Selective Angiotensin II AT_2 Receptor Agonists: Arylbenzylimidazole Structure−Activity Relationships

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Structural alterations in the 2- and 5-positions of the first drug-like selective angiotensin II AT_2 receptor agonist (**1**) have been performed. The imidazole ring system was proven to be a strong determinant for the AT_2 selectivity, and with few exceptions all variations gave good AT_2 receptor affinities and with retained high AT_2/AT_1 selectivities. On the contrary to the findings with AT_1 receptor agonists, the impact of structural modifications in the 5-position of the AT_2 selective compounds were less pronounced regarding activation of the AT_2 receptor. The butyloxyphenyl (**56**) and the propylthienyl (**50**) derivatives were found to exert a high agonistic effect as deduced from their capacity to induce neurite elongation in neuronal cells, as does angiotensin II.

Introduction

Angiotensin II (Ang II), recognized as the most important bioactive peptide of the rennin−angiotensin system, is the endogenous activator of the AT_1 and the AT_2 receptors. The AT_1 receptor is closely associated with the regulation of blood pressure, fluid, and electrolyte balance, but the role of the AT_2 receptor is less clear due to the low level of AT_2 expression in healthy adults.[1] One notable feature of the AT_2 receptor is the high level of expression in most fetal tissues, including the brain. The AT_2/AT_1 receptor ratio decreases dramatically after birth,[2,3] which may support a significant involvement of the AT_2 receptor in fetal development. In cells of neuronal origin, AT_2 receptor activation induces neurite outgrowth and modulates cell excitability. In adults, activation of the AT_2 receptor lowers blood pressure, inhibitits cell proliferation, and induces programmed cell death, extracellular matrix remodeling, and axonal regeneration.[4−9] In addition, it has recently been demonstrated that activation of the AT_2 receptor stimulates alkaline secretion by the duodenal mucosa in rats.[10] However, most remarkably, the 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.[7,11−13]

The signaling pathways activated after AT_2 receptor stimulation are still controversial. This seven-transmembrane domain receptor is not coupled to any of the classical, well established, second messengers, such as cAMP or inositol phosphates, and its coupling to a $G_{\alpha i}$ protein, reported by several authors, is not viewed in consensus.[4−9] However, various mediators, which could individually exert opposite effects, such as cGMP, tyrosine, or serine/threonine phosphatases, and the extracellular signal-regulated kinases p42/$p44^{mapk}$ have been associated with activation of the AT_2 receptor, depending on the cell types and experimental conditions used. A sustained increase in p42/$p44^{mapk}$ activity is found associated with neuronal differentiation. The activation of the p42/$p44^{mapk}$ pathway is essential to promote neurite outgrowth and the ability of AT_2 receptor ligands to induce neurite outgrowth and can serve as a robust AT_2 agonist assay.[14,15] The first nonpeptidic selective AT_2 receptor agonist (**1**), that we reported recently, activates p42/$p44^{mapk}$ as does Ang II.[16]

The agonist **1** was derived from the nonselective agonist **58** (L-162,313)[17−19] after the nitrogen-containing heterocycle of the latter had been altered (Figure 1). It is known from literature that removal of a methyl group from the isobutyl side-chain of the biphenyl analogue of **58** converts this AT_1/AT_2 nonselective agonist to an AT_1 antagonist (**59**, L-162,389).[20] Furthermore, if the terminal part of the propyl side chain of the latter antagonist is replaced by a 3-methoxybenzyl group, the compound is transformed into an AT_1 selective agonist (**60**, L-163,491).[21] We previously observed that minor alterations of the sulfonyl carbamate side chain of **58** had a dramatic negative impact on the binding affinity to the AT_2 receptor.[19] Thus, it seems apparent that relatively small structural modifications of **58** affect the biological outcome significantly.

Our preliminary study suggested that the imidazole ring of the AT_2 agonist **1** constitutes an important determinant for AT_2 receptor selectivity.[16] Herein, we report an extended SAR study where the alkoxysulfonylamide and isobutyl groups as well as the thienylphenyl scaffold of **1** were subjected to alterations. Selective AT_2 receptor ligands with a high agonistic potency were identified.

Results

Chemistry. The thiopheneboronic acid **2**, used as the starting material for compounds **5**−**14** (Scheme 1), was prepared essentially as described by Kevin et al.[22,23] Thus, thiophene-2-sulfonyl chloride was first converted to the *N-tert*-butylsulfonamide. Subsequent alkylation followed by selective 3-lithiation/boronation delivered the key-intermediate **2**. The 1-(4-bromobenzyl)-1*H*-imidazole **3** was obtained by N-alkylation of imidazole with 4-bromobenzyl bromide, utilizing KOH as base.[16] The aryl bromide **3** was thereafter reacted with the boronic acid **2** under Suzuki coupling conditions with $Pd(PPh_3)_4$

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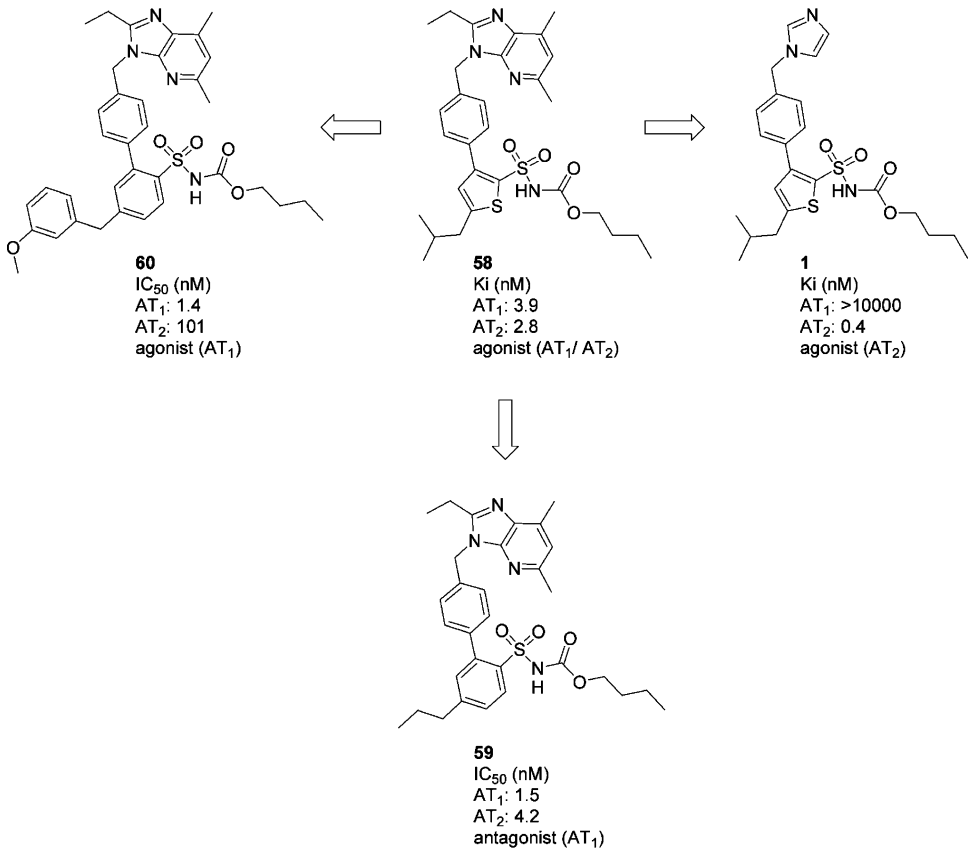
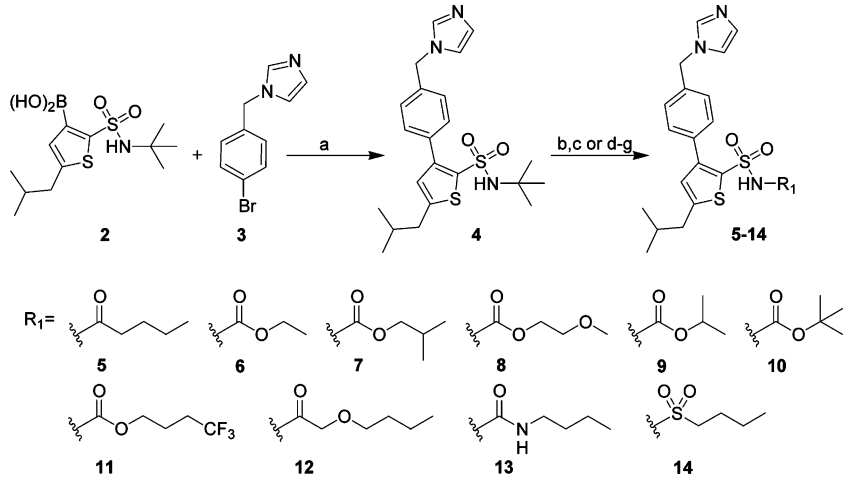


Figure 1.

Scheme 1[a]

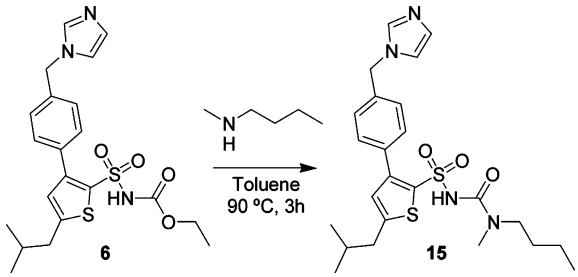


[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, NaOH (aq), ethanol/ toluene, thermal heating or microwave heating; (b) TFA, anisole or BCl_3 in CH_2Cl_2; (c) acyl chloride (**5**−**9**), di-*tert*-butyl carbonate (**10**), with pyrrolidinopyridine, pyridine; (d) 4,4,4-triflurobutanol, CDI, DBU, THF; (e) butoxyacetic acid, CDI, THF; (f) butyl isocyanate, acetone, NaOH; (g) butanesulfonyl chloride, NaOH, THF.

as the source of palladium and with KOH as base to give compound **4**.[16] Deprotection of **4** by TFA or alternatively by BCl_3, a milder deprotecting reagent,[24] delivered the primary sulfonamide that was subsequently reacted with acyl chlorides or alkyl chloroformates, at ambient temperature, in pyridine with a 4-pyrrolidin-1-ylpyridine nucleophilic catalyst, to afford the target compounds **5**−**9**.

In the case where isobutyl chloroformate and isopropyl chloroformate were employed, a reaction temperature of 50 °C was required to obtain the compounds **7** and **9** in good yields. For the preparation of compound **10**, di-*tert*-butyl carbonate was applied as the electrophile. In order to synthesize **11** with the 4,4,4-trifluorobutyloxy side chain, 4,4,4-trifluorobutanol was first reacted with CDI in THF at 50 °C for 3.5 h, whereafter this mixture was added to the deprotected sulfonamide in THF with DBU, as base. Compound **11** was afforded in 64% yield. The combination of CDI and DBU was also used for the acylation with butoxyacetic acid in the preparation of compound **12**. The amide **13** and the sulfone **14** were obtained by treating the deprotected sulfonamide with NaOH and subsequent addition of butyl isocyanate or butanesulfonyl chloride, respectively. As outlined in Scheme 2, the introduction of the *N*-methyl-*N*-butylamino side chain in compound **15** was accomplished by reacting compound **6** with *N*-methylbutylamine at 90 °C for 3 h.

Scheme 2



To synthesize compound **21**, a sulfonamide-carbonyl switched version of compound **5**, thiophene was used as starting material (Scheme 3). The lithiation of thiophene was conducted with n-butyllithium, and isobutyl iodide was used as alkylating agent. The reaction was sluggish and yielded only 30% of the 2-isobutylthiophene **16**. A second lithiation and subsequent treatment with *tert*-butyl isocyanate resulted in the *tert*-butyl protected amide **17** in 70% yield. Compound **17** was converted to the boronic acid **18** via lithiation and treatment with triisopropyl borate and was subsequently reacted with **3** under Suzuki conditions to obtain compound **19** in high yield. To remove the *tert*-butyl protection of the amide, rather harsh conditions were required. After 17 h of heating at 70 °C in TFA, the deprotected primary amide **20** could be isolated in 83% yield. The primary amide **20** was deprotonated with NaH and reacted with butanesulfonyl chloride to obtain the carbonylsulfonyl target compound **21**.

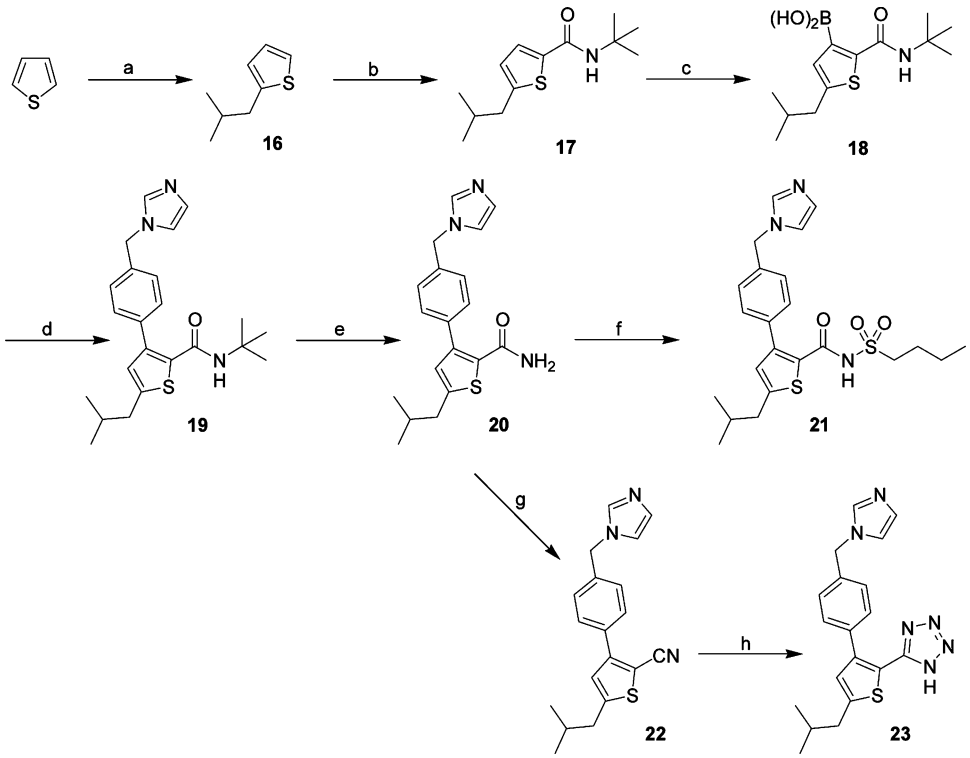
The tetrazole analogue was prepared by dehydration of the primary amide **20** with trifluoroacetic anhydride followed by treatment of the obtained nitrile **22** with sodium azide and Et_3NHCl. This procedure provided the tetrazole compound **23** in high yield (Scheme 3).

For the preparation of the target compounds **49**−**57** (Scheme 7) a series of different sulfonamides were required as precursors. The *N*-*tert*-butyl-5-alkylthiophene-2-sulfonamides (Scheme 4) were prepared by deprotonation of *N*-*tert*-butylthiophene-sulfonamide with n-butyllithium and thereafter adding the proper alkyl halides, to yield the compounds **24**−**26** in 45−81% yield. To provide compound **27**, 2-thiophene-2-ylethanol was first reacted with methyl iodide and with sodium hydride as base (Scheme 5). Compound **27** was thereafter slowly added to a mixture of chlorosulfonic acid, phosphorus pentachloride, and *tert*-butylamine to obtain compound **28** in 35% yield. The alkyl- and alkoxyphenylsulfonamides (Scheme 6) were synthesized from their corresponding alkyl- or alkoxyphenylsulfonyl chloride (the isobutylphenylsulfonyl chloride was synthesized form isobutylbenzene with chlorosulfonic acid) by reacting them with *tert*-butylamine.

The *N*-*tert*-butyl-aryl-sulfonamides **24**−**26**, **28**−**32** were converted to their corresponding boronic acids and subsequently reacted with **3** under Suzuki conditions to obtain the compounds **41**−**48**, in 20−96% yields (Scheme 7). Deprotection of *tert*-butylsulfonamide by TFA or BCl_3[24] delivered the primary sulfonamide that was subsequently treated with butyl chloroformate or ethyl chloroformate, at ambient temperature, in pyridine with 4-pyrrolidin-1-ylpyridine, to afford the target compounds **49**−**57**.

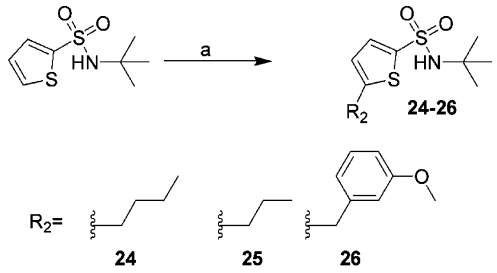
Binding Assays. Compounds **5**−**15**, **21**, **23**, **49**−**57** were evaluated in radioligand-binding assays by displacement of [^{125}I]Ang II from AT_1 receptors in rat liver membranes and from AT_2 receptors in pig uterus membranes as described previously.[25,26] The natural substrate Ang II and the selective AT_1 receptor antagonist losartan[27] were used as reference substances. The results are summarized in Table 1 and 2.

Scheme 3[a]



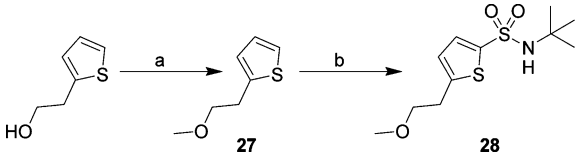
[a] Reagents and conditions: (a) n-BuLi, THF, 2-methylpropyl Iodide; (b) n-BuLi, THF, *tert*-butyl isocyanate; (c) n-BuLi, THF, triisopropyl borate; (d) $Pd(PPh_3)_4$, NaOH (aq), ethanol/ toluene, **3**; (e) TFA, anisole; (f) NaH, THF, butanesulfonyl chloride; (g) THF, pyridine, trifluorideacetic anhydride; (h) NaN_3, Et_3NHCl, toluene.

Scheme 4[a]



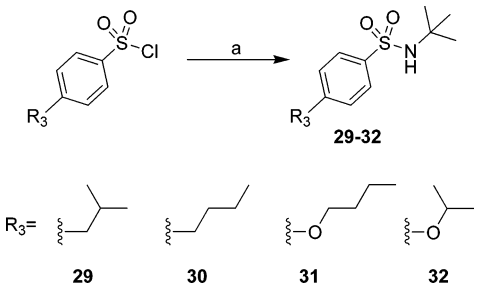
[a] Reagents and conditions: (a) n-BuLi, THF, iodo-butane (**24**), iodo-propane (**25**), 1-bromomethyl-3-methoxy-benzene (**26**).

Scheme 5[a]



[a] Reagents and conditions: (a) MeI, NaH, THF; (b) $ClSO_3H$, PCl_5, t-$BuNH_2$.

Scheme 6[a]

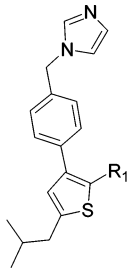


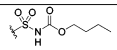
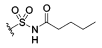
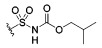
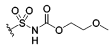
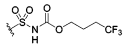
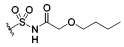
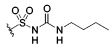
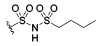
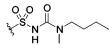
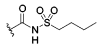
[a] Reagents and conditions: (a) *tert*-butylamine, CH_2Cl_2

In the first series, the effects of modifications of the sulfonamide side chain of the lead structure **1** (K_i AT_2: 0.4 nM, AT_1: >10000 nM)[16] were examined. As seen in Table 1, none of the side chain modifications gave any detectable increase in the AT_1 affinity for the tested compounds, but the affinities for the AT_2 receptor were affected by the structural modifications. Removing the carbamate oxygen from the side chain gave a compound (compound **5**) with a 100-fold decrease in affinity for the AT_2 receptor as compared to **1**. Shortening or branching of the butyloxy group rendered a 25−190 times decrease of the affinity for the AT_2 receptor (compound **6**−**10**), and surprisingly, introduction of a terminal trifluoromethyl group resulted in an inactive compound (compound **11**). On the other hand, extension of the chain as in the case with the ether **12** did not affect the binding to the AT_2 receptor significantly as compared to compound **1**. Substitution of the sulfonyl carbamate for sulfonyl ureas (**13** and **15**) also resulted in fairly potent ligands while considerable reduction in affinity was observed after replacement with either a sulfonyl sulfonamide group as in **14**, a reverse sulfonamide as in **21**, or a tetrazole as in **23**.

In the second series, the isobutyl side chain and the thionyl-phenyl scaffold of **1** were altered (Table 2). While a butyl or a propyl instead of an isobutyl group rendered compounds **49** and **50** approximately ten times less active than **1**, replacement of one methylene group of **49** by an oxygen atom as in **52** led to an additional loss of the AT_2 receptor affinity of more than 1 order of magnitude. Furthermore, a compound containing a methoxybenzyl group (**51**) was completely devoid of activity. Compound **53** exhibited the same binding profile as **1** (K_i AT_2: 0.6 nM, AT_1: >10000 nM). In the biphenyl series all side chain

Table 1



Entry	Compound	R_1	K_i[a] (nM)	
			AT_1	AT_2
1	**1**		>10000	0.4
2	**5**		>10000	34
3	**6**		>10000	10
4	**7**		>10000	37
5	**8**		>10000	31
6	**9**		>10000	75
7	**10**		>10000	63
8	**11**		>10000	>10000
9	**12**		>10000	1
10	**13**		>10000	13
11	**14**		>10000	76
12	**15**		>10000	4
13	**21**		>10000	79
14	**23**		>10000	189

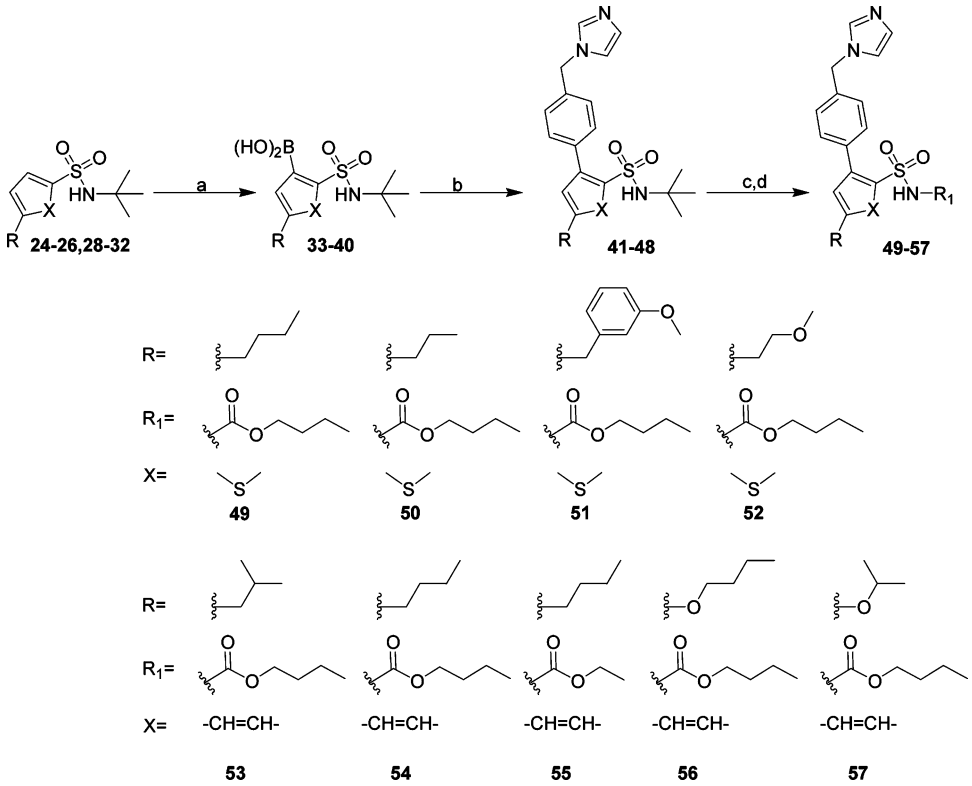
[a] K_i values are an average from three determinations. Standard deviations are less than 15% in all cases.

variations (**54**, **55**, and **57**), except in the case where the benzene ring was substituted with a butyloxy side chain (**56**), led to derivatives that were poorer AT_2 ligands than the parent compound **53**.

In Vitro Morphological Effects Induced by 56 and 50 in NG 108-15 Cells. To study the effects of compound **56** and **50** 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[28,29] and that a 3-day treatment with Ang II or the selective peptidic AT_2 receptor agonist CGP-42112 induces neurite outgrowth.[29] The mechanisms involve a sustained increase in p42/ $p44^{mapk}$ activity[15] and activation of the nitric oxide/guanylyl cyclase/cGMP pathway[30] (for a review see ref 9).

The cells were plated at the same initial density (4×10^4 cells/35 mm Petri dish) and were treated in the absence or presence of Ang II, **56**, or **50**. After 3 days of culture, cells were examined under a phase contrast microscope, and micrographs were taken. We first tested compounds **56** and **50** at concentrations ranging from 1 pM to 1 μM. Except for the

Scheme 7[a]



[a] Reagents and conditions: (a) n-BuLi, THF, triisopropyl borate; (b) $Pd(PPh_3)_4$, NaOH (aq), ethanol/toluene, **3**; (c) TFA, anisole or BCl_3 in CH_2Cl_2; (d) butyl chloroformate or ethyl chloroformate (**55**), with pyrrolidinopyridine, pyridine.

higher concentration of 1 μM, none of the other doses induced cell death. As shown in Figure 2, treatment for 3 days with concentrations of 0.1 μM of compounds **56** and **50** induced neurite outgrowth, comparable with that of Ang II. This effect was mediated through the AT_2 receptor, since co-incubation of **56** and **50** with the AT_2 receptor antagonist, **61** (PD 123,319)[22] (1 μM), virtually abolished neurite elongation (Figure 2), while alone, **61** did not alter the morphology compared to the untreated cells (data not shown).

Discussion

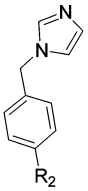
The benzylimidazole fragment seems not to be tolerated by the AT_1 receptor. Thus, neither of the sulfonyl carbamates nor the sulfonyl ureas nor the tetrazole derivative **21** were binding to the AT_1 receptor. This is notable, since the tetrazole ring structure, that serves as a carboxylic acid bioisostere, is a commonly used structure in AT_1 receptor selective antagonists, as in the sartans in clinic (e.g., losartan, valsartan, and candersartan).[31] Furthermore, it has previously been reported that with compounds structurally related to the sartans and with bicyclic heterocycles or other larger groups rather than an imidazole ring linked to the biaryl scaffold, strong AT_1 receptor binding can be achieved with a variety of modified alkoxy sulfonamide groups.[32] Considering AT_2 receptor affinities, all sulfonamide side chain modifications afforded derivatives that were weaker AT_2 receptor binders than **1** (Table 1). However, all the compounds in the first series showed affinity for to the AT_2 receptor and exhibited K_i values in the nanomolar range. Only one exception, the trifluoromethyl compound **11** lacked all affinity, an observation that we find difficult to rationalize. In contrast, less subtle modifications, such as replacement of the sulfonyl carbamate for a sulfonyl urea, in particular in the case where one of the nitrogens in the urea portion of the molecule was methylated (**15**, $K_i = 4$ nM), were apparently well accepted by the AT_2 receptor.

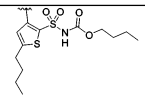
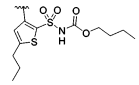
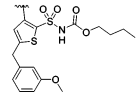
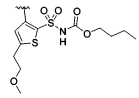
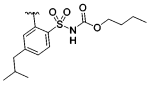
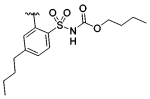
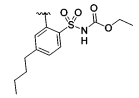
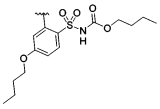
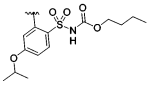
Alteration of the isobutyl side chain is known to affect the AT_1 agonistic properties of AT_1/AT_2 balanced compounds[20] and also to change the AT_1/AT_2 selectivity in favor of the AT_1 receptor.[22] As could have been expected,[22] the n-butyl analogue **49** was less potent (5 nM) than the isobutyl analogue **1** (0.4 nM). A similar attenuation of the affinity for the AT_2 receptor was also observed with the propyl analogue **50**. The replacement of the isobutyl with a *m*-methoxybenzyl moiety, a group found in the first AT_1 selective agonist disclosed (**60**)[21] lead to a totally inactive compound (**51**) at both the AT_1 and the AT_2 receptors.

The exchange of the thiophene for a phenyl ring did not affect the binding to the AT_2 receptor significantly (cf. **1** and **53**), a finding that is corroborated by results from previous studies of nonselective AT_1/AT_2 compounds.[33] This was also true for the n-butyl analogues (c.f **49** and **54**). By shortening the sulfonamide side chain, the affinity to the AT_2 receptor could be increased slightly (**55**, 2 nM). Surprisingly, the n-butyloxy side chain analogue (**56**) was as potent as the lead structure **1**, while the oxygen analogue of the isobutyl was much less active (**57**, 15 nM). We concluded that the imidazole ring strongly discriminates between the two receptor subtypes, and ligands with this ring system favor binding to the AT_2 receptor.

The most potent AT_2 binder, compound **56**, and the propylthiophene **50** were chosen for further in vitro studies to determine if the compounds acted as AT_2 selective agonists or antagonists. Although the receptor subtypes only share 34% homology, it is known from literature that removal of a methyl group from the isobutyl side-chain rendered an AT_1 antagonist (**59**). Thus, was a deletion of a methyl group from the branched

Table 2



Entry	Compound	R_2	K_i[a] (nM) AT_1	K_i[a] (nM) AT_2
1	**49**		>10000	5
2	**50**		>10000	6
3	**51**		>10000	>10000
4	**52**		>10000	86
5	**53**		>10000	0.6
6	**54**		>10000	5
7	**55**		>10000	2
8	**56**		>10000	0.5
9	**57**		>10000	15

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

side chain transforming these ligands from agonists to antagonists as was the case among the AT_1 receptor ligands? (cf. Figure 1).

Our results demonstrate that the two compounds induce neurite outgrowth (Figure 2), one of the first step of neuronal differentiation, as does Ang II and CGP-42112.[15,29,30] The butyloxy compound **56** was found to act as an agonist at the AT_2 receptor with a high potency, and even more remarkable the propylthiophene derivative **50** was also found to possess agonistic properties.

Conclusion

In summary, we have presented a structure−activity relationship study where the 2- and 5-positions of **1**, and its phenyl analogue, were altered. With few exceptions, all variations gave good AT_2 receptor affinities and with retained high AT_2/AT_1 selectivities. The trifluoroalkyl compound **11** and the *m*-methoxyphenyl compound **51** were inactive at both receptors. The butyloxyphenyl derivative **56** was found to exert a high agonistic potency as deduced from a neurite elongation assay.

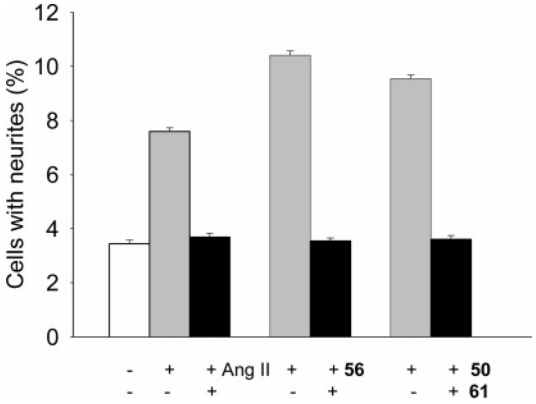


Figure 2. Effect of **50** and **56** on neurite outgrowth in NG108-15 cells. NG108-15 cells were plated at a density of 4×10^4 cells per dish in 35 mm Petri dishes and were cultured for 3 days in the absence or in the presence of 0.1 μM Ang II, compound **50** or compound **56**, alone or in the presence of 1 μM **61**. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. The number of cells with neurites represent the percentage of the total number of cells in the micrographs (from 50 to 100 cells according to the experiment).

Furthermore, we concluded that removal of a methyl group from the side chain of **1** delivers a compound (**50**) that acts as an AT_2 agonist with still a high agonistic potency. This finding is notable considering that the structurally related AT_1 agonist L-162,782 is transformed into the AT_1 antagonist **59** after the same alteration.[33]

Experimental Section

Chemistry. 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 δ values (ppm) downfield from tetramethylsilane. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden, or Analytische Laboratorien, Lindlar, Germany. Flash column 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 4.0 mL/min (H_2O/CH_3CN/0.05% HCOOH). All the organic phases were dried over $MgSO_4$, unless otherwise is stated. All chemicals were purchased from commercial suppliers and used directly without further purification.

General Procedure for Compound 5−9. Compound **4**[16] (150 mg, 0.311 mmol) and anisole (150 μL) were dissolved in TFA (5 mL) and stirred overnight. The reaction mixture was evaporated under vacuum and coevaporated with acetonitrile twice. The residue was dissolved in pyridine (1.6 mL), and pyrrolidinopyridine (52.1 mg, 0.35 mmol) was added. The solution was cooled on an ice bath, and acyl chloride (20 equiv) was added under N_2 (g) atmosphere. The reaction was stirred at ambient temperature overnight. The solvent was removed under vacuum and coevaporated with acetonitrile twice. The residue was dissolved in ethyl acetate (50 mL) and washed with citric acid (10% aq) and water. The organic phase was dried, evaporated, and purified by column chromatography to give the pure compounds **5**−**9**.

***N*-Butylcarbonyl-3-(4-imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-sulfonamide (5).** Compound **4** was used according to the general procedure and reacted with valeryl chloride (738 μL, 6.22 mmol), which after purification (CH_2Cl_2:MeOH 20:1) gave compound **5** in 50% yield (72 mg, 0.156 mmol). ^1H NMR ($CDCl_3$) δ 7.80 (br s, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.1 Hz, 2H), 6.84 (br s, 2H), 6.73 (s, 1H), 5.10 (br s, 2H), 2.69 (d, J = 7.1 Hz, 2H), 2.11 (t, J = 7.3 Hz, 2H), 1.93 (m, 1H), 1.44 (m, 2H),

1.22 (m, 2H), 0.98 (d, J = 6.6 Hz, 6H), 0.81(t, J = 7.1 Hz, 3H). Anal. ($C_{23}H_{29}N_3O_3S_2$) C, H, N.

***N*-(*N*-Butyl-methylaminocarbonyl)-3-(4-imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-sulfonamide (15).** A solution of **6** (29 mg, 0.065 mmol) and methylbutyl amine (11.6 mg, 0.133 mmol) in toluene was stirred at 90 °C for 3 h. The reaction mixture was concentrated and purified by prep-HPLC (AcCN in H_2O 0.5% HCOOH, 20 to 80%) to give compound **15** (19 mg, 0.039 mmol) in 60% yield. ^{1}H NMR ($CDCl_3$) δ 8.18 (br s, 1H), 7.84 (br s, 1H), 7.46 (d, J = 8.2 Hz, 2H), 7.16 (d, J = 8.2 Hz, 2H), 7.06 (br s, 1H), 6.99 (br s, 1H), 6.68 (s, 1H), 5.16 (s, 2H), 3.07 (br t, 2H), 2.67 (d, J = 7.1 Hz, 2H), 2.56 (br s, 3H), 1.91 (m, 1H), 1.35 (m, 2H), 1.19 (m, 2H), 0.96 (d, J = 6.6 Hz, 6H), 0.84 (t, J = 7.2 Hz, 3H). Anal. ($C_{24}H_{32}N_4O_3S_2 \times 1/2\ H_2O$) C, H, N.

2-Isobutylthiophene (16). To a solution of thiophene (5.7 mL, 0.071 mol) in dry THF (80 mL), at −78 °C, was added slowly n-BuLi (54 mL, 1.6 M in hexane, 0.086 mol), under a N_2 atmosphere. The mixture was stirred at −40 °C for 2 h. The solution was cooled to −78 °C, and 2-methylpropyl iodide (9.94 mL, 0.0864 mol) was added slowly. The reaction mixture was stirred at 0 °C for 2 h and then at r.t. overnight. The reaction was quenched with water (25 mL) and extracted with petroleum ether. The organic phase was dried and concentrated. The residue was distilled under vacuum (54−55 °C at 12 mmHg) to give compound **16** (3.0 g, 0.021 mol) in 30% yield. ^{1}H NMR ($CDCl_3$), δ 7.14 (dd, J = 5.1, 1.2 Hz, 1H), 6.95 (dd, J = 5.1, 3.3 Hz, 1H), 6.79 (dd, J = 3.3, 1.2 Hz, 1H), 2.72 (d, J = 7.1 Hz, 2H), 1.92 (m, 1H), 0.97 (d, J = 6.7 Hz, 6H). Anal. ($C_8H_{12}S$) C, H.

***N*-*tert*-Butyl-5-isobutylthiophene-2-carboxamide (17).** To a solution of **16** (1.0 g, 7.1 mmol) in dry THF (15 mL), at −78 °C, was added slowly n-BuLi (5.3 mL, 1.6M in hexane, 8.5 mmol), under a N_2 atmosphere. The mixture was stirred at 0 °C for 2 h. The solution was cooled to −78 °C, and *tert*-butyl isocyanate (0.896 mL, 7.84 mmol) was added slowly. The reaction mixture was stirred at 0 °C for 2 h. The reaction was quenched with water (15 mL) and extracted with EtOAc. The organic phase was dried and concentrated. The crude product was purified on column chromatography (P-ether: EtOAc 9:1) to provide compound **17** (1.2 g, 5.0 mmol) as white needles, in 70% yield. ^{1}H NMR ($CDCl_3$) δ 7.24 (d, J = 3.6 Hz, 1H), 6.69 (d, J = 3.6 Hz, 1H), 5.72 (br s, 1H), 2.65 (d, J = 7.2 Hz, 2H), 1.88 (m, 1H), 1.44 (s, 9H), 0.93 (d, J = 6.6 Hz, 6H). Anal. ($C_{13}H_{21}NOS$) C, H, N.

3-(4-Imidazol-1-ylmethylphenyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-carboxamide (19). To a solution of **17** (0.50 g, 2.1 mmol) in dry THF (50 mL), at −78 °C, was added slowly n-BuLi (3.3 mL, 1.6 M in hexane, 5.3 mmol), under a N_2 atmosphere. The mixture was stirred at −20 °C for 4 h. The solution was cooled to −78 °C, and triisopropyl borate (0.724 mL, 3.136 mmol) was added slowly. The reaction mixture was stirred at rt overnight. The reaction was quenched with HCl (2 mL, 2 M) and extracted with EtOAc. The organic phase was dried and concentrated. The resulting boronic acid **18** was used without further purification. A reaction tube was charged with the boronic acid **18** (200 mg, 0.706 mmol), **3**[16] (80.0 mg, 0.337 mmol), $Pd(PPh_3)_4$ (16.3 mg, 0.0141 mmol), NaOH (0.84 mL, 1.63M, 1.38 mmol), toluene (10 mL), and ethanol (2 mL). The reaction tube was flushed with $N_{2(g)}$ and sealed with a screw cap. The reaction mixture was heated at 100 °C for 3 h. The reaction mixture was diluted with water (15 mL) and extracted with EtOAc. The organic phase was dried and concentrated. The crude product was purified on column chromatography (CH_2Cl_2:MeOH 20:1) to provide compound **19** (129.0 mg, 0.327 mmol) in 97% yield. ^{1}H NMR ($CDCl_3$) δ 7.55 (br s, 1H), 7.41 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.1 Hz, 2H), 7.09 (br s, 1H), 6.89 (br s, 1H), 6.63 (s, 1H), 5.25 (br s, 1H, NH), 5.16 (s, 2H), 2.63 (d, J = 6.9 Hz, 2H), 1.89 (m, 1H), 1.13 (s, 9H), 0.95 (d, J = 6.6 Hz, 6H). Anal. ($C_{23}H_{29}N_3OS \times H_2O$) C, H, N.

3-(4-Imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-carboxamide (20). To **19** (165.0 mg, 0.417 mmol) were added TFA (2.5 mL) and anisole (150 μL). The reaction mixture was stirred at 70 °C for 17 h. The reaction mixture was concentrated, and the crude product was purified on column chromatography (CH_2Cl_2, MeOH 10:1) to give compound **20** (117.0 mg, 0.344 mmol) in 83% yield. ^{1}H NMR (CD_3OD) δ 9.05 (br s, 1H), 7.63 (br s, 1H), 7.55 (br s, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 6.81 (s, 1H), 5.47 (s, 2H), 2.68 (d, J = 7.1 Hz, 2H), 1.90 (m, 1H), 0.96 (d, J = 6.6 Hz, 6H). Anal. ($C_{19}H_{21}N_3OS$) C, H, N.

***N*-Butylsulfonyl-3-(4-imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-carboxamide (21).** To NaH (12.0 mg, 55% washed with isopentane) was added a solution of **20** (46.0 mg, 0.136 mmol) in THF (1 mL). The mixture was stirred at 50 °C for 0.5 h. To the reaction mixture was added butanesulfonyl chloride (35.0 mg, 0.223 mmol) slowly. The reaction was stirred at rt for 1 h and then concentrated. The crude product was purified by prep-HPLC (AcCN in H_2O 0.5% HCOOH, 10 to 80%) to give compound **21** (31.0 mg, 0.0674 mmol) in 50% yield. ^{1}H NMR ($CDCl_3$) δ 7.82 (br s, 1H), 7.32 (br s, 2H), 7.06 (d, J = 7.4 Hz, 2H), 6.95 (br s, 2H), 6.64 (s, 1H), 5.09 (br s, 2H), 4.79 (br s, 1H, NH), 2.97 (br s, 2H), 2.62 (d, J = 6.9 Hz, 2H), 1.90 (m, 1H), 1.65 (br s, 2H), 1.29 (m, 2H), 0.95 (d, J = 6.5 Hz, 6H), 0.81 (t, J = 6.6 Hz, 3H). Anal. ($C_{23}H_{29}N_3O_3S_2 \times 1/2$ HCOOH) C, H, N.

3-(4-Imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-carbonitrile (22). To a solution of **20** (133.3 mg. 0.3927 mmol) in THF (5 mL) was added pyridine (1.0 mL), and the mixture was stirred for 0.5 h at rt. Trifluoroacetic anhydride (66.6 μL, 0.471 mmol) was added at 0 °C. The reaction mixture was stirred at rt for 2 h. Ice was added to the solution and extracted with EtOAc. The organic phase was concentrated and purified on column chromatography (CH_2Cl_2:MeOH 24:1) to give compound **22** (50.0 mg, 0.156 mmol) in 40% yield. ^{1}H NMR ($CDCl_3$) δ 7.72−7.58 (m, 3H), 7.23 (d, J = 8.4 Hz, 2H), 7.10 (s, 1H), 6.93 (s, 2H), 5.17 (s, 2H), 2.70 (d, J = 6.6 Hz, 2H), 1.92 (m, 1H), 0.97 (d, J = 6.6 Hz, 6H). Anal. ($C_{19}H_{19}N_3S$) C, H, N.

5-[3-(4-Imidazol-1-ylmethylphenyl)-5-isobutylthiophen-2-yl]-1*H*-tetrazole (23). A mixture of **22** (48.1 mg, 0.150 mmol), sodium azide (156.0 mg, 2.40 mmol), and Et_3NHCl (330 mg, 2.40 mmol) in toluene (toluene 5 mL) was heated to 110 °C for 48 h. Water (8 mL) was added, and the reaction mixture was extracted with $CHCl_3$. The organic phase was dried and concentrated. The crude product was purified by prep-HPLC (AcCN in H_2O 0.5% HCOOH, 10 to 80%) to give compound **23** (50.8 mg, 0.139 mmol) in 93% yield. ^{1}H NMR ($CDCl_3$) δ 14.10 (br s, 1H), 8.18 (s, 1H), 6.97 (br d, 4H), 6.78 (d, J = 7.6 Hz, 2H), 6.64 (s, 1H), 5.02 (s, 2H), 2.61 (d, J = 6.3 Hz, 2H), 1.86 (m, 1H), 0.93 (d, J = 6.4 Hz, 6H). Anal. ($C_{19}H_{20}N_6S \times 1/2$ HCOOH) C, H, N.

***N*-*tert*-Butyl-5-propylthiophene-2-sulfonamide (25).** To a solution of *N*-*tert*-butylthiophene-2-sulfonamide (2.29 g, 10.44 mmol) in dry THF (20 mL), at −78 °C, was added slowly n-BuLi (12.7 mL, 1.6M in hexane, 20.3 mmol), under a N_2 atmosphere. The mixture was stirred at −40 °C for 2 h. The solution was cooled to −78 °C and iodo-propane (1.12 mL, 11.5 mmol) was added slowly. The reaction mixture was stirred at rt overnight. The reaction was quenched with NH_4Cl (aq) (10 mL) and extracted with EtOAc. The organic phase was dried and concentrated. The crude product was purified on column chromatography (P-ether; EtOAc 12:1) to give compound **25** (2.21 g, 8.45 mmol) in 81% yield. ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 3.8 Hz, 1H), 6.69 (d, J = 3.8 Hz, 1H), 4.89 (br s, 1H), 2.79 (t, J = 7.5 Hz, 2H), 1.70 (m, 2H), 1.26 (s, 9H), 0.96 (t, J = 7.3 Hz, 3H). Anal. ($C_{11}H_{19}NO_2S_2$) C, H, N.

***N*-*tert*-Butyl-4-butyloxybenzenesulfonamide (31).** To the solution of 4-n-butoxybenzene sulfonyl chloride (206.6 mg, 0.8306 mmol) in dry CH_2Cl_2 (12 mL) was *tert*-butylamine (900 μL, 8.56 mmol) added. The reaction mixture was stirred at rt for 1 h. Water (5 mL) was added, and the reaction mixture was extracted with CH_2Cl_2. The combined organic phase was washed with brine and water. The organic phase was dried and concentrated to give the pure compound **31** (230.8 mg, 0.8087 mmol) in 97% yield. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 9.0 Hz, 2H), 6.93 (d, J = 9.0 Hz, 2H), 4.54 (br s, 1H), 4.00 (t, J = 6.5 Hz, 2H), 1.79 (m, 2H), 1.50 (m, 2H), 1.21 (s, 9H), 0.98 (t, J = 7.4 Hz, 3H). Anal. ($C_{14}H_{23}NO_3S$) C, H, N.

***N*-*tert*-Butyl-3-(4-imidazol-1-ylmethylphenyl)-5-propylthiophene-2-sulfonamide (42).** To a solution of **25** (2.00 g, 7.65 mmol)

in dry THF (40 mL), at −78 °C, was added slowly n-BuLi (12.0 mL, 1.6M in hexane, 19.1 mmol), under a N_2 atmosphere. The mixture was stirred at −20 °C for 4 h. The solution was cooled to −78 °C, and triisopropyl borate (9.72 mL, 42.1 mmol) was added slowly. The reaction mixture was stirred at rt overnight. The reaction was quenched with HCl (15 mL, 2 M) and extracted with EtOAc. The organic phase was dried and concentrated. The resulting boronic acid **34** was used without further purification. A reaction tube was charged with the boronic acid **34** (420 mg, 1.38 mmol), 1-(4-bromobenzyl)-1*H*-imidazol(ref) (188 mg, 0.793 mmol), $Pd(PPh_3)_4$ (64 mg, 0.055 mmol), NaOH (1.64 mL, 1.54 M, 2.53 mmol), toluene (15 mL), and ethanol (2 mL). The reaction tube was flushed with $N_{2(g)}$ and sealed with a screw cap. The reaction mixture was heated at 100 °C for 3 h. The reaction mixture was diluted with water (15 mL) and extracted with EtOAc. The organic phase was dried and concentrated. The crude product was purified on column chromatography (CH_2Cl_2:MeOH 40:1) to provide compound **42** (278.1 mg, 0.6660 mmol) in 84% yield. ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.59 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.2 Hz, 2H), 7.11 (s, 1H), 6.93 (s, 1H), 6.75 (s, 1H), 5.18 (s, 2H), 4.33 (br s, 1H), 2.78 (t, J = 7.4 Hz, 2H), 1.72 (m, 2H), 0.99 (m, 12H). Anal. ($C_{21}H_{27}N_3O_2S_2$) C, H, N.

***N*-*tert*-Butyl-2-(4-imidazol-1-ylmethylphenyl)-4-butoxybenzenesulfonamide (47).** To a solution of **31** (230.8 mg, 0.8087 mmol) in dry THF (20 mL), at −78 °C, was added slowly n-BuLi (1.26 mL, 1.6M in hexane, 2.02 mmol), under a N_2 atmosphere. The mixture was stirred at −20 °C for 4 h. The solution was cooled to −78 °C, and triisopropyl borate (0.28 mL, 1.2 mmol) was added slowly. The reaction mixture was stirred at rt overnight. The reaction was quenched with HCl (5 mL, 2 M) and extracted with EtOAc. The organic phase was dried and concentrated. The resulting boronic acid **39** was flushed trough a silica column (CH_2Cl_2:MeOH 40:1) before further use. A reaction tube was charged with the boronic acid **39** (76.3 mg, 0.232 mol), **3**[16] (39.3 mg, 0.166 mmol), $Pd(PPh_3)_4$ (10.0 mg, 0.00865 mmol), NaOH (0.602 mL, 1.54 M, 0.927 mmol), toluene (12 mL), and ethanol (2.0 mL). The reaction tube was flushed with $N_{2(g)}$ and sealed with a screw cap. The reaction mixture was heated at 100 °C for 4.5 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc. The organic phase was dried and concentrated. The crude product was purified on column chromatography (CH_2Cl_2:MeOH 40:1) to provide compound **47** (52.0 mg, 0.118 mmol) in 71% yield. ^{1}H NMR ($CDCl_3$) δ 8.04 (d, J = 8.9 Hz, 1H), 7.78 (br s,1H), 7.49 (d, J = 8.1 Hz, 2H), 7.23 (d, J = 8.1 Hz, 2H), 7.12 (br s, 1H), 6,96 (br s, 1H), 6.91 (dd, J = 8.8, 2.6 Hz, 1H), 6.74 (d, J = 2.6 Hz, 1H), 5.21 (s, 2H), 3.99 (t, J = 6.4 Hz, 2H), 3.61 (s, 1H), 1.76 (m, 2H), 1.47 (m, 2H), 1.10−0.85 (m, 12H). Anal. ($C_{24}H_{31}N_3O_3S \times 1/2\ H_2O$) C, H, N.

***N*-Butyloxycarbonyl-3-(4-imidazol-1-ylmethylphenyl)-5-propylthiophene-2-sulfonamide (50).** Compound **42** (680.8 mg, 0.1934 mmol) was dissolved in CH_2Cl_2 (5 mL) under N_2 (g) atmosphere, BCl_3 (1.0 mL, 1 M CH_2Cl_2) was added, and the reaction mixture was stirred for 3 h. To the reaction mixture was added Na_2CO_3 (10% aq, 50 mL), and the aqueous phase was extracted with EtOAc. The combined organic phase was washed with brine and water and then dried and evaporated. The residue was dissolved in pyridine (2 mL), and pyrrolidinopyridine (57.3 mg, 0.387 mmol) was added. The solution was cooled on an ice bath, and butyl chloroformate (49.2 μL, 0.387 mmol) was added under N_2 (g) atmosphere. The reaction was stirred at ambient temperature overnight. The solvent was removed under vacuum and coevaporated with acetonitrile twice. The residue was dissolved in ethyl acetate (50 mL) and washed with citric acid (10% aq) and water. The organic phase was dried, evaporated, and purified by column chromatography (CH_2Cl_2:MeOH 15:1) to give the entitled compound in 56% yield (50.0 mg, 0.108 mmol). ^{1}H NMR (30%CD_3OD in $CDCl_3$) δ 7.79 (br s, 1H), 7.49 (d, J = 7.7 Hz, 2H), 7.17 (d, J = 7.7 Hz, 2H), 7.01 (br s, 2H), 6.72 (s, 1H), 5.15 (s, 2H), 3.94 (t, J = 6.4 Hz, 2H), 2.77 (t, J = 7.4 Hz, 2H), 1.70 (m. 2H), 1.46 (m, 2H), 1.21 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H). Anal. ($C_{22}H_{27}N_3O_4S_2 \times 1/2H_2O$) C, H, N.

***N*-Butyloxycarbonyl-2-(4-imidazol-1-ylmethylphenyl)-4-butoxybenzenesulfonamide (56).** Compound **47** (34.7 mg, 0.0785 mmol) and anisole (50 μL) were dissolved in TFA (2.0 mL) and stirred overnight. The reaction mixture was evaporated under vacuum and coevaporated with acetonitrile twice. The residue was dissolved in pyridine (3.0 mL) and pyrrolidinopyridine (23 mg, 0.16 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (20.0 μL, 0.157 mmol) was added under N_2 (g) atmosphere. The reaction was stirred at ambient temperature overnight. The solvent was removed under vacuum and coevaporated with acetonitrile twice. The residue was dissolved in ethyl acetate (50 mL) and washed with citric acid (10% aq) and water. The organic phase was dried, evaporated and purified by column chromatography (CH_2Cl_2:MeOH 15:1) to give the entitled compound in 53% yield (20.2 mg, 0.0416 mmol). ^{1}H NMR ($CDCl_3$) δ 8.53 (br s, 1H), 8.18 (d, J = 8.9 Hz, 1H), 7.36 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 7.17 (br s, 1H), 7.12 (br s, 1H), 6.98 (dd, J = 8.9, 2.5 Hz, 1H), 6.74 (d, J = 2.5 Hz, 1H), 6.53 (br s, 1H), 5.32 (s, 2H), 4.01 (t, J = 6.5 Hz, 4H), 1.77 (m, 2H), 1.48 (m, 4H), 1.22 (m, 2H), 0.96 (t, J = 7.1 Hz, 3H), 0.86 (t, J = 7.3 Hz, 3H). Anal. ($C_{25}H_{31}N_3O_5S$) C, H, N.

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Supporting Information Available: Experimental procedures including the synthesis of compounds **6**−**14**, **24**, **26**−**30**, **32**−**41**, **43**−**46**, **48**, **49**, **51**−**55**, and **57** with characterization data, NMR data for compounds presented in the experimental part, and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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