Design, Synthesis, and Biological Evaluation of the First Selective Nonpeptide AT_2 Receptor Agonist

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Received April 13, 2004

The first druglike selective angiotensin II AT_2 receptor agonist (**21**) with a K_i value of 0.4 nM for the AT_2 receptor and a K_i >10 μM for the AT_1 receptor is reported. Compound **21**, with a bioavailability of 20−30% after oral administration and a half-life estimated to 4 h in rat, induces outgrowth of neurite cells, stimulates p42/p44^{mapk}, enhances in vivo duodenal alkaline secretion in Sprague−Dawley rats, and lowers the mean arterial blood pressure in anesthetized, spontaneously hypertensive rats. Thus, the peptidomimetic **21** exerts a similar biological response as the endogenous peptide angiotensin II after selective activation of the AT_2 receptor. Compound **21**, derived from the prototype nonselective AT_1/AT_2 receptor agonist L-162,313 will serve as a valuable research tool, enabling studies of the function of the AT_2 receptor in more detail.

Introduction

The octapeptide angiotensin II (Ang II), the active component of the renin−angiotensin system, mediates its action via two major receptors, namely, AT_1 and AT_2. While the AT_1 receptor is closely associated with the regulation of blood pressure, fluid and electrolyte balance, and thirst, the role of the AT_2 receptor has been more difficult to elucidate due to its low level of expression.[1] However, one of the most remarkable features of the AT_2 receptor is its high level of expression in most fetal tissues, including the brain. The receptor ratio AT_2/AT_1 decreases dramatically after birth,[2,3] suggesting an involvement of the AT_2 receptor in fetal development. Indeed, in cells of neuronal origin, activation of the AT_2 receptor was shown to induce neurite outgrowth and elongation, modulate neuronal excitability, and promote cellular migration in the adult. The expression of the AT_2 receptor is mostly limited to certain tissues, such as the adrenal gland and specific areas of the brain, but importantly is up-regulated in pathological conditions such as heart failure, renal failure, myocardial infarction, brain lesions, vascular injury, and wound healing. In the adult, activation of the AT_2 receptor results in vasodilatation, inhibition of cell proliferation, induction of programmed cell death, extracellular matrix remodeling, and axonal regeneration.(for reviews see refs 4−9) Recently, we demonstrated that activation of the AT_2 receptor stimulates alkaline secretion by the duodenal mucosa in rats.[10]

The precise nature of the signaling pathways activated by AT_2 receptor stimulation is 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_{α^i} protein, reported by several authors, is not viewed in consensus.(for reviews see refs 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 ERK1/ERK2 (p42/p44^{mapk}), have been associated with activation of the AT_2 receptor, depending on the cell types and experimental conditions used. More precisely, a sustained increase in p42/p44^{mapk} activity is found associated with neuronal differentiation.[11,12]

The AT_2 receptor often mediates *opposing* effects to the AT_1 receptor. While there are several AT_1 receptor *antagonists* in the clinic (e.g., losartan, Figure 2), few selective AT_2 receptor *agonists* are available as research tools and are all peptides [e.g. CGP-42112 and (*p*-amino-Phe^6)Ang II].[13−15]

Therefore metabolically stable, druglike AT_2 receptor agonists are highly desirable as research tools and as potential therapeutics. We herein report that the nonselective AT_1 and AT_2 receptor agonist L-162,313 (**1**)[16−18] indeed can be modified to yield the highly selective AT_2 receptor agonist **21** (Figure 1).

Compound **21**, which has a bioavailability of 20−30% after oral administration in rat, is to the best of our

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10.1021/jm049715t CCC: $27.50
Published on Web 10/15/2004

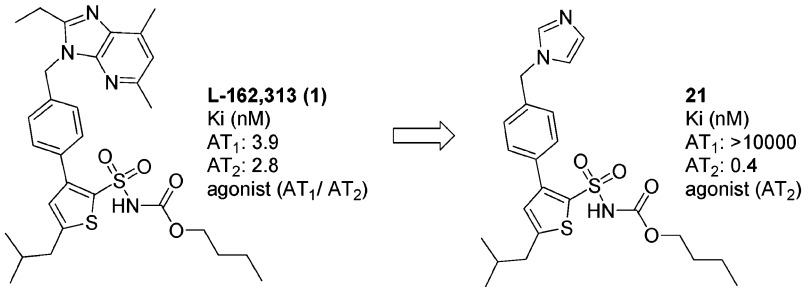


Figure 1.

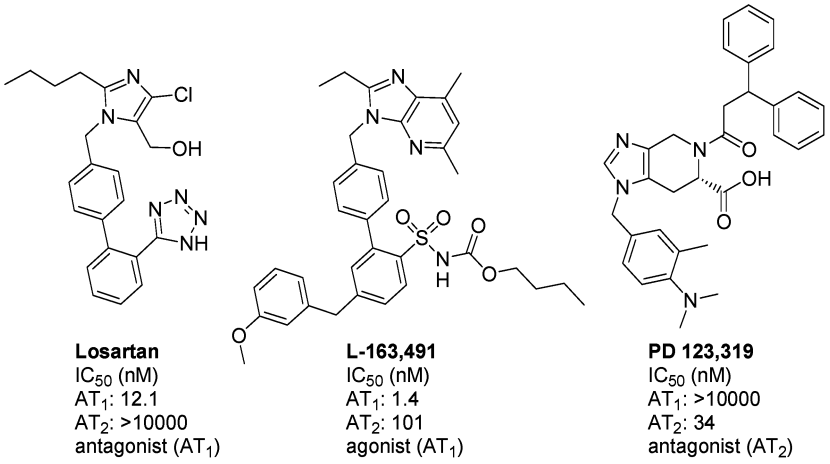


Figure 2.

knowledge the first selective nonpeptide AT_2 receptor agonist reported. Compound **21** (a) induces neurite outgrowth, (b) stimulates MAPK, (c) increases duodenal mucosal alkalization in the rat, and (d) decreases mean arterial blood pressure in spontaneously hypertensive rats (SHR), effects that are all suppressed in the presence of the selective AT_2 receptor antagonist PD 123,319 (Figure 2).[19]

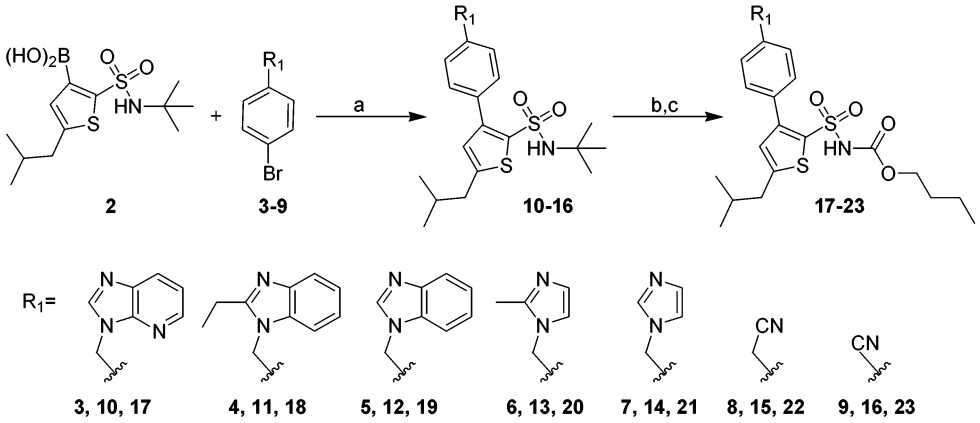
Results

Chemistry. Previous studies have demonstrated that replacement of the isobutyl group of **1** yielded compounds with low AT_2 receptor selectivity.[20] Note that replacement of the isobutyl group with a *m*-methoxybenzyl group in a structurally related series provided the first selective nonpeptide AT_1 receptor agonist L-163,491 (Figure 2).[21]

We decided initially to keep both the butyloxycarbonyl sulfonamide and the isobutyl groups unaltered and first focus our research efforts on the bicyclic nitrogen heterocycle.

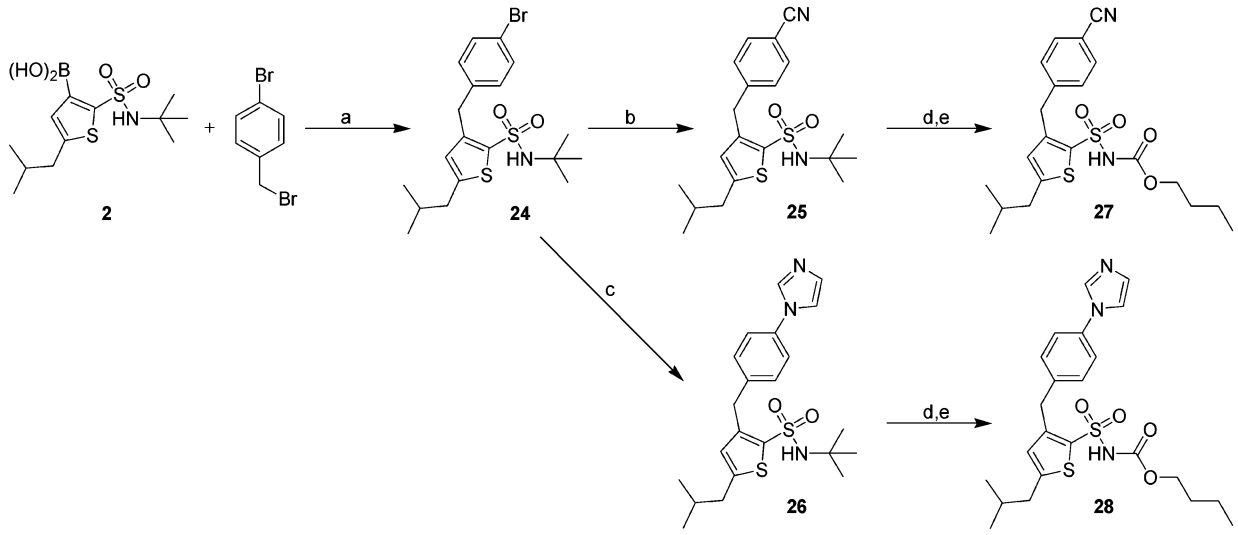
The compounds were prepared as shown in Schemes 1 and 2. The thiopheneboronic acid **2**, used as the starting material for all the compounds, was prepared, in essence, as described by Kevin et al.[20,22] Thus, thiophene-2-sulfonyl chloride was first converted to the *N-tert*-butylsulfonamide. Subsequent alkylation followed by selective 3-lithiation/boronation delivered the key intermediate **2**. The aryl bromides, **3**−**7**, were obtained by *N*-alkylation of the appropriate nitrogen-containing heterocycle with 4-bromobenzyl bromide, utilizing KOH as base. The aryl bromides **3**−**7** were thereafter reacted with the boronic acid **2** under Suzuki coupling conditions with $Pd(PPh_3)_4$ as the source of palladium and KOH as

Scheme 1[a]



[a] Conditions: (a) $Pd(PPh_3)_4$, NaOH (aq), ethanol/toluene, thermal or microwave heating; (b) TFA or BCl_3 in CH_2Cl_2; (c) butylchloroformate with pyrrolidinopyridine, pyridine or K_2CO_3 in CH_2Cl_2/water (3:1).

Scheme 2[a]



[a] Conditions: (a) $Pd(PPh_3)_4$, NaOH (aq), ethanol/toluene, microwave heating; (b) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, microwave heating; (c) imidazole, CuI, Cs_2CO_3, 1,2-diaminocyclohexane, DMF, microwave heating; (d) TFA or BCl_3 in CH_2Cl_2; (e) butylchloroformate, pyrrolidinopyridine, pyridine.

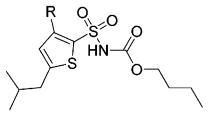
base to give compounds **10**−**14** (Scheme 1). Analogously, the sulfonamides **15** and **16** were obtained after Suzuki coupling with 4-bromophenylacetonitrile (**8**) and 4-bromobenzonitrile (**9**), respectively. The biaryl compounds **10**−**16** were isolated in moderate to good yields. Compound **14** was alternatively prepared by a fast microwave-promoted Suzuki reaction.[23,24] Deprotection by TFA or BCl_3, a milder deprotecting reagent,[25] delivered the primary sulfonamide, and subsequent reactions with butyl chloroformate in pyridine with 4-pyrrolidin-1-yl-pyridine as nucleophilic catalyst afforded the target compounds **17**−**19** and **21**−**23** (Scheme 1). To obtain the methylimidazole compound **20**, a minor modification was required, since an acylimidazolium salt seemed to have been formed using 4-pyrrolidin-1-ylpyridine in pyridine. To circumvent this obstacle, the reaction was performed in a CH_2Cl_2/water (3:1) mixture with Na_2CO_3 as base, which provided the compound **20** in a good yield.

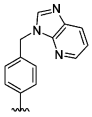
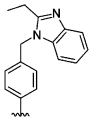
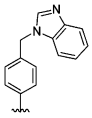
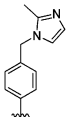
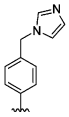
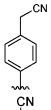
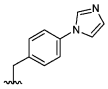
To obtain the compounds **27** and **28**, the boronic acid **2** was first treated with 4-bromobenzyl bromide under Suzuki coupling conditions with $Pd(PPh_3)_4$ and K_2CO_3 as base to give the aryl bromide **24**, isolated in 80% yield (Scheme 2). A small-scale microwave-mediated version of the Suzuki benzylation reaction required 30 min at 110 °C and provided a yield of 78%. The weaker carbonate base was used rather than KOH to prevent the benzylic bromide from being converted to the corresponding benzyl alcohol. The cyanide group was introduced by a microwave-promoted palladium-catalyzed cyanation reaction.[26] The reaction was complete after 2 min at 180 °C and provided the nitrile **25** in 75% yield. To introduce the imidazole ring, we aimed to use Buchwald−Hartwig conditions[27,28] with microwave heating. This reaction was first explored using 4-bromotoluene as a model substrate in DMF to optimize the reaction conditions. To our surprise, 4-tolylimidazole was not obtained. Instead, *N*,*N*-dimethyltolyl amide was the predominant product. The carbonyl moiety was proven to be derived from DMF, serving as an excellent carbon monoxide source.[29,30] Only with electron-deficient aryl bromides could the arylimidazoles be obtained.[31] Therefore, the imidazole was instead attached to the aromatic ring by a microwave-promoted Ullman coupling.[32,33] The reaction was sluggish and yielded only 27% of compound **26** after 40 min at 180 °C. Finally **25** and **26** were deprotected with BCl_3 to give the primary sulfonamide, and subsequent reaction with butyl chloroformate gave the target compounds **27** and **28**.

Binding Assays. Compounds **17**−**23**, **27**, and **28** 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 in essence as described previously (Table 1).[34,35] The natural substrate Ang II, the selective AT_1 receptor antagonist losartan,[36] and the selective AT_2 receptor antagonist PD 123,319[19] were used as reference substances.

The K_i values for **1** in Table 1 were determined to be 3.9 nM for AT_1 and 2.8 nM for the AT_2 receptors (lit.[17] IC_{50} = 1.1 and 2.0 nM, respectively). As apparent from Table 1, removal of the alkyl groups from the bicyclic heterocycle resulted in a dramatic loss of affinity to the AT_1 receptor, while the AT_2 receptor affinity was only slightly affected (entry 2, compound **17**). A similar response was encountered after replacement of the imidazopyridine with a 2-ethyl-1*H*-benzimidazole (entry 3, compound **18**). Unexpectedly, the AT_1 affinity was increased by the removal of the ethyl group from the benzimidazole (entry 4, compound **19**). Notably, with 2-methylimidazole or imidazole (entries 5 and 6) as heterocycles, the affinity to the AT_1 receptor remained poor, but a 10-fold improvement of the AT_2 receptor affinity was observed. On the other hand, further simplification of the structure, i.e. changing from an imidazole to a nitrile (entry 7, compound **22**) led to a 100-fold improved AT_1 affinity, while the affinity to the AT_2 receptor now dropped more than 100-fold. Thus, this alteration delivered a nonselective ligand. Removal of the benzylic methylene group of **22** (entry 8) resulted in the inactive arylnitrile **23**. Furthermore, employing a benzylthiophene instead of a phenylthiophene scaffold, in combination with attachment of the imidazole direct on the aromatic ring, had a deleterious effect on the

Table 1.



Entry	Compound	R-group	K_i[a] (nM)		AT_1/AT_2
			AT_1	AT_2	
1	**1**		3.9	2.8	1.4
2	**17**		>10000	7.1	>1400
3	**18**		>10000	3.2	>3125
4	**19**		500	4.0	125
5	**20**		>10000	0.5	>20000
6	**21**		>10000	0.4	>25000
7	**22**		100	70	1.4
8	**23**		>10000	>10000	-
9	**27**		>10000	>10000	-
10	**28**		>10000	>10000	-

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

affinities (entry 10). Compound **21**, with the highest affinity and receptor selectivity, was chosen for further in vitro and in vivo studies.

In Vitro Morphological Effects Induced by 21 in NG 108-15 Cells. To study the effects of compound **21** on differentiation, we used NG108-15 cells. 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[37,38] and that a three-day treatment with Ang II or the selective peptidic AT_2 receptor agonist CGP-42112 induces neurite outgrowth.[38] The mechanisms involve a sustained increase in p42/p44^{mapk} activity[12] and activation of the nitric oxide/guanylyl cyclase/cGMP pathway[39](for review, see ref 9).

For all experimental conditions, cells were plated at the same initial density (30×10^4 cells/35 mm Petri dish) and were treated without or with Ang II or compound **21**. After 3 days of culture, cells were examined under a phase-contrast microscope and micrographs were taken. We first tested compound **21** at various concentrations ranging from 1 pM to 1 μM. Except for the higher concentration of 1 μM, none of the other doses induced cell death. In addition, cells stimulated for 3 days with concentrations of compound **21** higher than 0.1 nM induced neurite outgrowth. As shown by phase-contrast microscopy, nontreated control cells had rounded cell bodies, without or with some thin processes (Figure 3A). After a 3-day treatment with **21** (0.1 μM), most cells extended one or two neurite processes, while the cell body retained a rounded appearance (Figure 3B). In comparison to Ang II (0.1 μM) (Figure 3C), compound **21** induces the same morphological changes, with the longer processes exhibiting a growth cone at the tip[38] (Figure 3D). This effect was mediated through the AT_2 receptor, since coincubation of **21** with the AT_2 receptor antagonist PD 123,319 (1 μM) virtually halted neurite elongation (Figure 3F), while alone PD 123,319 did not alter the morphology of the untreated cells (Figure 3E).

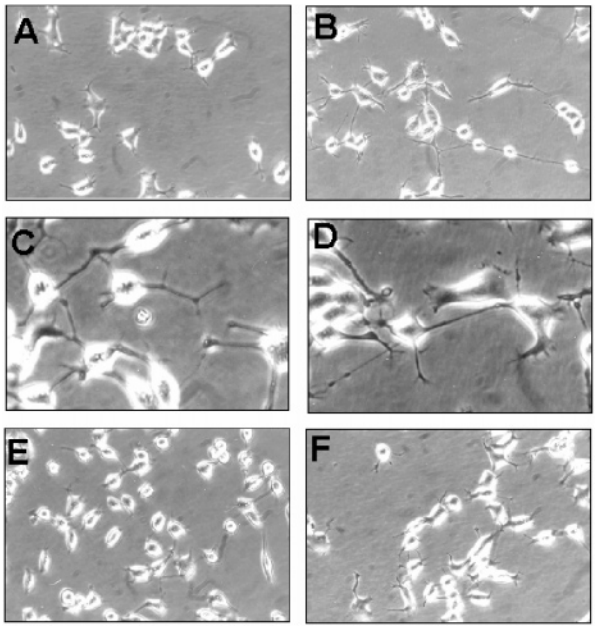


Figure 3. Effect of **21** and angiotensin II (Ang II) on neurite outgrowth in NG108-15 cells. NG108-15 cells were plated at a density of 3×10^4 cells per dish in 35 mm Petri dishes and were cultured for 3 days in the absence (control, A), or in the presence of 0.1 μM compound **21** (B, D), 0.1 μM Ang II (C), 1 μM PD 123,319 (E) or **21**, and 0.1 μM PD 123,319 (F). Panels A, B, E, and F are seen at the same magnification, and panels C and D at higher magnification (D is a magnification of B).

To further explore if compound **21** shares the same signaling transduction as Ang II,[12,39] cells were preincubated for 3 days with 10 μM PD 98,059: a dose that abolished MAPK activity. Alone, PD 98,059 induced a rounding up appearance of the cell body (compare Figure 4C to Figure 3A) and, when coincubated with **21**, decreased length and number of neurites was also observed (Figure 4 D). Cells were also preincubated with LY-83,583 (0.5 μM), an inhibitor of soluble guanylyl cyclase (sGC), or with KT 5823 (1 μM), an inhibitor of cGMP-dependent protein kinases, which were added every day, 30 min before compound **21**. KT 5823- or LY-83,583-treated cells had the same morphological appearance as the untreated control cells (Figure 4E,G). However, cells coincubated with **21** and LY-83,583 or

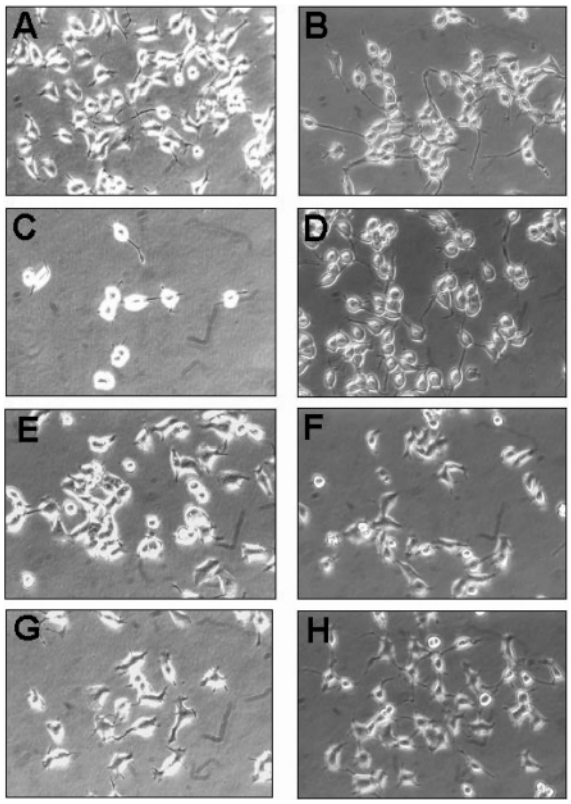


Figure 4. Effect of various inhibitors on **21**-induced neurite outgrowth in NG108-15 cells. NG108-15 cells were plated at a density of 3×10^4 cells per dish in 35 mm Petri dishes and were cultured for 3 days in the absence (control, A, C, E, and G) or in the presence of 0.1 μM **21** (B, D, F, H). Cells were cultured in the presence of 10 μM PD 98,059 (C, D), 0.5 μM LY-83,583 (E, F), or 1 μM KT 5823 (G, H). All panels are seen at the same magnification.

KT 5823 have only one or two thin processes (Figures 4F,H). Quantification of these results (Figure 5) indicated that a 3-day treatment with **21** increased the number of cells with neurites (from 5.5 ± 0.39% in control cells to 15.6 ± 0.7% in Ang II-treated cells and 19.9 ± 1.10% in the compound **21**-treated cells). This effect was abolished in cells coincubated with PD 123,319 (Figure 5A), PD 98,059, LY-83,583, and KT 5823 (Figure 5B).

Compound **21** also induced tyrosine phosphorylation of p42/p44^{mapk}, as determined by using an antibody directed against the tyrosine phosphorylated form of p42/p44^{mapk}. This effect is observed within 30 min of compound **21** application (2.2 ± 0.4-fold increase over control) and then decreases to basal level after 60 min. The stimulation observed after 30 min was abolished in cells preincubated with 10 μM PD 123,319 (Figure 6).

In Vivo Assays. The in vivo experiments were performed on anaesthetized nonfasted male Sprague−Dawley rats (S−D) and spontaneously hypertensive rats (SHR). A femoral artery and one or two veins were catheterized for subsequent blood pressure measurements and drug infusions, respectively. Duodenal mucosal alkaline secretion (HCO_3^- secretion) was measured by a pH-stat titration technique.[40] Alkaline secretion to the perfusate was continuously titrated to pH 7.4 with 0.02 M aqueous hydrochloric acid controlled by a pH-stat device.

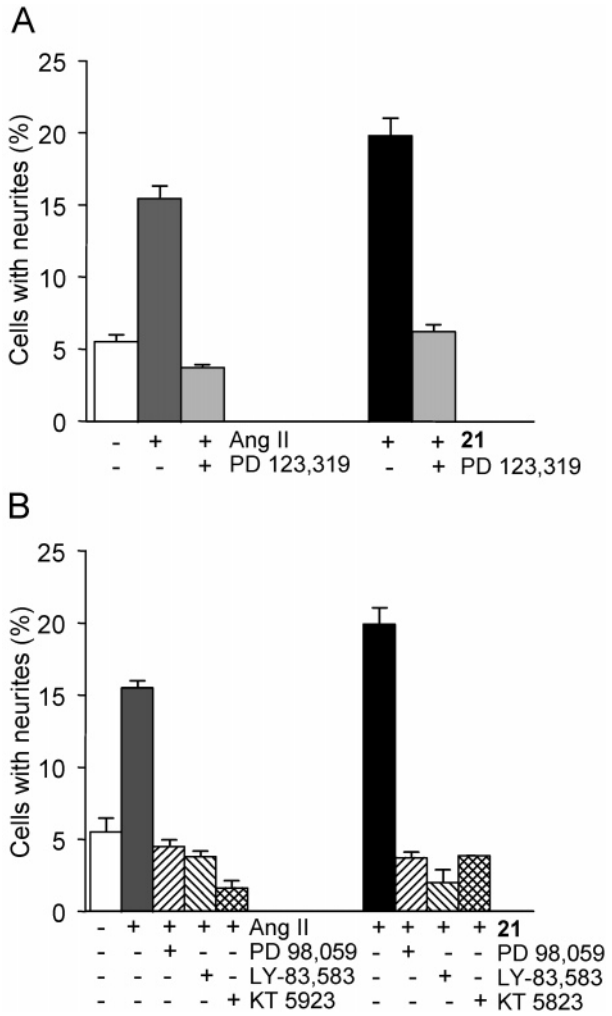


Figure 5. Effect of various inhibitors on **21**-induced neurite outgrowth in NG108-15 cells. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth The number of cells with neurites represent the percentage of the total number of cells in the micrographs (from 50 to 100 cells according to the experiment). (A) Control, with Ang II (0.1 μM) stimulation, Ang II (0.1 μM) in the presence of PD 123,319 (0.1 μM), **21** (0.1 μM), and **21** (0.1 μM) in the presence of PD 123,319 (0.1 μM). (B) control, Ang II (0.1 μM), Ang II (0.1 μM) in the presence of PD 98,059 (10 μM), Ang II (0.1 μM) in the presence of LY-83,583 (0.5 μM), Ang II (0.1 μM) in the presence of KT 5823 (1.0 μM), **21** (0.1 μM), **21** (0.1 μM) in the presence of PD 98,059 (10 μM), **21** (0.1 μM) in the presence of LY-83,583 (0.5 μM), and **21** (0.1 μM) in the presence of KT 5823 (1.0 μM).

Intravenous doses of compound **21** raised the duodenal alkaline secretion dose-dependently with a threshold for response at an infusion rate of 0.03 mg kg^{-1} h^{-1} (Figure 7). The secretory response to the highest dose was significantly inhibited by the AT_2-receptor antagonist PD 123,319 (0.3 mg kg^{-1} h^{-1}) (Figure 8, left panel). Compound **21** also stimulated secretion when exposed topically to the mucosa by administering it into the intraluminal perfusate at a concentration of 100 μM. In addition, this response was sensitive to PD 123,319 (150 μM intraluminally) (Figure 8, right panel). Mean arterial pressure was not significantly influenced during the secretion experiments in the S−D rat strain (data not shown). As AT_2-related hypotensive effects have been reported, particularly in SHR,[41] a limited number of experiments were performed in this rat strain. Basal

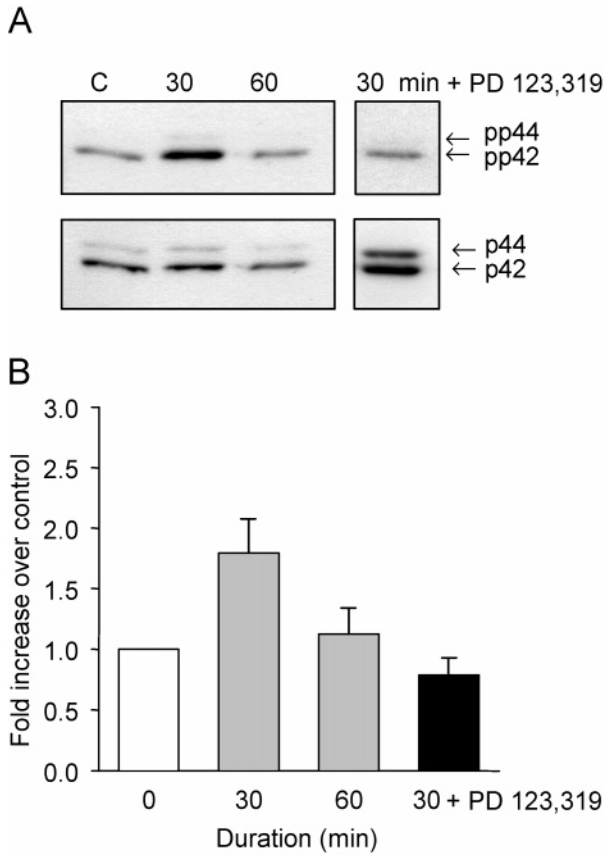


Figure 6. Effect of **21** on p42/p44^{mapk} activation. NG108-15 cells were stimulated without or with 0.1 μM **21** for 30 and 60 min alone or in the presence of 1 μM PD 123,319. Upper panels for each condition show Western blot analysis of phosphorylated p42/p44^{mapk} and lower panels represent the same blots reprobed for total p42/p44^{mapk}. Results are representative of at least three different experiments.

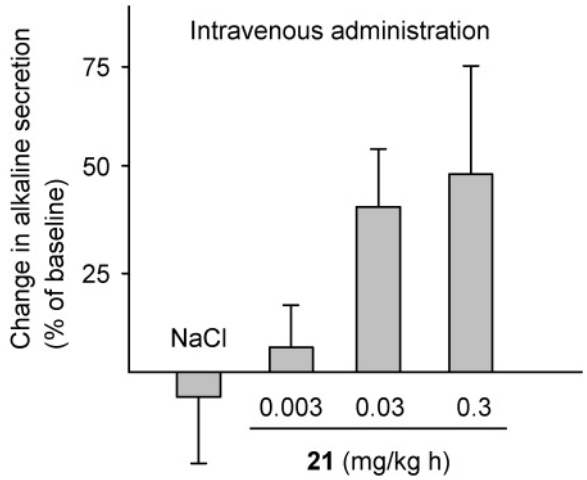


Figure 7. The effect of increasing iv doses of compound **21** on rat duodenal mucosal alkaline secretion in vivo. Data are expressed as the percent change from baseline and plotted as mean ± SEM, with $n = 5$ in each **21** group, $n = 4$ in the time controls (NaCl).

mean arterial pressure before drug administration ranged between 158 and 203 mmHg (mean value 182 mmHg, $n = 41$), and a wide dose range was studied (0.008−4 mg/kg intravenously). Compound **21** at low doses (0.008 and 0.05 mg/kg) decreased MAP, a response that was attenuated in animals treated with PD 123,319 (0.3 mg kg^{-1} h^{-1} iv) (Figure 9). Changes in MAP at the higher dose levels did not reach statistical significance as compared to vehicle. PD 123,319 had no intrinsic effect on MAP (data not shown).

Pharmacokinetics. The oral absorption study was performed on male Sprague−Dawley rats in triplicate with three oral concentrations (30, 100, and 300 μg/kg) and one intravenously (30 μg/kg). The orally administrated doses were given by gastric gavage, and the intravenous doses were administrated as a single bolus injection into the tail vein. All dose weights were adjusted to take into account the weight of the animal at the time of administration. A blood sample (200 μL) was taken from the animals by venepuncture of the tail vein at the following time points postdose: 30 min and 1, 2, 4, 6, 8, and 24 h. The concentration of compound **21** was analyzed with LC−MS/MS.

An approximate dose-proportional increase in the systematic exposure of **21** was observed with increasing dose levels. Total clearance of **21** from plasma was nominally similar to hepatic plasma flow rate in rats. The apparent volume of distribution was up to 2.9-fold greater than total body water in rats, suggesting that **21** may be sequestered widely into the tissues. Plasma concentrations of **21** declined with apparent terminal half-lives ranging from 3 to 6 h following oral administration. Following intravenous administration, plasma concentrations declined with apparent terminal half-lives of 0.5−2.5 h. Estimates of absolute bioavailability of **21** following oral administration ranged from 20 to 30%.

Discussion

The present results demonstrate that compound **21** induces neurite outgrowth, one of the first steps of neuronal differentiation, as does Ang II or CGP-42112.[38] In addition, **21** like Ang II stimulates MAPK, with a maximal activity observed at 30 min, and as previously shown,[12] this sustained activation of the p42/p44^{mapk} pathway is essential to promote neurite outgrowth. These effects are attributable to the AT_2 receptor, since coincubation with PD 123,319 abolishes the effect of **21**. These results indicate that compound **21** acts as an agonist at the AT_2 receptor and is the first nonpeptidic AT_2 agonist to our knowledge.

Moreover, inhibition of PKG with KT 5823 or sGC with LY-83,583 indicates that cGMP participates in the early steps of neurite elongation. All these results corroborate previous observations made with Ang II or the peptide agonist CGP-42112.[12,38,39]

The existence of AT_2 receptors in the rat duodenal mucosa has been reported recently.[10] When the endogenous hormone Ang II or the nonpeptide receptor agonist L-162,313 was administered in vivo in the presence of the AT_1 receptor antagonist losartan, duodenal mucosal alkaline secretion increases markedly. These responses were inhibited by the AT_2 receptor antagonist PD 123,319,[10,18] indicating mediation through the AT_2 receptor. Also the selective peptidic AT_2 receptor agonist CGP-42112 was shown to increase mucosal alkaline secretion to the same extent, and the response was sensitive to PD 123,319 but not to losartan. From these experiments it is apparent that duodenal mucosal alkaline secretion is accelerated by activation of the AT_2 receptors located in the duodenal mucosa/submucosa,

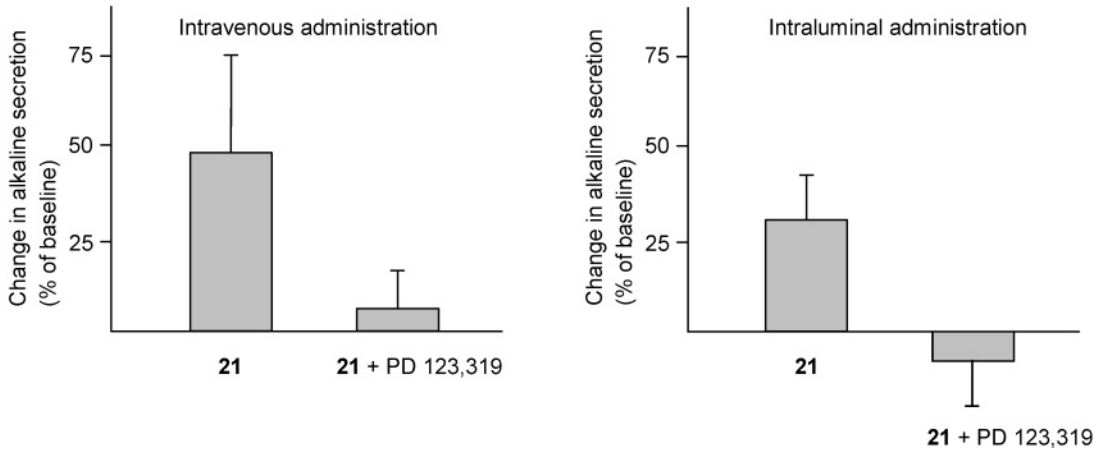


Figure 8. The effect of compound **21** on rat duodenal mucosal alkaline secretion in vivo at 0.3 mg kg^{-1} h^{-1} intravenously (left panel) or at 100 μM intraluminally (right panel). The figure also shows sensitivity to the selective AT_2 receptor antagonist PD 123,319 given intravenously at 0.3 mg kg^{-1} h^{-1} bolus (left panel) or intraluminally at 150 μM (right panel). Data are expressed as the percent change from baseline and are plotted as mean ±SEM, $n = 5$ in each group.

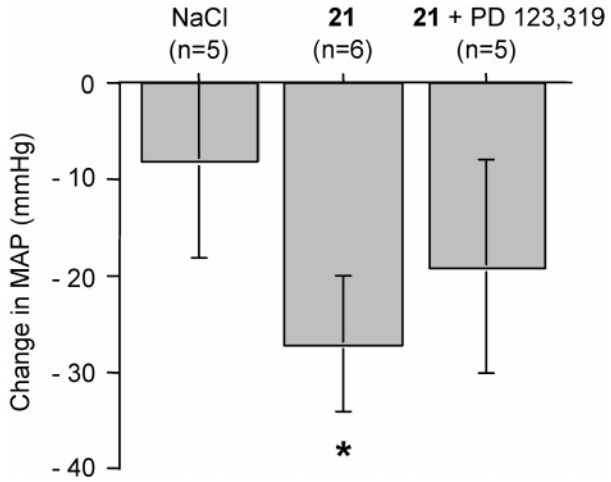


Figure 9. The acute effect of compound **21** (0.05 mg/kg intravenously) alone or together with the selective AT_2 receptor antagonist PD 123,319 (0.3 mg/kg h intravenously) on mean arterial pressure in anesthetized spontaneously hypertensive rats. Data are the absolute change from baseline pressure (MAP) within 30 min and are plotted as mean ±SEM; ∗ denotes a significant difference from the NaCl group.

and this model could therefore preferably be used to validate the agonistic properties of compound **21**. The present investigation shows that both when infused intravenously (0.3 mg kg^{-1} h^{-1}) or given topically in the luminal perfusate (100 μM), **21** markedly increased the mucosal alkaline secretion. Such responses were absent or greatly reduced in the presence of PD 123,319 (0.3 mg/kg iv bolus), strongly suggesting interaction with AT_2 receptors. Furthermore, increasing iv doses of **21** demonstrated a dose−response relationship, giving additional support to the agonistic nature of the compound in vivo.

It should be noted that the secretory effect could be obtained by both a systemic and local route of administration of **21**. The latter opens up the possibility of luminal (per oral) administration and local mucosal action with limited need for systemic concentrations of the drug, in turn reducing the risk of side effects on distant organs. The staining in the lamina propria of the villi for both AT_1 and AT_2 receptors suggests receptor localization in proximity to blood vessels.[10] Moderate changes in local blood flow cannot be excluded but seem unlikely to influence duodenal mucosal alkaline secretion.[42] It was reported recently that local bradykinin BK2-receptors, possibly by a paracrine bradykinin action, are activated when the peptidic AT_2 receptor agonist CGP-42112 stimulates rat duodenal mucosal alkaline secretion.[43] However, further studies are needed to investigate in detail the mediation of the increased alkaline secretion following AT_2 receptor stimulation.

In the spontaneously hypertensive rat (SHR), AT_2 receptor stimulation with CGP-42112 induces a depressor response during simultaneous AT_1 receptor blockade.[41] Selective AT_2 receptor stimulation was also found to mediate a hypotensive response in conscious normotensive rats, an effect dependent on an intact L-arginine−nitric oxide axis.[44] Activation of AT_2 receptors by endogenous Ang II has been shown to mediate flow-induced dilation of rat mesenteric resistance arteries, and AT_2 receptors modulate Ang II-induced contractions of small mesenteric arteries in SHR. In Ang II-dependent hypertension in rats, the hypotensive response to AT_1 receptor blockade has been shown to be attenuated by AT_2 receptor blockade (for review, see ref 45). It follows that from the actions described for the AT_2 receptors thus far, one would logically predict that a specific AT_2 receptor agonist should act hypotensive, particularly so in SHR. This was confirmed by the present experiments, where administration of **21** to S−D rats had no significant influence on arterial pressure, whereas a significant reduction occurred in the μg/kg dose range (0.008−0.05 mg/kg μg/kg) in SHR (N.B., the S−D rats were instrumented for secretion studies, which may have influenced the results). The hypotensive response to **21** in SHR was partially sensitive to the selective AT_2 receptor antagonist PD 123,319, indicating action through the AT_2 receptor. However, no effect on mean arterial pressure was observed at higher doses (mg/kg range), suggesting a bell-shaped dose−response curve. It may be that **21** acts as a partial agonist on the vasculature. It should also be noted that the effects observed were recorded only some 30−40 min after administration of the compound in anesthetized animals. Measurements of long term

blood pressure effects in conscious animals following chronic administration are needed to more completely characterize the hypotensive action of the compound.

Conclusion

Here we have reported the first druglike selective AT_2 receptor agonist, compound **21**, with 20−30% oral bioavailability and a half-life estimated to be 4 h in rat, which mimics Ang II in its actions on the AT_2 receptor. Thus, compound **21** induces outgrowth of neurite cells, enhances alkaline secretion from the rat intestine, and lowers the mean arterial blood pressure in spontaneously hypertensive rats. We believe that compound **21**, derived from the prototype nonselective AT_1/AT_2 receptor agonist **1** will serve as a valuable research tool for studies of the function of the AT_2 receptor. Although long-term clinical studies are lacking, numerous experimental studies indicate that the clinical improvements observed following treatment with selective AT_1 antagonists to a large extent are due to activation of the unopposed AT_2 receptor and an increased plasma level of Ang II.[45] It follows that direct activation of AT_2 receptors during, for example, hypertension and cardiovascular remodeling is an attractive pharmaceutical principle. The presently reported AT_2-selective agonist compound **21** could possibly be regarded as the first member of such a novel drug class.

Experimental Section

Chemistry. General Considerations. ^{1}H 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. Infrared spectra were recorded on a Perkin-Elmer model 1605 FT-IR and are reported as λ_{max} (cm^{-1}). For neat solids the instrument was equipped with a Microfocus Beam Condenser with ZnSe lenses in a Diasqueeze Pulse Diamond Compressor Cell (Graseby Specac Inc., Woodstock, GA). 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 1.5 mL/min (H_2O/CH_3CN/0.05% HCOOH). Temperature-controlled microwave heating was carried out using an automatic Smith single-mode synthesizer producing a radiation frequency of 2450 MHz (Personal Chemistry AB, Uppsala, Sweden). 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.

3-(4-Bromobenzyl)-3*H*-imidazo[4,5-*b*]pyridine (3).[46] Dimethyl sulfoxide (20 mL dried over 4 Å molecular sieves) was added to potassium hydroxide (2.24 g, 40 mmol, crushed pellets) and the mixture was stirred for 5 min. 3*H*-Imidazo-[4,5-*b*]pyridine (1.0 g, 8.4 mmol) was then added and the mixture was stirred for 2 h. 4-Bromobenzyl bromide (3.25 g, 13 mmol) was added and the mixture was cooled briefly and stirred for another hour before water (20 mL) was added. The mixture was extracted with ether and the combined organic phase was washed with water. The organic phase was dried over $CaCl_2$ and the solvent was removed in vacuo. The residue was chromatographed on silica gel with CH_2Cl_2:Et_2O (1:2) as eluent to yield the title compound (1.275 g, 4.4 mmol, 53% yield).[46] ^{1}H NMR ($CDCl_3$), δ, ppm: 8.42 (dd, J = 5.0, 1.5 Hz, 1H), 8.10 (dd, J = 8.1, 1.5 Hz, 1H), 8.07 (s, 1H), 7.45 (d, J = 8.1 Hz, 2H), 7.26 (dd, J = 8.1, 5.0 Hz, 1H), 7.18 (d, J = 8.1 Hz, 2H), 5.44 (s, 2H).

1-(4-Bromobenzyl)-2-ethyl-1*H*-benzoimidazole (4). DMSO (20 mL, dried over 4Å molecular sieves) was added to KOH (2.24 g, 40 mmol) and the mixture was stirred for 5 min. 2-Ethyl-1*H*-benzoimidazole (1.1 g, 7.5 mmol) was then added and the mixture was stirred for 2 h. 4-Bromobenzyl bromide (3.25 g, 13.0 mmol) was added and the mixture was cooled briefly and stirred for another hour before water (20 mL) was added. The mixture was extracted with ether and the combined organic phase was washed with water. The organic phase was dried over $CaCl_2$ and the solvent was removed in vacuo. The residue was chromatographed on silica gel with hexane as eluent, yielding the titled compound (2.30 g, 7.3 mmol, 97% yield). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.80 (d, J = 6.9 Hz, 1H), 7.43 (d, J = 8.6 Hz, 2H), 7.25−7.20 (m, 3H), 6.90 (d, J = 8.6 Hz, 2H), 5.29 (s, 2H), 2.87 (q, J = 7.6 Hz, 2H), 1.43 (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 156.1, 141.8, 135.0, 134.8, 132.2, 127.8, 122.6, 122.4, 121.9, 119.2, 109.3, 46.3, 20.8, 11.7. Anal. ($C_{16}H_{15}BrN_2$) C, H, N.

1-(4-Bromobenzyl)-1*H*-benzoimidazole (5).[47] Dimethyl sulfoxide (20 mL, dried over 4Å molecular sieves) was added to potassium hydroxide (2.24 g, 40 mmol, crushed pellets) and the mixture was stirred for 5 min. Benzimidazole (1.18 g, 0.01 mol) was then added and the mixture was stirred for a further 2 h. 4-Bromobenzyl bromide (5.0 g, 0.02 mol) was added, the mixture was cooled briefly and stirred for another hour before water (20 mL) was added. The mixture was extracted with ether and the combined organic phase was washed with water. The organic phase was dried over $CaCl_2$ and the solvent was removed in vacuo. The residue was chromatographed on silica gel with CH_2Cl_2:Et_2O (1:2) as eluent, yielding the title product (2.36 g, 8.2 mmol, yield: 82%).[47] ^{1}H NMR ($CDCl_3$ + CD_3OD), δ, ppm: 8.04 (s, 1H), 7.83 (dd, J = 6.5, 1.7 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.28 (m, 3H), 7.04 (d, J = 8.3 Hz, 2H), 5.32 (s, 2H).

1-(4-Bromobenzyl)-2-methyl-1*H*-imidazole (6). DMSO (40 mL, dried over 4 Å molecular sieves) was added to KOH (3.42 g, 61 mmol) and the mixture was stirred for 5 min. 2-Methyl-1*H*-imidazole (1.0 g, 12.1 mmol) was then added and the mixture was stirred for 2 h. 4-Bromobenzyl bromide (4.5 g, 18.3 mmol) was added and the mixture was cooled briefly and stirred for another hour before water (20 mL) was added. The mixture was extracted with ether and the combined organic phase was washed with water. The organic phase was dried over $CaCl_2$ and the solvent was removed in vacuo. The residue was chromatographed on silica gel with $CHCl_3$:MeOH (30:1) plus 0.05% formic acid as eluent, yielding the title compound (2.58 g, 10.3 mmol, 85% yield). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.46 (d, J = 8.3 Hz, 2H), 6.95 (s, 1H), 6.91 (d, J = 8.3 Hz, 2H), 6.82 (s, 1H), 5.00 (s, 2H), 2.31 (s, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 145.0, 135.5, 132.2, 128.4, 127.7, 122.0, 119.9, 49.2, 13.2. IR (compression cell), cm^{-1}: 3124, 3105, 2916. Anal. ($C_{11}H_{11}BrN_2$) C, H, N.

1-(4-Bromobenzyl)-1*H*-imidazole (7). DMSO (20 mL, dried over 4 Å molecular sieves) was added to KOH (2.24 g, 0.04 mol) and the mixture was stirred for 5 min. Imidazole (0.57 g, 8.4 mmol) was then added and the mixture was stirred for 2 h. 4-Bromobenzyl bromide (3.25 g, 13.0 mmol) was added and the mixture was cooled briefly and stirred for another hour before water (20 mL) was added. The mixture was extracted with ether and the combined organic phase was washed with water. The organic phase was dried over $CaCl_2$ and the solvent was removed in vacuo. The residue was chromatographed on silica gel with $CHCl_3$:MeOH (30:1) plus 0.05% formic acid as eluent, yielding the title compound (1.28 g, 5.4 mmol, 64% yield). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.58 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.10 (s, 1H), 7.02 (d, J = 8.3 Hz, 2H), 6.88 (s, 1H), 5.09 (s, 2H). ^{13}C NMR ($CDCl_3$), δ, ppm: 137.5, 135.3, 132.3, 130.2, 129.0, 122.4, 119.3, 50.2. IR (compression cell), cm^{-1}: 3138, 3091, 2985. Anal. ($C_{10}H_9BrN_2$) C, H, N.

3-(4-Imidazo[4,5-*b*]pyridin-3-yl-methylphenyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (10). Compound **2** (151.0 mg, 0.473 mmol), **3** (90.0 mg, 0.314 mmol), toluene

(15 mL), ethanol (1.3 mL), NaOH (1.3 mL, 1 M), and $Pd(PPh_3)_4$ (10.9 mg, 9 μmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (20 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (hexane:acetone 2:1) to give the title compound in 76% yield (115.8 mg, 0.240 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 8.43 (d, J = 4.9 Hz, 1H), 8.12−8.10 (m, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 7.28 (m, 1H), 6.71 (s, 1H), 5.53 (s, 2H), 4.09 (brs, 1H), 2.64 (d, J = 7.1 Hz, 2H), 1.90 (sep, J = 6.7 Hz, 1H), 0.95 (d, J = 6.7 Hz, 6H), 0.93 (s, 9H). ^{13}C NMR ($CDCl_3$), δ, ppm: 148.7, 147.0, 144.8, 143.8, 142.5, 136.8, 136.3, 135.2, 135.1, 129.8, 128.9, 128.2, 128.0, 118.8, 54.7, 47.0, 39.3, 30.6, 29.6, 22.3. IR (compression cell), cm^{-1}: 3095, 2954, 2872. Anal. ($C_{25}H_{30}N_4O_2S_2$) C, H, N.

3-[4-(2-Ethylbenzoimidazol-1-ylmethyl)phenyl]-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (11). Compound **2** (151.0 mg, 0.473 mmol), **4** (131.4 mg, 0.417 mmol), toluene (15 mL), ethanol (1.5 mL), NaOH (1.7 mL, 1 M), and $Pd(PPh_3)_4$ (14.5 mg, 12 μmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (20 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (hexane:acetone 4:1) to give the title compound in 54% yield (114.8 mg, 0.225 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.82 (d, J = 7.6 Hz, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.30−7.20 (m, 3H), 7.12 (d, J = 8.3 Hz, 2H), 6.70 (s, 1H), 5.39 (s, 2H), 4.05 (s, 1H), 2.94 (q, J = 7.4 Hz, 2H), 2.65 (d, J = 6.9 Hz, 2H), 1.90 (m, 1H), 1.45 (t, J = 7.4 Hz, 3H), 1.00 (s, 9H), 0.95 (d, J = 6.9 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 155.9, 148.6, 142.3, 135.8, 134.8, 129.7, 129.1, 128.8, 126.4, 126.1, 122.8, 122.7, 122.5, 119.1, 109.5, 54.6, 47.0, 39.2, 30.5, 29.4, 22.1, 20.8, 11.8. IR (compression cell), cm^{-1}: 3056, 2980, 2868. Anal. ($C_{28}H_{35}N_3O_2S_2$) C, H, N.

3-(4-Benzoimidazole-1-ylmethylphenyl)-5-isobutyl-*N*-*tert*-butylthiophenesulfonamide (12). Compound **2** (88.8 mg, 0.278 mmol), **5** (53.2 mg, 0.185 mmol), toluene (10 mL), ethanol (0.65 mL), NaOH (0.74 mL, 1 M) and $Pd(PPh_3)_4$ (6.4 mg, 5.5 μmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (20 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (hexane:acetone 2:1) to give the title compound in 95% yield (84.7 mg, 0.176 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 8.26 (d, J = 6.9 Hz, 1H), 7.89 (d, J = 8.6 Hz, 2H), 7.60−7.20 (m, 6H), 6.70 (s, 1H), 5.55 (s, 2H), 4.12 (s, 1H), 2.67 (d, J = 7.1 Hz, 2H), 1.90 (m, 1H), 0.94 (m, 15H). ^{13}C NMR ($CDCl_3$), δ, ppm: 148.7, 143.9, 143.2, 142.5, 136.8, 135.8, 135.2, 133.8, 129.9, 129.0, 127.3, 123.4, 122.6, 120.6, 110.1, 54.7, 48.8, 39.3, 30.7, 29.6, 22.3. IR (compression cell), cm^{-1}: 3063, 2964. Anal. ($C_{26}H_{31}N_3O_2S_2$) C, H, N.

3-[4-(2-Methylimidazol-1-ylmethyl)phenyl]-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (13). Compound **2** (479 mg, 1.50 mmol), **6** (251 mg, 1.00 mmol), toluene (12 mL), ethanol (3 mL), NaOH (160 mg, 4 mmol as pellets), PPh_3 (16 mg, 60 μmol), and $Pd(OAC)_2$ (6.7 mg, 0.03 mmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to 80 °C and stirred for 2 h. After dilution with EtOAc (20 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (CH_2Cl_2:MeOH 25:1) to give the title compound in 83% yield (368.8 mg, 0.828 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.59 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 6.98 (s, 1H), 6.86 (s, 1H), 6.73 (s, 1H), 5.09 (s, 2H), 4.06 (s, 1H), 2.67 (d, J = 7.1 Hz, 2H), 2.34 (s, 3H), 1.91 (m, 1H), 0.99 (s, 9H), 0.97 (d, J = 6.6 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 148.6, 144.8, 142.4, 136.7, 136.5, 134.8, 129.7, 128.9, 127.6, 126.7, 119.9, 54.6, 49.4, 39.2, 30.5, 29.5, 22.1, 13.1. IR (compression cell), cm^{-1}: 3053, 2958. Anal. ($C_{23}H_{31}N_3O_2S_2$) C, H, N.

3-(4-Imidazol-1-ylmethylphenyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (14). Compound **2** (200.5 mg, 0.628 mmol), **7** (98.8 mg, 0.416 mmol), toluene (15 mL), ethanol (1.5 mL), NaOH (1.5 mL, 1 M), and $Pd(PPh_3)_4$ (14.5 mg, 12.5 μmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (50 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography ($CHCl_3$:MeOH 20:1) to give the title compound in 63% yield (113.9 mg, 0.264 mmol).

Alternatively, a dried heavy-walled Pyrex tube was charged with **2** (49.2 mg, 0.154 mmol), **7** (21.9 mg, 92.4 μmol), toluene (1.56 mL), ethanol (0.22 mL), Na_2CO_3 (2.0 M, 0.22 mL), and $Pd(PPh_3)_4$ (8 mg, 7 μmol). The reaction mixture was flushed with nitrogen and the cap tightened thoroughly. The reaction mixture was exposed to microwave heating (150 °C, 5 min). The reaction mixture was diluted with EtOAc (15 mL), washed with water and brine, and dried. The solvent was removed and the residue was separated by column chromatography (CH_2Cl_2:MeOH 20:1) to give the title compound in 75% yield (30.0 mg, 0.0695 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.60−7.57 (m, 3H), 7.22 (d, J = 8.1 Hz, 2H), 7.08 (s, 1H), 6.90 (s, 1H), 6.73 (s, 1H), 5.15 (s, 2H), 4.35 (m, 1H), 2.66 (d, J = 7.3 Hz, 2H), 1.90 (m, 1H), 0.98 (s, 9H), 0.96 (d, J = 6.6 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 148.7, 142.5, 137.5, 136.8, 136.5, 135.1, 130.0, 129.8, 129.0, 127.5, 119.3, 54.7, 50.6, 39.3, 30.6, 29.6, 22.3. IR (compression cell), cm^{-1}: 3060, 2996. Anal. ($C_{22}H_{29}N_3O_2S_2{\cdot}H_2O$) C, H, N.

3-(4-Cyanomethylphenyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (15). Compound **2** (200.5 mg, 0.628 mmol), (4-bromophenyl)acetonitrile (81.7 mg, 0.416 mmol), toluene (15 mL), ethanol (1.5 mL), NaOH (1.5 mL, 1 M), and $Pd(PPh_3)_4$ (18.0 mg, 15.6 μmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (50 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (hexane:acetone 4:1) to give the title compound in 57% yield (92.5 mg, 0.237 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.65 (d, J = 8.2 Hz, 2H), 7.42 (d, J = 8.2 Hz, 2H), 6.75 (s, 1H), 4.05 (brs, 1H), 3.81 (s, 2H), 2.70 (d, J = 7.1 Hz, 2H), 1.97 (m, 1H), 1.01 (s, 9H), 0.98 (d, J = 6.9 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 148.7, 142.3, 134.8, 130.1, 129.8, 128.8, 128.0, 119.9, 117.6, 54.6, 39.2, 30.6, 29.6, 23.5, 22.1. IR (compression cell), cm^{-1}: 3291, 2996, 2243. Anal. ($C_{20}H_{26}N_2O_2S_2{\cdot}{}^1/_2H_2O$) C, H, N.

3-(4-Cyanophenyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (16). Compound **2** (200.5 mg, 0.628 mmol), 4-bromobenzonitrile (75.8 mg, 0.416 mmol), toluene (15 mL), ethanol (1.5 mL), NaOH (1.5 mL, 1 M), and $Pd(PPh_3)_4$ (18.0 mg, 15.6 μmol) were mixed together under a N_2(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (50 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (hexane:acetone 5:1) to give the title compound in 64% yield (100.1 mg, 0.266 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.74 (m, 4H), 6.75 (s, 1H), 4.12 (brs, 1H), 2.68 (d, J = 7.1 Hz, 2H), 1.91 (m, 1H), 1.03 (s, 9H), 0.97 (d, J = 6.6 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 149.2, 141.2, 139.5, 137.6, 132.0, 129.9, 128.6, 118.5, 112.0, 54.9, 39.1, 30.6, 29.6, 22.1. IR (compression cell), cm^{-1}: 3260, 2985, 2224. Anal. ($C_{19}H_{24}N_2O_2S_2{\cdot}{}^1/_3H_2O$) C, H, N.

***N*-Butyloxycarbonyl-3-(4-imidazo[4,5-*b*]pyridin-3-ylmethylphenyl)-5-isobutylthiophene-2-sulfonamide (17).** Compound **10** (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 butyl chloroformate (447 μL, 3.49 mmol) was added under a 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 (hexane:acetone 3:2) to give the title compound in 90% yield (147.9 mg, 0.281 mmol). ^{1}H NMR ($CDCl_3$ + CD_3OD), δ, ppm: 8.42 (dd, J = 4.8, 1.3 Hz, 1H), 8.39 (s, 1H), 8.10 (dd, J = 8.1, 1.3 Hz, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H), 7.34 (m, 1H), 6.77 (s, 1H), 5.58 (s, 2H), 3.94 (t, J = 6.6 Hz, 2H), 2.70 (d, J = 7.1 Hz, 2H), 1.92 (m, 1H), 1.42 (m, 2H), 1.17 (m, 2H), 0.97 (d, J = 6.6 Hz, 6H), 0.82 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$ + CD_3OD), δ, ppm: 157.2, 152.3, 151.8, 147.3, 146.4, 145.7, 145.4, 137.2, 135.6, 135.1, 130.5, 130.4, 128.6, 128.4, 119.8, 66.9, 47.6, 39.8, 31.5, 31.3, 22.5, 19.5, 13.9. IR (compression cell), cm^{-1}: 3033, 2960, 2871, 1734. Anal. ($C_{26}H_{30}N_4O_4S_2$) C, H, N.

***N*-Butyloxycarbonyl-3-[4-(2-ethylbenzoimidazol-1-ylmethyl)phenyl]-5-isobutylthiophene-2-sulfonamide (18).** Compound **11** (140 mg, 0.275 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 (47.7 mg, 0.32 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (440 μL, 3.43 mmol) was added under a 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 (hexane:acetone 3:2) to give the title compound in 31% yield (47.6 mg, 86 μmol). ^{1}H NMR ($CDCl_3$ + CD_3OD), δ, ppm: 7.65 (m, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.40 (m, 1H), 7.27 (m, 2H), 7.13 (d, J = 8.3 Hz, 2H), 6.79 (s, 1H), 5.51 (s, 2H), 3.95 (t, J = 6.6 Hz, 2H), 2.94 (q, J = 7.6 Hz, 2H), 2.70 (d, J = 6.9 Hz, 2H), 1.92 (m, 1H), 1.43 (m, 2H), 1.39 (t, J = 7.6 Hz, 3H) 1.20 (m, 2H), 0.97 (d, J = 6.6 Hz, 6H), 0.84 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$ + CD_3OD), δ, ppm: 157.5, 152.7, 151.6, 150.5, 146.2, 137.2, 135.2, 134.9, 130.6, 130.4, 127.0, 123.9, 123.7, 118.6, 111.1, 66.9, 47.0, 39.8, 31.5, 31.4, 22.5, 21.3, 19.6, 13.9, 11.9. IR (compression cell), cm^{-1}: 2954, 2880, 1751. Anal. ($C_{29}H_{35}N_3O_4S_2$) C, H, N.

***N*-Butyloxycarbonyl-3-(4-benzoimidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-sulfonamide (19).** Compound **12** (92.2 mg, 0.191 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 (32.6 mg, 0.220 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (301 μL, 2.07 mmol) was added under a 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 30:1) to give the title compound in 79% yield (78.8 mg, 0.150 mmol). ^{1}H NMR ($CDCl_3$ + CD_3OD), δ, ppm: 8.34 (s, 1H), 7.70 (m, 1H), 7.51 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 7.29 (m, 3H), 6.84 (s, 1H), 5.55 (s, 2H), 3.91 (t, J = 6.6 Hz, 2H), 2.73 (d, J = 7.0 Hz, 2H), 1.92 (m, 1H), 1.41 (m, 2H), 1.21 (m, 2H), 0.98 (d, J = 6.6 Hz, 6H), 0.84 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$ + CD_3OD), δ, ppm: 157.3, 153.3, 151.8, 151.7, 146.3, 144.9, 143.9, 143.8, 137.7, 135.5, 130.8, 128.4, 124.6, 123.8, 120.0, 112.0, 67.0, 48.0, 39.9, 31.8, 31.7, 22.5, 19.9, 13.9. IR (compression cell), cm^{-1}: 3033, 2944, 2872, 1734. Anal. ($C_{27}H_{31}N_3O_4S_2$) C, H, N.

***N*-Butyloxycarbonyl-3-[4-(2-methylimidazol-1-ylmethyl)phenyl]-5-isobutylthiophene-2-sulfonamide (20).** Compound **13** (56.6 mg, 0.127 mmol) was dissolved in CH_2Cl_2 (2 mL) under a N_2(g) atmosphere and BCl_3 (130 +100 + 100 μL, 1 M CH_2Cl_2) was sequentially added for 0.5 h to the stirred reaction mixture. The solvent was evaporated under vacuum and coevaporated three times with dichloromethane. The residue was dissolved in CH_2Cl_2 (2 mL) and H_2O (0.7 mL), and Na_2CO_3 (61 mg, 0.57 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (23 μL, 0.18 mmol) was added under a N_2(g) atmosphere. The reaction was stirred at ambient temperature overnight. The reaction mixture was diluted with $CHCl_3$ (15 mL) and washed with water and brine. The organic phase was dried, evaporated, and purified by column chromatography ($CHCl_3$:MeOH 25:1) to give the title compound in 72% yield (44.6 mg, 91 μmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 10.42 (brs, 1H) NH-signal, 7.62 (d, J = 7.9 Hz, 2H), 7.05 (d, J = 8.0 Hz, 2H), 6.71−6.69 (m, 3H), 5.08 (s, 2H), 3.90 (t, J = 6.6 Hz, 2H), 2.65 (d, J = 7.1 Hz, 2H), 2.54 (s, 3H), 1.91 (m, 1H), 1.44 (m, 2H), 1.19 (m, 2H), 0.97 (d, J = 6.6 Hz, 6H), 0.82 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 155.7, 148.4, 144.1, 141.8, 136.3, 136.1, 133.0, 130.1, 128.4, 127.2, 121.4, 119.9, 65.0, 50.1, 39.2, 30.8, 30.4, 22.3, 18.9, 13.7, 11.1. IR (compression cell), cm^{-1}: 3145, 2952, 1665. Anal. ($C_{24}H_{31}N_3O_4S_2$·H_2O) C, H, N.

***N*-Butyloxycarbonyl-3-(4-imidazol-1-ylmethylphenyl)-5-isobutylthiophene-2-sulfonamide (21).** Compound **14** (113 mg, 0.262 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 (40.5 mg, 0.262 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (364 μL, 2.50 mmol) was added under a 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 10:1) to give the title compound in 46% yield (57.8 mg, 0.122 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 9.71 (brs, 1H), 7.95 (s, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.1 Hz, 2H), 6.86 (m, 2H), 6.74 (s, 1H), 5.16 (s, 2H), 4.03 (t, J = 6.6 Hz, 2H), 2.70 (d, J = 7.1 Hz, 2H), 1.94 (m, 1H), 1.51 (m, 2H), 1.25 (m, 2H), 0.98 (d, J = 6.6 Hz, 6H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 156.5, 152.6, 150.5, 144.1, 136.6, 134.8, 133.2, 130.0, 129.0, 127.5, 125.9, 119.7, 66.0, 51.3, 39.4, 30.8, 30.6, 22.4, 19.0, 13.8. IR (compression cell), cm^{-1}: 3555, 3120, 2956, 1694. Anal. ($C_{23}H_{29}N_3O_4S_2$·H_2O) C, H, N.

***N*-Butyloxycarbonyl-3-(4-cyanomethylphenyl)-5-isobutylthiophene-2-sulfonamide (22).** Compound **15** (60.0 mg, 0.154 mmol) was dissolved in CH_2Cl_2 (5 mL) under a N_2(g) atmosphere, BCl_3 (2 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 (23.3 mg, 0.154 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (195 μL, 1.34 mmol) was added under a 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 ($CHCl_3$:MeOH 45:1) to give the title compound in 72% yield (47.9 mg, 0.110 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 9.73 (brs, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 6.76 (s, 1H), 4.07 (t, J = 6.6 Hz, 2H), 3.81 (s, 2H), 2.72 (d, J = 7.1 Hz, 2H), 1.97 (m, 1H), 1.53 (m, 2H), 1.28 (m, 2H), 1.01 (d, J = 6.6 Hz, 6H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 157.8, 152.0, 150.4, 145.9, 133.9, 130.6, 129.7, 129.4, 128.0, 117.8, 67.1, 39.4, 30.6, 30.5, 23.5, 22.3, 18.9, 13.7. IR (compression cell), cm^{-1}: 3217, 2960, 2872, 2251, 1747. Anal. ($C_{21}H_{26}N_2O_4S_2$·1/3H_2O) C, H, N.

***N*-Butyloxycarbonyl-3-(4-cyanophenyl)-5-isobutylthiophene-2-sulfonamide (23).** Compound **16** (90.0 mg, 0.239 mmol) was dissolved in CH_2Cl_2 (5 mL) under a N_2(g) atmosphere, BCl_3 (2 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 (34.4 mg, 0.239 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (295 μL, 2.32 mmol) was added under a 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 ($CHCl_3$:MeOH 50:1) to give the title compound in 53% yield (53.1 mg, 0.126 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 8.20 (brs, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 6.76 (s, 1H), 4.09 (t, J = 6.6 Hz, 2H), 2.73 (d, J = 7.1 Hz, 2H), 1.99 (m, 1H), 1.53 (m, 2H), 1.23 (m, 2H), 0.98 (d, J = 6.6 Hz, 6H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 157.9, 152.3, 150.5, 144.4, 138.8, 132.0, 130.0, 129.0, 118.5, 112.2, 67.0, 39.3, 30.6, 30.5, 22.3, 18.9, 13.7. IR (compression cell), cm^{-1}: 3203, 2959, 2872, 2228, 1749. Anal. ($C_{20}H_{24}N_2O_4S_2$) C, H, N.

3-(4-Bromophenylmethyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (24). Compound **2** (159.6 mg, 0.5 mmol), 1-bromo-4-bromomethylbenzene (312.4 mg, 1.25 mmol), toluene (15 mL), ethanol (1.3 mL), K_2CO_3 (2.0 mL, 1 M), and $Pd(PPh_3)_4$ (17 mg, 15 μmol) were mixed together under a N_2-(g) atmosphere. The mixture was heated to reflux and stirred for 3 h. After dilution with EtOAc (50 mL), the reaction mixture was washed with water and brine. The organic phase was dried and concentrated under vacuum. The crude product was purified by column chromatography (hexane:acetone 4:1) to give the title compound in 80% yield (178.2 mg, 0.401 mmol).

Alternatively, a dried heavy-walled Pyrex tube was charged with **2** (32.7 mg, 0.103 mmol), 1-bromo-4-bromomethylbenzene (64 mg, 0.26 mmol), toluene (1.56 mL), ethanol (0.22 mL), Na_2CO_3 (2.0 M, 0.22 mL), and $Pd(PPh_3)_4$ (7 mg, 6 μmol)). The reaction mixture was flushed with nitrogen and the cap tightened thoroughly. The reaction mixture was exposed to microwave heating (110 °C, 30 min). The reaction mixture was diluted with EtOAc (15 mL), washed with water and brine, and dried. The solvent was removed and the residue was separated by column chromatography (hexane:acetone 8:1) to give the title compound in 78% yield (35.3 mg, 79 μmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.42 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 6.45 (s, 1H), 4.35 (brs, 1H), 4.12 (s, 2H), 2.59 (d, J = 7.3 Hz, 2H), 1.86 (m, 1H), 1.18 (s, 9H), 0.90 (d, J = 7.1 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 149.2, 143.2, 138.5, 135.6, 131.8, 130.8, 129.0, 120.5, 55.1, 39.4, 34.0, 30.7, 30.1, 22.3. IR (compression cell), cm^{-1}: 3274, 2961. Anal. ($C_{19}H_{26}BrNO_2S_2$) C, H, N.

3-(4-Cyanophenylmethyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (25). A dried heavy-walled Pyrex tube was charged with **24** (89 mg 0.20 mmol), $Zn(CN)_2$ (23.5 mg, 0.20 mmol), and $Pd(PPh_3)_4$ (6.9 mg, 6 μmol) in DMF (1 mL). The reaction mixture was flushed with nitrogen and the cap tightened thoroughly. The reaction mixture was exposed to microwave heating (180 °C, 2 min). The reaction tube was cooled to room temperature before the reaction mixture was diluted in EtOAc (60 mL) and washed with water. The organic phase was dried, and the solvent was evaporated. The crude product was purified by column chromatography (hexane: acetone 5:1) to give the title compound in 76% yield (59.5 mg, 0.152 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.60 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 6.43 (s, 1H), 4.43 (brs, 1H), 4.24 (s, 2H), 2.60 (d, J = 7.3 Hz, 2H), 1.87 (m, 1H), 1.21 (s, 9H), 0.91 (d, J = 7.3 Hz, 6H). ^{13}C NMR ($CDCl_3$), δ, ppm: 149.6, 145.1, 142.0, 136.2, 132.5, 129.8, 128.7, 119.0, 110.6, 55.3, 39.3, 34.6, 30.7, 30.1, 22.3. IR (compression cell), cm^{-1}: 3297, 2965, 2231. Anal. ($C_{20}H_{26}N_2O_2S_2$) C, H, N.

3-(4-Imidazol-1-ylphenylmethyl)-5-isobutyl-*N*-*tert*-butylthiophene-2-sulfonamide (26). A dried heavy-walled Pyrex tube was charged with **24** (156 mg 0.351 mmol), imidazole (238 mg, 3.50 mmol), CuI (6.7 mg, 35 μmol), 1,2-diaminocyclohexane (17.2 μL, 0.140 mmol), and Cs_2CO_3 (228 mg, 0.70 mmol) in DMF (1 mL). The reaction mixture was flushed with nitrogen and the cap tightened thoroughly. The reaction mixture was exposed to microwave heating (180 °C, 40 min). The reaction tube was cooled to room temperature before the reaction mixture was diluted in EtOAc (60 mL) and washed with water. The organic phase was dried, and the solvent was evaporated. The crude product was purified on column chromatography with $CHCl_3$:MeOH (10:1) to give the title compound in 27% yield (40.1 mg, 93 μmol). ^{1}H NMR ($CDCl_3$ + CD_3OD), δ, ppm: 8.15 (s, 1H), 7.41−7.35 (m, 6H), 6.41 (s, 1H), 4.21 (s, 2H), 2.55 (d, J = 7.3 Hz, 2H), 1.85 (m, 1H), 1.23 (s, 9H), 0.93 (d, J = 8.1 Hz, 6H). ^{13}C NMR ($CDCl_3$ + CD_3OD), δ, ppm: 151.3, 145.9, 139.4, 135.0, 134.9, 130.1, 128.5, 127.8, 121.5, 118.7, 113.5, 66.7, 39.1, 34.4, 30.6, 30.1, 22.3. Anal. ($C_{22}H_{29}N_3O_2S_2$) C, H, N.

***N*-Butyloxycarbonyl-3-(4-cyanophenylmethyl)-5-isobutylthiophene-2-sulfonamide (27).** Compound **25** (59.5 mg, 0.152 mmol) was dissolved in CH_2Cl_2 (5 mL) under a N_2(g) atmosphere, BCl_3 (1 mL, 1 M CH_2Cl_2) was added, and the reaction mixture was stirred for 1 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 (1 mL), and pyrrolidinopyridine (21.9 mg, 0.148 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (188 μL, 1.52 mmol) was added under a 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 ($CHCl_3$:MeOH 40:1) to give the title compound in 75% yield (49.7 mg, 0.114 mmol). ^{1}H NMR ($CDCl_3$), δ, ppm: 8.53 (brs, 1H), 7.54 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 8.3 Hz, 2H), 6.46 (s, 1H), 4.31 (s, 2H), 4.07 (t, J = 6.6 Hz, 2H), 2.59 (d, J = 7.3 Hz, 2H), 1.82 (m, 1H), 1.54 (m, 2H), 1.27 (m, 2H), 0.88 (d, J = 6.6 Hz, 6H), 0.85 (d, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ, ppm: 156.5, 152.9, 150.7, 146.1, 144.8, 132.5, 129.7, 128.8, 118.9, 110.5, 67.1, 39.4, 34.7, 30.6, 30.5, 22.3, 18.9, 13.7. IR (compression cell), cm^{-1}: 3223, 2959, 2871, 2228, 1748. Anal. ($C_{21}H_{26}N_2O_4S_2 \cdot 3H_2O$) C, H, N.

***N*-Butyloxycarbonyl-3-(4-imidazol-1-ylphenylmethyl)-5-isobutylthiophene-2-sulfonamide (28).** Compound **26** (40.1 mg, 93 μmol) was dissolved in CH_2Cl_2 (5 mL) under a N_2(g) atmosphere, BCl_3 (1 mL, 1 M CH_2Cl_2) was added, and the reaction mixture was stirred for 1 h. To the reaction mixture was 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 (1 mL), and pyrrolidinopyridine (15.1 mg, 0.102 mmol) was added. The solution was cooled on an ice bath and butyl chloroformate (126 μL, 0.93 mmol) was added under a 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 ($CHCl_3$:MeOH 12:1) to give the title compound in 54% yield (23.8 mg, 50 μmol). ^{1}H NMR ($CDCl_3$ + CD_3OD), δ, ppm: 8.15 (s, 1H), 7.41−7.35 (m, 6H), 6.47 (s, 1H), 4.32 (s, 2H), 4.10 (t, J = 6.6 Hz, 2H), 2.62 (d, J = 7.1 Hz, 2H), 1.85 (m, 1H), 1.59 (m, 2H), 1.34 (m, 2H), 0.93 (d, J = 6.6 Hz, 6H), 0.89 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$ + CD_3OD), δ, ppm: 151.7, 151.1, 146.0, 139.6, 134.9, 134.8, 130.3, 128.4, 127.7, 121.6, 118.7, 113.5, 66.3, 39.1, 33.7, 30.5, 30.3, 21.9, 18.6, 13.4. IR (compression cell), cm^{-1}: 3223, 2958, 2871, 1740. Anal. ($C_{23}H_{29}N_3O_4S_2 \cdot {}^1/_2H_2O$) C, H, N.

Rat Liver Membrane AT_1 Receptor Binding Assay. Rat liver membranes were prepared according to the method of Dudley et al.[34] 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-fiber filter sheets, which had been presoaked 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 was determined by using six different concentrations (0.03−5 nmol/L) of the labeled [^{125}I]-AngII. Nonspecific binding was determined in the presence of 1 μM Ang II. The specific binding was determined by subtracting the nonspecific binding from the total bound [^{125}I]AngII. The apparent dissociation constant K_i values were calculated from IC_{50} values using the Cheng−Prusoff equation (K_d = 1.7 ± 0.1 nM, [L] = 0.057 nM). The binding data were best fitted with a one-site fit. All determinations were performed in triplicate.

Porcine (Pig) Myometrial Membrane AT_2 Receptor Binding Assay. Myometrial membranes were prepared from porcine uteri according to the method by Nielsen et al.[35] A presumable interference by binding to AT_1 receptors was blocked by addition of 1 μM losartan. 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-fiber filter sheets, which had been presoaked 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 were determined by using six different concentrations (0.03−5 nmol/L) of the labeled [^{125}I]-Ang II. Nonspecific binding was determined in the presence of 1 μM Ang II. The specific binding was determined by subtracting the nonspecific binding from the total bound [^{125}I]-Ang II. IC_{50} was determined by Scatchard analysis of data obtained with Ang II by using GraFit (Erithacus Software, UK). The apparent dissociation constant K_i values were calculated from IC_{50} values using the Cheng−Prusoff equation (K_d = 0.7 ± 0.1 nM, [L] = 0.057 nM). The binding data were best fitted with a one-site fit. All determinations were performed in triplicates.

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), fetal bovine serum (FBS), HAT supplement (hypoxanthine, aminopterin, thymidine), and gentamycin were from Gibco BRL (Burlington, Canada); [Val^5]angiotensin II was from Bachem (Marina Delphen, CA); PD 123,319 was from RBI (Natick, MA); LY-83,583 and KT 5823 were from Calbiochem-Novabiochem (La Jolla, CA); PD 98,059, anti-phosphorylated p42/p44^{mapk}, and total p42/p44^{mapk} antibodies were from New England Biolabs (Beverly, MA); horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies were from Amersham Pharmacia Biotech Inc. (NJ); and complete protease inhibitor, poly(vinylidene difluoride) (PVDF) membranes, and the enhanced chemiluminescence (ECL) detection system were from Roche Laboratories (Montreal, Canada). All other chemicals were of grade A purity.

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% fetal bovine serum (FBS, Gibco BRL, Burlington, 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.[37] Subcultures were performed at subconfluency. Under these conditions, cells express only the AT_2 receptor subtype.[37,38] According to experiments, cells were stimulated for periods ranging from minutes (MAPK) to 3 days (neurite elongation) (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 3 × 10^4 cells/35 mm Perti dish. Cells were untreated (control cells) or treated with Ang II (100 nM) or **21** (100 nM), in the absence or in the presence of the various inhibitors: PD 123,-319 (1 μM), an AT_2 receptor antagonist; PD 98,059 (10 μM), an inhibitor of MEK; LY-83,583 (0.5 μM), an inhibitor of soluble guanylyl cyclase; or KT 5823 (1 μM), an inhibitor of cGMP-dependent protein kinases (each introduced daily with inhibitors applied 30 min prior to Ang II or **21**).

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.[39]

Western Blotting for p42/p44^{mapk}. After 3 days of culture, cells were washed with Hanks buffered saline (HBS: 130 mM NaCl, 3.5 mM KCl, 2.3 mM $CaCl_2{\cdot}2H_2O$, 0.98 mM $MgCl_2{\cdot}6H_2O$, 5 mM HEPES, 0.5 mM EGTA) and then incubated for 10 min with 800 μL of stabilization buffer (100 nM staurosporine, 1 mM Na_3VO_4 in HBS) and finally lysed in 50 μL of lysis buffer (50 mM HEPES pH 7.8, 100 nM staurosporine, 1mM Na_3VO_4, 1% Triton-X100, protease inhibitors). Samples were separated on 10% SDS−polyacrylamide gels. Proteins were transferred electrophoretically to PVDF membranes. Membranes were blocked with 1% gelatin, 0.05% Tween 20 in TBS buffer (pH 7.5). After washing with TBS−Tween 20 (0.05%), membranes were incubated overnight at 4 °C with anti-phosphorylated p42/p44^{mapk} (1:1000) or anti-p42/p44^{mapk} (1:1000), diluted in TBS−Tween 20 (0.05%) plus BSA (0.1%). After washing with TBS−Tween 20, detection was accomplished using horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:2000) and an enhanced chemiluminescence (ECL) detection system, as described previously.[12]

Data Analysis. The data are presented as mean ± 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.

In Vivo Rat Studies. General. All in vivo experiments were approved by the Animal Ethics Committee of Gothenburg University. The secretion experiments were performed on nonfasted male Sprague−Dawley rats (Möllegård Breeding Center Ltd., Ejby, Denmark). For induction of anaesthesia, pentobarbital sodium, a barbiturate with a medium duration of action, was injected intraperitoneally (60 mg/kg of body wt) followed by continuous infusion (30 mg kg^{-1} h^{-1}).

Spontaneously hypertensive rats (SHR, Charles River, Germany) were anesthetized using 150 mg/kg Inactin (Byk-Gulden, Germany) by a single bolus ip.

A catheter was inserted into the trachea to ensure free airways. A femoral artery was catheterized for subsequent blood pressure measurements and the continuous infusion of the anaesthetic. One or two femoral veins were catheterized for intravenous drug administration. The body temperature was maintained at 38 °C with a heating pad and lamp, both controlled thermostatically.

Blood Pressure Recording (All Experiments). Arterial pressure was measured by a Statham *P*23Dc transducer (Statham, Hato Rey, PR) connected to a PE-50 catheter in the right femoral artery. Pressure data were integrated by a microcomputer to mean arterial pressure (MAP) over 5 min.

Secretion (Only S−D Rats). Duodenal mucosal alkaline (HCO_3^-) secretion was measured by a pH-stat titration technique.[40] After midline laparotomy, a segment of the duodenum (with its proximal end 0.5 cm distal to the pylorus) with intact vascular supply was isolated between two glass

tubes connected to a reservoir containing isotonic saline maintained at 38 °C by a water jacket. Saline was recirculated through the segment by means of a gas lift (pure air). The common bile duct was catheterized ~5 mm proximal to the papilla of Vater to avoid contamination of the segment by bile and pancreatic juice. Alkaline secretion to the perfusate was continuously titrated to pH 7.4 with 0.02 M HCl controlled by a pH-stat device.

Experimental Protocol. After surgery, the animals were left undisturbed to recover, after which baseline values were monitored during a 30 min control period. The study compounds or vehicle were then administered and the animals were monitored for an additional 45 min period.

Statistics. Secretion data obtained during the last 15 min before administration of drug were regarded as basal conditions. Net change was defined as the difference between basal conditions and data obtained between 30 and 45 min following onset of each drug administration. Values given in the figures are means ± SEM. Comparisons between groups were made by ANOVA and the Bonferroni post hoc test.

Oral Absorption Study. All materials and reagents used throughout the study were of standard analytical grade or equivalent. Twenty-four male Sprague−Dawley rats (age ca. 6−7 weeks at the time of dosing) were obtained from Harlan UK Ltd, a recognized supplier of laboratory animals. All animals were individually identified by tail markings with indelible ink. The oral absorption study was performed in triplicates with three oral concentrations (30, 100, and 300 μg/kg) and one intravenously (30 μg/kg). The orally administrated doses were given via gastric gavage and the intravenously were administrated as a single bolus injection into the tail vein. All dose weights were altered to take into account the weight of the animal at the time of administration. The actual dose received by each animal was calculated from the calculated dose concentration, the animal weight, and the weight of dose administered. A blood sample (200 μL) was taken from the animals via venepuncture of the tail vein at the following time points post dose: 30 min and 1, 2, 4, 6, 8, and 24 h. All blood samples were collected into heparinized blood tubes and centrifuged to prepare plasma, within 2 h of collection. The plasma was transferred into plain tubes and frozen at ca −20 °C until analyzed. Plasma (50 μL) was transferred into an Eppendorf tube. For calibration samples, 20 μL of an appropriate calibration spiking solution prepared in acetonitrile:water (1:1) was added, and for all study samples, 20 μL of acetonitrile:water (1:1) was added. To all samples, 150 μL of acetonitrile was then added, vortex mixed for approximately 2 s, and then centrifuge at 4000 rpm for 5 min at ~4 °C. Aliquots (50 μL) of supernatant were transferred to autosampler vials containing 20 μL of deionized water and vortex mixed. The samples were analyzed by LC−MS/MS using a Perkin-Elmer Series 200 Micropump and Series 200 Autosampler (Perkin-Elmer, Beaconsfield, UK) coupled to a Turbo IonSpray (TIS) source of an Applied Biosystems API 365 triple quadropole mass spectrometer (Applied Biosystems, Warrington, UK). Compound **21** was analyzed on a Metachem Polaris (5 μm, 50 × 2 mm) column using a gradient HPLC method. The mobile phases comprised of A, acetonitrile, and B, aqueous ammonium acetate (10 mM). The flow from the column was not split prior to entering the source of the mass spectrometer; however, the first minute of flow was directed to waste, with the remaining 4.2 min directed into the mass spectrometer. The injection volume was 40 μL and the autosampler needle was washed with acetonitrile:water (1:1). The mass spectrometer was operated in positive ion mode and compound **21** was analyzed by selected reaction monitoring (SRM) using the specific precursor to product ion transitions (476.3 → 293.2). Peak areas were compared against a calibration line of peak area versus concentration. The absolute bioavailability and dose proportionality were calculated using AUC_{0-8h} instead of $AUC_{0-\infty}$, as $AUC_{0-\infty}$ could not be calculated in many cases. Plasma concentrations below the limit of quantification of the assay, BLQ (<1 ng/mL), were taken as zero for all calculations. If the concentration reached BLQ before 8 h postdose, AUC_{0-8h} was calculated by extrapolation of the last measurable concentration to BLQ.

Acknowledgment. We thank Erik Helgstrand and Ann Christin Kvarnström for valuable discussions. We gratefully acknowledge support from the Swedish Research Council (VR), the Swedish Foundation for Strategic Research (SSF), the Knut and Alice Wallenberg Foundation (VRmedicine 8663), the Canadian Institutes of Health Research to N.G.P. and MDP (MOP 37891), and Vicore Pharma AB. N.G.P. is a holder of a Canada Research Chair in Endocrinology of the Adrenal Gland. We thank Biotage AB for providing the microwave synthesizer.

Supporting Information Available: Analysis data for **4**, **6**, **7**, and **10**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM049715T