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Aminoalkoxybenzyl pyrrolidines as novel human urotensin-II receptor antagonists

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Abstract—High throughput screening of the corporate compound collection led to the discovery of a novel series of substituted aminoalkoxybenzyl pyrrolidines as human urotensin-II receptor antagonists. The synthesis, initial structure–activity relationships, and optimization of the initial hit that led to the identification of a truncated sub-series, represented by SB-436811 (**1a**), are described.

Urotensin-II (U-II), a cyclic undecapeptide, was first isolated in the 1960s from goby urophysis[1] where it has been proposed to be involved primarily in osmoregulation.[2] Human urotensin-II (hU-II) and other orthologs including mouse, rat, and monkey were subsequently identified and cloned.[3] In 1999, hU-II was identified as a cognate ligand of human GPR-14 (hUT), an ''orphan'' 7TM receptor predominantly expressed in the cardiovascular tissue.[4] hU-II was found within vascular and cardiac tissue and effectively constricted isolated arteries from non-human primates.[4] The potency of hU-II as a vasoconstrictor was 10-times greater than that of endothelin-1, making hU-II the most potent mammalian vasoconstrictor identified to date.[4] More recently, hU-II was found to induce profound cardiohemodynamic effects upon systemic administration in cat[5] and in human.[6] It also influenced the cardiorenal function by acting as a potent regulator of cardiac contractility[7] and as a natriuretic factor.[8] hU-II and hUT are therefore proposed to be involved in the (dys)regulation of cardiorenal function[9], and have been implicated in the etiology of numerous cardiorenal and metabolic diseases including hypertension,[10] heart failure,[7,11] atherosclerosis,[12] renal failure,[13] and diabetes.[14]

Several non-peptidic UT ligands have recently been reported.[15] Herein, we describe the identification, synthesis, and initial structure–activity relationships (SAR) of a novel series of substituted 3-amino-*N*-(alkoxybenzyl)pyrrolidines as hUT antagonists. Optimization of the initial screening hit ultimately led to the identification of a truncated sub-series, represented by SB-436811 (**1a**).

High throughput screening (HTS) of our in-house compound collection using a fluorometric imaging plate reader (FLIPR) assay (measuring the inhibition of

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hU-II-mediated $[Ca^{2+}]_i$ mobilization in HEK293 cells expressing human recombinant UT receptor)[16] led to the identification of SB-328872 (**2a**), a single diastereoisomer (vide infra), as an antagonist with a pIC_{50} of 6.2 (Fig. 1). The compound was subsequently found to have good hUT receptor binding affinity with a pK_i of 6.9 in a [^{125}I]hU-II radioligand binding assay using HEK293 cell membranes stably expressing human recombinant UT receptors.[16,17] For comparison, hU–II had a pEC_{50} of 9.2 in the FLIPR assay and a pK_i of 8.7 in the radioligand binding assay.[4]

SB-328872 (**2a**) and its analogs were prepared using a general solid-phase synthetic route outlined in Scheme 1.[18] Optically pure 3(*S*)- or 3(*R*)-(*tert*-butoxycarbonyl-amino)pyrrolidine (**3**)[19] was converted to the corresponding nosyl-protected diamine (**4**) HCl salts in two steps. The diamines **4** were reacted with commercially available 2,6-dimethoxy-4-polystyrenebenzyloxy-benzaldehyde resin (DMHB resin)[20] via reductive amination to afford resin-bound amines **5**. Amines **5** were coupled with various optically pure L- or D-Fmoc-protected amino acids,[21] followed by Fmoc removal, to produce resin-bound amines **6**. Intermediates **6** were then coupled with various acids, followed by nosyl-group removal, to afford resin-bound intermediates **7**. Reductive amination of intermediates **7** and subsequent resin cleavage produced the targeted compounds **2** in good yield and purity. This solid-phase synthesis was readily amenable to rapid array-based optimization. Using the synthetic route, multiple regions of this chemical series were explored to improve potency and demonstrate tractable SAR.

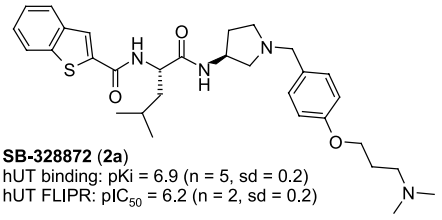
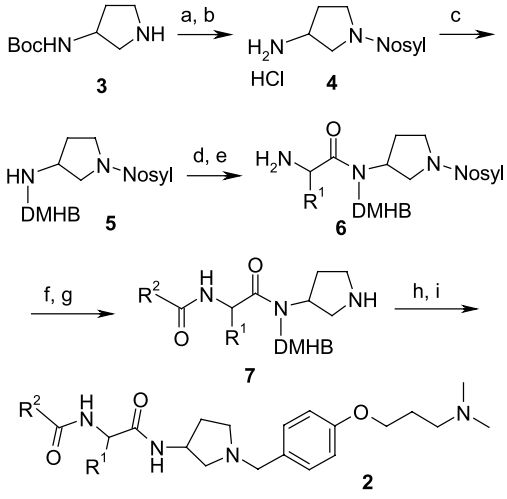


Figure 1. Structure of HTS hit SB-3288872 (**2a**).



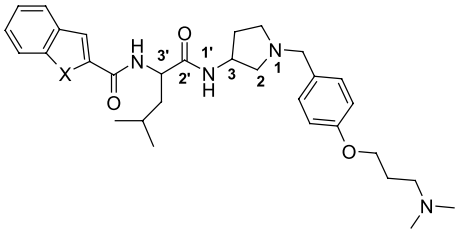
Scheme 1. Reagents and conditions: (a) 2-nitrobenzenesulfonyl chloride, pyridine, CH_2Cl_2, 0 °C to rt; (b) 4 M HCl in 1,4-dioxane, MeOH, rt; (c) 2,6-dimethoxy-4-polystyrenebenzyloxy-benzaldehyde (DMHB resin), $Na(OAc)_3BH$, diisopropylethylamine, 1% of HOAc in NMP, rt; (d) Fmoc-HNCH(R^1)CO_2H, 1,3-diisopropylcarbodiimide (DIC), 1-hydroxy-7-azabenzotriazole (HOAt), NMP, rt; (e) 20% of piperidine in NMP, rt; (f) R^2CO_2H, DIC, HOAt, NMP, rt; (g) K_2CO_3, PhSH, NMP, rt; (h) $Me_2N(CH_2)_3OPhCHO$, $Na(OAc)_3BH$, 10% of HOAc in NMP, rt; (i) 50% of TFA in DCE, rt.

The preferred stereochemistry at the 3-position of the pyrrolidine and the amino acid chiral center was first investigated by preparing the corresponding diastereoisomerically pure[22] compounds, **2a** (3*S*, 3′*S*), **2b** (3*R*, 3′*S*), **2c** (3*S*, 3′*S*), **2d** (3*R*, 3′*S*), and **2e** (3*S*, 3′*R*), from commercially available 3(*S*)- and 3(*R*)-(*tert*-butoxycarbonyl-amino)pyrrolidine (**3**), and L- and D-Fmoc-Leu-OH. As shown in Table 1, the preferred stereochemistry was (3*S*, 3′*S*). In subsequent optimization, only 3(*S*)-(*tert*-butoxycarbonyl-amino)pyrrolidine (**3**) and L-amino acids were used.

We next turned our attention to finding the optimum left-hand side (LHS) moiety. A number of amide, urea, and carbamate analogs were prepared and evaluated in the radioligand binding assay (Table 2). In general, modifications in this region were well tolerated with a variety of fused bicyclic and monocyclic aromatic groups having moderate binding affinity (pK_i 6.2–6.9). The benzothiophen-2-yl (**2a**) and 3,4-dichlorophenyl (**2j**) groups were slightly preferred. The simple methyl analog **2r** was significantly less potent. Carbamate **2q**[23] was slightly more potent than its amide analog **2o**, while urea **2p**[23] was slightly less potent.

We then investigated the central amino acid moiety (Table 3). The SAR in this region indicated a limited degree of tolerability for structural variation. A lipophilic group such as isobutyl (**2j**), benzyl (**2s**), and isopropyl (**2t**) was preferred, with the isobutyl group being optimal. Replacing the isobutyl group with hydrogen (**2u**),

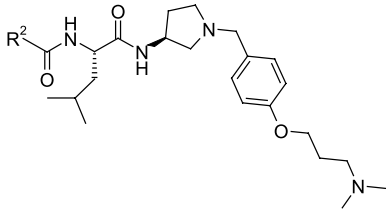
Table 1. Optimal stereochemistry



Compound	X	Diastereoisomer	hUT binding (pK_i)[a]
2a	S	(3*S*, 3′*S*)	6.9
2b	S	(3*R*, 3′*S*)	<5.0
2c	O	(3*S*, 3′*S*)	6.8
2d	O	(3*R*, 3′*S*)	<5.0
2e	O	(3*S*, 3′*R*)	5.5

[a] Mean of at least two determinations with standard deviation of <±0.3.

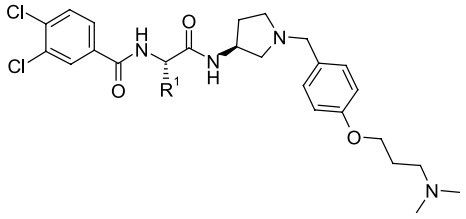
Table 2. hUT binding affinity of LHS amide, urea, and carbamate analogs **2f**–**2r**



Compound	R^2	hUT binding (pK_i)[a]
2a	Benzothiophen-2-yl	6.9
2c	Benzofuran-2-yl	6.8
2f	Indol-2-yl	6.3
2g	Quinolin-2-yl	6.2
2h	3,4-Methylenedioxyphenyl	6.3
2i	3,4-Methylenedioxybenzyl	6.4
2j	3,4-Dichlorophenyl	6.9
2k	4-Bromophenyl	6.7
2l	Thiophen-2-yl	6.4
2m	Furan-2-yl	6.2
2n	Pyridin-4-yl	5.7
2o	Phenyl	6.3
2p	Anilino	6.0
2q	Phenoxy	6.6
2r	Methyl	5.6

[a] Mean of at least two determinations with standard deviation of <±0.3.

Table 3. hUT binding affinity of the middle amino acid analogs **2j**, **2s**–**2w**

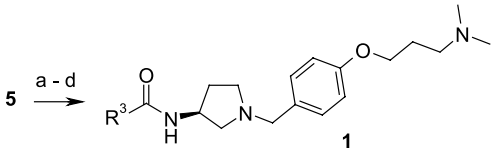


Compound	R^1	hUT binding (pK_i)[a]
2j	Isobutyl	6.9
2s	Benzyl	6.4
2t	Isopropyl	6.1
2u	H	<5.0
2v	4-Aminobutyl	<5.0
2w	2-Carboxyethyl	<5.0

[a] Mean of at least two determinations with standard deviation of <±0.3.

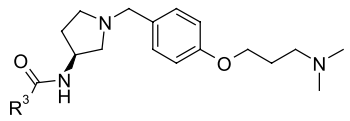
an aminobutyl group (**2v**), or a carboxyethyl group (**2w**) completely abolished hUT binding affinity.

While exploring the central amino acid moiety, we also prepared a number of truncated analogs (Table 4), in which the amino acid moiety was eliminated, using the synthetic route outlined in Scheme 2 involving the direct coupling of resin-bound amine **5** with various acids.[24] We were pleased to find that several truncated analogs, **1a**, **1c**, **1f**, and **1g**, showed moderate binding affinity with a pK_i range from 6.2 to 6.7 (Table 4). The SAR at the LHS moiety suggested a relatively restricted scope for



Scheme 2. Reagents and conditions: (a) R^3COOH, DIC, HOAt, NMP, rt; (b) K_2CO_3, PhSH, NMP, rt; (c) 4-$(Me_2N(CH_2)_3O)$PhCHO, $Na(OAc)_3$ BH, 10% of HOAc in NMP, rt; (d) 50% of TFA in DCE, rt.

Table 4. hUT binding affinity of truncated analogs **1a**–**1r**

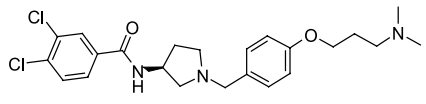


Compound	R^3	hUT binding (pK_i)[a]
1a	3,4-Dichlorophenyl	6.7
1b	3,5-Dichlorophenyl	5.7
1c	4-Chloro-3-methylphenyl	6.2
1d	3,4-Dimethoxyphenyl	<5.0
1e	3,4-Dimethylphenyl	<5.0
1f	4-Bromophenyl	6.5
1g	4-Bromo-3-methylphenyl	6.2
1h	4-Cyanophenyl	<5.0
1i	4-Trifluoromethylphenyl	<5.0
1j	4-Biphenyl	<5.0
1k	Phenyl	5.6
1l	Thiophen-2-yl	<5.0
1m	Furan-2-yl	<5.0
1n	Pyridin-4-yl	<5.0
1o	Methyl	<5.0
1p	Benzofuran-2-yl	5.6
1q	3,4-Methylenedioxyphenyl	5.6
1r	2-Naphthyl	<5.0

[a] Mean of at least two determinations with standard deviation of <±0.3.

modification in the truncated sub-series. For example, while the 3,4-dichlorophenyl analog **1a** and 4-chloro-3-methylphenyl analog **1c** had moderate affinity, **1d** (3,4-dimethoxyphenyl) and **1e** (3,4-dimethylphenyl) had no hUT receptor affinity. Similarly, 4-bromophenyl analogs **1f** and **1g** were moderately potent, but other 4-substituted analogs **1h**, **1i**, and **1j** had no binding affinity. Analogs containing an unsubstituted monocyclic aromatic (**1k**, **1l**, **1m**, and **1n**), bicyclic aromatic (**1p**, **1q**, and **1r**), or simple methyl group (**1o**) had little or no binding affinity.

In the functional FLIPR assay, SB-436811 (**1a**) (Fig. 2) was an antagonist with a pIC_{50} of 6.8, similar in potency to SB-328872 (**2a**). The FLIPR potency of both compounds was also commensurate with their receptor binding affinities further supporting the proposition that the observed inhibition of intracellular calcium mobilization in these cells was a result of hUT blockade. SB-436811 (**1a**) was also tested in monkey and rat UT binding assays[25], and found to have moderate monkey UT binding affinity ($pK_i = 6.6$) but poor rat UT binding affinity ($pK_i = 5.5$). Although it is not clear what contributes



SB-436811 (**1a**)
hUT binding: pKi = 6.7 (n = 7, sd = 0.2)
hUT FLIPR: pIC_{50} = 6.8 (n = 6, sd = 0.3)
monkey UT binding: pKi = 6.6 (n = 2, sd = 0.1)
rUT binding: pKi = 5.5 (n = 2, sd = 0.2)

Figure 2. Structure of truncated analog SB-436811 (**1a**).

to the binding affinity difference between primate and rodent receptors, the significant sequence differences between primate and rodent receptors[3] might be a reason for the observed receptor binding affinity difference.

In summary, a novel hU-II receptor antagonist series represented by SB-328872 (**2a**) was identified via HTS. The SAR was explored for several regions of this series and that led to the identification of a novel truncated sub-series represented by SB-436811 (**1a**). Further optimization of other regions, especially the right-hand side aminoalkoxybenzyl and central aminopyrrolidine moieties, of both series will be the subject of future publications.

Acknowledgments

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References and notes

1. (a) Bern, H. A.; Lederis, K. *J. Endocrinol.* **1969**, *45*, xi; (b) Bern, H. A.; Pearson, D.; Larson, B. A.; Nishioka, R. S. *Recent Prog. Horm. Res.* **1985**, *41*, 533.
2. (a) Ichikawa, T.; McMaster, D.; Lederis, K.; Koyabashi, H. *Peptides* **1982**, *3*, 859; (b) Loretz, C. A.; Howard, M. E.; Siegel, A. J. *J. Physiol.* **1985**, *249*, G284.
3. For a recent review, see: Dhanak, D.; Neeb, M. J.; Douglas, S. A. *Annu. Rep. Med. Chem.* **2003**, *38*, 99.
4. Ames, R. S.; Sarau, H. M.; Chambers, J. K.; Willette, R. N.; Aiyar, N. V.; Romanic, A. M.; Louden, C. S.; Foley, J. J.; Sauermelch, C. F.; Coatney, R. W.; Ao, Z.; Disa, J.; Holmes, S. D.; Stadel, J. M.; Martin, J. D.; Liu, W. S.; Glover, G. I.; Wilson, S.; McNulty, D. E.; Ellis, C. E.; Elshourbagy, N. A.; Shabon, U.; Trill, J. J.; Hay, D. W.; Ohlstein, E. H.; Bergsma, D. J.; Douglas, S. A. *Nature* **1999**, *401*, 282.
5. Behm, D. J.; Doe, C. P.; Johns, D. G.; Maniscalco, K.; Stankus, G. P.; Wibberley, A.; Willette, R. N.; Douglas, S. A. *Naunyn Schmiedeberg's Arch. Pharmacol.* **2004**, *369*, 274.
6. Böhm, F.; Pernow, J. *Br. J. Pharmacol.* **2002**, *135*, 25.
7. Russell, F. D.; Meyers, D.; Galbraith, A. J.; Bett, N.; Toth, I.; Kearns, P.; Molenaar, P. *Am. J. Physiol.* **2003**, *285*, H1576.
8. Song, W.; Ashton, N.; Balment, R. J. *J. Physiol.* **2003**, *552*, P107.
9. Douglas, S. A.; Dhanak, D.; Johns, D. G. *Trends Pharmacol. Sci.* **2004**, *25*, 76.
10. (a) Matsushita, M.; Shichiri, M.; Imai, T.; Iwashina, M.; Tanaka, H.; Takasu, N.; Hirata, Y. *J. Hypertens.* **2001**, *19*, 2185; (b) Cheung, B. M.; Leung, R.; Man, Y. B.; Wong, L. Y. *J. Hypertens.* **2004**, *22*, 1341.
11. (a) Douglas, S. A.; Tayara, L.; Ohlstein, E. H.; Halawa, N.; Giaid, A. *Lancet* **2002**, *359*, 1990; (b) Ng, L. L.; Loke, I.; O'Brien, R. J.; Squire, I. B.; Davies, J. E. *Circulation* **2002**, *106*, 2877; (c) Richards, A. M.; Nicholls, M. G.; Lainchbury, J. G.; Fisher, S.; Yandle, T. G. *Lancet* **2002**, *360*, 545; (d) Lapp, H.; Boerrigter, G.; Costello-Boerrigter, L. C.; Jaekel, K.; Scheffold, T.; Krakau, I.; Schramm, M.; Guelker, H.; Stasch, J. P. *Int. J. Cardiol.* **2004**, *94*, 93.
12. (a) Bousette, N.; Patel, L.; Douglas, S. A.; Ohlstein, E. H.; Giaid, A. *Atherosclerosis* **2004**, *176*, 117; (b) Maguire, J. J.; Kuc, R. E.; Wiley, K. E.; Kleinz, M. J.; Davenport, A. P. *Peptides* **2004**, *25*, 1767.
13. (a) Totsune, K.; Takahashi, K.; Arihara, Z.; Sone, M.; Satoh, F.; Ito, S.; Kimura, Y.; Sasano, H.; Murakami, O. *Lancet* **2001**, *358*, 810–811; (b) Shenouda, A.; Douglas, S. A.; Ohlstein, E. H.; Giaid, A. *J. Histochem. Cytochem.* **2002**, *50*, 885; (c) Langham, R. G.; Gow, R.; Thomson, N. M.; Dowling, J. K.; Gilbert, R. E. *Am. J. Kidney Dis.* **2004**, *44*, 826.
14. (a) Totsune, K.; Takahashi, K.; Arihara, Z.; Sone, M.; Satoh, F.; Ito, S.; Murakami, O. *Clin. Sci.* **2003**, *104*, 1; (b) Wenyi, Z.; Suzuki, S.; Hirai, M.; Hinokio, Y.; Tanizawa, Y.; Matsutani, A.; Satoh, J.; Oka, Y. *Diabetologia* **2003**, *46*, 972.
15. (a) Flohr, S.; Kurz, M.; Kostenis, E.; Brkovich, A.; Fournier, A.; Klabunde, T. *J. Med. Chem.* **2002**, *45*, 1799; (b) Croston, G. E.; Olsson, R.; Currier, E. A.; Burstein, E. S.; Weiner, D.; Nash, N.; Severance, D.; Allenmark, S. G.; Thunberg, L.; Ma, J.; Mohell, N.; O'Dowd, B.; Brann, M. R.; Hacksell, U. *J. Med. Chem.* **2002**, *45*, 4950(c) For patents, see Ref. 3.
16. (a) For $[Ca^{2+}]_i$ mobilization and radioligand binding assay details, see: Dhanak, D.; Knight, S. D.; Jin, J.; Keenan, R. M. WO Patent 2001045711-A1, 2001; *Chem. Abstr.* **2001**, *135*, 76785; (b) Dhanak, D.; Knight, S. D.; Warren, G. L.; Jin, J.; Widdowson, K. L.; Keenan, R. M. WO Patent 2001045710-A1, 2001; *Chem. Abstr.* **2001**, *135*, 71313.
17. The radioligand binding assay was subsequently used as the primary assay to generate SAR.
18. For experimental details, see Ref. 16b.
19. Purchased from TCI America, catalog number: A1172 or A1171.
20. Purchased from Polymer Laboratories, part number: 1466-6689, 150–300 μM, 1.5 mmol/g loading.
21. Purchased from Novabiochem.
22. Confirmed via NMR studies. All new compounds in this paper were characterized via LC/MS and/or ^{1}H NMR.
23. **2p** and **2q** were prepared via urea formation and carbamate formation of intermediate **6** with phenyl isocyanate and phenyl chloroformate, respectively, and subsequent reactions in Scheme 1.
24. For experimental details, see Ref. 16a.
25. For monkey and rat UT receptor radioligand binding assay details, see: Elshourbagy, N. A.; Douglas, S. A.; Shabon, U.; Harrison, S.; Duddy, G.; Sechler, J. L.; Ao, Z.; Maleeff, B. E.; Naselsky, D.; Disa, J.; Aiyar, N. V. *Br. J. Pharmacol.* **2002**, *136*, 9.