A COMBINATORIAL APPROACH TO THE CHEMICAL SYNTHESIS AND BIOLOGICAL EVALUATION OF 3,4,5-TRISUBSTITUTED FURAN-2(5*H*)-ONES

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Many important natural products contain the furan-2(5H)-one structure. The structure of this molecule lends itself to manipulation using combinatorial techniques due to the presence of more than one site for the attachment of different substituents. By developing different reaction schemes at the three sites available for attachment on the furan-2(5H)-one scaffold, combinatorial chemistry techniques can be employed to assemble libraries of novel furan-2(5H)-ones. These libraries can then be entered into various biological screening programmes. This approach will enable a vast diversity of compounds to be examined, in the hope of finding new biologically active lead structures.

The work in this thesis has investigated the potential that combinatorial chemistry has in the quest for new biologically active lead structures based on the furan-2(5H)-one structure. Different reactions were investigated with respect to their suitability for inclusion in a library. Once sets of reactions at the various sites had been established, the viability of these reactions in the assembly of combinatorial libraries was investigated. Purification methods were developed, and the purified products entered into suitable biological screening tests. Results from some of these tests were optimised using structure activity relationships, and the resulting products re-screened. The screening tests performed were for anticancer and antimicrobial activity, cholecystokinin (CCK-B) antagonism and anti-inflammatory activity (in the quest for novel cyclo-oxygenase (COX-2) selective non-steroidal anti-inflammatory drugs).

It has been shown that many reactions undergone by the furan-2(5H)-one structure are suitable for the assembly of a combinatorial library. Investigation into the assembly of different libraries has been carried out with initial screening results included. From this work, further investigation into combinatorial library assembly and structure activity relationships of screened reaction products can be undertaken.

Additional key words:

Anticancer, Butenolides, Cholecystokinin, Cyclo-oxygenase and Methicillin-resistant *Staphylococcus aureus*.

Dedicated to my parents Denise and Alan, my sister Jackie and my grandparents Violet and Douglas Marshall.

Acknowledgements

Publications arising from this work

1. Synthesis of Combinatorial Libraries of 3,4,5-Trisubstituted 2(5*H*)-Furanones. Part One: Construction of a Sub-Library of Halogenated 5-Alkoxy-2(5*H*)-Furanones. E. Lattmann, D. C. Billington and C. A. Langley. Drug Design and Discovery, 1999, 16, pp 237 – 242.

2. Synthesis of Combinatorial Libraries of 3,4,5-Trisubstituted 2(5*H*)-Furanones. Part Two: Construction of a Library of 4-amino-5-alkoxy-2(5*H*)-Furanones. E. Lattmann, D. C. Billington and C. A. Langley. Drug Design and Discovery, 1999, 16, pp 243 – 250.

Contents

Abbreviations

APCI	Atmospheric Pressure Chemical Ionisation
CCK	Cholecystokinin
COX	Cyclo-oxygenase
CSM	Committee on the Safety of Medicines
DMF	N,N-Dimethylformamide
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid
EGTA	Ethylene Glycol-bis(β-aminoethyl Ether) N,N,N',N'-Tetraacetic Acid
EI	Electron Impact
ES	Electrospray
FG	Functional Group
FT-IR	Fourier Transform – Infrared
5-FU	5-Fluorouracil
GABA	γ-Aminobutyric Acid
HPETE	Hydroperoxyeicosatetraenoic Acid
HETE	Hydroxyeicosatetraenoic Acid
HIV	Human Immunodeficiency Virus
5-HT	5-Hydroxytryptamine (Serotonin)
IC	Inhibitory Concentration
IR	Infrared
LT	Leukotriene
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
NSAID(s)	Non-steroidal Anti-inflammatory Drug(s)
PAF	Platelet Activating Factor
PBS	Phosphate Buffered Saline

PCC	Pyridinium Chlorochromate
PG	Prostaglandin
PGI_2	Prostacyclin
PLA_2	Phospholipase A_2
PPM	Part Per Million
Rf	Retention Factor
RPM	Revolutions Per Minute
SPS	Solid Phase Synthesis
TLC	Thin Layer Chromatography
TX	Thromboxane

Chapter 1 : Introduction

1.1 Furanone and butenolide nomenclature

1.1.1 The different naming systems

The nomenclature of five-membered rings containing an oxygen atom and a single double bond, along with a substituted carbonyl group, has been subject to a certain amount of confusion. Nowadays, two systems are mainly used, based on the two core names furanone and butenolide. The term furanone is the preferred of the two; however, butenolide was used first back in 1898 by Klobb. Before this, butenolides were generally referred to as crotonolactones.[1] Throughout this thesis, the term furanone will be used, as it is the term now adopted by Chemical Abstracts.

Three different types of furanone can be formed. The compounds within this work are based on the furan-2(5H)-one structure (2). It is so called because the carbonyl group appears at the C2 carbon (the 2-one part) and the double bond is between carbon three and four, allowing an extra hydrogen atom at carbon five (the 5H part) when compared with furan (1). An alternative to this, based on the core name butenolide is Δ^2-butenolide (3) (Figure 1.1).

Furan (1)   Furan-2(5*H*)-one (2)   Δ²-Butenolide (3)
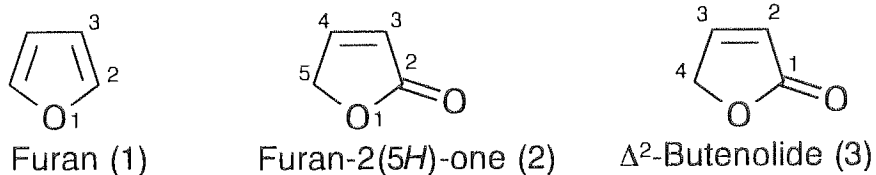
Figure 1.1 The structure of furan, furan-2(5H)-one and its equivalent butenolide.

1.1.2 Differences in furanone structure

Furan (1), along with other heterocycles, prefers to undergo electrophilic substitution reactions, rather than addition reactions, to maintain the stability of the aromatic ring. Substitution reactions occur more readily at the C2 carbon than the C3 carbon of furan (and other five membered heterocycles such as pyrrole and thiophene), as this is the most electron-rich (i.e. most nucleophilic) position on the ring. This can be explained by examining the intermediate cation formed for substitution at both the two and three positions. Substitution at the two position on the ring results in three resonance forms for the intermediate cation, whereas substitution at the three position only results in two (Figure 1.2). Substitution is therefore favoured at the C2 position as the intermediate cation stabilises the positive charge over three centres as opposed to only two centres for substitution at the C3 position.[2]
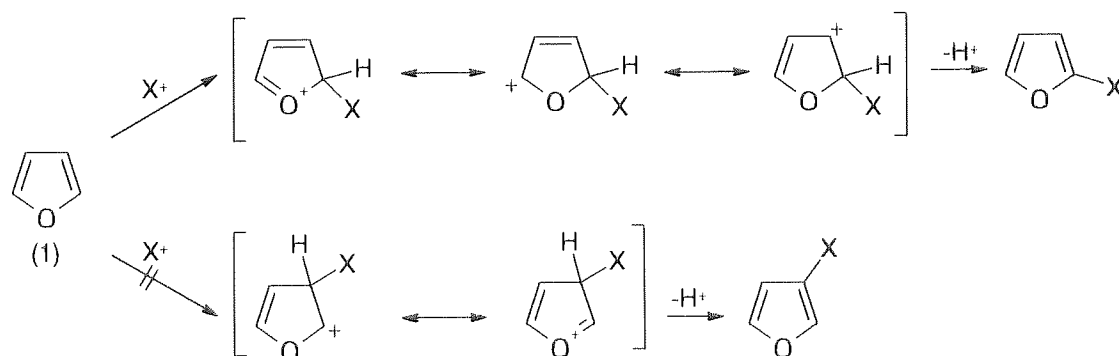
Figure 1.2 Electrophilic substitution of furan. The intermediate produced by reaction at C2 is more stable than that produced by reaction at C3.

The introduction of a carbonyl group to the furan ring and the removal of one of the two double bonds alters the electronic nature of the ring. The aromatic character is lost and the structure now favours substitution at the four position of furan-2(5H)-one (2) due to the creation of a Michael system (an α,β-unsaturated carbonyl) (Figure 1.3). The Michael acceptor character of the C4 carbon can be enhanced if a strongly electronegative atom is bonded to the C3 carbon. This increases the pull of electrons away from the C4 carbon and occurs in a variety of molecules derived from the mucohalic acids (mucochloric and mucobromic acids).
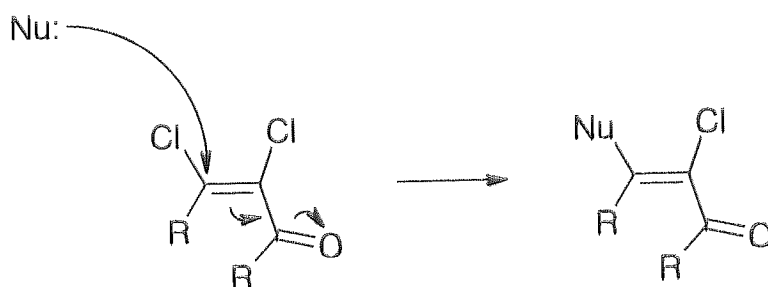
Figure 1.3 Furan-2(5H)-one contains a Michael system.

The other two furanones that can be formed are the furan-2(3H)-ones (4) and the furan-3(2H)-ones (5). The differing structures of the three molecules are shown below (Figure 1.4).[3]

Furan-2(5H)one (2)   Furan-2(3H)-one (4)   Furan-3(2H)-one (5)
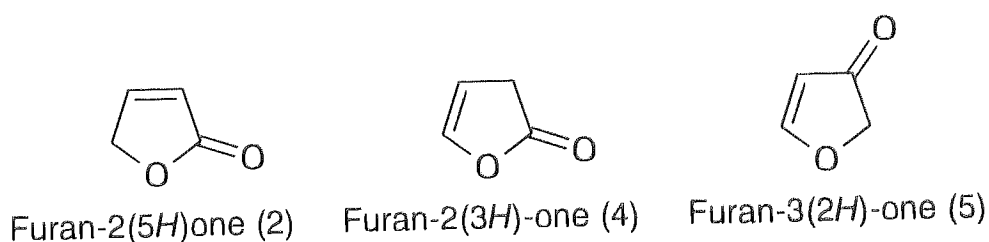
Figure 1.4 The structure of the three different furanones which can be formed.

There are many published methods for the synthesis of the three different furanones as either small molecules, or as part of larger natural products containing a furanone substructure.[4 - 35] The formation of small furanone molecules will be detailed further

in section 1.7.1. Larger natural products containing the furanone substructure are examined in the next section (section 1.2).

1.2 Natural products containing the furanone substructure

1.2.1 Manoalide and the inflammatory pathway

1.2.1.1 Manoalide

There are numerous naturally occurring molecules containing the furanone substructure. Furanones therefore lend themselves to further study and manipulation in the attempt to develop more biologically active compounds. One of the most widely studied of these molecules is manoalide (6) (Figure 1.5). Manoalide was originally extracted from the soft sponge, *Luffariella variabilis*, which is a dense dark brown animal, found in Palau, Western Carolines, at −20 to −35 metres.[36] This molecule has well known anti-inflammatory properties due to its inhibition of phospholipase A$_2$.[37]
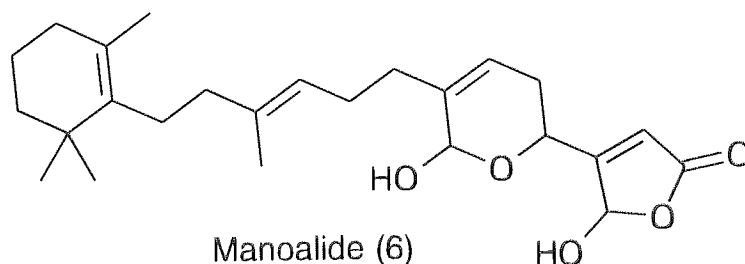
Manoalide (6)

Figure 1.5 The structure of manoalide – a natural product containing the furan-2(5H)-one substructure.

Many syntheses of manoalide have been published and most of them have in common the fact that the furanone end group is synthesised in the final step, due to its chemical sensitivity.[38 - 42]

1.2.1.2 The action of phospholipase A_2

Phospholipase A_2 is the first enzyme in the inflammatory pathway (Figure 1.7), which starts with phospholipids and ends with the formation of many of the main inflammatory mediators, including prostaglandins, leukotrienes and thromboxanes. Collectively, these products are known as the eicosanoids. This term derives from the fact that they contain twenty (eicosa) carbon atoms and at least one double bond (enoic). Platelet-activating factor (PAF) although not classed as an eicosanoid as it is not derived from arachidonic acid (5,8,11,14-eicosatetraenoic acid), is also formed by the action of phospholipase A_2. Phospholipase A_2 is also the site of inhibition for glucocorticoids, well-known and widely used anti-inflammatory steroids. The other main class of anti-inflammatory drugs, the non-steroidal anti-inflammatory drugs (NSAIDs), works further down the inflammatory chain, inhibiting the enzyme cyclo-oxygenase.[43]

The inhibition of phospholipase A_2 by manoalide (6) can be rationalised by looking more closely at its structure. Current inhibitors of phospholipase A_2 are all anti-inflammatory steroids, and the four core rings of the steroid molecule (7) can be mimicked by manoalide (Figure 1.6).[44]
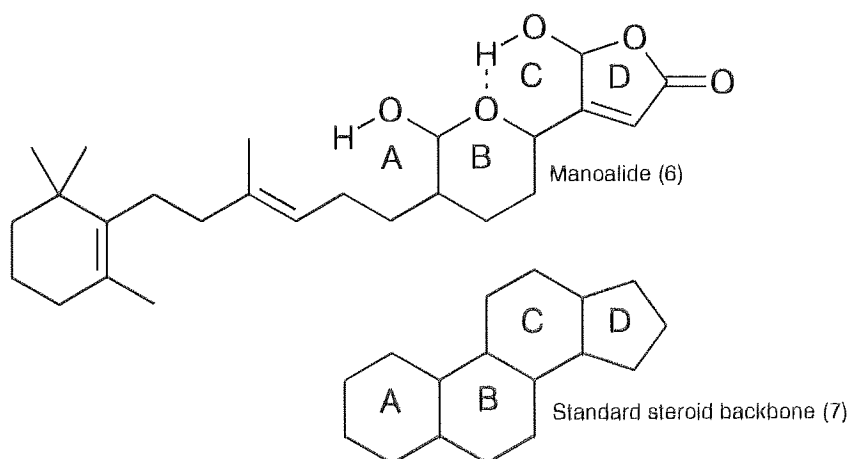
Figure 1.6 Manoalide can be shown to contain a mimic of the steroid backbone found in conventional anti-inflammatory steroids.

The biological metabolism of phospholipids can be summarised in Figure 1.7. The diagram has been adapted from Pharmacology 2nd edition, edited by H. P. Rang and M. M. Dale, page 262.[43] The diagram shows the sites of action of established drugs along with those under test. Enzymes are underlined.
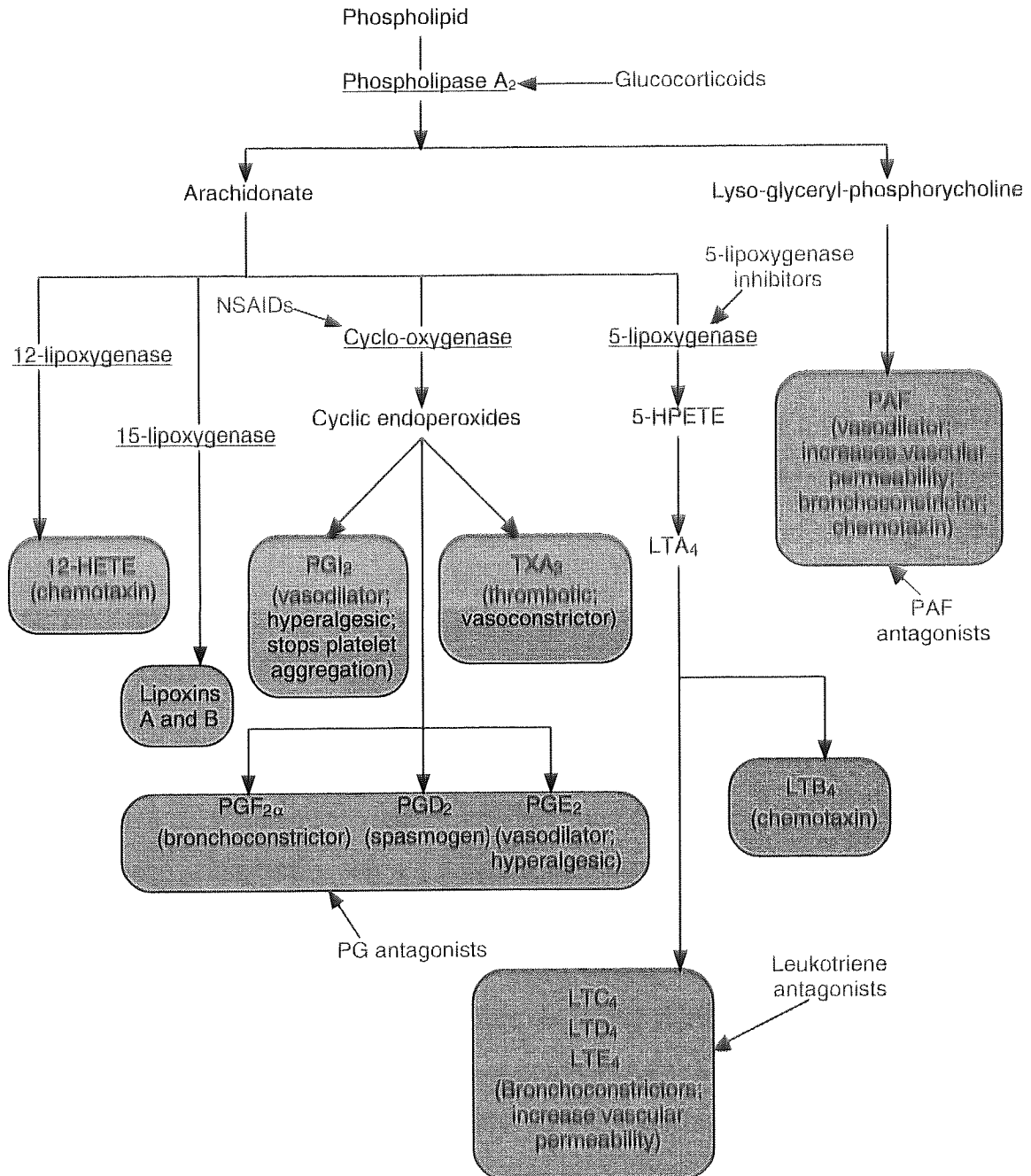
Figure 1.7 The biological metabolism of phospholipids and the resulting effect within the body. (The following abbreviations apply: PG = prostaglandin; PGI$_2$ = prostacyclin; TX = thromboxane; LT = leukotriene; HETE = hydroxyeicosatetraenoic

acid; HPETE = hydroperoxyeicosatetraenoic acid; PAF = platelet-activating factor;

NSAIDs = non-steroidal anti-inflammatory drugs.)

The eicosanoids are not found pre-formed in tissues. They are generated when required from phospholipids. Their presence has been detected in almost every tissue in the body. The name prostaglandin arises from initial studies performed in the 1930s when there were reports that semen contained a substance that caused the contraction of uterine tissue. The substance was believed to originate from the prostate and was named prostaglandin. Twenty years later it was discovered that prostaglandin was not just one compound but a whole collection of substances. The crystal structures of PGE and $PGF_{2\alpha}$ were elucidated in the 1960s.[43]

The main eicosanoids are the prostaglandins, the leukotrienes and the thromboxanes. There are other metabolites formed from arachidonate, for example the lipoxins. The biological effects of each of the different eicosanoids are summarised in Figure 1.7 along with the action of platelet-activating factor.

1.2.1.3 Action of cyclo-oxygenase

As shown above, the non-steroidal anti-inflammatory drugs (NSAIDs) work one step further down the inflammatory chain, inhibiting the enzyme cyclo-oxygenase. This is an extremely important group of drugs which is most frequently prescribed for rheumatic musculoskeletal complaints. These conditions account for an estimated 23% of all doctor consultations in the United Kingdom. There are now over fifty different NSAIDs on the market and more are being developed all the time (Figure 1.8). This is due to the fact that no current NSAID is ideal for controlling the symptoms of inflammation, and virtually all NSAIDs have unwanted side effects, especially in the elderly.[44, 45]
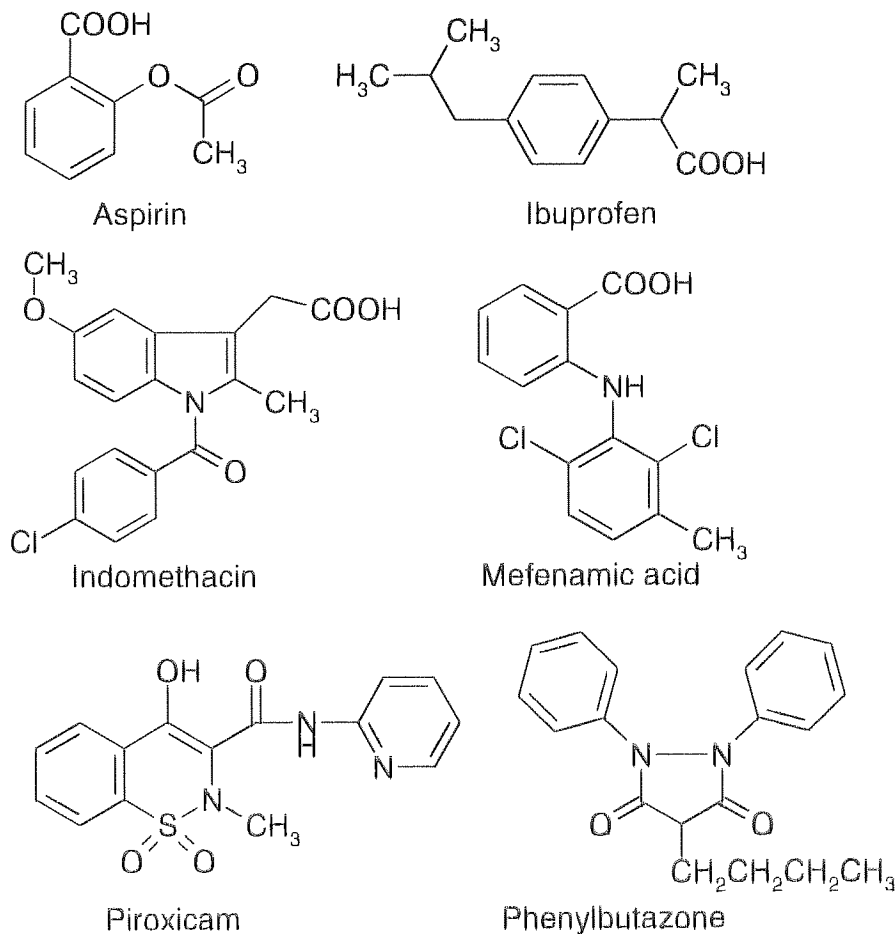
Figure 1.8 The different structures of a selection of well established non-steroidal anti-inflammatory drugs.

The NSAIDs have three main biological effects. These are:

Antipyretic effect   - lowering a raised body temperature

Analgesic effect   - reduction of some types of pain

Anti-inflammatory effect   - modification of the inflammatory reactions

Not every NSAID exhibits all three effects. For example, aspirin and indomethacin are highly anti-inflammatory whereas the atypical NSAID paracetamol has essentially no anti-inflammatory effect.

There are three main groups of side effects that the NSAIDs can exhibit. The different NSAIDs exhibit the three side effects to varying degrees, but overall this poses a

major problem. Nearly a quarter of all the adverse drug reactions reported to the Committee on the Safety of Medicines (CSM) in the United Kingdom are due to NSAIDs. The three main groups of side effects are:[44, 45]

1. Gastrointestinal side effects
2. Renal disease
3. Skin reactions

The vast problem of side effects with NSAIDs has been addressed with the attempted development of cyclo-oxygenase 2 (COX-2) selective NSAIDs.[46] The discovery of the two different types of COX enzyme has changed the way that new non-steroidal anti-inflammatory drugs are developed. It is now thought that COX-1 predominates in the stomach where it produces protective prostaglandins, whereas COX-2 is induced in inflammation, giving rise to pain, swelling and stiffness. This theory is not without its problems though. The first of these is that large amounts of NSAIDs are prescribed for conditions that are not inflammatory-based. There is therefore an additional analgesic property to the compounds (although it has been shown that the placebo effect can play a major role here).

Secondly, the human gastrointestinal tract does contain COX-2 expressing cells, for example, macrophages, neutrophils, myofibroblasts and endothelial cells. The gut can therefore mount inflammatory responses and express COX-2. In the gastritis caused by *Helicobacter pylori*, COX-2 expression does occur and so problems would arise in inhibiting the production of protective prostaglandins by COX-2 in these circumstances. Luckily, it does appear that COX-1 is the major producer of gastric mucosal prostaglandins even with *Helicobacter pylori* infection. This is still a matter to bear in mind, as any new COX-2 selective inhibitor would need to demonstrate safety in this patient group.

Some current NSAIDs may well have some COX-2 preference but they are by no means COX-2 selective. Meloxicam has been shown to have a 3 to 77 fold preference to COX-2 depending on the type of assay used. Nimesulide has been shown to have a

lower preference of between 5 and 16 fold. Two further NSAIDs, etodalac and nabumetone, may have a preference for COX-2 inhibition but the evidence is less convincing.

Two new NSAIDs have just recently been launched onto the market. These are said to be the first selective COX-2 inhibitors and are claimed to be so selective for COX-2 that loss of selectivity with increased dosing which can occur with some of the COX-2 preferring compounds is not seen. The two compounds, celecoxib and rofecoxib, will be examined in more detail in chapters two and five.

Selectively inhibiting the COX-2 enzyme is not exclusively linked to anti-inflammatory activity. It could also play a role in colon cancer, where it is thought that antagonising the expression of COX-2 could retard or prevent the disease. Alzheimer's disease may also benefit from a selective blockage of COX-2 as there appears to be an increase of COX-2, leading to an inflammatory process in the brain. Epidemiological surveys have initially shown a delayed expression of the disease and/or slow progression with NSAID use.

COX-2 selective inhibition is not, however, without its problems. Since the development of COX-2 inhibitors, there have been at least six postulated problems that could occur.

1. A major concern is whether COX-2 inhibitors can be safely used in individuals with gastritis caused by *Helicobacter pylori* or other cause, for example, inflammatory bowel disease. The factor that will ultimately decide this is if COX-2 induction occurs to such an extent as to become the predominant source of protective gastric prostaglandins. The degree of predominance of COX-2 will undoubtedly vary as to which inflammatory condition is causing the gastritis. In gastritis caused by *Helicobacter pylori*, the contribution of prostaglandins by COX-2 is probably minor, but it may be a more significant factor in conditions such as ulcerative colitis.

2. It is possible that COX-2 inhibitors may retard ulcer healing. The basis for this concern is linked to the fact that COX-2 is induced at the rim of ulcers in individuals with gastric injury. It has been shown in animal studies that COX-2 inhibitors can retard ulcer healing.

3. Linked with point two, is the concern that COX-2 inhibitors may actually cause ulcers in a subgroup of patients. This may occur, for example, in patients who have erosions or those who have previously had ulcers, where COX-2 induction could play an important role in preventing the occurrence or reoccurrence of an ulcer. Connected with this point, is the worry that if COX-2 inhibitors are portrayed and perceived as being safe, they will be given to patients who have a high risk of developing an ulcer for reasons which were unrelated to the use of COX-1 inhibitors, for example, infection with *Helicobacter pylori*. This in turn could lead to COX-2 inhibitors being labelled as unsafe as they will be perceived to be causing ulcers in subgroups of individuals who have developed an ulcer for a reason unrelated to drug use.

4. Unwanted renal effects could also be a major problem with COX-2 inhibitors. There is speculation that COX-2 inhibitors may cause fluid retention, which in turn could induce renal failure or exacerbate hypertension. It will be important to investigate to what extent COX-2 inhibitors demonstrate effects on renal function compared to non-selective NSAIDs.

5. It has been postulated that selective COX-2 inhibitors may affect ovulation and parturition. As cyclical induction of COX-2 has an important role in ovulation, and uterine COX-2 is induced at the end of pregnancy, where it is important for the onset of labour, COX-2 inhibitors are likely to have the same type of effects as non-selective NSAIDs.

6. Finally, there is the concern that COX-2 inhibitors may have an effect on the incidence of vascular disease. It has been identified that COX-2 has a role to play in sustaining vascular prostacyclin production.

The above points indicate that in addition to increasing the safety of NSAIDs, COX-2 specific inhibitors may also be limited as to the number of applications they can be used in. They will not replace the cardiovascular protective effects that aspirin possesses, which are mediated through COX-1. In addition to this, it has been suggested that COX-1 is induced at sites of inflammation implying that COX-2 selective inhibitors may actually be less effective as anti-inflammatories or analgesics. Despite this, selective COX-2 inhibition still remains a therapeutic goal for the medicinal chemist as selective COX-2 inhibition is very likely to offer substantial advantages over non-selective COX inhibition. This will be further investigated in chapters two and five.

1.2.2 Other natural products containing the furanone substructure

Although manoalide was the first compound extracted from the sea sponge *Luffariella variabilis*, other manoalide-related sesterterpenoids have been found, most of them with useful bioactivities. There is a group of five luffariolides referred to as luffariolides A-E. These all exhibit cytotoxic activity against murine lymphoma L1210 cells *in vitro* along with another group of compounds extracted, the neomanoalides. A further furanone, 5-hydroxy-4-methoxy-2(5H)-furanone (narthogenin), which is the aglycon of narthecide, exhibits antibiotic activity. A final furanone, thorectolide (11) can also be isolated.[47-49]

As many of these compounds, along with manoalide, contain the pyranofuranone substructure, ring-chain tautomerism of the keto cyclol type is possible. For example, the opening of the six membered ring of manoalide (6) leads to seco manoalide (8) and luffariellin B (10) from luffariellin A (9) (Figure 1.9).

Seco manoalide has similar IC$_{50}$ values, for the inhibition of phospholipase A$_2$, to manoalide and also has the additional property of inhibition of the enzyme aldose reductase. Inhibition of this enzyme causes diabetic cataracts due to the accumulation of sorbitol by reduction of glucose. It has been shown that a modified form of

manoalide, where the polyisoprenoid side chain has been replaced with a methyl group, is also active.[47]
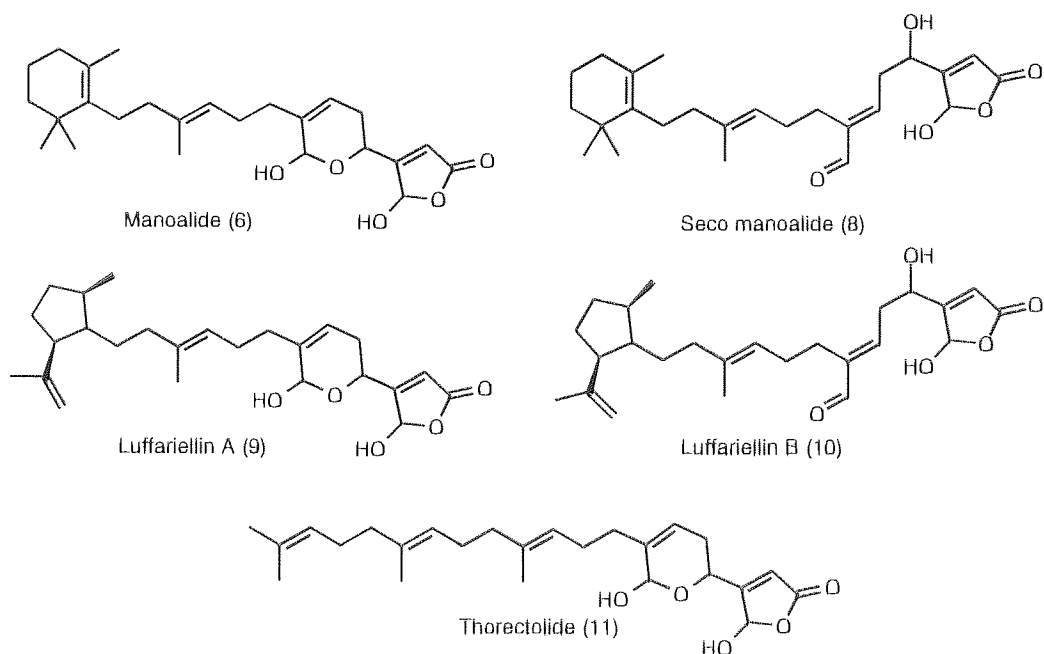
Figure 1.9 Some natural products containing the furanone end group. Ring-chain tautomerism has been shown for two of these molecules.

In addition to the compounds show above, there are many additional natural products containing the furan-2(5H)-one substructure.[50 - 62]

1.3 Combinatorial chemistry

As outlined above, the furan-2(5H)-one structure would be a suitable molecule for further adaptation in the search for new therapeutic molecules and due to the presence of more than one site for attachment, may be suitable for combinatorial chemistry. In this section, the differing techniques of combinatorial chemistry are reviewed, with examples of where each technique has been successful in the identification of biologically active molecules.[63 - 67]

Combinatorial chemistry enables us to generate large numbers of diverse compounds in order to find lead compounds in a fraction of the time it would take to prepare them by orthodox methods. The main feature of combinatorial chemistry is that a range of analogues can be produced using similar reaction conditions, either as a mixture in the same reaction vessel, or individually in parallel, using semi-automated synthesis.

New biological targets are being discovered all the time. Using traditional methods, for each new biological target found, separate research would need to be conducted in order to find new agonists and antagonists to the target. By using combinatorial chemistry, libraries of compounds can be tested to find possible leads, which can then be optimised. These libraries may be developed specifically for the receptor by modifying existing compounds (e.g. furanones for the PLA_2 receptor), or be already synthesised libraries which can then be re-screened on the new receptor.

Investigation into the different types of reaction which furan-2(5H)-one can undergo lends itself to combinatorial chemistry, due to the presence of more than one possible site for the attachment of various groups. Once the different types of reactions that the basic furanone structure can undergo have been evaluated, the assembly of various libraries by semi-automatic parallel synthesis can be undertaken. The products of these libraries can then be submitted for any biological testing required (not just against PLA_2).

If any successful leads are found, these can then be optimised by conventional methods or by the continued use of combinatorial chemistry. Although combinatorial chemistry may not be used at this point in the research, it will have speeded up the process of arriving at this point by enabling us to screen a larger number of test compounds. Combinatorial chemistry can, however, have an impact at the point of lead optimisation if the receptor structure is known. Here, it can be used to design a library of directly related analogues, which can then be submitted for further testing.

Combinatorial chemistry can be divided into two main groups called solution phase combinatorial chemistry and solid phase combinatorial chemistry. Conventionally,

chemists use the process of one pot, one reaction chemistry to discover lead compounds. This has been successful in the past but has a few major drawbacks. The main problem with this method is that it is heavily time consuming and so only a limited number of compounds can be synthesised and tested over a given period. The second limitation is that every biological receptor can only be worked on individually. Normally, when a new receptor arises, the entire process will need to be started again. Combinatorial chemistry attempts to get around this problem by focussing on techniques which have the potential to generate large numbers of compounds, either separately, producing many compounds in parallel or simultaneously in mixtures. Any library of compounds, once made, can be kept and tested against any existing or new receptors as and when necessary. This provides a faster, more efficient and cheaper method for lead discovery or optimisation (Figure 1.10).[63]

$$A + B \longrightarrow AB$$

$$\begin{matrix} A_1 & & B_1 \\ A_2 & & B_2 \\ A_3 & & B_3 \\ A_4 & & B_4 \\ \vdots & & \vdots \\ A_n & & B_n \end{matrix} \longrightarrow A_{1-n}B_{1-n}$$

Orthodox synthesis   Combinatorial synthesis
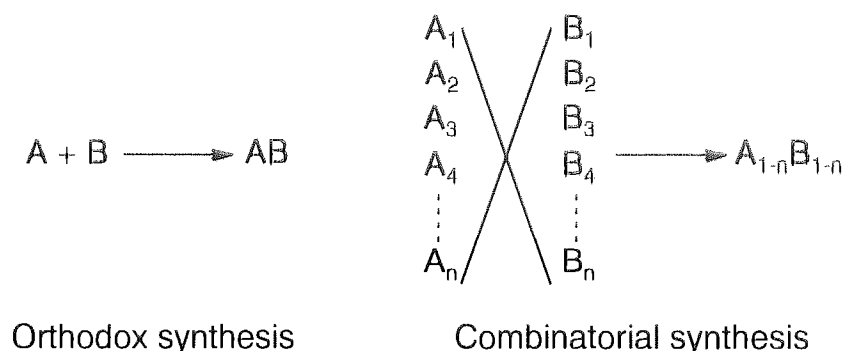
Figure 1.10 Demonstrating the difference between orthodox and combinatorial synthesis.

The next two sections will address these two techniques, and highlight the advantages and disadvantages that they offer to furanone research.

1.3.1   Solution phase combinatorial chemistry

Solution phase chemistry is best used if the reactions are performed in parallel, possibly accelerated by the use of semi-automatic methods. This will enable any lead structures to be identified easily, as the structure of the molecules under test in each of the test solutions is known. Performing many reactions in the same vessel using

solution phase chemistry is possible, but only if conditions are employed which minimise the production of by-products. The use of solid phase chemistry can simplify the process and eliminates any by-products that are formed. This will be explained fully in section 1.3.2. The main advantage of using solution phase chemistry is that no effort is needed to develop a suitable linker. Although solid phase combinatorial chemistry has been the preferred technique, there have been a few examples of successfully synthesised and screened solution phase libraries, with some groups preferring the technique to avoid the steps involved in the connection and removal of a linker (see the examples given later).

Concentrating on solution phase chemistry, the next point to be addressed is the question of how to screen the products of any combinatorial library. Obviously, any library produced in parallel, either by hand or by using semi-automation can be tested biologically in the conventional way. The problem of screening becomes far greater if attention is switched to solutions of mixtures of compounds. Solution phase chemistry, unlike solid phase chemistry does not allow the easy separation of each of the individual compounds. Instead, techniques are employed that can identify any active compounds by the comparison of different mixtures of compounds.

This can be explained better by the examination of two successful examples of the use of solution phase combinatorial chemistry, which have been disclosed. In both examples the groups concerned, Glaxo and Pirrung, have synthesised dimeric compounds using amide, ester or carbamate bond-forming reactions. In the example published by Glaxo, forty acid chlorides were reacted with forty amines or alcohols to produce the relevant amides or esters. Each library was formed twice in mixtures of different composition.[63]

In the first set, each of the forty acid chlorides was reacted with a stoichiometric amount of an equimolar mixture of all forty nucleophiles under investigation. In the second set, each amine or alcohol was reacted with an equimolar mixture of the acid chlorides. The eighty mixtures of forty components each were screened against a wide

variety of pharmacological targets. Any positive result would identify half of any active dimeric compound.

Pirrung's group used a similar technique to synthesise a library of fifty-four carbamates from nine alcohols and six isocyanates. As before, the first library set reacted an equimolar mixture of isocyanates with each of the alcohols and the second library set reacted each isocyanate with an equimolar mixture of alcohols. Each of the mixtures was tested biologically against electric eel acetyl cholinesterase. The results were entered into a two-dimensional matrix and from this, the structure of the most active compound could be identified (12) (Figure 1.11).

(12)
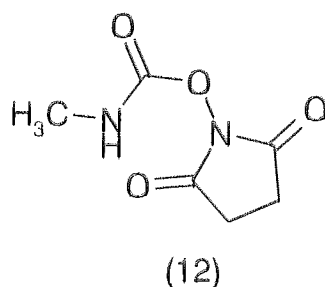
Figure 1.11 The structure of the most active compound against electric eel acetyl cholinesterase identified by solution phase combinatorial chemistry

1.3.2 Solid phase combinatorial chemistry

A different approach that can be taken to combinatorial chemistry is the use of solid-phase synthesis (SPS). In this method, each substrate is linked to a solid phase such as a resin bead. Surplus reagents and any by-products can then be removed by washing, as they won't be linked to the bead. The other advantage of this method is that a varying degree of reactivity of substrates can be accommodated as several equivalents of reagents can be used to drive the reaction to completion, and then removed by washing with an inert, volatile solvent.[63]

Solid phase combinatorial chemistry, as with solution phase combinatorial chemistry can be performed either in parallel or in a single or set of mixtures. It has the

advantage that reactions can be driven to completion as excess reagents can be used and then simply washed away. The main drawbacks with solid phase combinatorial chemistry are, however, that methods need to be established to link the molecule to a solid support and the problem of analysis of a reaction mixture where one component is still attached to the solid support. The use of a solid support for organic synthesis is therefore reliant on three requirements.

1. A cross-linked, insoluble, polymeric material that is inert to the conditions of synthesis is needed.

2. There needs to be some method for linking the substrate to the solid phase that permits the selective cleavage of some or all the product from the solid support during synthesis for analysis of the extent of reaction(s), and ultimately to give the final product of interest.

3. There also needs to be a chemical protection strategy to allow selective orthogonal protection and deprotection of groups in the monomers.

The whole process of solid phase combinatorial chemistry, whether it in performed in parallel (using conventional or semi-automatic techniques) or in a mixture can be represented by the following diagram (Figure 1.12).
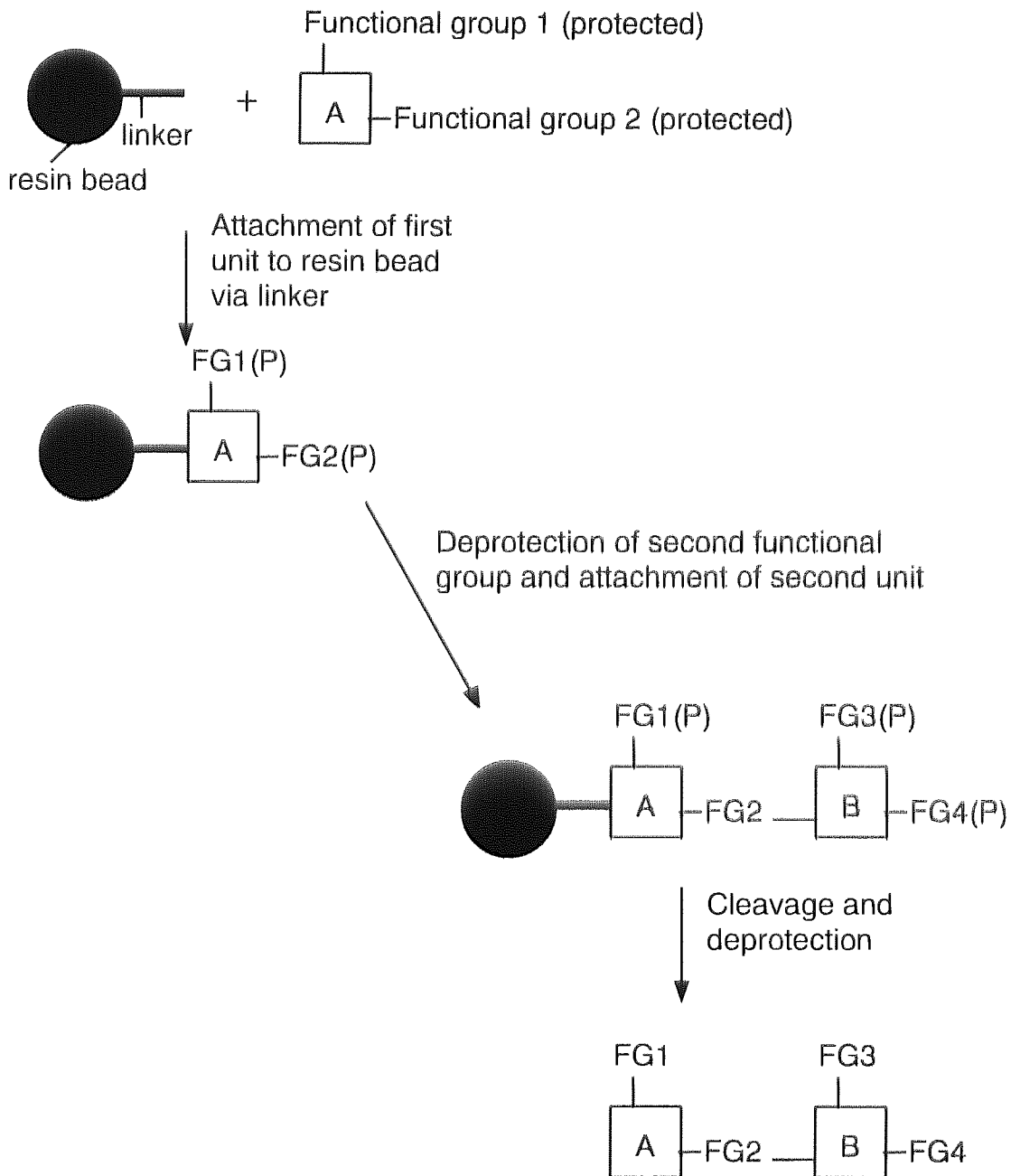
Figure 1.12 A graphical representation of the main features of solid phase combinatorial chemistry.

Once the desired compound has been synthesised on the bead, it can be cleaved and deprotected. If the library has been assembled by manual or semi-automatic parallel synthesis, after cleavage each molecule can be biologically tested independently in the standard fashion. If however, the library has been prepared as one or more mixtures, one of two things can be attempted. The first of these is that after cleavage, each of

the mixtures can be biologically tested and any active compound found by iterative re-synthesis and re-screening.

This can be explained using the following example. If a library of compounds is made based on a compound that has three sites for variability, with each site either linked to X, Y or Z, this will give a possible twenty-seven compounds (3^3). If three pots are synthesised with a constant in each pot in the first position, testing the three pots can identify the active ligand in that position. Re-synthesis of the compounds in that pot, keeping position one constant throughout and a set value for position two in each pot, can then identify the second ligand. Repetition of this process for the final ligand can identify the active molecule in less time than it would take to synthesise and test all the molecules (Figure 1.13).[63]
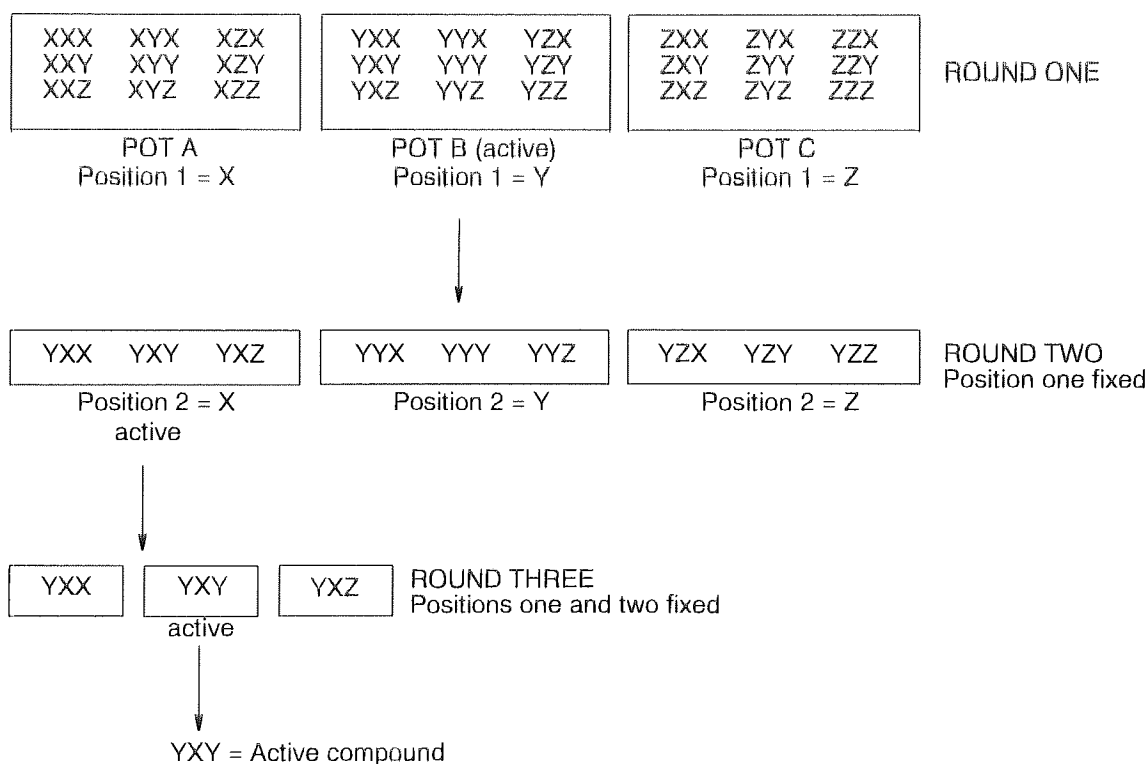
Figure 1.13 The identification of the most active compound from a collection of twenty-seven compounds using iterative re-synthesis and re-screening.

The alternative method for establishing the identity of an active compound is to use tags. Here, an encoding molecule is assembled on the same resin bead in parallel with the desired molecule. Each bead has a unique chemical tag, which encodes the chemical history of the bead. Thus the structure of the compound assembled on the bead may be deduced by reading the chemical tag. The three main forms of tags are peptides, oligonucleotides and halogenated aromatic molecules that encode a 'binary' sequence which can be deciphered by electron-capture capillary gas chromatography.[63]

The final analysis method in this section is to test the molecules while still attached to the resin bead. Any active compounds can then be sequenced later in the case of compounds such as peptides, or identified by using a tagging code as explained above. Obviously this method is not suitable for all applications due to conformational constraints put on the molecule by the resin bead.

This technique has been pioneered by Merrifield who was the first to use it in the synthesis of peptides. The synthesis of peptide libraries lends itself to solid phase combinatorial synthesis due to the limited range of synthetic transformations which are required for synthesis. This approach was utilised by Owens and co-workers at Lilly, who through the synthesis and screening of peptidic mixtures consisting of precursors including L- and D-α-amino acids and statine, successfully identified HIV protease inhibitors. This technique has also been successfully employed by different research groups to identify a whole range of biologically active compounds including antigenic determinants, antimicrobials, enzyme inhibitors and ligands for opioid receptors.[63]

Once the types of reaction, which the furan-2(5H)-one molecule can undergo, have been evaluated (see chapters two and three), biological screening can be undertaken on any suitable library components (see chapter four). Further use of combinatorial synthesis will be included in chapter five. The main screening test used in this thesis will be anti-inflammatory screening (COX-2 inhibition – see section 1.2.1.3), cancer

screening (see section 1.4), antibiotic screening (see section 1.5) and cholecystokinin receptor antagonism screening (see section 1.6).

1.4 Cancer screening

Cancer is a major disease which causes death in at least one in five people in the developed nations. It is characterised by the rapid and uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells. Worryingly, it has been shown that cancer is on the increase. This is largely due, however, to the fact that cancer is a disease of old age and thanks to the advances in public health, the general population is living longer. Treatment of cancer is largely determined by the type of cancer being treated and the clinical stage at which the cancer has reached. The three main approaches to dealing with cancer are surgical excision, irradiation and chemotherapy. Of the three, chemotherapy with cytotoxic drugs is the main method for treatment of all but a few cancers, and is commonly used as an adjuvant to surgery or irradiation.[68]

1.4.1 The problems in cancer treatment

The problems faced by the medicinal chemist in treating cancer chemically are due to the lack of variation between the cancer cells and the patients' own cells. Compared, for example, to the treatment of biological infection where the infecting organism has exploitable differences between it and the host (see section 1.5), cancer cells do not possess these differences. There are, however, three characteristics which cancer cells show which are not seen in normal cells.[68]

1. It is *generally* true that cancer cells proliferate at a much higher rate than normal body cells. Some cancer cells do multiply slowly so this is not true for all cancer cells. The main difference between the division of normal and cancerous cells is that the cancerous division is not subjected to any regulatory process. Some normal body cells may grow in certain situations at a very rapid rate. For example,

if a large portion of the liver is removed during surgery, it will rapidly re-grow to its original size. Once the desired size has been achieved, the regulatory mechanisms will kick in and slow the growth to normal levels. Cancerous cells do not possess this mechanism and grow at a fast rate most of the time.

2. Cancer cells invade the tissues of other organs. Normal body cells may grow side by side at the same site but they develop certain spatial relationships with respect to each other. These spatial awareness characteristics are maintained even during repair processes where one or both of the organs may be dividing rapidly. Cancerous cells do not possess this spatial awareness. Cancers can invade other organs and cause major problems.

3. The final characteristic of cancer cells, which is not shown by other cells, is secondary tumours, which can be caused by cells released by the primary tumour. These growths, called metastases, are caused by cancerous cells being taken to other sites by the blood or lymphatic system or as a result of being shed into body cavities, and establishing new colonies of cancer cells.

Most anticancer drugs are antiproliferative and so work by preventing the rapid dividing of cancer cells. Due to the similarity between the cancer cells and the host cells, the antiproliferative drugs will also affect the growth of rapidly dividing host cells. Bone marrow depression, impaired healing, growth retardation, sterility, hair loss and teratogenic effects are all common in patients on anticancer chemotherapy.

1.4.2   The general principle of action of cytotoxic drugs

The aim in cytotoxic chemotherapy is to produce as near as possible total elimination of cancerous cells. This is because even if an anticancer drug kills 99.99% of all the cancerous cells in a tumour with 10^{11} cells, this will still leave ten million (10^7) malignant cells. One of the main problems with cancer treatment is that therapy can only commence after diagnosis. As tumours, especially in their early stage, can proliferate very quickly, diagnosis may only occur after the tumour has reached a

certain size, which in some cases may be too late. The growth will, however, slow down in a lot of cases because the tumour outgrows its blood supply, resulting in necrosis or death of part of its bulk, and because not all cells proliferate continuously.[68]

The cells in a tumour mass can be divided into three main compartments, A, B and C. Cells in compartment A are dividing cells, possibly being continuously in cell cycle. Compartment B consists of cells that are resting (G_0 phase). These cells are not dividing but have the capability of doing so. The final compartment, compartment C, consists of cells which are no longer able to divide but still contribute to the overall tumour mass. The main problem with treating tumours with the main available cytotoxic agents is that they are only effective against cells in compartment A which may be as little as 5% of the tumour's mass. This means that the drugs are antiproliferative but they have no specific effect on invasiveness or the tendency to metastasise. This is why cytotoxic agents are used in combination with other techniques for the treatment of cancer and not on their own.[68]

Coupled with the problems mentioned above, is the effect the drugs have on normal dividing tissue. This results in the following effects:[68]

- Myelotoxicity with decreased leucocyte production leading to decreased resistance against infection.
- Impaired wound healing.
- Depression of growth in children.
- Sterility.
- Teratogenicity.
- Loss of hair (alopecia).

1.4.3   The main groups of cytotoxic agents

Drugs which are used to treat cancerous conditions can be divided into two main groups, cytotoxic agents and hormones. The cytotoxic agents can be further subdivided into four distinct subgroups.[68]

• Cytotoxic drugs

1. Alkylating agents. The alkylating agents, which include the nitrogen mustards, the nitrosoureas and busulphan, act by forming covalent bonds with DNA and thus impeding DNA replication.

2. Antimetabolites. The antimetabolites work by blocking or subverting one or more of the metabolic pathways involved in the synthesis of DNA. This group includes the folate antagonists (for example, methotrexate), and the purine and pyrimidine analogues such as 5-fluorouracil.

3. Cytotoxic antibiotics. This group of compounds contains those substances of microbiological origin which prevent eukaryotic cell division. This includes the cytotoxic drugs doxorubicin and mitomycin.

4. Vinca alkaloids. This last group of cytotoxic drugs contains the compounds, for example, vincristine and vinblastine, which come from plants and affect microtubule function, hence depressing the formation of the mitotic spindle, and thus inhibiting cell division.

• Hormones

The most important hormones used in the treatment of cancerous conditions are the glucocorticoids, oestrogens and androgens. These are mainly used for tumours, which are hormone dependent; i.e. the tumour growth is either inhibited by different hormones or by hormone antagonists. For example, the glucocorticoids have an inhibitory effect on lymphocyte proliferation and so are used in leukaemias and lymphomas and the oestrogen antagonist tamoxifen has been highly successful in the treatment and prevention of breast cancer.

Any small intermediate molecules formed as building blocks in the assembly of furan-2(5*H*)-one combinatorial libraries will also be suitable for and entered into anticancer screening programmes (see section 2.3) in the hope of identifying possible anticancer lead structures.

1.5 Antibiotic screening

The discovery that chemical compounds can inhibit the growth of micro-organisms within the body was a major breakthrough in the fight against infection and death. The advantage that medicinal chemists have in targeting compounds against micro-organisms is that there are usually some important exploitable differences between the infecting organism and the host. This is not the case with cancer cells and so the treatment of cancer with chemotherapeutic agents still remains difficult (see section 1.4). The ideal chemical agent to target infections is one that is toxic for the parasitic cell but innocuous to the host. The bigger the biochemical difference between the host and the parasitic cell, the easier this will be to achieve. Infections can be caused by a whole variety of organisms. The simplest of these, the prokaryotes (cells without nuclei – for example bacteria), will be the easiest to target as, biochemically they differ most greatly from the host cells. The eukaryotes (cells with nuclei), are harder to treat as they are, from a biochemical point of view, closer to the host. This category of infecting organism includes both single-celled organisms (for example, protozoa) and multicellular organisms (for example, helminths).[69]

In a separate category to the above organisms are the viruses. These organisms are not cells strictly speaking, as they have to use the cell replication machinery of the host to reproduce. Because of this fact, viral infections are usually harder than other types of micro-organism infection to treat chemically.[70]

A good site on the parasitic cell is the biochemical reactions that take place to form the various structures within the cell. These reactions can be divided into three distinct groups.[69]

- Class 1 reactions are those reactions which use glucose or other carbon source to form energy and simple carbon compounds, to be used as precursors for the next class of reactions.

- Class 2 reactions utilise the energy and small carbon compounds to manufacture the essential small molecules, for example, amino acids and nucleotides.

- Class 3 reactions assemble the small molecules generated in the class 2 reactions into larger macromolecules, for example, proteins and DNA.

The three different classes of reactions are different in their suitability for targeting. Class 1 reactions do not provide good targets, as there is no exploitable difference between how bacteria and human cells obtain energy from glucose. Both organisms use the Embden-Meyerhof pathway and the citric acid cycle. In addition to this point, is the fact that even if the pathway for obtaining energy from glucose was blocked, bacteria could utilise an alternative energy source, for example, amino acids or lactate.

Class 2 reactions provide a better target for the medicinal chemist, as there are differences between the pathways that exist in the bacterial cell compared to those in a human cell. One main difference is that over the course of evolution, humans have lost the ability to synthesise some amino acids and must obtain them from the diet. These so-called 'essential' amino acids can still be synthesised in the bacterial cell. Knocking out the synthesis of one of these amino acids would therefore be disastrous for the infecting organism but would have no effect on the host.

Class 3 reactions probably provide the best target, as every cell has to make its own macromolecules. There are distinct differences between bacterial and host pathways providing many points for selectively targeting, for example, the synthesis of peptidoglycan which constitutes the cell wall of bacteria but not eukaryotes.

Along with the biochemical pathways, there are other potential targets for the treatment of infection. Chemotherapeutic agents can attack the formed structures of

the cell (for example, the cell membrane or the microtubules in higher organisms). They can also be targeted against specific structures of higher organisms, for example, the muscle tissue in helminths.

The purified furan-2(5H)-ones prepared in this work will be screened against a variety of different organisms (see chapter four). This will give some idea as to the antibacterial potential that these molecules possess. Optimisation of any successful result can then be undertaken.

1.6   Cholecystokinin (CCK) screening

The biological hormone cholecystokinin (CCK) is distributed within the body in two main areas. It is a major intestinal hormone where it controls pancreatic secretion and bile ejection. Secondly, it plays an important role within the brain where it is one of the most widely distributed brain neuropeptides. Its presence in the brain was first detected in 1976. Since this point, major advances have been made in the understanding of the role of CCK in the alimentary tract and brain. At least two subtypes of CCK receptor have been discovered CCK-A and CCK-B. These are classified as to the location they are predominantly found at – CCK-A (alimentary) and CCK-B (brain). Evidence for this was obtained when it was found that the benzodiazepine CCK antagonist L-364,718 was highly selective for peripheral versus brain CCK receptors. Extensive evidence does now indicate, however, that CCK-A receptors are found in the brain and CCK-B receptors are also found in the alimentary canal, but the original nomenclature remains.[71]

Within the brain, CCK is mainly found as either the sulphated or desulphated form of the shorter octapeptide CCK-8. Sulphated CCK is widely distributed within the brain and is found in neurones in the hippocampus, nucleus accumbens, caudate nucleus, cerebral cortex and other brain regions. It has also been found that in some neurones it can coexist with other neurotransmitters, for example, dopamine, serotonin (5-HT), γ-aminobutyric acid (GABA) or other neuropeptides.

1.6.1 Possible benefits of CCK receptor antagonism.

CCK antagonists were originally developed to aid understanding as to the physiological role of CCK and to help identify any receptor subtypes. There are, however, a number of roles which CCK antagonists could be used in. These are detailed below:[71]

Peripheral roles:

1. They may play a role in the treatment of pancreatitis. This is linked with the inhibitory effect CCK antagonists have on pancreatic amylase secretion. Coupled with this is the ability that CCK has to promote growth of the pancreas and the blocking effect CCK antagonists have on this effect.

2. As CCK is believed to be a major regulator of gall bladder contraction, CCK antagonists will reduce the secretions of the gall bladder. Biliary colic, which is believed to be caused by intense contractions of the gall bladder when a gallstone blocks the outlet, can cause recurrent abdominal pain. CCK antagonists would therefore have a role to play in the alleviation of biliary colic pain.

3. CCK antagonists should also be able to reverse the gastric emptying delay controlled by CCK. If this is the case, CCK antagonists could be used to treat disorders of gastric emptying and also reduce the development of satiety by this mechanism and enhance appetite. This could play an important role in the treatment of anorectic conditions.

4. The CCK-B receptor is similar, if not identical, to the gastrin receptor. This means that any CCK-B antagonists would also be highly likely to antagonise the gastrin receptor. This may play an important role in the control of gastric acid secretion and in the treatment of gastrin-dependent proliferative disorders and gastrin-dependent tumours.

5. CCK antagonists can cause the potentiation of the effect of opiate analgesia. This effect is coupled with their protection against the development of narcotic tolerance. There may, therefore, be a role for CCK antagonists to play in the reduction of doses of opiates needed in the management of chronic pain. This potentiation is achieved without the potentiation of the side effects that can accompany opiate administration, namely, respiratory depression and constipation.

Central nervous system roles:[71]

1. It has been postulated that CCK antagonists may have a role to play in the treatment of schizophrenia. This theory has arisen due to the coexistence of CCK and dopamine in the midbrain. It is known clinically that neuroleptics and dopamine receptor blockers are effective in the treatment of schizophrenia, and agonists or indirect agonists (for example, amphetamine) produce or exacerbate the psychotic symptoms.

2. CCK-B receptor antagonists may well possess anxiolytic properties. This theory was tested using the two CCK-B receptor antagonists CI-988 and PD 135158. Each compound was tested for its anxiolytic effect using the rat elevated X-maze, the rat social interaction test and the mouse black-white box test. Anxiolytic activity was found in both compounds.

3. Finally, CCK antagonists could have an important role to play in the treatment of benzodiazepine dependence. This is linked with the fact that CCK antagonists do not appear to produce a withdrawal anxiogenesis and that CCK-B antagonists suppress the benzodiazepine-induced rebound anxiety. CCK-B antagonists may even have an extended role to perform in the withdrawal of other drugs of abuse such as cocaine, alcohol and nicotine.

As it can be seen from the above text, CCK antagonism may become an important goal in the development of further drugs. In many of the above sections, the potential benefit of CCK antagonism is theoretical or based on a limited knowledge of the effects of receptor antagonism. Nevertheless, CCK antagonism remains an important goal for the medicinal chemist and would act as a suitable screening test to enter any libraries of purified furan-2(5H)-ones into.

1.6.2   Current CCK antagonists

Current CCK receptor antagonists can be divided into five main categories. These are as follows:[71-76]

* Derivatives of cyclic nucleotides.
* Derivatives of amino acids.
* Partial sequences and derivatives of the C-terminal sequence heptapeptides of CCK.
* Benzodiazepine derivatives.
* Nonpeptide "peptoids" based on fragments in the CCK molecule.

Purified furan-2(5H)-one libraries can be entered into a CCK screening programme and any positive results optimised in parallel with the results from the antibacterial screening programme. In this way, it will be possible to pursue a further line of biological activity screening with the minimum amount of chemical synthesis needed. This may lead to the identification of a novel CCK antagonist.

1.7 Important published furanone chemistry

As the furanone structure has many possibilities for substitution (at positions three, four and five), many research groups have studied various different reactions at the three positions. Other groups have studied the different methods for the formation of the furanone structure itself. [4-35]

1.7.1 Formation of simple furanone building blocks

In his PhD dissertation, Michael Henningsen prepared many simple furanone building blocks from furfural (13). The main reactions involved in this can be summarised overleaf (Figure 1.14). [77]
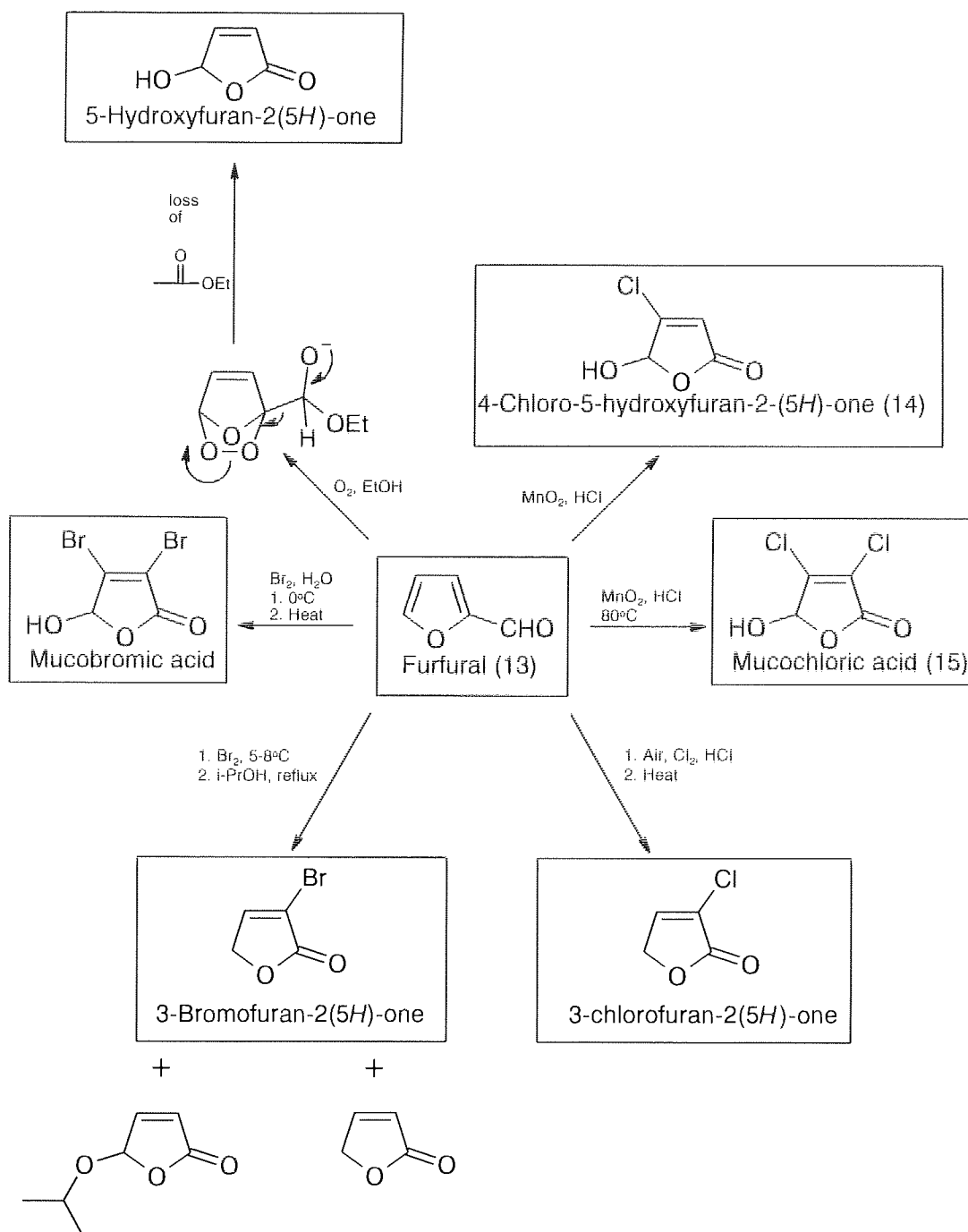
Figure 1.14 The formation of many simple furan-2(5H)-one building blocks.

One point worth noting from this work is that 4-chloro-5-hydroxyfuran-2(5*H*)-one (14) and 3,4-dichloro-5-hydroxyfuran-2(5*H*)-one (mucochloric acid) (15) use the same reaction conditions except for a difference in temperature. This is an important difference, which will be referred to later when the assembly of a building block with only a chlorine atom in the four position will be attempted. During this reaction, it

will be important to keep the temperature low so as to reduce the formation of the dichlorinated product (mucochloric acid).

1.7.2 Bicyclic systems

The bicyclic system of manoalide has been shown to be important in the irreversible inhibition of phospholipase A_2[37], and may also be important in the inhibition of cyclo-oxygenase. Lattmann *et al.* investigated the use of different dienes in the formation of bicyclic systems by Diels-Alder reaction. Chemoselective vinylogous glyoxylates are used in the reaction along with four differently reactive dienes. The least reactive diene (2,3-dimethylbuta-1,3-diene) needed a very strong Lewis acid ($TiCl_4$). These reactions can be summarised in the following diagram adapted from reference 47 (Figure 1.15).
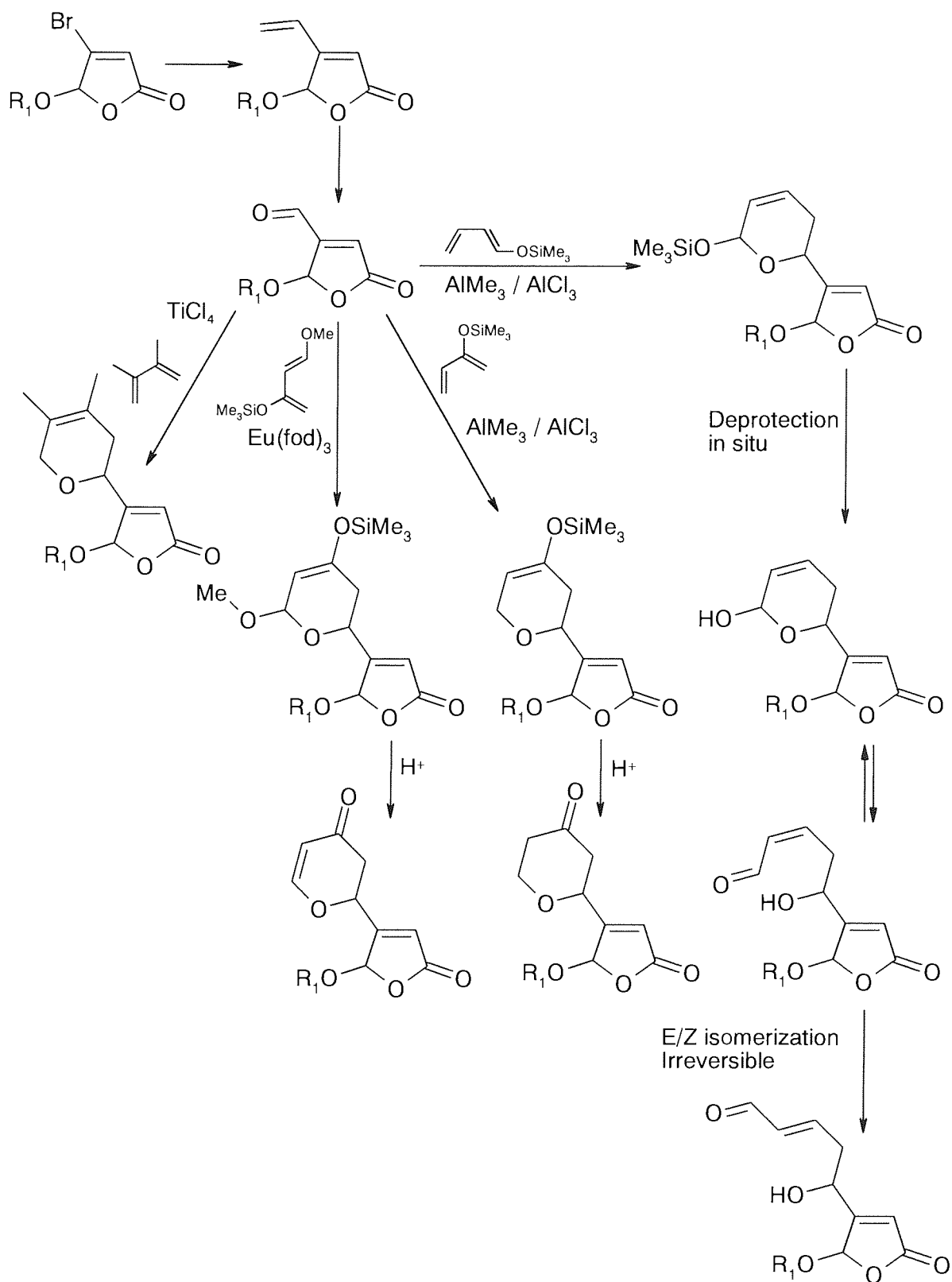
Figure 1.15 The assembly of many bicyclic systems based on the furan-2(5H)-one structure.

The reactions in Figure 1.15 were performed with 4-formyl-5-methoxy-2(5*H*)-furanone as well as 4-formyl-2(5*H*)-furanone. This provided a flexible route to many different pyranofuranones containing one or two sensitive acetal triggers.

The synthesised compounds were derived by first forming the relevant 4-bromo compound with the correct alkoxy group at position five. This was best achieved by forming the 5-methoxy-4-bromofuran-2(5*H*)-one and then substituting the desired alcohol by transacetalization. In the formation of the 4-vinylated products (17), the 5-alkoxy-4-bromofuran-2(5*H*)-one (16) was then reacted with tributylvinylstannane. This reaction was optimised with respect to temperature, solvent and palladium catalyst (Figure 1.16).[78]

Br
\
[chemical structure]
RO   O   O
(16)

Bu$_3$SnCH=CH$_2$
Palladium Catalyst
THF / MeCN / 35°C / 2 days

[chemical structure]
RO   O   O
(17)
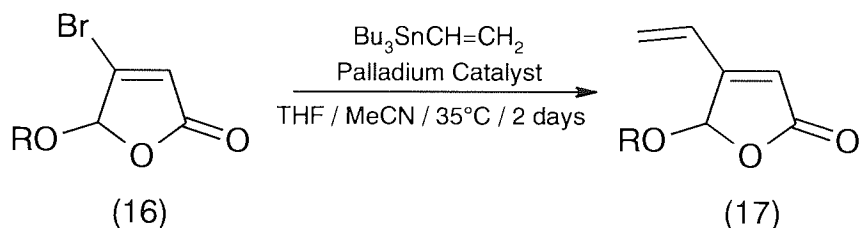
Figure 1.16 Formation of 4-vinylated furanones with the use of tributylvinylstannane.

4-Formyl-2(5*H*)-furanone (19) can be synthesised by two main routes. Oxidation of the parent alcohol (18) or oxidative cleavage of 4-vinyl-2(5*H*)-furanone (17). The parent alcohol was synthesised by three possible routes, outlined in the paper. The alcohol (18) is then oxidised to the relevant 4-formyl furanone (19) by pyridinium chlorochromate (PCC) (Figure 1.17), although over-oxidation to the carboxylic acid is a problem, especially in the presence of water. This problem was overcome by the use of MnO$_2$ on Na$_2$SO$_4$ as a solid support, and drying agent. This approach cannot be generalised for the preparation of 4-formyl-5-alkoxy derivatives. These products are best achieved by oxidative cleavage with ozone and work up with thiourea.[78]
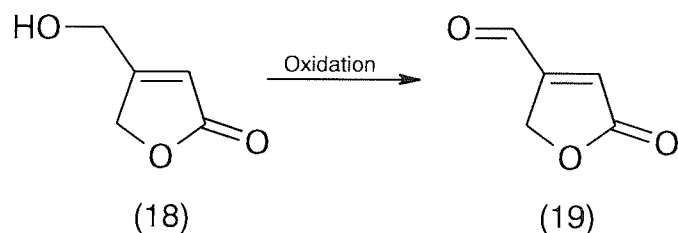
Figure 1.17 The formation of 4-formylfuranones by oxidation of the parent alcohol.

Other methods for the formation of bicyclic furanones have also been investigated, using the process of 3+2 cycloadditions with the external double bond of 4-vinyl-2(5H)-furanone (20). The additions were performed with nitrones as well as diazoacetic ester. Here, the external double bond functions as a dipolarophile producing, for example, isoxazolidinyl-2(5H)-furanones (21) and (22) (Figure 1.18).[79]
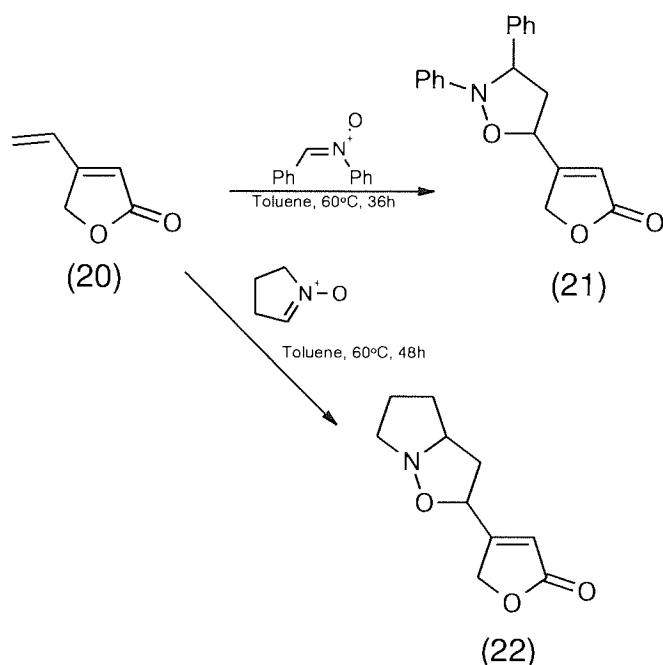
Figure 1.18 3+2 cycloadditions on the external double bond of 4-vinyl-2(5H)-furanone (20).

It was found that the scheme for the 3+2 cycloadditions could be extended to 5-alkoxy-4-vinyl-2(5H)-furanones.

1.8 Rationale for current work

Furanones can be easily synthesised from furfural (13), which is a cheap, readily available compound. Using published methods, detailed in this and subsequent chapters, and the techniques of combinatorial chemistry, a programme of furanone synthesis can be attempted with a many-fold objective. It will be shown that simple furanone building blocks (23) can be entered into a screening programme for anti-cancer activity with more complex furanones (24) being screened for anti-inflammatory activity. Disubstituted (4,5) furanones (25) can be easily prepared by combinatorial techniques and then submitted for any screening deemed appropriate (e.g. antibiotic activity). This plan can be outlined as follows (Figure 1.19).
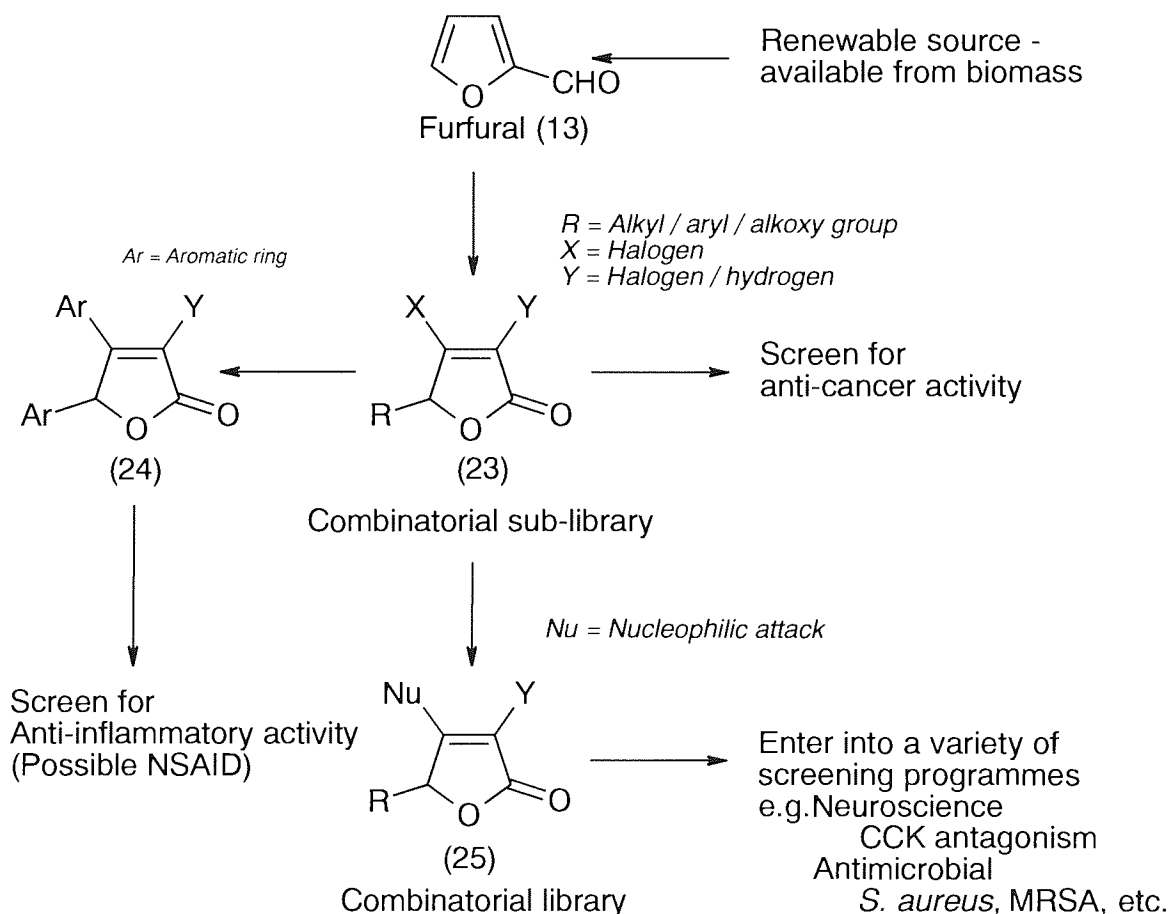
Figure 1.19 The many objectives within the project.

1.9 Summary

In summary, it has been shown that starting from manoalide (6), current research groups have progressed up to a certain point. Methods for the complete synthesis of manoalide have been published and then a further step has been taken in the publication of methods for forming bicyclic systems which are important in the inhibition of phospholipase A_2 and possibly cyclo-oxygenase. Some of the different types of reaction undergone by the furanone ring have been evaluated with further details being given in subsequent chapters. In the work reported in this thesis, combinatorial synthesis on the furan-2(5H)-one scaffold (26) will be employed to generate libraries in the attempt to find possible lead structures (Figure 1.20).

PLA$_2$ Inhibition

Manoalide (6)
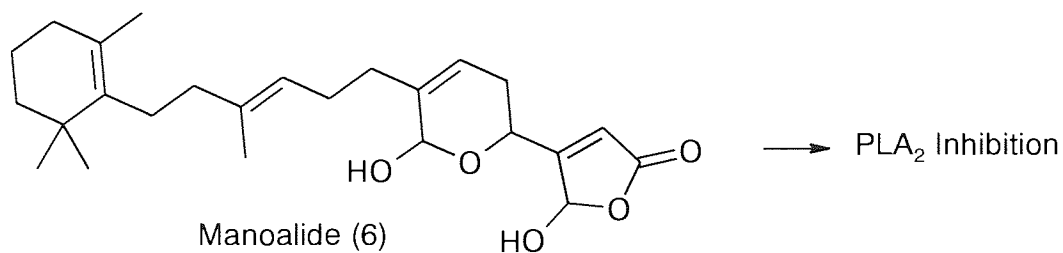
Structure simplification

Currently published furan-2(5H)-one reactions

Synthetic bis-heterocyclic
furan-2(5H)-ones
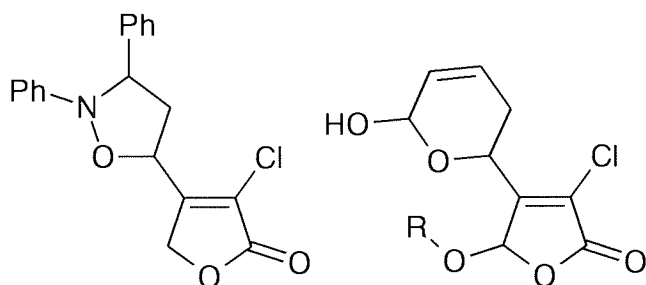
Reaction development

Programme of combinatorial
synthesis using furan-2(5H)-one scaffold

Furan-2(5H)-one
Scaffold (26)

☐ Different sites for
○ attachment to
△ furan-2(5H)-one
    scaffold
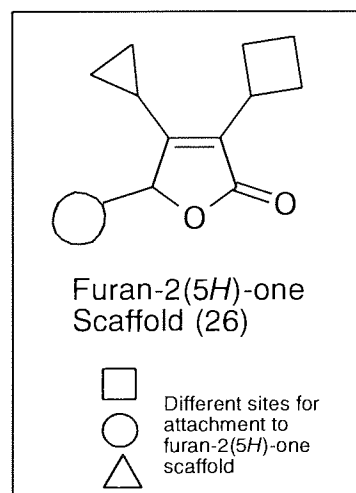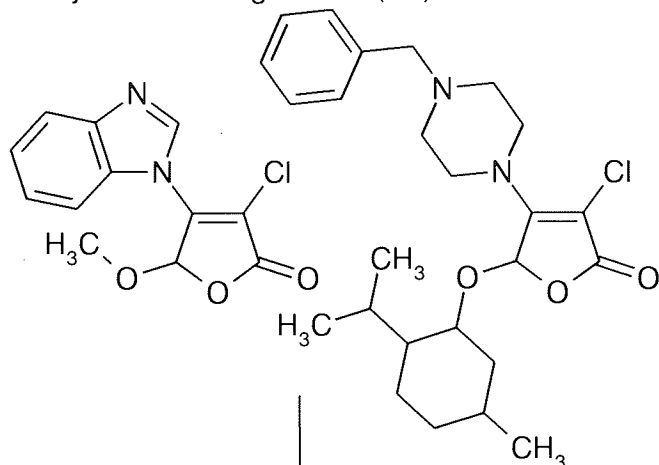
Possible lead
structure identification ⟶ Structure refinement

Figure 1.20 From manoalide, a natural product, to the construction of a library using solution phase combinatorial chemistry.

Chapter 2 : Results and Discussion

Furan-2(5*H*)-one building blocks

2.1 The furan-2(5*H*)-one core

2.1.1 Obtaining the core structure

The quest for more varied furan-2(5*H*)-ones has led to the study of mucochloric (15) and mucobromic acids (29). Mucochloric acid was initially studied in the late 1800s. It is the half aldehyde of dichloromaleic acid and is thought to exist in two forms (27) and (28) (Figure 2.1).[80]
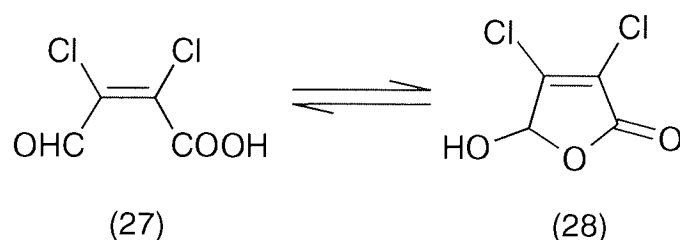
Figure 2.1 The two possible extreme forms mucochloric acid is believed to exist in.

Initially, it was decided to investigate the structure of the two mucohalic acids commercially available (mucobromic and mucochloric acid) by examining the structure in chloroform. A sample of each of the two acids was dissolved in deuterated chloroform and analysed by proton nuclear magnetic resonance spectroscopy (see section 7.1.1). It was found that both of the acids existed wholly in the closed ring form (Figure 2.2). This was inferred by the lack of any peak in the spectrum corresponding to either an aldehyde or carboxylic acid group, which would be present, if the molecules were partly or entirely in the open chain form.

Mucochloric acid (15)   Mucobromic acid (29)
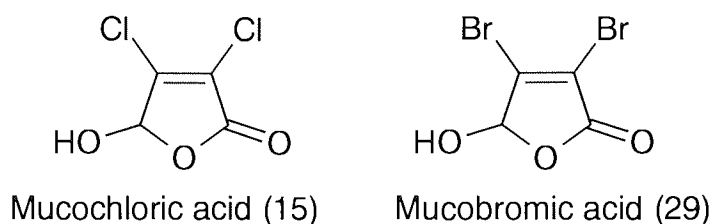
Figure 2.2 The structure of mucochloric and mucobromic acid.

In addition to the two commercially available mucohalic acids, it was decided to synthesise a further furan-2(5*H*)-one with a hydrogen atom at the three position. This was achieved by using a method, which converts furfural (2-furaldehyde) (13) into the relevant furan-2(5*H*)-one building block (30) containing a chlorine atom at the four position and a hydrogen at the three position (Figure 2.3) in a Ludwig (continuous extraction) unit (Figure 2.4) (see section 7.1.2).
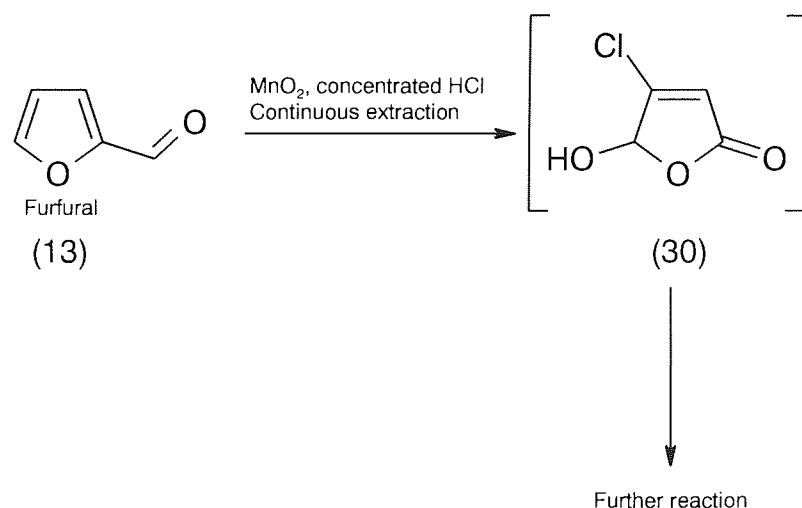
Figure 2.3 The synthesis of 4-chloro-5-hydroxyfuran-2(5H)-one for further reaction.

The extraction unit works by initially cooling concentrated hydrochloric acid in a long-necked vessel using an ice bath. A mixture of furfural (13) and manganese dioxide is slowly added while stirring continuously. After it has been left for forty minutes, a long thin glass tube with a hole at the bottom is connected to the long neck section of the vessel (see Figure 2.4). This tube has a hole in the side where a side arm leaves the outer section of the neck, linked to a second vessel containing ether. A condenser is connected to the top. The ether in the vessel on the right is heated and will evaporate and pass up the side arm. It will enter the inner glass tube, through the hole above the side arm and pass into the condenser where it will cool. After condensing, it will fall down the inner tube and through the hole at the bottom into the reaction vessel. The reaction product should enter the solvent phase, which will then lie on top of the aqueous phase. Once the reaction vessel has started to fill, the solvent phase on the top will pass back along the arm into the ether vessel. The whole process can then cycle again, extracting most of the reaction product into the ether vessel.
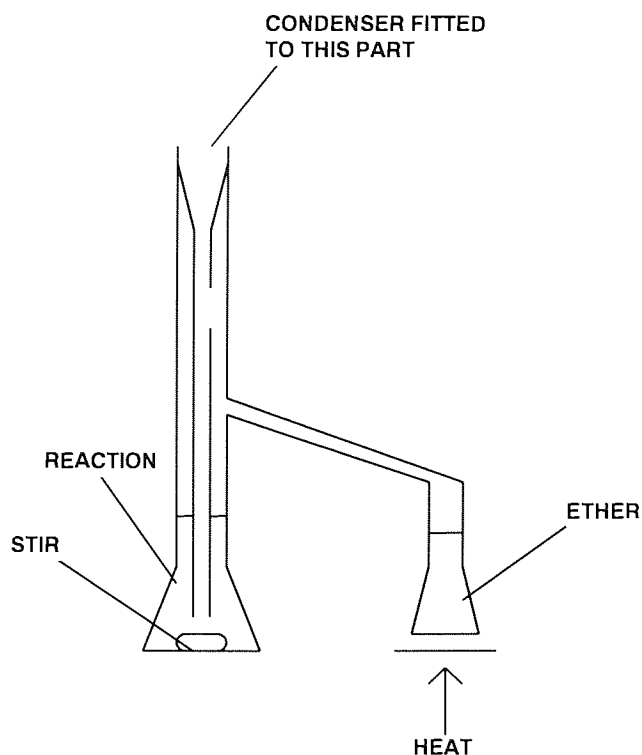
Figure 2.4 The extraction unit used in the formation of 4-chloro-5-hydroxyfuran-2(5H)-one (the ice bath and condenser are not shown in this diagram).

The solvent phase is then dried with magnesium sulphate and the ether removed by rotary evaporation under vacuum. This produces a yellow oil containing the desired product (30) (along with other by-products) suitable for further reaction.

There are now three core furan-2(5H)-ones available for use in the quest for suitable further reactions for combinatorial library assembly. Although it is possible to synthesise all three from furfural (13), two are commercially available, making synthesis unnecessary (Figure 2.5).

Furfural (13)

MnO₂, concentrated HCl
Continuous extraction

MnO₂, HCl
80°C

1. Br₂, 5-8°C
2. i-PrOH, reflux

(30)

Mucochloric acid (15)
Commercially available
Lancaster £6.90 / 100 g

Mucobromic acid (29)
Commercially available
Lancaster £31.70 / 100 g
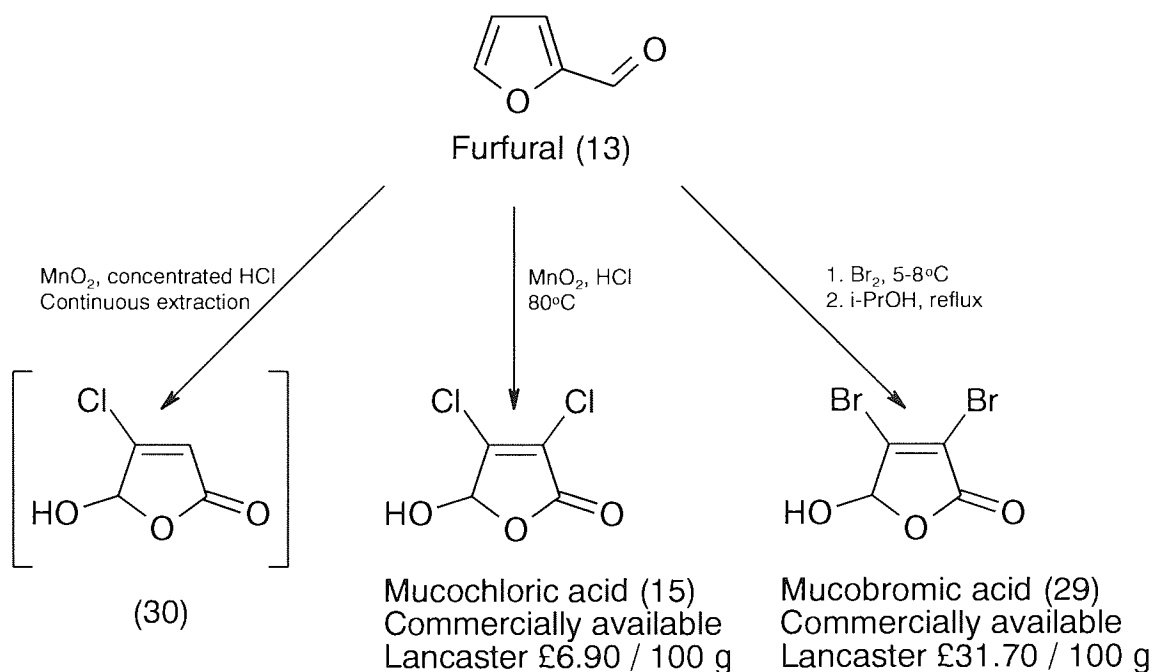
Figure 2.5 The synthesis of the three main furan-2(5H)-one core molecules.

2.1.2 Reaction of the aldehyde group (open-ring furanones)

The reactions that mucochloric acid undergoes can be divided into two sets. These sets correspond to the two extreme forms that the molecule is believed to exist in (see above). In a publication in the 1950s by Mowry, he divided the different reactions that he observed the molecule underwent into two groups, as to whether they occurred to the aldehyde group (27) (detailed in this section) or to the pseudo-acid group (28) (see section 2.1.3).[80, 81]

2.1.2.1 Condensation (aldol) reactions

In the second of his two papers on mucochloric acid, Mowry examines the reactions of the aldehyde group (27).[81] He starts with the reaction with acetophenone. This is performed in cold alkali to give the product 3,4-dichloro-5-(2-oxo-2-phenylethyl)furan-2(5H)-one (31) (Figure 2.6). This approach was then extended to produce further reaction products using nitromethane, nitroethane, 2-nitropropane,

cyanoacetamide and phenylacetonitrile. Attempts to repeat the reaction under less basic or acidic conditions were unsuccessful, indicating that basic conditions are required for the ring to open in order for reactions to take place at the aldehyde group.
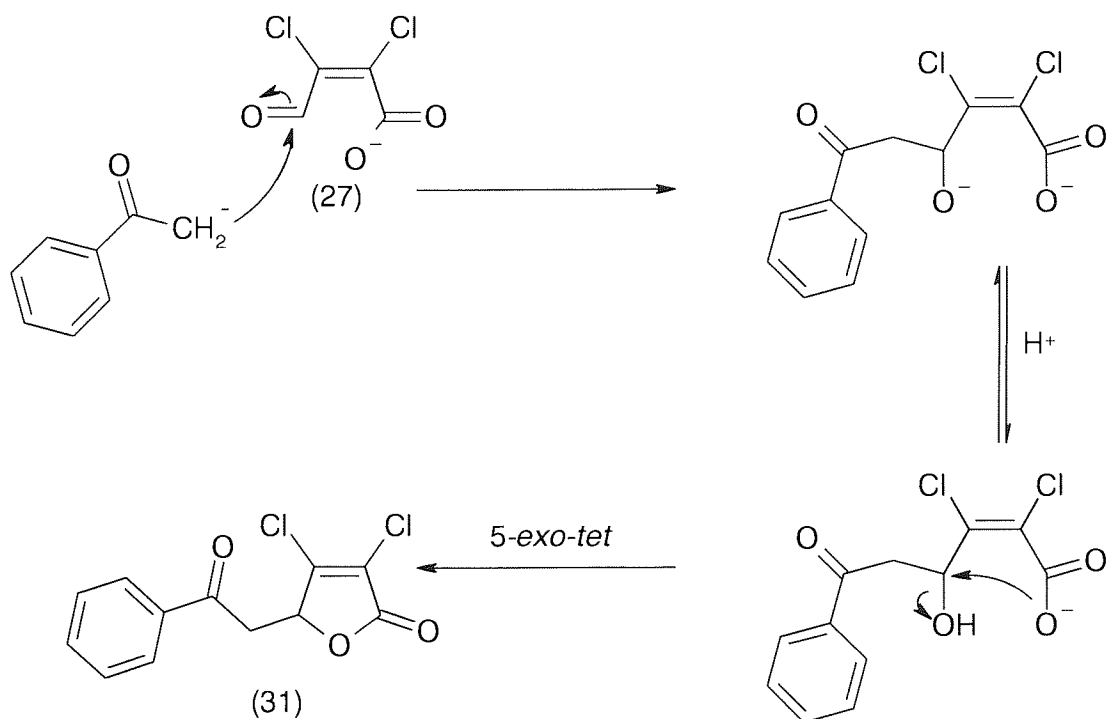
Figure 2.6 Proposed mechanism for the reaction of mucochloric acid and acetophenone.

Repetition of the reactions with acetophenone and nitromethane was attempted, following the same reaction conditions that were reported by Mowry above. After leaving both reactions overnight at room temperature, the reaction mixture was poured into ice water containing excess hydrochloric acid. The oily precipitate formed was dried and extracted in ethanol (Figure 2.7) (see section 7.2.1).
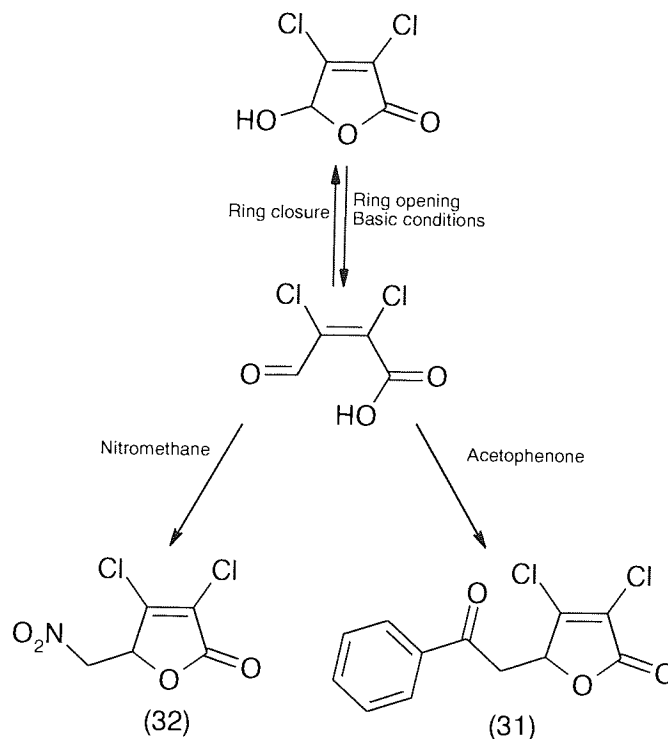
Figure 2.7 The use of basic conditions in the assembly of further furan-2(5H)-one building blocks.

Samples of each reaction product (31 and 32) were submitted for proton (and in the case of the acetophenone product, carbon) nuclear magnetic resonance spectroscopy in deuterated chloroform. This showed the presence of the desired product in both cases.

2.1.2.2 The role of the aldehyde in electrophilic aromatic substitution reactions

Because of the potentially serious side effects linked with current non-steroidal anti-inflammatory drugs (NSAIDs) (see section 1.2.1.3), new NSAIDs are still being developed. The NSAIDs are a very diverse group of molecules containing a variety of different structural groups (see section 1.2.1.3) (Figure 1.8).

Recent additions to the drug market include the COX-2 selective NSAIDs rofecoxib (33) and celecoxib (34) (Figure 2.8).[82 - 85]

Rofecoxib
(33)

Celecoxib
(34)

Bis-substituted Furan-2(5 H)-one
(35)
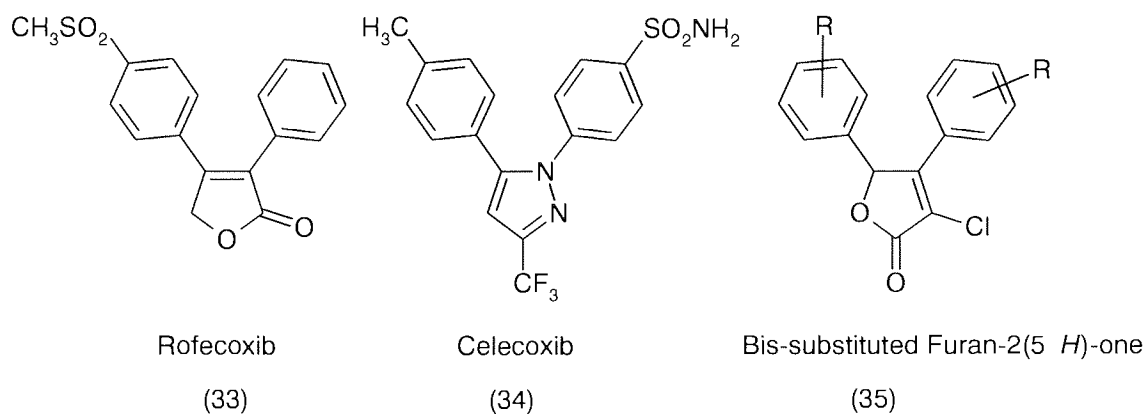
Figure 2.8 The differences in structure between rofecoxib and celecoxib (two recently marketed COX-2 selective inhibitors) and a 4,5-bis-arylated furan-2(5H)-one.

Addition of an aromatic molecule to the five position on the furan-2(5*H*)-one ring followed by a second addition at the C4 Michael position would provide molecules (35) that bear a resemblance to currently marketed compounds, suitable for entering into a COX-2 selective NSAID screening programme (Figure 2.9). After initial screening, further adaptation of the two rings could possibly result in a COX-2 selective antagonist lead structure.

(28)   (36)   (35)
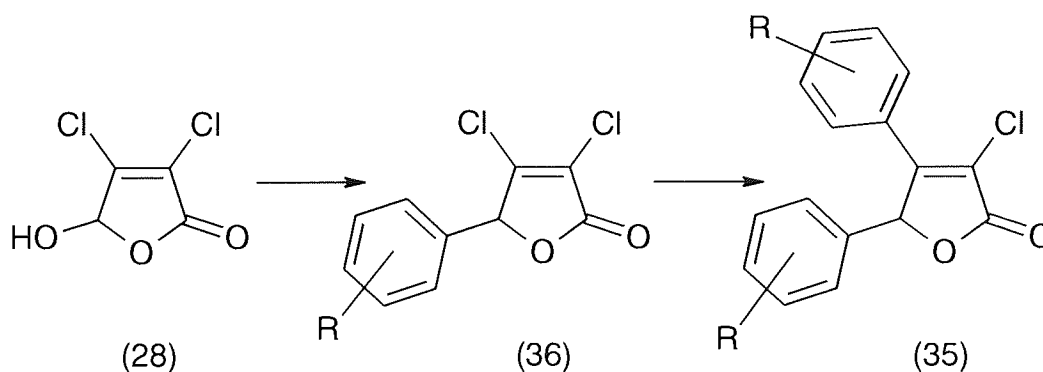
Figure 2.9 The attempted synthesis of 4,5-bisarylated furan-2(5H)-ones

Substitution at the five position can be attempted by using a method published in 1961 by Semonský *et al.* In this method, aluminium chloride is used to convert mucochloric acid (28) to 3,4-dichloro-5-phenylfuran-2(5*H*)-one (37), using benzene as the reactant

and solvent.[86] Repetition of the experiment produced a white crystalline solid (Figure 2.10) (see section 7.2.2).
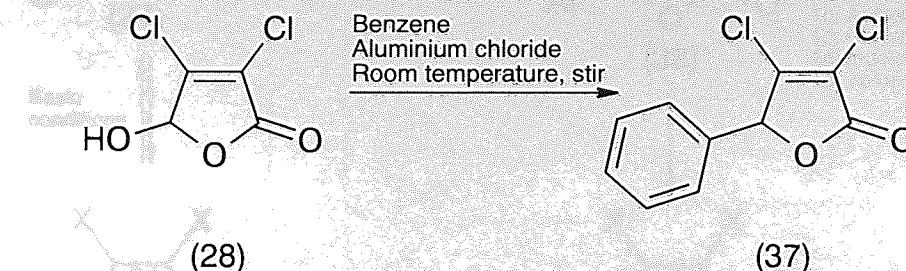
Figure 2.10 The conversion of mucochloric acid to 3,4-dichloro-5-phenylfuran-2(5H)-one

Detection of the 3,4-dichloro-5-phenylfuran-2(5H)-one (37) was attempted by APCI+ (atmospheric pressure chemical ionisation) mass spectroscopy and the molecular ion peak although small, was detectable. Confirmation of the synthesis was achieved by proton and carbon nuclear magnetic resonance studies (see section 7.2.2).

Repetition of the reaction, replacing mucochloric acid (28) with mucobromic acid (29) produced an orange solid. Analysis by nuclear magnetic resonance studies as above confirmed the structure as being the expected 3,4-dibromo-5-phenylfuran-2(5H)-one (see section 7.2.2).

It was decided to attempt the same method with other suitable aromatic electrophiles (Figure 2.11). The differing electrophiles had to be split into two sets of reactions. Initially, all the electrophiles that could act as both a reagent and solvent were attempted (using two different methods depending on the reactivity of the electrophile) (see section 7.2.2, Methods A & B). Then, interest was switched to concentrate on one remaining electrophile, indole, using 1.5 equivalents of the electrophile in 1,2-dichloroethane as the solvent (see section 7.2.2, Method C).

X = Cl / BR

Room temperature
Section 7.2.2 Method A / B / C

(27)

Basic conditions

(28)

Ar = aromatic ring

1a: Benzene
1b: Anisole
1c: Chlorobenzene
1d: Indole
1e: Toluene

(38)

(39)

2a: o-Xylene
2b: m-Xylene
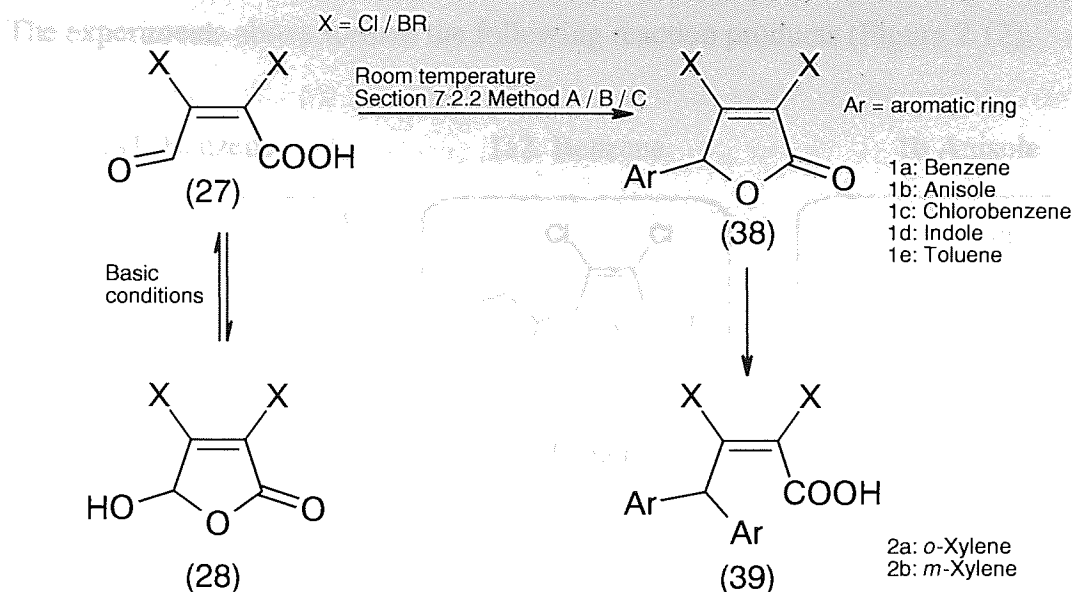
Figure 2.11 The reaction sequence followed for the reaction between different aromatic molecules and the two mucohalic acids.

The results for the electrophilic substitution reactions are:

Entry	X	Aromatic group	Yield
1a1	Br	Benzene	61%
1a2	Cl	Benzene	69%
1b	Cl	Anisole	76%
1c	Cl	Chlorobenzene	Unreactive
1d	Cl	Indole	30%
1e	Br	Toluene	69%
2a	Cl	o-Xylene	88%
2b	Cl	m-Xylene	85%

The experiments above formed the following reaction products (Figure 2.12):

1a1. Benzene   1a2. Benzene   1b Anisole

1d. Indole   1e. Toluene   2a. o-Xylene

2b. m-Xylene
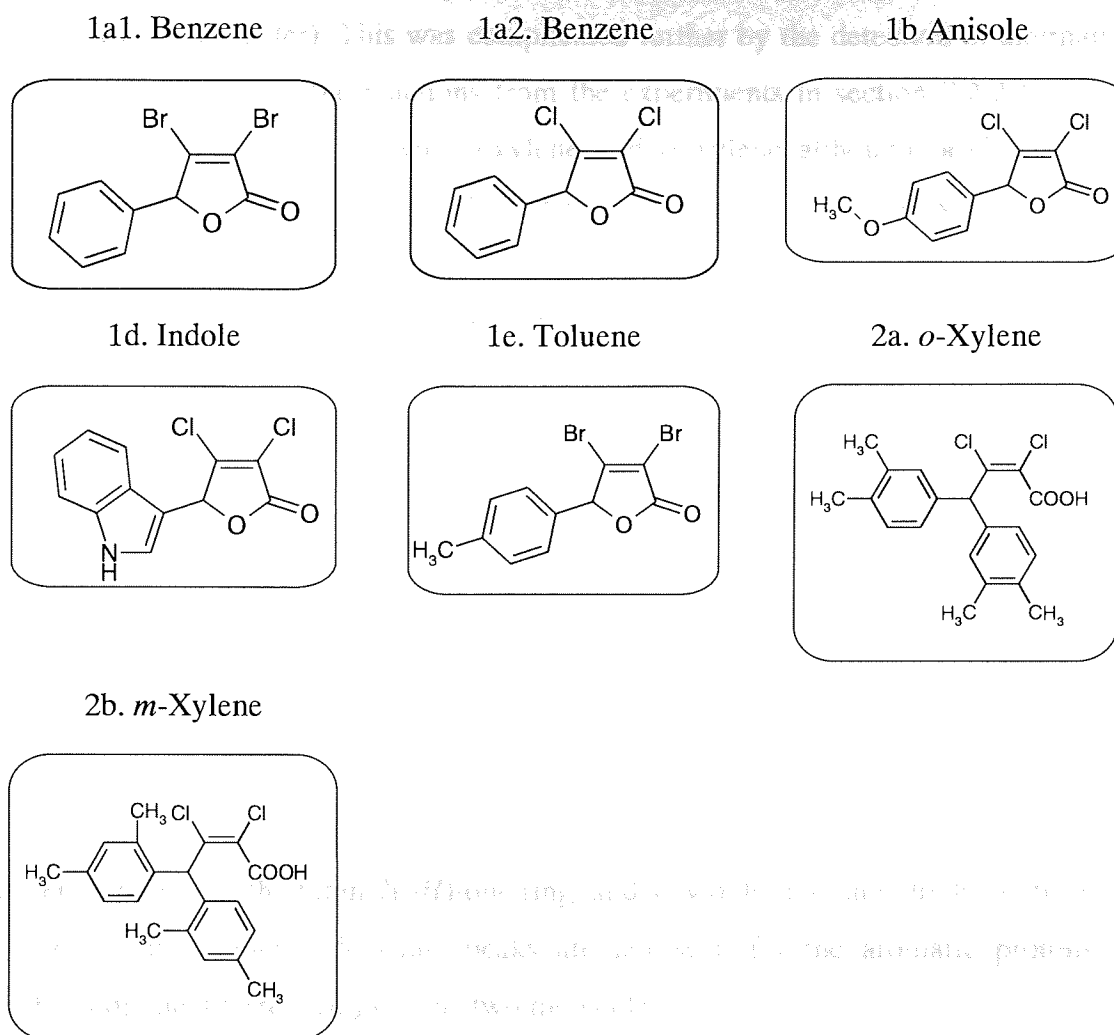
Figure 2.12 The structures of the seven reaction products formed by electrophilic aromatic substitution reaction at the C5 centre.

Initially, the results were to be simply divided into two groups indicating the success of the reaction (i.e. the detection of the expected peaks in the nuclear magnetic resonance spectrometer). This was complicated further by the detection of alternative compounds for two of the reactions from the experiments in section 7.2.2 (39) (see Figure 2.11). The reactions with o-xylene and m-xylene although producing pure products, did not appear to form the expected product. The proton NMR spectrum produced, appeared to be bis-substituted by the electrophile due to the integration of the two methyl groups on the aromatic ring being twice the value expected. APCI+ mass spectroscopy performed on the two molecules suggested that 4,5-bis-substitution may have occurred due to the correct molecular ion peaks being present and the presence of a single chlorine isotopic pattern. This would, it seemed initially, have provided a simple way to produce the desired 4,5 bis-substituted furan-2(5H)-ones, provided that the aromatic groups required at the four and five positions are the same.

Unfortunately, the proton and carbon nuclear magnetic resonance spectra could only confirm this predicted structure if the two additional aromatic rings are considered to be magnetically identical. This is unlikely as the two rings would be attached at different points on the furan-2(5H)-one ring, and so would undoubtedly have different magnetic environments. Separate peaks are not seen for the aromatic protons or carbons on the different rings in the two molecules.

The best method for the unequivocal determination of the structure is by x-ray crystallography. An attempt was made to re-crystallise a sample of each of the two products from ethanol. Only the m-xylene product produced crystals suitable for crystallographic analysis and was submitted. During data collection, the crystal appeared to degrade, giving only a partial set of data. This problem was initially addressed by preparing new crystals and analysing one immediately. This would hopefully prolong the life of the crystal during data collection.

Although submitting for x-ray analysis immediately after re-crystallisation preserved the life of the crystal, degradation still occurred during data collection. This problem was finally overcome by collecting five different sets of data, with each data set

beginning just before the crystal degraded during the previous data collection. Combination of these data sets, using a scaling factor, produced one complete set of data for analysis.

The x-ray data suggested that the furan-2(5*H*)-one had not formed the 4,5-bis substituted form as indicated by the APCI+ mass spectroscopy data. What had in fact happened was an opening of the furan-2(5*H*)-one ring and the substitution by the aromatic ring at the C5 centre twice. The crystal structure of the resulting product is shown in Figure 2.13.

Figure 2.13 The structure of the product of mucochloric acid and m-*xylene identified by x-ray crystallography.*

X-ray crystallography gives us a definitive structure for the product, which crystallised out from ethanol. The structure fits the NMR data, as the two aromatic rings are magnetically identical. The only part of the molecule, which is not evident in the proton NMR spectrum, is the carboxylic acid group. Even when re-crystallised product is analysed with an increased scan time, the carboxylic acid group cannot be seen. The major anomaly occurs with the APCI+ mass spectroscopy data. It is known that some furan-2(5H)-ones do not show up in the mass spectrometer using the APCI+ mass spectroscopy technique. The lack of a peak for the resulting product is therefore not unexpected. What is unusual, however, is the presence of the correct peak, with the correct isotopic pattern, if 4,5-bis substitution had occurred. Re-submission for electron impact mass spectroscopy of the m-xylene product did produce the correct molecular ion peak, confirming the structure predicted by the proton and carbon NMR and x-ray crystallography data (see section 7.2.2).

This means that the 4,5-bis-substituted furan-2(5H)-one (40) is not attainable by this method. The sequence with highly reactive electron rich aromatic systems is pushed to bis-substitution at the 5 position (41) despite the suitability for attack at the four position due to the presence of a Michael system (Figure 2.14). This has resulted in the formation of an open-chain molecule (41), suitable for further reaction from the closed-chain furan-(2H)-ones.

Further reaction of the formed bis-substituted product into a bicyclic closed-ring system (42) is not seen in the proton or carbon nuclear magnetic resonance spectrometer (Figure 2.14). Any attempt to identify the bicyclic product by reaction with a nitrogen nucleophile and subsequent analysis by APCI+ mass spectroscopy was also unsuccessful (43). The reaction between furan-2(5H)-ones and nucleophiles at the C4 Michael system is explored more fully in section 2.2.1.
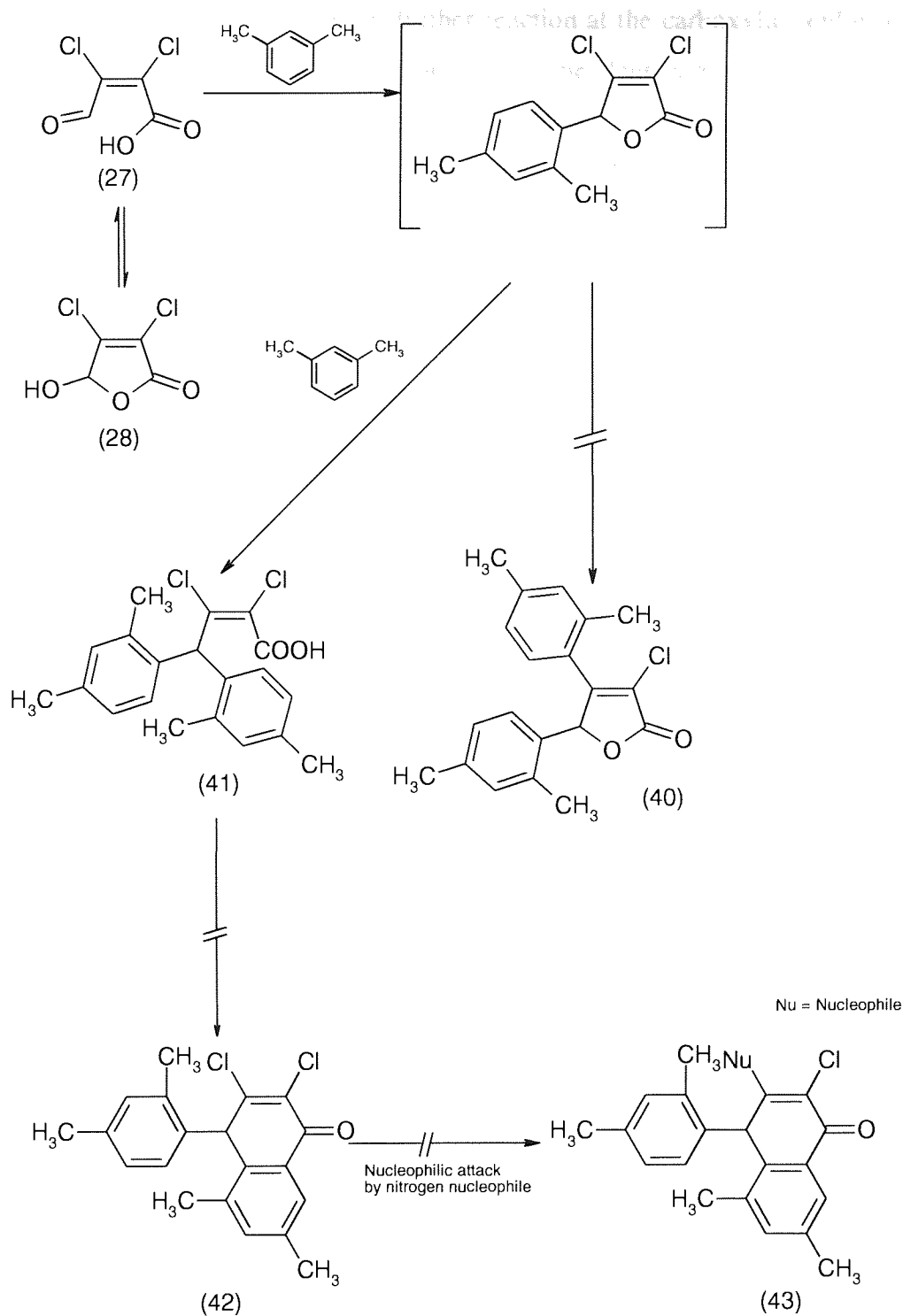
Figure 2.14 The second substitution occurs at the C5 and not the C4 carbon centre as expected. Further cyclisation is not detectable by nitrogen nucleophilic attack and subsequent detection by APCI+ mass spectroscopy.

These molecules may be suitable for further reaction at the carboxylic acid group for entry into an antihistamine (H_1) screening programme. This is more evident when the structure of the synthesised molecules is compared to the general structure of H_1 antagonists (45) (Figure 2.15).[87]

(40)

Reaction at the carboxylic acid group with suitable reagent

Ar = aromatic group
The carbon chain may be saturated, branched or part of a ring
NR1R2 is generally a tertiary amino group

(44)   Compared to   General structure
(45)
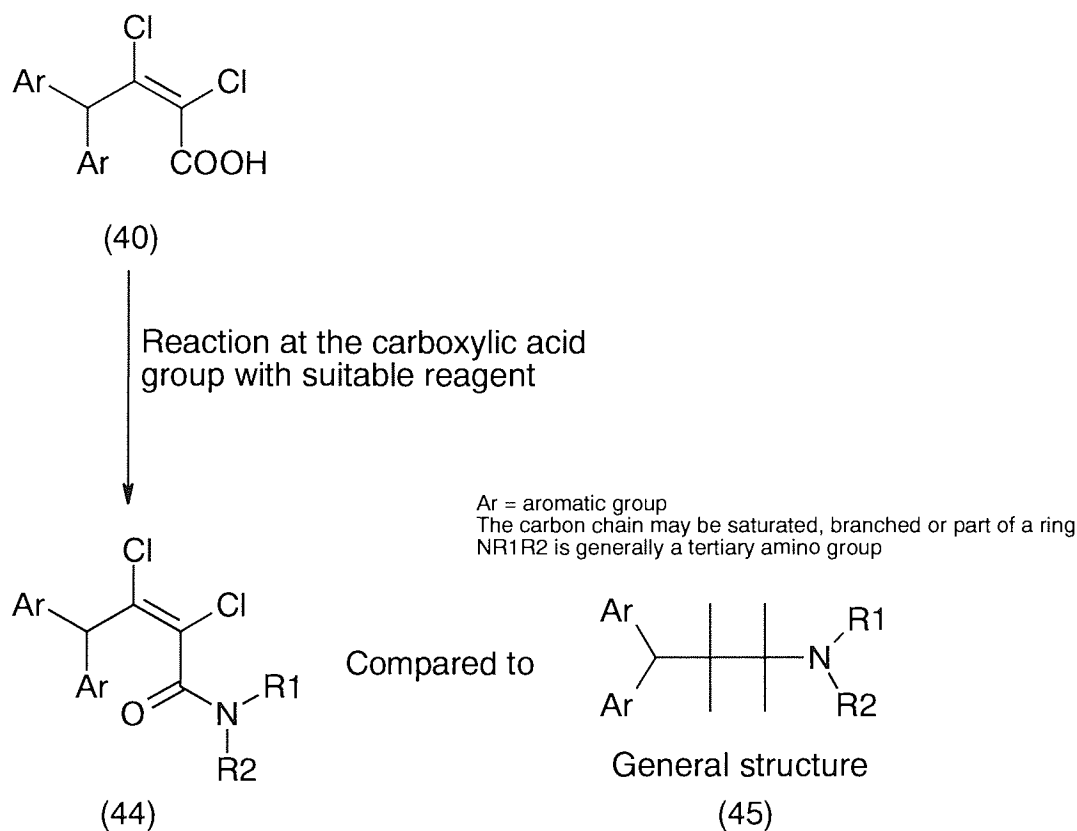
Figure 2.15 On further reaction, the open chain molecules formed above (44) are similar in structure to the general antihistamine (H_1) antagonist structure (45).

2.1.2.3 The effect of time on electrophilic aromatic substitution patterns

The reactions between mucochloric acid and chlorobenzene and mucochloric acid and toluene were examined with respect to time of reaction. The reaction was repeated and this time the length of reaction was increased from 24 hours to over 48 hours. No difference was seen for the detection of the expected product for the reaction with chlorobenzene, but the reaction with toluene progressed from the 5-monosubstituted product to the 5,5-bis-substituted (open-chain) product (see section 7.2.2, Method D).

This suggests that the bis-substitution reaction proceeds via a ring-closed intermediate to the ring-open 5,5-bis-substituted product, which does not re-close (Figure 2.14).

In summary, a method has been developed for the formation of 5-arylated furanones suitable for further reactions. These building blocks could be used in the first axis of a combinatorial library, due to the presence of another site for the attachment of suitable substituents. In addition, some bis-arylated open-chain furan-2(5H)-ones have been identified. Unfortunately, the desired 4,5-bis-substituted furan-2(5H)-ones have not been possible due to the bis-substitution at the C5 centre of the furan-2(5H)-one ring. The bis-substituted furan-2(5H)-ones will be revisited in chapter five.

2.1.2.4 Other reactions of the aldehyde group

In his paper, Mowry managed to reduce mucochloric acid directly to 3,4-dichloro-2(5H)-furanone (46) using aluminium isopropoxide.[81] This reaction proceeded with 52% yield (Figure 2.16). This was a useful new reaction, as previously the furanone had only been available through the reduction of mucochloryl bromide with tin and hydrochloric acid.
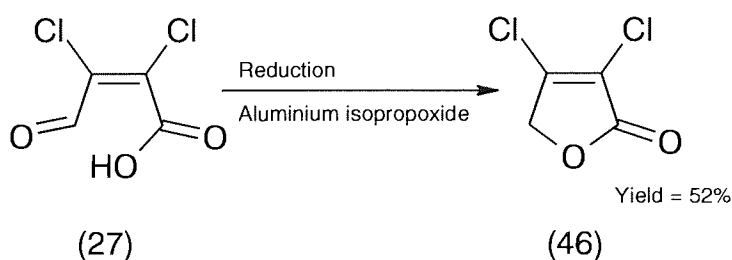
Figure 2.16 The reduction of mucochloric acid using aluminium isopropoxide.

Importantly, he found that the reaction with phenol did not occur at the aldehyde group but replaced the α-chlorine with a phenoxy group (47) (Figure 2.17). This pattern was extended to other phenols such as hydroquinone monomethyl ether, *p*-chlorophenol and β-naphthol.[81]
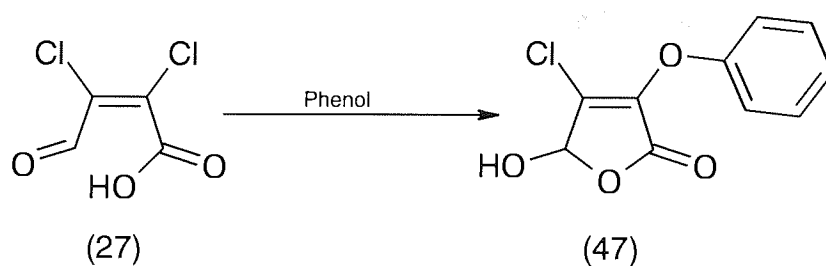
Figure 2.17 The substitution of the α-chlorine by phenol.

Mucochloric acid was found to form a semicarbazone and phenylhydrazone in the expected fashion and on heating in acetic acid solution, the products formed 4,5-dichloro-3-pyridazone (48) and 4,5-dichloro-2-phenyl-3-pyridazone (49) respectively in good yields (Figure 2.18).
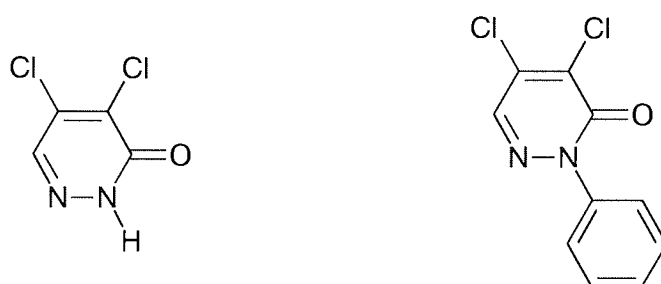
4,5-dichloro-3-pyridazone (48)   4,5-dichloro-2-phenyl-3-pyridazone (49)

Figure 2.18 The two products obtained from reacting mucochloric acid with semicarbazide and phenylhydrazine respectively.

It was found that the pyridazone was the only product isolated when two moles of mucochloric acid were condensed with one mole of hydrazine to form an azine. Finally, Mowry stated that although previously the aldehyde group had been condensed with benzene to form α,β-dichloro-γ,γ-diphenylcrotonic acid in the presence of aluminium chloride, the use of sulphuric acid or benzenesulphonic acid catalysts gave only mucochloric anhydrides.[81]

2.1.3 Reactions of the pseudo-acid group (closed-ring furanones)

In his other paper on mucochloric acid, Mowry examined the characteristics and reactions of the pseudo-acid group (28). The structure of 3,4-dichloro-5-methoxy-2(5*H*)-furanone (50) was studied and the molecule was found to exist in the cyclic form (Figure 2.19) due to its lack of reaction with hydroxylamine.[80]

(50)
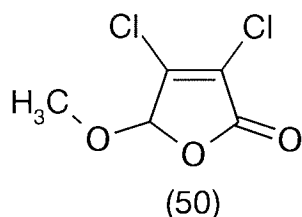
Figure 2.19 The methoxy ester of mucochloric acid is in the cyclic form.

This characterisation of the methyl ester as the pseudo structure was been found to be accurate by examination of the ultraviolet absorption structure. It was found that the spectrum has no absorption in the 260-280 mμ region, indicating the absence of a chromophore containing both a carboxyl and a carbonyl group conjugated with a double bond. It has also been found that mucochloric acid itself in alcoholic solution exists mainly in the cyclic form. Within this group of reactions, he also prepared the vinyl, n-amyl and n-dodecyl esters.[80]

Also within this group of reactions, Mowry examined the reactions of mucochloric acid with phenyl isocyanate and thionyl chloride. He found that refluxing mucochloric acid (28) with phenyl isocyanate in benzene gave mucochloric carbanilic anhydride (51). This produced a relatively stable, white solid in 82% yield (Figure 2.20).[80]
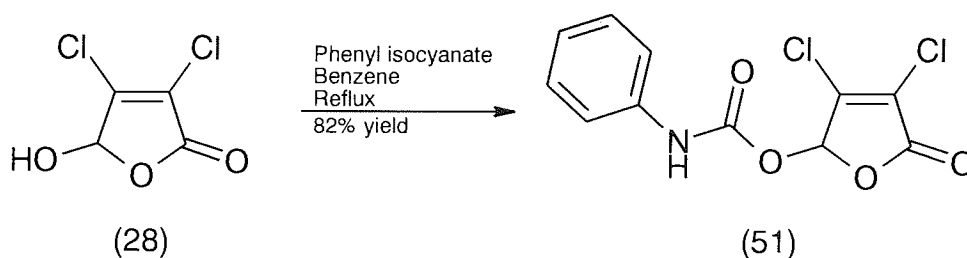
Figure 2.20 The reaction of mucochloric acid with phenyl isocyanate.

Mucochloric acid reacted much more slowly with thionyl chloride than ordinary acids do, but did react in the end to produce a 51% yield of mucochloryl chloride along with 26% of the α-isomer of the anhydride. It was found to possess less of a lachrymatory odour than normal acid chlorides of a similar volatility. Mowry stated that the compound appeared, by absorption data, to have a 3,4,5-trichloro-2(5*H*)-furanone (52) structure (Figure 2.21).[80]
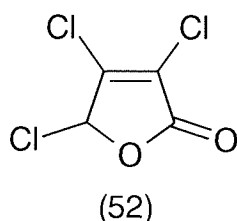
Figure 2.21 The structure of 3,4,5-trichloro-2(5H)-furanone.

Finally in this section, Mowry dehydrated the acid to its anhydride. This was achieved by refluxing in benzene or chlorobenzene with a trace of sulphuric or benzenesulphonic acid. This produced two forms of the anhydride, an α-isomer (which melts at 141-143 °C) and a β-isomer (which melts at 180 °C). The ultraviolet spectra of both isomers indicated that both exist in the cyclic form. The structures were presumed to be racemic and *meso* modifications of bis-(3,4-dichloro-2(5)-furanonyl) ether, which has two asymmetrical carbon atoms.[80]

2.2 Formation of 5-alkoxyfuran-2(5*H*)-ones

2.2.1 Reaction with methanol and the detection of the reaction products

Initially, it was decided to try and replicate the formation of the methoxy esters of both mucochloric and mucobromic acids (53) (Figure 2.22) (see section 7.3.2, Method A).[80, 88, 89] Although success appeared to have been achieved on analysis by thin layer chromatography in a mixture of 50% ether and 50% petrol ether 40-60, no correct molecular ion peak could be detected in the mass spectrometer under the APCI+ technique. As the APCI+ spectra did not show the M+ peak for either compound, the samples were resubmitted for EI (electron impact) mass spectroscopy. Peaks were seen in these spectra for the expected products with the loss of either the halogen atom or the methoxy group. As expected, a small peak was seen in the electron impact spectrum around the M+ region.
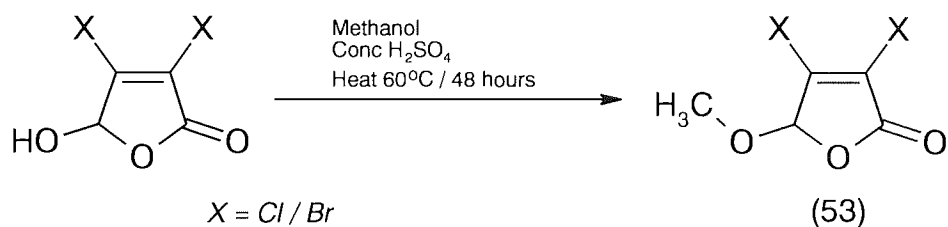
Figure 2.22 The formation of 5-methoxy-2(5H)-furanones.

Evidently, a better method for the characterisation would be needed if an entire series of 5-alkoxy products were to be synthesised. Ideally, this method would use APCI+ mass spectroscopy as this could positively identify the molecular ion peak. One of the easiest types of molecules to detect with APCI+ mass spectroscopy are those containing a nitrogen. It is know from studies by Fariña that the halogen atom at the four position of furanones is susceptible to nucleophilic attack by nitrogen nucleophiles as it acts as a Michael acceptor.[90 - 92]

Initially, work started with the five position to investigate which other alcohols formed alkoxy derivatives and which did not. A similar method to the above method

in forming the 5-methoxy derivatives was employed. Due to the ability of APCI+ mass spectroscopy to detect the molecular ion peak for a reaction product containing a nitrogen atom, and the inability to detect the 5-alkoxyfuranones, it was decided that after the reaction of mucochloric acid (28) with an alcohol to the desired 5-alkoxy product (54), a sample would be further reacted with a suitable nitrogen nucleophile in N,N-dimethylformamide (55). Samples could then be submitted directly, diluted in methanol, without any purification, for detection of the molecular ion peak (Figure 2.23). This method was also used in the previous section in an attempt to detect a possible bicyclic reaction product (see section 2.1.2.2).
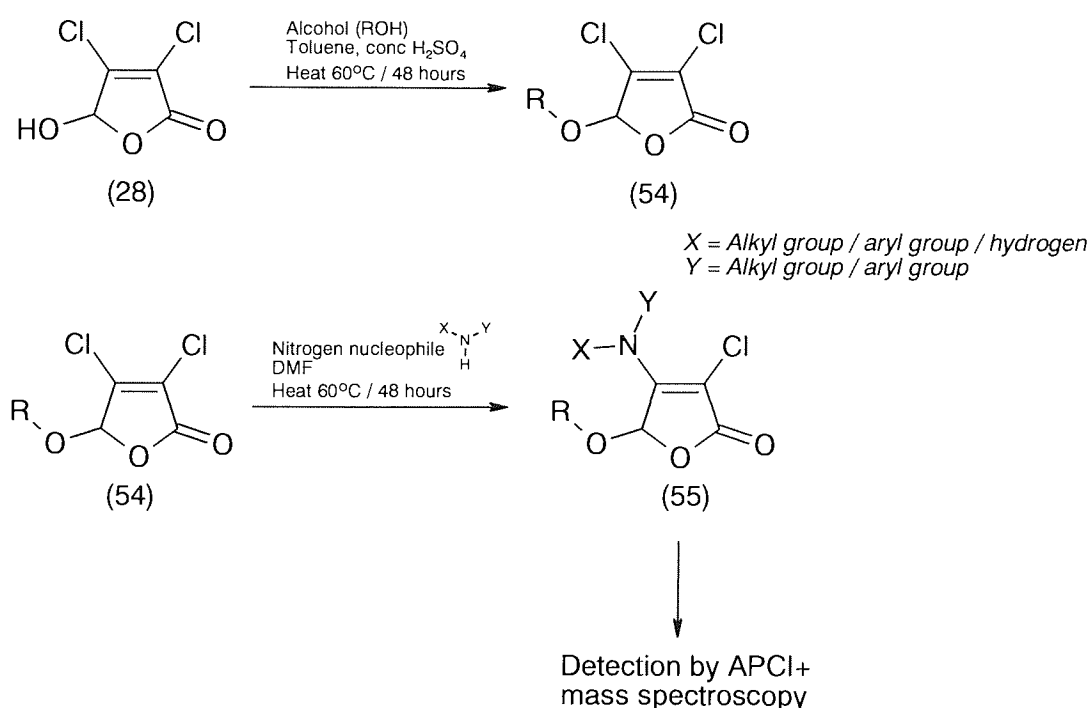
Figure 2.23 The identification of suitable alcohols for use in forming 5-alkoxy building blocks by the use of nitrogen nucleophilic attack on the Michael system.

Mechanistically, the nucleophilic attack on the four position on the furan-2(5*H*)-one ring proceeds as follows (Figure 2.24):
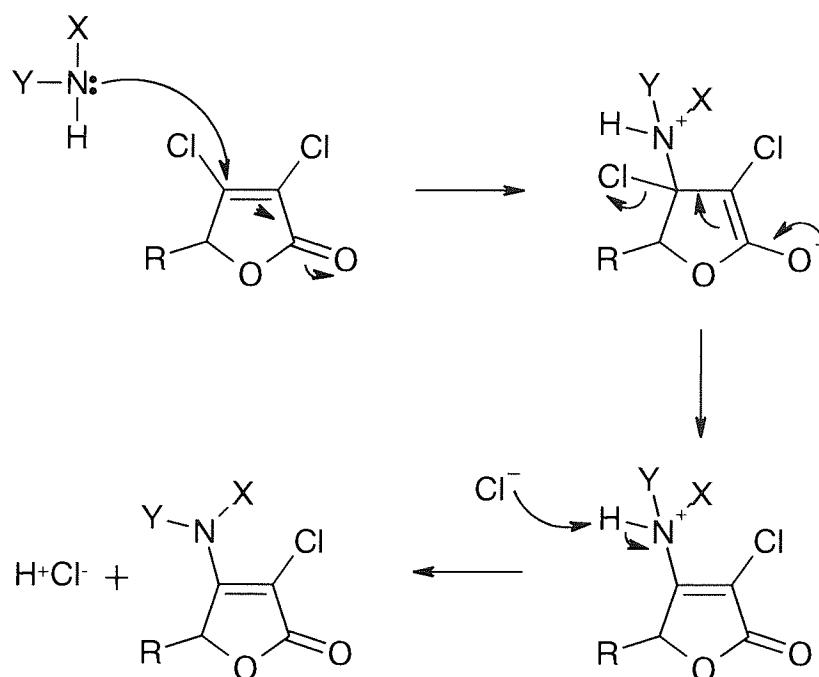
Figure 2.24 The C4 centre is more susceptible to nucleophilic attack than the C3 centre due to the presence of a Michael system.

2.2.2 Further reactions with alcohols and small library formation

In order to attempt construction of a combinatorial library, two suitable reaction series at different points on the molecule will be required. From the above, it seems logical to commence the assembly of a combinatorial library using pseudo-ester formation with alcohols at the five position, and nitrogen nucleophilic attack at the four position. Figure 2.25 shows the reaction scheme that was followed in the assembly of a small combinatorial library. The four nitrogen nucleophiles selected for the experiment were benzylmethylamine, 1-benzylpiperazine, 2,6-dimethylmorpholine and pyrrolidine. These were selected as they were all liquids, and so easier to manipulate, and they all gave very clear APCI+ mass spectra after reaction with 3,4-dichloro-5-methoxyfuran-2(5H)-one (50). The library was assembled as follows; All twenty-four alcohols were reacted with the four amines using mucochloric acid (15) as the core furan-2(5H)-one. A further selection of eight alcohols were reacted with the four amines using mucobromic acid (29) as the core furan-2(5H)-one and a final set of four alcohols

were reacted with the four amines using 4-chloro-5-hydroxyfuran-2(5*H*)-one (30) as the core furan-2(5*H*)-one (Figure 2.25) (see section 7.3.1).

Alcohols used
1) Benzyl alcohol
2) 4-Biphenylmethanol
3) Borneol
4) Cetyl Alcohol
5) 4-Chloro-3,5-dimethylphenol
6) *m*-Cresol
7) Cyclohexylmethanol
8) 2-Cyclohexylphenol
9) Cyclopentanol
10) Ethanol
11) Guaiacol
12) Menthol
13) Methanol
14) *m*-Methoxyphenol
15) 1-Nonanol
16) Pentanol
17) 2-Phenylethanol
18) 3-Phenylpropyl Alcohol
19) 3-Phenyl-2-propyn-1-ol
20) i-Propanol
21) Propargyl Alcohol
22) 3-(2-Pyridyl)-1-propanol
23) 1-Tetradecanol
24) Tetrahydrofurfuryl Alcohol

Figure 2.25 The reaction scheme followed for the assembly of a small combinatorial library and the subsequent characterisation by nitrogen nucleophilic attack and APCI+ mass spectroscopy.

The twenty-four alcohols were selected by hand in order to gain a reasonable diversity spread of structures, below a certain molecular weight (<250). Examples of long and short chain aliphatic alcohols were chosen along with aromatic and cyclic alcohols.

The alcohols chosen formed the following 5-alkoxyfuran-2(5H)-ones (Figure 2.26) (X = Br / Cl, Y = Br / Cl / H, Z = Cl / H).
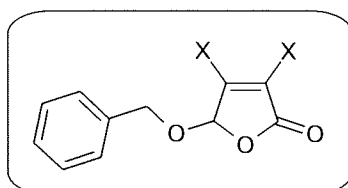
1. Benzyl alcohol	2. 4-Biphenolmethanol	3. Borneol
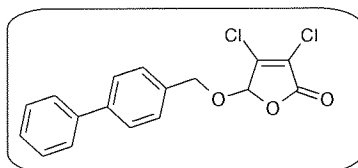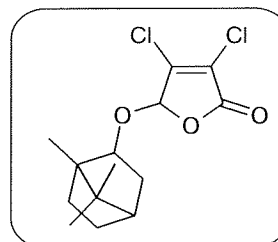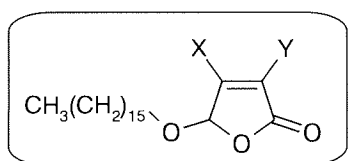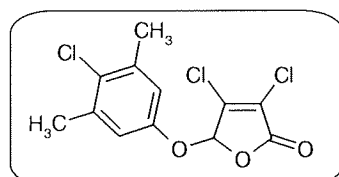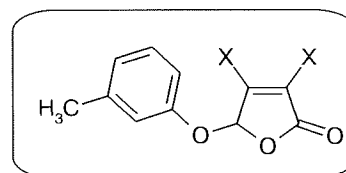
4. Cetyl alcohol	5. 4-Chloro-3,5-dimethylphenol	6. *m*-Cresol

7. Cyclohexylmethanol	8. 2-Cyclohexylphenol	9. Cyclopentanol

10. Ethanol	11. Guaiacol	12. Menthol

13. Methanol

14. *m*-Methoxyphenol

15. 1-Nonanol

16. 2-Pentanol

17. 2-Phenylethanol

18. 3-Phenylpropyl alcohol

19. 3-Phenyl-2-propyn-1-
ol

20. i-Propanol

21. Propargyl alcohol

22. 3-(2-Pyridyl)-1-
propanol

23. 1-Tetradecanol
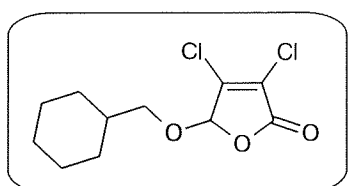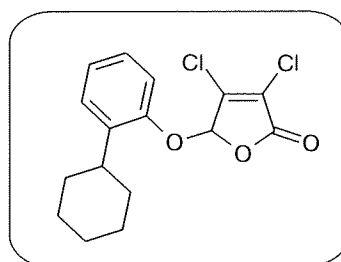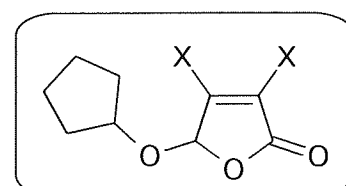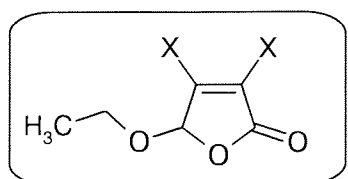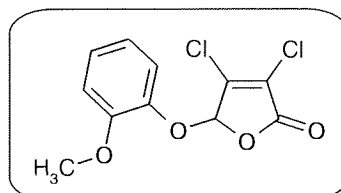
24. Tetrahydrofurfuryl
alcohol
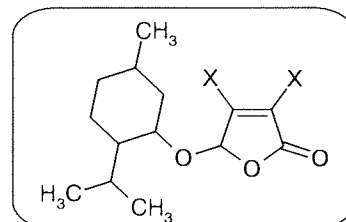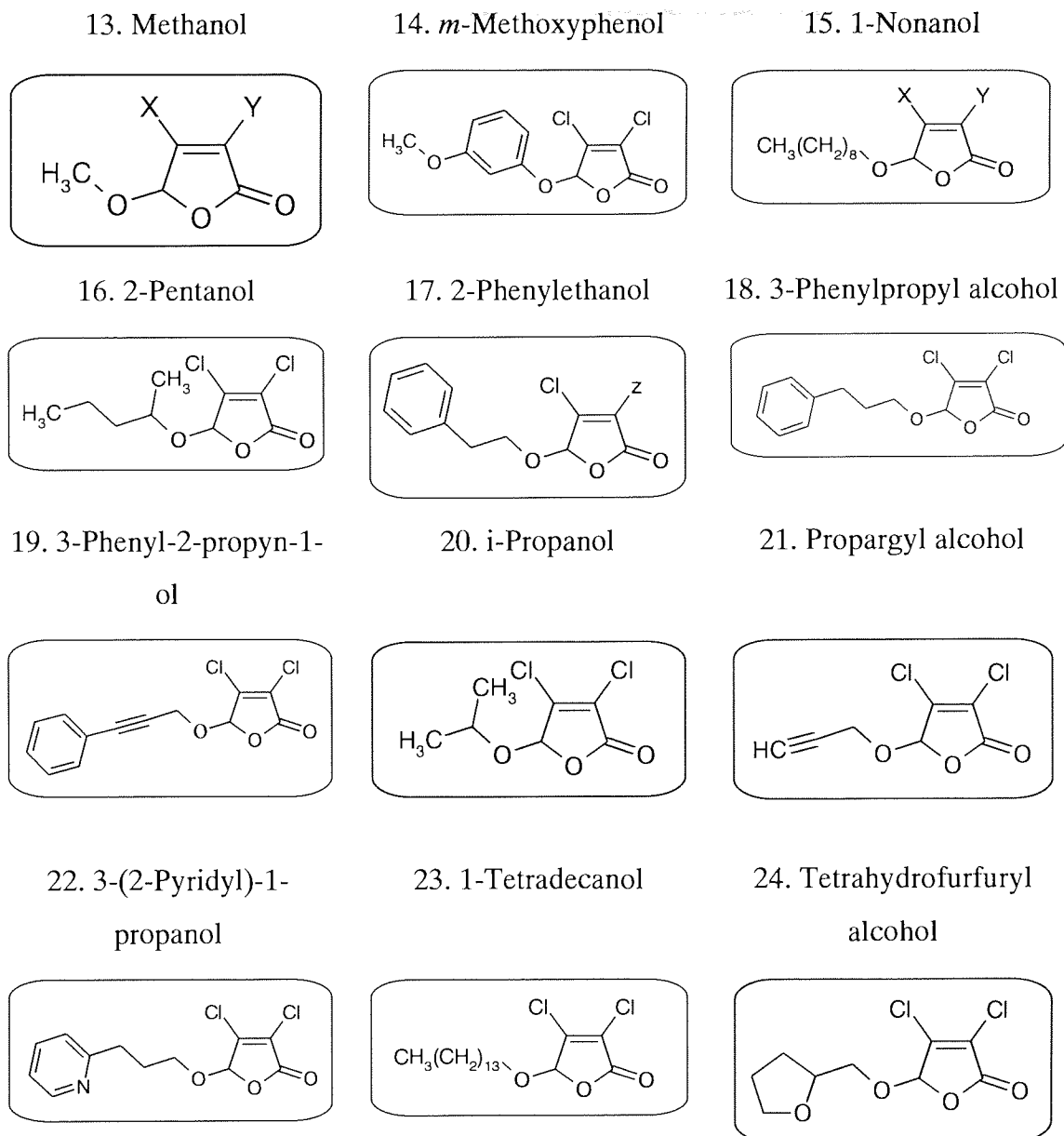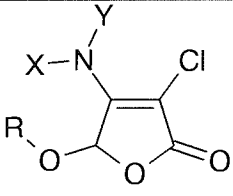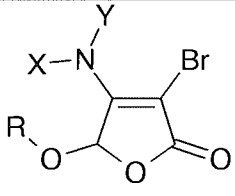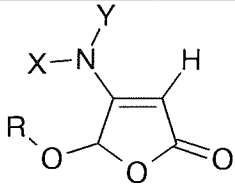
Figure 2.26 The structures of the twenty-four different 5-alkoxyfuran-2(5H)-ones possible.

The results from the APCl+ mass spectroscopic analysis of the 5-alkoxyfuran-2(5*H*)-one library attempted are depicted below (the four position code is 1 = benzylmethylamine, 2 = 1-benzylpiperazine, 3 = 2,6-dimethylmorpholine and 4 = pyrrolidine). A tick indicates the presence of the correct molecular ion peak in the APCl+ mass spectrometer and a cross denotes its absence.

	3-Position→	Chlorine				Bromine				Hydrogen			
5-Position ↓	4-Position →	1	2	3	4	1	2	3	4	1	2	3	4
1) Benzyl Alcohol		✓	✓	✓	✓	✓	✓	✓	✓				
2) 4-Biphenolmethanol		✓	X	✓	X								
3) Borneol		✓	✓	✓	✓								
4) Cetyl Alcohol		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5) 4-Chloro-3,5-dimethylphenol		✓	✓	✓	✓								
6) m-Cresol		✓	✓	✓	✓	✓	✓	✓	✓				
7) Cyclohexylmethanol		✓	✓	✓	✓								
8) 2-Cyclohexylphenol		✓	✓	✓	✓								
9) Cyclopentanol		✓	✓	✓	✓	✓	✓	✓	✓				
10) Ethanol		✓	✓	✓	✓	✓	✓	✓	✓				
11) Guaiacol		✓	✓	✓	✓								
12) Menthol		✓	✓	✓	✓	✓	✓	✓	✓				
13) Methanol		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
14) m-Methoxyphenol		✓	X	✓	X								
15) 1-Nonanol		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
16) 2-Pentanol		✓	✓	✓	✓								
17) 2-Phenylethanol		✓	✓	✓	✓					✓	✓	✓	✓
18) 3-Phenylpropyl Alcohol		✓	✓	✓	✓								
19) 3-Phenyl-2-propyn-1-ol		✓	X	✓	X								
20) i-Propanol		✓	✓	✓	✓								
21) Propargyl Alcohol		✓	✓	✓	✓								
22) 3-(2-Pyridyl)-1-propanol		✓	X	✓	X								
23) 1-Tetradecanol		✓	✓	✓	✓								
24) Tetrahydrofurfuryl Alcohol		✓	✓	✓	✓								

A list of twenty-four alcohols, which have been successfully included in a small combinatorial library, is now available. Further development of the four position arm

of the library in the next chapter will greatly enhance the potential for lead structure discovery.

2.3 Cancer studies

2.3.1 Furan-2(5*H*)-ones as anticancer drugs

Due to the distressing nature of the disease and the large number of people affected by it (as patients or relatives of sufferers), many research groups are searching for suitable molecules to enter into anticancer screening programmes.

Many anticancer drugs are small organic molecules, commonly containing a halogen atom, for example, chlorambucil (56) or cyclophosphamide (57) (Figure 2.27).[68]

Chlorambucil (56)   Cyclophosphamide (57)
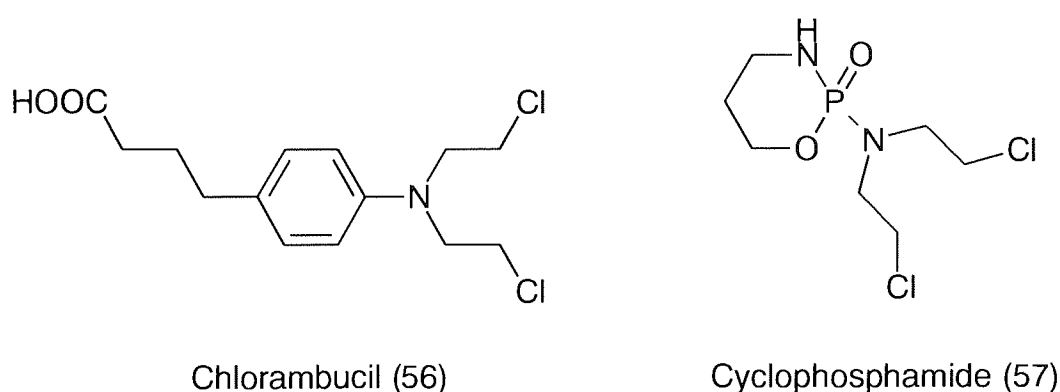
Figure 2.27 The structure of chlorambucil and cyclophosphamide – two commercially available anticancer drugs.

In the quest for suitable reactions on the furan-2(5*H*)-one molecule for inclusion into a combinatorial library, a selection of 5-alkoxyfuran-2(5*H*)-ones has been assembled for further reaction. Some of these molecules, being small organic molecules with two halogen atoms, are suitable for inclusion into an anticancer screening programme.

There are some known furan-2(5H)-ones which possess a degree of antitumour activity. Basidalin (58) (Figure 2.28) which has shown antitumour activity against L1210 mouse leukaemia[93] and the Luffariolides A-E have already show a degree of antitumour activity in the introduction (see section 1.2.2).[47 - 49] Penicillic acid (59) (Figure 2.28), another furan-2(5H)-one, is reported to have cytotoxic, cardiotoxic and carcinogenic activity.[94]

(58)   (59)
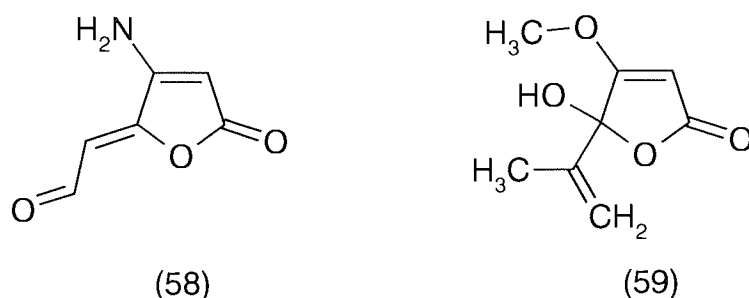
Figure 2.28 The structure of basidalin and penicillic acid.

A selection of the smaller 5-alkoxyfuran-2(5H)-ones were sent away to be tested against two cell lines and the IC_{50} value recorded. All the *in vitro* and *in vivo* anticancer screening was performed by D. Kinchington at the Department of Virology, St Bartholomew's and the Royal London School of Medicine and Dentistry, 51 – 53 Bartholomew Place, West Smithfield, London, EC1A 7BE. The *in vitro* cytotoxic assays were conducted using murine carcinoma cell lines (MAC13 and MAC16). The procedure followed is summarised below.[95]

The culture medium used was RPMI 1640 containing hepes, glutamine, antibiotics and 5% foetal calf serum for MAC13 cells and 10% foetal calf serum for MAC16 cells. On day 0, media from 250 ml flasks containing either 70% confluent MAC13 or MAC16 cells, were poured off and the cells washed with 10 ml PBS. 5 ml of versene was added to the flasks for three minutes. The detached cells were pipetted into plastic universals and spun down for five minutes at 1100 rpm. The media were poured off and 10 ml of fresh culture media was added to the cells. The cells were counted using the trypan blue exclusion method using plastic Kova counting chambers. The MAC13 and MAC16 cells were re-suspended in appropriate volumes and were seeded at 0.5 x

10^4 and 2 x 10^4 / 200 µl respectively in flat bottomed 96 well plates. The compounds were dissolved in dimethyl sulphoxide at a concentration of 100 mmol. Dilution series from 10^4 to 10^9 mol were made so that each compound was tested at six concentrations and in triplicate. 5-Fluorouracil (5-FU), a known anticancer agent, was used as a control and tested in the 20-0.02 µM range. Plates were then incubated at 37°C, 5% CO_2 for three days. Each compound was tested on at least two separate occasions. On day three, 20 µl of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (7.5 mg MTT / ml PBS) was added to each well and the plates were left to incubate for a further two hours.

The assay works by utilising the ability of the cells to reduce MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product. The formazan product can be measured spectrophotometrically following solubilization. The supernatant was carefully removed and acidified isopropanol containing 10% Triton-X100 was added to each well. Each plate was then agitated for ten minutes at 800 rpm on a plate shaker. All plates were then read, within fifteen minutes, on an Anthos AW200 plate reader at 540 nm with a reference wavelength of 590 nm.[95, 96]

The following table shows the 5-alkoxyfuran-2(5H)-ones tested along with the results obtained.
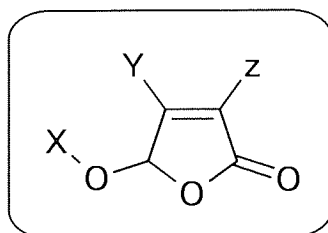
Entry	X	Y	Z	MAC13[*]	MAC16[*]
1	Methyl	Br	Br	50	70
2	Ethyl	Br	Br	6	8
3	Methyl	Cl	Cl	6	7
4	Ethyl	Cl	Cl	8	16
5	Benzyl	Br	Br	7	16
6	Menthol	Br	Br	3	40
7	i-Propanol	Cl	Cl	5	20

[*] = IC_{50} in µM

The above results can be combined with some results obtained by colleagues working on similar small furan-2(5H)-one molecules. These results were focused around furan-2(5H)-ones with increasing group size at the five position.[95]

These results are as follows:
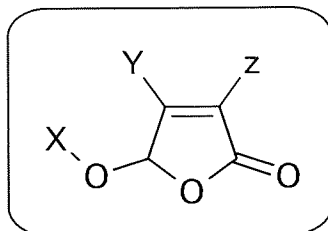
Entry	X	Y	Z	MAC13[*]	MAC16[*]
8	Vinyl	Cl	Cl	20	80
9	Allyl	Cl	Cl	8	50
10	Propargyl	Cl	Cl	2	3
11	n-Butyl	Cl	Cl	3	3
12	n-Hexyl	Br	Br	4	4
13	n-Nonyl	Cl	Cl	20	30
14	Dodecyl	Cl	Cl	20	40

[*] = IC_{50} in μM

Combination of the two sets of results help to identify the optimum length of 5-alkoxy side chain for *in vitro* activity of 5-alkoxy-3,4-dihalogenatedfuran-2(5H)-ones.

Entry	5-Alkoxy chain length (Number of C atoms)	Activity[*]	
		MAC 13	MAC16
1	1	50	70
2	2	6	8
3	1	6	7
4	2	8	16
7	3	5	20
8	2	20	80
9	3	8	50
10	3	2	3
11	4	3	3
12	6	4	4
13	9	20	30
14	12	20	40

[*] = IC_{50} in μM

As the chain value increases up to a maximum value of 4 carbon atoms, the *in vitro* activity improves. Increasing the chain length further increases the IC_{50}. It appears that the optimum 5-alkoxyfuran-2(5H)-one chain length is around 4 carbon atoms long for both MAC 13 and MAC 16 cell lines (Figure 2.29 and Figure 2.30).

Graph to show the optimum 5-alkoxy chain length for dihalogenated furan-2(5H)-ones against MAC13 cell lines
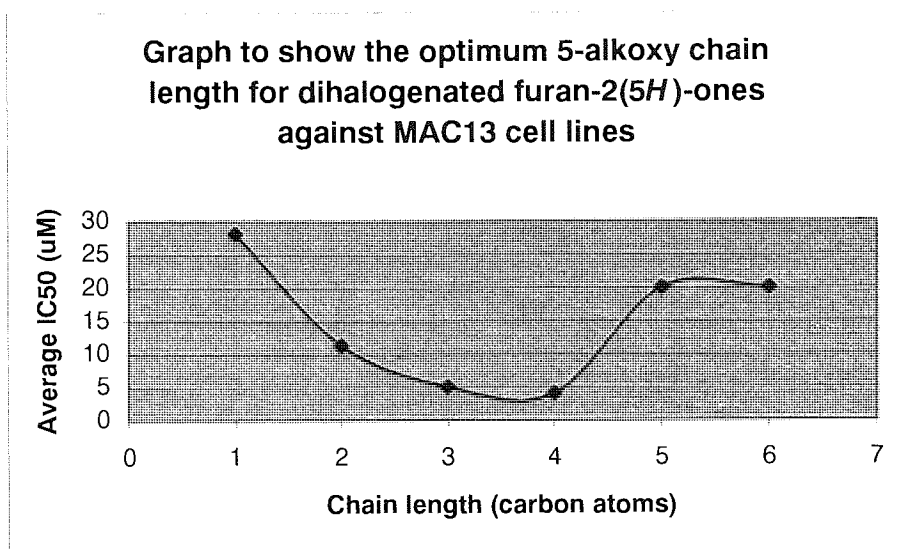
Figure 2.29 A graphical representation of the optimum 5-alkoxy chain length of dihalogenated furan-2(5H)-ones from averaged MAC13 in vitro studies.

Graph to show the optimun 5-alkoxy chain length for dihalogenated furan-2(5H)-ones against MAC 16 cell lines
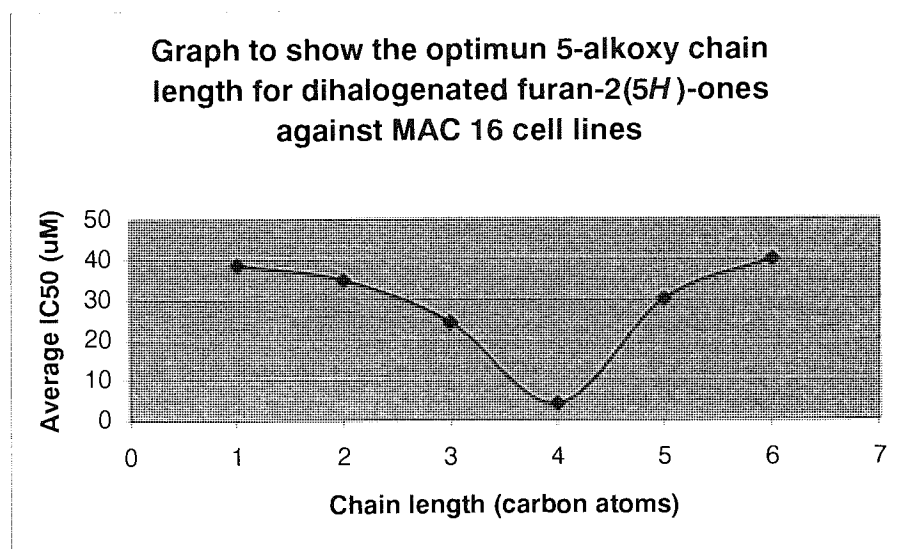
Figure 2.30 A graphical representation of the optimum 5-alkoxy chain length of dihalogenated furan-2(5H)-ones from averaged MAC16 in vitro studies.

In vitro anticancer studies were also performed on four of the 5-arylfuran-2(5*H*)-ones. The assay was performed as before on murine carcinoma cell lines MAC13 and MAC16.

The following table shows the 5-arylfuran-2(5*H*)-ones tested along with the results obtained. As before, each IC_{50} value is a mean of three results.
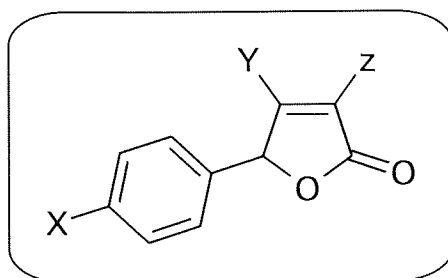
Entry	X	Y	Z	MAC13[*]	MAC16[*]
1	H	Cl	Cl	50	50
2	H	Br	Br	30	40
3	Me	Br	Br	30	50
4	OMe	Cl	Cl	30	40

[*] $= IC_{50}$ in μM

Results from initial *in vivo* testing on the 5-alkoxy-3,4-dihalofuran-2(5*H*)-ones indicated that the compounds are highly toxic and so are unlikely to be suitable as anticancer drugs.[95]

2.3.2 Cancer screening summary

In summary, a small number of the furan-2(5*H*)-one building blocks that have been assembled for further inclusion into a combinatorial synthesis programme, have been screened against two cancer cell lines. The results from this screening have identified the optimum chain length for anticancer activity for the 5-alkoxy-3,4-dihalofuran-2(5*H*)-ones for *in vitro* anticancer activity against both MAC13 and MAC16 cell lines. Further adaptation at the other sites on the furan-2(5*H*)-one ring may improve the *in vitro* activity. In addition to this, the techniques for screening reaction

intermediates have been refined. Combinatorial synthesis is based around the idea of producing large numbers of compounds to screen on mass. Further studies on bi (or tri) substitution of the furan-2(5H)-one scaffold will, as a by-product, produce suitable compounds to be entered into an anticancer screening programme. This approach of using combinatorial library assembly intermediates for screening purposes may produce a suitable lead structure with minimal extra cost or effort.

The problems of the *in vivo* toxicity of the compounds could be tackled by the development of a pro-drug (60). This would possibly combat the *in vivo* toxicity problems by removing the vinylic acid chloride, whilst once activated, retaining the anticancer activity (Figure 2.31).
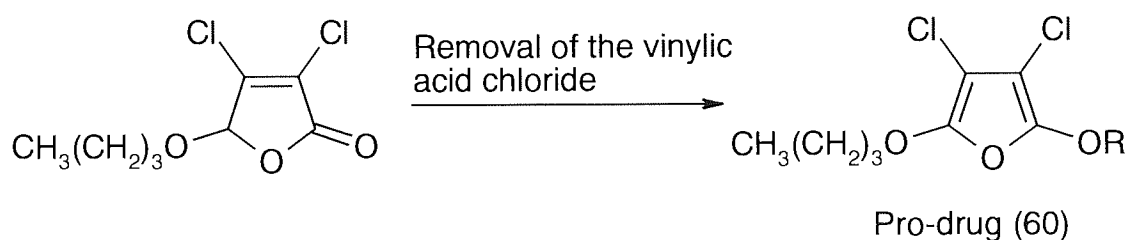
Figure 2.31 The development of a pro-drug could combat the in vivo *toxicity problems.*

2.4 Summary

In summary, a variety of different reactions have been investigated at the five position on the furan-2(5H)-one ring. Two of these, the reactions with alcohols and the reaction with electron rich aromatic systems, have been explored with the idea of further use for combinatorial library assembly. Initially, the alcohol products were concentrated on, with the assembly of a small combinatorial library investigating the possible use of twenty-four different alcohols for combinatorial library assembly. Detection methods have been developed using the ability that nitrogen containing nucleophiles have for attack at the C4 Michael position on the furan-2(5H)-one ring. This approach in adding diversity to the ring will be developed further in chapter three

to make further use of the second arm of the library, ultimately to produce a wider diversity of compounds. The reaction with electron rich aromatic systems will be studied in more detail in chapter five where it may become useful in the quest for novel COX-2 selective NSAIDs.

In addition to this, some small dihalogenated furan-2(5H)-ones have been entered into an anticancer screening programme and the optimum 5-alkoxy chain length for *in vitro* activity against MAC13 and MAC16 cell lines has been found. This approach used combinatorial library intermediates in a separate screening programme, thus increasing the chance any combinatorial library has to identify a biologically active lead structure.

Chapter 3 : Results and Discussion

Nucleophilic attack at the four position

3.1 Further reactions with nitrogen nucleophiles

3.1.1 Library formation

In order to develop a suitable second axis of the library, it was decided to test a larger number of amines for their ability to form nucleophilic products at the four position on the furan-2(5H)-one scaffold. A further twenty amines were used bring the total number of nitrogen nucleophiles tested to twenty-four (benzylmethylamine, 1-benzylpiperazine, 2,6-dimethylmorpholine and pyrrolidine have already been tested in the previous chapter). The amines were selected manually, as before with the alcohol selection, to provide a reasonable variety of structures below a certain molecular weight (<250). This library would react the twenty-four different amines with four different alkoxy building blocks, each divided into three separate reactions with either a chlorine, a bromine or a hydrogen atom at the three position. The four alcohols chosen for the initial reaction were selected so there was an example from four main diversity groups (short chain, long chain, cyclic and aromatic). This library would

contain 288 compounds (24x4x3). A small sample of each compound can be submitted directly, without any purification, for detection in the APCI+ mass spectrometer. This can be represented schematically in Figure 3.1 (see section 7.4.1).
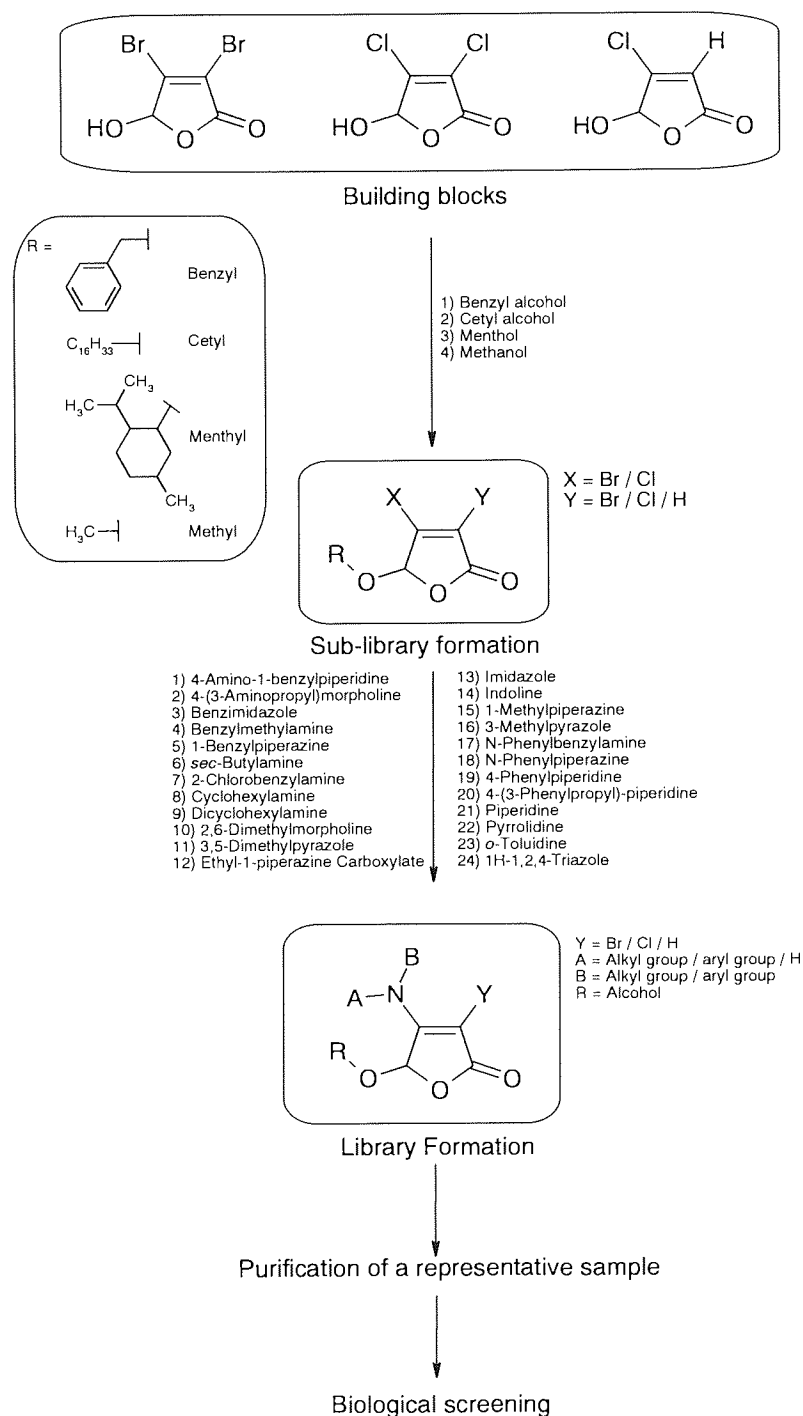
Figure 3.1 Schematic representation of the assembly of the library.

As with the selection of alcohols in the previous chapter, the amines were chosen for inclusion in the second library so a wide variety of structural groups were represented.

The amines chosen formed the following 4-aminofuran-2(5H)-ones (Y = Br / Cl / H) (R = Benzyl / Cetyl / Menthyl / Methyl) (Figure 3.2).
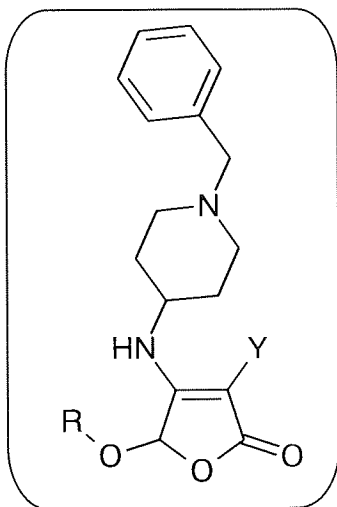
1. 4-Amino-1-benzylpiperidine	2. 4-(3-Aminopropyl)morpholine	3. Benzimidazole
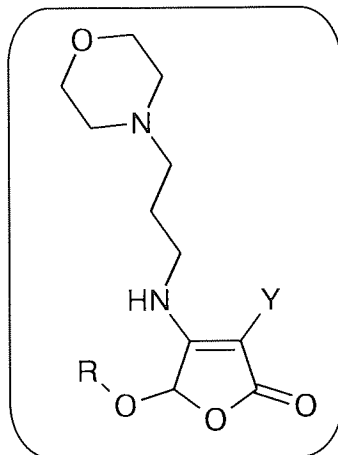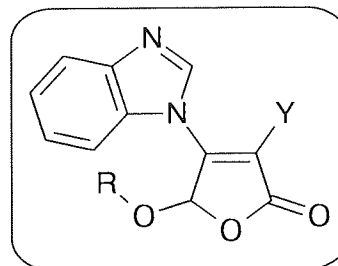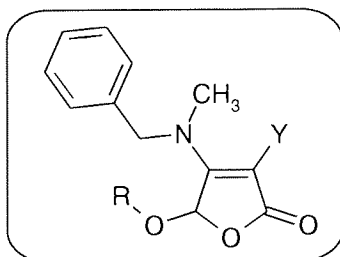
4. Benzylmethylamine	5. 1-Benzylpiperazine	6. *sec*-Butylamine
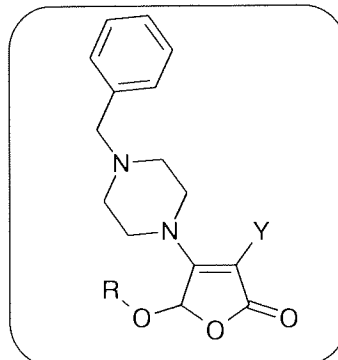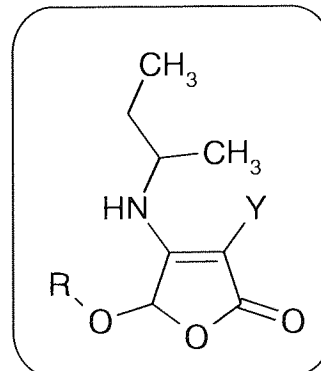
7. 2-Chlorobenzylamine
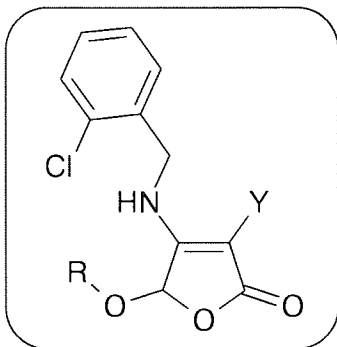
8. Cyclohexylamine
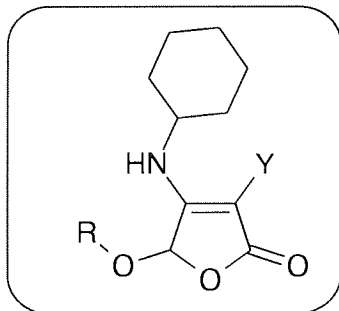
9. Dicyclohexylamine
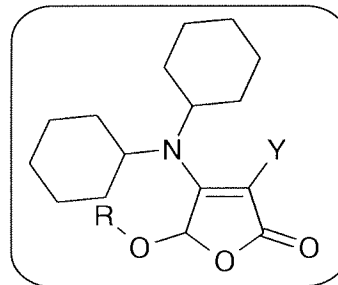
10. 2,6-Dimethylmorpholine
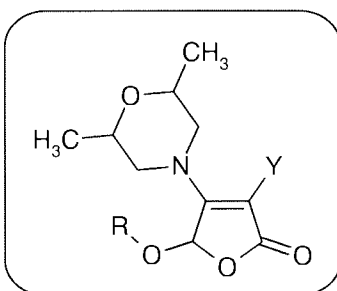
11. 3,5-Dimethylpyrazole
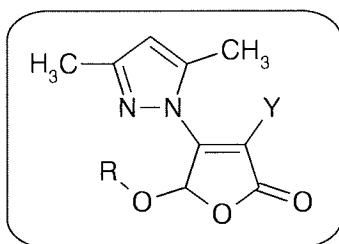
12. Ethyl-1-piperazine Carboxylate
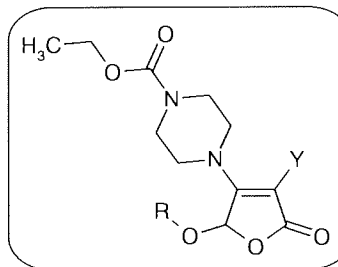
13. Imidazole
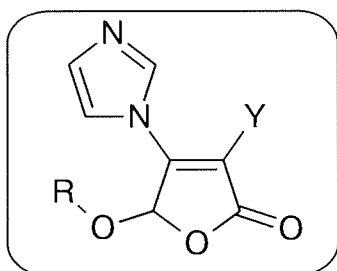
14. Indoline
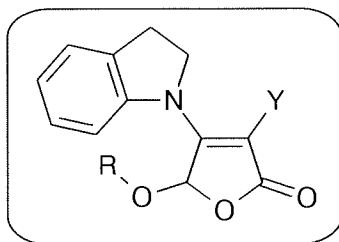
15. 1-Methylpiperazine
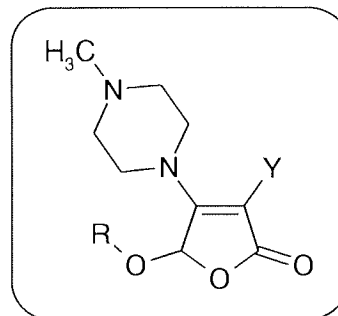
16. 3-Methylpyrazole
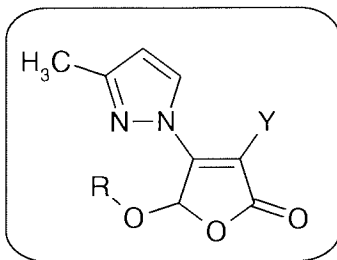
17. N-Phenylbenzylamine
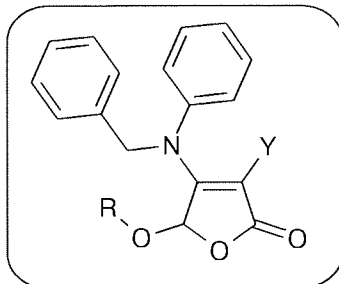
18. N-Phenylpiperazine
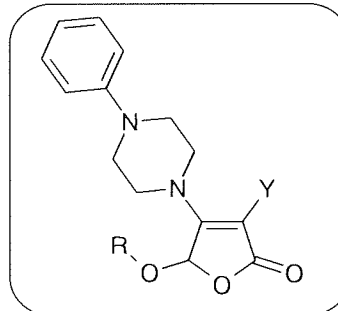
19. 4-Phenylpiperidine
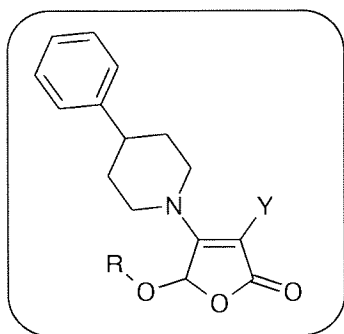
20. 4-(3-Phenylpropyl)piperidine
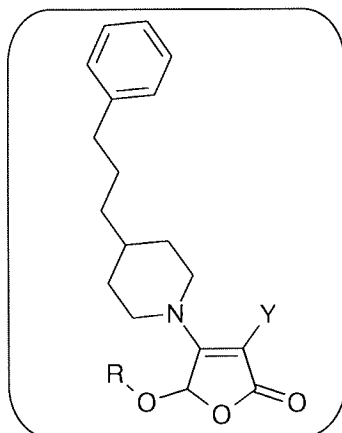
21. Piperidine
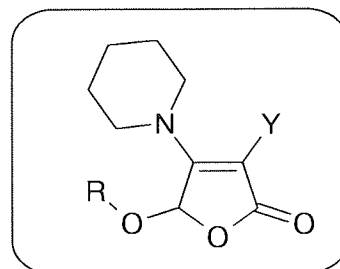
22. Pyrrolidine
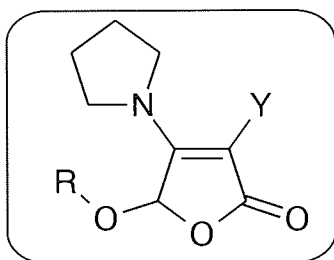
23. *o*-Toluidine
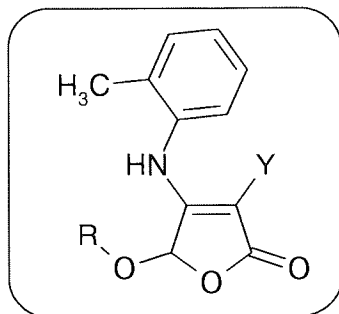
24. 1H-1,2,4-Triazole
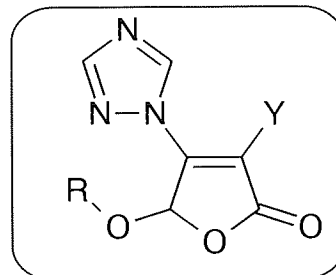
Figure 3.2 The structures of the twenty-four different 4-aminofuran-2(5H)-ones possible.

The results from the APCI+ mass spectroscopic analysis of the 4-aminofuran-2(5*H*)-one library are tabulated below (a shaded box denotes that the compound has been purified and fully characterised (see section 7.4.2)). As with the previous combinatorial library in section 2.2.2, a tick in the following table indicates the presence of the correct molecular ion peak in the APCI+ mass spectrometer and a cross denotes its absence.

4-position ↓	3-position →	(1) Methoxy			(2) Cetyloxy			(3) Benzyloxy			(4) Menthyloxy		
	5-position →	Br	Cl	H	Br	Cl	H	Br	Cl	H	Br	Cl	H
1) 4-Amino-1-benzylpiperidine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
2) 4-(3-Aminopropyl)morpholine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
3) Benzimidazole		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
4) Benzylmethylamine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓
5) 1-Benzylpiperazine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓
6) *sec*-Butylamine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
7) 2-Chlorobenzylamine		✓	✓	X	✓	✓	✓	✓	✓	X	✓	✓	X
8) Cyclohexylamine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
9) Dicyclohexylamine		✓	✓	✓	✓	✓	X	✓	✓	X	✓	✓	X
10) 2,6-Dimethylmorpholine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓
11) 3,5-Dimethylpyrazole		✓	✓	X	✓	✓	✓	✓	✓	X	✓	✓	X
12) Ethyl-1-piperazine carboxylate		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
13) Imidazole		✓	✓	✓	✓	X	✓	✓	✓	X	✓	✓	X
14) Indoline		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
15) 1-Methylpiperazine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
16) 3-Methylpyrazole		✓	✓	X	✓	✓	✓	✓	✓	X	✓	✓	X
17) N-Phenylbenzylamine		✓	✓	✓	✓	✓	X	✓	✓	X	✓	✓	X
18) N-Phenylpiperazine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
19) 4-Phenylpiperidine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
20) 4-(3-Phenylpropyl)piperidine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
21) Piperidine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
22) Pyrrolidine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓
23) *o*-Toluidine		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X
24) 1H-1,2,4-Triazole		✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X

This larger library further investigates the potential that the furan-2(5*H*)-one scaffold has for incorporation into a combinatorial library. A majority of the attempted 288 compounds were detected within the APCI+ mass spectrometer and a number of the undetected compounds could possibly be obtained if re-synthesis was attempted under different reaction conditions.

There are now two complete sets of reactions which could potentially be put together into a library, producing a vast number of compounds. Using the above information, there are twenty-four reacting nitrogen nucleophiles and twenty-four reacting alcohols. As there is the possibility of either a chlorine atom, a bromine atom or hydrogen at position three on the ring, there is the potential to synthesise 1728 compounds (24x24x3).

Rather than synthesise vast numbers of compounds at this stage, it was decided to purify some of the furan-2(5*H*)-ones within the libraries already assembled and perform biological testing on these purified products. This may identify any lead compound upon which structure refinement can be attempted. This should hopefully avoid the need to synthesise large volumes of compounds to arrive at the same point.

3.1.2   Purification methods

As a number of furan-2(5*H*)-ones are required to be purified from the library in order to gain a reasonable spread of diversity, traditional column chromatography methods are unacceptability slow. It was decided to use preparative thin layer chromatography plates made to a thicker specification. As commercially available preparative thin layer chromatography plates have quite limited loading, it was decided to make our own. This was achieved by making a paste with silica gel containing a fluorescent indicator, which was visible under ultraviolet light at 254 nm. 75 ml of the silica gel was placed in a 250 ml beaker. Water was added to the 100 ml mark and mixed to form a paste. Once the paste was at an even consistency, 50 ml was poured over each of two 20 cm x 20 cm dry glass plate. Once the paste had dried, the plate was left overnight to dry fully in an oven at around 100°C.

As the plates were very fragile, commercially available preparative thin layer chromatography plates (made to a thickness of 1000 μm instead of the standard thickness of 250 μm) were used for low volume samples. The plates selected were the Aldrich (Z26582-9) 1000 μm silica gel on glass (20 cm x 20 cm).

The separation procedure followed for both styles of plate is as follows:

Initially, a little of each of the samples was spotted onto an ordinary, small TLC plate and run in ether. This solvent gave sufficient separation of the different zones and the furan-2(5H)-one zone could be predicted by dipping the plate in a 1% solution of potassium permanganate solution, where an almost immediate decolouration of the purple to a brown colour was seen on removal. Each sample was then pipetted along the bottom of a preparative TLC plate about 3 cm from the lower edge. When the solvent had fully evaporated, the plates were run in an ether tank until the face of the solvent was about 3 cm from the top of the plate. The plate was then left to dry and the correct zone scraped off. The correct zone was taken to be the one (when viewed under ultra-violet light) that corresponded to the zone on the smaller TLC plate that had the fastest decolouration of the potassium permanganate solution.

Once the correct zone had been scraped off, it was placed in a beaker of methanol and gently heated to allow the furan-2(5H)-one to dissolve in the solvent. The solution was then filtered and left overnight to allow the methanol to evaporate. After drying in a desiccator under vacuum for a few hours, a little was dissolved in methanol and submitted for APCI+ mass spectroscopy. Some of the remainder was dissolved in deuterated chloroform and then proton (and in some cases carbon) nuclear magnetic resonance spectra were run. A little of each sample was also dissolved in chloroform and submitted for infrared analysis.

The compounds isolated for biological testing were as follows:
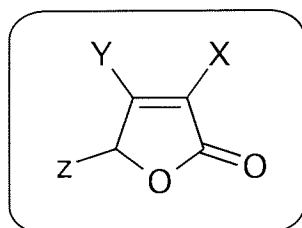
X = 3 Position
Y = 4 Position
Z = 5 Position

Compound Number	3-Position	4-Position	5-Position
1	Chlorine	Indoline	Methoxy
2	Chlorine	3-Methylpyrazole	Methoxy
3	Chlorine	4-(3-Aminopropyl)morpholine	Methoxy
4	Chlorine	4-Phenylpiperidine	Methoxy
5	Chlorine	Pyrrolidine	Methoxy
6	Chlorine	2,6-Dimethylmorpholine	Methoxy
7	Chlorine	4-Amino-1-benzylpiperidine	Methoxy
8	Bromine	Benzylmethylamine	Cetyloxy
9	Chlorine	1H-1,2,4-Triazole	Methoxy
10	Bromine	Imidazole	Menthyloxy
11	Bromine	Pyrrolidine	Cetyloxy
12	Chlorine	sec-Butylamine	Methoxy
13	Chlorine	N-Phenylpiperazine	Methoxy
14	Bromine	Piperidine	Menthyloxy
15	Bromine	3,5-Dimethylpyrazole	Benzyloxy
16	Chlorine	Benzimidazole	Cetyloxy
17	Chlorine	4-(3-Phenylpropyl)piperidine	Methoxy
18	Chlorine	1H-1,2,4-Triazole	Cetyloxy
19	Chlorine	Benzylmethylamine	Methoxy
20	Chlorine	1-Benzylpiperazine	Methoxy
21	Chlorine	o-Toluidine	Methoxy
22	Bromine	Pyrrolidine	Methoxy
23	Chlorine	1-Benzylpiperazine	Cetyloxy
24	Chlorine	2-Chlorobenzylamine	Cetyloxy
25	Bromine	Piperidine	Benzyloxy
26	Bromine	Piperidine	Methoxy
27	Bromine	Benzylmethylamine	Menthyloxy
28	Chlorine	Cyclohexylamine	Cetyloxy
29	Bromine	Benzimidazole	Cetyloxy
30	Bromine	Piperidine	Cetyloxy
31	Bromine	Benzimidazole	Methoxy
32	Chlorine	Benzylmethylamine	Menthyloxy
33	Bromine	Pyrrolidine	Menthyloxy
34	Chlorine	2,6-Dimethylmorpholine	Menthyloxy
35	Chlorine	3,5-Dimethylpyrazole	Cetyloxy
36	Chlorine	Benzimidazole	Methoxy
37	Chlorine	Dicyclohexylamine	Methoxy
38	Bromine	Benzimidazole	Menthyloxy
39	Chlorine	Ethyl-1-piperazinecarboxylate	Cetyloxy

All the purified library members were tested biologically and the results from this testing appears in chapter four.

3.2 Other possible nucleophilic substitutions to further increase diversity

Nucleophilic attack at the four position (the Michael position) on the furan-2(5H)-one ring is an important reaction. Extensive use of the reaction with nitrogen nucleophiles has been explored earlier in this chapter and the reaction was also used in section 2.2.1 for the indirect characterisation of the 5-alkoxyfuran-2(5H)-ones by APCl+ mass spectroscopy. In this section, the four main nucleophilic displacements (with nitrogen, sulphur, carbon and oxygen containing nucleophiles) will be examined.

3.2.1 Nitrogen nucleophilic attack

The main principle behind the addition of a nitrogen nucleophile to the four position on the furan-2(5H)-one ring has been examined earlier in chapter 2 (see section 2.2.1).[90 - 92] One area of published work which still remains to be mentioned is that performed by Jähnisch *et al.* on dihalogenated furanones. They looked at the different types of nucleophilic substitution reaction different dichlorofuran-2(5H)-ones can undergo with aniline, especially with respect to different solvents (Figure 3.3).[97]

They discovered that the solvent plays an important role in dictating the configuration of the furanone (whether it is in the open chain or ring form) and so the type of reaction the furanone undergoes. In dimethyl sulphoxide, aniline will act as a nucleophile and react at the four position (the Michael position) of the closed ring form of the furanone (61), to produce a monosubstituted product (62). Further reaction at the five position of the ring will occur if a suitable group (for example, chlorine) is present, producing a disubstituted furanone (63). When the initial furanone (61) is dissolved in chloroform, it is mainly in the open chain form (64) and so will follow one of two reaction sequences. Either the aniline will add to the ring in

place of the oxygen atom only (65), or if enough aniline is present it will add three equivalents as shown in Figure 3.3 (66).[97]
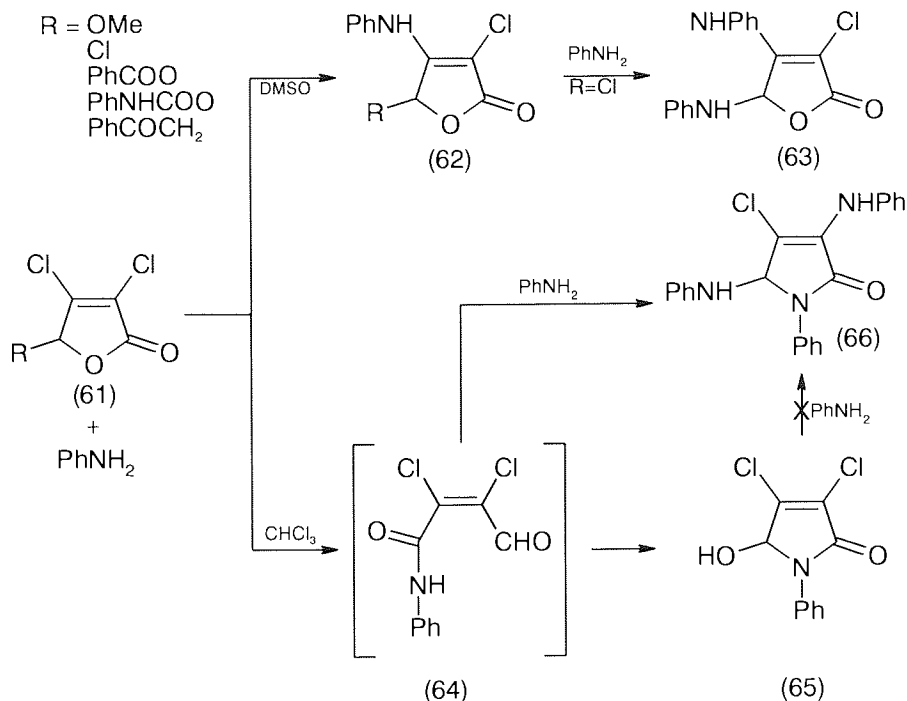
Figure 3.3 The different reactions that dichlorofuran-2(5H)-ones can undergo with aniline with respect to the solvent used.

3.2.2 Sulphur nucleophilic attack

Other research groups have expanded on the idea of developing further methods for forming derivatives of the furanone substructure. Fariña *et al.* investigated the halogen addition to 5-alkoxy-2(5*H*)-furanones followed by HX elimination.[90] This, performed under different conditions, offered a regioselective synthesis of different 3- and 4-halogenated 5-alkoxyfuran-2(5*H*)-ones. It was found that these (especially the 4-halogenated furanones), appeared to be suitable substrates for the addition of other functional groups by nucleophilic substitution of the halogen atom. The nucleophiles used in these experiments were nucleophiles containing nitrogen or sulphur atoms. The reactions were found to proceed in very good yields suitable for preparative processes.

Nucleophilic attack with sulphur containing nucleophiles would occur mechanistically similar as to nucleophilic attack with nitrogen containing nucleophiles (Figure 3.4).
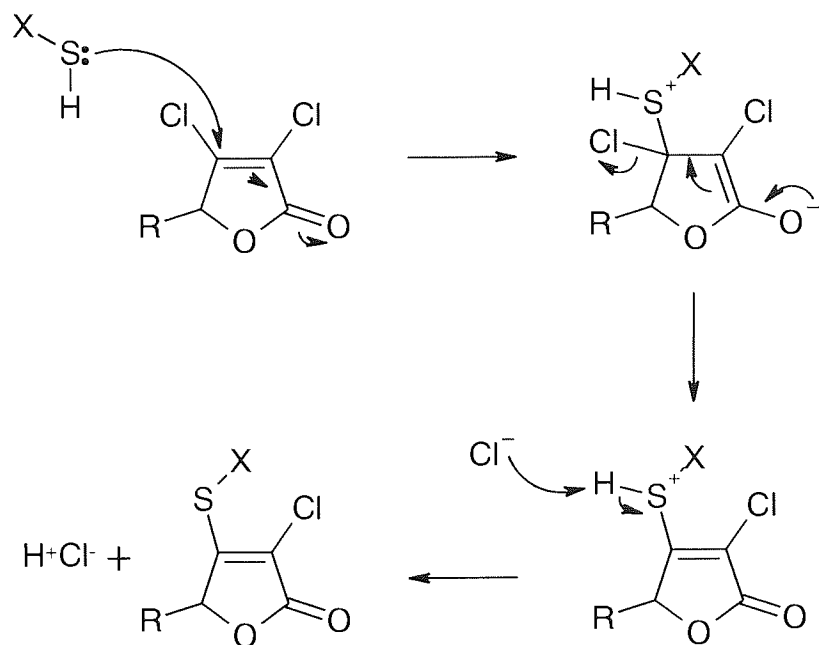
Figure 3.4 The nucleophilic attack at the C4 position on the furan-2(5H)-one ring by sulphur nucleophiles.

The halogenated furanones were reacted with primary or secondary amines and sodium thiolates in tetrahydrofuran or carbon tetrachloride to prevent the opening of the ring (Figure 3.5). The products (67) & (68) were achieved in very good yields. It was also found that the reaction with thiolates proceeded quicker in methanol than in carbon tetrachloride or tetrahydrofuran. A final point worth noting from this work is that when chlorofuranone reacts with 2-propanethiolate, the expected reaction product is only obtained in low yield (29%). The main reaction is ring opening to produce the normal aldehyde-ester, followed by conjugate addition of thiolate to the —CH=CX—CHO system and subsequent HX elimination yielding (Z)-2-isopropylthio-4-oxobut-2-enoate and its cyclic isomer, 3-isopropylthio-5-methoxy-2(5H)-furanone.[90]

X = Br / Cl

THF / CCl$_4$ R1\N–H /R2   R1–SNa   Methanol

(67)   (68)

Figure 3.5 The synthesis of 4-alkylamino and 4-alkylthio-5-methoxy-2(5H)-furanones.

Similar reactions have also been investigated by Ducher *et al.* published in a French paper in the Bulletin de la Société Chimique de France.[98] She and her co-workers investigated the reaction between sulphur nucleophiles and 3,4-dichlorofuranones. They stated that in dihalogenated, as well as mono halogenated furanones, nucleophiles prefer to react with the halogen at position four. This observation has been noted by other research groups.[99]

It was found, however, that certain compounds after reaction at the four position,[100] continue to react further at the three position. For example, Bobrova *et al.* found that the reaction between mucochloric acid (28) and thiophenol occurs at both the four and three positions (69) (Figure 3.6).[101]

Figure 3.6 The reaction between mucochloric acid and thiophenol occurs at both the four and three positions.

This reaction was extended to 4-chlorothiophenol as well as thiophenol.[101, 102]

3.2.3 Carbon nucleophilic attack

The reaction of carbon nucleophiles at the four position on the furan-2(5H)-one ring was investigated by Lattmann.[103] He found that some carbon nucleophiles attack at the four position of 3,4-dichloro-5-menthyloxyfuran-2(5H)-one (70) (Figure 3.7).
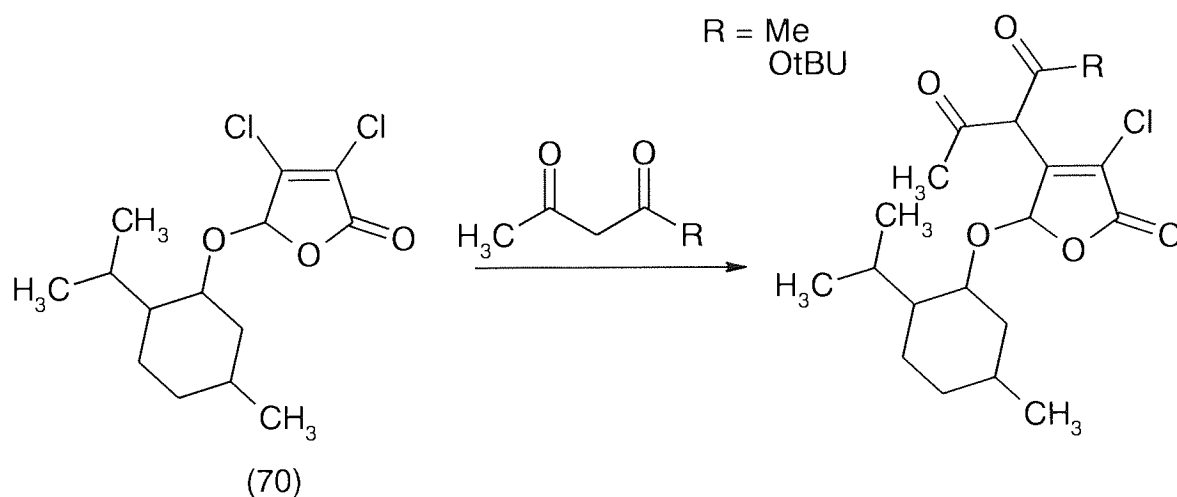
Figure 3.7 The reaction at the four position of 3,4-dichloro-5-menthyloxyfuran-2(5H)-one with carbon nucleophiles.

3.2.4 Oxygen nucleophilic attack

In section 2.1.2.4, it was seen that oxygen nucleophiles react at the three position of the open-chain form of mucochloric acid (27). This idea is expanded further in chapter five in the quest for trisubstituted furan-2(5H)-ones (Figure 3.8).
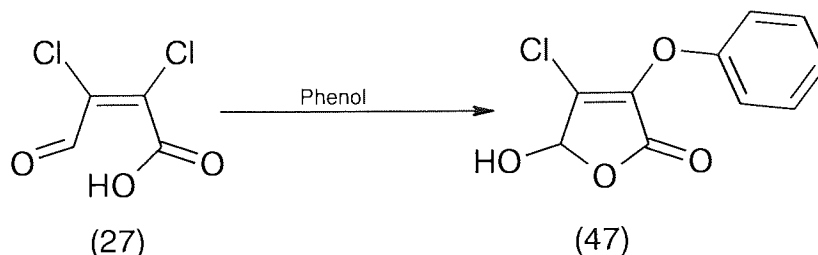
Figure 3.8 Oxygen nucleophiles attack at the three position of the open-chain form of mucochloric acid.

3.3 Summary

In summary, it has been shown in this chapter that nitrogen nucleophiles will make an excellent second reaction arm for combinatorial library synthesis. The ability of certain nitrogen nucleophiles to react at the four position on the furan-2(5H)-one ring was already known, as it was used in the indirect detection of the 5-alkoxyfuran-2(5H)-ones by APCI+ mass spectroscopy in the previous chapter. Now a series of twenty-four reacting nitrogen nucleophiles have been identified with a large structural diversity. This, coupled with their ease of reaction with 5-alkoxyfuran-2(5H)-ones is ideal for combinatorial library assembly. A larger combinatorial library with 288 members was synthesised and selected members of this library were purified by preparative thin layer chromatography for further biological analysis in the next chapter.

There is a great number of amines commercially available and so the scope for the addition of diversity at the four position is much greater than investigated here.

Coupled with this, is the additional substitution reactions outlined in section 3.2 which could be used alongside the reaction with nitrogen nucleophiles for increasing the size of any attempted combinatorial library. Without any selection criteria, it would be almost impossible to attempt to use all possible reactants at the four position. Diversity analysis, where each separate reactant is examined by computer would need to be employed. This technique identifies the least number of compounds that need to be used to produce a set of products representing the whole structural diversity set.

Chapter 4 : Results and Discussion

Biological screening

4.1 Screening results

4.1.1 Results from the antibacterial screening

Thirty-nine of the purified furan-2(5*H*)-ones from the combinatorial library in chapter three were biologically tested on a number of different organisms. Each compound was made up to a concentration of 10 mg in 1 ml of dimethyl sulphoxide and zones of inhibition were measured against *Staphylococcus aureus* NCTC 6571. This is a 'soft' organism, which can easily be killed by any antibiotic. Any compound, which fails to show a zone of inhibition against *Staphylococcus aureus* NCTC 6571, would therefore not warrant further investigation. This will eventually result in the identification of lead structures suitable for further chemical adaptation (Figure 4.1).

Combinatorial library of
288 compounds → Purification of a
representative sample

Pre-screening against
Staphylococcus aureus

Compound set for further
biological analysis

Other biological testing
e.g. CCK receptor antagonism

Further microbiological
screening tests

Lead structures for structure refinement experiments
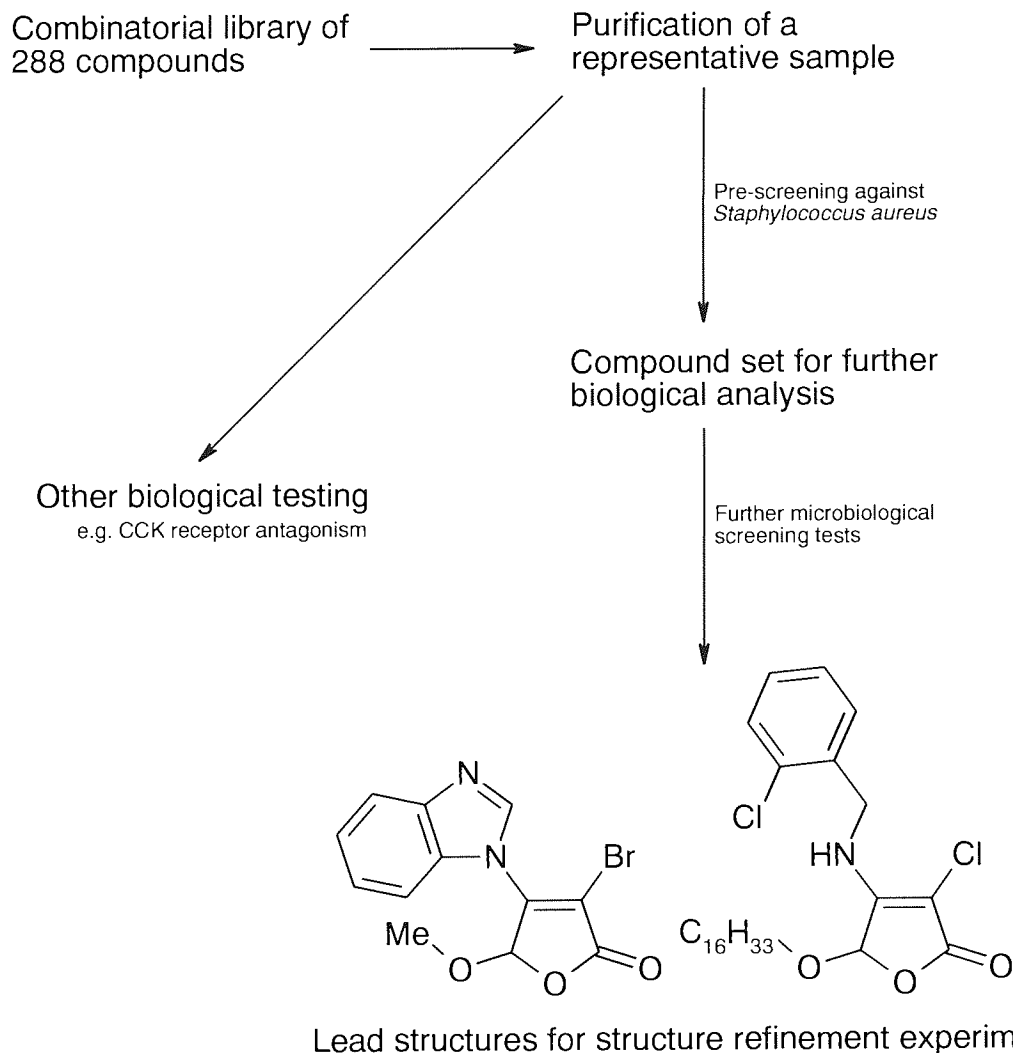
Figure 4.1 The proposed plan for the identification of lead structures for further chemical adaptation.

Initially, the organism under test was grown up on Columbia blood agar plates. Stock solutions of the organism were made in a broth solution containing 10% glycerol (the glycerol is to protect the organism if the stock solution is frozen for use later). The screening was undertaken on Mueller-Hinton agar plates, which had been fully covered with 100 µl of the stock solution of the organism under test. Wells were cut in the agar with the wide end of a glass pipette and 40 µl of the test solution (10 mg per ml of DMSO) was added to each well. The plates were left overnight in a warm room (at 37 °C) and zones of inhibition measured after twenty-four hours.

Of the 39 compounds tested, 8 gave measurable zones of inhibition and so warranted further investigation with a variety of different organisms. As mentioned above, any compound, which did not produce a suitable zone of inhibition with *Staphylococcus aureus* NCTC 6571, would be unlikely to inhibit the growth of other organisms and so was not investigated further.

The results are as follows:

Number	Zone	Number	Zone	Number	Zone	Number	Zone
1	2 mm	2	-	3	-	4	2 mm
5	-	6	-	7	-	8	-
9	-	10	3 mm	11	-	12	-
13	2 mm	14	-	15	-	16	-
17	-	18	-	19	-	20	-
21	-	22	-	23	-	24	10 mm
25	-	26	-	27	-	28	-
29	-	30	-	31	11 mm	32	-
33	-	34	-	35	-	36	9 mm
37	7 mm	38	-	39	-		

The eight compounds, which showed positive antibacterial activity, contain a reasonable diversity of structures (Figure 4.2).

Compound 1 Compound 4 Compound 10

Compound 13 Compound 24 Compound 31

Compound 36 Compound 37
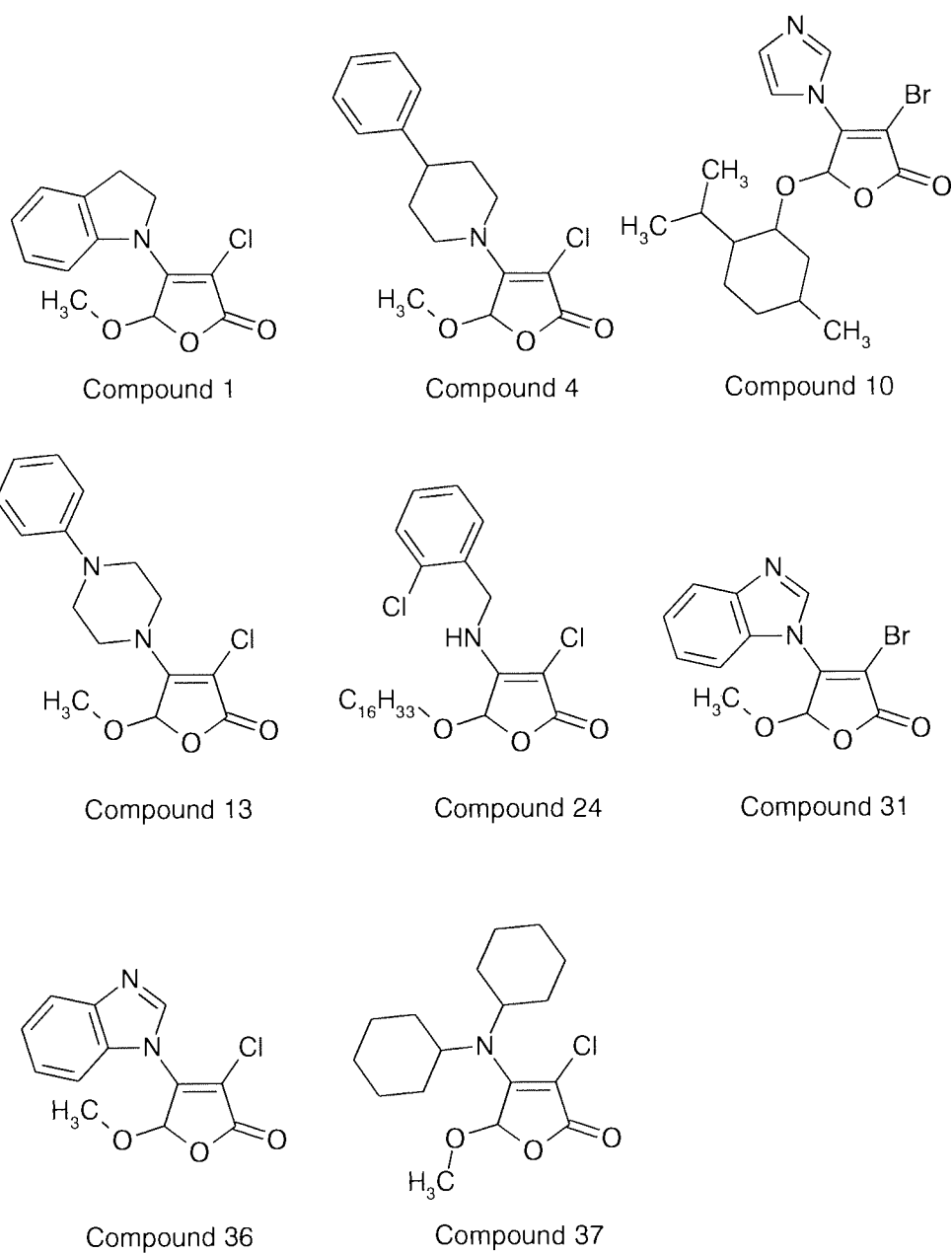
Figure 4.2 The structure of the eight compounds, which exhibited antibacterial activity against Staphylococcus aureus *NCTC 6571.*

The eight compounds shown in Figure 4.2 were then tested against the following organisms:

- *Enterococcus faecium* ATCC 10541 (*E. faecium*)
- Methicillin-resistant *Staphylococcus aureus* 96-7778 (MRSA)
- *Escherichia coli* DC0 (*E. coli*)
- *Candida albicans* 398M (*C. albicans*)

Each screening test was performed exactly as before, using stock solutions of the organism under test and Mueller-Hinton agar plates.

The results from this screening is as follows:

Compound	*E. faecium*	MRSA	*E. coli*	*C. albicans*
1	-	2 mm	-	-
4	-	1 mm	-	-
10	-	2 mm	-	-
13	-	-	-	-
24	-	3 mm	-	5 mm
31	-	10 mm	-	4 mm
36	-	-	-	-
37	3 mm	4 mm	-	1 mm

Compounds 24 and 31 show them most promising activity (*Candida albicans* activity for 24 and MRSA activity for 31). These structures will form the basis of structure refinement experiments (see section 4.2) (Figure 4.3).

Compound 24   Compound 31
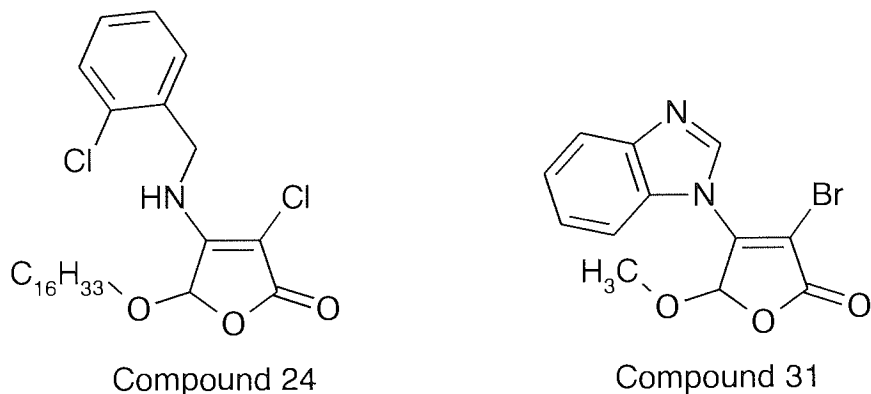
Figure 4.3 The structure of the two most active compounds on which structure refinement experiments will be performed.

It was decided to calculate the minimum inhibitory concentrations (MIC) for the two compounds identified for further structure refinement (Figure 4.3). Compound 24 had an MIC with Methicillin-resistant *Staphylococcus aureus* 96-7778 (MRSA) of 8 – 16 µg / ml and compound 31 had an MIC of 16 – 32 µg / ml. These values, although reasonably low, are much higher than currently available commercial antibiotics. Structure refinement at the four and five position may improve these values

4.1.2   Results from the CCK screening

Samples of each of the purified furan-2(5*H*)-ones tested for antibacterial activity were also submitted for CCK-B receptor antagonism testing. This was performed by Dr David Poyner from the pharmacology research group at Aston University.

The CCK binding assay was performed as follows:[104]

Tissue preparation: The tissue was weighed after dissection and then homogenised in 25 ml of ice cold 0.32 mol sucrose for 15 strokes at 500 rpm. It was centrifuged at 1000 G (3000 rpm) for 10 minutes and then the supernatant was re-centrifuged at 20,000 G (13000 rpm) for 20 minutes. The pellet produced was re-dispersed in the required volume of assay buffer (5 strokes of homogeniser at 500 rpm). The final

tissue concentration was 1 g original weight to 120 ml buffer. The tissue was stored in aliquots at –70 °C.

Binding assay: Radioligand (^{125}I-Bolton Hunter labelled CCK, NEN) at 25 pmol and drugs were incubated with membranes (0.1 mg/ml) in 20 mmol Hepes, 1 mmol EGTA, 5 mmol MgCl$_2$, 150 mmol NaCl, 0.25 mg/ml bacitracin, pH 6.5 for two hours at room temperature. Incubations were terminated by centrifugation. The membrane pellets were washed twice with water and bound radioactivity was measures in a γ-counter.

Initial screening tests were performed on the thirty-nine purified compounds but unfortunately, no compound exhibited any detectable CCK-B receptor inhibition.

4.2 Exploitation of the antibacterial screening results and further sub-libraries

As the antibacterial studies produced the best lead structure from the three screening tests undertaken so far (anticancer, antibacterial and CCK-B antagonism), this area was concentrated on for structure refinement studies.

4.2.1 Optimisation at the four position

It was evident from the results in the previous section that two amine groups (benzimidazole and 2-chlorobenzylamine) at the four position were important in the inhibition of bacterial growth. Benzimidazole was the amine in compound 31, which showed the largest zone of inhibition for MRSA. 2-chlorobenzylamine was the amine in compound 24, which showed the largest inhibition for *Candida albicans* and had the better MIC value against MRSA. It was decided to synthesise two small libraries based on these two amines with two different 5-alkoxyfuran-2(5*H*)-one building blocks. By varying the structure of the amine group at the four position slightly in each compound, optimisation can be attempted. This will also serve to demonstrate

the potential that combinatorial chemistry has to assist in the structure refinement of lead structures. The amines selected are shown in the next few pages (Figures 4.4, 4.5 and 4.6). Repetition of a number of compounds already synthesised and biologically tested, along with the synthesis and testing of a range of new compounds was attempted. In this way, full structural analysis of the two amine groups can be investigated, avoiding any screening errors which may have occurred earlier.

Initially, a library of twelve 5-cetyloxyfuran-2(5*H*)-ones and six 5-methoxyfuran-2(5*H*)-ones was attempted. All eighteen compounds were detectable in the APCI+ mass spectrometer after synthesis in the standard manner. After purification by preparative thin layer chromatography (see section 3.1.2), only ten of the 5-cetyloxyfuran-2(5*H*)-ones and five of the 5-methoxyfuran-2(5*H*)-ones were suitable for further biological analysis. As the structures are very similar within each of the sub-libraries, it was not decided worthwhile to invest a large amount of time purifying the remaining three. Any potential activity could be predicted by examination of the result of similar structures within the sub-library. The spectroscopic data from these experiments appears in section 7.4.3.1.

These compounds were then entered into screening programmes for antibacterial activity.

The twelve amines used in the 5-cetyloxy experiment were:

Based on the 2-chlorobenzylamine structure (Figure 4.4):

1. 2-Chlorobenzylamine
2. Benzylamine
3. Benzylmethylamine
4. N-Phenylbenzylamine
5. Aniline
6. 2-Phenylethylamine
7. Cyclohexylamine

8. Dicyclohexylamine (failed to purify satisfactorily)

1. Compound 40   2. Compound 41   3. Compound 42

4. Compound 43   5. Compound 44   6. Compound 45

7. Compound 46   8. (Failed to purify)
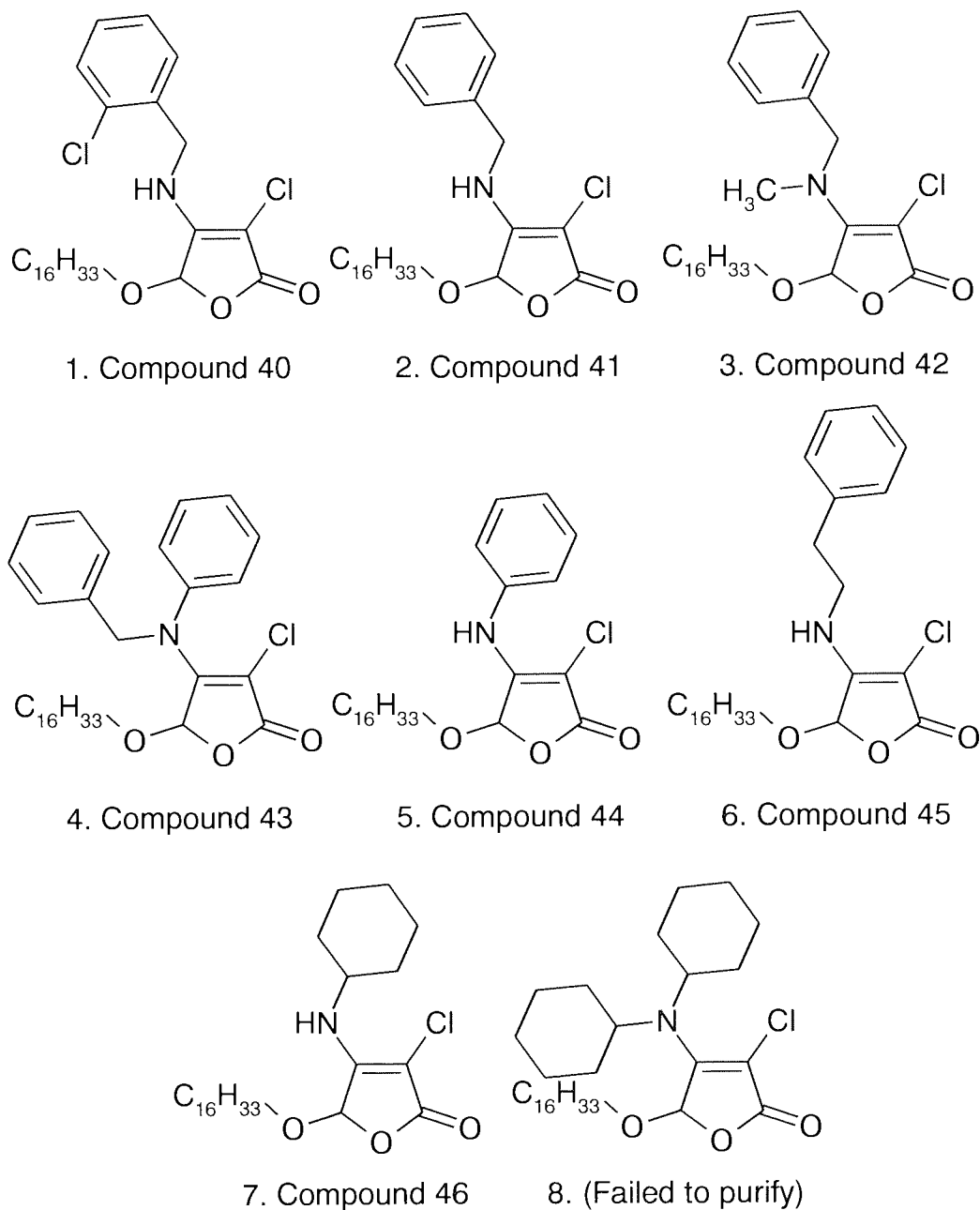
Figure 4.4 The eight amines selected based on the 2-chlorobenzylamine structure formed the above furan-2(5H)-ones.

Based on the Benzimidazole structure (Figure 4.5):

9. Imidazole

10. Pyrazole

11. 1H-1,2,4-Triazole

12. 2,6-Dimethylmorpholine (failed to purify satisfactorily)
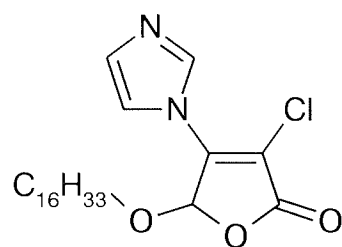
9. Compound 47
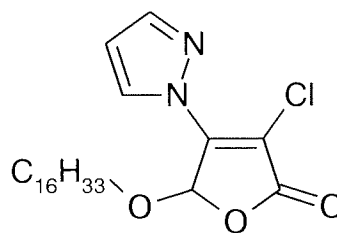
10. Compound 48
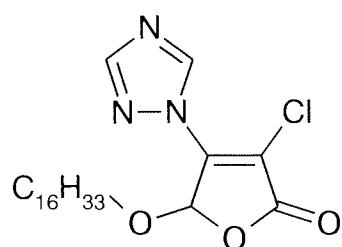
11. Compound 49
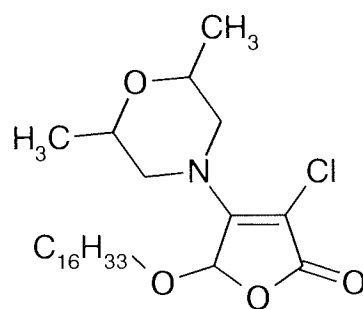
12. (Failed to purify)

Figure 4.5 The four amines selected based on the benzimidazole structure formed the above furan-2(5H)-ones.

The six amines used in the 5-methoxy experiment were (all based on the benzimidazole structure) (Figure 4.6):

1. Benzimidazole
2. Indoline
3. Imidazole
4. Pyrrolidine (failed to purify satisfactorily)
5. Pyrrole
6. 3,5-Dimethylpyrazole

1. Compound 50   2. Compound 51   3. Compound 52

4. (Failed to purify)   5. Compound 53   6. Compound 54
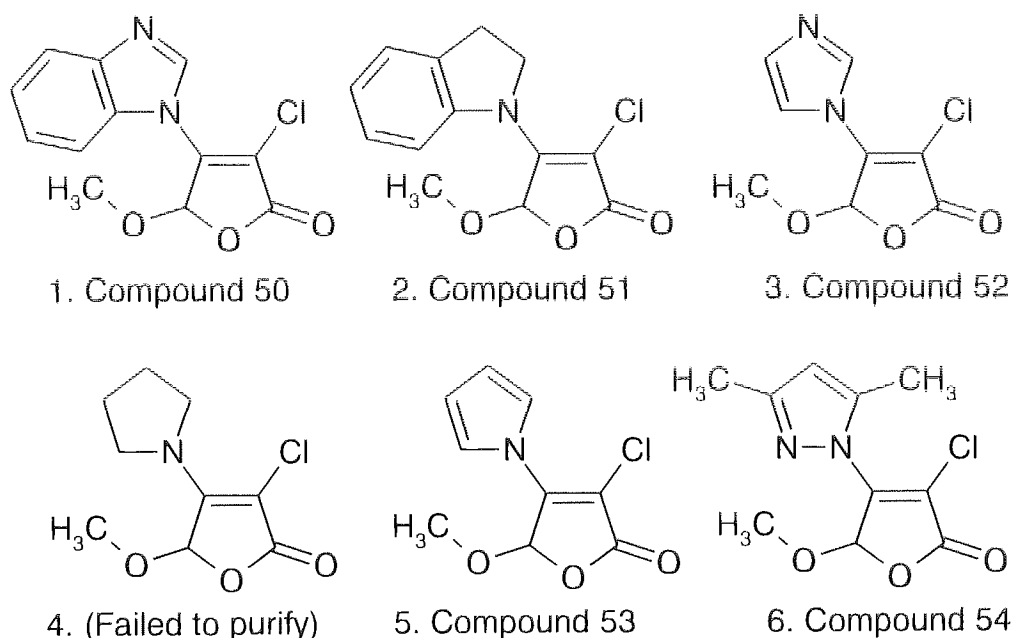
Figure 4.6 The six amines selected based on the benzimidazole structure formed the above furan-2(5H)-ones.

4.2.1.1   Results of the antibacterial screening

Initially, all fifteen compounds under test (compounds 40 to 54) were screened against four of the same organisms as before. Each was made up to a concentration of 10 mg

in 1 ml DMSO and again, 40 µl was used in each well of the agar plate. Zones of inhibition were noted and these are shown below.

The four organisms used were:

- *Staphylococcus aureus* NCTC 6571 (*S. aureus*)
- *Enterococcus faecium* ATCC 10541 (*E. faecium*)
- Methicillin-resistant *Staphylococcus aureus* 96-7778 (MRSA)
- *Escherichia coli* DC0 (*E. coli*)

Compound	S. aureus	E. faecium	MRSA	E. coli
40	-	-	-	-
41	-	-	-	-
42	-	-	-	1 mm
43	-	-	-	-
44	-	-	-	-
45	-	-	-	-
46	-	-	-	-
47	-	-	-	-
48	-	-	-	-
49	-	-	-	-
50	7 mm	7 mm	7 mm	-
51	-	-	1 mm	-
52	3 mm	-	2 mm	2 mm
53	-	-	-	-
54	6 mm	-	5 mm	1 mm

What can bee seen from the above table is that the structure refinement has not been successful. The compound with the largest zone of inhibition (compound number 50) (Figure 4.7), is the furan-2(5H)-one with benzimidazole at the four position. It only differs from compound 31 in the original series by the change of halogen at position three to a chlorine atom from a bromine atom (in fact, it is identical to compound 36 in the original series, which also had favourable zones of inhibition). (Interestingly, compound 40, which is identical to compound 24 in the original series, failed to exhibit any form of inhibition. This was assumed to be a screening anomaly as the original compound exhibited inhibition of various organisms, more than once.)
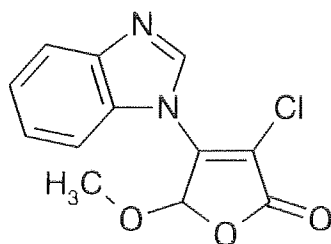
Figure 4.7 The structure of the most active furan-2(5H)-one after structure refinement at the four position (Compound 50).

4.2.1.2   Further antimicrobial testing

It was decided to further test twelve of the series of fifteen optimised compounds on a yeast (three of the compounds were not tested due to the stocks being used up in the previous screening tests). This screening tests was chosen mainly due to the presence of nitrogen containing rings (for example, imidazole) at the four position on some of the furan-2(5*H*)-ones. It is well known that some imidazole containing compounds are antifungal and so testing these compounds would be sensible. The compounds screened were as follows.
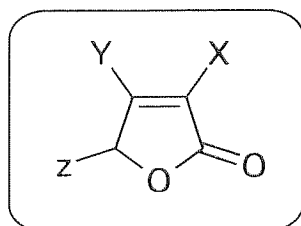
X = 3 Position
Y = 4 Position
Z = 5 Position

Compound Number	3-Position	4-Position	5-Position
40	Chlorine	2-Chlorobenzylamine	Cetyloxy
41	Chlorine	Benzylamine	Cetyloxy
42	Chlorine	Benzylmethylamine	Cetyloxy
44	Chlorine	Aniline	Cetyloxy
46	Chlorine	Cyclohexylamine	Cetyloxy
47	Chlorine	Imidazole	Cetyloxy
48	Chlorine	Pyrazole	Cetyloxy
49	Chlorine	1H-1,2,4-Triazole	Cetyloxy
50	Chlorine	Benzimidazole	Methoxy
51	Chlorine	Indoline	Methoxy
52	Chlorine	Imidazole	Methoxy
53	Chlorine	Pyrrole	Methoxy

The above compounds were tested as outlined in section 4.1.1 against four strains of the yeast *Saccharomyces cerevisiae*:

The results obtained are as follows:

Compound	630G	TP7	839Y	017J
40	2 mm	-	2 mm	-
41	1 mm	-	1 mm	-
42	-	-	1 mm	1 mm
44	-	-	1 mm	-
46	1 mm	-	2 mm	-
47	1 mm	-	2 mm	-
48	1 mm	2 mm	2 mm	-
49	1 mm	2 mm	2 mm	-
50	2 mm	5 mm	5 mm	3 mm
51	1 mm	2 mm	2 mm	2 mm
52	1 mm	3 mm	2 mm	2 mm
53	3 mm	6 mm	6 mm	-

The indication of a good inhibitor, which warrants further investigation, would be one that exhibits a zone of inhibition of around 7mm or above. As all the above

compounds contain zones of inhibition below this value, antimicrobial success has not been demonstrated for these compounds. What has been shown, is the way that combinatorial libraries can be entered into various screening programmes, any lead structures identified, and then optimisation and re-screening undertaken using the techniques already developed.

4.2.2 Optimisation at the five position

Varying the structure of the substituent at the four position on the furan-2(5H)-one ring did not increase the antibacterial activity of the molecule (see sections 4.2.1.1 and 4.2.1.2). The next logical approach would be to pick the best amine substituent (in this case one of the original amine tested) and vary the substituent at the five position. Compound number 24 was selected (due to its success in a variety of different microbiological challenges and its favourable MIC) and it was decided to vary the length of the cetyloxy side chain. Five new 5-alkoxyfuran-2(5H)-ones were synthesises using the methods developed previously with lengths of 2, 4, 6, 9 and 12 carbon atoms (see section 7.3.3). Further reaction with 2-chlorobenzylamine in DMF produced the desired compound (see section 7.4.3.2). After purification, the compounds can be tested to determine the effect varying the side chain has on the antibacterial success of the molecule.

The compounds tested were as follows (Figure 4.8):

Compound 55 Compound 56 Compound 57

Compound 58 Compound 59
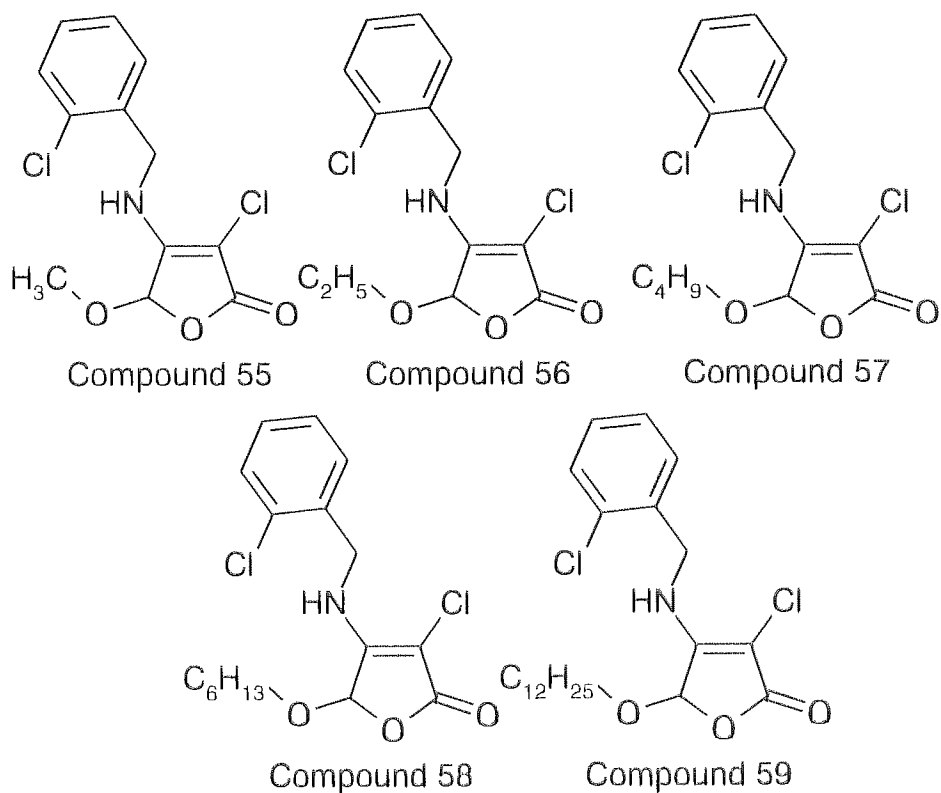
Figure 4.8 The structure of the five compounds synthesised for optimisation at the five position.

The four organisms used for the biological screening tests were:

- *Staphylococcus aureus* NCTC 6571 (*S. aureus*)
- *Enterococcus faecium* ATCC 10541 (*E. faecium*)
- Methicillin-resistant *Staphylococcus aureus* 96-7778 (MRSA)
- *Escherichia coli* DC0 (*E. coli*)

The results for the measured zones of inhibition are as follows:

Compound	*S. aureus*	*E. faecium*	MRSA	*E. coli*
55	2 mm	-	2 mm	-
56	2 mm	-	3 mm	-
57	2 mm	-	2 mm	-
58	-	-	-	-
59	-	-	-	-

In conclusion, the results from both of the optimisation studies have shown two things. Firstly, neither attempt to increase the antimicrobial success of the initial compounds synthesised has resulted in a greater zone of inhibition. Further work will be needed if the initial lead structure is to be refined any further for increased biological success. Secondly is the successful demonstration that this technique is suitable for the rapid synthesis of related analogues to potential lead structures. Although the newly synthesised compounds have not proved successful in this instance, further studies may prove more fruitful.

4.3 X-ray crystallographic studies resulting from this work

In the assembly and purification of the libraries of furan-2(5*H*)-ones within this section of work, a number of crystalline products have been formed. Selections of these have been suitable for analysis by x-ray crystallography. Each structure when fully elucidated will be published. The data for the structures has been collected by Dr Phil Lowe and Dr Carl Schwalbe at Aston University and will be published in due course.

These are the structures that have been submitted for x-ray crystallographic analysis:

3-Chloro-5-methoxy-4-(3-methyl-1*H*-pyrazol-1-yl)furan-2(5*H*)-one
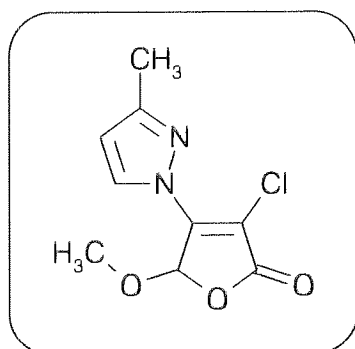
4-[Benzyl(methyl)amino]-3-bromo-5-menthyloxyfuran-2(5*H*)-one
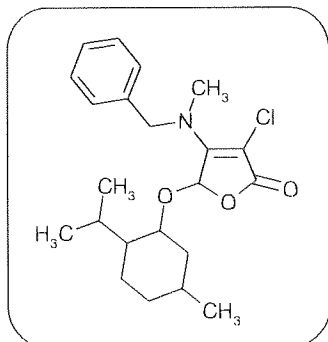
5-(Benzyloxy)-3-chloro-4-(3-methyl-*1H*-pyrazol-1-yl)-furan-2(5*H*)-one
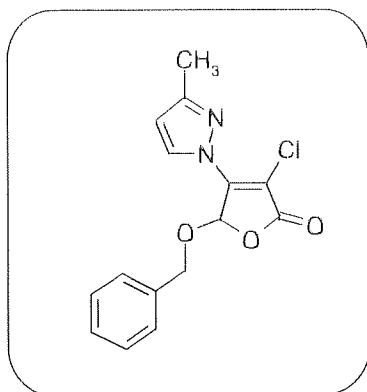
3-Bromo-5-menthyloxy-4-piperidin-1-ylfuran-2(5*H*)-one
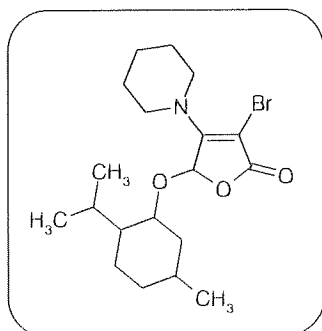
4-[Benzyl(methyl)amino]-3-bromo-5-menthyloxyfuran-2(5*H*)-one
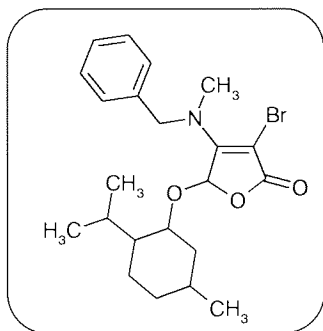
4.4 Summary and conclusions

In summary, it has been shown that the furan-2(5*H*)-one structure is a good template for use in the assembly of combinatorial libraries for the production of compounds for biological evaluation. Methods for the rapid purification of a selection of products from the two libraries have been developed, and the purified compounds entered for biological screening. Both antibacterial and CCK-B receptor antagonism studies have been performed, showing the versatility a combinatorial library has to differing biological tests.

Once the screening results had been analysed, the approach was switched to using the techniques gained in chapters two and three to refine the lead structures for re-screening. Although the refined structures tested from adaptation at both the four and five positions did not show increased antimicrobial activity, the methods developed were promising as they produced many compounds for screening, in sufficient purity and in the minimum amount of time possible.

Chapter 5 : Results and Discussion

Novel synthesis of 3-alkoxy-4-amino-5-arylfuran-2(5*H*)-ones

5.1 Development of the chemical approach towards the synthesis of 3,4,5-trisubstituted furan-2(5*H*)-ones

5.1.1 Mimicking the NSAID template with furanones

In chapter one, it has been seen that the development of new NSAIDs still remains an important chemical goal. In chapter two, the mimicking of two commercially available COX-2 selective compounds (33) & (34) was attempted (see section 2.1.2.2). This chapter will combine that work, with the additional goal of attempting to use the furan-2(5*H*)-one template to mimic another important NSAID moiety – the arylalkanoic acid. The arylalkanoic acids can be represented by the following moiety (71) (Figure 5.1).[45] This moiety could be mimicked using furanones by linking the furanone group to an aromatic ring via the five position and attaching a hydroxyl group to the four position (72).

Rofecoxib (33)   Celecoxib (34)   Attempted bis-substituted Furan-2(5*H*)-one (35)

(71)   (72)

Figure 5.1 Using the same approach as taken in chapter two for the mimicking of commercially available COX-2 NSAIDs, the arylalkanoic acid template (71) could be mimicked in furan-2(5H)-ones (72).

It has already been shown in chapter two that it is possible to directly attach an aromatic group to the five position of the furan-2(5*H*)-one ring in an attempt to mimic the COX-2 selective NSAIDs (see section 2.1.2.2). If it is possible to replace the chlorine atom at the four position on the ring with a hydroxyl group, a library of different 5-arylated-4-hydroxyfuran-2(5*H*)-ones could be prepared and tested for COX (and selective COX-2) inhibition.

5.1.2   Attempted formation of 4-hydroxyfuran-2(5*H*)-ones

The first part of the synthesis of the desired structure for NSAID screening has been achieved in chapter two (37). All that remains is to replace the chlorine atom at the four position with a hydroxyl group (73). This was attempted in a variety of different ways, of which the main one is detailed in section 7.5.1. Initially it appeared a simple mater of replacing the C4 chlorine atom with a hydroxyl group. It was speculated that this could be easily achieved by using a base, for example sodium hydroxide. This would form the desired 4-hydroxy group and the corresponding salt (Figure 5.2).
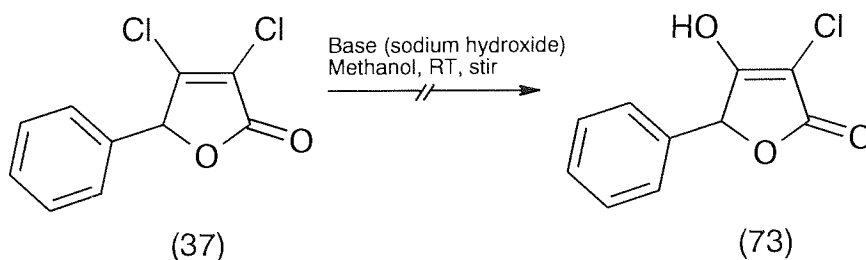
Figure 5.2 The attempted formation of 3-chloro-4-hydroxy-5-phenylfuran-2(5H)-one

In no case could the correct molecular ion peak be found in the APCl+ or APCl- mass spectrum. In both mass spectra, compound 37 could no longer be detected, suggesting that a reaction had taken place. Further analysis was performed and this is detailed in section 5.1.3.

5.1.3 The resulting product and its identification by ^{13}C-NMR prediction software

The desired product failed to be formed in all cases, but in most cases an alternative product was formed. This had a peak in the proton NMR at around δ 3.5, which integrated to three protons. APCl+ mass spectroscopy studies indicated that one of the chlorine atoms had been replaced (due to a change in the isotopic pattern). It was initially thought that the methanol that was used as a solvent had acted as a nucleophile and reacted at the Michael position forming the 4-methoxy product. This theory was supported by the mass spectrum, as the correct molecular ion peak was present for this product. This theory also fitted with the proton nuclear magnetic resonance data with respect to δ values and integrals.

This theory was only found to be incorrect on examination of the carbon nuclear magnetic resonance spectrum. When the spectrum for the new product was compared to the spectrum for the starting compound, the change in δ values for the C3 and C4 carbons were not as expected. Both of the changes in δ values changed in the opposite direction to what was predicted by computer simulation. This can be best illustrated below (Figure 5.3).

Starting material   4-Methoxy product
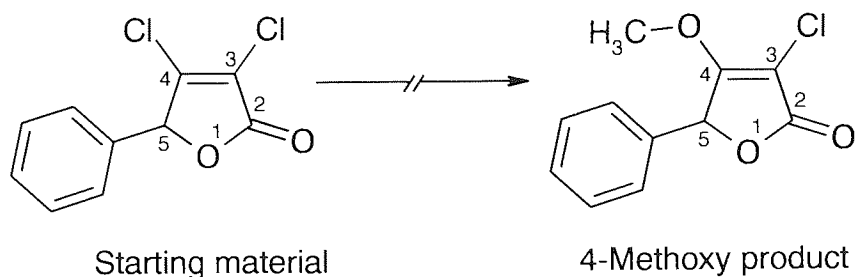
Ring Position	Starting Material	4-Methoxy product	Actual Product
2	157.76	159.93	163.36
3	124.32	107.11 (↓)	145.17 (↑)
4	156.76	173.77 (↑)	132.92 (↓)
5	77.83	70.50	121.60

Figure 5.3 The expected and observed changes in ^{13}C-NMR chemical shift for the carbons of the furan-2(5H)-one ring.

It is clear from mass spectroscopy studies that the resultant product contains one chlorine atom due to the characteristic isotopic pattern. If the chlorine atom at the C4 centre is not substituted (indicated by the incorrect changes in the δ value of the two carbon centres (the C3 and C4 centres) in the carbon nuclear magnetic resonance spectrum), could the methoxy group be at the C3 position? Re-running the prediction for the δ values for the two carbon centres this time produced the correct changes in the δ values (Figure 5.4).

Starting material   3-Methoxy product
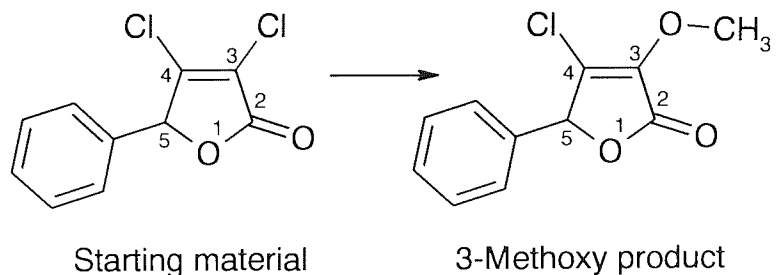
Ring Position	Starting Material	3-Methoxy product	Actual Product
2	157.76	161.10	163.36
3	124.32	141.38 (↑)	145.17 (↑)
4	156.76	134.02 (↓)	132.92 (↓)
5	77.83	79.62	121.60

Figure 5.4 Prediction software suggests substitution at the C3 centre of the furan-2(5H)-one ring.

In addition, this theory can be supported by further reaction by nitrogen nucleophiles at the four position of the ring. If further reaction occurs, detectable by APCI+ mass spectroscopy, the C4 chlorine atom is still present in the molecule. This means that the methoxy group must be at the three position on the ring. If, however, no further reaction is seen on the addition of a nitrogen nucleophile, it can be postulated that the methoxy group may be at the C4 centre (Figure 5.5). This idea is further investigated in section 5.2.
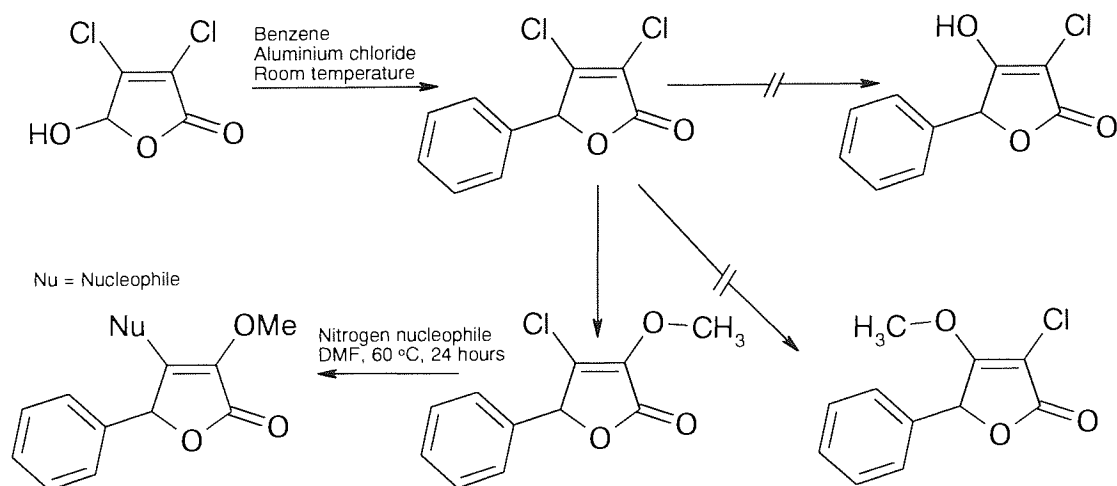
Figure 5.5 The attachment of the methoxy group at the three position on the furan-2(5H)-one ring and the subsequent attack of nitrogen nucleophiles at the free four position.

It was postulated that the oxygen nucleophilic attack at the three position on the ring was caused by the ring becoming open by the addition of a base. This correlates nicely with Mowry's work, which was reviewed in chapter two. He found out that phenol could react with the open-chain form of mucochloric acid and form the 3-alkoxy closed-ring product.[81] If in this case the sodium hydroxide could open the ring (even without the 5-hydroxy group that is present in mucochloric acid), 3-alkoxy substitution with oxygen containing nucleophiles would be expected. It is known from work completed by Jähnisch *et al.* (see section 3.2.1) that opening of the furanone ring containing a 5-alkoxy substituent is possible.[97] Mechanistically, the reaction would proceed as outlined in Figure 5.6.
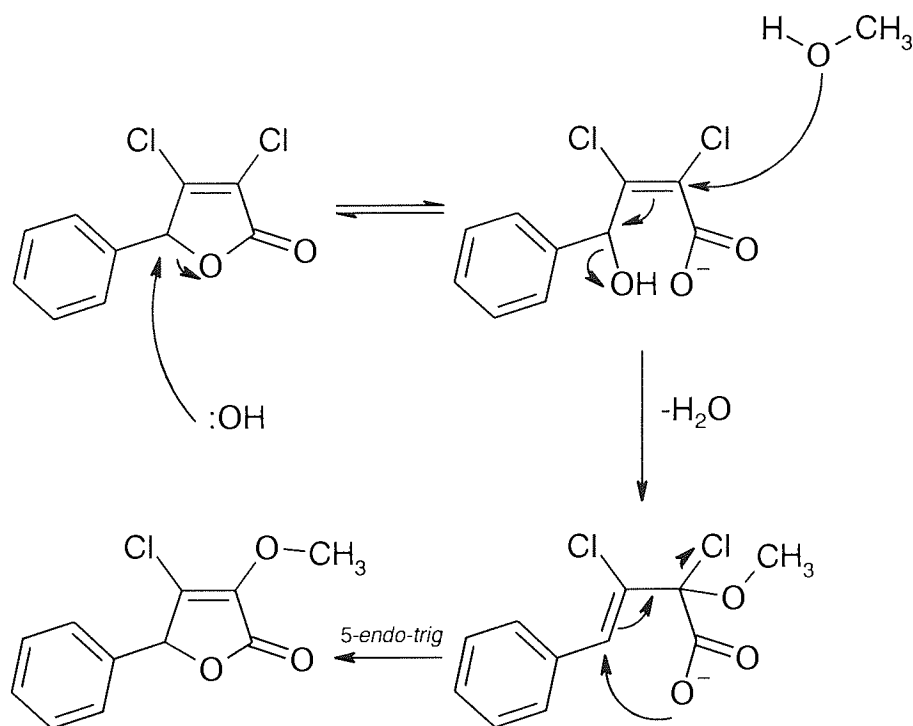
Figure 5.6 Proposed mechanism for the nucleophilic attack of oxygen nucleophiles at the C3 centre of furan-2(5H)-ones by using base to open the ring.

5.2 Formation of 3,4,5-trisubstituted furan-2(5*H*)-ones

5.2.1 Initial investigation into trisubstitution

To confirm the structure of the 3-methoxyfuran-2(5*H*)-one produced in section 5.1, further reaction with a nitrogen nucleophile (benzylmethylamine) was attempted (Figure 5.5). Analysis by APCI+ mass spectroscopy was successful in detecting the presence of the molecular ion peak (see section 7.5.3).

As the structure of the resulting product is now known, it has enabled the introduction of diversity to the three positions on the ring. Previously, diversity could only be

introduced in two of the three positions (the four and five positions), with variation at the three position limited to a chlorine, a bromine or a hydrogen atom, depending on which starting furan-2(5*H*)-one was used. This may be an important discovery as it may be necessary to introduce diversity at this position after preliminary screening results have been undertaken.

To investigate the scope of the introduction of diversity to the three position on the ring, it was first beneficial to try alternative oxygen containing molecules other than methanol. Using the method of using the alcohol as both solvent and nucleophile, four other oxygen nucleophiles were tested. These four nucleophiles were ethanol, propan-2-ol, butan-1-ol and 2-pentanol. After reaction with the relevant oxygen containing nucleophile, further reaction with benzylmethylamine was attempted to aid detection in the APCl+ mass spectrometer. In addition to the four alcohols, phenol was also attempted. This, however, was not used as the solvent so 1,2-dichloroethane was used instead and one and a half equivalents of phenol were added. This was detected in the same manner as the other alcohols (Figure 5.7) (see section 7.5.2).
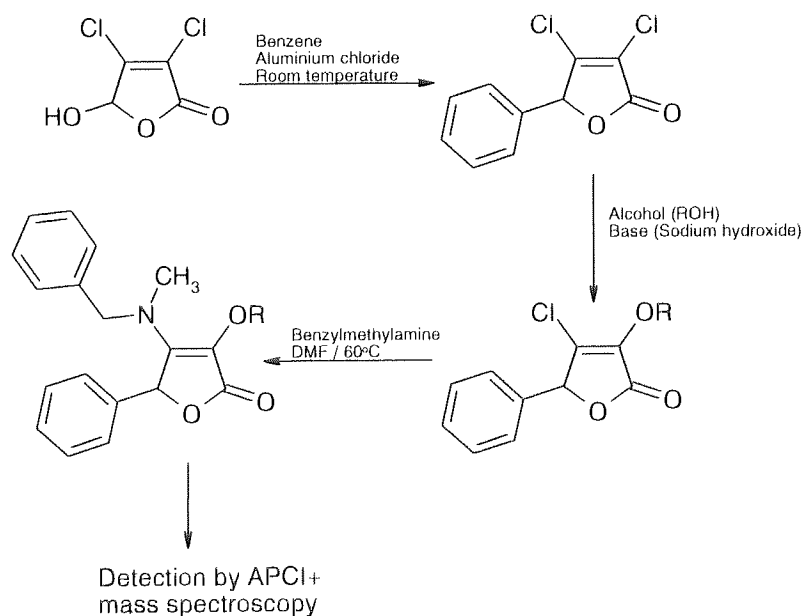

Figure 5.7 The reaction with oxygen containing nucleophiles at the 3 position and the subsequent detection by APCI+ mass spectroscopy, using the nitrogen nucleophile benzylmethylamine.

Success was achieved in two of the five cases (with phenol and butan-1-ol). This indicated that this method is suitable for the introduction of a range of diversity to the three position (see section 7.5.2). Further studies will be needed to ascertain the full range of diversity that can be added.

5.2.2 Synthesis of a series of trisubstituted furan-2(5H)-ones

The ability to produce a series of trisubstituted compounds needs to be further investigated. It has been shown in section 5.2.1 that a range of alkoxy substituents can be added to the three position of the ring. Attention now needs to be switched to find out if nucleophilic attack at the four position by an entire series of nitrogen nucleophiles is still possible with an alkoxy group at the three position. To investigate this, it was decided to form a series of trisubstituted furan-2(5H)-ones using a variety of different amines. Primarily, a phenyl group will be introduced to the five position of the ring. Secondly, the ring will be opened with the use of base, and diversity can be added to the three position of the ring by reaction with an oxygen nucleophile, followed by closure of the ring by acid. Lastly, reacting with different nitrogen nucleophiles on the closed ring will introduce diversity to the remaining four position on the ring (Figure 5.8).
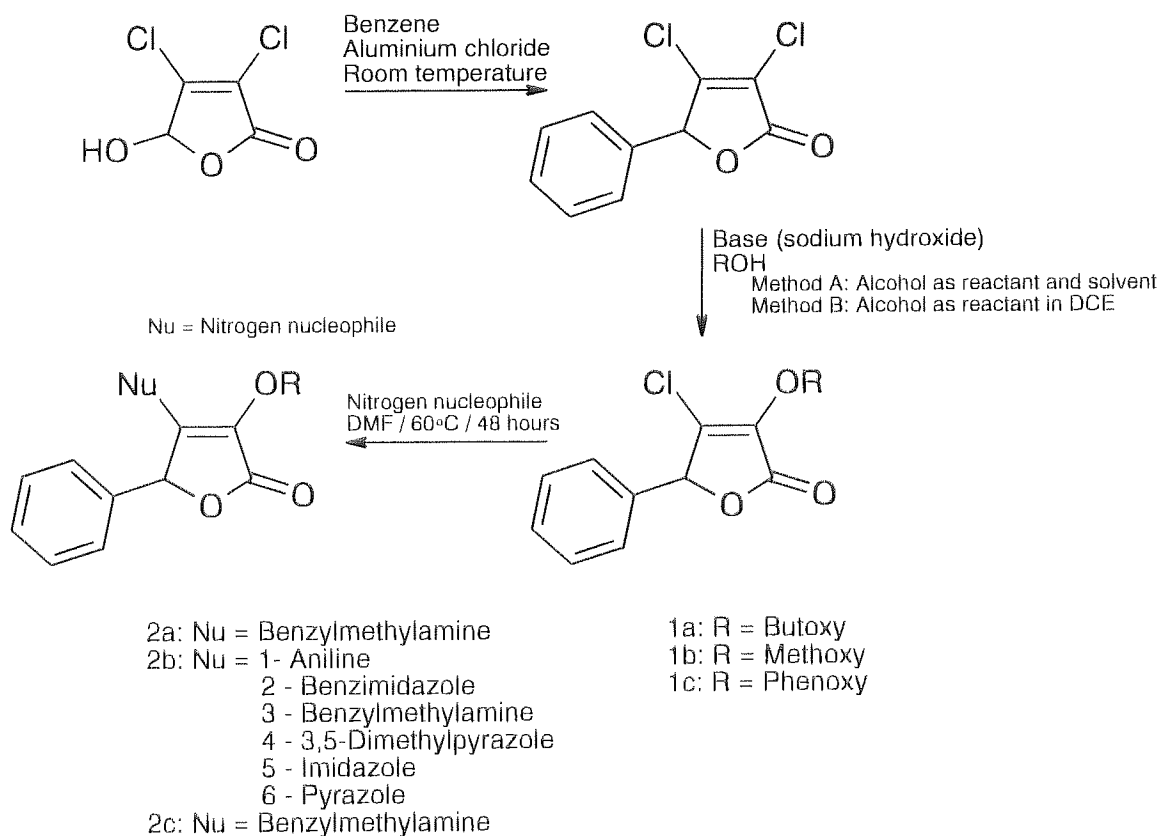
2a: Nu = Benzylmethylamine
2b: Nu = 1- Aniline
        2 - Benzimidazole
        3 - Benzylmethylamine
        4 - 3,5-Dimethylpyrazole
        5 - Imidazole
        6 - Pyrazole
2c: Nu = Benzylmethylamine

1a: R = Butoxy
1b: R = Methoxy
1c: R = Phenoxy

Figure 5.8 A summary of the reaction sequence to produce a mini series of trisubstituted furan-2(5H)-ones.

A sample of each of the reactions was submitted for APCI+ mass spectroscopy for identification of the molecular ion peak (see section 7.5.3). The results are tabulated below (including the successful results from the previous section):

Entry	3-Position	4-Position	Detection of product
1a	Butoxy	Chlorine	✓
1b	Methoxy	Chlorine	✓
1c	Phenoxy	Chlorine	✓
2a	Butoxy	Benzylmethylamine	✓
2b1	Methoxy	Aniline	✓
2b2	Methoxy	Benzimidazole	✓
2b3	Methoxy	Benzylmethylamine	✓
2b4	Methoxy	3,5-Dimethylpyrazole	✓
2b5	Methoxy	Imidazole	✓
2b6	Methoxy	Pyrazole	✓
2c	Phenoxy	Benzylmethylamine	✓

As it can be seen from the above table, all eight of the trisubstituted reaction products (along with the three intermediates) were detected in the APCI+ mass spectrometer (see section 7.5.3), indicating the success of this method in the formation of a series of trisubstituted furan-2(5*H*)-ones. Two of the series, (74) & (75), were selected for purification by a combination of column chromatography and preparative thin layer chromatography and submitted for proton nuclear magnetic resonance spectroscopy (and carbon nuclear magnetic resonance spectroscopy in one case) (Figure 5.9) (section 7.5.3).
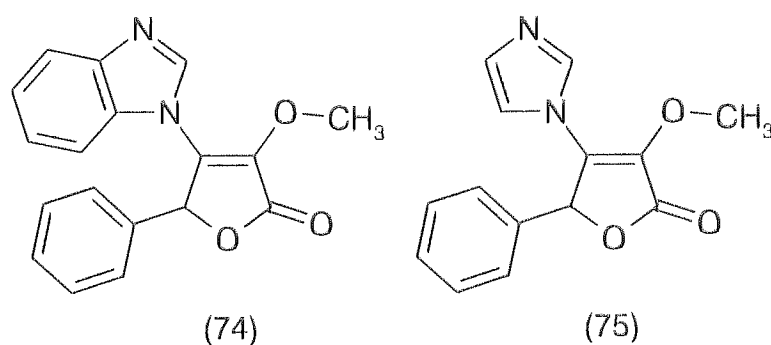

(74)　　　　　　　　(75)

Figure 5.9 The structure of the two 3,4,5-trisubstituted furan-2(5H)-ones purified and characterised by nuclear magnetic resonance spectroscopy.

It is clear, therefore, that the introduction of a more bulky group to the three position on the ring does not appear to hamper the subsequent reaction with nitrogen nucleophiles at the four position. This has been demonstrated to be true for both the mini series investigating the possibility of trisubstitution with different amines with a methoxy group at the three position, and with butan-1-ol and phenol at the three position and benzylmethylamine at the four position.

5.3 Biological screening

5.3.1 NSAID screening

To investigate the possible anti-inflammatory activity of the arylated furanones, screening will be necessary. Initially, one compound was submitted for screening and further screening can be attempted if deemed appropriate (Figure 5.10). The compound selected was the bis-substituted *m*-xylene product as this was one of the purer compounds formed and the only arylated furanone that the crystal structure is known. The screening was performed by Associate Professor Jintana Sattayasai, from the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand 40002.
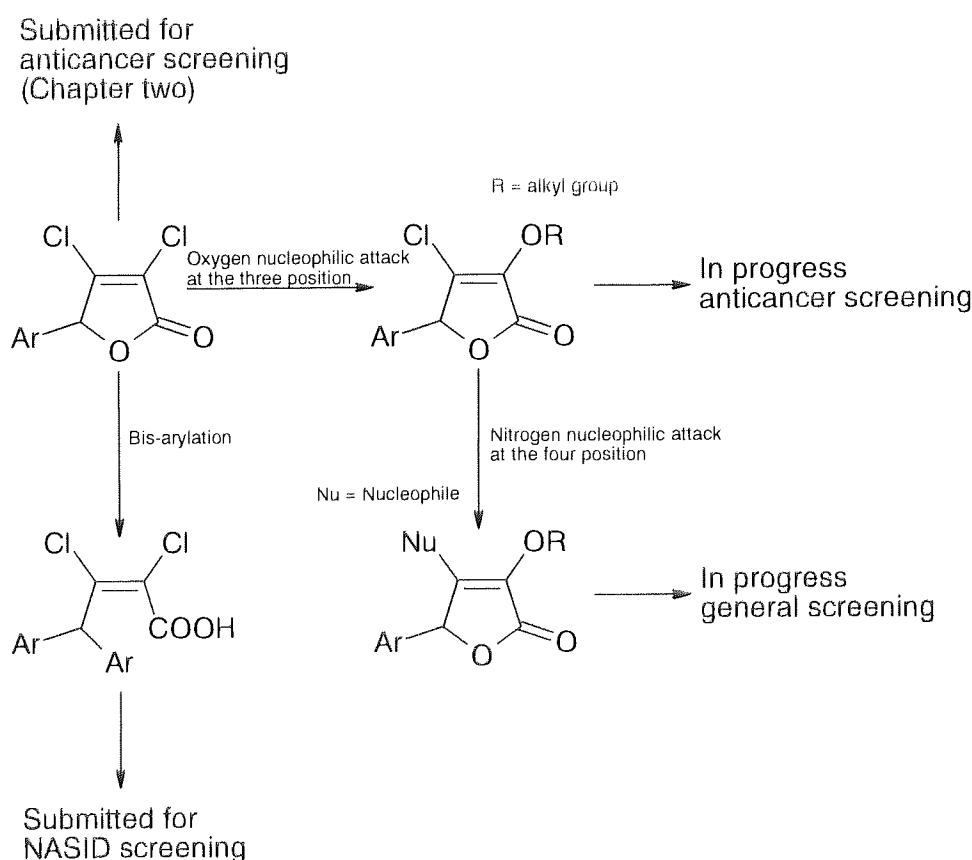

Figure 5.10 The different biological screening tests the arylated furan-2(5H)-ones can be entered into.

Initial anti-inflammatory screening tests were performed on the bis-substituted *m*-xylene compound comparing its activity with ibuprofen. Unfortunately, as no anti-inflammatory activity was seen, it wasn't deemed appropriate to perform COX-2 selective studies.

5.3.2   General screening

The mini series of trisubstituted furan-2(5*H*)-ones will be entered into a programme of general screening (Figure 5.10). Results from this, once completed, may indicate if further optimisation would be beneficial. The general screening will be performed by The Janssen Research Foundation at Janssen Pharmaceuticals N. V., Turnhoutseweg 30, B2340, Beerse, Belgium.

The 3-alkoxy-5-aryl-4-halofuran-2(5*H*)-ones can also be isolated and entered into an anticancer screening programme. This, as with the 5-alkoxyfuran-2(5*H*)-ones in chapter two will utilise small reaction intermediates to increase the chance of finding anticancer lead structures (see section 2.3).

In addition to this, the results from the biological screening on the libraries in chapter four may be optimised further by using the methods developed here to add diversity at the three position of the furanone ring.

5.4   Summary and conclusions

In summary, in this and the previous three chapters, it has been shown that the furan-2(5*H*)-one ring can undergo a variety of reactions. Although it has not been possible in this chapter to synthesise the desired compounds for NSAID testing (the 4-hydroxyfuran-2(5*H*)-ones), an interesting collection of novel 3,4,5-trisubstituted furan-2(5*H*)-ones has been produced. These compounds, once evaluated biologically, may be a useful source for lead structure discovery. In the assembly of the trisubstituted series, a number of compounds suitable for entry into the anticancer

screening programme have also been synthesised. These compounds can be analysed as before.

In addition to the variety of compounds synthesised, the technique of adding diversity to the three position on the furan-2(5*H*)-one ring has been explained. This may have an important role to play in the optimisation of lead structures obtained from the initial biological screening in chapter four, through structure activity relationships.

Chapter 6 : Thesis conclusion and further work

6.1 Thesis conclusion

In conclusion, the furan-2(5H)-one ring has proved to be a very versatile molecule. It has been shown that many important natural products contain the furan-2(5H)-one structure and that a large number of these products possess biological activity. Methods for adding diversity at all three sites have been investigated with the intention of using these reactions in combinatorial library synthesis. Initial investigation into library synthesis was positive, leading to a larger library being attempted.

Rapid purification methods were developed, and fully characterised products entered into a programme of antimicrobial and CCK-B antagonistic activity screening. Structure refinement of potential antimicrobial lead structures was attempted, demonstrating the ability the reaction sequences have for structure refinement experiments.

In addition to this, some of the smaller building blocks synthesised in the first stage were entered into an anticancer screening programme. This, although unsuccessful due to the high toxicity of the compounds found during *in vivo* testing, did

demonstrate that anticancer screening could be successfully performed on the small building blocks that would be inevitably made during the synthesis of any larger combinatorial library. This increases the likelihood of identifying a lead structure with minimal extra cost and time. However, the optimum 5-alkoxy chain length for *in vitro* activity against MAC13 and MAC16 cell lines for 5-alkoxy-3,4-dihalofuran-2(5*H*)-ones was successfully identified by combining data from compounds synthesised in this work, with the results from other groups.

Finally, methods for trisubstitution were identified during the attempted synthesis of potential COX-2 selective NSAIDs. These molecules were an important discovery as previously, diversity was limited at the three position by the starting mucohalic acid. These trisubstituted furan-2(5*H*)-ones will be entered into a general screening programme. One bis-substituted furan-2(5*H*)-one was screened for NSAID activity but this was unsuccessful. Reaction intermediates in this section, as with the initial libraries, will also be screened for anticancer activity.

This work has been an important start in the development of the synthesis of novel 3,4,5-trisubstituted furan-2(5*H*)-one libraries and their subsequent screening. Many further lines of research have been identified (see section 6.2) and the potential now exists to synthesise a novel biologically active furan-2(5*H*)-one.

6.2 Further work arising from this thesis

Over the last few chapters, it has been shown that the furan-2(5*H*)-one structure is highly suitable for use in the assembly of combinatorial libraries, produced for subsequent biological screening. In the initial investigations into possible reactions that could be used, and the screening of purified reaction products, many further lines of research have been identified. This section will highlight these points, enabling further work to be undertaken, based on the results in this thesis.

6.2.1 Further investigation into suitable reactions for inclusion into a combinatorial library

Initially, work needs to be focused towards looking at further reactions, which can be included in the assembly of various combinatorial libraries. This investigation has a two-fold objective. Primarily, new reactions can be found to increase the number and diversity of compounds it is possible to synthesise by combinatorial methods and subsequently include in biological screening programmes. Secondary to this, is the increased structure refinement ability that will be available if the number of viable reactions at the various sites on the furan-2(5H)-one scaffold is increased.

It will be necessary to increase the number of different viable reactions at all three of the sites for attachment on the furan-2(5H)-one scaffold. For example, developing a method for the use of isocyanates at the five position, more reliable methods for the nucleophilic attack of sulphur and carbon nucleophiles at the four position and increasing the number of reactions at the three position will all greatly increase the potential success of furan-2(5H)-ones in biological screening tests. Also, it may be beneficial to develop new reactions, in an attempt to produce an anticancer prodrug based on the combined *in vitro* testing data for the optimum 5-alkoxy chain length of 5-alkoxy-3,4-dihalofuran-2(5H)-ones (see section 2.3.2, Figure 2.32).

6.2.2 Increasing the range of biological screening tests

So far, interest has concentrated on screening for antimicrobial action and cholecystokinin receptor antagonism. A few compounds have been entered into screening tests for anti-cancer activity and the possibility of COX-2 inhibition has been examined for one furan-2(5H)-one. If the number of biological screening tests is increased, the possibility of obtaining a lead structure for further structure refinement would be greatly increased. There will be a great number of screening tests, suitable for investigation with the advantage that once the library has been synthesised in sufficient quantity, any new screening test deemed suitable would not require re-synthesising of the library. This approach, while decreasing the time taken to acquire

a lead structure, would also keep the cost down. In addition to this, derivatives of the open chain bis-substituted furan-2(5H)-ones could be synthesised and entered into an antihistamine (H1) screening programme (see section 2.1.2.2, Figure 2.15).

6.2.3 Increase the number of suitable compounds for COX-2 screening

Currently, only one compound has been selected for COX-2 screening. This was partly due to the failure of the initial line of research which attempted to attach a hydroxyl group to the four position on the furan-2(5H)-one ring, thus mimicking the structure of the arylalkanoic acid non-steroidal anti-inflammatory drugs. In the quest for suitable electron rich molecules to attach to the furan-2(5H)-one ring, it was found that bis-substitution occurs in a number of cases. This produced compounds that bear a similarity in structure to the modern COX-2 selected compounds rofecoxib and celecoxib (see section 2.1.2.2). Although the synthesised compound that was submitted for COX-2 screening failed to produce any activity, derivatives of this compound may be more successful.

Methods, therefore, need to be developed to form derivatives of the bis-substituted furan-2(5H)-ones (preferably using a combinatorial approach) and the resulting compounds entered into a COX-2 screening programme. If the work in section 6.2.2 has been completed, any purified compound obtained from the work in this section can also be entered into a variety of screening tests, increasing the possibility of lead structure discovery and possibly aiding structure activity refinement.

6.2.4 Following up of any outstanding screening results

Finally, any screening results which are outstanding at the writing of this thesis need to be followed up. These are the anticancer screening results of the intermediates in the trisubstituted series, and the general screening results for the purified trisubstituted furan-2(5H)-ones (see section 5.3.2).

Chapter 7 : Experimental

The nuclear magnetic resonance spectra were all recorded on a Bruker AC250 spectrometer at 250.1 MHz for proton spectra and 62.9 MHz for carbon spectra. (The following abbreviations apply: s = singlet, bs = broad singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet.) All nuclear magnetic resonance spectra were referenced to the deuterated chloroform used as the solvent. Mass spectroscopy was performed in either atmospheric pressure chemical ionisation (APCI) mode, electrospray (ES) mode or electron impact (EI) mode on a Hewlett-Packard HP5989B MS Engine apparatus using a HP 59987A API-electrospray LC/MS interface. Infrared spectra were recorded on a Mattson 3000 FT-IR Spectrophotometer. All infrared spectra were performed in chloroform and data is given for all peaks with a wavenumber greater than 1500 cm^{-1}. Column chromatography was performed using Prolabo 60 silica gel. Thin layer chromatography (TLC) was performed using aluminium backed Whatman silica gel plates containing a fluorescent indicator, which was visible under ultraviolet light at 254 nm. Preparative thin layer chromatography was performed on Aldrich 1000 μm silica gel glass backed (20 cm x 20 cm) plates containing a fluorescent indicator, which was visible under ultraviolet light at 254 nm. A 1% solution of potassium permanganate was used when appropriate to develop the thin layer chromatography plates. Elemental analysis was performed by Butterworth Laboratories (Middlesex). Where general compound names are given, the term 3,4-dihalo is used to refer to the

4-chlorofuran-2(5*H*)-ones as well as the 3,4-dichloro and 3,4-dibromofuran-2(5*H*)-ones. The same principle also applies to general names containing the term 3-halo, which is used to refer to compounds containing 3-hydro as well as 3-chloro and 3-bromo groups. Reference numbers for the biological analysis are given for each purified product where appropriate and are summarised in the following table.

Biologically tested molecules reference table:

Reference number	Compound name	Experimental page number
1	3-Chloro-4-(2,3-dihydro-1*H*-indol-1-yl)-5-methoxyfuran-2(5*H*)-one	227
2	3-Chloro-5-methoxy-4-(3-methyl-1*H*-pyrazol-1-yl)furan-2(5*H*)-one	228
3	3-Chloro-5-methoxy-4-[(3-morpholin-4-ylpropyl)amino]furan-2(5*H*)-one	219
4	3-Chloro-5-methoxy-4-(4-phenylpiperidin-1-yl)furan-2(5*H*)-one	230
5	3-Chloro-5-methoxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one	232
6	3-Chloro-4-(2,6-dimethylmorpholin-4-yl)-5-methoxyfuran-2(5*H*)-one	225
7	4-[(1-Benzylpiperidin-4-yl)amino]-3-chloro-5-methoxyfuran-2(5*H*)-one	218
8	4-[Benzyl(methyl)amino]-3-bromo-5-cetyloxyfuran-2(5*H*)-one	196
9	3-Chloro-5-methoxy-4-(1*H*-1,2,4-triazol-1-yl)furan-2(5*H*)-one	234
10	3-Bromo-4-(1*H*-imidazol-1-yl)-5-menthyloxyfuran-2(5*H*)-one	201
11	3-Bromo-5-cetyloxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one	198
12	4-(*sec*-Butylamino)-3-chloro-5-methoxyfuran-2(5*H*)-one	223
13	3-Chloro-5-methoxy-4-(4-phenylpiperazin-1-yl)furan-2(5*H*)-one	229
14	3-Bromo-5-menthyloxy-4-piperidin-1-ylfuran-2(5*H*)-one	204
15	5-(Benzyloxy)-3-bromo-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)furan-2(5*H*)-one	192
16	4-(1*H*-Benzimidazol-1-yl)-5-cetyloxy-3-chlorofuran-2(5*H*)-one	209
17	3-Chloro-5-methoxy-4-[4-(3-phenylpropyl)piperidin-1-yl]furan-2(5*H*)-one	231

| 18	5-Cetyloxy-3-chloro-4-(1*H*-1,2,4-triazol-1-yl)furan-2(5*H*)-one	215
19	4-[Benzyl(methyl)amino]-3-chloro-5-methoxyfuran-2(5*H*)-one	221
20	4-(4-Benzylpiperazin-1-yl)-3-chloro-5-methoxyfuran-2(5*H*)-one	222
21	3-Chloro-5-methoxy-4-(2-toluidino)furan-2(5*H*)-one	233
22	3-Bromo-5-methoxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one	208
23	4-(4-Benzylpiperazin-1-yl)-5-cetyloxy-3-chlorofuran-2(5*H*)-one	210
24	5-Cetyloxy-3-chloro-4-[(2-chlorobenzyl)amino]furan-2(5*H*)-one	211
25	5-(Benzyloxy)-3-bromo-4-piperidin-1-ylfuran-2(5*H*)-one	193
26	3-Bromo-5-methoxy-4-piperidin-1-ylfuran-2(5*H*)-one	207
27	4-[Benzyl(methyl)amino]-3-bromo-5-menthyloxyfuran-2(5*H*)-one	200
28	5-Cetyloxy-3-chloro-4-(cyclohexylamino)furan-2(5*H*)-one	212
29	4-(1*H*-Benzimidazol-1-yl)-3-bromo-5-cetyloxyfuran-2(5*H*)-one	195
30	3-Bromo-5-cetyloxy-4-piperidin-1-ylfuran-2(5*H*)-one	197
31	4-(1*H*-Benzimidazol-1-yl)-3-bromo-5-methoxyfuran-2(5*H*)-one	206
32	4-[Benzyl(methyl)amino]-3-chloro-5-menthyloxyfuran-2(5*H*)-one	216
33	3-Bromo-5-menthyloxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one	205
34	3-Chloro-4-(2,6-dimethylmorpholin-4-yl)-5-menthyloxyfuran-2(5*H*)-one	217
35	5-Cetyloxy-3-chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)furan-2(5*H*)-one	213
36	4-(1*H*-Benzimidazol-1-yl)-3-chloro-5-methoxyfuran-2(5*H*)-one	220
37	3-Chloro-4-(dicyclohexylamino)-5-methoxyfuran-2(5*H*)-one	224
38	4-(1*H*-Benzimidazol-1-yl)-3-bromo-5-menthyloxyfuran-2(5*H*)-one	199
39	Ethyl 4-(2-cetyloxy-4-chloro-5-oxo-2,5-dihydrofuran-3-yl)piperazine-1-carboxylate	214
40	5-Cetyloxy-3-chloro-4-[(2-chlorobenzyl)amino]furan-2(5*H*)-one	238
41	4-(Benzylamino)-5-cetyloxy-3-chlorofuran-2(5*H*)-one	236

7.1 Furan-2(5*H*)-one building blocks

7.1.1 Determination of the tautomeric configuration of the two commercially available mucohalic acids by proton nuclear magnetic resonance spectroscopy

Samples of the two starting mucohalic acids were analysed by proton nuclear magnetic resonance spectroscopy to determine their tautomeric structure in deuterated chloroform.
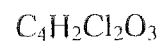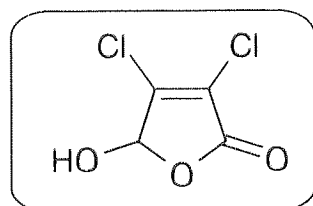
Mucobromic Acid:

$C_4H_2Br_2O_3$
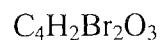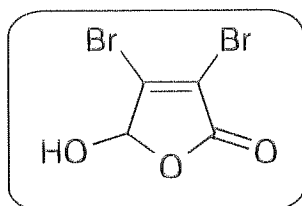
Molecular Weight: 257.87.

^{1}H NMR (CDCl$_3$): δ = 6.08 (s, 1H, C*H*), 4.07, (bs, 1H, O*H*) ppm.

Mucochloric Acid:


$C_4H_2Cl_2O_3$

Molecular Weight: 168.96.

^{1}H NMR (CDCl$_3$): δ = 6.09 (s, 1H, C*H*), 4.07, (bs, 1H, O*H*) ppm.

7.1.2 The synthesis of 4-chloro-5-hydroxyfuran-2(5*H*)-one

Method:

5 ml (5.8 g, 60 mmol) of furfural (2-furaldehyde) was mixed with 5 g of silica gel and 20 g of activated manganese (IV) oxide to form a grey powder. This powder was added to 100-200 ml of concentrated hydrochloric acid at 5-8 °C. The mixture was stirred and cooled with ice for 40 minutes. After this period, the product was extracted overnight with ether in a specially designed glass extraction unit (see section 2.1.1, Figure 2.4). The ether was then removed and dried with magnesium sulphate, and evaporated to dryness by rotary evaporation under vacuum to leave a yellow oil containing the desired furan-2(5*H*)-one along with many by-products.

4-Chloro-5-hydroxyfuran-2(5*H*)-one:

$C_4H_3ClO_3$
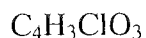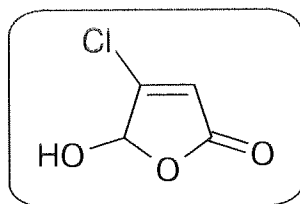
Molecular weight: 134.52.

## 7.2 Reactions of the aldehyde form of the mucohalic acids

7.2.1 Condensation (aldol) reactions

Method:

42 g (0.25 mol) of mucochloric acid was mixed with 1 equivalent of each of the compounds under investigation (acetophenone or nitromethane), in 150 ml of methanol. 15 g of sodium hydroxide in 150 ml of water was prepared and added slowly to each of the above mixtures. Each reaction was left to stand at room temperature overnight. Each reaction was then poured into a beaker of ice water containing excess hydrochloric acid. The oily precipitate formed in each case was dried and extracted in ethanol. Analysis was performed by proton (and in one case carbon) nuclear magnetic resonance spectroscopy.

1. Acetophenone:

3,4-Dichloro-5-(2-oxo-2-phenylethyl)furan-2(5*H*)-one:
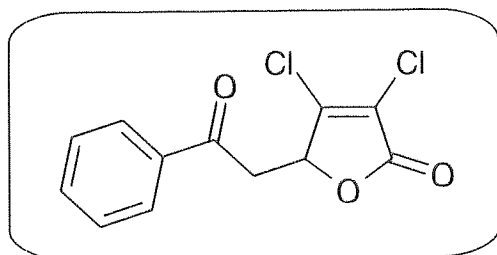
$C_{12}H_8Cl_2O_3$
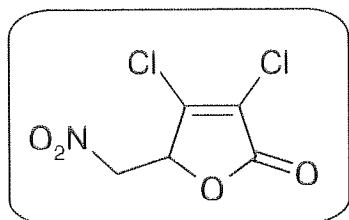
Molecular weight: 271.10.

APCI+ MS: m/z = 271/273/275.

^{1}H NMR (CDCl$_3$): δ = 7.95-7.91 (m, 2H, *o*-H̲), 7.65-7.58 (m, 1H, *p*-H̲), 7.52-7.45 (m, 2H, *m*-H̲), 5.73 & 5.69 (dd, *J* = 7.8 & 7.9 Hz, 1H, CH̲), 3.61-3.35 (m, 2H, CH̲$_2$) ppm.

^{13}C-NMR (CDCl$_3$): δ = 193.71 (CH$_2$C=O), 164.85 (OC=O), 151.95 (CHCCl), 135.70 (aryl-CC=O), 134.05 (p-C), 128.84 (m-C), 128.13 (o-C), 121.35 (ClCC=O), 78.01 (OCH), 40.00 (CH$_2$) ppm.

2. Nitromethane:

3,4-Dichloro-5-(nitromethyl)furan-2(5H)-one:

C$_5$H$_3$Cl$_2$NO$_4$

Molecular weight: 211.99.

^{1}H NMR (CDCl$_3$): δ = 5.65-5.61 (m, 1H, CH), 5.00-4.62 (m, 2H, CH$_2$) ppm.

7.2.2 Electrophilic aromatic substitution reactions

Method A: Readily reactive aromatic systems

A mixture of 15.7 g (111 mmol) phosphorous pentoxide and 92.8 g (947 mmol) of phosphoric acid was prepared. 17.1 g (101 mmol) of mucochloric acid was added along with 10.8 g (100 mmol) of anisole. The reaction mixture was left to stir overnight at room temperature. After this period 19 g (521 mmol) of concentrated hydrochloric acid was added and the whole mixture poured onto 62.5 g of ice. The product was extracted in toluene and dried with magnesium sulphate. The excess solvent was removed by rotary evaporation and analysed by proton nuclear magnetic resonance spectroscopy.

3,4-Dichloro-5-(4-methoxyphenyl)furan-2(5*H*)-one:
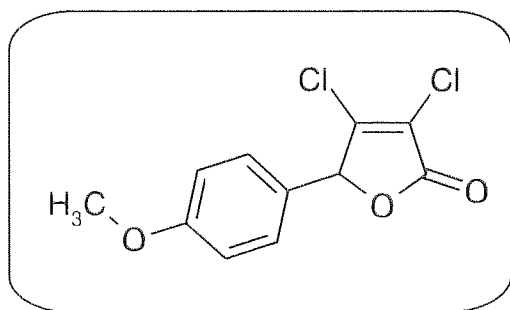
$C_{11}H_8Cl_2O_3$

Molecular weight: 259.09.

^{1}H NMR (CDCl$_3$): δ = 7.33-7.12 (m, 2H, *m*-H̲), 6.96-6.90 (m, 2H, *o*-H̲), 5.79 (s, 1H, CH̲), 3.80 (s, 3H, *p*-OCH̲$_3$) ppm.

Yield = 76%.

Method B: Less reactive aromatic systems acting as both reactant and solvent

16.38 g (96.9 mmol) of mucochloric acid (or 25 g of mucobromic acid) was placed in a 500 ml conical flask. Around 250 ml of the desired solvent was added and the mixture was stirred. 20 g (150 mmol) of powdered aluminium chloride was slowly added to the mixture whilst stirring and a drying tube was attached to the top of the conical flask. The reaction was left overnight and then the whole mixture was poured into a beaker containing 125 g of ice and 38 g (1.04 mol) of concentrated hydrochloric acid. The organic phase was separated from the aqueous phase and then a second extraction was performed on the aqueous phase using a further portion of solvent. The two organic layers were combined and dried with magnesium sulphate and the solvent removed under vacuum by rotary evaporation. Each of the products was fully dried in a desiccator and analysed by proton (and in some cases carbon) nuclear magnetic resonance spectroscopy.

1. Mucobromic acid and benzene:

3,4-Dibromo-5-phenylfuran-2(5H)-one:
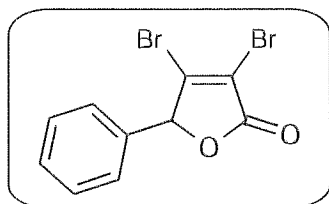
$C_{10}H_6Br_2O_2$

Molecular Weight: 317.96.

^{1}H-NMR (CDCl$_3$): δ = 7.54-7.40 (m, 3H, aryl-H), 7.32 - 7.29 (m, 2H, aryl-H), 5.86 (s, 1H, CH) ppm.

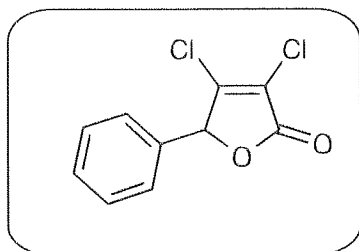^{13}C-NMR (CDCl$_3$): δ = 166.41 (C=O), 148.26 (CHCBr), 131.84 (aryl-CCH), 130.34 & 129.23 & 127.28 (o, m & p-aryl-C), 114.77 (CC=O), 86.61 (CH) ppm.

IR: ν = 3562, 3029, 3010, 2358, 2339, 1779, 1606 cm^{-1}.

Yield = 61%.

2. Mucochloric acid and benzene:

3,4-Dichloro-5-phenylfuran-2(5H)-one:

$C_{10}H_6Cl_2O_2$

Molecular Weight: 229.06.

APCI+ M/S: 229/231.

^{1}H-NMR (CDCl$_3$): δ = 7.50-7.41 (m, 3H, aryl-$\underline{H}$), 7.34 - 7.28 (m, 2H, aryl-$\underline{H}$), 5.86 (s, 1H, C$\underline{H}$) ppm.

^{13}C-NMR (CDCl$_3$): δ = 165.33 ($\underline{C}$=O), 152.24 (CH$\underline{C}$Cl), 131.56 (aryl-$\underline{C}$CH), 130.40 & 129.18 & 127.13 (o, m & p-aryl$\underline{C}$), 121.02 ($\underline{C}$C=O), 83.63 ($\underline{C}$H) ppm.

IR: ν = 3541, 3027, 1795, 1776, 1633, 1604 cm^{-1}.

Yield = 69%.

3. Mucobromic acid and toluene:

3,4-Dibromo-5-(4-methylphenyl)furan-2(5H)-one:
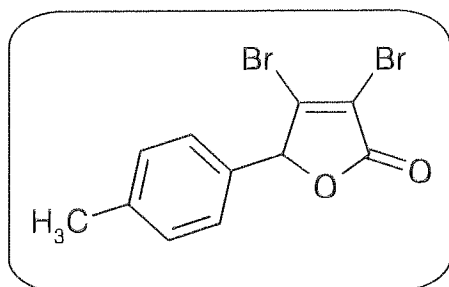
$C_{11}H_8Br_2O_2$

Molecular weight: 331.99.

^{1}H-NMR (CDCl$_3$): δ = 7.70-7.12 (m, 4H, aryl-$\underline{H}$), 5.69 (s, 1H, OC$\underline{H}$), 2.61-2.50 (s, 3H, C$\underline{H}_3$) ppm.

Yield = 69%.

4. Mucochloric acid and *o*-Xylene:

2,3-Dichloro-4,4-bis(3,4-dimethylphenyl)but-2-enoic acid:

C$_{20}$H$_{20}$Cl$_2$O$_2$
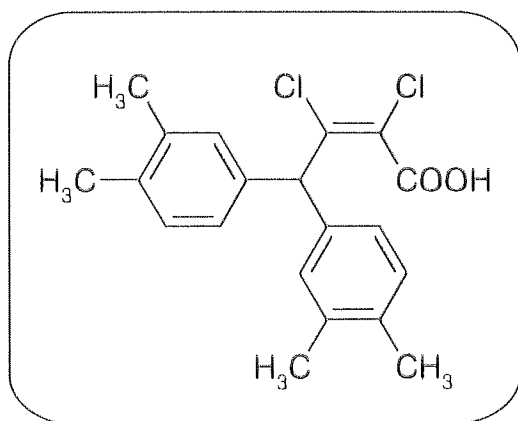
Molecular Weight: 363.28.

APCI+ M/S: 326/327.

^{1}H-NMR (CDCl$_3$): δ = 7.18-6.98 (m, 6H, aryl-$\underline{H}$), 6.59 (s, 1H, C$\underline{H}$), 2.34-2.21 (m, 12H, C$\underline{H}_3$) ppm (carboxylic acid group not detectable).

^{13}C-NMR (CDCl$_3$): δ = 166.17 ($\underline{C}$OOH), 153.67 (Cl$\underline{C}$CH), 136.87 & 136.57 & 135.62 & 130.28 & 129.54 & 126.53 (aryl-$\underline{C}$), 121.97 ($\underline{C}$COOH), 50.02 ($\underline{C}$H), 19.84 (aryl-$\underline{C}$H$_3$), 19.38 (aryl-$\underline{C}$H$_3$) ppm.

IR: ν = 2971, 2947, 2921, 2652, 2510, 1730, 1695 cm^{-1}.

Yield = 88%.

5. Mucochloric acid and *m*-Xylene:

2,3-Dichloro-4,4-bis(2,4-dimethylphenyl)but-2-enoic acid:
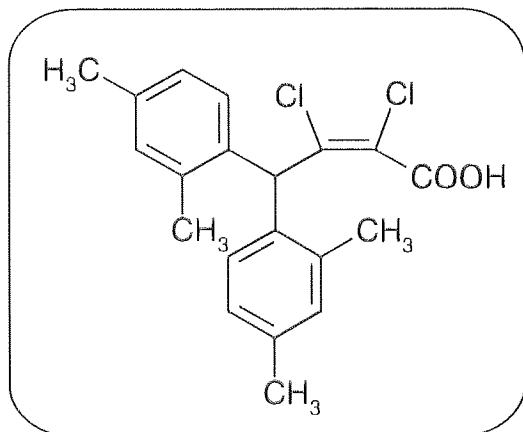
$C_{20}H_{20}Cl_2O_2$

Molecular Weight: 363.28.

APCI+ M/S: 326/327.

EI MS: m/z = 2362/364 (M+), 326, 291, 247, 232, 215.

^{1}H-NMR (CDCl$_3$): δ = 7.01-6.88 (m, 6H, aryl-H), 6.62 (s, 1H, CH), 2.31 (s, 6H, *o*-CH$_3$), 2.11 (s, 6H, *p*-CH$_3$) ppm (carboxylic acid group not detectable).

^{13}C-NMR (CDCl$_3$): δ = 166.41 (COOH), 155.23 (ClCCH), 136.83 & 136.35 & 135.62 & 131.21 & 128.29 &126.63 (aryl-C), 122.36 (CCOOH), 49.02 (CH), 20.95 (aryl-CH$_3$), 19.22 (aryl-CH$_3$) ppm.

IR: ν = 2969, 2925, 2865, 2649, 1730, 1693 cm^{-1}.

Elemental analysis: Expected: C (66.12%), H (5.55%). Obtained: C (63.87%), H (5.66%).

Yield = 85%.

Method C: Less reactive aromatic systems in 1,2-dichloroethane as the solvent

8.19 g (48.5 mmol) of mucochloric acid was dissolved in around 200 ml of 1,2-dichloroethane and stirred in a 500 ml conical flask. 1.5 equivalents of indole were added. Slowly, 10 g of powdered

aluminium chloride was added and the flask capped with a drying tube. The mixture was left stirring overnight. After this time, the mixture was poured into a beaker containing 125 g of ice and 38 g of concentrated hydrochloric acid. The organic phase was removed and any remaining product was removed from the aqueous phase by repeated extraction with 1,2-dichloroethane. After the organic phase was dried with magnesium sulphate, the solvent was removed by rotary evaporation and then the product dried under vacuum in a desiccator and submitted for spectroscopic analysis. by APCl+ mass spectroscopy and proton nuclear magnetic resonance spectroscopy.

3,4-Dichloro-5-(1*H*-indol-3-yl)furan-2(5*H*)-one:
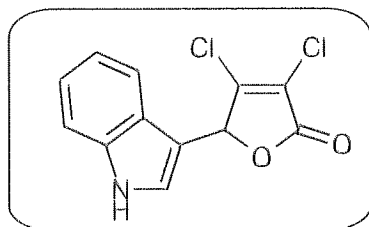
$C_{12}H_7Cl_2NO_2$

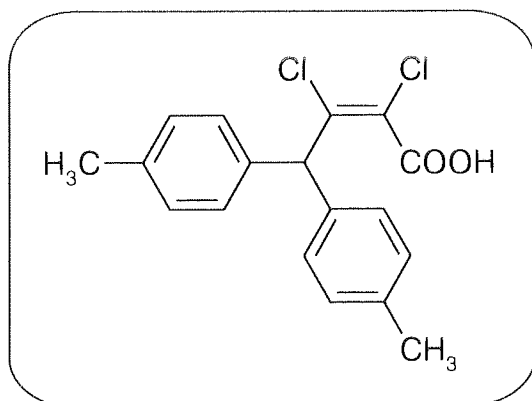Molecular Weight: 268.10.

APCl+ M/S: 268/270.

^{1}H-NMR (CDCl$_3$): δ = 9.31 (bs, 1H, N$\underline{H}$), 7.41-7.10 (m, 5H, aryl-$\underline{H}$), 6.20 (s, 1H, C$\underline{H}$) ppm.

Yield = 30%.

Method D: Increasing the reaction time for less reactive aromatic systems acting as reactant and solvent

8.19 g (48.5 mmol) of mucochloric acid was dissolved in around 200 ml of toluene by stirring in a 500 ml conical flask. Slowly, 10 g of powdered aluminium chloride was added and the flask capped with a drying tube. The mixture was left stirring for over 48 hours. After this time, the mixture was poured into a beaker containing 125 g of ice and 38 g of concentrated hydrochloric acid. The organic phase was removed and any remaining product was removed from the aqueous phase by repeated extraction with toluene. After the organic phase was dried with magnesium sulphate, the solvent was removed by rotary evaporation and then the product dried under vacuum in a desiccator.

2,3-Dichloro-4,4-bis(4-methylphenyl)but-2-enoic acid:

$C_{18}H_{16}Cl_2O_2$

Molecular Weight: 335.22.

APCI+ MS: m/z = 298/299.

^{1}H-NMR (CDCl$_3$): δ = 7.31-7.15 (m, 8H, aryl-$\underline{H}$), 6.62 (s, 1H, C$\underline{H}$), 2.38 (s, 6H, C$\underline{H}_3$) ppm (carboxylic acid group not detectable).

^{13}C-NMR (CDCl$_3$): δ = 166.24 (COOH), 153.83 (ClCCH), 136.97 & 136.45 & 129.04 & 129.00 (aryl-C), 128.16 (CCOOH), 53.24 (CH), 21.02 (CH$_3$) ppm.

7.3 Reactions of the hydroxy form of the mucohalic acids

7.3.1 Formation of the pseudo-esters

Method A: The reaction with liquid alcohols

1 mmol of mucochloric or mucobromic acid was dissolved in two equivalents (2 mmol) of each of the liquid alcohols under investigation. One or two drops of concentrated sulphuric acid were added to each reaction mixture. Each reaction was left at 60 °C for 24 hours. Thin layer chromatography was performed after this time and indicated that a reaction had occurred in some cases. A sample of each reaction was submitted for APCI+ mass spectroscopy.

The resulting spectra did not show the presence of the expected molecular ion peak. It was decided that the best way to detect the 5-alkoxy product was to further react each reaction product with a suitable nitrogen nucleophile. If the correct reaction product has been formed, on further reaction with an amine it would form a detectable product, as the amine group would be attached to the four position of the ring (see section 2.2.1, Figure 2.24). The alternative method would be to purify each of the reaction products either by preparative thin layer chromatography or column chromatography and then analyse the resulting product by nuclear magnetic resonance spectroscopy. This method was less favoured as it is much more costly and time consuming.

The four nitrogen nucleophiles that were selected to characterise the different 5-alkoxy-3,4-dihalofuran-2(5*H*)-ones were benzylmethylamine, 2,6-dimethylmorpholine, pyrrolidine and 1-benzylpiperazine. Each of the reaction mixtures formed above was split into four and 1 mmol of the amines added to each respectively. These reaction mixtures were then left for 24 hours at 60 °C. A sample of each reaction was then submitted for APCI+ mass spectroscopy in methanol. The correct molecular ion peak was detectable in some cases. It was decided that if a molecular ion peak was detected in at least two of the four cases, that the initial 5-alkoxy-3,4-dichlorofuran-2(5*H*)-one had been successfully synthesised. After method B, is a list of positively identifiable 5-alkoxy building blocks with their corresponding APCI+ mass spectroscopy results for the indirect identification. The code for the mass spectroscopy tables for the amine group at position X is 1: benzylmethylamine, 2: 1-benzylpiperazine, 3: 2,6-dimethylmorpholine and 4: pyrrolidine.

Method B: The reaction with solid alcohols

0.17 g (1 mmol) of mucochloric acid was dissolved in 2 ml of toluene. Two equivalents (2 mmol) of each of the solid alcohols under investigation were added along with one or two drops of concentrated sulphuric acid. Each reaction was left at 60 °C 24 hours. Thin layer chromatography was performed after this time and indicated that a reaction had occurred in some cases. Each of the reaction mixtures formed above was split into four and 1 mmol of a nitrogen nucleophile (see Method A) was added to each. These reaction mixtures were then left for 24 hours at 60 °C. A sample of each reaction was then submitted for APCI+ mass spectroscopy in methanol. A molecular ion peak was detectable in some cases. As with the liquid alcohols, it was decided that if a molecular ion peak was detected in at least two of the

four cases, that the initial 5-alkoxy-3,4-dichlorofuran-2(5*H*)-one had been successfully synthesised. (See Method A for the amine codes.)

The following results appear in the same order in which they occur in the results table in section 2.2.2. A shaded entry denotes that the compound has been purified and characterised in section 7.4.2.
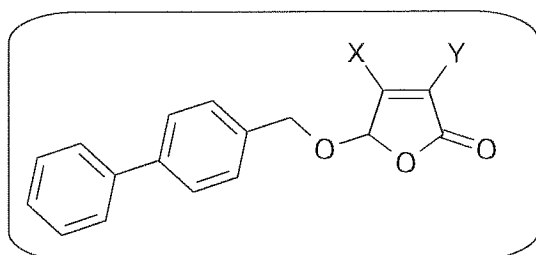
1. Benzyl alcohol:

5-(Benzyloxy)-3,4-dihalofuran-2(5*H*)-one:
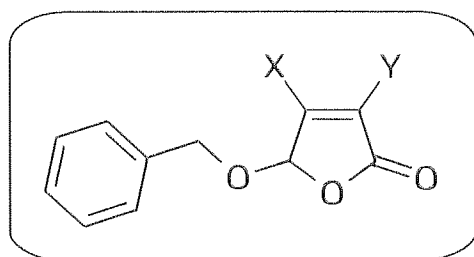
X	Y	Expected	Obtained
1	Br	388.26	388/390
2	Br	443.33	443/445
3	Br	382.25	382/384
4	Br	338.20	338/340
1	Cl	343.80	344/346
2	Cl	398.88	399/401
3	Cl	337.80	338/340
4	Cl	293.75	294/296

2. 4-Biphenolmethanol:

5-([1,1'-Biphenyl]-4-ylmethoxy)-3,4-dihalofuran-2(5*H*)-one:

X	Y	Expected	Obtained
1	Cl	419.90	420/422
2	Cl	474.98	-
3	Cl	413.89	414/416
4	Cl	369.84	-

3. Borneol:

5-Borneoloxy-3,4-dihalofuran-2(5*H*)-one:
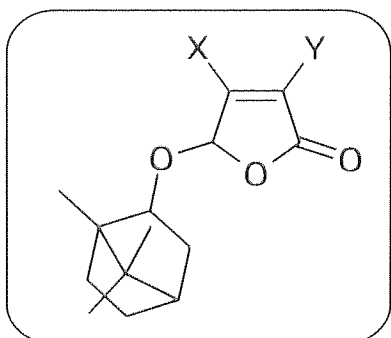
X	Y	Expected	Obtained
1	Cl	389.92	390/392
2	Cl	444.99	445/447
3	Cl	383.91	384/386
4	Cl	339.86	340/342

4. Cetyl alcohol:

5-Cetyloxy-3,4-dihalofuran-2(5*H*)-one:

$CH_3(CH_2)_{15}$
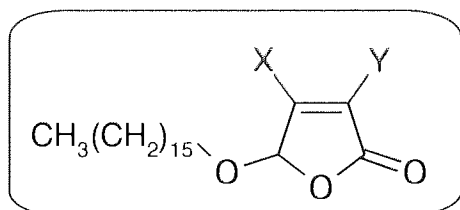
X	Y	Expected	Obtained
1	Br	522.56	522/524
2	Br	577.64	577/579
3	Br	516.55	516/518
4	Br	472.50	472/474
1	Cl	478.11	478/480
2	Cl	533.19	533/535
3	Cl	472.10	472/474
4	Cl	428.05	428/430
1	H	443.66	444
2	H	498.74	499
3	H	437.66	438
4	H	393.60	394

5. 4-Chloro-3,5-dimethylphenol:

5-(4-Chloro-3,5-dimethylphenoxy)-3,4-dihalofuran-2(5*H*)-one:
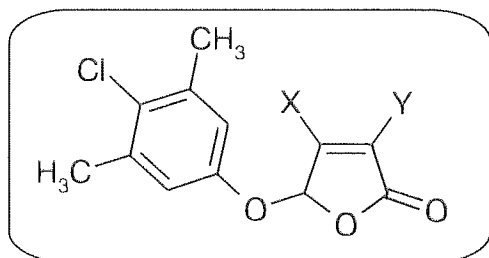
X	Y	Expected	Obtained
1	Cl	392.28	392/394
2	Cl	447.35	447/449
3	Cl	386.27	386/388
4	Cl	342.22	342/344

6. *m*-Cresol:

3,4-Dihalo-5-(3-methylphenoxy)furan-2(5*H*)-one:
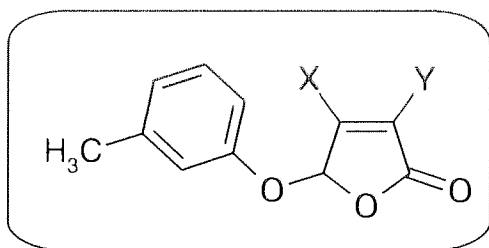
X	Y	Expected	Obtained
1	Br	388.26	388/390
2	Br	443.33	443/445
3	Br	382.25	382/384
4	Br	338.20	338/340
1	Cl	343.80	344/346
2	Cl	398.88	399/401
3	Cl	337.80	338/340
4	Cl	293.75	294/296

7. Cyclohexylmethanol:

5-(Cyclohexylmethoxy)-3,4-dihalofuran-2(5*H*)-one:
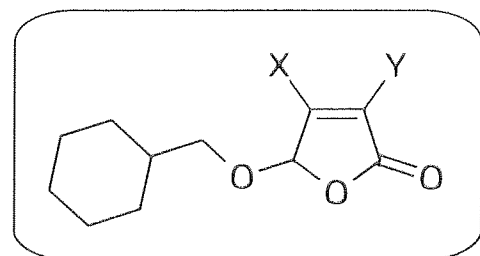
X	Y	Expected	Obtained
1	Cl	349.85	350/352
2	Cl	404.93	405/407
3	Cl	343.85	344/346
4	Cl	299.79	300/302

8. 2-Cyclohexylphenol:

5-(2-Cyclohexylphenoxy)-3,4-dihalofuran-2(5H)-one:
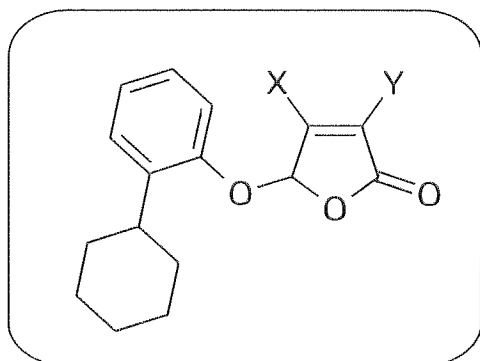
X	Y	Expected	Obtained
1	Cl	411.92	412/414
2	Cl	467.00	467/469
3	Cl	405.92	406/408
4	Cl	361.86	362/364

9. Cyclopentanol:

5-(Cyclopentyloxy)-3,4-dihalofuran-2(5H)-one:
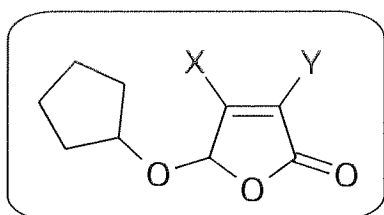
X	Y	Expected	Obtained
1	Br	366.25	366/368
2	Br	421.33	421/423
3	Br	360.24	360/362
4	Br	316.19	316/318
1	Cl	321.80	322/324
2	Cl	376.88	377/379
3	Cl	315.79	316/318
4	Cl	271.74	272/274

10. Ethanol:

5-Ethoxy-3,4-dihalofuran-2(5*H*)-one:
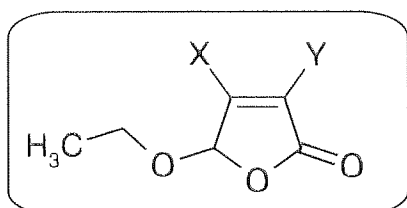
X	Y	Expected	Obtained
1	Br	326.19	326/328
2	Br	381.26	381/383
3	Br	320.18	320/322
4	Br	276.13	276/278
1	Cl	281.73	282/284
2	Cl	336.81	337/339
3	Cl	275.73	276/278
4	Cl	231.68	232/234

11. Guaiacol:

3,4-Dihalo-5-(2-methoxyphenoxy)furan-2(5*H*)-one:
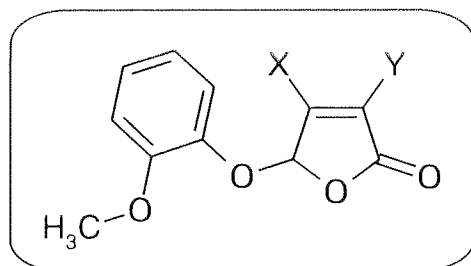
X	Y	Expected	Obtained
1	Cl	359.80	360/362
2	Cl	414.88	415/417
3	Cl	353.80	354/356
4	Cl	309.75	310/312

12. Menthol:

3,4-Dihalo-5-menthyloxyfuran-2(5H)-one:
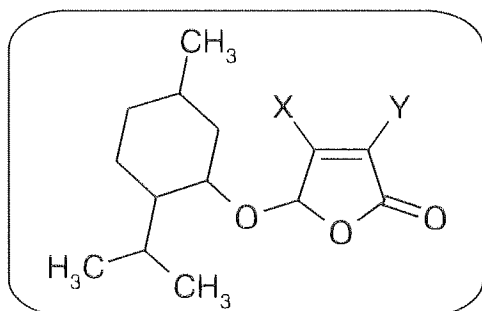
X	Y	Expected	Obtained
1	Br	436.38	436/438
2	Br	491.46	491/493
3	Br	430.38	430/432
4	Br	386.32	386/388
1	Cl	391.93	392/394
2	Cl	447.01	447/449
3	Cl	385.93	386/388
4	Cl	341.87	342/344

13. Methanol:

3,4-Dihalo-5-methoxyfuran-2(5H)-one:
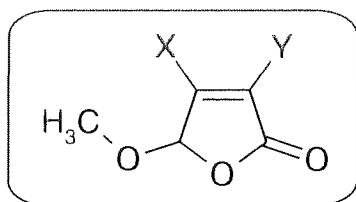
X	Y	Expected	Obtained
1	Br	312.16	312/314
2	Br	367.24	367/369
3	Br	306.15	306/308
4	Br	262.10	262/264
1	Cl	267.71	268/270
2	Cl	322.79	323/325
3	Cl	261.70	262/264
4	Cl	217.65	218/220
1	H	233.26	234
2	H	288.34	289
3	H	227.26	228
4	H	183.20	184

14. *m*-Methoxyphenol:

3,4-Dihalo-5-(3-methoxyphenoxy)furan-2(5*H*)-one:
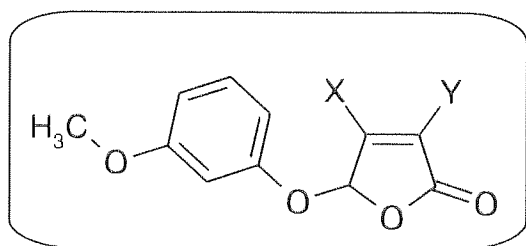
X	Y	Expected	Obtained
1	Cl	359.80	360/362
2	Cl	414.88	-
3	Cl	353.80	354/356
4	Cl	309.75	-

15. 1-Nonanol:

3,4-Dihalo-5-(nonyloxy)furan-2(5*H*)-one:
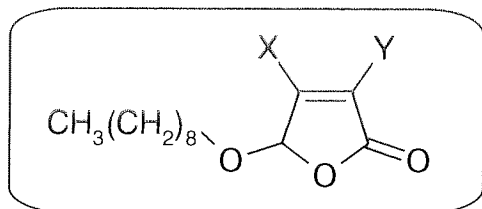
X	Y	Expected	Obtained
1	Br	424.37	424/426
2	Br	479.45	479/481
3	Br	418.37	418/420
4	Br	374.31	374/376
1	Cl	379.92	380/382
2	Cl	435.00	435/437
3	Cl	373.91	374/376
4	Cl	329.86	330/332
1	H	345.48	346
2	H	400.55	401
3	H	339.47	340
4	H	295.42	296

16. 2-Pentanol:

3,4-Dihalo-5-(1-methylbutoxy)furan-2(5*H*)-one:
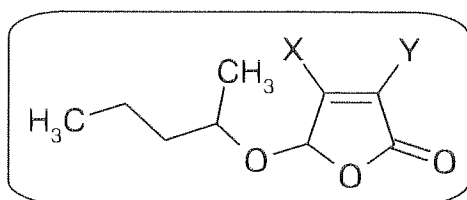
X	Y	Expected	Obtained
1	Cl	323.81	324/326
2	Cl	378.89	379/381
3	Cl	317.81	318/320
4	Cl	273.76	274/276

17. 2-Phenylethanol:

3,4-Dihalo-5-(phenethyloxy)furan-2(5*H*)-one:
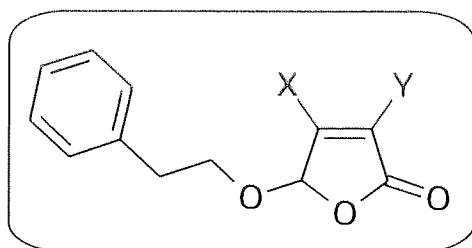
X	Y	Expected	Obtained
1	Cl	357.84	358/360
2	Cl	412.91	413/415
3	Cl	351.82	352/354
4	Cl	307.77	308/310
1	H	323.39	324
2	H	378.46	379
3	H	317.38	318
4	H	273.33	274

18. 3-Phenylpropyl alcohol:

3,4-Dihalo-5-(3-phenylpropoxy)furan-2(5*H*)-one:
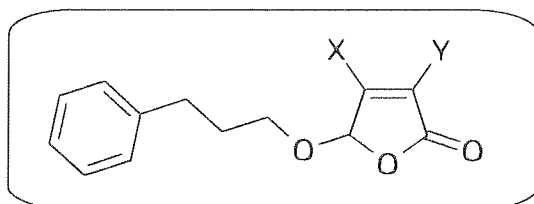
X	Y	Expected	Obtained
1	Cl	371.86	372/374
2	Cl	426.94	427/429
3	Cl	365.85	366/368
4	Cl	321.80	322/324

19. 3-Phenyl-2-propyn-1-ol:

3,4-Dihalo-5-[(3-phenylprop-2-ynyl)oxy]furan-2(5H)-one:
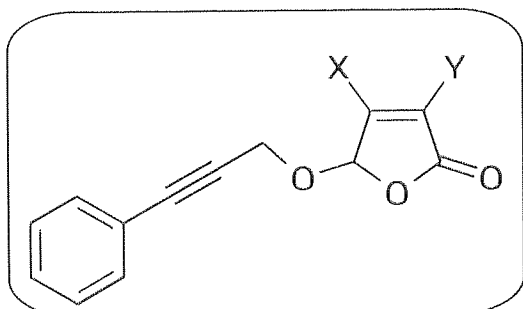
X	Y	Expected	Obtained
1	Cl	367.83	368/370
2	Cl	422.90	-
3	Cl	361.82	362/364
4	Cl	317.77	-

20. i-Propanol:

3,4-Dihalo-5-isopropoxyfuran-2(5H)-one:
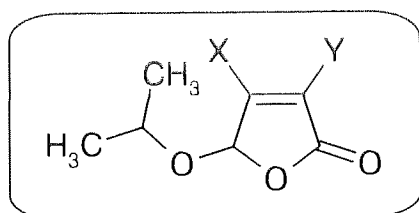
X	Y	Expected	Obtained
1	Cl	295.76	296/298
2	Cl	350.84	351/353
3	Cl	289.76	290/292
4	Cl	245.70	246/248

21. Propargyl alcohol:

3,4-Dihalo-5-(prop-2-ynyloxy)furan-2(5H)-one:
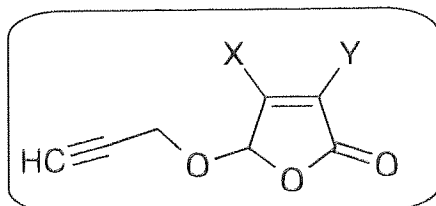
X	Y	Expected	Obtained
1	Cl	291.73	292/294
2	Cl	346.81	347/349
3	Cl	285.72	286/288
4	Cl	241.67	242/244

22. 3-(2-Pyridyl)-1-propanol:

3,4-Dihalo-5-(3-pyridin-2-ylpropoxy)furan-2(5H)-one:
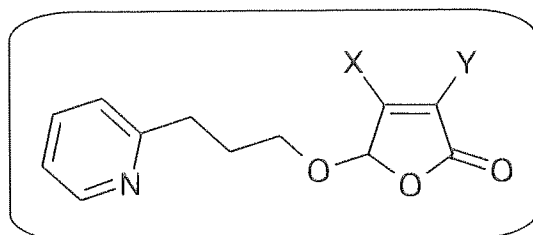
X	Y	Expected	Obtained
1	Cl	372.85	373/375
2	Cl	427.92	-
3	Cl	366.84	367/369
4	Cl	322.79	-

23. 1-Tetradecanol:

3,4-Dihalo-5-(tridecyloxy)furan-2(5H)-one:
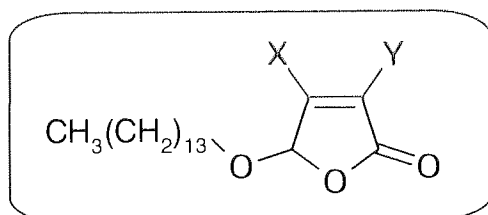
X	Y	Expected	Obtained
1	Cl	450.06	450/452
2	Cl	505.14	505/507
3	Cl	444.05	444/446
4	Cl	400.00	400/402

24. Tetrahydrofurfuryl alcohol:

3,4-Dihalo-5-(tetrahydrofuran-2-ylmethoxy)furan-2(5H)-one:
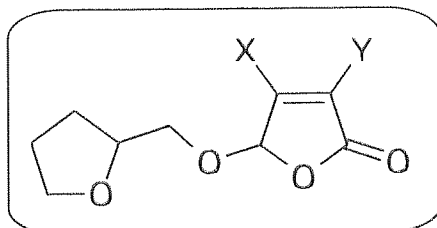
X	Y	Expected	Obtained
1	Cl	337.80	338/340
2	Cl	392.88	393/395
3	Cl	331.79	332/334
4	Cl	287.74	288/290

7.3.2 Purification of a selection of the 5-alkoxyfuran-2(5*H*)-ones

A selection of the building blocks synthesised in the previous section was purified by one of two methods.

Method A: Purification by solvent extraction

10 g of mucochloric or mucobromic acid was heated along with 70 ml of methanol, ethanol or isopropyl alcohol and a little concentrated sulphuric acid, over 48 hours under reflux. Thin layer chromatography (TLC) was performed in a 1:1 mixture of ether and petroleum ether 40-60 and this showed that the reaction had progressed sufficiently. Any remaining mucochloric acid was neutralised with an aqueous solution of sodium hydrogen carbonate and the desired product then extracted in ether. In total, three extractions were performed and then the solvent was removed by rotary evaporation.

1. Mucobromic acid and methanol:

3,4-Dibromo-5-methoxyfuran-2(5*H*)-one:
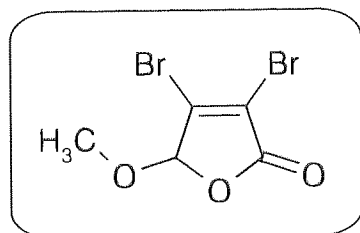
$C_5H_4Br_2O_3$

Molecular Weight: 271.89.

APCI+ MS: m/z = 287/289.

EI MS: m/z = 287, 272 (M+), 241, 193, 147.

^{1}H NMR (CDCl$_3$): δ = 5.79 (s, 1H, C$\underline{H}$), 3.59 (s, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2335, 1791, 1641, 1616 cm^{-1}.

Rf (50% Ether, 50% Petrol ether 40-60) = 0.40.

Yield = 67%.

2. Mucochloric acid and methanol:

3,4-Dichloro-5-methoxyfuran-2(5*H*)-one:
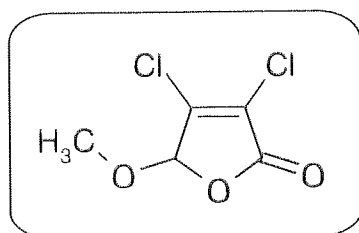
$C_5H_4Cl_2O_3$

Molecular Weight: 182.99.

APCI+ MS: m/z = 197/199.

EI MS: m/z = 193, 183 (M+), 151, 147.

^{1}H NMR (CDCl$_3$): δ = 5.78 (s, 1H, C$\underline{H}$), 3.60 (s, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 162.92 ($\underline{C}$=O), 147.27 ($\underline{C}$CH), 124.03 ($\underline{C}$C=O), 101.41 ($\underline{C}$H), 56.24 ($\underline{C}$H$_3$) ppm.

IR: ν = 2939, 2848, 2452, 2420, 1840, 1807, 1738, 1637, 1585 cm^{-1}.

Rf (50% Ether, 50% Petrol ether 40-60) = 0.38.

Yield = 80%.

3. Mucochloric acid and ethanol:

3,4-Dichloro-5-ethoxyfuran-2(5*H*)-one:
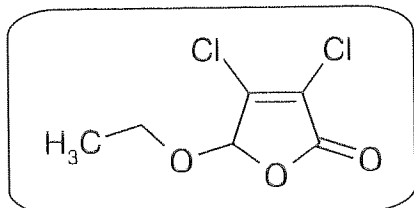
$C_6H_6Cl_2O_3$

Molecular weight: 197.02.

^1H NMR (CDCl$_3$): δ = 5.80 (s, 1H, C*H*), 3.82 (q, *J* = 7.8 Hz, 2H, C*H*$_2$), 1.26 (t, *J* = 7.1 Hz, 3H, C*H*$_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 163.09 (*C*=O), 147.57 (*C*CH), 123.80 (*C*C=O), 100.81 (*C*H), 66.06 (*C*H$_2$), 14.70 (*C*H$_3$) ppm.
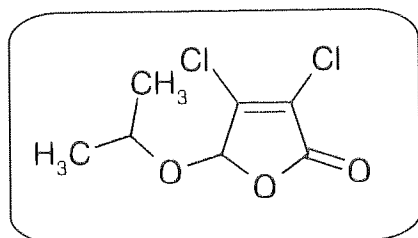
IR: ν = 2979, 2938, 2900, 2339, 1809, 1637 cm^{-1}.

Rf (50% Ether, 50% Petrol ether 40-60) = 0.46.

Yield = 84%.

4. Mucochloric acid and isopropyl alcohol:

3,4-Dichloro-5-isopropoxyfuran-2(5*H*)-one:

$C_7H_8Cl_2O_3$

Molecular weight: 211.04.

^{1}H NMR (CDCl$_3$): δ = 5.83 (s, 1H, OC**H**O), 4.13-4.03 (m, 1H, C**H**CH$_3$), 1.27-1.14 (m, 6H, C**H**$_3$) ppm.

IR: ν = 2975, 2921, 1793, 1643 cm^{-1}.

Rf (50% Ether, 50% Petrol ether 40-60) = 0.51.

Yield = 79%.

Method B: Purification by preparative thin layer chromatography

A selection of the 5-alkoxyfuran-2(5*H*)-ones made in section 7.3.1 was purified by preparative thin layer chromatography in ether. This was performed as follows:

Each sample was first run on an ordinary aluminium backed thin layer chromatography plate along with the starting mucohalic acid for comparison. The plate was dipped into a 1% solution of potassium permanganate and rapid decolouration of both the product and the starting mucohalic acid zones was observed. By comparison with the mucohalic acid tract, the required furan-2(5*H*)-one zone could be identified. As the alcohol zone does not show a rapid decolouration on the application of potassium permanganate, the required zone is easily identifiable by this method.

Once it had been established which was the required zone for each sample, purification could take place. A separate 1mm thick glass-backed preparative thin layer chromatography plate was used for each sample. Sufficient sample in DMF was pipetted along the bottom of the plate about 3 cm from the edge. Each plate was then left for the DMF to fully evaporate. Once the plate had dried, it could be place in a thin layer chromatography glass tank and left to run. Each purification in this section was performed in ether. Once the solvent front was approximately 3 cm from the top edge of the plate the plate was removed and left to dry. Examination under ultra violet light showed

the location of the different zones. The zone corresponding to the furan-2(5*H*)-one zone on the thin layer chromatography plate was scraped off and put in a 50 ml beaker containing approximately 35 ml of methanol. The mixture was gently warmed and stirred to allow the product to fully dissolve in the methanol. After a few minutes, the mixture was filtered to remove all the silica gel. This solution was then left overnight to allow the methanol to evaporate. Any remaining methanol was removed by placing the sample under vacuum in a desiccator for a few hours. Each sample could then be analysed by mass spectroscopy and proton (and carbon) nuclear magnetic resonance spectroscopy.

1. Mucobromic acid and benzyl alcohol:

5-Benzyloxy-3,4-dibromofuran-2(5*H*)-one:

$C_{11}H_8Br_2O_3$
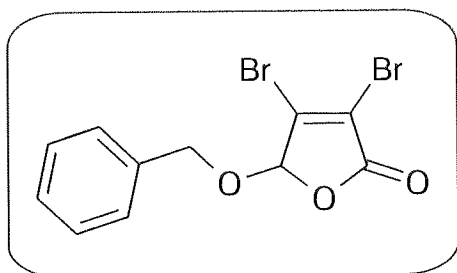
Molecular Weight: 347.99.

^{1}H NMR (CDCl$_3$): δ = 7.45-7.35 (m, 5H, aryl-$\underline{H}$), 5.86 (s, 1H, OC$\underline{H}$), 4.97 & 4.92 (d, J = 11.5 Hz, 1H, C$\underline{H}_2$), 4.81 & 4.76 (d, J = 11.5 Hz, 1H, C$\underline{H}_2$) ppm.

IR: ν = 2995, 2919, 2397, 2335, 1952, 1787, 1620 cm^{-1}.

Rf (Ether) = 0.83.

2. Mucobromic acid and menthol:

3,4-Dibromo-5-menthyloxyfuran-2(5*H*)-one:

$C_{14}H_{20}Br_2O_3$
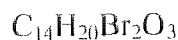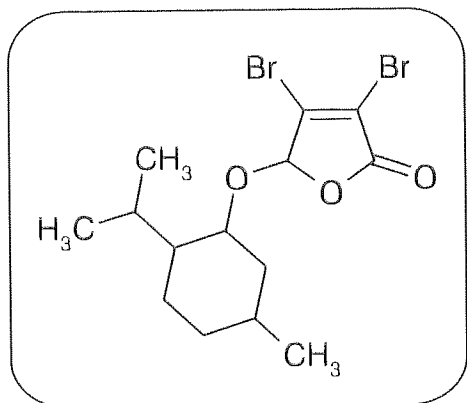
Molecular weight: 396.12.

^1H NMR (CDCl$_3$): δ = 5.89 & 5.82 (s, 1H, OC$\underline{H}$O (different isomers)), 3.72-3.54 (m, 1H, $\underline{H}$-menthol), 2.35-2.10 (m, 2H, $\underline{H}$-menthol), 1.72-1.66 (m, 2H, $\underline{H}$-menthol), 1.41-0.75 (m, 14H, $\underline{H}$-menthol) ppm.

IR: ν = 2964, 2929, 2869, 2404, 1782, 1618 cm^{-1}.

Rf (Ether) = 0.85.

3. Mucochloric acid and cetyl alcohol:

5-Cetyloxy-3,4-dichlorofuran-2(5*H*)-one:

$C_{20}H_{34}Cl_2O_3$
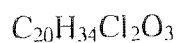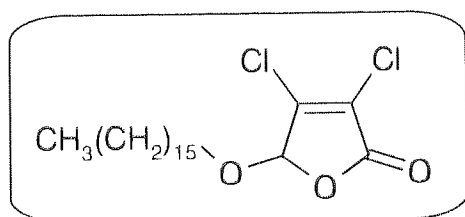
Molecular Weight: 393.39.

^{1}H NMR (CDCl$_3$): δ = 5.81 (s, 1H, CH), 3.90-3.60 (m, 2H, OCH$_2$), 1.72-1.61 (m, 2H, OCH$_2$CH$_2$), 1.26 (m, 26H, OCH$_2$CH$_2$(CH$_2$)$_{13}$), 0.88 (t, J = 6.6 Hz, 3H, CH$_3$) ppm.

IR: ν = 2925, 2852, 2335, 1792, 1641 cm^{-1}.

Rf (Ether) = 0.92.

7.3.3 Synthesis of a series of aliphatic 5-alkoxy-3,4-dichlorofuran-2(5H)-ones for biological diversity analysis

Using the methods detailed in section 7.3.1 for the synthesis and 7.3.2 for the purification, the following series of aliphatic 5-alkoxy-3,4-dichlorofuran-2(5H)-one building blocks was synthesised.

1. Ethanol:

3,4-Dichloro-5-ethoxyfuran-2(5H)-one:
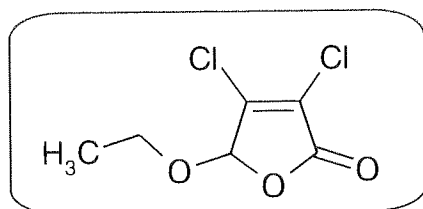
C$_6$H$_6$Cl$_2$O$_3$

Molecular weight: 197.02.

^{1}H NMR (CDCl$_3$): δ = 5.80 (s, 1H, CH), 3.82 (q, J = 7.8 Hz, 2H, CH$_2$), 1.26 (t, J = 7.1 Hz, 3H, CH$_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 163.09 (C=O), 147.57 (CCH), 123.80 (CC=O), 100.81 (CH), 66.06 (CH$_2$), 14.70 (CH$_3$) ppm.

IR: ν = 2979, 2938, 2900, 2339, 1809, 1637 cm^{-1}.

Rf (50% Ether, 50% Petrol ether 40-60) = 0.46.

2. Butan-1-ol:

5-Butoxy-3,4-dichlorofuran-2(5H)-one:
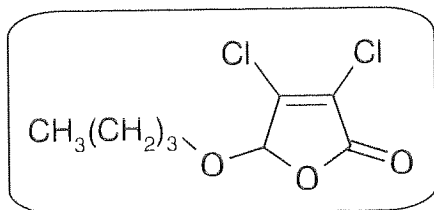
$C_8H_{10}Cl_2O_3$

Molecular weight: 225.07.

^{1}H NMR (CDCl$_3$): δ = 5.80 (s, 1H, C$\underline{H}$), 3.86-3.59 (m, 2H, OC$\underline{H}_2$), 1.66-1.35 (m, 4H, OCH$_2$C$\underline{H}_2$C$\underline{H}_2$), 0.92 (t, J = 7.3 Hz, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 163.24 ($\underline{C}$=O), 147.48 ($\underline{C}$CH), 124.12 ($\underline{C}$C=O), 100.94 ($\underline{C}$H), 70.05 (O$\underline{C}$H$_2$), 31.15 (OCH$_2\underline{C}$H$_2$), 18.84 ($\underline{C}$H$_2$CH$_3$), 13.59 ($\underline{C}$H$_3$) ppm.

IR: ν = 2958, 2873, 2335, 1809, 1637 cm^{-1}.

Rf (50% Ether, 50% Petrol ether 40-60) = 0.48.

3. Hexanol:

3,4-Dichloro-5-(hexyloxy)furan-2(5H)-one:
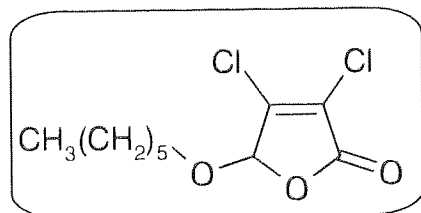
$C_{10}H_{14}Cl_2O_3$

Molecular weight: 253.12.

^{1}H NMR (CDCl$_3$): δ = 5.80 (s, 1H, C$\underline{H}$), 3.87-3.59 (m, 2H, OC$\underline{H}_2$), 1.67-1.29 (m, 8H, OCH$_2$(C$\underline{H}_2$)$_4$), 0.87 (t, J = 6.7 Hz, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 163.22 (C=O), 147.47 (CCH), 124.13 (CC=O), 100.93 (CH), 70.34 (OCH$_2$), 31.29 (OCH$_2$CH$_2$), 29.10 (OCH$_2$CH$_2$CH$_2$), 25.28 (CH$_3$CH$_2$CH$_2$), 22.41 (CH$_3$CH$_2$), 13.89 (CH$_3$) ppm.

IR: ν = 2860, 2335, 1801, 1637 cm^{-1}.

Rf (Ether) = 0.73.

4. Nonanol:

3,4-Dichloro-5-(nonyloxy)furan-2(5*H*)-one:
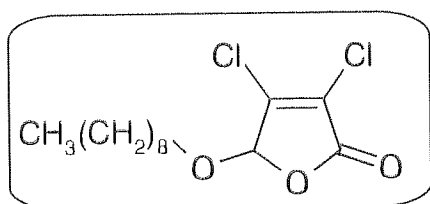
C$_{13}$H$_{20}$Cl$_2$O$_3$

Molecular weight: 295.20.

^{1}H NMR (CDCl$_3$): δ = 5.80 (s, 1H, CH), 3.87-3.61 (m, 2H, OCH$_2$), 1.71-1.49 (m, 2H, OCH$_2$CH$_2$), 1.25 (m, 12H, OCH$_2$CH$_2$(CH$_2$)$_6$), 0.86 (t, *J* = 6.5 Hz, 3H, CH$_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 163.17 (C=O), 147.45 (CCH), 124.13 (CC=O), 100.93 (CH), 70.35 (OCH$_2$), 31.75 (OCH$_2$CH$_2$), 29.35 (OCH$_2$CH$_2$CH$_2$), 29.15 (OCH$_2$CH$_2$CH$_2$CH$_2$), 29.12 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$), 26.11 (CH$_3$CH$_2$CH$_2$CH$_2$), 25.61 (CH$_3$CH$_2$CH$_2$), 22.57 (CH$_3$CH$_2$), 14.00 (CH$_3$) ppm.

IR: ν = 2927, 2860, 2335, 1793, 1641 cm^{-1}.

Rf (Ether) = 0.75.

5. Dodecyloxy:

3,4-Dichloro-5-(dodecyloxy)furan-2(5*H*)-one:

$C_{16}H_{26}Cl_2O_3$
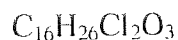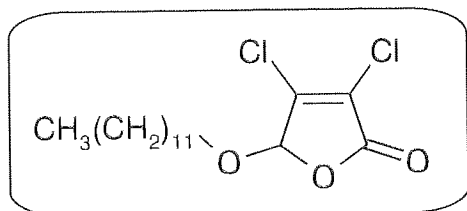
Molecular weight: 337.28.

^{1}H NMR (CDCl$_3$): δ = 5.81 (s, 1H, C$\underline{H}$), 3.86-3.70 (m, 2H, OC$\underline{H}_2$), 1.69-1.56 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.26 (m, 18H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_9$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 163.22 ($\underline{C}$=O), 147.42 ($\underline{C}$CH), 124.22 ($\underline{C}$C=O), 100.91 ($\underline{C}$H), 70.37 (O$\underline{C}$H$_2$), 31.84 (OCH$_2$$\underline{C}H_2$), 29.70 (OCH$_2CH_2$$\underline{C}H_2$), 29.60 (OCH$_2CH_2CH_2$$\underline{C}H_2$), 29.55 (OCH$_2CH_2CH_2CH_2$$\underline{C}H_2$), 29.40 (OCH$_2CH_2CH_2CH_2CH_2$$\underline{C}H_2$), 29.27 (CH$_3CH_2CH_2CH_2CH_2$$\underline{C}H_2$), 29.16 (CH$_3CH_2CH_2CH_2$$\underline{C}H_2$), 26.12 (CH$_3CH_2CH_2$$\underline{C}H_2$), 25.64 (CH$_3CH_2$$\underline{C}H_2$), 22.62 (CH$_3$$\underline{C}H_2$), 14.05 ($\underline{C}H_3$) ppm.

IR: ν = 2925, 2858, 2335, 1787, 1641 cm^{-1}.

Rf (Ether) = 0.75.

7.4 Nucleophilic attack at the four position on the furan-2(5*H*)-one ring

7.4.1 Synthesis of 5-alkoxy-4-amino-3-halofuran-2(5*H*)-ones

Method:

Initially, sufficient 5-alkoxy-3,4-dihalofuran-2(5*H*)-one was synthesised for the following reactions, according to the experimental in section 7.3.1. The twelve 5-alkoxy-3,4-dihalofuran-2(5*H*)-ones formed used methanol, benzyl alcohol, menthol or cetyl alcohol at the five position and chlorine, bromine or hydrogen at the three position. 1 mmol of each building block was used for each reaction and was dissolved in 1 ml of N,N-dimethylformamide (DMF). Two equivalents of each of the nitrogen nucleophiles under test was then added and each reaction kept at 60 °C for 24 hours. A sample of each reaction mixture was then submitted in methanol for analysis by APCl+ mass spectroscopy. Detection of the correct molecular ion peak in a majority of the twelve samples analysed was taken to be a direct indication of the success of the nitrogen nucleophile to react.

The following results appear in the same order in which they occur in the results table in section 3.1.1. The R position code is as follows: Bz: benzyl, Ce: cetyl, Mn: menthol and Me: methyl. A shaded entry denotes that the compound has been purified and characterised in section 7.4.2.

1. 4-Amino-1-benzylpiperidine:

5-Alkoxy-4-[(1-benzylpiperidin-4-yl)amino]-3-halofuran-2(5*H*)-one:
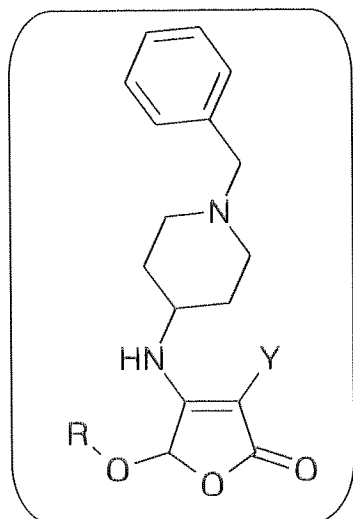
R	Y	Expected	Obtained
Bz	Br	457.37	457/459
Ce	Br	591.67	591/593
Mn	Br	505.49	505/507
Me	Br	381.27	381/383
Bz	Cl	412.91	413/415
Ce	Cl	547.22	547/549
Mn	Cl	461.04	461/463
Me	Cl	336.82	337/339
Bz	H	378.47	-
Ce	H	512.77	513
Mn	H	426.60	-
Me	H	302.37	303

2. 4-(3-Aminopropyl)morpholine:

5-Alkoxy-3-halo-4-[(3-morpholin-4-ylpropyl)amino]furan-2(5*H*)-one:
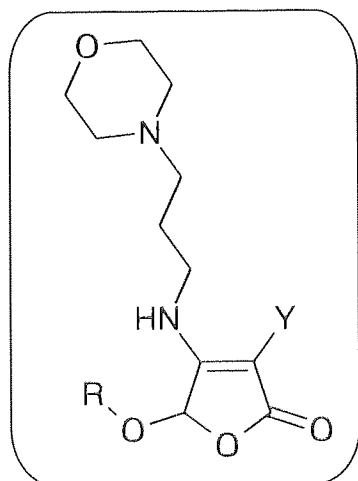
R	Y	Expected	Obtained
Bz	Br	411.29	411/413
Ce	Br	545.60	545/547
Mn	Br	459.42	459/461
Me	Br	335.20	335/337
Bz	Cl	366.84	367/369
Ce	Cl	501.15	501/503
Mn	Cl	414.97	415/417
Me	Cl	290.74	291/293
Bz	H	332.40	-
Ce	H	466.70	467
Mn	H	380.53	-
Me	H	256.30	257

3. Benzimidazole:

5-Alkoxy-4-(1*H*-benzimidazol-1-yl)-3-halofuran-2(5*H*)-one:
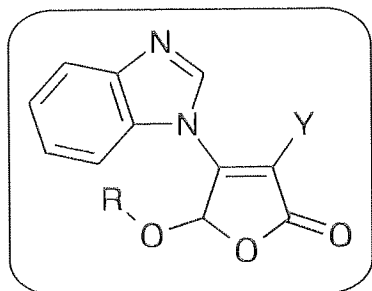
R	Y	Expected	Obtained
Bz	Br	385.22	385/387
Ce	Br	519.52	519/521
Mn	Br	433.34	433/435
Me	Br	309.12	309/311
Bz	Cl	340.76	341/343
Ce	Cl	475.07	475/477
Mn	Cl	388.89	389/391
Me	Cl	264.67	265/267
Bz	H	306.32	-
Ce	H	440.62	441
Mn	H	354.45	-
Me	H	230.22	231

4. Benzylmethylamine:

5-Alkoxy-4-[benzyl(methyl)amino]-3-halofuran-2(5*H*)-one:
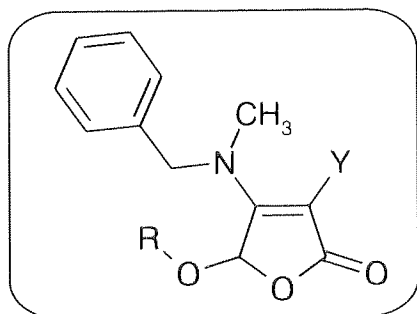
R	Y	Expected	Obtained
Bz	Br	388.26	388/390
Ce	Br	522.56	522/524
Mn	Br	436.39	436/438
Me	Br	312.16	312/314
Bz	Cl	343.81	344/346
Ce	Cl	478.11	478/480
Mn	Cl	391.94	392/394
Me	Cl	267.71	268/270
Bz	H	309.36	-
Ce	H	443.67	444
Mn	H	357.49	538
Me	H	233.27	234

5. 1-Benzylpiperazine:

5-Alkoxy-4-(4-benzylpiperazin-1-yl)-3-halofuran-2(5*H*)-one:
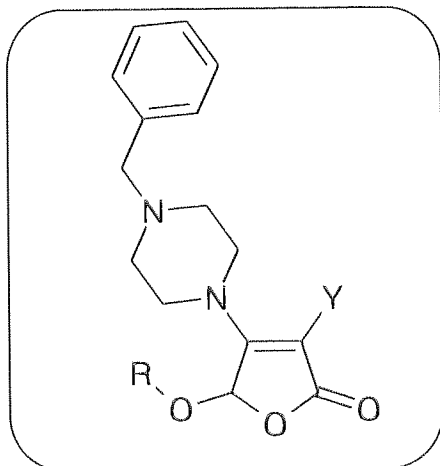
R	Y	Expected	Obtained
Bz	Br	443.34	443/445
Ce	Br	577.64	577/579
Mn	Br	491.47	491/493
Me	Br	367.24	367/369
Bz	Cl	398.89	399/401
Ce	Cl	533.19	533/535
Mn	Cl	447.02	447/449
Me	Cl	322.79	323/325
Bz	H	364.44	-
Ce	H	498.75	499
Mn	H	412.57	413
Me	H	288.34	289

6. *sec*-Butylamine:

5-Alkoxy-4-(*sec*-butylamino)-3-halofuran-2(5*H*)-one:
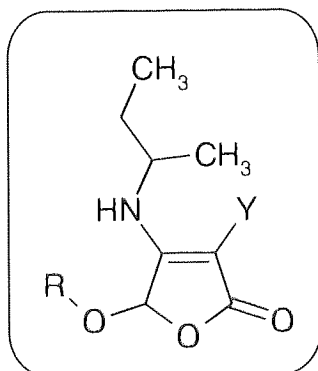
R	Y	Expected	Obtained
Bz	Br	340.22	340/342
Ce	Br	474.52	474/476
Mn	Br	388.34	388/390
Me	Br	264.12	264/266
Bz	Cl	295.76	296/298
Ce	Cl	430.07	430/432
Mn	Cl	343.89	344/346
Me	Cl	219.67	220/222
Bz	H	261.32	-
Ce	H	395.62	396
Mn	H	309.45	-
Me	H	185.22	186

7. 2-Chlorobenzylamine:

5-Alkoxy-4-[(2-chlorobenzyl)amino]-3-halofuran-2(5*H*)-one:
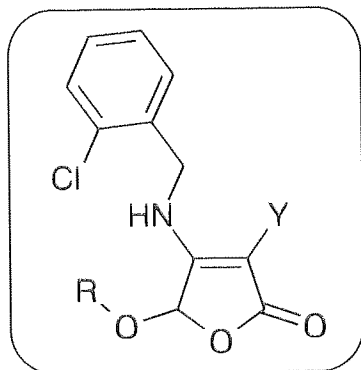
R	Y	Expected	Obtained
Bz	Br	408.68	408/410
Ce	Br	542.98	542/544
Mn	Br	456.81	456/458
Me	Br	332.58	332/334
Bz	Cl	364.23	364/366
Ce	Cl	498.53	498/500
Mn	Cl	412.35	413/415
Me	Cl	288.13	288/290
Bz	H	329.78	-
Ce	H	464.09	464/466
Mn	H	377.91	-
Me	H	253.68	-

8. Cyclohexylamine:

5-Alkoxy-4-(cyclohexylamino)-3-halofuran-2(5*H*)-one:
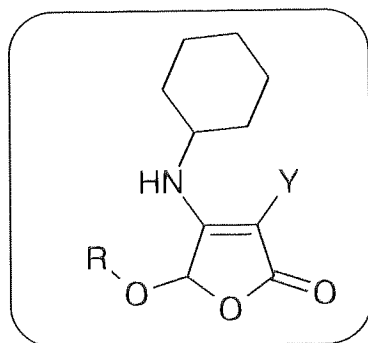
R	Y	Expected	Obtained
Bz	Br	366.25	366/368
Ce	Br	500.56	500/502
Mn	Br	414.38	414/416
Me	Br	290.16	290/292
Bz	Cl	321.80	322/324
Ce	Cl	456.11	456/458
Mn	Cl	369.93	370/372
Me	Cl	245.70	246/248
Bz	H	287.36	-
Ce	H	421.66	422
Mn	H	335.48	-
Me	H	211.26	212

9. Dicyclohexylamine:

5-alkoxy-4-(dicyclohexylamino)-3-halofuran-2(5*H*)-one:
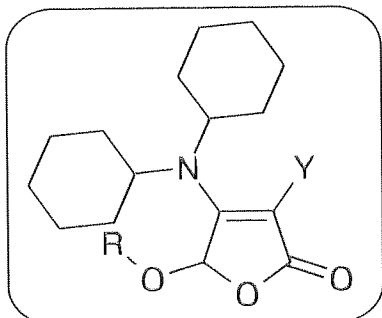
R	Y	Expected	Obtained
Bz	Br	448.40	448/450
Ce	Br	582.70	582/584
Mn	Br	496.53	496/498
Me	Br	372.30	372/374
Bz	Cl	403.95	404/406
Ce	Cl	538.25	538/540
Mn	Cl	452.07	452/454
Me	Cl	327.85	328/330
Bz	H	369.50	-
Ce	H	503.81	-
Mn	H	417.63	-
Me	H	293.40	294

10. 2,6-Dimethylmorpholine:

5-Alkoxy-4-(2,6-dimethylmorpholin-4-yl)-3-halofuran-2(5*H*)-one:
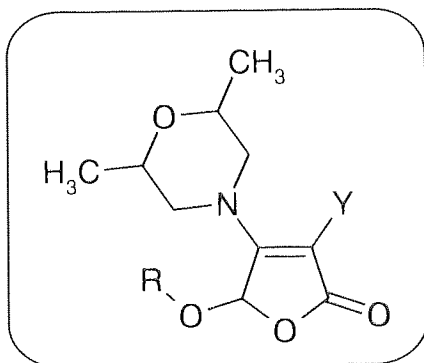
R	Y	Expected	Obtained
Bz	Br	382.25	382/384
Ce	Br	516.56	516/518
Mn	Br	430.38	430/432
Me	Br	306.15	306/308
Bz	Cl	337.80	338/340
Ce	Cl	472.11	472/474
Mn	Cl	385.93	386/388
Me	Cl	261.70	262/264
Bz	H	303.36	-
Ce	H	437.66	438
Mn	H	351.48	352
Me	H	227.26	228

11. 3,5-Dimethylpyrazole:

5-Alkoxy-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-halofuran-2(5*H*)-one:
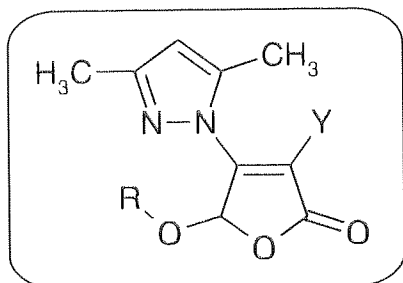
R	Y	Expected	Obtained
Bz	Br	363.21	363/365
Ce	Br	497.51	497/499
Mn	Br	411.34	411/413
Me	Br	287.11	287/289
Bz	Cl	318.76	319/321
Ce	Cl	453.06	453/455
Mn	Cl	366.89	367/369
Me	Cl	242.66	243/245
Bz	H	284.31	–
Ce	H	418.62	419
Mn	H	332.44	–
Me	H	208.22	–

12. Ethyl-1-piperazinecarboxylate:

Ethyl 4-(2-alkoxy-4-halo-5-oxo-2,5-dihydrofuran-3-yl)piperazine-1-carboxylate:
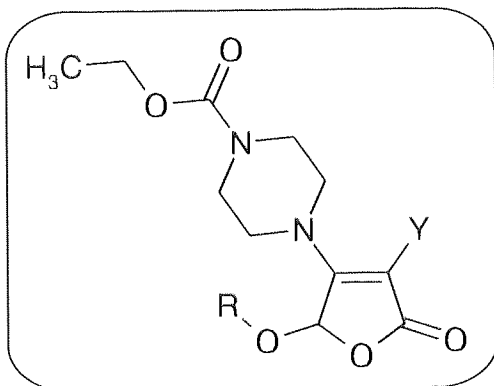
R	Y	Expected	Obtained
Bz	Br	425.28	425/427
Ce	Br	559.58	559/561
Mn	Br	473.40	473/475
Me	Br	349.18	349/351
Bz	Cl	380.83	381/383
Ce	Cl	515.13	515/517
Mn	Cl	428.95	429/431
Me	Cl	304.73	305/307
Bz	H	346.38	–
Ce	H	480.68	481
Mn	H	394.51	–
Me	H	270.28	271

13. Imidazole:

5-Alkoxy-3-halo-4-(1*H*-imidazol-1-yl)furan-2(5*H*)-one:
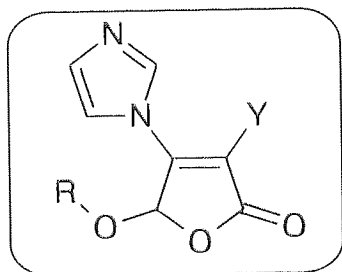
R	Y	Expected	Obtained
Bz	Br	335.16	335/337
Ce	Br	469.46	469/471
Mn	Br	383.28	383/385
Me	Br	259.06	259/261
Bz	Cl	290.70	291/293
Ce	Cl	425.01	-
Mn	Cl	338.83	339/341
Me	Cl	214.61	215/217
Bz	H	256.26	-
Ce	H	390.56	391
Mn	H	304.39	-
Me	H	180.16	181

14. Indoline:

5-Alkoxy-4-(2,3-dihydro-1*H*-indol-1-yl)-3-halofuran-2(5*H*)-one:
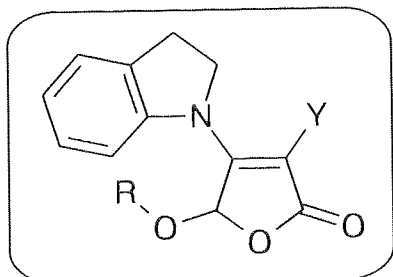
R	Y	Expected	Obtained
Bz	Br	386.24	386/388
Ce	Br	520.55	520/522
Mn	Br	434.37	434/436
Me	Br	310.15	310/312
Bz	Cl	341.79	342/344
Ce	Cl	476.10	476/478
Mn	Cl	389.92	390/392
Me	Cl	265.69	266/268
Bz	H	307.35	-
Ce	H	441.65	442
Mn	H	355.48	-
Me	H	231.25	232

15. 1-Methylpiperazine:

5-Alkoxy-3-halo-4-(4-methylpiperazin-1-yl)furan-2(5*H*)-one:
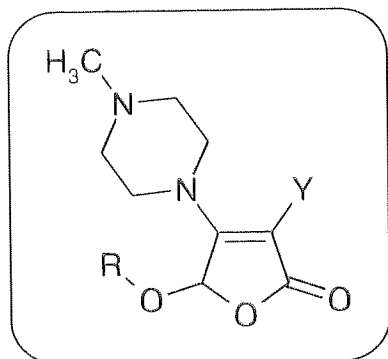
R	Y	Expected	Obtained
Bz	Br	367.24	367/369
Ce	Br	501.54	501/503
Mn	Br	415.37	415/417
Me	Br	291.14	291/293
Bz	Cl	322.79	323/325
Ce	Cl	457.09	457/459
Mn	Cl	370.92	371/373
Me	Cl	246.69	247/249
Bz	H	288.34	-
Ce	H	422.65	423
Mn	H	336.47	-
Me	H	212.25	213

16. 3-Methylpyrazole:

5-Alkoxy-3-halo-4-(3-methyl-1*H*-pyrazol-1-yl)furan-2(5*H*)-one:
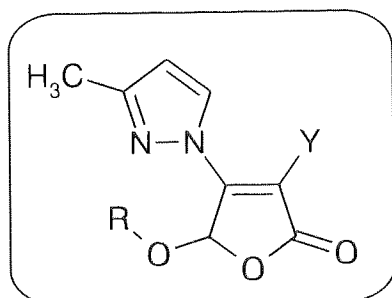
R	Y	Expected	Obtained
Bz	Br	349.18	349/351
Ce	Br	483.49	483/485
Mn	Br	397.31	397/399
Me	Br	273.09	273/275
Bz	Cl	304.73	305/307
Ce	Cl	439.04	439/441
Mn	Cl	352.86	353/355
Me	Cl	228.63	229/231
Bz	H	270.29	-
Ce	H	404.59	405
Mn	H	318.41	-
Me	H	194.19	-

17. N-Phenylbenzylamine:

5-Alkoxy-4-(benzylanilino)-3-halofuran-2(5*H*)-one:
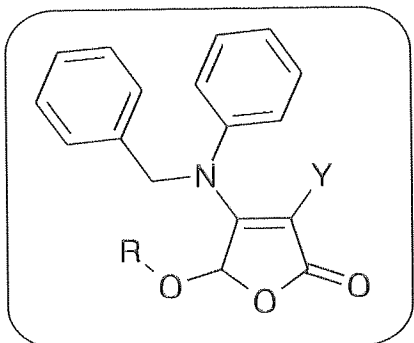
R	Y	Expected	Obtained
Bz	Br	450.33	450/452
Ce	Br	584.63	584/586
Mn	Br	498.46	498/500
Me	Br	374.23	374/376
Bz	Cl	405.88	406/408
Ce	Cl	540.18	540/542
Mn	Cl	454.01	455/457
Me	Cl	329.78	330/332
Bz	H	371.43	-
Ce	H	505.74	-
Mn	H	419.56	-
Me	H	295.34	296

18. N-Phenylpiperazine:

5-Alkoxy-3-halo-4-(4-phenylpiperazin-1-yl)furan-2(5*H*)-one:
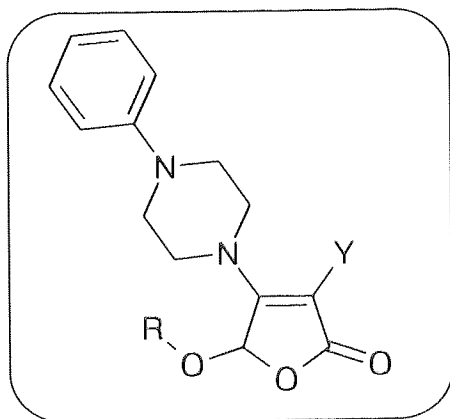
R	Y	Expected	Obtained
Bz	Br	429.31	429/431
Ce	Br	563.62	563/565
Mn	Br	477.44	477/479
Me	Br	353.21	353/355
Bz	Cl	384.86	385/387
Ce	Cl	519.17	519/521
Mn	Cl	432.99	433/435
Me	Cl	308.76	309/311
Bz	H	350.42	-
Ce	H	484.72	485
Mn	H	398.54	-
Me	H	274.32	275

19. 4-Phenylpiperidine:

5-Alkoxy-3-halo-4-(4-phenylpiperidin-1-yl)furan-2(5*H*)-one
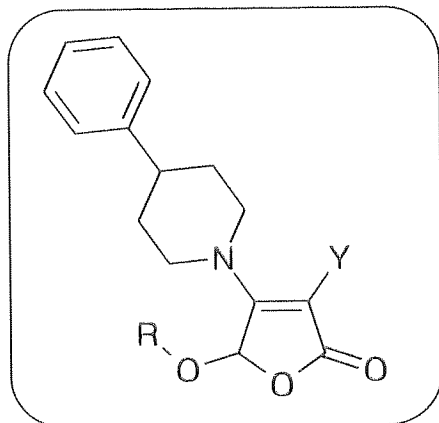
R	Y	Expected	Obtained
Bz	Br	428.32	428/430
Ce	Br	562.63	562/564
Mn	Br	476.45	476/478
Me	Br	352.23	352/354
Bz	Cl	383.87	384/386
Ce	Cl	518.18	518/520
Mn	Cl	432.00	432/434
Me	Cl	307.77	308/310
Bz	H	349.43	-
Ce	H	483.73	484
Mn	H	397.56	-
Me	H	273.33	274

20. 4-(3-Phenylpropyl)piperidine:

5-Alkoxy-3-halo-4-[4-(3-phenylpropyl)piperidin-1-yl]furan-2(5*H*)-one:
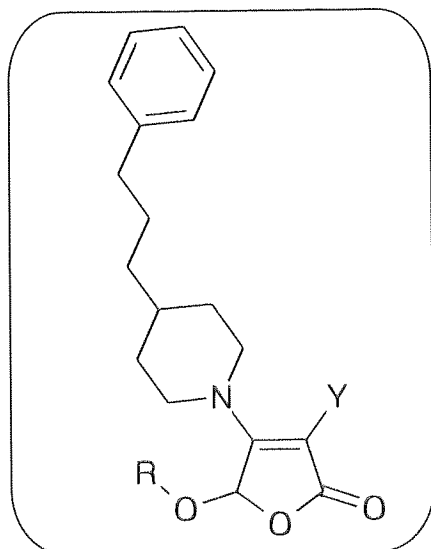
R	Y	Expected	Obtained
Bz	Br	470.40	470/472
Ce	Br	604.71	604/606
Mn	Br	518.53	518/520
Me	Br	394.31	394/396
Bz	Cl	425.95	426/428
Ce	Cl	560.26	560/562
Mn	Cl	474.08	474/476
Me	Cl	349.86	350/352
Bz	H	391.51	-
Ce	H	525.81	526
Mn	H	439.64	-
Me	H	315.41	316

21. Piperidine:

5-Alkoxy-3-halo-4-piperidin-1-ylfuran-2(5*H*)-one:
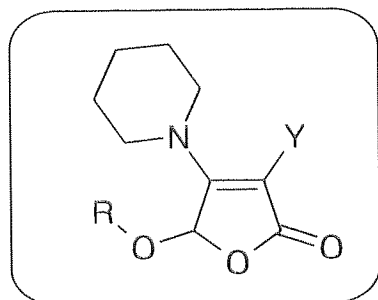
R	Y	Expected	Obtained
Bz	Br	352.23	352/354
Ce	Br	486.53	486/488
Mn	Br	400.35	400/402
Me	Br	276.13	276/278
Bz	Cl	307.78	308/310
Ce	Cl	442.08	442/444
Mn	Cl	355.90	356/358
Me	Cl	231.68	232/234
Bz	H	273.33	-
Ce	H	407.63	408
Mn	H	321.46	-
Me	H	197.23	198

22. Pyrrolidine:

5-Alkoxy-3-halo-4-pyrrolidin-1-ylfuran-2(5*H*)-one:
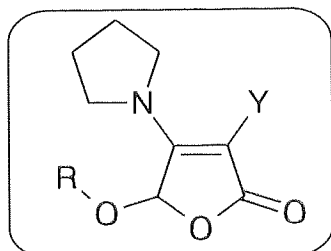
R	Y	Expected	Obtained
Bz	Br	338.20	338/340
Ce	Br	422.50	422/424
Mn	Br	386.33	386/388
Me	Br	262.10	262/264
Bz	Cl	293.75	294/296
Ce	Cl	428.05	428/430
Mn	Cl	341.88	342/344
Me	Cl	217.65	218/220
Bz	H	259.30	-
Ce	H	393.61	394
Mn	H	307.43	308
Me	H	183.21	184

23. *o*-Toluidine:

5-Alkoxy-3-halo-4-(2-toluidino)furan-2(5*H*)-one:
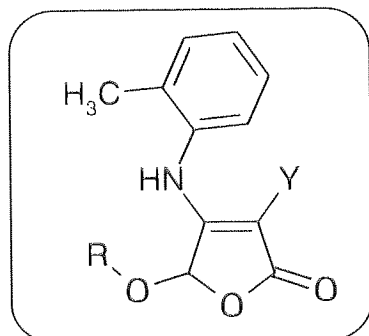
R	Y	Expected	Obtained
Bz	Br	374.23	374/376
Ce	Br	508.54	508/510
Mn	Br	422.36	422/424
Me	Br	298.14	298/300
Bz	Cl	329.78	330/332
Ce	Cl	464.09	464/466
Mn	Cl	377.91	378/380
Me	Cl	253.68	254/256
Bz	H	295.34	-
Ce	H	429.64	430
Mn	H	343.46	-
Me	H	219.24	220

24. 1H-1,2,4-Triazole:

5-Alkoxy-3-halo-4-(1*H*-1,2,4-triazol-1-yl)furan-2(5*H*)-one:
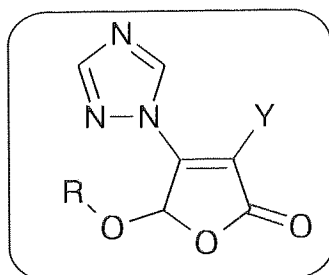
R	Y	Expected	Obtained
Bz	Br	336.14	336/338
Ce	Br	470.45	470/472
Mn	Br	384.27	384/386
Me	Br	260.05	260/262
Bz	Cl	291.69	292/294
Ce	Cl	426.00	426/428
Mn	Cl	339.82	340/342
Me	Cl	215.59	216/218
Bz	H	257.25	-
Ce	H	391.55	392
Mn	H	305.38	-
Me	H	181.15	182

7.4.2 Purification of a representative sample of the compounds synthesised in section 7.4.1

A representative sample of the compounds synthesised in section 7.4.1 was purified by preparative thin layer chromatography in ether (see section 7.3.2). Each compound once purified was submitted for APCI+ mass spectroscopy, proton (and in some cases carbon) nuclear magnetic resonance spectroscopy and infrared spectroscopy.

The results are detailed in the following sections. The sections are arranged in alphabetical order based on the 5-alkoxyfuran-2(5H)-one parent name. Within each section, the purified products appear in the same order in which they occur in the results table in section 3.1.1.

The 5-benzyloxy-3-bromofuran-2(5H)-ones

1. 3,5-Dimethylpyrazole:

5-(Benzyloxy)-3-bromo-4-(3,5-dimethyl-1H-pyrazol-1-yl)furan-2(5H)-one:

(Biological test compound number 15)
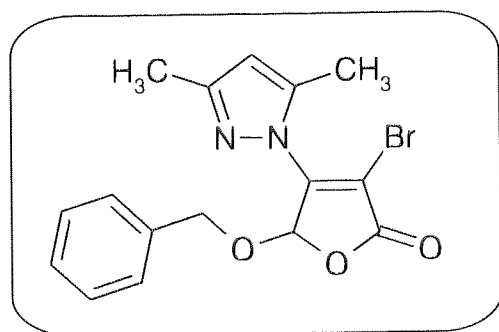
$C_{16}H_{15}BrN_2O_3$

Molecular Weight: 363.21.

APCI+ MS: m/z = 363/365.

^{1}H NMR (CDCl$_3$): δ = 7.41-7.07 (m, 5H, aryl-$\underline{H}$), 6.45 (s, 1H, NCC$\underline{H}$), 6.08 (s, 1H, OC$\underline{H}$), 4.90 & 4.86 (d, J = 11.6 Hz, 1H, C$\underline{H}_2$), 4.80 & 4.75 (d, J = 11.6 Hz, 1H, C$\underline{H}_2$), 2.41 (s, 3H, BrC=CNCC$\underline{H}_3$), 2.30 (s, 3H, BrC=CNNCC$\underline{H}_3$) ppm.

IR: ν = 3594, 3008, 2929, 2861, 2358, 2339, 1782, 1670, 1576 cm^{-1}.

Rf (Ether) = 0.30.

2. Piperidine:

5-(Benzyloxy)-3-bromo-4-piperidin-1-ylfuran-2(5H)-one:

(Biological test compound number 25)
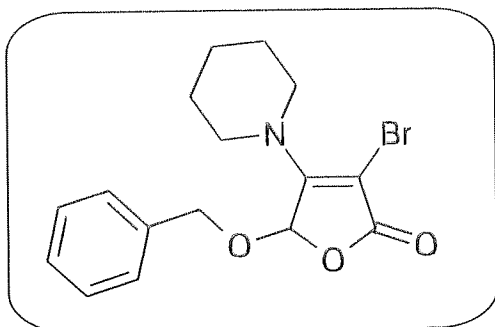
C$_{16}$H$_{18}$BrNO$_3$

Molecular Weight: 352.23.

APCI+ MS: m/z = 352/354.

^{1}H NMR (CDCl$_3$): δ = 7.40-7.09 (m, 5H, aryl-$\underline{H}$), 5.78 (s, 1H, C$\underline{H}$), 4.84 & 4.80 (d, J = 11.2 Hz, 1H, OC$\underline{H}_2$), 4.70 & 4.66 (d, J = 11.3 Hz, 1H, OC$\underline{H}_2$), 3.57 (m, 4H, NC$\underline{H}_2$), 1.66 (m, 6H, NCH$_2$(C$\underline{H}_2$)$_3$) ppm.

IR: ν = 3016, 2942, 2866, 1751, 1621 cm^{-1}.

Rf (Ether) = 0.44.

The 5-benzyloxy-3-chlorofuran-2(5*H*)-ones

1. 3-Methylpyrazole:

5-(Benzyloxy)-3-chloro-4-(3-methyl-*1H*-pyrazol-1-yl)-furan-2(5*H*)-one:
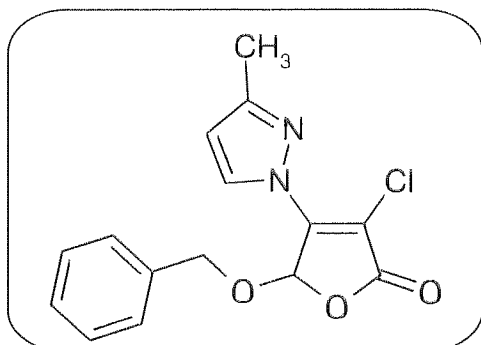
$C_{15}H_{13}ClN_2O_3$

Molecular weight: 304.73.

APCl+ MS: m/z = 305/307.

^{1}H NMR (CDCl$_3$): δ = 8.24 (d, *J* = 2.7 Hz, 1H, NC$\underline{H}$), 7.44-7.33 (m, 5H, aryl-$\underline{H}$), 6.46 (s, 1H, OC$\underline{H}$), 6.41 (d, *J* = 2.8 Hz, 1H, NCHC$\underline{H}$), 5.00 & 4.96 (d, *J* = 11.3 Hz, 1H, C$\underline{H}_2$), 4.94 & 4.90 (d, *J* = 11.2 Hz, 1H, C$\underline{H}_2$), 2.39 (s, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 165.66 ($\underline{C}$=O), 153.67 (ClC=$\underline{C}$N), 147.17 ($\underline{C}$CH$_3$), 135.40 (aryl-$\underline{C}$CH$_2$), 130.83 (ClC=CN$\underline{C}$), 128.58 (*m*-$\underline{C}$), 128.51 (*p*-$\underline{C}$), 128.47 (*o*-$\underline{C}$), 111.24 (ClC=CNC$\underline{C}$), 102.61 ($\underline{C}$Cl), 98.04 (O$\underline{C}$H), 72.46 ($\underline{C}$H$_2$), 13.64 ($\underline{C}$H$_3$) ppm.

IR: ν = 2879, 2362, 2339, 1788, 1672, 1552 cm^{-1}.

Rf (Ether) = 0.51.

The 3-bromo-5-cetyloxyfuran-2(5*H*)-ones

1. Benzimidazole:

4-(1*H*-Benzimidazol-1-yl)-3-bromo-5-cetyloxyfuran-2(5*H*)-one:

(Biological test compound number 29)
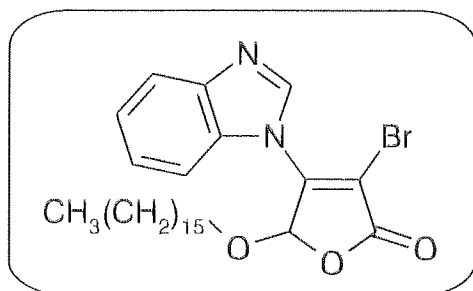
$C_{27}H_{39}BrN_2O_3$

Molecular Weight: 519.52.

APCI+ MS: m/z = 519/521.

^{1}H NMR (CDCl$_3$): δ = 8.62 (s, 1H, NC$\underline{H}$), 7.91-7.87 (m, 1H, aryl-$\underline{H}$), 7.48-7.40 (m, 3H, aryl-$\underline{H}$), 6.38 (s, 1H, OC$\underline{H}$), 3.96-3.76 (m, 2H, OC$\underline{H}_2$), 1.59-1.51 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.5 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 3008, 2925, 2852, 1790, 1650 cm^{-1}.

Rf (Ether) = 0.51.

2. Benzylmethylamine:

4-[Benzyl(methyl)amino]-3-bromo-5-cetyloxyfuran-2(5H)-one:

(Biological test compound number 8)
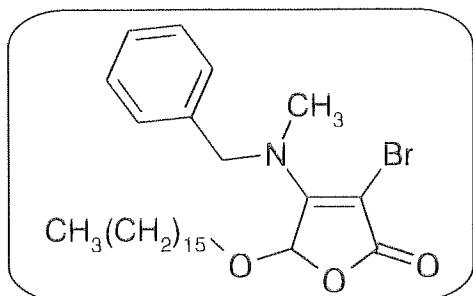
$C_{28}H_{44}BrNO_3$

Molecular Weight: 522.56.

APCI+ MS: m/z = 522/524.

^{1}H NMR (CDCl$_3$): δ = 7.42-7.21 (m, 5H, aryl-$\underline{H}$), 5.77 (s, 1H, C$\underline{H}$), 3.81-3.54 (m, 4H, 2H-NC$\underline{H}_2$ & 2H-OC$\underline{H}_2$), 3.12 (s, 3H, NC$\underline{H}_3$), 1.55 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.87 (t, J = 6.6 Hz, 3H, CH$_2$C$\underline{H}_3$) ppm.

IR: ν = 3010, 2925, 2852, 1755, 1625 cm^{-1}.

Rf (Ether) = 0.63.

3. Piperidine:

3-Bromo-5-cetyloxy-4-piperidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 30)

C$_{25}$H$_{44}$BrNO$_3$

Molecular Weight: 486.53.

APCI+ MS: m/z = 486/488.

^{1}H NMR (CDCl$_3$): δ = 5.70 (s, 1H, C*H*), 3.82-3.53 (m, 6H, 2H-OC*H*$_2$ & 4H-NC*H*$_2$), 1.74-1.47 (m, 8H, 2H-OCH$_2$C*H*$_2$ & 6H-NCH$_2$(C*H*$_2$)$_3$), 1.24 (m, 26H, OCH$_2$CH$_2$(C*H*$_2$)$_{13}$), 0.87 (t, *J* = 6.6 Hz, 3H, C*H*$_3$) ppm.
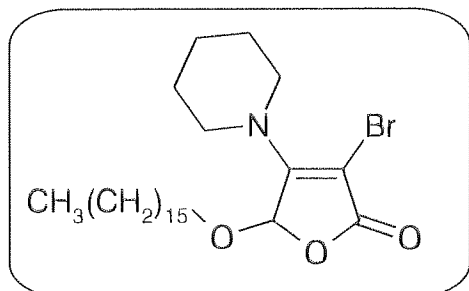
IR: ν = 3016, 2925, 2858, 1749, 1621 cm^{-1}.

Rf (Ether) = 0.49.

4. Pyrrolidine:

3-Bromo-5-cetyloxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 11)
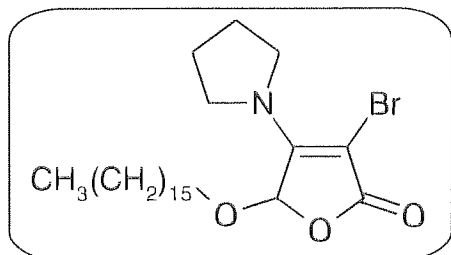
$C_{24}H_{42}BrNO_3$

Molecular Weight: 472.50.

APCI+ MS: m/z = 472/474.

^{1}H NMR (CDCl$_3$): δ = 5.67 (s, 1H, C$\underline{H}$), 4.04-3.45 (m, 6H, 2H-OC$\underline{H}_2$ & 4H-NC$\underline{H}_2$), 2.05-1.86 (m, 4H, NCH$_2$C$\underline{H}_2$), 1.68-1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.87 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 3010, 2925, 2852, 1751, 1631 cm^{-1}.

Rf (Ether) = 0.58.

The 3-bromo-5-menthyloxyfuran-2(5*H*)-ones

1. Benzimidazole:

4-(1*H*-Benzimidazol-1-yl)-3-bromo-5-menthyloxyfuran-2(5*H*)-one:

(Biological test compound number 38)
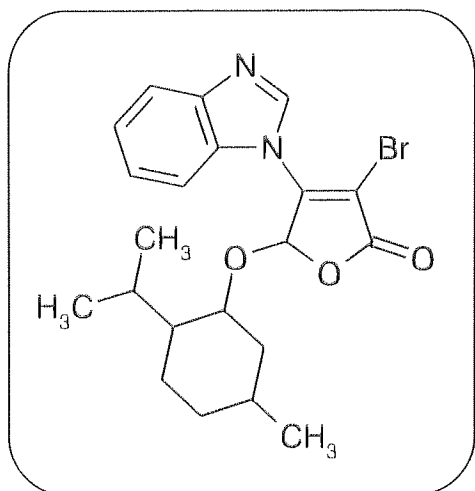
$C_{21}H_{25}BrN_2O_3$

Molecular Weight: 433.34.

APCI+ MS: m/z = 433/435.

^{1}H-NMR (CDCl$_3$): δ = 8.43 & 8.35 (s, 1H, NC*H* (different isomers)), 7.91-7.87 (m, 1H, aryl-*H*), 7.52-7.39 (m, 3H, aryl-*H*), 6.47 & 6.39 (s, 1H, OC*H*O (different isomers)), 3.84-3.41 (m, 1H, *H*-menthol), 2.32-1.91 (m, 2H, *H*-menthol), 1.74-1.52 (m, 2H, *H*-menthol), 1.38-0.69 (m, 14H, *H*-menthol) ppm.

IR: ν = 3018, 2962, 2925, 2869, 2358, 2345, 1788, 1652 cm^{-1}.

Rf (Ether) = 0.51.

2. Benzylmethylamine:

4-[Benzyl(methyl)amino]-3-bromo-5-menthyloxyfuran-2(5*H*)-one:

(Biological test compound number 27)
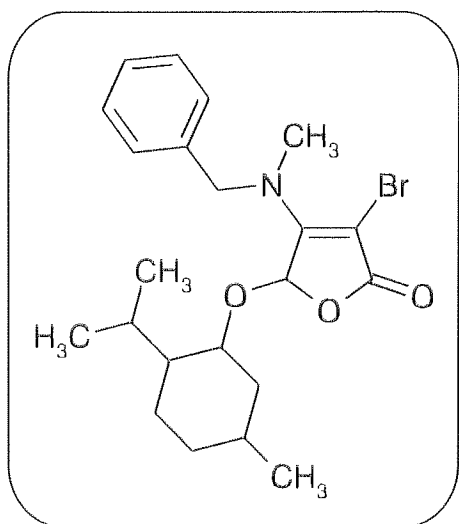
$C_{22}H_{30}BrNO_3$

Molecular Weight: 436.39.

APCI+ MS: m/z = 436.

^{1}H-NMR (CDCl$_3$): δ = 7.37-7.21 (m, 5H, aryl-$\underline{H}$), 5.94 & 5.86 (s, 1H, OC$\underline{H}$O (different isomers)), 4.86 & 4.80 (d, *J* = 15.8 Hz, 1H, NC$\underline{H}_2$), 4.68 & 4.61 (d, *J* = 15.9 Hz, 1H, NC$\underline{H}_2$), 3.70-3.55 (m, 1H, $\underline{H}$-menthol), 3.12 & 3.09 (s, 3H, NC$\underline{H}_3$ (different isomers)), 2.23-2.07 (m, 2H, $\underline{H}$-menthol), 1.65-1.60 (m, 2H, $\underline{H}$-menthol), 1.33-0.66 (m, 14H, $\underline{H}$-menthol) ppm.

IR: ν = 3018, 2956, 2923, 2869, 2362, 2329, 1749, 1630 cm^{-1}.

Rf (Ether) = 0.63.

3. Imidazole:

3-Bromo-4-(1*H*-imidazol-1-yl)-5-menthyloxyfuran-2(5*H*)-one:

(Biological test compound number 10)
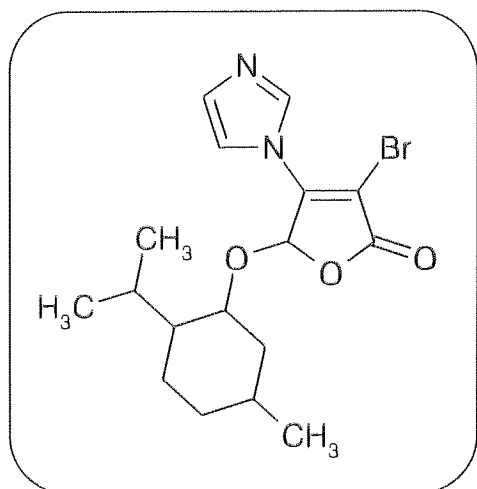
$C_{17}H_{23}BrN_2O_3$

Molecular Weight: 383.28.

APCI+ MS: m/z = 383/385.

^{1}H-NMR (CDCl$_3$): δ = 8.35 & 8.29 (s, 1H, BrC=CNCC<u>H</u> (different isomers)), 7.83 & 7.68 (s, 1H, BrC=CNC<u>H</u>N (different isomers)), 6.29 & 6.25 (s, 1H, BrC=CNC<u>H</u>C (different isomers)), 5.82 & 5.66 (s, 1H, OC<u>H</u>O (different isomers), 3.90-3.42 (m, 1H, <u>H</u>-menthol), 2.36-1.98 (m, 2H, <u>H</u>-menthol), 1.79-1.60 (m, 2H, <u>H</u>-menthol), 1.54-0.78 (m, 14H, <u>H</u>-menthol) ppm.

IR: ν = 3020, 2956, 2925, 2869, 2356, 2335, 1782, 1652, 1598 cm^{-1}.

Rf (Ether) = 0.67.

4. 1-Methylpiperazine:

3-Bromo-5-menthyloxy-4-(4-methylpiperazin-1-yl)furan-2(5*H*)-one:
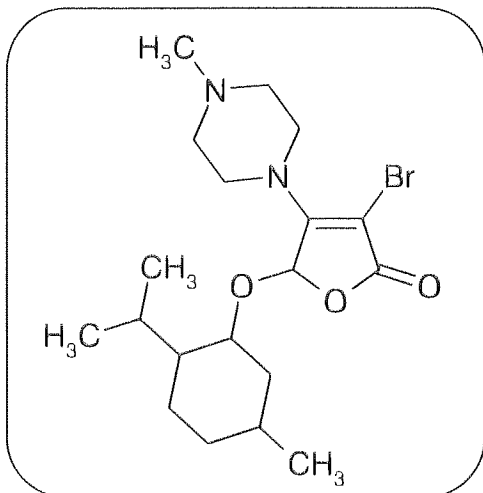
$C_{19}H_{31}BrN_2O_3$

Molecular Weight: 415.37.

APCI+ MS: m/z = 415/417.

^{1}H-NMR (CDCl$_3$): δ = 5.86 & 5.80 (s, 1H, OC*H*O (different isomers)), 3.84-3.49 (m, 5H, 4H-CH$_3$NCH$_2$C*H*$_2$ & 1H-*H*-menthol), 2.55-2.51 (m, 4H, CH$_3$NC*H*$_2$), 2.34 (s, 3H, NC*H*$_3$), 2.25-2.07 (m, 2H, *H*-menthol), 1.68-1.63 (m, 2H, *H*-menthol), 1.36-0.74 (m, 14H, *H*-menthol) ppm.

IR: ν = 3012, 2954, 2929, 2869, 2802, 2362, 2345, 1753, 1628 cm^{-1}.

5. 4-Phenylpiperidine:

3-Bromo-5-menthyloxy-4-(4-phenylpiperidin-1-yl)furan-2(5*H*)-one:
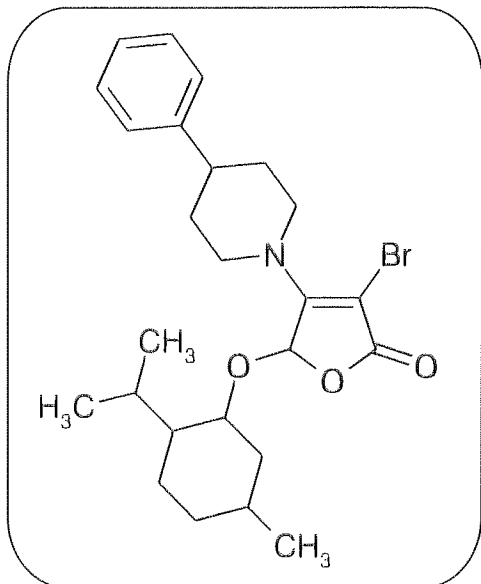
$C_{25}H_{34}BrNO_3$

Molecular Weight: 476.45.

APCl+ MS: m/z = 476/478.

^{1}H-NMR (CDCl$_3$): δ = 7.37-7.20 (m, 5H, aryl-H), 5.91 & 5.85 (s, 1H, OCHO (different isomers)), 4.62-4.31 (m, 2H, NCH$_2$), 3.75-3.53 (m, 1H, H-menthol) 3.22-3.06 (m, 2H, NCH$_2$), 2.86-2.77 (m, 1H, NCH$_2$CH$_2$CH), 2.29-2.12 (m, 2H, H-menthol), 1.99-1.76 (m, 4H, NCH$_2$CH$_2$), 1.69-1.64 (m, 2H, H-menthol), 1.38-0.76 (m, 14H, H-menthol) ppm.

IR: ν = 3016, 2960, 2929, 2869, 2362, 2329, 1753, 1626 cm^{-1}.

6. Piperidine:

3-Bromo-5-menthyloxy-4-piperidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 14)
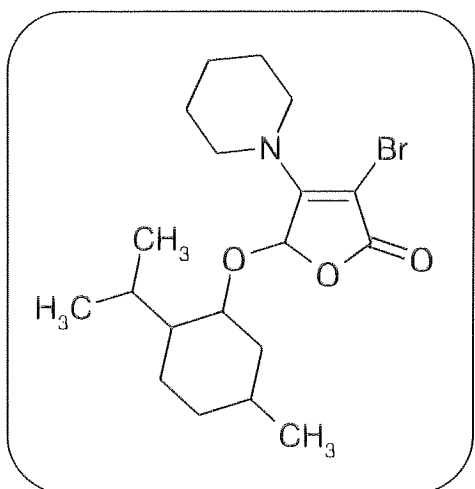
$C_{19}H_{30}BrNO_3$

Molecular Weight: 400.35.

APCI+ MS: m/z = 400/402.

^{1}H-NMR (CDCl$_3$): δ = 5.85 & 5.78 (s, 1H, OC$\underline{H}$O (different isomers)), 3.75-3.51 (m, 5H, 4H-NC$\underline{H}_2$ & 1H-$\underline{H}$-menthol), 2.26-2.07 (m, 2H, $\underline{H}$-menthol), 1.68 (m, 8H, 6H-NCH$_2$(C$\underline{H}_2$)$_3$ & 2H-$\underline{H}$-menthol), 1.34-0.74 (m, 14H, $\underline{H}$-menthol) ppm.

IR: ν = 3016, 2948, 2931, 2871, 2358, 2333, 1751, 1626 cm^{-1}.

Rf (Ether) = 0.63.

7. Pyrrolidine:

3-Bromo-5-menthyloxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 33)
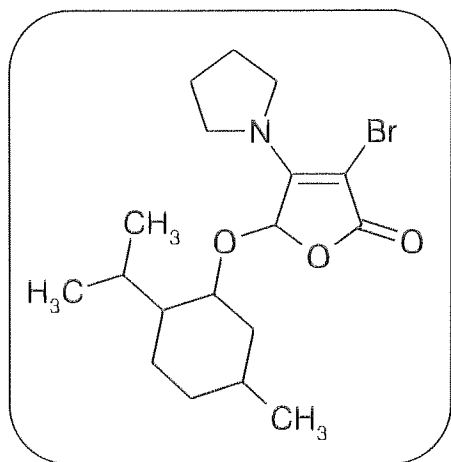
$C_{18}H_{28}BrNO_3$

Molecular Weight: 386.33.

APCI+ MS: m/z = 386/388.

^{1}H-NMR (CDCl$_3$): δ = 5.82 & 5.76 (s, 1H, OC$\underline{H}$O (different isomers)), 4.16-3.22 (m, 5H, 4H-NC$\underline{H}_2$ & 1H-$\underline{H}$-menthol), 2.22-2.08 (m, 2H, $\underline{H}$-menthol), 1.95-1.93 (m, 4H, NCH$_2$C$\underline{H}_2$), 1.67-1.64 (m, 2H, $\underline{H}$-menthol), 1.36-0.75 (m, 14H, $\underline{H}$-menthol) ppm.

IR: ν = 3016, 2956, 2925, 2875, 1747, 1625 cm^{-1}.

Rf (Ether) = 0.60.

The 3-bromo-5-methoxyfuran-2(5*H*)-ones

1. Benzimidazole:

4-(1*H*-Benzimidazol-1-yl)-3-bromo-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 31)
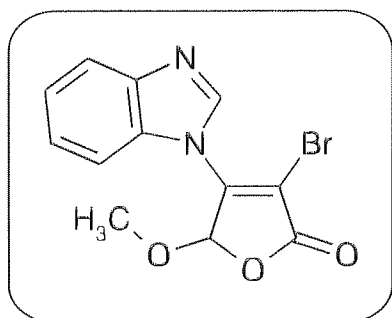
$C_{12}H_9BrN_2O_3$

Molecular Weight: 309.12.

APCI+ MS: m/z = 309/311.

^{1}H-NMR (CDCl$_3$): δ = 8.73 (s, 1H, NC$\underline{H}$), 7.92-7.89 (m, 1H, aryl-$\underline{H}$), 7.51-7.43 (m, 3H, aryl-$\underline{H}$), 6.37 (s, 1H, OC$\underline{H}$), 3.68 (s, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2999, 2945, 2362, 2339, 1792, 1652, 1508 cm^{-1}.

Rf (Ether) = 0.21.

2. Piperidine:

3-Bromo-5-methoxy-4-piperidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 26)
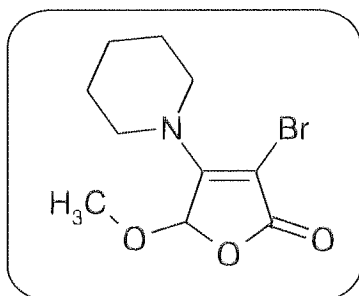
$C_{10}H_{14}BrNO_3$

Molecular Weight: 276.13.

APCl+ MS: m/z = 276/278.

^{1}H-NMR (CDCl$_3$): δ = 5.70 (s, 1H, C$\underline{H}$), 3.66 (m, 4H, NC$\underline{H}_2$), 3.48 (s, 3H, C$\underline{H}_3$), 1.70 (m, 6H, NCH$_2$(C$\underline{H}_2$)$_3$) ppm.

IR: ν = 3016, 2942, 2866, 1757, 1628 cm^{-1}.

Rf (Ether) = 0.33.

3. Pyrrolidine:

3-Bromo-5-methoxy-4-pyrrolidin-1-ylfuran-2(5H)-one:

(Biological test compound number 22)
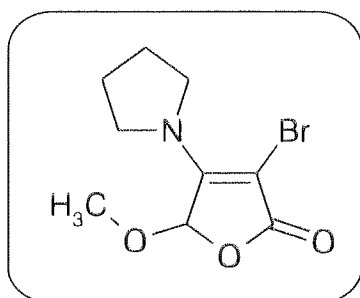
$C_9H_{12}BrNO_3$

Molecular Weight: 262.10.

APCI+ MS: m/z = 262/264.

^{1}H-NMR (CDCl$_3$): δ = 5.65 (s, 1H, C$\underline{H}$), 4.04-3.40 (m, 4H, NC$\underline{H}_2$), 3.48 (s, 3H, C$\underline{H}_3$), 1.96 (m, 4H, NCH$_2$C$\underline{H}_2$) ppm.

IR: ν = 3010, 2983, 2887, 2836, 1753, 1627 cm^{-1}.

Rf (Ether) = 0.30.

The 5-Cetyloxy-3-chlorofuran-2(5*H*)-ones

1. Benzimidazole:

4-(1*H*-Benzimidazol-1-yl)-5-cetyloxy-3-chlorofuran-2(5*H*)-one:

(Biological test compound number 16)
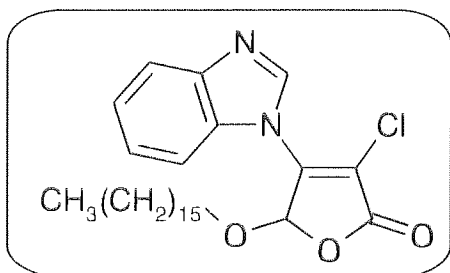
$C_{27}H_{39}ClN_2O_3$

Molecular Weight: 475.07.

APCI+ MS: m/z = 475/477.

^{1}H NMR (CDCl$_3$): δ = 8.59 (s, 1H, NC$\underline{H}$), 7.92-7.83 (m, 1H, aryl-$\underline{H}$), 7.51-7.39 (m, 3H, aryl-$\underline{H}$), 6.41 (s, 1H, OC$\underline{H}$), 4.00-3.76 (m, 2H, OC$\underline{H}_2$), 1.66-1.51 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.87 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 3604, 2925, 2852, 2362, 2339, 1793, 1718, 1662, 1608, 1508 cm^{-1}.

Rf (Ether) = 0.48.

2. 1-Benzylpiperazine:

4-(4-Benzylpiperazin-1-yl)-5-cetyloxy-3-chlorofuran-2(5*H*)-one:

(Biological test compound number 23)
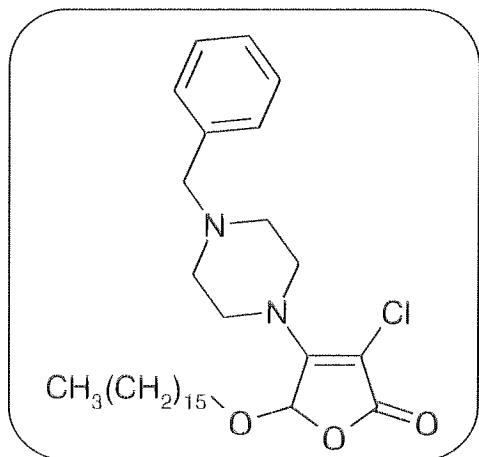
$C_{31}H_{49}ClN_2O_3$

Molecular Weight: 533.19.

APCI+ MS: m/z = 533/535.

^{1}H NMR (CDCl$_3$): δ = 7.34-7.30 (m, 5H, aryl-H̲), 5.69 (s, 1H, CH̲), 3.82-3.51 (m, 8H, 2H-OCH̲$_2$ & 6H-aryl-CH̲$_2$NCH$_2$CH̲$_2$), 2.54 (t, *J* = 4.9 Hz, 4H, aryl-CH$_2$NCH̲$_2$), 1.65-1.46 (m, 2H, OCH$_2$CH̲$_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(CH̲$_2$)$_{13}$), 0.87 (t, *J* = 6.6 Hz, 3H, CH̲$_3$) ppm.

IR: ν = 3627, 3016, 2925, 2852, 2364, 2343, 1761, 1631 cm^{-1}.

Rf (Ether) = 0.41.

3. 2-Chlorobenzylamine:

5-Cetyloxy-3-chloro-4-[(2-chlorobenzyl)amino]furan-2(5*H*)-one:

(Biological test compound number 24)
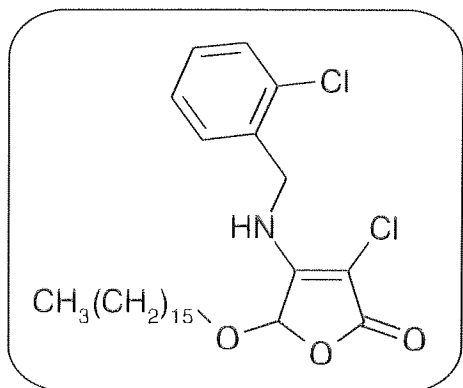
C$_{27}$H$_{41}$Cl$_2$NO$_3$

Molecular weight: 498.53.

APCI+ MS: m/z = 498/500.

^{1}H NMR (CDCl$_3$): δ = 7.45-7.30 (m, 4H, aryl-$\underline{H}$), 5.75 (s, 1H, C$\underline{H}$), 5.14 (m, 1H, N$\underline{H}$), 4.84-4.70 (m, 2H, NC$\underline{H}_2$), 3.82-3.58 (m, 2H, OC$\underline{H}_2$), 1.56 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.26 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, J = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 2405, 2364, 1762, 1658 cm^{-1}.

Rf (Ether) = 0.64.

4. Cyclohexylamine:

5-Cetyloxy-3-chloro-4-(cyclohexylamino)furan-2(5*H*)-one:

(Biological test compound number 28)
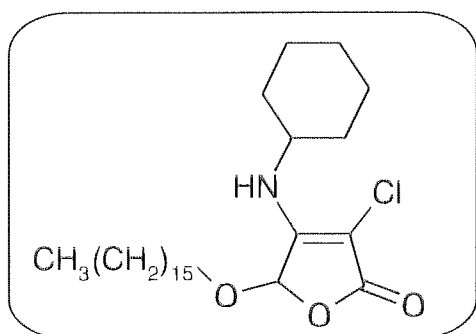
C$_{26}$H$_{46}$ClNO$_3$

Molecular weight: 456.11.

APCl+ MS: m/z = 456/458.

^{1}H NMR (CDCl$_3$): δ = 6.00 (s, 1H, OC$\underline{H}$), 5.70 (m, 1H, N$\underline{H}$), 3.67-3.58 (m, 2H, OC$\underline{H}_2$), 2.07-1.46 (m, 13H, 2H-OCH$_2$C$\underline{H}_2$ & 11H-NC$\underline{H}$(C$\underline{H}_2$)$_5$), 1.26 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: v = 2925, 2858, 2399, 2358, 1765, 1657 cm^{-1}.

Rf (Ether) = 0.63.

5. 3,5-Dimethylpyrazole:

5-Cetyloxy-3-chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)furan-2(5*H*)-one:

(Biological test compound number 35)
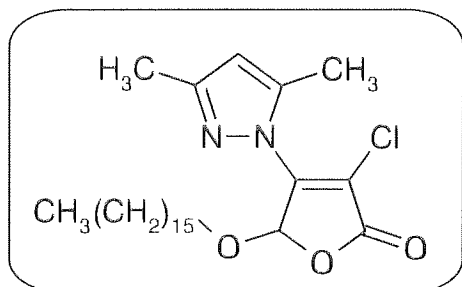
C$_{25}$H$_{41}$ClN$_2$O$_3$

Molecular Weight: 453.06.

APCI+ MS: m/z = 453/455.

^{1}H NMR (CDCl$_3$): δ = 6.37 (s, 1H, NCC$\underline{H}$C), 6.04 (s, 1H, OC$\underline{H}$), 3.79-3.56 (m, 2H, OC$\underline{H}_2$), 2.39 (s, 3H, ClC=CNCC$\underline{H}_3$), 2.25 (s, 3H, ClC=CNNCC$\underline{H}_3$), 1.57-1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.24 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.86 (t, *J* = 6.6 Hz, 3H, CH$_2$C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 1785, 1680, 1571 cm^{-1}.

Rf (Ether) = 0.73.

6. Ethyl-1-piperazine carboxylate:

Ethyl 4-(2-cetyloxy-4-chloro-5-oxo-2,5-dihydrofuran-3-yl)piperazine-1-carboxylate:

(Biological test compound number 39)
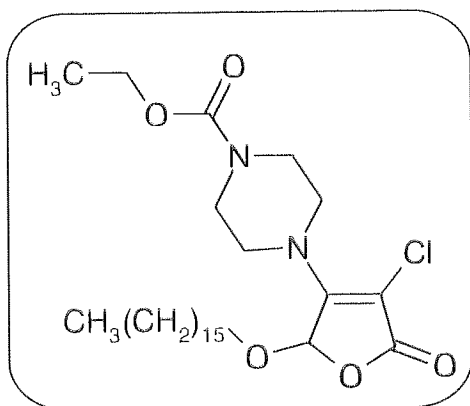
$C_{27}H_{47}ClN_2O_5$

Molecular Weight: 515.13.

APCl+ MS: m/z = 515/517.

^{1}H NMR (CDCl$_3$): δ = 5.71 (s, 1H, C$\underline{H}$), 4.16 (q, J = 7.1 Hz, 2H, CH$_3$C$\underline{H}_2$O), 3.85-3.51 (m, 10H, 8H-N(C$\underline{H}_2$C$\underline{H}_2$)N & 2H-OC$\underline{H}_2$CH$_2$), 1.66-1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.31-1.25 (m, 29H, 26H-OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$ & 3H-OCH$_2$C$\underline{H}_3$), 0.87-0.85 (t, J = 6.6 Hz, 3H, CH$_2$CH$_2$C$\underline{H}_3$) ppm.

IR: ν = 3627, 3008, 2925, 2852, 2362, 2339, 1758, 1699, 1637 cm^{-1}.

Rf (Ether) = 0.36.

7. 1H-1,2,4-Triazole:

5-Cetyloxy-3-chloro-4-(1*H*-1,2,4-triazol-1-yl)furan-2(5*H*)-one:

(Biological test compound number 18)
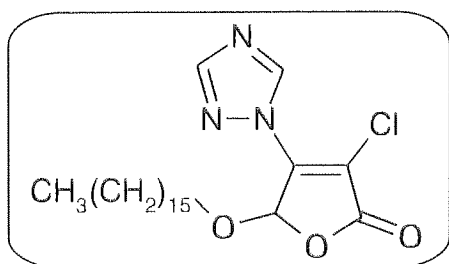
$C_{22}H_{36}ClN_3O_3$

Molecular weight: 426.00.

APCl+ MS: m/z = 426/428.

^{1}H NMR (CDCl$_3$): δ = 9.02 (s, 1H, ClC=CNC$\underline{H}$), 8.18 (s, 1H, ClC=CNNC$\underline{H}$), 6.35 (s, 1H, OC$\underline{H}$), 4.00-3.60 (m, 2H, OC$\underline{H}_2$), 1.69-1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.24 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.87 (t, J = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2931, 2858, 1787, 1687 cm^{-1}.

Rf (Ether) = 0.46.

The 3-chloro-5-menthyloxyfuran-2(5*H*)-ones

1. Benzylmethylamine:

4-[Benzyl(methyl)amino]-3-chloro-5-menthyloxyfuran-2(5*H*)-one:

(Biological test compound number 32)
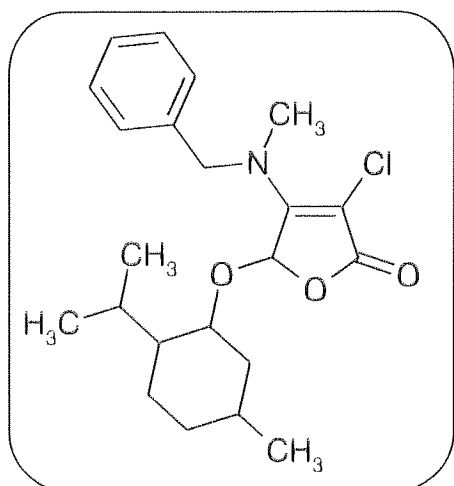
$C_{22}H_{30}ClNO_3$

Molecular Weight: 391.94.

APCI+ MS: m/z = 392/394.

^{1}H-NMR (CDCl$_3$): δ = 7.42-7.23 (m, 5H, aryl-H̲), 5.92 & 5.84 (s, 1H, OCH̲O (different isomers)), 4.86 & 4.80 (d, *J* = 15.8 Hz, 1H, NCH̲$_2$), 4.68 & 4.61 (d, *J* = 15.9 Hz, 1H, NCH̲$_2$), 3.61-3.51 (m, 1H, H̲-menthol), 3.12 & 3.09 (s, 3H, NCH̲$_3$ (different isomers)), 2.29-2.08 (m, 2H, H̲-menthol), 1.66-1.62 (m, 2H, H̲-menthol), 1.35-0.67 (m, 14H, H̲-menthol) ppm.

IR: ν = 3010, 2962, 2925, 2869, 2359, 2339, 1753, 1637 cm^{-1}.

Rf (Ether) = 0.61.

2. 2,6-Dimethylmorpholine:

3-Chloro-4-(2,6-dimethylmorpholin-4-yl)-5-menthyloxyfuran-2(5*H*)-one:

(Biological test compound number 34)
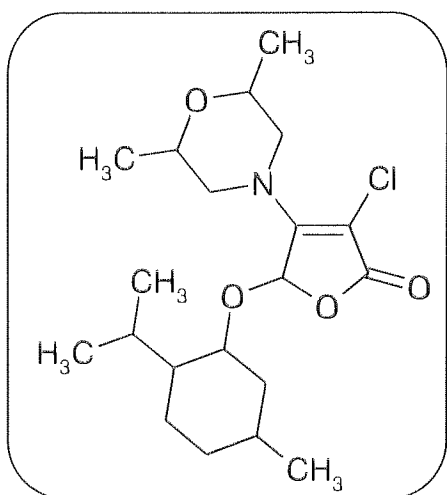
$C_{20}H_{32}ClNO_4$

Molecular Weight: 385.93.

APCl+ MS: m/z = 386/388.

^{1}H-NMR (CDCl$_3$): δ = 5.82 & 5.80 (s, 1H, OC<u>H</u>O (different isomers)), 4.15-3.50 (m, 5H, 1H-<u>H</u>-menthol & 2H-NC<u>H</u>$_2$ & 2H-NCH$_2$C<u>H</u>), 2.89-2.68 (m, 2H, NC<u>H</u>$_2$), 2.28-2.11 (m, 2H, <u>H</u>-menthol), 1.67-1.62 (m, 2H, <u>H</u>-menthol), 1.30-0.75 (m, 20H, 14H-<u>H</u>-menthol & 6H-OCHC<u>H</u>$_3$) ppm.

IR: ν = 3006, 2958, 2933, 2875, 2358, 2339, 1761, 1631 cm^{-1}.

Rf (Ether) = 0.59.

The 3-chloro-5-methoxyfuran-2(5*H*)-ones

1. 4-Amino-1-benzylpiperidine:

4-[(1-Benzylpiperidin-4-yl)amino]-3-chloro-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 7)
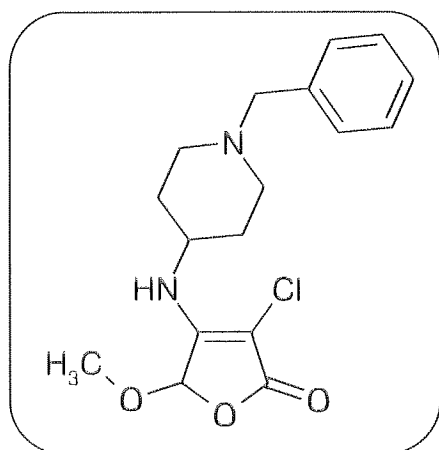
$C_{17}H_{21}ClN_2O_3$

Molecular Weight: 336.82.

APCI+ MS: m/z = 337/339.

^{1}H-NMR (CDCl$_3$): δ = 7.33-7.26 (m, 5H, aryl-H̲), 5.64 (s, 1H, OCH̲), 4.94 (d, *J* = 9.0 Hz, 1H, NH̲), 3.97-3.60 (m, 1H, NCH̲), 3.53 (s, 2H, CH̲$_2$-aryl), 3.48 (s, 3H, CH̲$_3$), 2.96-2.86 (m, 4H, CHCH$_2$CH̲$_2$), 2.17-2.07 (m, 2H, CHCH̲$_2$), 1.67-1.52 (m, 2H, CHCH̲$_2$) ppm.

IR: ν = 3390, 3010, 2945, 2810, 2763, 2356, 1762, 1658, 1522 cm^{-1}.

Rf (Ether) = 0.26.

2. 4-(3-Aminopropyl)morpholine:

3-Chloro-5-methoxy-4-[(3-morpholin-4-ylpropyl)amino]furan-2(5*H*)-one:

(Biological test compound number 3)
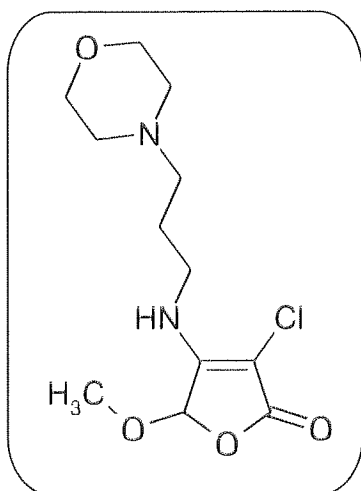
$C_{12}H_{19}ClN_2O_4$

Molecular Weight: 290.75.

APCI+ MS: m/z = 291/293.

^{1}H NMR (CDCl$_3$): δ = 7.77 (m, 1H, N_H_), 5.69 (s, 1H, C_H_), 3.76-3.73 (m, 4H, NCH$_2$C_H_$_2$O), 3.50 (s, 3H, C_H_$_3$), 2.60-2.51 (m, 8H, 4H-NC_H_$_2$CH$_2$O & 4H-NHC_H_$_2$CH$_2$C_H_$_2$), 1.82-1.73 (m, 2H, NHCH$_2$C_H_$_2$) ppm.

IR: ν = 3018, 2939, 2860, 2823, 2358, 2343, 1758, 1654 cm^{-1}.

Rf (Ether) = 0.13.

3. Benzimidazole:

4-(1*H*-Benzimidazol-1-yl)-3-chloro-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 36)
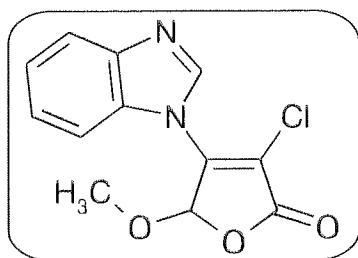
$C_{12}H_9ClN_2O_3$

Molecular Weight: 264.67.

APCI+ MS: m/z = 265/267.

^{1}H NMR (CDCl$_3$): δ = 8.51 (s, 1H, NC$\underline{H}$), 7.79-7.74 (m, 1H, aryl-$\underline{H}$), 7.47-7.31 (m, 3H, aryl-$\underline{H}$), 6.37 (s, 1H, OC$\underline{H}$), 3.60 (s, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 164.26 ($\underline{C}$=O), 145.51 ($\underline{C}$CH), 143.18 (ClC=CNCHN$\underline{C}$), 140.46 (N$\underline{C}$H), 131.30 (ClC=CNaryl-$\underline{C}$), 125.14 (ClC=CNCCHCH$\underline{C}$H), 124.63 (ClC=CNCCH$\underline{C}$H), 121.08 (ClC=CNCHNC$\underline{C}$H), 112.54 (ClC=CNC$\underline{C}$H), 111.35 ($\underline{C}$Cl), 99.40 (O$\underline{C}$H), 57.04 ($\underline{C}$H$_3$) ppm.

IR: ν = 2939, 2840, 2401, 1793, 1662, 1608 cm^{-1}.

Rf (Ether) = 0.20.

4. Benzylmethylamine:

4-[Benzyl(methyl)amino]-3-chloro-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 19)
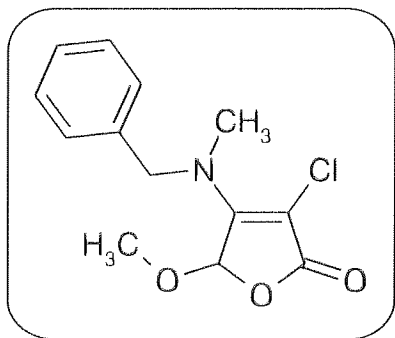
$C_{13}H_{14}ClNO_3$

Molecular Weight: 267.71.

APCl+ MS: m/z = 268/270.

^{1}H-NMR (CDCl$_3$): δ = 7.43-7.21 (m, 5H, aryl-H), 5.73 (s, 1H, CH), 4.84 & 4.78 (d, *J* = 15.7 Hz, 1H, CH$_2$), 4.70 & 4.64 (d, *J* = 14.5 Hz, 1H, CH$_2$), 3.52 (s, 3H, OCH$_3$), 3.11 (s, 3H, NCH$_3$) ppm.

IR: ν = 3024, 2358, 2343, 1756, 1701, 1633 cm^{-1}.

Rf (Ether) = 0.34.

5. 1-Benzylpiperazine:

4-(4-Benzylpiperazin-1-yl)-3-chloro-5-methoxyfuran-2(5H)-one:

(Biological test compound number 20)
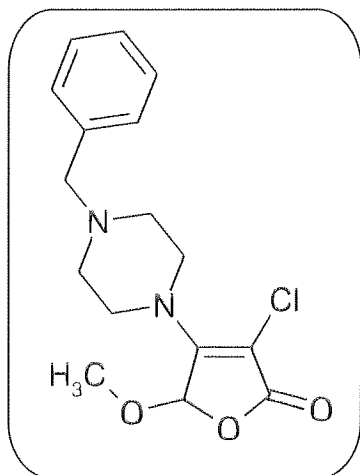
$C_{16}H_{19}ClN_2O_3$

Molecular Weight: 322.79.

APCI+ MS: m/z = 323/325.

^{1}H-NMR (CDCl$_3$): δ = 7.34-7.25 (m, 5H, aryl-$\underline{H}$), 5.68 (s, 1H, C$\underline{H}$), 3.71 (m, 4H, ClC=CNC$\underline{H}_2$), 3.55 (s, 2H, C$\underline{H}_2$-aryl), 3.47 (s, 3H, C$\underline{H}_3$), 2.56-2.52 (m, 4H, C$\underline{H}_2$NCH$_2$-aryl) ppm.

IR: ν = 3018, 2937, 2813, 2769, 2358, 2339, 1756, 1631, 1550 cm^{-1}.

Rf (Ether) = 0.17.

6. *sec*-Butylamine:

4-(*sec*-Butylamino)-3-chloro-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 12)
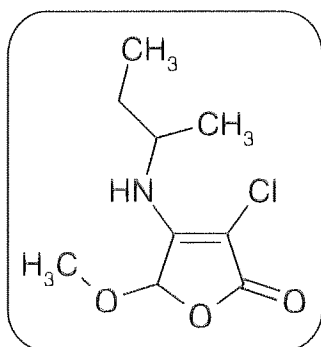
$C_9H_{14}ClNO_3$

Molecular Weight: 219.67.

APCI+ MS: m/z = 220/222.

^{1}H NMR (CDCl$_3$): δ = 6.67 (m, 1H, N$\underline{H}$), 5.66 (s, 1H, OC$\underline{H}$), 4.91 (m, 1H, NC$\underline{H}$), 3.48 (s, 3H, OC$\underline{H}_3$), 1.61-1.53 (m, 2H, C$\underline{H}_2$), 1.26-1.22 (m, 3H, CHC$\underline{H}_3$), 0.98-0.87 (m, 3H, CH$_2$C$\underline{H}_3$) ppm.

IR: ν = 3392, 3022, 2976, 2935, 2877, 2838, 2358, 2339, 1765, 1720, 1658, 1517 cm^{-1}.

Rf (Ether) = 0.38.

7. Dicyclohexylamine:

3-Chloro-4-(dicyclohexylamino)-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 37)
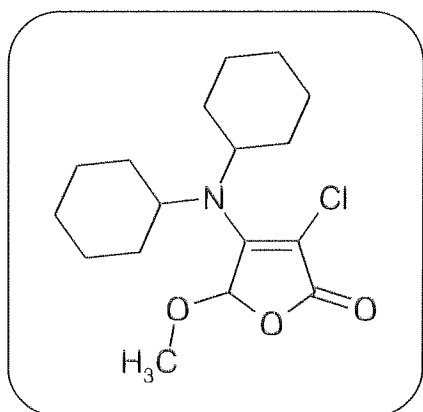
$C_{17}H_{26}ClNO_3$

Molecular Weight: 327.85.

APCI+ MS: m/z = 328/330.

^{1}H NMR (CDCl$_3$): δ = 5.63 (s, 1H, OC$\underline{H}$), 3.82 (s, 3H, C$\underline{H}_3$), 3.87-3.86 (m, 2H, NC$\underline{H}$), 1.78-1.13 (m, 20H, NCH(C$\underline{H}_2$)$_5$) ppm.

IR: ν = 3012, 2935, 2856, 1718, 1627 cm^{-1}.

Rf (Ether) = 0.55.

8. 2,6-Dimethylmorpholine:

3-Chloro-4-(2,6-dimethylmorpholin-4-yl)-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 6)
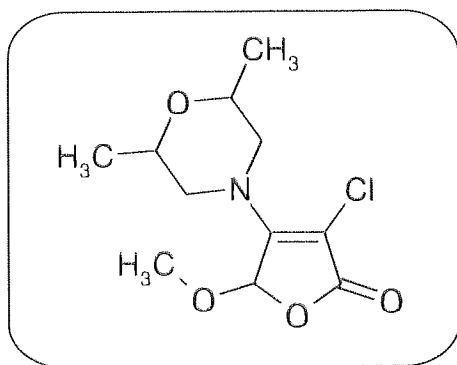
$C_{11}H_{16}ClNO_4$

Molecular Weight: 261.70.

APCI+ MS: m/z = 262/264.

^{1}H-NMR (CDCl$_3$): δ = 5.69 (s, 1H, OC$\underline{H}$O), 4.18-3.93 (m, 2H, NCH$_2$C$\underline{H}$), 3.77-3.61 (m, 2H, NC$\underline{H}_2$), 3.47 (s, 3H, OC$\underline{H}_3$), 2.87-2.71 (m, 2H, NC$\underline{H}_2$), 1.25-1.13 (m, 6H, CC$\underline{H}_3$) ppm.

IR: ν = 3010, 2981, 2939, 2898, 2358, 2343, 1763, 1657 cm^{-1}.

Rf (Ether) = 0.36.

9. 3,5-Dimethylpyrazole:

3-Chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-methoxyfuran-2(5*H*)-one:

$C_{10}H_{11}ClN_2O_3$

Molecular Weight: 242.66.

APCI+ MS: m/z = 243/245.

^{1}H-NMR (CDCl$_3$): δ = 6.33 (s, 1H, CH$_3$CC<u>H</u>), 6.07 (s, 1H, OC<u>H</u>), 3.57 (s, 3H, OC<u>H</u>$_3$), 2.40 (s, 3H, ClC=CNCC<u>H</u>$_3$), 2.28 (s, 3H, ClC=CNNCC<u>H</u>$_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 165.53 (<u>C</u>=O), 153.82 (ClC=<u>C</u>N), 142.91 (ClC=CNN<u>C</u>), 129.29 (Cl<u>C</u>=CN<u>C</u>), 115.96 (<u>C</u>Cl), 109.95 (ClC=CNNC<u>C</u>H), 100.57 (O<u>C</u>H), 57.24 (O<u>C</u>H$_3$), 13.57 (ClC=CNNC<u>C</u>H$_3$), 12.57 (ClC=CNC<u>C</u>H$_3$) ppm.

IR: ν = 2941, 2403, 1792, 1680, 1635, 1571 cm^{-1}.

Rf (Ether) = 0.60.

10. Indoline:

3-Chloro-4-(2,3-dihydro-1*H*-indol-1-yl)-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 1)

C$_{13}$H$_{12}$ClNO$_3$

Molecular Weight: 265.70.

APCI+ MS: m/z = 266/268.

^{1}H NMR (CDCl$_3$): δ = 7.23-6.96 (m, 4H, aryl-$\underline{H}$), 6.21 (s, 1H, C$\underline{H}$), 4.56-4.34 (m, 2H, NC$\underline{H}_2$), 3.53 (s, 3H, C$\underline{H}_3$), 3.36-3.10 (m, 2H, NCH$_2$C$\underline{H}_2$) ppm.

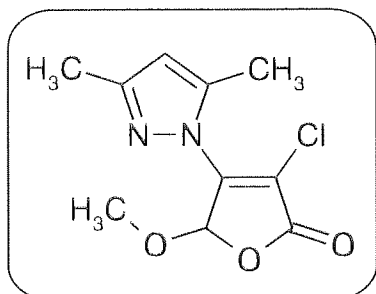^{13}C NMR (CDCl$_3$): δ = 167.19 ($\underline{C}$=O), 151.15 ($\underline{C}$CH), 142.49 (ClC=CNaryl-$\underline{C}$), 131.82 (NCH$_2$CH$_2$$\underline{C}$), 127.45 (NCH$_2CH_2C\underline{C}$H), 125.57 (ClC=CNCCH$\underline{C}$H), 123.69 (ClC=CNCCHCH$\underline{C}$H), 113.49 (ClC=CNC$\underline{C}$H), 102.50 ($\underline{C}$Cl), 97.99 (O$\underline{C}$H), 55.26 ($\underline{C}H_3$), 51.70 (N$\underline{C}H_2$), 28.47 (NCH$_2$$\underline{C}H_2$) ppm.

IR: ν = 2969, 2935, 2860, 2838, 1766, 1662, 1628, 1593 cm^{-1}.

Rf (Ether) = 0.43.

11. 3-Methylpyrazole:

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

(Biological test compound number 2)
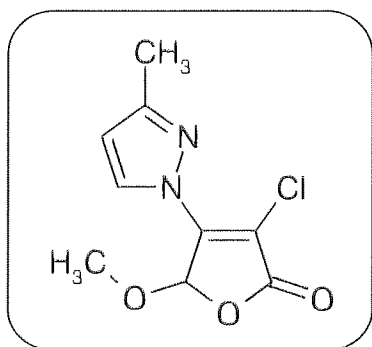
$C_9H_9ClN_2O_3$

Molecular Weight: 228.63.

APCI+ MS: m/z = 229/231.

^{1}H NMR (CDCl$_3$): δ = 8.26 (d, *J* = 2.7 Hz, 1H, NC*H*), 6.40 (d, *J* = 2.8 Hz, 1H, NCHC*H*), 6.30 (s, 1H, OC*H*), 3.65 (s, 3H, OC*H*$_3$), 2.35 (s, 3H, CC*H*$_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 165.49 (*C*=O), 153.95 (ClC=*C*N), 146.75 (*C*CH$_3$), 130.85 (ClC=CN*C*), 111.28 (CH$_3$C*C*), 102.87 (*C*Cl), 99.53 (O*C*H), 57.02 (O*C*H$_3$), 13.69 (C*C*H$_3$) ppm.

IR: ν = 3024, 2941, 2846, 2362, 2339, 1780, 1672, 1556 cm^{-1}.

Rf (Ether) = 0.51.

12. N-Phenylpiperazine:

3-Chloro-5-methoxy-4-(4-phenylpiperazin-1-yl)furan-2(5*H*)-one:

(Biological test compound number 13)
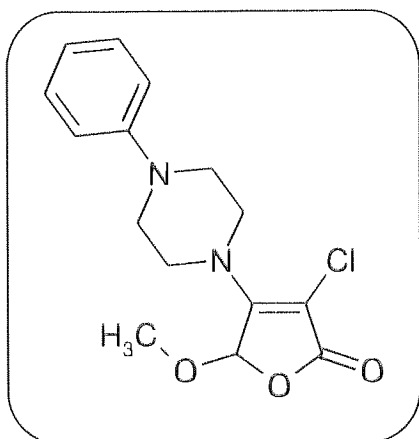
$C_{15}H_{17}ClN_2O_3$

Molecular Weight: 308.76.

APCI+ MS: m/z = 309/311.

^{1}H-NMR (CDCl$_3$): δ = 7.35-7.26 (m, 2H, *m*-H̲), 6.98-6.89 (m, 3H, *o*- & *p*-H̲), 5.75 (s, 1H, CH̲), 3.90-3.86 (m, 4H, aryl-NCH$_2$CH̲$_2$), 3.53 (s, 3H, CH̲$_3$), 3.30-3.23 (m, 4H, aryl-NCH̲$_2$) ppm.

IR: ν = 3010, 2839, 2358, 2343, 1758, 1639, 1603, 1535 cm^{-1}.

Rf (Ether) = 0.30.

13. 4-Phenylpiperidine:

3-Chloro-5-methoxy-4-(4-phenylpiperidin-1-yl)furan-2(5*H*)-one:

(Biological test compound number 4)
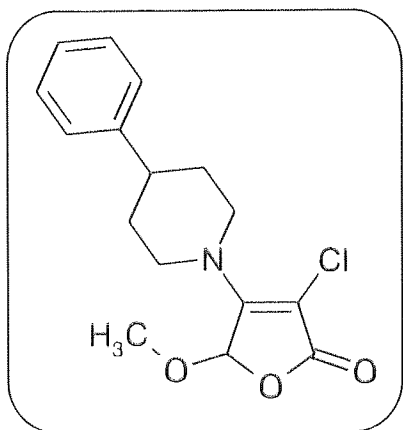
$C_{16}H_{18}ClNO_3$

Molecular Weight: 307.78.

APCI+ MS: m/z = 308/310.

^{1}H-NMR (CDCl$_3$): δ = 7.37-7.21 (m, 5H, aryl-H̲), 5.73 (s, 1H, OCH̲), 4.59-4.26 (m, 2H, NCH̲$_2$), 3.54 (s, 3H, CH̲$_3$), 3.25-3.10 (m, 2H, NCH̲$_2$), 2.89-2.77 (m, 1H, NCH$_2$CH$_2$CH̲), 2.01-1.75 (m, 4H, NCH$_2$CH̲$_2$) ppm.

IR: ν = 3030, 3010, 2941, 2843, 1761, 1629, 1552 cm^{-1}.

Rf (Ether) = 0.39.

14. 4-(3-Phenylpropyl)piperidine:

3-Chloro-5-methoxy-4-[4-(3-phenylpropyl)piperidin-1-yl]furan-2(5*H*)-one:

(Biological test compound number 17)
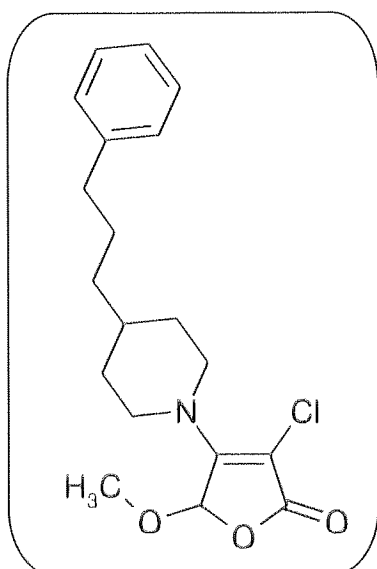
$C_{19}H_{24}ClNO_3$

Molecular Weight: 349.86.

APCI+ MS: m/z = 350/352.

^{1}H-NMR (CDCl$_3$): δ = 7.31-7.15 (m, 5H, aryl-H̲), 5.65 (s, 1H, OCH̲), 4.35-4.10 (m, 2H, NCH̲$_2$), 3.47 (s, 3H, CH̲$_3$), 3.02-2.97 (m, 2H, NCH̲$_2$), 2.63-2.57 (m, 2H, CH̲$_2$-aryl), 1.81-1.76 (m, 1H, NCH$_2$CH$_2$CH̲), 1.70-1.43 (m, 4H, NCH$_2$CH̲$_2$), 1.35-1.30 (m, 4H, CH̲$_2$CH̲$_2$CH$_2$-aryl) ppm.

IR: ν = 3016, 2938, 2856, 2358, 2339, 1759, 1629, 1546 cm^{-1}.

Rf (Ether) = 0.43.

15. Pyrrolidine:

3-Chloro-5-methoxy-4-pyrrolidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 5)
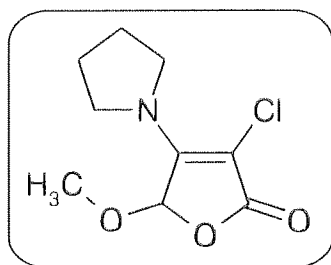
$C_9H_{12}ClNO_3$

Molecular Weight: 217.65.

APCI+ MS: m/z = 218/220.

^{1}H NMR (CDCl$_3$): δ = 5.59 (s, 1H, C$\underline{H}$), 3.83 (m, 4H, NC$\underline{H}_2$), 3.43 (s, 3H, C$\underline{H}_3$), 1.88 (m, 4H, NCH$_2$C$\underline{H}_2$) ppm.

^{13}C NMR (CDCl$_3$): δ = 168.33 ($\underline{C}$=O), 154.06 ($\underline{C}$CH), 97.82 (O$\underline{C}$H), 85.22 ($\underline{C}$Cl), 55.04 ($\underline{C}$H$_3$), 48.92 (N$\underline{C}$H$_2$), 24.95 (NCH$_2$$\underline{C}H_2$) ppm.

IR: ν = 2984, 2960, 2933, 2887, 2836, 1761, 1633 cm^{-1}.

Rf (Ether) = 0.30.

16. *o*-Toluidine:

3-Chloro-5-methoxy-4-(2-toluidino)furan-2(5*H*)-one:

(Biological test compound number 21)

$C_{12}H_{12}ClNO_3$

Molecular Weight: 253.68.

APCI+ MS: m/z = 254/256.

^{1}H-NMR (CDCl$_3$): δ = 7.29-7.14 (m, 4H, aryl-H̲), 6.43 (bs, 1H, NH̲), 5.76 (s, 1H, CH̲), 3.44 (s, 3H, OCH̲$_3$), 2.31 (s, 3H, aryl-CH̲$_3$) ppm.

IR: ν = 3386, 1770, 1656 cm^{-1}.

Rf (Ether) = 0.43.

17. 1H-1,2,4-Triazole:

3-Chloro-5-methoxy-4-(1*H*-1,2,4-triazol-1-yl)furan-2(5*H*)-one:

(Biological test compound number 9)

$C_7H_6ClN_3O_3$

Molecular Weight: 215.56.

APCI+ MS: m/z = 216/218.

^{1}H-NMR (CDCl$_3$): δ = 9.05 (s, 1H, ClC=CNC$\underline{H}$), 8.22 (s, 1H, ClC=CNNC$\underline{H}$), 6.31 (s, 1H, OC$\underline{H}$), 3.73 (s, 3H, C$\underline{H}_3$) ppm.

IR: ν = 3147, 3032, 3008, 2939, 1789, 1687, 1512 cm^{-1}.

Rf (Ether) = 0.19.

7.4.3 Synthesis of compounds based on initial screening results

7.4.3.1 Structure refinement at the four position

The 5-cetyloxyfuran-2(5*H*)-one series

1. Aniline:

4-Anilino-5-cetyloxy-3-chlorofuran-2(5*H*)-one:

(Biological test compound number 44)

$C_{26}H_{40}ClNO_3$

Molecular weight: 450.06.

APCI+ MS: m/z = 450/452.

^{1}H NMR (CDCl$_3$): δ = 7.42 - 7.06 (m, 5H, aryl-$\underline{H}$), 6.52 (s, 1H, N$\underline{H}$), 5.96 (s, 1H, C$\underline{H}$), 3.81-3.43 (m, 2H, OC$\underline{H}_2$), 1.59 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.26 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, J = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: v = 2925, 2852, 2402, 2364, 1768, 1660, 1601 cm^{-1}.

Rf (Ether) = 0.61.

2. Benzylamine:

4-(Benzylamino)-5-cetyloxy-3-chlorofuran-2(5*H*)-one:

(Biological test compound number 41)

$C_{27}H_{42}ClNO_3$

Molecular weight: 464.09.

APCI+ MS: m/z = 464/466.

^{1}H NMR (CDCl$_3$): δ = 8.62-8.59 (m, 2H, aryl-$\underline{H}$), 7.71-7.64 (m, 1H, aryl-$\underline{H}$), 7.31-7.25 (m, 2H, aryl-$\underline{H}$), 6.75 (s, 1H, C$\underline{H}$), 4.27-4.16 (m, 2H, NC$\underline{H}_2$), 3.63 (m, 1H, N$\underline{H}$), 3.42-3.33 (m, 2H, OC$\underline{H}_2$), 1.81-1.45 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.24 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.87 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 2404, 2368, 1766, 1662 cm^{-1}.

Rf (Ether) = 0.52.

3. Benzylmethylamine:

4-[Benzyl(methyl)amino]-5-cethyloxy-3-chlorofuran-2(5*H*)-one:

(Biological test compound number 42)

$C_{28}H_{44}ClNO_3$

Molecular weight: 478.11.

APCl+ MS: m/z = 478/480.

^{1}H NMR (CDCl$_3$): δ = 7.40-7.24 (m, 5H, aryl-H̲), 5.75 (s, 1H, CH̲), 4.73 (m, 2H, NCH̲$_2$), 3.86-3.55 (m, 2H, OCH̲$_2$), 3.12 (s, 3H, NCH̲$_3$), 1.58 (m, 2H, OCH$_2$CH̲$_2$), 1.26 (m, 26H, OCH$_2$CH$_2$(CH̲$_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, CH̲$_3$) ppm.

IR: ν = 2925, 2852, 2403, 1755, 1639 cm^{-1}.

Rf (Ether) = 0.52.

4. 2-Chlorobenzylamine:

5-Cetyloxy-3-chloro-4-[(2-chlorobenzyl)amino]furan-2(5*H*)-one:

(Biological test compound number 40)

$C_{27}H_{41}Cl_2NO_3$

Molecular weight: 498.53.

APCI+ MS: m/z = 498/500.

^{1}H NMR (CDCl$_3$): δ = 7.45-7.30 (m, 4H, aryl-$\underline{H}$), 5.75 (s, 1H, C$\underline{H}$), 5.14 (m, 1H, N$\underline{H}$), 4.84-4.70 (m, 2H, NC$\underline{H}_2$), 3.82-3.58 (m, 2H, OC$\underline{H}_2$), 1.56 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.26 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 2405, 2364, 1762, 1658 cm^{-1}.

Rf (Ether) = 0.64.

5. Cyclohexylamine:

5-Cetyloxy-3-chloro-4-(cyclohexylamino)furan-2(5*H*)-one:

(Biological test compound number 46)

$C_{26}H_{46}ClNO_3$

Molecular weight: 456.11.

APCI+ MS: m/z = 456/458.

^{1}H NMR (CDCl$_3$): δ = 6.00 (s, 1H, OC$\underline{H}$), 5.70 (m, 1H, N$\underline{H}$), 3.67-3.58 (m, 2H, OC$\underline{H}_2$), 2.07-1.46 (m, 13H, 2H-OCH$_2$C$\underline{H}_2$ & 11H-NC$\underline{H}$(C$\underline{H}_2$)$_5$), 1.26 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2858, 2399, 2358, 1765, 1657 cm^{-1}.

Rf (Ether) = 0.63.

6. Imidazole:

5-Cetyloxy-3-chloro-4-(1*H*-imidazol-1-yl)furan-2(5*H*)-one:

(Biological test compound number 47)

$C_{23}H_{37}ClN_2O_3$

Molecular weight: 425.01.

APCI+ MS: m/z = 425/427.

^{1}H NMR (CDCl$_3$): δ = 8.26 (m, 1H, ClC=CNCHC$\underline{H}$), 7.71 (m, 1H, ClC=CNC$\underline{H}$N), 7.29 (m, 1H, ClC=CNC$\underline{H}$C), 6.18 (s, 1H, OC$\underline{H}$), 3.95-3.62 (m, 2H, OC$\underline{H}_2$), 1.75-1.63 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 2405, 2364, 1791, 1676, 1604 cm^{-1}.

Rf (Ether) = 0.57.

7. N-Phenylbenzylamine:

4-(Benzylanilino)-5-cetyloxy-3-chlorofuran-2(5*H*)-one:

(Biological test compound number 43)

$C_{33}H_{46}ClNO_3$

Molecular weight: 540.18.

APCI+ MS: m/z = 540/542.

^{1}H NMR (CDCl$_3$): δ = 7.42-7.06 (m, 10H, aryl-$\underline{H}$), 5.96 (s, 1H, C$\underline{H}$), 3.81-3.43 (m, 4H, 2H-OC$\underline{H}_2$ & 2H-NC$\underline{H}_2$), 1.60 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.26 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 2403, 2360, 1762, 1660 cm^{-1}.

Rf (Ether) = 0.57.

8. 2-Phenylethylamine:

5-Cetyloxy-3-chloro-4-(phenethylamino)furan-2(5*H*)-one:

(Biological test compound number 45)

C$_{28}$H$_{44}$ClNO$_3$

Molecular weight: 478.11.

APCI+ MS: m/z = 478/480.

^{1}H NMR (CDCl$_3$): δ = 7.38-7.18 (m, 5H, aryl-H), 5.68 (s, 1H, CH), 4.81 (m, 1H, NH), 3.83-3.53 (m, 2H, OCH$_2$), 2.98-2.89 (m, 2H, NCH$_2$), 1.66-1.57 (m, 4H, 2H-NCH$_2$CH$_2$ & 2H-OCH$_2$CH$_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(CH$_2$)$_{13}$), 0.88 (t, *J* = 6.4 Hz, 3H, CH$_3$) ppm.
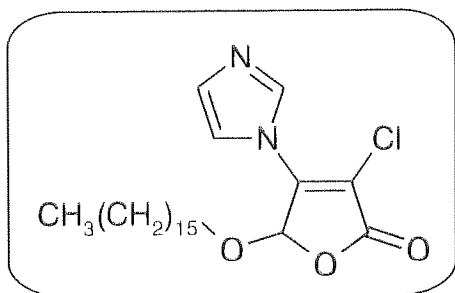
IR: ν = 2925, 2858, 2399, 2366, 1758, 1656, 1647 cm^{-1}.

Rf (Ether) = 0.55.

9. Pyrazole:

5-Cetyloxy-3-chloro-4-(1*H*-pyrazol-1-yl)furan-2(5*H*)-one:

(Biological test compound number 48)

$C_{23}H_{37}ClN_2O_3$

Molecular weight: 425.01.

APCI+ MS: m/z = 425/427.

^{1}H NMR (CDCl$_3$): δ = 8.40 (d, *J* = 2.8 Hz, 1H, ClC=CNNC$\underline{H}$), 7.84 (d, *J* = 1.6 Hz, 1H, ClC=CNC$\underline{H}$), 6.63-6.61 (m, 1H, ClC=CNCC$\underline{H}$), 6.41 (s, 1H, OC$\underline{H}$), 3.96-3.62 (m, 2H, OC$\underline{H}_2$), 1.69-1.49 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.25 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.88 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2925, 2852, 2399, 1780, 1680 cm^{-1}.

Rf (Ether) = 0.62.

10. 1H-1,2,4-Triazole:

5-Cetyloxy-3-chloro-4-(1*H*-1,2,4-triazol-1-yl)furan-2(5*H*)-one:

(Biological test compound number 49)

$C_{22}H_{36}ClN_3O_3$

Molecular weight: 426.00.

APCI+ MS: m/z = 426/428.

^{1}H NMR (CDCl$_3$): δ = 9.02 (s, 1H, ClC=CNC$\underline{H}$), 8.18 (s, 1H, ClC=CNNC$\underline{H}$), 6.35 (s, 1H, OC$\underline{H}$), 4.00-3.60 (m, 2H, OC$\underline{H}_2$), 1.69-1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.24 (m, 26H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_{13}$), 0.87 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2931, 2858, 1787, 1687 cm^{-1}.

Rf (Ether) = 0.46.

The 5-methoxyfuran-2(5H)-one series

1. Benzimidazole:

4-(1H-Benzimidazol-1-yl)-3-chloro-5-methoxyfuran-2(5H)-one:

(Biological test compound number 50)

$C_{12}H_9ClN_2O_3$

Molecular weight: 264.67.

APCl+ MS: m/z = 265/267.

^{1}H NMR (CDCl$_3$): δ = 8.51 (s, 1H, NC$\underline{H}$), 7.79-7.74 (m, 1H, aryl-$\underline{H}$), 7.47-7.31 (m, 3H, aryl-$\underline{H}$), 6.37 (s, 1H, OC$\underline{H}$), 3.60 (s, 3H, C$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 164.26 ($\underline{C}$=O), 145.51 ($\underline{C}$CH), 143.18 (ClC=CNCHN$\underline{C}$), 140.46 (N$\underline{C}$H), 131.30 (ClC=CNaryl-$\underline{C}$), 125.14 (ClC=CNCCHCH$\underline{C}$H), 124.63 (ClC=CNCC$\underline{H}$CH), 121.08 (ClC=CNCHNC$\underline{C}$H), 112.54 (ClC=CNC$\underline{C}$H), 111.35 ($\underline{C}$Cl), 99.40 (O$\underline{C}$H), 57.04 ($\underline{C}$H$_3$) ppm.

IR: ν = 2939, 2840, 2401, 1793, 1662, 1608 cm^{-1}.

Rf (Ether) = 0.20.

2. 3,5-Dimethylpyrazole:

3-Chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 54)

$C_{10}H_{11}ClN_2O_3$

Molecular weight: 242.66.

APCI+ MS: m/z = 243/245.

^{1}H NMR (CDCl$_3$): δ = 6.33 (s, 1H, CH$_3$CC$\underline{H}$), 6.07 (s, 1H, OC$\underline{H}$), 3.57 (s, 3H, OC$\underline{H}_3$), 2.40 (s, 3H, ClC=CNCC$\underline{H}_3$), 2.28 (s, 3H, ClC=CNNCC$\underline{H}_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 165.53 ($\underline{C}$=O), 153.82 (ClC=$\underline{C}$N), 142.91 (ClC=CNN$\underline{C}$), 129.29 (ClC=CN$\underline{C}$), 115.96 ($\underline{C}$Cl), 109.95 (ClC=CNNC$\underline{C}$H), 100.57 (O$\underline{C}$H), 57.24 (O$\underline{C}$H$_3$), 13.57 (ClC=CNNC$\underline{C}$H$_3$), 12.57 (ClC=CNC$\underline{C}$H$_3$) ppm.

IR: ν = 2941, 2403, 1792, 1680, 1635, 1571 cm^{-1}.

Rf (Ether) = 0.60.

3. Imidazole:

3-Chloro-4-(1*H*-imidazol-1-yl)-5-methoxyfuran-2(5*H*)-one:

(Biological test compound number 52)

$C_8H_7ClN_2O_3$

Molecular weight: 214.61.

APCI+ MS: m/z = 215/217.

^{1}H NMR (CDCl$_3$): δ = 8.18-8.17 (m, 1H, ClC=CNCHC<u>H</u>), 7.65-7.63 (m, 1H, ClC=CNC<u>H</u>N), 7.17-7.16 (m, 1H, ClC=CNC<u>H</u>C), 6.16 (s, 1H, OC<u>H</u>), 3.58 (s, 3H, C<u>H</u>$_3$) ppm.

^{13}C NMR (CDCl$_3$): δ = 164.28 (<u>C</u>=O), 144.07 (<u>C</u>CH), 136.04 (ClC=CNC<u>H</u>N), 131.21 (ClC=CNCH<u>C</u>H), 116.81 (ClC=CN<u>C</u>HCH), 107.30 (<u>C</u>Cl), 98.58 (O<u>C</u>H), 56.15 (<u>C</u>H$_3$) ppm.

IR: ν = 2441, 2405, 1790, 1766, 1668, 1628, 1597 cm^{-1}.

Rf (Ether) = 0.36.

4. Indoline:

3-Chloro-4-(2,3-dihydro-1*H*-indol-1-yl)-5-methoxyfuran-2(*5H*)-one:

(Biological test compound number 51)

$C_{13}H_{12}ClNO_3$

Molecular weight = 265.70.

APCI+ MS: m/z = 266/268.

^{1}H NMR (CDCl$_3$): δ = 7.23-6.96 (m, 4H, aryl-$\underline{H}$), 6.21 (s, 1H, C$\underline{H}$), 4.56-4.34 (m, 2H, NC$\underline{H}_2$), 3.53 (s, 3H, C$\underline{H}_3$), 3.36-3.10 (m, 2H, NCH$_2$C$\underline{H}_2$) ppm.

^{13}C NMR (CDCl$_3$): δ = 167.19 ($\underline{C}$=O), 151.15 ($\underline{C}$CH), 142.49 (ClC=CNaryl-$\underline{C}$), 131.82 (NCH$_2$CH$_2$$\underline{C}$), 127.45 (NCH$_2CH_2C\underline{C}$H), 125.57 (ClC=CNCCH$\underline{C}$H), 123.69 (ClC=CNCCHCH$\underline{C}$H), 113.49 (ClC=CNC$\underline{C}$H), 102.50 ($\underline{C}$Cl), 97.99 (O$\underline{C}$H), 55.26 ($\underline{C}H_3$), 51.70 (N$\underline{C}H_2$), 28.47 (NCH$_2$$\underline{C}H_2$) ppm.

IR: ν = 2969, 2935, 2860, 2838, 1766, 1662, 1628, 1593 cm^{-1}.

Rf (Ether) = 0.43.

5. Pyrrolidine:

3-Chloro-5-methoyx-4-pyrrolidin-1-ylfuran-2(5*H*)-one:

(Biological test compound number 53)

C$_9$H$_{12}$ClNO$_3$

Molecular weight: 217.65.

APCl+ MS: m/z = 218/220.

^{1}H NMR (CDCl$_3$): δ = 5.59 (s, 1H, C$\underline{H}$), 3.83 (m, 4H, NC$\underline{H}_2$), 3.43 (s, 3H, C$\underline{H}_3$), 1.88 (m, 4H, NCH$_2$C$\underline{H}_2$) ppm.

^{13}C NMR (CDCl$_3$): δ = 168.33 ($\underline{C}$=O), 154.06 ($\underline{C}$CH), 97.82 (O$\underline{C}$H), 85.22 ($\underline{C}$Cl), 55.04 ($\underline{C}$H$_3$), 48.92 (N$\underline{C}$H$_2$), 24.95 (NCH$_2$$\underline{C}H_2$) ppm.
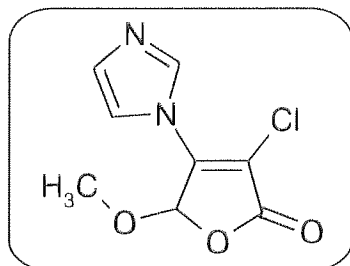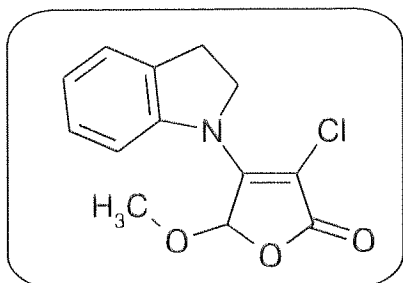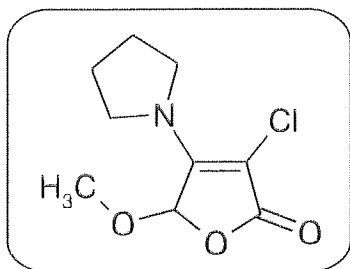
IR: ν = 2984, 2960, 2933, 2887, 2836, 1761, 1633 cm^{-1}.

Rf (Ether) = 0.30.

7.4.3.2   Structure refinement at the five position

Method:

> 2 mmol of each of the 5-alkoxy building blocks synthesised in section 7.3.3 was reacted with 1.5 equivalents (425 mg) of benzylmethylamine in DMF and heated to 60 °C for forty-eight hours. Thin layer chromatography in ether was performed on each of the reactions after this time, indicating the formation of a new product. A sample of each

reaction was then submitted in methanol for analysis by APCI+ mass spectroscopy. The resulting spectra all showed the correct molecular ion peak.

Purification was performed by placing the contents of each reaction vessel in a separating funnel with around 150 ml of water. To this mixture, an equal amount of dichloromethane was added and the funnel stoppered and shaken. The dichloromethane (lower) layer was removed and the extraction repeated with a fresh portion of solvent. The combined organic layers were then shaken in a clean funnel with an equal amount of acidified water (pH 4-5 with hydrochloric acid) to remove any excess amine into the aqueous layer. The organic layer was then removed, dried with magnesium sulphate and then the solvent removed by rotary evaporation. The five resulting products were all characterised by APCI+ mass spectroscopy and proton nuclear magnetic resonance spectroscopy.

1. 3,4-Dichloro-5-ethoxyfuran-2(5*H*)-one:

3-Chloro-4-[(2-chlorobenzyl)amino]-5-ethoxyfuran-2(5*H*)-one:

(Biological test compound number 55)
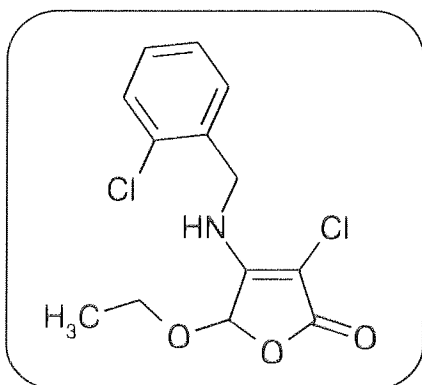
$C_{13}H_{13}Cl_2NO_3$

Molecular weight: 302.15.

APCI+ MS: m/z = 302/304.

^{1}H NMR (CDCl$_3$): δ = 7.46-7.30 (m, 4H, aryl-$\underline{H}$), 5.72 (s, 1H, C$\underline{H}$), 5.21 (m, 1H, N$\underline{H}$), 4.85-4.76 (m, 2H, NC$\underline{H}_2$), 3.79, (q, J = 10.3 Hz, 2H, OC$\underline{H}_2$), 1.26 (t, J = 7.1 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2896, 2399, 2339, 1768, 1658 cm^{-1}.

2. 5-Butoxy-3,4-dichlorofuran-2(5H)-one:

5-Butoxy-3-chloro-4-[(2-chlorobenzyl)amino]furan-2(5H)-one:

(Biological test compound number 56)
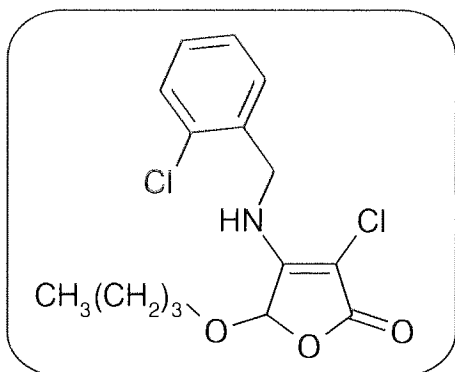
C$_{15}$H$_{17}$Cl$_2$NO$_3$

Molecular weight: 330.21.

APCI+ MS: m/z = 330/332.

^{1}H NMR (CDCl$_3$): δ = 7.41-7.26 (m, 4H, aryl-$\underline{H}$), 5.72 (m, 2H, 1H-C$\underline{H}$ & 1H-N$\underline{H}$), 4.76-4.66 (m, 2H, NC$\underline{H}_2$), 3.78-3.56 (m, 2H, OC$\underline{H}_2$), 1.59-1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.39-1.24 (m, 2H, CH$_3$C$\underline{H}_2$), 0.88 (t, J = 7.3 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2960, 2937, 2873, 1762, 1658 cm^{-1}.

3. 3,4-Dichloro-5-(hexyloxy)furan-2(5H)-one:

3-Chloro-4-[(2-chlorobenzyl)amino]-5-(hexyloxy)furan-2(5H)-one:

(Biological test compound number 57)
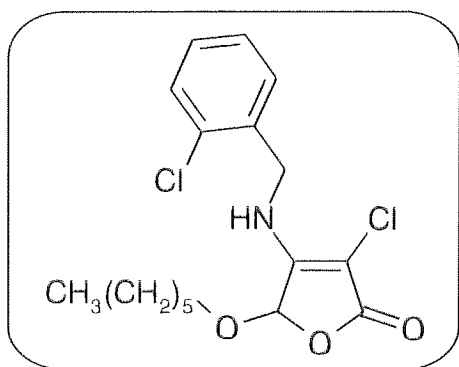
$C_{17}H_{21}Cl_2NO_3$

Molecular weight: 358.26.

APCI+ MS: m/z = 358/360.

^{1}H NMR (CDCl$_3$): δ = 7.31-7.21 (m, 4H, aryl-H), 6.20 (m, 1H, NH), 5.68 (s, 1H, CH), 4.85-4.60 (m, 2H, NCH$_2$), 3.85-3.50 (m, 2H, OCH$_2$), 1.52 (m, 2H, OCH$_2$CH$_2$), 1.21 (m, 6H, OCH$_2$CH$_2$(CH$_2$)$_3$), 0.82 (t, J = 6.3 Hz, 3H CH$_3$) ppm.

IR: ν = 2960, 2929, 2875, 2335, 1762, 1662 cm^{-1}.

4. 3,4 Dichloro-5-(nonyloxy)furan-2(5*H*)-one:

3-Chloro-4-[(2-chlorobenzyl)amino]-5-(nonyloxy)furan-2(5*H*)-one:

(Biological test compound number 58)
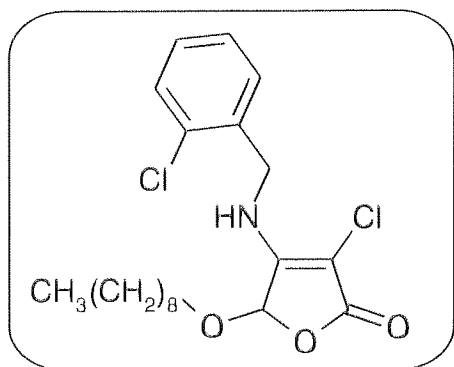
$C_{20}H_{27}Cl_2NO_3$

Molecular weight: 400.34.

APCl+ MS: m/z = 400/402.

^{1}H NMR (CDCl$_3$): δ = 7.33-7.19 (m, 4H, aryl-$\underline{H}$), 6.29 (m, 1H, N$\underline{H}$), 5.67 (s, 1H, C$\underline{H}$), 4.74-4.59 (m, 2H, NC$\underline{H}_2$), 3.70-3.53 (m, 2H, OC$\underline{H}_2$), 1.51 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.21 (m, 12H, OCH$_2$CH$_2$(C$\underline{H}_2$)$_6$), 0.81 (t, *J* = 6.4 Hz, 3H, C$\underline{H}_3$) ppm.

IR: ν = 2931, 2854, 1762, 1658 cm^{-1}.

5. 3,4-Dichloro-5-(dodecyloxy)furan-2(5*H*)-one:

3-Chloro-4-[(2-chlorobenzyl)amino]-5-(dodecyloxy)furan-2(5*H*)-one:

(Biological test compound number 59)
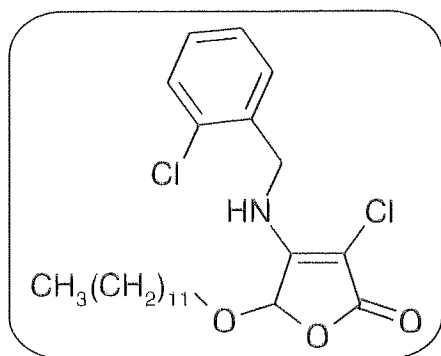
$C_{23}H_{33}Cl_2NO_3$

Molecular weight: 442.42.

APCI+ MS: m/z = 442/444.

^{1}H NMR (CDCl$_3$): δ = 7.39-7.25 (m, 4H, aryl-H̲), 5.71 (m, 2H, 1H-CH̲ & 1H-NH̲),
4.83-4.44 (m, 2H, NCH̲$_2$), 3.78-3.55 (m, 2H, OCH̲$_2$), 1.57 (m, 2H, OCH$_2$CH̲$_2$), 1.24
(m, 18H, OCH$_2$CH$_2$(CH̲$_2$)$_9$), 0.87 (t, *J* = 6.5 Hz, 3H, CH̲$_3$) ppm.

IR: ν = 2925, 2858, 2399, 2335, 1762, 1662 cm^{-1}.

7.5 The attempted synthesis of 4-hydroxyfuran-2(5*H*)-one and the resulting product identification by carbon nuclear magnetic resonance spectroscopy

7.5.1 The initial reaction

Method:

14.33 g (62.6 mmoles) of 3,4-dichloro-5-phenylfuran-2(5*H*)-one
(synthesised in section 7.2.2) was put in a 500 ml conical flask and

around 200 ml of methanol was added. The solution was stirred to dissolve all of the starting material. 1.5 equivalents of potassium hydroxide (3.5 g) were added and the mixture was stirred again until all the base had dissolved. During the stirring, the solution changed colour from colourless, through yellow, orange and red to a dark red/brown colour. The methanol was then removed by allowing the solvent to evaporate off over a 48 hour period. The resulting orange solid was then dried under vacuum in a desiccator and submitted for proton NMR analysis. The resulting spectrum showed that the compound contained impurities. TLC studies in ether, when compared to the starting material, indicated that the highest zone on the TLC plate is the desired compound (as this zone, in addition to the starting material zone, decolourised quickly on the application of a 1% potassium permanganate solution). Using this information, the compound was purified by column chromatography in ether. NMR studies indicated the presence of the desired compound in increased purity. Full structure identification was now possible (see section 5.1.3) using the changes in carbon NMR shifts for the product when compared to the starting material. These results showed that the compound underwent substitution at the three position resulting in the formation of 4-chloro-3-methoxy-5-phenylfuran-2(5H)-one.

4-Chloro-3-methoxy-5-phenylfuran-2(5H)-one:
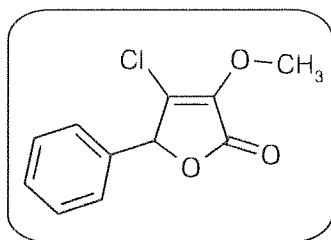
$C_{11}H_9ClO_3$

Molecular Weight: 224.64.

APCI+ MS: m/z = 225/227.

^{1}H-NMR (CDCl$_3$): δ = 7.97-7.43 (m, 5H, aryl-$\underline{H}$), 6.47 (s, 1H, C$\underline{H}$), 3.59 (s, 3H, C$\underline{H}_3$).

^{13}C-NMR (CDCl$_3$): δ = 163.36 ($\underline{C}$=O), 145.17 ($\underline{C}$OCH$_3$), 132.92 ($\underline{C}$Cl), 130.00 (CHaryl-$\underline{C}$) 129.21 & 128.95 & 128.74 (o, m & p-aryl-$\underline{C}$), 121.60 ($\underline{C}$H), 52.08 ($\underline{C}$H$_3$).

IR: ν = 3029, 2952, 2356, 2329, 1786, 1719, 1683, 1623, 1596 cm^{-1}.

Rf (Ether) = 0.48.

7.5.2 The reaction with various alcohols at the three position

Method A : Using the alcohol as reactant and solvent

0.22 g (0.96 mmol) of 3,4-dichloro-5-phenylfuran-2(5H)-one was dissolved in around 10 ml of each of the alcohols under test. 1.5 equivalents of potassium hydroxide were added (0.081 g) and each of the mixtures kept at 60 °C for twelve hours. After this time, each solution was neutralised with hydrochloric acid and 1.5 equivalents of benzylmethylamine was added to aid characterisation. Each of the mixtures was kept at 60 °C for twelve hours and then samples from each reaction were submitted for APCI+ mass spectroscopy and the presence or absence of the expected molecular ion peak noted. The four alcohols under test were ethanol, propan-2-ol, butan-1-ol and 2-pentanol.

Method B: Using the alcohol in 1,2-dichloroethane as the solvent

0.22g (0.96 mmol) of 3,4-dichloro-5-phenylfuran-2(5H)-one was dissolved in around 10 ml of 1,2-dichloroethane and 1.5 equivalents of phenol (0.135 g) and 1.5 equivalents of potassium hydroxide (0.081 g) were added. The reaction was kept at 60 °C for twelve hours. Subsequent reaction with benzylmethylamine and analysis by APCI+ mass spectroscopy was performed as in Method A.

Of the five reactions sampled, two of the produced detectable molecular ion peaks on analysis by APCI+ mass spectroscopy. These were the reactions with butan-1-ol and phenol. Each of the samples submitted was, however, heavily contaminated with excess amine, which could have masked any reaction product for the remaining alcohols.

The products, which were indirectly identifiable by APCI+ mass spectroscopy of the nucleophilic reaction product (with benzylmethylamine), were:

1. Butan-1-ol:

3-Butoxy-4-chloro-5-phenylfuran-2(5H)-one:
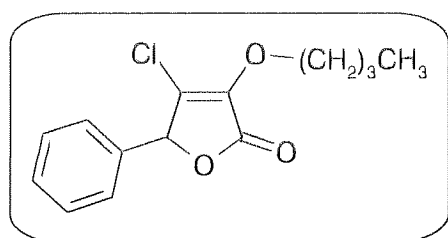
	Expected	Obtained
	351.44	352

2. Phenol:

4-Chloro-3-phenoxy-5-phenylfuran-2(5H)-one:
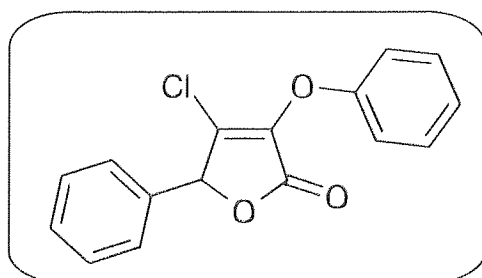
	Expected	Obtained
	371.43	372

7.5.3 A mini series of 3,4,5-trisubstituted furan-2(5H)-ones

Method:

Sufficient 4-chloro-3-methoxy-5-phenylfuran-2(5H)-one was synthesised as detailed in section 7.5.1 and re-crystallised from ethanol for further reaction. 0.23 g (1.0 mmol) of the compound was dissolved in DMF in six aliquots and 1.5 equivalents of the relevant amine added to each. Each reaction was kept at 60 °C for twelve hours and then samples of each reaction submitted in methanol for detection of the molecular ion peak by APCI+ mass spectroscopy. Two of the series were purified further by column chromatography in a mixture of 90% ethyl acetate and 10% petrol ether 40-60, and submitted for proton nuclear magnetic resonance studies. The resulting spectra indicated the presence of the product, although the compound was impure. Further purification by preparative thin layer chromatography in ether on the compounds produced pure products identifiable by both proton nuclear magnetic resonance studies and APCI+ mass spectroscopy in methanol. One of the purified compounds was further analysed by carbon nuclear magnetic resonance spectroscopy (see below).

1. Aniline:

4-Anilino-3-methoxy-5-phenylfuran-2(5*H*)-one:
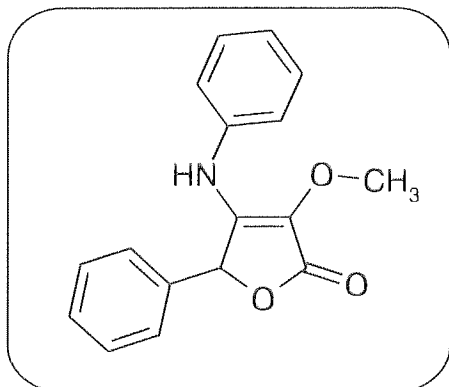
$C_{17}H_{15}NO_3$

Molecular weight: 281.31.

APCI+ MS: m/z = 282.

2. Benzimidazole:

4-(1*H*-Benzimidazol-1-yl)-3-methoxy-5-phenylfuran-2(5*H*)-one:
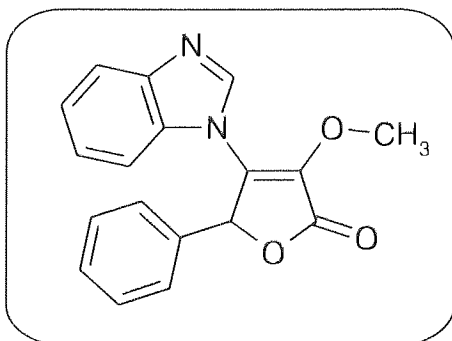
$C_{18}H_{14}N_2O_3$

Molecular Weight: 306.32.

APCI+ MS: m/z = 307.

¹H-NMR (CDCl₃): δ = 8.15-7.32 (m, 10H, 9H-aryl-$\underline{H}$ & 1H-NC$\underline{H}$N), 6.60 (s, 1H, OC$\underline{H}$), 3.67 (s, 3H, C$\underline{H}_3$) ppm.

IR: ν = 1772, 1685, 1629, 1594 cm⁻¹.

Rf (90% Ethyl acetate, 10% Petrol ether 40-60) = 0.55.

3. Benzylmethylamine:

4-[Benzyl(methyl)amino]-3-methoxy-5-phenylfuran-2(5*H*)-one:
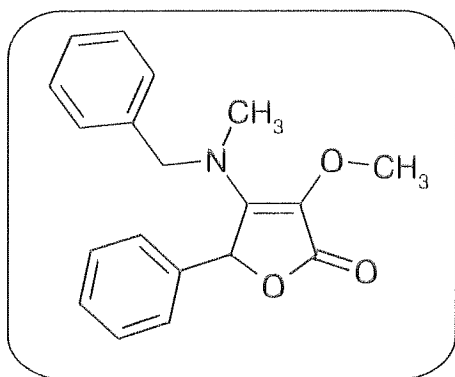
$C_{19}H_{19}NO_3$

Molecular weight: 309.36.

APCI+ MS: m/z = 310.

4. 3,5-Dimethylpyrazole:

4-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-3-methoxy-5-phenylfuran-2(5*H*)-one:
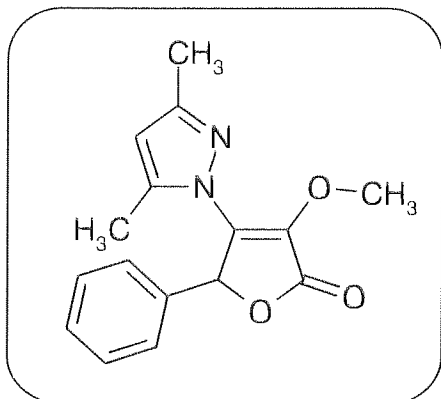
$C_{16}H_{16}N_2O_3$

Molecular weight: 284.31.

APCI+ MS: m/z = 285.

5. Imidazole:

4-(1*H*-Imidazol-1-yl)-3-methoxy-5-phenylfuran-2(5*H*)-one:
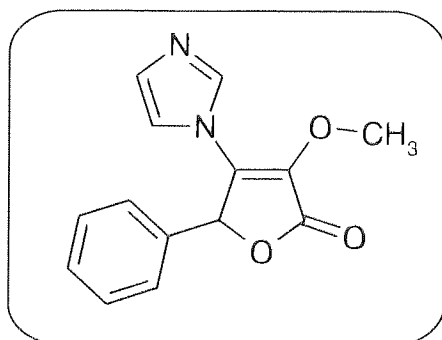
$C_{14}H_{12}N_2O_3$

Molecular weight: 256.26.

APCI+ MS: m/z = 257.

¹H-NMR (CDCl$_3$): δ = 7.97-7.93 (m, 2H, OCHCNCHNC<u>H</u>), 7.68-7.61 (m, 2H, *o*-<u>H</u>), 7.54-7.47 (m, 2H, *m*-<u>H</u>), 7.16 (m, 2H, 1H-*p*-<u>H</u> & 1H-OCHCNC<u>H</u>CHN), 6.30 (s, 1H, OC<u>H</u>), 3.63 (s, 3H, C<u>H</u>$_3$) ppm.

¹³C-NMR (CDCl$_3$): δ = 189.67 (<u>C</u>=O), 164.85 (<u>C</u>CH), 145.95 (<u>C</u>OCH$_3$), 135.78 (N<u>C</u>N), 134.78 (OCHCNC<u>C</u>), 134.47 (CHaryl-<u>C</u>), 131.54 (*p*-<u>C</u>), 129.21 (*o*-<u>C</u>), 128.85 (*m*-<u>C</u>), 116.53 (OCHCN<u>C</u>C), 107.02 (O<u>C</u>H), 52.16 (<u>C</u>H$_3$) ppm.

IR: ν = 1741, 1718, 1675, 1633, 1602 cm^{-1}.

Rf (90% Ethyl acetate, 10% Petrol ether 40-60) = 0.38.

6. Pyrazole:

3-Methoxy-5-phenyl-4-(1*H*-pyrazol-1-yl)furan-2(5*H*)-one:
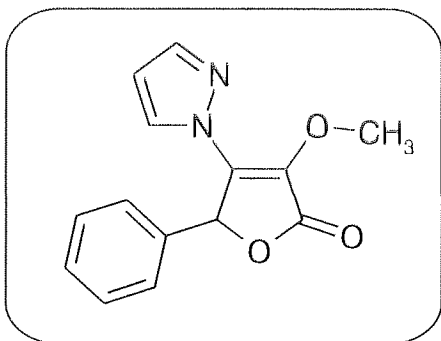
C$_{14}$H$_{12}$N$_2$O$_3$

Molecular weight: 256.26.

APCI+ MS: m/z = 257.

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