receptor. As an explanation for the different results from the mutagenesis experiments, they suggested that HOE140 makes weaker but more numerous contact to the receptor than BK, which makes a fewer number of specific contacts.

As confirmation to these ideas, Jarnagin *et al.*[126] synthesised Ala[9]-BK and studied its conformation by 1H-nmr spectroscopy. They found that the C-terminal β-turn was still present but that the molecule's biological affinity was reduced by a factor of 27 000 compared to BK. They then synthesised Ala[9]-HOE140 and studied its conformation by 1H-nmr spectroscopy. The C-terminal β–turn was again still present, but this time the biological affinity of the molecule was only reduced by a factor of seven compared to the natural peptide. In a

further experiment, des-Arg[9]-BK was synthesised and found to be 150 000 times less potent at the BK B2 receptor than BK. However, des-Arg[9]-HOE140 was only 450 times less potent than HOE140.

A possible reason for the lack of biological activity in our compounds is that we are attempting to mimic the β–turn in HOE140 and this portion of the molecule may not be as crucially important for its biologically activity as we first presumed. For bradykinin, this is not the case, as this portion of the molecule is of critical importance for its biological activity. One could argue that a different overlay model over the C-terminal β–turn of BK, and not HOE140, may allow for the design and synthesis of a more active series of BZD BK analogues.

However, the work of Ripka *et al.* shows that the C-terminal β-turn in BK can be mimicked by the BZD scaffold used in our HOE140 overlay model (Figure 25, p 48). In their example, the β-turn present in the cyclic octapeptide **47** was mimicked by the BZD **207**. The C-terminal β-turn of BK contains the same amino acids, proline and phenylalanine, at the (*i* + 1) and (*i* + 2) corner of the β-turn. Using the BZD scaffold with the opposite stereochemistry at C-3 means that the β-turn of BK can be mimicked by the BZD scaffold **208** as shown in Figure 55.

Figure 55. Benzodiazepines as β-turn mimetics
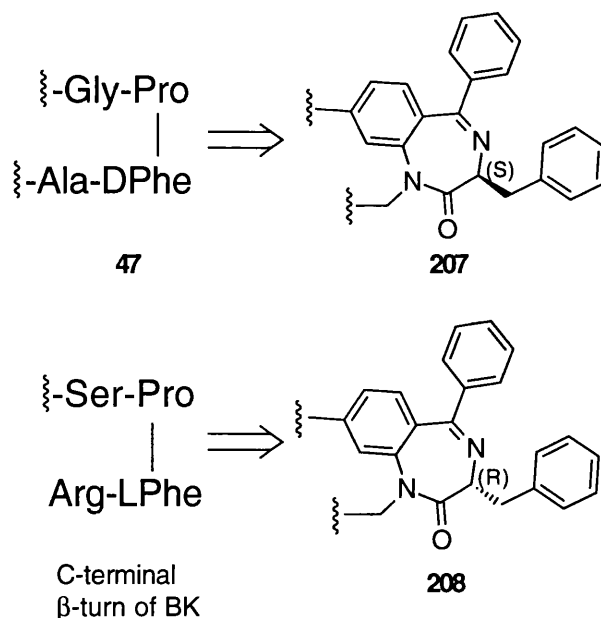
A more valid criticism, and one of which we were aware, is that the assumption we have made, namely that our non-peptidal antagonists bind to the same receptor site as the peptidal antagonists, is incorrect. Although our hypothesis

may not be true, a preliminary model was needed to begin a rational exploration of the factors contributing to the binding of a BZD-based BK non-peptidal antagonist.

Many pharmaceutical companies have spent a lot of time, effort and money in creating compound libraries by applying the developments in combinatorial chemistry. Progress in multi-throughput screening has enabled thousands of compounds to be assayed against enzymes or receptors within a relatively short time-frame. The efforts described here, in the search for non-peptidal BK B2 receptor antagonists, illustrates that rational drug design can be effective in the search for new lead compounds.

Table 3: Human Bradykinin B2 Receptor Affinities (K_i) of BZD Ligands.[a, b]
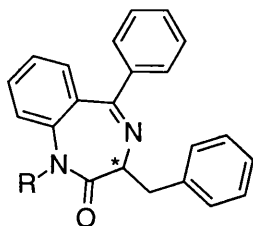
Compound	R	Stereochemistry (*)	K_i (μM)
51	H	R/S	> 100[b]
203	3-aminobenzyl	R/S	> 100[b]
188	CH_2CO_2H	R/S	>100[b]
61	4-guanidinophenyl	R/S	9.2 ± 5.4
64	3-guanidinobenzyl	R/S	8.9 ± 4.3
70	3-guanidinobenzyl	S	15.8 ± 7.6
67	3-guanidinobenzyl	R	11.9 ± 3.8
73	4-guanidinobenzyl	R/S	6.4 ± 1.5
79	4-ureidobutyl	R/S	>100[b]
85	4-guanidinobutyl	R/S	21.3 ± 4.0
82	3-aminopropyl	R/S	>100[b]
88	3-amidinobenzyl	R/S	14.5 ± 6.3
89	4-amidinobenzyl	R/S	13.5 ± 3.2

[a] [^{3}H]-BK binding assay. K_i values were determined from concentration-response curves at the human B2 BK receptor (membranes from Cos-7 cells expressing the human B2 receptor). The values shown are the mean ± s.e.m. (n ≥ 3).

[b] IC_{50} values

Table 4: Human Bradykinin B2 Receptor Affinities of BZD Ligands Where the K_i was not Determined.
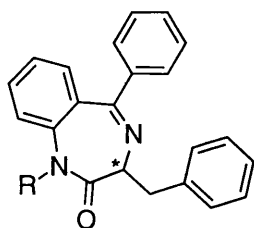
Compound	R	Stereochemistry (*)	% inhibition at 10 μM
83	4-aminobutyl	R/S	(26 ± 5)
74	2-guanidinobenzyl	R/S	(<10)
84	3-guanidinopropyl	R/S	(<10)

## Table 5: Human Bradykinin B2 Receptor Affinities: Variation in 1,4 Benzodiazepin-2-one Core Structure.[a,]
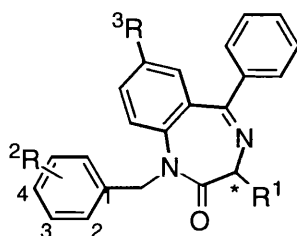
| Compd.	R^1	R^2	stereochemistry	R^3	Ki (μM)
175	H	4-guanidino	R/S	H	>100[b, c]
92	methyl	3-guanidino	R/S	H	21.9 ± 10.3
121	methyl	4-guanidino	R/S	H	0.95 ± 0.2
122	n-butyl	4-guanidino	R/S	H	15.7 ± 5.7[b]
123	i-butyl	4-guanidino	R/S	H	3.26 ± 0.54
124	phenethyl	4-guanidino	R/S	H	4.5 ± 0.3[b]
125	3-indolylmethyl	4-guanidino	R/S	H	10-100[b, c]
177	H	4-guanidino	R/S	Cl	23.8 ± 3.4[b]
163	methyl	4-guanidino	R/S	Cl	>100[c]
164	butyl	4-guanidino	R/S	Cl	6.9 ± 0.5[b]
162	benzyl	4-guanidino	R/S	Cl	3.4 ± 0.98
186	4-guanidinobenzyl	H	R/S	H	6.1 ± 1.8
181	$(CH_2)_3NHCO_2CH_2Ph$	4-guanidino	R/S	H	2.5 ± 0.31[b]
187	4-guanidinobenzyl	4-guanidino	R/S	H	10-100[b, c]

[a] [^{3}H]-BK binding assay. K_i values were determined from concentration-response curves at the human B2 BK receptor (membranes from Cos-7 cells expressing the human B2 receptor unless otherwise indicated). The values shown are the mean ± s.e.m. (n ≥ 3).

[b] Binding assay with membranes from CHO cells expressing the human B2 receptor

[c] IC_{50} value

Table 6: Human Bradykinin B2 Receptor Affinities: Variation in 1,4 Benzodiazepin-2-one Core Structure.[a, d]
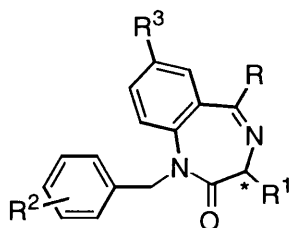
Compd.	R	R^1	R^2	R^3	K_i (μM)
95	methyl	benzyl	3-guanidino	H	>100[c]
128	2-naphthyl	benzyl	4-guanidino	H	2.42 ± 1.16
135	4-tolyl	methyl	4-guanidino	H	>100[b, c]
136	4-tolyl	butyl	4-guanidino	H	10-100[b, c]
142	4-MeO phenyl	butyl	4-guanidino	H	4.6 ± 0.7[b]
143	4-MeO phenyl	benzyl	4-guanidino	H	2.8 ± 0.5[b]
148	4-Cl phenyl	methyl	4-guanidino	H	9.6 ± 2.1[b]
149	4-Cl phenyl	benzyl	4-guanidino	H	>100[b, c]
152	2-Cl phenyl	methyl	4-guanidino	H	>100[b, c]
176	2-Cl phenyl	H	4-guanidino	Cl	11.1±0.92[b]
165	2-Cl phenyl	methyl	4-guanidino	Cl	10-100[c]

[a] [^{3}H]-BK binding assay. K_i values were determined from concentration-response curves at the human B2 BK receptor (membranes from Cos-7 cells expressing the human B2 receptor unless otherwise indicated). The values shown are the mean ± s.e.m. (n ≥ 3).

[b] Binding assay with membranes from CHO cells expressing the human B2 receptor

[c] IC_{50} value

[d] All compounds tested are racemic

Table 7. Human Bradykinin B2 Receptor Affinities Where the K_i was not Determined: Variation in 1,4 Benzodiazepin-2-one Core Structure.[a, b]
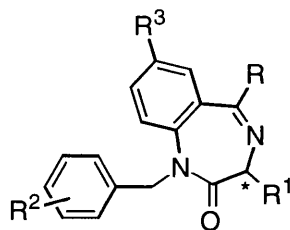
Compd.	R	R^1	R^2	R^3	% inhibition at 100 μM
137	4-tolyl	benzyl	4-guanidino	H	(49)

[a] [^{3}H]-BK binding assay (membranes from CHO cells expressing the human B2 receptor).

[b] All compounds are racemic

Table 8: Human Bradykinin B2 Receptor Affinities: Overlay Model II.[a, c]
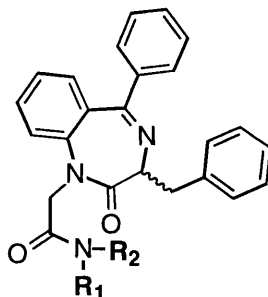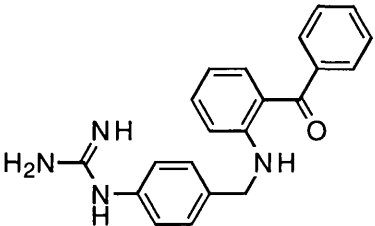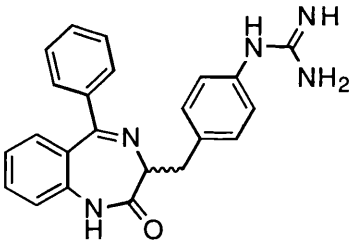
Compound	R_1	R_2	Ki (μM)
190	4-aminobutyl	H	11.4 ± 3.0
192	4-guanidinobutyl	H	15.6 ± 5.6
196	4-guanidinophenyl	H	5.4 ± 0.42[b]
197	$Me_2N(CH_2)_3-$	$Me_2N(CH_2)_3-$	29.1 ± 7.6

[a] [^{3}H]-BK binding assay. K_i values were determined from concentration-response curves at the human B2 BK receptor (membranes from Cos-7 cells expressing the human B2 receptor unless otherwise indicated). The values shown are the mean $\pm$ s.e.m. ($n \geq 3$).

[b] Binding assay with membranes from CHO cells expressing the human B2 receptor

[c] All compounds tested are racemic

Table 9: Human Bradykinin B2 Receptor Affinities of Miscellaneous Ligands[a, b]

Compound	K_i (µM)
200	16.3 ± 5.6
201	$> 100^b$

[a] [^{3}H]-BK binding assay. K_i values were determined from concentration-response curves at the human B2 BK receptor (membranes from Cos-7 cells expressing the human B2 receptor). The values shown are the mean $\pm$ s.e.m. ($n \geq 3$).

[b] IC_{50} value

3. EXPERIMENTAL

3.1 Instrumentation and Experimental Techniques

Microanalyses were carried out by the Microanalytical Section of the Chemistry Department, University College London and at Sandoz Pharma AG, Basel. The infra-red (I.R.) spectra were recorded on a Perkin-Elmer PE-983 spectrophotometer using potassium bromide (KBr) pellets (unless otherwise stated); absorptions are recorded in wavenumbers (v_{max} in cm^{-1}). Nuclear magnetic resonance (nmr) spectra were recorded using a Varian Gemini 200 (200 MHz), Bruker AC300 (300 MHz), Varian VX400 (400 MHz) and a Bruker 360 (360 MHz) spectrometer. ^{1}H nmr spectra are reported in parts per million (ppm, ∂), with residual protic solvent as the internal standard. Coupling constants (J) are given in hertz (Hz). The following abbreviations are used in signal assignments: s (singlet); d (doublet); t (triplet), q (quartet); m (multiplet). ^{13}C nmr spectra were recorded at 100 MHz on the Varian VXR-400 spectrometer. Mass spectra were recorded either on a VG7070H mass spectrometer with Finnigan Incos II data system at University College or on a VG ZAB SE mass spectrometer at Sandoz Pharma AG, Basel. Molecular ion peaks (M$^+$), base peaks and any other major peaks are reported. High resolution mass spectra (HRMS) were performed by Mr M. Cocksedge at the London School of Pharmacy using a VG ZAB SE mass spectrometer and fast atom bombardment (FAB) ionization. Commercially available Merck Kieselgel 60 F$_{254}$ plates were used for analytical thin layer chromatography (tlc). Reaction components were visualised with ultra-violet light (254 nm) or iodine vapour. Flash column chromatography was performed using Merck flash silica (200-400 mesh) as the stationary phase.

All experiments using moisture-sensitive reagents were carried out under an atmosphere of dry nitrogen. All solvents and reagents were obtained from commercial sources and used without further purification except where noted. Tetrahydrofuran (THF) was freshly distilled from sodium-benzophenone-ketyl under nitrogen prior to use. Glassware and syringes for moisture-sensitive reactions were dried in an oven at 130 °C. Temperatures below 0 °C were obtained by cooling acetone with solid carbon dioxide pellets.

High performance liquid chromatography (HPLC) was carried out using a Waters 600 system (μ-Bondapak C18 column). Preparative HPLC was performed on a Waters Delta Preparative 3000 system equipped with a

Dynamax 300A C18 12 μm particle size column (83-243), dimensions 41.4 x 250 mm. Solvents were HPLC grade and used without purification. The mobile phase was composed of a mixture of water, acetonitrile, and 0.1% trifluoroacetic acid (TFA). Yields of final products after HPLC purification are not optimised. The compounds obtained after HPLC purification are isolated as the salts and contain differing amounts of TFA and water. For clarity, the structures of these compounds are named and represented as the neutral species.

Energy minimised molecular modelling structures were obtained using the MM2 force field calculations described by Schnur *et al.*[126b].

3.2. General Synthetic Procedures

For experimental procedures of a general nature, a complete general description is given and subsequent details for actual examples include quantities of reagent, yield and characterisation details.

Method 1 :   Coupling of 2-aminobenzophenone derivatives to N-Boc amino acids[89]
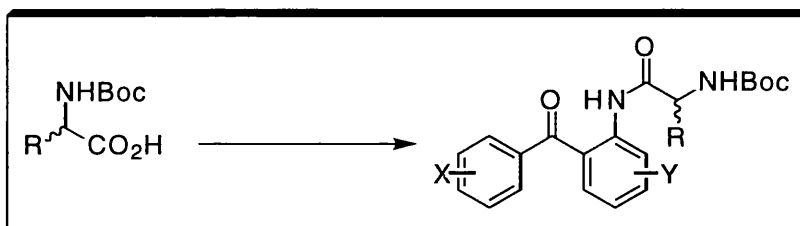
The racemic Boc-protected α–amino acid (12.17 mmol, 1.2 eq) was dissolved in THF (30 ml) and the solution cooled to -10 °C. The colourless solution was treated with triethylamine (1.26g, 12.48 mmol, 1.22 eq), followed by ethyl chloroformate (1.32g, 12.17 mmol, 1.2 eq). After 30 min the 2-aminobenzophenone derivative (10.14 mmol, 1 equiv) in THF (10 ml) was added dropwise. The vessel was allowed to warm to room temperature. After 24 h the reaction mixture was diluted with EtOAc (50 ml) and washed with water (50 ml). The aqueous phase was re-extracted with EtOAc (2 x 50 ml). The combined organic phases were washed with NaOH (0.1M; 2 x 50 ml) and then brine (1 x 50 ml). The organic phase was then dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The crude yellow syrup was used in Method 2 without purification.

Method 2:   Boc deprotection and benzodiazepinone formation[89]
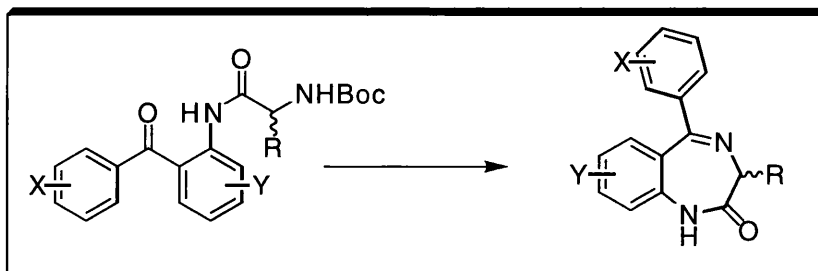
The crude syrup from Method 1 was dissolved in EtOAc (50 ml) and cooled to 0 °C. HCl (g) was admitted *via* a syringe for 20 min and after 24 h the solution

was concentrated *in vacuo* to afford a yellow oil. The oil was redissolved in MeOH (20 ml) and the pH was adjusted to 8. The cloudy suspension was allowed to stir for 24 h. The reaction mixture was concentrated *in vacuo* and then diluted with ethyl acetate (100 ml). The organic phase was washed with water (50 ml), brine (50 ml) and dried over $MgSO_4$. The organic phase was then filtered and concentrated *in vacuo*. The crude yellow gum was purified by flash column chromatography (60-80 pet spirit : EtOAc 4:1 to 1:1) to afford the product.

Method 3 : Alkylation of benzodiazepinone lactam nitrogen atom.
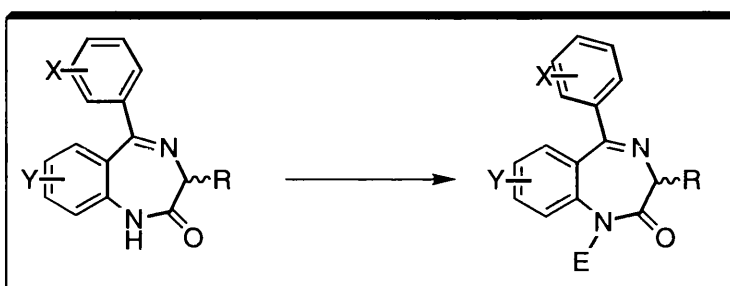
The BZD (3 mmol) was dissolved in THF (30 ml) and cooled to -10 °C. KOᵗBu in THF (1 M soln, 4.5 ml, 1.5 eq) was added and the vessel was allowed to stir at -10 °C for 30 min. The vessel was allowed to warm to room temperature before a solution of the electrophile (E-Br, 4.5 mmol) in THF (10 ml) was added dropwise. After 24 h the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (100 ml) and washed with HCl (70 ml, 1 M) and then brine (100 ml). The organic phase was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (EtOAc : hexane; 1:3).

Method 4   Reduction of nitro-containing benzodiazepinone derivatives[108]
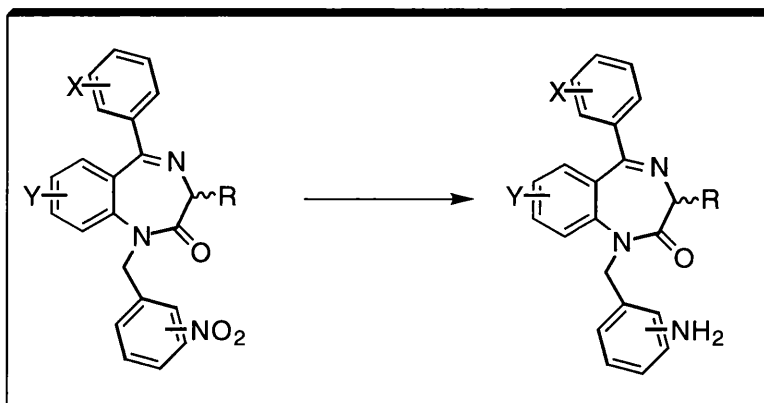
The nitro-group containing BZD (1.7 mmol; 1 equiv) was dissolved in EtOAc (50 ml). Tin (II) chloride dihydrate (1.9g; 8.6 mmol; 5 equiv) was added to the vessel which was then heated at 70 °C for 4 h. The reaction mixture was poured into ice (50 g) and diluted with EtOAc (200 ml). NaHCO$_3$ soln (satd) was added to the vessel until the pH was 9. The aqueous phase was then separated and extracted with more EtOAc (2 x 200 ml). The combined organic layers were washed with brine (300 ml), dried over MgSO$_4$, filtered and concentrated *in vacuo* to afford the aryl amine as a foam.

Method 5:   Guanylation of aryl amino benzodiazepinone derivatives[110].
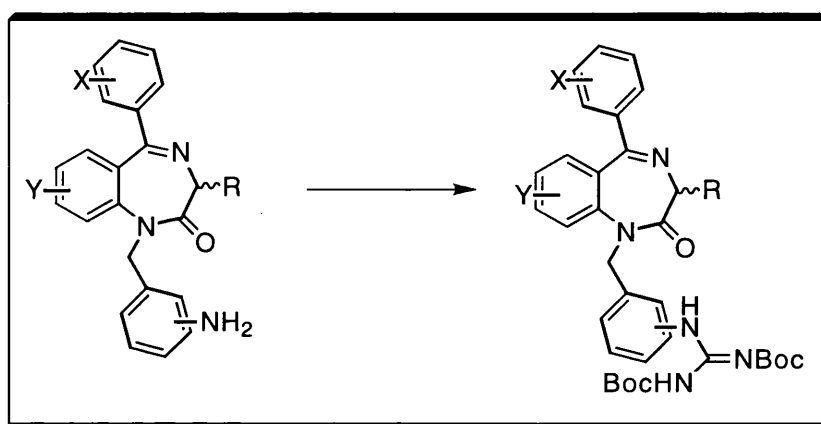
To a solution of the aryl amine (1.36 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (383 mg; 1.36 mmol) and triethylamine (445 mg; 4.4 mmol) in DMF (20 ml) at 0 °C was added mercury (II) chloride (370 mg, 1.36 mmol) with stirring. The resultant bright yellow solution was stirred at 0 °C for 20 min and allowed to warm to ambient temperature. After stirring at room temperature for 6 h, the reaction mixture was diluted with ethyl acetate (20 ml) and filtered through a pad of Celite. The filtrate was washed with water (75 ml) and brine (70 ml), dried over MgSO$_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with EtOAc : hexane (1 : 4) to give the bis-Boc protected guanidine as a foam.

**Method 6   Guanylation of aliphatic amino benzodiazepinone derivatives[110].**
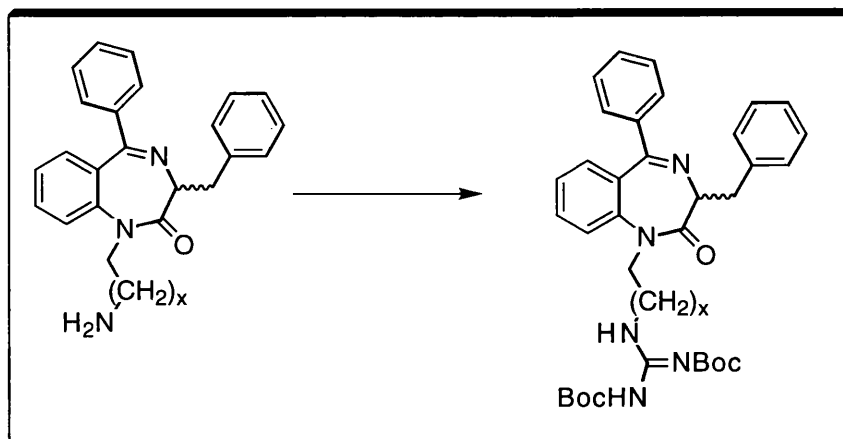
To a solution of the aliphatic amine (1.36 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (383 mg; 1.36 mmol) and triethylamine (445 mg; 4.4 mmol) in DMF (20 ml) at 0 °C was added mercury (II) chloride (370 mg, 1.36 mmol) with stirring. The resultant bright yellow solution was stirred at 0 °C for 20 min and allowed to warm to ambient temperature. After stirring at room temperature for 6 h, the reaction mixture was diluted with ethyl acetate (20 ml) and filtered through a pad of Celite. The filtrate was washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with EtOAc : hexane (1 : 4) to give the bis-Boc protected guanidine as a foam.

## Method 7   Deprotection of Boc protected guanidines
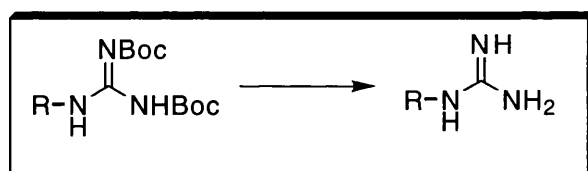
The Boc guanidine (0.96 mmol) was dissolved in dichloromethane (50 ml) and stirred at room temperature. Trifluoroacetic acid (4 ml) was added and the vessel was allowed to stir at room temperature for 48 h. The solution was concentrated *in vacuo* and the residue was purified by preparative HPLC. All pure product-containing fractions were evaporated *in vacuo* to afford the TFA salt after high vacuum drying.

## Method 8:   Synthesis of substituted 2-aminobenzophenone benzamides[82].
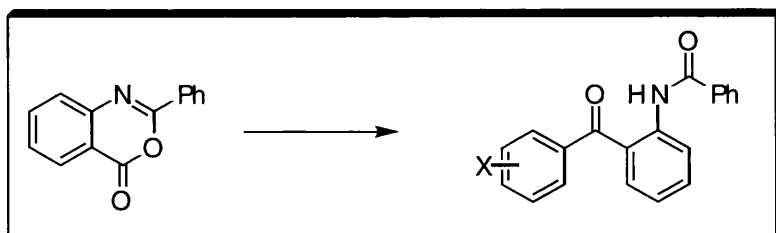
To a suspension of Mg turnings (680 mg; 28.3 mmol) in diethyl ether (200 ml) was added dropwise a solution of aryl bromide (28.3 mmol) in diethyl ether (50 ml). The magnesium was activated by grinding with a glass rod. After the Grignard reagent had formed the reaction mixture was heated to reflux for a further 2 h. The vessel was cooled to -40 °C before a solution of 2-phenyl-4H-3,1-benzoxazin-4-one[115] (2.8 g; 12.5 mmol) in diethyl ether (100 ml) was added dropwise. After stirring for 3 h, saturated NH_4Cl solution (20 ml) was added. After a further 15 min of stirring, Na_2SO_4 powder, (6 g) was added. The suspension was stirred for 10 min and then filtered. The collected solid was washed with diethyl ether, and the combined filtrate and washes were concentrated to dryness. The crude product was used in Method 9 without further purification.

Method 9: Saponification of benzamides of afford 2-amino benzophenone derivatives[82].
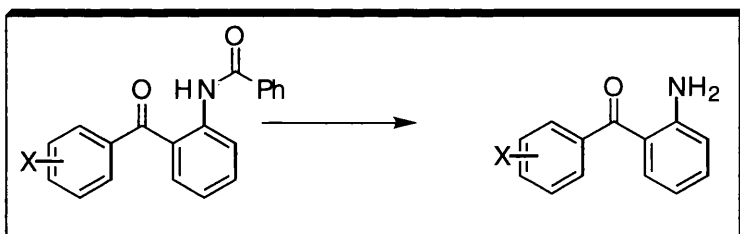

A mixture of the benzamide (1.52 mmol), 10 M NaOH (2 ml) and MeOH (30 ml) was heated at reflux overnight. Water (50 ml) was added and the mixture was slowly cooled to room temperature and stirred for 3 h. The reaction mixture was diluted with EtOAc (200 ml) and water (50 ml). The organic phase was separated, washed with brine (50 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude yellow gum was purified by flash column chromatography (60-80 pet spirit : EtOAc 7:1) to yield the desired benzophenone.

Method 10: Coupling of a carboxylic acid containing benzodiazepinone to an amine.
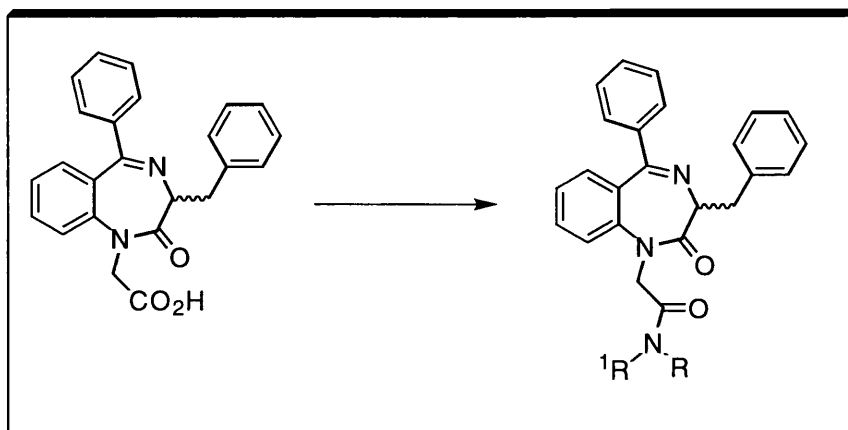
To a solution of the carboxylic acid (1 mmol) in DCM (30 ml) was added HOBT (176 mg; 1.3 mmol), DIPC (151 mg; 1.2 mmol) and the amine (2.5 mmol). After stirring for 24 h the reaction mixture was concentrated *in vacuo* and diluted with EtOAc (100 ml). The organic phase was washed with water (2 x 100 ml), HCl (1M; 100 ml), brine (100 ml) and dried over $MgSO_4$. The organic phase was filtered and the filtrate concentrated *in vacuo*. The isolated gum was purified by flash column chromatography (EtOAc : hexane 1:5) to afford the desired amide.

Method 11:   Alternative Benzodiazepinone synthesis route[116]
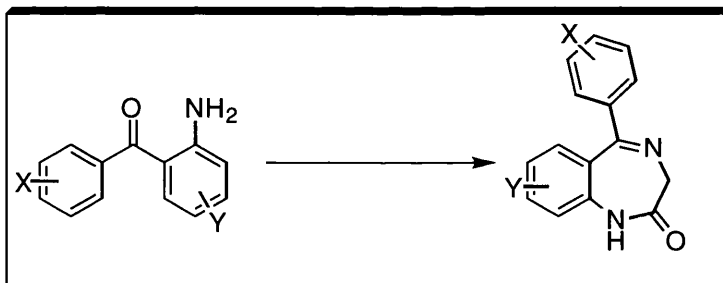
To a solution of the 2-aminobenzophenone (32.5 mmol) in DCM (200 ml) at -10 °C was added triethylamine (35.6 mmol) followed by careful addition of bromoacetyl bromide (35.6 mmol). After warming to room temperature the vessel was allowed to stir for 20 h. The cloudy suspension was then diluted with water (200 ml) and DCM (100 ml), the organic phase was separated and washed with water (100 ml), HCl (1M, 100 ml), brine (100 ml) and dried over $MgSO_4$. The organic phase was filtered and the filtrate was transferred to a large round-bottomed flask. After cooling the vessel to -10 °C, liquid ammonia (80 ml) was added portionwise over 20 min. After 48 h the reaction mixture was diluted with DCM (200 ml) and water (100 ml). The organic phase was separated and washed with water (100 ml), HCl (1M, 100 ml), brine (100 ml) and dried over $MgSO_4$.The organic phase was filtered and the filtrate

concentrated *in vacuo.* The isolated gum was purified by flash column chromatography (DCM : hexane 1:5) to afford the desired benzodiazepinone.

Method 12: Alkylation of the benzodiazepinone C-3 carbon atom
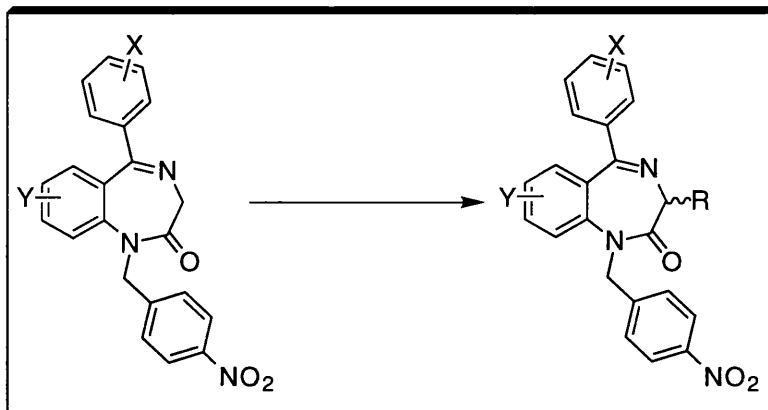

The BZD (1 mmol) was dissolved in THF (30 ml) and cooled to -10 °C. KO^{t}Bu in THF (1 M soln, 1.5 ml, 1.5 eq) was added and the vessel was allowed to warm to room temperature. After 30 min the dark red solution was cooled to -50 °C before a solution of the electrophile (R-Br, 1.5 mmol) in THF (10 ml) was added dropwise. After 24 h the reaction mixture was concentrated *in vacuo.* The residue was dissolved in EtOAc (100 ml) and washed with HCl (70 ml, 1 M) and then brine (100 ml). The organic phase was then dried over MgSO$_4$, filtered and concentrated *in vacuo.* The crude product was purified by flash column chromatography (EtOAc : Hexane; 1:3).

## X3 Benzodiazepinone Overlay Model (I)

3.3 Variation in Phenylalanine-derived BZD side chain

3-Benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (51)
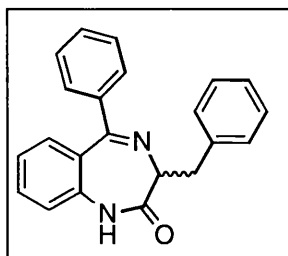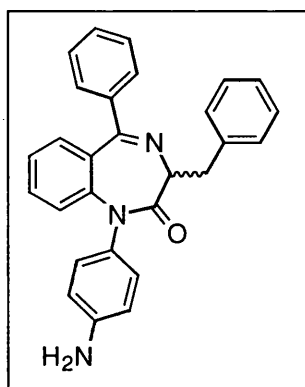

Compound **51** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (2.0 g; 10.14 mmol), ethyl chloroformate (1.32 g; 12.17 mmol), triethylamine (1.26 g, 12.47 mmol) and (±) N-Boc phenylalanine (3.2 g; 12.2 mmol).

yield	1.5 g, 4.6 mmol (45%), colourless crystals
Mp	197.5-199.5 °C (EtOAc-Hexane); lit 192.5-193°C[127]
^{1}H nmr (400 MHz, CDCl$_3$) ∂	3.59 (d, 2H, J = 8.0 Hz, CHCH_2Ph), 3.79 (t, 1H, J = 7.2 Hz, CHCH$_2$Ph), 7.1-7.6 (m, 14H, Ph), 8.83 (s, 1H, NH)
^{13}C-nmr. (CDCl$_3$) ∂	37.6, 64.9, 121.0, 123.3, 126.1, 127.5, 128.1, 129.7, 129.8, 129.9, 130.3, 131.7, 138.1, 139.2, 139.2, 139.3, 169.2, 171.6
I.R. (v_{max})	3432, 3060, 1682, 1608, 1322, 1301 cm^{-1}
MS(FAB) (m/z, %)	327 (MH$^+$, 100)
C$_{22}$H$_{18}$N$_2$O (326.40)	calc: C, 80.96; H, 5.56; N, 8.58. found: C,80.58; H, 5.60; N, 8.38.

## 3-Benzyl-1-(4-nitrophenyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (55)
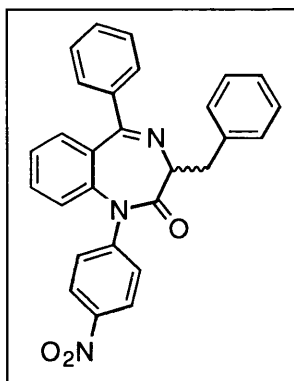
To a solution of **51** (975 mg; 3 mmol) in DMF (30 ml) was added copper powder (400 mg; 9.4 mmol), 4-iodonitrobenzene (400 mg;1.7 mmol) and sodium acetate (400 mg; 4.9 mmol). The mixture was heated for 4 h at 140 °C with stirring before being allowed to stir at room temperature. After 24 h the reaction mixture was concentrated *in vacuo*, diluted with EtOAc (50 ml) and filtered through a pad of Celite. The organic phase was washed with HCl (50 ml; 1M), water (50 ml) and then brine (50 ml). The organic phase was dried over MgSO$_4$, filtered and concentrated *in vacuo* to a brown glass. This was purified by flash column chromatography eluting with hexane : EtOAc (10 : 1) to afford the desired product.

yield:	400 mg; 0.89 mmol; 30% (brown glass)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.6 (d, 2H, J = 6 Hz, CHC*H$_2$*Ph), 3.9 (t, 1H, J = 6 Hz, C*H*CH$_2$Ph), 6.9-7.6 (m, 18H, Ph)
I.R. (v$_{max}$)	3343, 1680, 1595, 1521, 1345 cm^{-1}
MS(EI), (m/z %)	446 (MH$^+$ 100)

1-(4-Aminophenyl)-3-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (57)

Compound **57** was synthesised from **55** (400 mg, 0.89 mmol) and tin (II) chloride (675 mg, 3 mmol) using the procedure outlined in Method 4.

|	
yield:	350 mg, 0.84 mmol, 85 % (dark brown glass)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.5 (d, 2H, J = 6 Hz, CHCH_2Ph), 3.9 (t, 1H, CHCH$_2$Ph), 7.0-7.6 (m, 18H, Ph)
I.R. (v$_{max}$) film	3400, 1684, 1604, 1514, 784, 697 cm^{-1}
MS(FAB) (m/z %)	418 (MH$^+$ 100)

N-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (60)
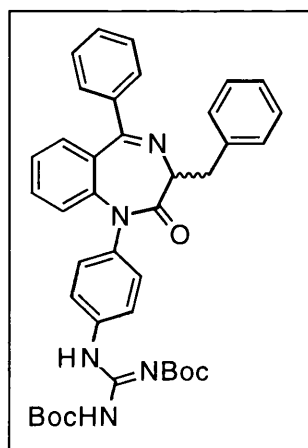
Compound **60** was synthesised from **57** (340 mg, 0.82 mmol), triethylamine (273 mg, 2.7 mmol), mercury (II) chloride (244 mg, 0.90 mmol), N,N' bis-*tert*-

butoxycarbonyl thiourea (248 mg, 0.90 mmol) using the procedure outlined in Method 5.

yield:	335 mg, 0.50 mmol, 62 % (yellow foam)
^{1}H-nmr (CDCl$_3$, 200 MHz) ∂	1.5 (s, 18H, (CH_3)$_3$C), 3.6 (d, 2H, CHCH_2Ph), 4.0 (t, 1H, CHCH$_2$Ph), 6.9-7.8 (m, 18H, Ph), 10.4 (s, 1H, NH), 11.6 (s, 1H, NH)
I.R. (v_{max}) film	1695, 1640, 1368, 1151 cm^{-1}
MS(FAB) (m/z %)	660 (MH$^+$, 22), 560 (MH$^+$-Boc, 10), 460 (MH$^+$-2Boc, 100)

N-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)phenyl] guanidine (61)
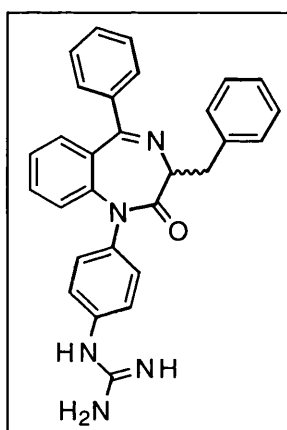

Compound **61** was synthesised from **60** (335 mg, 0.50 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	76.9 mg, 0.11 mmol, 21% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.4 (d, 2H, J = 6 Hz, CHCH_2Ph), 3.9 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 7.0-7.6 (m, 18H, Ph), 9.8 (s, 1H, NH)
I.R. (v_{max}) (film)	3339, 3166, 1685, 1600, 1203, 1140, 721 cm^{-1}
MS(FAB) (m/z, %)	460 (MH$^+$-CF$_3$CO$_2^-$, 100)

analysis: $C_{29}H_{25}N_5O.2.2TFA.H_2O$
effective molecular weight [728.41]

calc: C, 55.07; H, 4.04; N, 9.6;
O, 14.06; F, 17.2
found: C, 55.0; H, 4.0; N, 9.7;
O, 13.6; F, 17.6

(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) acetic acid ethyl ester (209)
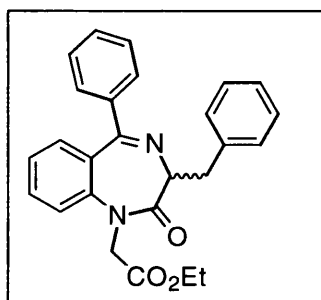
Compound **209** was prepared from **51** (1.00 g, 3.1 mmol), ethyl bromoacetate (512 mg, 3.1 mmol) and KO^{t}Bu in THF (1 M soln, 3.1 ml, 3.1 mmol) using the procedure outlined in Method 3.

|	
yield:	1.18g, 2.76 mmol, 89% (white foam)
^{1}H nmr (400 MHz, CDCl$_3$) ∂	1.3 (t, 3H, J = 5 Hz, C*H$_3$*CH$_2$), 3.6 (d, 2H, J = 8 Hz, CHC*H$_2$*Ph), 3.9 (t, 1H, J = 8 Hz, C*H*CH$_2$Ph), 4.1 (q, 2H, J = 5 Hz, CH$_3$C*H$_2$*O), 4.50 (d, 1H, J = 18 Hz, NC*H*HOOR), 4.65 (d, 1H, J = 18 Hz, NCH*H*OOR), 7.2-7.6 (m; 14H, Ph)
^{13}C-nmr (CDCl$_3$) ∂	14.0, 14.2, 21.1, 37.8, 50.6, 61.6, 121.3, 124.3, 126.1, 128.1, 128.3, 129.3, 129.5, 129.9, 130.3, 131.5, 138.8, 139.2, 142.4, 168.6, 168.7, 169.9
I.R. (v$_{max}$)	3500, 3250, 3200, 1750, 1700, 1600, 1550, 1200 cm^{-1}
MS(FAB) (m/z %)	428 (M$^+$, 100)

(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) acetic acid (188)
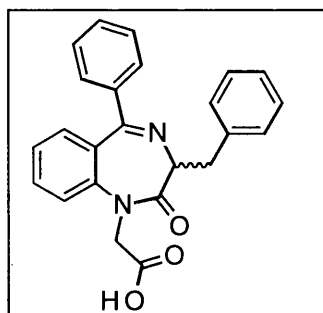
To a solution of **209** (454 mg, 1 mmol) in EtOH (10 ml) was added 10 ml of KOH solution (1 M). After 24 h the reaction was concentrated *in vacuo,* diluted with EtOAc (50 ml) and washed with HCl solution (2 M; 50 ml) then brine (50 ml). The organic phase was dried over $MgSO_4$, filtered and the filtrate concentrated *in vacuo* to give the acid **188** (428 mg, 100%, off-white foam). The crude product was then purified by preparative HPLC.

yield:	127.4 mg, 0.25 mmol, 25% (light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.4 (dd, 2H, CHCH_2Ph, J = 6 Hz and 9 Hz), 3.8 (dd, 1H, J = 6 Hz and 9 Hz, CHCH$_2$Ph), 4.5 (d, 1H, J^2 = 18 Hz, NCHHCOOH), 4.7 (d, 1H, J^2 = 18 Hz, NCHHCOOH), 7.1-7.6 (m, 14H, Ph)
^{13}C-nmr (CDCl$_3$) ∂	37.3, 38.8, 48.8, 64.2, 122.2, 124.5, 126.0, 128.0, 128.1, 129.2, 129.4, 129.6, 130.2, 131.6, 138.5, 138.9, 141.9, 168.0, 168.8, 170.6
I.R. (v_{max})	3600, 1780, 1750, 1600, 1210, 1100 cm^{-1}
MS(FAB) (m/z %)	384 (M$^+$-CF$_3$CO$_2$$^-$, 100)
analysis: C$_{24}$H$_{20}$N$_2$O$_3$.TFA.0.4H$_2$O effective molecular weight [505.65]	calc: C, 61.7; H, 4.3; N, 5.5; O, 17; F, 11.3 found: C, 61.5; H, 4.3; N, 5.8; O, 16.5; F, 11.4

## 3-Benzyl-1-(3-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (62)
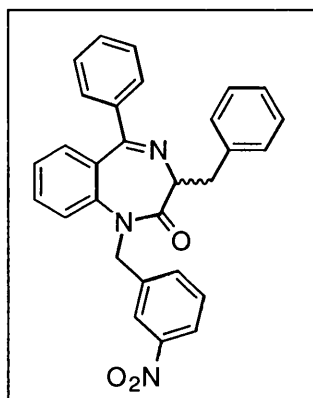
Compound **62** was synthesised from **51** (650 mg, 2 mmol), 3-nitrobenzyl bromide (532 mg, 2 mmol) and KO^{t}Bu in THF (1 M soln, 2 ml, 2 mmol) using the procedure outlined in Method 3.

yield:	400 mg, 0.86 mmol, 43% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	3.6 (d, 2H, J = 6 Hz, CHCH_2Ph), 3.8 (d, 1H, J = 6 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 6.9-7.6 (m, 18H, Ph)
I.R. (v_{max})	3026, 1650, 1603, 1521, 1344 cm^{-1}
MS(FAB) (m/z %)	462 (MH$^+$, 100)

1-(3-Aminobenzyl)-3-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (203)
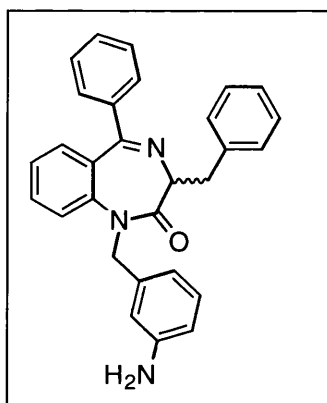
Compound **203** was synthesised from **62** (400 mg, 0.86 mmol) and tin (II) chloride (825 mg, 3 mmol) using the procedure outlined in Method 4.
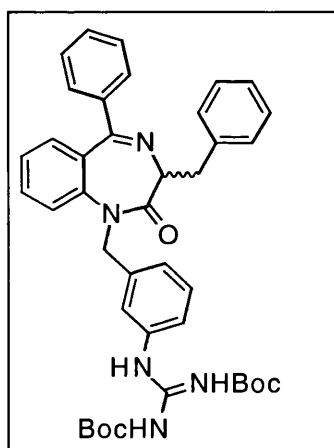
yield:	301 mg, 0.7 mmol, 81%, (orange foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.6 (d, 2H, J = 5 Hz, CHCH_2Ph), 3.9 (d, 2H, J = 5 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.4 (d, 1H, J = 18 Hz, NCHHPh), 7.0-7.6 (m, 18H, Ph)
I.R. (ν$_{max}$)	3450, 3400, 1674, 1603, 1447, 698 cm^{-1}
MS(FAB) (m/z %)	432 (MH$^+$, 100), 327 (15)

N-[3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonylguanidine (63)


Compound **63** was synthesised from **203** (588 mg, 1.36 mmol), mercury (II) chloride (374 mg, 1.38 mmol), triethylamine (445 mg, 4.45 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (383 mg, 1.38 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	650 mg, 0.96 mmol, 70% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 18H, (CH_3)$_3$C), 3.4 (m, 2H, CHCH_2Ph), 3.7 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.4 (d, 1H, J = 18 Hz, NCHHPh), 6.9-7.6 (m, 20H, Ph, NH)

I.R. (v_{max})	3320, 3010, 2976, 1720, 1682, 1640, 1608 cm^{-1}
MS(FAB) (m/z %)	674 (MH$^+$, 30), 574 (MH$^+$-Boc, 5), 474 (MH$^+$-2 Boc, 100)

N-[3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (64)
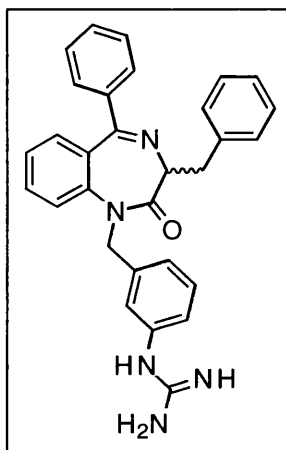
Compound **64** was synthesised from **63** (650 mg, 0.96 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

| yield:	141.7 mg, 0.21 mmol, 22% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.40 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz), 3.85 (dd, 1H, J = 6 Hz and 9 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NC*H*HPh), 5.5 (d, 1H, J = 18 Hz, NC*H*HPh), 6.8-7.7 (m, 21H, Ph and NH), 9.65 (s, 1H, NH)
I.R. (v_{max})	3357, 3164, 1678, 1600, 1204 cm^{-1}
MS(FAB) (m/z %)	474 (MH$^+$-CF$_3$CO$_2^-$, 100), 457 (M$^+$-NH$_2$, 25), 327 (10)
analysis: C$_{30}$H$_{27}$N$_5$O.1.7TFA.H$_2$O effective molecular weight [685.42]	calc: C, 58.5; H, 4.5; N, 10.2; O, 12.6; F, 14.1 found: C, 58.3; H, 4.4; N, 10.3; O, 12.1; F, 14.2

3-(R)-Benzyl-1-(3-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (210)
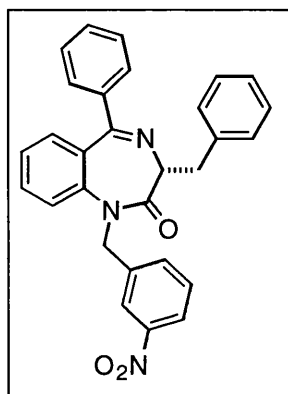
Compound **210** was synthesised from 3-(R)-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (326 mmol, 1 mmol), 3-nitrobenzyl bromide (216 mg, 1 mmol) and KO^{t}Bu in THF (1 M soln, 1 ml, 1 mmol) using the procedure outlined in Method 3. (278 mg, 0.60 mmol, 60%). The spectral data obtained for **210** were identical to those obtained for **62**.

1-(3-Aminobenzyl)-3-(R)-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (66)
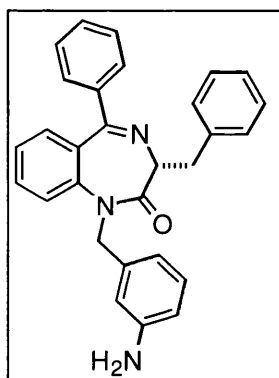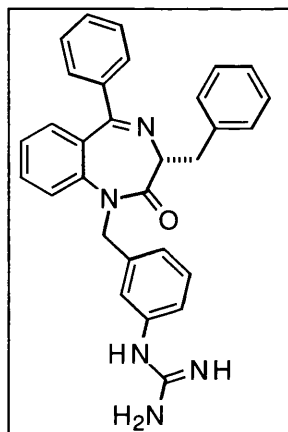

Compound **66** was synthesised from **210** (278 mg, 0.65 mmol) and tin (II) chloride (1.13 g, 5 mmol) using the procedure outlined in Method 4. (233 mg, 0.54 mmol, 90%). The spectral data obtained for **66** were identical to those obtained for **203**.

N-[3-(3-(R)-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (211)
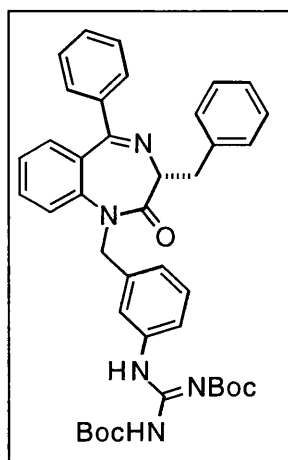
Compound **211** was synthesised from **66** (233 mg, 0.54 mmol), mercury (II) chloride (163 mg, 0.6 mmol), triethylamine (182 mg, 1.8 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (166 mg, 0.6 mmol) and DMF (20 ml) using the procedure outlined in Method 5 (275 mg, 0.41 mmol, 76%). The spectral data obtained for **211** were identical to those obtained for **63**.

N-[3-(3-(R)-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (67)
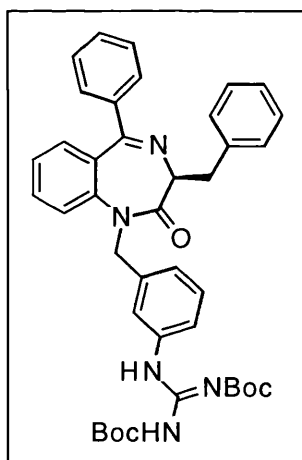

Compound **67** was synthesised from **211** (275 mg, 0.41 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield: 227 mg, 0.33 mmol, 80% (yellow foam)

136

^{1}H nmr (360 MHz, DMSO) ∂	3.45 (dd, 2H, J = 6 Hz and 10 Hz, CHCH_2Ph), 3.9 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 6.9-7.6 (m, 21H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (ν_{max})	3306, 3034, 1676, 1601, 1203 cm^{-1}
$[\alpha]_D^{20}$	+8.9 ° (c = 1.007, methanol)
MS(FAB) (m/z %)	474 (MH$^+$-CF$_3$CO$_2^-$, 100), 457 (MH$^+$-NH$_2$, 22)
analysis: C$_{30}$H$_{27}$N$_5$O.2.8TFA.0.6H$_2$O effective molecular weight [803.64]	calc: C, 53.2; H, 3.88; N, 8.71; O, 14.3; F, 19.8 found: C, 52.7; H, 3.7; N, 8.7; O, 14.0; F, 19.1

N-[3-(3-(S)-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (212)


Compound **212** was synthesised from 1-(3-aminobenzyl)-3-(S)-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (250 mg, 0.58 mmol), mercury (II) chloride (175 mg, 0.65 mmol), triethylamine (192 mg, 1.9 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (163 mg, 0.59 mmol) and DMF (20 ml) using the procedure outlined in Method 5 (300 mg, 0.45 mmol, 77%). The spectral data obtained for **212** were identical to those obtained for **63**

N-[3-(3-(S)-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (70)
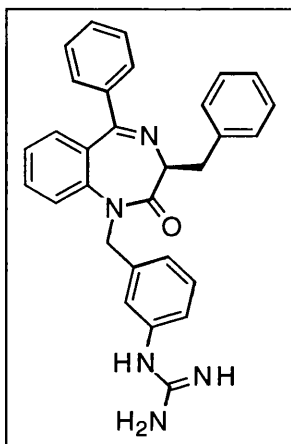
Compound **70** was synthesised from **212** (300 mg, 0.45 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	153.7 mg, 0.19 mmol, 33% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.45 (dd, 2H, J = 6 Hz and 10 Hz, CHCH_2Ph), 3.9 (t, 1 H, J = 6 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 6.9-7.6 (m, 19H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (ν_{max})	3158, 1685, 1600, 1203 cm^{-1}
$[\alpha]_D^{20}$	-8.7 ° (c = 1.01, methanol)
MS(FAB) (m/z %)	474 (MH$^+$-CF$_3$CO$_2^-$, 100), 457 (MH$^+$-NH$_2$, 70)
analysis: C$_{30}$H$_{27}$N$_5$O.2.8TFA.H$_2$O effective molecular weight [810.85]	calc: C, 52.72; H, 3.95; F, 19.68; N, 8.64; O, 14.99 found: C, 52.31; H, 3.80; F, 19.9; N, 8.55; O, 14.8

## 3-Benzyl-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (71)
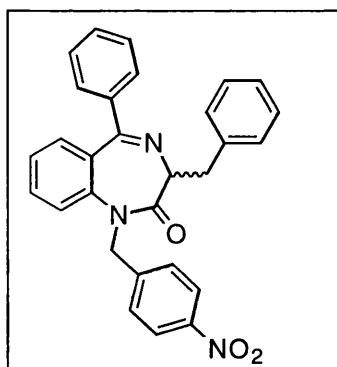
Compound **71** was synthesised from **51** (975 mg, 3 mmol), 4-nitrobenzyl bromide (648 mg, 3 mmol) and KO^{t}Bu in THF (1 M soln, 3.3 ml, 3.3 mmol) using the procedure outlined in Method 3.

|	
yield:	1.30 g, 2.82 mmol, 94% (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.6 (d, 2H, J = 5 Hz, CHC*H*$_2$Ph), 3.9 (t, 1H, J = 5 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NC*H*HPh), 5.5 (d, 1H, J = 18 Hz, NC*H*HPh), 7.0-7.6 (m, 18H, Ph)
I.R. (ν_{max})	1678, 1607, 1520, 1340 cm^{-1}
MS(FAB) (m/z %)	462 (MH$^+$, 100)

1-(4-Aminobenzyl)-3-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (213)
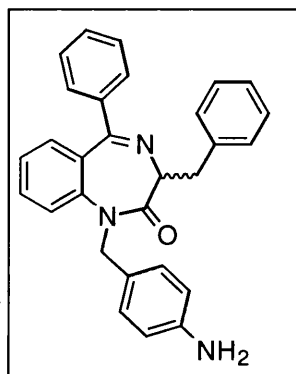
Compound **213** was synthesised from **71** (1.3 g, 2.82 mmol) and tin (II) chloride (3.3 g, 15 mmol) using the procedure outlined in Method 4.

|	
yield:	970 mg, 2.25 mmol, 79% (light brown foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂=	3.6 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 4.6 (d, 1H, J = 15 Hz, NCHHPh), 5.6 (d, 1H, J = 15 Hz, NCHHPh), 6.8 (d, 2H, J = 8 Hz), 7.0-7.4 (m, 16H, Ph)
I.R. (ν_{max})	3320, 1672, 1604, 1518 cm^{-1}
MS(FAB) (m/z %)	432 (MH$^+$, 100), 327 (15)

N-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (214)
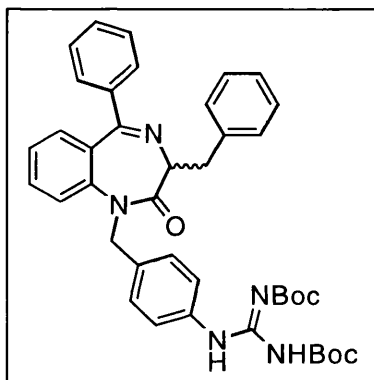
Compound **214** was synthesised from **213** (970 mg, 2.3 mmol), mercury (II) chloride (670 mg, 2.5 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (670 mg, 2.4 mmol), triethylamine (707 mg, 7 mmol) and DMF (25 ml) using the procedure outlined in Method 5.

|	
yield:	780 mg, 1.16 mmol, 50% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.5 (s, 18H, (CH_3)$_3$C), 3.6 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 4.9 (d, 1H, J = 15 Hz, NCHHPh), 5.5 (d, 1H, J = 15 Hz, NCHHPh), 7.1-7.4 (m, 18H, Ph)
I.R. (ν_{max})	3266, 2925, 1721, 1679, 1635 cm^{-1}
MS(FAB) (m/z %)	674 (MH$^+$, 20), 474 (MH$^+$-2 Boc, 100)

140

N-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl)phenyl] guanidine (73)
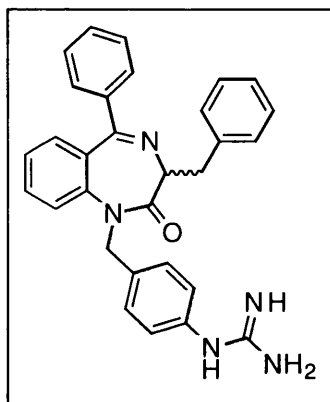
Compound **73** was synthesised from **214** (700 mg, 1.04 mmol) and trifluoroacetic acid (2 ml) using the procedures outlined in Method 7.

|	
yield:	165.9 mg, 0.22 mmol, 21% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.5 (m, 2H, CHCH_2Ph), 3.9 (t, 1 H, J = 6 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 6 Hz, NCHHPh), 5.5 (d, 1H, J = 6 Hz, NCHHPh), 6.9 (m, 4H), 7.1-7.6 (m, 18H, Ph)
I.R. (v_{max})	3335, 3141, 3105, 1676, 1602, 697 cm^{-1}
MS(EI) (m/z %)	473 (M$^+$-CF$_3$CO$_2^-$, 30), 431 (MH$^+$-CH$_3$N$_2$, 10), 106 (CH$_2$PhNH$_2$, 100)
analysis: C$_{30}$H$_{27}$N$_5$O.2.5TFA.0.5H$_2$O effective molecular weight [767.63]	calc: C, 54.75; H, 4.00; F, 18.56; N, 9.12; O, 13.5 found: C, 54.41; H, 4.12; F, 18.7; N, 8.98; O, 13.6

5-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) pentanoic acid ethyl ester (75)
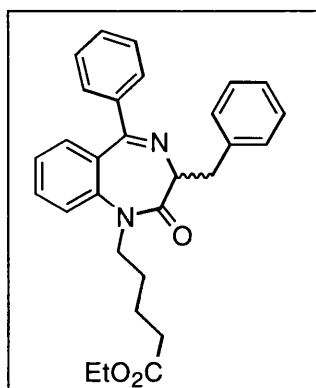
Compound **75** was synthesised from **51** (975 mg, 3 mmol), ethyl 5-bromovalerate (940 mg, 4.5 mmol) and KO^tBu in THF (1 M soln, 4.5 ml, 4.5 mmol) using the procedure outlined in Method 3.

|	
yield:	640 mg, 1.5 mmol, 50% (white foam)
^1H nmr (400 MHz, $CDCl_3$) ∂	1.15 (t, 3H, J = 8 Hz, CH_3CH_2), 1.4-1.6 (m, 4H, RCH_2CH_2R), 2.15 (m, 2H, $RCH_2CH_2CO_2R$), 3.6 (m, 3H, $CHCH_2Ph$ and $NCHHCH_2R$), 3.76 (t, 1H, J = 7 Hz, $CHCHPh$), 4.0 (q, 2H, J = 8 Hz, CH_3CH_2O), 4.4 (m, 1H, $NCHHCH_2R$), 7.1-7.5 (m, 14H, Ph)
^{13}C nmr ($CDCl_3$) ∂	14.3, 21.8, 27.3, 33.6, 38.0, 46.7, 60.2, 65.2, 122.2, 124.2, 126.0, 127.5, 128.1, 128.2, 129.5, 129.8, 130.3, 131.3, 138.7, 139.3, 139.4, 142.1, 168.3, 169.6, 173.1
I.R. (ν_{max})	3100, 1750, 1680, 1600, 1450, 1280 cm^{-1}
MS(FAB) (m/z %)	455 (MH+, 100)

5-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) pentanoic acid (76)
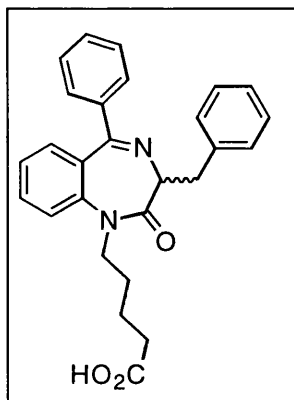
To a solution of **75** (454 mg, 1 mmol) in EtOH was added KOH solution (10 ml, 1 M). After 24 h the reaction mixture was concentrated *in vacuo*, diluted with EtOAc (50 ml), washed with HCl solution (50 ml, 2 M) and then brine (50 ml). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* giving the product.

|	
yield:	428 mg, 1 mmol, 100%, (yellow foam)
¹H nmr (400 MHz, CDCl₃,) ∂	1.3-1.6 (m, 4H, RC*H₂*C*H₂*R), 2.2 (t, 2 H, J = 6 Hz, RCH₂C*H₂*CO₂H), 3.6 (d, 2H, J = 8 Hz, CHC*H₂*Ph), 3.65 (m, 1H, NCH*H*CH₂R), 3.8 (t, 1H, J = 8 Hz, C*H*CH₂Ph), 4.4 (m, 1H, NC*H*HCH₂R), 7.2-7.6 (m, 14H, Ph)
I.R. (νmax)	3600-3200, 1750, 1700, 1650, 1600, 1100 cm⁻¹
MS(FAB) (m/z %)	427 (MH⁺, 100)

143

[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) butyl] carbamic acid benzyl ester (77)
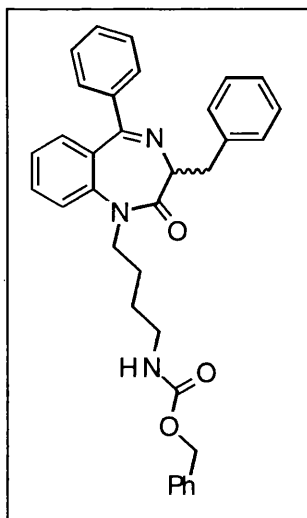
A mixture of **76** (0.23 g, 0.53 mmol), diphenyl phosphoryl azide (145 mg, 0.53 mmol) and triethylamine (53 mg, 0.53 mmol) in benzene was stirred at reflux for 2 h. Benzyl alcohol (0.5 ml) was added to the mixture, which was then refluxed for 18 h. The mixture was concentrated *in vacuo* and diluted with ethyl acetate (50 ml). The solution was then successively washed with 5% citric acid solution (30 ml), water (30 ml), satd NaHCO$_3$ (30 ml) and satd NaCl solution (30 ml). The organic phase was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude product (500 mg) was then purified by flash column chromatography (EtOAc : hexane 5:1) to give the desired product.

|	
yield:	200 mg; 0.38 mmol; 71%, (light brown foam)
^{1}H nmr (400 MHz, CDCl$_3$) ∂	1.2-1.6 (m, 4H, RCH_2CH_2R), 3.0 (m, 2H, RCH$_2$CH_2NR), 3.6 (d, 2H, J = 6 Hz, CHCH_2Ph), 3.7 (m, 1H, NCHHCH$_2$R), 3.8 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 4.4 (m; 1H, NCHHCH$_2$R), 5.0 (m, 2H, OCH_2Ph), 7.1-7.6 (m; 19H, Ph)
I.R. (v$_{max}$)	3400, 1720, 1675, 1604, 1524, 1496, 1242, 749, 697, 676 cm^{-1}
MS(FAB) (m/z %)	532 (MH$^+$, 100)

[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) butyl] urea (79)
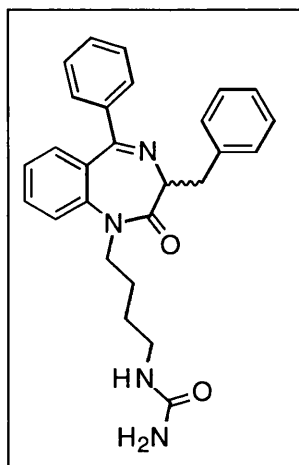
To a solution of **77** (660 mg; 1.25 mmol) in methanol (40 ml) was added ammonium formate (315 mg; 5 mmol) followed by palladium on charcoal (10%; 100 mg). After 8 h the catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc (50 ml) and the solution was then successively washed with water (50 ml) then brine (50 ml). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was then purified by preparative HPLC.

yield:	125.9 mg, 0.19 mmol, 15% (pale yellow foam)
^1H nmr (DMSO, 360 MHz) ∂	1.1-1.4 (m, 4H, RCH_2CH_2R), 2.7 (t, 2H, J = 6 Hz, RCH_2CH_2NHR), 3.4 (m, 2H, $CHCH_2Ph$), 3.7 (t, 2H, J = 8 Hz, $CHCH_2Ph$), 4.3 (m, 1H, $NCHHCH_2R$), 7.1-7.3 (m, 7H, Ph), 7.4-7.5 (m, 5H, Ph), 7.6-7.7 (m, 4H, Ph and NH)
I.R. (ν_{max})	3379, 1697, 1682, 1625, 1061, 785, 720, 698 cm^{-1}
MS(FAB) (m/z %)	441 (MH^+-$CF_3CO_2^-$, 100), 424 (M^+-NH_2, 50)
analysis: $C_{27}H_{28}N_4O_2$.1.8TFA.0.5H_2O effective molecular weight [654.78]	calc: C, 56.1; H, 4.74; F, 15.67; N, 8.56; O, 14.9 found: C, 55.86; H, 4.66; F, 15.9; N, 8.44; O, 14.9

2-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) butyl] isoindole-1,3-dione (81)
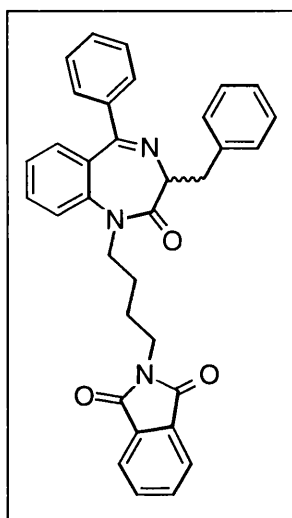
Compound **81** was synthesised from **51** (975 mg, 3 mmol), N-(4-bromobutyl) phthalimide (1.270 g, 4.5 mmol) and KO^{t}Bu in THF (1 M soln, 4.5 ml, 4.5 mmol) using the procedure outlined in Method 3. The crude product isolated (650 mg) was used in the next step without further purification.

## 1-(4-Aminobutyl)-3-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (83)
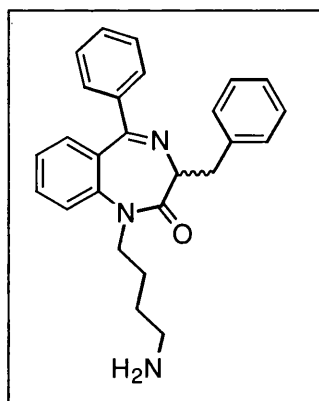
To a solution of **81** (650 mg) in DCM (30 ml) was added hydrazine hydrate (82 mg, 1.6 mmol). After 24 h the reaction mixture was diluted with more DCM (200 ml) and washed with water (100 ml) and then brine (100 ml). The organic phase was then separated, dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude product isolated was then purified by preparative HPLC.

yield: 314.5 mg, 0.45 mmol, 15% (over two steps; white foam)

146

^{1}H nmr (360 MHz, DMSO) ∂	1.3-1.5 (m, 4H, RCH_2CH_2R), 2.7 (m, 2H, RCH$_2$CH_2NH$_2$), 3.40 (dd, 1H, J = 6 Hz and 12 Hz, CHCHHPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHCHHPh), 3.7 (m, 2H, CHCH$_2$Ph and NCHHCH$_2$R), 4.3 (m, 1H, NCHHCH$_2$R), 7.1-7.7 (m, 17H, Ph and NH)
I.R. (ν_{max})	2944, 1696, 1601, 1203 cm^{-1}
MS(FAB) (m/z %)	398 (MH$^+$-CF$_3$CO$_2^-$, 100)
analysis: C$_{26}$H$_{27}$N$_3$O.2.4TFA.1.5H$_2$O effective molecular weight [698.19]	calc: C, 52.98; H, 4.67; F, 19.59; N, 6.01; O, 16.7
	found: C, 52.60; H, 4.26; F, 19.3; N, 5.90; O, 15.48

N-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) butyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (215)
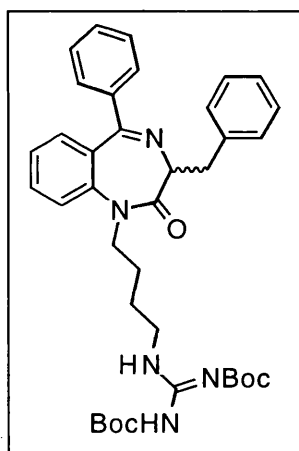

Compound **215** was synthesised from **83** (269 mg, 0.68 mmol), mercury (II) chloride (180 mg, 0.66 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (185 mg, 0.67 mmol), triethylamine (200 mg, 1.98 mmol) and DMF (20 ml) using the procedure outlined in Method 6.

yield:	340 mg, 0.53 mmol, 78% (cream foam)

^{1}H nmr (400 MHz, CDCl$_3$) ∂	1.2 (m, 4H, RCH_2CH_2R), 1.5 (s, 18H, (CH_3)$_3$C), 3.3 (m, 2H, RCH$_2$CH_2NHR), 3.5 (m, 1H, NCHHCH$_2$R), 3.6 (dd, 2H, J = 6 Hz and 12 Hz, CHCH_2Ph), 3.7 (dd, 1H, J = 6 Hz and 8 Hz, CHCH$_2$Ph), 4.4 (m, 1H, NCHHCH$_2$R), 7.1-7.6 (m; 14H, Ph), 8.2(m, 1H, NH)
I.R. (v$_{max}$)	3400, 2927, 1722, 1681, 1639, 1639, 1617, 1577, 1448, 1417, 1368, 1328, 1134 cm^{-1}
MS(FAB) (m/z %)	640 (MH$^+$, 32), 440 (MH$^+$-2 Boc, 100)

## N-[4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) butyl] guanidine (85)
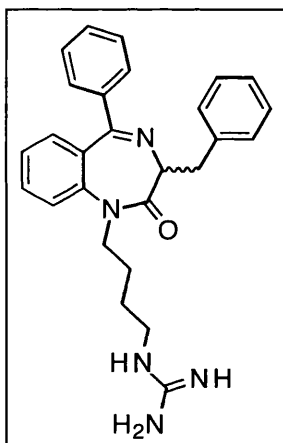
Compound **85** was synthesised from **215** (340 mg, 0.53 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

|	
yield:	90.9 mg, 0.16 mmol, 30% (white foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.3-1.5 (m, 4H, RCH_2CH_2R), 3.0 (t, 2H, J = 6 Hz, RCH$_2$CH_2NHR), 3.40 (dd, 1H, J = 6 Hz and 12 Hz, CHCHHPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHCHHPh), 3.8 (m, 2H, CHCH$_2$Ph and NCHHCH$_2$R), 4.8 (m, 1H, NCHHCH$_2$R), 7.1-7.6 (m, 14H, Ph)

I.R. (v_{max})	3380, 3181, 1675, 1603, 1203 cm^{-1}
MS(FAB) (m/z %)	440 (MH$^+$-CF$_3$CO$_2^-$, 100), 423 (M$^+$-NH$_2$, 20)
analysis: C$_{27}$H$_{29}$N$_5$O.TFA.0.9H$_2$O effective molecular weight [569.78]	calc: C, 61.13; H, 5.62; F, 10.0; N, 12.29; O, 10.95
	found: C, 61.06; H, 5.48; F, 9.6; N, 11.84; O, 10.42

3-Benzyl-1-(2-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (72)
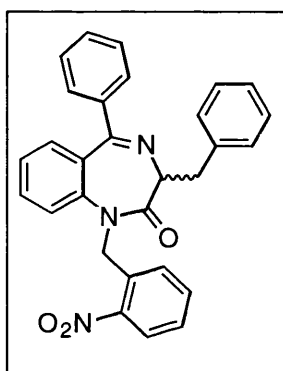
Compound **72** was synthesised from **51** (654 mg, 2 mmol), 2-nitrobenzyl bromide (648 mg, 3 mmol) and KO^{t}Bu in THF (1 M soln, 3 ml, 3 mmol) using the procedure outlined in Method 3.

yield:	897 mg, 1.95 mmol, 97% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.65 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 4.9 (d, 1H, J = 15 Hz, NCHHPh), 5.7 (d, 1H, J = 15 Hz, NCHHPh), 7.1-7.5 (m, 18H, Ph)
I.R. (v_{max})	3059, 1681, 1602, 1520, 1344 cm^{-1}
MS(FAB) (m/z %)	462 (MH$^+$, 100), 327 (35)

1-(2-Aminobenzyl)-3-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (216)
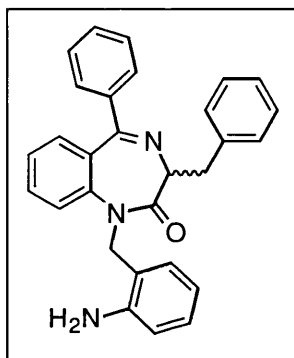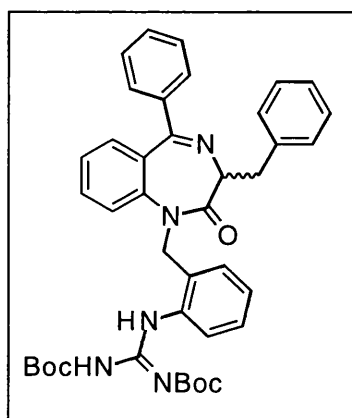

Compound **216** was synthesised from **72** (640 mg, 1.38 mmol) and tin (II) chloride (937 mg, 4.16 mmol) using the procedure outlined in Method 4.

|	
yield:	378 mg, 0.88 mmol, 64% (orange foam)
¹H nmr (200 MHz, CDCl₃) ∂	3.65 (m, 2H, CHCH₂Ph), 3.85 (t, 1H, J = 6 Hz, CHCH₂Ph), 4.6 (d, 1H, J = 16 Hz, NCHHPh), 5.6 (d, 1H, J = 16 Hz, NCHHPh), 7.0-7.5 (m, 18H, Ph)
I.R. (νmax)	3320, 1672, 1604, 1518 cm⁻¹
MS(FAB) (m/z %)	432 (MH⁺, 100)

N-[2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (217)

Compound **217** was synthesised from **216** (230 mg, 0.53 mmol), mercury (II) chloride (143 mg, 0.53 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (146 mg, 0.53 mmol), triethylamine (180 mg, 1.76 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	140 mg, 0.21 mmol, 39% (orange foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 18H, (C*H*$_3$)$_3$C), 3.65 (m, 2H, CHC*H*$_2$Ph), 3.9 (t, 1H, J = 5 Hz, C*H*CH$_2$Ph), 4.9 (d, 1H, J = 15 Hz, NC*H*HPh), 5.5 (d, 1H, J = 15 Hz, NC*H*HPh), 6.9-7.5 (m, 19H, Ph and NH), 10.2 (bs, 1H, NH)
I.R. (v$_{max}$)	2926, 1721, 1680, 1636, 1156 cm^{-1}
MS(FAB) (m/z %)	674 (MH$^+$, 30), 474 (MH$^+$-2Boc, 100)

N-[2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (74)
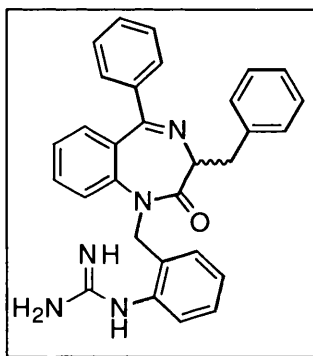

Compound **74** was synthesised from **217** (140 mg, 0.21 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	125.1 mg, 0.16 mmol, 78% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.40 (dd, 1H, J = 6 Hz and 9 Hz, CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 9 Hz, CHC*H*HPh), 3.9 (dd, 1H, J = 6 Hz and 7 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NC*H*HPh), 5.2 (d, 1H, J = 18 Hz, NC*H*HPh), 6.6 (d, 1H, J = 7 Hz), 7.0 (t, 1H, J = 6 Hz, Ph), 7.2-7.6 (m, 20H, Ph and NH), 9.5 (s, 1H, NH)
I.R. (v$_{max}$)	3354, 3169, 1684, 1599, 1203 cm^{-1}

MS(FAB) (m/z %)   474 (MH$^+$-CF$_3$CO$_2^-$, 100), 457 (M$^+$-NH$_2$, 50), 327 (35)

analysis: C$_{30}$H$_{27}$N$_5$O.2.5TFA.0.5H$_2$O   calc: C, 54.75; H, 4.0; F, 18.56;

effective molecular weight [767.64]   N, 9.12; O, 13.55

found: C, 54.71; H, 3.94; F, 18.2; N, 9.10; O, 13.69

2-[3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) propyl] isoindole-1,3-dione (80)
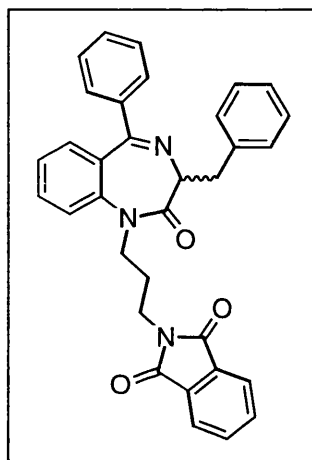
Compound **80** was synthesised from **51** (326 mg, 1 mmol), N-(3-bromopropyl) phthalimide (295 mg, 1.1 mmol) and KO^{t}Bu in THF (1 M soln, 1.1 ml, 1.1 mmol) using the procedure outlined in Method 3. The crude product isolated (340 mg) was used in the next step without further purification.

1-(3-Aminopropyl)-3-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (82)
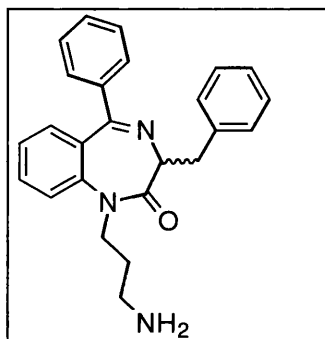

To a solution of **80** (340 mg) in DCM (30 ml) was added hydrazine hydrate (164 mg, 3.2 mmol). After 24 h the reaction mixture was diluted with more DCM (200 ml) and washed with water (100 ml) and then brine (100 ml). The organic phase

was then separated, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product isolated was then purified by preparative HPLC.

yield:	298.9 mg, 0.43 mmol, 43% over two steps (white foam)
¹H nmr (360 MHz, DMSO) ∂	1.8 (m, 2H, RCH_2R), 2.7 (t, 2H, J = 6 Hz, RCH$_2$CH_2NH$_2$), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHCHHPh), 3.50 (dd, 1H, J = 6 Hz and 12 Hz, CHCHHPh), 3.7 (m, 2H, CHCH$_2$Ph and NCHHCH$_2$R), 4.3 (m, 1H, NCHHCH$_2$R), 6.3 (bs, 1 H, NH), 7.1-7.6 (m, 14H, Ph)
I.R. (v_{max})	3066, 1675, 1601, 1203 cm⁻¹
MS(EI) (m/z %)	384 (MH⁺-CF$_3$CO$_2$⁻, 25), 327 (35), 45 (100)
analysis: $C_{25}H_{25}N_3O.2.6TFA.0.7H_2O$ effective molecular weight [692.55]	calc: C, 52.37; H, 4.22; F, 21.39; N, 6.06; O, 15.94 found: C, 52.25; H, 3.90; F, 21.4; N, 5.95; O, 16.32

N-[3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) propyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (218)
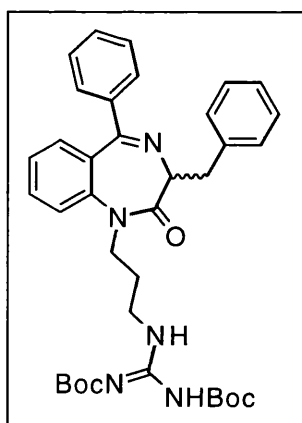
Compound **218** was synthesised from **82** (177 mg, 0.46 mmol), mercury (II) chloride (128 mg, 0.47 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (128 mmol, 0.46 mmol), triethylamine (153 mg, 1.53 mmol) and DMF (20 ml) using the procedure described in Method 6. The crude product isolated (185 mg) was used in the next step without purification.

N-[3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) propyl] guanidine (84)
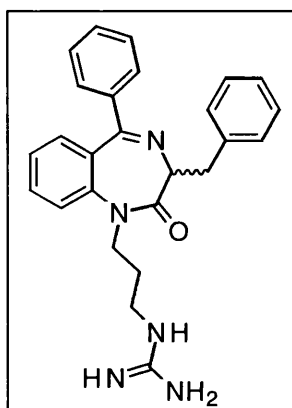
Compound **84** was synthesised from **218** (185 mg, 0.29 mmol) and trifluoroacetic acid (2 ml) using the procedure described in Method 7.

yield:	128.9 mg, 0.18 mmol, 39% (over two steps; light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.4-1.6 (m, 2H, RCH_2R), 3.0 (m, 2H, RCH$_2$CH_2NHR), 3.4 (dd, 2H, J = 6 Hz and 7.5 Hz, CHCH_2Ph), 3.7(m, 2H, NCHHCH$_2$R and CHCH$_2$Ph), 4.3 (m, 1H, NCHHCH$_2$R), 7.1-7.7 (m, 18H, Ph and NH)
I.R. (ν_{max})	3370, 3191, 1700, 1600, 1203 cm^{-1}
MS(FAB) (m/z %)	426 (MH$^+$-CF$_3$CO$_2^-$, 100), 409 (M$^+$-NH$_2$, 15)
analysis: C$_{26}$H$_{27}$N$_5$O.2.5TFA.0.8H$_2$O effective molecular weight [724.99]	calc: C, 51.35; H, 4.32; F, 19.6; N, 9.66; O, 15.00 found: C, 51.03; H, 4.21; F, 19.5; N, 9.41; O, 14.67

3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl) benzonitrile (86)
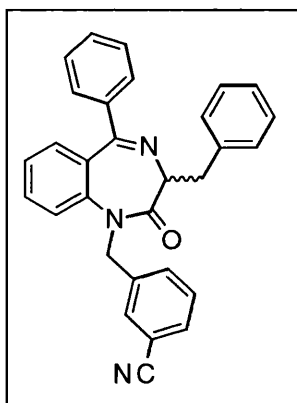
Compound **86** was synthesised from **51** (1.300 g, 3.99 mmol), 3-bromomethyl benzonitrile (804 mg, 4.1 mmol) and KO^{t}Bu in THF (1 M soln, 4.2 ml, 4.2 mmol) using the procedure described in Method 3.
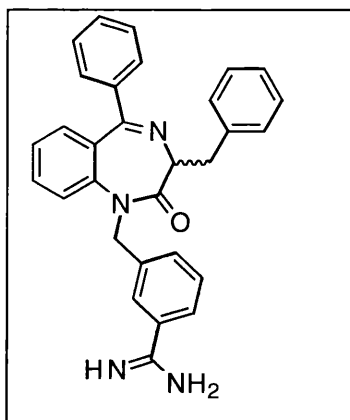
yield:	947 mg, 2.15 mmol, 54% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	3.6 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 4.8 (d, 1H, J = 15 Hz, NCHHPh), 5.7 (d, 1H, J = 15 Hz, NCHHPh), 7.0-7.5 (m, 18H, Ph)
I.R. (ν_{max})	3027, 2229, 1678, 1601, 1485 cm^{-1}
MS(FAB) (m/z %)	442 (MH$^+$, 100), 327 (25)

## 3-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl) benzamidine (88)

Compound **86** (150 mg, 0.34 mmol) was dissolved in EtOH (30 ml) and saturated with HCl (g) for 20 min. The mixture was stirred for 4 d and then

155

concentrated *in vacuo* and redissolved in methanol saturated with ammonia (30 ml). The mixture was stirred for 3 d and then concentrated *in vacuo* . The crude product was purified by preparative HPLC.

yield:	50.9 mg, 0.07 mmol, 20% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.40 (dd, 1H, J = 6 Hz and 12 Hz CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.9 (dd, 1H, J = 6 Hz and 9 Hz, C*H*CH$_2$Ph), 5.1 (d, 1H, J = 18 Hz, NC*H*HPh), 5.5 (d, 1H, J = 18 Hz, NC*H*HPh), 7.1-7.7 (m, 18H, Ph), 8.9 (s, 1H, NH), 9.2 (s, 1H, NH)
I.R. (v_{max})	3068, 1680, 1600, 1203 cm^{-1}
MS(FAB) (m/z %)	459 (MH$^+$-CF$_3$CO$_2^-$, 100), 442 (M$^+$-NH$_2$, 40)
analysis: C$_{30}$H$_{26}$N$_4$O.2.2TFA.1.2H$_2$O effective molecular weight [731.02]	calc: C, 56.51; H, 4.21; F, 17.1; N, 7.66; O, 14.44 found: C, 56.11; H, 3.87; F, 16.8; N, 7.56; O, 13.29

4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl) benzonitrile (87)
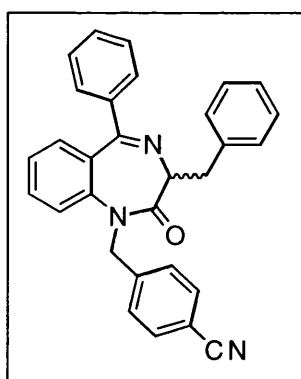

Compound **87** was synthesised from **51** (650 mg, 2 mmol), 4-bromomethyl benzonitrile (431 mg, 2.2 mmol) and KO^{t}Bu in THF (1 M soln, 2.2 ml, 2.2 mmol) using the procedure outlined in Method 3.

yield:	743 mg, 1.68 mmol, 84% (light yellow foam)

^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.6 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 4.8 (d, 1H, J = 16 Hz, NCHHPh), 5.7 (d, 1H, J = 16 Hz, NCHHPh), 7.0-7.5 (m, 18H, Ph)
I.R. (ν_{max})	3026, 2228, 1678, 1601, 1447 cm^{-1}
MS(FAB) (m/z %)	442 (MH$^+$, 100)

4-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl) benzamidine (89)
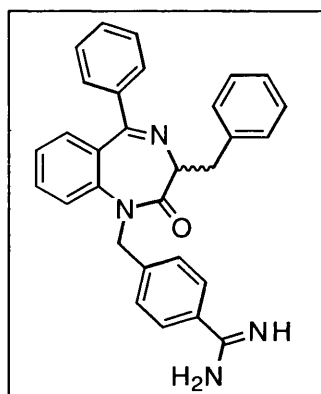
Compound **87** (700 mg, 1.58 mmol) was dissolved in EtOH (30 ml) and saturated with HCl (g) for 20 min. The mixture was stirred for 5 d and then concentrated *in vacuo* and redissolved in methanol saturated with ammonia (30 ml). The mixture was stirred for 3 d and then concentrated *in vacuo* . The crude product was purified by preparative HPLC.

yield:	128.9 mg, 0.16 mmol, 10% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.4 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 5.1 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 7.1-7.6 (m, 18H, Ph), 8.9 (s, 1H, NH), 9.2 (s, 1H, NH)
I.R. (ν_{max})	3370, 3066, 1679, 1203 cm^{-1}
MS(FAB) (m/z %)	459 (MH$^+$-CF$_3$CO$_2^-$, 100), 327 (100)
analysis: C$_{30}$H$_{26}$N$_4$O.2.7TFA.0.9H$_2$O effective molecular weight [782.63]	calc: C, 54.32; H, 3.92; F, 19.66; N, 7.16; O, 14.92
	found: C, 54.69; H, 3.73; F, 19.6; N, 7.16; O, 14.65

## 3.4 Variation in 1,4-Benzodiazepin-2-one Core Structure

3-Methyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (90)
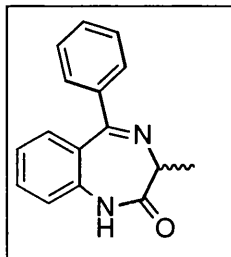
Compound **90** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (5.0 g; 25.4 mmol), ethyl chloroformate (3.91 g; 36 mmol), triethylamine (1.26 g, 37.4 mmol) and (±) N-Boc alanine (5.67 g; 30 mmol).

| yield	5.43 g, mmol, 21.7 mmol, 86% (colourless crystals)
Mp	201-202 °C(EtOAc-Hexane); lit 203-204 °C (PhH)[128]
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 7.0-7.5 (m, 9H), 8.6 (s, 1H)
I.R. (ν_{max})	3021, 1678, 1611, 1478 cm^{-1}
MS(FAB) (m/z %)	251 (MH$^+$, 100)
HRMS: (C$_{16}$H$_{15}$N$_2$O, MH$^+$)	requires: 251.1184, found: 251.1170

3-Methyl-1-(3-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (91)
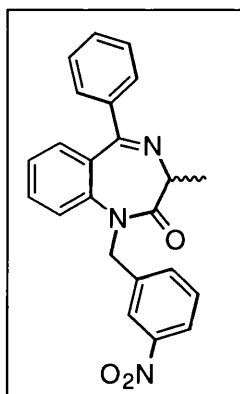
Compound **91** was synthesised from **90** (500 mg, 2 mmol), 3-nitrobenzyl bromide (432 mg, 2 mmol) and KO^{t}Bu in THF (1 M soln, 2.2 ml, 2.2 mmol) using the procedure outlined in Method 3.

yield:	750 mg, 1.95 mmol, 97% (light yellow foam)
^{1}H nmr (CDCl$_3$, 300 MHz) ∂	1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 4.9 (d, 1H, J = 17 Hz), 5.7 (d, 1H, J = 17 Hz), 7.2-7.6 (m, 13H)
I.R. (ν_{max})	1670, 1605, 1530, 1448, 1346 cm^{-1}
MS(FAB) (m/z %)	386 (MH$^+$, 100)

1-(3-Aminobenzyl)-3-methyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (219)
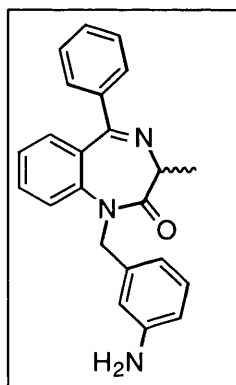
Compound **219** was synthesised from **91** (750 mg, 1.95 mmol) and tin (II) chloride (1.200 g, 5.3 mmol) using the procedure outlined in Method 4.

yield:	330 mg, 0.93 mmol, 48%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 4.7 (d, 1H, J = 16 Hz), 5.6 (d, 1H, J = 16 Hz), 6.5 (m, 2H), 7.0 (m, 1H), 7.1-7.6 (m, 12H)
I.R. (ν_{max})	3448, 3341, 1609, 1601, 1446 cm^{-1}
MS(FAB) (m/z %)	356 (MH$^+$, 100),
HRMS: (C$_{23}$H$_{22}$N$_3$O, MH$^+$)	requires: 356.1763, found: 356.1750

N-[3-(3-Methyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (220)
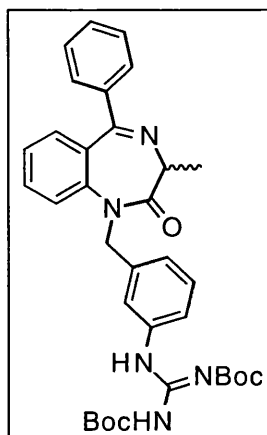
Compound **220** was synthesised from **219** (320 mg, 0.90 mmol), mercury (II) chloride (276 mg, 1.02 mmol), triethylamine (300 mg, 2.97 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (276 mg, 1.00 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

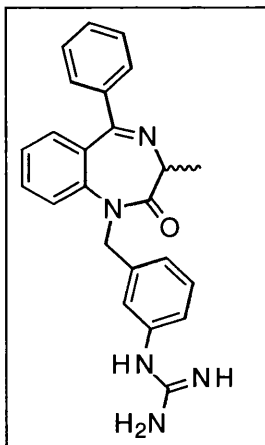|	
yield:	328 mg, 0.55 mmol, 71% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.7 (d, 1H, J = 15 Hz), 7.1-7.6 (m, 13H)
I.R. (v$_{max}$)	3414, 2978, 1792, 1685, 1638, 1607, 1239, 1150 cm^{-1}
MS(FAB) (m/z %)	598 (MH$^+$, 34), 398 (MH$^+$-2 Boc, 100)
HRMS: (C$_{34}$H$_{40}$N$_5$O$_5$, MH$^+$)	requires: 598.3029, found: 598.3040

N-[3-(3-Methyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (92)

Compound **92** was synthesised from **220** (320 mg, 0.54 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

|	
yield:	83.9 mg, 0.11 mmol, 21% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.6 (d, 3H, J = 6 Hz, CHCH_3), 3.8 (q, 1H, J = 6 Hz, CHCH$_3$), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 6.8 (s, 1H, Ph), 6.9 (d, 1H, J = 7 Hz, Ph), 7.0 (d, 1H, J = 7 Hz, Ph), 7.2-7.7 (m, 14H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (v_{max})	3413, 1700, 1600, 1204 cm^{-1}
MS(FAB) (m/z %)	398 (MH$^+$-CF$_3$CO$_2^-$, 100), 381 (46)
analysis: C$_{24}$H$_{23}$N$_5$O.2.8TFA.1.1H$_2$O effective molecular weight [736.56]	calc: C, 48.26; H, 3.83; F, 21.6; N, 9.50; O, 16.72 found: C, 47.86; H, 3.44; F, 21.5; N, 9.35; O, 16.27

3-Benzyl-5-methyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (93)
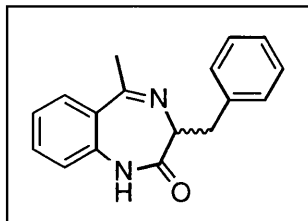
Compound **93** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminoaceteophenone (5.0 g; 37 mmol), ethyl chloroformate (5.75 g; 53 mmol), triethylamine (5.54 g, 54 mmol) and (±) N-Boc phenylalanine (11.7 g; 44 mmol).

yield	6.32 g, 23.9 mmol, 65% (colourless crystals)
Mp	203-205°C (EtOAc-hexane)
^{1}H nmr (400 MHz, CDCl$_3$) ∂	2.44 (s, 3H), 3.32 (dd, 1H, J = 9.2 Hz and 16.8 Hz), 3.62 (dd, 2H, J = 7.6 Hz and 16.8 Hz), 7.03 (d, 1H, J = 7.2 Hz), 7.1-7.45 (m, 7H), 7.53 (d, 1H, J = 9.2 Hz), 9.18 (s, 1H)
^{13}C nmr (CDCl$_3$) ∂	25.9, 37.3, 64.3, 121.3, 123.9, 126.1, 128.1, 128.3, 129.0, 129.3, 129.7, 131.3, 136.3, 139.1, 168.1, 171.2
I.R. (v_{max})	3202, 3044, 1678 cm^{-1}
MS(FAB) (m/z, %)	265 (MH$^+$, 100)
HRMS: (C$_{17}$H$_{17}$N$_2$O, MH$^+$)	requires: 265.1341, found: 265.1350

**3-Benzyl-5-methyl-1-(3-nitrobenzyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (94)**
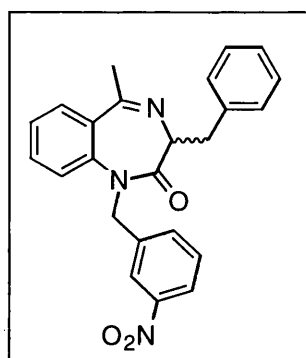
Compound **94** was synthesised from **93** (792 mg, 3 mmol), 3-nitrobenzyl bromide (648 mg, 3 mmol) and KO^{t}Bu in THF (1 M soln, 3.3 ml, 3.3 mmol) using the procedure outlined in Method 3.

yield:	410 mg, 1.03 mmol, 34% (yellow foam)

¹H nmr (CDCl₃, 400 MHz) ∂	2.43 (s, 3H), 3.30-3,35 (dd, 1H, J = 5.6 Hz and 8 Hz), 3.67-3.78 (m, 2H), 4.97 (d, 1H, J = 16 Hz), 5.36 (d, 1H, J = 16 Hz), 7.1-7.6 (m, 11H), 7.81 (s, 1H), 8.04 (m, 1H)
¹³C-nmr. (CDCl₃) ∂	25.3, 37.7, 50.3, 64.7, 121.6, 122.4, 125.3, 126.1, 127.7, 128.2, 129.5, 129.7, 131.3, 131.6, 133.0, 138.9, 139.8, 167.9, 169.2
I.R. (v$_{max}$)	1679, 1601, 1510, 1345 cm⁻¹
MS(FAB) (m/z %)	400 (MH⁺, 100)

1-(3-Aminobenzyl)-3-benzyl-5-methyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (221)
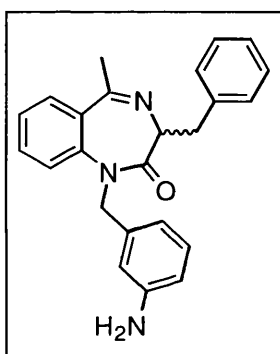
Compound **221** was synthesised from **94** (400 mg, 1 mmol) and tin (II) chloride (825 mg, 3 mmol) using the procedure outlined in Method 4.

yield:	320 mg, 0.87 mmol, 87%, (orange foam)
¹H nmr (400 MHz, CDCl₃) ∂	2.41 (s, 3H), 3.30 (dd, 1H, J = 9.6 Hz and 17.2 Hz), 3.74 (dd, 2H, J = 7.6 Hz and 16.4 Hz), 4.97 (s, 2H), 6.18 (s, 1H), 6.4 (m, 2H), 6.9 (t, 1H, J = 7.6 Hz), 7.1-7.5 (m, 9H)
I.R. (v$_{max}$)	3412, 1702, 1604, 1536 cm⁻¹
MS(FAB) (m/z %)	370 (MH⁺, 100)

N-[3-(3-Benzyl-5-methyl-2-oxo-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (222)
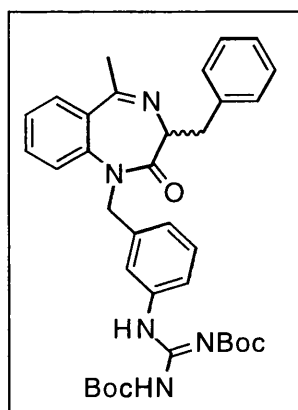
Compound **222** was synthesised from **221** (300 mg, 0.81 mmol), mercury (II) chloride (241 mg, 0.89 mmol), triethylamine (270 mg, 2.67 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (246 mg, 0.89 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	230 mg, 0.38 mmol, 47% (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 2.4 (s, 3H), 3.3 (dd, 2H, J = 7 Hz and 9 Hz), 3.8 (t, 1H, J = 7 Hz), 5.0 (d, 1H, J = 16 Hz), 5.2 (d, 1H, J = 16 Hz), 7.0-7.6 (m, 13H)
I.R. (ν_{max})	2952, 1715, 1605, 1151 cm^{-1}
MS(FAB) (m/z %)	612 (MH$^+$, 13), 412 (MH$^+$-2 Boc, 100)

N-[3-(3-Benzyl-5-methyl-2-oxo-2,3-dihydrobenzo[e][1,4]diazepin-1-yi methyl)phenyl] guanidine (95)
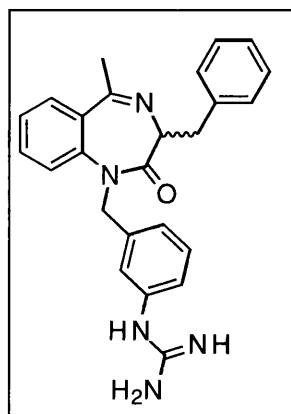
Compound **95** was synthesised from **222** (230 mg, 0.38 mmol) and trifluoroacetic acid (2 ml) using the procedure described in Method 7.

|	
yield:	57.2 mg, 0.08 mmol, 20% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	2.4 (s, 3H, RCCH_3), 3.2 (dd, 1H, J = 7 Hz and 12 Hz, NCHCHHPh), 3.4 (dd, 1H, J = 7 Hz and 12 Hz, NCHCHHPh), 3.8 (t, 1H, J = 7 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.3 (d, 1H, J = 18 Hz, NCHHPh), 6.8 (s, 1H, Ph), 7.1-7.7 (m, 12H, Ph), 9.7 (s, 1H, NH)
I.R. (v_{max}) film	3350, 3178, 1696, 1603, 1202 cm^{-1}
MS(FAB) (m/z %)	412 (MH$^+$-CF$_3$CO$_2^-$, 100), 395 (20), 265 (15), 149 (30)
analysis: $C_{25}H_{25}N_5O.2.7TFA.1.9H_2O$ effective molecular weight [753.60]	calc: C, 48.45; H, 4.21; F, 20.4; N, 9.29; O, 17.6 found: C, 48.5; H, 3.76; F, 20.0; N, 8.83; O, 16.04

(2-Aminophenyl)-(naphthalen-2-yl)-methanone (102)
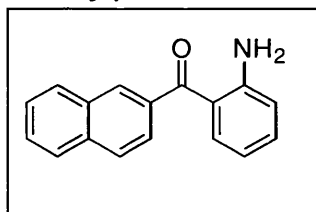
Compound **102** was synthesised in two steps from 2-bromonaphthalene (2.5 g, 12.1 mmol), magnesium (319 mg, 13.3 mmol) and 2-phenyl-4H-3,1-benzoxazin-4-one (2.79 g, 12.5 mmol) using the procedures outlined in Methods 8 and 9.

yield:	328 mg, 1.33 mmol, 11%, canary yellow foam
^{1}H nmr (300 MHz, CDCl$_3$) ∂	6.6-6.8 (m, 2H), 7.3-8.0 (m, 9H)
I.R. (v_{max})	3475, 3376, 1636, 1299 cm^{-1}
MS(FAB) (m/z %)	248 (MH$^+$, 90), 155 (45), 120 (100)
HRMS: ($C_{17}H_{14}NO$, MH$^+$)	requires: 248.1075, found: 248.1070

3-Benzyl-5-naphthalen-2-yl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (126)
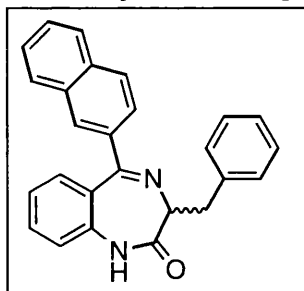
Compound **126** was synthesised from **102** (325 mg, 1.3 mmol), (±) N-Boc phenylalanine (398 mg, 1.5 mmol) and triethylamine (160 mg, 1.6 mmol) using the procedures outlined in Methods 1 and 2.

yield:	249 mg, 0.66 mmol, 51%, (white powder)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.7 (d, 2H, J = 4 Hz), 3.9 (t, 1H, J =4 Hz), 7.1-7.6 (m, 12H), 7.8-8.0 (m, 4H), 8.7 (s, 1H)
I.R. (v_{max})	2953, 1674, 1593 cm^{-1}
MS(FAB) (m/z %)	377 (MH$^+$, 100)

3-Benzyl-5-naphthalen-2-yl-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (127)
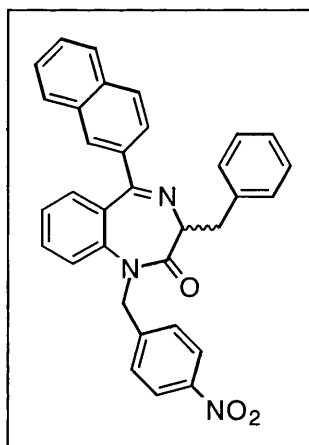
Compound **127** was synthesised by the procedures outlined in Method 3 using **126** (245 mg, 0.66 mmol), KO^{t}Bu (1 M soln, 0.73 ml, 0.73 mmol) and 4-nitrobenzyl bromide (157 mg, 0.73 mmol).

yield:	330 mg, 0.64 mmol, 98%, (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.7 (d, 2H, J = 4 Hz), 4.0 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 14 Hz), 5.7 (d, 1H, J = 14 Hz), 7.1-8.0 (m, 20H)

| I.R. (v$_{max}$) | 1678, 1600, 1524, 1342 cm^{-1} |
| MS(FAB) (m/z %) | 512 (MH$^+$, 100) |

1-(4-Aminobenzyl)-3-Benzyl-5-naphthalen-2-yl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (223)
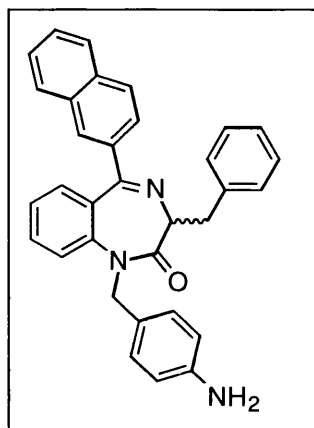
Compound **223** was synthesised from **127** (320 mg, 0.63 mmol) and tin (II) chloride (500 mg, 2.22 mmol) using the procedure described in Method 4.

yield:	263 mg, 0.62 mmol, 99% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.6 (m, 2H), 3.9 (m, 1H), 4.6 (d, 1H, J = 12 Hz), 5.6 (d, 1H, J = 12 Hz), 7.1-7.9 (m, 20H)
I.R. (v$_{max}$)	3412, 1674, 1601, 1251 cm^{-1}
MS(EI) (m/z %)	422 (MH$^+$, 49), 377 (100)

N-[4-(3-Benzyl-5-naphthalen-2-yl-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-yimethyl) phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (224)
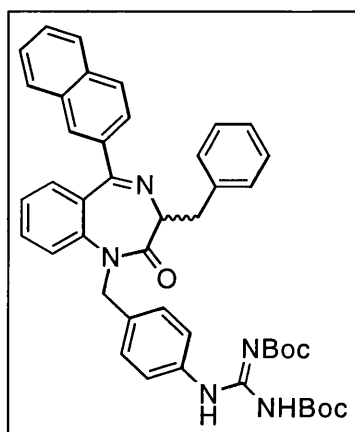
Compound **224** was synthesised from **223** (263 mg, 0.62 mmol), triethylamine (267 mg, 2.64 mmol), mercury (II) chloride (217 mg, 0.8 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (221 mg, 0.8 mmol) and DMF (20 ml) using the procedure described in Method 5.

yield:	215 mg, 0.29 mmol, 46% (light brown foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.7 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 7.0-7.8 (m, 20H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (ν_{max})	1782, 1718, 1637, 1543, 1150 cm^{-1}
MS(FAB) (m/z %)	741 (MH$^+$, 50), 524 (20), 382 (100)
HRMS: (C$_{45}$H$_{46}$N$_5$O$_5$, MH$^+$)	requires: 741.3890, found: 741.3870

N-[4-(3-Benzyl-5-naphthalen-2-yl-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl)phenyl] guanidine (128)

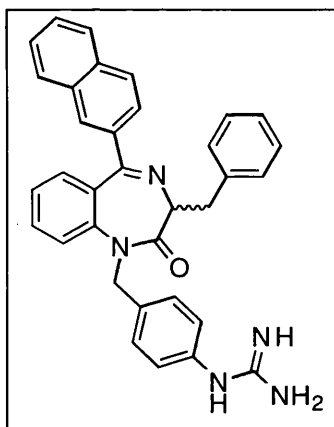Compound **128** was synthesised from **224** (215 mg, 0.29 mmol) and trifluoroacetic acid (2 ml) using the procedure described in Method 7.

yield:	93 mg, 0.10 mmol, 34% (yellow foam)

^{1}H nmr (360 MHz, DMSO) ∂	3.45 (dd, 1H, J = 6 Hz and 12 Hz, NCHC*H*HPh), 3.55 (dd, 1H, J = 6 Hz and 12 Hz, NCHC*H*HPh), 3.9 (dd, 1H, J = 6 Hz and 7 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NC*H*HPh), 5.5 (d, 1H, J = 18 Hz, NC*H*HPh), 6.9 (d, 2H, J = 8 Hz, Ph), 7.0 (d, 2H, J = 8 Hz, Ph), 7.2-7.45 (m, 10H, Ph), 7.5-7.85 (m, 5H, Ph), 7.9 (d, 1H, J = 9 Hz, Ph and NH), 8.0 (m, 2H, Ph), 9.6 (s, 1H, NH)
I.R. (ν_{max})	3369, 1698, 1601, 1516, 1203, 703 cm^{-1}
MS(FAB) (m/z %)	524 (MH$^+$-CF$_3$CO$_2^-$, 95), 377 (65), 148 (100)
analysis: C$_{34}$H$_{29}$N$_5$O.3.1TFA.H$_2$O effective molecular weight [895.12]	calc: C, 53.94; H, 3.84; F, 19.74; N, 7.82; O, 14.66 found: C, 53.74; H, 3.71; F, 19.6; N, 8.02; O, 14.29

3-Methyl-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (116)
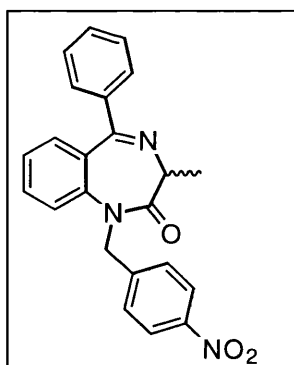

Compound **116** was synthesised from **90** (500 mg, 2 mmol), 4-nitrobenzyl bromide (432 mg, 2 mmol), KO^{t}Bu in THF (1 M soln, 2.2 ml, 2.2 mmol) using the procedure described in Method 3.

yield:	450 mg, 1.17 mmol, 59% (light yellow foam)

¹H nmr (200 MHz, CDCl₃) ∂	1.8 (d, 3H, J = 5 Hz), 3.8 (q, 1H, J = 5 Hz), 4.9 (d, 1H, J = 15 Hz), 5.7 (d, 1H, J = 15 Hz), 7.1-7.6 (m, 13H)
I.R. (νmax)	1686, 1654, 1607, 1523, 1345 cm⁻¹
MS(FAB) (m/z %)	386 (MH⁺, 100)
HRMS: (C₂₃H₂₀N₃O₃, MH⁺)	requires: 386.1505, found: 386.1520

1-(4-Aminobenzyl)-3-methyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (225)
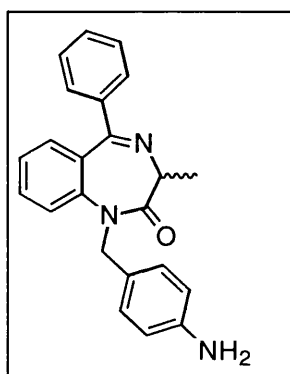
Compound **225** was synthesised from **116** (450 mg, 1.17 mmol) and tin (II) chloride (1.540 g, 6.84 mmol) using the procedure described in Method 4.

yield:	320 mg, 0.9 mmol, 76% (orange foam)
¹H nmr (300 MHz, CDCl₃) ∂	1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 4.6 (d, 1H, J = 17 Hz), 5.6 (d, 1H, J = 17 Hz), 7.0-7.6 (m, 13H)
I.R. (νmax)	3364, 1676, 1604, 1516 cm⁻¹
MS(FAB) (m/z %)	356 (MH⁺, 17), 251 (35), 106 (100)
HRMS: (C₂₃H₂₂N₃O, MH⁺)	requires: 356.1763, found: 356.1750

N-[4-(3-Methyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (226)
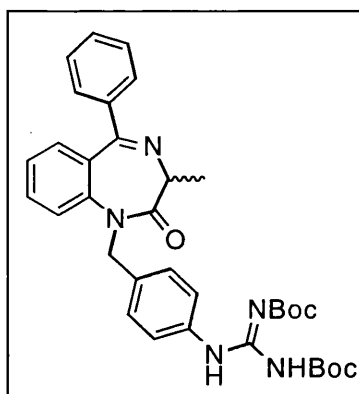
Compound **226** was synthesised from **225** (320 mg, 0.90 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (276 mg, 1 mmol), mercury (II) chloride (271 mg, 1 mmol), triethylamine (333 mg, 3.3 mmol) and DMF (20 ml) using the procedure described in Method 5.

yield:	250 mg, 0.42 mmol, 47% (light brown foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 1.8 (d, 3H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 7.0-7.6 (m, 13H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (v_{max})	1720, 1671, 1628, 1150 cm^{-1}
MS(FAB) (m/z %)	598 (MH$^+$, 20), 498 (6), 398 (100)
HRMS: (C$_{34}$H$_{40}$N$_5$O$_5$, MH$^+$)	requires: 598.3029, found: 598.3040

N-[4-(3-Methyi-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (121)
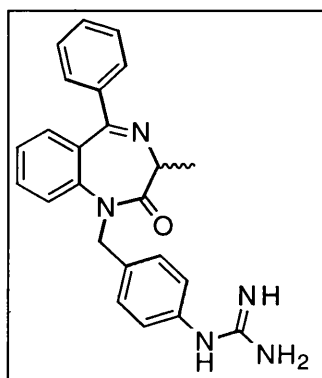
Compound **121** was synthesised from **226** (250 mg, 0.42 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

171

yield:	213.2 mg, 0.27 mmol, 65% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.6 (d, 3H, J = 6 Hz, RCCH_3), 3.85 (q, 1H, J = 6 Hz, CHCH$_3$), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 7.0-7.7 (m, 16H, Ph and NH), 9.7 (s, 1H, NH)
I.R. (ν_{max})	3349, 3178, 1700, 1517, 1202 cm^{-1}
MS(EI) (m/z %)	397 (M$^+$-CF$_3$CO$_2$⁻, 35), 249 (30), 148 (100), 106 (82)
analysis: C$_{24}$H$_{23}$N$_5$O.3.2TFA.H$_2$O effective molecular weight [780.37]	calc: C, 46.78; H, 3.64; F, 23.37; N, 8.97; O, 17.22 found: C, 46.79; H, 3.52; F, 23.4; N, 8.99; O, 16.81

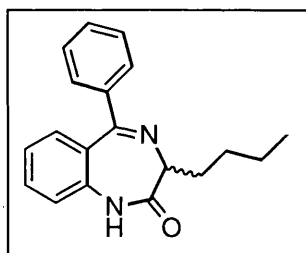## 3-Butyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (112)

Compound **112** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (1.18 g, 6 mmol), ethyl chloroformate (765 mg; 7 mmol), triethylamine (707 mg 7 mmol) and (±) N-Boc norleucine (1.5 g, 6.5 mmol).

yield:	1.42 g; 3.46 mmol; 58% (light yellow crystals)
Mp	164-167°C (EtOAc-hexane)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	0.9 (t, 3H, J = 6 Hz), 1.6 (m, 2H), 1.8 (m, 2H), 2.0 (m, 2H), 3.4 (t, 1H, J =6 Hz), 7.15 (t, 2H, J = 6 Hz), 7.2-7.6 (m, 7H), 10.5 (s, 1H)
I.R. (ν_{max})	3204, 3061, 2953, 1682, 1604, 1478 cm^{-1}
MS(FAB) (m/z %)	293 (MH$^+$, 100)
HRMS: (C$_{19}$H$_{21}$N$_2$O, MH$^+$)	requires: 293.1654, found: 293.1660

3-Butyl-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (117)
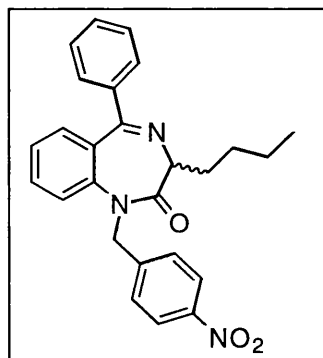
Compound **117** was synthesised from **112** (200 mg, 0.68 mmol), 4-nitrobenzyl bromide (220 mg, 1.02 mmol) and KO^{t}Bu in THF (1 M soln, 1 ml, 1 mmol) using the procedure outlined in Method 3.

yield:	203 mg, 0.48 mmol, 71%, (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	0.9 (t, 3H, J = 5 Hz), 0.95-1.0 (m, 4H), 3.8 (m, 1H), 5.1 (d, 1H, J = 15 Hz), 5.4 (d, 1H, J = 15 Hz), 7.1-7.6 (m, 13H)
I.R. (ν_{max})	2952, 2928, 1680, 1604, 1521, 1345 cm^{-1}
MS(FAB) (m/z %)	428 (MH$^+$, 100)

1-(4-Aminobenzyl)-3-butyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (227)
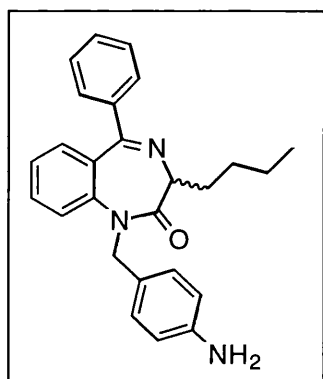
Compound **227** was synthesised from **117** (200 mg; 0.48 mmol) and tin (II) chloride (326 mg: 1.45 mmol) using the procedure outlined in Method 4.

yield:	144 mg, 0.36 mmol, 76%, (brown foam)

¹H nmr (300 MHz, CDCl₃) ∂	1.0 (t, 3H, J = 6 Hz), 1.2-1.5 (m, 4H), 2.3 (m, 2H), 3.6 (t, 1H, J = 7 Hz), 4.6 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.4 (d, 2H, J = 7.5 Hz), 6.8 (d, 2H, J = 7.5 Hz), 7.1-7.6 (m, 9H)
I.R. (ν_max)	2952, 2928, 1680, 1604, 1521, 1345 cm⁻¹
MS(FAB) (m/z %)	428 (MH⁺, 100)

N-[4-(3-Butyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (228)
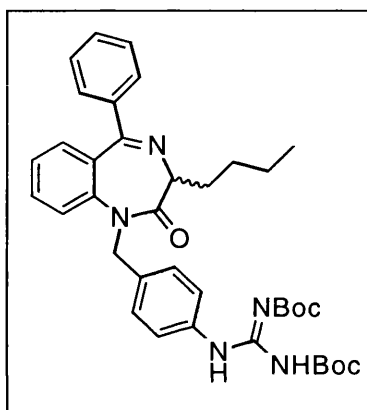
Compound **228** was synthesised from **227** (140 mg, 0.36 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (111 mg, 0.4 mmol), HgCl₂ (116 mg, 0.4 mmol) and triethylamine (123 mg, 1.2 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	135 mg, 0.21 mmol, 59%, (yellow foam)
¹H nmr (300 MHz, CDCl₃) ∂	0.9 (t, 3H, J = 6 Hz), 1.4-1.7 (m, 22H), 2.3 (m, 2H), 3.6 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.9-7.5 (m, 13H), 10.2 (bs, 1H)
I.R. (ν_max)	2979, 1721, 1684, 1643, 1151 cm⁻¹
MS(FAB) (m/z %)	640 (MH⁺, 10), 440 (100)

N-[4-(3-Butyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (122)
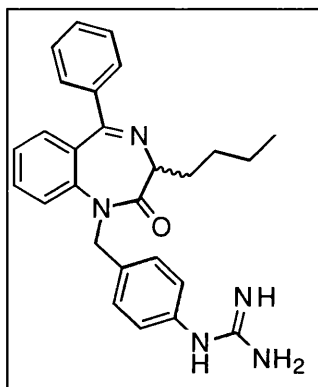
Compound **122** was synthesised from **228** (135 mg, 0.21 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield:	87.3 mg, 0.12 mmol, 55% (light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	0.9 (t, 3H, J = 6 Hz, RCH$_2$CH_3), 1.3-1.5 (m, 4H, RCH_2CH_2R), 2.1 (m, 2H, RCHCH_2R), 3.6 (t, 1H, J =6 Hz, CHCH$_2$R), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh) 7.0 (m, 4H, Ph), 7.2-7.7 (m, 9H, Ph), 9.5 (s, 1H, NH)
I.R. (v_{max}) film	3165, 2963, 1696, 1601, 1202 cm^{-1}
MS(FAB) (m/z %)	440 (MH$^+$-CF$_3$CO$_2$$^-$, 100), 293 (55), 148 (83)
analysis: C$_{27}$H$_{29}$N$_5$O.2.6TFA.H$_2$O effective molecular weight [754.04]	calc: C, 51.28; H, 4.49; F, 19.65; N, 9.28; O, 15.27 found: C, 51.06; H, 4.23; F, 19.3; N, 9.02; O, 15.0

3-Isobutyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (113)
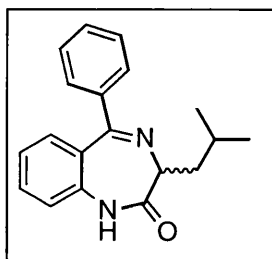
Compound **113** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (887 mg, 4.5 mmol), ethyl chloroformate (601 mg; 5.5 mmol), triethylamine (556 mg, 5.5 mmol) and (±) N-Boc leucine (1.130 g, 4.9 mmol).

yield:	705 mg, 2.41 mmol, 49% (colourless crystals)
Mp	193-195 °C(EtOAc-Hex); lit 196-197 °C[127]
^{1}H nmr (400 MHz, CDCl$_3$) ∂	0.82 (d, 3H, J = 6.4 Hz, RCHCH_3), 1.02 (d, 3H, J = 6.4 Hz, RCHCH_3), 1.88-2.06 (m, 2H, RCHCH_2R), 2.30-2.37 (m, 1H, CH(CH$_3$)$_2$), 3.60 (dd, 1H, J = 4 Hz and 9.2 Hz, CHCH$_2$R), 7.11-7.51 (m, 9H, Ph), 9.14 (s, 1H, NH)
^{13}C-nmr (CDCl$_3$) ∂	21.7, 23.6, 24.6, 39.7, 61.3, 121.1, 123.2, 127.6, 128.1, 129.7, 130.1, 131.1, 131.6, 138.5, 139.5, 169.2, 172.5
I.R. (v_{max})	3059, 2961, 1678, 1609, 1482 cm^{-1}
MS(FAB) (m/z %)	293 (MH$^+$, 100)
HRMS: (C$_{19}$H$_{21}$N$_2$O, MH$^+$)	requires: 293.1654, found: 293.1646

3-Isobutyl-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (118)
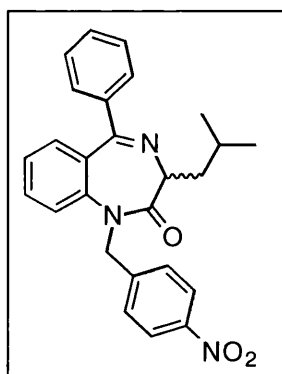
Compound **118** was synthesised from **113** (240 mg, 1.44 mmol), 4-nitrobenzyl bromide (404 mg, 1.87 mmol) and KO^{t}Bu in THF (1 M soln, 1.9 ml, 1.9 mmol) using the procedure outlined in Method 3.

yield:	483 mg, 1.13 mmol, 79% (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	0.9 (d, 3H, J = 4 Hz), 1.1 (d, 3H, J = 4 Hz), 1.9-2.1 (m, 2H), 2.3-2.4 (m, 1H), 3.6 (m, 1H), 7.1-7.5 (m, 13H)
I.R. (v_{max})	2952, 1681, 1604, 1522, 1345 cm^{-1}
MS(FAB) (m/z %)	428 (MH$^+$, 100)
HRMS: (C$_{26}$H$_{26}$N$_3$O$_3$, MH$^+$)	requires: 428.1974, found: 428.1960

1-(4-Aminobenzyl)-3-isobutyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (229)
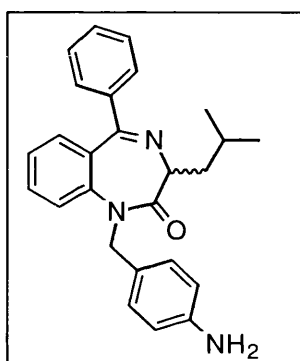
Compound **229** was synthesised from **118** (483 mg, 1.13 mmol) and tin (II) chloride (763 mg: 3.39 mmol) using the procedure outlined in Method 4.

yield	329 mg, 0.83 mmol, 73% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	0.9 (d, 3H, J = 6 Hz), 1.0 (d, 3H, J = 6 Hz), 2.0-2.1 (m, 2H), 2.4 (m, 1H), 3.6 (t, 1H, J = 5 Hz), 4.6 (d, 1H, J = 15 Hz), 5.7 (d, 1H, J = 15 Hz), 6.4 (d, 2H, J = 7.5 Hz), 6.8 (d, 2H, J = 7.5 Hz), 7.1-7.5 (m, 9H)
I.R. (v_{max})	3447, 3358, 2953, 1673, 1604, 1518 cm^{-1}
MS(FAB) (m/z %)	398 (MH$^+$, 40), 293 (65)
HRMS: (C$_{26}$H$_{28}$N$_3$O, MH$^+$)	requires: 398.2232, found: 398.2220

N-[4-(3-Isobutyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (230)
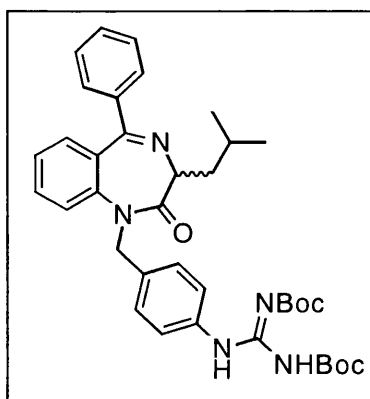
Compound **230** was synthesised from **229** (329 mg, 0.83 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (224 mg, 0.81 mmol), $HgCl_2$ (219 mg, 0.81 mmol), triethylamine (244 mg, 2.42 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	234 mg, 0.37 mmol, 44% (light yellow foam)
^1H nmr (300 MHz, $CDCl_3$) ∂	0.8 (d, 3H, J = 6 Hz), 1.0 (d, 3H, J = Hz), 1.6 (s, 18H), 2.0 (m, 2H), 2.3 (m, 1H), 3.7 (t, 1H, J = 6 Hz), 4.8 (d, 1H, J = 17 Hz), 5.6 (d, 1H, J = 17 Hz), 6.9-7.5 (m, 13H)
I.R. (v_{max})	3424, 2978, 1722, 1684, 1642, 1607, 1151 cm^{-1}
MS(FAB) (m/z %)	640 (MH$^+$, 13), 440 (75), 382 (70), 189 (90), 143 (100)
HRMS: ($C_{37}H_{46}N_5O_5$, MH$^+$)	requires: 640.3499, found: 640.3480

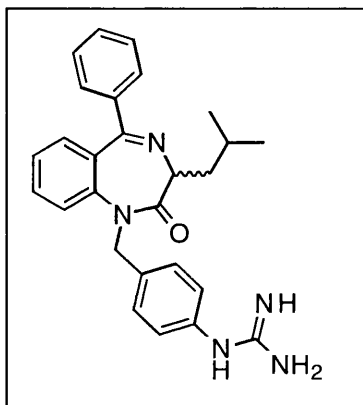## N-[4-(3-Isobutyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (123)

Compound **123** was synthesised from **230** (234 mg, 0.37 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield	71.3 mg, 0.14 mmol, 38% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	0.8 (d, 3H, J = 7 Hz, RCHCH_3), 1.0 (d, 3H, J = 7 Hz, RCHCH_3), 2.0 (m, 2H, RCHCH_2R), 2.3 (m, 1H, CH(CH$_3$)$_2$), 3.7 (dd, 1H, J = 6 Hz and 12 Hz, CHCH$_2$R), 4.8 (d, 1H, J = 18 Hz, NCHHPh), 5.6 (d, 1H, J = 18 Hz, NCHHPh), 6.9-7.5 (m, 13H, Ph)
I.R. (v$_{max}$)	3350, 2954, 1676, 1603, 1203 cm^{-1}
MS(EI) (m/z %)	439 (MH$^+$-CF$_3$CO$_2^-$, 15), 291 (60), 148 (65), 106 (100)
analysis: C$_{27}$H$_{29}$N$_5$O.0.4TFA.1.5H$_2$O effective molecular weight [512.18]	calc: C, 65.18; H, 6.37; F, 4.45; N, 13.67; O, 10.31 found: C, 64.95; H, 6.05; F, 4.3; N, 13.63; O, 7.06

3-Phenethyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (114)
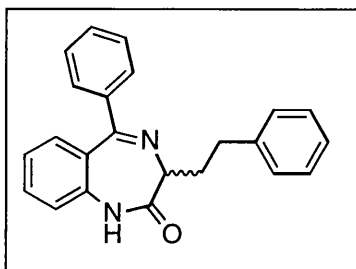
Compound **114** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (621 mg, 3.15 mmol), ethyl chloroformate (227 mg; 2.1 mmol), triethylamine (212 mg, 2.1 mmol) and (±) N-Boc homophenylalanine (540 mg, 1.94 mmol)

yield	249 mg, 0.74 mmol, 38% over two steps (white powder)
Mp	225-230°C (EtOAc-hexane)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	2.4-3.0 (m, 4H), 3.6 (dd, 1H, J = 2 Hz and 4 Hz), 7.1-7.6 (m, 14H), 8.6 (s, 1H)
I.R. (v$_{max}$)	3414, 2957, 1677, 1607, 1476 cm^{-1}
MS(FAB) (m/z %)	341 (MH$^+$, 100), 154 (65), 136 (50)
HRMS: (C$_{23}$H$_{21}$N$_2$O, MH$^+$)	requires: 341.1654, found: 341.1640

1-(4-Nitrobenzyl)-3-phenethyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (119)
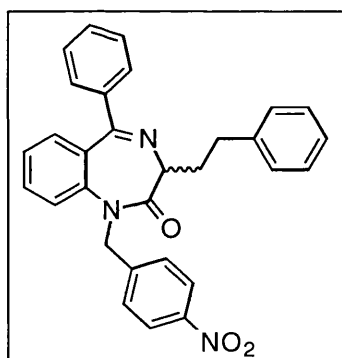
Compound **119** was synthesised from **114** (249 mg, 0.74 mmol), 4-nitrobenzyl bromide (239 mg, 1.1 mmol) and KO^{t}Bu in THF (1 M soln, 1.1 ml, 1.1 mmol) using the procedure outlined in Method 3.

yield:	340 mg, 0.72 mmol, 97% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	2.4-2.5 (m, 2H), 2.6-3.0 (m, 2H), 3.9 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 17 Hz), 5.8 (d, 1H, J = 17 Hz), 7.2-7.8 (m, 18H)
I.R. (v$_{max}$)	2957, 1678, 1603, 1521, 1344 cm^{-1}
MS(FAB) (m/z %)	476 (MH$^+$, 100), 341 (80), 307 (85)
HRMS: (C$_{30}$H$_{26}$N$_3$O$_3$, MH$^+$)	requires: 476.1974, found: 476.1960

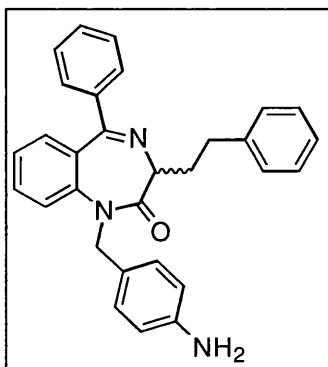## 1-(4-Aminobenzyl)-3-phenethyl-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (231)

Compound **231** was synthesised from **119** (340 mg, 0.72 mmol) and tin (II) chloride (743 mg, 3.3 mmol) using the procedure outlined in Method 4.

yield:	310 mg, 0.7 mmol, 97% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	2.4-2.5 (m, 2H), 2.6-3.0 (m, 2H), 3.9 (t, 1H, J = 5 Hz), 4.9 (d, 1H, J = 16 Hz), 5.6 (d, 1H, J = 16 Hz), 7.0-7.6 (m, 18H)
I.R. (v_{max})	3440, 1671, 1600, 1410 cm^{-1}
MS(FAB) (m/z %)	446 (MH$^+$, 100)
HRMS: (C$_{30}$H$_{28}$N$_3$O, MH$^+$)	requires: 446.2232, found: 446.2247

N-[4-(2-Oxo-3-phenethyl-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (232)
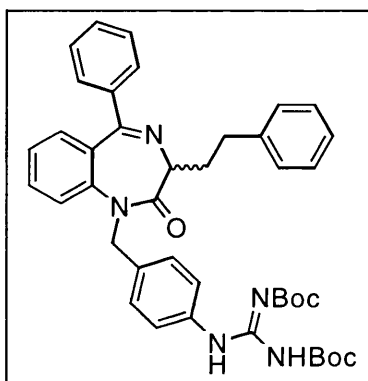
Compound **232** was synthesised from **231** (310 mg, 0.70 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (305 mg, 1.1 mmol), HgCl$_2$ (298 mg, 1.1 mmol) and triethylamine (333 mg, 3.3 mmol) using the procedure outlined in Method 5.

181

yield:	229 mg, 0.33 mmol, 48% (light brown foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 2.4-3.0 (m, 4H), 3.7 (t, 1H, J = 7 Hz), 4.9 (d, 1H, J = 16 Hz), 5.6 (d, 1H, J = 16 Hz), 7.0-7.6 (m, 18H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (v_{max})	3419, 1719, 1685, 1638, 1601, 1411, 1151 cm^{-1}
MS(FAB) (m/z %)	688 (MH$^+$, 10), 588 (10), 488 (100), 341 (60)
HRMS: (C$_{41}$H$_{46}$N$_5$O$_5$, MH$^+$)	requires: 688.3499, found: 688.3480

N-[4-(2-Oxo-3-phenethyl-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (124)
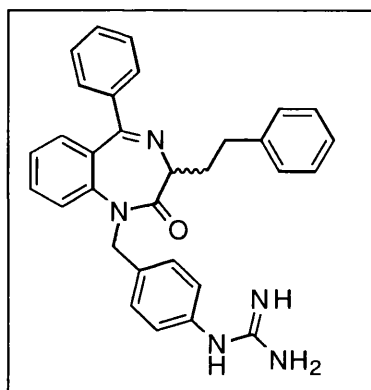
Compound **124** was synthesised from **232** (229 mg, 0.33 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield	40 mg, 0.05 mmol, 15% (light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	2.3-2.5 (m, 2H, RCH_2R), 2.65-2.80 (m, 2H, RCH_2R), 3.6 (dd, 1H, J = 6 Hz and 9 Hz, CHCH$_2$R), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 7.0 (m, 4H, Ph), 7.1-7.7 (m, 14H, Ph), 9.7 (s, 1H, NH)
I.R. (v_{max})	3162, 1695, 1601, 1202 cm^{-1}
MS(CI) (m/z %)	488 (MH$^+$-CF$_3$CO$_2^-$, 40), 244 (MH^{2+}, 100)

analysis: $C_{31}H_{29}N_5O.2.8TFA.H_2O$

effective molecular weight [824.88]

calc: C, 53.29; H, 4.13; F, 19.34;

N, 8.49; O, 14.74

found: C, 52.95; H, 3.91; F, 19.7;

N, 8.69; O, 14.45

5-Phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (169)
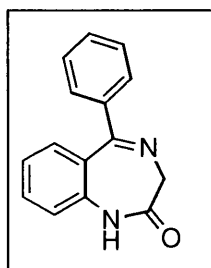
Compound **169** was synthesised from 2-aminobenzophenone (6.400 g, 32.5 mmol), bromoacetyl bromide (7.218 g, 35.6 mmol) and triethylamine (3.596 g, 35.6 mmol) using the procedure described in Method 11.

yield:	4.680 g, 19.8 mmol, 61% (colourless crystals)
Mp	174-176 °C(EtOAc-Hexane); lit 178-179°C (EtOH)[129]
^1H nmr (400 MHz, $CDCl_3$) ∂	4.3 (bs, 2H), 7.1-7.6 (m, 9H)
^{13}C-nmr ($CDCl_3$) ∂	56.6, 121.1, 123.4, 127.2, 128.2, 129.7, 130.3, 131.4, 138.7, 139.3, 171.1, 171.9
I.R. (ν_{max})	3184, 2897, 1685, 1611, 1364 cm^{-1}
MS(FAB) (m/z %)	237 (MH$^+$, 100)
HRMS: ($C_{15}H_{13}N_2O$, MH$^+$)	requires: 237.1028, found: 237.1020

1-(4-Nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (172)
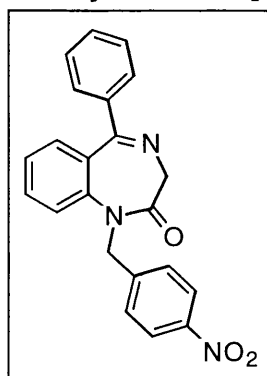
Compound **172** was synthesised from **169** (1.000 g, 4.24 mmol), 4-nitrobenzyl bromide (1.007 g, 4.66 mmol) and KO^{t}Bu in THF (1M soln, 4.6 ml, 4.6 mmol) using the procedure outlined in Method 3.

yield:	794 mg, 2.14 mmol, 50% (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.8 (d, 1H, J = 10 Hz), 4.9 (m, 2H), 5.6 (d, 1H, 15 Hz), 7.1-7.5 (m, 11H), 7.9 (d, 2H, J = 10 Hz)
I.R. (ν_{max})	1679, 1519, 1343 cm^{-1}
MS(FAB) (m/z %)	372 (MH$^+$, 100)
HRMS: (C$_{22}$H$_{18}$N$_3$O$_3$, MH$^+$)	requires: 372.1348, found: 372.1337

1-(4-Aminobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (233)
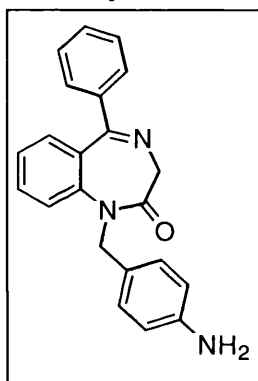
Compound **233** was synthesised from **172** (794 mg; 2.14 mmol) and tin (II) chloride (326 mg: 1.45 mmol) using the procedure outlined in Method 4.

yield:	640 mg, 1.87 mmol, 88% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.8 (d, 1H, J = 10 Hz), 4.6 (d, 1H, J = 15 Hz), 4.8 (d, 1H, J = 10 Hz), 5.4 (d, 1H, J = 15 Hz), 6.4 (d, 2H, J = 7.5 Hz), 6.8 (d, 2H, J = 7.5 Hz), 7.0-7.5 (m, 9H)
I.R. (ν_{max})	3440, 1672, 1616, 1481 cm^{-1}
MS(FAB) (m/z %)	342 (MH$^+$, 60), 237 (100)

N-[4-(2-Oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl) phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (234)
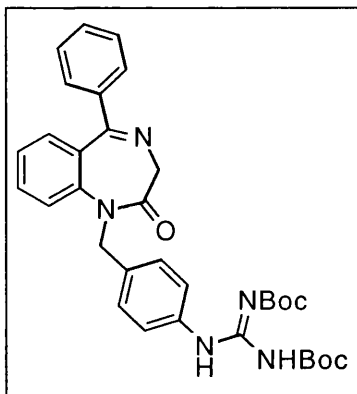
Compound **234** was synthesised from **233** (200 mg, 0.59 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (180 mg, 0.65 mmol), $HgCl_2$ (176 mg, 0.65 mmol) and triethylamine (197 mg, 1.95 mmol) using the procedure described in Method 5.

yield:	174 mg, 0.30 mmol, 51% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.9 (d, 1H, J = 13 Hz), 4.8-4.9 (m, 2H), 5.5 (d, 1H, J = 15 Hz), 7.0-7.5 (m, 13H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (v_{max})	2978, 1718, 1685, 1638, 1607, 1151 cm^{-1}
MS(FAB) (m/z %)	584 (MH$^+$, 15), 384 (100), 237 (50), 148(100)
HRMS: ($C_{33}H_{38}N_5O_5$, MH$^+$)	requires: 584.2873, found: 584.2860

N-[4-(2-Oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl) phenyl] guanidine (175)
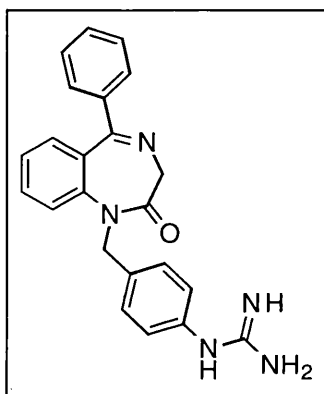
Compound **175** was synthesised from **234** (174 mg, 0.30 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield	66.9 mg, 0.09 mmol, 30% (yellow foam)
¹H nmr (360 MHz, DMSO) ∂	3.9 (d, 1H, J = 12 Hz, RCHH), 4.6 (d, 1H, J = 12 Hz, RCHH), 5.0 (d, 1H, J = 15 Hz, NCHHPh), 5.4 (d, 1H, J = 15 Hz, NCHHPh), 7.0-7.7 (m, 13H, Ph), 9.6 (s, 1H, NH)
I.R. (v_{max})	3341, 1675, 1602, 1202 cm⁻¹
MS(FAB) (m/z %)	384 (MH⁺-CF₃CO₂⁻, 100), 192 (MH²⁺, 100)
analysis: C₂₃H₂₁N₅O.3.2TFA.0.75H₂O effective molecular weight [761.84]	calc: C, 46.35; H, 3.4; N, 9.19; O, 17.1 found: C, 46.24; H, 3.22; N, 9.22; O, 16.54

3-(1H-Indol-3-yl methyl)-5-phenyl-1,3-dihydrobenzo [e][1,4]diazepin-2-one (115)
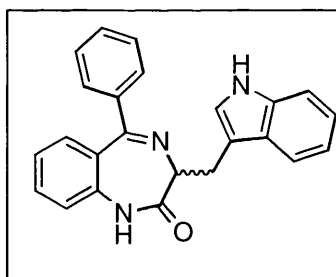
Compound **115** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (1.182 g, 6 mmol), ethyl chloroformate (714 mg; 6.58 mmol), triethylamine (664 mg 6.58 mmol) and (±) N-Boc tryptophan (2.0 g, 6.58 mmol)

yield	422 mg, 1.16 mmol, 19% (light brown powder)
Mp	258-260 °C(EtOAc-Hexane); lit 260-263 °C[74]
¹H nmr (200 MHz, CDCl₃) ∂	3.6-3.8 (m, 3H), 7.0-7.5 (m, 13H), 7.7 (d, 1H, J = 6 Hz), 8.0 (bs, 1H), 8.5 (s, 1H)

I.R. (ν_{max})	3348, 3054, 1686, 1599 cm^{-1}
MS(FAB) (m/z %)	366 (MH$^+$, 100)
HRMS: ($C_{24}H_{20}N_3O$, MH$^+$)	requires: 366.1606, found: 366.1600

3-(1H-Indol-3-yl methyl)-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo [e][1,4]diazepin-2-one (120)
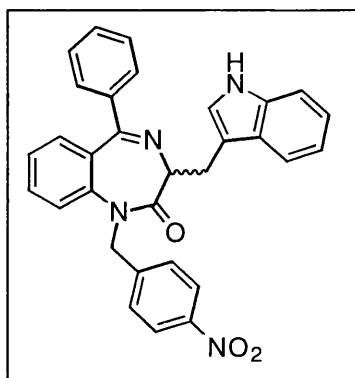
Compound **120** was synthesised from **115** (422 mg, 1.16 mmol), 4-nitrobenzyl bromide (374 mg, 1.73 mmol) and KO^{t}Bu in THF (1 M soln, 1.7 ml, 1.7 mmol) using the procedure outlined in Method 3.

yield	178 mg, 0.36 mmol, 31% (light brown foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.7-4.0 (m, 3H), 4.9 (d, 1H, J =12 Hz), 5.7 (d, 1H, J = 12 Hz), 7.1-7.5 (m, 16H), 7.9 (d, 2H, J = 8 Hz), 8.1 (bs, 1H)
I.R. (ν_{max})	3421, 1677, 1601, 1522, 1344 cm^{-1}
MS(FAB) (m/z %)	501 (MH$^+$, 20), 309 (10), 130 (100)
HRMS: ($C_{31}H_{25}N_4O_3$, MH$^+$)	requires: 501.1927, found: 501.1940

1-(4-Aminobenzyl)-3-(1H-indol-3-yl methyl)-5-phenyl-1,3-dihydrobenzo [e][1,4]diazepin-2-one (235)
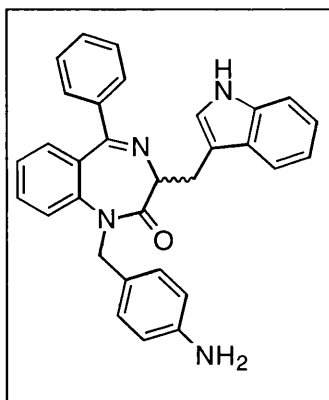
Compound **235** was synthesised from **120** (178 mg; 0.36 mmol) and tin (II) chloride (244 mg: 1.09 mmol) using the procedure outlined in Method 4.

yield:	142 mg, 0.30 mmol, 84% (brown foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.7 (dd, 2H, J = 6 Hz and 10 Hz), 3.95 (dd, 1H, J = 6 and 10 Hz), 4.7 (d, 1H, J = 15 Hz), 5.6 (d, 1H, 15 Hz), 6.4 (d, 2H, J = 6 Hz), 6.8 (d, 2H, J = 6 Hz), 7.0-7.7 (m, 14H), 8.1 (s, 1H)
I.R. (v_{max})	3419, 1663, 1601, 1516, 1447 cm^{-1}
MS(FAB) (m/z %)	471 (M$^+$, 65), 366 (100)
HRMS: (C$_{31}$H$_{26}$N$_4$O, MH$^+$)	requires: 471.2185, found: 471.2170

N-{4-[3-(1H-Indol-3-yl methyl)-2-oxo-5-phenyl-2,3-dihydrobenzo [e][1,4] diazepin-1-yl methyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (236)
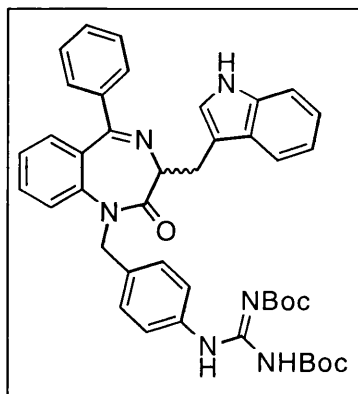
Compound **236** was synthesised from **235** (142 mg, 0.30 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (91 mg, 0.33 mmol), $HgCl_2$ (89 mg, 0.33 mmol) and triethylamine (100 mg, 0.99 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	170 mg, 0.24 mmol, 81% (brown foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.6 (d, 2H, J = 6 Hz), 3.8 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 7.0-7.6 (m, 18H)
I.R. (ν_{max})	3417, 1720, 1685, 1601, 1150 cm^{-1}
MS(FAB) (m/z %)	713 (MH$^+$, 6), 513 (MH$^+$-2Boc, 100)
HRMS: (C$_{42}$H$_{45}$N$_6$O$_5$, MH$^+$)	requires: 713.3451, found: 713.3440

N-{4-[3-(1H-Indol-3-yl methyl)-2-oxo-5-phenyl-2,3-dihydrobenzo [e][1,4] diazepin-1-yl methyl]phenyl} guanidine (125)
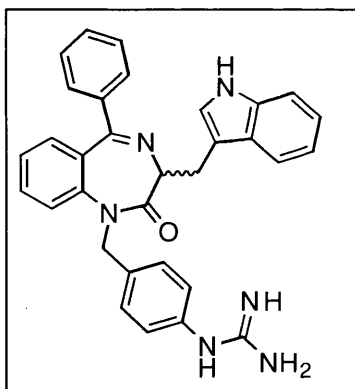
Compound **125** was synthesised from **236** (170 mg, 0.24 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield:	17.8 mg, 0.03 mmol, 14% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.5 (dd, 1H, J = 6 Hz and 12 Hz, NCHC*H*HPh), 3.6 (dd, 1H, J = 6 Hz and 12 Hz, NCHC*H*HPh), 3.85 (t, 1H, J = 6 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 15 Hz, NCH*H*Ph), 5.5 (d, 1H, J = 15 Hz, NCH*H*Ph), 6.9-7.6 (m, 22H, Ph and NH), 9.5 (s, 1H, NH), 10.8 (s, 1H, NH)

I.R. (v$_{max}$)	3325, 1672, 1599, 1178, 1139 cm^{-1}
MS(FAB) (m/z %)	513 (MH$^+$-CF$_3$CO$_2^-$, 100)
HRMS: (C$_{32}$H$_{29}$N$_6$O, MH$^+$-CF$_3$CO$_2^-$)	requires: 513.2403, found: 513.2420

(2-Aminophenyl)-*p*-tolyl methanone (103)
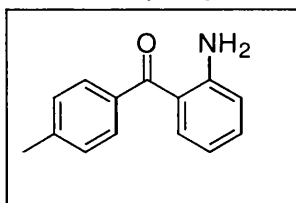
Compound **103** was synthesised in two steps from 4-bromotoluene (1.881 g, 11 mmol), magnesium (264 mg, 11 mmol) and 2-phenyl-4H-3,1-benzoxazin-4-one (2.100 g, 9.42 mmol) using the procedures outlined in Methods 8 and 9.

yield:	740 mg, 3.51 mmol, 37% (yellow crystals)
Mp	87-89 °C; lit 93-94 °C[130]
I.R. (v$_{max}$)	3355, 2980, 1719, 1599, 1511, 1253 cm^{-1}
MS(FAB) (m/z %)	212 (MH$^+$, 100)
HRMS: (C$_{14}$H$_{14}$NO, MH$^+$)	requires: 212.1075, found: 212.1080

3-Methyl-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (129)
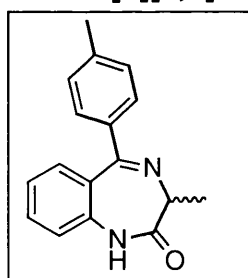
Compound **129** was synthesised by the procedures outlined in Methods 1 and 2 using **103** (211 mg, 1 mmol), ethyl chloroformate (152 mg; 1.4 mmol), triethylamine (141 mg, 1.4 mmol) and (±) N-Boc alanine (246 mg, 1.3 mmol).

yield:	210 mg, 0.74 mmol, 79% (light yellow powder)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.8 (d, 3H, J = 5 Hz), 2.4 (s, 3H), 3.85 (q, 1H, J = 5 Hz), 7.1-7.5 (m, 8H), 8.6 (s, 1H)
I.R. (v$_{max}$)	2951, 1678, 1601 cm^{-1}

| MS(FAB) (m/z %) | 265 (MH$^+$, 100) |
| HRMS: (C$_{17}$H$_{17}$N$_2$O, MH$^+$) | requires: 265.1341, found: 265.1332 |

3-Methyl-1-(4-nitrobenzyl)-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (132)
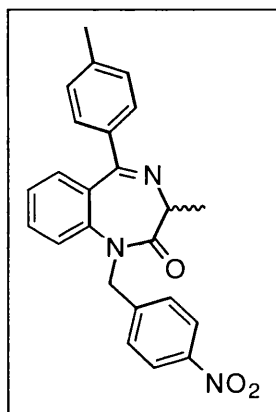
Compound **132** was synthesised from **129** (210 mg, 0.79 mmol), 4-nitrobenzyl bromide (194 mg, 0.9 mmol) and KO^{t}Bu in THF (1 M soln, 0.9 ml, 0.9 mmol) using the procedure outlined in Method 3.

yield	180 mg, 0.45 mmol, 57% (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (d, 3H, J = 6 Hz), 2.4 (s, 3H), 3.8 (q, 1H, J = 6 Hz), 4.9 (d, 1H, J = 12 Hz), 5.0 (d, 1H, J = 12 Hz), 7.2-7.5 (m, 12H)
I.R. (ν$_{max}$)	2988, 1678, 1602, 1525, 1346 cm^{-1}
MS(FAB) (m/z %)	400 (MH$^+$, 100)
HRMS: (C$_{24}$H$_{22}$N$_3$O$_3$, MH$^+$)	requires: 400.1661, found: 400.1650

1-(4-Aminobenzyl)-3-methyl-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (237)
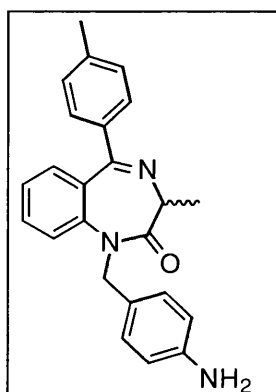
Compound **237** was synthesised from **132** (180 mg; 0.45 mmol) and tin (II) chloride (509 mg: 2.26 mmol) using the procedure outlined in Method 4.

yield	150 mg, 0.41 mmol, 90% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.85 (d, 3H, J = 6 Hz), 2.4 (s, 3H), 3.9 (q, 1H, J = 6 Hz), 4.7 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.4 (d, 2H, J = 8 Hz), 6.9 (d, 2H, J = 8 Hz), 7.1-7.5 (m, 8H)
I.R. (ν_{max})	3437, 3347, 1608, 1529, 1517 cm^{-1}
MS(FAB) (m/z %)	370 (MH$^+$, 55), 265 (100)
HRMS: (C$_{24}$H$_{24}$N$_3$O, MH$^+$)	requires: 370.1919, found: 370.1930

N-[4-(3-Methyl-2-oxo-5-*p*-tolyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (238)
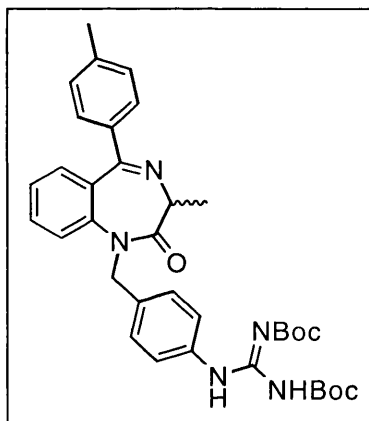
Compound **238** was synthesised from **237** (150 mg, 0.41 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (138 mg, 0.5 mmol), HgCl$_2$ (136 mg, 0.5 mmol) and triethylamine (152 mg, 1.5 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	172 mg, 0.28 mmol, 58% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 1.8 (d, 3H J = 6 Hz), 2.4 (s, 3H), 3.9 (q, 1H, J = 6 Hz), 4.8 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 7.0-7.4 (m, 12H), 10.2 (bs, 1H), 11.6 (bs, 1H)

I.R. (v_{max})	2980, 1719, 1686, 1639, 1606, 1370, 1151 cm^{-1}
MS(FAB) (m/z %)	612 (MH^+, 4.5), 512 (2), 412 (50), 382 (25), 192 (100)
HRMS: ($C_{35}H_{42}N_5O_5$, MH^+)	requires: 612.3186, found: 612.3170

N-[4-(3-Methyl-2-oxo-5-*p*-tolyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (135)
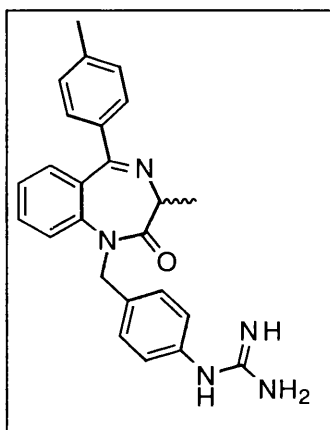
Compound **135** was synthesised from **238** (172 mg, 0.28 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield:	52.4 mg, 0.07 mmol, 25% (pale yellow foam)
^{1}H nmr (300 MHz, $CDCl_3$) ∂	1.6 (d, 3H, J = 6 Hz, RCHCH_3), 2.35 (s, 3H, PhCH_3), 3.8 (q, 1H, J = 6 Hz, CHCH$_3$), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 7.0 (m, 4H, Ph), 7.2-7.7 (m, 12H, Ph and NH), 9.7 (s, 1H, NH)
I.R. (v_{max})	3343, 3168, 1695, 1603, 1201 cm^{-1}
MS(EI) (m/z %)	411 (M^+-$CF_3CO_2^-$, 15), 369 (23), 263 (85), 106 (100)
analysis:	calc: C, 48.82; H, 4.1; F, 20.89;
$C_{25}H_{25}N_5O.2.75TFA.1.4H_2O$	N, 9.33; O, 16.85
effective molecular weight [750.29]	found: C, 48.43; H, 3.68; F, 20.6; N, 9.19; O, 16.09

3-Butyl-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (130)
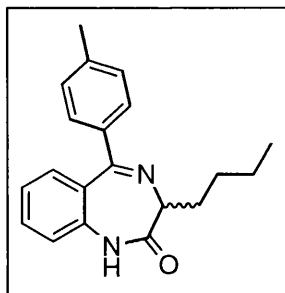
Compound **130** was synthesised by the procedures outlined in Methods 1 and 2 using **103** (211g, 1 mmol), ethyl chloroformate (152 mg; 1.4 mmol), triethylamine (141 mg, 1.4 mmol) and (±) N-Boc norleucine (300 mg, 1.3 mmol).

yield:	160 mg, 0.52 mmol, 52% (colourless crystals)
Mp	166-168°C (EtOAc-hexane)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.0 (t, 3H, J = 7 Hz), 1.4-1.6 (m, 4H), 2.2-2.4 (m, 2H), 2.45 (s, 3H), 3.6 (t, 1H, J = 6 Hz), 7.1-7.6 (m, 8H)
I.R. (v_{max})	2947, 1678, 1594 cm^{-1}
MS(FAB) (m/z %)	307 (MH$^+$, 100)
HRMS: (C$_{20}$H$_{23}$N$_2$O, MH$^+$)	requires: 307.1816, found: 307.1820

3-Butyl-1-(4-nitrobenzyl)-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (133)
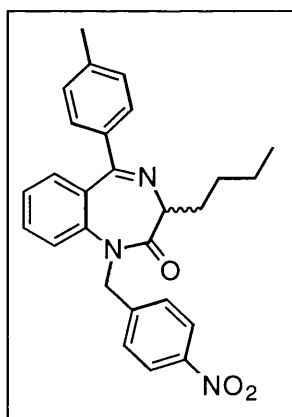
Compound **133** was synthesised from **130** (160 mg, 0.52 mmol), 4-nitrobenzyl bromide (162 mg, 0.75 mmol) and KO^{t}Bu in THF (1 M soln, 0.8 ml, 0.8 mmol) using the procedure outlined in Method 3.

yield:	171 mg, 0.39 mmol, 75% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	0.9 (t, 3H, J = 6 Hz), 1.2-1.5 (m, 4H), 2.2-2.3 (m, 5H), 3.8 (t, 1H, J = 5 Hz), 4.9 (d, 1H, J = 16 Hz), 5.8 (d, 1H, J = 16 Hz), 7.2-7.6 (m, 12H)
I.R. (v_{max})	2926, 1677, 1601, 1521, 1345 cm^{-1}
MS(FAB) (m/z %)	442 (MH$^+$, 100)
HRMS: (C$_{27}$H$_{28}$N$_3$O$_3$, MH$^+$)	requires: 442.2131, found: 442.2120

1-(4-Aminobenzyl)-3-butyl-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (239)
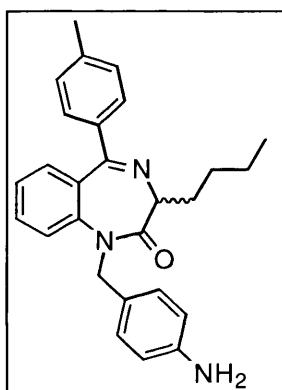
Compound **239** was synthesised from **133** (171 mg; 0.39 mmol) and tin (II) chloride (439 mg: 1.95 mmol) using the procedure outlined in Method 4.

yield	121 mg, 0.29 mmol, 74% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	0.9 (t, 1H, J = 6 Hz), 1.2-1.4 (m, 4H), 2.2 (m, 2H), 2.4 (s, 3H), 3.6 (t, 1H, J = 6 Hz), 4.6 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.5 (d, 2H, J = 8 Hz), 6.8 (d, 2H, J = 15 Hz), 7.1-7.5 (m, 8H)
I.R. (v_{max})	2954, 1678, 1601, 1521, 1345, 1252 cm^{-1}
MS(FAB) (m/z %)	412 (MH$^+$, 30), 307 (100)
HRMS: (C$_{27}$H$_{30}$N$_3$O, MH$^+$)	requires: 412.2389, found: 412.2400

N-[4-(3-Butyl-2-oxo-5-*p*-tolyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (240)
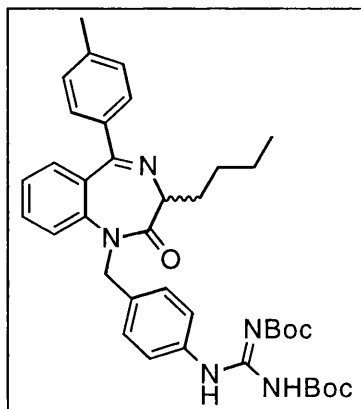
Compound **240** was synthesised from **239** (121 mg, 0.29 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (138 mg, 0.5 mmol), $HgCl_2$ (136 mg, 0.5 mmol) and triethylamine (152 mg, 1.5 mmol) using the procedure outlined in Method 5.

yield:	101 mg, 0.15 mmol, 53% (light yellow foam)
^{1}H nmr (300 MHz, $CDCl_3$) ∂	1.0 (t, 3H, J = 6 Hz), 1.2-1.7 (m, 22H), 2.2 (m, 2H), 2.4 (s, 3H), 3.6 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 7.1-7.5 (m, 12H)
I.R. (v_{max})	3416, 1718, 1638, 1560, 1152 cm^{-1}
MS(FAB) (m/z %)	654 (MH$^+$, 10), 454 (40), 382 (100), 192 (61)
HRMS: ($C_{38}H_{48}N_5O_5$, MH$^+$)	requires: 654.3655, found: 654.3640

N-[4-(3-Butyl-2-oxo-5-*p*-tolyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (136)
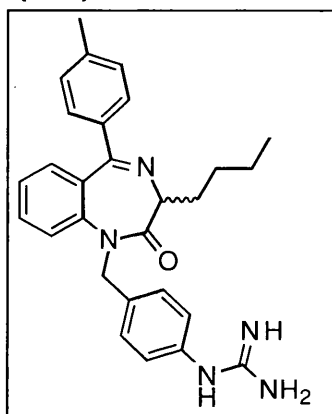
Compound **136** was synthesised from **240** (101 mg, 0.15 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield	20.6 mg, 0.03 mmol, 20% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	0.9 (t, 3H, J = 6 Hz, CH_3CH$_2$R), 1.2-1.5 (m, 4H, RCH_2R), 2.1 (m, 2H, RCHCH_2R), 2.45 (s, 3H, PhCH_3), 3.6 (t, 1H, J = 6 Hz, CHCH$_2$R), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 7.0 (s, 4H, Ph), 7.2-7.6 (m, 10H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (ν_{max})	3130, 2952, 1696, 1602, 1202 cm^{-1}
MS(CI) (m/z %)	454 (MH$^+$-CF$_3$CO$_2$$^-$, 40), 227 (MH^{2+}, 100)
analysis: $C_{28}H_{31}N_5O$.2.34TFA.1.6H_2O effective molecular weight [749.22]	calc: C, 52.39; H, 4.9; F, 17.8; N, 9.4; O, 15.5 found: C, 52.01; H, 4.5; F, 17.9; N, 9.8; O, 14.02

3-Benzyl-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (131)
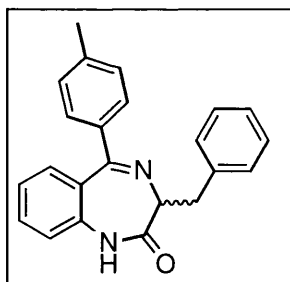
Compound **131** was synthesised by the procedures outlined in Methods 1 and 2 using **103** (211 mg, 1 mmol), ethyl chloroformate (152 mg; 1.4 mmol), triethylamine (141 mg, 1.4 mmol) and (±) N-Boc phenylalanine (345 mg, 1.3 mmol).

yield:	200 mg, 0.59 mmol, 59% (light yellow crystals)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	2.4 (s, 3H), 3.6 (d, 2H, J = 3 Hz), 3.8 (t, 1H, J = 3 Hz), 7.1-7.6 (m, 13H)
I.R. (ν_{max})	3404, 2962, 1684, 1601, 1322 cm^{-1}

| MS(EI) (m/z %) | 341 (MH$^+$, 100) |
| HRMS: (C$_{23}$H$_{21}$N$_2$O, MH$^+$) | requires: 341.1654, found: 341.1640 |

## 3-Benzyl-1-(4-nitrobenzyl)-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (134)
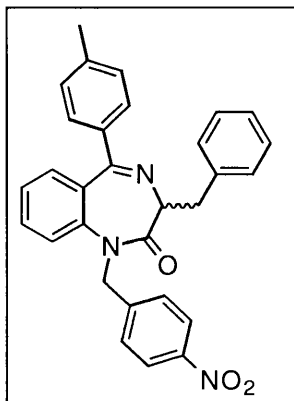
Compound **134** was synthesised from **131** (200 mg, 0.59 mmol), 4-nitrobenzyl bromide (162 mg, 0.75 mmol) and KO^{t}Bu in THF (1 M soln, 0.8 ml, 0.8 mmol) using the procedure outlined in Method 3.

yield:	240 mg, 0.51 mmol, 86% (light yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	2.4 (s, 3H), 3.6 (m, 2H), 3.9 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 8 Hz), 5.7 (d, 1H, J = 8 Hz), 7.1-7.6 (m, 17H)
I.R. (v$_{max}$)	2930, 1678, 1590, 1345 cm^{-1}
MS(FAB) (m/z %)	476 (MH$^+$, 100)
HRMS: (C$_{30}$H$_{26}$N$_3$O$_3$, MH$^+$)	requires: 476.1974, found: 476.1960

1-(4-Aminobenzyl)-3-benzyl-5-*p*-tolyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (241)
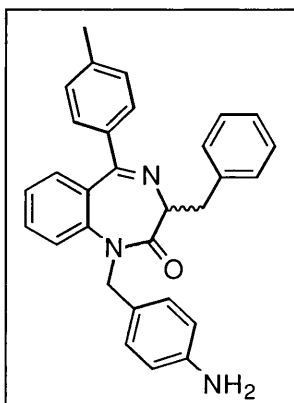
Compound **241** was synthesised from **134** (240 mg; 0.50 mmol) and tin (II) chloride (568 mg: 2.53 mmol) using the procedure outlined in Method 4

yield:	210 mg, 0.47 mmol, 94% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	2.4 (s, 3H), 3.6 (d, 2H, J = 4 Hz), 3.9 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 8 Hz), 5.7 (d, 1H, J = 8 Hz), 7.0-7.6 (m, 17H)
I.R. (ν_{max})	3412, 1682, 1601 cm^{-1}
MS(FAB) (m/z %)	446 (MH$^+$, 46), 341 (100)
HRMS: (C$_{30}$H$_{28}$N$_3$O, MH$^+$)	requires: 446.2232, found: 446.3344

N-[4-(3-Benzyl-2-oxo-5-*p*-tolyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (242)
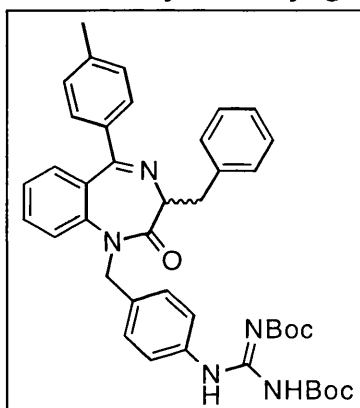
Compound **242** was synthesised from **241** (*210* mg, 0.47 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (167 mg, 0.60 mmol), HgCl$_2$ (163 mg, 0.60 mmol) and triethylamine (182 mg, 1.80 mmol) using the procedure outlined in Method 5.

yield:	147 mg, 0.21 mmol, 46% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 2.4 (s, 3H), 3.7 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.9-7.4 (m, 17H)
I.R. (ν_{max})	1677, 1601, 1522, 1344 cm^{-1}
MS(FAB) (m/z %)	688 (MH$^+$, 3), 488 (10), 382 (85), 192 (100)
HRMS: (C$_{41}$H$_{46}$N$_5$O$_5$, MH$^+$)	requires: 688.3499, found: 688.3480

N-[4-(3-Benzyl-2-oxo-5-*p*-tolyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (137)
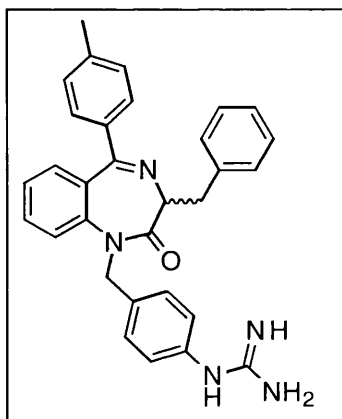
Compound **137** was synthesised from **242** (147 mg, 0.21 mmol) and trifluoroacetic acid (1 ml) using the procedure outlined in Method 7.

yield	23.3 mg, 0.03 mmol, 14% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	2.45 (s, 3H, PhCH_3), 3.40 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.85 (dd, 1H, J = 6 Hz and 7 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCH*H*Ph), 5.5 (d, 1H, J = 18 Hz, NCH*H*Ph), 7.0 (s, 4H, Ph), 7.1-7.7 (m, 17H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (v_{max})	3164, 1684, 1601, 1202 cm^{-1}
MS(EI) (m/z %)	488 (MH$^+$-CF$_3$CO$_2^-$, 2), 445 (15), 339 (35), 297 (60), 249 (75), 106 (100)
analysis: C$_{31}$H$_{29}$N$_5$O.2.5TFA.1.1H$_2$O effective molecular weight [792.47]	calc: C, 54.56; H, 4.29; F, 17.98; N, 8.84; O, 14.3 found: C, 54.19; H, 4.13; F, 17.9; N, 8.88; O, 13.8

(2-Aminophenyl)-(4-methoxyphenyl)-methanone (104)
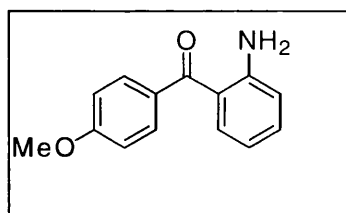
Compound **104** was synthesised in two steps from 4-bromoanisole (935 mg, 5 mmol), magnesium (139 mg, 5.8 mmol) and 2-phenyl-4H-3,1-benzoxazin-4-one (1.19 g, 5.3 mmol) using the procedures outlined in Methods 8 and 9.

yield:	625 mg, 2.75 mmol, 55%, yellow crystals
Mp	74-75 °C(EtOAc-Hexane); lit 78-80°C[131]
I.R. (v_{max})	3363, 1719, 1599, 1511, 1253 cm^{-1}
MS(FAB) (m/z %)	228 (MH$^+$, 100)
HRMS: ($C_{14}H_{14}NO_2$, MH$^+$)	requires: 228.1024, found: 228.1015

3-Benzyl-5-(4-methoxyphenyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (139)
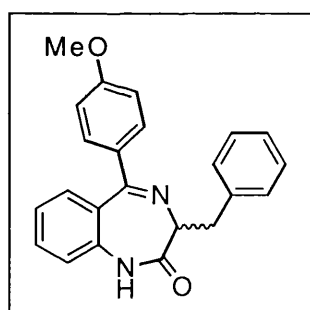
Compound **139** was synthesised by the procedures outlined in Methods 1 and 2 using **104** (227 mg; 1 mmol), ethyl chloroformate (152 mg; 1.4 mmol), triethylamine (141 mg, 1.4 mmol) and (±) N-Boc phenylalanine (318 mg; 1.2 mmol).

yield	248 mg, 0.70 mmol, 70% (light yellow crystals)
Mp	203-205°C (EtOAc-hexane)
^{1}H nmr (200 MHz, CDCl$_3$) δ	3.65 (m, 2H), 3.8 (m, 1H), 7.2-7.6 (m, 13H)

I.R. (v$_{max}$)	3178, 3055, 2955, 1667, 1593, 1249 cm^{-1}
MS(FAB) (m/z, %)	357 (MH$^+$, 100)
HRMS: (C$_{23}$H$_{21}$N$_2$O$_2$, MH$^+$)	requires: 357.1603, found: 357.1610

3-Benzyl-5-(4-methoxyphenyl)-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (141)
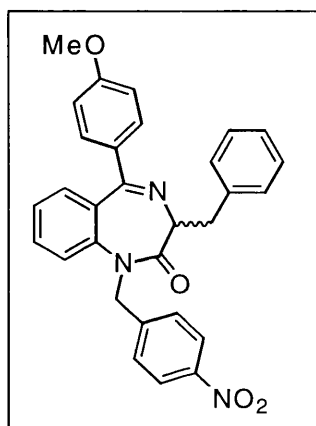
Compound **141** was synthesised from **139** (248 mg, 0.70 mmol), 4-nitrobenzyl bromide (196 mg, 0.90 mmol) and KO^{t}Bu in THF (1 M soln, 0.90 ml, 0.90 mmol) using the procedure outlined in Method 3.

yield:	334 mg, 0.68 mmol, 97% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.65 (dd, 2H, J = 6 Hz and 7 Hz), 3.8 (m, 4H), 4.9 (d, 1H, J = 15 Hz), 5.7 (d, 1H, J = 15 Hz), 7.2-7.6 (m, 17H)
I.R. (v$_{max}$)	1678, 1601, 1522, 1346, 1253 cm^{-1}
MS(FAB) (m/z %)	492 (MH$^+$, 100)
HRMS: (C$_{30}$H$_{26}$N$_3$O$_4$, MH$^+$)	requires: 492.1923, found: 492.1910

1-(4-Aminobenzyl)-3-benzyl-5-(4-methoxyphenyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (243)
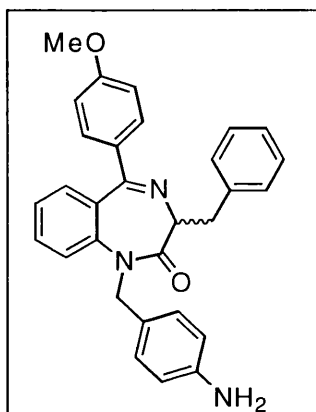
Compound **243** was synthesised from **141** (334 mg, 0.68 mmol) and tin (II) chloride (450 mg, 2 mmol) using the procedure outlined in Method 4.

yield:	300 mg, 0.65 mmol, 96%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.7 (t, 2H, J = 7 Hz), 3.8-3.9 (m, 4H), 4.7 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.8-7.0 (m, 4H), 7.05-7.4 (m, 13H)
I.R. (v_{max})	3364, 2932, 1675, 1600, 1511, 1252 cm^{-1}
MS(FAB) (m/z %)	462 (MH$^+$, 100), 357 (100)
HRMS: (C$_{30}$H$_{28}$N$_3$O$_2$, MH$^+$)	requires: 462.2182, found: 462.2170

N-{4-[3-Benzyl-5-(4-methoxyphenyl)-2-oxo-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (244)
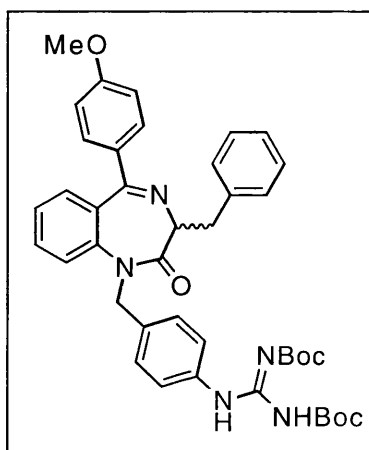
Compound **244** was synthesised from **243** (300 mg, 0.90 mmol), mercury (II) chloride (266 mg, 0.98 mmol), triethylamine (295 mg, 2.94 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (270 mg, 0.98 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	340 mg, 0.48 mmol, 54% (light brown foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.6 (d, 2H, J = 6 Hz), 3.8 (t, 1H, J = 6 Hz), 3.85 (s, 3H), 4.9 (d, 1H, J = 15 Hz), 5.5 (d, 1H, J = 15 Hz), 7.2-7.5 (m, 17H)
I.R. (ν$_{max}$)	2921, 1782, 1717, 1641, 1542, 1311, 1128 cm^{-1}
MS(FAB) (m/z %)	704 (MH$^+$, 2), 504 (MH$^+$-2 Boc, 10), 382 (100)
HRMS: (C$_{41}$H$_{46}$N$_5$O$_6$, MH$^+$)	requires: 704.3448, found: 704.3470

N-{4-[3-Benzyl-5-(4-methoxyphenyl)-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-yl methyl]phenyl} guanidine (143)
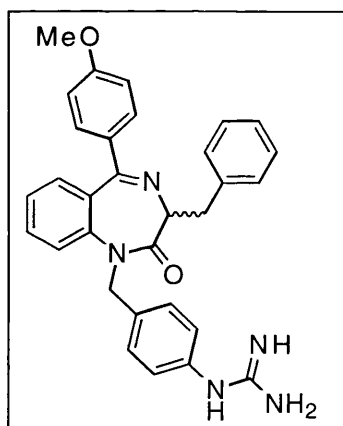
Compound **143** was synthesised from **244** (340 mg, 0.48 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	34.8 mg, 0.06 mmol, 12% (white foam)

^{1}H nmr (360 MHz, DMSO) ∂	3.40 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHCH*H*Ph), 3.8 (m, 4H, OC*H₃* and C*H*CH₂Ph), 5.0 (d, 1H, J = 18 Hz, NCH*H*Ph), 5.4 (d, 1H, J = 18 Hz, NCH*H*Ph), 6.9-7.7 (m, 18H, Ph and NH)
I.R. (ν$_{max}$)	3152, 1676, 1601, 1513, 1254 cm^{-1}
MS(FAB) (m/z %)	504 (MH$^+$-CF₃CO₂$^-$, 100)
analysis: C₃₁H₂₉N₅O₂.0.5TFA.2H₂O effective molecular weight [596.63]	calc: C, 64.41; H, 5.66; F, 4.77; N, 11.74; O, 13.41 found: C, 64.20; H, 5.33; F, 4.4; N, 11.64; O, 10.27

3-Butyl-5-(4-methoxyphenyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (138)
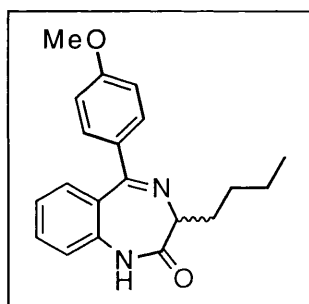
Compound **138** was synthesised by the procedures outlined in Methods 1 and 2 using **104** (601 mg; 2.65 mmol), ethyl chloroformate (382 mg; 3.5 mmol), triethylamine (354 mg, 3.5 mmol) and (±) N-Boc norleucine (739 mg; 3.2 mmol).

yield	535 mg, mmol, 1.66 mmol, 63% (light yellow crystals)
Mp	160-164°C (EtOAc-hexane)
^{1}H nmr (400 MHz, CDCl₃) δ	0.95 (t, 3H, J = 7.2 Hz), 1.36-1.62 (m, 4H), 2.14-2.28 (m, 2H), 3.49 (dd, 1H, J = 5.6 Hz and 8.4 Hz), 3.83 (s, 3H), 6.88 (m, 2H), 7.1-7.5 (m, 6H), 9.04 (s, 1H)

^{13}C-nmr (CDCl$_3$) ∂	14.2, 22.8, 28.5, 30.9, 55.3, 63.1, 113.5, 121.1, 123.1, 127.8, 131.1, 131.3, 131.4, 132.1, 138.5, 161.2, 168.4, 172.6
I.R. (v_{max})	3174, 2959, 2923, 1678, 1595, 1478, 1251 cm^{-1}
MS(FAB) (m/z, %)	323 (MH$^+$, 100)
HRMS: (C$_{20}$H$_{23}$N$_2$O$_2$, MH$^+$)	requires: 323.1760, found: 323.1770

3-Butyl-5-(4-methoxyphenyl)-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (140)
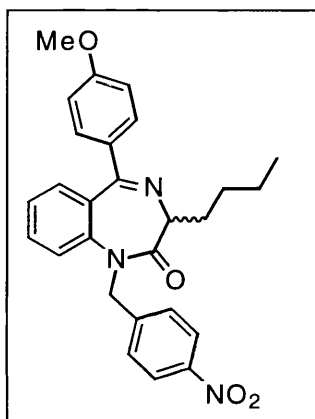
Compound **140** was synthesised from **138** (322 mg, 1 mmol), 4-nitrobenzyl bromide (281 mg, 1.3 mmol) and KO^{t}Bu in THF (1 M soln, 1.3 ml, 1.3 mmol) using the procedure outlined in Method 3.

yield:	425 mg, 0.93 mmol, 93% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.0 (t, 3H, J = 6 Hz), 1.3-1.6 (m, 4H), 2.2-2.3 (m, 2H), 3.6 (t, 1H, J = 6 Hz), 3.9 (s, 3H), 4.9 (d, 1H, J = 15 Hz), 5.8 (d, 1H, J = 15 Hz), 6.8 (d, 2H, J = 7 Hz), 7.2-7.6 (m, 8H), 8.0 (d, 2H, J = 8 Hz)
I.R. (v_{max})	2953, 1678, 1602, 1522, 1345, 1251 cm^{-1}
MS(FAB) (m/z %)	458 (MH$^+$, 100)
HRMS: (C$_{27}$H$_{28}$N$_3$O$_4$, MH$^+$)	requires: 458.2080, found: 458.2070

1-(4-Aminobenzyl)-3-butyl-5-(4-methoxyphenyl)-1,3-dihydrobenzo [e][1,4]diazepin-2-one (245)
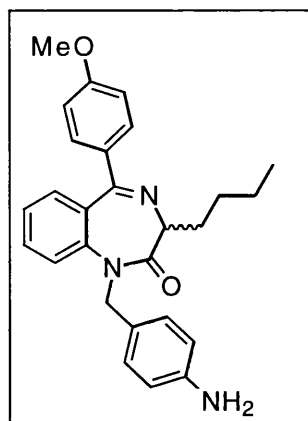
Compound **245** was synthesised from **140** (425 mg, 0.93 mmol) and tin (II) chloride (628 mg, 2.79 mmol) using the procedure outlined in Method 4.

yield:	358 mg, 0.84 mmol, 90%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.0 (t, 3H, J = 6 Hz), 1.2-1.4 (m, 4H), 2.2 (m, 2H), 3.6 (t, 1H, J = 6 Hz), 3.9 (s, 3H), 4.7 (d, 1H, J = 17 Hz), 5.6 (d, 1H, J = 17 Hz), 6.4 (d, 2H, J = 8 Hz), 6.8 (d, 2H, J = 8 Hz), 6.9 (d, 1H, J = 7 Hz), 7.1-7.5 (m, 7H)
I.R. (v$_{max}$)	3448, 2954, 1678, 1601, 1522, 1252 cm^{-1}
MS(FAB) (m/z %)	428 (MH$^+$, 70), 323 (100)
HRMS: (C$_{27}$H$_{30}$N$_3$O$_2$, MH$^+$)	requires: 428.2338, found: 428.2350

N-{4-[3-Butyl-5-(4-methoxyphenyl)-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-yl methyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (246)
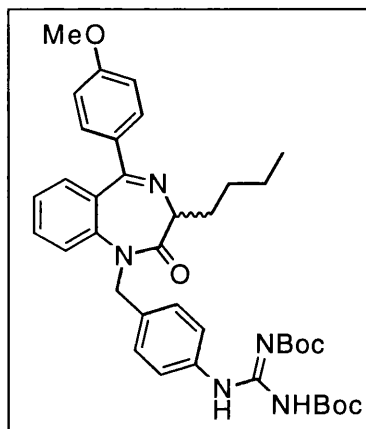
Compound **246** was synthesised from **245** (358 mg, 0.84 mmol), mercury (II) chloride (249 mg, 0.92 mmol), triethylamine (279 mg, 2.77 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (255 mg, 0.92 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	288 mg, 0.41 mmol, 49% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.0 (t, 3H, J = 6 Hz), 1.2-1.6 (m, 22H), 2.2 (m, 2H), 3.6 (d, 2H, J = 6 Hz), 3.8-3.9 (m, 4H), 4.9 (d, 1H, J = 16 Hz), 5.6 (d, 1H, J = 16 Hz), 6.8-7.4 (m, 12H), 10.2 (bs, 1H)
I.R. (v$_{max}$)	2980, 1782, 1718, 1683, 1642, 1151 cm^{-1}
MS(FAB) (m/z %)	670 (MH$^+$, 20), 470 (MH$^+$-2 Boc, 100)
HRMS: (C$_{38}$H$_{48}$N$_5$O$_6$, MH$^+$)	requires: 670.3605, found: 670.3620

208

N-{4-[3-Butyl-5-(4-methoxyphenyl)-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-yl methyl]phenyl} guanidine (142)
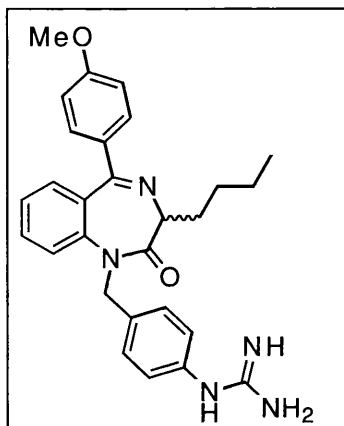
Compound **142** was synthesised from **246** (288 mg, 0.41 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	57.6 mg, 0.10 mmol, 25% (pale yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	0.9 (t, 3H, J = 6 Hz, RCH$_2$CH_3), 1.2-1.5 (m, 4H, RCH_2CH_2R), 2.1 (m, 2H, RCHCH_2R), 3.6 (t, 1H, J = 6 Hz, CHCH$_2$R), 3.8 (s, 3H, OCH_3), 5.0 (d, 1H, J = 18 Hz, NCHHPh),5.4 (d, 1H, J = 18 Hz, NCHHPh), 7.0-7.1 (m, 6H, Ph), 7.2-7.7 (m, 7H, Ph and NH)
I.R. (ν$_{max}$)	3377, 2955, 1676, 1601, 1253 cm^{-1}
MS(EI) (m/z %)	469 (M$^+$-CF$_3$CO$_2^-$, 3), 427 (5), 321 (60), 279 (50), 236 (35), 106 (100)
analysis:	calc: C, 63.43; H, 5.78; N, 12.58;
C$_{28}$H$_{31}$N$_5$O$_2$.0.7TFA.0.4H$_2$O	O, 10.06
effective molecular weight [556.59]	found: C, 63.25; H, 6.02; N, 12.63; O, 10.12

(2-Aminophenyl)-(4-chlorophenyl) methanone (105)
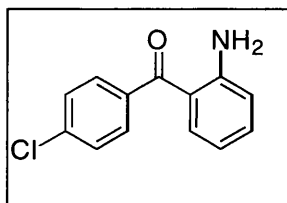
Compound **105** was synthesised in two steps from 4-bromochlorobenzene (2.292 g, 12 mmol), magnesium (288 mg, 12 mmol) and 2-phenyl-4H-3,1-benzoxazin-4-one (2.5 g, 11.4 mmol) using the procedures outlined in Methods 8 and 9.

yield:	1.204 g, 5.21 mmol, 46% (yellow crystals)
Mp	92-94 °C(EtOAc-Hexane); lit 98-99°[132]
I.R. (v_{max})	3417, 3313, 1617, 1534, 1239, 1151 cm^{-1}
MS(FAB) (m/z %)	232 (MH$^+$, 100), 234 (30), 154 (30), 105 (50)
HRMS: (C$_{13}$H$_{11}$^{35}ClNO, MH$^+$)	requires: 232.0529, found: 232.0540

5-(4-Chlorophenyl)-3-methyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (144)
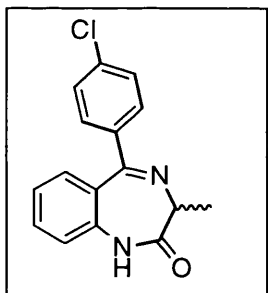
Compound **144** was synthesised by the procedures outlined in Methods 1 and 2 using **105** (400 mg; 1.73 mmol), ethyl chloroformate (227 mg; 2.1 mmol), triethylamine (212 mg, 2.1 mmol) and (±) N-Boc alanine (378 mg, 2.0 mmol).

yield	350 mg, 1.23 mmol, 71% (light yellow powder)
Mp	231-233 °C(EtOAc-Hexane); lit 234-235°C (DCM-MeOH)[133]
^{1}H nmr (200 MHz, CDCl$_3$) δ	1.75 (d, 2H, J = 4 Hz), 3.7 (q, 1H, J = 4 Hz), 7.1-7.6 (m, 8H), 9.1 (s, 1H)
I.R. (v_{max})	3201, 3878, 1678, 1610 cm^{-1}
MS(FAB) (m/z, %)	287 (MH$^+$, 30), 285 (MH$^+$, 100)
HRMS: (C$_{16}$H$_{14}$^{35}ClN$_2$O, MH$^+$)	requires: 285.0795, found: 285.0786

5-(4-Chlorophenyl)-3-methyl-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (146)
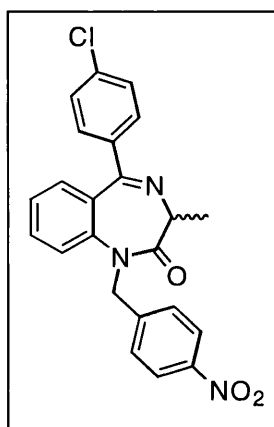
Compound **146** was synthesised from **144** (350 mg, 1.23 mmol), 4-nitrobenzyl bromide (324 mg, 1.5 mmol) and KO^{t}Bu in THF (1 M soln, 1.5 ml, 1.5 mmol) using the procedure outlined in Method 3.

yield:	292 mg, 0.69 mmol, 57% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	1.8 (d, 3H, J = 4 Hz), 3.9 (q, 1H, J = 4 Hz), 4.9 (d, 1H, J = 12 Hz), 5.7 (d, 1H, J = 12 Hz), 7.1-7.6 (m, 12H)
I.R. (v$_{max}$)	1677, 1605, 1518, 1345 cm^{-1}
MS(FAB) (m/z %)	422 (MH$^+$, 30), 420 (MH$^+$, 100), 285 (30), 154 (65), 136 (71)
HRMS: (C$_{23}$H$_{19}$^{35}ClN$_3$O$_3$, MH$^+$)	requires: 420.1115, found: 420.1130

1-(4-Aminobenzyl)-5-(4-chlorophenyl)-3-methyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (247)
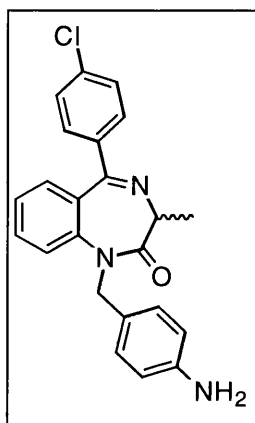
Compound **247** was synthesised from **146** (255 mg, 0.61 mmol) and tin (II) chloride (414 Mg, 1.83 mmol) using the procedure outlined in Method 4.

yield:	233 mg, 0.60 mmol, 98%, (yellow foam)
¹H nmr (200 MHz, CDCl₃) ∂	1.75 (d, 1H, J = 6 Hz), 3.8 (q, 1H, J = 6 Hz), 4.9 (d, 1H, J = 10 Hz), 5.6 (d, 1H, J = 10 Hz), 7.1-7.6 (m, 12H)
I.R. (v_{max})	3353, 1670, 1607, 1607, 1517 cm⁻¹
MS(FAB) (m/z %)	392 (MH⁺, 9), 390 (MH⁺, 34), 287 (30), 285 (100)
HRMS: ($C_{23}H_{21}{}^{35}ClN_3O$, MH⁺)	requires: 390.1373, found: 390.1360

**N-{4-[5-(4-Chlorophenyl)-3-methyl-2-oxo-2,3-dihydrobenzo[e][1,4]
diazepin-1-ylmethyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine
(248)**
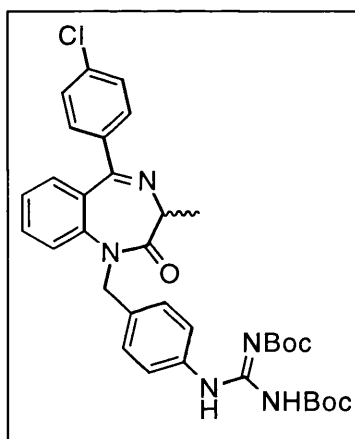
Compound **248** was synthesised from **247** (233 mg, 0.60 mmol), mercury (II) chloride (184 mg, 0.68 mmol), triethylamine (207 mg, 2.05 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (188 mg, 0.68 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	179 mg, 0.28 mmol, 47% (yellow foam)
¹H nmr (300 MHz, CDCl₃) ∂	1.6 (s, 18H), 1.8 (d, 3H, J = 6 Hz), 4.8 (d, 1H, J = 15 Hz), 5.7 (d, 1H, J = 15 Hz), 7.0-7.5 (m, 12H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (v_{max})	2979, 2932, 1719, 1686, 1638, 1607, 1151 cm⁻¹

MS(FAB) (m/z %)   632 (MH$^+$, 10), 434 (20), 432 (MH$^+$-2 Boc, 100)

HRMS: (C$_{34}$H$_{39}$^{35}ClN$_5$O$_5$, MH$^+$)   requires: 632.2640, found: 632.2651

N-{4-[5-(4-Chlorophenyl)-3-methyl-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl]phenyl} guanidine (148)
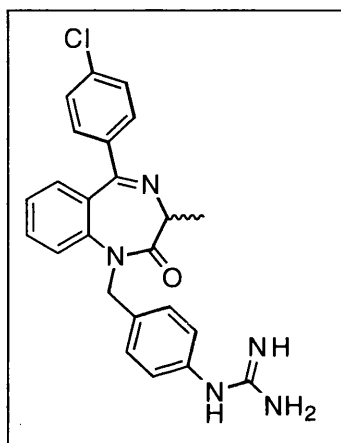
Compound **148** was synthesised from **248** (179 mg, 0.28 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	107.8 mg, 0.14 mmol, 50 % (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.6 (d, 3H, J = 6 Hz, CHCH_3), 3.8 (q, 1H, J = 6 Hz, CHCH$_3$), 5.0 (d, 1H, J = 12 Hz, NCHHPh), 5.5 (d, 1H, J = 12 Hz, NCHHPh), 7.0 (s, 4H, Ph), 7.2 (m, 2H, Ph), 7.3-7.8 (m, 10H, Ph and NH), 9.7 (s, 1H, NH)
I.R. (v_{max})	3382, 1700, 1601, 1203 cm^{-1}
MS(FAB) (m/z %)	434 (MH$^+$-CF$_3$CO$_2^-$, 36), 432 (MH$^+$-CF$_3$CO$_2^-$, 100), 257 (36), 237 (32)
analysis:	calc: C, 45.32; H, 3.56; F, 20.93;
C$_{24}$H$_{22}$ClN$_5$O.2.9TFA.1.5H$_2$O	N, 8.87; O, 16.82
effective molecular weight [789.62]	found: C, 45.32; H, 3.28; F, 21.0; N, 8.97; O, 17.62

3-Benzyl-5-(4-chlorophenyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (145)
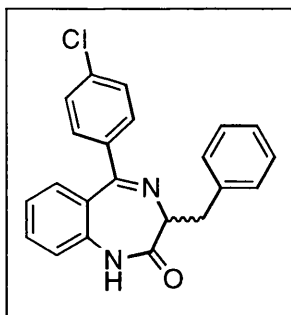
Compound **145** was synthesised by the procedures outlined in Methods 1 and 2 using **105** (351 mg; 1.52 mmol), ethyl chloroformate (227 mg, 2.1 mmol), triethylamine (212 mg, 2.1 mmol) and (±) N-Boc phenylalanine (530 mg; 2 mmol).

|	
yield	191 mg, 0.53 mmol, 35% (light yellow crystals)
Mp	243-245°C (EtOAc-hexane)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.6 (d, 2H, J = 5 Hz), 3.8 (t, 1H, J = 5 Hz), 7.0-7.6 (m, 13H)
I.R. (v_{max})	3429, 1681, 1605, 1322, 1301 cm^{-1}
MS(FAB) (m/z, %)	363 (MH$^+$, 35), 361 (MH$^+$, 100)
HRMS: (C$_{22}$H$_{18}$^{35}ClN$_2$O, MH$^+$)	requires: 361.1108, found: 361.1100

3-Benzyl-5-(4-chlorophenyl)-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (147)
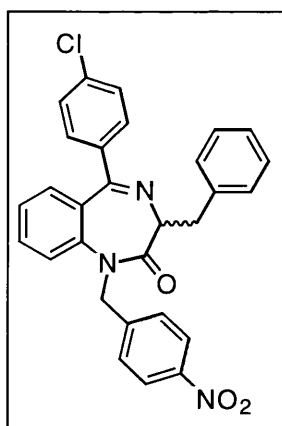
Compound **147** was synthesised from **145** (191 mg, 0.53 mmol), 4-nitrobenzyl bromide (173 mg, 0.8 mmol) and KO^{t}Bu in THF (1 M soln, 0.8 ml, 0.8 mmol) using the procedure outlined in Method 3.

yield:	260 mg, 0.52 mmol, 98% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	3.6 (d, 2H, J = 4 Hz), 3.9 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 12 Hz), 5.65 (d, 1H, J = 12 Hz), 7.1-7.4 (m, 17H)
I.R. (v_{max})	3060, 1679, 1610, 1520, 1343 cm^{-1}
MS(FAB) (m/z %)	498 (MH$^+$, 40), 496 (MH$^+$, 100)
HRMS: (C$_{29}$H$_{23}$^{35}ClN$_3$O$_3$, MH$^+$)	requires: 496.1428, found: 496.1440

1-(4-Aminobenzyl)-3-benzyl-5-(4-chlorophenyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (249)
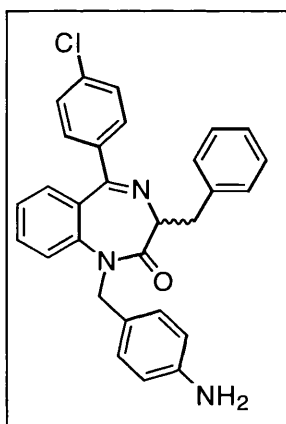
Compound **249** was synthesised from **147** (260 mg, 0.52 mmol) and tin (II) chloride (355 mg, 1.57 mmol) using the procedure outlined in Method 4.

yield:	252 mg, 0.51 mmol, 98%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.7 (dd, 2H, J = 5 Hz and 7 Hz), 3.9 (dd, 1H, J = 5 Hz and 7 Hz), 4.9 (d, 1H, J = 17 Hz), 5.7 (d, 1H, J = 17 Hz), 7.1-7.5 (m, 17H)
I.R. (v_{max})	3446, 3341, 1669, 1600, 1450 cm^{-1}
MS(FAB) (m/z %)	468 (MH$^+$, 15), 466 (MH$^+$, 50), 363 (35), 365 (100)
HRMS: (C$_{29}$H$_{25}$^{35}ClN$_3$O, MH$^+$)	requires: 466.1686, found: 466.1670

N-{4-[3-Benzyl-5-(4-chlorophenyl)-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (250)
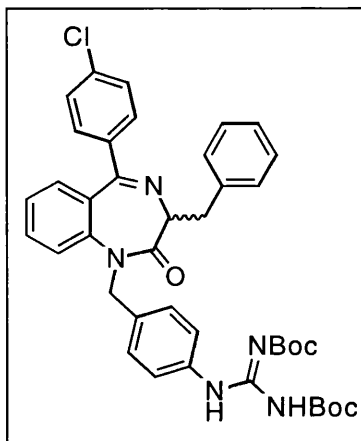

Compound **250** was synthesised from **249** (252 mg, 0.51 mmol), mercury (II) chloride (165 mg, 0.61 mmol), triethylamine (185 mg, 1.83 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (169 mg, 0.61 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	112 mg, 0.16 mmol, 31% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.6 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 14 Hz), 5.6 (d, 1H, J = 14 Hz), 7.0-7.4 (m, 17H), 10.2 (bs, 1H)
I.R. (ν$_{max}$)	3414, 2979, 1686, 1607, 1240, 1150 cm^{-1}
MS(FAB) (m/z %)	708 (MH$^+$, 3), 510 (MH$^+$-2 Boc, 10), 508 (MH$^+$-2 Boc, 25), 382 (100)
HRMS: (C$_{40}$H$_{43}$^{35}ClN$_5$O$_5$)	requires: 708.2953, found: 708.2940

N-{4-[3-Benzyl-5-(4-chlorophenyl)-2-oxo-2,3-dihydrobenzo[e][1,4]diazepin-1-ylmethyl]phenyl} guanidine (149)
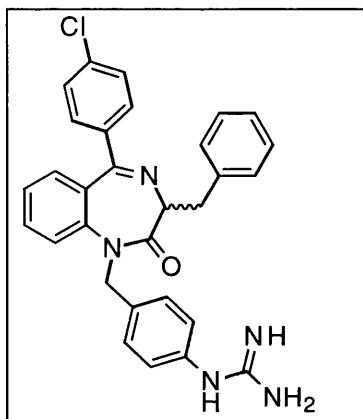
Compound **149** was synthesised from **250** (112 mg, 0.16 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	13.6 mg, 0.02 mmol, 10% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.40 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.9 (dd, 1H, J = 6 Hz and 9 Hz, C*H*CH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCH*H*Ph), 5.5 (d, 1H, J = 18 Hz, NCH*H*Ph), 7.0 (s, 4H, Ph), 7.1-7.8 (m, 17H, Ph and NH), 9.55 (s, 1H, NH)
I.R. (v_{max})	3163, 1675, 1601, 1201 cm^{-1}
MS(FAB) (m/z %)	510 (MH$^+$-CF$_3$CO$_2^-$, 36), 508 (MH$^+$-CF$_3$CO$_2^-$, 100), 275 (24)
analysis:	calc: C, 50.56; H, 3.72; N, 8.28.
C$_{30}$H$_{26}$ClN$_5$O.2.8TFA.1.2H$_2$O effective molecular weight [845.7]	found: C, 50.67; H, 3.39; N, 8.58.

(2-Aminophenyl)-(2-chlorophenyl) methanone (106)
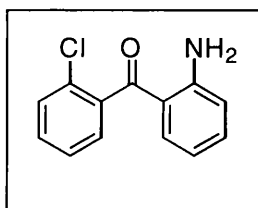
Compound **106** was synthesised in two steps from 2-bromochlorobenzene (10.18 g, 54.3 mmol), Mg (1.303 g, 54.3 mmol) and 2-phenyl-4H-3,1-benzoxazin-4-one (10.00 g, 45.6 mmol) using the procedures outlined in Methods 8 and 9.

yield:	2.478 g, 10.7 mmol, 24% (yellow crystals)
Mp	55-56 °C(EtOAc-Hexane); lit 58-60°[134]
I.R. (v_{max})	3434, 3327, 1617, 1592, 1532, 1239 cm^{-1}
MS(FAB) (m/z %)	234 (MH$^+$, 30), 232 (MH$^+$, 100), 196 (35)
HRMS: ($C_{13}H_{11}{}^{35}ClNO$, MH$^+$)	requires: 232.0529, found: 232.0540

5-(2-Chlorophenyl)-3-methyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (150)
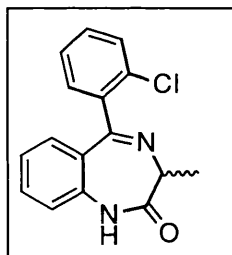
Compound **150** was synthesised by the procedures outlined in Methods 1 and 2 using **106** (1.00 g; 4.33 mmol), ethyl chloroformate (456 mg; 4.2 mmol), triethylamine (424 mg, 4.2 mmol) and (±) N-Boc alanine (794 mg; 4.2 mmol).

yield	200 mg, mmol, 0.70 mmol, 17% (white powder)
Mp	81-84 °C(EtOAc-Hexane); lit 82-85°C d (EtOAc-Hexane)[133]
^{1}H nmr (200 MHz, CDCl$_3$)	1.6 (d, 3H, J = 5 Hz), 3.8 (q, 1H, J = 5 Hz), 7.1-7.5 (m, 8H), 9.1 (bs, 1H)
I.R. (v_{max})	3200, 3078, 1679, 1611, 1479, 1330 cm^{-1}
MS(FAB) (m/z, %)	287 (MH$^+$, 32), 285 (MH$^+$, 100)
HRMS: ($C_{16}H_{14}{}^{35}ClN_2O$, MH$^+$)	requires: 285.0795, found: 285.0785

5-(2-Chlorophenyl)-3-methyl-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (151)
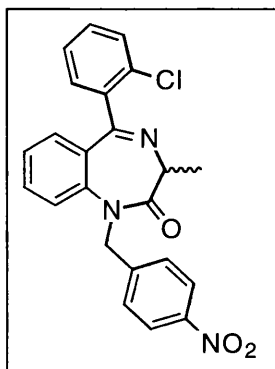
Compound **151** was synthesised from **150** (200 mg, 0.7 mmol), 4-nitrobenzyl bromide (228 mg, 1.05 mmol) and KO^{t}Bu in THF (1 M soln, 1.1 ml, 1.1 mmol) using the procedure outlined in Method 3.

yield:	750 mg, 1.95 mmol, 97% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	1.8 (d, 3H, J = 5 Hz), 3.9 (q, 1H, J = 5 Hz), 5.2 (d, 1H, J = 15 Hz), 5.25 (d, 1H, J = 15 Hz), 7.1-7.5 (m, 10H), 8.1 (d, 2H, J = 10 Hz)
I.R. (ν$_{max}$)	2982, 1685, 1605, 1584, 1341 cm^{-1}
MS(FAB) (m/z %)	422 (MH$^+$, 35), 420 (MH$^+$, 100), 285 (25)
HRMS: (C$_{23}$H$_{19}$^{35}ClN$_3$O$_3$, MH$^+$)	requires: 420.1115, found: 420.1125

1-(4-Aminobenzyl)-5-(2-chlorophenyl)-3-methyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (251)
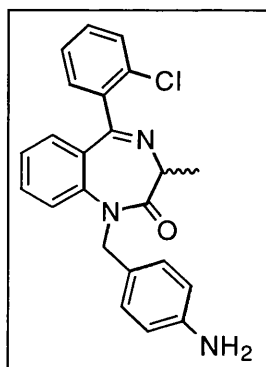
Compound **251** was synthesised from **151** (750 mg, 1.95 mmol) and tin (II) chloride (1.200 g, 5.3 mmol) using the procedure outlined in Method 4.

yield:	330 mg, 0.93 mmol, 48%, (yellow foam)
¹H nmr (300 MHz, CDCl₃) ∂	1.8 (d, 3H, J = 5 Hz), 3.85 (q, 1H, J = 5 Hz), 5.1 (d, 1H, J = 15 Hz), 5.25 (d, 1H, J = 15 Hz), 7.0-7.6 (m, 12H)
I.R. (νmax)	3357, 1670, 1607, 1516 cm⁻¹
MS(FAB) (m/z %)	392 (MH⁺, 31), 390 (MH⁺, 100),

N-{4-[5-(2-Chlorophenyl)-3-methyl-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (252)
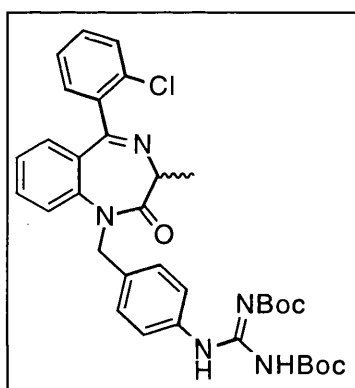
Compound **252** was synthesised from **251** (320 mg, 0.90 mmol), mercury (II) chloride (276 mg, 1.02 mmol), triethylamine (300 mg, 2.97 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (276 mg, 1.00 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	328 mg, 0.55 mmol, 71% (yellow foam)
¹H nmr (200 MHz, CDCl₃) ∂	1.6 (s, 18H), 1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 5.0 (d, 1H, J = 15 Hz), 5.1 (d, 1H, J = 15 Hz), 7.0-7.5 (m, 12H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (νmax)	2978, 1718, 1684, 1636, 1410, 1150 cm⁻¹
MS(FAB) (m/z %)	632 (MH⁺, 15), 434 (MH⁺-2 Boc, 15), 432 (MH⁺-2 Boc, 100), 285 (55)
HRMS: (C₃₄H₃₉³⁵ClN₅O₅, MH⁺)	requires: 632.2640, found: 632.2660

N-{4-[5-(2-Chlorophenyl)-3-methyl-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl]phenyl} guanidine (152)
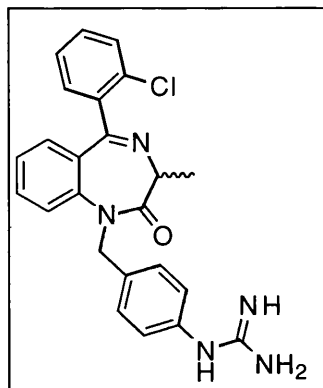
Compound **152** was synthesised from **252** (320 mg, 0.54 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	46.4 mg, 0.06 mmol, 12% (light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.6 (d, 3H, J = 6 Hz, CHCH_3), 3.75 (q, 1H, J = 6 Hz, CHCH$_3$), 5.2 (bs, 2H, NCH_2Ph), 7.0-7.6 (m, 14H, Ph and NH), 9.7 (s, 1H, NH)
I.R. (ν_{max})	3391, 1696, 1601, 1203 cm^{-1}
MS(CI) (m/z %)	434 (MH$^+$-CF$_3$CO$_2^-$, 32)), 432 (MH$^+$-CF$_3$CO$_2^-$, 100), 216 (MH^{2+}, 100)
analysis:	calc: C, 47.98; H, 3.76; Cl, 4.95;
C$_{24}$H$_{22}$ClN$_5$O.2.3TFA.1.2H$_2$O	F, 18.3; N, 9.78; O, 15.19
effective molecular weight [715.8]	found: C, 47.69; H, 3.44; Cl, 4.9; F, 18.0; N, 9.55; O, 14.19

## 3-Benzyl-7-chloro-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (154)
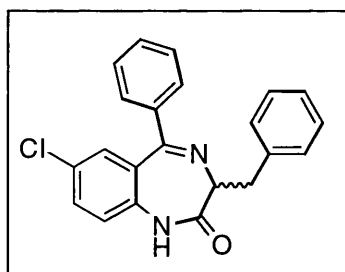
Compound **154** was synthesised by the procedures outlined in Methods 1 and 2 using 2-amino-5-chlorobenzophenone (2.387 g; 10.3 mmol), ethyl chloroformate (1.34 g; 12.3 mmol), triethylamine (1.23 g, 12.3 mmol) and (±) N-Boc phenylalanine (3.0 g; 11.3 mmol).

221

yield	1.845 g, 5.12 mmol, 55% (light yellow powder)
Mp	107-109 °C(EtOAc-Hexane); lit 108-110°C[135]
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.6 (d, 2H, J = 8 Hz), 3.85 (t, 1H, J = 8 Hz), 7.0-7.5 (m, 13H), 8.2 (s, 1H)
I.R. (ν_{max})	3203, 3060, 1682, 1604, 1478 cm^{-1}
MS(FAB) (m/z, %)	363 (MH$^+$, 30), 361 (MH$^+$, 100)
HRMS: (C$_{22}$H$_{18}$^{35}ClN$_2$O, MH$^+$)	requires: 361.1108, found: 361.1100

3-Benzyl-7-chloro-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (158)
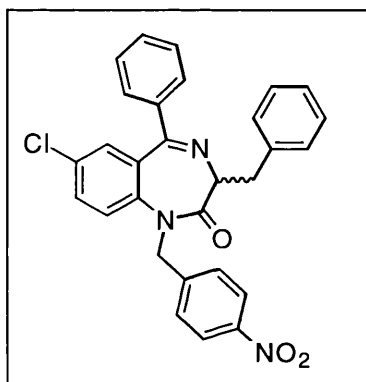
Compound **158** was synthesised from **154** (361 mg, 1 mmol), 4-nitrobenzyl bromide (238 mg, 1.1 mmol) and KO^{t}Bu in THF (1 M soln, 1.1 ml, 1.1 mmol) using the procedure outlined in Method 3.

yield:	480 mg, 0.97 mmol, 97% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.7 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 5.0 (d, 1H, J = 16 Hz), 5.7 (d, 1H, J = 16 Hz), 7.0-7.5 (m, 17H)
I.R. (ν_{max})	1679, 1605, 1520, 1340 cm^{-1}
MS(FAB) (m/z %)	498 (MH$^+$, 30), 496 (MH$^+$, 100)
HRMS: (C$_{29}$H$_{23}$^{35}ClN$_3$O$_3$, MH$^+$)	requires: 496.1428, found: 496.1441

1-(4-Aminobenzyl)-3-benzyl-7-chloro-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (253)
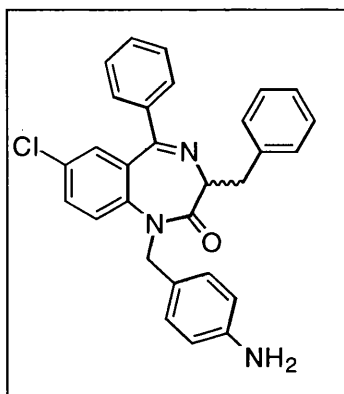
Compound **253** was synthesised from **158** (480 mg, 0.97 mmol) and tin (II) chloride (506 mg, 2.25 mmol) using the procedure outlined in Method 4.

yield:	420 mg, 0.90 mmol, 93%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.7 (d, 1H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.6 (d, 1H, J = 16 Hz), 5.6 (d, 1H, J = 16 Hz), 6.4 (d, 2H, J = 8 Hz), 6.8 (d, 2H, J = 8 Hz), 7.0-7.5 (m, 17H)
I.R. (ν_{max})	3331, 1671, 1604, 1518 cm^{-1}
MS(FAB) (m/z %)	468 (MH$^+$, 30), 466 (MH$^+$, 82), 363 (35), 361 (100)
HRMS: (C$_{29}$H$_{25}$^{35}ClN$_3$O, MH$^+$)	requires: 466.1686, found: 466.1670

N-[4-(3-Benzyl-7-chloro-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-2-ylmethyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (254)
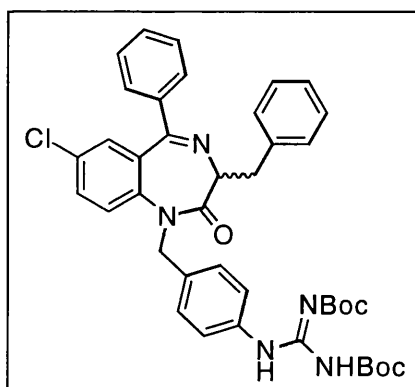
Compound **254** was synthesised from **253** (320 mg, 0.90 mmol), mercury (II) chloride (271 mg, 1.00 mmol), triethylamine (333 mg, 3.3 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (276 mg, 1.00 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	243 mg, 0.34 mmol, 38% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.5 (s, 18H), 3.6 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.7 (d, 1H, J = 12 Hz), 5.5 (d, 1H, J = 12 Hz), 6.9 (d, 2H, J = 6 Hz), 7.2-7.5 (m, 15H), 8.0 (bs, 1H), 10.2 (s, 1H)
I.R. (v_{max})	2979, 1718, 1686, 1636, 1607, 1238, 1150 cm^{-1}
MS(FAB) (m/z %)	708 (MH$^+$, 5), 510 (29), 508 (MH$^+$-2 Boc, 100)
HRMS: (C$_{40}$H$_{43}$^{35}ClN$_5$O$_5$, MH$^+$)	requires: 708.2953, found: 708.2942

N-[4-(3-Benzyl-7-chloro-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-2-ylmethyl)phenyl] guanidine (162)
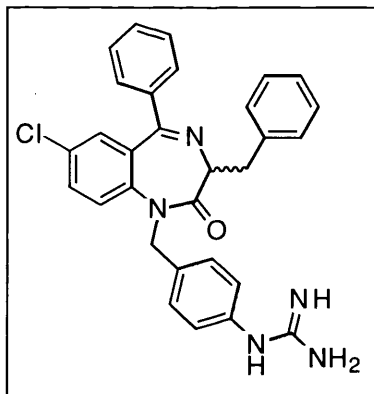
Compound **162** was synthesised from **254** (243 mg, 0.34 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	101.6 mg, 0.12 mmol, 36% (light yellow foam)

^{1}H nmr (360 MHz, DMSO) ∂	3.40 (dd, 1H, J = 6 Hz and 9 Hz, CHC*H*HPh), 3.45 (dd, 1H, J = 6 Hz and 9 Hz, CHC*H*HPh), 3.9 (dd, 1H, J = 6 Hz and 7 Hz, C*H*CH₂Ph), 4.95 (d, 1H, J = 18 Hz, NCH*H*Ph), 5.5 (d, 1H, J = 18 Hz, NCH*H*Ph), 7.0 (s, 4H, Ph), 7.1-7.7 (m, 16H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (v_{max})	3370, 1680, 1203, 701 cm⁻¹
MS(FAB) (m/z %)	510 (MH⁺-CF₃CO₂⁻, 35), 508 (MH⁺-CF₃CO₂⁻, 100), 363 (20), 361 (50), 191 (20)
analysis: C₃₀H₂₆ClN₅O.2.7TFA.1.1H₂O effective molecular weight [835.69]	calc: C, 50.87; H, 3.72; Cl, 4.24; F, 18.4; N, 8.38; O, 14.35 found: C, 50.50; H, 3.49; Cl, 4.1; F, 18.2; N, 8.14; O, 14.52

7-Chloro-3-methyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (155)
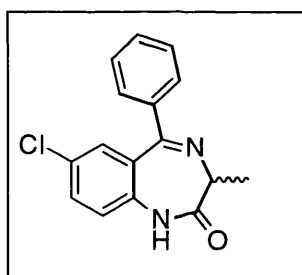
Compound **155** was synthesised by the procedures outlined in Methods 1 and 2 using 2-amino-5-chlorobenzophenone (1.622 g; 7.0 mmol), ethyl chloroformate (872 mg; 8 mmol), triethylamine (808 mg, 8 mmol) and (±) N-Boc alanine (1388 mg; 7.34 mmol).

yield	870 mg, 2.37 mmol, 34% (white powder)
Mp	221-223 °C(EtOAc-Hexane); lit 220-221°C (benzene-pet ether)[129]
^{1}H nmr (300 MHz, CDCl₃) ∂	1.85 (d, 3H, J = 6 Hz), 3.85 (q, 1H, J = 6 Hz), 7.1-7.6 (m, 8H), 8.3 (bs, 1H)
I.R. (v_{max})	3199, 3078, 1679, 1611, 1470 cm⁻¹
MS(FAB) (m/z, %)	287 (MH⁺, 32), 285 (MH⁺, 100)
HRMS: (C₁₆H₁₄³⁵ClN₂O, MH⁺)	requires: 285.0795, found: 285.0785

## 7-Chloro-3-methyl-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (159)
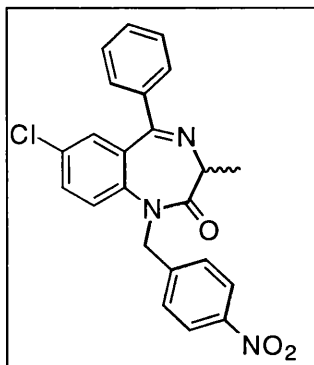
Compound **159** was synthesised from **155** (200 mg, 0.7 mmol), 4-nitrobenzyl bromide (173 mg, 0.8 mmol) and KO^{t}Bu in THF (1 M soln, 0.8 ml, 0.8 mmol) using the procedure outlined in Method 3.

yield:	230 mg, 0.55 mmol, 78% (light yellow foam)
^{1}H nmr (CDCl$_3$, 300 MHz) ∂	1.8 (d, 3H, J = 5 Hz), 3.9 (q, 1H, J = 5 Hz), 4.9 (d, 1H, J = 15 Hz), 5.8 (d, 1H, J = 15 Hz), 7.1-7.6 (m, 12H)
I.R. (v$_{max}$)	3347, 1684, 1605, 1581, 1341 cm^{-1}
MS(FAB) (m/z %)	422 (MH$^+$, 35), 420 (MH$^+$, 100)

1-(4-Aminobenzyl)-7-chloro-3-methyl-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (255)
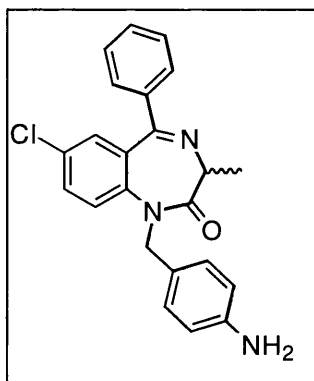
Compound **255** was synthesised from **159** (230 mg, 0.55 mmol) and tin (II) chloride (618 mg, 2.75 mmol) using the procedure outlined in Method 4.

yield:	213 mg, 0.54 mmol, 99% (yellow foam)

|	
¹H nmr (200 MHz, CDCl₃) ∂	1.8 (d, 3H, J = 6 Hz), 3.8 (q, 1H, J = 6 Hz), 4.8 (d, 1H, J = 12 Hz), 5.8 (d, 1H, J = 12 Hz), 7.1-7.5 (m, 12H)
I.R. (ν$_{max}$)	3355, 1670, 1607, 1516 cm⁻¹
MS(FAB) (m/z %)	392 (MH⁺, 30), 390 (MH⁺, 100),

N-[4-(7-Chloro-3-methyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (256)
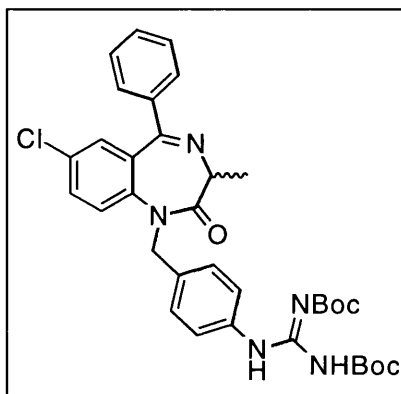
Compound **256** was synthesised from **255** (213 mg, 0.54 mmol), mercury (II) chloride (167 mg, 0.6 mmol), triethylamine (182 mg, 1.8 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (166 mg, 0.6 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	184 mg, 0.29 mmol, 54% (yellow foam)
¹H nmr (300 MHz, CDCl₃) ∂	1.6 (s, 18H), 1.8 (d, 3H, J = 6 Hz), 3.8 (q, 1H, J = 6 Hz), 4.9 (d, 1H, J = 16 Hz), 5.5 (d, 1H, J = 16 Hz), 7.0-7.6 (m, 12H)
I.R. (ν$_{max}$)	2997, 2932, 1721, 1682, 1639, 1607, 1150 cm⁻¹
MS(FAB) (m/z %)	632 (MH⁺, 5), 434 (MH⁺-2 Boc, 22), 432 (MH⁺-2 Boc, 100)

N-[4-(7-Chloro-3-methyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl)phenyl] guanidine (163)
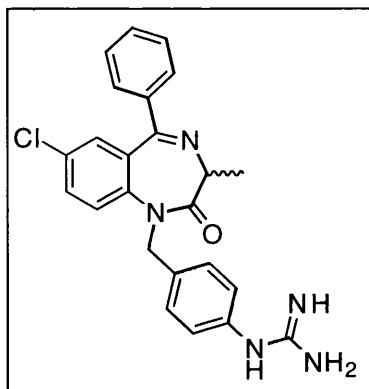

Compound **163** was synthesised from **256** (184 mg, 0.29 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	109.5 mg, 0.13 mmol, 46% (yellow foam)
¹H nmr (360 MHz, DMSO) ∂	1.6 (d, 3H, J = 6 Hz, CHCH_3), 3.85 (q, 1H, J = 6 Hz, CHCH₃), 5.0 (d, 1H, J = 17 Hz, NCHHPh), 5.5 (d, 1H, J = 17 Hz, NCHHPh), 7.0 (s, 4H, Ph), 7.2 (s, 1H, Ph), 7.3-7.8 (m, 10H, Ph and NH), 9.7 (s, 1H, NH)
I.R. (v_{max})	3346, 3177, 1699, 1202 cm⁻¹
MS(FAB) (m/z %)	434 (MH⁺-CF₃CO₂⁻, 35), 432 (MH⁺-CF₃CO₂⁻, 100), 285 (35), 148 (100)
analysis: C₂₄H₂₂ClN₅O.3.2TFA.H₂O effective molecular weight [814.81]	calc: C, 44.8; H, 3.36; Cl, 4.35; F, 22.38; N, 8.59; O, 16.49
	found: C, 44.41; H, 3.23; Cl, 4.3; F, 22.3; N, 8.63; O, 16.13

## 7-Chloro-5-(2-chlorophenyl)-3-methyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (157)
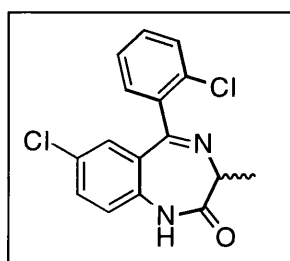
Compound **157** was synthesised by the procedures outlined in Methods 1 and 2 using 2-amino-2',5-dichlorobenzophenone (1.330 g; 5.0 mmol), ethyl chloroformate (648 mg; 6.0 mmol), triethylamine (606 mg, 6 mmol) and (±) N-Boc alanine (1.055 g; 5.58 mmol).

|	
yield	300 mg, 0.69 mmol, 14% (colourless crystals)
Mp	171-173 °C(EtOAc-Hexane); lit 172-173°C (acetone-H_2O)[136]
^{1}H nmr (300 MHz, $CDCl_3$) ∂	1.8 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 7.0-7.6 (m, 7H), 9.4 (s, 1H)
^{13}C-nmr ($CDCl_3$) ∂	16.8, 58.7, 122.5, 127.0, 128.9, 129.1, 129.5, 130.1, 130.9, 131.1, 131.9, 133.3, 136.3, 138.2, 167.3, 172.1
MS(FAB) (m/z, %)	323 (MH^+, 15), 321 (MH^+, 52), 319 (MH^+, 100)

7-Chloro-5-(2-chlorophenyl)-3-methyl-1-(4-nitrobenzyl)-1,3-dihydrobenzo [e][1,4]diazepin-2-one (161)
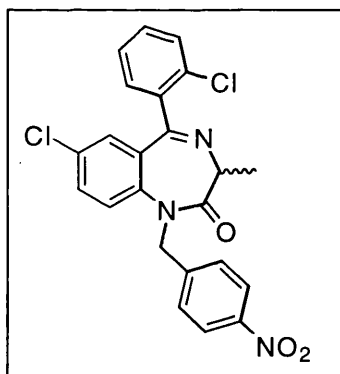
Compound **161** was synthesised from **157** (273 mg, 0.86 mmol), 4-nitrobenzyl bromide (203 mg, 0.94 mmol) and KO^{t}Bu in THF (1 M soln, 1.0 ml, 1.0 mmol) using the procedure outlined in Method 3.

yield:	350 mg, 0.77 mmol, 90% (light yellow foam)
^{1}H nmr ($CDCl_3$, 200 MHz) ∂	1.8 (d, 3H, J = 4 Hz), 3.9 (q, 1H, J = 4 Hz), 5.1 (d, 1H, J = 15 Hz), 5.4 (d, 1H, J = 15 Hz), 7.2-7.5 (m, 10H), 8.1 (d, 1H, J = 7 Hz)

I.R. (v_{max})	3071, 1678, 1517, 1348 cm^{-1}
MS(FAB) (m/z %)	457 (16), 456 (64), 455 (30), 454 (MH$^+$, 100)
HRMS: ($C_{23}H_{18}{}^{35}Cl_2N_3O_3$, MH$^+$)	requires: 454.0725, found: 454.0740

## 1-(4-Aminobenzyl)-7-chloro-5-(2-chlorophenyl)-3-methyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (257)
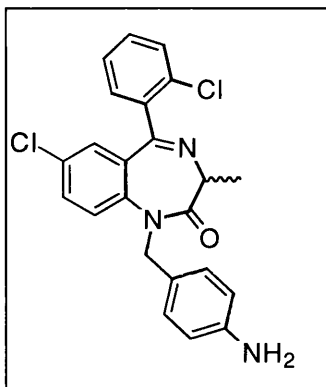
Compound **257** was synthesised from **161** (350 mg, 0.77 mmol) and tin (II) chloride (520 mg, 2.31 mmol) using the procedure outlined in Method 4.

|	
yield:	290 mg, 0.67 mmol, 87% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.85 (d, 3H, J = 6.5 Hz), 3.9 (q, 1H, J = 6.5 Hz), 4.9 (d, 1H, J = 17 Hz), 5.1 (d, 1H, J = 17 Hz), 7.0-7.5 (m, 11H)
I.R. (v_{max})	3437, 3347, 1609, 1602 cm^{-1}
MS(FAB) (m/z %)	426 (MH$^+$, 10), 424 (MH$^+$, 15), 323 (60), 321 (100)
HRMS: ($C_{23}H_{20}{}^{35}Cl_2N_3O$, MH$^+$)	requires: 424.0983, found: 424.0970

N-{4-[7-Chloro-5-(2-chlorophenyl)-3-methyl-2-oxo-2,3-dihydrobenzo [e][1,4]diazepin-1-ylmethyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (258)
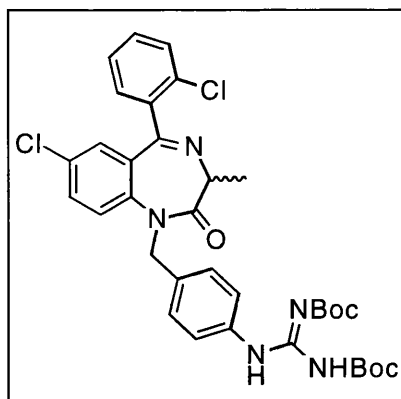
Compound **258** was synthesised from **257** (290 mg, 0.67 mmol), mercury (II) chloride (186 mg, 0.74 mmol), triethylamine (223 mg, 2.21 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (186 mg, 0.74 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	78 mg, 0.12 mmol, 17% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 1.9 (d, 3H, J = 6 Hz), 3.9 (q, 1H, J = 6 Hz), 5.05 (d, 1H, J = 17 Hz), 5.2 (d, 1H, J = 17 Hz), 7.0-7.6 (m, 11H), 10.3 (bs, 1H), 11.6 (bs, 1H)
I.R. (v$_{max}$)	3414, 2978, 1718, 1637, 1239, 1150 cm^{-1}
MS(FAB) (m/z %)	666 (MH$^+$, 2), 468 (MH$^+$-2 Boc, 10), 466 (MH$^+$-2 Boc, 10), 382 (100)
HRMS: (C$_{34}$H$_{38}$^{35}Cl$_2$N$_5$O$_5$, MH$^+$)	requires: 666.2250, found: 666.2270

N-{4-[7-Chloro-5-(2-chlorophenyl)-3-methyl-2-oxo-2,3-dihydrobenzo [e][1,4]diazepin-1-ylmethyl]phenyl} guanidine (165)
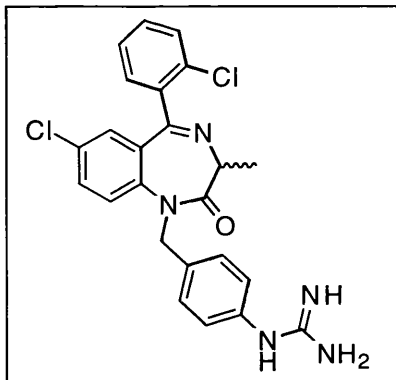
Compound **165** was synthesised from **258** (78 mg, 0.12 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	28.8 mg, 0.06 mmol, 50% (yellow foam)
^{1}H nmr (360 MHz, DMSO) δ	1.6 (d, 3H, J = 6 Hz, CHC*H₃*), 3.9 (q, 1H, J = 6 Hz, C*H*CH₃), 5.1 (d, 1H, J = 12 Hz, NCH*H*Ph), 5.2 (d, 1H, J = 12 Hz, NCH*H*Ph), 6.9 (s, 1H, Ph and NH), 7.1-7.6 (m, 11H, Ph)
I.R. (ν_{max}) film	3342, 3180, 1684, 1202 cm^{-1}
MS(FAB) (m/z %)	468 (MH$^+$-CF₃CO₂⁻, 30), 466 (MH$^+$-CF₃CO₂⁻, 40), 148 (100)
HRMS: (C₂₄H₂₂^{35}Cl₂N₅O, MH$^+$-CF₃CO₂⁻)	requires: 466.1201, found: 466.1220

7-Chloro-5-(2-chlorophenyl)-1,3-dihydrobenzo[e][1,4]diazepin-2-one (170)
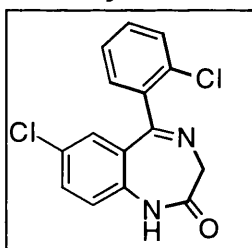
Compound **170** was synthesised from 2-amino-2',5-dichlorobenzophenone (4.6 g, 17.29 mmol), bromoacetyl bromide (3.842 g, 19.02 mmol) and triethylamine (1.921 g, 19.02 mmol) using the procedure described in Method 11.

yield	2.742 g, 9.0 mmol, 52% (colourless crystals)

Mp	196-197 °C(EtOAc-Hexane); lit 199-201 °C (EtOH)[129]
^{1}H nmr (300 MHz, CDCl$_3$) δ	4.4 (bs, 2H), 7.2-7.6 (m, 7H), 9.1 (s, 1H)
I.R. (v_{max})	3183, 3050, 1686, 1610, 1478, 1304 cm^{-1}
MS(FAB) (m/z, %)	309 (MH$^+$, 10), 307 (MH$^+$, 60), 305 (MH$^+$, 100)
HRMS: (C$_{15}$H$_{11}$^{35}Cl$_2$N$_2$O, MH$^+$)	requires: 305.0248, found: 305.0240

## 7-Chloro-5-(2-chlorophenyl)-1-(4-nitrobenzyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (173)
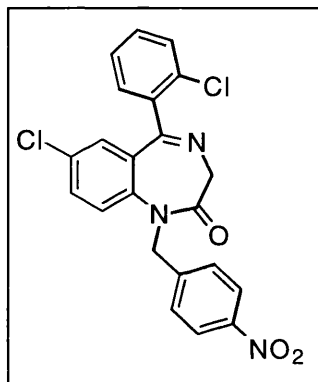
Compound **173** was synthesised from **170** (1.0 g, 3.28 mmol), 4-nitrobenzyl bromide (779 mg, 3.61 mmol) and KO^{t}Bu in THF (1 M soln, 3.6 ml, 3.6 mmol) using the procedure outlined in Method 3.

| yield:	754 mg, 1.71 mmol, 52% (light yellow foam)
^{1}H nmr (CDCl$_3$, 300 MHz) ∂	3.9 (d, 1H, J = 10 Hz), 5.0 (d, 1H, J = 10 Hz), 5.1 (d, 1H, J = 15 Hz), 5.4 (d, 1H, J = 15 Hz), 7.2-7.6 (m, 11H)
I.R. (v_{max})	1676, 1608, 1517, 1347 cm^{-1}
MS(FAB) (m/z %)	443 (MH$^+$, 20), 442 (MH$^+$, 65), 441 (MH$^+$, 40), 440 (MH$^+$, 100)
HRMS: (C$_{22}$H$_{16}$^{35}Cl$_2$N$_3$O$_3$, MH$^+$)	requires: 440.0569, found: 440.0580

1-(4-Aminobenzyl)-7-chloro-5-(2-chlorophenyl)-1,3-dihydrobenzo[e][1,4] diazepin-2-one (259)
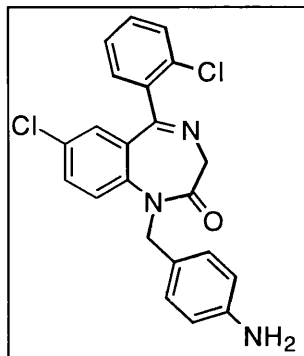
Compound **259** was synthesised from **173** (754 mg, 1.71 mmol) and tin (II) chloride (1.2 g, 5.3 mmol) using the procedure outlined in Method 4.

yield:	694 mg, 1.69 mmol, 99% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.9 (d, 1H, J = 10 Hz), 4.9-5.0 (m, 2H), 5.2 (d, 1H, J = 15 Hz), 6.6 (m, 2H), 6.8-7.4 (m, 9H)
I.R. (ν_{max})	3363, 1672, 1616, 1516 cm^{-1}
MS(FAB) (m/z %)	412 (MH$^+$, 25), 411 (MH$^+$, 20), 410 (MH$^+$, 35), 307 (80), 305 (100)
HRMS: (C$_{22}$H$_{18}$^{35}Cl$_2$N$_3$O, MH$^+$)	requires: 410.0827, found: 410.0840

## N-{4-[7-Chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (260)
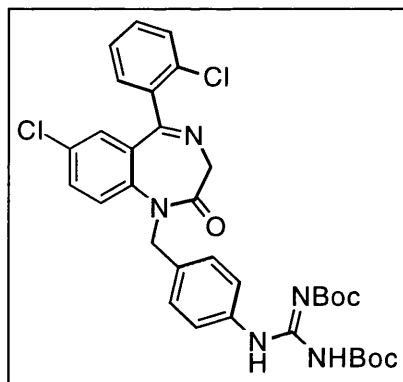
Compound **260** was synthesised from **259** (295 mg, 0.72 mmol), mercury (II) chloride (214 mg, 0.79 mmol), triethylamine (240 mg, 2.37 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (218 mg, 0.79 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	228 mg, 0.35 mmol, 49% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.9 (d, 1H, J = 10 Hz), 4.9-5.0 (m, 2H), 5.1 (d, 1H, J = 15 Hz), 7.1-7.6 (m, 11H), 10.2 (bs, 1H), 11.6 (bs, 1H)
I.R. (ν_{max})	3412, 2977, 1719, 1637, 1150 cm^{-1}
MS(FAB) (m/z %)	652 (MH$^+$, 6) 454 (65), 453 (35), 452 (MH$^+$-2 Boc, 100)
HRMS: (C$_{33}$H$_{36}$^{35}Cl$_2$N$_5$O$_5$, MH$^+$)	requires: 652.2094, found: 652.2080

N-{4-[7-Chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl]phenyl} guanidine (176)
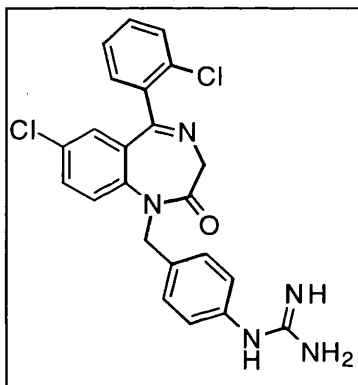
Compound **176** was synthesised from **260** (228 mg, 0.35 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	76.8 mg, 0.10 mmol, 29% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	4.0 (d, 1H, J = 12 Hz, R$_2$CHH), 4.7 (d, 1H, J = 12 Hz, R$_2$CHH), 5.1 (d, 1H, J = 15 Hz, NCHHPh), 5.2 (d, 1H, J = 15 Hz, NCHHPh), 6.9 (s, 1H, Ph), 7.15 (d, 2H, J = 6 Hz, Ph), 7.3 (d, 2H, J = 6 Hz, Ph), 7.4-7.7 (m, 11H, Ph and NH), 9.75 (s, 1H, NH)
I.R. (ν_{max})	3373, 1682, 1516, 1203 cm^{-1}
MS(FAB) (m/z %)	456 (15), 454 (65), 452 (MH$^+$-CF$_3$CO$_2^-$, 100), 305 (15)

analysis:
$C_{23}H_{19}Cl_2N_5O.2.45TFA.1.3H_2O$
effective molecular weight [755.12]

calc: C, 44.37; H, 3.21; Cl, 9.39;
F, 18.49; N, 9.27; O, 15.25
found: C, 43.99; H, 3.14; Cl, 9.1;
F, 18.0; N, 10.01; O, 14.88

3-Butyl-7-chloro-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (156)
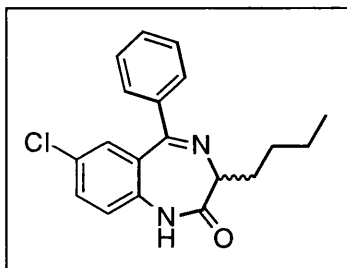
Compound **156** was synthesised by the procedures outlined in Methods 1 and 2 using 2-amino-5-chlorobenzophenone (1.622 g; 7 mmol), ethyl chloroformate (872 mg; 8 mmol), triethylamine (808 mg, 8 mmol) and (±) N-Boc norleucine (1.695 g; 7.34 mmol).

yield	533 mg, 1.63 mmol, 23% (light yellow powder)
Mp	140-145°C (EtOAc-hexane)
^{1}H nmr (200 MHz, CDCl$_3$)	0.9 (t, 3H, J = 5 Hz), 1.3-1.6 (m, 4H), 2.1-2.2 (m, 2H), 3.5 (t, 1H, J = 5 Hz), 7.1-7.6 (m, 8H)
I.R. (v_{max})	3203, 2954, 1682, 1604 cm^{-1}
MS(FAB) (m/z, %)	329 (MH$^+$, 30), 327 (MH$^+$, 100)
HRMS: ($C_{19}H_{20}{}^{35}ClN_2O$, MH$^+$)	requires: 327.1264, found: 327.1270

3-Butyl-7-chloro-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (160)
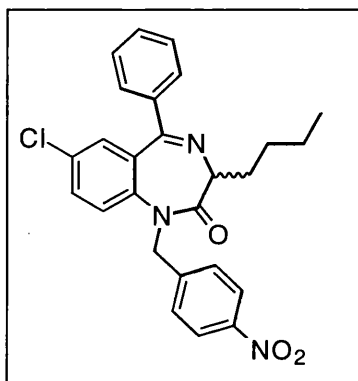
Compound **160** was synthesised from **156** (400 mg, 1.23 mmol), 4-nitrobenzyl bromide (344 mg, 1.6 mmol) and KO^{t}Bu in THF (1 M soln, 1.6 ml, 1.6 mmol) using the procedure outlined in Method 3.

yield:	370 mg, 0.80 mmol, 65% (light yellow foam)
^{1}H nmr (CDCl$_3$, 300 MHz) ∂	1.0 (t, 3H, J = 5 Hz), 1.4-1.6 (m, 4H), 2.2 (m, 2H), 3.6 (t, 1H, J = 6 Hz), 4.8 (d, 1H, J = 16 Hz), 5.9 (d, 1H, J = 16 Hz), 7.1-7.6 (m, 12H)
I.R. (v_{max})	2953, 1680, 1603, 1522, 1346 cm^{-1}
MS(FAB) (m/z %)	464 (MH$^+$, 35), 462 (MH$^+$, 100)
HRMS: (C$_{26}$H$_{25}$^{35}ClN$_3$O$_3$, MH$^+$)	requires: 462.1584, found: 462.1570

1-(4-Aminobenzyl)-3-butyl-7-chloro-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (261)
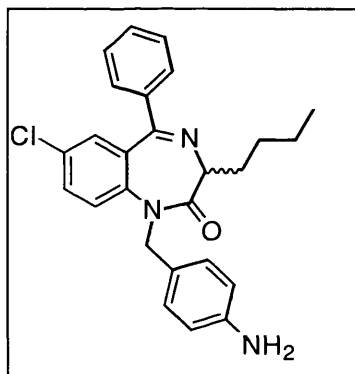
Compound **261** was synthesised from **160** (370 mg, 0.80 mmol) and tin (II) chloride (540 mg, 2.4 mmol) using the procedure outlined in Method 4.

yield:	283 mg, 0.66 mmol, 82%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.0 (t, 3H, J = 5 Hz), 1.3-1.6 (m, 4H), 2.2-2.4 (m, 2H), 3.6 (t, 1H, J = 5 Hz), 4.6 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.4 (d, 2H, J = 8 Hz), 6.6 (d, 2H, J = 8 Hz), 7.1-7.5 (m, 8H)
I.R. (v_{max})	3360, 2959, 1677, 1607, 1479 cm^{-1}
MS(FAB) (m/z %)	434 (MH$^+$, 15), 432 (MH$^+$, 40), 327 (100),
HRMS: (C$_{26}$H$_{27}$^{35}ClN$_3$O, MH$^+$)	requires: 432.1843, found: 432.1830

## N-[4-(3-Butyl-7-chloro-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (262)
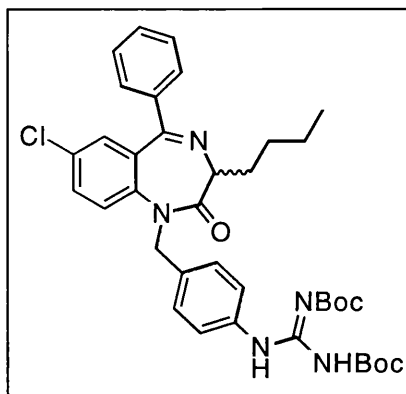
Compound **262** was synthesised from **261** (283 mg, 0.66 mmol), mercury (II) chloride (198 mg, 0.73 mmol), triethylamine (220 mg, 2.18 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (201 mg, 0.73 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

|	
yield:	275 mg, 0.41 mmol, 62% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.0 (t, 3H, J = 6 Hz), 1.3-1.7 (m, 22H), 2.3 (m, 2H), 3.6 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 7.0-7.4 (m, 12H), 10.2 (bs, 1H)
I.R. (v_{max})	3419, 1782, 1718, 1686, 1642, 1607, 1309, 1151 cm^{-1}
MS(FAB) (m/z %)	674 (MH$^+$, 5), 476 (20), 474 (55), 382 (100)
HRMS: (C$_{37}$H$_{45}$^{35}ClN$_5$O$_5$, MH$^+$)	requires: 674.3109, found: 674.3120

N-[4-(3-Butyl-7-chloro-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-1-ylmethyl)phenyl] guanidine (164)
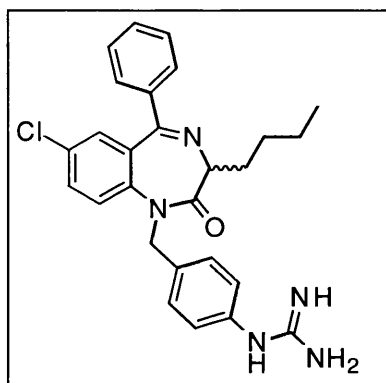
Compound **164** was synthesised from **262** (275 mg, 0.41 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	50.2 mg, 0.11 mmol, 27% (white foam)
¹H nmr (360 MHz, DMSO) ∂	0.9 (t, 3H, J = 6 Hz, CH_3CH$_2$R), 1.3-1.4 (m, 4H, RCH_2CH_2R), 2.1 (m, 2H, RCHCH_2R), 3.65 (t, 1H, J = 6 Hz, CHCH$_2$R), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 7.0-7.3 (m, 5H, Ph and NH), 7.35-7.75 (m, 7H, Ph), 10.3 (bs, 1H, NH), 11.2 (bs, 1H, NH)
I.R. (ν$_{max}$)	3365, 2959, 1680, 1607, 1150 cm^{-1}
MS(EI) (m/z %)	475 (M$^+$-CF$_3$CO$_2^-$, 5), 473 (M$^+$-CF$_3$CO$_2^-$, 10), 327 (15), 325 (30), 148 (70), 106 (100)
HRMS: (C$_{27}$H$_{29}$^{35}ClN$_5$O, MH$^+$)	requires: 474.2061, found: 474.2050

7-Chloro-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (171)
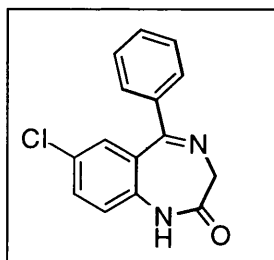
Compound **171** was synthesised from 2-amino-5-chlorobenzophenone (2.643 g, 11.44 mmol), bromoacetyl bromide (2.542 g, 12.6 mmol) and triethylamine (1.273 g, 12.6 mmol) using the procedure described in Method 11.

yield:	1.944 g, 7.16 mmol, 63% (colourless crystals)
Mp	215-217 °C(EtOAc-Hexane); lit 216-217 °C (acetone)[137]
^{1}H nmr (200 MHz, CDCl$_3$)	4.3 (s, 2H), 7.1-7.6 (m, 8H), 8.7 (s, 1H)
I.R. (ν_{max})	3210, 2934, 1686, 1482, 1234 cm^{-1}
MS(FAB) (m/z, %)	273 (MH$^+$, 37), 271 (MH$^+$, 100)
HRMS: (C$_{15}$H$_{12}$^{35}ClN$_2$O, MH$^+$)	requires: 271.0638, found: 271.0630

7-Chloro-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (174)
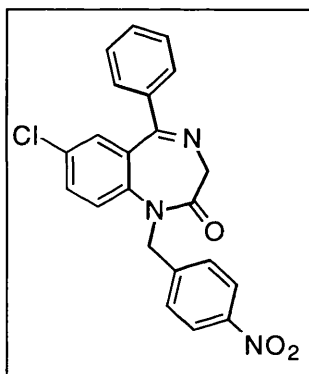
Compound **174** was synthesised from **171** (812 mg, 3.0 mmol), 4-nitrobenzyl bromide (713 mg, 3.3 mmol) and KO^{t}Bu in THF (1 M soln, 3.3 ml, 3.3 mmol) using the procedure outlined in Method 3.

|	
yield:	820 mg, 2.02 mmol, 67% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	3.9 (d, 1H, J = 10 Hz), 4.9 (d, 1H, J = 10 Hz), 5.1 (d, 1H, J = 15 Hz), 5.4 (d, 1H, J = 15 Hz), 7.2-7.5 (m, 10H), 8.1 (d, 2H, J = 7.5 Hz)
I.R. (ν_{max})	3071, 1678, 1607, 1517, 1348 cm^{-1}
MS(FAB) (m/z %)	408 (MH$^+$, 35), 406 (MH$^+$, 100)
HRMS: (C$_{22}$H$_{17}$^{35}ClN$_3$O$_3$, MH$^+$)	requires: 406.0958, found: 406.0970

1-(4-Aminobenzyl)-7-chloro-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (263)
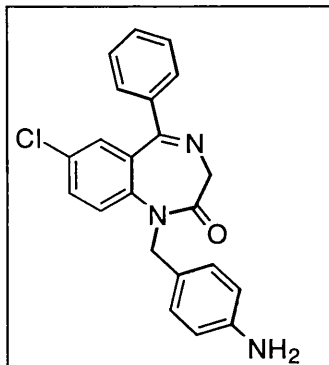
Compound **263** was synthesised from **174** (820 mg, 2.02 mmol) and tin (II) chloride (1.200 g, 5.3 mmol) using the procedure outlined in Method 4.

yield:	732 mg, 1.95 mmol, 96%, (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.9 (d, 1H, J = 10 Hz), 5.0 (d, 1H, J = 10 Hz), 5.2 (d, 1H, J = 16 Hz), 5.4 (d, 1H, J = 16 Hz), 7.2-7.6 (m, 10H), 8.2 (d, 2H, J = 8 Hz)
I.R. (ν_{max})	1677, 1607, 1517, 1348 cm^{-1}
MS(FAB) (m/z %)	378 (MH$^+$, 34), 376 (MH$^+$, 100)
HRMS: (C$_{22}$H$_{19}$^{35}ClN$_3$O, MH$^+$)	requires: 376.1217, found: 376.1210

N-[4-(7-Chloro-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (264)
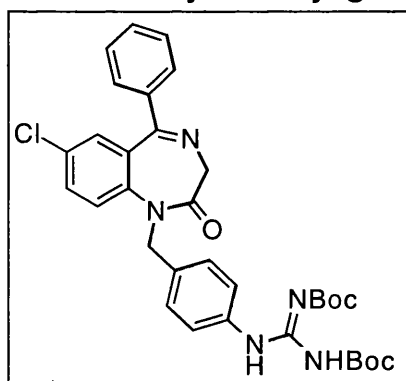
Compound **264** was synthesised from **263** (290 mg, 0.77 mmol), mercury (II) chloride (230 mg, 0.85 mmol), triethylamine (256 mg, 2.54 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (235 mg, 0.85 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	194 mg, 0.31 mmol, 41% (yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.9 (d, 1H, J = 10 Hz), 4.9 (d, 1H, J = 10 Hz), 5.2 (d, 1H, J = 16 Hz), 5.4 (d, 1H, J = 16 Hz), 7.0-7.6 (m, 12H), 10.2 (bs, 1H)
I.R. (v_{max})	2978, 1718, 1684, 1636, 1406, 1150 cm^{-1}
MS(FAB) (m/z %)	618 (MH$^+$, 10), 420 (35), 418 (MH$^+$-2 Boc, 100)
HRMS: (C$_{33}$H$_{37}$^{35}ClN$_5$O$_5$)	requires: 618.2483, found: 618.2470

N-[4-(7-Chloro-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl methyl)phenyl] guanidine (177)
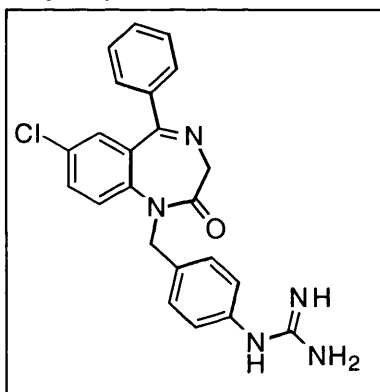
Compound **177** was synthesised from **264** (194 mg, 0.31 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	70.9 mg, 0.17 mmol, 55% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.9 (d, 1H, J = 12 Hz, R$_2$C*H*H), 4.7 (d, 1H, J = 12 Hz, R$_2$C*H*H), 5.0 (d, 1H, J = 18 Hz, NCH*H*Ph), 5.4 (d, 1H, J = 18 Hz, NCH*H*Ph), 7.0 (m, 4H, Ph), 7.15 (d, 1H, J = 2 Hz, Ph), 7.35-7.8 (m, 12H, Ph and NH), 9.8 (s, 1H, NH)
I.R. (v_{max})	3391, 1698, 1203, 1140 cm^{-1}
MS(FAB) (m/z %)	420 (MH$^+$-CF$_3$CO$_2^-$, 30), 418 (MH$^+$-CF$_3$CO$_2^-$, 100), 271 (22)

HRMS: ($C_{23}H_{21}{}^{35}CIN_5O$, MH+-CF₃CO₂⁻)

requires: 418.1435, found: 418.1430

(3S)-[3-(2-Oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl) propyl] carbamic acid benzyl ester (179)
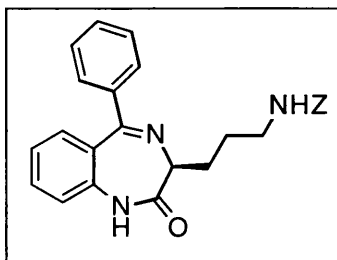
Compound **179** was synthesised by the procedures outlined in Methods 1 and 2 using 2-aminobenzophenone (1.19 g; 6.0 mmol), ethyl chloroformate (820 mg; 7.5 mmol), triethylamine (758 mg, 7.5 mmol) and (2S)-5-benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-pentanoic acid (2.5 g; 6.82 mmol).

yield	974 mg, 2.28 mmol, 38% (light brown foam)
¹H nmr (200 MHz, CDCl₃)	1.6-1.8 (m, 2H), 2.1-2.2 (m, 2H), 3.3 (m, 2H), 3.6 (t, 1H, J = 5 Hz), 5.1 (s, 2H), 7.1-7.6 (m, 14H), 9.3 (s, 1H)
I.R. (v_{max})	3237, 3060, 2929, 1686, 1607, 1244 cm⁻¹
MS(FAB) (m/z, %)	428 (MH+, 100)
HRMS: ($C_{27}H_{28}N_3O_3$, MH+)	requires: 428.1974, found: 428.1980

(3S)-{3-[1-(4-Nitrobenzyl)-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4] diazepin-3-yl] propyl} carbamic acid benzyl ester (265)
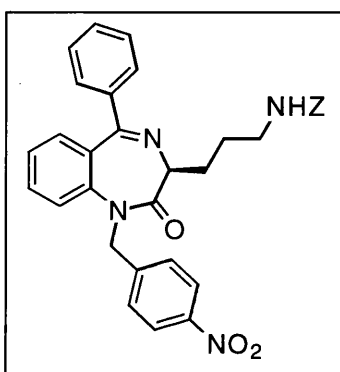
Compound **265** was synthesised from **179** (300 mg, 0.70 mmol), 4-nitrobenzyl bromide (173 mg, 8 mmol) and KO^{t}Bu in THF (1 M soln, 0.80 ml, 0.80 mmol) using the procedure outlined in Method 3.

yield:	250 mg, 0.44 mmol, 64% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	1.3-1.5 (m, 4H), 3.3 (m, 2H), 3.7 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 12 Hz), 5.1 (s, 2H), 5.7 (d, 1H, J = 12 Hz) 7.1-7.5 (m, 18H)
I.R. (v_{max})	3500, 1718, 1679, 1604, 1521, 1346 cm^{-1}
MS(FAB) (m/z %)	563 (MH$^+$, 100)

(3S)-{3-[1-(4-Aminobenzyl)-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-3-yl] propyl} carbamic acid benzyl ester (266)
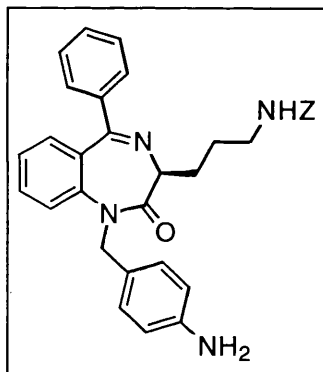
Compound **266** was synthesised from **265** (250 mg, 0.44 mmol) and tin (II) chloride (500 mg, 2.2 mmol) using the procedure outlined in Method 4.

yield:	136 mg, 0.26 mmol, 58%, (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.3-1.5 (m, 2H), 2.2 (m, 2H), 3.4 (m, 2H), 3.8 (t, 1H, J = 5 Hz), 4.9 (d, 1H, J = 12 Hz), 5.1 (s, 1H), 5.6 (d, 1H, J = 12 Hz), 6.9-7.6 (m, 18H)
I.R. (v_{max})	3356, 1714, 1670, 1518, 1252, 698 cm^{-1}
MS(FAB) (m/z %)	533 (MH$^+$, 100), 428 (45)

(3S)-{3-[1-(4-N',N''-bis-*tert*-butoxycarbonyl guanidinobenzyl)-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4] diazepin-3-yl] propyl} carbamic acid benzyl ester (180)
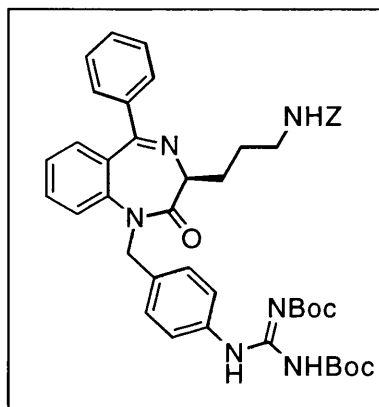
Compound **180** was synthesised from **266** (136 mg, 0.26 mmol), mercury (II) chloride (80 mg, 0.30 mmol), triethylamine (87 mg, 0.86 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (80 mg, 0.29 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	158 mg, 0.20 mmol, 79% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 2.2 (m, 2H), 2.8 (m, 2H), 3.4 (m, 2H), 3.8 (t, 1H, J = 5 Hz), 4.8 (d, 1H, J = 12 Hz), 5.15 (s, 2H), 5.6 (d, 1H, J = 12 Hz), 7.0-7.5 (m, 18H), 10.2 (s, 1H)
I.R. (v_{max})	3421, 2977, 1718, 1685, 1637, 1607, 1150 cm^{-1}
MS(FAB) (m/z %)	797 (MH$^+$+Na, 6), 575 (MH$^+$-2 Boc, 8), 543 (14), 283 (70)
HRMS: (C$_{44}$H$_{51}$N$_6$NaO$_7$, MH$^+$+Na$^+$)	requires: 797.3639, found: 797.3620

(3S)-{3-[1-(4-Guanidinobenzyl)-2-oxo-5-phenyl-2,3-dihydro-1H-benzo [e][1,4]diazepin-3-yl] propyl} carbamic acid benzyl ester (181)
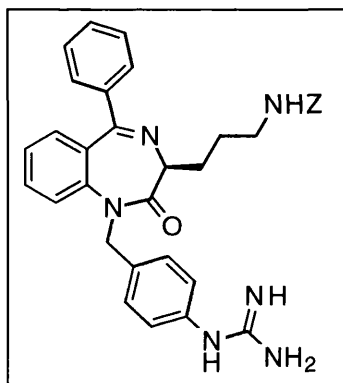

Compound **181** was synthesised from **180** (158 mg, 0.20 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	80.4 mg, 0.14 mmol, 70% (white foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.4-1.5 (m, 1H, RC*H*HR), 1.6-1.7 (m, 1H, RC*H*HR), 2.1 (m, 2H, RC*H$_2$*NHR), 3.0 (m, 2H, J = 6 Hz, RCHC*H$_2$*R), 3.6 (dd, 1H, J = 6 Hz and 7.5 Hz, C*H*CH$_2$R), 5.0 (m, 3H, NCH*H*Ph and C*H$_2$*Ph), 5.5 (d, 1H, J = 18 Hz, NCH*H*Ph), 7.0 (m, 4H, Ph), 7.2-7.7 (m, 19H, Ph and NH), 9.6 (s, 1H, NH)
I.R. (ν$_{max}$)	3386, 1696, 1600, 1203, 1137, 698 cm^{-1}
MS(FAB) (m/z %)	575 (MH$^+$-CF$_3$CO$_2^-$, 100)
HRMS: (C$_{34}$H$_{35}$N$_6$O$_3$, MH$^+$-CF$_3$CO$_2^-$)	requires: 575.2771, found: 575.2790

1,3-Bis-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (185)
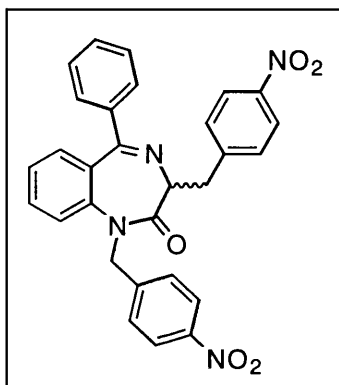
Compound **185** was synthesised from **183** (141 mg, 0.38 mmol), 4-nitrobenzyl bromide (90 mg, 0.42 mmol) and KO^{t}Bu in THF (1 M soln, 0.4 ml, 0.4 mmol) using the procedure outlined in Method 3.

yield:	150 mg, 0.32 mmol, 84% (light yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	3.7 (dd, 2H, J = 4 Hz and 2 Hz), 3.9 (t, 1H, J = 4 Hz), 4.9 (d, 1H, J = 14 Hz), 5.8 (d, 1H, J = 14 Hz), 7.1-7.6 (m, 14H), 7.9 (d, 1H, J = 6 Hz), 8.2 (m, 2H)
I.R. (v$_{max}$)	1678, 1607, 1519, 1346 cm^{-1}
MS(FAB) (m/z %)	507 (MH$^+$, 100)

1,3-Bis-(4-aminobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (267)
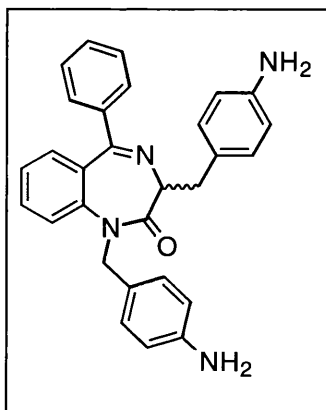
Compound **267** was synthesised from **185** (150 mg, 0.32 mmol) and tin (II) chloride (550 mg, 2.0 mmol) using the procedure outlined in Method 4.

yield:	112 mg, 0.25 mmol, 78%, (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.5 (m, 2H), 3.8 (t, 1H, J = 6 Hz), 4.6 (d, 1H, J = 12 Hz), 5.6 (d, 1H, J = 12 Hz), 7.1-7.5 (m, 17H)
I.R. (v$_{max}$)	3358, 2924, 1670, 1517 cm^{-1}
MS(FAB) (m/z %)	447 (MH$^+$, 100),

N-[4-(3-(4-N',N''-Bis-*tert*-butoxycarbonyl guanidinobenzyl)-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine 268
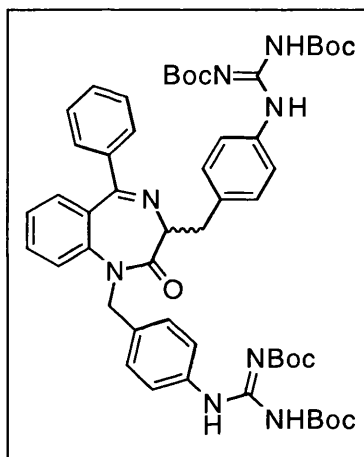
Compound **268** was synthesised from **267** (112 mg, 0.25 mmol), mercury (II) chloride (152 mg, 0.56 mmol), triethylamine (170 mg, 1.68 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (155 mg, 0.56 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	140 mg, 0.15 mmol, 60% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 36H), 3.6 (m, 2H), 3.8 (t, 1H, J =5 Hz), 4.7 (d, 1H, J = 12 Hz, NCH*H*Ph), 5.6 (d, 1H, J = 12 Hz, NCH*H*Ph), 6.9-7.5 (m, 17H)
I.R. (v$_{max}$)	3335, 2982, 1784, 1720, 1642, 1152 cm^{-1}

MS(FAB) (m/z %) 931 (MH$^+$, 15), 831 (MH$^+$-Boc, 25), 731 (MH$^+$-2 Boc, 15), 631 (10), 531 (55), 384 (55), 248 (35), 192 (100)

N-[4-(3-(4-Guanidinobenzyl)-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl methyl)phenyl]guanidine 187
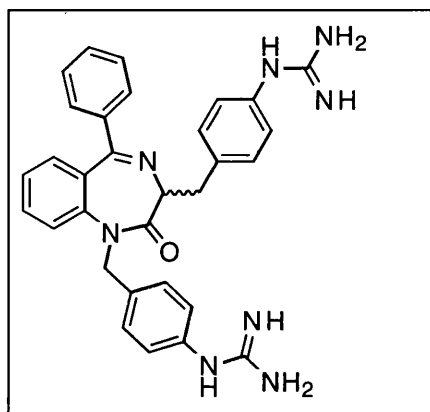
Compound **187** was synthesised from **268** (140 mg, 0.15 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	52.7 mg, 0.10 mmol, 66% (light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.45 (d, 2H, J = 6 Hz, CHCH_2Ph), 3.8 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 5.0 (d, 1H, J = 18 Hz, NCHHPh), 5.5 (d, 1H, J = 18 Hz, NCHHPh), 6.9 -7.7 (m, 25H, Ph and NH), 9.5-9.7 (m, 2H, NH)
I.R. (v_{max})	3343, 3181, 1675, 1602, 1203 cm^{-1}
MS(FAB) (m/z %)	531 (MH$^+$-CF$_3$CO$_2^-$, 100), 384 (44)
HRMS: (C$_{31}$H$_{31}$N$_8$O, MH$^+$-CF$_3$CO$_2^-$)	requires: 531.2621, found: 531.2640

249

1-Benzyl-3-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (184)
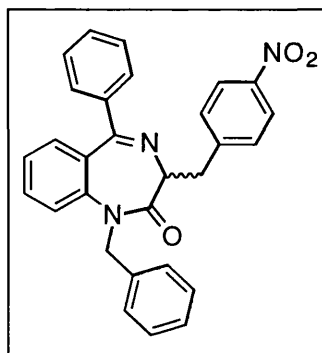
Compound **184** was synthesised from **183** (520 mg, 1.4 mmol), benzyl bromide (257 mg, 1.5 mmol) and KO^tBu in THF (1 M soln, 1.5 ml, 1.5 mmol) using the procedure outlined in Method 3.

yield:	470 mg, 1.02 mmol, 73% (light yellow foam)
^{1}H nmr (300 MHz, CDCl$_3$) ∂	3.8 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.8 (d, 1H, J = 15 Hz), 7.0-7.6 (m, 18H)
I.R. (v_{max})	1678, 1605, 1517, 1345 cm^{-1}
MS(FAB) (m/z %)	462 (MH$^+$, 100)
HRMS: (C$_{29}$H$_{24}$N$_3$O$_3$, MH$^+$)	requires: 462.1818, found: 462.1810

3-(4-Aminobenzyl)-1-benzyl-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (269)
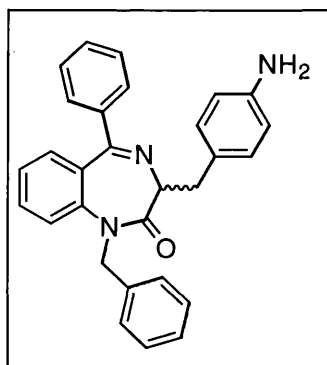
Compound **269** was synthesised from **184** (350 mg, 0.76 mmol) and tin (II) chloride (1.200 g, 5.3 mmol) using the procedure outlined in Method 4.

yield:	200 mg, 0.46 mmol, 31%, (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.65 (d, 2H, J = 5 Hz), 3.9 (t, 1H, J = 5 Hz), 4.9 (d, 1H, J = 15 Hz), 5.7 (d, 1H, J = 15 Hz), 7.0-7.6 (m, 18H)
I.R. (v$_{max}$)	3321, 1671, 1601, 1514 cm^{-1}
MS(FAB) (m/z %)	432 (MH$^+$, 100),

N-[4-(1-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl methyl)phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (270)
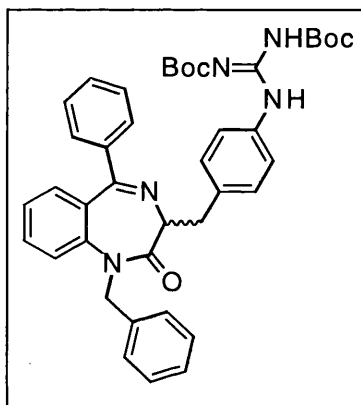
Compound **270** was synthesised from **269** (200 mg, 0.46 mmol), mercury (II) chloride (128 mg, 0.48 mmol), triethylamine (154 mg, 1.54 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (128 mg, 0.46 mmol) and DMF (20 ml) using the procedure outlined in Method 5.

yield:	190 mg, 0.28 mmol, 61% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 18H), 3.7 (d, 2H, J = 6 Hz), 3.9 (t, 1H, J = 6 Hz), 4.9 (d, 1H, J = 15 Hz), 5.6 (d, 1H, J = 15 Hz), 6.9-7.5 (m, 18H)
I.R. (v$_{max}$)	2954, 1720, 1680, 1636, 1156 cm^{-1}
MS(FAB) (m/z %)	674 (MH$^+$, 20), 474 (MH$^+$-2 Boc, 100)

N-[4-(1-Benzyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl methyl)phenyl] guanidine (186)
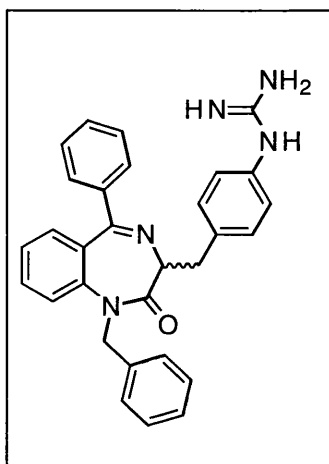
Compound **186** was synthesised from **270** (190 mg, 0.28 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	148.9 mg, 0.19 mmol, 68% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.4 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.5 (dd, 1H, J = 6 Hz and 12 Hz, CHC*H*HPh), 3.9 (dd, 1H, J = 6 Hz and 7 Hz, C*H*CH$_2$Ph), 4.9 (d, 1H, J = 12 Hz, NCH*H*Ph), 5.5 (d, 1H, J = 12 Hz, NCH*H*Ph), 6.9-7.7 (m, 20H, Ph and NH), 9.7 (s, 1H, NH)
I.R. (ν_{max})	3369, 1698, 1600, 1203 cm^{-1}
MS(FAB) (m/z %)	474 (MH$^+$-CF$_3$CO$_2^-$, 100)
analysis: C$_{30}$H$_{27}$N$_5$O.2.9TFA effective molecular weight [804.24]	calc: C, 53.46; H, 3.74; F, 20.55; N, 8.70; O, 13.52 found: C, 53.84; H, 3.72; F, 20.7; N, 8.48; O, 9.22

3.5 Benzodiazepin-2-one Overlay Model (II)

{4-[2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)acetylamino]butyl} carbamic acid *tert*-butyl ester (189)
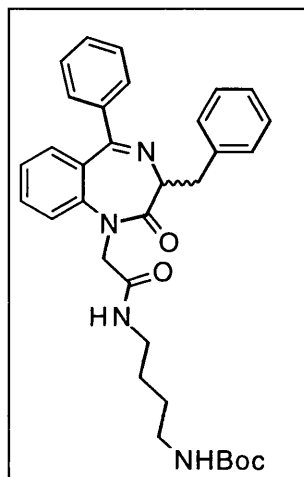
Compound **189** was synthesised from **188** (384 mg, 1 mmol), DIPC (151 mg, 1.2 mmol), N-Boc-1,4-diaminobutane (470 mg, 2.5 mmol) and HOBT (176 mg, 1.3 mmol) using the procedure described in Method 10.

yield:	200 mg, 0.52 mmol, 52% (white foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.1-1.2 (m, 3H), 1.3-1.8 (m, 10H), 3.0-3.2 (m, 4H, RCH$_2$CH_2NHR and CONHCH_2C H $_2$R), 3.6 (m, 2H, CHCH_2Ph), 3.8-4.0 (m, 2H, CHCH$_2$Ph and NCHHCONHR), 4.5 (m, 1H, NCHHCONR), 6.3 (bs, 1H, NH), 7.2-7.7 (m, 14H, Ph)
I.R. (v_{max})	3340, 2970, 1681, 1570, 1169 cm^{-1}
ms (FAB) (m/z, %)	555 (MH$^+$, 100), 455 (MH$^+$-Boc, 25), 311 (30)

N-(4-Aminobutyl)-2-(3-benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-1-yl)acetamide (190)
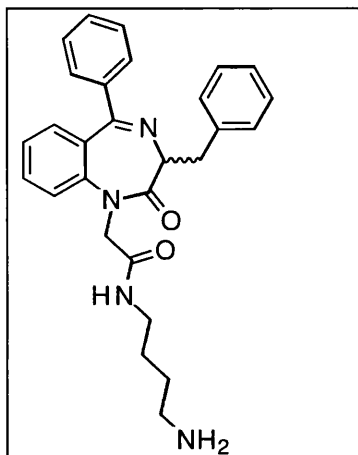
Compound **190** was synthesised from **189** (290 mg, 0.52 mmol) and trifluoroacetic acid (2 ml) using the procedure described in Method 7.

yield:	72.6 mg, 0.10 mmol, 19% (light yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.4-1.6 (m, 4H, RCH_2CH_2R), 2.8 (m, 2H, CONHCH_2CH$_2$R), 3.0 (m, 2H, RCH$_2$CH_2N H $_2$), 3.4 (m, 2H, CHCH_2Ph), 3.75 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 4.4 (d, 1H, J = 18 Hz, NCHHCONR), 4.5 (d, 1H, J = 18 Hz, NCHHCONR), 7.2-7.7 (m, 17H, Ph and NH), 8.1 (t, 1H, J = 3 Hz, NH)
I.R. (v$_{max}$)	3066, 1679, 1601, 1203 cm^{-1}
MS(FAB) (m/z, %)	455 (MH$^+$-CF$_3$CO$_2^-$, 100), 327 (10)
analysis: C$_{28}$H$_{30}$N$_4$O$_2$.2.2TFA.H$_2$O effective molecular weight [723.43]	calc: C, 53.78; H, 4.76; F, 17.3; N, 7.74; O, 16.36 found: C, 53.49; H, 4.59; F, 16.7; N, 7.67; O, 15.97

2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)-N-(4-N',N''-bis-*tert*-butoxycarbonyl guanidinobutyl)acetamide (191)
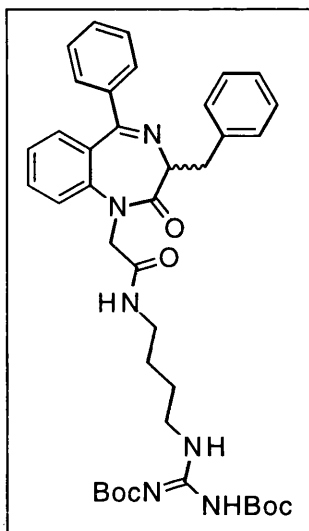
Compound **191** was synthesised from **190** (140 mg, 0.25 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (83 mg, 0.3 mmol), triethylamine (100 mg, 1 mmol), mercury (II) chloride (85 mg, 0.31 mmol) and DMF (20 ml) using the procedure described in Method 6.

yield:	160 mg, 0.23 mmol, 92% (white foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.3-1.7 (m, 22H), 3.0-3.2 (m, 4H, RCH$_2$CH_2NHR and CONHCH_2CH$_2$R), 3.5 (m, 2H, CHCH_2Ph), 3.8 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 4.45 (d, 1H, J = 18 Hz NCHHCONR), 4.50 (d, 1H, J = 18 Hz NCHHCONR), 7.1-7.6 (m, 15 H, Ph and NH), 9.6 (s, 1H, NH), 10.2 (s, 1H, NH)
I.R. (v$_{max}$)	3339, 2970, 1680, 1619, 1561, 1249, 1169 cm^{-1}
ms (FAB) (m/z, %)	697 (MH$^+$, 15), 597 (10), 497 (MH$^+$-2 Boc, 100)

2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)-N-(4-guanidinobutyl)acetamide (192)
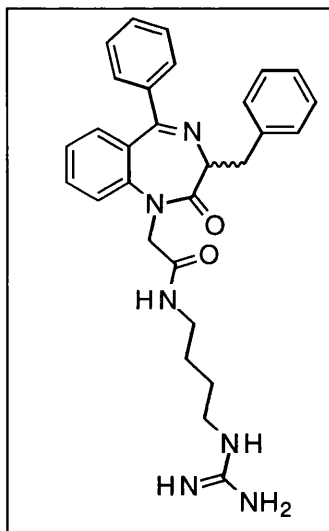
Compound **192** was synthesised from **191** (150 mg, 0.22 mmol) and trifluoroacetic acid (2 ml) using the procedure described in Method 7.

yield:	108.6 mg, 0.13 mmol, 58% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.3-1.5 (m, 4H, RC*H$_2$*C*H$_2$*R), 3.1 (m, 4H, CONHC*H$_2$*C H $_2$R and RCH$_2$C*H $_2$*NHR), 3.4 (m, 2H, CHC*H$_2$*Ph), 3.8 (dd, 1H, J = 6 Hz and 9 Hz, C*H*CH$_2$Ph), 4.45 (d, 1H, J = 18 Hz, NC*H*HCONR), 4.55 (d, 1H, J = 18 Hz, NC*H*HCONR), 7.1-7.6 (m, 18H, Ph and NH), 8.1 (t, 1H, J = 3 Hz, NH)
I.R. (v$_{max}$)	3353, 2946, 1669, 1567, 1201 cm^{-1}
MS(EI) (m/z, %)	497 (MH$^+$-CF$_3$CO$_2^-$, 10), 455 (MH$^+$-CH$_2$N$_2$, 15), 384 (20), 44 (C(NH)(NH$_3$), 100)
analysis: C$_{29}$H$_{32}$N$_6$O$_2$.2.9TFA.H$_2$O effective molecular weight [845.27]	calc: C, 49.44; H, 4.40; F, 19.55; N, 9.94; O, 16.6 found: C, 49.18; H, 4.13; F, 19.5; N, 9.77; O, 16.2

{4-[2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl) acetylamino]phenyl}carbamic acid *tert*-butyl ester (193)
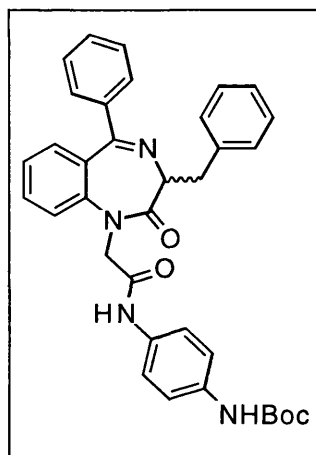
Compound **193** was synthesised from **188** (384 mg, 1 mmol), *tert*-butyl-4-aminophenylcarbamate (416 mg, 2 mmol), DIPC (151 mg, 1.2 mmol) and HOBT (176, mg, 1.3 mmol) using the procedure described in Method 10.

yield:	400 mg, 0.84 mmol, 84% (dark brown foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 9H, (CH$_3$)$_3$C), 3.5-3.7 (m, 2H, CHCH_2Ph), 3.85 (t, 1H, J = 5 Hz, CHCH$_2$Ph), 4.2 (d, 1H, J = 14 Hz, NCHHCONR), 4.7 (d, 1H, J = 14 Hz, NCHHCONR), 6.8 (d, 2H, J = 8 Hz), 7.1-7.6 (m, 18H, Ph), 8.6 (s, 1H, NH)
I.R. (v_{max})	3340, 2969, 1684, 1519, 1161 cm^{-1}
ms (FAB) (m/z, %)	575 (MH$^+$, 15), 335 (100), 279 (40)

N-(4-Aminophenyl)-2-(3-benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4] diazepin-1-yl)acetamide (194)
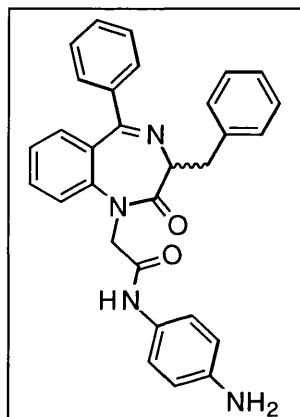
Compound **194** was synthesised from **193** (400 mg, 0.84 mmol) and trifluoroacetic acid (2 ml) using the procedure described in Method 7. The crude residue isolated was purified by flash column chromatography (EtOAc-hexane; 1:4) to afford the desired product.

yield:	141 mg, 0.3 mmol, 35% (brown foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.5-3.7 (m, 2H, CHCH_2Ph), 3.9 (t, 1H, J = 4 Hz, CHCH$_2$Ph), 4.3 (d, 1H, J = 12 Hz, NCHHCONR), 4.8 (d, 1H, J = 12 Hz, NCHHCONR), 6.5 (d, 2H, J = 8 Hz), 7.1-7.6 (m, 18H, Ph), 8.6 (s, 1H, NH)
I.R. (ν_{max})	3340, 2974, 1722, 1681, 1613, 1161 cm^{-1}
ms (FAB) (m/z, %)	475 (MH$^+$, 100)

2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)-N-(4-N',N''-bis-*tert*-butoxycarbonyl guanidinophenyl)acetamide (195)
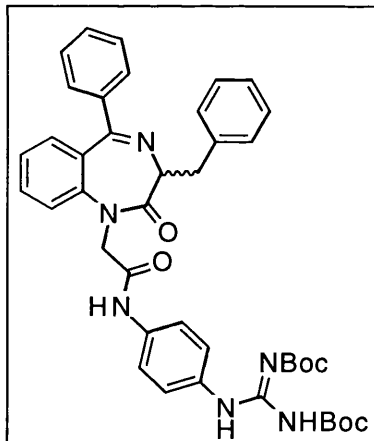
Compound **195** was synthesised from **194** (141 mg, 0.3 mmol), triethylamine (90 mg, 0.9 mmol), mercury (II) chloride (89 mg, 0.33 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (91 mg, 0.33 mmol) and DMF (20 ml) using the procedure described in Method 5.

yield:	137 mg, 0.19 mmol, 64% (light brown foam)

¹H nmr (200 MHz, CDCl₃) ∂	1.5 (s, 18H, (C*H*₃)₃C), 3.6-3.7 (m, 3H, CHC*H₂*Ph and C*H*CH₂Ph), 4.3 (d, 1H, J = 12 Hz, NCH*H*CONR), 4.8 (d, 1H, J = 12 Hz, NC*H*HCONR), 7.1-7.6 (m, 18H, Ph), 8.3 (s, 1H, NH), 10.2 (s, 1H, NH)
I.R. (ν_max)	3340, 2973, 1723, 1681, 1612, 1520. 1152 cm⁻¹
ms (FAB) (m/z, %)	717 (MH⁺, 25), 617 (5), 517 (MH⁺-2 Boc, 100)

2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)-N-(4-guanidinophenyl)acetamide (196)
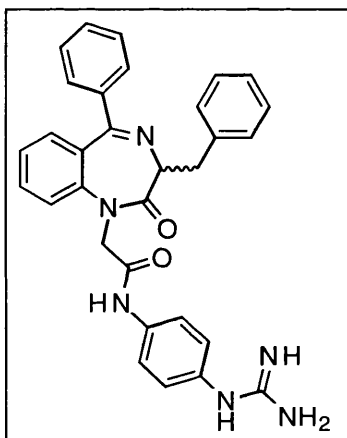
Compound **196** was synthesised from **195** (137 mg, 0.19 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	100.8 mg, 0.12 mmol, 66% (white foam)
¹H nmr (360 MHz, DMSO) ∂	3.4 (d, 2H, J = 6 Hz, CHC*H₂*Ph), 3.8 (t, 1H, J = 6 Hz, C*H*CH₂Ph), 4.65 (d, 1H, J = 12 Hz, NCH*H*COOH), 4.8 (d, 1H, J = 12 Hz, NCH*H*CONR), 7.1-7.7 (m, 18H, Ph), 9.5 (s, 1H, NH), 10.4 (s, 1H, NH)
I.R. (ν_max)	3391, 1684, 1600, 1202 cm⁻¹
MS(FAB) (m/z, %)	517 (MH⁺-CF₃CO₂⁻, 100), 327 (10)

analysis: $C_{31}H_{28}N_6O_2.2.4TFA.H_2O$ calc: C, 53.19; H, 4.04; F, 16.9;

effective molecular weight [808.27]   N, 10.39; O, 15.44

found: C, 53.08; H, 3.78; F, 16.8;

N, 10.18; O, 15.41

2-(3-Benzyl-2-oxo-5-phenyl-2,3-dihydrobenzo[e][1,4]diazepin-1-yl)-N,N-bis-(3-dimethylaminopropyl) acetamide (197)
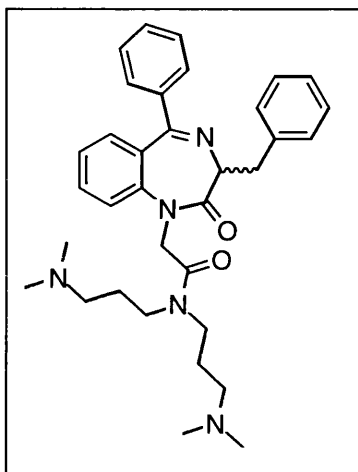
Compound **197** was synthesised from **188** (150 mg, 0.39 mmol), DIPC (60 mg, 0.47 mmol), HOBT (70 mg, 0.51 mmol) and 3,3'-iminobis(N,N--dimethyl-propylamine) (184 mg, 0.98 mmol) using the procedure described in Method 10. The crude product isolated was purified by preparative HPLC.

yield:	73.4 mg, 0.08 mmol, 21% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	1.8-2.0 (m, 4H, 2xRCH_2R), 2.7 (d, 6H, J = 3 Hz, (CH_3)$_2$NR), 2.8 (d, 6H, J = 3 Hz, (CH_3)$_2$NR) 2.9 (m, 2H, RCH$_2$CH_2NR), 3.1 (m, 2H, RCH$_2$CH_2NR), 3.4 (m, 6H, CHCH_2Ph and 2xRCH$_2$CH_2NCOR), 3.8 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 4.7 (d, 1H, J = 18 Hz, NCHHCONR), 4.8 (d, 1H, J = 18 Hz, NCHHCONR), 7.1-7.6 (m, 14H, Ph), 9.4 (bs, 1H, NH), 9.9 (bs, 1H, NH)
I.R. (ν_{max})	3435, 3032, 1677, 1604, 1202 cm^{-1}
MS(FAB) (m/z, %)	554 (MH$^+$-CF$_3$CO$_2^-$, 100), 311 (21)

analysis: $C_{34}H_{43}N_5O_2.2.7TFA.H_2O$

effective molecular weight [879.62]

calc: C, 53.79; H, 5.46; F, 17.49;
N, 7.96; O, 15.28

found: C, 53.43; H, 5.25; F, 17.5;
N, 7.85; O, 15.51

3.6 Miscellaneous Compounds

The structures of the compounds synthesised in this section differ from the compounds described under General Synthetic Procedures. The functional group interconversions are, however, identical to the examples described in the general methods.

[2-(4-Nitrobenzylamino)phenyl] phenylmethanone (199)
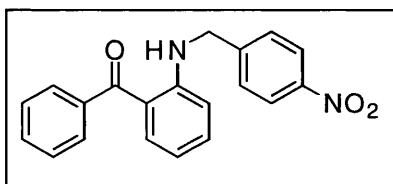
A mixture of 2-aminobenzophenone (900 mg, 4.47 mmol), 4-nitrobenzyl bromide (994 mg, 4.6 mmol), calcium carbonate (474 mg, 4.7 mmol) dioxane (40 ml) and water (4 ml) was heated at reflux for 48 h. The reaction mixture was concentrated *in vacuo* , diluted with EtOAc (100 ml) and HCl (100 ml, 1 M). The organic phase was separated, dried over $MgSO_4$, filtered and the filtrate concentrated *in vacuo*. The crude oil isolated (1.450 g) was used in the next step without purification.

[2-(4-Aminobenzylamino)phenyl] phenylmethanone (271)
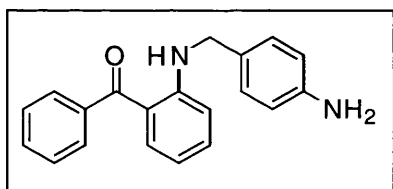
Compound **271** was synthesised from **199** (1.450 g) and tin (II) chloride dihydrate (2.000 g, 8.89 mmol) using the procedure described in Method 4.

yield:	600 mg, 2 mmol, 43% over two steps (yellow oil)
[1]H nmr (200 MHz, $CDCl_3$) ∂	4.6 (s, 2H), 6.6 (d, 1H, J = 6 Hz), 6.8 (d, 1H, J = 6 Hz), 7.1-7.6 (m, 11H)
I.R. (ν_{max})	3342, 1675, 1201 cm^{-1}
ms (FAB) (m/z, %)	302 (MH$^+$, 100)

## N-{4-[(2-Benzoylphenylamino)methyl]phenyl}-N',N''-bis-*tert*-butoxycarbonyl guanidine (272)
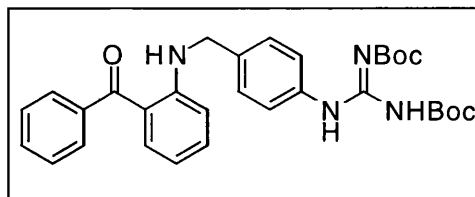
Compound **272** was synthesised from **271** (600 mg, 2 mmol), triethylamine (666 mg, 6.6 mmol), mercury (II) chloride (542 mg, 2 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (558 mg, 2 mmol) and DMF (20 ml) using the procedure described in Method 5.

yield:	330 mg, 0.63 mmol, 31% (yellow foam)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	1.6 (s, 18H, (CH_3)$_3$C), 4.5 (s, 2H), 6.7 (d, 1H, J = 5 Hz), 6.9 (d, 2H, J = 5 Hz), 7.1-7.6 (m, 13H)
I.R. (v_{max})	3312, 2979, 1719, 1641, 1150 cm^{-1}
ms (FAB) (m/z, %)	545 (MH$^+$, 10), 345 (MH$^+$-2 Boc, 100)

N-{4-[(2-Benzoylphenylamino)methyl]phenyl} guanidine (200)
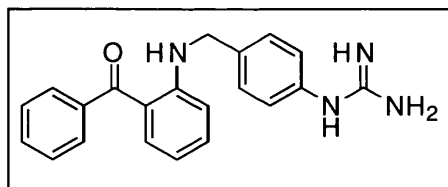
Compound **200** was synthesised from **272** (330 mg, 0.63 mmol) and trifluoroacetic acid (2 ml) using the procedure outlined in Method 7.

yield:	98.6 mg, 0.15 mmol, 24% (yellow foam)
^{1}H nmr (360 MHz, DMSO ∂	3.75 (s, 2H), 6.8 (d, 2H, J = 8 Hz), 7.1-7.2 (m, 6H), 7.3-7.6 (m, 5H), 9.7 (s, 1H, NH)
I.R. (v_{max})	3391, 1679, 1204 cm^{-1}
ms (FAB) (m/z, %)	345 (MH$^+$-CF$_3$CO$_2^-$, 100)

analysis: $C_{21}H_{20}N_4O.2.5TFA.0.8H_2O$ calc: C, 48.49; H, 3.77; F, 22.1;
effective molecular weight [643.88] N, 8.70; O, 16.89

found: C, 48.31; H, 3.47; F, 21.7;

N, 8.51; O, 16.27

3-(4-Nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (183)
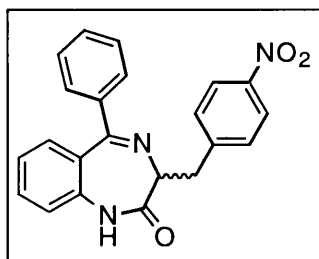
Compound **183** was synthesised from 2-aminobenzophenone (2.370 g, 12.03 mmol), triethylamine (1.410 g, 13.96 mmol), ethyl chloroformate (1.530 g, 14.10 mmol) and (±) N-Boc-4-nitrophenyl alanine (4.000 g, 12.90 mmol) using the procedures described in Methods 1 and 2.

yield:	1.245g, 3.36 mmol, 28% over two steps (white powder)
^{1}H nmr (200 MHz, CDCl$_3$) ∂	3.6 (d, 2H, J = 6 Hz, CHCH_2Ph), 3.8 (t, 1H, J = 6 Hz, CHCH$_2$Ph), 7.0-7.5 (m, 13H), 9.1 (s, 1H, NH)
I.R. (ν$_{max}$)	3074, 1689, 1673, 1342 cm^{-1}
HRMS: (C$_{22}$H$_{18}$N$_3$O$_3$, MH$^+$)	requires: 372.1348, found: 372.1340

3-(4-Aminobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4]diazepin-2-one (273)
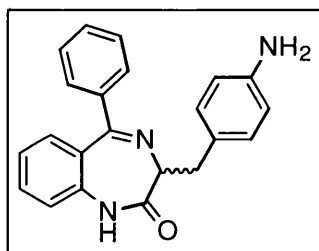
Compound **273** was synthesised from **183** (82 mg, 0.22 mmol) and tin (II) chloride dihydrate (225 mg, 1 mmol) using the procedure described in Method 4.

yield	70 mg, 0.21 mmol, 93% (light yellow foam)

¹H nmr (200 MHz, CDCl₃) ∂	3.6 (d, 2H), 3.8 (t, 1H), 7.0-7.5 (m, 13H), 9.1 (s, 1H)
I.R. (ν$_{max}$)	3386, 1679, 1607 cm⁻¹
ms (FAB) (m/z, %)	342 (MH⁺, 100)

N-[4-(2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-ylmethyl) phenyl]-N',N''-bis-*tert*-butoxycarbonyl guanidine (274)
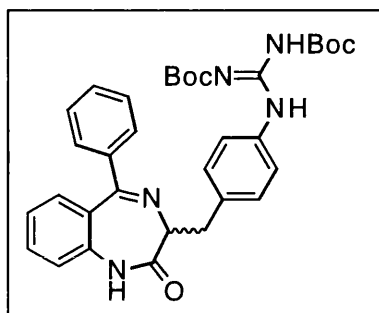
Compound **274** was synthesised from **273** (70 mg, 0.22 mmol), triethylamine (73 mg, 0.73 mmol), mercury (II) chloride (67 mg, 0.25 mmol), N,N' bis-*tert*-butoxycarbonyl thiourea (67 mg, 0.24 mmol) and DMF (15 ml) using the procedure described in Method 5.

yield:	64 mg, 0.11 mmol, 50% (yellow foam)
¹H nmr (200 MHz, CDCl₃) ∂	1.5 (s, 18H), 3.5 (d, 2H), 3.8 (t, 1H), 7.0-7.5 (m, 13H), 9.1 (s, 1H), 10.2 (s, 1H), 11.6 (bs, 1H)
I.R. (ν$_{max}$)	3300, 2979, 1719, 1641, 1608 cm⁻¹
ms (FAB) (m/z, %)	584, (MH⁺, 100)

N-[4-(2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-ylmethyl) phenyl] guanidine (201)
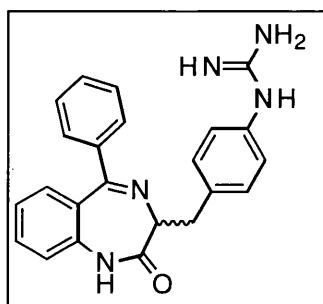
Compound **201** was synthesised from **274** (64 mg, 0.11 mmol) and trifluoroacetic acid (1 ml) using the procedure described in Method 7.

yield	75.5 mg, 0.10 mmol, 89% (yellow foam)
^{1}H nmr (360 MHz, DMSO) ∂	3.4 (dd, 2H, J = 6 and 18 Hz, CH*H₂*Ph), 3.7 (dd, 1H, J = 6 Hz and 9 Hz, C*H*CH₂Ph), 7.1-7.6 (m, 13H, Ph), 9.6 (s, 1H, NH), 10.7 (s, 1H, NH)
I.R. (ν_{max})	3180, 1675, 1609, 1202 cm^{-1}
ms (FAB) (m/z, %)	384 (MH$^+$-CF$_3$CO$_2^-$, 100)
analysis: C$_{23}$H$_{21}$N$_5$O.3.2TFA.1.5H$_2$O effective molecular weight [775.36]	calc: C, 45.53; H, 3.54; F, 23.52; N, 9.03; O, 18.36 found: C, 45.41; H, 3.27; F, 23.5; N, 9.19; O, 18.54

3.7 Alkylation studies on BZD intermediates 174 and 169

7-Chloro-3-methyl-1-(4-nitrobenzyl)-5-phenyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (159)
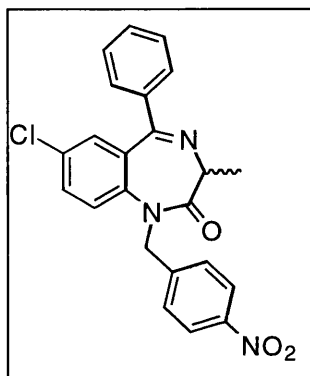
Compound **159** was synthesised from **174** (406 mg, 1.00 mmol), methyl iodide (213 mg, 1.5 mmol) and KO^{t}Bu in THF (1 M soln, 1.5 ml, 1.5 mmol) using the procedure described in Method 12.

yield:	270 mg, 0.55 mmol, 64% (yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	1.8 (d, 3H, J = 5 Hz), 3.9 (q, 1H, J = 5 Hz), 4.9 (d, 1H, J = 15 Hz), 5.8 (d, 1H, J = 15 Hz), 7.1-7.6 (m, 12H)
I.R. (v$_{max}$)	3347, 1684, 1605, 1581, 1341 cm^{-1}
MS(FAB) (m/z %)	422 (MH$^+$, 35), 420 (MH$^+$, 100)

7-Chloro-5-(2-chlorophenyl)-1-(4-nitrobenzyl)-3-propyl-1,3-dihydrobenzo [e][1,4]diazepin-2-one (202)
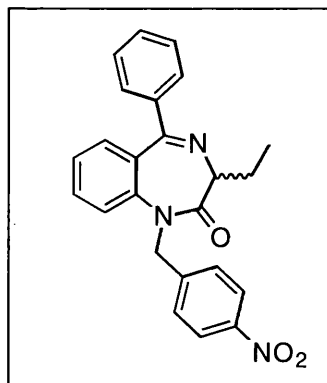
Compound **202** was synthesised from **169** (440 mg, 1.19 mmol), iodoethane (228 mg, 1.78 mmol) and KO^{t}Bu in THF (1 M soln, 1.8 ml, 1.8 mmol) using the procedure described in Method 12.

yield:	210 mg, 0.53 mmol, 44% (yellow foam)
^{1}H nmr (CDCl$_3$, 200 MHz) ∂	1.1 (t, 3H, J = 5 Hz), 1.6-1.7 (m, 2H), 3.8 (d, 1H, J = 10 Hz), 4.3 (dd, 1H, J = 5 Hz and 10 Hz), 4.8 (d, 1H, J = 10 Hz), 7.1-7.6 (m, 13H)
I.R. (v$_{max}$)	2957, 1684, 1654, 1603, 1521, 1340 cm^{-1}
MS(FAB) (m/z %)	400 (MH$^+$, 100)

REFERENCES

(1) Melzack, R.; Wall, P. D. In *The Challenge of Pain*; 3rd ed.Penguin: London, 1982.

(2) Lawrence, D. R.; Bennett, P. N. In *Clinical Pharmacology*; 6th ed.Churchill Livingstone: London, 1987; pp 284-285.

(3) Vane, J. R. *Nature, New Biol.* **1971**, *231*, 232.

(4) Frolich, J. C. *Trends Pharmacol. Sci.* **1997**, *18*, 30.

(5) Lipton, S. In *The Control of Chronic Pain*; S. Lipton, Ed.; Arnold: London, 1979; pp 22-26.

(6) Frey, E. K. *Langenbecks Arch. Klin. Chirurg.* **1926**, *142*, 663.

(7) Roche e Silva, M.; Beraldo, W. T.; Rosenfeld, G. *Am. J. Physiol.* **1949**, *156*, 261.

(8) Elliot, D. F.; Lewis, G. P.; Horton, E. W. *Biochem. Biophys. Res. Commun.* **1960**, *3*, 87.

(9) Boissonnas, R. A.; Guttmann, S.; Jaquenoud, P. A.; Konzett, H.; Sturmer, E. *Experientia* **1960**, *16*, 326.

(10) Pierce, J. V.; Webster, M. E. *Biochem. Biophys. Res. Commun.* **1961**, *5*, 353.

(11) Nicolaides, E. D.; De Wald, H. A.; McCarthy, D. S. *Biochem. Biophys. Res. Commun.* **1961**, *6*, 210.

(12) Sharma, J. D.; Watson Buchanan, W. *Exp. Toxic. Pathol.* **1994**, *46*, 421.

(13) Bhoola, K. D.; Figueroa, C. D.; Worthy, K. *Pharmacol. Rev.* **1992**, *44*, 1.

(14) Whalley, E. T.; Nwator, I. A.; Stewart, J. M.; Vavrek, R. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *336*, 430.

(15) Liebmann, C.; Schnittler, M.; Stewart, J. M.; Reissmann, S. *Eur. J. Pharmacol.* **1991**, *199*, 363.

(16) Farmer, S. G.; Burch, R. M. *Ann. Rev. Pharmacol. Toxicol.* **1992**, *32*, 511.

(17) Dray, A.; Perkins, M. *Trends Neurosci.* **1993**, *16*, 99.

(18) Perkins, M. N.; Campbell, E.; Dray, A. *Pain* **1993**, *53*, 191.

(19) Moncada, S.; Palmer, R. M. S.; Higgs, E. A. *Pharmacol. Rev.* **1991**, 109.

(20) Ljunggren, O.; Vavrek, R. J.; Stewart, J. M.; Lerner, U. H. *Bone Mineral Res.* **1991**, *6*, 807.

(21) Erdos, E. G. *J. Cardiovasc. Pharmacol. (Supl. 6.)* **1990**, *15*, 520.

(22) Erdos, E. G.; Skidgel, R. A. *FASEB J.* **1989**, *3*, 145.

(23) Dorer, F. E.; Ryan, J. W.; Stewart, J. M. *Biochem. J.* **1974**, *14*, 915.

(24) Cuthbert, A. W.; Margolius, H. S. *Br. J. Pharmacol.* **1982**, *75*, 587.

(25) Barnes, P. J.; Chung, K. F.; Page, C. P. *Pharmacol. Rev.* **1988**, *40*, 49.

(26) Smits, G. J.; McGraw, D. E.; Trapani, A. J. *Am. J. Physiol* **1990**, *258*, F1417.

(27) Benetos, A.; Gavras, H.; Stewart, J. M.; Vavrek, R. J.; Hatinoglou, S.; Gavras, I. *Hypertension* **1986**, *8*, 971.

(28) Carbonell, L. F.; Carreterro, O.; Stewart, J. M.; Scicli, A. G. *Hypertension* **1988**, *11*, 239.

(29) Yoshimura, Y.; Espey, L.; Hosoi, Y.; Adachi, T.; Atlas, S. J.; Ghodgaonkar, R. B.; Dubin, N. H.; Wallach, E. E. *Endocrinology* **1988**, *122*, 2540.

(30) Steranka, L. R.; Manning, D. C.; DeHaas, C. J.; Ferkany, J. W.; Borosky, S. A.; Connor, J. R.; Vavrek, R. J.; Stewart, J. M.; Snyder, S. H. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 3245.

(31) Kurjan, J.; Taylor, B. L. *Signal Transduction*; Academic Press: San Diego, CA, 1993.

(32) Lembeck, F. In *Substance P and Neurokinins*; J. L. Henry; R. Couture; A. C. Cuello; G. Pelletier; R. Quirion and D. Regoli, Ed.; Springer-Verlag: New York, 1987; pp 380-387.

(33) Keele, C. A.; Armstrong, D. *Substances producing pain and itch*; Arnold: 1964.

(34) Oyvin, I. A.; Gaponyuk, P. Y.; Oyvin, V. I.; Tokarev, O. Y. *Experimentia* **1970**, *26*, 843.

(35) Vavrek, R. J.; Stewart, J. M. *Peptides* **1985**, *6*, 161.

(36) Stewart, J. M.; Vavrek, R. J. In *Bradykinin Antagonists*; R. M. Burch, Ed.; Pergamon: Oxford, 1990; pp 51-96.

(37) Regoli, D.; Drapeau, G.; Rovero, P.; Dion, S.; D'Orleans-Juste, P. *Eur. J. Pharmacol.* **1986**, *123*, 61.

(38) Togo, J.; Burch, R. M.; DeHaas, J.; Connor, J. R.; Steranka, L. R. *Peptides* **1989**, *10*, 109.

(39) Hock, F. J.; Wirth, K.; Albus, H.; Linz, W.; Gerhards, H. J.; Wiemer, G.; S.T., H.; Breipohl, G.; Konig, W.; Knolle, J.; Scholkens, B. A. *Br. J. Pharmacol.* **1991**, *102*, 769.

(40) Cheronis, J. C.; Whalley, E. T.; Nguyen, K. T.; Eubanks, S. R.; Allen, L. G.; Duggan, M. J.; Loy, S. D.; Bonham, K. A.; Blodgett, J. K. *J. Med. Chem.* **1992**, *35*, 1563.

(41) Cheronis, J. C.; Whalley, E. T.; Allen, L. G.; Loy, S. D.; Elder, M. W.; Duggan, M. J.; Gross, K. L.; Blodgett, J. K. *J. Med. Chem.* **1994**, *37*, 348.

(42) Stewart, J. M. *Biopolymers* **1995**, *37*, 143.

(43) Abraham, W. M.; Burch, R. M.; Farmer, S. G. *Am. Rev. Respir. Dis.* **1991**, *143*, 787.

(44) Ellis, E. F.; Heizer, M. L.; Hambrecht, G. S.; Holt, S. A.; Stewart, J. M.; Vavrek, R. J. *Stroke* **1987**, *18*, 792.

(45) Whalley, E. T.; Solomon, J. A.; Madafferi, D. M.; Bonham, K. A.; Cheronis, J. C. In *Recent Progress on Kinins*; G. Bonner; H. Fritz; B. Schoelkens; G. Dietze and K. Lupetz, Ed.; Birkhauser: Basle, 1992; pp 413-420.

(46) Lowe, V. C.; Kyle, D. J.; Hiner, R. N.; Martin, J. A.; Burch, R. M.; Noronha-Blob, L. *Am. Fed. Clin. Res. Abstracts* **1992**, *40*, p. A 156

(47) Neugebauer, V.; Schaible, H. G.; Schmidt, R. F. *Pfluegers Arch.* **1989**, *415*, 330.

(48) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244.

(49) Moore, G. J. *Trends Pharmacol. Sci* **1994**, *15*, 124.

(50) Morgan, B. A.; Gainor, J. A. *Annu. Rep. Med. Chem.* **1989**, *24*, 243.

(51) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699.

(52) Luthman, K.; Hacksell, U. In *A Textbook of Drug Design and Development*; 2nd ed.; P. Krogsgaard-Larsen; T. Liljefors and U. Madsen, Ed.; Harwood Academic Publishers: Amsterdam, 1996.

(53) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656.

(54) Zabrocki, J.; Smith, G. D.; Dunbar Jr, J. B.; Iijima, H.; Marshall, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 5875.

(55) Wiley, R. A.; Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327.

(56) Plow, E. F.; Ginsberg, M. H. *Prog. Hemostasis Thrombosis* **1989**, *9*, 117

(57) Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Smith III, A. B. *Tetrahedron* **1993**, *49*, 3665.

(58) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Grant Spoors, P.; Shakespeare, W. C.; Sprengeler, P. A.; Hamley, P.; Smith III, A. B.; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550.

(59) Freidinger, R. M. *Trends Pharmacol. Sci.* **1989**, 270.

(60) Hirschmann, R. *Angew. Chem. Engl. Intl. Ed. Engl.* **1991**, *30*, 1278.

(61) Schwyzer, R. *Ann. NY Acad. Sci.* **1977**, *297*, 3.

(62) Kiefer, B. L.; Befort, K.; Gaveriaux, C.; Hirth, C. G. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 12048.

(63)   Breslow, R. In *Design and Synthesis of Organic Molecules Based on Molecular Recognition*; G. van Binst, Ed.; Springer-Verlag: Berlin, 1986; p 24.

(64)   Farmer, P. S. In *Drug Design*; E. J. Ariens, Ed.; Academic Press Inc.: London, 1980; Vol. X; pp 119-143.

(65)   Ariens, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. In *The Receptors: A Comprehensive Treatise*; R. D. O`Brien, Ed.; Plenum: New York, 1979; Vol. 1; pp 33-91.

(66)   Tanford, C. *The Hydrophobic effect: Formation of micelles and biological membranes.*; Wiley-Interscience: New York, 1980.

(67)   Beck-Sickinger, A. G. *Drug Discovery Today* **1996**, *1*, 502.

(68)   Tota, M. R.; Candelore, M. R.; Dixon, R. A. F.; Strader, C. D. *Trends Pharmacol. Sci.* **1991**, *12*, 4.

(69)   Bemis, G. W.; Murcko, M. A. *J. Med. Chem.* **1996**, *39*, 2887.

(70)   Sternbach, L. H. *J. Med. Chem.* **1978**, *22*, 1.

(71)   Hamor, T. A.; Martin, I. L. *Progress in Medicinal Chemistry* **1983**, *20*, 157.

(72)   Sangamameswaran, L.; Fales, H. M.; Friedrich, P.; De Blas, A. L. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 9236.

(73)   Stephenson, F. A. *Trends Neurosci.* **1987**, *10*, 185.

(74)   Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, N. P.; Gould, N. P.; Lundell, G. F.; Homnick, C. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; King, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem.* **1987**, *30*, 1229.

(75)   Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, N. P.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem.* **1988**, *31*, 2235.

(76)   Bondinell, W. E.; Keenan, R. M.; Miller, W. H.; Ali, F. E.; Allen, A. C.; De Brosse, C. W.; Eggleston, D. S.; Erhard, K. F.; Haltiwanger, R. C.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Koster, P. F.; Ku, T. W.; Lee, C. P.; Nichols, A. J.; Ross, S. T.; Samanen, J. M.; Valocik, R. E.; Vasko-Moser, J. A.; Venslavsky, J. W.; Wong, A. S.; Yuan, C. K. *Bioorg. Med. Chem.* **1994**, *2*, 897.

(77)   McDowell, R. S.; Gadek, T. R.; Barker, P. L.; Burdick, D. J.; Chan, K. S.; Quan, C. L.; Skelton, N.; Struble, M.; Thorsett, E. D.; Tischler, M.; Tom, J. Y. K.; Webb, T. R.; Burnier, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 5069.

(78) McDowell, R. S.; Blackburn, B. K.; Gadek, T. R.; McGee, L. R.; Rawson, T.; Reynolds, M. E.; Robarge, K. D.; Somers, T. C.; Thorsett, E. D.; Tischler, M.; Webb II, T. R.; Venuti, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 5077.

(79) Sugg, E. In *Gordon Research Conference on Medicinal Chemistry*; Colby Sawyer College, New London, New Hampshire, USA, 1996.

(80) Papageorgiou, C.; Borer, X. *BioMed. chem. Lett.* **1996**, *6*, 267.

(81) Johnson, R. E.; Baizman, E. R.; Becker, C.; Bohnet, E. A.; Bell, R. H.; Birsner, N. C.; Busacca, C. A.; Carabateas, P. M.; Chadwick, C. C.; Gruett, A. D.; Hane, J. T.; Herrmann Jr., J. L.; Josef, K. A.; Krafte, D. S.; Kullnig, R. K.; Michne, W. F.; Pareene, P. A.; Perni, R. B.; O'Connor, B.; Salvador, U. J.; Sanner, M. A.; Schlegel, D. C.; Silver, P. J.; Swestock, J.; Stankus, G. P.; Tatlock, J. H.; Volberg, W. A.; Weigelt, C. C.; Erzin, A. M. *J. Med. Chem.* **1993**, *36*, 3361.

(82) Okabe, M.; Sun, R. C. *Tetrahedron* **1995**, *51*, 1861.

(83) Venkatachalam, V. M. *Biopolymers* **1968**, *6*, 1425.

(84) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein. Chem.* **1985**, *37*, 1.

(85) Oka, M.; Mortelione, G. T.; Scheraga, H. A. *J. Am. Chem. Soc* **1984**, *106*, 7959.

(86) Smith, J. A.; Pease, L. G. *CRC Crit. Rev. Biochem.* **1980**, *8*, 315.

(87) Prasad, B. V. V.; Ravi, A.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 1138.

(88) Rao, B. N. N.; Kumar, A.; Balaram, H.; Ravi, A.; Balaram, P. *J. Am. Chem. Soc.* **1983**, *105*, 7423.

(89) Ripka, W. C.; DeLucca, G. V.; Bach II, A. C.; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *49*, 3593.

(90) Kopple, K. D.; Parameswarean, K. N.; Yonan, J. *J. Am. Chem. Soc.* **1984**, *106*, 7212.

(91) Izumiya, N.; Kato, T.; Aoyagi, H.; Waki, M.; Kondo, M. *Synthetic aspects of biologically active peptides- Gramicidin S and Tyrocidines*; Wiley and Sons: New York, 1979.

(92) Ripka, W. C.; DeLucca, G. V.; Bach II, A. C.; Pottorf, R. S.; Blaney, J. M. *Tetrahedron* **1993**, *49*, 3609.

(93) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters Jr, J. C. *Science* **1993**, *260*, 1937.

(94) Baracid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779.

(95)   Bokoch, G. M.; Der, C. J. *FASEB J.* **1993**, *7*, 750.

(96)   Hancock, J. F. *Current Biology* **1993**, *3*, 770.

(97)   Marsters Jr, J. C.; McDowell, R. S.; Reynolds, M. E.; Somers, T. C.; Goldstein, J. L.; Brown, M. S.; James, G. L. *Chemistry and Biology* **1994**, *1*, 8.

(98)   Hancock, J. F.; Magee, A. I.; Childs, J. E.; Marshall, C. J. *Cell* **1989**, *57*, 1167.

(99)   Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. *Cell* **1990**, *62*, 81.

(100) Pompliano, D. L.; Rands, E.; Schaber, M. D.; Mosser, S. D.; Anthony, N. J.; Gibbs, J. B. *Biochemistry* **1992**, *31*, 3800.

(101) Reiss, Y.; Brown, M. S.; Goldstein, J. L. *J. Biol. Chem.* **1992**, *267*, 6403.

(102) Reiss, Y.; Stradley, S. J.; Gierasch, L. M.; Brown, M. S.; Goldstein, J. L. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 732.

(103) Moores, S. L.; Schaber, M. D.; Mosser, S. D.; Rands, E.; O'Hara, M. B.; Garsky, V. M.; Marshall, M. S.; Pompliano, D. L.; Gibbs, J. B. *J. Biol. Chem* **1991**, *266*, 146.

(104) Wirth, K.; Hock, F. J.; Albus, H.; Linz, W.; Alpermann, H. G.; Anagnostopoulos, H.; S.T., H.; Breipohl, G.; Konig, W.; Knolle, J.; Scholkens, B. A. *Br. J. Pharmacol.* **1991**, *102*, 774.

(105) Krstenansky, J. L.; Baranowki, R. L.; Currie, B. L. *Biochem. Biophys. Commun.* **1982**, *109*, 1368.

(106) Stewart, J. M.; Wooley, D. W. *Nature (Lond.)* **1965**, *207*, 1160.

(107) Bauer, A.; Rhein, I. A.; Weber, K. H.; Algesheim, G. *US Patent* **1974**, 3,786,051.

(108) Bellamy, F. D.; Ou, K. *Tetrahedron lett.* **1984**, *25*, 839.

(109) Miller, A. E.; Bischoff, J. J. *Synthesis* **1986**, 777.

(110) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.

(111) Mulzer, J.; Schroder, F.; Lobbia, A.; Buschmann, J.; Luger, P. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1737.

(112) Ninomiya, K.; Shioiro, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.

(113) Walsh, D. A. *Synthesis* **1980**, 677.

(114) Zhang, W.; Liu, R.; Huang, Q.; Zhang, P.; Koehler, K. F.; Harris, B.; Skolnick, P.; Cook, J. M. *Eur. J. Med. Chem.* **1995**, *30*, 483.

(115) Bain, D. I.; Smalley, R. K. *J. Chem. Soc. (C)* **1968**, 1593.

(116) Marsters Jr, J. C.; McDowell, R. S.; Reynolds, M. E.; Oare, D. A.; Somers, T. C.; Stanley, M. S.; Rawson, T. E.; Struble, M. E.; Burdick, D.

J.; Chan, K. S.; Duarte, C. M.; Paris, K. J.; Tom, J. Y. K.; Dairian, D. T.; Xue, Y.; Burnier, J. P. *Bioorg. Med. Chem.* **1994**, *2*, 949.

(117) Salvino, J. M.; Seoane, P. R.; Douty, B. D.; Awad, M. M. A.; Dolle, R. E.; Houck, W. T.; Faunce, D. M.; Sawutz, D. G. *J. Med. Chem.* **1993**, *36*, 2583.

(118) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2024.

(119) Walser, A.; Ian Fryer, R. In *Bicyclic Diazepines*; R. Ian Fryer, Ed.; John Wiley & Sons: New York, 1991; Vol. 50; pp 667-668.

(120) Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.

(121) McPherson, G. A.; Molenaar, P.; Raper, C.; Malta, E. *J. Pharmacol. Meth.* **1983**, *10*, 231.

(122) Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Panzer-Knodle, S. G.; Nicolson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *J. Med. Chem.* **1993**, *36*, 1811.

(123) Nishio, M.; Umezawa, Y.; Hirota, M.; Takeuchi, Y. *Tetrahedron* **1995**, *51*, 8865.

(124) Doherty, A. M.; Patt, W. C.; Edmunds, J. J.; Berryman, K. A.; Reisdorph, B. R.; Plummer, M. S.; Shahripour, A.; Lee, C. L.; Cheng, X. M.; Walker, D. M.; Haleen, S. J.; Keiser, J. A.; Flynn, M. A.; Welch, K. M.; Hallak, H.; Taylor, D. G.; Reynolds, E. E. *J. Med. Chem.* **1995**, *38*, 1259.

(125) Dziadulewicz, E., personal communication.

(126) Jarnagin, K.; Bhakta, S.; Zuppan, P.; Yee, C.; Ho, T.; Phan, T.; Tahilraman, R.; Pease, J. H. B.; Miller, A.; Freedman, R. *J. Biol. Chem.* **1996**, *271*, 28277.

(126b) Schnur, D.M.; Grieshaber, M.V.; Bowen, J.P. *J. Comput. Chem.* **1991**, *12*, 844.

(127) Kogyo, P. T. *Japanese Patent 26302* **1969**,

(128) Bogatskii, A. V.; Andronati, S. A.; Gul'tyai, V. P.; Vikhyalaev, Y. I.; Galatin, A. F.; Zhilina, Z. I.; Klygul', T. A. *J. Gen. Chem. USSR (Engl. Trans.)* **1971**, *41*, 1364.

(129) Sternbach, L. H.; Ian Fryer, R.; Metlesics, W.; Reeder, E.; Sach, G.; Saucy, G.; Stempel, A. *J. Org. Chem.* **1962**, *27*, 3788.

(130) Misra, B. K.; Rao, Y. R.; Mahapotra, S. N. *Indian J. Chem. [B]* **1979**, *18*, 19.

(131) Simpson, J. C. E.; Atkinson, C. M.; Schofield, K.; Stephenson, O. *J. Chem. Soc.* **1945**, 646.

(132) Reeder, E.; Sternbach, L. H. *C.A.* **1966**, *64*, 19498.

(133) Walser, A.; Ian Fryer, R. In *Bicyclic Diazepines*; R. Ian Fryer, Ed.; John Wiley & Sons: New York, 1991; Vol. 50; pp 718.

(134) Chen, G. S. J.; Gibson, M. S. *J. Chem. Soc. Perkin Trans. 1* **1975**, 1138.

(135) Sunjic, V.; Kajfez, F.; Stromar, I.; Blasevic, N.; Kolbah, D. *J. Heterocycl. Chem.* **1973**, *10*, 591.

(136) Kajfez, F.; Sunjic, V. *US Patent 4,010,153* **1977**,

(137) Zinic, M.; Kolbah, D.; Blasevic, N.; Kajfez, F.; Sunjic, V. *J. Heterocycl. Chem.* **1977**, *14*, 1225.

APPENDIX - 1

Details of the crystal structure determination of $C_{22}H_{18}N_2O_3$.

A colourless single crystal of approximate size 0.44×0.46×0.28 mm was mounted on a glass fibre. All geometric and intensity data were taken from this sample using an automated four-circle diffractometer (Nicolet R3mV) equipped with Mo-Kα radiation (λ=0.71073 Å). Important crystallographic parameters are summarised in the table.

The lattice vectors were identified by application of the automatic indexing routine of the diffractometer to the positions of 28 reflections taken from a rotation photograph and centred by the diffractometer. The ω-2θ technique was used to measure 8743 reflections (8109 unique) in the range $5°\leq2\theta\leq55°$. Three standard reflections (remeasured every 97 scans) showed no significant loss in intensity during data collection. The data were corrected for Lorentz and polarisation effects. The 4197 unique data with I$\geq$3.0σ(I) were used to solve and refine the structure in the monoclinic space group P2$_1$/c.

The structure was solved by direct methods and developed by using alternating cycles of least-squares refinement and difference-fourier synthesis. The asymmetric unit contains two complete molecules. The non-hydrogen atoms were refined anisotropically while hydrogens were placed in idealised positions (C-H 0.96 Å) and assigned a common isotropic thermal parameter (U=0.08 Å^2). The final cycle of least-squares refinement included 451 parameters for 4197 variables and did not shift any parameter by more than 0.001 times its standard deviation. The final R values were 0.0590 and 0.0724, and the final difference-fourier was featureless with no peaks greater than 0.47 eÅ^{-3}.

Structure solution used the SHELXTL PLUS program package on a microVax II computer.
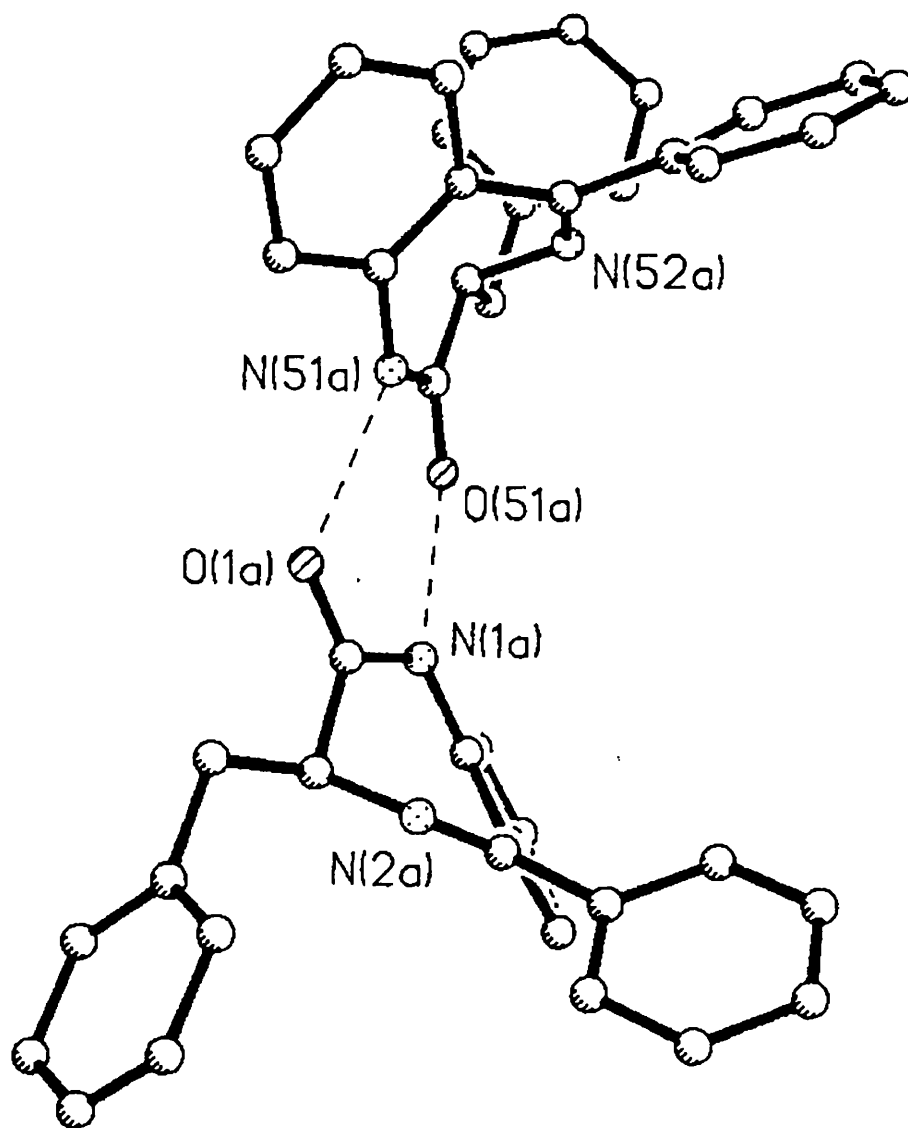
Table 1. Crystallographic data for $C_{22}H_{18}N_2O_1$.

Formula	$C_{22}H_{18}N_2O_1$
Space group	$P2_1/c$
a, Å	17.497(3)
b, Å	12.910(2)
c, Å	17.868(4)
α, deg	90.0
β, deg	118.91(2)
γ, deg	90.0
V, Å^3	3533
Z	8
F(000)	1376
d_{calc}, g/cm^3	1.23
Cryst. size, mm	0.44x0.46x0.28
μ(Mo-K$_\alpha$), cm^{-1}	0.71
Data collection instrument	Nicolet R3mV
Radiation	Mo-K$_\alpha$ (λ = 0.71073 Å)
Orientation reflections: no.;	28
range (2θ)	16$\leq$2$\theta$$\leq$26°
Temp., K	293
Data measured	8743
Unique data	8109
No. of unique with I$\geq$3.0σ(I)	4197
No. of parameters	451
R ^{a}	0.0590
R$_w$ ^{b}	0.0724
Weighting scheme	w^{-1} = σ^2(F) + 0.007239F^2
Largest shift/esd, final cycle	0.001
Largest peak, e/Å^3	0.47

^{a} R = $\Sigma[|F_o| - |F_c|]/\Sigma |F_o|$

^{b} R$_w$ = $\Sigma w^{\frac{1}{2}}.[|F_o| - |F_c|]/\Sigma w^{\frac{1}{2}}. |F_o|$

Table 1. Atomic coordinates (x10^4 Å) and equivalent isotropic displacement parameters (Å^{2}x10^3) for $C_{22}H_{18}N_2O_1$.

	x	y	z	U(eq)
O(2A)	7265(2)	5383(2)	-148(2)	64(1)
N(1)	6888(2)	6261(2)	711(2)	49(1)
N(4)	8288(2)	7585(2)	732(1)	44(1)
C(2)	7219(2)	6213(2)	160(2)	47(1)
C(3)	7518(2)	7234(2)	-40(2)	41(1)
C(10)	7743(2)	7101(3)	-760(2)	51(1)
C(11)	7980(2)	8103(3)	-1025(2)	48(1)
C(12)	7352(3)	8701(3)	-1676(2)	62(2)
C(13)	7552(4)	9592(3)	-1943(3)	88(3)
C(14)	8380(6)	9921(4)	-1584(4)	103(4)
C(15)	9038(4)	9372(5)	-934(4)	103(3)
C(16)	8847(3)	8448(3)	-636(2)	70(2)
C(9A)	6772(2)	7168(2)	1086(2)	41(1)
C(9)	6019(2)	7221(3)	1168(2)	50(1)
C(8)	5867(2)	8058(3)	1547(2)	57(2)
C(7)	6468(2)	8863(3)	1865(2)	58(2)
C(6)	7227(2)	8801(2)	1814(2)	48(1)
C(5)$_\alpha$	7392(2)	7963(2)	1415(2)	40(1)
C(5)	8217(2)	7923(2)	1368(2)	39(1)
C(1')	9028(2)	8313(2)	2112(2)	45(1)
C(2')	9587(2)	8983(3)	1999(2)	54(2)
C(3')	10354(2)	9307(3)	2698(2)	66(2)
C(4')	10592(3)	8957(3)	3501(2)	74(2)
C(5')	10063(3)	8272(4)	3622(2)	77(2)
C(6')	9265(2)	7969(3)	2931(2)	64(2)
O(52A)	5953(2)	4498(2)	853(2)	64(1)
N(51)	6782(2)	3573(2)	445(2)	49(1)
N(54)	6761(2)	2307(2)	1821(2)	43(1)
C(52)	6224(2)	3651(2)	773(2)	47(1)
C(53)	5988(2)	2640(2)	1041(2)	43(1)
C(60)	5224(2)	2752(3)	1217(2)	52(1)
C(61)	4981(2)	1743(3)	1465(2)	48(1)
C(62)	4492(2)	1011(3)	848(2)	57(2)
C(63)	4263(3)	80(3)	1076(3)	73(2)
C(64)	4507(3)	-132(3)	1892(4)	84(3)
C(65)	5002(4)	571(4)	2528(3)	88(3)
C(66)	5243(3)	1505(3)	2318(2)	69(2)
C(59A)	7167(2)	2648(2)	358(2)	43(1)
C(59)	7290(2)	2589(3)	-356(2)	55(2)
C(58)	7701(3)	1749(3)	-468(2)	62(2)
C(57)	7994(3)	950(3)	118(2)	60(2)
C(56)	7894(2)	1024(2)	838(2)	50(1)
C(55A)	7476(2)	1863(2)	971(2)	40(1)
C(55)	7411(2)	1942(2)	1769(2)	38(1)
C(51')	8162(2)	1579(2)	2591(2)	40(1)
C(52')	7999(2)	1119(2)	3200(2)	47(1)
C(53')	8685(2)	837(3)	3989(2)	57(2)
C(54')	9529(2)	1021(3)	4169(2)	62(2)
C(55')	9699(2)	1478(3)	3570(2)	66(2)
C(56')	9020(2)	1749(3)	2777(2)	55(1)

Equivalent isotropic U defined as one third of the trace of the orthogonalize⌐ ᴜ ⌐ᴇᴎᴄᴏʀ

Table 3. Bond angles (°) for $C_{22}H_{18}N_2O_1$.

C(2)-N(1)-C(9A)	126.0(3)		C(3)-N(4)-C(5)	120.4(3)
O(2A)-C(2)-N(1)	120.4(3)		O(2A)-C(2)-C(3)	123.3(4)
N(1)-C(2)-C(3)	116.4(3)		N(4)-C(3)-C(2)	108.2(2)
N(4)-C(3)-C(10)	109.6(3)		C(2)-C(3)-C(10)	111.0(3)
C(3)-C(10)-C(11)	113.1(3)		C(10)-C(11)-C(12)	121.1(3)
C(10)-C(11)-C(16)	121.0(3)		C(12)-C(11)-C(16)	117.9(4)
C(11)-C(12)-C(13)	122.3(4)		C(12)-C(13)-C(14)	120.3(4)
C(13)-C(14)-C(15)	120.8(6)		C(14)-C(15)-C(16)	120.0(6)
C(11)-C(16)-C(15)	118.6(4)		N(1)-C(9A)-C(9)	116.7(3)
N(1)-C(9A)-C(5)	123.3(3)		C(9)-C(9A)-C(5)	119.8(3)
C(9A)-C(9)-C(8)	120.7(3)		C(9)-C(8)-C(7)	120.3(4)
C(8)-C(7)-C(6)	119.5(4)		C(7)-C(6)-C(5)	121.4(3)
C(9A)-C(5)-C(6)	118.2(3)		C(9A)-C(5)-C(5)	121.8(3)
C(6)-C(5)-C(5)	120.0(3)		N(4)-C(5)-C(5)	125.2(2)
N(4)-C(5)-C(1')	116.7(3)		C(5)-C(5)-C(1')	118.1(3)
C(5)-C(1')-C(2')	121.0(3)		C(5)-C(1')-C(6')	120.3(3)
C(2')-C(1')-C(6')	118.6(3)		C(1')-C(2')-C(3')	120.0(3)
C(2')-C(3')-C(4')	121.3(4)		C(3')-C(4')-C(5')	119.5(3)
C(4')-C(5')-C(6')	120.2(4)		C(1')-C(6')-C(5')	120.2(4)
C(52)-N(51)-C(59A)	125.9(3)		C(53)-N(54)-C(55)	119.1(3)
O(52A)-C(52)-N(51)	120.9(3)		O(52A)-C(52)-C(53)	123.4(4)
N(51)-C(52)-C(53)	115.6(3)		N(54)-C(53)-C(52)	106.7(2)
N(54)-C(53)-C(60)	109.3(3)		C(52)-C(53)-C(60)	112.6(3)
C(53)-C(60)-C(61)	112.4(3)		C(60)-C(61)-C(62)	120.6(3)
C(60)-C(61)-C(66)	121.5(3)		C(62)-C(61)-C(66)	117.9(3)
C(61)-C(62)-C(63)	120.7(4)		C(62)-C(63)-C(64)	120.8(4)
C(63)-C(64)-C(65)	120.4(5)		C(64)-C(65)-C(66)	119.9(5)
C(61)-C(66)-C(65)	120.3(4)		N(51)-C(59A)-C(59)	116.0(3)
N(51)-C(59A)-C(55A)	123.4(3)		C(59)-C(59A)-C(55A)	120.3(3)
C(59A)-C(59)-C(58)	120.2(3)		C(59)-C(58)-C(57)	120.6(4)
C(58)-C(57)-C(56)	119.1(4)		C(57)-C(56)-C(55A)	121.8(3)
C(59A)-C(55A)-C(56)	117.9(3)		C(59A)-C(55A)-C(55)	121.4(3)
C(56)-C(55A)-C(55)	120.5(3)		N(54)-C(55)-C(55A)	125.4(2)
N(54)-C(55)-C(51')	115.8(3)		C(55A)-C(55)-C(51')	118.8(3)
C(55)-C(51')-C(52')	119.3(3)		C(55)-C(51')-C(56')	121.4(3)
C(52')-C(51')-C(56')	119.2(2)		C(51')-C(52')-C(53')	120.4(3)
C(52')-C(53')-C(54')	119.8(4)		C(53')-C(54')-C(55')	120.4(3)
C(54')-C(55')-C(56')	120.3(4)		C(51')-C(56')-C(55')	120.0(4)

Table 4. Anisotropic displacement parameters ($Å^2 \times 10^3$) for $C_{22}H_{18}N_2O_1$.

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(2A)	93(2)	45(1)	77(2)	-5(1)	59(2)	4(1)
N(1)	59(2)	43(1)	55(1)	1(1)	36(1)	6(1)
N(4)	38(1)	59(2)	38(1)	4(1)	21(1)	8(1)
C(2)	53(2)	44(2)	46(2)	3(1)	25(2)	10(1)
C(3)	41(2)	48(2)	38(1)	4(1)	21(1)	7(1)
C(10)	65(2)	53(2)	43(2)	-6(1)	32(2)	4(2)
C(11)	57(2)	60(2)	36(1)	-9(1)	31(1)	-1(2)
C(12)	77(2)	66(2)	50(2)	0(2)	35(2)	1(2)
C(13)	134(5)	78(3)	80(3)	14(2)	74(3)	9(3)
C(14)	180(6)	79(3)	115(4)	-18(3)	122(5)	-30(4)
C(15)	105(4)	128(5)	120(4)	-65(4)	90(4)	-65(4)
C(16)	61(2)	101(3)	63(2)	-26(2)	41(2)	-9(2)
C(9A)	45(2)	39(2)	43(1)	8(1)	25(1)	7(1)
C(9)	51(2)	52(2)	56(2)	7(1)	33(2)	3(1)
C(8)	56(2)	62(2)	72(2)	9(2)	46(2)	11(2)
C(7)	73(2)	51(2)	72(2)	-1(2)	53(2)	8(2)
C(6)	61(2)	44(2)	57(2)	-2(1)	42(2)	2(1)
C(5)	43(2)	44(2)	40(1)	6(1)	25(1)	6(1)
C(5)	40(1)	45(2)	39(1)	6(1)	25(1)	8(1)
C(1')	46(2)	54(2)	40(2)	-1(1)	26(1)	4(1)
C(2')	56(2)	61(2)	54(2)	1(2)	33(2)	-1(2)
C(3')	56(2)	82(3)	68(2)	-15(2)	36(2)	-11(2)
C(4')	54(2)	104(3)	57(2)	-25(2)	22(2)	-9(2)
C(5')	74(3)	111(3)	36(2)	-1(2)	18(2)	-4(2)
C(6')	66(2)	90(3)	41(2)	1(2)	29(2)	-10(2)
O(52A)	68(2)	42(1)	92(2)	3(1)	45(1)	6(1)
N(51)	55(2)	44(1)	52(1)	2(1)	29(1)	2(1)
N(54)	43(1)	49(1)	42(1)	-5(1)	23(1)	-2(1)
C(52)	43(2)	42(2)	49(2)	0(1)	17(1)	2(1)
C(53)	37(2)	45(2)	42(2)	-2(1)	16(1)	2(1)
C(60)	43(2)	56(2)	57(2)	-1(2)	25(1)	6(1)
C(61)	37(2)	58(2)	53(2)	3(1)	24(1)	8(1)
C(62)	42(2)	57(2)	65(2)	7(2)	22(2)	5(1)
C(63)	61(2)	60(2)	104(3)	1(2)	45(2)	3(2)
C(64)	98(3)	69(3)	118(4)	22(3)	77(3)	12(2)
C(65)	107(4)	104(4)	85(3)	38(3)	71(3)	39(3)
C(66)	68(2)	85(3)	63(2)	1(2)	39(2)	10(2)
C(59A)	48(2)	39(2)	40(1)	0(1)	20(1)	-1(1)
C(59)	73(2)	51(2)	47(2)	3(1)	33(2)	-4(2)
C(58)	88(3)	60(2)	51(2)	-6(2)	43(2)	-2(2)
C(57)	79(2)	52(2)	64(2)	-3(2)	47(2)	4(2)
C(56)	55(2)	49(2)	52(2)	1(1)	32(2)	4(1)
C(55A)	41(2)	42(2)	38(1)	-2(1)	19(1)	-8(1)
C(55)	40(2)	36(1)	39(1)	0(1)	20(1)	-4(1)
C(51')	41(2)	39(1)	38(1)	-2(1)	18(1)	-3(1)
C(52')	48(2)	55(2)	41(2)	-3(1)	24(1)	-4(1)
C(53')	65(2)	65(2)	40(2)	5(1)	25(2)	-1(2)
C(54')	51(2)	69(2)	49(2)	13(2)	10(2)	3(2)
C(55')	40(2)	77(3)	66(2)	10(2)	13(2)	-5(2)
C(56')	43(2)	64(2)	56(2)	10(2)	22(2)	-4(2)

Table 5.   H-Atom coordinates (x10^4) and isotropic displacement parameters (Å^{2}x10^3) for $C_{22}H_{18}N_2O_1$.

	x	y	z	U
H(4)	8815	7555	761	80
H(3)	7063	7740	-209	80
H(10A)	7256	6795	-1247	80
H(10B)	8229	6635	-567	80
H(12)	6759	8462	-1941	80
H(13)	7095	9987	-2392	80
H(14)	8515	10551	-1781	80
H(15)	9630	9613	-682	80
H(16)	9295	8055	-178	80
H(9)	5610	6658	962	80
H(8)	5341	8091	1589	80
H(7)	6352	9456	2118	80
H(6)	7656	9340	2056	80
H(2')	9431	9215	1433	80
H(3')	10725	9790	2617	80
H(4')	11125	9186	3984	80
H(5')	10244	7990	4180	80
H(6')	8878	7523	3020	80
H(54)	6773	2359	2329	80
H(53)	5846	2129	602	80
H(60A)	4726	3021	720	80
H(60B)	5385	3239	1676	80
H(62)	4318	1159	261	80
H(63)	3921	-410	636	80
H(64)	4334	-774	2036	80
H(65)	5186	403	3115	80
H(66)	5581	1996	2758	80
H(59)	7090	3143	-764	80
H(58)	7785	1712	-960	80
H(57)	8270	356	28	80
H(56)	8108	476	1253	80
H(52')	7408	991	3073	80
H(53')	8573	509	4408	80
H(54')	10006	841	4718	80
H(55')	10291	1606	3699	80
H(56')	9136	2052	2351	80

APPENDIX - 2

Figure **56**: Energy minimised conformation of **73**[*]
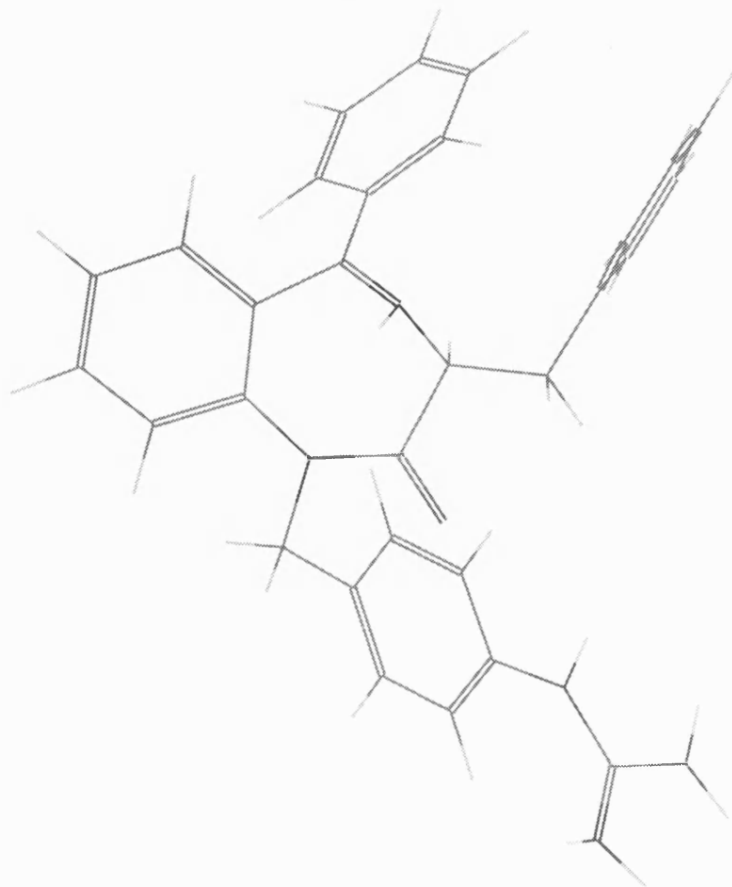
Figure **57**: Energy minimised conformation of **121***
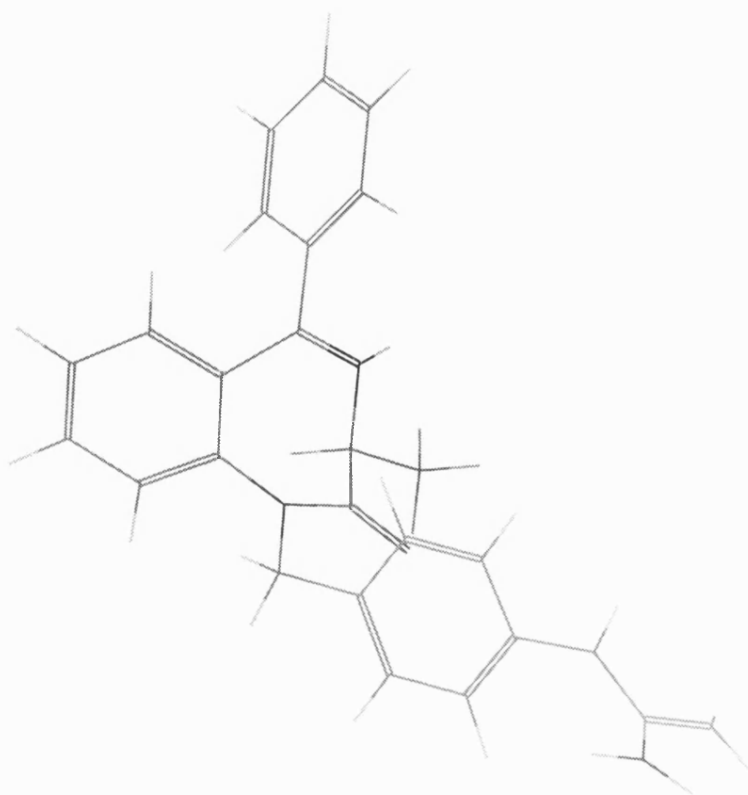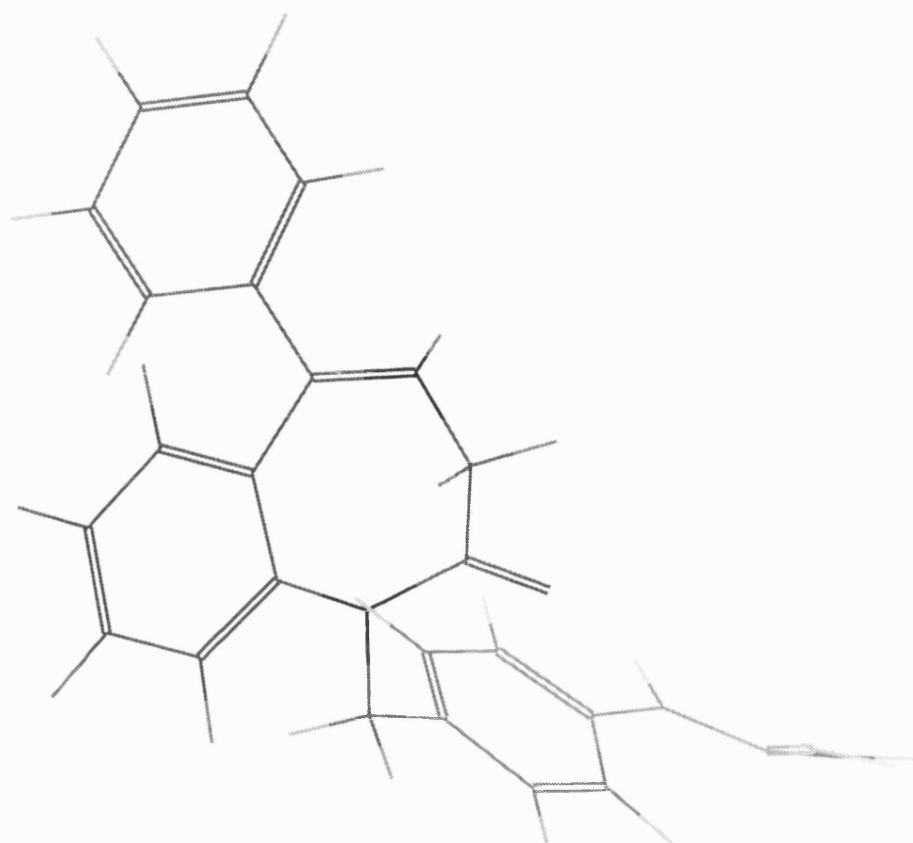
Figure **58:** Energy minimised conformation of **175***

Figure **59**: Energy minimised conformation of **177***