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N-Aryl Isoleucine Derivatives as Angiotensin II AT_2 Receptor Ligands

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A novel series of ligands for the recombinant human AT_2 receptor has been synthesized utilizing a fast and efficient palladium-catalyzed procedure for aminocarbonylation as the key reaction. Molybdenum hexacarbonyl [$Mo(CO)_6$] was employed as the carbon monoxide source, and controlled microwave heating was applied. The prepared *N*-aryl isoleucine derivatives, encompassing a variety of amide groups attached to the aromatic system, exhibit binding affinities at best with K_i values in the low micromolar range versus the recombinant human AT_2 receptor. Some of the new nonpeptidic isoleucine derivatives may serve as starting points for further structural optimization. The presented data emphasize the importance of using human receptors in drug discovery programs.

Introduction

The octapeptide angiotensin II (Ang II) is the major effector peptide of the renin-angiotensin system (RAS). It acts via two receptors, the AT_1 and the AT_2 receptor (AT_1R and AT_2R). The effects mediated by AT_1R are well known and include regulation of blood pressure and fluid/electrolyte balance.[1] When AT_2R is expressed together with AT_1R, its activation results in several effects that oppose those mediated by the latter. Thus, stimulation induces vasodilatation, antiproliferation and apoptosis. Conversely, when expressed alone in undifferentiated cells, AT_2R stimulation is involved in cell differentiation.[1–2] In fact, AT_2R is abundant in fetal tissues but its expression drops rapidly after birth, an observation in agreement with its role in cell differentiation. In the healthy adult, aside from a few specific tissues, the expression is at barely detectable levels.[2b,3] However, a re-expression of the receptor occurs in some pathological states, such as heart and renal failure, myocardial infarction, hypertension, brain disorders or obesity disorders.[4] There is piling evidence that AT_2R is involved in tissue repair. Therefore, AT_2R has attracted special interest in connection with cardiac remodeling, and has now been addressed as a new target for drug intervention.[5]

We have conducted two projects in parallel with the common objective to identify selective drug-like agonists to AT_2R. The first project commenced with the endogenous peptide ligand Ang II (**A**) and subsequent stepwise modifications, including minimizations/truncations, rigidifications and incorporation of turn mimetics resulted in series of AT_2R-selective analogues, for example **C** (Figure 1).[6] This approach has led us to a new unique lead structure (**E**) that we anticipated could serve as a starting point for a new class of selective AT_2R agonists (Figure 1).[6f] A parallel project focused on transforming the nonpeptidic but nonselective AT_1R agonist L-162,313, disclosed by Merck, into a nonpeptidic AT_2R-selective agonists.[7] We demonstrated that L-162,313 acts as an agonist also at the AT_2R, and stepwise structural modifications of L-162,313 led to the identification of the first selective drug-like nonpeptide AT_2R agonist C21/M024 (**D**) that has been extensively studied in various in vitro and in vivo models (Figure 1).[8] When comparing the two lead structures **D** and **E**, the structural similarities seem obvious, despite the different origins of the molecules. Hence, we hypothesize that both of the leads, **D** and **E**, mimic the C terminus of Ang II (**A**) and the truncated analogues **B** (Ang IV) and **C** (Figure 1).[6f] As indicated in Figure 1, the imidazole group would thus correspond to the histidine side chain, the sulfonyl carbamate would provide an acidic proton corresponding to the C-terminal carboxylic acid, and either the isobutyl or the *n*-butyl chain in C21/M024 would be able to mimic the hydrophobic Phe/Ile side chain of the C terminus of the peptide analogues.

Both lead structures comprise an imidazole group, which is frequently associated with undesired interactions with cytochrome P450 (CYP) enzymes. This issue was addressed, and CYP inhibition could successfully be minimized be replacement

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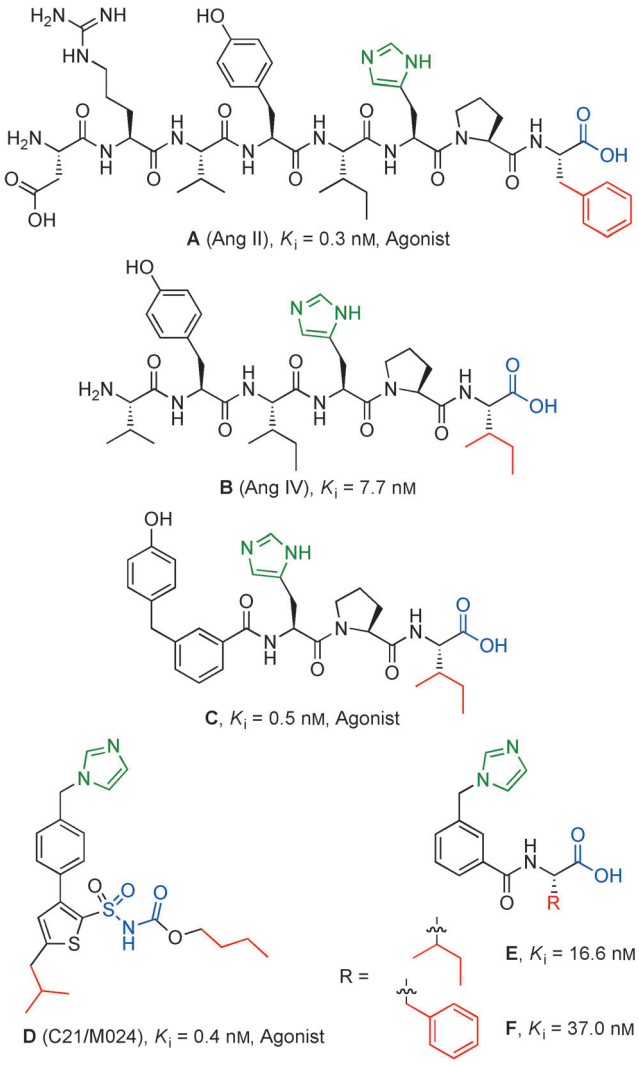


Figure 1. Selective AT_2R ligands with common and potentially important structural motifs indicated, K_i values are derived from radioligand binding assay by displacement of radiolabeled Ang II from AT_2R in pig uterus membranes. **E** originates from the endogenous peptide Ang II (**A**) while the nonpeptide C21/M024 (**D**) originates from the nonpeptide AT_1/AT_2 receptor agonist L-162,313.

of the imidazole in C21/M024 (**D**) with various amide groups, providing ligands with retained activity and function.[9]

With the ambition to assess the potential of lead **E**, as an entry to a new class of selective AT_2R agonists, we aimed at replacing the imidazole with a substituent less prone to bind to CYP enzymes, for example various amide groups. We decided to evaluate this new class of ligands towards the human AT_2R using transfected HEK-293 cells (HEK293-hAT_2R)[8e] rather than AT_2R in pig myometrial membranes, which had been used previously.

Herein we report a convenient synthesis and pharmacological evaluation of a series of benzamides derived from **E**, comprising an isoleucine residue at the C terminus and with the generic structure depicted in Figure 2. We further conclude that the amides synthesized as well as **E** exhibit only a weak affinity towards human AT_2R, while C21/M024 (**D**) binds with high affinity.

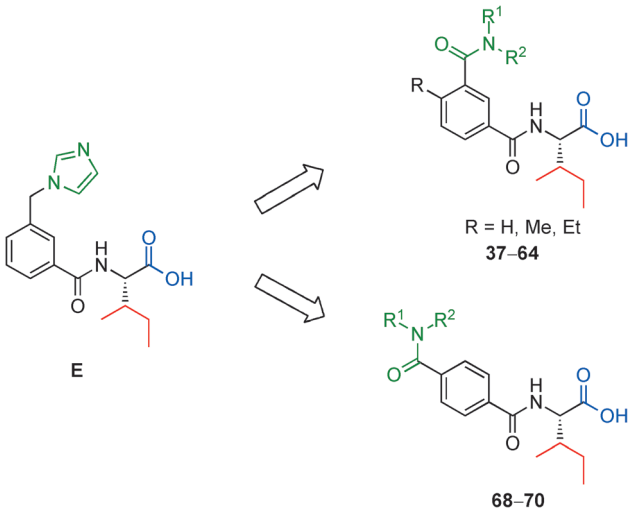


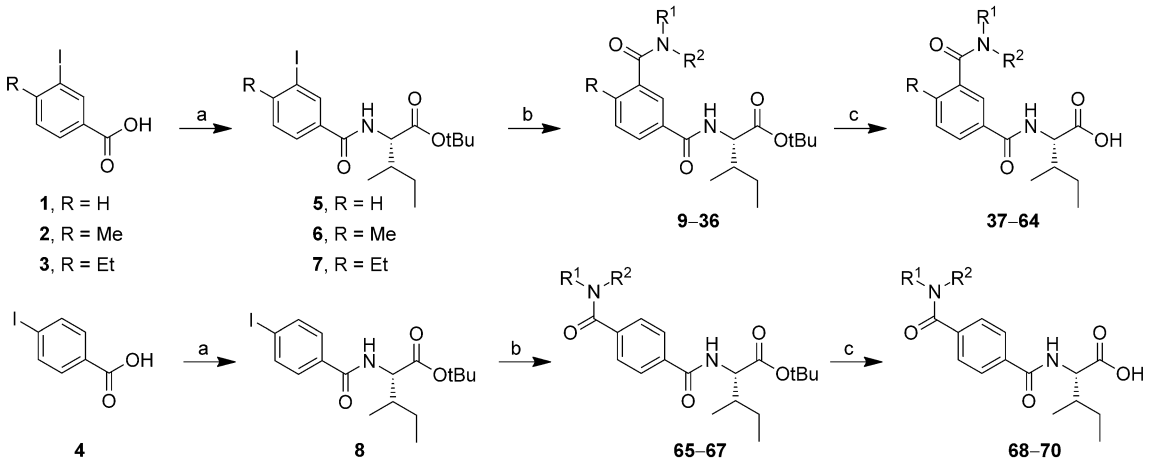
Figure 2. Lead structure **E** and the generic structures of the synthesized benzamides.

Results and Discussion

Chemistry

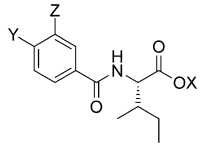
The synthesis of the new potential AT_2R ligands was performed by palladium-catalyzed aminocarbonylation reactions starting from the corresponding iodo compounds under microwave heating. To allow this reaction to be conducted in sealed vials under CO gas-free conditions, molybdenum hexacarbonyl ($Mo(CO)_6$) was chosen as the carbon monoxide source.[10] This rather recent method allows an efficient, fast and straightforward benzamide synthesis in air, and it has previously been used for the synthesis of biologically active compounds.[11] Although gaseous CO is advantageous for aminocarbonylations in large scale,[12] solid CO sources[13] such as $Mo(CO)_6$[14] are safer and more convenient for lab-scale chemistry since no gas tubes and high pressure equipment are required.

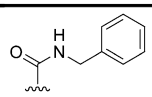
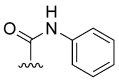
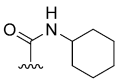
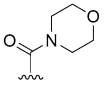
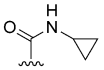
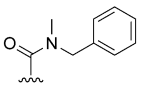
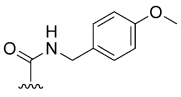
The aryl iodides **1**–**4** were converted by a standard coupling with isoleucine-*tert*-butyl ester to afford **5**–**8** (Scheme 1). After purification, moderate to excellent yields of 38–98% were achieved. The aryl iodides coupled with the isoleucine-*tert*-butyl ester residue were subsequently MW irradiated for 15 min at 100 °C in the presence of palladium catalyst with $Mo(CO)_6$ and a selection of primary and secondary amines bearing aliphatic, aromatic, cyclic as well as heterocyclic groups with diverse steric and lipophilic properties (**9**–**36** and **65**–**67**, Table 1). The yields of the aminocarbonylation step varied much depending on the steric hindrance of the nucleophilic amine and its electronic properties and ranged from 14% (**19**; Table 1, entry 11) to 85% (**17** and **22**; entries 9 and 14). After hydrolysis with trifluoroacetic acid (TFA), target compounds **37**–**64** and **68**–**70** were afforded in mostly good to very good isolated yields, between 52% (**63**, Table 1, entry 27) and 96% (**47**, entry 11). In a few cases the hydrolysis resulted in yields below 50% (entries 1, 14, 26, 28).



Scheme 1. Synthesis of novel benzamides as AT_2R ligands. *Reagents and conditions*: a) IleO*t*Bu, HATU, DIEA, DMF, RT, 16 h; b) HNR^1R^2, $Mo(CO)_6$, DBU, $Pd(OAc)_2$, THF, MW 100 °C, 15 min; c) TFA/DCM (1:1), RT, 2 h.

Table 1. Yields of synthesized benzamides and affinity towards human AT_2R in HEK-293 cells by displacement of either [^{125}I]CGP-42112A or [^{125}I]Sarile, and reference K_i values from displacement of [^{125}I]Ang II from the AT_2R in pig myometrial membrane.

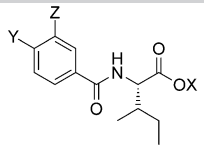


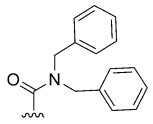
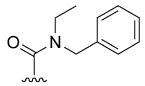
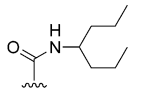
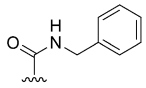
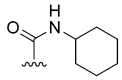
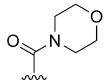
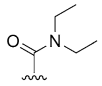
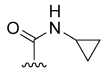
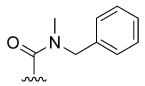
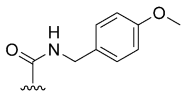
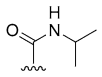
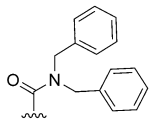
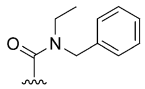
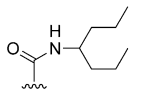
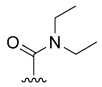
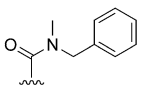
Entry	Y	Z	Yield[a] X = *t*Bu	Yield[b] X = H	Inhibition [%] of [^{125}I]CGP binding 1 μM	Inhibition [%] of [^{125}I]CGP binding 10 μM	K_i AT_2R [μM] Human [^{125}I]CGP	K_i AT_2R [μM] Human [^{125}I]Sarile	K_i AT_2R [μM] Pig [^{125}I]Ang II
1	H		78% **9**	21% **37**[e]	6	10			
2	H		66% **10**	56% **38**[e]	3	11			
3	H		70% **11**	75% **39**[e]	4	13			
4	H		76% **12**	82% **40**[e]	–[c]	12			
5	H		57% **13**	68% **41**[e]	5	6			
6	H		24% **14**	73% **42**[e]	6	23	22.0	19.4	
7	H		42% **15**	69% **43**[e]	2	4			
8	H		75% **16**	65% **44**[e]	6	32	9.0	11.0	
9	H		85% **17**	62% **45**[e]	6	7			
10	H		55% **18**	95% **46**[e]	6	7			

In vitro pharmacology: Binding assays

All free acids shown in Table 1 (**37**–**64** and **68**–**70**) were evaluated in a first radioligand binding assay relying on the displacement of [^{125}I]CGP-42112A (CGP-42112A; N_α-nicotinoyl-Tyr-(N_α-Cbz-Arg)-Lys-His-Pro-Ile), a selective but peptidic AT_2 receptor agonist[15] from human AT_2R expressed in HEK-293 cells (HEK293-hAT_2R). Ang II was used as the reference substance.[16] The majority of the new benzamides shown in Table 1 (20 compounds) were also evaluated for binding toward human AT_1R.[17] None of the evaluated compounds showed any affinity toward AT_1R, and therefore the remaining compounds were evaluated only towards AT_2R. In this first assay, the compounds were initially screened for binding activity (% inhibition of [^{125}I]CGP-42112 A binding) at a concentration of 1 μM and 10 μM. The re-

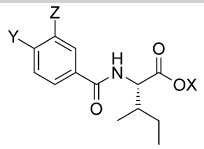
Table 1. (Continued)

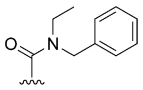
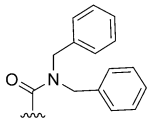
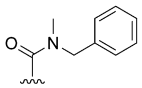
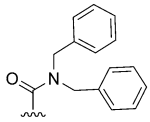


Entry	Y	Z	Yield[a] X = *t*Bu	Yield[b] X = H	Inhibition [%] of [^{125}I]CGP binding 1 μM	10 μM	K_i AT$_2$R [μM] Human [^{125}I]CGP	Human [^{125}I]Sarile	Pig [^{125}I]Ang II
11	H		14% **19**	96% **47**[e]	13	43	7.5	9.6	
12	H		43% **20**	64% **48**	14[d]	31[d]	11.0	3.1	
13	H		70% **21**	89% **49**	–[c]	12			
14	Me		85% **22**	47% **50**[e]	–[c]	7			
15	Me		75% **23**	77% **51**[e]	7	13			
16	Me		53% **24**	75% **52**[e]	4	7			
17	Me		22% **25**	72% **53**[e]	28	62	2.4	2.6	
18	Me		30% **26**	84% **54**[e]	1	18			
19	Me		45% **27**	68% **55**[e]	16	34	7.6	11.2	
20	Me		83% **28**	65% **56**[e]	1	7			
21	Me		74% **29**	75% **57**[e]	1	4			
22	Me		22% **30**	82% **58**[e]	18	62	2.5	2.3	
23	Me		48% **31**	71% **59**	4[d]	54[d]	3.4	1.5	
24	Me		75% **32**	76% **60**	–[c]	6			
25	Et		48% **33**	58% **61**	6[d]	29[d]	10.0		
26	Et		66% **34**	49% **62**	–[c]	26[d]	16.0		

sults from the initial compound screen indicated that noncyclic disubstituted benzamides (CONR^{1}R^2) showed better interaction with AT$_2$R. More lipophilic substituents on the benzamide function led to higher affinity towards AT$_2$R, and all compounds with activity in the initial screen had at least one benzyl group as substituent, except the diethyl benzamide, **42** and **53**. Based on the activities found in the screen, compounds were selected for K_i value determinations. The ligands bearing a methyl substituent in *para* position showing a displacement of more than 30% in the affinity screen [Table 1, entries 17 (**53**), 19 (**55**) and 22 (**58**)] were selected for K_i determinations. Additionally, the *para*-unsubstituted derivatives [Table 1, entries 6 (**42**), 8 (**44**) and 11 (**47**)] were also included to evaluate the influence of substitution in this position of the aromatic ring, even though they did not fully reach the same displacement. For the same reason, the *para*-ethyl derivatives [Table 1, entries 25 (**61**), 26 (**62**), 28 (**64**)] and the derivatives with the benzamides in the *para* position [Table 1, entries 29 (**68**), 30 (**69**), 31 (**70**)] were submitted directly for K_i determination, despite not being included in the initial screen. Furthermore, it was decided to include the benzylethyl benzamides (**48**, **59**, **63**) based on the preliminary results of the diethyl (**42**, **53**) and dibenzyl compounds (**47**, **58**).

Table 1. (Continued)



Entry	Y	Z	Yield[a] X = *t*Bu	Yield[b] X = H	Inhibition [%] of [^{125}I]CGP binding 1 μM	10 μM	K_i AT$_2$R [μM] Human [^{125}I]CGP	Human [^{125}I]Sarile	Pig [^{125}I]Ang II
27	Et		50% **35**	52% **63**	11[d]	40[d]	8.3		
28	Et		62% **36**	46% **64**	11[d]	50[d]	4.4		
29		H	68% **65**	74% **68**	–[c]	–[c]	n.c.[f]		
30		H	73% **66**	88% **69**	–[c]	7[d]	> 100		
31		H	16% **67**	57% **70**	–[c]	5[d]	35.0		
	Compound								
32	**E**						110.0	47.0	0.0166
33	**F**						63.0		0.0370
34	**C**						0.025		0.0005
35	**B** (Ang IV)						0.035		0.0077
36	**A** (Ang II)						0.044×10^{-3}	0.0028	0.0003
37	**D** (C21/M024)						0.0098	0.0076	0.0004

[a] Isolated yield after the aminocarbonylation reaction, > 95% purity. [b] Isolated yield after the deprotection, > 95% purity. [c] Not active. [d] Compound not included in the affinity screen, value taken from K_i determination. [e] Evaluated for AT$_1$R affinity, none of the compounds exhibited any binding. [f] Not calculable—less than 25% displacement at highest concentration.

The K_i values were determined from at least six data points with test concentrations ranging from 30 pM to 1 mM. The concentration range was adjusted to be appropriate for the expected K_i values. The results are summarized in Table 1.

The *para*-H and *para*-methyl benzamide analogues, compounds **42**/**53**, **44**/**55**, **47**/**58** and **48**/**59**, were also evaluated in a second radioligand binding assay, relying on the displacement of the AT$_1$R/AT$_2$R balanced peptide [^{125}I]Sarile (Sarile; [Sar^1, Ile^8]Ang II) instead of the AT$_2$R selective [^{125}I]CGP-42112 A from human AT$_2$R (HEK293-hAT$_2$R) as well as human AT$_1$R (HEK293-hAT$_1$R).[8e,18] No binding to the AT$_1$R was observed, and the K_i values towards AT$_2$R are summarized and compared with K_i values from the first HEK-293 binding assay in Table 1. As can be seen, the K_i values from these two assays correspond very well.

When comparing the affinity results given in Table 1, two trends become apparent. First, within each of the three sets of compounds differing only in the substituent in *para* position (i.e., H, Me, Et), the methyl substituted ligands show the highest affinities. The second trend is that the larger the substituents on the benzamide, the smaller the difference in K_i value within each group. In the case of the diethyl substituted compounds, the values are 22.0 μM ([^{125}I]CGP-42112A) for **42** as compared to 2.4 μM ([^{125}I]CGP-42112A) for **53**, and in the case of the dibenzyl substituted compounds, the difference is as low as 5 μM between the *para*-methylated ligand **58** and the unsubstituted compound **47**. Moving the benzamide group to the *para* position leads to a significant loss in affinity. Only compound **70** still shows some affinity towards the AT$_2$R with a K_i value of 35.0 μM, but the compound is inferior to its *meta* analogues (**47**, **58** and **64**). The dibenzyl substituted benzamides were the most consistent in affinity in all sets of analogues. The benzamides showing the best K_i values correlate partly to the most potent benzamide analogues of C21/M024. The diethyl substituted analogues are among the compounds with highest affinities in both series, which could suggest that the amide functions of the two classes of compounds interact with the same environment in the receptor.[9b]

To be able to correlate the results from these new ligands targeting the human AT$_2$R to our previous studies performed with AT$_2$R in membranes from pig uterus, a few reference compounds were selected and included in the K_i determination. To our surprise, lead compound **E** (Table 1, entry 32) only exhibited a K_i value of 110.0 μM. In our previous studies using the pig AT$_2$R, the K_i value was found to be 16.6 nM.[6f] Furthermore, reduced binding affinities were encountered also for the other peptide analogues, that is, **F** with 63.0 μM (pig AT$_2$R 37.0 nM),[6f] **C** with 25 nM (pig AT$_2$R 0.5 nM),[6e] and Ang IV (**B**) with a K_i value of 35 nM (pig AT$_2$R 7.7 nM; see Table 1, entries 33–35). The nonpeptide agonist C21/M024 (**D**) displayed a reduced binding but not to the same extent (K_i human AT$_2$R 9.8 nM versus K_i pig AT$_2$R 0.4 nM). The values were verified by the second binding assay, also targeting the human AT$_2$R, but

with displacement of [^{125}I]Sarile instead of [^{125}I]CGP-42112A. In this assay, lead compound **E** exhibited a K_i value of 47.0 μM, C21 M024 (**D**) showed a K_i value of 7.6 nM, while Ang II (**A**) showed a 60 times higher K_i value (2.8 nM; see Table 1). C21/M024 (**D**) has also been evaluated for binding towards the human AT_2R by Bosnyak et al. with a reported $IC_{50} = 2.29$ nM (Ang II (**A**); $IC_{50} = 0.522$ nM).[19] Thus, while the drug-like C21/M024 (**D**) binds with high affinity to AT_2R both from human and pig, the lead **E** exhibits a remarkable difference in affinity in the two species.

The data suggest a species difference in the interaction of the peptide analogues and the nonpeptidic substances, respectively, with regard to their binding to AT_2R, and emphasizes the importance of performing affinity studies on human AT_2R. The differences could of course be due to experimental conditions, in addition to species variations, for example, cell and receptor origin (endogenous membranes from uterus versus transfected kidney cells) and/or differences in the experimental design to measure ligand binding. However, the assays seem comparable, as the reference compounds exhibit the same relative order of affinity in both assays (Table 1, entries 32–37).

A comparison of the sequence of AT_2R from human and pig reveals that the receptors are very similar (95% homology in the amino acid sequence),[20] and very fine-tuned receptor models on a molecular level or mutation studies are required to investigate whether the observed discrepancy in binding data originates from species differences at the receptor level. It is conceivable that binding could be modulated by various levels of interferences in the two assays with partner proteins as AT_2R-interacting proteins (ATIP), the promyelocytic zinc finger protein (PLZF), the phosphatase SHP-1 or alpha subunit of G proteins. Such interactions might account for the incongruity reported herein.[21] Functional diversity of highly homologous proteins is rare (>90% amino acid identity), although it has been shown for AT_2R orthologues when comparing rabbit and human AT_2R.[22] To verify if our results originates from functional diversity (i.e., species differences) much more studies must be performed that are outside of the scope of this report. The AT_2R exists as a single copy, localized on the X chromosome and contains no intron in its coding region, and we hypothesize that it is more likely that the different binding data obtained are related to variations in tissues rather than species.

Georgsson et al. described the possibility to reduce the ligand size from **C** to the structures **E** and **F** without a major loss of affinity (Figure 1).[6f] Interestingly, these smaller ligands showed much higher K_i values when tested towards the human AT_2 receptor. The herein presented new ligands are of comparable size as **E** and **F** but show improved affinity towards the human receptor. Thus, these new compounds will serve as a new starting point for further improvement of this new class of AT_2R-selective ligands.

Conclusions

In summary, an efficient palladium-catalyzed procedure for aminocarbonylation of aryl iodides utilizing molybdenum hexacarbonyl ($Mo(CO)_6$) as carbon monoxide source has been employed to make a series of AT_2R ligands. Even though the K_i values presented in this work are comparably high (micromolar range), 13 of the 15 evaluated compounds demonstrate higher affinities towards the human AT_2R (HEK293-hAT_2R) than the original lead structure **E**. The large drop in affinity going from pig to human AT_2 receptor assay was unexpected, and the discrepancy is more pronounced for the peptides and pseudopeptides than for the nonpeptidic drug-like structure **D**. The presented data emphasize the importance of using human receptors in drug discovery programs. However, with the synthesized benzamides, we have obtained new starting points for targeting the human AT_2R, but significantly more efforts are required until equally high potency at the human AT_2R as our previously reported selective nonpeptide AT_2R agonist C21/M024 (**D**), is achieved.

Experimental Section

Chemistry

General information and materials: The microwave heating was performed in a Biotage Initiator single mode reactor, which produces controlled irradiation at 2450 MHz. The reaction temperature was determined using the built-in online IR sensor. Microwave mediated reactions were performed in sealed Smith process vials designed for 2–5 mL reaction volumes. Analytical TLC was performed using Merck aluminium-backed 0.2 mm silica gel 60 F-254 plates, and visualization was performed with UV light ($\lambda = 254$ nm). Silica gel 60 was purchased from Merck. NMR spectra were recorded on a Varian Mercury plus at 25 °C and 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts (δ) are reported in ppm and referenced indirectly to TMS via the solvent (or residual solvent) signals. Analytical RP-HPLC–MS was performed on a Gilson-Finnigan ThermoQuest AQA system (Onyx monolithic C18 column, 50×4.6 mm) and a Dionex Ultimate 3000 (C18 column, 50×3 mm) using a MeCN/H_2O gradient with 0.05% HCOOH. Detection was performed using UV ($\lambda = 214$ nm and 254 nm) and MS detection in ESI mode. Preparative RP-HPLC was performed on a Dionex Ultimate 3000 system (SB-C8 column, 21.2×150 mm; MeCN/H_2O gradient with 0.05% HCOOH) using UV detection ($\lambda = 214$ nm and 254 nm). Molecular masses were determined on a mass spectrometer equipped with an electrospray ion source (ESI-HRMS; 7-T hybrid linear ion trap (LTQ) FT mass spectrometer modified with a nanoelectrospray ion source). The optical rotation was determined using a PerkinElmer 241 polarimeter. Specific rotations ($[\alpha]^{25}_{589}$) are reported in $10^{-1} \times \text{deg} \times \text{cm}^2\,\text{g}^{-1}$, and the samples were prepared at a concentration of 1.0 g/100 mL in $CHCl_3$. All starting materials, reagents and solvents are commercially available and were used as received.

General procedure A: Synthesis of iodoaryl O*t*Bu-Ile derivatives 5–8: The iodo benzoic acid (**1**–**4**; 1 equiv), L-isoleucine *tert*-butyl ester hydrochloride (IleO*t*Bu·HCl; 1.1 equiv) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU; 1.1 equiv) were dissolved in *N*,*N*-dimethylformamide (DMF; 4 mL mmol^{-1}). *N*,*N*-Diisopropylethylamine (DIEA; 3.3 equiv) was added, and the reaction mixture was stirred at RT

overnight. The mixture was poured into H_2O and extracted with EtOAc (4×40 mL mmol^{-1}). The combined organic layers were washed with saturated NH_4Cl (1×40 mL mmol^{-1}), H_2O (4×40 mL mmol^{-1}) and brine (2×40 mL mmol^{-1}). After drying over Na_2SO_4, the solvent was evaporated in vacuo. Purification by column chromatography provided the pure products in moderate to excellent yields (**5**: 94%, **6**: 98%, **7**: 38%, **8**: 88%).

(2*S*,3*R*)-*tert*-Butyl 2-(3-iodobenzamido)-3-methylpentanoate (5): According to general procedure A, 3-iodo benzoic acid (**1**, 1.24 g, 5.0 mmol) was reacted with IleO*t*Bu (1.23 g, 5.5 mmol), HATU (2.09 g, 5.5 mmol) and DIEA (2.9 mL, 16.5 mmol) in DMF (20 mL). Purification by column chromatography (*i*-hexane/EtOAc, 0–100%) afforded **5** as a white semi-solid (1.96 g, 94%): ^{1}H NMR ($CDCl_3$): δ = 0.96 (d, J = 6.9 Hz, 3H), 0.95–1.00 (m, 3H), 1.22–1.31 (m, 1H), 1.45–1.58 (m, 1H), 1.49 (s, 9H), 1.94–2.02 (m, 1H), 4.68 (dd, J = 8.2 Hz, 4.4 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 7.74 (ddd, J = 7.8 Hz, 1.6 Hz, 1.1 Hz, 1H), 7.82 (ddd, J = 7.9 Hz, 1.7 Hz, 1.1 Hz, 1H), 8.12 ppm (t, J = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 11.8, 15.4, 25.5, 28.1, 38.5, 57.1, 82.4, 94.2, 126.1, 130.2, 136.1, 136.3, 140.5, 165.3, 171.1 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(4-methyl-3-iodobenzamido)-3-methylpentanoate (6): According to general procedure A, 3-iodo-4-methyl-benzoic acid (**2**, 1.31 g, 5.00 mmol) was reacted with IleO*t*Bu (1.23 g, 5.50 mmol), HATU (2.09 g, 5.50 mmol) and DIEA (2.90 mL, 16.50 mmol) in DMF (20 mL). Purification by column chromatography (*i*-hexane/EtOAc, 0–100%) afforded **6** as a white semi-solid (2.12 g, 98%): ^{1}H NMR ($CDCl_3$): δ = 0.93 (d, J = 6.9 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H), 1.18–1.32 (m, 1H), 1.46 (s, 9H), 1.47–1.55 (m, 1H), 1.95 (ddd, J = 9.0 Hz, 4.5 Hz, 2.1 Hz, 1H), 2.41 (s, 3H), 4.66 (dd, J = 8.3 Hz, 4.6 Hz, 1H), 6.79 (d, J = 7.8 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 7.61 (dd, J = 7.9 Hz, 1.8 Hz, 1H), 8.18 ppm (d, J = 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 11.6, 15.3, 25.5, 27.98, 28.02, 38.3, 57.0, 82.2, 100.8, 126.6, 129.4, 133.3, 137.5, 145.1, 165.0, 171.2 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(4-ethyl-3-iodobenzamido)-3-methylpentanoate (7): According to general procedure A, 3-iodo-4-ethyl-benzoic acid (**3**, 2.00 g, 7.25 mmol) was reacted with IleO*t*Bu (1.78 g, 7.97 mmol), HATU (3.03 g, 7.97 mmol) and DIEA (4.20 mL, 23.91 mmol) in DMF (25 mL). Purification by column chromatography (*i*-hexane/EtOAc, 0–100%) afforded **7** as a white semi-solid (1.20 g; 38%): ^{1}H NMR ($CDCl_3$): δ = 0.98 (t, J = 7.3 Hz, 6H), 1.22–1.34 (m, 1H), 1.29 (t, J = 7.5 Hz, 3H), 1.49 (s, 9H), 1.54 (ddd, J = 13.1 Hz, 7.6 Hz, 3.7 Hz, 1H), 1.99 (dddd, J = 11.4 Hz, 6.8 Hz, 4.5 Hz, 2.3 Hz, 1H), 2.95 (q, J = 7.5 Hz, 2H), 4.70 (dd, J = 8.2 Hz, 4.4 Hz, 1H), 6.79 (d, J = 7.9 Hz, 1H), 7.43 (dd, J = 8.1 Hz, 2.1 Hz, 1H), 7.94 (dt, J = 8.0 Hz, 1.7 Hz, 1H), 8.27 ppm (d, J = 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 11.8, 14.7, 15.4, 25.6, 26.1, 28.1, 38.4, 57.2, 82.6, 110.0, 123.2, 131.1, 131.6, 133.3, 142.3, 164.5, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(4-iodobenzamido)-3-methylpentanoate (8): According to general procedure A, 4-iodo-benzoic acid (**4**, 2.48 g, 10.0 mmol) was reacted with IleO*t*Bu (2.46 g, 10.0 mmol), HATU (4.18 g, 10.0 mmol) and DIEA (5.75 mL, 33.0 mmol) in DMF (30 mL). Purification by column chromatography (*i*-hexane/EtOAc, 0–100%) afforded **8** as a white semi-solid (3.69 g, 88%): ^{1}H NMR ($CDCl_3$): δ = 0.94 (d, J = 7.4 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.20–1.32 (m, 1H), 1.47 (s, 9H), 1.49–1.57 (m, 1H), 1.92–2.01 (m, 1H), 4.66 (dd, J = 8.2 Hz, 4.5 Hz, 1H), 6.74 (d, J = 8.1 Hz, 1H), 7.48–7.52 (m, 2H), 7.73–7.77 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 11.8, 15.3, 25.6, 28.1, 38.4, 57.1, 82.3, 98.5, 128.6, 133.7, 137.7, 166.1, 171.1 ppm.

General procedure B: Aminocarbonylation reactions: Iodoaryl O*t*Bu-Ile derivative (**5**–**8**; 1 equiv, 0.5–1.0 mmol), amine (3 equiv), $Pd(OAc)_2$ (0.1 equiv) and $Mo(CO)_6$ (1 equiv) were dissolved in tetrahydrofuran (THF; 2.5 mL mmol^{-1}) in a 2–5 mL Smith process vial. The mixture was stirred for 2 min at RT. Diazabicycloundecene (DBU; 3 equiv) was added, and the vial was immediately sealed and irradiated in a microwave reactor at 100 °C for 15 min. After cooling to RT, MeOH was added, and the suspension was filtered through a short plug of Celite®. The solvent was evaporated, and the crude mixture was purified by column chromatography (*i*-hexane/EtOAc, 0–100%), giving the desired products in yields between 14% and 85%.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(diethylcarbamoyl)benzamido)-3-methylpentanoate (14): According to general procedure B, reaction of derivative **5** afforded **14** as a colorless oil (45 mg, 24%): ^{1}H NMR ($CDCl_3$): δ = 0.93 (d, J = 6.9 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H), 1.02–1.15 (m, 3H), 1.16–1.30 (m, 4H), 1.45 (s, 9H), 1.43–1.56 (m, 1H), 1.95 (ddt, J = 11.4 Hz, 6.8 Hz, 2.2 Hz, 1H), 3.20 (s, 2H), 3.51 (s, 2H), 4.65 (dd, J = 8.2 Hz, 4.5 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H, CH_{Ar}), 7.47 (ddd, J = 7.6 Hz, 1.6 Hz, 1.1 Hz, 1H, CH_{Ar}), 7.76 (t, J = 1.6 Hz, 1H, CH_{Ar}), 7.80 ppm (dt, J = 7.3 Hz, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 11.7, 12.7, 14.1, 15.3, 25.5, 28.0, 38.3, 39.3, 43.3, 57.1, 82.2, 124.9, 127.7, 128.7, 129.2, 134.6, 137.6, 166.2, 170.2, 170.9 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(benzyl(methyl)carbamoyl)benzamido)-3-methylpentanoate (16): According to general procedure B, reaction of derivative **5** afforded **16** as a white semi-solid (165 mg, 75%): ^{1}H NMR ($CDCl_3$): δ = 0.90–1.00 (m, 6H), 1.18–1.32 (m, 1H), 1.48 (s, 9H), 1.46–1.58 (m, 1H), 1.93–2.04 (m, 1H), 2.84–3.08 (m, 3H), 4.43–4.73 (m, 2H), 4.76 (s br, 1H), 6.63–6.78 (m, 1H), 7.13–7.22 (m, 1H), 7.27–7.32 (m, 1H), 7.35 (s br, 3H), 7.41–7.53 (m, 1H), 7.58 (d, J = 7.5, 1H), 7.82–7.87 (m, 1H), 7.89 ppm (s br, 1H); ^{13}C NMR ($CDCl_3$): δ = 11.8, 15.4, 25.5, 28.1, 33.3, 37.0, 38.4, 50.8, 55.1, 57.1, 82.3, 125.7, 126.6, 127.6, 128.0, 128.2, 128.4, 128.7, 128.9, 130.0, 134.8, 136.8, 138.8, 166.1, 168.3, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(dibenzylcarbamoyl)benzamido)-3-methylpentanoate (19): According to general procedure B, reaction of derivative **5** afforded **19** as a white semi-solid (72 mg, 14%): ^{1}H NMR ($CDCl_3$): δ = 0.94 (d, J = 7.0 Hz, 3H), 0.95–1.00 (m, 3H), 1.17–1.30 (m, 1H), 1.46–1.58 (m, 1H), 1.49 (s, 9H), 1.92–2.02 (m, 1H), 4.39 (s br, 2H), 4.67 (dd, J = 8.3 Hz, 4.5 Hz, 1H), 4.68–4.82 (m, 2H), 6.67 (d, J = 8.2 Hz, 1H), 7.08–7.18 (m, 2H), 7.26–7.39 (m, 8H), 7.45 (t, J = 7.8 Hz, 1H), 7.59–7.64 (m, 1H), 7.83 (ddd, J = 7.8 Hz, 1.7 Hz, 1.2 Hz, 1H), 7.92 ppm (t, J = 1.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 11.8, 15.4, 25.5, 28.1, 38.4, 47.1, 51.5, 57.1, 82.3, 125.4, 126.9, 127.7, 128.2, 128.4, 128.7, 128.9, 129.6, 134.8, 136.1, 136.7, 166.0, 170.9, 171.3 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(benzyl(ethyl)carbamoyl)benzamido)-3-methylpentanoate (20): According to general procedure B, reaction of derivative **5** afforded **20** as a white semi-solid (97 mg, 43%): ^{1}H NMR ($CDCl_3$): δ = 0.92–1.00 (m, 6H), 1.02–1.15 (m, 1H), 1.15–1.27 (m, 3H), 1.44–1.56 (m, 1H), 1.47 (s, 9H), 1.91–2.01 (m, 1H), 3.18 (s br, 1H), 3.51 (s br, 1H), 4.46 (s, 1H), 4.66 (s br, 1H), 4.76 (s, 1H), 6.70 (d, J = 33.1 Hz, 1H), 7.10–7.38 (m, 5H), 7.50–7.38 (m, 1H), 7.54 (s, 1H), 7.79–7.89 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 11.7, 12.1, 13.6, 15.3, 25.5, 28.0, 38.4, 40.0, 42.9, 46.9, 52.1, 57.1, 82.2, 125.1, 126.6, 127.4, 127.9, 128.1, 128.4, 128.6, 128.8, 129.4, 133.1, 134.7, 137.1, 162.5, 166.1, 170.9 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(diethylcarbamoyl)-4-methylbenzamido)-3-methylpentanoate (25): According to general procedure B, reaction of derivative **6** afforded **25** as a white semi-solid (43 mg, 22%): ^{1}H NMR ($CDCl_3$): δ = 0.94 (d, J = 6.8 Hz, 3H), 0.95 (t, J = 7.2 Hz, 3H), 1.02 (t, J = 7.1 Hz, 3H), 1.17–1.23 (m, 1H), 1.25 (t, J = 7.1 Hz, 3H), 1.47 (s, 9H), 1.49–1.56 (m, 1H), 1.96 (ddt, J = 9.2 Hz, 6.8 Hz, 4.6 Hz, 1H), 2.32 (s, 3H), 3.06–3.14 (m, 2H), 3.22–3.81 (m, 2H), 4.66

(dd, J=8.3 Hz, 4.5 Hz, 1H), 6.65 (d, J=8.2 Hz, 1H), 7.26 (dd, J=8.0 Hz, 0.4 Hz, 1H), 7.59 (d, J=1.7 Hz, 1H), 7.68 ppm (dd, J=8.0 Hz, 1.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ=11.7, 12.8, 14.0, 15.3, 18.9, 25.5, 28.0, 38.4, 38.8, 42.7, 57.0, 82.2, 124.2, 127.1, 130.6, 132.1, 137.4, 138.0, 166.2, 169.8, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(benzyl(methyl)carbamoyl)-4-methylbenzamido)-3-methylpentanoate (27): According to general procedure B, reaction of derivative **6** afforded **27** as a white semi-solid (102 mg, 45%): ^{1}H NMR ($CDCl_3$): δ=0.91–1.00 (m, 6H), 1.18–1.34 (m, 1H), 1.49 (s, 9H), 1.51–1.58 (m, 1H), 1.92–2.03 (m, 1H), 2.36 (s, 3H), 2.70 (s, 2H), 3.08 (s, 1H), 4.35 (s, 1H), 4.68 (dd, J=8.3 Hz, 4.5 Hz, 1H), 4.78 (s, 1H), 6.67 (d, J=8.3 Hz, 1H), 7.09 (d, J=7.6 Hz, 1H), 7.25–7.36 (m, 3H), 7.36–7.39 (m, 2H), 7.66 (d, J=1.7 Hz, 1H), 7.71 ppm (t, J=7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ=11.8, 15.4, 19.0, 25.5, 28.1, 32.6, 35.7, 38.5, 50.2, 54.6, 57.0, 82.3, 124.6, 127.0, 127.6, 128.4, 128.7, 128.8, 130.9, 132.3, 136.0, 136.7, 138.0, 166.2, 170.5, 171.1 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(dibenzylcarbamoyl)-4-methylbenzamido)-3-methylpentanoate (30): According to general procedure B, reaction of derivative **6** afforded **30** as a white semi-solid (115 mg, 22%): ^{1}H NMR ($CDCl_3$): δ=0.93 (d, J=6.9 Hz, 3H), 0.97 (t, J=7.4 Hz, 3H), 1.16–1.29 (m, 1H), 1.49 (s, 9H), 1.50–1.56 (m, 1H), 1.91–1.99 (m, 1H), 2.36 (s, 3H), 4.22 (s, 2H), 4.29–4.57 (m, 1H), 4.64 (dd, J=8.3 Hz, 4.5 Hz, 1H), 4.87–5.34 (m, 1H), 6.59 (d, J=8.2 Hz, 1H), 7.05–7.10 (m, 2H), 7.26–7.39 (m, 9H), 7.67–7.71 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ=11.8, 15.3, 19.2, 25.5, 28.1, 38.4, 46.7, 50.9, 57.0, 82.2, 124.6, 127.2, 127.5, 127.7, 127.8, 128.7, 128.8, 128.8, 130.9, 132.1, 135.8, 136.4, 136.7, 138.5, 166.0, 170.96, 170.99 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(benzyl(ethyl)carbamoyl)-4-methylbenzamido)-3-methylpentanoate (31): According to general procedure B, reaction of derivative **6** afforded **31** as a white semi-solid (101 mg; 45%): ^{1}H NMR ($CDCl_3$): δ=0.90–1.00 (m, 7H), 1.12–1.33 (m, 3H), 1.48 (s, 9H), 1.50–1.58 (m, 1H), 1.92–2.02 (m, 1H), 2.36 (s, 3H), 3.01–3.10 (m, J=7.0 Hz, 2H), 4.31–4.55 (m, 2H), 4.68 (dd, J=8.2 Hz, 4.4 Hz, 1H), 6.67 (d, J=8.2 Hz, 1H), 7.10 (d, J=7.9 Hz, 1H), 7.21–7.41 (m, 5H), 7.60–7.70 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ=11.7, 12.2, 13.4, 15.3, 19.1, 25.5, 28.0, 38.4, 42.1, 51.5, 57.0, 82.2, 124.3, 127.0, 127.4, 127.7, 128.1, 128.6, 128.7, 130.6, 132.2, 136.3, 137.2, 138.1, 162.5, 166.0, 170.4 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(diethylcarbamoyl)-4-ethylbenzamido)-3-methylpentanoate (33): According to general procedure B, reaction of derivative **7** afforded **33** as a white semi-solid (100 mg, 48%): ^{1}H NMR ($CDCl_3$): δ=0.99–0.93 (m, 6H), 1.04 (t, J=7.1 Hz, 3H), 1.29–1.20 (m, 7H), 1.48 (s, 9H), 1.59–1.49 (m, 1H), 2.02–1.92 (m, 1H), 2.65 (q, J=7.6 Hz, 2H), 3.11 (q, J=6.7 Hz, 2H), 3.83–3.29 (m, 2H), 4.67 (dd, J=8.3 Hz, 4.5 Hz, 1H), 6.64 (d, J=8.2 Hz, 1H), 7.33 (d, J=8.1 Hz, 1H), 7.58 (s, 1H), 7.73 ppm (d, J=6.7 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ=11.8, 12.8, 14.0, 14.8, 15.4, 25.5, 25.9, 28.1, 38.5, 38.8, 42.9, 57.0, 82.2, 124.0, 127.0, 129.0, 132.1, 136.9, 144.1, 166.2, 169.8, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(benzyl(methyl)carbamoyl)-4-ethylbenzamido)-3-methylpentanoate (34): According to general procedure B, reaction of derivative **7** afforded **34** as a white semi-solid (155 mg, 66%): ^{1}H NMR ($CDCl_3$): δ=0.89–0.99 (m, 6H), 1.16–1.28 (m, 4H), 1.47 (s, 9H), 1.49–1.56 (m, 1H), 1.89–2.02 (m, 1H), 2.65 (q, J=7.5 Hz, 3H), 2.68 (s, 2H), 3.06 (s, 1H), 4.20–4.53 (m, 1H), 4.67 (dd, J=8.2 Hz, 4.5 Hz, 1H), 4.71–4.88 (m, 1H), 6.69 (d, J=7.8 Hz, 1H), 7.09 (d, J=7.9 Hz, 1H), 7.32 (m, 5H), 7.63 (d, J=1.8 Hz, 1H), 7.70–7.78 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): δ=11.7, 14.8, 15.3, 25.5, 25.9, 28.0, 32.6, 35.9, 38.4, 50.2, 54.7, 57.0, 82.2, 124.4, 126.9, 127.6, 127.7, 128.3, 128.6, 128.8, 129.0, 132.2, 136.0, 136.7, 144.2, 166.0, 170.4, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(benzyl(ethyl)carbamoyl)-4-ethylbenzamido)-3-methylpentanoate (35): According to general procedure B, reaction of derivative **7** afforded **35** as a white semi-solid (120 mg, 50%): ^{1}H NMR ($CDCl_3$): δ=0.93–1.04 (m, 9H), 1.19–1.33 (m, 4H), 1.49 (s, 9H), 1.54 (ddd, J=13.0 Hz, 7.5 Hz, 5.1 Hz, 1H), 1.93–2.02 (m, 1H), 2.68 (q, J=7.5 Hz, 2H), 3.05 (q, J=7.1 Hz, 2H), 4.32 (d, J=7.9 Hz, 2H), 4.68 (dd, J=8.3 Hz, 4.5 Hz, 1H), 6.67 (d, J=8.3 Hz, 1H), 7.11 (d, J=7.3 Hz, 1H), 7.21–7.41 (m, 7H), 7.59–7.67 (m, 1H), 7.75 ppm (d, J=7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ=11.8, 13.3, 15.0, 15.4, 25.5, 26.0, 28.1, 38.4, 42.2, 51.7, 57.0, 82.2, 124.2, 127.0, 127.5, 127.7, 128.3, 128.6, 128.8, 129.1, 132.1, 136.3, 137.3, 144.2, 166.0, 170.4, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(3-(dibenzylcarbamoyl)-4-ethylbenzamido)-3-methylpentanoate (36): According to general procedure B, reaction of derivative **7** afforded **36** as a white semi-solid (339 mg, 62%): ^{1}H NMR ($CDCl_3$): δ=0.89–0.94 (m, 3H), 0.96 (t, J=7.4 Hz, 3H), 1.15–1.27 (m, 1H), 1.23 (t, J=7.6 Hz, 3H), 1.47–1.55 (m, 1H), 1.49 (s, 9H), 1.94 (s, 1H), 2.61–2.75 (m, 2H), 4.10–4.27 (m, 2H), 4.27–4.45 (m, 1H), 4.64 (dd, J=8.3 Hz, 4.5 Hz, 1H), 5.04–5.27 (m, 1H), 6.57 (d, J=8.0 Hz, 1H), 7.07–7.11 (m, 2H), 7.24–7.39 (m, 9H), 7.68 (d, J=1.9 Hz, 1H), 7.75 ppm (dd, J=8.0 Hz, 1.9 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ=11.8, 15.0, 15.4, 25.5, 26.1, 28.1, 38.4, 46.6, 51.0, 57.0, 82.2, 124.5, 127.1, 127.7, 127.8, 128.7, 128.8, 128.9, 129.2, 132.1, 135.8, 136.0, 136.7, 144.5, 166.0, 170.9, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(4-(diethylcarbamoyl)benzamido)-3-methylpentanoate (65): According to general procedure B, reaction of derivative **8** afforded **65** as a white semi-solid (133 mg, 68%): ^{1}H NMR ($CDCl_3$): δ=0.94–0.99 (m, 6H), 1.05–1.14 (m, 3H), 1.22–1.27 (m, 3H), 1.28–1.35 (m, 1H), 1.48 (s, 9H), 1.49–1.61 (m, 1H), 1.94–2.02 (m, 1H), 3.14–3.26 (m, 2H), 3.49–3.58 (m, 2H), 4.69 (dd, J=8.2 Hz, 4.4 Hz, 1H), 6.71 (d, J=7.6 Hz, 1H), 7.40–7.46 (m, 2H), 7.80–7.85 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ=11.8, 14.2, 15.4, 25.5, 28.1, 38.5, 39.4, 43.2, 57.1, 82.4, 126.5, 127.2, 134.9, 140.4, 166.3, 170.3, 171.1 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(4-(benzyl(methyl)carbamoyl)benzamido)-3-methylpentanoate (66): According to general procedure B, reaction of derivative **8** afforded **66** as a white semi-solid (160 mg, 73%): ^{1}H NMR ($CDCl_3$): δ=0.94 (s br, 6H), 1.18–1.32 (m, 1H), 1.46 (s, 9H), 1.49–1.63 (m, 1H), 1.91–2.02 (m, 1H), 2.80 (s, 2H), 3.02 (s, 1H), 4.44 (s, 1H), 4.68 (s br, 1H), 4.73 (s, 1H), 6.78 (s br, 1H), 7.12 (d, J=6.6 Hz, 1H), 7.37–7.25 (m, 4H), 7.48 (t, J=6.4 Hz, 2H), 7.74–7.85 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ=11.7, 15.3, 25.5, 28.0, 33.2, 36.8, 38.4, 50.7, 54.9, 57.1, 82.3, 126.5, 126.9, 127.2, 127.7, 128.2, 128.7, 128.8, 135.3, 136.1, 139.1, 166.1, 170.5, 171.0 ppm.

(2*S*,3*R*)-*tert*-Butyl 2-(4-(dibenzylcarbamoyl)benzamido)-3-methylpentanoate (67): According to general procedure B, reaction of derivative **8** afforded **67** as a white semi-solid (40 mg, 16%): ^{1}H NMR ($CDCl_3$): δ=0.96 (d, J=7.0 Hz, 3H), 0.97 (t, J=7.6 Hz, 3H), 1.21–1.30 (m, 1H), 1.48 (s, 9H), 1.50–1.58 (m, 1H), 1.98 (ddt, J=9.2 Hz, 4.7 Hz, 2.4 Hz, 1H), 4.72 (s, 2H), 4.36 (s, 2H), 4.68 (dd, J=8.2 Hz, 4.4 Hz, 1H), 6.71 (d, J=8.2 Hz, 1H), 7.12 (d, J=6.6 Hz, 2H), 7.27–7.39 (m, 8H), 7.53–7.57 (m, 2H), 7.79–7.83 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ=11.8, 15.4, 25.5, 28.1, 38.5, 47.0, 51.4, 57.1, 82.4, 126.8, 126.9, 127.3, 127.7, 127.8, 128.5, 128.7, 128.9, 135.4, 136.0, 136.7, 139.2, 166.0, 171.1, 171.3 ppm.

General procedure C: Hydrolysis of *tert*-butyl esters: The ester (1 equiv, 0.08–0.6 mmol) was dissolved in CH_2Cl_2 (15 μL/μmol). Trifluoroacetic acid (TFA; 7.7 μL/μmol) added and the mixture was

stirred at RT for 3 h. The solvent was evaporated, and the crude mixture was purified using HPLC (MeCN/H_2O, 0–100%). The pure products were isolated in 46–96% yield.

(2*S*,3*R*)-2-(3-(Diethylcarbamoyl)benzamido)-3-methylpentanoic acid (42): According to general procedure C, reaction of ester **14** gave **42** as a white semi-solid (31 mg; 73%): $[\alpha]_{589}^{25} = +10.3$; ^{1}H NMR ($CD_3OD$): $\delta = 0.97$ (t, $J = 7.4$ Hz, 3H), 1.03 (d, $J = 6.8$ Hz, 3H), 1.14 (t, $J = 6.4$ Hz, 3H), 1.27 (t, $J = 6.4$ Hz, 3H), 1.30–1.41 (m, 1H), 1.56–1.68 (m, 1H), 1.98–2.10 (m, 1H), 3.25–3.37 (m, 2H), 3.57 (d, $J = 6.8$ Hz, 2H), 4.57 (d, $J = 6.3$ Hz, 1H), 7.56 (dt, $J = 5.8$ Hz, 3.2 Hz, 2H), 7.86 (s, 1H), 7.91–7.97 ppm (m, 1H); ^{13}C NMR (CD_3OD): $\delta =$ 11.7, 13.1, 14.4, 16.1, 26.6, 38.1, 41.0, 45.0, 59.0, 126.5, 129.6, 130.0, 130.4, 135.9, 138.3, 169.4, 172.6, 174.9 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{26}N_2O_4$: 335.1971, found: 335.1972.

(2*S*,3*R*)-2-(3-(Benzyl(methyl)carbamoyl)benzamido)-3-methylpentanoic acid (44): According to general procedure C, reaction of ester **16** gave **44** as a white semi-solid (51 mg, 65%): $[\alpha]_{589}^{25} = +8.0$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.3$ Hz, 3H), 1.03 (d, $J = 6.9$ Hz, 3H), 1.25–1.40 (m, 1H), 1.54–1.68 (m, 1H), 1.97–2.08 (m, 1H), 2.89 (s, 2H), 3.02 (s, 1H), 4.52 (s br, 1H), 4.57 (d, $J = 6.0$ Hz, 1H), 4.76 (s br, 1H), 7.17 (d, $J = 6.8$ Hz, 1H), 7.25–7.42 (m, 4H), 7.49–7.59 (m, 1H), 7.62 (d, $J = 6.5$ Hz, 1H), 7.89–7.98 ppm (m, 2H); ^{13}C NMR (CD_3OD): $\delta =$ 11.8, 16.3, 26.7, 33.9, 37.7, 38.3, 52.0, 56.3, 59.1, 127.2, 128.1, 128.8, 129.3, 129.8, 130.0, 130.1, 131.1, 136.1, 137.8, 138.2, 169.6, 173.0, 175.0 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{26}N_2O_4$: 383.1971, found: 383.1974.

(2*S*,3*R*)-2-(3-(Dibenzylcarbamoyl)benzamido)-3-methylpentanoic acid (47): According to general procedure C, reaction of ester **19** gave **47** as a white semi-solid (37 mg, 96%): $[\alpha]_{589}^{25} = +5.5$; ^{1}H NMR ($CD_3OD$): $\delta = 0.95$ (t, $J = 7.4$ Hz, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 1.23–1.37 (m, 1H), 1.59 (dqd, $J = 14.9$ Hz, 7.5 Hz, 4.3 Hz, 1H), 1.95- 2.08 (m, 1H), 4.44 (s br, 2H), 4.55 (d, $J = 6.3$ Hz, 1H), 4.63–4.77 (m, 2H), 7.14 (s, 2H), 7.29–7.44 (m, 8H), 7.52 (t, $J = 7.7$ Hz, 1H), 7.64 (dt, $J =$ 7.7 Hz, 1.4 Hz, 1H), 7.89–7.93 ppm (m, 1H), 7.94 (t, $J = 1.5$ Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 16.3, 26.7, 38.3, 48.7, 53.3, 59.1, 127.0, 128.3, 128.9, 129.0, 129.5, 130.0, 130.1, 130.16, 130.21, 130.8, 136.2, 137.5, 137.7, 138.1, 169.6, 173.8, 175.0 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{30}N_2O_4$: 459.2284; found: 459.2287.

(2*S*,3*R*)-2-(3-(Benzyl(ethyl)carbamoyl)benzamido)-3-methylpentanoic acid (48): According to general procedure C, reaction of ester **20** gave **48** as a white semi-solid (47 mg, 64%): $[\alpha]_{589}^{25} = +6.4$; ^{1}H NMR ($CD_3OD$): $\delta = 0.97$ (t, $J = 7.4$ Hz, 3H), 1.03 (d, $J = 6.7$ Hz, 3H), 1.06–1.25 (m, 2H), 1.27–1.39 (m, 1H), 1.54–1.69 (m, 1H), 1.97–2.09 (m, 1H), 3.22–3.29 (m, 1H), 3.45–3.60 (m, 1H), 4.54 (s br, 1H), 4.56 (d, $J = 6.4$ Hz, 1H), 4.80 (s, 1H), 7.16–7.43 (m, 5H), 7.49–7.65 (m, 2H), 7.88–7.98 ppm (m, 2H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 12.7, 14.1, 16.3, 26.8, 38.3, 41.8, 44.9, 48.9, 53.7, 59.2, 126.7, 128.2, 128.7, 128.9, 129.2, 129.9, 130.1, 130.2, 130.6, 136.2, 138.1, 138.7, 169.6, 173.4, 175.1 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}N_2O_4$: 397.2122, found: 397.2122.

(2*S*,3*R*)-2-(3-(Diethylcarbamoyl)-4-methylbenzamido)-3-methylpentanoic acid (53): According to general procedure C, reaction of ester **25** gave **53** as a white semi-solid (30 mg, 72%): $[\alpha]_{589}^{25} = +9.7$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.4$ Hz, 3H), 1.02 (d, $J = 6.8$ Hz, 3H), 1.07 (t, $J = 7.1$ Hz, 3H), 1.29 (t, $J = 7.1$ Hz, 3H), 1.31–1.39 (m, 1H), 1.56–1.68 (m, 1H), 1.08–2.08 (m, 1H), 2.34 (s, 3H), 3.08–3.28 (m, 2H), 3.44–3.77 (m, 2H), 4.55 (d, $J = 6.5$ Hz, 1H), 7.39 (d, $J = 8.0$ Hz, 1H), 7.70 (s, 1H), 7.83 ppm (dd, $J = 8.0$ Hz, 1.8 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 13.2, 14.3, 16.3, 19.1, 26.8, 38.2, 40.7, 44.7, 59.0, 126.0, 129.3, 131.9, 133.3, 138.2, 139.5, 169.5, 172.4, 175.1 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{28}N_2O_4$: 349.2127, found: 349.2136.

(2*S*,3*R*)-2-(3-(Benzyl(methyl)carbamoyl)-4-methylbenzamido)-3-methylpentanoic acid (55): According to general procedure C, reaction of ester **27** gave **55** as a white semi-solid (46 mg, 68%): $[\alpha]_{589}^{25} = +7.3$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.4$ Hz, 3H), 1.02 (d, $J = 6.9$ Hz, 3H), 1.24–1.39 (m, 1H), 1.54–1.67 (m, 1H), 1.97–2.07 (m, 1H), 2.32 (s, 3H), 2.75 (s, 2H), 3.08 (s, 1H), 4.39 (s br, 1H), 4.56 (d, $J = 6.7$ Hz, 1H), 4.66–4.87 (m, 1H), 7.12 (d, $J = 7.1$ Hz, 1H), 7.23–7.35 (m, 2H), 7.35–7.44 (m, 3H), 7.71–7.78 (m, 1H), 7.80–7.86 ppm (m, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 16.3, 19.2, 26.8, 33.5, 36.6, 38.2, 51.4, 55.8, 59.1, 126.2, 128.5, 129.0, 129.4, 129.5, 130.0, 130.1, 131.9, 133.6, 137.6, 138.2, 139.5, 169.5, 173.0, 175.1 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}N_2O_4$: 397.2127; found: 397.2117.

(2*S*,3*R*)-2-(3-(Dibenzylcarbamoyl)-4-methylbenzamido)-3-methylpentanoic acid (58): According to general procedure C, reaction of ester **30** gave **58** as a white semi-solid (67 mg, 82%): $[\alpha]_{589}^{25} = +5.5$; ^{1}H NMR ($CD_3OD$): $\delta = 0.95$ (t, $J = 7.4$ Hz, 3H), 0.99 (d, $J = 6.8$ Hz, 3H), 1.23–1.35 (m, 1H), 1.58 (ddt, $J = 11.7$ Hz, 7.5 Hz, 4.3 Hz, 1H), 1.96–2.05 (m, 1H), 2.29 (s, 3H), 4.28 (s, 2H), 4.54 (d, $J = 6.4$ Hz, 1H), 4.92 (s br, 2H), 7.04–7.07 (m, 2H), 7.20–7.44 (m, 9H), 7.78 (s, 1H), 7.81 ppm (dd, $J = 7.9$ Hz, 1.9 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 16.3, 19.4, 26.7, 38.3, 48.8, 52.8, 59.0, 126.4, 128.7, 129.0, 129.1, 129.5, 129.8, 129.95, 130.02, 132.1, 133.3, 137.2, 137.5, 138.1, 134.0, 169.3, 173.5, 175.0 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{32}N_2O_4$: 473.2440; found: 473.2445.

(2*S*,3*R*)-2-(3-(Benzyl(ethyl)carbamoyl)-4-methylbenzamido)-3-methylpentanoic acid (59): According to general procedure C, reaction of ester **31** gave **59** as a white semi-solid (48 mg, 71%): $[\alpha]_{589}^{25} = +6.4$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.4$ Hz, 3H), 0.99 (d, $J = 6.9$ Hz, 3H), 1.01–1.26 (m, 3H), 1.26–1.39 (m, 1H), 1.54–1.67 (m, 1H), 1.95–2.07 (m, 1H), 4.40 (s br, 1H), 4.55 (d, $J = 6.5$ Hz, 1H), 2.34 (s, 3H), 3.07–3.40 (m, 2H), 4.69–4.96 (m, 1H), 7.11–7.16 (m, 1H), 7.25–7.46 (m, 5H), 7.69–7.77 (m, 1H), 7.82 ppm (ddd, $J = 13.8$ Hz, 8.0 Hz, 1.9 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 12.7, 13.8, 16.3, 19.3, 26.8, 38.4, 41.4, 44.3, 48.3, 53.2, 59.3, 126.1, 128.6, 128.9, 129.0, 129.3, 129.5, 129.9, 130.0, 132.0, 133.5, 137.9, 138.8, 139.7, 169.3, 173.0, 175.3 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{30}N_2O_4$: 411.2278, found: 411.2289.

(2*S*,3*R*)-2-(3-(Diethylcarbamoyl)-4-ethylbenzamido)-3-methylpentanoic acid (61): According to general procedure C, reaction of ester **33** gave **61** as a white semi-solid (31 mg, 58%): $[\alpha]_{589}^{25} = +9.7$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.4$ Hz, 3H), 1.02 (d, $J = 6.9$ Hz, 3H), 1.08 (t, $J = 7.1$ Hz, 3H), 1.25 (t, $J = 7.6$ Hz, 3H), 1.28 (t, $J = 7.1$ Hz, 3H), 1.31–1.39 (m, 1H), 1.62 (ddq, $J = 14.9$ Hz, 7.5 Hz, 4.3 Hz, 1H), 1.97–2.08 (m, 1H), 2.61–2.72 (m, 2H), 3.09–3.27 (m, 2H), 3.39–3.56 (m, 1H), 3.71 (s br, 1H), 4.56 (d, $J = 6.5$ Hz, 1H), 7.45 (d, $J = 8.1$ Hz, 1H), 7.69 (s, 1H), 7.88 ppm (dd, $J = 8.1$ Hz, 2.0 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 13.1, 14.3, 15.4, 16.3, 26.8, 27.1, 38.3, 40.6, 44.8, 59.1, 126.1, 129.5, 130.4, 133.3, 137.7, 145.6, 169.4, 172.4, 175.0 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{30}N_2O_4$: 363.2278, found: 363.2278.

(2*S*,3*R*)-2-(3-(Benzyl(methyl)carbamoyl)-4-ethylbenzamido)-3-methylpentanoic acid (62): According to general procedure C, reaction of ester **34** gave **62** as a white semi-solid (45 mg, 49%): $[\alpha]_{589}^{25} = +4.2$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.4$ Hz, 3H), 1.02 (d, $J = 6.9$ Hz, 3H), 1.18–1.28 (m, 3H), 1.28–1.39 (m, 1H), 1.54–1.67 (m, 1H), 1.97–2.08 (m, 1H), 2.60–2.73 (m, 2H), 2.76 (s, 2H), 3.08 (s, 1H), 4.38 (s, 1H), 4.55 (d, $J = 6.5$ Hz, 1H), 4.60–4.80 (m, 1H), 7.11–7.16 (m, 1H), 7.23–7.36 (m, 2H), 7.37–7.47 (m, 3H), 7.72 (t, $J = 7.5$ Hz, 1H), 7.87 ppm (dt, $J = 8.1$ Hz, 1.8 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta =$

11.8, 15.5, 16.3, 26.8, 27.1, 33.5, 37.0, 38.2, 51.5, 56.0, 59.1, 126.4, 128.5, 129.0, 129.6, 129.8, 130.0, 130.1, 130.5, 133.6, 137.4, 138.2, 145.7, 169.6, 173.0, 175.1 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{24}H_{30}N_2O_4$: 411.2284; found: 411.2276.

(2*S*,3*R*)-2-(3-(Benzyl(ethyl)carbamoyl)-4-ethylbenzamido)-3-methylpentanoic acid (63): According to general procedure C, reaction of ester **35** gave **63** as a white semi-solid (46 mg, 52%): $[\alpha]_{589}^{25} = +5.7$; ^{1}H NMR ($CD_3OD$): $\delta = 0.95$ (t, $J = 7.4$ Hz, 3H), 1.02 (t, $J = 7.1$ Hz, 3H), 1.20 (t, $J = 7.6$ Hz, 3H), 1.24 (t, $J = 7.6$ Hz, 3H), 1.27–1.38 (m, 1H), 1.51–1.66 (m, 1H), 1.93–2.08 (m, 1H), 2.59–2.70 (m, 2H), 3.04–3.17 (m, 1H), 3.61–3.86 (m, 1H), 4.36 (d, $J = 4.6$ Hz, 1H), 4.54 (d, $J = 6.5$ Hz, 1H), 4.57–5.03 (m, 1H), 7.10–7.16 (m, 1H), 7.21–7.33 (m, 2H), 7.34–7.45 (m, 3H), 7.67–7.75 (m, 1H), 7.86 ppm (dd, $J = 8.1$ Hz, 2.0 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 12.6, 15.5, 16.3, 26.8, 27.2, 38.3, 41.3, 44.4, 48.1, 53.4, 59.1, 126.2, 128.6, 128.9, 129.0, 129.5, 129.9, 130.0, 130.5, 133.4, 137.4, 138.8, 145.7, 169.3, 173.0, 175.1 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{25}H_{32}N_2O_4$: 425.2440; found: 425.2442.

(2*S*,3*R*)-2-(3-(Dibenzylcarbamoyl)-4-ethylbenzamido)-3-methylpentanoic acid (64): According to general procedure C, reaction of ester **36** gave **64** as a white semi-solid (138 mg, 46%): $[\alpha]_{589}^{25} = +5.5$; ^{1}H NMR ($CD_3OD$): $\delta = 0.96$ (t, $J = 7.4$ Hz, 3H), 0.98–1.03 (m, 3H), 1.20 (t, $J = 7.6$ Hz, 3H), 1.23–1.36 (m, 1H), 1.53–1.65 (m, 1H), 1.95–2.06 (m, 1H), 2.63 (q, $J = 7.4$ Hz, 2H), 4.29 (s, 2H), 4.33–4.49 (m, 1H), 4.52 (d, $J = 6.3$ Hz, 1H), 4.90–5.22 (m, 1H), 7.10 (d, $J = 7.2$ Hz, 2H), 7.22–7.41 (m, 8H), 7.43 (d, $J = 8.1$ Hz, 1H), 7.76 (s br, 1H), 7.85 ppm (dd, $J = 8.1$ Hz, 1.9 Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.9$, 15.5, 16.3, 26.7, 27.2, 38.5, 48.7, 53.0, 59.3, 126.2, 128.7, 129.05, 129.09, 129.7, 129.9, 130.0, 130.1, 130.6, 133.5, 137.0, 137.2, 138.2, 146.0, 169.2, 173.5, 175.5 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{34}N_2O_4$: 487.2591, found: 487.2606.

(2*S*,3*R*)-2-(4-(Diethylcarbamoyl)benzamido)-3-methylpentanoic acid (68): According to general procedure C, reaction of ester **65** gave **68** as a white semi-solid (70 mg, 74%): $[\alpha]_{589}^{25} = +15.4$; ^{1}H NMR ($CD_3OD$): $\delta = 0.97$ (t, $J = 7.4$ Hz, 3H), 1.04 (d, $J = 6.9$ Hz, 3H), 1.12 (t, $J = 7.0$ Hz, 3H), 1.26 (t, $J = 7.0$ Hz, 3H), 1.30–1.41 (m, 1H), 1.63 (ddq, $J = 14.9$ Hz, 7.5 Hz, 4.3 Hz, 1H), 1.98–2.11 (m, 1H), 3.27 (q, $J = 7.0$ Hz, 2H), 3.56 (q, $J = 6.9$ Hz, 2H), 4.58 (d, $J = 6.4$ Hz, 1H), 7.44–7.49 (m, 2H), 7.90–7.94 ppm (m, 2H); ^{13}C NMR (CD_3OD): $\delta =$ 11.9, 13.2, 14.5, 16.3, 26.7, 38.3, 41.0, 45.0, 59.0, 127.5, 129.1, 136.6, 141.2, 169.7, 172.7, 174.9 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{26}N_2O_4$: 335.1965, found: 335.1967.

(2*S*,3*R*)-2-(4-(Benzyl(methyl)carbamoyl)benzamido)-3-methylpentanoic acid (69): According to general procedure C, reaction of ester **66** gave **69** as a white semi-solid (42 mg, 88%): $[\alpha]_{589}^{25} = +13.2$; ^{1}H NMR ($CD_3OD$): $\delta = 0.90$–0.98 (m, 3H), 0.98–1.05 (m, 3H), 1.25–1.38 (m, 1H), 1.60 (dt, $J = 12.4$ Hz, 7.5 Hz, 1H), 1.96–2.06 (m, 1H), 2.87 (s, 2H), 3.02 (s, 1H), 4.49–4.53 (m, 1H), 4.55 (d, $J = 6.5$ Hz, 1H), 4.75 (s br, 1H), 7.16 (d, $J = 7.4$ Hz, 1H), 7.24–7.39 (m, 4H), 7.53 (d, $J = 8.1$ Hz, 2H), 7.86 (d, $J = 7.9$ Hz, 1H), 7.92 ppm (d, $J = 8.1$ Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 11.8$, 16.3, 26.7, 33.8, 37.7, 38.3, 51.9, 56.2, 59.1, 128.1, 128.2, 128.9, 129.0, 129.1, 129.3, 130.0, 130.1, 137.0, 137.7, 138.2, 140.5, 169.8, 173.0, 175.0 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{26}N_2O_4$: 383.1965, found: 383.1968.

(2*S*,3*R*)-2-(4-(Dibenzylcarbamoyl)benzamido)-3-methylpentanoic acid (70): According to general procedure C, reaction of ester **67** gave **70** as a white semi-solid (20 mg, 57%): $[\alpha]_{589}^{25} = +11.9$; ^{1}H NMR ($CD_3OD$): $\delta = 0.95$ (t, $J = 7.4$ Hz, 3H), 1.01 (d, $J = 6.9$ Hz, 3H), 1.25–1.38 (m, 1H), 1.60 (ddq, $J = 14.9$ Hz, 7.5 Hz, 4.3 Hz, 1H), 1.95–2.07 (m, 1H), 4.43 (s br, 2H), 4.54 (d, $J = 6.3$ Hz, 1H), 4.71 (s br, 2H), 7.14 (d, $J = 6.9$ Hz, 2H), 7.26–7.40 (m, 8H), 7.54–7.59 (m, 2H), 7.86–7.91 ppm (m, 2H); ^{13}C NMR (CD_3OD): $\delta = 11.9$, 16.3, 26.7, 38.4, 48.7, 53.2, 59.2, 127.9, 128.3, 128.9, 129.2, 129.4, 130.0, 130.1, 137.1, 137.5, 138.1, 140.4, 169.7, 173.8, 175.2 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{30}N_2O_4$: 459.2278, found: 459.2289.

Biology

Cell cultures: The human embryonic kidney 293 (HEK-293) cell line stably expressing human Flag-AT_1 receptor was a generous gift from Dr. Richard Leduc (Department of Pharmacology, Université de Sherbrooke, Sherbrooke, Quebec, Canada) and prepared as previously described.[23] The native 293/FRT cell line (HEK-293 cell line with single genome-integrated *Flp* recombinase target site (FRT)) was maintained in high-glucose DMEM with 7% FBS, 2 mM GlutaMAX and 100 μg mL^{-1} zeocin.

The stable cell line stably expressing the human AT_2 receptor was established as described previously.[24] First, the forward primer containing a *Hind*III restriction site and a Myc epitope (ttaaacttaagcttaccatggaacaaaaactcatctcagaagaggatctgatgaagggcaactccacc) was used with a reverse primer containing a *Sal*I site (agcaagcaagacacatgtcgacttaagacacaaaggtctcc) with the Expand High Fidelity^{PLUS} PCR System (Roche) to create a *Hind*III-Myc-AT_2 receptor-*Sal*I fragment, which was cloned into pcDNA5/FRT between its *Hind*III and *Xho*I sites, thus creating the pcDNA5/FRT/Myc-AT_2 R vector. Then, the cell line stably expressing Myc-human AT_2 receptor (293/FRT/Myc-hAT_2R) was generated by *Flp* recombinase-mediated homologous recombination system (Flp-In^{TM}) and was maintained with 100 μg mL^{-1} hygromycin B.[24]

Binding experiments

First radioligand binding assay: This study was performed at Cerep (France) according to literature.[16–17] The assays were performed in HEK-293 cells transfected with recombinant human AT_2R or AT_1R and relying on the displacement of [^{125}I]CGP-42112 A (AT_2R)[16] and [^{125}I][Sar^1, Ile^8]Ang II (AT_1R)[17] with radiolabeled Ang II as reference compound in the AT_2R assay and radiolabeled saralasin [Sar^1, Val^5, Ile^8]Ang II in the AT_1R assay. Unlabeled Ang II was used for nonspecific binding in both the AT_2R (1 μM) and AT_1R (10 μM) assay. In the initial screen the % inhibition of [^{125}I]CGP-42112 A (AT_2R) or [^{125}I][Sar^1, Ile^8]Ang II (AT_1R) binding was measured at 1 and 10 μM of the compounds. The K_i values were determined from at least six data points with test concentrations ranging from 30 pM to 1 mM. The concentration range was adjusted to be appropriate for the expected K_i values.

Second radioligand binding assay: Binding studies were conducted in HEK-293 transfected cells with human AT_1R or AT_2R and were performed as recently described.[8e] Briefly, the analogue [Sar^1, Ile^8]Ang II was iodinated by the Iodogen method, and binding assays were performed on cultured cells. The hormone binding reaction was initiated by addition of 0.1 nM of [^{125}I][Sar^1, Ile^8]Ang II (1000 Ci mmol^{-1}) to each Petri dish (1.0×10^6 cells/Petri dish) either alone (total binding) or in the presence of increasing concentrations of Ang II or the ligands under investigation, including 10 μM Ang II for nonspecific binding (which represents less than 10% of total binding). Incubations were performed in duplicate for 30 min at RT (22 °C). After incubation, cells were rapidly detached from the substratum with a rubber policeman; cells and media were filtered through Whatman GF/C filters (presoaked overnight in 2% BSA), rinsed three times and counted in a Beckman γ-counter. The endogenous ligand Ang II, the selective nonpeptide AT_1 antagonist losartan, the selective AT_2 agonist CGP42112 A, and the selective

nonpeptide AT_2 antagonist PD 123,319 were used as reference compounds for the binding studies. In radioligand binding experiments, IC_{50} values were obtained by fitting radioligand competition data to a sigmoidal function by use of a nonlinear least-squares program (GraphPad Software Inc., San Diego, CA). K_i values were determined using the Cheng–Prusoff equation: $K_i = IC_{50}/(1+H/K_D)$ where H is the radioligand concentration and K_D is the K_D value for the radioligand.

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