The Synthesis and Evaluation of Small Organic molecules as Cholecystokinin Antagonists

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Abstract: Cholecystokinin (CCK) is a peptide hormone, present in the alimentary and the CNS. It is the most abundant peptide in the brain. CCK has been implicated in a number of disorders. The link between CCK and anxiety was the basis for this research. A comprehensive discussion on the many types of CCK receptor antagonists is included. For the drug discovery process, a number of synthetic approaches have been investigated and alternative chemical approaches developed.

1,4-Benzodiazepine analogues were prepared, with substitutents in the 1,2 & 3-position of the benzodiazepine scaffold varied, and substituted 3-anilino benzodiazepines exhibited the greatest *in vitro* activity towards the CCK_A receptor subtype. Through extensive screening, pyrazolinone-ureido derivatives were identified, optimised, SAR studied and re-screened. A comprehensive *in vivo* study on the most active analogue is included, which has a number of common structural features with L-365, 260 including activity. Pyrazolinone-amide derivatives, bearing the tryptophan moiety were equally active. A number of existing and novel furan-2(5H)-one building blocks were prepared, from which a selected mini-library of 4-amino-substituted furan-2(5H)-ones were prepared and evaluated. All synthesised compounds were evaluated in a CCK radiolabelled binding assay (CCK_A & CCK_B), with compounds demonstrating receptor selectivity and lead structures being discovered. The work in this thesis has identified a number of highly active prime structures, from which further investigations are essential in providing more *in vitro* & *in vivo* data and the need to prepare more analogues.

Keywords: Cholecystokinin, ($CCK_{A/1}$ & $CCK_{B/2}$) receptor antagonists, Anxiety, 1,4-Benzodiazepine template, Pyrazolyl/Pyrazolinone ureido-amide derivatives, Furan-2(5)-one anlogues, Lead structure.

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Abbreviations

Aq	Aqueous
Ar	Aromatic
APCI(+)	Atmospheric pressured chemical ionisation (positive mode)
APCI(-)	Atmospheric pressured chemical ionisation (negative mode)
Z	Benzyloxycarbonyl
Boc	tert-Butoxycarbonyl
BuLi	n-Butyl lithium
BuOH	n-Butanol
BZD	Benzodiazepine
C	Carbon
CCl_4	Carbontetrachloride
CCK	Cholecystokinin
$CDCl_3$	Deuterated chloroform
$DMSO\text{-}_{d6}$	Deuterated dimethylsulphoxide
$CHCl_3$	Chloroform
Cl	Chlorine
CNS	Central Nervous System
Cpd	Compound
DEA	Diethylamine
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DIC	Dicyclohexylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
EtOAc	Ethylacetate
Et_2O	Diethylether
EtOH	Ethanol
Eq	Equivalent
FC	Flash chromatography
Fmoc	9-Fluorenylmethoxycarbonyl

GABA	Gamma aminobutyric acid
GAD	Generalised panic disorder
GAS	Gastric acid secretion
HCl	Hydrochloric acid
IC_{50}	Inhibitory concentration, 50 %
IR	Infra-red
μM	Micromolar
N	Nitrogen
NaH	Sodium hydride
Nm	Nanomolar
NaOH	Sodium hydroxide
MS	Mass spectometery
MeOH	Methanol
MF	Molecular formula
MP	Mobile phase
MW	Molecular weight
NMR	Nuclear magnetic resonance
P.P.M	Parts per million
PD	Panic disorder
Phe	Phenethylamine
PTSD	Post-traumatic stress disorder
i-PrOH	iso-Propanol
Pyr	Pyridine
R_f	Retention factor
RT	Room temperature
SAR	Structure-activity-relationship
SP	Social phobia
$Sn(OH)_2$	Tin hydroxide
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer-chromatography
Trp	Tryptophan

Chapter 1: Introduction

1.1. Drug Discovery

The initial starting point in any drug discovery process is the identification of a biological target and then the study of the interactions with the target. A careful choice has to be made about which target to pursue, because the large number of new drug targets in this area. This may be as high as 3,000[1].

Compounds are screened against biological targets and active compounds also called "hits" are then subjected for further optimisation in order to identify "lead" compounds as potential drug candidates[2,3]. Lead compounds are subjected to extensive studies in a development phase before entry into clinical trials.
The development phase includes; toxicology, distribution, metabolism and excretion from the body. For every drug approved in the US, in the mid-1990s, an average of 21 compounds out of 6,200 were put into the development phase. On average 6.5 of these were tested in humans and only 2.5 made it to the phase III clinical trials. Up to this point the process cost an average of 350 million dollars[4] and takes an average of 12.8 years[4].
Typical reasons for the failure of new compounds, in the development phase include:

1) Toxicity,
2) Poor pharmaceutical properties,
3) Lack of efficiency and
4) Other market reasons, e.g. no marketable or no competitive new drugs.

To decrease these possible failures and the resulting enormous costs in development, pharmaceutical companies have had to re-evaluate the drug discovery process and a discussion has now commenced on how to accelerate the drug discovery process, in order to increase the chance of finding a lead compound. Combinatorial chemistry in solution or on solid support is being developed to increase the efficiency of organic syntheses. Furthermore, successful applications of such methods, leading to the discovery of therapeutic candidates have been reported[5]. Figure 1.1 outlines the key steps for the drug discovery process.

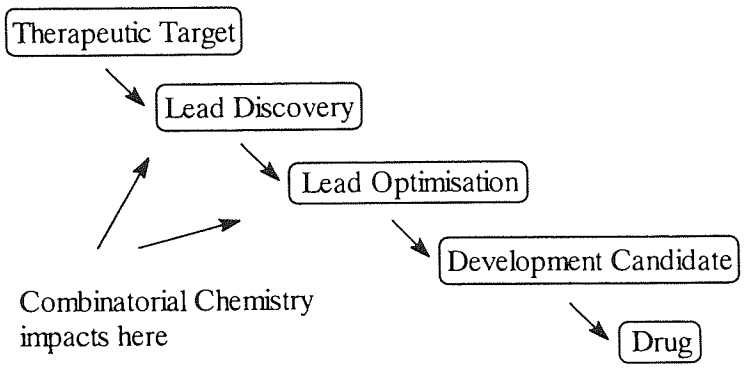


Figure 1.1: The key steps in the drug discovery process.

1.2. Central Nervous System (CNS) disorders

The pace of life in the highly developed industrial nations leads many to become anxious and depressed in view of the high demands placed on them by their peers and society at large. The success ethic and the high price of failure contribute in no small way to this malaise. Anxiety disorders are among the most prevalent and debilitating illnesses in medical practice today. More than 20% of alcoholics and 10% of the general population suffer significantly from anxiety[6]. Panic disorder occurs in about 2% of the population and is punctuated by such attacks, often following a chronic course. The phenomenon of panic, of extreme fear accompanied by physical

symptoms, has had numerous descriptions and different names in the psychiatric literature.

As a consequence of anxiety disorders, the cost to society is substantial. Quality of life studies have shown rates of functional impairment comparable to depression and chronic medical illnesses such as diabetes, arthritis and back, lung or gastrointestinal disorders[7]. Several other studies[7] support the premise that treatment of anxiety disorders is highly cost-effective because of decreases in indirect costs (i.e., loss of productivity, missed work days and use of non-psychiatric medical care).

First, Klein[8] proposed that "anxiety neurosis" should differentiate panic disorder (PD) from other forms of anxiety. Since then, other categories have been defined, such as generalised panic disorder (GAD), phobias (P), and post-traumatic stress disorder (PTSD). Anxiety in general is the emotional condition that is experienced by all humans. It is characterised by the unpleasant and diffuse sense of apprehension, accompanied by symptoms such as headache palpitations, restlessness, muscle pain, respiratory distress just to name a few. It is important to distinguish between fear and anxiety. Fear is considered to be the response of a threat, which is known, whereas anxiety relates to the threat, which is unknown[9]. The common somatic symptoms of panic attacks tend to consist of palpitations, dizziness, nausea and choking.

Results of studies would seem to suggest a genetic link to the disorder and, due to the significant impairment of social functioning associated with the disorder, this has caused much interest within medical research.

For determination and, for a better understanding of the emotional condition, anxiety and panic researchers used substrates such as *Carbon Dioxide* inhalation[10] and *Sodium Lactate* infusions[11] or Flumazenil[12], a benzodiazepine derivative, which can produce anxiety or panic attacks in humans.

However, studies have led to the realisation that the underlying biochemical and physiological mechanisms are more complex than was originally thought.

This thesis addresses the development of potential drug compounds relevant for the treatment of central nervous system (CNS) disorders such as anxiety, fear, analgesia or schizophrenia.

1.3. Cholecystokinin (CCK) The biological target

A potential new approach has been suggested by the findings that fragments of the amino acid peptide cholecystokinin (CCK_4) provokes panic attacks in healthy volunteers[13,14,15] shortly after injection. Bradwejn et al.[16] (1991) has also found that, compared to normal volunteers, patients with panic disorder had increased sensitivity to its administration.

These results have lead to the conclusion that one type of CCK receptors, which occurs mainly in the brain (CCK_B/CCK_2 receptors) are involved in the regulation of anxiety and experimental results[17], with new and selective CCK_B antagonists suggests the possibility that CCK antagonists might have a role in the treatment of CNS disorders. CCK is widely used as a biological target in order to develop new and highly selective CCK antagonists and to explore the functional role of CCK.

1.4. Cholecystokinin

Cholecystokinin (CCK) is a major intestinal hormone with a key role in regulating the control of pancreatic secretion and bile release. Ivy and Oldberg[18] (1928) were the first to describe "a substance released from the upper intestine and produced gallbladder contractions".

There is considerable evidence[19] for a physiological role in the regulation of motor function, at various levels in the gastro-intestinal/alimentary tract. Recent advances in peptide chemistry have resulted in a greater understanding of the physiological role of these gastrointestinal hormones. An important step was the development of potent and

specific receptor antagonists. Of these gastrointestinal hormones, only the gastrin and Cholecystokinin (CCK) antagonists have been tested in humans

Gastrin was the first gastrointestinal peptide that had its structure determined. It is produced by G cells, which are located in the gastric mucosa and upper small intestine. Gastrin mainly stimulates gastric acid secretion (GAS) from parietal cells and promotes the growth of gastric mucosa[20].

Many different structural forms of gastrin have since been discovered[21] and they all share the five-amino acid C-terminal that is responsible for the biological activity of gastrin (Table 1.4). The five-amino acid C-terminus of gastrin is also common to Cholecystokinin (CCK), a structurally related gastroinstestinal peptide, which, despite being similar to the C-terminus pentapeptide sequence exhibits different biological effects[22,23].

Cholecystokinin (CCK) is produced by I cells of the duodenal and jejunal mucosa and exists most prominently as an eight amino-acid hormone (CCK-8). CCK has been long been recognised as having an effect on the regulation of pancreatic secretion[24] and of gall bladder contraction[18]. Cholecystokinin has also been found in the brain, where it is widely distributed and may therefore have an effect as a neuromodulator or perhaps as a neurotransmitter.

CCK is characterised by the α-aminated terminus Trp-Met-Asp-Phe-NH_2 aminated sequence. It was initially identified as a 33 amino acid chain[25] and was later synthesised[26]. Subsequent studies have revealed the existence of multiple forms[27,28]. CCK is derived from a primary prepro-CCK polypeptide of 115 residues. After transcription, enzymatic cleavage results in the formation of many different fractions.

CCK_{58}, CCK_{39}, CCK_{33}, CCK_{22}, CCK_{8s} (sulphated), CCK_{8ns} (non-sulphated), CCK_7, CCK_5, CCK_4 all of them demonstrate biochemical activity[29]. The predominant circulating form has a sulphated tyrosine residue at position 7.

CCK 8s	Asp-Tyr-[SO_3H]-Met-Gly-Trp-Met-Asp-Phe-NH_2
CCK 8	Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2
CCK 4	H-Trp-Met-Asp-Phe-NH_2
Pentagastrin	Gly-Trp-Met-Asp-Phe-NH_2

Table 1.4: Amino acid sequence of CCK and Pentagastrin fragments

It is important to distinguish between the CCK tetrapeptide[30] and octapeptide (Sincalide)[31] as shown in Table 1.4. Both of them have been extensively studied, particularly in relation to food intake regulation, and they have brought a great deal of confusion when it came to anxiety and panic. They have differential affinity for CCK receptors[32,33] different distribution in both the periphery and the brain[34,35] and have various effects on behaviour.

CCK and its receptors[36] are also widely distributed in the central nervous system (CNS) and contribute to the regulation of satiety, anxiety, analgesia, and dopamine-mediated behaviour. Its presence in the brain was first conclusively demonstrated in 1976[37]. Gastrin and CCK-8 have identical -COOH terminal penta-peptide sequences. Most gastrin-like activity in the brain is present as CCK-8, which exists in sulphated (CCK-S) and desulphated forms. CCK-containing neurones are widely distributed in the brain. In some neurones, CCK-8 coexists with other neurotransmitters.

The role of CCK in the CNS has been an area of immense investigation over the past 20 years. As a result of this intense investigation, CCK has been implicated in e.g. feeding and satiety[38], pain perception[39], psychiatric diseases[40], and anxiety disorders[41] (Table 1.5). This suggests an interaction with other neurotransmitter systems. Evidence has been found for interaction of CCK with multiple receptors[42] such as dopamine (DA)[43], gamma-aminobutyric acid (GABA)[44,45], serotonin (5-hydroxytryptamine, HT), noradrenaline (NA) and opioid peptides[46,47,48]. Thus, it is expected that its actions and effects are various and complex.

High affinity CCK binding sites were initially demonstrated in rat pancreatic acini[49] and the cerebral cortex[50]. Derivatives of cyclic nucleotides were shown to antagonize the actions of CCK in the guinea pig pancreatic acini and ileum[49]. Although, an important physiological role was recognized for CCK receptors in the periphery, the function of CCK in the brain was not well understood.

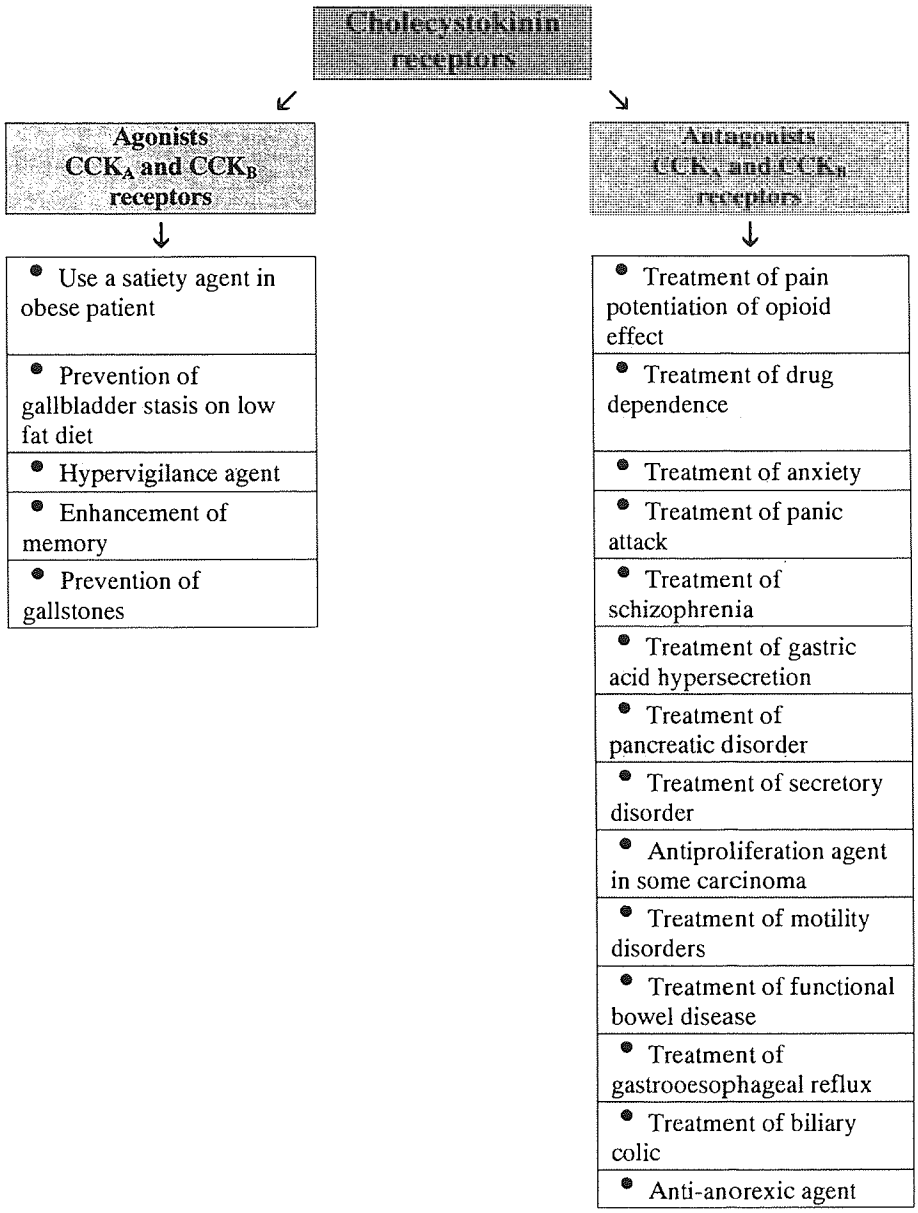


Table 1.5: Potential therapeutic applications of CCK-receptor ligands linked to a specific action on the two CCK sub-receptors[51].

Given the location of CCK receptors, they were classified according to their position. Type *A* (alimentary) and type *B* (brain)[52]. The existence of these receptors was confirmed by cloning by De Weeth et al.[53]. The various CCK fragments showed different affinity for each of the binding sites, periphery and the brain. Peripheral type CCK_A/CCK_1 receptors show the greatest affinity for sulphated CCK_8[54] and have a 100-fold lower affinity for desulphated CCK_8 and CCK_4[55]. CCK_B receptors show the same affinity for sulphated CCK_8, desulphated CCK_8[56] and CCK_4 (Lee et al.[57]).

In humans the distribution of CCK_A receptors has been found in the alimentary tract and certain regions in the brain e.g. area postrema, nucleus solitarius, hypothalamus and the vagus nerve complex. CCK_A receptors occur predominantly at the peripheral level where they are responsible for the digestive effects of CCK: intestinal and biliary smooth muscle contraction, pancreatic enzyme secretion, trophic effects on gastric and intestinal mucosa and regulation of feeding. CCK_B receptors are much more widely distributed in the CNS and high densities are present in cortical and limbic areas such as the amygdala, hippocampus and the hypothalamus.

However, some brain CCK-receptors belong to the A-type, but the majority of them are CCK_B receptors. Furthermore, extensive studies[58] have shown the original classification was oversimplified. B type receptors have also been isolated in the periphery of rat lung cells. At the peripheral level, CCK_B receptor antagonists are active on gastrin receptors; these two receptors are similar, and to date, no compounds have been developed that will clearly distinguish between these two receptors.

Both the human CCK_A and CCK_B receptors have been cloned and the results have shown they belong to the G-Protein-Coupled-Receptor (GPCR) superfamily[59]. Signal transduction is mediated via activation of Phospholipase C and the formation of Inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol[59]. Experimental evidence suggests involvement of brain CCK processes in 4 domains: modulation of dopaminergic function, control of pain sensation, anxiety and memory formation. Thus, CCK_B antagonists may be useful to treat certain neuropathological conditions associated with CCK dysfunction.

In recent years specific and highly potent CCK antagonists have been developed including some that are highly selective for CCK receptor subtypes. The availability of these compounds has prompted investigations into the functional role of CCK in the brain. This has opened up new possibilities for the treatment of central nervous system disorders.

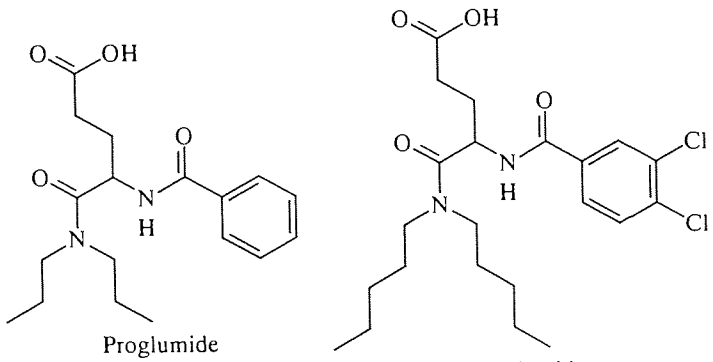
1.5. Therapeutic applications of cholecystokinin receptor antagonists

Many scientists have discovered specific peptide and non-peptide antagonists of CCK_B/gastrin receptors. As a result, a number of new chemical entities appeared, exhibiting high selectivity for specific population of CCK_B/gastrin receptors. The various compounds under development belong to the following main chemical classes:

- Amino acid derivatives;
- Cyclic nucleotide derivatives;
- Tryptophan dipeptoid derivatives;
- Peptides;
- Pyrazolidinones;
- Ureidoacetamides;
- Ureidophenoxyacetanilides;
- Ureidomethylcarbamoylphenylketones;
- Dibenzobicyclo[2.2.2] octane and bicyclic hetroaromatic derivatives;
- Benzodiazepine derivatives;
- Ureidobenzodiazepine derivatives;
- Quinazolinone-based compounds and
- Indol-2-based compounds.

1.5.1. Amino acid derivatives

During the 1970's amino acid derivatives (Figure 1.5.1) were found to possess antigastrin activity[60]. The chemical similarities of gastrin and CCK made it possible for such derivatives to demonstrate CCK antagonist activity. Proglumide, the first putative gastrin antagonist clinically available, has long been used in the treatment of peptic ulcers, because of its antisecretory and gastroprotective activities. Several studies have subsequently demonstrated that proglumide is also a weak CCK_A receptor antagonist[61] and despite its low potency, it has been the reference CCK and gastrin antagonist for several years.



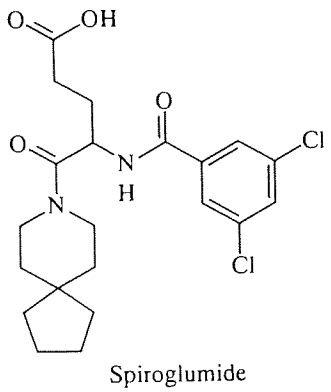


Figure 1.5.1: Structures of early amino acid derivatives as CCK antagonist

Rotta research group produced analogues of proglumide, which showed varying degrees of selectivity for CCK_A receptors and even suggested possible sub-types of the peripheral receptors. Some derivatives had a higher affinity for pancreatic CCK

receptors mediating gallbladder contraction. Lorglumide showed up to a 26-fold increase in potency for blocking CCK-stimulated gallbladder contraction but only a two-fold increase for blocking CCK-stimulated pancreatic amylase secretion[62,63]. Intravenous administration of lorglumide[64] antagonized the CCK-induced reduction of gastric emptying in rats, acceleration of intestinal transport in mice, increase in ileal motility in rabbits, gallbladder contraction in guinea pigs and acceleration of gallbladder emptying in mice but showed reduced activity when orally administered[64]. Further structural modifications to lorglumide resulted in CR2194 (spiroglumide). Spiroglumide exhibited CCK_B/gastrin antagonist in the micromolar range, with excellent oral bioavaiability. However, it has poor selectivity for CCK_B/gastrin receptor, which raises doubts of its potential therapeutic usefulness.

1.5.2. Cyclic nucleotide derivatives

Dibutyryl cyclic guanosine monophosphate (Bt_2cGMP) was the first competitive antagonist of CCK-mediated action to be discovered[65].

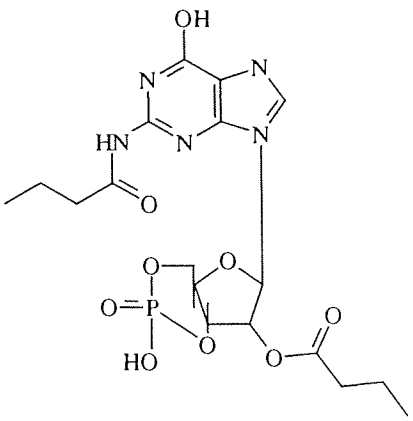
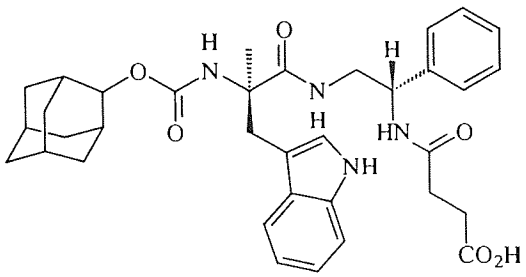


Figure 1.5.2: Structure of (Bt_2cGMP)

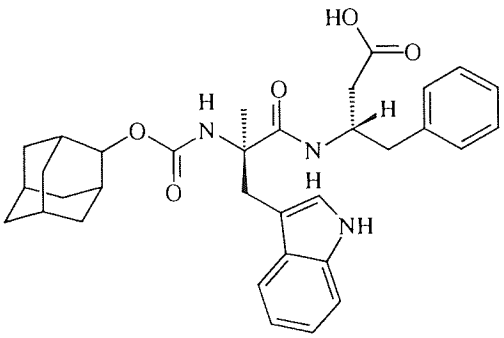
It was found to cause both reversible and selective inhibition of CCK-stimulated amylase secretion from rat pancreatic cells. Subsequently it was found to block the effects of CCK at many peripheral sites. However, Bt_2cGMP failed to inhibit CCK binding in mouse cerebral cortex[35].

1.5.3. Tryptophan dipeptoid derivatives

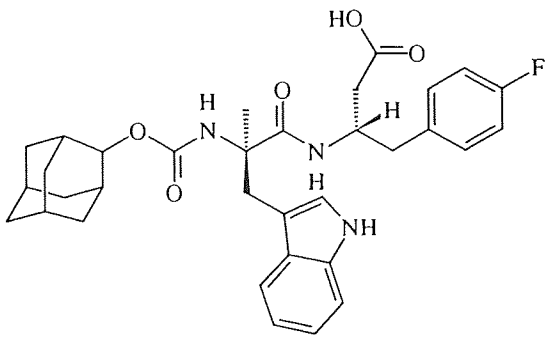
A research group at Parke-Davis[66,67] examined the activity of CCK-30-33 fragments, in binding experiments on CCK_B/ gastrin receptors. This led to the development of C1988 (Figure 1.5.3), which exhibited 1600-fold selectivity for CCK_B over CCK_A receptors. Structural C-terminal modifications led to alternative compounds, which demonstrated sub-nanomolar affinity for CCK_B/gastrin receptors. Compound (**1**) displays high affinity (IC_{50} = 0.3 nM). Further structural modifications, to optimise the substitution on the phenyl ring of (**1**) led to the analogue (**2**). This showed exceptionally high affinity for CCK_B/gastrin receptors, (IC_{50} = 0.08 nM) and was 940 times more selective over the CCK_A receptor. However, due to the high molecular weight and the dipeptoid structure, these derivatives have a low bioavailability, and therefore are not suitable drugs for oral therapy.



C1988



(1)



(2)

Figure 1.5.3: Tryptophan dipeptoid CCK_B antagonists

1.5.4. Peptides

The first CCK related peptide found to demonstrate CCK receptor agonist was CCK-27-32-NH_2. This inhibited CCK-induced pancreatic enzyme secretion. Clark et al. [68]claimed the C-terminal phenylalanine is essential for intrinsic activity but not for binding. The L-tryptophan residue is important also for binding to both central and peripheral CCK receptors.

In another study[69], a synthetic peptide derivative of CCK-7, t-butyloxycarbonyl-Tyr-(SO_3)-Met-Gly-D-Try-Nle-Asp2-phenylethyl ester inhibited binding of labelled CCK-9 to both pancreatic acini and cerebral cortical membranes, in addition to blocking agonist-stimulated amylase secretion. The lack of oral bioavailability of these peptide CCK antagonists severely restricts their potential therapeutic use.

1.5.5. Pyrazolidinones

Scientists from Lilly identified, through a bulk random screening technology, a series of functionalised pyrazolidinone derivatives. Structure-activity relationship studies[70] led to the identification of compounds such as (A, LY262691) (Table1.5.5), with binding affinities of 31nM and 11,600 nM for the CCK_B & CCK_A receptors respectively. It showed more than 350-fold selectivity for CCK_B receptors[71,72].

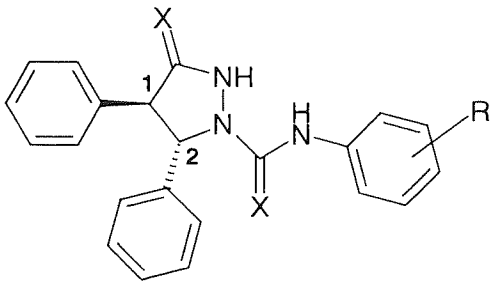


Figure 1.5.5: Pyrazolidinone derivative LY288513

Example	X	R	1	2	CCK_A IC_{50}	CCK_B (nM)
A	O	p-Br	R/S	R/S	11600	31
B	O	p-Br	R	S	10400	370
C	O	p-Br	S	R	20500	19
D	S	m-CF_3, p-Cl	R/S	R/S	42	880
E	S	m-CF_3, p-Cl	R	S	17	1900
F	S	m-CF_3, p-Cl	S	R	810	550

Table 1.5.5: Pyrazolidinone CCK_B antagonists

As observed with the other series, binding affinity & selectivity for the receptor subtypes was dependent on the absolute stereochemistry of the chiral centres in the molecule. LY288513 (C) having 4S, 5R stereochemistry at the two-phenyl centres retains most of the binding affinity of the reacemate (A), whereas the corresponding enantiomer LY288512 (B) is significantly less potent.

Stereo-dependent interactions with the CCK receptors were further substantiated within the same series of compounds having CCK_A receptor selectivity. Therefore the racemic thiourea analogue (D) had binding affinities of 880 nM and 42 nM for the CCK_B & CCK_A receptors respectively. The CCK_A binding affinity was improved for the enantiomer (E), having 4R, 5S configuration at the two-phenyl centres. However, the corresponding enantiomer (F) was significantly less active and showed modest CCK_B selectivity. Further development of compound (C) has been discontinued due to adverse effects in preclinical toxicological studies[73].

1.5.6. Ureidoacetamides

Developed by Rhone-Poulenc, these nonpeptide ureidoacetamides are potent and selective ligands for CCK_B/gastrin receptors. Compound RP69758 demonstrated nanomolar activity for CCK_B/gastrin receptors, whilst exhibiting 100-1000 fold selectivity for CCK_B/gastrin receptors over CCK_A receptors[74].

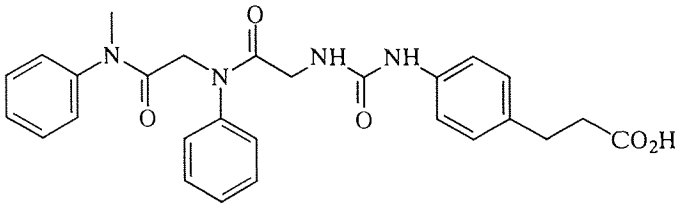


Figure 1.5.6: Ureidoacetamide derivative RP69758

1.5.7. Ureidophenoxyacetanilides

In order to avoid adverse effects derived from CCK_A receptor antagonist activity with gastrin/CCK_B receptor antagonists (exemplified for Proglumide, Figure 1.5.7) Scientists from Japan[75] have developed a series of phenoxyacetanilide derivatives, which were linked with the ureido-phenyl moiety. The most active compound of this series is DZ-3514, which demonstrated nanomolar activity for CCK_B/gastrin receptors (0.8 nM) with 250-500 fold selectivity over CCK_A receptors.

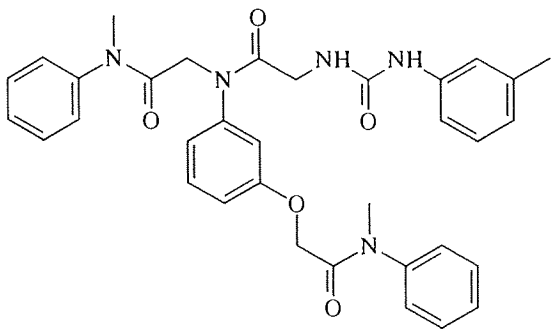
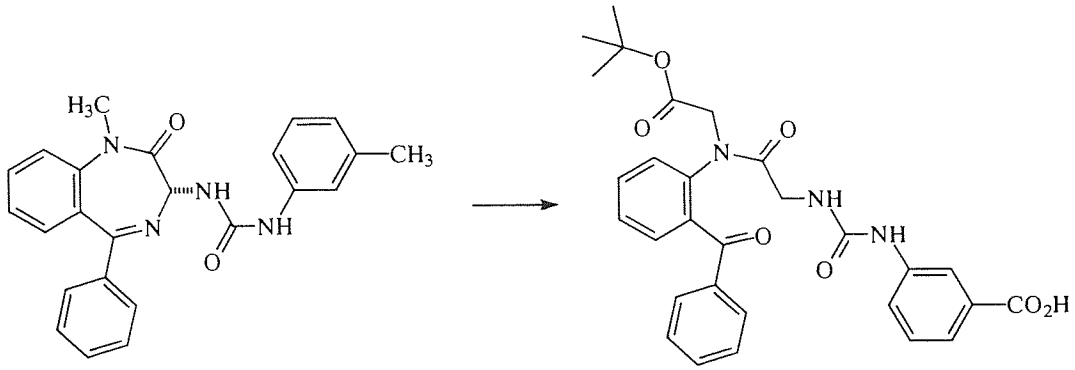


Figure 1.5.7: Ureidophenoxyacetanilide derivative DZ-3514

1.5.8. Ureidomethylcarbamoylphenylketones

Shiogoni has developed a series of ureidomethylcarbamoylphenylketones[76] as selective CCK_B receptor antagonists. This series of compounds exemplified by the urea, was derived by cleavage of the C-3/N-4 bond of the 1,4-benzodiazepine L-365,260 (**3**). The highlight of this series was S-0509 (**4**), which had 120-fold selectivity for CCK_B receptors over CCK_A.



(3) (4)

Figure 1.5.8: Structures of 3-ureido-1,4-benzodiazepine derivative L-365,260 (3) and ureidomethylcarbamoylphenylketone derivative S-0509 (4)

1.5.9. Dibenzobicyclo [2.2.2] octane and bicyclic heteroaromatic derivatives

Based on the dibenzobicyclo [2.2.2] octane skeleton (Figure 1.5.9), the James Black foundation[77] synthesised potent and selective CCK_B/gastrin antagonist. The most potent compound was (**5**). Given intravenously at the dose of 0.025 μm/kg it gave a peak inhibition of 79% of GAS observed for a submaximal infusion of pentagastrin (i.e. it was at least 40 times more potent than L-365.260). Compound (**6**), a 5,6-disubstituted-indole derivative was the optimal compound of this new series. It totally inhibited (97%) pentagastrin-stimulated GAS in the rat, when administered

intravenously at the dose of 0.025 μm/kg and exhibited a comparable activity in the dog assay.

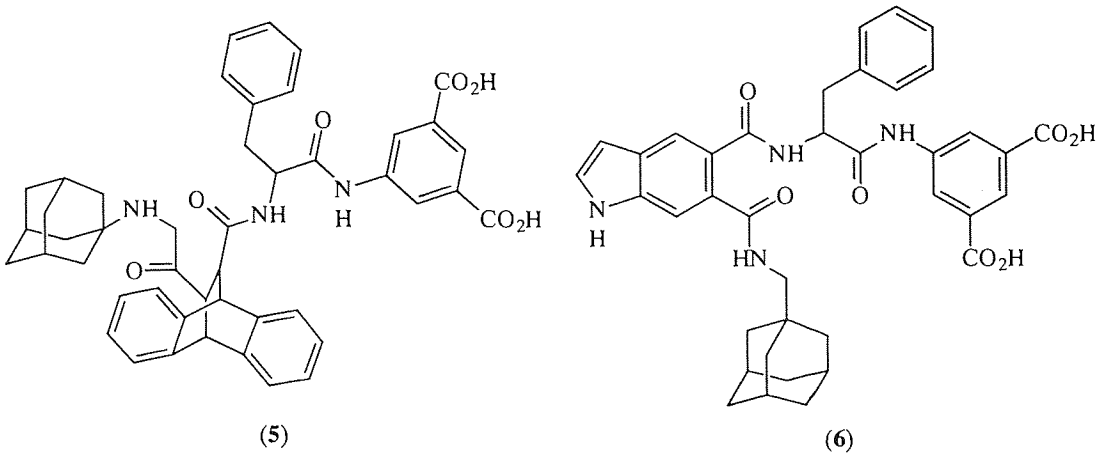


Figure 1.5.9: Structures of selected dibenzobicyclo [2.2.2] octane (5) and bicyclic heteroaromatic (6) CCK_B antagonists

Recently[78] the James Black Foundation has described a novel series of gastrin and CCK ligands based on a pyrrole or imidazole ring system[78]135. 2,4,6-Trisubstituted imidazole-based compounds are the more potent from the initial data (IC_{50} = 2.95 nM). They also appear to be more selective over CCK_A receptors. There is no *in vivo* data yet reported.

1.5.10. Benzodiazepine derivatives

Chlordiazepoxide (Figure 1.5.10.1) was discovered by Sternbach[79] and co-workers in the mid 1950s and introduced in 1960 as the first benzodiazepine drug[80]. In the thirty years that followed the discovery of this compound, an intense investigation by medicinal chemists[81] in search of compounds with useful anxiolytic activity has started.

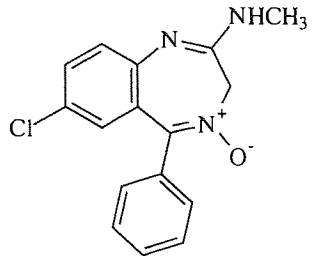
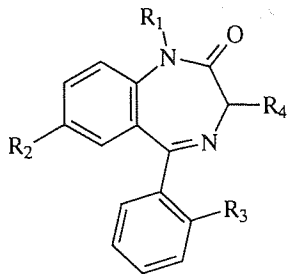


Figure 1.5.10.1: Structure of the first 1,4-benzodiazepine

Benzodiazepines (BZD) are the primary agents used to treat anxiety and they are one of the most successful compound classes worldwide. Around 50 benzodiazepine derivatives have been on the market worldwide. They are all classed as anxiolytics, anticonvulsants, sedatives and muscle relaxants. They mainly act by binding to a specific regulatory site on the $GABA_A$ (γ-amino butyric acid) receptor, thus increasing the inhibitory effect of GABA[82].

Many schemes have been proposed to classify the various benzodiazepines (Table 1.5.10.2). In pharmacokinetics, a useful framework is the categorization according to the range of elimination half-life (ultrashort, short, intermediate or long). Some authors classify the benzodiazepines as chloro- and nitrobenzodiazepines. It is also possible to classify the benzodiazepines to their pharmacological activity. It is remarkable that a "small" change of a functional group leads to different pharmaceutical categories and some agents exhibiting marked hypnotic activity. This observation stimulated a systematic study[82] of other derivates of benzodiazepines, from which a number of these compounds have had effects on the central nervous system. The toxicity is generally very low[83], so low that the LD_{50}'s has often been difficult to determine and have not been an important factor in the pharmacological evaluation of individual compounds.



1,4-Benzodiazepine template

Compound	R_1	R_2	R_3	R_4	Therapeutic Category
Diazepam	Methyl	Cl	H	-	anxiolytic, muscle relaxant (skeletal)
Nitrazepam	H	Nitro	H	-	anticonvulsant, hypnotic
Oxazepam	H	Cl	H	OH	anxiolytic
Temazepam	Methyl	Cl	H	OH	sedative, hypnotic
Lorazepam	H	Cl	Cl	OH	anxiolytic

Table 1.5.10.2: Selected 1,4-benzodiazepines

Four main substituents groups can be modified without significant loss of activity. The seven-membered hetero ring system is bent[84] and is essential for activity[85].

In a quantitative study on peripheral CCK receptors, chlordiazepoxide, medazepam and diazepam were shown to antagonise the contractile response to CCK in isolated guinea pig gallbladder strips[86, 87]. Although the potency of these compounds was not high, lorazepam and chlordiazepoxide also inhibited nerve mediated responses of ileal longitudinal muscle.

However, benzodiazepines were very weak in displacing CCK in mouse brain (IC_{50} = 10 μM)[88]. In a study from Japan[89] it was noted that anthramycin, a benzodiazepine

derivative, produced by streptomyces microorganisms were reported to be potent antagonists of CCK in mice. Anthramycin reversed CCK-8 induced satiety and was shown to displace [^{125}I] CCK-8 binding in different brain regions, especially in the cortex. Further investigations are underway to elucidate the pharmacological potential of this compound.

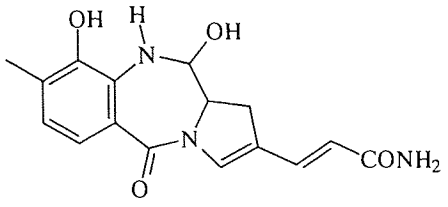


Figure 1.5.10.3: Structure of the natural 1,4-benzodiazepine derivative, Anthramycin

The discovery of Asperlicin, as already stated, was important because it led to new potent and specific CCK_A and CCK_B receptor antagonists. This new and naturally occurring benzodiazepine was isolated from the fungus *Aspergillus alliaceus*. It was discovered using an alternative approach, searching through microbial broths, using a radioreceptors assay technique[90]. Asperlicin showed selectivity for CCK_A receptors and at the time of its discovery was the most potent nonpeptide CCK antagonist known (IC_{50} = 1.4 μM pancreas binding).

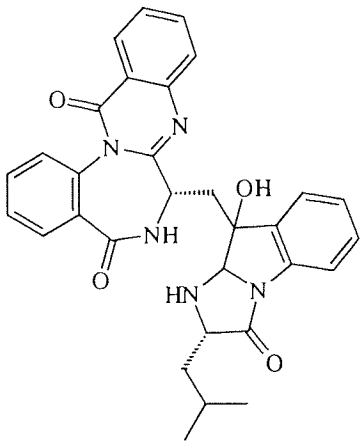


Figure 1.5.10.4: Structure of the natural 1,4-benzodiazepine derivative Asperlicin

Asperlicin represented a major advance in the development of CCK receptor antagonists. It demonstrated 300-400 times more affinity for pancreatic and gallbladder CCK receptors than proglumide. Asperlicin is therefore selective for the CCK_A receptor as opposed to CCK_B or gastrin receptors. However, this compound demonstrated scare stability and poor oral bioavailability. Many efforts to prepare Asperlicin modifications devoid of disadvantages of the parent compound were unsuccessful[91], due both to lack of oral activity and low compound potency. By combining the elements of Asperlicin, L-364, 286 was the first successful synthetic analogue, in which the diazepam-like structure is linked with a 3-amido group.

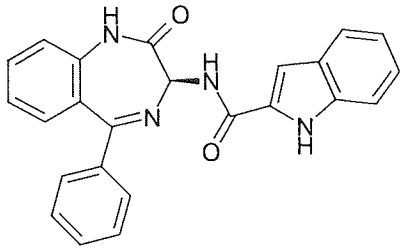


Figure 1.5.10.5: 3-amido-1,4-benzodiazepine derivative L-364, 286

New efforts to optimise the CCK_A antagonist activity of these benzodiazepine derivatives were very successful and led to devazepide (MK-329, formerly L-364,718, Figure 1.5.10.6) an extremely potent and orally active CCK_A antagonist (IC_{50} = 0.1 nM inhibition of ^{125}I-CCK-8 rat pancreas binding). This compound had a longer lasting efficacy *in vitro* and *in vivo* has more than 1000-fold selectivity for the CCK_A receptor with respect to the CCK_B receptor.

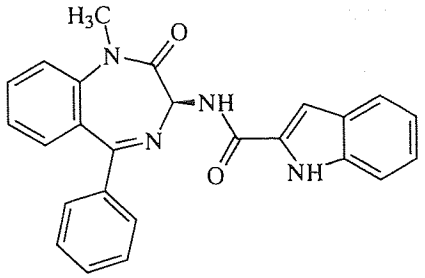


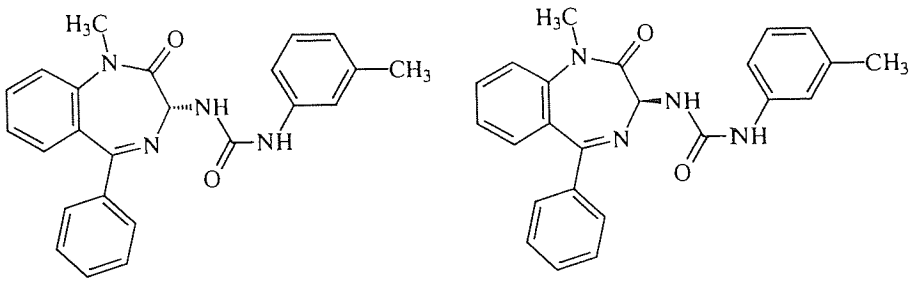
Figure 1.5.10.6: 3-Amido-1,4-benzodiazepine derivative L-364, 718, MK-329/devazepide

Devazepide possess potent CCK_A blocking activity in different tissues[92,93]. Pancreatic amylase secretion is antagonised with a potency of 2,000,000 times more than proglumide (Figure: 1.5.1). Devazepide has been claimed[94] to be a selective antagonist, with the effects of CCK-8 (Sincalide) on food intake. In contrast, when CCK-8, present in the intestine and brain and secreted from the gastric mucosa, stimulates the release of both bile from the gallbladder, and the release of digestive enzymes from the pancreas[95]. Although devazepide shows selectivity for CCK_A over CCK_B receptors it is nevertheless a potent CCK_B antagonist. Devazepide was a key tool in the autoradiographical demonstration of the presence of CCK_A receptors in the various regions of the brain[96]. During the extensive development of L-364, 718 it was noted that some analogues lost their selectivity for CCK_A.

1.5.11. Ureidobenzodiazepine derivatives

When the 3-amido linkages were replaced with a benzamido urea, the CCK_A affinity decreased and the CCK_B affinity increased substantially. The most interesting compound developed by Merck scientists in these studies was L-365,260[97]. L-365, 260[95] showed high affinity for CCK_B receptors in rats, mice and in humans. L-364, 718 was reported[98] to have a 125 fold greater affinity for pancreatic CCK_A receptors, than for gastrin receptors. L-365,260 shows only an 80 fold greater affinity for gastrin/CCK receptors than for pancreatic CCK_A.

Both L-364,718 and L-365, 260 (Figure 1.5.11.1) were investigated[99] whether the satiety response to CCK is mediated by CCK_A or CCK_B receptors. L-365, 260 was reported to be 100 times more potent than devazepide in increasing feeding frequency and preventing satiated rats. The conclusion from the study was that endogenous CCK causes satiety by interaction with CCK_B receptors in the brain.



(3R)-Isomer
CCK_B-selective

(3S)-Isomer
CCK_A selective

Figure 1.5.11.1: Isomers of 3-ureido-1,4-benzodiazepine derivative L-365, 260

The high affinity CCK_B-selective urea L-365,260 and related analogues is dependent upon the stereochemistry at C-3 of the benzodiazepine ring, the (3S)-enantiomer generally being CCK_A selective and the (3R)-isomer CCK_B selective[100]. L-365, 260 shows high affinity for CCK_B receptors in rats, mice and in humans[99]. Although L-365,260 is a benzodiazepine in structure, it has no affinity for GABA receptors and does not reduce tolerance and withdrawal in animal models. However, during phase 1 clinical trials it was found that L-365,260 had a limited oral bioavailability due to its low aqueous solubility and bio-distribution studies in mice,[101] showing a very low brain uptake (<0.8% dose/gram) after intravenous injections.

One of the most potent and selective CCK_B receptor ligand is L-708,474[102]. L-708,474 (Figure 1.5.11.2) is about thirty-fold higher in affinity than the L-365,260 enantiomer (IC_{50} = 8.5 nM) at the CCK_B receptor and is markedly more selective for

CCK_B receptors over CCK_A (6,500-fold v. 87-fold). The binding affinities of the cyclohexyl benzodiazepines demonstrate the importance of the size of the lipophilic substituent at C-5 of the benzodiazepine, and point to an advantage of cyclohexyl over phenyl for effective binding at the CCK_B receptor. L-708,474 (IC_{50} = 0.28 nM) was an exceptionally high affinity ligand at the CCK_B receptor. L-708,474 is considerably more potent than either the cyclopentyl (IC_{50} = 16 nM) or cyclobutyl (IC_{50} = 29.9 nM) analogues. This compound showed by increasing lipophilicity in comparison to L-365,260 an increase in potency and selectivity for CCK_B receptor but the bioavailability was reduced.

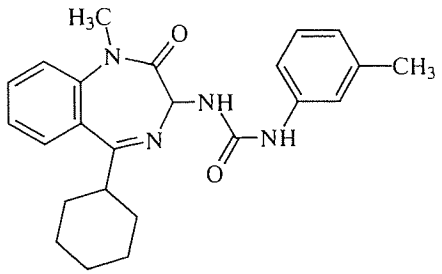


Figure 1.5.11.2: 3-Ureido-1,4-benzodiazepine derivative L-708,474

Results from the phase 1 clinical trials with L-365,260 prompted Merck chemists to develop a second generation of CCK_B/gastrin receptor antagonists. By introducing a group with high water-enhancing properties, the chemists at Merck hoped to increase the oral bioavailability of the new compounds, which were synthesised.

One of the compounds with increased bioavailability, synthesised by Merck[103,104] was named L-740,093, an amidine derivative with basic character, that was found to be extremely potent. L-740,093 (Figure 1.5.11.3) showed an aqueous solubility around one hundred times greater (as HCl salt) than that displayed by the parent compound L-365,260. L-740,093 showed high CCK_B/gastrin affinity (IC_{50} = 0.1 nM) whilst displaying excellent selectivity for the receptor subtypes, it had a CCK_A/CCK_B ratio of approximately 16000. Thus L-740,093 seems to be suitable for oral treatment in humans.

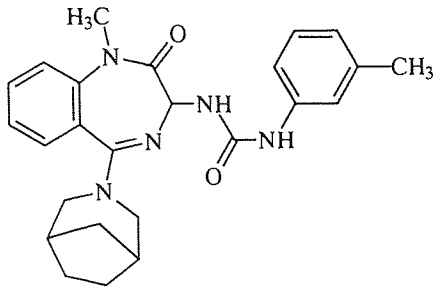


Figure 1.5.11.3: 3-ureido-1,4-benzodiazepine derivative L-740,093

Another approach to increase the water solubility of L-365,260 in order to achieve good levels of oral bioavailability, was successfully performed by incorporating acidic solubilising groups into the phenyl ring of the acylurea moiety of the parent compound[105].

The C5-cyclohexyl derivatives incorporating aminotetrazole group (L-737,425) was the most potent and selective (CCK_A/CCK_B = 37000) antagonists so far reported for CCK-B/gastrin receptors (Figure 1.5.11.4). However, the preparation of this compound includes synthetic complexity.

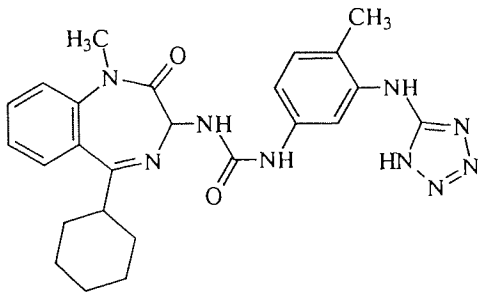


Figure 1.5.11.4: 3-ureido-1,4-benzodiazepine derivative L-737,425

A novel series of 1-aroylmethyl analogues of L-365,260 was prepared and evaluated for activity as CCK_B/gastrin receptor antagonist by the Yamanouchi group[106]. YM022[107,108,109] has shown to be a significantly more potent antagonist of pentagastrin than L-365,260. YM022 (Figure 1.5.11.5) was the optimal compound of this series,

exhibiting very high CCK_B/gastrin receptor affinity (IC_{50} = 0.11 nM) and very good receptor subtype selectivity, the CCK_A/CCK_B ratio being about 1300. YM022 showed, compared to L-365,260, a better bioavailability and is a compromise between the lipophilicity and selectivity for CCK_B receptor. However, the improvement in potency obtained did not compensate for the increase in synthetic complexity in this series.

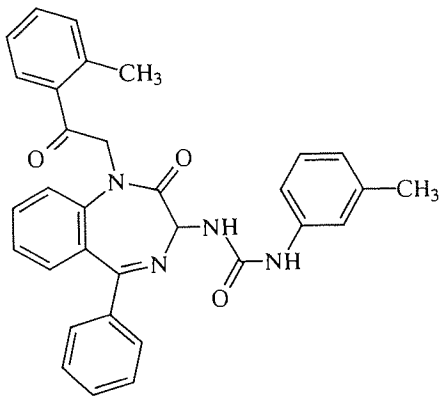
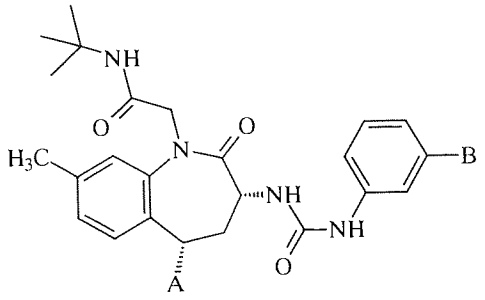


Figure 1.5.11.5: 1-benzoylmethyl 3-ureido-1,4-benzodiazepine derivative YM022

Scientists from Pfizer[110] modified the benzodiazepine nucleus of L-365, 260 to a benzazepin-2-one moiety. These compounds demonstrate typical subnanomolar CCK_B/gastrin receptors affinity (IC_{50} = 0.48 nM) and have a CCK_A/CCK_B ratio of 350 (Table 1.5.11.6). Compound (**7**) (CP212452) potently inhibited pentagastrin induced GAS, with an ED_{50} of 0.8 mg/kg compared with 1.5 mg/kg subcutaneously for L-365, 260. Despite its potent and selective CCK_B/gastrin receptor affinity, it has poor oral bioavailability, resulting from its low water solubility. The focus of the program turned to identifying a more water-soluble derivative, with the potassium salt of the carboxylic acid derivative

CP-310,713 (**8**) has improved aqueous solubility and *in vivo* efficacy (ED_{50} = 0.03 mg/kg s.c in the pentagastrin induced GAS model). The discrepancy between the concentration of drug required for efficacy in the animal models was attributed to low CNS penetration of CP-310,713. In view of the efficacy and bioavailability problems Pfizer have reportedly terminated the project.

In addition Merck scientists synthesised novel ureidobenzazepines containing a basic cationic substituent at the 5-position. Compound (**9**) was the optimal molecule of the series, exhibiting a relatively high affinity at the CCK_B receptor (IC_{50} = 15.7 nM) but with low selectivity.



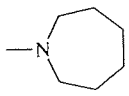
Compound	A	B
7	Phenyl	Cl
8	Cyclohexyl	CO_2H
9		Methyl

Table 1.5.11.6: Structures of selected 3-ureido-1,4-benzazepine CCK_B/gastrin receptor antagonists

A series of potent CCK_B antagonists based on the 1,5-benzodiazepin-2,4-dione skeleton possessing a C-3 ureido have been reported by Glaxo Wellcome scientists[111, 112,113] (Figure 1.5.11.7). Sterically large groups at N-1 are important for achieving high CCK_B receptor affinity and good selectivity over the CCK_A receptor, and 1-adamantylmethyl was found to be optimal.

The best compound from this series GV150013X, showed affinity to CCK_B at 7.5 nM. However, based on the bulky substituent, the bioavailability was reduced but nevertheless GV150013X was selected for exploratory development in the treatment of panic attacks and anxiety.

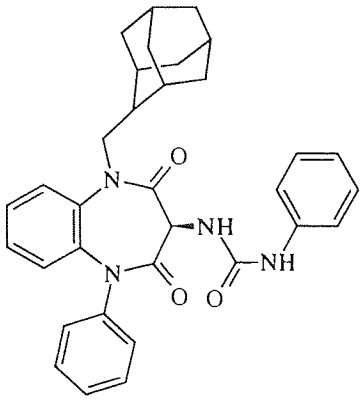
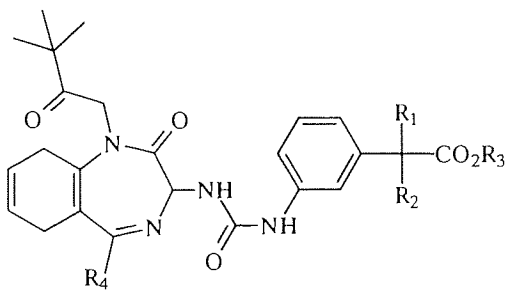


Figure 1.5.11.7: 1-adamantylmethyl-3-ureido-1,5-benzodiazepine derivative GV150013X

Recently, the company Zeria Pharma Co. Ltd claimed a patent[114] on 3-phenylureido-1,4-benzodiazepine derivatives (Table 1.5.11.8), which contains a cyclohexenyl ring, annulated to the heterocyclic seven membered ring. Information of the biological potential of the series of these compounds has not been published.



R_1	Lower alkyl
R_2, R_3	H or lower alkyl
R_4	Cyclohexyl or phenyl

Table 1.5.11.8: Structures of recently developed 3-ureido-1,4-benzodiazepine derivatives

1.5.12. Quinazolinone-based compounds

A quinazolino-1,4-benzodiazepin-5,13-dione also provided the starting point for a series of quinazolinone-based CCK_B receptor antagonists, devised by Eli Lilly[78].

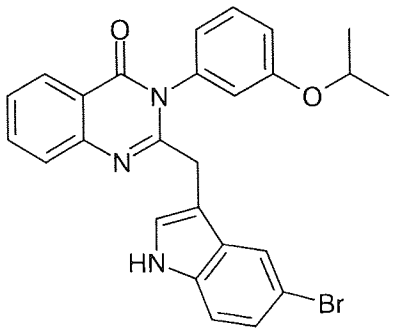


Figure 1.5.12: LY-202769

LY-202769 (Figure. 1.5.12) potently inhibited [^{125}I]-CCK-8(S) binding to the mouse cortical membrane (IC_{50} = 9.3 nM). Members of this series displayed up to a 20-fold higher affinity for CCK_B receptor in cortical membranes than those in gastric tissues[78].

1.5.13. Indol-2-based compounds

3,3-Disubstituted indol-2-ones, described by Chugai[78], inhibited [^{125}I]-gastrin binding to guinea pig gastric glands and was selective over CCK_A sites in pancreatic tissue. Only by replacing the geminal bisurea with a geminal acetamido-urea and through optimisation of the substituent at N-1, to yield AG-041R (Figure.1.5.13), was the compound adequately orally active[78].

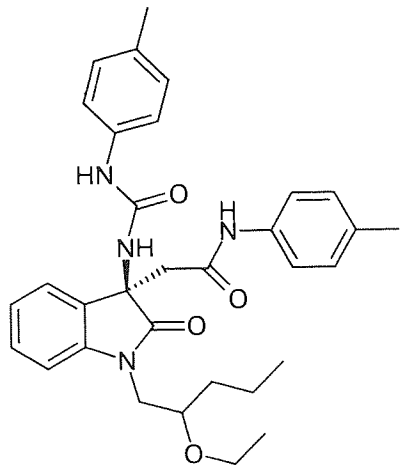


Figure.1.5.13: Structure of AG-041R

AG-041R inhibited specific binding of [^{125}I]-gastrin binding to guinea pig gastric glands (IC_50 = 1.11 nM), which was 500-fold greater than its affinity for CCK_A receptors in the guinea pig pancreas. In the pylorus-ligated rat, AG-041R inhibited pentagastrin stimulated acid secretion by iv administration (ID_{50} = 5 nmol/kg) and had no inhibitory effect on carbachol or histamine stimulated secretion.

The effect of intraduodenal administration of AG-041R on pentagastrin stimulated acid secretion has not been reported but it has been shown to inhibit basal acid secretion (ID_{50} = 10 μmol/kg) by this route, achieving prolonged duration (12 hrs at 10 mg/kg p.o.)[78]. AG-041R also exhibited greater potency than L-365,260 in water immersion stress (600 fold) and indomethacin-induced ulcer (6-fold) models[78].

1.6. Summary

Cholecystokinin[115] (CCK) a 33 amino acid peptide and its various circulating forms act both as a central neurotransmitter/neuromodulator and as a gut hormone[116]. Its peripheral effects are mediated mainly through the A(1) receptor subtype, while the central effects are correlated with the B(2) receptor subtype[117].

There have been numerous studies, in which the administration of CCK peptide agonists produce anxiogenic-like effects in animals, therefore concluding that CCK antagonists have a beneficial use as anxiolytics agents. The only effective way to evaluate potential antagonists as anxiolytics, is through clinical studies in man.

The CCK receptors are involved in many pathological situations, and their antagonists may possess great therapeutic potential in humans. The improvement in potency, specificity, oral-bioavailability and low toxicity of new CCK antagonists has increased hopes of producing therapeutically useful compounds.

Agents which possess CCK_A blocking activity shows effects on food intake; they stimulate the release of both bile from the gallbladder, and the release of digestive enzymes from the pancreas[94]. These inhibitors might have a therapeutic use for the treatment of diabetes melitus and chronic pancreatitis.

The CCK_B receptor is involved in many pathological situations as well, and amongst these, anxiety and panic are particularly relevant targets for therapeutic interventions. The best evidence that CCK is strongly related to panic attacks is based on experiments in which CCK_4 was administered to healthy volunteers who panicked shortly after injection[118]. These physiological reactions were significantly blocked or reduced by the administration of selective CCK_B antagonists[119].

Peptides and non-peptides as CCK_B antagonists have been developed by research groups and pharmaceutical companies. However, peptides as CCK antagonists have their own particular difficulties, which includes metabolic lability and poor oral absorption. This would seem to be a far from perfect therapy, with further work needed to eliminate these complications.

Non-peptidal analogues might circumvent these liabilities and academic research groups as well as pharmaceutical companies have synthesised numerous different compound classes. Most of these compounds contain the phenylurealyl moiety, which might be responsible for CCK activity[120].

Merck scientists have chemically modified the low-toxic benzodiazepine template. The 3-amido and 3-ureido benzodiazepines are the most outstanding non-peptidal CCK antagonists. These compounds have changed successfully from $GABA_A$/benzodiazepine receptor-selective benzodiazepine, such as Diazepam into CCK_A and CCK_B/gastrin selective antagonists. MK-329 or Devazepide was the first purely synthetic 3-amido-benzodiazepine, acting as a CCK_A antagonist. The change from an amido to the urea linkage resulted in the CCK_B selective antagonist L-365.260 to be prepared. Although this potent and selective compound had shown limited oral bioavailability, due to its low aqueous solubility, it served as a lead structure[119] and was excessively modified to enhance its bioavailability,[107] with cationic amidines[105] being introduced at the 5-position of the benzodiazepine template.

Yamanoushi[106] developed 1,4-benzodiazepines further by various alkylation reactions[108] and other major pharmaceutical companies claimed patents on compounds which are ureas but contain a 1,5-benzodiazepine template[121] (Figure 2.19) or an azazepine scaffold.

In summary, the role of CCK in many different physiological processes has led to intense interest regarding the biological as well as possible therapeutic roles of CCK receptor ligands. Despite many different studies, the literature concerning the behavioural actions of the peptide CCK and CCK receptor ligands appears sometimes broad, with many inconsistencies and discrepancies. The possible applications of CCK receptor ligands in treating diseases remains promising and interesting. However, the complex biological role of CCK complicates the issue of CCK receptor agonists or antagonists for therapeutic use.

1.7. Aims and Objectives

This thesis attempts to address the development of chemically diverse structures as potent and selective cholecystokinin antagonists and seeks to establish if these compounds might be comparable or even more potent CCK binding ligands than the 3-amido or 3-ureido-1,4-benzodiazepines. While the preparation of 3-amido or 3-ureido-1,4-benzodiazepines includes the synthetic complexity[122,123,124], the new compounds should be less complicated to synthesise, which is a major important criteria, also they should ideally not have a chiral centre.

This class of 1,4-benzodiazepines has a well established template, because of its low toxicity rate therefore is ideal for using it in this drug discovery process. However, the classical 1,4-benzodiazepines bind, with high affinity, to the $GABA_A$/benzodiazepine receptor complex[48,125,126]. In order to avoid the resulting side effects such as sedation and tolerance, commonly associated with $GABA_A$/benzodiazepine binding, the drug discovery process is focused on the development of CCK_B/CCK_A antagonists for the possible treatment of anxiety and panic[127]. The CCK receptors are localised with other multiple receptors[42] and the known interaction of CCK with GABA[48, 128] has to be taken into account.

Initially the research can focus on compounds with a hybrid combination, composed of a 1,4-benzodiazepine scaffold with the most actively derived Park-Davis dipeptide on the 3-position. This unique strategy could offer a more potent and selective ligand. In accordance with biological results the CCK antagonist potencies of benzodiazepines depend critically on the 3-position of the benzodiazepine skeleton[98]. The N_1 alkylation with esters and ketones, especially with the t-butyl ketone[129] has enhanced bioactivity of 3-ureido-benzodiazepines, whereas a substituent on the N1 position blocks $GABA_A$ binding[130]. The 1,4-benzodiazepines have been shown to enhance the binding at the $GABA_A$/benzodiazepine receptor complex, by an electron-withdrawing group at the 7-position. Based on all this information a further approach was to synthesise a diverse range of 3-amino substituted-1,4-benzodiazepines. A full SAR can thus be deduced, with the most active analogue being optimised further. The

7-membered benzodiazepine ring has 4 sites (1, 3, 5 and 7-position), which can all be potentially modified.

Simple novel ureas can be generated, to derive structurally different derivatives by combining amines and isocyanates. This hypothesis is based on observing 3-ureido compounds, which are currently the most active compounds[103-105]. Optimisation of the lead "urea" compound could result in the classification of a new class of compounds. For the lead optimisation process the classical synthesis in solution can be used for the preparation of furan-2(5)-one building blocks and the subsequent nucleophilic addition, by amines, at the 4-position of the template. This can provide a totally new class of non-urea, non-benzodiazepine based ligands. A small library could be initially generated, using a small number of selected building blocks and amines.

All novel agents can be evaluated in a CCK radiolabelled binding assay for CCK_B (brain) and also CCK_A (alimentary). The biological evaluation on both CCK receptor subtypes might lead to a better understanding for the essential structural pharmacophores important to exhibit bioactivity, such as size, shape, hydrogen bond donors, hydrogen bond acceptors, positive charge centres, aromatic ring centres or hydrophobic centres, needed to provide active and selective CCK receptor antagonists.

Results and Discussion

Chapter 2. Dipeptides

2.1. Peptides as CCK antagonists

It has been shown that compound L365, 260 blocks panic attacks, when induced by CCK-4, in both the healthy and patients suffering from panic disorders. This has led researchers at Warner-Lambert to develop a rational drug design (PD134308)[68] based on a peptoid analogue of the CCK_B receptor ligand CCK-4.

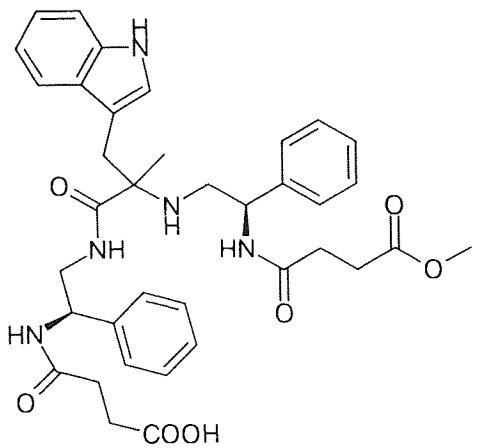


Fig. 2.1.1: PD134308

The discovery at Park-Davis of dipeptoid, was based on the systematic chemical modifications of the N and C terminal ends of the CCK-4 fragment[69]. The main structural

feature of PD134308 (Fig. 2.1.1) is the α methyl substituted Trp residue. This has been shown to be important for CCK_B receptor affinity by acting as a conformational modifier and stabilizing the peptide bond against acidic and enzymatic degradation *in vivo*.

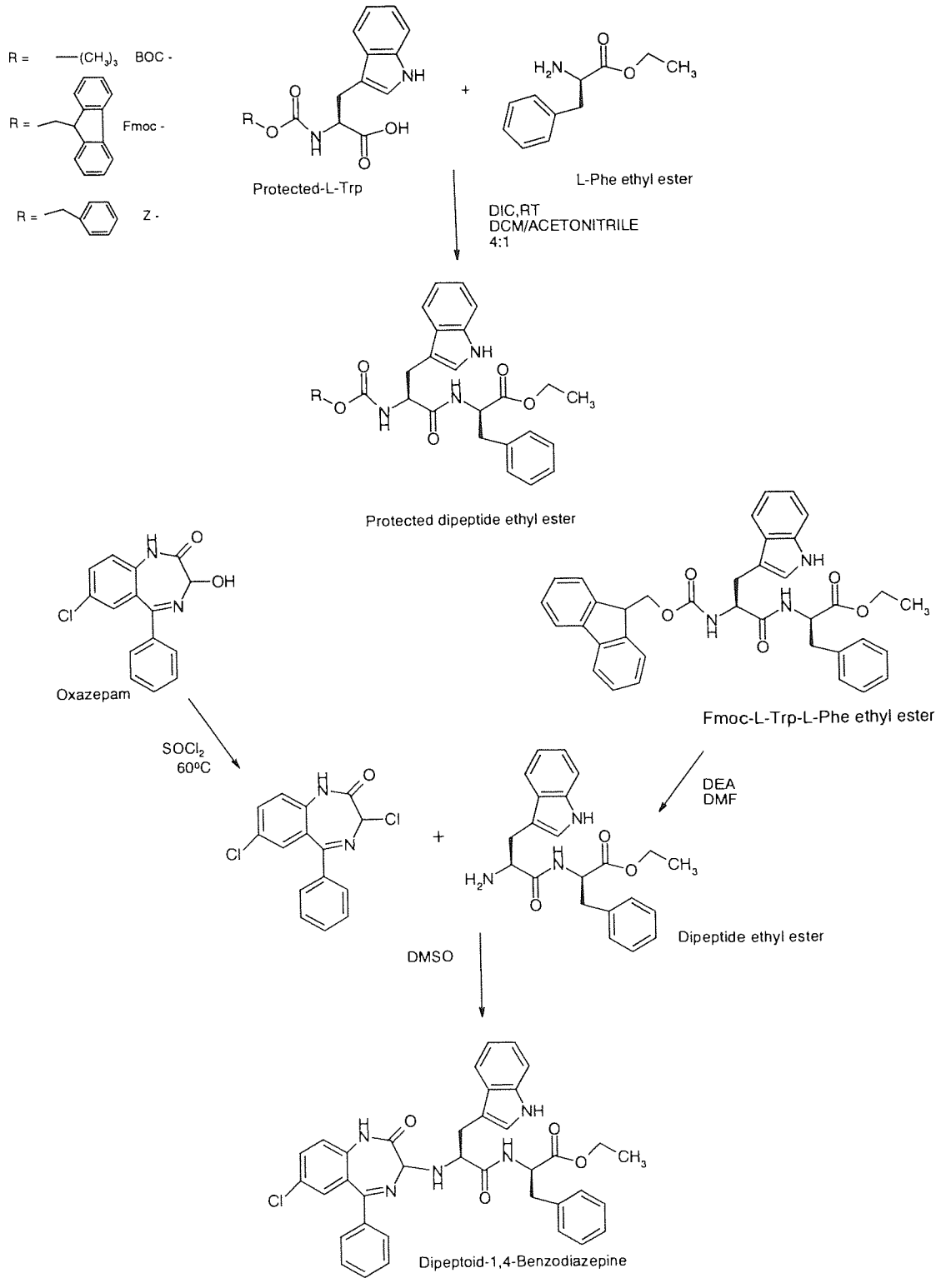
2.2. Why synthesise an dipeptoid-1,4-benzodiazepine ?

CCK receptor antagonists can be grouped into a number of broad categories. To date dipeptoids have been widely manipulated. The primary aim was to synthesise near non-peptidal peptidomimetrics. This new approach involved the combination of the most active dipeptide fragment onto the 3-position, of the key benzodiazepine nucleus. Research has identified important structural characteristics necessary for antagonist activity[131], with the non-essential role for the indole moiety[32]. The C-terminal and the L-Tryptophan residue, has been found to be important for binding to both central and peripheral receptors. The simple dipeptide, Boc-Try-Phe-NH_2 has CCK affinity comparable with the modified tetrapeptide.

Molecular modelling studies[68] have already shown the necessary features of the benzodiazepine template are the 3-substituents and a hydrophobic substituent at the N-1 or C-5 position. The role of the benzodiazepine template remains unclear. It may serve as a conventional structural mimic of the natural ligands or it may exploit some yet unidentified feature, common to peptide receptors. However, the ring system has provided very effective ligands for both receptors, suggesting a generality that could be used in the design of further ligands.

In general peptide synthesis is more difficult, compared with simple amides as amide bonds must be formed in a specific order, rather than at random. This can be achieved by using protecting groups, which allow protecting the non-reacting centres of the amine and carboxylic acid groups, except the reacting ones.

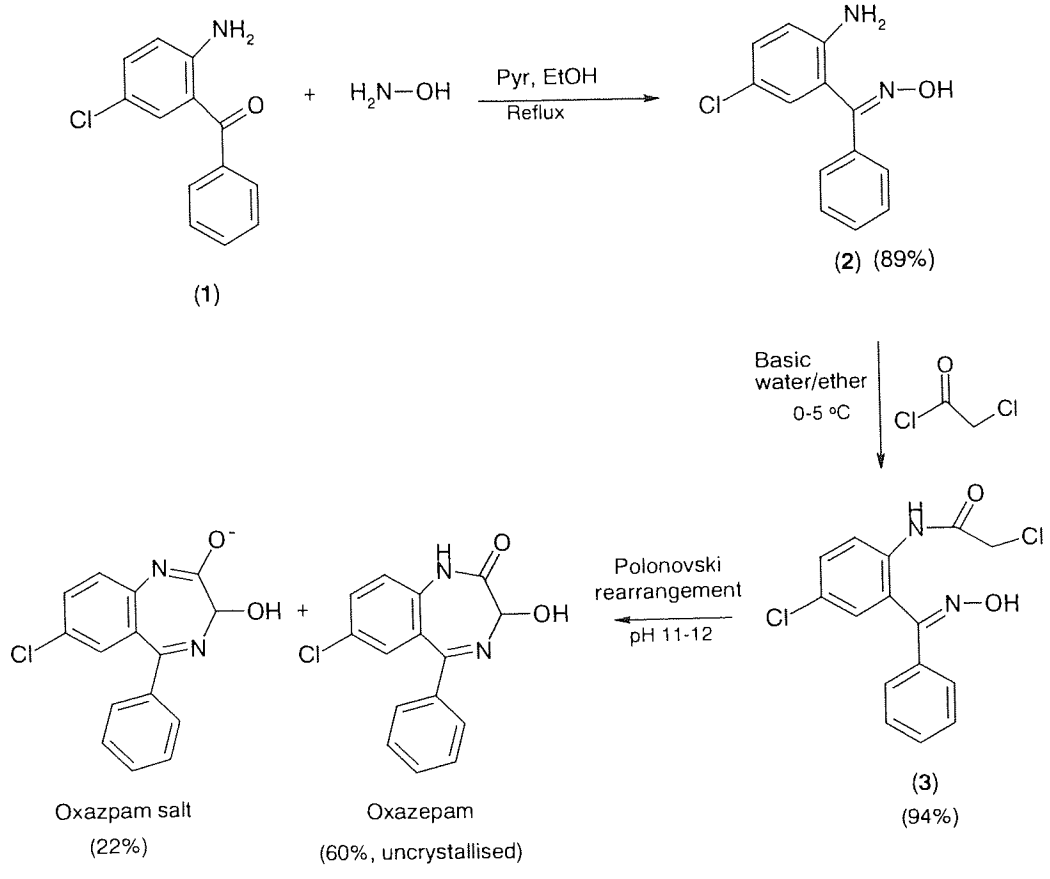
2.3. Synthetic overview of the dipeptoid-1,4-benzodiazepine formation



Scheme 2.3.1: An overview reaction pathway of the protected dipeptide formation, cleavage and addition to the 1,4-benzodiazepine template

2.4. Synthesis of 7-chloro-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one (Oxazepam)

Oxazepam, a metabolite of Diazepam, demonstrates typical anxiolytic activity[80]. Increasing interest over the years has led research groups to synthesise 3-substituted 1,4-benzodiazepines.



Scheme 2.4: Synthetic steps in the formation of Oxazepam and its corresponding salt.

The addition of hydroxylamine (Scheme 2.4) to 2 amino-5-chlorobenzophenone (**1**) gave the oxime (**2**) product. Reacting this (**2**) under cooling conditions gave 2-chloroacetamido-5-chlorobenzophenone (**3**). Keeping the solution basic, to neutralise the by-product HCl, on stirring overnight resulted in the formation of Oxazepam salt. This

was acidified to give Oxazepam and the undissolved salt. Each synthetic yield was high. However, in the final crystallisation in methanol or 1,4-dioxane, it was low. The method was modified to enhance both yield and purity from the original paper[132-134].

The mechanistic formation of Oxazepam involves 2-chloroacetamido-5-chlorophenone, in basic conditions, to form a benzodiazocrine, an eight membered hetrocyclic ring system. This subsequently undergoes an intramolecular ring contraction. Attack of hydroxyl ions on the imine nitrogen forms an anion. This then adds onto the aldehyde carbon to give Oxazepam. This type of compound is usually prepared by the *Polonovski Rearrangement*[135].

2.4.1. Attempts to modify the ketone building block

It has been known[68] that the C5-phenyl ring can be advantageously replaced by more lipophilic groups. This combats the problems of low aqueous solubility. In this approach seven different ketone building blocks were used, to modify the template, which included:

(1) 2-amino-5-nitrobenzophenone;
(2) 2-amino-2'-fluro-5-benzophenone;
(3) 2-amino-benzophenone;
(4) 2-amino-5-chloro-2'-flurobenzophenone;
(5) 2-amino acetophenone;
(6) 2-amino-4',5'methylene dioxyacetophenone and
(7) 2-amino-2',5-dichlorobenzophenone.

However the final cyclisation step was unsuccessful, given the current conditions, for all intermediates. Solubility problems and low yields as well as trace, product peaks in the MS. Due to these difficulties, no further work was performed on modifying the existing ketone building block.

2.4.2. Attempted reactions of Oxazepam

A number of different reactions of Oxazepam were investigated, to react nucleophiles on the 3-position. Conversion to tosylates, 3,5-dinitrobenzoates as well as the Mitsunobu reaction were all unsuccessful. It was deduced that the hydroxyl group was too basic to function as an effective leaving group in nucleophilic reactions, either in a SN^1 or SN^2 reaction. It was found it could be converted into a better leaving group through the use of thionyl chloride. Thionyl chloride was reacted neat with the alcohol functionality to give the corresponding Ar-Cl. Then the Ar-Cl group was easily displaced by nucleophiles.

2.5. Synthesis of N α-protected-L-Tryptophan-L-Phenylanine ethyl ester

Initial work involved synthesising three protected dipeptides (Boc, Z and Fmoc L-Trp-L-Phe ethylester), then the cleavage of the protecting group. Then the subsequent attempt to attach the dipeptide onto the 3-position of the Oxazepam ring and bioevaluation. Due to the high cost of reactants, protected dipeptides were synthesised on a smaller scale, with the methodology being optimised and then synthesised on a slightly larger scale.

There were problems (fragmentation of product) in removing DMF, prior to performing a chromatographic separation. A different solvent system was investigated, which would be sufficiently polar as DMF but with a lower bp, to enable the removal of the solvent without fragmentation. DCM/Acetonitrile (4:1) was found to be an ideal ratio. Treating either DCC or DIC with the amino acid carboxylic end formed the peptide bond. DCC/DIC functions by converting the carboxylic acid group into a reactive acylating agent, that undergoes a further nucleophilic acyl substitution with the amine. This resulted in the formation of the peptide bond and the by-product, diisopropyl urea.

An efficient method to synthesise the protected dipeptides was the removal of one third of the solvent and allowing the flask was allowed to stand for 3 days. This gave a white crystalline product, which was highly pure. This method eliminated the need to perform an column chromatographic separation.

2.6. Pharmacology

$^{[125]}$I-CCK-8 receptor binding essay:

CCK_A and CCK_B receptor binding assays were performed, by using guinea pig cerebral cortex (CCK_B) or rat pancreas (CCK_A). Male guinea pig brain tissues were prepared according to the modified method described by Saita et al.[136]. Pancreatic membranes were prepared in a similar way but by Charpentier et al.[137]. The *in vivo* CCK binding essay: Tissues were homogenized in ice cold sucrose (0.32 M, 25 ml) for 15 strokes at 500 rpm and centrifuged at 13000 rpm for 10 mins. The supernatant was re-centrifuged at 13000 rpm for 20 mins. The resulting pellet was re-dispersed to the required volume of buffer at 500 rpm and stored in aliquots at 70^0C.

Binding was achieved using radioligand ^{125}I-Bolton-Hunter labeled CCK, NEN at 25 pM. The samples were incubated {with membranes (0.1 mg/ml)} in 20 mM Hepes, 1mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, at pH 6.5 for 2 hrs at RT and then centrifuged at 11000 rpm for 5 minutes. The membrane pellets were washed twice with water and the bound radioactivity was measured in a Packard Cobra Auto-gamma counter (B5005). All binding assays were carried out with L-363, 260 as an internal non-specific control. Controls (no compound) were also added. All samples were made in duplicate and repeated twice. All compounds were initially screened for percentage inhibition at 20 μM. Samples showing an average inhibition of <35% were diluted to 2μM and re-screened and if active diluted again. This enabled the IC_{50} of the most active compounds to be calculated, by plotting the percentage inhibition against concentration and reading the value at 50% inhibition.

2.7. Biological evaluation of protected dipeptide esters

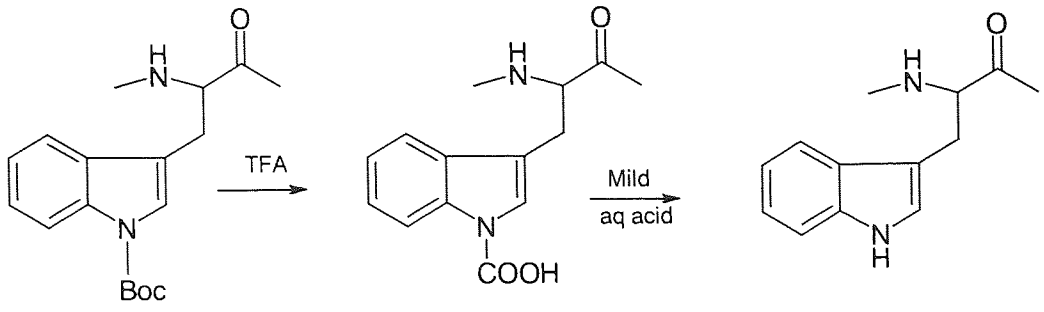
Compound	IC50 μM	Yield [%]
Boc-LTrp-LPhe	1.6	64.6
Fmoc-LTrp-LPhe	0.88	78.0
Z-LTrp-LPhe	0.7	67.6

Table 2.7.1: Biological screening results of the protected dipeptides

To summarise N α-protected dipeptide synthesis proceeded at RT under dry conditions. Compounds were produced in good yields and purity. The reaction conditions were optimised. Optimisation was achieved by selecting a more volatile solvent and then removing off the excess solvent. This ensured crystallised products to be produced. Bioevaluation (Table 2.7.1), by *in vitro* binding to CCK_B receptors, showed good binding especially the N α-Z-L-Trp-L-Phe at 700 nM.

2.8 Cleavage of the N α-protecting group

The next stage was to cleave off one of the protecting group. Cleavage of the Boc group with TFA was complete. N α-Boc dipeptide was cleaved off using a mild reliable method with TFA. However, the indole side chain of tryptophan is electron rich and reacted with the electrophilic species generated during the acidic deprotection. The indole ring captures electrophiles with great ease, especially the 2-position. One solution was to drain much of the π density, by using a Boc group to protect the nitrogen. This is a reversible protection and one that is commercially available.



Scheme 2.8.1: Draining π density, by using a Boc group

The above scheme (2.8.1) shows the removal of the Boc group after TFA treatment. The electron withdrawing influence remains and protects the ring, while the protecting groups are removed. However, a much simpler solution was to include additives like methoxybenzene and dimethylsulphide. These act as scavengers by sequestering electrophiles but also by acting as direct nucleophiles in the cleavage reaction.

The reaction was repeated, firstly using methoxybenzene, then the stronger scavenger dimethylsulphide, using increasing concentrations. However, although cleavage was complete electrophilic addition was still persistent, even when cleaving the Z-protected peptide.

An alternative approach was selected which involved the base catalysed cleavage of the Fmoc-dipeptide. Base catalysed removal of the 9-fluorenylmethyl-oxycarboxyl group was complete and efficient, to give the free dipeptide in a reasonable yield (50%). The powerful cleavage reagent, piperidine was replaced by diethylamine, which could be easily removed.

2.9. Summary

The final step was the combination of the free dipeptide with the activated Oxazepam. Solubility problems were encountered with the free amino-dipeptide and Oxazepam-chloride and it was finally found that the solvent DMSO was ideal. Mass spectrometry of the final solution did indicate the presence of M+1 peak at 648 m/z but this was small and a number of impurities were present (Scheme 2.3.1). Separation was unsuccessful due to the high boiling point of the solvent and instability of the final compound. Due to the large molecular weight of the final product, it now does not seem feasible to simply attach two large fragments together in a high yield. A retrosynthetic approach would have been advantageous.

A second approach to synthesise the dipeptoid-1,4-benzodiazepine was attempted by coupling each amino acid onto the benzodiazepine template. However, although it was possible, yields were extremely low and not enough material was left for the next subsequent stages. It was finally decided to leave this approach, as peptide derivatives would make poor drugs, due to the poor oral bioavailability and the ease of cleavage by peptidases. This approach has concentrated on working from the amino acid sequence of tetragastrin, which has led to a number of potent compounds. However, these types of peptoid antagonists, designed from natural peptide ligands, potentially exert some partial agonist activity *in vivo*. A recent hypothesis has been proposed to describe how the various classes of peptide agonists bind to the G-protein-coupled CCK_A receptors[70].

Chapter 3. Benzodiazepine derivatives

3.1. Why synthesise benzodiazepine derivatives ?

Peptidomeinetrics approaches, to provide antagonists for peptide hormone receptors have become increasingly popular, overcoming problems such as poor oral bioavailability. The existence of a common feature of structure and conformation among segments of many peptides implies that the 5-phenyl-1,4-benzodiazepine ring system might have a correspondingly broader use in the construction of ligands.

Here, the rational for designing improved CCK antagonists is based on the 5-phenyl-1,4-benzodiazepine ring and directly attaching amines (primary and secondary) to the 3-position. This should enhance both the activity and to increase the bio-availability of such compounds. Thousands of benzodiazepines analogues have been synthesised to date but not a great deal of work has been performed on 3-amino-substituted benzodiazepines. Previous findings[138,139]on a small selected number of compounds have found only modest anxiety activity on the $GABA_A$ receptor but not on the CCK receptors.

3.2. Synthesis of 2-substituted-1,4-benzodiazepines

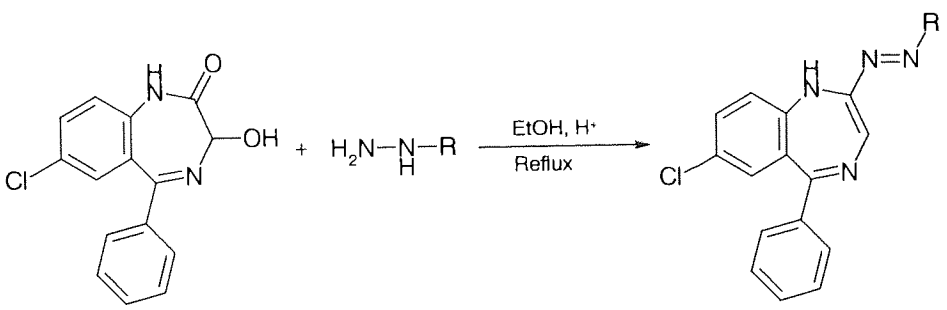
A number of different methods were investigated to attempt to react Oxazepam and amines on the 3-position. One such method by Kulkarni et al.[140] claimed to react simple amines successfully in the 3-position, whilst refluxing in slightly acidic conditions. This was not reproducible and only caused fragmentation of the benzodiazepine system. However, using this method but refluxing longer (2 days) in acidic conditions, with

hydrazine's or other conjugated systems enabled addition to the ketone functionality, providing novel compounds (Scheme 3.2.1.1). These compounds had reacted in the 2-position, as the I.R showed no ketone/amide linkage at ~1600-1750 cm^{-1}. The MS gave a single peak at 18 m/z units less than that expected for the 3-position, suggesting the loss of water from the 2-position.

Although the amide is relatively stable, acidic conditions causes the formation of an typical imine type reactions, resulting in the formation of hydrazone, semicarbazone derivatives, which were easily prepared in good yields in ethanol and were highly pure, with high R_f values.

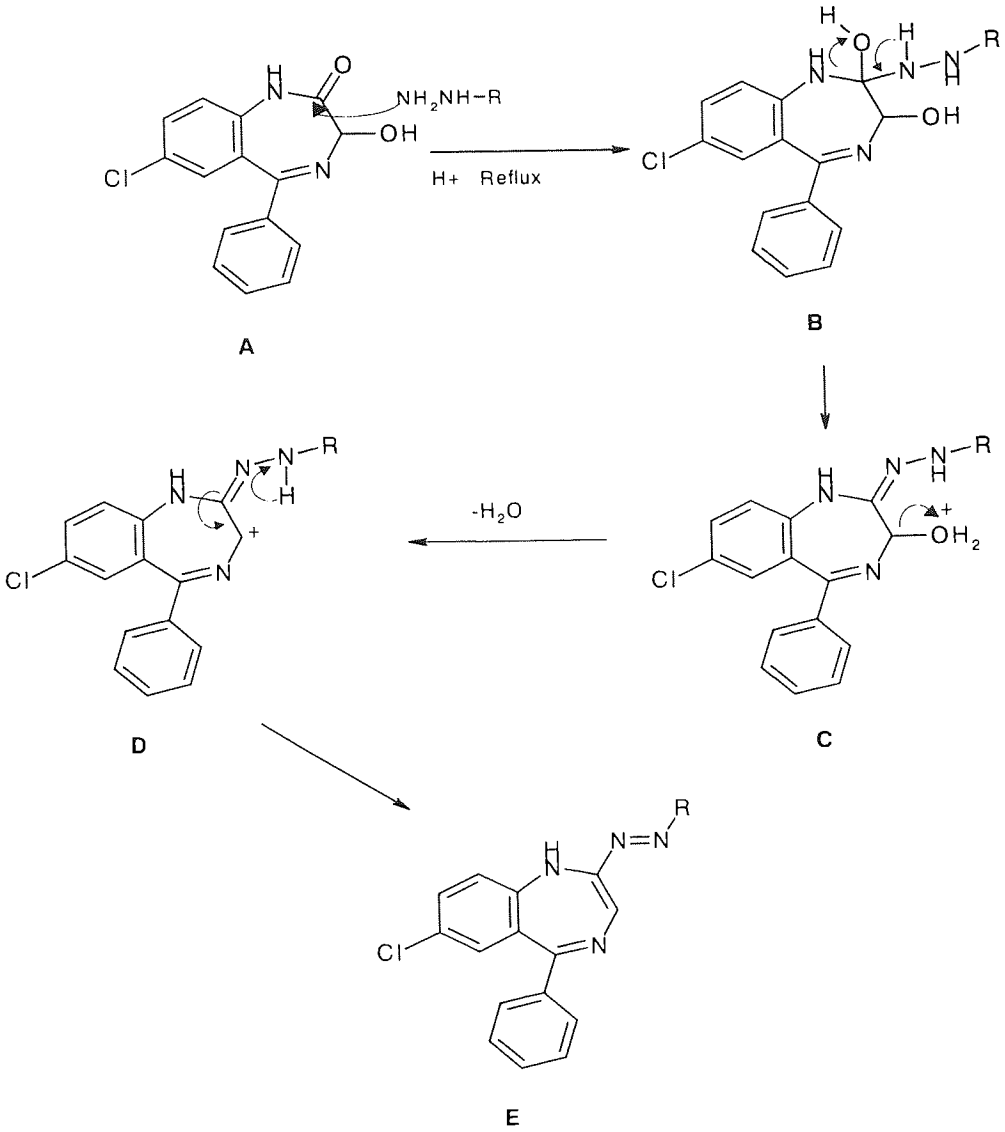
Reagents used:

1) 2,4-Dinitrophenylhydrazine.HCl
2) Phenylhydrazine
3) Semicarbazide.HCl
4) 4-Nitrophenylhydrazine.HCl
5) 4-Chlorophenylhydrazine.HCl
6) Benzoylhydrazine
7) 3,4-Dimethylphenylhydrazine.HCl
8) p-Tolylhydrazine.HCl
9) Aminoguanidine nitrate
10) Hydrazine hydrate
11) p-Toluenesulphonylhydrazine.HCl
12) 4-Methoxyphenylhydrazine.HCl
13) 1,1-Diphenylhydrazine.HCl
14) N,N'-Dimethyl-p-phenylenediamine



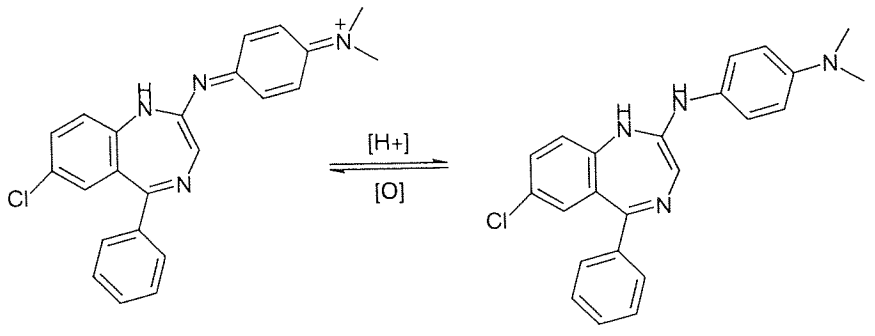
Scheme 3.2.1.1: Illustrating the general route in the preparation of 2-substituted-1, 4-benzodiazepines

Scheme 3.2.1.2: Proposed formation mechanism of 2-substituted-1, 4-benzodiazepines



Scheme 3.2.1.2 depicts the nucleophilic attack on the ketone (**A**) by the amine leads to the formation of a carbinolamine (**B**). The acid catalyst aids the formation of the imine (**C**), through the loss of water. Imine formation is maximum at weakly acidic conditions. Product (**C**) is very unstable and protonation of the hydroxyl group results in the formation of a positive charge on carbon-3. Loss of a nitrogen proton proceeds in the formation of a conjugated, stable product (**E**).

Compounds prepared via this method display good stability and coloration. All working amines were sufficiently nucleophilic, with conjugated forms and therefore typical anilines were non-working. Compound 3.2.14 (Scheme 3.2.1.3) exhibited indicator properties. It turned, from yellow, to purple in acidic media and back to yellow, with the addition of base.



Scheme 3.2.1.3 Transformation of compound 3.2.14 under different conditions

When p-toluenesulphonyl hydrazide was reacted (reagent 11), the acidic conditions caused the cleavage of the p-toluenesulphonyl group and only the hydrazine part of the compound reacted (compound 3.2.11). Initially impurities were observed on the TLC plate. After purification, the MS showed a peak of 284 m/z, the cleaved product. This was subsequently confirmed by ^{1}H nmr. For comparison and diversity, the reaction was attempted using thiourea. However, this approach was not working.

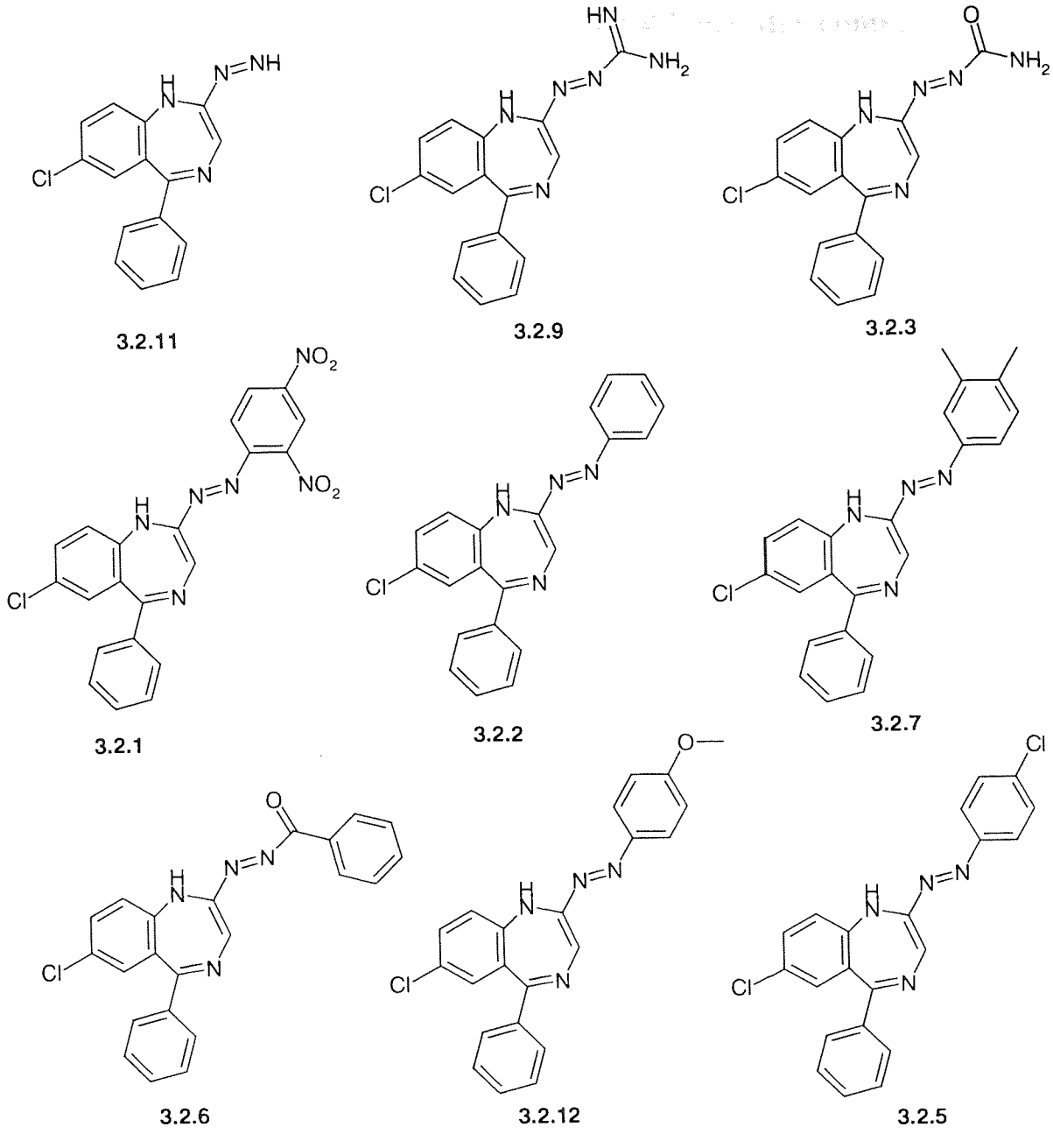


Fig 3.2.1.4: Selected 2-substituted-1,4-benzodiazepines analogues

3.2.1. Biological evaluation of 2-substituted-1,4-benzodiazepines

Table 3.2.1.1: Shows *in vitro* binding affinity of 2-substituted benzodiazepines

Compound Entry	MF	MW	MS+H [m/z]	Yield [%]	IC_{50} [μM]
3.2.1*	$C_{21}H_{13}ClN_6O_4$	449	449	67	>20
3.2.2*	$C_{21}H_{15}ClN_4$	358	359	47	>50
3.2.3*	$C_{16}H_{12}ClN_5O$	325	326	50	>15
3.2.4*	$C_{21}H_{14}ClN_5O_2$	404	404	70	>20
3.2.5*	$C_{21}H_{14}Cl_2N_4$	393	393	65	>20
3.2.6*	$C_{22}H_{15}ClN_4O$	386	387	41	>20
3.2.7*	$C_{23}H_{19}ClN_4$	386	387	61	>15
3.2.8*	$C_{22}H_{17}ClN_4$	272	373	66	>20
3.2.9*	$C_{16}H_{13}ClN_6$	324	308	53	>20
3.2.10*	$C_{15}H_{11}ClN_4O$	298	299	47	>20
3.2.11*	$C_{15}H_{11}ClN_4$	283	284	41	>15
3.2.12	$C_{22}H_{17}ClN_4O$	388	389	34	>20
3.2.13	$C_{27}H_{20}ClN_4$	436	437	69	>20
3.2.14	$C_{23}H_{20}ClN_4$	387	388	67	>20

*** = fully characterised**

The 2-substituted-1,4-benzodiazepines analogue series was evaluated, *in vitro,* on the CCK_B receptor subtype. Most compounds displaced no CCK_B activity, whilst compounds 3.2.3, 3.2.7 & 3.2.11 displayed extremely weak binding.

3.2.2. Summary

Novel compounds have been prepared from Oxazepam, resulting in the formation of 2-substituted 1,4 benzodiazepines, in reasonable yields. However, this series of unique compounds have been found to be inactive, with IC_{50}'s of >15 μM. It was observed that the addition of a nitro group on the ring reduces potency, while two reduce it further. The unsubstituted phenyl hydrazine (3.2.2) displayed the most activity from the series. Aliphatic amines show no activity at all. It can be deduced, from the data, that 2-substituted-1, 4-benzodiazepine analogues have a detrimental effect on activity. It can be concluded, that the ketone functionality of the benzodiazepine is the optimal group. However, these new class of compounds have the potential of being drug candidates in a general screening program.

3.3.3. Amino substituted 1,4-benzodiazepine-2-one analogues

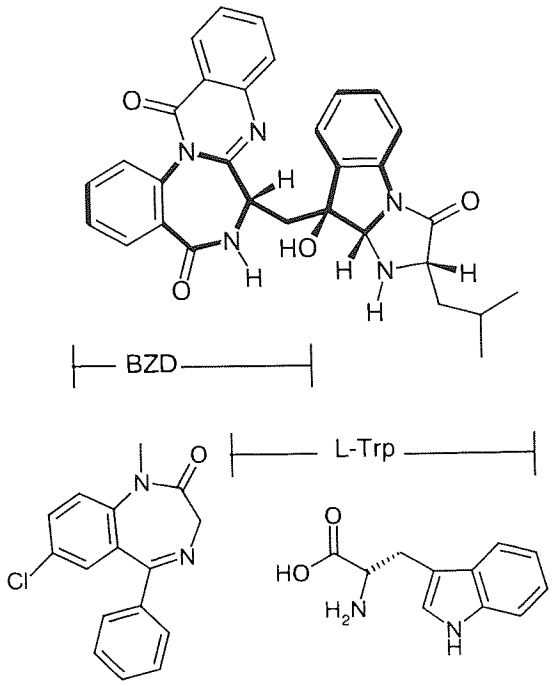


Figure 3.3.0.1: Asperlicin, with the two key fragments for activity

As shown from Figure 3.3.0.1, Asperlicin contains the key 1, 4-benzodiazepine (BZD) ring system, which is found in typical anti-anxiety agents. The 3-hydroxyindoline is a distant molecular analogue of the L-tryptophan side chain. L-Tryptophan is one of the key amino acids of the required carboxyl-terminal sequence of CCK. It has already been shown that a number of 3-substituted 5-phenyl-1, 4-benzodiazepines have already been synthesised[115,141,142], the 3-alkyl series, L-364, 718, a potent 3-amido derivative and also L-365, 260.

The aim was to synthesise potent non-peptidal CCK antagonists, based around the L-365,260, structure. The Oxazepam nucleus was reacted with various amines; Primary, secondary, hetrocyclic, hydrazine's, aromatic and aliphatic were reacted on the 3-position

(Figure 3.3.0.2). Nitrogen nucleophiles were chosen to compare the biological effect of directly attaching the amines to the ring, without using an amide or urea linkage. A diverse range of nucleophiles were selected, where nitrogen was the main nucleophile as to reduce by-products and to provide novel compounds. By combining these two elements, new synthetic analogues were prepared and assessed, which would hopefully overcome the current problem of potential CCK drugs, of poor bioavailability.

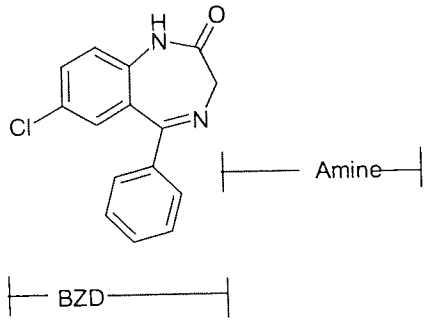
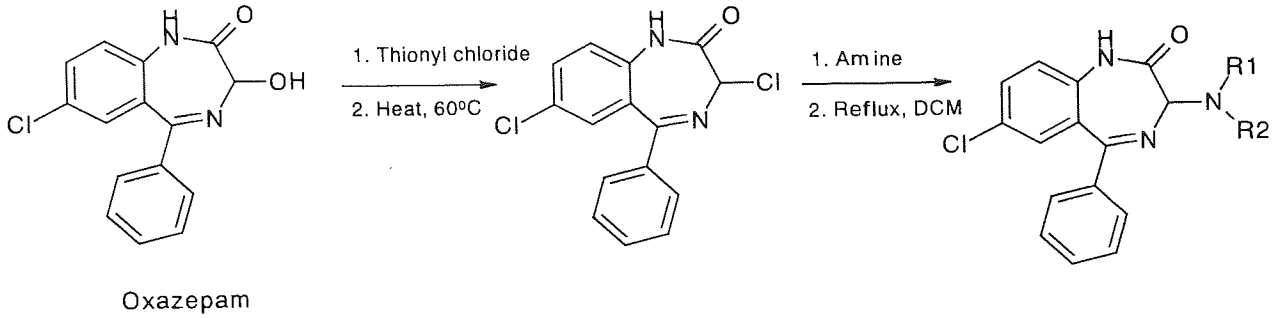


Figure 3.3.0.2: New synthetic approach

Oxazepam was chosen to represent the BZD part of the molecule. It has a similar half-life to Lorazepam but is not so dependence inducing and is less sedating than Diazepam. It is shorter acting and has less adverse side effects. Also the synthesis has been established and is a working approach.

Initially a first series of diverse compounds were synthesised and screened (without purification but analysed by TLC & MS). The results would give a potential lead structure, which can be purified and optimised further.



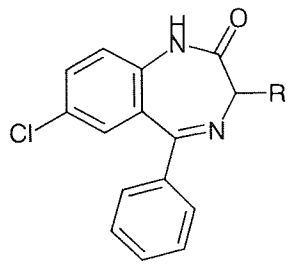
Oxazepam

Scheme 3.3.0.3: Formation of substituted 3-amino-benzodiazepines via thionyl chloride

A reliable route (Scheme 3.3.0.3), from chapter 2, to synthesis 3-amino substituted benzodiazepines was to react Oxazepam with an excess (4 Eq) of thionyl chloride at RT, then heating the mixture to 60^0C, to give a yellow solid. This could be purified, by washing with dry ether. The appropriate amine (2.5 Eq) was added, with a few drops of TEA in dry DCM, to keep the solution basic. It was essential to keep the mixture dry, as to reduce the reformation back to Oxazepam. The mixture was refluxed for 2-3 hrs and then subsequently allowed to cool. For work up, the organic phase was washed with dilute HCl and then with water, to remove any unreacted amine. DCM was dried and evaporated to dryness, to give the desired product.

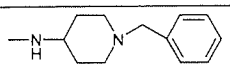
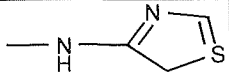
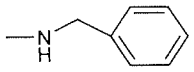
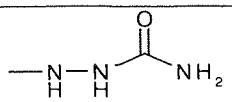
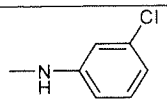
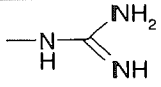
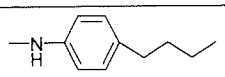
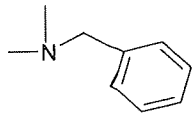
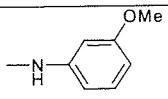
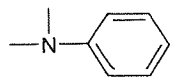
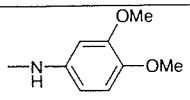
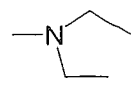
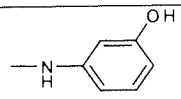
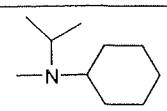
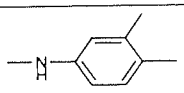
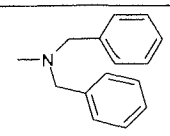
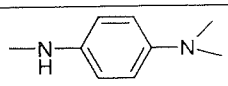
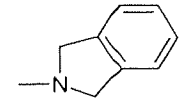
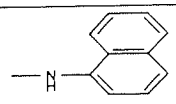
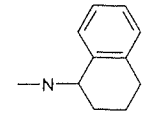
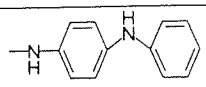
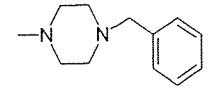
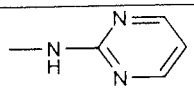
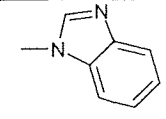
3.3.1. Biological evaluation of 3-amino substituted 1,4-benzodiazepin-2-one analogues

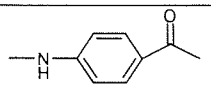
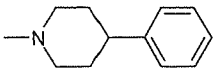
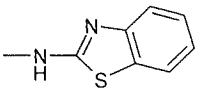
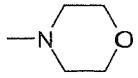
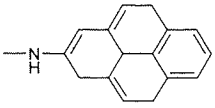
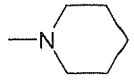
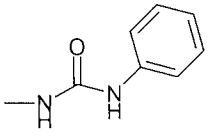
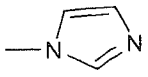
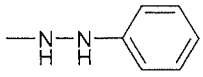
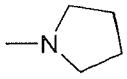
Table 3.3.1.1: Structure and activity of the synthesised analogues



General structure of the analogues

All synthesised analogues were composed of the core Oxazepam template but containing a varying 3-positioned substitutents (primary and secondary amines). All synthesised compounds were initially screened on the CCK_B receptor subtype.

Entry	R	MS (M+H) [m/z]	CCK_B IC_{50} [μM]	Entry	R	MS (M+H) [m/z]	CCK_B IC_{50} [μM]
1		459	-	18		-	-
2		376	1.2	19		-	-
3		396	0.32	20		-	-
4		418	0.58	21		390	0.86
5		391	-	22		390	0.10
6		422	-	23		342	-
7		378	-	24		410	0.19
8		390	0.66	25		466	0.26
9		391	-	26		387	-
10		412	-	27		402	0.42
11		453	-	28		445	-
12		368	-	29		387	-

13		404	-		30		430	-
14		419	-		31		356	2.7
15		486	0.84		32		354	-
16		-	-		33		-	-
17		-	-		34		340	-

(-) IC_{50}> 10 μM or not available due to solubility problems/complexation with the radioactive ligand.

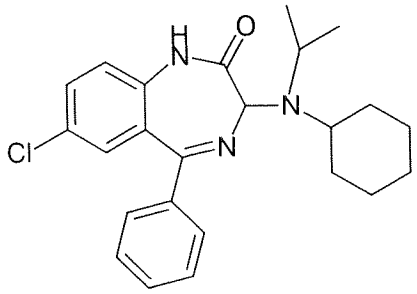
From Table 3.3.1.1, a series of 3-amino-substituted-1,4-benzodiazepines were successfully synthesised and evaluated. Not all combinations were working, especially with hydrazines and urea derivatives. The *in vitro* activity was encouragingly good and these results justify a further set of purer analogues to be synthesised and screened on both receptor-subtypes.

3.3.2. Summary

A number of diverse amines were selected in this mini library approach to drug discovery. An excess of amine & TEA were used to neutralise HCl produced in the course of the reaction. Conditions were kept dry, if water was present this would reform Oxazepam. Product formation was dependent on the nucleophilic amine. Hydrazine's, semicarbazone's and some hetrocyclic systems were non-working, with this method.

Biological evaluation showed good CCK_B binding affinities (IC_{50} = in the nanomolar range). This initial screening result suggests that anilines, particularly secondary, demonstrate the highest CCK_B binding activity.

The most active compound in this series was **24**. It is composed of a cyclohexylamine and an alkyl isopropyl group (IC50 = 190 nM).



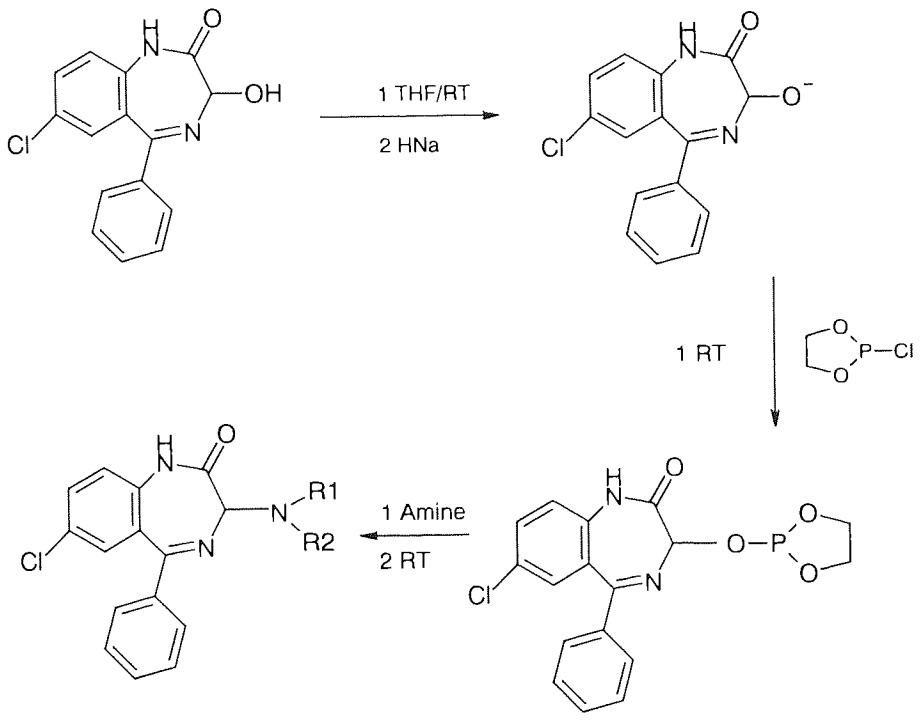
24

These results justify a second series of purified compounds to be prepared of the most active and also different analogues of compound 24.

3.4. Synthesis of selected 3-amino substituted 1, 4-benzodiazepin-2-ones derivatives

From the initial screening results, aniline analogues and cyclohexylamine derivatives showed the best *in vitro* activity (Section 3.3.2). A second series of compounds and other 3-amino substituted 1, 4-benzodiazepine-2-ones derivatives were synthesised. This should reconfirm and may even show potentially enhanced activity. All 3-amino substituted 1, 4-benzodiazepine-2-ones derivatives were prepared via route A, the thionyl chloride or by route B, an phospho-oxy derivative. When route A was used, after the work up with water, the compounds were isolated by precipitation with hexane. This was achieved by allowing the solution to stand overnight, with an excess of hexane and then washing and drying the product.

Scheme 3.4.0.1: Formation of 3-amino substituted 1, 4-benzodiazepine-2-ones via a phospho-oxy derivative (route B)



The synthetic route B[138,139] (Scheme 3.4.0.1) was investigated to compare yields and reliability, compared with route A. Under inert conditions Oxazepam was treated with sodium hydride, to abstract the hydroxyl proton. 2-Chloro-1, 3, 2-dioxaphospholane was then allowed to stir at room temperature. The appropriate amine was added and the mixture was left overnight at room temperature. TLC suggested formation of product was optimal, when left overnight. The filtrate was purified by flash chromatography, using ethyl acetate as the mobile phase. Mass spectrometeric analysis of the compounds showed, as well an M+1, a strong 269+ m/z peak, suggesting the loss of the amine group. R_f values were higher than the Oxazepam but lower than the amine reactant. Yields of formation were generally higher with the precipitation method (A) but lower when using route (B).

3.4.1. Biological evaluation of selected 3-amino substituted 1, 4-benzodiazepin-2-ones analogues

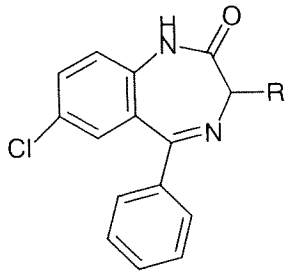
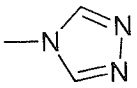
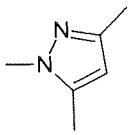
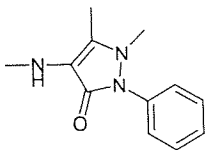
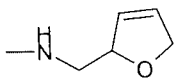
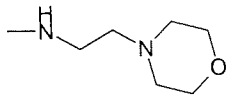
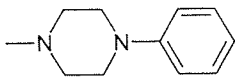
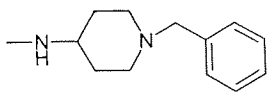
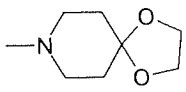
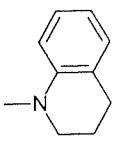
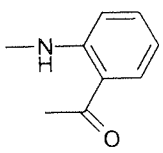
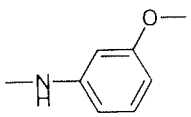
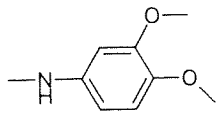
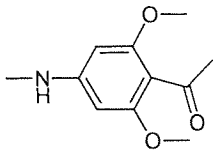
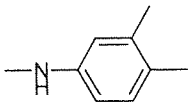
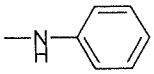
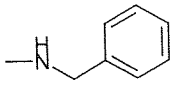
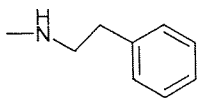
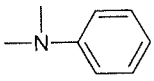
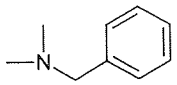
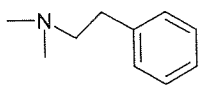
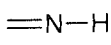
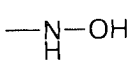
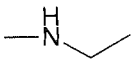
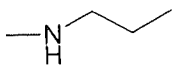
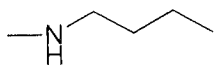
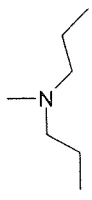
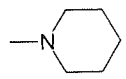
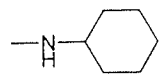


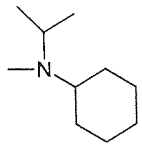
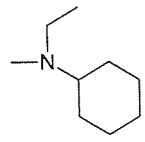
Figure 3.4.1.1: Structure of benzodiazepine analogues (Table 3.4.1.2)

The amines were selected into five main series (0-4). Series 0, was composed of substituted anilines, heterocyclic and large bulky amines. Series 1, contained an unsubstituted aniline with 0,1,2 carbon spacers, between the amino group and the phenyl ring. Series 2, comprised of amines in series 1 but each with a N-methyl subsituent. Series 3, contained various small groups and varying alkyl side chains. Series 4, was composed of analogues of compound 24, which was the most active from the previous screening result.

Table 3.4.1.2: Activity of benzodiazepine analogues

Entry	Structure	Route A/B	MW	FW	MS (M+1) [m/z]	Yield [%]	IC_{50} [μM]
	Series 0						
3.4.1		A	337	$C_{17}H_{12}ClN_5O$	338	52	>20
3.4.2*		A	364	$C_{20}H_{22}ClN_5O_3$	365	69	>20
3.4.3*		A	471	$C_{26}H_{22}ClN_5O_2$	472	58	9.3
3.4.4		B	367	$C_{20}H_{18}ClN_3O_2$	368	20	>20
3.4.5		B	398	$C_{21}H_{23}ClN_4O_2$	399	21	>20
3.4.6*		B	430	$C_{25}H_{23}ClN_4O$	431	28	>15
3.4.7*		B	458	$C_{27}H_{27}ClN_4O$	459	35	>15
3.4.8*		A	411	$C_{22}H_{22}ClN_3O_3$	412	62	>15
3.4.9*		A	401	$C_{24}H_{20}ClN_3O$	402	66	7.2
3.4.10*		B	403	$C_{23}H_{18}ClN_3O$	404	33	8.4
3.4.11*		A	391	$C_{22}H_{18}ClN_3O_2$	392	45	1.5
3.4.12*		A	421	$C_{23}H_{20}ClN_3O_3$	422	50	1.2

3.4.13 *		B	363	$C_{25}H_{22}ClN_3O_4$	364	30	5.3
3.4.14 *		A	389	$C_{23}H_{20}ClN_3O$	390	58	0.92
Series 1							
3.4.15 *		A	361	$C_{21}H_{16}ClN_3O$	362	80	4.1
3.4.16 *		A	375	$C_{22}H_{18}ClN_3O$	376	62	6.0
3.4.17		A	389	$C_{23}H_{20}ClN_3O$	390	60	>20
Series 2							
3.4.18 *		A	375	$C_{22}H_{18}ClN_3O$	376	39	0.15
3.4.19 *		A	389	$C_{23}H_{20}ClN_3O$	390	36	2.9
3.4.20		A	403	$C_{24}H_{22}ClN_3O$	404	36	9.8
Series 3							
3.4.21		A	283	$C_{15}H_{10}ClN_3O$	284	39	>20
3.4.22 *		A	301	$C_{15}H_{12}ClN_3O_2$	302	44	>20
3.4.23 *		A	313	$C_{17}H_{16}ClN_3O$	314	37	>15
3.4.24 *		A	327	$C_{18}H_{18}ClN_3O$	328	39	8.9
3.4.25 *		B	341	$C_{19}H_{20}ClN_3O$	342	25	>15
3.4.26		B	369	$C_{21}H_{24}ClN_3O$	370	19	>20
Series 4							
3.4.27		B	353	$C_{20}H_{20}ClN_3O$	354	21	>15
3.4.28 *		A	367	$C_{21}H_{22}ClN_3O$	368	33	10.1

3.4.29		A	409	$C_{24}H_{28}ClN_3O$	410	31	>15
3.4.30		A	395	$C_{23}H_{26}ClN_3O$	396	30	>15

*** = fully characterised: TLC, IR, Mass-Spectrometry, ^1H & ^{13}C NMR. The remainder, being characterised by TLC and Mass-Spectrometry.**

Most compounds, prepared through route A (via the thionyl chloride intermediate), were generally of a higher yield and were quicker to synthesise, unlike route B, which was via the phospho-oxy derivative. This second set of compounds were not as active as the first, IC_{50}'s being much lower (Fig. 3.4.1.3), mostly in the low micromolar range rather than the low nanomolar, as in the previous set of results, except compound **3.4.18**. This second set of compounds were purified and prepared to 1 μM and not screened at a higher concentration, without purification.

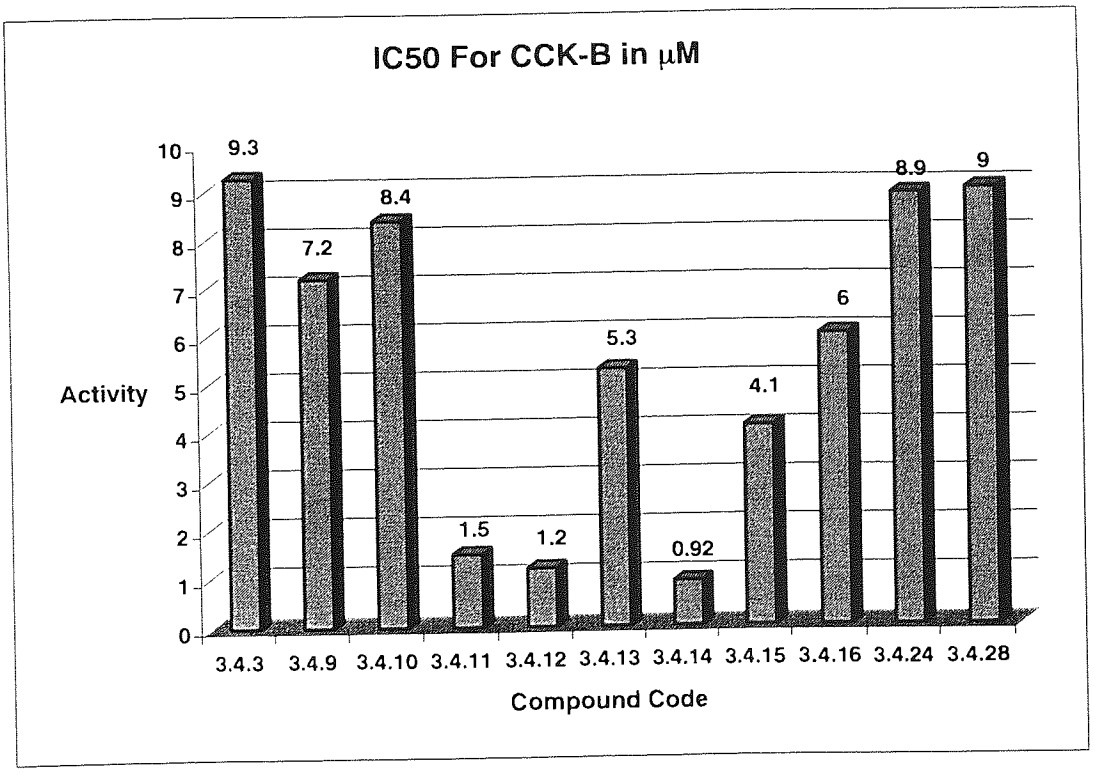


Fig. 3.4.1.3: Selected biological activity of 3 amino-substituted 1,4-benzodiazepine analogues

3.4.2. Summary

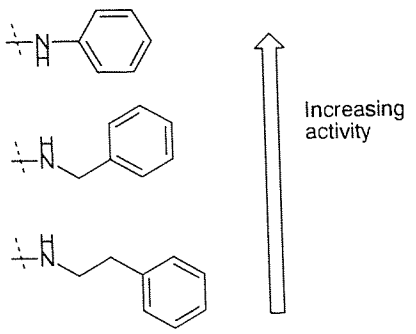


Figure 3.4.1.5: Series 1

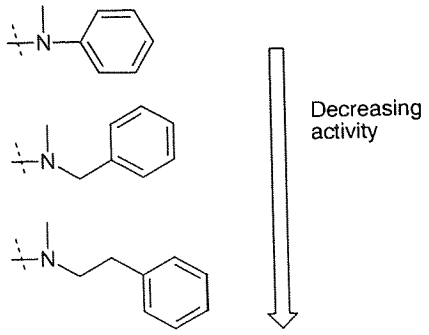


Figure 3.4.1.6: Series 2

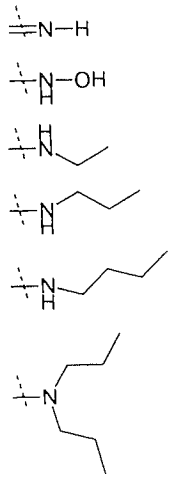


Figure 3.4.1.7: Series 3

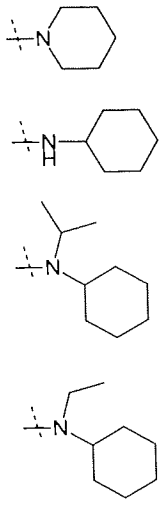


Figure 3.4.1.8: Series 4

From observing these sets of results, a structure activity relationship can be determined. Increasing the carbon chain length, from the 3-position nitrogen to the phenyl reduces activity, this can be observed for compounds **3.4.15** and **3.4.16** & **3.4.17** (series 1). An unsubstituted aniline proved to be active, whilst a cyclohexane ring **3.4.28** reduces potency. The most active compound from the first series **3.4.29** shows no activity, neither does its analogues **3.4.27** and **3.4.30** (series 4).

Alkyl chains have no affect on activity but a reduction from 4 to 3 carbons does show slight increase in activity, **3.4.25** to **3.4.24** (series 3). Hetrocyclic amines, generally

demonstrate no potency but **3.4.3** exhibits good displacement. The most active compound was found to be **3.4.18**, an unsubstituted aniline with a N-methyl group. The addition of only 1 carbon spacer **3.4.19**, between the N-methyl and the phenyl ring has the affect of reducing activity, this is further confirmed by compound **3.4.20**, with 2 carbon spacers (series 2).

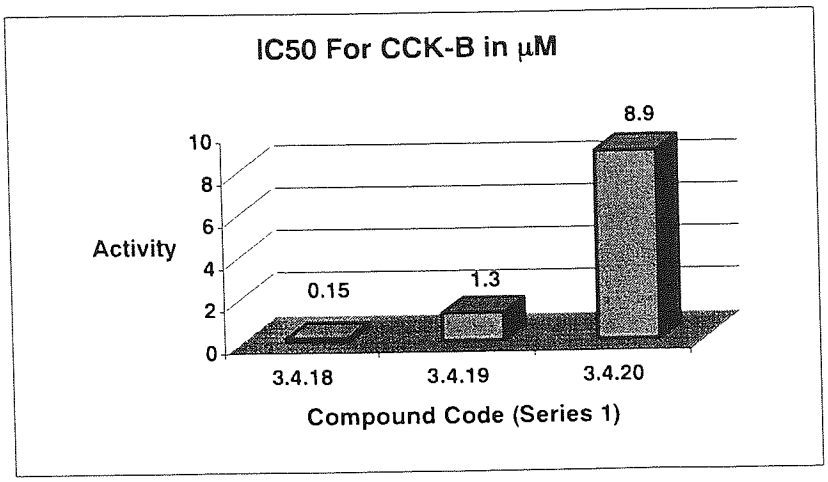


Fig. 3.4.1.4: The effect of increasing carbon chain length on the activity of 3 amino-substituted 1,4-benzodiazepine analogues

All non-bulky substituted anilines showed activity, particularly meta substituted anilines. The addition of a second methoxy group in compound **3.4.12**, reduces potency from compound **3.4.11**, with only one meta substituted methoxy group. Compounds with large bulky groups on the ring system are less active. This is probably due to steric hindrance, when trying to interact with the receptor.

To summarise, it can be determined that large bulky and hetrocyclic ring systems have little or no effect in displacing the radioactive ligand. Small aromatic, meta substituted anilines exhibit good activity. Activity is lost by introducing an alkyl spacer unit but is enhanced by the addition of an N-methyl group. From the results, compounds **3.4.14** & **3.4.18** are the most active from the series at 920 & 150 nM respectively. The next logical step would be to synthesize substituted anilines around the two most active lead structures.

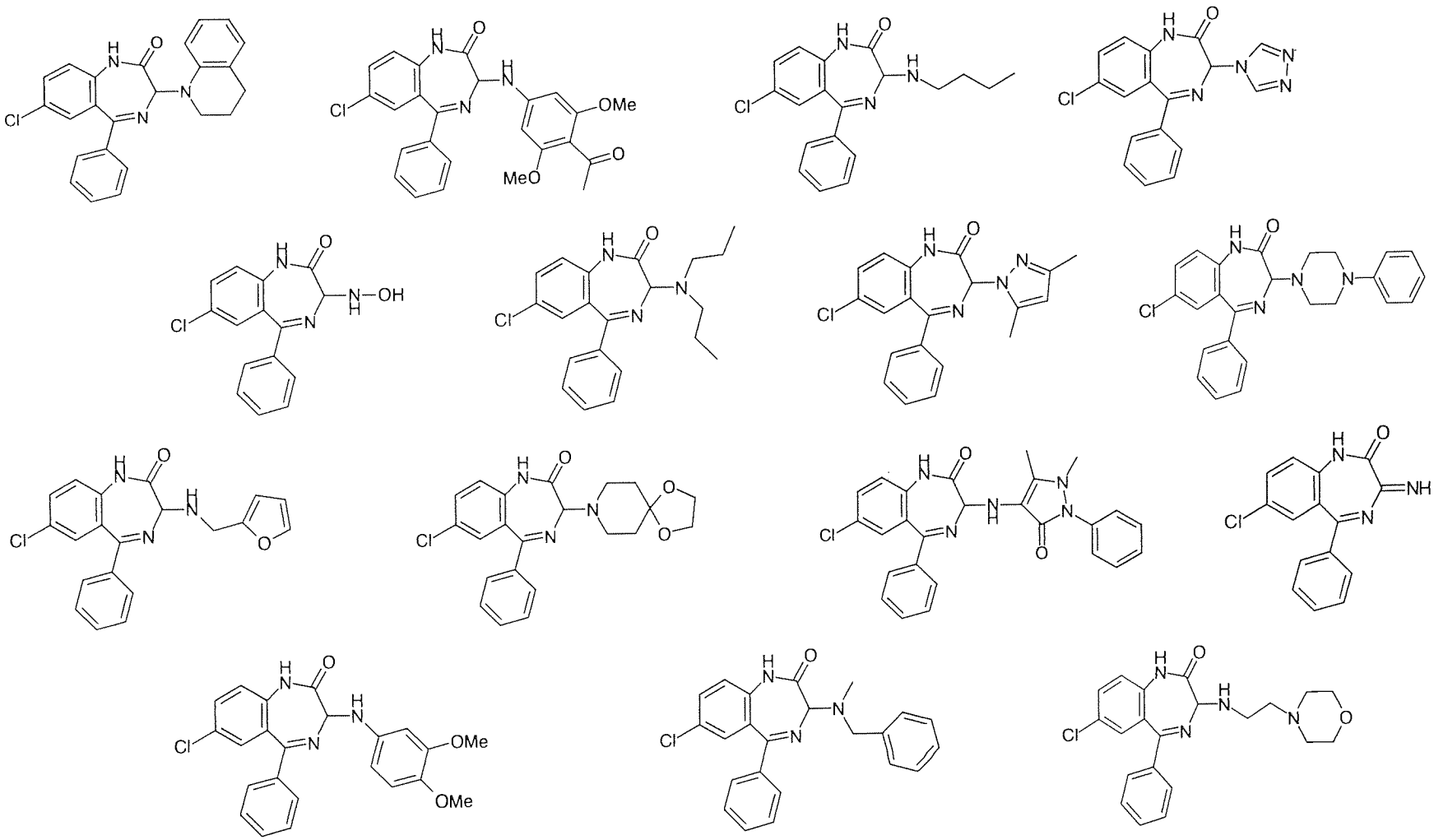
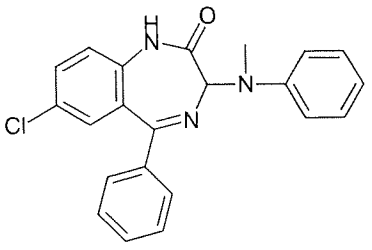


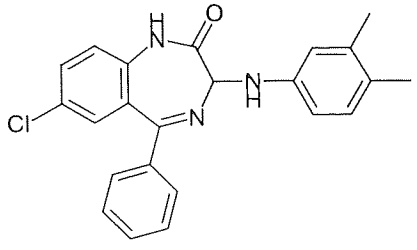
Figure 3.4.1.9: Structures of selected 3-amino substituted 1,4-benzodiazepines

3.5. Structure Activity Relationship of substituted anilines

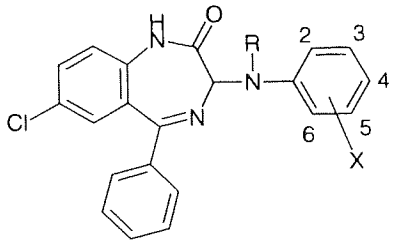
From the previous section compounds 3.4.14 & 3.4.18 demonstrated good *in vitro* activity. By combining the common features of both compounds, it was possible to synthesise analogues of compound 3.5.1a. These would, potentially, show an enhanced binding for the CCK receptor. The selectivity of each analogue can be measured by comparing the ratio of CCK_B over CCK_A receptor subtypes.



Compound 3.4.18



Compound 3.4.14



Compound 3.5.1a

From Table 3.5.1.1, no reaction yields are provided. This is because only a small portion of mixture, of the synthesised compounds, was isolated. They were separated by preparative TLC (MP = ether), via the thionyl chloride route (A). Nitro-anilines were generally difficult to isolate, as the amine was difficult to remove through washing with dilute acid and water. In general, from the table, substituted anilines were extremely potent and selective towards the CCK_A receptor subtype, rather than the CCK_B receptor subtype.

3.5.1. Biological evaluation of substituted anilines

Table 3.5.1.1: SAR of aniline subsitutents

Cpd Entry	R	X					R_f	MS (M+1) [m/z]	IC 50		Ratio CCK_B / CCK_A
		2	3	4	5	6			CCK_B [μM]	CCK_A [μM]	
3a*	H	NO_2	H	H	H	H	0.45	407	7.5	>20	-
3b	H	H	NO_2	H	H	H	0.21	407	6	0.011	545
3c	H	H	H	NO_2	H	H	0.37	407	8.5	>20	-
3d	H	Cl	H	H	H	H	0.44	396	9.3	>20	-
3e*	H	H	Cl	H	H	H	0.40	396	6.2	0.27	23
3f	H	H	H	Cl	H	H	0.38	396	2.9	20	-
3g	H	OMe	H	H	H	H	0.37	392	1.5	>20	-
3h*	H	H	OMe	H	H	H	0.30	392	4.5	0.010	450
3i*	H	H	H	OMe	H	H	0.34	392	>10	20	-
3j	H	CH_3	H	H	H	H	0.38	376	7.1	10	-
3k	H	H	CH_3	H	H	H	0.40	376	3.8	0.011	380
3l	H	H	H	CH_3	H	H	0.29	376	4.6	0.29	16
3m	H	CH_3	H	H	H	CH_3	0.48	390	>10	10	-
3n	H	H	CH_3	CH_3	H	H	0.25	390	0.92	0.015	61
3o	H	CH_3	CH_3	H	H	H	0.36	390	6	0.018	333
3p	H	H	CH_3	H	CH_3	H	0.20	390	2.9	0.009	322
3q*	H	CH_3	H	CH_3	H	H	0.42	390	6.1	0.39	16
3r	H	CH_3	H	H	CH_3	H	0.48	390	6.0	1.0	6
3s*	H	H	H	H	H	H	0.37	362	4.1	0.24	17
3t*	CH_3	H	H	H	H	H	0.57	376	0.15	0.014	11
3u*	CH_3	H	CH_3	H	H	H	0.52	390	0.07	0.008	9
3v	CH_2-CH_3	H	CH_3	H	H	H	0.60	404	0.65	0.33	2

*** Fully characterized**

3.5.2. Summary

The entire combination of substituted anilines was successfully synthesised and separated, based on the two initial lead compounds (3.4.14 & 3.4.18). The results, from Table 3.5.1.1, show that the synthesised compounds are very potent (Fig. 3.5.1.1) towards the CCK_A rather than the CCK_B receptor (Fig. 3.5.1.2 & 3.5.2.1). Nitroanilines displayed weak CCK_B binding. However, compound **3b**, a meta-nitroaniline showed excellent CCK_A binding at IC_{50} = 11 nM, whilst the ortho & para groups were inactive (IC_{50} = >20 μM). This same result was observed for the m-chloroanilines (**3e**, IC_{50} = 270 nM), the m-methoxyaniline (**3h**, IC_{50} = 10 nM) and the m-toluidine (**3k**, IC_{50} = 11 nM). Dimethylanilines were equally active, when at least one group was at the 3-position (Compounds **3n**, **3o**, **3p**), whilst the N-methylaniline (**3t**) had an IC_{50} value of 14 nM.

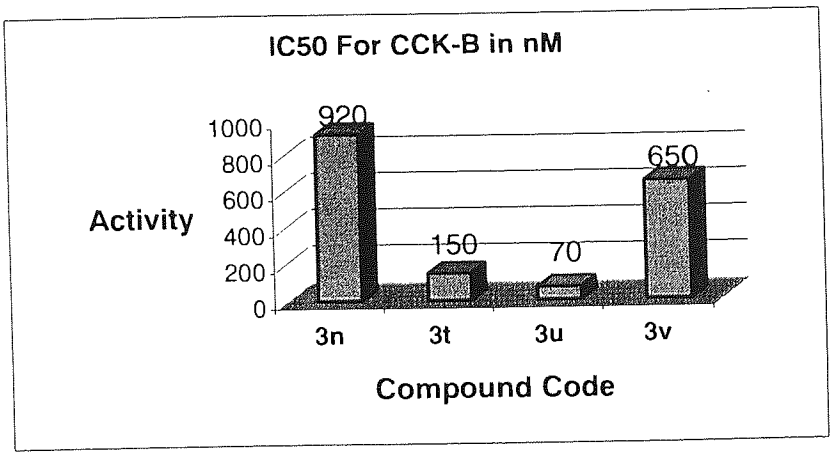


Fig. 3.5.1.1: Selected IC_{50}'s of substituted anilines

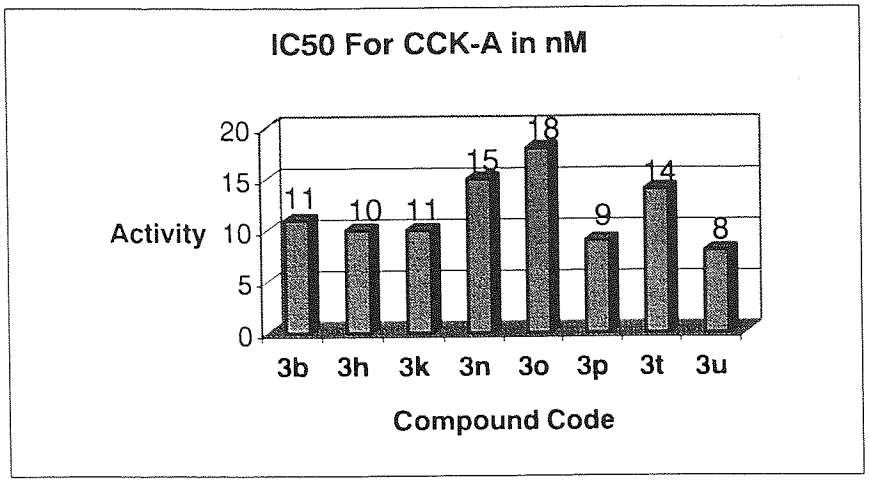


Fig. 3.5.1.2: Comparison of CCK_A binding affinity of the most active compounds

Compound **3u** is similar to L-365, 260, the methyl group being in the meta position of the phenyl ring. This was the most active compound in the series for both receptors, at 70 & 8 nM for the CCK_B & CCK_A receptor subtypes respectively. It can be deduced that removing the urea functionality produces analogues that are less potent towards the CCK_B receptor. However, activity is greatly enhanced when screened towards the CCK_A receptor, especially the meta-positioned substitutents. Selectivity is extraordinarily high, providing the opportunity for selective application. The amine group has been fully optimised in this position. The next approach would be to alkylate the nitrogen of compound **3u**, as this could potentially enhance activity further.

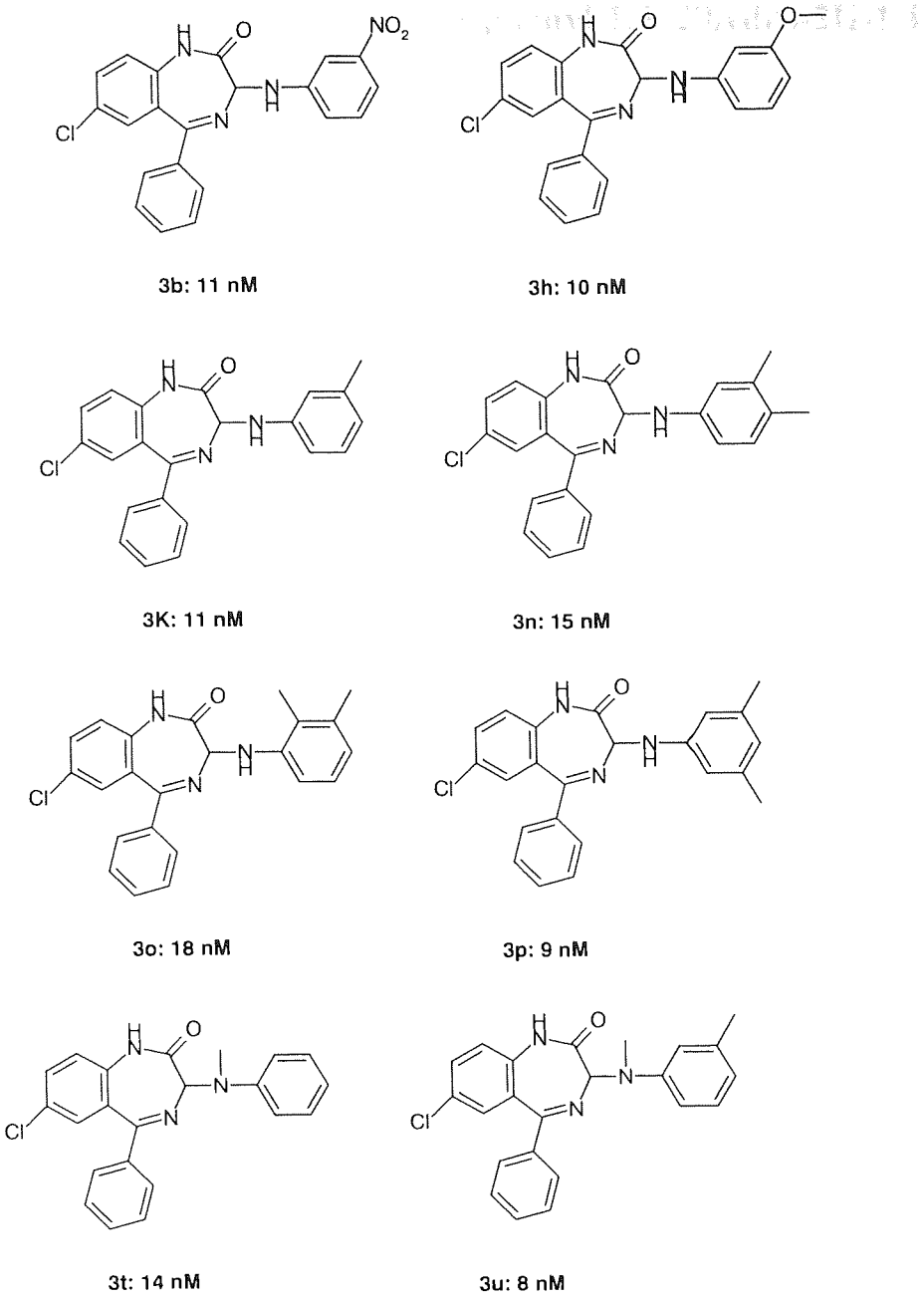
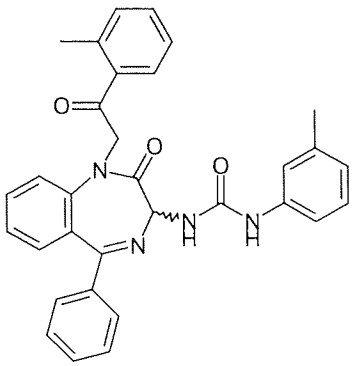


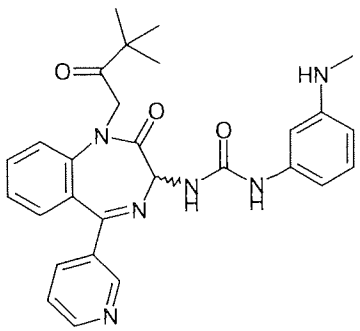
Fig. 3.5.2.1: Structures of the most potent CCK_A antagonists, with IC_{50}'s given.

3.6. Introduction to alkylation of 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (Oxazepam)

To further boost the receptor binding potential of compound **3u**, introducing a lipophilic group may result in superior interactions with the receptor. Researchers at Yamanouchi Pharmaceuticals[106,143] have discovered potent CCK_B ligands, related to the archetype analogue L-365, 260 but with sub-nanomolar affinities for the receptor.



YM022

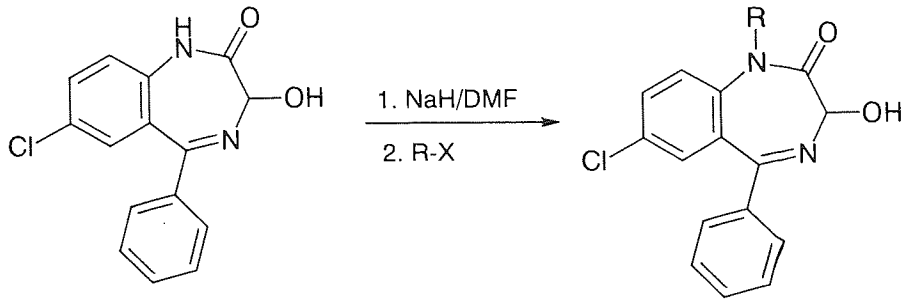


YF476

Initially compound YM022 was the optimal structure in the series. However, further improvements in the *in vitro* activity and bioavailability of these derivatives was attained by incorporating a 1-alkylcarbonylmethyl and 5-(2-pyridyl) substituent. This produced a novel and potent CCK_B antagonist (YF476), which is currently undergoing clinical trials.

3.6.1. Alkylation of 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (Oxazepam)

Alkylation (Scheme 3.6.1.1) was performed selectively, with the most active, diverse and readily available alkylating groups, rather than selecting a large number of alkyl halides at this initial stage.



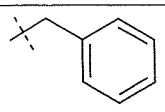
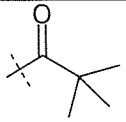
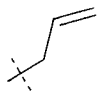
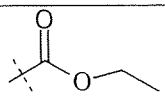
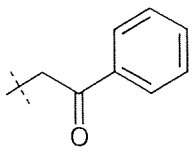
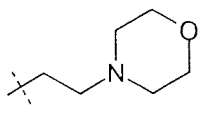
Scheme 3.6.1.1: Alkylation of Oxazepam

Alkylation[144,145] was achieved using a 50% suspension of NaH, in dry DMF with Oxazepam (Scheme 3.6.1.1). After stirring at RT, the appropriate alkylating agent was added in drops and left for 45 minutes. Work up was accomplished with ethyl acetate and then washed with water and brine. Column chromatography (ether/petrol ether 1:2) yielded the pure products. Synthetic yields (Table 3.6.2.1) were low to good with compound 2 being the highest at 81%, whilst compound 1 being the lowest at 40%. Compound 7 was a non-working reagent, probably due to the salt form not reacting. Mass spectrometric analysis of the alkylated products was achieved using the negative mode of the APCI instrument, since the positive mode failed to detect the M+1 product peaks.

3.6.2. Biological evaluation of alkylated Oxazepam series

All synthesised compounds were screened on the CCK_B receptor subtype.

Table 3.6.2.1: Structure and activity of alkylated Oxazepam

Cpd Entry	Alkylating agent	R	MF	MW	R_f	MS (M-1) [m/z]	Yield [%]	IC_{50} CCK_B [nM]
3.6.1	Benzyl chloride		$C_{22}H_{17}ClN_2O_3$	376	0.42	375	40	760
3.6.2*	Trimethylacetyl chloride		$C_{20}H_{19}ClN_2O_3$	370	0.51	369	81	190
3.6.3*	Propargyl bromide		$C_{18}H_{13}ClN_2O_2$	324	0.38	323	67	960
3.6.4	Ally bromide		$C_{18}H_{15}ClN_2O_2$	326	0.35	325	68	980
3.6.5	Ethyl chloro-formate		$C_{18}H_{115}ClN_2O_4$	358	0.47	357	29	690
3.6.6	Phenacyl chloride		$C_{23}H_{17}ClN_2O_3$	404	0.44	403	25	200
3.6.7	4-(2-chloro-ethyl)morpoline HCl		-	-	-	-	-	-

*** = fully characterised**

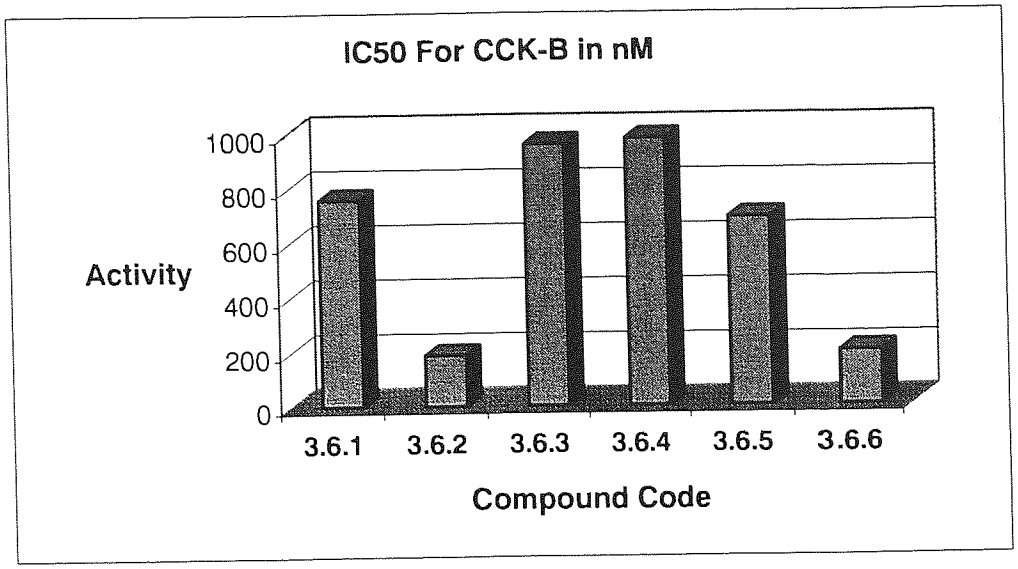
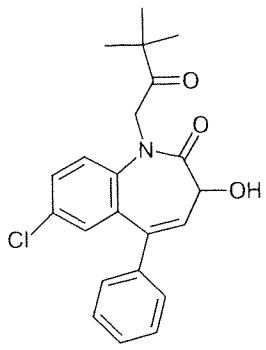


Fig.3.6.2.2: Comparison of biological activity of the alkylated Oxazepam series

Compound 3.6.2 demonstrated the highest binding activity, with an IC_{50} value of around 190 nM. A wide selection of alkylating agents containing aryl-, saturated and unsaturated alkyl groups were selected. Generally the alkylated series (Fig. 3.6.2.2) were active, with even the Oxazepam template demonstrating an IC_{50} of around 1.5 μM. This result has confirmed the most active N1-substituent from the Yamanouchi researchers.



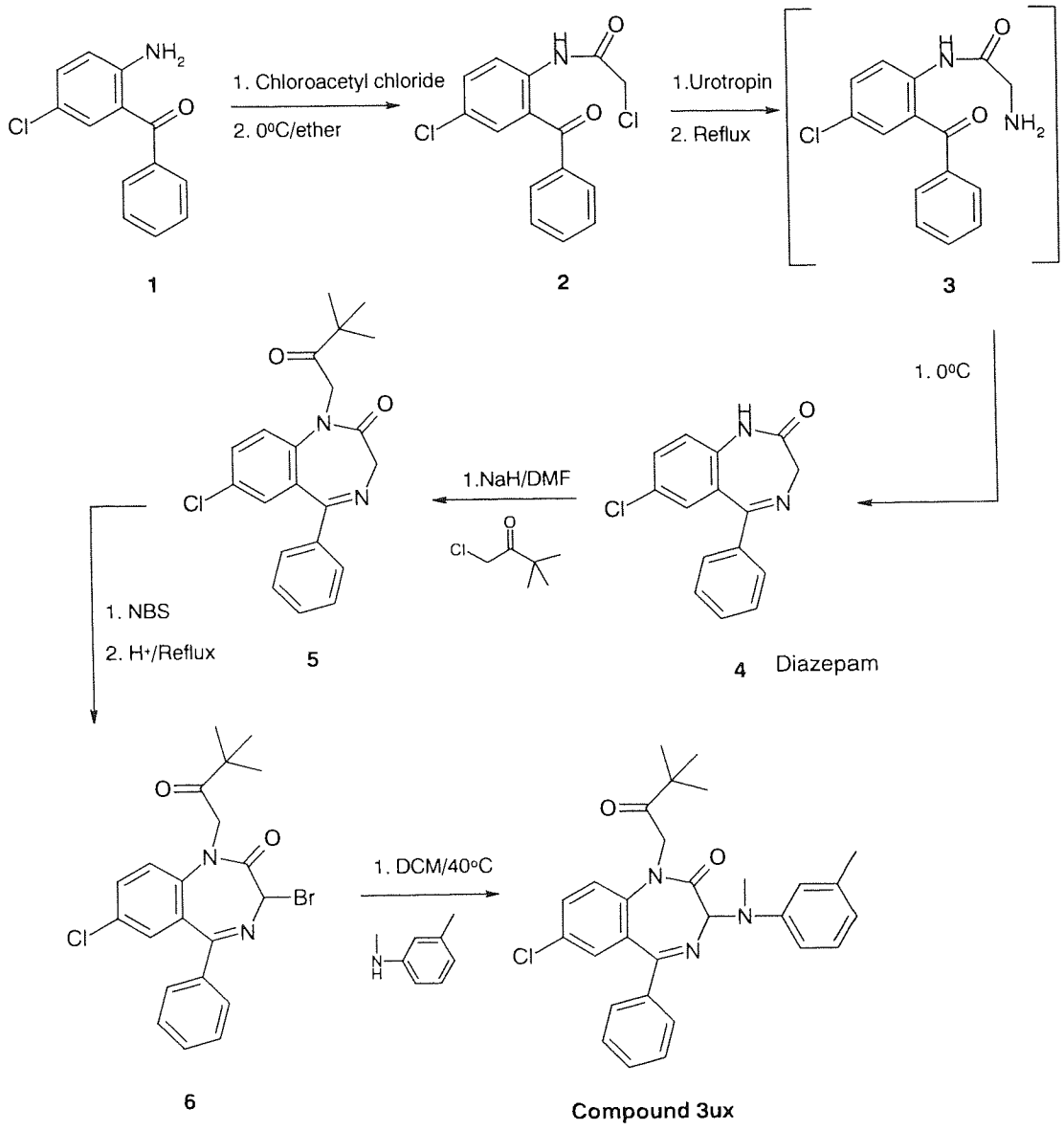
Compound 3.6.2

3.6.3. Summary

The next synthetic approach was to combine the most active N1-substituent, pinacolone, with the most active 3-positioned amine, N-methyl-m-toluidine. This was initially attempted via the thionyl chloride route. However, the thionyl chloride route caused the N1 substituent to be decomposed, with only fragments being observed in the mass-spectrum. It was concluded that this route was very harsh and destructive and a milder route needed. When the amine was reacted first with Oxazepam and then subsequently the alkyl halide, a precipitate was formed. This had occurred due to the lone pair of electrons on the amine nitrogen complexing with the alkyl reagent, rather than the amide nitrogen. Conversion into a phospho-oxy derivative and then displacement with the amine was also found to be unsatisfactory, with a number of impurities and trace amounts of the final product being observed.

3.7. Preparation of an N1-alkylated-3-anilino-benzodiazepin-2-one via Diazepam

A new synthetic strategy had to be developed to overcome the problem of combining the amine to the alkylated Oxazepam compound. One alternative approach was to synthesise Diazepam, then alkylate with chloropinacolone, bromination and then the chemoselective displacement with the amine (Scheme 3.7.0.1).



Scheme 3.7.0.1: Formation of Diazepam, subsequent alkylaton, bromination and nucleophilic displacement.

Diazepam (4) was synthesised according to the standard literature procedure[146,147]. The ketone building block (1) was acetylated with chloroacetyl chloride in anhydrous ether at 0°C (2) and was not isolated. This was then refluxed with urotropin (hexamethylenetetramine) for 16 hrs to enable the cyclization step (the Delepine reaction) to give the aminoacetoamide compound (3), which was not isolated. The whole mixture was cooled, with the diazepam crystals (4) precipitating out in a yield of 64%.

Diazepam was alkylated, using the standard literature conditions[148]. The bromination procedure[149], involved the use of NBS, CCl_4 and a halocarboxylic acid (TFA) in a radical reaction. The reaction was initially stirred at RT, then refluxed vigorously for 1-1.5 hrs. The residue was decanted, washed with CCl_4, to give an unstable yellowy brown oil, in a high yield of 91%. N-methyl-m-toluidine (2.5 Eq) was added in dry DCM, with drops of TEA and left stirring at 40°C overnight. The mixture was washed with water and dried, with the DCM removed in vacuo.

A number of impurities were observed on the TLC plate. The mass-spectrum of the mixture indicated the formation of the desired product at 489 m/z, as well as a number of minor and major by-products. These by-products were identified as the amine reacting with the N-1 alkyl groups. The bromination reaction was not selective towards the 3-position. The bromine atom was a better leaving group than the chlorine. However, after a column chromatography on the mixture (ether:petro ether 1:2), the product (yellow powder) was isolated in just under 2% yield.

3.7.1. Summary

The target compound 3ux was successfully synthesised, although the yield was extremely low and the product was very difficult to separate. The reaction conditions still have scope for improvement. The *in vitro* activity was disappointingly low, with an IC_{50}, for both the CCK_B & CCK_A receptor at around 800 & 245 nM respectively. Therefore, it was deduced that an alkylation at the N-1 position, and the amine in the 3-position result in a

loss of binding, although each fragment shows better binding on their own. Compound 3u has an IC_{50} of 150 nM, the Merck compound, L-365, 260, at ~10 nM and the most active Yamanouchi compound (YF476) at ~0.1 nM. It can be noted that compound 3u is less active. However, it has the potential of demonstrating a better bioavailability, (especially over the Merck compound) with the nitrogen being easily protonated for oral administration. Compound 3u was evaluated as a racemic mixture. One isomer may be more potent than the other and selective over either receptor subtype. Under the current reaction conditions it would be difficult to control the stereoselectivity of the reaction.

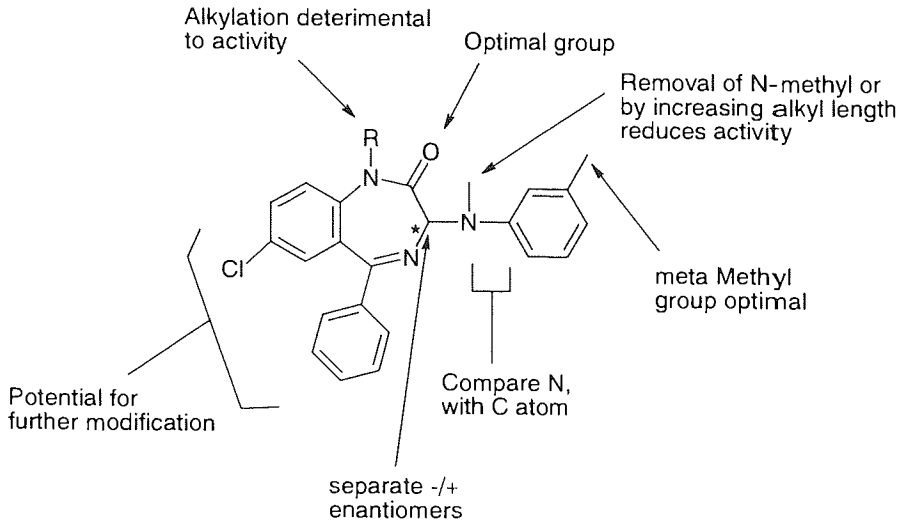
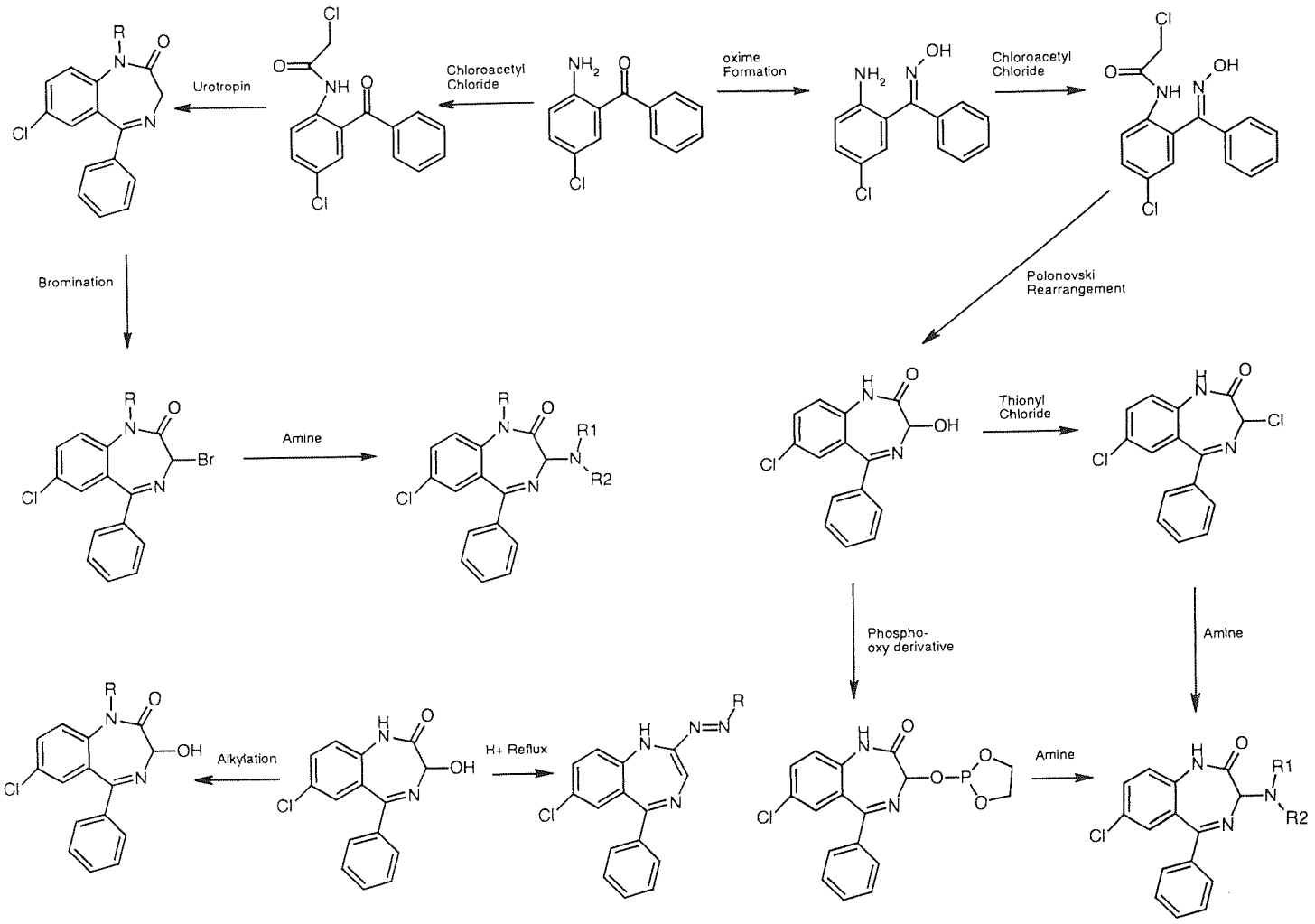


Fig. 3.7.1.1: Summary of the results obtained and the potential for further modification

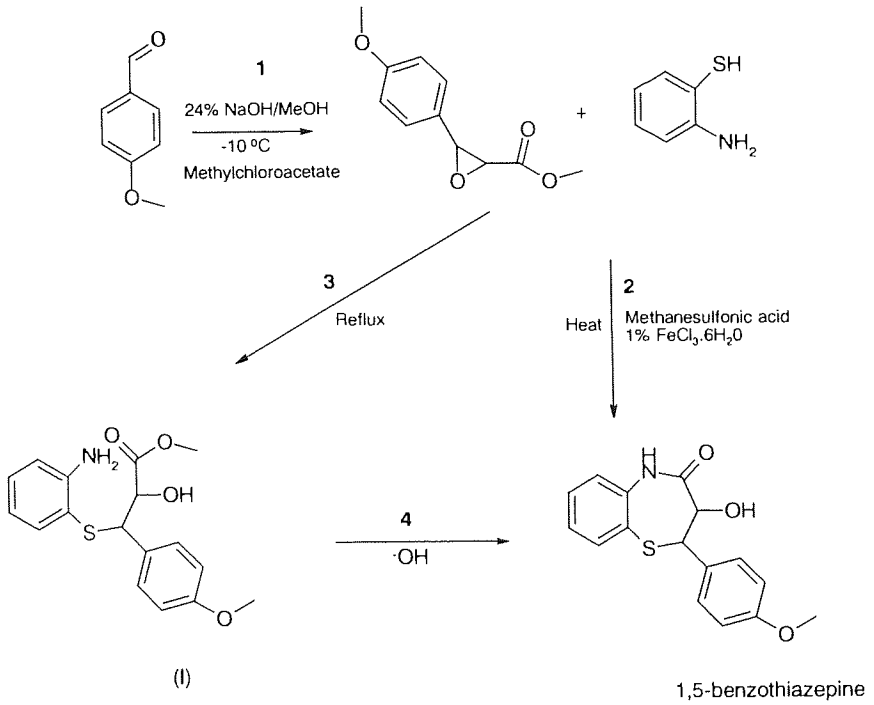
From all the various data obtained from modifying the 1,4-benzodiazepine template, the results can be summarised in Fig. 3.7.1.1. It was decided to discontinue this approach due to the limited scope for further improvements. Instead of a polar atom like the nitrogen at the 3-position, they may be the need for a more lipophilic atom, like carbon. Although the active compounds are novel, further analogues, if active, would be difficult to patent, because of extensive overlap with pre-existing patents. Many thousands of benzodiazepine compounds have been synthesised by leading research groups around the world, who have extensively studied the template. It was decided a completely new approach was required, providing a novel structural class of CCK antagonists.



Scheme 3.7.1.2: Overall reaction summary of the benzodiazepine template formation and subsequent further reactions

3.8. Synthesis of 3-hydroxy-4-(4-methoxyphenyl)-1,3,4,5- tetrahydro-2H-1-benzothiazepin-4-one

Another project was to evaluate analogues of 1,5-benzothiazepines, which could be compared to the 1,4-benzodiazepine analogues. The 1,5-benzothiazepine structure differs in that it consists of a sulphur atom in the 5-position and has a methoxy-phenyl ring on the 4-position. 1,5-benzothiazepine derivatives are commonly associated as anti-cancer, fungal and bacterial agents[150]. This additional new approach would evaluate if the geometry of substituted 3-amino-1, 5-benzothiazepines analogues allows for a more or less potent CCK antagonist, than the 1,4 benzodiazepines.



Scheme 3.8.0.1: Formation of the new 1,5-benzothiazepine template

Methyl 3-(4-methoxyphenyl) glycidate (**1**) was prepared via a Darzens Condensation reaction (Scheme 3.8.0.1) with the corresponding aldehyde and methyl chloroacetate at – 10 ^{0}C, in a high yield of 93%. This was achieved according to the modified method of Paolo Crotti et al.[151]

According to the method from Swati, Prackash and Sharma[150] (**3**) and S.Yamador et al.,[152] a one pot synthesis, did not result in the formation of 1,5-benzothiazepine. However, only the intermediate (**I**) was isolated in a low yield (25%). Cyclization of this intermediate in the presence of base (**4**) /or DBU (catalyst) in acetonitrile aided the formation of the desired product.

The method from Shin-ichi Yamada et al was successful (**2**). The research group used $FeCl_3.6H_2O$ as a catalyst and methanesulfonic acid to aid the cyclization process. The use of dry reagents and conditions was essential to prevent the oxidisation of sulphur. Refluxing the mixture using an air condenser or distilling one third of the solvent removed methanol, the by-product, and gave the desired product (30%). Without removing methanol, this hindered the cyclization process and therefore only the intermediate was formed.

3.8.1 Attempted reactions on the 1,5-benzothiazepine template

A number of different reactions were performed on the 1,5-benzothiazepine to form carbamates, react amines and hydrazines but each were unsuccessful. It was concluded that this new template was much more stable than the 1,4-benzodiazepine and would require much harsher conditions to enable it to react. Provisional screening data on the template showed it to be completely inactive for CCK application. Any further attempts were abandoned and a completely new approach investigated.

Chapter 4. Synthesis of simple ureas

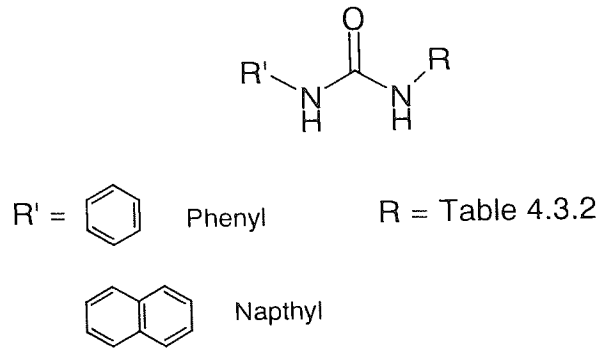
4.1. Basis to synthesise urea analogues

It was decided that a different approach was required to produce a novel, starting compound. The aim was to derive a novel template, connected via a urea linkage. It was noted that the common functionality of key CCK_B antagonists was the urea link, which is the key component for activity. A completely new approach was to synthesise urea analogues, rather than following similar known approaches. Most active compounds have a common feature, they are composed of a urea linkage between the BZD and the aromatic system, making them potent CCK receptor antagonists.

It has already been stated that the benzodiazepine structure is not essential for activity and thus can be replaced by an alternative aromatic system to mimic its moiety and effect. In this approach, a vast number of aromatic amines, with various substituents present (Table 4.2.2), were reacted with aromatic isocyanates (phenyl and napthyl). Phenyl and napthyl, although are not structurally diverse, would hopefully reconfirm any potential activity and therefore reduce the possibility of false positives. These compounds would be relatively simple but they do however, provide a focal starting point to synthesise more complex compounds. Throughout the experiment it was essential to keep conditions dry, so as to avoid the formation of di-phenyl/di-napthyl urea, a major side product. A solution of the relevant amine in dry acetonitrile was stirred at room temperature in a carousel reaction station. The appropriate isocyanate (1-phenyl/ 1-napthyl, 1.1 Eq) in dry acetonitrile was added slowly over 5 minutes, allowed to stir at

room temperature or heated to 60^{0}C and left overnight. The precipitate that formed was filtered, washed (twice), with cold acetonitrile and dried, to give the corresponding urea (Table 4.2.3).

4.2. Structure and characterization of the urea analogues



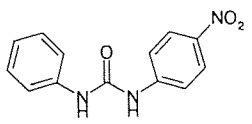
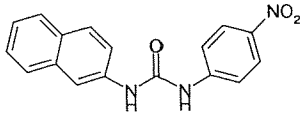
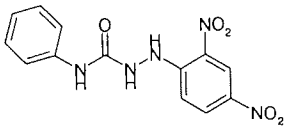
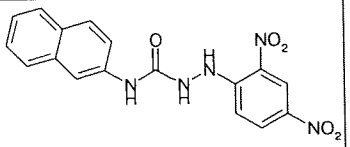
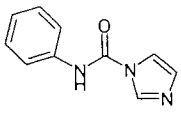
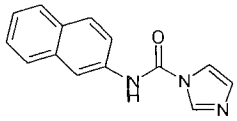
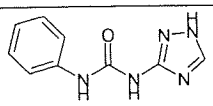
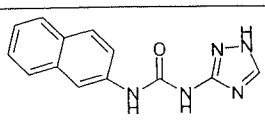
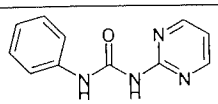
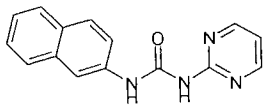
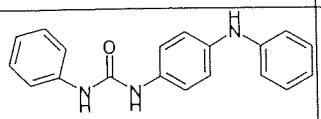
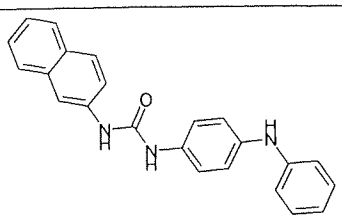
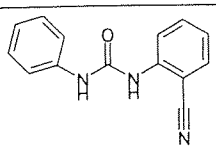
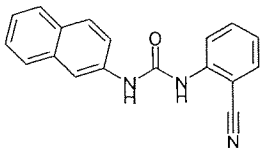
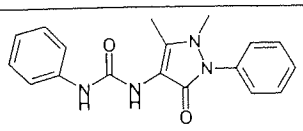
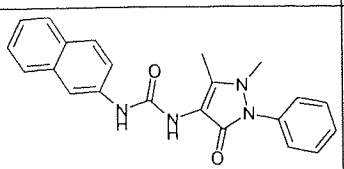
4.2.1: General compound structure (Table 4.3.2)

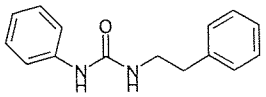
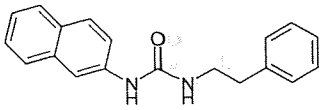
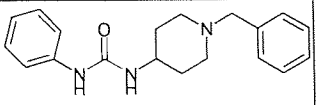
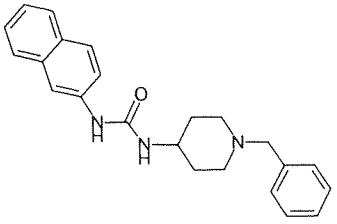
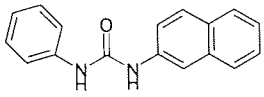
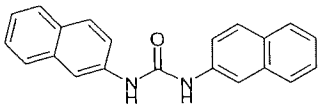
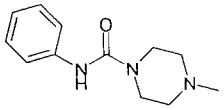
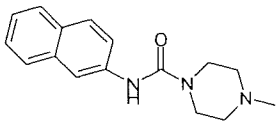
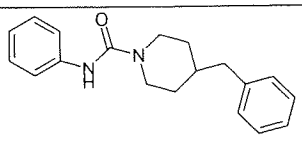
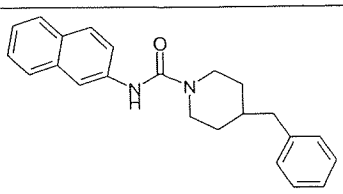
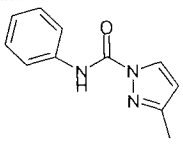
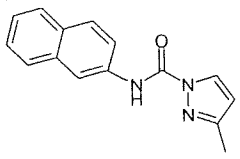
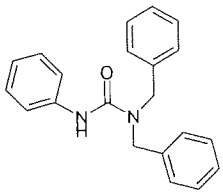
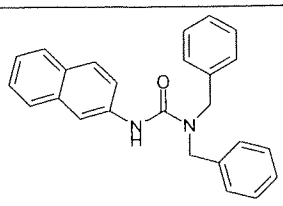
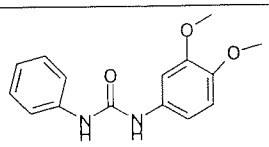
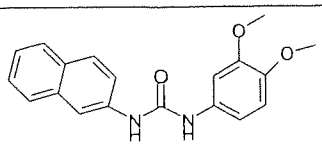
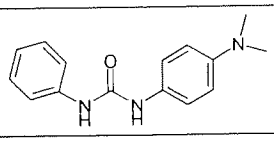
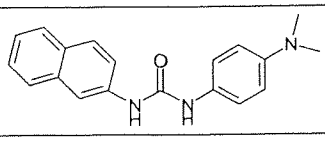
Table 4.2.2: Selection of amines chosen

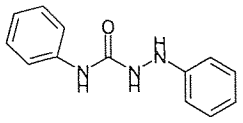
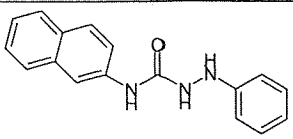
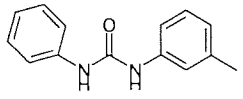
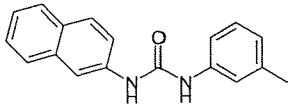
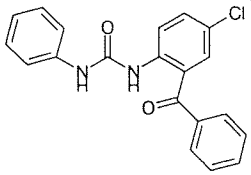
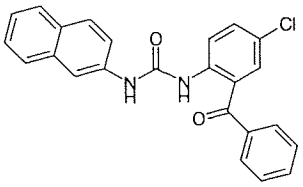
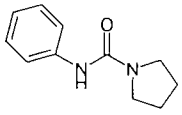
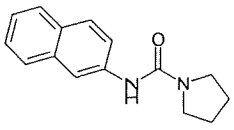
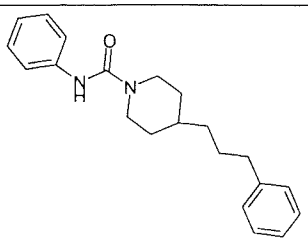
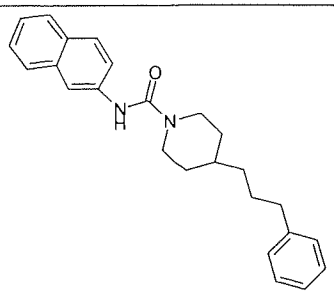
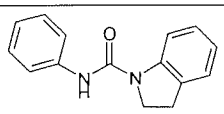
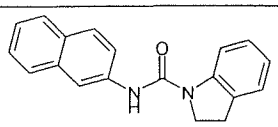
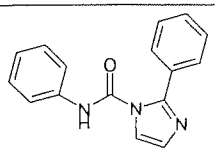
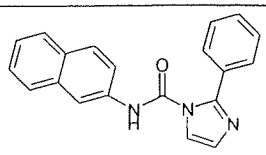
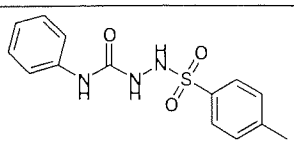
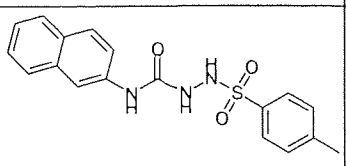
Amines used:

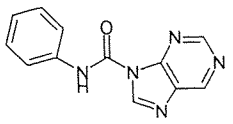
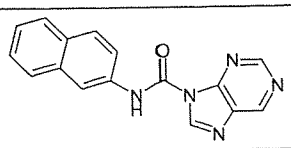
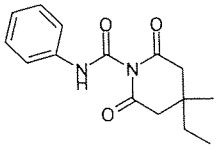
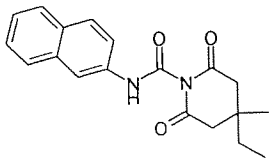
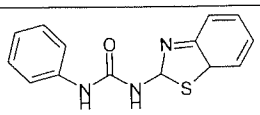
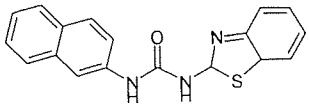
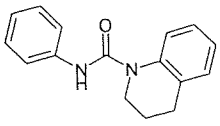
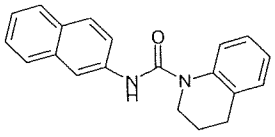
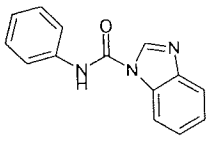
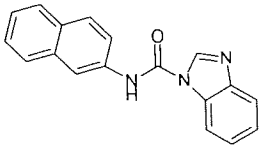
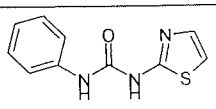
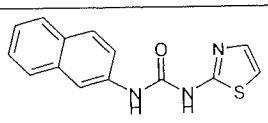
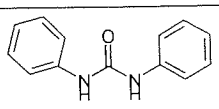
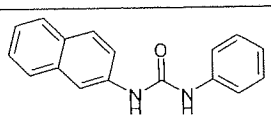
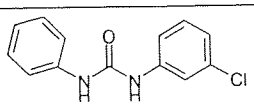
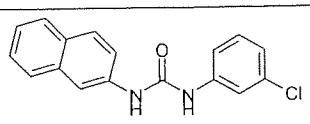
(1) 4-Nitro-phenylhydrazine
(2) 2,4-Dinitro-phenylhydrazine
(3) Imidazole
(4) 3-Amino-1H-1,2,4-triazole
(5) 2-Amino pyrimidine
(6 N-Phenyl-1,4-phenylenediamine
(7) o-Amino-benzonitrile
(8) 4-Amino-antipyrine
(9) Phenethylamine
(10) 1- Benzylpiperazine
(11) 1- Aminonaphthalene
(12) N-methyl-piperazine
(13) 1- Benzylpiperazine
(14) 3-Methylpyrazole
(15) Dibenzylamine
(16) 3,4-Dimethoxyaniline
(17) N,N-Dimethyl-p-phenylenediamine
(18) Phenylhydrazine
(19) o-Toluidine
(20) 2-Amino-5-chlorobenzophenone
(21) Pyrrolidine
(22) 4-(3-Phenyl-propyl)piperidine
(23) Indoline
(24) 2-Phenylimidazole
(25) Toluene-4-sulphonhydrazide
(26) Purine
(27) 3-Ethyl-3-methylglutarimide
(28) 2-Aminobenzothiazole
(29) 1,2,3-4 Tetrahydroquinoline
(30) Benzimidazole
(31) 3-Amino-2-thiazoline
(32) Aniline
(33) 3-Chloroaniline

Table 4.2.3: Structures of the urea analogues

Cpd	R' = Ph, R	MS+H [m/z]	Cpd	R' = Naphthyl, R	MS+H [m/z]
4.1.a		213	4.1.b		313
4.2.a		318	4.2.b		-
4.3.a		-	4.3.b		-
4.4.a		-	4.4.b		-
4.5.a		215	4.5.b		265
4.6.a		304	4.6.b		354
4.7.a		238	4.7.b		-
4.8.a		323	4.8.b		373

4.9.a		241	4.9.b		291
4.10.a		310	4.10.b		360
4.11.a		263	4.11.b		313
4.12.a		220	4.12.b		270
4.13.a		296	4.13.b		346
4.14.a		-	4.14.b		352
4.15.a		317	4.15.b		267
4.16.a		273	4.16.b		323
4.17.a		256	4.17.b		306

4.18.a		228	4.18.b		278
4.19.a		227	4.19.b		277
4.20.a		-	4.20.b		401
4.21.a		191	4.21.b		241
4.22.a		323	4.22.b		373
4.23.a		239	4.23.b		289
4.24.a		-	4.24.b		-
4.25.a		306	4.25.b		356

4.26.a		-	4.26.b		-
4.27.a		-	4.27.b		-
4.28.a		270	4.28.b		320
4.29.a		353	4.29.b		303
4.30.a		-	4.30.b		-
4.31.a		-	4.31.b		-
4.32.a		-	4.32.b		263
4.33.a		-	4.33.b		297

(-) Diphenyl/napthyl urea and/or solubility problems

All urea analogues were initially characterized by MS APCI(+), dissolved in methanol with a few drops of DMSO, because solubility was difficult, when using 100% methanol. It was noted that not all combinations of amines and isocyanate were working. The synthetic yields were generally very high, with most of the urea analogues.

4.3. Biological evaluation

Table 4.3.1: Biological evaluation of urea analogues

Cpd	Yield [%]	IC_{50} CCK_A [μM]	Cpd	Yield [%]	IC_{50} CCK_B [μM]
4.1.a	63	>20	4.1.b	22	>20
4.2.a	71	>20	4.5.b	97	>20
4.5.a	65	>20	4.6.b	70	>20
4.6.a	96	>20	*4.8.b	91	>20
4.7.a	43	>20	4.9.b	76	>20
*4.8.a	94	5	4.10.b	96	>20
4.9.a	72	>20	4.11.b	96	>20
4.10.a	82	>20	4.12.b	48	>20
4.11.a	95	>20	4.13.b	83	>20
4.12.a	17	>20	4.14.b	23	>20
4.13.a	83	>20	4.15.b	68	>20
4.15.a	14	>20	4.16.b	95	>20
4.16.a	73	>20	4.17.b	69	>20
4.17.a	94	>20	4.18.b	91	6
4.18.a	66	7	4.19.b	83	>20
4.19.a	89	>20	4.20.b	97	>20
4.21.a	83	>20	4.21.b	40	6
4.22.a	95	>20	4.22.b	94	6
4.23.a	09	10	4.23.b	60	>20
4.25.a	47	10	4.25.b	75	>20
4.28.a	86	>20	4.28.b	90	>20
4.29.a	34	>20	4.29.b	89	>20
			4.32.b	67	>20
			4.33.b	94	>20

*** = Fully characterised**

All synthesised urea analogues were screened on the CCK_B receptor subtype. From Table 4.3.1, most analogues were judged to be inactive, displaying IC_{50} values of >20 μM. However, compound 4.8.a displayed good binding activity.

4.4. Summary

A small number of selected compounds (Fig. 4.3.2) showed weak to promising CCK_B activity.

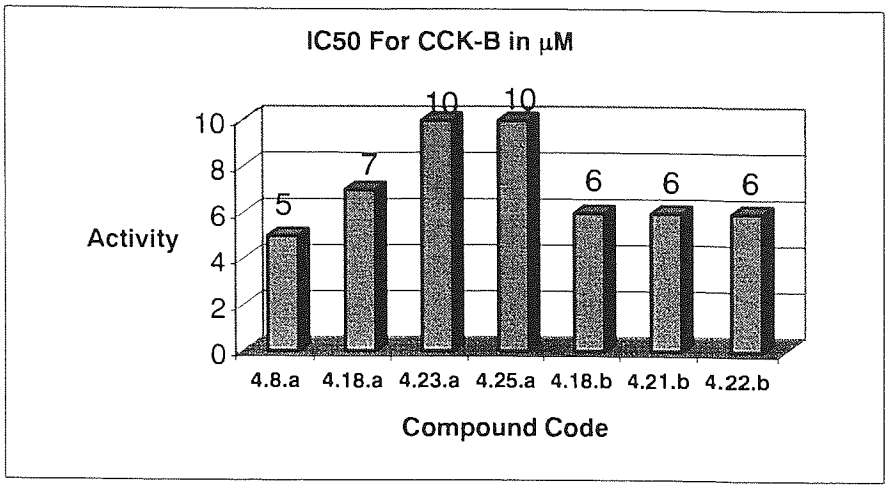


Fig. 4.3.2: Activity of selected ureas

Synthetic yields were generally high, with the methodology being relatively easy. Amines, which were expected to react with the isocyanates but did not, this was probably due to the age of the reactant and the possiblity of water being present. The products were exceptionally pure and did not require further purification.

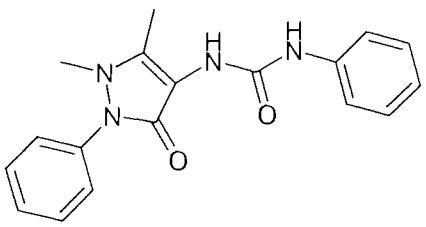


Fig 4.8.a

This higher risk approach has given promising results (Fig. 4.3.2). It has been successful in finding a potential lead template, the amino-antipyrine. Compound **4.8.a** gave an IC_{50} value of 5 μM. Compounds **4.18.a**, **4.23.a,** from the phenyl isocyanate series and **4.18.b** **4.21.b** & **4.22.b** from the napthyl series displayed weak to good activity.

However, these additional leads would not be developed further, as they are less active and judged to be too simple, than the amino-antipyrine derivative. They also show common components of ureido-acetamides, amino acid and dipeptoid derivatives. Repeating the screening procedure with napthyl instead of phenyl, with amino-antipyrine, gave an inactive compound **4.8.b**. It can be observed that the phenyl substituent is more active than the naphthyl. The next step would be to react amino-antipyrine with a series of phenyl analogues, to determine the optimal active group from the series.

Chapter 5. Pyrazole ureido-amide derivatives

5.1 Introduction to pyrazolinones

Traditionally the pyrazolinone template (Fig. 5.1.1) has been used for anti-pyretic, rheumatic and analgesic drugs. It has been suggested[70] that both the pyrazolidinone and diazepine rings provide a scaffold to orient the key aromatic residues and the urea functionality in a similar region, therefore possessing similar potency and selectivity for the CCK_B receptor.

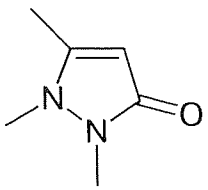


Fig. 5.1.1: Pyrazolinone template

Both the L-365,260 and Lilly's diphenylpyrazolidinones compounds contain several common substructures, despite their independent development. Both series contain the phenyl urea side chains, as well as two other phenyl rings, either pendant from or fused to their respective heterocyclic system. Molecular modeling study[68] has shown the correspondence of several domains within these two series. The low energy conformations predicted by modeling showed three-dimensional homology between their structures. This has led to the hypothesis of a common pharmacophore for the binding to the CCK family of receptors.

After undergoing clinical trials, compound LY288513 was withdrawn due to major adverse effects. Although the lead urea template (Compound **4.8.a**) is similar, it is sufficiently different from compound LY288513 to overcome its side effect. This would provide novel compounds. It was also decided to deviate away from the benzodiazepine template, as much work has been already been carried out. The amino-antipyrine template would hopefully combine the structural features of both series to form a hybrid structure.

5.2. Preparation of pyrazolyl analogues

Compound 4.8.a was found to demonstrate the highest CCK_B antagonist activity from the urea series, from Chapter 4. The next logical approach was to synthesise a series of similar analogues (Fig. 5.2.0.1), where the phenyl urea substituent was modified. The result will enable the most optimum, active group to be deduced. This series of compounds have already been prepared but not screened in any program or fully characterised.

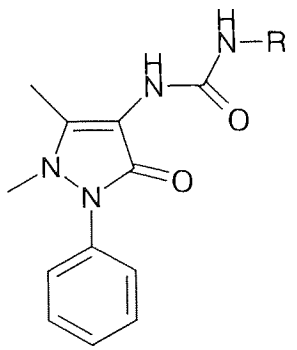
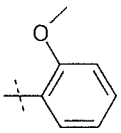
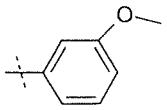
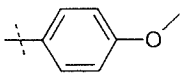
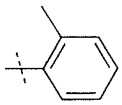
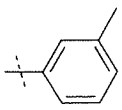
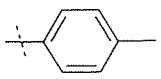
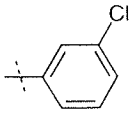
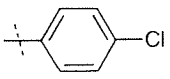
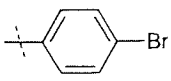
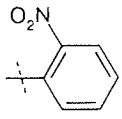
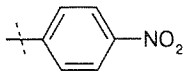
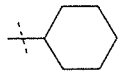


Fig. 5.2.0.1: Optimization of the pyrazolyl template (Table 5.2.1.1)

5.2.1. Structure and biological evaluation of pyrazolyl derivatives

Table 5.2.1.1: Structure and *in vitro* activity of pyrazolyl derivatives

Entry Cpd	Group R	Yield [%]	MW	FW	MS+H [m/z]	IC_{50} CCK_B [μM]
5.2.1		-	-	-	-	-
5.2.2		93	352	$C_{19}H_{20}N_4O_3$	353	>10
5.2.3		97	352	$C_{19}H_{20}N_4O_3$	353	>10
5.2.4		90	336	$C_{19}H_{20}N_4O_2$	337	7
5.2.5		95	336	$C_{19}H_{20}N_4O_2$	337	5
5.2.6		92	336	$C_{19}H_{20}N_4O_2$	337	>20
*5.2.7		89	356	$C_{18}H_{17}ClN_4O_2$	357	>10
5.2.8		75	356	$C_{18}H_{17}ClN_4O_2$	357	2.5
5.2.9		82	401	$C_{18}H_{17}BrN_4O_2$	402	7
5.2.10		84	367	$C_{18}H_{17}N_5O_4$	368	>20

5.2.11		94	367	$C_{18}H_{17}N_5O_4$	368	>20
5.2.12		85	328	$C_{18}H_{24}N_4O_2$	329	>10
5.2.13		78	302	$C_{16}H_{22}N_4O_2$	303	8

*** = Fully characterised**

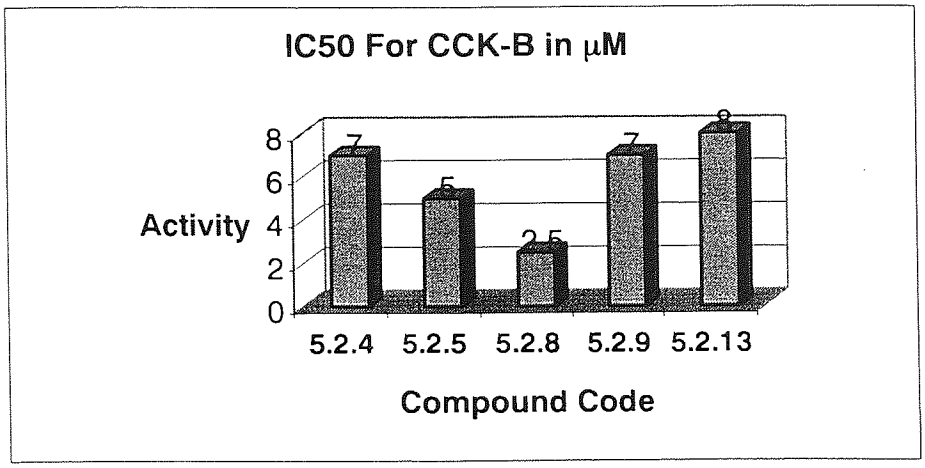


Fig. 5.2.1.2: Comparison of biological activity of selected pyrazolyl derivatives

A number of the pyrazolyl derivatives (Fig. 5.2.1.2) demonstrated encouraging to modest CCK_B activity. The amino-antipyrine compound was reacted further with indolyl-2-carboxylic acid and also with a series of 3-indolyl acids to form an amide linkage, with the aid of the reagent DIC. This would enable the analogues to be evaluated as potential CCK_A antagonists and compare the potency with Devazepide (Table 5.2.2.1).

5.2.2. Structure and biological evaluation of pyrazolylamide derivatives

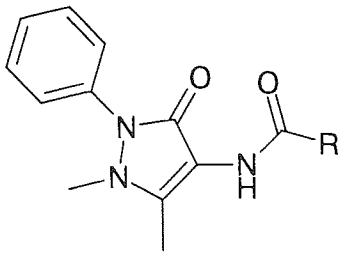
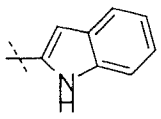
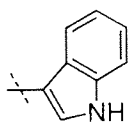
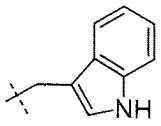
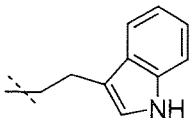
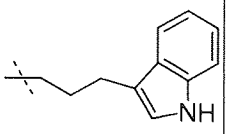


Fig. 5.2.2.1: Structure of pyrazolylamide analogues

The pyrazolylamides series (Fig. 5.2.2.1) were prepared by reacting 4-amino-antipyrine, with 1.25 Eq of the indole acid in the presence of 3 Eq of DIC, in dry acetonitrile. The mixture was heated to 60°C and left overnight. The precipitated crystals were washed and dried with cold acetonitrile and were exceptionally pure. Compounds 5.2.14 and 5.2.17 are reported for the first time, whilst the remaining compounds have been previously prepared.

Table 5.2.2.2: Evaluation of Pyrazol-4yl amide analogues

Entry Cpd	Group R	Yield [%]	MW	FW	MS+H [m/z]	IC_{50} CCK_B [μM]	IC_{50} CCK_A [μM]	Ratio A/B
***5.2.14**		76	346	$C_{20}H_{18}N_4O_2$	347	0.9	0.080	11.3
5.2.15		80	346	$C_{20}H_{18}N_4O_2$	347	15	2	7.5

5.2.16		82	360	$C_{21}H_{20}N_4O_2$	361	9	2	4.5
***5.2.17**		75	374	$C_{22}H_{22}N_4O_2$	375	4	1	4
5.2.18		78	388	$C_{23}H_{24}N_4O_2$	389	20	20	1

*** = Fully characterised**

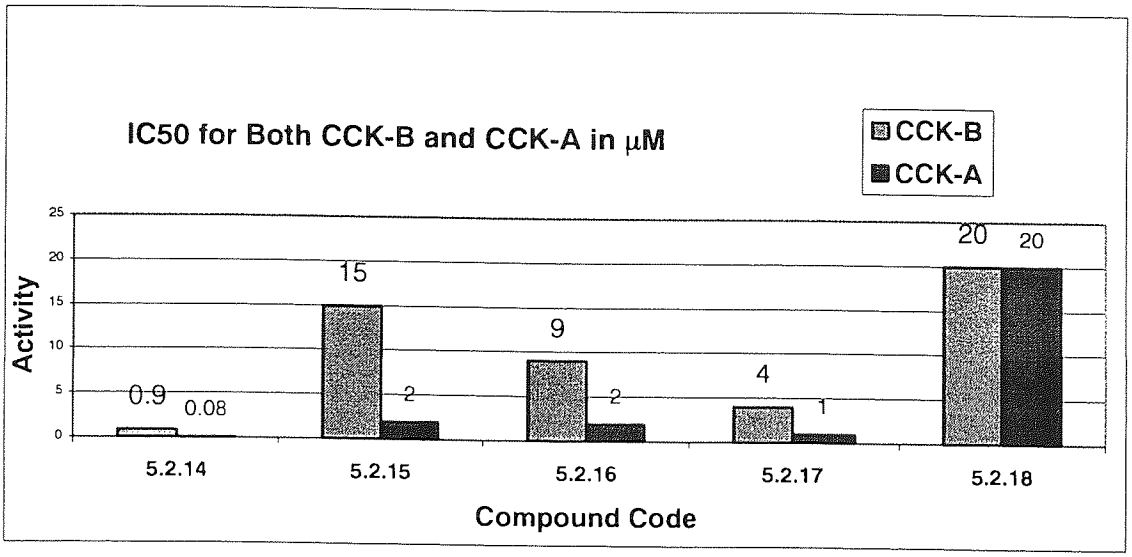


Fig.5.2.2.3: Evaluation of biological activity of pyrazolyl-amide analogues, for both CCK_B and CCK_A activity.

5.3. Summary

The synthetic method was relatively simple and straightforward, in synthesising high yield, active urea and amide analogues (Fig. 5.2.1.2 & 5.2.2.3). Compound 5.2.8, from the urea series demonstrated the highest activity, a para-chlorine substitutent, while the meta positioned chlorine showed no activity. Both the ortho and meta toluidines showed modest activity, while the para group and the nitro groups showed no activity. Compound

5.2.14, a 2-indolyl pyrazolyl-amide showed the highest activity for both CCK_B and CCK_A, 900 & 80 nM respectively, with a good selectivity ratio of 11.3. The remaining 3-indolyl series exhibited low micromolar activity towards the CCK_A receptor, whilst compound 5.2.18, with a butyl group spacer, showed no activity for either receptor subtype.

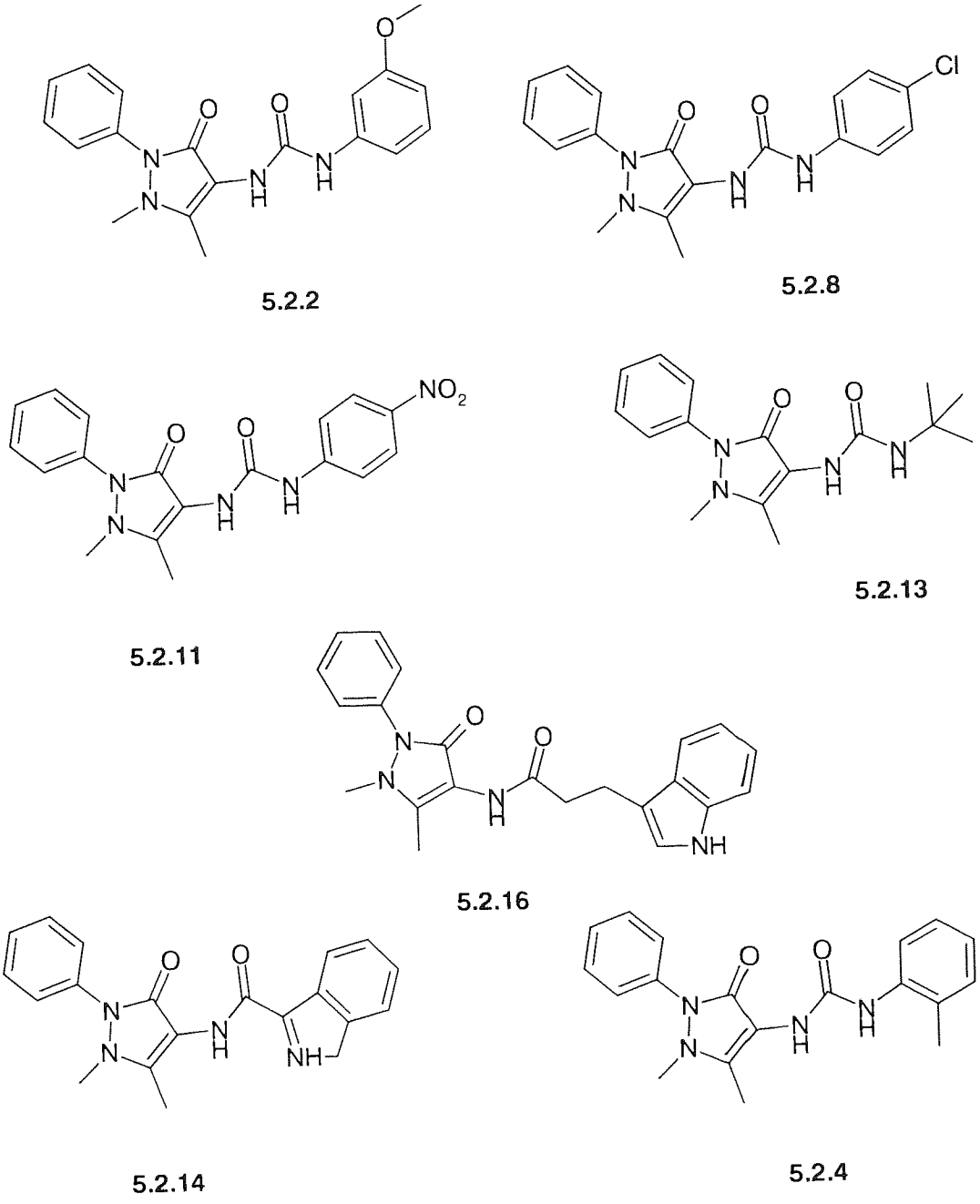


Fig. 5.3.1: Selected structures of both pyrazole ureido and amide derivatives

5.4. Optimisation of the pyrazolyl template

From the previous findings compound 5.2.8 was found to be the most active antagonist of the series but not very potent. It was decided that a diphenyl system would be a more active in trying mimic the benzodiazepine type system. The diphenyl-2,3-dihydro-1H-pyrazol-4-yl template was synthesised to assess this hypothesis.

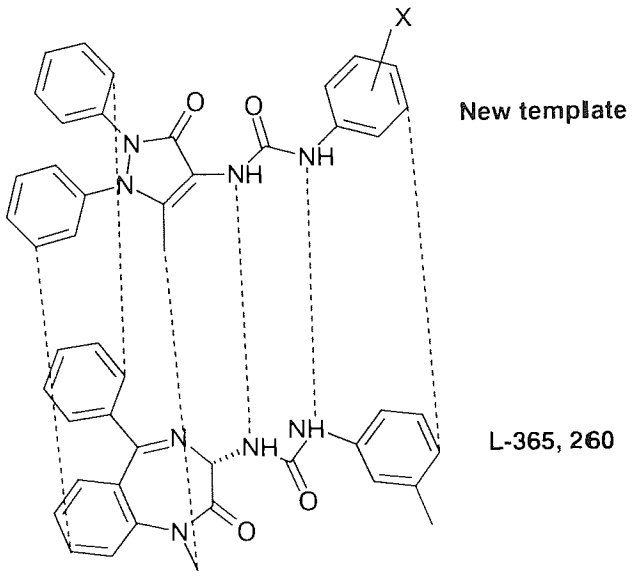
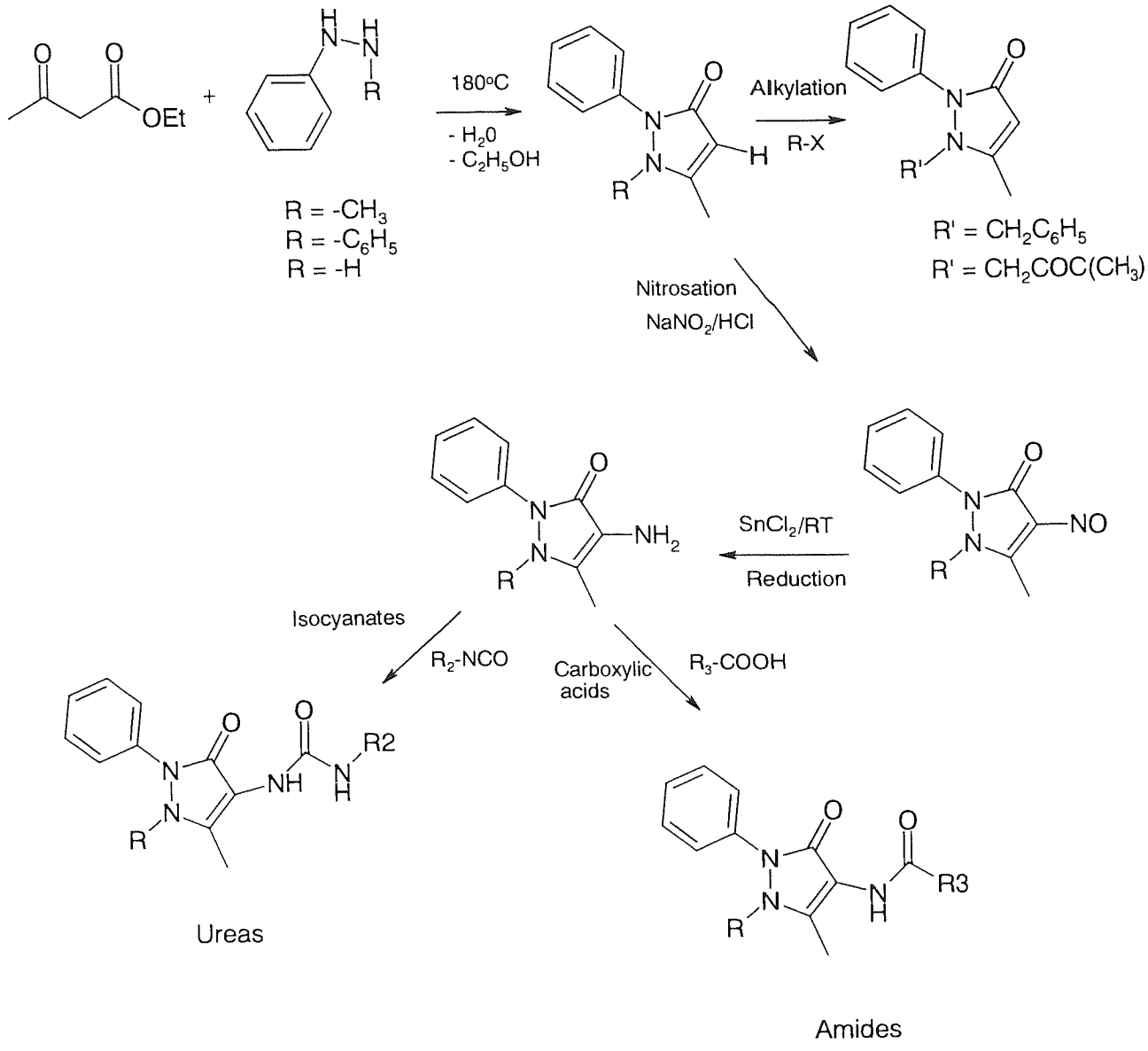


Fig. 5.4.1: Common structural features present in the new template and L-365, 260

Investigation of this novel class of pyrazolyl template is extremely attractive, in view of combining the high flexibility in the substitution arrangement and the straight-forward synthesis.

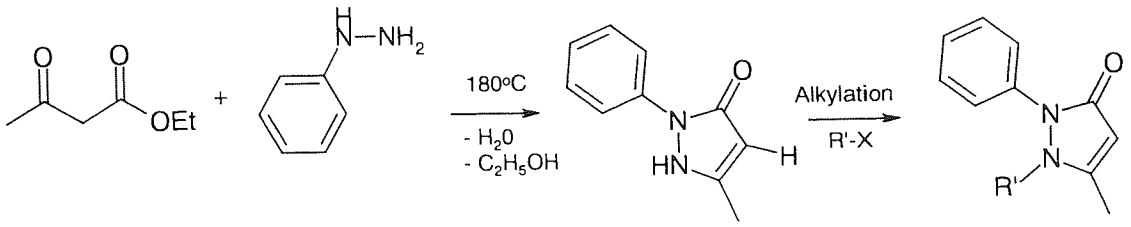
Scheme 5.4.2: An synthetic overview of the new reaction pathway to be undertaken

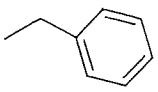
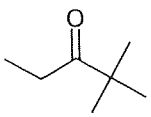


An synthetic overview of the various chemical transformation and reactions to be undertaken, to synthesize novel and potentially potent "Ureas" and "Amides".

5.5. Preparation of 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one

The phenazone ring structure[153] was built up from ethylacetoacetate and phenylhydrazine at 180°C. Then alkylation of the remaining free NH-group gave compounds 5.4.3a & 5.4.3b. A reason for investigating this approach was to assess if this method was easier to attach a subsituent directly onto the nitrogen.



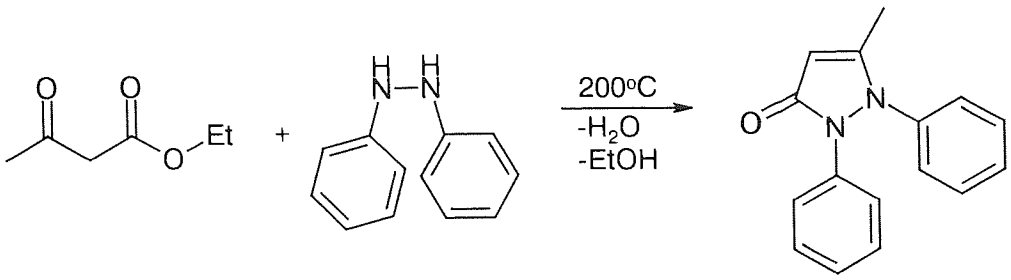
R'	Yield [%]
	37
	42

Scheme 5.5.0.1: Synthesis of pyrazolinone precursor

Alkylation was achieved by a suspension of NaH, in mineral oil, under inert conditions. The appropriate alkylating agent was added at RT, left for 35 mins, then washed with brine and extracted with AcOEt. Column chromatography isolated the pure product. Both benzyl bromide and chloropinolone were good examples, the latter giving a higher yield, despite the bromine being a better leaving group than the chlorine. The benzyl bromide (alkylated product) being already synthesized previously. Phenyl chloride did not react, this was probably due to the electrons in the aromatic ring being stabilized. The alkylation method would not be viable in a larger scale synthetic method to prepare pyrazolinone derivatives.

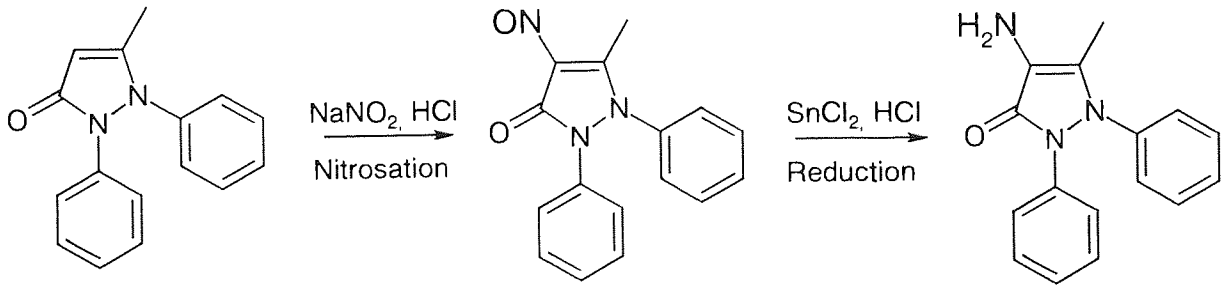
Diphenyl-pyrazolone was synthesised according to the reference[154], where the diphenyl-hydrazine was reacted with neat acetic acid ester at 130-150°C under a Dean Stark Trap,

for 2 hrs and then at 180°C for 1.5 hrs. The remaining mixture was distilled off at 230-250°C to remove any unreacted starting material. The method was modified from the literature[154] by increasing the reaction times and also by using a powerful pump to minimize decomposition of the product. For safety reasons, sand was used instead of oil because of the high temperatures involved. Several recrystallisations of the brown solid, from toluene, instead of benzene, gave white crystals, which were subsequently filtered, washed and dried.



Scheme 5.5.0.2: Formation of 5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one

The next step was to nitrosate the 4-position (Scheme 5.5.0.3). This was achieved using conc HCl with sodium nitrite, in water, at 0°C. After 30 mins a precipitate had formed which was filtered and washed immediately with water. Leaving the mixture to stand causes decomposition of the unstable product, a colour change can be observed, from green to brown to black.



Scheme 5.5.0.3: Nitrosation and reduction to give 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one

The nitro-diphenyl-pyrazolone was dissolved in ethanol, while separately a mixture of tin chloride in 20% conc HCl was heated to 90°C. Once dissolved the mixture was poured carefully into the alcoholic solution and allowed to cool to RT. Concentrated ammonia solution was added until no further precipitate formed. It was found that leaving the precipitate overnight increased the yield greatly and minimized the reactants left over.

The mixture was filtered and extracted several times with EtOH. The ethanol was removed in vacuo to give a mixture of product and $Sn(OH)_2$, a yellow solid by-product. The product was dissolved in acetonitrile, while the tin hydroxide remained undissolved, filtration and removal of acetonitrile in vacuo gave bright yellow crystals, with a low R_f value. 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one was reacted in a similar way as with 4-amino-antipyrine to construct novel urea and amide analogues.

5.5.1. Evaluation of pyrazolinone analogues

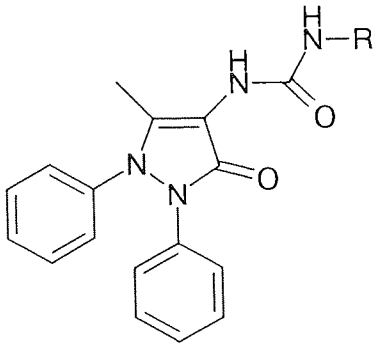
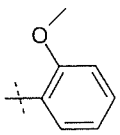
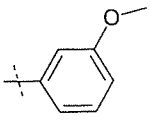
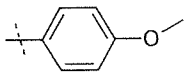
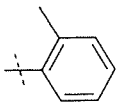
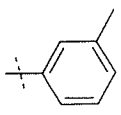
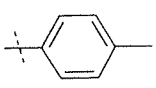
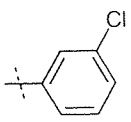
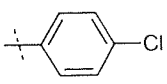
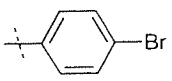
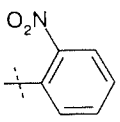
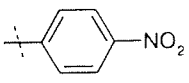


Fig. 5.5.1.1: General structure of the diphenylpyrazol-3-one analogues (Table 5.5.1.2)

All compounds were initially examined by APCI(+) mass spectroscopy analysis. Products were judged pure and screened for initial CCK_B activity. Activity for the diphenylpyrazol-3-one analogues was mixed, with most being inactive, except 3-methoxy & 3-methyl analogues (5.5.2 & 5.5.5). These compounds demonstrated very good binding activity and were further screened on the CCK_A receptor, where they were equally potent.

Table 5.5.1.2: Structure and *in vitro* activity of pyrazolinone derivatives

Entry Cpd	Group R	Yield [%]	MW	FW	MS+H [m/z]	IC_{50} CCK_B [µM]	IC_{50} CCK_A [µM]
5.5.1		-	-	-	-	-	-
***5.5.2**		67	414	$C_{24}H_{22}N_4O_3$	415	0.035	0.010
5.5.3		57	414	$C_{24}H_{22}N_4O_3$	415	>20	-
5.5.4		88	398	$C_{24}H_{22}N_4O_2$	399	>20	-
***5.5.5**		91	398	$C_{24}H_{22}N_4O_2$	399	0.025	0.020
5.5.6		89	398	$C_{24}H_{22}N_4O_2$	399	>20	-
***5.5.7**		73	418	$C_{23}H_{19}ClN_4O_2$	419	>20	-
5.5.8		81	418	$C_{23}H_{19}ClN_4O_2$	419	>20	-
***5.5.9**		92	463	$C_{23}H_{19}BrN_4O_2$	464	>20	-
5.5.10		65	430	$C_{23}H_{19}N_5O_4$	431	7.5	-
***5.5.11**		77	430	$C_{23}H_{19}N_5O_4$	431	>20	-

*5.5.12		91	384	$C_{23}H_{20}N_4O_2$	385	3	-
5.5.13		75	434	$C_{27}H_{22}N_4O_2$	435	>20	-
*5.5.14		86	390	$C_{23}H_{23}N_4O_2$	391	0.85	-
5.5.15		60	364	$C_{21}H_{24}N_4O_2$	365	1	-

*** = Fully characterised**

- = Data not available

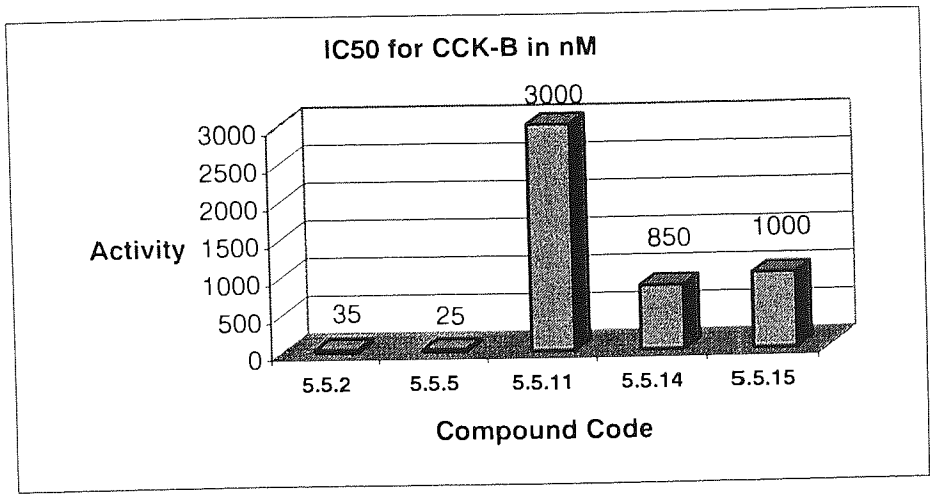


Fig. 5.5.1.3: Comparison of biological activity, of selected pyrazolinone derivatives

5.5.2. Biological evaluation of pyrazolinone-amide analogues

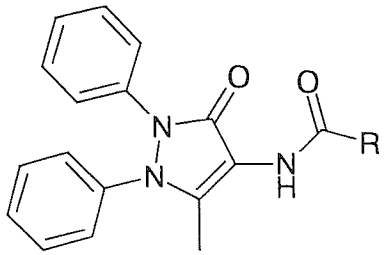
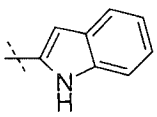
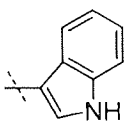
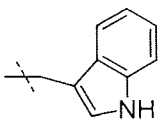
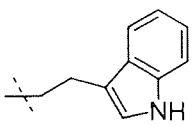
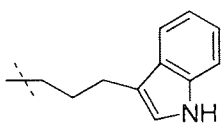


Fig. 5.5.2: General structure of pyrazolinone-amide analogues (Table 5.5.2.2)

Entry Cpd	Group R	Yield [%]	MW	FW	MS+H [m/z]	IC_{50} CCK_B [μM]	IC_{50} CCK_A [μM]	Ratio A/B
***5.5.16**		65	408	$C_{25}H_{20}N_4O_2$	409	0.03	0.020	1.5
***5.5.17**		78	408	$C_{25}H_{20}N_4O_2$	409	3.5	2	1.8
***5.5.18**		66	422	$C_{26}H_{22}N_4O_2$	423	2.5	0.020	125
***5.5.19**		79	436	$C_{27}H_{24}N_4O_2$	437	2	0.025	80
***5.5.20**		80	450	$C_{28}H_{26}N_4O_2$	451	20	20	1

*** = Fully characterised**

Table 5.5.2.2: Evaluation of pyrazolinone-amide analogues

The diphenylpyrazolylamides series (Fig. 5.5.2.2) were prepared similarly to the pyrazolylamides series. 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one was reacted with 1.25 Eq of the indole acid in the presence of 3 Eq of DIC, in dry acetonitrile. The mixture was heated to 60°C and left overnight. The precipitated crystals were washed and dried with cold acetonitrile and were exceptionally pure for analysis.

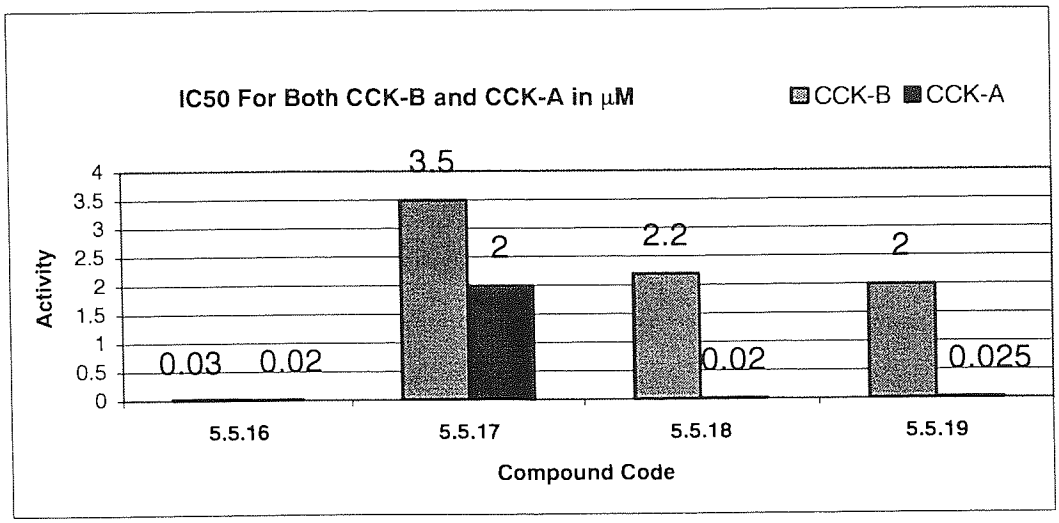


Fig. 5.5.2.3: Evaluation of biological activity of pyrazolinone-amide analogues, for both CCK_B and CCK_A Activity.

5.6. Summary

Template precursors showed no CCK activity. High purity of reactants was essential for reactivity, lower grade diphenylhydrazine distilled out unreacted. The o-methoxyphenyl isocyanate was nonworking for both series. Synthetic yields were overall good but much higher when the amide analogues were formed. Isocyanates, that were readily available at a reasonable cost, were chosen to give a comprehensive SAR Both the diphenyl-pyrazolinone ureido and amide derivatives were much more active (Fig. 5.5.1.3 & Fig. 5.5.2.3) than the single phenyl template. Compound 5.5.5, a meta toluidine substitutent

the highest CCK_B activity of 25 nM, while 20 nM towards the CCK_A receptor. However, ortho and para toluidine groups showed no activity. Compound 5.5.2, a meta methoxy group demonstrated the highest CCK_A at 10 nM, with 35 nM towards the CCK_B receptor. In general all para- substitutents were inactive with IC_{50}'s of >20 μM. Even compound 5.5.8 (para-chlorine group) showed no activity, unlike in the first series. Cyclohexyl and t-butyl groups exhibited modest activity of ~1 μM.

Compound 5.5.16, a 2-indolyl pyrazolinone-amide was the most active structure for both receptor subtypes at around 20 nM. However, the compound showed extremely poor solubility while screening it. It was therefore not possible to obtain both a proton or a carbon NMR. Compounds 5.5.18 & 5.5.19, 3-indolyl amides, with 1 & 2 carbon spacers, were equally active towards the CCK_A receptor but with a low μM activity towards the (B) receptor. Receptor selectivity was extremely good, showing values of 125 & 80 respectively. Compound 5.5.20, with 3 carbon spacers showed no activity.

It can be concluded that the diphenyl template has been very successful in increasing activity (Fig. 5.5.1.3 & 5.5.2.3), trying to mimic the aromatic regions of the benzodiazepine system. Highly potent compounds have been synthesised, with the further potential of modification, making them selective targets for drug treatment. It was decided that the most active CCK_B compound (5.5.5) be further evaluated with a general *in vivo* study.

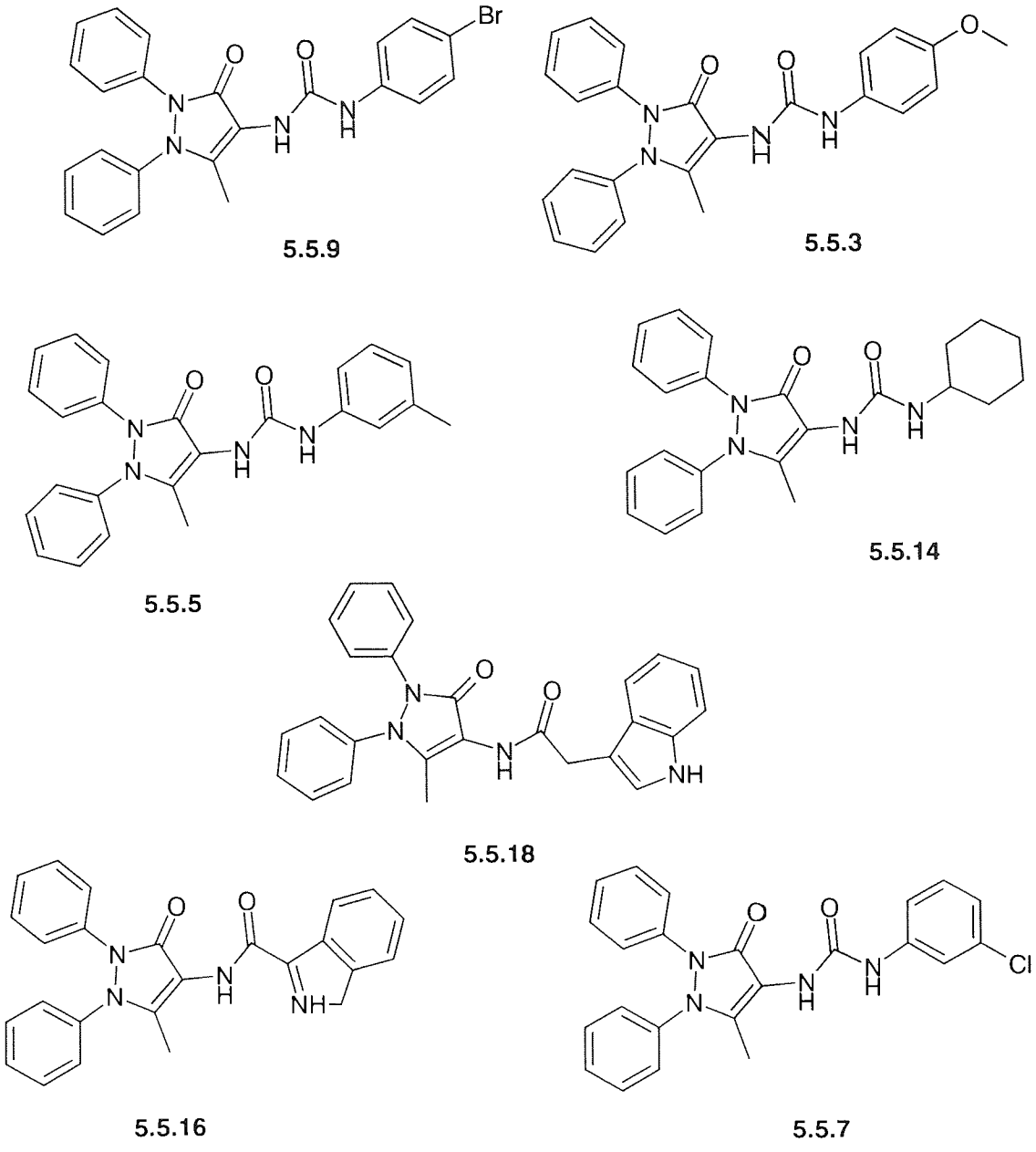


Fig. 5.6.1: Selected structures of diphenyl-pyrazolinone ureido and amide derivatives

5.7. Animal Studies (*in vivo* screening) on Compound 5.5.5

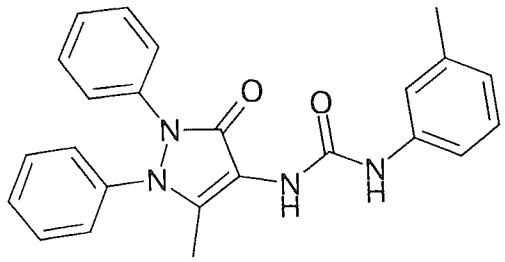


Fig. 5.7.0.1: Structure of compound 5.5.5

It was decided to screen compound 5.5.5 further, in animal studies at Khon Kaen University in Thailand. Associate Professor J. Sattayasai's research group kindly provided all the data, each result was repeated three times, with groups of 7 adult mice and then the results averaged, Morphine being as a Standard drug. Pain, depression, anxiety and neuroleptic potential were all evaluated using an X-Maze & Black and White Test (anxiety), Immobility Time Test (depression), Tail Flick & Hot Plate Test (pain) and Climbing Score Test (neuroleptic).

5.7.1. Elevated plus maze test (X-maze)

This test[155] has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds have the effect of decreasing anxiety and therefore increase the open arm exploration of the mice[156]. Anxiogenic compounds have the opposite effect.

5.7.2. Anti-anxiety test (black and white test)

This simple behaviour model[157] detects compounds with anxiolytic effects. Mice tend to explore a novel environment but retreat from the aversive properties of a brightly lit open field. In a two-chambered system, mice can freely move between a brightly lit open field and a dark corner, animals show more crossings between the two chambers and more loco motor activity after treatment with anxiolytics.

5.7.3. Despair swim test (Immobility time test)

It has been suggested[155] that rodents forced to swim in a restricted space, from which they cannot escape are induced to the characteristic behavior of immobility[158]. This reflects a state of despair, which can be reduced by several agents, which are therapeutically effective in human depression.

5.7.4. Tail immersion test (tail flick test)

This procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal effect in mice[159], induced by immersing the end of the tail in warm water of 55°C[160].

5.7.5. Hot plate method

The paws of mice are very sensitive to heat at temperatures, which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicyclic acid or phenyl-acetic

type do not generally affect these responses. The method was originally described by Woolfe and MacDonald[161].

5.7.6. Climbing in mice (climbing test)

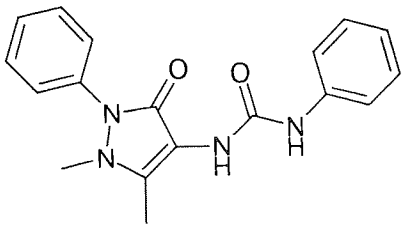
Administration of morphine to mice results in a peculiar climbing behavior characterized, initially by rearing and the full-climbing activity[162], predominantly mediated by the mesolimbic dopamine system. The ability of a drug to antagonize morphine-induced climbing behavior in the mouse has been correlated with neuroleptic potential.

5.8. Summary

The test compound (5.5.5) has been fully evaluated in the four main animal test studies, with further work on the dosage range needed on the active studies. The test drug exhibited potential as a new neuroleptic and as an antidepressant drug. Disappointingly the compound showed no anti-anxiety potential in both test methods (X-maze & Black and White).

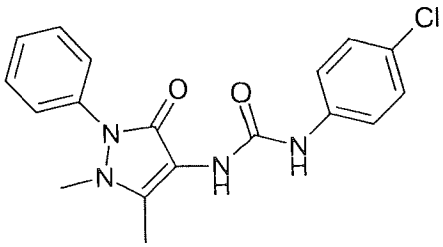
5.9. Further work

Fig. 5.9.1: Compound overview



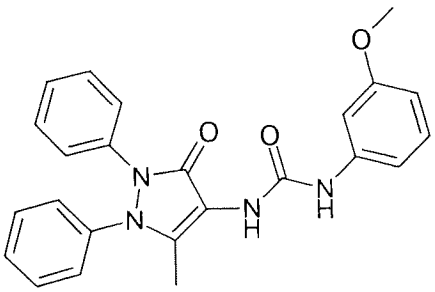
4.8.a

Initial compound found from the urea analogues.

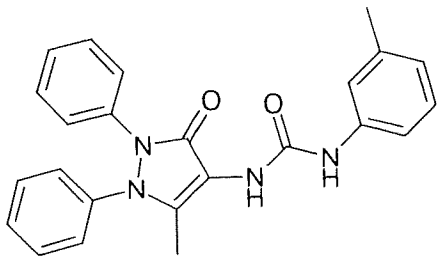


5.2.8

Optimised from the initial compound.



Replacement of the methyl with A phenyl ring enhances activity.



Most active compound from series.

A series[163] based on a 1,5-benzodiazepin-2,4-dione skeleton, possessing a C-3 ureido substituent and a N-1 admamantymethyl group has been shown to be a potent and CCK_B selective antagonist. The bulky methyl substituent present in compound 5.5.5 can be replaced with a ketone group, to form a 2,4-dione system, which has been shown to be active, in the seven-membered benzodiazepine system.

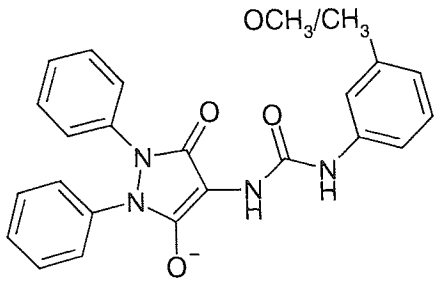
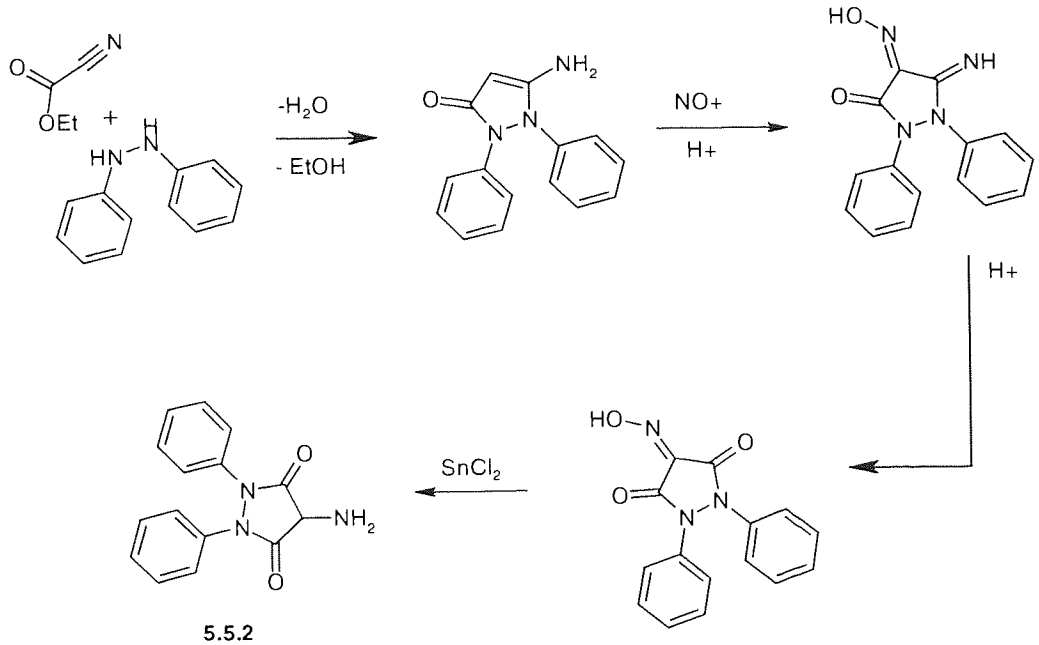


Fig. 5.9.2: Proposed new template

Attempts to react diethylamino malonate with 1,2-diphenylhydrazine were unsuccessful, due to solubility problems of the diethylamino malonate. The use of diethylacetamido malonate improved solubility and eliminated bi-products associated with the unprotected amino group. The acetyl group could be subsequently cleaved under basic/acidic conditions. This approach only gave trace amounts of the product, by MS and therefore a new approach has to be devised (Scheme 5.9.3).



Scheme 5.9.3: Proposed new synthetic approach[164]

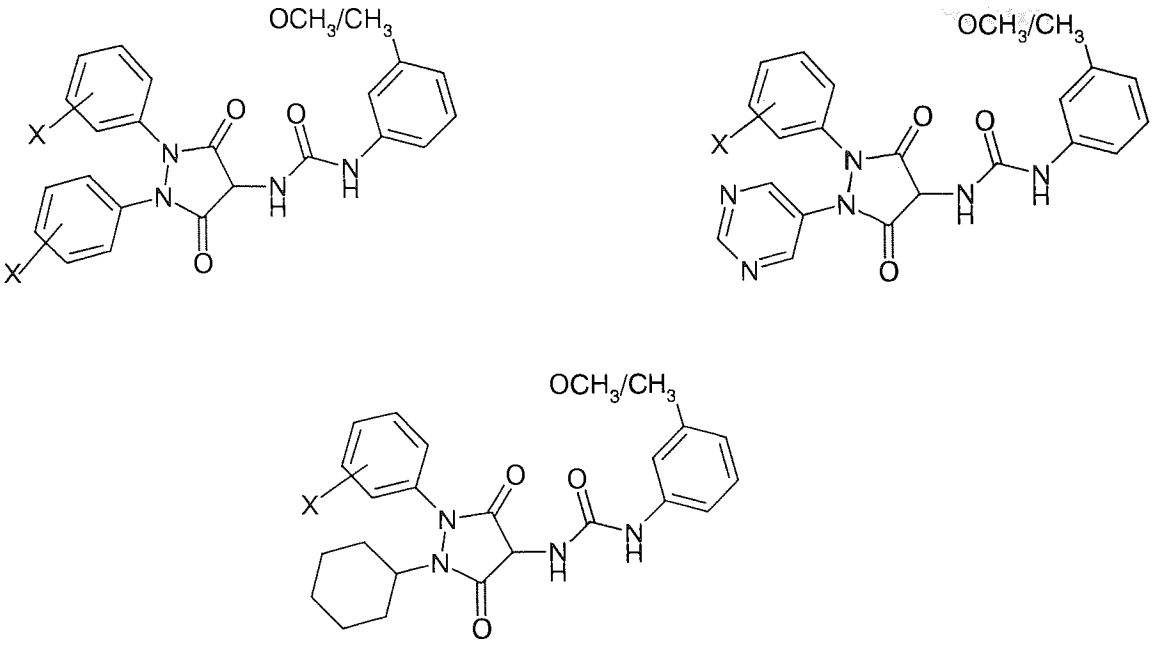


Fig. 5.9.4: Potential compounds, which may provide an more potent ligand, with good bioavailability

The m-toluidine substituent can be further optimised, by introducing heterocyclic rings and charged ions, to enhance both solubility and potency.

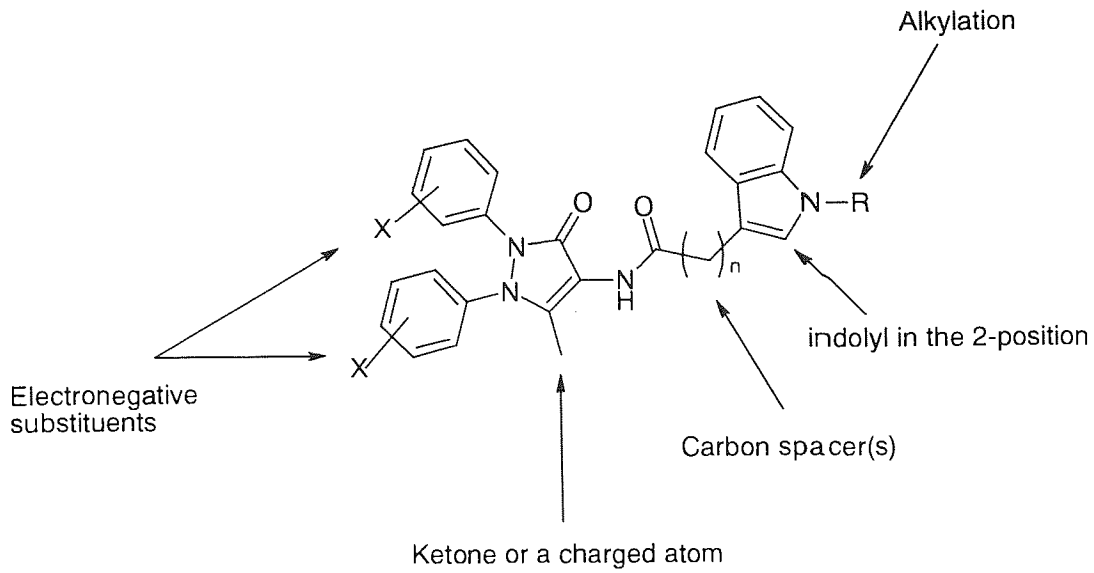


Fig. 5.9.5: Possibilities of further optimisation of the pyrazolinone-amide analogues

It has been demonstrated that the 2-indolyl amides are highly potent in both series, especially in the diphenyl template. The next logical step would be to synthesise a series of 2-indolyl amides with various carbon spacers, between the indolyl ring and the template (Fig. 5.9.5). The 2-indolyl-acid reagents would have to be synthesised, as they are not commercially available. Alkylation of the indolyl group could be further investigated, as to whether biological activity is enhanced or decreased. Any lead compound would be a racemic mixture and separation of each enantiomer may potentially be more selective over either sub-receptor.

Chapter 6. Formation of furan-2(5)-one building blocks

6.1. Introduction to furan-2(5)-ones

Intensive investigations were performed on the chemistry of mucochloric acid, from 1880 to 1905 by Hill and Simonis[165], but was not again studied further until the 1950's by Mowry. Mowry[166] stated that mucochloric acid is thought to be in the half aldehyde state of dichloromaleic acid and is thought to exist in the open and closed ring forms (Scheme 6.1.1)

Scheme 6.1.1: The two forms of mucochloric acid.

Two naming systems are mainly used, based on the two core names, furanone and butenolide. The term furanone is preferred of the two, however, butenolide was used first back in 1898 by Klobb[166]. In recent years there has been much interest focused on furan-2(5)-ones because of their wide occurrence in a variety of biologically active products and their use as valuable synthetic intermediates[167,168,169].

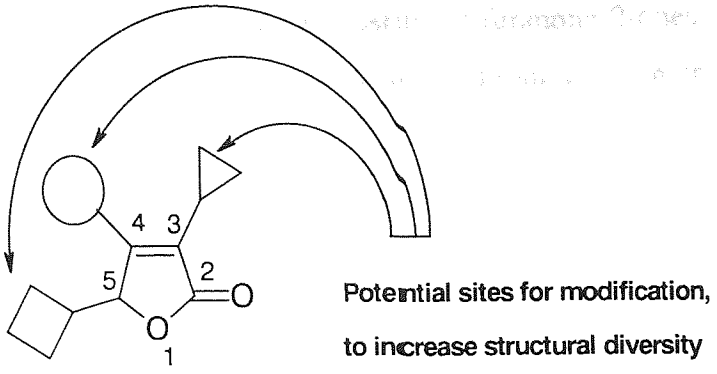


Fig. 6.1.2: Sites of modification in the 5H-furan-2-one structure

Mucochloric acid, is derived from furfural, which is obtained from biomass. It is a relatively inexpensive, readily available compound. The bromo-compounds, although known, do not appear to be used by organic chemists, probably due to the toxicity of the dibrom functionality. The aim of this investigation seeks to establish a variety of synthetic methods for the construction of more diverse, more functionalised 2-(5)-furanone molecules, starting from the mucochloric acid building block. Reactions can be perfomed on the open and closed forms of mucochloric acid, in order to synthesise a large range of 2-(5)-furanones bearing different groups.

Once the 5-subsituted-furanones have been formed, nucleophiles containing nitrogen can be reacted at the 4-position. It was noted these reactions proceed in good yields and the products were relatively stable and easy to isolate. Initial work has been performed in Christopher Langley's Ph.D. dissertation[170], which found antibiotic activities but no CCK activity. However, only a small selected number of compounds were screened on the CCK_B receptor. There is still the potential of developing a potential CCK antagonist, in this higher risk approach, with the careful selection of structurally diverse amines to react at the 4-position.

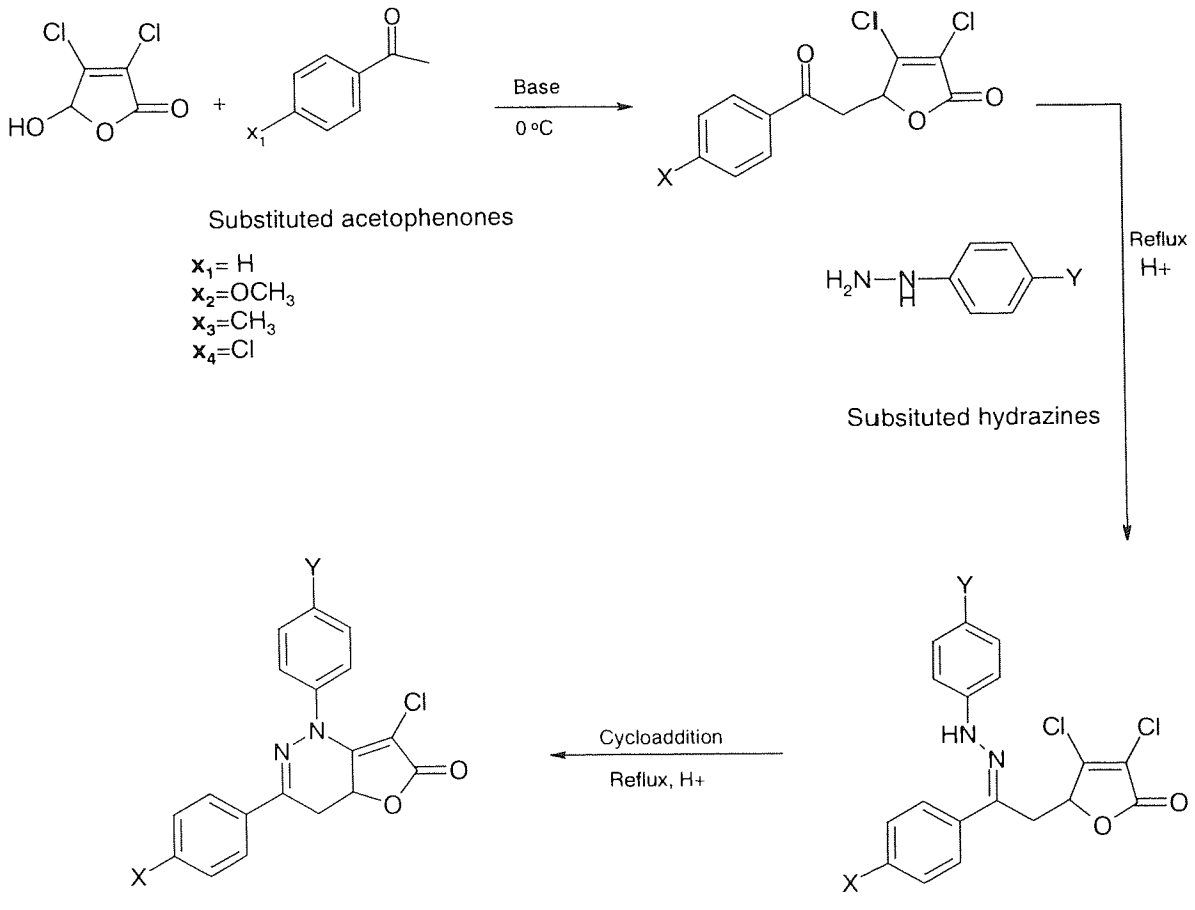
Due to the expertise of the research group in the field of 5-substituted-furanone-2-ones, new and existing building blocks can be used to derive novel 4-amino-furanones, which could be potentially more potent and selective CCK receptor antagonists.

The reactions that mucochloric acid undergoes can be divided into two sets, depending on the two extreme forms in which the molecule is thought to exist. Mowry divided the different reactions that occur in the molecule, into two groups;

(i) Reactions of the aldehyde group and
(ii) Reactions of the pseudo-acid group

6.2. Reactions of the aldehyde group

6.2.1. Preparation of 3,4-dichloro-5-[2-(4-subsituted-phenyl)-2-oxoethyl] furan-2(5H)-ones and subsequent synthesis to pyridazinone furanones

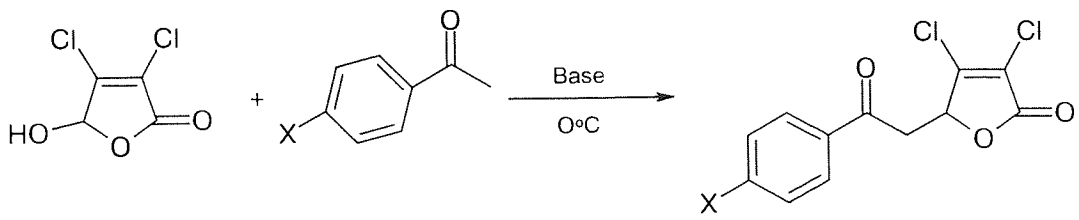


Scheme 6.2.1: Synthesis of novel, cyclized and non-cyclized pyridazinone-furanone derivatives

6.2.2. Condensation (aldol) reactions

Mowry[166] investigated condensation reactions of mucochloric acid with compounds containing a reactive methylene and hydrogens α to a carbonyl, nitro or a cyano group. This was achieved in a cold alkaline solution to form 3,4-dichloro-(2)-furanones substituted in the 5-position. Attempts to react the aldehyde moiety of mucochloric acid under less basic or acidic conditions were successful. This working approach, with acetophenone, was expanded further to react with substituted acetophenones. This produced novel 3,4-dichloro-(2)-furanones.

Scheme 6.2.2.1: Synthesis of 3,4-dichloro-5-[2-(sub-phenyl)-2-oxoethyl]furan-2(5H)-ones



Compound	Group	Solvent	Yield [%]
6.2.1	X = H	Methanol	46.5
6.2.2	X = OCH_3	Methanol	17.5
6.2.3	X = CH_3	Propan-2-ol	42.8
6.2.4	X = Cl	Propan-2-ol	70.9

Mucochloric acid (Scheme 6.2.2.1) with the appropriate substituted acetophenone was dissolved in methanol/propan-2-ol, cooled to 0°C and the base added, dropwise. The solution was allowed to stand for at least 3 hrs and then poured into ice-water containing an excess of HCl conc. An oily precipitate formed for compound 6.2.1, while a solid

formed for the remainder. All precipitates were recrystallised from ethanol. Reacting nitro-acetophenone was non-working because of solubility problems. It was not soluble in water, ethanol, methanol, propan-2-ol, DCM or aceotonitrile. It was observed that a deviation away from methanol, a very polar solvent system towards propan-2-ol, less polar solvent enabled products to form in good yields.

6.2.3. 3,4-Dichloro-5-[2-(4-substituted-phenyl)-2-oxoethyl]furan-2(5H)-ones derivatives (bicyclic furanones)

To further investigate the reactions on the ketone functionality of 3,4-dichloro-5-[2-(sub-phenyl)-2-oxoethyl]furan-2(5H)-ones, the four building blocks were reacted with a number of different hydrazines. Refluxing the appropriate nucleophile in ethanol, with drops of conc sulphuric acid, over 18-20 hrs allowed imines to initially form with only aromatic hydrazines. Depending on the phenyl hydrazine substitutent, an additional cyclization occurred to form a novel series of bicyclic, six membered rings, which precipitated out of solution on cooling (Scheme 6.2.3.1). This was not observed for the nitro-phenylhydrazines. This was probably due to the nitro group being strongly electron withdrawing, causing the compounds to precipitate out of solution in the first step. Sulphuric acid was the ideal acidic media, as it tended to bind with the water formed in the reaction.

Hydrazines used in this working approach

(A) Phenylhydrazine

(B) p-Tolylhydrazine.HCl

(C) 4-Chlorophenylhydrazine.HCl

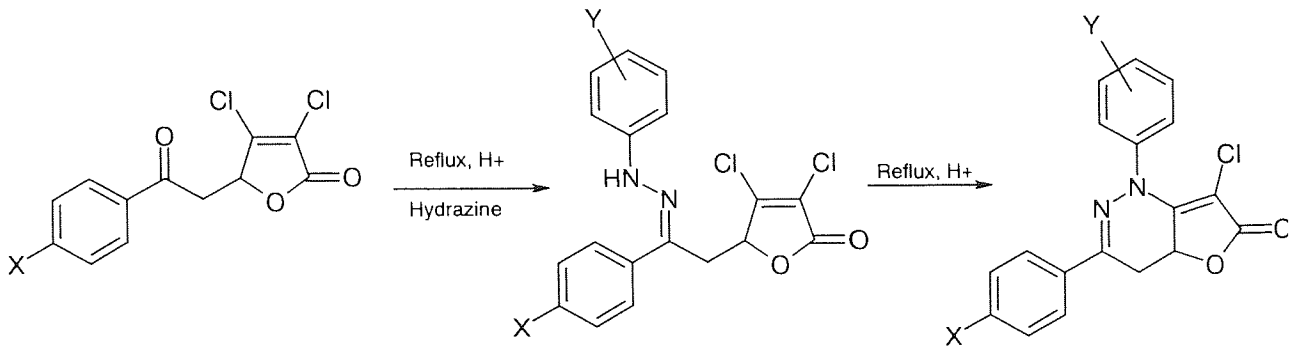
(D) 4-Methoxyphenylhydrazine.HCl

(E) 2,4-Dinitrophenylhydrazine.HCl

(E) 4-Nitrophenylhydrazine.HCl

Reactions with non-aromatic hydrazines, semicarbazides or hydroxylamine were non-working. Diamines were also reacted to form 5,6,7 and 8 membered rings. This ring expansion approach was also not working.

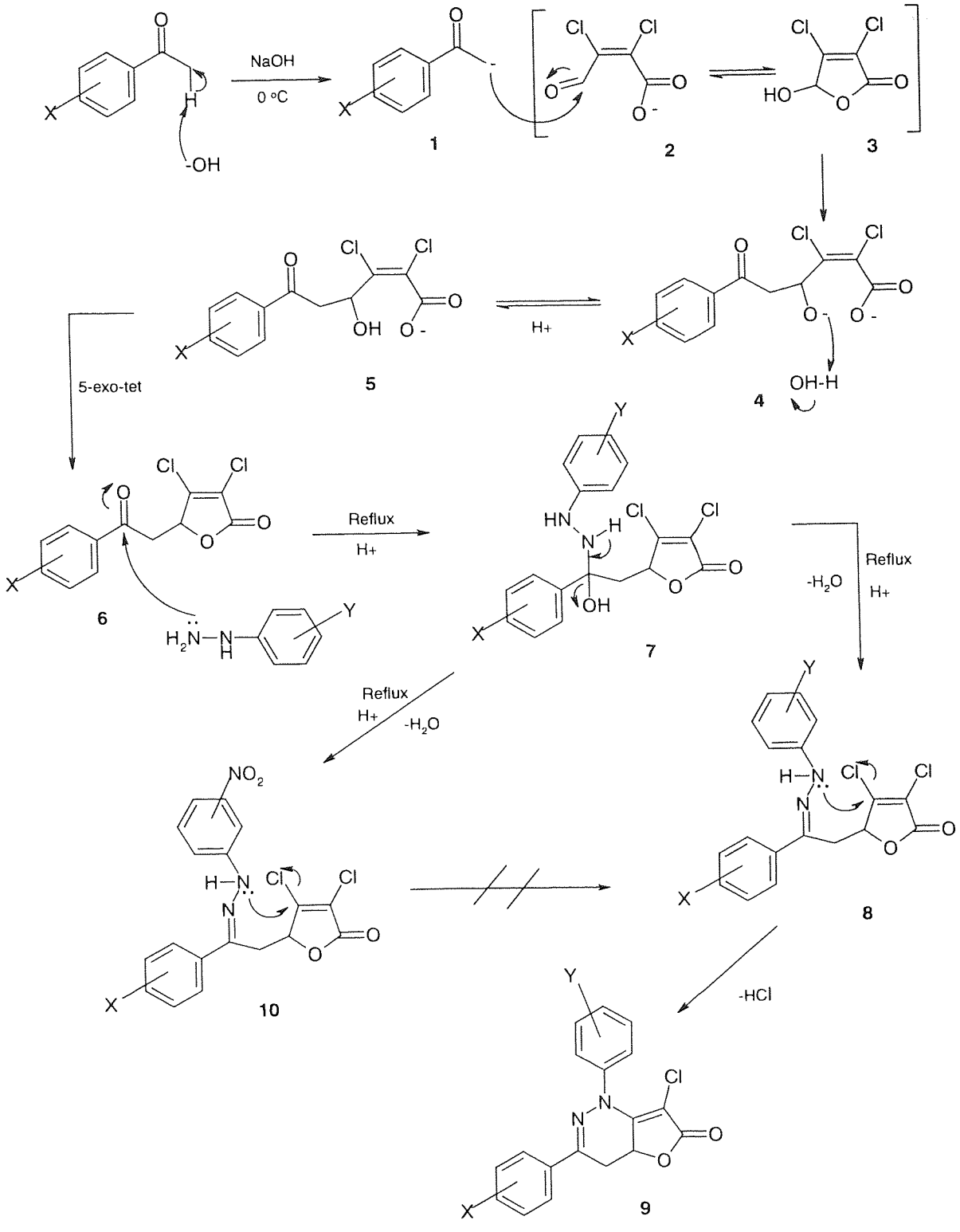
Reaction yields were rather low for the bicyclic products, after recrystallisation with EtOH, whilst the uncyclized products were formed in a much higher yield. All compounds were analysed by MS and TLC to check purity and a selected number of interesting compounds were fully characterized.



Scheme 6.2.2.1 **Table 6.2.3.4** **Table 6.2.3.3**

Scheme 6.2.3.1: Formation of bicyclic and uncyclized products

Scheme 6.2.3.2: Proposed mechanism of bicyclic furanone formation



Strongly basic conditions are essential for the reactions to occur in the open ring position of mucochloric acid (Scheme 6.2.3.2). The methyl α-proton is abstracted by base, from the substituted acetophenone, to form a nucleophilic carbanion (**1**). This reacts with the aldehyde group of the ring opened form of mucochloric acid (**2**), rather than the closed ring system (**3**). Protonation of the anion gives an free hydroxyl functionality (**5**). A 5-exo-tet, intramolecular reaction allows the ring to reform (**6**). Hydrazines, under acidic conditions, react readily with the ketone group to form a carbinolamine (**7**). This subsequently forms an imine with the elimination of water (**8**). Further heating allows the lone pair of electrons in the nitrogen to cyclize, with the loss of hydrochloric acid and the formation of a bicyclic structure (**9**). This, cyclisation, was not observed with the electron withdrawing subsituent, the nitro group.

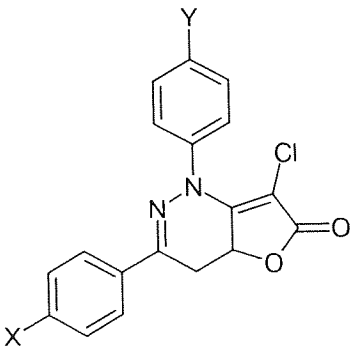


Table 6.2.3.3: Characterized structures of bicyclic furanones

Compound	X	Y	MF	MW	Yield [%]	MS
6.2.a*	-H	-H	$C_{18}H_{13}ClN_2O_2$	324	33.5	325
6.2.b*	-H	$-CH_3$	$C_{19}H_{15}ClN_2O_2$	339	35.5	339
6.2.c	-H	-Cl	$C_{18}H_{12}Cl_2N_2O_2$	358	35.3	359
6.2.d*	-H	$-OCH_3$	$C_{19}H_{15}ClN_2O_3$	355	30.1	355
6.2.e	$-OCH_3$	-H	$C_{19}H_{15}ClN_2O_3$	355	12.3	355
6.2.f	$-OCH_3$	$-CH_3$	$C_{20}H_{17}ClN_2O_3$	369	41.8	369
6.2.g*	$-OCH_3$	-Cl	$C_{19}H_{14}Cl_2N_2O_3$	389	21.7	389
6.2.h	$-OCH_3$	$-OCH_3$	$C_{20}H_{17}ClN_2O_4$	384	13.7	384

*** = Fully characterised**

Compound	X	Y	MF	MW	Yield [%]	MS
6.2.i*	$-CH_3$	-H	$C_{19}H_{15}ClN_2O_2$	339	22.4	339
6.2.j	$-CH_3$	$-CH_3$	$C_{20}H_{17}ClN_2O_2$	353	4.1	353
6.2.k	$-CH_3$	-Cl	$C_{19}H_{14}Cl_2N_2O_2$	373	45.5	373
6.2.l*	$-CH_3$	$-OCH_3$	$C_{20}H_{17}ClN_2O_3$	369	12.3	369
6.2.m	-Cl	-H	$C_{18}H_{12}Cl_2N_2O_2$	358	26.4	359
6.2.n*	-Cl	$-CH_3$	$C_{19}H_{14}Cl_2N_2O_2$	373	38.6	373
6.2.o*	-Cl	-Cl	$C_{18}H_{11}Cl_3N_2O_2$	393	38.1	393
6.2.p	-Cl	$-OCH_3$	$C_{19}H_{14}Cl_2N_2O_3$	389	19.2	389

*** = Fully characterised**

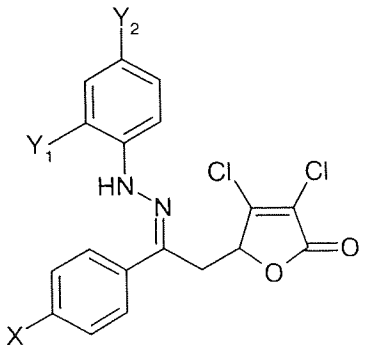


Table 6.2.3.4: Characterized structures of uncyclized furanones

Compound	X	Y1	Y2	MF	MW	Yield [%]	MS
6.2.q*	-H	$-NO_2$	$-NO_2$	$C_{18}H_{12}Cl_2N_2O_6$	450	76.1	451
6.2.r	-H	-H	$-NO_2$	$C_{18}H_{13}Cl_2N_3O_4$	405	82.9	406
6.2.t	$-OCH_3$	$-NO_2$	$-NO_2$	$C_{19}H_{14}Cl_2N_4O_7$	480	82.2	482
6.2.s	$-OCH_3$	-H	$-NO_2$	$C_{19}H_{15}Cl_2N_3O_5$	435	52.1	437

*** = Fully characterised**

Compound	X	Y1	Y2	MF	MW	Yield [%]	MS
6.2.u	-CH3	$-NO_2$	$-NO_2$	$C_{19}H_{14}Cl_2N_4O_6$	464	67.2	465
6.2.v	-CH3	-H	$-NO_2$	$C_{19}H_{15}Cl_2N_3O_4$	419	62.2	420
6.2.w	-Cl	$-NO_2$	$-NO_2$	$C_{18}H_{11}Cl_3N_4O_6$	484	76.5	486
6.2.x	-Cl	-H	$-NO_2$	$C_{18}H_{12}Cl_3N_3O_4$	439	45.4	441

A selected number of uncyclized and bicyclic furanones structures were fully characterized, as indicated from the Tables (6.2.3.3 & 6.2.3.4). All compounds were initially characterized by MS APCI(+) and TLC (MP = 10% MeOH in ether). Both ^{13}C & ^{1}H NMR gave concise, clear peaks.

6.2.4. Summary

By using a established procedure a series of 3,4-dichloro-5-[2-(sub-phenyl)-2-oxoethyl]furan-2(5H)-ones were synthesised in good yields. These were subsequently reacted further with substituted aromatic hydrazines to form novel uncyclized and bicyclic compounds. All products displayed good stability and colouration but poor solubility. A selected number of compounds were put in both CCK_A and CCK_B screening. Disappointingly they was no activity in both assays. These natural-product like structures may show activity in a general screening procedure.

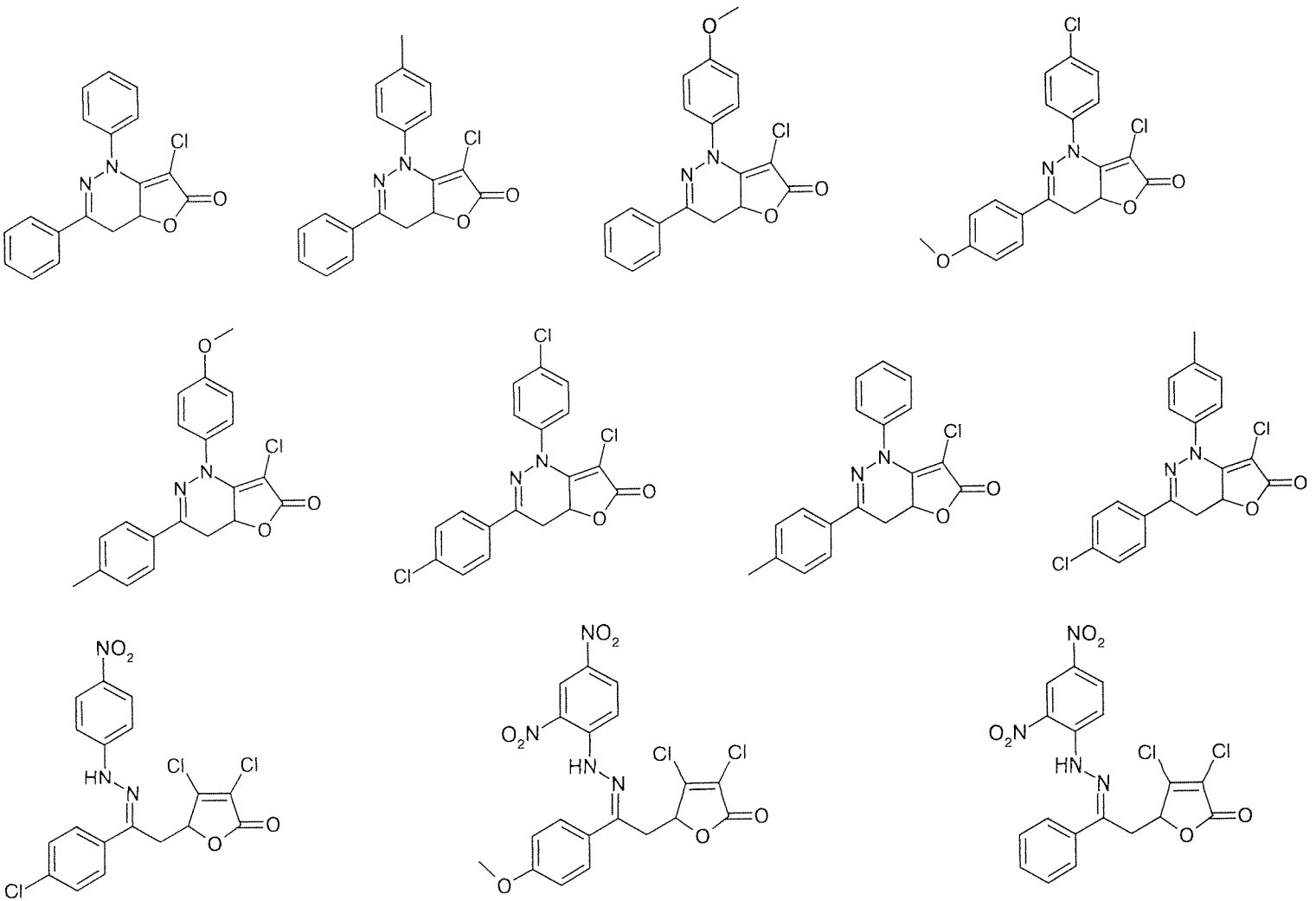


Figure 6.2.4.1: Structures of cyclic and uncylized pyridazinone derivatives

6.3. Preparation of 5-(3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl) imidazolidine-2,4-dione (aldol)

It was decided to attempt the same type of condensation method with different reactants containing a cyclic alkyl functionality. An equimolar amount of mucochloric acid and hydantoin was dissolved in DCE and cooled to 0^0C. A solution of NaOH was added slowly and then the mixture was allowed to stand for 4 hrs. The whole mixture was then poured into ice-water, containing an excess of HCl conc. After 45 mins the precipitate was filtered and recrystallised from dilute ethanol to give a white powder. The yield was quite low at 20% (Fig. 6.3.1).

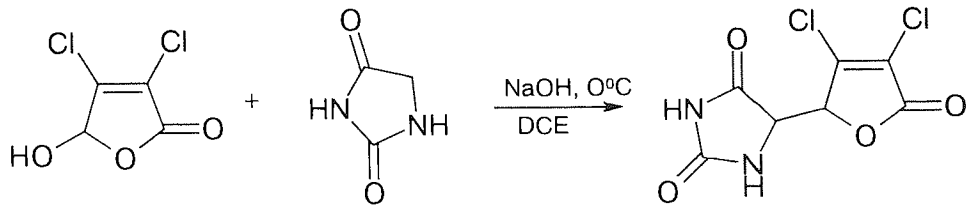


Fig. 6.3.1: The conversion of mucochloric acid to 5-(3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl)imidazolidine-2,4-dione

The reaction conditions were modified to overcome initial solubility problems. The reaction was carried out in a two-phase system of water and DCE. The novel product was fully characterized with sharp and concise ^{1}H & ^{13}C peaks. Attempts to react fluorene and rhodanine were unsuccessful. This was probably due to the lack of reactivity, of the cyclic alkyl protons.

6.4. Preparation of 3,4-dichloro-5-phenylfuran-2(5H)-one (S_E reaction)

The Friedel Crafts reaction conditions were utilised to prepare 3,4-dichloro-5-phenylfuran-2 (5H)-one according to the published method by Semonsky et al.[171] Mucochloric acid was dissolved in benzene, which acts a both solvent and reagent and also aluminium chloride. The mixture was allowed to stir in RT for 3 days, under inert conditions. After work up, the brown oil was recrystallised from ethanol to give white crystals, 54% yield and fully characterised (Fig. 6.4.1).

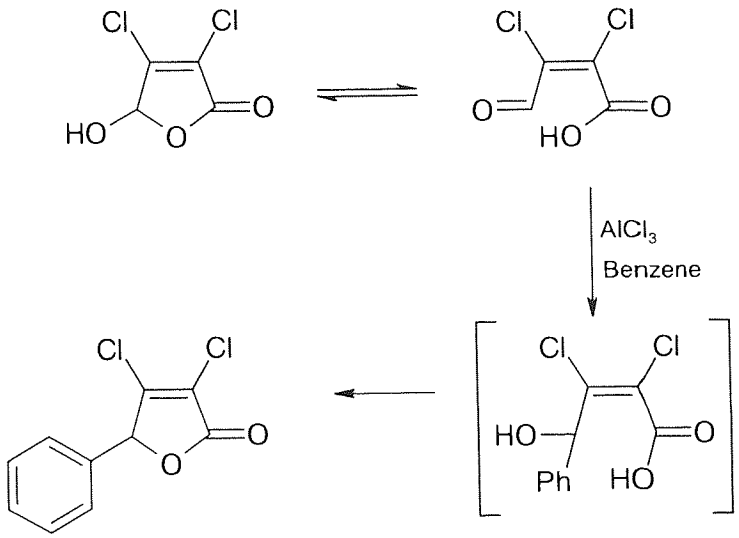


Fig. 6.4.1: Formation of 3,4-dichloro-5-phenylfuran-2 (5H)-one

Analysis of the product was initially achieved by APCI+ mass-spectrometry, where the MS+H was just detectable and subsequently confirmed by both ^{1}H & ^{13}C NMR spectroscopy.

6.5. Preparation of 3,4-dichlorofuran-2(5H)-one (Reduction)

Mowry[166] managed to reduce mucochloric acid, to prepare 3,4-dichlorofuran-2 (5H)-one. Mucochloric acid and aluminium isoproxide were dissolved in isopropanol and refluxed, using a vigreux column. Excess isopropanol was distilled off and the remaining mixture was poured into a mixture of ice-water, containing an excess of HCl conc. After the extraction and washing stages, the crude product was recrystallised in dilute ethanol to give a white crystalline solid in 33% yield. The product was fully characterised (Fig. 6.5.1).

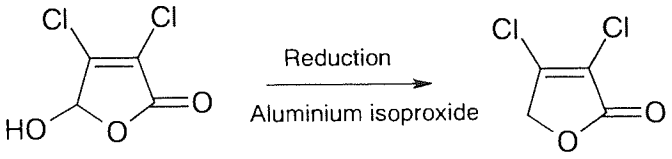
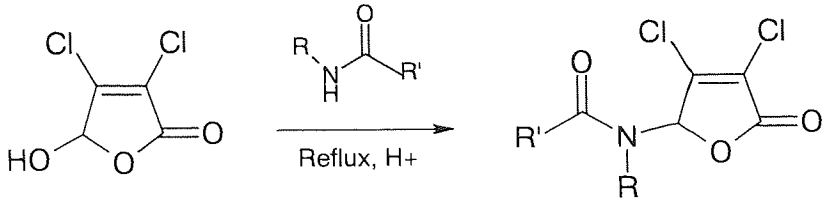


Fig. 6.5.1: The reduction of mucochloric acid to 3,4-dichlorofuran-2 (5H)-one

6.6. Preparation of 3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl amides (Pseudo amides)

In a new approach various amides were reacted with mucochloric acid, under refluxing conditions with a trace of acid. Amides are generally much less reactive than acid chlorides, anhydrides and esters. However, the amide linkage is stable enough to serve as a basic unit. It was found that amide formation proceeded via the aldehyde group of mucochloric acid, rather than the pseudo acid. Amides in general have a lower bascity

than amines, because they are resonance stabilised, due to the nitrogen lone pair of electrons. However, an amide protonated on its nitrogen lacks this resonance stabilisation and therefore can react via a nucleophilic subsitution type reaction (Fig. 6.6.1)

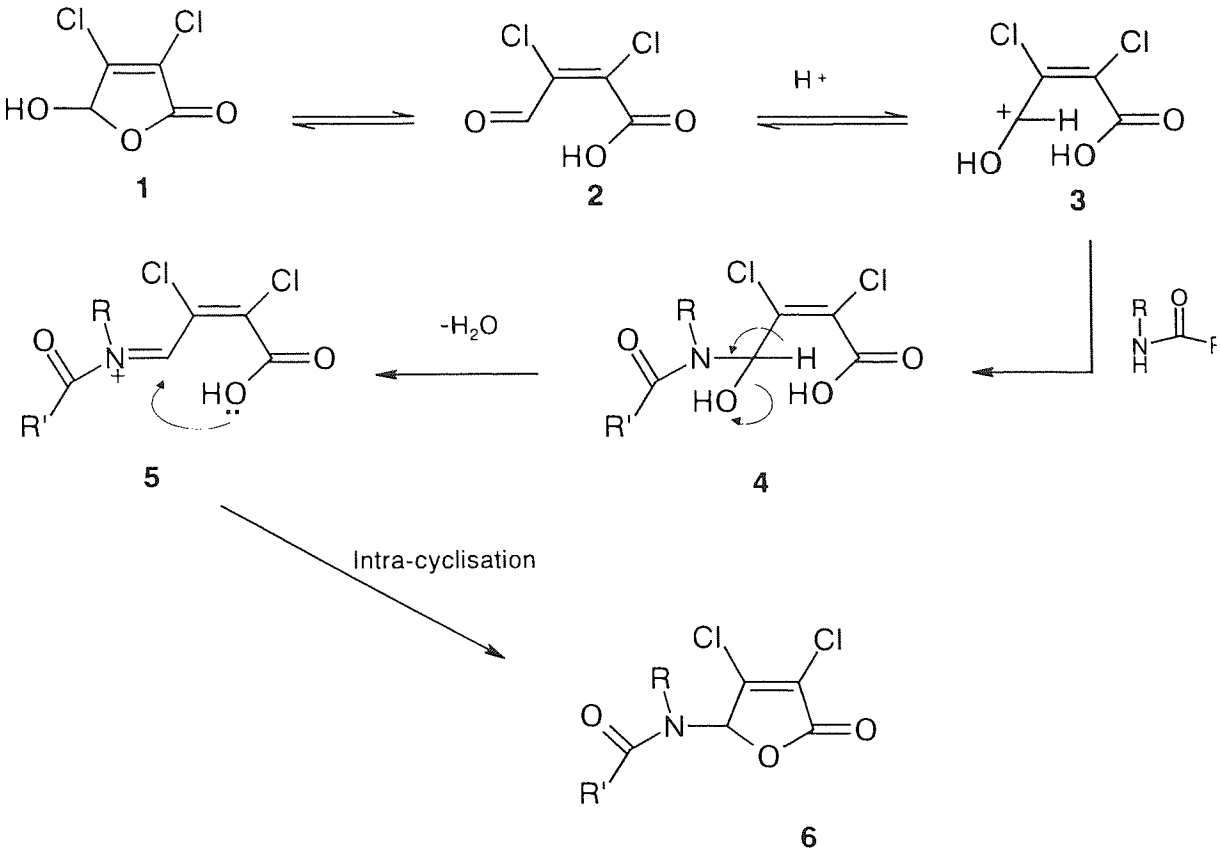


Compound	R	R'	Yield [%]
6.6.a	$-CH_3$	-H	10.5
6.6.b	$-(CCH_3)_3$	-H	11.9
6.6.c	$-CH_3$	$-CH_3$	6.0
6.6.d	$-CH_2C_6H_5$	-H	15.0
6.6.e	$-C_6H_5$	-H	48.5

Fig. 6.6.1: Synthesis of amide derivatives from mucochloric acid

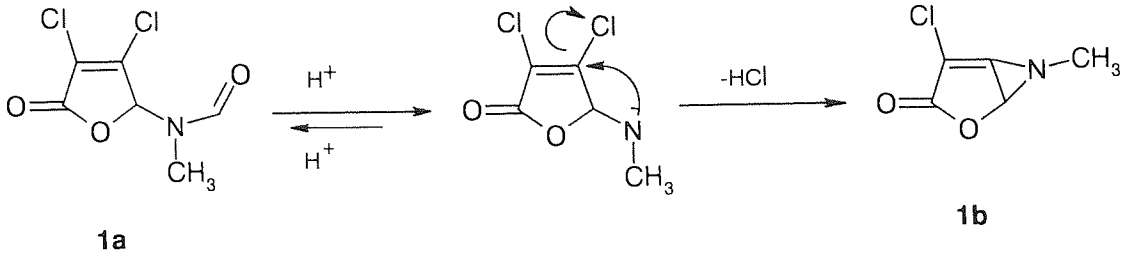
6.6.2: Proposed mechanism

Mucochloric acid exists in the ring closed **1** and the ring open form **2**. In the ring open form, the aldehyde protonates into the free hydroxyl group, with the formation of a carbocation **3**. The basicity of the amide nitrogen allows it to react with the carbocation **4**, rather than to displace the relatively stable chlorine atoms. Elimination of water gives the imine intermediate **5** and subsequent intra-molecular cyclization gives the desired amide product **6** (Scheme 6.6.3).



Scheme 6.6.3: Formation of 3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl amides

These compounds have been noted for being generally less stable in acidic/alcoholic solutions. When N-methylformamide (6.6.a) was reacted with mucochloric acid, two products were obtained, depending on the reaction time. The greater the time refluxed, the higher the yield is, with respect to the closed azabicyclo-one structure than the normal open structured formamide form. Interestingly this additional cyclization product was not isolated with rest of the amide series, particularly with the free aldehyde functionality. However, traces were observed in mass-spectometry analysis. Perhaps a much more harsher, prolonged heating may have been needed to cyclize the remaining aldehyde derivatives (Fig. 6.6.4).



1a **1b**

Fig. 6.6.4: Formation of the bicyclic system

Under the acidic conditions, 3-4-dichloro-5-oxo-2,5-dihydrofuran-yl(methyl)-formamide (**1a**) undergoes hydrolysis to form formic acid (Fig. 6.6.4) and the subsequent intra-molecular cyclization yields 4-chloro-6-methyl-2-oxa-6-azabicyclo[3.1.0]hex-4-en-3-one (**1b**). One possible mechanism could be the conversion of **1a** to a carboxylic acid and the subsequent decarboxylation. However, although the temperature criterion is present, this is unlikely, as oxygen needs to be present for converting the aldehyde group into the acid. Decarbonylation is not favourable as this needs extremely high temperature (500°C), with the presence's of a palladium or a Wilkinson's catalyst ($RhCl(Ph_3P)_3$), or heating with peroxides, or light induced reactions.

Refluxing mucochloric acid with the relevant amide, in the presence of a catalytic amount of acid, over 3-4 days synthesised a new class of exciting compounds. A Dean Stark apparatus was essential to remove the by-products produced, notably water. Column chromatography was needed for most of the polar compounds. The formation of 3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl(phenyl)formamide (6.6.e) did not require chromato-graphic separations, the compound precipitated out of the solution, as a bright yellow crystalline solid. However, it was not possible to form the amide derivative using benzanilide (a diphenyl amide). This may have been due to fact that the reactant was too stable to react or/and it was too sterically bulky to react. Reaction yields were generally

very low, reflecting the difficulty of reacting stable amides at the 5-position of the furanone structure.

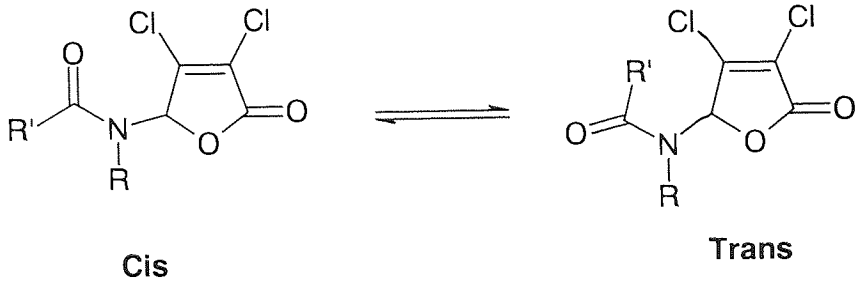


Fig. 6.6.5: Cis-Trans conformation of the amide furanones

Geometric isomers were obtained (cis & trans) with these novel compounds (Fig. 6.6.5). These conformational isomers may be easily inter-converted by rotation about the bond. The staggered, low energy conformation is more favourable as shown by the NMR data (^{1}H & ^{13}C), ratio 3:1 (trans:cis).

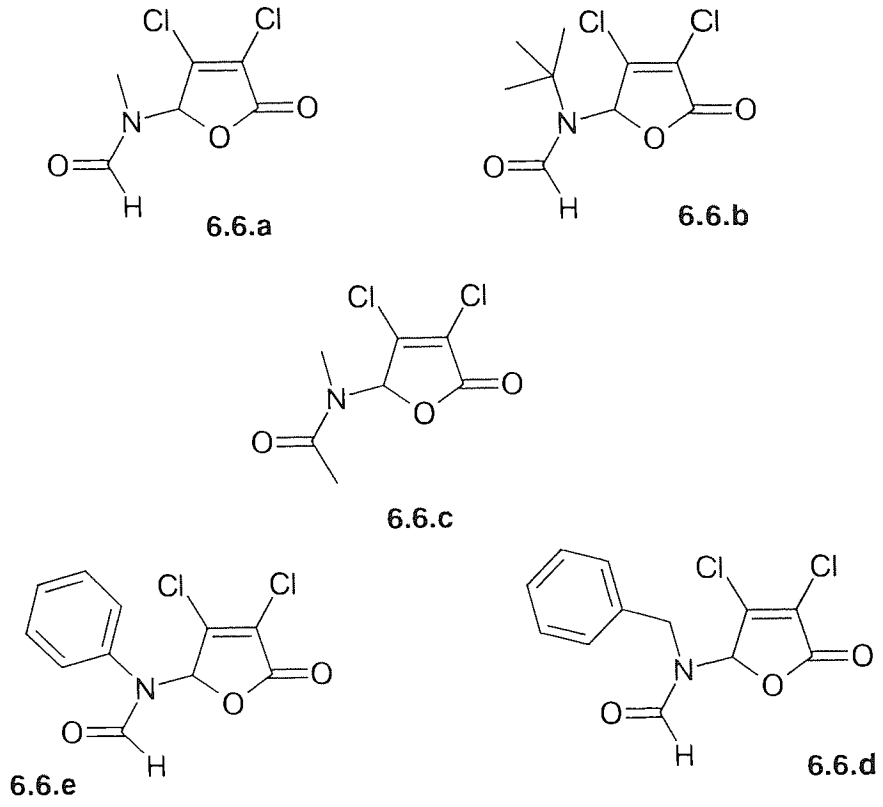
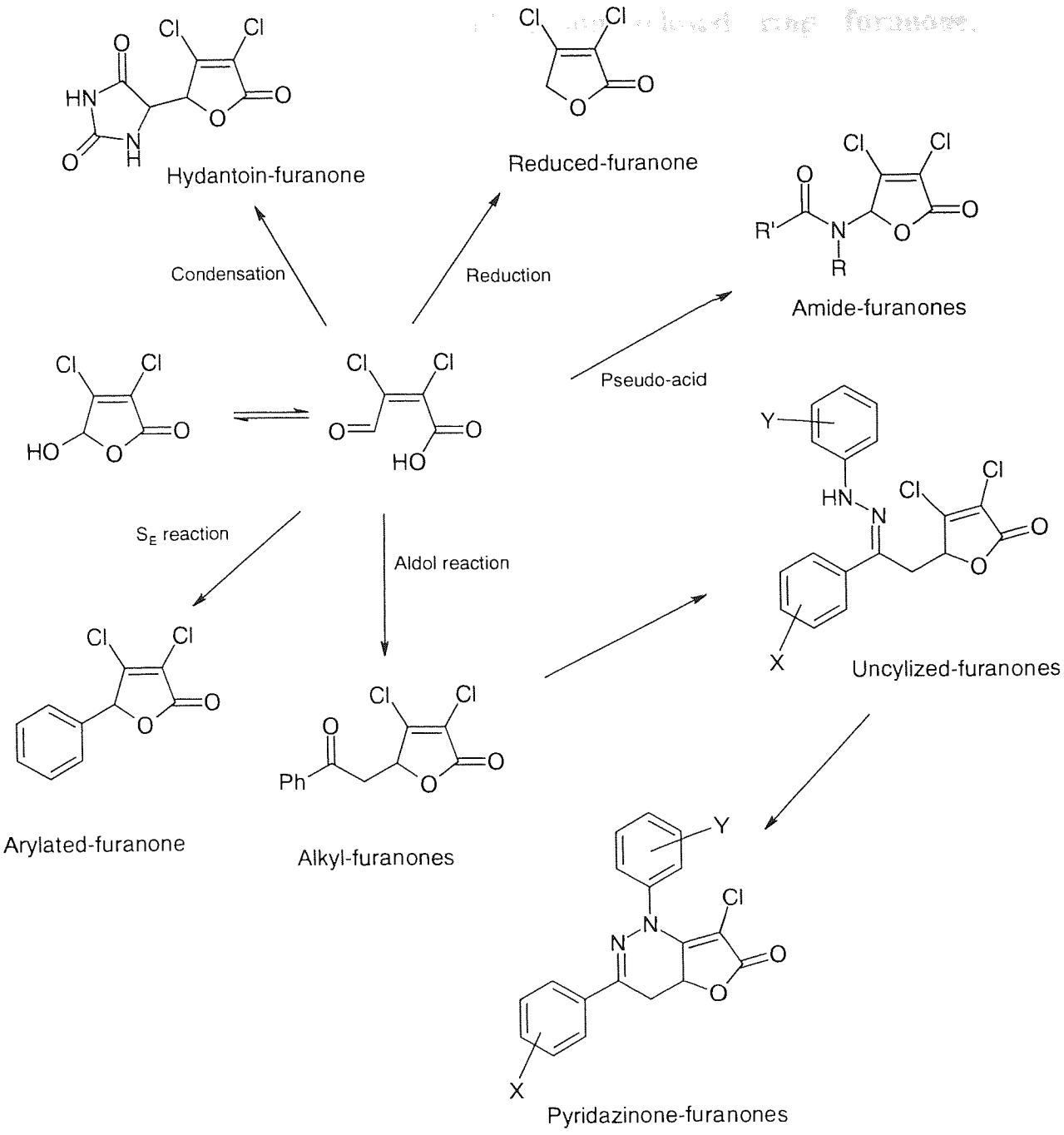


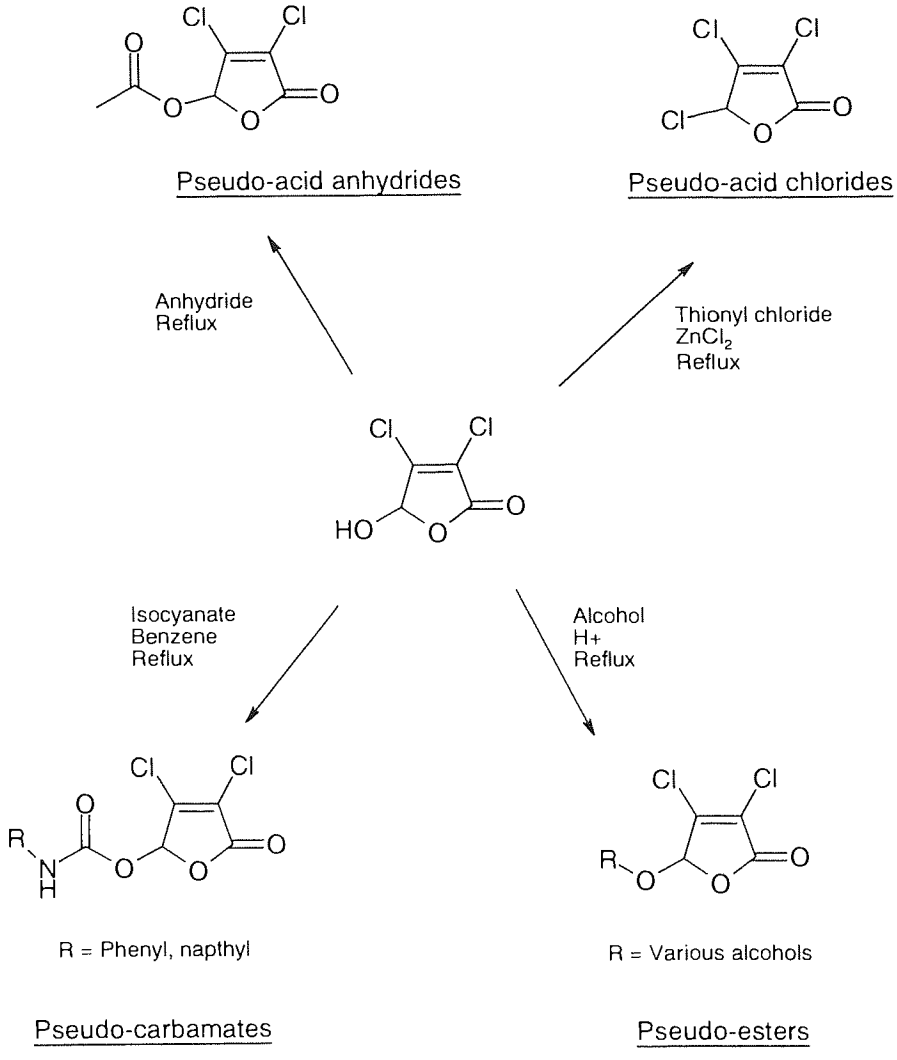
Fig. 6.6.6: Structures of the amide-furanones



Scheme 6.6.7: Reaction summary of the open form of mucochloric acid

6.7. Reaction of the pseudo-acid group (closed ring furanone, pseudoesters)

In Mowry's original paper, he studied the characteristics and reactions of the pseudo-acid group[165,166] (mucochloric acid in the closed form). He found that refluxing mucochloric acid with various reagents resulted in the formation of pseudo esters, anhydrides, acid chlorides, which were all in the cyclic form (Scheme 6.7.0.1).



Scheme 6.7.0.1. Reaction summary of the pseudo-acid group

A large number of alcohols, which included: methanol, ethanol, isopropanol, n-butanol, 1-nanonol, menthol, cetyl, vinyl acetate, allyl and propargyl were included as potential building blocks. Phenyl and naphthyl were the two carbamates chosen.

All the above pseudo-acid anhydride, esters, chloride and carbamates were kindly provided by the medicinal chemistry research group. These compounds were remaining after being screened for potential anti-cancer activity. All the potential building blocks were analysed by MS to check both the purity and stability.

6.7.1. Summary

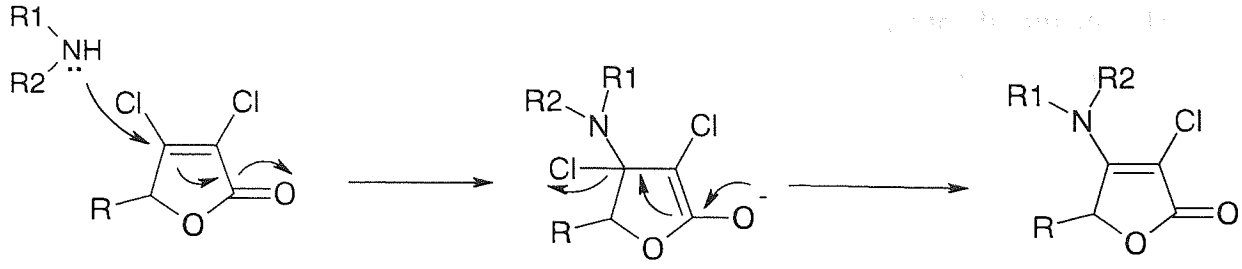
New and existing furan-2(5)-one building blocks were constructed in low to high synthetic yields. A variety of synthetic methods on the open form of mucochloric acid were performed. Condensation, reduction, S_E reaction and aldol reactions were all successfully achieved. Further utilization of existing building blocks (anhydride, chloride, carbamates and esters), with nucleophilic amines offer the opportunity to develop new and potential CCK receptor antagonists.

Chapter 7. Preparation of 4-substituted amino furanon-2(H)-ones

7.1. Nucleophilic attack at the 4-position (IPSO-Substitution)

A large selection of 5-substituted furanone building blocks were prepared and assembled in the previous chapter (chapter 6) and now were assembled for further reaction. A small library was primarily constructed, which initially assessed the working ability of these building blocks Fig. 7.2.1), against two good nucleopilic amines. The selected number of working building blocks were then reacted with a more diverse range of amines, depending on the quantity of the initial building block.

The C_4-centre on the furan-2(5H)-one molecule is more susceptible towards nucleophilic attack than the C_3 centre, due to the presence of a Michael system (Scheme 7.1.1) Mechanistically, the nucleophilic attack at the four position on the furan-2(5H)-one ring proceeds as follows (Scheme: 7.1.1). Nucleophilic attack at the four position is an important reaction, it has been extensively studied by Jahnisch et. al.[172] Who examined different types of nucleophilic substitution reactions, that can occur in dihalogenated furanones, especially with respect to different solvents. The research group found that the type of solvent plays a important role in dictating the configuration of the furanone (whether it is in the open or closed ring form). In DMSO, the relevant amine will act as a nucleophile and react at the four position of the closed ring form of the furanone, to give monosubstituted products.



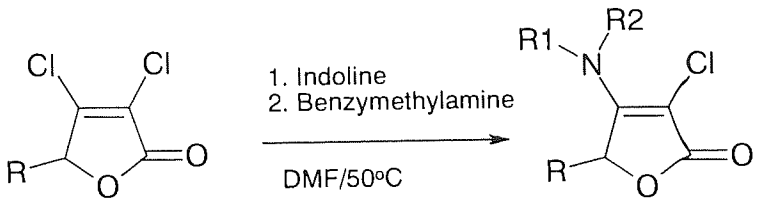
Scheme 7.1.1: Mechanistic representation of the nucleophilic attack on the four-position

Other research groups [172,173] developed methods for forming 4-substituted furanones with various nucleophiles, under different conditions. Mechanistically, nucleophilic attack with sulphur containing nucleophiles, proceed similarly as to nucleophilic attack with nitrogen containing nucleophiles. Reacting sodium thiolates in THF or CCl_4 gives similar yields as with amines. Carbon nucleophiles are also known to react in the four position of the furan-2(5H)-one ring. However, oxygen nucleophiles react exclusively at the three position.

7.2. Chemical evaluation of furan-2(5H)-one building blocks

All building blocks were reacted with indoline and benzylmethylamine (Scheme 7.2.1), which were judged to be good nucleophilic amines. They are both liquids and so are easier to manipulate and also give a very precise APCI(+) mass spectra. DMF was chosen as a universal solvent to dissolve all reactants and being sufficiently nucleophilic to aid product formation. A 12-test-tube reaction carousel was used. The appropriate furanone building block and 3 Eq of amine was sufficient The mixtures were heated and stirred up to 50 °C and left overnight. Leaving the mixtures longer caused the product to decompose

into sticky black liquid. TLC analysis was used to monitor the reaction progress. It was decided, that in general product formation was optimal after 15-20 hours. After 15-20 hours, excess water was added to each test tubes and allowed to stand for 30 minutes. The work up phase removed any excess amine in the mixture. The products were extracted with DCM and washed with dilute HCl (pH 5). The organic layer was further washed with water, dried and removed in vacuo.



R = 27 assembled building blocks (Fig. 7.2.2)

4-Substituted amino furanones (Table. 7.2.3)

Scheme 7.2.1: General scheme for the formation of 4-substituted amino furan-2(5H)-one

The samples were submitted directly, diluted in methanol, without any purification, for detection of the molecular ion peak.

Assembled building blocks

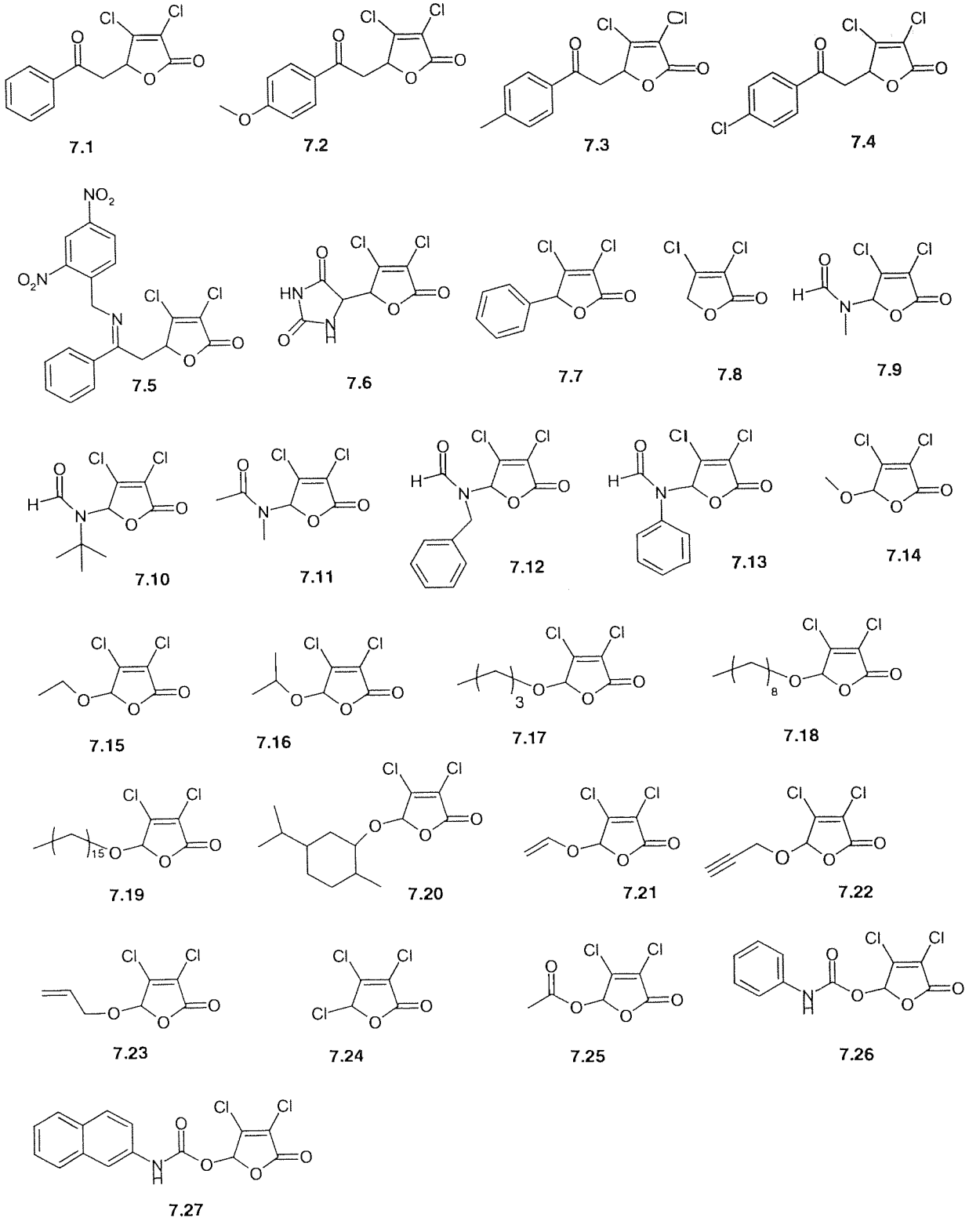


Fig 7.2.2: Chemical structures of the 27 furan-2(5)-one building blocks

Table 7.2.3: Depicts the initial reaction of indoline & benzylmethylamine with all the assembled building blocks. Most building blocks were successfully reacted with indoline & benzylmethylamine. However, the amide series and the naphthyl compound, from the carbamate series were non-working, when using both amines, under the current reaction conditions.

Building block	Product formation	Building block	Product formation
7.1	✔	7.15	✔
7.2	✔	7.16	✔
7.3	✔	7.17	✔
7.4	✔	7.18	✔
7.5	✔	7.19	✔
7.6	✔	7.20	✔
7.7	✔	7.21	✔
7.8	✔	7.22	✔
7.9	✘	7.23	✔
7.10	✘	7.24	✔
7.11	✘	7.25	✔
7.12	✘	7.26	✔
7.13	✘	7.27	✘
7.14	✔		

Table 7.2.3: Testing of each building block

The amide series 7.9-7.13 were nonworking, this was probably due to the nitrogen on the 5-position. The lone pair of electrons on the nitrogen resonance into and stabilizes the ring system and therefore preventing any nucleophilic attack at the four position. All pseudo esters and anhydrides worked successfully. The building block 7.24 (a chlorine at

the 5-position) gave predominantely a mono-substituted product but traces of a disubstituted product were also produced (nucleophilic attack at both the 4 & 5 position).

7.3. Preparation of a chemical library of selected 4-substituted amino-furan-2-(H)-ones

A selected number of building blocks were further reacted. Mono nucleophilic amines were chosen, where the main/ only reacting group was a primary or secondary amine, as to reduce bi-product formation. A selected number of eight diverse building blocks were chosen to react up to 26 different amines. Amines were manually selected and included straight chained, cyclic, hetrocyclic and aromatic groups, with a relatively small molecular weight.

At this initial exploratory stage, it was decided not to produce a vast number of compounds within the assembled library of 26 amines and 8 building blocks. The synthesised compounds were evaluated for initial CCK_B screening and if active then screened on the CCK_A receptor. This approach should identify any potential lead compound(s), upon which further analogues can be prepared and the remaining combinations, of the library be synthesised and evaluated.

The same chemical procedure was used, as in the chemical evaluation of the furan-2(5H)-one building blocks. This enabled the preparation of 4-substituted amino-furan-2-(H)-ones. After the products were extracted with DCM and washed with dilute HCl (pH 5). The organic layer was further washed with water, dried and removed in vacuo. Three different isolating techniques were employed;

(1) The target compound precipitated out of solution, was washed with water and dried to give a pure compound. This method was used to isolate isopropyl-substituted furanone analogues.

(2) The target compound was extracted with DCM, the organic phase was washed with water, then with diluted HCl (pH 5). The organic layer was dried and removed in vacuo. This method was used to isolate cetyl-substituted furanone analogues and other lipophilic groups.

(3) In addition to method (2), chromatographic separation was used (Preparartive TLC), with either 100% ether or 10% MeOH in ether as the mobile phase. This was the common technique employed for isolating the majority of the compounds.

It was observed that secondary amines (non-cyclic form) exhibited two isomeric forms, depending on the position of the nitrogen substituent. Both ^{13}C & ^{1}H NMR on compound A_6B_6 (Fig. 7.3.1) showed the varying interconvertable positions of the methyl substituents.

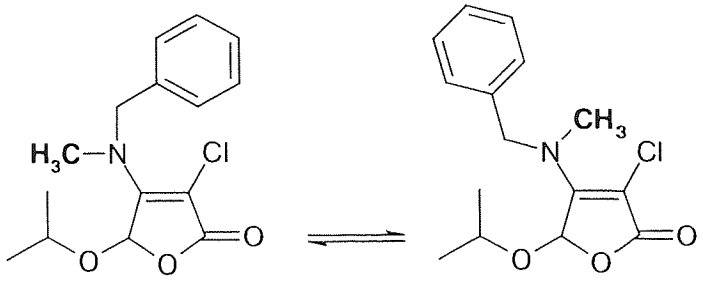
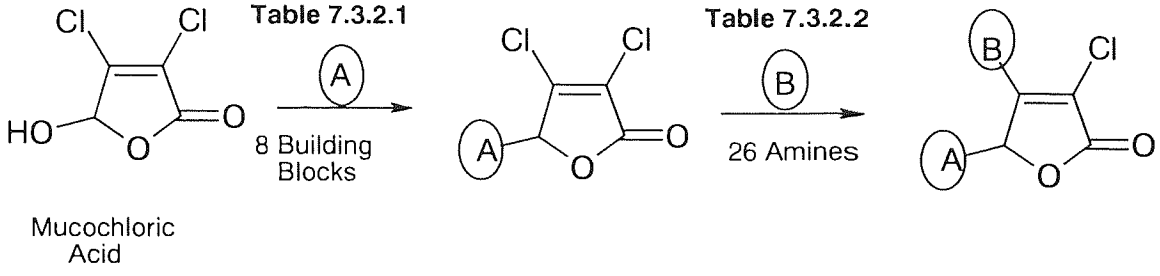


Fig. 7.3.1: Two isomeric forms of compound A_6B_6

Table 7.3.2.1

Building Blocks (A)

A_1 = Methyloxy (7.20)
A_2 = Acetoxy (7.25)
A_3 = Propargyloxy (7.22)
A_4 = Vinyloxy (7.21)
A_5 = Chloro (7.24)
A_6 = Isopropyloxy (7.16)
A_7 = Cetylyloxy (7.19)
A_8 = Methoxyl (7.14)



Mucochloric Acid

Table 7.3.2.2

Amines (B)

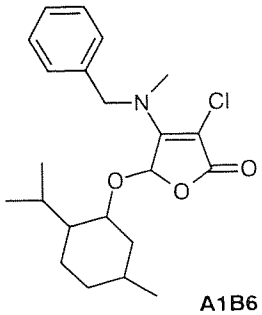
B_1 = 4-aminoantipyrine
B_2 = Indoline
B_3 = Benzimidazole
B_4 = m-Toluidine
B_5 = *sec*-Butylamine
B_6 = Benzylmethylamine
B_7 = Dimethylmorpholine
B_8 = N-Phenylpiperazine
B_9 = Pyrrolidine
B_{10} = n-Dodecylamine
B_{11} = 4-Phenylpiperidine
B_{12} = n-Butylamine
B_{13} = Benzylamine
B_{14} = Aniline
B_{15} = Benzylpiperazine
B_{16} = 4-Amino-1-benzylpiperidine
B_{17} = Aminopropylmorpholine
B_{18} = 2-Chlorobenzylamine
B_{19} = Dibenzylamine
B_{20} = 2,6-Dimethylpiperidine
B_{21}= N,N'-Isopropylcyclohexylamine
B_{22} = Phenethylamine
B_{23} = 3,5-Dimethyl pyrazole
B_{24} = Ethyl-1-piperazine carboxylate
B_{25} = 4-(3-Phenylpropyl) piperidine
B_{26} = 3-Methyl pyrazole

Fig. 7.3.2: Synthetic scheme of building block formation and subsequent reaction with amines at the 4-position

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24	B25	B26
A1	-	-	-	-	-	✔	✔	-	✔	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A2	-	✔	-	-	-	-	-	-	-	-	-	-	-	-	✔	-	-	-	-	-	-	-	-	-	-	-
A3	-	✔	-	-	-	-	✔	-	-	-	-	-	✔	-	✔	-	-	-	-	✔	✔	-	-	-	-	-
A4	✔	✔	✔	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A5	✔	✔	-	-	-	-	-	-	-	-	-	-	-	✔	-	-	-	-	-	-	-	-	-	-	-	-
A6	-	✔	-	-	-	✔	-	-	-	-	-	-	-	-	✔	-	-	✔	✔	-	-	✔	-	-	-	-
A7	-	-	✔	-	-	-	-	-	-	-	-	-	-	-	✔	-	-	✔	-	-	-	-	✔	✔	-	-
A8	✔	✔	✔	✔	✔	-	✔	✔	-	✔	✔	✔	✔	-	✔	✔	✔	-	-	-	-	✔	-	-	✔	✔

Fully characterized ✔ = TLC & MS APCI(+)

Table 7.3.2: Construction and chemical evaluation of the partial mini library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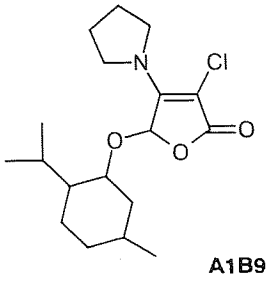
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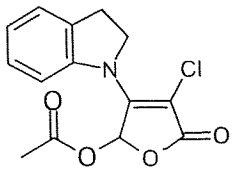
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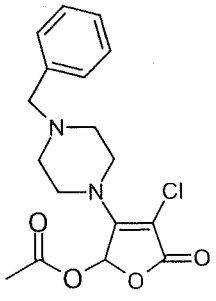
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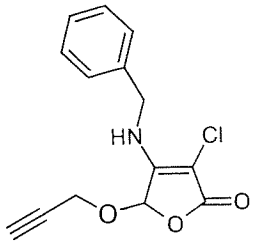
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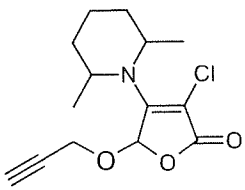
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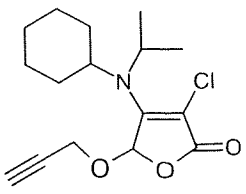
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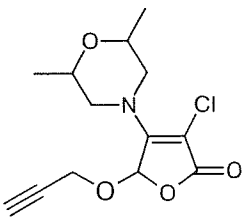
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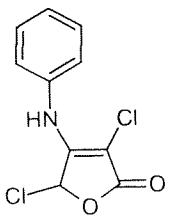
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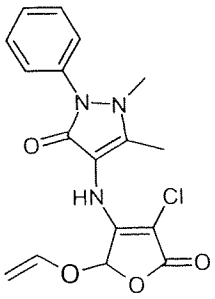
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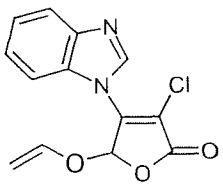
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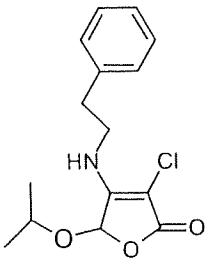
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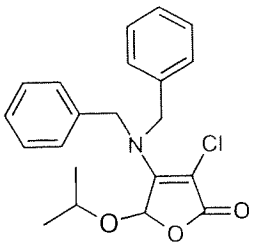
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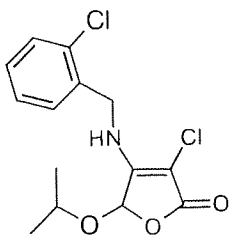
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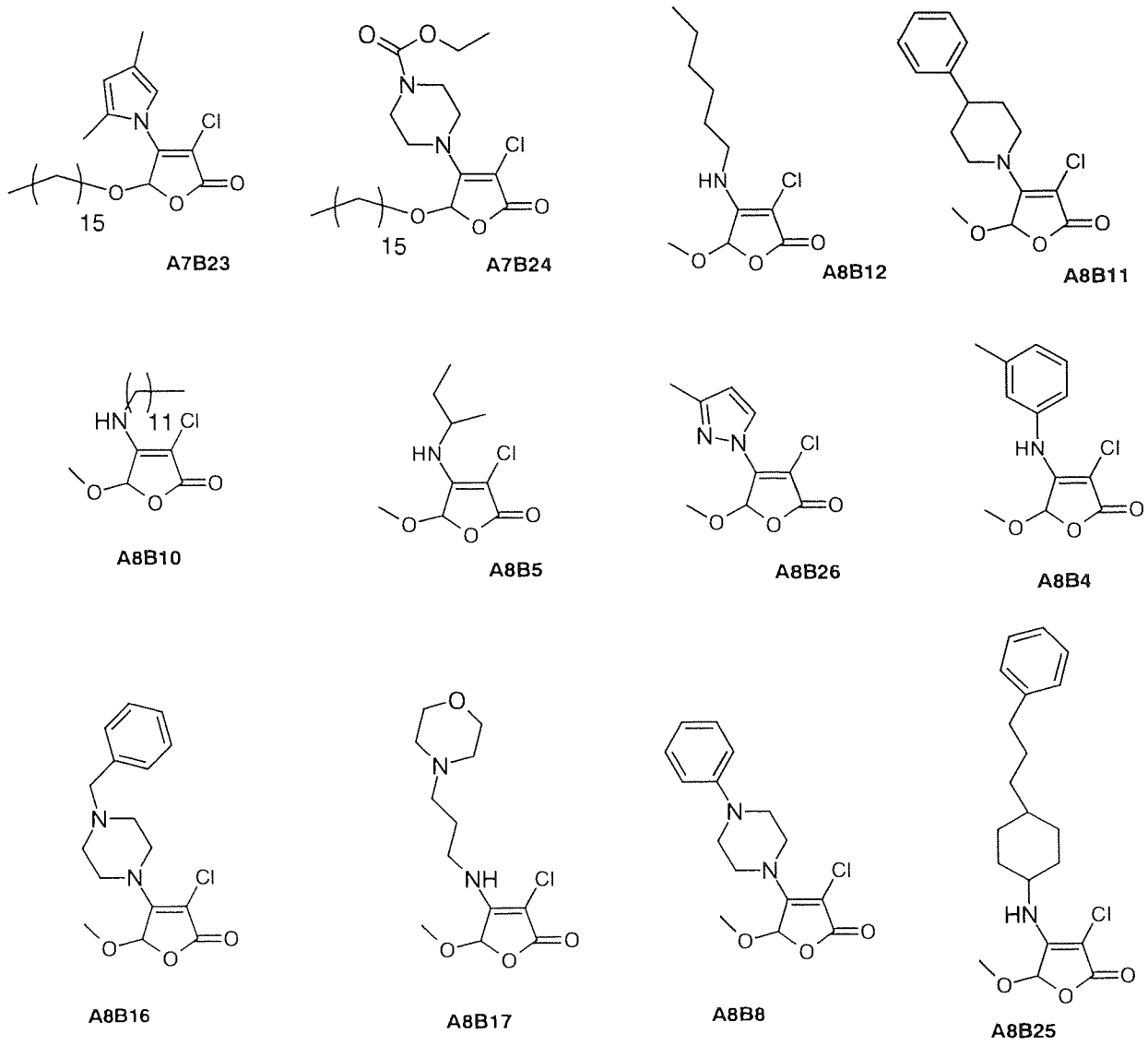


Fig.7.3.3: Structures of selected 4-substituted amino-furan-2(H)-ones

All compounds were initially examined by MS APCI(+) analysis. The synthesised compounds were selected for initial CCK_B screening. All selected combination of furanone building blocks reacted with the selection of amines. They were no nonworking combinations in the series. Synthetic yields were good but were not calculated. This synthetic approach is particularly suitable for a complete, larger scale library construction.

7.4. Biological Evaluation

Table 7.4.1: Shows *in vitro* activity (IC_{50}'s) of 4-substituted amino-furan-2-(H)-ones

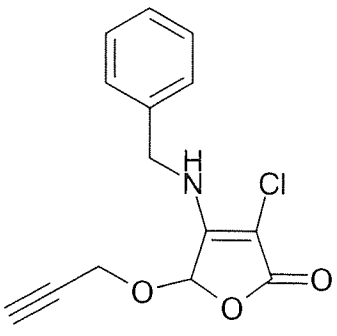
Compound Code	MS [m/z]	Activity CCK-B [μM]	Compound Code	MS [m/z]	Activity CCK-B [μM]
A_1B_6	392	0.31	A_7B_3	475	1.2
A_1B_7	386	10.3	A_7B_{15}	533	13.9
A_1B_9	342	2.2	A_7B_{18}	499	0.24
A_2B_2	294	>20	A_7B_{23}	452	0.86
A_2B_{15}	351	>20	A_7B_{24}	515	12.4
A_3B_2	290	>20	A_8B_1	350	18
A_3B_7	286	12.6	A_8B_2	266	1.5
A_3B_{13}	278	0.014	A_8B_3	265	2.3
A_3B_{15}	347	>20	A_8B_4	254	2.5
A_3B_{20}	284	11.2	A_8B_5	220	1.2
A_3B_{21}	312	0.008	A_8B_7	262	15
A_4B_1	362	3.3	A_8B_8	309	14
A_4B_2	278	18.5	A_8B_{10}	332	2.5
A_4B_3	277	>20	A_8B_{11}	308	3
A_5B_1	355	>20	A_8B_{12}	220	0.012
A_5B_2	270	>20	A_8B_{13}	254	1
A_5B_{14}	244	16	A_8B_{15}	323	14
A_6B_2	294	2.5	A_8B_{16}	323	16
A_6B_6	296	12	A_8B_{17}	291	15
A_6B_{15}	351	>20	A_8B_{22}	268	0.9
A_6B_{18}	316	0.95	A_8B_{25}	364	16
A_6B_{19}	372	17	A_8B_{26}	229	11
A_6B_{22}	296	1.1			

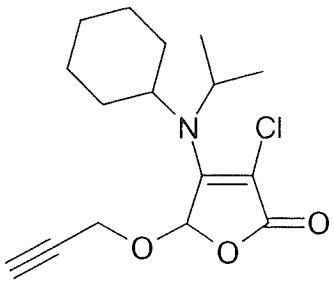
The *in vitro* activity (IC_{50}'s) of the prepared 4-substituted amino-furan-2-(H)-ones (Table 7.4.1) was very superior and outstanding. The majority of compounds exhibited modest to excellent activity, from the high micromolar to low nanomolar range. From the results, three compounds can be highlighted in terms of showing the greatest activity. Compounds A_3B_{13}, A_3B_{21} & A_8B_{13} were further evaluated on the CCK_A receptor subtype. Binding affinities were below 10 nM for each compound.

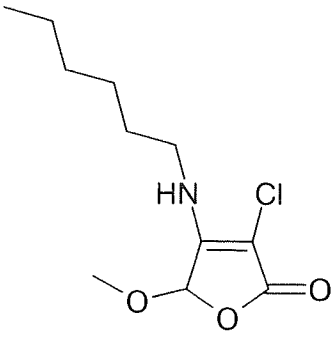
7.5. Summary

This has been the first time that 4-substituted amino-furan-2-(H)-ones, non urea, non benzodiazepine compounds, have shown CCK activity at the low nM range (compounds A_3B_{13}, A_3B_{20} & A_8B_{12}).

Fig. 7.5.1: Structures of the most active ligands from the 4-substituted amino-furan-2-(H)-one library.







	A_3B_{13}	A_3B_{20}	A_8B_{12}
CCK_B (nM):	14	8	12
CCK_A (nM):	9	6	8
Selectivity:	1.5	1.3	1.5

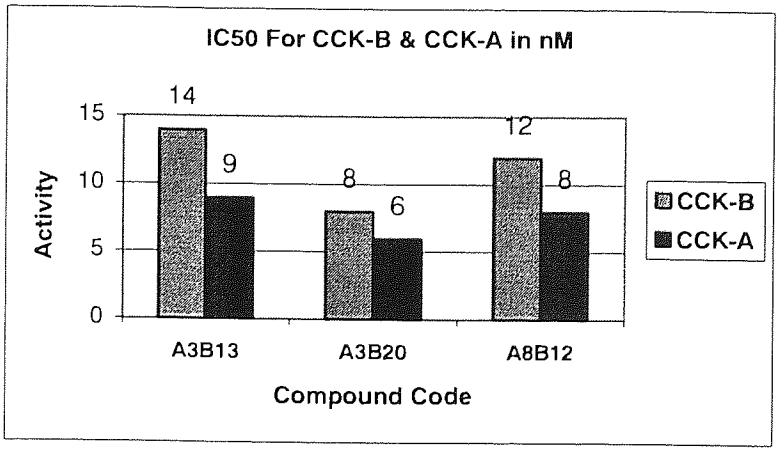


Fig. 7.5.1: Comparison of both CCK_B & CCK_A activity of the three analogues

In general a selected number of 4-substituted amino-furan-2-(H)-ones exhibited a range of modest to high activity. Large bulky substituents on the 4-position caused a reduction in activity. The results suggest that smaller ligands exerted excellent receptor affinity. This may probably due to the easy access of the drug into the receptor site. Amino substituted acetic anhydride (A_2), vinyl acetate (A_4), and the chloride (A_5) building blocks showed none to weak CCK activity.

No clear SAR can be derived from the data, as more compounds need to be synthesised. This higher risk strategy has been hugely successful in deriving a novel class of potent CCK antagonists. Three compounds displayed the greatest activity towards both receptor subtypes, with a slight selectivity towards the CCK_A receptor (Fig. 7.5.1). There is great potential to synthesise further potent compounds around the main ligands.

7.6. Further Work

From the results it justifies the reaction of the remaining building blocks with the selected amines. There is further scope for combining different building blocks and amines to enable a full SAR to be determined, with the aid of molecular modelling. The methodology is quite straight-forward and can be easily applied to produce hundreds of compounds, through the use of a computerised robot.

Chapter 8. Conclusion

Through extensive, innovative efforts of medicinal chemists, a range of receptor antagonists have been identified, enabling the physiological roles of both CCK_A & CCK_B receptors being clarified. However, the mechanism by which anxiolytic effects of CCK_B receptor antagonists are mediated, remain to be elucidated. It appears more likely that the function of CCK, in anxiety, is through its interaction with other neurotransmitter systems.

This in-depth study has been hugely successful in utilising a diverse range of different chemical approaches to synthesise potent CCK antagonists. The main aim of this work was to design novel and potent CCK_B antagonists to rival current existing compounds. The primary requirement was that any final compounds should be selective for one receptor type over the other. It should show significant anxiolytic properties without the side effects such as sedation, which is common for $GABA_A$ binding. Also the structures had to be sufficiently different as to avoid the recognised problems associated with using peptides as drugs; namely poor bioavailability, rapid cleavage and clearance *in vivo*.

The initial strategy was to design a hybrid structure of a 1,4-benzodiazepine template with a dipeptide fragment on the 3-position. However, this was unsuccessful but the 1,4-benzodiazepine template became a platform on which to design a new range of 3-amino substituted-1,4-benzodiazepines, to overcome the problem poor oral bioavailability. A comprehensive SAR was determined, with 3-substituted anilines showing significant activity (low nM range) towards the CCK_A receptor, rather than the CCK_B receptor subtype, with excellent selectivity. Novel 2-substituted-1,4-benzodiazepine analogues were developed accidentally but were not active *in vitro*. The most active 1,4-

benzodiazepine compound was combined with the most active 3-anilino compound but activity was lost. However, these anilino-1,4-benzodiazepines would show excellent solubility properties. This would potentially give a very high bioavailability of the drug *in vivo*.

Simple non-peptide urea antagonists provided a basis for deriving a new template. This strategy was used to design novel ligands for the CCK peptide receptors. Pyrazolinone ureido-amide derivatives (diphenyl system) provided active analogues, especially the amide series, which has the possibilities for further optimisation. Animal study data, on the most active pyrazolinone-ureido analogue (Compound 5.5.5) showed that the test drug having tremendous potential as a neuroleptic and antidepressant drug but not as a anxiolytic agent.

The furan-2(5H)-one ring proved to be a very versatile molecule to manipulate. Methods for adding diversity on the 5-position were investigated, namely reactions of the aldehyde and the pseudo-acid group. Pyridazinone derivatives (bicyclic) and uncyclized derivatives were successfully developed and made. No CCK activity was found but these natural product-like structures should potentially possess biological activity in a general screening program. A selected number of 4-amino furan-2(5H)-one analogues were prepared, from which three potent ligands were identified, with the possibility for further extensive work.

Extensive work has been undertaken in identifying lead structures. There is still the scope for further work; to optimise the analogues and further *in vitro* studies. Molecular modelling should provide structural information and lead to a better understanding of ligand-receptor interactions. This should aid in defining a precise pharmacophores for both the CCK receptor subtypes. To conclude, a number of selective and extremely potent antagonists were prepared for the peptide hormone, cholecystokinin. These compounds are of potential use as both pharmacological tools and as possible therapeutic agents. However, it should be noted that developing non-peptide ligands, may not necessarily translate into "bioavailable" drug candidates.

Chapter 9. Experimental Section

9.1. General methods

The majority of chemicals used were obtained from the laboratory and chemical stores. The remainder were ordered from Aldrich Catalogue Handbook of Fine Chemicals and Lancaster 1999/2000/2001.

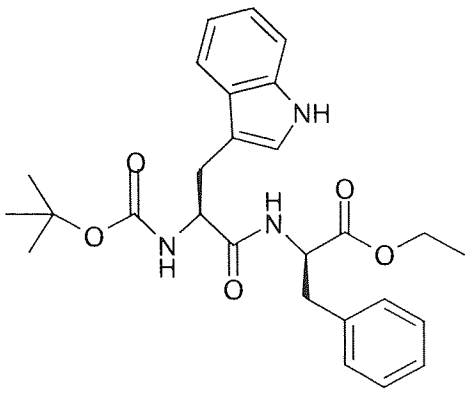
Mass spectrometric analyses was obtained by Atmospheric Pressure Chemical Ionisation (APCI), negative or positive mode, using a Hewlett-Packard 5989b quadrupole instrument. This was connected to an electrospray 59987A unit with an automatic injection (Hewlett-Packard 1100 series autosampler). Samples were dissolved in HPLC grade methanol, toluene or acetonitrile. Both Proton and Carbon NMR spectra were obtained on a brucker AC 250 instrument, operating at 250 MHz, calibrated with the solvent reference peak or TMS.

IR spectra were plotted from KBr discs on a Mattson 300 FTIR Spectrophotometer. Melting points were recorded from a Stuart Scientific Melting Point (SMP1) and are uncorrected. Analytical Thin Layer Chromatography was obtained using aluminium sheets, silica gel_{60} F254 and visualized using ultraviolet light. Preparative chromatography was performed on 250 μm, 20 x 20 cm silica gel TLC plates, obtained from Aldrich. Jencons sonomatic sonicator (SO175) was used to prepare samples for screening. All compounds, for screening, were prepared to 1 μM in HPLC grade DMSO.

Small scale solution syntheses was carried out on a carousel reaction stations (RR 98030), with 12 place carousel reaction station and reflux head and 12 x flexible tubing from Radleys, on a RCT basic hotplate from IKA Labortechnik with IKATRON ETS D3 temperature controller or by using heating blocks (TECHNE Dri-block DB-3A).

9.2. Experiments to Chapter 2

(1) N α-Boc-L-Tryptophan-L-Phenylanine ethyl ester



N α-Boc-L-Tryptophan (0.73g, 2.4 mmol) was dissolved in DCM/DMF: (4:1, 15ml). DIC (0.45 ml, 1.5 Eq) was added, while stirring the mixture. Once dissolved, L-Phenylalanine ethyl ester.HCl (0.60 g, 2.6 mmol) was added. Additional DIC (0.30 ml, 1.0 Eq) was added and left at ambient temperature for 14 hrs, with a drying tube. TLC (diethyl ether) indicated the presence of a new, less polar compound and reduction of the reactants. Column chromatography of the remaining mixture yielded a pure white powder.

Yield: 64.6 %.

R_f (ether) = 0.29.

Mp: 91-101 ^{0}C.

Mol. Formula: $C_{27}H_{32}N_3O_5$.

Mol. Weight: 478.

IR (KBr-disc) υ: 3430, 3354, 2971, 2930, 1723, 1660, 1518, 1479, 1281, 1168 cm^{-1}.

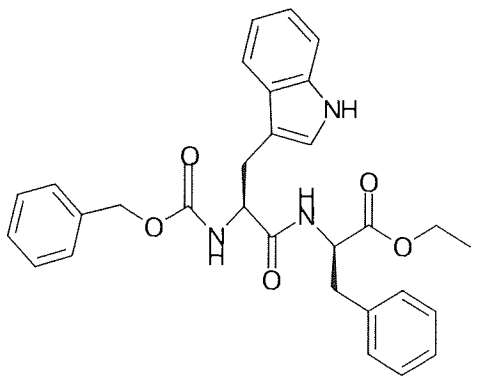
MS (APCI(+)): 479 (M+H), 380 (M-boc group), 368 (M+) m/z;

^{1}H NMR (DMSO-d_6) 300K δ: 1.30 (s, ($C\underline{H}_3$)$_3$), 1.09 (t, CH_3, J= 3.7, 3.9 Hz), 4.05 (q, $C\underline{H}_2$-CH_3, 7.0, 7.1, 7.1 Hz), 4.21 (dd, -CH-phenyl, 7.2, 7.3 Hz), 4.30 (t, -CH-ester, J= 5.8, 6.0 Hz), 4.32 (d, -CH_2-Ar, J= 6.9 Hz), 4.35 (t, -CH-Ar, J= 6.5, 6.7 Hz), 6.71 (d, Ar-H, 8.5

Hz), 7.00 (t, Ar-H, J= 7.7, 7.4 Hz), 7.08 (s, phenyl-5H), 7.30 (t, Ar-H, J= 7.8, 7.5 Hz), 7.56 (d, Ar-H, 7.2 Hz), 7.24 (m, Ar-4H), 8.07 (d, NH-ester, J= 7.9 Hz), 8.30 (d, NH-boc, J= 7.6 HZ), 10.80 (s, NH), p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 14.99 (CH_2-$\underline{C}H_3$) 29.1 ((CH_3)$_3$), 38.4 (-CH_2-Ar), 51.9 (CH-ester), 55.3 (-$\underline{C}$H-CH_2-), 61.1 ($\underline{C}H_2$-CH_3), 79.9 ($\underline{C}$-(CH_3)$_3$), 105.9, 113.1, 120.9, 121.0, 121.9, 123.3, 126.9, 128.0, 128.6 (2 C), 129.3 (2 C), 137.9, 138.8 (Ar C), 155.9 (C=O-boc group), 172.3 (C=O-ester), 172.7 (C=O-NH) p.p.m.

(2) N α-Z-L-Tryptophan-L-Phenylanine ethyl ester



In a dry flask, connected with a drying tube, N α-Z-L-Tryptophan (1.06g, 3.0 mmol) and DIC (0.73 ml, 1.5 Eq) were dissolved and stirred in acetonitrile/DMF: (4:1, 20ml). L-Phenylalanine ethyl ester.HCl (0.83 g, 3.6 mmol) were added with a further amount of DIC (0.73 ml, 1.5 Eq) and kept at RT. The reaction was judged complete after 24 hrs. Two thirds of the solvent was removed. Column chromatography (diethyl ether) of the remaining residue gave a white crystalline product.

Yield: 78.0 %

R_f (ether) = 0.45.

Mp: 79-83 ^{0}C.

Mol. Formula: $C_{30}H_{31}N_3O_5$.

Mol. Weight: 513.

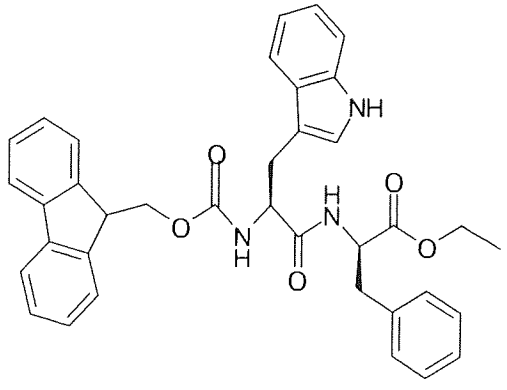
IR (KBr-disc) υ: 3396, 3308, 3059, 2972, 1715, 1670, 1534, 1447, 1210, 1027 cm^{-1}.

MS (APCI(+)): 514 (M+H), 470(M-z group), 453, 380 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 1.09 (t, CH_3, J= 7.2, 7.2 Hz), 2.88 (m, CH_2), 4.04 (q, $C\underline{H}_2$-CH_3, 7.0, 7.1, 7.1 Hz), 3.00 (m, CH_2), 4.32 (m, -CH-), 4.56 (m, -CH-), 4.93 (s, Ar-CH_2-COO-), 6.97 (t, Ar-H, J= 6.9, 6.8), 7.06 (t, Ar-H, 7.0, 6.8), 7.11 (s, Ar-H), 7.23 (m, phenyl), 7.26 (d, Ar-H, 7.9 Hz), 7.30 (m, phenyl-5H), 7.33 (d, Ar-H, J= 8.3 Hz), 7.64 (d, NH-ester, J= 7.7 Hz), 8.43 (d, NH-z, J= 7.5 Hz), 10.81(s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 14.5 (CH_2-$\underline{C}H_3$) 29.1 (-CH_2-Ar), 38.4 (-CH_2-phenyl), 52.1 (CH-ester), 55.6 (-$\underline{C}$H-CH_2-), 61.2 ($\underline{C}H_2$-CH_3), 67.7 (CH_2-Ar), 106.1, 133.3, 127.2,127.7 (2xC), 127.9 (2xC), 128.1, 128.6 (2xC), 129.0 (2xC), 136.5, 137.8, 138.6, 158.1.3 (C=O-ester), 171.5 (C=O-z-group), 171.2 (NH-C=O) p.p.m.

(3) N α-Fmoc-L-Tryptophan-L-Phenylanine ethyl ester



Method (A):

N α-Fmoc-L-Tryptophan (0.051g, 0.12 mmol) was dissolved in DCM/DMF: (4:1, 15ml). DIC (0.025 ml, 1.5 Eq) was added, while stirring. L-Phenylalanine ethyl ester.HCl (0.023 g, 0.1 mmol) was added. Additional DIC (0.015 ml, 1.0 Eq) was added and left at RT. After 10 hrs, the reactants were still present. Additional N α-Fmoc-L-Tryptophan (0.015g, 0.05 mmol) and DIC (0.015 ml, 1.0 Eq) were added in DCM/DMF: (4:1, 5ml). After 8 hrs the reaction was judged complete.

Method (B):

N α-Fmoc-L-Tryptophan (2.13g, 5mmol) in a dry flask and DIC (1.2 ml, 1.5 Eq) were dissolved and stirred at RT in DCM/acetonitrile (4:1, 50ml). L-Phenylalanine ethyl ester.HCl (1.72 g, 7.5 mmol) was added with DIC (1.2 ml, 1.5 Eq). Reaction was judged complete after 20 hrs. One third of the solvent was evaporated and the flask was allowed to stand for 3 days. The product was filtered, washed and dried to give a highly pure product.

Yield: 67.6 % (based on method B)

R_f (ether) = 0.48.

Mp: 153-163 ^{0}C.

Mol. Frmula: $C_{37}H_{35}N_3O_5$.

Mol. Weight: 601.

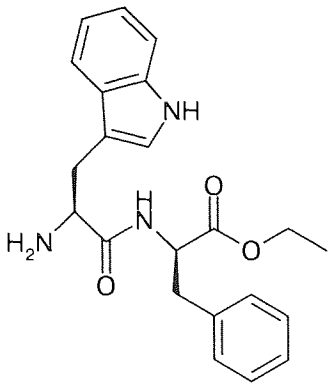
IR (KBr-disc) υ: 3388, 2969, 2921, 2360, 1733, 1644, 1540, 1510, 1447, 1217, 1038, 740, 506 cm^{-1}.

MS (APCI(+)): 602 (M+H), 380 (M-fmoc group) m/z.

^{1}H NMR ($CDCl_3$) 300K δ: 1.08 (t, CH_3, J= 7.1, 7.1 Hz) 3.17 (m, CH_2), 3.74 (m, CH_2), 4.07 (q, $\underline{C}H_2$-CH_3, J= 7.1, 7.2, 7.2 Hz), 4.20 (t, -CH-fmoc), 4.42 (m, -CH_2-fmoc), 4.51 (m, CH), 4.70 (m, CH), 6.98 (t, Ar-H, 7.0, 7.3 Hz), 7.07 (t, Ar-H, 6.8, 7.2 Hz), 7.15 (s, Ar-H), 7.23 (m, phenyl-5H), 7.31 (t, Ar-2H), 7.39 (t, Ar-2H), 7.64 (d, fmoc-2H, J= 7.5 Hz), 7.86 (d, fmoc-2H, J= 7.7 Hz), 8.47 (d, Ar-H, J= 7.7 Hz), 8.04 (NH), 10.81(s, Ar-NH), p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 14.7 (CH_2-$\underline{C}H_3$) 29.2 (-CH_2-Ar), 38.3 (-CH-phenyl), 48.1 (CH-fmoc), 52.5 (CH-ester), 55.1 (-$\underline{C}$H-CH_2-), 61.9 ($\underline{C}H_2$-CH_3), 70.3 (CH_2-fmoc), 106.5, 113.1, 120.2, 120.4 (2xC), 121.2, 122.3, 122.9, 124.8 (2xC), 127.0 (2xC), 127.3, 127.6, 127.7 (2xC), 128.7 (2xC), 129.9 (2xC), 138.2, 139.2, 142.0 (2xC), 149.6 (2xC), 157.4 (C=O-fmoc), 170.9 (C=O-ester), 172.1 (NH-C=O) p.p.m.

L-Tryptophan-L-Phenylalanine ethyl ester



A solution of N α-Fmoc-Ltryptophan-L-phenylanine ethyl ester (0.06 g, 1.0 mmol) was dissolved in dimethylformamide (10 ml). Diethylamine (1.0 ml) was added dropwise and the reaction was allowed to proceed at room temperature. After 1.5 hrs, the solvent was removed and the remaining residue was triturated with diethyl ether (2 x 25 ml) and allowed to stand 12 hrs. The solid was collected, washed with diethyl ether (20 ml x 2), and dried.

Yield: 50 %.

R_f (ether) = 0.39.

Mp: decomposes.

Mol. Formula: $C_{22}H_{25}N_3O_3$.

Mol. Weight: 379.

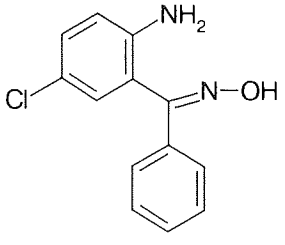
IR (KBr-disc) υ: 3337, 3058, 2931, 1682, 1538, 1344, 1211, 1026, 805, 740, 699 cm^{-1}.

MS (APCI(+)): 380 (M+H), 368 (M-OH) m/z.

^1H NMR (DMSO-d_6) 300K δ:1.11 (t, CH_3, J= 7.0, 6.9 Hz) 3.01 (m, CH_2), 3.78 (m, CH_2), 3.98 (m, CH), 4.06 (q, $C\underline{H}_2$-CH_3, 7.0, 7.1, 7.3 Hz), 4.53 (m, CH), 6.96 (d, Ar-H, J= 7.5 Hz), 7.05 (t, Ar-H, J= 7.0, 7.5 Hz), 7.08 (d, Ar-H, J= 7.3 Hz), 7.17 (m, phenyl-4H), 7.34 (t, Ar-H, 7.3, 7.0 Hz), 7.48 (d, Ar-H, J= 7.9 Hz), 7.65 (d, Ar-H, J= 7.4 Hz), 7.70 (s, NH), 7.90 (s, NH), 10.91 (s, Ar-NH), p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 14.4 (CH_2-$\underline{C}H_3$) 32.1 (-CH_2-Ar), 37.2 (-CH_2-phenyl), 53.0 (CH-ester), 54.5 (CH-NH_2), 61.3 ($\underline{C}H_2$-CH_3), 107.3, 112.0, 118.9, 121.6, 125.5, 126.8, 127.2, 127.6, 128.9 (2xC), 129.6 (2xC), 136.8, 137.2, 169.5 (C=O-ester), 171.3 (C=O-NH) ppm.

(2-amino-5-chlorophenyl)(phenyl)methanone oxime



2-Amino-5-chlorobenzophenone (160 mmol, 37.08g) and hydroxylamine.HCl (320 mmol, 11.12g) were placed in a dry round bottom flask. Ethanol (230 ml) and pyridine (50 ml) were added and the mixture were refluxed for 72 hrs. The solution was allowed to cool and one third of the solvent was removed.

(A): The remaining residue was partitioned first with ether then water. The organic phase was washed with water and dried over magnesium sulphate and then concentrated to dryness. The yellow solid was heated with a minimum amount of toluene and then cooled to room temperature and allowed to crystallise.

(B): The remaining residue was precipitated with water. The solid was filtered, washed with water and then heated with a minimum amount of toluene and then cooled to room temperature and allowed to crystallise.

Yield: 89% (including second crop).

Mp: 162-165 ^{0}C.

Mol. Formula: $C_{13}H_{11}N_2OCl$.

Mol. Weight: 246.7.

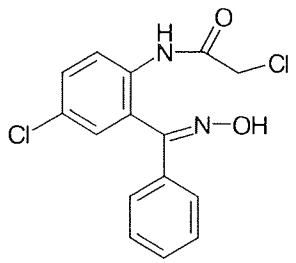
IR (KBr-disc) υ: 3398, 3376, 3018, 2950, 1605, 826, 744 cm^{-1}.

MS (APCI (+)): 247, 249 (M+H), 229, 231 (M-H_2O) m/z.

^1H NMR (DMSO-d_6) 300K δ: 4.80 (s, OH), 6.77 (d, Ar-H, J=5.3 Hz), 7.13 (d, Ar-H, J=6.3 Hz), 6.80 (s, Ar-H), .37 (m, Phenyl-H), 11.57 (s, NH) p.p.m.

^{13} C NMR (DMSO-d_6) 300K δ: 116.7, 121.1, 126.9, 127.1, 127.8, 128.0, 128.6, 130.0, 130.9, 132.7 (Ar- C), 149.2 (C-NH_2), 156.6 (C=N) p.p.m.

2-chloro-N-{4-chloro-2-[(hydroxyimino)(phenyl)methyl] phenyl} acetamide



A solution of 2-amino-5-chlorobenzophenone syn-oxime (142 mmol, 35.18g) in diethylether (1000 ml) and water (300 ml) were stirred in an ice bath at 0-5 ^{0}C. Chloroacetyl-chloride (160 mmol, 12.8 ml) was added dropwise, over 30 mins, whilst maintaining a slightly basic solution with the addition of 15% aqueous sodium hydroxide. After the addition of chloroacetyl chloride the reaction was stirred for additional 3 hrs at room temperature. The organic phase was washed with water, dried over magnesium sulphate and concentrated to dryness to yield a white powder.

Yield: 94%.

Mp: 174-176 ^{0}C.

Mol. Formula: $C_{15}H_{12}N_2O_2Cl_2$.

Mol. Weight: 323.2.

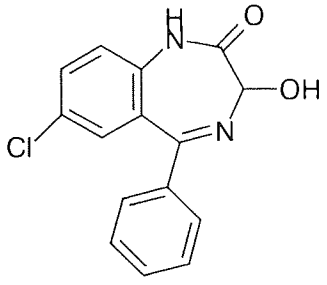
IR (KBr-disc) υ: 3388, 3305, 3015, 2989, 2874, 1660, 820 cm^{-1}.

MS (APCI(+)): 323, 325 (M+H), 305, 307 ($M-H_20$) m/z.

^1H NMR (DMSO-d_6) 300K δ: 4.06 (s, -CH_2-), 7.20 (s, Ar-H), 7.39 (s, Phenyl-H), 7.53 (d, Ar-H, J=8.7 Hz), 7.83 (d, Ar-H, J=8.8 Hz), 9.25 (s, N-OH), 11.92 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 43.1 (-CH_2-), 124.9, 125.8, 127.9, 130.1, 130.8, 132.2, 132.8, 133.1, 133.9, 136.0, 136.9 (Ar-C), 137.1 (C-NH-), 157.2 (C=N-OH) p.p.m.

7-chloro-5-phenyl-1, 3-dihydro2H-1,4-benzodiazepin-2-one (Oxazepam)



A solution of 2-chloroacetamido-5-chlorobenzephenone (134 mmol, 43.28g) in ethanol (900 ml) and sodium hydroxide (2M, 280 ml) were stirred at room temperature over night. The precipitate that formed was separated by filtration and dissolved in a minium amount of ethanol, water 60:40 mix, (undissolved Oxazepam salt was collected and dried). The mixture was acidified to pH 1.0-2.0 by the addition of concentrated hydrochloric acid. The filtrate was cooled in a ice bath to 0^0C-10^0C over night. The precipitate was filtered and dried to give the crude product (light brown solid).

Yield: 59.8% (uncrystallised).

Mp: 191-193 ^0C.

Mol. Formul: $C_{15}H_{11}N_2O_2Cl$.

Mol. Weight: 286.7.

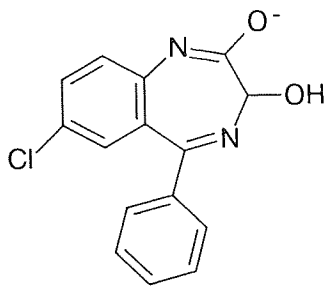
IR (KBr-disc) υ: 3422, 3383, 3025, 3319, 3049, 2877, 1704, 1590, 746 cm^{-1}.

MS (APCI(+)): 287, 289 (M+H), 269, 271 (M-H_2O) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 4.78 (d, C_3-H, J= 8.7 Hz), 6.33 (d, OH, J= 8.7 Hz), 7.64 (d, Ar-H, J= 8.7 Hz), 7.23 (s, Ar-H), 7.25 (d, Ar-H, J= 8.8 Hz), 7.48 (s, Phenyl-H), 10.81 (s, N-H), p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 83.3 (C_3-OH), 123.7, 127.1, 128.3, 128.9, 128.5 (2xC), 129.7, 129.8 (2xC), 131.0, 132.3, 138.5 (Ar-C), 165.9 (C=N), 168.7 (C=O), p.p.m.

7-chloro-5-phenyl-3H-1, 4-benzodiazepin-2, 3-diol (Oxazepam salt)



Yield: 22.3%.

Mp: 202-208 ^{0}C.

Mol. Formula: $C_{15}H_{11}N_2O_2Cl$.

Mol. Weight: 286.7.

IR (KBr-disc) υ: 3347, 3032, 2949, 1687, 1594, 1206, 760 cm^{-1}.

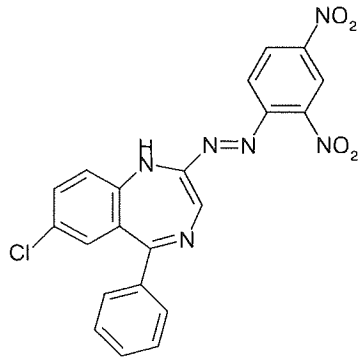
APCI(+): 287 (M+H), 269 (-H_2O) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 4.25 (s, C_3-H), 5.90 (s, C-OH), 6.94 (d, Ar-H, J= 8.6 Hz), 6.97 (s, Ar-H), 7.30 (d, Ar-H, J= 8.8 Hz), 7.87 (m, Phenyl–H), p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 83.5 (CH-OH), 122.7, 126.5, 127.2, 128.7 (2xC), 129.2, 129.6, 129.9 (2xC), 131.3, 132.8, 139.5 (Ar-C), 168.7 (C=N), 194.0 (C=O) p.p.m.

9.3 Experiments to Chapter 3

(3.2.1) 7-chloro-2-[(E)-2-(4-dinitrophenyl) diazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Method: A solution of Oxazepam (0.1 g, 3.5 $x10^{-4}$ mol) in ethanol (10-20 ml), with 3-5 drops of glacial acetic acid was refluxed for 20-25 hours with the appropriate amine/hydrazine ($3.56x10^{-4}$ mol). TLC monitored the reaction progress (MP: diethyl ether). The precipitate was filtered, washed with ethanol (twice), dried and was exceptionally pure.

Yield: 70 %

Mol. Weight: 449.8

Mol. Formula: $C_{21}H_{13}ClN_6O_4$

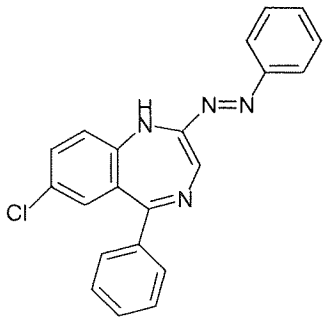
MS (APCI(+)): 404, 406 (M+1) m/z.

IR (KBr-disc) υ max: 3445, 3197, 3033, 1595, 1579, 1558, 1528, 1502, 1342, 1281, 1265, 1147, 1110 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 11.86 (s, NH), 8.24 (s, Ar-H), 8.19 (d, Ar-2H, J= 9.3 Hz), 8.12 (s, C3-H), 8.04 (dd, Ar-H, J= 9.0 Hz), 7.92 (d, Ar-H, J= 2.3 Hz), 7.81-7.85 (m, phenyl-2H), 7.67-7.69 (m, phenyl-3H), 7.26 (d, Ar-H, J= 9.2 Hz) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 112.7 (2xC), 122.3, 126.0, 126.5 (2xC), 129.3 (2xC), 130.3 (2xC), 130.8, 131.2, 132.7, 135.4, 136.7, 140.0, 140.5, 149.9 (Ar-C), 150.3 (C3), 158.1(C-N=), 168.2 (C=N) p.p.m.

(3.2.2) 7-chloro-2-[(E)-2-(4-chlorophenyl)diazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Yield: 65%

Mol. Weight: 393.3

Mol. Formula: $C_{21}H_{14}Cl_2N_4$

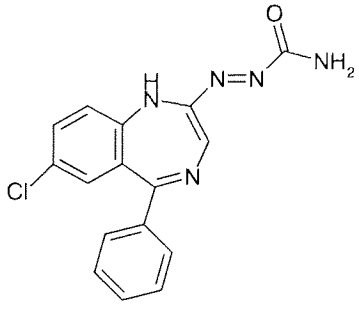
MS (APCI(+)): 393, 395 (M+1) m/z.

IR (KBr-disc) υ max:3436, 2929, 1611, 1490, 1390, 1280, 1143, 1085, 823, 703 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 11.30 (s, NH), 8.11 (s, C3-H), 8.01-8.10 (d, Ar-H, J= 8.8 Hz), 7.99-8.04 (dd, Ar-H, J= 9.0 Hz), 7.90 (s, Ar-H), 7.77-7.85 (m, phenyl-2H), 7.65-7.69 (m, phenyl-3H), 7.30-7.35 (d, Ar-2H, J=8.9 Hz), 7.15-7.18 (d, Ar-2H, J=8.9 Hz) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 124.1, 126.0, 126.9 (2xC), 128.3, 128.5, 129.3 (2xC), 130.4, 130.6 (2xC), 132.1 (2xC), 132.2, 133.5, 133.8, 143.7, 150.2 (Ar-C), 152.9 (C3), 152.9 (C-N=N), 169.2 (C=N) p.p.m.

(3.2.3) (E)-2-(7-chloro-5-phenyl-1H-1, 4-benzo-diazepin-2-yl)diazene-1-carboxamide



Yield: 50 %

Mol. Weight: 325.8

Mol. Formula: $C_{16}H_{12}ClN_5O$

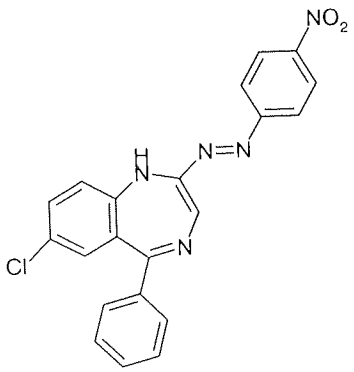
MS (APCI(+)): 326, 328 (M+1), 309, 311 (-NH_2), 283+ m/z.

IR (KBr-disc) υ max: 3430, 2975, 2925, 2360, 1720, 1680, 1585, 1535, 1410, 1390, 1345, 1180, 1100, 835 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 10.89 (s, NH), 8.13 (d, Ar-2H, J= 9.0 Hz), 8.04 (d, Ar-H, J= 2.3 Hz), 7.95 (d, Ar-H, J= 2.1 Hz), 7.80-7.85 (m, phenyl-2H), 7.63-7.67 (m, phenyl-3H), 6.52 (s, NH_2) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 125.3 (2xC), 126.7, 127.1, 130.5, 130.7, 131.0, 131.8 (2xC), 132.3, 136.2, 140.9 (Ar-C), 146.4 (C3), 164.8 (C-N=), 166.1(C=O), 167.2 (C=N) p.p.m.

(3.2.4) 7-chloro-2-[(E)-2-(2,4-dinitro-phenyl)diazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Yield: 67 %

Mol. Weight: 403.8

Mol. Formula: $C_{21}H_{14}ClN_5O_2$

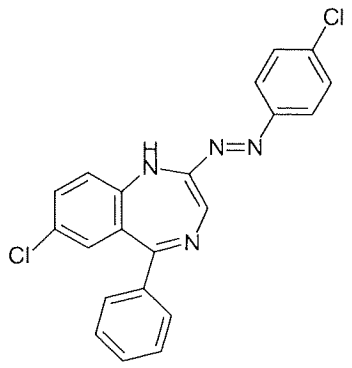
MS (APCI(+)): 404, 406 (M+1) m/z.

IR (KBr-disc) υ max: 3440, 3290, 2915, 2360, 1620, 1505, 1330, 1320, 1135, 1085, 830 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 11.96 (s, NH), 8.82 (d, Ar-H, J= 2.7 Hz), 8.43 (dd, Ar-H, J= 9.5 Hz), 8.14 (d, Ar-H, J= 9.1 Hz), 8.07 (d, Ar-H, J= 7.7 Hz), 8.05 (d, Ar-H (overlapping), J= 8.9 Hz), 7.93 (d, Ar-H, J= 2.1 Hz), 7.83-7.87 (m, phenyl-2H), 7.66-7.70 (m, phenyl-3H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 120.9, 121.8, 122.5, 124.7, 125.1 (2xC), 127.9, 128.5, 130.3, 131.2, 131.7 (2xC), 132.6, 133.8, 134.9, 136.4, 140.2 (Ar-C), 144.6 (C3), 153.5, 154.3 (C-N=), 168.0 (C=N) p.p.m.

(3.2.5) 7-chloro-2-[(E)-2-(4-chlorophenyl)diazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Yield 65%

Mol. Weight: 393.3

Mol. Formula: $C_{21}H_{14}Cl_2N_4$

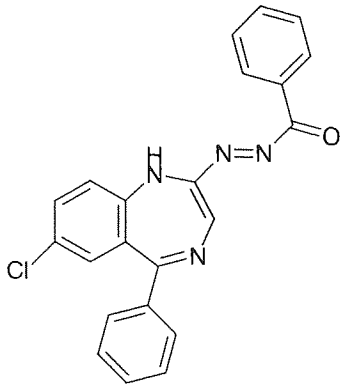
MS (APCI(+)): 393, 394, 395 (M+1) m/z.

IR (KBr-disc) υ max:3436, 2929, 1611, 1490, 1390, 1280, 1143, 1085, 823, 703 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 11.30 (s, NH), 8.11 (s, C3-H), 8.01-8.10 (d, Ar-H, J= 8.8 Hz), 7.99-8.04 (dd, Ar-H, J= 9.0 Hz), 7.90 (s, Ar-H), 7.77-7.85 (m, phenyl-2H), 7.65-7.69 (m, phenyl-3H), 7.30-7.35 (d, Ar-2H, J=8.9 Hz), 7.15-7.18 (d, Ar-2H, J=8.9 Hz) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 124.1, 126.0, 126.9 (2xC), 128.3, 128.5, 129.3 (2xC), 130.4, 130.6 (2xC), 132.1 (2xC), 132.2, 133.5, 133.8, 143.7, 150.2 (Ar-C), 152.9 (C3), 152.9 (C-N=N), 169.2 (C=N) p.p.m.

(3.2.6) [(E)-2-(7-chloro-5-phenyl-1H-1,4-benzodiazepin-2-yl)diazenyl] (phenyl)-methanone



Yield: 41%

Mol. Weight: 386.8

Mol. Formula: $C_{22}H_{15}ClN_4O$

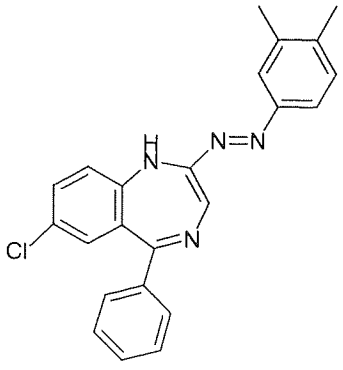
MS (APCI(+)): 387, 388, 389 (M+1) m/z.

IR (KBr-disc) υ max: 3395, 3060, 2358, 1661, 1560, 1531, 1474, 1386, 1264, 1131, 1074, 835, 701 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 12.28 (s, NH), 8.69 (s, C3-H), 8.17-8.21 (d, Ar-H, J= 8.9 Hz), 8.07-8.11 (dd, Ar-H, J= 9.0, 8.9 Hz), 7.95-7.99 (m, phenyl-2H), 7.81-7.85 (m, phenyl-3H), 7.53-7.69 (m, Ar-H & s, Ar-H overlapping) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 122.7, 126.1 (2xC), 128.4, 129.1, 129.3 (2xC), 130.4 (2xC), 130.5, 130.9, 131.5, 131.6, 133.4 (2xC), 133.6, 135.7, 136.5 (Ar-C), 150.0 (C3), 158.0 (C-N=N) 168.3(C=O), 182.8 (C=N) p.p.m.

(3.2.7) 7-chloro-2-[(E)-2-(3,4-dimethylphenyl)diazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Yield: 61%

Mol. Weight: 386

Mol. Formula: $C_{23}H_{19}ClN_4$

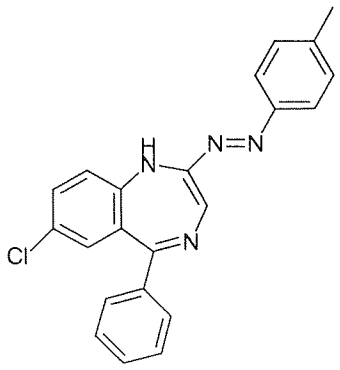
MS (APCI(+)): 387, 389 (M+1) m/z.

IR (KBr-disc) υ max:3443, 2921, 2856, 2364, 1569, 1517, 1382, 1261, 832, 699 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 13.85 (s, NH), 8.37-8.45 (dd, Ar-H, J=8.9 Hz), 8.05-8.14 (dd, Ar-H, J=9.0 Hz) 8.01-8.08 (d, Ar-H, J=8.9 Hz), 7.97 (s, C3-H), 7.79-7.91 (m, phenyl-2H), 7.62-7.74 (m, phenyl-3H & overlapping Ar-H), 7.41 (s, Ar-H), 7.20 (s, Ar-H), 2.25 (s, CH_3), 2.18 (s, CH_3) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 19.3 (CH_3), 20.2 (CH_3), 121.4, 122.0, 125.9, 127.5, 128.2, 129.2 (2xC), 129.3, 129.9 (2xC), 130.3, 130.3, 130.7, 132.9, 133.0, 134.6, 142.7, 148.7 (Ar-C), 150.2 (C3), 159.3 (C-N=N), 167.8 (C=N) p.p.m.

(3.2.8) 7-chloro-2-[(E)-2-(4-methylphenyl)diazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Yield: 66%

Mol. Weight: 372.8

Mol. Formula: $C_{22}H_{17}ClN_4$

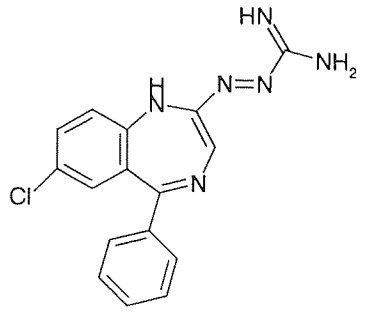
MS (APCI(+)): 373, 375 (M+1) m/z.

IR (KBr-disc) υ max:3428, 3060, 2923, 2364, 1560, 1519, 1390, 1253, 811, 699 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 13.86 (s, NH), 8.38-8.41 (dd, Ar-H, J=9.0 Hz), 8.08-8.14 (dd, Ar-H, J=9.0 Hz), 7.96 (s, Ar-H), 7.78-7.85 (m, phenyl-2H), 7.62-7.69 (m, phenyl-3H), 7.42 (s, C3-H), 7.26-7.30 (d, Ar-2H, J=8.4 Hz), 7.13-7.17 (d, Ar-2H, J=8.2 Hz), 2.23 (s, CH_3) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 19.5 (CH_3), 126.2, 126.4 (2xC), 128.1 (2xC), 129.2, 129.4, 131.3 (2xC), 130.6, 131.2 (2xC), 132.8, 132.9, 133.3, 133.6, 149.0, 149.9 (Ar-C), 149.7 (C3), 158.8 (C-N=N), 167.2 (C=N) p.p.m.

(3.2.9) (E)-2-(7-chloro-5-phenyl-1H-1, 4-benzodiazepin-2-yl)diazene-1-carboximidamide



Yield: 53 %

Mol. Weight: 324.8

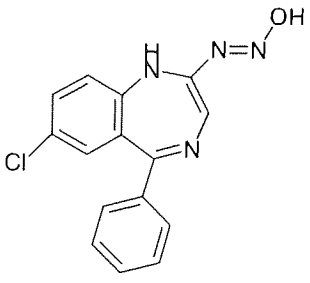
Mol. Formula: $C_{16}H_{13}ClN_6$

MS (APCI(+)): 325, 327 (M+1), 308, 310 (-NH_2) m/z.

IR (KBr-disc) υ max: 3423, 2923, 2360, 1627, 1519, 1388, 1121, 620 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 11.04 (s, NH), 8.33 (s, C3-H), 8.08-8.12 (d, Ar-H, J= 9.0 Hz), 8.01-8.06 (dd, Ar-H, J=8.9,9.0 Hz), 7.90 (s, Ar-H), 7.79-7.83 (m, phenyl-2H), 7.64-7.69 (m, phenyl-3H), 7.20 (s, NH_2) p.p.m.

(3.2.10) 7-chloro-2-[(E)-2-hydroxydiazenyl]-5-phenyl-1H-1, 4-benzodiazepine



Yield: 47 %

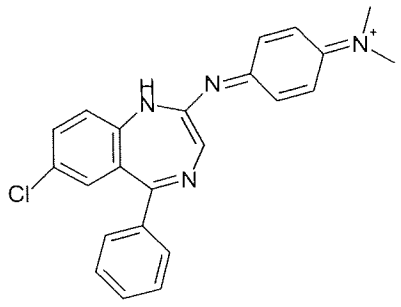
Mol. Weight: 298.7

Mol. Formula: $C_{15}H_{11}ClN_4O$

MS (APCI(+)): 299, 301 (M+1), 281, 283 m/z.

IR (KBr-disc) υ max: 3448, 2921, 2849, 2358, 1550, 1484, 1388, 1106, 840, 799, 705 cm^{-1}. ^1H NMR (DMSO-d_6) 300K δ: 12.14 (s, NH), 8.29 (s, C3-H), 8.11-8.15 (d, Ar-H, J= 9.0 Hz), 7.97-8.09 (dd, Ar-H, J=8.9 Hz), 7.98 (s, Ar-H), 7.78-7.82 (m, phenyl-2H), 7.64-7.67 (m, phenyl-3H) p.p.m.

(3.2.14) 7-chloro-N-[1-(dimethylamino)pyridin-4(1H)-ylidene]-5-phenyl-1H-1,4-benzo diazepin-2-amine



Yield: 62.0 %

Mol. Weight: 387.9

Mol. Formula: $C_{23}H_{20}ClN_4$

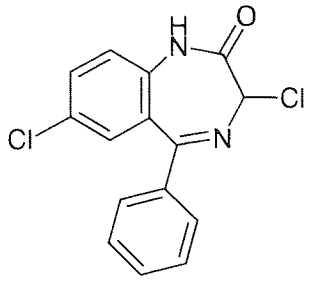
MS (APCI(+)): 387, 389 (M+1) m/z.

IR (KBr-disc) υ max: 3433, 2889, 2803, 1624, 1573, 1518, 1477, 1438, 1500, 1347, 1280, 1159, 826 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 8.95 (s, NH), 8.24 (d, Ar-H, J= 9.0 Hz), 8.09 (d, Ar-H, J= 2.3 Hz), 7.86 (dd, Ar-H, J= 9.1 Hz), 7.81-7.84 (m, phenyl-2H), 7.61-7.63 (m, phenyl-3H), 7.55 (d, Ar-H, J= 6.9 Hz), 7.27 (s, C3-H), 6.76 (d, Ar-H, J= 7.0 Hz), 3.04 (s, 2x CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 40.3 (2x CH_3), 112.1 (2xC), 122.8, 123.8 (2xC), 125.9, 128.8 (2xC), 129.9 (2xC), 130.3, 131.3, 133.9, 134.9, 136.5, 138.9, 150.2, 150.5, 153.3 (Ar-C), 158.7 (C-N=), 168.3 (C=N) p.p.m.

3,7-dichloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Oxazepam (0.5 g, 1.75 $x10^{-3}$ mol) was treated with thionyl chloride (4 Eq, 0.4 ml) and heated to 60 ^{O}C for 1.5 hours. The resulting intermediate, yellow solid, was washed with dry diethyl ether (twice), to remove any excess thionyl cholride.

Yield: 93.4%.

Mol. Formula: $C_{15}H_{10}N_2O_2Cl_2$

Mol. Weight: 305.2.

IR (KBr-disc) υ max: 3418, 3220, 3060, 2919, 1704, 1606, 1476, 1322, 1226, 902, 823, 693 cm^{-1}.

MS (APCI(+)): 305, 306, 307 (M+1), 269, 270, 271 m/z.

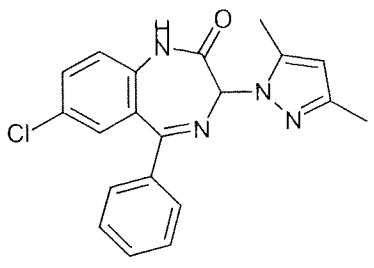
^{1}H NMR ($CDCl_3$) 300K δ: 9.89 (s,NH), 7.26-7.80 (m,Ar-H, 8H), 5.64 (s, C3-H) p.p.m.

Method for preparing 3-amino-substituted 1,4-benzodiazepin-2-ones

Route A: Oxazepam (0.1 g, 3.5 $x10^{-4}$ mol) was treated with thionyl chloride (4 Eq, 0.1 ml) and heated to 60 ^{O}C for 1.5 hours. The resulting intermediate, yellow solid, was washed with dry diethyl ether (twice) to remove any excess thionyl cholride. The appropriate amine 2.5 Eq, 1.1 $x10^{-3}$ mol), with TEA (drops) was added with dry DCM or dry 2-methoxyethyl ether (15 ml) and refluxed for two hours. The organic phase was washed with hydrochloric acid (pH 4.0–5.0) and dried over sodium sulphate. Excess hexane was added and the mixture was allowed to stand overnight. The precipitate was filtered, washed with hexane and dried.

Route B: Oxazepam (0.2 g, 6.8 $x10^{-4}$ mol) in dry THF (13 ml), and sodium hydride (60% in mineral oil, 0.052 g, 1.0 $x10^{-3}$ mol) was stirred for 1 hour at room temperature under argon. The solution turned light brown in colouration. After 1hour 2-chloro-1, 3, 2-dioxaphospholane (1.0 $x10^{-3}$ mol) was added drop-wise and stirred at room temperature for 2.5 hours. The appropriate amine (1.8 $x10^{-3}$ mol) was added and left overnight at room temperature under argon. The resulting precipitate was filtered, solvent evaporated and the residue purified by preparative chromatography (MP: ethyl acetate).

A (3.4.2) 7-chloro-3-(3, 5-dimethyl-1H-pyrazol-1-yl)-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one



Yield: 69.0 %.

Mol. Formula: $C_{20}H_{17}ClN_4O$.

Mol. Weight: 364.8.

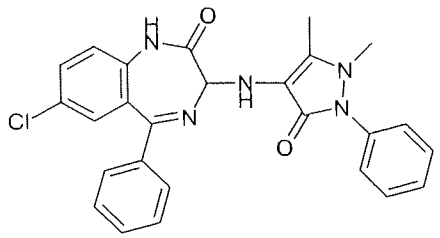
IR (KBr-disc) υ max: 3400, 3020, 2930, 2970, 1695, 1320, 1215, 1100, 790 cm^{-1}.

MS (APCI(+)): 365, 367 (M+1), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.13 (s, NH), 7.73 (dd, Ar-H, J= 8.8 Hz), 7.46-7.56 (m, phenyl-5H), 7.38 (d, Ar-H, J= 8.8 Hz), 7.32 (s, Ar-H), 5.71 (s, Ar-H), 4.79 (s, C3-H), 2.36 (s, $-OCH_3$), 2.09 (s,$-OCH_3$) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 11.0 (CH_3), 13.7 (CH_3), 71.6 (C3), 106.3, 122.9, 125.5 (2xC), 128.2, 129.3, 130.3 (2xC), 130.8, 131.2, 134.1, 135.0, 139.2, 140.7, 156.1, (Ar-C), 162.2 (C=O), 170.9 (C=N) p.p.m.

A (3.4.3) 7-chloro-3-(4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one - 5-phenyl-1,3-dihydro-2-H-1,4-benzodiazepine-2-one



Yield: 58%.

Mol. Formula: $C_{26}H_{22}ClN_5O_2$.

Mol. Weight: 471.94.

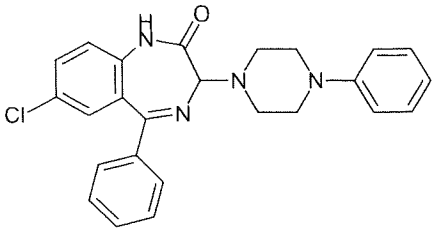
IR (KBr-disc) υ max: 3448, 3254, 2927, 1716, 1625, 1448, 1313, 1168, 696 cm^{-1}.

MS (APCI(+)): 472, 474 (M+1), 454, 456 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 10.95 (s, NH), 7.66-7.70 (dd, Ar-H, J=9.0 Hz), 7.22-7.53 (m, Phenyl-13H), 5.08 (s, C_3-H), 2.86 (s, N-CH_3), 2.20 (s, C-CH_3) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 11.0 (C-$\underline{C}H_3$), 38.2 (N-CH_3), 70.1 (C_3), 118.4 (2xC), 122.9 (2xC), 123.9, 126.1, 127.2, 128.3, 129.0, 129.5 (2xC), 129.8, 129.9, 131.1, 132.5, 135.8 (2xC), 138.2, 138.6, 143.0 (Ar-C), 162.0 (C=O), 164.9 (NH-C=O), 169.0 (C=N) p.p.m.

B (3.4.6) 3-(1-phenylpiperazine)-7-chloro-5-phenyl-1,3-dihydro-2-H-1,4-benzodiazepine-2-one



R_f (ethylacetate) = 0.37.

Yield: 58%.

Mol. Formula: $C_{25}H_{23}ClN_4O$.

Mol. Weight: 430.94.

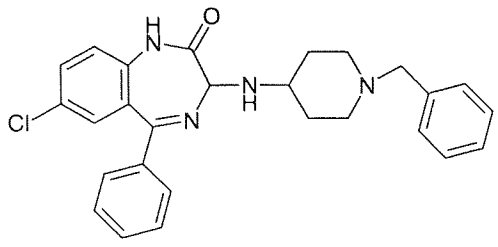
IR (KBr-disc) υ max: 3434, 3049, 2921, 2417, 1704, 1596, 1482, 1324, 1091, 685 cm^{-1}.

MS (APCI(+)): 431, 433 (M+1), 269, 271 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 11.79 (s, NH), 7.76-7.82 (dd, Ar-H, J=9.8 Hz), 7.49-7.65 (m, Phenyl-6H), 7.34 (s, Ar-H), 7.26-7.32 (d, Ar-2H, J=8.3 Hz), 7.02-7.05 (d, Ar-2H, J= 8.1 Hz), 6.85-6.91 (t, Ar-H, J= 7.3, 7.2 Hz), 5.30 (s, C_3-H), 3.40-3.53 (m, -CH_2- 8H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 58.5 (2x-CH_2-N-C_3), 70.0 (2x-CH_2-N-Ar), 71.8 (C_3), 116.4 (2xC), 120.5, 124.7, 128.1, 128.3, 129.2, 129.7 (2xC), 130.4 (2xC), 132.2, 133.3, 134.9, 137.6, 137.8 (2xC), 150.0 (Ar-C), 164.2 (C=O), 167.9 (C=N) p.p.m.

B (3.4.7) 3-benzylpiperidin-4-anilino-7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ethylacetate) = 0.44.

Yield: 35.0 %

Mol. Formula: $C_{27}H_{27}ClN_4O$.

Mol. Weight: 458.9.

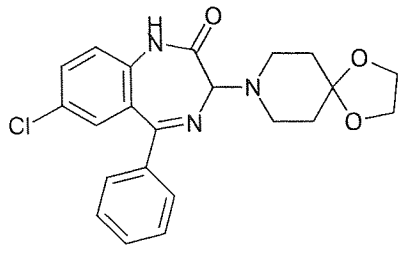
IR (KBr-disc) υ max: 3434, 2828, 2358, 1994, 1602, 1481, 1318, 1120, 742, 699 cm^{-1}.

MS (APCI(+)): 459, 461 (M+1), 269, 271 (M +) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.59 (s, NH), 7.66-7.74 (m, Ar-H, 13H), 4.30 (s, C3-H), 2.74-3.04 (m, CH), 2.66-2.83 (m,-CH_2-Ar), 1.92-2.09 (m,-CH_2-, 4H), 1.40-1.55 (m,-CH_2-, 4H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 32.0 (-CH_2-x2), 38.9 (-CH_2-N x2), 52.5 (CH), 73.1 (C3), 123.8, 127.3, 128.3 (2xC), 128.6, 128.7, 128.9 (2xC), 129.3 (2xC), 129.7 (2xC), 129.9, 131.0, 132.2, 138.3, 138.8, 139.1 (Ar-C), 164.9 (C=O), 167.2 (C=N) p.p.m.

A (3.4.8) 1,4-diaoxaspiro [4.5] decan-8-anilino-7-chloro-5-phenyl-1,3-dihydro-2-H-1,4-benzodiazepine-2-one



Yield: 62%.

Mol. Formula: $C_{22}H_{22}ClN_3O_3$.

Mol. Weight: 411.87.

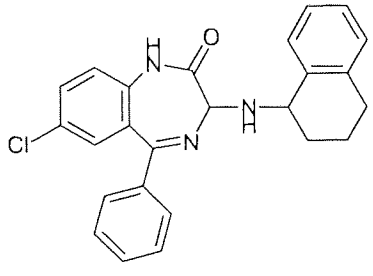
IR (KBr-disc) υ max: 3436, 2915, 2473, 2358, 1706, 1606, 1478, 1081, 695 cm^{-1}.

APCI(+): 412, 414 (M+1), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.65 (s, NH), 7.76-7.80 (dd, Ar-H, J=9.0 Hz), 7.49-7.69 (m, Phenyl-5H), 7.40-7.43 (d, Ar-H, J=9.0 Hz), 7.31 (s, Ar-H), 5.24 (s, C_3-H), 3.39-3.52 (m, -CH_2- 12H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 58.6 (2x-CH_2-C), 70.1 (2x-CH_2-N), 71.8 (2x-CH_2-O), 77.8 (C_3), 104.7 (C-O), 124.7, 128.1 (2xC), 128.3, 129.2 (2xC), 130.4, 132.1, 133.2, 137.5, 137.7 (Ar-C), 164.5 (C=O), 167.8 (C=N) p.p.m.

A (3.4.9) 7-chloro-3-[3, 4-dihydroquinolin-1(2H)-yl]-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one



Yield: 66.0 %.

Mol. Formula: $C_{24}H_{20}ClN_3O$.

Mol. Weight: 401.9.

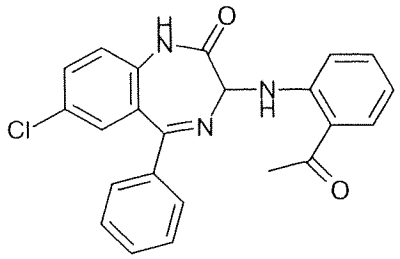
IR (KBr-disc) υ max: 3440, 3050, 2930, 2850, 2360, 1700, 1610, 1480, 830, 740 cm^{-1}.

MS (APCI(+)): 402, 404 (M+1), 384, 386 ($-H_2O$), 269, 271(M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 10.91 (s, NH), 7.72 (dd, Ar-H, J= 8.7 Hz), 7.47-7.55 (m, phenyl-5H), 7.37 (d, Ar-H, J= 8.8 Hz), 7.28 (s, Ar-H), 6.95 (d, Ar-H, J= 7.2 Hz), 6.86 (d, Ar-H, J= 7.4, 7.6 Hz), 6.52 (t, Ar-H, J= 7.4, 7.2 Hz), 6.22 (d, Ar-H, J= 8.2 Hz), 5.22 (s, C3-H), 4.09 (m, -CH-), 3.62 (m, -CH-), 2.77 (m, $-CH_2-$), 1.98 (m, $-CH_2-$) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 22.0, 28.5, 45.5 ($-CH_2-$), 78.5 (C3), 111.5, 117.5, 122.6, 122.7, 125.6 (2xC), 128.1, 128.2, 128.3, 129.3, 129.9, 130.0 (2xC), 130.5, 131.7, 136.9, 138.1, 148.1, (Ar-C), 165.1 (C=O), 166.3 (C=N) p.p.m.

B (3.4.10) 3-(2 acetylanilino)-7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ethylacetate) = 0.35.

Yield: 43.0 %.

Mol. Formula: $C_{23}H_{18}ClN_3O$.

Mol. Weight: 403.8.

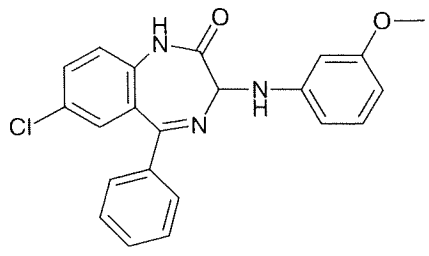
IR (KBr-disc) υ max: 3432, 2923, 2364, 1696, 1612, 1465, 1318, 1094, 742, 699 cm^{-1}.

MS (APCI(+)): 404, 406 (M+1), 386, 388 ($-H_2O$), 269, 271 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 11.50 (s, NH), 7.74-7.68 (m, Ar-H, 12H), 4.30 (s, C3-H), 3.42 (s, CH_3) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 33.8 (CH_3), 73.2 (C3), 123.8, 127.2, 127.3, 128.3, 128.6 (2xC), 128.7, 128.8, 128.9 (2xC), 129.3, 129.7, 129.9, 130.1, 132.2, 138.3, 138.9, 139.1, (Ar-C), 162.5, 164.7 (C=O), 169.2 (C=N) p.p.m.

A (3.4.11) 7-chloro-3-(3-methoxyanilino)-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one



Yield: 45.0 %.

Mol. Formula: $C_{22}H_{18}ClN_3O_2$.

Mol. Weight: 391.9.

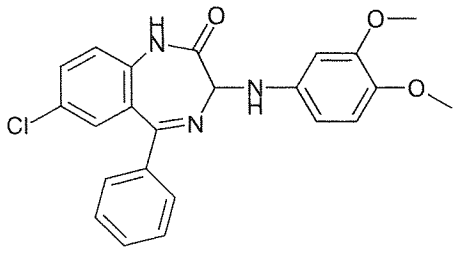
IR (KBr-disc) υ max: 3445, 3210, 3080, 2940, 1690, 1520, 1495, 1230, 1140, 1030 cm^{-1}.

MS (APCI(+)): 392, 394 (M+1), 374, 376 (-H_2O), 269, 271 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 11.20 (s, NH), 7.65 (dd, Ar-H, J= 8.8 Hz), 7.43-7.51 (m, phenyl-5H), 7.31 (s, Ar-H), 7.30 (d, Ar-H, J= 8.7 Hz), 6.98 (t, Ar-H, J= 8.0, 8.0 Hz), 6.45 (d, Ar-H, J= 7.5 Hz), 6.27 (s, Ar-H), 6.22 (m, Ar-H), 4.89 (d, C3-H, J= 7.5 Hz), 3.66 (s, OCH_3) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 55.7 (OCH_3), 66.9 (C3), 104.5, 107.9, 113.0, 122.7, 125.7 (2xC), 128.0, 128.5, 129.8 (2xC), 129.9, 130.8, 131.5, 137.1, 138.2, 142.1 (Ar-C), 160.1 (Ar-O) 164.1(C=O), 167.9 (C=N) p.p.m.

A (3.4.12) 7-chloro-3-(3,4-dimethoxyanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Yield: 50.0 %.

Mol. Formula: $C_{23}H_{20}ClN_3O_3$.

Mol. Weight: 421.9.

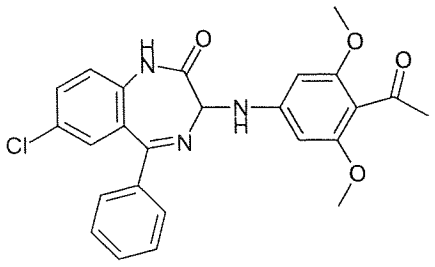
IR (KBr-disc) υ max: 3450, 3215, 3070, 2940, 1695, 1515, 1495, 1230, 700 cm^{-1}.

MS (APCI(+)): 422, 426 (M+1), 404, 406 ($-H_2O$), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.15 (s, NH), 7.70 (dd, Ar-H, J= 8.7 Hz), 7.43-7.48 (m, phenyl-5H), 7.36 (s, Ar-H), 7.33 (d, Ar-H, J= 8.8 Hz), 6.80 (d, Ar-H, J= 8.7 Hz), 6.19 (dd, Ar-H, J= 8.7 Hz), 6.06 (d, Ar-H, J= 7.1 Hz), 5.97 (s, Ar-H), 5.03 (d, C3-H, J= 7.1 Hz), 3.82 (s, OCH_3), 3.61(s, OCH_3) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 55.1, 65.5 (OCH_3), 67.5 (C3), 105.1, 111.7, 122.9, 125.4 (2xC), 128.1, 128.6, 129.9 (2xC), 130.0, 130.8, 132.1, 136.5, 137.1, 140.2 (Ar-C), 152.7, 153.1 (Ar-O-), 165.2 (C=O), 165.9 (C=N) p.p.m

B (3.4.13) 3-(4-acetylanilino)-7-chloro-3-(3, 5-dimethoxy)-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one



R_f (ethylacetate) = 0.35.

Yield: 70.0 %.

Mol. Formula: $C_{25}H_{22}ClN_3O_4$

Mol. Weight: 463.9.

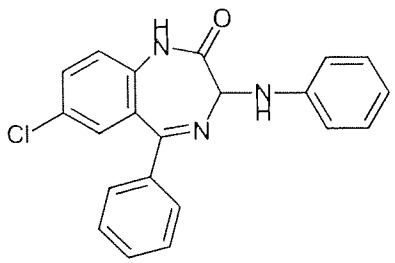
IR (KBr) υ max: 3435, 3025, 2970, 2915, 1700, 1330, 1220, 7200 cm^{-1}.

MS (APCI(+)): 464, 466 (M+1), 269, 271(M+) m/z.

^1H NMR ($CDCl_3$) 300K δ: 12.85 (s, NH), 8.99 (d, NH, J= 8.9 Hz), 7.80 (d, Ar-H, J= 7.0 Hz), 7.53-7.67 (m, phenyl-5H), 7.27-7.53 (m, Ar-3H), 7.09 (s, Ar-H), 6.10 (s, C3-H), 4.08 (s, CH_3), 3.88 (s, OCH_3), 3.84 (s, OCH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 32.3 (CH_3), 55.7, 55.9 (OCH_3), 70.1 (C3), 93.8 (2xC), 107.6, 122.6, 125.8 (2xC), 128.1, 128.7, 129.5, 129.9 (2xC), 130.6, 132.6, 135.9, 136.4, 149.7, 165.1 (2xC), (Ar-C), 165.1 (C=O), 168.2, 168.9 (Ar-CO), 200.1 (C=N) p.p.m.

A (3.4.15) 3-anilino-7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Yield: 80.0 %.

Mol. Formula: $C_{21}H_{16}ClN_3O$.

Mol. Weight: 361.8.

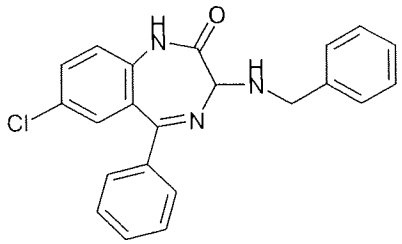
IR (KBr-disc) υ max: 3425, 3025, 3065, 2930, 1710, 1590, 1440, 1340, 1100, 730, 700 cm^{-1}.

MS (APCI(+)): 362, 364 (M+1), 344, 346 ($-H_2O$), 269, 271 (M +) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.09 (s, NH), 7.70 (dd, Ar-H, J= 8.7 Hz), 7.42-7.50 (m, phenyl-5H), 7.36 (d, Ar-H, J= 8.8 Hz), 7.32 (s, Ar-H), 7.23 (t, Ar-2H, J= 7.7, 7.8 Hz), 7.09 (t, Ar-H, J= 7.6, 7.3 Hz), 6.96 (d, Ar-2H, J= 7.7 Hz), 4.98 (s, C3-H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 67.4 (C3), 114.2 (2xC), 117.9, 122.8, 125.5 (2xC), 127.1, 128.8, 129.9 (2xC), 131.7, 136.9, 138.0, 150.2, (Ar-C), 165.1 (C=O), 167.9 (C=N) p.p.m.

A (3.4.16) 3-(benzylamino)-7-chloro-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one



Yield: 62.0 %.

Mol. Formula: $C_{22}H_{18}ClN_3O$.

Mol. Weight: 375.9.

IR (KBr-disc) υ max: 3430, 3335, 2975, 1705, 1580, 1330, 1095, 705 cm^{-1}.

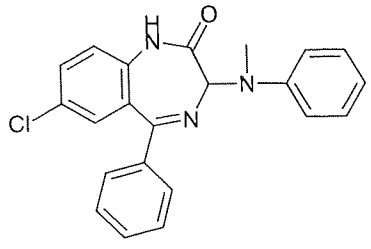
MS (APCI(+)): 276, 278 (M+1), 358, 360 ($-H_2O$), 269, 270 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.57 (s, NH), 7.77 (dd, Ar-H, J= 8.7 Hz), 7.53-7.62 (m, phenyl-5H), 7.53 (d, Ar-H, J= 8.8 Hz), 7.40-7.44 (m, amine phenyl-5H), 7.30 (s, Ar-H), 5.75 (s, $-CH_2-$), 5.11 (s, C_3-H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 52.1 (CH_2), 74.2 (C3), 121.2, 125.8 (2xC), 126.5, 128.3,

128.5, 129.2 (2xC), 130.1 (2xC), 130.3, 130.6, 130.8 (2xC), 131.0, 134.2, 136.1, 136.9 (Ar-C), 162.1 (C=O), 167.1 (C=N) p.p.m.

A (3.4.18) 7-chloro-3-(methylanilino)-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one



R_f (ether)= 0.59.

Mol. Formula: $C_{22}H_{18}ClN_3O$.

Mol. Weight: 375.9.

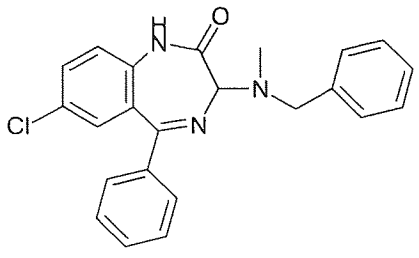
IR (KBr-disc) υ max: 3430, 3216, 3129, 2923, 2851, 2358, 1708, 1596, 1496, 1318, 114, 693 cm^{-1}.

MS (APCI(+)): 376, 378 (M+1), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 3.40 (s, CH_3), 5.24 (s, C3-H), 6.68-6.71 (d, Ar-2H, J= 8.8 Hz), 7.12-7.18 (t, Ar-2H, J= 7.3, 8.5 Hz), 7.28 (s, Ar-H), 7.34-7.38 (d, Ar-H, J= 8.8 Hz), 7.48-7.55 (m, phenyl-5H), 7.68-6.73 (dd, Ar-H, J= 8.7 Hz), 11.89 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 70.1, 70.8 (CH_3 isomers), 83.4 (C3), 117.8, 123.2, 123.3, 128.2 (2xC), 128.3, 128.9 (2xC), 129.4, 129.4 (2xC), 130.0, 130.3 (2xC), 131.7, 136.9, 138.1 (Ar-C), 165.1(C=O), 169.4 (C=N) p.p.m.

A (3.4.19) 7-chloro-3-(methylbenzyl)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.63.

Mol. Formula: $C_{23}H_{20}ClN_3O$.

Mol. Weight: 389.9.

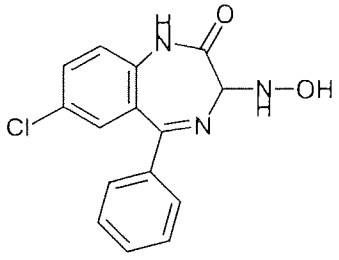
IR (KBr-disc) υ max: 3398, 3324, 3128, 2899, 2832, 1767, 1522, 1477, 1320, 1150, 683 cm^{-1}.

MS (APCI(+)): 390, 391 (M+1), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 3.43 (s, CH_3), 4.21 (m, 2H,-CH_2-), 5.13 (s, C3-H), 6.65-6.70 (d, Ar-2H, J= 8.8 Hz), 7.11-7.19 (t, Ar-2H, J= 7.9, 8.5 Hz), 7.29 (s, Ar-H), 7.34-7.40 (d, Ar-H, J= 8.9 Hz), 7.47-7.58 (m, phenyl-5H), 7.68-6.73 (dd, Ar-H, J= 8.8 Hz), 11.59 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 66.7 (-CH_2-), 70.4, 71.2 (CH_3 isomers), 84.4 (C3), 117.83, 123.0, 123.1, 128.2 (2xC), 128.5, 128.8 (2xC), 129.2, 129.3 (2xC), 130.2, 130.1 (2xC), 131.7, 136.8, 138.8 (Ar-C), 166.2(C=O), 169.8 (C=N) p.p.m.

A (3.4.22) 7-chloro-3-(hydroxyamino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Yield: 44.0 %.

Mpl. Formula: $C_{15}H_{12}ClN_3O_2$.

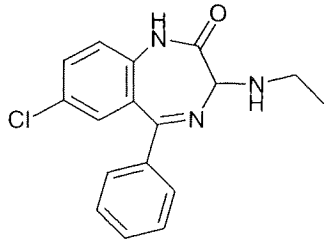
Mol. Weight: 301.7.

IR (KBr-disc) υ max: 3413, 3193, 3100, 2911, 2358, 1728, 1615, 1472, 1328, 1220, 1025, 693 cm^{-1}.

MS (APCI(+)): 301, 303 (M+1), 269, 271 (M +) m/z.

^1H NMR (DMSO-d_6) 300K δ: 10.67 (s, NH), 7.64-7.68 (dd, Ar-H, J= 8.8 Hz), 7.46-7.50 (m, phenyl-5H), 7.28-7.32 (d, Ar-H, J= 8.8 Hz), 7.21-7.23 (sd, Ar-H, J= 2.5 Hz), 5.73 (s, OH), 4.81 (C3-H) ppm; ^{13}C NMR (DMSO-d_6) 300K δ: 75.6 (C_3), 121.6, 125.3 (2xC), 127.5, 128.9, 128.7 (2xC), 129.1, 130.2, 132.8, 137.2, 137.8 (Ar-C), 163.1(C=N), 167.1 (C=O) p.p.m.

A (3.4.23) 3-(ethylamino)-7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.40.

Mol. Formula: $C_{17}H_{16}ClN_3O$.

Mol. Weight: 313.8.

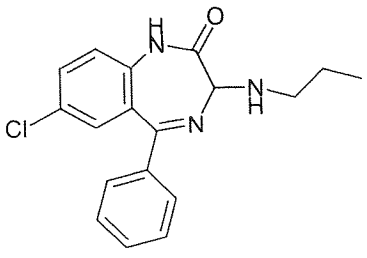
IR (KBr-disc) υ max: 3430, 3121, 2977, 2855, 1654, 1607, 1478, 1320, 693 cm^{-1}.

MS (APCI(+)): 314, 315 (M+1), 296, 297 (M+), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 1.16-1.24 (t, 3H, J= 7.1 Hz), 2.70-3.0 (m,-CH_2-), 4.34 (s, C3-H), 7.24-7.61 (m, Ar-8H), 11.19 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 14.7 (CH_3), 42.4 (-CH_2-), 71.3 (C3), 123.4, 125.8 (2xC), 128.3, 129.7 (2xC), 130.6, 131.1, 136.3, 137.7, 166.5 (C=O), 169.0 (C=N) p.p.m.

A (3.4.24) 3-(propylamino)-7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.44.

Mol. Formula: $C_{18}H_{18}ClN_3O$

Mol. Weight: 327.8.

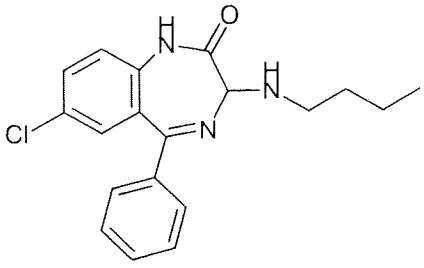
IR (KBr-disc) υ max: 3433, 3108, 2980, 2851, 1764, 1471 1315, 699 cm^{-1}.

MS (APCI(+)): 326, 327 (M+1), 308, 309 (M+), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 1.11-1.20 (t, 3H, J= 7.0 Hz), 2.51-2.72 (m, 4H, -CH_2-), 4.45 (s, C3-H), 7.25-7.83 (m, Ar-8H), 11.12 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.9 (CH_3), 34.0 & 42.4 (-CH_2-), 71.1 (C3), 123.4, 125.1 (2xC), 128.5, 129.9 (2xC), 130.0, 131.3, 136.4, 137.7 (Ar-C), 166.2 (C=O), 169.5 (C=N) p.p.m.

B (3.4.25) 3-(butylamino)-7-chloro-5-phenyl-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one



R_f (ethylacetate) = 0.54.

Yield: 25.0 %.

Mol. Formula: $C_{19}H_{20}ClN_3O$.

Mol. Weight: 341.8.

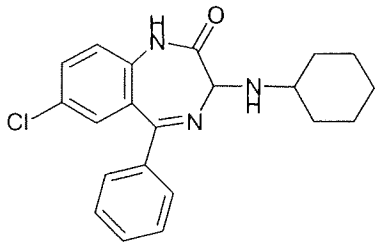
IR (KBr-disc) υ max: 3425, 3100, 2930, 2850, 1700, 1640, 1615, 1480, 1330, 1080 cm^{-1}.

MS (APCI(+)): 342, 344 (M+1), 324, 326 (-H_2O), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 11.57 (s, NH), 9.69 (s, NH), 7.77 (dd, Ar-H, J= 8.7 Hz), 7.60-7.49 (m, phenyl-5H), 7.42 (d, Ar-H, J= 8.8 Hz), 7.30 (s, Ar-H), 5.12 (s, C3-H), 3.21 (m, -CH-), 3.02 (m, -CH-), 1.73 (m, -CH_2-), 1.39 (m, -CH_2-), 0.92 (t, CH_3, J= 7.2, 7.4 Hz) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 13.7 (CH_3), 21.9, 30.2, 45.2 (CH_2), 70.5 (C3), 121.9, 125.1 (2xC), 127.6, 128.2, 129.9 (2xC), 130.8, 131.3, 132.0, 136.8, 138.2 (Ar-C), 167.5(C=O), 168.1 (C=N) p.p.m.

A (3.4.28) 7-chloro-3-(cyclohexylamino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Yield: 33.0 %.

Mol. Formula: $C_{21}H_{22}ClN_3O$.

Mol. Weight: 367.9.

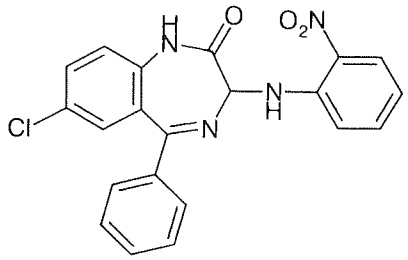
IR (KBr-disc) υ max: 3403, 3092, 3033, 2927, 2863, 2358, 1700, 1623, 1474, 1322, 695 cm^{-1}.

MS (APCI(+)): 368, 370 (M+1), 350, 352 (-H_2O), 269, 271 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 11.57 (s, NH), 9.58 (s, NH), 7.74-7.79 (dd, Ar-H, J= 8.7 Hz), 7.43-7.63 (m, phenyl-5H), 7.40-7.43 (d, Ar-H, J= 8.8 Hz), 7.30-7.31 (sd, Ar-H, J=2.4 Hz), 5.15 (s, C3-H), 1.07-2.22 (m, -CH_2-, 11H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 24.7, 25.3, 28.6, 29.8, 30.8 (-CH_2-), 54.6 (-CH-), 124.4 (2xC), 127.9, 128.0, 129.1, 130.3, 130.7, 131.9, 133.2, 137.8 (2xC), 137.9 (Ar-C), 165.7 (C=O), 167.8 (C=N) p.p.m.

(3a) 7-chloro-3-(2-nitroanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Oxazepam (0.1 g, 3.5x10^{-4} mol) was treated with thionyl chloride (4 Eq, 0.1 ml) and heated to 60 ^{O}C for 1.5 hours. The resulting intermediate, yellow solid, was washed with dry diethyl ether (twice) to remove any excess thionyl cholride. The appropriate substituted aniline 2.5 Eq, 1.1x10^{-3} mol), with TEA (drops) was added with dry DCM (15 ml) and refluxed for two hours. The organic phase was washed with hydrochloric acid (pH 4.0–5.0) and dried over sodium sulphate. Excess DCM was removed and preparative TLC (MP: ether, 6% Methanol in ether) isolated the desired product.

R_f (ether)= 0.30.

Mol. Formula: $C_{21}H_{15}ClN_4O_2$.

Mol. Weight: 390.8.

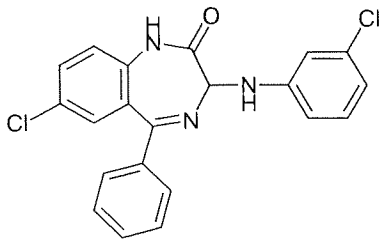
IR (KBr-disc) υ max: 3349, 3279, 1702, 1590, 1527, 1469, 1316, 1297, 1114, 830, 693 cm^{-1}.

MS (APCI(+)): 391, 393 (M+1), 269, 271 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 5.19-5.22 (d, C3-H, J=7.0 Hz), 6.82-6.86 (d, Ar-H, J=9.2 Hz), 7.10-7.18 (m, Ar-2H), 7.33 (s, Ar-H), 7.44-7.55 (m, phenyl-5H), 7.70-7.75 (dd, Ar-H, J=8.8 Hz), 7.09-8.02 (d, Ar-H, J=9.3 Hz), 7.04-7.07 (d, Ar-H, J= 7.1 Hz), 11.15 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 70.7 (C3), 113.2, 124.1, 126.3, 127.2 (2xC), 128.9, 129.9, 130.3, 131.3, 132.6, 137.5, 138.2 (2xC), 138.6, 153.2 (2xC), 166.8 (C=O), 167.7 (C=N) p.p.m.

(3e) 7-chloro-3-(3-chloroanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.31.

Mol. Formula: $C_{21}H_{15}Cl_2N_3O$.

Mol. Weight: 396.3.

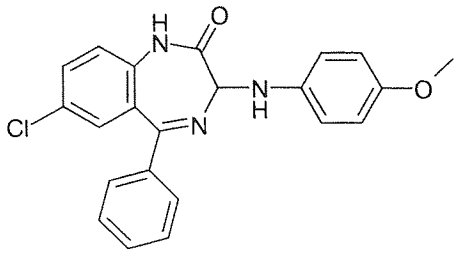
IR (KBr-disc) υ max: 3438, 2919, 2856, 2362, 2338, 1653, 1594, 1318, 1014, 671 cm^{-1}.

MS (APCI(+)): 396, 397, 398 (M+1), 378, 379, 380 (-H_2O), 269, 271 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300K δ: 5.05 (s, C3-H), 6.80-6.60 (d, Ar-H, J=8.5 Hz), 6.64-6.69 (d, Ar-H, J=8.0 Hz), 6.80 (s, Ar-H), 7.05-7.11 (t, Ar-H, J= 8.1, 8.1 Hz), 7.32 (s, Ar-H), 7.33-7.35 (d, Ar-H, J= 8.7 Hz), 7.43-7.53 (m, phenyl-5H), 7.68-7.73 (dd, Ar-H, J= 8.8 Hz), 11.06 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 67.9 (C3), 117.5, 118.1, 122.6, 122.8, 125.8 (2xC), 128.4, 128.7, 130.1, 130.5 (2xC), 130.8, 130.9, 132.0, 133.5, 136.1, 137.2, 142.5 (Ar-C), 164.2 (C=O), 168.1 (C=N) p.p.m.

(3i) 7-chloro-3-(4-methoxyanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.38

Mol. Formula: $C_{22}H_{18}ClN_3O_2$.

Mol. Weight: 391.9.

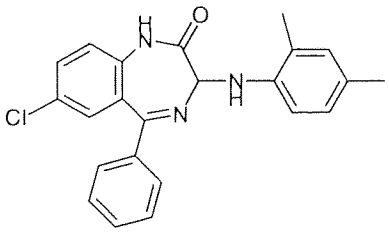
IR (KBr-disc) υ max: 3426, 3193, 3058, 2935, 1687, 1519, 1476, 1320, 1220, 698 cm^{-1}.

MS (APCI(+)): 392, 394 (M+1), 374, 376 ($-H_2O$), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 3.77 (s, OCH_3) 4.80 (s, C3-H), 7.00-7.05 (d, Ar-2H, J=7.8 Hz), 7.28-7.31 (d, Ar-H, J= 7.7 Hz), 7.32-7.36 (d, Ar-H, J=7.9 Hz), 7.46-7.55 (m, phenyl-5H), 7.63-7.68 (dd, Ar-H, J= 8.8 Hz), 10.16 (s, NH), 10.85 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 53.3 (OCH_3), 68.1 (C3), 116.3 (2xC), 117.2 (2xC), 122.3, 124.5 (2xC), 124.9, 126.6, 126.9, 129.8 (2xC), 129.9, 130.6, 137.0, 137.4, 139.7, 153.6, 165.3 (C=O), 167.1 (C=N) p.p.m.

(3q) 7-chloro-3-(2,3-dimethylanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.44.

Mol. Formula: $C_{23}H_{20}ClN_3O$.

Mol. Weight: 389.9.

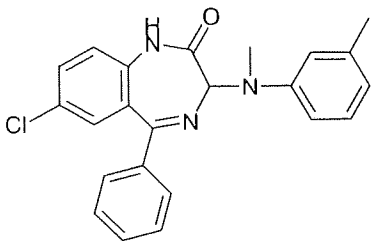
IR (KBr-disc) υ max: 3436, 3182, 2919, 2618, 1690, 1606, 1473, 1222, 1147 cm^{-1}.

MS (APCI(+)): 390, 392, (M+1), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 2.32 (s, CH_3), 2.78 (s, CH_3), 4.80 (s, C3-H), 7.08-7.18 (m, Ar-2H), 7.22-7.23 (s,d Ar-H, J=2.5 Hz), 7.28-7.31 (d, Ar-H, J=8.5 Hz), 7.32-7.35 (d, Ar-H, J=7.5 Hz), 7.46-7.53 (m, phenyl-5H), 7.63-7.68 (dd, Ar-H, J= 8.7, 8.8 Hz), 10.18 (s, NH), 10.84 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 17.5 (CH_3), 21.0 (CH_3), 83.2 (C3), 123.8 (2xC), 125.8, 127.2, 127.8, 128.1, 128.7, 129.0 (2xC), 129.4, 129.9, 131.2, 132.3, 132.4, 132.5, 138.2, 138.4 (Ar-C), 163.5 (C=O), 170.1 (C=N) p.p.m.

(3u) 7-chloro-3-(3-dimethylanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



R_f (ether)= 0.66.

Mol. Formula: $C_{23}H_{20}ClN_3O$.

Mol. Weight: 389.9.

IR (KBr-disc) υ max: 3420, 2925, 1700, 1600, 1481, 1320, 1121, 699 cm^{-1}.

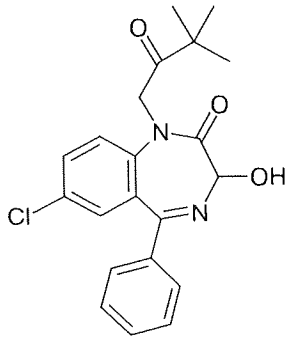
MS (APCI(+)): 390, 392, (M+1), 269, 271 (M+) m/z.

^1H NMR (DMSO-d_6) 300K δ: 2.22 (s, CH_3), 3.25 (s, N-CH_3), 5.61 (s, C3-H), 7.00-7.06 (t, Ar-H, J=7.8, 7.9 Hz), 7.23 (s, Ar-H), 7.22-7.30 (d, Ar-H, J=7.4 Hz), 7.31 (s, Ar-H),

7.37-7.40 (d, Ar-H, J=8.7 Hz), 7.49-7.56 (m, phenyl-5H), 7.64-7.66 (dd, Ar-H, J= 8.8 Hz), 7.69-7.74 (dd, Ar-H, J= 8.7 Hz), 10.89 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 22.0 (CH_3), 58.6 (N-CH_3), 71.8 (C3), 122.3, 124.6 (2xC), 125.2, 125.7, 127.0, 127.6, 128.6, 128.1, 128.9, 129.0, 129.3, 129.8 127.2, 127.8, 128.1, 128.7, 129.0, 129.3, 129.8 (2xC), 129.9, 138.4, 138.4, 138.6, 149.5 (Ar-C), 165.1 (C=O), 169.4 (C=N) p.p.m.

(3.6.2) 7-chloro-1-(3,3-dimethyl-2-oxobutyl)-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Alkylation method: A 50% suspension of NaH in mineral oil (0.06 mol) was added in drops to a solution of Oxazepam (0.05 mol) in dry DMF (100 ml). After stirring for 15 mins at RT, the alkylating agent (0.06 mol) was added in drops to the mixture, with ice cooling. The solution was stirred for additional 30-45 mins at RT. For workup: Water was added (75 ml) and the suspension was added to ethylacetate (75 ml). The extract was washed with brine (100 ml x 2), dried over sodium sulphate, with the solvent evaporated. Column chromatography, with ether/petrolether 1:2 as the eluent.

Yield: 81 %.

R_f (ether/petrolether 1:2) = 0.51

Mol. Weight: 384.9.

Mol. Formula: $C_{21}H_{21}ClN_2O_3$.

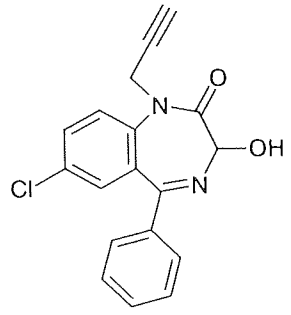
MS (APCI(-)): 383, 385 (M-1), 285, 287 (M+) m/z.

IR (KBr-disc) υ max: 3450, 2933, 2358, 1710, 1677, 1596, 1482, 1322, 1131 & 693 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.23 (s, $(CH_3)_3$), 4.81 (s, C3-H), 5.04-5.12 (m, $-CH_2-$), 7.05-7.67 (m, Ar-9H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 26.3 ($(\underline{C}H_3)_3$), 43.5 ($(\underline{C}CH_3)_3$), 53.2 (CH_2), 82.0 (C3), 122.9, 128.3 (2xC), 128.4, 129.6, 129.8 (2xC), 130.4, 130.8, 131.9, 137.4, 140.1 (Ar-C), 155.3 (C=O), 166.9 (C=N), 169.4 (C=O) p.p.m.

(3.6.3) 7-chloro-3-hydroxy-5-phenyl-1-prop-2-ynyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



Yield: 67 %.

R_f (ether/petrolether 1:2) = 0.38

Mol. Weight: 324.8.

Mol. Formula: $C_{18}H_{13}ClN_2O_2$.

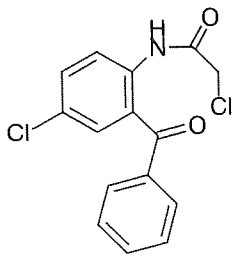
MS (APCl(-)): 323, 325 (M-1), 284, 286 (M+) m/z.

IR (KBr-disc) υ max: 3418, 3291, 3225, 2923, 1700, 1634, 1478, 1415, 1324, 1131, 1002 & 695 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 2.10-2.34 (t, CH, J = 24.7, 25.0 Hz), 4.51-4.66 (m, $-CH_2-$), 5.04 (C3), 7.21-7.63 (Ar-H) p.p.m.

^{13}C NMR (($CDCl_3$)) 300K δ: 37.0 ($-CH_2-$), 73.5, 75.19 (CH), 86.6 (C3), 123.4, 128.3 (2xC), 128.3, 129.4 (2xC), 130.3, 130.7, 131.1, 132.1, 137.1, 139.5 (Ar-H), 164.6 (C=O), 166.1 (C=N) p.p.m.

N-(2-benzoyl-4-chlorophenyl)-2-chloroacetamide



A solution of 2-amino-5-chlorobenzophenone (11.6 g, 50 mmol) in anhydrous ether (75 ml) was stirred and cooled in an ice bath to 0-5°C. Chloroacetyl chloride (55 mmol, 4.4 ml) in ether (25 ml) was added dropwise. Precipitation of the title compound occurred. The suspension was stirred for half an hour at 0-5°C and for 2 hours at room temperature. The solid product was collected by filtration and crystallised with toluene.

Yield: 91%.

R_f (ether) = 079

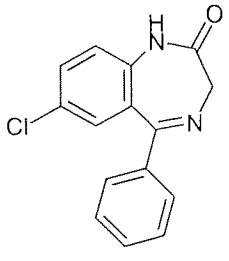
Mol. Weight: 308.1.

Mol. Formula: $C_{15}H_{11}Cl_2N_2O$.

MS (APCI (+)) m/s: = 308 M+H, 231 M+ m/z.

^{1}H-NMR ($CDCl_3$) 300K δ 4.2 (s, 2H, NHCOC$\underline{H}_2$Cl), 7.3-7.8 (m, Ar-8H), 11.5 (s, 1H, N$\underline{H}$COCH$_2$Cl) p.p.m.

7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (Diazepam)



A mixture of the precursor 2-chloro-N-{4-chloro-2-[(hydroxyimino)(phenyl)methyl] phenyl} acetamide (13 g, 42 mmol, 11.9 g of urotropine (85 mmol), HCl, (20 ml 2N aqueous), methanol (80 ml) and water (10 ml) were added (pH of solvent mixture was ≅ 5) and refluxed for 16 hours. The mixture was cooled in an ice bath and the precipitated crystals were filtered. The crystals were washed with a 10 ml ice-cold mixture of methanol/water (1:1). The product was dried at 60°C under reduced pressure overnight.

Yield: 82 %.

R_f (ether) = 0.42

Mol. Weight: 270.2.

Mol. Formula: $C_{15}H_{11}ClN_2O$.

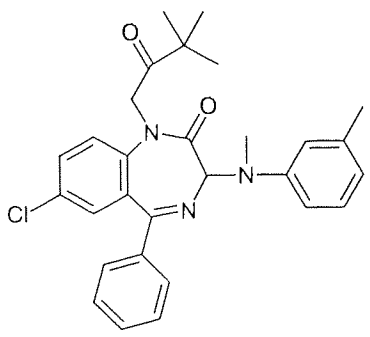
MS (APCI(+)): 271, 272 (M+1) m/z.

IR (KBr-disc) υ max: 3420, 3312, 3207, 2960, 1679, 1534, 1213 & 794cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 4.38 (s, 2 H, C_3), 7.65 (m, Ar-8H), 10.0 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 55.2 (C3), 121.9, 128.6, 129.5, 129.7, 130.8, 131.4, 137.3, 139.4(Ar-C), 164.8(C=N), 168.7 (C=O) p.p.m.

(3ux)7-chloro-1-(3,3-dimethyl-2-oxobutyl)-3-(3-dimethylanilino)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one



A suspension of compound 3.6.2 (7-chloro-1-(3,3-dimethyl-2-oxobutyl)-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) (2g, 7.1 mmols) and NBS (N-bromosuccinimde) (1.53g, 8.6 mmols) in carbon tetrachloride (80 ml) was stirred at ambient temperature for 20 mins. Trifluoroacetic acid (70 mg, 0.6 mmols) was added and then mixture was vigorously stirred and heated, under reflux, for 1.5 hours. The hot solution was cooled, separated from the yellow, sticky precipitate by decantation. The residue was washed with carbon tetrachloride (2 x30 ml). The combined solution was evaporated to dryness to give the bromo-intermediate 91%.

2g of this material, with N-methyl-m-toluidine (2.5 Eq, 0.95 ml) was stirred in dry DCM (30 ml), with a few drops of TEA for 15 mins. The mixture was refluxed for 2 hours. Afterwards the organic phase was washed with hydrochloric acid (pH 4.0–5.0) and dried over sodium sulphate and the residue purified by preparative chromatography (MP: ether/petrolether) to give a yellow powder.

Yield: 1.9 %.

R_f (1:2) = 0.54.

Mol. Weight: 488.0.

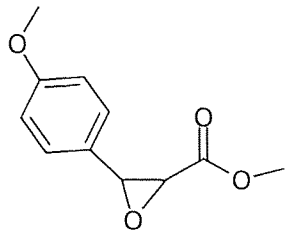
Mol. Formula: $C_{29}H_{30}ClN_3O_2$.

MS (APCI(-)): 487, 389 (M-1), 366, 368 (M+) m/z.

IR (KBr disc) υ max: 3460, 3325, 2823, 2418, 1721, 1643, 1602, 1571, 1266 & 697 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.21 (s, $(CH_3)_3$), 2.19 (CH_3), 3.24 (N-CH_3), 4.88 (s, C3-H), 5.06-5.15 (m, -CH_2-), 7.01-7.42 (m, Ar-5H), 7.49-7.77 (m, Ar-7H) p.p.m.

Preparation of methyl-3-(4-methoxyphenyl) glycidate



A solution of 4-methoxybenzaldehyde (0.18 mol, 21.9 ml) and methyl chloroacetate (0.27 mol, 23.4 ml) were cooled to -10^0C in a dry-ice bath. Sodium methoxide (24 % wt, 60 ml, 25 % in methanol) was added dropwise, over 45 mins, whilst maintaining the temperature at -10^0C. The mixture was allowed to stir for 2 hrs at 0^0C and for a further 2hrs at room temperature. The solid that formed was poured into a solution of ice-water (400 ml) containing glacial acetic acid (3 ml) and stirred for 30 mins. The precipitate was filtered, washed with water and dried, to give a pure white powder.

Yield: 93.2 %.

Mp: 68-70 ^{0}C.

Mol. Weight: 208.2.

Mol. Formula: $C_{11}H_{12}O_4$.

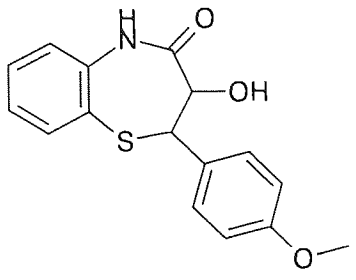
IR (KBr-disc) υ: 3010, 2960, 1250, 1760, 1610, 815 cm^{-1}.

MS (APCI(-)): 207 (M-H), 192, 175, 149 (M+) m/z.

^{1}H NMR ($CDCl_3$) 300K δ: 3.51 (d, CH, J= 2.4 Hz), 3.80 (s, $COOCH_3$) 3.81 (s, Ar-OCH_3), 4.09 (d, CH, J= 2.3 Hz), 6.88 (d, Ar-H, J= 8.8 Hz), 7.20 (d, Ar-2H, J=8. 9 Hz), p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 52.5 ($OOCH_3$), 55.3 (Ar-OCH_3), 56.5 (CH- $COOCH_3$), 57.9 (Ar-CH), 114.0 (Ar-C), 126.6 (Ar-2C), 127.1 (Ar-2C), 160.2 (C=O) p.p.m.

Preparation of 3-hydroxy-4-(4-methoxyphenyl)-1,3,4,5- tetrahydro-2H-1-benzothiazepin-4-one



A solution of methyl-trans-3-(4-methoxyphenyl) (0.104 mol, 20.8 g) and 1 % aqueous $FeCl_3.6H_2O$ (2 drops) in chlorobenzene (120 ml) were heated to 80-85^{0}C. 2-Aminothiophenol (1.04 mol, 11.30 ml) was added over 30 mins. The resulting mixture was then stirred for 50 mins at 115 ^{0}C and methanesulfonic acid (0.001 mol, 65µl) was added. The mixture was refluxed, using an air condenser to remove methanol, for 10 hrs and then stirred for 15 hrs under cooling. The precipitate was collected by filtration and washed with chlorobenzene to give the crude product. The crude product was refluxed for 2 hrs in methanol and then cooled to room temperature. Filtering gave a white powder, corresponding to the pure product.

Yield: 30.3 %

Mp: 168-170 ^{0}C.

Mol. Weight: 301.4.

Mol. Formula: $C_{16}H_{15}NO_3S$

MS (APCI(-)): 300 (M-H), 282 (M-H_2O) m/z.

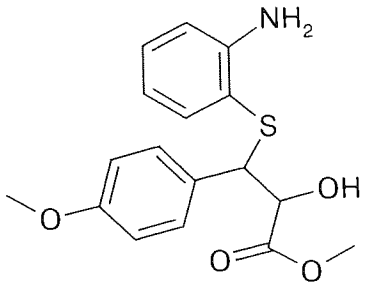
MS (APCI(+)): 302 (M+H), 284 (M-H_2O) m/z.

IR (KBr-disc) υ: 3400, 3300, 3030, 2950, 1690, 1580, 1500, 750 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 3.75 (s, CH_3), 4.32 (d, C3-H, J= 6.7 Hz), 5.06 (d, CH-Ar, J= 6.7 Hz), 6.89 (d, Ar-2H J= 8.7 Hz), 7.18 (t, Ar-2H, J= 7.5, 7.8 Hz), 7.40 (d, Ar-2H, J= 8.4 Hz), 7.41 (d, Ar-H, J= 7.7 Hz), 7.60 (d, Ar-H, J= 7.6 Hz), 10.33 (s, NH), p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 55.6 (CH_3), 57.8 (C3), 70.0 (S-C-Ar), 113.8, 123.1, 126.1, 126.8 (2xC), 128.9, 130.4, 131.4 (2xC), 134.4, 142.4, 159.5 (Ar-C), 173.0 (C=O) p.p.m.

Preparation of the intermediate (uncyclised) of 3-hydroxy-4-(4-methoxyphenyl)-1,3,4,5- tetrahydro-2H-1-benzothiazepin-4-one



A mixture of 2-amino thiophenol (0.03 mol, 3.0 ml) and of methyl-3-(4-methoxyphenyl) glycidate (0.03 mol, 6.24 g) were stirred in dry xylene (70 ml) at 160^{0}C for 30 hrs. TLC (diethyl ether) confirmed the presence of a new product. 50 ml of the solvent was removed and the mixture was cooled in a ice bath and left overnight. The product was filtered, washed with ethanol and dried to give the intermediate (1). The intermediate can be cyclized to yield the desired product on stirring in basic solution (sodium hydroxide, 2M) for 2 days filtered and washed with ethanol to give a white powder.

Yield: 25.1 %

Mp: 91-92 ^{0}C.

Mol. Weight: 333.4

Mol. Formula: $C_{17}H_{18}NO_4S$

MS (APCI(+)): 334 (M+H), 302 (M-$COOCH_3$), 284 (M-H_2O), 209 (M+) m/z.

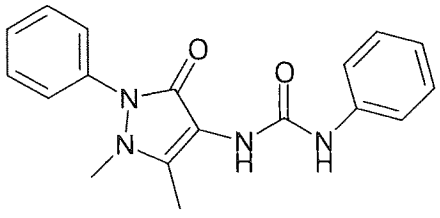
IR (KBr-disc) υ: 3520, 3400, 3350, 2950, 2350, 1730, 1610, 1510, 1480, 1290 , 1120, 750 cm^{-1}.

^{1}H NMR ($CDCl_3$) 300K δ: 3.79 (s, Ar-OCH_3), 3.99 (s, $\underline{C}OOCH_3$) 4.51 (s, C_3-H, S-CH-ester), 6.65 (t, Ar-H, J= 7.7, 7.5 Hz), 6.80 (d, Ar-H J= 8.3 Hz), 6.82 (d, Ar-2H, J= 8.8 Hz), 7.12 (t, Ar-H, J= 7.6, 7.8 Hz), 7.14 (d, Ar-H, J= 8.5 Hz), 7.34 (d, Ar-2H, J= 8.8 Hz), 10.83 (s, NH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 52.5 (CHOH), 55.5 ($COO\underline{C}H_3$), 55.6 (Ar-OCH_3), 74.0 (S-C-Ar), 113.7 (2xC), 115.5, 116.4, 188.9, 129.5 (2xC), 130.5, 131.6, 137.5, 148.4, 159.0 (Ar-C), 172.8 (C=O) p.p.m.

9.4. Experiments to Chapter 4

(4.8.a) N-(1,5-dimethyl-3-oxo-2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-N'-phenylurea



General method:

A solution of the relevant amine in dry acetonitrile was stirred at room temperature. The appropriate isocyanate (1-phenyl/ 1-napthyl, 1.1 Eq) in dry acetonitrile (20 ml) was added slowly over 5 minutes, allowed to stir at room temperature or heated to 60^0C and left overnight. The precipitate that formed was filtered, washed (twice) and dried, to give the corresponding urea product.

Yield: 94 %.

Mol. Weight: 322.4.

Mol. Formula: $C_{18}H_{18}N_4O_2$.

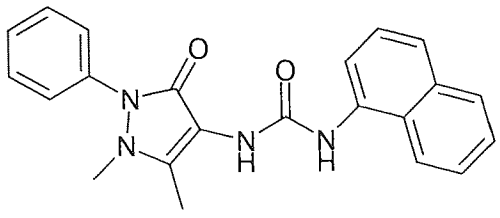
MS (APCI(+)): 323 (M+1) m/z.

IR (KBr-disc) υ max: 3318, 3279, 3139, 1700, 1642, 1586, 1550, 1496, 1311, 1210, 737, & 699 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 2.21 (s, CH_3), 3.04 (s, N-CH_3), 6.91-6.97 (t, Ar-H, J=7.3 Hz), 7.22-7.53 (m, Ar-9H), 8.80 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 11.7 (CH_3), 36.6 (N-CH_3), 108.7 (CH-NH), 118.6 (2xC), 122.2, 123.9 (2xC), 126.7, 129.2 (2xC), 129.6 (2xC), 135.5 (C-CH_3), 140.2, 152.2 (Ar-C), 154.2, 162.7 (C=O) p.p.m.

(4.8.b) N-(1,5-dimethyl-3-oxo-2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-N-(1-naphthyl)urea



Yield: 91 %.

Mol. Weight: 372.4.

Mol. Formula: $C_{22}H_{20}N_4O_2$.

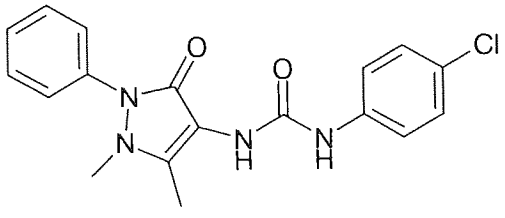
MS (APCI(+)): 373 (M+1) m/z.

IR (KBr-disc) υ max: 3280, 3044, 1663, 1638, 1565, 1496, 1317, 1253, 780 & 668 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.26 (s, CH_3), 3.04 (s, N-CH_3), 7.29-7.32 (t, Ar-H, J=7.2 Hz), 7.38-7.67 (m, Ar-8H) 7.93-7.99 (t, Ar-H, 7.4, 7.6 Hz), 7.99-8.02 (d, Ar-H, J=7.4 Hz), 8.14-8.17 (d, Ar-H, J=7.9 Hz), 8.95 (s, NH), 9.20 (s, NH) p.p.m.

9.5. Experiments to Chapter 5

(5.2.8) N-(4-chlorophenyl)-N'-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea



General method:

A solution of 4-amino-antipyrine (0.5g, 2.45 mmols) in dry acetonitrile was stirred at room temperature. The appropriate isocyanate (1.1 Eq) in dry acetonitrile (20 ml) was added slowly over 5 minutes, allowed to stir at room temperature or heated to 60^0C and left overnight. The precipitate that formed was filtered, washed (twice) and dried, to give the corresponding urea product.

Yield: 75 %.

Mol. Weight: 418.9.

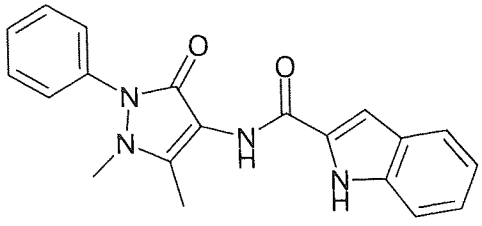
Mol. Formula: $C_{23}H_{19}ClN_4O_2$.

MS (APCI(+)): 419, 421 (M+1) m/z.

IR (KBr-disc) υ max: 3371, 3210, 3059, 1698, 1656, 1512, 1319, 747 & 655 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 2.19 (s, CH_3), 3.04 (s, N-CH_3), 7.27-7.53 (m, Ar-9H), 8.95 (s, NH) p.p.m.

(5.2.14) N-(1,5-dimethyl-3-oxo-2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-3-carboxamide



General method:

A solution of 4-amino-antipyrine (0.5g, 2.45 mmols) was dissolved in dry acetonitrile (20 ml). The appropriate indole acid (1.25 Eq) was added, with DIC (3 Eq). The mixture was heated to 60 °C and left overnight. The resulting precipitated crystals were filtered, washed and dried.

Yield: 76%.

Mol. Weight: 346.4.

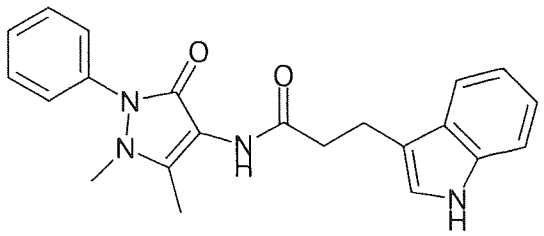
Mol. Formula: $C_{20}H_{18}N_4O_2$.

MS (APCI(+)): 347 (M+1), 329 (M+) m/z.

IR (KBr-disc) υ max: 3337, 3307, 2965, 1696, 1696, 1623, 15557, 1363, 1251 & 826 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.18 (s, CH_3), 3.10 (s, N-CH_3), 5.50 (s, C=CH-), 7.02-7.06 (t, Ar-H, J= 7.4 Hz), 7.18-7.22 (t, Ar-H, J= 7.2, 7.3 Hz), 7.33-7.51 (m, Ar-6H), 7.62-7.65 (d, Ar-H, J= 8.0 Hz), 9.51 (s, NH), 11.56 (s, NH) p.p.m.

(5.2.17) N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(1H-indol-3-yl)propanamide



Yield: 75 %.

Mol. Weight: 374.4.

Mol. Formula: $C_{22}H_{22}N_4O_2$.

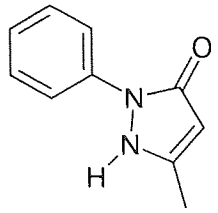
MS (APCI(+)): 375 (M+1) m/z.

IR (KBr-disc) υ max: 3421, 3311, 3059, 2843, 1676, 1645, 1543, 1529, 1487, 1395, 1240 & 704 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.01 (s, CH_3), 2.61-2.69 (t, CH_2, J= 8.1, 7.9 Hz), 3.02 (s, N-CH_3), 3.57-3.74 (m, CH_2), 6.93-7.58 (m, Ar-9H), 8.22-8.26 (d, Ar-H, J= 7.8 Hz), 9.08 (s, NH), 10.76 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 22.2 (CH_3), 20.8 (CH_2), 23.8 (CO-CH_2), 36.5 (N-CH_3), 108.3 (C-NH), 111.9, 118.7, 122.5, 122.8, 123.9, 126.7 (2xC), 127.5, 127.6 (2xC), 129.6, 135.6, 136.7 (Ar-C), 162.4, 170.2 (C=O) p.p.m.

5-methyl-2-phenyl-1,2-dihdyro-3H-pyrazol-3-one



Phenyl hydrazine (15.0 g, 0.14 mol, 1 Eq.) was added slowly to neat acetic acid ester (2 Eq, 36.0 ml, 0.28 mol) at 180 °C. The mixture was allowed to heat over 3 hours and then cooled to room temperature. The mixture was washed with ethanol to remove any unreacted starting materials. It was then filtered to give a white precipitate. This was subsequently recrystallised from ethanol.

Yield: 71 %.

Mol. Weight: 174.2

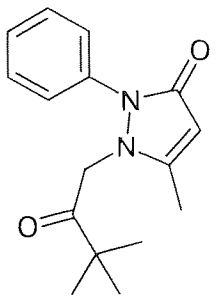
Mol. Formula: $C_{10}H_{10}N_2O$.

MS (APCI(+)): 175 (M+1) m/z.

IR (KBr-disc) υ max: 3438, 3063, 2685, 1592, 1557, 1492, 1455, 1407, 1293, 750 & 703 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.11 (s, CH_3), 5.62 (s, CH), 7.37-7.43 (t, Ar-2H, J=7.6, 7.9 Hz), 7.53-7.59 (t, Ar-H, J=7.4, 7.5 Hz), 7.68-7.72 (d, Ar-2H, J=8.7 Hz) p.p.m.

1-(3,3-dimethyl-2-oxobutyl)-5-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one



Alkylating method:

A suspension of 50% sodium hydride in mineral oil (0.03 mol) was added in drops to a solution of 5-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (4.35 g, 0.025 mol) in dry DMF (50.0 ml). After stirring for 20 mins at RT, under inert conditions, the relevant alkylating agent (0.03 mol) was added in drops to the mixture, under ice cooling. The mixture was stirred for an additional 35 mins at RT. After 35 mins, water was added and ethylacetate was added to the suspension. The organic extract was washed with brine & water, dried over sodium sulphate and the solvent removed in vacuo. Column chromatography afforded the pure products.

Yield: 42.5 %.

R_f (50% ether in 40-60 petroleum ether) = 0.63.

Mol. Weight: 272.3.

Mol. Formula: $C_{16}H_{20}N_2O_2$.

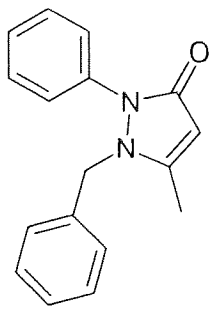
MS (APCI(+)): 273 (M+1) m/z.

IR (KBr-disc) υ max: 3442, 2963, 1721, 1600, 1511, 1476, 1451, 1394, 1162, 1054, 918 & 762 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 0.97 (s, CH_3), 1.18 (s, $(CH_3)_3$), 5.01 (s, CH_2), 7.23-7.28 (t, Ar-H, J=7.4, 7.5 Hz), 7.38-7.44 (t, Ar-2H, J=7.5, 7.8 Hz), 7.70-7.74 (d, Ar-2H, J=8.0 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 26.1 ($C(\underline{C}H_3)_3$), 45.5 ($\underline{C}(CH_3)_3$), 51.3 (s, CH_2), 122.0 (2xC), 126.4, 128.7 (2xC), 138.3 (Ar-C), 149.1 (CH), 162.5 (C=O), 194.0 (C=O) p.p.m.

1-benzyl-5-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one



R_f (50% ether in 40-60 petroleum ether) = 0.61.

Yield: 37.1 %.

Mol. Weight: 264.3.

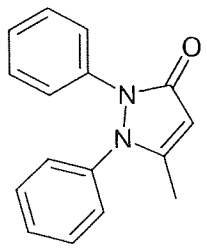
Mol. Formula: $C_{17}H_{16}N_2O$.

MS (APCI(-)): 263 (M-1) m/z.

IR (KBr-disc) υ max: 3201, 3062, 3010, 2915, 2866, 1754, 1694, 1542, 1476, 1205 & 747 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.31 (s, CH_3), 4.50 (s,-CH_2-), 4.77 (s, CH), 7.12-7.56 (m, Ar-10H) p.p.m.

5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one



Diphenyl hydrazine (50.0g, 0.27 mol) and acetic acid ester (2 Eq. 69.0 ml, 0.52 mol) was heated at 130-150 °C for 2 hours, with a Dean stark trap. The mixture was then heated additionally for 1.5 hours at 180 °C, to remove water, ethanol and acetic acid ester. The remaining solution was distilled at 230-250 °C at 2mm Hg. This removed any unreacted diphenyl hydrazine and gave a viscous black liquid. The mixture was allowed to cool to RT and then ether was added to precipitate out the crude black crystals. These were subsequently recrystallised twice from toluene.

Yield: 32.8 %.

Mol. Weight: 250.3.

Mol. Formula: $C_{16}H_{14}N_2O$.

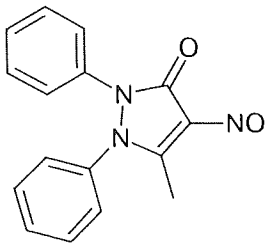
MS (APCI(+)): 251 (M+1) m/z.

IR (KBr disc) υ max: 3465, 3090, 1671, 1590, 1490, 1380, 1349, 1241, 971, 753 & 688 cm^{-1}.

^{1}H NMR ($CDCl_3$) 300K δ: 2.07 (s, CH_3), 5.55 (s, CH), 7.05-7.37 (m, Ar-10H) p.p.m.

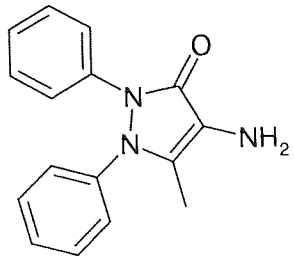
^{13}C NMR ($CDCl_3$) 300K δ: 13.7 (CH_3), 99.2 (CH), 123.6 (2xC), 125.5 (2xC), 125.9 (2xC), 128.0, 128.6 (2xC), 129.3, 135.7, 139.0 (Ar-C), 156.3 (C-N), 166.5 (C=O) p.p.m.

4-nitro-5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one



5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one (10.0g, 0.04 mol) was warmed in HCl (conc) (60.0 ml), when dissolved, the solution was diluted with water (up to 400 ml) sodium nitrite (2.8 g, 0.041 mol) in water (50.0 ml) was added in drops to the mixture at 0 °C, whilst stirring. A green precipitate was produced, which was allowed to stand for 45 mins, then filtered, washed with cold water and dried.

4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one



4-nitro-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one (0.04 mol) was dissolved in ethanol (250 ml). A mixture of tin chloride (20.4g, 0.11 mol) in 20 % HCl (120 ml) was heated to 90 °C. When dissolved the hot mixture was added to the alcoholic solution and allowed to cool to RT & left to stand overnight. Ammonia solution (conc 33%) was added to the mixture until no further precipitation occurred. The mixture was filtered, dried and extracted several times with ethanol. The ethanol was removed in vacuo and the crude mixture was recrystallised in ethanol to give bright yellow crystals.

Yield: 37.0 %.

Mol. Weight: 265.3.

Mol. Formula: $C_{16}H_{15}N_3O$.

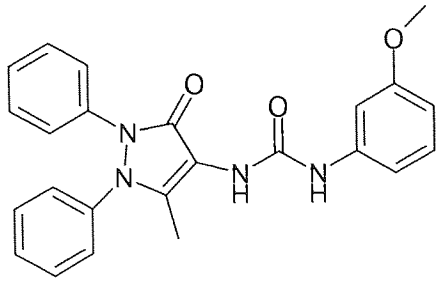
MS (APCI(+)): 266 (M+1), 251 (M+) m/z.

IR (KBr-disc) υ max: 3407, 3210, 1654, 1592, 1492, 1351, 1262, 751 & 690 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 1.88 (s, CH_3), 5.57 (s, CH), 7.05-7.12 (tt, Ar-H, J=7.3 Hz), 7.20-7.45 (m, Ar-9H) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 11.09 (CH_3), 120.3, 122.5 (2xC), 123.8, 125.5 (2xC), 128.0, 129.1 (2xC), 129.8 (2xC) (Ar-C), 136.4 (CH), 142.7 (Ar-C), 156.3, 166.3 (C=O) p.p.m.

(5.5.2) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)N'-3-methoxyphenylurea



4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one (0.1g, 3.8 x 10^{-4} mol) in dry acetonitrile (10-15 ml) was stirred at room temperature. The appropriate substituted isocyanate (1.3 Eq) in dry acetonitrile was added slowly over 5 minutes, allowed to stir at room temperature or heated to 60 ^{0}C and left overnight. The precipitate that formed was filtered, washed (twice) and dried, to give the corresponding pure urea product.

Yield: 67 %.

Mol. Weight: 414.6.

Mol. Formula: $C_{24}H_{22}N_4O_3$.

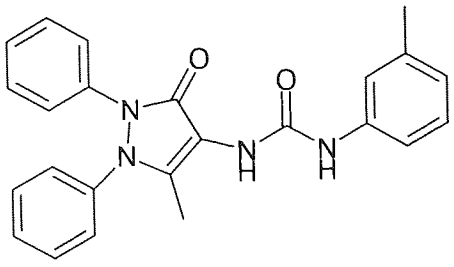
MS (APCI(+)): 415 (M+1), 266 (M+) m/z.

IR (KBr-disc) υ max: 3207, 1708, 1646, 1619, 1594, 1540, 1488, 1453, 1282, 761 & 697 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 2.02n(s, C-$\underline{C}H_3$), 3.72 (s, OCH_3), 6.50-6.55 (dd, Ar-H, J= 8.2 Hz), 6.88-6.92 (Ar-H, J= 8.1 Hz), 7.12-7.18 (m, Ar-3H), 7.26-7.44 (m, Ar-9H), 7.57 (s, NH), 8.88 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.6 (C-$\underline{C}H_3$), 55.4 (OCH_3), 99.7 ($\underline{C}$-CH_3), 104.2, 107.7, 109.7, 110.8, 123.6 (2xC), 125.6 (2xC), 126.2, 128.6, (2xC), 130.0 (2xC), 136.1, 139.2, 141.6, 143.0, 151.2, 153.8 (Ar-C), 160.2, 163.0 (C=O) p.p.m.

(5.5.5) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)N'-3-methylphenylurea



Yield: 91 %.

Mol. Weight: 398.5.

Mol. Formula: $C_{24}H_{22}N_4O_2$.

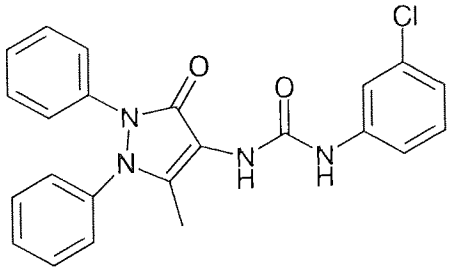
MS (APCI(+)): 399 (M+1), 266 (M+) m/z.

IR (KBr-disc) υ max: 3322, 1698, 1644, 1625, 1538, 1490, 1285, 1211, 759 & 697 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.01 (s, CH_3), 2.25 (s, C-$\underline{C}H_3$), 6.75-7.78 (d, Ar-H, J= 7.2 Hz), 7.10-7.44 (m, Ar-13H), 7.59 (s, NH), 8.80 (NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.7 ($\underline{C}$-CH_3), 21.7 (CH_3), 109.9, 115.7, 119.1, 123.0, 123.7 (2xC), 126.4, 128.7, 129.1 (2xC), 130.1 (2xC), 136.1, 138.4, 139.9, 140.2, 142.9, 151.1 (Ar-C), 153.9, 163.0 (C=O) p.p.m.

(5.5.7) N-(2-chlorophenyl)-N'-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)urea



Yield: 73 %.

Mol. Weight: 418.9.

Mol. Formula: $C_{23}H_{19}N_4O_2$.

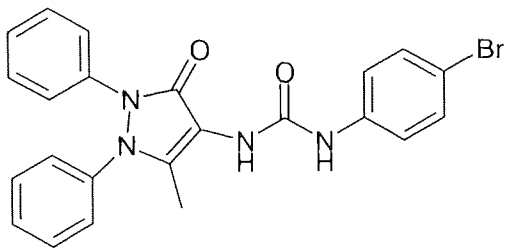
MS (APCI(+)): 418, 420 (M+1), 266 (M+) m/z.

IR (KBr-disc) υ max: 3293, 3212, 1710, 1621, 1590, 1530, 1488, 1422, 1291, 1191, 761 & 680 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.01 (s, CH_3), 6.97-7.03 (tt, Ar-H, J= 6.8 Hz), 7.11-7.18 (tt, Ar-H, J= 6.9, 6.8 Hz), 7.22-7.44 (m, Ar-12H), 7.89 (s, NH), 9.09 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.6 (CH_3), 109.5, 117.4, 118.3, 122.2, 123.7 (2xC), 126.2 (2xC), 128.7, 129.3 (2xC), 130.8 (2xC), 130.9, 133.7, 139.8, 141.5, 141.9, 151.4 (Ar-C), 153.8, 162.9 (C=O) p.p.m.

(5.5.9) N-(4-bromophenyl)-N'-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)urea



Yield: 92 %.

Mol. Weight: 463.3.

Mol. Formula: $C_{23}H_{19}BrN_4O_2$.

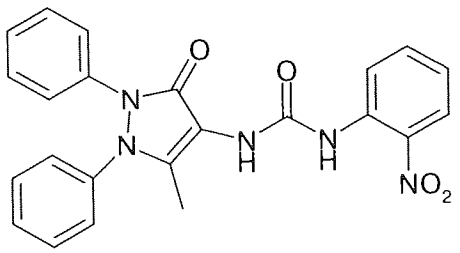
MS (APCI(+)): 464, 466 (M+1), 266 (M+) m/z.

IR (KBr-disc) υ max: 3285, 3062, 1704, 1644, 1490, 1534, 1486, 1288, 1209, 757 & 705 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.01 (s, CH_3), 6.50-6.52 (d, Ar-H, J= 6.9 Hz), 7.01-7.17 (m, Ar-2H), 7.29-7.43 (m, Ar-11H), 7.65 (s, NH), 9.02 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.6 (CH_3), 109.6, 113.9, 116.3, 120.7, 123.7 (2xC), 125.9 (2xC), 126.2, 128.7 (2xC), 129.3 (2xC), 130.5, 131.9, 132.0 (2xC), 136.1 (2xC), 139.8, 153.8 (Ar-C), 157.8, 162.9 (C=O) p.p.m.

(5.5.10) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)N'-2-nitrophenylurea



Yield: 65 %.

Mol. Weight: 430.4.

Mol. Formula: $C_{23}H_{20}N_5O_4$.

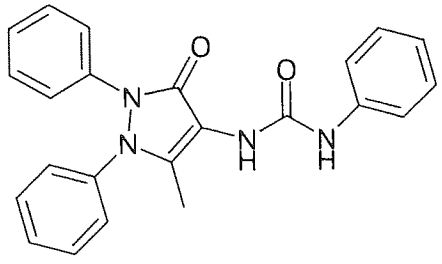
MS (APCI(+)): 431 (M+1), 266 (M+) m/z.

IR (KBr-disc) υ max: 3318, 3181, 3010, 1712, 1658, 1635, 1588, 1502, 1432, 1344, 1272, 1201, 759 & 688 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 2.02 (s, CH_3), 7.12-7.44 (m, Ar-11H), 7.64-7.71 (t, Ar-H, J= 7.3, 7.4 Hz), 8.06-8.10 (d, Ar-H, J= 8.4 Hz), 8.28-8.32 (d, Ar-H, J= 8.5 Hz), 8.90 (s, NH), 9.71 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.4 (CH_3), 122.7, 123.9, 124.0, 125.9, 126.3 (2xC), 126.6, 127.3 (2xC), 128.8, 128.9, 129.3 (2xC), 130.1 (2xC), 133.2, 135.6, 136.0, 138.0, 139.6 (Ar-C), 153.5, 162.8 (C=O) p.p.m.

(5.5.12) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-N'-phenylurea



Yield: 91 %.

Mol. Weight: 384.4.

Mol. Formula: $C_{23}H_{20}N_4O_4$.

MS (APCI(+)): 385 (M+1), 266 (M+) m/z.

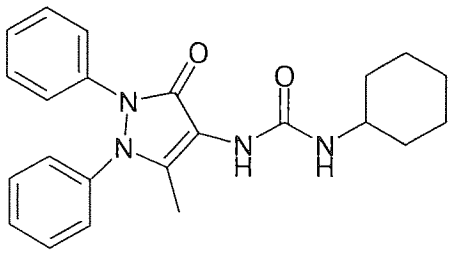
IR (KBr-disc) υ max: 3420, 3297, 3072, 3065, 1706, 1640, 1544, 1492, 1448, 1297, 1202, 755 & 697 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.02 (s, CH_3), 6.91-6.97 (tt, Ar-H, J=7.3 Hz), 7.11-7.17 (tt, Ar-H, 7.0, 7.1 Hz), 7.22-7.45 (m, Ar-13H), 7.60 (s, NH), 8.87 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.6 (CH_3), 109.8 ($\underline{C}$-CH_3), 118.5 (2xC), 122.2, 122.4 (2xC), 123.7 (2xC), 125.9 (2xC), 126.2, 128.6 129.1 (2xC), 129.8 (2xC), 136.1, 139.9, 140.3, 151.1 (Ar-C), 153.8, 163.0 (C=O) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 11.9 (CH_3), 36.6 (N-CH_3), 108.9 (CH-N), 118.0, 121.9, 123.4, 124.0, 126.2, 126.3 (2xC), 126.4, 126.5, 126.7 (2xC), 134.2, 134.9 ($\underline{C}$-CH_3), 135.2, 135.5, 151.1 (Ar-C), 154.6, 162.6 (C=O) p.p.m.

(5.5.14) N'-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-N'-cyclohexylurea



Yield: 86 %.

Mol. Weight: 390.5.

Mol. Formula: $C_{23}H_{23}N_4O_2$.

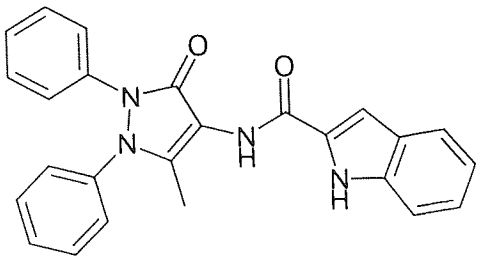
MS (APCI(+)): 391 (M+1), 266 (M+) m/z.

IR (KBr-disc) υ max: 3359, 3299, 2929, 2849, 1636,1694, 1596, 1538, 1488, 1276, 1228, 763 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 1.10-1.88 (m, -CH, -CH_2-, 11H), 1.95 (s, CH_3), 6.27-6.30 (d, Ar-H, J= 7.9 Hz), 7.12-7.16 (tt, Ar-H, J=6.8, 6.9 Hz), 7.24-7.42 (m, Ar-8H), 7.63 (s, NH), 8.86 (NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.9 (CH_3), 24.9 (-CH_2-x2), 25.8 (-CH_2-), 33.5 (-CH2-x2), 48.5 (-CH-NH), 99.7 (C-CH_3), 110.0 (C-N), 123.5 (2xC), 126.1 (2xC), 128.5, 129.2 (2xC), 130.0 (2xC), 136.1, 140.3, 150.2 (Ar-C), 155.6, 163.1 (C=O) p.p.m.

(5.5.16) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-2-carboxamide



General method:

A solution of 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one (0.2g, 0.76 mmols) was dissolved in dry acetonitrile (20 ml). The appropriate indole acid (1.25 Eq) was added, with DIC (3 Eq). The mixture was heated to 60 °C and left overnight. The resulting precipitated crystals were filtered, washed and dried.

Yield: 65%.

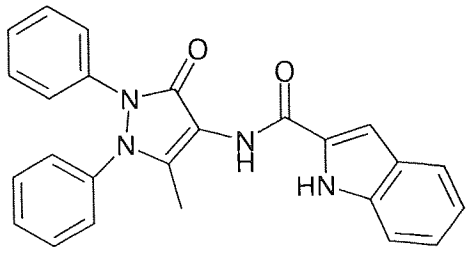
Mol. Weight: 408.5.

Mol. Formula: $C_{22}H_{20}N_4O_2$.

MS (APCI(-)): 407 (M+1), 364 (M+), 237 (M+) m/z.

IR (KBr-disc) υ max: 3401, 3339, 2965, 2358, 1710, 1615, 1583, 1454, 1361, 1172 & 748 cm^{-1}.

(5.5.17) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-3-carboxamide



Yield: 78 %.

Mol. Weight: 408.5.

Mol. Formula: $C_{25}H_{20}N_4O_2$.

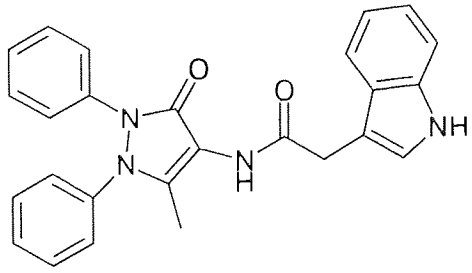
MS (APCI(+)): 409 (M+1) m/z.

IR (KBr-disc) υ max: 3343, 2965, 1615, 1581, 1535, 1494, 1453, 1318, 1249, 1191 & 750 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.04 (s, CH_3), 7.09-7.20 (m, Ar-3H), 7.27-7.45 (m, Ar-10H), 7.44-7.47 (d, Ar-H, J= 7.0 Hz), 7.99 (s, Ar-H), 9.16 (s, NH), 11.69 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.6 (CH_3), 109.7 (C-NH), 112.4, 121.0, 121.1, 121.5, 122.6, 123.6, 126.1(2xC), 126.3, 126.9 (2xC), 128.6 (2xC), 129.2, 129.3 (2xC), 130.1, 132.7, 136.4, 139.9, 152.8 (Ar-C), 164.5, 171.9 (C=O) p.p.m.

(5.5.18) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-(1H-indol-3-yl)acetamide



Yield: 66 %.

Mol. Weight: 422.5.

Mol. Formula: $C_{26}H_{22}N_4O_2$.

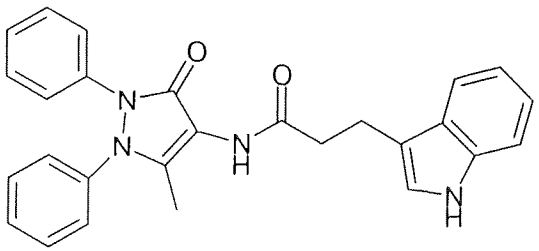
MS (APCI(+)): 423 (M+1) m/z.

IR (KBr-disc) υ max: 3337, 2965, 1679, 1648, 1629, 1592, 1525, 1488, 1312, 1243 & 749 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 1.86 (s, CH_3), 3.73 (s, CH_2), 6.94-7.00 (t, Ar-H, J= 8.0, 7.9 Hz), 7.03-7.09 (t, Ar-H, J= 8.2, 8.1 Hz), 7.10-7.17 (t, Ar-H, J= 6.8, 6.7 Hz), 7.27-7.38 (m, Ar-10H), 7.61-7.64 (d, Ar-H, J= 7.7 Hz), 9.38 (s, NH), 10.88 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.5 (CH_3), 23.8 (CH_2), 109.1 (C-NH), 109.4, 111.8, 118.4, 119.2, 121.5, 123.7 (2xC), 124.4 (2xC), 126.1, 126.4 (2xC), 127.7, 128.6 (2xC), 129.3, 130.0, 136.1, 136.6, 139.7, 151.9 (Ar-C), 162.7, 170.8 (C=O) p.p.m.

(5.5.19) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(1H-indol-3-yl)propanamide



Yield: 79 %.

Mol. Weight: 436.5.

Mol. Formula: $C_{27}H_{24}N_4O_2$.

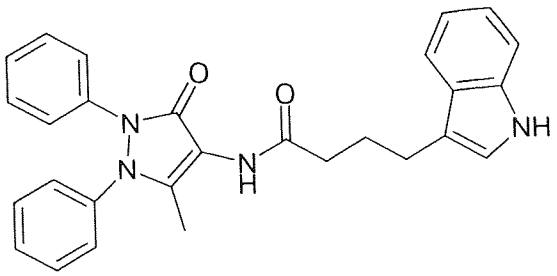
MS (APCI(+)): 375 (M+1) m/z.

IR (KBr-disc) υ max: 3436, 3284, 1640, 1590, 1548, 1490, 1459, 1317 & 753 cm^{-1}..

^{1}H NMR (DMSO-d_6) 300K δ: 1.84 (s, CH_3), 2.65-2.71 (t, CH_2, J= 7.2, 7.1 Hz), 2.98-3.04 (t, CH_2, J= 7.3, 7.4 Hz), 6.94-7.00 (t, Ar-H, J= 6.8, 6.8 Hz), 7.03-7.09 (t, Ar-H, J= 6.9, 6.9 Hz), 7.11-7.17 (m, Ar-2H), 7.27-7.41 (m, Ar-11H), 7.55-7.58 (d, Ar-H, J= 7.7 Hz), 9.27 (s, NH), 10.77 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.5 (CH_3), 21.4, 23.8 (CH_2), 109.3 (C-NH), 111.8, 114.1, 118.6, 118.8, 121.4 (2xC), 122.8, 123.7, 126.1 (2xC), 126.4, 127.6 (2xC), 128.6 (2xC), 129.3, 130.1, 136.2, 136.7, 139.9, 151.9 (Ar-C), 162.7, 172.1 (C=O) p.p.m.

(5.5.20) N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-(1H-indol-3-yl)butanamide



Yield: 80 %.

Mol. Weight: 450.5.

Mol. Formula: $C_{28}H_{26}N_4O_2$.

MS (APCI(+)): 450 (M+1) m/z.

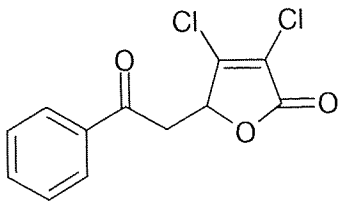
IR (KBr-disc) υ max: 3235, 3046, 1656, 1635, 1590, 1544, 1494, 1432, 1276 & 699 cm^{-1}.

^{1}H NMR (DMSO-d_6) 300K δ: 1.92-1.98 (m, CH_3, CH_2 (overlapping), 2.35-2.40 (t, CH_2, J= 7.3, 7.3 Hz), 2.71-2.77 (t, CH_2, J= 7.4, 7.5 Hz), 6.93-6.99 (t, Ar-H, J= 6.9, 7.2 Hz), 7.02-7.10 (t, Ar-H, J= 6.9, 6.9 Hz), 7.13-7.16 (t, Ar-2H, J= 7.3, 7.1 Hz), 7.24-7.42 (m, Ar-11H), 7.51-7.54 (d, Ar-H, J= 7.7 Hz), 9.20 (s, NH), 10.75 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 12.5 (CH_3), 23.8, 24.8, 26.7 (CH_2), 109.4 (C-NH), 111.8, 114.6, 118.4, 118.8, 121.3 (2xC), 122.8, 123.7, 126.1 (2xC), 126.4, 127.7 (2xC), 128.6 (2xC), 129.5, 130.1, 136.2, 136.8, 139.8, 152.0 (Ar-C), 162.8, 172.4 (C=O) p.p.m.

9.6. Experiments to Chapter 6

a: (6.2.1) 3,4-dichloro-5-(-2-oxo-2-phenylethyl]furan-2(5H)-one



Mucochloric acid (21.0 g, 0.125 mol) and (a) acteophenone and (b) 4-methoxyacetophenone were each dissolved in methanol (200 ml) and cooled to 0°C. A solution of NaOH (8.0 g in 70 ml water, 2.5 M) was added slowly, whilst stirring at 0-5 °C. After the addition of NaOH, the mixture was allowed to stand at RT for 3 hrs. The brown mixture was poured into ice-water, containing an excess of conc HCl and allowed to stand for 45 mins. A yellow oily liquid/solid was decanted and washed with water to give a crude yellow product. Refluxing from dilute ethanol gave a pure white powder.

Yield: 46.5 %

R_f (ether) = 0.26

Mol. Weight: 271.1

Mol. Formula: $C_{12}H_8Cl_2O_3$

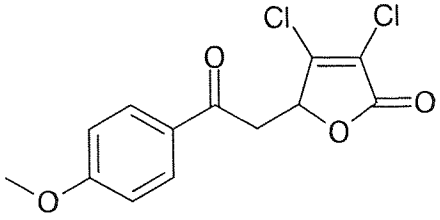
MS (APCl(+)): 271 (M+) m/z

IR (KBr-disc) υ max: 3010, 1773, 1683, 1648, 1210, 1033, 767 & 692 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 3.35-3.61 (m, CH_2), 5.69-5.73 (dd, CH, J= 3.6 Hz), 7.45-7.52 (t, Ar-2H, J= 7.8, 7.2 Hz), 7.56-7.65 (tt, Ar-H, J= 7.4, 7.3 Hz), 7.91-7.95 (d, Ar-2H, J= 7.1 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 40.0 (CH_2), 78.0 (CH), 121.4 (C-Cl), 128.1 (2xC), 134.1, 135.7 (2xC) (Ar-C), 151.9 (C-Cl), 164.8 169.9, 193.7 (C=O) p.p.m.

b: (6.2.2) 3,4-dichloro-5-[2-(4-methoxyphenyl)-2-oxoethyl]furan-2(5H)-one



Yield: 17.5 %

R_f (ether) = 0.43

Mol. Weight: 301.1

Mol. Formula: $C_{13}H_{10}Cl_2O_4$

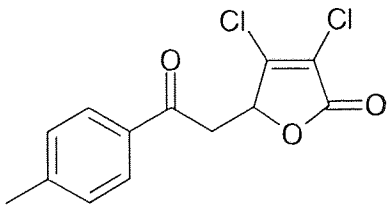
MS (APCI(+)): 301 (M+) m/z

IR (KBr-disc) υ max: 3448, 1775, 1665, 1629, 1598, 1258, 1209, 1174, 1031, 983 & 744 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 3.29-3.57 (m, CH_2), 3.86 (s, CH_3), 5.68-5.72 (dd, CH, J= 3.7 Hz), 6.89-6.96 (d, Ar-2H, J= 9.0 Hz), 7.87-7.93 (d, Ar-2H, J= 9.0 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 39.6 (CH_2), 55.3 (CH_3), 78.2 (CH), 113.9 (2xAr-C), 121.2 (C-Cl), 128.7, 130.5 (2xC), 152.1 (C-Cl), 164.2 (Ar-C), 169.9, 192.0 (C=O) p.p.m.

a: (6.2.3) 3,4-dichloro-5-[2-(4-methylphenyl)-2-oxoethyl]furan-2(5H)-one



Mucochloric acid (21.0 g, 0.125 mol) and (a) 4-methylacteophenone and (b) 4-chloroacetohenone were each dissolved in propan-2-ol (250 ml) and cooled to 0°C. A solution of NaOH (8.0 g in 70 ml water, 2.5 M) was added slowly, whilst stirring at 0-5 °C. After the addition of NaOH, the mixture was allowed to stand at RT for 3 hrs. The crude precipitate was poured into ice-water containing an excess of conc HCl and allowed to stand for 45 mins. The solid was filtered, washed with water and recrystallised from dilute propan-2-ol to give a white powder.

Yield: 42.8 %

R_f (ether) = 0.77

Mol. Weight: 285.1

Mol. Formula: $C_{13}H_{10}Cl_2O_3$

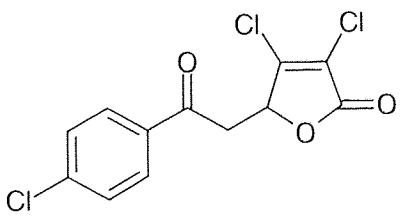
MS (APCI(+)): 285 (M+), 187 (M+) m/z

IR (KBr-disc) υ max: 3420, 1783, 1677, 1631, 1602, 1368, 1183, 1019, 948 & 915 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 2.41 (s, CH_3), 3.32-3.57 (m, CH_2), 5.68-5.70 (dd, CH, J= 3.6 Hz), 7.26-7.29 (d, Ar-2H, J= 8.3 Hz), 7.80-7.84 (d, Ar-2H, J= 8.2 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 21.6 (CH_3), 39.8 (CH_2), 78.1 (CH), 121.3 (C-Cl), 128.2 (2xC), 129.5 (2xC), 133.2, 145.1 (Ar-C), 152.0 (C-Cl), 164.8, 193.2 (C=O) p.p.m.

b: (6.2.4) 3,4-dichloro-5-[2-(4-chlorophenyl)-2-oxoethyl]furan-2(5H)-one



Yield: 70.9 %

R_f (ether) = 0.70

Mol. Weight: 305.5

Mol. Formula: $C_{12}H_7Cl_3O_3$

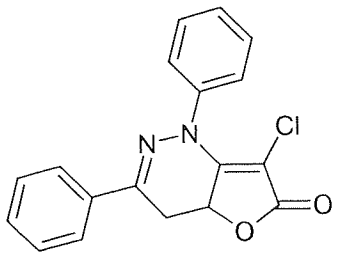
MS (APCI(+)): 225 (M+), 207 (M+) m/z

IR (KBr-disc) υ max: 3430, 1777, 1687, 1633, 1584, 1390, 1203, 1087, 1027, 834 & 747 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 3.32-3.57 (m, CH_2), 5.68-5.73 (dd, CH, J= 3.6 Hz), 7.45-7.50 (d, Ar-2H, J= 8.7 Hz), 7.85-7.90 (d, Ar-2H, J= 8.8 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 40.0 (CH_2), 77.8 (CH), 121.5 (C-Cl), 129.2 (2xC), 129.5 (2xC), 133.9, 140.7 (Ar-C), 151.7 (C-Cl), 164.7, 192.5 (C=O) p.p.m.

(6.2.a) 7-chloro-1,3-diphenyl-4,4a,7,7a-tetrahydrofuro[3,2-c]pyridazin-6(1H)-one



The appropriate 3,4-dichloro-5-[2-(4-substituted-phenyl)-2-oxoethyl]furan-2(5H)-one (0.1 g, 1.0 Eq) was dissolved in ethanol (20 ml), with the appropriate hydrazine (2.5 Eq). Concentrated HCl acid (0.5 ml) was added and the mixture was refluxed for up to 20 hrs. The solution was allowed to cool to RT, with the precipitate being filtered, washed and dried.

Yield: 33.5 %

Mol. Weight: 326.8

Mol. Formula: $C_{18}H_{15}ClN_2O_2$

MS (APCI(+)): 327, 329 (M+) m/z

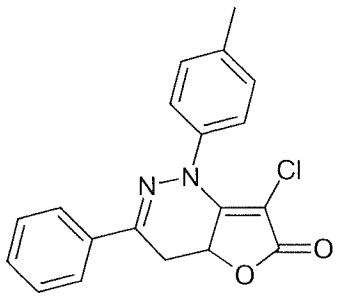
IR (KBr-disc) υ max: 1758, 1627, 1588, 1490, 1330, 1195, 1094, 1004, 767 & 691 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 2.81-2.94 (dd, CH, J= 13.6 Hz), 3.63-3.74 (dd, CH, J= 7.5

Hz), 5.19-5.28 (dd, CH-O, J= 7.4 Hz), 7.31-7.50 (m, Ar-8H), 7.78-7.82 (m, Ar-2H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 29.3 (CH_2), 70.1 (CH), 90.2 (C=N), 123.4 (2xC), 125.7 (2xC), 128.6 (2xC), 128.7 (2xC), 129.9, 133.6, 135.8, 139.3, 148.0, 148.9 (Ar-C), 167.9 (C=O) p.p.m.

(6.2.b) 7-chloro-1-(4-methylphenyl)-3-phenyl-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 35.5 %

Mol. Weight: 338.8

Mol. Formula: $C_{19}H_{15}ClN_2O_2$

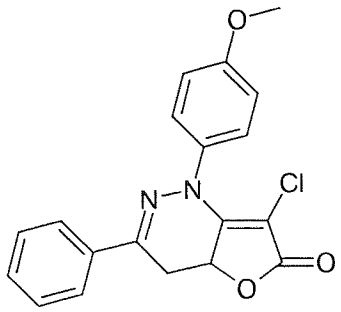
MS (APCI(+)): 339, 341 (M+), 303, 305 (M+) m/z

IR (KBr-disc) υ max: 1771, 1640, 1610, 1511, 1310, 1247, 1174, 1085, 1012 & 823 cm^{-1}.

^1H NMR (DMSO) 300K δ: 2.35 (s, CH_3), 2.93-3.05 (dd, CH, J= 13.7 Hz), 3.71-3.81 (dd, CH, J= 7.7 Hz), 5.50-5.58 (dd, CH-O, J= 7.6, 7.7 Hz), 7.26-7.34 (m, Ar-4H), 7.44-7.47 (m, Ar-3H), 7.81-7.85 (m, Ar-2H) p.p.m.

^{13}C NMR (DMSO) 300K δ: 21.1 (CH_3), 29.0 (CH_2), 70.3 (CH), 89.7 (C=N), 124.2 (2xC), 126.5 (2xC), 129.1 (2xC), 129.6 (2xC), 130.5, 135.9, 136.8, 138.6, 148.0, 149.9 (Ar-C), 168.7 (C=O) p.p.m.

(6.2.d) 7-chloro-1-(4-methoxyphenyl)-3-phenyl-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 30.1 %

Mol. Weight: 354.8

Mol. Formula: $C_{19}H_{15}ClN_2O_3$

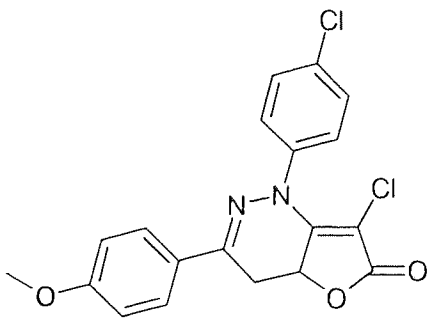
MS (APCI(+)): 355, 357 (M+), 319, 321 (M+) m/z

IR (KBr-disc) υ max: 1762, 1627, 1507, 1320, 1257, 1193, 1091 & 746 cm^{-1}.

^1H NMR (DMSO) 300K δ: 2.91-3.03 (dd, CH, J= 13.9 Hz), 3.69-3.80 (dd, CH, J= 7.6 Hz), 3.80 (s, OCH_3), 5.48-5.57 (dd, CH-O, J= 7.5, 7.6 Hz), 7.00-7.05 (d, Ar-2H, J= 9.0 Hz), 7.35-7.40 (d, Ar-2H, J= 9.0 Hz), 7.43-7.46 (m, Ar-3H), 7.80-7.84 (m, Ar-2H) p.p.m.

^{13}C NMR (DMSO) 300K δ: 28.7 (CH_2), 55.9 (OCH_3), 70.3 (CH), 88.9 (C=N), 114.3 (2xC), 126.2 (2xC), 126.5 (2xC), 129.1 (2xC), 130.5, 134.2, 135.9, 148.0, 150.1, 158.6, (Ar-C), 168.7 (C=O) p.p.m.

(6.2.g) 7-chloro-1-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 21.7 %

Mol. Weight: 389.2

Mol. Formula: $C_{19}H_{14}Cl_2N_2O_3$

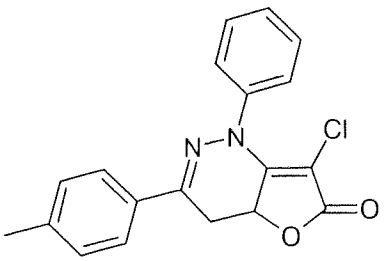
MS (APCI(+)): 389 (M+), 353 (M+), 345 (M+) m/z

IR (KBr-disc) υ max: 1744, 1617, 1511, 1492, 1330, 1253, 1175, 1089, 1006 & 828 cm^{-1}.

^1H NMR (DMSO) 300K δ: 3.42-3.01 (dd, CH, J= 13.6 Hz), 3.37-3.83 (dd, CH, J= 7.7 Hz), 3.80 (s, CH_3), 5.49-5.57 (dd, CH-O, J= 7.6 Hz), 7.00-7.04 (d, Ar-2H, J= 8.9 Hz), 7.42-7.47 (d, Ar-2H, J= 8.9 Hz), 7.52-7.57 (d, Ar-2H, J= 8.9 Hz), 7.80-7.84 (d, Ar-2H, J= 8.9 Hz) p.p.m.

^{13}C NMR (DMSO) 300K δ: 29.2 (CH_2), 55.8 (OCH_3), 70.3 (CH), 90.4 (C=N), 114.5 (2xC), 125.6 (2xC), 128.0, 128.3 (2xC), 129.1 (2xC), 131.1, 139.8, 148.2, 149.7, 161.4 (Ar-C), 168.5 (C=O) p.p.m.

(6.2.i) 7-chloro-3-(4-methylphenyl)-1-phenyl-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 22.4 %

Mol. Weight: 338.8

Mol. Formula: $C_{19}H_{15}ClN_2O_2$

MS (APCI(+)): 339, 341 (M+), 303, 305 (M+) m/z

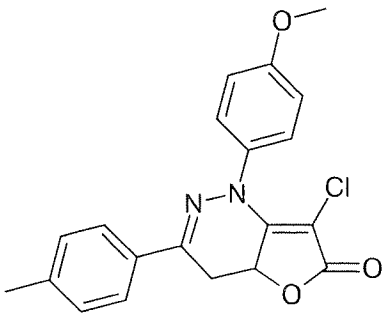
IR (KBr-disc) υ max: 1762, 1625, 1590, 1496, 1395, 1330, 1193, 1098, 998 & 747 cm^{-1}.

^1H NMR (DMSO) 300K δ: 2.34 (s, CH_3), 2.90-3.03 (dd, CH, J= 13.7 Hz), 3.71-3.81 (dd, CH, J= 7.7 Hz), 5.50-5.59 (dd, CH-O, J= 7.7, 7.6 Hz), 7.25-7.52 (m, Ar-6H), 7.73-7.76 (d, Ar-2H, J= 8.3 Hz) p.p.m.

^{13}C NMR (DMSO) 300K δ: 21.4 (CH_3), 29.1 (CH_2), 70.3 (CH), 90.1 (C=N), 124.2 (2xC),

126.5 (2xC), 127.2 (2xC), 129.1 (2xC), 129.7, 133.1, 140.4, 140.9, 148.1, 149.9 (Ar-C), 168.6 (C=O) p.p.m.

(6.2.l) 7-chloro-1-(4-methoxyphenyl)-3-(4-methylphenyl)-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 12.3 %

Mol. Weight: 368.8

Mol. Formula: $C_{20}H_{17}ClN_2O_3$

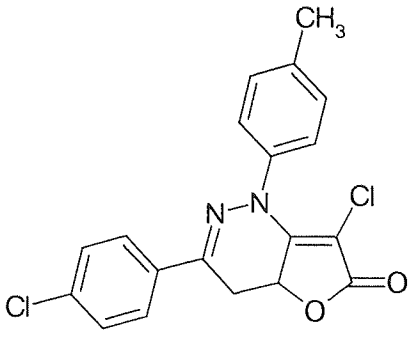
MS (APCI(+)): 369, 271 (M+), 333, 335 (M+) m/z

IR (KBr-disc) υ max: 2358, 1737, 1623, 1507, 1399, 1322, 1228, 1091, 998 & 834 cm^{-1}.

^1H NMR (DMSO) 300K δ: 2.33 (s, CH_3), 2.86-2.98 (dd, CH, J= 14.0 Hz), 3.67-3.77 (dd, CH, J= 7.6 Hz), 3.80 (s, OCH_3), 5.46-5.55 (dd, CH-O, J= 7.5 Hz), 7.02-7.07 (d, Ar-2H, J= 9.0 Hz), 7.24-7.28 (d, Ar-2H, J= 8.2 Hz), 7.32-7.39 (d, Ar-2H, J= 8.9 Hz), 7.70-7.74 (d, Ar-2H, J= 8.2 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 21.4 (CH_3), 28.7 (CH_2), 55.9 (OCH_3), 70.3 (CH), 88.6 (C=N), 114.2 (2xC), 125.3 (2xC), 126.5 (2xC), 129.7 (2xC), 133.1, 134.2, 140.3, 148.0, 150.0, 158.6 (Ar-C), 168.8 (C=O) p.p.m.

(6.2.n) 7-chloro-3-(4-chlorophenyl)-1-(4-methylphenyl)-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 38.6 %

Mol. Weight: 373.2

Mol. Formula: $C_{19}H_{14}Cl_2N_2O_2$

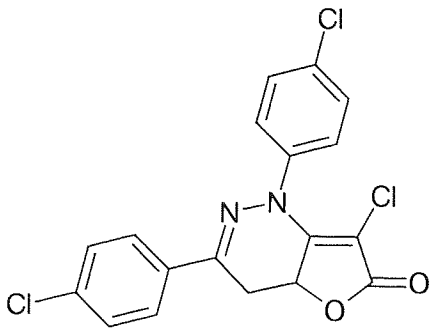
MS (APCI(+)): 373 (M+), 337 (M+) m/z

IR (KBr-disc) υ max: 3450, 1773, 1643, 1513, 1492, 1396, 1324, 1199, 1010 & 817 cm^{-1}.

^1H NMR (DMSO) 300K δ: 2.35 (s, CH_3), 2.93-3.05 (dd, CH, J= 13.7 Hz), 3.70-3.80 (dd, CH, J= 7.7 Hz), 5.50-5.58 (dd, CH-O, J= 7.6, 7.7 Hz), 7.26-7.34 (m, Ar-4H), 7.50-7.53 (d, Ar-2H, J= 8.7 Hz), 7.83-7.86 (d, Ar-2H, J= 8.7 Hz) p.p.m.

^{13}C NMR (DMSO) 300K δ: 21.1 (CH_3), 28.9 (CH_2), 70.2 (CH), 90.2 (C=N), 124.2 (2xC), 128.3 (2xC), 129.2 (2xC), 129.6 (2xC), 134.8, 135.2, 136.8, 138.5, 146.8, 149.8 (Ar-C), 168.6 (C=O) p.p.m.

(6.2.o) 7-chloro-1,3-bis(4-chlorophenyl)-4,4a-dihydrofuro[3,2-c]pyridazin-6(1H)-one



Yield: 38.1 %

Mol. Weight: 393.7

Mol. Formula: $C_{18}H_{11}Cl_3N_2O_2$

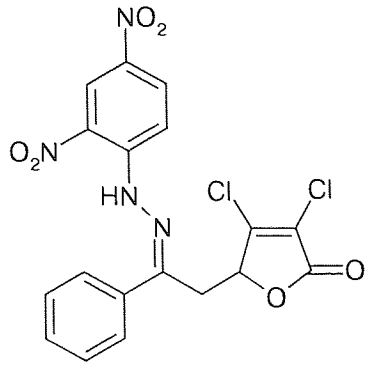
MS (APCI(+)): 393 (M+), 357 (M+) m/z

IR (KBr-disc) υ max: 1760, 1623, 1486, 1390, 1328, 1199, 1083, 1004 & 823 cm^{-1}.

^1H NMR (DMSO) 300K δ: 2.95-3.07 (dd, CH, J= 13.5, 13.4 Hz), 3.73-3.83 (dd, CH, J= 7.7 Hz), 5.51-5.59 (dd, CH-O, J= 7.6, 7.7 Hz), 7.42-7.58 (m, Ar-6H), 7.85-7.88 (d, Ar-2H, J= 8.7 Hz) p.p.m.

^{13}C NMR (DMSO) 300K δ: 29.2 (CH_2), 70.1 (CH), 91.4 (C=N), 125.5 (2xC), 129.1, 129.2 (2xC), 131.2 (2xC), 134.6, 135.3, 139.7, 147.3, 149.5 (Ar-C), 168.5 (C=O) p.p.m.

(6.2.q) 3,4-dichloro-5-{2-[(Z)-2(2,4-dinitrohenyl) hydrazono]-2-phenylethylfuran-2(5H)-one



The appropriate 3,4-dichloro-5-[2-(4-substituted-phenyl)-2-oxoethyl]furan-2(5H)-one (0.5 g, 1.0 Eq) was dissolved in ethanol (50 ml), with the appropriate nitro-substituted hydrazine (2.0 Eq) added. Concentrated HCl acid (1.0 ml) was added and the mixture was refluxed for up to 2 hrs. The solution was allowed to cool to RT, with the precipitate being filtered, washed and dried.

Yield: 76.1 %

Mol. Weight: 451.2

Mol. Formula: $C_{18}H_{12}N_4Cl_2O_6$

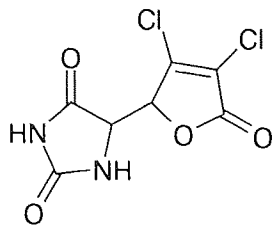
MS (APCI(+)): 451 (M+) m/z

IR (KBr-disc) υ max: 3461, 3295, 3102, 1779, 1590, 1490, 1417, 1328, 1029 & 712 cm^{-1}.

^1H NMR (DMSO) 300K δ: 3.51-3.79 (m, CH_2), 5.72-5.77 (dd, CH, J= 3.7 Hz), 7.49-7.64 (m, Ar-5H), 8.05-8.08 (d, Ar-H, J= 9.5 Hz), 8.42-8.46 (dd, Ar-H, J= 9.6 Hz), 8.89-8.92 (m, Ar-H), 11.27 (s, NH) p.p.m.

^{13}C NMR (DMSO) 300K δ: 33.6 (CH_2), 76.2 (CH), 90.3 (C=N), 113.6, 120.2, 123.0 (2xC), 124.1, 128.7, 129.6 (2xC), 133.7, 145.7, 145.9, 150.3, 155.9 (Ar-C), 165.8 (C=O) p.p.m.

5-(3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl)imidazolidine-2,4-dione



Mucochloric acid (8.45 g, 0.05 mol) and hydantoin (5.0 g, 0.05 mol) were dissolved in dichloroethane (60 ml) and cooled to 0°C. A solution of sodium hydroxide (6.0 g in 75 ml water, 2 M) was slowly added, whilst stirring at 0-5°C. After the addition of sodium hydroxide, the mixture was allowed to stand at room temperature for 4 hrs. The solution was poured into ice water containing an excess of concentrated hydrochloric acid and allowed to stand for 45 mins. The precipitate was filtered, washed with water and dried to give a crude light brown product. Recrystallisation from dilute ethanol gave a pure white powder.

Yield: 20 %.

Mol. Weight: 251.0.

Mol. Formula: $C_7H_4Cl_2N_2O_4$

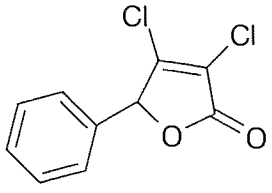
MS (APCI(+): 251, 253 (M+1) m/z.

IR (KBr-disc) υ: 3286, 3171, 3054, 1787, 1723, 1652, 1419, 1234, 1023 & 811 cm^{-1}

^1H NMR (DMSO-d_6) 300K δ: 4.70 (t, 1H, -CH-furan, J = 1.6, 1.6 Hz), 5.66 (d, 1H, -CH-hydantoin, J = 1.7 Hz), 8.25 (s, -NH), 11.03 (s, -NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 57.4 (CH-furan), 80.1 (CH-hydantoin),121.5 (CH-CCl), 158.2 (C-furan), 165.3 (C=O-furan), 165.3 & 172.4 (C=O-hydantoin) p.p.m.

3,4-dichloro-5-phenylfuran-2 (5H)-one



Mucochloric acid (16.38 g, 0.1 mol) was dissolved in benzene (250 ml). Powdered aluminium chloride (20 g) was slowly added to the mixture, whilst stirring. The solution was left for 3 days under inert conditions. The whole mixture was poured into an acidic-ice solution comprising of (130 g ice, 40 g HCl con). The organic phase was separated and washed with water. The benzene layer was dried over magnesium sulphate and removed in vacu*o*. An viscous brown oil was recrystallised from ethanol to yield white crystals.

Yield: 53.6 %.

Mol. Weight: 229.0.

Mol. Formula: $C_{10}H_6Cl_2O_3$.

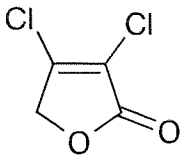
IR (KBr-disc) υ: 3526, 1772, 1625, 1287, 1228, 1025, 909, 765, 700 cm^{-1}.

MS (APCI(+)): 229, 231 (M+1) m/z.

^1H NMR ($CDCl_3$) 300K δ: 5.86 (s, 1H, -CH), 7.25-7.49 (m, 5H, aryl-H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 83.6 (CH), 121.0 (C=OC<u>C</u>Cl), 127.1, 127.2 & 130.4 (o,m & p-aryl C), 131.6 (CH-aryl-C), 152.2 (C=O<u>C</u>Cl), 156.3 (C=O) p.p.m.

3,4-dichlorofuran-2 (5H)-one



Mucochloric acid (33.8 g, 0.2 mol) and aluminium isopropoxide (50.0 g, 0.25 mol) was dissolved in isopropanol (200 ml) and refluxed using a vigreux column, until acetone ceased distilling. The excess isopropanol was removed by distillation and the mixture poured into a mixture of ice (300 g) and concentrate hydrochloric acid (100 ml). The resulting slurry was heated to 50°C and extracted with chloroform. After washing with water, sodium carbonate and hydrochloric acid solutions twice, the extract was distilled to give a crude product. Recrystallised from dilute ethanol gave a white solid.

Yield: 33.1 %.

Mol. Weight: 152.9.

Mol. Formula: $C_4H_2Cl_2O_2$.

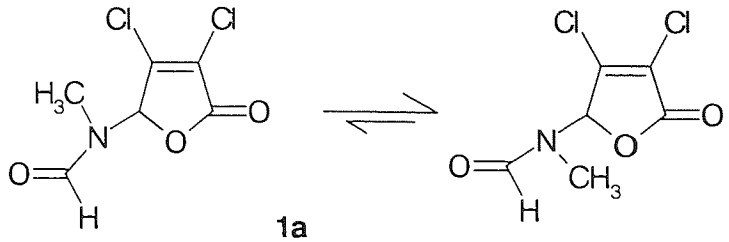
IR (KBr-disc) υ: 1781, 1631, 1442, 1351, 1243, 1013, 913, 747 cm^{-1}.

MS (APCI(+)): 153, 155 (M+1) m/z.

^1H NMR ($CDCl_3$) 300K δ: 4.86 (s, 1H, -CH) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 72.0 (CH), 120.6 (C=OC<u>C</u>Cl), 149.3 (C=O<u>C</u>Cl), 165.9 (C=O) p.p.m.

(6.6.a) 3-4-dichloro-5-oxo-2,5-dihydrofuran-yl(methyl)formamide



1a

Mucochloric acid (15.0 g, 88.8 mmol) and N-methylformamide (4.73 g, 90 mmol) were refluxed in toluene (180 ml), under a Dean stark trap, with 8-10 drops of H_2SO_4 conc. After 50-55 hrs the mixture was cooled to room temperature. Chloroform and water was added, with the organic layer separated and washed with a further portion of water. The organic layer was dried over magnesium sulphate and removed in vacuo. A viscous crude liquid was obtained. Column chromatography (MP = 10% methanol in ether) gave a white crystalline product, (the azabicyclo-one **1b**)
and an yellow oil, (the formamide product **1a**).

Yield: 10.5 %.

R_f (10% MeOH/ether)= 0.53.

Mol. Formula: $C_6H_5Cl_2NO_3$.

Mol. Weight: 210.

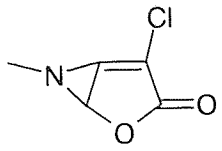
IR (KBr-disc) υ max: 2961, 1806, 1701, 1408, 1299, 1030, 913, 747 cm^{-1}.

MS (APCI(+)): 210 (M+1) m/z.

^1H NMR (DMSO-d_6) 300 K δ: (Isomers) 2.60, 2.84 (s, CH_3), 6.22, 6.80 (s, CH), 8.37, 8.52 (s, COH) p.p.m.

^{13}C NMR (DMSO-d_6) 300 K δ: (Isomers) 24.4, 28.3 (CH_3), 81.5, 88.6 (CH), 124.0, 124.9 (C-Cl), 146.3, 147.1 (C-Cl-CO), 161.8, 162.5 (CO-O), 163.8, 167.4 (C=O) p.p.m.

4-chloro-6-methyl-2-oxa-6-azabicyclo[3.1.0]hex-4-en-3-one



1b

Yield: 15.2 %.

R_f (ether)= 0.63.

Mol. Formula: $C_5H_4ClNO_2$.

Mol. Weight: 145.54.

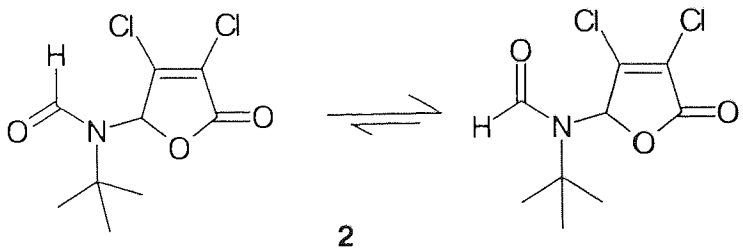
IR (KBr-disc) υ max: 2985, 2844, 1790, 1624, 1248, 1167, 954 cm^{-1}.

MS (APCI+): 146, 148 (M+H) m/z.

^{1}H-NMR ($CDCl_3$) 300K δ: 3.07 (s, 3H, $-CH_3$), 6.65 (s, 1H, -CH) p.p.m.

^{13}C-NMR ($CDCl_3$) 300K δ: 24.4 (N-CH_3), 126.8 (N-C-O), 140.9 (CCl), 164.9 (=C-N), 167.8 (C=O) p.p.m.

(6.6.b) *tert*-butyl(3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl)formamide



2

Mucochloric acid (15.0 g, 88.8 mmol) and the relevent amide (2: N-tert-butyl-formamide, 3: N-methylacetamide, 4: N-benzylformamide) (133.2 mmol) were refluxed in toluene (180 ml), under a Dean stark trap, with 8-10 drops of H_2SO_4 conc. After 48-60 hrs the mixture was cooled to room temperature. Chloroform and water was added, with the organic layer separated and washed with a further portion of water. The organic layer was dried over magnesium sulphate and removed in vacuo. A viscous crude liquid was obtained. Column chromatography (MP = 10%, MeOH in ether) yielded the corresponding crystalline formamide product.

Yield: 11.9 %.

R_f (10% MeOH/ether)= 0.61.

IR (KBr-disc) υ max: 3279, 2971, 1679, 1614, 1392, 1346, 1266, 1195, 1006 cm^{-1}.

Mol. Formula: $C_9H_{11}ClNO_3$.

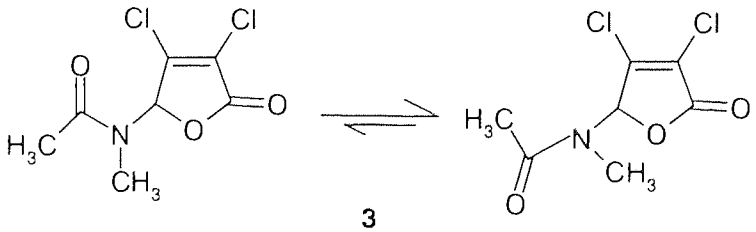
Mol. Formula: 252.1.

MS (APCI(+)): 253 (M+1), 162, 163, 164 (M+) m/z.

^1H NMR ($CDCl_3$) 300 K δ: (Isomers) 1.25-1.32 (m, CH_3, 9H), 7.27, 8.14 (s, CH), 7.82, 8.89 (s, COH) p.p.m.

^{13}C NMR ($CDCl_3$) 300 K δ: (Isomers)(28.7, 29.9, 30.17), (50.3, 51.1, 53.0) (CH_3), 61.6, 63.1 ($\underline{C}(CH_3)_3$), 106.4 (CH), 148.8 (C-Cl), 160.8 (C-Cl-CO), 180.4 (CO-O), 192.9 (C=O) p.p.m.

(6.6.c) N-(3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl)-N-methylacetamide



3

Yield: 6.0 %.

R_f (10% MeOH/ether)= 0.73.

Mol. Formula: $C_7H_7Cl_2NO_3$.

Mol. Weight: 224.0.

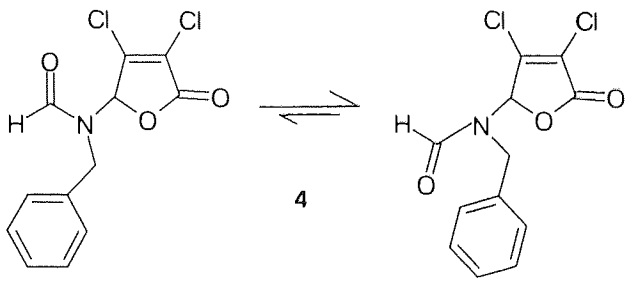
IR (KBr-disc) υ max: 3372, 2963, 1769, 1640, 1447, 1233, 1150, 1023, 946, 886, 748 cm^{-1}.

MS (APCI(+)): 224 (M+1), 182, 183, 184 (M+) m/z.

^1H NMR (DMSO-d_6) 300 K δ: (Isomers) 2.18, 2.34 (s, CH_3), 2.59, 2.79 (s, N- CH_3), 6.23 (s, CH) p.p.m.

^{13}C NMR ($CDCl_3$) 300 K δ: (Isomers) 22.0 (CH_3), 28.9 (N- CH_3), 83.3 (CH), 124.2 (C-Cl), 148.0 (C-Cl-CO), 163.5 (CO-O), 172.3 (C=O) p.p.m.

(6.6.d) Benzyl(3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl)formamide



4

Yield: 15.0 %.

R_f (10% MeOH/ether)= 0.71.

Mol. Formula: $C_{12}H_9Cl_2NO_3$.

Mol. Weight: 286.1.

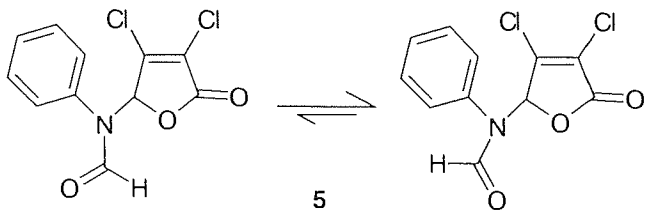
IR (KBr-disc) υ max: 3281, 3052, 2882, 2358, 1648, 1530, 1451, 1386, 1241, 753, 695 cm^{-1}.

MS (APCI(+)): 287 (M+1), 196, 197, 198 (M+) m/z.

^{1}H NMR (DMSO-d_6) 300 K δ: (Isomers) 4.32-4.34, 4.70-4.50 (sd, -CH_2-, J=6.1 Hz), 7.22-7.41 (m, phenyl-5H), 7.85, 7.90 (s, CH), 8.52, 8.90 (s, COH) p.p.m.

^{13}C NMR (DMSO-d_6) 300 K δ: (Isomers) 41.3, 45.1 (m, CH_2), 100.4, 105.0 (s, CH), 127.4 (2xC), 127.5, 127.6, 127.8, 127.8 (2xC), 127.9, 128.8 (2xC), 128.9, 129.0 (2xC), 129.1 (Ar-C), 139.3 (C-Cl), 140.1 (C-Cl-CO), 155.7, 161.6 (CO-O), 165.5 (C=O) p.p.m.

(6.6.e) 3,4-dichloro-5-oxo-2,5-dihydrofuran-2-yl(phenyl)formamide



5

Mucochloric acid (15.0 g, 88.8 mmol) and formanilide 21.51 g, 133.2 mmol) were refluxed in toluene (180 ml), under a Dean stark trap, with 8-10 drops of H_2SO_4 conc. After 48-60 hrs the mixture was cooled to room temperature. A dark yellow precipitate was filtered, washed with toluene and dried, to give a yellow crystalline powder.

Yield: 48.5 %.

R_f (10% MeOH/ether)= 0.90.

Mol. Formula: $C_{11}H_7Cl_2NO_3$.

Mol. Weight: 272.1.

IR (KBr-disc) υ max: 3426, 3048, 2971, 1627, 1581, 1484, 1328, 1266, 1187, 757, 684 cm^{-1}.

MS (APCI(+)): 273(M+1) m/z.

^{1}H NMR (DMSO-d_6) 300 K δ: (Isomers) 7.05-7.10 (t, Ar-H, J=7.2 Hz), 7.29-7.52 (m, Ar-H), 7.66-7.69 (d, Ar-H, J=7.9 Hz), 9.22, 9.48 (s, CH), 11.81 (s, COH) p.p.m.

^{13}C NMR (DMSO-d_6) 300 K δ: (Isomers) 105.8, 113.4 (CH), 122.1, 124.2 (2xC), 128.1, 128.7, 132.0, 132.8, 134.5, 134.8 (2xC) (Ar-C), 145.5(C-Cl), 152.5 (C-Cl-CO), 160.5 (CO-O), 187.4 (C=O) p.p.m.

9.7. Experiments to Chapter 7

General method:

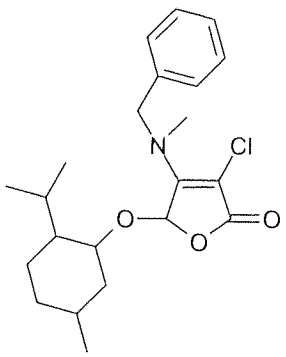
The appropriate building block (A1-A8), 0.2g was dissolved in DMF (10-20 ml) and placed into test tubes in the reaction carousel. The suitable amine (3 Eq) was added to each tube and was allowed to stir at a temperature of 50°C. The mixtures were left overnight and monitored by TLC. Water (25 ml) was added to each mixture, allowed to stand for 30 mins. Then;

Method 1 The precipitated compound was filtered, washed with water and dried.

Method 2 The compound was extracted with DCM and washed with dilute HCl (pH 5) and water twice. The organic layer was dried and removed in vacuo to give the desired product.

Method 3 The compound was extracted with DCM and washed with dilute HCl (pH 5) and water twice. The organic layer was dried and removed in vacuo. Chromatographic separation was achieved with either 100% ether or 10% MeOH in ether, as the mobile phase.

2: (A_1B_6) 4-[benzyl(methyl)amino]-3-chloro-5-[(2-isopropyl-5-methylcyclohexyl)oxo] furan-2(5*H*)-one



R_f (ether)= 0.33

Mol. Weight: 291.9.

Mol. Formula: $C_{22}H_{30}ClNO_3$.

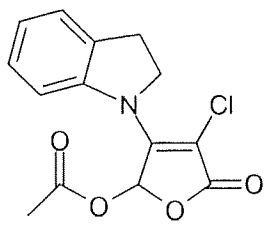
MS (APCI(+)): 392, 394 (M+1), 254, 256 (M+) m/z.

IR (KBr-disc) υ max: 3472, 2954, 2867, 1746, 1629, 1449, 1342, 1270, 1108, 1025, 979, 738 & 699 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: (isomers) 0.81-1.19 overlapping (m, CH_3), CH_2, CH, 14H), 1.60-1.66 (m, CH_2), 2.08-2.41 (m, CH_2), 3.07 (s, CH_2), 3.10 (s, N-CH_3), 3.55-3.72 (m, CH), 5.80 (s, CH), 7.21-7.39 (m, Ar-5H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 15.7 (2xCH_3), 20.7 (CH_3), 21.0 (CH), 23.2 (CH), 25.2 (CH_2), 31.5 (CH_2), 38.1 (N-CH_3), 42.2 (CH), 47.7 (CH), 55.4 (CH_2-Ar), 80.6 (alkyl-CH-O), 94.3 (CH-O), 97.3 (C-Cl), 127.0 (2xC), 127.8 (2xC), 135.6, 135.7 (Ar-C), 156.5 (C-N), 168.3 (C=O) p.p.m.

2: (A_2B_2) 4-chloro-3-(2,3-dihydro-1H-indol-1-yl)-5-oxo-2,5-dihydrofuran- 2-yl acetate



R_f (ether)= 0.83

Mol. Weight: 293.7.

Mol. Formula: $C_{14}H_{12}ClNO_4$.

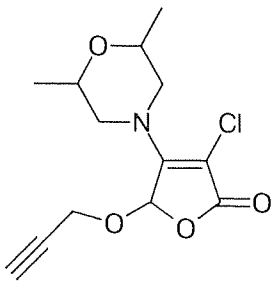
MS (APCI(+)): 294, 296 (M+1), 252, 254 (M+), 234, 236 (M+) m/z.

IR (KBr-disc) υ max: 2933, 1764, 1629, 1590, 1488, 1409, 1205, 1062, 977, 911 & 752 cm^{-1}.

^{1}H NMR ($CDCl_3$) 300K δ: 1.98 (s, CH_3), 3.08-3.49 (m, CH_2), 4.39-4.49 (m, CH_2), 5.76-5.79 (d, Ar-1H, J= 8.1 Hz), 6.99-7.05 (t, Ar-1H, J= 7.3, 7.2 Hz), 7.12-7.18 (t, Ar-1H, J= 8.0, 7.9 Hz), 7.26-7.23 (d, Ar-1H, J= 8.2 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 20.3 (CH_3), 28.5 (CH_2), 51.9 (CH_2-N), 88.4 (CH), 112.8 (Ar-C), 114.3 (C-Cl), 124.0, 125.8, 127.6, 131.9, 142.1 (Ar-C), 151.0 (C-N), 166.6, 168.9 (C=O) p.p.m.

3: (A_3B_7) 3-chloro-4-(2,6-dimethylmorpholin-4-yl)-5-(prop-2-ynyloxy)furan-2(5*H*)-one



R_f (ether)= 0.53

Mol. Weight: 285.7.

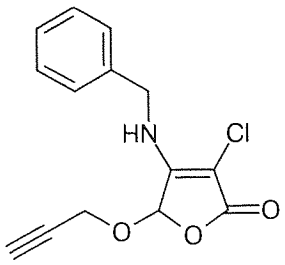
Mol. Formula: $C_{13}H_{16}ClNO_3$.

MS (APCI(+)): 286, 288 (M+1), 230, 232 (M+) m/z.

IR (KBr-disc) υ max: 3210, 2988, 2853, 1786, 1699, 1552, 1409, 1348, 1277, 1100 & 744 cm^{-1}.

^{1}H NMR ($CDCl_3$) 300K δ: 1.17 (CH_3), 1.20 (CH_3), 2.57-2.59 (t, CH≡C, J= 2.3, 2.4 Hz), 2.70-2.88 (m, CH), 3.44-3.48 (m, CH), 3.65-3.76 (m, CH_2), 4.09-4.34 (m, CH_2), 4.43 (s, CH_2-O), 5.95 (s, CH-O) p.p.m.

3: (A_3B_{13}) 4-(benzylamino)-3-chloro-5-(prop-2-ynyloxy)furan-2(5*H*)-one



R_f (ether)= 0.51

Mol. Weight: 277.7.

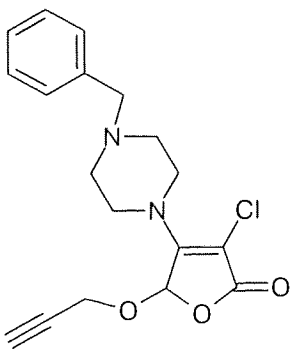
Mol. Formula: $C_{14}H_{12}ClNO_3$.

MS (APCI(+)): 278, 280 (M+1) m/z.

IR (KBr-disc) υ max: 3380, 3283, 2358, 2338, 1752, 1646, 1455, 1326, 1123, 971 & 695 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 2.54-2.56 (t, CH≡C, J= 2.4 Hz), 4.44-4.47 (m, CH), 4.66 (s, CH_2), 5.20 (s, NH), 5.98 (s, CH), 7.26-7.44 (m, Ar-5H) p.p.m.

3: (A_3B_{15}) 4-(4-benzylpiperazin-1-yl) 3-chloro-5-(prop-2-ynyloxy)furan-2(5*H*)-one



R_f (10% MeOH/ether) = 0.23

Mol. Weight: 346.8.

Mol. Formula: $C_{18}H_{19}ClN_2O_3$.

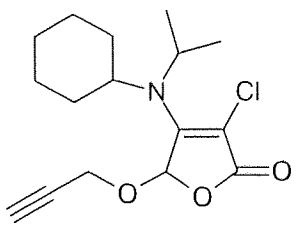
MS (APCI(+)): 347, 349(M+1) m/z.

IR (KBr-disc) υ max: 3253, 2938, 2815, 2125, 1756, 1623, 1452, 1349, 1276, 1228, 1106, 983, 742 & 698 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ:2.29 (s, C≡CH), 3.27-3.56 (m, CH_2-N, 8H), 3.72 (s, CH_2-Ar), 4.41 (s, CH_2-O), 5.94 (s, CH), 7.26-7.33 (m, Ar-5H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 47.4 (CH_2-O), 52.6 (CH_2-Nx2), 55.6 (CH_2-Nx2), 62.6 (CH_2-Ar), 76.8 (C≡CH), 86.3 (C≡CH), 94.2 (CH), 103.2 (C-Cl), 127.4, 128.3 (2xC), 129.1 (2xC), 137.0 (Ar-C), 153.9 (C-N), 168.3 (C=O) p.p.m.

3: (A_3B_{20}) 3-chloro-4-[cyclohexyl(isopropyl)amino]-5-(prop-2-ynyloxy)furan-2(5*H*)-one



R_f (ether)= 0.58

Mol. Weight: 311.8.

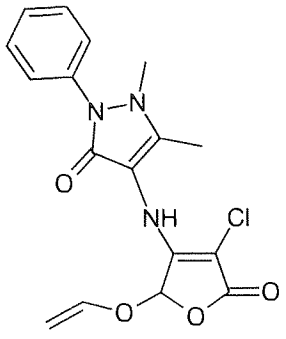
Mol. Formula: $C_{16}H_{22}ClNO_3$.

MS (APCI(+)): 312, 314 (M+1) m/z.

IR (KBr-disc) υ max: 3440, 2927, 2362, 2338, 1702, 1636, 1552, 1447, 1128, 1098 & 1044 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: overlapping 1.11-2.06 (m, CH_3, CH_2, CH, 16H), 2.09-2.16 (t, CH≡C, J= 2.4 Hz), 3.46-3.97 (m, CH_2, 6H), 5.99 (s, CH) p.p.m.

2: (A_4B_1) 3-chloro-4-[(1,5-dimethyl-2-phenyl-1,3-dihydro-3*H*-pyrazol-3-one)amino]-5-(vinyloxy)furan-2(5*H*)-one



R_f (10% MeOH/ether)= 0.2

Mol. Weight: 361.8.

Mol. Formula: $C_{17}H_{16}ClN_3O_4$.

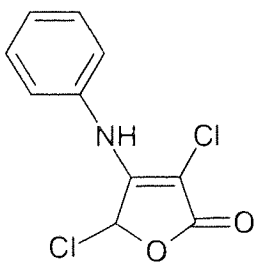
MS (APCI(+)): 362, 664 (M+1), 336, 338 (M+), 318, 320 (M+) m/z.

IR (KBr-disc) υ max: 3448, 2927, 2358, 1766, 1658, 1488, 1372, 1310, 1222, 1154 & 968 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.22 (s, CH_3), 2.68-2.73 (d, CH, J= 13.9 Hz), 2.89-2.97 (d, CH, J= 19.6 Hz), 3.36 (s, N-CH_3), 5.72 (s, CH), 6.23-6.34 (d, CH-O, J= 17.8 Hz), 7.32-7.55 (m, Ar-5H), 9.25 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 10.86 (CH_3), 36.0 (N-CH_3), 82.5 (CH_2), 96.3 (CH), 105.3 (C-Cl), 109.2 (C-N), 122.9, 124.3 (2xC), 129.7 (2xC) (Ar-C), 136.2 (C-CH_3), 136.4 (Ar-C), 154.7 (CH_2-O), 159.9 (C-N), 168.4, 170.2 (C=O) p.p.m.

1: (A_5B_{14}) 4-anilino-3,5-dichlorofuran-2(5*H*)-one



R_f (ether)= 0.62

Mol. Weight: 241.1.

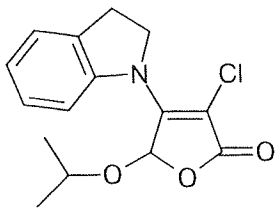
Mol. Formula: $C_{10}H_7Cl_2NO_2$.

MS (APCI(+)): 244 (M+1) m/z.

IR (KBr-disc) υ max: 3434, 3183, 2923, 1750, 1641, 1598, 1405, 1201 & 979 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 6.25 (s, CH), 7.10-7.41 (m, Ar-5H), 9.66 (s, NH) p.p.m.

1: (A_6B_2) 3-chloro-4-(2,3-dihydro-1H-indol-1-yl)-5-isopropoxy-2(5*H*)-one



R_f (ether)= 0.58

Mol. Weight: 293.7.

Mol. Formula: $C_{15}H_{16}ClNO_3$.

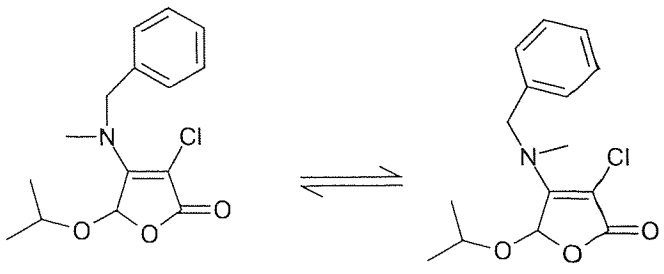
MS (APCI(+)): 294, 296 (M+1), 252, 254 (M+) m/z.

IR (KBr-disc) υ max: 2979, 2927, 1741, 1589, 1488, 1303, 1243, 1104, 950 & 757 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.10 (s, CH_3), 1.24 (s, CH_3), 3.05-3.36 (m, CH_2), 4.03-4.13 (q, $\underline{C}H$-$(CH_3)_2$, J=6.2 Hz), 4.27-4.49 (m, CH_2), 6.29 (s, CH-O), 6.95-7.04 (m, Ar-2H), 7.15-7.26 (m, Ar-2H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 22.0 (CH_3), 23.2 (CH_3), 28.7 $\underline{C}H$-$(CH_2)_2$), 51.2 (CH_2), 73.9 (CH_2), 97.2 (CH), 113.9 (C-Cl), 123.5 (2xC), 125.4, 127.0 (2xC), 131.9 (Ar-C), 142.6 (C-N), 152.2 (C=O) p.p.m.

1: (A_6B_6) 4-[benzyl(methyl)amino]-3-chloro-5-isopropoxyfuran-2(5*H*)-one



R_f (ether)= 0.50

Mol. Weight: 295.8.

Mol. Formula: $C_{15}H_{18}ClNO_3$.

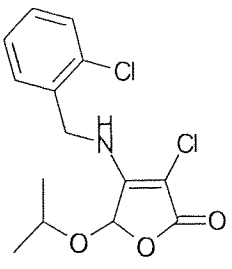
MS (APCI(+)): 296, 298 (M+1), 254, 256 (M+) m/z.

IR (KBr-disc) υ max: 2971, 2919, 1749, 1636, 1461, 1407, 1345, 1322, 1208, 1110, 981, 958 & 752 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: (isomers) 1.18-1.20 (d, CH_3, J=6.2 Hz), 1.26-1.28 (d, CH_3, J=6.2 Hz), 1.82 (s, -CH_2-), 2.95, 3.19 (s, N-CH3), 4.05 (q, C<u>H</u>-(CH_3) J=6.2 Hz), 4.69 (s, CH-O), 7.23-7.42 (m, Ar-H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 21.5 (CH_3), 23.2 (CH_3), 37.8 (N-CH_3), 55.4 (CH_2), 72.9 (C<u>H</u>-(CH_3)$_2$), 96.0 (CH-O), 102.3 (C-Cl), 127.2, 127.9 (2xC), 128.9 (2xC), 135.6 (Ar-C), 155.9 (C-N), 168.4 (C=O) p.p.m.

1: (A_6B_{18}) 3-chloro-4-[(2-chlorobenzyl)amino]-5-isopropoxy-4-2(5*H*)-one



R_f (ether)= 0.51

Mol. Weight: 316.2.

Mol. Formula: $C_{15}H_{18}ClN_2O_3$.

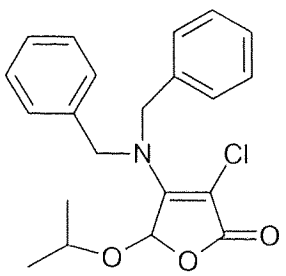
MS (APCI(+)): 316, 317, 318 (M+1), 274, 275, 276 (M+), 125, 127 (M+) m/z.

IR (KBr-disc) υ max: 3280, 3085, 2975, 1739, 1644, 1556, 1430, 1305, 1230, 944 & 744 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.20-1.23 (d, CH_3), J=6.2 Hz), 1.24-1.27 (d, CH_3, J=6.2 Hz), 4.03-4.13 (q, $\underline{C}H$-$(CH_3)_2$, J=6.2 Hz), 4.68-4.82 (m, CH_2), 4.93 (s, NH), 5.79 (s, CH-O), 7.26-7.43 (m, Ar-4H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 21.7 (CH_3), 23.1 (CH_3), 45.2 (CH_2), 73.4 CH-$(CH_2)_2$), 95.8 (CH-O), 102.3 (C-Cl), 127.3, 128.7, 129.4, 129.8, 132.9, 134.6 (Ar-C), 156.1 (C-N), 168.7 (C=O) p.p.m.

1: (A_6B_{19}) 3-chloro-4-(dibenzylamino)-5-isopropoxyfuran-2(5*H*)-one



R_f (ether)= 0.64

Mol. Weight: 371.9.

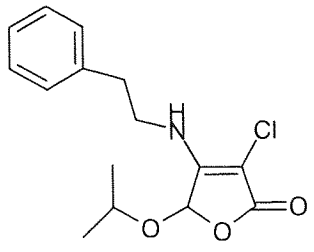
Mol. Formula: $C_{21}H_{22}ClNO_3$.

MS (APCI(+)): 372, 374 (M+1), 330, 332 (M+) m/z.

IR (KBr-disc) υ max: 3309, 3216, 3060, 2821, 2802, 1722, 1698, 1560, 1272, 1213, 1063 & 748cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.08-1.10 (d, CH_3, J= 6.2 Hz), 4.03-4.18 (q, CH, J= 6.2 Hz), 4.64 (s, CH_2, 4H, 5.85 (s, CH-O), 7.19-7.49 (m, Ar-H) p.p.m.

1: (A_6B_{22}) 3-chloro-5-isopropoxy-4-(phenethylamine)furan-2(5*H*)-one



R_f (ether)= 0.56

Mol. Weight: 295.8.

Mol. Formula: $C_{15}H_{18}ClN_2O_3$.

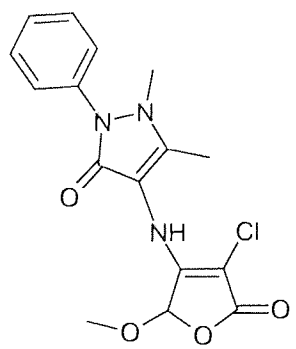
MS (APCI(+)): 296, 298 (M+1), 254, 256 (M+) m/z.

IR (KBr-disc) υ max: 3274, 3073, 2971, 1733, 1644, 1556, 1337, 1284, 1241, 1008, 900 & 744 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 1.25 (s, CH_3), 1.27 (s, CH_3), 2.86-2.94 (m, CH_2), 3.70 (s, CH_2), 4.00-4.15 (m, CH), 5.03 (s, NH), 5.54 (s, CH), 7.18-7.35 (m, Ar-5H) p.p.m.

^{13}C NMR ($CDCl_3$) 300K δ: 21.9 (CH_3), 23.2 (CH_3), 37.0 (CH_2-Ar), 44.8 (CH_2-N), 73.3 (C<u>H</u>-(CH_3)$_2$), 95.9 (CH), 100.2 (C-Cl), 126.9 (2xC), 128.4, 128.8 (2xC), 137.5 (Ar-C), 150.0 (C-N), 168.2 (C=O) p.p.m.

2: (A_8B_1) 3-chloro-4-[(1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one)amino-5-mthoxy-furan-2(5*H*)-one



R_f (ether)= 0.78

Mol. Weight: 349.8

Mol. Formula: $C_{16}H_{16}ClN_3O_4$.

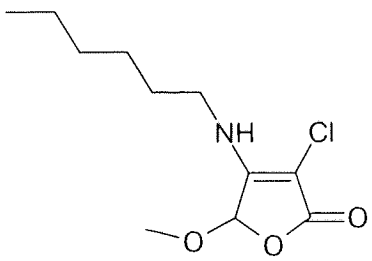
MS (APCI(+)): 350, 351 (M+1), 318, 320 (M+) m/z.

IR (KBr-disc) υ max: 3432, 3173, 3065, 2919, 1756, 1664, 1488, 1401, 1314, 1228, 1141, 1018 & 952 cm^{-1}.

^1H NMR (DMSO-d_6) 300K δ: 2.23 (s, CH_3), 3.10 (s, N-CH_3), 3.51 (s, CH-O), 5.99 (s, CH), 7.33-7.55 (m, Ar-5H), 9.09 (s, NH) p.p.m.

^{13}C NMR (DMSO-d_6) 300K δ: 11.1 (CH_3), 35.9 (N-CH_3), 56.4 (CH_2-O), 98.5 (CH), 102.0 (C-Cl), 107.7 (C-N), 124.7 (2xC), 127.3, 129.7 (2xC) (Ar-C), 135.0 (C-CH_3), 135.3 (Ar-C), 154.8 (C-N), 161.6, 168.2 (C=O) p.p.m.

3: (A_8B_{12}) 3-chloro-4-(hexylamino)-5-methoxyfuran-2(5*H*)-one



R_f (ether)= 0.46

Mol. Weight: 219.7.

Mol. Formula: $C_9H_{14}ClNO_3$.

MS (APCI(+)): 220, 222 (M+1) m/z.

IR (KBr-disc) υ max: 3326, 2933, 2855, 1746, 1646, 1445, 1475, 1125, 1013 & 961 cm^{-1}.

^1H NMR ($CDCl_3$) 300K δ: 0.83-0.86 (m, CH_3), 1.25-1.28 (m, CH_2), 1.56-1.61 (m, CH_2), 3.37-3.46 (m, CH_2), 3.57 (s, CH_3-O), 5.32 (s, CH-O), 5.65 (NH) p.p.m.

References

1. Greenlee, W.J. and Desai, M., (1998), Combinatorial chemistry library design using pharmacophore diversity, *Current Opinion in Drug Discovery and Development*, **1** (1), 3-18.

2. Wang, G.T.; Li, S., Wideburg, N., Krafft, G.A. and Kempf, D.J., (1995), Synthetic chemical diversity: Solid phase synthesis of libraries of C2 symmetrical inhibitors of HIV protease containing diamino diol and diamino alcohol cores., *J. Med. Chem.*, **38**, 2995-3002.

3. Walters, W.P. and Murcko, M.A., (1999), Recognizing molecules with drug-like properties, *Current Opinion in Chemical Biology,* **3** *(4),* 384-387.

4. Petsko, G.A; Verdine, G.L.; Broach, J.R.; Thorner, J.; Hogan, J.C. and Blundell,
T.L., (1996), Developments in techniques of pharmaceutical chemistry are poised to revolutionize the design of drugs beyond previous expectations. But what are our current expectations and what does the future hold?, *Nature*, **384**, 1-26.

5. Gordon, E.M.; Barret, R.W.; Dower, W.J.; Fodor, S.P.A. and Gallop, M.A., (1994), Applications of combinatorial technologies to drug discovery. Combinatorial organic synthesis, library screening strategies, *J. Med. Chem.*, **37** (9), 1385-1401 and Gallop, M.A., (1994), Applications of combinatorial technologies to drug discovery. Combinatorial organic synthesis, library screening strategies, and future directions., *J. Med. Chem.*, **37**, 1233-1251

6. Montgomery, S.A., (1993), Psychopharmacology of Panic, British Association for Psychopharmacology Monograph No. 12, pp, 75-76.

7. Earle E. Bain and Philip J. Candilis, (2000), New dirctions in the treatment of anxiety disorders. *Exp. Opin. Ther. Patents.* **10**, (4), 389-402.

8. Klein, D.F., (1964), Anxiety: New research and changing concepts (Klein, D.F. and Rabkin, J.G., eds.), Raven Press, pp. 235-263.

9. Millet B.; Bayle F.J. and Olie J.P., (1988), Prospects for anxiolytic therapy: a reflection from different viewpoints, *DDT*, Vol. **3**, no 10, 471-479.

10. Gorman, J.M.; Askanazi, J.; Liebowitz, M.R.; Fryer, A.J.; Stein, J.; Kinney, J.M. and Klein, D.F., (1984), Response to hyperventilation in a group of patients with panic disorders., *Amer. Journal of Psychiatry*, **141**, 857-891.

11. Margraf, J.; Ehlers, A. and Roth, W.T., (1986), Sodium lactate and panic attacks: a review and critique, *Psychosomatic Medicine*, **48**, 23-51.

12. Nutt, D.J.; Glue, P.; Lawson, C.W. and Wilson, S., (1990), Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder., *Archives of General Psychiatry*, **47**, 917-925.

13. Wilson, T.M.; Henke, B.R.; Momtahen, T.M.; Myers, P.L.; Sugg, E.E.; Unwalla, R.J.; Croom, D.K.; Dougherty, R.W.; Grizzele, M.K.; Johnson, M.F.; Queen, K.L.; Rimele, T.J.; Yingling, J.D. and James, M.K., (1996), 3-[2-(N-Phenylacetamide)]-1,5-benzodiazepines: Orally active, binding selective CCK-A agonists, *J. Med. Chem.*, **39**, 3030-3034.

14. de Montigny, C., (1989), Cholecystokinin tetrapeptide induces panic-like attacks in healthy volunteers., *Arch. Gen. Psychiatry*, **46**, 511-517.

15. Bradwejn, J.; Koszycki, D. and Meterissian, G., (1990), Cholecystokinin tetrapeptide induces panic attacks in patients with panic disorder., *Can. J. Psychiaty*, **35**, 83-85.

16. Bradwejn, J.; Koszycki, D. and Shriqui, C., (1991), Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorder., *Arch. Gen. Psychiatry*, **48**, 603-610.

17. Ravard, S. and Dourish, C.T., (1990), Cholecystokinin and anxiety., *Trends Pharmacol. Sci.*, **11**, 271-273.

18. Ivy, A.C. and Goldberg, E.A., (1928), The Discovery of a substance released from the upper intestine and produced gallbladder contractions, *Am. J. Physiol.*, **86**, 599-606.

19. D'Amato, M.; Varro, A. and Dimaline, R., (1996), CCK_B/gastrin receptor antagonists, *Physiol. Res.*, **76**, 767-798 and Olsson, C. and Holmgren, S., (2001), The control of gut motility, *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology,* **128** *(3),* 479-501.

20. Yassin, R.R., (1999), Signaling pathways mediating gastrin's growth-promoting effects, *Peptides*, **20** (7), 885-898.

21. Wilborg, O., (1984), Gastrin. Isolation, characterisation and functions. In: Gastrointestinal hormones., *Proc. Nat. Acad. Sci. USA*, **81**, 1067-1069.

22. Stening, G.F. and Grossman, M.I., (1969), Gastrin-related peptides as stimulant of pancreatic and gastric secretion, *Amer. J. Physiol.* **217**, 262-266.

23. Man, V. and Yorpes, J.E, (1968), Secretion, cholecystokinin, pancreozinine and gastrin, *Eur. J. Biochem.,* **6**, 156-162.

24. H. Mineo, H., Iwaki, N., Kogishi, K., Zabielski, R., Onaga, T., and Kato, S., (1995), Effects of intravenous infusions of cholecystokinin (CCK)-8 on exocrine and endocrine pancreatic secretion in conscious sheep., *Comparative Biochemistry and Physiology Part A: Physiology.*, 111 (1), 133-138.

25. Mutt, J. and Jorpes, J.E.; (1971), Hormonal polypeptides of the upper intestine., *Biochem. J.,* **125**, 57-58.

26. Horwell, D.C.; Beeby, A.; Clark, C.R. and Hughes, J., (1987), Synthesis and binding affinities of cholecystokinin (30-33) as probes for central nervous system cholecystokinin receptors., *J. Med. Chem.*, **30**, 729-732.

27. Eysselein, V.E.; Reeve, J.R. and Eberlein, G., (1986), Cholecystokinin gene structure and molecular forms in tissue and blood., *Gastroenterology*, **24**, 645-659.

28. Böhme, G.A. and Blanchard, J.C., (1992), Cholecystokinins and their receptors. Functional aspects, *Therapie*, **47** (6), 541-548.

29. Lattmann, E. and Singh, H., (2000), Small organic molecules as cholecystokinin antagonists., *J. Sc. (KKU)*, **34** (in the press).

30. Lin, C.W.; Shiosaki, K.; Miller, T.R.; Witte, D.G.; Bianchi, B.R.; Wolfram, C.A.; Kopecka, H.; Craig, R.; Wagenaar, F. and Nadzan, A.M., (1991), Characterization of two novel cholecystokinin tetrapeptide (30-33), *Mol. Pharmacol.*, **39** (3), 346-351.

31. Noble, F. and Roques, B.P., (1999), CCK-B receptor: Chemistry, molecular biology, biochemistry and pharmacology, *Progress in Neurobiology*, **58** (4) 349-379.

32. Clark, C.R.; Dauni, P. and Hughes, J., (1986), A study of the cerebral cortex cholecystokinin receptor using two radiolabelled probes: Evidence for a common CCK 8 and CCK 4 cholecystokinin receptor binding Site., *J. Neurochem.*, **46**, 1094-1101.

33. Takeda, Y.; Hoshino, M.; Yanaihara, N.; Yanaihara, C. and Isobe, J., (1989), Comparison of CCK-8 receptors in the pancreas and brain of rats using CCK-8 analogues., *Jpn. J. Pharmacol.*, **49**, 471-481.

34. Dirieux, C.; Pelaprat, D.; Charpentier, B.; Morgat, J.L. and Roqucs, B.P., (1988), Characterization of [^{3}H] CCK binding sites in mouse and rat brain., *Neuropeptides*, **12**, 141-148.

35. Saito, A.; Goldfine, I.D. and Williams, J.A., (1981), Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex., *J. Neurochem.*, **37**, 483-490.

36. Wank, S.A, (1995), Cholecystokinin receptors, *Amer. J. Physiol.*, **269** (5), 628-646.

37. Dockray, G.J.R., (1976), Immunochemical evidence of cholecystokinin-likepeptides in brain, *Nature*, **264**, 568-570.

38. Morley, V.E., (1990), Appetite regulation by gut peptides, *Annu. Rev. Nutr.*, **10**, 383-395.

39. Wang, X.J.; Wang, X.H. and Hans, S., (1990), Cholecystokinin octapeptide antagonised opioid analgesia mediated by kappa-receptors in the spinal cord of the rat, *Brain Res.*,**523**, 5-10.

40. Payeur, K., (1993), Psychiatric comorbidity in patients with generalized anxiety disorder, *Eur. Psychiatry*, **21**, 101-105.

41. Bradwejn, J.; Koszychi, D.; Annable, L.; Couetoux du Tertre, A.; Bourin, M.; Palmour, R. and Ervin, F., (1992), The cholecystokinin hypothesis of panic anxiety disorders, *J. Psychopharmacol.*, **6**, 354-361.

42. Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W.L.; 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. and Hirshfield, J., (1988), Methods for drug discovery: Development of potent, selective, orally effective cholecystokinin antagonists, *J. Med. Chem.*, **31**, 2235-2246.

43. Lodge, D.J.; Short, J.L.; Mercer, L.D.; Beart, P.M. and Lawrence, A.J., (2000), CCK/dopamine interactions in fawn-hooded and wistar–kyoto rat brain, *Peptides*, **21** (3), 379-386.

44. Harro, J.; Marcusson, J. and Oreland, L., (1992), Changes at cholecystokinin receptors induced by long-term treatment with diazepam and haloperidol, *European Neuropshychopharmacology*, **2**, 57-63 and Acosta, G.B., (2000), A possible interaction between CCKergic and GABAergic systems in the rat brain, *Comparative Biochemistry and Physiology-Part C: Pharmacology, Toxicology and Endocrinology*, **128** (1), 11-17.

45. Mohler, H., (1992), $GABA_A$-receptor subtypes differing in alpha-subunit composition display unique pharmacological properties, *Arzneim.-Forsch./Drug Res.*, **42** (2a), 211-218.

46. Fuxe, K.; Anderson, K.; Locatelli, V.; Agnat, L.F.; Hokfelt, T.; Skirball, L. and Mutt, V., (1980), Cholecystokinin peptides produce marked reduction of dopamine turnover in discrete areas in the rat brain, following intraventricular injection., *Eur. J. Pharmacol.*, **67**, 325-331.

47. Harro, T.; Jossan, S.S. and Oreland, L., (1992), Changes in cholecystokinin receptor binding in rat brain after selective damage of the locus coeruleus projections by DSP-4 treatment, *Arch. Pharmacol.*, **364**, 425-431.

48. Pittaway, K.M. and Hill, R.G., (1987), Cholecystokinin and pain. Pain and headache., *J. Psychopharmacol.*, **9**, 213-246.

49. Hays, S.E.; Beinfeld, M.C.; Jensen, R.T.; Goodwin, F.K. and Paul, S.M.; (1980), Demonstration of a putative receptor site for cholecystokinin in rat brain., *Neuropeptides*, **1**, 53-52.

50. Altar, C.A.; (1989), Cholecystokinin receptor subtypes and neuromodulation., *Prog. NeuroPsychopharmacol. Biol. Psychiat.*, **13**, 381-383.

51. Pascal de Tullio, Jacques Delarge and Bernard Pirotte, (2000), Therapeutic and chemical developments of cholecystokinin receptor ligands. *Exp. Opin. Invest. Drugs*. **9**, (1), 129-146.

52. Moran, T.H., (1986), Behavioral recovery following acid lesions and fetal implants of the striatum occurs independent of dopaminergic mechanisms., *Brain Research*, **362**, 175-179.

53. de Weerth, A.; Pisegna, J.R.; Huppi, K. and Wank, A., (1993), Molecular cloning, functional expression and chromosomal localisation on the human cholecystokinin type A receptor., *Biochem. Biophys. Res. Commun.*, **2**, 811-818.

54. Huang, S.C.; Yu, D-H.; Wank, S.A.; Mantey, S.; Gardener, J. D. and Jensen, R.T., (1989), Importance of sulfation of gastrin or cholecystokinin (CCK) on affinity for gastrin and CCK receptors, *Peptides,* **10**, 785-789.

55. Innis, R.B. and Synder, S.H., (1986), Distinct cholecystokinin receptors in brain and pancreas., *Proc. Natl. Acad. Sci. USA.*, **77**, 6917-6921.

56. Knight, M.; Tamminga, C.A.; Steardo, L.; Beck, M.E.; Barone, P. and Chase, T.N., (1984), Cholecystokinin-octapeptide framents: binding to brain cholecystokinin receptors., *Eur. J. Pharmacol.,* **105**, 49-55.

57. Lee, Y.M.; Beinborn, M.; Mcbride, E.W.; Miller, L.J.; Lu, M.; Kolakovki, L.F. and Kopin, A.S., (1993), The human brain cholecystokinin-B/gastrin receptor-cloning and characterisation., *J. Biol. Chem.,* **268**, 8164-8169.

58. Yoder, D.G. and Moody, T.W., (1987), High affinity binding of cholecystokinin to small cell lung cells., *Peptides*, **8**, 103-107.

59. King, F.D. and Oxford, A.W., CCK-B Antagonists in the control of anxiety and gastric acid secretion. *Progress In medicinal chemistry* (Elsevier). **37**, 44-80.

60. Vidal, Y.; Plana, R.R.; Cifarelli, A. and Bizzarri, D., (1980), Effects of antigastrin drugs on the interaction of ^{125}I-human gastrin with rat gastric mucosa membranes., *Hepata-Gastroenterol.*, **27**, 41-47.

61. Stening, G.F. and Grossman, M.I., (1969), Gastrin-related peptides as stimulant of pancreatic and gastric secretion, *Amer. J. Physiol.* **217**, 262-266.

62. Jensen, R.T.; Zhou, Z.C.; Murphy, R.B.; Jones, S.W. and Setnikar, I., (1986), Structural features of various proglumide-related cholecystokinin receptor antagonists., *Am. J. Physiol.*, **251**, 839-846.

63. Makovec, F.; Bani, M.; Chiste, R.; Revel, L. and Rovati, L.C., (1986), Differentiation of central and peripheral cholecystokinin receptors by new glutaramic acid derivatives and cholecystokinin-antagonistic activity., *Arzmeim-Forsch./Drug Res.*, **36**, 98-102.

64. Wakatsuki, K,; Saito, T.; Saeki, M.; Ninomiya, K.; Kasai H.; Kimura, K. and Fuji, M., (1997), Cholecystokinin antagonistic activities of loxiglumide., *Arzneim-Forsch./Drug Res.*, **47** (10), 1130-1133.

65. Peikin, S.R.; Costenbader, C.L. and Gardner, J.D., (1979), Actions of derivatives of cyclic nucleotides on dispersed acini from guinea pig pancreas., *J. Biol. Chem.*, **254** (24), 5321-5327.

66. Boden, P.R.; Higginbottom, M.; Hill, D.R.; Horwell, D.C.; Hughes, J., Rees, D.C.; Roberts,E.; Singh, L.; Suman-Chauhan, N. and Woodruff, G.N., (1993), Cholecystokinin dipeptoid antagonists: Design, synthesis and anxiolytic profile of some novel CCK-A and CCK-B and "mixed" CCK-A/CCK-B antagonists, *J. Med. Chem.*, **36**, 552-565.

67. Kerwin, J.F., Wagenaar, F.; Kopecka, H.; Lin, hun Wel and Miller, T., (1991), Cholecystokinin antagonists: (R)-Tryptophan-based hybrid antagonists of high affinity and selectivity for CCK-A receptors, *J. Med.Chem.* **34** (12), 3350-3359.

68. Clark, C.R.; Dauni, P. and Hughes, J., (1986), A study of the cerebral cortex cholecystokinin receptor using two radiolabelled probes: Evidence for a common CCK 8 and CCK 4 cholecystokinin receptor binding Site., *J. Neurochem.*, **46**, 1094-1101.

69. Stark, H.A.; Sharp, C.M.; Sutliff, V.E.; Martinez, J. and Jensen, R.T., (1989), CCK-JMV-180: Peptides that distinguishes high affinity cholecystokinin receptors from low affinity cholecystokinin., *Biochem. Biophys. Acta.*, **1010**, 145-150.

70. Roth, H.J. and Kleemann, A., Drug Synthesis. *Pharmaceutical Chemistry* (Ellis Horwood). **1**, 202-247.

71. Rasmussen, K.; Czachura, J.F.; Stockton, M.E. and Howbert, J.J., (1993), Electrophysiological effects of diphenylpyrazolidinone cholecystokinin-B and cholecystokinin-A antagonists on midbrain dopamine neurons., *J. Pharmacol. Exp. Ther.*, **264**, 480-488.

72. Howbert, J.J.; Lobb, K.L.; Britton, T.C.; Mason, N.R. and Bruns, R.F., (1993), Diphenylpyrazolidinone and benzodiazepine cholecystokinin antagonists: A case of convergent evolution in medicinal chemistry., *Bioorg. Med. Chem. Lett.*, **3**, 875-880.

73. Bertrand, P.; Guyon, D.; Dubroeucq, M.C.; Doble, A., and Barreau, M., (1994), Evaluation of brain penetration of CCK-B antagonists using an ex-vivo binding assay., *Eur. J. Pharmacol.*, **262**, 233-245.

74. Rasmussen, K.; Stockton, M.E.; Czachura, J.F. and Howbert, J.J., (1995), Cholecystokinin and schizophrenia: The selective CCK-B antagonists decreases midbrain dopamine unit activity., *Expert Opin. Invest. Drugs.*, **4**, 313-322.

75. Takeda, Y.; Kawagoe, K.; Yokomizo, A.; Yokomizo, Y.; Hosokami, T. Shimoto, Y.; Tabuchi, Y.; Ogihara, Y.; Otsubo, R.; Honda, Y. and Yokohama, S., (1998), Synthesis of phenoxyacetic acid derivatives as highly potent antagonists of gastrin/ cholecystokinin-B receptors, *Chem. Pharm Bull.*, **46** (6), 951-961.

76. Hagishita, S.; Murakami, Y.; Seno, K.; Kamata, S. and Haga, N., (1997), *Bioorg. Med.Chem.*, **5**, 1695-1714.

77. Kalindjian, S.B.; Harper, E.A. and Pether, M.J., (1995), The synthesis of a radioligand with high potency and selectivity for CCK-B/gastrin receptors., *J. Med. Chem.*, 38, 4294-4302.

78. Iain M. McDonald, (2001), CCK2 receptor antagonists. *Exp. Opin. Ther. Patents*. **11**, (3), 445-462.

79. Sternbach, L.H. and Reeder, E., (1961), Quinazolines and 1,4-benzodiazepines. The rearrangement of 6-chloro-2-chloromethyl-4-phenylquinazoline 3-oxide into 2-amino derivatives of 7-chloro-5-phenyl-3H-1,4-benzodiazepine 4-oxide, *J. Org. Chem.*, **26**, 1111-1118.

80. Haefely, W., (1983), Alleviation of anxiety - the benzodiazepine saga In: Parnham, M.J. and Bruinvels, J. (eds). Discoveries in Pharmacology., *Psycho- and Neuropharmacology*, **1**, 269-292, Amsterdam: Elsevier

81. Archer, G.A. and Sternbach, L.H., (1968), *Chem. Rev.*, **68**, 747-742 and Elderfeld, R.C., (1967), *Heterocycl. Compounds*, **9**, 332-372.

82. Puia, G., (1991), Influence of recombinant gamma-aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of gamma-aminobutyric acid-gated Cl-currents., *Molecular Pharm.*, **39**, 691-698.

83. Sioufi, A. and Dubois, P., (1990) Chromatography of benzodiazepines., *J. Chromatogr.*, **531**, 459-480.

84. Camerman, A., (1972), 3D-Structure of Diazepam, *Journal of Amer. Soc.*, **94**, 268-270.

85. Masafumi, F.; Yuji, T.; Katsumi, H. and Kazuo, K., (1980), Detection and determination of pharmacologically active benzodiazepines in rat brain after the administration of 2-o-chlorobenzoyl-4-chloro-N-methyl-N'-glycylglycinanilide, using a combination of high-pressure liquid chromatography and radioreceptor assay, *Chem.Pharm.Bull.*, **28** (5), 1378-1386.

86. Kubota, K.; Sugaya, K.; Sunagane, N. and Matsuda, F., (1985), Cholecystokinin antagonism by benzodiazepines in the contractile response of the isolated guinea pig gallbladder., *Eur. J. Pharmacol.*, **110**, 225-231.

87. Kubota, K.; Sugaya, K.; Fuji, F. and Sunagane, N., (1985), Inhibition of cholecystokinin response in the gallbladder by dibenamine and its protection by benzodiazepines., *Jpn. J. Pharmaco.*, **39**, 274-276.

88. Sugaya, K.; Matsuda, I.; Uruna, T. and Kubota, K., (1984), Studies on the CCK antagonism by benzodiazepines: Displacement of CCK by benzodiazepines in the binding in mouse brain CCK receptor., *Jpn. J. Pharmacol.*, **40**, 114p.

89. Kubota, K.; Sugaya, K.; Koizumi, Y. and Toda, M., (1989), Cholecystokinin antagonism by anthramycin in the central nervous system in mice., *Brain Res.*, **485**, 62-66.

90. Miller, L.J., (1984), Characterisation of cholecystokinin receptors on human gastric smooth muscle tumours., *Am. J. Physiol.*, **247**, 402-410.

91. Bock, M.G.; DiPardo, R.M.; Rittle, K.E., et al., (1986), Cholecystokinin antagonists. Synthesis of asperlicin analogues with improved potency and water solubility, *J. Med. Chem.*, **29**, 1941-1952.

92. Hosontanti, R.; Chowdhury, P.; McKay, D. and Rayford, P.L., (1988), Effect of L-364,718, a new CCK antagonist, on amylase scretion in isolated rat pancreatic acini., *Pancreas*, **3**, 95-98.

93. Anderson, L. and Dockray, G.L., (1988), The cholecystokinin antagonist L-364,718 inhibits the action of cholecystokinin but not bombesin on rat pancreatic secretion in vivo., *Euro. J. Pharmacol.*, **146**, 307-311.

94. Silver, A.J.; Flood, J.F.; Song, A.M. and Morley, J.E., (1989), Evidence for a physiological role for CCK in the regulation of food intake in mice., *Am. J. Physiol.*, **256**, 646-652.

95. Dunlop, J.; (1998), CCK receptor antagonists, *Gen Pharmacol.* **31** (4), 519-524.

96. Hill, D.R.; Shaw, T.M.; Graham, W. and Woodruff, G.N., (1989), Autoradiographical detection of cholecystokinin-A receptors in primate brain using ^{125}I bolton Hunter CCK-8 and 3H-MK-329., *J. Neurosci.*, **10**, 1070-1081.

97. Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Veber, D.F. and Anderson, P.S., (1986), Design of potent, orally effective, non peptidal antagonists of the peptide hormone cholecystokinin, *Proc. Natl. Acad. Sci. USA*, **83**, 4918-4922.

98. Dourish, C.T.; Rycroft, W. and Iversen, S.D., (1989), Postponement of satiety by blockade of brain cholecystokinin-B receptors., *Science.*, **245**, 1509-1511.

99. Kubota, K.; Sugaya, K.; Koizumi, Y. And Toda, M., (1989), Cholecystokinin antagonism by anthramycin in the central nervous system in mice., *Brain Res.*, **485**, 62-66.

100. Bock, M.G.; DiPardo, R.M. and Evans B.E., (1989), Benzodiazepine gastrin and brain cholecystokinin receptor ligands. L-365,260., *J. Med. Chem.*, **32**, 13-24.

101. Haradahira, T.; Inoue, O.; Kobayashi, K. and Suzuki, K., (1998), Synthesis and evaluation of 11C-labeled nonpeptide antagonists for cholecystokinin receptors: [11C] L-365,260 and [11C]L-365,346., *Nucl. Med. Biol.*, **25** (3), 203-208.

102. Chamber, M.S.; Hobbs, S.C.; Flecher, S.R.; Matassa, V.G.; Mitchell, P.J.; Watt, A.P.; Baker R.; Freedman, S.B.; Patel, S. and Smith, A.J., (1993), L-708,474: The C5-cyclohexyl analogue of L-365,260, a selective high affinity ligand for the CCK-B/gastrin receptor, *Bioorganic & Medicinal Chemistry Letters*, **3**, 1919-1924.

103. Showell, G.A.; Bourrain, S.; Neduvelil, J.G.; Fletcher, A.E.; Baker, R.; Watt, A.P.; Fletcher, A.E.; Freedman, S.P.; Kemp, J.A.; Marshall, G.R.; Patel, S.; Smith, A.J. and Matassa, V.G., (1994), High affinity and potent, water soluble 5-amino-1,4-benzodiazepine CCK_B/gastrin receptor antagonists containing a cationic solubilizing group, *J. Med. Chem.*, **37**, 719-721.

104. Castro, L.C.; Broughton, H.B.; Russell, M.G.N.; Rathbone, R. and Watt, A.P., (1997), 5-(Piperidin-2-yl)- and 5-(homopiperidin-2-yl)-1,4-benzodiazepines: High-affinity, basic ligands for the cholecystokinin-B receptor., *J. Med. Chem.*, **40** (16), 2491-2501.

105. Bock, M.G.; DiPardo, R.M.; Mellin, E.C. and Newton, N.C., (1994), Second-generation benzodiazepine CCK-B antagonists. Development of subnanomolar analogues with selectivity and water solubility., *J. Med. Chem.*, **37**, 722-724.

106. Semple, G.; Ryder, H.; Kendrick, D.A.; Szelke, M.; Ohta, M.; Satoh, M.; Nisida, A.; Akuzawa, S.; Miyata, K.; Rooker, D.P. and Batt, A.R., (1997), (3R)-N(tert- butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1H-1,4-benzodiazepin-3-yl)-N'-(3-(methylamino)phenyl)urea (YF476): A potent and orally active gastrin/CCK-B antagonist, *J. Med. Chem.*, **40**, 331-341.

107. Yuji, S.; Hidenori, Y.; Yumiko, H.; Akito, N.; Keito, M. and Kazuo, H., (1994), Characterisation of YM022: its CCK_B/gastrin receptor binding profile and antagonism to CCK-8-induced Ca^{2+} mobilization; *European Journal of Pharmacology, Molecular Pharmacology Section,* **269**, 249-254.

108. Semple, G.; Ryder, H.; Kendrick, D.A.; Szelke, M.; Ohta, M.; Satoh, M.; Nisida, A.; Akuzawa, S. and Miyata, K., (1996), Synthesis and biological activity of 1-alkylcarbonylmethyl analogues of YM022, *Bioorganic and Medicinal Chemistry Letters*, **6**, 51-54.

109. Masato, S.; Yutaka, K.; Yoshinori, O.; Akito, N. and Keiji, M., (1995), New 1,4-benzodiazepin-2-one derivatives as gastrin/cholecystokinin-B antagonists., *Chem.*

Pharm.Bull, ,**43** (12), 2159-2167.

110. Padia, J.K.; Chilvers, H.; Daum, P.; Pinnock, R.; Suman-Chauhan, N.; Webdale, L. and Trivedi, B.K., (1997), Design and synthesis of novel non-peptides CCK-B receptor antagonists, *Bioorganic and Medicinal Chemistry Letters*, **7**, 805-810.

111. Finizia, G.; Donati, D.; Oliosi, B.; Tranquillini, M.E. and Ursini, A., (1996), Synthesis and evaluation of novel 1,5-benzodiazepines as potent and selective CCK-B ligands. Effect of the substitution of the N-5 phenyl with alkyl groups, *Bioorganic and Medicinal Chemistry Letters*, **24** (6), 2957-2962.

112. Wilson, T.M.; Henke, B.R.; Momtahen, T.M.; Myers, P.L.; Sugg, E.E.; Unwalla, R.J.; Croom, D.K.; Dougherty, R.W.; Grizzele, M.K.; Johnson, M.F.; Queen, K.L.; Rimele, T.J.; Yingling, J.D. and James, M.K., (1996), 3-[2-(N-Phenylacetamide)]-1,5-benzodiazepines: Orally active, binding selective CCK-A agonists, *J. Med. Chem.*, **39**, 3030-3034 and Bailey, N.; Box, P.C.; Car, R.A.E.; Cooke, J.W.B.; Evans, B.; Finch, H.; Head, J.E.; Pass, M.; Shah, P. and Wheatcroft, J.R., (1997), Novel 1,5-benzodiazepindione gastrin/CCK_B antagonists, *Bioorganic and Medicinal ChemistryLetters*, **7**, 381-286.

113. Hirst, G.C.; Aquino, C.; Birkemo, L.; Croom, G.K. and Dezube, M., (1996), Discovery of 1,5-benzodiazepines with peripheral cholecystokinin (CCK-A) receptor agonist activity (II): Optimization of the C3 amino substituent., *J. Med. Chem.*, **39** (26), 5236-5245.

114. PCT-WO 99 64 403; Int. Cl.: C 07 D – 243/12. Zeria Pharma Co. LtD., Saitama (JP); Ind.: [166] 7 (2000).

115. Trivedi, K. and Bharat, J., (1994), Cholecystokinin receptor antagonists: Current status, *Current Medicinal Chemistry*, **1**, 313-327 and Francesco, M., (1993), CCK-B/gastrin-receptor antagonists, *Drugs of the Future*, **18**, 919-931.

116. Makovec, F. and D'Amato, M., (1997), CCK_B/gastrin receptor antagonists as potential drugs for peptic ulcer therapy, *DDT*, **2**, 283-293.

117. Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Lundell, G.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Gilbert, K.F.; Garsky, V.M.; Leighton, J.L.; Carson, K.l.; Mellin, E.C.; Smith, A.J. and Patel, S., (1993), Development of 1,4-benzodiazepine cholecystokinin type B antagonists, *J. Med. Chem.*, **36**, 4276-4292.

118. Wilson, T.M.; Henke, B.R.; Momtahen, T.M.; Myers, P.L.; Sugg, E.E.; Unwalla, R.J.; Croom, D.K.; Dougherty, R.W.; Grizzele, M.K.; Johnson, M.F.; Queen, K.L.; Rimele, T.J.; Yingling, J.D. and James, M.K., (1996), 3-[2-(N-Phenylacetamide)]- 1,5-benzodiazepines: Orally active, binding selective CCK-A agonists, *J. Med. Chem.*, **39**, 3030-3034 and deMontigny, C., (1989), Cholecystokinin tetrapeptide induces panic-like attacks in healthy volunteers., *Arch. Gen. Psychiatry*, **46**, 511-517 and Bradwejn, J.; Koszycki, D. and Meterissian, G., (1990), Cholecystokinin tetrapeptide induces panic attacks in patients with panic disorder., *Can. J. Psychiaty*, **35**, 83-85.

119. Ravard, S. and Dourish, C.T., (1990), Cholecystokinin and anxiety., *Trends Pharmacol. Sci.*, **11**, 271-273.

120. Parsons, W.H.; Patchett, A.A.; Hollaway, M.K.; Smith, G.M. and Davidson, J.L., (1989), Cholecystokinin antagonist. Synthesis and biological evaluation of 2-substituted benzolactams., *J. Med. Chem.*, **32**, 1681-1685.

121. Bailey, N.; Box, P.C.; Car, R.A.E.; Cooke, J.W.B.; Evans, B.; Finch, H.; Head, J.E.; Pass, M.; Shah, P. and Wheatcroft, J.R., (1997), Novel 1,5-benzodiazepindione gastrin/CCK_B antagonists, *Bioorganic and Medicinal Chemistry Letters*, **7**, 381-286.

122. Padia, J.K.; Chilvers, H.; Daum, P.; Pinnock, R.; Suman-Chauhan, N.; Webdale, L. and Trivedi, B.K., (1997), Design and synthesis of novel non-peptides

CCK-B receptor antagonists, *Bioorganic and Medicinal Chemistry Letters*, **7**, 805-810.

123. Yuji, S.; Hidenori, Y.; Yumiko, H.; Akito, N.; Keito, M. and Kazuo, H., (1994), Characterisation of YM022: its CCK_B/gastrin receptor binding profile and antagonism to CCK-8-induced Ca^{2+} mobilization; *European Journal of Pharmacology, Molecular Pharmacology Section,* **269**, 249-254.

124. Masato, S.; Yutaka, K.; Yoshinori, O.; Akito, N. and Keiji, M., (1995), New 1,4-benzodiazepin-2-one derivatives as gastrin/cholecystokinin-B antagonists., *Chem. Pharm. Bull.,* **43** (12), 2159-2167.

125. Puia, G., (1991), Influence of recombinant gamma-aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of gamma-aminobutyric acid-gated Cl-currents., *Molecular Pharm.*, **39**, 691-698.

126. Haefely, W., (1985), *Advances in Drug Research,* Academic Press, vol. **14**, pp 165-322.

127. Hughes, J.; Boden, P.; Costall, B.; Domeney, A. and Kelly, E., (1990), Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity., *Proc. Natl. Acad. Sci. USA*, **87**, 6728-6732.

128. Harro, J.; Marcusson, J. and Oreland, L., (1992), Changes at cholecystokinin receptors induced by long-term treatment with diazepam and haloperidol, *European Neuropshychopharmacology*, **2**, 57-63 and Acosta, G.B., (2000), A possible interaction between CCKergic and GABAergic systems in the rat brain, *Comparative Biochemistry and Physiology - Part C: Pharmacology, Toxicology and Endocrinology*, **128** (1), 11-17.

129. Satoh, M.; Okamoto, Y.; Koshio, H.; Ohta, M., Nisida, A.; Akuzawa, S.; Miyata, K.; Mase, T. and Semple, G., (1996), Biological activity of YM022. Novel (3-amino substituted phenyl) urea derivatives of 1,4-benzodiazepin-2-one as gastrin/ cholecystokinin-B receptor agonists, *Chem. Pharm. Bull.*, **44**, 1412-1414.Semple, G.;

Ryder, H.; Kendrick, D.A.; Szelke, M.; Ohta, M.; Satoh, M.; Nisida, A.; Akuzawa, S. and Miyata, K., (1996), Synthesis and biological activity of 1-alkylcarbonylmethyl analogues of YM022, *Bioorganic and Medicinal Chemistry Letters*, **6**, 51-54.

130. Garattini, S.; Mussini, E. and Randall, L.O.; (1969), The Benzodiazepines, Raven Press, New York, pp 13-26.

131. Evans, B.E., Rittle, K.E., Bock, M.G., DiPardo, R.M., Freidinger, R.M., Whitter, W.L., Lundell, G.F., Veber, D.F., Anderson, P.S., Chang, R.S. and Cerino, D.J., (1988), Methods for drug discovery: Development of potent, selecive, orally effective cholecystokinin-A antagonists, *J. Med. Chem.*, **31**, 2235-2246.

132. Bell, C.S.; Sulkowski, T.S.; Gochman, C. and Childress, S., (1962), 1,3-Dihydro-2H-1,4-benzodiazepin-2-ones and their 4-oxides; *J. Org. Chem.*, **27**, 562-566.

133. Stempel, A.; Douvan, I.; Reeder, E. and Sternbach, L.H., (1967), Quinazolines and 1,4-benzodiazepines. 4,1,5-Benzoxadiazocin-2-ones, a novel ring system, *J. Org. Chem.*, **32**, 2417-2425.

134. Sternbach, L.H., Fryer, R.I., Metlesics, W., Sach, G. and Stempel, A., (1962), Quinazolines and 1,4-benzodiazepines. o-Aminobenzophenones., *J. Org. Chem.* **27**, 3781-3788.

135. Stempel, A.; Reeder, E. and Sternbach, L.H., (1965), Quinazolines and 1,4-benzodiazepines. Mechanism for ring enlargement of quinazoline 3-oxide with alkali to 1,4-benzodiazepin-2-one 4-oxides., *J. Org. Chem.*, **30**, 4267-4271.

136. Saita Y., Yazawa H., Honma, Y., Nishida A., Miyata K. and Honda K., (1994),Characterization of YM022: its CCKB/gastrin receptor binding profile and antagonism to CCK-8-induced Ca2+ mobilization., *Eur. J. Pharmacol.*,**269**, 249-254.

137. Charpentier, B.; Pelaprat, D.; Durieux, C.; Dor, A.; Reibaud, M.; Blanchard, J.C. and Roques, B.P., (1988), Cyclic cholecystokinin analogues with high selectivity for central receptors., *Proc Natl Acad Sci U S A*, **85**, 1968-1972.

138. Gatta, F.; Del Giudice M.R and Settimj, G., (1979), New Synthesis of 3-(disubstituted-amino)-1,4-benzodiazepin-2-ones. *Commu.* **12**. 718-719.

139. Gatta, F.; Del Giudice, M.R.; Pandolfi, C and Settimj, G. (1982), Sintesi di 2-E 3-amino 1,4–benzodizepine. *Il Farmaco Ed. Sc.*, **27**, (5), 343-352.

140. Kulkarni, Y.D.; Abdi, S.H.R and Sharma, V.L., (1986), Synthesis of 3-substituted-amino/arcyloxy-7-chloro-5-phenyl-1,3-dihydro-2H-1,1,4-benzodiazepin-2-ones as possible anxiolytics. *J. Indian Chem. Soc.* **15a**, 425-426.

141. Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Veber, D.F. and Anderson, P.S., (1986), Design of potent, orally effective, non-peptidal antagonists of the peptide hormone cholecystokinin, *Proc. Natl. Acad. Sci. USA*, **83**, 4918-4922.

142. Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M. and Freidinger, R.M., (1987), Design of nonpeptidal ligands for a peptide receptor: Cholecystokinin antagonists, *J. Med. Chem.*, **30** (7), 1229-1239.

143. Satoh, M.; Okamoto, Y.; Koshio, H.; Ohta, M., Nisida, A.; Akuzawa, S.; Miyata, K.; Mase, T. and Semple, G., (1996), Biological activity of YM022. Novel (3-amino substituted phenyl)urea derivatives of 1,4-benzodiazepin-2-one as gastrin/cholecystokinin-B receptor agonists, *Chem. Pharm. Bull.*, **44**, 1412-1414.

144. Bogat-skii, A.V., Andronati, S.A., Zhilina, Z.I., Kobzareva, O.V., Shabatyran, P.A., Ivanova, R.Yu. and Chumachenko, T.K., (1975), 1,4-Benzodiazepines, their cyclic homologs and analogs: Synthesis and properties of 3-(arymethylene)- and 3-(heterylmethylene)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-ones; *Zhurnal Obshchei Khimii*, **45** (2), 396-401.

145. Elvidge, J.A.; Jones, J.A.; O`Brien, C.; Evans, E.A. and Sheppard, H.C., (1974), Base catalysed hydrogen exchange; *Adv.Heterocycl.Chem.*, **16**, 1-18.

146. F. Hoffmann-La Roche & Co. Aktiengesellschaft, Basel/Schweiz, Swiss Patent 2.233.482.

147. Berghot, M.A., (1992), Heterocyclic groups [b]-annulated to the 1,4-benzodiazepine system., *Arch. Pharm. (Weinheim),* **325**, 285-289.

148. Reiter, B.E., Sachdeva, Y.P. and Wolfe, J.F., (1981), Metallation of Diazepam and use of the resulting carbanion intermediate in a new synthesis of 3-substituted derivatives., *J. Org. Chem.,* **46**, 3945-3949.

149. Kovac, T.; Oklobdzija, M.; Sunjie, V. and Kajfez, F., (1979), Comparative study on the preparation of C(3)-hydroxy-1,3-dihydro-2H-1,4-benzodiazepine-2-ones. *Hetrocyclic. chem.* **16**, 1449-1454.

150. Swati, A.; Sharma, K and Prackash, L., (1996), A convient one pot Synthesis of some 1,5-benzothiazepine derivatives as possible potential drugs for cancer metastatis. *Indian. J. Chemistry.* **35B**, 1221-1224.

151. Crotti, P.; Ferretti, M.M and Stoppioni, A., (1986), Ring opening reactions of cis and trans 2,3-bis(4-methoxybenzyl)oxirane: Competition between assistance by and migration of an aryl group. *J.Org. Chem.* **51**, 2795-2766.

152. Yamada, S.; Yoshioka, R. and Shibatani, T., (1997), Optical resolution of a 1,5-benzothiazepine derivative, a synthetic intermediate of diltiazepam, by preferential crystallization and diastereomeric salt formation. *Chem. Pharm. Bull.* **45**, (12), 1922-1927.

153. Hans Ruhkopf, (1940), Uber einige oxy-pyrazolidine. *Chem. Ber. Chbeam.* **73**, 820-822.

154. Stenzl, H.; Staub, A.; Simon, C.H. and Baumann, W., (1950), Zur Kenntnis der 3-Amino-pyrazolone-(5). *Helv. Chim. Acta.* **33**, 1183-1195.

155. Vogel, H.G. and Vogel, W.H., (1997), Pharmacological assays. *Drug Discovery and Evaluation* (Springer). 232-372.

156. Silverman, P. (1978), Approach to a conditioned stimulus mazes. *Animal behaviour in the laboratory* (Chapman and Hall). 110-119.

157. Kilfoil, T.; Michel, A.; Montgomery, D. and Whithing, R.L., (1989), Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. *Neuropharmacol.* **28**, 901-905.

158. Porsolt, R.D.; Le Pichon, M. and Jalfre, M., (1977), Depression: A new animal model sensitive to antidepressant treatments. *Nature.* (266), 730-732.

159. Walker, J.M. and Dixon, W.C., (1985), A solid state device for the measuring sensitivity to thermal pain. *Physiol. Behav.* **30**, 481-483.

160. Cowan, A., (1990), Modern methods in pharmacology: Recent approaches in the testing of analgesics in animals. *Testing and Evaluation of Drugs of Abuse* (Wiley-Liss Inc.). 33-42.

161. Kitchen, I. And Crowder, I., (1985), Assessment of the hot-plate antinociceptive test in mice: A new method for the statistical treatment of graded data. *J. Pharmacol.* Meth. **13**, 1-7.

162. Costall, B.; Naylor, R.J. and Nohria, V., (1978), Climbing behaviour induced by apomorphine in mice: A potent model for the detection of neuroleptic activity. *Eur. J. Pharmacol.* **50**, 39-50.

163. Ursini, A.; Capelli, A.M.; Carr, R.A.E.; Cassara, P.; Corsi, M.; Curcuruto, O.; Curotto, G.; Dal Cin, M.; Davalli, S.; Donati, D.; Feriani, A.; Finch, H.; Finizia, G.; Gaviraghi, G.; Marien, M.; Pentassuglia, G.; Polinelli, S., Ratti, E.; Reggiani, A.;

Tarzia, G.; Tedesco, G.; Tranquillini, M.E.; Trist, D.G. and Van Amsterdam, F.T.M., (2000), Synthesis and S.A.R. of new 5-phenyl-3-ureido-1,5-benzodiazepines as cholecystokinin-B receptor antagonists. *J. Med. Chem.* **43**, 3596-3613.

164. Tokuichi Tsumaki, (1931), 3,5-Diketo-Pyrazolidine derivatives. *Bull. Chem. Soc. Jap.* **6**, 1-7.

165. Mowry, D.T., (1950), Muchloric acid I: Reactions of the pseudo-acid group. *J. Am. Chem. Soc.*2535-3537.

166. Mowry, D.T., (1953), Muchloric acid II: Reactions of the aldehyde group. *J. Am. Chem. Soc.*1909-1910

167. E. Dilip de Silva and Paul J. Scheuver, (1980), Manoalide, an antibiotic sesterterpenoid from the marine songe *Luffariella variabilis* (Polejaeff). *Tetrahedron Letters.* **21**, 1611-1614.

168. Clemo, N.G. and Pattenden, G., (1982), Naturally occurring 5-methoxy-3(2H)-furanones: Re-assignment of structures to the aspertetronin group of natural products. *Tetrahedron Letters.* **23**, (5), 589-592.

169. McCombs, J.D.; Blunt, J.W.; Chamber, M.V.; Munro, M.H.G. and Robinson, W.T., (1988), Novel 2(5H)-furanones from the red marine alga *Delisea alegans* (Lamouroux). *Tetrahedron.* **44**, (5), 1489-4502.

170. Christopher A. Langley. (2000), The combinatorial approach to the chemical synthesis and biological evaluation of 3,4,5-trisubstituted furan-2(5H)-ones. Ph.D. Dissertation. University of Aston.

171. Semonsky, M.; Rockova, E.; Cerny, A.; Kakac, B. and Macek, K. (1961), Substanzen mit antineoplastishcher wirksamkeit IV: Einige γ-aryl-α,β-substituierte-crotolactone. *Collec. Czech. Chem. Commun.* **27**, 1939-1954.

172. Farina, F.; Martin, M. V. and Sanchez, F. (1983), Peseudoesters and Derivatives: XVII Synthesis of 4-Alkylamino- and 4-Alkylthio-5-methoxyfuran-2(5H)-ones. *Synthesis communications*. 397-398.

173. Martin, M. R. and Mateo, A. I. (1994). Synthesis of homochiral polyfunctionalized furan-2(5H)-ones. *Tetrahedron Asymmetry*. **5**, (7), 1385-1392.

174. E. Lattmann. Private communication.