Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 7166–7183
Bioorganic & Medicinal Chemistry

# Selective angiotensin II $AT_2$ receptor agonists devoid of the imidazole ring system

A. M. S. Murugaiah,[a] Chalotta Wallinder,[a] A. K. Mahalingam,[a] Xiongyu Wu,[a] Yiqian Wan,[a] Bianca Plouffe,[b] Milad Botros,[c,d] Anders Karlén,[a] Mathias Hallberg,[c,d] Nicole Gallo-Payet[b,†] and Mathias Alterman[a,*]

[a]*Department of Medicinal Chemistry, BMC, Uppsala University, PO Box 574, SE-751 23 Uppsala, Sweden*
[b]*Service of Endocrinology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Que., Canada J1H 5N4*
[c]*Department of Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Que., Canada J1H 5N4*
[d]*Department of Biological Research on Drug Dependence, BMC, Uppsala University, PO Box 591, SE-751 23 Uppsala, Sweden*

Received 19 February 2007; revised 28 June 2007; accepted 6 July 2007
Available online 22 August 2007

**Abstract**—A versatile parallel synthetic method to obtain three series of non-cyclic analogues of the first drug-like selective angiotensin II $AT_2$ receptor agonist (**1**) has been developed. In analogy with the transformation of losartan to valsartan it was demonstrated that a non-cyclic moiety could be employed as an imidazole replacement to obtain $AT_2$ selective compounds. In all the three series, $AT_2$ receptor ligands with affinities in the lower nanomolar range were found. None of the analogues exhibited any affinity for the $AT_1$ receptor. Four compounds, **17**, **22**, **39** and **51**, were examined in a neurite outgrowth cell assay. All four compounds were found to exert a high agonistic effect as deduced from their capacity to induce neurite elongation in neuronal cells, as does angiotensin II.


## 1. Introduction

Angiotensin II (Ang II), recognized as the most important bioactive peptide of the renin-angiotensin system, is the endogenous ligand to the $AT_1$ and the $AT_2$ receptors where it acts as an agonist. The $AT_1$ receptor is closely associated with the regulation of blood pressure, fluid and electrolyte balance, while the role of the $AT_2$ receptor has been less clear due to the low level of $AT_2$ expression in healthy adults.[1] One interesting feature of the $AT_2$ receptor is the high level of expression in most foetal tissues, including the brain. Notably, the $AT_2/AT_1$ receptor ratio decreases dramatically after birth,[2,3] which suggests that the $AT_2$ receptor may be involved in foetal development. This is further supported by the observed induction of neurite outgrowth, elongation and the modulated neuronal excitability upon $AT_2$ receptor activation in cells of neuronal origin.[4,5] In adults, activation of the $AT_2$ receptor exhibits effects that could be considered more 'anti $AT_1$', for example, activation lowers blood pressure, inhibits cell proliferation, induces programmed cell death and extracellular matrix remodelling, as well as axonal regeneration.[6–11] Moreover, $AT_2$ receptor expression is up-regulated in pathological conditions such as heart failure, renal failure, myocardial infarction, brain lesions, vascular injury and wound healing.[9,12–14] In addition, it has been demonstrated that activation of the $AT_2$ receptor stimulates alkaline secretion by the duodenal mucosa in rats.[15]

The $AT_1$ selective antagonist losartan (Fig. 1)[16] was the first drug to be registered for the treatment of high blood pressure via blockage of the $AT_1$ receptor. The hydroxymethyl group of the losartan is metabolized in vivo to the active corresponding carboxylic acid. Valsartan is one of the successors of losartan.[17] An interesting feature of valsartan is that it does not contain the commonly used nitrogen containing heterocycle, found in many of the 'sartans'.[18] In valsartan the imidazole ring is replaced by a tertiary amide and remarkably both the $AT_1$ affinity and the selectivity were maintained with this open chain structural element.

*Keywords*: Angiotensin; $AT_2$; Agonist.
* Corresponding author. Tel.: +46 18 4714905; fax: +46 18 4714474; e-mail addresses: nicole.gallo-payet@usherbrooke.ca; mathias.alterman@ orgfarm.uu.se
† Tel.: +1 819 5645243; fax: +1 819 5645292.

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.07.026

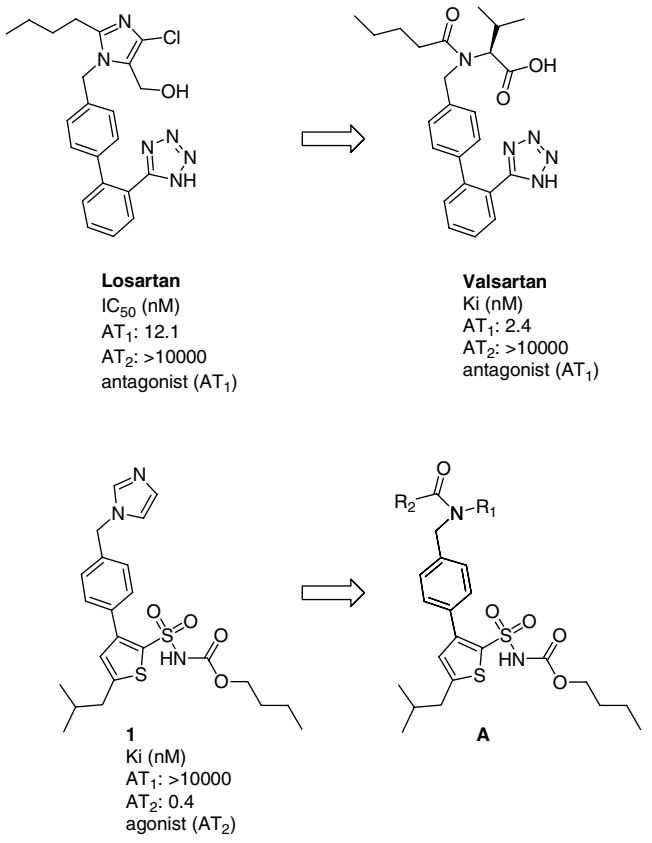



**Figure 1.**

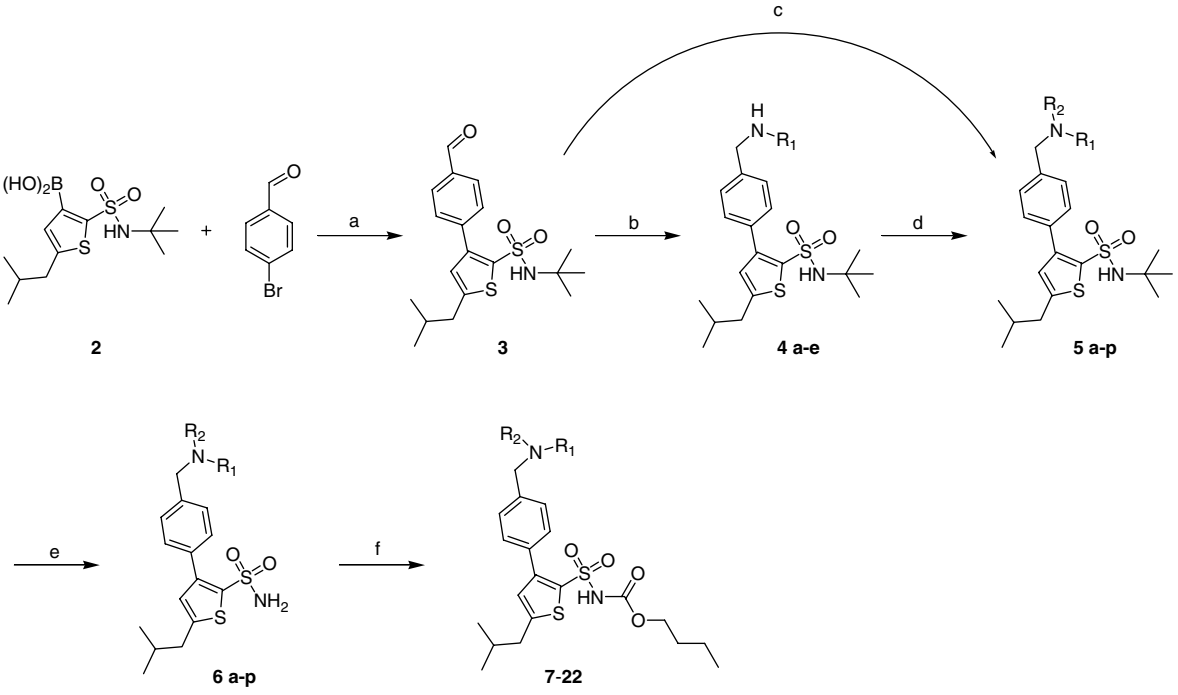
We have recently reported the first drug-like selective $AT_2$ receptor agonist (**1**, Fig. 1).[19] We have also demonstrated that alterations in the 2- and 5-positions of the thiophene moiety modulate the affinity for the $AT_2$ receptor, but did not render any compounds with affinity for the $AT_1$ receptor.[20] Thus, we postulated that the imidazole was a strong determinant for the $AT_2$ selectivity.

The successful conversion of losartan to valsartan (Fig. 1) encouraged us to explore the impact of a tertiary amide as a replacement for the imidazole structure. The question arose whether the imidazole ring, in some system associated with CYP-P450 inhibition, could be favourably displaced and whether $AT_2/AT_1$ selectivity, $AT_2$ receptor agonism and $AT_2$ receptor affinity could be achieved with open chain amide analogues (General structure A, Fig. 1). Herein, we report a synthetic strategy that conveniently gives access to a series of tertiary amide analogues. In addition, a number of these analogues act as high affinity selective $AT_2$ receptor ligands as deduced from the structure–activity relationship data enclosed.

## 2. Results

### 2.1. Chemistry

The thiopheneboronic acid **2** (Scheme 1) was prepared, in essence, as described by Kevin et al.[19–23] Thus, thiophene-2-sulfonyl chloride was first converted to the

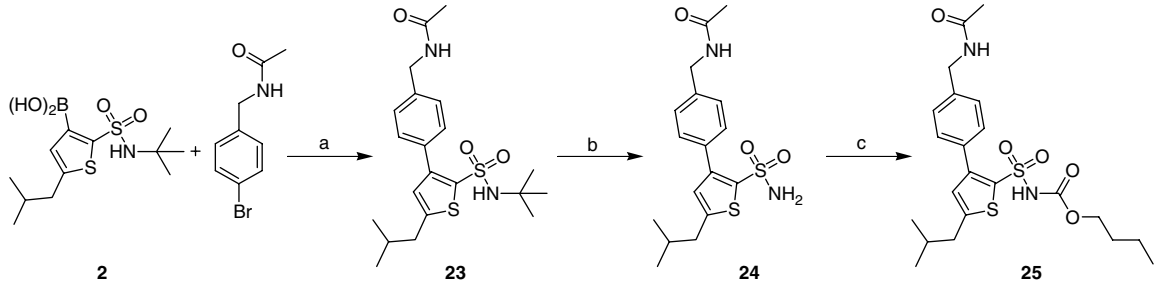



**Scheme 1.** Reagents: (a) $Pd(OAc)_2$, $P(Ph)_3$, $K_2CO_3$, THF/DME/EtOH/$H_2O$; (b) primary amine, $NaBH_4$, MeOH; (c) secondary amine, $NaB(OAc)_3H$, MeOH; (d) acid chloride, $N(Et)_3$, DMAP, $CH_2Cl_2$; (e) TFA, anisole; (f) *n*-butyl chloroformate, pyrrolidinopyridine, $Et_3N$, $CH_2Cl_2$. **4a**: benzylamine, **b**: 3-picolylamine, **c**: 4-aminotoluene, **d**: ethylamine, **e**: methylamine. **5a**: benzylamine, valeroyl chloride, **b**: benzylamine, acetyl chloride, **c**: 3-picolylamine, benzoyl chloride, **d**: 3-picolylamine, acetyl chloride, **e**: 4-aminotoluene, acetyl chloride, **f**: 4-aminotoluene, benzoyl chloride, **g**: ethylamine, acetyl chloride, **h**: ethylamine, thiophene-2-carbonyl chloride, **i**: methylamine, valeroyl chloride, **j**: methylamine, ethyl chloroformate, **k**: methylamine, acetyl chloride, **l**: methylamine, methansulfonyl chloride, **m**: methylamine, ammonium formiate, **n**: dimethylamine, **o**: methylaminoacetonitrile, **p**: *N*-methoxymethylamine.

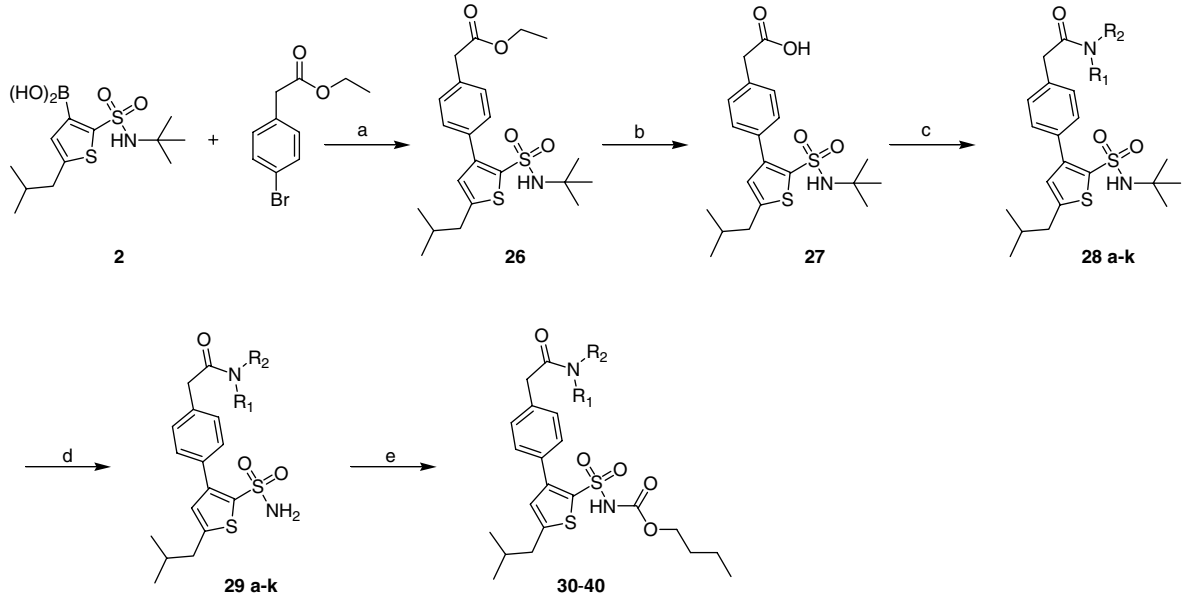
corresponding *N-tert*-butylsulfonamide. Subsequent alkylation followed by selective 3-lithiation/boronation delivered the boronic acid **2**. The boronic acid **2** was then reacted with 4-bromobenzaldehyde under Suzuki coupling conditions with in situ prepared palladium tetrakis as catalyst and $K_2CO_3$ as base to give the aldehyde **3**. This aldehyde (**3**) was used as a key intermediate for the first series (compounds **7**–**22**, Scheme 1).

In the preparation of the first series, the aldehyde **3** was dissolved in methanol and added to sample vials. A diverse set of amines were dispensed to the vials and $NaBH_4$ was added as reducing agent to achieve the reductive amination, with full conversion. In the cases where secondary amines were used, $NaB(OAc)_3H$ was applied as the reducing agent (**5n**–**p**, Scheme 1). The workup was conveniently performed with a diatomaceous earth plug (solid–liquid extraction) in a polypropylene column and eluted with ethyl acetate. The secondary amines (**4a**–**e**, Scheme 1) were obtained in high purity and were therefore used in the second step, or third step for the secondary amines, without further purification. In the second step, the secondary amines (**4a**–**e**) were dissolved in dichloromethane and triethyl amine and DMAP were added. Acid chlorides were added to the reaction vials and the reaction mixtures were stirred overnight at ambient temperature to achieve the tertiary amides **5a**–**p** (to synthesise the formyl compound **5m** ammonium formate was refluxed with the secondary amine **4e**). The same workup as in the previous step was also applied in this step to obtain the tertiary amides (**5a**–**p**) in high yields and with high purity. The *tert*-butyl protecting group was removed from the sulfonamide with TFA and *n*-butyl chloroformate was added to accomplish the final products **7**–**22**, in 45–92% yields after purification on preparative HPLC. To synthesise compound **25**, *N*-(4-bromo-benzyl)-acetamide was coupled directly to the boronic acid **2** under Suzuki conditions. The product was then deprotected and reacted with *n*-butyl chloroformate, as above, to obtain **25** (Scheme 2). This procedure was applied to avoid the reductive amination with ammonia.

To obtain the amides in the second series (compound **30**–**40**) the thiopheneboronic acid **2** was coupled to ethyl-4-bromophenyletanoate using $Pd(OAc)_2$ and




**Scheme 2.** Reagents: (a) $Pd(OAc)_2$, $P(Ph)_3$, $K_2CO_3$, THF/DME/EtOH/$H_2O$; (b) TFA, anisole; (c) *n*-butyl chloroformate, pyrrolidinopyridine, $Et_3N$, $CH_2Cl_2$.



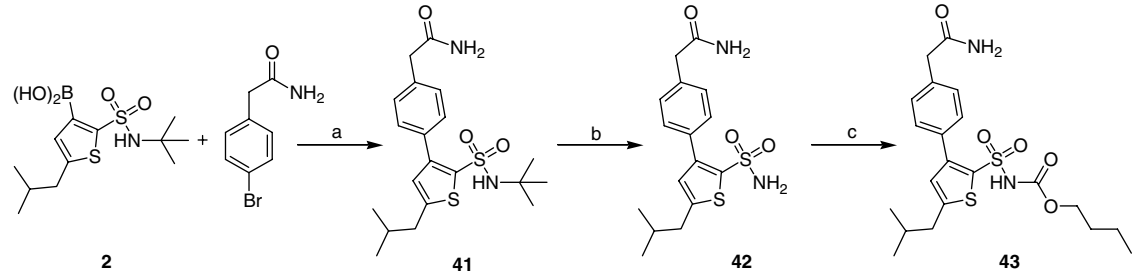

**Scheme 3.** Reagents: (a) $Pd(OAc)_2$, $P(Ph)_3$, $K_2CO_3$, CsF, DME; (b) LiOH, THF/$H_2O$/MeOH; (c) amine, EDC, HOBT, $N(Et)_3$, DMF; (d) TFA, anisole; (e) *n*-butyl chloroformate, pyrrolidinopyridine, $Et_3N$, $CH_2Cl_2$. **28a**: methylbutylamine, **b**: benzylamine, **c**: ethylbenzylamine, **d**: dibenzylamine, **e**: diphenylamine, **f**: morpholine, **g**: thiazolidine, **h**: 4-tolylamine, **i**: hexylamine, **j**: dimethylamine, **k**: methylamine.

DPPF as catalyst. The mild base CsF was employed in the Suzuki reaction to avoid the hydrolysis of the ester functionality. Compound **26** was isolated in 95% yield (Scheme 3). The ester was then hydrolyzed with LiOH to afford the carboxylic acid **27** that was used as a key intermediate (Scheme 3). The carboxylic acid **27** was thereafter coupled to a selection of amines using EDC and HOBT as coupling reagents. Though the reaction was run in parallel, we did not yield products of sufficient purity after solid phase extraction work up as in the aforesaid step, presumably due to the use of DMF as solvent. Hence, liquid extraction was applied to obtain the compounds pure enough to carry forward to the next step. The subsequent deprotection with TFA and final reaction of the sulfonamides with *n*-butyl chloroformate afforded the compounds **30**–**40** in 46–77% yields after purification on preparative HPLC. For similar reasons as those for compound **25**, compound **43** was synthesised by directly coupling 2-(4-bromo-phenyl)-acetamide with the boronic acid **2** (Scheme 4). The resulting compound **41** was thereafter deprotected and reacted with *n*-butyl chloroformate, as above, to obtain **43**.

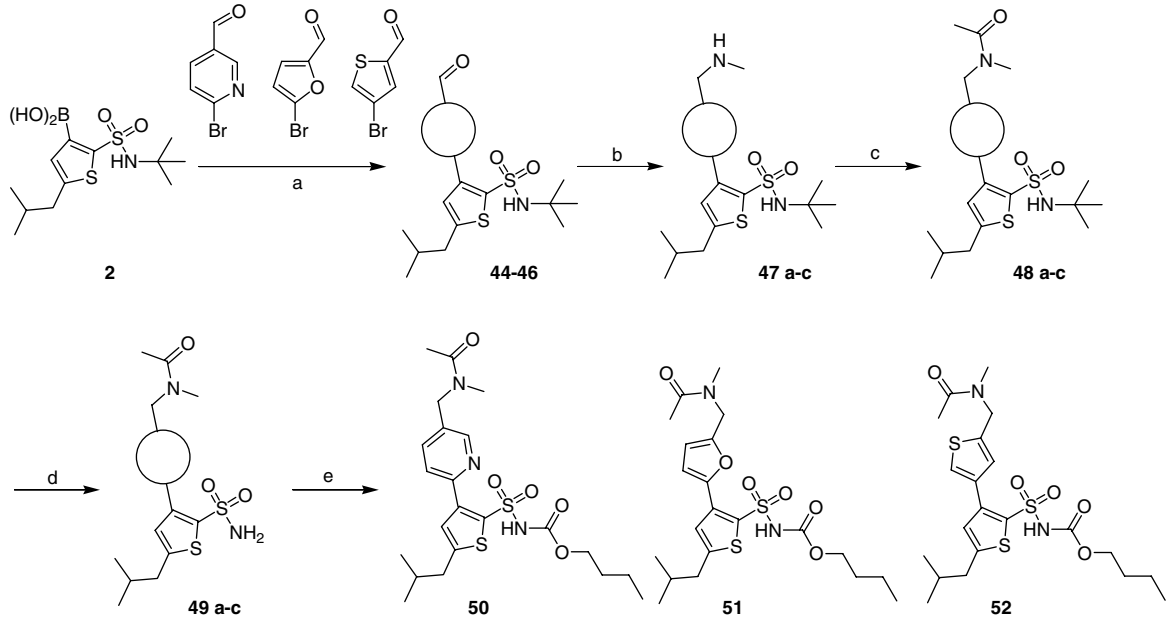
In the third series, the central phenyl ring was exchanged with five- and six-membered heterocycles. The thiopheneboronic acid **2** was coupled with 6-bromo-pyridine-3-carbaldehyde, 5-bromofuran-2-carboxaldehyde and 4-bromothiophene-2-carboxaldehyde using the same Suzuki coupling conditions as in the second series (Scheme 5). This protocol gave the three aldehydes **44**–**46** in modest yields (51–63%). The aldehydes were then reacted with methylamine and $NaBH_4$ followed by acetyl chloride as described for series one. TFA treatment and reaction with *n*-butyl chloroformate delivered the compounds **50**–**52** in 51–68% yields.

## 2.2. Binding assays

Compounds **7**–**22**, **25**, **30**–**40**, **43** and **50**–**52** were evaluated in radioligand-binding assays by displacement of [$^{125}$I]Ang II from $AT_1$ receptors in rat liver membranes and from $AT_2$ receptors in pig uterus membranes as described previously (Tables 1–3).[24,25] The natural substrate Ang II, the selective $AT_1$ receptor antagonist losartan,[16] and the selective $AT_2$ receptor antagonist PD 123,319[26] were used as reference substances.

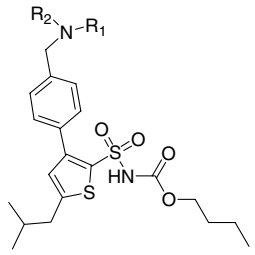


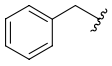
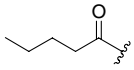
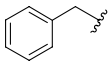
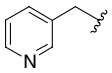
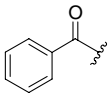
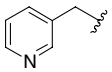
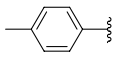
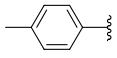
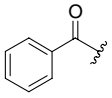
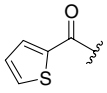
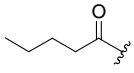
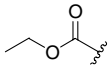

**Scheme 4.** Reagents: (a) $Pd(OAc)_2$, $P(Ph)_3$, $K_2CO_3$, DME; (b) TFA, anisole; (c) *n*-butyl chloroformate, pyrrolidinopyridine, $Et_3N$, $CH_2Cl_2$.




**Scheme 5.** Reagents: (a) $Pd(OAc)_2$, DPPF, $K_2CO_3$, DME/EtOH/$H_2O$; (b) $MeNH_2$, $NaBH_4$, MeOH; (c) acetyl chloride, $Et_3N$, DMAP, $CH_2Cl_2$; (d) TFA, anisole; (e) *n*-butyl chloroformate, pyrrolidinopyridine, $Et_3N$, $CH_2Cl_2$.

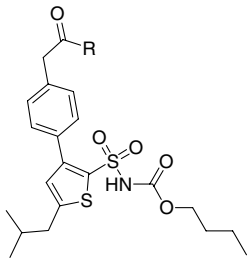
**Table 1.**

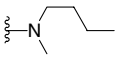
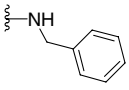
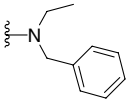
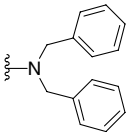
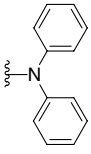
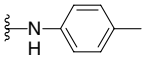
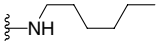


| Entry | Compound | $R_1$ | $R_2$ | $K_i$[a] (nM) | |
|---|---|---|---|---|---|
| | | | | $AT_2$ | $AT_1$ |
| 1 | **7** |  |  | 84 | >10,000 |
| 2 | **8** |  |  | 200 | >10,000 |
| 3 | **9** |  |  | 404 | >10,000 |
| 4 | **10** |  |  | 59 | >10,000 |
| 5 | **11** |  |  | 60 | >10,000 |
| 6 | **12** |  |  | 46 | >10,000 |
| 7 | **13** |  |  | 126 | >10,000 |
| 8 | **14** |  |  | 138 | >10,000 |
| 9 | **15** |  |  | 12 | >10,000 |
| 10 | **16** |  |  | 9.7 | >10,000 |
| 11 | **17** |  |  | 8.8 | >10,000 |
| 12 | **18** |  |  | 32 | >10,000 |
| 13 | **19** |  |  | 3.1 | >10,000 |
| 14 | **20** |  |  | >10,000 | >10,000 |
| 15 | **21** |  |  | >10,000 | >10,000 |
| 16 | **22** |  |  | 2.8 | >10,000 |
| 17 | **25** |  |  | 5.8 | >10,000 |

[a] $K_i$ values are an average from three determinations. Standard deviations are less than 15% in all cases.

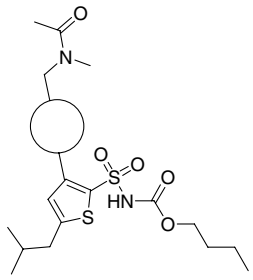
**Table 2.**

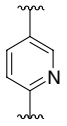
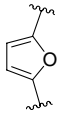
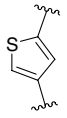


| Entry | Compound | R | $K_i$[a] (nM) | |
|---|---|---|---|---|
| | | | $AT_2$ | $AT_1$ |
| 1 | **30** |  | 367 | >10,000 |
| 2 | **31** |  | 221 | >10,000 |
| 3 | **32** |  | 322 | >10,000 |
| 4 | **33** |  | 185 | >10,000 |
| 5 | **34** |  | 219 | >10,000 |
| 6 | **35** |  | 124 | >10,000 |
| 7 | **36** |  | 133 | >10,000 |
| 8 | **37** |  | 58 | >10,000 |
| 9 | **38** |  | 158 | >10,000 |
| 10 | **39** |  | 7.0 | >10,000 |
| 11 | **40** |  | 80 | >10,000 |
| 12 | **43** |  | 51 | >10,000 |

[a] $K_i$ values are an average from three determinations. Standard deviations are less than 15% in all cases.

**Table 3.**



| Entry | Compound | Aromatic structure | $K_i$[a] (nM) | |
|---|---|---|---|---|
| | | | $AT_2$ | $AT_1$ |
| 1 | **50** |  | >10,000 | >10,000 |
| 2 | **51** |  | 9.1 | >10,000 |
| 3 | **52** |  | >10,000 | >10,000 |

[a] $K_i$ values are an average from three determinations. Standard deviations are less than 15% in all cases.

In the first series, the imidazole ring of compound **1** was substituted with a tertiary amide, sulfonamide or amine. As seen in Table 1 none of the compounds in the first series showed any $AT_1$ affinity, which is notable considering the high $AT_1$ selectivity that valsartan exhibits. All of the compounds except the tertiary amines exhibited moderate to high affinity towards the $AT_2$ receptor. A pattern that could be seen from the first series was that the smallest substituents gave the highest affinity for the $AT_2$ receptor. This trend is corroborating with our earlier observations regarding the nitrogen containing heterocycles.[19] Although the trend was not linear and fully consistent; for example, compound **11** has a larger substituent in $R_1$ than compound **13**, but is twice as active. By removing a methyl from compound **17**, either compound **19** or **25** could be obtained depending if $R_1$ or $R_2$ lacks a methyl group. Independently of which side that lacks the methyl group the affinity was improved, but with a slight advantage in affinity for compound **19**. The introduction of a nitrogen into the phenyl ring gave a fourfold increased affinity for the $AT_2$ receptor (cf. **8** and **10**), although the 3-pyridyl moiety in combination with benzoyl in $R_2$ gave the least potent compound in the series (compound **9**).

In the first series, four compounds lacked an amide as structural element. Compound **20** encompassing the tertiary amine in the sidechains was found to be inactive against the $AT_2$ receptor. This was also the case for the acetonitrile compound **21**. However, the sulfonamide compound **18** showed good affinity for the $AT_2$ receptor (32 nM), although not as high as for the comparable amide compound (**17**, 8.8 nM). The *N*-methoxymethylamide compound (**22**, 2.8 nM) exhibited the highest affinity in the first series.

In the second series, the amide moiety is reversed. This series was prepared to allow an assessment of impact of the position of the carbonyl and nitrogen atom. Inter-

estingly, the dimethyl compound **39** (Table 2) was found to display a slightly inferior $K_i$ value than the corresponding compound in the first series (compound **17**, Table 1). However, deletion of the methyl groups, that is, compound **43**, leads to a substantial decrease in the affinity. This was also the case when only one of the methyl groups was replaced with a hydrogen (**40**). The introduction of larger or heterocyclic amine substituents did not render any highly potent compounds (compounds **30**–**38**, Table 2).

By modifying the central phenyl ring, not only are the electrostatic properties of the compounds altered, but also the angles of which the benzylic amide substituents are presented to the $AT_2$ receptor. As seen in Table 3 only the furanyl compound **51** showed any affinity for the $AT_2$ receptor. It is notable that this compound exhibits similar affinity as the related compound **17**.

### 2.3. In vitro morphological effects induced by 17, 22, 39 and 51 in NG108-15 cells

To study the effects of compounds **17**, **22**, **39** and **51** on $AT_2$ induced differentiation, NG108-15 cells were used. In their undifferentiated state, neuroblastoma × glioma hybrid NG108-15 cells have a rounded shape and divide actively. We have shown previously that these cells express only the $AT_2$ receptor[27,28] and that a 3-day treatment with Ang II or the selective peptidic $AT_2$ receptor agonist CGP-42112 induces neurite outgrowth.[28] The mechanisms involve a sustained increase in $p42/p44^{mapk}$ activity[5] and activation of the nitric oxide/guanylyl cyclase/cGMP pathway (for a review see Ref. 11).

The cells were plated at the same initial density ($3.6 \times 10^4$ cells/35 mm Petri dish) and were treated in with or without of Ang II, compound **17**, **22**, **39** or **51**. Ang II, compounds **17** and **22** were tested at one occasion and Ang II, compounds **39** and **51** on a second occasion. After 3 days of culture, cells were examined under a phase contrast microscope and micrographs were taken. The compounds **17**, **22**, **39** and **51** were first examined at concentrations ranging from 1 pM to 1 μM. Except for the higher concentration of 1 μM, none of the other doses induced cell death. As shown in Figures 2 and 3, treatment for 3 days with compounds **17** (1 nM), **22** (1 nM), **39** (0.1 μM) and **51** (1 nM) induced neurite outgrowth, comparable with Ang II. This effect was mediated through the $AT_2$ receptor, since co-incubation of **17**, **22**, **39** and **51** with the $AT_2$ receptor antagonist, PD 123,319,[21] virtually abolished neurite elongation (Figs. 2 and 3), while alone, PD 123,319 did not alter the morphology compared to the untreated cells (data not shown).

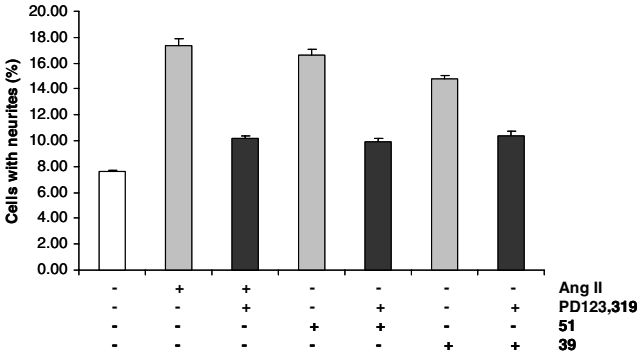



**Figure 2.** Effect of **17** and **22** on neurite outgrowth in NG108-15 cells. NG108-15 cells were plated at a density of $3.6 \times 10^4$ cells per dish in 35 mm Petri dishes and were cultured for 3 days in the absence or in the presence of 0.1 μM Ang II, compound **17** (1 nM) or compound **22** (1 nM), alone or in the presence of 1 μM PD 123,319. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. The number of cells with neurites represents the percentage of the total number of cells in the micrographs (at least 290 cells according to the experiment).

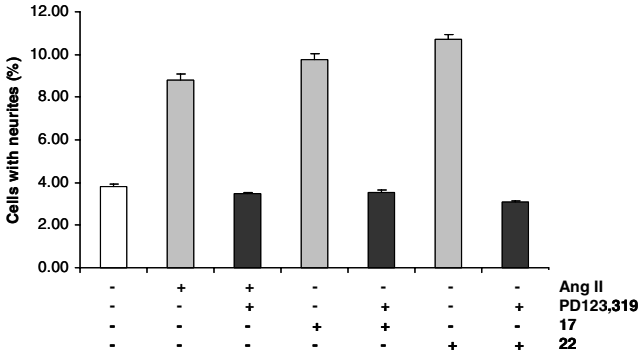



**Figure 3.** Effect of **51** and **39** on neurite outgrowth in NG108-15 cells. NG108-15 cells were plated at a density of $3.6 \times 10^4$ cells per dish in 35 mm Petri dishes and were cultured for 3 days in the absence or in the presence of 0.1 μM Ang II, compound **51** (1 nM) or compound **39** (0.1 μM), alone or in the presence of 10 μM PD 123,319. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. The number of cells with neurites represents the percentage of the total number of cells in the micrographs (at least 290 cells according to the experiment).

## 3. Discussion

Replacement of the imidazole in compound **1** with a tertiary amide rendered analogues with high affinity for the $AT_2$ receptor. Thus, a modification resembling the losartan to valsartan transformation was also allowed with $AT_2$ ligands. Our previous results suggested that the imidazole might be a strong determinant for the $AT_2$ selectivity.[20] The fact that the nitrogen and oxygen of the amide could occupy the same positions as the two nitrogens in the imidazole ring provides a rationale for the high $AT_2$ receptor affinities found with analogues bearing small substituents. However, when considering the balanced nature of the compound L-162,313[23,29,30] ($K_i$ $AT_1$: 3.9 nM, $AT_2$: 2.8 nM), which was used as a template for compound **1**, it was unexpected to find that none of the compounds in the first series possessed any $AT_1$ receptor affinity, especially compounds with larger substituents, for example, **7**–**12**. The non-cyclic amide structure cannot fully explain the lack of $AT_1$ affinity since valsartan, with its non-cyclic structure in the benzylic position, is an $AT_1$ selective antagonist. It seems more likely that a combination of a substituent in the

5-position of the thiophene and the structure of the benzylic moiety is responsible for the $AT_2$ selectivity of the structures.

Considering $AT_2$ receptor affinities, the trend was that smaller substituents were more favorable than larger lipophilic structures. In addition, a hydrogen bond-acceptor atom has to be present in the structure and at the right distance from the benzylic nitrogen to obtain any $AT_2$ receptor affinity (cf. compounds **20** and **21**). It was therefore interesting to find that the *N*-methoxymethyl amine analogue (**22**, 2.8 nM) showed the highest affinity for the $AT_2$ receptor of the compounds in the first series. It seems that the position of the oxygen in the amide is not the optimal distance for the $AT_2$ receptor.

To further study the effect of the position of the heteroatoms the second series, with reversed amide bonds, were examined. Our hypothesis was that the reversed amide might adopt the position of the oxygen in the compound **22**. As seen from the result a high affinity analogue was obtained in this series (**39**, 7.0 nM), which displayed a slight improvement in affinity compared to the non-switched compound **17** (8.8 nM), although the affinity was not as good as for the compound **22**. As in the first series, the preference in the second series was for smaller substituents, although when one or two methyl groups were removed from compound **39** (compounds **40** and **43**) a large drop in affinity was encountered.

With the introduction of a heterocycle in the central phenyl position, as exemplified in the third series, the angle of which the amide is presented to the receptor was changed slightly and the electrostatic properties of the aromatic ring were also altered. Interestingly, the furanyl compound **51** (9.1 nM) was found to exert a high affinity for the $AT_2$ receptor whereas both the thiophene and the pyridine were inactive. The affinity of the furanyl compound was similar to that of the corresponding phenyl compound **17**. This suggests that a thienylfuran bicyclic system could provide an alternative core structure to be considered in further optimization processes.

To study the effect of the structural modifications on the agonistic properties, the most potent $AT_2$ ligands in the three series, compounds **22**, **39** and **51**, and as a reference to the compounds **39** and **51**, that is, compound **17**, were selected for further in vitro studies to determine if the compounds acted as $AT_2$ agonists. Our results demonstrate that all four compounds induce neurite outgrowth (Figs. 2 and 3), one of the first steps of neuronal differentiation, as do Ang II and the $AT_2$ selective peptide CGP-42112.[28] Hence, as deduced from these data the determinant for the agonistic property appears not to be located in benzylic region of the structure.

## 4. Conclusion

In summary, we have presented a versatile parallel synthetic route to obtain series of selective $AT_2$ receptor ligands. In addition, we demonstrated that the imidazole heterocycle can be displaced with a small non-cyclic moiety and that high $AT_2$ receptor selectivity and agonism is retained after these modifications. In all the three series, $AT_2$ receptor ligands with affinity in the lower nanomolar range were identified. None of the analogues, regardless of the substituents, exhibited any affinity for the $AT_1$ receptor. This finding is notable considering the structural relationship to the $AT_1$ selective antagonist valsartan. The presence as well as the distance of a hydrogen bond accepting atom, in the amine substituents of the first series, appears to be important for the binding to the $AT_2$ receptor as deduced from the compounds **20** and **21**, that lack affinity for the $AT_2$ receptor. Furthermore, all of the four compounds **17**, **22**, **39** and **51** were found to induce neurite elongation in NG108-15 cells and served as potent $AT_2$ selective agonists.

## 5. Experimental

### 5.1. Chemistry

**5.1.1. General considerations.** $^1H$ and $^{13}C$ NMR spectra were recorded on a JEOL JNM-EX 270 spectrometer at 270.2 and 67.8 MHz, respectively. Chemical shifts are given as $\delta$ values (ppm) downfield from tetramethylsilane. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden or Analytische Laboratorien, Lindlar, Germany. Flash chromatography was performed on silica gel 60 (0.04–0.063 mm, E. Merck). Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm, E. Merck) and was visualized with UV light. Analytical RP-LC/MS was performed on a Gilson HPLC system with a Zorbax SB-C8, 5 μm 4.6 × 50 mm (Agilent Technologies) column, with a Finnigan AQA quadropole mass spectrometer at a flow rate of 1.5 mL/min ($H_2O$/$CH_3CN$/0.05% HCOOH). All the organic phases were dried over $MgSO_4$, unless otherwise stated. All chemicals were purchased from commercial suppliers and used directly without further purification.

**5.1.1.1. 3-(4-Formylphenyl)-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (3).** Palladium acetate (69.6 mg, 0.31 mmol) and triphenylphosphine (0.33 g, 1.24 mmol) in THF (5 mL) were stirred for 30 min under $N_{2(g)}$. The solvent was removed in vacuo and the residue was dissolved in DME (5 mL). The catalyst was then transferred into a nitrogen-flushed mixture of 2 (1.11 g, 3.13 mmol), 4-bromobenzaldehyde (1.45 g, 7.84 mmol), and $K_2CO_3$ (1.73 g, 12.5 mmol) in a solvent mixture of DME (10 mL), ethanol (3 mL), and water (2 mL). After stirring for 20 h at reflux under $N_2$ atmosphere, the reaction mixture was diluted with 1 M NaOH solution (20 mL) followed by ethyl acetate (70 mL). The organic layer was washed with water, and brine, dried over anhydrous $MgSO_4$, concentrated in vacuo, and the residue subjected to flash chromatographic purification (20% ethyl acetate in pet. ether) to afford 3 as colourless solid (0.76 g, 64%). $^1H$ NMR ($CDCl_3$), $\delta$: 0.97 (d, $J$ = 6.6 Hz, 6H), 1.01 (s, 9H), 1.94 (m, 1H), 2.68 (d, $J$ = 6.6 Hz, 2H), 4.24 (s, 1H),

6.78 (s, 1H), 7.78 (d, $J$ = 8.3 Hz, 2H), 7.95 (d, $J$ = 6.6 Hz, 2H), 10.04 (s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 22.1, 29.6, 30.5, 39.1, 54.7, 128.7, 129.6, 129.8, 135.7, 137.5, 141.0, 141.8, 149.0, 191.8; IR (compression cell), cm$^{-1}$: 3284, 2963, 1702, 1606; Anal. Calcd for $C_{19}H_{25}NO_3S_2$: C, 60.13; H, 6.64, N, 3.69; O, 12.65; S, 16.9. Found: C, 59.9; H, 6.6; N, 3.6; O, 12.4; S, 16.8.

### 5.1.2. General procedure to compound 7–22

*Step 1.* To a solution of **3** (30 mg, 0.08 mmol) in methanol (1.5 mL) taken in a sample vial (5 mL size), amine (1.1 equiv, 0.09 mmol) was added. After being stirred for 2 h, $NaBH_4$ (6.1 mg, 0.16 mmol, or $NaB(OAc)_3H$ for the secondary amines) was added and the stirring continued for 2 h. The mixture was acidified with dilute HCl (5 M, 0.1 mL), stirred for 10 min, neutralised with saturated $NaHCO_3$ solution (~0.5 mL) and diluted with ethyl acetate (10 mL). The contents were poured into diatomaceous earth (solid–liquid extraction catridge) in a polypropylene column (packed for 1.5 cm, 24 mL size) and eluted with ethyl acetate (30 mL). Concentration under vacuum afforded the crude product.

*Step 2.* The preceding product was dissolved in dry $CH_2Cl_2$ (1.5 mL) in a sample vial (5 mL size). Triethylamine (0.033 mL, 0.24 mmol), *N*,*N*-dimethylaminopyridine (1 mg, 0.008 mmol) and acid chloride (2 equiv, 0.16 mmol) were then added sequentially. The sample vial was tightly closed. The mixture was stirred overnight, quenched with aqueous saturated $NaHCO_3$ solution (0.5 mL), stirred for 30 min, and filtered through diatomaceous earth (packed for 1.5 cm in the column of 24 mL capacity) on elution with $CH_2Cl_2$ (30 mL). Concentration in vacuo afforded the crude product.

*Step 3.* The mixture of the above product and anisole (~2 drops) in trifluoroacetic acid (1.5 mL) in a sample vial (5 mL size) was stirred at 30 °C overnight. After the removal of the solvent in vacuo, the residue was dissolved in acetonitrile (2 mL) and evaporated (2×).

*Step 4.* To a mixture of the preceding product in dry $CH_2Cl_2$ (1.5 mL), pyrrolidinopyridine (17.8 mg, 0.12 mmol) and triethylamine (0.5 mL, 0.36 mmol), *n*-butyl chloroformate (0.04 mL, 0.3 mmol) were sequentially added. The solution was stirred for 12 h, concentrated in vacuo and the crude product was purified by preparative LC–MS to afford the products **7**–**22**.

**5.1.2.1. *N*-Butyloxycarbonyl-3-[4-(*N*-benzyl-pentylamidomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (7).** The compound **7** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (benzylamine and valeroyl chloride). The crude product in the final step was purified by LC–MS (30% aqueous acetonitrile to pure acetonitrile, reverse phase) to afford **7** as a colourless solid (26 mg, 55%).

Spectroscopic data of step 1: $^1$H NMR ($CDCl_3$), $\delta$: 0.82–1.08 (m, 15H), 1.82–2.1 (m, 2H), 2.69 (d, $J$= 6.9 Hz, 2H), 3.76 (d, $J$ = 11.9 Hz, 4H), 4.08 (s, 1H), 6.77 (s, 1H), 7.23–7.48 (m, 5H), 7.44 (d, $J$ = 7.9 Hz, 2H), 7.59 (d, $J$ = 8.3 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 22.1, 29.4, 30.5, 39.1, 52.6, 53.0, 54.4, 125.7, 127.0, 128.1, 128.3, 128.4, 128.8, 129.0, 133.6, 136.1, 139.8, 140.5, 143.0, 148.2; IR (compression cell, cm$^{-1}$) $v$: 3294, 2962, 1465, 1391, 1367, 1312, 1051. Anal. ($C_{26}H_{34}N_2O_2S_2$): C, H, N.

Spectroscopic data of step 2 after column chromatography purification (pet. ether/EtOAc 7:3): $^1$H NMR ($CDCl_3$), $\delta$: 0.8–1.08 (m, 18H), 1.28–1.44 (m, 2H), 1.62–2.0 (m, 3H), 2.36–2.50 (m, 2H), 2.63–2.73 (m, 2H), 4.25 (s, 1H), 4.47 (d, $J$ = 3.6 Hz, 2H), 4.61 (d, $J$ = 6.3 Hz, 2H), 6.75 (d, $J$ = 4.3 Hz, 1H), 7.12–7.42 (m, 7H), 7.52–7.65 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.9, 22.1, 22.5, 27.5, 29.4, 30.5, 32.9, 39.1, 47.8, 48.1, 49.6, 50.1, 54.4, 126.3, 126.5, 127.4, 127.6, 128.2, 128.6, 128.9, 128.9, 129.2, 129.6, 133.9, 134.3, 136.4, 137.0, 137.3, 137.9, 142.6, 142.8, 148.3, 148.5, 173.6, 173.8; IR (compression cell, cm$^{-1}$) $v$: 3292, 2958, 1650, 1434, 1312, 1206, 1143, 1051. Anal. ($C_{31}H_{42}N_2O_3S_2$): C, H, N.

Spectroscopic data of step 3 after column chromatography purification (pet. ether/EtOAc 7:3): $^1$H NMR ($CDCl_3$), $\delta$: 0.91 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.28–1.44 (m, 2H), 1.62–1.76 (m, 2H), 1.84–2.0 (m, 1H), 2.38–2.48 (m, 1H), 2.63–2.72 (m, 2H), 4.45–4.80 (m, 6H), 6.77 (d, $J$ = 6.3 Hz, 1H), 7.13–7.42 (m, 8H), 7.50–7.61 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.9, 22.2, 22.5, 27.5, 30.5, 32.9, 39.1, 48.2, 49.8, 50.5, 126.3, 126.6, 128.2, 128.6, 128.9, 129.1, 129.3, 129.5, 133.4, 133.7, 134.8, 136.4, 137.2, 137.3, 138.1, 143.3, 143.4, 148.3, 148.5, 173.7, 174.0; IR (compression cell, cm$^{-1}$) $v$: 2958, 1634, 1466, 1432, 1341, 1161. Anal. (C27H34N2O3S$_2$ × 1/3 $H_2O$): C, H, N.

Spectroscopic data of compound **7**: $^1$H NMR ($CDCl_3$), $\delta$: 0.83–1.03 (m, 12H), 1.17–1.44 (m, 6H), 1.68 (m, 2H), 1.95 (m, 1H), 2.44 (dt, $J$ = 1.25, 8.3 Hz, 2H), 2.66–2.74 (m, 2H), 4.05 (q, $J$ = 5.3 Hz, 2H), 4.43–4.68 (m, 4H), 6.76 (d, $J$ = 7.9 Hz, 1H), 7.1–7.50 (m, 9H), 7.60–7.95 (br s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 13.9, 18.7, 22.2, 22.5, 27.5, 30.4, 30.5, 33.0, 39.3, 48.1, 49.7, 50.4, 66.8, 66.9, 126.4, 127.4, 127.7, 128.0, 128.3, 128.6, 128.9, 129.1, 129.5, 130.6, 133.0, 133.3, 136.3, 137.2, 137.3, 138.0, 146.1, 146.2, 150.2, 151.4, 151.6, 173.9, 174.1; IR (compression cell), cm$^{-1}$: 2959, 2871, 1748, 1626, 1453; Anal. Calcd for $C_{32}H_{42}N_2O_5S_2$: C, 64.18; H, 7.07; N, 4.68. Found: C, 63.8; H, 7.0; N, 4.70.

**5.1.2.2. *N*-Butyloxycarbonyl-3-[4-(*N*-acetyl-*N*-benzyl-aminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (8).** The compound **8** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (benzylamine and acetyl chloride). The crude product in the final step was purified by LC–MS (25% aqueous acetonitrile to pure acetonitrile, reverse phase) to afford **8** as a colourless solid (30 mg, 68%). $^1$H NMR ($CDCl_3$), $\delta$: 0.86 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 7.4 Hz, 6H), 1.25 (m, 2H), 1.49 (m, 2H), 1.94 (m, 1H), 2.2 (d, 3 H,

$J$ = 3.6 Hz), 2.66–2.75 (m, 2H), 3.98–4.08 (m, 2H), 4.46–4.60 (m, 4H), 6.75 (d, 1 H, $J$ = 8.6 Hz), 7.14–7.51 (m, 9H), 8.25 (br d, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 21.5, 22.2, 29.6, 30.4, 30.5, 39.3, 48.0, 50.5, 51.1, 66.7, 66.8, 126.3, 127.4, 127.7, 128.0, 128.3, 128.6, 129.0, 129.1, 129.4, 129.5, 130.7, 133.2, 133.4, 136.0, 136.9, 137.0, 137.6, 145.9, 146.1, 150.3, 151.3, 151.5, 171.4, 171.6; IR (compression cell), cm$^{-1}$: 2955, 1747, 1629, 1451; Anal. Calcd for $C_{29}H_{36}N_2O_5S_2$: C, 62.56; H, 6.52; N, 5.03. Found: C, 62.8; H, 6.7; N, 5.0.

**5.1.2.3. *N*-Butyloxycarbonyl-3-{4-[*N*-(pyridin-3-ylmethyl)-benzylamidomethyl]phenyl}-5-*iso*-butylthiophene-2-sulfonamide (9).** The compound **9** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (3-picolylamine, benzoyl chloride). The crude product in the final step was purified by LC–MS (45% aqueous acetonitrile to pure acetonitrile, reverse phase) to afford **9** as a colourless solid (46 mg, 92%). $^1$H NMR ($CDCl_3$), $\delta$: 0.89 (t, $J$=7.3 Hz, 3H), 0.97 (d, $J$ = 6.6 Hz, 6H), 1.30 (m, 2H), 1.55 (m, 2H), 1.92 (m, 1H), 2.68 (d, $J$ = 6.9 Hz, 2H), 4.08 (t, $J$ = 6.6 Hz, 2H), 4.58–4.82 (m, 4H), 6.6 (br s, 1H), 6.9–7.54 (m, 11H), 8.23–8.43 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.7, 18.9, 22.2, 30.5, 30.7, 39.2, 48.1, 50.9, 53.5, 55.9, 65.9, 123.5, 123.9, 126.1, 126.5, 128.3, 128.8, 129.3, 129.7, 133.0, 134.1, 134.8, 135.9, 136.7, 139.9, 144.4, 146.5, 147.5, 147.3, 148.3, 148.4, 150.3, 152.0, 172.1; IR (compression cell), cm$^{-1}$: 2959, 1740, 1635, 1457, 1410; Anal. Calcd for $C_{33}H_{37}N_3O_5S_2$: C, 63.95; H, 6.02; N, 6.78. Found: C, 63.6; H, 6.2; N, 6.4.

**5.1.2.4. *N*-Butyloxycarbonyl-3-{4-[*N*-acetyl-*N*-(pyridin-3-ylmethyl)-aminomethyl]phenyl}-5-*iso*-butylthiophene-2-sulfonamide (10).** The compound **10** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (3-picolylamine, benzoyl chloride). The crude product in the final step was purified by LC–MS (45% aqueous acetonitrile to 90% acetonitrile, reverse phase) to afford **10** as a colourless syrup (38 mg, 86%). $^1$H NMR ($CDCl_3$), $\delta$: 0.89 (t, $J$ = 7.6 Hz, 3H), 0.97 (d, $J$ = 6.6 Hz, 6H), 1.26 (m, 2H), 1.53 (m, 2H), 1.93 (m, 1H), 2.27 (m, 3H), 2.68 (d, $J$ = 6.9 Hz, 2H), 4.07 (m, 2H), 4.49 (s, 2H), 4.65 (t, $J$ = 6.9 Hz, 2H), 6.68 (m, 1H), 6.93–7.36 (m, 6H), 7.61–8.45 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.8, 22.2, 30.4, 30.6, 39.2, 48.2, 50.8, 52.2, 54.9, 65.8, 123.6, 127.2, 128.0, 128.5, 129.3, 129.7, 132.0, 132.5, 133.1, 133.6, 133.9, 134.7, 136.1, 136.4, 138.7, 139.9, 144.3, 145.0, 146.2, 147.4, 147.9, 148.2, 150.3, 151.6, 152.0, 170.6, 170.8, IR (compression cell), cm$^{-1}$: 2960, 1742, 1650, 1429; Anal. Calcd for $C_{28}H_{35}N_3O_5S_2 \times 1/2H_2O$: C, 59.30; H, 6.4; N, 7.4. Found: C, 59.5; H, 6.1; N, 7.4.

**5.1.2.5. *N*-Butyloxycarbonyl-3-[4-(*N*-acetyl-*N*-*p*-tolylaminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (11).** The compound **11** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (*p*-tolylamine and acetyl chloride). The crude product in the final step was purified by LC–MS (40% aqueous acetonitrile to pure acetonitrile, reverse phase) to afford **11** as a colourless solid (30 mg, 68%). $^1$H NMR ($CDCl_3$), $\delta$: 0.86 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.24 (m, 2H), 1.48 (m, 2H), 1.82–12.1 (m, 4H), 2.33 (s, 3H), 2.69 (d, $J$ = 7.3 Hz, 2H), 4.02 (t, $J$ = 6.6 Hz, 2H), 4.83 (s, 2H), 6.76 (s, 1H), 6.88 (d, $J$ = 8.3 Hz, 2H), 7.13 (d, $J$ = 8.3 Hz, 2H), 7.23 (d, $J$ = 7.9 Hz, 2H), 7.39 (d, $J$ = 8.3 Hz, 2H), 8.36 (s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.8, 21.1, 22.2, 22.5, 29.7, 30.5, 39.3, 52.6, 66.7, 127.8, 128.7, 128.9, 129.3, 130.2, 130.9, 133.1, 138.0, 140.0, 146.0, 150.3, 151.2, 171.1; IR (compression cell), cm$^{-1}$: 2960, 1747, 1636, 1513, 1465; Anal. Calcd for $C_{29}H_{36}N_2O_5S_2 \times 1/2H_2O$: C, 61.6; H, 6.6; N, 4.9. Found: C, 61.7; H, 6.5; N, 4.8.

**5.1.2.6. *N*-Butyloxycarbonyl-3-[4-(*N*-*p*-tolyl-benzylamidomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (12).** The compound **12** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (*p*-tolyamine, benzoyl chloride). The crude product in the final step was purified by LC–MS (45% aqueous acetonitrile to pure acetonitrile, reverse phase) to afford **12**as a colourless solid (46 mg, 92%). $^1$H NMR ($CDCl_3$), $\delta$: 0.85 (t, $J$ = 7.6 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.24 (m, 2H), 1.48 (m, 2H), 1.93 (m, 1H), 2.23 (s, 3H), 2.69 (d, $J$ = 6.9 Hz, 2H), 3.99 (t, $J$ = 6.6 Hz, 2H), 5.12 (s, 2H), 6.76 (s, 1H), 6.82 (d, $J$ = 8.6 Hz, 2H), 6.95 (d, $J$ = 7.9 Hz, 2H), 7.1–7.24 (m, 3H), 7.31–7.44 (m, 6H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 20.9, 22.3, 30.4, 39.5, 53.8, 66.8, 127.4, 127.8, 128.2, 128.8, 129.0, 129.3, 129.7, 133.0, 135.7, 136.7, 138.2, 140.8, 146.1, 146.9, 151.4, 170.6; IR (compression cell), cm$^{-1}$: 2960, 1748, 1628, 1511, 1447; Anal. Calcd for $C_{34}H_{38}N_2O_5S_2$: C, 65.99; H, 6.19; N, 4.53. Found: C, 65.8; H, 6.4; N, 4.3.

**5.1.2.7. *N*-Butyloxycarbonyl-3-[4-(*N*-acetyl-*N*-ethylaminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (13).** The compound **13** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (ethylamine, acetyl chloride). The crude product in the final step was purified by LC–MS (25% aqueous acetonitrile to 90% acetonitrile, reverse phase) to afford **13** as a colourless solid (18 mg, 46%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, 6.6 Hz, 6H), 1.08–1.34 (m, 5H), 1.50 (m, 2H), 1.94 (m, 1H), 2.14 (m, 3H), 2.69 (m, 2H), 3.38 (m, 2H), 4.04 (m, 2H), 4.58 (m, 2H), 6.75 (m, 1H), 7.14–7.60 (m, 4H); $^{13}$C NMR ($CDCl_3$), $\delta$: 12.7, 13.6, 18.8, 21.2, 21.8, 22.2, 30.5, 39.3, 40.9, 42.9, 47.7, 51.3, 66.7, 66.8, 126.2, 127.7, 127.9, 129.2, 129.4, 130.9, 133.1, 133.4, 137.6, 138.4, 146.1, 150.4, 151.3, 151.5, 170.7; IR (compression cell), cm$^{-1}$: 2960, 1747, 1627, 1463; Anal. Calcd for $C_{24}H_{34}N_2O_5S_2$: C, 58.27; H, 6.93; N, 5.66. Found: C, 58.7; H, 7.1; N, 5.8.

**5.1.2.8. *N*-Butyloxycarbonyl-3-[4-(*N*-ethyl-thiophenecarboylaminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (14).** The compound **14** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (ethylamine, thiophenecarboxyl chloride). The crude product in the final step was purified by LC–MS (45%

aqueous acetonitrile to 90% acetonitrile, reverse phase) to afford **14**as a colourless syrup (20 mg, 45%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.99 (d, $J$ = 6.6 Hz, 6 H), 1.24 (m, 5H), 1.50 (m, 2H), 1.95 (m, 1H), 2.71 (d, $J$ = 7.3 Hz, 2H), 3.58 (q, $J$ = 6.9 Hz, 2H), 4.03 (t, $J$ = 6.6 Hz, 2H), 6.78 (s, 1H), 7.02 (t, $J$ = 4.1 Hz, 1H), 7.29–7.50 (m, 6H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.5, 39.3, 66.9, 126.9, 127.1, 128.6, 129.1, 129.3, 130.6, 133.2, 137.8, 146.1, 150.0, 151.6, 164.6; IR (compression cell), cm$^{-1}$: 2960, 1748, 1604, 1436; Anal. Calcd for $C_{27}H_{34}N_2O_5S_3$: C, 57.62; H, 6.09; N, 4.98. Found: C, 58.1; H, 6.5; N, 4.6.

**5.1.2.9. *N*-Butyloxycarbonyl-3-[4-(*N*-methyl-pentyl-amidomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (15).** The compound **15** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (methylamine, valeroyl chloride). The crude product in the final step was purified by LC–MS (30% aqueous acetonitrile to 85% acetonitrile, reverse phase) to afford **15** as a colourless syrup (26 mg, 63%). $^1$H NMR ($CDCl_3$), $\delta$: 0.82–1.02 (m, 12H), 1.17–1.56 (m, 6H), 1.66 (m, 2H), 1.93 (m, 1H), 2.38 (m, 2H), 2.65–2.74 (d, $J$ = 6.9 Hz, 2H), 2.95 (m, 3H), 4.02 (t, $J$ = 6.6 Hz, 2H), 4.57 (s, 2H), 6.75 (m, 1H), 7.22 (m, 2H), 7.44 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 13.9, 18.7, 22.2, 22.6, 27.2, 27.5, 30.4, 30.5, 32.9, 33.2, 33.9, 35.2, 39.3, 50.6, 53.1, 66.7, 66.8, 126.2, 127.7, 129.1, 129.4, 130.7, 133.0, 133.3, 137.3, 138.0, 146.1, 150.3, 151.3, 151.5, 173.6, 173.7; IR (neat, cm$^{-1}$) $v$ 2959, 1747, 1628, 1466; Anal. Calcd for $C_{26}H_{38}N_2O_5S_2$: C, 59.74; H, 7.33; N, 5.36. Found: C, 59.8; H, 7.5; N, 5.5.

**5.1.2.10. *N*-Butyloxycarbonyl-3-[4-(*N*-(ethyloxy-carbonyl)-*N*-methyl-aminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (16).** The compound **16** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (methylamine, ethyl chloroformate). The crude product in the final step was purified by LC–MS (45% aqueous acetonitrile to 75% acetonitrile, reverse phase) to afford **16** as a colourless syrup (30 mg, 74%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.17–1.30 (m, 5H), 1.49 (m, 2H), 1.94 (m, 1H), 2.70 (d, $J$ = 7.3 Hz, 2H), 2.87 (s, 3H), 4.02 (t, $J$ = 6.6 Hz, 2H), 4.18 (q, $J$ = 7.3 Hz, 2H), 4.49 (s, 2H), 6.76 (s, 1H), 7.26 (m, 2H), 7.44 (d, $J$ = 8.3 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 14.7, 18.7, 22.2, 30.4, 30.5, 33.7, 34.3, 39.3, 52.1, 61.6, 66.8, 127.2, 127.6, 129.1, 129.4, 130.5, 133.0, 138.2, 146.2, 150.1, 151.5, 156.5, 157.0; IR (compression cell), cm$^{-1}$: 2960, 1750, 1678, 1465, 1348, 1222, 1158; Anal. Calcd for $C_{24}H_{34}N_2O_6S_2$: C, 56.5; H, 6.7; N, 5.5. Found: C, 56.9; H, 7.1; N, 5.4.

**5.1.2.11. *N*-Butyloxycarbonyl-3-[4-(*N*-acetyl-*N*-methyl-aminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (17).** The compound **17** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (methylamine, acetyl chloride). The crude product in the final step was purified by LC–MS (35% aqueous acetonitrile to 85% acetonitrile, reverse phase) to afford **17** as a colourless syrup (19 mg, 50%). $^1$H NMR ($CDCl_3$), $\delta$: 0.81 (t, $J$ = 7.3 Hz, 3H), 0.92 (d, $J$ = 6.6 Hz, 6H), 1.18 (m, 2H), 1.44 (m, 2H), 1.87 (m, 1H), 2.09 (m, 3H), 2.65 (m, 2H), 2.90 (m, 3H), 3.97 (m, 2H), 4.52 (m, 2H), 6.69 (m, 1H), 7.17 (m, 2H), 7.39 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.8, 21.4, 21.7, 22.2, 30.4, 30.5, 33.8, 35.8, 39.3, 50.5, 54.0, 66.7, 66.8, 126.2, 127.8, 129.2, 129.4, 129.5, 130.9, 133.2, 133.5, 137.0, 137.7, 146.0, 150.5, 151.2, 151.5, 171.2; IR (neat, cm$^{-1}$) $v$ 2960, 1746, 1628, 1466; Anal. Calcd for $C_{23}H_{32}N_3O_5S_2$: C, 57.47; H, 6.71; N, 5.83. Found: C, 57.0; H, 6.7; N, 5.8.

**5.1.2.12. *N*-Butyloxycarbonyl-3-[4-(*N*-methanesulfonyl-*N*-methyl-aminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (18).** The compound **18** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine and acid chloride (methylamine, methansulfonyl chloride). The crude product in the final step was purified by LC–MS (35% aqueous acetonitrile to 70% acetonitrile, reverse phase) to afford **18** as a colourless syrup (24 mg, 59%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.99 (d, $J$ = 6.6 Hz, 6H), 1.25 (m, 2H), 1.50 (m, 2H), 1.94 (m, 1H), 2.71 (d, $J$ = 7.3 Hz, 2H), 2.80 (s, 3H), 2.88 (s, 3H), 4.04 (t, $J$ = 6.6 Hz, 2H), 4.34 (s, 2H), 6.77 (s, 1H), 7.35 (s, 1H), 7.40 (d, $J$ = 8.5 Hz, 2H), 7.47 (d, $J$ = 8.5 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.4, 30.5, 34.5, 36.0, 39.3, 53.5, 66.9, 128.2, 129.3, 129.4, 130.6, 133.8, 136.3, 146.0, 150.0, 151.7; IR (compression cell), cm$^{-1}$: 3208, 2960, 1749, 1458; Anal. Calcd for $C_{22}H_{32}N_2O_6S_3$: C, 51.14; H, 6.24; N, 5.42. Found: C, 51.0; H, 5.8; N, 5.7.

**5.1.2.13. *N*-Butyloxycarbonyl-3-[4-(*N*-formyl-*N*-methyl-aminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (19).** The compound **19** was synthesised from **3** in the same fashion following step 1 in the above procedure with the appropriate choice of amine (methylamine). To the preceding product from step 1 (32 mg, 0.08 mmol) was added ammonium formate (120 mg, 0.57 mmol) in $CH_3CN$ (1.5 mL). The reaction mixture was refluxed for 12 h, cooled and evaporated. Work up as in step 2 was applied to the crude product. The preceding product was then treated as in steps 3 and 4 in the above procedure. The crude product in the final step was purified by LC–MS (35% aqueous acetonitrile to 75% acetonitrile, reverse phase) to afford **19** as a colourless solid (29 mg, 77%) $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.24 (m, 2H), 1.50 (m, 2H), 1.94 (m, 1H), 2.69 (d, $J$ = 6.9 Hz, 2H), 2.78 and 2.86 (s, 3H), 4.04 (td, $J$ = 2.3 Hz, $J$ = 6.6 Hz, 2H), 4.42 (s, 1H), 4.53 (s, 1H), 6.76 (d, $J$ = 3.0 Hz, 1H), 7.26 (t, $J$ = 7.9 Hz, 2H), 7.47 (t, $J$ = 7.9 Hz, 2H), 8.11 and 8.23 (s, 1H), 8.6–8.9 (br m, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 29.6, 30.4, 30.5, 34.3, 39.3, 47.6, 53.3, 66.7, 126.8, 127.3, 128.0, 129.3, 129.5, 131.0, 133.5, 133.9, 136.1, 136.3, 145.6, 145.7, 150.4, 151.3, 151.5, 163.1; IR (compression cell), cm$^{-1}$: 2960, 1746, 1662, 1466, 1346, 1158; Anal. Calcd for $C_{22}H_{30}N_2O_5S_2$: C, 56.63; H, 6.48; N, 6.00. Found: C, 56.4; H, 6.59; N, 5.88.

**5.1.2.14. *N*-Butyloxycarbonyl-3-[4-(dimethylaminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (20).** The compound **20** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine (dimethylamine). The crude product in the final step was purified by LC–MS (40% aqueous acetonitrile to 80% acetonitrile, reverse phase) to afford **20** as a colourless syrup (30 mg, 80%). $^1$H NMR ($CD_3CN + D_2O$), $\delta$: 0.84 (t, $J = 7.3$ Hz, 3H), 0.97 (d, $J = 6.6$ Hz, 6H), 1.20 (m, 2H), 1.37 (m, 2H), 1.98 (m, 1H), 2.71 (m, 8H), 3.79 (t, $J = 6.3$ Hz, 2H), 4.14 (s, 2H), 6.78 (s, 1H), 7.39 (d, $J = 8.3$ Hz, 2H), 7.67 (d, $J = 8.3$ Hz, 2H); $^{13}$C NMR ($CD_3CN + D_2O$), $\delta$: 13.2, 18.9, 21.5, 30.4, 30.9, 38.5, 42.1, 60.5, 64.4, 128.7, 129.2, 130.0, 130.4, 137.1, 139.0, 140.7, 146.7, 158.4; IR (compression cell), cm$^{-1}$: 2958, 2476, 2159, 2025, 1663, 1465, 1273, 1091, 880, 756; Anal. Calcd for $C_{22}H_{32}N_2O_4S_2 \times 1/3H_2O$: C, 57.6; H, 7.2; N, 6.1. Found: C, 57.6; H, 7.0; N, 6.1Anal.

**5.1.2.15. *N*-Butyloxycarbonyl-3-{4-[*N*-(cyanomethyl)-*N*-methyl-aminomethyl]phenyl}-5-*iso*-butylthiophene-2-sulfonamide (21).** The compound **21** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine (methylamino-acetonitrile). The crude product in the final step was purified by LC–MS (45% aqueous acetonitrile to 85% acetonitrile, reverse phase) to afford **21** as a colourless syrup (20 mg, 51%). $^1$H NMR ($CDCl_3$), $\delta$: 0.88 (t, $J = 7.3$ Hz, 3H), 0.99 (d, $J = 6.6$ Hz, 6H), 1.25 (m, 2H), 1.49 (m, 2H), 1.94 (m, 1H), 2.45 (s, 3H), 2.71 (d, $J = 7.3$ Hz, 2H), 3.46 (s, 2H), 3.67 (s, 2H), 4.03 (t, $J = 6.6$ Hz, 2H), 6.77 (s, 1H), 7.35 (d, $J = 8.3$ Hz, 2H), 7.44 (d, $J = 8.3$ Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.4, 30.5, 39.3, 42.2, 44.0, 59.8, 66.8, 114.3, 129.1, 129.2, 129.3, 130.9, 133.8, 136.9, 145.9, 150.1, 151.5; IR (compression cell), cm$^{-1}$: 2960, 1749, 1465, 1347, 1221, 1158; Anal. Calcd for $C_{23}H_{31}N_3O_4S_2$: C, 57.84; H, 6.54; N, 8.80. Found: C, 57.8; H, 6.6; N, 8.6.

**5.1.2.16. *N*-Butyloxycarbonyl-3-[4-(*N*-methoxy-*N*-methylaminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (22).** The compound **22** was synthesised from **3** in the same fashion following the above procedure with the appropriate choice of amine (*N*-methoxymethylamine). The crude product in the final step was purified by LC–MS (45% aqueous acetonitrile to 85% acetonitrile, reverse phase) to afford **22** as a colourless syrup (30 mg, 79%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J = 7.3$ Hz, 3H), 0.98 (d, $J = 6.6$ Hz, 6H), 1.25 (m, 2H), 1.49 (m, 2H), 1.95 (m, 1H), 2.64 (s, 3H), 2.71 (d, $J = 6.9$ Hz, 2H), 3.40 (s, 3H), 3.81 (s, 2H), 4.02 (t, $J = 6.6$ Hz, 2H), 6.78 (s, 1H), 7.43 (m, 4H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.4, 30.5, 39.3, 44.8, 59.9, 64.2, 66.9, 128.7, 129.5, 130.5, 133.0, 138.1, 146.3, 149.9, 151.5; IR (compression cell), cm$^{-1}$: 2958, 1751, 1436, 1347, 1159, 1047; Anal. Calcd for $C_{22}H_{32}N_2O_5S_2$: C, 56.4; H, 6.9; N, 6.0. Found: C, 56.8; H, 7.1; N, 5.7.

**5.1.2.17. 3-[4-(*N*-acetyl-aminomethyl)phenyl]-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (23).** To a nitrogen-flushed solution of $Pd(OAc)_2$ (17.5 mg, 0.078 mmol) in DME (2 mL), triphenylphosphine (82 mg, 0.31 mmol) was added. The solution was flushed with nitrogen again and stirred under $N_2$ atmosphere for 30 min. The brownish suspension was transferred to the nitrogen-flushed mixture of **2** (0.44 g 1.25 mmol), *N*-(4-bromobenzyl)acetamide (0.71 g, 3.12 mmol) and $K_2CO_3$ (0.86 g, 6.2 mmol) in DME/$H_2O$/EtOH (3.5–1.5–1 mL). The mixture was refluxed overnight under $N_2$ atmosphere, washed with aqueous NaOH solution (1 M), water and brine, dried over anhydrous $MgSO_4$ and concentrated to afford the residue ,which was purified by circular chromatography (50% acetone in pet. ether) to afford the colourless solid as the pure product (0.43 g, 65%): $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ 0.97 (d, $J = 6.6$ Hz, 6H), 1.0 (s, 9H), 1.91 (m, 1H), 2.07 (s, 3H), 2.67 (d, $J = 6.9$ Hz, 2H), 4.47 (d, $J = 5.6$ Hz, 2H), 6.20 (br s, 1H), 6.73 (s, 1H), 7.37 (d, $J = 7.9$ Hz, 2H), 7.53 (d, $J = 7.9$ Hz, 2H); $^{13}$C NMR (67.5 MHz, $CDCl_3$): $\delta$ 22.1, 23.2, 29.5, 30.5, 39.2, 43.3, 54.5, 127.8, 128.9, 129.3, 133.9, 136.1, 138.6, 143.1, 148.5, 170.2; IR (neat, cm$^{-1}$): $\nu$ 3314, 2963, 1650, 1535, 1432, 1308, 1136. Anal. Calcd for $C_{21}H_{30}N_2O_3S_2$: C, 59.68; H, 7.16; N, 6.63. Found: C, 59.4; H, 7.16; N, 6.0.

**5.1.2.18. *N*-Butyloxycarbonyl-3-[4-(*N*-acetyl-aminomethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (25).** A solution of **23** (40 mg, 0.09 mmol) and anisole (0.1 mL) in trifluoroacetic acid (2.5 mL) was stirred for 12 h at room temperature, evaporated and the residue reevaporated with acetonitrile (2× 6 mL). The crude product was dissolved in $CH_2Cl_2$ (2 mL). Triethylamine (0.037 mL, 0.27 mmol), *n*-butyl chloroformate (0.023 mL, 0.18 mmol) and pyrrolidinopyridine (1.5 mg, 0.009 mmol) were successively added. The mixture was stirred at room temperature for 30 min, and evaporated. The residue was purified by preparative LC–MS on gradual elution from 40% aqueous acetonitrile over 30 min duration to afford the pure product as a colourless solid (31 mg, 70%). $^1$H NMR ($CD_3COCD_3 + D_2O$), $\delta$: 0.84 (t, $J = 7.3$ Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 6H), 1.24 (m, 2H), 1.46 (m, 2H), 1.87–2.02 (m, 4H), 2.75 (d, $J = 7.3$ Hz, 2H), 3.56 (br s, 2H), 3.97 (t, $J = 6.3$ Hz, 2H), 4.38 (s, 2H), 6.92 (s, 1H), 7.32 (d, $J = 8.3$ Hz, 2H), 7.47 (d, $J = 8.3$ Hz, 2H); $^{13}$C NMR ($CD_3COCD_3 + D_2O$), $\delta$: 12.5, 18.0, 21.0, 21.4, 29.9, 38.0, 41.8, 65.2, 126.2, 126.7, 128.5, 129.2, 131.3, 132.2, 139.2, 145.0, 149.8, 150.4, 169.7; IR (compression cell), cm$^{-1}$: 2954, 2496, 1748, 1612, 1458; Anal. Calcd for $C_{22}H_{30}N_2O_5S_2 \times 1/3H_2O$: C, 55.91; H, 6.54; N, 5.93. Found: C, 55.9; H, 6.6; N, 5.9Anal.

**5.1.2.19. {4-[2-(*tert*-Butylsulfamoyl)-5-isobutyl-thiophen-3-yl]-phenyl}-acetic acid ethyl ester (26).** $Pd(OAc)_2$ (120 mg, 0.25 mmol) and DPPF (280 mg, 0.5 mmol) were dissolved in DME (5 mL) under $N_2$-bubbling. After stirring for 20 min, the brownish suspension was cannulated into a pre-$N_2$-flushed mixture of 2 (2.0 g, 6.27 mmol), ethyl-4-bromophenylethanoate (1.52 g, 6.27 mmol) and CsF (2.86 g, 18.9 mmol) in DME (20 mL). The mixture was heated to reflux under $N_2$ atmosphere for 12 h, cooled to room temperature, and partitioned between ethyl acetate (75 mL) and water (25 mL). The organic layer was washed with water, and brine, dried over anhydrous $MgSO_4$, concentrated

in vacuo and the residue purified by flash chromatography (ethyl acetate/petroleum ether, 1:7) to afford compound **26** as colourless solid (2.6 g, 95%). $^1$H NMR ($CDCl_3$), $\delta$: 0.97 (m, 15H), 1.25 (t, $J$ = 6.9 Hz, 3H), 1.91 (m, 1H), 2.67 (d, $J$ = 7.3 Hz, 2H), 3.65 (s, 2H), 4.10–4.20 (m, 3H), 6.75 (s, 1H), 7.35 (d, $J$ = 8.3 Hz, 2H), 7.57 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 14.1, 22.1, 29.4, 30.5, 39.2, 41.1, 54.4, 60.9, 128.8, 129.1, 129.4, 133.7, 134.5, 136.3, 142.7, 148.3, 171.2; IR (compression cell), cm$^{-1}$: 3290, 2961, 1738, 1513, 1368, 1312, 1144, 1050; Anal. Calcd for $C_{22}H_{31}NO_4S_2$: C, 60.38; H, 7.14; N, 3.20. Found: C, 60.38; H, 7.14; N, 3.20.

**5.1.2.20. 3-(4-Carboxymethyl-phenyl)-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (27).** A mixture of **26** (1.0 g, 2.3 mmol) and LiOH (140 mg, 5.72 mmol) in a solvent mixture of THF/$H_2O$/MeOH (4.5 mL, 1:1:1) was refluxed for 3 h, acidified with dilute hydrochloric acid (2 mL) and diluted with cold water (30 mL). The precipitated solid was filtered and dried to afford the pure compound **27** as colourless solid (0.93 g, 99%). $^1$H NMR ($CD_3OD$), $\delta$: 0.91–1.01 (m, 15H), 1.91 (m, 1H), 2.70 (d, $J$ = 7.3 Hz, 2H), 3.65 (s, 2H), 6.83 (s, 1H), 7.34 (d, $J$ = 8.3 Hz, 2H), 7.54 (d, $J$ = 8.3 Hz, 2H); $^{13}$C NMR ($CD_3OD$), $\delta$: 22.5, 29.8, 31.8, 39.9, 41.8, 54.8, 129.8, 130.3, 130.4, 130.6, 134.8, 136.3, 137.9, 144.7, 149.4, 175.5; IR (compression cell), cm$^{-1}$: 3283, 2963, 1709, 1310, 1144, 756; Anal. Calcd for $C_{20}H_{27}NO_4S_2$: C, 58.65; H, 6.64; N, 3.42. Found: C, 58.65; H, 6.64; N, 3.42.

### 5.1.3. General procedure for compound 30–40

*Step 1.* To a mixture of **27** (50 mg, 0.12 mmol), EDC (29.3 mg, 0.15 mmol), HOBT (26 mg, 0.15 mmol) and triethylamine (150 μL, 0.021 mmol) in DMF (2 mL), amine (0.15 mmol) was added. The mixture was stirred at room temperature overnight. The reaction mixture was partitioned between ethyl acetate and ether (2.5 mL + 7.5 mL) and water (10 mL). The organic layer was washed with dilute hydrochloric acid, aqueous sodium hydroxide (1 M), water and brine, dried over anhydrous $MgSO_4$ and evaporated to afford the crude product, which was directly used in the next step.

*Step 2.* To a solution of the product from step 1 in trifluoroacetic acid (2.5 mL) was added anisole (0.05 mL) and the solution was stirred for 12 h at room temperature. The residue was evaporated and reevaporated with acetonitrile (2× 5 mL) to give the crude product.

*Step 3.* The crude product from step 2 was dissolved in $CH_2Cl_2$ (2 mL). Triethylamine (0.5 mL, 0.33 mmol), *n*-butyl chloroformate (0.04 mL, 0.3 mmol) and pyrrolidinopyridine (1.5 mg, 0.009 mmol) were successively added. The mixture was stirred at room temperature for 12 h and evaporated. The residue was purified by preperative LC–MS to afford the products **30**–**40**.

**5.1.3.1. *N*-Butyloxycarbonyl-3-[4-(*N*-butyl-*N*-methylcarbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (30).** The compound **30** was synthesised as stated in the above general procedure using methylbutylamine. The crude product in the final step was purified by LC–MS (50–80% aqueous acetonitrile, reverse phase) to afford **30** as a colourless solid (45 mg, 71%). $^1$H NMR ($CDCl_3$), $\delta$: 0.84–0.98 (m, 12H), 1.17–1.58 (m, 8H), 1.93 (m, 1H), 2.69 (d, $J$ = 6.9 Hz, 2H), 2.93 and 2.99 (s, 3H), 3.29 and 3.37 (t, $J$ = 7.9 Hz, $J$ = 7.6 Hz, 2H), 3.68 (d, $J$ = 3.6 Hz, 2H), 4.02 (t, $J$ = 6.6 Hz, 2H), 6.73, 6.74 (s, 1H), 7.25 (d, $J$ = 6.6 Hz, 2H), 7.40 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 48.5, 48.7, 53.6, 54.8, 54.9, 57.1, 64.1, 65.3, 68.5, 70.6, 74.1, 74.9, 75.6, 82.7, 85.0, 101.4, 163.4, 163.8, 164.0, 164.2, 165.9, 167.4, 170.5, 170.7, 180.9, 185.5, 185.9; IR (compression cell), cm$^{-1}$: 2959, 1747, 1627, 1466, 1346, 1222, 1158; Anal. Calcd for $C_{26}H_{38}N_2O_5S_2$: C, 59.7; H, 7.33; N, 5.36. Found: C, 59.6; H, 7.1; N, 5.4.

**5.1.3.2. *N*-Butyloxycarbonyl-3-[4-(*N*-benzylcarbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (31).** The compound **31** was synthesised as stated in the above general procedure using benzylamine. The crude product in the final step was purified by LC–MS (45–75% aqueous acetonitrile, reverse phase) to afford **31** as a colourless solid (30 mg, 46%). $^1$H NMR ($CDCl_3$), $\delta$: 0.86 (t, $J$ = 7.3 Hz, 3H), 0.99 (d, $J$ = 6.6 Hz, 6H), 1.24 (m, 2H), 1.47 (m, 2H), 1.93 (m, 1H), 2.69 (d, $J$ = 7.3 Hz, 2H), 3.56 (s, 2H), 3.99 (d, $J$ = 6.6 Hz, 2H), 4.38 (d, $J$ = 5.9 Hz, 2H), 6.1 (br s, 1H), 6.74 (s, 1H), 7.18–7.32 (m, 7H), 7.41 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.4, 39.2, 43.2, 66.7, 127.4, 127.5, 128.6, 129.3, 130.9, 132.9, 135.3, 138.1, 145.9, 150.4, 151.4, 170.8; IR (compression cell), cm$^{-1}$: 2960, 1746, 1650, 1454, 1345, 1158; Anal. Calcd for $C_{28}H_{34}N_2O_5S_2$: C, 62.0; H, 6.3; N, 5.2. Found: C, 62.06; H, 6.42; N, 5.32.

**5.1.3.3. *N*-Butyloxycarbonyl-3-[4-(*N*-benzyl-*N*-ethylcarbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (32).** The compound **32** was synthesised as stated in the above general procedure using ethylbenzylamine. The crude product in the final step was purified by LC–MS (48–77% aqueous acetonitrile, reverse phase) to afford **32** as a colourless solid (53 mg, 77%). $^1$H NMR ($CDCl_3$), $\delta$: 0.86 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.12 (dt, $J$ = 1.3 Hz, $J$ = 7.3 Hz, 3H), 1.24 (m, 2H), 1.50 (m, 2H), 1.93 (m, 1H), 2.70 (d, $J$ = 7.3 Hz, 2H), 3.31 and 3.45 (q, $J$ = 7.3 Hz, 2H), 3.68 and 3.77 (s, 2H), 4.02 (t, $J$ = 6.6 Hz, 2H), 4.55 and 4.61 (s, 2H), 6.72 and 6.74 (s, 1H), 7.14–7.44 (m, 9H); $^{13}$C NMR ($CDCl_3$), $\delta$: 12.5, 13.6, 18.7, 22.2, 30.4, 39.2, 40.0, 40.4, 41.3, 41.8, 47.9, 50.9, 66.5, 126.2, 127.3, 127.6, 128.0, 128.5, 128.9, 129.0, 129.1, 129.4, 131.1, 132.6, 135.5, 135.6, 137.5, 145.9, 150.6, 151.0, 170.7, 171.0; IR (compression cell), cm$^{-1}$: 2960, 1747, 1628, 1452, 1347, 1158; Anal. Calcd for $C_{30}H_{38}N_2O_5S_2$: C, 63.1; H, 6.71; N, 4.91. Found: C, 62.91; H, 6.73; N, 5.01.

**5.1.3.4. *N*-Butyloxycarbonyl-3-[4-(*N*,*N*-dibenzylcarbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (33).** The compound **33** was synthesised as stated in the above

general procedure using dibenzylamine. The crude product in the final step was purified by LC–MS (55–85% aqueous acetonitrile, reverse phase) to afford **33** as a colourless solid (54 mg, 70%). $^1$H NMR ($CDCl_3$), $\delta$: 0.84 (t, $J$ = 7.3 Hz, 3H), 0.97 (d, $J$ = 6.6 Hz, 6H), 1.21 (m, 2H), 1.47 (m, 2H), 1.92 (m, 1H), 2.68 (d, $J$ = 6.9 Hz, 2H), 3.75 (s, 2H), 3.99 (t, $J$ = 6.6 Hz, 2H), 4.45 (s, 2H), 4.59 (s, 2H), 6.71 (s, 1H), 7.1–7.4 (m, 14H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.37, 30.42, 39.2, 40.3, 48.4, 50.1, 66.5, 126.3, 127.4, 127.7, 128.3, 128.6, 129.0, 129.2, 129.3, 131.0, 132.7, 135.3, 136.0, 137.0, 145.9, 150.5, 151.0, 171.5; IR (compression cell), cm$^{-1}$: 2959, 1747, 1628, 1452, 1348, 1222, 1158; Anal. Calcd for $C_{35}H_{40}N_2O_5S_2$: C, 66.4; H, 6.37; N, 4.43. Found: C, 66.2; H, 6.2; N, 4.4.

**5.1.3.5. *N*-Butyloxycarbonyl-3-{4-[*N*-(diphenylmethyl)-carbamoylmethyl]phenyl}-5-*iso*-butylthiophene-2-sulfonamide (34).** The compound **34** was synthesised as stated in the above general procedure using diphenylmethylamine. The crude product in the final step was purified by LC–MS (55–85% aqueous acetonitrile, reverse phase) to afford **34** as a colourless solid (39 mg, 54%). $^1$H NMR ($CDCl_3$), $\delta$: 0.84 (t, $J$ = 7.3 Hz, 3H), 0.95 (d, $J$ = 6.6 Hz, 6H), 1.20 (m, 2H), 1.44 (m, 2H), 1.90 (m, 1H), 2.67 (d, $J$ = 7.1 Hz, 2H), 3.60–3.74 (m, 3H), 3.97 (t, $J$ = 6.6 Hz, 2H), 6.22 (s, 2H), 6.71–6.80 (m, 1H), 6.96–7.30 (m, 12H), 7.34–7.40 (m, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.4, 30.5, 39.3, 43.3, 55.2, 55.9, 56.9, 66.8, 113.6, 126.2, 127.3, 127.5, 128.2, 128.6, 129.2, 129.4, 130.3, 130.7, 133.0, 135.3, 141.2, 146.1, 150.1, 151.6, 169.7; IR (compression cell), cm$^{-1}$: 2958, 1751, 1654, 1509,1458, 1345, 1157; Anal. Calcd for $C_{34}H_{38}N_2O_5S_2$: C, 65.99; H, 6.19; N, 4.53. Found: C, 66.1; H, 6.2; N, 4.5.

**5.1.3.6. *N*-Butyloxycarbonyl-3-[4-(2-morpholin-4-yl-2-oxo-ethyl)-phenyl]-5-*iso*-butylthiophene-2-sulfonamide (35).** The compound **35** was synthesised as stated in the above general procedure using morpholine. The crude product in the final step was purified by LC–MS (38–68% aqueous acetonitrile, reverse phase) to afford **35** as a colourless solid (37 mg, 59%). $^1$H NMR ($CDCl_3$ + $CD_3OD$), $\delta$: 0.73 (t, $J$ = 7.3 Hz, 3H), 0.85 (d, $J$ = 6.6 Hz, 6H), 1.04–1.18 (m, 2H), 1.29–1.41 (m, 2H), 1.72–1.88 (m, 1H), 2.48 (t, $J$ = 4.3 Hz, 4H), 2.56 (d, $J$ = 6.6 Hz, 2H), 3.45 (s, 2H), 3.61 (t, $J$ = 4.6 Hz, 4H), 3.84 (s, 2H), 6.60 (s, 1H), 7.21(d, $J$ = 8.3 Hz, 2H), 7.33 (d, $J$ = 8.3 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$), $\delta$: 13.6, 18.5, 22.0, 30.0, 30.5, 52.3, 60.8, 64.3, 65.0, 129.0, 129.2, 129.5, 129.8, 133.6, 134.2, 134.3, 141.7, 146.1, 146.9, 154.0, 154.3; IR (compression cell), cm$^{-1}$: 2964, 1657, 1461, 1253, 1138, 1089, 1033; Anal. Calcd for $C_{25}H_{34}N_2O_6S_2$: C, 57.5; H, 6.6; N, 5.4. Found: C, 57.2; H, 6.8 ; N, 5.5.

**5.1.3.7. *N*-Butyloxycarbonyl-3-[4-(2-thiazolidine-3-yl-2-oxo-ethyl)-phenyl]-5-*iso*-butylthiophene-2-sulfonamide (36).** The compound **36** was synthesised as stated in the above general procedure using thiazolidine. The crude product in the final step was purified by LC–MS (38–68% aqueous acetonitrile, reverse phase) to afford **36** as a colourless solid (39 mg, 62%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.99 (d, $J$ = 6.6 Hz, 6H), 1.27–1.31 (m, 2H), 1.4–1.52 (m, 2H), 1.86–2.04 (m, 1H), 2.7 (d, $J$ = 6.9 Hz, 2H), 2.9 (t, $J$ = 5.6 Hz, 2H), 3.08 (t, $J$ = 5.9 Hz, 2H), 3.58 (s, 2H), 3.95–4.07 (m, 4H), 6.76 (s, 1H), 7.35 (d, $J$ = 8.3 Hz, 2H), 7.43 (d, $J$ = 8.3 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 29.4, 30.4, 30.5, 39.2, 56.2, 57.3, 59.6, 66.7, 128.0, 129.0, 129.4, 130.9, 133.3, 138.5, 145.9, 150.4, 151.2; IR (compression cell), cm$^{-1}$: 2958, 1748, 1444, 1345, 1289, 1223, 1157; Anal. Calcd for $C_{24}H_{32}N_2O_5S_3$: C, 54.9; H, 6.2; N, 5.3. Found: C, 55.0; H, 6.4; N, 5.2.

**5.1.3.8. *N*-Butyloxycarbonyl-3-{4-[*N*-(4-methylphenyl)-carbamoylmethyl]phenyl}-5-*iso*-butylthiophene-2-sulfonamide (37).** The compound **37** was synthesised as stated in the above general procedure using 4-tolylamine. The crude product in the final step was purified by LC–MS (40–70% aqueous acetonitrile, reverse phase) to afford **37** as a colourless solid (43 mg, 65%). $^1$H NMR ($CDCl_3$), $\delta$: 0.71 (t, $J$ = 7.3 Hz, 3H), 0.86 (d, $J$ = 6.6 Hz, 6H), 1.08 (m, 2H), 1.32 (m, 2H), 1.81 (m, 1H), 2.14 (s, 3H), 2.57 (d, $J$ = 6.9 Hz, 2H), 3.55 (s, 2H), 3.86 (t, $J$ = 6.6 Hz, 2H), 6.63 (s, 1H), 6.93 (d, $J$ = 8.3 Hz, 2H), 7.13–7.29 (m, 6H), 7.48 (br s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.5, 18.7, 22.2, 29.8, 30.3, 30.5, 39.2, 44.1, 66.8, 120.0, 128.5, 129.3, 130.6, 132.9, 133.9, 135.2, 135.3, 146.2, 150.4, 151.6, 169.0; IR (compression cell), cm$^{-1}$: 3367, 2960, 1748, 1664, 1464, 1353, 1222, 1158; Anal. Calcd for $C_{28}H_{34}N_2O_5S_2$: C, 62.0; H, 6.3; N, 5.2. Found: C, 61.71; H, 6.38; N, 5.09.

**5.1.3.9. *N*-Butyloxycarbonyl-3-[4-(*N*-hexylcarbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (38).** The compound **38** was synthesised as stated in the above general procedure using hexylamine. The crude product in the final step was purified by LC–MS (38–68% aqueous acetonitrile, reverse phase) to afford **38** as a colourless solid (35 mg, 54%). $^1$H NMR ($CDCl_3$), $\delta$: 0.83–0.90 (m, 6H), 0.99 (d, $J$ = 6.6 Hz, 6H), 1.18–1.32 (m, 8H), 1.38–1.55 (m, 4H), 1.94 (m, 1H), 2.70 (d, $J$ = 7.3 Hz, 2H), 3.19 (q, $J$ = 6.9 Hz, 2H), 3.54 (s, 2H), 4.03 (t, $J$ = 6.6 Hz, 2H), 5.65 (s, 1H), 6.75 (s, 1H), 7.28 (d, $J$ = 8.9 Hz, 2H), 7.42 (d, $J$ = 8.3 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 14.0, 18.7, 22.2, 22.5, 26.5, 29.4, 30.4, 30.5, 32.38, 39.29, 39.81, 43.33, 66.8, 129.32, 130.9, 132.9, 135.6, 146.0, 150.3, 151.44, 170.7; IR (compression cell), cm$^{-1}$: 2958, 1747, 1650, 1543, 1465, 1346, 1221, 1158; Anal. Calcd for $C_{27}H_{40}N_2O_5S_2$: C, 60.4; H, 7.5; N, 5.2. Found: C, 60.5; H, 7.4; N, 5.2.

**5.1.3.10. *N*-Butyloxycarbonyl-3-[4-(*N*,*N*-dimethylcarbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (39).** The compound **39** was synthesised as stated in the above general procedure using dimethylamine. The crude product in the final step was purified by LC–MS (45–80% aqueous acetonitrile, reverse phase) to afford **39** as a colourless solid (38 mg, 65%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.96 (d, $J$ = 6.6 Hz, 6H), 1.22 (m, 2H), 1.47 (m, 2H), 1.91 (m, 1H), 267 (d, $J$ = 7.3 Hz, 2H), 2.93 (s, 3H), 3.01 (s, 3H), 3.67 (s, 2H), 4.0 (t, $J$ = 6.6 Hz, 2H), 6.72 (s, 1H), 7.24 (d, $J$ = 7.6 Hz, 2H), 7.39 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.5, 18.6, 22.1, 29.7, 30.3, 35.6, 37.7, 39.1, 40.2, 66.4, 129.8, 129.1, 129.3, 130.8, 132.4,

135.3, 145.9, 150.5, 150.9, 171.1; IR (compression cell), $cm^{-1}$: 2959, 1638, 1500, 1393, 1313, 1144; Anal. Calcd for $C_{23}H_{32}N_2O_5S_2 \times 2/3H_2O$: C, 56.07; H, 6.82; N, 5.7. Found: C, 55.9; H, 6.7; N, 5.8.

**5.1.3.11. *N*-Butyloxycarbonyl-3-[4-(*N*-methylcarbamoylmethyl)henyl]-5-*iso*-butylthiophene-2-sulfonamide (40).** The compound **40** was synthesised as stated in the above general procedure using methylamine. The crude product in the final step was purified by LC–MS (40–70% aqueous acetonitrile, reverse phase) to afford **40** as a colourless solid (37 mg, 65%). $^1H$ NMR ($CDCl_3$), $\delta$: 0.87 (t, $J = 7.3$ Hz, 3H), 0.99 (d, $J = 6.6$ Hz, 6H), 1.24 (m, 2H), 1.50 (m, 2H), 1.93 (m, 1H), 2.69–2.73 (m, 5H), 3.55 (s, 2H), 4.02 (t, $J = 6.6$ Hz, 2H), 5.81 (br s, 1H), 6.75 (m, 1H), 7.27 (m, 2H), 7.43 (m, 2H); $^{13}C$ NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 26.5, 30.4, 30.5, 39.3, 43.1, 66.7, 129.3, 129.4, 130.9, 133.0, 135.4, 145.9, 150.4,151.4, 171.6; IR (compression cell), $cm^{-1}$: 3401, 2960, 1746, 1650, 1465, 1345, 1158; Anal. Calcd for $C_{22}H_{30}N_2O_5S_2$: C, 56.63; H, 6.48; N, 6.00. Found: C, 56.4; H, 6.59; N, 5.88.

**5.1.3.12. 3-[4-(Carbamoylmethyl)phenyl]-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (41).** A suspension of Pd(0) catalyst was generated in situ by stirring a mixture of $Pd(OAc)_2$ (20.0 mg, 0.09 mmol) and triphenylphosphine (95 mg, 0.36 mmol) in DME (2 mL) under $N_2$ atmosphere (3× vacuum and 3× nitrogen flush). After stirring for 30 min, the suspension was introduced via syringe into a nitrogen-flushed mixture of **2** (0.57 g, 1.8 mmol), 4-bromophenylacetamide (0.39 g, 1.8 mmol) and $K_2CO_3$ (0.96 g, 7.2 mmol) in a solvent mixture of DME, ethanol and water (7 + 2 + 3 mL). After stirring for 12 h at reflux under $N_2$ atmosphere, the reaction mixture was diluted with 1 M NaOH solution (50 mL) followed by ethyl acetate (150 mL). The organic layer was washed with water, and brine, dried over anhydrous $MgSO_4$, concentrated in vacuo and the residue was purified by preparative LC–MS (40–80% aqueous acetonitrile, 35 min duration) to afford the pure product as a colourless solid (0.1 g, 14%). $^1H$ NMR ($CDCl_3$): $\delta$ 0.97 (d, 6H), 1.00 (s, 9H), 1.91 (m, 1H), 2.68 (d, $J = 6.9$ Hz, 2H), 3.61 (s, 2H), 4.72 (br s, 1H), 5.70 (br d, 2H), 6.74 (s, 1H), 7.35 (d, $J = 7.9$ Hz, 2H), 7.58 (d, $J = 8.3$ Hz, 2H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 22.1, 29.5, 30.5, 39.2, 42.9, 54.5, 128.1, 129.0, 129.5, 134.0, 135.1, 136.4, 142.8, 148.5, 173.1; IR (neat, $cm^{-1}$): $\nu$ 3296, 2960, 1670, 1311, 1142; Anal. Calcd for $C_{20}H_{28}N_2O_3S_2$: C, H, N. 58.79; H, 6.91; N, 6.86. Found: C, 58.8; H, 7.0; N, 6.6

**5.1.3.13. *N*-Butyloxycarbonyl-3-[4-(carbamoylmethyl)phenyl]-5-*iso*-butylthiophene-2-sulfonamide (43).** A solution of **41** (0.045 g, 0.11 mmol) and anisole (0.05 mL) in trifluoroacetic acid (2.5 mL) was stirred for 12 h at room temperature, evaporated and the residue reevaporated with acetonitrile (2× 5 mL). The crude product was dissolved in $CH_2Cl_2$ (2 mL). Triethylamine (0.05 mL, 0.33 mmol), *n*-butyl chloroformate (0.028 mL, 0.22 mmol) and pyrrolidinopyridine (1.5 mg, 0.009 mmol) were successively added. The mixture was stirred at room temperature for 30 min and evaporated. The residue was purified by preparative LC–MS on gradual elution from 40% to 80% aqueous acetonitrile (0.05% HCOOH, 35 min duration) to afford the pure product as a colourless solid (37 mg, 74%). $^1H$ NMR ($CDCl_3$), $\delta$: 0.86 (t, $J = 7.3$ Hz, 3H), 0.98 (d, $J = 6.6$ Hz, 6H), 1.26 (m, 2H), 1.51 (m, 2H), 1.94 (m, 1H), 2.70 (d, $J = 6.9$ Hz, 2H), 3.60 (s, 2H), 4.03 (t, $J = 6.9$ Hz, 2H), 5.85 (br s, 1H), 6.1 (br s, 1H), 6.74 (s, 1H), 7.22 (d, $J = 7.9$ Hz, 2H), 7.42 (d, $J = 7.9$ Hz, 2H); $^{13}C$ NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 22.2, 30.4, 39.3, 42.4, 66.6, 129.0, 129.3, 129.5, 131.3, 133.3, 134.7, 145.5, 151.1, 151.3, 174.6; IR (compression cell), $cm^{-1}$: 3456, 3360, 2960, 1745, 1663, 1466, 1345, 1158; Anal. Calcd for $C_{21}H_{28}N_2O_5S_2$: C, 55.73; H, 6.24; N, 6.19. Found: C, 55.6; H, 6.2; N, 6.0.

**5.1.4. General procedure for compound 44–46.** Palladium acetate (69.6 mg, 0.31 mmol) and DPPF (349 mg, 0.63 mmol) in DME (5 mL) were stirred for 30 min under $N_{2(g)}$. The suspension was then cannulated into a nitrogen-flushed mixture of **2** (1.0 g, 3.13 mmol), $K_2CO_3$ (1.29 mg, 9.36 mmol) and bromo-aryl-carbaldehyde (2 equiv, 6.26 mmol) in a solvent mixture of DME/$H_2O$/EtOH (7:3:2 ratio, 12 mL). The reaction mixture was refluxed for overnight, diluted with ethyl acetate (25 mL), washed with aqueous NaOH solution (1 M), brine, dried over anhydrous $MgSO_4$ solution and concentrated.

**5.1.4.1. 3-(5-Formylpyridin-2-yl)-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (44).** The compound **44** was synthesised from **2** in the same fashion following the above procedure with 6-bromo-pyridine-3-carbaldehyde. The crude product was purified by column chromatography (15% EtOAc/petroleum ether) to afford **44** as a colourless syrup (0.78 g, 63%). $^1H$ NMR ($CDCl_3$), $\delta$: 0.98 (d, $J = 6.6$ Hz, 6H), 1.33 (s, 9H), 1.93 (s, 1H), 2.71 (d, $J = 6.9$ Hz, 2H), 7.09 (s, 1H), 7.40 (br s, 1H), 7.76 (d, 8.3 Hz, 1H), 8.25 (dd, $J = 2.0$ Hz, $J = 8.3$ Hz, 1H), 9.07 (d, $J = 2.3$ Hz, 1H), 10.12 (s, 1H); $^{13}C$ NMR ($CDCl_3$), $\delta$: 22.1, 30.0, 30.5, 39.1, 55.0, 123.7, 127.7, 129.9, 137.2, 137.8, 142.2, 148.3, 150.7, 157.2, 189.7; IR (compression cell), $cm^{-1}$: 2962, 1701, 1594, 1443, 1324, 1198. Anal. Calcd for $C_{18}H_{24}N_2O_3S_2$: C, 56.82; H, 6.36; N, 7.36. Found: C, 57.2; H, 6.5; N, 7.5.

**5.1.4.2. 3-(5-Formyl-furan-2-yl)-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (45).** The compound **45** was synthesised from **2** in the same fashion following the above procedure with 5-bromo-furan-2-carbaldehyde. The crude product was purified by column chromatography (10% EtOAc/petroleum ether) to afford **45** as a colourless syrup (0.73 g, 51%). $^1H$ NMR ($CDCl_3$), $\delta$: 0.96 (d, $J = 6.6$ Hz, 6H), 1.28 (s, 9H), 1.84–1.99 (m, 1H), 2.67 (d, $J = 7.3$ Hz, 2H), 5.82 (s, 1H), 6.91 (d, $J = 3.8$ Hz, 1H), 7.05 (s, 1H), 7.29 (d, $J = 4.0$ Hz, 1H), 9.61 (s, 1H); $^{13}C$ NMR ($CDCl_3$), $\delta$: 22.1, 29.9, 30.5, 39.0, 54.9, 111.3, 123.4, 126.1, 128.7, 139.7, 149.2, 151.7, 153.0, 176.9; IR (compression cell), $cm^{-1}$: 3326, 2963, 1664, 1509, 1331, 1148, 1046. Anal. Calcd for $C_{17}H_{23}NO_4S_2$: C, 55.26; H, 6.27; N, 3.79. Found: C, 55.0; H, 6.1; N, 3.7.

**5.1.4.3. 3-(2-Formyl-thiophen-4-yl)-5-*iso*-butyl-*N*-*tert*-butylthiophene-2-sulfonamide (46).** The compound **46**

was synthesised from **2** in the same fashion following the above procedure with 5-bromo-furan-2-carbaldehyde. The crude product was purified by column chromatography (5% EtOAc/petroleum ether) to afford **46** as a colourless syrup (0.68 g, 61%). $^1$H NMR ($CDCl_3$), $\delta$: 0.97 (d, $J$ = 6.6 Hz, 6H), 2.08 (s, 9H), 1.84–2.0 (m, 1H), 2.69 (d, $J$ = 6.9 Hz, 2H), 4.4 (s, 1H), 6.83 (s, 1H), 8.8 (d, $J$ = 9.9 Hz, 2H), 9.96 (s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 22.1, 29.7, 30.6, 39.1, 54.9, 128.4, 133.9, 136.1, 136.2, 136.4, 136.9, 143.8, 149.2, 182.9; IR (compression cell), cm$^{-1}$: 3283, 2961, 1671, 1466, 1391, 1315, 1142. Anal. Calcd for $C_{17}H_{23}NO_3S_3$: C, 52.96; H, 6.01; N, 3.63. Found: C, 53.1; H, 6.1; N, 3.5.

### 5.1.5. General procedure to compound 50–52

*Step 1.* To a solution of **44**, **45** or **46** (30 mg, 0.08 mmol) in methanol (1.5 mL) taken in a sample vial (5 mL size), methylamine (2 M in methanol, 0.09 mmol) was added. After being stirred for 2 h, sodium borohydride (6.1 mg, 0.16 mmol) was added and the stirring continued for 2 h. The mixture was acidified with dilute HCl (5 M, 0.1 mL), stirred for 10 min, neutralised with saturated $NaHCO_3$ solution (~0.5 mL) and diluted with ethyl acetate (10 mL). The contents were poured into diatomaceous earth (liquid–liquid extraction catridge) in a polypropylene column (packed for 1.5 cm, 24 mL size) and eluted with ethyl acetate (30 mL). Concentration under vacuum afforded the crude product.

*Step 2.* The preceding product was dissolved in dry $CH_2Cl_2$ (1.5 mL) in a sample vial (5 mL size). Triethylamine (0.033 mL, 0.24 mmol), *N*,*N*-dimethylaminopyridine (1 mg, 0.008 mmol) and acetyl chloride (2 equiv, 0.16 mmol) were then added sequentially. The sample vial was tightly closed. The mixture was stirred overnight, quenched with aqueous saturated $NaHCO_3$ solution (0.5 mL), stirred for 30 min and filtered through diatomaceous earth (packed for 1.5 cm in the column of 24 mL capacity) on elution with $CH_2Cl_2$ (30 mL). Concentration in vacuo afforded the crude product.

*Step 3.* The mixture of the above product and anisole (~2 drops) in trifluoroacetic acid (1.5 mL) in a sample vial (5 mL size) was stirred at 30 °C overnight. After the removal of the solvent in vacuo, the residue was dissolved in acetonitrile (2 mL) and evaporated (2×).

*Step 4.* To a mixture of the preceding product in dry $CH_2Cl_2$ (1.5 mL), pyrrolidinopyridine (17.8 mg, 0.12 mmol) and triethylamine (0.5 mL, 0.36 mmol), *n*-butyl chloroformate (0.04 mL, 0.3 mmol) were sequentially added. The solution was stirred for 12 h, concentrated in vacuo and the crude purified by LC–MS to afford the products **50**–**52**.

**5.1.5.1. *N*-Butyloxycarbonyl-3-[5-(*N*-acetyl-*N*-methylaminomethyl)pyridin-2-yl]-5-*iso*-butylthiophene-2-sulfonamide (50).** The compound **50** was synthesised from **44** in the same fashion following the above procedure. The crude product in the final step was purified by LC–MS (35% to 70% aqueous acetonitrile) to afford **50** as a colourless syrup (26 mg, 68%). $^1$H NMR ($CDCl_3$), $\delta$: 0.87 (t, $J$ = 7.3 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.33 (m, 2H), 1.58 (m, 2H), 1.93 (m, 1H), 2.16 (s, 3H), 2.70 (d, $J$ = 6.9 Hz, 2H), 2.91–2.99 (m, 3H), 4.11 (t, $J$ = 5.3 Hz, 2H), 4.60 (s, 2H), 7.04 (m, 1H), 7.54–7.74 (m, 2H), 8.55 (m, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.8, 21.4, 21.7, 22.1, 30.4, 33.7, 35.9, 39.2, 48.1, 51.6, 66.7, 123.1, 123.5, 123.7, 127.7, 128.1, 131.9, 132.9, 133.5, 135.3, 137.3, 141.6, 147.2, 148.3, 150.7, 151.0, 151.2, 151.5, 170.8, 171.0; IR (compression cell), cm$^{-1}$: 2960, 1746, 1643, 14566, 1348, 1159, 1047; Anal. Calcd for $C_{22}H_{31}N_3O_5S_2$: C, 54.86; H, 6.49; N, 8.72. Found: C, 54.8; H, 6.1; N, 8.7.

**5.1.5.2. *N*-Butyloxycarbonyl-3-[5-(*N*-acetyl-*N*-methylaminomethyl)furan-2-yl]-5-*iso*-butylthiophene-2-sulfonamide (51).** The compound **51** was synthesised from **45** in the same fashion following the above procedure. The crude product in the final step was purified by LC–MS (35% aqueous acetonitrile to 70% acetonitrile, reverse phase) to afford **51** as a colourless syrup (22 mg, 58%). $^1$H NMR ($CDCl_3$), $\delta$: 0.86 (t, $J$ = 7.3 Hz, 3H), 0.96 (d, $J$ = 6.6 Hz, 6H), 1.22–1.37 (m, 2H), 1.53–1.64 (m, 2H), 1.84–1.98 (m, 1H), 2.19 (s, 3H), 2.63 (d, $J$ = 6.9 Hz, 2H), 2.99 (s, 3H), 4.10 (t, $J$ = 6.6 Hz, 2H), 4.58 (s, 2H), 6.33 (d, $J$ = 3.3 Hz, 2H), 6.54 (d, $J$ = 3.3 Hz, 1H), 6.89 (s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.8, 22.2, 30.5, 35.6, 39.2, 43.9, 66.3, 109.8, 110.2, 124.9, 129.8, 131.8, 148.7, 151.2, 151.4, 173.8; IR (compression cell), cm$^{-1}$: 2945, 1742, 1618, 1460, 1343, 1219, 1152, 1039; Anal. Calcd for $C_{21}H_{30}N_2O_6S_2$: C, 53.60; H, 6.43; N, 5.95. Found: C, 53.3; H, 6.5; N, 5.8.

**5.1.5.3. *N*-Butyloxycarbonyl-3-[2-(*N*-acetyl-*N*-methylaminomethyl)thiophen-4-yl]-5-*iso*-butylthiophene-2-sulfonamide (52).** The compound **52** was synthesised from **46** in the same fashion following the above procedure. The crude product in the final step was purified by LCMS (35% aqueous acetonitrile to 70% acetonitrile, reverse phase) to afford **52** as a colourless syrup (20 mg, 51%). $^1$H NMR ($CDCl_3$), $\delta$: 0.85 (t, $J$ = 7.6 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 6H), 1.14–1.29 (m, 2H), 1.40–1.52 (m, 2H), 1.85–1.98 (m, 1H), 2.08–2.24 (m, 3H), 2.66 (d, $J$ = 7.3 Hz, 2H), 2.95–3.13 (m, 3H), 3.98 (t, $J$ = 6.27 Hz), 4.63–4.69 (m, 2H), 6.84 (s, 1H), 7.37 (s, 1H), 7.43 (s, 1H), 8.88 (s, 1H); $^{13}$C NMR ($CDCl_3$), $\delta$: 13.6, 18.7, 21.4, 21.8, 22.2, 30.4, 30.5, 36.6, 39.3, 46.8, 49.6, 66.8, 124.5, 125.3, 126.8, 128.2, 128.9, 130.5, 133.4, 139.3, 139.6, 140.1, 150.5, 151.1, 151.5, 171.7; IR (compression cell), cm$^{-1}$: 2959, 1747, 1620, 1466, 1344, 1221, 1146, 1045; Anal. Calcd for $C_{21}H_{30}N_2O_5S_3$: C, 51.83; H, 6.21; N, 5.76. Found: C, 51.5; H, 6.1; N, 5.5.

## 5.2. Rat liver membrane $AT_1$ receptor binding assay

Rat liver membranes were prepared according to the method of Dudley et al.[24] Binding of [$^{125}$I]Ang II to membranes was conducted in a final volume of 0.5 mL containing 50 mM Tris–HCl (pH 7.4), 100 mM NaCl,

10 mM $MgCl_2$, 1 mM EDTA, 0.025% bacitracin, 0.2% BSA (bovine serum albumin), liver homogenate corresponding to 5 mg of the original tissue weight, [$^{125}$I]Ang II (80,000–85,000 cpm, 0.03 nM ) and variable concentrations of test substance. Samples were incubated at 25 °C for 2 h, and binding was terminated by filtration through Whatman GF/B glass-fibre filter sheets, which had been pre-soaked overnight with 0.3% polyethylamine, using a Brandel cell harvester. The filters were washed with 3× 3 mL of Tris–HCl (pH 7.4) and transferred to tubes. The radioactivity was measured in a γ-counter. The characteristics of the Ang II binding $AT_1$ receptor were determined by using six different concentrations (0.03–5 nmol/L) of the labelled [$^{125}$I]Ang II. Non-specific binding was determined in the presence of 1 μM Ang II. The specific binding was determined by subtracting the non-specific binding from the total bound [125I]Ang II. The apparent dissociation constant $K_i$ values were calculated from $IC_{50}$ values using the Cheng–Prusoff equation ($K_d = 1.7 \pm 0.1$ nM, [L] = 0.057 nM). The binding data were best fitted with a one-site fit. All determinations were performed in triplicate.

### 5.3. Porcine (pig) myometrial membrane $AT_2$ receptor binding assay

Myometrial membranes were prepared from porcine uteri according to the method by Nielsen et al.[25] A presumable interference by binding to $AT_1$ receptors was blocked by addition of 1 μM losartan.[16] Binding of [$^{125}$I]Ang II to membranes was conducted in a final volume of 0.5 mL containing 50 mM Tris–HCl (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.025% bacitracin, 0.2% BSA, homogenate corresponding to 10 mg of the original tissue weight, [$^{125}$I]Ang II (80,000–85,000 cpm, 0.03 nM) and variable concentrations of test substance. Samples were incubated at 25 °C for 1.5 h, and binding was terminated by filtration through Whatman GF/B glass-fibre filter sheets, which had been pre-soaked overnight with 0.3% polyethylamine, using a Brandel cell harvester. The filters were washed with 3× 3 mL of Tris–HCl (pH 7.4) and transferred to tubes. The radioactivity was measured in a γ-counter. The characteristics of the Ang II binding $AT_2$ receptor was determined by using six different concentrations (0.03–5 nmol/L) of the labelled [125I]Ang II. Non-specific binding was determined in the presence of 1 μM Ang II. The specific binding was determined by subtracting the non-specific binding from the total bound [$^{125}$I]Ang II. The apparent dissociation constant $K_i$ values were calculated from $IC_{50}$ values using the Cheng–Prusoff equation ($K_d = 0.73 \pm 0.6$ nM, [L] = 0.057 nM). The binding data were best fitted with a one-site fit. All determinations were performed in triplicate.

### 5.4. In vitro morphological effects: General

The chemicals used in the present study were obtained from the following sources: Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), HAT supplement (hypoxanthine, aminopterin, thymidine), gentamycin from Gibco BRL (Burlington, Ont., Canada); [Val5]angiotensin II from Bachem (Marina Delphen, CA, USA). Compound **61** was from RBI (Natick, MA, USA). All other chemicals were of grade A purity.

### 5.5. Cell culture

NG108-15 cells (provided by Drs. M. Emerit and M. Hamon; INSERM, U. 238, Paris, France) were cultured (passage 7–30) in DMEM with 10% foetal bovine serum (FBS, Gibco BRL, Burlington, Ont., Canada), HAT supplement and 50 mg/L gentamycin at 37 °C in 75 $cm^2$ Nunclon Delta flasks in a humidified atmosphere of 93% air and 7% $CO_2$, as previously described.[27] Subcultures were performed at subconfluency. Under these conditions, cells express only the $AT_2$ receptor subtype.[27,28] Cells were stimulated once a day for 3 days (first stimulation 24 h after plating). Cells were cultured for three subsequent days under these conditions. For all experiments, cells were plated at the same initial density of $4 \times 10^4$ cells/35 mm Petri dish. Cells were treated without (control cells), with [Val5]Ang II (0.1 μM) or **50**, **56** (0.1 μM), in the absence or in the presence of the inhibitor, **61** (1 μM), an $AT_2$ receptor antagonist (each introduced daily with inhibitors applied 30 min prior to Ang II or **50**, **56**).

### 5.6. Determination of cells with neurites

Cells were examined daily under a phase contrast microscope and micrographs were taken after 3 days under the various experimental conditions. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. At least 140 cells were counted in three independent experiments.[31]

### 5.7. Data analysis

The data were presented as means ± SEM of the number of experiments indicated in the text, each performed in duplicate or triplicate. Statistical analyses of the data were performed using the one-way analysis of variance (ANOVA) test. Homogeneity of variance was assessed by Bartlett's test and $p$ values were obtained from Dunnett's tables.

## Acknowledgments

We gratefully acknowledge support from the Swedish Research Council (VR), the Swedish Foundation for Strategic Research (SSF), Knut and Alice Wallenberg Foundation (VRmedicine 8663), the Canadian Institutes of Health Research to N.G.P. and M.D.P. (MOP 37891), and Vicore Pharma AB. N.G.P. is a holder of a Canada Research Chair in Endocrinology of the Adrenal Gland.

## References and notes

1. Lenkei, Z.; Palkovits, M.; Corvol, P.; Llorens-Cortes, C. *Front. Neuroendocrinol.* **1997**, *18*, 383–439.

2. Tsutsumi, K.; Saavedra, J. M. *Am. J. Physiol.* **1991**, *261*, R209–R216.
3. Grady, E. F.; Sechi, L. A.; Griffin, C. A.; Schambelan, M.; Kalinyak, J. E. *J. Clin. Invest.* **1991**, *88*, 921–933.
4. Stroth, U.; Blume, A.; Mielke, K.; Unger, T. *Brain Res. Mol. Brain Res.* **2000**, *78*, 175–180.
5. Gendron, L.; Laflamme, L.; Rivard, N.; Asselin, C.; Payet, M. D.; Gallo-Payet, N. *Mol. Endocrinol.* **1999**, *13*, 1615–1626.
6. Horiuchi, M.; Akishita, M.; Dzau, V. *Hypertension* **1999**, *33*, 613–621.
7. Inagami, T.; Kambayashi, Y.; Ichiki, T.; Tsuzuki, S.; Eguchi, S.; Yamakawa, T. *Clin. Exp. Pharmacol. Physiol.* **1999**, *26*, 544–549.
8. Nouet, S.; Nahmias, C. *Trends Endocrinol. Metab.* **2000**, *11*, 1–6.
9. de Gasparo, M.; Catt, K. J.; Inagami, T.; Wright, J. W.; Unger, T. *Pharmacol. Rev.* **2000**, *52*, 415–472.
10. Stoll, M.; Unger, T. *Regul. Pept.* **2001**, *99*, 175–182.
11. Gendron, L.; Payet, M. e.; Gllo-Payet, N. *J. Mol. Endocrinol.* **2003**, *31*, 359–372.
12. Kaschina, E.; Unger, T. *Blood Pressure* **2003**, *12*, 70–88.
13. Widdop, R. E.; Jones, E. S.; Hannan, R. E.; Gaspari, T. A. *Br. J. Pharmacol.* **2003**, *140*, 809–824.
14. Jöhren, O.; Dendorfer, A.; Dominiak, P. *Cardiovasc. Res.* **2004**, *62*, 460–467.
15. Johansson, B.; Holm, M.; Ewert, S.; Casselbrant, A.; Pettersson, A.; Fändriks, L. *Am. J. Physiol.* **2001**, *280*, G1254–G1260.
16. Carini, D. J.; Duncia, J. V.; Aldrich, P. E.; Chiu, A. T.; Johnson, A. L.; Pierce, M. E.; Price, W. A.; Santella, J. B., 3rd; Wells, G. J.; Wexler, R. R.; Wong, P. C.; Yoo, W.-E.; Timmermans, P. B. M. W. M. *J. Med. Chem.* **1991**, *34*, 2525–2547.
17. Buhlmayer, P.; Furet, P.; Criscione, L.; de Gasparo, M.; Whitebread, S.; Schmidlin, T.; Lattmann, R.; Wood, J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 29–34.
18. Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D.; Timmermans, P. B. M. W. M. *J. Med. Chem.* **1996**, *39*, 625–656.
19. Wan, Y.; Wallinder, C.; Plouffe, B.; Beaudry, H.; Mahalingam, A. K.; Wu, X.; Johansson, B.; Holm, M.; Botoros, M.; Karlen, A.; Pettersson, A.; Nyberg, F.; Fändriks, L.; Gallo-Payet, N.; Hallberg, A.; Alterman, M. *J. Med. Chem.* **2004**, *47*, 5995–6008.
20. Wu, X.; Wan, Y.; Mahalingam, A. K.; Murugaiah, A. M. S.; Plouffe, B.; Botros, M.; Karlen, A.; Hallberg, M.; Gallo-Payet, N.; Alterman, M. *J. Med. Chem.* **2006**, *49*, 7160–7168.
21. Kevin, N. J.; Rivero, R. A.; Greenlee, W. J.; Chang, R. S. L.; Chen, T. B. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 189–194.
22. Kivlighn, S.; Lotti, V. J.; Rivero, R. A.; Siegl, P. K. S.; Zingaro, G. J. In *Brit. UK Pat. Appl.*; (Merck and Co., Inc. Rahway, NJ, USA): GB 2281298, 1995, p 21.
23. Wan, Y.; Wallinder, C.; Johansson, B.; Holm, H.; Mahalingam, A. K.; Wu, X.; Botros, M.; Karlén, A.; Pettersson, A.; Nyberg, F.; Fändriks, L.; Hallberg, A.; Alterman, M. *J. Med. Chem.* **2004**.
24. Dudley, D. T.; Panek, R. L.; Major, T. C.; Lu, G. H.; Bruns, R. F.; Klinkefus, B. A.; Hodges, J. C.; Weishaar, R. E. *Mol. Pharmacol.* **1990**, *38*, 370–377.
25. Nielsen, A. H.; Schauser, K.; Winther, H.; Dantzer, V.; Poulsen, K. *Clin. Exp. Pharmacol. Physiol.* **1997**, *24*, 309–314.
26. Blankley, C. J.; Hodges, J. C.; Klutchko, S. R.; Himmelsbach, R. J.; Chucholowski, A.; Connolly, C. J.; Neergaard, S. J.; Van Nieuwenhze, M. S.; Sebastian, A.; Quin, J., 3rd *J. Med. Chem.* **1991**, *34*, 3248–3260.
27. Buisson, B.; Bottari, S. P.; De Gasparo, M.; Gallo-Payet, N.; Payet, M.-D. *FEBS Lett.* **1992**, *309*, 161–164.
28. Laflamme, L.; Gasparo, M.; Gallo, J. M.; Payet, M. D.; Gallo-Payet, N. *J. Biol. Chem.* **1996**, *271*, 22729–22735.
29. Perlman, S.; Schambye, H. T.; Rivero, R. A.; Greenlee, W. J.; Hjorth, S. A.; Schwartz, T. W. *J. Biol. Chem.* **1995**, *270*, 1493–1496.
30. Kivlighn, S. D.; Huckle, W. R.; Zingaro, G. J.; Rivero, R. A.; Lotti, V. J.; Chang, R. S. L.; Schorn, T. W.; Kevin, N.; Johnson, R. G., Jr.; Greenlee, W. J.; Siegl, P. K. S. *Am. J. Physiol.* **1995**, *268*, R820–R823.
31. Gendron, L.; Cote, F.; Payet, M. D.; Gallo-Payet, N. *Neuroendocrinology* **2002**, *75*, 70–81.