Synthesis of a New Class of Druglike Angiotensin II C-Terminal Mimics with Affinity for the AT_2 Receptor

Jennie Georgsson,[†] Christian Sköld,[†] Milad Botros,[‡] Gunnar Lindeberg,[†] Fred Nyberg,[‡] Anders Karlén,[†] Anders Hallberg,[†] and Mats Larhed*,[†]

Department of Medicinal Chemistry, Division of Organic Pharmaceutical Chemistry, BMC, Uppsala University, P.O. Box 574, SE-751 23 Uppsala, Sweden, and Department of Pharmaceutical Sciences, Division of Biological Research on Drug Dependence, BMC, Uppsala University, P.O. Box 591, SE-751 24 Uppsala, Sweden

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Four tripeptides corresponding to the C-terminal region of angiotensin II were synthesized. One of these peptides (Ac-His-Pro-Ile) showed moderate binding affinity for the AT_2 receptor. Two aromatic histidine-related scaffolds were synthesized and introduced in the tripeptides to give eight new peptidomimetic structures. Three of the new peptide-derived druglike molecules exhibited selective, nanomolar affinity for the AT_2 receptor. These ligands may become lead compounds in the future development of novel classes of selective AT_2 receptor agonists.

Introduction

The peptide hormone angiotensin II (Ang II,[a] Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) activates two receptors, angiotensin II type I and angiotensin II type 2 (AT_1 and AT_2, respectively). The receptors are both G-protein coupled, but differ considerably in amino acid sequence and in the physiological effects. Knowledge of the AT_1 receptor has been available for 35 years and it is mostly recognized for its important role in the regulation of blood pressure, salt and water retention, and cellular growth.[1,2] The AT_2 receptor, on the other hand, was not characterized until the early 1990s.[3,4] The AT_2 receptor often exerts opposite effects, as compared to that of the AT_1 receptor; its activation results in vasodilatation and suppressed cellular growth.[1,5,6] It appears to have a regulatory role in the renin−angiotensin system, balancing the effects of the AT_1 receptor especially in pathological conditions. The receptor also promotes antiproliferation, neurite outgrowth in certain cell types, apoptosis, and cell differentiation.[5,7,8] It has been proposed that the AT_2 receptor could be an important target in the therapeutic area of hypertension and cardiac remodeling.[5,9]

Using the endogenous peptide ligand as a starting point for the development of a new lead series of druglike compounds is, to us, an appealing approach, and over the years, we have made some progress in our efforts to identify selective AT_2 receptor agonists. For example, we have designed and synthesized a number of bioactive AT_2 receptor selective analogues of similar size to Ang II, where the dipeptide fragment Tyr[4]-Ile[5] was replaced by various γ-turn mimetic scaffolds.[10−12] In 1991, de Gasparo et al. showed that removal of three amino acids from the N-terminus of Ang II ([Val[5]]Ang II (4−8) and [Val,[5]Ile[8]]Ang II (4−8)) resulted in AT_2 receptor selectivity.[13] Inspired by these results, we synthesized and evaluated the structurally similar compound **I** (Figure 1) and the corresponding analogue with Phe as the C-terminal amino acid. Interestingly, both of them were AT_2 receptor agonists.[14] In the same study, we also demonstrated that replacement of the Tyr-Ile unit by a single aromatic core produced ligand **II** (Figure 1) with AT_2 receptor affinity and agonistic activity comparable to Ang II.[14] In parallel, nonpeptide AT_2 receptor ligands have been developed in our group, originating from the first reported nonpeptide AT_1 receptor agonist L-162,313 disclosed by Merck.[15,16] L-162,-313 was found to also exert agonistic properties toward the AT_2 receptor,[17] and structural modifications have resulted in the first selective nonpeptide AT_2 receptor agonist M024 (Figure 1).[18]

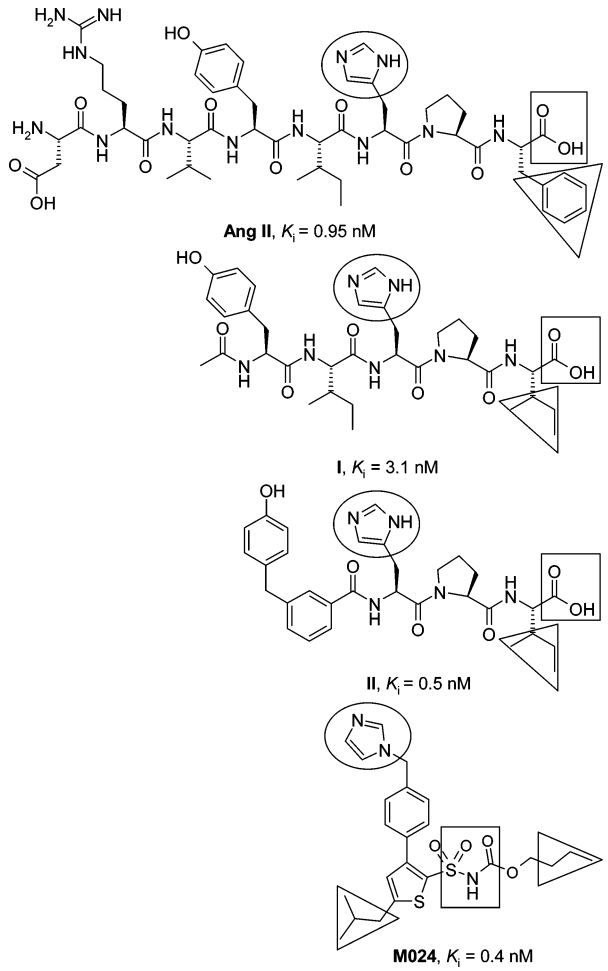

Figure 1. AT_2 receptor agonists with common and potentially important structural elements indicated.

* To whom correspondence should be sent. Phone: +46-184714667. Fax: +46-184714474. E-mail: mats@orgfarm.uu.se.
[†] Department of Medicinal Chemistry.
[‡] Department of Pharmaceutical Sciences.
[a] Abbreviations: Ang II, angiotensin II; AT_1, angiotensin II type 1; AT_2, angiotensin II type 2.

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Chart 1

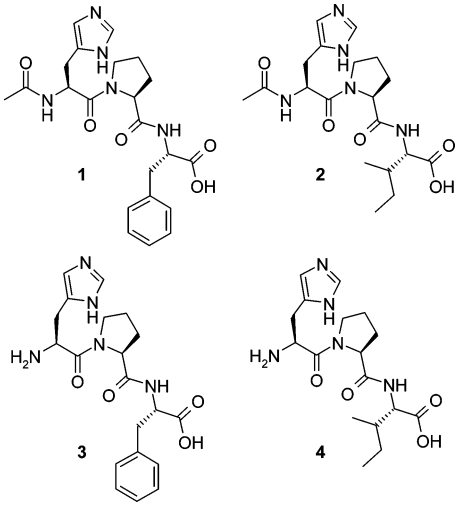
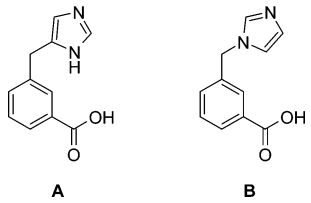


Chart 2



Based on structural comparison, we suggest that the nonpeptidic agonist M024 mimics the C-terminal of Ang II and the truncated analogues **I** and **II**, as illustrated in Figure 1. Thus, the imidazole group of M024 corresponds to the histidine side chain, the acylsulfonamide functionality provides an acidic proton similar to the carboxylic acid, and either the isobutyl chain or the *n*-butyl chain of M024 shares the same interaction as the C-terminal Phe/Ile side chain. However, no apparent structural motif can be found in M024 corresponding to a Tyr side chain, which is present in Ang II and the truncated analogues. Thus, the following question arises: Is a Tyr side chain really necessary in the case of the more Ang II-like analogues or can it be removed to further reduce the molecular size and peptide character? Herein, we address this question by presenting the synthesis of four novel tripeptide analogues (**1**−**4**), the synthesis of two aromatic scaffolds (**A** and **B**), and their incorporation, providing eight peptidomimetic analogs (**5**−**12**) as well as the biological evaluation of all 12 compounds.

Results and Discussion

Chemistry. The four peptides **1**−**4** (Chart 1) were obtained from commercially available resin-bound protected tripeptides. Scaffolds **A** and **B** (Chart 2) were prepared as described below and were thereafter coupled to one or two amino acids to generate eight pseudopeptides, **5**−**12** (Chart 3). For the synthesis of scaffold **A**, a benzoic acid with a histidine side chain, an orthogonally diprotected imidazole was required. To obtain this, *N*,*N*-dimethylimidazole-1-sulfonamide was TIPS-protected in position 2 using *n*-BuLi to deprotonate, followed by addition of triisopropylsilyl triflate (TIPS-OTf) to afford compound **13** (Scheme 1). The subsequent preparation of scaffold **A** (as sulfonamide **19**) is outlined in Scheme 2.

First, the free carboxylic acid of the commercially available monomethyl isophthalate was selectively reduced using BH_3−THF to yield the benzyl alcohol.[19] The alcohol was directly protected by TIPS-OTf to form compound **14**.[20] The TIPS protecting group was chosen because of the high stability in basic conditions and the possibility to selectively cleave it using tetrabutylammonium fluoride (TBAF) in the presence of the sulfonamide.[21] The methyl ester was next reduced with $LiAlH_4$ in dry THF. The resulting alcohol was treated with mesyl chloride (MsCl) overnight.[22] As a result of the prolonged reaction time, the benzyl chloride **15** was formed instead of the mesylate via a double substitution reaction. To obtain a better leaving group, the benzyl iodide **16** was prepared immediately before usage by classic treatment with NaI in acetone. An ortho-directed lithiation of the protected imidazole **13** with *n*-BuLi in THF at −78 °C followed by the slow addition of benzyl iodide **16** afforded structure **17**.[23] The two silyl protecting groups were easily cleaved by treatment with TBAF to furnish free **18** in an 83% yield. A mild two-step oxidation of benzyl alcohol **18** was utilized to reduce the risk of oxidation at the second benzylic position. First, the aldehyde was generated by a Swern oxidation at the alcohol. The intermediate aldehyde was further oxidized to the benzoic acid by sodium chlorite and sodium dihydrogen phosphate.[24] Benzoic acid **19** was then coupled to the appropriate *tert*-butyl ester protected amino acid using standard amide coupling conditions, with HATU as the coupling reagent. Finally, removal of the remaining protecting groups with HCl in dioxane generated compounds **5**, **9**, **6**, and **10** as salts.

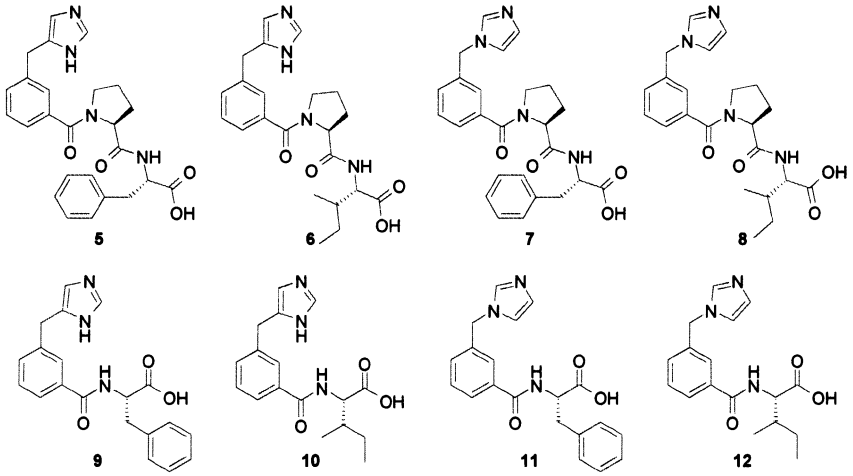
The synthetic route to scaffold **B**[25] is presented above (Scheme 3). As for compound **14**, the free benzoic acid of monomethyl isophthalate was selectively reduced by BH_3·THF to give monoester **20**. The alcohol was converted to the benzyl bromide (**21**) by the standard reaction with PBr_3. Treatment of **21** with imidazole in DMF followed by hydrolysis of the methyl ester with LiOH gave scaffold **B**, which was isolated as the HCl salt. To generate the final products **7**, **8**, **11**, and **12**, scaffold **B** was coupled to the amino acids using the same conditions as above. No loss of the stereochemistry was detected.

Binding Assay. Compounds **1**−**12** were evaluated in a radioligand binding assay relying on displacement of [^{125}I]Ang II from AT_1 receptors in rat liver membranes[26] and AT_2 receptors in pig uterus membranes.[27] Ang II and [4-NH_2-Phe^6]-Ang II were used as reference substances. These results are presented in Table 1. Tripeptide **2** with an N-terminal Ile residue binds selectively to the AT_2 receptor with a K_i value of 1.1 μM, while peptide **3** was the only analogue that displayed affinity for the AT_1 receptor. Of the evaluated eight pseudopeptides (**5**−**12**), compounds **9**, **11**, and **12** had nanomolar affinity for the AT_2 receptor, with K_i values ranging from 16.6 to 40.6 nM. Despite being very good AT_2 receptor ligands, the compounds did not display AT_1 receptor binding.

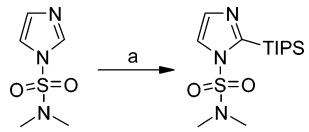
Discussion

We have previously reported on the development of a set of pentapeptides and pseudopeptides, starting from the octapeptide Ang II. Most of the ligands synthesized by us displayed high affinity for the AT_2 receptor.[14] Four of the compounds were also tested in a functional assay based on neurite outgrowth from NG 108−15 cells and proved to be agonists. In parallel and using a more classical medicinal chemistry approach starting from the AT_1 agonist L-162,313,[15,16] the nonpeptidic selective AT_2 receptor agonist M024 was developed in our group.[18] As mentioned above, the nonpeptidic AT_2 receptor agonists may mimic the C-terminal part of Ang II, and if this hypothesis is correct, removal of the Tyr residue should be possible without loss of activity. To evaluate this hypothesis, a set of four tripeptides (**1**−**4**) lacking the Tyr residue were synthesized and tested for AT_1 and AT_2 receptor affinity (Chart 1). Only

Chart 3

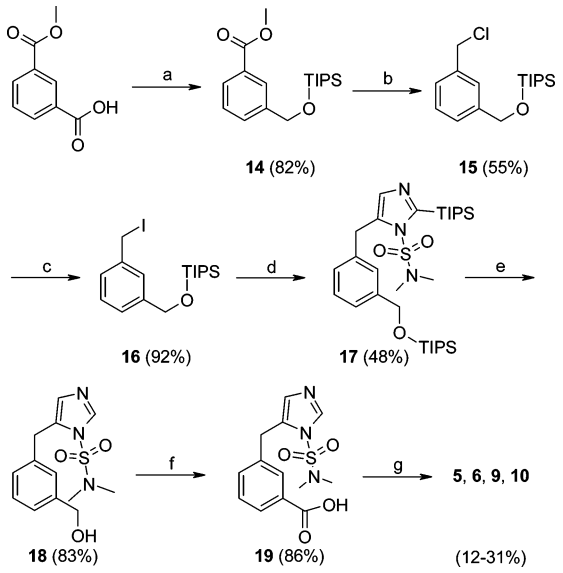


Scheme 1[a]



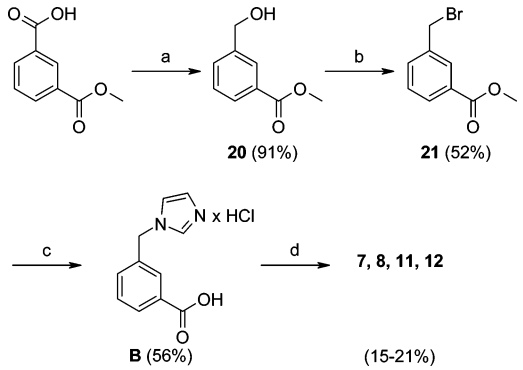
[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, TIPS-OTf.

Scheme 2[a]



[a] Reagents and conditions: (a) (i) BH_3·THF, THF; (ii) TIPS-OTf, PPTS, DMAP, pyridine, DCM; (b) (i) $LiAlH_4$, THF; (ii) NaOH/THF; (iii) MsCl, NEt_3, DCM; (c) NaI, acetone; (d) *n*-BuLi in hexane, dry THF, −78 °C, **13**; (e) TBAF, THF; (f) (i) oxalyl chloride, NEt_3, DMSO, DCM; (ii) $NaClO_2$, NaH_2PO_4·H_2O, cyclohexene, *tert*-butyl alcohol; (g) (i) amino acid, HATU, DIEA, DMF; (ii) HCl in dioxane.

Scheme 3[a]



[a] Reagents and conditions: (a) (i) BH_3−THF, THF; (b) PBr_3, diethyl ether; (c) (i) imidazole, DMF; (ii) LiOH, H_2O/MeOH/THF; (iii) aq. HCl; (d) (i) amino acid, HATU, DIEA, DMF; (ii) HCl in dioxane.

Table 1. Binding Affinities for the AT_1 and AT_2 Receptors

compound	AT_1[a] $K_i \pm$ SEM (nM)	AT_2[b] $K_i \pm$ SEM (nM)
Ang II	0.5	0.3
[4-NH_2-Phe[6]] Ang II	>10000	0.9
1	>10000	>10000
2	>10000	1110 ± 17
3	1598 ± 66	>10000
4	>10000	>10000
5	>10000	>10000
6	>10000	>10000
7	>10000	>10000
8	>10000	>10000
9	>10000	40.6 ± 1.0
10	>10000	>10000
11	>10000	37.5 ± 0.6
12	>10000	16.6 ± 0.5

[a] Binding assay relying on displacement of [^{125}I] Ang II from rat liver membranes. [b] Binding assay relying on displacement of [^{125}I] Ang II from pig uterus myometrium.

analogue **2**, with the Ile residue in the C-terminal, showed moderate AT_2 receptor binding affinity (K_i = 1110 nM). In fact, Ile in this position has previously been found to enhance the AT_2 receptor binding affinity when compared to the corresponding Phe analogue.[14] Furthermore, it also seemed that the N-terminal acetyl group was necessary for receptor binding because primary amine **4** lacked affinity. The reasonable binding affinity of the N-acetylated tripeptide **2** suggests that it could serve as a lead compound in producing less peptidic compounds.

With this in mind, a series of analogues were synthesized based on the 1,3-disubstituted benzene core structure. This simple unit has previously proved successful as a mimic of Tyr-Ile when incorporated into Ang II related pseudopeptides.[12,14] However, after appropriate functionalizations, this scaffold might also be worthwhile investigating as a mimic of His or His-Pro. Thus, compound **19** (protected **A**) equipped with a His mimicking side chain was synthesized (Scheme 2). In addition, isomer **B** containing an *N*-imidazolemethyl side chain similar to that found in the nonpeptide AT_2 receptor agonist M024 was prepared (Scheme 3, Figure 1). Because a direct atom-to-atom comparison in the His region was not possible between **2** and scaffolds **A** or **B**, it was difficult to decide which amino acid

should be coupled to the C-terminal end of the scaffold. To circumvent the problem, two series of compounds were produced. In the first set of ligands (**5**−**8**), scaffolds **A** and **B** substitute His, while in the second series (**9**−**12**) they occupy the position of the dipeptide His-Pro. The resulting compounds in the first series showed no detectable affinity up to 10 μM (Table 1). It can be concluded that in the series of monoamides (**9**, **11**, and **12**) both scaffolds **A** and **B** effectively replace the dipeptide fragment His-Pro, as indicated by their reasonably high AT_2 receptor affinities. Compound **12** with an Ile C-terminal showed slightly better affinity (K_i = 16.6 nM) than the corresponding Phe analogue (K_i = 37.5 nM), which is in accordance with previous observations.[14] No great difference in binding affinity was observed when scaffolds **A** and **B** were coupled to Phe (compare **9** and **11**). Surprisingly, structure **10** showed no detectable binding up to 10 μM. It is remarkable that substitution of Phe with Ile when having a histidine side chain (compare **9** and **10**) results in loss of AT_2 receptor affinity when the same substitution increases the affinity in the analogues with an N-imidazolemethyl group (compare **11** and **12**). However, similar sensitivity in affinity to the AT_2 receptor has been found previously, where relatively small structural modifications of the sulfonylcarbamate side chain in a series of M024 analogues resulted in inactive compounds.[17] This sensitivity was also present but not equally profound in compounds having the same N-imidazolemethyl group as M024.[28] In **10**, the combination of the Ile and His side chain is clearly not suitable for a favorable AT_2 receptor interaction, which seems to reflect a similar sensitivity in this series.

To examine if **12** and M024 could adopt a similar binding mode at the AT_2 receptor, pharmacophore modeling was performed. As a result of their similarity, it was hypothesized that the imidazole and the acidic groups, respectively, correspond to pharmacophore groups and are superimposed upon binding. A third possible pharmacophore group in **12** corresponds to the Ile side chain. However, it is not obvious which of the isobutyl or *n*-butyl groups of M024 should share the same receptor interaction as the Ile side chain in that case. To address this issue, these compounds were modeled using the DISCOtech[29] program in Sybyl.[30] Because it has been proposed that Lys[215] in the AT_2 receptor is involved in an ionic interaction with the C-terminal of Ang II,[31] the acidic groups in **12** and M024 were defined as a pharmacophore group that all models were forced to contain. Using this approach, DISCOtech found 194 models. Guided by the superimposed features and the overall structural overlap of the superimposed structures in each model, two reasonable models were identified. In these models, the imidazole groups and the acidic groups overlay nicely, while the Ile side chain can be either superimposed on the *n*-butyl group (Figure 2a) or in the vicinity of the isobutyl group (Figure 2b). It is shown in Figure 2b that the Ile side chain is not perfectly matched to the isobutyl group but is oriented more toward the thiophene ring (Figure 2b). Based on the present modeling, it therefore seems that **12** and M024 should be able to adopt similar binding modes in general when binding to the AT_2 receptor. However, M024 will have additional interactions with the AT_2 receptor, considering that **12** only has one hydrophobic side chain.

Conclusion

In summary, two aromatic scaffolds functionalized with either a histidine side chain or an N-imidazolemethyl group were successfully synthesized and coupled to C-terminal peptide fragments of Ang II. Based on these scaffolds, a novel set of compounds was prepared, and three of them were found to possess nanomolar affinity for the AT_2 receptor. These ligands were developed based on the Ang II octapeptide and may serve as unique lead structures in the development of a novel and selective class of AT_2 receptor agonists.

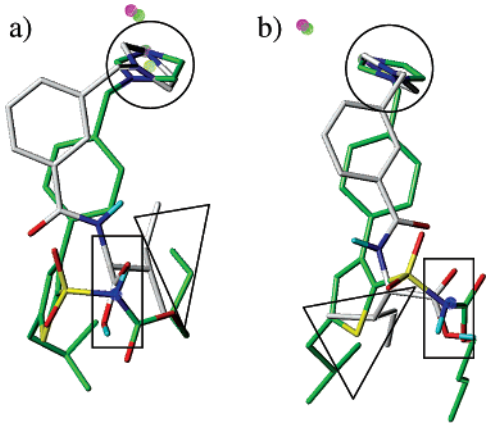


Figure 2. Possible pharmacophore models of **12** (white carbons) and M024 (green carbons), with important structural elements indicated (Figure 1). The colored spheres correspond to DISCOtech features. (a) Model in which the Ile side chain of **12** and the *n*-butyl group of M024 are overlaid. (b) Model in which the Ile side chain of **12** and the isobutyl group are overlaid.

Experimental Section

Preparation of Peptides 1−4. Fmoc-His(Trt)-Pro-Phe-Wang resin[10] or Fmoc-His(Trt)-Pro-Ile-Wang resin[10] was swelled in DMF. The Fmoc-group was removed by treatment with 20% piperidine in DMF, and the resin was washed with DMF, DCM, and MeOH before being dried under vacuum overnight. For the acetylated analogues, an aliquot of the resin was swelled in DMF. The acetic acid was dissolved together with PyBOP or HBTU and DIEA in DMF. The solution was added to the resin and agitated by rotation overnight. The resin was deprotected, washed, and dried as above. The peptides were cleaved from the resin using triethylsilane and 95% aqueous TFA. The polymer was filtered off and washed with TFA. The filtrate was evaporated in a stream of nitrogen, and the product was precipitated by addition of diethyl ether. The precipitate was collected by centrifugation, washed with ether, and dried. The crude peptide was purified by RP-HPLC and isolated at yields of 11−50%.

General Procedure for Amide Coupling and Deprotection. Scaffold **19** or **B**, HATU, the appropriate amino acid, and DIEA were dissolved in DMF. After stirring in ambient temperature overnight, the reaction mixture was poured into water and extracted with ethyl acetate. The combined organic phase was washed with saturated NH_4Cl, water, and brine and dried with Na_2SO_4. The crude product was purified on preparative RP-LC-MS. The product was dissolved in 4 M HCl in dioxane and stirred at room temperature overnight (or compounds with dimethylsulfonamide protecting group present, at 70 °C for 5 h). The dioxane was evaporated under vacuum. The crude products were purified by RP-LC-MS and isolated in yields of 12−31%.

Compound 9. Isolated yield of **9** was 12.5 mg (14%). ^{1}H NMR (CD_3CN + D_2O) δ 8.50 (d, J = 1.5 Hz, 1H), 7.59 (m, 1H), 7.57 (m, 1H), 7.44−7.37 (m, 2H), 7.29−7.18 (m, 5H), 7.16 (m, 1H), 4.79 (dd, J = 5.1, 9.4 Hz, 1H), 4.07 (s, 2H), 3.29 (dd, J = 5.1, 14.0 Hz, 1H), 3.08 (dd, J = 9.4, 14.0 Hz, 1H); ^{13}C NMR (CD_3CN + D_2O) δ 174.3, 168.7, 138.5, 138.4, 135.2, 134.6, 133.6, 133.1, 130.2, 130.1, 129.4, 128.5, 127.8, 126.8, 117.3, 55.1, 37.7, 30.8; LC/MS (M, 349.1) (350.1; M + H^+). HRMS (M + 1) calcd, 350.1505; found, 350.1501. $[\alpha]^{20}_D$ = −20.5 (*c* 0.44, DMSO-d_6)

Compound 10. Isolated yield of **10** was 18.1 mg (23%). Diasteromeric mixture of 1:0.11 Ile/*allo*-Ile determined by amino acid analysis. ^{1}H NMR (DMSO-d_6) δ (major) 8.30 (d, J = 7.4 Hz,

NH), 7.73 (m, 1H), 7.69 (m, 1H), 7.53 (m, 1H), 7.37 (m, 1H), 7.35 (m, 1H), 6.76 (m, 1H), 4.27 (t, $J = 7.4$ Hz, 1H), 3.89 (s, 2H), 1.93 (m, 1H), 1.50 (m, 1H), 1.24 (m, 1H), 0.91 (d, $J = 6.8$ Hz, 3H), 0.86 (t, $J = 7.4$ Hz, 3H) (the signal from the 2-pos in the imidazol is missing due to broadening); ^{13}C NMR (DMSO-d_6) δ 173.2, 166.5, 140.6, 136.2, 134.9, 134.1, 131.4, 128.0, 127.6, 125.0, 57.3, 35.8, 32.7, 25.0, 15.6, 11.1 (The signal from the 5-pos in the imidazol is missing due to broadening. It was identified by HMBC in CD_3CN and 5% D_2O at δ 116.7). LC/MS (M, 315.2) (316.1; M + H^+). HRMS (M + 1) calcd, 316.1661; found, 316.1655.

Compound 11. Isolated yield of **11** was 18.2 mg (21%). ^{1}H NMR (CD_3CN + D_2O) δ 8.70 (m, 1H), 7.67 (m, 1H), 7.65 (m, 1H), 7.52−7.37 (m, 4H), 7.26−7.16 (m, 5H), 5.37 (s, 2H), 4.75 (dd, $J = 5.0$, 9.4 Hz, 1H), 3.28 (dd, $J = 5.0$, 14.0 Hz, 1H), 3.05 (dd, $J = 9.4$ 14.0 Hz, 1H); ^{13}C NMR (CD_3CN + D_2O) δ 175.1, 168.5, 138.4, 136.0, 135.6, 135.5, 132.8, 130.6, 130.2, 129.4, 128.6, 128.3, 127.7, 122.8, 121.5, 55.6, 53.0, 37.8; LC/MS (M, 349.1) (350.1; M + H^+). HRMS (M + 1) calcd, 350.1505; found, 350.1508. $[\alpha]^{20}_D = -41.2$ (*c* 0.51, DMSO-d_6).

Compound 12. Isolated yield of **12** was 15.0 mg (19%). ^{1}H NMR (CD_3CN + D_2O) δ 8.74 (m, 1H), 7.79 (m, 1H), 7.75 (m, 1H), 7.55−7.49 (m, 2H), 7.42 (m, 2H), 5.40 (s, 2H), 4.42 (d, $J = 6.4$ Hz, 1H), 1.95 (m, 1H), 1.50 (m, 1H), 1.24 (m, 1H), 0.94 (d, $J = 6.9$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (CD_3CN + D_2O) δ 175.3, 169.5, 135.8, 135.41, 135.39, 132.9, 130.6, 128.9, 128.5, 122.8, 121.2, 58.8, 53.1, 37.4, 26.0, 15.9, 11.6; LC/MS (M, 315.2) (316.1; M + H^+). HRMS (M + 1) calcd, 316.1661; found, 316.1659. $[\alpha]^{20}_D = -10.9$ (*c* 0.46, DMSO-d_6).

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Supporting Information Available: Experimental details, HPLC tracings and spectroscopic data for compounds **1**−**21** and **B**, procedures for AT_1 and AT_2 receptor binding assays, and description of the molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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