Bioorganic & Medicinal Chemistry Letters 18 (2008) 3716–3719

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potent and selective small-molecule human urotensin-II antagonists with improved pharmacokinetic profiles

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ARTICLE INFO

Article history:
Received 18 April 2008
Revised 9 May 2008
Accepted 15 May 2008
Available online 20 May 2008

Keywords:
Urotensin
HUT
HUT-II
UT
GPR-14
Antagonist
7-TM
7-Transmembrane

ABSTRACT

Lead compound **1** was successfully redesigned to provide compounds with improved pharmacokinetic profiles for this series of human urotensin-II antagonists. Replacement of the 2-pyrrolidinylmethyl-3-phenyl-piperidine core of **1** with a substituted *N*-methyl-2-(1-pyrrolidinyl)ethanamine core as in compound **7** resulted in compounds with improved oral bioavailability in rats. The relationship between stereochemistry and selectivity for hUT over the κ-opioid receptor was also explored.

Human urotensin-II (hU-II), a cyclic undecapeptide, has been identified as a powerful vasoconstrictor.[1,2] In 1999, hU-II was identified as a cognate ligand of human GPR-14 (hUT), an 'orphan' 7-TM receptor predominantly expressed in vascular and cardiac tissue.[2] hU-II and hUT are thought to be involved in the (dys)regulation of cardiorenal function,[3] and have been implicated in the etiology of numerous cardiorenal and metabolic diseases including hypertension,[4] heart failure,[5,6] atherosclerosis,[7] renal failure,[8] and diabetes.[9] Several non-peptidic UT ligands have recently been reported.[10] hUT antagonists are of interest as potential drugs to address these cardiorenal and metabolic conditions.

Recently, we reported the development of potent and selective hUT antagonists based on the 2-pyrrolidinylmethyl-3-phenyl-piperidine core of **1**[11] (Table 1). Within this 'piperidine-core' series, lead optimization led to several improvements to compound **1** in terms of hUT-binding affinity (K_i)[11,12] and selectivity against the κ-opioid receptor,[11] the $Na_V1.5$ cardiac sodium channel,[11] the rat brain batrachotoxinin (BTX) sensitive sodium channel,[11] and P450 inhibition for CYP2D6. However, the rat pharmacokinetic profile of these compounds remained poor, especially with regard to oral bioavailability. In addition, CYP3A4 inhibition was still generally problematic for this chemical series. In seeking a structural modification that might allow for a new direction in lead optimization, a strategy was designed which incorporated two elements. The first element of the strategy explored potential structural and synthetic simplifications to the amide–amine–aryl structural

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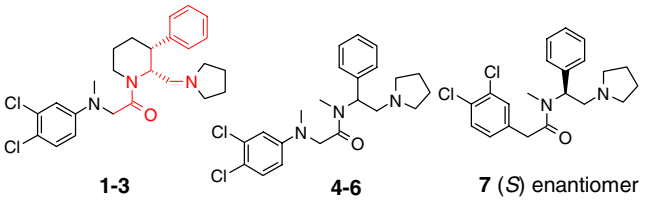
doi:10.1016/j.bmcl.2008.05.058

Table 1
Stereochemical comparison of chemical series



Compound	Stereochemistry	hUT K_i[a] (nM)	Kappa EC_{50}[b] (nM)
1	Racemate	16	3200
2	(*R*,*R*)	6	6300
3	(*S*,*S*)	1600	2000
4	Racemate	15	0.2
5	(*R*)	13	4
6	(*S*)	2500	0.1
7	(*S*)	4000	0.4

[a] Means of at least two determinations with a standard deviation of <±0.3 log units.
[b] Single determination or a mean of two determinations with a standard deviation of <±0.3 log units.

motif highlighted in structure **1** (Table 1). Lead optimization of the piperidine-core series[11] established that a basic amine linked to an amide group as in compound **1** was essential for hUT-binding affinity in this chemical series. Analogs with the pendant phenyl ring had significantly greater hUT-binding affinity than those lacking a phenyl ring.[11] Based on this information, compound **4** was proposed as an alternative scaffold, which shares the amide–amine–aryl structural motif with **1**, but has one less stereocenter and is more synthetically accessible. The second element of the strategy involved several observations related to the stereochemistry of the piperidine core template (**1**–**3**) as it relates to hUT-binding affinity and κ-opioid receptor agonism. In the piperidine-core series, (*R*,*R*) enantiomer **2** was found to have more than 200-fold higher binding affinity for hUT than the (*S*,*S*) enantiomer **3**.[11] Interestingly, κ-opioid receptor agonism exhibited the opposite stereochemical trend with the (*S*,*S*) enantiomer being the more active one. At the same time, exploration of the chemical literature revealed that compounds such as **7** based on a substituted *N*-methyl-2-(1-pyrrolidinyl)ethanamine core (referred to herein as the 'ethane-diamine core' for convenience) have been previously reported as κ-opioid agonists.[3,14] The importance of the (*S*) stereochemistry for kappa receptor agonism in compound **7** and structurally related analogs has been demonstrated.[13,14] The fact that compound **7** shares the amide–amine–aryl structural motif with **1** and also exhibits a stereochemical preference for the (*S*) enantiomer with regard to κ-opioid agonism prompted the evaluation of whether or not the same stereochemical trends in orthogonal κ-opioid agonism and hUT antagonism held for the ethane-diamine core series. If true, then the (*R*) stereochemistry in compounds containing the ethane-diamine core should provide hUT antagonists with selectivity over the κ-opioid receptor. Together, pharmacophore-driven rational design and the stereochemical observations from the chemical literature led to the discovery of compound **5** with an ethane-diamine core as a potent urotensin antagonist Table 1.

Compounds **5** and **6** were obtained by chiral HPLC separation of the racemate **4**. While (*R*) enantiomer **5** was the more potent hUT antagonist, κ receptor agonism resided predominantly with the (*S*) enantiomer **6**. Although **5** remained a potent kappa agonist (4 nM), the separation between hUT and κ activity for these enantiomers provided confidence that further optimization might identify a compound with acceptable selectivity for progression (Fig. 2 and Table 2).

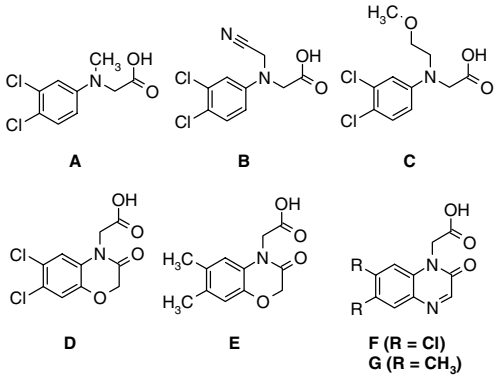


Fig. 1. Acid precursors to side-chain amide groups.[11]

Although these data suggested that the (*R*) stereochemistry would be advantageous for both hUT-binding affinity and for selectivity against the κ-opioid receptor, initial SAR development was performed with racemic compounds for ease of synthesis. Follow-up of selected analogs as the (*R*) enantiomer to maximize selectivity over the κ-opioid receptor would then be pursued if necessary. Adding a phenyl ring to **4** (Fig. 2) to generate the biphenyl analog **8** resulted in a compound with significantly reduced CYP2D6 inhibition and an 80-fold reduction in κ-opioid agonism (Table 2). However, **8** still retained sodium channel activity.[11d] Interestingly, some of the SAR trends that were observed in the piperidine core series[11] were also observed in the ethane-diamine series. It was found that replacing the pyrrolidine moiety of **8** with a morpholine group (compound **9**) resulted in several improvements in the in vitro profile: reduced sodium channel activity, a 100-fold reduction in κ-opioid agonist potency, and reduced CYP2D6 inhibition. However, hUT-binding affinity dropped by more than 30-fold with this change. It was also observed that hUT-binding affinity could be regained by employing one of the improved amide groups[11] (Fig. 1) as exemplified by compound **10**. The opposing stereo-

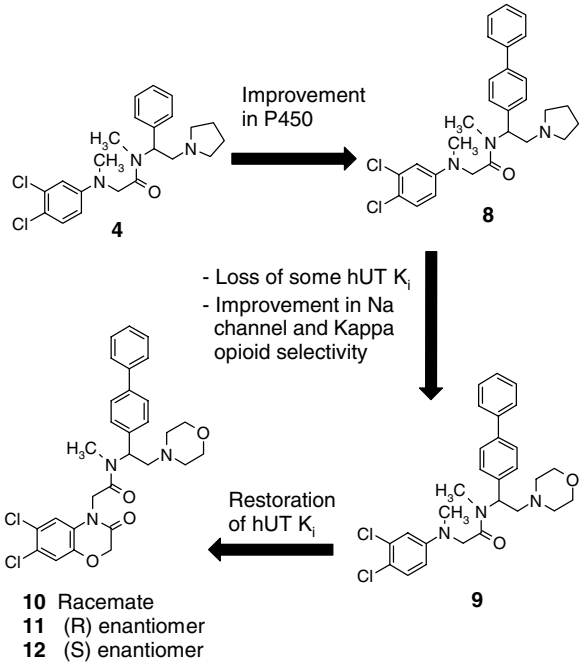


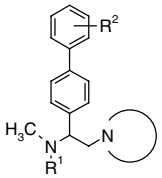
Fig. 2. Initial optimization of ethane-diamine core analogs.

Table 2
Optimization of ethane-diamine core analogs

Compound	In vitro data				
	hUT K_i[a] (nM)	Kappa EC_{50}[b] (nM)	Na channel K_i[a,c] (nM)	CYP 2D6 IC_{50}[d] (μM)	CYP 3A4 DEF IC_{50}[d]. (μM)
1	16	3200	2500	0.75	1.4
4	15	0.2	NA[e]	0.16	3.3
8	20	16	1200	5.9	4.9
9	630	1600	>30,000	16	2.1
10	13	320	11,130	33	4.6
11	16	800	7700	33	9.1
12	630	40	9400	33	4.2

[a] Means of at least two determinations with a standard deviation of <±0.3 log units.
[b] Single determination or a mean of two determinations with a standard deviation of <±0.3 log units.
[c] Rat brain batrachotoxinin (BTX)-sensitive sodium channel assay.
[d] Single determination, Cypex Bactosomes.
[e] Na channel data ARE not available for **7**, but for (*R*)-**4**, Na channel K_i = 2000 nM.

Table 3
Further development of the ethane-diamine series



Compound	R^1	R^2	Amine	hUT K_i[a] (nM)	Kappa EC_{50}[b] (nM)	Na Channel K_i[a,c] (nM)	CYP 2D6 IC_{50}[d] (μM)	CYP 3A4 DEF IC_{50}[d] (μM)
13	G	3-$CONH(CH_3)$	Morpholine	6	4000	30,000	33	9
14	C	4-$CONH_2$	Pyrrolidine	3	100	17,000	6.2	2.6
15	B	3-$CONH_2$	Pyrrolidine	0.3	32	22,000	3.5	2.2
16	F	3-$CONH(CH_3)$	Morpholine	4	2000	30,000	33	5
17	E	4-$NHCOCH_3$	Morpholine	3	800	30,000	30	6.4
18	D	3-OCH_3	Morpholine	5	160	22,000	17	1

[a] Means of at least two determinations with a standard deviation of <±0.3 log units.
[b] Single determination or a mean of two determinations with a standard deviation of <±0.3 log units.
[c] Rat brain batrachotoxinin (BTX)-sensitive sodium channel assay.
[d] Single determination, *Cypex Bactosomes.*

chemical SAR trends for hUT-binding affinity and κ-opioid agonism are also valid for this new biphenyl-ethanediamine series, as shown by the divergent profiles of **11** and **12**. Indeed, the (*R*) enantiomer **11** is 50-fold selective for hUT over the κ-opioid receptor.

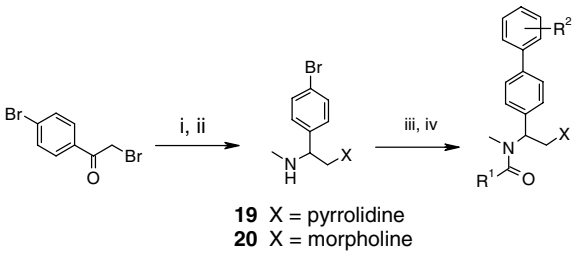
Further development of this compound series focused on variations in the substituents of the distal ring of the biphenyl group, exploration of different side-chain amides, and utilization of either the pyrrolidine or morpholine moieties as the basic amine group. Table 3 summarizes the in vitro profiles of representative examples. It was noted that polar functional groups on the distal ring sufficiently improved selectivity for hUT over the sodium channel such that even pyrrolidine analogs were still highly selective. Thus, biphenyl amide **15** has acceptable selectivity over the sodium channel and has improved binding affinity (40-fold) compared to compound **10**. Even as racemates, several of these analogs such as **13**, **15**, and **17** demonstrate selectivity against the κ-opioid receptor that exceeds that of the (*R*) enantiomer **11**. Furthermore, these improvements were achieved without any degradation of the P450 profile. Most significant was the finding that the pharmacokinetic profile for the ethane-diamine series is substantially improved over the original piperidine-core series (Table 4). In addition, these compounds are functionally potent in the rat aortic ring contraction assay[15] at levels comparable to the in vitro rat binding affinity. For example, compound **18** has a rat aorta K_b of 21 nM.

While the greatest overall improvement was achieved with regard to oral bioavailability, it was possible to identify compounds such as **13** and**16** in which all of the pharmacokinetic parameters except half-life were improved relative to the piperidine core series.[11] One important result with this series was the general reduction in the volume of distribution compared to the piperidine core series. In several cases, such as compounds **13**, **16**, and **17** the volume of distribution was an order of magnitude lower than the average for the piperidine core analogs. Since the urotensin receptor population that is relevant to blood pressure regulation is primarily expressed in the vascular smooth muscle, compounds with a high volume may be less desirable than low-to-moderate volume compounds. Interestingly, stereochemistry did not seem to affect the pharmacokinetic profile to the extent that it influenced hUT-binding affinity or κ-opioid agonism. There was little difference in the rat pharmacokinetic profiles of the (*R*) enantiomer **11** or the (*S*) enantiomer **12** compared to the racemate **10**.

Table 4
Rat PK data for representative analogs

Compound	PK data[a]				
	C_{max} (ng/mL)	$T_{1/2}$ (h)	CL (mL/min/kg)	Vdss (L/kg)	Oral *F* (%)
1	100	3.8	97	23	0-3
10	160	2.4	150	23	44
11	200	2.2	120	11	43
12	230	2.6	130	19	17
13	890	1.0	60	1.7	39
15	340	3.4	100	20	36
16	1100	1.5	36	2	29
17	600	1.7	94	2.8	83
18	290	3.4	140	20	30

[a] Rat PK data based on 2 mg/kg iv dose and 4 mg/kg solution oral dose.



Scheme 1. Reagents and conditions: (i) pyrrolidine or morpholine, ether, 96%; (ii) CH_3NH_2, CH_3COOH, $NaBH_3(CN)$, THF, 99%; (iii) carboxylic acid from Figure 1, BOP reagent, Et_3N, DMF, 70–90%; (iv) phenyl boronic acid, 1 M Na_2CO_3, $Pd(dppf)Cl_2$, dioxane, 160 °C, microwave, 360 s, 20–70%.

To prepare the diamine core for this series, 2,4′-dibromoacetophenone was treated with either morpholine or pyrrolidine to give a high yield of the α-amino ketones, which were subjected to reductive amination with methylamine to give ethane-diamine intermediates **19** and **20**. Amide coupling of the side-chain acid groups (syntheses reported in a previous publication[11]) shown in Figure 1 to either **19** or **20** yielded the aryl bromide intermediates for the Suzuki coupling in the final step (Scheme 1). The (*R*) and (*S*) enantiomers, **11** and **12**, respectively, were obtained by chiral HPLC separation of racemic **10**.

In summary, replacing the piperidine core of lead compound **1** with the substituted ethane-diamine core resulted in compounds with superior pharmacokinetic profiles, particularly with regard to the improved oral bioavailability. It was discovered that the stereochemical SAR trends for hUT-binding affinity and κ-opioid agonism were in direct opposition. As a result, several compounds from the ethane-diamine series were identified with high hUT-binding affinity, selectivity against both the sodium channel and the κ-opioid receptor, and a developable pharmacokinetic profile. Many of these compounds have good selectivity over the κ-opioid receptor even as the racemates, but compounds such as **14** and **15** could be further improved by capitalizing on this stereochemical differentiation in hUT and κ activity via the synthesis of the *R* enantiomer of each of these compounds.

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