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Original article

Synthesis and serotonergic activity of variously substituted (3-amido)phenylpiperazine derivatives and benzothiophene-4-piperazine derivatives: novel antagonists for the vascular 5-HT_{1B} receptor

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Abstract

The synthesis and vascular 5-HT_{1B} receptor activity of a novel series of substituted 3-amido phenylpiperazine and 4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene derivatives is described. Modifications to the amido linked sidechains of the 3-amidophenyl-piperazine derivatives and to the 2-sidechain of the 1-benzo[b]thiophene derivatives have been explored. Several compounds were identified which exhibited affinity at the vascular 5-HT_{1B} receptor of $pK_B > 7.0$. From the 3-amidophenyl-piperazine series, *N*-(4-(4-chlorophenyl)thiazol-2-yl-3-(4-methyl-1-piperazinyl)benzamide (**30**) and from the benzo[b]thiophene-4-piperazine series *N*-(2-ethylphenyl)-4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**38**) were identified as a highly potent, silent (as judged by the inability of angiotensin II to unmask 5-HT_{1B} receptor mediated agonist activity in the rabbit femoral artery) and competitive vascular 5-HT_{1B} receptor antagonist. The affinity of compounds from these two series of compounds for the vascular 5-HT_{1B} receptor is discussed as well as a proposed mode of binding to the receptor pharmacophore.

Keywords: Antagonist; 5-HT_{1B} receptor; Synthesis; Molecular modeling

1. Introduction

In recent times, serotonin (5-HT) receptors have been extensively investigated and classified into seven distinct receptor classes, 5-HT_1 to 5-HT_7 [1–3]. Within these classes, 14 different 5-HT receptor subtypes have been identified [1–3]. In some cases i.e. 5-ht_{1E}, 5-ht_{1F}, 5-ht_5 and 5-ht_6 only the gene products encoding putative serotonin receptor proteins have been identified and although the recombinant proteins are functionally active when transfected into a mammalian host cell, true physiological roles have not been demonstrated. For this reason, these gene products are provisionally referred to using a lower case notation [4]. The 5-HT_1 class is diverse and comprises 5-HT_{1A}, 5-HT_{1B} (formally 5-$HT_{1D\alpha}$), [5] 5-HT_{1D} (formally 5-$HT_{1D\alpha}$), [5] 5-ht_{1E} and 5-ht_{1F} subtypes. Increasing evidence has indicated that the 5-HT_{1B} receptor is likely to be the 5-HT receptor mediating vasoconstriction, but in the absence of ligands to make a definitive classification, it is referred to here as 5-HT_{1B}. The 5-HT_{1B} and 5-HT_{1D} receptors have attracted considerable attention in recent times as putative targets for novel anti-migraine drugs, leading to the development of 5-$HT_{1B/1D}$ receptor agonists such as sumatriptan (GR 43175) [6–8] and more recently zolmitriptan [9], rizatriptan, eletriptan, avitriptan and others [10,11].

Until recently, efforts to unambiguously characterise vascular 5-HT_{1B} receptors have been frustrated by the lack of

Abbreviations: DIAD, Diisopropyl azidicarboxylate; TBTU, *O*-Benzothiazol-1-yl-*N*,*N*,*N*′,*N*′,-tetramethyluronium tetrafluoroborate; DIPEA, Diisopropylethylamine; $NaCNBH_3$, Sodium cyanoborohydride; K^{t}BuO, Potassium tertiarybutoxide; CH_2O, Formaldehyde; Ph_3P, Triphenylphosphine; Et_3N, Triethylamine; TFA, Trifluoroacetic acid; Et_3N, Triethylamine; $SOCl_2$, Thionyl chloride.

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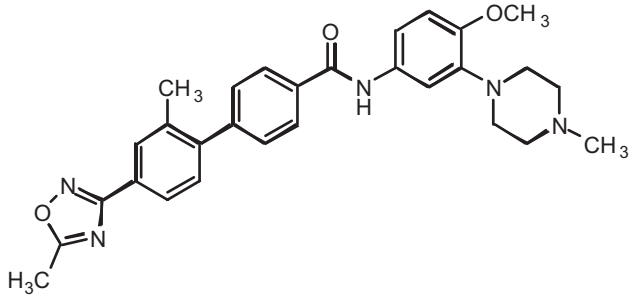
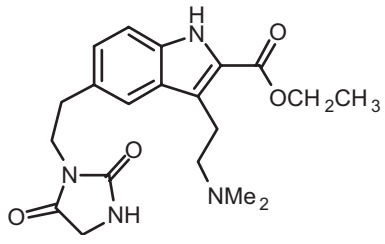
selective antagonists. A series of benzanilides [12], exemplified by GR 127935, have been described as potent antagonists at 5-HT_{1B} and 5-HT_{1D} receptors and have been shown to block both peripheral vascular and central responses mediated by both of these receptor types [13]. However, this compound is not a silent antagonist and behaves as a partial agonist at recombinant human 5-HT_{1B} and 5-HT_{1D} receptor [14] Moreover, the drug exhibits pseudo-irreversible pharmacodynamics, making it less than ideal for the quantitative study of 5-HT_{1B} and 5-HT_{1D} receptors [13].



The objective of our research program was to develop a novel, silent (as judged by the inability of angiotensin II to unmask 5-HT_{1B} receptor mediated agonist activity in the rabbit femoral artery) and highly selective antagonist at vascular 5-HT_{1B} receptors with good oral bioavailability, a plasma half-life of at least 4 h and low central penetration. Such a compound may have potential as a prophylactic treatment for unstable angina, [15,16] Raynaud's syndrome [17–20] and a variety of other vasospastic conditions in which a pathophysiological role for 5-HT has been implicated. We recently published work leading to the discovery of ethyl 3-[2-(dimethylamino)ethyl]-5-[2-(2,5-dioxo-1-imidazolidinyl)ethyl]-1H-indole-2-carboxylate (**1**) [21].



Compound **1** is a unique molecule in that it displays silent (as judged by the inability of angiotensin II to unmask 5-HT_{1B} receptor mediated agonist activity in rabbit femoral artery), competitive antagonism at the vascular 5-HT_{1B} receptor with moderate affinity and shows no significant affinity at a wide variety of G-protein coupled receptors in the periphery and/or the CNS [21]. Unfortunately, pharmacokinetic studies revealed the poor stability of the 2-ester group in animal plasma, the major metabolic by-product being the corresponding tryptamine-2-carboxylic acid derivative, which was found to be relatively inactive. **1** was shown to be rapidly metabolised by plasma esterases in lower mammals ($t_{1/2}$ = 2–10 min) but showed reasonable stability in plasma from humans, dogs and primates ($t_{1/2}$ ~ 2 h). **1** had good oral bioavailability in the conscious dog (~80%) but an elimination half-life of only 1 h. The poor pharmacokinetic properties of **1** precluded further development. Our chemical effort was therefore directed towards stable isosteres of the 2-ester group and this work lead to the development of a family of 2-*N*-benzylcarboxamido tryptamine derivatives.

A recent patent published by Merrell-Dow pharmaceuticals [22] reported a series of 1-benzo[b]thiophene-4-piperazine derivatives as high affinity vascular 5-HT_{1B} (formally 5-HT_{1D}) antagonists. These compounds lack selectivity over $5HT_{1A}$ receptors and although several had pK_B's > 7 most compounds had below average potency. Previous work carried out at The Wellcome Research Laboratories in Beckenham on a series of indole-based ligands [21,23] suggested that the long sidechain connected to the piperazine nitrogen may be the cause of the high affinity at $5HT_{1A}$. A series of 1-benzo[b]thiophene derivatives incorporating a (4-methyl-1-piperazinyl) moiety were therefore investigated incorporating a variety of sidechains connected at the benzo[b]thiophene-2-position including amido and oxadiazole linked sidechains.

We describe here the synthesis and vascular 5-HT_{1B} receptor activity of a series of (3-amido)phenylpiperazine derivatives and 4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene derivatives and related analogs. The importance of the carbonyl group and the *N*-substituent of the amide moiety for binding will be investigated. Discussion is made regarding the proposed mode of interaction of molecules of this type with the proposed pharmacophore as well explanation for the serotonergic activity of molecules of these classes.

2. Synthesis

The synthesis of the amide derivatives follows a simple method outlined in Fig. 1. An amino benzoic acid **2** derivative was reacted with the alkyl chloride **3** to form the (4-methyl-1-piperazinyl) ring derivative **4**. This (4-methyl-1-piperazinyl) benzoate derivative **4** was hydrolysed to afford the corresponding (4-methyl-1-piperiazinyl) benzoic acid derivative **5** which was amide coupled to a chosen amine derivative to produce the desired benzamide derivative **6**, Fig. 1.

All of the amines used to synthesise the compounds tested shown in Table 1 were commercially available.

The oxadiazole derivative **8** was synthesised in one step by reaction of the methyl ester derivative **7** with a previously synthesised oxime intermediate in THF in the presence of sodium hydride and 3 Å sieves, Fig. 2.

The synthesis of the benzo[b]thiophene-based derivatives followed a simple method outlined in Fig. 3.

Reaction of *N*-methyl piperazine **9** with 2,6-difluorobenzaldehyde afforded a mixture of the mono **12** and

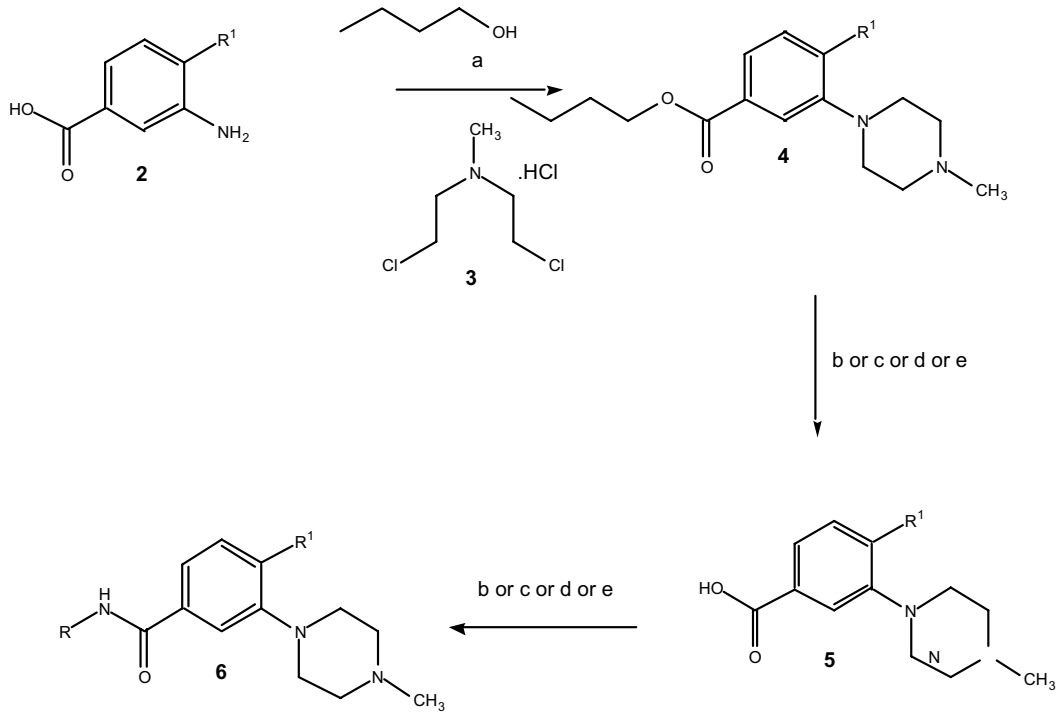


Fig. 1. Reagents: (a) *n*-butanol. (b) *O*-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyl-uronium-tetrafluoroborate, dimethylformamide, diisopropylethylamine. (c) CDI, THF. (d) *O*-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyl-uronium-tetrafluoroborate, dimethylformamide, diisopropylethylamine. (e) $C_6H_8ClN_2O_5P$, triethylamine, dichloromethane.

di-substituted piperazine **11** benzaldehyde derivatives. Condensation of **12** with ethyl-mercaptoacetate afforded the 2,4-substituted-1-benzo[b]thiophene derivative **14**. Hydrolysis with 2 N NaOH afforded the benzo[b]thiophene-2-carboxylic acid **16**. Amide coupling with the desired amine using TBTU and DIPEA in DMF afforded the desired amide **18**.

The oxadiazole derivative **22** was synthesised in one step by reaction of the ethyl ester derivative **20** with a previously synthesised oxime intermediate **21** in THF in the presence of sodium hydride and 3 Å sieves, Fig. 4.

The oxime intermediate **21** was synthesised as previously reported [24].

The amine intermediates used to form the benzo[b]thiophene-2-amides were commercially available.

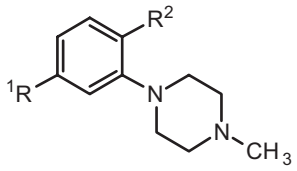
3. Results and discussion

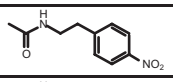
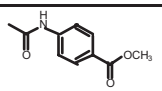
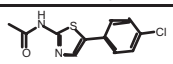
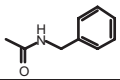
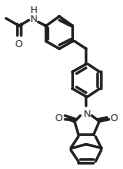
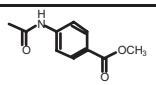
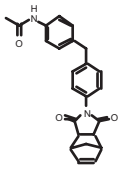
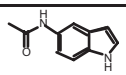
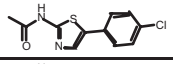
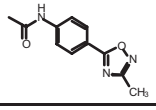
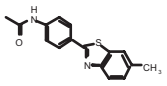
The compounds synthesised and their biological results are shown in Tables 1 and 2.

In order to explain the biological data we referred to our theoretical receptor model for the vascular 5-HT_{1B} receptor as shown in Fig. 5. The theoretical receptor model, which is composed of a protonated amine, an aromatic binding site, a hydrogen-bond acceptor site, a 'selectivity' site for 5-HT_{1B} over 5-HT_{2A}, a hydrophobic site and an additional hydrogen-bonding donor/acceptor site with associated inter-group distances was generated using systematic conformational searching of a series of analogs having a range of affinities and efficacies at both the 5-HT_{1B} and 5-HT_{2A} receptors [24]. This model proved to be qualitatively predictive for both affinity and selectivity and enabled the design of analogs having both affinity and selectivity at 5-HT_{1B} receptors. Compounds which were selective for the 5-HT_{1B} receptor over 5-HT_{2A} were found to have occupied the 'selectivity site' with some part of the molecule [24]. Additionally, due to the structural nature of the pharmacophore model, it was possible to use this model to design novel analogs (e.g. other than indole based compounds) while maintaining affinity and selectivity for the 5-HT_{1B} receptor.

The principal regions responsible for affinity are overlaid using zolmitriptan[22] another 5-HT_{1B} agonist (compound **37** from Glen et al. [24]) and methysergide. The distances between each site are shown in angstroms. Fig. 6 shows the classical 2D structures of zolmitriptan, methysergide and compound **37** (Glen et al. [24]). Methysergide (pA_{50}/α = 6.7/0.64 at 5-HT_{1B}) was one of the structures chosen with restricted conformational freedom about the ethylamine sidechain to deduce the theoretical model [24]. This was one of a larger number of structures used to deduce the relative positions of pharmacophoric groups. It is shown here as a reference structure for comparison purposes, with relevant new structures overlayed. Zolmitriptan is a selective 5-HT_{1B} agonist (pA_{50}/α = 6.8/0.77 at 5-HT_{1B}) with no substituent at the 2-position of the indole ring system. It is a good example of a tryptamine derivative, which possesses functionality that can interact with important pharmacophore binding sites. Compound **37** from Glen et al. [24], Fig. 6, represents a selective 5-HT_{1B} agonist (pA_{50}/α = 7.4/0.8 at

Table 1
Biological data for the (3-amido)phenyl-piperazine derivatives



Compound Number	R^1	R^2	5-HT_{1B} RbSV[a] (pK_B)	5-HT_{2A} RbA (pK_B)
23		OCH_3	6.97	< 5.0
24		OCH_3	6.85	< 5.0
25		OCH_3	8.25	< 5.0
26		OCH_3	6.19	< 5.0
27		OCH_3	6.41	< 5.0
7		H	5.96	< 5.0
28		H	6.06	< 5.0
29		H	6.71	5.22
30		H	7.16	5.0
8		H	6.71	< 5.0
31		H	6.27	5.0

[a] Affinity (pK_B: $-\log_{10}K_B$, the dissociation equilibrium constant) estimates for novel compounds at the vascular 5-HT_{1B} and 5-HT_{2A} receptors in the rabbit saphenous vein (RbSV) and aorta (RbA), respectively. Affinity values are the means of at least three separate estimates. Standard errors are omitted for clarity, but in all cases were ≤ 0.2 $\log_{10}$ units. In each case, affinity estimates were determined using the Gaddum-Schild equation and 5-HT as the receptor agonist.

5-HT_{1B}) which can also interact with the binding sites of the theoretical 5-HT_{1B} receptor model including the secondary binding sites, the hydrogen bonding donor/acceptor sites and hydrophobic binding site [24].

With several notable exceptions the affinity of this series of phenyl piperazine derivatives for the vascular 5-HT_{1B} receptor was below our desired level. One compound from the methoxy substituted series **25** has an amido phenylthiazole sidechain and has excellent affinity at the 5-HT_{1B} receptor (pK_B = 8.25). The proposed mode of binding for **25** is shown in Fig. 7.

Molecular modelling studies indicate that the carbonyl of the amide group cannot interact with our proposed hydrogen-bonding site. The sulfur of the thiazole group however can

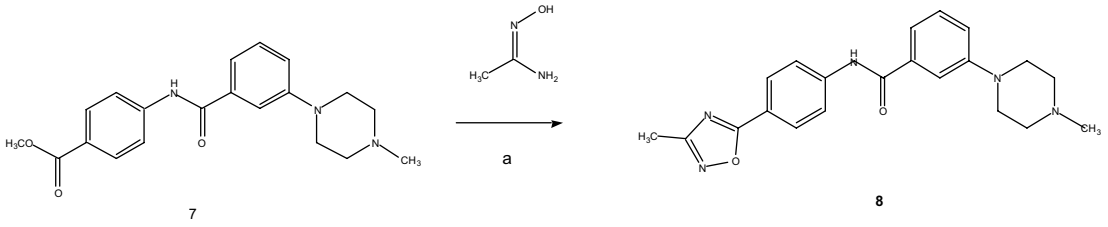


Fig. 2. Reagents: (a) 3 Å sieves, NaH, THF.

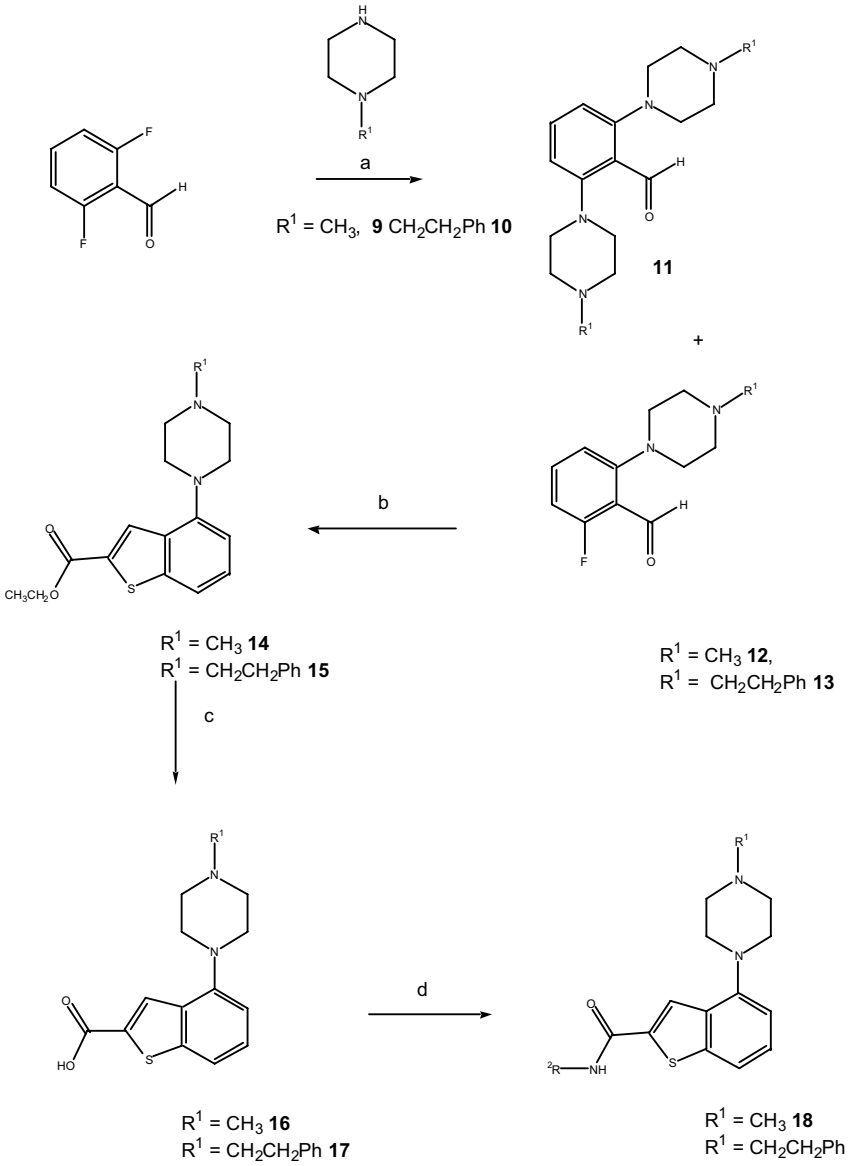


Fig. 3. Reagents: (a) K_2CO_3, DMF. (b) $HSCH_2CO_2Et$. (c) 2 N NaOH, THF. (d) DMF, DIPEA, TBTU, R^2NH_2.

interact and positions the attached phenyl group into a proposed hydrophobic binding site, (Fig. 3). **25** is currently being tested in the RbFA and based on previous results for 2-methoxy phenyl piperazine derivatives would be expected to be a low efficacy agonist. However incorporation of an electron withdrawing reverse amide linking group may have the effect of nullifying the effect of the electron donating 2-methoxy group and producing a silent (as judged by the inability of angiotensin II to unmask 5-HT_{1B} receptor mediated agonist activity in the rabbit femoral artery) compound.

Compounds **24, 26, 27** and **32** all have low affinity for the 5-HT_{1B} receptor. These results provide further confirmation that a hydrogen-bond acceptor is required in the sidechain for good affinity.

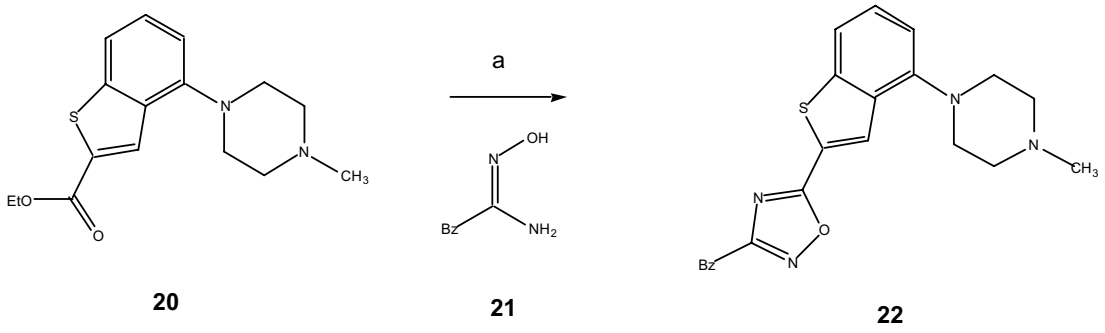


Fig. 4. Reagents: (a) 3 Å sieves, THF, NaH (60%).

3.1. Des-methoxy amide derivatives

Given the low efficacy agonism detected for the 2-methoxy phenyl piperazine derivatives with an amide-linked sidechain at the 5-position, a series of des-methoxy amides were investigated, Table 1.

A trend of lower affinity has been observed for the des-methoxy derivatives compared with the corresponding 2-methoxy derivatives. As expected **7** (pK_B = 5.96) and 28 (pK_B = 6.06) had low affinity for the 5-HT_{1B} receptor. **7** also has a short sidechain and the phenyl ring is unable to access the proposed hydrophobic pocket. The biphenyl sidechain of **28** may be too flexible and adopt unfavourable conformations.

Compound **29** with a 5-indole amide sidechain had a slightly higher potency (pK_B = 6.71) however it is still below our desired affinity of pK_B = 7.0.

Compound **30** which is a des-methoxy analogue of **25** has a phenyl substituted thiazole group attached to the amide linking group has good affinity for the 5-HT_{1B} receptor (pK_B = 7.16). Fig. 8 shows how the five membered thiazole ring orientates so as to allow the phenyl group to position in the proposed hydrophobic binding site.

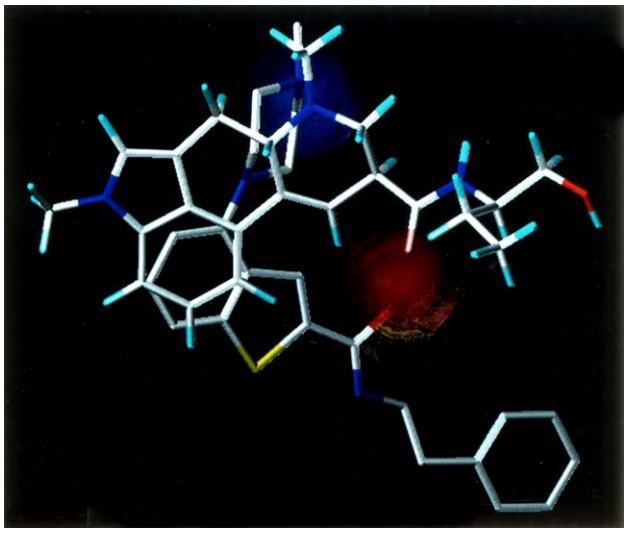


Fig. 9. Proposed conformation of *N*-(2-ethylphenyl)-4-(*N*-methyl)piperazino-1-benzo[b]thiophene-2-carboxamide (**38**) fitted to the pharmacophore model with methysergide as background. The blue sphere represents the binding site for the piperazine nitrogen and the red sphere represents the hydrogen bonding site.

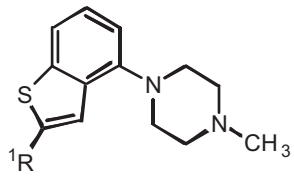
Compound **30** proved to be silent (as judged by the inability of angiotensin II to unmask 5-HT_{1B} receptor mediated agonist activity in the rabbit femoral artery) in the RbFA assay consistent with other des-methoxy phenyl piperazine derivatives. Compound **30** is currently undergoing further investigation.

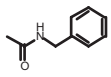
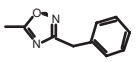
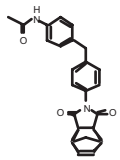
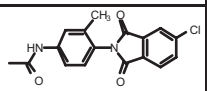
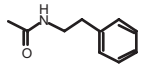
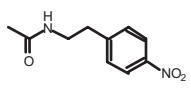
From the benzothiophene-4-piperazine series of compounds shown in table 2.0 the most potent antagonist was *N*-(2-ethylphenyl)-4-(*N*-methyl)piperazino-1-benzo[b]thiophene-2-carboxamide (**38**) and the proposed conformation this molecule adopts to fit the pharmacophore model is illustrated in Fig. 9. The nitrogen of the piperazin ring interacts with the receptor as indicated allowing the rest of the molecule to adopt a conformation allowing the carbonyl group of the benzothiophene-2-carboxamide to interact with the hydrogen bonding site.

4. Conclusion

In this study we have identified a novel series of 3-(*N*-methyl) piperazinobenzamide derivatives and 4-(*N*-methyl)piperazino-1-benzo[b]thiophene derivatives as silent (as judged by the inability of angiotensin II to unmask 5-HT_{1B} receptor mediated agonist activity in the rabbit femoral artery) antagonists of the vascular 5-HT_{1B} receptor. Several compounds including **25** and **30** were discovered to have good 5-HT_{1B} receptor affinity **25** (pK_B = 8.25), **30** (pK_B = 7.16). Several 1-benzo[b]thiophene based derivatives including compounds **34** and **38** were discovered to have good 5-HT_{1B} receptor affinity **34** (pK_B = 7.22) and **38** (pK_B = 7.78). These results were encouraging because they further supported our claims that we can design compounds which are not tryptamine-based compounds such as 4-(*N*-methyl) piperazino-1-benzo[b]thiophene derivatives which possess functionality which are able to interact with important binding sites within the 5-HT_{1B} receptor can be developed as efficient 5-HT_{1B} receptor antagonists. Compounds such as **25, 30, 34** and **38** will prove to be useful pharmacological probes for the vascular 5-HT_{1B} receptor.

Table 2
Biological data of benzo[b]thiophene-4-piperazine derivatives



Compound Number	R^1	$5HT_{1B}$[a] RbSV (pK_B)	$5HT_{2A}$ RbA (pK_B)
33	CO_2H	5.34	< 5.0
34		7.22	< 5.0
35		7.59	< 5.0
36		6.79	< 5.0
37		6.9	5.0
38		7.78	< 5.0
39		8.99 non-surmountable	< 5.0

[a] Affinity (pK_B: $-\log_{10}K_B$, the dissociation equilibrium constant) estimates for novel compounds at the vascular 5-HT_{1B} and 5-HT_{2A} receptors in the rabbit saphenous vein (RbSV) and aorta (RbA), respectively. Standard errors are omitted for clarity, but in all cases were ≤ 0.2 $\log_{10}$ units. In each case, affinity estimates were determined using the Gaddum-Schild equation and 5-HT as the receptor agonist.

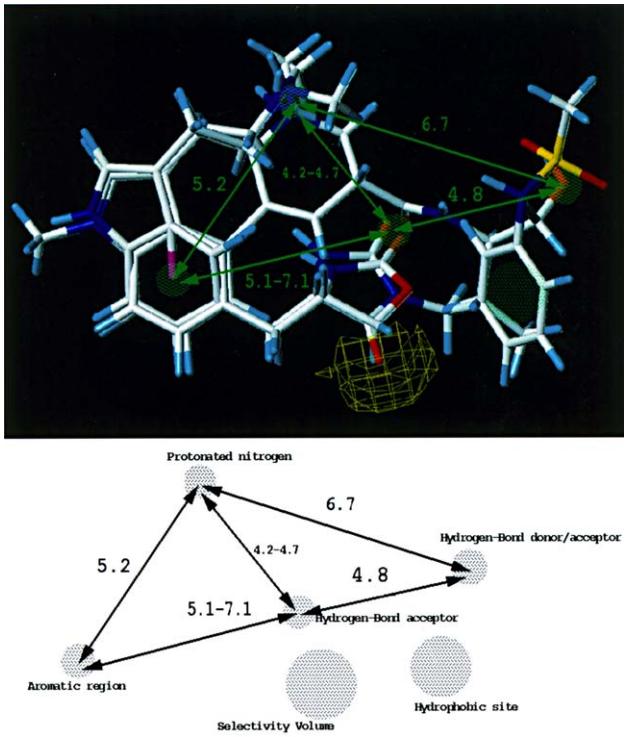


Fig. 5. Theoretical 5-HT_{1B} receptor model using zolmitriptan [24] and compound **37** from Glen et al. [24] as references and with methysergide as background.

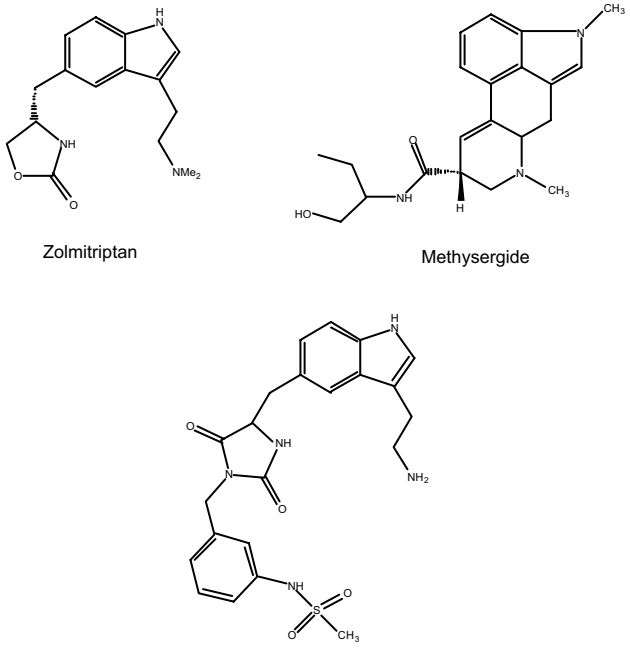


Fig. 6. Compound **37** from Glen et al. [24].

5. Experimental

5.1. Biological methods

Definition; 'Intrinsic activity': the maximum effect of the test agonist relative to a standard (usually a full agonist).

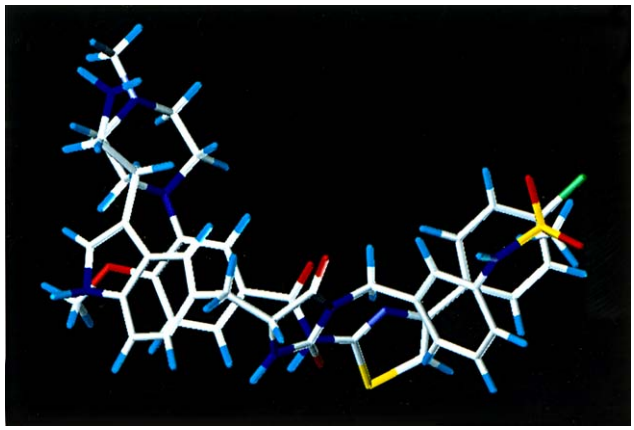


Fig. 7. Monoview of the preferred mode of binding for 3-(4-methyl-1-piperazinyl-4-methoxy-*N*-(4-chlorophenyl)-2-thiazole) benzamide (**25**) overlaid on compound **37** from Glen et al. [24].

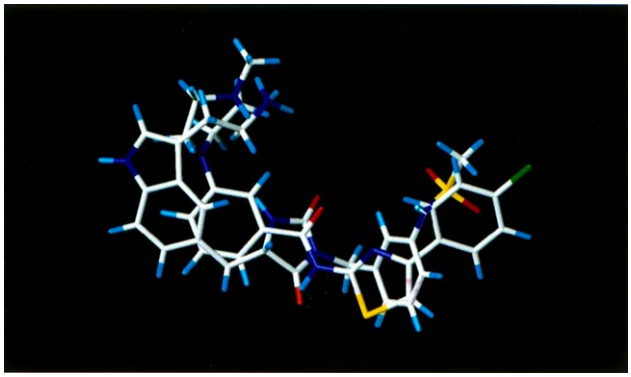


Fig. 8. Monoview of the preferred mode of binding of 3-(4-methyl-1-piperazinyl)-*N*-(4-(4-chlorophenyl)-2-thiazole) benzamide (**30**) overlapped with compound **37** from Glen et al. [24]. The amide carbonyl oxygen is able to interact with the hydrogen bonding site and the phenyl ring can orientate so as to allow the π electrons are available to interact with a secondary hydrogen bonding site.

5.1.1. Rabbit saphenous vein (RbSV) preparation

The vascular 5-HT_{1B} receptor affinities of compounds were assessed using ring preparations of rabbit saphenous vein [25]. Vessels were removed from male New Zealand White rabbits killed by injecting pentobarbitone (80 mg/kg, i.v.) followed by exsanguination. After removing adhering connective tissue, ring segments (4–5 mm) were prepared and mounted between parallel tungsten wires. Tissues were suspended in 20 ml organ baths containing Krebs-Henseleit buffer at 37 °C, pH 7.4 and constantly gassed with 95% O_2 / 5% CO_2. The Krebs-Henseleit solution used had the following composition: (mM) NaCl 118.41, $NaHCO_3$ 25.00, KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ 1.19, glucose 11.10 and $CaCl_2$ 2.50. After application of a passive force (2 g) tissues were exposed to pargyline (500 μM) to inactivate monoamine oxidase. In order to prevent the direct or indirect activation of α_1-adrenoreceptors, saphenous veins were simultaneously exposed to phenoxybenzamine (0.3 μM). After 30 min excess inhibitors were removed by several exchanges of the organ bath buffer and the tissues challenged with 5-HT (1 μM) to determine viability. In the saphenous vein a cumulative

concentration-effect (*E*/[*A*]) curve to 5-HT was constructed followed by washout and after 60 min recovery, by a second curve to the test compound. When the test compound failed to produce agonism, it was evaluated as a 5-HT antagonist, potency being determined as an apparent pK_B. When the test produced vascular contraction, potency estimates were determined as p$[A]_{50}$ and intrinsic activity (α) values determined from the ratio Test maximum response / 5-HT maximum.

5.1.2. Rabbit femoral artery (RbFA) preparation

Rings (2 mm) of rabbit femoral artery were exposed to pargyline (500 μM) for 30 min during which time they were progressively tensioned to 2.6 g. The tissues were exposed to 80 mM KCl to assess tissue viability and provide a reference contracture for subsequent data analysis. After washout, angiotension II was titrated to provide contraction equivalent to ~45% of the KCl response. Once this was achieved a cumulative *E*/[*A*] curve to the novel compound (or 5-HT as a reference) was constructed to determine vascular 5-HT_{1B} agonist activity. Krebs solution containing prazosin, mepyramine and spiperone (0.3 μM of each) was used throughout to block possible effects mediated by α_1 adrenergic, H_1 histaminergic and 5-HT_{2A} serotonergic receptor activation, respectively.

5.1.3. Rabbit aorta (RbA) preparation

Rings (3 mm) of rabbit thoracic aorta were exposed to pargyline (500 μM) for 30 min during which they were tensioned twice to 3.0 g. Exposure to L-phenylephrine (L-Phe, 10 μM) enabled tissue viability to be assessed and provided a reference contracture for subsequent data analysis. Following washout tissues were exposed to novels (30 μM) for 60 min prior to a cumulative *E*/[*A*] curve to L-Phe being constructed.

6. Chemical methods: general directions

Computational chemistry was performed on a Silicon Graphics Iris indigo II using the Sybyl [26] molecular modelling software.

Unless otherwise stated, all ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer or at 300 MHz on a Bruker AM 300 spectrometer. Chemical shifts are in δ ppm relative to TMS. Deuterated dimethylsulphoxide (99.9%) was used as solvent unless otherwise stated. Mass spectra and high resolution mass spectra (HRMS) were obtained on a Kratos Concept IS (EIMS), a Kratos MS50 (FAB) mass spectrometer or a Joel JMX DX-300 double focussing instrument. Melting points were determined on a Gallencamp melting point apparatus and are uncorrected. Methanol and ethanol were distilled from iodine and magnesium and stored over type 3 Å molecular sieves. Anhydrous THF was freshly distilled over potassium and benzophenone. Anhydrous DMF, ether and toluene were stored over type 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}). Infra red spectra were run in KBr disks on a Bruker IFS66 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 Millenium system comprising a 490E Multiwavelength detector, 600 Controller, a series 600 pump with a 717 Plus autosampler. A Zorbax 4.6 × 250 mm, 5 μm column was used for analytical work while a 22.4 × 250 mm, 7 μm C18 column was used for preparative work. A 10% H_2O/AcCN (10–90% gradient elution) (A) 0.1 M NH_4OAc (pH 4) (90–10%) (B) solvent system was used.

6.1. N-*[4-(3-Methyl-1,2,4-oxadiazol-5-yl)phenyl]-3-(4-methylpiperazino) benzenecarboxamide (**8**)*

N-Hydroxyacetamidine (19.0 mg, 0.25 mmol) was added to a suspension of powdered 3 Å sieves in anhydrous THF (3.0 ml) under an atmosphere of nitrogen. After 15 min sodium hydride (60% dispersion) (20.5 mg, 0.51 mmol) was added and stirring was continued for further 40 min. 3-*N*-(methyl)piperazine-*N*-(methyl-4-benzoate)benzamide (50.0 mg, 0.13 mmol) dissolved in anhydrous THF (3.0 ml) was added and the reaction mixture was refluxed for 2 h. The reaction mixture was cooled, filtered through a bed of celite and evaporated to dryness under reduced pressure. The residue was partitioned between dichloromethane and water (basified) and the aqueous phase was extracted three times (3 × 20.0 ml). The combined organic phase was washed with water (3 × 20 ml), dried, filtered and evaporated under reduced pressure afford (34.3 mg, 70.8%) of the desired *N*-[4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl]-3-(4-methylpiperazino) benzenecarboxamide as a white powder. The free base was converted to the hydrochloride salt which was freeze dried to afford the desired *N*-[4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl]-3-(4-methylpiperazino) benzenecarboxamide (**8**) as a white lyophylate. M.S. *m/z* 378 $(M + 1)^+$ ^{1}H NMR δ 2.39 (3H, s, CH_3), 2.85–3.22 (8H, m, 4 × CH_2), 7.25 (1H, d, ArH, J = 7.2 Hz), 7.26–7.51 (3H, m, 3 × ArH), 5.35 (2H, s, CH_2O), 7.12 (1H, d, H6, J = 8.7 Hz), 7.3–7.5 (7H, m, H-7, H-4, 5 × ArH), 8.02 (2H, d, 2 × ArH, J = 9.0 Hz), 8.09 (2H, d, 2 × ArH, J = 8.7 Hz). Found $(M)^+$ = 377.18514 $C_{21}H_{23}N_5O_2$·HCl requires $(M)^+$ = 377.18515. t.l.c. (SiO_2) R_f = 0.35 (dichloromethane/ethanol/ammonia) (100/8/1) one component.

*6.2. Butyl-3-(4-methyl-1-piperazinyl)-4-methoxybenzoate (**19**)*

3-Amino-4-methoxybenzoic acid (4.5 g, 27.0 mmol) was dissolved in *n*-butanol (70.0 ml) and 2-chloro-*N*-(2-chloroethyl)-*N*-methylethanamine (5.18 g, 27.0 mmol) was added and the reaction mixture was heated to reflux for 20 h.

The reaction mixture was cooled and anhydrous sodium carbonate (1.43 g, 13.5 mmol) was added and the reaction mixture was heated to reflux overnight. The reaction mixture was cooled and the solvent was evaporated under reduced pressure and water (135.0 ml) and 2 N HCl (127.5 ml) was added and the aqueous phase was extracted with ethyl acetate and the aqueous phase was basified with sodium bicarbonate and re-extracted with ethyl acetate, dried, filtered and evaporated under reduced pressure to afford a black/brown oil, which was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (400/8/1) to afford the desired butyl-3-(*N*-methylpiperazino-4-methoxybenzoate (**19**) as a viscous yellow oil. M.S. *m/z* 307 $(M+1)^+$ ^{1}H NMR (D_2O) δ 0.92 (3H, t, CH_3, J = 7.3 Hz), 1.41 (2H, m, CH_2), 1.66 (2H, m, CH_2), 2.48 (4H, m, 2 × CH_2), 2.956 (4H, 2 × CH_2), 3.84 (3H, s, N–CH_3), 4.22 (3H, t, CH_2, J = 6.54 Hz), 7.03 (1H, d, H-2, J = 8.55 Hz), 7.41 (1H, d, H-5, J = 2.0 Hz), 7.59 (1H, dd, H-3, J = 2.0 Hz, J = 8.5 Hz).

*6.3. Butyl (4-methyl-3-piperazinyl)benzoate (**20**)*

3-Amino benzoic acid (2.19 g, 0.16 mmol) was added to *n*-butanol (40.0 ml) and 2-chloro-*N*-(2-chloroethyl)-*N*-methylethanamine (3.07 g, 0.16 mmol) was also added. The suspension was heated to reflux for 20 h. Sodium carbonate (840.0 mg, 7.9 mmol) was then added and the suspension was heated to reflux for a further 12 h. The reaction mixture was cooled and stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the resulting residue was taken up in water (40.0 ml) and 2 N HCl (80.0 ml) and the aqueous phase was extracted with ethyl acetate and the ethyl acetate phase was dried, filtered and the ethyl acetate was evaporated under reduced pressure to afford a black oil, which was purified with column chromatography eluting with (dichloromethane/ethanol/ammonia) (300/8/1) to (100/8/1) to afford (958.0 mg, 21.7%) of the desired butyl (4-methyl-3-piperazinyl)benzoate (**20**) as a brown oil. M.S. *m/z* 277 $(M + 1)^+$ ^{1}H NMR (DMSO-d_6) δ 0.92 (3H, t, CH_3, J = 7.2 Hz), 1.38 (2H, m, CH_2), 1.67 (2H, m, CH_2), 1.89 (2H, m, CH_2), 2.21 (3H, s, CH_3), 2.46 (4H, m, 2 × CH_2), 3.31–3.44 (4H, m, 2 × CH_2), 4.23 (2H, t, CH_2, J = 6.2 Hz), 7.21–7.43 (4H, m, 4 × ArH).

*6.4. 3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid (**21**)*

Butyl-3-(4-methyl-1-piperazinyl)-4-methoxybenzoate (1.69 g, 5.5 mmol) was dissolved in methanol (11.0 ml) and 1 N NaOH (22.0 ml) was added and the reaction mixture was heated to reflux for 5 h. The reaction mixture was cooled and water was added and the aqueous phase was extracted with diethyl ether, then the aqueous phase was acidified to pH 2 with concentrated hydrochloric acid and re-extracted with diethyl ether, dried, filtered and evaporated under reduced pressure to afford a white solid, which was triturated with ethanol to afford (882.0 mg, 63.6%) of the desired 3-(*N*-methylpiperazino-4-methoxybenzoic acid (**21**) as a white powder. M.S. *m/z* 251 $(M + 1)^+$ ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s, OCH_3), 2.95 (4H, m, 2 × CH_2), 3.03 (7H, m, 2 × CH_2, CH_3), 7.22 (1H, d, ArH, J = 7.9 Hz), 7.32–7.41 (2H, m, 2 × ArH), 7.47 (1H, m, OH). Found $(M)^+$ = 250.13171 $C_{13}H_{18}N_2O_3$·HCl requires $(M)^+$ = 250.13172.

*6.5. 3-(4-Methyl-1-piperazinyl)benzoic acid (**22**)*

Butyl (4-methyl-3-piperazinyl)benzoate (950.0 mg, 3.47 mmol) was dissolved in methanol (7.0 ml) and 1 N aqueous NaOH (14.0 ml) was added. The resulting solution was refluxed for 24 h. The reaction mixture was cooled and water (140.0 ml) was added and the aqueous layer extracted with diethyl ether (3 × 100.0 ml). The organic layer was dried, filtered and the diethyl ether was discarded as TLC indicated all the desired product was in the water phase, which was evaporated under reduced pressure and the resulting solid was dried in the presence of phosphorus pentoxide to afford (523.0 mg, 68.0%) the desired 3-(4-methyl-1-piperazinyl) benzoic acid (**22**) as a white powder. m.p. = 220 °C (decomposed). M.S. *m/z* 220 $(M)^+$ ^{1}H NMR δ ($CDCl_3$) 2.86 (3H, s, NCH_3), 3.06–3.24 (4H, m, 2 × CH_2), 3.46 (2H, m, CH_2), 3.80–3.87 (2H, m, CH_2), 7.2–7.5 (5H, m, 4 × ArH, NH^+).

*6.6. Methyl 4-{[3-(4-methyl-1-piperazinyl)benzoyl]amino} benzenecarboxylate (**7**)*

A mixture of the ethyl 4-aminobenzoate hydrochloride (402.0 mg, 2.15 mmol) and 3-(4-methylpiperazino)benzoic acid (500.0 mg, 1.95 mmol) were dissolved in anhydrous DMF (10.0 ml) and TBTU (686.0 mg, 2.15 mmol) was added and the reaction mixture was stirred at room temperature for 10 min. Diisopropylethylamine (302.0 mg, 2.34 mmol) was then added and the reaction mixture was stirred at room temperature for 18 h. The solution was concentrated under reduced pressure to afford a gum, which was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (300/8/1) to (60/8/1) to afford (70.0 mg, 90.0%) of the desired methyl 4-{[3-(4-methylpiperazino) benzoyl]amino} benzene carboxylate (**7**) as a powder which was converted to the hydrochloride salt to afford the desired methyl 4-{[3-(4-methylpiperazino) benzoyl]amino} benzenecarboxylate (**7**) as a white lyophylate after freeze–drying. M.S. *m/z* 335 $(M + 1)^{+.1}$H NMR δ 2.0 (6H, s, 2 × NCH_3), 2.2 (2H, m, CH_2Nme_2), 2.98 (4H, m, 5-CH_2, 3-CH_2), 3.83 (2H, t, CH_2Phth, J = 7.0 Hz), 5.35 (2H, s, CH_2O), 7.12 (1H, d, H6, J = 8.7 Hz), 7.3–7.5 (7H, m, H7, H4, 5 × ArH), 7.79 (4H, s, 4 × PhthH), 11.45 (1H, s, NH). t.l.c (SiO_2) R_f = 0.14 (dichloromethane/ethanol/ammonia) (150/8/1) one component.

6.7. N-*(2-(4-Nitrophenyl)ethyl)-3-(4-methyl-1-piperazinyl)-4-methoxybenzamide (**23**)*

3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid hydrochloride (50.0 mg 0.17 mmol) was dissolved in anhy-

drous DMF (2.0 ml) and 4-nitrophenethylamine (31.8 mg, 0.19 mmol) and TBTU (61.6 mg, 0.19 mmol) was added and the solution stirred for 10 min. Diisopropylethylamine (72.9 µl, 0.42 mmol) was added to the mixture and the solution was stirred over night at room temperature. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (400/8/1) to afford (43.6 mg, 56.5%) of the desired *N*-(2-(4-nitrophenyl)ethyl-3-(4-methyl-1-piperazinyl)-4-methoxybenzamide (**23**) as a yellow powder. The free base was converted into the hydrochloride salt, which was freeze dried to afford the desired *N*-(2-(4-nitrophenyl)ethyl-3-(4-methyl-1-piperazinyl)-4-methoxybenzamide hydrochloride (**23**) as a yellow lyophylate. M.S. *m/z* 399 $(M + 1)^{+\cdot}$ $_1$H NMR δ 2.95–3.18 (9H, m 4 × CH_2, CH), 3.49 (2H, dd, CH_2, J = 6.1 Hz, J = 12.9 Hz), 3.62 (1H, m, CH), 3.82 (3H, s, OCH_3), 6.98 (1H, d, ArH, J = 8.5 Hz), 7.31 (1H, d, ArH, J = 1.9 Hz), 7.46 (1H, dd, ArH, J = 1.8 Hz, J = 8.5 Hz), 7.51 (1H, d, ArH, J = 8.7 Hz), 8.15 (1H, d, ArH, J = 8.7 Hz), 8.37 (1H, t, NH, J = 5.5 Hz). Found C, 53.85, H, 6.41, N, 11.65%, $C_{21}H_{26}N_4O_4$·HCl requires C, 53.55, H, 6.64, N, 11.89%.

*6.8. Methyl 4-{(4-methoxy-3-(4-methylpiperazino) benzoyl]amino} benzenecarboxylate (**24**)*

3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid hydrochloride (250.0 mg, 0.9 mmol) was dissolved in anhydrous DMF (10.0 ml) and methyl (4-amino) benzoate (144.9 mg, 0.96 mmol) and HBTU (363.5 mg, 0.96 mmol) was added and the solution stirred for 10 min. Diisopropylamine (546.7 µl) was added to the mixture and the solution was stirred over night at room temperature and under an atmosphere of nitrogen for 3 h. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (91.8 mg, 10.0%) of the desired methyl 4-{(4-methoxy-3-(4-methylpiperazino)benzoyl]amino} benzenecarboxylate (**24**) as a yellow powder. The free base was converted into the hydrochloride salt, which was freeze-dried to afford the desired methyl 4-{(4-methoxy-3-(4-methylpiperazino)benzoyl]amino} benzene carboxylate hydrochloride (**24**) as a white lyophylate. M.S. *m/z* 384 $(M + 1)^{+.1}$H NMR δ 2.48–3.3 (8H, m, 4 × CH_2), 3.83 (3H, 2, NCH_3), 3.87 (3H, s, OCH_3), 7.1 (1H, d, ArH, J = 8.73 Hz), 7.48 (1H, s), 7.71 (1H, d, ArH, J = 8.1 Hz), 7.89–7.96 (4H, m, 4 × ArH), 10.36 (1H, s, NH). Found C, 54.69, H, 6.96, N, 9.12%, $C_{21}H_{25}N_3O_4$·HCl 2.25 H_2O requires C, 54.78, H, 6.68, N, 9.13%.

6.9. N-*{[5-(4-Chlorophenyl)-1,3-thiazol-2-yl]methyl}-4-methoxy-3-(4-methylpiperazino)benzenecarboxamide (**25**)*

3-(4-Methyl)-4-methoxybenzoic acid hydrochloride, (107.8 mg 0.37 mmol) was dissolved in anhydrous DMF (4.0 ml) and 5-(4-chlorophenyl)-2-amino-1,3-thiazine (83.8 mg, 0.4 mmol) and HBTU (150.7 mg, 0.4 mmol) was added and the solution stirred for 10 min. Diisopropylethylamine (151.2 µl, 0.87 mmol) was added to the mixture and the solution at room temperature and under an atmosphere of nitrogen for 3 h. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (58.6 mg, 35.2%) of the desired *N*-[5-(4-chlorophenyl)-1,3-thiazol-2-yl]-4-methoxy-3-(4-methylpiperazino)benzenecarboxamide (**25**) as a yellow powder. h.p.l.c. retention time = 16.72 min (Gradient elution 0.2% TFA/CH_3CN·TFA/H_2O over 20 min. C-8 column. M.S. *m/z* 443 $(M + 1)^{+.1}$H NMR δ 2.2–2.3 (2H, m, CH_2), 2.93–3.1 (6H, m, 3 × CH_2), 4.24 (3H, s, NCH_3), 4.27 (3H, s, OCH_3), 7.08 (1H, d, ArH, J = 8.4 Hz), 7.49 (2H, d, ArH, J = 8.5 Hz), 7.81 (1H, d, ArH, J = 6.2 Hz), 12.64 (1H, s, NH). Found C, 53.23, H, 6.25, N, 12.0%, $C_{20}H_{24}N_4O_4$·HCl 1.75 H_2O requires C, 53.09, H, 6.35, N, 12.38%.

6.10. N-*Benzyl-4-methoxy-3-(4-methylpiperazino) benzamide (**26**)*

3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid hydrochloride (107.2 mg, 0.37 mmol) was dissolved in anhydrous DMF (5.0 ml) and 4-benzylamine (41.1 mg, 0.38 mmol) and TBTU (123.1 mg, 0.38 mmol) were added and the solution stirred for 10 min. Diisopropylethylamine (146.0 µl, 0.84 mmol) was added to the mixture and the solution was stirred at room temperature and under an atmosphere of nitrogen overnight. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (117.0 mg, 96.0%) of the desired *N*-benzyl-3-(4-methyl-1-piperazinyl)-4-methoxybenzamide (**26**) as a yellow powder. The free base was converted into the hydrochloride salt, which was freeze dried to afford the desired amide *N*-benzyl-4-methoxy-3-(4-methyl-1-piperazinyl)benzamide (**26**) as a white lyophylate after freeze-drying. MS *m/z* 340 $(M + 1)^{+.1}$H NMR (DMSO-d_6) δ 1.23 (3H, s, NCH_3, J = 6.60 Hz), 2.37 (2H, br s, CH_2), 2.68 (2H, br s, CH_2), 3.29 (4H, br s, 2 × CH_2), 4.44 (2H, d, CH_2, J = 5.9 Hz), 6.99 (1H, d, ArH, J = 8.5 Hz), 7.2–7.36 (5H, m, 5 × ArH), 7.43 (1H, s, ArH), 7.57 (1H, dd, ArH, J = 1.9 Hz, J = 8.5 Hz), 8.85 (1H, t, NH, J = 5.9 Hz). Found $(M)^+$ = 339.19466 $C_{20}H_{25}N_3O_2$ requires $(M)^+$ = 339.19466. t.l.c. (SiO_2) R_f = 0.57 (CH_2Cl_2/EtOH/NH_3 (60/8/1) one component.

6.11. N-*{4-[4-(3,5-Dioxo-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)benzyl]phenyl}-4-methoxy-3-(4-methylpiperazino) benzenecarboxamide (**28**)*

3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid hydrochloride (150.0 mg 0.52 mmol) was dissolved in anhydrous DMF (10.0 ml). 5-(4-Methylenephenylnorbornane (199.0 mg, 0.57 mmol) and HBTU (218.0 mg, 0.57 mmol)

were added and the solution stirred for 10 min. Diisopropylethylamine (81.0 mg, 0.63 mmol) was added to the mixture and the solution was stirred at room temperature and under an atmosphere of nitrogen overnight. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (166.0 mg, 55.1%) of the desired *N*-{4-[4-(3,5-dioxo-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)benzyl]phenyl}-4-methoxy-3-(4-methylpiperazino)benzenecarboxamide (**27**) as a yellow powder. M.S. *m/z* 577 $(M + 1)^{+.}$ ^{1}H NMR (DMSO-d_6) δ 1.58 (2H, s), 2.56 (4H, m, 2 × CH_2), 3.04 (4H, m, 2 × CH_2), 3.45 (2H, m, 2 × CH), 3.85 (3H, s, CH_3), 3.92 (2H, s, CH_2), 6.19 (2H, m, 2 × CH), 6.99–7.05 (3H, m, 3 × ArH), 7.19 (2H, d, 2 × ArH, *J* = 8.5 Hz), 7.27 (2H, d, 2 × ArH, *J* = 8.4 Hz), 7.44 (1H, d, ArH, *J* = 2.1 Hz), 7.6–7.67 (3H, m, 3 × ArH), 9.96 (1H, s, NH). Found $(M)^+$ = 576.27361 $C_{35}H_{36}N_4O_4$ requires $(M)^+$ = 576.27362.

6.12. N-*(4-Carboxymethylphenyl)-3-(4-methyl-1-piperazinyl) benzamide (**7**)*

4-Methoxy-3-(4-methyl-1-piperazinyl)benzoic acid hydrochloride (252.4 mg, 0.9 mmol) was dissolved in anhydrous DMF (10.0 ml) and methyl 4-amino benzoate hydrochloride (180.6 mg, 1.0 mmol) and HBTU (364.0 mg, 0.96 mmol) were added and the reaction mixture was stirred at room temperature for 30 min. Diisopropylethylamine (546.7 μl, 3.14 mmol) was added and stirring was continued for a further 3 h. The DMF was evaporated under reduced pressure and the resultant residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (250/8/1) to (60/8/1) to afford (32.5 mg, 9.7%) of the desired *N*-(4-carboxymethylphenyl)-3-(4-methyl-1-piperazinyl) benzamide (**7**). M.S. *m/z* 384 $(M + 1)^+$ ^{1}H NMR δ 2.64–3.12 (8H, m, 4 × CH_2), 3.83 (3H, s, NCH_3), 3.87 (3H, s, OCH_3), 7.1 (1H, d, ArH, *J* = 8.7 Hz), 7.48 (1H, s, ArH), 7.71 (1H, d, ArH, *J* = 8.7 Hz), 7.89–7.96 (4H, m, 4 × ArH), 10.36 (1H, s, NH).

6.13. N-*{4-[4-(3,4-Dioxo-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)benzyl]phenyl}-3-(4-methylpiperazino) benzenecarboxamide (**28**)*

3-(4-Methyl-1-piperazinyl) benzoic acid hydrochloride (250.0 mg 0.98 mmol) was dissolved in anhydrous DMF (7.0 ml). *N*-[4-(4-Aminobenzyl) phenyl]-5-norbornene-2,3-dicarboximide (369.0 mg, 1.0 mmol) and TBTU (343.0 mg, 1.0 mmol) were added and the solution stirred for 10 min. Diisopropylethylamine (151.0 mg, 1.2 mmol) was added to the mixture and the solution was stirred at room temperature and under an atmosphere of nitrogen overnight. The DMF was evaporated under reduced pressure and water (50.0 ml) was added and the aqueous phase was basified to pH 10 with potassium carbonate and was then extracted with ethyl acetate, dried, filtered and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to (100/8/1) to (60/8/1) to afford the desired *N*-{4-[4-(3,4-dioxo-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)benzyl]phenyl}-3-(4-methylpiperazino) benzenecarboxamide (**28**) as a yellow powder. M.S. *m/z* 547 $(M + 1)^{+.}$ ^{1}H NMR δ 2.81 (3H, s, CH_3), 3.16–3.47 (8H, m, 4 × CH_2), 3.93 (4H, s, Norbornene aliphatics), 6.2 (2H, d, *J* = 1.8 Hz), 7.0 (2H, d, *J* = 8.4 Hz), 7.2 (2H, d, *J* = 8.4 Hz), 7.28 (2H, d, *J* = 8.3 Hz), 7.36–7.42 (3H, m 3 × ArH), 7.68 (2H, d, 2 × ArH, *J* = 8.5 Hz), 10.29 (1H, s, NH), 10.72 (1H, s, NH). t.l.c. (SiO_2) R_f = 0.2 (dichloromethane/ethanol/ammonia) (150/8/1) one component.

6.14. N-*(1H-Indol-5-yl)-3-(4-methylpiperazino) benzenecarboxamide (**29**)*

3-(*N*-Methylpiperazino benzoic acid hydrochloride (376.0 mg, 1.47 mmol) was dissolved in anhydrous DMF (3.0 ml). 5-aminoindole (213.0 mg, 1.6 mmol) and TBTU (516.0 mg, 1.6 mmol) were added and the solution stirred for 10 min. Diisopropylamine (306.0 μl, 1.75 mmol) was added to the reaction mixture and the solution was stirred at room temperature and under an atmosphere of nitrogen for 18 h. The DMF was evaporated under reduced pressure and water was added and the aqueous phase was basified with potassium carbonate and extracted with ethyl acetate, dried, filtered and evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to (60/8/1) to afford (169.0 mg, 34.5%) of the desired *N*-(1*H*-indol-5-yl)-3-(4-methylpiperazino) benzenecarboxamide (**29**) as a grey powder. m.p. = 97–99 °C (softens at m.p. = 90 °C), M.S. *m/z* 335 $(M + 1)^+$ ^{1}H NMR δ 2.23 (3H, s, NCH_3), 3.19–3.21 (8H, m, 4 × CH_2), 6.39 (1H, m, ArH), 7.11 (1H, d, ArH, *J* = 7.5 Hz), 7.3–7.37 (4H, m, 4 × ArH), 7.46 (1H, s, ArH), 7.93 (1H, s, ArH), 9.95 (1H, s, ArH), 11.0 (1H, s, NH). Found $(M)^+$ = 334.17934 $C_{20}H_{22}N_4O$ requires $(M)^+$ = 334.17934. t.l.c. (SiO_2) R_f = 0.2 (dichloromethane/ethanol/ammonia) (150/8/1) one component.

6.15. N-*[5-(4-Chlorophenyl)-1,3-thiazol-2-yl]-3-(4-methylpiperazino) benzenecarboxamide (**30**)*

3-(4-Methyl-1-piperazinyl) benzoic acid hydrochloride (246.0 mg, 0.9 mmol) was dissolved in anhydrous DMF (5.0 ml). To this stirring solution was added 2-amino-4-(4-chlorophenyl)thiazole (222.1 mg, 1.05 mmol) and TBTU (338.0 mg, 1.0 mmol) were added and the solution stirred for 10 min. Diisopropylethylamine (149.0 mg, 1.1 mmol) was added to the mixture and the solution was stirred at room temperature and under an atmosphere of nitrogen for 72 h. The DMF was evaporated under reduced pressure and water was added to the residue and the aqueous phase was extracted with ethyl acetate, dried, filtered and evaporated under reduced

pressure and the resulting brown oil was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (105.0 mg, 26.6%) of the desired *N*-[5-(4-chlorophenyl)-1,3-thiazol-2-yl]-3-(4-methylpiperazino)-3-(4-methylpiperazino)benzenecarboxamide (**30**) as a yellow powder. The free base was converted into the hydrochloride salt, which was freeze dried to afford the desired *N*-[5-(4-chlorophenyl)-1,3-thiazol-2-yl]-3-(4-methylpiperazino)-3-(4-methylpiperazino)benzenecarboxamide (**16**) as a white lyophylate. M.S. *m/z* 413 $(M + 1)^+$ ^{1}H NMR δ 2.07 (6H, s, 2 × NCH_3), 3.04 (8H, m, 4 × CH_2), 7.18 (1H, d, ArH, J = 8.07 Hz), 7.37 (1H, t, ArH, J = 8.0 Hz), 7.39–7.47 (2H, m, 2 × ArH), 7.59 (1H, d, ArH, J = 8.9 Hz), 7.91 (1H, d, ArH, J = 8.5 Hz). Found $(M)^+$ = 393.16075 $C_{23}H_{24}ClN_3O$ requires $(M)^+$ = 393.16077. t.l.c. (SiO_2) R_f = 0.74 (dichloromethane/ethanol/ammonia) (60/8/1) one component.

6.16. N-*[4-(6-Methyl-1,3-benzothiazol-2-yl)phenyl]-3-(4-methylpiperazino) benzenecarboxamide (**31**)*

3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid hydrochloride (100.0 mg 0.35 mmol) was dissolved in anhydrous DMF (10.0 ml). 2-(4-aminophenyl)-4-methylbenzenesulfonamide (92.0 mg, 0.38 mmol) and TBTU (123.0 m, 0.38 mmol) were added and the solution stirred for 10 min. Diisopropylethylamine (54.0 mg, 0.42 mmol) was added to the mixture and the solution was stirred at 80 °C and under an atmosphere of nitrogen for 4 h. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (23.0 mg, 14.0%) of the desired *N*-[4-(6-methyl-1,3-benzothiazol-2-yl)phenyl]-3-(4-methylpiperazino) benzenecarboxamide (**31**) as a yellow powder. The free base was converted into the hydrochloride salt, which was freeze dried to afford the desired *N*-[4-(6-methyl-1,3-benzothiazol-2-yl)phenyl]-3-(4-methylpiperazino) benzenecarboxamide (**17**) as a yellow lyophylate. M.S. *m/z* 473 $(M+1)^{+\cdot}$ ^{1}H NMR δ 2.45 (3H, s, $ArCH_3$), 2.58–3.08 (8H, m, 4 × CH_2), 3.87 (3H, s, NCH_3), 7.08 (1H, d, ArH, J = 8.7 Hz), 7.34 (1H, s, ArH, J = 8.1 Hz), 7.48 (1H, d, ArH, J = 1.8 Hz), 7.69 (1H, dd, ArH, J = 2.1 Hz, J = 8.7 Hz), 7.88–8.07 (4H, m, 4 × ArH), 10.31 (1H, s, NH). Found $(M)^+$ = 472.19326 $C_{27}H_{28}N_4O_2S$ requires $(M)^+$ = 472.19327, t.l.c. (SiO_2) R_f = 0.64 (CH_2Cl_2/EtOH/NH_3 (60/8/1) one component.

6.17. 3-(4-Methyl-1-piperazinyl)-4-methoxy-N-*(4-nitrophenyl)benzamide (**18**)*

3-(4-Methyl-1-piperazinyl)-4-methoxybenzoic acid, (50.0 mg, 0.17 mmol) was dissolved in anhydrous DMF (2.0 ml) and 4-nitrobenzylamine (36.2 mg, 0.19 mmol) and TBTU (61.6 mg, 0.19 mmol) were added and the solution stirred for 10 min. Diisopropylethylamine (81.1 mg) was added to the mixture and the solution was stirred over night at room temperature and under an atmosphere of nitrogen for 3 h. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with a solution of (dichloromethane/ethanol/ammonia) (300/8/1) to afford (44.9 mg, 67.0%) of the desired 3-(4-methyl-1-piperazinyl)-4-methoxy-N-(4-nitrophenyl) benzamide (**18**) as a yellow powder. The free base was converted into the hydrochloride salt, which was freeze dried to afford the desired 3-(4-methyl)-4-methoxy-*N*-(4-nitrophenyl) benzamide (**18**) as a white lyophylate. M.S. *m/z* 385 $(M + 1)^{+\cdot}$ ^{1}H NMR δ 2.92–3.0 (8H, m, 4 × CH_2), 3.83 (3H, s, NCH_3CH_3), 3.85 (3H, s, OCH_3CH_3), 4.56 (2H, d, CH_2, J = 5.9 Hz), 7.02 (1H, d, ArH, J = 8.7 Hz), 7.42 (1H, d, ArH, J = 2.0 Hz), 7.55 (2H, d, 2 × ArH, J = 8.7 Hz), 8.19 (1H, d, ArH, J = 8.7 Hz), 8.99 (1H, t, NH, J = 5.7 Hz). Found C 53.23, H 6.25, N 12.0%, $C_{20}H_{24}N_4O_4$·HCl·1.75 H_2O requires C, 53.09, H, 6.35, N, 12.38%.

*6.18. 2-Fluoro-6-(*N-*methylpiperazino) benzaldehyde (**4**)*

2,6-Difluorobenzaldehyde (5.0 g, 35.2 mmol) was dissolved in anhydrous DMF (10.0 ml) and *N*-methylpiperazine (4.22 g, 42.2 mmol) and potassium carbonate (5.83 g, 42.2 mmol) were added and the reaction mixture was heated to 80 °C for 4 h. The reaction mixture was then stirred overnight at room temperature. The reaction mixture was quenched with water (100.0 ml) and the aqueous phase was extracted with ethyl acetate (3 × 100.0 ml). The ethyl acetate phase was washed with saturated ammonium chloride solution, dried, filtered and evaporated under reduced pressure to afford a yellow oil, which was purified by column chromatography eluting with (ethyl acetate/hexane) (5/95) to afford (4.67 g, 59.7%) of the desired 2-fluoro-6-(*N*-methylpiperazino) benzaldehyde (4). M.S. *m/z* 223 $(M)^+$ ^{1}H NMR (DMSO-d_6) δ 2.22 (3H, s, CH_3), 2.47 (4H, m, 4 × CH_2), 3.03 (4H, m, 4 × CH_2), 6.86 (1H, dd, ArH, J = 8.4 Hz, J = 10.8 Hz), 6.98 (1H, d, ArH, J = 8.4 Hz), 7.62 (1H, m, ArH), 10.08 (1H, s, CHO).

*6.19. Ethyl 4-(*N-*methylpiperazino)-1-benzo[b]thiophene-2-carboxylate (**6**)*

2-Fluoro-6-(4-methyl-1-piperazinyl)benzaldehyde (2.19 g, 9.9 mmol) was dissolved in anhydrous DMF (50.0 ml) and stirred under an atmosphere of nitrogen. Ethyl-2-mercaptoacetate (1.78 g, 14.8 mmol) and sodium hydride (60% dispersion) (592.0 mg, 14.8 mmol) were added and the reaction mixture was stirred at room temperature for 18 h. 2 N NaOH was added and the aqueous phase was extracted with diethyl ether and the diethyl ether phase was washed with water, dried, filtered and evaporated under reduced pressure to afford (247.0 mg, 62.7%) of the desired ethyl 4-(*N*-methylpiperazino)-1-benzo[b]thiophene-2-carboxylate (**6**) as an orange oil. M.S. *m/z* 305 $(M)^+$ ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, CH_3, J = 7.2 Hz), 2.24 (3H, s, CH_3), 2.52 (4H, m, 2 × CH_2), 3.08 (4H, m, 2 × CH_2), 4.34 (2H, q, CH_2,

J = 6.9 Hz), 6.96 (1H, d, ArH, J = 7.5 Hz), 7.43 (1H, t, ArH, J = 7.8 Hz), 7.65 (1H, d, ArH, J = 8.1 Hz), 7.94 (1H, s, ArH), 7.66 (1H, s, ArH), 9.73 (1H, s, ArH).

*6.20. 4-(4-Methylpiperazino)-1-benzo[b]thiophene-2-carboxylic acid (**8**)*

Ethyl (4-methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylate (770.0 mg, 2.54 mmol) was dissolved in methanol (5.0 ml) and 1 N NaOH (10.2 ml) and the reaction mixture was heated to reflux for 6.0 h. Water (150.0 ml) was added and the aqueous phase was extracted with ethyl acetate (3 × 100.0 ml), the aqueous phase was acidified with concentrated hydrochloric acid and the aqueous phase was extracted with ethyl acetate, dried, filtered and concentrated under reduced pressure until the desired 4-(4-methyl piperazino)-1-benzo[b]thiophene-2-carboxylic acid (**8**) crashed out of solution as a yellow solid (230.0 mg, 32.9%). m.p. = 270 °C (decomposed), M.S. *m/z* 277 $(M)^+$ ^{1}H NMR (DMSO-d_6) δ 2.85 (3H, s, CH_3), 3.2–3.49 (8H, m, 4 × CH_2), 7.04 (1H, d, ArH, J = 7.5 Hz), 7.45 (1H, t, ArH, J = 7.8 Hz), 7.72 (1H, d, ArH, J = 8.1 Hz), 8.03 (1H, s, ArH), 10.68 (1H, br s, N^+H), 13.45 (1H, br s, OH). t.l.c. (SiO_2) R_f = 0.15 ((CH_2Cl_2/EtOH/NH_3) (30/8/1)).

*6.21. 1-[2-(3-Benzyl-1,2,4-oxadiazol-5-yl)-1-benzo[b]thiophen-4-yl]-4-methylpiperazine (**32**)*

Ethyl (4-methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylate (142.0 mg, 0.46 mmol) was dissolved in anhydrous THF (5.0 ml) and *N,N′*-carbonyl diimidazole (83.3 mg, 0.51 mmol) was added and the solution was stirred at room temperature for 2.5 h. In a separate flask *N*-hydroxy benzylimidine (128.9 mg, 0.93 mmol) and crushed 3 Å sieves (155.0 mg) were stirred in anhydrous THF (5.0 ml) for 45 min at room temperature under an atmosphere of nitrogen. The *N*-hydroxybenzylimidine solution was then added to the benzo[b]thiophene-2-carboxylate solution. The resulting reaction mixture was stirred at room temperature overnight. THF was evaporated under reduced pressure and water (100.0 ml) was added to the residue and the aqueous phase was extracted with dichloromethane, dried, filtered and evaporated under reduced pressure to afford a yellow oil, which was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (350/8/1) to (200/8/1) to afford (59.7 mg, 32.7%) of the desired 2-(3-benzyl-5-oxadiazolyl)-4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene (**32**) as off-white solid. M.S. *m/z* 391 $(M + 1)^{+\cdot}$ ^{1}H NMR (D_2O) δ 2.49 (3H, s, CH_3), 4.18 (2H, s, CH_2), 7.09 (1H, d, ArH, J = 7.62 Hz), 7.26–7.36 (6H, m, 6 × ArH), 7.49 (1H, t, ArH, J = 7.83 Hz), 7.8 (1H, d, ArH, J = 8.2 Hz), 8.28 (1H, s, NH). H.p.l.c. retention time = 17.36 min. (Gradient elution 0.2% TFA/CH_3CH_3CN/0.2% TFA/H_2O over 20 min).

*6.22. N-Benzyl-4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**15**)*

(4-Methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid (50.0 mg, 0.16 mmol) was dissolved in anhydrous DMF (2.0 ml) and benzylamine (19.2 μl, 0.17 mmol) and TBTU (56.5 mg, 0.17 mmol) was added and the reaction mixture was stirred under an atmosphere of nitrogen at room temperature for 1 h. Diisopropylethylamine (666.8 μl, 0.38 mmol) was added and the reaction mixture was stirred at room temperature overnight. The DMF was evaporated under reduced pressure and the resultant yellow residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (200/8/1) to (60/8/1) to afford (42.0 mg, 71.9%) of the desired *N*-benzyl-4-(4-methyl-1- piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**15**) as a yellow solid. M.S. *m/z* 366 $(M + 1)^+$ ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s, CH_3), 2.59–2.6 (4H, m, 2 × CH_2), 3.07–3.34 (4H, m, 2 × CH_2), 4.51 (2H, d, CH_2, J = 6.0 Hz), 6.92 (1H, d, ArH, J = 7.8 Hz), 7.25–7.42 (6H, m, 6 × ArH), 7.59 (1H, d, ArH, J = 7.8 Hz), 8.15 (1H, s, ArH). Found $(M)^+$ = 365.15615 $C_{21}H_{23}N_3OS$ requires $(M)^+$.= 365.15616, t.l.c. (SiO_2) R_f = 0.57 ((dichloromethane/ethanol/ammonia) (60/8/1)).

*6.23. N-{4-[4-(3,5-Dioxo-4-azatricyclo[5.2.1.0]dec-8-en-4-yl)benzyl]phenyl}-4-(4-methylpiperazino)-1-benzo[b]thiophene-2-carboxamide (**16**)*

(4-Methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid hydrochloride (50.1 mg, 0.16 mmol), *N*-[4-(4-aminobenzyl)phenyl]-5-norbornene-2,3-dicarboximide (62.2 mg, 0.18 mmol) and TBTU (58.8 mg, 0.18 mmol) were dissolved in anhydrous DMF (2.0 ml) and stirred at room temperature under an atmosphere of nitrogen for 15 min. Diisopropylethylamine (67.0 μl, 0.38 mmol) was added and the reaction was stirred overnight. The DMF was evaporated under reduced pressure and the residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (300/8/1) to (60/8/1) to afford (41.2 mg, 42.7%) of the desired *N*-{4-[4-(3,5-dioxo-4-aza tricyclo[5.2.1.0] dec-8-en-4-yl)benzyl]phenyl}-4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**16**) as a yellow solid. The free base was converted to the hydrochloride salt. M.S. *m/z* 603 $(M + 1)^{+\cdot}$ ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s, NCH_3), 2.66 (3H, m, 3 × H), 3.12 (6H, m, 6 × H), 3.46 (2H, m, 2 × H), 3.95 (2H, s CH_2), 6.19 (2H, s, 2 × H), 6.95 (1H, d, ArH, J = 7.53 Hz), 7.01 (1H, d, ArH, J = 8.3 Hz), 7.24 (1H, d, ArH, J = 8.6 Hz), 7.29 (1H, d, ArH, J = 8.6 Hz), 7.29 (1H, d, ArH, J = 8.22 Hz), 7.37 (1H, t, ArH, J = 7.92 Hz), 7.58 (1H, t, ArH, J = 3.7 Hz), 7.67 (1H, d, ArH, J = 8.3 Hz), 8.27 (1H, s, NH). Found $(M)^+$ = 602.23512 $C_{36}H_{34}N_4SO_3$·HCl requires $(M)^{+\cdot}$= 602.23513, t.l.c. (SiO_2) R_f = 0.39 ((dichloromethane/ethanol/ammonia) (60/8/1)).

6.24. N-*[4-(5-Chloro-1,3-dioxo-1,3-dihydro-2*H-*isoindol-2-yl)-3-methylphenyl]-4-(4-methylpiperazino)-1-benzo[b]thiophene-2-carboxamide (**17**)*

(4-Methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid (40.4 mg, 0.13 mmol) and *N*-(4-amino-2-methylphenyl)-4-chlorophthalimide (40.7 mg, 0.14 mmol) and TBTU (45.5 mg, 0.14 mmol) were dissolved in anhydrous DMF (3.0 ml) and stirred at room temperature under an atmosphere of nitrogen for 30 min and diisopropylethylamine (49.4 μl, 0.28 mmol) was added and the reaction mixture was stirred for a further 12 h. The DMF was evaporated under reduced pressure and water was added and the aqueous phase was extracted ethyl acetate which was dried, filtered and evaporated under reduced pressure to afford a yellow oil, which was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (100/8/1) to afford (38.1 mg, 54.1%) of the desired *N*-[4-(5-chloro-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-methylphenyl]-4-(4-methylpiperazino)-1-benzo[b]thiophene-2-carboxamide (**17**) as a yellow powder. The free base was converted into the hydrochloride salt to afford the desired *N*-[4-chloro-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-methylphenyl]-3-methylphenyl]-4-(4-methylpiperazino)-1-benzo[b]thiophene-2-carboxamide (**17**) as a light brown lyophylate after freeze-drying. M.S. *m/z* 545 $(M)^+$ ^{1}H NMR (DMSO-d_6) δ 2.14 (3H, s, CH_3), 2.30 (3H, s, CH_3), 2.64 (4H, m, 2 × CH_2), 3.14 (4H, m, 2 × CH_2), 6.97 (1H, d, ArH, J = 7.8 Hz), 7.33–7.42 (2H, m, 2 × ArH), 7.63–7.78 (3H, m, 3 × ArH), 7.98 (2H, s, 2 × ArH), 8.34 (1H, s, ArH), 10.69 (1H, s, NH). t.l.c. (SiO_2) R_f = 0.25 ((CH_2Cl_2/EtOH/NH_3) (100/8/1)).

*6.25. 4-(4-Methyl-1-piperazinyl)-*N*-phenethyl-1-benzo[b]thiophene-2-carboxamide (**18**)*

(4-Methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid hydrochloride (100.0 mg, 0.32 mmol) and 2-phenethylamine (44.2 μl, 0.35 mmol) and TBTU (110.0 mg, 0.35 mmol) were dissolved in anhydrous DMF (4.0 ml) and the reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 30 min. Diisopropylethylamine (122.5 μl, 0.7 mmol) was added and stirring was continued for a further 4 h. The DMF was evaporated under reduced pressure and the residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (300/8/1) to (60/8/1) to afford (45.4 mg, 37.4%) of the desired 4-(4-methyl-1-piperazinyl)-*N*-phenethyl-1-benzo[b]thiophene-2-carboxamide (**18**) as a yellow powder. The free base was converted into the hydrochloride salt to afford the desired 4-(4-methyl-1-piperazinyl)-*N*-phenethyl-1-benzo[b]thiophene-2-carboxamide (**18**) as a white lyophylate after freeze-drying. M.S. *m/z* 380 $(M + 1)^+$ ^{1}H NMR (DMSO-d_6) δ 2.59 (4H, m, 2 × CH_2), 2.86 (2H, t, CH_2, J = 7.9 Hz), 3.08 (4H, m, 2 × CH_2), 3.45–3.52 (2H, m, CH_2), 6.91 (1H, d, ArH, J = 7.6 Hz), 7.20–7.36 (5H, m, 5 × ArH), 7.58 (1H, d, ArH, J = 8.0 Hz), 8.0 (1H, s, ArH), 8.94 (1H, m, NH). h.p.l.c. retention time = 12.47 min. Linear trace 10% *B*/90% *D* (*B* = 90% CH_3CN/10% H_2O), (*D* = 0.1 N NH_4OAc (pH 4). Found $(M)^{+\cdot}$ = 379.1718 $C_{22}H_{25}N_3OS$ .HCl requires $(M)^{+\cdot}$ = 379.17181. t.l.c. (SiO_2) R_f = 0.27 (CH_2Cl_2/EtOH/NH_3) (100/8/1).

6.26. N-*(4-Nitrobenzyl)-4-(4-methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**19**)*

(4-Methyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid hydrochloride (100.0 mg, 0.32 mmol), 4-nitrophenethylamine (71.2 mg, 0.35 mmol) and TBTU (112.8 mg, 0.35 mmol) were dissolved in anhydrous DMF (5.0 ml) and the solution was stirred under an atmosphere of nitrogen at room temperature for 60 min. Diisopropylethylamine (200.0 μl, 1.15 mmol) was added and the reaction mixture was stirred for a further 3 h. The DMF was evaporated under reduced pressure and water was added and the aqueous phase was extracted with ethyl acetate, dried, filtered and evaporated under pressure and the resultant residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (200/8/1) to afford the desired *N*-(4-nitrophenethyl)-4-(4-methyl-1-piperazinyl)O-1-benzo[b]thiophene-2-carboxamide (**19**) as a yellow powder m.p. = 180–184 °C, M.S. *m/z* 425 $(M + 1)^+$ ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s, CH_3), 2.58 (4H, m, 2 × CH_2), 2.99–3.07 (6H, m, 3 × CH_2), 3.52–3.59 (2H, m, CH_2), 6.91 (1H, d, ArH, J = 7.5 Hz), 7.33 (1H, t, ArH, J = 7.8 Hz), 7.53–7.59 (3H, m, 3 × ArH), 7.95 (1H, s, ArH), 8.17 (1H, d, ArH, J = 8.7 Hz), 8.92 (1H, m, NH), h.p.l.c. retention time = 12.0 min. Linear trace 10% *B*/90% *D* (*B* = 90% CH_3CN/10% H_2O), (*D* = 0.1 N NH_4OAc (pH 4).

*6.27. 4-(*N*-2-Phenethylpiperazino)-1-benzo[b]thiophene-2-carboxylic acid (**9**)*

2-Fluoro-6-(4-phenethyl-1-piperazinyl)benzaldehyde (2.15 g, 6.9 mmol) was dissolved in anhydrous DMF (60.0 ml) and stirred at room temperature under an atmosphere of nitrogen. Ethyl-2-mercaptoacetate (1.13 ml, 10.32 mmol) and sodium hydride (60% dispersion) (412.8 mg, 10.32 mmol) were added and the reaction was stirred at room temperature for 18 h. The DMF was evaporated under reduced pressure and 2 N NaOH was added, which had the effect of causing complete hydrolysis of the ethyl ester to form the benzo[b]thiophene-2-carboxylic acid. As a result the aqueous phase was acidified with concentrated hydrochloric acid. The aqueous phase was evaporated to dryness under reduced pressure to afford a white solid, which was taken up in boiling ethanol and a hot filtration was performed. The ethanol was evaporated under reduced pressure to afford (1.68 g, 66.7%) of the desired 4-(4-2-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid (**9**) as a white powder. M.S. *m/z* 367 $(M + 1)^{+\cdot}$ ^{1}H NMR (DMSO-d_6d_6) δ 2.64–2.69 (8H, m, 4 × CH_2), 3.11 (4H, m, 2 × CH_2), 6.94 (1H, d, ArH, J = 7.5 Hz), 7.16–7.31 (5H, m, 5 × ArH), 7.39 (1H, t, ArH,

J = 7.8 Hz), 7.62 (1H. d, ArH, *J* = 8.1 Hz), 7.91 (1H, s, ArH). h.p.l.c. retention time = 8.15 min. Linear trace 10% *B*/90% *D* (*B* = 90% CH_3CN/10% H_2O) (*B* = 0.1 N NH_4OAc (pH 4)). t.l.c. (SiO_2) R_f = 0.74 (dichloromethane/ethanol/ammonia) (20/8/1).

6.28. N-*Benzyl-4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**20**)*

4-(4-Phenethyl-1-piperazinyl)benzo[b]thiophene-2-carboxylic acid hydrochloride (60.0 mg, 0.15 mmol) and benzylamine (17.8 μl, 0.16 mmol) and TBTU (52.5 mg, 0.16 mmol) were dissolved in anhydrous DMF (5.0 ml) and stirred at room temperature under an atmosphere of nitrogen for 1.0 h. Diisopropylethylamine (57.1 μl, 0.33 mmol) was then added and the reaction mixture was stirred for a further 12 h. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (200/8/1) to afford (67.3 mg, 99.0%) of the desired *N*-benzyl-4-(4-phenethyl-1-piperazinyl)-1-benzo[b] thiophene-2-carboxamide (**20**) as a yellow powder. The free base was converted into the hydrochloride salt to afford the desired *N*-benzyl-4-(4-phenethyl-1-piperazinyl)-1-benzo[b] thiophene-2-carboxamide (**20**) as a cream lyophylate after freeze-drying. M.S. *m/z* 456 $(M)^+$ ^1H NMR (DMSO-d_6) δ 2.59–2.82 (8H, m, 4 × CH_2), 3.1 (4H, m, 2 × CH_2), 4.52 (2H, d, CH_2, *J* = 6.0 Hz), 6.94 (1H, d, ArH, *J* = 7.62 Hz), 7.18–7.39 (11H, m, 11 × ArH), 7.62 (1H, d, ArH, *J* = 8.1 Hz), 8.17 (1H, s, ArH), 9.43 (1H, t, NH, *J* = 5.9 Hz). h.p.l.c. retention time = 15.77 min. Linear trace 10% *B*/90% *D* (*B* = 90% CH_3CN/10% H_2O) (*D* = 0.1N NH_4OAc (pH 4)).

*6.29. Ethyl 4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylate (**7**)*

2-(4-Phenethyl-1-piperazinyl)-6-fluorobenzaldehyde (1.23 g, 3.93 mmol) was dissolved in anhydrous DMF (30.0 ml) and the reaction mixture was stirred at room temperature under an atmosphere of nitrogen. Sodium hydride (236.0 mg, 5.9 mmol) and ethyl-2-mercaptoacetate (647.0 μl, 5.9 mmol) were added and the reaction mixture was stirred a further 18 h. The DMF was evaporated under reduced pressure and water was added and the aqueous phase was extracted with ethyl acetate (5 × 50.0 ml), dried, filtered and evaporated under reduced pressure to afford a yellow oil, which was purified by column chromatography eluting with (hexane/ethyl acetate) (6/4) to afford a yellow solid, which was further purified by recrystallisation from ethanol to afford (941.0 mg, 60.6%) of the desired ethyl 4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylate (**7**). M.S. *m/z* 395 $(M+1)^+$ ^1H NMR (DMSO-d_6) δ 1.33 (3H, t, CH_3, *J* = 7.2 Hz), 2.6–2.82 (8H, m, 4 × CH_2), 3.1–3.11 (4H, m, 2 × CH_2), 4.34 (2H, q, CH_2, *J* = 7.2 Hz), 6.97 (1H, d, ArH, *J* = 7.7 Hz), 7.16–7.31 (5H, m, 5 × ArH), 7.43 (1H, t, ArH, *J* = 7.9 Hz), 7.65 (1H, d, ArH, *J* = 8.2 Hz), 7.99 (1H, s, ArH).

*6.30. 4-(4-Phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid (**9**)*

2-(4-Methyl-1-piperazinyl)-6-fluorobenzaldehyde (2.9 g, 12.8 mmol) was dissolved in anhydrous DMF (80.0 ml) under an atmosphere of anhydrous nitrogen. Ethyl-2-mercaptoacetate (2.11 ml, 19.3 mmol) and sodium hydride (60% dispersion) (770.0 mg, 19.3 mmol) was added and the reaction was stirred for 18 h at room temperature. Aqueous 2 N sodium hydroxide (45.0 ml) was added and the aqueous phase was extracted with diethyl ether. TLC of the aqueous and organic phases indicated that the aqueous sodium hydroxide had caused hydrolysis of the ethyl ester to afford the desired 4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid. The aqueous phase was acidified with concentrated hydrochloric acid after which a white precipitated formed, which was filtered and the filtrate was evaporated to dryness under reduced pressure. The white residue was boiled in ethanol and a hot filtration was performed to afford (3.5 g, 98.0%) of the desired 4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid. (**9**) M.S. *m/z* 277 $(M)^+$ ^1H NMR (DMSO-d_6) δ 2.86 (3H, s, CH_3), 3.03 (4H, m, 2 × CH_2), 3.17 (4H, m, 2 × CH_2), 6.86 (1H, d, ArH, *J* = 7.7 Hz), 7.26 (1H, t, ArH, *J* = 7.8 Hz), 7.49 (1H, d, ArH, *J* = 7.9 Hz), 7.68 (1H, s, ArH), 7.87 (1H, s, ArH), 8.27 (1H, m, NH^+). h.p.l.c. retention time = 3.53 min. Linear trace 10% *B*/90% *D* (*B* = 90% CH_3CN/10% H_2O) (*D* = 0.1 N NH_4OAc (pH 4). t.l.c. (SiO_2) R_f = 0.74 (dichloromethane/ethanol/ammonia) (20/8/1).

*6.31. 4-Methyl-4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**21**)*

4-(4-Phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid hydrochloride (100.0 mg, 0.25 mmol), methylamine hydrochloride (18.4 mg, 0.27 mmol) and TBTU (87.6 mg, 0.27 mmol) were dissolved in anhydrous DMF (4.0 ml) and the reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 30 min. Diisopropylethylamine (156.0 μl, 0.89 mmol) was added and the reaction mixture was stirred for a further 3 h. The DMF was evaporated under reduced pressure and the resultant residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (300/8/1) to afford (48.7 mg, 51.7%) of the desired 4-methyl-4-(4-phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxamide (**21**) as a white powder. m.p. = 177–180 °C, found C, 69.09, H, 6.74, N, 10.87%, $C_{22}H_{25}N_3OS{\cdot}0.2\,H_2O$ requires C, 68.96, H, 6.68, N, 10.96%. M.S. *m/z* 380 $(M)^+$ ^1H NMR (DMSO-d_6) δ 2.48 (3H, s, CH_3), 2.65 (2H, m, CH_2), 2.74 (4H, m, 2 × CH_2), 2.8 (3H, s, CH_3), 2.81 (2H, m, CH_2), 3.09 (4H, m, 2 × CH_2), 6.91 (1H, d, ArH, *J* = 7.6 Hz), 7.34 (1H, m, ArH), 7.57 (1H, d, ArH, *J* = 8.1 Hz), 8.05 (1H, s, ArH), 8.77 (1H, m, NH).

6.32. 4-(4-Phenethyl-1-piperazinyl)-N-*phenyl-1-benzo[b]thiophene-2-carboxamide (**22**)*

4-(4-Phenethyl-1-piperazinyl)-1-benzo[b]thiophene-2-carboxylic acid hydrochloride (60.0 mg, 0.15 mmol), aniline

(15.0 μl, 0.16 mmol) and TBTU (52.6 mg, 0.16 mmol) were dissolved in anhydrous DMF (3.0 ml) and stirred at room temperature under an atmosphere of nitrogen for 30 min. Diisopropylethylamine (57.0 μl, 0.33 mmol) was added and the reaction mixture was stirred a further 3 h. The DMF was evaporated under reduced pressure and the resulting residue was purified by column chromatography eluting with (dichloromethane/ethanol/ammonia) (300/8/1) to (60/8/1) to afford the desired 4-(4-phenethyl-1-piperazinyl)-*N*-phenyl-1-benzo[b]thiophene-2-carboxamide (**22**) as a yellow powder. M.S. *m/z* 442 $(M + 1)^+$ ^{1}H NMR DMSO-d_6) δ 2.64 (2H, m, CH_2), 2.78 (4H, m, $2 \times CH_2$), 3.15 (4H, m, $2 \times CH_2$), 6.97 (1H, d, ArH, J = 7.6 Hz), 7.13 (1H, m, ArH), 7.74 (1H, d, ArH, J = 7.9 Hz), 8.31 (1H, s, ArH), 10.54 (1H, s, NH). h.p.l.c. retention time = 15.97 min. Linear trace 10% *B*/90% *D* (*B* = 90% CH_3CN/10% H_2O) (*D* = 0.1 N NH_4OAc (pH 4)).

References

[1] D. Hoyer, D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena, P.P.A. Humphrey, International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46 (1994) 157–204.

[2] G.R. Martin, P.P.A. Humphrey, Receptors for 5-hydroxy tryptamine: current perspectives on classification and nomenclature, Neuropharmacology 33 (1994) 261–273.

[3] D. Hoyer, G.R. Martin, 5-HT receptor classification and nomenclature: towards a harmonisation with the human genome, Neuropharmacology 36 (1997) 419–428.

[4] P.M. Vanhoutte, P.P.A. Humphrey, M. Spedding, Recommendations for nomenclature of new receptor subtypes, Pharmacol. Rev. 48 (1996) 1–2.

[5] P.R. Hartig, D. Hoyer, P.P.A. Humphrey, G.R. Martin, Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes, Trends Pharmacol. Sci. 17 (1996) 103–105.

[6] P.P.A. Humphrey, W. Feniuk, M.J. Perren, H.E. Connor, A.W. Oxford, I.H. Coates, D. Butina, GR43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein, Br. J. Pharmacol. 94 (1988) 1123–1132.

[7] K.L. Dechant, S.P. Clissold, Sumatriptan. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the acute treatment of migraine and cluster headache, Drugs 43 (1992) 776–798.

[8] P.C. North, Migraine therapy—serotonin to sumatriptan, in: F.G. King (Ed.), Medicinal Chemistry: Principles and Practice, The Royal Society of Chemistry, 1994.

[9] R.C. Glen, A.P. Hill, G.R. Martin, A.D. Robertson, Computer-aided design of 5-HT_{1D} agents for the acute treatment of migraine headache, Headache 34 (1994) 307.

[10] E. Hamel, 5-HT_{1D} receptors: pharmacology and therapeutic potential, Serotonin ID Research Alert 1 (1996) 19–29.

[11] G.R. Martin, Serotonin Receptor Involvement in the Pathogenesis and Treatment of Migraine, in: S. Silberstein, P.J. Goadsby (Eds.), Blue Books on Neurology, Butterworth-Heinemann, Boston,1997.

[12] (a) European Patent EP 0 533 266 A1, (1992); (b) European Patent EP 0 533 267 A1, (1992); (c) European Patent EP 0 533 268 A1, J.W. D.I.C. (1992); (d) Clitherow, M. Scopes, C.C. Skingle, W. Jordan, I.B. Feniuk, M.C. Campbell, E.W. Carter, H.E. Collington, G.A. Connor, D. Higgins, H.A. Beattie, W.L. Kelly, A.W. Mitchell, A. Oxford, H. Wadsworth, M.B. Tyers, Evolution of a Novel Series of [(*N,N*-Dimethylamino)propyl]- and piperazinylbenzanilides as the first selective 5-HT_{1D} antagonists, J. Med. Chem. 37 (1994) 2253–2257.

[13] M. Skingle, A.J. Sleight, S. Feniuk, Effects of the 5-HT_{1D} receptor antagonist GR127935 on extracellular levels of 5-HT in the guinea-pig frontal cortex as measured by microdialysis, Neuropharmacology 34 (1995) 377–382.

[14] D.M. Walsh, D.T. Beattie, H.E. Connor, The Activity of 5-HT_{1D} receptor ligands at cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, Eur. J. Pharmacol. 287 (1995) 79–84.

[15] E.P. McFadden, J.G. Clarke, G.J. Davies, J.C. Kaski, A.W. Haider, A. Maseri, Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina, New. Eng. J. Med. 324 (1991) 648–654.

[16] P. Golino, F. Piscione, J.T. Willerson, M. Cappelli-Bigazzi, A. Focaccio, B. Villari, C. Indolfi, E. Russolillo, M. Condorelli, M. Chiariello, Divergent effects of serotonin on coronary artery dimensions and blood flow in patients with coronary atherosclerosis and control patients, New Eng. J. Med. 324 (1991) 641–648.

[17] A. Maseri, Pathogenetic components of acute ischemic syndromes. Focus on acute ischemic stimuli, Circulation 81 (1 suppl.) (1990) I1–I3.

[18] D.J. Fitzgerald, L. Roy, F. Catella, G.A. Fitzgerald, Platelet activation in unstable coronary disease, New Eng. J. Med. 315 (1986) 983–989.

[19] P.B. Bradley, G. Engel, W. Feniuk, J.R. Fozard, P.P.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson, P.R. Saxena, Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine, Neuropharmacology 25 (1986) 563–576.

[20] A.L. Scherbel, J.N. Harrison, Response to serotonin and its antagonists in patients with rheumatoid arthritis and related diseases, Angiology 10 (1959) 29–38.

[21] G.P. Moloney, A.D. Robertson, G.R. Martin, S. MacLennan, N. Mathews, S. Dodsworth, P.Y. Sang, C. Knight, R.C. Glen, A novel series of 2,5-substituted tryptamine derivatives as vascular 5-$HT_{1B/1D}$ receptor antagonists, J. Med. Chem. 40 (1997) 2347–2362.

[22] Merrell Dow Pharmaceuticals, PCT/US93/08865.

[23] (a) G.P. Moloney, G.R. Martin, N. Mathews, A. Milne, H. Hobbs, S. Dodsworth, P.Y. Sang, C. Knight, M. Williams, M. Maxwell, R.C. Glen, Synthesis and serotonergic activity of substituted 2,*N*-benzylcarboxamido-5-(2-ethyl-1-dioxoimidazolidinyl)-*N,N*-dimethyltryptamine derivatives: novel antagonists for the vascular 5-HT_{1B}-like receptor, J. Med. Chem. 42 (1999) 2504–2526(b) G.P. Moloney, G.R. Martin, N. Mathews, H. Hobbs, S. Dodsworth, P.Y. Sang, C. Knight, R.C. Glen, Synthesis and serotonergic activity of a series of 2,*N*-benzylcarboxamido-5-substituted-*N,N*-dimethyltryptamine derivatives: novel antagonists for the vascular 5-HT_{1B}-like receptors, J. Chem. Soc. Perkins Trans. 1 (1999) 2173–2723.

[24] R.C. Glen, G.R. Martin, A.P. Hill, R.M. Hyde, P.M. Woollard, J.A. Salmon, A.D. Robertson, Computer-aided design and synthesis of 5-substituted tryptamines and their pharmacology at the 5-HT_{1D} receptor: discovery of compounds with potential anti-migraine properties, J. Med. Chem. 38 (1995) 3566–3580.

[25] G.R. Martin, S.J. MacLennan, Analysis of the 5-HT receptor in rabbit saphenous vein exemplifies the problems of using exclusion criteria for receptor classification, Naunyn. Schmiedebergs. Arch. Pharmacol. 342 (1990) 111–119.

[26] Sybyl 6.1 molecular modelling package, Tripos Associates, St. Louis, MO, 1992.