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Deconstruction of sulfonamide inhibitors of the urotensin receptor (UT) and design and synthesis of benzylamine and benzylsulfone antagonists

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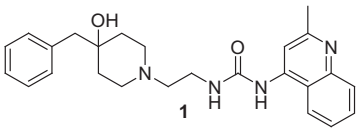
ABSTRACT

Potent small molecule antagonists of the urotensin receptor are described. These inhibitors were derived via systematically deconstructing a literature inhibitor to understand the basic pharmacophore and key molecular features required to inhibit the protein receptor. The series of benzylamine and benzylsulfone antagonists herein reported display a combination of nanomolar molecular and cellular potency as well as acceptable in vitro permeability and metabolic stability.

Urotensin II (U-II) is a small cyclic peptide originally isolated in 1967 from the fish species *Gillichtys mirabilis*.[1] Subsequent to its discovery, U-II was identified as an extremely powerful vasoconstrictor, many times more potent than endothelin.[2] In 1999, human U-II was cloned and identified as the natural ligand of the G-protein coupled receptor GPR14 (urotensin receptor, UT).[3] Upon binding of U-II to UT, a variety of intracellular signaling molecules are released in the cell that effect a wide range of physiological conditions, primarily related to cardiovascular function.[4] The physiology of UT antagonism is complex; in some tissues binding of U-II to the UT results in vasoconstriction induced by phospholipase C and RhoA, whereas in others U-II binding and activation of UT leads to vasodilatation, hypothesized to be mediated by nitrous oxide and prostacyclin.[5]

Based upon the reported variable pharmacological effects of U-II, a number of research groups have pursued small molecule inhibitors of UT, primarily for cardiovascular indications.[6] The most advanced antagonist, Palosuran (Fig. 1, **1**), reached Phase II clinical trials for diabetic neuropathy in 2004.[7] Other research teams have also disclosed non-peptidic small molecule inhibitors of the UT,[7b] however to our knowledge none have been advanced to clinical evaluation.

To determine the effect of UT antagonism in vivo it is critical to develop chemical tools that can provide the appropriate receptor coverage. Towards that goal we examined a number of literature-based compounds to better understand the pharmacophoric elements critical for non-peptidic, small molecule receptor antagonists of the UT. Initially we chose to deconstruct the known sulfonamide inhibitors, **2** and **3** (Fig. 2), to determine the importance of the various regions of the molecule, and to aid in the construction of novel chemical matter.[8] This series of inhibitors was chosen



UT IC_{50} (nM)	UT Cell IC_{50} (nM)	HLM $\%Q_h$
<5	39	53

Figure 1. Structure of the urotensin receptor antagonist Palosuran.

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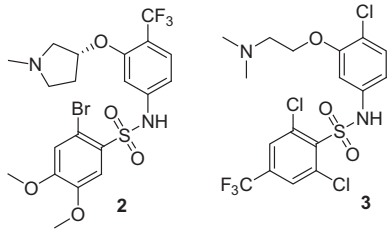
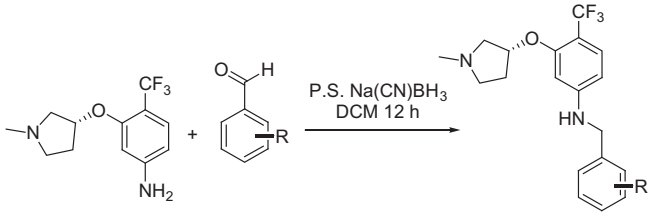


Figure 2. Sulfonamide antagonists of the urotensin receptor.

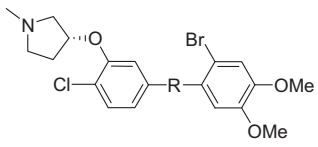


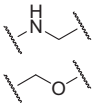
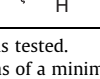
Scheme 1. Reagents and conditions: polymer supported $Na(CN)BH_3$, dichloromethane, rt, 12 h.

based upon their conformational rigidity as well as reported potency and in vivo efficacy.[9] These compounds are characterized by two substituted lipophilic aromatic groups linked together by a sulfonamide moiety. One of the aryl rings is consistently tethered to an ionizable amine, often restrained into an aliphatic ring system (**2**). We were further interested in the linear amine **3** as this compound (with an obvious linage to **2**), was reported to display efficacy in a disease-relevant model of heart failure in rodents (coronary arterial ligation).[10]

Our initial structure–activity relationship (SAR) campaign focused on determining the role of the sulfonamide linker, as this was hypothesized to serve as a spacer, primarily positioning the flanking aryl groups and basic amine in the proper orientation for antagonist activity. As such, we chose to systematically examine a variety of two-atom replacements for this moiety. Compounds were profiled for their ability to displace a radiolabeled ligand, or inhibit calcium flux in a cell line overexpressing the UT receptor. Replacement of the nitrogen atom of the sulfonamide linker with a carbon resulted in a compound that was almost equipotent to the analogous literature compound **4** (Table 1, entries 1 and 2), implying that the amine hydrogen is not involved in a direct hydrogen bond with the receptor. Reduction of the sulfone to either the sulfoxide **7** or the thio ether **8** resulted in a 10-fold loss in potency (Table 1, entries 4 and 5). The simple benzyl amine **9** was an order of magnitude less potent than baseline sulfonamide **4**. Furthermore, translocation of the amine to the right-hand side aromatic group resulted in an additional 10-fold loss in potency (Table 1, entry 9), suggesting a role for a hydrogen bond acceptor at this site. This hypothesis was confirmed by profiling of the ether analog **11** that partially recovered the potency lost with analog **12**. A strong enantiomeric preference was observed favoring the (*R*) enantiomer of the pyrrolidine ether side chain in both the benzylamine and benzylsulfone analogs (Table 1, entries 2, 3, 6 and 7). The initial sets of analogs were also profiled in human liver microsomes as a predictor of in vivo clearance. Of the analogs tested, benzylamine **9** showed a preferred in vitro metabolic profile and was therefore used as a probe substrate for further exploration of the SAR on the right hand side of the molecule.

Table 1
Data for linker replacements/sulfonamide replacements[a,b]



Entry	Compd	Linker (R)	UT IC_{50} (nM)	UT cell IC_{50} (nM)	HLM %Q_h
1	**4**		23	43	65
2	**5**		34	60	85
3	**6**[*]		1280	742	83
4	**7**		245	nt	72
5	**8**		440	nt	76
6	**9**		237	1328	28
7	**10**[*]		1350	1450	36
8	**11**		669	nt	53
9	**12**		3060	nt	11

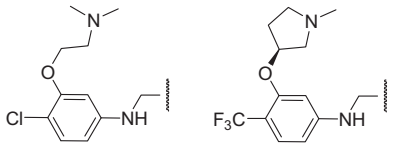
[*] (*S*) enantiomer was tested.
[a] Values are means of a minimum of two experiments (nt = not tested).
[b] (*S*) Enantiomer tested. UT IC_{50} assay determines the ability of a ligand to inhibit binding of ^{125}I-U-II to the receptor. UT Cell determines the ability to inhibit U-II stimulated Ca^{2+} mobilization in a CHO cell line expressing the human U-II receptor. HLM in vitro metabolism of compounds in the presence of human liver microsomes, represented a percentage of total liver blood flow. For a complete description of the assays see Ref. 11.

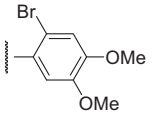
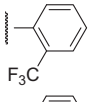
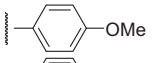
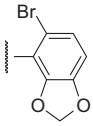
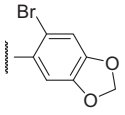
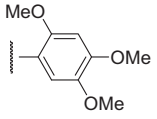
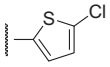
A high-throughput method was developed to evaluate the effect of substitution of the benzyl aromatic ring of the baseline molecule **9** (Scheme 1). Combining the aniline and a variety of aldehydes at room temperature and employing polystyrene-supported sodium cyanoborohydride amines were cleanly obtained by simple filtration followed by silica gel purification. Using this method, more than 30 analogs were rapidly synthesized, representatives of which are shown in Table 2.

Removal of all substitution of the benzyl ring resulted in complete loss of activity against the enzyme (Table 2, entry 3). Mono-substitution at the 2-, 3- or 4- position (compounds **17**–**20**) of the aryl ring regained some of the activity that was lost with compound **15**. Cyclization of the two pendant methoxy groups in **13** resulted in a deterioration in activity against the UT receptor (Table 2, entries 9 and 10). Additionally, isosteric replacement of the phenyl ring with a thiophene was not tolerated either (**24**), however activity could be restored by the addition of a halogen to the thiophene (Table 2, entry 13). Substituted phenyl analogs were consistently more active when coupled with the pyrrolidine-CF_3 aniline **B**, compared with the straight chain amine chloride core **A** (Table 2, entries 1 vs 2 and 4 vs 5).

The difference in potency between compound pairs **13**–**14**, and **16**–**17** suggested that the basic amine could be sensitive to simple modifications and should be explored further. When the sulfonamide linker was employed in place of the amine linker, the SAR of the basic amine tracked with the previous rank order observations (Table 3 compd **26**–**27**, and **13**–**14**). Conformational restriction of the amine by cyclication lead to compounds that were five times more potent in both the UT binding and functional assays (Table 3, entries 1 and 2). Ring expansion from the pyrrolidine

Table 2
Data for benzyl substituents on the aniline cores[a]



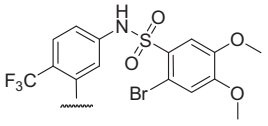
Entry	Compd	LHS	Aromatic	UT IC_{50} (nM)	UT cell IC_{50} (nM)
1	**13**	A		1200	1300
2	**14**	B		217	191
3	**15**	A		>10,000	nt
4	**16**	A		3500	6000
5	**17**	B		821	1870
6	**18**	A		520	765
7	**19**	B		714	3100
8	**20**	B		691	1130
9	**21**	B		620	1400
10	**22**	B		450	950
11	**23**	B		520	550
12	**24**	B		2700	2000
13	**25**	B		180	600

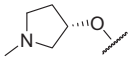
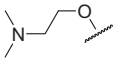
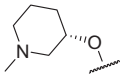
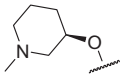
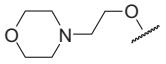
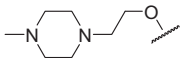
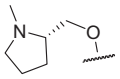
[a] Values are means of a minimum of two experiments (nt = not tested). UT IC_{50} assay determines the ability of a ligand to inhibit binding of ^{125}I-U-II to the receptor. UT Cell determines the ability to inhibit U-II stimulated Ca^{2+} mobilization in a CHO cell line expressing the human U-II receptor. For a complete description of the assays see Ref. 11.

to the piperidine moiety however was not tolerated (**28**). As previously observed with the benzylamine and sulfone analogs, there was a strong enantiomeric preference for the (*R*) stereoisomer (Table 3, entries 3 and 4). Reducing the pK_a of the amine also was detrimental to activity (Table 3, entries 5 vs 6). Maintaining the same distance between the amine and the phenoxy group but using an alternative pyrrolidine led to a compound (**32**) that was significantly less active with the sulfonamide (**26**) implying subtle steric constraints at this site. Not unexpectedly, complete removal of the basic amine resulted in a complete loss in activity (Table 3 and **34**) as has been observed with other UT inhibitors.

Two of the top analogs generated by examining linker replacements were further profiled in more advanced in vitro assays to determine other potential liabilities with these classes of compounds (Table 4). The benzylsulfone **5** was a more potent antagonist of UT in all species tested, including human, rat, and mouse

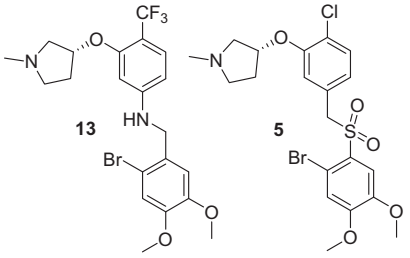
Table 3
Data for basic amine modifications of sulfonamide inhibitors[a]



Entry	Compd	LHS linker	UT IC_{50} (nM)	UT cell IC_{50} (nM)
1	**26**		6	14
2	**27**		134	100
3	**28**		160	120
4	**29**		>5000	5500
5	**30**		6200	nt
6	**31**		840	nt
7	**32**		800	350
8	**33**[*]		2100	nt
9	**34**		>10,000	nt

[*] (*S*) enantiomer was tested.
[a] Values are means of a minimum of two experiments (nt = not tested). UT IC_{50} assay determines the ability of a ligand to inhibit binding of ^{125}I-U-II to the receptor. UT Cell determines the ability to inhibit U-II stimulated Ca^{2+} mobilization in a CHO cell line expressing the human U-II receptor. For a complete description of the assays see Ref. 11.

Table 4
Advanced profiling of benziylamines and benzylsulfone analogs[a]



Assay	13	5
IC_{50} UT binding (μM)	0.22	0.03
UT cell human (μM)	0.19	0.06
UT cell murine (μM)	6.4	4.7
UT cell rat (μM)	3.9	2.0
HLM (%Q_h)	43	85
RLM (%Q_h)	<6	66
Solubility pH 4.5 (μg/mL)	>52	>87
Solubility pH 7.4 (μg/mL)	>52	>87
CAC0-2 PEAB (cm/s)	2.9	7.8
CACO-2 PEBA (cm/s)	0.22	32

[a] Values are means of a minimum of two experiments (nt = not tested). UT IC_{50} assay determines the ability of a ligand to inhibit binding of ^{125}I-U-II to the receptor. UT Cell determines the ability to inhibit U-II stimulated Ca^{2+} mobilization in a CHO cell line expressing the human U-II receptor. HLM in vitro metabolism of compounds in the presence of human liver microsomes, represented a percentage of total liver blood flow (%Q_h). For a complete description of the assays see Ref. 11.

than the corresponding benzylamine analog **13**. Compounds **13** and **5** both displayed a significant potency shift between the rodent and human receptors. Permeability for the sulfone analog was acceptable as assessed by CACO2 permeability, whereas the amine displayed a less attractive profile. The amine however, was more stable to in vitro metabolism. Solubility for both **5** and **13** were excellent at pH 4.5 and 7.4 (Table 4).

In summary, a potent inhibitor of UT was systematically deconstructed to determine the generic pharmacophore features necessary for activity. As with many GPCR's, the basic amine as well as a properly substituted aromatic right hand side proved critical for activity. Modification of the central linking atoms was tolerated although only specific locations of donor and acceptor functionalities were tolerated. The linker atoms also played an important divergent role in in vitro metabolism and permeability assays. We have applied this information towards designing novel chemotypes capable of inhibiting the UT receptor to better understand the in vivo pharmacological relevance of long term inhibition.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.105.

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