Synthesis and evaluation of furan-2(5H)-one based CCK antagonists.

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Master of Philosophy

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The furan based template, furan-2(5H)-one, is a relatively well known and important structure in nature. The structural arrangement of this template allows it to be susceptible to nucleophilic attack at different sites of this molecule, which can help in the formation of a new lead structure. This thesis had been compiled to document synthesis and evaluation of different classes of compound in the hope of finding new furanone based CCK antagonists.

Initially a new nociceptic assay involving tramadol potentiation, was developed using two CCK active pyrazolones which have previously been shown to possess CCK antagonist properties from *in vitro* receptor binding assays. Here these compounds have revealed significant tramadol potentiation in an *in vivo* 'tail flick' pain assay. The results have also shown a direct correlation between the receptor binding and the tramadol potentiation for these pyrazolones. This new assay was used in relation to newly synthesised compounds, in order to identify CCK active agents.

A range of 4-amino-5-alkoxy-furan-2(5H)-ones were formed via 5-alkoxy-furan-2(5H)-one building blocks based on a previously known lead structure, and were biologically evaluated using the fore-mentioned pain assay. Selected CCK antagonists showing good tramadol potentiation were submitted for other pharmacological tests. These indicated that compounds in this class possessed anxiolytic and / or antidepressant properties.

Pyrrol-2,5-diones, formed as a by-product, during the synthesis of an anti-cancer agent, were developed and synthesised as the main product during this reaction. These compounds were also synthesised using an alternative route, which proceeded via the same mechanistic pathway, using the above mentioned 5-alkoxy-furan-2(5H)-ones.

A further development of this pyrrole scaffold lead to the formation of a new class of 5-hydroxy-5-phenyl-pyrrol-2-ones. CCK antagonists were identified using the potentiation pain assay. Some of these compounds showed antidepressant and anxiolytic properties.

A novel class of but-2-enoic acid amides were designed and developed via molecular modelling studies using Merck's L-365,260 antagonist as a comparison. Selected compounds were identified, via the nociceptic assay developed here and showed results comparable to existing standard CCK active pyrazolones CCK activity. These non-chiral CCK antagonist amides could possess anti-cancer properties as they contain a dichlorinated vinylic unit, which could help mimic the anti-cancer agent cisplatin.

The furan-2(5H)-one building block used here has been shown to be a very important and exciting template which has yielded 3 different classes of compounds, each of which contained potent CCK antagonists.

Additional key words:

Cholecystokinin, Antagonist, Antidepressant, Anxiolytic, Tramadol, Potentiation.

Dedicated to my family, especially to my Mother and Father, Patricia and Clifford, for the help and support they have given me throughout my life. I would also like to dedicated this work as a mark of respect to my pet Labrador Lucky who recently past away.

Acknowledgements.

I would like to thank numerous people for making it possible for me to complete the work present in this thesis. First and foremost I would like to thank my research supervisor Dr Eric Lattmann who has helped me throughout my time at Aston University and without his support none of this would have been possible.

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Publications.

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Contents

Tables, Figures and Schemes.

Abbreviations.

$AlCl_3$	Aluminium Chloride
APCI	Atmospheric Pressure Chemical Ionisation.
BW	Body Weight
CCK	Cholecystokinin
$CDCl_3$	Deuterated Chloroform
cm	Centimetre
CNS	Central Nervous System.
DIC	Dicyclohexylcarbodiimide
DNA	Deoxyribonucleic Acid.
DMF	*N*,*N*-Dimethylformamide
DMSO-d_6	Deuterated dimethylsulphoxide
eq.	Equivalent
GABA	γ-Aminobutyric Acid.
GI	Gastrointestinal.
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
IC_{50}	Inhibitory Concentration, 50%
IR	Infra-red
ml	Millilitre
M.P.	Melting Point
MPE	Maximum Possible Effect
MS	Mass Spectroscopy
μM	Micromolar
NMR	Nuclear Magnetic Resonance.
NSAID(s)	Non-steroidal Anti-inflammatory Drug(s)
PNS	Peripheral Nervous System
p.p.m.	Parts Per Millon
R_f	Retention Factor
RT	Room Temperature
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
X-maze	Elevated plus-maze

Aim.

This thesis has been compiled to document synthesis, development and biological evaluation of a structurally diverse range of potent cholecystokinin antagonists. These compounds should be formed with simplicity and optimisation in mind, i.e. any new CCK active agents should be synthesised in the minimum number of steps and in the highest yield possible. These antagonists should also ideally have no chiral centre present in the molecule.

Objectives.

- To develop and apply a nociceptic assay to filter out novel CCK antagonists.
- To show *in vivo* efficacy.
- To synthesise and evaluate the biological activities of 4-amino-5-alkoxy-furanones.
- To design and explore a pyrrol-2,5-dione template.
- To synthesise 5-hydroxy-5-aryl-pyrrol-2-ones direct from simple furanones and to evaluate them in the search for a novel lead structure.
- To prepare non-chiral bis-arylated but-2-enoic acid amides based on molecular modelling studies.

Chapter 1: Introduction.

1.0. Developing new drugs.

In order to develop new drugs a biological target needs to be identified, followed by a careful look at the interaction with that target. Compounds of interest are screened, and then further optimised in the hope of identifying new lead compounds[1] which can be studied in a development phase (toxicology, distribution, metabolism and excretion) before being put into clinical trials as potential medical drugs. During the 1990s it was found that less than 0.5% of over 6000 drugs approved in the USA per year were put into the development phase of which less than 7 on average were tested on humans and less than 3 on average made it into phase 3 clinical trials.
Generally the main reasons for these new potential drug candidates not continuing past the development phase include:

- Poor pharmaceutical properties
- Lack of efficacy
- Toxicity
- Other reasons such as, non-marketable / non-competitive drugs

1.1. Central Nervous System (CNS) and associated disorders.

Control and communication within the body come mainly from the brain and spinal column (more commonly known as the central nervous system – CNS), which receives and sends messages via a vast network of nerves. The CNS represents the largest part of the nervous system and is contained within the dorsal cavity. Together with the peripheral nervous system (PNS)[2], it has a fundamental role in the control of behaviour[3].
CNS disorders such as anxiety[4] and depression[5] are among the more commonly known and debilitating illnesses occurring in today's highly stressed, quick paced society.

Around 25% of alcoholics and 15% of the general population suffer from anxiety. Approximately 2% of the population are known to suffer from panic disorder.

Anxiety disorders have resulted in high financial costs having to be paid by society, but several studies[6] have found the treatment of such disorders highly cost-effective due to the decrease in indirect costs (i.e. missed work days, loss of productivity and non-psychiatric medical care).

Anxiety is an emotional condition that is experienced by all humans and is characterised by the feeling of apprehension as well as symptoms such as headache, palpitations, restlessness, muscle pain and respiratory problems. Studies have shown that the numbers of cases of anxiety known are comparable to those of depression and chronic illnesses such as diabetes, arthritis and back, lung or gastrointestinal disorders.

Klein[7] suggested that 'anxiety neurosis' should differentiate panic disorder (PD) from other forms of anxiety. These have since been characterised as generalised panic disorder (GAD), phobias (P) and post-traumatic stress disorder (PTSD).

Researchers have over the last few decades tried to gain a better understanding of this emotional condition by using substrates such as carbon dioxide for inhalations[8] and sodium lactate infusions or Flumazenil[9] (a benzodiazepine derivative anxiety and panic inducing drug).

1.2. Cholecystokinin.

Cholecystokinin (CCK) is a naturally occurring 33 amino acid peptide[10] abundant in the gastrointestinal system, the pancreas and the central nervous system. It is produced by I cells of the duodenal and jejunal mucosa and is found mainly as an eight amino acid hormone (CCK_8). CCK is characterised by the α-aminated terminus Trp-Met-Asp-Phe-NH_2 sequence and numerous studies have shown its existence in various forms[11, 12]. Cholecystokinin is derived from a primary prepro-CCK polypeptide of 115 residues. After transcription, enzymatic cleavage results in the formation of many different fractions including CCK_{8s} (sulphated), CCK_{8ns} (non-sulphated), CCK_7, CCK_5 and CCK_4. Both CCK_8 (octapeptide)[13] and CCK_4 (tetrapeptide)[14] have been explored, mainly with respect to food intake regulation, panic and anxiety, the latter two of which have brought about a great deal of confusion. These two fractions have different

affinities for CCK receptors[15, 16], different distribution in both the periphery and the brain[17, 18] and have various effects on behaviour.

D'Amato *et al.*[19] have in recent years provided evidence for this major intestinal hormone having a physiological role in the regulation of motor function at various levels of the gastrointestinal/alimentary tract such as in the control of bile release and pancreatic secretion.

CCK and its receptors[20] are also known to be widely distributed in the CNS and contribute to the regulation of satiety, analgesia and dopamine-mediated behaviour. Its presence in the brain was first shown by Dockray in 1976 and since then there has been some evidence to suggest that CCK may have an effect as a neuromodulator or possibly as a neurotransmitter.

Gastrin and CCK_8 have identical –COOH terminal penta-peptide sequences. It is this C-terminal that gives gastrin, which mainly stimulates gastric secretion for parietal cells and promotes the growth of gastric mucosa, its biological activity. Most gastrin like activity in the brain is present as both CCK_{8s} and CCK_{8ns}.

A number of researchers have suggested a potential new approach, by where they found that the fragment of cholecystokinin, CCK_4, provoked panic attacks in healthy volunteers[21] shortly after injection. Bradwejn *et al.*[22] found that patients with existing panic disorders had increased sensitivity to its administration compared to normal volunteers. These results suggest that one type of CCK receptor, occurring mainly in the brain (CCK_B/CCK_2) is involved in the regulation of anxiety. Some results[23] suggest that selective CCK_B antagonists might be able to help in the treatment of CNS disorders.

Over the last 25 years the role of CCK in the central nervous system has been researched vastly and has been connected to conditions such as pain perception[24], anxiety disorders[25], feeding and satiety[26] and psychiatric illnesses[27], thus suggesting an interaction with other receptors. Evans *et al.*[28] have shown this to be the case, i.e. that CCK interacts with numerous receptors such as gamma-aminobutyric acid (GABA)[29], noradrenaline (NA), opioid peptides[30], serotonin (5-hydroxytryptamine, HT) and dopamine (DA)[31]. For example, depending on the conditions, CCK was found to facilitate and inhibit dopamine activity[32]. A link has also been found between CCK and serotonin and the satiety mechanism. When CCK was injected into the hypothalamus it exerted a direct influence over satiety and reduced food consumption. When rats were fed 5-hydroxy-L-tryptophan they produced serotonin, which increased the inhibitory

effects of CCK-8 on food consumption[33]. This link has been investigated extensively in an attempt to synthesise CCK ligands that would act as satiety agents in obese patients. Cyclic nucleotide derivatives were shown to antagonise the actions of CCK in rat pancreatic acini[34] and the cerebral cortex[35] as well as in guinea pig acini and ileum in the guinea pig pan confirming the presence of high affinity CCK binding sites. This indicated an important physiological role for CCK receptors in the periphery, but its function in the brain was not as well understood. These CCK receptors were classified according to their location, i.e. type A (alimentary) and type B (brain)[36] and their existence was confirmed by cloning by de Weeth[37].

CCK-RECEPTOR AGONISTS	CCK-RECEPTOR ANTAGONISTS
Satiety agent in obese patients	Treatment of drug dependence
Prevention of gallbladder stasis on low fat diet	Treatment of pain potentiation of opioid effect
Hypervigilance agent	Treatment of CNS disorders
Memory enhancement	Treatment of gastric acid hypersecretion
Prevention of gallstones	Treatment of pancreatic disorder
	Treatment of motility disorders
	Treatment of biliary colic
	Treatment of panic attack
	Treatment of anxiety
	Treatment of schizophrenia
	Treatment of functional bowel disease
	Treatment of gastro oesophageal reflux disease
	Anti-proliferating agent in some carcinoma
	Anti-anorexic agent

Table 1.2. Potential therapeutic and pharmacological applications of CCK-receptor ligands.

CCK_A/CCK_1 receptors have been shown to have the greatest affinity for CCK_{8s}[38] (100-fold higher than for CCK_{8ns} and CCK_4[39]) and predominantly occur at the peripheral level where they are responsible for the digestive effects of CCK. These include intestinal and biliary smooth muscle contraction, pancreatic enzyme secretion and regulation of feeding.

Lee *et al.*[40] showed that CCK_B/CCK_2 receptors show the same affinity for CCK_{8s}[41], CCK_{8ns} and CCK_4 and have been shown to be more widely spread in the CNS, with high amounts present in libric and cortical regions e.g. the hippocampus, hypothalamus and the amygdala. Both of these receptor types have been shown to belong to the G-Protein-Coupled-Receptor (GPCR) superfamily[42].

Due to CCK's role in many physiological processes there is much potential for the use of CCK receptor ligands therapeutically[43] (Table 1.2.) and so efforts have been made to synthesise highly selective agents for a specific receptor subtype. As well as serving a therapeutic role, another application of these ligands that is being researched at present, is as pharmacological tools. Herranz[44] has recently reviewed both the therapeutic and pharmacological potential of CCK ligands. This review suggested that CCK_A antagonists might have both therapeutic and pharmacological potential for the treatment of pancreatic disorders and as prokinetics for the treatment of gastroesophageal reflux disease, bowel disorders and gastroparesis. CCK_B antagonists were found to potentially have application for the treatment of gastric acid secretion and anxiety disorders.

Hayes *et al.*[45] recently found evidence to support that serotonin-3 ($5\text{-}HT_3$) receptors have a role in the modulation of CCK induced satiation. Their findings suggested that CCK_A and 5-HT3 receptors cooperate independently in control of short term food intake and that interconnection exists through a feed-forward parallel model arising from CCK_A and 5-HT3 receptors, where activation of one system engages the other to intensify the overall satiety signal.

Experimental research has suggested that CCK_B[42] antagonists may be useful in the treatment of certain neuropathological conditions associated with CCK dysfunction, such as modulation of dopaminergic function, control of pain, anxiety and memory formation.

1.2.0. CCK antagonists: chemical classes and therapeutic applications.

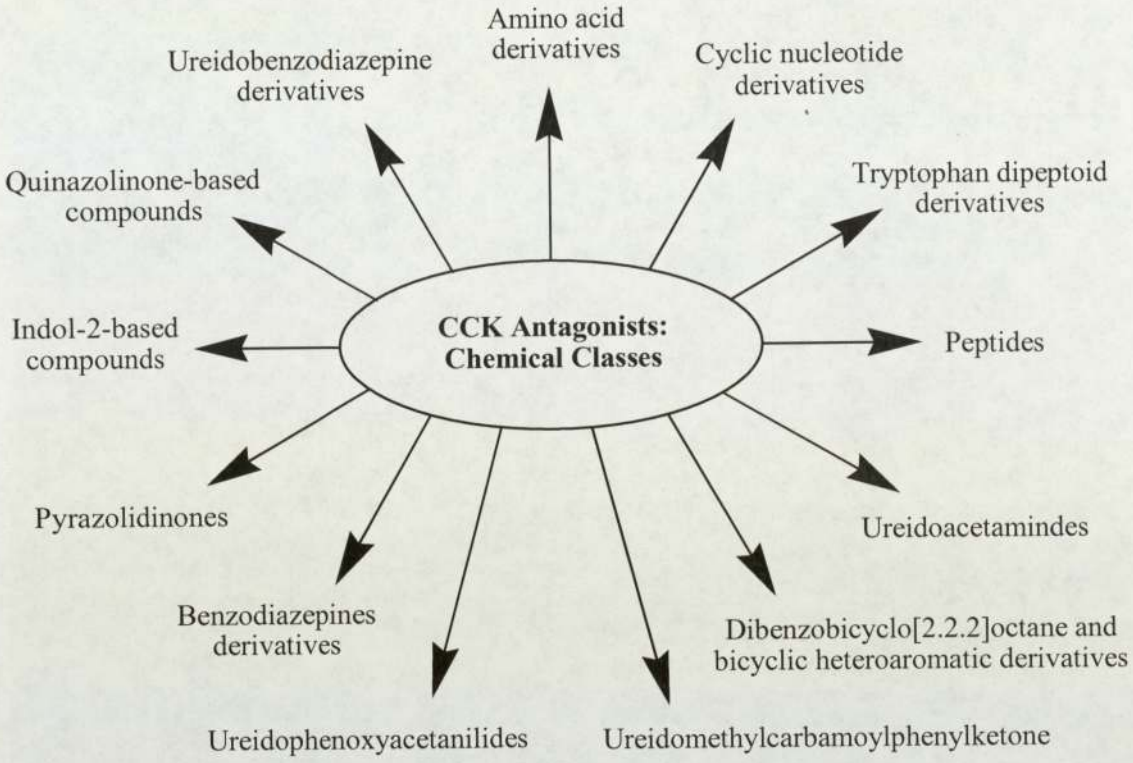


Figure 1.2.0. Chemical classes of CCK antagonists.

Over the past few decades new CCK peptide and non-peptide antagonists have been discovered. Many exhibit high selectivity for CCK_B/gastrin receptors, most predominantly belonging to one of the classes shown in figure 1.2.0.

1.2.1. Peptides.

Spanarkel *et al.*[46] synthesised cholecystokinin-27-32-amide (CCK-27-32-NH_2), the first peptide to show CCK antagonistic properties. They tested the ability of this compound to stimulate amylase secretion from dispersed acini prepared from guinea pig pancreas and found that CCK-27-32-NH_2 instead of stimulating enzyme secretion it acted as a full competitive CCK receptor antagonist.

Hruby *et al.*[47] have provided a new approach in the drug design for pathological conditions such as neuropathic pain and opioid analgesic tolerance. They modified a potent and selective peptide ligand for the CCK_B receptor to give a peptide that had potent agonist binding affinity and bioactivity at Delta and Mu opioid receptors and simultaneous antagonist activity at CCK receptors.

1.2.2. Amino acid derivatives.

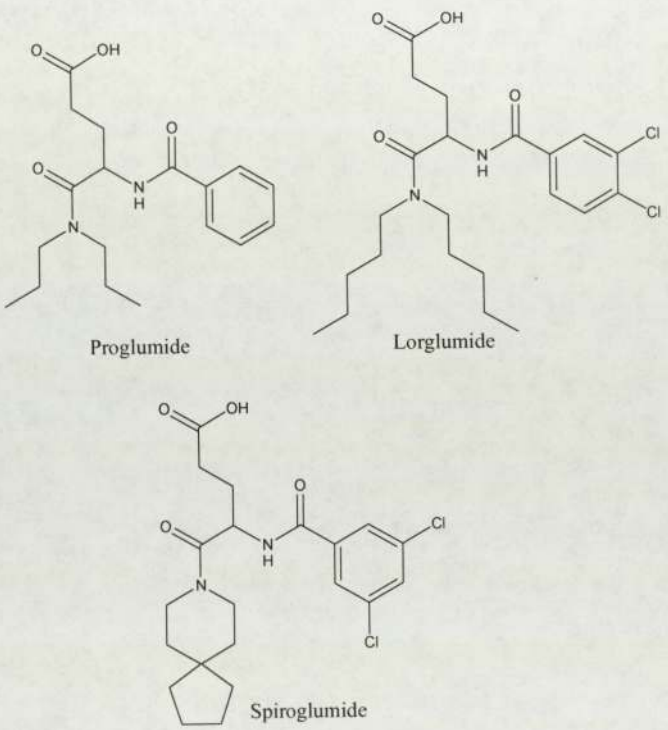


Figure 1.2.2. CCK antagonist amino acid derivatives.

Around 30 years ago amino acid derivatives were found to possess antigastrin activity[48]. These derivatives showed CCK antagonist activity due to the fact that CCK and gastrin have similar chemical properties. Proglumide (figure 1.2.2.), a putative gastrin and weak CCK_A antagonist[49], is used for the treatment of peptic ulcers (because of its antisecretory and gastroprotective activities) and has been the reference CCK and gastrin antagonist for many years. Analogues of this compound have been produced

showing varying degrees of selectivity for CCK_A receptors. Lorglumide (figure 1.2.2.) gave around a 26-fold and a 2-fold increase in potency for blocking CCK-stimulated gallbladder contraction and pancreatic amalyse secretion[50] respectively. Spiroglumide (figure 1.2.2., structurally modified Lorglumide) demonstrated CCK_B/gastrin antagonist properties in micromolar range, but although it has excellent oral bioavailability it has poor CCK_B/gastrin receptor selectivity indicating doubts about its possible therapeutic uses.

1.2.3. Pyrazolidinones.

A series of functionalised pyrazolidinone derivatives was identified and synthesised, via a large random screening programme, by a research group at Lilly. Structure-activity relationship (SAR) studies[51] resulted in the finding of compounds such as LY288513 (figure 1.2.3.), which has 2 chiral centres and binding affinities of 19 nM and 20500 nM for the CCK_B and CCK_A receptors respectively (showing more than 1000-fold selectivity for CCK_B receptor)[52]. After undergoing clinical trials, compound LY288513 was discontinued due to major adverse effects[53].

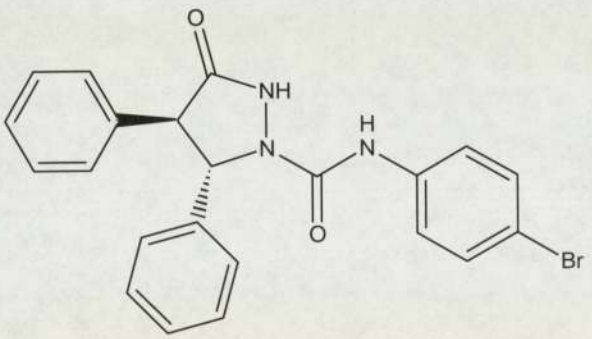


Figure 1.2.3. Pyrazolidinone derivative: LY288513.

1.2.4. Cyclic nucleotide derivatives.

Bt_2cGMP[54], dibutyryl cyclic guanosine monophosphate (figure 1.2.4.), was the first competitive antagonist of CCK-mediated action to be discovered and caused both selective and reversible inhibition of CCK-stimulated amylase secretion from rat pancreatic cells. It was found to block the effects of CCK at a high number of peripheral sites, but was found to be unsuccessful in inhibiting CCK binding in mouse cerebral cortex.

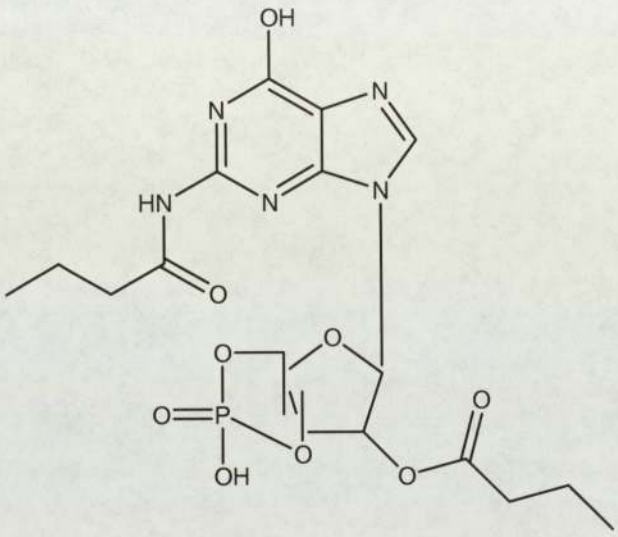


Figure 1.2.4. Bt_2cGMP: a cyclic nucleotide derivative.

1.2.5. Ureidoacetamides.

Rhône-Poulenc developed non-peptide ureidoacetamides[55], which are potent and selective ligands for CCK_B/gastrin receptors, such as RP69758 (figure 1.2.5.) which possess nanomolar activity (around 500-fold more selectivity for CCK_B/gastrin receptors over CCK_A receptors).

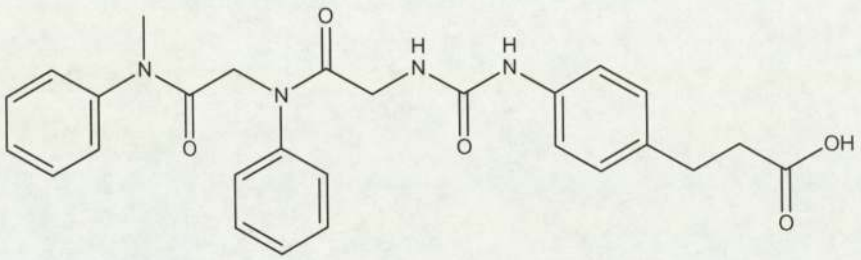


Figure 1.2.5. RP69758: a ureidoacetamide derivative.

1.2.6. Ureidomethylcarbamoylphenylketones.

A series of selective CCK_B receptor antagonists have been developed by Shiogoni. These ureidomethylcarbamoylphenylketones[56] were derived by cleavage of the C-3 to N-4 bond of Merck's L-365,260 compound led to the selective CCK_B receptor compound S-0509 (figure 1.2.6.).

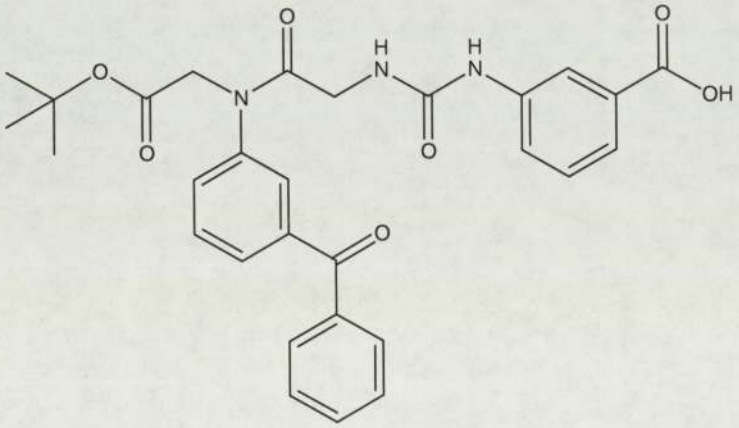


Figure 1.2.6. S-0509: a ureidomethylcarbamoylphenylketone derivative.

1.2.7. Ureidophenoxyacetanilides.

DZ-3514 (figure 1.2.7.), a ureidophenoxyacetanilide derivative, was developed by Japanese scientists[57], in order to prevent adverse effects i.e. as seen with Proglumide – a CCK_B/gastrin antagonist with weak CCK_A receptor antagonist activity. DZ-3514

showed nanomolar activity for CCK_B/gastrin receptors (0.8 nM, 250-500 higher selectivity over CCK_A receptors).

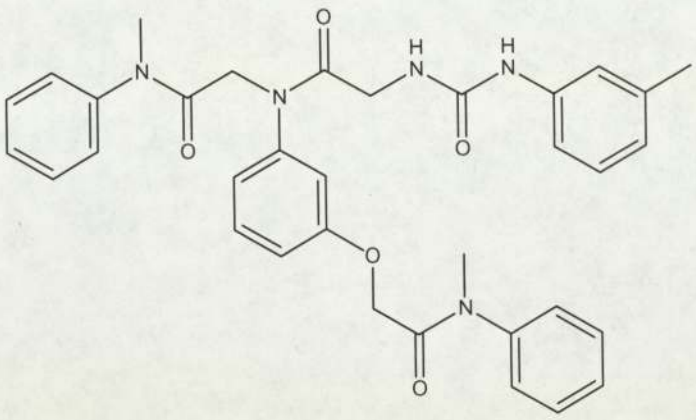
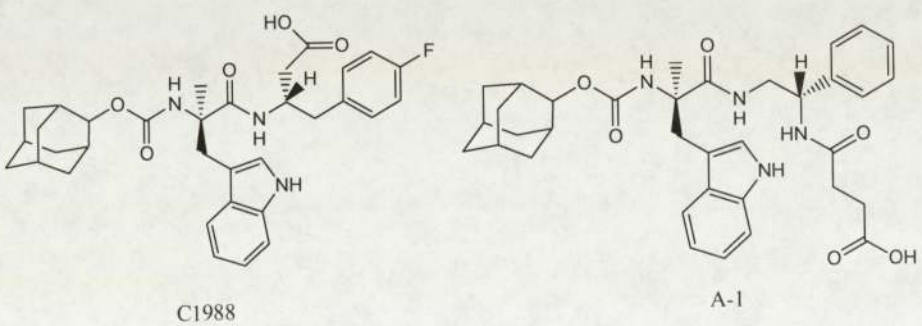


Figure 1.2.7. DZ-3514: a ureidophenoxyacetanilide derivative.

1.2.8. Tryptophan dipeptoid derivatives.

The activity of CCK-30-33 fragments was looked at by Parke-Davis[58] in binding experiments on CCK_B/gastrin receptors and led to the development of C1988 (figure 1.2.8.) which had a 1600-fold selectivity for CCK_B receptors compared to CCK_A receptors. Structurally modified compounds were derived from C1988, possessing low nanomolar affinity for CCK_B/gastrin receptors. One compound in particular A-1 (figure 1.2.8., IC_{50} 0.08 nM) was around 1000 times more selective for the CCK_B receptor, but displayed low bioavailability due to its high molecular weight and dipeptide-like structure.



C1988 A-1

Figure 1.2.8. C1988 and A-1: tryptophan dipeptoid CCK_B antagonists.

1.2.9. Dibenzobicyclo [2.2.2] octane and bicyclic heteroaromatic derivatives.

Potent and selective CCK_B/gastrin antagonists have been developed by the James Black foundation, based upon a dibenzobicyclo [2.2.2] octane template and on a pyrrole or imidazole ring system[59]. B-1 (a dibenzobicyclo [2.2.2] octane derivative figure 1.2.9.), when given intravenously (0.025 μM/kg) inhibited 79% of Gastric Acid Secretion (GAS) for a submaximal infusion, whereas compound B-2 (a 5,6-disubstituted-indole derivative, figure 1.2.9.) almost fully inhibited pentagastrin-stimulated GAS at the same dose.

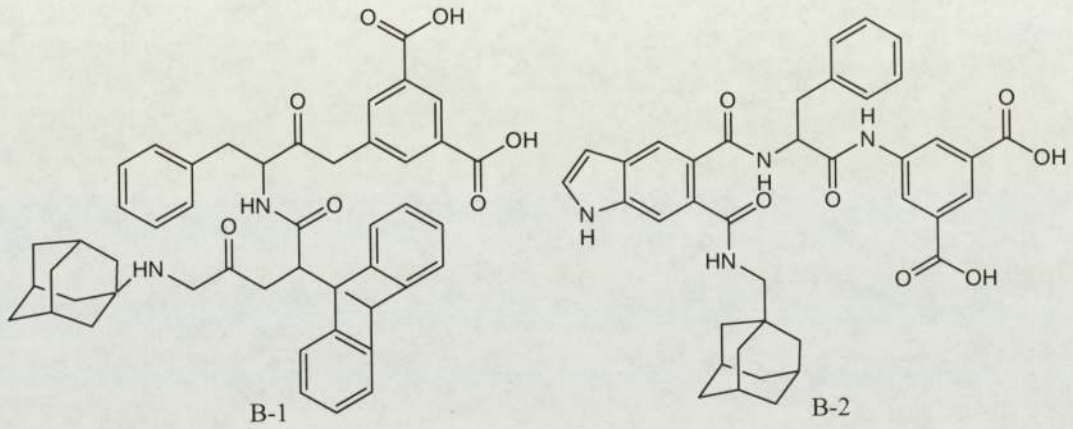


Figure 1.2.9. B-1 and B-2: dibenzobicyclo [2.2.2] octane and heteroaromatic CCK_B antagonists.

1.2.10. Quinazolinone-based compounds.

A series of quinazolinone-based CCK_B antagonists, derived by Lilly, were synthesised around a quinazolino-1,4-benzodiazepin-5,13-dione and displayed around a 20-fold higher affinity for CCK_B receptors in the cortical membrane than those in gastric tissues[59]. One of these compounds, LY-202769 (figure 1.2.10.), potently inhibited [^{125}I]CCK_{8s} binding to the mouse cortical (IC_{50} = 9.3 nM).

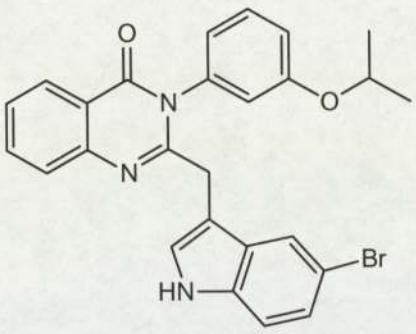


Figure 1.2.10. LY-202769: a quinazolinone-based CCK_B antagonist.

1.2.11. Benzodiazepine derivatives.

During the past 40-50 years an in depth study of benzodiazepine derivatives has occurred, starting with the discovery of chlorodiazepine by Sternbach *et al.*[60] during the 1950's. There are approximately 50-60 benzodiazepine derivatives in medical use today with their primary use in the treatment of anxiety, but are also classed as anticonvulsants, sedatives and muscle relaxants. They mainly act by binding to a specific regulatory site on the γ-amino butyric acid ($GABA_A$) receptor therefore increasing the inhibitory effect of GABA[61, 62]. All benzodiazepines and derivatives contain a seven-membered heterocyclic ring system which is bent[63] which has been shown to be essential for activity[64].

A number of benzodiazepine derivatives have been developed by only replacing functional groups. This alteration has led to the synthesis of drugs which have an effect on the CNS, as well as having a low toxicity. Examples are shown in figure 1.2.11. Diazepam has been found to possess anxiolytic and muscle relaxant properties, while nitrazepam is an anticonvulsant and hypnotic agent. Oxazepam and Lorazepam are both anxiolytic drugs, while temazepam is an anxiolytic, sedative and hypnotic agent. Four main substituent groups have been found (chloro, hydrogen, nitro and hydroxyl) that can be modified without losing much activity in relation to benzodiazepine derivatives.

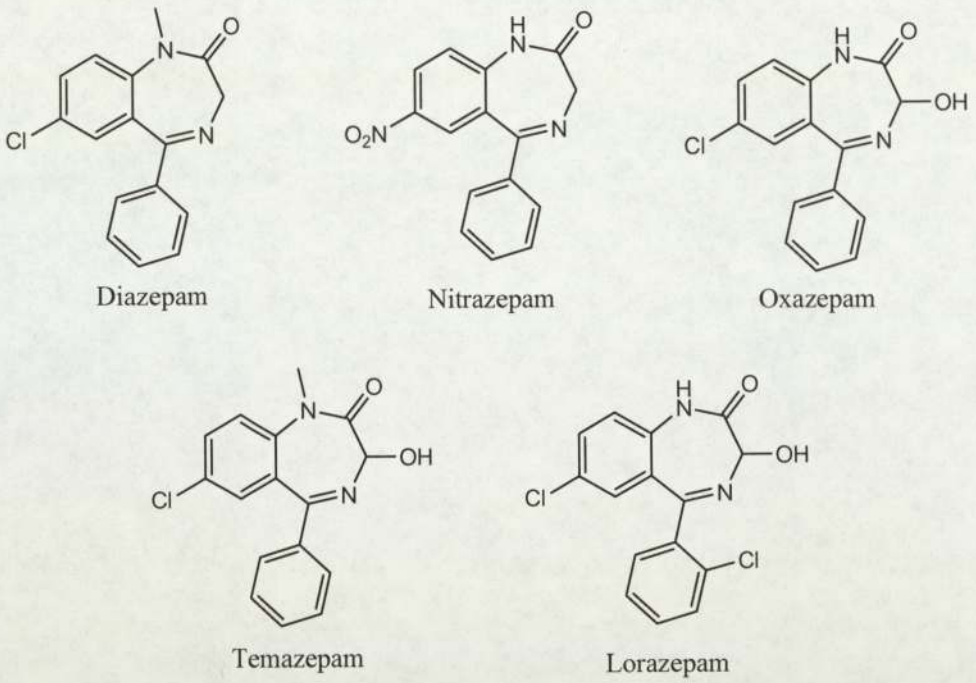
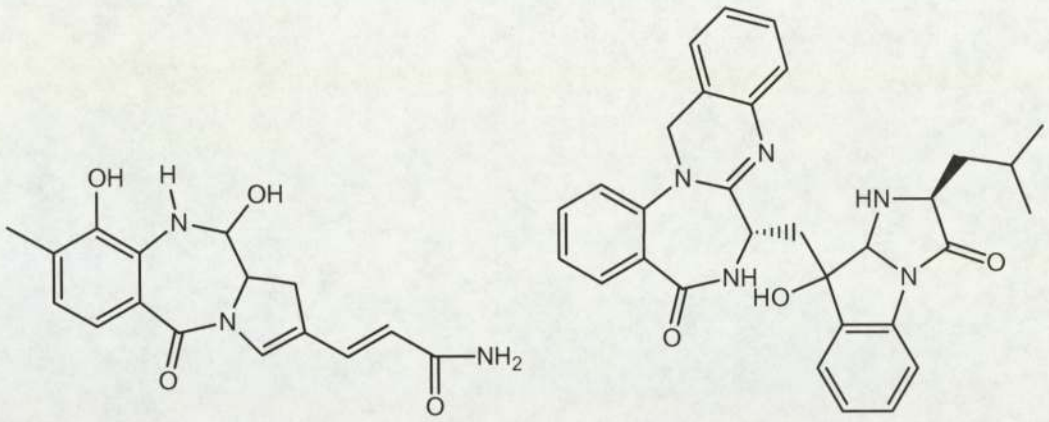


Figure 1.2.11. Benzodiazepine derivatives possessing various biological properties.

Anthramycin (figure 1.2.11.1.), reportedly a potent antagonist of CCK in mice[65], is produced by Streptomyces microorganisms and has been to found to reverse CCK_8 induced satiety and displace ^{125}I-CCK_8 binding in different brain regions (mainly in the cortex).

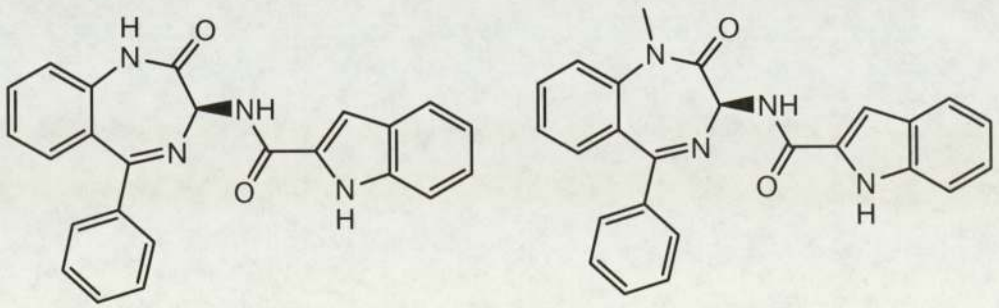
Asperlicin (figure 1.2.11.1.), isolated from *Aspergillus alliaceus*, was discovered whilst analysing microbial broths using a radioreceptor assay[66], showing selectivity for CCK_A receptors. This naturally occurring 1,4-benzodiazepine antagonist, which has around 300 times more affinity for pancreatic and gallbladder CCK receptor over proglumide (IC_{50} = 1.4 μM – pancreas binding), was an important discovery as it has since led to potent and specific CCK_A and CCK_B receptor antagonists.



Anthramycin Asperlicin

Figure 1.2.11.1. Anthramycin and Asperlicin: naturally occurring 1,4-benzodiazepine derivatives.

Although modifications of asperlicin were tried and failed[67], due to low oral activity and compound potency, L-364,286 (diazepam like structure linked with a 3-amino group) was synthesised successfully based upon the structural make up of asperlicin. Further optimisation of these CCK_A antagonists led to the extremely potent and orally active devazepide (figure 1.2.11.2., IC_{50} = 0.1 nM inhibition of ^{125}I-CCK_8 rat pancreas binding) which is also known as MK-329 and L-364,718 and has a longer lasting efficacy *in vitro* and *in vivo* and has more than 1000-fold selectivity for the CCK_A receptor over CCK_B.



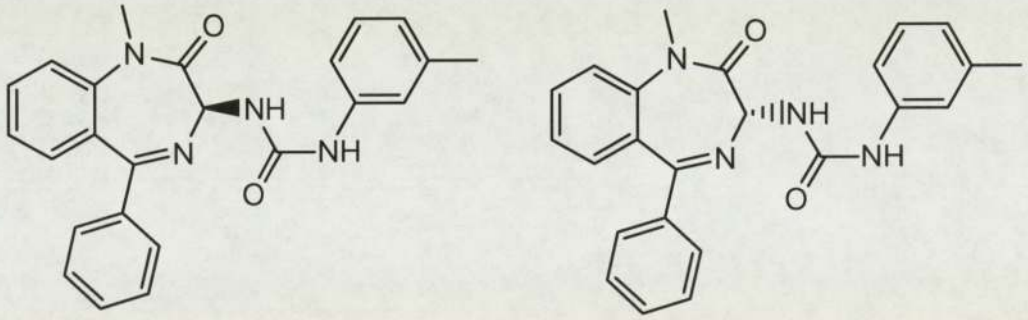
L,364,286 Devazepide

Figure 1.2.11.2. L-364,286 and devazepide: 3-amino-1,4-bezodiazepine derivatives.

Devazepide, which shows selectivity for CCK_A over CCK_B receptors (but still a potent CCK_B antagonist), had been stated[68] to be a selective antagonist possessing the effects of CCK_8 (Sincalide) on the intake of food[69] and to possess potent blocking activity in different tissues[70], such as the antagonism of pancreatic amylase secretion (2 million times more potent compared to proglumide).

1.2.12. Ureidobenzodiazepine derivatives.

Compound L-365,260 (figure 1.2.12.) is a highly selective CCK_B receptor antagonist in rats, mice and humans (80 fold greater affinity for gastrin/CCK_B receptors over pancreatic CCK_A)[71], that was developed by Merck scientists[72], which contains a benzamido urea linkage. It has been shown in general that by replacing the 3-amino linkage with a benzamido urea that a large increase in CCK_B affinity over CCK_A affinity occurs. Due to there being stereochemistry at the C-3 position of the benzodiazepine ring of this compound (and related derivatives), the (3*R*)-isomer tends to be more CCK_A selective compound to the (3*S*)-isomer which is prone to be CCK_B selective.



L-365,260: CCK_A-selective (3*S*)-Isomer

L-365,260: CCK_B-selective (3*R*)-Isomer

Figure 1.2.12. L-365,260: isomers of the ureidobenzodiazepine deriviative.

Singh[73] synthesised, optimised and biologically evaluated a series of 3-amino-1,4-benzodiazepine-2-ones based around L-365,260, most of which showed good CCK_B binding affinities with IC_{50} values in the nanomolar region. One particular compound, racemic 3U (figure 1.2.12.1.), which is similar to L-365,260 (methyl group in the *meta* position of the phenyl ring) was found to be the most active compound in the series for both CCK_A and CCK_B receptor subtypes (IC_{50} = 8 nM and 70 nM respectively), but was more potent towards the CCK_A receptor. It was concluded that by removing the urea functionality (as seen with L-365,260), activity is greatly enhanced towards the CCK_A receptor subtype.

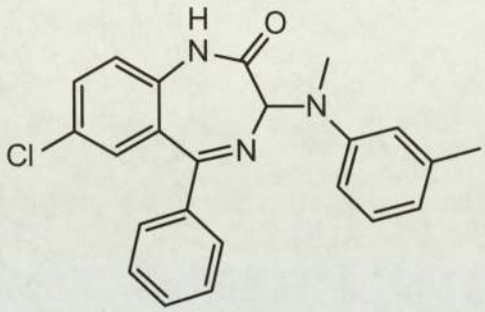


Figure 1.2.12.1. 3U: a potent CCK_A 3-amino-1,4-benzodiazepine-2-one antagonist.

An investigation into whether the satiety response to CCK is mediated by CCK_A or CCK_B receptors was carried out by Kubota *et al.*[65] using and comparing devazepide and L-365,260. The latter of these was found to be in excess of 100 times more potent in increasing feeding frequency and preventing satiated rats, indicating that endogenous CCK causes satiety via interaction with CCK_B receptors in the brain. A major concern with this compound was that it had limited oral bioavailability due to its low aqueous solubility and bio-distribution[74]. During a phase I clinical trial using mice, a very low brain uptake (<0.8% dose/gram) after intravenous injections was shown.

Merck scientists tried to overcome this problem, by developing a second generation of compounds[75] based around L-365,260. One amidine derivative L-740,093 (figure 1.2.12.2.), was found to be extremely potent and showed around 100 times better aqueous solubility (as HCl salt) than the parent molecule. It showed high CCK_B/gastrin

affinity (IC_{50} = 0.1 nM) and therefore appears to possess the necessary pharmaceutical properties to be used orally in humans.

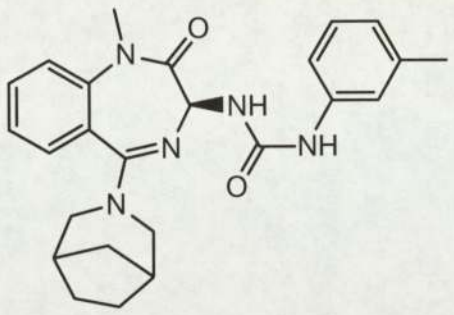


Figure 1.2.12.2. L-740,093: a ureidobenzodiazepine derivative.

Other attempts have been made to improve bioavailability by synthesising new ureidobenzodiazepine derivatives of L-365,260, for example Merck's L-737,425[76] and the Yamanouchi groups' YM022[77, 78] but this has been hindered by the complexity in synthesising the compound in question. In the other direction, compounds such as Pfizer's CP-310,713[79], Merck's L-708,474[80] and Glaxo Wellcome's GV150013X[81] synthesised in good yields, were found to be potent and showed high CCK_B affinity and good selectivity over CCK_A, but possessed poor oral bioavailability.

1.2.13. Indol-2-based compounds.

Chugai developed and optimised 3,3-disubstituted indol-2-ones which inhibited [^{125}I]-gastrin binding[59] to guinea pig gastric glands and were selective towards pancreatic CCK_A sites. This optimisation led to the average orally active AG-041R, which contains a geminal acetamido-urea (figure 1.2.13.) and was found to inhibit specific binding of [^{125}I]-gastrin binding to guinea pig gastrin glands (IC_{50} = 1.11 nM). This compound also inhibited pentagastrin stimulated acid secretion in pylorus ligated rats by intravenous injection (IC50 = 5 nM/kg), but had no inhibitory effect on carbacol or histamine stimulated secretion. AG-041R has also been shown to exhibit greater potency

compared to L-365,260 for water immersion stress (>600 fold) and indomethacin-induced ulcer (6-fold) models.

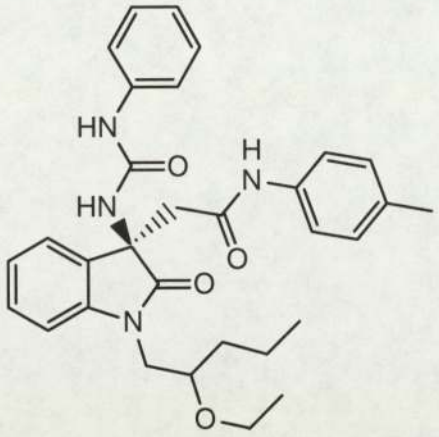


Figure 1.2.13. AG-041R: an indol-2-based CCK active agent.

1.2.14. Pyrazolone urea and amide derivatives.

Singh[73] followed a completely different approach, away from the benzodiazepine structure, by developing simple ureas. He found that the most active compounds contained a urea linkage between the benzodiazepine and the aromatic system, making them potent CCK_B receptor antagonists. From his deductions he decided to replace the benzodiazepine structure with alternative aromatic systems in order to mimic its moiety and effect. Most compounds were synthesised in high yield, with one amino-antipyrine compound in particular showing good CCK_B activity, but only average potency (4.8b, IC_{50} = 5 μM, figure 1.2.14.).

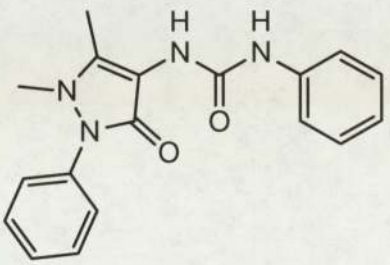


Figure 1.2.14. 4.8b: a pyrazolone urea

Singh developed and optimised this pyrazolyl template further by using a diphenyl system in order to more closely mimic the Merck's L-365,260 (section 1.2.12.). Compound 5e, a *meta*-toluidine substituent which does not contain any stereo centre, showed the highest CCK_B activity of IC_{50} = 25 nM, while showing 20 nM towards the CCK_A receptor (figure 1.2.14.1.). *In vivo* screening showed this agent to possess tremendous potential as new neuroleptic, anxiolytic and antidepressant drugs.

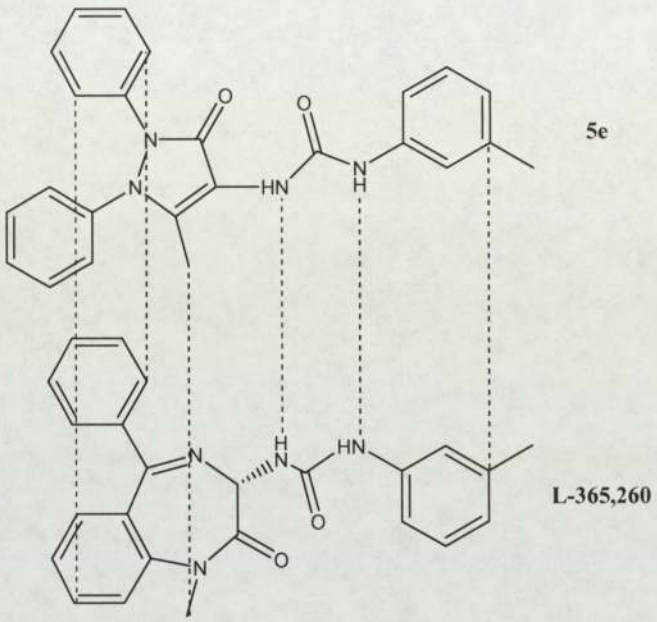


Figure 1.2.14.1. Common features present in 5e and Merck's antagonist L-365,260.

Singh also synthesised, developed and evaluated pyrazolone amides [82] as cholecystokinin antagonists based around devazepide (section 1.2.12.), a potent CCK_A antagonist, which contains a 1,4-benzodiazepine template and an indole moiety. He found the best amide 4d (figure 1.2.14.2.) of this series displayed an IC_{50} of approximately 25 nmol/L for the CCK_A receptor. This compound was also shown to possess both anxiolytic and anti-depressant properties.

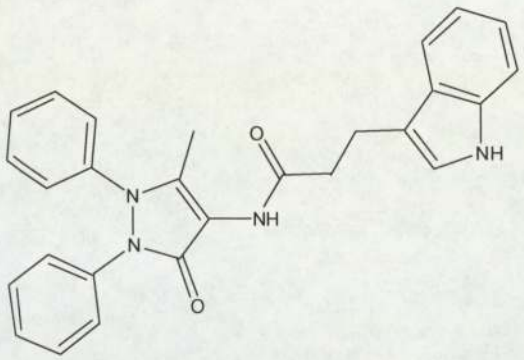


Figure 1.2.14.2. 4d: an amido-pyrazolone CCK antagonist.

1.2.15. Summary.

The 33 amino acid peptide CCK[83] (and associated forms) acts as both a gut hormone[84] and as a central neurotransmitter or neuromodulator, with its CNS effects mainly mediated through the CCK_B / CCK_2 receptor subtype[85], while its peripheral effects are mainly related with the CCK_A / CCK_1 receptor subtype.

Antagonist compounds possessing CCK_A blocking activity show effects on food intake by stimulating the release of both bile (from the gallbladder) and digestive enzymes (from the pancreas)[68], whereas both peptide and non-peptide CCK_B antagonists have therapeutic uses for many pathological situations such as anxiety and panic disorders[23]. However, peptides such as CCK antagonists tend to have poor oral absorption and metabolic lability.

The use of non-peptide analogues such as Merck's low toxic 3-amido- and 3-ureido-benzodiazepine CCK antagonists have alleviated these problems. Devazepide was the first fully synthesised 3-amido-benzodiazepine acting as a CCK_A antagonist instead of at the GABA receptor where typical benzodiazepines, such as Diazepam, act. Merck also found that by changing from an amido to a urea linkage, such as seen with the antagonist L-365,260, CCK_B selectivity resulted. This compound has shown limited oral bioavailability (due to low aqueous solubility), but was still used as a lead structure in order to modify and improve this bioavailability[77]. Singh has developed and synthesised

ureido and amido-pyrazolones, based on certain benzodiazepines. These CCK antagonists have been found to possess both anxiolytic and anti-depressant properties.

To conclude, a high interest with regards to the biological and therapeutic roles of cholecystokinin (CCK) receptor ligands has been shown over the past few decades due to the numerous physiological processes involving the CCK receptor. Although the complicated biological role of CCK is still to be fully understood, the potential application of CCK ligands for the treatment of such diseases as anxiety, depression, gastrointestinal disorders, cancer and pain is still at the forefront of many chemical and biological companies.

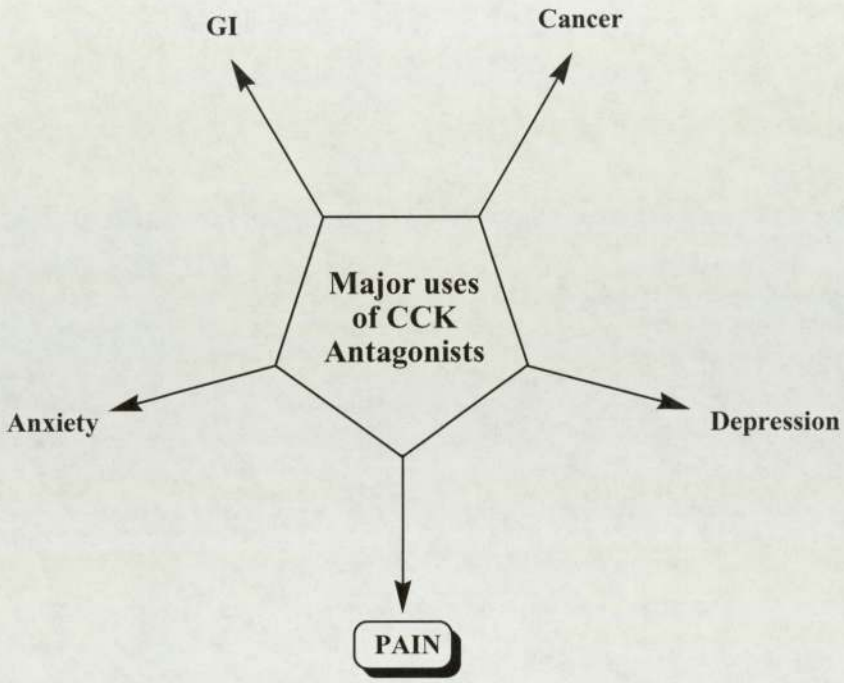


Figure 1.15. Major uses of CCK antagonists.

Both anxiety and depression in general are quite difficult to measure, in comparison to pain, as there are numerous undesired errors that can occur within these assays, whilst there have already been numerous studies carried out regarding CCK and the GI system (figure 1.15.). Here, a nociception (pain) assay involving opioid potentiation has been developed using both a full and partial opioid agonist, in combination with standard pyrazolone CCK antagonists (discussed in chapter 1b). A comparison of this data with data from new classes of compounds can hopefully yield further CCK antagonists,

which can be tested in a broader range of assays. It is the intention that once these selected biologically active compounds have been identified, they could be tested in cytotoxic assays, to reveal any anti-cancer properties.

Chapter 1b: Synthesis of Pyrazolones for use as pharmacological standards.

1b.1. Introduction.

Pyrazolones, which contain a 5-membered lactam ring, have been found to be clinically useful. They have the general structure shown below (figure1b.1.). Pyrazolone class NSAID (nonsteroidal anti-inflammatory drugs)[86] include phenylbutazone (Butazolidin), oxphenbutazone (Oxalid) and aminopyrine (Pyramidon). These drugs, alongside antipyrine (Felsol, figure 1b.1.), also possess analgesic and antipyretic properties.
Phenylbutazone and its related compounds were used worldwide until the early 1980's when numerous drugs from this class of compound, were withdrawn from the medical market due to adverse and serious toxic side effects, including agranulcytosis[87], blood dyscrasias[88] and lymphadenopathy[89].

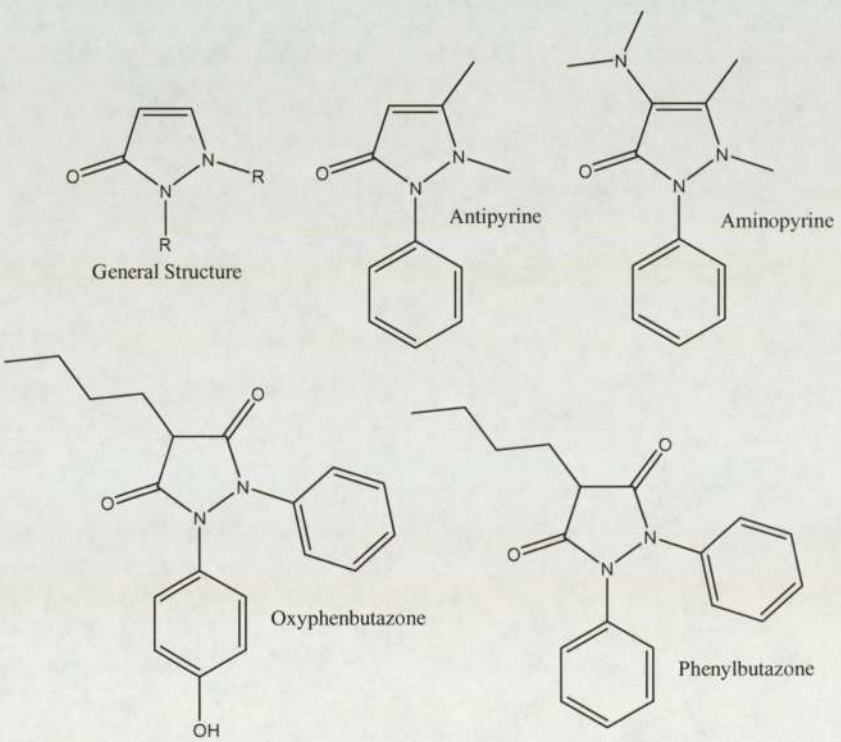
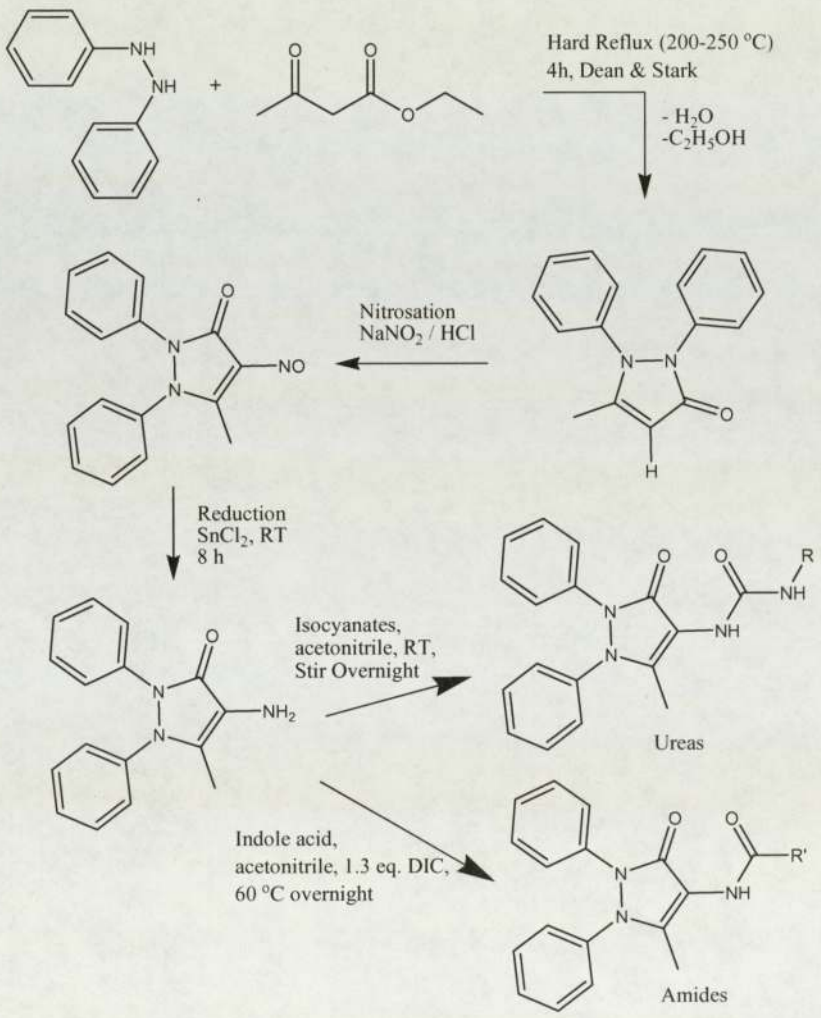


Figure 1b.1. Biologically active pyrazolones.

There are also numerous patents covering these pyrazolones and their biological activities for example as inhibitors of diabetes[90]. Therefore further development of these compounds would not be very feasible as they are not novel compounds and there may be patent related issues which either delay or stop any biological testing or clinical trials. Another reason for not continuing the development of these compounds is due to the fact that they are known analgesic drugs[91] and therefore it would be beneficial to develop other novel compounds as analgesics based on Cholecystokinin antagonism.

1b.2. Synthetic overview for the synthesis of pyrazolone ureas and amides.



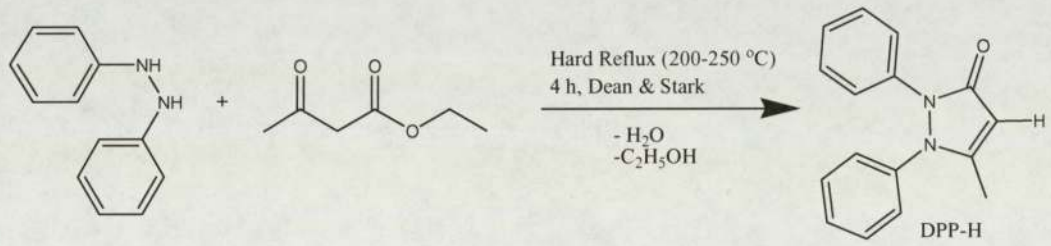
Scheme 1b.2. Synthetic scheme for the synthesis of diphenyl-pyrazole ureas and indoles.

Scheme 1b.2. shows an overview towards the synthesis of both pyrazolone ureas and amides, which were formed in a four-step synthesis via a 4-amino-pyrazolone, from the starting materials diphenylhydrazine and ethyl 3-oxobutanoate.

A range of both ureido- and amido-pyrazolone compounds had previously been formed[73], but were to be re-synthesised, in order to be used as pharmacological standards. Compounds from both of these classes had previously been shown to possess CCK antagonist properties and were therefore required in order to make a comparison with the compounds synthesised and biologically tested herein.

1b.2.1. Synthesis of 3-methyl-1,2-diphenyl-1,2-dihydropyrazol-5-one.

Scheme 1b.2.1. shows the synthetic route developed for the synthesis of 3-methyl-1,2-diphenyl-1,2-dihydropyrazol-5-one (**DPP-H**). This compound had previously been formed as a black semisolid[73]. Here after optimisation, it was recrystallised, forming black needle shaped crystals in 52% yield with melting point 152-155 °C.

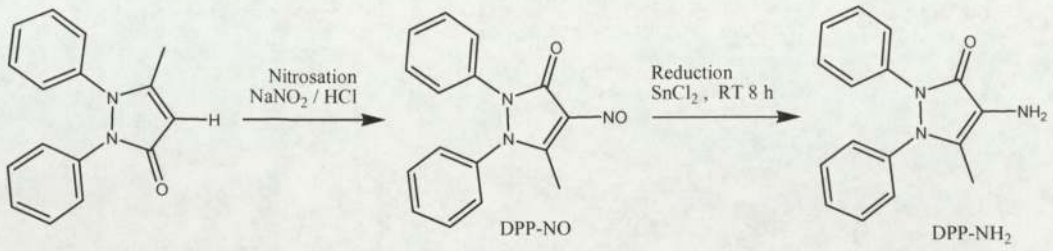


Scheme 1b.2.1. Synthetic route for the synthesis of 5-methyl-1,2-diphenyl-1,2-dihydro-pyrazol-3-one.

1b.2.2. Synthesis of 4-amino-3-methyl-1,2-diphenyl-1,2-dihydro pyrazol-5-one.

Scheme 1b.2.2. shows the synthetic route for the formation of 4-amino-3-methyl-1,2-diphenyl-1,2-dihydropyrazol-5-one (**DPP-NH_2**). This compound was synthesised via nitrosation and reduction and was formed via a nitroso-pyrazolone intermediate (**DPP-**

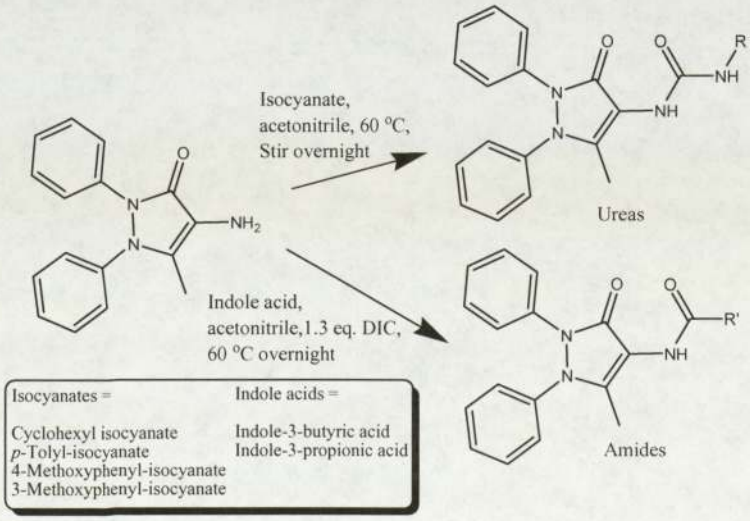
NO), which was confirmed by MS and proton NMR. **DPP-NH_2** was formed as bright yellow crystals in 42% yield.



Scheme 1b.2.2. Nitrosation and reduction for the synthesis of diphenyl-pyrazole amine.

1b.2.3. Synthetic route for the synthesis of ureido- and amido-pyrazolones.

Scheme 1b.2.3. shows the synthetic routes developed for the synthesis of both pyrazolone ureas and amides. A range of isocyanates and indole acids were chosen, with the intent on synthesising low, medium and high activity CCK antagonist compounds to be used as biological standards. Both reaction mixtures were stirred overnight and DIC (diisopropylcarbodiimide) was added as an activating reagent, along with the indole acid, in order to convert the acid to a reactive acylating agent so that the desired amide could be formed.



Scheme 1b.2.3. Synthetic route for the synthesis of diphenyl-pyrazole ureas and amides.

Figure 1b.2.4. shows the yields and general binding affinity for the synthesised ureido- and amido-pyrazolones. All compounds were formed in good yield, with the urea, **UR-CyH**, being formed in the highest yield (85%), while its bioisostere **UR-Me(p)** was formed in 73%. The amide **AM-IND(2)**, was synthesised in 74%, while its homologue **AM-IND(3)**, was formed in a slightly lower yield (70%). The pyrazolone urea **UR-MeO(m)** was synthesised in a lower yield (70%) than its isomer **UR-MeO(p)** which was synthesised in a 75% yield. All of these compounds were fully characterised before being used in any pharmacological experiments.

All of these pyrazolones have previously been subjected to binding affinity assays[82, 92]. Compounds **AM-IND(2)** and **UR-MeO(m)** displayed high binding affinity, with both having an IC_{50} value of approximately 25 nmol/L, while **UR-CyH** has exhibited a modest binding affinity of approximately 1 μmol/L. Compounds **UR-MeO(p)** and **AM-IND(3)** were both found to be inactive, with binding affinities of greater than 20 μmol/L. The CCK antagonists **AM-IND(2)** and **UR-MeO(m)** were chosen for use as standards in the development of a nociception assay (section 1b.3) because of their high binding affinities. Significant tramadol potentiation in the following pain assay using these pyrazolone should show that there is a correlation between this *in vivo* nociceptive assay and *in vitro* CCK receptor binding.

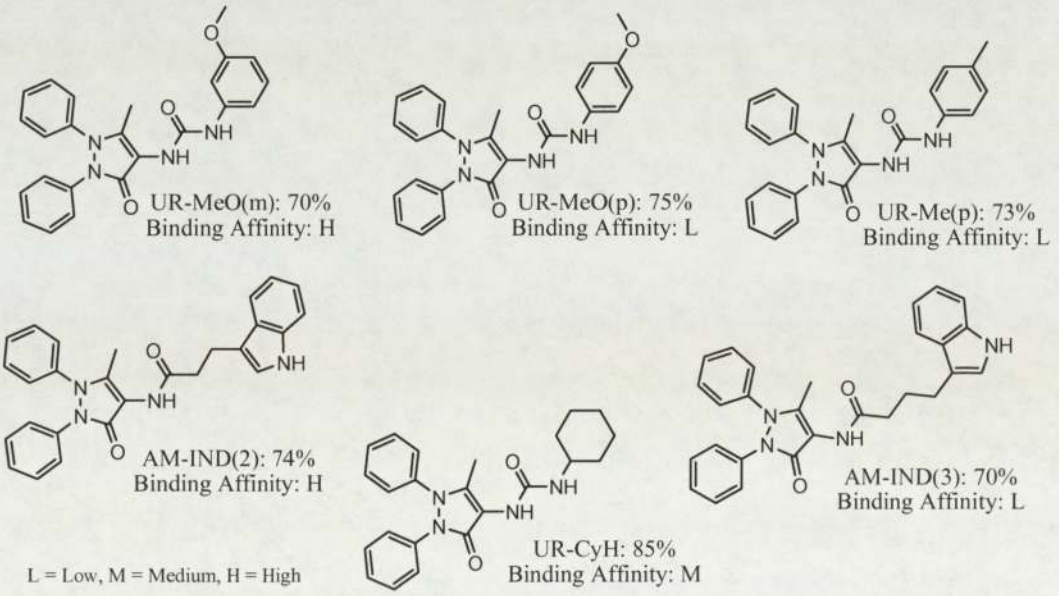


Figure 1b.2.4. Structures of synthesised pyrazolone ureas and amides to be used as biological standards.

1b.3. Development of an opioid nociception assay using pyrazolone standards.

One of the main physiological actions of CCK in the CNS includes the modulation of pain[93]. There is evidence that some biological actions of CCK are opposite to those triggered by opioids, suggesting that CCK might function in the CNS as a physiological opioid antagonist[94]. For example it has been shown that CCK_B receptor antagonists enhance the effect of morphine[95].

Here, a nociception assay (pain; tail flick, experimental chapter 6.6) was developed using full or partial opioid agonists in combination with standard pyrazolone CCK antagonists (synthesised in the previous section). The idea behind this assay was that there should be a larger enhancement in potentiation[96] with regards to a partial opioid agonist compared to that of a full agonist, i.e. an animal should have a significant pain threshold when using the partial agonist in combination with the standard CCK antagonist pyrazolones. Male mice were obtained from the animal house at Khon Kaen University. These experiments were conducted in collaboration with Khon Kaen University. Each experimental group consisted of six mice, which were injected intraperitoneally with either test or standard compounds dissolved in DMSO at a volume of not more than 0.2 ml per animal. This assay consisted of a mouse having its tail immersed in hot water (50 ^{O}C, for no more than 45 seconds in order to avoid tail damage) before the time was recorded for it to remove it when pain was felt. The full agonist, morphine, was used first. Morphine potentiation using **AM-IND(2)** and **UR-MeO(p)** as pyrazolone standards was recorded at 30, 60 and 90 minutes, using doses of morphine at 2, 4, 8 and 16 mg/kg. The standard amount of compound, 0.5 mg/kg, was used for each experiment. The same assay using the partial agonist, tramadol and the same standards and conditions was carried out over the same time period. Figure 1b.3. shows the results using these opioids at 30 minutes.

As expected there is a bigger maximum possible effect (MPE) seen when the partial agonist, tramadol, is used in conjunction with the standard antagonist pyrazolones, when compared to the results for the morphine potentiation. The differences in MPEs between morphine used alone and when used with the standards are significant. There is a very large difference in the MPE with relation to the use of tramadol alone and when used alongside the pyrazolone standards. In general significant morphine potentiation is

given when a lower dose (2 mg/kg) of morphine is used, whereas a very strong effect in tramadol potentiation is seen over a wide range of 2-16 mg/kg with the use of tramadol.

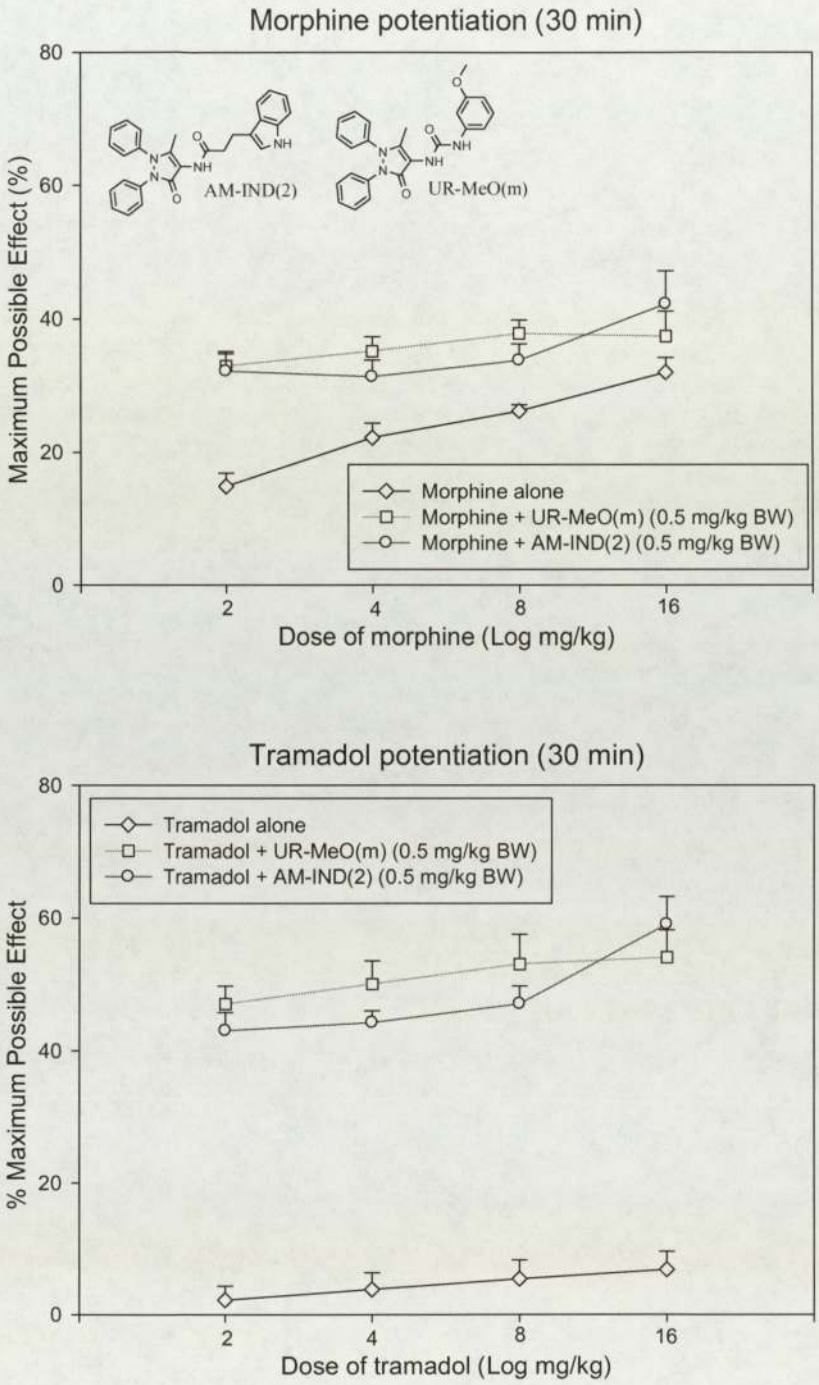


Figure 1b.3. Morphine and tramadol potentiation at 30 minutes.

At 60 minutes (figure 1b.3.1) there was not a significant increase in morphine potentiation when compared with the results obtained at 30 minutes. There was however

a further increase in tramadol potentiation at this time compared to the 30 minute point. Again, tramadol at 16 mg/kg in combination with 0.5 mg/kg of both standards gave the best potentiation, in particular with **UR-MeO(m)** (where MPE values of around 60%).

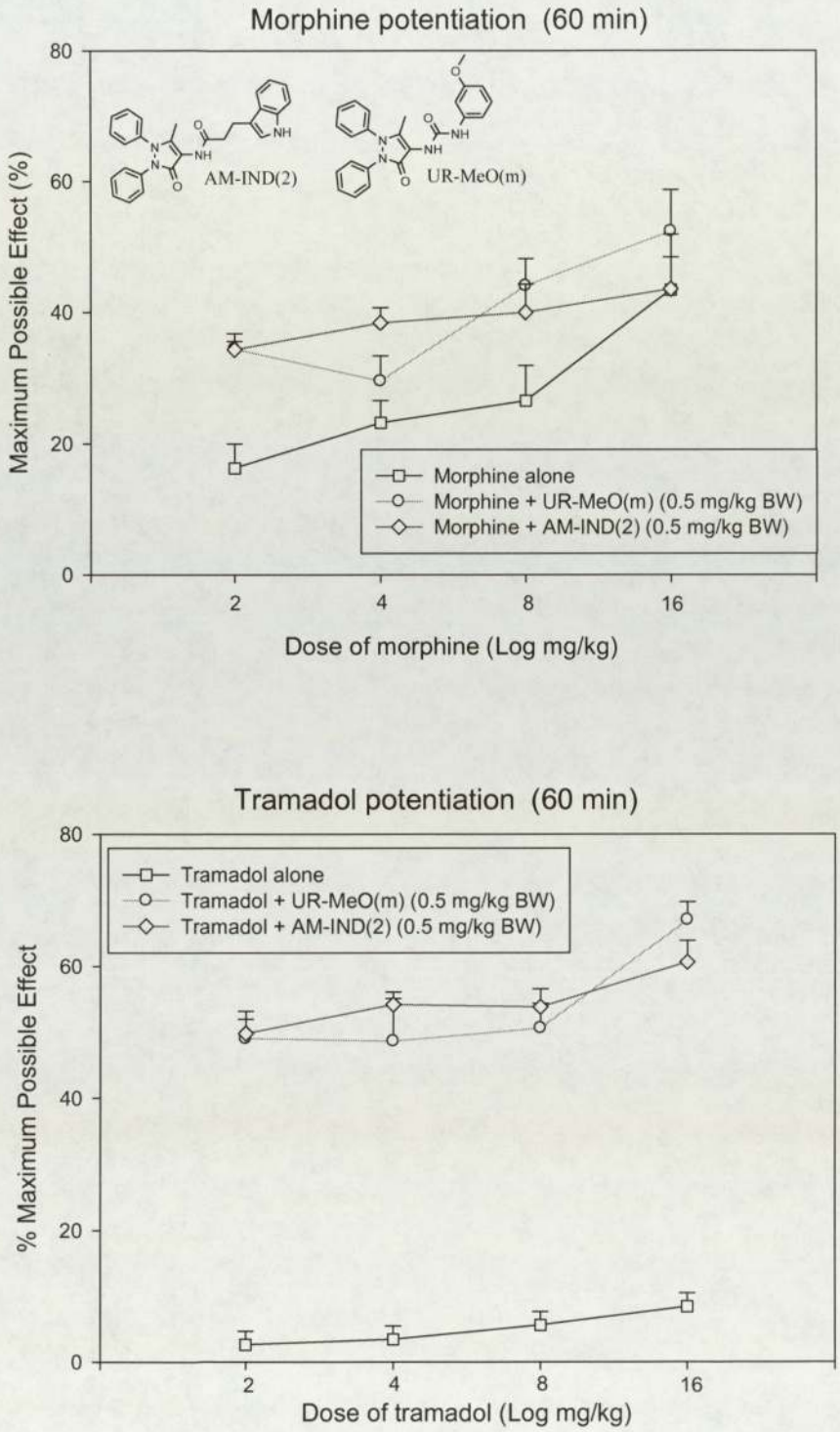


Figure 1b.3.1. Morphine and tramadol potentiation at 60 minutes.

At 90 minutes (figure 1b.3.2.) there was no significant difference in comparison to the corresponding data given for 60 minutes for either tramadol or morphine potentiation. There did however appear to be a slight decrease in MPE at this time over the dose range for both opioid potentations, suggesting that the 60 minute duration yielded the best results. Tramadol in combination with the standards still had an overall higher MPE compared to that of morphine and the best tramadol potentiation was seen at doses of 8 and 16 mg/kg of tramadol.

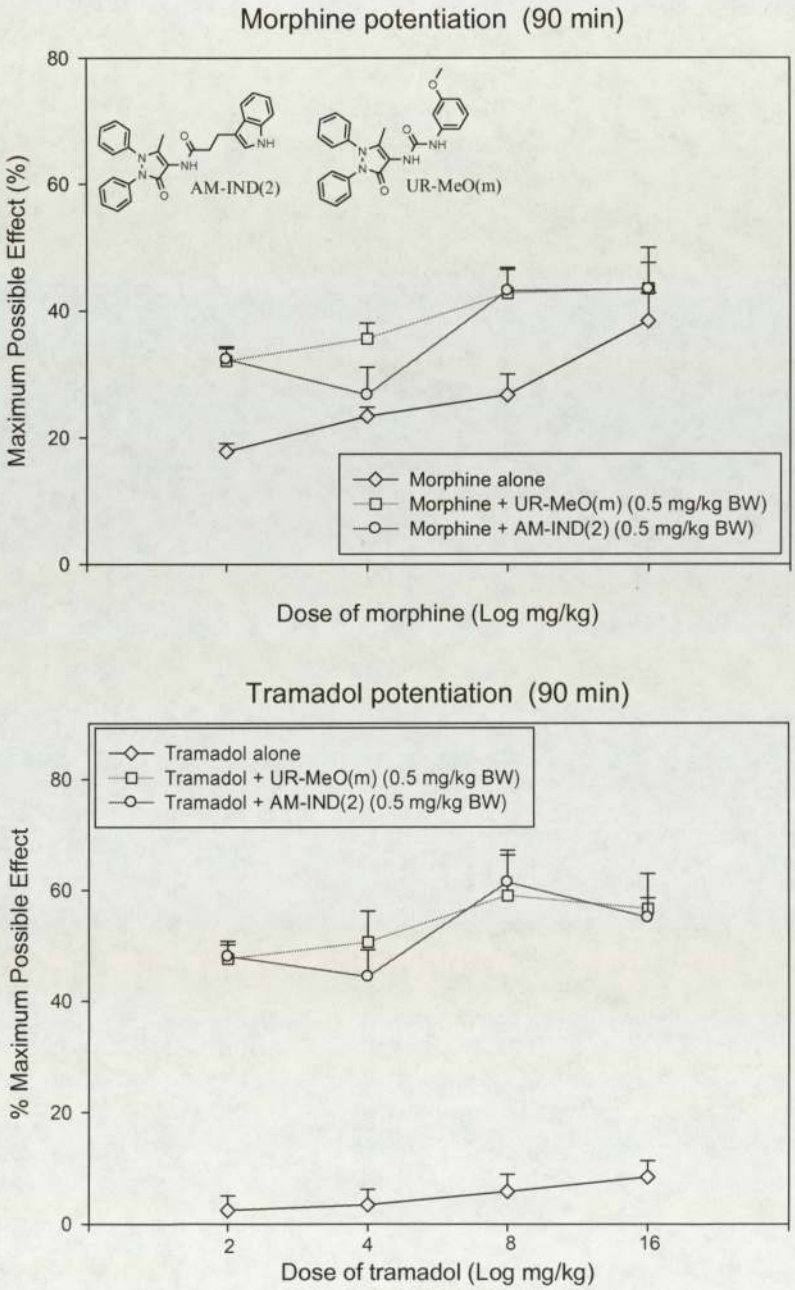


Figure 1b.3.2. Morphine and tramadol potentiation at 90 minutes.

In conclusion the results from the tramadol potentiation, as expected, have a higher and better effect than the corresponding morphine potentiation. The partial agonist, tramadol, has been shown to give a larger enhancement towards the relief of pain. In general the most significant potentiation is seen at the 60 minute point across the whole range (2-16 mg/kg) of tramadol doses and in particular at a dose of 16 mg/kg.

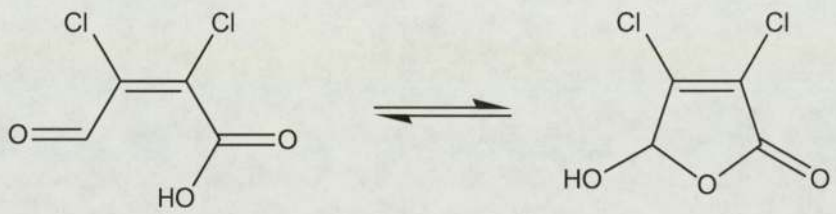
1b.4. Summary.

A nociception assay has been developed and used in collaboration with researcher at Khon Kaen University and has produced reliable and significant data in showing a greater enhancement in potentiation when using tramadol (in combination with pyrazolone CCK standard antagonists) in relation to the corresponding results obtained when using morphine. These results also show that there is a correlation between *in vitro* CCK receptor binding and this *in vivo* tramadol potentiation pain assay. This nociceptic assay can therefore be used in conjunction with new classes of compounds formed herein and will now be applied as an initial pharmacological starting point to identify, non-benzodiazepine, non-urea and non-indol based compounds possessing CCK antagonist properties. Evidence of CCK active agents will result from a comparison of the MPE values of newly synthesised compounds with those of the standard pyrazolones.

Chapter 2: Synthesis and evaluation of 5-alkoxy-4-amino-furanones as CCK antagonists.

2.1. Mucochloric acid as a template.

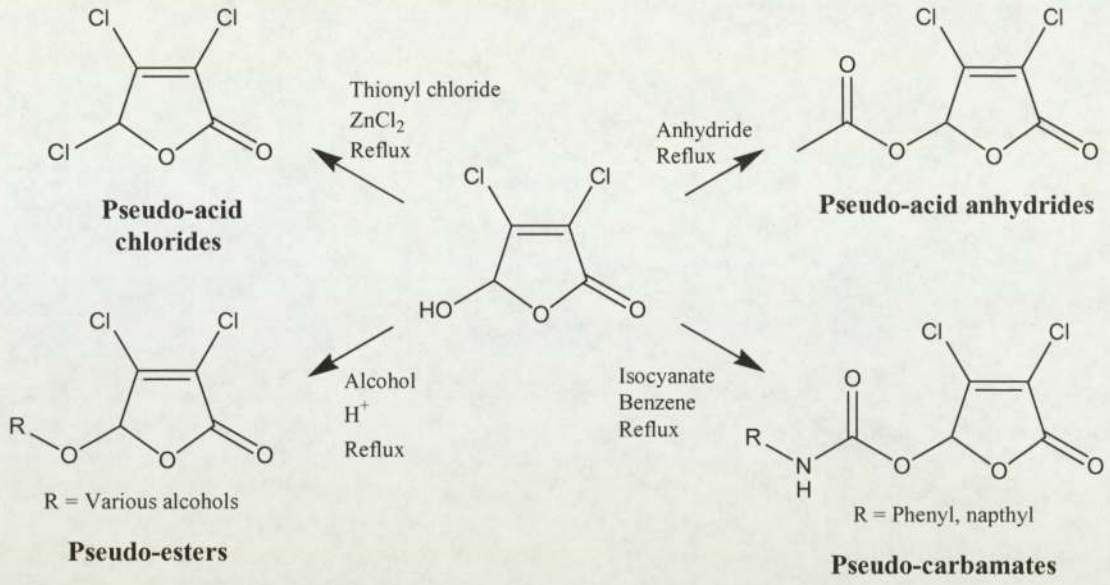
Between 1880 and 1905, Hill[97] and Simon investigated, in some detail, the chemistry of mucochloric acid. For some reason this compound was not re-investigated until the 1950's, when Mowry[98] presented his finding, stating that mucochloric acid was thought to be in the half aldehyde state of dichloromaleic acid and exists in both the open and closed ring forms (scheme 2.1.).



Scheme 2.1. The two forms of mucochloric acid.

Of the two main naming systems, one based on the butenolide[99] and the other on the furanone core names, it is the term furanone that is preferred. There has been considerable interest in furan-2-ones, over the last few decades, because of their presence in numerous biologically active products, as well as their important use as synthetic intermediates[100].

Mowry carried out extensive research on this template, and included the characteristics and reactions of the pseudo-acid group (mucochloric acid in the closed form). By heating mucochloric acid with a variety of reagents, he found that, pseudo esters, anhydrides, acid chlorides, were all formed in the cyclic form (scheme 2.1.1.).



Scheme 2.1.1. Overview of the reaction of the pseudo-acid group.

Furfural, an inexpensive, readily available compound (obtained from biomass)[101], is the initial starting material from which mucochloric acid is derived. Mucobromic acid is not that widely used in comparison with mucochloric acid. This is most likely due to the toxicity of the dibromo-functionality. Figure 2.1.2. shows the possible sites that can potentially be modified in the furan-2(5H)-one structure.

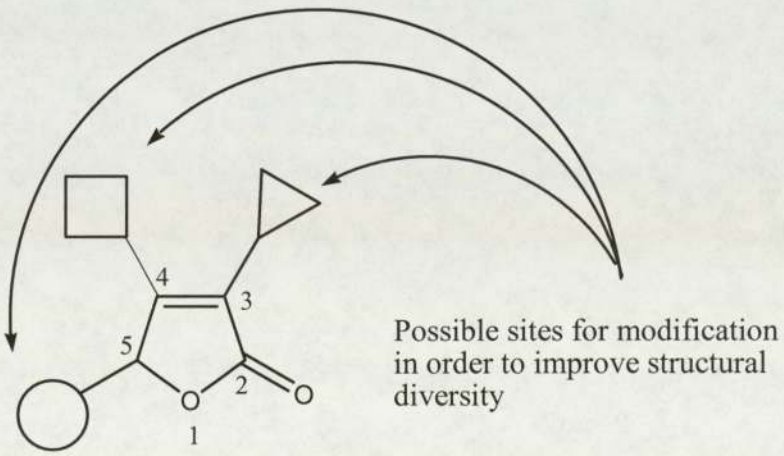
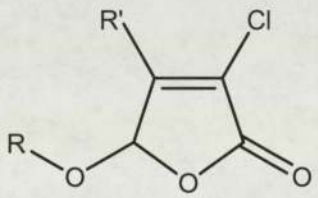


Figure 2.1.2. Potential sites for the modification of furan-2(5H)-one.

For this investigation, a range of pseudo esters are to be chemically synthesised from mucochloric acid, using the appropriate alcohol. Once these 5-substituted alkoxy-furan-2(5H)-ones have been formed, nitrogen containing nucleophiles can be used to displace halogens at the 4-position, in order to obtain 4-amino-5-alkoxy-furan-2(5H)-ones (figure 2.1.3.). A range of these compounds has been successful synthesised before in good yield, with biological activity in mind, during the previous work performed by both Langley[102] and Singh[73].

The initial work carried out during this research was to develop a range of these compounds containing this furanone template as shown in figure 2.1.3., as anti-bacterial agent[103]. Mucohalogen acids were used with various alcohols and amines in the construction of a chemical library. One of these compounds, a benzimidazole furanone, showed anti-bacterial activity against MRSA (*methicillin-resistant Staphylococcus aureas*). These biological findings however did not fit in with work carried out here on CCK and so it was decided to synthesise new compounds, based around this biologically active 4-amino-5-alkoxy-furanone template and to use the same chemical synthetic approach in order to yield and evaluate potential CCK antagonist furanones.

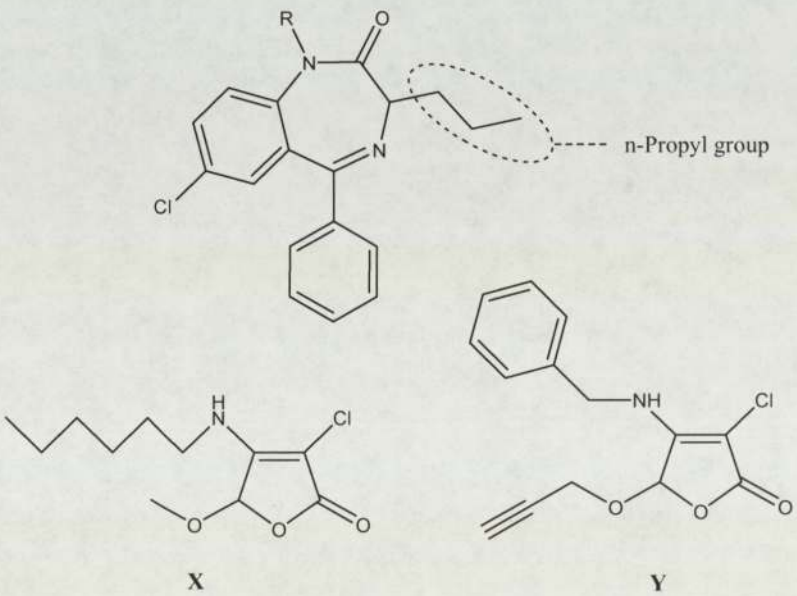


R = Various alkyl, aryl and aralkyl substituents derived from alcohols
R' = Various alkyl, aryl and aralkyl substituents derived from amines

Figure 2.1.3. Template for 4-amino-5-alkyl-furan-2(5H)-ones.

2.2. The furanone lead structure as a starting point for the development of CCK antagonists.

Previous work on CCK has been carried out by Harjit Singh[73] using mucochloric acid as starting material. He synthesised a number of 4-amino-5-alkoxy-furan-2(5H)-ones via 5-alkoxy-furan-2(5H)-ones, using a combinatorial chemistry approach. This strategy was considered "high risk" due to potential problems such as lack of purity, but had been hugely successful in deriving a novel class of potent CCK antagonists. Some of these non-urea, non-benzodiazepine compounds displayed activity at the low nM range towards both receptor subtypes, with a slight selectivity towards the CCK-A receptor. Figure 2.2.0. shows the structures of two of the most active ligands (**X** and **Y**, active in the nanomolar region) that were synthesised in this library. He found in general that large bulky substituents on the 4 and 5 positions caused a reduction in activity and that smaller ligands exerted excellent receptor affinity.



X **Y**

Figure 2.2.0. Active CCK antagonists previously synthesised.

A range of amines and alcohol were to be used in the synthesis of new 4-amino-5-alkoxy-furan-2-ones based around the lead structures shown in figure 2.2.0. Other factors influencing the choice of alcohols and amines were mainly based upon the

structure of asperlicin[72], the first non-peptidal CCK antagonist[104], which contains an isobutyl side chain, and CCK antagonist work carried out by Lattmann *et al*[105]. His team synthesised and evaluated a group of 1,4-benzodiazepine-2-ones as CCK receptor ligands using small side chains. They found that inclusion of small alkyl groups such as a propyl group (figure 2.2.0.) was required for these compounds to show an affinity on the CCK_B receptor. Other known benzodiazepine drugs not possessing this simple alkyl chain, such as diazepam and oxazepam, have shown poor or no binding affinity.

The aforementioned biologically active compounds, shown in figure 2.2.0. contain short alkyl side chain groups. Therefore as a starting point for the synthesis of new 4-amino-5-alkoxy-furanones, short chain alkyl, cyclic-alkyl and aryl based amines and alcohols were to be chosen in order to synthesise, develop and optimised simple CCK antagonists.

To develop and optimise these compounds, certain criteria need to be fulfilled. The compounds will need to be highly crystalline, be synthesised in high yield and be biologically active. The overlapping area of the three circles shown in figure 2.2.0.1. shows the properties required for these compounds.

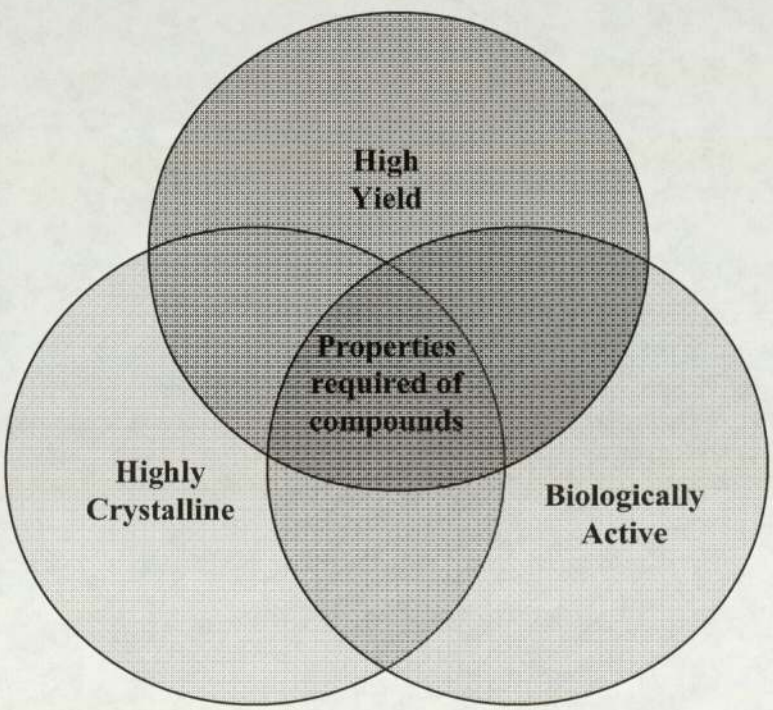
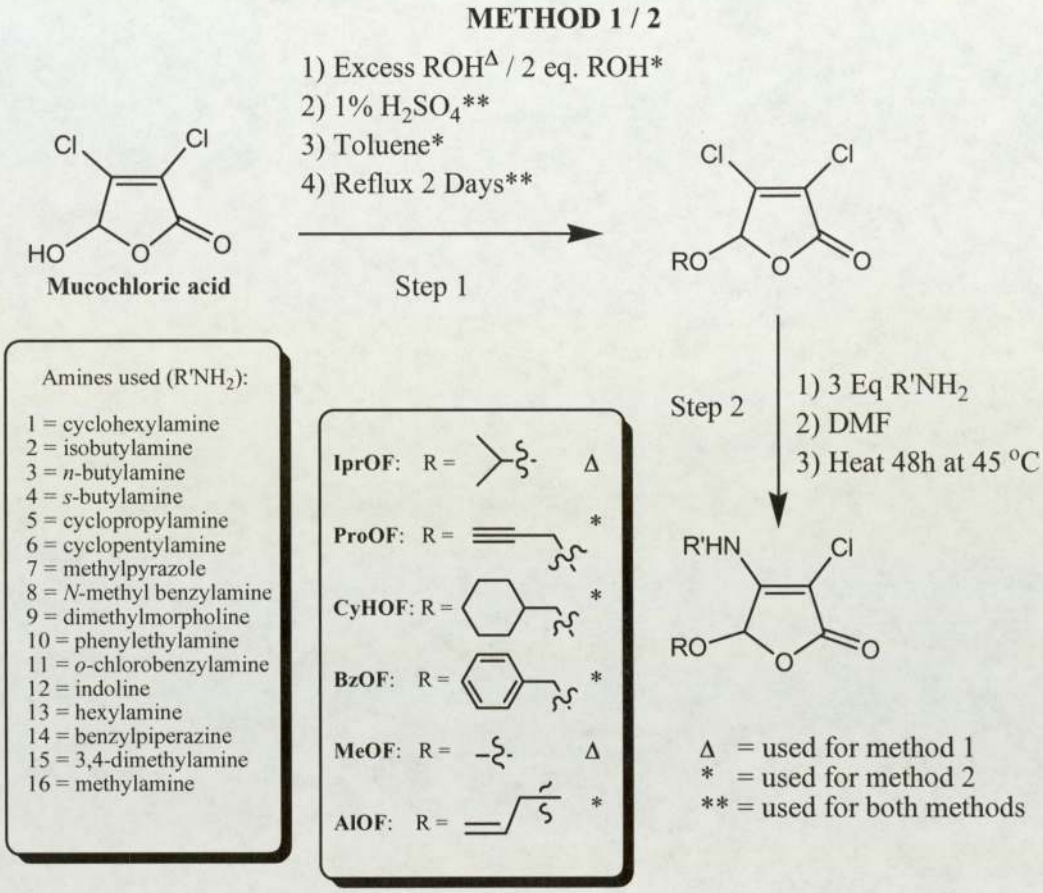


Figure 2.2.0.1. Properties required for the synthesised compounds.

2.2.1. The synthesis of 4-amino-5-alkoxy-furan-2(5H)-ones via 5-alkoxy-furan-2(5H)-ones.



Scheme 2.2.1. Synthetic route for the formation of 4-amino-5-alkoxy-furan-2(5H)-ones.

Scheme 2.2.1. shows the reaction pathway for the synthesis of 5-alkoxy-furan-2(5H)-ones using a variety of alcohols as well as the formation of the targeted 4-amino-5-alkoxy-furan-2(5H)-ones.

It is a major challenge to convert a compound with high affinity for a biological target (i.e. a lead molecule) to a successful drug on the market. A lead compound with desirable pharmacological activity may have unwanted characteristics that limit its bioavailability or it may have structural features which adversely have an effect on its metabolism and excretion from the body. It may also have undesirable side effects or be

toxic. Bioisosterism[106] and the use of homologues is a route which we can try to follow to combat these problems, by modify lead compounds to give safer and more clinically effective drugs.

Bioisosteres and homologues based around the 5-prop-2-ynyloxy group, which is part of the lead structure **Y** (figure 2.2.0.), were to be synthesised in order to form closely related structures. These groups are shown in figure 2.2.1.1. The methoxyl group present in the other lead structure **X**, (figure 2.2.0.) was also used.

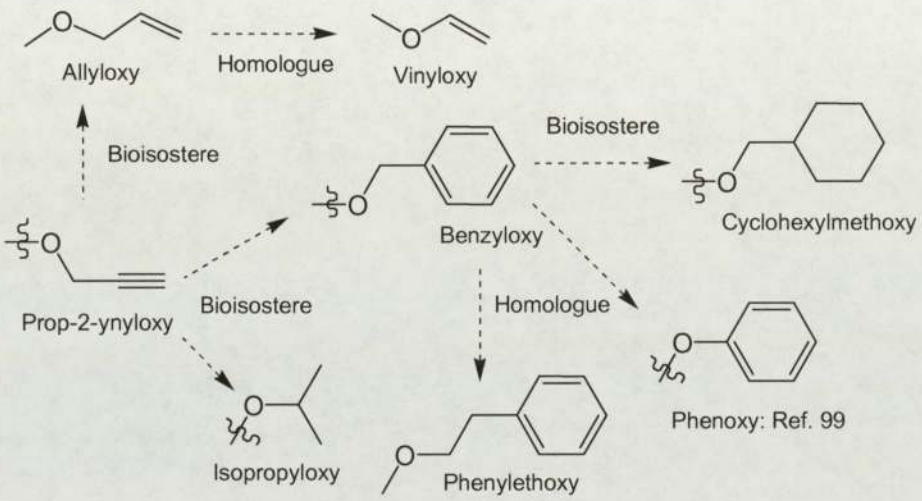
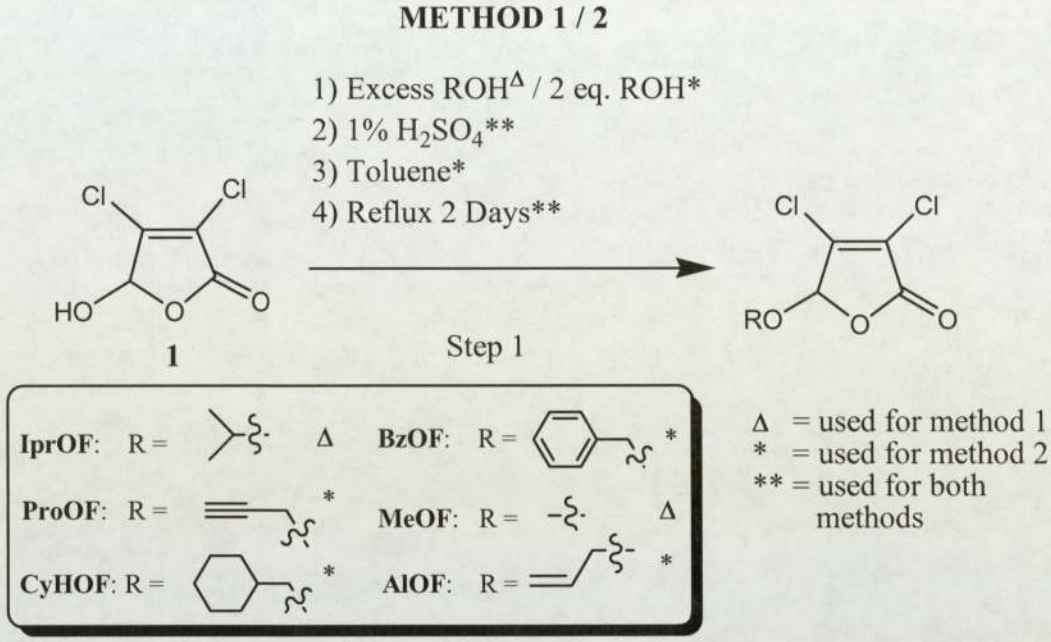


Figure 2.2.1.1. Bioisosteres and homologues based around the prop-2-ynyloxyl group.

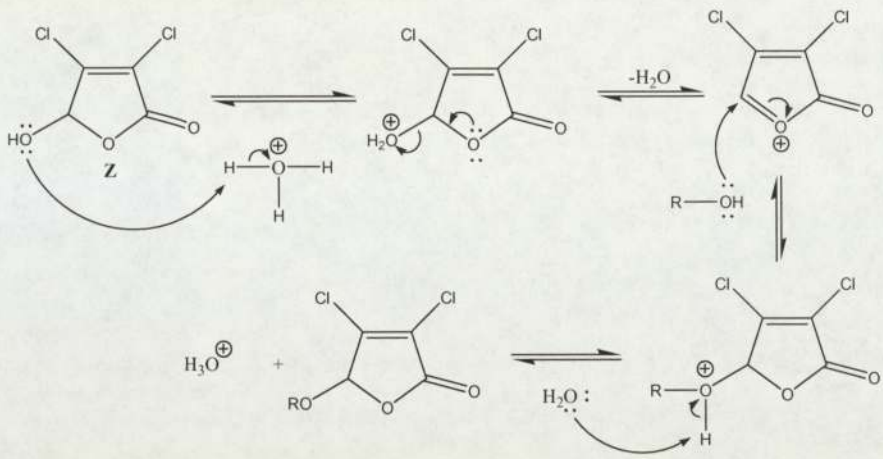
A phenoxyl group (a homologue of the benzyloxyl group) was to be used, but it has been reported by Mowry that when reacting mucochloric acid with phenol the expected 5-aryloxy furanone did not form, but in fact the 3-chloro group is displaced yielding a 3-phenoxy-5-hydroxy-furanone[99]. This group was declined at this point. Other alkoxyl groups considered included were ethoxyl, butoxyl and *n*-propoxyl, but have been found to be inactive. Vinyloxyl, a bioisostere of the prop-2-ynyl group was found to polymerise, while phenylethoxyl, a homologue of the benzyloxyl group, was more difficult to synthesise and was formed in low yield.

2.2.2. Synthesis of building blocks: Synthesis of 3,4-dichloro-5-alkoxy-furan-2(5H)-ones.



Scheme 2.2.2. Synthetic scheme for the formation of 5-alkoxy-furanones.

Two methods were used for the synthesis of 5-alkoxy-furan-2(5H)-ones from mucochloric acid (scheme 2.2.2.). Method 1 used the appropriate alcohol (methanol and isopropanol) as the solvent, in excessive amounts. In the second method, 2 equivalents of the appropriate alcohol (remaining alcohols) were used. In this instance toluene was used as the solvent. In both cases 1% H_2SO_4 was used to catalyse the reaction. It was found that refluxing the reaction for 2 days optimised the yield of the product.



Scheme 2.2.2.1. Mechanistic pathway for the synthesis of 5-alkoxy-furan-2(5H)-ones.

The proposed mechanism for this step, the formation of a pseudoester, can be seen in scheme 2.2.2.1. and can be classed as a hemiacetal[107] like reaction. Compound **Z** acts like a hydroxyl ether called a hemiacetal. In the presence of an acid, protonation of the hydroxyl group, followed by an E1-like loss of water leads to an oxonium ion, $R_2C{=}O^+$-R. This ion reacts with the alcohol to yield the acetal. Due to this reaction being reversible, the conditions set up for the reaction are important. Heating under reflux will favour the production of the acetal, as too will the removal of water, by the use of a Dean and Stark apparatus.

The building blocks **IprOF** and **BzOF** (figure 2.2.2.2.) which are bioiosteres of **ProOF** were produced in the best yields (73% and 77% respectively), while **MeOF**, one of the lead structure building blocks, was also synthesised in high yield. **CyHOF**, a bioisostere of **BzOF**, was formed in good yield. Another bioisostere of **ProOF** (which itself was produced in the lowest yield, 56%), **AlOF**, was synthesised in 60% yield. All compounds were fully characterised using, IR, MS and NMR spectroscopy to confirm that each of these compounds had been made. The purity of the compounds was established by distillation.

All of these 5-alkoxy-furan-2(5H)-one templates were formed as oils (no melting point could be obtained) and were to be reacted with a range of amines, the majority of which were liquids, in the formation of crystalline compounds. This leads to the ideal situation

in terms of purification, as excess starting materials should be able to be removed easily from the required product during recrystallisation.

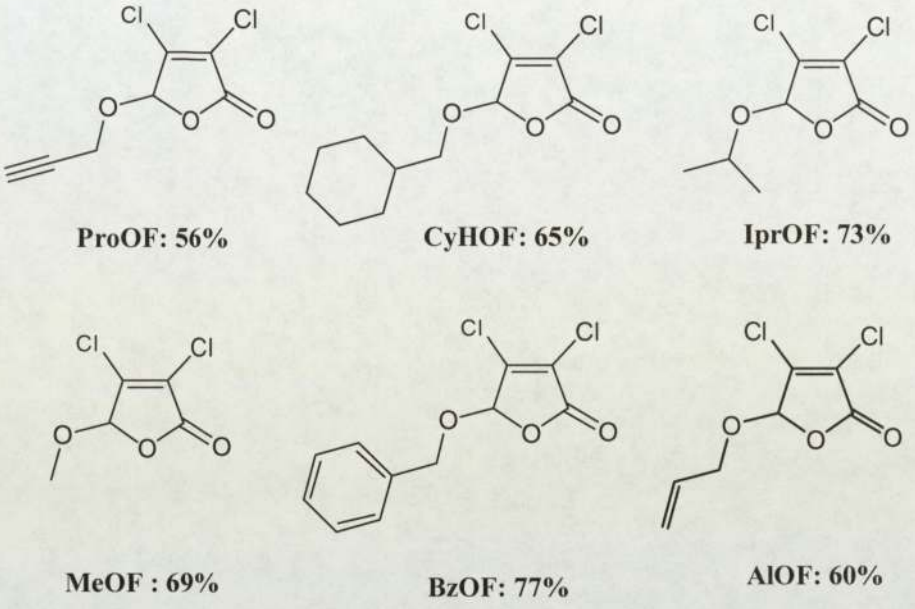


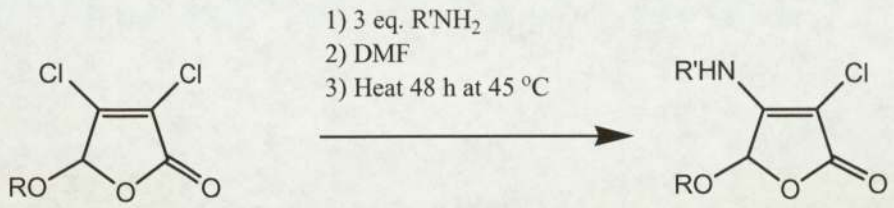
Figure 2.2.2.2. Synthesised 3,4-dichloro-5-alkoxyfuran-2(5H)-ones.

2.2.3. Synthesis of 4-amino-5-alkoxy-furan-2(5H)-ones.

A selection of sixteen diverse amines (scheme 2.2.3.) were chosen to undergo nucleophilic attack at the 4 position of 5-alkoxy-furanone ring system in a Michael-type reaction. These included aryl and cyclic amines and amines with simple alkyl- and cyclic alkyl side chains. Homologues of certain amines were used, i.e. cyclo propyl, pentyl and hexylamines to try and mimic the benzyl group present in lead structure **Y** (figure 2.2.0.), while isomers of butylamine were used to try and mimic the isobutyl side chain present in asperlicin.

These amines were reacted with the desired 5-alkoxy-furan-2-one template which was dissolved in DMF and heated at 45 °C for 48 hours. Scheme 2.2.3. shows an overview of the compounds formed. One other amine was used with the **ProOF** template, benzylamine, in order to try and re-synthesise one of the lead structures (**Y**, figure 2.2.1.). Previously this compound had been formed using a combinatorial approach.

Here it was formed in low yield as a mixture. It was decided that due to its poor formation and purity and the fact that the other lead structure had been formed (**X**, figure 2.2.0.), this lead structure would be disregarded at this point as a compound with optimum properties was required.



Section 2.2.2. Figure 2.2.3.3.

Amines: NHR' =

A = Isobutylamine	I = 3-Methylpyrazole
B = *n*-Butylamine	J = 2-Phenylethylamine
C = *sec*-Butylamine	K = *o*-Chlorobenzylamine
D = Methylamine	L = *N*-Methylbenzylamine
E = Hexylamine	M = 3,4-Dimethylaniline
F = Cyclopropylamine	N = Benzyl piperazine
G = Cyclopentylamine	O = 2,6-Dimethyl morpholine
H = Cyclohexylamine	P = Indoline

Scheme 2.2.3. Reaction scheme for the synthesis of 4-amino-5-alkoxy-furan-2-ones.

Each of these synthesised furan-2-ones were analysed in terms of their crystallinity and yield, so that compounds could be selected in order to find compounds possessing optimum properties. Table 2.2.3.1. shows these characteristic properties. Compounds were classed as being high yield (red boxes) if they were in excess of 65% and medium yield (green boxes) if their yield was between 50-64%. From the table below, it can be observed that all compounds that were synthesised in high yield were also formed as crystals. Another observation was that all of these afore-mentioned compounds were formed from either the 5-isopropyloxy- or 5-benzyloxy-furan-2-one template. Only 4 compounds were formed in yields lower than 50%, two of these were derived from the **PrOF** template, suggesting this to be one of the less reactive templates. In conclusion these high yield compounds possess two of the three optimum properties required as discussed in section 2.2. (figure 2.2.0.1.), with only biological activity left to measure.

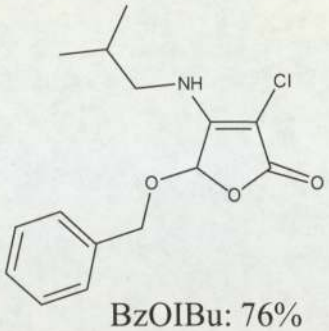
	Alkoxy-furanones					
Amine	**ProOF**	**CyHOF**	**IprOF**	**MeOF**	**BzOF**	**AlOF**
A	✓	✓*	✓*	✓*	✓*	✓
B				✓	✓*	✓
C				✓	✓*	✓
D		✓*				
E						
F			✓*		✓*	
G			✓*		✓*	
H			✓*		✓	
I				✓*	✓*	
J			✓			
K			✓*			
L	✓					
M			✓*			
N	✓*					
O	✓					
P			✓*			

High Yield (over 65%) = ■ Crystalline = * Synthesised and fully characterised = ✓

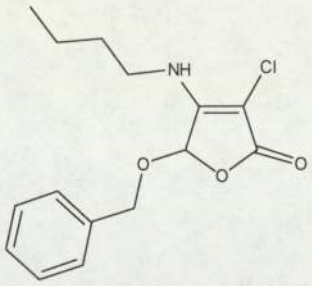
Medium Yield (50-64%) = ■ ■ = Lead Structure (yield < 50%)

Table 2.2.3.1. Characteristic overview for the newly synthesised 4-amino-5-alkoxy-furanones.

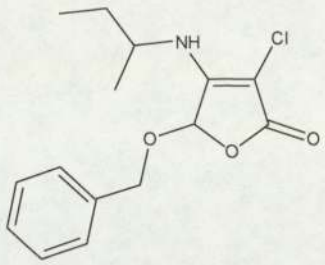
It would therefore be of interest to biologically test both the high yield crystalline compounds discussed as well as medium yield and crystalline compounds, i.e. the red and green shaded compounds in figure 2.2.3.1., which cover the majority of amines and all of the 5-alkoxy-furanones used. This will hopefully lead to a new lead structure. The blue shaded box represents **MeOHx** (formed in less that 50% yield), which was chosen for testing as it was the lead compound.



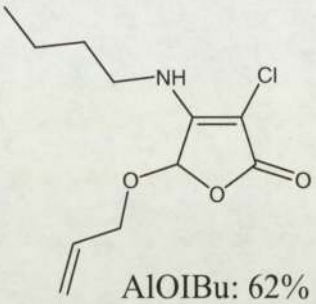
BzOIBu: 76%



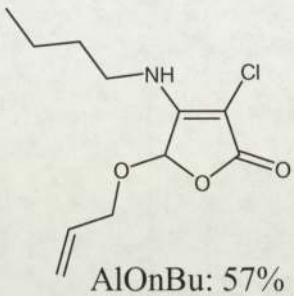
BzOnBu: 68%



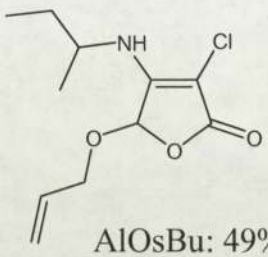
BzOsBu: 64%



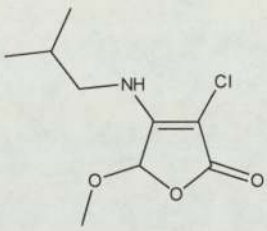
AlOIBu: 62%



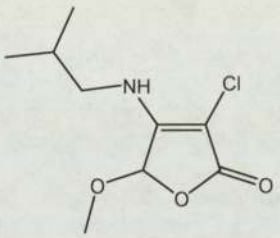
AlOnBu: 57%



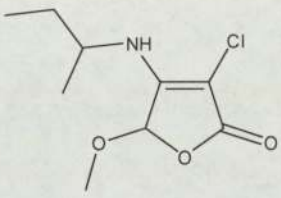
AlOsBu: 49%



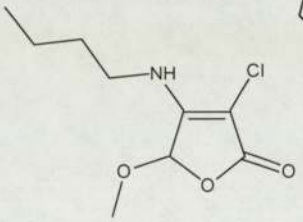
MeOIBu: 61%



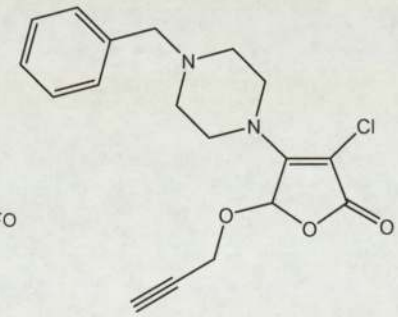
MeOnBu: 56%



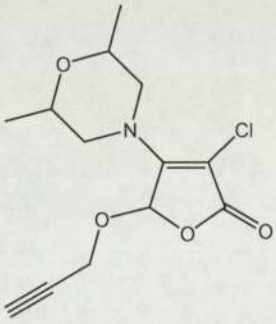
MeOsBu: 53%



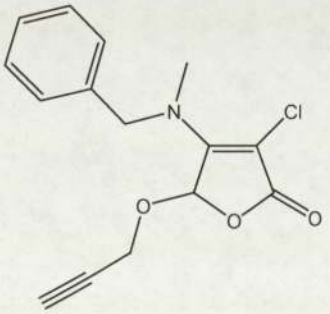
PrOIBu: 52%



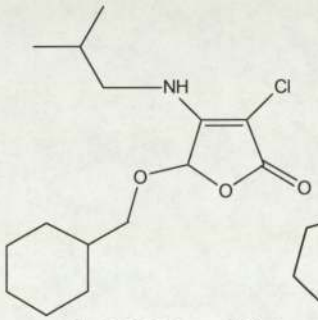
PrOBzpip: 50%



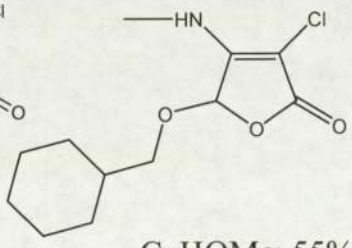
PrODMM: 47%



PrOMNBz: 49%



CyHOIBu: 59%



CyHOMe: 55%

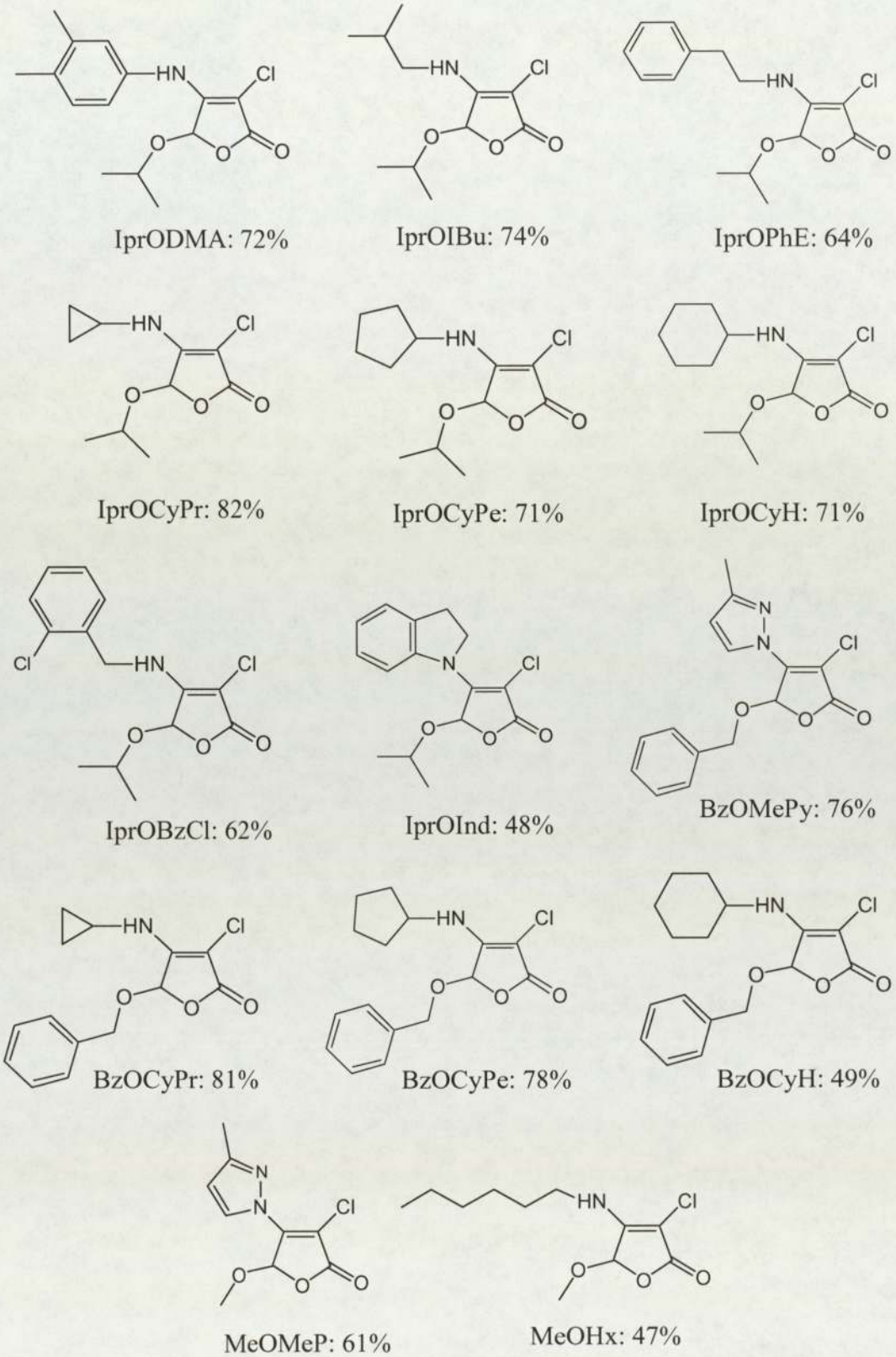
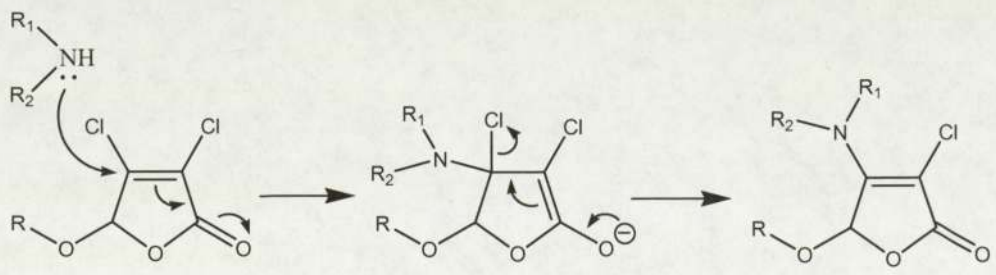


Figure 2.2.3.2. 4-amino-5-alkoxy-furan-2-ones synthesised and their yields.

A range of six 5-alkoxy-furanones and sixteen amines were used during the synthesis of twenty-eight 4-amino-5-alkoxy-furanones. Figure 2.2.3.2. shows the range of furanones synthesised and the yield they were formed in generally good to high yield. **IprOCyPr** and **BzOCyPr** containing the cyclopropylamino unit were formed in best yields, 82% and 81% respectively, while homologues of these compounds such as **IprOCyPe** (71%) and **BzOCyPe** (78%) were formed in slightly lower yields. In general compounds containing aromatic amines were formed in lower yields, with the exception of **IprODMA** (72%), while compounds using the aromatic template **BzOF** were formed in high yield. Compounds containing isobutylamine, e.g. **BzOIbu** (76%) and **IprOIbu** (74%) were formed in the highest yield compared with other compounds formed using the alternative butylamine isomers. A crystal structure was obtained for **BzOIbu** using X-ray crystallography (see experimental chapter 6.2 for structure and corresponding data), to confirm the general structure of the 4-amino-5-alkoxy-furanone template synthesised here.

Twenty-four of these compounds (1 being the lead compound) were selected based on crystallinity and yield and put forward for biological testing using the assay described in chapter 1b (nociceptic tail flick assay: chapter 6.6). **MeOHx**, one of the twenty-four compounds, was synthesised in poorer yield (47%) as a viscous oil. Despite the low yield, this compound was included in the assay as it was the previous lead structure and was required as a comparison to other potential CCK antagonists. These compounds are highlighted in table 2.2.3.1.

2.2.4. Mechanism of IPSO – substitution: Nucleophilic attack at the 4-position of 5-alkoxy-furan-2(5H)-ones.



Scheme 2.2.4. Mechanistic overview for the nucleophilic attack on the 4-position.

On the 5-alkoxy-furan-2(5H)-one, there are two carbon centres (C_3 and C_4), with adjoining chlorine atoms, which are the most susceptible to nucleophilic attack. Although these two positions look similar, it is the C_4 centre which is more prone towards attack. This is due to the presence of a Michael system[108] (scheme 2.2.4.). Fariña *et al*[109]. confirmed that this was the case. They found that the carbon atom at the 4-position of furanones undergoes nucleophilic attack (rather than at the 3-position) by nitrogen nucleophiles, as it acts as a Michael acceptor.

This Michael-type reaction takes place because of the presence of the electron withdrawing carbonyl group present on the furan-2(5H)-one and the alkene bond. Usually with this type of reaction conjugated addition[110] is seen, but it is thought that because of the stability of the conjugated system of the furan-2(5H)-one ring, the lactone and not the enol tautomer is preformed

2.3. Pharmacology of the 4-amino-5-alkoxy-furanones.

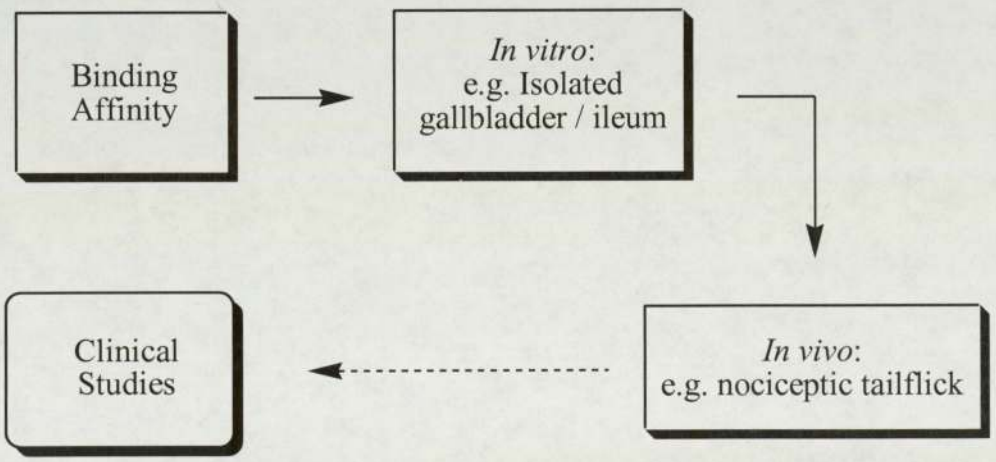


Figure 2.3.0. Classical pharmacological approach for the evaluation of potential drugs.

The classical approach during the pre-clinical phase for the pharmacological evaluation of a compound, usually involves receptor binding, *in vitro* and *in vivo* studies (figure 2.3.0.). Receptor binding affinity[111] can be measured using radioactive ligands, which are designed to bind to a specific receptor target. One major drawback with the use of radioactive ligands is their cost, which can be upwards of £1000. *In vitro* studies

involve the isolation of an organ, such as a gallbladder, in order to see if a biological response is detected via the targeted receptor, when used in conjunction with selected compounds. This method can lead to errors in experimental data: for example the isolated organ may be damaged, or if used for numerous samples, it may become less sensitive. *In vivo* studies involve compounds being directly tested on animals and compared with standard drugs. Although some people disapprove of *in vivo* assays, they can be very valuable tools for producing reliable data about potential drugs. If a compound at this stage shows the required activity, it will be put forward for clinical studies. One obvious disadvantage in using this classical pharmacology route, is the time required to perform all of these individual tasks.

Here a new alternative route was used to identify selected CCK active agents using the optimised nociceptic tailflick assay developed in chapter 1b, which bypasses the CCK binding assay process (figure 2.3.0.1.), saving both time and money. This assay

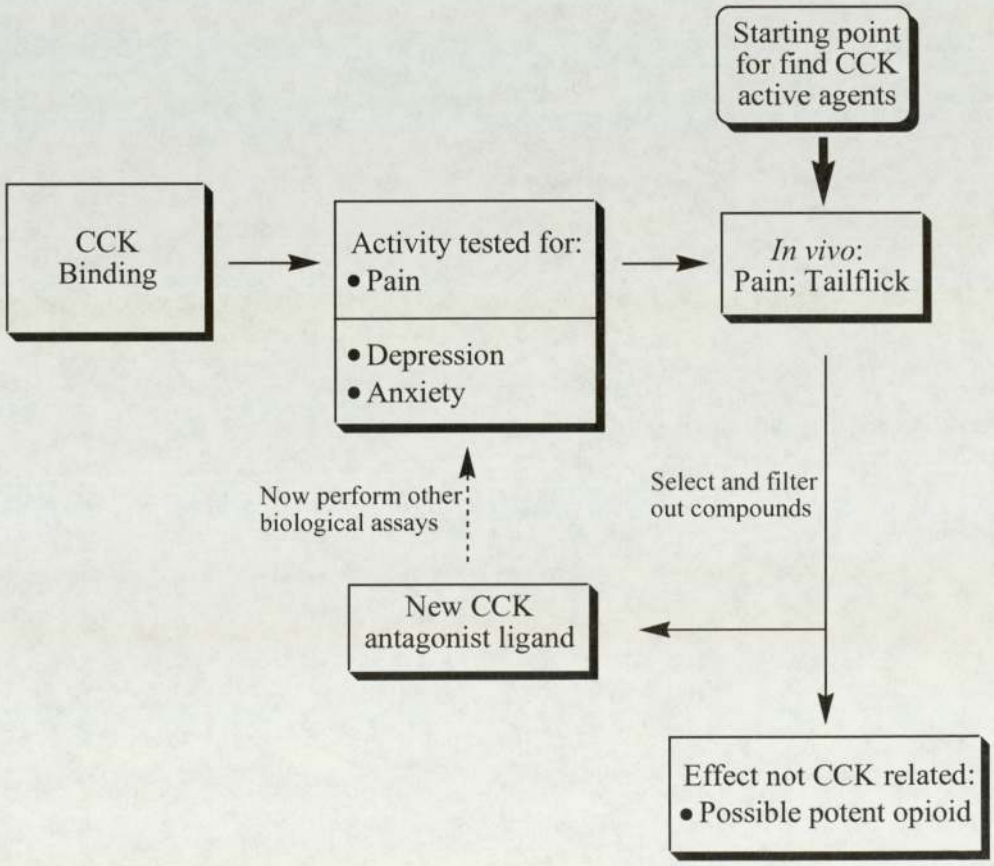


Figure 2.3.0.1. New optimised pharmacological route used to select CCK active agents.

identifies CCK antagonists due to there being a correlation between *in vitro* receptor binding data and the *in vivo* studies performed here using CCK active pyrazolone standard compounds. Selected compounds that have been selected from this pain assay that possess CCK antagonist activity, can be tested for other biological activities such as depression and anxiety. This route again allows for financial saving as not all compounds have to be tested. This assay also has a major advantage over the classical method, in that any compounds showing CCK antagonist activity will automatically have good bioavailability, lipophilicity, solubility and suitable pharmacokinetic properties. Any compounds that are selected which show an effect that is not CCK related, i.e. false positive compounds, could potentially be potent opiates, as only a low dose of 0.5 mg/kg is to be used. These compounds could at this or at a later stage be reassessed.

As discussed in section 2.2. a range of high yield and crystalline 4-amino-5-alkoxy-furanones as highlighted (red and green) in table 2.2.3.1. are to be biologically tested using the pain assay developed in chapter 1b. The idea behind this assay is to compare the results from these twenty-four furanone compounds against the standard pyrazolones synthesized and biologically evaluated in chapter 1b. Sample compounds with a similar maximum possible effect (MPE) to the standard compounds will be selected and tested further in other biological assays. These twenty-four compounds have been synthesized using all six of the 5-alkoxy-furanone templates and the majority of the sixteen amines employed.

As a starting point to filter out these CCK active agents, 0.5 mg/kg of each sample and standard compound were used in conjunction with 20 mg/kg tramadol. Measurements were made after a 30 minute period, as this was considered a good duration to evaluate the pharmacokinetics of these compounds. Much longer or shorter times may result in undesired effects, such as premature excretion or prolonged storage within the body. These experiments were conducted in collaboration with Khon Kaen University.

The **BzOF** series in general gave the best data (figure 2.3.0.2.), in particular **BzOIbu** (26.7%) and **BzOCyPe** (25.5%), which had similar maximum possible effects (MPE) compared to the standards **UR-MeO(m)**; 30.0% and **AM-IND(2)**; 28.2%. Tramadol alone gave an MPE of 5.7%, indicating good tramadol potentiation using some of the **BzOF** series. The homologue **BzOCyPr**, of **BzOCyPe**, had an MPE of 23.5%, while **BzOMePy**, a bioisostere of **BzOCyPe**, had an MPE of 24.2%. There are no other

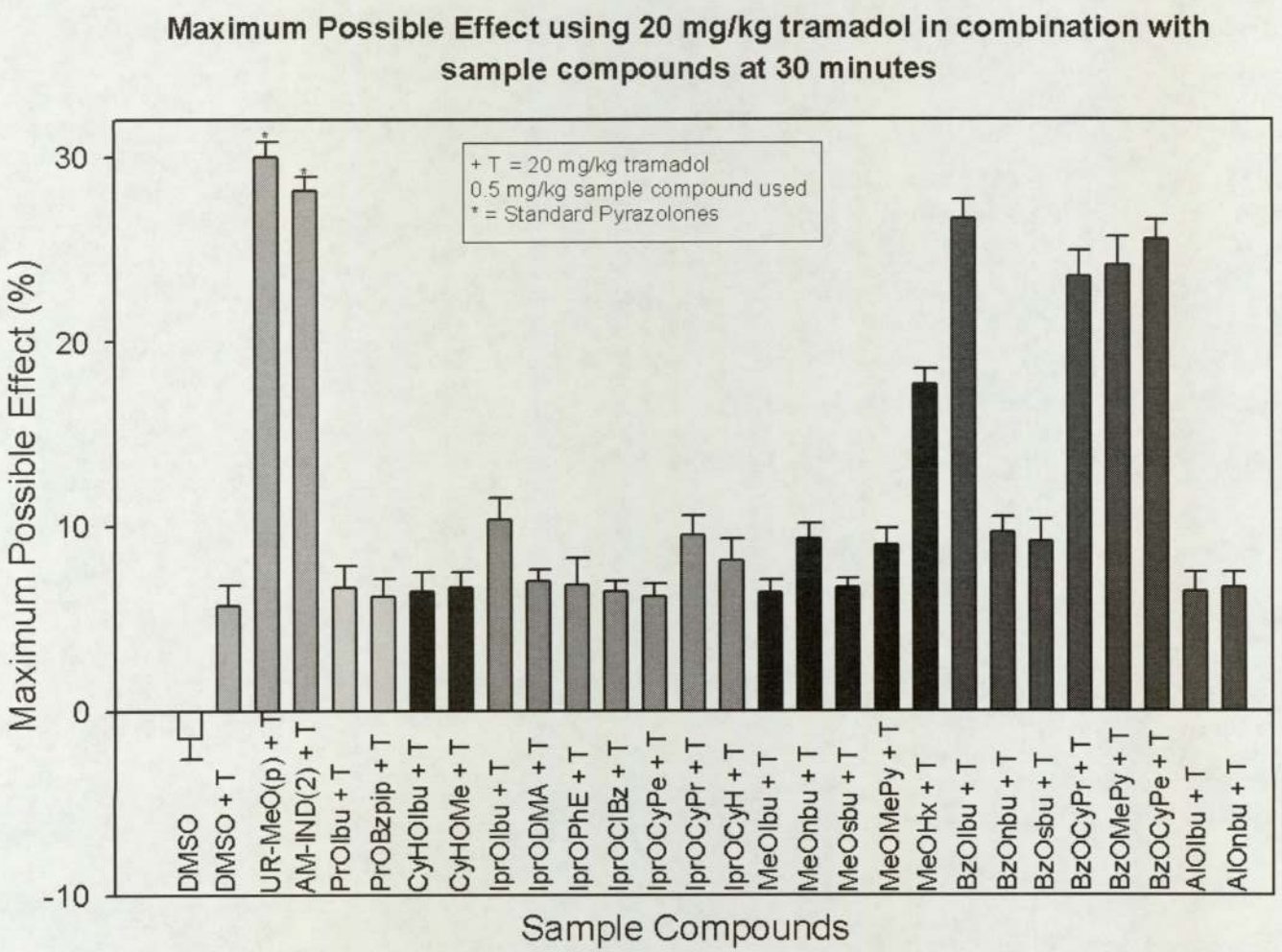


Figure 2.3.0.2. MPE using tramadol in combination with sample compounds at 30 minutes

compounds with a significant MPE, with the exception of **MeOHx** (17.7%), the lead compound and **IprOIbu** (10.3%), which had low to medium values, suggesting some CCK activity. This finding suggests that the **BzOF** series potentially has similar or better CCK antagonist properties compared to the lead structure **MeOHx**.

The best compounds were filtered out at this stage, these being **BzOIbu**, **BzOCyPe**, **BzOCyPr** and **BzOMePy** (figure 2.3.0.3.). These compounds, which were all formed in high yield as white or off-white crystalline samples, were to be biologically tested in a second round using the same assay, but with various tramadol concentrations over a range of different times. This assay was used with the aim of finding the best tramadol potentiation, the optimum time duration (pharmacokinetics) and also to select further sample compounds with the intent of finding a potential new lead structure. Although **MeOHx** (the original lead compound) produced a moderate MPE approaching the standard pyrazolones and was formed in good yield, it was synthesized as a viscous oil, which is not one of the optimized requirements (figure 2.2.0.). This compound was disregarded at this stage.

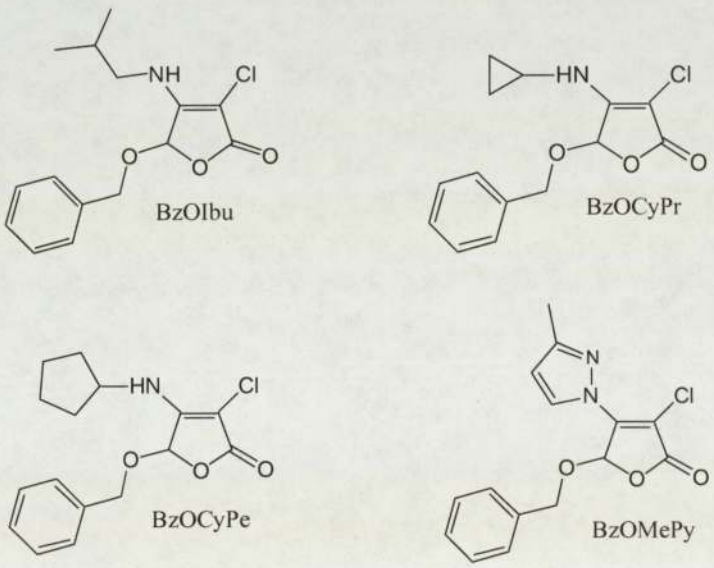


Figure 2.3.0.3. Selected compounds used in a nociception assay.

The three graphs in figure 2.3.0.4. show the results of the maximum possible effect (MPE) for the 4 compounds (dose of 0.5 mg/kg) from the **BzO** series used in combination with tramadol of various doses over a 30, 60 and 90 minute period. The

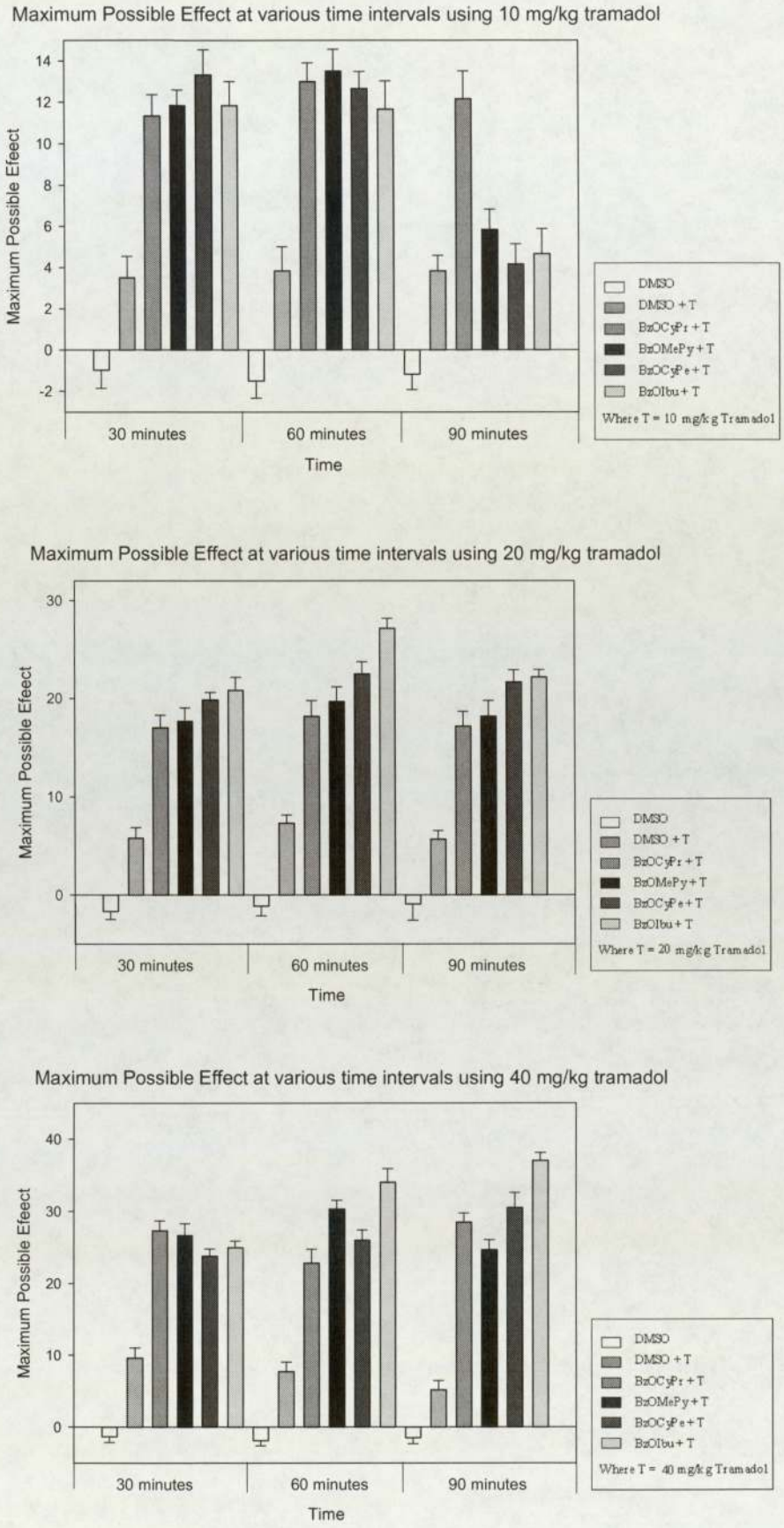


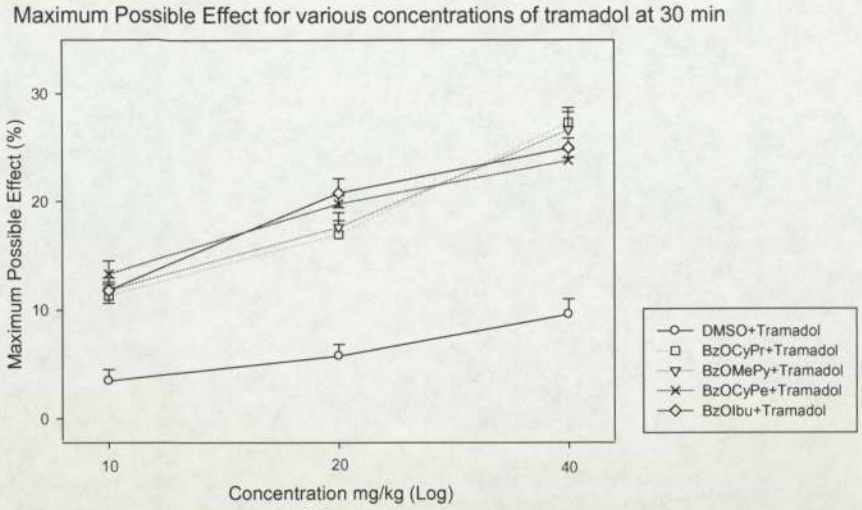
Figure 2.3.0.4. MPE of sample compounds using tramadol at various times.

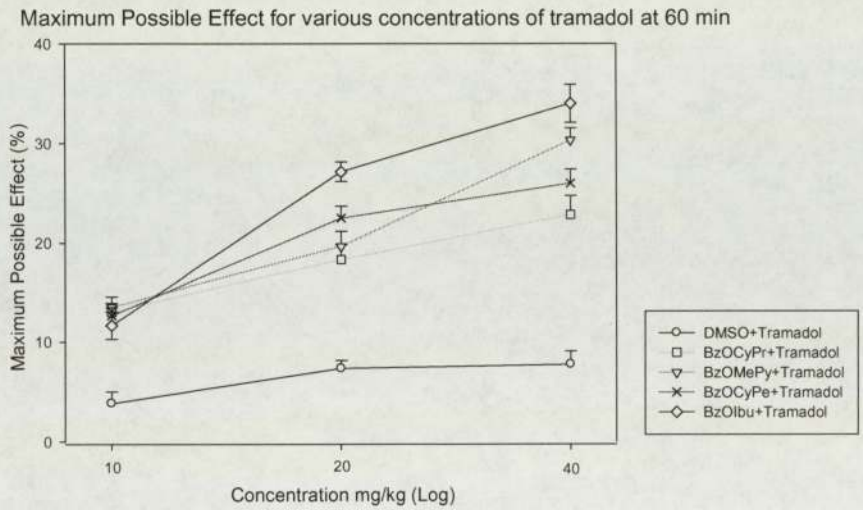
first graph uses tramadol at a dose of 10 mg/kg and shows compounds **BzOCyPe** (the highest MPE at 30 min), **BzOMePy** and **BzOIbu** with MPEs of approximately 11-14% after the 60 minutes duration. This effect decreases fairly rapidly after the 60 minutes period to around 5%, similar to that of tramadol alone. **BzOCyPr** has a slightly lower MPE at 30 minutes and after an initial increase at 60 minutes, only decreased slightly, from approximately 13% to 12% over the 90 minute period. In general this compound gives the better tramadol potentiation over the 90 minutes using 10 mg/kg tramadol.

The second graph (tramadol: 20 mg/kg) shows that this series of compounds, as expected, has an overall higher MPE compared to tramadol at 10 mg/kg. **BzOIbu** appears to the best of the series as the MPE is highest at all three times and after an initial increase in MPE (20.8% to 27.2%) from 30 to 60 minutes. This effect only decreased slightly after 90 minutes duration. A similar pattern is seen with the other 3 samples. With tramadol at 40 mg/kg the MPE using **BzOIbu** increases significantly over the 90 minutes from 25.0% (30 minutes) to 37.1% (90 minutes), but in particular after 60 minutes period. Although the MPEs for **BzOMePy**, **BzOCyPe** and **BzOCyPr** are generally higher than the corresponding data using 20 mg/kg tramadol, there is no significant increase in potentiation.

The three graphs in figure 2.3.0.5. show the results of the MPE for the same compounds over the three different time periods with varying doses of tramadol. The first thing that can be noticed is that again **BzOIbu** appears to give the best overall MPE. This compound stands out in the second (60 minutes) graph, as the MPE increases quite significantly from 10 mg/kg to 40 mg/kg (11.6% to 33.1%) in comparison to the other compounds. There is also no significant increase between the results shown in the 60 and 90 minutes graphs, indicating that a time period of 60 minutes appears to result in the best overall MPE. At 30 minutes there is no real significant increase in MPE for any of the test compounds at any of the three concentrations, when compared to tramadol alone. In general there is approximately a 10% increase in MPE from at any concentration for each compound (compared to tramadol) at this time.

From the results discussed previously regarding figures 2.3.0.4. and 2.3.0.5., **BzOIbu** was taken as the best compound tested in combination with the partial opioid agonist, tramadol. The data obtained for this compound has been interpreted to give an idea about the pharmacokinetics of **BzOIbu**, by analysing the effect this compound has on tramadol potentiation with respect to time. The Pharmacokinetics[112] of a drug, i.e. how





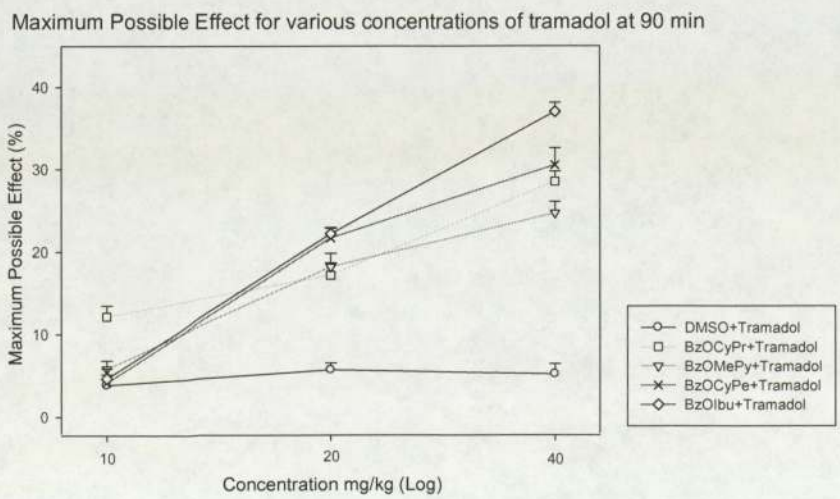
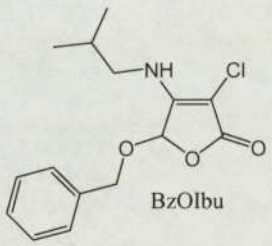


Figure 2.3.0.5. MPE for sample compounds using tramadol at various doses.

the drug is absorbed, metabolised, distributed and eliminated in the body, is an important property. If a compound is too hydrophilic, it may be eliminated by the body too quickly (by the kidneys) to take effect, or may not be able to pass the blood-brain barrier. If it is too lipophilic, it will be poorly absorbed from the GI tract and be stored in the fatty tissues of the body for longer periods, which can lead to health problems. The assay here eliminates this potential problem as a highly lipophilic or highly hydrophilic compound will not cause any tramadol potentiation.

Figure 2.3.0.6. shows a final representation of the data collected for this compound in terms of MPE versus time using the three different doses of tramadol. From these results it can be seen that there is a significant increase in MPE between tramadol at 10 and 20 mg/kg, but only a slight increase between 20 and 40 mg/kg, suggesting that 20 mg/kg of tramadol in combination with 0.5 mg/kg **BzOIbu** is the best one overall, especially with regards to cost effectiveness. Once more the previous conclusions



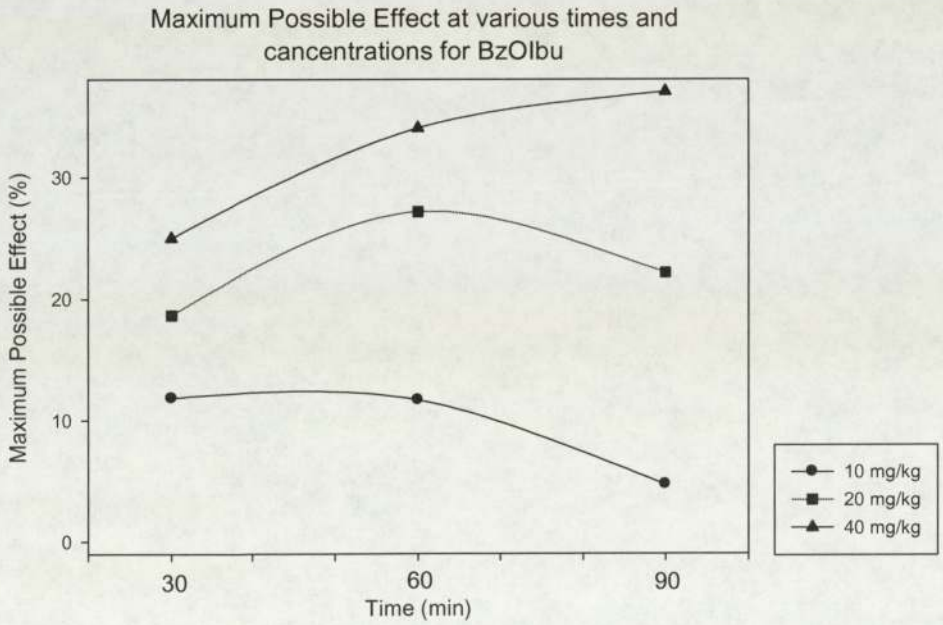


Figure 2.3.0.6 .The maximum possible effect for compound BzOIbu, at various times in combination with tramadol.

drawn from figures 2.3.0.4. and 2.3.0.5. have been confirmed, i.e. a 60 minute period appears to give the best results in terms of time efficiency and pharmacokinetics.
A nociceptic assay developed in chapter 1b was used here to select compounds with CCK antagonist properties. Compounds from the **BzOF** series have shown significant tramadol potentiation in this assay. These CCK active agents will now be used in other biological assays to show their usefulness as anxiolytic and antidepressant compounds.

2.3.1. Anxiolytic assay: X-maze.

When placed in an elevated plus-maze (X-maze) for the first time, a mouse's behaviour is largely based on its anxiety level. Normal mice that have not received any anxiolytic drugs will become moderately anxious in this new environment. Thus, they tend to prefer the closed arms over the less secure open arms posture[113]. Meanwhile, mice treated with anxiolytic drugs such as diazepam the standard compound used in this

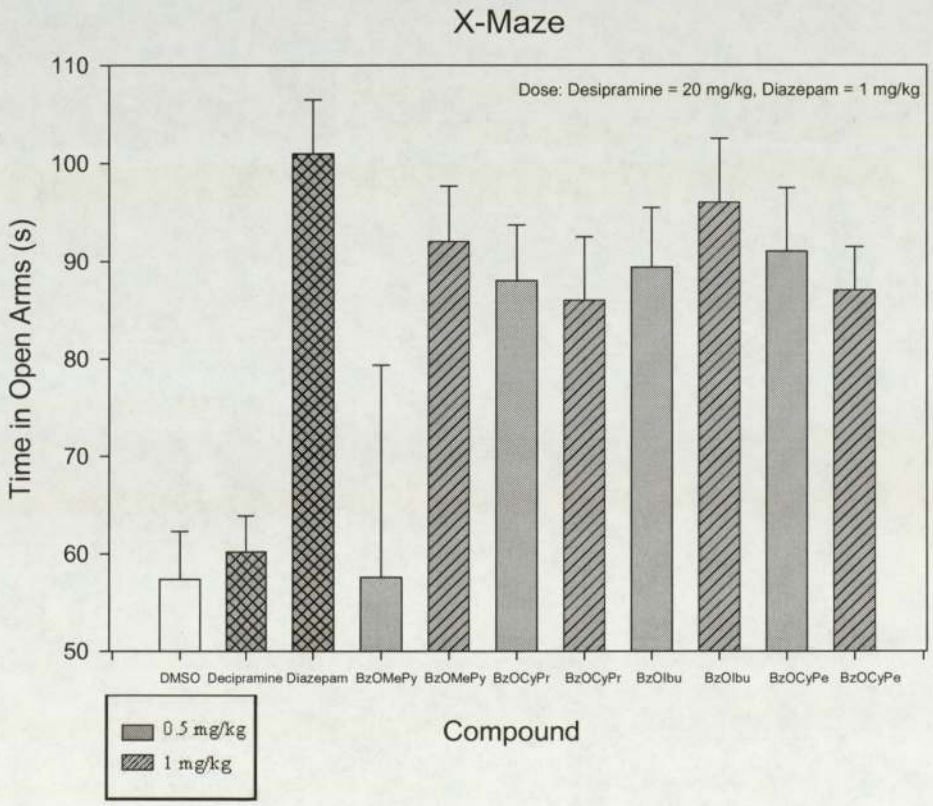


Figure 2.3.1. Results from the anxiolytic X-maze test.

assay, tend to be less anxious, so they spend more time on the open arms platform compared to normal mice[114], and they are generally less active[115]. This experiment was carried out using two different doses of the **BzOF** compounds (figure 2.3.1.), 0.5 and 1 mg/kg. 1 mg/kg Diazepam, an anxiolytic drug, was used as a positive control, while DMSO and desipramine (positive control for the antidepressant assay) served as negative controls.

The results (figure 2.3.1.) of the elevated plus maze test (X-maze)[116], shows that a greatly enhanced exploration of the open arm platforms was seen with all the **BzOF** series at both concentrations (with the exception of **BzOMePy** at 0.5 mg/kg). An increased number of total crossings within the maze was also observed with each of these sample compounds, indicating a lowering of anxiety in the mice. **BzOMePy** has a large bar associated with it, with standard deviation of 21.8 (compared to approximately 4-7 for other compounds), suggesting either very inconsistent data, or more likely an inputting data error. The results of **BzOIbu** (96 seconds in open arms) at 1 mg/kg are similar to those of the anxiolytic drug diazepam, indicating that this member of the **BzOF** series possess anxiolytic properties. At the lower dose of 0.5 mg/kg, **BzOCyPe**, provided the best results with an open arm platform exploration time of 91 seconds compared to 101 seconds for diazepam.

Evidence has shown that anxiolytic effects of cholecystokinin are mediated by CCK_B receptors. It has recently been reported that CCK_A[117] receptors have been found in the brain and so CCK_A antagonists have could have anxiolytic properties, but this is still to be confirmed. This finding suggests that the compounds synthesised and evaluated here could be mixed CCK antagonists, i.e. they could possess both CCK_A and CCK_B selectivity. This idea could be tested further at a later date using the **BzOF** series formed and used here in receptor binding assays, as receptor sub-type selectivity was not relevant to this work.

2.3.2. Antidepressant assay: Swimming test.

Antidepressant drugs have the effect of reducing the duration of immobility in the despair swim test (immobility time test)[118]; the subject tries to fight to survive and keeps

swimming for longer. Desipramine, a tricyclic antidepressant drug served as positive control. DMSO and diazepam were used as negative controls.

The results from figure 2.3.2. in general show that all of the **BzOF** series possess varying degrees of antidepressant properties. Two compounds clearly stand out from the others, these being **BzOCyPr** and **BzOCyPe** (at 1 mg/kg), which both caused a lower immobility time (81 and 80 seconds respectively) compared to the standard desipramine (86 seconds). These results suggest that compounds **BzOCyPe** and **BzOCyPr** are better antidepressants than the standard positive control used here. At the lower dose of 0.5 mg/kg, **BzOMePy**, has a slightly lower immobility time of 83 seconds, again suggesting significant antidepressant properties. These results suggest that these potential antidepressant compounds are selective towards the CCK_B receptor sub-type.

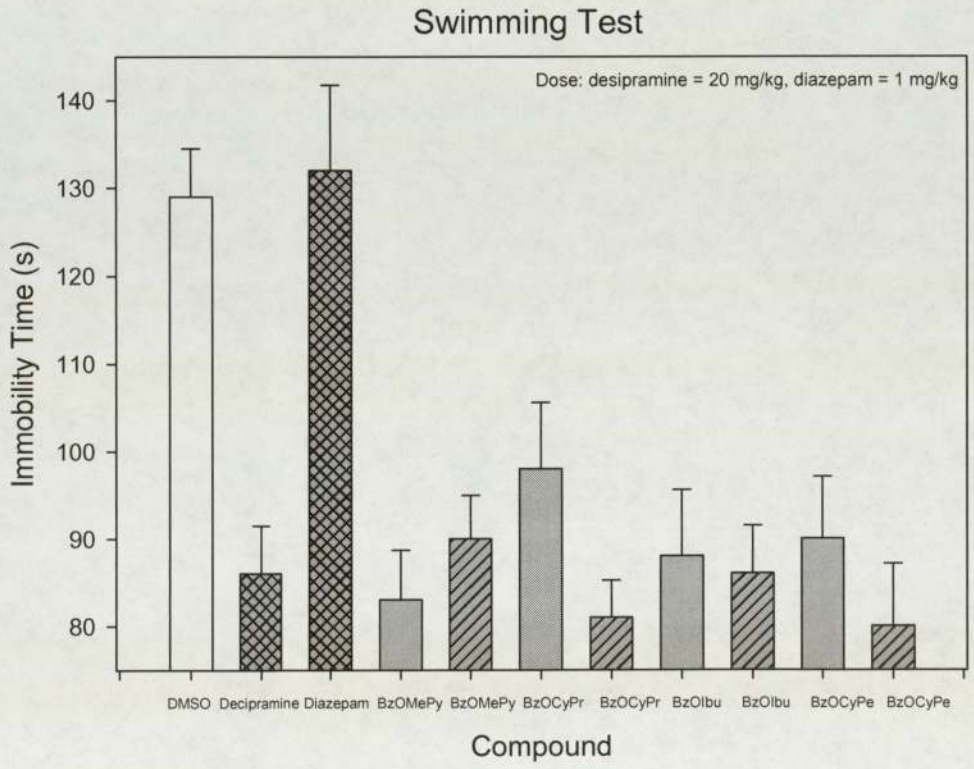


Figure 2.3.2. Results from the antidepression swimming test.

2.4. Conclusion.

A diverse range of twenty-eight 4-amino-5-alkoxy-furan-2-ones have been synthesised via selected 5-alkoxy-furan-2-one building blocks in a two step synthesis using sixteen amines and six alcohols. 4-Amino-5-alkoxy-furanones were developed around previously synthesised CCK active lead structures, in order to find an optimum CCK antagonist. Twenty-four of these compounds were selected with regards to yield and crystallinity and were biologically tested for tramadol potentiation in combination with tramadol using the nociceptic assay, which was developed in chapter 1b. Compounds from the **BzOF** series were found to have the optimum properties required and produced biological results similar to those of standard pyrazolones, suggesting these compounds possessed CCK antagonist properties. Selected compounds from this series were further tested in anxiolytic and antidepressant assays. These results yielded potential anxiolytic and antidepressant drugs, with similar or better biological properties to those of the positive control standard drugs used. Compound **BzOIbu** at 1 mg/kg and **BzOCyPe** at 0.5 mg/kg performed well in the anxiolytic X-maze with respective times of 96 and 91 seconds in the open arms stance. Compound **BzOMePy** at 0.5 mg/kg and **BzOCyPe** at 1 mg/kg yielded the best results in the antidepression swimming test with respective immobility times of 83 and 80 seconds. The results from these biological assays suggest that these compounds could have selectivity for both CCK_A and CCK_B receptor sub-types.

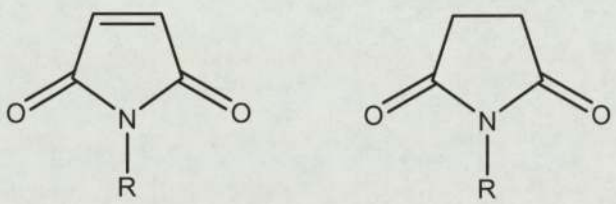
All of the 4-amino-5-alkoxy-furanones synthesised here contain a chiral centre at the 5-C-position and therefore will be formed as a pair of enantiomers. This property may cause problems for a potential marketable drug; such as one of the enantiomer may be toxic. Another possible problem may arise if these enantiomeric furanones are formed in a 1:1 ratio, one being an agonist and the other being an antagonist. If such a compound is used in a biological assay there will be no overall effect and therefore a potential CCK antagonist may be overlooked.

For these reasons bis-arylated compounds synthesised in chapter 5 have been developed so that no chiral centre is present and so that pharmacological problems associated with chirality cannot occur.

Chapter 3: Novel synthesis and preparation of pyrrole-2,5-diones.

3.1. Introduction.

Cyclic imides such as succinimides and maleimides (figure 3.1.) and their derivatives contain an imide ring and have been found to be biologically and pharmaceutically useful[119], i.e. as antibacterial[120], anticonvulsant[121] and antitumor drugs[122]. Maleimides, also known as pyrrole-2,5-diones, can be synthesised by a number of routes. The majority of these routes are based on reactions of the corresponding maleic anhydride with an amine[123]. Another alternative one-step method involves the action of ammonium acetate on the maleic anhydride in boiling acid[124].



Maleimide Succinimide

Figure 3.1. General structures for maleimides and succinimides.

This class of pyrroles contain a number of biologically active compounds including *N*-(carboxyalkyl)-maleimides, which are rapid and time-dependant inhibitors of prostaglandin endoperoxide synthase (PGHS)[125], anti-fungicidal[126] active chlorinated 1-arylamino-1H-pyrrole-2,5-diones and *N*-substituted imides, which show both antimicrobacterial[127], antidepressant[128], anxiolytic[129] and analgesic activity[130].

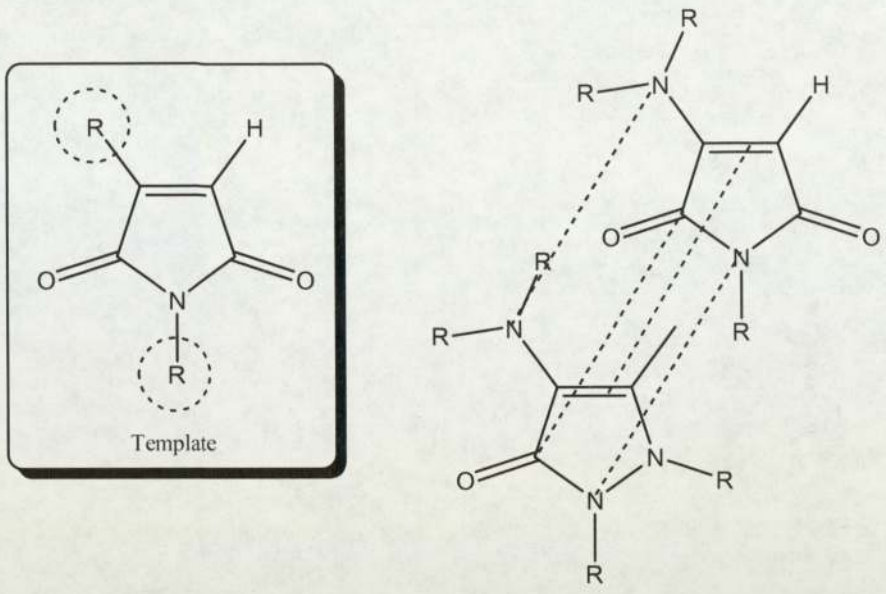
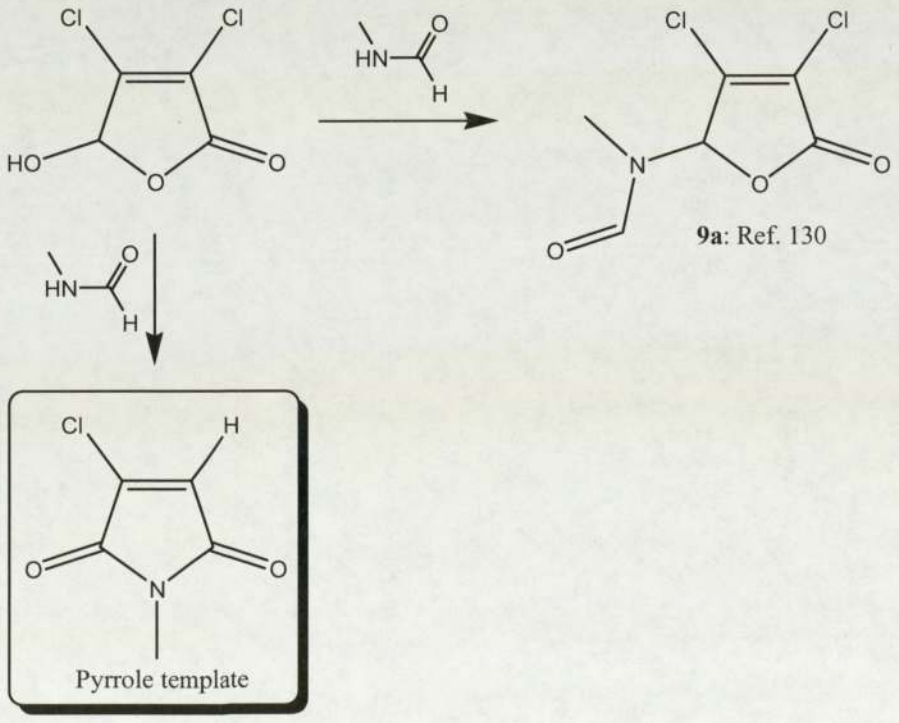


Figure 3.1.1. 3-Amino-pyrrol-2,5-dione template and its structural similarities to pyrazolones.

The pyrrol-2,5-dione template (figure 3.1.1.), which is to be synthesised here, has structural similarities to both the 4-amino-5-alkoxy-furan-2-ones (synthesised in chapter 2) and to other known CCK antagonists i.e. pyrazolones, which are known to possess antidepressant[131] and anxiolytic properties[92]. Pyrazolones and pyrrol-2,5-diones have a similar structural motif. Both have a 5 membered nitrogen containing ring system, a carbonyl group, a vinylic group and an amino side chain (figure 3.1.1.). One noticeable difference between these molecules is that the pyrazolone contains a methyl group, compared to the proton of the pyrrol-2,5-diones in the equivalent position. During the synthesis of these pyrazolones proton replacement for this methyl group was undertaken by using the corresponding aldehyde to ethyl acetoacetate (ethyl glyoxylate), but this was found to be too reactive and did not form the desired compound[82].

3.2. From amido-furanones to *N*-substituted-pyrrol-2,5-dione.

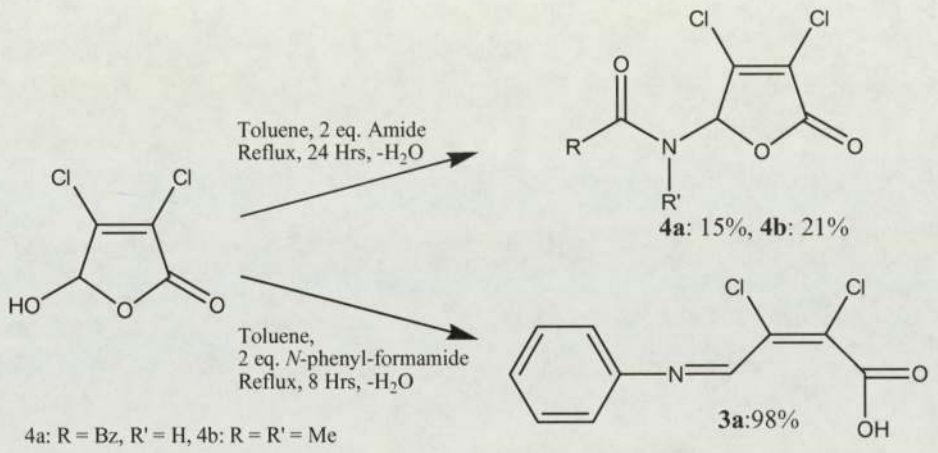
A pseudo-amide anti-cancer lead structure[132] (**9a**, scheme 3.2.), which was originally synthesised by Lattmann *et al.* in a very low yield, was taken as the starting point for this work. The idea was to re-synthesise this compound and explore why this low yield had occurred. This amido-furanone was synthesised here, using similar conditions[133] (mucochloric acid, 2 eq. amide and heated under reflux for 24 hrs in toluene with a catalytic amount of sulphuric acid). TLC analysis indicated that apart from the expected pseudo-amide compound, a by-product had been formed in low yield. After isolating and fully characterising this compound, it was deduced that this by-product, was a pyrrol-2,5-dione template (3-chloro-pyrrol-2,5-dione, scheme 3.2.), which had not been discovered or identified from previous syntheses. The reaction conditions for the synthesis of this pyrrole template were optimised by varying the equivalents of NMF used and the time. Other amides were used with mucohalogen acids in order to try and optimise this template further (section, 3.2.3.). After optimisation of this template, a further reaction involving amines and sodium alkoxides was carried out in order to synthesise a range of 3-amino- or 3-alkoxy-pyrrol-2,5-dione (section 3.2.3.).



Scheme 3.2. From a by-product to a new synthesis of pyrrol-2,5-diones.

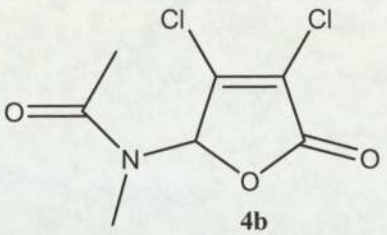
3.2.1. Synthesis of amido-furanones.

Various amides were added to mucochloric acid in toluene, containing a catalytic amount of H_2SO_4 and heated under reflux overnight, using a Dean and Stark trap. The resulting viscous liquid was subject to column chromatography. These compounds were fully characterised. **4a** and **4b** (scheme 3.2.1.), 5-amido-furanones were synthesised in 15% and 21% yield respectively. Compound **4a** did not react further to form the corresponding pyrrol-2,5-dione and **4b** could not form this pyrrole due to the nature of the amide (*N*-methyl-acetamide).



Scheme 3.2.1. *N*-(3,4-Dichloro-5-oxo-2,5-dihydro-furan-2-yl)-*N*-methyl-formamide.

Compound **3a** was formed in a very high yield as a green powder. The expected 5-amido-furanone did not formed but instead it was thought that an imine was formed and precipitated out as **3a**, before the ring could cyclise to form the furanone. This structure appears to have the qualities of a good building block, i.e. two chlorines, a carboxylic acid and an imine group. At this point it was decided not to investigate this template further. Although it does resemble the bis-arylated but-2-enoic acid investigated in chapter 5, it does not have the structural characteristics of the pyrazolones, to which the acids are compared.



4b

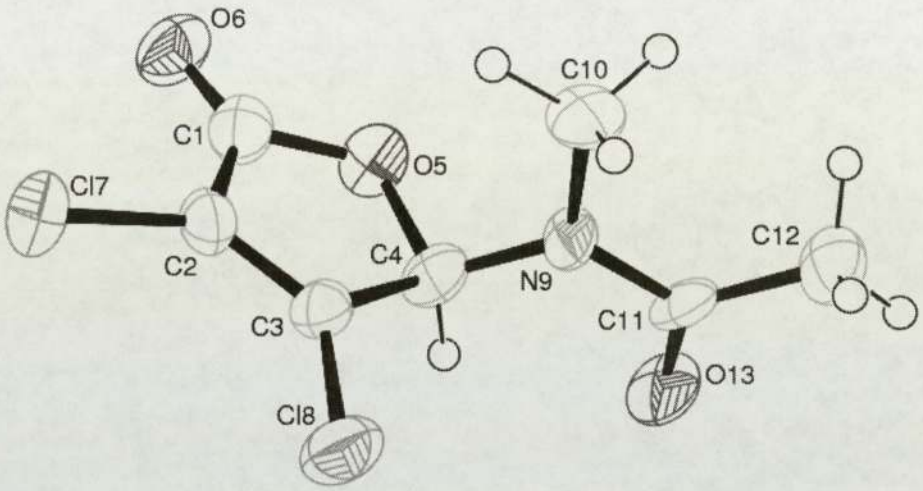


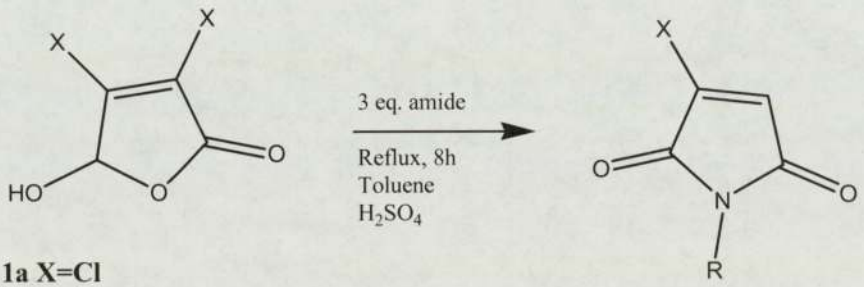
Figure 3.2.1.1. Crystal structure of *N*-(3,4-dichloro-5-oxo-2,5-dihydro-furan-2-yl)-*N*-methyl-acetamide.

X-ray crystallography was carried out using **4b** in order to confirm the structure of the amido-furanones (crystal data can be found in chapter 6.3. This compound was also fully characterised using ^{1}H and ^{13}C NMR, MS and IR spectroscopy. These spectra along side this crystal structure (figure 3.2.1.1.) confirmed that the structure of this compound was as expected *N*-(3,4-dichloro-5-oxo-2,5-dihydro-furan-2-yl)-*N*-methyl-acetamide.

3.2.2. Formation of *N*-alkylated halogenated pyrrole-2,5-diones.

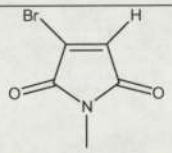
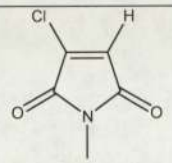
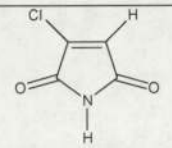
Mucohalogen acids[134], such as the mucochloric acid **1a** and mucobromic acid **1b** are commercially available and are synthesised from furfural on an industrial scale. Furfural is obtained by heating biomass with sulphuric acid. The findings here are particularly useful, as any chemical application of furfural present an important example of using a renewable resource from biomass.

Amides were reacted with mucohalogen acids to give various derivatives as shown in scheme 3.2.2.These reactions were carried out with the absence of a Dean and Stark trap for 8 hours and purified using column chromatography. *N*-Benzylformamide was also used as a reagent. Varying reaction conditions were used, but no pyrrol-2,5-dione was detected (TLC analysis).



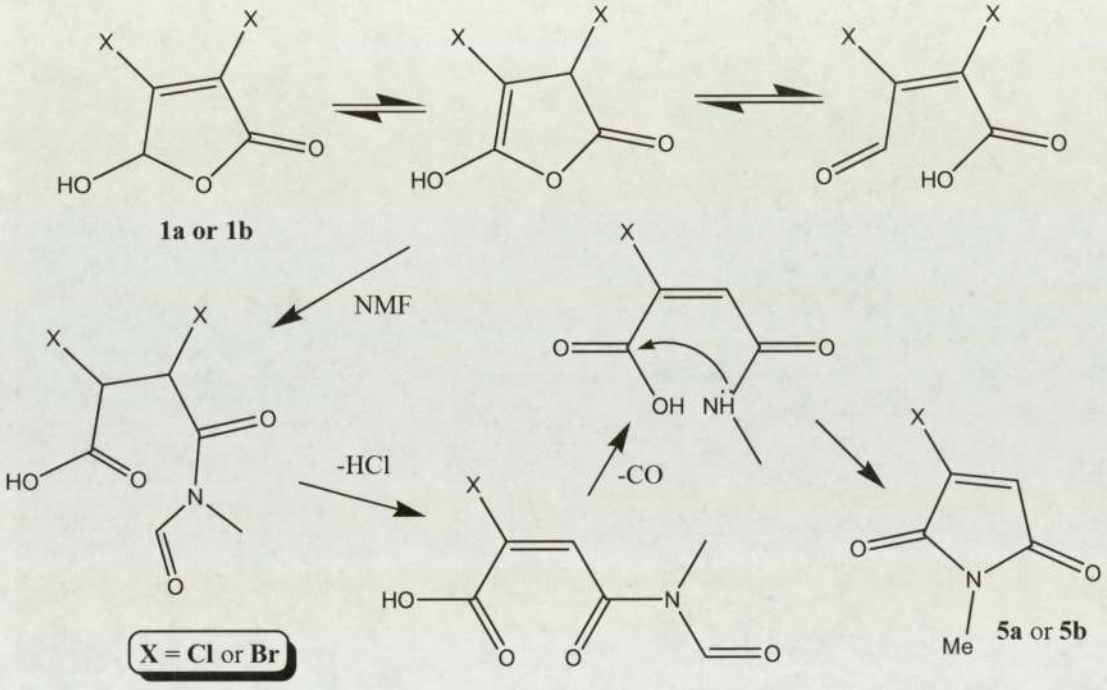
1a X=Cl
1b X=Br

5a-5c

Code	Reagent used	Structure	Purification by:	m.p. / °C	Yield (%)
5a	*N*-methyl-formamide		Column Chromatography	90-92	41
5b	*N*-methyl-formamide		Column Chromatography	88-90	22
5c	Formamide		Column Chromatography	113-115	11

Scheme 3.2.2. Synthetic route and results for 3-halo-1-alkyl-pyrrole-2,5-diones.

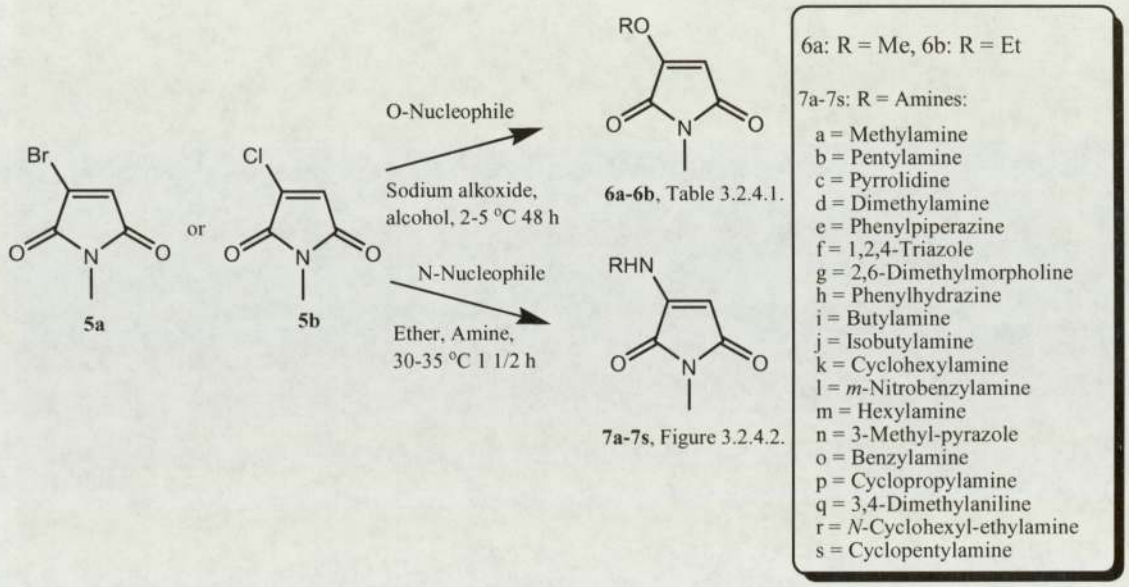
The proposed mechanism for the transformation of mucohalogen acids **1a** or **1b** into the 3-halo-1-methyl-pyrrole-2,5-dione **5a** and **5b**, is outlined in Scheme 3.2.2.1. The hydroxyl-form of acid **1a** or **1b** reacted to highly diverse dihalogenated 2(5H)-furanones[135], the aldehyde-form provided pyridazines, evaluated as anticancer agents[136]. Here, the enol-form, in which the ring opening is preformed, reacted with methyl formamide to form the acyclic intermediate. Elimination of HCl, loss of CO and cyclisation, provided the targets **5a** and **5b** in medium yields.



Scheme 3.2.2.1. Mechanism for the formation of 3-halo-1-methyl-pyrrole-2,5-dione.

3.2.3. Synthesis of 3-alkoxy- and 3-Amino-pyrrol-2,5-diones.

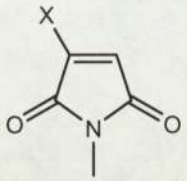
Scheme 3.2.3. shows the synthetic routes for the synthesis of both 3-alkoxy- and 3-amino-pyrrol-2,5-diones. The rational behind the synthetic routes shown below, was to begin with an oxygen nucleophile (alkoxide) as this is a softer nucleophile than the hard nucleophile nitrogen (amine). It was proposed that if series **6** (3-alkoxy-pyrrol-2,5-diones) formed, then the series **5** 3-halo-pyrrol-2,5-diones should readily react with amines to form 3-amino-pyrrol-2,5-diones (series **7**).



Scheme 3.2.3. Synthetic routes for the synthesis of 3-alkoxy- and 3-amino-pyrrol-2,5-diones.

Both **5a** and **5b** 3-halo-pyrrol-2,5-dione templates were used during the synthesis of the 3-alkoxy-pyrrol-2,5-diones, in order to observe which template was the better reagent. Template **5c** was disregarded at this point as a template due to its formation in low yield and the fact that both **5a** and **5b** were formed in better yields. It was predicted that the bromo-template should be more reactive and therefore form series **6** compounds in higher yields. The reaction was carried out using the corresponding alcohol and sodium alkoxide and was refrigerated for 48 hours. Table 3.2.3.1. shows the results from the

synthesis of these 3-alkoxy-pyrrole compounds. Structures **6a** and **6b** were formed in low-medium yields using both 3-halo-pyrrole templates. The bromo-template, as predicted, gave the higher yield in both cases. **6a** was formed in best yield (31%) as a crystalline solid, compared to **6b** which was a semisolid.



Template: **5a** or **5b**

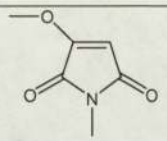
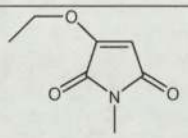
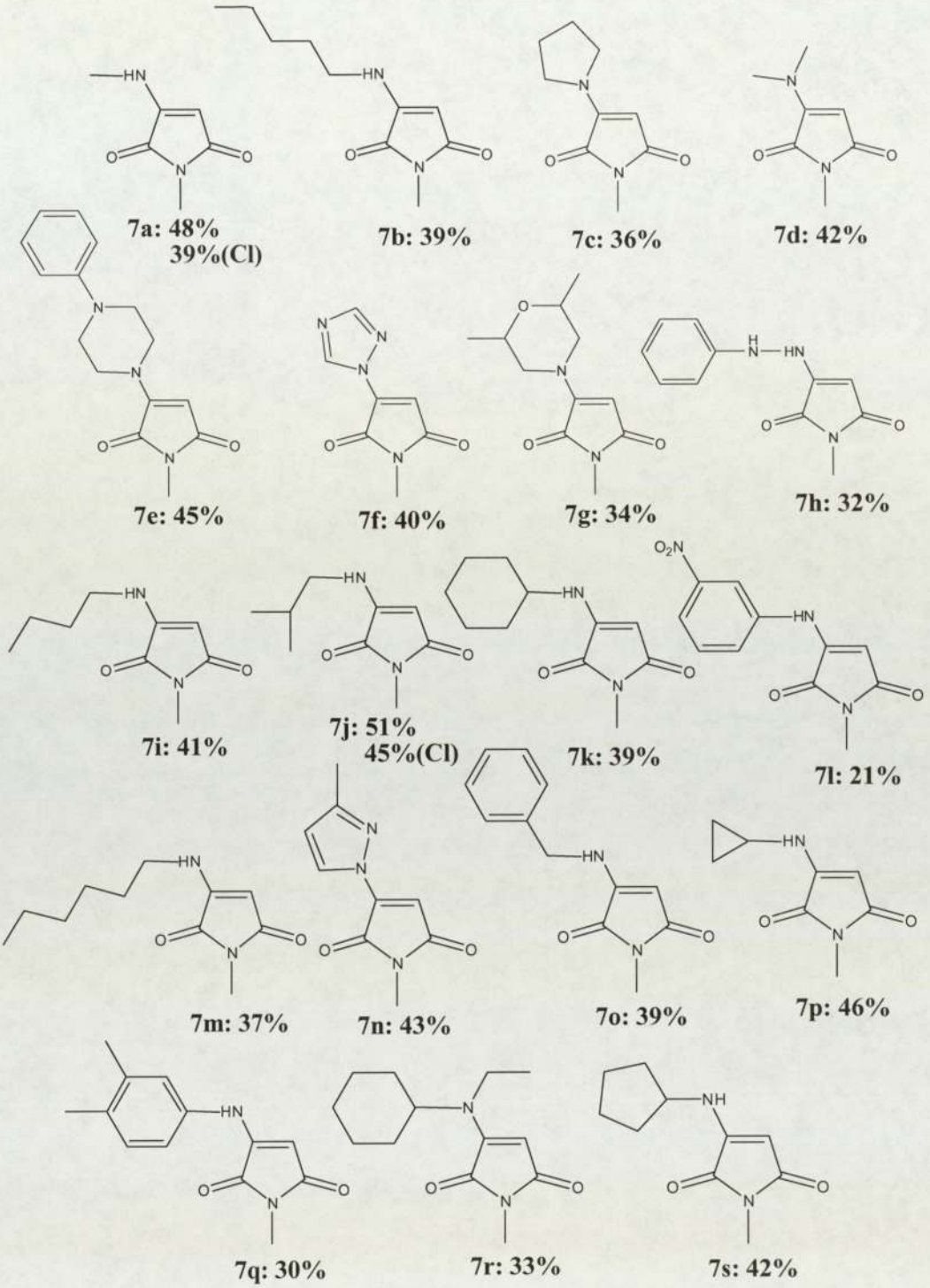
Code	Product Structure	Reagent used	Template: X =	Yield (%)
6a		Sodium Methoxide	Br	31
		Sodium Methoxide	Cl	25
6b		Sodium Ethoxide	Br	24
		Sodium Ethoxide	Cl	19

Table 3.2.3.1. Results for the formation of 3-alkoxy-pyrrol-2,5-diones.

Gill *et al*[137] had previous synthesised a series of maleimides including **6a** by using an alternative route by reacting maleic anhydride with the corresponding amine. Analytical data from the 3-alkoxy-1-methyl-pyrrole-2,5-dione synthesised here was compared to the data obtained by Gill *et al.* to help confirm the identity of this compound.

Due to the success of the oxygen nucleophile (alkoxide), a range of amines (nitrogen nucleophiles) were chosen to react with the 3-bromo-pyrrol-2,5-diones, as this was found to be the more reactive template. As a comparison and confirmation of this finding, the 3-chloro-pyrrol-2,5-dione template was also reacted with certain amines including isobuyl- and methylamine, which have produced compounds in good yield in other chapters of this work. A diverse range of amines were chosen to react with this 3-bromo-template, including alkyl, cyclo-alkyl, aryl and cyclic amines, as well as both primary and secondary amines. The 3-halo-pyrrole was dissolved in ether and reacted



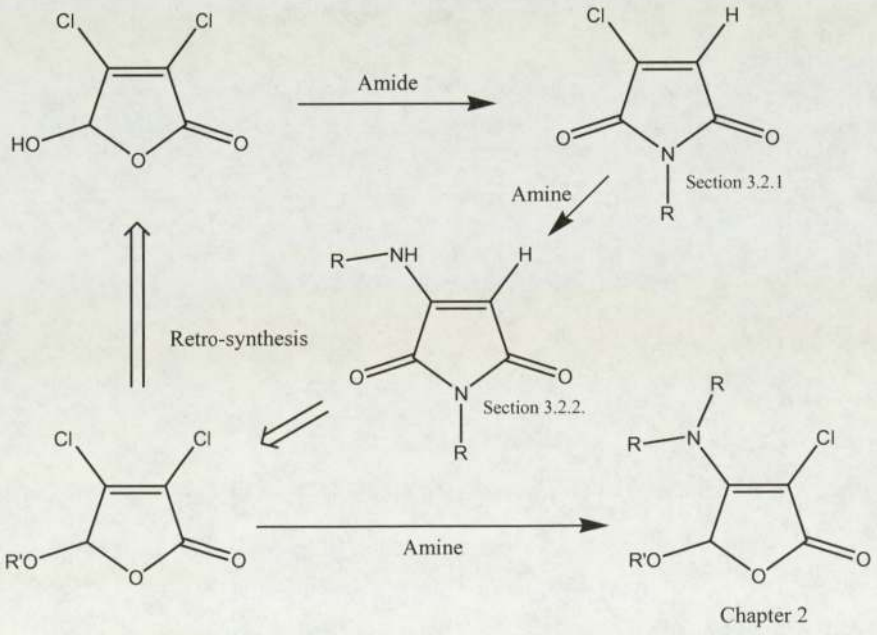
***(Cl)** = Template **5b** was used instead of **5a**.

Figure 3.2.3.2. Synthesised 3-amino-pyrrol-2,5-diones.

with the appropriate amine for 1 ½ hours at 30-35 °C. Figure 3.2.3.2. shows the structures and yields of the 3-amino-pyrrole compounds formed.

All of these compounds were synthesised in moderate yields, with compounds **7a** (methyl-amino unit) and **7j** (isobutyl-amino unit) being formed in the best yields (48% and 51% respectively). The corresponding compounds synthesised using the 3-chloro-pyrrole template were formed in 39% and 45% yields, confirming that the 3-bromo-pyrrole template is more reactive. In general 3-aryl-aminopyrroles were formed in lower yields, with the exception of **7e**, which was formed in a medium 45% yield. X-ray crystallography was performed using **7e** to confirm the structure of this class of compound, the results of which can be seen in the experimental section (chapter 6.3). **7l** was synthesised in the lowest yield (21%) out of all of the compounds formed. This was thought to be due to the nitro-benzene *meta*-deactivating properties. Cyclic-alkyl-amino-pyrroles were also synthesised in good yields, as in the case of **7p**, which contains the cyclopropyl-amino unit and was formed in 46% yield. There also appears to be a decline in yields as the alkyl side-chain length increases, i.e. **7i** (butyl-amino unit, 41%), **7b** (pentyl-amino unit, 39%) and **7m** (hexyl-amino unit, 37%).

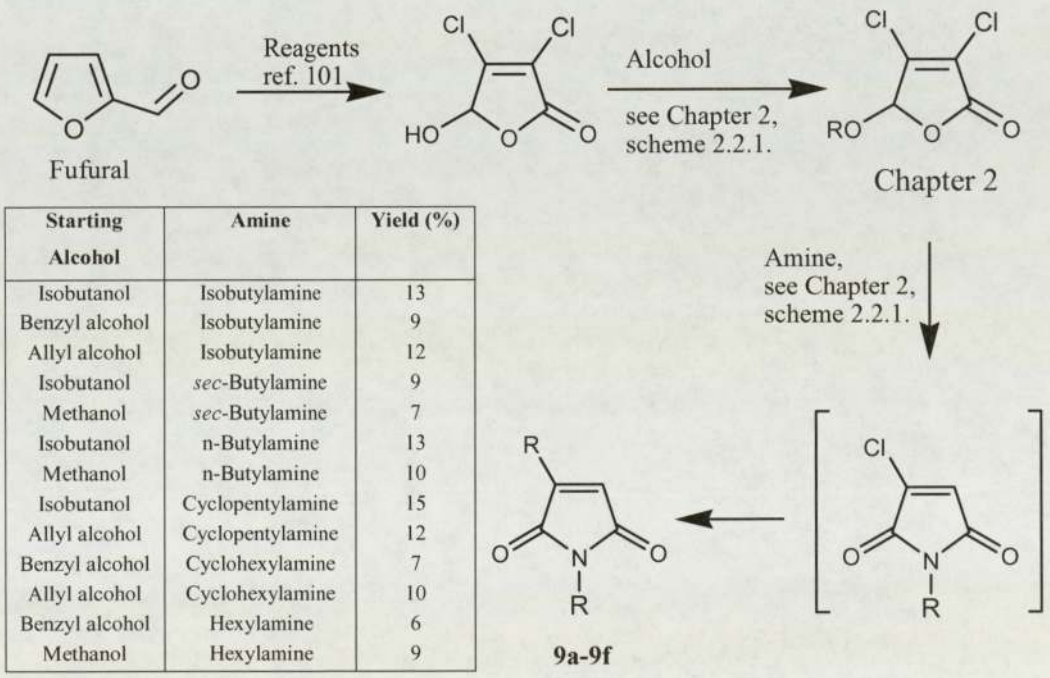
3.2.4. Retro-synthetic approach towards the formation of 1,3-diamino-pyrrol-2,5-diones.



Scheme 3.2.4. Synthetic routes for the synthesis of 3-amino-pyrrol-2,5-diones.

The 3-amino-pyrrol-2,5-dione synthesised in section 3.2.3., here underwent a retro-synthetic approach, to find an alternative strategy for the formation of these compounds. The retro-synthesis of this pyrrole, derives a 5-alkoxy-furanone, i.e. replacing the 3-amino group with a chlorine atom and the pyrrole ring nitrogen with oxygen (scheme 3.2.4.). This furanone template has previously been synthesised here in chapter 2. The second retro-synthetic step is now obvious as 5-alkoxy-furanones have already been formed here in chapter 2 and in other literature sources[102, 103] from mucohalogen acids. Therefore an alternative route for the synthesis of these pyrrole compounds is from mucochloric acid via a 5-alkoxyl-furan-2-one.

3.3. Synthesis of 1,3-diamino-pyrrol-2,5-diones.



Starting Alcohol	Amine	Yield (%)
Isobutanol	Isobutylamine	13
Benzyl alcohol	Isobutylamine	9
Allyl alcohol	Isobutylamine	12
Isobutanol	*sec*-Butylamine	9
Methanol	*sec*-Butylamine	7
Isobutanol	n-Butylamine	13
Methanol	n-Butylamine	10
Isobutanol	Cyclopentylamine	15
Allyl alcohol	Cyclopentylamine	12
Benzyl alcohol	Cyclohexylamine	7
Allyl alcohol	Cyclohexylamine	10
Benzyl alcohol	Hexylamine	6
Methanol	Hexylamine	9

Scheme 3.3. Synthetic scheme and yields of 1,3-diamino-pyrrol-2,5-diones

During the synthesis of numerous 4-amino-5-alkoxy- or 5-aryloxy-furanon-2-ones (chapter 2, section 2.2.3.), from mucochloric acid via 5-alkoxy-furanones, a by-product was isolated in each case and purified using column chromatography. These by-

products were fully characterised and were found to be 1,3-diamino-pyrrol-2,5-diones (scheme 3.3.). Although the pyrroles synthesised here were formed via the starting material mucochloric acid, due to its low cost and availablility, they can actually be obtained from environmentally friendly crop waste[101], as too can most of the compounds synthesised throughout this work. This in principle gives a synthetic approach towards making biologically active drugs from natural renewable resources. Mucochloric acid can be synthesised from the chlorination of furfural (using manganese oxide in hydrochloric acid), which itself is formed from pentosans (polysaccharides extracted from plants).

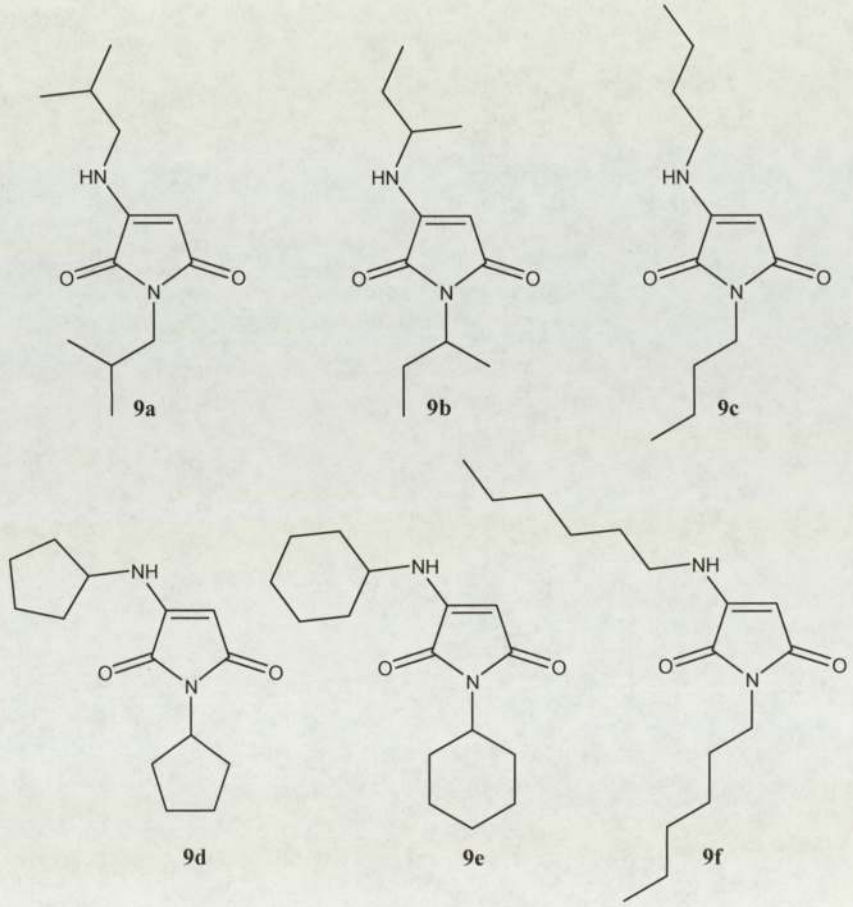
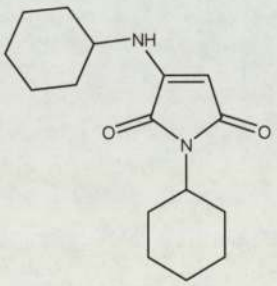


Figure 3.3.0. Synthesised structures of the 1,3-diamino-pyrrol-2,5-diones.

Each of the compounds shown in figure 3.3.0. were synthesised as a by-product using at least two 5-alkoxy-furanones in order to provide evidence for this synthetic pathway

(scheme 3.3.). These compounds were formed in relatively low yields. **9d** was formed the highest yield (15%), when isobutanol was used as the starting alcohol. In general this alcohol formed compounds in the best yields. Pyrroles derived from benzyl alcohol were isolated in poor yields for example **9f** (6%). This may be due to the corresponding 4-amino-5-alkoxy-furanone (major product) in each of these cases being formed in a high yield (chapter 2). **9a** was formed in better yields with respect to the alcohols used (13% using isobutanol and 12% using allyl alcohol). **9b**, an isomer of **9a** was formed in poorer yields (9% using isobutanol and 7% using methanol), while **9e** was formed in a lower yield (10% using allyl alcohol) than its cyclic homologue **9d.** These diamino-pyrroles, in principle, can be formed as a by-product during the synthesis of any 4-amino-5-alkoxy-furanone where primary amines are used. The reaction is thought to proceed via the same mechanism shown in section 3.2.2.1., but with the expulsion of a 5-alkoxy- or 5-aryl-group instead of the hydroxyl group. The molecule then undergoes Michael addition at the 4-position resulting in these diamino-pyrroles.



9e

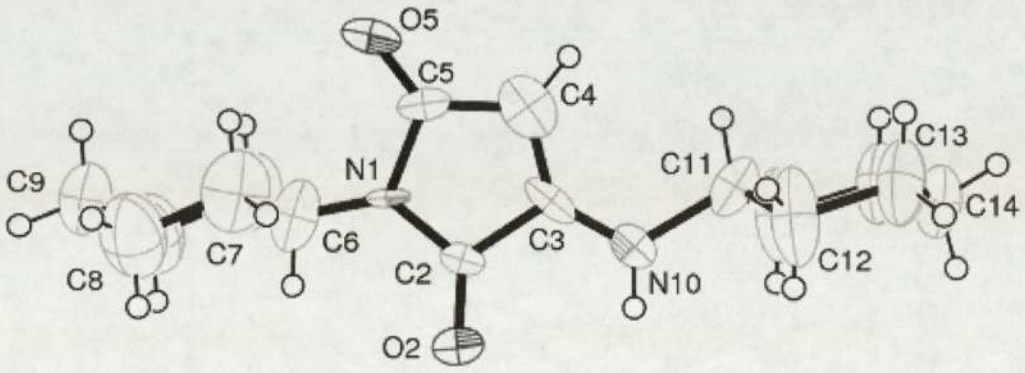


Figure 3.3.0.1. Crystal structure of 1-cyclohexyl-3-(cyclohexylamino)-1H-pyrrole-2,5-dione.

X-ray crystallography was carried out using compound **9e** in order to confirm the structure of the amido-furanones. Figure 3.3.0.1. shows and confirms, alongside ^{1}H and ^{13}C NMR, IR and MS analysis, that the structure of this compound was as expected 1-cyclohexyl-3-(cyclohexylamino)-1H-pyrrole-2,5-dione. The corresponding data for this compound can be found in experimental chapter 6.3.

Further optimisation of these compounds was declined at this point, firstly because these compounds were being formed in low yields (some as semisolids) and secondly due to there being a better alternative method developed earlier in this chapter (section 3.2.3.). There are however drawbacks with both of the previously described routes carried out here for the synthesis of pyrrol-2,5-diones.

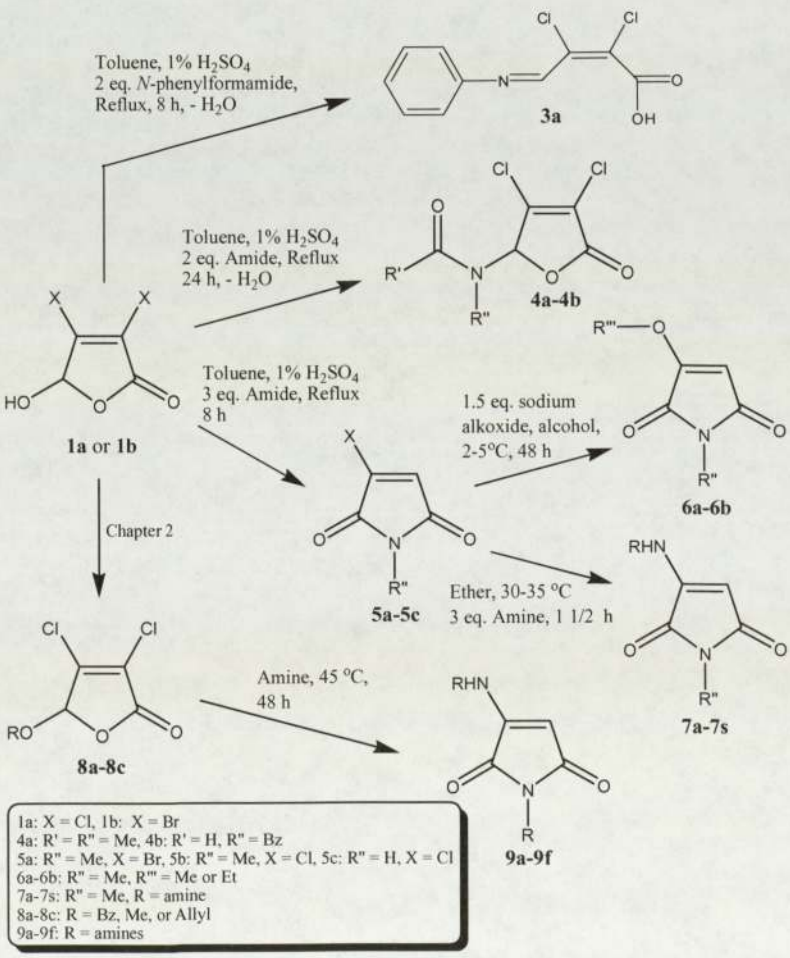
The number of Pyrrole templates derived from *N*-substituted formamides as discussed in section 3.2.3 is limited, mainly to the *N*-methyl-pyrrole template. The other route by where the pyrroles are obtained as a by-product of 4-amino-5-alkoxy-furanones, is not as limited, but these compounds can only be obtained as diamino-pyrroles. This may not always be an advantage if different side chains are required. For these reasons a third pyrrole template was developed, which will be discussed in chapter 4.

All of the aforementioned bis-amino-pyrrol-2-ones have been synthesised using primary amines. A secondary amine was reacted here with one of the 5-alkoxy-furan-2-one (**PrOF**) synthesised in chapter 2, to observe what would happen, as no cyclisation can occur with secondary amines. It was predicted that if di-substitution occurred, as seen with primary amines, the product would be synthesised following the same mechanism as mentioned above, but would stop and be formed as a butenoic acid amide.

TLC analysis indicated that a mixture had been formed. MS, IR and NMR analysis suggested that this mixture contained a maleimide, which was isolated. Due to this maleimide structure being of little relevance here, no further reactions or analysis was carried out using secondary amines.

3.4. Synthetic overview towards the synthesis of pseudo-amideo-furan-2-ones and pyrrole-2,5-diones.

Scheme 3.4. shows a reaction overview for the synthesis of pseudo-amides, pyrrole-2,5-diones and a but-2-enoic acid using different ratios of the reagent *N*-methyl-formamide with mucohalogen acid. **3a**, a but-2-enoic acid was formed in high yield from mucochloric acid, but did not cyclise to the expect amido-furanones of the type **4a**. Amido-furanones, **4a** and **4b** were formed in low-moderate yields, as too were the pyrrol-2,5-diones **5a-5c**. The pyrroles **5a** and **5b** were used to synthesise a range of 3-amino- and 3-alkoxy-pyrrol-2,5-diones (**7a-7s** and **6a-6b** respectively), which led to an investigation of diamino-pyrroles (**9a-9f**) formed as by-product of 4-amino-5-alkoxy-furanones synthesis.



Scheme 3.4. Synthesis of amido-furanones and pyrrole-2,5-diones.

3.5. Discussion of pyrrole-2,5-diones.

The pyrrol-2,5-diones (maleimides) synthesised here, have been formed via two new alternative routes to the ones already known. One reaction forms these compounds via a new 3-halo-pyrrol-2,5-dione template, while the other is as a by-product via 5-alkoxy-furan-2-ones synthesised in chapter 2. These compounds, in the near future, are to be explored further in terms of their biological activity as CCK antagonists.

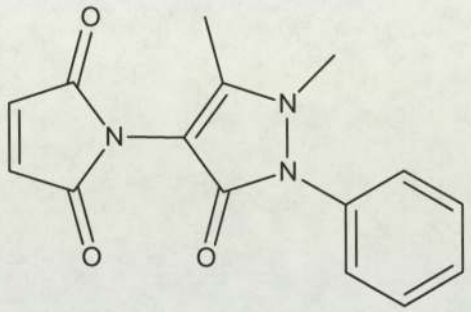


Figure 3.5. The active but highly toxic antipyrino-pyrrole-2,5-dione.

Pyrrol,2-5-dione compounds, which are structurally related to the cyclic imides synthesised here, are already known as biological agents. One class has been synthesised in order to explore analgesic properties against acetic acid-induced writhing in mice. The most active agent tested was a compound containing antipyrine directly attached to the imido ring (figure 3.5.)[138]. It was found to be approximately 50-fold more active than the standard drugs, aspirin and paracetamol. However, all animals died after treatment with this compound, suggesting a high toxicity. The introduction of two chlorine atoms into the double bond of the imido ring gave a high analgesic action (figure 3.5.1.), which was about 30-fold more potent than aspirin and paracetamol.

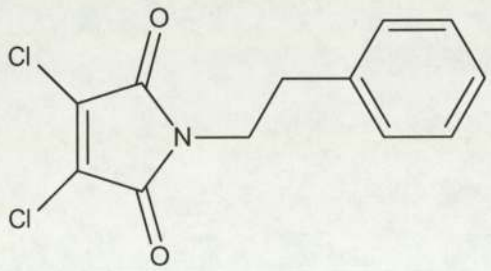


Figure 3.5.1. The analgesic dichloro-*N*-aryl-pyrrole-2,5-dione.

These findings suggest that the 3-halo-pyrrole-2,5-diones derived for this chapter could potentially contain groups which contribute to them being biologically active.

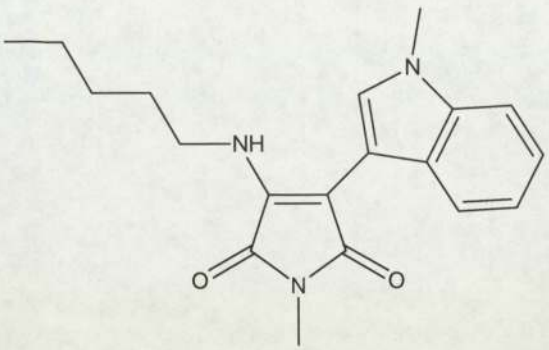
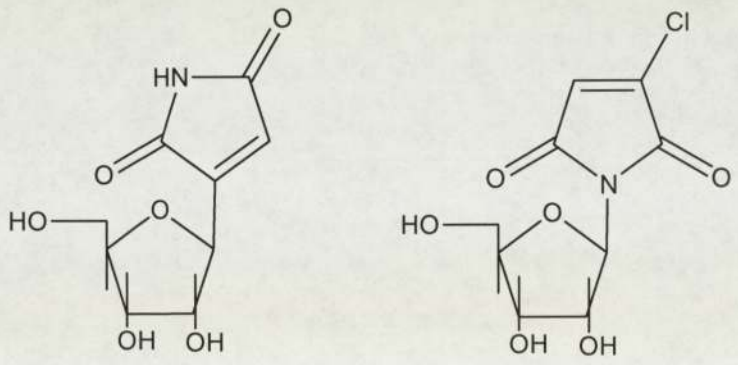


Figure 3.5.2. IM-54 - Necrotic cell death inhibitor.

Dodo *et al.* [139] have recently synthesised novel analogues of indolylmaleimide derivatives which were tested for hydrogen peroxide necrotic cell death-inhibitory activity. They found IM-54 (2-(1H-indol-3-yl)-3-pentylamino-maleimide, figure 3.5.2.) to be the most effective cell death inhibitor among the compounds tested, showing low (~ 10 μM in HL60 cells exposed to 100 μM H_2O_2) inhibitory activity towards kinases.



Showdomycin 3-Chloro-*N*-ribosylmaleiminde

Figure 3.5.3. The antitumor antibiotic showdomycin and one of its derivatives.

Showdomycin (figure 3.5.3.), a pyrrole-2,5-dione derivative, is an antitumor antibiotic. It is thought that the ribose moiety of this molecule contributes to a facilitated entry into the cell, where the showdomycin exerts its alkylating capability on the intramolecular sulfhydryl groups. Numao *et al.* [140] synthesised and evaluated analogues of this antibiotic, one of which, is a directly related to the chloro-pyrrole-2,5-dione synthesised in this work, with the exception of the ribose group.

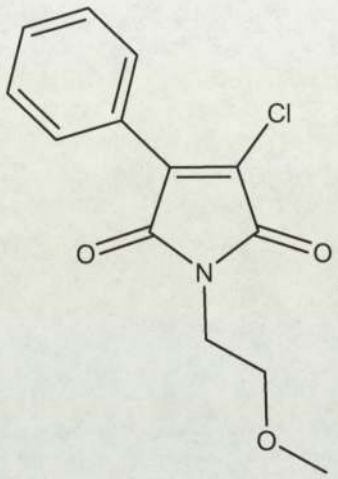


Figure 3.5.4. A 2-alkoxy-4-aryl-pyrrole-2,5-dione derivative with useful biological properties.

2-Alkoxy-4-phenyl-pyrrol-2,5-diones[141] (figure 3.5.4.) have been prepared as liver X receptor modulators, with the intent of treating or preventing clinical conditions such as inflammatory diseases and Alzheimer's disease. Structurally related 3-anilino-4-arylmaleimides (Smith *et al.* [142]) and 3-indolyl-4-phenyl-1H-pyrrole-2,5-dione derivatives (Gong *et al.*[143]) and have been prepared with the intention to be used for the treatment or prophylaxis of conditions associated with a need for inhibition of glycogen synthase kinase-3 (GSK-3), especially diabetes and chronic neurodegenerative conditions including dementias (Alzheimer's diseases), neurotraumatic diseases (acute stroke), brain injury, manic depression, obesity and cardiovascular diseases. These novel GSK-3 inhibitors showed high potency and selectivity.

The cyclic imide antibiotic D-cycloserine[144] (figure 3.5.5.) which is also known as Oxamycin or D-4-amino-3-isoxazolidone was discovered and isolated as a metabolic

product of a new species of Streptomyces and was found to possess broad spectrum antibiotic activity[145]. It was later synthesised from DL-serine[146].

D-Cycloserine has been used for over 30 years in tuberculosis[147] and urinary tract infections[148]. It is also used against schizophrenia[149], psychosis[150], anxiety[151, 152] and depression[153], and has been found to facilitate learning[154] and memory and improve visual memory in animals with cases associated to Parkinson's disease[155].

The pyrrol-2,5-diones synthesised here have a very similar structural template to that of cycloserine (figure 3.5.5.). The slight difference is that cycloserine has an internal oxygen within the ring, compared to the pyrrole-dione which has an external oxygen. These similarities suggest this pyrrole template could lead to new anti-tuberculosis agents.

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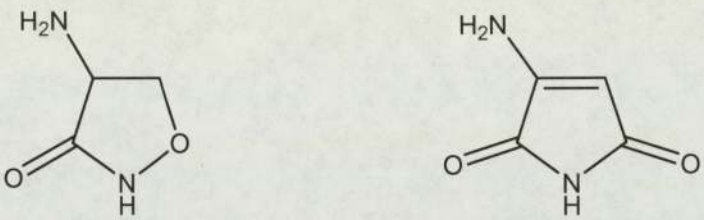
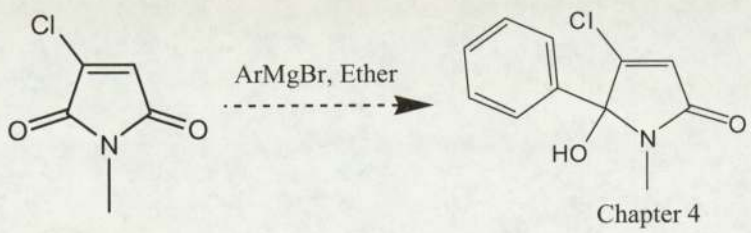


Cycloserine Potential anti-tuberculosis agent

Figure 3.5.5. The antibiotic cycloserine and potential anti-tuberculosis pyrrole agent.

There is also the potential of reacting the 3-halo-*N*-methyl-pyrrole templates synthesised here with a Grignard reagent, in a similar manner to the reaction discussed in chapter 4[171] (figure 4.1.3.). This should yield the same or similar 5-hydroxy-5-aryl-pyrrol-2-ones (**Ar-4**, figure 4.2.2.1), as synthesised in chapter 4 from a 5-arylated-furanone.



Scheme 3.5.6. Proposed reaction pathway for the synthesis of 5-hydroxy-5-aryl-pyrrol-2-ones.

Scheme 3.5.6 shows the proposed reaction pathway. This reaction is however limited. Firstly due to the fact that only an *N*-methyl-pyrrol-2,5-dione derivative can be synthesised via this reaction, whereas in chapter 4 a variety on *N*-alkyl- and *N*-aryl-pyrrol-2-ones can be formed (figure 4.2.2.1.). Secondly, although it would be expected to achieve a high yield for these pyrrol-2-ones via the above reaction using a Grignard reagent, the *N*-methyl-pyrrol-2,5-dione formed here was in a low yield. Therefore this method is not really feasible for the synthesis of 5-hydroxy-5-aryl-pyrrol-2-ones, in comparison to the method used in chapter 4.

3.6 Conclusion.

A 3-alkylated *N*-alkyl-pyrrol-2,5-dione (**5c**) was isolated as a by-product during the synthesis of an amido-furanone anti-cancer lead structure (**9a**) from mucochloric acid and NMF. The reaction conditions were optimised leading to the formation of this pyrrole as a main product. This pyrrol-2,5-dione template was then optimised using commercially available amides and mucohalogen acids leading to **5a-5c**.

The halogen of selected halogenated pyrroles was displaced by an oxygen nucleophile to give 3-alkoxylated *N*-alkyl-pyrrole-2,5-diones **6a-6b** using a system of sodium alkoxide or alcohol under mild reaction conditions in low to average yields. The same halogen was also displaced by amino groups (nitrogen nucleophiles) to give 3-amino-pyrrol-2,5-diones **7a-7s** using ether and a diverse selection of amines. These compounds were formed in moderate yields. 1,3-Diamino-pyrrol-2,5-diones **9a-9f** of the same pyrrole template were formed using an alternative synthetic approach. These compounds were synthesised in low yield, as a by-product of 4-amino-5-alkoxy-furanones which were formed via a series of 5-alkoxy-furan-2-ones in chapter 2. No further optimisation occurred as the scope of these compounds was limited. These diamino-pyrrol-2,5-diones were thought to form by the same mechanism as the **7a-7s** series. Further development and optimisation of this pyrrole template, which is present in various biological agents, may help in the formation of new lead structures. This development had already begun here in terms of the synthesis and evaluation of 5-hydroxy-5-arylated-pyrroles, which will be discussed in chapter 4.

Chapter 4: - Synthesis of 5-phenyl-5-hydroxy-pyrrol-2-ones.

4.1. Introduction.

Tetronic and tetramic acid (figure 4.1.) derivatives are an important class of oxygen and nitrogen containing heterocycles respectively, whose motifs are present in many natural products[156, 157], such as penicillic acid and ascorbic acid (vitamin C). Tetronic acid has been chemically synthesised from the reduction of enaminones followed by the hydrolysis of the corresponding ester[158]. Svendsen and Boll[159] reported that tetronic acid could be prepared by the cyclisation of halogenated acylmalonic esters to 3-ethoxycarbonyltetronic acids followed by subsequent alkaline hydrolysis to remove the ethoxycarbonyl group. Tetramic acid has also been formed synthetically from the cyclisation of ethyl ethoxycarbonylacet-aminoacetate followed by the hydrolysis and neuralisation of the corresponding compound[160].

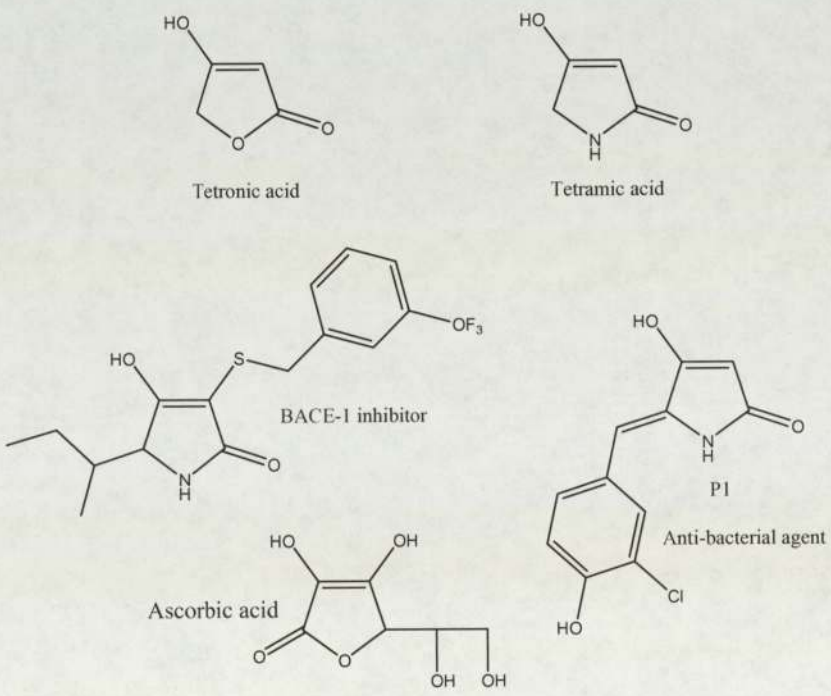


Figure 4.1. Tetronic and tetramic acid present in selected natural products.

Derivatives of both classes of compounds exhibit a wide range of biological activities[161] including antibiotic [162], antitumor [163], antiviral, antifungal, cytotoxic and enzyme inhibitory properties. Acetyl tetramic acid derivatives are known to act as anti HSV and HIV agents with potent tyrosine phosphatase inhibitory activites[164] whereas tetronic acid derivatives are known HIV-1 protease inhibitors[165]. *N*-Substituted tetramic acids, formed using solid phase approach on Wang resin, have been identified as inhibitors of BACE-1 (β-secrease), which is involved in the generation of Alzheimer's disease[166] (figure 4.1). Another tetramic acid derivative **P1** (figure 4.1), which was isolated from a natural product has exhibited anti-bacterial activity against *Bacillus subtilis*.

5-Hydroxy-1,5-dihydropyrrol-2-one derivatives are important building blocks (figure 4.1.1.) for the preparation of a wide variety of natural products with potential pharmaceutical application[167]. In general these derivatives have to date been synthesised by photooxygenation of pyrrole derivatives[168].

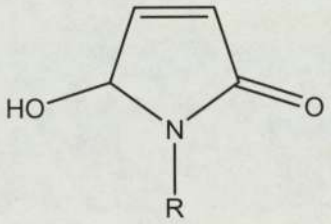


Figure 4.1.1. 5-Hydroxy-pyrrol-2-one building block.

Jatropham ((*R*)-(-)-5-hydroxy-3-methyl-3-pyrrolin-2-one), an antitumor[169] alkaloid, is both a natural and synthetic compound, possessing similar structural properties to the series to be synthesised herein. Mase *et al.*[170] synthesised this tetramic acid derivative in three steps from a tetronic acid derived compound (figure 4.1.2.) via regioselective reduction and kinetic resolution.

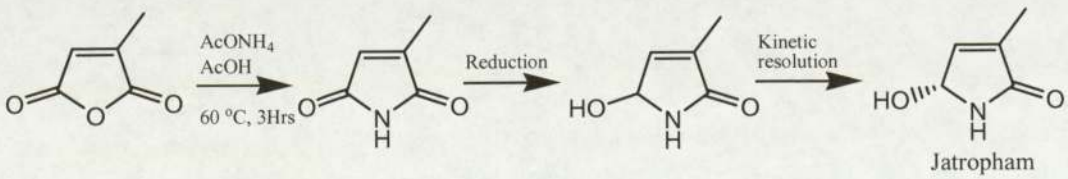
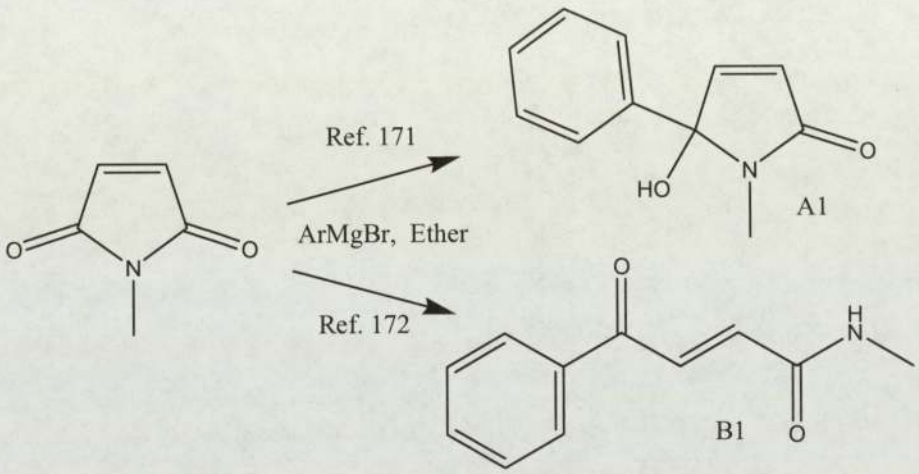


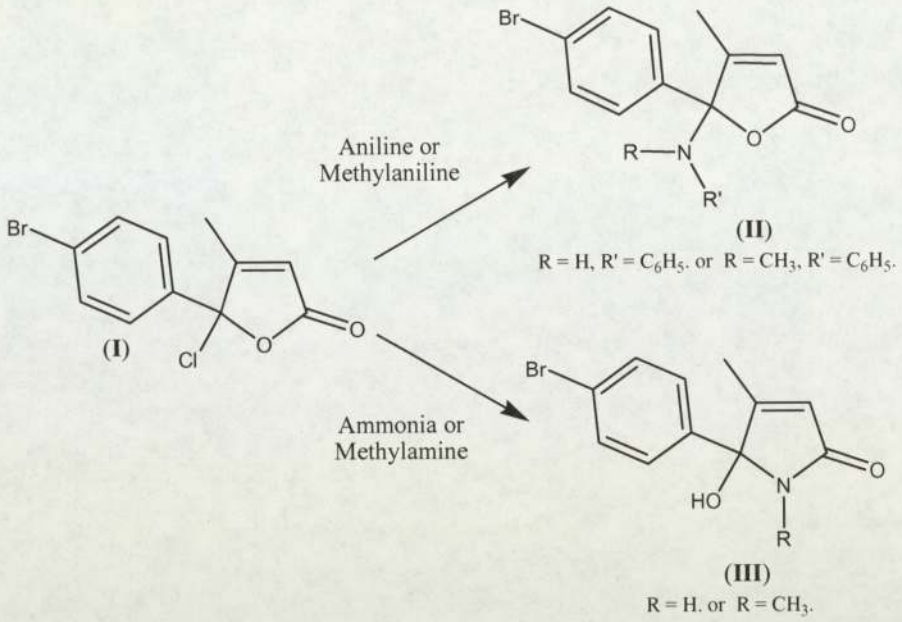
Figure 4.1.2. Synthesis of Jatropham, an antitumor tetramic acid derivative.

A 5-aryl-5-hydroxy-pyrrol-2-one, again similar to the series to be synthesised herein (excluding the 4-chloro group) had been synthesised from an *N*-substituted maleimide using a Grignard reagent (arylmagnesium bromide)[171]. This compound (figure 4.1.3., **A1**) was synthesised in 76% yield. Awad *et al.*[172] did not believe that this pyrrolinone template was formed, but in fact the corresponding open chain β-aroylacrylamide (figure 4.1.3., **B1**). In both cases it was unclear how the maleimide starting material template was synthesised.



Scheme 4.1.3. Synthesis of a 5-aryl-5-hydroxy-pyrrol-2-one.

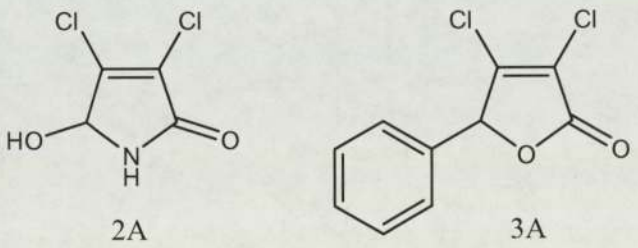
During an investigation carried out by Lutz *et al.*[173] on the ring tautomerism of a 5-aryl-furan-2(5H)-ones (5-(4-Bromo-phenyl)-5-hydroxy-4-methyl-5H-furan-2-one), they found that the corresponding chloride of this compound, 5-(4-Bromo-phenyl)-5-chloro-4-methyl-furan-2(5)-one (**I**, figure 4.1.4.), underwent different reactions with various amines or ammonia, to give cyclic amides. When this 5-chloro-furanone was reacted with ammonia and methylamine, 5-hydroxy-pyrrol-2-ones (**III**) were formed, whereas when aniline and methylaniline were used 5-amino-furanones (**II**) were synthesised.



Scheme 4.1.4. Amides derived from the acid chloride (**I**).

4.2. Investigation of 3,4-dichloro-5-phenyl-2(5H)-furanone and its derivatives.

The starting point for this investigation was to design and develop CCK antagonists, with potential anti-cancer properties (dual action drugs) based on initial work (not included here) regarding two compounds which have been synthesised and evaluated for cytotoxicity. The first compound, a 5-hydroxy-pyrrol-2-one (**2A** figure 4.2), was found to possess anti-cancer activity[136], but was formed in low yield. The second, a 5-aryl-furanone (**3A**, figure 4.2.), showed good *in vitro* cytotoxicity, but was toxic *in vivo*[136]. This was thought to be due to the two reactive chlorines. Jamshidipour *et al.*[174] have shown CCK antagonists, for example devazepide (chapter 1, figure 1.2.11.2.) can enhance the effects of the cytotoxic drug cisplatin. They found that a combination of these two compounds resulted in an increased tumour delay, compared to cisplatin alone.

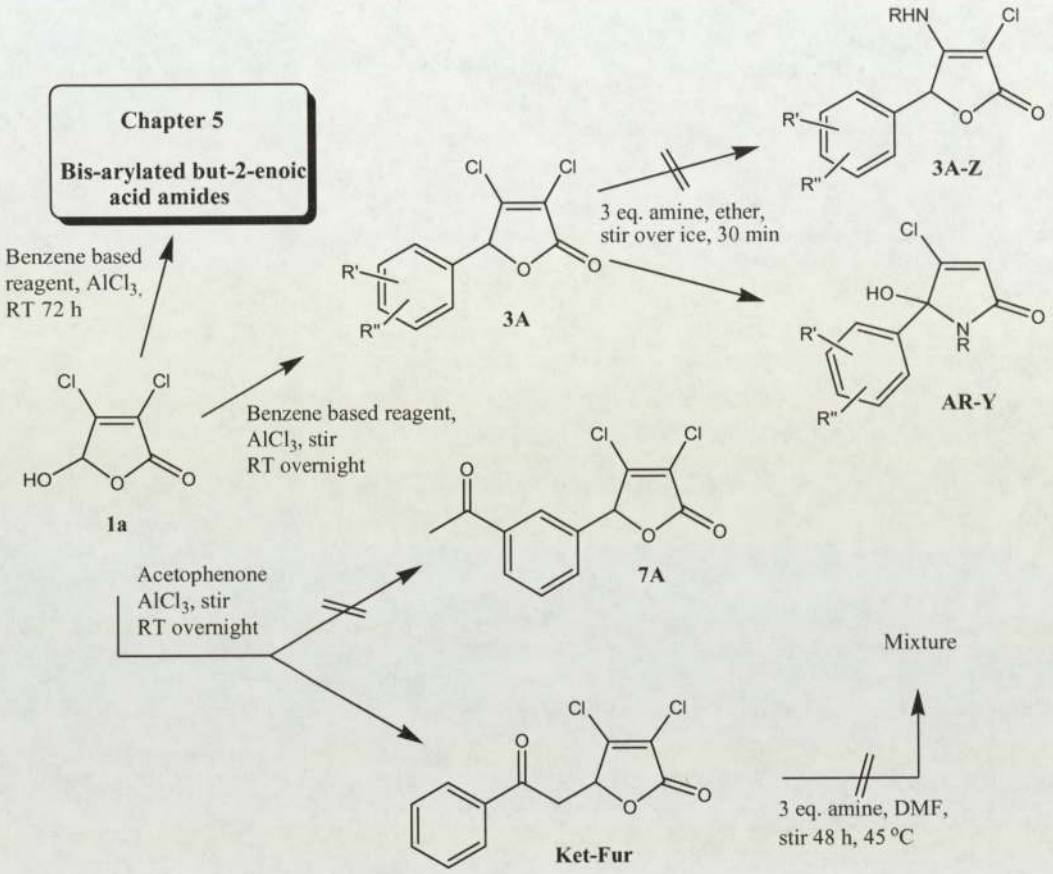


2A 3A

Figure 4.2. Previously synthesised and evaluated cytotoxic compounds.

From the analytical results for **2A** and **3A**, the idea here was to design compounds possessing structural characteristics of both of the aforesaid compounds in order to achieve the required biological activity. The first step was to re-synthesise the stable 5-aryl-furanone template, and develop other compounds around this templates (**3A**, scheme 4.2.0.) using a range of substituted benzene compounds such as chlorobenzene and toluene. This was followed by the formation of targeted 5-hydroxy-5-aryl-pyrrol-2-ones **(AR-Y)** using a diverse range of amines. One alternative reaction that may have occurred at this stage but did not, was the formation of 4-amino-5-phenyl-furan-2(5H)-one compounds (**3A-Z)** via nucleophilic attack at the 4-position of **3A** in a Michael type reaction (as seen in chapter 2). Instead development and optimisation of **AR-Y**

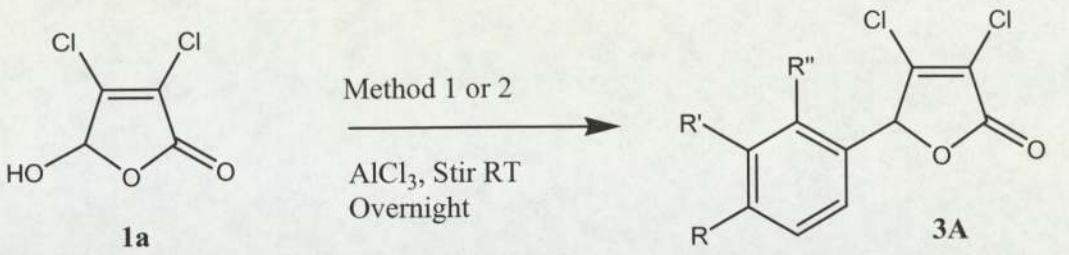
compounds was undertaken, after which these potential CCK antagonists were structurally and biologically evaluated.



Scheme 4.2.0. Reaction overview for the synthesis of the class of compounds 5-phenyl-5-hydroxy-pyrrol-2-ones.

4.2.1. Preparation of 3,4-dichloro-5-phenyl-furan-2(5H)-one.

5-Phenyl-furan-2(5H)-one was first synthesised by Ettel *et al.*[175] in 1952, where mucochloric acid was subject to condensation with benzene using $AlCl_3$ as a Lewis acid. They obtained an off white crystalline compound in 89% yield (m.p. 79-80 °C). Due to the success of this procedure it was concluded that this method would be used for the basis of the synthesis carried out during this investigation.



Solvent / Reagent	Co-solvent	R	R'	R''	Method	Yield (%)
Benzene	-	H	H	H	1	66
Benzene	THF	H	H	H	2	78
Chlorobenzene	-	Cl	H	H	1	58
Chlorobenzene	THF	Cl	H	H	2	69
Toluene	-	Me	H	H	1	NDR
Xylene	-	Me	H	Me	1	NDR
Anisole	-	MeO	H	H	1	NDR
Veritrole	-	MeO	MeO	H	2*	NDR
Nitrobenzene	-	H	NO_2	H	1	NDR

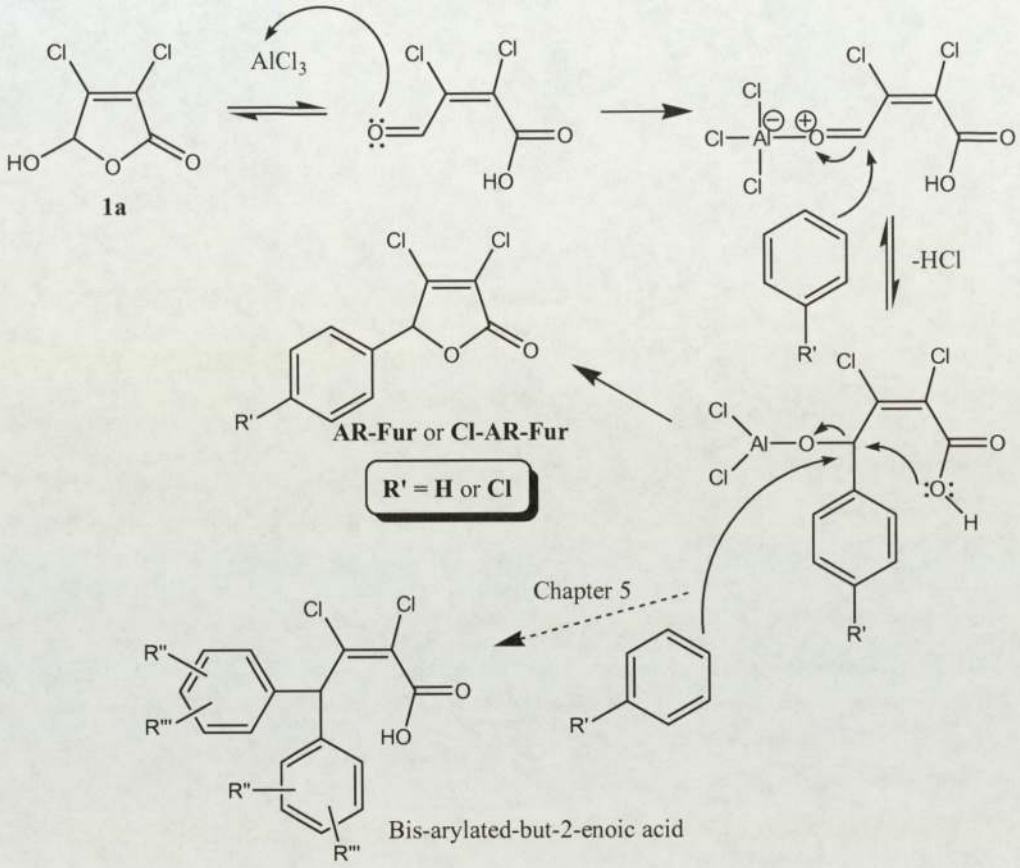
NDR = No Desired Reaction * = THF used as main solvent

Scheme 4.2.1. Synthetic pathway for the formation of 3,4-dichloro-5-aryl-furan-2(5H)-ones.

Mucochloric acid was dissolved in a range of benzene derived reagents or solvents (and co-solvents where appropriate) followed by the addition of $AlCl_3$ and left overnight at RT. Friedel Crafts reaction conditions were used for this reaction. The reagents or solvents used and the results obtained can be seen if scheme 4.2.1.

Only 3,4-dichloro-5-phenyl-furan-2(5H)-one (**AR-Fur**) and 3,4-Dichloro-5-(4-chloro-phenyl)-furan-2(5H)-one (**Cl-AR-Fur**) were successfully prepared based on the publicised method by Semonsky *et al.*[176] Other reagents gave undesired results, i.e. toluene, xylene and anisole gave bis-arylated but-2-enoic acids in low yields of the type

seen in chapter 5, while nitrobenzene did not react at either RT or at an elevated temperature (60 °C). This was thought to be due to its meta-deactivating properties.



Scheme 4.2.1.0. Proposed mechanism for the formation of **AR-Fur** or **Cl-AR-Fur**.

The reactions were monitored by TLC (8:1 ether, petroleum ether). **AR-Fur** and **Cl-AR-Fur** (no co-solvent) were formed, after work up, as semisolid residues which were recrystallized in n-hexane to give white crystals, 66% and 58% yield respectively. Scheme 4.2.1.0. shows the proposed Friedel Crafts type mechanism[177] by which these aryl-furanones and the bis-arylated but-2-enoic acids (chapter 5) were synthesised.

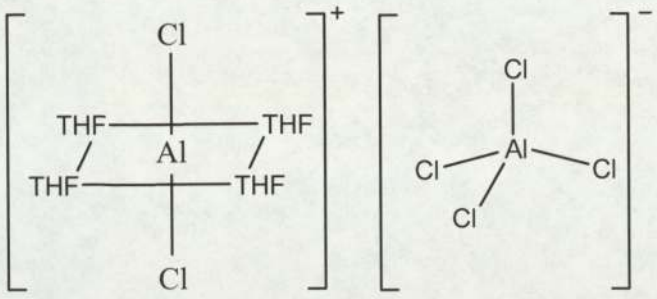


Figure 4.2.1.0.1. Activating complex of $AlCl_3$ and THF formed as $[AlCl_4][AlCl_2(THF)_4]$.

Both of the working examples were re-synthesised using a co-solvent, tetrahydrofuran (THF), which is a weak Lewis base. **AR-Fur** and **Cl-AR-Fur** were synthesised in a higher yield, 78% and 69% respectively, compared to the corresponding reactions where THF was not used. It has been shown in recent years[178, 179] that electron-pair donors (Lewis bases) can enhance the electrophilic character of the electron-pair acceptor (Lewis acids), i.e. the Lewis base can prompt activation of the Lewis acid within a reaction leading to higher yields. Extensive research has been carried out on the interaction of $AlCl_3$ with THF. Means *et al.*[180] isolated and structurally characterised an $AlCl_3$-THF complex, using a ratio 1:2 anhydrous aluminium chloride to THF (as used here) in aromatic solvents such as toluene and found the complex (figure 4.2.1.0.1.) to exist as $[AlCl_4][AlCl_2(THF)_4]$.

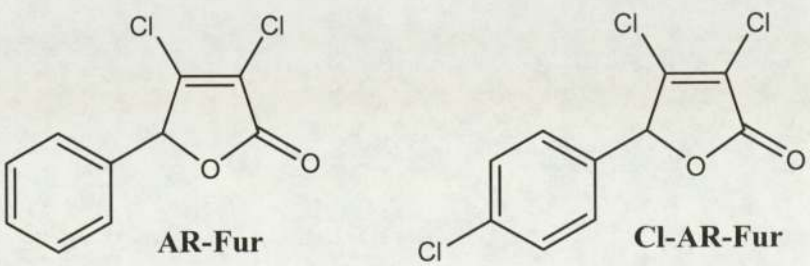
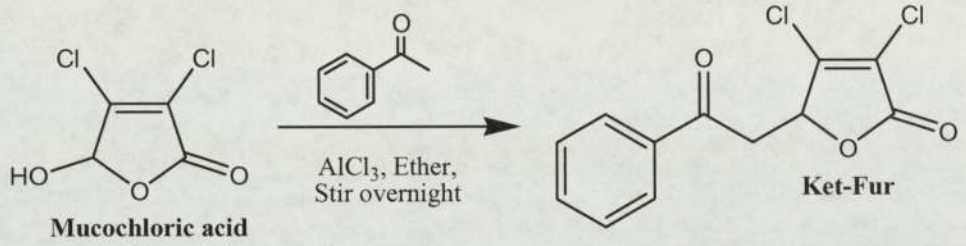


Figure 4.2.1.0.2. Synthesised 5-arylated furanones.

Following the successful synthesis and full characterisation of these 5-phenyl-furanone templates (figure 4.2.1.0.2.), a series of chemically diverse amines were then chosen to react with **AR-Fur** and **Cl-AR-Fur**. The choice of amines were based upon the structure of asperlicin[28, 104], the first non-peptidal CCK antagonist, which contains an isobutyl side chain, and the findings by Lattmann *et al.*[105] who found that the use of small side chains such as a propyl group, in a series of 1,4-benzodiazepine-2-ones enhance their biological properties as CCK receptor ligands.

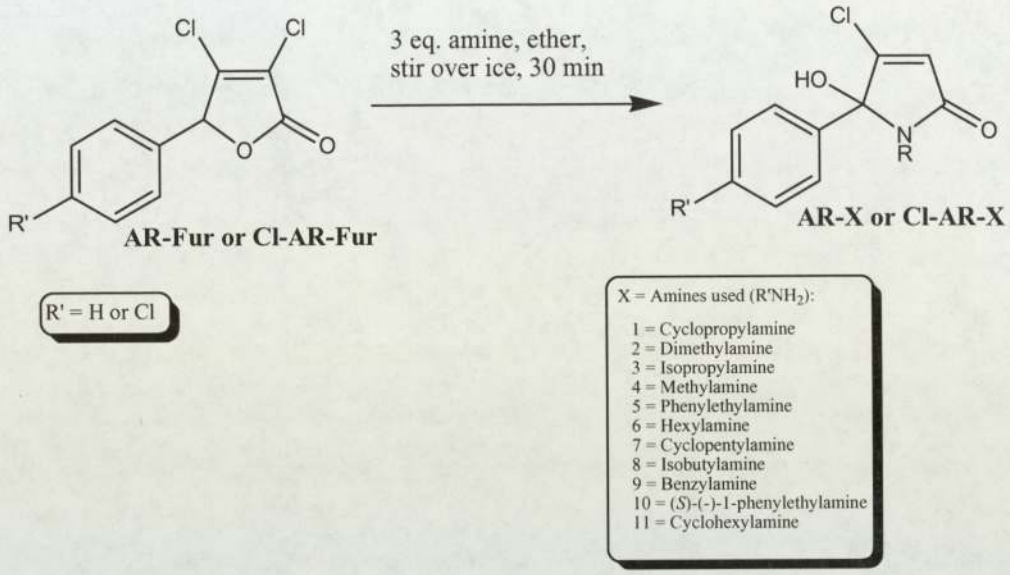
4.2.1.1. Synthesis of 3,4-dichloro-5-(2-oxo-2-phenylethyl)furan-2(5H)-one.



Scheme 4.2.1.1. Synthesis of 3,4-dichloro-5-(2-oxo-2-phenylethyl)furan-2(5H)-one.

During the synthesis of 5-arylated-furanones, formed in section 4.2.1., acetophenone was used in order to try and synthesise a *meta*-substituted keto-arylated furanone (**7A**, scheme 4.2.0.), in a Friedel Crafts type reaction. After analysing this compound it was found to be the furanone (**Ket-Fur**), shown in scheme 4.2.1.1. above. This compound has previously been formed by Langley[102], using a base-catalysed aldol condensation reaction. He suggested that this compound was not formed under acid conditions. Here it is shown that this compound was formed via an acid-catalysed aldol condensation reaction, where $AlCl_3$ activates the aldehyde of mucochloric acid (in the aldehyde form) and acetophenone tautomerises into the enol-form before an electron pair from the vinylic bond attacks the carbonyl carbon of the aldehyde, followed by ring closure forming the furanone.

4.2.2. Synthesis of 5-hydroxy-5-aryl-pyrrol-2-ones.



Scheme 4.2.2. Reaction pathway for the synthesis of the class of compounds **AR-X** or **Cl-AR-X**.

A selection of eleven diverse amines (scheme 4.2.2.) were chosen to undergo nucleophilic attack at the carbonyl group of the 5-arylated-furan-2-one ring of **AR-X** and **Cl-AR-X**, leading to ring opening and recyclisation as a pyrrol-2-ones. These included aryl amines, one of which has a chiral centre and amines with simple alkyl- and cyclic alkyl side chains. The 5-arylated-5-hydroxy-pyrrol-2-ones being formed here all contain a chiral centre, at the C_5 position of the molecule and therefore each will exist as a pair of enantiomers. In order to control the stereochemistry, a chiral amine (*S*)-(-)-phenylethylamine was to be used. Diasteromers would be formed, which could be isolated and biologically tested to see if the chirality of these compounds played a part in any CCK activity observed. Homologues of cyclic-amines were used, i.e. cyclo propyl, pentyl and hexylamines as CCK antagonist activity was shown from 4-amino-5-alkoxy-furanones incorporating cyclic-amines (chapter 2). Isobutylamine and its homologue isopropylamine were also used as the aforementioned amine again has shown good activity in relation to pain, depression and anxiolytic assays.

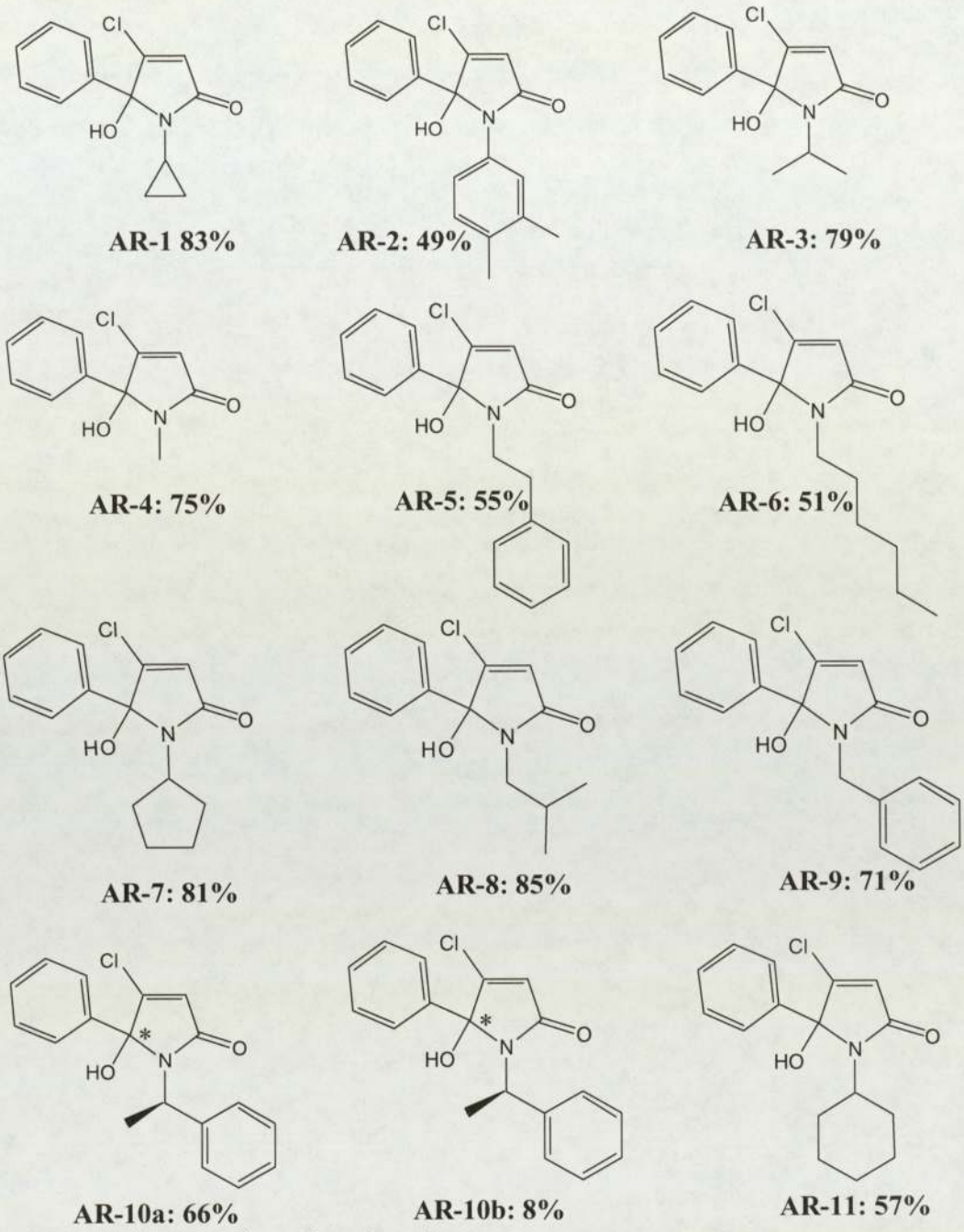


Figure 4.2.2.1. Synthesised compounds of the class **AR-X**.

3,4-Dichloro-5-phenyl-5H-furan-2-ones were dissolved in ether and cooled on ice. The appropriate amine was added to the cold solution and stirred for 30 minutes, allowing the reaction mixture to slowly warm up to room temperature. After work up, the desired compound was extracted using column chromatography (80% ether, 20% petroleum

ether) and analysed using NMR, IR and MS. Scheme 4.2.2. shows the chemical formation of these amines (**AR-X**). Figure 4.2.2.1. shows the compounds synthesised from 3,4-dichloro-5-phenyl-furan-2(5H)-one. All compounds were white or off white and crystalline. **AR-1** and **AR-8** were formed in the highest yields (83% and 85% respectively). The homologue **AR-7** with respect to **AR-1**, was formed in 81% yield, while another homologue of this compound (**Ar-11**) was synthesised in 57%. **AR-3**, a bioisostere of **AR-1** and homologue of **AR-8**, was formed in 79% yield. Diastereomers **AR-10a** and **AR-10b** were formed from using (*S*)-(-)-phenylethylamine, a chiral amine. **AR-10a** was isolated as the major stereoisomer in 66% yield, while **AR-10b** was isolated as the minor component in 8% yield. The exact diastereomeric forms for **AR-10** are yet to be determined. **AR-9**, an *N*-arylated pyrrol-2-one was formed in good yield (71%), while its homologue **AR-5**, was formed in modarate yield (55%). Figure 4.2.2.2. shows the compounds synthesised from 3,4-dichloro-5-(4-chlorophenyl)furan-2(5H)-one. The same amines were used during the formation of these compounds, with the exception of cyclohexylamine and 3,4-dimethylaniline which gave a lower yield in combination with **AR-Fur** (**AR-2**; 49%, **AR-6**; 51%) and the chiral amine, (*S*)-(-)-phenylethylamine, due to difficult isolation and no available biological data for **AR-10**, at the point of synthesis. All compounds were white or off-white and crystalline. **Cl-AR-7** and **Cl-AR-8** were formed in highest yield in 73% and 76% respectively. **Cl-AR-1**, a homologue of **Cl-AR-7** and bioisostere of **Cl-AR-3**, was synthesised in 72%, while **Cl-AR-3** itself, a homologue of **Cl-AR-8**, was formed in 69% yield. The *N*-arylated-pyrrole **Cl-AR-9** was synthesised in 59% yield, while its homologue **Cl-AR-5**, which contained an extra CH_2 unit, was formed in a lower yield of 45%. In general cyclic and short chained alkylamine were synthesised in the highest yields and the **Cl-AR-X** template produced compounds in lower yields compared to the **AR-X** series. These tetramic acid derived compounds, which have been synthesised by a new novel synthetic route, will be included in the nociceptic assay developed in chapter 1b, so that compounds possessing CCK antagonist activity can be selected and biologically tested further.

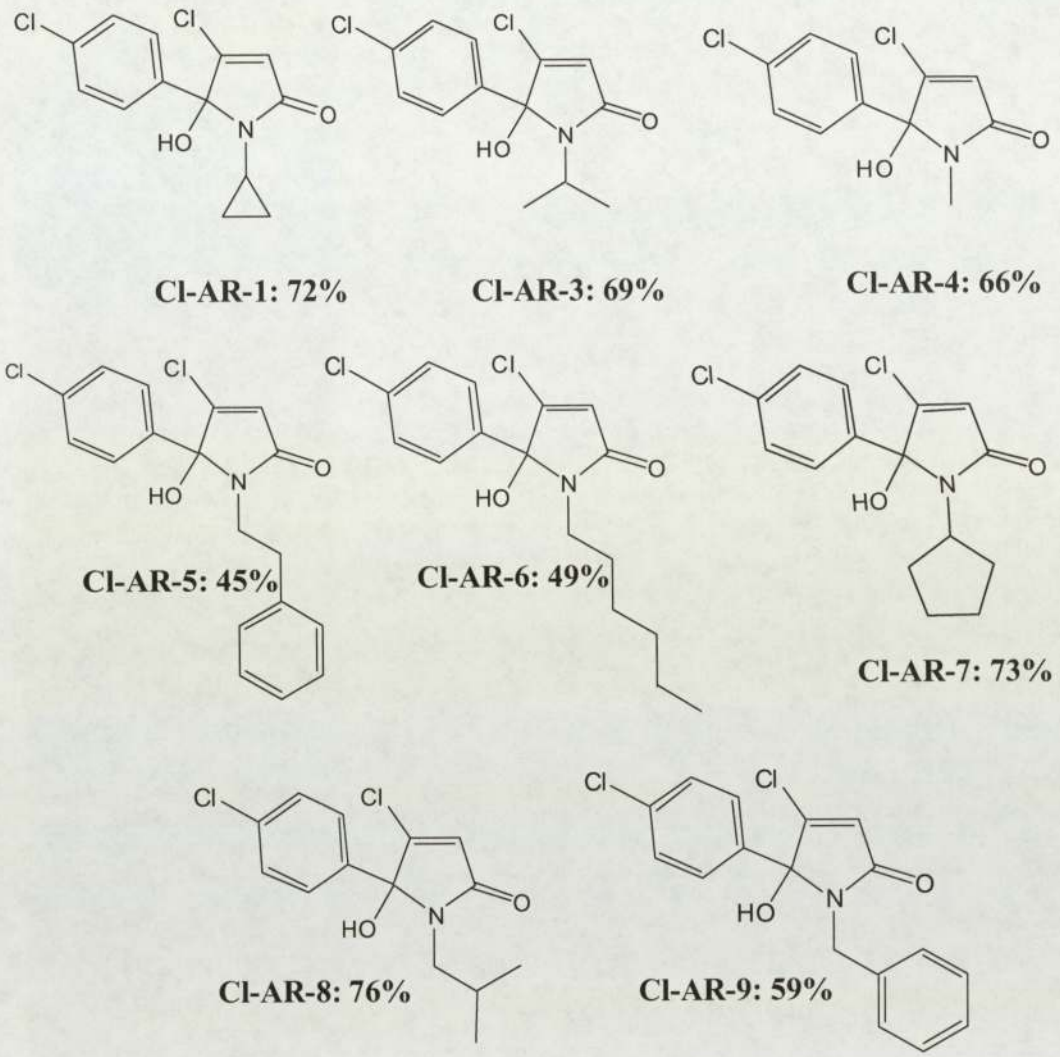
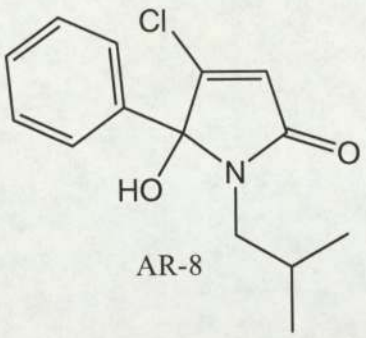


Figure 4.2.2.2. Synthesised compounds of the series **Cl-Ar-X**.

Compound **AR-8** was put forward for X-ray crystallography. Figure 4.2.2.3. shows the results from this study and confirms as predicted, that **AR-8** exists as a pair of enantiomers of the 5-arylated-5-hydroxy-pyrrol-2-one template shown below. Further X-ray crystallography data can be found in the experimental chapter (section 6.4). This compound alongside the other compounds synthesised here were fully characterised using ^{1}H and ^{13}C NMR, MS and IR spectroscopy. The spectra obtained for **AR-8** provided further evidence that this general class of compound had been formed.



AR-8

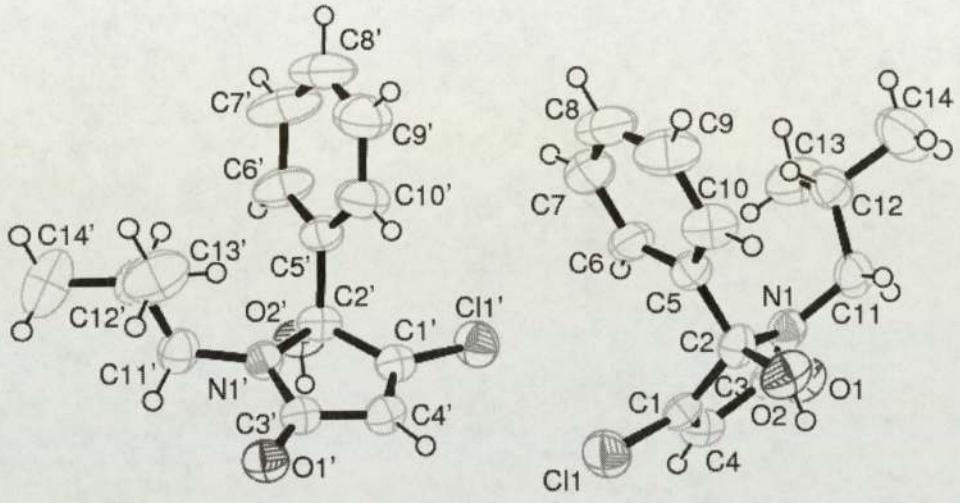
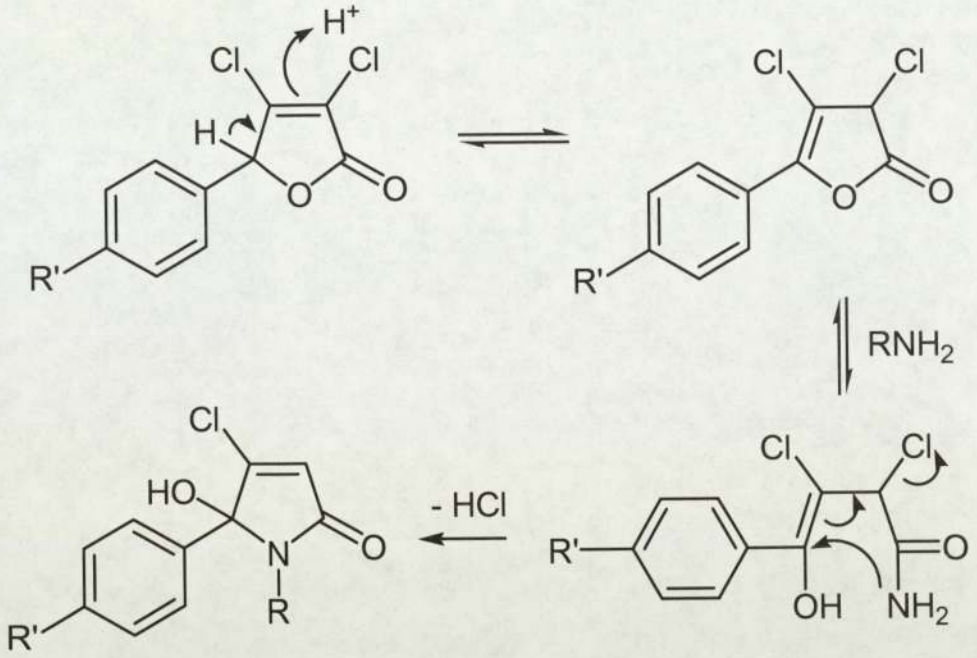


Figure 4.2.2.3. Crystal structure of the independent molecules of **AR-8**.

4.2.3. Proposed Mechanism for the synthesis of 5-hydroxy-5-aryl-pyrrol-2-ones.

The proposed mechanism for the formation 5-hydroxy-5-phenyl-pyrrol-2-ones can be seen in scheme 4.2.3. It is believed that after a tautomeric shift of the vinylic bond, the amine attacks the carbonyl carbon. This results in the breakage of the furanone ring, which undergoes nucleophilic attack and loss of HCl during the cyclisation and formation of the 5-hydroxy-5-aryl-pyrrol-2-ones (**AR-X** and **Cl-AR-X**).



Scheme 4.2.3. Proposed mechanism for the formation of the class of compounds **AR-X** and **Cl-AR-X**.

4.2.4. Reactivity of the 4-chloro- and 5-hydroxy-groups of 5-hydroxy-5-aryl-pyrrol-2-ones.

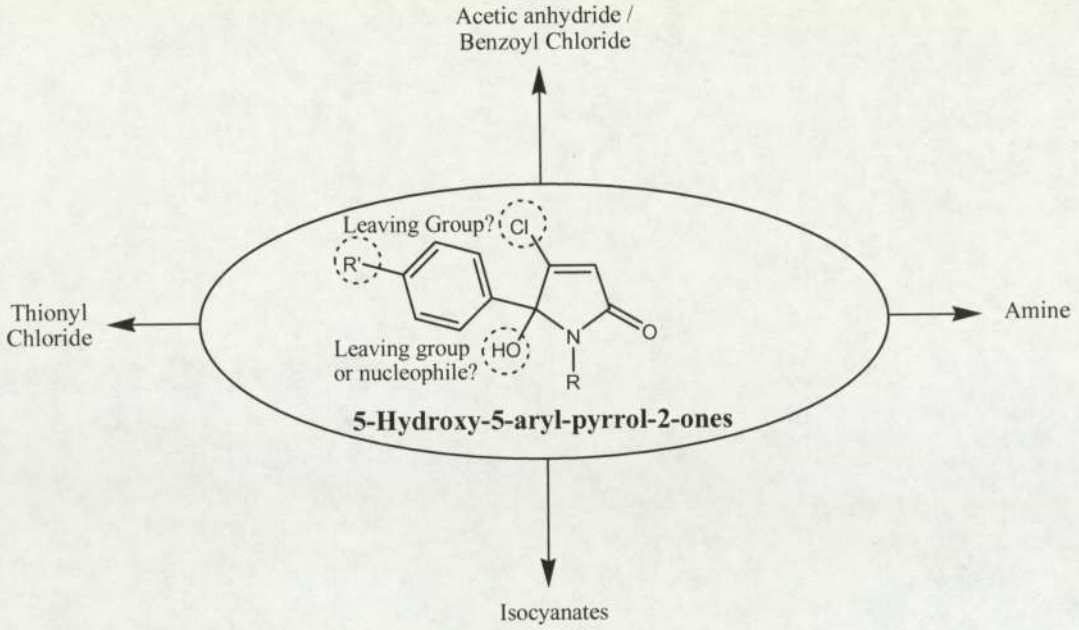


Figure 4.2.4. Reagents used to show the reactivity of the 5-hydroxy and 4-chloro groups of 5-hydroxy-5-aryl-pyrrol-2-ones.

A range of reagents (figure 4.2.4.) were used to find out if any of the 5-hydroxy, 4-chloro or chloro *para*-arylated groups present in the synthesised pyrrol-2-ones were reactive groups. This information can be used to predict if any unwanted biological reactions (involving these groups) in animals or humans would occur. Acetic anhydride, benzoyl chloride and and isocyanate were added to see if the hydroxyl group had nucleophilic properties, while thionyl chloride was used to see if the hydroxyl group could be turned into a good leaving group. Excess amine was also added to see if the 4-chloro group or aryl chlorine (R' = Cl) was a leaving group. It was predicted that no reaction would occur here, as during the formation of these pyrrol-2-ones 3 equivalents of amine had been added and no reaction had been seen at that time. All reagents were added in excess to the pyrrol-2-ones and monitored for 3hrs at RT and 1hr under refluxing conditions. In each case no reaction had taken place. This was confirmed by TLC, MS and ^{1}H NMR which showed starting material. These tests confirmed that the above mentioned groups were not reactive.

4.3. Biological evaluation of compound 5-hydroxy-5-phenyl-pyrrol-2-ones.

Selected 5-hydroxy-5-phenyl-pyrrol-2-ones, synthesised in the previous section (figure 4.2.2.1. and 4.2.2.2.) were incorporated into the nociceptic (thermal tail flick; chapter 6.6) tramadol potentiation assay developed in chapter 1b to assess and select compounds showing CCK antagonist activity. Some compounds from the **Cl-AR-X** series were not tested at this point, due to cost and time efficiency, but were to be tested at a later date, if the **AR-X** series showed activity. Two compounds from the **Cl-AR-X** series, **Cl-AR-1** and **Cl-AR-8**, were chosen specifically for testing and for a comparison to **AR-8** and **AR-1**, due to the 3-amino-side chains present in these molecules showing good CCK activity in other biologically active compounds including certain 4-amino-5-alkoxy-furanones synthesised in chapter 2. These assays were conducted in collaboration with Khon Kaen University

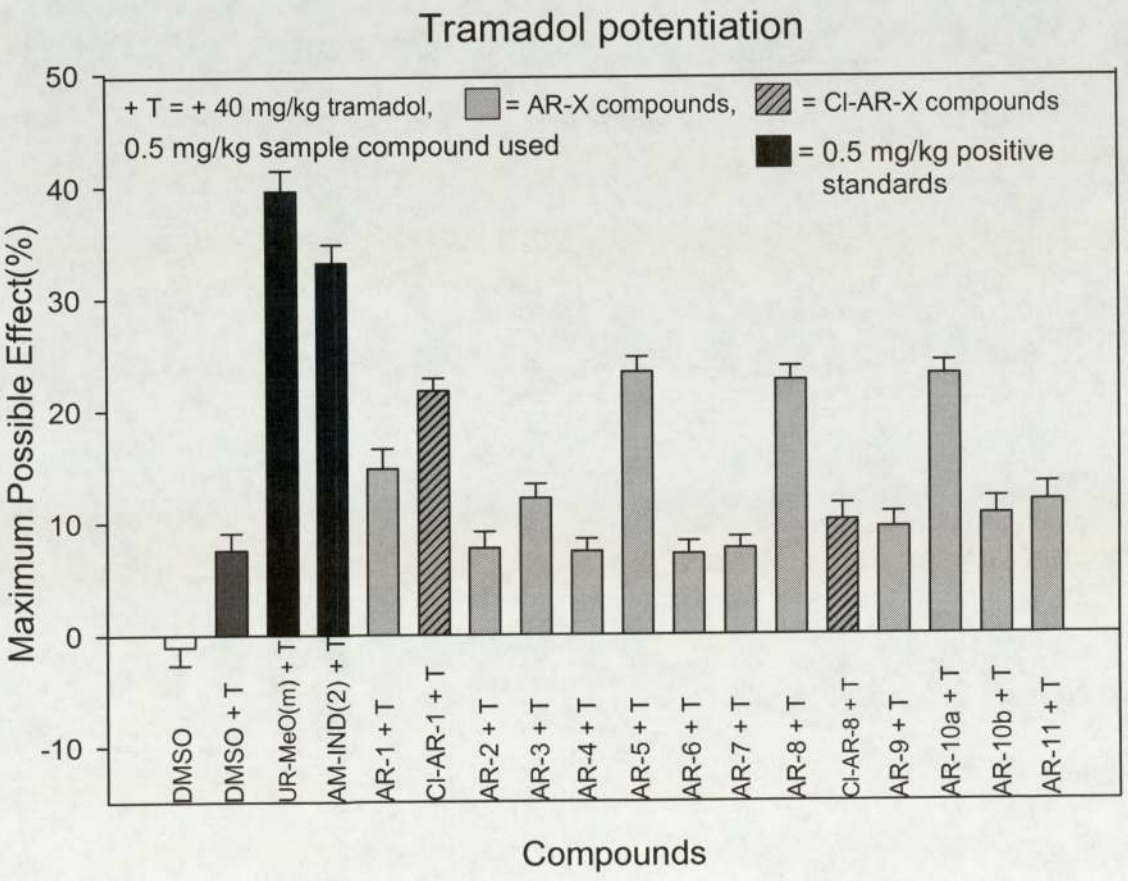


Figure 4.3. MPE of **AR-X** and **Cl-AR-X** compounds during tramadol potentiation.

Figure 4.3. shows the results obtained for series **AR-X** (figure 4.2.1.) and **Cl-AR-X** (figure 4.2.2.) from the nociception assay involving tramadol potentiation. **AR-8**, **AR-5**, **AR-10a** and **Cl-AR-1** gave the highest tramadol potentiation of the compounds tested, but showed only a moderate MPE (20-24%) in comparison to the standards tested here (**UR-MeO(m)**; 39.6%, **AM-IND(2)**; 33.2%). **AR-5** gave the highest MPE at 23.4%. **Cl-AR-1** gave a medium MPE of 21.8%, while the corresponding bioisostere **AR-1** gave a medium to low MPE of 14.8%. **AR-8** gave one of the better MPEs seen (22.7%), while it bioisostere **Cl-AR-8** gave a low effect of 10.2%, similar to that seen with tramadol alone (7.6%). **AR-3**, a homologue of **AR-8** and **AR-11**, a homologue of **AR-7**, gave medium to low MPEs of 12.1% and 11.8% respectively, while other compounds gave effects comparible to tramadol alone. These biological results and those obtained for the furanones synthesised in chapter 2, suggest that compounds incorporating either an isobutyl or a cyclopropyl-amino side chain in general yield agents possessing CCK antagonist properties. The two chloro-aryl pyrrolones **Cl-AR-1** and **Cl-AR-8**, which are more lipophilic than compounds in the **AR-X** series, also give medium high MPEs, suggesting that this chloro *para*-aromatic substituent maybe required for optimising CCK activity.

AR-10a, a diastereomer, also achieved a high MPE (23.2%), whilst the other diastereomer **AR-10b** showed a low medium MPE (10.7%). It is possible that **AR-10a**, mimics the diphenyl system of the pyrazolone standards (figure 4.3.0.) therefore showing CCK antagonist activity.

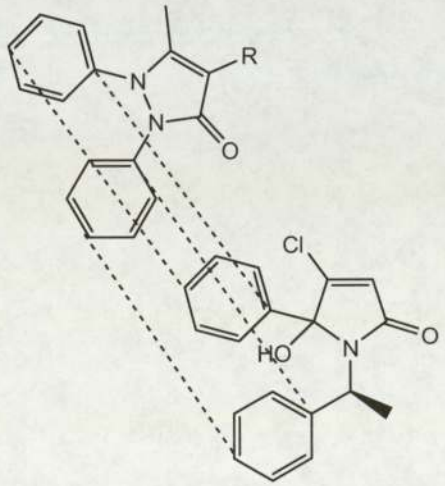


Figure 4.3.0. How **AR-10a** could mimic the diphenyl system of pyrazolones.

AR-8 and **AR-10a**, have been selected for further biological testing. **AR-8**, although displaying a slightly lower MPE than **AR-5**, was chosen due to its high yield (85%, compared to 55% for **AR-5**) and crystallinity. It was also chosen due to the presence of the isobutyl group which is present in other active CCK antagonists synthesised in chapter 2 and in other active compounds such as asperlicin[104]. The diastereomer **AR-10a** was chosen due to its stereochemistry, as unlike other **AR-X** synthesised here, it does not exist as a pair of enantiomers and therefore any activity is due to this diastereomer alone. **Cl-AR-1**, although synthesised in a slightly higher yield (72%) than **AR-10a**, had a lower MPE value than **AR-8** and **AR-10a** and therefore due this reason and due to financial restrictions will be tested at a later date after further data has been obtained for the **Cl-AR-X**.

4.3.1. Antidepressant assay: Swimming Test.

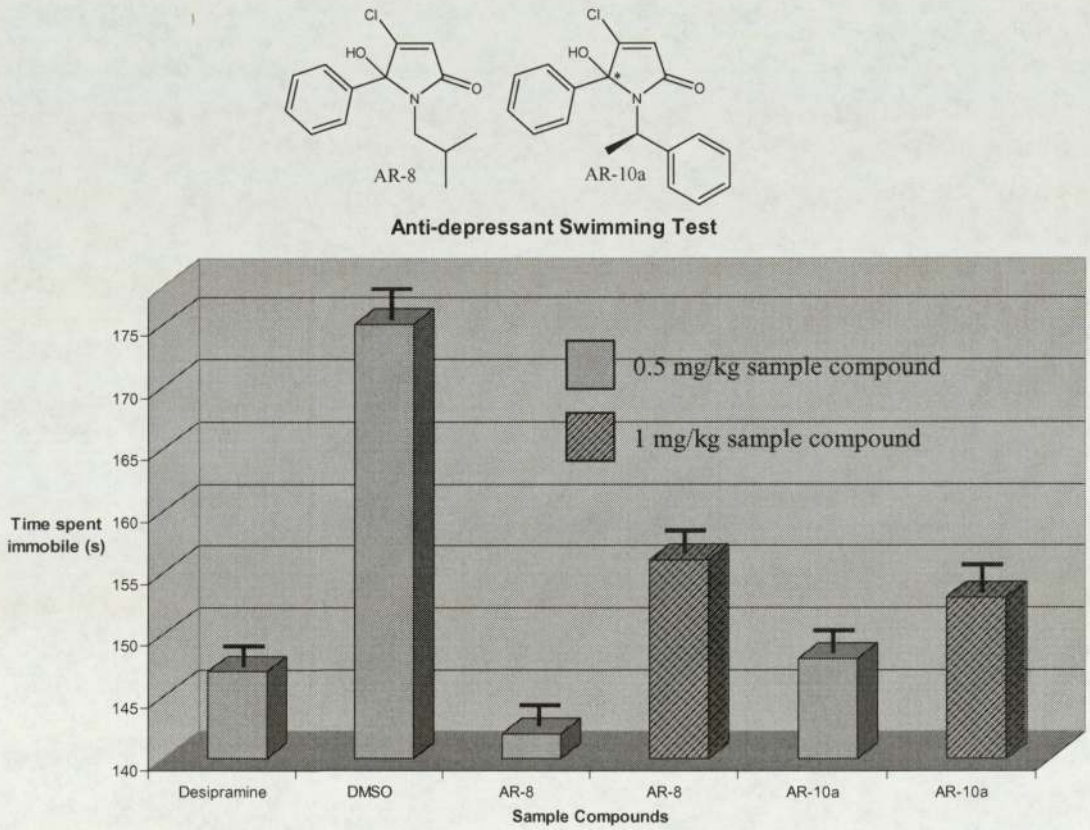


Figure 4.3.1. Results from the antidepressant swim test for **AR-8** and **AR-10a**.

Antidepressant drugs have the effect of reducing the duration of immobility in the despair swim test (immobility time test)[118, 181]. This experiment was carried out using two different doses of the **AR-X** compounds (figure 4.3.1.), 0.5 and 1 mg/kg. 1 mg/kg Desipramine, an antidepressant drug, was used as a positive control. DMSO was used as a negative control (zero concentration of sample compound) and had an immobility time of 175 seconds. The ligand **AR-8** resulted in mice having a lower immobility time (142 seconds) when compared to the standard (147 seconds) at a very low dose (0.5 mg/kg), showing that **AR-8** could possess better antidepressant properties than desipramine. (figure 4.3.1.).**Ar-10a** showed a slightly greater immobility time (156 seconds) than desipramine, suggesting lower antidepressant activity than both **AR-8** and the standard. At the higher dose of 1mg/kg immobility time increased significantly for both **AR-8** and **AR-10a**, indicating no antidepressant properties at doses above 0.5 mg/kg.

4.3.2. Anxiolytic assay: X-maze.

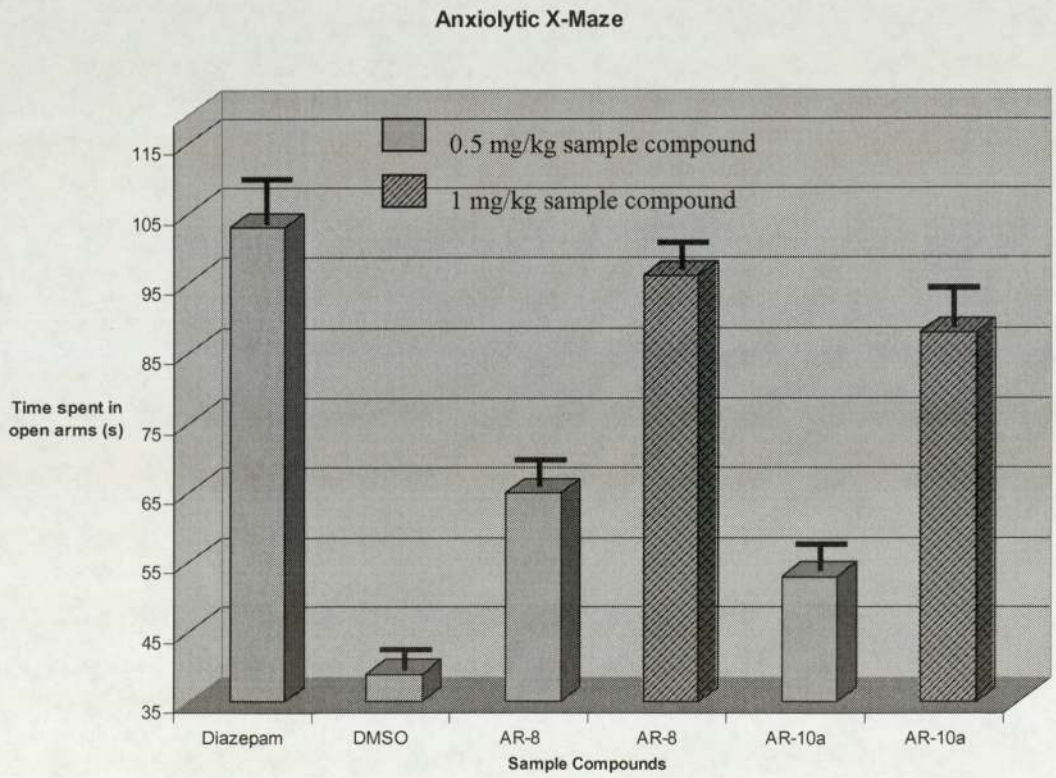


Figure 4.3.2. Results of the X-maze test for **AR-8** and **AR-10a**.

Mice were placed in an elevated plus-maze (X-maze)[116] as described in chapter 6.6 (section 6.6.5.) to monitor a mouse's anxiety level. The anxiolytic drug diazepam was used as a standard positive control in this assay. DMSO was used as a negative control (zero concentration of sample compound). Two concentration of the sample compounds, 0.5 and 1 mg/kg, were used. At the lower dose (0.5 mg/kg), animals injected with **AR-10a** showed a minimal increase in the time spent in the open arm platform (53 seconds) compared to the negative control (DMSO, 39 seconds). The results of this assay are shown in figure 4.3.2. Mice injected with **AR-10a**, resulted in a slightly higher open arm platform time (64 seconds). This suggests that both of these sample compounds do not possess any significant anxiolytic properties at doses lower that 0.5 mg/kg. At the higher dosage (1 mg/kg), there was a significant increase in the time spent in the open arms posture for both both of the ligands **AR-8** (96 seconds) and **AR-10a** (88 seconds). **AR-8**, in particular, showed an open arms platform time approaching that of the standard positive control drug, diazepam (103 seconds), indicating anxiolytic properties at 1 mg/kg.

4.4. Conclusion.

5-Hydroxy-5-aryl-pyrrol-2-ones have been synthesised and optimised via 5-aryl-furanone templates, using a diverse range of amines and substituted benzenes. A selected range of these compounds were biologically evaluated using the nocicetic assay developed in chapter 1b, to select compounds possessing CCK antagonist properties. **AR-8**, **AR-5**, **AR-10a** and **Cl-AR-1** were selected as having the best CCK antagonist activity, although only moderate activity with respect to the standard pyrazolones. **AR-8** and **AR-10a** were biologically tested further in both anxiolytic and antidepressant assays. **AR-8** showed positive results in both of these tests suggesting it possesses both anxiolytic and antidepressant properties in the low μmol region, while the diastereomer **AR-10a**, showed weak activity in both of these assays. **AR-8** could therefore be a new lead structure from which further CCK active agents can be developed. Some of the newer developed compounds in the **Cl-AR-X** series, were not tested in the nocicetic assay due to cost and time restrictions and so it would be of interest to test these for tramadol potentiation at a later date, to observe if the chloro *para*-aromatic substituent has an increased effect in CCK antagonist activity as already seen with **Cl-AR-1**.

Chapter 5: Synthesis and evaluation of but-2-enoic acid amides.

5.1. Introduction.

But-2-enoic acids are relatively well known. This class of compound contains an α,β-unsaturated ketone structure (figure 5.1.), which is a common feature to several natural antibiotics, such as penicillic acid[182] and clavacin[183]. It has also been reported that α,β-unsaturated ketones containing an adjacent aromatic group show bacteriostatic activity[184].

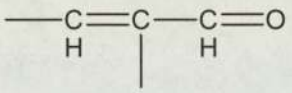


Figure 5.1. α,β-unsaturated ketone structure found in some natural antibiotics.

Papa *et al.*[185] investigated the antibacterial action of α,β-unsaturated keto acids and their derivatives of the general formula seen in figure 5.1.0., where R is aliphatic, aryl or heterocyclic, X is hydrogen or halogen and n = 1 or 2. They found that some of these compounds showed a good *in vitro* activity against several strains of Staphylococcus aureus, as well as a pronounced fungistatic activity.

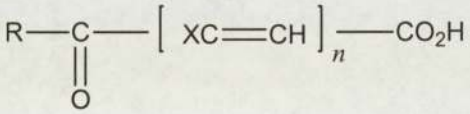
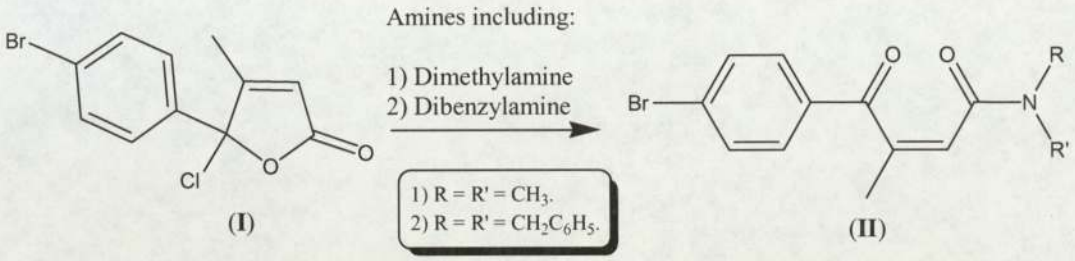


Figure 5.1.0. General structure for α,β-unsaturated keto acids and its derivatives.

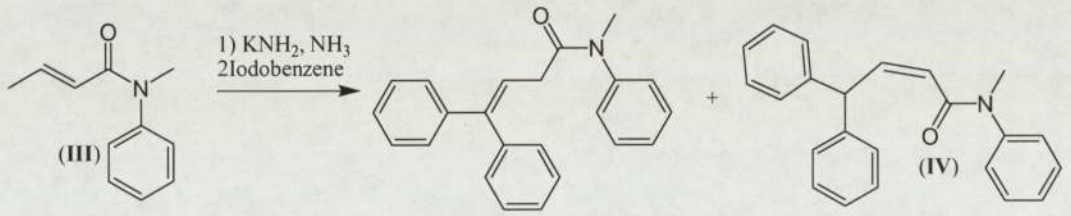
Lutz *et al.*[173] found that the corresponding acid chloride of the compound 5-(4-Bromo-phenyl)-5-chloro-4-methyl-furan-2(5H)-one (**I**, scheme 5.1.0.1), reacted with range of

amines including dimethylamine and dibenzylamine to give acyclic but-2-enoic acid amides (**II**).



Scheme 5.1.0.1. But-2-enoic acid amines from 5-arylated-furanones.

Wong *et al.*[186] formed a bis-arylated but-2-enoic acid amide (**IV**) as a by-product in 8% yield (scheme 5.1.0.2.) via the reaction between a potassium dienolate of *N*-methyl-*N*-phenyl-2-butenamide intermiediate and iodobenzene using photostimulation. This intermediate was formed as a carbanion generated from *N*-methyl-*N*-phenyl-2-butenamide (**III**) reacting with KNH_2 in NH_3.



Scheme 5.1.0.2. Synthesis of a bis-arylated but-2-enoic acid amide via photostimulation.

5.1.1. Bioisosteric similarities between but-2-enoic acid amides and ureidobenzodiazepines.

From an initial structural comparison between certain derivatives of the class of but-2-enoic acid amides and ureidobenzodiazepines it has been deduced that there are structural similarities, suggesting that these but-2-enoic acid amides could be potential CCK antagonists. Figure 5.1.1. below shows the structural similarities of the antagonist **L-365,260** and **3A-1**, which appeared to be the best amide to mimic Merck's compounds. Amide terminal groups with bioisosteric similarities to the aromatic amide group featured in **3A-1**, i.e. cyclohexyl, benzyl piperazyl and isobutyl groups, were chosen for this comparison, as it was predicted that these types of groups would be required in order to closely mimic the biological properties of **L-365,260**.

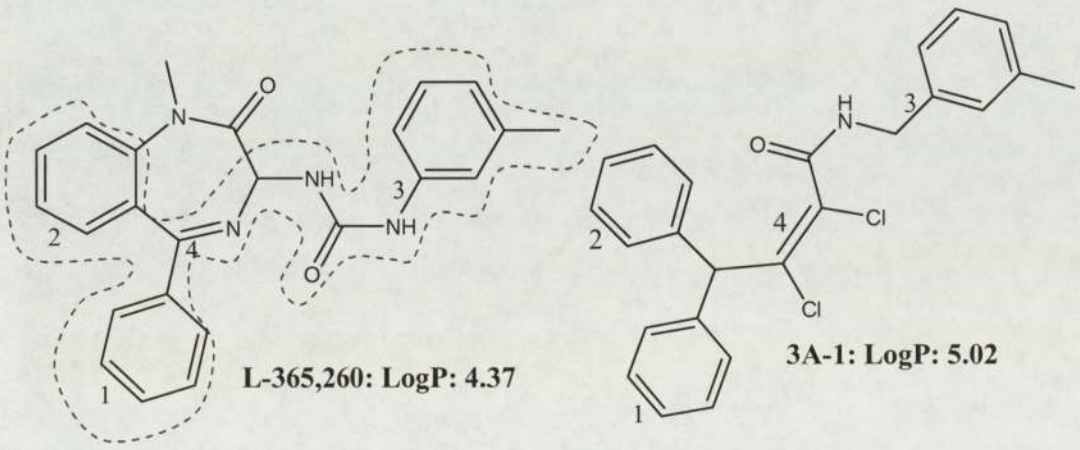


Figure 5.1.1. A comparison of the structural similarities of **L-365,260** and **3A-1**.

From a comparison of the above two structures (figure 5.1.1) it is evident that there are a number of bioisosteric similarities, for example an aromatic amide or aromatic urea group (**3**) and two aromatic rings (**1**) and (**2**). **L-365,260** contains an imide group (**4**), whereas **3A-1** contains a vinylic group (**4**). There are also 6 linker units between the left and right aromatic rings in both compounds, suggesting similar structural lengths. These

initial results lead to a further comparison using molecular modelling to look for spatial as well as structural similarities between these compounds (section 5.1.2.1.).

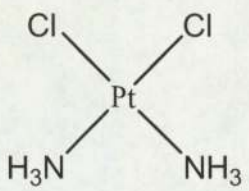
Fundamental physiochemical features of CNS drugs[187] are related to their ability to penetrate the blood-brain barrier and exhibit CNS activity[188]. One of these properties, the lipophilicity (LogP)[189] of a compound, is expressed as a ratio of solubility in octanol to its aqueous solubility and is a useful tool for the prediction and optimization of a potential drug. Lipophilicity was the first descriptor identified as important for CNS penetration and is one of the "Rule of Five", which predict that good absorption or permeation for a compound is more likely when:

- Oil / water distribution coefficient (LogP) is ≤ 5,
- Molecular weight is ≤ 500,
- Hydrogen bond donors ≤ 5 (expressed as a sum of OHs and NHs),
- Hydrogen bond acceptors ≤ 10 (expressed as a sum of Ns and Os),
- Number of rotational bonds < 10.

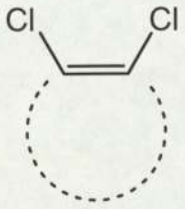
Figure 5.1.1. also shows the LogP, of these two compounds (**L-365,260** = 4.37 and **3A-1** = 5.02), both values being around 5, indicating good absorption or permeation. These five rules will be taken into consideration during the synthesis of any bis-arylated but-2-enoic acid amides.

There is also a possibility that this group of but-2-enoic acid amides could possess properties similar to the anti-cancer drug cisplatin[190], due to the presence and positioning of the two chlorines (figure 5.1.1.1.). These potential CCK antagonist agents could therefore act as dual action drugs.

Cisplatin is widely used for the treatment of various cancers including testicular, ovarian[191], lung, bladder, head and neck cancers[192]. Cisplatin is believed to kill cancer cells by binding to DNA by chloride displacement and interfering with its repair mechanism – leading to cell death[193]. It was first used to treat patients in 1971 and is used in combination with other drugs[194] to treat solid tumours. This cancer agent is one of the most successful chemotherapeutics to date. One major drawback with cisplatin is the number of undesirable side effects including nausea, renal toxicity, and neurotoxicity[190].



Cisplatin



Vinylic bond of dichloro-but-2-enoic acid amide

Figure 5.1.1.1. Diagram to show the reactive chlorines present in cisplatin and but-2-enoic acid amides.

5.1.2. Molecular modelling and drug design.

Intimate knowledge of the 3D structure and analysis of the structural chemistry of a target are essential for the process of structure based design. Strong and selective interactions between drug and target require both of these to be chemically and structurally similar and therefore reliable structural information is needed[195].

Desktop modelling tools are now generally affordable and widely available. A combination of the vast increase in speed and power of computers and the improvements in computational techniques has helped in the discovery of lead structures, with the aid of detailed receptor or active site information. It is now possible to use computer tools to build and dock ligands or inhibitor (new leads) prior to investigating time and resources for synthesising or testing and it is thought that molecular modelling is essential for understanding and exploring the structure-function relationship.

Molecular modelling[196] and docking techniques are becoming increasingly important in drug design for therapy treatment of diseases such as AIDS, cancer and Alzheimer[197]. Targeted studies, which seek to understand the basic chemistry and physiology of these diseases, are more promising. For example human immunodeficiency virus (HIV) proteinase inhibitor development[198] is one of the most important findings with regards to structure-based drug design. By possessing the structure of Rev and Rex

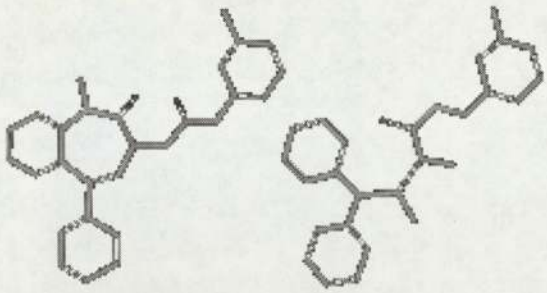
transactivator proteins of HIV-1 and T-cell leukaemia virus[199], it was possible to compare structures of normal and pathological molecules in order to design inhibitors of pathogenic enzymes and receptors using molecular modelling.

Alzheimer's disease[200] is the most common cause of dementia in adults, affecting approximately 10% and 50% of the population over the ages of 65 and 85 respectively. It is characterised by senile plaquets and cholinergic deficits as the disease progresses. Improvement of cholinergic neurotransmission is the basis of some of the drugs currently used; by where they inhibit acetylcholinesterase (AChE), which is the enzyme responsible for acetylcholine hydrolysis. Among the drugs currently used for the treatment of this disease are tacrine (Cognex) and donepezil (Aricept). Based on the orientation of how these anticholinesterase compounds are linked to the receptor, it is possible to suggest molecular modifications with the aim of optimising them, whilst still considering their pharmacodynamic and pharmacokinetic properties and synthetic viability etc[201].

5.1.2.1 Molecular modelling of L-365,260 and bis-arylated but-2-enoic acid amides.

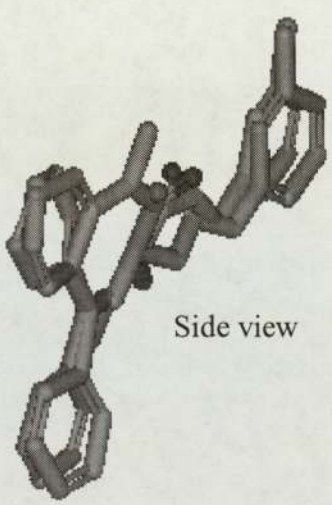
A selection of structures were chosen in section 5.1.1. based on a bis-arylated but-2-enoic acid amide template, in order to find a molecule that could mimic Merck's ureidobenzodiazepines **L-365,260**. The best closely matched molecule was **3A-1**, which contained a terminal aryl amide group. This compound will now undergo a comparison to **L-365,260** using molecular modelling studies (webviewer 5.0).

Figure 5.1.2.1. shows the molecular modelling results and comparison of Merck's **L-365,260** and **3A-1**. Both the plan and side views of **3A-1** show a good overlap with respect to **L-365-260**, especially in relation to the terminal aromatic groups. The butenyl unit of **3A-1** also appears to mimic part of the benzodiazepine ring of **L-365,260**. Any regions of **3A-1** that do not fully overlap with the corresponding areas of **L-365,260**, can be compensated for by the sp3 hybidised carbons which are present in this amide, i.e. numerous bonds of **3A-1** have the ability to rotate and mimic **L-365,260** in order

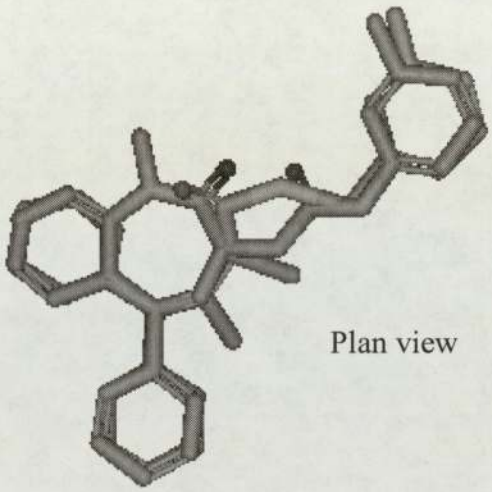


L-365,260 3A-1

Atom	L-365,260	3A-1 (Amide)
Carbon		
Nitrogen		
Oxygen		
Chlorine		



Side view

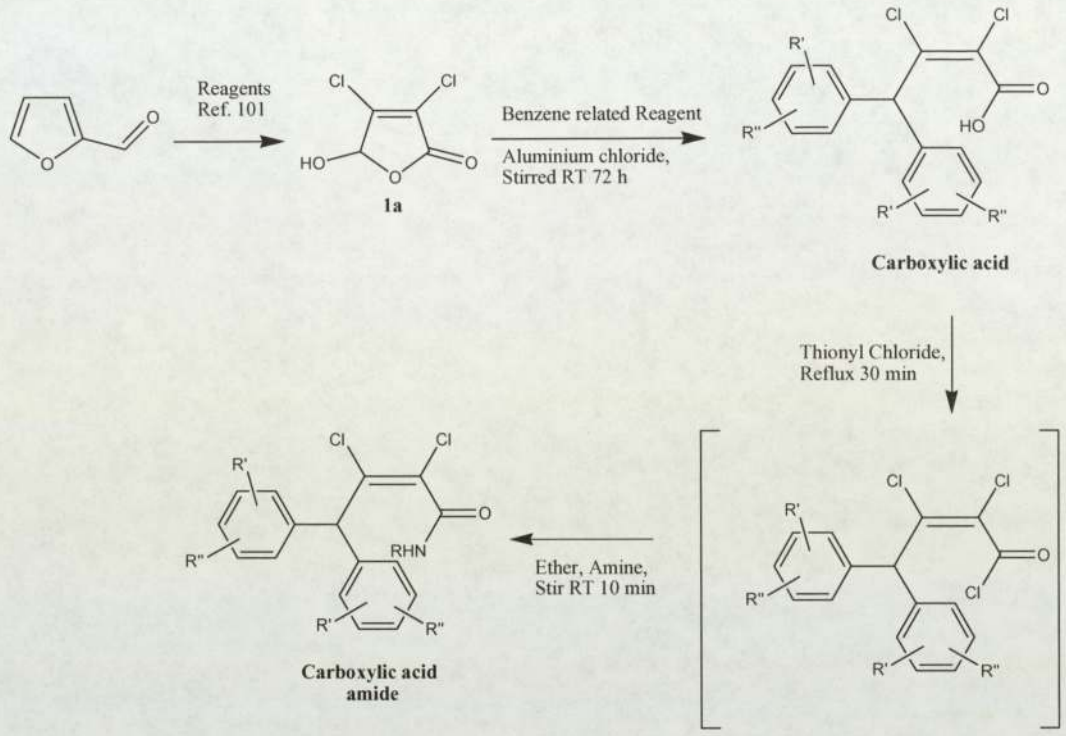


Plan view

Figure 5.1.2.1. Molecular modelling results from Merck's **L-365,260** and a **3A-1**.

to fit in to the specified receptor. In conclusion the overall shape of these molecules appears to be very similar and therefore there is a good probability that these new bis-arylated but-2-enoic acid amides could possess CCK antagonist properties.

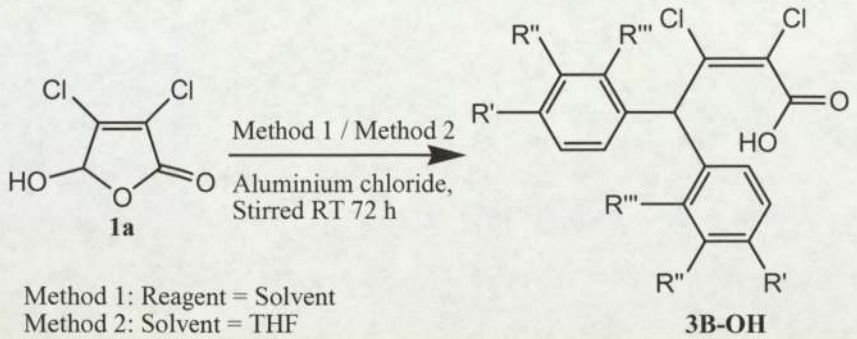
5.2. Synthesis of 2,3-dichloro-4,4-di-*p*-tolyl-but-2-enoic acid amides.



Scheme 5.2. Reaction overview for the synthesis of carboxylic acid amides.

As a starting point a range of bis-arylated but-2-enoic acids were to be synthesised using various benzene substituted compounds, based on the molecular modelling results (section 5.1.2.1.). These carboxylic acids would then be reacted further to form bis-arylated but-2-enoic acid amides via an acid chloride intermediate. Scheme 5.2. shows a reaction overview for the synthesis of bis-arylated but-2-enoic acid amides via a carboxylic acid and an acid chloride intermediate.

5.2.1. Synthesis of but-2-enoic acids.



Method 1: Reagent = Solvent
Method 2: Solvent = THF

Code	Method	Reagent	R'	R''	R'''	Yield (%)
Z-OH	1	Toluene	Me	H	H	75
X-OH	1	Xylene	Me	H	Me	71
AR-OH	1	Benzene	H	H	H	33
Cl-AR-OH	1	Chlorobenzene	Cl	H	H	51
NO_2-AR-OH	1	Nitrobenzene	H	NO_2	H	No reaction
MeO-OH	2	Anisole	MeO	H	H	47
$(MeO)_2$-OH	2	Veritrole	MeO	MeO	H	No reaction

Figure 5.2.1. Synthetic route for the synthesis of bis-substituted aryl-but-2-enoic acids.

A range of benzene substituted compounds were reacted with mucochloric acid in the presence of aluminium chloride at room temperature for 72 hours to form various bis-subsubstituted-aryl-but-2-enoic acids (**3B-OH**) as shown in figure 5.2.1. With the exception of nitrobenzene (meta-deactivating substituted benzene) and veritrole, which did not react, all compounds were formed as white or off-white powders, with **Z-OH** and **X-OH** being formed in the highest yields (75% and 71% respectively). These reactions were also carried out at 60 °C, to observe if an elevated temperature would yield **NO_2-AR-OH** or **$(MeO)_2$-OH**. Neither compound was formed. Other but-2-enoic acids (figure 5.2.1.) were also synthesised at this higher temperature, but in each case a lower yield was obtained.

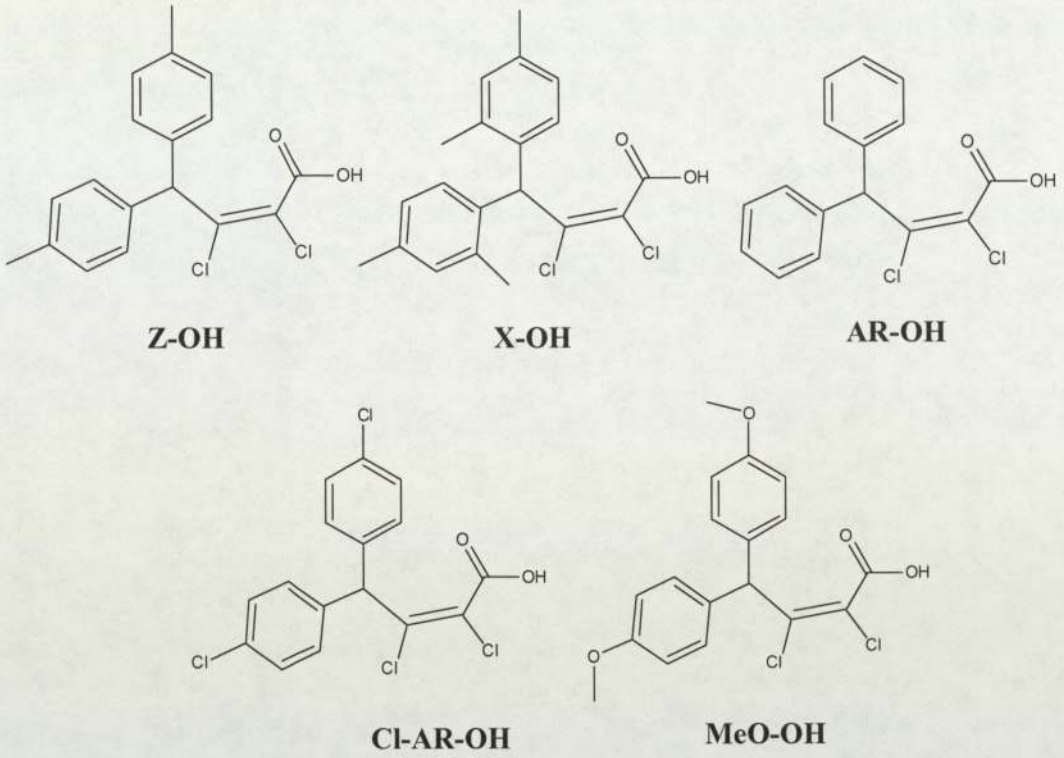
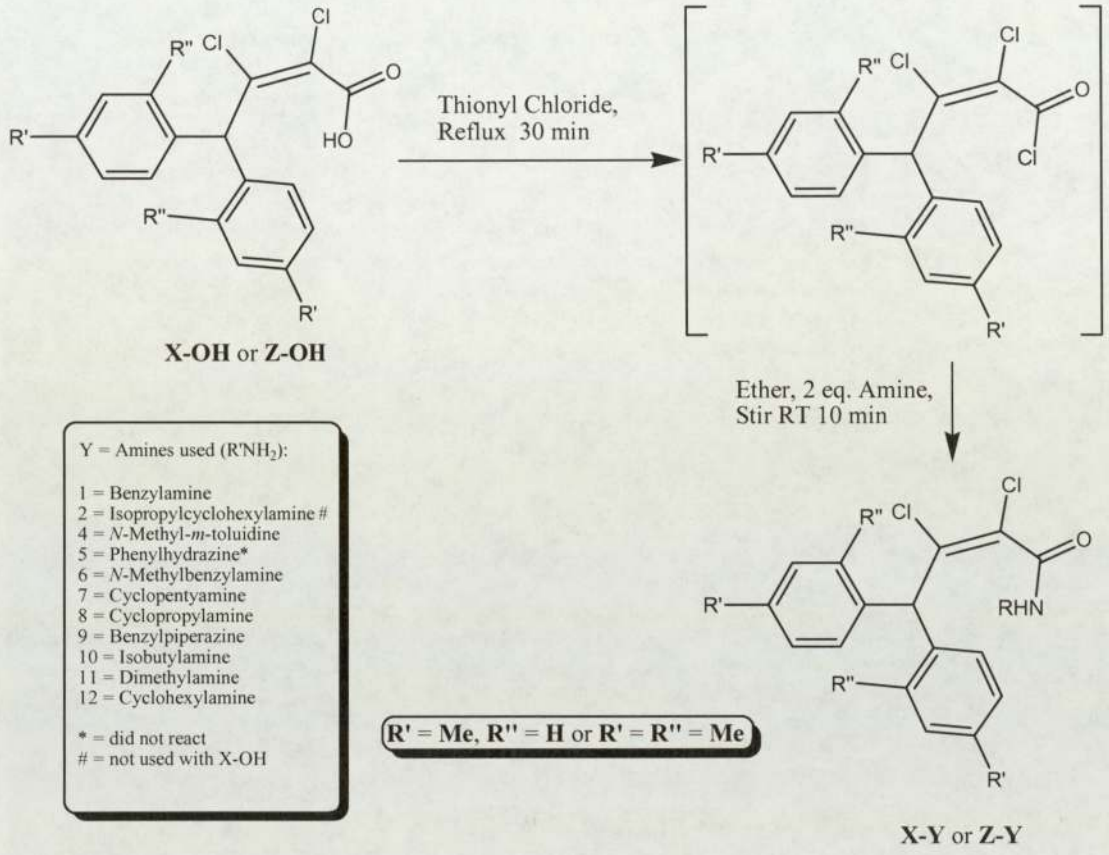


Figure 5.2.1.1. Synthesised bis-arylated but-2-enoic acids.

All of the compounds synthesised in this reaction were novel carboxylic acids with the exception of **Z-OH** and **X-OH**, which had previously been synthesised by Langley and were the starting point for the formation of these compounds. The synthesis of the template **AR-OH** had previously been attempted by Langley, but no compound had been formed. Here it had been successfully formed in 33% yield. Figure 5.2.1.1. shows the bis-arylated but-2-enoic acids synthesised here. The least lipophilic template **MeO-AR-OH**, was formed in a moderate yield (51%), while the more lipophilic chlorobenzene derivative, **Cl-AR-OH**, was formed in 47% yield. The best template to mimic Merck's **L-365,260**, is **AR-OH**, as it does not possess any aryl-substituents. A slight drawback with this compound, is its formation in a moderate to low yield. In comparison **X-OH** (71%) was formed in a higher yield, but has two methyl groups on each of the aromatic rings, thus it is expected that any receptor binding will be hindered. **Z-OH** (75%), which is formed in the highest yield, would have a slight hindrance from the single *para*-substituted methyl group, as too would **Cl-AR-OH** from the chloro-group.

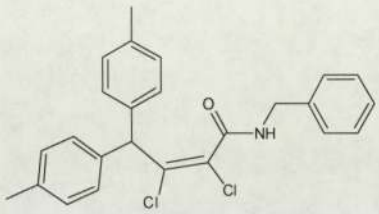
5.3. Synthesis of 2,3-dichloro-4,4-di-*p*-tolyl-but-2-enoic acid amides.



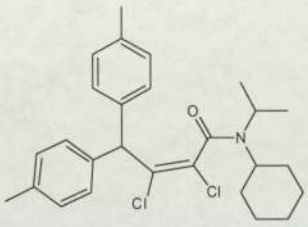
Scheme 5.3. Reaction pathway for the synthesis of the series of compounds **Z-Y** and **X-Y**.

Each of the bis-arylated but-2-enoic acids formed in section 5.2.1., were heated under reflux with thionyl chloride to form an acid chloride of the corresponding compound. A few selected amines (isobutyl- and cyclopropylamine) were instantly added to these unstable light or dark brown oils to see if deriviatives of each acid chloride would form. These two amines were chosen as they had previously been used to form analogues of other classes of compound in good yield (chapters 2, 3 and 4). Reactions were allowed to proceed at both RT in ether and at 60 °C in THF for 10 and 90 minutes, using these amines, whilst being monitored by TLC analysis. **X-OH** and **Z-OH** were the only templates which reacted to form novel but-2-enoic acid amides, with optimum yields

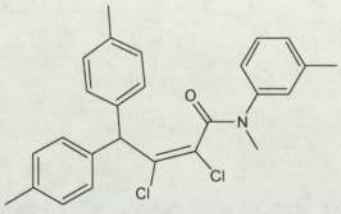
being obtained at RT in ether for 10 minutes. TLC analysis suggested that starting material was present for the other templates and that no reaction had taken. This was confirmed by proton NMR spectroscopy. In principle these non-working templates should react under the same conditions as used with **Z-OH**, as it has been shown that the same method, used in synthesising the **Z-Y** series (figure 5.3.1.), was used for the formation of the **X-Y** but-2-enoic acid amides series (figure 5.3.1.1.). Following this optimisation, a diverse range of alkyl, cyclo-alkyl, aryl, primary and secondary amines were used to synthesise a series of but-2-enoic acid amides. Scheme 5.3. shows the



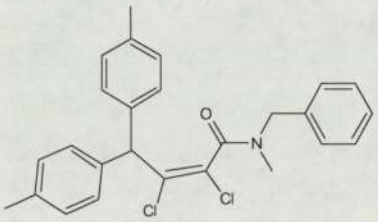
Z-1: 61% / LogP: 5.99



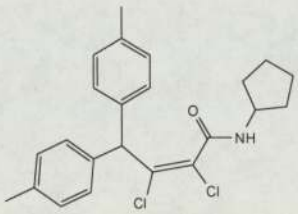
Z-2: 62% / LogP: 6.70



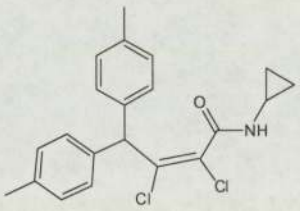
Z-4: 65% / LogP: 6.65



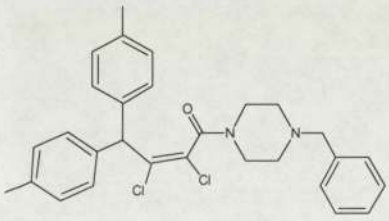
Z-6: 59% / LogP: 6.72



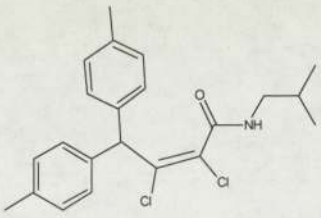
Z-7: 76% / LogP: 5.39



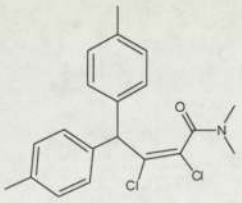
Z-8: 82% / LogP: 4.56



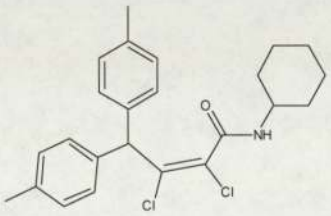
Z-9: 72% / LogP: 5.99



Z-10: 84% / LogP: 5.48



Z-11: 67% / LogP: 4.50

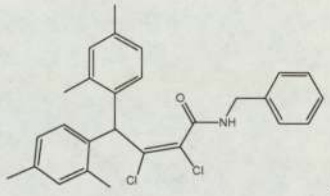


Z-12: 68% / LogP: 5.81

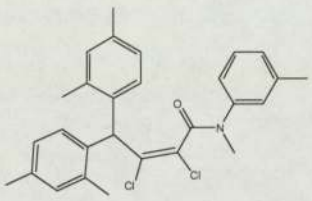
Figure 5.3.1. Synthesised compounds of the series **Z-Y**.

reaction pathway for the formation of these amides in order to try and imitate Merck's CCK antagonist **L-365,260** (figure 5.1.2.1.). The reaction was monitored using TLC and the purified products were fully characterised using IR, NMR and MS analysis.

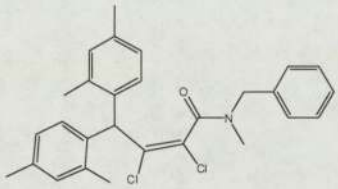
Figure 5.3.1. shows the amides formed from using **Z-OH**. All of the **Z-Y** series of compounds were formed as crystalline compounds in good yield, with the exception of phenylhydrazine, which gave a mixture and so was disregarded at this point. **Z-8** and **Z-10** were formed in the highest yields (82% and 84% respectively). **Z-7** (76%) and **Z-12** (68%), homologues of **Z8**, were also formed in good yield, although as the length of the cyclic chain increases, a decline in yield was seen. The arylated compound **Z-4** (65%), had a higher yield than its homologue **Z-6** (removal of CH_2 unit), which itself has a lower yield than **Z-1** (61%), a bioisostere of **Z-6** and structurally similar to the molecule suggested from molecular modelling studies. In general arylated amides were formed in lower yields, with the exception of **Z-9** (72%). X-ray crystallography was performed



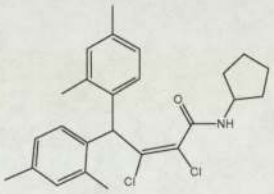
X-1: 66% / LogP: 6.97



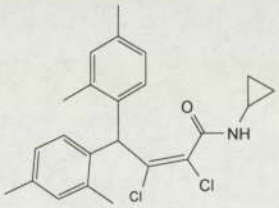
X-4: 58% / LogP: 7.62



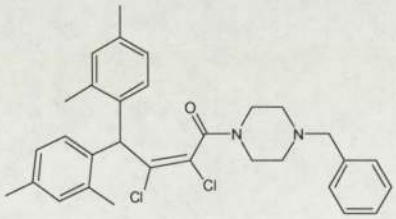
X-6: 61% / LogP: 7.20



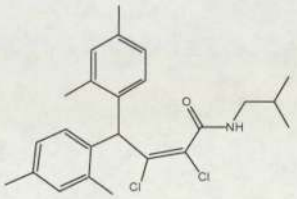
X-7: 73% / LogP: 5.53



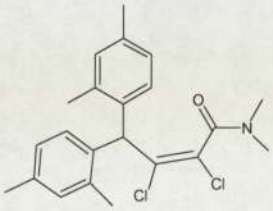
X-8: 79% / LogP: 6.37



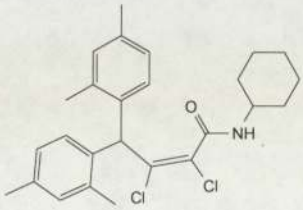
X-9: 65% / LogP: 6.96



X-10: 68% / LogP: 6.46



X-11: 59% / LogP: 5.47



X-12: 61% / LogP: 6.78

Figure 5.3.1.1. Synthesised compounds of the **X-Y** series.

using compound **Z-6** in order to confirm the general structure of these amides (see section 6.5. for structure and data).

All amines reacted with **X-OH** and were formed in good yields (figure 5.3.1.1.), but in general were formed in lower yields, when compared to the **Z-Y** series. **X-7** and **X-8** were formed in the highest yields (73% and 79% respectively), while a homologue of these compounds, **X-12**, was formed in a slightly lower yield (61%). The arylated amide, **X-1**, was formed in 66% yield, while the bioiostere of this compound, **X-6**, was formed in a lower yield of 61%. **X-4**, a homologue of **X-6**, was formed in 58%. The secondary amides, **X-9** and **X-11**, were synthesised in moderate yields of 65% and 59% respectively.

From a comparison of LogP for both **Z-Y** and **X-Y** compounds, the general trend shown was that the **X-Y** series had higher LogP values that those of the **Z-Y** series (typically above 6 for **X-Y** and below 6 for **Z-Y**), suggesting that the **Z-Y** series would have better absorption or permeation. This trend is probably due to the extra methyl group present within the xylene ring system, causing the molecule to become more lipophilic. From the **Z-Y** series compounds **Z-7**, **Z-8** and **Z-11** have LogP values closest to 5 (5.39, 4.56 and 4.50 respectively) and therefore it would be expected for these compounds to have the better absorption or permeation.

Unlike **L-365,260**, which has a chiral centre and hence results in stereoisomers (figure 5.1.2.1.), none of these novel compounds in both the **Z-Y** or **X-Y** series contain a chiral centre, therefore theoretically no enantiomers can be formed during their synthesis, making these amides both potentially chemically and biologically better. Due to the **AR-OH** template not reacting, it was hypothesised at this point that amides synthesised from using the slightly more sterically hindered **Z-OH** template would now be the closest, structurally, to match this CCK antagonist benzodiazepine and therefore the most likely series in which to find activity.

The synthesised compounds for both **Z-Y** and **X-Y** were to be put forward for nociceptic pharmacological testing in order to find CCK antagonist activity. Tramadol, a synthetic analogue of codeine, is a weak (partial) opioid agonists (analgesic) which binds at the Mu receptor, was to be used in these assays in combination with the but-2-enoic acid amides and pyrazolone standards so that tramadol potentiation could be measured. Tramadol has been shown not to cause the significant side effects such as respiratory depression, constipation or sedation as seen with common opioids[202].

5.4. Biology of but-2-enoic acid amides.

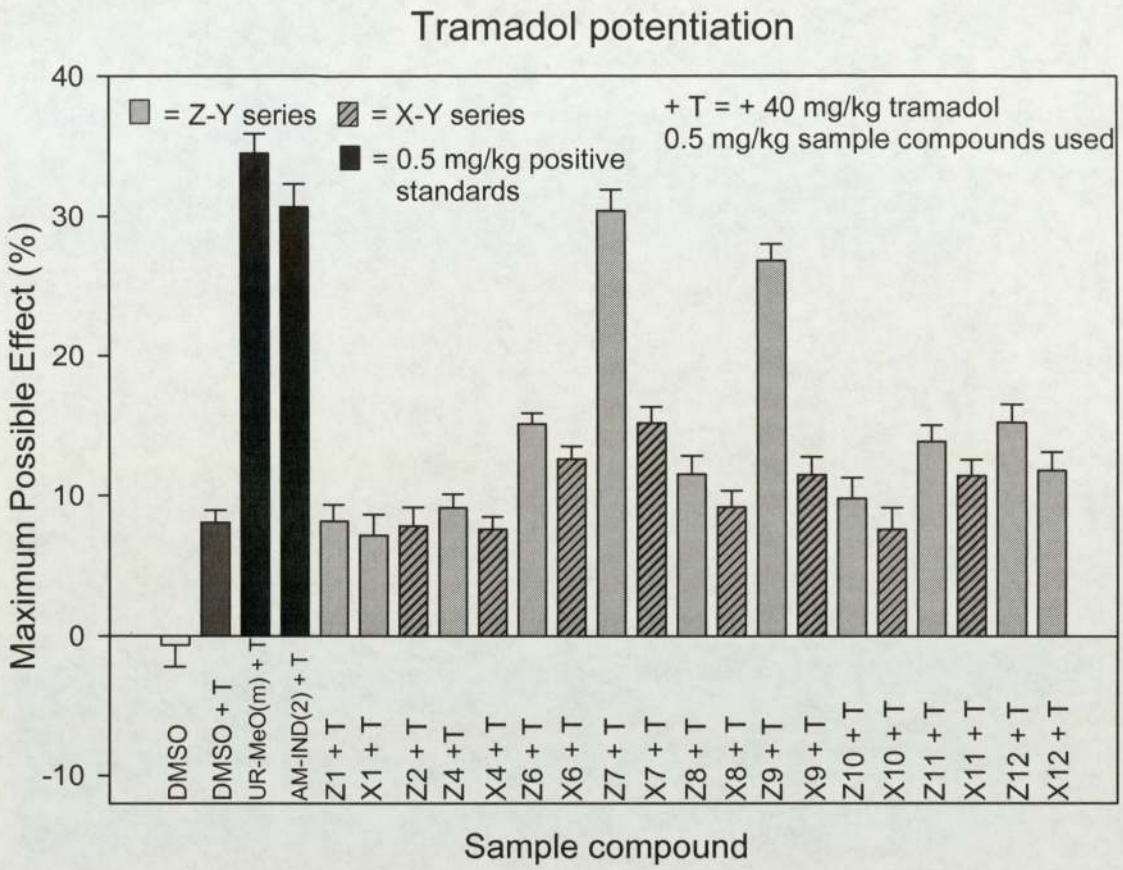
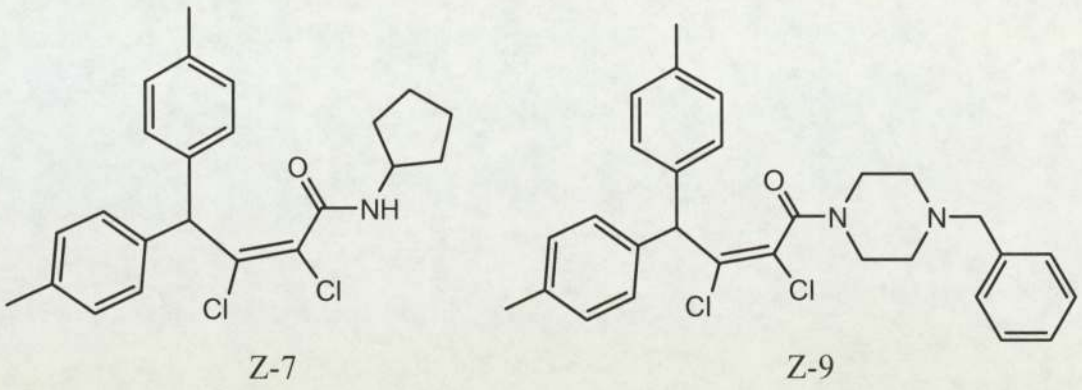


Figure 5.4. Tabulated results from nociception tests.

Both of the **Z-Y** and **X-Y** series of compounds were subject to animal studies. These assays were carried in conjunction with Khon Kaen University Male mice (six per groups) were used in a nociception (thermal tail flick test; experimental chapter 6.6) tramadol potentiation assay. The animal's tail was immersed in warm water (50 °C) and the time response for the animal to withdraw its tail from the water was measured. The maximum duration of each test was 45 seconds to avoid tissue damage.

The results from this assay can be seen in figure 5.4. As hypothesised, molecules from the **Z-Y** series in particular, **Z-7** (30.4%) and **Z-9** (26.8%), gave the highest maximum possible effect (MPE) out of the compounds tested and were noticeable better than tramadol alone. The amide containing a cyclopentyl unit, **Z-7**, had an MPE similar to

the standard pyrazolone (UR-MeO(m); 34.5%, AM-IND(2); 30.7%), whereas its bioisostere, **X-7**, had a lower MPE of 15.2%. In general the **Z-Y** series showed higher



Z-7 Z-9

Figure 5.4.1. CCK active but-2-enoic acid amides as lead structures.

MPEs, compared to the corresponding compounds in the **X-Y** series. This is thought to be due to the presence of an extra aryl methyl group. It would be hypothesised from this trend and as expected, that an unsubstituted bis-arylated amide would yield better pharmacological results, i.e. if compounds had have formed from the **AR-OH** but-2-enoic acid series. This **AR-OH** template did not however react. **Z-1** (8.2%), a similar compound to the one suggested by molecular modelling studies to mimic **L-365,260**, only had an MPE similar to tramadol alone (8.1%) and therefore poor CCK antagonist activity would be expected, whereas its bioisostere, **Z-6**, had a higher MPE (15.1%) and therefore better activity than **Z-1**. The arylated amide, **Z-9**, which contains a piperazine ring is a surprise new lead structure. This compound has the potential to be optimised and evaluated further, for example by replacing the piperazine unit for a piperadine or a pyrimidine ring system. This alteration may yield better biologically active compounds. The LogP of both **Z-7** and **Z-9** were 5.39 and 5.99 respectively suggesting reasonable lipophilicity, which was confirmed from the biological results obtained here. These lead structures (figure 5.4.1.) should now be biologically evaluated further to see if they possess anxiolytic or antidepressant properties. It would also be of interest to test these compounds as anti-cancer agent as they contain a dichlorinated vinylic unit which may mimic the structure of cisplatin and therefore bind to DNA leading to the death of cancer cells.

5.5. Summary & Further work.

During this investigation a new class of but-2-enoic acid amides were formed via but-2-enoic acids based on molecular modelling studies. Amides from this class of compound were compared to Merck's CCK antagonist **L-365,260** and were found to have similar structural and spatial properties. These findings led to the synthesis of a diverse range of bis-arylated but-2-enoic acid amides which were formed via their corresponding acid chloride. These compounds were synthesised in good to high yields, with **Z-10** and **Z-8** being formed in the best yields. These synthesised amides from the **X-Y** and **Z-Y** series (in combination with tramadol) were subject to the nociceptic assay developed in chapter 1b, to find compounds which cause tramadol potentiation and therefore possess CCK antagonist activity. Compounds **Z-7** and **Z-9** were found to cause the highest tramadol potentiation, a similar effect to that caused by the standard pyrrazolones. It would be of interest for these new lead structures to be biologically evaluated further in assay for conditions such as anxiety and depression. **Z-1** was predicted as a compound from molecular modelling to mimic **L-365,260**, but was found to be inactive. The new lead structure, **Z-9**, contains a piperazine ring, which appears to enhance the CCK antagonist activity of these amides. Further optimisation and evaluation of this piperazine unit may yield more active CCK compounds.

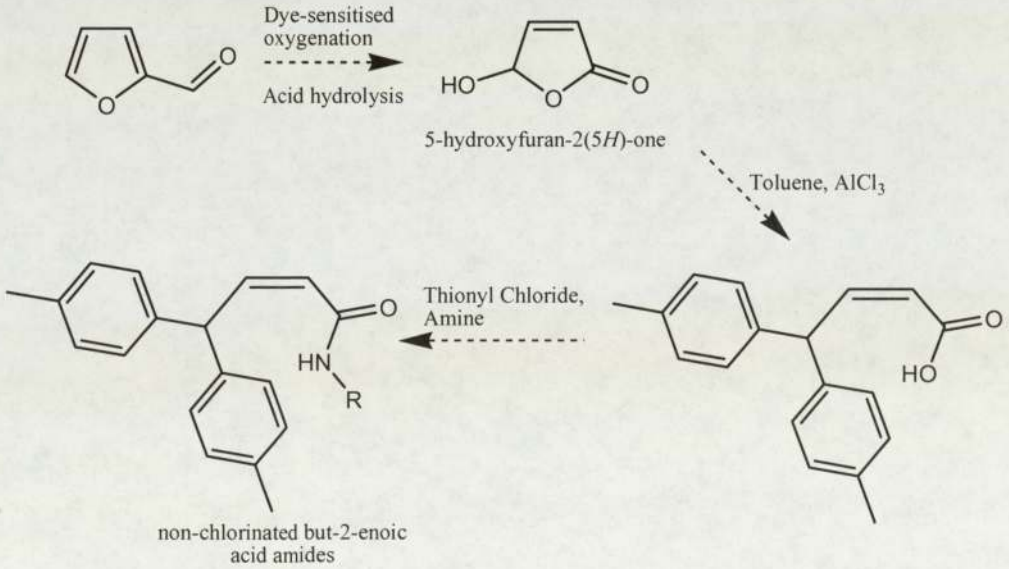


Figure 5.5. Proposed scheme for the synthesis of but-2-enoic acid amides.

The same two chlorines as previously mentioned, which are part of the but-2-enoic acid amide structure synthesised here, could potentially cause *in vivo* toxicity[132] and it would therefore be of interest to synthesis and evaluate amide derivatives of this class of compound (in particular deriviatives of **Z-7** and **Z-9**), which do not have these atoms present. These new but-2-enoic acid amides could potentially be synthesised from furfural, via 5-hydroxy-2(5H)-furanone. Furfural can be obtained from biomass. Various methods for the synthesis of 5-hydroxy-2(5H)-furanone have been reported in the literature. This furanone has been prepared by the oxidation of 2-furoic acid via singlet oxygen generated by photochemistry[203], by the reaction of furfural and hydrogen peroxide in the presence of a catalyst consisting of group V and VI metals[204] and by the oxidation of furan over a TS-1/H_2O_2 system. Another route which has been reported for the synthesis of 5-hydroxy-2(5H)-furanone is by the dye-sensitised oxygenation of furfural in ethanol followed by acid hydrolysis of the 5-ethoxy analogue[205]. This furanone can then be reacted under the same conditions as used here, using toluene and $AlCl_3$, forming the corresponding but-2-enoic acid, which itself can be reacted with amines giving non-chlorinated but-2-enoic acid amides (scheme 5.5).

Chapter 6: Experimental.

6.0. General Methods.

The majority of chemicals were either obtained from the laboratory, chemical stores or were ordered from Lancaster, Aldrich, Fischer or Avocado 2003/2004/2005/2006 chemical catalogues.

Nuclear magnetic resonance proton and carbon spectra were obtained on a Bruker AC250 spectrometer operating at 250 MHz and 62.9 MHz respectively which was calibrated with the solvent reference peak (deuterated chloroform or DMSO). Mass spectrometry was performed by Atmospheric Pressure Chemical Ionisation (APCI), positive or negative mode, using a Hewlett-Packard 5989B quadrupole instrument, which was connected to an electrospray 59987A unit with automatic injection (Hewlett-Packard 1100 series autosampler). Samples were dissolved in HPLC grade methanol.

Infra-red spectra were obtained from using KBr discs on a Mattson FTIR spectrophotometer. Column chromatography was performed using Prolabo silica gel_{60}. Thin layer chromatography was obtained using aluminium sheets, silica gel_{60} F254 and visible ultraviolet light. A 1% solution of potassium permanganate was prepared and used where appropriate to develop the thin layer chromatography plates. Melting points were recorded from a Stuart Scientific Melting Point (SMP1) apparatus. Unless otherwise stated all compounds were formed as solids.

X-ray crystallography was performed using an Enraf-Nonius CAD-4 diffractometer. Samples were recrystallised using methanol. Data collection and cell refinement occurred using CAD-4 software. SHELXS97 software was used to solve the structure, for structure refinement and for presentation.

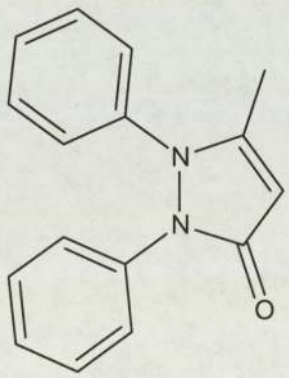
6.1. Experiments to Chapter 1b.

(CHECKED BY S.DUNN)

(DPP-H): 5-Methyl-1,2-diphenyl-1,2-dihydro-pyrazol-3-one.

Method:

Diphenyl hydrazine (20g, 0.11 mol) and ethyl acetoacetate (3 eq. 42 ml, 0.31 mol) were stirred and then heated under reflux for 4 hours with a Dean and Stark trap, in order to remove water, ethanol and some of the excess ethyl acetoacetate. The off black solution was subject to vacuum to remove any remaining ethyl acetoacetate. Ether (30 ml) was added to the resulting crude black viscous liquid to precipitate out black crystals which were filtered This washing process was repeated 3 times to remove any unreacted diphenyl hydrazine. The compound was recrystallised from toluene, resulting in off black needle shaped crystals.



Yield = 52%

Melting Point: 152-155 ^{o}C

Rf (Ether) = 0.12

Molecular Weight: 250.3

Molecular Formula: $C_{16}H_{14}N_2O$

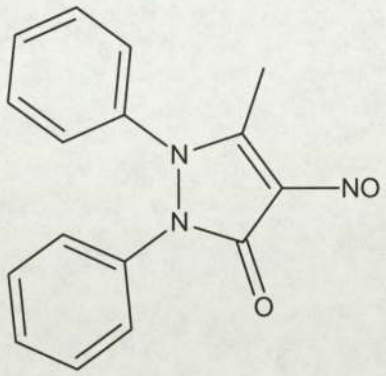
MS (APCI(+)): 251 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.09-7.41 (m, 10H, Ar-H), 5.55 (CH), 2.13 (S, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: 166.7 (C=O), 156.5 (C-CH_3), 139.1, 135.9, 129.5, 128.8, 128.1, 126.0, 125.6, 123.7 (10 x Ar-C), 99.3 (CH), 13.8 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3445, 3101, 1675, 1496, 1599, 1381, 1347, 1244, 973, 700 cm^{-1}.

(DPP-NO): 5-Methyl-4-nitroso-1,2-diphenyl-1,2-dihydro-pyrazol-3-one.



5-Methyl-1,2-diphenyl-1,2-dihydro-pyrazol-3-one (10.0g, 0.04 mol) was stirred and gently heated (30 min) in conc. HCl (60.0 ml). The resulting solution was diluted with H_2O (300 ml) and stirred on ice. A solution of sodium nitrite (2.8g, 0.04 mol) in 40 ml water was slowly added over 5-10 minutes. The greenish brown precipitate was formed and after a further 30 minutes of standing was filtered and washed with cold water and dried in a vacuum decicator.

Yield = 89%

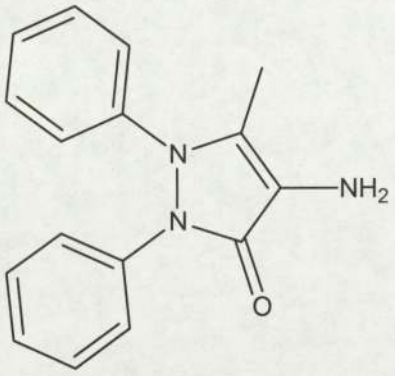
Molecular Weight: 279.3

Molecular Formula: $C_{17}H_{14}ClNO_2$

MS (APCI(-)): 278 (M-1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.04-7.89 (m, Ar-H, 10H), 2.06 (s, CH_3) p.p.m.

(DPP-NH2): 4-Amino-5-methyl-1,2-diphenyl-1,2-dihydro-pyrazol-3-one.



Tin chloride (20.4g, 0.11 mol) was stirred and heated to 90-95 °C in 20 % HCl (120 ml) until dissolved and then added to a solution of 5-methyl-4-nitroso-1,2-diphenyl-1,2-dihydro-pyrazol-3-one dissolved in ethanol (250 ml). The resulting solution was allowed to cool to RT and left to stand for 8 hours. Concentrated ammonia solution was added to the mixture until pH 6-7 was achieved. The precipitate was filtered using Buckner filtration, dried and dissolved 3 times with 30 ml ethanol. The ethanol was removed under vacuum and the yellow crude compound was recrystallised in ethanol giving bright yellow crystals.

Yield = 42%

Melting Point: 156-159 °C

R_f (ether) = 0.10

Mol. Weight: 265.3

Mol. Formula: $C_{16}H_{15}N_3O$

MS (APCI(+)): 251 (M+), 266 (M+1) m/z

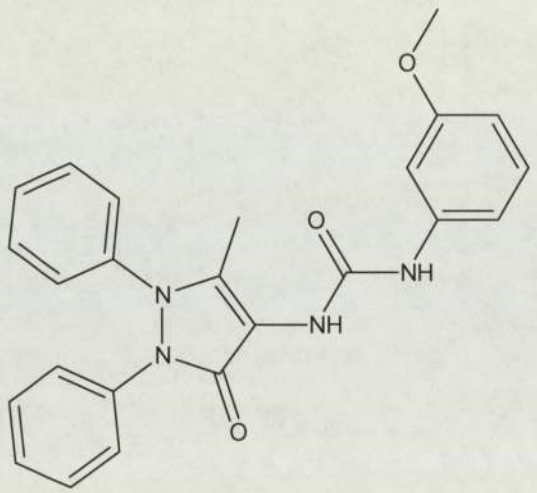
^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.06-7.56 (m, ArH, 10H), 5.58 (s, CH), 2.11 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 162.7 (C=O), 141.5 (C-CH_3), 137.8, 135.8, 129.3, 128.8, 128.2, 127.9, 126.1, 125.7, 123.8, 122.7 (10 x Ar-C), 99.3 (C-NH_2), 10.9 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3669, 3420, 3311, 3206, 1986, 1957, 1656, 1598, 1496, 1358, 1272, 760, 708, 692 cm^{-1}

(UR-MeO(m)): 1-(3-Methoxy-phenyl)-3-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-urea.

4-Amino-5-methyl-1,2-diphenyl-1,2-dihydro-pyrazol-3-one (0.2g, 0.00075 mol) was stirred and dissolved in dry acetonitrile (25 ml) at RT. The appropriate isocyanate (1.3 eq., see page 46) was dissolved in the minimum amount dry acetonitrile and added to the aforementioned solution. The mixture was stirred and heated to 55-60 °C for 24 hours. Acetonitrile was removed under vacuum until approximately 1-2 ml of solution was left. Ether (20 ml) was added to remove excess isocyanate. The resulting precipitate was filtered and washed with a further 2 portions of ether (10 ml). The corresponding pure urea was dried under vacuum.



Yield = 71%

Melting Point: 224-227 °C

R_f (ether) = 0.11

Mol. Weight: 414.6

Mol. Formula: $C_{24}H_{22}N_4O_3$

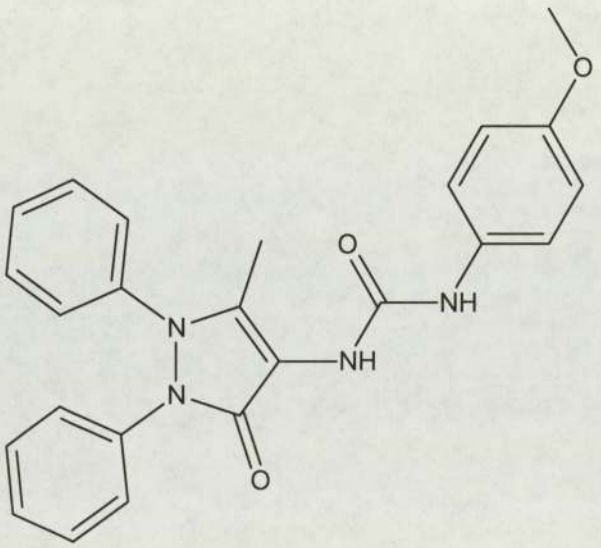
MS (APCI(+)): 266 (M+), 415 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 8.41-8.49 (d, NH, 2H), 7.12-7.49 (m, 11H, Ar-H), 6.88-6.97 (t, Ar-H, *J* = 8.6 Hz, 1H), 6.48-6.54 (d, Ar-H), 6.22-6.26 (d, Ar-H), 3.71 (s, CH_3-O), 2.19 (s, CH_3-C) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.0 (C-C=O), 160.0 (NH-C=O), 154.1 (ArC), 140.7 (ArC), 138.4 (2xArC), 134.7 (C-CH_3), 129.5 (2xArC), 129.2 (2xArC), 128.6 (ArC), 127.2 (ArC), 126.5 (ArC), 124.9 (ArC), 123.1 (2xArC), 111.2 (ArC), 113.9 (ArC), 109.5 (C-C=O), 108.4 (ArC), 104.3 (ArC), 55.2 (CH_3-O), 12.9 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3308, 3217, 2929, 1713, 1645, 1629, 1596, 1547, 1491, 1459, 1292, 1207, 1154, 860, 755, 702 cm^{-1}

(UR-MeO(p)): 1-(4-Methoxy-phenyl)-3-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-urea



Yield = 75%

Melting Point: 244-246 °C

R_f (ether) = 0.14

Mol. Weight: 414.6

Mol. Formula: $C_{24}H_{22}N_4O_3$

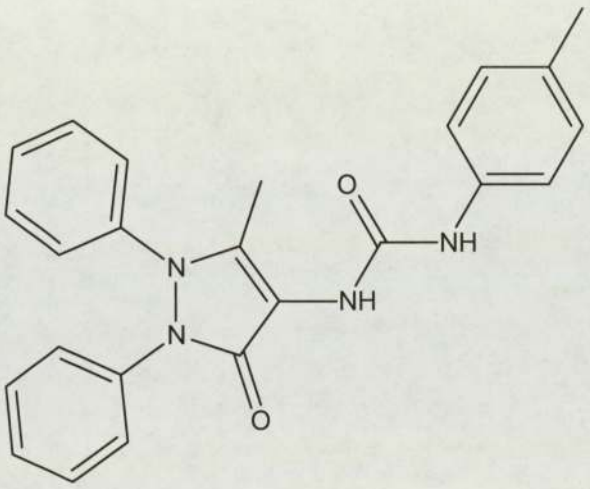
MS (APCI(+)): 266 (M+), 415 (M+1) m/z

^1H NMR (DMSO-d_6) 250 MHz: δ = 8.70 (s, NH), 7.57 (s, NH), 7.24-7.49 (m, 11H, Ar-H), 7.11-7.20 (m, Ar-H), 6.79-6.90 (d, Ar-H), 3.72 (s, CH_3-O), 2.02 (s, CH_3-C) p.p.m.

^{13}C NMR (DMSO-d_6) 62.9 MHz: δ = 162.5 (C-C=O), 150.6 (NH-C=O), 154.3 (ArC), 153.6 (2xArC), 139.4, 135.6 (2xArC), 132.9 (C-CH_3), 129.5 (2xArC), 128.7, 128.6 (2xArC), 128.1 (2xArC), 125.8 (2xArC), 123.1 (2xArC), 119.8 (2xArC), 113.9 (ArC), 109.5 (C-C=O), 55.1 (CH_3-O), 12.1 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3300, 3261, 2929, 1708, 1642, 1618, 1552, 1512, 1420, 1250, 1207, 1018, 833, 763, 697 cm^{-1}

(UR-Me(p)): 1-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-p-tolyl-urea



Yield = 73%

Melting Point: 250-252 °C

R_f (ether) = 0.15

Mol. Weight: 414.6

Mol. Formula: $C_{24}H_{22}N_4O_2$

MS (APCI(+)): 266 (M+), 399 (M+1) m/z

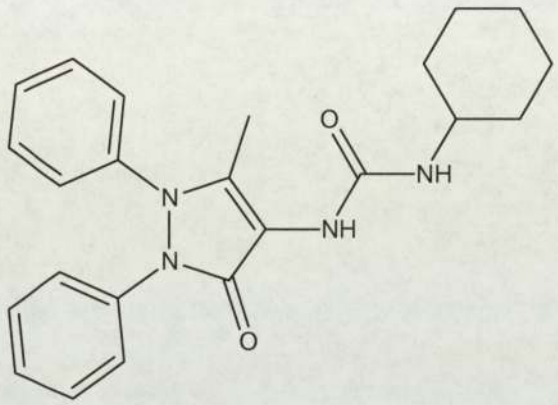
^1H NMR (DMSO-d_6) 250 MHz: δ = 8.80 (s, NH), 7.57 (s, NH), 7.24-7.49 (m, 11H, Ar-H), 7.06-7.28 (m, Ar-H), 2.27 (s, Ar-CH_3), 2.02 (s, CH_3-C) p.p.m.

^{13}C NMR (DMSO-d_6) 62.9 MHz: δ = 162.6 (C-C=O), 150.6 (NH-C=O), 153.5 (2xArC), 139.4, 137.2, 135.5 (3xArC), 130.5 (C-CH_3), 129.5 (2xArC), 129.1 (2xArC), 128.7

(ArC), 128.1 (2xArC), 125.7 (2xArC), 123.1 (2xArC), 118.1 (2xArC), 109.4 (C-C=O), 20.3 (Ar-CH_3), 12.1 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3299, 3058, 2924, 2857, 2358, 2334, 1711, 1648, 1624, 1601, 1540, 1506, 1416, 1292, 1202, 756, 693 cm^{-1}

(UR-CyH): 1-Cyclohexyl-3-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-urea.



Yield = 85%

Melting Point: 238-241 °C

R_f (ether) = 0.17

Mol. Weight: 390.5

Mol. Formula: $C_{23}H_{23}N_4O_2$

MS (APCI(+)): 266 (M+), 391 (M+1) m/z

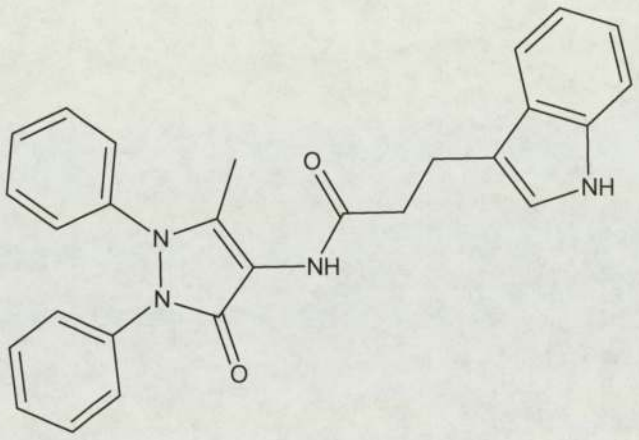
^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.81 (s, NH), 7.16-7.44 (m, 10H, Ar-H), 5.99-6.06 (d, N<u>H</u>-CH), 3.42-3.62 (m, C<u>H</u>-NH), 2.12 (s, CH_3-C), 0.61-1.91 (m, 10H, CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.0 (C-<u>C</u>=O), 156.1 (NH-C=O), 148.7, 139.3, (2xArC), 135.1 (<u>C</u>-CH_3), 129.4 (ArC), 128.8, 128.4 (2xArC), 126.6, 126.3 (ArC), 124.2 (ArC), 111.2 (<u>C</u>-C=O), 48.8 (CH-NH), 33.3 (CH-<u>C</u>H_2, 4xC), 31.0 (CH-CH_2-CH_2-<u>C</u>H_2, 4xC), 25.5 (CH-CH_2-<u>C</u>H_2, 4xC), 13.0 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3359, 3300, 2930, 2855, 1700, 1662, 1642, 1595, 1546, 1492, 1277, 1231, 768, 702 cm^{-1}

(AM-IND(2)): 3-(1H-Indol-3-yl)-*N*-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-propionamide

4-Amino-5-methyl-1,2-diphenyl-1,2-dihydro-pyrazol-3-one (0.2g, 0.00075 mol) was stirred and dissolved in dry acetonitrile (25 ml) at RT. The appropriate indole acid (1.3 eq., see page 46) was dissolved in minimum amount dry acetonitrile and added with diisopropylcarbodiimide (3 eq.) to the afore-mentioned solution. The mixture was stirred and heated to 55-60 °C for 24 hours. Acetonitrile was removed under vacuum until approximately 1-2 ml of solution was left. Ether (20 ml) was added. The resulting precipitate was filtered and washed with water and a further 2 portions of ether (10 ml) to remove DIC and indole acid. The corresponding pure amide was dried under vacuum.



Yield = 74%

Melting Point: 255-257 °C

R_f (ether) = 0.14

Mol. Weight: 436.5

Mol. Formula: $C_{27}H_{24}N_4O_2$

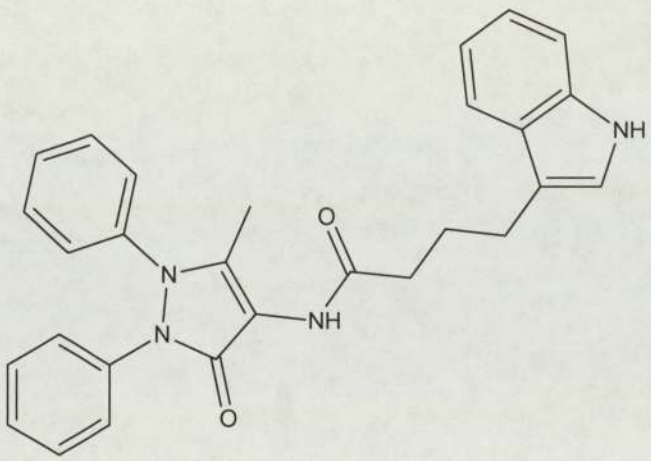
MS (APCI(+)): 423 (M+1), 437 (M+) m/z

^{1}H NMR (DMSO-d_6) 62.9 MHz: δ = 10.93 (s, NH-CO), 9.47 (s, NH), 7.61-7.72 (d, Ar-H), 7.26-7.49 (m, 10xAr-H + C<u>H</u>-NH), 6.98-7.21 (m, Ar-H, 3H), 3.75 (s, CO-CH_2, 2H), 2.52 (s, C-CH_2, 2H), 1.90 (s, CH_3-C), p.p.m.

^{13}C NMR (DMSO-d_6) 62.9 MHz: δ = 170.3 (NH-C=O), 162.2 (C-C=O), 151.3 (2xArC), 139.2 (ArC), 136.1 (ArC), 135.6 (C-CH_3), 129.5 (ArC), 128.7 (2xArC), 128.1 (2xArC), 125.8 (2xArC), 125.6 (2xArC), 123.8, 123.1, 118.7, 118.3, 111.3 (5xArC), 127.2 (C-CO), 120.1 (CH-NH), 108.6 (C-NH), 39.4 (CO-CH_2) 32.5 (C-CH_2), 12.0 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3320, 3215, 3170, 3010, 2925, 1680, 1655, 1592, 1522, 1493, 1309, 1242, 1155, 755 cm^{-1}

(AM-IND(3)): 4-(1H-Indol-3-yl)-*N*-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-butyramide



Yield = 70%

Melting Point: 233-236 °C

R_f (ether) = 0.14

Mol. Weight: 450.5

Mol. Formula: $C_{28}H_{26}N_4O_2$

MS (APCI(+)): 451 (M+1) m/z

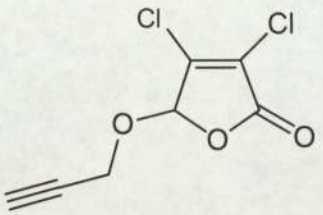
^1H NMR (DMSO-d_6) 62.9 MHz: δ = 10.87 (s, NH-CO), 9.25 (s, NH), 7.52-7.60 (d, Ar-H), 7.26-7.50 (m, 10xAr-H + C-NH), 6.88-7.22 (m, 3xAr-H), 2.71-2.82 (t, CH_2, *J* = 7.4 Hz), 2.38-2.49 (t, CO-CH_2, *J* = 7.3 Hz), 1.89-2.01 (m, CH_3 + CO-CH_2-CH_2, overlapping) p.p.m.

^{13}C NMR-APT (DMSO-d_6) 250 MHz: δ = 171.8 (NH-C=O), 162.2 (C-C=O), 151.4 (2xArC), 139.2 (ArC), 136.3 (ArC), 135.6 (C-CH_3), 129.5 (ArC), 128.7 (2xArC), 128.1 (2xArC), 125.8 (2xArC), 125.6 (2xArC), 123.1, 122.3 118.2, 118.0, 111.3 (5xArC), 127.1 (C-CO), 120.8 (CH-NH), 108.7 (C-NH), 34.9 (CO-CH_2), 26.1 (CO-CH_2-CH_2), 24.2 (C-CH_2), 12.0 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3279, 3229, 3192, 3041, 2926, 2848, 1654, 1623, 1594, 1539, 1489, 1406, 1016, 699 cm^{-1}

6.2. Experiments to Chapter 2.

(PropOF): Preparation of 3,4-dichloro-5-propynoxy-furan-2-(5H)-one.



To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 ml), propargyl alcohol (2 eq. 6.5 g, 0.12 mol) and concentrated sulphuric acid (33%, 0.2 ml) were added. The resulting solution was left for 2 days to reflux using Dean and Stark apparatus. The brown oily tar like crude product was analysed by TLC and then quenched with saturated sodium bicarbonate solution (50 ml). After leaving the crude mixture to settle for 10 minutes, the product was extracted with ether (3 x 50 ml). The organic layers were combined and dried using magnesium sulphate. The solvent was removed in vacuo, resulting in a dark brown coloured oil.

Yield = 61%

Rf (1:1 ether / petroleum ether) = 0.54

Molecular Weight: 207.0

Molecular Formula: $C_7H_4Cl_2O_3$

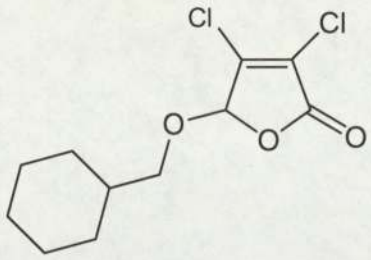
MS (APCI(+)): Corresponding peak was absent

^{1}H NMR ($CDCl_3$) 250MHz: δ = 6.11 (s, CH-O), 4.45-4.58 (m, 2H, C$\underline{H}_2$-O), 2.61-2.69 (t, J = 2.4 Hz, CH≡C).

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.1 (C=O), 147.5 (CH-$\underline{C}$Cl), 128.2 (CO-$\underline{C}$Cl), 98.6 (CH-O), 77.7 ($\underline{C}$-CH_2), 57.4 ($\underline{C}H_2$), (C≡$\underline{C}$H absent) p.p.m.

IR (KBr-disc) υ max: 3578, 3293, 2938, 2881, 2122, 1799, 1634, 1450, 1355, 1232, 1143, 1017, 903, 748, 687 cm^{-1}.

(CyHOF): Preparation of 3,4-dichloro-5-cyclohexylmethoxy-5H-furan-2-one.



To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 ml), cyclohexyl methanol (2 eq. 13.5 g, 0.12 mol) and concentrated sulphuric acid (0.2 ml) were added. The resulting solution was left for 2 days to reflux using Dean and Stark apparatus to collect excess water. The brown viscous oil crude product was analysed by TLC and then quenched with saturated sodium bicarbonate solution (50 ml). After leaving the crude mixture to settle for 10 minutes, the product was extracted with ether (3 x 50 ml). The organic layers were combined and dried using magnesium sulphate. The solvent was removed in vacuo, resulting in a dark brown coloured oil, which was distilled under vacuum to give an orange liquid.

Yield = 57%

Rf (1:1 ether / petroleum ether) = 0.58

Molecular Weight: 265.1.

Molecular Formula: $C_{11}H_{14}Cl_2O_3$

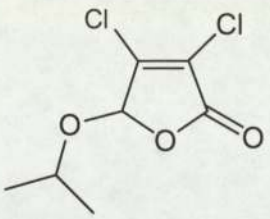
MS (APCI(+)): Corresponding peak was absent

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.73 (s, CH-O), 3.90-3.95 (m, 2H, CH_2-O), 1.39-1.76 (m, overlapping, C<u>H</u>-CH_2 & CH_2-CH_2, 11H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.4 (C=O), 147.6 (CH-<u>C</u>-Cl), 125.3 (CO-<u>C</u>-Cl), 101.2 (CH-O), 68.6 (CH_2-O), 40.3 (<u>C</u>H-CH_2), 29.5 (CH-<u>C</u>H_2, 2xC), 26.7 (CH-CH_2-<u>C</u>H_2, 2xC), 25.7 (CH-CH_2-CH_2-<u>C</u>H_2) p.p.m.

IR (KBr-disc) υ max: 3334, 2926, 2851, 2669, 1794, 1732, 1634, 1444, 1177, 1018, 729, 697 cm^{-1}.

(IprOF): Preparation of 3,4-dichloro-5-isopropoxy-5H-furan-2-one.



To a solution of (10 g, 0.06 mol) mucochloric acid in excess isopropanol (60 ml), concentrated sulphuric acid (0.2 ml) was added. The resulting solution was left for 2 days to reflux. The brown crude product was analysed by TLC and then quenched with saturated sodium bicarbonate solution (50 ml). After leaving the crude mixture to settle for 10 minutes, the product was extracted with ether (3 x 50 ml). The organic layers were combined and dried using magnesium sulphate. The solvent was removed in vacuo, resulting in a light dark brown coloured oil, which was distilled under vacuum giving a colourless liquid.

Yield = 73%

Rf (1:1 ether / petroleum ether) = 0.57

Molecular Weight: 211.1

Molecular Formula: $C_7H_8Cl_2O_3$

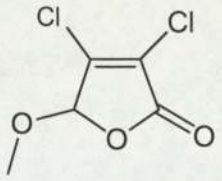
MS (APCI(+)): Corresponding peak was absent

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.90 (s, CH-O), 4.11-4.25 (m, J = 4.9 Hz, C<u>H</u>-CH_3), 1.29-1.42 (d, 6H, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.7 (C=O), 148.0 (CH-<u>C</u>-Cl), 123.9 (CO-<u>C</u>-Cl), 100.3 (CH-O), 75.1 (<u>C</u>H-CH_3), 22.9 (<u>C</u>H_3-CH), 22.0 (<u>C</u>H_3-CH) p.p.m.

IR (KBr-disc) υ max: 3392, 2975, 2927, 1797, 1645, 1460, 1380, 1326, 1234, 1160, 1118, 951, 892, 746 cm^{-1}.

(MeOF): Preparation of 3,4-dichloro-5-methoxy-5H-furan-2-one.



To a solution of (10 g, 0.06 mol) mucochloric acid in excess methanol (60 ml), concentrated sulphuric acid (0.2 ml) was added. The resulting solution was left for 2 days to reflux. The light brown crude product was analysed by TLC and then quenched with saturated sodium bicarbonate solution (50 ml). After leaving the crude mixture to settle for 10 minutes, the product was extracted with ether (3 x 50 ml). The organic layers were combined and dried using magnesium sulphate. The solvent was removed in vacuo, resulting in an orange brown coloured oil, which was distilled under vacuum giving a light yellow liquid.

Yield = 69%

Rf (1:1 ether / petroleum ether) = 0.52

Molecular Weight: 183.0

Molecular Formula: $C_5H_4Cl_2O_3$

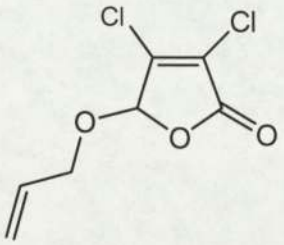
MS (APCI(+)): Corresponding peak was absent

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.77 (s, CH-O), 3.59-3.68 (CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.2 (C=O), 147.4 (CH-C-Cl), 124.5 (CO-C-Cl), 101.6 (CH-O), 56.5 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3569, 3291, 3019, 2987, 2948, 2864, 1794, 1642, 1451, 1367, 1332, 1148, 1025, 967, 905, 747, 659 cm^{-1}.

(AlOF): Preparation of 5-allyloxy-3,4-dichloro-5H-furan-2-one.



To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 ml), allyl alcohol (2 eq., 7.1 g, 0.12 mol) and concentrated sulphuric acid (0.2 ml) were added. The resulting solution was left for 2 days to reflux using Dean and Stark apparatus to collect excess water. The brown crude product was analysed by TLC and then quenched with saturated sodium bicarbonate solution (50 ml). After leaving the crude mixture to settle for 10 minutes, the product was extracted with ether (3 x 50 ml). The organic layers were combined and dried using magnesium sulphate. The solvent was removed in vacuo, resulting in a dark brown coloured oil, which was distilled under vacuum to give an orange liquid.

Yield = 69%

Rf (1:1 ether / petroleum ether) = 0.55

Molecular Weight: 209.0

Molecular Formula: $C_7H_6Cl_2O_3$

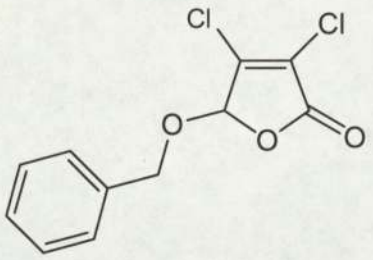
MS (APCI(+)): Corresponding peak was absent

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.78-5.99 (m, overlapping CH-O & O-CH_2-C<u>H</u>, 2H), 5.27-5.48 (O-CH_2-CH-C<u>H_2</u>, 2H), 4.22-4.48 (O-CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.3 (C=O), 147.9 (CH-<u>C</u>-Cl), 131.9 (O-CH_2-<u>C</u>H), 124.2 (CO-<u>C</u>-Cl), 119.5 (O-CH_2-CH-<u>C</u>H_2), 99.7 (CH-O), 71.1 (O-<u>C</u>H_2) p.p.m.

IR (KBr-disc) υ max: 3413, 3092, 2935, 2870, 2365, 2339, 1796, 1642, 1334, 1236, 1157, 1020, 899, 778, 748 cm^{-1}.

(BzOF): Preparation of 5-benzyloxy-3,4-dichloro-5H-furan-2-one.



To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 ml), benzyl alcohol (2 eq., 13.8 g, 0.12 mol) and concentrated sulphuric acid (0.2 ml) were added. The resulting solution was left for 2 days to reflux using Dean and Stark apparatus to collect excess water. The brown crude product was analysed by TLC and then quenched with saturated sodium bicarbonate solution (50 ml). After leaving the crude mixture to settle for 10 minutes, the product was extracted with ether (3 x 50 ml). The organic layers were combined and dried using magnesium sulphate. The solvent was removed in vacuo, resulting in a light brown coloured oil, which was distilled under vacuum giving a yellow liquid.

Yield = 77%

Rf (1:1 ether / petroleum ether) = 0.50

Molecular Weight: 259.1

Molecular Formula: $C_{11}H_8Cl_2O_3$

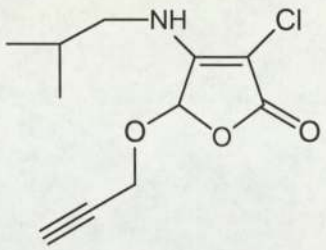
MS (APCI(+)): Corresponding peak was absent

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.42-7.55 (m, Ar-H, 2H), 5.94 (s, CH), 4.78-5.07 (m, CH_2, 2H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.3 (C=O), 147.7 (CH-C-Cl), 135.2 (ArC)), 128.9 (2xArC), 128.5 (2xArC), 126.2 (ArC), 124.5 (CO-C-Cl), 99.7 (CH-O), 71.4 (O-CH_2) p.p.m.

IR (KBr-disc) υ max: 3432, 3034, 2928, 2371, 2344, 1794, 1642, 1452, 1330, 1231, 1148, 1022, 909, 746, 697 cm^{-1}.

(PrOIBu): 3-Chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one.



Method:

Compound **PropOF** (0.5g) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and removed in vacuo to give a dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether / petroleum ether) to give the desired product.

Yield = 52%

M.P: N/A – Viscous Oil

Rf (1:1 ether / petroleum ether) = 0.23

Molecular Weight: 243.7

Molecular Formula: $C_{11}H_{14}ClNO_3$

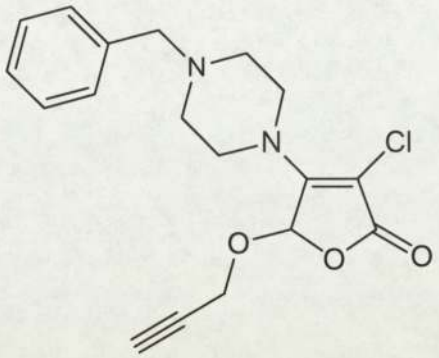
MS (APCI(+)): 244/246 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.92 (s, CH-O), 5.01-5.19 (bs, NH), 4.41 (s, CH_2-O), 3.12-3.38 (m, N-CH_2), 2.50-2.55 (m, C≡CH, J = 2.4 Hz), 1.70-1.94 (m, C<u>H</u>-CH_3, J = 6.3 Hz), 1.07-1.09 (d, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 166.2 (C=O), 155.3 (<u>C</u>N=CCl), 104.5 (C-<u>C</u>Cl), 94.5 (CH-O), 76.7 (<u>C</u>≡CH), 56.1 (<u>C</u>H_2-NH), 51.1 (CH_2-O), 29.6 (<u>C</u>H-CH_3), 19.7 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3306, 3103, 2967, 2937, 2881, 2370, 1748, 1646, 1542, 1458, 1434, 1335, 1126, 969, 747, 701 cm^{-1}.

(PrOBzpip): 4-(4-Benzyl-piperazin-1-yl)-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one



Yield = 50%

M.P: 120-123 °C

Rf (1:1 ether / petroleum ether) = 0.19

Molecular Weight: 346.8

Molecular Formula: $C_{18}H_{19}ClN_2O_3$

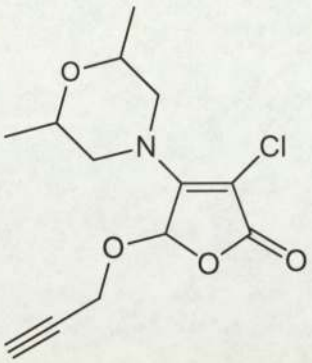
MS (APCI(+)): 346/348 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.18-7.38 (m, Ar-H, 5H), 5.87 (s, CH-O), 4.36 (s, Ar-CH_2), 3.68-3.76 (m, N-CH_2, 4H), 3.51 (s, O-CH_2), 2.39-2.45 (m, overlapping N-CH_2 & C≡CH, 5H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.1 (C=O), 154.0 (CN=CCl), 137.2 (ArC), 129.2 (2xArC), 128.4 (2xArC), 127.5 (Ar-C), 103.0 (C-Cl), 94.3 (CH-O), 76.9 (C-CH_2), 62.7 (Ar-CH_2), 55.7 (Ar-CH_2-N-CH_2, 2xC), 52.7 (O-CH_2), 47.5 (Ar-CH_2-N-CH_2-CH_2) p.p.m.

IR (KBr-disc) υ max: 3253, 2935, 2815, 2126, 1758, 1446, 1347, 1277, 1111, 985, 849, 740, 693 cm^{-1}.

(PrODMM): 3-Chloro-4-(2,6-dimethyl-morpholin-4-yl)-5-prop-2-ynyloxy-5H-furan-2-one.



Yield = 47%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.21

Molecular Weight: 285.7

Molecular Formula: $C_{13}H_{16}ClNO_4$

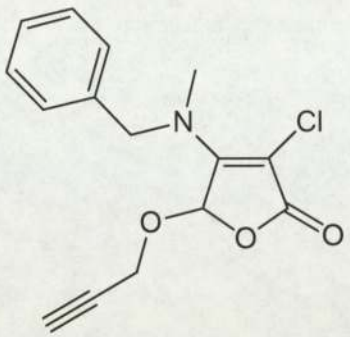
MS (APCI(+)): 286/288 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.94 (s, CH-O), 4.36 (s, O-CH_2), 3.89-4.25 (m, CH_3-CH, 2H), 3.58-3.85 (m, N-CH_2), 2.51-2.98 (m, overlapping C≡CH & N-CH_2, 3H), 1.01-1.46 (m, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 170.4 (C=O), 153.7 (CN=CCl), 104.2 (C-Cl), 94.3 (CH-O), 71.8 (C-CH_2), 66.0 (CH≡C), 55.8 (N-CH_2), 52.6 (O-CH_2), 18.5 (CH_3), 18.4 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3484, 3255, 2981, 2928, 2883, 2366, 2108, 1743, 1639, 1267, 1083, 981, 751, 697 cm^{-1}.

(PrOMeNBz): 4-(Benzyl-methyl-amino)-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one.



Yield = 49%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.23

Molecular Weight: 291.7

Molecular Formula: $C_{15}H_{14}ClNO_3$

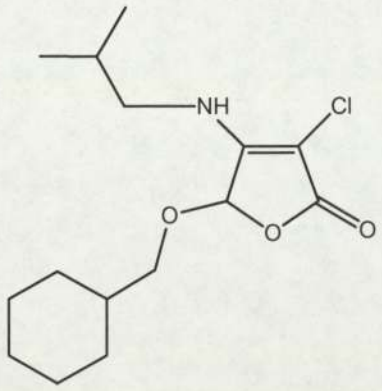
MS (APCI(+)): 292/294 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.16-7.41 (m, Ar-H, 5H), 5.96 (s, CH-O), 4.58-4.79 (m, N-CH_2), 4.38-4.45 (m, O-CH_2), 3.04 (s, N-CH_3), 2.46-2.51 (t, C≡C<u>H</u> *J* = 3.1 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.2 (C=O), 155.6 (<u>C</u>N=CCl), 135.7 (ArC), 129.0 (2xArC), 128.6 (2xArC), 127.3 (Ar-C), 107.3 (C-Cl), 94.6 (CH-O), 76.9 (<u>C</u>-CH_2), 55.9 (CH_2), 38.1 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3441, 3296, 3037, 2930, 2374, 2343, 2128, 1766, 1643, 1460, 1419, 1353, 1270, 1229, 1116, 983, 747, 703 cm^{-1}.

(CyHOIBu): 3-Chloro-5-cyclohexylmethoxy-4-isobutylamino-5H-furan-2-one.



Method:

Compound **CyHOF** (0.5g) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and removed in vacuo to give a dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether / petroleum ether) to give the desired product.

Yield = 59%

M.P: 119-122 °C

Rf (1:1 ether / petroleum ether) = 0.31

Molecular Weight: 301.8

Molecular Formula: $C_{15}H_{24}ClNO_3$

MS (APCI(+)): 206/208 (M+), 302/304 (M+1) m/z

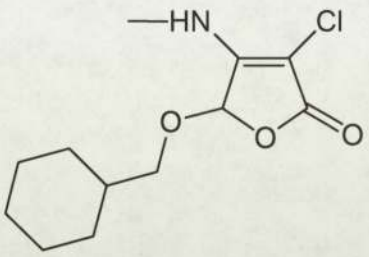
^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.75 (s, CH-O), 4.89-5.08 (bs, NH), 3.53-3.63 (m, CH_2-O, 1H), 3.39-3.51 (m, CH_2-O, 1H), 3.11-3.37 (bs, $C\underline{H}_2$-NH), 2.76-2.91 (bs, CH_3-C$\underline{H}$), 1.53-1.79 (m, overlapping O-CH_2-C$\underline{H}$, O-CH_2-CH-C$\underline{H}_2$, 5H), 0.81-1.42 (m, O-CH_2-CH-CH_2-C$\underline{H}_2$, O-CH_2-CH-CH_2-CH_2-C$\underline{H}_2$, 2xCH_3, 12H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.5 (C=O), 153.6 (C$\underline{N}$=CCl), 104.2 (C-Cl), 97.0 (CH-O), 74.0 (O-CH_2), 51.1 (N-CH_2), 37.7 (O-CH_2-C$\underline{H}$), 29.8 (O-CH_2-CH-C$\underline{H}_2$, 2xC),

29.7 (CH_3-$\underline{C}H$), 27.0 (O-CH_2-CH-CH_2-$\underline{C}H_2$, 2xC), 25.7 (O-CH_2-CH-CH_2-CH_2-$\underline{C}H_2$), 19.74 (CH_3), 19.71 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3285, 2971, 2933, 2863, 2370, 1745, 1678, 1630, 1470, 1333, 1256, 1147, 1025, 948, 750, 718 cm^{-1}.

(CyHOMe): 3-Chloro-5-cyclohexylmethoxy-4-methylamino-5H-furan-2-one.



Yield = 55%

M.P: 117-120 °C

Rf (1:1 ether / petroleum ether) = 0.27

Molecular Weight: 259.7

Molecular Formula: $C_{12}H_{18}ClNO_3$

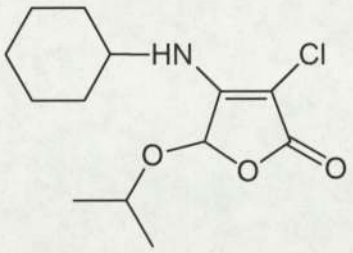
MS (APCI(+)): 164/166 (M+1), 260/262 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.72 (s, CH-O), 4.89-5.02 (bs, NH), 3.49-3.60 (m, CH_2-O, 1H), 3.37-3.47 (m, CH_2-O, 1H), 3.03-3.23 (d, $C\underline{H}_3$-NH), 2.76-2.91 (bs, CH_3-$C\underline{H}$), 1.47-1.72 (m, overlapping O-CH_2-$C\underline{H}$, O-CH_2-CH-$C\underline{H}_2$, 5H), 0.77-1.39 (m, O-CH_2-CH-CH_2-$C\underline{H}_2$, O-CH_2-CH-CH_2-CH_2-$C\underline{H}_2$, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 164.1 (C=O), 151.3 ($\underline{C}$N=CCl), 102.6 (C-Cl), 97.0 (CH-O), 74.2 (O-CH_2), 37.7 (O-CH_2-$\underline{C}$H), 30.6 (CH_3), 29.7 (O-CH_2-CH-$\underline{C}H_2$, 2xC), 27.0 (O-CH_2-CH-CH_2-$\underline{C}H_2$, 2xC), 25.7 (O-CH_2-CH-CH_2-CH_2-$\underline{C}H_2$) p.p.m.

IR (KBr-disc) υ max: 3267, 2925, 2848, 2370, 2338, 1742, 1684, 1632, 1451, 1329, 1255, 1159, 1016, 949, 755, 716 cm^{-1}.

(IprOCyH): 3-Chloro-4-cyclohexylamino-5-isopropoxy-5H-furan-2-one.



General Method:

Compound **IprOF** (0.5g) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and solvent removed in vacuo to give a dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether / petroleum ether) to give the desired product.

Yield = 71%

M.P: 84-86 °C

Rf (1:1 ether / petroleum ether) = 0.24

Molecular Weight: 273.8

Molecular Formula: $C_{13}H_{20}ClNO_3$

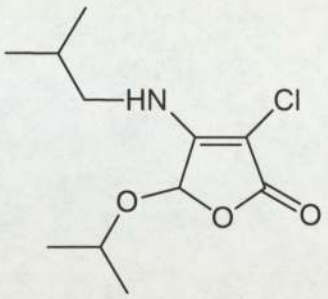
MS (APCI(+)): 232/234 (M+1), 274/276 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.76 (s, CH-O), 4.75-5.11 (bs, NH), 4.01-4.18 (m, C<u>H</u>-CH_3), 1.50-2.12 (m, overlapping NH-C<u>H</u> & NH-CH-C<u>H</u>$_2$, 5H), 1.02-1.48 (m, overlapping CH_2, CH_3, 12H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 172.2 (C=O), 150.0 (<u>C</u>N=CCl), 102.9 (C-Cl), 96.1 (CH-O), 73.4 (O-CH), 50.9 (N-CH), 34.3 (N-CH-<u>C</u>H_2, 2xC), 25.1 (N-CH-CH_2-<u>C</u>H_2, 2xC), 24.6 (N-CH-CH_2-CH_2-<u>C</u>H_2), 23.3 (CH_3), 22.0 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3272, 3087, 2939, 2856, 1745, 1636, 1560, 1454, 1336, 1227, 1118, 948, 750, 696 cm^{-1}.

(IprOIBu): 3-Chloro-4-isobutylamino-5-isopropoxy-5H-furan-2-one.



Yield = 74%

M.P: 104-107 °C

Rf (1:1 ether / petroleum ether) = 0.20

Molecular Weight: 247.7

Molecular Formula: $C_{11}H_{18}ClNO_3$

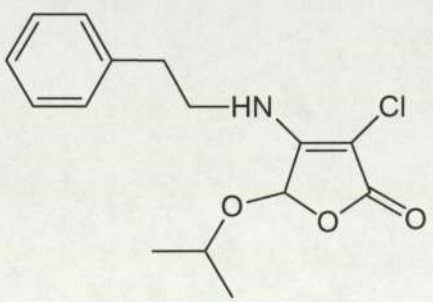
MS (APCI(+)): 206/208 (M+1), 248/250 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.75 (s, CH-O), 4.72-4.93 (bs, NH), 3.96-4.18 (m, *J* = 5.0 Hz, C<u>H</u>-CH_3), 3.04-3.26 (bs, NH-C<u>H</u>$_2$), 1.68-1.90 (m, *J* = 4.9 Hz, C<u>H</u>-CH_2), 1.21-1.35 (m, CH-CH_3, 6H), 0.82-0.97 (d, CH_2-C<u>H</u>$_3$, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.3 (C=O), 153.9 (<u>C</u>N=CCl), 107.0 (C-Cl), 95.9 (CH-O), 73.2 (O-CH), 51.0 (N-CH_2), 29.6 (N-CH_2-<u>C</u>H), 23.3 (O-CH_3), 22.0 (O-CH_3), 19.7 (N-CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3263, 3119, 2975, 2937, 2868, 1741, 1678, 1632, 1469, 1431, 1325, 1256, 1115, 1021, 958, 748, 723 cm^{-1}.

(IprOPhE): 3-Chloro-5-isopropoxy-4-phenethylamino-5H-furan-2-one



Yield = 64%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.22

Molecular Weight: 295.7

Molecular Formula: $C_{15}H_{18}ClNO_3$

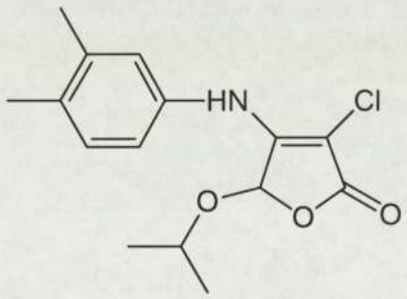
MS (APCI(+)): 254/256 (M+1), 296/298 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.04-7.39 (m, 5H Ar-H), 5.45 (s, CH-O), 4.98 (bs, NH), 3.89-4.08 (m, O-C<u>H</u>, *J* = 6.9 Hz), 3.52-3.71 (m, NH-C<u>H</u>$_2$), 2.78-2.93 (t, *J* = 5.4 Hz, NH-CH_2-C<u>H</u>$_2$), 1.17-1.26 (m, C<u>H</u>$_3$, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.8 (C=O), 156.2 (<u>C</u>N=CCl), 104.9 (C-Cl), 94.8 (C-<u>C</u>H-O), 72.1 (O-CH), 43.6 (N-CH_2), 35.9 (N-CH_2-<u>C</u>H_2), 22.0 (O-CH_3), 20.7 (O-CH_3) p.p.m.

IR (KBr-disc) υ max: 3268, 3076, 2963, 2935, 2870, 2366, 1743, 1643, 1632, 1302, 1115, 1020, 934, 745, 697 cm^{-1}.

(IprODMA): 3-Chloro-4-(3,4-dimethyl-phenylamino)-5-isopropoxy-5H-furan-2-one.



Yield = 72%

M.P: 122-124 °C

Rf (1:1 ether / petroleum ether) = 0.19

Molecular Weight: 295.8

Molecular Formula: $C_{15}H_{18}ClNO_3$

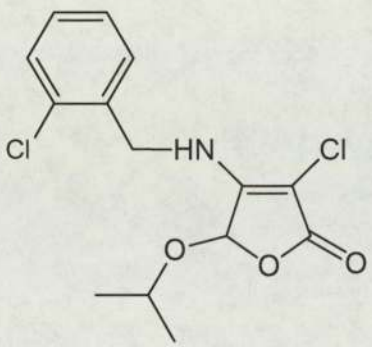
MS (APCI(+)): 236/238 (M+1), 254/256 (M+), 296/298 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.11-7.18 (d, Ar-H), 6.90-7.02 (m, 2H, Ar-H), 6.49-6.58 (bs, NH), 5.95 (s, CH-O), 3.78-3.99 (m, *J* = 4.9 Hz, C<u>H</u>-CH_3), 2.28 (s, Ar-CH_3, 6H) 1.19-1.26 (d, CH-C<u>H</u>$_3$), 0.90-0.99 (d, CH-C<u>H</u>$_3$) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 170.4 (C=O), 154.7 (<u>C</u>N=CCl), 137.7, 135.1, 134.3, 130.2, 125.2, 121.3 (6xAr-C), 102.1 (<u>C</u>-CCl), 96.7 (O-<u>C</u>H-C-CCl), 74.1 (<u>C</u>H-CH_3), 23.1 (<u>C</u>H_3-CH), 21.5 (<u>C</u>H_3-CH), 19.8 (Ar-CH_3), 19.3 (Ar-CH_3) p.p.m.

IR (KBr-disc) υ max: 3287, 3083, 2993, 2936, 2859, 2374, 2342, 1745, 1646, 1559, 1451, 1339, 1119, 1087, 946, 748, 697 cm^{-1}.

(IprOClBz): 3-Chloro-4-(2-chloro-benzylamino)-5-isopropoxy-5H-furan-2-one.



Yield = 62%

M.P: 107-110 °C

Rf (1:1 ether / petroleum ether) = 0.16

Molecular Weight: 316.2

Molecular Formula: $C_{14}H_{15}Cl_2NO_3$

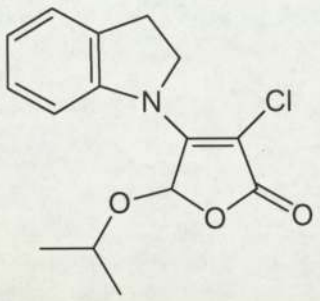
MS (APCI(+)): 274/276/278 (M+1), 316/318/320 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.21-7.42 (m, Ar-H, 4H), 5.73 (s, CH-O), 5.21-5.34 (bs, NH), 4.51-4.76 (m, CH_2), 3.95-4.10 (m, *J* = 7.5 Hz, C<u>H</u>-CH_3), 1.11-1.27 (m, C<u>H</u>$_3$-CH, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 171.2 (C=O), 156.4 (<u>C</u>N=CCl), 134.7, 132.0, 129.9, 129.6, 128.9, 127.4 (6xAr-C), 105.7 (C-Cl), 95.9 (<u>C</u>H-O), 73.5 (<u>C</u>H-CH_3), 45.3 (CH_2), 23.2 (<u>C</u>H_3-CH), 21.9 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3271, 3076, 2974, 2910, 1727, 1639, 1550 1307, 927, 750 cm^{-1}.

(IprOIND): 3-Chloro-4-(2,3-dihydro-indol-1-yl)-5-isopropoxy-5H-furan-2-one.



Yield = 48%

M.P: 140-143 °C

Rf (1:1 ether / petroleum ether) = 0.20

Molecular Weight: 293.8

Molecular Formula: $C_{15}H_{16}ClNO_3$

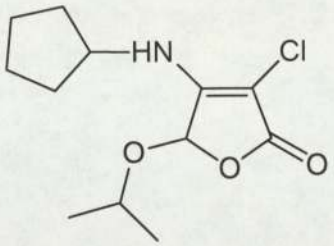
MS (APCI(+)): 252/254 (M+1), 294/296 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.01-7.25 (m, Ar-H, 2H), 6.81-7.00 (m, Ar-H, 2H), 6.22 (s, CH-O), 4.18-4.45 (m, N-CH_2), 3.89-4.16 (m, C<u>H</u>-CH_3), 3.01-3.33 (m, N-CH_2-C<u>H</u>$_2$), 0.98-1.25 (dd, C<u>H</u>$_3$-CH, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.5 (C=O), 152.3 (<u>C</u>N=CCl), 142.7, 132.0, 127.1, 125.5, 123.6, 114.0 (6xAr-C), 104.1 (C-Cl), 97.2 (CH-O), 74.0 (<u>C</u>H-CH_3), 52.2 (N-CH_2), 28.8 N-CH_2-<u>C</u>H_2), 23.3 (<u>C</u>H_3-CH), 22.1 (CH_3-C) p.p.m.

IR (KBr-disc) υ max: 3466, 2981, 2928, 2359, 2310, 1750, 1623, 1587, 1486, 1243, 1106, 956, 749, 678 cm^{-1}.

(IprOCyPe): 3-Chloro-4-cyclopentylamino-5-isopropoxy-5H-furan-2-one.



Yield = 71%

M.P: 85-88 °C

Rf (1:1 ether / petroleum ether) = 0.24

Molecular Weight: 273.8

Molecular Formula: $C_{12}H_{18}ClNO_3$

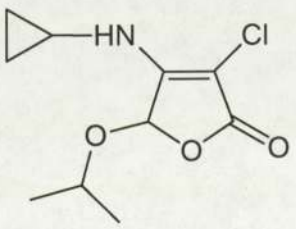
MS (APCI(+)): 218/220 (M+), 260/262 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.78 (s, CH-O), 4.82-5.03 (bs, NH), 4.01-4.27 (m, overlapping C<u>H</u>-CH_3 & NH-CH, 2H), 1.95-2.12 (m, NH-CH-C<u>H$_2$</u>), 1.41-1.81 (m, overlapping CH_2, 6H), 1.23-1.37 (m, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.6 (C=O), 155.1 (<u>C</u>N=CCl), 105.3 (C-Cl), 96.1 (CH-O), 73.3 (O-CH), 55.3 (N-CH), 34.7 (N-CH-<u>C</u>H_2, 2xC), 34.5 (N-CH-<u>C</u>H_2, 2xC), 23.8 (N-CH-CH_2-<u>C</u>H_2), 23.3 (CH_3), 22.0 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3284, 3077, 2984, 2871, 2378, 2340, 1743, 1634, 1544, 1456, 1344, 1231, 1141, 962, 750, 703 cm^{-1}.

(IprOCyPr): 3-Chloro-4-cyclopropylamino-5-isopropoxy-5H-furan-2-one.



Yield = 82%

M.P: 89-91 °C

Rf (1:1 ether / petroleum ether) = 0.25

Molecular Weight: 231.7

Molecular Formula: $C_{10}H_{14}ClNO_3$

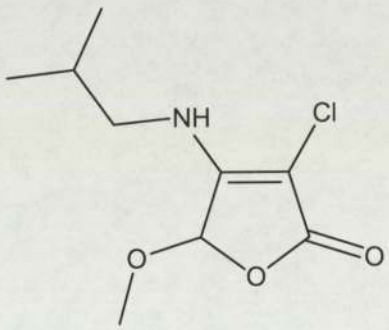
MS (APCI(+)): 190/192 (M+), 232/234 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.47-5.81 (m, overlapping CH-O & NH), 3.98-4.22 (m, C<u>H</u>-CH_3), 2.78-2.99 (m, NH-C<u>H</u>), 1.23-1.40 (m, CH_3, 6H), 0.57-0.94 (m, overlapping CH_2, 4H), p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.7 (C=O), 158.2 (<u>C</u>N=CCl), 106.3 (C-Cl), 96.4 (CH-O), 73.7 (O-CH), 25.4 (N-CH), 23.2 (CH_3), 21.9 (CH_3), 8.4 (N-CH-<u>C</u>H_2), 8.2 (N-CH-<u>C</u>H_2) p.p.m.

IR (KBr-disc) υ max: 3479, 3258, 3052, 2987, 2928, 2372, 2348, 1739, 1649, 1542, 1461, 1396, 1335, 1238, 1144, 989, 750, 695 cm^{-1}.

(MeOIbu): 3-Chloro-4-isobutylamino-5-methoxy-5H-furan-2-one.



General Method:

Compound **MeOF** (0.5g) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and solvent removed in vacuo to give a

dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether / petroleum ether) to give the desired product.

Yield = 61%

M.P: 93-95 °C

Rf (1:1 ether / petroleum ether) = 0.24

Molecular Weight: 219.7

Molecular Formula: $C_9H_{14}ClNO_3$

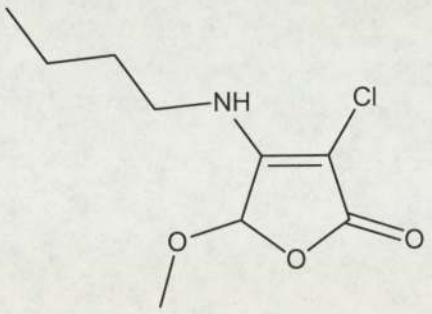
MS (APCI(+)): 220/222 (M+), 246/248 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 5.19-5.69 (m, overlapping O-CH & NH, 2H), 3.48 (s, O-CH_3), 3.08-3.42 (s, N-CH_2), 1.73-1.95 (m, C<u>H</u>-CH_2, *J* = 6.5 Hz), 0.75-1.00 (d, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.2 (C=O), 157.2 (C<u>N</u>=CCl), 106.8 (C-Cl), 97.7 (CH-O) 55.0 (O-CH_3), 51.0 (NH-CH_2), 29.6 (C<u>H</u>-CH_2), 19.7 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3352, 3059, 2975, 2921, 2371, 1743, 1653, 1540, 1456, 1369, 1340, 1222, 1137, 1012, 966, 747, 713 cm^{-1}.

(MeOnBu): 4-Butylamino-3-chloro-5-methoxy-5H-furan-2-one.



Yield = 56%

M.P: N/A – Viscous Oil

Rf (1:1 ether / petroleum ether) = 0.28

Molecular Weight: 219.7

Molecular Formula: $C_9H_{14}ClNO_3$

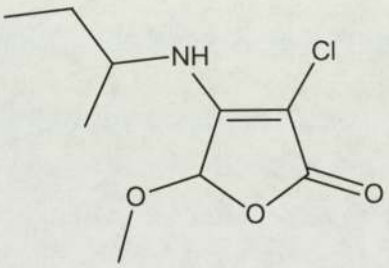
MS (APCI(+)): 188/190 (M+), 220/222 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.65 (s, CH), 5.09-5.36 (bs, NH), 3.32-3.44 (m, overlapping O-CH_3 & NH-C$\underline{H}_2$, 5H), 1.44-1.63 (m, N-CH_2-C$\underline{H}_2$, J = 6.7 Hz), 1.21-1.41 (m, CH_3-C$\underline{H}_2$, J = 7.9 Hz), 0.80-0.98 (t, CH_3, J = 8.3 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.9 (C=O), 156.5 (C$\underline{N}$=CCl), 105.4 (C-Cl), 97.6 (CH-O) 55.0 (O-CH_3), 43.6 (NH-CH_2), 32.7 (NH-CH_2-C$\underline{H}_2$), 19.7 (CH_3-C$\underline{H}_2$), 13.7 (C$\underline{H}_3$-CH_2) p.p.m

IR (KBr-disc) υ max: 3310, 3091, 2968, 2948, 2871, 1752, 1658, 1539, 1370, 1332, 1209, 1135, 1076, 1025, 973, 753, 701 cm^{-1}.

(MeOsBu): 4-*sec*-Butylamino-3-chloro-5-methoxy-5H-furan-2-one.



Yield = 53%

M.P: N/A – Viscous Oil

Rf (1:1 ether / petroleum ether) = 0.27

Molecular Weight: 219.7

Molecular Formula: $C_9H_{14}ClNO_3$

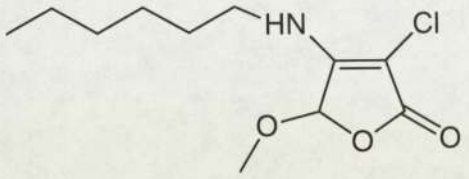
MS (APCI(+)): 164/166 (M+), 220/222 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.66 (s, CH), 5.05-5.23 (bs, NH), 3.46 (s, O-CH_3), 3.16-3.38 (m, NH-C$\underline{H}$), 1.48-1.77 (m, N-CH-C$\underline{H}_2$), 1.33-1.42 (d, C$\underline{H}_3$-CH), 1.19-1.30 (m, C$\underline{H}_3$-CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 171.6 (C=O), 156.7 (C$\underline{N}$=CCl), 103.8 (C-Cl), 97.6 (CH-O) 54.8 (O-CH_3), 49.8 (NH-CH), 27.7 (NH-CH-C$\underline{H}_2$), 17.9 (C$\underline{H}_3$-CH), 10.0 (C$\underline{H}_3$-CH_2) p.p.m

IR (KBr-disc) υ max: 3439, 3230, 2994, 2935, 2719, 1750, 1642, 1511, 1462, 1331, 1207, 1135, 1013, 971, 748, 709 cm^{-1}.

(MeOHx): 3-Chloro-4-hexylamino-5-methoxy-5H-furan-2-one.



Yield = 47%

M.P: N/A – Viscous Oil

Rf (1:1 ether / petroleum ether) = 0.26

Molecular Weight: 247.7

Molecular Formula: $C_{11}H_{18}ClNO_3$

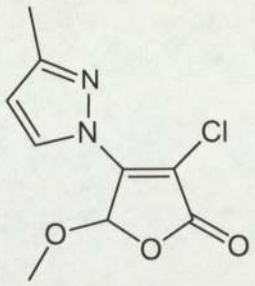
MS (APCI(+)): 216/218 (M+), 248/250 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 5.62 (s, CH) 5.02-5.19 (bs, NH), 3.43 (s, O-CH_3), 1.18-1.81 (m, overlapping CH_2, 10H), 0.72-0.89 (m, CH_2-$C\underline{H}_3$) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.2 (C=O), 155.6 (CH-$\underline{C}$-Cl), 107.6 (C-Cl), 97.5 (CH-O), 54.9 (O-CH_3), 40.3 (NH-CH_2), 31.4 (NH-CH_2-$\underline{C}H_2$), 30.7 (NH-CH_2-CH_2-$\underline{C}H_2$), 26.1 (CH_3-CH_2-$\underline{C}H_2$), 22.5 (CH_3-$\underline{C}H_2$), 14.0 ($\underline{C}H_3$-CH_2) p.p.m.

IR (KBr-disc) υ max: 3426, 2937, 2864, 2381, 1751, 1654, 1544, 1463, 1376, 1212, 1132, 1078, 1021, 967, 750, 706 cm^{-1}.

(MeOMePy): 3-Chloro-5-methoxy-4-(3-methyl-pyrazol-1-yl)-5H-furan-2-one.



Yield = 61%

M.P: 88-90 °C

Rf (1:1 ether / petroleum ether) = 0.18

Molecular Weight: 228.6

Molecular Formula: $C_9H_9ClN_2O_3$

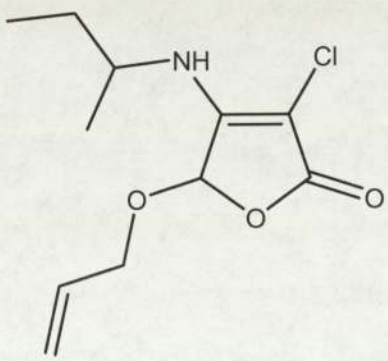
MS (APCI(+)): 197/199 (M+1), 229/231 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 8.21-8.25 (d, N-CH), 6.40-6.48 (d, N-CH-C<u>H</u>), 6.27 (s, CH-O), 3.59 (s, O-CH_3), 2.30 (s, CH_3-C) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.6 (C=O), 154.0 (CH-<u>C</u>-Cl), 146.8 (CH_3-<u>C</u>), 130.9 (N-CH), 111.4 (N-CH-<u>C</u>H), 103.0 (CH-O), 99.6 (CO-<u>C</u>-Cl), 57.1 (<u>C</u>H-CH_3), 13.8 (<u>C</u>H_3-C) p.p.m.

IR (KBr-disc) υ max: 3436, 2932, 2863, 2372, 2341, 1749, 1639, 1538, 1456, 1265, 1123, 972, 773, 698 cm^{-1}.

(AlOsBu): 5-Allyloxy-4-*sec*-butylamino-3-chloro-5H-furan-2-one.



General Method:

Compound **AlOF** (0.5g) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and solvent removed in vacuo to give a dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether / petroleum ether) to give the desired product.

Yield = 49%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.30

Molecular Weight: 245.7

Molecular Formula: $C_{11}H_{16}ClNO_3$

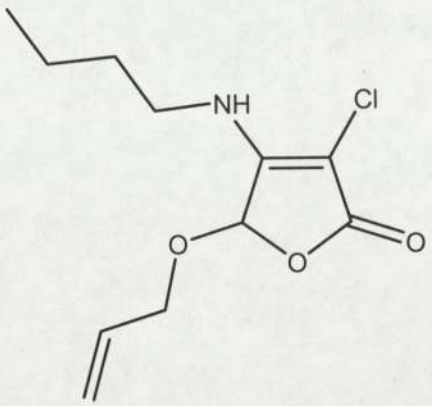
MS (APCI(+)): 190/192 (M+), 246/248 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.80-5.98 (m, C<u>H</u>-CH_2), 5.74 (s, CH), 5.18-5.39 (m, O-CH_2-CH-C<u>H</u>$_2$), 3.56-4.27 (m, overlapping O-CH_2 & NH-C<u>H</u>, 2H), 1.43-1.68 (m, CH_3-C<u>H</u>$_2$), 1.01-1.82 (m, CH-C<u>H</u>$_3$), 0.55-0.99 (t, CH_3-CH_2, *J* = 4.6 Hz), (NH absent) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.3 (C=O), 153.5 (<u>C</u>N=CCl), 132.5 (CH_2-<u>C</u>H), 119.0 (O-CH_2-CH-<u>C</u>H$_2$), 106.3 (C-Cl), 96.5 (CH-O), 69.6 (O-CH_2), 49.6 (NH-CH), 30.5 (CH-<u>C</u>H$_2$), 21.4 (CH-<u>C</u>H$_3$), 10.3 (<u>C</u>H$_3$-CH_2) p.p.m.

IR (KBr-disc) υ max: 3296, 3088, 2978, 2978, 2939, 2881, 1753, 1649, 1546, 1467, 1338, 1150, 972, 709 cm^{-1}.

(AlOnBu): 5-Allyloxy-4-butylamino-3-chloro-5H-furan-2-one.



Yield = 57%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.33

Molecular Weight: 245.7

Molecular Formula: $C_{11}H_{16}ClNO_3$

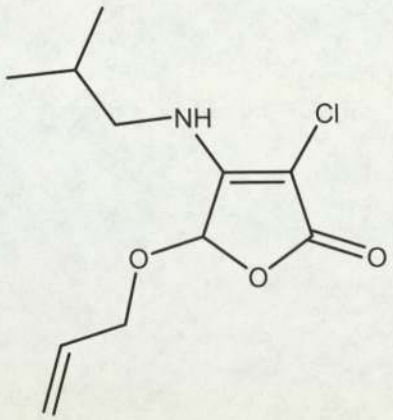
MS (APCI(+)): 188/190 (M+), 246/248 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.80-6.02 (m, C<u>H</u>-CH_2), 5.76 (s, CH), 5.24-5.40 (m, O-CH_2-CH-C<u>H</u>$_2$), 4.09-4.40 (m, O-CH_2), 3.33-3.58 (bs, NH-C<u>H</u>$_2$, 2H), 1.53-1.68 (m, CH_3-CH_2-C<u>H</u>$_2$, *J* = 6.9 Hz), 1.28-1.49 (m, CH_3-C<u>H</u>$_2$, *J* = 7.0 Hz), 0.88-1.01 (t, CH_3, *J* = 8.2 Hz), (NH absent) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.9 (C=O), 151.3 (<u>C</u>N=CCl), 132.4 (CH_2-<u>C</u>H), 119.4 (O-CH_2-CH-<u>C</u>H_2), 105.6 (C-Cl), 96.2 (CH-O), 69.6 (O-CH_2), 43.6 (NH-CH_2), 32.7 (N-CH_2-<u>C</u>H_2), 19.7 (N-CH_2-CH_2-<u>C</u>H_2), 13.7 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3332, 3095, 2974, 2941, 2892, 1754, 1655, 1540, 1457, 1333, 1227, 1138, 1087, 969, 748, 700 cm^{-1}.

(AlOIBu): 5-Allyloxy-3-chloro-4-isobutylamino-5H-furan-2-one.



Yield = 62%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.29

Molecular Weight: 245.7

Molecular Formula: $C_{11}H_{16}ClNO_3$

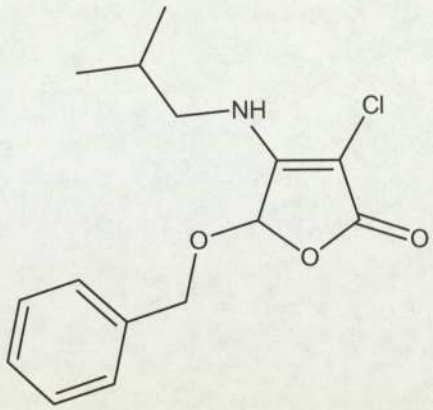
MS (APCI(+)): 188/190 (M+), 246/248 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.79-5.99 (m, C<u>H</u>-CH_2), 5.73 (s, CH), 5.24-5.40 (m, O-CH_2-CH-C<u>H_2</u>), 4.69-4.89 (bs, NH), 4.07-4.38 (m, O-CH_2), 3.12-3.29 (bs, NH-C<u>H_2</u>, 2H), 1.73-1.91 (m, CH_3-C<u>H</u>, *J* = 7.1 Hz), 0.88-1.01 (d, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 172.1 (C=O), 156.7 (<u>C</u>N=CCl), 132.2 (CH_2-<u>C</u>H), 119.6 (O-CH_2-CH-<u>C</u>H_2), 108.0 (C-Cl), 95.9 (CH-O), 69.3 (O-CH_2), 51.0 (NH-CH_2), 29.6 (N-CH_2-<u>C</u>H), 19.7 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3285, 3088, 2965, 2940, 2872, 1736, 1641, 1557, 1465, 1341, 1236, 1147, 1085, 968, 755, 702 cm^{-1}.

(BzOIBu): 5-Benzyloxy-3-chloro-4-isobutylamino-5H-furan-2-one.



Method:

Compound **BzOF** (0.5g) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and solvent removed in vacuo to give a dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether / petroleum ether) to give the desired product.

Yield = 76%

M.P: 105-107 °C

Rf (1:1 ether / petroleum ether) = 0.23

Molecular Weight: 295.8

Molecular Formula: $C_{15}H_{18}ClNO_3$

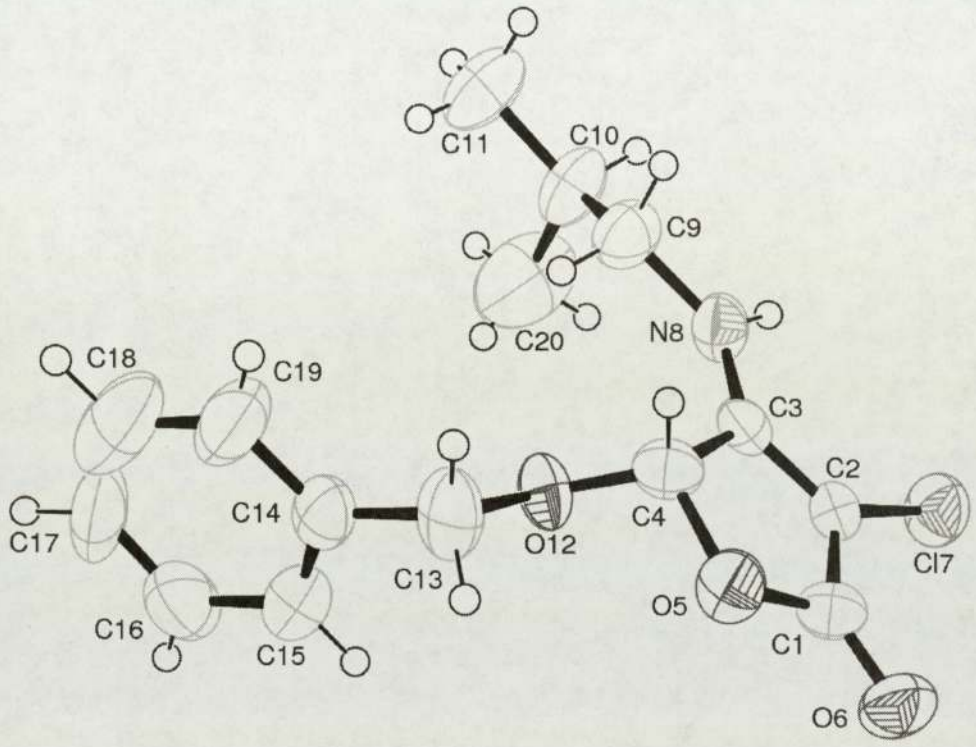
MS (APCI(+)): 188/190 (M+), 296/298 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.01-7.49 (m, ArH, 5H), 5.79 (s, CH), 5.00-5.20 (bs, NH), 4.61-4.91 (bs, CH_2-O), 3.02-3.39 (m, NH-CH_2), 1.69-1.92 (m, C̲H-CH_2), 0.87-1.02 (d, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 170.4 (C=O), 170.4 (C̲N=CCl), 135.4 (ArC), 128.8 (5xArC), 106.3 (C-Cl), 95.6 (CH-O), 70.4 (O-CH_2), 51.0 (NH-CH), 29.5 (C̲H-CH_2), 19.7 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3460, 3252, 2977, 2937, 2877, 2368, 2341, 1741, 1631, 1433, 1353, 1329, 1255, 1128, 1028, 961, 750, 703 cm^{-1}.

Crystal Data - (sample recrystallised from methanol):



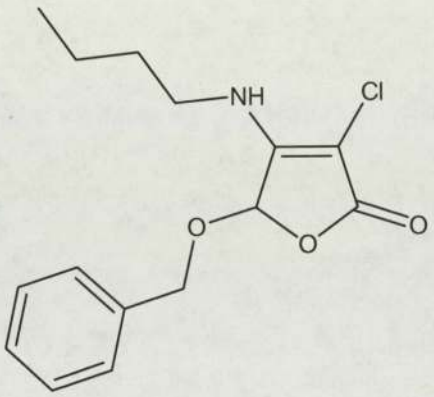
$C_{15}H_{18}ClNO_3$
$M_r = 295.75$
T = 293(2) K
Tabular
0.20 x 0.15 x 0.05 mm
Colourless
Mo Kα radiation: λ = 0.71073 Å
Monoclinic
$P2_1/c$
a = 11.420(5) Å
b = 10.736(2) Å
c = 12.798(5) Å
β = 102.83(4) °

V = 1529.9(9) $Å^3$
Z = 4
$D_x = 1.284$ Mg/m^{-3}
D_m not measured
R $[F^2 > 2\sigma(F^2)] = 0.0791$
$wR(F^2) = 0.1845$
3207 reflections
187 parameters

Selected geometric parameters (Å, °)

Cl(7)-C(2)	1.707(7)	O(12)-C(4)	1.386(7)
O(5)-C(1)	1.365(8)	C(3)-N(8)	1.331(7)
O(5)-C(4)	1.432(7)	C(2)-C(3)	1.354(9)
O(6)-C(1)	1.202(7)		
C(4)-O(12)-C(13)	113.7(5)	O(6)-C(1)-O(5)	120.3(5)
O(12)-C(13)-C(14)	108.2(6)	C(1)-C(2)-Cl(7)	122.9(5)
N(8)-C(3)-C(4)	124.5(6)	C(3)-C(2)-Cl(7)	127.0(5)
O(12)-C(4)-O(5)	111.5(5)	C(3)-N(8)-C(9)	124.5(6)

(BzOnBu): 5-Benzyloxy-4-butylamino-3-chloro-5H-furan-2-one.



Yield = 68%

M.P: 109-112 °C

Rf (1:1 ether / petroleum ether) = 0.26

Molecular Weight: 295.8

Molecular Formula: $C_{15}H_{18}ClNO_3$

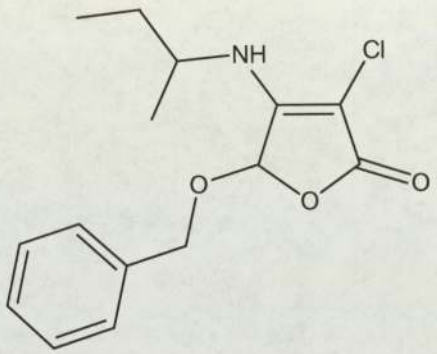
MS (APCI(+)): 188/190 (M+), 296/298 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.29-7.48 (m, ArH, 5H), 5.74 (s, CH), 4.54-5.15 (m, overlapping NH &, CH_2-O, 3H), 3.11-3.49 (bs, NH-CH_2), 1.46-1.59 (m, N-CH_2-$\underline{C}H_2$, *J* = 5.7 Hz, 2H), 1.19-1.41 (m, CH_3-$\underline{C}H_2$, *J* = 5.0 Hz, 2H), 0.79-0.93 (t, CH_3, *J* = 6.6 Hz, 3H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 171.4 (C=O), 153.4 (CH-<u>C</u>Cl), 135.5 (ArC), 128.8 (5xArC), 107.5 (CO-<u>C</u>Cl), 95.5 (CH-O), 70.3 (O-CH_2), 43.7 (NH-CH_2), 29.5 (N-CH_2-<u>C</u>H_2), 19.7 (CH_3-CH_2) 13.7 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3419, 2067, 3028, 2928, 2371, 2338, 1794, 1642, 1502, 1456, 1327, 1234, 1144, 1022, 972, 902, 750, 707 cm^{-1}.

(BzOsBu): 5-Benzyloxy-4-*sec*-butylamino-3-chloro-5H-furan-2-one.



Yield = 64%

M.P: 93-96 °C

Rf (1:1 ether / petroleum ether) = 0.26

Molecular Weight: 295.8

Molecular Formula: $C_{15}H_{18}ClNO_3$

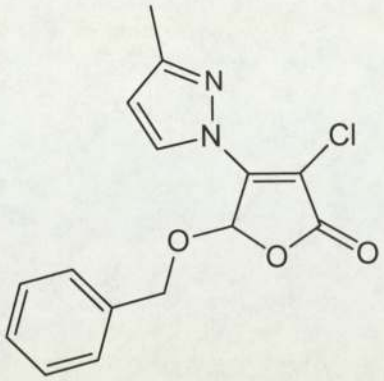
MS (APCI(+)): 188/190 (M+), 296/298 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.30-7.43 (m, ArH, 5H), 5.73 (s, CH), 5.01-5.20 (bs, NH), 4.58-4.79 (m, CH_2-O), 4.40-4.53 (bs, NH), 3.49-3.99 (bs, NH-C<u>H</u>), 1.26-1.68 (m, CH-C<u>H</u>$_2$), 1.06-1.19 (m, CH-C<u>H</u>$_3$, J = 6.5 Hz, 3H), 0.74-0.91 (m, CH_2-C<u>H</u>$_3$) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.3 (C=O), 155.5 (<u>C</u>N=CCl), 135.6 (ArC), 128.7 (5xArC), 105.9 (C-Cl), 95.7 (CH-O), 70.2 (O-CH_2), 51.1 (NH-CH), 30.5 (<u>C</u>H-CH_2), 21.6 (CH-<u>C</u>H_3), 21.6 (CH_2-<u>C</u>H_3) p.p.m.

IR (KBr-disc) υ max: 3272, 3085, 2977, 2929, 2880, 2368, 1732, 1632, 1560, 1454, 1348, 1231, 1123, 966, 749, 700 cm^{-1}.

(BzOMePy): 5-Benzyloxy-3-chloro-4-(3-methyl-pyrazol-1-yl)-5H-furan-2-one.



Yield = 76%

M.P: 89-91 ^{o}C

Rf (1:1 ether / petroleum ether) = 0.23

Molecular Weight: 304.7

Molecular Formula: $C_{15}H_{13}ClN_2O_3$

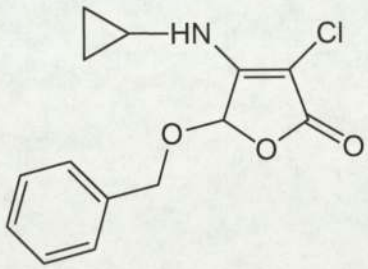
MS (APCI(+)): 305/307 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 8.25-8.29 (d, N-CH), 7.31-7.50 (m, Ar-H, 5H), 6.49 (s, CH-O) 6.40-6.47 (d, N-CH-C<u>H</u>), 4.94-5.06 (m, O-CH_2), 2.41 (s, CH_3-C) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.7 (C=O), 153.7 (CH-<u>C</u>-Cl), 147.3 (CH_3-<u>C</u>), 135.5 (ArC) 130.9 (N-CH), 128.7 (2xArC), 128.6 (2xArC), 128.55 (ArC), 111.3 (N-CH-<u>C</u>H), 102.7 (C-Cl), 99.1 (CH-O), 72.6 (O-CH_2), 13.7 (<u>C</u>H_3-C) p.p.m.

IR (KBr-disc) υ max: 3440, 3176, 2950, 2941, 2885, 2362, 2336, 1790, 1674, 1551, 1443, 1324, 1264, 1122, 1016, 983, 735, 695 cm^{-1}.

(BzOCyPr): 5-Benzyloxy-3-chloro-4-cyclopropylamino-5H-furan-2-one.



Yield = 81%

M.P: 104-107 °C

Rf (1:1 ether / petroleum ether) = 0.19

Molecular Weight: 279.7

Molecular Formula: $C_{14}H_{14}ClNO_3$

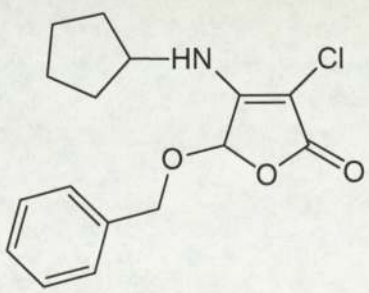
MS (APCI(+)): 278/280 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.25-7.80 (m, Ar-H, 5H), 5.95 (s, CH-O), 4.74-5.12 (m, overlapping NH & O-CH_2, 3H), 2.80-2.99 (m, NH-C<u>H</u>), 0.49-0.98 (m, 3H, C<u>H</u>$_2$-CH, 4H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.4 (C=O), 153.4 (<u>C</u>N=CCl), 133.6 (ArC), 128.8 (4xArC), 128.7 (ArC), 106.1 (C-Cl), 95.7 (CH-O), 70.8 (<u>C</u>H-CH_3), 25.5 (NH-CH), 8.3 (<u>C</u>H_2-CH, 2xC) p.p.m.

IR (KBr-disc) υ max: 3453, 3265, 3071, 2935, 2864, 2366, 1788, 1742 1636, 1442, 1348, 1245, 983, 753, 701 cm^{-1}.

(BzOCyPe): 5-Benzyloxy-3-chloro-4-cyclopentylamino-5H-furan-2-one.



Yield = 78%

M.P: 101-103 °C

Rf (1:1 ether / petroleum ether) = 0.23

Molecular Weight: 307.8

Molecular Formula: $C_{16}H_{18}ClNO_3$

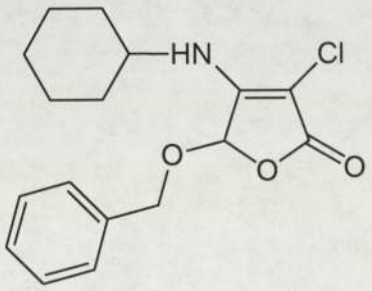
MS (APCI(+)): 284/286 (M+), 306/308 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.39-7.51 (m, Ar-H, 5H), 5.78 (s, CH-O), 4.68-4.89 (m, O-CH_2), 4.01-4.28 (bs, NH), 1.33-2.15 (m, overlapping NH-C<u>H</u> & CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.2 (C=O), 151.3 (CH-<u>C</u>-Cl), 135.5 (ArC), 128.8 (5xAr-C), 104.2 (C-Cl), 95.6 (CH-O), 70.2 (<u>C</u>H-CH_3), 55.3 (NH-CH), 34.6 (<u>C</u>H_2-CH, 2xC), 23.7 (<u>C</u>H_2-CH_2-CH, 2xC) p.p.m.

IR (KBr-disc) υ max: 3459, 3306, 3070, 2961, 2859, 2367, 2342, 1735, 1626, 1547, 1352, 1231, 1144, 1007, 959, 758, 707 cm^{-1}.

(BzOCyH): 5-Benzyloxy-3-chloro-4-cyclohexylamino-5H-furan-2-one.



Yield = 49%

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.25

Molecular Weight: 321.8

Molecular Formula: $C_{17}H_{20}ClNO_3$

MS (APCI(+)): 322/324 (M+) m/z

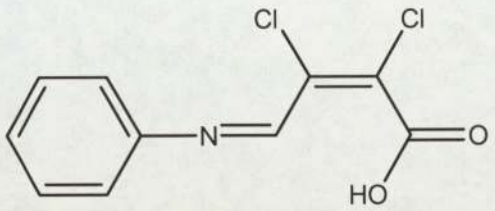
^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.30-7.49 (m, Ar-H, 5H), 5.78 (s, CH-O), 4.96-5.04 (bs, NH), 4.68-4.85 (m, O-CH_2), 1.04-2.18 (m, overlapping NH-C<u>H</u> & CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.5 (C=O), 155.5 (CN=CCl), 135.5 (ArC), 128.7 (5xAr-C), 104.5 (C-Cl), 95.6 (CH-O), 67.0 ($\underline{C}H\text{-}CH_3$), 52.7 (NH-CH), 34.5 ($\underline{C}H_2\text{-}CH$, 2xC), 25.5 ($\underline{C}H_2\text{-}CH_2\text{-}CH$, 2xC), 25.1 ($\underline{C}H_2\text{-}CH_2\text{-}CH_2\text{-}CH$) p.p.m.

IR (KBr-disc) υ max: 3293, 3067, 2941, 2861, 2375, 2355, 1754, 1648, 1550, 1446, 1337, 1231, 1134, 1091, 955, 746, 700 cm^{-1}.

6.3. Experiments to Chapter 3.

(3a): 2,3-Dichloro-4-phenylimino-but-2-enoic acid



Method: Dry mucochloric acid (15.0 g, 88.8 mmol) and 2 equivalents *N*-phenyl-formamide (21g, 175 mmol) were heated under reflux in toluene (100 ml) with conc. H_2SO_4 (1% v/w), using a Dean and Stark apparatus. TLC was used to monitor the reaction progress (1:1 ether / petroleum ether). After 8 hours, a green powder precipitated out in solution. The resulting mixture was filtered under Buchner filtration. The resulting product was dried under vacuum resulting in a pure green crystalline compound.

Yield = 98%

M.P: 185-187 °C

R_f (1:1 ether / petroleum ether) = 0.15

Molecular Weight: 244.1

Molecular Formula: $C_{10}H_7Cl_2NO_2$

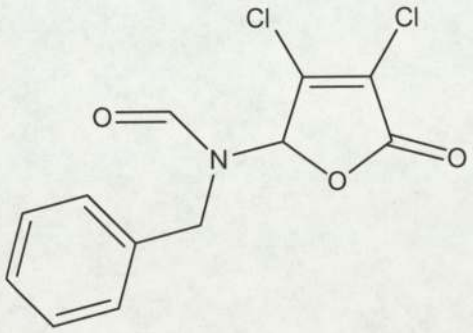
MS (APCI(-)): Corresponding peak was absent

^{1}H NMR (DMSO-d_6) 250 MHz: δ = 11.30-12.20 (br s, 1H, OH), 9.50 (s, CH), 7.84-7.90 (m, ArH, 2H), 7.75-7.81 (m, ArH, 2H), 7.68-7.74 (m, ArH) p.p.m.

^{13}C NMR (DMSO-d_6) 62.9 MHz: 182.2 (C=O), 155.3 (C=N), 138.5 (ArC), 129.7 (ArC, 2xC), 126.9 (CH-C-Cl), 119.5 (ArC, 2xC), 116.9 (ArC), 100.6 (CO-C-Cl) p.p.m.

IR (KBr-disc) υ max: 3413, 2974, 1674, 1626, 1580, 1486, 1329, 1263, 1192, 755, 686 cm^{-1}.

(4a): *N*-Benzyl-*N*-(3,4-dichloro-5-oxo-2,5-dihydro-furan-2-yl)-formamide.



Method: Dry mucochloric acid (15.0 g, 88.8 mmol) and 2 equivalents of the appropriate amide (175 mmol) were heated under reflux in toluene (100 ml) with conc. H_2SO_4 (1% v/w), using a Dean and Stark apparatus. TLC was used to monitor the reaction progress. After 24 hours, the liquid phase of the mixture was poured off and silicagel was added to the remaining dark brown thick oily layer until a light brown fine powder was obtained. The mixture was extracted using solid extraction column chromatography (1:9 methanol / ether) and remaining solvent was evaporated off under vacuum to give a yellow semisolid compound.

Yield = 15%

M.P: 189-91 ^{o}C

Rf (1:9 methanol / ether) = 0.28

Molecular Weight: 286.1

Molecular Formula: $C_{12}H_9NO_3Cl_2$

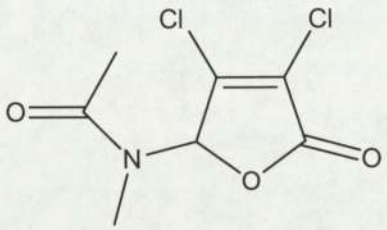
MS (APCI(+)): 136/138 (M+1), 285/287 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 8.18 (s, CHO), 7.15-7.59 (m, Ar-H, 5H), 6.03 (s, CH), 4.39-4.61 (m, CH_2, 2H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 164.9 (N-CO), 161.4 (CO), 154.6 (CH-C-Cl), 137.7 (Ar-C), 135.2 (C-Cl), 128.9 (2xAr-C), 128.0 (2xAr-C), 127.0 (Ar-C), 101.7 (CH), 42.1 (CH_2), 22.1 p.p.m.

IR (KBr-disc) υ max: 3428, 3281, 3052, 2924, 2883, 2356, 2338, 1655, 1637, 1527, 1390, 1235, 739, 698 cm^{-1}

(4b): *N*-(3,4-Dichloro-5-oxo-2,5-dihydro-furan-2-yl)-*N*-methyl-acet- amide.



Yield = 21%

M.P: 119-121 °C

Rf (1:9 methanol / ether) = 0.37

Molecular Weight: 224.0

Molecular Formula: $C_7H_7NO_3Cl_2$

MS (APCI(+)): 182/184/186 (M+1), 224/226/228 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.48 (s, CH), 2.69 (s, N-CH_3), 2.44 (s, CO-CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 172.4 (N-CO), 163.5 (CO), 148.1 (CH-C-Cl), 124.3 (C-Cl), 83.4 (CH), 29.1 (N-CH_3), 22.1 (CO-CH_3) p.p.m.

IR (KBr-disc) υ max: 3436, 2949, 2843, 1787, 1663, 1631, 1397, 1314, 988, 745 cm^{-1}

Crystal Data - (see section 3.2.2. for structure, sample recrystallised from methanol):

$C_7H_7Cl_2NO_3$
$M_r = 224.04$
T = 293(2) K
Tabular
0.30 x 0.10 x 0.05 mm
Colourless
Mo Kα radiation: λ = 0.71073 Å
Triclinic
P-1
a = 6.4718(15) Å
b = 8.3329(9) Å
c = 9.708(2) Å
α = 93.049(17) °
β = 94.791(12) °
γ = 107.651(19) °

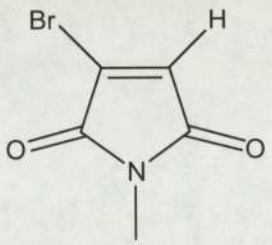
$V = 2772.4(5)$ Å^3
Z = 2
$D_x = 1.604$ Mg/m^{-3}
D_m not measured
$R [F^2 > 2\sigma(F^2)] = 0.0615$
$wR(F^2) = 0.1289$
2138 reflections
120 parameters

Selected geometric parameters (Å, °)

Cl(7)-C(2)	1.698(7)	O(5)-C(4)	1.467(9)
Cl(8)-C(3)	1.671(7)	C(2)-C(3)	1.327(9)
O(6)-C(1)	1.175(8)	N(9)-C(4)	1.408(9)
O(13)-C(11)	1.211(8)	N(9)-C(10)	1.474(8)
O(5)-C(1)	1.378(9)	O(13)-C(11)	1.211(8)
C(1)-C(2)-Cl(7)	120.6(5)	C(2)-C(3)-Cl(8)	128.3(6)
C(4)-C(3)-Cl(8)	121.6(5)	C(4)-N(9)-C(10)	118.8(5)
O(6)-C(1)-C(2)	131.0(7)	O(13)-C(11)-N(9)	120.2(7)
O(6)-C(1)-O(5)	122.2(7)		

(5a): 3-Bromo-1-methyl-pyrrole-2,5-dione.

Method: Dry mucochalic acid (15.0 g, 88.8 mmol) and 3 equivalents of the appropriate amide (287 mmol) were heated under reflux in toluene (100 ml) with conc. H_2SO_4 (1% v/w). TLC was used to monitor the reaction progress (1:1 ether / petroleum ether). After 8 hours, the liquid phase of the mixture was poured off and silicagel was added until a light brown fine powder was obtained. The mixture was extracted using solid extraction column chromatography (1:4 ether / petroleum ether) and remaining solvent was evaporated off under vacuum to give a pure crystalline compound.



Yield = 41%

M.P: 90-92 °C

R_f (1:1 ether / petroleum ether) = 0.72

Molecular Weight: 190

Molecular Formula: $C_5H_4BrNO_2$

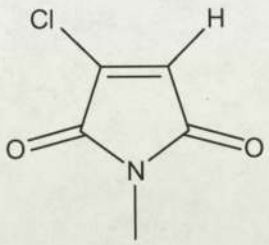
MS (APCI(+)): 190/192 (M+1), 222/224 (M+MeOH) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 6.89 (s, CH), 3.11 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.6 (CH-C=O), 165.6 (C-Br-C=O), 131.9 (CH), 129.4 (C-Br), 25.4 (CH) p.p.m.

IR (KBr-disc) υ max: 3464, 3105, 2947, 1778, 1714, 1707, 1585, 1429, 1371, 1287, 1139, 1102, 997, 962, 867, 817, 796, 756, 698 cm^{-1}

(5b): 3-Chloro-1-methyl-pyrrole-2,5-dione.



Yield = 22%

M.P: 88-90 °C

R_f (1:1 ether / petroleum ether) = 0.81

Mol. Weight: 145.5

Mol. Formula: $C_5H_4ClNO_2$

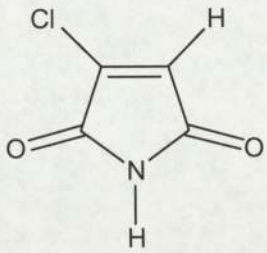
MS (APCI(+)): 146/148 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 6.65 (s, CH), 3.11 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.8 (CH-C=O), 165.7 (CCl-C=O), 140.9 (C-Cl), 126.8 (CH), 24.4 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3470, 3099, 2956, 1826, 1780, 1723, 1703, 1600, 1440, 1386, 1246, 980, 875, 852, 709 cm^{-1}

(5c): 3-Chloro-pyrrole-2,5-dione.



Yield = 11%

M.P: 113-115 °C

R_f (1:1 ether / petroleum ether) = 0.76

Mol. Weight: 131.5

Mol. Formula: $C_4H_2ClNO_2$

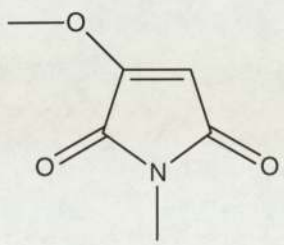
MS (APCI(-)): 130/132 (M-1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.45-7.95 (br s, NH), 6.71 (s, CH) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.8 (CH-C=O), 161.3 (C-Cl-C=O), 140.6 (C-Cl), 128.7 (CH) p.p.m.

IR (KBr-disc) υ max: 3481, 3243, 3107, 2705, 1847, 1774, 1712, 1612, 1595, 1338, 1239, 1137, 1052, 1038, 879, 735, 664, 557, 483 cm^{-1}

(6a): 3-Methoxy-1-methyl-pyrrole-2,5-dione.



Method 1: 1 ½ equivalents sodium alkoxide (5.2 mmol) was added to 0.5 g (3.4 mmol) 3-bromo-1-methyl-pyrrole-2,5-dione (**5a**) in 10 ml of alcohol (methanol or ethanol) and

stirred at room temperature for 10 minutes. The mixture was transferred to the fridge for a further 48 hours. TLC control was used to monitor the reaction (1:1 ether / petroleum ether). 10 ml water was added to this solution and washed with 3 x 15 ml ether. The combined organic layers were dried under magnesium sulphate. Solvent was removed from the remaining solution under vacuum until approximately 3-5 ml of solution remained. This liquid was allowed to evaporate off under argon resulting in a white or yellow compound.

Yield = 31%

25% (method 2)

M.P: 128-130 °C

R_f (4:1 ether / petroleum ether) = 0.73

Mol. Weight: 141.1

Mol. Formula: $C_6H_7NO_3$

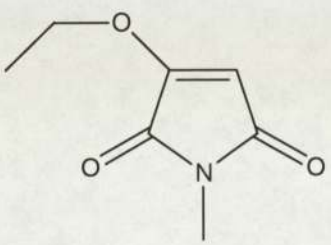
MS (APCI(+)): 142 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.45 (s, CH), 3.95 (s, 3H, O-CH_3), 3.05 (s, 3H, N-CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 170.5 (CH-C=O), 164.6 (O-C-C=O), 155.2 (CH-C-O), 94.6 (CH), 59.5 (O-CH_3), 24.5 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3435, 3110, 2944, 2358, 2345, 1722, 1635, 1326, 1246, 1118 cm^{-1}

(6b): 3-Ethoxy-1-methyl-pyrrole-2,5-dione.



Method 2: The same method as above was used, with the exception that (**5b**) 3-chloro-pyrrol-2,5-dione was used as reagent.

Yield = 24%

19% (method 2)

M.P: N/A – Semisolid

R_f (4:1 ether / petroleum ether) = 0.76

Mol. Weight: 155.1

Mol. Formula: $C_7H_9NO_3$

MS (APCI(+)): 128 (M+), 156 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.35 (s, CH), 4.08-4.20 (q, CH_2, J = 7.0 Hz, 2H), 3.01 (s, N-CH_3), 1.19-1.30 (t, CH_3, J = 7.0 Hz, 3H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.2 (CH-C=O), 161.0 (O-C-C=O), 153.9 (CH-C-O), 94.9 (CH), 61.8 (CH_2), 23.0 (N-CH_3), 16.7 CH_2-CH_3) p.p.m.

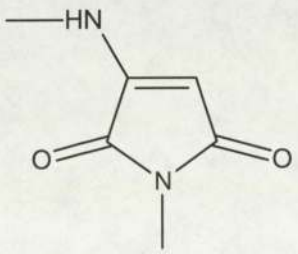
IR (KBr-disc) υ max: 3449, 3107, 2942, 2371, 2327, 1716, 1630, 1446, 1323, 1237, 952, 809, 679 cm^{-1}

(7a): 1-Methyl-3-methylamino-pyrrole-2,5-dione.

Method 1:

3-Bromo-1-methyl-pyrrole-2,5-dione (**5a**, 3.5x10^{-3} mol) was added to 3 ml ether. 3 equivalents of the appropriate amine (0.015 mol) were added and left to stir at 30-35 °C for 1 ½ hours. The mixture was worked up and extracted with ether and water. The resultant crystals were carefully re-crystallised from petroleum ether.

Method 2: The same method as above was used, with the exception that (**5b**) 3-chloro-1-methyl-pyrrol-2,5-dione was used as reagent.



Yield = 48%

39% (method 2)

M.P: 141-144 °C

R_f (3:2 ether / petroleum ether) = 0.33

Molecular Weight: 140.1

Molecular Formula: $C_6H_8N_2O_2$

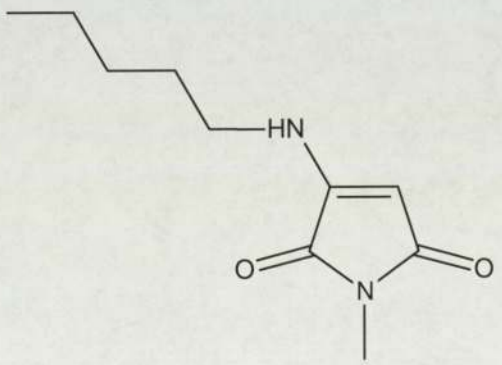
MS (APCI(+)): 141 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 5.21-5.42 (bs, NH), 4.78 (s, CH), 2.92 (m, overlapping, 2xCH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.6 (N-CO), 165.4 (CH-CO), 131.9 (CH), 131.3 (NH-C), 25.5 (NH-CH_3), 24.6 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3339, 3114, 2935, 2365, 1758, 1701, 1642, 1449, 1422, 985, 782 cm^{-1}.

(7b): 1-Methyl-3-pentylamino-pyrrole-2,5-dione.



Yield = 39%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.42

Molecular Weight: 196.3

Molecular Formula: $C_{10}H_{16}N_2O_2$

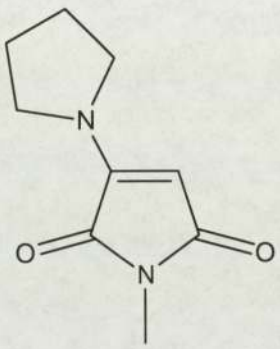
MS (APCI(+)): 197 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.31-5.52 (bs, NH), 4.81 (s, CH), 3.06-3.28 (m, NH-C$\underline{H}_2$), 3.02 (s, NH-CH_3), 1.58-1.79 (m, CH_3-C$\underline{H}_2$), 1.19-1.54 (m, overlapping, CH_3-CH_2-C$\underline{H}_2$, CH_3-CH_2-CH_2-C$\underline{H}_2$), 0.82-1.01 (m, C$\underline{H}_3$-CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.9 (N-CO), 160.8 (CH-CO), 145.2 (C-NH), 87.4 (CH), 47.6 (NH-CH_2), 32.2 (NH-CH_2-$\underline{C}H_2$), 28.9 (NH-CH_2-CH_2-$\underline{C}H_2$), 22.6 (N-CH_3), 20.9 (CH_3-$\underline{C}H_2$), 15.1 ($\underline{C}H_3$-CH_2) p.p.m.

IR (KBr-disc) υ max: 3438, 3321, 2927, 2852, 2364, 2336, 1699, 1633, 1375, 996 cm^{-1}

(7c): 1'-Methyl-2,3,4,5-tetrahydro-[1,3']bipyrrolyl-2',5'-dione.



Yield = 36%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.45

Molecular Weight: 180.2

Molecular Formula: $C_9H_{12}N_2O_2$

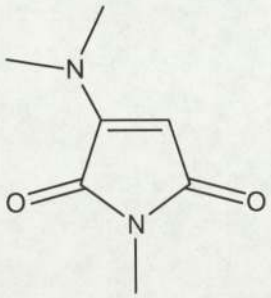
MS (APCI(+)): 181 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.05 (s, CH), 2.81-3.51 (m, overlapping N-CH_2, N-CH_3, 7H), 1.69-2.40 (m, N-CH_2-C$\underline{H}_2$, 4H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.1 (N-CO), 160.7 (CH-CO), 146.6 (C-N), 88.8 (CH-O), 48.4 (N-CH_2, 2xC), 37.5 (N-CH_2-$\underline{C}H_2$, 2xC), 25.7 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3433, 2926, 2848, 2361, 2325, 1694, 1616, 1443, 1369, 1340, 1253, 1167, 738 cm^{-1}

(7d): 3-Dimethylamino-1-methyl-pyrrole-2,5-dione.



Yield = 42%

M.P: 103-105 °C

R_f (3:2 ether / petroleum ether) = 0.40

Molecular Weight: 154.1

Molecular Formula: $C_7H_{10}N_2O_2$

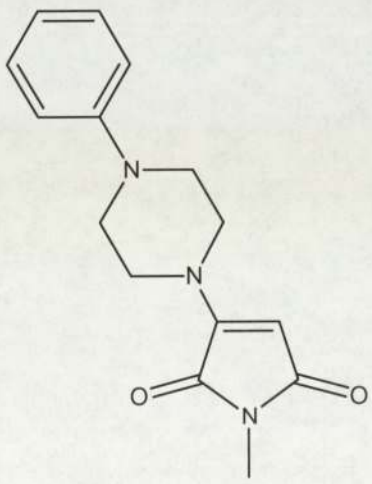
MS (APCI(+)): 155 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 4.72 (s, CH), 2.81-3.58 (m, 3xCH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.7 (N-CO), 161.6 (CH-CO), 144.3 (CH), 87.1 (CH-N), 44.0 (N-CH_3, 2xC), 23.3 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3449, 3118, 2924, 2359, 2337, 1706, 1627, 1442, 1375, 758 cm^{-1}

(7e): 1-Methyl-3-(4-phenyl-piperazin-1-yl)-pyrrole-2,5-dione.



Yield = 45%

M.P: 174-176 °C

R_f (3:2 ether / petroleum ether) = 0.29

Molecular Weight: 271.3

Molecular Formula: $C_{15}H_{17}N_3O_2$

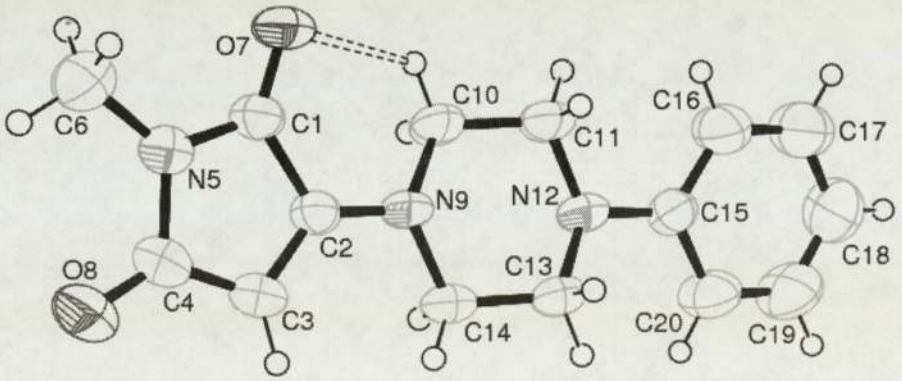
MS (APCI(+)): 272 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.41 (m, 2H, Ar-H), 6.92-7.25 (m, 3H, Ar-H), 4.98 (s, CH), 3.80-4.05 (m, ArC-N-CH_2, 4H) 3.28-3.41 (m, C-N-CH_2, 4H), 2.98 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.5 (N-CO), 160.4 (CH-CO), 151.3 (CH), 134.3 (ArC), 129.5 (2xArC), 128.0 (2xArC), 125.4 (ArC), 84.4 (CH-N), 51.5 (C-N-$\underline{C}H_2$, 2xC), 46.8 (ArC-N-$\underline{C}H_2$, 2xC), 24.7 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3432, 2919, 2854, 2366, 1701, 1620, 1452, 1384, 1236, 945 758 cm^{-1}

Crystal Data - (sample recrystallised from methanol):



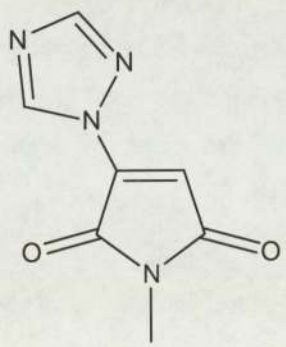
$C_{15}H_{17}N_3O_2$
$M_r = 271.32$
T = 293(2) K
Needle
0.80 x 0.20 x 0.15 mm
Yellow, transparent
Mo Kα radiation: λ = 0.71073 Å
Monoclinic
C2/c
a = 20.6250(17) Å
b = 6.1567(9) Å
c = 21.840(2) Å
β = 91.424(8) °

$V = 2772.4(5)$ Å^3
$Z = 8$
$D_x = 1.300$ Mg/m^{-3}
D_m not measured
$R [F^2 > 2\sigma(F^2)] = 0.0516$
$wR(F^2) = 0.1639$
2699 reflections
182 parameters

Selected geometric parameters (Å, °)

N(5)-C(6)	1.444(3)	C(2)-C(3)	1.345(3)
O(7)-C(1)	1.211(3)	N(9)-C(2)	1.344(3)
O(8)-C(4)	1.216(3)	N(12)-C(15)	1.406(3)
C(1)-N(5)-C(4)	110.0(2)	N(9)-C(2)-C(3)	129.7(2)
O(7)-C(1)-N(5)	125.0(2)	C(3)-C(2)-C(1)	106.5(2)
O(8)-C(4)-N(5)	122.2(3)	C(10)-N(9)-C(14)	112.6(2)

(7f): 1-Methyl-3-[1,2,4]triazol-1-yl-pyrrole-2,5-dione.



Yield = 40%

M.P: 166-168 °C

R_f (3:2 ether / petroleum ether) = 0.28

Molecular Weight: 178.2

Molecular Formula: $C_7H_6N_4O_2$

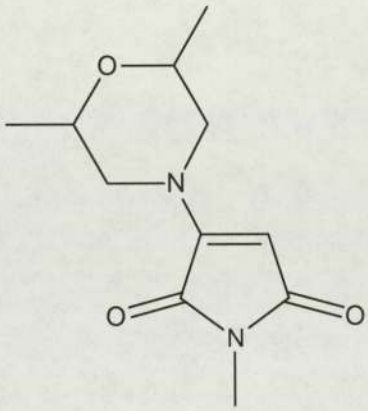
MS (APCI(+)): 179 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 8.09-8.31 (d, C-N-CH), 8.18 (d, N-N-CH), 4.83 (s, CH), 3.05 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.7 (N-CO), 161.2 (CH-CO), 153.3 (C-N-CH), 145.3 (N-N-CH), 133.3 (CH), 86.5 (CH-N), 24.2 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3439, 3124, 2929, 2864, 2363, 2340, 1769, 1645, 1472, 1273, 1143, 980 cm^{-1}

(7g): 3-(2,6-Dimethyl-morpholin-4-yl)-1-methyl-pyrrole-2,5-dione.



Yield = 34%

M.P: 103-106 °C

R_f (3:2 ether / petroleum ether) = 0.32

Molecular Weight: 224.3

Molecular Formula: $C_{11}H_{16}N_2O_3$

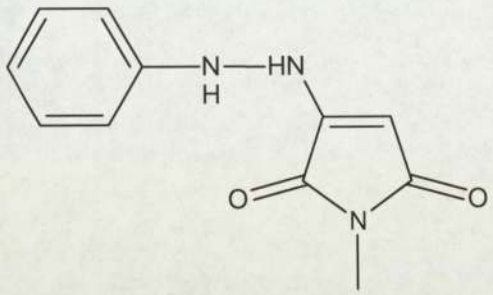
MS (APCI(+)): 225 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 4.82 (s, CH), 3.58-3.79 (m, O-CH, 2H), 2.90 (s, CH_3), 2.55-2.69 (m, N-CH_2, 4H), 1.16-1.22 (d, overlapping O-CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.6 (N-CO), 161.2 (CH-CO), 144.8 (CH-C-N), 89.1 (CH-CO), 64.0 (O-CH, 2xC), 54.9 (CH_2, 2xC), 20.4 (N-CH_3), 19.4 (CH-CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3439, 2975, 2924, 2855, 2367, 2335, 1709, 1607, 1444, 1379, 1133, 1082, 762 cm^{-1}

(7h): 1-Methyl-3-(*N'*-phenyl-hydrazino)-pyrrole-2,5-dione.



Yield = 32%

M.P: 179-181 °C

R_f (3:2 ether / petroleum ether) = 0.25

Molecular Weight: 217.2

Molecular Formula: $C_{11}H_{11}N_3O_2$

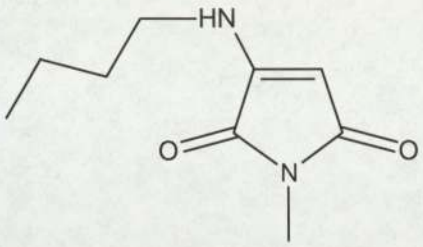
MS (APCI(+)): 218 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.24-7.51 (m, 3H, Ar-H), 6.79-7.06 (m, 2H, Ar-H), 6.25-6.35 (bs, ArC-NH), 5.31 (s, CH), 4.49-5.33 (bs, C-NH), 3.03 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.6 (N-CO), 160.9 (CH-CO), 153.3 (C-NH), 138.0 (ArC), 127.9 (2xArC), 124.0 (2xArC), 121.9 (ArC), 89.3 (CH-CO), 24.0 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3444, 3281, 3204, 2919, 2361, 2352, 1771, 1698, 1599, 1432, 1006, 762 cm^{-1}

(7i): 3-Butylamino-1-methyl-pyrrole-2,5-dione.



Yield = 41%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.35

Molecular Weight: 182.2

Molecular Formula: $C_9H_{14}N_2O_2$

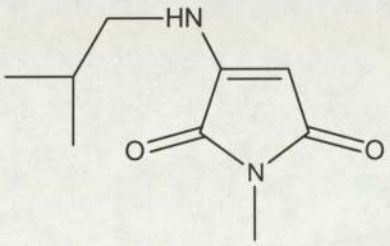
MS (APCI(+)): 183 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.35-5.45 (bs, NH), 4.82 (s, CH), 3.07-3.15 (q, NH-CH_2, *J* = 5.1 Hz), 2.98 (s, N-CH_3), 1.59-1.78 (m, NH-CH_2-CH_2), 1.35-1.56 (m, CH_3-$C\underline{H}_2$, *J* = 4.2 Hz), 0.96-1.02 (t, $C\underline{H}_3$-CH_2, *J* = 8.2 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.7 (N-CO), 160.4 (CH-CO), 148.0 (CH-N-$\underline{C}$), 85.8 ($\underline{C}$H-CO), 44.0 (NH-CH_2), 30.5 (NH-CH_2-$\underline{C}H_2$), 23.7 (N-CH_3), 22.8 (CH_3-$\underline{C}H_2$), 13.9 (CH_2-$\underline{C}H_3$) p.p.m.

IR (KBr-disc) υ max: 3434, 3328, 2927, 2860, 2372, 2332, 1710, 1644, 1446, 1380, 1279, 1122, 1005, 775 cm^{-1}

(7j): 3-Isobutylamino-1-methyl-pyrrole-2,5-dione.



Yield = 51%

45% (method 2)

M.P: 174-177 °C

R_f (3:2 ether / petroleum ether) = 0.37

Molecular Weight: 182.2

Molecular Formula: $C_9H_{14}N_2O_2$

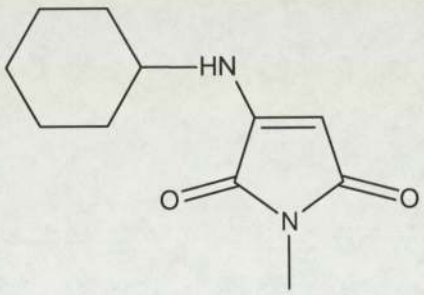
MS (APCI(+)): 183 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 5.27-5.43 (bs, NH), 4.75 (s, CH), 2.91-3.17 (m, overlapping NH-CH_2 & N-CH_3, 5H), 1.42-1.62 (m, CH_2-C<u>H</u>), 0.69-1.03 (m, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.3 (N-CO), 161.1 (CH-CO), 143.6 (CH-N-<u>C</u>), 88.1 (<u>C</u>H-CO), 49.4 (NH-CH_2), 31.9 (NH-CH_2-<u>C</u>H), 23.5 (N-CH_3), 16.9 (CH_2-<u>C</u>H_3) p.p.m.

IR (KBr-disc) υ max: 3448, 3305, 3105, 2957, 2914, 2864, 2367, 2335, 1699, 1639, 1435, 1282, 994, 758 cm^{-1}

(7k): 3-Cyclohexylamino-1-methyl-pyrrole-2,5-dione.



Yield = 39%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.32

Molecular Weight: 208.3

Molecular Formula: $C_{11}H_{16}N_2O_2$

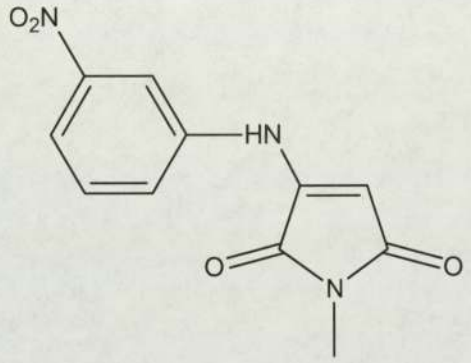
MS (APCI(+)): 209 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 4.99 (s, CH), 4.01-4.22 (bs, NH-C<u>H</u>), 2.98 (s, N-CH_3), 0.98-1.55 (m, overlapping CH-C<u>H</u>$_2$, CH-CH_2-C<u>H</u>$_2$, CH-CH_2-CH_2-C<u>H</u>$_2$, 10H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.7 (N-CO), 161.3 (CH-<u>C</u>O), 145.2 (CH-N-<u>C</u>), 84.4 (<u>C</u>H-CO), 53.6 (NH-CH), 31.7 (NH-CH-<u>C</u>H$_2$, 2xC), 25.4 (CH-CH_2-<u>C</u>H$_2$, 2xC), 24.4 (CH-CH_2-CH_2-<u>C</u>H$_2$), 23.3 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3442, 3339, 3112, 3009, 2924, 2361, 2329, 1718, 1699, 1629, 1441, 1392, 1115, 1044, 1026, 994, 767 cm^{-1}

(7l): 1-Methyl-3-(3-nitro-phenylamino)-pyrrole-2,5-dione.



Yield = 21%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.22

Molecular Weight: 247.2

Molecular Formula: $C_{11}H_{19}N_3O_4$

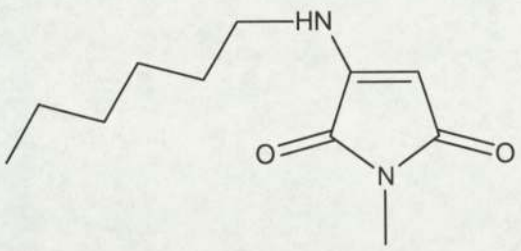
MS (APCI(+)): 248 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.79-8.38 (m, 4H, Ar-H), 5.18-5.35 (bs, NH-C<u>H</u>), 4.78 (s, CH), 3.05 (s, N-CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.2 (N-CO), 160.6 (CH-CO), 147.6 (CH-N-<u>C</u>), 137.8 (ArC), 125.7 (ArC), 121.2 (2xArC), 120.3 (ArC), 87.7 (<u>C</u>H-CO), 24.0 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3462, 3286, 3200, 3042, 2914, 2363, 2341, 1770, 1711, 1594, 1436, 1260, 1224, 1016, 749 cm^{-1}

(7m): 3-Hexylamino-1-methyl-pyrrole-2,5-dione.



Yield = 37%

M.P: 141-143 °C

R_f (3:2 ether / petroleum ether) = 0.37

Molecular Weight: 210.3

Molecular Formula: $C_{11}H_{18}N_2O_2$

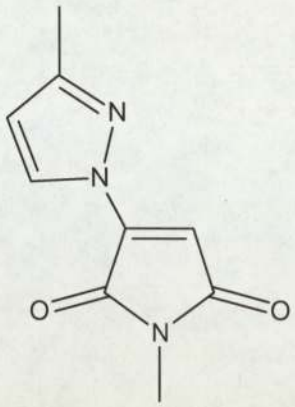
MS (APCI(+)): 211 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.18 (s, CH), 4.71-4.95 (bs, NH-C<u>H</u>), 3.49-3.61 (t, *J* = 5.5 Hz, NH-CH_2), 3.03 (s, N-CH_3), 1.21-1.69 (m, overlapping, NH-CH_2-C<u>H</u>$_2$, NH-CH_2-CH_2-C<u>H</u>$_2$, NH-CH_2-CH_2-CH_2-C<u>H</u>$_2$, NH-CH_2-CH_2-CH_2-CH_2-C<u>H</u>$_2$, 8H), 0.72-0.99 (t, *J* = 6.5 Hz, CH_2-CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.6 (N-CO), 161.3 (CH-CO), 143.4 (CH-N-<u>C</u>), 87.4 (<u>C</u>H-CO), 44.0 (NH-CH_2), 37.0 (NH-CH_2-<u>C</u>H$_2$), 33.8 (NH- CH_2-CH_2-<u>C</u>H$_2$), 30.5 (NH-CH_2-CH_2-CH_2-<u>C</u>H$_2$), 22.7 (N-CH_3), 23.7 (NH-CH_2-CH_2-CH_2-CH_2-C<u>H</u>$_2$), 14.2 (CH_2-<u>C</u>H$_3$) p.p.m.

IR (KBr-disc) υ max: 3438, 3325, 2937, 2362, 2335, 1708, 1634, 1442, 1107, 784 cm^{-1}

(7n): 1-Methyl-3-(3-methyl-pyrazol-1-yl)-pyrrole-2,5-dione.



Yield = 43%

M.P: 169-171 ^{o}C

R_f (3:2 ether / petroleum ether) = 0.37

Molecular Weight: 191.2

Molecular Formula: $C_9H_9N_3O_2$

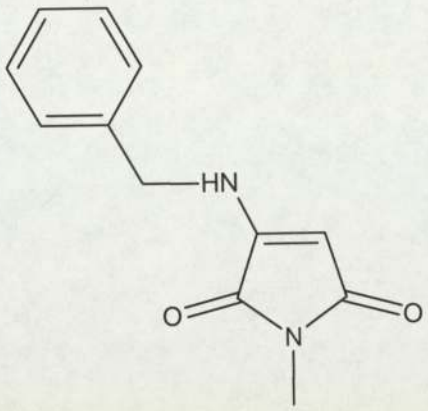
MS (APCI(+)): 192 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.10-7.18 (d, N-CH), 6.60-6.69 (d, N-CH-C<u>H</u>), 6.49 (s, CH), 3.00 (s, N-CH_3), 2.55-2.66 (m, C-CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.9 (N-CO), 161.3 (CH-CO), 150.6 (N-N-C), 144.3 (CH-N-<u>C</u>), 130.5 (N-CH), 111.2 (N-CH-<u>C</u>H), 88.8 (<u>C</u>H-CO), 24.1 (N-CH_3), 13.9 (C-<u>C</u>H_3) p.p.m.

IR (KBr-disc) υ max: 3437, 2929, 2860, 2365, 2338, 1784, 1719, 1701, 1436, 1376, 1289, 1074, 1042, 762 cm^{-1}

(7o): 3-Benzylamino-1-methyl-pyrrole-2,5-dione.



Yield = 39%

M.P: 143-145 °C

R_f (3:2 ether / petroleum ether) = 0.27

Molecular Weight: 216.2

Molecular Formula: $C_{12}H_{12}N_2O_2$

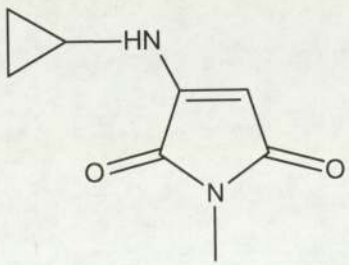
MS (APCI(+)): 217 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.25-7.65 (m, Ar-H, 5H), 5.58-5.89 (bs, NH), 4.79 (s, CH), 4.32-4.48 (d, CH_2), 2.98 (s, N-CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.3 (N-CO), 161.1 (CH-CO), 143.1 (CH-N-C), 133.4 (ArC), 128.2 (2xArC), 126.6 (2xArC), 125.1 (Ar-C), 89.0 (N-CH-CH), 44.0 (NH-CH_2), 24.0 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3322, 3023, 2965, 2363, 2336, 1694, 1626, 1450, 1120, 694 cm^{-1}

(7p): 3-Cyclopropylamino-1-methyl-pyrrole-2,5-dione.



Yield = 46%

M.P: 125-127 °C

R_f (3:2 ether / petroleum ether) = 0.34

Molecular Weight: 166.2

Molecular Formula: $C_8H_{10}N_2O_2$

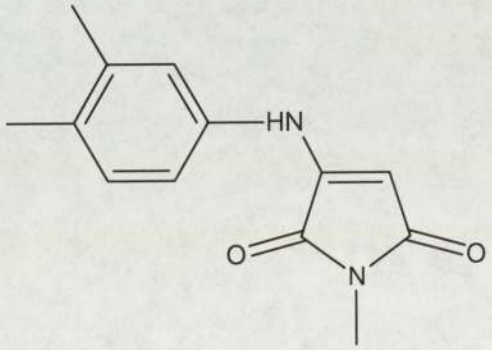
MS (APCI(+)): 167 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.41-5.61 (bs, NH), 5.02 (s, CH), 2.95 (s, N-CH_3), 2.45-2.62 (m, NH-CH), 0.58-1.10 (m, NH-CH-C$\underline{H}_2$, 4xH) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 170.7 (N-CO), 161.3 (CH-CO), 143.3 (CH-N-$\underline{C}$), 89.8 (N-CH-$\underline{C}$H), 23.7 (N-CH_3), 23.0 (NH-CH), 6.8 (NH-CH-CH_2) p.p.m.

IR (KBr-disc) υ max: 3445, 3333, 3105, 3011, 2957, 2921, 2366, 2335, 1708, 1632, 1448, 1390, 1256, 1018, 763 cm^{-1}

(7q): 3-(3,4-Dimethyl-phenylamino)-1-methyl-pyrrole-2,5-dione.



Yield = 30%

M.P: 185-187 °C

R_f (3:2 ether / petroleum ether) = 0.26

Molecular Weight: 230.3

Molecular Formula: $C_{13}H_{14}N_2O_2$

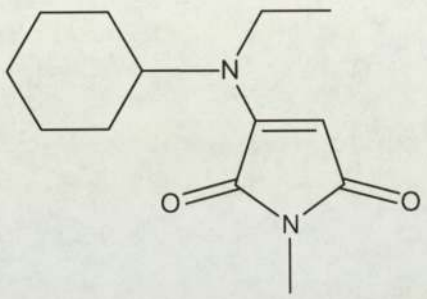
MS (APCI(+)): 231 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.79-7.42 (m, Ar-H, 3H), 5.39 (s, CH), 4.52-4.71 (bs, NH), 3.09 (s, N-CH_3), 2.55-2.68 (m, overlapping CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.0 (N-CO), 161.6 (CH-CO), 142.4 (CH-N-C), 137.3 (ArC), 135.0 (ArC) 132.0 (ArC), 124.2, (ArC) 120.3 (ArC), 117.6 (Ar-C), 87.8 (N-CH-CH), 23.0 ($N-CH_3$), 18.6 (ArC-CH_3, *o*-position) 17.2 (ArC-CH_3, *p*-position) p.p.m.

IR (KBr-disc) υ max: 3444, 3299, 2924, 2856, 2368, 2336, 1707, 1640, 1450, 1386, 1273, 1124, 595 cm^{-1}

(7r): 3-(Cyclohexyl-ethyl-amino)-1-methyl-pyrrole-2,5-dione.



Yield = 33%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.36

Molecular Weight: 236.3

Molecular Formula: $C_{13}H_{20}N_2O_2$

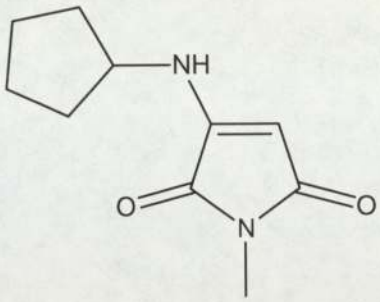
MS (APCI(+)): 237 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 4.80 (s, CH), 2.89 (s, $N-CH_3$), 2.49-2.73 (m, overlapping, N-CH, $N-CH_2$, 3H), 0.88-1.59 (m, overlapping, N-CH-CH_2, N-CH-CH_2-CH_2, N-CH-CH_2-CH_2-CH_2, CH_2-CH_3,13H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.1 (N-CO), 160.6 (CH-CO), 143.6 (CH-N-C), 89.1 (CO-CH), 60.9 (N-CH), 44.2 ($N-CH_2$), 30.7 (N-CH-CH_2, 2xC), 24.1 (N-CH-CH_2-CH_2, 2xC), 23.1 (N-CH-CH_2-CH_2-CH_2), 21.0 ($N-CH_3$), 13.5 (CH_2-CH_3) p.p.m.

IR (KBr-disc) υ max: 3425, 2933, 2855, 2363, 2335, 1699, 1602, 1440, 1379, 762 cm^{-1}

(7s): 3-Cyclopentylamino-1-methyl-pyrrole-2,5-dione.



Yield = 42%

M.P: N/A – Semisolid

R_f (3:2 ether / petroleum ether) = 0.32

Molecular Weight: 194.2

Molecular Formula: $C_{10}H_{14}N_2O_2$

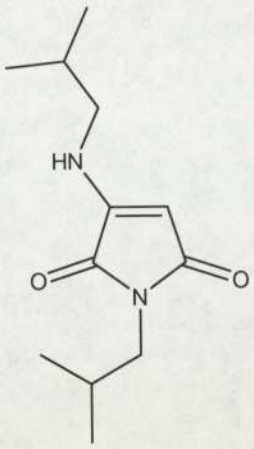
MS (APCI(+)): 195 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 5.38-5.58 (bs, NH), 4.83 (s, CH), 3.65-3.84 (m, N-CH_2), 3.00 (s, N-CH_3), 1.50-2.25 (m, overlapping, N-CH-C$\underline{H}_2$, N-CH-CH_2-C$\underline{H}_2$, 8H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 172.7.0 (N-CO), 167.8 (CH-CO), 148.8 (CH-N-$\underline{C}$), 84.4 (N-CH-$\underline{C}$H), 55.7 (N-CH), 43.9 (N-CH-$\underline{C}H_2$), 32.5 (N-CH-$\underline{C}H_2$, 2xC), 23.8 (N-CH-CH_2-$\underline{C}H_2$, 2xC), 23.4 (N-CH_3) p.p.m.

IR (KBr-disc) υ max: 3531, 3331, 3099, 2931, 2364, 2338, 1706, 1635, 1509, 1445, 1393, 1122, 1029, 768 cm^{-1}

(9a): 1-Isobutyl-3-isobutylamino-pyrrole-2,5-dione.



General Method:

0.5 g of the appropriate 5-alkoxy- or 5-aryloxy-furan-2-one (method 1-4) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and removed in vacuo to give a dark brown oil, which was purified using column chromatography (solvent system: 1:1 ether /petroleum ether) to give the desired product.

Methods 1-4: 5-alkoxy- or 5-aryl-furan-2-one used for the above synthesis:-

Method 1: (**IprOF**) 3,4-Dichloro-5-isopropoxy-5H-furan-2-one.
Method 2: (**BzOF**) 5-Benzyloxy-3,4-dichloro-5H-furan-2-one.
Method 3: (**AlOF**) 5-Allyloxy-3,4-dichloro-5H-furan-2-one.
Method 4: (**MeOF**) 3,4-Dichloro-5-methoxy-5H-furan-2-one.

Yield = 13% (method 1)
9% (method 2)
12% (method 3)
M.P: 130-133 °C

Rf (1:1 ether / petroleum ether) = 0.77

Molecular Weight: 224.3

Molecular Formula: $C_{12}H_{20}N_2O_2$

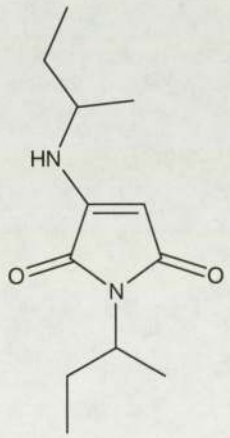
MS (APCI(+)): 181 (M+), 225 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.33-5.58 (bs, NH), 4.83 (s, CO-CH), 3.26-3.34 (d, N-CH_2), 2.97-3.08 (t, NH-CH_2, J = 6.0 Hz), 1.89-2.11 (m, overlapping C<u>H</u>-CH_2-N & C<u>H</u>-CH_2-NH, 2H), 0.87-1.09 (m, overlapping CH_3, 12H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 173.0 (CN-<u>C</u>=O), 167.8 (CH-<u>C</u>=O), 149.5 (CO-<u>C</u>N) 83.7 (CH-CCl), 52.0 (NH-CH_2) 45.0 (N-CH_2), 28.0 (<u>C</u>H-CH_2-NH), 27.9 (<u>C</u>H-CH_2-N), 20.2 (NH-CH_2-CH-<u>C</u>H_3, 2xC), 20.0 (N-CH_2-CH-<u>C</u>H_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3339, 2961, 2941, 2882, 2365, 1705, 1635, 1519, 1449, 1416, 1297, 1131, 1035, 786 cm^{-1}.

(9b): 1-sec-Butyl-3-sec-butylamino-pyrrole-2,5-dione.



Yield = 9% (method 1)

7% (method 4)

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.80

Molecular Weight: 224.3

Molecular Formula: $C_{12}H_{20}N_2O_2$

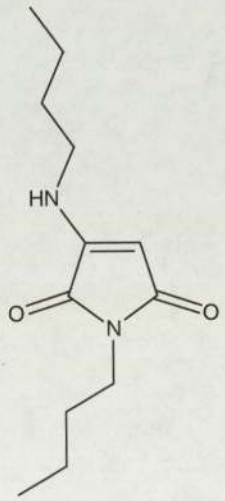
MS (APCI(+)): 169 (M+), 225 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.79 (s, CO-CH), 5.04-5.25 (bs, NH), 3.82-4.16 (m, overlapping N-CH & NH-CH, 2H), 1.46-1.73 (m, overlapping CH_2-C<u>H</u>-N & CH_2-C<u>H</u>-NH, 2H), 1.19-1.31 (m, overlapping CH-<u>C</u>H_3, 6H), 0.72-0.97 (m, overlapping CH_2-<u>C</u>H_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 173.0 (CN-<u>C</u>=O), 168.3 (CH-<u>C</u>=O), 148.0 (CO-<u>C</u>N), 83.4 (CH-CCl), 51.9 (NH-CH), 48.3 (N-CH), 29.0 (<u>C</u>H_2-CH-NH), 27.1 (<u>C</u>H_2-CH-N), 19.4 (NH-CH-<u>C</u>H_3), 18.4 (N-CH-<u>C</u>H_3), 11.3 (NH-CH-CH_2-<u>C</u>H_3), 10.3 (N-CH-CH_2-<u>C</u>H_3) p.p.m.

IR (KBr-disc) υ max: 3428, 2977, 2932, 2867, 2370, 2338, 1703, 1642, 1461, 1393 1229, 1106, 1019, 900 cm^{-1}.

(9c): 1-Butyl-3-butylamino-pyrrole-2,5-dione.



Yield = 13% (method 1)

10% (method 4)

M.P: 135-137 °C

Rf (1:1 ether / petroleum ether) = 0.81

Molecular Weight: 224.3

Molecular Formula: $C_{12}H_{20}N_2O_2$

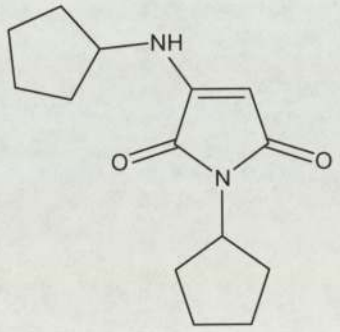
MS (APCI(+)): 225 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.11-5.30 (bs, NH), 4.69 (s, CO-CH), 3.40-3.52 (t, N-CH_2, *J* = 6.9 Hz), 3.05-3.18 (q, NH-CH_2, *J* = 4.9 Hz), 1.18-1.67 (m, overlapping CH_2, 8H), 0.82-1.03 (m, overlapping CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ =170.4 (CN-C=O), 165.1 (CH-C=O), 150.4 (CO-CN), 84.9 (CH-CCl), 39.8 (NH-CH_2), 39.4 (N-CH_2), 31.3 (CH_2-CH_2-NH), 29.5 (CH_2-CH_2-N), 20.1 (NH-CH_2-CH_2-CH_2), 19.7 (N-CH_2-CH_2-CH_2), 13.7 (NH-CH_2-CH_2-CH_2-CH_3), 13.6 (N-CH_2-CH_2-CH_2-CH_3) p.p.m.

IR (KBr-disc) υ max: 3431, 2966, 2925, 2867, 2361, 2338, 1701, 1638, 1458, 1399 1232, 1105, 1019, 893 cm^{-1}

(9d): 1-Cyclopentyl-3-cyclopentylamino-pyrrole-2,5-dione.



Yield = 15% (method 1)

12% (method 3)

M.P: 142-144 °C

Rf (1:1 ether / petroleum ether) = 0.78

Molecular Weight: 248.3

Molecular Formula: $C_{14}H_{20}N_2O_2$

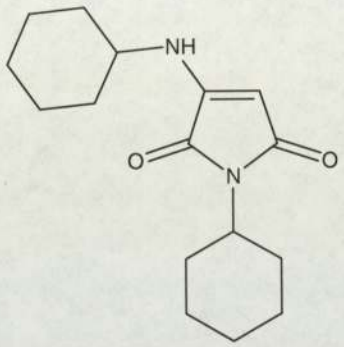
MS (APCI(+)): 249 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.26-5.41 (bs, NH), 4.82 (s, CH-O), 4.31-4.49 (m, N-CH, J = 8.8 Hz), 3.62-3.79 (m, NH-CH), 1.51-2.13 (m, overlapping CH_2, 16H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.6 (CN-C=O), 161.0 (CH-C=O), 148.3 (CH-CCl), 84.3 (CH-CO), 55.6 (NH-CH), 50.5 (N-CH), 32.1 (NH-CH-CH_2, 2xC), 29.5 (NH-CH-CH_2, 2xC), 24.7 (NH-CH-CH_2-CH_2, 2xC), 23.8 (NH-CH-CH_2-CH_2, 2xC) p.p.m.

IR (KBr-disc) υ max: 3441, 3252, 2924, 2855, 2376, 2338, 1698, 1651, 1623, 1399, 1125, 1075, 989, 892, cm^{-1}.

(9e): 1-Cyclohexyl-3-cyclohexylamino-pyrrole-2,5-dione.



Yield = 7% (method 2)

10% (method 3)

M.P: 146-148 °C

Rf (1:1 ether / petroleum ether) = 0.80

Molecular Weight: 276.4

Molecular Formula: $C_{16}H_{24}N_2O_2$

MS (APCI(+)): 195 (M+), 277 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 5.19-5.40 (bs, NH), 4.66 (s, CH-O), 3.74-3.99 (m, N-CH), 2.99-3.23 (m, NH-CH), 1.53-2.15 (m, overlapping CH_2, 12H), 1.07-1.49 (m, overlapping CH_2, 8H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 169.2 (CN-C=O), 162.0 (CH-C=O), 147.3 (CH-CCl), 83.4 (CH-CO), 53.1 (NH-CH), 50.0 (N-CH), 32.0 (NH-CH-CH_2, 2xC), 30.1 (NH-CH-CH_2, 2xC), 26.0 (NH-CH-CH_2-CH_2, 2xC), 25.3 (NH-CH-CH_2-CH_2, 2xC), 24.7 (NH-CH-CH_2-CH_2-CH_2), 24.4 (NH-CH-CH_2-CH_2-CH_2) p.p.m.

IR (KBr-disc) υ max: 3431, 2966, 2925, 2867, 2361, 2338, 1701, 1638, 1521, 1458, 1399, 1232, 1105, 1019, 893 cm^{-1}.

Crystal Data - (see section 3.3. for structure, sample recrystallised from methanol):

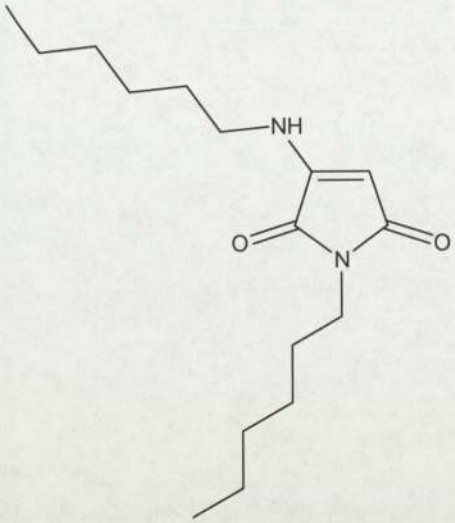
$C_{16}H_{24}N_2O_2$
$M_r = 276.37$
T = 293(2) K
Block
0.50 x 0.50 x 0.50 mm
Yellow, transparent
Mo Kα radiation: λ = 0.71073 Å
Tetragonal
$I4_1md$
a = 12.826(3) Å
b = 12.826(3) Å
c = 18.910(3) Å

$V = 3110.8(5)$ $Å^3$
Z = 8
$D_x = 1.180$ Mg/m^{-3}
D_m not measured
$R [F^2 > 2\sigma(F^2)] = 0.0753$
$wR(F^2) = 0.2020$
3226 reflections
157 parameters

Selected geometric parameters (Å, °)

C(6)-N(1)	1.328(15)	C(2)-O(2)	1.218(12)
C(3)-N(10)	1.333(14)	N(1)-C(2)	1.361(15)
C(5)-O(5)	1.220(11)	N(1)-C(5)	1.418(11)
C(3)-C(4)	1.357(15)		
C(6)-C(7)-C(8)	112.4(8)	O(5)-C(5)-N(1)	121.3(11)
C(7)-C(8)-C(9)	110.1(7)	O(5)-C(5)-C(4)	129.2(12)
C(13)-C(12)-C(11)	111.7(7)	O(2)-C(2)-C(3)	126.4(13)
C(14)-C(13)-C(12)	113.6(7)	N(10)-C(3)-C(4)	133.0(11)
O(2)-C(2)-N(1)	126.4(12)		

(9f): 1-Hexyl-3-hexylamino-pyrrole-2,5-dione.



Yield = 6% (method 2)

9% (method 4)

M.P: N/A – Semisolid

Rf (1:1 ether / petroleum ether) = 0.84

Molecular Weight: 280.4

Molecular Formula: $C_{16}H_{28}N_2O_2$

MS (APCI(+)): 281 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 5.22-5.42 (bs, NH), 4.82 (s, CO-CH), 3.10-3.66 (m, overlapping N-CH_2 & NH, 3H), 2.83-2.99 (m, NH-CH_2), 1.18-1.67 (m, overlapping N-CH_2-C$\underline{H_2}$ & NH-CH_2-C$\underline{H_2}$ 4H), 1.06-1.38 (m, overlapping CH_2, 12H), 0.64-0.98 (m, overlapping CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.0 (CN-$\underline{C}$=O), 163.0 (CH-$\underline{C}$=O), 143.9 (CO-$\underline{C}$N), 84.4 (CH-CCl), 40.0 (NH-CH_2), 39.7 (N-CH_2), 31.4 ($\underline{C}H_2$-CH_2-NH), 31.3 ($\underline{C}H_2$-CH_2-N), 29.7 (NH-CH_2-CH_2-$\underline{C}H_2$), 29.2 (N-CH_2-CH_2-$\underline{C}H_2$), 26.6 (NH-CH_2-CH_2-CH_2-$\underline{C}H_2$), 26.5 (N-CH_2-CH_2-CH_2-$\underline{C}H_2$), 22.6 (NH-CH_2-CH_2-CH_2-CH_2-$\underline{C}H_2$), 22.5 (N-CH_2-CH_2-CH_2-CH_2-$\underline{C}H_2$), 14.0 (NH-CH_2-CH_2-CH_2-CH_2-$\underline{C}H_3$), 13.96 (N-CH_2-CH_2-CH_2-CH_2-$\underline{C}H_3$) p.p.m.

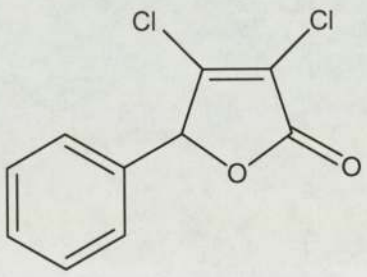
IR (KBr-disc) υ max: 3447, 2931, 2853, 2370, 2350, 1716, 1638, 1524, 1462, 1220, 1040, 972, 711 cm^{-1}.

6.4. Experiments to Chapter 4.

(AR-Fur) Synthesis of 3,4-dichloro-5-phenyl-5H-furan-2-one.

Method 1: Dry and powdered aluminium chloride (20g, 0.15 mol) was added slowly to a mixture of mucochloric acid (16.9g, 0.1 mol) and benzene or chlorobenzene (250 ml). The reaction mixture was stirred overnight at room temperature. It was then poured into a mixture of ice (100g) and concentrated HCl (32 ml). The organic layer was separated by separating funnel and washed with H_2O (3 x 100 ml). The combined organic layers were dried over magnesium sulphate and the solvent was removed under vacuum. The residue was crystallized in n-hexane.

Method 2: The same as method 1, except THF (12 ml, 0.30 mol) was used as a co-solvent along side benzene or chlorobenzene.



Yield = 66% (Method 1)

78% (Method 2)

M.P: 78-79 °C

R_f (4:1 ether / petroleum ether) = 0.62

Molecular Weight: 229.1

Molecular Formula: $C_{10}H_6Cl_2O_2$

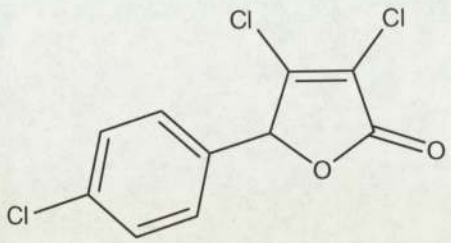
MS (APCI(+)): 195/197 (M+), 230/232 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.22-7.51 (m, ArH, 5H), 5.81 (CH) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.3 (C=O), 152.2 (CH-C-Cl), 139.8 (ArC), 130.5, 129.3, 128.5, 127.4, 127.2 (5xArC), 121.2 (C=O-C-Cl), 83.5 (CH) p.p.m.

IR (KBr-disc) υ max: 3445, 3074, 3035, 2959, 2056, 1768, 1630, 1499, 1457 1294, 1224, 1028, 910, 772, 705 cm^{-1}.

(Cl-AR-Fur): 3,4-Dichloro-5-(4-chloro-phenyl)-5H-furan-2-one.



Yield = 58% (Method 1)

69% (Method 2)

M.P: 76-78 °C

R_f (4:1 ether / petroleum ether) = 0.55

Molecular Weight: 263.5

Molecular Formula: $C_{10}H_5Cl_3O_2$

MS (APCI(+)): 227/229/231 (M+1), 262/263/265 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.42-7.55 (m, ArH, 2H), 7.28-7.40 (m, ArH, 2H), 5.91 (CH) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.3 (C=O), 152.0 (CH-C-Cl), 136.6 (ArC), 130.1 (ArC), 129.6 (2xArC), 128.7 (2xArC), 121.3 (CO-C-Cl), 82.9 (CH) p.p.m.

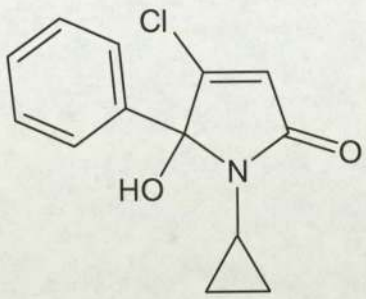
IR (KBr-disc) υ max: 3451, 3075, 2952, 2051, 1769, 1636, 1497, 1419, 1289, 1231, 1027, 927, 826, 748, 720 cm^{-1}.

General Method:

The relevant amine (3 eq.) was added to a solution of **Ar-Fur** or **Cl-Ar-Fur** (0.7 mol) in ether (10 ml) and stirred on ice for 30 minutes, allowing to warm up to RT over the time. The resultant mixture was poured into 5 ml water and separated using a separating funnel. The mixture was washed with H_2O (3 x 5 ml). The organic layer was dried over

magnesium sulphate and the solvent was removed under vacuum. All compounds gave a semi-solid. These were passed through a column (4:1 ether / petroleum ether). The resulting combined fractions were dried from excess solvent under vacuum to yield crystals.

(AR-1): 4-Chloro-1-cyclopropyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 83 %

Melting Point: 177-179 °C

R_f (4:1 ether / petroleum ether) = 0.24

Molecular Weight: 249.7

Molecular Formula: $C_{13}H_{12}ClNO_2$

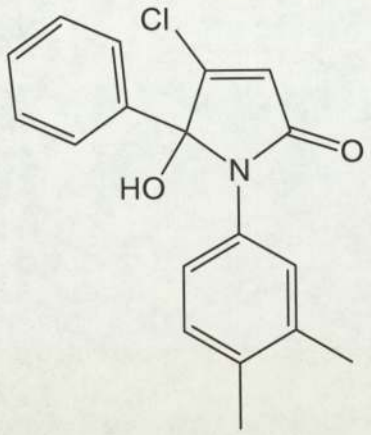
MS (APCI(+)): 193/195 (M+1), 250/252 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.49 (ArH, 5H), 6.09 (s, CH), 3.41-3.50 (C-OH), 2.08-2.21 (m, N-CH), 0.95-1.04 & 0.38-0.69 (m, CH_2, 4H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.4 (C=O), 154.8 (C-Cl), 135.2 (ArC), 129.2 (2xArC), 128.8 (2xArC), 126.1 (ArC), 122.2 (CH-CCl), 93.5 (C-OH), 22.6 (N-CH), 3.8, 5.1 (CH_2, 2C) p.p.m.

IR (KBr-disc) υ max: 3416, 3260, 3105, 3011, 2363, 2338, 1671, 1602, 1490, 1450, 1409, 1369, 1256, 1144, 1032, 939, 833, 752, 702 cm^{-1}.

(AR-2): 4-Chloro-1-(3,4-dimethyl-phenyl)-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 49 %

M.P: 168-171 °C

R_f (4:1 ether / petroleum ether) = 0.19

Molecular Weight: 313.8

Molecular Formula: $C_{18}H_{16}ClNO_2$

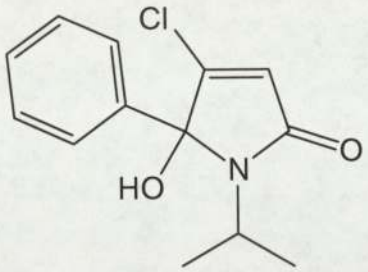
MS (APCI(+)): 193/195 (M+1), 314/316 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.41-7.52 (m, Ar-H, 2H), 7.30-7.38 (m, Ar-H, 3H), 7.18 (s, Ar-H, 1H), 6.92-7.01 (m, Ar-H, 2H), 6.38 (s, CH-O), 3.68-3.73 (bs, C-OH), 2.13-2.27 (m, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.9 (C=O), 159.7 (C-Cl), 136.9 (ArC), 135.1 (ArC), 132.4 (ArC), 129.9 (ArC), 129.0 (2xArC), 126.9 (ArC), 126.1 (2xArC), 123.0 (ArC) 122.2 (CH-CCl), 93.5 (C-OH), 19.9 (Ar-CH_3), 19.3 (Ar-CH_3) p.p.m.

IR (KBr-disc) υ max: 3517, 3357, 3114, 2840, 2674, 2361, 2342, 1678, 1607, 1464, 1412, 1361, 1208, 1138, 1071, 988, 755, 700 cm^{-1}.

(AR-3): 4-Chloro-5-hydroxy-1-isopropyl-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 79 %

Melting Point: 163-165 °C

R_f (4:1 ether / petroleum ether) = 0.26

Molecular Weight: 251.7

Molecular Formula: $C_{13}H_{14}ClNO_2$

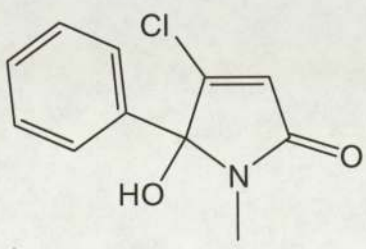
MS (APCI(+)): 193/195 (M+1), 252/254 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.40-7.51 (m, ArH, 5H), 6.14 (s, CH), 3.71-3.79 (bs, OH), 3.42-3.59 (m, N-CH, *J* = 7.5 Hz), 1.33-1.48 & 1.21-1.28 (d, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.5, 155.0, 135.0 (ArC), 129.1 (2xArC), 128.5 (2xArC), 126.4 (ArC), 122.4 (CH-CCl), 93.4 (C-OH), 45.6 (N-CH), 21.1, 20.0 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3227, 2990, 2940, 2365, 2350, 1956, 1693, 1615, 1456, 1428, 1247, 1131, 1072, 1009, 934, 847, 747, 697 cm^{-1}.

(AR-4): 4-Chloro-5-hydroxy-1-methyl-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 75 %

Melting Point: 146-148 °C

R_f (4:1 ether / petroleum ether) = 0.26

Molecular Weight: 223.7

Molecular Formula: $C_{11}H_{10}ClNO_2$

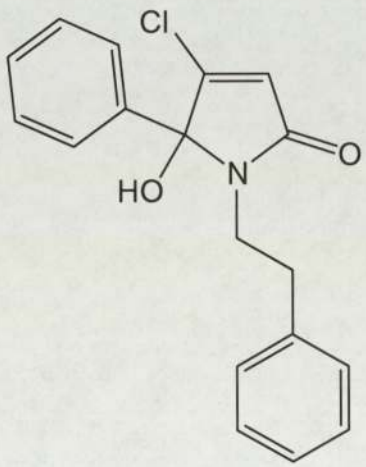
MS (APCI(+)): 193/195 (M+1), 224/226 (M+) m/z

^1H NMR ($CDCl_3$)) 250 MHz: δ = 7.29-7.48 (m, ArH, 5H), 6.49 (s, CH), 2.08 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.1 (C=O), 156.4 (C-Cl), 134.1 (ArC), 129.4 (2xArC), 128.9 (2xArC), 126.2 (ArC), 121.3 (CH-Cl), 92.6 (C-OH), 24.5 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3224, 3110, 2952, 2820, 2617, 2375, 2339, 1975, 1697, 1605, 1453, 1438, 1258, 1207, 1065, 992, 856, 764, 704 cm^{-1}.

(AR-5): 4-Chloro-5-hydroxy-1-phenethyl-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 49 %

Melting Point: 155-158 °C

R_f (4:1 ether / petroleum ether) = 0.21

Molecular Weight: 313.8

Molecular Formula: $C_{18}H_{16}ClNO_2$

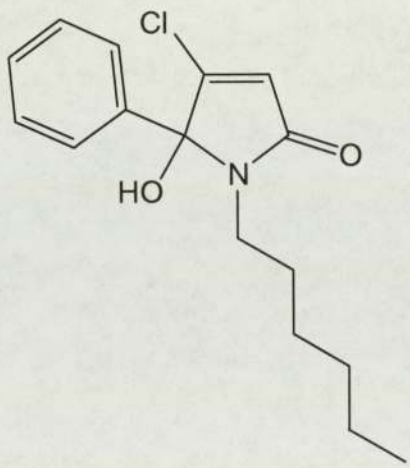
MS (APCI(+)): 193/195 (M+1), 314/316 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.09-7.53 (m, ArH, 10H), 6.20 (s, CH), 3.64-3.79 (m, N-CH_2, 1H), 2.88-3.29 (m, overlapping OH & Ar-CH_2, 3H), 2.60-2.75 (m, N-CH_2, 1H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.0 (C=O), 155.7 (C-Cl), 139.0 (ArC), 134.6 (ArC), 129.4 (2xArC), 128.85 (2xArC), 128.84 (2xArC), 128.6 126.6 (2xArC), 126.2 (2xArC), 121.8 (CH-CCl), 92.7 (C-OH), 41.9 (N-CH_2), 34.6 (Ar-CH_2) p.p.m.

IR (KBr-disc) υ max: 3433, 3246, 2929, 2366, 2334, 1681, 1658, 1607, 1455, 1406, 1251, 1151, 1128, 1066, 931, 753, 699 cm^{-1}.

(AR-6): 4-Chloro-1-hexyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 51 %

Melting Point: 173-175 °C

R_f (4:1 ether / petroleum ether) = 0.28

Molecular Weight: 293.8

Molecular Formula: $C_{16}H_{20}ClNO_2$

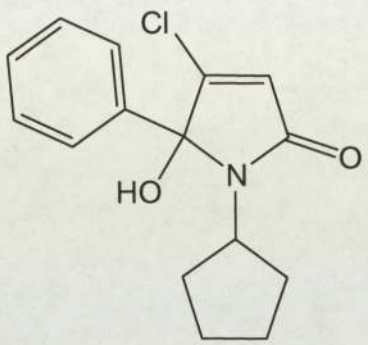
MS (APCI(+)): 193/195 (M+1), 294/296 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.40-7.52 (m, ArH, 5H), 6.15 (s, CH), 4.76-4.82 (bs, OH), 3.28-3.49 (m, CH_2, 1H), 2.91-3.08 (m, CH_2, 1H), 1.09-1.59 (m, CH_2, overlapping, 8H), 0.78-0.92 (t, CH_3, J = 7.1 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.0 (C=O), 155.6 (C-Cl), 134.9 (ArC), 129.2 (2xArC), 128.7 (2xArC), 126.2 (ArC), 121.8 (CH-Cl), 93.0 (C-OH), 40.2, 31.3, 28.7, 26.8, 22.5 (CH_2, 5xC), 14.0 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3245, 2930, 2865, 1689, 1658, 1494, 1453, 1412, 1365, 1321, 1150, 1069, 927, 753, 696 cm^{-1}.

(AR-7): 4-Chloro-1-cyclopentyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 81 %

Melting Point: 180-182 °C

R_f (4:1 ether / petroleum ether) = 0.26

Molecular Weight: 277.8

Molecular Formula: $C_{15}H_{16}ClNO_2$

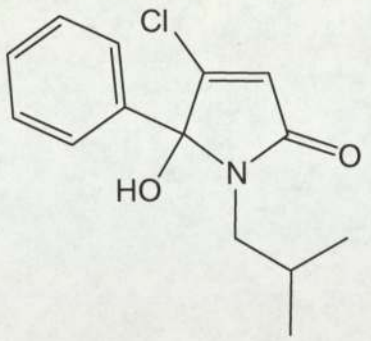
MS (APCI(+)): 193/195 (M+1), 278/280 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.39-7.61 (m, ArH, 5H), 6.08 (s, CH), 4.77-4.92 (bs, OH), 3.49-3.68 (m, N-CH, *J* = 8.9 Hz), 1.98-2.17 (m, CH_2), 1.71-1.96 (m, CH_2, 4H), 1.36-1.55 (m, CH_2, 4H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.2 (C=O), 155.0 (C-Cl), 135.2 (ArC), 129.1 (2xArC), 128.6 (2xArC), 126.5 (ArC), 122.2 (CH-CCl), 93.3 (C-OH), 54.3 (N-CH), 30.0 (CH_2), 28.8 (CH_2), 24.5, 24.4 (CH_2, 2xC) p.p.m.

IR (KBr-disc) υ max: 3220, 2961, 2877, 2373, 2341, 1684, 1613, 1448, 1426, 1248, 1199, 1141, 1070, 934, 850, 750, 701 cm^{-1}.

(AR-8): 4-Chloro-5-hydroxy-1-isobutyl-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 85 %

Melting Point: 167-169 °C

R_f (4:1 ether / petroleum ether) = 0.27

Molecular Weight: 264.7

Molecular Formula: $C_{14}H_{16}ClNO_2$

MS (APCI(+)): 193/195 (M+1), 266/268 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.38-7.51 (m, ArH, 5H), 6.24 (s, CH), 4.79-4.98 (bs, OH), 3.23-3.32 & 2.18-2.29 (dd, CH_2, *J* = 8.1 Hz, 2H), 1.71-1.90 (m, C<u>H</u>-CH_2, *J* = 7.4) Hz), 0.76-0.96 (m, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 168.5 (C=O), 155.7 (CH-<u>C</u>Cl), 137.1 (ArC), 129.2, 128.7, 126.2 (5xArC), 121.7 (<u>C</u>H-CCl), 93.1 (C-OH), 47.6 (CH_2), 27.5 (<u>C</u>H-CH_2), 20.4 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3237, 3114, 2965, 2926, 2881, 2374, 2343, 1675, 1614, 1460, 1416, 1299, 1251, 1202, 1150, 1072, 1027, 878, 758, 696 cm^{-1}.

Crystal data - (See section 4.2.2. for structure, sample recrystallised from methanol):

$C_{28}H_{32}Cl_2N_2O_4$
M_r = 531.46
T = 293(2) K
Tabular
0.20 x 0.15 x 0.10 mm
Colourless

V = 1371.5(5) $Å^3$
Z = 2
D_x = 1.287 Mg/m^{-3}
D_m not measured
R [$F^2 > 2\sigma(F^2)$] = 0.0541
w$R(F^2)$ = 0.1165

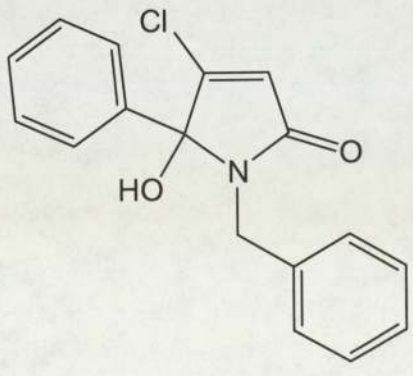
Mo Kα radiation: λ = 0.71073 Å
Triclinic
P-1
a = 8.3190(13) Å
b = 12.614(4) Å
c = 13.8106(18) Å
α = 93.049(17) °
β = 94.791(12) °
γ = 107.651(19) °

5136 reflections
331 parameters

Selected geometric parameters (Å, °)

Cl(1)-C(1)	1.696(4)	Cl(1')-C(1')	1.695(4)
C(1)-C(4)	1.310(5)	C(1')-C(4')	1.322(5)
C(2)-C(5)	1.511(5)	C(2')-C(5')	1.524(5)
C(2)-O(2)	1.410(4)	C(2')-O(2')	1.400(4)
C(3)-O(1)	1.224(5)	C(3')-O(1')	1.237(4)
N(1)-C(11)	1.448(5)	N(1)-C(11)	1.448(5)
C(3)-N(1)-C(11)	124.0(4)	C(3')-N(1')-C(11')	124.5(3)
O(1)-C(3)-N(1)	125.6(4)	O(1')-C(3')-N(1')	124.5(4)
O(2)-C(2)-C(5)	108.0(3)	O(2')-C(2')-C(5')	108.2(3)
C(4)-C(1)-Cl(1)	129.0(4)	C(4')-C(1')-Cl(1')	129.1(3)

(AR-9): 1-Benzyl-4-chloro-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 71 %

Melting Point: 165-167 °C

R_f (4:1 ether / petroleum ether) = 0.21

Molecular Weight: 299.8

Molecular Formula: $C_{17}H_{14}ClNO_2$

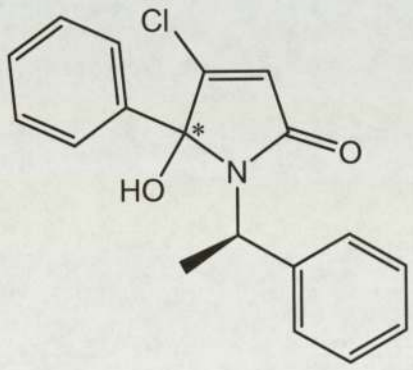
MS (APCI(+)): 193/195 (M+1), 300/302 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.42 (m, ArH, 5H), 7.14-7.27 (m, ArH, 5H), 6.08 (s, CH), 4.59-4.70 (d, CH_2, 1H), 3.93-4.09 (d, CH_2, 1H), 3.52-3.79 (bs, OH) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.9 (C=O), 155.9 (C-Cl), 137.6 (ArC), 134.4 (ArC), 129.3 (ArC), 128.7 (4xArC), 128.4 (2xArC), 128.4 (ArC), 127.3 (ArC), 126.4 (ArC), 93.2 (C-OH), 43.4 (CH_2) p.p.m.

IR (KBr-disc) υ max: 3446, 3279, 3098, 2931, 2850, 2374, 2334, 1684, 1611, 1456, 1413, 1349, 1276, 1205, 1128, 1051, 696 cm^{-1}.

(AR-10a) (major): 4-Chloro-5-hydroxy-5-phenyl-1-((*S*)-(-)-1-phenyl-ethyl)-1,5-dihydro-pyrrol-2-one.



Yield = 66 %

Melting Point: 162-164 °C

R_f (4:1 ether / petroleum ether) = 0.23

Molecular Weight: 313.8

Molecular Formula: $C_{18}H_{16}ClNO_2$

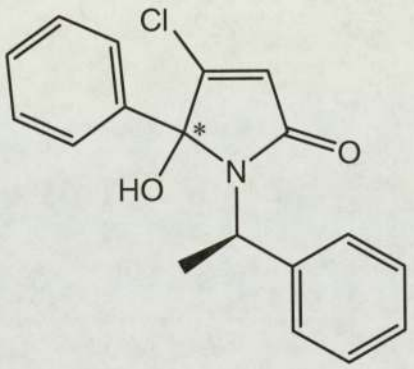
MS (APCI(+)): 193/195 (M+1), 314/316 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.34-7.53 (ArH, 7H), 7.08-7.25 (ArH, 3H), 5.96 (s, CH), 4.16-4.28 (q, N-CH, *J* = 7.9 Hz), 2.91-3.37 (bs, OH), 1.49-1.58 (d, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.3 (C=O), 154.3 (C-Cl), 142.5 (ArC), 134.7 (ArC), 129.4 (ArC), 128.7 (2xArC), 128.4 (2xArC), 127.7 (2xArC), 127.3 (2xArC), 126.4 (ArC), 123.0 (CH-CCl), 93.8 (C-OH), 53.5 (NH-CH), 18.8 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3241, 2983, 2932, 2863, 2366, 2347, 1686, 1661, 1614, 1494, 1456, 1425, 1356, 1258, 1202, 1025, 931, 855, 755, 692 cm^{-1}.

(AR-10b) (minor): 4-Chloro-5-hydroxy-5-phenyl-1-((*S*)-(-)-1-phenyl-ethyl)-1,5-dihydro-pyrrol-2-one.



Yield = 8 %

R_f (4:1 ether / petroleum ether) = 0.23

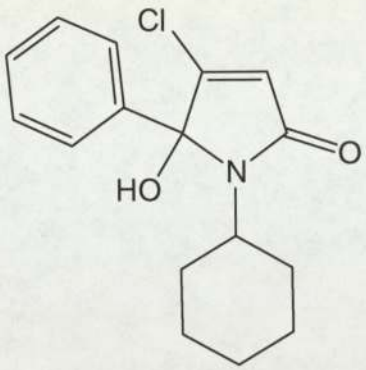
Molecular Weight: 313.8

Molecular Formula: $C_{18}H_{16}ClNO_2$

MS (APCI(+)): 193/195 (M+1), 314/316 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.29-7.53 (ArH, 7H), 6.95-7.21 (ArH, 3H), 6.08 (s, CH), 4.59-4.78 (q, N-CH, *J* = 7.9 Hz), 2.62-2.71 (bs, OH), 1.49-1.63 (d, CH_3) p.p.m.

(AR-11): 4-Chloro-1-cyclohexyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one.



Yield = 57 %

Melting Point: 170-172 °C

R_f (4:1 ether / petroleum ether) = 0.27

Molecular Weight: 291.8

Molecular Formula: $C_{16}H_{18}ClNO_2$

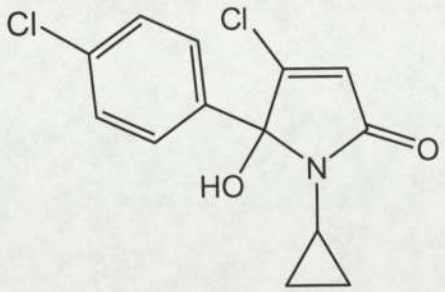
MS (APCI(+)): 193/195 (M+1), 292/294 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.26-7.61 (m, ArH, 5H), 6.08 (s, CH), 3.72-3.85 (bs, OH), 2.83-3.19 (m, N-CH), 1.21-2.07 (m, overlapping CH_2, 10H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.9 (C=O), 153.9 (C-Cl), 135.0 (ArC), 129.25 (2xArC), 128.9 (2xArC), 126.4 (ArC), 122.9 (CH-CCl), 96.0 (C-OH), 53.6 (N-CH), 32.8 (CH_2), 31.1 (CH_2), 29.8 (CH_2), 26.2 (2xCH_2), 24.2 (CH_2) p.p.m.

IR (KBr-disc) υ max: 3440, 2924, 2858, 2355, 2344, 1641, 1449, 1367, 1250, 1138, 1016, 996,742, 695 cm^{-1}.

(Cl-AR-1): 4-Chloro-5-(4-chloro-phenyl)-1-cyclopropyl-5-hydroxy-1,5-dihydro-pyrrol -2-one.



Yield = 72 %

Melting Point: 169-171 °C

R_f (4:1 ether / petroleum ether) = 0.19

Molecular Weight: 284.1

Molecular Formula: $C_{13}H_{11}Cl_2NO_2$

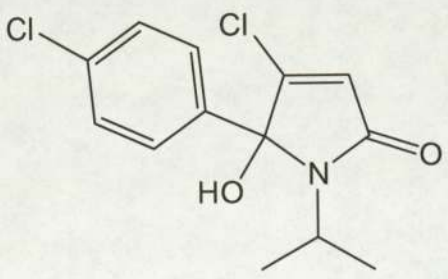
MS (APCI(+)):227/229/231 (M+1), 284/286/288 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.12-7.32 (m, ArH, 4H), 5.97 (s, CH), 3.98-4.16 (bs, OH, 1.67-1.82 (m, N-CH), 0.24-0.99 (m, overlapping CH_2, 4H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.8 (C=O), 155.4 (C-Cl), 144.2 (ArC), 133.7 (ArC), 129.0 (2xArC), 127.7 (2xArC), 122.2 (CH-CCl), 91.7 (C-OH), 22.6 (N-CH), 3.7 & 5.2 (CH_2, 2xC) p.p.m.

IR (KBr-disc) υ max: 3433, 3220, 3019, 2935, 2858, 1700, 1675, 1497, 1412, 1251, 1209, 1144, 1089, 1015, 940, 844, 802, 679 cm^{-1}.

(Cl-AR-3): 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-isopropyl-1,5-dihydro-pyrrol-2-one.



Yield =69 %

Melting Point: 127-130 °C

R_f (4:1 ether / petroleum ether) = 0.21

Molecular Weight: 286.2

Molecular Formula: $C_{13}H_{13}Cl_2NO_2$

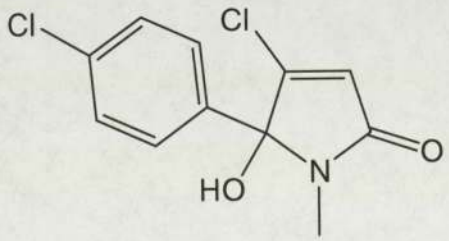
MS (APCI(+)):227/229/231 (M+1), 286/288/290 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.48 (m, ArH, 4H), 6.06 (s, CH), 3.33-3.52 (m, N-CH), 1.25-1.37 & 1.10-1.22 (d, CH_3, 6H), (OH not detected) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.1 (C=O), 154.0 (C-Cl), 136.7 (ArC), 133.4 (ArC), 128.9 (2xArC), 128.0 (2xArC), 123.2 (CH-CCl), 92.9 (C-OH), 45.6 (N-CH), 20.1 & 21.3 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3272, 2978, 2927, 1691, 1614, 1496, 1429, 1384, 1352, 1249, 1096, 1012, 936, 846, 801, 683 cm^{-1}.

(Cl-AR-4): 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one.



Yield = 66 %

Melting Point: 179-181 °C

R_f (4:1 ether / petroleum ether) = 0.24

Molecular Weight: 258.1

Molecular Formula: $C_{11}H_9Cl_2NO_2$

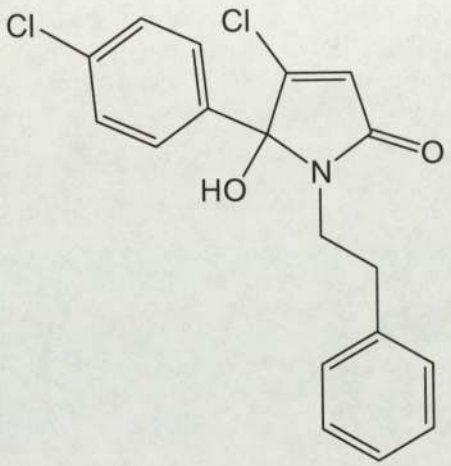
MS (APCI(+)): 227/229/231 (M+1), 258/260/262 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.42 (ArH, 4H), 6.06 (s, CH), 4.56-4.71 (bs, OH), 2.60 (s, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.8 (C=O), 156.0 (C-Cl), 135.5 (ArC), 132.8 (ArC), 129.1 (2xArC), 127.8 (2xArC), 121.6 (CH-CCl), 92.2 (C-OH), 24.4 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3429, 3102, 2970, 2932, 2857, 1677, 1611, 1494, 1475, 1431, 1202, 1151, 1091, 988, 928, 811, 692 cm^{-1}.

(Cl-AR-5): 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-phenethyl-1,5-dihydro-pyrrol-2-one.



Yield = 45 %

Melting Point: 145-148 °C

R_f (4:1 ether / petroleum ether) = 0.18

Molecular Weight: 348.2

Molecular Formula: $C_{18}H_{15}Cl_2NO_2$

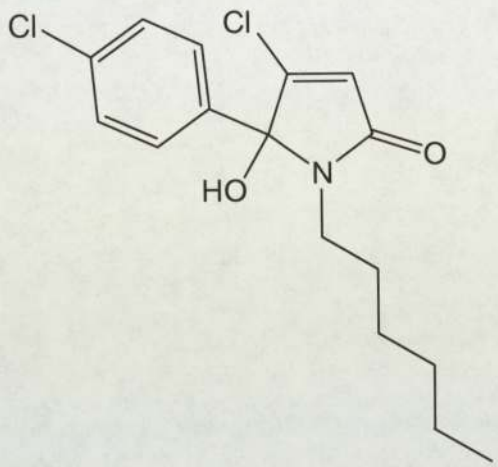
MS (APCI(+)): 227/229/231 (M+1), 348/350/352 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.22-7.49 (m, ArH, 7H), 7.12-7.18 (m, ArH, 2H), 6.13 (s, CH), 3.68-3.79 & 2.64-2.77 (m, N-CH_2), 2.88-3.29 (m, Ar-CH_2), (OH not detected) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.7 (C=O), 155.5 (C-Cl), 138.8 (ArC), 135.5 (ArC), 133.3 (ArC), 129.1 (2xArC), 128.8 (2xArC), 128,7 (2xArC), 127.7 (2xArC), 126.7 (ArC), 121.9 (CH-CCl), 92.3 (C-OH), 42.0 (N-CH_2), 34.5 (Ar-CH_2) p.p.m.

IR (KBr-disc) υ max: 3421, 3228, 2925, 2848, 2370, 2338, 1684, 1658, 1606, 1461, 1406, 1248, 1190, 1097, 935, 806, 697 cm^{-1}.

(Cl-AR-6): 4-Chloro-5-(4-chloro-phenyl)-1-hexyl-5-hydroxy-1,5-dihydro-pyrrol-2-one.



Yield = 49 %

Melting Point: 169-172 °C

R_f (4:1 ether / petroleum ether) = 0.25

Molecular Weight: 328.2

Molecular Formula: $C_{16}H_{19}Cl_2NO_2$

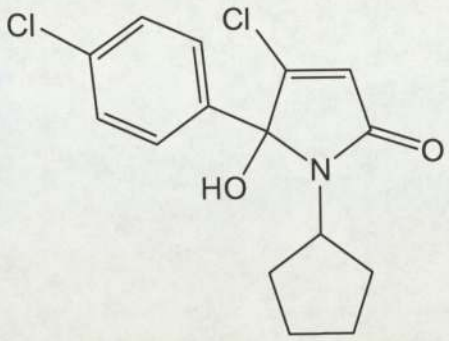
MS (APCI(+)): 227/229/231 (M+1), 328/330/332 (M+) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.43 (m, ArH, 4H), 6.15 (s, CH), 3.24-3.44 (m, CH_2, 1H), 2.67-2.91 m, CH_2, 1H), 1.04-1.69 (m, overlapping CH_2, 8H), 0.74-0.89 (t, CH_3, J = 6.3 Hz) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 165.8 (C=O), 155.7 (C-Cl), 140.8 (ArC), 136.9 (ArC), 129.1 (2xArC), 127.8 (2xArC), 91.6 (C-OH), 40.3 (N-CH_2), 30.8 (N-CH_2-$\underline{C}H_2$), 29.1 (N-CH_2-CH_2-$\underline{C}H_2$), 26.8 (NH-CH_2-CH_2-CH_2-$\underline{C}H_2$), 22.6 (CH_3-$\underline{C}H_2$), 15.2 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3446, 2935, 2863, 1698, 1413, 1252, 1200, 1138, 1092, 1013, 938, 846, 814, 702 cm^{-1}.

(Cl-AR-7): 4-Chloro-5-(4-chloro-phenyl)-1-cyclopentyl-5-hydroxy-1,5-dihydro-pyrrol-2-one.



Yield = 73 %

Melting Point: 157-159 °C

R_f (4:1 ether / petroleum ether) = 0.23

Molecular Weight: 312.2

Molecular Formula: $C_{15}H_{15}Cl_2NO_2$

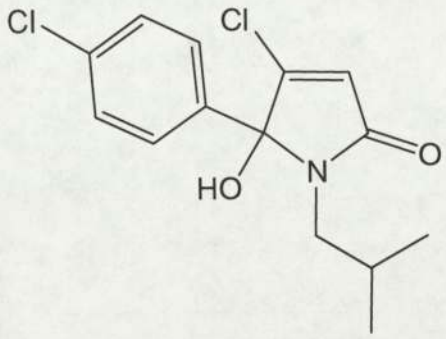
MS (APCI(+)): 227/229/231 (M+1), 312/314/316 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.32-7.51 (ArH, 4H), 6.03 (s, CH), 4.95-5.03 (bs, OH), 3.41-3.62 (m, N-CH, *J* = 9.26 Hz), 1.97-2.19 (m, CH_2), 1.68-1.93 (m, overlapping CH_2, 8H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.1 (C=O), 154.8 (CH-CCl), 135.2 (ArC), 133.9 (ArC), 128.9 (2xArC), 128.0 (2xArC), 122.3 (CH-CO), 93.0 (C-OH), 54.3 (N-CH), 30.0 & 28.9 (N-CH-CH_2, 4xC), 24.5 (N-CH-CH_2-CH_2, 2xC) p.p.m.

IR (KBr-disc) υ max: 3407, 3276, 2968, 2922, 2883, 2379, 2339, 1691, 1491, 1429, 1367, 1249, 1203, 1092, 1013, 932, 843, 787, 709 cm^{-1}.

(Cl-AR-8): 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-isobutyl-1,5-dihydro-pyrrol-2-one.



Yield = 76%

Melting Point: 155-158 °C

R_f (4:1 ether / petroleum ether) = 0.22

Molecular Weight: 300.2

Molecular Formula: $C_{14}H_{15}Cl_2NO_2$

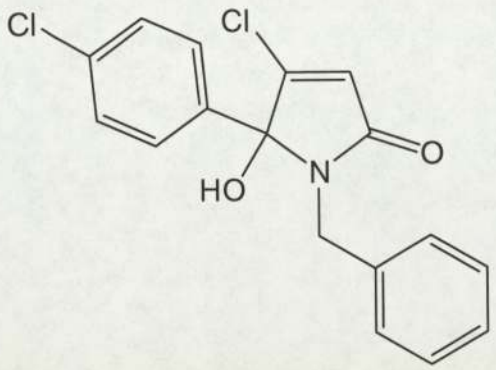
MS (APCI(+)): 227/229/231 (M+1), 300/302/304 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.30-7.41 (m, ArH, 4H), 6.19 (s, CH), 3.13-3.31 (dd, CH_2, *J* = 8.0 Hz, 1H), 2.49-2.62 (dd, CH_2, *J* = 8.0 Hz, 1H), 1.69-1.83 (m, CH, *J* = 5.8 Hz), 0.69-0.80 (t, CH_3, *J* = 4.5 Hz, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.3 (C=O), 156.3 (CH-CCl), 139.4 (ArC), 134.8 (ArC), 129.1 (2xArC), 127.7 (2xArC), 122.3 (CH-CCl), 95.0 (C-OH), 47.6 (CH_2), 27.6 (CH-CH_2), 20.4 (CH_3, 2xC) p.p.m

IR (KBr-disc) υ max: 3426, 3252, 2964, 2924, 2850, 1684, 1406, 1209, 1095, 817, 743, 703 cm^{-1}.

(Cl-AR-9): 1-Benzyl-4-chloro-5-(4-chloro-phenyl)-5-hydroxy-1,5-dihydro-pyrrol-2-one.



Yield = 59 %

Melting Point: 149-152 °C

R_f (4:1 ether / petroleum ether) = 0.18

Molecular Weight: 334.2

Molecular Formula: $C_{17}H_{13}Cl_2NO_2$

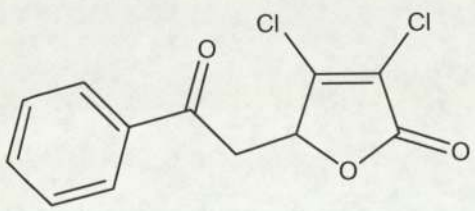
MS (APCI(+)): 227/229/231 (M+1), 334/336/338 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.29-7.36 (m, ArH, 4H), 7.06-7.25 (m, ArH, 5H), 6.09 (s, CH), 4.52-4.60 (d, CH_2, 1H), 3.89-3.98 (d, CH_2, 1H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.6 (C=O), 155.4 (C-Cl), 137.5 (ArC), 135.3 (ArC), 133.2 (ArC), 129.1 (ArC), 129.0 (ArC), 128.9 (2xArC), 128.6 (ArC), 128.4 (ArC), 127.9 (2xArC), 127.4 (ArC), 121.9 (CH), 92.6 (C-OH), 43.2 (CH_2) p.p.m.

IR (KBr-disc) υ max: 3442, 2931, 2849, 2365, 2339, 1674, 1616, 1492, 1406, 1349 1272, 1199, 1094, 1018, 817, 699 cm^{-1}.

(Ket-Fur): 3,4-Dichloro-5-(2-oxo-2-phenyl-ethyl)-5H-furan-2-one.



Mucochloric acid (4g, 0.024 mol) was dissolved in ether (35 ml). 2 ½ equivalents (7.2g, 0.06 mol) acetophenone was added to the mixture, followed by the careful addition of 1 ½ equivalents aluminium chloride (4.8g, 0.036 mol). A calcium guard tube was fitted to the flask and left to stir overnight at room temperature. The solution was carefully poured into a mixture of conc. HCl (33%, 8 ml) in crushed ice (25g). The organic layer was washed twice with water (10 ml) and dried over magnesium sulphate. Ether was removed under vacuum to leave a brown oil. The oil was washed with petroleum ether (20 ml) to remove excess acetophenone. The petroleum ether layer was decanted. This washing process was repeated 3 or 4 times. Ether (20 ml) was added to the remaining dark orange yellow semisolid. An off-white powder precipitated out, which was filtered and dried. The compound was recrystallised in methanol to give off-white crystals.

Yield = 75%

M.P: 123-125 °C

Rf (1:1 ether / petroleum ether) = 0.25

Molecular Weight: 271.1

Molecular Formula: $C_{12}H_8Cl_2O_3$

MS (APCI(+)): 191/193/195 (M+), 271/273/275 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.94-8.03 (d, Ar-H, 2H), 7.63-7.74 (m, Ar-H, 1H), 7.49-7.62 (m, Ar-H, 2H), 5.74-5.82 (dd, CH-O, *J* = 6.9 Hz), 3.39-3.62 (m, CH_2) p.p.m.

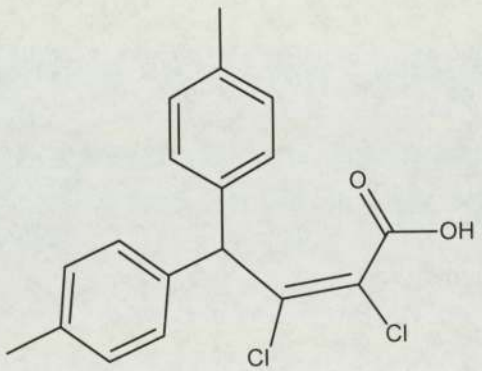
^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 193.8 (CH_2-C=O), 165.0 (C=O), 152.1 (CH=CCl), 135.8 (ArC), 134.1 (ArC), 128.9 (2xArC), 128.2 (2xArC), 121.4 (CO-CCl), 77.7 (CH-O), 25.4 (CH_2) p.p.m.

IR (KBr-disc) υ max: 3366, 3067, 2961, 2936, 1780, 1689, 1624, 1456, 1375, 1338, 1238, 1204, 1035, 919, 770, 689 cm^{-1}.

6.5. Experiments to Chapter 5.

(Z-OH) 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid.

8.19 g (48.5 mmol) mucochloric acid was dissolved in 200 ml of the appropriate benzene substituted solvent. 10 g powdered aluminium chloride was added slowly to the resultant solution, capped with a drying tube and stirred for 72 hours. The crude product was poured into a beaker containing ice (125 g) and conc. HCl (33%, 38 g). The organic phase was separated from the aqueous phase by extraction using toluene. The resulting solution was dried over magnesium sulphate and excess solvent was removed under vacuum, before the product was recrystallised from methanol and dried under vacuum in a decicator.



Yield = 75%

Melting Point: 189-191 °C

R_f (4:1 ether / petroleum ether) = 0.06

Mol. Weight: 335.2

Mol. Formula: $C_{18}H_{16}Cl_2O_2$

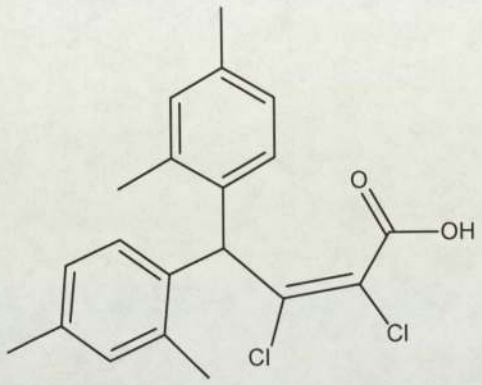
MS (APCI(-)): 218/220 (M-1), 297/299 (M-) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 11.35-11.61 (bs, COOH), 7.13-7.33 (m, ArH, 8H), 6.65 (s, CH-CCl), 2.41 (s, 6H, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 167.6 (COOH), 154.0 (CCl-CH), 137.2, 136.5 (4xArC), 129.22, 129.15 (8xAr-C), 122.4 (CCl-COOH), 53.5 (CH-CCl), 21.2 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3457, 3017, 2974, 2934, 2881, 2658, 2496, 1700, 1614, 1558, 1502, 1418, 1270, 1006, 926, 824, 705, 672 cm^{-1}.

(X-OH) 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid



Yield = 71%

Melting Point: 175-177 °C

R_f (4:1 ether / petroleum ether) = 0.08

Mol. Weight: 363.3

Mol. Formula: $C_{20}H_{20}Cl_2O_2$

MS (APCI(-)): 289/291 (M-1), 325/327 (M-) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 9.49-10.53 (bs, COOH), 6.89-7.08 (m, ArH, 6H), 2.41 (s, CH_3, *m*-position), 2.19 (s, CH_3, *o*-position) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 166.9 (COOH), 155.4 (CCl-CH), 136.9, 136.4, 135.7 (6xArC), 131.3, 128.4, 126.7 (6xAr-C), 122.4 (CCl-COOH), 49.1 (CH-CCl), 21.0 (CH_3, 2xC, *p*-position), 19.3, (CH_3, 2xC, *o*-position) p.p.m.

IR (KBr-disc) υ max: 3483, 2971, 2933, 2870, 2637, 2489, 1696, 1566, 1508, 1412, 1255, 1113, 1005, 913, 816, 699 cm^{-1}.

Yield = 51%

Melting Point: 155-158 °C

R_f (4:1 ether / petroleum ether) = 0.04

Mol. Weight: 376.1

Mol. Formula: $C_{16}H_{10}Cl_4O_2$

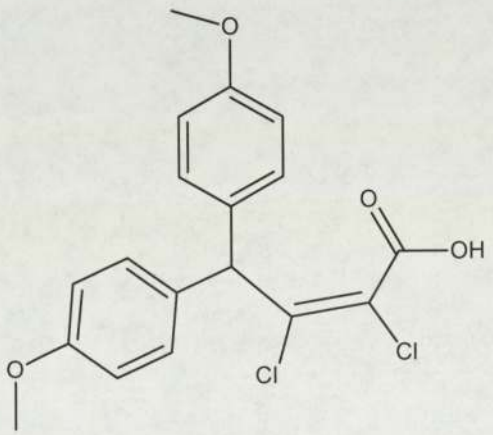
MS (APCI(-)): 258/260/262 (M-1), 337/339/341 (M-) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 9.99-10.51 (COOH), 7.28-7.41 (d, ArH, 4H), 7.19-7.25 (d, ArH, 4H), 6.69 (s, CH-CCl) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.0 (COOH), 151.8 (CCl-CH), 137.8 (2xArC), 132.0 (2xArC), 130.6 (2xArC), 129.8 (2xArC), 129.2 (2xArC), 128.6 (2xArC), 125.1 (CCl-COOH), 52.1 (CH) p.p.m.

IR (KBr-disc) υ max: 3458, 2938, 2854, 2641, 2493, 1773, 1687, 1629, 1564, 1493, 1413, 1261, 1097, 1013, 903, 822, 697 cm^{-1}.

(MeO-OH): 2,3-Dichloro-4,4-bis-(4-methoxy-phenyl)-but-2-enoic acid



8.19 g (48.5 mmol) mucochloric acid was dissolved in THF (150 ml) and 13.1g (2.5 eq., 120 mmol) anisole was added. 10 g powdered aluminium chloride was added slowly to the resultant solution, fitted with a drying tube and stirred for 72 hours. The crude product was poured into a beaker containing ice (125 g) and conc. HCl (33%, 38 g). THF was removed under vacuum to leave a brown viscous oil, which was washed twice with portions of petroleum ether (20 ml) to remove excess anisole. The resulting oil was

recrystallised from methanol to give a white powder, before the product was dried under vacuum in a decicator.

Yield = 47%

Melting Point: 114-115 °C

R_f (4:1 ether / petroleum ether) = 0.05

Mol. Weight: 367.2

Mol. Formula: $C_{18}H_{16}Cl_2O_4$

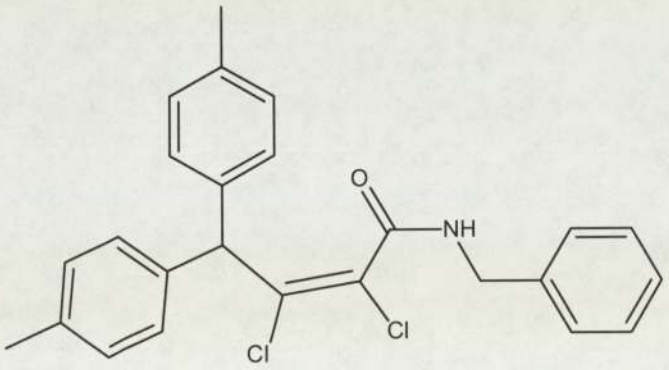
MS (APCI(-)): 250/252 (M-1), 329/331 (M-) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 11.42-11.68 (bs, COOH), 7.11-7.40 (d, ArH, 4H), 6.89-7.01 (d, ArH, 4H), 6.59 (s, CH), 3.92 (s, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 166.7 (COOH), 158.8 (2xArC), 154.0 (CCl-CH), 131.6 (2xArC), 130.3 (4xArC), 122.0 (CCl-COOH), 113.8 (4xArC), 55.3 (CH_3, 2xC), 52.6 (CH-CCl) p.p.m.

IR (KBr-disc) υ max: 3433, 3018, 2964, 2931, 2843, 2663, 2515, 2381, 1698, 1611, 1564, 1520, 1420, 1256, 1182, 1038, 914, 827, 699 cm^{-1}.

(Z-1): 2,3-dichloro-4,4-di-p-tolyl-but-2-enoic acid amides.



Z-OH or **X-OH** (1.49 mmol) was added to $SOCl_2$ (5ml) and heated under reflux for 30 minutes. The excess $SOCl_2$ was distilled off, leaving a medium brown viscous oil. Ether (2 ml) was added and the content of the beaker was stirred, after which 2 equivalents of

the appropriate amine (3.00 mmol) were added. The solution was stirred for a further 10 minutes. The reaction was monitored using TLC. H_2O (10 ml) was added to the resulting product and washed twice with ether (20 ml). After work up, the product which slowly precipitated out of the ether solution was collected by filtration. The sample was recrystallised from methanol and then dried in a vacuum decicator.

Yield = 71%

Melting Point: 165-167 °C

R_f (4:1 ether / petroleum ether) = 0.38

Mol. Weight: 424.4

Mol. Formula: $C_{25}H_{23}Cl_2NO$

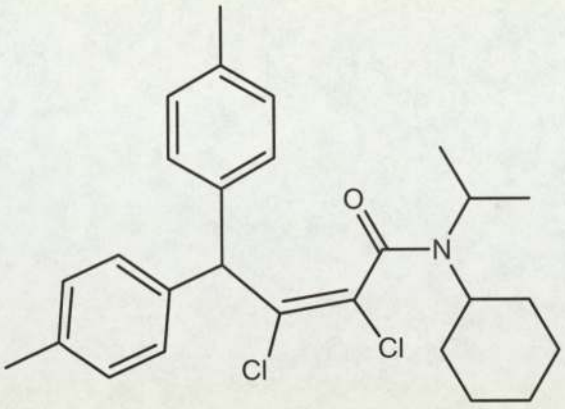
MS (APCI(+)): 352/354 (M+1), 424/426 (M+) m/z

^{1}H NMR (DMSO-d_6) 250 MHz: δ = 6.86-7.61 (m, ArH, 13H), 6.72 (m, 2H, CH & NH), 4.49-4.56 (d, 2H, CH_2), 2.21 (s, 6H, CH_3) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.6 (C=O), 146.8 (CH-<u>C</u>Cl), 137.1 (ArC), 137.0 (2xArC), 136.8 (2xArC), 129.1 (3xArC), 129.08 (2xArC), 128.9 (2xArC), 127.9 (2xArC), 127.8 (2xArC), 127.8 (2xArC), 124.2 (CO-CCl), 52.8 (CCl-<u>C</u>H), 44.3 (CH_2), 21.1 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3312, 3059, 3022, 2953, 2921, 2857, 2366, 2343, 1902, 1645, 1580, 1530, 1507, 1447, 1424, 1282, 1259, 1020, 818, 690 cm^{-1}.

(Z-2): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid cyclohexyl-isopropyl-amide.



Yield = 62%

Melting Point: 156-159 °C

R_f (4:1 ether / petroleum ether) = 0.45

Mol. Weight: 458.5

Mol. Formula: $C_{27}H_{33}Cl_2NO$

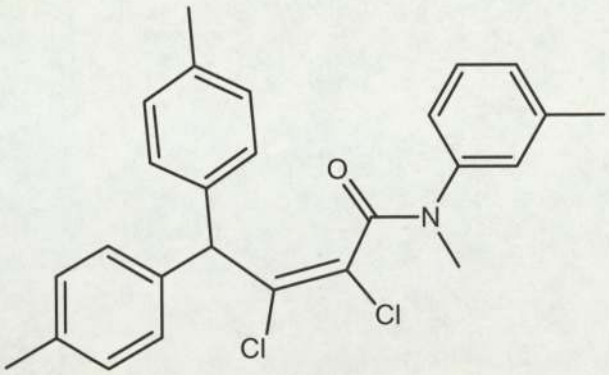
MS (APCI(+)): 458/460 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.05-7.27 (m, ArH, 8H), 5.51-5.54 (d, CH), 3.28-3.49 (m, N-CH), 2.50-2.71 (m, N-C<u>H</u>-CH_2), 2.33-2.42 (m, CH_3, 6H), 0.58-1.99 (m, overlapping, CH_2 & CH_3, 16H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 162.9 (C=O), 147.0 (2xArC), 137.1 (CH-<u>C</u>Cl), 136.8, 136.7, 136.5, 136.2 (4xArC), 129.5 (2xArC), 129.2 (2xArC), 128.9 (2xArC), 128.2 (2xArC), 125.2 (CO-CCl), 60.2 (N-CH-CH_2), 53.2 (N-CH-CH_3), 47.4 (<u>C</u>H-CCl), 30.7, 30.8 (CH_2, 4xC), 25.5, 25.6 (CH_2, 2xC), 25.1 (CH_2), 20.6, 20.1 (Ar-CH_3, 2xC), 19.5 (CH-CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3443, 2994, 2948, 2858, 2368, 2341, 1910, 1647, 1511, 1439, 1357, 1244, 1112, 985, 822, 670 cm^{-1}.

(Z-4): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid methyl-m-tolyl-amide.



Yield = 65%

Melting Point: 125-127 °C

R_f (4:1 ether / petroleum ether) = 0.36

Mol. Weight: 438.4

Mol. Formula: $C_{26}H_{25}Cl_2NO$

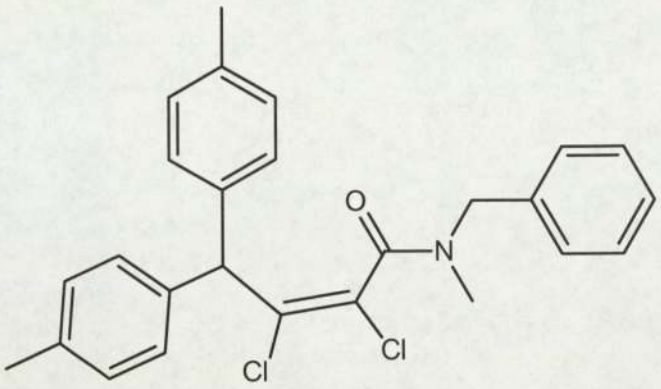
MS (APCI(+)): 438/440 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.03-7.42 (m, ArH, 10H), 6.90-6.97 (m, ArH), 6.76 (s, ArH), 5.66 (s, CH), 3.46 (s, N-CH_3), 2.45 (s, Ar-CH_3, 9H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.8 (C=O), 141.8 (CH-CCl), 139.4 (ArC), 138.9 (ArC), 136.7 (2xArC), 136.4 (2xArC), 129.2 (4xArC), 129.1 (4xArC), 129.0 (ArC), 128.7 (ArC), 127.2 (ArC), 124.5 (CO-CCl), 123.8 (ArC), 53.5 (CH-CCl), 38.0 (N-CH_3), 21.1 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3445, 3056, 3020, 2961, 2921, 2362, 2345, 1650, 1605, 1511, 1462, 1368, 1305, 1112, 812, 777, 696 cm^{-1}.

(Z-6): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid benzyl-methyl-amide.



Yield = 59%

Melting Point: 120-122 °C

R_f (4:1 ether / petroleum ether) = 0.37

Mol. Weight: 438.4

Mol. Formula: $C_{26}H_{25}Cl_2NO$

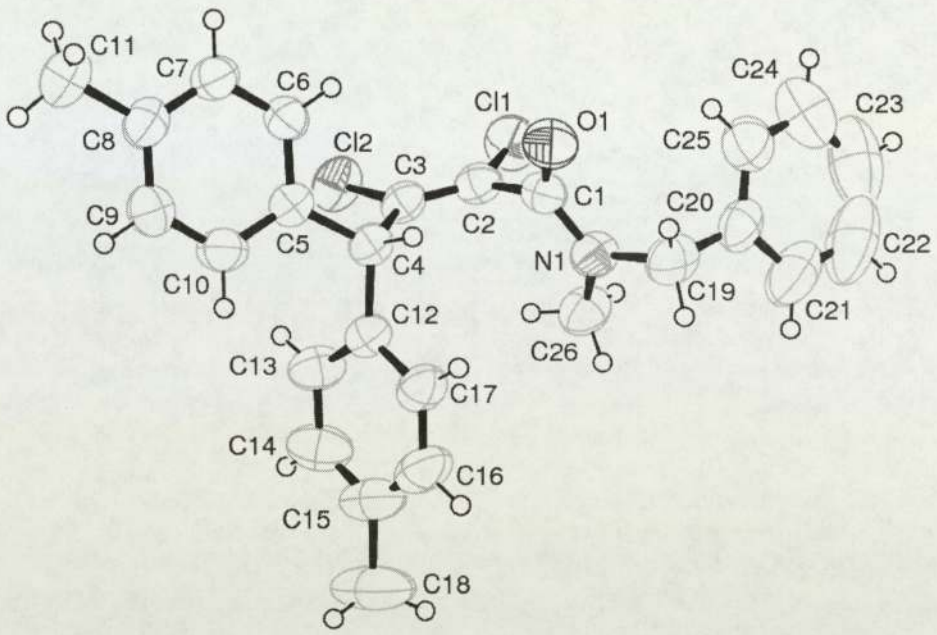
MS (APCI(+)): 366/368 (M+), 438/440 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.02-7.75 (m, ArH, 13H), 5.50 (s, CH), 4.66 (s, CH_2), 2.81 (s, N-CH_3), 2.41-2.52 (m, Ar-CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 164.1 (C=O), 141.8 (2xArC), 137.5, (CH-$\underline{C}$Cl), 136.9 (ArC), 136.1, 136.0 (2xArC), 129.15, 129.1 (2xArC), 128.9 (2xArC), 128,8 (2xArC), 128.7 (2xArC), 128.3 (2xArC), 127.9 (2xArC), 127.5 (ArC), 123.2 (CO-$\underline{C}$Cl), 53.9 (CH), 50.6 (CH_2), 35.6 (N-CH_3), 21.1 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3443, 3271, 3019, 2921, 2855, 2360, 2337, 1643, 1506, 1404, 1267, 1183, 1135, 1020, 895, 821, 706 cm^{-1}.

Crystal Data – (sample recrystallised from methanol):



$C_{26}H_{25}Cl_4NO$
$M_r = 437.13$
T = 293(2) K
Plate
0.40 x 0.40 x 0.12 mm
Colourless
Mo Kα radiation: λ = 0.71073 Å
Triclinic
P-1
a = 8.4597(5) Å
b = 11.1492(13) Å
c = 12.6662(10) Å
α = 81.102(8) °
β = 82.596(6) °
γ = 86.897(7) °

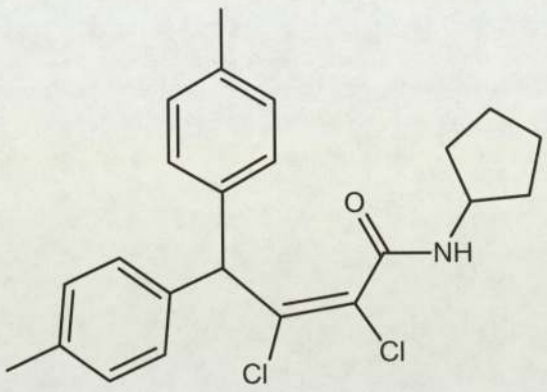
V = 1169.73(18) $Å^3$
Z = 8
$D_x = 1.245$ Mg/m^{-3}
D_m not measured
R $[F^2 > 2\sigma(F^2)] = 0.0412$
$wR(F^2) = 0.1129$
5570 reflections
274 parameters

Selected geometric parameters (Å, °)

C(4)-C(12)	1.520(3)	Cl(2)-C(3)	1.730(2)
C(4)-C(5)	1.522(3)	N(1)-C(1)	1.341(3)
Cl(1)-C(2)	1.732(2)	O(1)-C(1)	1.220(3)
C(12)-C(4)-C(5)	116.63(17)	C(3)-C(2)-C(1)	124.51(19)
C(3)-C(4)-C(5)	112.28(16)	C(2)-C(3)-C(4)	123.08(19)

C(3)-C(4)-C(12)	109.62(16)	C(1)-N(1)-C(19)	119.10(19)
C(2)-C(3)-Cl(2)	120.11(17)	N(1)-C(19)-C(20)	111.4(2)
C(4)-C(3)-Cl(2)	116.76(15)		

(Z-7): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid cyclopentylamide.



Yield = 76%

Melting Point: 156-158 °C

R_f (4:1 ether / petroleum ether) = 0.43

Mol. Weight: 402.4

Mol. Formula: $C_{23}H_{25}Cl_2NO$

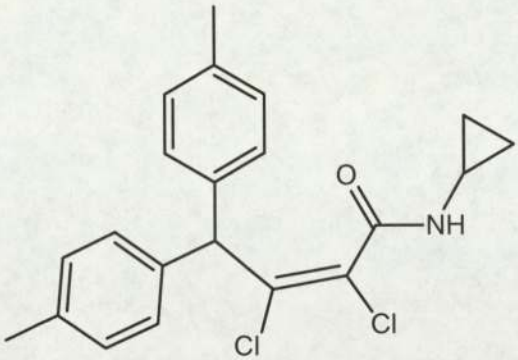
MS (APCI(+)): 330/332 (M+1), 402/404(M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.12-7.23 (m, ArH, 8H), 6.70 (s, CH), 6.22-6.39 (bs, NH), 4.16-4.38 (m, CH, *J* = 6.8 Hz), 2.40 (s, CH_3, 6H), 1.96-2.12 (m, CH_2), 1.62-1.78 (m, CH_2, 4H), 1.37-1.56 (m, CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.3 (C=O), 145.7 (CH-CCl), 137.1 (ArC), 136.8 (ArC), 129.1 (4xArC), 129.0 (4xArC), 124.7 (CO-CCl), 52.9 (CH-CCl), 52.1 (NH-CH), 32.9 (NH-CH-CH_2, 2xC), 23.7 (NH-CH-CH_2-CH_2, 2xC), 21.1 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3437, 3279, 3056, 2026, 2960, 2864, 2362, 2340, 1637, 1536, 1510, 1440, 1314, 1270, 1178, 1113, 817, 732, cm^{-1}.

(Z-8): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid cyclopropylamide.



Yield = 82%

Melting Point: 130-132 ^{o}C

R_f (4:1 ether / petroleum ether) = 0.42

Mol. Weight: 374.3

Mol. Formula: $C_{21}H_{21}Cl_2NO$

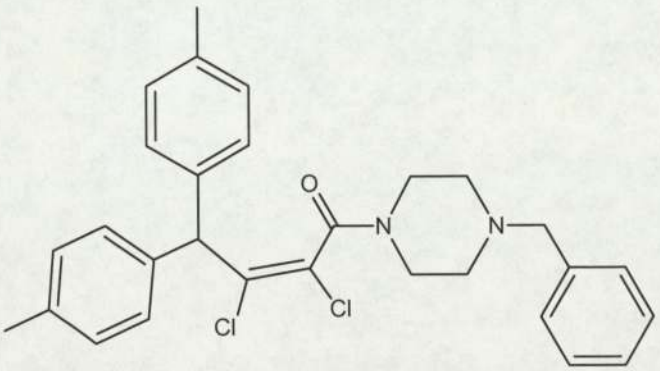
MS (APCI(+)): 302/304 (M+1), 374/376 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.07-7.41 (m, ArH, 8H), 6.78 (s, CH), 6.46-6.61 (bs, NH), 2.71-2.93 (m, NH-CH), 2.40 (s, CH_3, 6H), 1.88-1.99 (m, NH-CH-CH_2), 1.50-1.74 (m, NH-CH-CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.1 (C=O), 147.0 (CH-$\underline{C}$Cl), 137.1 (ArC), 136.8 (ArC), 124.1 (CO-$\underline{C}$Cl), 52.7 ($\underline{C}$H-CCl), 23.3 (NH-CH), 21.1 (CH_3, 2xC), 6.8 (CH_2, 2xC) p.p.m.

IR (KBr-disc) υ max: 3281, 3084, 3020, 2919, 2362, 2335, 1908, 1638, 1512, 1446, 1285, 1181, 1115, 1028, 823, 649 cm^{-1}.

(Z-9): 1-(4-Benzyl-piperazin-1-yl)-2,3-dichloro-4,4-di-p-tolyl-but-2-en-1-one.



Yield = 72%

Melting Point: 182-184 °C

R_f (4:1 ether / petroleum ether) = 0.36

Mol. Weight: 493.5

Mol. Formula: $C_{29}H_{30}Cl_2N_2O$

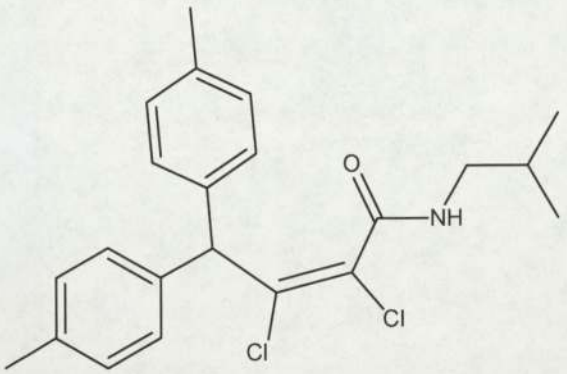
MS (APCI(+)): 493/495 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.41-7.62 (m, ArH, 5H), 7.00-7.27 (m, ArH, 8H), 5.43 (s, CH), 3.01-4.18 (m, overlapping CH_2, 8H), 2.41 (s, CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 162.2 (C=O), 147.1 (CH-CCl), 139.7 (2xArC), 137.4 (ArC), 131.3 (4xArC), 130.5 (2xArC), 129.5 (4xArC), 129.4 (2xArC), 128.8 (ArC), 123.0 (CO-CCl), 61.2 (Ar-CH_2), 46.8 (CH-CCl), 43.3 (Ar-CH_2-N-CH_2, 2xC), 38.4 (CO-N-CH_2, 2xC), 21.2 (CH_3, 2xC) p.p.m.

IR (KBr-disc) υ max: 3524, 3439, 3027, 2915, 2536, 2457, 2372, 1642, 1511, 1429, 1282, 951, 820, 755, 702 cm^{-1}.

(Z-10): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid isobutyl-amide.



Yield = 84%

Melting Point: 138-140 °C

R_f (4:1 ether / petroleum ether) = 0.42

Mol. Weight: 390.4

Mol. Formula: $C_{22}H_{25}Cl_2NO$

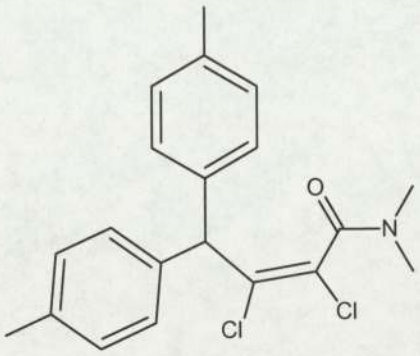
MS (APCI(+)): 318/320 (M+1), 390/392 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.01-7.23 (m, ArH, 8H), 6.71 (s, CCl-CH), 6.28-6.43 (bs, NH), 3.02-3.18 (d, C<u>H</u>$_2$-CH), 2.32 (s, 6H, C-CH_3), 1.77-1.98 (m, *J* = 4.9 Hz, 2H, C<u>H</u>-CH_3), 0.86-1.01 (m, 6H, CH-C<u>H</u>$_3$) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.8 (C=O), 146.2 (CH-<u>C</u>Cl), 137.1, 136.7 (4xArC), 129.1, 129.0 (8xAr-C), 124.6 (CO-<u>C</u>Cl), 52.8 (<u>C</u>H-CCl), 47.5 (NH-CH_2), 28.4 (<u>C</u>H-CH_2), 21.1 (Ar-C-<u>C</u>H_3), 20.1 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3293, 2967, 2929, 2872, 1642, 1549, 1515, 1464, 1278, 1118, 732 cm^{-1}.

(Z-11): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid dimethylamide.



Yield = 67%

Melting Point: 114-116 °C

R_f (4:1 ether / petroleum ether)) = 0.39

Mol. Weight: 362.3

Mol. Formula: $C_{20}H_{21}Cl_2NO$

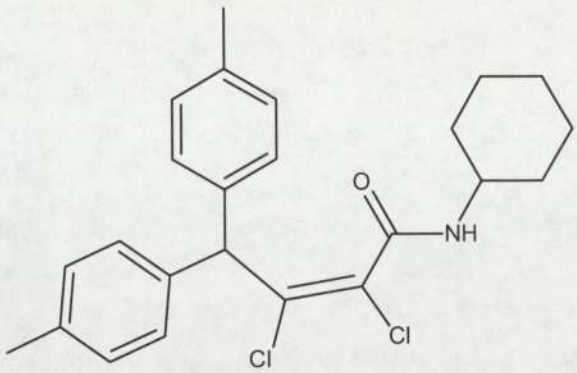
MS (APCI(+)): 270/272 (M+), 362/364 (M+1) m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.11-7.26 (m, Ar-H, 8H), 5.45 (s, CH), 3.05 (s, N-CH_3), 2.39 (s, N-CH_3), 2.41 (s, Ar-CH_3, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.2 (C=O), 145.7 (CH-$\underline{C}$Cl), 137.0, 136.2, 129.1, 129.0 (Ar-C), 54.0 ($\underline{C}$H-CCl), 38.3 (N-$\underline{C}H_3$), 34.9 (N-CH_3), 21.1 (Ar-C-$\underline{C}H_3$, 2xC) p.p.m.

IR (KBr-disc) υ max: 3425, 3022, 2924, 2850, 2363, 2340, 1653, 1514, 1398, 1259, 1180, 672 cm^{-1}.

(Z-12): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid cyclohexylamide.



Yield = 68%

Melting Point: 158-160 °C

R_f (4:1 ether / petroleum ether) = 0.45

Mol. Weight: 416.4

Mol. Formula: $C_{24}H_{27}Cl_2NO$

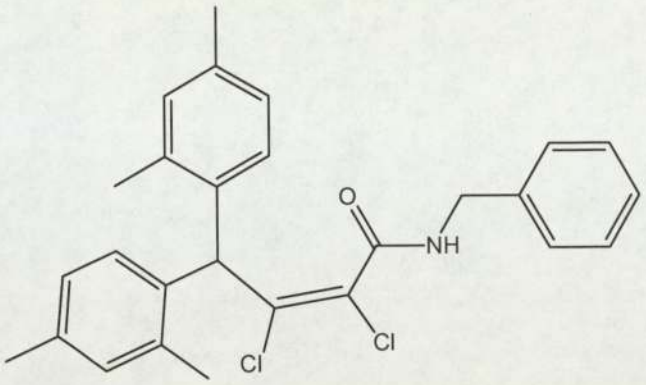
MS (APCI(+)): 344/346 (M+1), 416/418 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.01-7.51 (m, ArH, 8H), 6.70 (s, CCl-CH), 6.21-6.38 (bs, NH), 3.80-3.98 (m, 1H, NH-C<u>H</u>), 2.42 (s, 6H, CH_3), 1.09-2.06 (m, 10H, CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 160.8 (C=O), 142.8 (CH-<u>C</u>-Cl), 137.1 136.7 (4xArC), 129.0, 129.1 (8xAr-C), 124.7 (CO-<u>C</u>Cl), 52.8 (NH-CH), 49.2 (CCl-<u>C</u>-H), 32.7 (NH-CH-<u>C</u>H_2, 2xC), 25.4 (NH-CH-CH_2-<u>C</u>H_2, 2xC), 24.7 (NH-CH-CH_2-CH_2-<u>C</u>H_2), 21.1 (CH_3) p.p.m.

IR (KBr-disc) υ max: 3272, 2932, 2863, 1642, 1554, 1513, 1450, 1321, 1117, 890, 815, 726 cm^{-1}.

(X-1): 2,3-Dichloro-4,4-di-p-tolyl-but-2-enoic acid benzylamide.



Yield = 66%

Melting Point: 122-125 °C

R_f (4:1 ether / petroleum ether) = 0.46

Mol. Weight: 452.4

Mol. Formula: $C_{25}H_{23}Cl_2NO$

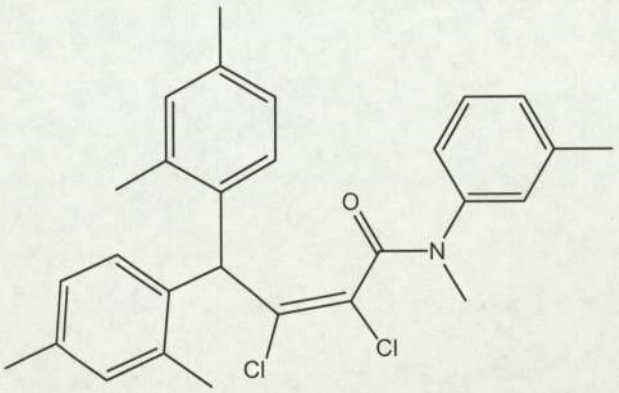
MS (APCI(+)): 380/382, 452/454 m/z

^1H NMR ($CDCl_3$) 250 MHz: δ = 7.40-7.46 (m, ArH, 3H), 7.08-7.20 (m, ArH, 2H), 6.97-7.04 (m, ArH, 6H), 6.71 (s, CH), 6.48-6.55 (bs, NH), 4.42-4.50 (d, CH_2), 2.41 (s, Ar-CH_3, *p*-position, 6H), 2.20 (s, Ar-CH_3, *o*-position, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.4 (C=O), 146.4 (CH-$\underline{C}$Cl), 137.1 (2xArC), 136.65 (2xArC), 136.61 (2xArC), 136.2 (2xArC), 131.3 (2xArC), 128.8 (2xArC), 128.4 (2xArC), 127.72 (2xArC), 129.66 (2xArC), 126.6 (ArC), 124.3 (CO-$\underline{C}$Cl), 48.4 (CH), 44.2 (CH_2), 21.1 (CH_3, *p*-position, 2xC), 19.4 (CH_3, *o*-position, 2xC) p.p.m.

IR (KBr-disc) υ max: 3392, 3293, 3021, 2921, 2862, 2365, 2345, 1764, 1672, 1592, 1592, 1512, 1496, 1254, 1231, 1032, 697 cm^{-1}.

(X-4): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid methyl-m-tolyl-amide.



Yield = 58%

Melting Point: 160-163 °C

R_f (4:1 ether / petroleum ether) = 0.45

Mol. Weight: 466.4

Mol. Formula: $C_{28}H_{29}Cl_2NO$

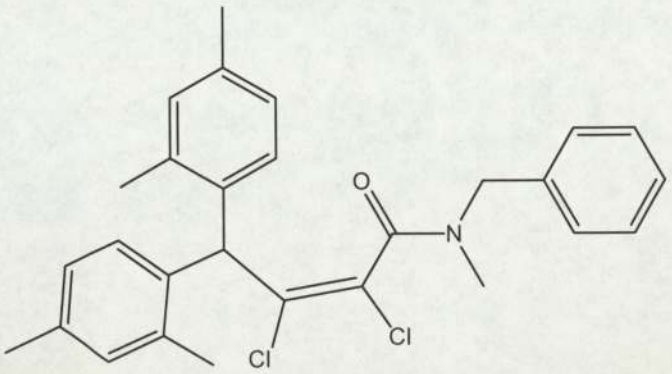
MS (APCI(+)): 360/362 (M+), 466/468 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.29-7.41 (m, ArH, 3H), 6.81-7.12 (m, ArH, 7H), 5.71 (s, CH), 2.04-2.60 overlapping CH_3, 18H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.9 (C=O), 149.2 (CH-CCl), 138.0 (ArC), 136.9 (ArC), 136.8 (ArC), 136.6 (ArC), 135.8 (ArC), 135.1 (ArC), 131.3 (ArC), 128.6 (2xArC), 127.8 (ArC), 127.6 (ArC), 127.55 (ArC), 127.5 (ArC), 126.7 ArC), 126.5 (ArC), 123.6 (CO-CCl), 48.4 (CH-CCl), 35.2 (N-CH_3), 21.1 (CH_3, *p*-position, 2xC), 19.4 (CH_3, *o*-position, 2xC) p.p.m.

IR (KBr-disc) υ max: 3446, 3040, 2922, 2372, 2333, 1649, 1599, 1498, 1455, 1432, 1406, 1246, 1135, 1082, 1010, 954, 820, 745, 686 cm^{-1}.

(X-6): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid benzyl-methyl-amide.



Yield = 61%

Melting Point: 130-132 °C

R_f (4:1 ether / petroleum ether) = 0.48

Mol. Weight: 466.4

Mol. Formula: $C_{28}H_{29}Cl_2NO$

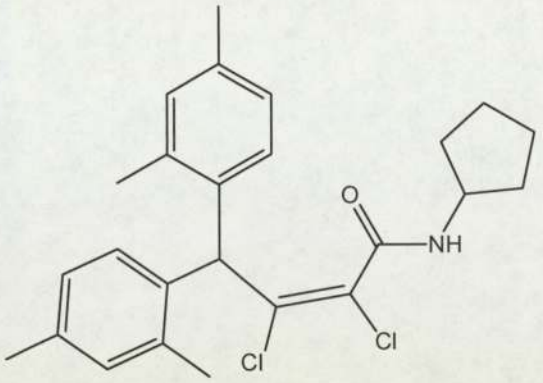
MS (APCI(+)): 394/396 (M+1), 466/468 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.69-7.24 (m, Ar-H, 11H), 6.15 (s, CH), 3.28 (s, N-CH_3), 2.75 (s, N-CH_2), 2.11-2.48 (m, CH_3, overlapping) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.7 (C=O), 141.7 (4xArC), 136.7 (2xArC), 135.4 (ArC), 128.9 (2xArC), 128.4 (2xArC), 126.6 (2xArC), 126.3 (2xArC), 123.6 (2xArC), 122.4 (ArC), 47.1 (CH-CCl), 38.2 (CH_2), 21.3 (N-CH_3), 21.0 (Ar-CH_3), 19.6 (Ar-CH_3) p.p.m.

IR (KBr-disc) υ max: 3440, 2918, 2859, 2370, 2343, 1651, 1491, 1298, 1161, 818, 786, 704 cm^{-1}.

(X-7): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid cyclopentyl-amide.



Yield = 73%

Melting Point: 101-103 °C

R_f (4:1 ether / petroleum ether) = 0.50

Mol. Weight: 430.4

Mol. Formula: $C_{25}H_{29}Cl_2NO$

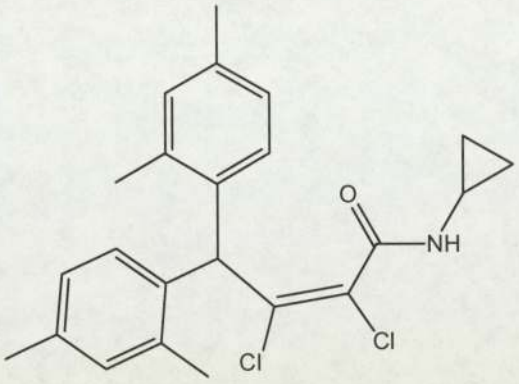
MS (APCI(+)): 358/360 (M+1), 430/432 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.95-7.19 (m, ArH, 6H), 6.52 (s, CH), 5.99-6.11 (bs, NH), 4.11-4.30 (m, CH, *J* = 6.8 Hz), 2.44 (s, Ar-CH_3, *p*-position, 6H), 2.23 (s, Ar-CH_3, *o*-position, 6H), 1.96-2.12 (m, CH_2), 1.48-1.73 (m, CH_2, 4H), 1.12-1.41 (m, CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.1 (C=O), 144.2 (CH-$\underline{C}$Cl), 136.7, 136.6 136.2, (6xArC), 131.4, 128.3, 126.6 (6xArC), 125.0 (CO-$\underline{C}$Cl), 51.7 ($\underline{C}$H-CCl), 48.6 (NH-CH), 32.8 (CH-$\underline{C}H_2$, 2xC) 23.6 (NH-CH_2-CH_2, 2xC), 21.0 (Ar-$C\underline{H}_3$, 2xC, *p*-position), 19.4 (Ar-$C\underline{H}_3$, 2xC, *o*-position) p.p.m.

IR (KBr-disc) υ max: 3303, 2960, 2912, 2863, 1628, 1565, 1520, 1496, 1287, 1249, 1005, 818, 667 cm^{-1}

(X-8): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid cyclopropyl-amide.



Yield = 79%

Melting Point: 140-143 °C

R_f (4:1 ether / petroleum ether) = 0.49

Mol. Weight: 402.4

Mol. Formula: $C_{23}H_{25}Cl_2NO$

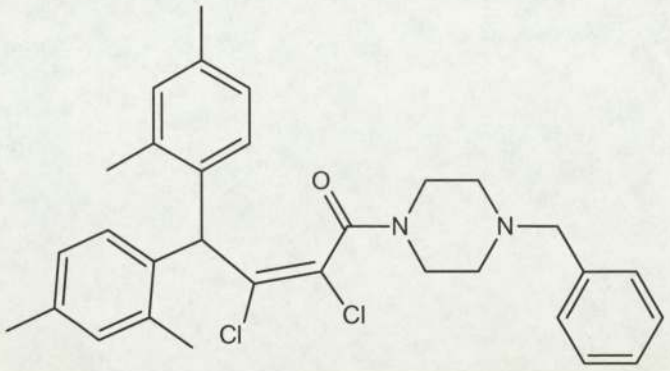
MS (APCI(+)): 330/332 (M+1), 402/404 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.91-7.13 (m, ArH, 6H), 6.64 (s, CH), 6.23-6.38 (bs, NH), 2.70-2.81 (m, CH, *J* = 2.4 Hz), 2.41 (s, Ar-CH_3, *p*-position, 6H), 2.23 (s, Ar-CH_3, *o*-position, 6H), 0.75-0.89 (m, CH_2, 4H), 1.48-1.73 (m, CH_2), 0.41-0.50 (m, CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 162.8 (C=O), 145.8 (CH-$\underline{C}$Cl), 136.6, 136.2 (6xArC), 131.3, 129.5, 128.8, 128.3, 126.6, 125.7 (6xArC), 124.4 (CO-$\underline{C}$Cl), 48.4 ($\underline{C}$H-CCl), 23.1 (NH-CH), 21.0 (Ar-C$\underline{H}_3$, 2xC, *p*-position), 19.4 (Ar-C$\underline{H}_3$, 2xC, *o*-position), 6.7 (CH-$\underline{C}H_2$, 2xC) p.p.m.

IR (KBr-disc) υ max: 3270, 2923, 2912, 2374, 2337, 1633, 1583, 1542, 1496, 1273, 1094, 1024, 819, 687, 655 cm^{-1}

(X-9): 1-(4-Benzyl-piperazin-1-yl)-2,3-dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-en-1-one.



Yield = 65%

Melting Point: 176-178 °C

R_f (4:1 ether / petroleum ether) = 0.46

Mol. Weight: 520.5

Mol. Formula: $C_{31}H_{34}Cl_2N_2O$

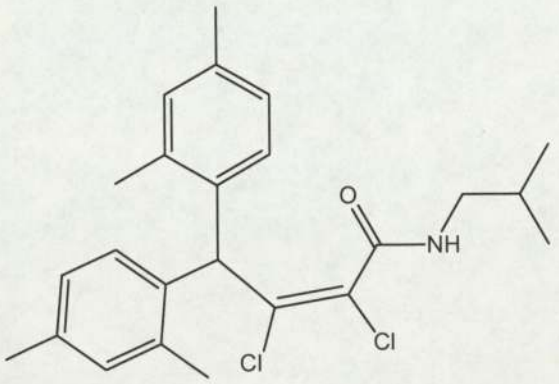
MS (APCI(+)): 521/523 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.41-7.70 (m, ArH, 5H), 6.68-7.27 (m, ArH, 6H), 5.75 (s, CH), 4.41-4.69 (m, Ar-CH_2), 2.87-4.05 (m, overlapping CH_2, 8H), 1.90-2.53 (m, CH_3, 12H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 162.0 (C=O), 140.0 (2xArC), 137.4 (4xArC), 131.5 (2xArC), 131.2 (2xArC), 130.5 (2xArC), 129.5 (2xArC), 129.4 (2xArC), 128.8 (2xArC), 127.3 (CO-CCl), 61.1 (Ar-CH_2), 50.9 (CH-CCl), 50.6 (Ar-CH_2-N-CH_2, 2xC), 38.1 (CO-N-CH_2, 2xC), 21.1 (Ar-CH_3, 2xC, *p*-position), 19.4 (Ar-CH_3, 2xC, *o*-position) p.p.m.

IR (KBr-disc) υ max: 3454, 2925, 2512, 2428, 2370, 1638, 1493, 1425, 1277, 1261, 948, 819, 751, 700 cm^{-1}.

(X-10): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid isobutyl-amide.



Yield = 68 %

Melting Point: 142-144 °C

R_f (4:1 ether / petroleum ether) = 0.48

Mol. Weight: 418.4

Mol. Formula: $C_{24}H_{29}Cl_2NO$

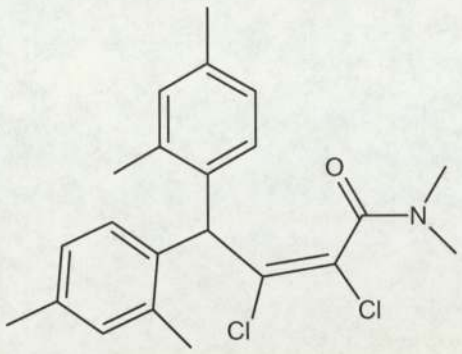
MS (APCI(+)): 346/348, 418/420 (M+1) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.96-7.08 (m, ArH, 6H), 6.69 (s, CCl-CH), 6.20-6.30 (bs, NH), 3.03-3.16 (t, C<u>H</u>$_2$-CH, *J* = 7.5 Hz), 2.40 (s, Ar-CH_3, *p*-position, 6H), 2.23 (s, Ar-CH_3, *o*-position, 6H), 1.60-1.78 (m, C<u>H</u>-CH_3, *J* = 4.9 Hz), 0.79-0.92 (d, CH-C<u>H</u>$_3$, 6H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 161.5 (C=O), 145.7 (CH-<u>C</u>Cl), 136.6 (4xArC), 136.3 (2xArC) 131.3 (2xArC), 128.4 (2xArC), 126.6 (2xArC), 124.7 (CO-<u>C</u>Cl), 48.7 (<u>C</u>H-CCl), 47.4 (NH-CH_2), 28.2 (<u>C</u>H-CH_2), 21.0 (Ar-C<u>H</u>$_3$, 2xC, *p*-position), 19.9 (CH-CH_3, 2xC), 19.4 (Ar-C<u>H</u>$_3$, 2xC, *o*-position) p.p.m.

IR (KBr-disc) υ max: 3333, 2961, 2930, 2879, 2374, 2343, 1636, 1586, 1498, 1280, 1132, 810, 668 cm^{-1}.

(X-11): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid dimethyl-amide.



Yield = 59%

Melting Point: 102-105 °C

R_f (4:1 ether / petroleum ether) = 0.45

Mol. Weight: 390.4

Mol. Formula: $C_{22}H_{25}Cl_2NO$

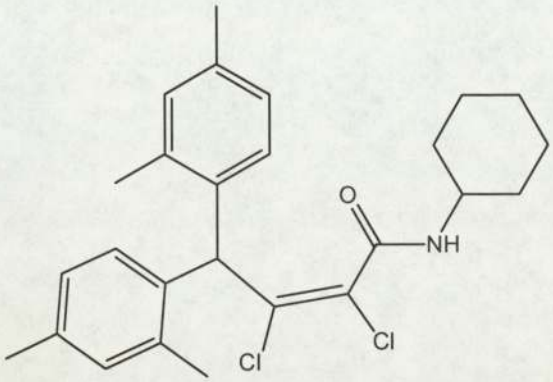
MS (APCI(+)): 284/286 (M+1), 390/392 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.81-7.04 (m, ArH, 6H), 5.53 (s, CH), 2.69 (s, N-CH3, 6H), 2.21-2.35 (m, Ar-C<u>H</u>$_3$, overlapping, 12H) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 163.8 (C=O), 147.3 (CH-<u>C</u>Cl), 136.8, 136.3, 134.8, (6xArC), 131.2, 128.9, 126.5 (6xArC), 123.6 (CO-<u>C</u>Cl), 48.5 (<u>C</u>H-CCl), 37.3, 34.4 (N-CH_3, 2xC), 21.0 (Ar-C<u>H</u>$_3$, 2xC, *p*-position), 19.4 (Ar-C<u>H</u>$_3$, 2xC, *o*-position) p.p.m.

IR (KBr-disc) υ max: 3441, 3030, 2935, 2375, 2338, 1649, 1500, 1397, 1262, 1179, 823, 677 cm^{-1}.

(X-12): 2,3-Dichloro-4,4-bis-(2,4-dimethyl-phenyl)-but-2-enoic acid cyclohexyl-amide.



Yield = 61%

Melting Point: 122-124 °C

R_f (4:1 ether / petroleum ether) = 0.52

Mol. Weight: 444.4

Mol. Formula: $C_{26}H_{31}Cl_2NO$

MS (APCI(+)): 372/374 (M+1), 444/446 (M+) m/z

^{1}H NMR ($CDCl_3$) 250 MHz: δ = 6.91-7.11 (m, ArH, 6H), 6.53 (CH), 5.98-6.09 (bs, NH), 3.70-3.82 (m, 1H, NH-C<u>H</u>), 2.40 (s, Ar-CH_3, *p*-position, 6H), 2.25 (s, Ar-CH_3, *o*-position, 6H), 0.77-1.94 (m, 10H, overlapping CH_2) p.p.m.

^{13}C NMR ($CDCl_3$) 62.9 MHz: δ = 160.6 (C=O), 144.6 (CH-<u>C</u>-Cl), 136.6 (2xArC), 136.58 (2xArC), 136.2 (2xArC), 131.3 (2xAr-C), 128.4 (2xArC), 126.6 (2xArC) 125.0 (CO-<u>C</u>-Cl), 48.8 (NH-CH), 48.6 (CCl-<u>C</u>-H), 32.5 (NH-CH-<u>C</u>H_2, 2xC), 25.4 (NH-CH-CH_2-<u>C</u>H_2, 2xC), 24.6 (NH-CH-CH_2-CH_2-<u>C</u>H_2), 21.0 (Ar-C<u>H</u>$_3$, 2xC, *p*-position), 19.4 (Ar-C<u>H</u>$_3$, 2xC, *o*-position) p.p.m.

IR (KBr-disc) υ max: 3426, 3305, 2923, 2856, 1631, 1589, 1531, 1492, 1443, 1251, 1085, 1012, 818, 708, 690 cm^{-1}.

6.6. Pharmacology.

Animal studies

Experiments were conducted with male IRC mice obtained from the Animal House, Faculty of Medicine, Khon Kaen University, Thailand. Each experimental group consisted of six animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, Khon Kaen University.

6.6.1. Potentiation of morphine andtramadol-induced analgesia (positive control).

Each of the six mice received the same dose, in this opioid potentiation assay. All mice received 2 injections. In the first injection, mice were intraperitoneally injected with 5 % DMSO and subcutaneous injected with either normal saline or morphine or tramadol (2, 4, 8 or 16 mg/kg BW) as the second injection. Thermal response latency of the animals were determined by the tail flick test before the first injection and the second injection. The antinociceptive-like effects of various doses of each opioid was expressed as the mean % maximum possible effect (MPE).

6.6.2 Potentiation effect of UR-MeO(m) and AM-IND(2) on opioid-induced analgesia in mice.

Pyrazolone standard CCK antagonists **UR-MeO(m)** and **AM-IND(2)** at a dose of 0.5 mg/kg BW were selected to be use in the study of tramadol potentiation effects using morphine potentiation as a direct comparison. Six mice were used per group. DMSO (5%), **UR-MeO(m)** or **AM-IND(2)** (in 5% DMSO) was intraperitoneally injected as the first injection. A second injection, twenty minutes later, was done by subcutaneously injecting with morphine or tramadol at either 2, 4, 8 or 16 mg/kg BW. Thermal response

latency of the animals were determined by the tail flick test (section 6.6.3.) before both injections. The results were expressed as % MPE.

6.6.3. Potentiation effect of sample compounds on tramadol-induced analgesia in mice.

Each selected sample compound at a dose of 0.5 mg/kg BW were use in the study of tramadol potentiation effects. Six mice were used per group. **UR-MeO(m)** or **AM-IND(2)** (in 5% DMSO) were used as a positive control. DMSO (5%), or sample compound (in 5% DMSO) was intraperitoneally injected as the first injection. A second injection, twenty minutes later, was done by subcutaneously injecting with tramadol at either 10, 20 or 40 mg/kg BW. Thermal response latency of the animals were determined by the tail flick test before both injections. The results were expressed as % MPE.

6.6.4. Nociceptic (pain) assay: Thermal tail flick.

Figure 6.6.4. Thermal tail flick assay.

Animals were injected with the appropriate dose of DMSO (5%), sample or standard compound (in 5% DMSO) as described in section the previous sections (6.6.1.-6.6.3.). The thermal response latency was measured by the tail flick test (figure 6.6.4.). The animals were placed into individual restraining cages leaving the tail hanging freely. The tail was immersed into water preset at 50°C. The response time, at which the animal reacted by withdrawing its tail from water, was recorded and the cut-off time was 45 seconds in order to avoid damaging the animal's tissue.

6.6.5. Anxiolytic assay: The elevated plus-maze (X-maze).

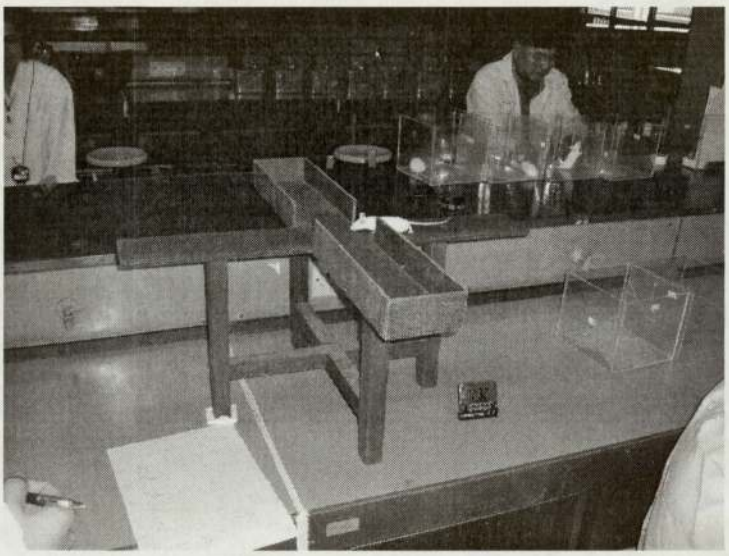


Figure 6.6.5. X-maze assay.

Mice were intraperitoneal injected with test compound at a dose of 0.5 or 1 mg/kg dissolved in 5% DMSO at the volume not more than 0.2 ml/animal. At 30 min after treatment, animals were tested as described in the following sections.

The wooden elevated plus-maze (X-maze, figure 6.6.5.) consisted of two open arms (30x10 cm) without any walls, two enclosed arms of the same size with 5-cm high side walls and end wall, and the central arena (10x10 cm) interconnecting all the arms. The maze was elevated approximately 30 cm height from the floor. At the beginning of the experiment the mouse was placed in the central arena facing one of the enclosed arms. During a 5 min interval, the time animals spent in the open arm platforms of the plus-

maze was recorded. The mouse was considered to be in the open part when it had clearly crossed the line between the central arena and the open arm area with its 4 legs.

6.6.6. Antidepressant assay: The forced swimming test.

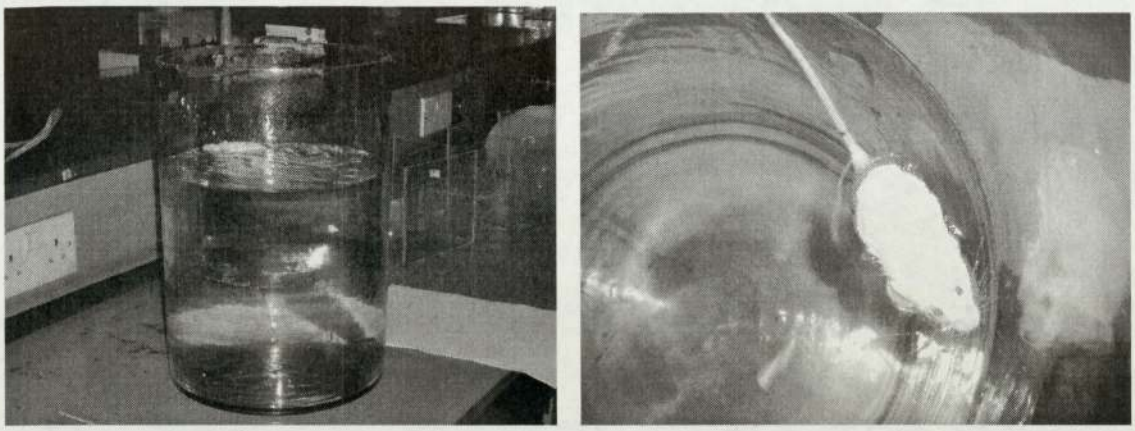


Figure 6.6.6. The forced swimming assay.

Mice were intraperitoneal injected with test compound at a dose of 0.5 or 1 mg/kg dissolved in 5% DMSO at the volume not more than 0.2 ml/animal. At 30 min after treatment, animals were tested as described in the following sections.

The forced swim test was carried out in a glass cylinder (20 cm diameter, 30 cm height) filled with water to the height of 30 cm. The water temperature was approximately 25-28°C. Mice were gently placed into the water and the immobility time was recorded by an observer during the period of 5 min (figure 6.6.6.). Immobility was defined as absence of all movement and remained floating passively in the water with its head just above the water surface.

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