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Synthesis and Cannabinoid Activity of a Variety of 2,3-Substituted 1-Benzo[*b*]thiophen Derivatives and 2,3-Substituted Benzofuran: Novel Agonists for the CB_1 Receptor

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An exploratory chemical effort has been undertaken to develop a novel series of compounds as selective CB_1 agonists. It is hoped that compounds of this type will have clinical utility in pain control and cerebral ischaemia following stroke or traumatic head injury. We report here medicinal chemistry studies directed towards the investigation of several classes of 1-benzo[*b*]thiophen and benzofuran derivatives as novel CB_1 agonists. We have discovered a novel series of compounds, which contain a 1-benzo[*b*]thiophen or a benzofuran group as the central aromatic group. Our investigation of this series of compounds has enhanced our understanding of the importance of binding sites within the CB_1 receptor for favourable CB_1 potency. Our understanding of these factors allowed us to modify the structure of a 1-benzothiophen derivative and improve its potency at the CB_1 receptor.

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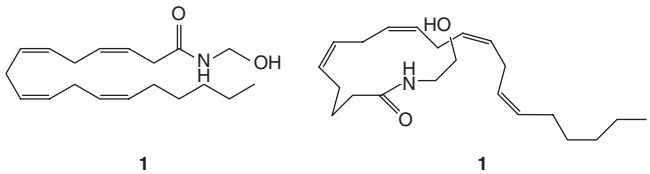
Introduction

There are two cannabinoid receptors designated neuronal (CB_1) and peripheral (CB_2).[1,2] Activation of both receptors leads to inhibition of adenylate cyclase and activation of mitogen-activated protein kinases. Activation of CB_1 receptors also leads to the gating of a variety of ion channels, including the inhibition of N-type voltage-dependent calcium channels. As a consequence of these cellular effects, together with the distribution of the receptors in neuronal tissues, cannabinoid CB_1 agonists have been suggested to offer significant therapeutic potential in various conditions including chronic neuropathic pain.[3–5] In 1992, it was discovered that the endogenous ligand for the CB_1 receptor is anandamide.[6] Anandamide **1** and a range of other structurally unrelated cannabinoid agonists have been shown to block the N-type calcium channel. Given that blockade of these channels by certain conotoxin peptides has already demonstrated clinical potential for the treatment of neuropathic pain,[7] there is considerable interest for the development of anandamide analogues as one means for achieving a similar therapeutic outcome.[8–13] Molecular modelling studies on anandamide[14] have resulted in a proposed conformation for activity at the CB_1 receptor (Scheme 1).[15,16]



1 **1**

Scheme 1.

In recent times, it has been demonstrated that the activity of a series of aminoalkylindole (AAI) antinociceptive agents, originally designed as non-ulcerogenic, non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a second mechanism of action manifested by potent activity at inhibiting electrically induced contractions of mouse vas deferens (MVD).[12,17–20] Studies on WIN-55,212–2 **2**, Pravadoline **3**, and related compounds have shown that compounds from this structurally unrelated indole-based series (relative to the non-classical

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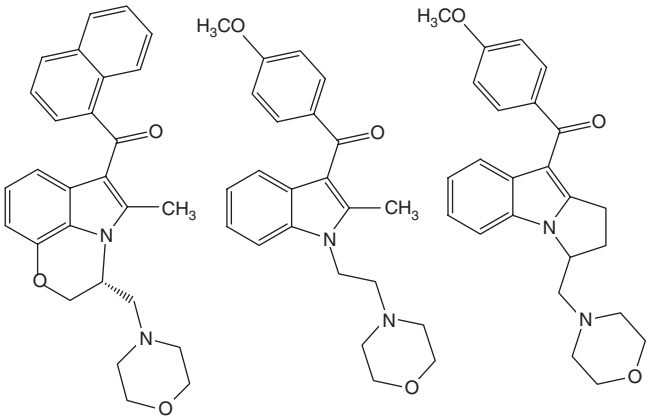
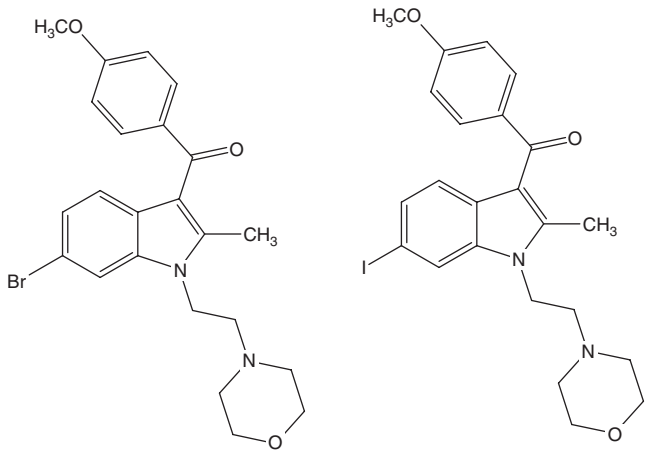


2 **3** **4**

Scheme 2.



Bromopravadoline
WIN-54,461

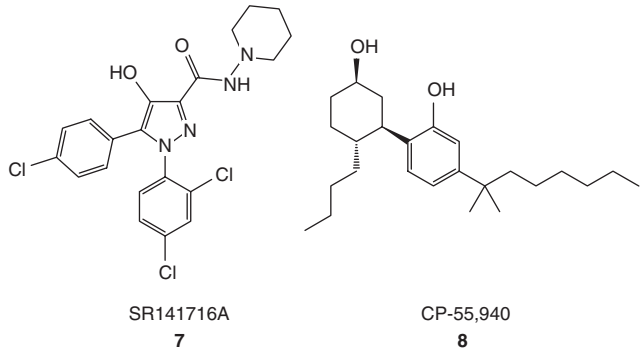
AM630

5 **6**

Scheme 3.

cannabinoids or to anandamide **1** and other arachidonic acid derivatives) are capable of producing pharmacological responses in a fashion resembling the profile exhibited by (−)-Δ^9-THC. Additionally the AAI drug class produces its cannabimimetic actions in a stereoselective manner as shown by the results for the optical isomers of WIN-55,212–2.[21]

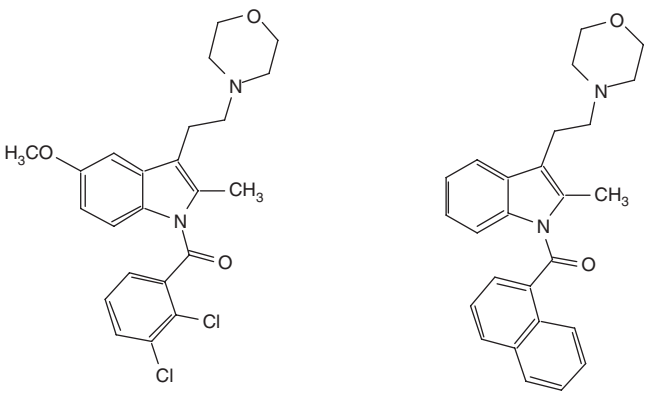
WIN-55,212–2 (CB_1, median inhibition concentration (IC_{50}) 2.0 nM) **2**, Pravadoline (CB_1, IC_{50} 453 nM) **3**, and (4-methoxyphenyl)(3-morpholinomethyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl)methanone **4**[17] (CB_1, IC_{50} 320.0 nM) are all potent CB_1 receptor agonists (Scheme 2).[2,17,22] The key structural features for potent cannabinoid activity in this series are a 3-keto group, an aryl or bicyclic (naphthyl) substituent at the 3-position, a small (H or CH_3) substituent at the 2-position, and an ethylene-linked morpholine group at the 1-position. Molecular modelling studies have been performed to develop a pharmacophore and to compare AAI structures with those of classical cannabinoids.[23] Within the work of Eissenstat et al., a CB_1 antagonist WIN-54,461 **5** (or bromopravadoline) was also identified. A separate group identified a related compound AM630 **6** (or iodopravadoline) (Scheme 3) that is also found to be a competitive cannabinoid receptor antagonist.[24]



SR141716A
7

CP-55,940
8

Scheme 4.



L-768,242
9

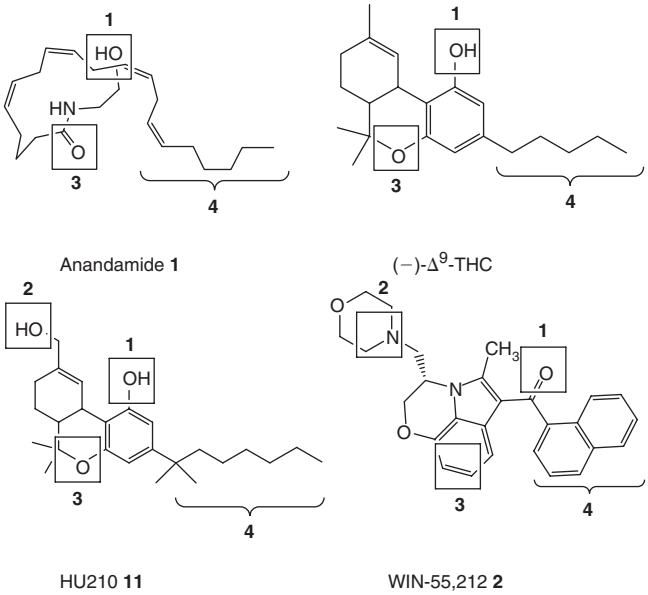
L-759,787
10

Scheme 5.

AM630 **6** behaved as a competitive antagonist of CP-55,940 **8**, WIN-55,212–2 **2**, and anandamide **1** but was more markedly potent as an antagonist of (−)-Δ^9-THC and CP-55,940 **8** (Scheme 4) than as an antagonist of WIN-55,212–2 **2** or anandamide. These differences imply that the MVD may contain more than one type of cannabinoid receptor. The results also indicate that the receptors for which AM-630 **6** has the highest affinity may not be the cannabinoid CB_1 receptors, as the CB_1 selective antagonist SR141716A **7** is known to be equally potent in attenuating the inhibitory effects of CP-55,940 **8** and anandamide **1** on the twitch response of the MVD.

In 1993, a human peripheral cannabinoid (CB_2) receptor was disclosed.[22] Several workers have put forward the hypothesis that a selective and potent ligand for the CB_2 receptor would show therapeutically useful effects,[19] including a series of analogues selected through a topological search using WIN-55,212–2 **2** as the template.[17,18] The work of D'Ambra et al. and Gallant et al. led to the discovery of a range of N1-benzoyl and N1-naphthoyl indole analogues.[17,18] Two of the more potent compounds from the research of Adams et al. included L-768,242 **9** and L-759,787 (Scheme 5).[12]

In conjunction with the synthesis of novel compounds as potential CB_1 agonists, we have performed preliminary modelling studies in an attempt to build up a pharmacophore for the CB_1 receptor. A comparison was made between the aminoalkylindoles, anandamide **1** and the classical and non-classical cannabinoids. We extrapolated from work carried out by Thomas and coworkers[15] using the proposed conformation



Scheme 6.

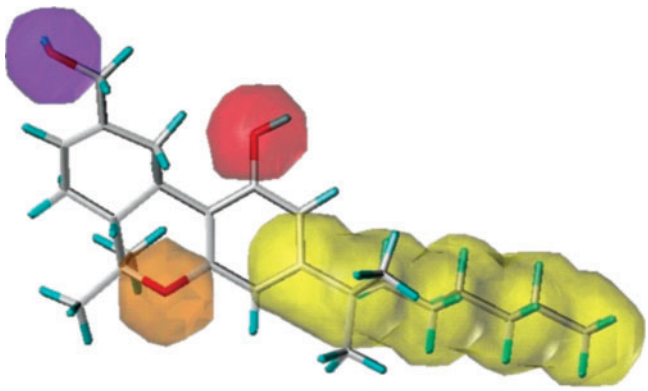


Fig. 1. Proposed mode of binding for a low-energy conformation of HU210 at the CB_1 pharmacophore model.

anandamide adopts for binding. Scheme 6 illustrates the four proposed important binding sites within the CB_1 pharmacophore using anandamide, (−)-Δ^9-THC, HU210 **11**, and WIN-55,212–2 **2**. The proposed binding sites are a hydrogen-bond donor or acceptor site 1, and two further hydrogen-bonding sites 2 and 3, and a hydrophobic pocket 4. The pharmacophore is further illustrated in Figs 1 and 2. The proposed mode of binding of a low energy conformer of HU210 **11** and anandamide **1** are shown in Figs 1 and 2 respectively.

We recently reported the results of a drug discovery program directed toward a series of 1-substituted indole-3-oxadiazole derivatives.[25] The work of Moloney and Robertson resulted in the discovery of several exciting compounds including 3-(3-benzyl-[1,2,4]oxadiazol-5-yl)-1-(2-pyrrolidin-1-ylethyl)-1*H*-indole **12** (Scheme 7), which had good CB_1 potency (pK_b 7.2).

As part of the present research program, it was our intention to investigate a variety of 1-benzo[*b*]thiophen- and benzofuran-based molecules in our effort to discover novel CB_1 ligands and understand fully the binding characteristics of CB_1 ligands.

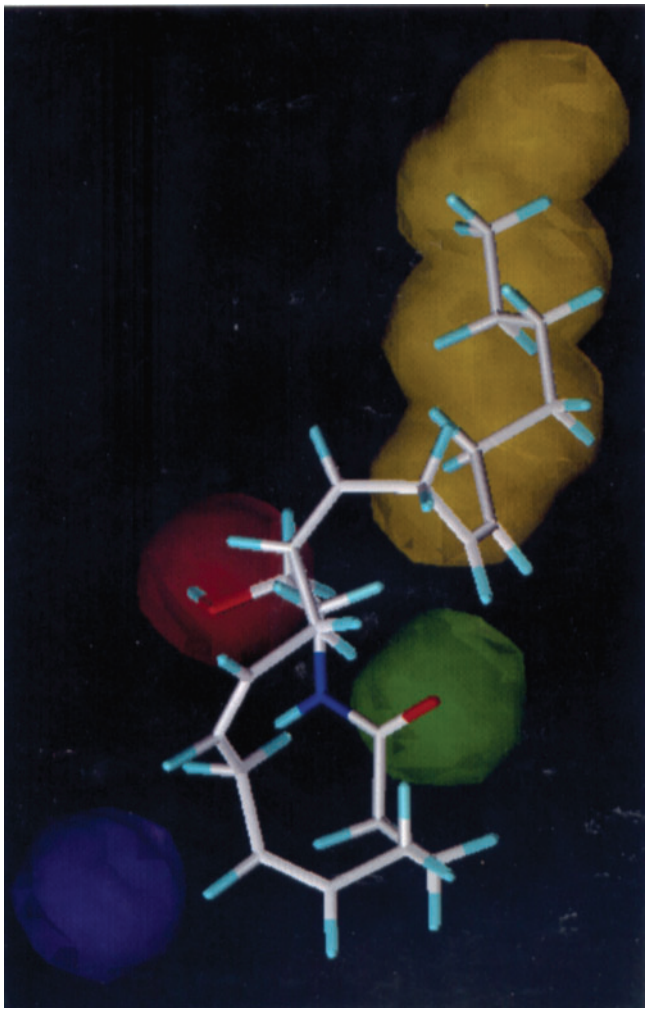
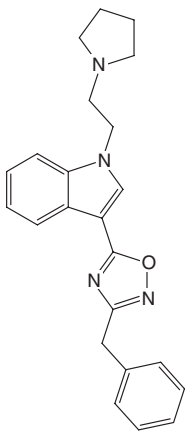


Fig. 2. Proposed mode of binding for a low-energy conformation of anandamide at the CB_1 pharmacophore model. The four proposed binding sites are shown as coloured spheres.



12

Scheme 7.

Results and Discussion

Synthesis

Our decision to investigate several benzofuran and 1-benzo[*b*]thiophen derivatives was based on several previously

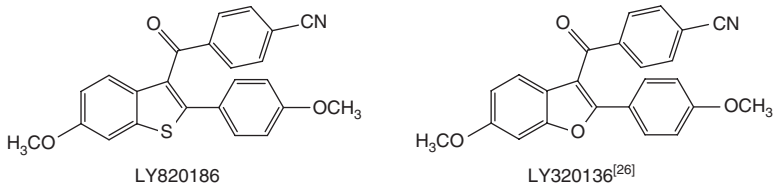
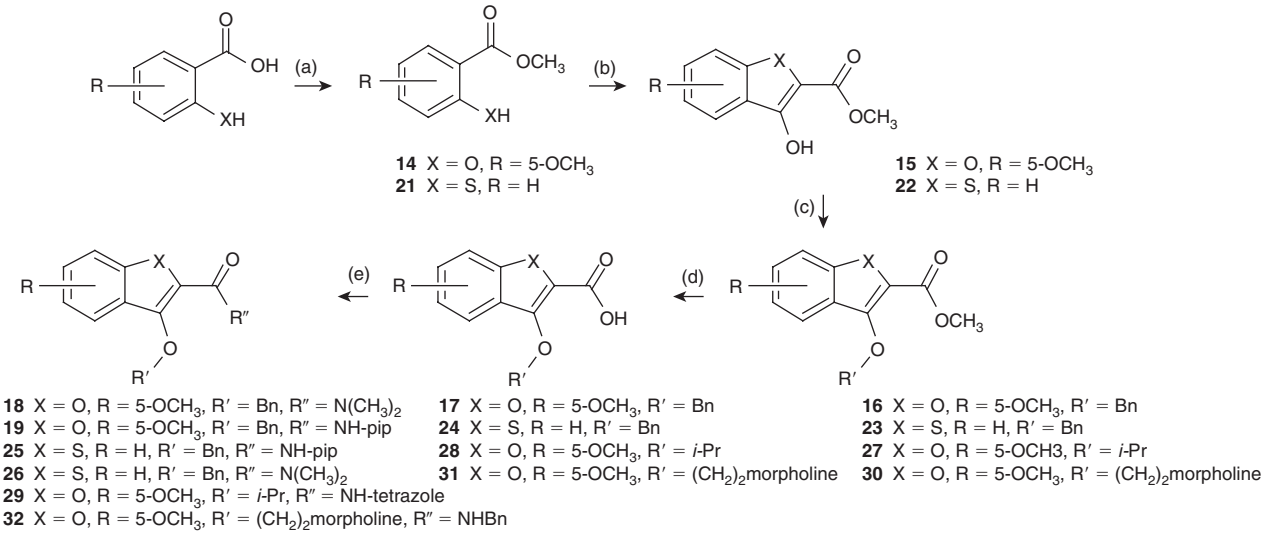


Fig. 3. 2,3,6-Trisubstituted-1-benzo[*b*]thiophen and benzofuran derivatives LY820186 and LY320136, which have been previously reported to inhibit cannabinoid receptors.[26]



Scheme 8. Reagents: (a) $SOCl_2$, MeOH; (b) $KOBu^t$, THF, $ClCH_2CO_2CH_3$; (c) DMSO, $KOBu^t$, alkyl halide; (d) KOH, MeOH; (e) amine, DMF, TBTU, DIPEA or CDI, THF, 5-aminotetrazole.

reported 1-benzo[*b*]thiophen and benzofuran derivatives that inhibit cannabinoid receptors in mammals (Fig. 3).[26]

The substituted benzofuran derivatives were synthesized by two general methods. In the first (Scheme 8), the substituted salicylic acid or thiosalicylic acid (**14**, **15**) derivatives were alkylated with methyl 2-chloroacetate in the presence of potassium *tert*-butoxide, with the di-ester intermediates undergoing a Diekmann-type condensation to afford the heterocyclic 2-carboxylates,[27] which were then alkylated and hydrolyzed to form the 3-alkoxybenzofuran and benzo[*b*]thiophen-2-carboxylic acids. The carboxylic acid derivatives could be readily amide-coupled with a range of amines to form the amide inhibitors **18**, **19**, **25**, **26**, **29**, and **32**.

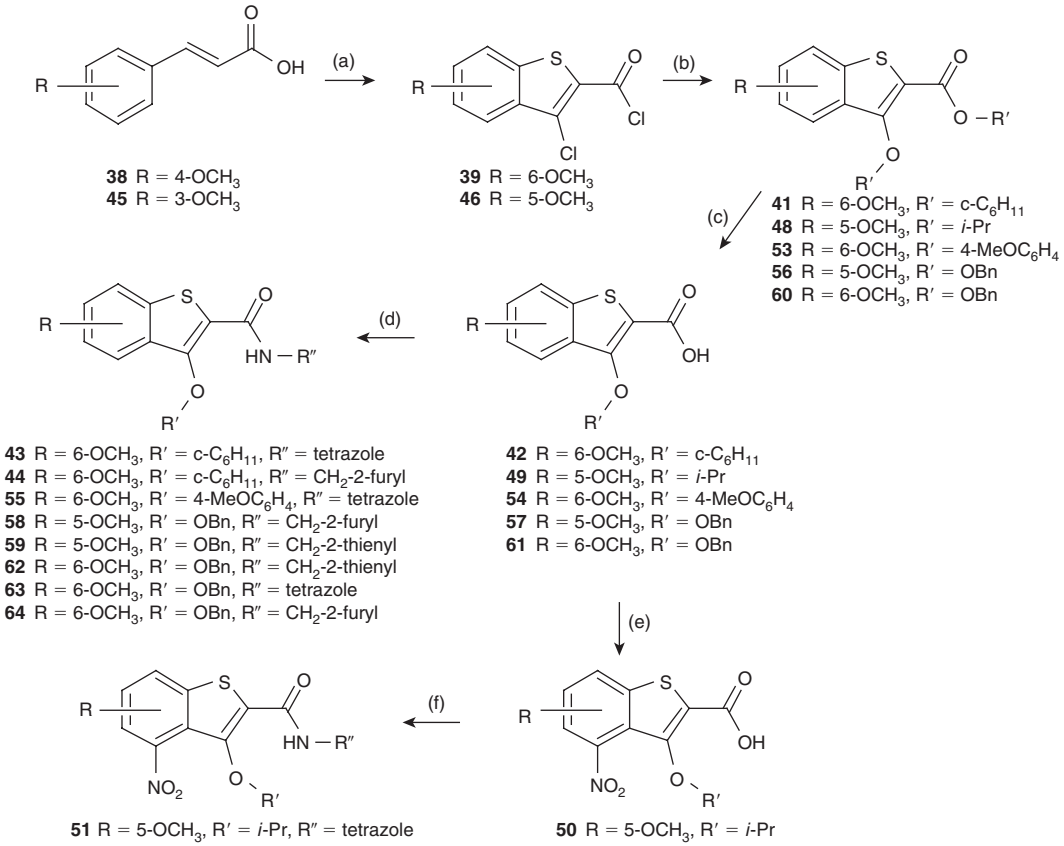
The second general method for the preparation of the 1-benzo[*b*]thiophen derivatives is illustrated in Scheme 9 and starts with the substituted cinnamic acids **38** and **45**. Reaction with thionyl chloride in pyridine/DMF generated the 3-chloro-1-benzo[*b*]thiophen-2-carbonyl chloride intermediates **29** and **46**. The carbonyl chloride intermediate could then be treated with the alcohol of choice to simultaneously introduce the alkoxy group at C3 and to form the ester intermediates **41**, **48**, **53**, **56**, and **60**. The esters were then hydrolyzed to form the 1-benzo[*b*]thiophen-2-carboxylic acid derivatives **42**, **49**, **54**, **57**, and **61**, which were then amide-coupled to the amine of choice to form the 1-benzo[*b*]thiophen-2-carboxamides **43**, **44**, **55**, **58**, and **62**–**64**.

The nitro analogue **51** was synthesized in the same method by first nitrating the carboxylic acid intermediate **49** followed by amide coupling with 5-aminotetrazole.[28]

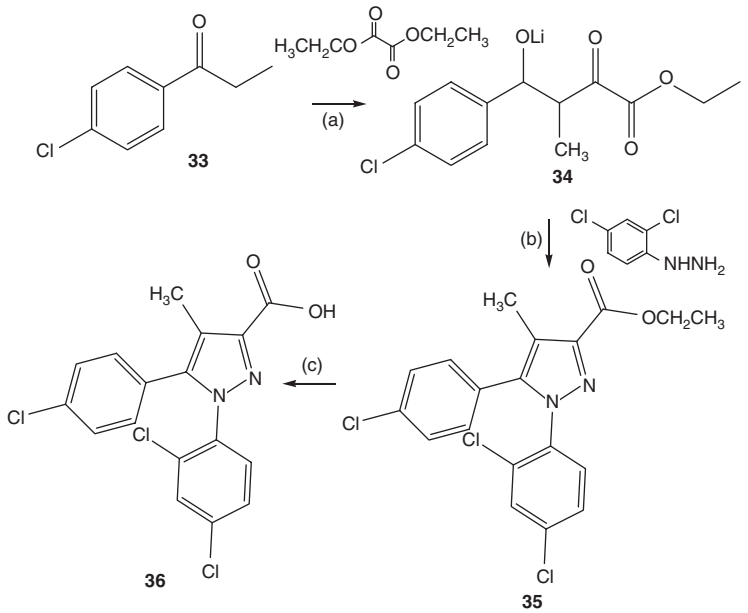
We also synthesized the known central cannabinoid antagonist SR141716 for comparison with our novel CB_1 agonists (Scheme 10). The synthesis begins with condensation of 4-chloropropiophenone **33** with diethyl oxalate, which is promoted by lithium hexamethydisilazide[29] to afford the lithium enolate **34**. Condensation of **34** with (2,4-dichlorophenyl) hydrazine in ethanol at reflux[30] afforded the pyrrole ester **35**. The pyrrole ester **35** was then heated with ethanolic sodium hydroxide to afford the pyrazole carboxylic acid **36**. Amide coupling of **36** with *N*-aminopiperidine afforded the desired SR141716 **37** in good yield.

Biological Results

The compounds were investigated and their biological data are shown in Table 1. To explain the biological data, we referred to our theoretical receptor model for the CB_1 receptor, shown in Fig. 1 using the preferred conformation of the cannabinoid mimetic HU210 (**11**) fitted to the proposed CB_1 receptor model. The theoretical receptor model is composed of four binding sites. To build our CB_1 receptor model, we compared potential commonality between aminoalkyl indoles and other cannabinoids. We initially built the structure for anandamide **1** and minimized the conformation anandamide adopts for binding to the CB_1 receptor. We then recognized that when we compared HU210 and anandamide **1**, it was possible to overlay the phenolic hydroxyl of HU210 with the amido ethyl alcohol of anandamide **1** (binding site 1). Binding site 1 is represented by the red sphere and is a hydrogen-bonding site, which the phenolic hydroxyl



Scheme 9. Reagents: (a) $SOCl_2$, DMF, pyridine; (b) R′OH, THF; (c) NaOH, MeOH; (d) amine, TBTU, DMF, DIPEA or CDI, THF, 5-aminotetrazole; (e) CH_3CO_2H, HNO_3; (f) CDI, THF, 5-aminotetrazole.

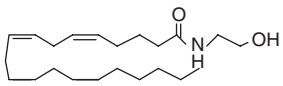
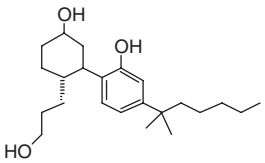
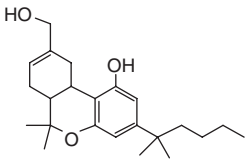
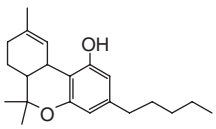
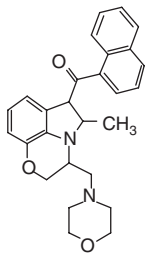
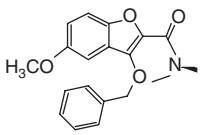
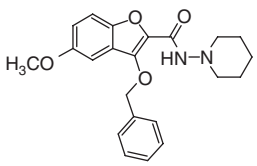
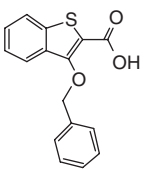
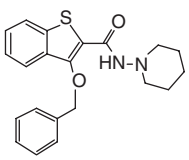


Scheme 10. Reagents and conditions: (a) LiHMS (1 M in THF), THF −78°C; (b) 2,4-dichlorophenyl hydrazine hydrochloride, ethanol; (c) 2 M NaOH, EtOH.

of HU210 can interact with (Fig. 1). Binding site 2 is represented by the purple sphere in Fig. 1 and the 9-hydroxymethyl group of HU210 interacts with this binding site and is an important binding site for the aminoalkyl indole series[12,20,26] and our novel 2,3-substituted-1-benzo[*b*]thiophen- and benzofuran-based compounds. Binding site 3 is a hydrogen-bonding site and is represented by the orange sphere (Fig. 1) and is accessed by the pyran oxygen of HU210 and the amide carbonyl of anandamide **1**

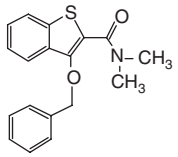
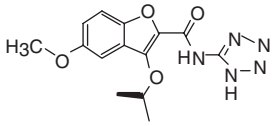
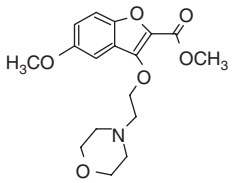
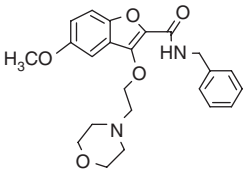
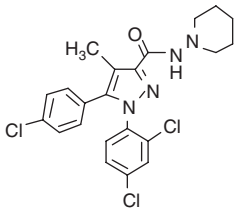
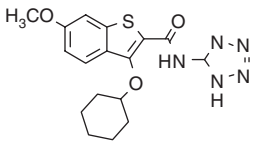
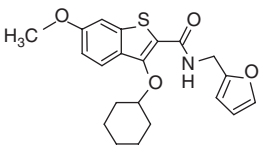
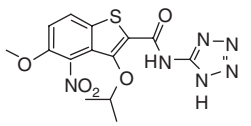
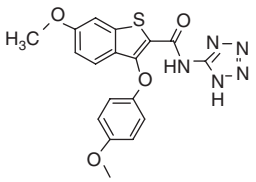
Table 1. Percentage inhibition of CB_1: 1-substituted-indole-3-oxadiazole derivatives

MVD, mouse vas deferens bioassay; IC_{50}, negative logarithm of the concentration of compound required to inhibit 50% of the specific binding of the radioligand. Affinity values are the average of at least four different estimates. Negative values connote stimulation rather than inhibition

Compound no.	Structure	CB_1 (MVD)[A]	CB_1 (Binding)
Anandamide **1**		pK_B 8.52	
CP-55,940			
HU210 **11**			IC_{50} 3.22 μM
(−)-Δ^9-THC		pK_B 8.74	
WIN-55,212 **2**		pK_B 8.6	IC_{50} 1.007 μM
18		12.2% inhibition at 1.0 μM	
19		11.0% inhibition at 1.0 μM	
24		17.5% inhibition at 1.0 μM	
25		18.8% inhibition at 1.0 μM	

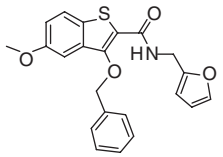
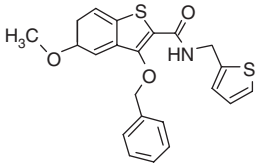
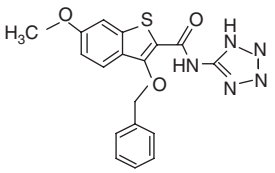
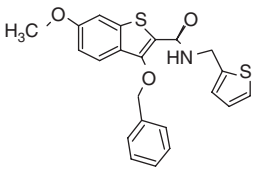
(*Continued*)

Table 1. (Continued)

Compound no.	Structure	CB_1 (MVD)[A]	CB_1 (Binding)
26		22% inhibition at 1.0 μM	
29		35.71% inhibition at 1.0 μM	30% inhibition at 1.0 μM
30		10.3% inhibition at 1.0 μM	
32		20.3% inhibition at 1.0 μM	IC_{50} 1.84 μM
SR141716A **37**		pK_B 8.4–8.6	IC_{50} 3.92 nM
43		25.0% inhibition at 1.0 μM	
44		31.1% inhibition at 1.0 μM	20% inhibition at 1.0 μM
51		29.3% inhibition at 1.0 μM	
55		3.33% inhibition at 1.0 μM	

(*Continued*)

Table 1. (Continued)

Compound no.	Structure	CB_1 (MVD)[A]	CB_1 (Binding)
58		−21.0% inhibition at 1.0 μM	
59		29.7% inhibition at 1.0 μM	20% inhibition at 1.0 μM
63		22.7% inhibition at 1.0 μM	Inactive
64		−59.2% inhibition at 1.0 μM	Inactive

[A]Affinity ($pK_B = -\log_{10} K_B$, the dissociation equilibrium constant) estimates for novel compounds at the CB_1 receptor in the MVD. Standard errors are omitted for clarity but in all cases were ≤ 0.2 $\log_{10}$ unit.

(Fig. 2). A low-energy conformation of HU210 is shown in Fig. 1.

The proposed conformation anandamide **1** adopts for binding to the CB_1 receptor is shown in Fig. 2.

In the current study, we decided to investigate a novel series of 1-benzo[*b*]thiophen-based compounds in an attempt to successfully replace the indole nucleus of the aminoalkyl indole series. A review of the structural requirements for good interaction with the CB_1 receptor binding sites revealed to us that it would be more difficult to produce compounds from this series that contained appropriate functionality to interact with the four main binding sites of the CB_1 receptor, Scheme 6. In a previous study,[25] we discovered an aminoalkyl indole, 3-benzyl-5-(1-pentyl-1*H*-indol-3-yl)-1,2,4-oxadiazole **12**, which inhibits CB_1 100% at 1.0 μM. **12** does not contain functionality to interact with binding site 2, Scheme 6. With this information, we decided to synthesize several 1-benzo[*b*]thiophen-based compounds that contained functionality to interact with binding sites 1, 3, and 4.

To begin our investigation of the benzo[*b*]thiophen series, we synthesized several simple 3-benzyloxy-1-benzo[*b*]thiophen derivatives. Our preliminary modelling studies suggested that compounds such as **24**, **25**, and **26** would be able to adopt conformations that allow the 3-benzyloxy substituent to occupy the hydrophobic pocket (binding site 4), whereas the 2-carbonyl group could interact with the hydrogen-bonding site (binding site 1) and the central 1-benzo[*b*]thiophen nucleus could interact with binding site 3. The results for these simple 1-benzo[*b*]thiophen derivatives were lower than expected, with **25** showing 18.8% inhibition of CB_1 at 1.0 μM. The dimethyl derivative **24** showed a similar lack of potency with 22.0% inhibition of CB_1. The 1-benzo[*b*]thiophen-2-carboxylic acid derivative **24** exhibited 17.5% inhibition of CB_1.

A similar lack of potency was observed for 5-methoxybenzofuran derivatives with the same substituents at the 2 position. **18**, a dimethylbenzofuran-2-carboxamide derivative, exhibited a similar lack of potency, with 12.2% inhibition of CB_1. Similarly the piperidin-1-yl benzofuran-2-carboxamide derivative **19** exhibited similar lack of activity, showing 11.0% inhibition of CB_1. It became clear that in order to obtain potent compounds within this series of compounds, we would be required to include functionality to interact with binding site 2, Scheme 6. Molecular modelling studies showed that if we incorporated a heterocyclic moiety into the 2-carboxamide substituent, a heteroatom would be able to interact with the hydrogen-bonding site designated as binding site 2. These preliminary modelling studies led us to the amido tetrazole derivative **29**. Our computational studies indicated that as **29** adopts a low-energy conformation for interaction with CB_1, functional groups are able to interact with binding sites 1, 3, and 4 whereas one of the tetrazole nitrogens should be able to interact with binding site 2. The biological results in Table 1 reveal that although **29** is not super-potent, inclusion of the amido tetrazole moiety increased the potency of this compound to up to 35.71% inhibition of CB_1 at 1.0 μM. The 3-isopropyl group is able to interact with binding site 4, the hydrophobic pocket, and the amide carbonyl is able to interact with binding site 1.

Similarly, the 4-nitro analogue **51** exhibited a similar profile to **29**, inhibiting CB_1 29.3% at 1.0 μM.

We then decided to explore some 3-cyclohexyloxy derivatives, as our molecular modelling studies suggested that the cyclohexyl group might be able to interact with the hydrophobic pocket, binding site 4 more accurately. The 3-cyclohexyloxy derivative **43** was observed to inhibit CB_1 with similar potency to **29** showing 25.0% inhibition. Our preliminary modelling studies suggested that the 2-carboxamidotetrazole moiety was not able to interact accurately with binding site 2. In an attempt to overcome this problem, we decided to replace the tetrazole group with a furan-2-ylmethyl group, which is able to interact with hydrogen-bonding sites such as binding site 2 while the cyclohexyl group interacts with the hydrophobic site. Our results showed that the inclusion of a 2-(furan-2-ylmethyl) carboxamide group did improve potency, with 31.1% inhibition of CB_1 observed. The marginally improved potency is likely to be due to the ability of the methyl-linked morpholine substituent to interact with the hydrogen-bonding site, binding site 2, while the 3-cyclohexyloxy group interacts with the hydrophobic pocket, binding site 4, as well as the ability of the more hydrophobic 2-(furan-2-ylmethyl) carboxamide group of **44** to interact with the proposed additional hydrophobic pocket[31] in the region between the central 1-benzo[*b*]thiophen moiety and the hydrogen-bonding site, binding site 2 (Scheme 6). We previously suggested that this proposed hydrophobic region may be where the six-membered rings of (−)-Δ^9-THC and HU210 are placed when these compounds are interacting with the CB_1 receptor.[31] We envisage that future work on compounds from this series will involve the synthesis and assessment of an analogue of **44** with an ethyl linking chain between the morpholine moiety and the 1-benzo[*b*]thiophen central nucleus. Such a compound would be expected to interact more closely with the hydrophobic pocket and binding site 2. In addition, such an analogue would be more hydrophobic in the region between the central 1-benzo[*b*]thiophen nucleus and binding site 2, which might enhance affinity at the CB_1 receptor.

We decided to investigate several 3-benzyloxy derivatives, with various 2-carboxamido substituents for interaction with binding site 2. Initially we examined the 2-carboxamidotetrazole derivative **63**, which displayed 22.7% inhibition of CB_1, which is a similar lack of potency to the 3-isopropoxy and 3-cyclohexyloxy derivatives. It was encouraging to observe that replacement of the tetrazole group with a (thiophen-2-yl)methyl group had the same effect of marginally improving CB_1 inhibition, as **59** was observed to inhibit CB_1 29.7%. We also investigated other hydrophobic groups to interact with the hydrophobic pocket, binding site 4.

We synthesized one 3-(4-methoxyphenoxy) derivative, which proved to be totally inactive. Clearly the 3-phenoxy group is not ideal to combine with a 2-carboxamido tetrazole group. It has become evident that for optimum CB_1 activity with this family of compounds, an extended 2-carboxamido side chain is required for optimal interaction with the four identified binding sites of the CB_1 receptor. Future work with the 3-phenoxy series would involve incorporation of alternative 2-carboxamido groups.

We decided at this point to examine several 1-benzo[*b*]thiophen derivatives incorporating an ethyl-linked morpholine side chain at the 3-position in an attempt to more closely mimic the aminoalkyl indole family of CB_1 inhibitors. Our modelling studies suggested that our first compound from this family, the 2-benzylamide derivative **32**, should be able to interact with the binding sites of the CB_1 receptor. Our results revealed that **32** lacked potency at CB_1, inhibiting CB_1 20.3%. We also examined one of the intermediates, the 2-methyl ester **30**, which was equally as inactive, inhibiting CB_1 10.3%. The lack of potency for this series of compounds appears to be due to inappropriate distance between the morpholine nitrogen, which interacts with binding site 2, and the benzoyloxy phenyl group, which interacts with the hydrophobic pocket binding site 4. Future work within this series of 2,3,5-substituted 1-benzo[*b*]thiophen derivatives will involve incorporating a longer linking chain between the morpholine moiety and the central 1-benzo[*b*]thiophen group. Alkylation with 4-(3-chloropropyl)morpholine will allow such an analogue to adopt a conformation that allows more accurate interaction with all the binding sites of the CB_1 receptor.

Conclusions

In the present study, we have identified a novel class of 1-benzo[*b*]thiophen and benzofuran derivatives as agonists of the CB_1 receptor. We have successfully designed and synthesized a novel series of compounds using a 1-benzo[*b*]thiophen group as the central aromatic group. The inclusion of suitable functionality to this central aromatic group has allowed us to produce a series of CB_1 agonists. The 1-benzo[*b*]thiophen and benzofuran ring systems have previously been used to produce CB_1 antagonists. Although many of the molecules in our study have not exhibited high CB_1 affinity in many cases, our design strategy based on our understanding of the CB_1 pharmacophore has allowed improvement in CB_1 affinity of the compounds. We were able to improve the affinity of a 3-cyclohexyloxy derivative with a 2-carboxamidotetrazole group **43**, which exhibited 25.0% inhibition, by replacing the 2-carboxamido group with a longer and more lipophilic 2-amide side chain, a 2-(furan-2-ylmethyl) carboxamide group, **44**. We propose that the heteroatom of the furan ring is able to interact with binding site 2 more accurately than the nitrogens. This was reflected in a marginally increased CB_1 inhibition of **44**, 31.1%. We envisage that this class of molecule with proposed structural improvements will be useful pharmacological probes for the CB_1 receptor.

Experimental

Biological Methods

Radioligand binding assays were conducted using rat cerebellum (CB_1 receptors[32,33]) or rat spleen (CB_1 and CB_2, with the latter predominating[34,35]). Functional activity of the compounds was assayed using the mouse electrically stimulated vas deferens bioassay, a measure of CB_1 receptor function.[36,37] Compounds showing appreciable activity in this assay and the ability to be antagonized by the CB_1-selective antagonist SR141716A **7** were deemed to be CB_1 agonists. The remaining compounds were classed as cannabinoid ligands. CB_2 functionality may be investigated using assays such as those described in US 6013648.[38]

Radioligand Binding

Sprague Dawley rats of either sex (200–300 g) were killed by gassing with 80% CO_2 in O_2 and decapitation, and the entire brain and spleen were excized rapidly and placed in ice-cold Tris–HCl buffer (Tris 50 mM, $MgCl_2$ 3 mM, ethylene glycol tetraacetic acid 0.2 mM, pH to 7.4 with HCl). On ice, the cerebellum was dissected away from the rest of the brain, the spleen was diced using a scalpel and both were weighed and

homogenized (PT-DA 1205/2EC Polytron Aggregate; Kinematica, Luzern, Switzerland) in 10 volumes of ice-cold Tris–HCl buffer. Homogenates were then made up to 20 volumes with Tris–HCl buffer and centrifuged at either 31000*g* for 15 min at 4°C (cerebellum) or 12600*g* for 5 min, 4°C (spleen). For the spleen membranes, the pellet was discarded, the supernatant was retained and was subjected to a second centrifugation step (23800*g*, 20 min, 4°C). Subsequently, supernatants from both cerebellar and spleen preparations were discarded, the pellets were resuspended in buffer, assayed for protein content using the method of Bradford[39] with bovine serum albumin as a standard, and stored in 500 μL aliquots at −80°C until used for the binding assays.

Ligand binding assays were performed in polypropylene tubes in a total volume of 1.0 mL containing Tris–HCl buffer, a final concentration of 1.0 mg mL^{-1} bovine serum albumen (BSA), and varying concentrations of drugs. Tubes contained 30.0 μg of membrane protein and incubation was started by the addition of 100 μL of either 0.5 nM [^{3}H]SR141716A or 0.2 nM [^{3}H]WIN-55,212–2. Experiments were carried out in triplicate at 30°C for 90 min with non-specific binding being defined as radioligand binding in the presence of 1.0 μM unlabelled WIN-55,212–2. Incubations were terminated by rapid filtration with an M-24 Cell Harvester (Brandel, Gaithersburg, MD, USA) using ice-cold Tris–HCl buffer containing 0.5 mg mL^{-1} BSA. Filters (Wattman GR/B) were soaked for 2 h before filtering in a solution of Tris–HCl buffer containing 3.0 mg mL^{-1} BSA and 0.5% w/w polyethyleneimine (PEI). Filters were left to dry thoroughly before placement into scintillation vials with 5.0 mL of scintillation cocktail (Ultima Gold LSC-cocktail; Packard Bioscience, Meridian, CT, USA) added. Vials were left to stand overnight before the radioactivity was determined using a model 1409 DSA Liquid Scintillation counter (EG&E Wallac, Gaithersburg, MD, USA).

The resulting radioligand binding curves were analyzed by non-linear regression using the program *PRISM 3.0* (Graphpad Software, San Diego, CA, USA) to derive ligand affinity estimates for the cannabinoid receptor(s).

Mouse Isolated Vasa Deferentia

Swiss white mice (35–50 g) were killed by exposure to 80% CO_2 in O_2 and exsanguination. Mouse vasa deferentia were dissected with capsular connective tissue intact and set up in 20-mL organ baths at 37°C in Mg^{2+}-free physiological salt solution.[36] The upper (epididymal) end was attached to an isometric force transducer (Grass FTO3C) and lower (prostatic) end tied to a fixed support between two parallel field electrodes (5 mm apart, 5 mm long). The tissue was initially stretched by 0.5*g* force and allowed to equilibrate for 10 min. The tissues were stimulated (Grass S88 stimulator) to contract using trains of electrical field stimulation of three pulses (4 Hz), 0.5 ms duration, 100 V (80% maximal voltage) every 20 s for 10 min. This electrical stimulation period was applied before and after both antagonist and agonist addition. Output from the transducer amplifier was recorded on a flatbed recorder (Gould BS 272, Cleveland, OH, USA). All drugs were dissolved in dimethyl sulfoxide (DMSO; Sigma, St Louis, MO, USA) and allowed to equilibrate with the tissue for 30 min before the responses to field stimulation were assessed. Drug effects were measured as the percentage decrease of the pre-drug twitch force. In experiments where only a single drug concentration was tested, the resulting effect was expressed as a percentage of that observed in the presence of vehicle.

Chemical Methods: General Directions

Computational chemistry was performed on a Silicon Graphics Iris indigo II using the *Sybyl*[40] molecular modelling software.

Unless otherwise stated, all ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AM 300 spectrometer. Chemical shifts are in ppm relative to TMS. Deuterated dimethylsulfoxide (99.9%) was used as solvent unless otherwise stated. Mass spectra and high-resolution mass spectra (HRMS) were obtained on a Kratos Concept IS (electron ionization mass spectrometer), a Kratos MS50 (fast atom bombardment mass spectrometer or a Joel JMX DX-300 double-focussing instrument. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Methanol and ethanol were distilled from iodine and magnesium and stored over 3 Å molecular sieves. Anhydrous THF was freshly distilled over potassium and benzophenone. Anhydrous DMF, diethyl ether, and toluene were stored over 4 Å molecular sieves. Triethylamine, diisopropylethylamine, and pyridine were stored over sodium hydroxide. All solutions were dried over $MgSO_4$ or Na_2SO_4 and concentrated on a Buchi rotary evaporator. Column chromatography was performed on silica gel (Merck Kieselgel 60 F_{254}). IR spectra were run in KBr disks on a Bruker IFS66 Fourier transform infrared (FTIR) spectrometer. Microanalyses were performed on a VG Platform spectrometer and are within 0.4% of the theoretical values unless otherwise stated. HPLC was performed on a Waters Millennium system comprising a 490E Multi-wavelength detector, 600 Controller, and a series 600 pump with a 717 Plus Autosampler. A Zorbax 4.6 mm × 250 mm, 5 μm column was used for analytical work whereas a 22.4 mm × 250 mm, 7 μm C_{18} column was used for preparative work.

Methyl 2-Hydroxy-5-methoxy Benzoate **14**[41]

Thionyl chloride (500.0 μL, 6.85 mmol) was added slowly to methanol (30.0 mL) followed by 5-methoxysalicylic acid (2.0 g, 11.9 mmol), and the reaction mixture was heated at reflux overnight. The reaction mixture was allowed to cool and the solvent was evaporated under reduced pressure to afford an oil, which was purified by column chromatography with ethyl acetate/hexane (1:9) as eluent to afford 2.03 g (94.0%) of the desired methyl 2-hydroxy-5-methoxy benzoate **14** as clear colourless oil. *m/z* 183 $(M + 1)^+$. δ_H ([D_6]DMSO) 3.78 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 6.91 (1H, d, *J* 9.0, H6), 7.08 (1H, dd, *J* 3.1, 9.0, H5), 7.29 (1H, d, *J* 3.2, H3).

Methyl 3-Hydroxy-5-methoxybenzofuran-2-carboxylate **15**[27]

Potassium *tert*-butoxide (1.1 g, 8.2 mmol) was added to methyl 5-methoxysalicylate (1.5 g, 8.2 mmol) in anhydrous THF (15.0 mL) at room temperature. The resulting yellow mixture was stirred at room temperature for 30 min. Methyl chloroacetate (800.0 μL, 9.12 mmol) was added and the reaction mixture was stirred at room temperature for 15 min. After this time, the reaction mixture was heated to reflux for 60 min. The reaction mixture was allowed to cool to room temperature and potassium *tert*-butoxide (1.1 g, 8.2 mmol) was added and the resulting mixture heated at reflux for 4 h and then allowed to stir at room temperature over 2 days. The thick slurry was poured into water and then acidified with concentrated hydrochloric acid and the resulting precipitate was collected by filtration. The resulting off-white powder was recrystallized from methanol to afford 367.2 mg (20.1%) of the desired methyl 3-hydroxy-5-methoxybenzofuran-2-carboxylate **15** as white crystals. *m/z*

223 $(M+1)^+$. δ_H ([D_6]DMSO) 3.87 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 7.04–7.17 (2H, m, ArH), 7.36 (1H, d, *J* 9.9, H7). HPLC retention time R_t 4.47 min, isocratic 50% B (90% CH_3CN/H_2O)/50% D (0.05% H_3PO_4/H_2O).

By the above method was also prepared methyl 3-hydroxy-1-benzo[*b*]thiophen-2-carboxylate **22**[42] as a pink powder, mp 106–108°C. *m/z* 209 $(M+1)^+$. δ_H ([D_6]DMSO) 2.48 (3H, s, CH_3), 6.06–6.11 (1H, m, ArH), 6.19 (1H, t, ArH, *J* 8.1,), 6.55–6.59 (1H, m, ArH), 6.63–6.69 (1H, m, ArH), 9.32 (1H, s, OH). Found C 57.3, H 3.8. $C_{10}H_8O_3S$ requires C 57.7, H 3.9%.

Methyl 3-(Benzyloxy)-5-methoxybenzofuran-2-carboxylate ***16***[42]

Methyl 3-hydroxy-5-methoxybenzofuran-2-carboxylate (333.0 mg, 1.5 mmol) and potassium *tert*-butoxide (202.0 mg, 1.8 mmol) were dissolved in anhydrous DMSO (5.0 mL). Benzyl bromide (399.0 µL, 3.3 mmol) was added and the reaction mixture was heated to 100°C for 2 h. The reaction mixture was allowed to cool, water was added, and the aqueous phase was extracted with ethyl acetate, dried, filtered, and evaporated under reduced pressure to afford a viscous yellow oil, which was purified by column chromatography with chloroform/methanol (99:1) as eluent to afford **16** (280.6 mg, 60%) as a pale yellow oil. *m/z* 313.1 $(M+1)^+$. δ_H ([D_6]DMSO) 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 5.45 (2H, s, CH_2), 6.91–7.54 (8H, m, ArH), 7.36 (1H, d, *J* 9.9, H7). R_t 10.14 min, isocratic conditions 50% B (90% CH_3CN/10% H_2O)/50% D (0.01% TFA in H_2O).

By the above method were also prepared the following compounds.

*Methyl 3-(Benzyloxy)-1-benzo[*b*]thiophen-2-carboxylate* ***23***[25]

Yellow powder. *m/z* 299 $(M+1)^+$. δ_H ([D_6]DMSO) 3.8 (2H, s, OCH_2), 3.81 (3H, s, OCH_3), 7.01–7.04 (2H, m, ArH), 7.05–7.4 (2H, m, 2 ArH), 7.57–7.67 (4H, m, 4 ArH). Found C 68.4, H 4.71. $C_{17}H_{14}O_3S$ requires C 68.44, H 4.73%. Found $(M+1)^+$ 299.06636. $C_{17}H_{14}O_3S$ requires $(M+1)^+$ 299.06637.

Methyl 3-Isopropoxy-5-methoxybenzofuran-2-carboxylate ***27***[42]

White powder, mp 66–67°C [lit. 66–68°C[18]]. *m/z* 265 $(M+1)^+$. δ_H ([D_6]DMSO) 1.39 (6H, d, *J* 6.0, $CH(CH_3)_2$), 3.89 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.17 (1H, m, *J* 6.0, $CH(CH_3)_2$), 7.14–7.79 (3H, m, ArH).

Methyl 3-(2-Morpholinoethoxy)-5-methoxybenzofuran-2-carboxylate ***30***

The crude product was purified by column chromatography with chloroform/methanol (99:1) as eluent to afford the *title compound* as a yellow waxy solid. *m/z* 336 $(M+1)^+$. δ_H ([D_6]DMSO) 3.16–3.23 (8H, m, 4 CH_2), 3.46 (2H, t, *J* 5.5, CH_2), 4.24 (4H, m, 2 CH_2), 4.47 (3H, s, OCH_3), 4.59 (3H, s, OCH_3), 5.23 (2H, t, *J* 5.5, OCH_2), 7.88 (1H, dd, *J* 2.6, 9.0, ArH), 7.9 (1H, d, *J* 2.8, ArH), 8.29 (1H, d, *J* 9.0, ArH). R_t 4.47 min, isocratic conditions 50% B (90% CH_3CN/H_2O)/50% D (0.05% H_3PO_4 in H_2O).

3-(Benzyloxy)-5-methoxybenzofuran-2-carboxylic Acid ***17***[42]

Methyl 3-(benzyloxy)-5-methoxybenzofuran-2-carboxylate (244.0 mg, 0.8 mmol) was dissolved in methanol (3.0 mL), and potassium hydroxide (96.0 mg, 0.2 mmol) was added. The reaction mixture was heated to reflux for 2 h. The reaction mixture was allowed to cool, the methanol was evaporated under reduced pressure, water was added, and the reaction mixture was acidified with concentrated hydrochloric acid. The aqueous phase was extracted with ethyl acetate, dried, filtered, and evaporated under reduced pressure to afford an orange oil, which was purified with preparative HPLC to afford **17** (174.2 mg, 75%) as an off-white solid. *m/z* 299 $(M+1)^+$. δ_H ([D_6]DMSO) 3.72 (3H, s, OCH_3), 5.39 (2H, s, CH_2), 7.04–7.89 (8H, m, 8 ArH). Found C 64.96, H 4.47. $C_{17}H_{14}O_4S$ requires C 64.95, H 4.49%. R_t 4.47 min, isocratic conditions 50% B (90% CH_3CN/10% H_2O)/50% D (0.01% TFA in H_2O).

By the above method were also prepared the following compounds.

*3-(Benzyloxy)-1-benzo[*b*]thiophen-2-carboxylic Acid* ***24***[25]

White powder. *m/z* 285 $(M+1)^+$. δ_H ($CDCl_3$) 5.19 (2H, s, OCH_2), 6.49 (1H, m, ArH), 6.7 (1H, m, ArH), 6.78–6.82 (2H, d, 2 ArH), 6.88–7.15 (3H, m, 3 ArH), 7.62 (1H, m, ArH), 10.05 (1H, s, OH). Found C 67.6, H 4.2. $C_{16}H_{12}O_3S$ requires C 67.6, H 4.3%. Found $(M+1)^+$ 285.0507. $C_{16}H_{12}O_3S$ requires $(M+1)^+$ 285.05071.

3-Isopropoxy-5-methoxybenzofuran-2-carboxylic Acid ***28***[27,42]

The crude product was recrystallized from methanol/water to afford **28** (311.0 mg, 28.0%) as cream crystals, mp 138–140°C. *m/z* 250 $(M)^+$. δ_H ([D_6]DMSO) 1.3 (6H, d, *J* 6.0, $CH(CH_3)_2$), 3.81 (3H, s, OCH_3), 4.79 (1H, m, *J* 6.0, $CH(CH_3)_2$), 7.08–7.11 (2H, m, ArH), 7.15–7.52 (1H, d, *J* 9.6, ArH), 13.16 (1H, s, OH). Found C 62.5, H 5.6. $C_{13}H_{14}O_5$ requires C 62.4, H 5.6%. HPLC R_t 7.33 min (10% B/90% D) to (90% B/10% D) over 20 min (B 90% CH_3CN/10% H_2O; D 0.1 M NH_4OAc (pH 4)).

3-(2-Morpholinoethoxy)-5-methoxybenzofuran-2-carboxylic Acid ***31***[43]

Light orange powder. *m/z* 322 $(M+1)^+$. δ_H ([D_6]DMSO) 2.35 (3H, m, CH_2, CH), 2.65 (2H, m, CH_2), 3.4 (3H, m, 2 CH_2), 3.81 (2H, d, *J* 4.2, $NHCH_2$), 4.53 (3H, m, CH_2, CH), 7.06 (1H, dd, *J* 2.2, 6.4, H6), 7.24 (1H, d, *J* 2.7, H4), 7.49 (1H, d, *J* 9.0, H7), 8.56 (1H, m, NH). R_t 8.87 min, gradient conditions 30% B/70% A to 90% B/10% A over 20 min (B 90% CH_3CN/10% H_2O; A 0.1% TFA in H_2O).

*3-Cyclohexyloxy-6-methoxy-1-benzo[*b*]thiophen-2-carboxylic Acid* ***42***

White powder. *m/z* 307 $(M+1)^+$ δ_H ($CDCl_3$) 1.2–1.39 (3H, m, CH_2, CH), 1.58–1.68 (3H, m, CH_2, CH), 1.8–1.85 (2H, m, CH_2), 2.0–2.14 (2H, m, CH_2), 3.88 (3H, s, OCH_3), 4.49 (1H, m, *J* 3.9, $CH(CH_3)_2$), 7.13 (1H, dd, *J* 2.5, 8.6, ArH), 7.23 (1H, m, ArH), 7.7 (1H, m, ArH). Found $(M+1)^+$ 307.09255. $C_{16}H_{18}O_4S$ requires $(M+1)^+$ 307.09258.

*3-Isopropoxy-5-methoxy-1-benzo[*b*]thiophen-2-carboxylic Acid* ***49***[28]

The crude product was recrystallized from acetonitrile to afford 140.8 mg (25%) of the desired 3-isopropoxy-5-methoxy-1-benzo[*b*]thiophen-2-carboxylic acid as a white powder, mp 76–80°C. *m/z* 266 $(M)^+$. δ_H ($CDCl_3$) 3.85 (3H, s, OCH_3), 7.09 (1H, dd, *J* 2.5, 8.8, ArH), 7.26 (1H, d, *J* 2.5, ArH), 7.59 (1H, d, *J* 8.8, ArH). Found C 54.5, H 4.6. $C_{13}H_{13}ClO_3S$ requires C 54.9,

H 4.5%. R_t 5.37 min, linear gradient over 10 min. 10% B/90% D to 90% B/10% D (B 90% CH_3CN, 10% H_2O; D 0.1 N NH_4OAc (pH 4)).

*3-(4-Methoxyphenoxy)-6-methoxy-1-benzo[*b*]thiophen-2-carboxylic Acid* ***54***

The crude product was recrystallized from methanol to afford 98.8 mg (65%) of the desired 3-(4-methoxyphenoxy)-6-methoxy-1-benzo[*b*]thiophen-2-carboxylic acid as a cream solid, mp 169–172°C (dec.). *m/z* 368 $(M+1)^+$. δ_H ([D_6]DMSO) 3.69 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 6.92 (3H, m, ArH), 7.3 (3H, m, ArH), 7.81 (1H, d, *J* 2.0, ArH). Found $(M+1)^+$ 331.05600. $C_{17}H_{14}O_5S$ requires $(M+1)^+$ 331.05619.

*3-(Benzyloxy)-5-methoxy-1-benzo[*b*]thiophen-2-carboxylic Acid* ***57****[27,44]*

Pink solid. *m/z* 315 $(M+1)^+$. δ_H ($CDCl_3$) 3.5 (3H, s, OCH_3), 5.0 (2H, s, OCH_2), 6.4–6.9 (5H, m, ArH), 7.2–7.3 (2H, m, 2 ArH), 7.4 (1H, d, *J* 8.5, ArH).

*3-(Benzyloxy)-6-methoxy-1-benzo[*b*]thiophen-2-carboxylic Acid* ***61****[44]*

Pink powder. *m/z* 315 $(M+1)^+$. δ_H ($CDCl_3$) 3.49 (3H, s, OCH_3), 4.95 (2H, s, CH_2), 6.57 (1H, m, ArH), 6.81 (2H, m, ArH), 6.98 (1H, m, ArH), 7.11 (1H, m, ArH), 7.23 (1H, d, *J* 8.9, H4).

*3-(Benzyloxy)-5-methoxy-*N,N*-dimethylbenzofuran-2-carboxamide* ***18***

3-(Benzyloxy)-5-methoxy-1-benzofuran-2-carboxylic acid (128.0 mg, 0.4 mmol), *N*-aminopiperidine (47.0 mg, 50.0 μL, 47.0 mmol), and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (TBTU) (152.0 mg, 0.5 mmol) were dissolved in anhydrous DMF (5.0 mL). The solution was stirred for 15 min under an atmosphere of nitrogen after which diisopropylethylamine (89.8 μL, 0.5 mmol) was added. The solution was stirred overnight at room temperature under nitrogen. The DMF was evaporated under reduced pressure to afford a residue. Water was added to the residue and the aqueous solution was extracted with ethyl acetate, then dried, filtered, and evaporated under reduced pressure to afford a yellow solid. The yellow solid was purified by column chromatography with chloroform as eluent to afford **18** as yellow oil. *m/z* 326 $(M+1)^+$. δ_H ($CDCl_3$) 3.02 (6H, s, 2 NCH_3), 3.81 (3H, s, OCH_3), 5.29 (2H, s, OCH_2), 6.87–7.65 (8H, m, ArH). R_t 9.99 min, isocratic conditions 50 B (90% CH_3CN/10% H_2O)/50 D (0.05% H_3PO_4 in H_2O).

By the above method were also prepared the following compounds.

*3-(Benzyloxy)-5-methoxy-*N,N*-(piperidin-1-yl)benzofuran-2-carboxamide* ***19***

The crude product was purified by column chromatography with chloroform as eluent to afford **19** (65.3 mg, 40%) as a yellow oil. *m/z* 381 $(M+1)^+$. δ_H ($CDCl_3$) 1.51–1.54 (6H, m, 3 CH_2), 2.8 (4H, m, 2 CH_2), 3.8 (3H, s, OCH_3), 5.1 (2H, s, CH_2), 7.0–7.48 (6H, m, ArH), 7.5 (1H, d, *J* 2.2, H4), 7.6 (1H, d, *J* 8.0, H7). Found $(M+1)^+$ 381.17363. $C_{22}H_{24}N_2O_4$ requires $(M+1)^+$ 381.17361.

*3-(Benzyloxy)-*N*-(piperidin-1-yl)-1-benzo[*b*]thiophen-2-carboxamide* ***25***

The crude product was purified by column chromatography with chloroform as eluent to afford **25** as clear viscous oil. *m/z* 367 $(M+1)^+$. δ_H ([D_6]DMSO) 3.31 (10H, m, 5 CH_2), 5.16 (2H, s, OCH_2), 7.32–7.92 (9H, m, ArH). R_t 12.01 min, isocratic conditions 50% B (90% CH_3CN/10% H_2O)/50% B/D (0.05% H_3PO_4/H_2O). Found $(M+1)^+$ 367.147. $C_{21}H_{22}N_2O_2S$ requires $(M+1)^+$ 367.14801.

*3-(Benzyloxy)-*N,N*-dimethyl-1-benzo[*b*]thiophen-2-carboxamide* ***26***

The crude product was purified by column chromatography with chloroform as eluent to afford 3-(benzyloxy)-*N*,*N*-dimethyl-1-benzo[*b*]thiophen-2-carboxamide as a white powder. *m/z* 312 $(M)^+$. δ_H ([D_6]DMSO) 2.96 (6H, s, 2 NCH_3), 5.16 (2H, s, OCH_2), 7.32–7.92 (9H, m, ArH), 9.66 (1H, s, OH). R_t 18.82 min, isocratic conditions 50 B (90% CH_3CN/H_2O)/50 D (0.05% H_3PO_4 in H_2O).

N-Benzyl-3-(2-morpholinoethoxy)-5-methoxybenzofuran-2-carboxamide ***32****[43]*

The crude product was purified by column chromatography with chloroform/methanol (99:1) as eluent to afford 21.4 mg (34%) of the desired *N*-benzyl-3-(2-morpholinoethoxy)-5-methoxybenzofuran-2-carboxamide as yellow crystals, mp 118–120°C. *m/z* 411 $(M+1)^+$. δ_H ([D_6]DMSO) 2.35 (3H, m, CH_2, CH), 2.65 (2H, m, CH_2), 3.4 (4H, m, 2 CH_2), 3.81 (2H, d, *J* 4.2, $NHCH_2$), 4.53 (3H, m, CH_2, CH), 7.06 (1H, dd, *J* 2.2, 6.4, H6), 7.24 (1H, d, *J* 2.6, H4), 7.49 (1H, d, *J* 9.0, H7), 8.56 (1H, m, NH). R_t 8.87 min, gradient conditions 30% B/70% A to 90% B/10% A over 20 min (B 90% CH_3CN, 10% H_2O; A 0.1% TFA in H_2O).

*3-Cyclohexyloxy-6-methoxy-*N*-(furan-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide* ***44***

The crude product was purified by column chromatography with ethyl acetate/hexane (1:9 to 1:1) as eluent to afford 130.0 mg (98.0%) of the desired 3-cyclohexyloxy-6-methoxy-*N*-(furan-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide as pale pink crystals, mp 115–117°C. *m/z* 386 $(M+1)^+$. δ_H ($CDCl_3$) 1.18 (2H, m, CH_2), 1.38 (1H, m, CH), 1.54 (3H, s, OCH_3), 3.87 (2H, s, CH_2), 4.33 (1H, m, CH), 4.64 (2H, d, *J* 8.9, $NHCH_2$). R_t 2.66 min, isocratic 100% B (90% CH_3CN/H_2O).

*3-Cyclohexyloxy-5-methoxy-*N*-(furan-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide* ***58***

The crude product was purified by column chromatography with hexane/ethyl acetate (1:9 to 1:1) as eluent to afford 55.5 mg (88.0%) of the desired 3-cyclohexyloxy-5-methoxy-*N*-(furan-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide as a yellow gum. *m/z* 386 $(M+1)^+$. δ_H ([D_6]DMSO) 1.04–1.24 (3H, m, 3 CH), 1.32–1.5 (3H, m, 3 CH), 1.63–1.67 (2H, m, 2 CH), 1.89–1.98 (2H, m, 2 CH), 3.83 (3H, s, OCH_3), 4.32 (1H, m, OCH), 4.52 (2H, d, *J* 5.5, CH_2NH), 6.34 (1H, s, furan ArH), 6.42 (1H, s, furan ArH), 7.12 (3H, m, ArH), 7.83–7.85 (1H, m, ArH), 8.17 (1H, t, *J* 5.9, NH). Found C 65.4, H 6.0, N 3.6. $C_{21}H_{23}NO_4S$ requires C 65.4, H 6.0, N 3.6%.

3-(Benzyloxy)-5-methoxy-N-*(thiophen-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide* ***59***

The crude product was purified by column chromatography with hexane/ethyl acetate (8:2 to 3:1) as eluent to afford 151.0 mg (57.0%) of the desired 3-(benzyloxy)-5-methoxy-*N*-(thiophen-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide as an off-white solid. *m*/*z* 410 $(M+1)^+$. δ_H ([D_6]DMSO) 3.79 (3H, s, OCH_3), 4.6 (2H, d, $NHCH_2$), 5.25 (2H, s, OCH_2), 6.85–7.45 (11H, m, 11 ArH), 8.43 (1H, t, NH). R_t 2.82 min, isocratic 100% B (90% CH_3CN/H_2O).

3-Benzyloxy-6-methoxy-N-*(thiophen-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide* ***62***

The crude product was purified by column chromatography with ethyl acetate/hexane (2:8) as eluent to afford 149.0 mg (57.0%) of the desired 3-benzyloxy-6-methoxy-*N*-(thiophen-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide as pale pink crystals. *m*/*z* 410 $(M+1)^+$. δ_H ($CDCl_3$) 3.88 (3H, s, OCH_3), 4.61 (2H, d, *J* 1.8, $NHCH_2$), 5.19 (2H, s, $PhCH_2$), 6.85–6.89 (2H, m, ArH), 7.21–7.23 (3H, m, 3 ArH), 7.31 (3H, m, ArH), 7.61 (1H, m, $NHCH_2$), 7.65 (2H, m, ArH).

3-(Benzyloxy)-6-methoxy-N-*(thiophen-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide* ***64***

The crude product was purified by column chromatography with ethyl acetate/hexane (2:8) as eluent to afford 150.0 mg (57.3%) of the desired 3-(benzyloxy)-6-methoxy-*N*-(thiophen-2-ylmethyl)-1-benzo[*b*]thiophen-2-carboxamide as pale pink crystals. *m*/*z* 411 $(M+1)^+$. δ_H ($CDCl_3$) 3.9 (3H, s, OCH_3), 4.62 (2H, d, $NHCH_2$), 5.19 (2H, s, OCH_2), 7.0 (4H, m, ArH), 7.36 (3H, m, ArH), 7.64 (2H, d, $NHCH_2$). R_t 2.85 min, 100% B (90% CH_3CN/H_2O).

3-Isopropoxy-5-methoxy-N-*(1*H*-1,2,3,4-tetrazol-5-yl)benzofuran-2-carboxamide* ***29***[27,42]

1,1′-Carbonyldiimidazole (120.0 mg, 0.74 mmol) was added to a solution of 3-isopropoxy-5-methoxybenzofuran-2-carboxylic acid (150.0 mg, 0.6 mmol) dissolved in anhydrous THF (5.0 mL) at room temperature. The reaction mixture was heated to reflux for 90 min. The reaction mixture was partially cooled, 5-aminotetrazole (60.0 mg, 0.7 mmol) was added, and the reaction mixture was heated to reflux overnight. The reaction mixture was allowed to cool, then poured into water (200.0 mL), and acidified with concentrated hydrochloric acid. The precipitate was collected by filtration and recrystallized from acetonitrile/water to afford **29** (158.0 mg, 83.0%) as a cream crystalline solid, mp 236–238°C. *m*/*z* 318 $(M+1)^+$. δ_H ($CDCl_3$) 1.37 (6H, d, *J* 6.0, 2 CH_3), 3.85 (3H, s, OCH_3), 5.07 (1H, m, $CH(CH_3)_2$), 7.17–7.21 (2H, m, H4, H6), 7.57 (1H, d, *J* 8.9, H7), 11.8 (1H, s, NH).

By the above method were also prepared the following compounds.

3-Cyclohexyloxy-6-methoxy-N-*(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide* ***43***

The crude product was recrystallized from methanol/water to afford 30.0 mg (87.0%) of the desired 3-cyclohexyloxy-6-methoxy-*N*-(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide as a white solid, mp 219–222°C. *m*/*z* 373 $(M)^+$. δ_H ($CDCl_3$) 1.28 (3H, m, 3 CH), 1.54–1.66 (3H, m, 3 CH), 1.85–1.88 (2H, m, 2 CH), 2.15–2.29 (2H, m, 2 CH), 3.91 (3H, s, OCH_3), 4.65 (1H, m, OCH), 7.05 (1H, dd, *J* 2.2, 8.9, ArH), 7.24 (1H, d, *J* 2.2, ArH), 7.71 (1H, d, *J* 8.9, ArH). Found C 54.7, H 5.4, N 18.6. $C_{17}H_{19}N_5O_3S$ requires C 54.7, H 5.1, N 18.8%. R_t 12.62 min, 10% B/90% D to 90% B/10% D over 20 min (B 90% CH_3CN, 10% H_2O; D 0.1 N NH_4OAc (pH 4)).

3-(4-Methoxyphenoxy)-6-methoxy-N-*(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide* ***55***

The crude product was recrystallized from methanol/DMF/water to afford 106.0 mg (86.0%) of the desired 3-(4-methoxyphenoxy)-6-methoxy-*N*-(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide as a fine white powder, mp 267–268°C. *m*/*z* 368 $(M+1)^+$. δ_H ($CDCl_3$) 1.18 (2H, m, CH_2), 1.38 (1H, m, CH), 1.54 (3H, s, OCH_3), 3.87 (2H, s, CH_2), 4.33 (1H, m, CH), 4.64 (2H, d, *J* 8.9, $NHCH_2$). R_t 2.16 min, isocratic 100% B (90% CH_3CN/H_2O).

3-(Benzyloxy)-6-methoxy-N-*(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide* ***63***[44]

Pale pink powder. *m*/*z* 382 $(M+1)^+$. δ_H ($CDCl_3$) 3.63 (3H, s, OCH_3), 5.19 (2H, s, OCH_2), 6.25–7.75 (8H, m, ArH), 10.01 (1H, s, NH). R_t 2.16 min, isocratic 100% B (90% CH_3CN/H_2O).

3-Isopropoxy-4-nitro-5-methoxy-N-*(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide* ***51***[28]

The crude product was recrystallized from methanol/DMF/water to afford 36.3 mg (30.0%) of the desired 3-isopropoxy-4-nitro-5-methoxy-*N*-(1*H*-1,2,3,4-tetrazol-5-yl)-1-benzo[*b*]thiophen-2-carboxamide as a white powder, mp 236–238°C. *m*/*z* 379 $(M+1)^+$. δ_H ([D_6]DMSO) 1.15 (6H, d, *J* 6.0, 2 CH_3), 3.97 (3H, s, OCH_3), 4.49 (1H, m, CH), 7.65 (1H, d, *J* 9.2, H7), 8.25 (1H, d, *J* 9.0, H6). Found C 55.6, H 5.1, N 4.9. $C_{13}H_{15}NO_4S$ requires C 55.5, H 5.4, N 5.0%. Found $(M+1)^+$ 282.08002. $C_{13}H_{15}NO_4S$ requires $(M+1)^+$ 282.08.

Lithium 1-(4-Chlorophenyl)-4-ethoxy-2-methyl-3,4-dioxobutan-1-olate ***34***[44]

Lithium hexamethyl disilazide (1.0 N in THF) (29.6 mL, 30.0 mmol) was added to anhydrous THF (120.0 mL). The solution was then cooled to −78°C under an atmosphere of nitrogen. After 30 min, 4-chloropropiophenone (5.0 g, 30.0 mmol) in anhydrous THF (25.0 mL) was added to the reaction mixture. The solution was then allowed to warm up to room temperature overnight. The solvent was evaporated under reduced pressure to afford a gum, which was recrystallized from acetone to afford 2.41 g (30.0%) of the desired lithium 1-(4-chlorophenyl)-4-ethoxy-2-methyl-3,4-dioxobutan-1-olate as yellow crystals. *m*/*z* 269 $(M+1)^+$. δ_H ([D_6]DMSO) 1.13 (3H, t, *J* 7.1, CH_3), 1.27 (3H, d, *J* 6.9, CH_3), 4.18 (2H, q, *J* 6.9, CH_2), 5.28 (1H, q, *J* 6.9, CH), 7.66 (1H, d, *J* 6.8, ArH), 7.69 (1H, d, *J* 7.8, ArH), 8.01 (1H, d, *J* 8.3, ArH), 8.03 (1H, d, *J* 8.6, ArH).

*Ethyl 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate* ***35***[44]

Ethyl 3-(4-chlorophenyl)-2-methyl-3-oxopropanoate (1.31 g, 4.9 mmol) was dissolved in anhydrous ethanol (40.0 mL) and 2,4-dichlorophenyl hydrazine hydrochloride (1.04 g, 4.9 mmol) was added. The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 48 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography with hexane/chloroform (1:1) leading to chloroform as eluent to afford 498.0 mg (24.0%) of the desired ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate as a yellow solid. *m*/*z* 431

$(M+1)^+$ (cyclizes in the source). δ_H ([D_6]DMSO) 1.05 (3H, t, *J* 6.9, CH_3), 1.25 (2H, q, *J* 6.9, CH_2), 1.36 (3H, d, *J* 4.2, CH_3), 3.05 (1H, q, *J* 4.2, CH), 7.2–8.02 (7H, m, ArH).

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic Acid* ***36***[44]

(*Z*)-Ethyl 4-(4-chlorophenyl)-2-(2,4-dichlorophenyl)hydrazone-3-methyl-4-oxobutanoate (33.5 mg, 0.08 mmol) was dissolved in ethanol (2.0 mL) and 2 M NaOH (980.0 μL, 1.9 mmol) was added and the reaction mixture was heated to reflux for 6 h, then allowed to stir at room temperature overnight. The solvent was evaporated under reduced pressure and water was added; the aqueous phase was acidified with 2 M HCl and extracted with ethyl acetate, dried, filtered and the solvent evaporated under reduced pressure to afford 3.1 mg (10.0%) of the desired 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid as a yellow solid. *m*/*z* 381 $(M)^+$. δ_H ([D_6]DMSO) 2.1 (3H, s, CH_3), 7.1 (2H, d, *J* 8.4, ArH), 7.34 (2H, d, *J* 8.4, ArH), 7.45 (1H, m, ArH), 7.58 (1H, m, ArH), 7.69 (1H, m, ArH).

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-piperidino-1*H*-pyrazole-3-carboxamide* ***37***[44]

1-(2,4-Dichlorophenyl)-4-methyl-5-(4-chlorophenyl) pyrazole-3-carboxylic acid (433.0 mg, 11.1 mmol) was dissolved in anhydrous DMF (25.0 mL) and *N*-aminopiperidine (125.0 mg, 1.3 mmol) and TBTU (400.0 mg, 1.3 mmol) were added and the reaction mixture was stirred under an atmosphere of nitrogen for 30 min, and diisopropylethylamine (238.0 μL, 1.4 mmol) was added and the reaction was stirred for 72 h. The DMF was evaporated under reduced pressure, water was added and the aqueous phase was extracted with ethyl acetate, dried, filtered, and evaporated under reduced pressure to afford a dark brown oil, which was purified by column chromatography with chloroform as eluent to afford 346.0 mg (68.0%) of the desired 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-piperidino-1*H*-pyrazole-3-carboxamide as a light yellow glassy solid. *m*/*z* 464 $(M+1)^+$. δ_H ([D_6]DMSO) 1.33 (2H, m, CH_2), 1.55 (3H, m, CH_3), 2.76 (4H, m, 2 CH_2), 7.21 (2H, d, *J* 8.4, ArH), 7.45 (2H, d, *J* 8.4, 2 ArH), 7.57 (1H, dd, *J* 2.3, 8.5, ArH), 7.74 (2H, m, 2 ArH), 9.03 (1H, s, NH).

*3-Chloro-6-methoxy-1-benzo[*b*]thiophen-2-carbonyl Chloride* ***39***[45–47]

To a mixture of 4-methoxycinnamic acid (10.0 g, 56.1 mmol) and anhydrous pyridine (560.0 μL, 6.9 mmol) was added dropwise with stirring thionyl chloride (30.0 mL, 0.41 mmol) and the reaction mixture was heated to 105°C for 48 h. The reaction mixture was allowed to cool and the volatiles were removed by evaporation under reduced pressure to afford a yellow solid, which was recrystallized from Bu^tOCH_3 to afford 6.75 g (46.0%) of the desired 3-chloro-6-methoxy-1-benzo[*b*]thiophen-2-carbonyl chloride as yellow needles, mp 120°C. *m*/*z* 261 $(M+1)^+$. δ_H ($CDCl_3$) 3.89 (3H, s, OCH_3), 7.19–7.3 (2H, m, ArH), 7.66 (1H, d, *J* 9.0, ArH).

By the above method was also prepared 3-chloro-5-methoxy-1-benzo[*b*]thiophen-2-carbonyl chloride **46**[28,48] as a yellow solid. *m*/*z* 260 $(M)^+$. δ_H ($CDCl_3$) 3.9 (3H, s, OCH_3), 7.2–7.3 (2H, m, ArH), 7.69 (1H, d, *J* 8.8, ArH).

*Cyclohexyl 3-Chloro-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate* ***40***

3-Chloro-6-methoxy-1-benzo[*b*]thiophen-2-carbonyl chloride (16.3 g, 62.4 mmol) was dissolved in anhydrous benzene (20.0 mL) and anhydrous cyclohexanol (20.0 mL) and the reaction mixture was heated to reflux for 3 h under an atmosphere of nitrogen and then stirred at room temperature for 17 h. After this time, the reaction mixture was evaporated under reduced pressure to afford a yellow oil, which was purified by column chromatography with hexane/dichloromethane (1:9) as eluent to afford a yellow solid, which was recrystallized from hexane to afford 19.94 g (98.0%) of the desired cyclohexyl 3-chloro-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate as a yellow powder. *m*/*z* 326 $(M+1)^+$. δ_H ($CDCl_3$) 1.4–1.44 (2H, m, CH_2), 1.46–1.49 (2H, m, CH_2), 1.62–1.73 (2H, m, CH_2), 1.8–1.86 (2H, m, CH_2), 1.9–1.97 (2H, m, CH_2), 3.9 (3H, s, OCH_3), 5.1 (1H, m, CH), 7.1 (1H, dd, *J* 2.0, 9.0, H6), 7.21 (1H, d, *J* 2.0, H4), 7.83 (1H, d, *J* 9.0, H7). Found $(M+1)^{+\cdot}$ 325.0586. $C_{16}H_{17}ClO_3S$ requires $(M+1)^+$ 325.05869.

*Cyclohexyl 3-Cyclohexyloxy-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate* ***41***

Cyclohexanol (8.3 mL, 80.0 mmol) was added dropwise to a stirred suspension of sodium hydride (60%, 3.2 g, 80.0 mmol) in anhydrous THF (60.0 mL) under an atmosphere of nitrogen. The reaction mixture was stirred for 90 min. After this time, a solution of cyclohexyl 3-chloro-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate (13.0 g, 40.0 mmol) in warm anhydrous THF (100.0 mL) was added slowly to the stirred sodium hydride mixture over 5 min. The reaction mixture was then heated to reflux for 17 h under an atmosphere of nitrogen. After this time, the reaction mixture was allowed to cool to room temperature and the cyclohexanol and THF were evaporated under reduced pressure to afford a brown solid, which was dissolved in diethyl ether (500.0 mL) and washed with water. The diethyl ether phase was then dried, filtered and evaporated under reduced pressure to afford 14.19 g (91.0%) of the desired cyclohexyl 3-cyclohexyloxy-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate as a yellow oil. *m*/*z* 389 $(M+1)^+$. δ_H ($CDCl_3$) 1.18–1.3 (10H, m, 5 CH_2), 1.46–1.99 (10H, m, 5 CH_2), 4.5 (1H, m, CH), 4.99 (1H, m, CH), 6.95 (1H, dd, *J* 2.2, 8.9, H6), 7.13 (1H, d, *J* 2.0, H4), 7.7 (1H, d, *J* 9.0, H7). Found $(M+1)^+$ 389.17081. $C_{22}H_{28}O_4S$ requires $(M+1)^+$ 389.17083.

*Isopropyl 5-Methoxy-3-chloro-1-benzo[*b*]thiophen-2-carboxylate* ***47***[28]

3-Chloro-5-methoxy-1-benzo[*b*]thiophen-2-carbonyl chloride (7.32 g, 28.0 mmol) was added to a mixture of anhydrous THF (30.0 mL) and isopropyl alcohol (30.0 mL) and the reaction mixture was stirred at reflux for 5 h. The reaction was allowed to cool and the solvent was evaporated under reduced pressure to afford a yellow residue, which was purified by column chromatography with dichloromethane/hexane (2:3) as eluent to afford a yellow solid, which was recrystallized from hexane to afford 1.39 g (17.0%) of the desired isopropyl 5-methoxy-3-chloro-1-benzo[*b*]thiophen-2-carboxylate as pale fluffy yellow crystals, mp 76–80°C. *m*/*z* 319 $(M+1)^+$. δ_H ([D_6]DMSO) 1.32 (6H, d, *J* 6.2, $CH(CH_3)_2$), 4.03 (3H, s, OCH_3), 5.15 (1H, m, *J* 6.2, $CH(CH_3)_2$), 7.55 (1H, d, *J* 9.0, ArH), 8.05 (1H, d, *J* 8.9, ArH). Found C 54.5, H 4.6. $C_{13}H_{13}ClO_3S$ requires C 54.9, H 4.5%.

Isopropyl 3-Isopropoxy-5-methoxy-1-benzo[b]thiophen-2-carboxylate ***48***[28]

Sodium hydride (60%, 600.0 mg, 4.0 mmol) was suspended in anhydrous THF (2.0 mL) and stirred under an atmosphere of nitrogen for 10 min. A solution of isopropyl alcohol (350.0 μL, 4.6 mmol) dissolved in anhydrous THF (2.0 mL) was slowly added to the sodium hydride in THF. The reaction mixture was then stirred at room temperature for 1.5 h under an atmosphere of nitrogen. After this time, isopropyl 3-chloro-5-methoxy-1-benzo[*b*]thiophen-2-carboxylate (600.0 mg, 2.1 mmol) in anhydrous THF (3.0 mL) was slowly added. The reaction mixture was heated to reflux for 17 h. After this time, the THF and isopropanol were evaporated under reduced pressure to afford a residue, which was partitioned between hexane and water. The aqueous phase was extracted with hexane and the hexane extracts were dried, filtered, and evaporated under reduced pressure to afford (325.0 mg, 50.0%) of the desired isopropyl 3-isopropoxy-5-methoxy-1-benzo[*b*]thiophen-2-carboxylate as yellow oil. *m*/*z* 309 $(M+1)+$. δ_H ($CDCl_3$) 1.24 (6H, d, *J* 3.8, 2 CH_3), 2.1 (6H, s, *J* 6.9, 2 CH_3), 3.89 (3H, s, OCH_3), 7.06 (1H, dd, *J* 2.4, 9.0, ArH), 7.24 (1H, d, *J* 2.4, ArH), 7.5 (1H, d, *J* 9.0, ArH).

3-Isopropoxy-4-nitro-5-methoxy-1-benzo[b]thiophen-2-carboxylic Acid ***50***[28]

A solution of 3-isopropoxy-5-methoxy-1-benzo[*b*]thiophen-2-carboxylic acid (500.0 mg, 1.87 mmol) in acetic acid (10.0 mL) was cooled in ice-water and concentrated nitric acid (1.5 mL) was added dropwise over 2 min. The reaction mixture was stirred at room temperature for 60 min. After this time, the reaction mixture was poured into water (150.0 mL) and a precipitate formed, which was collected by filtration and washed well with water, then dried under reduced pressure to afford a yellow solid, which was recrystallized from dichloromethane/hexane to afford 289.0 mg (50.0%) of the desired 3-isopropoxy-4-nitro-5-methoxy-1-benzo[*b*]thiophen-2-carboxylic acid as a yellow powder, mp 197–200°C. *m*/*z* 312 $(M+1)^+$. δ_H ($CDCl_3$) 1.32 (6H, d, *J* 6.0, 2 CH_3), 3.99 (3H, s, OCH_3), 5.04 (1H, m, $CH(CH_3)_2$), 7.32 (1H, d, *J* 9.0, H6), 7.8 (1H, d, *J* 8.9, H7). Found C 50.1, H 4.2, N 4.4. $C_{13}H_{13}NO_6S$ requires C 50.2, H 4.2, N 4.5%. R_t 6.39 min, 10% B/90% D to 90% B/10% D (B 90% CH_3CN/10% water; D 0.1 N NH_4OAc (pH 4)).

4-Methoxyphenyl 3-Chloro-6-methoxy-1-benzo[b]thiophen-2-carboxylate ***52***

2-Chloroethyl formate (250.0 μL, 2.61 mmol) was added to 3-chloro-6-methoxy-1-benzo[*b*]thiophen-2-carboxylic acid (500.0 mg, 2.1 mmol) and triethylamine (350.0 μL, 2.51 mmol) in acetone (5.0 mL) at 0°C. The reaction mixture was stirred at 0°C for 15 min, then a solution of 4-methoxyphenol (400.0 mg, 3.22 mmol) and triethylamine (450.0 μL, 3.23 mmol) in acetone (1.5 mL) was added slowly over 2 h. The reaction mixture was allowed to stir to room temperature for 2 h. The reaction mixture became very thick, so a further quantity of acetone (5.0 mL) was added and stirring was continued for a further 60 min. After this time, the reaction mixture was poured onto water (200.0 mL) and a precipitate formed, which was collected by filtration. The solid was then recrystallized from hexane/dichloromethane to afford 461.2 mg (64.0%) of the desired 4-methoxyphenyl 3-chloro-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate as white fluffy crystals, mp 160–161.5°C. *m*/*z* 349 $(M+1)^+$. δ_H ($CDCl_3$) 3.83 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.92 (2H, d, *J* 9.7, 2 ArH), 7.13 (1H, dd, *J* 2.3, 9.0, ArH), 7.18 (2H, d, *J* 9.0, 2 ArH), 7.26 (1H, d, *J* 2.2, ArH), 7.88 (1H, d, *J* 9.0, ArH). Found $(M+1)^+$ 349.02231. $C_{17}H_{13}ClO_4S$ requires $(M+1)^+$ 349.02231.

4-Methoxyphenyl 3-(4-Methoxyphenoxy)-6-methoxy-1-benzo[b]thiophen-2-carboxylate ***53***

Potassium hydroxide (100.0 mg, 1.78 mmol) and 4-methoxyphenol (240.0 mg, 1.93 mmol) in anhydrous toluene (60.0 mL) were heated to reflux under Dean–Stark conditions for 17 h. After this time, the reaction mixture was allowed to cool to room temperature, then DMSO (5.0 mL) was added and the toluene was evaporated under reduced pressure. The DMSO solution was brown. 4-Methoxyphenyl 3-chloro-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate (300.0 mg, 0.86 mmol) was added to the DMSO solution and the reaction mixture was heated to 100°C for 17 h. After this time, the reaction mixture was allowed to cool to room temperature, then poured into 0.25 N aqueous potassium carbonate solution (100.0 mL) and stirred for 2 h. A precipitate formed and was collected by filtration and dried under reduced pressure to afford 212.3 mg (57.0%) of the desired 4-methoxyphenyl 3-(4-methoxyphenoxy)-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate as a cream powder. *m*/*z* 437 $(M+1)^+$. δ_H ($CDCl_3$) 3.77 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.78–6.9 (5H, m, ArH), 6.91–7.04 (6H, m, ArH), 7.53 (1H, d, *J* 8.9, ArH). Found $(M+1)^+$ 437.09804. $C_{24}H_{24}O_6S$ requires $(M+1)^+$ 437.09806.

Benzyl 3-(Benzyloxy)-5-methoxy-1-benzo[b]thiophen-2-carboxylate ***56***[44]

Benzyl alcohol (9.0 mL, 70.0 mmol) in anhydrous THF (10.0 mL) was added dropwise to a suspension of sodium hydride (60%, 2.8 g, 70.0 mmol) in anhydrous THF (40.0 mL) under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 90 min. After this time, 3-chloro-5-methoxy-1-benzo[*b*]thiophen-2-carbonyl chloride (8.0 g, 30.0 mmol) was added and the reaction mixture was heated to reflux for 17 h under an atmosphere of nitrogen. After this time, the THF was evaporated under reduced pressure to afford a solid, which was purified by column chromatography, eluting with hexane/ethyl acetate (8:2) to afford 6.07 g (49.0%) of the desired benzyl 3-(benzyloxy)-5-methoxy-1-benzo[*b*]thiophen-2-carboxylate as a yellow solid, *m*/*z* 405 $(M+1)^+$. δ_H ([D_6]DMSO) 3.88 (3H, s, OCH_3), 5.32 (2H, s, OCH_2), 5.4 (2H, s, OCH_2), 7.0–7.22 (2H, m, 2 ArH), 7.28–7.29 (3H, m, ArH), 7.29 (1H, dd, *J* 2.5, 8.9, ArH), 7.34 (1H, d, *J* 2.3, ArH), 7.37–7.44 (2H, m, ArH), 7.45–7.58 (3H, m, ArH). Found $(M+1)^+$ 405.10821. $C_{24}H_{20}O_4S$ requires $(M+1)^+$ 405.10823.

By the above method was also prepared benzyl 3-benzyloxy-6-methoxy-1-benzo[*b*]thiophen-2-carboxylate **60**, which was purified by column chromatography, with ethyl acetate/hexane (5:95 to 2:8) as eluent, to afford **60** (6.78 g, 67.4%) as an orange oil. δ_H ($CDCl_3$) 3.76 (3H, s, OCH_3), 5.27 (2H, s, CH_2), 5.4 (2H, s, CH_2), 7.03 (1H, dd, *J* 2.1, 9.0, H5), 7.18–7.45 (11H, m, 11 ArH), 7.54 (1H, d, *J* 9.0, H4).

Biological Methods

Experiments using rat or mouse tissues were approved by the University of Melbourne Animal Ethics Committee in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (Australian Government, National Health and Medical Research Council, Canberra, 2004).

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