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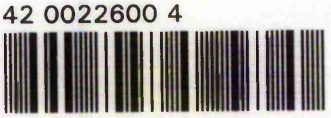

Synthesis & Evaluation of Some Anti-Proliferative Substances

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A thesis submitted in partial fulfilment of the requirements of The Nottingham Trent University for the degree of Doctor of Philosophy

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Abstract:

In previous patented investigations at The Nottingham Trent University, it was found that a number of compounds with the general structure $ArO(CH_2)_nNR_1R_2$ presented significant fungicidal potency. With sponsorship from the British Technology Group, the most promising compounds of the series were prepared in gram quantities, using established or improved methods, and were subjected to *in vitro* and *in vivo* toxicity testing. Some were confirmed as being potent against *Aspergillus* species *in vitro* and relatively non-toxic but none was active *in vivo*.

The major part of the investigation was the synthesis of analogues of an aminoalkyloxybromobenzofuran, **RWA2109**, prepared by a previous student, which had shown remarkable activity in an anti-invasion screen operated by Professor S. Mac Neil's group at Sheffield University.

A number of structural isomers of **RWA2109** were synthesized, and attempts were made to resolve an ambiguity in the structure of the parent compound. A water-soluble sulfoxide analogue, **KY30a**, was also prepared. The study was extended by the preparation of compounds in which the benzofuran nucleus was replaced by an indole or N-alkyl indole.

Considerable efforts were made to replicate the early anti-invasion results obtained with **RWA2109**, using of necessity a complex and laborious technique. It became obvious that the melanoma cell line originally used no longer gave reproducible results even as control, and was replaced by a more robust line, again with disappointing results.

It was however established that some of the indoles synthesized, and **KY30a** were potent in an anti-proliferation assay, with IC50 values of less than 5μM. There was also some indication that these compounds were active at very low concentration in the anti-invasion assay, but this result has to be regarded with caution.

In Loving Memory of My

Dearest Father

Acknowledgement

I would like to express my thanks to my supervisor, Dr. I. G. C. Coutts, for his invaluable advice, encouragement and ideas during the period of my research; to Professor John Wallis, Dr. Robert Allcock and Dr. Robert Saint for their technical help and support. I am also grateful to Professor S. Mac Neil and Mark Wagner of the Department of Clinical Science at Sheffield University for providing melanoma cell lines and teaching me the skills required to operate the anti-invasion assay.

I would like to acknowledge the British Technology Group and The Nottingham Trent University for sponsorship.

Thanks to all my friends who helped me during my PhD studying.

Finally, my thanks go to my mum, who has supported me in every way in my student career; my fiancé, Daniel A. Rintoul for his love and understanding; also Bill and Carol for their countless encouragement.

Glossary

Boc	*tert*-butyloxycarbonyl
n-BuLi	*n*-butyllithium
m-CPBA	*meta*-chloroperoxybenzoic acid
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
E_2	estrodiol
IC50	concentration required for 50% inhibition
EtOAc	ethyl acetate
LiHMDS	lithium hexamethyldisilazide
MDR	multi-drug-resistance
MIC	minimum inhibitory concentration
MLCK	myosin light chain kinase
m.p.	melting point
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBS	*N*-bromosuccinimide
NMR	Nuclear magnetic resonance
PBS	phosphate buffered saline
PDE	phosphodiesterase
PPTS	pyridinium *p*-toluenesulphonate
rt	room temperature
sat.	saturated
TBDPS	*tert*-butyldiphenylsilyl
TBAF	tetrabutylammonium fluoride
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
t.l.c.	thin layer chromatography
TMSCl	chlorotrimethylsilane

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Introduction

Chapter 1

General Introduction

The calcium ion, Ca^{2+}, is a key messenger in the regulation of a variety of cellular functions [1-3], such as excitation contraction coupling in muscles, hormonal secretion, enzyme regulation, mitosis, neurotransmitter biosynthesis and gene expression. Calmodulin (CaM) is a Ca^{2+} receptive protein, found in eukaryotic cells, which was initially recognized to be an activator protein of Ca^{2+}dependent cyclic nucleotide phosphodiesterase [4, 5]. It is a highly conserved protein of ubiquitous species and tissue distribution, and is different from other Ca^{2+} receptors including troponin C, the parvalbumins and a vitamin D inducible protein [6, 7]. This protein is implicated in a variety of cellular processes, previously established as Ca^{2+}dependent, which include cyclic nucleotides and Ca^{2+} metabolism, muscle contraction, secretary process, microtubule and mitotic apparatus assembly and glycogen metabolism [6, 7]. Calmodulins from various vertebrate sources seem to be highly conserved and show heat stability with virtually identified proteins obtained from sea urchins and mammals.

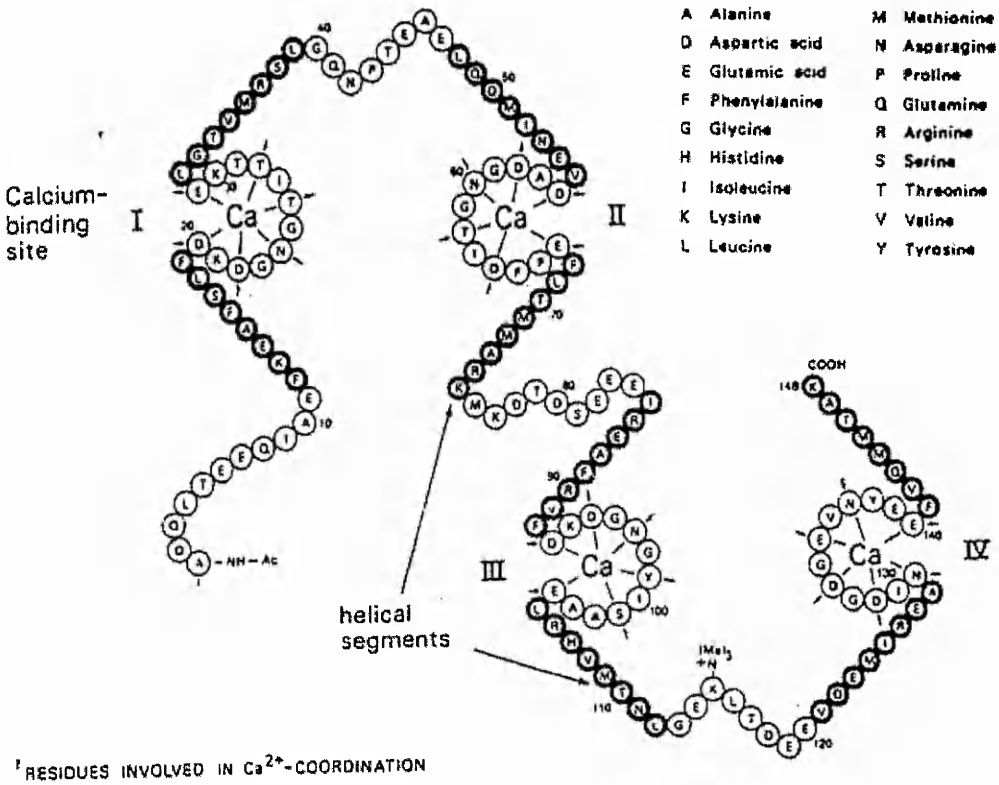
1.1 Structure

CaM is a monomeric protein composed of 148 amino acids (**Fig. 1**) [8-10]. Characteristics of the amino acid composition are the high content of glutamic and aspartic acid (about 33%), a high ratio of phenylalanine to tyrosine, the lack of cysteine and tryptophan as well as the presence of a trimethyllysine at position 115. CaM adopts a

dumb-bell like conformation characterized by two globular dicalcium binding domains which are linked by a predominantly acidic 2.0nm long 8-turn alpha-helical stretch.

CaM can be subdivided into four domains of similar amino acid sequence (**Fig. 1**). Each domain contains a helix-loop-helix structure, consisting of twelve residues, and six of which are involved in Ca binding, which binds one calcium ion within the loop [11]. This Ca^{2+}-binding structure has been described as the EF-hand by Krestsinger [10, 11], and similar structures have been recognized in other calcium-binding proteins [9, 10], like troponin C, muscle parvalbumin, regulatory light chains of myosin, brain calcium-binding protein, and intestinal calcium-binding protein [9, 10, 12-14].

Fig. 1. Sequence of bovine brain calmodulin (amino acids in one letter code) [8-10].



1.2 Calcium-Calmodulin Binding

There are two to four low affinity cation sites in CaM, which are involved in non-specific binding. Those four domains were numbered I to IV from the N-terminal region[15]. The affinity of these sites significantly depends on environmental conditions such as pH, ionic strength and the concentration of other cations present [15].

Two models of binding Ca^{2+} to CaM are widely accepted. Direct binding and microcalorimetric studies showed four independent sites with almost identical affinities; while the evidence derived from conformational studies proved that two pairs of sites exhibiting positive cooperativity within the pairs and pronounced differences in affinity between the pairs [16].

Ca^{2+} binding to CaM is accompanied by conformational changes transforming CaM from a less-ordered asymmetrical into a compact symmetrical structure. This conformational change is characterized by an increase in the alpha-helical content as well as the exposure of hydrophobic residues at the protein surface [16]. CaM is not specific for Ca^{2+}, it could also bind Mg^{2+} [17, 18]. However, the conformational changes of CaM induced by Mg^{2+} are less than those induced by calcium [19, 20].

1.3 Enzyme Binding to Calmodulin

Activation of a target enzyme by calmodulin has generally been found to require calcium ions which bind to calmodulin and change its conformation. This two-step process was first described by Teo and Wang [21] in a qualitative manner:

$$CaM + nCa^{2+} \rightleftharpoons CaM^{*}\text{-}Ca_n$$

$$CaM^{*}\text{-}Ca_n + E \rightleftharpoons E^{*}\text{-}CaM\text{-}Ca$$

A more precise model, additionally reflecting the interaction of the target with the different Ca-CaM complexes and the affinity of Ca for the enzyme-caM complex was proposed by Huang *et al.* [22]:

$$\begin{array}{ccccccccc} CaM & \rightleftharpoons & CaM\text{-}Ca_1 & \rightleftharpoons & CaM\text{-}Ca_2 & \rightleftharpoons & CaM\text{-}Ca_3 & \rightleftharpoons & CaM\text{-}Ca_4 \\ \downarrow\uparrow & & \downarrow\uparrow & & \downarrow\uparrow & & \downarrow\uparrow & & \downarrow\uparrow \\ E\text{-}CaM & \rightleftharpoons & E\text{-}CaM\text{-}Ca_1 & \rightleftharpoons & E\text{-}CaM\text{-}Ca_2 & \rightleftharpoons & E\text{-}CaM\text{-}Ca_3 & \rightleftharpoons & E\text{-}CaM\text{-}Ca_4 \end{array}$$

Take phosphodiesterase as a recent example on the enzyme activation by CaM. The enzyme exists as a dimer composed of two identical subunits in its native state, each of which contains two functional domains: a catalytic domain binding the substrate and a regulatory domain binding CaM. In the absence of CaM, the regulatory domain inhibits the catalytic domain and keeps the enzyme in basal activity state. Enzyme activation by the Ca^{2+}-CaM complex is expressed by binding to regulatory domains thereby removing their inhibitory influence. Additional catalytic sites are assumed to be thereby exposed resulting in an increased enzyme affinity for its substrates.

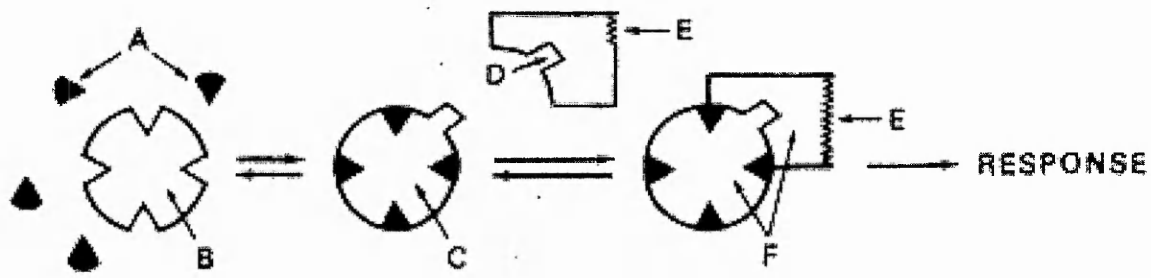
In another recent study on enzyme activation by CaM, Jarett and Madhavan [23] assumed a new type of model (the existence of a CaM binding site (CBS) and a CaM-like binding site (CLBS), on the target enzyme. The CBS region is presumed to be bound to the CLBS region without CaM and the enzyme is not active. Dissociation of the CBS from the CLBS- due to thermal motion- is a prerequisite for basal enzyme activity. CaM activation

is viewed as a dynamic competition between CaM and CLBS for the CBS region. The target enzymes can "flip-flop" between an activated and non-activated state depending upon what the CBS bound to.

1.4 Possible Modes of Drug Interactions with Calmodulin

Considering the cascade of events underlying the activator function of CaM the following modes of drug interferences (**Fig. 2**) are possible [24]:

Fig. 2. Modes of action by which a calmodulin antagonist might interfere with calmodulin-regulated processes [27].



Mode A: reduction of the concentration of intracellularly available activator calcium and thereby a prevention of the formation of the active Ca^{2+}-CaM complex. These include calcium entry blockers and calcium chelators.

Mode B: binding to Ca^{2+}-free CaM and an alteration of its ability to bind Ca^{2+}. These species might act by binding directly to the calcium binding sites on CaM or by

binding to some other site on the molecule and inducing conformational changes in the calcium binding regions. Several bi- and trivalent cations are included in this category.

Mode C: binding to the active Ca^{2+}-CaM complex and modifying its activity. A large number of compounds classified as CaM antagonists are assumed to act via binding to the Ca-CaM complex.

Mode D: binding to the CaM recognition site on the CaM sensitive enzyme, thus preventing the interaction of the Ca^{2+}-CaM complex with the target enzyme. Several irreversible CaM-drug adducts have been shown to inhibit CaM activation of target enzymes presumably by competing with CaM for recognition sites on the enzyme.

Mode E: interaction with the catalytic portion of the CaM-sensitive enzyme and altering its activity, e.g. methylxanthine PDE inhibitors were classified as indirect CaM antagonists since they do not interact with CaM or its binding sites. These agents are relatively non-specific because they can inhibit stimulated and non-stimulated enzymes [25]. They can also inhibit the activation of the enzyme or inhibit CaM-insensitive forms of the enzyme [25], e.g. trifluoroperazine (TFP) could bind not only to CaM/MLCK, but also to the myosin phosphorylation site, inhibiting its phosphorylation [26].

Mode F: interaction with the ternary Ca^{2+}-CaM enzyme complex; little is known about such agents.

1.5 Calmodulin Antagonists

Most compounds are described as exhibiting mode C as the CaM inhibitory mechanism. From the chemical point of view, the compounds in this subgroup could be divided into the following main classes: phenothiazines, naphthalenesulfonamides,

dihydropyridines, arylalkylamines, isoquinolines and quinolones. Several individual structures such as calmidazolium, and phenoxybenzamine are not attributable to one chemical class. The most important and best investigated calmodulin antagonists are described in the following section.

1.5.1 Antipsychotics and Antidepressants

The CaM antagonistic properties of drug molecules were detected for the first time by the group of Weiss [28] investigating the influence of phenothiazines (**Fig. 3**) on the metabolism of cyclic nucleotides. They found an effect of phenothiazines not only on phosphodiesterase activity, but also on brain adenylate cyclase. In further investigations, it was confirmed that the Ca^{2+}-CaM-stimulated subforms of these enzymes were inhibited, and it was established that this enzyme inhibition results from the direct binding of phenothiazines to the CaM molecule.

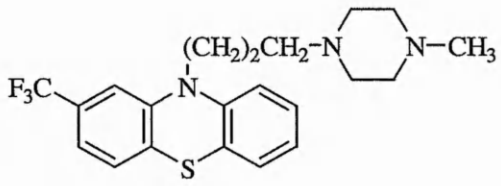
Among phenothiazine compounds, trifluoroperazine (TFP) is the most and best investigated drug. Levin and Weiss's [28] investigation found two high-affinity binding sites and four low-affinity binding sites, and also proved the binding to be Ca^{2+}- and pH-dependent. Binding could be decreased by raising pH from 7.5 to 8.5 or lowering it below 4.2 due to the alteration in the CaM binding sites or to changes in the ionized state of the drugs. If the latter mechanism holds, phenothiazine binding to CaM probably occurs via the protonated form. Two high-affinity binding sites are assumed to be localized in the C-terminal [29] and N-terminal [30] respectively. However, one more recent study by Masson et al. [31] using automated high performance liquid chromatography indicated that there are

four high-affinity binding sites. One molecule of the basic polypeptide displaces two TFP from CaM indicating two different classes of TFP binding sites.

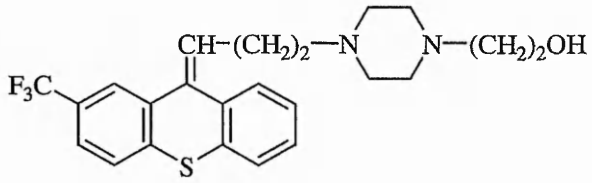
Gariepy et al. [32] in 1983 proposed a TFP binding site on troponin C represented by the helical region of amino acids 92-102 which are rich in hydrophobic (phenylalanine, methylgroups of leucine and alanine) as well as negatively charged side chains (carboxyl groups of glutamic acid). The corresponding homologous sequence in CaM is represented by the amino acids 82-92. In 1988, Strynadka and James [33] reported the amino acids involved in TFP binding within C-terminal and N-terminal. Hoeltje et al. [34] have also suggested two binding sites via molecular modelling investigations. In the first site an interaction between Glu 84 and the positively charged nitrogen of the phenothiazine side chain is assumed; additionally an interaction between Arg 86 and the electron-rich substituents in position 2 of the tricycle is postulated. In the second site the phenothiazine ring is placed in a hydrophobic cavity formed by amino acids 32-55; interacting residues of the central helix are Lys 75 and Glu 82. More recently, Cook et al. [35] showed by X-ray studies on the crystal of CaM-TFP complex that the tricyclic ring of TFP lies in the same hydrophobic pocket in the C-terminal domain which is utilised in binding peptides, and the piperazine ring lies between several hydrophobic residues.

Several other antipsychotics exhibit CaM antagonistic activity in addition to phenothiazine. Compounds with some similarity to phenothiazines, all containing three annellated rings, are flupenthixol [36], and clozapine [28]. Among the conformationally more flexible structures, pimozide has been established as a rather potent CaM inhibitor [37].

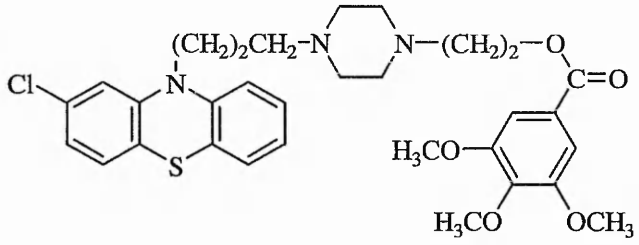
Fig. 3 CaM-Inhibitory Antipsychotics and antidepressants



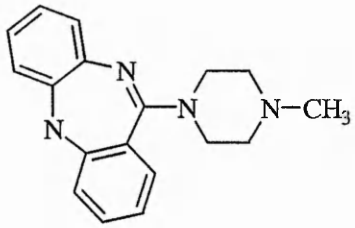
Trifluoperazine



Flupenthixol



Metofenazate



Clozapine

1.5.2 Naphthalenesulfonamides

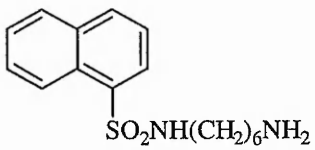
Naphthalenesulfonamides (**Fig. 4**), including W5, W7, W12, W13 were first developed by Hidaka et al. in 1978 [40] and have been intensively investigated by the same group [39a, b,]. Each of them has its own analogues and isomers, which were found also to inhibit calmodulin. It was claimed that calmodulin agents, such as W7 and its derivatives could inhibit Ca^{2+}-calmodulin activated phosphodiesterase selectively [40]. Especially, W7 (N-6-aminohexyl)-5-chloro-1-naphthalenesulfonamide), is one of the most widely used reagents in many tests involved calmodulin.

A kinetic analysis of W7-induced inhibition of activation of phosphodiesterase reveals that W7 inhibits this activity in a competitive fashion against calmodulin-induced

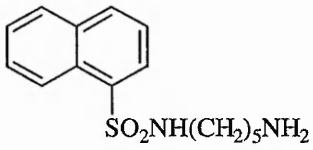
activation. It has been demonstrated that Ca^{2+}-calmodulin has two classes of W7 binding sites: three functional sites with a high affinity for W7 (K_D 11μM) and nine sites with a low affinity for the drug (K_D 200μM) [39].

The effect of W-7 on the calcium-bound conformation of calmodulin was studied by 1H-NMR at 400 MHz [41]. W-7 affected the resonances of Ile-27, Phe-68, Phe-92, Ile-100, His-107 and Val-142 and probably also affected Met-71, Met-72, Met-76, Phe-89 and Phe-141. These findings suggest that W-7 binds to hydrophobic amino acid residues, which nearly always occur in calcium-binding sites II, III and IV or their vicinity. The effect of W-7 on the structure of calmodulin was similar to that of other drugs, trifluoperazine, and oxmetidine. Thus, those residues in the high-field methyl region, the methionine methyl region and the phenylalanine aromatic region of calmodulin, which were similarly affected by all four drugs, may be important at the interface for binding of calmodulin to the regulatory sites on target enzymes.

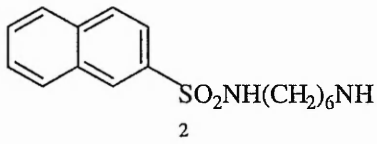
Fig. 4 Naphthalenesulfonamides



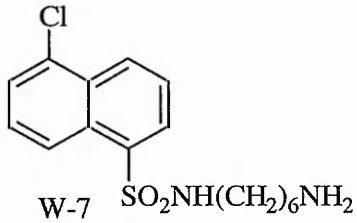
W-5



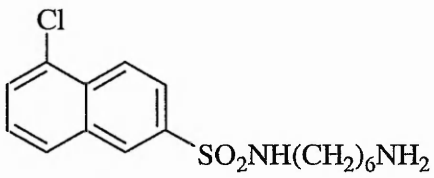
W-5 homologue



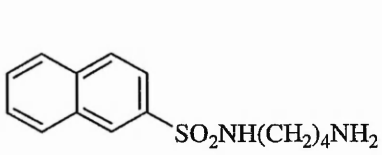
W-5 isomer



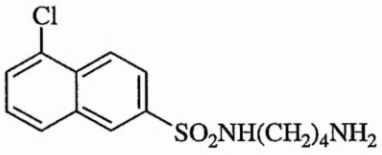
W-7



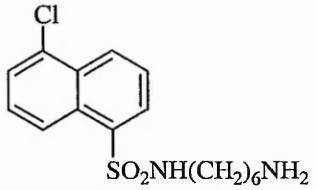
W-7 isomer



W-12



W-13



W-13 isomer

Small-angle X-ray scattering and nuclear magnetic resonance were used to investigate the structural change of calcium-bound calmodulin (Ca^{2+}/CaM) in solution upon binding to its antagonist W-7 [42]. The radius of gyration was 17.4+/-0.3 A for Ca^{2+}/CaM-W-7 with a molar ratio of 1:5 and 20.3+/-0.7 A for Ca2+/CaM. The results suggest a tendency for Ca2+/CaM to form a globular structure in solution, which is inducible by a small compound like W-7.

Displacement of [^{3}H]-W7 binding and inhibition of CaM-stimulated phosphodiesterase activity have been used by Hidaka et al. [43] to derived structure-activity relationships for naphthalenesulfonamide derivatives. They found a strong dependence of CaM inhibitory potency on the presence of the chlorine substituent, and the alkyl chain length being optimal with n=10 (**Table 1**).

Table 1. The effect of alkyl chain length on the naphthalenesulfonamede affinity for CaM [43]

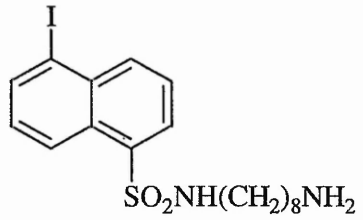
$-(CH_2)_n-$	**Displacement of [^{3}H]-W7 from calmodulin** IC50 μM	**Inhibition of PDE activation by calmodulin** IC50 μM
5	68	32
6	31	26
8	3.6	18
10	2.3	3.2

Naphthalenesulphonamide compounds were investigated in further work carried out by Blackburn and co-workers [44]. Compared to the pioneer study, an obvious improvement in calmodulin antagonistic potency was observed by replacing chlorine with iodine on the 5-position. The relationship between alkyl chain length and activity was also investigated. However, the correlation results obtained (**Table 2**) were not identical to those from Hidaka's structural-activity study described above [44]. A minimum activity for the C6 chain and a sudden five fold increase in activity going from C10 to C12 were observed in this study; the increase in potency had to be offset against the decrease in water solubility. However, N-(8-aminooctyl)-5-iodo-1-naphthalenesulfonamide, also known as J8 (**Fig. 5**), was considered to have the best combination of potency and solubility.

Table 2. Comparison of potency of W7 and 5-iodo-C_n derivatives of W7 for the inhibition of calmodulin-dependent beef heart phosphodiesterase and for the inhibition of [^{3}H]thymidine uptake in mouse B16 melanoma [44]

	Concentration of drugs required (μM) for 50% inhibition (means ±SEM)	
Agents	**Calmodulin-dependent PDE**	**Incorporation of ^{3}H]thymidine in cultured cells (B16)**
W7	29 ± 4	42 ± 10
C6	28 ± 4	35 ± 11
C7	7.0 ± 0.6	44 ± 6
C8 (J8)	3.0 ± 0.6	46 ± 6
C9	3.0 ± 0.4	45
C10	4.0 ± 0.9	36
C12	0.7 ± 0.1	50

Fig. 5 Structure of N-(8-aminooctyl)-5-iodo-1-naphthalenesulfonamide (J8)



J 8

The binding of J-8 to the C-terminal domain (tr2c) of calmodulin has been investigated by Craven and co-workers [45]. Using a combination of NMR methods, including NOESY data, mobility measurements, chemical shift and line-shape analysis, it is shown that the primary interaction between J-8 and tr2c is between the naphthalene ring of the antagonist and the hydrophobic pocket of the protein, similar to the binding of the hydrophobic side-chain residues of calmodulin target peptides. No significant change in the binding mode in J-8.CaM compared to J-8.tr2c, with one molecule binding to each domain, was observed during the NMR operation [45]. In particular, it is found that the mobility of the aliphatic amino "tail" of J-8 remains highly mobile in both systems. This contrasts with the notion that the tail may bridge between the two domains to give a "globular" form of CaM [45].

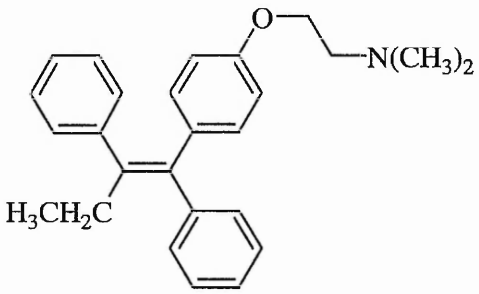
1.5.3 Tamoxifen

Tamoxifen, which was designed as an anti-estrogen reagent, is one of the most widely used anti-cancer drugs, especially with breast cancer. It was also found to have calmodulin antagonism. Calmodulin (CaM) was claimed to associate with the estrogen

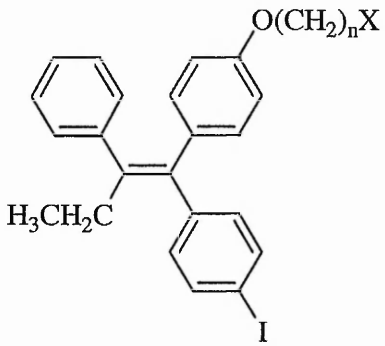
receptor [46]. The antiestrogen tamoxifen (**Fig. 6**) impedes this association suggesting that the latter would play an important role in CaM-dependent enzymatic catalyses [46]. Estrogen receptor, antiestrogen binding sites and calmodulin are potential targets of antiestrogen action.

Triphenylethylenes [Tamoxifen (TAM), TAM metabolites] were found to inhibit Ca^{2+}-calmodulin (CaM)-dependent cAMP phosphodiesterase (PDE) activity of the quail oviduct [47]. The Ca^{2+}-CaM-independent PDE activity was not affected by triphenylethylenes, suggesting that they do not interact directly with the active site of the enzyme. The order of growth inhibitory potency of tamoxifen and its metabolites was not correlated with estrogen receptor affinities, but was the same as that reported for PDE inhibition. This correlation suggests that interaction of antiestrogen with Ca^{2+}-CaM dependent PDE may be one of the mechanisms responsible for the estrogen antagonist activity of these drugs.

Fig. 6. Tamoxifen and its analogues



Tamoxifen



n=3 X = N Iodoxifene

n=4 X = $N(CH_3)_2$ Iodotamoxifene

Rowland et al. [48] suggested that inhibition of the estrogen receptor-CaM association by various triphenylethylene antiestrogens (tamoxifen and its derivatives) does not relate to antagonism of calmodulin function or their binding affinity for the estrogen receptor.

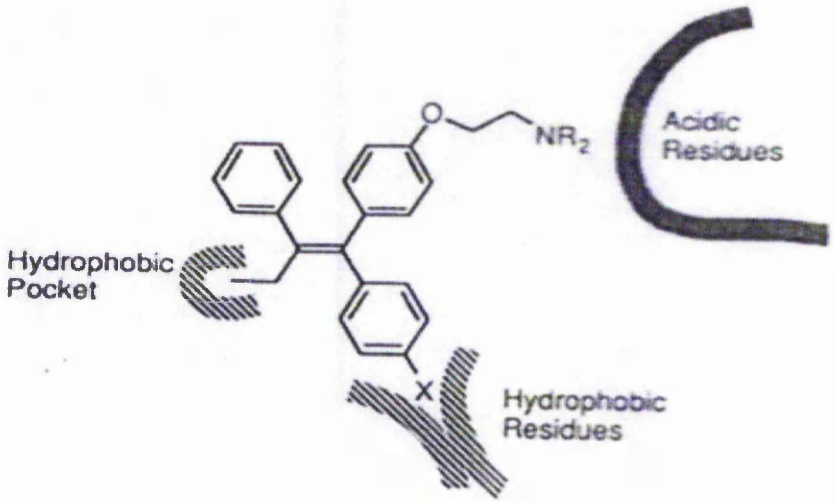
The design of more potent antagonists has been investigated in a molecular modelling study carried out by Hardcastle and co-workers [49]. This indicated that compounds with either three or four methylene units in the basic side chain or slim lipophilic 4-substituents were expected to be more potent. All compounds were tested for antagonism of the calmodulin dependent activity of cAMP phosphodiesterase and for binding affinity to the estrogen receptor. The compound possessing the optimal combination of calmodulin antagonism and estrogen receptor binding (IC50 = 1.1μM, receptor binding affinity = 23) is shown in **Figure 6**,.

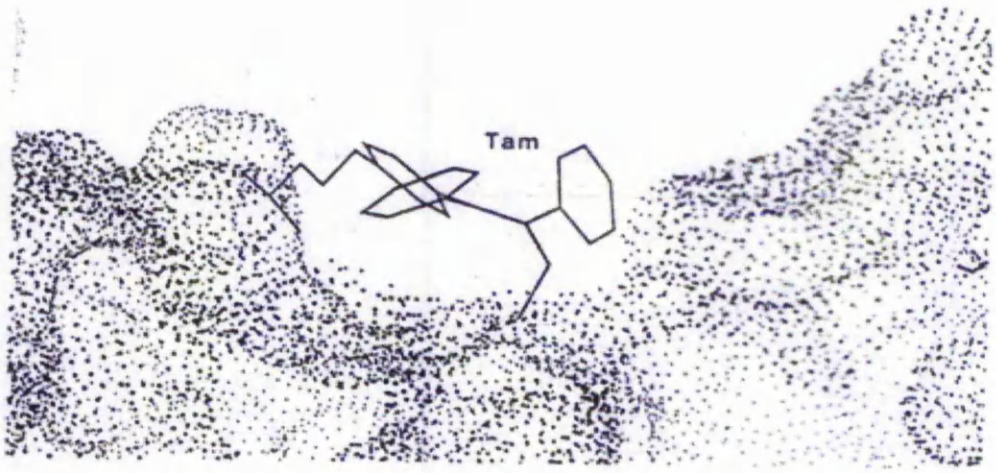
The molecular modeling study [50] also located a hydrophobic cavity in the C-terminal domain as the most likely binding site for tamoxifen, and it was possible to demonstrate a structure-activity relationship for the analogues. The model (**Fig. 7**) shows the importance of the interaction of the ethyl group with a small hydrophobic pocket, the interaction of the basic side chain, which is protonated at physiological pH, with a region of acidic residues, and the presence of a hydrophobic region close to the 4-position of the drug [49].

1.5.4 Miscellaneous Antagonists

In addition to the calmodulin antagonists already discussed, calmodulin antagonism has also been established for a diverse series of compounds. These compounds include

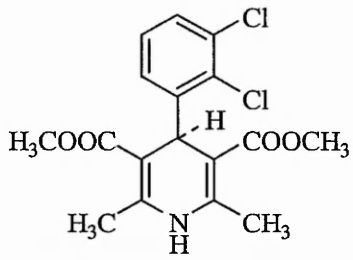
Fig. 7 (top) Schematic of the interactions of tamoxifen and its derivatives with calmodulin. (bottom) Plot of tamoxifen (Tam) bound in the high-affinity hydrophobic cavity of calmodulin (shown as solvent accessible surface), from the molecular modeling study [49]



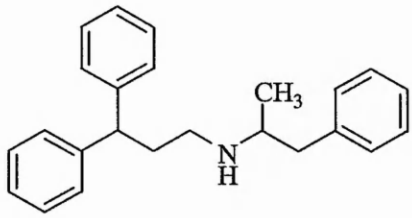


calcium channel ligands which were divided into two subgroups, dihydropyridine (**Fig. 8**) and arylalkylamines (**Fig. 8**) [51a], antiarrhythmics, such as ethmozine [51b] (**Fig. 8**), isoquinoline derivatives, wherein papaverine (**Fig. 8**) is the most active compound, and also calmidazolium (**Fig. 8**), an imidazolium derivative which was shown to be one of the most potent currently available CaM inhibitors [52, 53].

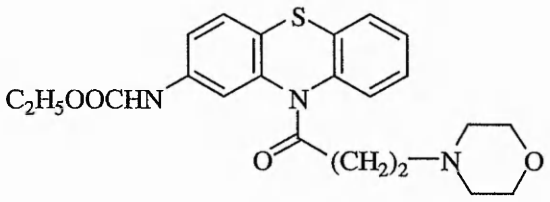
Fig. 8 Structure of some CaM-antagonists



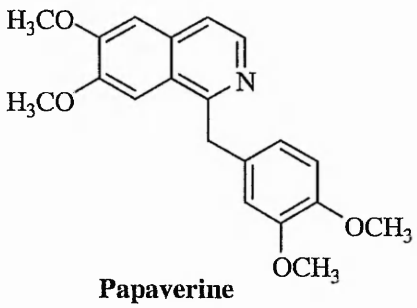
Felodipine



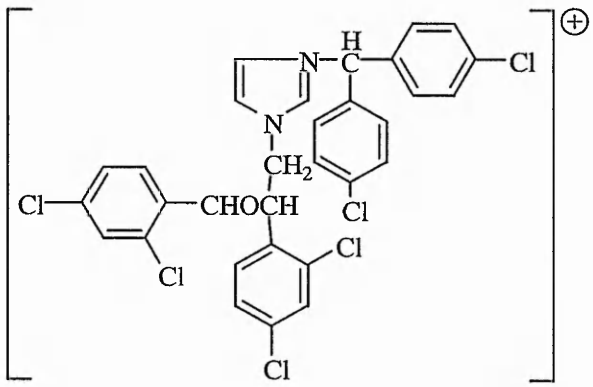
Prenylamine



Ethmozine



Papaverine



Calmidazolium

1.6 Putative Therapeutic Applications of Calmodulin Antagonists

Literature reports indicate a broadening spectrum of putative therapeutic applications of calmodulin antagonists. Some of these are discussed below.

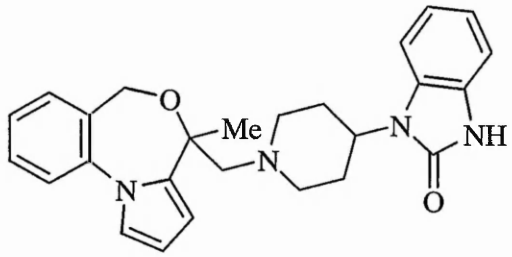
1.6.1 Calmodulin Antagonists in the Treatment of Diarrhoea

Calmodulin in the intestinal epithelium [54] plays a key role in the secretion of electrolytes and fluid in the intestinal tract [55]. Some investigations [56, 57] have shown that phenothiazine CaM-antagonists, such as trifluoperazine and chlorpromazine, have positive effect on diarrhea in rodents. However, these drugs cause sedation due to their neuroleptic property and are not suitable for in anti-diarrhea therapy [58].

To our knowledge, the only drug designed specifically as a CaM antagonist to proceed to phase III trial is the anti-diarrhea agent zaldaride maleate (**Fig. 9**), also called KW-5617 or CGS 9343B. It is a selective and potent inhibitor of calmodulin, which is even more potent than trifluoperazine [59] and cause less sedation [56]. Unlike TFP, KW-5617 does not inhibit protein kinase C and presents less affinity for dopamine receptors [59]. Moreover, it was demonstrated [56] that this drug could ameliorate an experimentally induced diarrhea situation without reducing the rate of intestinal propulsion.

It is suggested by some reports that KW-5617 is a potent drug for the treatment of acute secretary diarrhoea [60]. Furthermore, there is a possibility that KW-5617 can be used for the treatment of infectious and/or traveller's diarrhoea induced by bacteria or viruses, without the side effects of constipation caused by the gold standard drug, loperamide [61].

Fig. 9 the structure of zaldaried (1, 3-dihydro-1-[1-[(4-methyl-4H, 6H-pyrrolo[1, 2-a][4, 1]benzoxazepin-4-yl)methyl]-4-piperidinyl]-2H-benzimidazol-2-one)



Zaldaride

1.6.2 Calmodulin Antagonists as Possible Anti-fungal Reagents

Primarily as a result of the growing population of immuno-suppressed and immuno-compromised patients, arising from HIV infection and AIDS, transplant recipients, and cancer chemotherapy, both the number and severity of systemic fungal infections have increased [62, 63]. However, compared to anti-bacterial drugs, there are fewer anti-fungal agents available. It has been reported that although calmodulins are highly conserved in most higher eukaryotes, there is significant divergence in fungal CaMs, compared with those from other species, which raises the possibility that these might be suitable targets for novel antifungal drugs [64].

There are a few reports of the inhibitory effects of antifungal agents on calmodulin-mediated systems. Chloraniformethan was revealed to possess inhibitory effects on CaM-dependent cyclic nucleotide phosphodiesterase, and the clinically useful azole antifungal ketoconazole could also inhibit this enzyme at low micromolar concentrations [65].

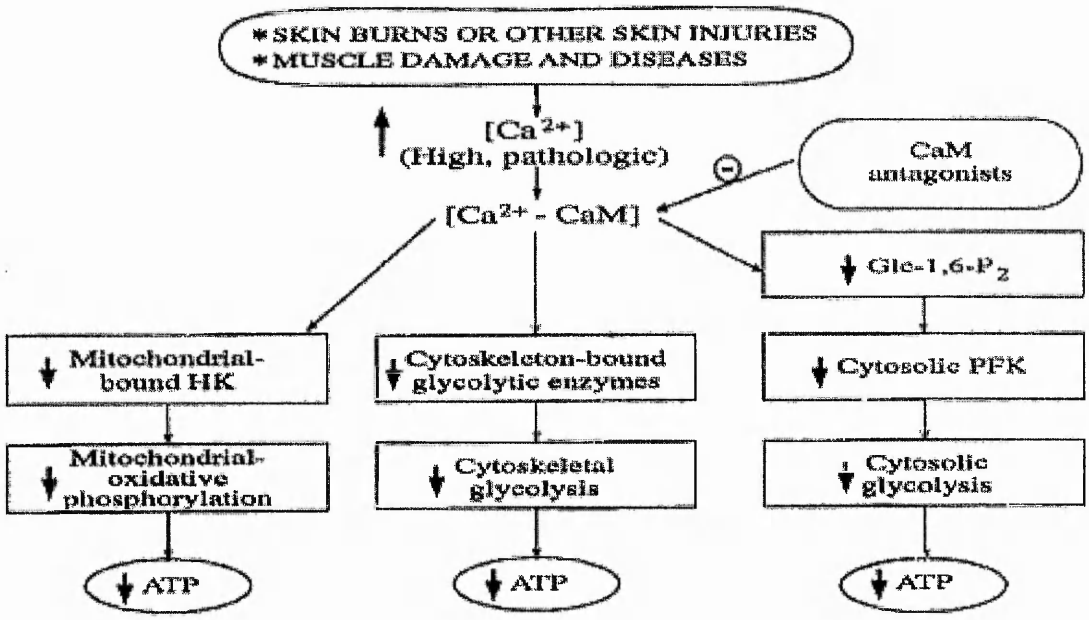
However, to our knowledge, no anti-fungal drug in clinical use was specifically designed as a CaM-antagonist. In research carried out at Nottingham Trent University, it was demonstrated that a series of compounds synthesized as novel specific calmodulin antagonists also showed antifungal potency against the plant pathogen, *Pythium ultimum.* This fungus is an oomycete, which does not synthesize ergosterol, and is thus unaffected by fungicides which inhibit sterol biosynthesis. It is suggested that these compounds could possibly be an important new class of antifungal agents [66]. Additionally, J8, a classic CaM-antagonist, was also found to have significant activity against this pathogen [67].

1.6.3 Calmodulin Antagonists in the Treatment of Skin Burns and Other Skin Injuries

It has been shown that some CaM antagonists counteracted the decrease in ATP level, and activities of certain enzymes, induced by burns in rat skin [68]. These antagonists also exhibit a protective action on blood capillaries and erythrocyte membranes [68]. It is suggested that CaM antagonists may be effective agents in the treatment of burns. More recently, it is reported that a rapid release of Ca^{2+}-mobilizing hormones, bradykinin, serotonin, and histamine play a pathogenic role in tissue injury and inflammation in skin burns or other skin injuries [69-72]. It is demonstrated that the CaM antagonists act by binding to the Ca^{2+}-CaM complex and thereby, eliminating its pathological effects on the ATP-producing systems in different cellular compartments (**Fig. 10**). The investigation shows the decrease in ATP level in skin, induced by burns or frostbite, could be reversed to normal level following treatment with different calmodulin antagonists. Therefore, it

was also proved that the CaM antagonists have both a therapeutic and a prophylactic action on skin burns, frostbite, and UV-induced burns [68].

Fig. 10 The reduction in cell energy metabolism in skin and muscle damage, induced by high pathological rise in free intracellular Ca^{2+}, and its prevention by CaM antagonists. ↑, increase; ↓, decrease; Θ, inhibition [68].



1.6.4 Calmodulin Antagonists in the Treatment of Cancer

The involvement of calmodulin in cell division, such as the control of DNA duplication and ATP level, actions of many important kinases, raised high interest in cancer therapeutic application. Additionally, a large number of *in vitro* and *in vivo* investigations support the putative therapeutic use of CaM antagonists as anticancer

agents. Both direct and indirect modes of antiproliferative action seem to underlie the antitumor efficacy of these compounds.

1.6.4.1 Calmodulin Antagonists and Cellular Proliferation

There is abundant evidence implicating calmodulin in a role in calcium-stimulated cellular proliferation [73-75]. For example, Whitfield, *et al.* demonstrated the requirement for calcium in the early stages of DNA synthesis in regenerating hepatocytes [76]. Since calmodulin appears to play a critical role in normal cellular proliferation, it is not surprising that alterations in this branch of the calcium messenger system may occur in and be responsible for states of abnormal cellular proliferation.

Early studies revealed that calmodulin is synthesized exclusively at the G_1/S boundary of the cell cycle [77]. Calmodulin antagonists were used to block cells specifically and reversibly at this point in the cell cycle. They also prevent the re-entry of quiescent cells into the cell cycle in response to mitogens [78]. Finally an elevated level of calmodulin prior to initiation of DNA synthesis has been proposed to be required for effective DNA repair [78].

More recently, accumulating evidence strongly suggests that CaM is the major mediator of Ca^{2+}-dependent cell proliferation, especially in later phases of cell cycle progression. Hidaka *et al.* [79] first reported that the naphthalenesulfonamide calmodulin antagonist W-7 potently inhibits DNA synthesis and cell proliferation in synchronized cells. Cells did not start DNA synthesis when Chinese hamster ovary (CHO-K_1) cells at mitosis were collected and replated in the presence of W-7. Upon removal of W-7, cells immediately entered S phase, followed by cell division [79]. When W-7 was added at a

slightly higher concentration to the same cells at the midst of S phase, cells did not divide when this compound was present. However, cell division resumed after removal of W-7[80]. These observations support the notion that CaM plays pivotal roles at two points in the cell cycle: the G_1/S and somewhere between the G_2 and the M phases.

Chafouleas *et al.* [78] also reported that another CaM antagonist W-13 blocked the cell cycle of CHO-K_1cells at the G_0/G_1interphase at a relatively high concentration. Even at this concentration, W-13 did not shut off the ongoing DNA synthesis when introduced to these cells after entry into the S phase, although it did inhibit completion of the S phase [77]. Therefore, the $G_0/G_1$and the S/G_2transitions are likely to be additional targets for the CaM action.

Measurements of the CaM content in a variety of transformed cells in culture and tumor tissues have revealed that these cells often show elevated levels of CaM [81-84]. Rasmussen and Means [85-88] have shown that CaM over-expression results in acceleration of cell proliferation featured by a selective shortening of the G_1 phase and increases in cell density at growth saturation. In contrast, an inducible transient reduction in the CaM level in exponentially proliferating cells caused concomitant transient cessation of cell proliferation at either the G1/S boundary or in the M phase at metaphase/anaphase transition point [86-88]. These results demonstrated that CaM is indispensable for cell cycle progression.

1.6.4.2 Calmodulin Antagonists and Cell Energy Metabolism

Cancer cells are characterized by a high rate of glycolysis, even under aerobic conditions, which is their primary energy source [89-92]. It has been found that different calmodulin antagonists reduce all the ATP-producing systems in different cell apartments of melanoma cells (**Fig. 11**), which eventually leads to cell death [93-96]. Calmodulin antagonists decreased the levels of glucose 1,6-bisphosphate and fructose 1,6-bisphosphate, the potent allosteric activator of cytosolic phosphofructokinase (PFK), the rate-limiting enzyme of glycolysis, and, thereby, reduced ATP production through cytosolic glycolysis in B16 melanoma cells [95].

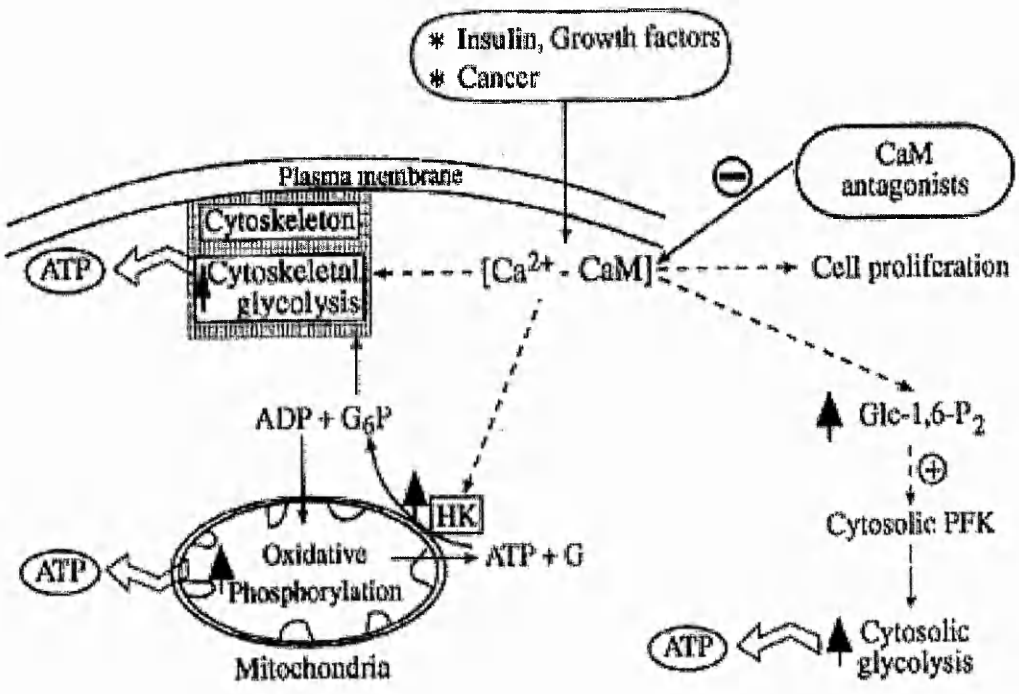
In addition to reducing cytosolic glycolysis in B16 melanoma cells, calmodulin antagonists also reduced cytoskeletal glycolysis by causing a detachment of glycolytic enzmes from cytoskeleton of melanoma cells [97]. This detachment would reduce the provision of local ATP in the vicinity of the cytoskeleton-membrane interaction and would affect cytoskeleton structure. The reduction in both cytosolic and cytoskeletal glycolysis, induced by CaM-antagonists, preceded the decrease in cell viability, which indicates that this is an early effect and not a result of cell death.

The levels of mitochondrial bound hexokinase in highly glycolytic tumor cells greatly exceed that of normal tissues. Mitochondrial binding increases the catalytic activity of hexokinase, and leads to the stimulation of glucose 6-phosphate production [98, 99]. Glucose 6-phosphate is a critical precursor not only for glycolysis for supplying cellular ATP but also for the pentose phosphate pathway, leading to synthesis of excessive amounts of nucleic acids and lipids, which would be available for maintenance of cancer cell proliferation [100]. The findings proved that the high mitochondrial-bound hexokinase

in cancer cells plays an important role in regulating cell energy metabolism and cell growth rate.

Therefore, the detachment of hexokinase from mitochondria of melanoma cells as well as the detachment of other glycolytic enzymes from cytoskeleton [97] and the reduction in cytosolic glycolysis [95] induced by CaM-antagonists, would lead to a reduction in cell metabolism, growth, and proliferation. The fall in ATP leads eventually to cell death [95]. It is shown that these reductions make these calmodulin antagonists most promising agents in the treatment of cancer.

Fig. 11 Calmodulin antagonists block the stimulatory effects of the growth-promoting effects of hormones, such as insulin and growth factors, and cancer on the energy-producing systems in different cellular compartments. ↑increase; ⊖ inhibition.



1.6.4.3 Calmodulin Antagonists as Modifiers of Cancer Drugs

CaM antagonists have also been reported to potentiate the effects of a number of chemotherapeutic agents used in cancer therapies. The most effective enhancement has been demonstrated by combining CaM antagonists with bleomycin or its derivative, peplomycin. Mizuno and Ishida [101] were the first to suggest that the action of CaM antagonists on cell membranes potentiated the toxicity of bleomycin.

Chafouleas et al. [102] implicated CaM as a possible mediator of specific DNA repair functions in mammalian cells, by demonstrating that the naphthalenesulfonamide CaM inhibitor, W-13, dramatically increased the toxicity of bleomycin for CHO cells [102], leukemic L1210 cells [103], and murine EMT6 [104]. Monitoring of cell survival demonstrated that CHO cells are capable of fully recovering from bleomycin-induced damage. However, the addition of a CaM antagonist to the recovery medium appeared to prevent a CaM regulated DNA repair process. As a single drug, bleomycin's beneficial use is limited by its pneumotoxicity which results in pulmonary fibrosis. However, the volume density of the bleomycin-induced lesion in the lung, including the volume of amorphous material, the degree of interstitial inflammation and the number of infiltrating monocytes were all reduced in the animals receiving trifluoperazine [105, 106].

The effect of calmodulin antagonists on other anti-cancer agents has also been tested. Yoshihiro *et al.* demonstrated that the combination of 1-(2-tetrahydrofuryl)-5-fluorouracil plus uracil and W5/W7 could result in a delay in tumour formation and inhibition in tumour growth [107].

1.6.4.4 Calmodulin Antagonists as Anti-Cancer Agents

Calmodulin antagonists have been studied not only as modifiers in cancer research; but as single agents in this field. Thus, the Hidaka compound, W7, has been reported to exhibit carcinostatic properties against several solid tumors in vivo [108]. More recently, the antimetastatic effect of W7 was examined in an experimental model of lung metastasis induced by Lewis lung carcinomas in C57BL/6crSlc mice [109]. Injection of W7 *i.v.* after the removal of the implanted primary tumor caused the greatest inhibition of development of lung metastases [109]. The results from the studies of W7 raise the possibility that W7 may have clinical value in the prevention of cancer metastasis.

The related compound, J8 (**Section 5.2**), and its analogues have been studied in some detail in Sheffield by the group of Professor Blackburn and Mac Neil. These compounds were found to inhibit cell proliferation and arrest cell cycle progression in a similar manner to W7 [110]. The greater potency and improved specificity of the longer 5-iodo-Cn (n= 6-12) analogues could help to exclude the possibility of the drugs binding to other hydrophobic proteins in addition to calmodulin [110]. More recently, MacNeil *et al.* demonstrated the effective inhibition of cutaneous and uveal melanoma cell invasion through fibronectin in vitro achieved with submicromolar concentration of J8 [111].

As a single anti-cancer agent, tamoxifen is possible one of the most widely used drugs, especially in breast cancer. Although it was designed as an anti-estrogen receptor, it was found that tamoxifen could inhibit cell growth in some solid tumors which do not have any estrogen receptor. These findings showed that tamoxifen might act as a calmodulin antagonist in these cases. Tamoxifen has been studied in a great detail [111-114] over various cell lines, including murine melanoma, human ocular melanoma, human breast tumor cells

(MCF7) etc. It is demonstrated that both tamoxifen and its metabolites (N-desmethyltamoxifen and 4-hydroxyltamoxifen) are capable of inhibiting attachment of murine and human neoplastic cells to plastic and to extracellular matrix proteins [113]. It was also proposed that tamoxifen given to patients after surgery for a primary breast tumor may reduce the chances of circulating breast tumor cells adhering at secondary sites and forming metastatic deposits. In their later study [111] against invasion assay on a melanoma cell line, N-desmethyltamoxifen was found to have better potency than its parent drug, tamoxifen, at submicromolar levels in inhibiting invasion whereas 4-hydroxytamoxifen was relatively ineffective in inhibiting invasion of A375-SM cells. The finding raised the research interest in Nottingham Trent University which will be introduced in the **Chapter 2.**

1.6.4.5 Multi-Drug-Resistance (MDR) and Calmodulin Antagonists in the Treatment of Cancer

The ability of malignant cells to develop resistance to chemotherapeutic drugs is a major obstacle to the successful treatment of clinical tumors. The phenomenon, MDR, in cancer cells results in cross-resistance to a broad range of structurally diverse antineoplastic agents, due to outward efflux of cytotoxic substrates by the multidrug resistance gene product, P-glycoprotein (P-gp) [115]. Numerous pharmacologic agents have been identified to inhibit the efflux pump and modulate MDR, including calmodulin antagonists [115].

It was reported that 21PT cells are tamoxifen resistant because there is no binding between estrogen receptor (ER) and the steroid hormone estradiol (E2) [116]. However, the

finding 21PT cells are sensitive to W7 suggested that the significance of an alternative therapeutic target for tamoxifen-resistant estrogen receptor positive human breast cancers with compounds, such as W7 [116]. It was also established that the combination of tamoxifen with trifluoperazine could increase the preventive and therapeutic effects of tamoxifen [117].

Some DNA-damaging agents, such as cisplatin, carmustine and dacarbazine, might be most effective when they are administered with tamoxifen. Recent data suggest that tamoxifen can inhibit human melanoma cell proliferation by interaction with type II oestrogen binding sites in human melanoma cells [118].

The effects of calmodulin antagonists (W7/W5) on cisplatin uptake were studied in human ovarian cancer cells by using KF cells derived from serous cystadenocarcinoma of the ovary and cisplatin-resistant cells (KFr). An increased cisplatin uptake by the KF and KFr tumors was shown when it was administered with calmodulin antagonists [119]. It is suggested that the co-administration of calmodulin antagonists and cisplatin may be useful in patients with refractory ovarian cancer.

Chapter 2

Background of Previous Studies Related to This Present Investigation

As discussed earlier, Hidaka et al. [79] established that W-7 is a significant calmodulin antagonist, which showed inhibitory potency in cell proliferation. This study of naphthalenesulfonamides was extended by Professor Blackburn [44]. The best compound in his investigation was found to be J-8. Both of W-7 and J-8 have been used widely in many biological tests involving calmodulin.

The alteration of inhibitory activity towards the protein arising from the change of the chemical structure has been further investigated by a group in Nottingham Trent University. Some compounds synthesized by them possess strong activity in anti-fungal screens, and show inhibition of the proliferation of melanoma cells.

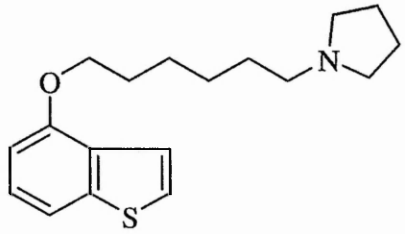
The aim of the original study by O'Donnell [120] to design and synthesize novel specific CaM antagonists was based on the lead compounds W7 and J8. They have the general formula $ArSO_2NH(CH_2)_nNH_2$, where ***Ar*** is a α- or β- naphthyl residue, preferably halogenated and ***n*** lies between 6 and 10 for maximum inhibitory potency. The question of whether the replacement of the sulphonamide linkage would change the compounds' biological activity stimulated an attempt to modify parent compounds by exchange for various linkages, including polar carboxamide, thiourea, or urea groups. However, the ability of the resulting compounds to inhibit CaM-dependent phosphodiesterase was much reduced in each case. Compared to the compounds containing ***polar*** linkages, the substitution of the sulphonamide moiety by a ***non-polar*** linkage, such as ether or thio-

ether, gave compounds equipotent with corresponding sulfonamides, and the ether group was further adopted in all subsequent compounds synthesized.

In addition to the investigation in linkage, the effect of the base strength of the terminal amine on calmodulin inhibition was also studied by O'Donnell. It was found that compounds with pyrrolidine or piperidine as terminal amine showed better potency. It is possibly because the pKa of N-alkylpyrrolidines (10.32) [120], and piperidines (10.08) [122] is close to that of primary alkylamines (10.64) [123], whereas incorporation of imidazole (pKa 7.33) [124] or morpholine (pKa 7.41) [125] gave poorer antagonists. This might be because pyrrolidine and piperidine residue can be protonated at physiological pH.

Comparing compounds that differ in only one respect, it is found that the benzothiophene derivatives are more potent than their naphthalene derivatives. Benzothiophene, a biostere for naphthalene, could thus successfully replace naphthalene as the aryl moiety.

The most potent compound synthesized by O'Donnell appeared to be 4-(N-pyrrolidinohexyloxy)benzo[b]thiophene, **JSD35**, with an IC50 value of ~0.3μM against CaM-stimulated phosphodiesterase enzyme assay, which is significantly more potent than both W7 (IC50 31μM) and J8 (IC50 3μM) against the same enzyme [118], and is also one of the most potent compounds described at that time.

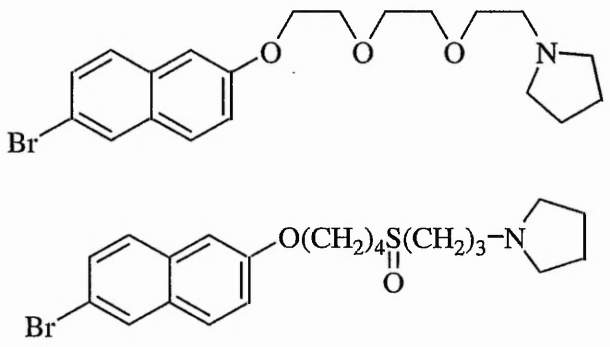


JSD 35

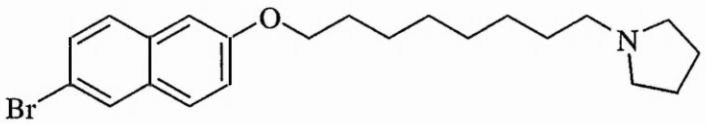
In a further study carried out by Bulpitt [126a], benzothiophene derivatives were converted to analogues by halogention, especially bromination, in the 3, 5, and 7-position, and by exchange of the side-chain position on the aryl moiety. The investigation of aryl nucleus was also extended to benzofuran, which could be comparable to corresponding benzothiophene derivatives, and to dibenzofuran, which resembles a number of existing CaM-antagonists, e.g. phenothiazines which possess a tricyclic aromatic moiety. It was shown that dibenzofuran derivatives have reasonable potency from the results of biological testing. However, the poor solubility of these compounds stopped the further investigation of dibenzofuran derivatives.

Because both W-7 and J-8 are poorly soluble compounds, Bulpitt attempted to increase the solubility by changing the side chain. Initially, a polyether side chain was introduced into the 2-position (**Fig. 12**). Disappointingly, this resulted in a poorly active compound. However, both solubility and potency were increased with the introduction of sulfoxide group (**Fig. 12**).

Fig.12 Compounds with polyether side chain and sulfoxide side chain



At this stage, the laborious and difficult enzyme assay against phosphodiesterase used by O'Donnell's was replaced with a plant pathogen *Pythium ultimum* screen devised by Dr Buckley from Nottingham Trent University. It was shown that some of the compounds synthesized by Bulpitt possessed antifungal activity. In further anti-fungal testing in a broad-spectrum human pathogenic screen carried out by Zeneca Pharmaceuticals, compound **PCAB300** showed significantly activity. That it was a Cam antagonist was established by its inhibition of Cam-dependent myosin light chain kinase (MLCK), with an IC 50 value of 10μM. (**Table 3**) [126b].



PCAB 300

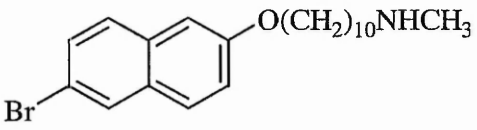
Table 3. Antifunal activity of PCAB 300 [127]

Pathogen	*Candida albicans*	*C. Abicans Azole resist.*	*Sacch. cerevisiae*	*Aspergillus fumigatus*	*Trichophyton quinkeanum*
MIC (μg/ml)	0.5-4	4	8	4	1

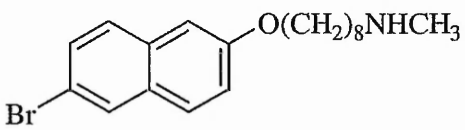
Professor Mac Nleil informed the group that one of tamoxifen's metabolites, N-desmethyltamoxifen is more potent than its parent compound in an invasion assay (**described in section 6.44**). A new research student, Allcock, synthesized a number of

analogues of PCAB 300, in which the pyrrolidine residue was replaced by a methylamino group.

All Allcock's [127] compounds were tested for anti-fungal activity by Astra Zeneca Pharmaceutical. It is found that a significant increase in potency was achieved by the substitution of the pyrrolidine moiety of **PCAB300** with methylamine (**Table 4**). The IC 50 of the best anti-fungal compound (**RWA1143**) is around 0.02μg/ml which is very close to a well-know fungicidal drug, amphotericin, especially against *Candida* species. Therefore, it could be concluded that the structural features required for optimal *in vitro* antifungal activity are a naphthalene ring substituted in the 6-position by bromine and in the two position by an alkyloxy side chain of eight to ten carbon atoms, possessing a terminal N-methyl substituent (**RWA1143** and **RWA9**).



RWA1143



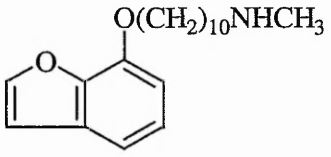
RWA 9

Table 4 IC50 (μg/ml) of Compounds against various pathogenic fungi

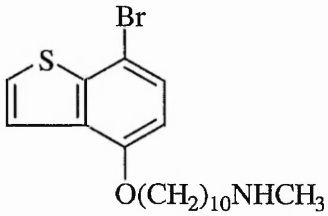
	Candida	*Cryptococcus neoformans*	*Sacch. cerevisiae*	*Aspergillus fumigatus*	*Trichophyton quinkeanum*
Amphotericin	0.06	0.06	0.06	0.06	0.06
RWA1143	0.06	0.13	0.06	0.25	0.13
RWA9	0.06	0.13	0.13	0.13	0.06

However, the really encouraging result was from further biological tests conducted *in vivo* in Sheffield. A number of compounds were tested against the melanoma cell line, A375-SM, in inhibiting invasion, attachment and proliferation (**Table 5**). The compounds tested in this melanoma assay are shown below. It was established that these antifungal compounds showed expected inhibitory activity, with IC50 values in the 5-10μM. Benzofuran derivative, **RWA2109**, which is not an outstanding anti-fungal reagent, showed good potency, especially in anti-invasion assay at nano-molar concentration. Compared to compounds W7 and J8, which were reported to possess anti-proliferation activity, the newly developed series of compounds presented equal or better potency.

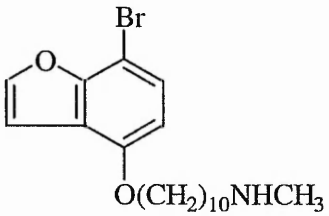
It was therefore determined to investigate the development of analogues of the benzofuran derivative to explore further their efficacy as anti-invasive agents. Methylamine was also adopted as terminal amine in the side chain due to the recent results obtained in Sheffield.



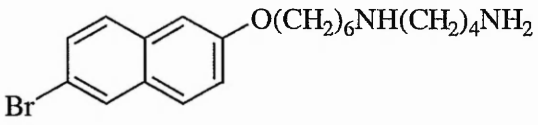
RWA245



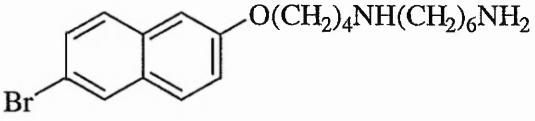
RWA2137



RWA2109



RWA205



RWA2165

Table 5. Comparison of potency of drugs in inhibiting invasion, attachment and proliferation of A375-SM melanoma cells

	Concentration required for 50% inhibition (μM) mean ± SEM (n)		
	Invasion	Attachment	Proliferation
RWA245	6.6 ± 3.3 (3)	7.3 ± 0.5 (2)	6.6 ± 6.6 (3)
RWA2137	5.9 ± 3.0 (3)	5.8 ± 0.5 (3)	8.9 ± 1.1 (3)
J8	0.2 ± 0.2 (3)	11.0 ± 2.0 (3)	> 20.0 (3)
RWA1143	0.06 ± 0.03 (4)	5.5 ± 1.2 (3)	7.2 ± 0.3 (2)
RWA2165	0.03 ± 0.03 (4)	2.4 ± 0.1 (2)	10.6 ± 4.7 (3)
RWA205	0.02 ± 0.01 (4)	2.7 ± 0.15 (2)	7.1 ± 0.4 (3)
RWA2109	0.0012 ± 0.002 (2)	4.0 ± 1.4 (2)	7.2 ± 0.57 (4)

Chemistry

Chapter 3

Preparation of Known and Novel Aryloxalkylamines

In the previous studies by Nottingham Trent University students P. J. O'Donnell [120], P. C. A. Bulpitt [126], and R. W. Allcock [127], it had been established that compounds of the general formula (**Fig. 1**) have calmodulin antagonist properties and also possess significant antifungal or anti-proliferative activity, although the link between calmodulin antagonist and the other properties may not be causal.

$$Ar—O—(CH_2)_m—X—(CH_2)_n—NR^1R^2$$

Fig. 1

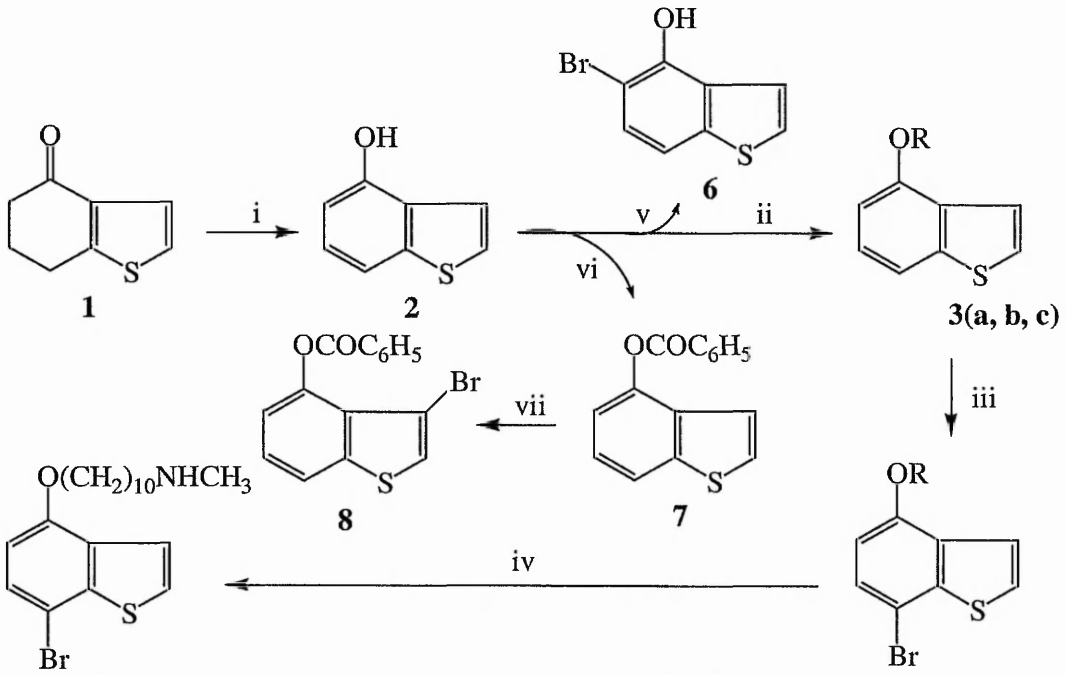
The striking finding that compound **RWA 2109** possesses inhibitory potency in an invasion assay at low nanomolar concentration stimulated further investigation of this series of compounds. To synthesize more analogues of **RWA 2109** and to test their activity against melanoma cell line were the major aims in this extended study. Moreover, it was intended to repeat and improve the synthesis of **RWA 2109**.

To carry out toxicity and *in vivo* efficacy animal studies, discussed later in **Chapter 6,** one gram quantities of each compound were required. Although several re-preparations of these compounds were straightforward, the synthesis of some compounds warrants some discussion because of the different route used in the synthesis.

3.1 Preparation of Compounds for Toxicity Studies

3.1.1 Preparation of 7-bromo-4-(10-methylaminodecyloxy)benzothiophene) (5)

This preparation of product **5** is straightforward and shown in **Scheme 1**. Although 4-substituted benzo[b]thiophenes were known as early as 1886 [128], the phenol **2** was unavailable until recent years [129, 130]. It is readily prepared from the commercially available 4-keto-4,5,6,7-tetrahydrathianaphthene (**1**) as shown in **Scheme 1** [129, 130].



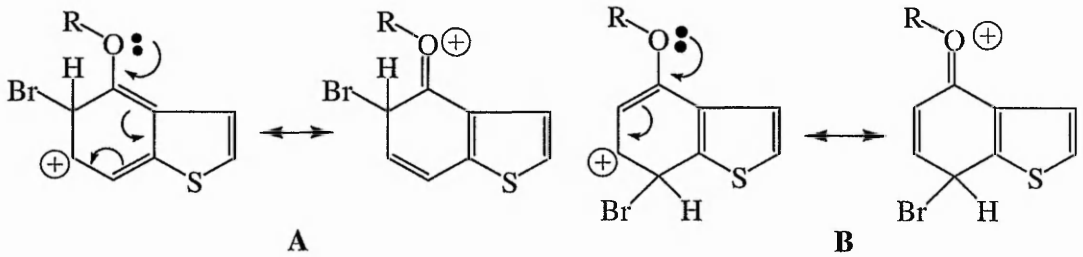
3a: R= CH_3; 3b: R= $(CH_2)_6Br$; 3c: R=$(CH_2)_{10}Br$
4a: R= CH_3; 4b: R= $(CH_2)_6Br$; 4c: R=$(CH_2)_{10}Br$

i. sulfur powder, diphenyl ether, 240°C; ii. $Br(CH_2)_{10}Br$, K_2CO_3, 2-butanone, 85°C; iii. NBS, CH_3CN, rt; iv. NH_2CH_3 (33% in ethanol), DMF, rt; v. NBS/$(PhCO_2)_2$, CCl_4; vi. PhCOCl, pyridine; vii. Br_2, CCl_4.

Scheme 1

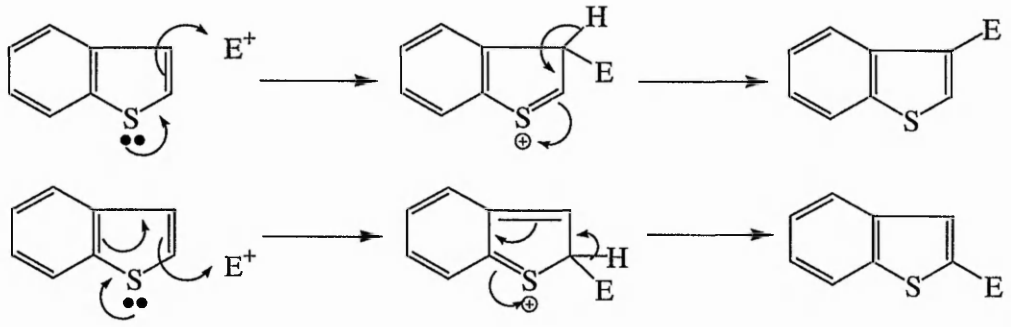
Substitution reactions of 4-substituted benzo[b]thiophene have been rarely reported compared to those of the 2, 3, 5, and 6 substituted isomers. However, in an intensive investigation of psilocin analogues carried out by Campaign and co-workers, this type of reaction, including bromination was studied in great detail [131].

It was established that reaction of **2** with a combination of NBS/ benzoyl peroxide rather unexpectedly gave a single product, the 5-bromo-4-hydroxybenzothiophene (**6**). Treatment of **2** with bromine in carbon tetrachloride resulted in a complex mixture, but the methyl ether **3a** of free phenol **2** was transformed cleanly to the 7-bromo derivatives (**4a**) under the same reaction conditions. Bulpitt claimed a similar reaction was successful, in which **4b** was obtained from bromination of the more complex ether (**3b**) in carbon tetrachloride. Allcock, however reported that the related ether **3c** in carbon tetrachloride treated with bromine gave a mixture of products, not surprising in a reaction probably involving radical intermediates of similar energies. The 7-bromo ether (**4c**) was best obtained by reaction of **3c** with NBS in the polar solvent acetonitrile [131]. The possible reason of this achievement is shown in **Mechanism 1**. The donation of lone pair electrons from oxygen stabilises the σ-complex resulting from electrophilic attack on the benzene ring which lead to two possible products. However, the steric hindrance from the alkyl residue on oxygen could decrease the 5-position substitution effectively.



Mechanism 1

An added complication is that the alkoxy or hydroxyl-substituted benzene ring and the thiophene ring are both electron rich. Introduction of an electron-withdrawing substituent (COC_6H_5), by ester formation renders the benzene ring less prone to electrophilic attack (**step vi, vii**). Thus bromination of the benzoate ester **7** resulted in attack at the 3-position to give compound **8**. The preference for the 3-position is that the reaction simply involved the rather isolated π-system in the five-membered ring, and does not disturb the aromaticity of the benzene ring. Although the positive charge in the intermediate is delocalized round the benzene ring, it gets its main stabilization from the sulfer atom (**Mechanism 2**).



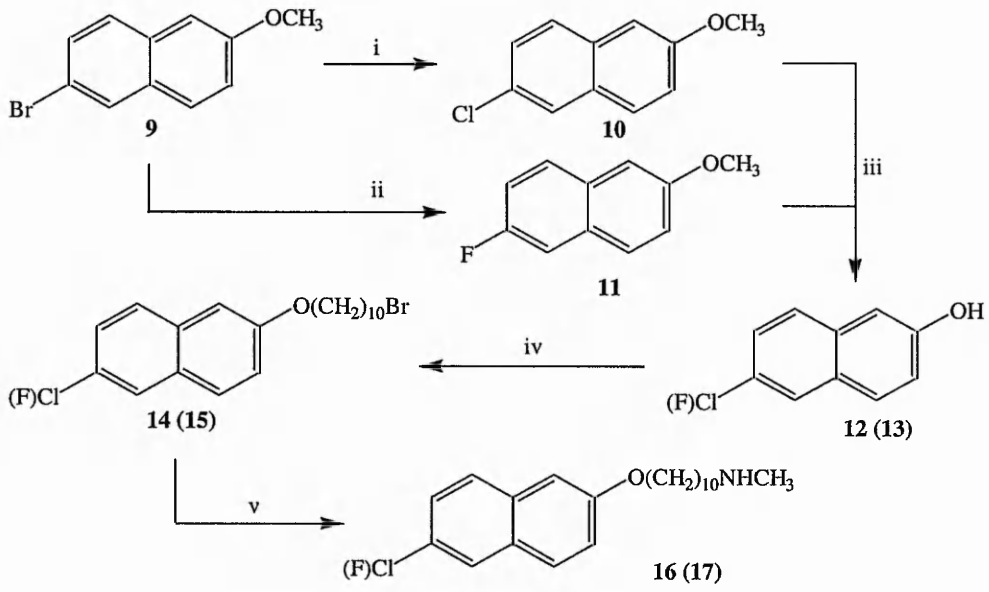
Mechanism 2

3.1.2 Preparation of Chloro- (16) or Fluoro (17)-Substituted Analogues of RWA 1143

These compounds had been prepared in small quantity by Allcock from commercially available 6-bromo-2-methoxynaphthalene **9**. Generally, the preparation (**Scheme. 2**) of these two compounds is the same, apart from the first stage of chlorination or fluorination. The preparation of chloro-compound *via* the formation of nucleophile with

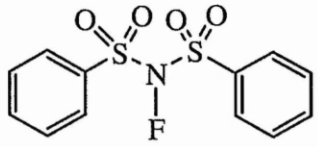
n-butyllithium followed by the addition of electrophile, hexachloroethane [132] as shown in **Scheme 2** gave a good yield of pure **10** which was then converted to the amine **16** by standard routes.

However it was more of struggle to prepare the product with fluorine as substituent. The reaction of an electrophilic reagent, *N*-fluorobenzenesulfonimide [133] (**18**), with an appropriate organo-metallic intermediate was explored. The original route [127] in which the bromonaphthalene was transformed to the corresponding freshly made Grignard reagent and reacted with electrophilic reagent, **18,** at -30°C (acetonitrile/ dry ice) gave a mixture of fluoro and bromo compounds, as shown by GC/ MS analysis. The method was abandoned due to the extreme difficulty separating this mixture. Therefore the preparation route was improved by choosing a different organo-metallic intermediate, the 6-lithio derivative. This derivative may be formed by halogen-metal exchange, using n-butyllithium [134].



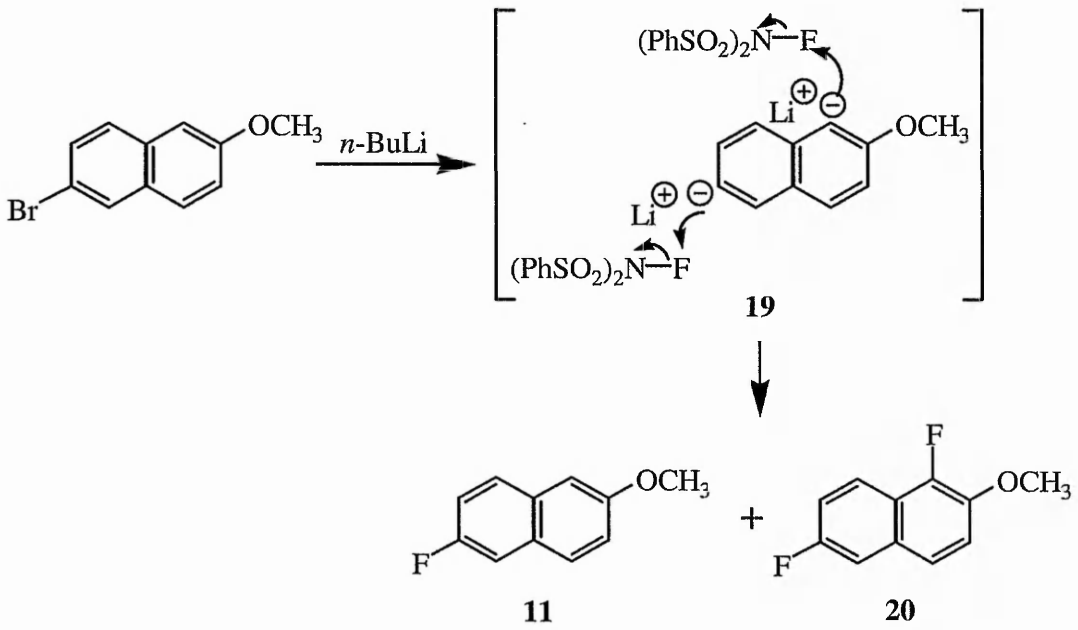
i. *n*-BuLi, Cl_3C-CCl_3, THF (-78°C); ii. *n*-BuLi, $(PhSO_2)_2NF$, THF (-78°C); iii. BBr_3, DCM (-78°C); iv. $Br(CH_2)_{10}Br$, K_2CO_3, 2-butanone; v. CH_3NH_2, DMF (rt).

Scheme 2



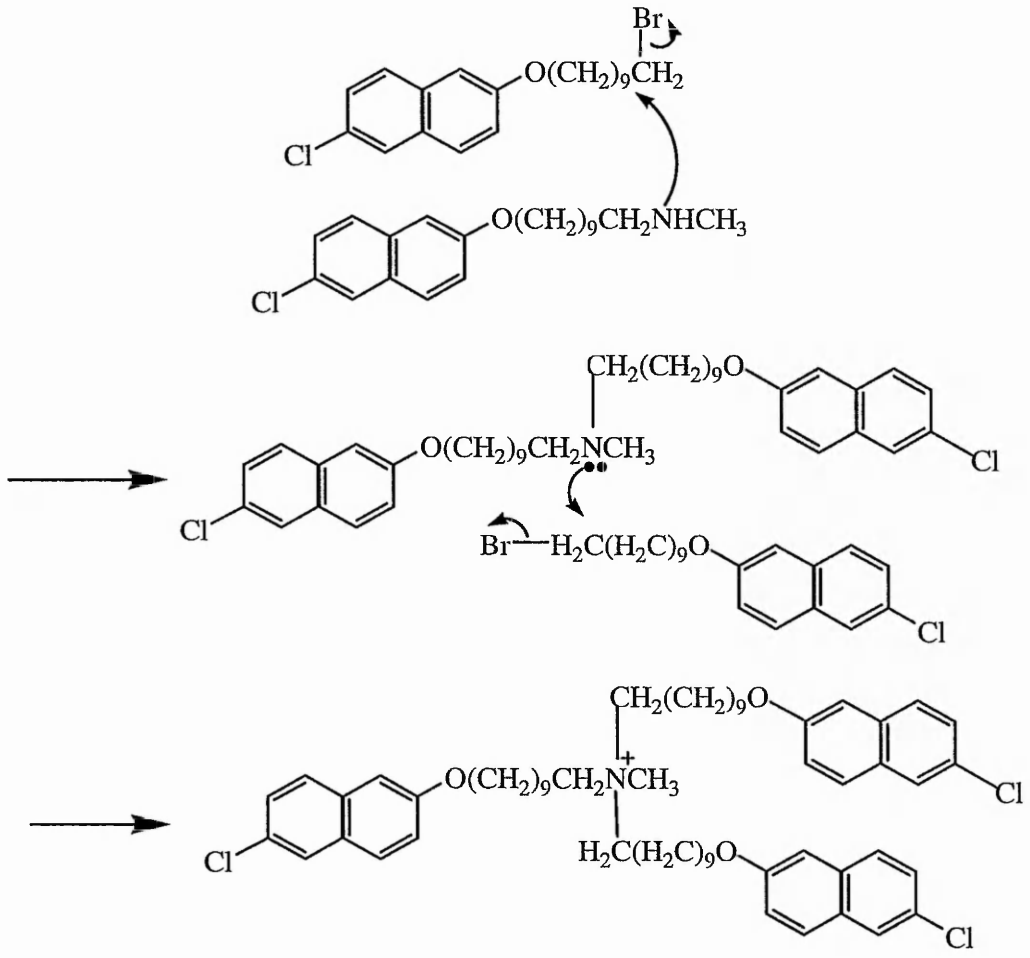
18

There is the possibility of a competing reaction, an "ortho-lithiation" [134], in which a proton α to a co-ordinating species can be removed to give, in this case the 1-lithio intermediate. Indeed, when the starting material **9** was treated with n-butyllithium at -45°C, followed by the addition of *N*-fluorosulfonimide, the product obtained was shown by GC/ MS examination to consist of a mixture of mono- and difluoro ethers. Repeating the reaction at -78°C gave a single monofluoro ether **11**, free of bromine.



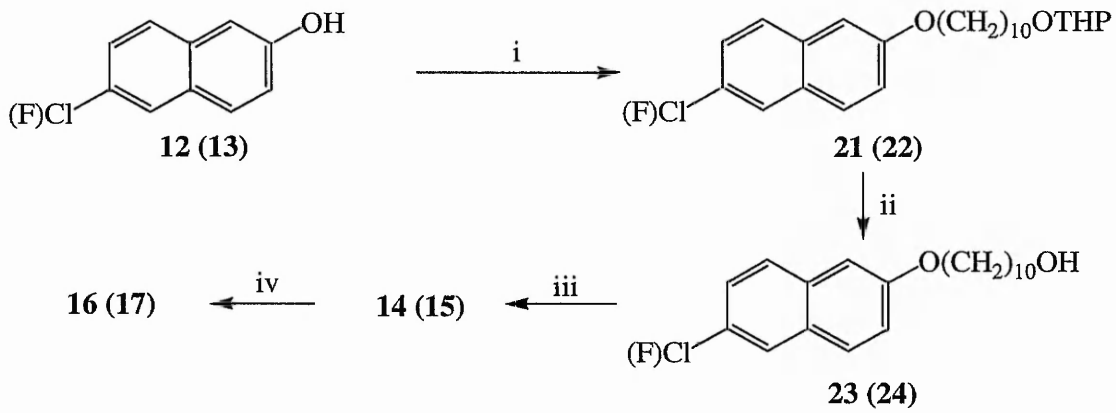
Scheme 3

An unexpected problem was encountered in the final stage, **v,** in **Scheme 2**. A polymer-like product formed instead of the expected oily compound in the removal of solvent, DMF, in a 60-80°C water bath with the "cold-finger". The possible reason for this side reaction is that methylamine is a very good nucleophile. In liquid phase, for example, the intermediate **14** can be attacked by another nucleophile due to the lone pair electrons on **N** in methylamine residue of final product **16**, existing in the reaction. This procedure might be able to repeat in the reaction system, as shown in **Scheme 4**. Conditions were established to minimize this side reaction, giving a satisfactory result.



Scheme 4

These two compounds were also prepared via another modified route. The final compounds **16**& **17** were prepared with a bifunctional reagent, $Br(CH_2)_{10}OTHP$ (**Scheme 5**) which would be discussed in **section 8**. The advantage of this method is that the formation of the diether, in step **iv** of **Scheme 2,** could be easily avoided.

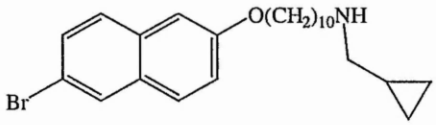


i. $Br(CH_2)_{10}OTHP$, K_2CO_3, 2-butanone; ii. PPTS, EtOH; iii. CBr_4, Ph_3P; NH_2CH_3 (33% in ethanol), DMF.

Scheme 5

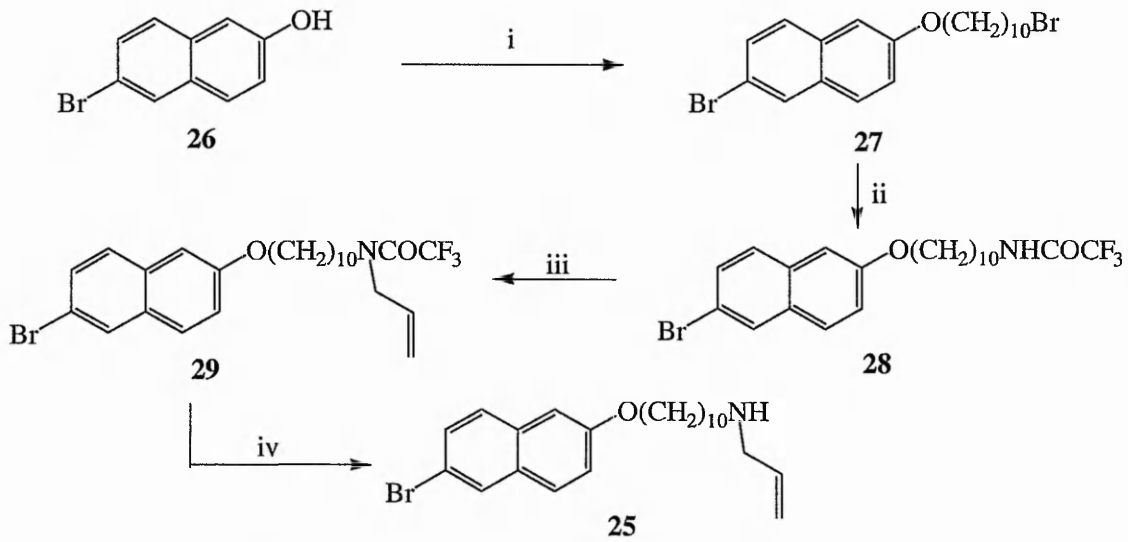
3.2 Preparation of an Allylamine Compounds (25)

The replacement of the N-methyl group in the most potent anti-fungal compound **RWA 1143** by a N-cyclopropylmethyl gave a compound, **RWA 265** which showed strong potency in the test of against *A. fumigatus*, but with much less activity against *Candida* species [127]. This indicated that this series of compound had a more subtle mode of antifungal action, instead of being merely detergent ambiphiles. It also suggested that another compound, 6-bromo-2-(10-allylaminodecyloxy) naphthalene (**25**), might share the same/similar antifungal activity due to the biosteric exchange of allyl for cyclopropyl methyl group, and this was an early target molecule.



RWA 265

However the synthesis of this compound presented unexpected difficulty via standard synthesis. A polymerisation phenomenon was observed because of the existence of allylamine in the reaction system. A new route, **Scheme 6,** was developed to avoid this polymerization. After the conversion of phenol **26** to ether **27**, compound **27** was treated with trifluoroacetamide instead of the direct introduction of allylamine to give the *N*-alkyltrifluoroacetamide **28** [135a, b]. Then the alkylation of **28** with allyl bromide gave a key intermediate **29** which could be hydrolysed in basic solution to give the final compound **25**.



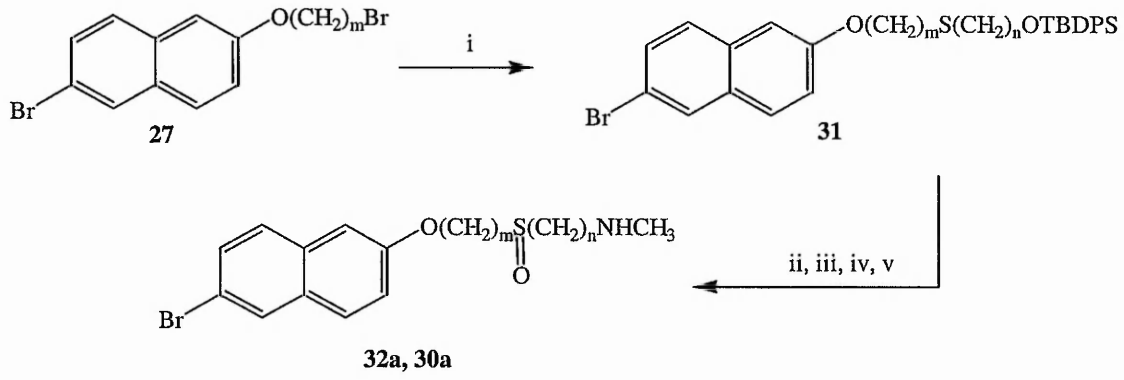
i. $Br(CH_2)_{10}Br$, K_2CO_3, 2-butanone, 80°C; ii. CF_3CONH_2, $[CH_3(CH_2)_3]_4NBr$, K_2CO_3, DMF; iii. $BrCH_2CH{=}CH_2$, K_2CO_3, CH_3CN; iv. OH^-, H_2O

Scheme 6

3.3 Preparation of a Sulfoxide Compound (30a)

From previous research at The Nottingham Trent University, it had been established that introduction of a sulfoxide linkage into the side chain increases solubility in aqueous media to a marked degree [126]. Bulpitt had made compound **32b** (m=6, n=3) by the route shown in **Scheme 8**, while Allcock had synthesized **32a** (m=6, n=6) by **Scheme 7**. It was envisaged that similar side chain would be introduced into benzofurans and indoles, and since they are less available than naphthalenes, it was decided to gain experience in their synthesis.

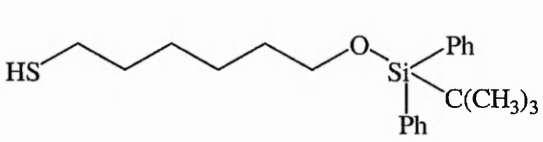
Initially, compound **30a** (m=10, n=6) was chosen as the target, using the Allcock approach (**Scheme 7**). However it proved impossible to repeat the reported clean formation of the O-silylated thiol **33a**, from a very expensive precursor, 6-mercaptohexanol.



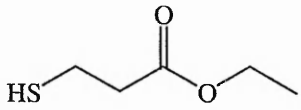
32a: m=6, n=6; **30a**: m=10, n=3.

i. $Na_2S_2O_5$, K_2CO_3, 2-butanone, $TBDPSO(CH_2)_6SH$; ii. *m*-CPBA, DCM; iii. TBAF, DCM; iv. CH_3SO_2Cl, TEA; v NH_2CH_3 (33% in ethanol), DMF.

Scheme 7

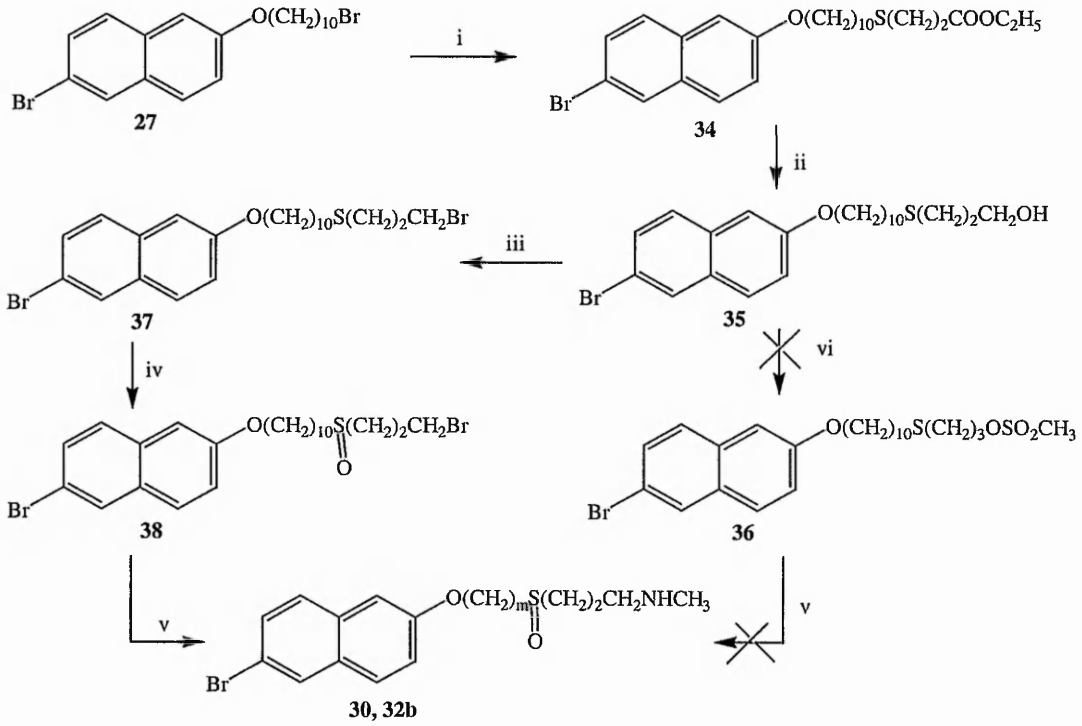


33a



33b

Attention turned to the Bulpitt route, and the preparation of compound **30** (m=10, n= 3) was attempted (**Scheme 8**). This sulfoxide **30**, with the dihedral at sulfur has approximately the same chain length as the potent anti-fungal **RWA1143** (page 35).

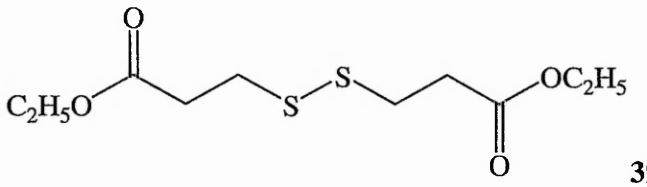


30: m= 10, n=3; 32b: m= 6, n=3

i. $HS(CH_2)_2COOCH_2CH_3$, K_2CO_3, $Na_2S_2O_5$, 2-butanone (80°C); ii. $NaBH_4$, EtOH, rt; iii. CBr_4, EtOH, Ph_3P, 0°C; iv. *m*-chloroperoxybenzoic acid, DCM (-10°C); v. NH_2CH_3 (33% in EtOH), DMF; vi. CH_3SO_2Cl, TEA.

Scheme 8

Thus intermediate **27** was reacted with thiol (**33b**) to give thioether **34**. Exclusion of oxygen and addition of dithionite both considerably reduced the formation of disulfide by-product (**39**). The ester **34** was subsequently smoothly reduced to the corresponding alcohol **35** using sodium borohydride. Attempts to convert this alcohol to compound 36 containing mesylate group (**36**) prior to reaction with methylamine were unsuccessful. Therefore the key intermediate **35** was treated with the combination reagent of carbon tetrabromide and triphenylphosphine to afford the bromide **37.** This was oxidized in high yield to the sulfoxide **38** which was then converted to the aminosulfoxide **30**.



39

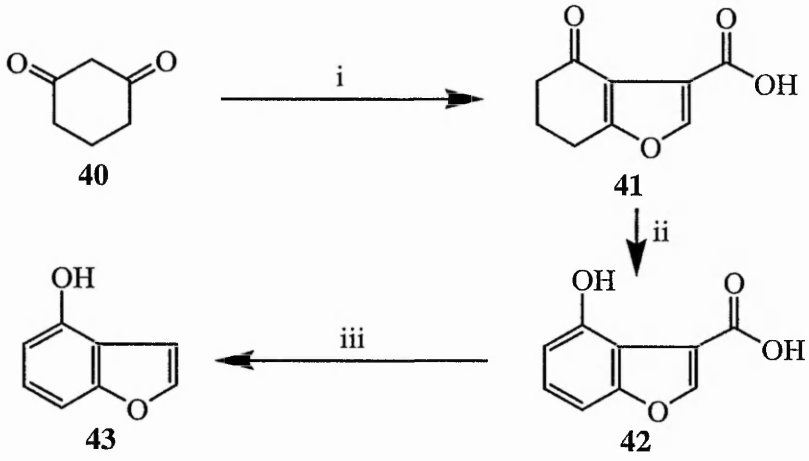
Time did not allow the synthesis of heterocyclic analogues of this compound **30**, but it is proved to be a potent anti-proliferation agent as discussed in the **Biology Section**.

3.4 Re-preparation of RWA 2109 (49a)

Although **RWA 2109** did not show outstanding activity in the anti-fungal assays, it is extremely potent compared to other RWA compounds in the anti-invasive assay (discussed on P38), which made it very important for a larger quantity to be re-prepared in order to carry out further research. Moreover, the structure of **RWA 2109** was not absolutely established, due to an ambiguity in the bromination step of the original synthesis.

3.4.1 Routes to Re-prepare Karanjol (43)

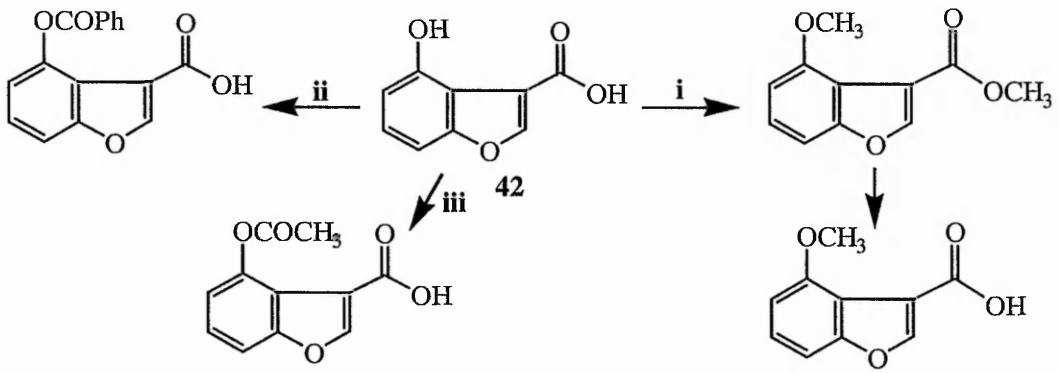
At the time of the investigation, there had been two routes reported for the synthesis of karanjol, 4-hydroxybenzofuran **43**. The first starting with the very expensive 2-hydroxy-6-methoxybenzaldehyde gives the target compound in low overall yield [136]. The second route [137] to prepare **45**, which had been used once by Allcock is shown in **Scheme 9**. Condensation of bromopyruvic acid ($BrCH_2COCOOH$) with 1, 3-cyclohexanedione **40** gives acid **41** in good yield. The following step in which **41** is aromatized to benzofuran **42** by reaction of palladium on activated carbon, has proved difficult to reproduce. It was found that it was necessary to dry both the solvent decalin and the hydrogen transfer agent dodecene scrupulously. The source of the catalyst, palladium on activated carbon, was also crucial.



i. aqueous KOH, $BrCH_2COCOOH$, MeOH, 0°C, 2h; H^+, 100°C, 2h; ii. Pd/C, dodecene, decalin; iii. copper, quinoline.

Scheme 9

The final stage, decarboxylation, carried out by heating with copper catalyst and quinoline was also troublesome. Varying the reaction temperature, time and catalyst all failed to improve the poor yield. It was suspected that the free phenol group in product **42** may be sensitive to the copper during the high temperature reaction, and attempts were made to protect the hydroxyl group (**Scheme 10**) by methylation **i**, or esterification **ii&iii**, prior to decarboxylation.

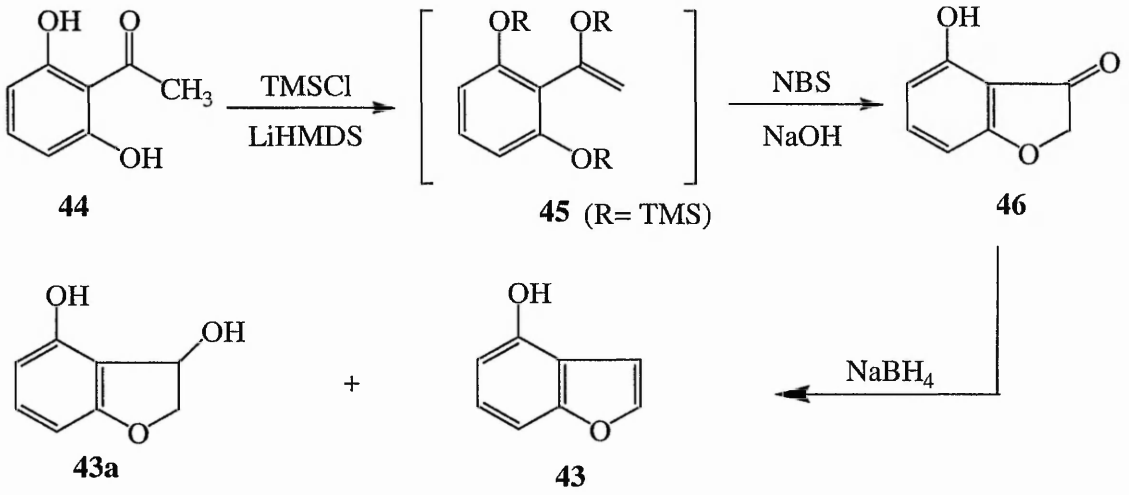


i. CH_3I, K_2CO_3; ii. PhCOCl; iii. $(CH_3CO)_2O$

Scheme 10

However, the protection routes investigated failed to be satisfactory apart from method **iii**. Meanwhile, it had been noticed that the decarboxylation can be carried out straightforwardly from compound **42** provided that atmospheric oxygen is rigorously excluded. In this stage, the reaction was required to be degassed by pump under nitrogen. The product could be purified by Kugelrohr distillation to give a pale yellow low melting solid in reasonable yield.

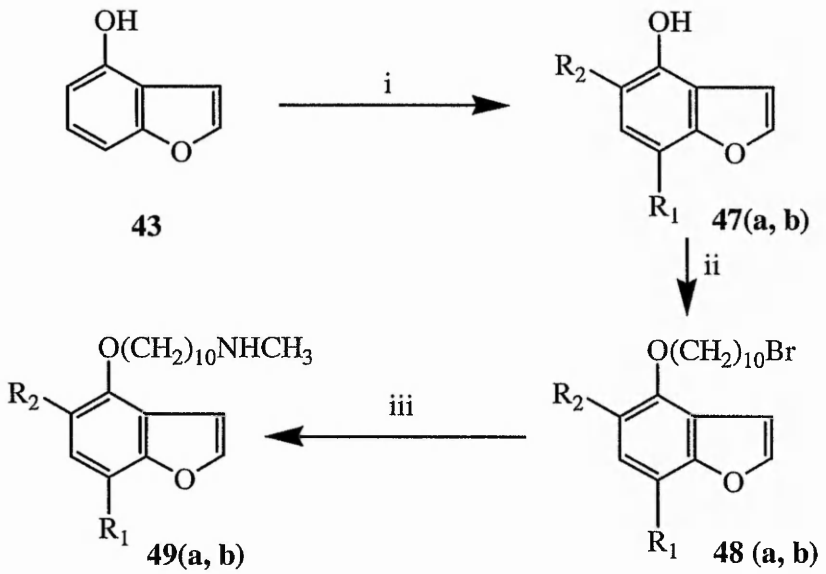
A new route (**Scheme 11**) to prepare karanjol **43** has been accidentally developed in a very recent investigation [138]. The key intermediate **46** was obtained (**Scheme 11**) from 2,6-dihydroxyacetophenone **44** by persilylation, followed by treatment with NBS/ NaOH. Then the intermediate was reduced with $NaBH_4$ to afford a mixture of **43a** and **43** with the latter as the major product.



Scheme 11

3.4.3 Bromination of Karanjol

This reaction has not been described in the literature. In the original preparation by Allcock, treatment of karanjol **43** with *N*-bromosuccinimide (NBS) [139] gave a complex mixture, which by careful chromatography yielded a mono-brominated product, which from its NMR spectra was assigned the structure 7-bromo-4-hydroxybenzofuran **47a** or 5-bromo-4-hydroxybenzofuran **47b**. This ambiguous compound was then converted to **49(a or b)** by standard methods (**Scheme 12**).



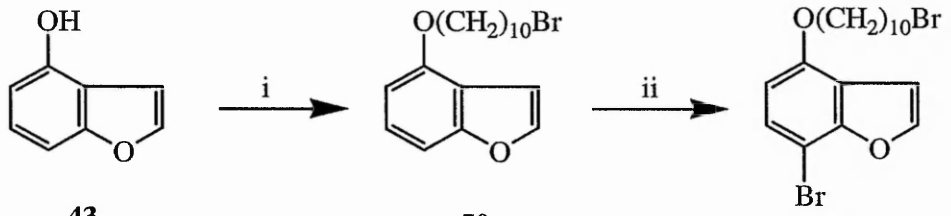
47a, 48a, 49a: R_1=Br, R_2= H; **47b, 48b, 49b**: R_1= H, R_2= Br;

i. NBS, dry CH_3CN; ii. $Br(CH_2)_{10}Br$, K_2CO_3, 2-butanone; iii. CH_3NH_2 (33% in EtOH)

Scheme 12

It was hoped that a cleaner product would result from the NBS bromination of the 10-bromodecyl derivative **50**. Steric hindrance might also guide the bromine into the seven position instead of five. However, a mixture of products was again obtained. Use of the bulky brominating agents pyridinium hydrobromide perbromide [140], and 2,4,4,6-tetrabromocyclohexa-2, 5-dienone (**51**) [141] was then applied respectively. The former reagent failed to react, but the dienone **51** appears to give a clean product **48a** (**Scheme 13**). The NMR spectra of this compound, however, do not appear to coincide with those reported by Allcock. The product obtained at stage **ii** in **Scheme 13** was presumed to be **48a** due to the steric hindrance from long side chain in intermediate **50** and the bulky brominating reagent. This implies that the compound obtained by Allcock from the

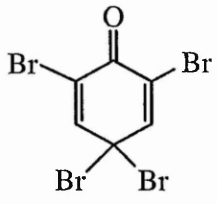
reaction of his bromokaranjol with 1, 10-dibromodecane must have structure **48b**, and thus compound **RWA2109** should now be formulated as **49b**.



43 **50** **48a**

i. $Br(CH_2)_{10}Br$, 2-butanone (85°C); ii. dienone, DCM (-70°C).

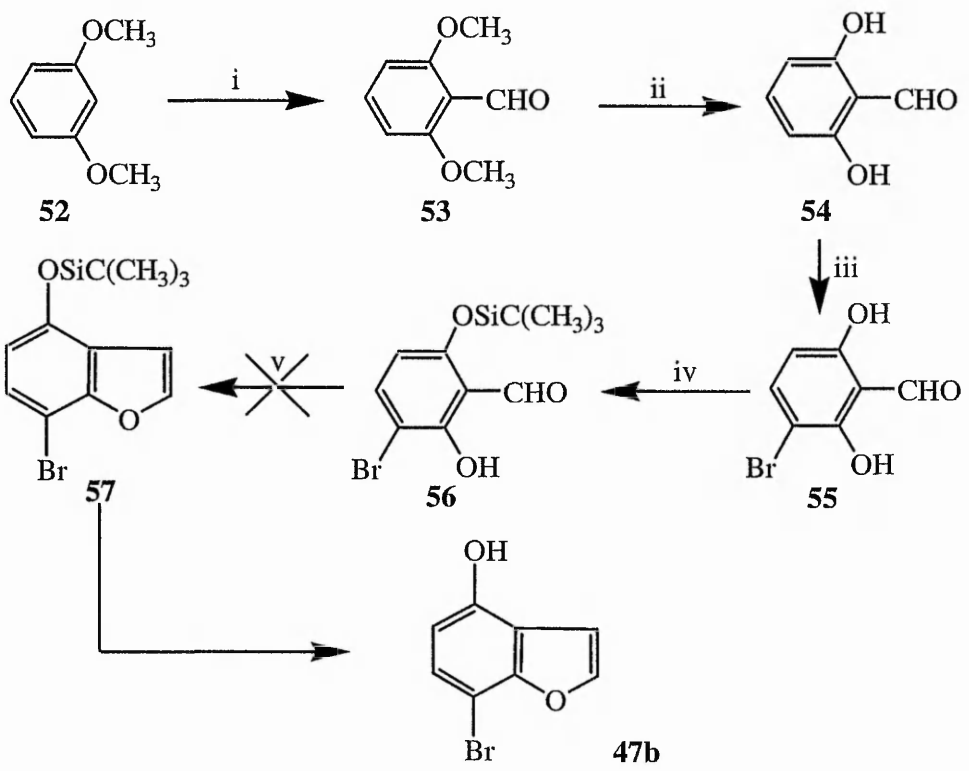
Scheme 13



51

Attempts to grow crystals of ambiguous compound **48b** or its derived methylamine hydrochloride, suitable for X-ray determination of structure, have so far been fruitless.

New synthesis routes were explored in an attempt to determine the bromination position. An alternative, unambiguous synthesis of 7-bromokaranjol (**47b**) is shown in **Scheme 14**. The formation of 2, 6-dihydroxybenzaldehyde **54** and the bromination with dienone afforded only small quantities of the novel bromo-compound **55**. Treatment of **55** with $(CH_3)_3CSiCl$ gave crude silyl ether **56**, which decomposed on a silica chromatography. The synthesis was abandoned due to the dangerous reagents and low yield.

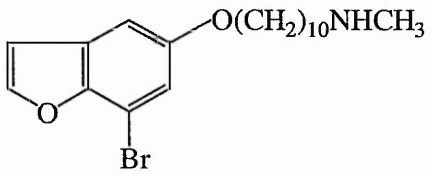


i. phenyllithium, *N*-methylformanilide; ii. $AlBr_3$, CS_2; iii. dienone, -20°C; iv. *t*-butylchlorosilane; v. $ClCH_2COOEt$.

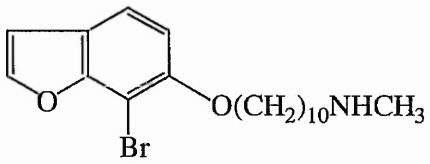
Scheme 14

3.5 Preparation of RWA2109 Analogues

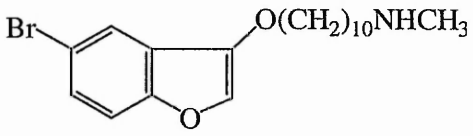
In treating **RWA 2109** as the lead compound, it is obvious that the importance of the relative positions of the bromine and side chain on the benzofuran nucleus should be established. The synthesis of the analogues **58**, **59** and **60** were therefore investigated.



58



59



60

3.5.1 Preparation of [10-(7-bromobenzofuran-6-yloxy)decyl]methylamine (58)

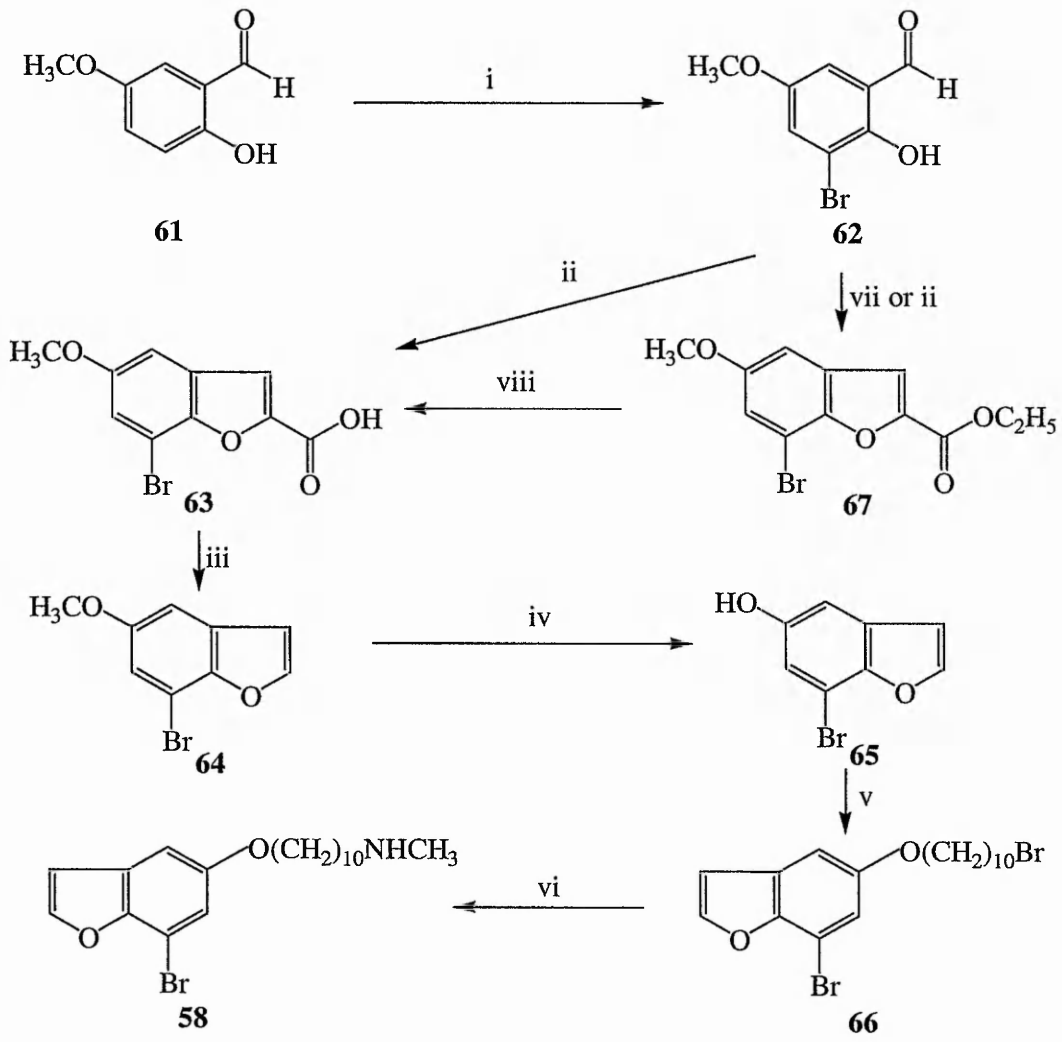
The preparation of **58** has proved to be straightforward and is shown in **Scheme 15**. Bromination (**step i**) was easily carried out by dropwise addition of bromine in glacial acetic acid, following Rubenstein method [142], improved by Swenton [143], to give the 2-hydroxy-3-bromo-5-methoxybenzaldehyde **62** in good yield.

The preparation of 5-methoxy-7-bromobenzofuran-2-carboxylic acid **63** was carried out using the method developed by Rene and Royer [136]. Condensation of benzaldehyde **62** with ethyl chloroacetate under reflux conditions gave a mixture of acid **63** and a small amount of neutral compound **64** which could readily be separated. The ethyl ester **67** of acid **63** (**Scheme 15**) can also be found in this step sometimes, depending on the reaction time. Proton, carbon 13 NMR and IR spectra confirmed that **63** was the expected acid. However, the melting point (227-230°C) does not match that reported for acid **63** (210°C) in an earlier paper [144a]. In this publication, the acid was obtained from ester which was prepared by the treatment of **62** with $BrCH(COOC_2H_5)_2$ (**step vii**), which

has subsequently been reported [144b] to give good yield of 5-bromobenzofuran. Attempting to repeat this gave no products with the expected spectral properties. However the ethyl ester **67** from the Royer and Rene route [136] had a melting point of 103-105°C, which is the same as that reported by the other group [144]. Again the product obtained from saponification of ester **67,** with expected spectral properties, had a melting point of 238-240°C.

The following step, in which decarboxylation of acid **63** was carried out with copper powder and quinoline, was fast and easy to give 5-methoxy-7-bromobenzofuran **64** in a reasonable yield, provided that atmospheric oxygen is excluded. The product can be combined with the neutral compound **64** obtained in the cyclization process (**step ii**).

In the original work [126], demethylation to give free phenol **65** is accomplished with pyridine hydrochloride. It has now been established that this reaction can be cleanly effected with boron tribromide (BBr_3) in dry dichloromethane at low temperature in good yield [145]. After the alkylation of **65**, intermediate **66** could be easily led to the final product amine **58** with the standard synthetic route.



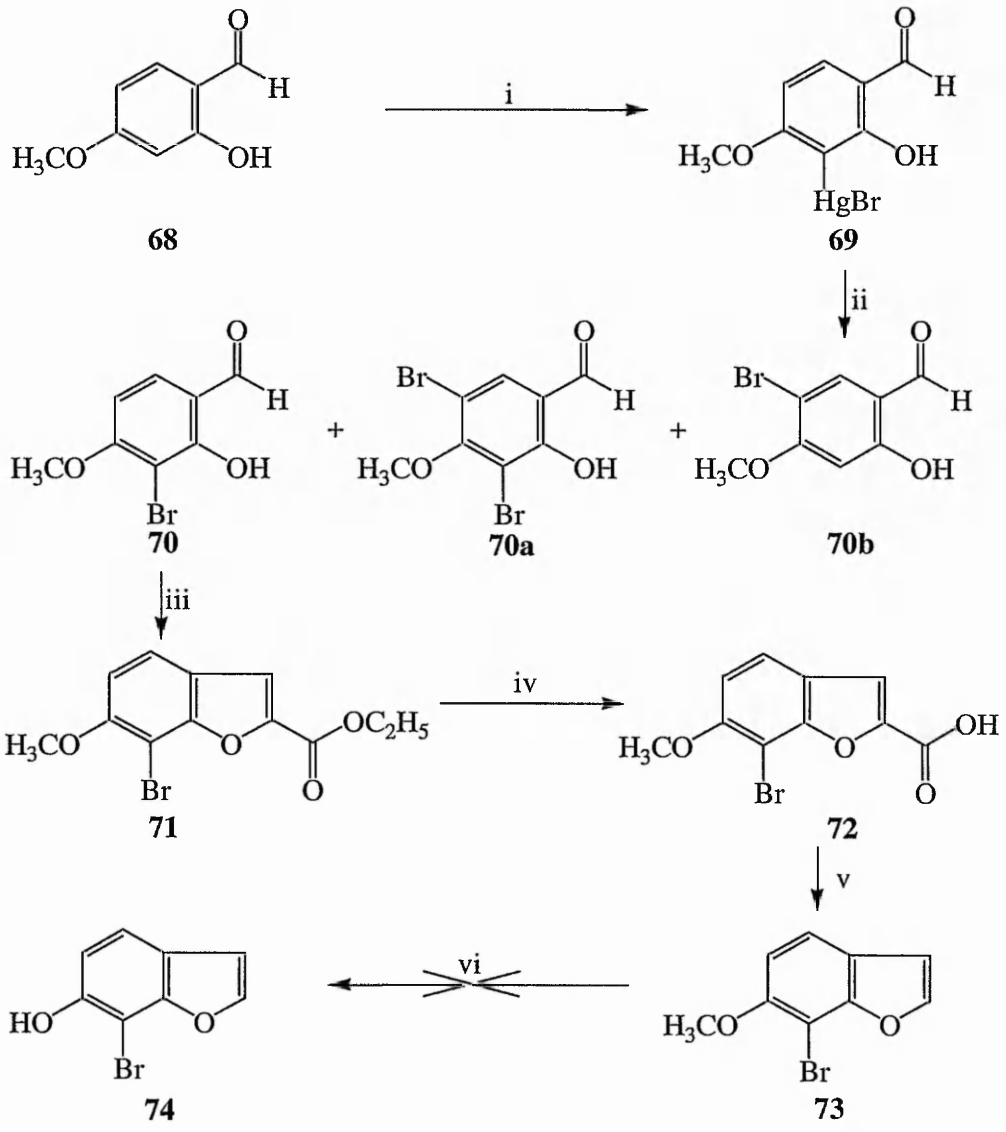
i. Br_2, CH_3COONa, CH_3COOH; ii. $CH_2ClCOOC_2H_5$, K_2CO_3, DMF (160°c); iii. Cu, quinoline (220°c); iv. BBr_3,. DCM (-78°c); v. $Br(CH_2)_{10}Br$, K_2CO_3, 2-butanone; vi. CH_3NH_2 (33% in EtOH), DMF; vii. $BrCH(COOC_2H_5)_2$, K_2CO_3, DMF; viii. OH^-, EtOH, H^+.

Scheme 15

3.5.2 Preparation of [10-(7-bromobenzofuran-6-yloxy)decyl]methylamine HCl (59)

The synthesis of another isomeric compound **59** (**Scheme 16**) by a related route required the preparation of the aldehyde **70**, which is different from that obtained by direct bromination in the synthesis of compound **62** in **Scheme 15**. The hydroxyl group on position 2 of starting material can offer co-ordination to a Hg^{++} salt, and therefore, can be expected to direct the electrophilic reagent, mercury (II) acetate, to the *ortho* position, giving an organo-mercury intermediate which can undergo *ipso* bromination [146, 147]. However, clean preparation of **70** proved difficult, as a considerable amount of dibromo by-product **70a** and small quantities of 5-bromo by-product **70b** were always produced in this stage. Careful flash chromatography allowed the isolation of required compound **70** from the other two by-products, although not in good yield. The following compound **71** could also be prepared by Rene and Royer cyclization [136] with ethyl chloroacetate. After saponification and decarboxylation via standard route, compound **73** was produced in a reasonable yield as greenish oil, which turned to solid at room temperature. However, demethylation of **73** with boron tribromide (BBr_3) [145], which successfully removed the methyl group in most similar reactions, failed to produce a key intermediate **74**. The reason is not very clear, but it is possibly because the demethylation reagent BBr_3, possessing three bromines, is a big molecule which is not able to coordinate to the 6-methoxy next to the 7-bromo substituent.

Another reagent HBr (45%) in acetic acid, widely used in demethylation in many cases, was also applied in this stage [148]. Unfortunately, the starting material was decomposed in this hot strongly acidic solution. Thus the route starting with 4-methoxy-2-hydroxybenzaldehyde **68** was abandoned.

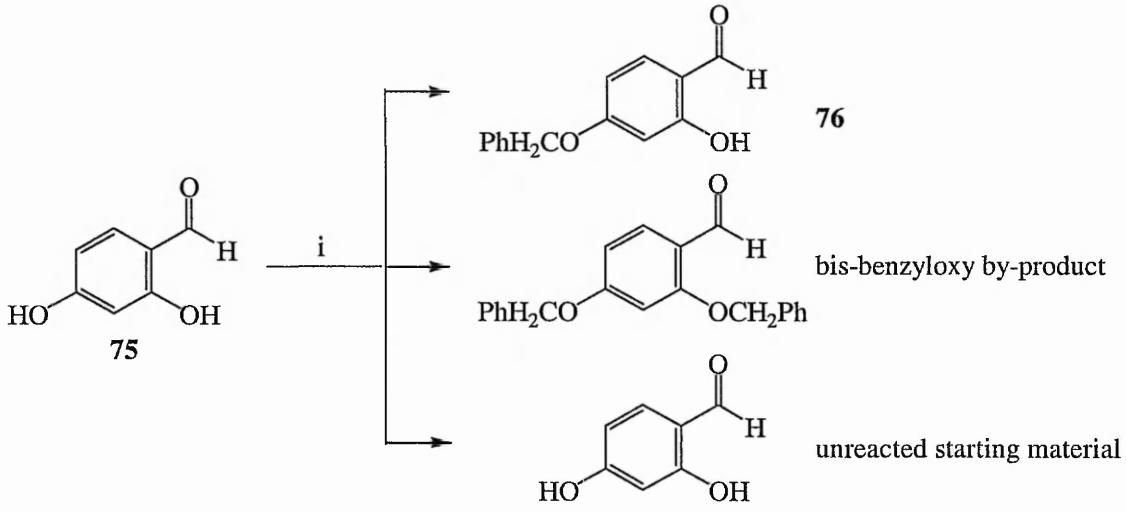


i. $Hg(OAc)_2$, ethanol, NaBr, CH_3COOH; ii. bromine, CH_3COOH, DCM; iii. $CH_2ClCOOC_2H_5$, K_2CO_3, DMF; iv. NaOH, ethanol; v. copper, quinoline; vi. BBr_3, DCM.

Scheme 16

The novel compound **77**, 4-benzyloxy-3-bromo-2-hydroxybenzaldehyde appeared to be a promising alternative. Its synthesis is shown in **Scheme 18**. Although the

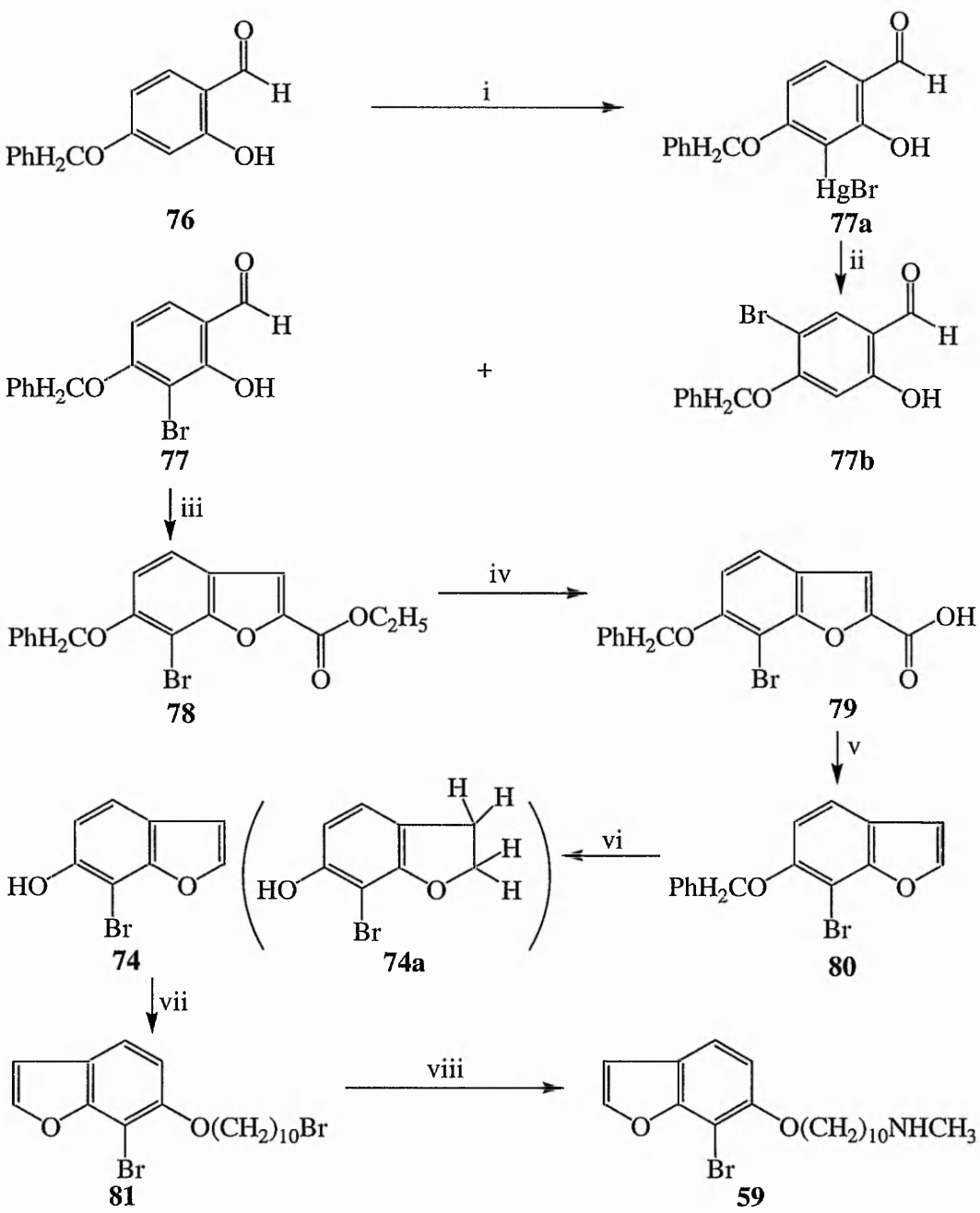
benzyloxysalicaldehyde **76** is commercially available, it is expensive. It was prepared in good yield from 2, 4-dihydroxybenzaldehyde **75** and benzyl chloride in the presence of KF and acetonitrile [149] (**Scheme 17**), and was separated from starting material and bis-benzyloxy by-product by fractional crystallisation, using *t*-butyl methyl ether.



i. benzyl chloride, acetonitrile, KF, reflux.

Scheme 17

The subsequent synthetic steps are shown in **Scheme 18**. The *ipso* bromination of the sterically crowded mercury (II) acetate **77a**, potentially the most problematic step, proved to be remarkably simple, with only a trace of the isomeric bromo-compound **77b** produced, and no evidence of dibromo by-product according to analysis by GC/ MS. Desired product **77** could be separated chromatographically from **77b**, and had two characteristic doublets at δ7.43 and δ6.67 in the proton NMR spectrum.



i. $Hg(OAc)_2$, ethanol, NaBr, CH_3COOH; ii. bromine, CH_3COOH, DCM; iii. $CH_2ClCOOC_2H_5$, K_2CO_3, DMF; iv. NaOH, ethanol, H^+; v. copper, quinoline; vi. Pd/C, H_2; vii. $Br(CH_2)_{10}Br$, K_2CO_3; viii. CH_3NH_2 (33% in ethanol), DMF.

Scheme 18

The following steps, Rene and Royer cyclization (**iii**), saponification (**iv**), decarboxylation (**v**), gave a key intermediate, 7-bromo-6-benzyloxybenzofuran **80**. To remove the benzyl group, hydrogenation with palladium (10%) on carbon was chosen. However, after work-up and chromatographic purification, NMR showed the expected product **74** also contained a by-product **74a**, a dihydrobenzofuran, whose two triplets from 2, 3-H appeared at high field, suggesting that hydrogenation could not only remove benzyl group, but also reduce the furan ring. Without further purification, this mixture was carried forward to the next stage, alkylation, by treatment with 1, 10-dibromodecane to prepare product **81** which could be converted to the final product **59** with the standard method. At stage **vii**, both NMR and MS showed no evidence of the dihydrobenzofuran derivative. It is possibly because that the dihydrobenzofuran derivative was transformed to the aromatic benzofuran during heating.

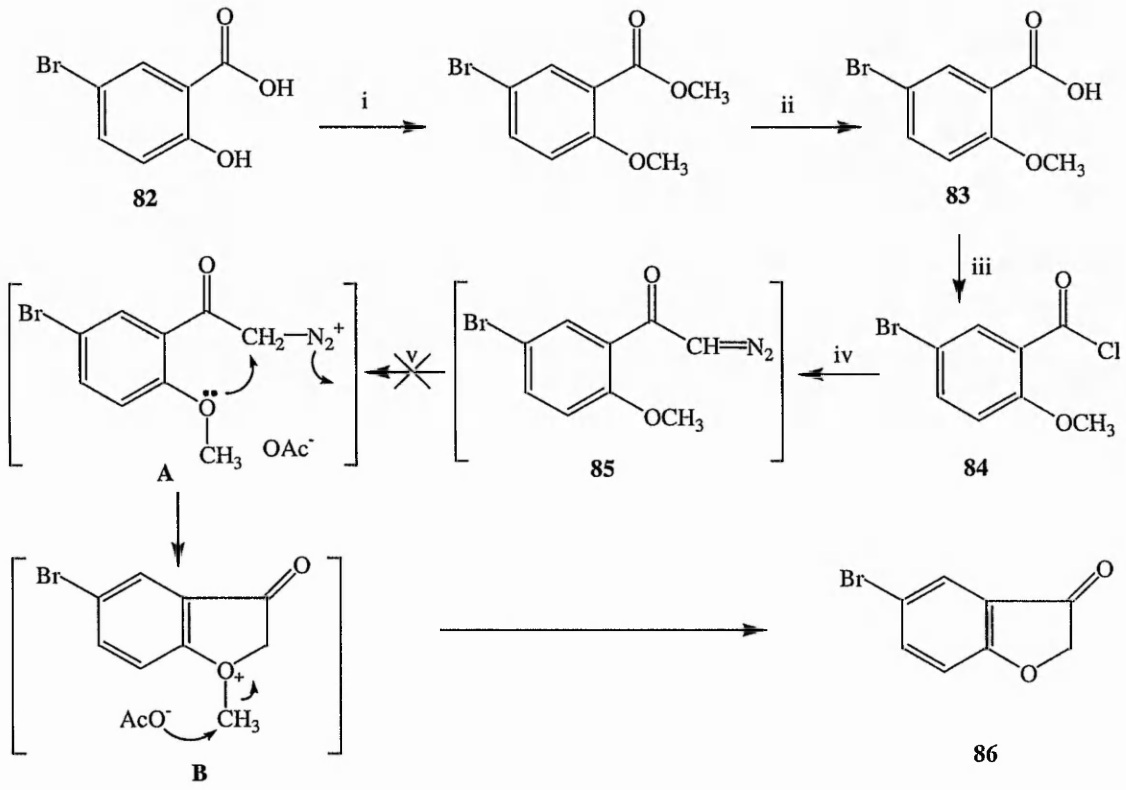
3.5.3 Preparation of [10-(5-bromobenzofuran-3-yloxy)decyl]methylamine HCl (60)

3.5.3.1 Preparation of 5-bromo-3-benzofuranone (86)

A more interesting challenge is presented by the synthesis of analogue (**60**) of **RWA2109** in which the side chain is on the furan ring. The key intermediate in this preparation is 5-bromo-3-benzofuranone which has previously been prepared by several different routes. Among these routes, two had been followed and modified (**Scheme 19& 20**).

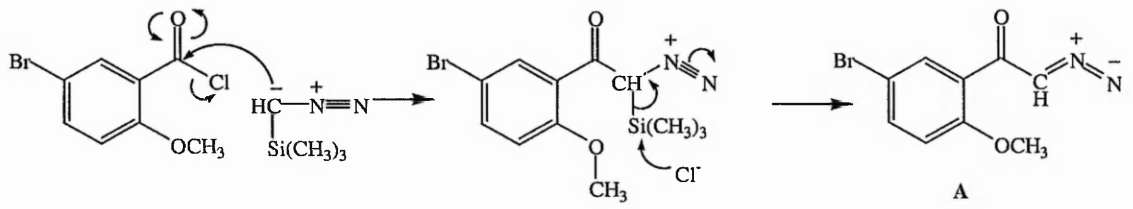
The route to prepare **86** via diazoketone introduced by Jung et al. [150a] proved to be disappointing. 5-Bromosalicylic acid **82** was converted by standard methods to the corresponding methyl ether **83**, and acid chloride **84**. Treatment of **84** with

trimethylsilyldiazomethane (**Mechanism 3**), a safe substitute for diazomethane [150b], gave a product. The infrared spectrum of this compound showed peaks at 2098cm^{-1} and 1606cm^{-1} together with a one-proton peak (***CH***N_2) at δ6.3ppm, characteristic of a diazoketone, presumed to be compound **85**.



i. iodomethane, K_2CO_3, 2-butanone; ii. KOH, ethanol; iii. $SOCl_2$, dry DCM; iv. $(CH_3)_3SiCHN_2$ (2M) in hexane, dry THF.

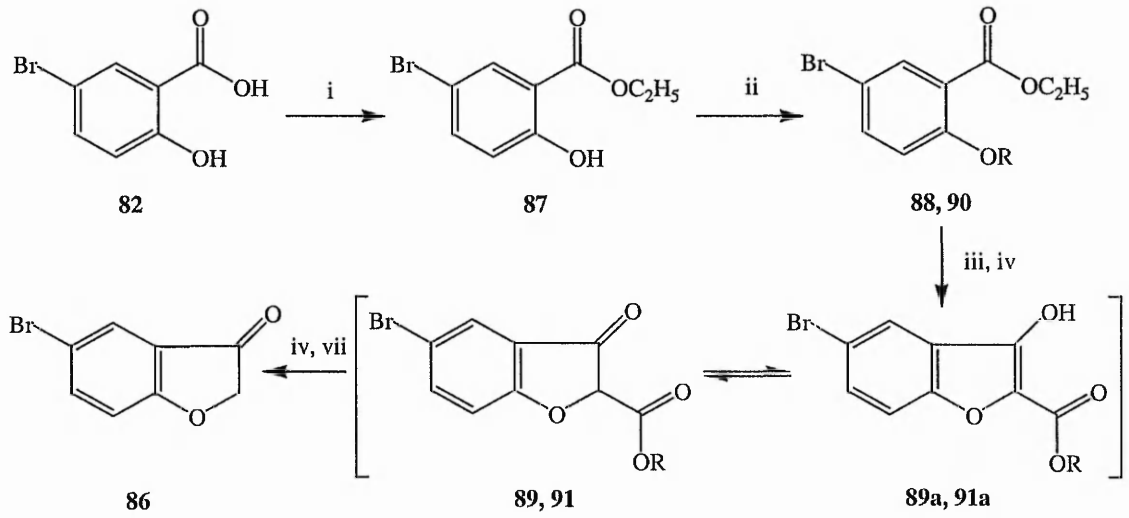
Scheme 19



Mechanism 3

Intramolecular displacement of nitrogen from acyl diazonium acetate **A** **(Scheme 19),** produced by protonation of the initially formed diazo ketone **85,** by the lone pair of the *O*-methoxy group would give the oxonium acetate **B**, which would then lead to benzofuranone by loss of methyl acetate. However, the conversion from **85** to **A** followed by stirring the solution with glacial acetic acid at room temperature failed to afford the key intermediate **86**. The constant failure of protonation in the following step stopped the further investigation of this method.

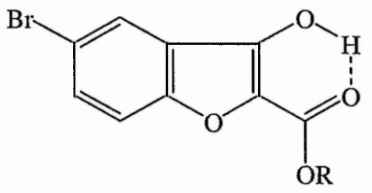
Another route (**Scheme 20**) applied in the investigation was designed by Schroeder and co-workers [151] in 1961 who found the most effective method was a Dieckmann reaction performed on ethyl *O*-carbethoxymethylsalicylate (**88**). From NMR and IR spectra analysis, compound **89** exists as a mixture due to the tautomerism. Additionally, the carbonyl group on position two is another precursor for the existence of compound **89a**, because the formation of an intramolecular hydrogen bond between O (from carbonyl) and H (from hydroxyl) (**Fig. 2**) could lead to a six-membered ring, which would stabilize compound **89a**. Moreover, it was observed that there are not only **89** and **89a** in this mixture sometimes, but a small amount of key synthon **86** according to GC/ MS, which might result from the excess base.



88: R= $CH_2COOC_2H_5$; **89**, **89a**: R=C_2H_5; **90**: R= $CH_2COOC(CH_3)_3$; **91**, **91a**: R= $C(CH_3)_3$

i. dry ethanol, conc. H_2SO_4; ii. $BrCH_2COOC_2H_5$, KOH, dry ethanol; iii. CH_3CH_2ONa, dry benzene; iv. 5% NaOH aqueous solution; v. $ClCH_2COOC(CH_3)_3$, K_2CO_3, DMF, 100°C; vi. NaH, THF, 40°C; vii. CF_3COOH, DCM, rt.

. Scheme 20

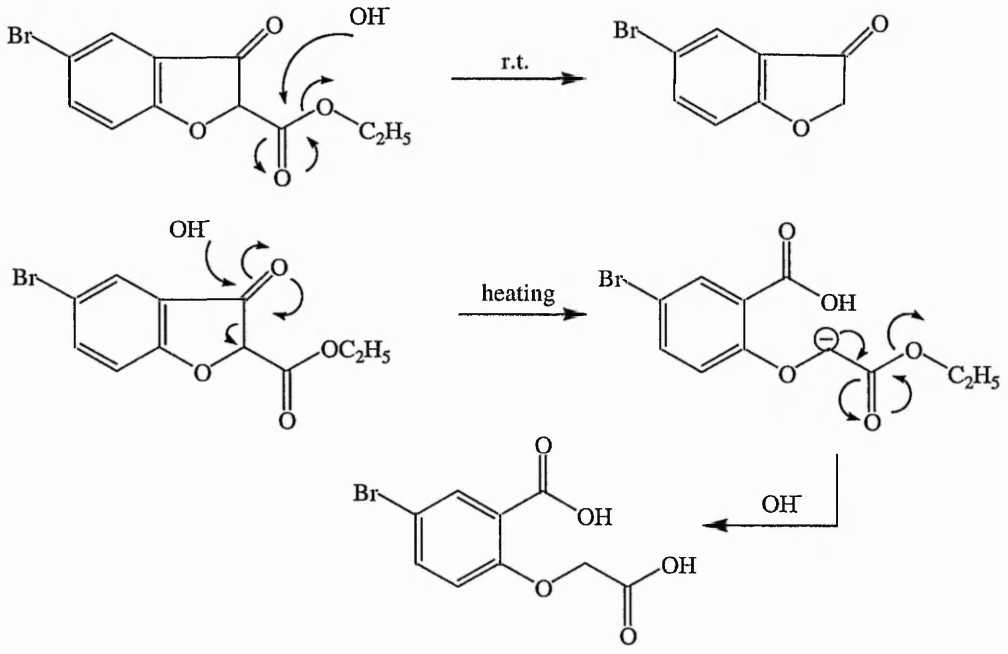


89a: R= C_2H_5; **91a**: R= $C(CH_3)_3$

Fig 2

However, to prepare the important intermediate, compound **86**, the mixture obtained from step **iii** was suspended in 5% sodium hydroxide solution, and kept stirring until all solid dissolved, which needed approximately four weeks. Obviously, although the

route introduced above could lead to intermediate **86**, the long period is a major drawback. When gentle heating was employed to speed up the reaction, decomposition of the furan ring occurred, forming a di-acid by a retro-aldol process (**Mechanism 4**). It is possibly because the nucleophile, OH^-, prefers the electrophile from ketone to that from ester under the heating condition.

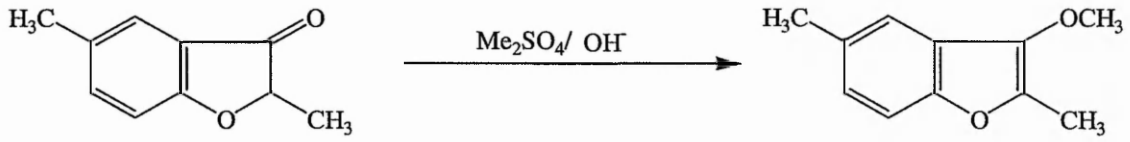


Mechanism 4

To get round these problems, a modified approach (**Scheme 20**) was used in which ethyl chloroacetate was replaced by the t-butyl ester to give ketoester **91** in reasonable yield. Although the spectra were consistent with the keto-enol mixture (**91**& **91a**), elemental analysis was not satisfactory. However brief treatment of the mixture with an excess of trifluoroacetic acid resulted in 5-bromo-3-benzofuranone (**86**), identical to that previously obtained.

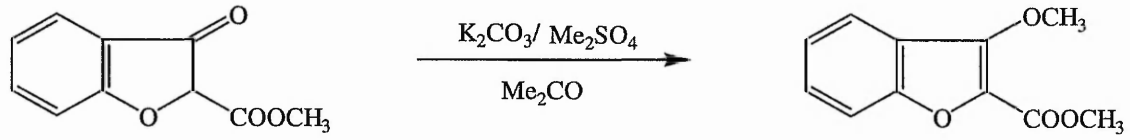
3.5.3.2 Alkylation of 5-Bromo-3-Benzofuranone

The approach to the target product is by the *O*-alkylation of 5-bromobenzofuran-3-one, followed by elaboration of the resulting side chain. An early report by Auwers suggested that "coumaranone", 3-benzofuranone, formed predominantly the O-ether when it was treated with alkaline dimethyl sulfate [152] (**Scheme 21**).



Scheme 21

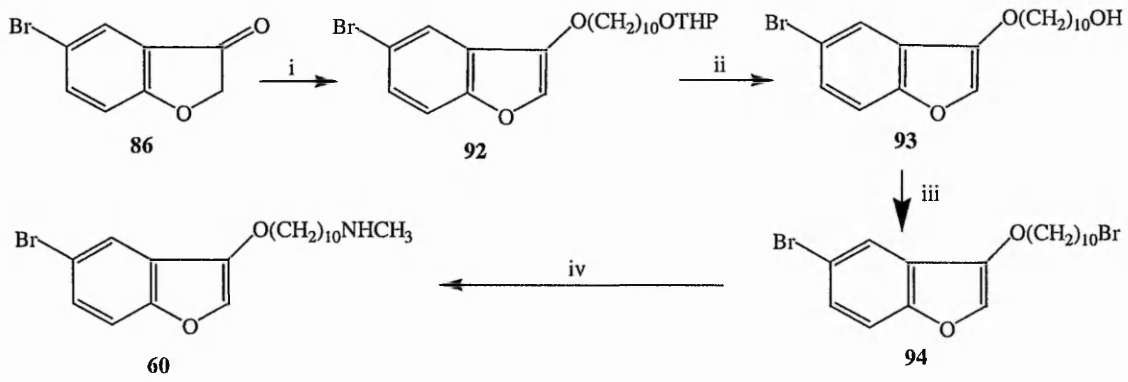
Most subsequent alkylations have been carried on benzofuranones with an alkoxycarbonyl substituent on the 2-position, which would exist mainly in the enol form. An example of this is shown in **Scheme 22**, which was used by Royer [153].



Scheme 22

A preliminary experiment in which commercially available 3-benzofuranone was treated with 1, 10-dibromodecane resulted in a complex mixture of products, possibly *O*- and *C*-alkylated materials, together with products resulting from displacement of both bromines of the dibromodecane. It is obvious that the reagent, 1,10-dibromodecane containing two equivalent electrophilic carbons caused this major problem. Therefore, it

seems very important to replace this alkylating reagent with one containing two different functional groups. The alkylating reagent, 2-(10-bromodecyloxy)-tetrahydropyran ($Br(CH_2)_{10}OTHP$), which is discussed in the next section, was applied to produce a cleaner product **92** (**Scheme 23**).



i. $Br(CH_2)_{10}OTHP$, NaH, DMF, 80°C; ii. PPTS, ethanol, 50°C; iii. Ph_3P, CBr_4, DCM, ice bath, then warmed up to rt; iv. NH_2CH_3 33% in ethanol, DMF, rt.

Scheme 23

Intermediate **92** obtained in **Scheme 23** was examined carefully by NMR. O-alkylation would yield a benzofuran derivative giving a singlet for the 2-proton with a δ value around 7, while C-alkylation would produce a triplet at about δ5.2ppm. No evidence of C-alkylation was observed.

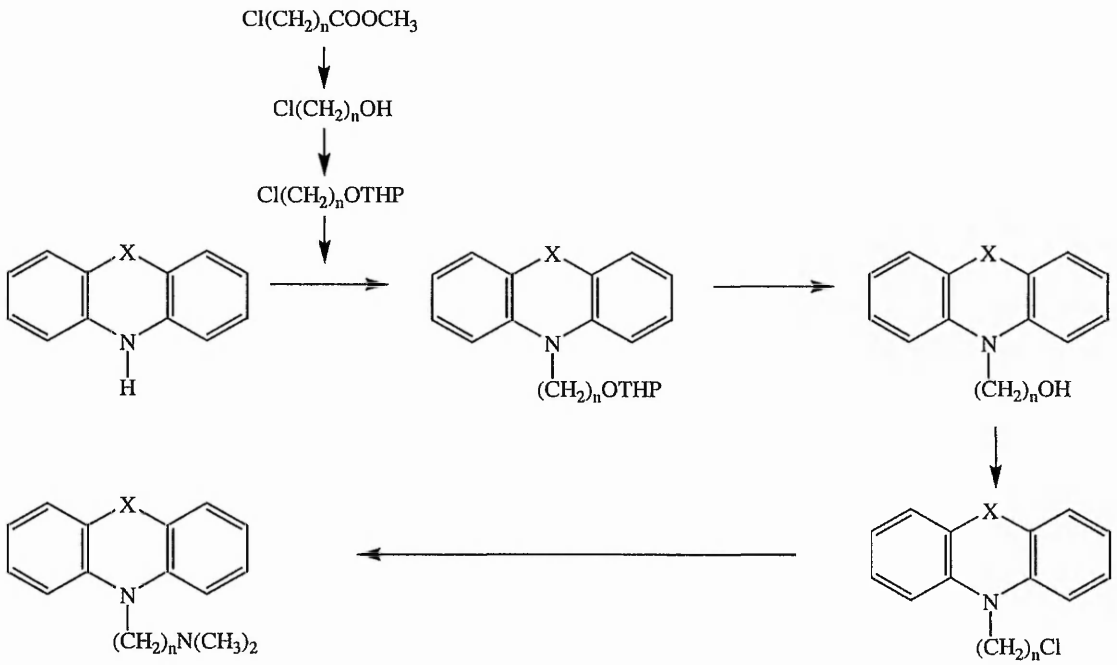
3.6 Preparation of 2-(10-bromodecyloxy)tetrahydropyran (95)

With more synthesis of **RWA2109** analogues, the Williamson method, which has been applied widely within the group, showed drawbacks, especially in the preparation of compound **60** discussed above. Excess of 1,10-dibromodecane, the low-cost alkylating reagent, could be easily removed by Kugelrohr distillation. However, the requirement of pressure and temperature of Kugelrohr distillation does not make it work in every case, e.g. indole derivatives could decompose under this condition; and also two electrophilic carbons existing in the original alkylating reagent were causing some side reactions. Chromatography is not very efficient in the separation of desired product and main by-product due to the large amount of unreacted dibromoalkane. Additionally, the by-product diether (**96**) which presents a similar polarity is difficult to separate from the target product on a silica column.

$$Ar — O(CH_2)_{10}O — Ar$$

96

Hence, the utilization of a differentially bifunctional reagent becomes a primary choice. This reagent should be easy to prepare, have no/fewer side reactions, and easily converted to terminal amine after alkylation. A very recent report [154] also employed a similar synthetic route in the research of antimalarial drugs (**Scheme 24**), which supported the concept of using this type of bifunctional reagent. Alcohol with chlorol residue on the other end was protected simply by forming a tetrahydropyranyl ether. The protected alkylating reagent reacted with the heterocylic ring to give a target product after few efficient steps.



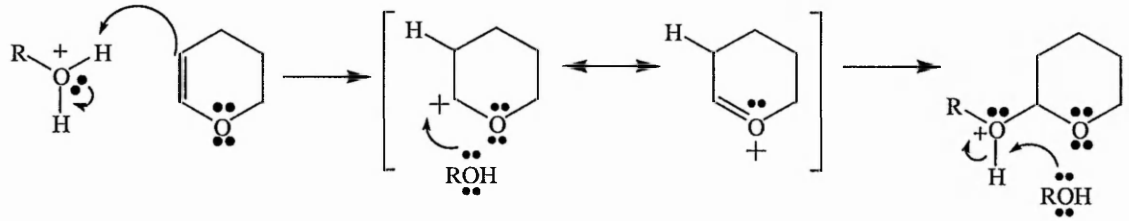
Scheme 24

There are many routes, such as silylation, acylation, and tetrahydropyranylation, which have been investigated to prepare a bi-functional reagent. Among all these well-known methods, tetrahydropyranyl ether is one of the most widely used protective groups employed in chemical synthesis because of its low cost, ease of introduction, general stability to most non-acidic agents (strong basic media, oxidative conditions, reduction with hydrides, and reaction involving Grignard reagents, lithium alkyls and alkylating and acylating reagents), and the ease with which it can be removed [155].

However, 2-(10-bromo-decyloxy)-tetrahydropyran **95** is not commercially available, and although the other possible starting material $Br(CH_2)_{10}OH$ could be purchased, it is very expensive. Therefore, a cheap starting material 1, 10-decandiol (**97**) was chosen to prepare this bi-functional reagent.

The crucial step in the production of the bifunctional reagent is often the mono-protection of the diol. While numerous methods have been investigated selective mono-protection still remains a problem in symmetrical diols. In general, if stoichiometric equivalents of protecting reagents are utilized with a symmetric α, ω-diol, the mixture of un-protected, mono-protected, di-protected products were observed as 1: 2: 1 [156]. This statistical pitfall was circumvented easily by employing a large excess of starting material, which gave an acceptable yield. The excess diol, if not expensive, can be simply discarded, or, if expensive, can be recycled via chromatography.

There are a number of reagents available for tetrahydropyranylation of alcohols, which include the use of Brønsted and Lewis acid [157], iodine-microwave irradiation [158], ion-exchange resins [159, 160], sulphuric acid on silica gel [161], zinc chloride [162] or $AlPO_4$ [163] on alumina, tributylammonium bromide [164], toluenesulfonic acid (TsOH), PPTS [165] etc.. The general **Mechanism 5** of the formation of THP-ether is considered to be as follows: the formation of a mesomerically stabilized carbocation leads to a regioselective addition of the alcohol to the alkene.

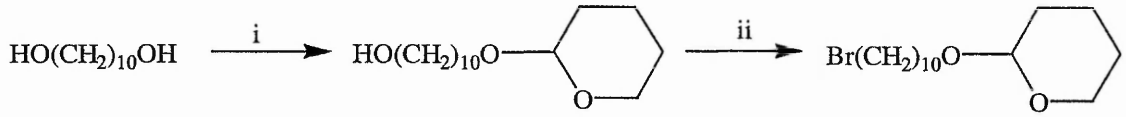


Mechanism 5

In an attempt to prepare a mono-THP protected diol in this study, silica chloride was primarily chosen as a catalyst among all the applicable reagents because a very good

yield was claimed via this route [166]. Silica chloride was easily prepared from the readily available chemicals, thionyl chloride and oven dried silica gel [167].

However, **Scheme 25** employed with freshly made catalyst, silica chloride, did not give as good a yield as claimed in the paper [166]. A noticeable amount of unreacted diol, and di-protected by-product reduced the yield of target compound **98**. The possible reason is scaling up of the method, using a diol which is poorly soluble in dichloromethane. Fortunately, the mixture can be separated by flash chromatography and the starting material, 1, 10-decandiol, can also be recycled.



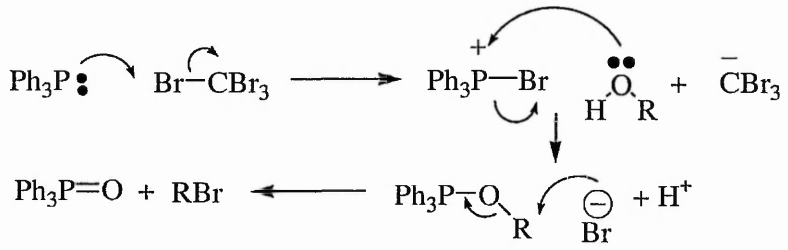
97 **98** **96**

i. silica chloride, 3, 4-dihydro-2H-pyran; ii. CBr_4, Ph_3P.

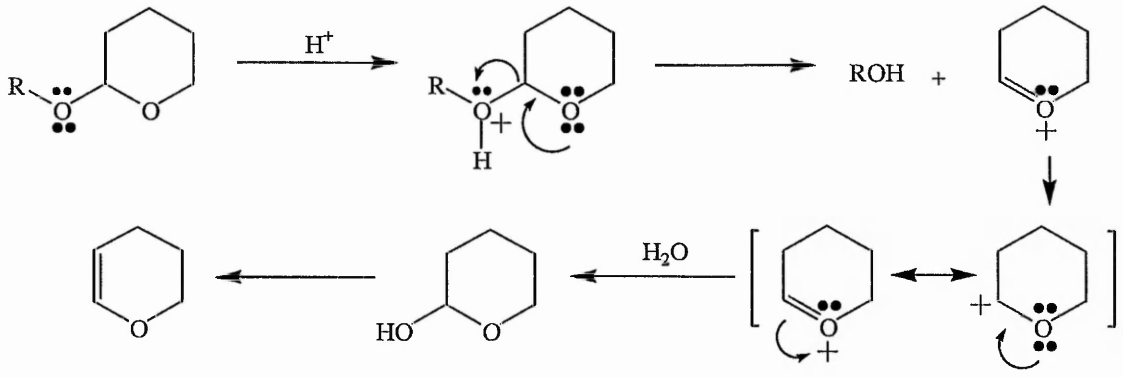
Scheme 25

Meanwhile, a more serious problem arose in the conversion of alcohol **98** to a brominated compound **96**. The reagents at this stage are triphenylphosphine (Ph_3P) and carbon tetrabromide (CBr_4) [168], the S_N2 reaction of Ph_3P on bromine in CBr_4 led to the completion of bromination (**Mechanism 6**). After normal working-up, formation of 1, 10-dibromodecane was observed in addition to **96** according to GC/ MS because of the cleavage of THP ether followed by bromination of hydroxyl group. As discussed previously, although THP-ether is fairly stable under most conditions, it is susceptible to hydrolysis under acidic conditions (**Mechanism 7**). According to **Mechanism 6**, the

proton produced from the conversion of **98** to **96** is possibly acidic enough to cleave the THP-ether.

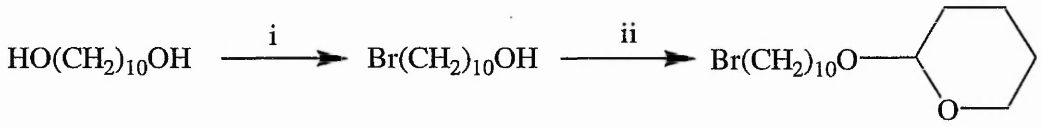


Mechanism 6



Mechanism 7

The obvious method to avoid this loss of THP group is to introduce it after the conversion of one alcohol function to bromide (**Scheme 26**). Reaction of 1, 10-decanediol (**97**) with aqueous HBr under Dean& Stark condition [169] gave a moderate yield of the 10-bromodecanol (**99**). A modification, simply heating together the diol with aqueous HBr, actually gave a better yield of alcohol **99** which was easily converted to the desired compound **96** [170], with a readily prepared catalyst, silica chloride [166]. The disadvantage of a Dean-Stark apparatus is that it can lead to the recovery of considerable amounts of starting diol, and also acid [170].



97 **99** **96**

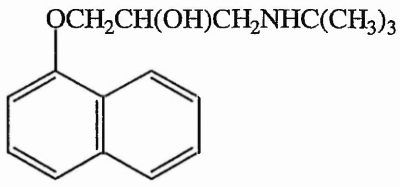
i. CBr_4, Ph_3P; ii. silica chloride, 3, 4-dihydro-2H-pyran.

Scheme 26

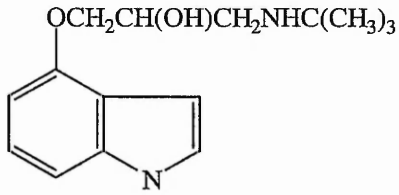
3.7 Preparation of Indole Derivatives

In previous studies, benzothiophene and benzofuran derivatives have been synthesized and studied *in vivo* and *in vitro*, it seems that it is very necessary and interesting to extend this investigation to some indole analogues.

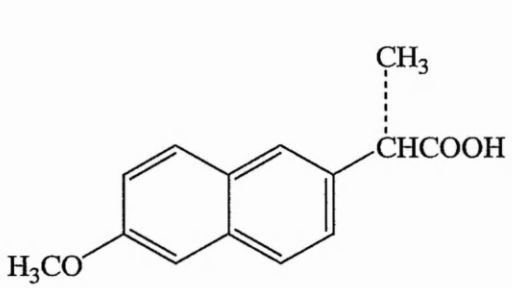
The synthesis and reactivity of indole derivatives has been a topic of research for the pharmaceutical industries for well over a century. Significantly the replacement of a naphthalene residue by an indole had resulted in chemically useful drugs. A couple of examples are shown in the **Figure 3**, a β-adrenergic blocker, pindolol and non-steroidal anti-inflammatory drug, indomethacin and their pioneering drugs propranolol and naproxen.



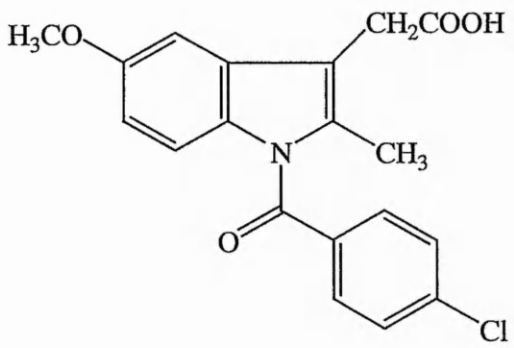
Propranolol



Pindolol



Naproxen



Indomethacin

Fig. 3

Indole is classified as a π-excessive aromatic compound. It is isoelectronic with naphthalene, with the heterocyclic nitrogen atom donating two of the ten π-electrons. As an electron-rich heteroaromatic, indole is subject to oxidative processes. Many indoles are readily oxidized by exposure to atmospheric oxygen, with the initial product being a 3-hydroperoxy-3H-indole (**Fig. 4**). From the perspective of laboratory practice, the sensitivity of many indoles to acids, oxygen and light prescribes the use of an inert atmosphere for most reactions involving indoles and the avoidance of storage with exposure to light.

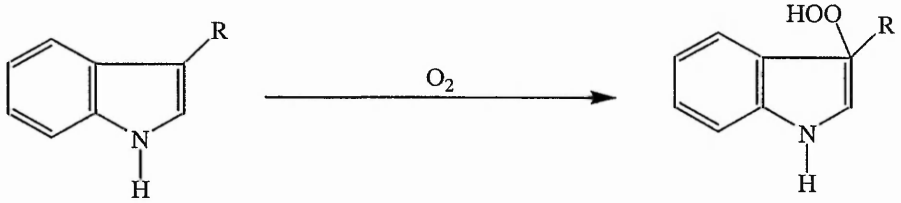


Fig. 4

In the present investigation, some classic indole syntheses have been applied, which are introduced in the following sections.

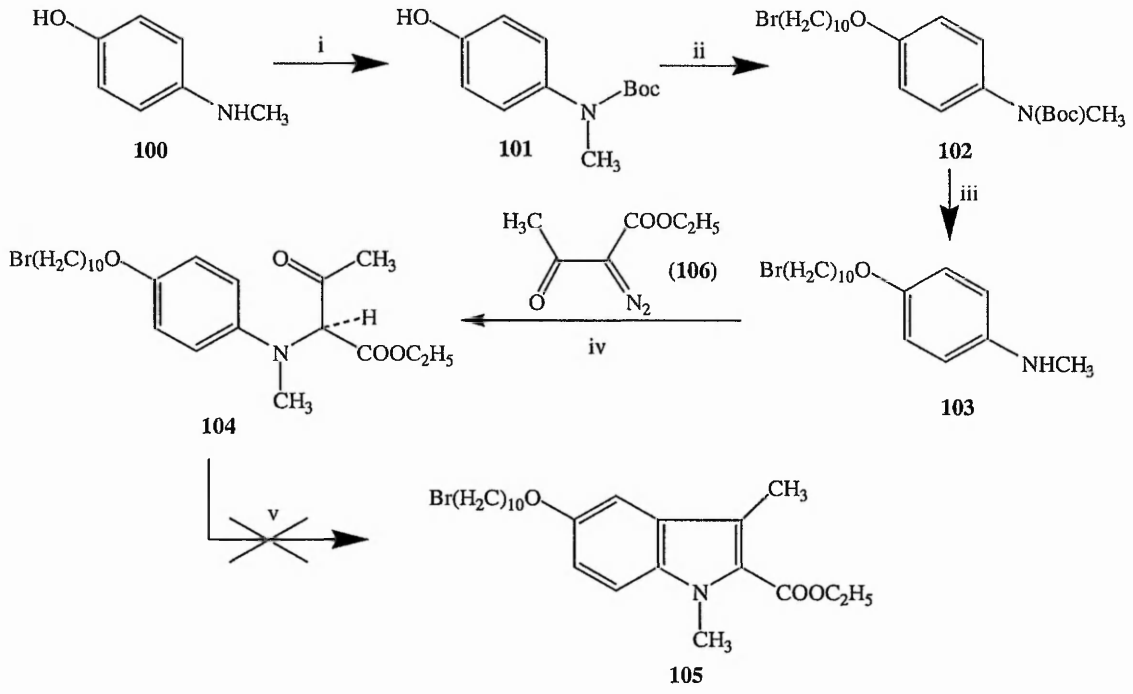
3.7.1 Bischler Indole Synthesis

The Bischler indole synthesis, discovered over 100 years ago [171], involves the reaction of anilines with α-halo-ketones or –acetals and the subsequent acid catalysed cyclisation of the resulting α-(N-arylamino)-ketones or –acetals (the Norlander modification in which a number of Lewis acids have been used to effect the cyclisation) [172, 173]. Moody and Swann have described a modification of the Bischler synthesis wherein the key step is the N-H insertion reaction [174] of anilines with rhodium carbenoids generated from diazocarbonyl compounds to give α-(N-arylamino)ketones for subsequent cyclisation [175].

The method [176] was applied to the attempted synthesis of compound **105** starting with 4-methylamino phenol (**Scheme 27**). Of the two nucleophilic (OH and NH) centres present in starting material **100**, the secondary amine, was selectively protected with a *t*-butyloxycarbonyl (Boc) group followed by the alkylation of hydroxyl group. After the removal of the -Boc group, the N-H insertion reaction was carried by heating a mixture of the intermediate **103** and the ethyl diazoacetoacetate (**106**) in boiling toluene in the presence of rhodium (II) acetate (2mol%), which should lead to the production of a key intermediate **104** in a reasonable yield.

However, the NMR spectra analysis did not show the existence of the proton on the carbon between two carbonyls in compound **85**; the reason is not clear. It is possibly because of either the formation of intramolecular hydrogen bond or of the acidity of this hydrogen.

The acidic ion-exchange resin Amberlyst 15 was adopted in the **stage v**, cyclization, which was heated with intermediate **104** in toluene to prepare indole **105**. However, there is no evidence from tlc of the formation of new compound in the reaction system after overnight heating. This was surprising, because Moody had successfully prepared the corresponding compound with methoxy in the 5-position. Because of this continued failure, the instability of **104,** and the expense of the diazoacetate and rhodium acetate, this approach was not pursued further.

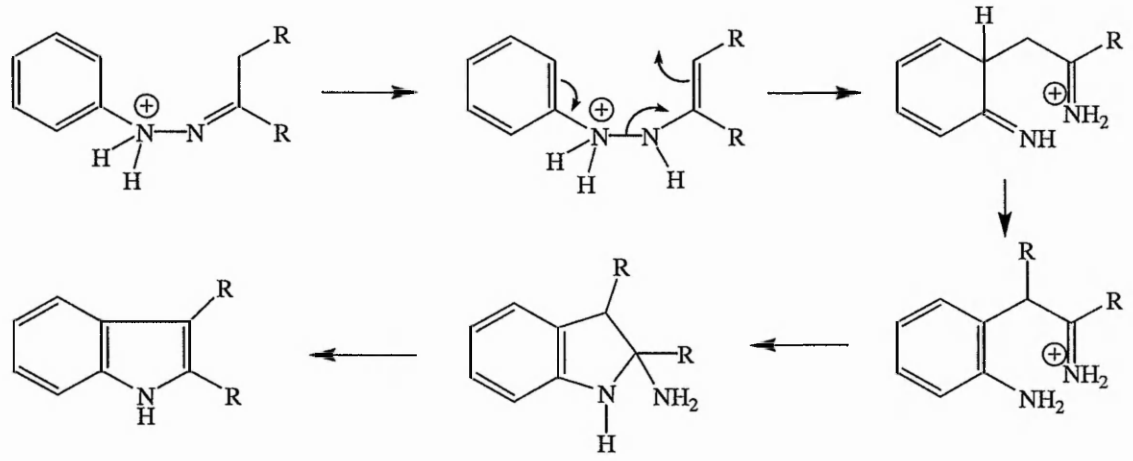


i. di-tert-butyldicarbonate, THF; ii. 1, 10-dibromodecane, 2-butanone, K_2CO_3; iii. TFA, DCM; iv. ethyl diazoacetoacetate, rhodium (II) acetate, toluene; v. Amberlyst 15, toluene.

Scheme 27

3.7.2 Fischer Indole synthesis

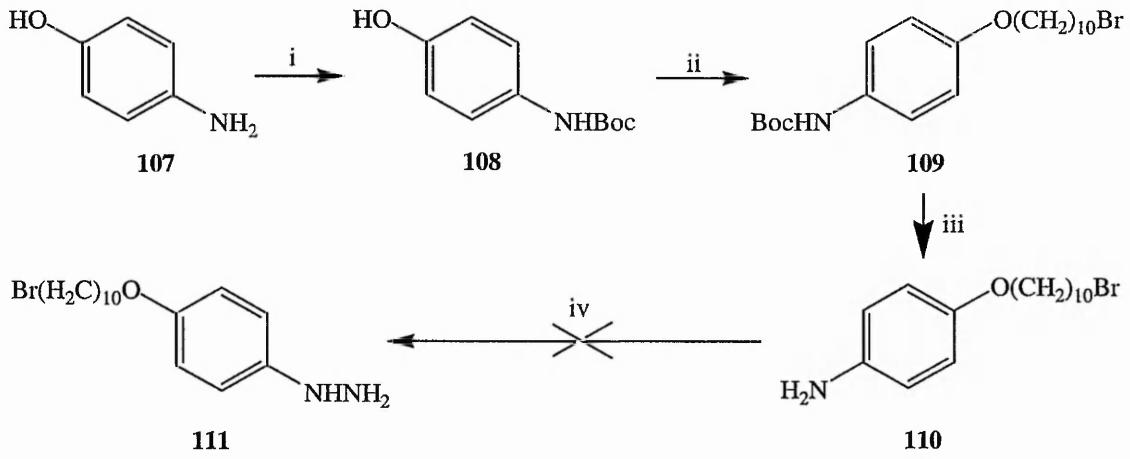
The venerable Fischer indole synthesis [177, 178] has played a prominent role as a route to indoles and to the large-scale production of indole pharmaceutical intermediates. The Fischer cyclization converts arylhydrazones of aldehydes or ketones into indoles by a process (**Mechanism 8**) which involves *ortho*-substitution via a sigmatropic rearrangement. The rearrangement generates an imine of an O-aminobenzyl ketone which cyclizes and aromatizes by loss of ammonia.



Mechanism 8

The preparation of a key intermediate, phenylhydrazine (**111**), in Fischer indole synthesis was attempted following standard procedures. Starting material, 4-aminophenol (**107**) was protected with *t*-butyloxycarbonyl group followed by alkylation with 1, 10-dibromodecane and deprotection (**Scheme 28**). Compound **110** was treated with concentrated hydrochloric acid and sodium nitrite to afford a diazonium cation which could be converted to a phenylhydrazine by reduction with dithionite. However, the

desired hydrazine was not detected in the reaction system. It is noteworthy that the method used was carried out in aqueous conditions. Unlike aniline, **110** possess a bulky hydrophobic residue, which could decrease the solubility of **110** dramatically. Therefore, the reaction cannot proceed because the reactants stay in two different phases.



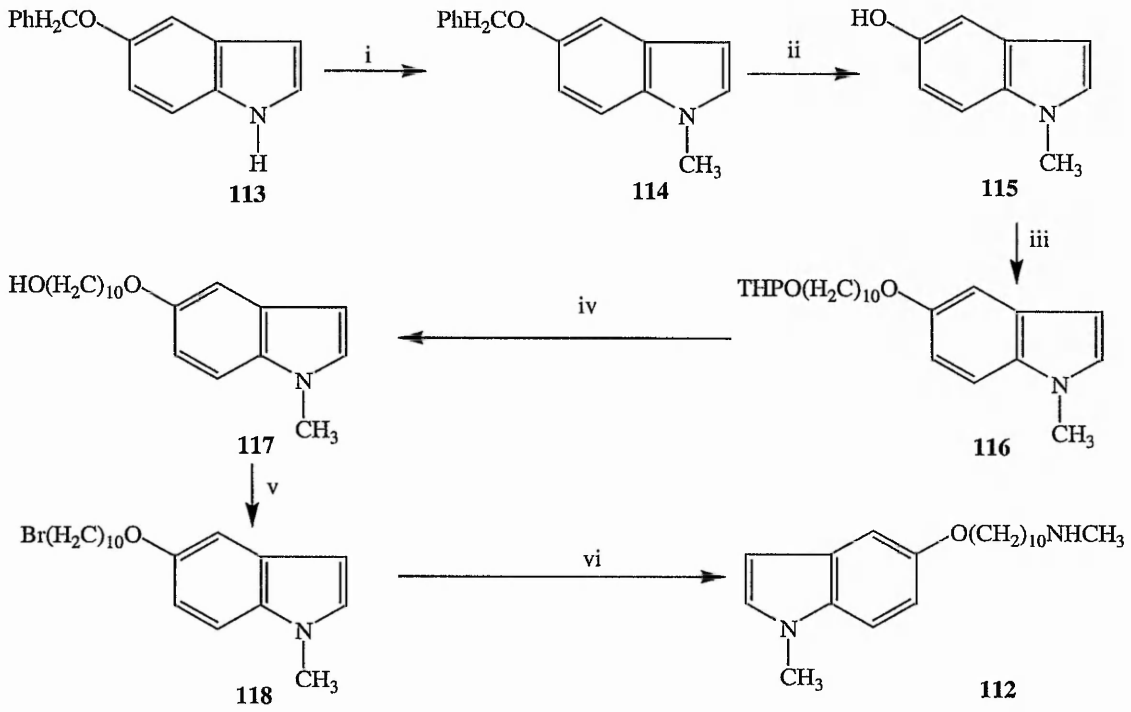
i. di-tert-butyldicarbonate, THF; ii. 1, 10-dibromodecane, 2-butanone, K_2CO_3; iii. TFA, DCM; iv. $NaNO_2$, conc. HCl, $Na_2S_2O_5$, OH^-.

Scheme 28

3.7.3 Preparation of Methyl-[10-(1-methyl-1H-indole-5-yloxydecyl)amine (112)

The next approach was by the modification of a preformed indole. Commercially available 5-benzyloxyindole, was N-methylated by removal of the acidic indole hydrogen with sodium hydride followed by addition of iodomethane (**Scheme 29**) [179]. The benzyloxy group was removed by hydrogenation with palladium on activated carbon (10%) in ethanol at room temperature. Alkylation with the reagent, 2-(10-bromo-

decyloxy)-tetrahydropyran (**96**) discussed in **section 6**, was successfully applied to give ether **116**. After the conversion from compound **116** to **118**, followed by the treatment of methylamine, the final product was obtained in good yield. In spite of the notorious sensitivity of indoles towards acid, reaction with ethereal HCl gave a hydrochloric salt of **112**.

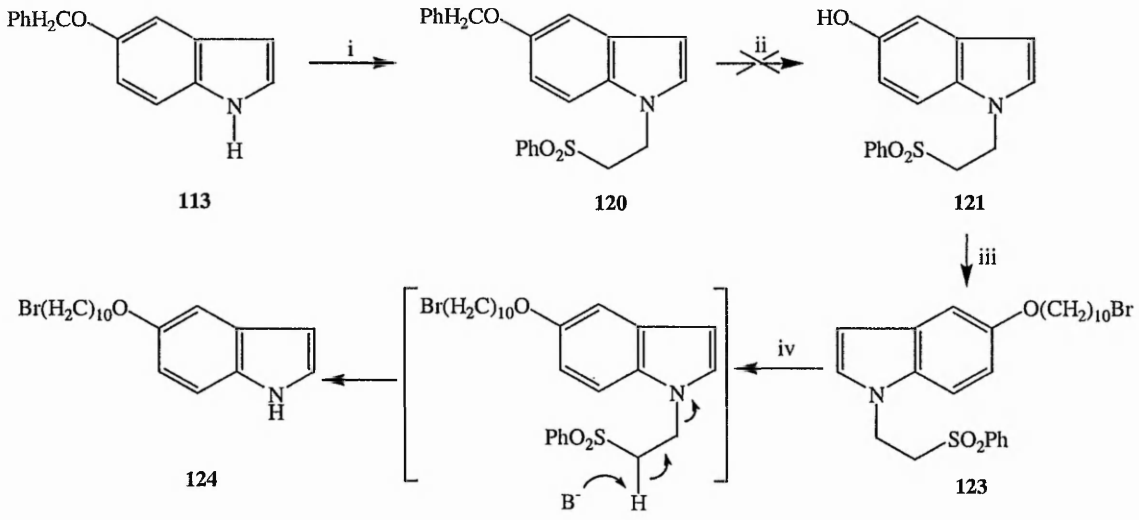


i. MeI, NaH, DMF; ii. Pd/C, H_2; iii. $Br(CH_2)_{10}OTHP$, K_2CO_3, 2-butanone; iv. PPTS, ethanol; v. CBr_4, Ph_3P, DCM; vi. NH_2CH_3 (33% in ethanol), DMF.

Scheme 29

3.7.4 Preparation of an Indole Derivative with an Unsubstituted Indole Nitrogen (119)

In the proposed synthesis of the target molecule **119**, it was necessary to protect the indole nitrogen prior to *O*-alkylation. An early attempt used benenesulfonylethyl group, introduced by Moody [180]. Formation of the protected species **120** was easy, but to remove this protecting group by hydrogenation was unsuccessful, possibly because of poisoning of the palladium catalyst by traces of sulfur from the blocking group (**Scheme 30**).

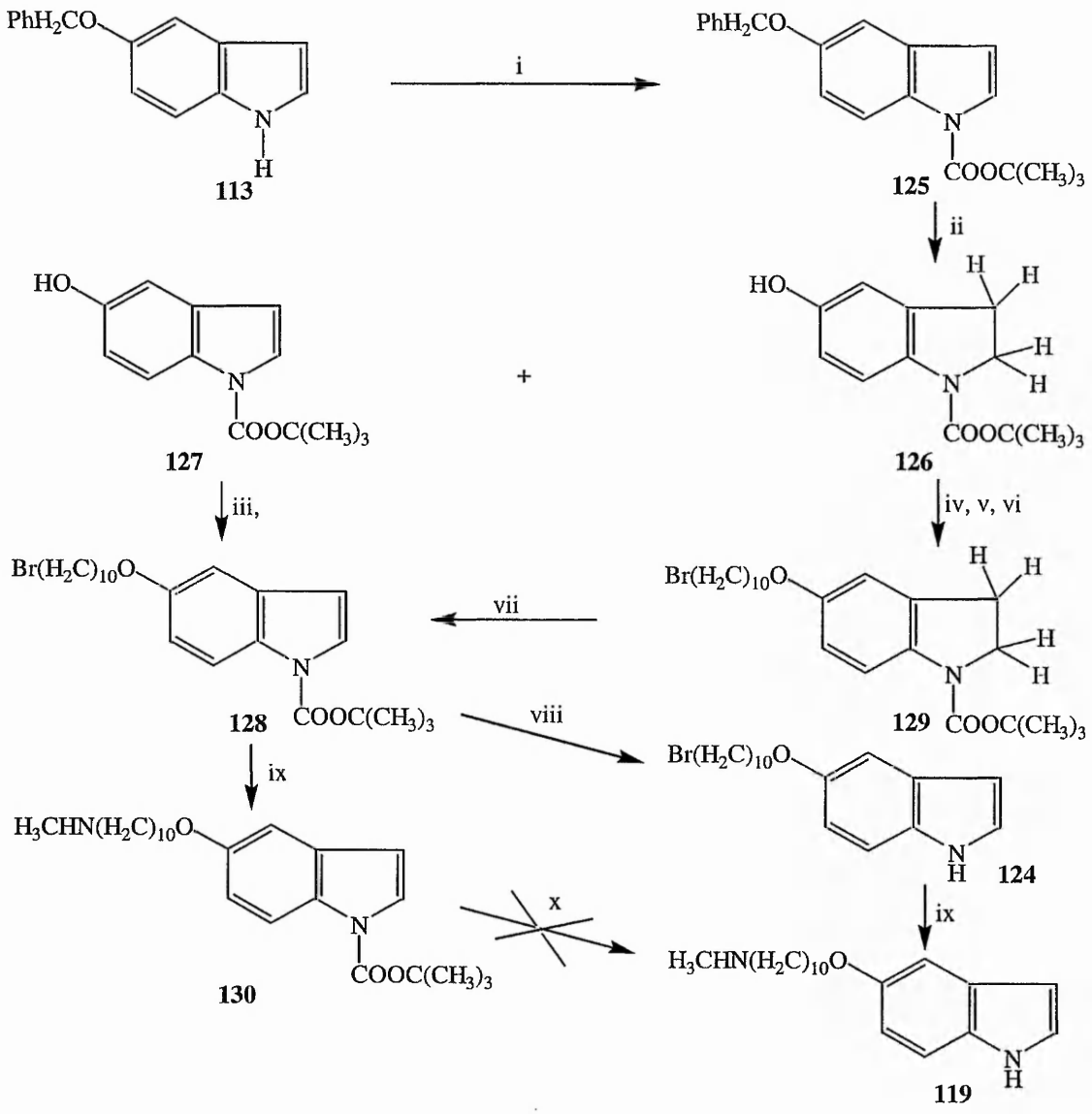


i. $ClCH_2CH_2SO_2Ph$, NaH, DMF; ii. Pd/C (10%), H_2; iii. $Br(CH_2)_{10}OTHP$; PPTS, EtOH; CBr_4, Ph_3P; iv. t-BuOK, DMF.

Scheme 30

Another common protecting group t-butyloxycarbonyl was chosen due to its fast and easy removal (**Scheme 31**). In this route, the benzyl group could be easily removed with Pd/C under hydrogen. However, unlike **step ii** in **Scheme 30**, a mixture of products **126** and **127** was obtained from the reaction. It is possibly because that the electron-

withdrawing group on the nitrogen makes the lone pair electrons of nitrogen move towards carbonyl, decreasing the stability of aromatic system. After separation by chromatography, the expected intermediate **127** was converted to **128** by standard synthetic procedure. To improve the yield of this reaction, the by-product **126** could also be converted to intermediate **128** by dehydrogenation with active manganese dioxide [181].



i. di-t-butyldicarbonate, NaH; ii. Pd/C, H_2; iii. $Br(CH_2)_{10}Br$, K_2CO_3, 2-butanone; iv. $Br(CH_2)_{10}OTHP$, K_2CO_3, 2-butanone; v. PPTS, ethanol; vi. CBr_4, Ph_3P; vii. MnO_2, benzene; viii. TFA, DCM; ix. NH_2CH_3 (33% in ethanol), DMF; x. $NaOCH_3$, CH_3OH, THF.

Scheme 31

To remove the Boc group, trifluoroacetic acid has been generally used. However, in the present investigation with indole derivatives, strong acidic conditions had to be avoided if possible. Instead of using TFA, sodium methoxide in methanol was applied [182]. Prior to the attempted deblocking, the side chain of intermediate **128** was transformed to the methylamine terminal to prevent the formation of an ether between **128** and neucleophile methoxide. However, unfortunately, there is no evidence of the formation of new compound **119**. Surprisingly, the desired intermediate **124** could be obtained by using TFA in a reasonable yield [183]. Then the final product **119** could be produced after standard synthesis. Another by-product **130** obtained accidentally from step ix could also be used in biological testing to complete the indole derivatives group.

Chapter 4

Experimental

NMR spectra were measured on a JEOL EX 270 machine at 270 MHz for ^{1}H and at 67.8 MHz for ^{13}C using $CDCl_3$ as solvent, unless otherwise indicated, and with tetramethylsilane (TMS) as the internal standard. Coupling constants (J) are given in Hz. Fourier Transform InfraRed (FTIR) spectrometer were recorded on a Perkin-Elmer 1600 Series FTIR machine either by liquid films (nujol mulls, or dissolved in chloroform), or by the preparation of a KBr disc. Low resolution Electrospray Ionization (ESI) mass spectra were recorded on LCQ Finnigan spectrometer. Elemental analysis were performed by the Microanalytical Department of Nottingham University

Chromatography is flash column chromatography and was performed using Merk silica gel 60 using the eluant specified. Thin layer chromatography was carried out using pre-prepared plates, Merk silica gel 60 F-254. Technical grade petroleum ether (bp. 60-80°C) was distilled prior to use, and is hereafter designated as petroleum ether. Anhydrous diethyl ether and dichloromethane were dried over calcium chloride. Aldrich anhydrous dimethylformide (99.8%) was used as supplied. Tetrahydrofuran was dried by distillation from sodium-benzophenone ketyl. Triethylamine was dried by distillation from potassium hydroxide. *N*-Bromosuccinimide was purified by recrystallisation from water and dried over silica gel *in vacuo*. All other reagents were purchased from commercial sources and used without further purification.

General Method

A. Ether synthesis

To the appropriate phenol (1 eq) and powdered anhydrous potassium carbonate (3 eq) suspended in 2-butanone (0.3 M) at room temperature under an atmosphere of nitrogen was added the appropriate 1,10-dibromoalkane (5eq). The heterogeneous mixture was stirred vigorously and gently refluxed until the reaction was judged complete by t.l.c., allowed to cool, and the solvent was removed *in vacuo*. The residue was suspended in dichloromethane, filtered, and sequentially partitioned with 2M NaOH and saturated sodium chloride. The organic fraction was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Generally, excess 1,10-dibromoalkane was removed by Kugelrohr distillation at reduced pressure and the residue was triturated with 1 to 5% ethanolic petroleum ether to give a precipitate that was recrystallised from an appropriate solvent. Alternatively, the excess dibromodecane can be removed with flash chromatograpy with an appropriate eluent in some cases.

B. Alkylation of amines

To the appropriate alkyl halide (1eq) and triethylamine (2eq) dissolved in dimethylformamide (0.1 M), at room temperature under an atmosphere of nitrogen, was added the appropriate amine (50 eq)*. The mixture was stirred at room temperature for 18hr. and the solvent removed *in vacuo*. The residue was dissolved in ethyl acetate and sequentially partitioned, twice with water, and saturated sodium chloride. The organic fraction was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by chromatography eluting with ethyl acetate: methanol: 880 NH_3, 90:(10 to 15):

3. Fractions containing the product were combined and concentrated *in vacuo* to give the desired amine as the free base.

* Methylamine was used as a 33% solution in ethanol.

C. Preparation of hydrochloride salts

To the appropriate amine dissolved in the minimum amount of dichloromethane at room temperature was slowly added an excess of ethereal hydrogen chloride. The mixture was cooled to 0°C, filtered, and the hydrochloride salt was purified by recrystallisation from an appropriate solvent.

D. Formation of 1-aryloxy-10-tetrahydropyranyloxydecanes

2-(10-Bromodecyloxy)tetrahydropyran (1.05eq) was added to a refluxing solution of the phenolic compound (1eq) dissolved in 2-butanone containing powdered anhydrous K_2CO_3 (1.25eq). After the full conversion of starting material, the mixture was concentrated under reduced pressure, and the residue was suspended in DCM, and filtered. The filtrate was washed with 2M NaOH, H_2O and sat. NaCl, dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by chromatography with an appropriate eluent to give a single product.

E. Removal of THP blocking group

A solution of THP ether (1eq) obtained from general method **D** and pyridinium p-toluenesulphonate (PPTS) (0.1eq) dissolved in ethanol (ether/ ethanol 0.1M) was stirred at 55°C (bath temperature) for 3 hours until t.l.c. showed the full conversion of starting

material to a new compound. The reaction was cooled down to room temperature and the solvent was removed under reduced pressure to give a desired product in acceptable yield.

F. Conversion of alcohols to ω-bromoalkanes

Triphenylphosphine (1.5eq) in dry DCM in ice bath was added into a similarly cooled mixture of the alcohol (1eq) and carbon tetrabromide (1.25eq) dissolved in dry DCM. The reaction was kept in an ice bath for 15min and warmed up to room temperature and the progress was monitored by t.l.c.. The solvent was removed *in vacuo* when the reaction was judged complete by tlc. The residue was purified by flash chromatography with an appropriate eluent to give product in an acceptable yield.

4.1 Preparation of 7-bromo-4-(10-methylaminodecyloxy)benzothiophene (5)

(1) Preparation of 4-hydroxybenzothiophene (2)

4-Keto-5,6,7-tetrahydrothionaphthlene (obtained from Aldrich) (6.5g, 42.7mmol) and sulfur (1.8g, 55.5mmol) were suspended in diphenyl ether (60ml) at room temperature under nitrogen. The mixture was heated to reflux and kept stirring vigorously to give a deep red homogenous mixture. T.l.c (petroleum ether: EtOAc: MeOH/ 8:2:0.5) indicated the completion of reaction. The reaction was stopped cooled down to room temperature, and diluted with diethyl ether. The mixture was extracted with 2M NaOH (60ml×2). The aqueous layer was acidified with 2M HCl until pH 3, and extracted with DCM (100ml×2). All the organic layers were combined, and washed with sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure to give crude oily

product. The residue was purified by flash chromatography (pet ether 60-80°C: EtOAc: MeOH/ 8:2:0.25) to give product as a yellow solid (1.8g, 28%).

m.p. 76-80°C (Lit. [184] m.p. 80-82°C)

δ_H ($CDCl_3$): 7.51 (7-***H***, d, J 7.87Hz), 7.32 (2-***H***, d, J 7.67 Hz), 7.27 (3-***H***, d, J 7.69Hz), 7.13 (6-***H***, m), 6.77 (5-***H***, d, J 7.91Hz), 2.74 (***OH***, brs, disappeared after D_2O shake); δ_C ($CDCl_3$): 152.6 (4-***C***), 141.1 (7a-***C***), 130.8 (3a-***C***), 125.1 (2-***C***), 123.4 (6-***C***), 120.4 (3-***C***), 113.2 (7-***C***), 108.4 (5-***C***); ν_{max}/cm^{-1} (KBr disc): 3203.7cm^{-1}.

(2) Preparation of 4(10-bromodecyloxy)benzothiophene (3c)

Compound **3c** was prepared from phenol (1.5g, 10mmol) and 1,10-dibromodecane (15g, 50mmol) by general method **A** with purification carried out by flash chromatography to give the bromoether as a colourless oil (2g, 54%).

δ_H ($CDCl_3$): 7.50 (7-***H***, dd, J 0.89 Hz, 7.44Hz), 7.45 (2-***H***, d, J 6.70Hz), 7.31 (3-***H***, d, J 6.69Hz), 7.27 (6-***H***, m), 6.74 (5-***H***, dd, J 0.89Hz 7.92Hz), 4.08 ($OCH_2(CH_2)_9Br$), t, J 6.42Hz), 3.39 ($O(CH_2)_9CH_2Br$, t, J 6.97Hz), 1.84 ($CH_2CH_2(CH_2)_6CH_2CH_2Br$, 4H, m), 1.30-1.32 ($O(CH_2)_2$ ***(CH$_2$)$_6$***$(CH_2)_2Br$, 12H, m); δ_C ($CDCl_3$): 154.6 (4-***C***), 141.2 (7a-***C***), 130.6 (2-***C***), 125.2 (3a-***C***), 124.3 (6-***C***), 120.7 (3-***C***), 114.6 (7-***C***), 104.6 (5-***C***), 68.1 ($OCH_2(CH_2)_9$), 34.0 ($O(CH_2)_9CH_2Br$), 32.8 ($O(CH_2)_8CH_2CH_2Br$), 30.1 ($OCH_2CH_2(CH_2)_8Br$), 29.4-28.7 ($O(CH_2)_3(CH_2)_5(CH_2)_2Br$), 26.1 ($O(CH_2)_2CH_2(CH_2)_7Br$).

(3) 7-Bromo-4(10-bromodecyloxy)benzothiophene (4c)

The bromoether **3c** (1.9g, 5mmol) was dissolved in dry acetonitrile (30ml) at room temperature under an atmosphere of nitrogen. *N*-bromosuccinimide (0.9g, 5mmol) was added in one portion to give a yellow homogeneous mixture. The reaction was kept stirring overnight. T.l.c. (petroleum ether 60-80: EtOAc/ 40:1) indicated the disappearance of the starting material (Rf 0.25) and the formation of the new product (Rf 0.42). The solvent was removed *in vacuo* to give a brown oil. This crude compound was dissolved in EtOAc, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography with petroleum ether 60-80°C: EtOAc (40:1) to give product as a colourless solid (1.9g, 81%).

m.p. 44-46°C

δ_H ($CDCl_3$): 7.59 (2-***H***, d, J 5.57Hz), 7.37 (3-***H***, 6-H, m), 6.34 (5-***H***, d, J 8.21Hz), 4.05 ($OCH_2(CH_2)_9Br$), t, J 6.42Hz), 3.39 ($O(CH_2)_9CH_2Br$, t, J 6.94Hz), 1.83($CH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.48 ($O(CH_2)_2$ $(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 153.9 (4-***C***), 142.6 (7a-***C***), 131.4 (6-***C***), 127.6 (3a-***C***), 125.3 (2-***C***), 121.8 (3-***C***), 106.5 (5-***C***), 105.0 (7-***C***), 68.4 ($OCH_2(CH_2)_9Br$), 34.0 ($O(CH_2)_9CH_2Br$), 32.8 ($O(CH_2)_8CH_2CH_2Br$), 29.4 ($OCH_2CH_2CH_2(CH_2)_5(CH_2)_2Br$), 26.1 ($O(CH_2)_2CH_2(CH_2)_7Br$).

(4) Preparation of 7-bromo-4-(10-methylaminodecyloxy)benzothiophene HCl (5)

The titled product **5** (1.1g, 80%) was obtained from the treatment of intermediate **4c** (1.5g, 3.3mmol) and an excess of methylamine in ethanol (1.4ml, 33.3mmol) by general methods **B&C** repectively.

m.p. 97-99°C (Lit.[127] m.p. 98-101°C)

δ_H (DMSO): 7.59 (2-***H***, d, J 5.44Hz), 7.37 (3-***H***, 6-H, m), 6.34 (5-***H***, d, J 8.41Hz), 4.05 ($OCH_2(CH_2)_9NHCH_3$), t, J 6.43Hz), 3.64 (***NH***, bs, disappeared after D_2O shake), 2.55 ($O(CH_2)_9CH_2NHCH_3$, t, J 6.94Hz), 1.83($CH_2CH_2(CH_2)_6CH_2CH_2NHCH_3$, m), 2.42 ($NHCH_3$, s) 1.48 ($O(CH_2)_2(CH_2)_6(CH_2)_2NHCH_3$, m); δ_C ($CDCl_3$): 153.9 (4-***C***), 139.8 (7a-***C***), 130.4 (6-***C***), 127.6 (3a-***C***), 125.3 (2-***C***), 121.8 (3-***C***), 106.5 (5-***C***), 106.0 (7-***C***), 68.4 ($OCH_2(CH_2)_9NHCH_3$), 52.3 ($O(CH_2)_9CH_2NHCH_3$), 36.6 ($O(CH_2)_{10}NHCH_3$), 32.8 ($O(CH_2)_8CH_2CH_2$), 29.4 ($OCH_2CH_2CH_2(CH_2)_6CH_2$), 26.1 ($O(CH_2)_2CH_2(CH_2)_7NHCH_3$).

4.2 Preparation of 6-chloro-2-(10-methylaminodecyloxy)naphthalene (16)

(1) Preparation of 6-chloro-2-methoxynaphthalene (10)

To a solution of 6-bromo-2-methoxynaphthalene (6.0g, 25.3mmol) dissolved in THF (120ml) at -78°C (acetone/dry ice) under an atmosphere of nitrogen was added *n*-butyllithium (10.5ml, 26.4mmol) (2.5M in hexane), over a period of 20min. The pale yellow homogeneous mixture was stirred for a further 30min. A solution of hexachloroethane (12.0g, 50.7mmol) in THF (15ml) was added dropwise over a period of 20min. The mixture was stirred at -78°C for one hour, and allowed to attain room temperature. The reaction was quenched by addition to a mixture of ice and 2M HCl, and diluted with water. The mixture was extracted with EtOAc. All the organic layers were combined and partitioned with water, sat. NaCl, and dried over $MgSO_4$, filtered. The solvent was removed under reduced pressure. The residue was recrystallised from ethanol to give the chloronaphthoether as white needle-like crystal (3.7g, 77%).

m.p. 64-65°C (Lit. [185] m.p. 64-65°C)

δ_H ($CDCl_3$): 7.74 (1-***H***, d, J 2.23Hz), 7.68 (8-***H***, d, J 8.91Hz), 7.66 (4-***H***, d, J 8.41Hz), 7.39 (3-***H***, dd, J 2.23Hz, 8.65Hz), 7.14 (7-***H***, dd, 2.47Hz, 8.91Hz), 7.10 (5-***H***, d, J 2.48Hz), 3.90 (O***CH***$_3$, 3H, s); δ_C ($CDCl_3$): 157.8 (6-***C***), 132.8 (4a-***C***), 129.5 (8a-***C***), 129.1 (2-***C***), 128.3 (8-***C***), 128.2 (4-***C***), 127.2 (3-***C***), 126.4 (1-***C***), 119.8 (7-***C***), 105.7 (5-***C***), 55.3 (O***CH***$_3$).

(2) Preparation of 6-chloro-2-hydroxynaphthalene (12)

To a solution of boron tribromide (1M in DCM, 20ml, 20mmol) in DCM (50ml) at -78°C, was added a similarly cooled solution of 6-chloro-2-methoxynaphthalene (3.3g, 17.3mmol) in dry DCM (20ml) with needle-transfer. The reaction was protected by a calcium chloride drying tube. The mixture was allowed to attain room temperature and kept stirring overnight. T.l.c (petroleum ether 60-80°C: EtOAc/ 10:3) indicated the disappearance of starting material (Rf 0.38), and the formation of product (Rf 0.06). The reaction was quenched by adding water, kept stirring for half hour. The mixture was extracted with ether. All the organic layers were combined and washed with sat. NaCl, dried over $MgSO_4$, filtered. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 10:3) and crystallized from ethanol to give the chloronaphthol (1.8g, 61%).

m.p. 119-121°C (Lit. [185] m.p. 115°C)

δ_H (DMSO): 9.09 (***OH***, brs, disappeared after D_2O shake), 7.69 (1-***H***, d, J 1.73Hz), 7.63 (4-***H***, d, J 9.65Hz), 7.59 (8-***H***, d, J 8.66Hz), 7.32 (3-***H***, dd, J 2.22Hz, 8.66Hz), 7.16 (7-***H***, dd, J 2.23Hz, 9.37Hz) 7.12 (5-***H***, d, J 2.23Hz); δ_C (DMSO): 155.0 (6-***C***), 132.5 (4a-***C***), 127.9 (8a-***C***), 127.7 (2-***C***), 127.2 (8-***C***), 127.1 (4-***C***), 126.0 (3-***C***), 125.5 (1-***C***), 119.1 (7-***C***), 108.5 (5-***C***); ν_{max}/cm^{-1} (KBr disc): 3286.1, 1627.7, 1592.9 cm^{-1}.

(3) Preparation of 6-chloro-2-(10-tetrahydropyranyloxydecyloxy)naphthalene (21)

The treatment of phenol **12** (0.7g, 3.8mmol) with alkylating reagent **96** (1.2g, 3.8mmol) by general method D gave the desired product as a colourless oil (1.2g, 76%).

δ_H ($CDCl_3$): 7.71 (1-***H***, d, J 1.98Hz), 7.63 (4-***H***, 8-***H***, d, J 8.66Hz), 7.35 (3-***H***, dd, 1.98Hz, 8.66Hz), 7.16 (7-***H***, dd, J 2.47Hz, 8.90Hz), 7.06 (5-***H***, d, J 2.47Hz), 4.56 (2'-***H***, t, J 4.20Hz), 4.04 (OCH_2, t, J 6.43Hz), 3.70 (6'-***H***, m), 3.35 ($(CH_2)_9CH_2O$, m), 1.77 (OCH_2CH_2, m), 1.53 (4', 5'-***H***, m), 1.30 ($O(CH_2)_2(CH_2)_7CH_2O$, m); δ_C ($CDCl_3$): 157.3 (6-***C***), 132.8 (4a-***C***), 129.3 (8a-***C***), 128.8 (2-***C***), 128.4 (8-***C***), 128.1 (4-***C***), 127.0 (3-***C***), 126.3 (1-***C***), 120.1 (7-***C***), 106.4 (5-***C***), 98.8 (2'-***C***), 68.0 (OCH_2), 67.6 ($(CH_2)_9CH_2O$), 62.3 (6'-***C***), 30.7 (3'-***C***), 29.7-29.1 ($OCH_2CH_2CH_2(CH_2)_4CH_2CH_2$), 26.2 (5'-***C***), 26.0 ($O(CH_2)_2CH_2$), 25.5 ($O(CH_2)_7CH_2$), 19.7 (4'-***C***); m/z (EI): 419.5 $[M+H]^+$ ($C_{25}H_{35}ClO_3$ requires [M] 418.2).

(4) Preparation of 6-chloro-2-(10-hydroxydecyloxy)naphthalene (23)

Intermediate **21** (1.1g, 2.5mmol) was treated with PPTS (0.06g, 0.25mmol) following general method **E** to give alcohol as a colourless solid (0.8g, 92%).

m.p. 67-69°C

δ_H ($CDCl_3$): 7.66 (1-***H***, d, J 1.58Hz), 7.59 (4-***H***, 8-***H***, d, J 8.69Hz), 7.35 (3-***H***, dd, 1.58Hz, 8.68Hz), 7.16 (7-***H***, dd, J 2.47Hz, 8.91Hz), 7.06 (5-***H***, d, J 2.23Hz), 4.73 (***OH***, bs, disappeared after D_2O shake) 3.98 (OCH_2, t, J 6.44Hz), 3.57 ($(CH_2)_9CH_2O$, t, J 6.43Hz), 1.77 (OCH_2CH_2, m), 1.38 ($O(CH_2)_8CH_2$, m) 1.25 ($O(CH_2)_2(CH_2)_6(CH_2)_2OH$, m); δ_C ($CDCl_3$): 157.3 (6-***C***), 132.9 (4a-***C***), 128.9 (8a-***C***), 128.4 (2-***C***), 128.1 (8-***C***), 127.1 (4-***C***), 126.4 (3-***C***), 125.4 (1-***C***), 120.1 (7-***C***), 106.5 (5-***C***), 68.1 (OCH_2), 63.1 (CH_2OH), 32.8

(***CH₂***CH_2OH), 29.5-29.1 (OCH_2***CH_2***CH_2***$(CH_2)_4$***), 26.1 ($O(CH_2)_2$***CH_2***), 25.7 ($O(CH_2)_7$***CH_2***$(CH_2)_2OH$); ν_{max}/cm^{-1}: 3286.0, 1637.1, 1592.2, 1213.8, 819.0cm^{-1}; m/z (EI): $[M+H]^+$ 335, ($C_{20}H_{27}ClO_2$ requires [M] 334).

(5) Preparation of 2-chloro-6-(10-bromodecyloxy) naphthalene (14)

The treatment of alcohol **23** (0.8g, 2.3mmol) with a combination reagent CBr_4(0.1g, 2.9mmol)/ Ph_3P (0.9g, 3.5mmol) led to the formation of bromide **14** (0.9g, 97%) as a colourless oil by general method **F**. Bromide **14** was obtained directly from phenol **12** (1.8g, 10mmol) with an excess of dibromodecane (1.5g, 50mmol) by general method **A** (2.4g, 62%).

δ_H ($CDCl_3$): 7.73 (1-***H***, d, J 1.98Hz), 7.65 (4-***H***, 8-***H***, d, J 8.90Hz), 7.35 (3-*H*, dd, J 2.23Hz, 8.90Hz), 7.16 (7-***H***, dd, J 2.23Hz, 8.90Hz), 7.09 (5-***H***, d, J 2.47Hz), 4.05 (**OCH_2**, t, J 6.43Hz), 3.57 (($CH_2)_9$***CH_2***Br, t, J 6.68Hz), 1.84 (OCH_2***CH_2***, m), 1.42 ($O(CH_2)_8$***CH_2***, m) 1.32 ($O(CH_2)_2$***$(CH_2)_6$***$(CH_2)_2OH$, m); δ_C ($CDCl_3$): 157.4 (6-***C***), 132.9 (4a-***C***), 129.3 (8a-***C***), 128.9 (2-***C***), 128.4 (8-***C***), 128.1 (4-***C***), 127.1 (3-***C***), 126.3 (1-***C***), 120.1 (7-***C***), 106.5 (5-***C***), 68.0 (OCH_2), 34.0 (***CH_2***Br), 32.8 (***CH_2***CH_2Br), 29.5-29.1 (OCH_2***CH_2***CH_2***$(CH_2)_5$***), 26.1 ($O(CH_2)_2$***CH_2***).

(6) Preparation of 2-chloro-6-(10-methylaminodecyloxy)naphthalene HCl (16)

The title compound **16** (0.4g, 61%) was prepared from bromide **14** (0.8g, 2mmol) and an excess of methylamine (33%) in ethanol (2.5ml, 20mmol) by general methods **B** & **C.**

m.p. 140-141°C (Lit. [127] m.p. 141-143°C)

δ_H (DMSO): 9.45 (***NH***, bs, disappeared after D_2O shake), 7.73 (1-***H***, d, J 1.83Hz), 7.64 (4-***H***, 8-***H***, d, J 8.66Hz), 7.35 (3-***H***, dd, J 2.23Hz, 8.66Hz), 7.16 (7-***H***, dd, J 2.72Hz, 8.91Hz), 7.09 (5-***H***, d, J 2.47Hz), 4.05 (OCH_2, t, J 6.67Hz), 2.98 ($NHCH_3$, s) 2.48 ($(CH_2)_9CH_2NHCH_3$, t, J 7.92Hz), 1.84 (OCH_2CH_2, m), 1.42 ($O(CH_2)_2(CH_2)_7$, m); δ_C (DMSO) : 158.3 (6-***C***), 132.9 (4a-***C***), 129.3 (8a-***C***), 128.9 (2-***C***), 128.4 (8-***C***), 128.2 (4-***C***), 127.0 (3-***C***), 126.3 (1-***C***), 120.1 (7-***C***), 106.5 (5-***C***), 74.9 (OCH_2), 50.3 ($(CH_2)_9CH_2NH$) 33.9 ($NHCH_3$), 31.4 ($(CH_2)_8CH_2CH_2NH$), 29.5-28.1 ($OCH_2CH_2CH_2(CH_2)_4$), 27.3 ($O(CH_2)_7CH_2$) 26.1 (OCH_2CH_2); m/z (EI): 348 $[M+H]^+$ ($C_{21}H_{30}ClNO$ requires [M] 347).

4.3 Preparation of 6-fluoro-2-(10-methylaminodecyloxy)naphthalene (17)

(1) Preparation of 6-fluoro-2-methoxynaphthalene (11)

n-Butyllithium (6.7ml, 16.5mmol) in hexane (2.5M) was added slowly to the 6-bromo-2-methoxynaphthalene (3.6g, 15mmol), dissolved in dry THF (40ml) over 20min at -78°C. The yellow homogeneous mixture was stirred for further 30min. *N*-Fluorobenzenesulphonimide (5.2g, 16.5mmol) in THF (15ml) was added dropwise over 15 min at the same temperature under nitrogen. The red → yellow homogeneous mixture was kept stirring at -78°C for another 1 hour and quenched by adding to ice (50g) and 2M HCl (20ml), diluted with water (25ml). The mixture was extracted with EtOAc (60ml×2). All the organic layers were combined, and washed with water, sat. NaCl, dried over $MgSO_4$, filtered. The solvent was removed *in vacuo*. The residue was purified by flash chromatography with petroleum ether 60-80°C: EtOAc (10:1) to give a colourless product (1.8g, 70%).

m.p. 59-61°C (Lit.[186] m.p. 59-60°C)

δ_H ($CDCl_3$): 7.72 (1-***H***, d, J 2.23Hz), 7.70 (4-***H***, 8-H, m), 7.38(3-***H***, dd, J 2.47Hz, 8.40Hz), 7.20 (7-***H***, dd, J 2.47Hz, 8.90Hz), 7.13 (5-***H***, d, J 2.72Hz), 3.92 (O***CH***$_3$, 3H, s); δ_C ($CDCl_3$): 161.1 (2-***C***), 157.0 (6-***C***), 131.4 (4a-***C***), 129.4 (8a-***C***), 129.2 (4-***C***), 128.8 (8-***C***), 119.8 (7-***C***), 116.7 (3-***C***), 110.7 (1-***C***), 105.7 (5-***C***), 55.3 (O***CH***$_3$).

(2) Preparation of 6-fluoro-2-hydroxynaphthalene (13)

To a solution of borontribromide (1M in DCM, 11ml, 11mol) in DCM (35ml) at -78°C, was added a similarly cooled solution of 6-fluoro-2-methoxynaphthalene (1.8g, 10mmol) in dry DCM (35ml) slowly with a needle-transfer. The mixture was allowed to attain room temperature and kept stirring overnight, and protected by a calcium chloride drying tube. T.l.c (petroleum ether 60-80°C: EtOAc/ 10:3) indicated the disappearance of starting material (Rf 0.36), and the formation of a new product (Rf 0.08). The reaction was quenched by adding water (50ml), kept stirring for half hour. The mixture was extracted with diethyl ether. All the organic layers were combined and washed with sat. NaCl, dried over $MgSO_4$, filtered. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 10:3) to give the colourless fluoronaphthol (1.3g, 78%).

m.p. 115-116°C (Lit.[186] m.p. 116-117.5°C)

δ_H ($CDCl_3$): 7.70 (1-***H***, d, J 2.97Hz), 7.65 (4-***H***, 8-H, m), 7.37 (3-***H***, dd, J 2.72Hz, 9.89Hz), 7.22 (7-***H***, dd, J 2.47Hz, 8.91Hz), 4.92 (***OH***, brs, disappeared after D_2O shake); δ_C ($CDCl_3$): 157.5 (2-***C***), 152.7 (6-***C***), 131.4 (4a-***C***), 129.9 (8a-***C***), 129.1 (8-***C***), 128.5 (4-***C***),

118.1 (7-***C***), 116.7 (3-***C***), 110.7 (1-***C***), 109.5 (5-***C***); ν_{max}/cm^{-1}(KBr disc): 3266.0, 1608.7, 1514.5, 1231.6, 872.4, 806.8cm^{-1}; m/z (EI): 163 $[M+H]^+$ ($C_{10}H_7FO$ requires [M] 162).

(3) Preparation of 6-fluoro-2-(10-tetrahydropyranyloxydecyloxy)naphthalene (22)

Intermediate **22** (0.6g, 70%), a colourless oily product, was obtained by the treatment of fluoronaphthol **13** (0.4g, 2.2mmol) with alkylating reagent $Br(CH_2)_{10}OTHP$ (0.8g, 2.4mmol) following general method **D**.

δ_H ($CDCl_3$): 7.63 (4-***H***, 8-***H***, m), 7.28 (3-***H***, dd, J 2.44Hz, 9.64Hz), 7.16-7.08 (1-***H***, 3-***H***, 7-H, m), 4.50 (2'-***H***, t, J 4.20Hz), 3.97 (OCH_2, t, J 6.44Hz), 3.70 ($O(CH_2)_9CH_2OTHP$, m), 3.31 (6'-***H***, m), 1.76 ($OCH_2CH_2(CH_2)_8$, 3'-H, m), 1.52-1.20 ($O(CH_2)_2(CH_2)_6(CH_2)_2$, 4'-***H***, 5'-H, m); δ_C($CDCl_3$): 161.0 (2-***C***), 157.4 (6-***C***), 131.5 (4a-***C***), 129.2 (8a-***C***), 129.1 (4-***C***), 128.7 (8-***C***), 120.1 (7-***C***), 116.6 (3-***C***), 110.9 (1-***C***), 106.7 (5-***C***), 98.9 (2'-***C***), 68.1 (OCH_2), 67.7 ($O(CH_2)_9CH_2OTHP$), 62.3 (6'-***C***), 31.9 (3'-***C***), 30.8 ($O(CH_2)_8CH_2CH_2OTHP$), 29.7-29.2 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3OTHP$), 26.2 (5'-***H***), 26.1 ($O(CH_2)_2CH_2(CH_2)_7OTHP$), 25.5 ($O(CH_2)_7CH_2(CH_2)_2OTHP$), 19.7 (4'-***C***); ν_{max}/cm^{-1} (liquid film): 1608.7, 1232.5, 866.2cm^{-1}; m/z (EI): 403 $[M+H]^+$ ($C_{25}H_{35}FO_3$ requires [M] 402).

(4) Preparation of 6-fluoro-2-(10-hydroxydecyloxy)naphthalene (24)

THP ether **22** (0.5g, 1.3mmol) was converted to alcohol **24** with PPTS (0.035g, 0.13mmol) by general method **E** in good yield (0.4g, 98%)

m.p.: 66-68°C

δ_H ($CDCl_3$): 8.07 (***OH***, brs, disappeared after D_2O shake), 7.63 (4-***H***, 8-***H***, m), 7.28 (3-***H***, dd, J 2.73Hz, 9.37Hz), 7.16-7.08 (1-***H***, 3-*H*, 7-***H***, m), 4.04 (OCH_2, t, J 6.68Hz), 3.55

($O(CH_2)_9\boldsymbol{CH_2}OH$, t, J 6.68Hz), 1.77 ($OCH_2\boldsymbol{CH_2}(CH_2)_8$, m), 1.48 ($O(CH_2)_8\boldsymbol{CH_2}CH_2OH$, m), 1.21 ($O(CH_2)_2\boldsymbol{(CH_2)_6}(CH_2)_2OH$, m); δ_C ($CDCl_3$): 159.0 (2-***C***), 158.4 (6-***C***), 131.5 (4a-***C***), 128.7 (8a-***C***), 128.6 (4-***C***), 128.5 (8-***C***), 120.1 (7-***C***), 116.6 (3-***C***), 110.9 (1-***C***), 106.7 (5-***C***), 68.1 ($O\boldsymbol{CH_2}(CH_2)_9OH$), 63.1 ($O(CH_2)_9\boldsymbol{CH_2}OH$), 32.8 ($O(CH_2)_8\boldsymbol{CH_2}CH_2OH$), 29.4 ($OCH_2\boldsymbol{CH_2}CH_2\boldsymbol{(CH_2)_4}(CH_2)_3OH$), 26.1 ($O(CH_2)_2\boldsymbol{CH_2}(CH_2)_7OH$), 25.5 ($O(CH_2)_7\boldsymbol{CH_2}(CH_2)_2OH$); ν_{max}/cm^{-1} (KBr disc): 3364.1, 1609.0, 1512.1, 1244.1, 872.8, 857.2cm^{-1}; m/z (EI): 319 $[M+H]^+$ ($C_{20}H_{27}FO_2$ requires [M] 318).

(5) Peparation of 6-fluoro-2-(10-bromodecyloxy)naphthalene (15)

Bromide **15** was prepared from alcohol **24** (0.4g, 1.2mmol), and combination bromination reagent CBr_4 (0.5g, 1.45mmol)/ Ph_3P (0.5g, 1.7mmol) by general method **F** in good yield (0.4g, 99%). Alkylating of phenol **13** (1.2g, 7.1mmol) with an excess of bromodecane (10.7g, 35.7mmol) by general method **A** also led bromide **15** (1.7g, 62%).

m.p. 59-61°C (Lit. [127] m.p. 65-66°C)

δ_H ($CDCl_3$): 7.63 (4-***H***, 8-***H***, m), 7.39 (3-***H***, dd, J 2.22Hz, 9.65Hz), 7.16-7.08 (1-***H***, 3-***H***, 7-***H***, m), 4.04 ($O\boldsymbol{CH_2}$, t, J 6.44Hz), 3.55 ($O(CH_2)_9\boldsymbol{CH_2}OH$, t, J 6.93Hz), 1.77 ($OCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}CH_2Br$, m), 1.29 ($O(CH_2)_2\boldsymbol{(CH_2)_6}(CH_2)_2Br$, m); δ_C ($CDCl_3$): 158.6 (2-***C***), 156.3 (6-***C***), 131.4 (4a-***C***), 129.3 (8a-***C***), 129.1 (4-***C***), 128.5 (8-***C***), 120.1 (7-***C***), 116.6 (3-***C***), 110.9 (1-***C***), 106.7 (5-***C***), 68.1 ($O\boldsymbol{CH_2}(CH_2)_9Br$), 34.0 ($O(CH_2)_9\boldsymbol{CH_2}Br$), 32.8 ($O(CH_2)_8\boldsymbol{CH_2}CH_2Br$), 29.4 ($OCH_2\boldsymbol{CH_2}CH_2\boldsymbol{(CH_2)_5}(CH_2)_2Br$), 26.1 ($O(CH_2)_2\boldsymbol{CH_2}(CH_2)_7Br$).

(6) Preparation of 6-fluoro-2-(10-methylaminodecyloxy) naphthalene (17)

The title compound **17** (0.2g, 53%) was prepared from bromide **15** (0.4g, 1.2mmol) and an excess of 33% methylamine in ethanol (1.4ml, 11.5mmol) by general methods **B** & **C** respectively.

m.p. 138-140°C (Lit. [127] m.p. 140-143°C)

δ_H (DMSO): 8.67 (***NH***, brs, disappeared after D_2O shake), 7.86-7.76 (4-***H***, 8-***H***, m), 7.35 (3-***H***, dd, J 2.72Hz, 10.39Hz), 7.30 (1-***H***, 5-***H***, m), 7.17 (7-***H***, dd, J 1.98Hz, 8.66Hz), 5.67 (***NH***, brs, appeared after D_2O shake), 4.04 ($OCH_2(CH_2)_9Br$, 2H, t, J 2.47Hz), 3.31 ($NHCH_3$, s) 2.78 ($O(CH_2)_9CH_2$, 2H, t, J 7.42Hz), 1.74 ($OCH_2CH_2(CH_2)_6CH_2CH_2$, 4H, m), 1.41 ($O(CH_2)_8CH_2CH_2$, m), 1.22 ($O(CH_2)_2(CH_2)_6(CH_2)_2NHCH_3$, m); δ_C (DMSO): 158.6 (2-***C***), 156.3 (6-***C***), 131.4 (4a-***C***), 129.3 (8a-***C***), 129.1 (4-***C***), 128.5 (8-***C***), 120.1 (7-***C***), 116.6 (3-***C***), 110.9 (1-***C***), 106.7 (5-***C***), 67.5 ($OCH_2(CH_2)_9NHCH_3$), 48.1 ($O(CH_2)_9CH_2NHCH_3$), 32.3 ($NHCH_3$), 28.7 ($OCH_2CH_2CH_2(CH_2)_4CH_2CH_2CH_2$), 25.8 ($O(CH_2)_7CH_2(CH_2)_2$), 25.5 ($O(CH_2)_2CH_2(CH_2)_7NHCH_3$); ν_{max}/cm^{-1}: 3454.1, 1609.7, 1240.9cm^{-1}; m/z(EI): 332 $[M+H]^+$ ($C_{21}H_{30}FNO$ requires [M] 331).

4.4 Preparation of *N*-allyl-[10-(6-bromonaphthalen-2-yloxy)decyl]amine HCl (25)

(1) Preparation of *N*-[10-(6-bromonaphthalen-2-yloxy)decyl]-2,2,2-trifluoroacetamide (28)

A DMF (5.4ml) solution of trifluoroacetamide (1.0g, 9mmol), tetra-*n*-butylammonium bromide (0.15g, 0.45mmol) and powdered K_2CO_3 (1.2g, 9mmol) placed in a flask with reflux condenser with an attached drying tube was treated with 2-bromo-6-

(10-bromodecyloxy)naphthalene (2g, 4.5mmol). The mixture was heated to 50°C (bath temperature) and kept stirring overnight. Tlc (petroleum ether 60-80°C: EtOAc/ 4:1) showed the full conversion of starting material (Rf 0.65) to a new compound (Rf 0.43). The reaction was cooled down to room temperature and filtered. The filter cake (inorganic precipitate) was washed with DCM and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether 60-80°C: ethyl acetate/ 8:2) to give acetoamide as a colourless solid (1.4g, 68%).

m.p. 88-90°C

δ_H ($CDCl_3$): 7.89 (1-***H***, d, J 1.98Hz), 7.65 (8-***H***, d, J 8.91Hz), 7.59 (4-***H***, d, J 8.78Hz), 7.49 (3-***H***, dd, J 1.98Hz, 8.58Hz), 7.16 (7-***H***, dd, J 2.64Hz, 8.91Hz), 7.07 (5-***H***, d, J 2.63Hz), 6.27 (***NH***, brs, disappeared after D_2O shake), 4.05 ($OCH_2(CH_2)_9$, t, J 6.27Hz), 3.36 ($O(CH_2)_9CH_2$, t, J 6.93Hz), 1.81 ($OCH_2CH_2(CH_2)_8$, m), 1.49 ($O(CH_2)_2(CH_2)_7CH_2$, m); δ_C ($CDCl_3$): 171.3 (*C*OCF_3), 158.6 (6-***C***), 138.2 (4a-***C***), 129.7 (1, 3-***C***), 128.8 (4, 8-***C***), 127.6 (8a-***C***), 123.2 ($COCF_3$), 118.2 (7-***C***), 116.3 (2-***C***), 103.2 (5-***C***), 68.2 ($OCH_2(CH_2)_9$), 40.0 ($O(CH_2)_9CH_2NH$), 29.4 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3$), 28.1 ($O(CH_2)_7CH_2(CH_2)_2$), 25.7 ($O(CH_2)_2CH_2(CH_2)_7$); ν_{max}/cm^{-1}: 3311.4, 1695.9cm^{-1}; m/z (EI): 474/476 $[M+H]^+$ ($C_{22}H_{27}BrF_3NO_2$ requires [M] 473/ 475).

(2) Preparation of *N*-allyl-*N*-[1-(6-bromonaphthalene-2-yloxy)decyl]-2,2,2-trifluoro acetamide (29)

A solution of trifluoroacetamide **28** (0.9g, 2.0mmol), allyl bromide (0.36ml, 4.1mmol), *tetra-n*-butylammoniumbromide (0.2g, 0.6mmol), and powdered anhydrous K_2CO_3 (2.5g, 18mmol) in dry acetonitrile (10ml) was heated to 80°C with stirring. Tlc

(petroleum ether 60-80°C: diethyl ether/ 5:1) indicated the disappearance of starting material (Rf 0.11) and the formation of a new compound (Rf 0.32) after 3 hours. The cooled mixture was filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (Et_2O: petroleum ether 60-80°C/ 1:5) to give product as pale yellow oil (0.6g, 63%).

δ_H ($CDCl_3$): 7.89 (1-***H***, d, J 1.96Hz), 7.65 (8-***H***, d, J 8.91Hz), 7.59 (4-***H***, d, J 8.44Hz), 7.49 (3-***H***, dd, J 1.98Hz, 8.45Hz), 7.16 (7-***H***, dd, J 2.64Hz, 8.91Hz), 7.07 (5-***H***, d, J 2.64Hz), 5.80 (CH_2***CH***=CH_2, m), 5.38 (CH_2CH=***CH***$_2$, m), 4.05 (O***CH***$_2(CH_2)_9$, ***CH***$_2CH$=CH_2, m), 3.30 ($O(CH_2)_9$***CH***$_2N$), 1.86 (OCH_2***CH***$_2(CH_2)_8$, m), 1.47 ($O(CH_2)_8$***C***H_2CH_2, m), 1.20 ($O(CH_2)_2$(***CH***$_2$)$_6(CH_2)_2N$); δ_C ($CDCl_3$): 157.4 (***C***OCF_3), 156.3 (6-***C***), 133.0 (4a-***C***), 132.0 (CH_2***CH***=CH_2), 131.2 (1, 3-***C***), 129.4 (4, 8-***C***), 128.3 (8a-***C***), 120.0 (CO***CF***$_3$), 118.8 (7-***C***), 116.7 (2-***C***), 114.4 (CH_2CH=***CH***$_2$), 106.3 (5-***C***), 67.9 (O***CH***$_2(CH_2)_9$), 49.7 (***CH***$_2CH$=CH_2), 47.8 ($O(CH_2)_9$***CH***$_2$), 29.8 (OCH_2***CH***$_2CH_2$(***CH***$_2$)$_4CH_2$***CH***$_2CH_2$), 26.4 ($O(CH_2)_7$***CH***$_2(CH_2)_2$), 26.0 ($O(CH_2)_2$***CH***$_2(CH_2)_7$); ν_{max}/cm^{-1} (liquid film): 1690.6 cm^{-1}; m/z (EI): 514/516 $[M+H]^+$ ($C_{25}H_{31}BrF_3NO_2$ requires [M] 513/ 515).

(3) Preparation of *N*-allyl-[10-(6-bromonaphthalen-2-yloxy)decyl]amine (25)

20% Aqueous NaOH (1ml) was added into a solution of trifluoroacetamide (0.9g, 1.8ml) in ethanol (10ml). The reaction was kept shaking overnight. T.l.c. (Et_2O: petroleum ether 60-80°C/1:5) indicated that reaction completed. Ethanol was removed under reduced pressure and the residue was suspended in a small amount of DCM. The resulting white precipitate was filtered off and dried in the air (0.7g, 92%). The hydrochloric acid salt was made with ethereal hydrochloric acid by general method **C**.

m.p. 52-53°C

δ_H ($CDCl_3$): 7.87 (1-***H***, d, J 1.99Hz), 7.68 (8-***H***, d, J 8.90Hz), 7.59 (4-***H***, d, J 8.44Hz), 7.43 (3-***H***, dd, J 1.90Hz, 8.47Hz), 7.16 (7-***H***, dd, J 2.64Hz, 8.91Hz), 7.07 (5-***H***, d, J 2.64Hz), 5.80 (CH_2***CH***=CH_2, m), 5.15 (CH_2CH=***CH_2***, m), 4.04 (O***CH_2***$(CH_2)_9$,t, J 6.59Hz), 3.22 (***CH_2***$CH=CH_2$, d, J 6.84Hz), 2.55 $(O(CH_2)_9$***CH_2***NH, t, J 7.59Hz), 1.89 (OCH_2***CH_2***$(CH_2)_8$, m), 1.30 $(O(CH_2)_8$***CH_2***CH_2, m), 1.20 $(O(CH_2)_2$***$(CH_2)_6$***$(CH_2)_2NH)$; δ_C ($CDCl_3$): 157.4 (***C***OCF_3), 137.1 (6-***C***), 133.0 (4a-***C***), 132.0 (CH_2***CH***=CH_2), 131.2 (1, 3-***C***), 129.4 (4, 5, 8-***C***), 128.3 (8a-***C***), 120.0 (CO***CF_3***), 118.8 (7-***C***), 116.7 (2-***C***), 115.9 (CH_2CH=***CH_2***), 68.1 (O***CH_2***$(CH_2)_9$), 52.6 (***CH_2***$CH=CH_2$), 49.5 $(O(CH_2)_9$***CH_2***), 30.1 $(O(CH_2)_8$***CH_2***CH_2), 29.6-29.2 (OCH_2***CH_2***CH_2***$(CH_2)_4$***), 27.4 $(O(CH_2)_7$***CH_2***$(CH_2)_2$), 26.1 $(O(CH_2)_2$***CH_2***$(CH_2)_7$); ν_{max}/cm^{-1}: 2919.6, 1625.7, 1587.3cm^{-1}; m/z (EI): 418.5/420.5 $[M+H]^+$ ($C_{23}H_{32}BrNO$ requires [M] 417/419); (Found: C 66.0%, H 7,3%, N 3.2%; $C_{23}H_{32}BrNO$ requires: C 66.2%, H 7.7%, N 3.4%).

4.5 Preparation of {3-[10-(6-bromonaphthalen-2-yloxy)decane-1-sulfinyl]propyl} methylamine HCl (30)

(1) Preparation of 2-bromo-6-(10-bromodecyloxy)naphthalene (27)

Bromide **27** was converted from 2-bromo-6-naphthol (2.5g, 11.2mmol) with an excess of 1,10-dibromodecane (16.8g, 56.1mmol) via general method **A** (3.3g, 67%).

m.p. 67-69°C (Lit [127] m.p. 70-72°C)

δ_H ($CDCl_3$): 7.89 (1-***H***, d, J 1.49Hz), 7.64 (8-***H***, d, J 9.15Hz), 7.55 (4-***H***, d, J 8.90Hz), 7.49 (3-***H***, dd, J 1.73Hz, 8.91Hz), 7.15 (7-***H***, dd, J 2.23Hz, 9.16Hz), 7.05 (5-***H***, d, J 2.23Hz),

4.04 ($OCH_2(CH_2)_9Br$, t, J 6.44Hz), 3.41 ($O(CH_2)_9CH_2Br$, t, J 6.93Hz), 1.82 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.40 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_c ($CDCl_3$): 155.9 (6-*C*), 137.8 (4a-*C*), 129.8 (1-*C*, 3-*C*), 128.7 (4-*C*, 8-*C*), 126.5 (8a-*C*), 118.7 (7-*C*), 116.2 (2-*C*), 103.9 (5-*C*), 67.8 ($OCH_2(CH_2)_9Br$), 33.9 ($O(CH_2)_9CH_2Br$), 32.8 ($O(CH_2)_8CH_2CH_2Br$), 29.8 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3Br$), 28.1 ($O(CH_2)_8CH_2CH_2Br$), 25.9 ($O(CH_2)_2CH_2(CH_2)_7Br$).

(2) Preparation of 3-[10-(6-bromonaphthalen-2-yloxy)decylsulfanyl]propionic acid ethyl ester (34)

Ethyl 3-mercaptopropionate (2.2ml, 17mmol) was added to a mixture of 6-bromo-2-(10-bromodecyloxy)naphthalene (3.0g, 6.8mmol), powdered anhydrous potassium carbonate (2.3g, 17mmol), and small amount of sodium metabisulfate (0.24g) suspended in dry 2-butanone (34ml) at room temperature under an atmosphere of nitrogen. The mixture was heated to reflux and kept stirring until t.l.c (petroleum ether 60-80°C: EtOAc/ 4:1) showed the disappearance of starting material (Rf 0.66) and the formation of product (Rf 0.54). The reaction was stopped and cooled down to room temperature. 2-Butanone was removed *in vacuo,* the residue was suspended in DCM (50ml) and filtered. The filtrate was washed with water, sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 10:1) to give the product ether as a colourless solid (2.4g, 70%).

m.p. 63-65°C

δ_H ($CDCl_3$): 7.90 (1-***H***, d, J 1.73Hz), 7.65 (8-***H***, d, J 9.16Hz), 7.60 (4-***H***, d, J 8.90Hz), 7.49 (3-***H***, dd, J 1.98Hz, 8.91Hz), 7.18 (7-***H***, dd, J 2.48Hz, 9.16Hz), 7.08 (5-***H***, d, J 2.47Hz), 4.16 ($COO\boldsymbol{CH_2}CH_3$, q, J 7.18Hz), 4.04 ($O\boldsymbol{CH_2}(CH_2)_9$, t, J 6.44Hz), 2.78 ($S\boldsymbol{CH_2}CH_2COOC_2H_5$, t, J 7.18Hz), 2.59 ($O(CH_2)_9\boldsymbol{CH_2}SCH_2\boldsymbol{CH_2}$, m), 1.83 ($OCH_2\boldsymbol{CH_2}(CH_2)_8$, m), 1.67-1.25 ($O(CH_2)_2\boldsymbol{(CH_2)_7}CH_2S(CH_2)_2COOCH_2\boldsymbol{CH_3}$, m); δ_C ($CDCl_3$): 172.0 ($\boldsymbol{C}OOC_2H_5$), 157.4 (6-***C***), 133.1 (4a-***C***), 129.5 (1-***C***, 3-***C***), 128.4 (4-***C***, 8-***C***), 120.1 (7-***C***), 116.9 (2-***C***), 106.5 (5-***C***), 68.1 ($O\boldsymbol{CH_2}(CH_2)_9$), 60.6 ($COO\boldsymbol{CH_2}CH_3$), 35.0 ($SCH_2\boldsymbol{CH_2}COOC_2H_5$), 32.8 ($O(CH_2)_9\boldsymbol{CH_2}S(CH_2)_2$), 32.3 ($O(CH_2)_8\boldsymbol{CH_2}CH_2S(CH_2)_2$) 29.6($OCH_2\boldsymbol{CH_2}CH_2\boldsymbol{(CH_2)_5}(CH_2)_2$), 28.9 ($S\boldsymbol{CH_2}CH_2$), 26.1 ($OCH_2\boldsymbol{CH_2}(CH_2)_8$), 14.2 ($COOCH_2\boldsymbol{CH_3}$); ν_{max}/cm^{-1}: 1724.2, 1588.9cm^{-1}; m/z (EI): 495/497 $[M+H]^+$ ($C_{25}H_{35}BrO_3S$ requires [M] 494/ 496); (Found: C 60%, H 7.1%; $C_{25}H_{35}BrO_3S$ requires: C 60.3%, H 7.1%).

(3) Preparation 3-[10-(6-bromonaphthalen-2-yloxy)decylsulfanyl]propan-1-ol (35)

To a solution of ester **34** (2.4g, 4.8mmol) and methanol (4ml, 9.5mmol) in dry THF (25ml) at room temperature under nitrogen, was added sodium borohydride (0.4g, 9.5mmol) slowly. The reaction was heated to reflux. T.l.c. (petroleum ether 60-80°C: EtOAc/ 4:1) showed the disappearance of starting material (Rf 0.68) and the formation of a new compound (Rf 0.22). The reaction was quenched by the addition of 2M HCl (50ml). The mixture was extracted by EtOAc (50ml×2), and the organic layers were partitioned by water, sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 4:1) to give the alcohol as a colourless solid (1.5g, 70%).

m.p. 71-72°C

δ_H ($CDCl_3$): 7.90 (1-***H***, d, J 1.73Hz), 7.61 (8-***H***, d, J 8.91Hz), 7.56 (4-***H***, d, J 8.66Hz), 7.49 (3-***H***, dd, J 1.73Hz, 8.66Hz), 7.18 (7-***H***, dd, J 2.23Hz, 8.91Hz), 7.08 (5-***H***, d, J 2.23Hz), 4.75 (***OH***, brs, disappeared after D_2O shake), 4.05 ($OCH_2(CH_2)_9$, t, J 6.43Hz), 3.76 ($S(CH_2)_2CH_2OH$, t, J 6.18Hz), 2.63($SCH_2(CH_2)_2OH$, t, J 7.17Hz), 2.52 ($O(CH_2)_9CH_2S$, t, J 4.51Hz), 1.83 ($OCH_2CH_2(CH_2)_8SCH_2CH_2CH_2OH$, m), 1.58 ($O(CH_2)_8CH_2CH_2S$, 2H, m) 1.37 ($O(CH_2)_2(CH_2)_6(CH_2)_2$, m); δ_C ($CDCl_3$):157.4 (6-***C***), 133.1 (4a-***C***), 129.9-128.3 (1, 3, 4, 8, 8a-***C***), 120.1 (7-***C***), 116.9 (2-***C***), 106.4 (5-***C***), 68.1 ($OCH_2(CH_2)_9$), 62.0 ($S(CH_2)_2CH_2OH$), 35.7 ($SCH_2CH_2CH_2OH$), 32.1 ($O(CH_2)_9CH_2S(CH_2)_3OH$), 29.6 ($OCH_2CH_2CH_2(CH_2)_6CH_2SCH_2(CH_2)_2OH$), 26.1 ($O(CH_2)_2CH_2(CH_2)_7$); ν_{max}/cm^{-1} (KBr disc): 3406.2, 1587.1 cm^{-1}; m/z (EI): 453/455 [M+H] ($C_{23}H_{33}BrO_2S$ requires [M] 452/454); (Found: C 60.6%, H 7.2%; $C_{23}H_{33}BrO_2S$ requires: C 61.0%, H 7.2%).

(4) Preparation 6-bromo-2-[10-(3-bromopropylsulfanyl)decyloxy]naphthalene (37)

To a magnetically stirred solution of the alcohol **35** (0.6g, 1.4mmol), and carbon tetrabromide (0.6g, 1.7mmol) dissolved in dry DCM (5ml), was added portionwise with ice-bath cooling triphenylphosphine (0.5g, 2.0mmol) DCM solution with needle-transfer following general method **F** to give bromide **37** (1.2g, 94%) as a colourless solid.

m.p. 52-53°C

δ_H ($CDCl_3$): 7.90 (1-***H***, d, J 1.97Hz), 7.61 (8-***H***, d, J 8.90Hz), 7.56 (4-***H***, d, J 8.91Hz), 7.49 (3-***H***, dd, J 1.98Hz, 8.66Hz), 7.18 (7-***H***, dd, J 2.47Hz, 8.91Hz), 7.08 (5-***H***, d, J 2.47Hz), 4.05 ($OCH_2(CH_2)_9$, t, J 6.50Hz), 3.52 ($S(CH_2)_2CH_2Br$, t, J 6.38Hz), 2.65($SCH_2(CH_2)_2Br$, t, J 6.92Hz), 2.51 ($O(CH_2)_9CH_2S$, , t, J 7.34Hz), 2.11 ($SCH_2CH_2CH_2Br$, m), 1.84

($OCH_2\boldsymbol{CH_2}(CH_2)_8S(CH_2)_3Br$, m), 1.50 ($O(CH_2)_2\boldsymbol{(CH_2)_7}CH_2CH_2S$, m); δ_C ($CDCl_3$): 157.4 (6-***C***), 133.1 (4a-***C***), 129.9-128.3 (1, 3, 4, 8, 8a-***C***), 120.1 (7-***C***), 116.9 (2-***C***), 106.4 (5-***C***), 68.1 ($O\boldsymbol{CH_2}(CH_2)_9$), 36.3 ($SCH_2\boldsymbol{CH_2}CH_2Br$), 32.4 ($O(CH_2)_9\boldsymbol{CH_2}S$), 32.3($O(CH_2)_8\boldsymbol{CH_2}CH_2S$), 32.1 ($S\boldsymbol{CH_2}CH_2\boldsymbol{CH_2}Br$), 29.6 ($OCH_2\boldsymbol{CH_2}CH_2\boldsymbol{(CH_2)_5}(CH_2)_2S$), 26.1 ($O(CH_2)_2\boldsymbol{CH_2}(CH_2)_7S$); ν_{max}/cm^{-1} (KBr disc): 1622.6, 1585.4, 1256.2cm^{-1}; m/z (EI): 515/517/519 $[M+H]^+$ ($C_{23}H_{32}Br_2OS$ requires [M] 514/516/518).

(5) Preparation of 6-bromo-2-[10-(3-bromopropane-1-sulfinyl)decyloxy]naphthalene (38)

Bromide **37** (0.5g, 1.0mmol) was dissolved in dry DCM (20ml), cooled to -10°C (ice/salt). To this stirred and cooled solution was added in one portion of *meta*-chloroperoxybenzoic acid (0.17g, 1mmol). The reaction was allowed to attain room temperature after 1hour. T.l.c (DCM: EtOH/ 50:1) showed the disappearance of starting material (Rf 0.9) and the formation of product (Rf 0.3). The mixture was diluted by DCM (20ml) and washed with sat. $NaHCO_3$ solution, sat. NaCl solution, dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give product as a colourless solid (0.4g, 80%).

m.p. 93-95°C

δ_H ($CDCl_3$): 7.90 (1-***H***, d, J 1.97Hz), 7.61 (8-***H***, d, J 8.16Hz), 7.56 (4-***H***, d, J 8.90Hz), 7.49 (3-***H***, dd, J 1.98Hz, 8.91Hz), 7.18 (7-***H***, dd, J 2.47Hz, 8.90Hz), 7.08 (5-***H***, d, J 2.48Hz), 4.05 ($O\boldsymbol{CH_2}(CH_2)_9$, t, J 6.44Hz), 3.56 ($S(CH_2)_2\boldsymbol{CH_2}Br$, t, J 6.42Hz), 2.78 ($O(CH_2)_9\boldsymbol{CH_2}SO\boldsymbol{CH_2}(CH_2)_2Br$, m), 2.37 ($SCH_2\boldsymbol{CH_2}CH_2Br$, 2H, m), 1.80 ($OCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}CH_2Br$, m), 1.50 ($O(CH_2)_2\boldsymbol{(CH_2)_6}(CH_2)_2S$, m); δ_C ($CDCl_3$): 157.4 (6-

C), 133.1 (4a-***C***), 129.9-128.3 (1, 3, 4, 8, 8a-***C***), 120.1 (7-***C***), 116.9 (2-***C***), 106.4 (5-***C***), 68.1 ($O\boldsymbol{C}H_2(CH_2)_9$), 52.8 ($O(CH_2)_9\boldsymbol{C}H_2SO$), 50.4 ($SO\boldsymbol{C}H_2(CH_2)_2Br$), 31.9-31.7 ($SOCH_2\boldsymbol{C}H_2\boldsymbol{C}H_2Br$), 29.4 ($OCH_2\boldsymbol{C}H_2CH_2(\boldsymbol{C}H_2)_5(CH_2)_2SO$), 26.1 ($O(CH_2)_8\boldsymbol{C}H_2CH_2SO$); ν_{max}/cm^{-1} (KBr disc): 1627.6, 1590.0, 1209.4, 1067.6cm^{-1}; m/z (EI): 531/533/535 $[M+H]^+$ ($C_{23}H_{32}Br_2O_2S$ requires 530/532/534); (Found: C 51.9%, H 5.9%; $C_{23}H_{32}Br_2O_2S$ requires: C 52.1%, H 6.0%).

(6) Preparation of {3-[10-(6-bromonaphthalen-2-yloxy)decane-1-sulfinyl]propyl} methylamine hydrochloride salt (30)

The title compound **30** (0.3g, 60%) was prepared from sulfoxide **38** (0.5g, 0.9mmol) and an excess of 33% methylamine in ethanol (1ml, 9.4mmol) by general methods **B** & **C** respectively.

m.p. 66-67°C

δ_H ($CDCl_3$): 7.90 (1-***H***, d, J 1.98Hz), 7.61 (8-***H***, d, J 8.16Hz), 7.56 (4-***H***, d, J 8.90Hz), 7.49 (3-***H***, dd, J 1.98Hz, 8.91Hz), 7.18 (7-***H***, dd, J 2.47Hz, 8.90Hz), 7.08 (5-***H***, d, J 2.48Hz), 4.67 ($\boldsymbol{NH}CH_3$, brs, disappeared after D_2O shake), 4.05 ($O\boldsymbol{C}H_2(CH_2)_9$, t, J 6.68Hz), 2.76-2.45 ($O(CH_2)_9\boldsymbol{C}H_2SO\boldsymbol{C}H_2CH_2\boldsymbol{C}H_2NHCH_3$, m), 2.43 ($NH\boldsymbol{C}H_3$, s), 2.07 ($OCH_2\boldsymbol{C}H_2(CH_2)_6\boldsymbol{C}H_2CH_2SOCH_2\boldsymbol{C}H_2CH_2$, m), 1.48-1.33 ($O(CH_2)_2(\boldsymbol{C}H_2)_6(CH_2)_2S$, m); δ_C ($CDCl_3$): 157.4 (6-***C***), 133.1 (4a-***C***), 129.9-128.3 (1, 3, 4, 8, 8a-***C***), 120.1 (7-***C***), 116.9 (2-***C***), 106.4 (5-***C***), 68.1 ($O\boldsymbol{C}H_2(CH_2)_9$), 52.6 ($O(CH_2)_9\boldsymbol{C}H_2$), 50.7 ($SO(CH_2)_2\boldsymbol{C}H_2NHCH_3$), 50.2 ($SO\boldsymbol{C}H_2$), 36.3 ($NH\boldsymbol{C}H_3$), 29.5 ($OCH_2\boldsymbol{C}H_2CH_2(\boldsymbol{C}H_2)_5(CH_2)_2SO\boldsymbol{C}H_2CH_2\boldsymbol{C}H_2NH$), 26.1 ($O(CH_2)_8\boldsymbol{C}H_2CH_2SO$), 23.0 ($O(CH_2)_2\boldsymbol{C}H_2(CH_2)_7SO$); ν_{max}/cm^{-1}: 3424.6, 2789.4, 2848.1, 1587.7, 1210.9cm^{-1}; m/z (EI): 482/484 $[M+H]^+$ ($C_{24}H_{36}BrNO_2S$ requires [M]

481/483), 453 $[M+H-NHCH_3]^+$; (Found: C 55.4%, H 7.1%, N 2.9%; $C_{24}H_{37}BrClNO_2S$ requires: C 55.5%, H 7.2%, N 2.7%).

4.6 Preparation of RWA2109 (49a)

(1) Preparation of 3-carboxy-4-oxo-4,5,6,7-tetrahydrobenzenzo(b)-furan (41)

1,3-Cyclohexanedione (6.7g, 59.8mol) was added to aqueous KOH (3.5g in 15ml of H_2O) of solution in ice bath. A freshly prepared solution of bromopyruvic acid (10.0g, 59.9mmol) in methanol (100ml) was added dropwise over period of 10min. The reaction was warmed up to room temperature after two-hour stirring at 5°C. Most methanol was removed under pressure; the resulting slurry was diluted with H_2O (90ml) and acidified to pH1 by the dropwise of concentrated HCl. The acidic mixture was stirred and heated to 100°C for another two hours followed by the cooling down in ice bath and filtration. The pale brown product was recrystallized from ethanol to give compound as buff coloured solid (8.1g, 75%).

m.p. 144.8°C (Lit. [137] m.p. 141-143°C)

δ_H (DMSO): 13.29 (-CO***OH***, brs, disappeared after D_2O shake), 8.12 (2-***H***, s), 3.00 (5-***H***, t, J 6.18Hz), 2.72 (7-***H***, t, J 5.93Hz), 2.30 (6-**H**, m); δ_C (DMSO): 199.3 (4-***C***), 170.7 (7a-***C***), 161.7(***C***OOH), 150.3 (2-***C***), 118.7 (3a-***C***), 117.3 (3-***C***), 36.5 (5-***C***), 23.2 (7-***C***), 22.4 (6-**C**); ν_{max}/cm^{-1} (KBr disc): 3433.9, 3157.3, 2962.8, 1727.8, 1624.6, 1549.8 cm^{-1}.

(2) Preparation of 4-hydroxybenzofuran-3-carboxylic acid (42)

Tetrahydrobenzofuran **41** (6.0g, 33.3mmol), dodecene (9ml) and 10% palladium on carbon (3.0g) were heated in decalin (60ml) under reflux in a nitrogen atmosphere for 20 hours. To this cooled mixture (80°C), was added ethanol (180ml) and filtered under nitrogen. The catalyst was washed with ethanol (2 x 30ml) and ethyl acetate (60ml). The solvent was removed under pressure to give the resulting slurry cooled in ice bath. Filtration followed by washing with distilled petroleum (60-80°C) and air-drying gave the crude phenol as a white amorphous solid (3.0g, 51%).

m.p. 211.3°C (Lit. [137] m.p. 209-212°C)

δ_H (DMSO): 8.17 (2-***H***, s), 7.27 (6-***H***, m), 6.98 (7-***H***, dd, J 1.15Hz, 8.69Hz), 6.72 (5-***H***, dd, J 8.49Hz, 1.12Hz), 4.62 (***OH***, brs, disappeared after D_2O shake); δ_C (DMSO): 163.4 (***C***OOH), 156.9 (7a-***C***), 151.6 (2-***C***), 149.9 (4-***C***), 127.1 (6-***C***), 112.8 (3-***C***), 109.3 (3a-***C***), 102.7 (5-***C***), 101.3 (7-***C***); ν_{max}/cm^{-1} (KBr disc): 3427.9, 3128, 1695cm^{-1}.

(3) Preparation of 4-hydroxybenzo[b]furan (43)

Acid **42** (2.7g, 15.2mmol) and copper powder (2.7g, 42.2mmol) were suspended in quinoline (20ml) at room temperature under an atmosphere of nitrogen. The heterogeneous mixture was stirred vigorously and heated to 220°C. T.L.C. (ether: methanol/ 10:1) showed the full conversion of the starting material. The mixture was cooled to 100°C, poured onto crushed ice (150g) and diluted with ether (100ml). The mixture was filtered, and the two layers of the filtrate were separated. The organic fraction was partitioned with 2M HCl (100ml×3) and sat. NaCl solution, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give a black oil. The crude product was purified by

Kugelrohr distillation (130°C/ 0.5mmHg) to give the target phenol as a pale yellow solid (1.4g, 67%).

m.p. 53.7°C (Lit. [137] m.p. 55~56°C)

δ_H ($CDCl_3$): 7.54 (2-***H***, d, J 2.23Hz), 7.16 (6-***H***, 7-***H***, m), 6.86 (3-***H***, d, J 2.22Hz), 6.63 (5-***H***, d, J 8.42Hz), 6.19 (***OH***, brs, disappeared after D_2O shake); δ_C ($CDCl_3$): 156.7(7a-***C***), 149.2 (4-***C***), 143.8 (2-***C***), 124.9 (6-***C***), 116.8 (3a-***C***), 107.8 (5-***C***), 104.6 (3-***C***), 103.2 (7-***C***); ν_{max}/cm^{-1} (KBr disc): 3445.8 cm^{-1}.

(4) Preparation of 5-bromo-4-hydroxybenzofuran (47b)

4-Hydroxybenzofuran **43** (1.0g, 7.45mmol) was dissolved in dry acetonitrile (30ml) at room temperature under an atmosphere of nitrogen and *N*-bromosuccinimide (1.3g, 7.45mmol) was added in one portion to give a dark red homogeneous mixture. The mixture was stirred at room temperature for 3.5h. and gave a pale yellow solution. T.l.c. (petroleum ether 60-80°C: ethyl acetate/ 4:1) indicated the formation of new compounds. The solvent was removed under reduced pressure to give yellow viscous oil. The crude oil was dissolved in DCM (20ml) and partitioned with water (20ml) and saturated sodium chloride (20ml). The organic fraction was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo* to give a pale brown solid. This crude product was purified by flash chromatography (petroleum ether 60-80°C: ethyl acetate/ 5:1) to give the title compound with an Rf value of 0.27 as a pale yellow solid (0.5g, 30%).

m.p. 63-64°C (Lit.[127] m.p. 62-64°C)

δ_H ($CDCl_3$): 7.54 (2-***H***, d, J 2.23Hz), 7.30 (6-***H***, d, J 8.91Hz), 7.04 (5-***H***, dd, J 0.89Hz, 8.76Hz), 6.89 (3-***H***, d, J 0.99Hz, 2.23Hz), 5.79 (***OH***, brs disappeared after D_2O shake); δ_C

($CDCl_3$): 155.7 (7a-***C***), 145.8 (4-***C***), 144.7 (2-***C***), 126.9 (6-***C***), 117.2 (3a-***C***), 105.5 (5-***C***), 104.2 (3-***C***), 102.1 (7-***C***). These values are in accord with those recorded by Allcock.

(5) Preparation of 4-(10-bromodecyloxy)benzofuran (50)

To a mixture of phenol **43** (0.8g, 5.8mmol), K_2CO_3 (2.4g, 17.4mmol) suspended in dry 2-butanone (20ml), was added 1,10-dibromodecane (8.7g, 29mmol) following general method **A** to give product as a colourless solid (1.3g, 61%).

m.p. 55-56°C

δ_H ($CDCl_3$): 7.57 (2-***H***, d, J 2.49Hz), 7.08 (6, 7-***H***, m), 6.83 (3-***H***, d, J 2.51Hz), 6.61 (5-***H***, d, 6.78Hz), 4.22 ($OCH_2(CH_2)_9Br$, t, J 6.60Hz), 3.36 ($O(CH_2)_9CH_2Br$, t, J 5.28Hz), 1.82 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.42 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 157.9 (7a-***C***), 153.2 (4-***C***), 143.3 (2-***C***), 124.9 (6-***C***), 117.8 (4a-***C***), 104.3 (5-***C***), 104.0 (3-***C***), 103.6 (7-***C***), 65.6 ($OCH_2(CH_2)_9Br$), 34.7 ($O(CH_2)_8CH_2CH_2Br$), 31.8 ($O(CH_2)_9CH_2Br$), 29.6 ($OCH_2CH_2CH_2(CH_2)_5(CH_2)_2Br$), 26.9 ($O(CH_2)_2CH_2(CH_2)_7Br$); m/z (EI): 353/355 $[M+H]^+$, ($C_{18}H_{25}BrO_2$ requires [M] 352/354).

(6a) Preparation of 5-bromo-4-(10-bromodecyloxyl)benzofuran (48b)

Bromide **48b** (1.2g, 75%) was prepared from phenol **47b** (0.8g, 3.8mmol) with an excess of 1, 10-dibromodecane (4.5g, 15.0mmol) by general method **A** as a colourless oil.

δ_H ($CDCl_3$): 7.55 (2-***H***, d, J 2.23Hz), 7.42 (6-***H***, d, J 8.66Hz), 7.12 (5-***H***, dd, J 0.99Hz, 8.66Hz), 6.86 (3-***H***, dd, J 0.99Hz, 2.22Hz), 4.22 ($OCH_2(CH_2)_9Br$), t, J 6.43Hz), 3.41 ($O(CH_2)_9CH_2Br$, t, J 6.93Hz), 1.85 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.57 ($O(CH_2)_2CH_2(CH_2)_7Br$, m), 1.33 ($O(CH_2)_3(CH_2)_5(CH_2)_2Br$, m); δ_C ($CDCl_3$): 155.7 (7a-***C***),

149.4 (4-***C***), 144.7 (2-***C***), 128.4 (6-***C***), 121.1 (3a-***C***), 108.1 (5-***C***), 107.6 (3-***C***), 104.5 (7-***C***), 68.4 ($O\boldsymbol{C}H_2(CH_2)_9Br$), 34.1 ($O(CH_2)_9\boldsymbol{C}H_2Br$), 32.8 ($O(CH_2)_8\boldsymbol{C}H_2CH_2Br$), 28.1 ($OCH_2\boldsymbol{C}H_2CH_2(\boldsymbol{C}H_2)_5(CH_2)_2Br$) 25.9 ($O(CH_2)_2\boldsymbol{C}H_2(CH_2)_7Br$).

(6b) Preparation of 7-bromo-4-(10-bromodecyloxyl)benzofuran (48a)

This compound was prepared by the route introduced in **Scheme 13**. 2,4,4,6-Tetrabromocyclohexa-2,5-dienone (0.7g, 1.7mmol) in DCM (3ml) was added to a solution of ether **50** (0.6g, 1.7mmol) dissolved in DCM (3ml) at -20°C (acetonitrile/dry ice). The mixture was kept stirring at this temperature until tlc (petroleum ether 60-80°C: EtOAc/ 20:3) showed the full conversion of ether **50**. The by-product, tribromophenol, was removed by washing with 4M NaOH (10ml) solution and water. The organic phase was dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by flash chromatography to give the product as a pale purple oil (0.2g, 27%).

δ_H ($CDCl_3$): 7.63 (2-***H***, d, J 2.33Hz), 7.22 (6-***H***, d, J 8.25Hz), 6.92 (3-***H***, d, J 2.31Hz), 6.49 (5-***H***, d, J 8.25Hz), 4.08 ($O\boldsymbol{C}H_2(CH_2)_9Br$, t, J 6.43Hz), 3.43 ($O(CH_2)_9\boldsymbol{C}H_2Br$, t, J 6.98Hz), 1.76 ($OCH_2\boldsymbol{C}H_2(CH_2)_6\boldsymbol{C}H_2CH_2Br$, m), 1.30 ($O(CH_2)_2(\boldsymbol{C}H_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 151.8 (7a-***C***), 152.7 (4-***C***), 144.0 (2-***C***), 127.4 (6-***C***), 116.7 (4a-***C***), 105.6 (5-***C***), 105.1 (3-***C***), 93.8 (7-***C***), 68.6 ($O\boldsymbol{C}H_2(CH_2)_9Br$), 34.0 ($O(CH_2)_8\boldsymbol{C}H_2CH_2Br$), 32.7 ($O(CH_2)_9\boldsymbol{C}H_2Br$), 29.7-28.6 ($OCH_2\boldsymbol{C}H_2CH_2(\boldsymbol{C}H_2)_5(CH_2)_2Br$), 25.9 ($O(CH_2)_2\boldsymbol{C}H_2(CH_2)_7Br$).

(7) Preparation of 5-bromo-4(10-methylaminodecyloxy)benzofuran HCl (49b, RWA 2109)

The title compound **49b** was made from bromide **48b** (1.2g, 2.8mmol) and an excess of 33% methylamine in ethanol (3.5ml, 28mmol) by general methods **B**& **C** respectively as a white solid (0.9g, 86%).

m.p. 104-106°C (Lit. [127] m.p. 107-108°C)

δ_H (DMSO): 9.75 (***NH***, brs, disappeared after D_2O shake), 7.56 (2-***H***, d, J 2.48Hz), 7.43 (6-***H***, d, J 8.66Hz), 7.13 (5-***H***, dd, J 0.99Hz, 8.66Hz), 6.85 (3-***H***, dd, J 0.99Hz, 2.23Hz), 4.22 ($OCH_2(CH_2)_9NHCH_3$), t, J 6.58Hz), 2.92 ($O(CH_2)_9CH_2NHCH_3$, t, J 7.17Hz), 2.67 ($NHCH_3$, s), 1.83 ($O(CH_2CH_2(CH_2)_6CH_2CH_2NH$, m), 1.52 ($O(CH_2)_2CH_2(CH_2)_7NHCH_3$, m), 1.31 ($O(CH_2)_3(CH_2)_5(CH_2)_2Br$, m); δ_C (DMSO): 155.7 (7a-***C***), 149.6 (4-***C***), 144.7 (2-***C***), 128.4 (6-***C***), 121.1 (3a-***C***), 108.1 (5-***C***), 107.6 (3-***C***), 104.5 (7-***C***), 68.4 ($OCH_2(CH_2)_9NHCH_3$), 52.0 ($O(CH_2)_9CH_2NHCH_3$), 36.2 ($NHCH_3$), 28.1 ($CH_2CH_2CH_2(CH_2)_4CH_2CH_2CH_2$), 27.3 ($O(CH_2)_7CH_2(CH_2)_2$), 26.0 ($O(CH_2)_2CH_2(CH_2)_7$); m/z (EI): 418/ 420 $[M+H]^+$ ($C_{19}H_{29}BrClNO_2$ requires [M] 417/419). These spectral data are in agreement with those obtained from an authentic specimen of **RWA 2109** prepared by Allcock.

4.7 Attempt to prepare unambiguously 7-bromo-4-hydroxybenzofuran (47)

(1) Preparation of 2, 6-dimethoxybenzaldehyde (53)

A mixture of 1,3-dimethoxybenzene (13.8g, 0.1mol) and phenyllithium (1.9M solution in cyclohexane-ether) (8.4g, 0.1mol, 52.6ml) in anhydrous ether (50ml) was

allowed to stand at room temperature for sixty hours. The 2-litho derivative separates in large, colourless crystals. A solution of *N*-methylformanilide (13.5g, 0.23mol) in anhydrous ether (50ml) was added dropwise to the mixture. After the initial ebullition subsided, the mixture was allowed to stand for one-half hour and was poured into an excess of dilute sulphuric acid. The ethereal layer was separated and dried, and the solvent was removed *in vacuo* [188]. The residue was purified with flash chromatography (cyclohexane: EtOAc/ 5:2) to give the product as a yellow solid (8.5g, 51%).

m.p. 97-98°C (Lit. [187] m.p. 98-99°C)

δ_H ($CDCl_3$): 9.86 (C***H***O, s), 7.41 (4-***H***, t, J 8.56Hz), 6.57 (3, 5-***H***, d, J 8.45Hz), 3.74 (O***CH*****3**, s); δ_C ($CDCl_3$): 187.6 (***C***HO), 164.3 (2, 6-***C***), 141.2 (4-***C***), 105.7 (3, 5-***C***), 51.7 (O***CH*****3**); ν_{max}/cm^{-1} (KBr disc): 1634.7cm^{-1}.

(2) Preparation of 2,6-dihydroxybenzaldehyde (54)

To a solution of methoxybenzaldehyde **53** (5g, 30.1mmol) in carbon disulfide (250ml) in a 1000ml of round bottom flask fitted with a mechanical stirrer, aluminium bromide (25g, 94.8mmol) in carbon disulfide (250ml) was added quickly with stirring. The addition complex precipitated as a red gum. After stirring for one hour the carbon disulfide was decanted into a separatory funnel and crushed ice (100g), hydrochloric acid (3M, 150ml) and ether (200ml) was added to the residual gum in the flask and stirred until it was completely dissolved. The carbon disulfide in the separating funnel was washed with hydrochloric acid (3M, 50ml), and poured to a brown bottle, sealed and handled to technician. The acid layer was combined with mixture in the flask, and separated. The aqueous layer was extracted with more ether (2 x 200ml).

The combined ether solutions were extracted with 1M NaOH (3 x100ml) and the phenol **54** was precipitated from the alkaline solution by the addition of conc. HCl (30ml). The yellow product was filtered, washed with a small amount of water and crystallized from boiling water (125ml) to give the dihydroxybenzaldehyde, 1.1g (28%).

m.p. 156-158°C (Lit [189] m.p. 157-158°C)

δ_H (DMSO): 11.3 (OH, brs, disappeared after D_2O shake), 10.22 (C***H***O, s), 7.34 (4-***H***, t, J 8.56Hz), 6.33 (3, 5-***H***, d, J 8.57Hz); δ_C (DMSO): 194.9 (***C***HO), 162.6 (2, 6-***C***), 139.5 (4-***C***), 110.5 (1-***C***), 107.2 (3, 5-***C***); ν_{max}/cm^{-1} (KBr disc): 3451.9, 1634.7cm^{-1}.

(3) Preparation of 3-bromo-2,6-dihydroxybenzaldehyde (55)

2,4,4,6-Tetrabromocyclohexa-2,5-dienone (2.4g, 5.5mmol) in anhydrous DCM (10ml) was added to a solution of 2,6-dihydroxybenzaldehyde (0.8g, 5.5mmol) dissolved in anhydrous DCM (10ml) at -20°C. The reaction was kept stirring at this temperature for one hour before it was allowed to reach room temperature. The progress of the reaction was monitored by tlc (EtOAc: cyclohexane/ 2:1). The solvent of the reaction was removed under reduced pressure, and the residue was purified by flash chromatography (EtOAc: cyclohexane/ 2:1) to give the novel bromoaldehyde as a yellow solid (0.8g, 64%).

m.p.: 144-145°C

δ_H (DMSO): 10.31 (C***H***O, s), 7.09 (5-***H***, d, J 7.98Hz), 7.32 (4-***H***, d, J 7.65Hz); δ_C (DMSO): 191.5 (***C***HO), 163.2 (2-***C***), 151.3 (6-***C***), 140.1 (4-***C***), 112.9 (1-***C***), 103.5 (5-***C***), 97.8 (3-***C***); m/z (EI): 217/219 $[M+H]^+$, ($C_7H_5BrO_3$ requires [M] 216/218).

4.8 Preparation of [10-(7-bromo-benzofuran-5-yloxy)decyl]methylamine (58)

(1) Preparation of 2-hydroxy-3-bromo-5-methoxybenzaldehyde (62)

Powdered, freshly fused sodium acetate (1.8g, 21.9mmol) was added to the solution of 2-hydroxy-5-methoxybenzaldehyde (1.8g, 21.9mmol) dissolved in glacial acetic acid (15ml). The suspension was treated dropwise with a solution of bromine (0.96ml, 18.8mmol) in glacial acetic acid (6ml). After remaining for a few hours, the crude acetic acid solution was poured into three volumes of 5% stannous chloride dihydrate solution. Crystallization of the crude product from ethanol gave **62** as a yellow needle-like crystal (2.4g, 53%).

m.p. 110-112°C (Lit [142, 143] m.p. 109-110.5°C).

δ_H (CDCl3): 9.83 (C***H***O, s), 7.42 (6-***H***, d, J 2.97Hz), 7.04 (4-***H***, d, J 2.97Hz), 4.76 (***OH***, brs, disappeared after D_2O shake).3.82 (O***CH***$_3$, s); δ_C ($CDCl_3$): 195.6 (***C***HO), 152.8 (5-***C***), 152.4 (2-***C***), 127.4 (1-***C***), 120.4 (4-***C***), 115.8 (6-***C***), 111.5 (3-***C***), 56.1 (***OCH***$_3$); ν_{max}/cm^{-1} (KBr disc): 3410.3, 1632.8cm-1.

(2) Preparation of 7-Bromo-5-methoxy-2-benzofuran acid (63)

A mechanically stirred mixture of aldehyde **62** (2.7g, 11.8mmol), ethyl chloroacetate (1.3ml, 12mmol) and powdered anhydrous potassium carbonate (3.3g, 23.9mmol) in dry DMF (25ml) was heated to 160°C and kept stirring vigorously for 8hr. T.l.c (petroleum ether 60-80°C: EtOAc/ 5:1) was used to monitored the progress of the reaction. The disappearance of the starting material and the formation of two new compounds (one compound stays on the baseline, the other one's Rf 0.37). The mixture

was poured onto crushed ice (50g) and the aqueous layer was extracted with toluene (50ml×3). All the organic layers were combined and washed with H_2O, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a neutral product **64**. The aqueous phase was acidified with conc. HCl and filtered to give a dark brown solid **63**, which was air-dry (1.5g, 47%).

m.p.: 227-230°C (Lit. [144] 210°C);

δ_H (DMSO): 7.49 (3-***H***, s), 7.23 (4-***H***, d, J 2.43Hz), 7.08 (6-***H***, d, J 2.43Hz), 3.86 (s, O***CH*****3**), 2.84 (CO***OH***, brs, disappeared after D_2O shake); δ_C (DMSO): 160.2 (***C***OOH), 156.4 (5-***C***), 147.6 (7a-***C***), 147.4 (2-***C***), 128.0 (4a-***C***), 119.3 (3-***C***), 113.7 (6-***C***), 104.2, (7-***C***), 103.2 (4-***C***), 55.7 (O***CH*****3**); ν_{max}/cm^{-1} (KBr disc): 3446.6, 2925.1, 1697.3cm^{-1}.

(3) Preparation of 7-bromo-5-methoxybenzofuran (64)

The acid 63 (3.9g, 10mmol) was added to a mixture of copper powder (2.4g, 30mmol) suspended in quinoline (40ml) at room temperature under an atmosphere of nitrogen and the air in the reaction should be removed as much as possible. The mixture was heated to 210°C and the reaction was kept stirring vigorously until t.l.c (ether: methanol/ 10:1) showed that the full conversion of starting material to a new compound. The reaction was quenched by pouring onto crushed ice (50g), and diluted with ether (100ml). The mixture was filtered through glass wool, washed with 2M HCl (60ml×3), sat. NaCl solution, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The neutral compound **64** obtained from the preparation of acid **63** and the residue were combined and purified by flash chromatography with petroleum ether 60-

80°C: EtOAc (5:1) to give product as a pale yellow oil (2.0g, 61%), which crystallized overnight at room temperature.

m.p.: 45-47°C

δ_H ($CDCl_3$): 7.63 (2-***H***, d, J 2.23Hz), 7.09 (4-***H***, d, J 2.38Hz), 6.98 (6-***H***, d, J 2.38Hz), 6.74 (3-***H***, d, J 2.23Hz), 3.81 (O***CH***$_3$, s,); δ_C ($CDCl_3$): 156.4 (5-***C***), 147.3 (7a-***C***), 146.3 (2-***C***), 128.7 (4a-***C***), 115.9 (6-***C***), 107.6 (7-***C***), 104.0 (3-***C***), 103.2 (4-***C***), 56.1 (O***CH***$_3$); m/z (EI): 227/ 229 $[M+H]^+$ ($C_9H_7BrO_2$ requires [M] 226/228).

(4) Preparation of 7-bromo-5-hydroxybenzofuran (65)

A 5% w/v solution of 7-bromo-5-methoxybenzaldehyde (1.3g, 5.5mmol) in dry dichloromethane (25ml) was cooled to -78°C and added to a similarly cooled 10% v/v solution of borontribomide (5.5ml, 5.5mmol) in dichloromethane (5ml). The reaction, protected from moisture by a calcium drying tube, was allowed to reach to room temperature after one hour and left stirring over night. T.l.c (petroleum ether 60-80°C: EtOAc/ 10:1) showed the disappearance of the starting material (Rf 0.36) and the formation of a new compound (Rf 0.08) followed the addition of H_2O (50ml). The mixture was extracted with diethyl ether (50ml×2). The organic layers were washed with sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent was removed *in vacuo* to give product as a colorless solid (1.1g, 90%).

m.p. 108-109°C

δ_H ($CDCl_3$): 7.65 (2-***H***, d, J 1.98Hz), 7.03 (4-***H***, d, J 2.47Hz), 6.96 (6-***H***, d, J 2.47Hz), 6.73(3-***H***, d, J 1.98Hz).4.75 (***OH***, brs, disappeared after D_2O shake); δ_C ($CDCl_3$): 151.9 (5-***C***), 146.6 (7a-***C***), 138.9 (2-***C***), 128.9 (4a-***C***), 116.2 (6-***C***), 107.3 (4-***C***), 107.1 (7-***C***), 105.0

(3-***C***); ν_{max}/cm^{-1} (KBr disc): 3326.7cm^{-1}; GC/MS showed a single peak at 212/ 214, in agreement with $C_8H_5BrO_2$ [M] 212/214.

(5) Preparation of 7-bromo-5-(10-bromodecyloxyl)benzofuran (66)

Compound **66** was prepared by the treatment of phenol **65** (0.5g, 2.3mmol) with an excess of 1, 10-dibromodecane (3.5g, 11.5mmol) via general method A as a colourless solid (0.4g, 41%).

m.p. 47-49°C

δ_H ($CDCl_3$): 7.64 (2-***H***, d, J 1.98Hz), 7.09 (4-***H***, d, J 2.47Hz), 7.00 (6-***H***, d, J 2.38Hz), 6.75 (3-***H***, d, J 1.98Hz), 3.95 ($OCH_2(CH_2)_9Br$, t, J 6.68Hz), 3.41, ($(CH_2)_9CH_2Br$, t, J 6.93Hz), 1.82 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.42 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 155.9 (5-***C***), 146.2 (2-***C***), 139.7 (7a-***C***), 128.6 (4a-***C***), 116.6 (6-***C***), 113.8 (7-***C***), 107.6 (4-***C***), 103.9 (3-***C***), 69.1 ($OCH_2(CH_2)_9Br$), 34.0 ($(CH_2)_8CH_2CH_2Br$), 32.8 ($(CH_2)_9CH_2Br$), 29.4 ($OCH_2CH_2CH_2(CH_2)_5(CH_2)_2Br$), 26.0 ($O(CH_2)_2CH_2(CH_2)_7Br$); GC/MS showed peaks at 430/432/434 which is consistent with $C_{18}H_{24}Br_2O_2$ [M] 430/432/434.

(6) Preparation of N-methyl-10-(7-bromobenzofuran-5-ylxyl)decanamine HCl (58)

The title compound **58** was prepared from bromide **66** (0.4g, 0.88mmol) and an excess of methylamine (33%) in ethanol (1.1ml, 8.8mmol) by general methods B& C (0.2g, 63%).

m.p. 106-108°C

δ_H ($CDCl_3$): 7.64 (2-***H***, d, J 1.98Hz), 7.09 (4-***H***, d, J 2.22Hz), 7.00 (6-***H***, d, J 2.22Hz), 6.78 (3-***H***, d, J 1.98Hz), 4.00($OCH_2(CH_2)_9NHCH_3$, t, J 6.56Hz), 3.47 (***NH***, brs, disappear after

D_2O shake), 2.54 ($O(CH_2)_9\boldsymbol{CH_2}NHCH_3$, t, J 7.17Hz), 2.43 ($NH\boldsymbol{CH_3}$, s), 1.81 ($OCH_2\boldsymbol{CH_2}(CH_2)_8NH$, m), 1.48 ($O(CH_2)_8\boldsymbol{CH_2}CH_2NH$, m), 1.30 ($O(CH_2)_2\boldsymbol{(CH_2)_6}(CH_2)_2$, m); δ_C ($CDCl_3$): 155.9 (5-***C***), 148.9 (7a-***C***), 146.2 (2-***C***), 128.6 (4a-***C***), 116.6 (6-***C***), 107.6 (3-***C***), 104.1 (4-***C***), 99.4 (7-***C***), 69.1 ($O\boldsymbol{CH_2}(CH_2)_9NHCH_3$), 52.1 ($O(CH_2)_9\boldsymbol{CH_2}NHCH_3$), 36.3 ($NH\boldsymbol{CH_3}$), 29.8($OCH_2\boldsymbol{CH_2}CH_2\boldsymbol{(CH_2)_4}CH_2\boldsymbol{CH_2}CH_2NH$), 27.1($O(CH_2)_8\boldsymbol{CH_2}CH_2NH$), 26.0 ($OCH_2\boldsymbol{CH_2}(CH_2)_8NHCH_3$); m/z (EI): 382/ 384 $[M+H]^+$ ($C_{19}H_{28}BrNO_2$ requires [M] 381/383); (Found: C 54.4%, H 7.0%, N 3.5%; $C_{19}H_{29}BrClNO_2$ requires: C 54.4% H 7.0% N 3.3%).

4.9 Preparation of [10-(7-bromobenzofuran-6-yloxy)decyl]methylamine HCl (59)

(1) Preparation of 4-benzyloxy-2-hydroxybezaldehyde (76)

A 500ml 3-necked flask equipped with a temperature thermocouple was charged with 2,4-dihydroxybenzaldehyde (16g, 116mmol), dry potassium fluoride (13g, 230mmol) and dry acetonitrile (160ml). The reaction was stirred and heated, benzyl chloride (23ml, 203mmol) was added in one portion with a small amount of acetonitrile as a rinse when the temperature reached 55°C. The reaction was heated at reflux and stirred for 16-21 hours. T.l.c. (petroleum ether 60-80°C: ethyl acetate/ 2:1) indicated the full conversion of starting material (Rf 0.04) to a new compound (Rf 0.23). The reaction was stopped and most of the acetonitrile was removed by distillation at 40-45°C. The residue was treated with water (600ml), ethyl acetate (250ml). The layers were separated, and the aqueous layer was extracted with additional ethyl acetate (2× 220ml). The organic layers were combined and washed successively with 3% aqueous potassium carbonate (200ml), saturated with ethyl

acetate, diluted HCl (1.2ml of conc. hydrochloric acid in 200ml of water), brine (200ml), dried over Na_2SO_4 and filtered. The solvent was removed, and the solid residue was dried *in vacuo* at 30°C for 12 hours. The product was dissolved by stirring and heating in *tert*-butyl methyl ether (TBME) (75ml) at 45-50°C and then cooled to 30°C at which time hexane (95ml) was added. The resulting precipitate was stirred for 2 hours at 5-10°C, and the product collected by filtration. The flask was rinsed with cold TBME-hexane (35ml of a 1: 1 mixture). After air drying for 1.5 h, the yellow product (18g, 68%) was dried at below 35°C for 16h.

m.p. 77-79°C (Lit.[149] m.p. 75-77°C).

δ_H ($CDCl_3$): 11.42 (***OH***, brs, disappeared after D_2O shake), 9.57 (C***H***O, s), 7.35 (6-***H***, d, J 8.90Hz), 6.55 (5-***H***, dd, J 2.23Hz, 8.66Hz), 6.43 (3-***H***, d, J 2.47Hz), 7.29 (Ph-***H***, m), 5.04 (Ph***CH*****$_2$**, s|); δ_C: 194.4 (***C***HO), 165.9 (4-***C***), 164.4 (2-***C***), 135.6 (1'-***C***), 135.3 (6-***C***), 128.7-127.5 (2', 3', 4', 5', 6'-***C***), 115.3 (1-***C***), 108.9 (5-***C***), 101.6 (3-***C***), 70.4 (Ph***CH*****$_2$**); ν_{max}/cm^{-1} (KBr): 3444.4, 1628.5cm^{-1}; GC/MS showed a single peak at 228 in agreement with$C_{14}H_{12}O_3$ [M] 228.

(2) Preparation of 4-benzyloxy -2-hydroxybenzaldehyde-3-mercuribromide (77a)

Mercury (II) acetate (5g, 15.4mmol) was added to a solution of the benzaldehyde (2.8g, 12.3mmol) dissolved in EtOH (280ml) containing 1% acetic acid (2.8ml). The mixture was heated to reflux until the tlc (petroleum ether 60-80°C: EtOAc/ 10:1) showed the disappearance of starting material. On the addition of sodium bromide (1.6g, 15.4mmol) in water (20ml), a white precipitate appeared. The cooled mixture was filtered and the filter cake was dried in air (4.5g, 72%).

m.p. 168-174°C

δ_H (DMSO): 9.92 (C***H***O, s), 7.64 (6-***H***, d, J 8.43Hz), 7.29 (Ph-***H***, m), 6.87 (5-***H***, d, J 8.66Hz), 5.19 (Ph***CH***$_2$, s) 3.89 (***OH,*** brs, disappeared after D_2O shake); δ_C: 194.2 (***C***HO), 169.1 (4-***C***), 166.3 (2-***C***), 136.8 (1'-***C***), 135.3 (6-***C***), 127.8 (2', 3', 4', 5', 6'-***C***), 119.5 (1-***C***), 113.6 (5-***C***), 105.2 (3-***C***), 69.2 (Ph***CH***$_2$).

(3) Preparation of 4-benzyloxy-3-bromo-2-hydroxybenzaldehyde (77a)

The mercuribromide **77a** (4.5g, 8.86mmol) dissolved in DCM (220ml) without any further purification was treated slowly with bromine (0.46ml, 8.9mmol) in DCM (100ml) containing 1% of acetic acid. The reaction was kept stirring until t.l.c. showed the full conversion of starting material. The mixture was filtered and the filtrate was washed with H_2O until pH paper showed no evidence of acid in the organic layer, the organic layer was washed with sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure, and the residue was recrystllised from ethanol giving the product as pink needles (2.2g, 82%).

m.p. 137-140°C

δ_H ($CDCl_3$): 9.68 (C***H***O, s), 7.46 (6-***H***, d, J 8.70Hz), 7.30 (Ph-***H***, m), 6.61 (5-***H***, d, J 8.66Hz), 5.26 (Ph***CH***$_2$, s), 4.50 (***OH***, bs, disappeared after D_2O shake); δ_C ($CDCl_3$): 194.2 (C***H***O), 161.8 (4-***C***), 160.3 (2-***C***), 135.4 (1'-***C***), 134.3 (6-***C***), 128.7-126.9 (2', 3', 4', 5', 6'-***C***), 116.1 (1-***C***), 105.1 (5-***C***), 100.3 (3-***C***), 71.1 (Ph***CH***$_2$); ν_{max}/cm^{-1} (KBr): 3430.3, 1645.6, 1490.8, 745.0cm^{-1}; m/z (EI): 307/309 $[M+H]^+$ (75%) , 229 $[M+H-Br]^+$ ($C_{14}H_{11}BrO_3$ requires [M] 306);

(4) Preparation of 6-benzyloxy-7-bromobenzofuran-2-carboxylic acid ethyl ester (78)

Powdered dry potassium cabonate (1.0g, 7.5mmol) was added to a mixture of aldehyde **77**a (1.15g, 3.8mmol) and ethyl chloroacetate (0.44ml, 4.1mmol) dissolved in dry DMF (40ml). The reaction was heated to 150°C and the progress was monitored by t.l.c. (petroleum ether 60-80°C: EtOAc/ 1:1) (starting material Rf 0.53, product Rf 0.59). The reaction was quenched by pouring onto crushed ice (50g) and the mixture was extracted with toluene (60ml×3). All the organic layers were combined and washed with H_2O, sat. NaCl solution, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 3:1) to give product as a white solid (0.8g, 57%)

m.p. 149-152°C

δ_H ($CDCl_3$): 7.45 (4-***H***, d, J 8.66Hz), 7.43 (3-***H***, s), 7.18 (Ph-***H***, m), 6.95 (5-***H***, d, J 8.66Hz), 5.17 (Ph***CH*****$_2$**, s), 4.35 (COO***CH*****$_2$**CH_3, q, J 7.17Hz), 1.35 ($COOCH_2$***CH*****$_3$**, t, J 7.18Hz); δ_C ($CDCl_3$):159.1 (7a-***C***), 155.3 (6-***C***), 146.1 (***C***$OOCH_2CH_3$), 141.9 (4-***C***), 136.3 (1'-***C***), 128.6-127.1 (2', 3', 4', 5', 6'-***C***), 121.8 (3a-***C***), 121.5 (4-***C***), 114.3 (3-***C***), 116.7 (5-***C***), 95.1 (7-***C***), 71.9 (Ph***CH*****$_2$**), 61.5 (COO***CH*****$_2$**CH_3), 14.3 ($COOCH_2$***CH*****$_3$**); ν_{max}/cm^{-1} (KBr): 3421.1, 1713.1, 1272.0cm^{-1}; m/z (EI): 375/377 $[M+H]^+$ ($C_{18}H_{15}BrO_4$ requires [M] 374/376).

(5) Preparation of 6-benzyloxy-7-bromobenzofuran-2-carboxylic acid (79)

Ester **78** (0.8g, 2.1mmol) in ethanol (80ml) was treated with sodium hydroxide (0.1g, 2.5mmol), and the mixture was heated to reflux and magnetically stirred overnight. The mixture was cooled down to room temperature and concentrated *in vacu.* The residue

was dissolved in water and acidified with 4M HCl. The white precipiation was collected by filtration and the filter cake was dried in air to give a white solid (0.6g, 80%).

m.p.: 251-253°C

δ_H (DMSO): 7.66 (3-***H***, s), 7.35 (Ph-***H***, 4-***H***, m), 7.31 (5-***H***, d, J 8.66Hz), 5.23 (Ph***CH***$_2$, s), 3.29 (CO***OH***, bs, disappeared after D_2O shake); δ_C (DMSO): 159.7 (***C***OOH), 154.9 (6-***C***), 153.4 (7a-***C***), 146.2 (2-***C***), 136.5 (1'-***C***), 127.9 (2', 3', 4', 5', 6'-***C***), 122.5 (3a-***C***), 121.6 (4-***C***), 114.4 (3-***C***), 111.8 (5-***C***), 93.2 (7-***C***), 70.9 (Ph***CH***$_2$); ν_{max}/cm^{-1} (KBr): 3455.8, 2920.5, 1676.1, 1274.4cm^{-1}; m/z(EI): 347/ 349 $[M+H]^+$ (65%), 379/ 381 $[M+CH_3OH]^+$ ($C_{16}H_{11}BrO_4$ requires [M] 346/348).

(6) Preparation of 6-benzyloxy-7-bromobezofuran (80)

The mixture of acid **79** (0.5g, 1.45mmol) and copper powder (0.3g, 4.05mmol) suspended in quinoline (15ml) was was purged with nitrogen three times. The reaction was heated to 220°C under N_2 and kept stirring magnetically until t.l.c. (diethyl ether: MeOH/ 8:2) indicated the full conversion of starting material (Rf 0.15) to a new compound (Rf 0.92). The mixture was cooled down to 100°C, poured onto crushed ice (20g) and diluted with ether (50ml), filtered through glass wool. The filtrate was washed with 2M HCl (50ml×3), sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure to give a black oil. This crude product was purified by flash chromatography (petroleum ether 60-80°C: ethyl acetate/ 4:1) to give product as a yellow oil (0.3g, 60%).

δ_H ($CDCl_3$): 7.55 (2-***H***, d, J 2.23Hz), 7.44 (4-***H***, d, J 8.41Hz), 7.32 (Ph-***H***', m), 6.88 (5-***H***, d, J 8.42Hz), 6.68 (3-***H***, d, J 2.22Hz), 5.13 (Ph***CH***$_2$, s); δ_C ($CDCl_3$): 161.8 (6-***C***), 153.2 (7a-

C), 145.2 (2-*C*), 136.7 (1'-*C*), 127.2 (2', 3', 4', 5', 6'-*C*), 122.4 (3a-*C*), 119.7 (4-*C*), 111.1 (5-*C*), 107.0 (3-*C*), 95.1 (7-*C*), 72.2 (Ph*CH*$_2$); m/z (EI): 303/ 305 $[M+H]^+$ ($C_{15}H_{11}BrO_2$ requires 302/304); (Found: C 59.8%, H 3.4%; $C_{15}H_{11}BrO_2$ requires: C59.4%, H 3.7%).

(7) Preparation of 7-bromo-6-hydroxybenzofuran (74)

Benzofuran **80** (1.6g, 5.4mmol), and 10% palladium on carbon (0.3g) suspended in ethyl acetate (20ml) was purged with nitrogen followed by hydrogen (130ml×3) at room temperature (the reaction was carried in hydrogenation room). The mixture was kept stirring under hydrogen and the progress of the reaction was monitored by t.l.c. (petroleum ether 60-80°C: EtOAc/ 10:1). The reaction was stopped until the starting material (Rf 0.48) was fully converted to a new compound (Rf 0.27). The mixture was filtered through celite under nitrogen and the solvent was removed. The residue was purified by chromatography, washed with pet ether 60-80°C: ethyl acetae (5:1) to give a colourless mixture (1.1g) containing 7-bromo-6-hydroxybenzofuran and 7-bromo-2,3-dihydro-benzofuran-6-ol. The result was proved by NMR which showed two triplets at δ 4.63 and 3.17 and also by GC/MS.

(8) Preparation of 7-bromo-6(10-bromodecyloxy)benzofuran (81)

The mixture (1.0g) containing **74** and **74a** obtained from step vi (**Scheme 18**, P64) was treated with an excess of 1, 10-dibromodecane (7g, 23.4mmol) via general method **A** to give desired product, bromide **81**, as a colourless solid (0.9g, 88%).

m.p. 41-42°C

δ_H ($CDCl_3$): 7.54 (2-***H***, d J 2.23Hz), 7.33 (4-***H***, d, J 8.41Hz), 6.84 (5-***H***, d, J 8.38Hz), 6.69 (3-***H***, d, J 2.23Hz), 4.03 ($OCH_2(CH_2)_9Br$, t, J 6.44Hz), 3.33 ($O(CH_2)_9CH_2Br$, t, J 6.92Hz), 1.78 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.32 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 153.7 (7a-***C***), 153.4 (5-***C***), 145.7 (2-***C***), 121.9 (4a-***C***), 119.6 (4-***C***), 110.3 (5-***C***), 107.8 (3-***C***), 94.7 (7-***C***), 70.5 ($OCH_2(CH_2)_9Br$), 34.1 ($O(CH_2)_8CH_2CH_2Br$), 32.3 ($O(CH_2)_9CH_2Br$), 29.4 ($OCH_2CH_2CH_2(CH_2)_5(CH_2)_2Br$), 25.9 ($O(CH_2)_2CH_2(CH_2)_7Br$); GC/MS showed a single peak at 430/432/434 which is in agreement with $C_{18}H_{24}Br_2O_2$ [M] 430/432; (Found: C 50.8%, H 5.7%; $C_{18}H_{24}Br_2O_2$ requires: C 50.2%, H 5.6%).

(9) Preparation of [10-(7-bromobenzofuran-6-yloxy)decyl]methylamine HCl (59)

The title compound **59** was prepared from bromide **81** (0.9g, 2.1mmol) and an excess of 33% methylamine in ethanol (2.6ml, 20.8mmol) by general methods **B**& **C** as a yellow solid (0.7g, 87%).

m.p. 118-121°C.

δ_H ($CDCl_3$): 7.53 (2-***H***, d J 2.22Hz), 7.33 (4-***H***, d, J 8.41Hz), 6.84 (5-***H***, d, J 8.66Hz), 6.69 (3-***H***, d, J 2.23Hz), 4.00 ($OCH_2(CH_2)_9Br$, t, J 7.18Hz), 3.40 (***NH,*** brs, disappeared after D_2O shake), 2.48 ($O(CH_2)_9CH_2NHCH_3$, t, J 7.10Hz), 2.35 ($NHCH_3$, s), 1.77 ($OCH_2CH_2(CH_2)_8NHCH_3$, m), 1.42 ($O(CH_2)_8CH_2CH_2NHCH_3$, m), 1.18 (($O(CH_2)_2(CH_2)_6(CH_2)_2NHCH_3$, m); δ_C ($CDCl_3$): 153.7 (7a-***C***), 157.1 (6-***C***), 145.1 (2-***C***), 121.9 (4a-***C***), 119.6 (4-***C***), 110.4 (5-***C***), 106.9 (3-***C***), 94.8 (7-***C***), 70.5 ($OCH_2(CH_2)_9Br$), 52.2 ($O(CH_2)_9CH_2NHCH_3$), 36.5 ($NHCH_3$), 30.3 ($O(CH_2)_8CH_2CH_2NHCH_3$), 29.8 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3$), 27.3 ($O(CH_2)_7CH_2(CH_2)_2$), 25.9 ($O(CH_2)_2CH_2(CH_2)_7NH$);

m/z (EI): 382/ 384 $[M+H]^+$ ($C_{19}H_{28}BrNO_2$ requires [M] 381/383); (Found: C 54.9%, H 7.32%, N 3.7%; $C_{19}H_{29}BrClNO_2$ requires: C 54.5%, H 7.3%, N 3.3%).

4.10 Preparation of 7-bromo-6-methoxybenzofuran ((73)

(1) Preparation of 4-methoxy-3-bromo 2-hydroxybenzaldehyde (70)

Mercury (II) acetate (6.6g, 20.6mmol) was added to a solution of 4-methoxy-2-hydroxybenzaldehyde (3.1g, 20.6mmol), dissolved in ethanol (150ml) containing 1% acetic acid. The reaction was heated to reflux for 10 min and monitored by t.l.c. (petroleum ether 60-80°C: EtOAc/ 2:1). When t.l.c. showed the disappearance of the starting material, the aqueous sodium bromide (2.1g, 20.6mol) was added to give a white precipitate. The cool mixture was filtered to give a mixture of organo-mercury compounds, dried in the air. Treatment of this mixture of organo-mercury compounds with bromine (0.68ml, 13.1mmol) in dichloromethane containing 1% of acetic acid gave a yellow solution. T.l.c (EtOAc: hexane/ 3:7) showed the formation of desired compound **70** (Rf0.3). The reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography (EtOAc: hexane/ 3:7) to give product as a colourless solid (1.7g, 35%).

m.p. 113-116°C (Lit.[146, 147] m.p. 115-117°C).

δ_H (DMSO): 9.72 (C***H***O, s), 7.81 (6-***H***, d, J 8.71Hz), 6.64 (5-***H***, d, J 8.72Hz), 4.86 (***OH***, brs, disappeared after D_2O shake), 4.01 (O***CH₃***, s); δ_C (DMSO): 194.3 (***C***HO), 163.7 (4-***C***), 160.2 (3-***C***), 134.5 (6-***C***), 116.3 (1-***C***), 103.7 (5-***C***), 99.7 (3-***C***), 56.8 (O***CH₃***); ν_{max}/cm^{-1} (KBr disc): 3406.2, 1637.3cm^{-1}.

(2) Preparation of 7-bromo-6-methoxybenzofuran-2-carboxylic acid ethyl ester (71)

Powdered anhydrous K_2CO_3 (2.4g, 17.4mmol) was added to the solution of aldehyde **70** (2g, 8.7mmol), ethyl chloroacetate (1.1ml, 10.4mmol) dissolved in dry DMF (20ml) under an atmosphere of N_2. The reaction was heated to 160°C and the progress was monitored by t.l.c. (petroleum ether 60-80°C: EtOAc/ 5:1). The reaction was quenched by pouring onto crushed ice (30g) following the full conversion of starting material (Rf 0.27) to a new compound. The mixture was extracted with DCM (50ml×2) and the organic layer was washed with water, sat. NaCl solution, dried over $MgSO_4$, and filtered. The filtration was concentrated *in vacuo*, and the residue was purified with flash chromatography (petroleum ether 60-80°C: EtOAc/5:1) to give product as a pale yellow solid (1.8g, 69%).

m.p. 100-101°C.

δ_H ($CDCl_3$): 7.57 (4-***H***, d, J 8.66Hz), 7.52 (3-***H***, s), 6.96 (5-***H***, d, J 8.71Hz), 4.45 ($COO\boldsymbol{CH_2}CH_3$, q, J 7.18Hz), 3.99 ($O\boldsymbol{CH_3}$, s), 1.42 ($COOCH_2\boldsymbol{CH_3}$, t, J 7.18Hz); δ_C ($CDCl_3$): 159.1 (6-***C***), 156.4 ($\boldsymbol{C}OOC_2H_5$), 154.4 (7a-***C***), 124.3 (4a-***C***), 121.6 (4-***C***), 114.3 (3-***C***), 109.6 (5-***C***), 93.9 (7-***C***), 61.5 ($COO\boldsymbol{CH_2}CH_3$), 57.1 ($O\boldsymbol{CH_3}$), 14.4 ($COOCH_2\boldsymbol{CH_3}$); ν_{max}/cm^{-1} (KBr disc): 1718.4cm^{-1}; m/z (EI): 299/301 $[M+H]^+$, 331/333 $[M+CH_3OH]^+$, ($C_{12}H_{11}BrO_4$ requires [M] 298/300);

(3) Preparation of 7-bromo-6-methoxy-benzofuran-2-carboxylic acid (72)

Ester **71** (1.6g, 5.5ml), suspended in ethanol (55ml) was treated with KOH (0.3g, 5.5mmol) dissolved in ethanol (20ml), and heated to reflux. The reaction was cooled down to room temperature until t.l.c. (petroleum ether 60-80°C: etOAc/ 5:1) showed the full conversion of the starting material (Rf 0.25). The solvent was removed, and the residue

was dissolved in water, acidified with conc. HCl to give product as a white solid (1.3g, 94%).

m.p. 247-250°C

δ_H (DMSO): 7.57 (4-***H***, d, J 8.66Hz), 7.52 (3-***H***, s), 6.96 (5-***H***, d, J 8.71Hz), 4.50 (***OH***, brs, disappeared after D_2O shake), 3.99 (O***CH***$_3$, s); δ_C (DMSO): 165.9 (7a-***C***)), 162.4 (***C***OOH), 157.6 (6-***C***), 124.0 (4a-***C***), 121.6 (4-***C***), 116.4 (3-***C***), 112.7 (5-***C***), 93.7 (7-***C***), 57.3 (O***CH***$_3$); ν_{max}/cm^{-1} (KBr disc): 3449.6, 1683.0cm^{-1}; m/z(EI): 271/ 273 $[M+H]^+$ ($C_{10}H_7BrO_4$ requires [M] 270/272).

(4) Preparation of 7-bromo-6-methoxy-benzofuran (73)

Copper powder (0.2g, 11.1mmol) was added into the solution of acid **72** (1.3g, 4.7mmol) dissolved in quinoline (15ml). The mixture was purged with nitrogen at room temperature, and then heated to 210°C. T.l.c. (ether: petroleum ether 60-80°C/ 10:1) indicated the disappearance of the starting material (on the baseline) and the formation of a new compound (Rf 0.58). The rmixture was cooled down to 100°C, poured onto crushed ice (30g), and diluted with ether (50ml). The mixture was filtered through glass wool and separated. The organic layer was washed with 2M HCl (20ml×2), sat. NaCl solution, dried over $MgSO_4$, and filtered. The solvent of the filtration was removed under reduced pressure, and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 15:1) to give product as a pale green oil (0.6, 52%), which crystallised at room temperature.

m.p. 71-73°C

δ_H ($CDCl_3$): 7.61 (2-***H***, d, J 2.23Hz), 7.47 (4-***H***, d, J 8.66Hz), 6.98 (5-***H***, d, J 8.66Hz), 6.76 (3-***H***, d, J 2.23Hz), 3.96 (O***CH*$_3$**, s); δ_C ($CDCl_3$): 154.1 (6-***C***), 145.6 (2-***C***), 144.0 (7a-***C***), 122.1 (4a-***C***), 119.8 (4-***C***), 108.7 (5-***C***), 107.0 (3-***C***), 93.9 (7-***C***), 57.3 (O***CH*$_3$**); m/z (EI): 227/ 229 $[M+H]^+$ ($C_9H_7BrO_2$ requires [M] 226/228).

4.11 Preparation of [10-(5-bromobenzofuran-3-yloxy)decyl]methylamine (60)

(1) Preparation of 5-bromo-2-hydroxy-benzoic acid ethyl ester (87)

A few drops of concentrated sulphuric acid was added to a solution of 5-bromosalicylic acid (10g, 0.24mol) dissolved in dry ethanol (200ml), and heated to reflux over night. The mixture was cooled down to room temperature, and most of the solvent was removed under reduced pressure. The residue was suspended in EtOAc, washed with sat. $NaHCO_3$ till no more CO_2, water and brine, dried over $MgSO_4$, filtered. The filtrate was concentrated *in vacuo* to give product as a pale yellow solid in good yield (9.8g, 87%). m.p. 49-50°C (Lit. [190] m.p. 51-53°C)

δ_H ($CDCl_3$): 10.9 (***OH***, brs, disappeared after D_2O shake), 7.95 (6-***H***, d, J 2.47Hz), 7.53 (4-***H***, dd, J 2.44Hz, 8.91Hz), 6.86 (3-***H***, d, J 8.90Hz), 4.40 (COO***CH*$_2$**CH_3, q, J 6.93Hz), 1.42 ($COOCH_2$***CH*$_3$**, t, J 7.17Hz); δ_C ($CDCl_3$): 169.1 (***C***OOC_2H_5), 160.7 (2-***C***), 138.3 (4-***C***), 132.3 (6-***C***), 119.6 (1-***C***), 114.1 (3-***C***), 110.7 (5-***C***), 61.9 (COO***CH*$_2$**CH_3), 14.1 ($COOCH_2$***CH*$_3$**); ν_{max}/cm^{-1}: 3196.7, 1684.0cm^{-1}.

(2a) Preparation of 5-bromo-2-ethoxycarbonylmethoxybenzoic acid ethyl ester (88)

The ester **87** (2.7g, 12.3mmol) was added to a solution of potassium hydroxide (0.7g, 12.3mmol) dissolved in dry ethanol (40ml) and subsequently treated with ethyl bromoacetate (1.4ml, 12.3mmol,). The homogeneous mixture was heated to reflux for 12 hours. Tlc (petroleum ether 60-80°C: EtOAc/ 5: 1) indicated the disappearance of starting material (Rf 0.21) and the formation of a new compound (Rf 0.58). The reaction was stopped and concentrated under reduced pressure. The residue was purified by flash chromatography (pet ether 60-80°C: ethyl acetate/ 5:1) to give the product as a white solid (1.5g, 41%).

m.p. 74-75°C

δ_H ($CDCl_3$): 7.93 (6-***H***, d, J 2.47Hz), 7.53 (4-***H***, dd, J 2.42Hz, 8.97Hz), 6.86 (3-***H***, d, J 8.99Hz), 4.68 ($OCH_2COOC_2H_5$, s), 4.35 ($OCH_2COOCH_2CH_3$, q, J 7.19Hz), 4.25 ($COOCH_2CH_3$, q, J 7.21Hz), 1.32 ($OCH_2COOCH_2CH_3$, t, J 7.19Hz), 1.29 ($COOCH_2CH_3$, t, J 7.22Hz); δ_C ($CDCl_3$): 168.2 ($OCH_2COOCH_2CH_3$), 164.5 ($COOCH_2CH_3$), 156.8 (2-***C***), 135.8 (4-***C***), 134.4 (6-***C***), 123.4 (1-***C***), 116.2 (3-***C***), 113.9 (5-***C***), 66.9 ($OCH_2COOC_2H_5$), 61.5 ($OCH_2COOCH_2CH_3$), 61.2($COOCH_2CH_3$), 14.2 ($COOCH_2CH_3$), 14.1 ($OCH_2COOCH_2CH_3$); ν_{max}/cm^{-1} (KBr disc): 3439.1, 1765.5, 1688.7 cm^{-1}.

(2b) Preparation of 5-bromo-2-*tert*-butoxycarbonylmethoxybenzoic acid ethyl ester (90)

To a stirred mixture of ester **90** (2g, 8.2mmol), and powdered anhydrous K_2CO_3 (1.7g, 12.2mmol) in dry DMF (20ml), was added *tert*-butyl bromoacetate (1.4ml, 9.8mmol) in one portion, and the mixture was heated to 80°C. The process of the reaction

was monitored by t.l.c (petroleum ether 60-80°C: ethyl acetate/ 10:1). The reaction was quenched by pouring to the water (50ml) and extracted with ethyl acetate (50ml×3). All the organic layers were combined and washed with water, brine, dried over $MgSO_4$, and filtered. The filtration was concentrated *in vacuo*, and the residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 10:1) to give product as colourless oil, which crystallized at room temperature (2.3g, 79%).

m.p. 35-37°C

δ_H ($CDCl_3$): 7.88 (6-***H***, d, J 2.73Hz), 7.49 (4-***H***, dd, J 2.72Hz, 8.66Hz), 6.70 (3-***H***, d, J 8.81Hz), 4.54 ($OCH_2COOC(CH_3)_3$, s), 4.31 ($COOCH_2CH_3$, q, J 7.17Hz), 1.43 ($OCH_2COOC(CH_3)_3$, s), 1.34 ($COOCH_2CH_3$, t, J 7.18Hz); δ_C ($CDCl_3$):167.1 ($COOC(CH_3)_3$), 164.4 ($COOCH_2CH_3$), 156.6 (2-***C***), 135.6 (4-***C***), 134.2 (6-***C***), 123.0 (1-***C***), 115.6 (3-***C***), 113.4 (5-***C***), 82.5 ($OCH_2COOC(CH_3)_3$), 66.8 ($OCH_2COOC(CH_3)_3$), 61.2 ($COOCH_2CH_3$), 27.9 ($OCH_2COOC(CH_3)_3$), 14.2 ($COOCH_2CH_3$); ν_{max}/cm^{-1} (KBrdisc): 1735.2,1730.1cm^{-1}; m/z (EI): $[M+H]^+$ 359/361 ($C_{15}H_{19}BrO_5$ requires [M] 360/362).

(3a) Preparation of 5-bromo-3-oxo-2,3-dihydrobenzofuran-2-carboxylic acid ethyl ester (89)

A solution of compound **88** (5.6g, 17.0mmol) dissolved in 60ml of dry benzene (Care, carcinogen) was added dropwise to a mixture of freshly made sodium ethoxide (1.3g, 17.0mmol) suspended in dry benzene (26ml). Stirring was continued and the reaction was heated at reflux for 4 hours. Tlc (petroleum ether 60-80: EtOAc/ 7:2) showed the full conversion of starting material (Rf 0.26). The mixture was poured to water and sufficient dilute NaOH was added to make the solution alkaline to litmus. The benzene and

aqueous layers were separated followed by the addition of dilute hydrochloric acid to the aqueous portion caused the product to precipitate. The product was filtered and the filter cake was an equal mixture of compound (**89**) (284/286 [M+H]) and 5-bromo-3-benzofuranone (212/214[M+H]) in equal amount according to GC/MS.

(3b) Preparation of 5-bromo-3-oxo-2,3-dihydrobenzofuran-2-carboxylic acid *tert*-butyl ester (91)

Sodium hydride (0.4g, 9.52mmol) was added in one portion into the solution of ester **90** (2.3g, 6.35mmol) made from **2b** (P133) dissolved in DMF (40ml). The dark pink reaction was heated to 80°C and monitored by t.l.c. (petroleum ether 60-80°C: EtOAc/ 10:1). The reaction was quenched by pouring onto crushed ice (50g) until t.l.c. showed the full conversion of the starting material (Rf 0.13) to a new compound (Rf 0.38). The mixture was extracted with ethyl acetate (50ml×2), and the organic layers were combined followed by washing with water, brine, and dried over $MgSO_4$, filtered. The solvent was removed, and the residue was purified by flash chromatography to give this benzofuran ester as a creamy solid in poor yield (0.4g, 21%).

δ_H ($CDCl_3$): 7.78 (4-***H***, d, J 1.98Hz), 7.45 (6-***H***, dd, J 1.98Hz, 8.91Hz), 7.28 (7-***H***, d, J 8.91Hz), 1.55 (COOC(***CH₃***)₃, s); δ_C ($CDCl_3$): 157.0 (***C***OOC(CH_3)$_3$), 154.7 (7a-***C***), 142.9 (2-***C***), 135.9 (4a-***C***), 131.8 (6-***C***), 123.1 (4-***C***), 122.0 (5-***C***), 114.2 (7-***C***), 115.7 (3-***C***), 83.8 (COO***C***(CH_3)$_3$), 28.4 (COOC(***CH₃***)₃); ν_{max}/cm^{-1} (KBr disc): 3395.7, 1749.5, 1662.2cm^{-1}; m/z (EI): $[M+H]^+$ 313/315 ($C_{13}H_{13}BrO_4$ requires [M] 312/314).

(4a) Preparation of 5-bromo-3-benzofuranone (86)

The mixture (2.4g) obtained from stage 3a without further purification was suspended in approximately 40ml of 5% of aqueous NaOH. The mixture was allowed to stand at room temperature with occasional stirring until the entire solid had dissolved. Diluted sulphuric acid was added cautiously to the aqueous until there is no further evidence of decarboxylation. The product is extracted with toluene and isolated by removing the solvent *in vacuo*. The residue was recrystallized from ethanol and dried in the air to give a red compound (1.4g, 86%).

m.p. 111-113°C (Lit. [191] m.p. 102-106°C)

δ_H ($CDCl_3$): 7.72 (4-***H***, d, J 1.97Hz), 7.53 (6-***H***, dd, J 1.97Hz, 8.90Hz), 6.86 (7-***H***, d, J 8.90Hz), 4.60 (2-***H***, s); δ_C ($CDCl_3$): 183.9 (3-***C***), 155.1 (7a-***C***), 135.1 (6-***C***), 131.4 (4-***C***), 128.7(3a-***C***), 112.3 (5-***C***), 111.9 (7-***C***), 55.5 (2-***C***); ν_{max}/cm^{-1} (KBr disc): 3423.9 (enol form), 1709.8cm^{-1}; GC/MS showed a single peak at 212/214 which is consistent with $C_8H_5BrO_2$ [M] 212/214.

(4b) Preparation of 5-bromo-3-benzofuranone (86)

The solution of the ester **91** (0.8g, 2.69mmol), dissolved in dry DCM (15ml) was treated with 2 ml of trifluoroacetic acid. The formation of a new compound (Rf 0.22, pet ether 60-80: EtOAc/ 5:1) and the disappearance of starting material indicated that the reaction was complete. Saturated sodium bicarbonate was added cautiously until there is no more CO_2. The aqueous layer was extracted with DCM (20ml×2). The organic layers were combined, and washed with water, brine, and dried over $MgSO_4$, filtered. The

filtration was concentrated under reduced pressure to give a bright yellow solid in reasonable yield (79%).

m.p.: 111-112°C (Lit. [191] m.p.102-106°C).

δ_H (DMSO): 7.60 (4-***H***, d, J 1.73Hz), 7.56 (6-***H***, dd, J 1.69Hz, 8.66Hz), 7.10 (7-***H***, d, J 8.65Hz), 4.63 (2-***H***, s); δ_C (DMSO): 171.1 (3-***C***), 166.6 (7a-***C***), 155.9 (6-***C***), 134.4 (4-***C***), 132.4 (4a-***C***), 119.0 (7-***C***), 113.2 (5-***C***), 69.0 (2-***C***); ν_{max}/cm^{-1} (KBr disc): 1717.5 cm^{-1}; DC/MS showed a single peak at 212/214 in agreement with $C_8H_5BrO_2$ [M] 212/214).

(5) Preparation of 5-bromo-3-[10-(tetrahydropyran-2-yloxy)decyloxy]benzofuran (92)

Sodium hydride (60%) in mineral oil (0.1g, 2.5mmol) was added one portion into a solution of benzofuranone **86** (0.4g, 2.1mmol) dissolved in dry DMF (20ml) under an atmosphere of N_2, and heated to 80°C. The alkylating reagent **96** was added, and the process of the reaction was monitored by t.l.c. (petroleum ether 60-80°C:EtOAc/ 10:1). The reaction was stopped until starting material (Rf 0.21) was fully converted of to a new compound **92** (Rf 0.31). The mixture was poured to water (20ml), and extracted with ethyl acetate (50ml×2). Combined organic layers were washed with water, sat. NaCl solution, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified with flash chromatography to give product as a yellow oil (0.5g, 53%).

δ_H ($CDCl_3$): 7.72 (4-***H***, d, J 1.98Hz), 7.33 (6-***H***, dd, J 1.98Hz, 8.66Hz), 7.24 (7-***H***, d, J 8.90Hz), 7.19 (2-***H***, s), 4.55 ($OCH_2(CH_2)_9OTHP$, t, J 2.73Hz), 3.92 ($O(CH_2)_9CH_2OTHP$, t, J 6.43Hz), 3.87 (2'-***H***, m), 3.67 (3'-***H***, m), 1.76 ($OCH_2CH_2(CH_2)_8OTHP$, 6'-H, m), 1.53-1.20 ($O(CH_2)_2(CH_2)_7CH_2O$, 4'-***H***, 5'-***H***, m); δ_C ($CDCl_3$): 152.3 (7a-***C***), 143.6 (3-***C***), 127.6 (6-***C***), 125.9 (2-***C***), 123.9 (4a-***C***), 121.7 (4-***C***), 115.1 (5-***C***), 113.1 (7-***C***), 98.9 (2'-***C***), 71.1

($OCH_2(CH_2)_9OTHP$), 67.7 ($O(CH_2)_9CH_2OTHP$), 62.3 (6'-***C***), 31.9 (3'-***C***), 29.4 ($OCH_2CH_2CH_2(CH_2)_4CH_2CH_2CH_2OTHP$), 26.2 (5'-***C***), 25.6, 25.5 ($O(CH_2)_2CH_2(CH_2)_4CH_2(CH_2)_2OTHP$), 19.7 (4'-***C***); m/z (EI): [M+H] 453/455 ($C_{23}H_{33}BrO_4$ requires $C_{23}H_{33}BrO_4$ [M] 452).

(6) Preparation of 10-(5-bromobenzofuran-3-yloxy)decan-1-ol (93)

The intermediate **92** (0.5g, 1.1mmol) was treated with PPTS (0.027g, 0.11mmol) via general method **E** to give a desired product as a pale yellow solid (Rf 0.19) in good yield (0.3g, 85%).

m.p. 42-44°C

δ_H ($CDCl_3$): 7.67 (4-***H***, d, J 1.98Hz), 7.33 (6-***H***, dd, J 10.99Hz, 1.98Hz), 7.18 (7-***H***, d, J 11.09Hz), 7.15 (2-***H***, s), 3.88 ($OCH_2(CH_2)_9OH$, t, J 6.44Hz), 3.57 ($O(CH_2)_9CH_2OH$, t, J 6.68Hz), 1.75 ($O(CH_2CH_2(CH_2)_8OH$, m), 1.50 ($O(CH_2)_8CH_2CH_2OH$, m), 1.25 ($O(CH_2)_2(CH_2)_6(CH_2)_2OH$, m); δ_C ($CDCl_3$): 152.3 (7a-***C***), 143.5 (3-***C***), 127.8 (6-***C***), 125.9 (2-***C***), 125.2 (4a-***C***), 121.7 (4-***C***), 115.1 (5-***C***), 113.1 (7-***C***), 71.2 ($OCH_2(CH_2)_9OH$), 63.1 ($(CH_2)_9CH_2OH$), 33.2 ($(CH_2)_8CH_2CH_2OH$), 29.3 ($CH_2CH_2CH_2(CH_2)_4CH_2CH_2CH_2OH$), 25.9 ($O(CH_2)_2CH_2(CH_2)_7OH$), 25.2 ($O(CH_2)_7CH_2(CH_2)_2OH$); ν_{max}/cm^{-1} (KBr disc): $3348cm^{-1}$; m/z (EI): $[M+H]^+$ 369/371 ($C_{18}H_{25}BrO_3$ requires [M] 368/370).

(7) Preparation of 5-bromo-3-(10-bromodecyloxy)benzofuran (94)

The alcohol **93** (0.3g, 0.8mmol) was treated with bromination reagent CBr_4 (0.3g, 1.0mmol) and Ph_3P (0.3g, 1.2mmol) via general method **F** to give the product as a pale yellow oil (0.2g, 68%).

δ_H ($CDCl_3$): 7.66 (4-***H***, d, J 1.97Hz), 7.33 (6-***H***, dd, J 11.09Hz, 1.98Hz), 7.18 (7-***H***, d, J 11.09Hz), 7.15 (2-***H***, s), 3.88 ($OCH_2(CH_2)_9Br$, t, J 6.43Hz), 3.34 ($O(CH_2)_9CH_2Br$, t, J 6.68Hz), 1.76 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.22 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 151.9 (7a-***C***), 146.8 (3-***C***), 127.8 (6-***C***), 126.0 (2-***C***), 123.4 (4a-***C***), 121.7 (4-***C***), 115.8 (5-***C***), 113.1 (7-***C***), 71.2 ($OCH_2(CH_2)_9Br$), 33.9 ($O(CH_2)_8CH_2CH_2Br$), 32.8 ($O(CH_2)_9CH_2Br$), 29.3 ($OCH_2CH_2CH_2(CH_2)_3(CH_2)_4Br$), 28.7 ($O(CH_2)_6CH_2(CH_2)_3Br$), 28.1 ($O(CH_2)_7CH_2(CH_2)_2Br$), 25.9 ($O(CH_2)_2CH_2(CH_2)_7Br$); m/z (EI): $[M+H]^+$431/433/435 ($C_{18}H_{24}Br_2O_2$ requires [M] 430/432/434).

(8) Preparation of [10-(5-bromobenzofuran-3-yloxy)decyl]methylamine (60)

The title compound **60** was prepared from bromide **94** (0.2g, 0.5mmol) and an excess of methylamine (33%) in ethanol (0.6ml, 5mmol) via general method **B** to give the product as a yellow oil (0.12g, 60%). This compound was not treated with ethereal hydrochloric acid due to the possible hydrolysis of the enol ether and it also has to be protected under nitrogen and in the fridge. The result of elemental analysis is not consistent with the theoretical data. However, all the other spectra analysis showed it is the expected compound.

δ_H ($CDCl_3$): 7.66 (4-***H***, d, J 1.97Hz), 7.33 (6-***H***, dd, J 11.09Hz, 1.98Hz), 7.18 (7-***H***, d, J 11.09Hz), 7.15 (2-***H***, s), 5.68 (***NH***, brs, disappeared after D_2O shake), 3.88 ($OCH_2(CH_2)_9NH$, t, J 6.43Hz), 3.34 ($O(CH_2)_9CH_2NH$, t, J 6.68Hz), 2.57 ($NHCH_3$, s) 1.76 ($OCH_2CH_2(CH_2)_6CH_2CH_2NH$, m), 1.22 ($O(CH_2)_2(CH_2)_6(CH_2)_2NH$, m); δ_C ($CDCl_3$): 151.9 (7a-***C***), 146.8 (3-***C***), 127.8 (6-***C***), 126.0 (2-***C***), 123.4 (4a-***C***), 121.7 (4-***C***), 115.8 (5-***C***), 113.1 (7-***C***), 71.2 ($OCH_2(CH_2)_9NH$), 46.7 ($O(CH_2)_9CH_2NH$), 33.6 ($NHCH_3$, s)

31.7($O(CH_2)_8\boldsymbol{CH_2}CH_2NH$), 29.3 ($OCH_2\boldsymbol{CH_2}CH_2\boldsymbol{(CH_2)_3}(CH_2)_4NH$), 28.7 ($O(CH_2)_6\boldsymbol{CH_2}(CH_2)_3NH$), 28.1 ($O(CH_2)_7\boldsymbol{CH_2}(CH_2)_2NH$), 25.9 ($O(CH_2)_2\boldsymbol{CH_2}(CH_2)_7NH$); m/z (EI): 382/384 ($C_{13}H_{28}BrNO_2$ requires [M] 381/383).

4.12 Preparation of 2-(10-bromodecyloxy)tetrahydropyran (96)

(1) Formation of 10-(tetrahydropyran-2-yl)decan-1-ol (98)

Silica chloride [166] (500mg) was added to a mixture of 1,10-decanediol (8.7g, 50mmol,) and 3,4-dihydro-2H-pyran (4.6ml, 50mmol) dissolved in dry DCM (500ml) (heating was applied due to the poor solubility of the diol in DCM). Tlc (petroleum ether 60-80°C: EtOAc/ 5:1, Rf 0.26) indicated that the completion of the reaction and the mixture was filtered through celite. The filtrate was concentrated under reduced pressure anf the residue was purified by flash chromatography with petroleum ether 60-80°C: ethyl acetate (5:1) to give the mono-THP ether as a colorless oil (8.0g, 62%).

δ_H ($CDCl_3$): 4.70 (***OH***, brs, disappeared after D_2O shake), 4.25 (THPO$\boldsymbol{CH_2}(CH_2)_9OH$, t, J 2.62Hz), 3.64 (5'-***H***, m), 3.55 ($\boldsymbol{CH_2}OH$, t, J 6.70Hz), 3.33 (1'-***H***, m), 1.78 (2', 3', 4'-***H***, m), 1.49 (THPO$\boldsymbol{CH_2}(CH_2)_7\boldsymbol{CH_2}CH_2OH$, m), 1.23 (THPO$(CH_2)_2\boldsymbol{(CH_2)_7}CH_2OH$, m); δ_C ($CDCl_3$): 67.6 (1'-***C***), 62.7 ($\boldsymbol{CH_2}OH$), 62.1 (5'-***C***), 32.7 (THPO$\boldsymbol{CH_2}(CH_2)_9OH$), 30.6 (THPO$(CH_2)_8\boldsymbol{CH_2}CH_2OH$), 29.6 (2'-***C***), 29.4 (THPO$(CH_2)_3\boldsymbol{(CH_2)_5}(CH_2)_2OH$, 26.1 (4'-***C***), 25.6 (THPO$(CH_2)_7\boldsymbol{CH_2}(CH_2)_2$), 25.4 (THPO$CH_2\boldsymbol{CH_2}(CH_2)_8OH$), 19.5 (3'-***C***).

(2) Formation 10-bromo-decane-1-ol (99)

To a mixture of 1, 10-decanediol (8.7g, 50mmol) in toluene (100ml) was added hydrobromic acid (48%) (9 ml, 50mmol,), and the reaction was heated to reflux for 28 hours. A small amount of hydrobromic acid was added if t.l.c. (ether: hexan 7:5, starting material Rf 0.07; product Rf 0.33) showed the reaction had not yet reached the completion. Once the reaction finished, the mixture was cooled down to room temperature followed by the washings with 6M NaOH (100ml), 10% HCl (100ml), water (2 × 100ml), brine (100ml) and then dried over $MgSO_4$, filtered. The solvent was removed *in vacuo*. The residue was distilled by Kugelrohr distillation (150°C / 7torr) followed by purification by flash chromatography (ether: hexane/ 6:5) to give compound as colorless oil (7.2g, 61%).

δ_H ($CDCl_3$): 3.53 (***CH*$_2$**OH, t, J 6.68Hz), 3.30 (Br***CH*$_2$**, t, J 6.92Hz), 2.33 (***OH***, brs, disappeared after D_2O shake). 1.78 ($BrCH_2$***CH*$_2$**$(CH_2)_8OH$, m), 1.47 ($Br(CH_2)_8$***CH*$_2$**CH_2OH, m), 1.22 ($Br(CH_2)_2$***(CH*$_2$**$)_6(CH_2)_2OH$, m); δ_C ($CDCl_3$): 62.3 (**CH$_2$**OH), 33.9 ($BrCH_2$***CH*$_2$**$(CH_2)_8OH$), 32.5 ($Br(CH_2)_8$***CH*$_2$**CH_2OH), 31.7 (Br***CH*$_2$**), 29.3 ($Br(CH_2)_2$***(CH*$_2$**$)_5(CH_2)_3$), 25.8 ($Br(CH_2)_7$***CH*$_2$**$(CH_2)_2$).

(3) Preparation of 2-(10-bromo-decyloxy)-tetrahydro-pyran (96)

The title compound **96** was prepared from the reaction between ether **98** (1.9g, 7.5mmol) and the combination reagents CBr_4 (3.1g, 9.4mmol)/ Ph_3P (2.9g, 11.3mmol) following general method F in a poor yield (0.7g, 31%). However, this bifunctional alkylating reagent **96** (7.5g, 81%) was prepared in a relatively good yield by bromide **99** (6.8g, 28.7mmol) via the method introduced in the preparation of compound **98**.

δ_H ($CDCl_3$): 4.50 (1'-***H***, t, J 2.62Hz), 3.81 (5'-***H***, m), 3.64 (THPO***CH***$_2$(CH_2)$_9$, m), 3.32 (THPO(CH_2)$_9$***CH***$_2$Br, t, J 6.70Hz), 1.82 (2'-***H***, THPO(CH_2)$_8$***CH***$_2$$CH_2$Br, m), 1.55 (THPO$CH_2$***CH***$_2$($CH_2$)$_8$Br, 3', 4'-***H***, m), 1.30 (THPO(CH_2)$_2$(***CH***$_2$)$_6$(CH_2)$_2$Br, m); δ_C ($CDCl_3$): 98.9 (1'-***C***), 67.5 (THPO***CH***$_2$(CH_2)$_9$), 62.2 (5'-***C***), 34.5 (2'-***C***), 33.8 (THPO(CH_2)$_9$***CH***$_2$Br), 32.7 (THPO(CH_2)$_8$***CH***$_2$$CH_2$Br), 30.6 (THPO$CH_2$***CH***$_2$($CH_2$)$_8$Br), 29.2-29.6 (THPO($CH_2$)$_3$(***CH***$_2$)$_4$($CH_2$)$_3$Br), 28.6 (THPO($CH_2$)$_9$***CH***$_2$($CH_2$)$_2$Br), 28.0 (4'-***C***), 25.4 (THPO(CH_2)$_2$***CH***$_2$(CH_2)$_7$), 19.6 (3'-***C***).

4.13 Preparation of methyl-[10-(1-methyl-1H-indole-5-yloxy)decyl]amine (112)

(1) Preparation 5-benzyloxy-1-methyl-1H-indole (114)

Iodomethane (1.4ml, 22mmol) was added to a stirred mixture of 5-benzyloxyindole (obtained from Aldrich) (4.5g, 20mmol), and sodium hydride (60%) in mineral oil (0.9g, 22mmol) in dry DMF (200ml) at ambient temperature. T.l.c. (petroleum ether 60-80°C: EtOAc/ 10:1) showed the disappearance of starting material (Rf 0.09), and the formation of a new compound (Rf 0.27) 2.5h later. The mixture was cooled down to room temperature and concentrated under reduced pressure. The residue was suspended in DCM (100ml), and washed with sat. $NaHCO_3$, water, sat. NaCl, dried over $MgSO_4$, and filtered. The filtration was concentrated *in vacuo* to give the product as a pink crystal (2.9g, 61%).

m.p. 125-127°C (Lit. [192] m.p. 125-127°C)

δ_H ($CDCl_3$): 7.30 (***PhH***CH_2O, 5-**H**, m), 7.15 (7-***H***, d, J 8.91Hz), 7.09 (4-***H***, d, J 2.48Hz), 6.93 (2-***H***, d, J 2.97Hz), 6.90 (6-***H***, dd, J 8.91Hz, 2.48Hz), 6.30 (3-***H***, d, J 2.97Hz), 5.02 (Ph***CH***$_2$O, s), 3.68 (N-***CH***$_3$, s); δ_C ($CDCl_3$): 153.1 (5-***C***), 139.8 (1'-***C***), 132.4 (7a-***C***), 129.3

(2-***C***), 128.8 (4a-***C***), 126.7 (2', 3', 4', 5', 6'-***C***), 112.6 (7-***C***), 109.8 (4-***C***), 104.2 (6-***C***), 100.4 (3-***C***), 70.9 (Ph***C*H$_2$O), 32.9 (N-***CH*$_3$); ν_{max}/cm^{-1} (KBr disc): 2925.3, 2872.2, 1490.7cm^{-1}; m/z (EI): 238 $[M+H]^+$ ($C_{16}H_{15}NO$ requires [M] 237); (Found: C 80.61%, H 6.37%, 5.78%; $C_{16}H_{15}NO$ requires: C 80.98%, H 6.29%), N 5.90%).

(2) Preparation of 1-methyl-1H-indole-5-ol (115)

Palladium (10% on carbon) (0.2g) was added to a solution of N-methyl indole 114 (2.4g, 10mmol) dissolved in ethyl acetate (50ml). The mixture was purged with N_2 followed by H_2, and finally equipped with a balloon filled with hydrogen (250ml). The mixture was kept stirring at room temperature until t.l.c. (petroleum ether 60-80°C: ethyl acetate/ 4:1) indicated the full conversion of starting material (Rf 0.19) to a new compound (Rf 0.44). The suspension was filtered through celite under N_2 and the filter cake was washed with EtOAc (20ml). The filtrate was concentrated *in vacuo*, and the residue was purified by flash chromatography (petrolem ether 60-80°C: ethyl acetate/ 3:1) to give the product as a white solid (0.7g, 86%).

m.p. 132-134°C (Lit. [192] m.p. 129-130°C)

δ_H ($CDCl_3$): 7.11 (7-***H***, d, J 8.70Hz), 7.09 (2-***H***, d, J 2.97Hz), 7.07 (4-***H***, d, J 2.48Hz), 6.74 (6-***H***, dd, J 8.66Hz, 2.48Hz), 6.28 (3-***H***, d, J 2.97Hz), 4.41 (***OH***, brs, disappeared after D_2O shake), 3.68 (N-***CH*$_3$, s); δ_C ($CDCl_3$): 149.3 (5-***C***), 132.7 (7a-***C***), 129.8 (2-***C***), 129.0 (4a-***C***), 111.7 (7-***C***), 109.8 (4-***C***), 105.2 (6-***C***), 100.0 (3-***C***), 32.9 (N-***CH*$_3$); ν_{max}/cm^{-1} (KBr disc): 3435.4, 2921.6, 1490.4cm^{-1}; m/z (EI): 148 $[M+H]^+$ (C_9H_9NO requires [M] 147); (Found: C 72.94%, H 6.71%, N 9.10%; C_9H_9NO requires: C 73.45%, H 6.15%, N 9.52%).

(3) Preparation of 1-methyl-5-[10-(tetrahydropyran-2-yloxy)decyloxy]-1H-indole (116)

THP ether **116** was prepared from the phenol **115** (0.6g, 4.0mmol) and bi-functional alkylating reagent **96** (1.3g, 4.2mmol) by general method D to give product as a yellow-green oil (1.0g, 70%), which crystallised at room temperature.

m.p. 44-46°C

δ_H ($CDCl_3$): 7.10 (7-***H***, d, J 8.90Hz), 7.01 (4-***H***, d, J 2.37Hz), 6.93 (2-***H***, d, J 2.97Hz), 6.79 (6-***H***, dd, J 8.91Hz, 2.47Hz), 6.32 (3-***H***, d, J 2.97Hz), 4.59 (2'-***H***, m), 3.91 ($OCH_2(CH_2)_9OTHP$, t, J 6.68Hz), 3.76 (6'-***H***, m), 3.60 (N-CH_3, s), 3.30 ($O(CH_2)_9CH_2OTHP$, m), 1.74 (3'-***H***, $OCH_2CH_2(CH_2)_8OTHP$, m), 1.49 (4', 5'-***H***, $O(CH_2)_8CH_2CH_2OTHP$, m), 1.24 ($O(CH_2)_2(CH_2)_6(CH_2)_2OTHP$, m); δ_C ($CDCl_3$): 153.4 (5-***C***), 132.1 (7a-***C***), 129.2 (2-***C***), 128.7 (4a-***C***), 112.5 (7-***C***), 109.8 (4-***C***), 103.6 (6-***C***), 100.3 (3-***C***), 98.8 (2'-***C***), 68.9 ($OCH_2(CH_2)_9OTHP$), 67.7 ($O(CH_2)_9CH_2OTHP$), 62.3 (6'-***H***), 32.9 (N-CH_3), 31.3 (3'-***C***), 30.8 ($O(CH_2)_8CH_2CH_2O$), 29.4 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3O$), 26.2 ($O(CH_2)_2CH_2(CH_2)_4CH_2(CH_2)_2OTHP$), 25.5 (5'-***C***), 19.7 (4'-***C***); ν_{max}/cm^{-1} (KBr disc): 2924.5, 2853.5, 1492.5cm^{-1} ; m/z (EI): 388 $[M+H]^+$ ($C_{24}H_{37}NO_3$ requires [M] 387); (Found: C 74.43%, H 9.48%,N 3.60%; $C_{24}H_{37}NO_3$ requires: C 74.38%, H 9.62%, N 3.61%).

(4) Preparation of 10-(1-methyl-1H-indole-yloxy)decan-1-ol (117)

The treatment of THP ether 116 (1.0g, 2.56mmol) with PPTS (0.064g, 0.256mmol) by general method E led to the formation of alcohol 117 (0.8g, 97%).

m.p. 55-57°C

δ_H ($CDCl_3$): 7.18 (7-***H***, d, J 8.90Hz), 7.08 (4-***H***, d, J 2.23Hz), 7.01 (2-**H**, d, J 2.97Hz), 6.86 (6-***H***, dd, J 8.90Hz, 2.23Hz), 6.38 (3-***H***, d, J 2.97Hz), 3.99 ($OCH_2(CH_2)_9OH$, t, J 6.68Hz), 3.76 (N-CH_3, s), 3.63 ($O(CH_2)_9CH_2OH$, t, J 6.68Hz), 1.76 ($OCH_2CH_2(CH_2)_8OH$, m), 1.56 ($O(CH_2)_8CH_2CH_2OH$, m), 1.25 ($O(CH_2)_2(CH_2)_6(CH_2)_2OH$, m); δ_C ($CDCl_3$): 153.4 (5-***C***), 131.4 (7a-***C***), 129.2 (2-***C***), 128.7 (4a-***C***), 112.5 (7-***C***), 109.8 (4-***C***), 103.7 (6-***C***), 100.3 (3-***C***), 68.8 ($OCH_2(CH_2)_9OH$), 63.1 ($O(CH_2)_9CH_2OH$), 32.8 ($O(CH_2)_8CH_2CH_2OH$), 29.4 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3OH$), 26.1 ($O(CH_2)_2CH_2(CH_2)_7OH$), 25.8 ($O(CH_2)_7CH_2(CH_2)_2OH$); ν_{max}/cm^{-1} (KBr disc): 3326.5, 2922.1, 2850.9cm^{-1}; m/z (EI): 304 $[M+H]^+$ ($C_{19}H_{29}NO_2$ requires [M] 303); (Found: C 75.02%, H 9.62%, N 4.42%; $C_{19}H_{29}NO_2$ requires: C 75.21%, H 9.63 %, N 4.62%).

(5) Preparation 5-(10-bromodecyloxy)-1-methyl-1H-indole (118)

The alcohol **117** (0.7g, 2.3mmol) was converted to bromide, a dark red waxy solid **118**, with combination bromination reagents CBr_4 (0.9g, 2.8mmol) and Ph_3P (0.9g, 3.4mmol) via general method **F** (0.5g, 64%).

δ_H ($CDCl_3$): 7.18 (7-***H***, d, J 8.90Hz), 7.08 (4-***H***, d, J 2.23Hz), 7.01 (2-***H***, d, J 2.97Hz), 6.86 (6-***H***, dd, J 8.90Hz, 2.23Hz), 6.38 (3-***H***, d, J 2.97Hz), 3.99 ($OCH_2(CH_2)_9OH$, t, J 6.68Hz), 3.76 (N-CH_3, s), 3.41 ($O(CH_2)_9CH_2OH$, t, J 6.83Hz), 1.79 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.29 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 152.4 (5-***C***), 131.4 (7a-***C***), 129.2 (2-***C***), 128.7 (4a-***C***), 112.5 (7-***C***), 109.8 (4-***C***), 103.7 (6-***C***), 100.3 (3-***C***), 68.8 ($OCH_2(CH_2)_9Br$), 34.7 ($O(CH_2)_8CH_2CH_2Br$), 32.8 ($O(CH_2)_9CH_2Br$), 29.4 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3Br$), 28.1 ($O(CH_2)_7CH_2(CH_2)_2Br$), 26.1 ($O(CH_2)_2CH_2(CH_2)_7Br$); homogeneous by GC/ MS $[M]^+$ at 365/ 367; m/z (EI): 366/368 $[M+H]^+$ ($C_{19}H_{29}BrNO$ requires [M] 365/367).

(6) Preparation of methyl-[10-(1-methyl-1H-indole-5-yloxy)-decyl]-amine HCl (112)

The title amine salt **112** was prepared from the bromide **118** (0.5g, 1.34mmol) and an excess of methylamine (33%) in ethanol (1.7ml, 13.4mmol) by general methods B& C to give product as a red solid (0.3g, 80%).

m.p. 154-156°C

δ_H (DMSO): 8.79 (***NH***, brs, disappeared after D_2O shake), 7.30 (7-***H***, d, J 8.90Hz), 7.25 (4-***H***, d, J 2.23Hz), 7.01 (2-***H***, d, J 2.97Hz), 6.86 (6-***H***, dd, J 8.90Hz, 2.23Hz), 6.38 (3-***H***, d, J 2.97Hz), 3.94 ($OCH_2(CH_2)_9NHCH_3$, t, J 6.70Hz), 3.72 (N-CH_3, s), 2.89 ($O(CH_2)_9CH_2NHCH_3$, t, J 6.90Hz), 2.37 ($NHCH_3$, s), 1.78 ($OCH_2CH_2(CH_2)_8NHCH_3$, m), 1.56 ($O(CH_2)_8CH_2CH_2NHCH_3$, m), 1.23 ($O(CH_2)_2(CH_2)_6(CH_2)_2NHCH_3$, m); δ_C (DMSO): 152.7 (5-***C***), 131.7 (7a-***C***), 129.8 (2-***C***), 128.3 (4a-***C***), 111.5 (7-***C***), 110.2 (4-***C***), 103.0 (6-***C***), 99.7 (3-***C***), 67.8 ($OCH_2(CH_2)_9NHCH_3$), 48.1 ($O(CH_2)_9CH_2NHCH_3$), 44.5 (N-CH_3), 32.5 ($NHCH_3$), 32.2 ($O(CH_2)_8CH_2CH_2NHCH_3$), 28.8 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3NH$), 25.8 ($O(CH_2)_7CH_2(CH_2)_2NHCH_3$), 25.2 ($O(CH_2)_2CH_2(CH_2)_7NHCH_3$); m/z (EI): 317 $[M+H]^+$ ($C_{20}H_{32}N_2O$ requires [M] 316) ; (Found: C 78.1%, H 9.7%, N 8.1%; $C_{20}H_{32}N_2O$ requires: C 78.3%, H 9.5%, N 8.0%).

4.14 Preparation of [10-(1H-indole-5-yloxy)decyl]methylamine (119)

(1) Preparation of 5-benzyloxyindole-1-carboxylic acid *tert*-butyl ester (125)

A dry, 250ml, two-necked flask was charged with nitrogen, and NaH (60% in mineral oil) (2.1g, 52.5mmol), was added. The mineral oil was removed by washing three times with pentane. The grey NaH powder was dried by evacuation, and dry THF (10ml) was added. The suspension was stirred and cooled in ice bath. 5-Benzyloxy-1H-indole (7.8g,

35.0mmol) in dry THF (75ml) was added cautiously. After gas evolution ceased, di-*tert*-butyldicarbonate (8.0g, 36.7mmol) was slowly added. The reaction was kept stirring at room temperature overnight. T.l.c. (petroleum ether 60-80°C: EtOAc/ 2:1) showed the full conversion of starting material (Rf 0.43) to a new compound (Rf 0.67). The reaction was quenched by the addition of water (100ml) and the mixture was partitioned with ether (100ml×2). The organic layers were combined and washed with water, sat. NaCl solution, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure, the residue was purified by flash chromatography (petroleum ether 60-80°C: ethyl acetate/ 5:1) to give the carbamate **125** as a colourless oil (9.1g, 80%).

δ_H ($CDCl_3$): 7.96 (7-***H***, d, J 8.91Hz), 7.49 (2-***H***, d, J 3.71Hz), 7.36 (***PhH***CH_2O, m), 7.02 (4-***H***, d, J 2.48Hz), 6.93 (6-***H***, dd, J 9.10Hz, 2.47Hz), 6.43 (3-***H***, d, J 3.71Hz), 5.03 (Ph***C*****H_2**O, s), 1.60 (COOC(***CH_3***$)_3$, s); δ_C ($CDCl_3$): 155.0 (***C***$OOC(CH_3)_3$), 149.7 (5-***C***), 137.3 (1'-***C***), 131.3 (7a-***C***), 129.8 (4a-***C***), 128.7 (3', 5'-***C***), 127.6 (2', 4', 6'-***C***), 126.5 (2-***C***), 115.8 (7-***C***), 113.7 (4-***C***), 107.1 (6-***C***), 104.9 (3-***C***), 83.4 (COO***C***$(CH_3)_3$), 70.5 (Ph***C*****H_2**O), 28.2 (COOC(***CH_3***$)_3$); ν_{max}/cm^{-1} (liquid film): 1728.9cm^{-1}; m/z (EI): 302 $[M+H]^+$ (100%).

(2) Preparation of 5-hydroxyindole-1-carboxylic acid *tert*-butyl ester (127)

The solution of *N*-Boc indole **125** (9.0g, 27.9mmol) dissolved in ethyl acetate was treated with palladium (10%) on carbon (0.9g). The mixture was purged with N_2 followed by hydrogen (675ml×2) (the reaction was carried out in hydrogenation room) and kept stirring until t.l.c. (petroleum ether 60-80°C: EtOAc/ 5:1) showed that the starting material (Rf 0.52) was fully converted to two new compounds — 5-hydroxyindole-1-carboxylic

acid *tert*-butyl ester (Rf 0.28), and 5-hydroxyindole-2, 3-dihydro-1-carboxylic acid *tert*-butyl ester (Rf 0.16). The solvent was removed *in vacuo* and the mixture was separated by flash chromatography (petroleum ether 60-80°C: EtOAc/ 5:1), to give the phenolic carbamate **127** as colourless oil (4.7g, 73%), and the dihydroxy analogue **126** (1.34g) as a white solid.

m.p. 168°C

δ_H ($CDCl_3$): 7.95 (7-***H***, d, J 8.66Hz), 7.51 (2-***H***, d, J 3.72Hz), 6.94 (4-***H***, d, J 2.48Hz), 6.81 (6-***H***, dd, J 8.90Hz, 2.47Hz), 6.43 (3-***H***, d, J 3.71Hz), 5.13 (***OH***, brs, disappeared after D_2O shake), 1.57 ($COOC(\boldsymbol{CH_3})_3$, s); δ_C ($CDCl_3$): 157.0 ($\boldsymbol{C}OOC(CH_3)_3$), 151.5 (5-***C***), 131.5 (7a-***C***), 128.7 (4a-***C***), 126.7 (2-***C***), 115.8 (7-***C***), 112.9 (4-***C***), 106.9 (6-***C***), 106.0 (3-***C***), 83.6 ($COO\boldsymbol{C}(CH_3)_3$), 28.2 ($COOC(\boldsymbol{CH_3})_3$); ν_{max}/cm^{-1} (liquid film): 3414.3, 1730.4cm^{-1}; GC/ MS showed a peak at 233 in agreement with $C_{13}H_{15}NO_3$ [M] 233; (Found: C66.84%, H 6.41%, N 5.96%; $C_{13}H_{15}NO_3$ requires: C 66.94%, H 6.48%, N 6.00%.

(3) Preparation of 5-(10-bromodecyloxy)indole-1-carboxylic acid tert-butyl ester (128)

The phenolic carbamate **127** (2.3g, 9.9mmol) was treated with an excess of 1,10-dibromodecane (14.5g, 48.3mmol) to give the product **128** as an oil (3.1g, 70%) purified by flash chromatography following general method **A**.

δ_H ($CDCl_3$): 7.94 (7-***H***, d, J 8.66Hz), 7.47 (2-***H***, d, J 3.46Hz), 6.94 (4-***H***, d, J 2.48Hz), 6.86 (6-***H***, dd, J 8.79Hz, 2.47Hz), 6.40 (3-***H***, d, J 3.71Hz), 3.93 ($O\boldsymbol{CH_2}(CH_2)_9Br$, t, J 6.43Hz), 3.31 ($O(CH_2)_9\boldsymbol{CH_2}Br$, t, J 6.93Hz), 1.76 ($OCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}CH_2CH_2Br$, m), 1.34 ($O(CH_2)_2(\boldsymbol{CH_2})_6(CH_2)_2Br$, m), 1.27 ($COOC(\boldsymbol{CH_2})_3$, s); δ_C ($CDCl_3$): 155.3 ($\boldsymbol{C}OOC(CH_2)_3$),

149.8 (5-***C***), 131.3 (7a-***C***), 129.4 (4a-***C***), 126.3 (2-***C***), 115.7 (7-***C***), 113.5 (4-***C***), 107.1 (6-***C***), 104.4 (3-***C***), 83.4 ($COOC(CH_2)_3$), 68.5 ($OCH_2(CH_2)_9Br$), 34.0 ($OCH_2CH_2(CH_2)_8Br$), 32.8 ($O(CH_2)_9CH_2Br$), 29.7-28.1 ($OCH_2CH_2CH_2(CH_2)_5(CH_2)_2Br$, $COOC(CH_2)_3$), 26.0 ($O(CH_2)_2CH_2(CH_2)_7Br$); m/z(EI): 452/ 454 $[M+H]^+$ ($C_{23}H_{34}BrNO_3$ requires [M] 451/453).

(4) 5-(10-methylaminodecyloxy)indole-1-carboxylic acid *tert*-butyl ester (130)

The bromide **128** (1.8g, 4.0mmol) was converted to **130** with NH_2CH_3 (33%) in ethanol (5ml, 40mmol) via general method **B** to give the product as a brown oil (1.2g, 76%). No attempt was made to form a hydrochloric salt.

δ_H ($CDCl_3$): 7.99 (7-***H***, d, J 8.91Hz), 7.52 (2-***H***, d, J 3.71Hz), 6.99 (4-***H***, d, J 2.23Hz), 6.91 (6-***H***, dd, J 8.91Hz, 2.23Hz), 6.45 (3-***H***, d, J 3.71Hz), 3.95 ($OCH_2(CH_2)_9NHCH_3$, t, J 6.68Hz), 2.53 ($O(CH_2)_9CH_2NHCH_3$, t, J 5.94Hz), 2.49 ($NHCH_3$, s), 1.73 ($OCH_2CH_2(CH_2)_8NHCH_3$, m), 1.62 ($COOC(CH_2)_3$), 1.44 ($O(CH_2)_8CH_2CH_2NHCH_3$), 1.20 (($O(CH_2)_2(CH_2)_6(CH_2)_2NHCH_3$, m); δ_C ($CDCl_3$): 155.2 ($COOC(CH_3)_3$), 149.8 (5-***C***), 131.3 (7a-***C***), 129.7 (4a-***C***), 126.3 (2-***C***), 115.7 (7-***C***), 113.4 (4-***C***), 107.2 (6-***C***), 104.3 (3-***C***), 83.3 ($COOC(CH_2)_3$), 68.5 ($OCH_2(CH_2)_9Br$), 51.2 ($O(CH_2)_9CH_2NHCH_3$), 36.5 ($NHCH_3$), 29.9 ($OCH_2CH_2CH_2(CH_2)_4CH_2CH_2CH_2NHCH_3$), 28.7 ($COOC(CH_2)_3$), 27.3 ($O(CH_2)_7CH_2(CH_2)_2$), 26.0 ($O(CH_2)_2CH_2(CH_2)_7NHCH_3$); ν_{max}/cm^{-1} (liquid film): 1735.5cm^{-1}; m/z (EI): 403 $[M+H]^+$ ($C_{24}H_{38}N_2O_3$ requires [M] 402); the elemental analysis does not agree with the theoretical data due to the unstability. However, the other spectra analysis showed it is the desired product.

(5) Preparation of 5-(10-bromodecyloxy)-1H-indole (124)

A few drops of TFA was added to a solution of bromide **128** (0.3g, 0.71mmol) dissolved in DCM (15ml). T.l.c. (petroleum ether 60-80°C: EtOAc/ 4:1) showed the full conversion of starting material to a new compound **124** (Rf 0.42). The reaction was stopped, and $NaHCO_3$ was added cautiously until no more sing of CO_2. The organic layer was washed with water, sat. NaCl solution, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography (petroleum ether 60-80°C: ethyl acetate/ 5:1) to give the product as a yellow oil (0.2g, 87%).

δ_H ($CDCl_3$): 8.01 (***NH***, brs, disappeared after D_2O shake), 7.17 (7-***H***, d, J 8.71Hz), 7.09 (4-***H***, d, J 2.67Hz), 7.03 (2-***H***, d, J 2.23Hz), 6.80 (6-***H***, dd, J 8.91Hz, 2.47Hz), 6.40 (3-***H***, d, J 2.23Hz), 3.92 ($O\boldsymbol{CH_2}(CH_2)_9Br$, t, J 6.43Hz), 3.33 ($O(CH_2)_9\boldsymbol{CH_2}Br$, t, J 6.93Hz), 1.78 ($OCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}CH_2Br$, m), 1.24 ($O(CH_2)_2(\boldsymbol{CH_2})_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 153.6 (5-***C***), 130.9 (7a-***C***), 128.3 (4a-***C***), 124.7 (2-***C***), 119.9 (7-***C***), 115.6 (4-***C***), 103.5 (6-***C***), 102.3 (3-***C***), 68.8 ($O\boldsymbol{CH_2}(CH_2)_9Br$), 34.0 ($O(CH_2)_8\boldsymbol{CH_2}CH_2Br$), 32.8 ($O(CH_2)_9\boldsymbol{CH_2}Br$), 29.3 ($O(CH_2)_3(\boldsymbol{CH_2})_5(CH_2)_2Br$), 26.1 ($O(CH_2)_2\boldsymbol{CH_2}(CH_2)_7Br$); m/z(EI): $[M+H]^+$ 352/354 ($C_{18}H_{26}BrNO$ requires [M] 351/353).

(6) Preparation of [10-(1H-indole-5-yloxy)-decyl]-methylamine (119)

The title compound **119** was prepared from indole **124** (0.2g, 0.54mmol) and an excess of 33% methylamine in ethanol (0.7ml, 5.4mmol) via general method B to afford product as a brown oil (0.11g, 67%). There is no attempt to form a hydrochloric salt. The

elemental analysis is not consistent to the desired data due to the unstability, however, all the other spectra showed it is an expected compound.

δ_H ($CDCl_3$): 8.01 (***NH***, brs, disappeared after D_2O shake), 7.17 (7-***H***, d, J 8.71Hz), 7.09 (4-***H***, d, J 2.67Hz), 7.03 (2-***H***, d, J 2.23Hz), 6.80 (6-***H***, dd, J 8.91Hz, 2.47Hz), 6.40 (3-***H***, d, J 2.23Hz), 3.92 ($OCH_2(CH_2)_9NHCH_3$, t, J 6.43Hz), 2.55 ($(CH_2)_9CH_2NHCH_3$, t, J 6.70Hz), 2.47 ($NHCH_3$), 1.78 ($OCH_2CH_2(CH_2)_8NHCH_3$, m), 1.42 ($O(CH_2)_8CH_2CH_2NHCH_3$, m), 1.27 ($O(CH_2)_2(CH_2)_6(CH_2)_2NHCH_3$, m); δ_C ($CDCl_3$): 155.6 (5-***C***), 130.7 (7a-***C***), 127.3 (4a-***C***), 124.4 (2-***C***), 112.9 (7-***C***), 110.6 (4-***C***), 103.5 (6-***C***), 102.3 (3-***C***), 68.8 ($OCH_2(CH_2)_9Br$), 59.6 ($O(CH_2)_9CH_2NHCH_3$), 34.7 ($NHCH_3$), 29.5 ($OCH_2CH_2CH_2(CH_2)_4CH_2CH_2CH_2NHCH_3$), 26.7 ($O(CH_2)_7CH_2(CH_2)_2NHCH_3$), 24.3 ($O(CH_2)_2CH_2(CH_2)_7NHCH_3$); m/z (EI): 303.2 $[M+H]^+$ ($C_{19}H_{30}N_2O$ requires [M] 302).

4.15 Preparation of Miscellaneous Indole Precursors

(1) Preparation of (4-hydroxyphenyl)methyl carbamic acid *tert*-butyl ester (101)

di-tert-Butyldicarbonate (6.5g, 29.6mmol) was added to a solution of 4-methylaminophenol (3.44g, 28.2mmol) dissolved in dry THF (40ml) at temperature. Tlc (petroleum ether 60-80°C: EtOAc/ 2:1) showed the disappearance of starting material and the formation of a new compound (Rf 0.42). The solvent was removed and the residue was purified by flash chromatography to give the product as a pale purple solid (5.5g, 87%).

m.p. 138-140°C (Lit. [193] m.p. 144-145°C)

δ_H ($CDCl_3$): 7.13 (2, 6-***H***, d, J 6.78Hz), 6.90 (3, 5-***H***, d, J 6.78Hz), 3.11 (NCH_3, s), 1.39 ($C(CH_3)_3$, s); δ_C ($CDCl_3$): 154.4 (***C***O), 147.8 (4-***C***), 131.3 (1-***C***), 127.1 (2, 6-***C***), 115.9 (3, 5-***C***), 63.4 ($C(CH_3)_3$), 38.0 (NCH_3), 28.4 ($C(CH_3)_3$); ν_{max}/cm^{-1} (KBr disc): 3296, 1657cm^{-1}.

(2) Preparation of [4-(10-bromodecyloxy)phenyl]methyl carbamic acid *tert*-butyl ester (102)

1,10-Dibromodecane was added to a mixture of phenol **101** (2.2g, 10mmol) dissolved in dry 2-butanone (45ml) via general method **A** to give the product as a pale purple oil (3.9g, 88%).

δ_H ($CDCl_3$): 7.04 (2, 6-***H***, d, J 8.91Hz), 6.76 (3, 5-***H***, d, J 8.91Hz), 4.03 ($OCH_2(CH_2)_9Br$, t, J 6.43Hz), 3.32 ($O(CH_2)_9CH_2Br$, t, J 6.68Hz), 3.13 (NCH_3, s), 1.82 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.30 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, $C(CH_3)_3$m); δ_C ($CDCl_3$): 156.7 (4-C), 155.0 (***C***O), 136.6 (1-***C***), 126.7 (2, 6-***C***), 114.3 (3, 5-***C***), 79.7 ($OCH_2(CH_2)_9Br$), 68.0 ($C(CH_3)_3$), 37.5 (NCH_3), 33.9 ($O(CH_2)_8CH_2CH_2Br$) 32.7 ($O(CH_2)_9CH_2Br$), 29.7 ($OCH_2CH_2CH_2(CH_2)_5(CH_2)_2$, $C(CH_3)_3$); m/z(EI): 442/444 ($C_{22}H_{36}BrNO_3$ requires 441/443).

(3) Preparation of 4-(10-bromodecyloxy)phenyl methylamine (103)

A few drops of TFA was added to a solution of carbamate **102** (5.7g, 13.0mmol) dissolved in DCM (50ml). Tlc (petroleum ether 60-80°C: EtOAc: TEA/ 10:1:0.3) showed the disappearance of starting material and the formation of a new compound (Rf 0.2). The solvent was removed under reduced pressure, and the residue was purified by flash chromatography to give product as a pale yellow solid (2.5g, 57%).

m.p. 62-64°C

δ_H ($CDCl_3$): 8.02 (***NH***, brs, disappeared after D_2O shake), 6.70 (3, 5-***H***, d, J 8.44Hz), 6.51 (2, 6-***H***, d, J 8.44Hz), 4.03 ($OCH_2(CH_2)_9Br$, t, J 6.03Hz), 3.30 ($O(CH_2)_9CH_2Br$, t, J 6.68Hz), 2.60 (NCH_3, s), 1.76 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.30

$(O(CH_2)_2(\boldsymbol{CH_2})_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 146.7 (4-***C***), 136.6 (1-***C***), 113.7 (3, 5-***C***), 112.3 (2, 6-***C***), 69.7 ($O\boldsymbol{CH_2}(CH_2)_9Br$), 37.5 ($N\boldsymbol{CH_3}$), 34.9 ($O(CH_2)_8\boldsymbol{CH_2}CH_2Br$) 32.7 ($O(CH_2)_9\boldsymbol{CH_2}Br$), 29.7 ($OCH_2\boldsymbol{CH_2}CH_2(\boldsymbol{CH_2})_5(CH_2)_2$).

(4) Preparation of 2-{[4-(10-bromodecyloxy)phenyl]methylamino}-3-oxo-butyric acid ethyl ester (104)

The mixture of methylamine **103** (0.3g, 1mmol), ethyl diazoacetoacetate (0.20g, 1.3mmol) and rhodium (II) acetate (0.02mmol) in toluene (12.5ml) was heated to reflux for 1h under nitrogen. Tlc (petroleum ether 60-80°C: EtOAc/ 2:1) showed the disappearance of the starting material (Rf 0.36) and the formation of a new compound (Rf 0.68). The cooled mixture was cooled down to room temperature and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether 60-80°C: EtOAc/ 10:1) to give the product as yellow oil (0.1g, 51%).

δ_H ($CDCl_3$): 6.73 (3, 5-***H***, d, J 9.15Hz), 6.48 (2, 6-***H***, d, J 9.16Hz), 4.03 ($N(CH_3)\boldsymbol{CH}(COCH_3)COO\boldsymbol{CH_2}CH_3$, m) 3.80 ($O\boldsymbol{CH_2}(CH_2)_9Br$, t, J 6.63Hz), 3.35 ($O(CH_2)_9\boldsymbol{CH_2}Br$, t, J 6.68Hz), 2.91 ($N\boldsymbol{CH_3}$, s), 1.90 ($CO\boldsymbol{CH_3}$, s), 1.76 ($OCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}CH_2Br$, m), 1.35 ($O(CH_2)_2(\boldsymbol{CH_2})_6(CH_2)_2Br$, m), 1.04 ($COOCH_2\boldsymbol{CH_3}$, t, J 7.18Hz); δ_C ($CDCl_3$): 175.6 ($\boldsymbol{C}OCH_3$), 172.1 ($\boldsymbol{C}OOCH_2CH_3$), 151.7 (4-***C***), 143.6 (1-***C***), 115.7 (3, 5-***C***), 112.3 (2, 6-***C***), 111.7 ($\boldsymbol{CH}(COCH_3)COOC_2H_5$), 68.7 ($O\boldsymbol{CH_2}(CH_2)_9Br$), 60.4 ($COO\boldsymbol{CH_2}CH_3$), 39.7 ($N\boldsymbol{CH_3}$), 33.9 ($O(CH_2)_8\boldsymbol{CH_2}CH_2Br$) 32.7 ($O(CH_2)_9\boldsymbol{CH_2}Br$), 29.7 ($OCH_2\boldsymbol{CH_2}CH_2(\boldsymbol{CH_2})_5(CH_2)_2$), 26.1 ($O(CH_2)_2\boldsymbol{CH_2}(CH_2)_7$), 17.4 ($CO\boldsymbol{CH_3}$), 14.2 ($COOCH_2\boldsymbol{CH_3}$); ν_{max}/cm^{-1} (liquid film): 1650.7, 1619.4cm^{-1}.

(5) Preparation of 4-hydroxyphenyl carbamic acid *tert*-butyl ester (108)

di-tert-Butyldicarbonate (21.8g, 0.1mol) was added to a solution of 4-aminophenol (10.9g, 0.1mol) dissolved in dry THF (100ml) under nitrogen. The homogeneous solution was kept stirring until tlc (EtOAc: cyclohexane/ 1:1) showed the reaction completed. The solvent was removed *in vacuo*, and the residue was recrystallised from toluene to give the product as a pale purple needle crystal. The product was filtered off and washed with distilled petroleum ether (60-80°C), dried in air (15.5g, 74%).

m.p. 145-147°C (Lit. [193] m.p.)

δ_H ($CDCl_3$): 7.51 (3, 5-***H***, d, J 8.53Hz), 6.77 (2, 6-***H***, d, J 8.53Hz), 1.38 (C(***CH₃***)₃, s); δ_C ($CDCl_3$): 157.6 (***C***O), 144.7 (1-***C***), 137.6 (4-***C***), 123.6 (3, 5-***C***), 113.9 (2, 6-***C***), 64.3 (***C***$(CH_3)_3$), 27.8 (C(***CH₃***)₃); ν_{max}/cm^{-1} (KBr disc): 3365.8, 1693.9cm^{-1}.

(6) Preparation of 4-[10-bromodecyloxy)phenyl]carbamic acid *tert*-butyl ester (109)

To a mixture of phenol **108** (1g, 4.8mmol) and powdered dry K_2CO_3 suspended in dry 2-butanone (25ml), was added 1, 10-dibromodecane (7g, 24mmol) following general method **A** to give the product as a colourless solid (0.9g, 44%).

m.p. 101-103°C

δ_H ($CDCl_3$): 7.26 (3, 5-***H***, d, J 8.91Hz), 6.77 (2, 6-***H***, d, J 8.91Hz), 3.93 (O***CH₂***$(CH_2)_9$Br, t, J 6.69Hz), 3.40 (O$(CH_2)_9$***CH₂***Br, t, J 6.93Hz), 1.82 (OCH_2***CH₂***$(CH_2)_6$***CH₂***CH_2Br, m), 1.55 (C(***CH₃***)₃, s), 1.30 (O$(CH_2)_2$(***CH₂***)₆$(CH_2)_2$Br, m); δ_C ($CDCl_3$): 157.6 (***C***O), 144.7 (4-***C***), 130.6 (1-***C***), 121.6 (2, 6-***C***), 114.6 (3, 5-***C***), 70.2 (***C***$(CH_3)_3$), 27.8 (C(***CH₃***)₃); m/z(EI): 428/430 ($C_{21}H_{34}BrNO_3$ requires [M] 427/429).

(7) Preparation of 4-(10-bromodecyloxy)phenylamine (110)

A few drops of trifluoroacetic acid were added to a solution of ester **109** (0.4g, 1mmol) dissolved in DCM (10ml). The mixture was kept stirring until t.l.c. (EtOAc: petroleum ether 60-80°C/ 1:7) showed the full conversion of starting material. The solvent was removed under reduced pressure, and the residue was dissolved in ether and extracted with 10% sodium carbonate, sat. NaCl solution, dried over Na_2SO_4, and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by flash chromatography (EtOAc: petroleum ether 60-80°C/ 1:7) to give product as a pale purple solid (0.2g, 64%).

m.p. 92-95°C

δ_H ($CDCl_3$): 6.75 (3, 5-***H***, d, J 8.46Hz), 6.66 (2, 6-***H***, d, J 8.47Hz), 3.87 ($OCH_2(CH_2)_9Br$, t, J 6.69Hz), 3.40 ($O(CH_2)_9CH_2Br$, t, J 6.93Hz), 1.82 ($OCH_2CH_2(CH_2)_6CH_2CH_2Br$, m), 1.30 ($O(CH_2)_2(CH_2)_6(CH_2)_2Br$, m); δ_C ($CDCl_3$): 148.6 (4-***C***), 134.9 (1-***C***), 116.6 (2, 6-***C***), 115.6 (3, 5-***C***), 68.7 ($OCH_2(CH_2)_9Br$), 34.0 ($O(CH_2)_9CH_2CH_2Br$), 32.8 ($O(CH_2)_9CH_2Br$), 29.3 ($OCH_2CH_2CH_2(CH_2)_4(CH_2)_3$), 28.1 ($O(CH_2)_7CH_2(CH_2)_2$), 26.0 ($O(CH_2)_2CH_2(CH_2)_7Br$); ν_{max}/cm^{-1} (KBr disc): 3409.6, 3329.1cm^{-1}.

Biology

Chapter 5

Biological Introduction

5.1 Melanoma and Metastasis

There are two main types of skin cancer, melanoma and non-melanoma skin cancer. Melanoma is the most serious and the least common form (10%) of skin cancer; it begins when melanocytes become malignant in the skin. Even so, if diagnosed and removed while it is still thin and limited to the outermost skin layer, it is almost 100% curable. Once the cancer advances and metastasises (spreads) to other parts of the body, it is hard to treat and can be fatal. During the past 10 years the number of cases of melanoma has increased more rapidly than that of any other cancer, especially in most developed countries (e.g. the United States of America, Australia, Canada).

The process of cancer metastasis consists of a long series of linked, sequential, and selective steps. Metastasis begins when tumour cells detach from the primary neoplasm and the surrounding stroma is invaded by single cells or a group of cells with increased motility and secretion of degradative enzymes [194]. Thin-walled venues, like lymphatic channels, are easily penetrated by tumour cells and provide the most common pathways for tumour cell entry into the circulation. The tumour emboli must survive immune and non-immune defences and the turbulence of the circulation, where the vast majority of circulating tumour cells is destroyed rapidly. Arrest in the capillary bed of receptive organs follows next. After extravasation, development of vascularization and proliferation within the organ parenchyma completes the metastatic process. Tumour cells can also invade host

stroma, penetrate blood vessels, and enter the circulation to produce additional metastases [195, 196].

5.2 Treatment of melanoma

Treatment modalities for melanoma include surgery, chemotherapy, radiotherapy and immunotherapy. Although useful for treating early stages of melanoma, surgical intervention once the primary melanoma has metastasised is often not an option.

5.2.1 Chemotherapy

Chemotherapy is the use of special anti-cancer (cytotoxic) drugs to destroy cancer cells, which disrupt the growth of cancer cells. It may be taken by pill, or it may be put into the body by a needle in the vein or muscle. Chemotherapy is also called a systemic treatment because drugs can be put directly into the bloodstream of the arm or leg where the melanoma is found. Chemotherapy cannot usually cure melanoma, but may stop or slow its growth for a time. It alone has not shown to be particularly effective in treating melanoma, so it is sometimes used to relieve symptoms or to extend the life of patients with advanced cancer.

Chemotherapy regimes include the use of hormonal and DNA alkylating agents such as tamoxifen, decarbazine, carmustin and cis-platin. However, unfortunately, the response is limited because of the multi-drug-resistance (MDR) to the standard high dose monochemotherapy. In order to delay the onset of MDR, the drugs mentioned above may also be used in various combinations [197, 198]. Nevertheless, this kind of combination still remains resistance to chemotherapy treatment. The possible reason is that chemotherapeutic reagents kill cells by introducing them to suicide (apoptosis). However,

the inherent resistance to apoptosis makes melanoma cells to not act sensitively to the drugs [199]. Unfortunately, chemotherapy causes unpleasant side effects, such as nausea, vomiting, or even hair loss.

5.2.2 Immunotherapy

Immunotherapy is the use of naturally occurring or synthetically made biological products that can boost a person's immune system to fight off cancer. Along with chemotherapy, immunotherapy is considered an adjuvant therapy for patients with more advanced stages of melanoma. Cytokines therapy, for example, is the use of protein called cytokines that activate the immune system and can shrink 10-20% of all metastatic tumours in patients with stage III or IV melanoma. Researchers are studying many different types of products that could be used in immunotherapy, including a possible vaccine that could be injected to stimulate an immune response to melanoma.

5.2.3 Radiotherapy

Radiotherapy treats cancer by using high-energy rays which destroy the cancer cells while going as little harm as possible to normal cells. The primary use of radiation therapy for melanoma patients is as a palliative treatment (it treats the symptoms but not the actual cancer) for stage IV disease. Radiotherapy is not commonly used to treat malignant melanoma but it may be used to relieve symptoms if the melanoma has spread to other parts of the body.

Chapter 6

Results and Discussion of Biology Assays

6.1 Evaluation of anti-fungal Compounds

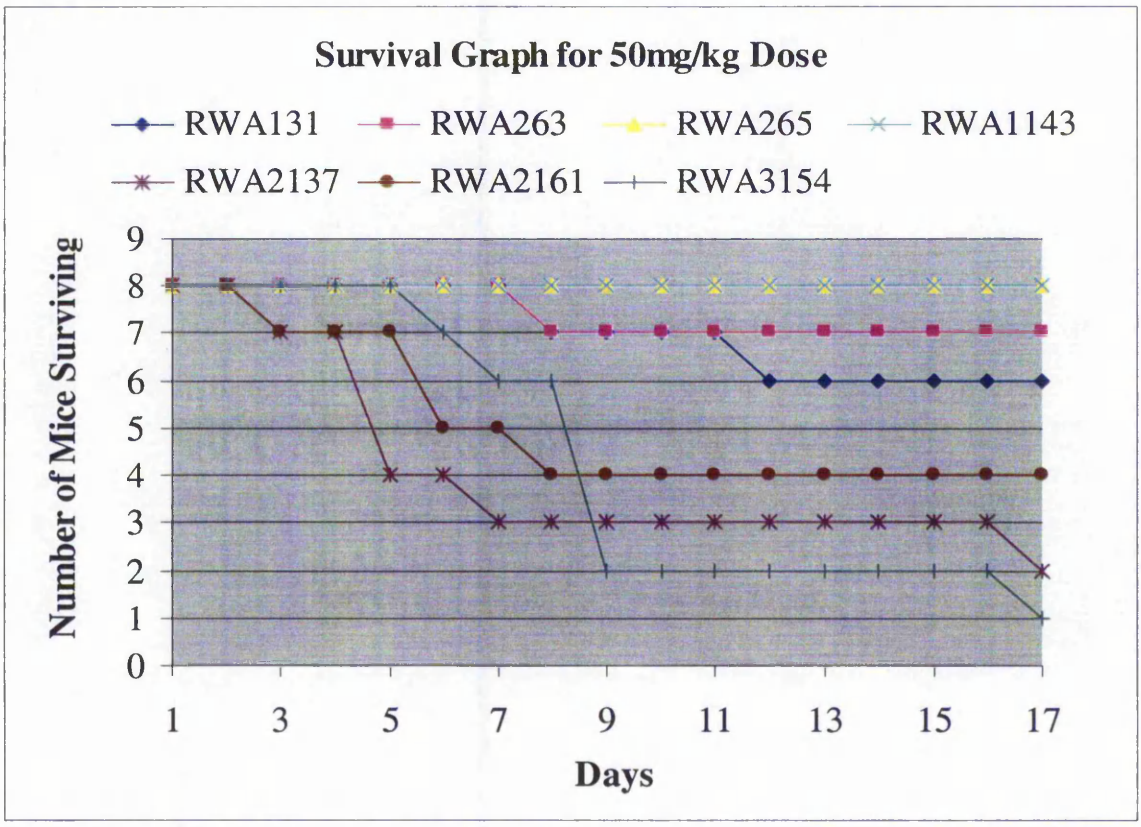
Some of Allcock's best anti-fungal compounds (mainly against *Candida* species), and **KY25**, a biostere of **RWA 265** (structure was shown on P48), were re-examined by the British Technology Group in an anti-fungal screen against *Aspergillus* species. The values of MIC (minimum inhibitory concentration) obtained are shown in **Table 1**. These results are in reasonable agreement with those previously obtained by Zeneca, and warranted examination of the compounds *in vivo*, starting with toxicity studies. These used some of the compounds whose larger scale preparation is discussed in an earlier section (**3.1**).

Table 1. the MIC(μM) of compounds tested in fungus xcreen against Aspergillus species.

	A. fumigatus		*A. flavus*	
	MIC24 (μM)	**MIC48 (μM)**	**MIC24 (μM)**	**MIC48 (μM)**
Amphotericin	0.25	0.25	1	1
Itraconazole	0.03	0.25	0.03	0.06
KY25	4	4	2	4
RWA131	1	1	1	2
RWA263	2	2	4	4
RWA265	2	2	2	2
RWA1143	1	1	1	1
RWA2137	2	2	2	2
RWA2161	4	8	1	1
RWA2134	2	2	1	1

Compared to the other compounds, **KY25** did not show any better results in this fungicidal test and was replaced by **RWA3154** (**KY16**), the chloroanalogue of **RWA1143**. The results are shown in **Figure 1**.

Figure 1: the number of survival mice for 50mg/kg dose.

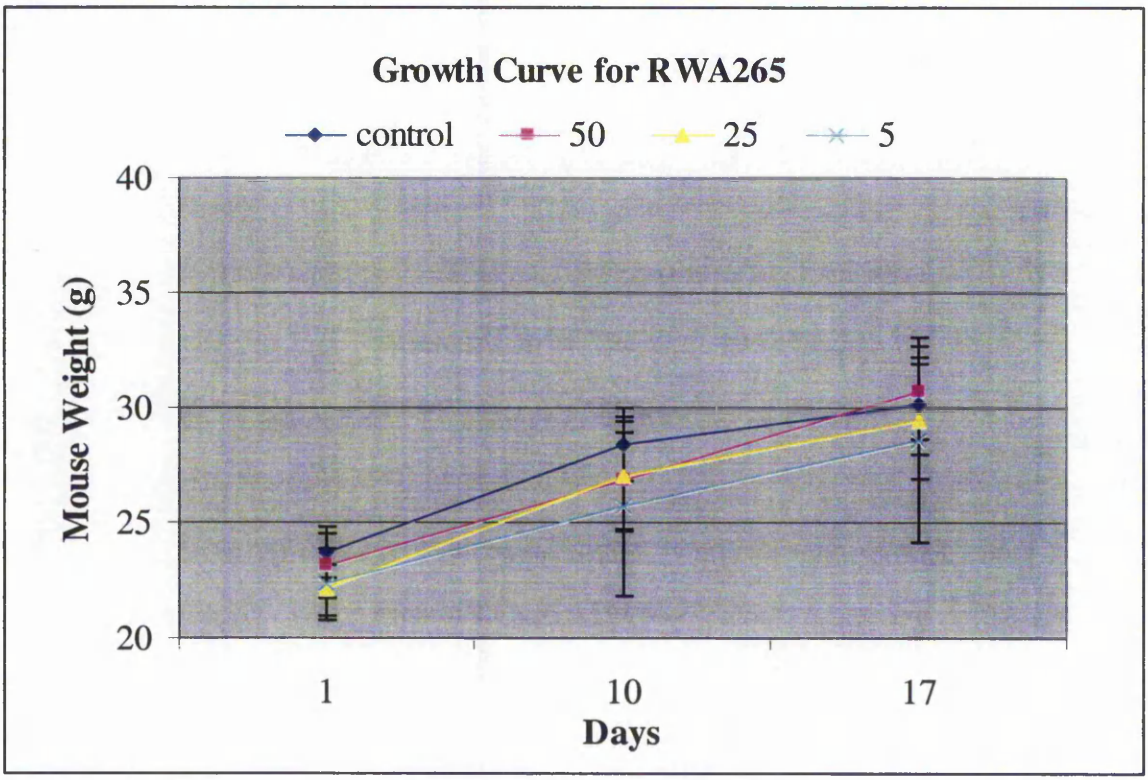


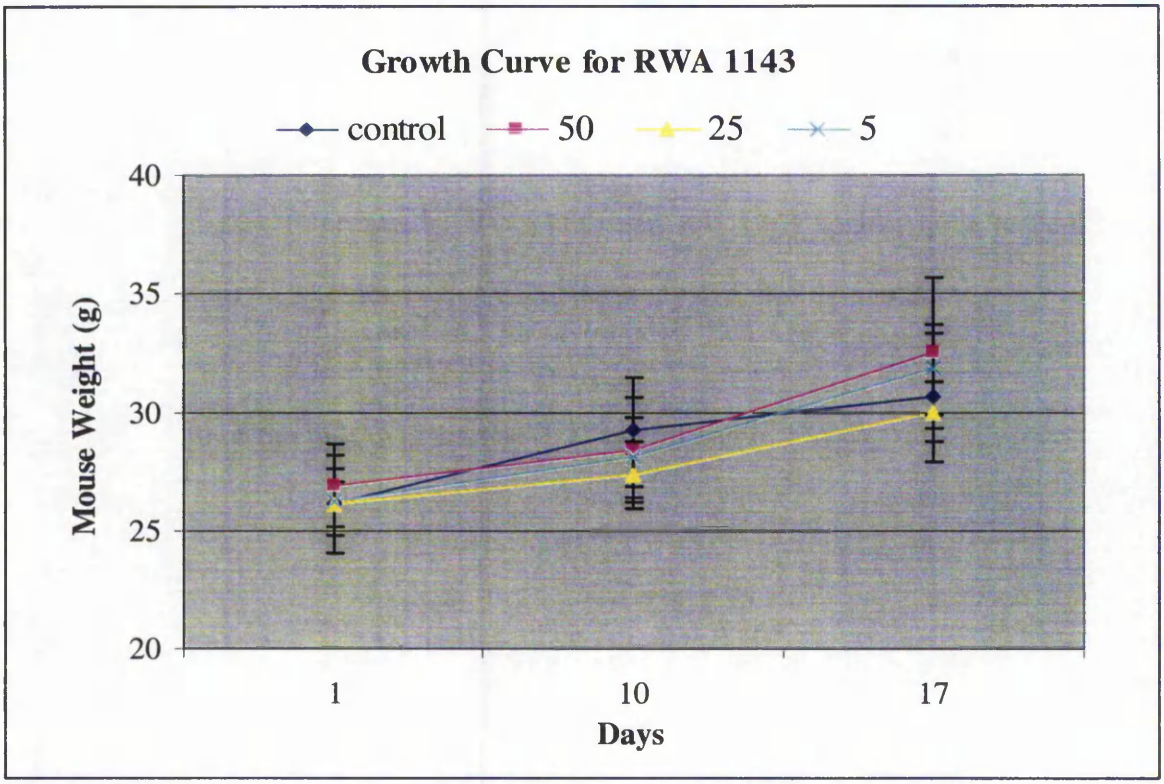
All the mice were divided into seven groups containing 8 mice each and every compound was injected into these mice with the dosage of 50mg/kg. It was found that the groups of mice subject to **RWA265** and **RWA1143** (structure was shown on P35) all survived after 17 days. It is clearly shown that these two anti-fungal compounds, especially active against *Candida* species, are non-toxic *in vivo*. To prove this result, the mouse

weight in these two groups in 17-day was further examined with, dosages of 5, 25 and 50mg/kg respectively. The resulting growth curves also showed that **RWA265** and **1143** are non-toxic. The graphs of the mouse weight experiment are shown in **Figure 2**.

Unfortunately, final tests of **RWA1143** and **RWA265** against mice loaded with *Aspergillus* showed no beneficial results, and the study was discontinued.

Figure 2: weight of the mouse using RWA 1143 and 265 in 17-day.





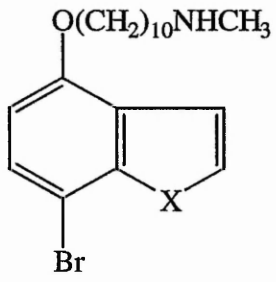
It is noteworthy that **RWA1143**, **2161** (**16**) and **3154** (**17**) basically possess the same chemical structure. However, the toxicity varies when the bromine in **RWA265** is replaced with fluorine (**RWA2161** or **KY17**) and chlorine (**RWA3154** or **KY16**). This results in much more toxic compounds (**Fig. 1**) with only one mice surviving after 17-day exposure to **KY16** (**RWA2161**). It was thought that this difference in toxicity might be reflected in the *in vitro* antiproliferation assay, discussed in the next section.

6.2 Tests Against Melanoma Cell Lines

Anticancer compounds can act by a variety of mechanisms. They may kill the cell or prevent its growth (anti-proliferation; this is particularly useful for dealing with

established tumors). The most deadly aspects of cancer cells is their ability to spread and colonise distant sites in the host. For this metastatic spread, the cell must be able to adhere to a matrix and to invade it. Thus compounds with anti-adhesive (attachment) or anti-invasive properties have potential as anticancer agents. It was established that a number of Allcock's compounds possess these activities at the end of the previous investigation. The table of test data and these compounds' structure are shown on P36 and 37.

More striking results were obtained in an anti-invasion assay developed by Professor Mac Neil's group. For two very similar compounds (**Figure 3**), the IC50 values were 6μM for X=S, but 1nM for X=O. Therefore the biology aim of this study was to evaluate analogues of **RWA2109** which showed extremely good potency in proliferation, attachment and invasion assays.



X=O RWA2109

X=S RWA2137

Fig. 3

To gain the experience with the anti-invasion assay, the effect on cell invasion of a number of compounds, **RWA1143**, **2165**, **2109** and **205**, prepared by Allcock was re-examined. The cell line originally used, A375SM, a highly metastatic cell line derived from a human ocular melanoma, was used again in the learning stage. However, the results obtained were not consistent with the early one. The reason is not very clear, because the reference compound J8 did not give a reasonable result either. The anti-invasion assay was

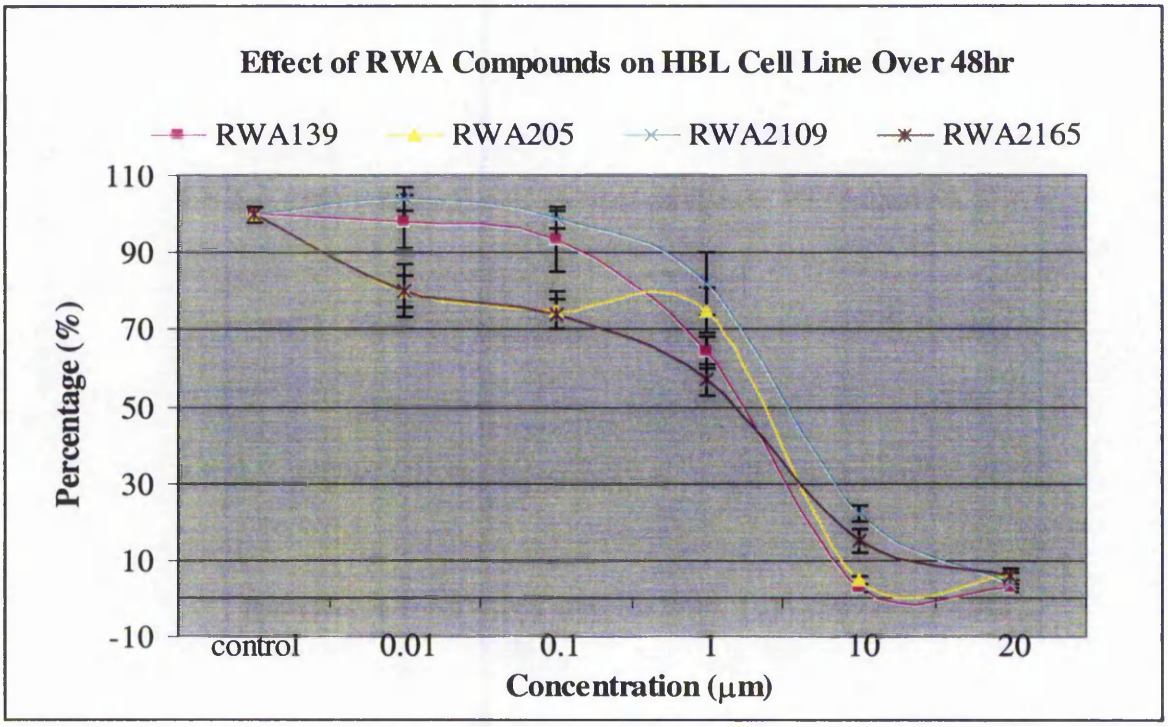
then repeated using a HBL cell line, with J8 as inhibitor, again with disappointing results. This failure of the assay may be due to a change in the cell cultures after many generations.

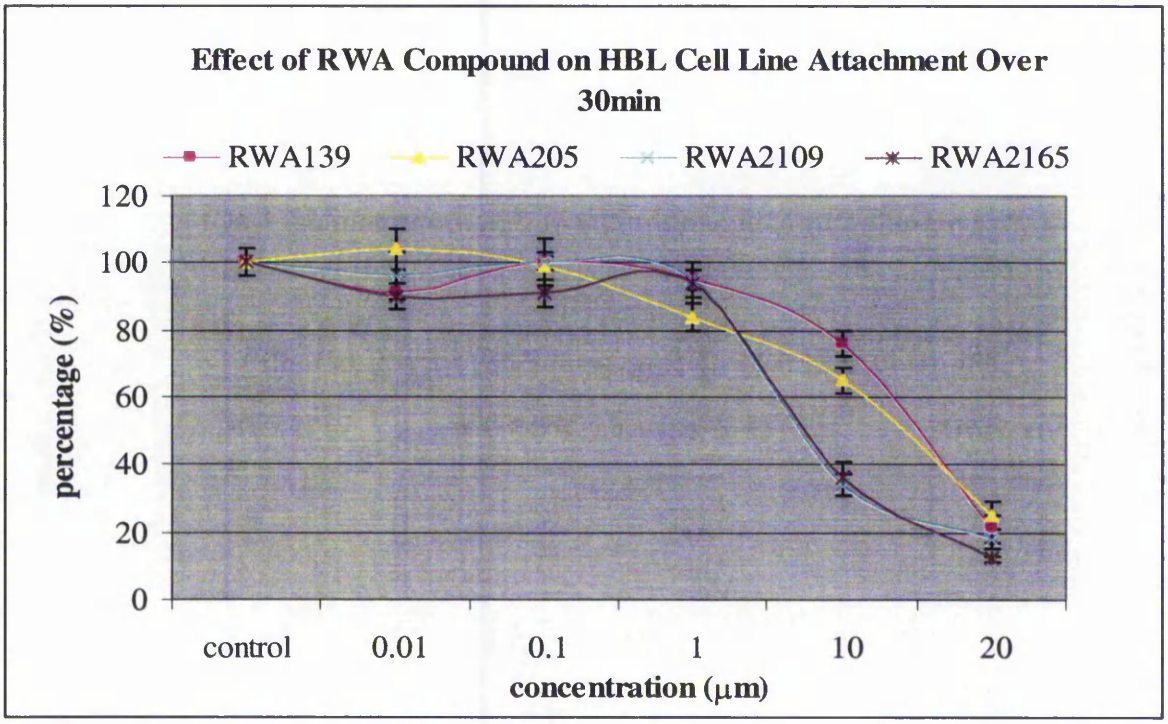
Later work by the Sheffield group confirmed that the loss of metastatic potency (invasion) by the cell line, A375SM is not due to viral, bacterial, or mycoplasmic infection and is independent of passage number. Therefore, this line can no longer be considered suitable for anti-invasion studies, but a new HBL line was proved to be able to give consistent results in this assay.

6.2.1 Result of an *in vitro* Proliferation Test

Proliferation and attachment assays were also required to be learned. Therefore Allcock's compounds were re-examined against this newly established cell line, HBL, and the results were in agreement with the original studies. These results are shown in **Figure 4** agreed with those from previous studies carried out by Professor Mac Neil's group showing that the IC50 values for anti-proliferation and attachment were of the same order of magnitude. Therefore no further attachment determinations were carried out.

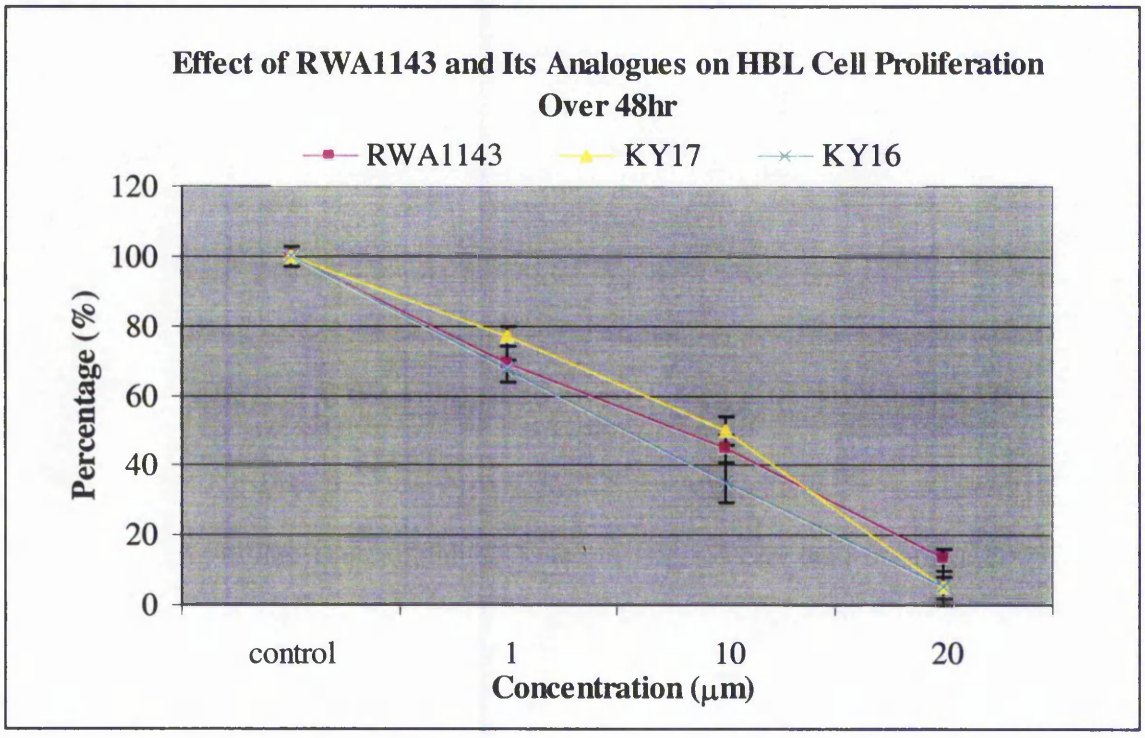
Fig 4. Effect of RWA compounds in anti-proliferation and anti-adhesion assays





Referring to the results obtained in the *in vivo* toxicity test, fluoro- (**RWA2161** or **KY17**) and chloro-compound (**RWA3154** or **KY16**) seem more toxic than **RWA1143**. This difference was presumed to reflect in the *in vitro* anti-proliferation assay. However, it is surprising to see that these three compounds act very similarly in this assay against melanoma cell line (**Fig 5**). The reason for this disparity is not clear.

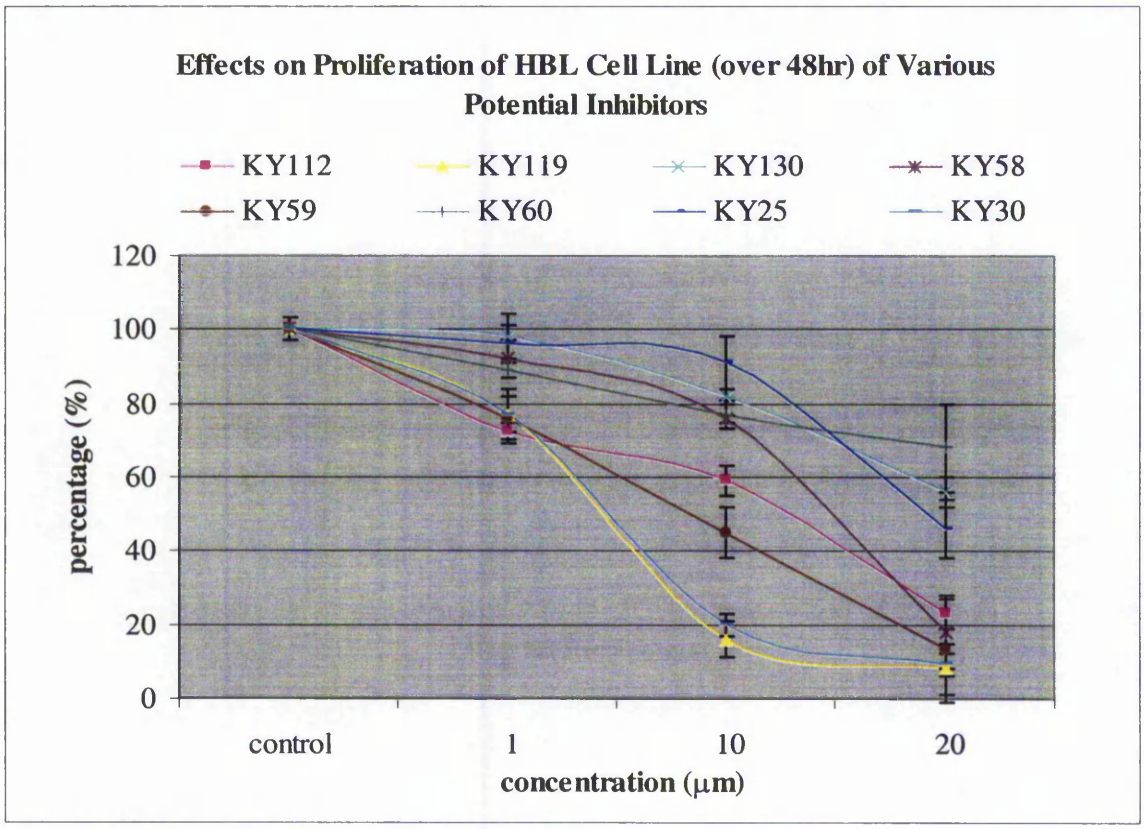
Fig 5. Effect of RWA1143, KY16 and KY17 on melanoma cell proliferation



* Results shown are means ± SEM of triplicates. Control values are shown as a percentage of cell proliferation in the absence of drugs.

The novel compounds synthesized in this programme were tested against HBL melanoma cell line in proliferation assays. The results obtained are shown in **Figure 6**.

Fig 6. Effect of some potential inhibitors on melanoma cell line proliferation assays

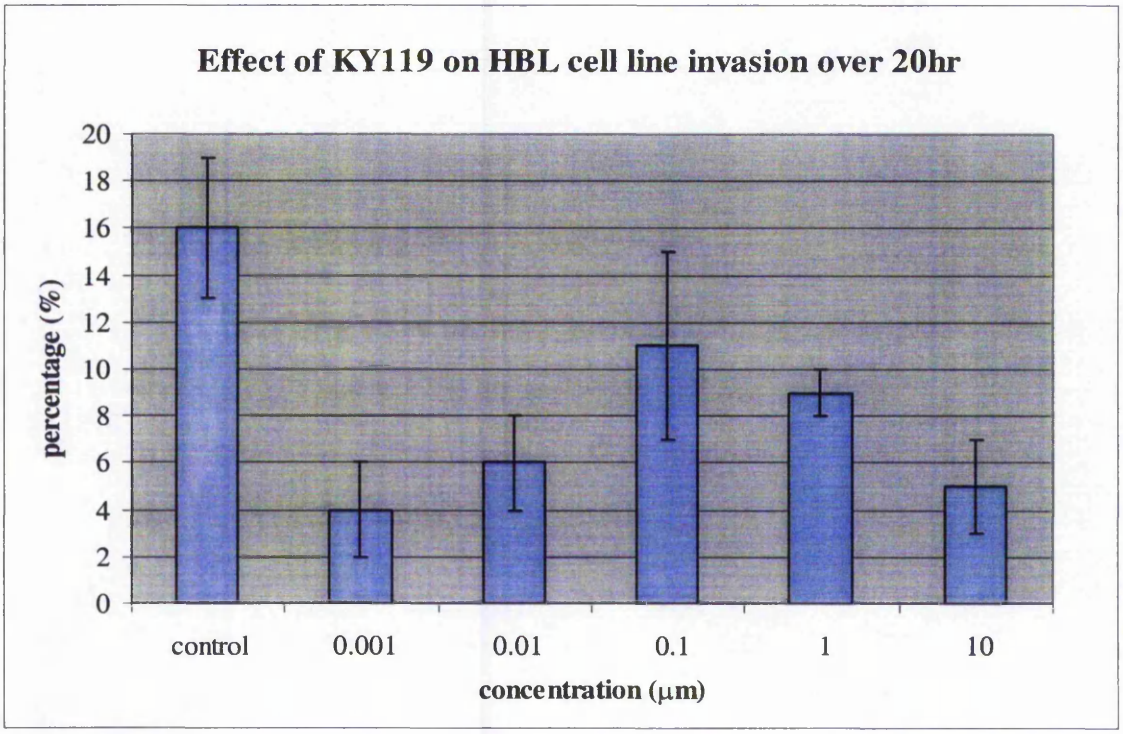


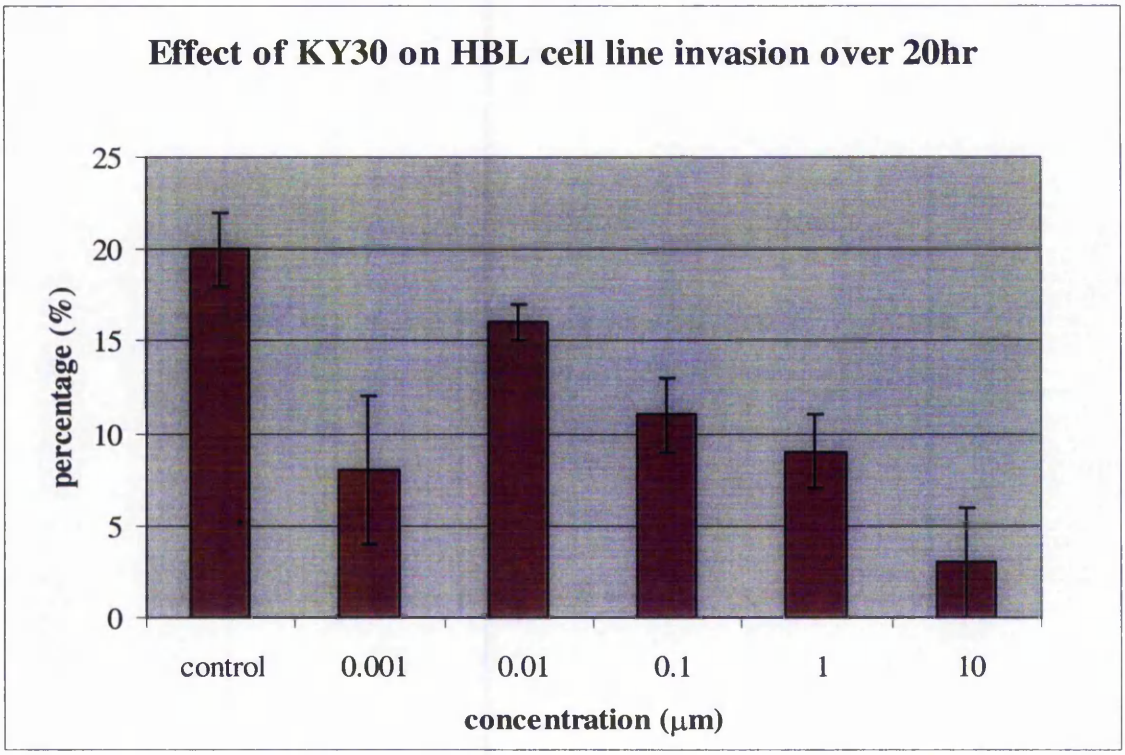
*Results shown are means ± SEM of triplicate wells of a single representative experiment. Control values are shown as a percentage of cell proliferation in the absence of drugs.

6.2.2 Results of KY119 and KY30a in Invasion Assay

Two most potent compounds, indole **KY119** and sulfoxide **KY30a** were carried out further in an invasion assay. The results were shown in **Figure 7**, which are mean ± SEM of experiments based on triplicates.

Fig 7. Effects of KY119 and KY30a on melanoma cell line invasion





6.3 Discussion of the Proliferation and Invasion Results

The cancer cells can attach to the matrix proteins followed by proliferation, and then some of these cells could finally invade through these matrix proteins, starting metastasis. Some compounds were found accidentally to possess the ability to inhibit these actions in previous study. It is possibly because these compounds are toxic to cancer cells or some of them act by inhibiting cells' ability to invade. In both anti-proliferation and anti-invasion assays, 96-well plate and transwells were pre-coated and incubated with fibronectin to form a layer of matrix which allowed cancer cells to attach, proliferate and invade.

As shown in **Figure 6**, all compounds tested tend to inhibit cell proliferation. However, among these potential inhibitors, the indole **KY119** and the sulfoxide **KY30a**

showed the best potency with IC50 values around 4μM. There are only 8& 9% of cells, which could survive in the exposure to 20μM of these two inhibitors in those proliferation assays.

Moreover, these two compounds' invasion assays gave unusual results, which are not concentration-dependent, but with sharp drops at the lowest concentration in both assays. Unfortunately, the conclusion cannot be simply drawn due to this abnormal finding. From the invasion percentage of control chamber (inhibitors were replaced with serum-free medium), cells applied in these assays are not very active with invasion percentage around 20.

It is noteworthy that **33b**, an analogue of **KY30a**, primarily prepared in Bulpitt's study to enhance the solubility showed very good potency with IC50 around 4μM in four different breast cancer cell lines with or without estrodiol (E_2) proliferation [R]. It is indicated that compounds with this type of structure possibly have great potential in the treatment of cancer.

Compared to unsubstituted indole **KY119**, although the other two indole derivatives, **KY112** and **KY130** are poorer inhibitors, **KY112** is more potent than **KY130** (**Figure 6**). What caused this difference in proliferative investigation is not clear. However from chemistry view, it is possibly due to the chemical structure. The Boc group substituted on nitrogen in indole would reduce indole electron density dramatically. The electron density of **KY112** with methyl group attached to "N" is obviously higher than that of **KY119**. Furthermore, the active inhibitor, **KY119**, is not a stable compound. Therefore, it is almost certain that the structure changed during the dilution of drug stock, and the long incubation period. It seems that **KY119**'s metabolites have some activities in these assays.

The allyl compound **KY25**, which did not show any particularly good results in anti-fungal screen, also did not present good effect on melanoma cell lines. It was not tested in the in vivo toxicity, but the proliferation result showed it might not be a toxic compound.

Among benzofuran derivatives, **KY60** is possibly the poorest compound in proliferation assays. According to **Figure 8, KY59** is a better inhibitor with IC50<10μM than **KY58** with IC50 values around 15μM.

Chapter 7

Biology Experimental

7.1 Reagents

Foetal calf serum, phosphate buffered saline, trypsin, serum free medium, and medium containing serum were prepared by laboratory technician Mark Wagner in the Clinical Science Department of Sheffield University. 3-[4, 5-Dimethylthiazol-2-yl]2, 5-di-phenyl-tetrazolium bromide (MTT), human fibronectin, ethylenediamine tetraacetic acid (EDTA) and ethylene glycol were purchased from Sigma Chemical Co. Led., UK. Transwell inserts were obtained from Costar UK. Chemical reagents tested were synthesized from current investigation.

7.2 Human melanoma cells

7.2.1 HBL cell line

The human metastatic HBL melanoma cell line was established in the laboratory from a lymph node metastasis of a nodular melanoma. Cells were maintained in Ham's F10 melanoma culture medium (HMCM), consisting of Ham's F10 medium supplemented with 5% FCS, 5% NBCS, 2 x 10^{-3} mol/l glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. Originally, as with fibroblasts and keratinocytes, HBL cells were routinely passaged (when 80-90% confluent) with 10ml sterile EDTA (without calcium and magnesium ions). After 5-15minutes incubation at 4°C followed by gentle tapping, HBL

cells were collected into sterile universals with 10ml HMCM and were spun at 200g for 5 minutes. For invasion, attachment and proliferation assays HBLs were resuspended in serum free invasion assay medium (SFIAM).

7.2.2 A375SM cell line

The human A375SM cell line was originally established in culture from a lymph node metastasis of a 54-year-old female. A375 cells are heterogeneous in nature and highly metastatic variants, such as A375SM have been successfully established in culture from lung metastases produced by parental A375 cells growing subcutaneously in nude mice. A375SM cells were maintained by serial passages in Eagle's modified essential medium (EMEM) supplemented with 10% FCS, 2 μM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin, 1.5% (100 x stock) vitamin concentrate, 1mM sodium pyruvate, 10% non-essential amino acids and 0.187% sodium bicarbonate at 37°C in a 5% carbon dioxide/ 95% air atmosphere. To avoid using trypsin, A375SM cells were routinely passaged using cell dissociation solution. When approximately 80-90% confluent, cells were washed twice with sterile PBS and incubated at 37°C with 6-10 ml cell dissociation solution for 10-20 minutes. Following gentle tapping A375SM cells were collected into sterile universals with 10ml of the above EMEM based melanoma culture medium (EMEM) and were spun at 200g for 5 minutes. As with the HBL cell line, A375SMs were resuspended in SFIAM from those three assays.

7.3 Cell Passage

The medium was taken off and discarded followed by the addition of 10ml of PBS to gently wash the medium and foetal calf serum away. The flask was left in the incubator for 10min after the treatment with 6-8ml of EDTA which was performing as a cell-lifting reagent. When all the cells were lifted up, 10ml of growth medium was added into the flask and the cell suspension was transferred into a universal. The medium was poured away after the transfugation (1000rpm for 5min at 20°C), and the cell lump was broken into the individual cells. The cells were suspended in serum free medium followed by cell counting.

7.4 Cell attachment and cell proliferation using MTT-eluted stain assay

After the appropriate incubation times cell attachment and proliferation were assessed using 3-[4, 5-dimethylthiaxol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-eluted stain assay (MTT-ESTA) which established by Ealey et. al. [200]. MTT is a hydrogen-acceptor substrate for dehydrogenase activity in the cell and the reduced MTT could form a coloured formazan product which is then eluted from the cells using acidified isopropanol. This cytobiochemical assay can provide an indirect reflection of cell number, in that dehydrogenase activity usually relates to cell number. Generally the intensity of staining increases with the number of live cells, which is further determined by using a Dynatech plate reader.

Measurement of cell attachment and cell proliferation assays run in Professor Mac Neil's group was determined by using MTT-ESTA. The method and procedure of these two assays were previously established by Mac Neil et al. [201] and Wagner et al.[202].

A 96-well tissue culture plate was coated with 50μl of diluted fibronectin in PBS (dilution ration 1/66), and the plate was incubated for at least 45 minutes at 37°C in a 5% carbon dioxide 95% air atmosphere. Any excess fibronectin was removed after the incubation but the blank column, and HBL were seeded into wells at a density of 2×10^4 cells/well followed by the addition of medium and drug solution into the control and experimental rows separately. The plates were incubated at 37°C in a 5% carbon dioxide 95% air atmosphere for further 30 minutes as an attachment assay and 48 hours as a proliferation assay.

After 30 minutes (attachment) or 48 hours (proliferation) incubation, the medium was replaced by 100μl of serum free medium and 10μl (attachment)/ 20μl (proliferation) of MTT apart from the blank column. The plates were left in incubator for another 40 minutes, then 100μl of ethylene glycol was added into each well including blank column after the removal of the mixture of serum free medium and MTT. For both attachment and proliferation assays, the final results were obtained by the plate reader.

7.5 Invasion assay

The fibronectin invasion assay has been established in Professor Mac Neil's group for a number of years and has been used to investigate the invasive ability of various human-derived cells, for example cutaneous melanoma cells (A375SM and HBL), uveal

melanocytes and melanoma cells and human fibroblasts. Transwell inserts, containing a polycarbonate filter with 8μm diameter pores randomly distributed over its surface, were inverted and placed in 6-well tissue culture plates. Human fibronectin (75μl) at 10μg/ml (diluted in PBS) was added to the underside of the polycarbonate filter and left for 1 hour at 37°C in a 5% carbon dioxide/95% air atmosphere. Following this incubation, the excess fibronectin was removed by gently tilting and touching the edge of the insert on the tissue culture plastic and then left to dry in the air for 1hr until a white coat formed on the filter. The drug stock could also be diluted down to desired concentration and cells could be counted during this period. Then the transwell were placed in the first and third rows of fresh 24 well tissue culture plates. Serum free invasion assay medium (600μl) was added to the second and fourth wells of the tissue culture plate.

Cell suspensions (50μl containing 0.6×10^5 [A375SM] or 1.7×10^5 [HBL and C8161]) resuspended in SFIAM, plus an equivalent volume of SFIAM (with or without drug) were then added to the transwell inserts in rows one and three. The inserts were then transferred to rows two and four, containing 600μl SFIAM and were incubated for 15 to 52 hours which depends on cell line at 37°C in a 5% carbon dioxide 95% air atmosphere. Each cell line was observed to invade at different rates hence, in order to obtain sensible control invasion levels, the HBL cell line was run for the standard 20 hours, the C8161 cell line was run for 15 hours and the A375SM cell line was run for 52 hours (due to this long incubation time a lower cell density [0.6×10^5] was used). In order to make the assay more accurate, tubes were weighed before and after the cells were harvested, hence enabling a more accurate estimation of the weight/ volume of the cell suspension.

Thus, following the relevant incubation period for each cell line, LP4 tubes were weighed (W1) and labelled as either upper or lower tubes. A 50: 50 mixture of PBS and FCS was added to each LP4 tube (600μl in the lower chambers and 100μl in the upper chambers) in order to neutralise the action of the trypsin. Briefly, medium containing cells was collected from the upper and lower chambers and placed into the corresponding upper and lower LP4 tubes. Equivalent volumes (600μl in the lower chambers and 100μl in the upper chambers) of trypsin were added, the cells were incubated for 5-10 minutes and the trypsin was removed into the relevant upper and lowerLP4 tubes. The above trysinisation step was repeated once more in order to ensure that all the cells had been harvested. After this second incubation in trypsin, the surface of the polycarbonate filter (in the upper chamber) was gently scraped and the cells were transferred to the relevant upper tubes. After the transfugation, medium in all tubes was poured away and the tubes were weighed again (W2). The cell lump in every LP4 tube was broken into individual cells followed by cell counting. The method to work out invasion percentage was described in **Table 2**:

Table 2: The example of calculating invasion percentage.

	W_E (g)	W_C (g)	ΔW (g)	Cell Counting
Top Chamber	3.3482	3.4259	0.0777	106
Bottom Chamber	3.4604	3.5409	0.0805	2

$106 \times 10^4 \times 0.078 = 82680$ Cells in the top chamber;

$2 \times 10^4 \times 0.081 = 1620$ Cells in the bottom chamber;

$82680 + 1620 = 84300$ Cells in total;

Invasion% = $(1620/ 84300) \times 100\% = 2\%$.

7.6 Drug dilution

Drug's concentration range was determined to be from 0.01 to 20 μM. Each drug was tested at five different concentrations in the range: 20, 10, 1, 0.1, 0.01μM. Drug stock was firstly prepared by dissolving compound in DMSO to obtain a 10mM solution. Then 20μl of 10mM drug stock was added into 5ml of serum-free medium to make 40μM solution and was sterilised by filtration. Then the following required concentration could be obtained by diluting down the sterilised 40μM solution with serum-free medium.

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