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Development of potent and selective small-molecule human Urotensin-II antagonists

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ABSTRACT

This work describes the development of potent and selective human Urotensin-II receptor antagonists starting from lead compound **1**, (3,4-dichlorophenyl)methyl{2-oxo-2-[3-phenyl-2-(1-pyrrolidinylmethyl)-1-piperidinyl]ethyl}amine. Several problems relating to oral bioavailability, cytochrome P450 inhibition, and off-target activity at the kappa opioid receptor and cardiac sodium channel were addressed during lead development. hUT binding affinity relative to compound **1** was improved by more than 40-fold in some analogs, and a structural modification was identified which significantly attenuated both off-target activities.

Urotensin-II (U-II), a cyclic undecapeptide, was first isolated in the 1960's from *goby urophysis*[1] and was proposed to be involved primarily in osmoregulation in fish.[2] Human Urotensin-II (hU-II) and other mammalian 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' G-protein-coupled receptor predominantly expressed in cardiovascular tissue.[4] Both U-II ligand and UT receptor were found within mammalian 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 hemodynamic effects upon local and systemic administration in cat[5] and in man.[6,7] U-II also influences cardiorenal function by acting as a potent regulator of cardiac contractility[7] and 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.[15–17] Human Urotensin-II receptor antagonists are of interest as potential drugs to address these cardiovascular conditions.

High-throughput screening (HTS) combined with hit-to-lead chemistry[16a] identified compound **1** (Table 1) as an antagonist of hUT. The binding affinity (K_i) of this compound was 16 nM in a [^{125}I]hU-II radioligand binding assay using HEK293 cell membranes stably expressing recombinant human UT receptors.[16b,c] Furthermore, this compound also exhibited potency (K_b = 28 nM) in an isolated rat aorta functional assay[17] which was comparable to the in vitro rat binding affinity. In addition to being functionally

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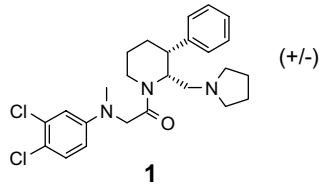
Table 1
In vitro and pharmacokinetic data for **1**



1

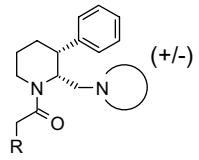
In vitro data	Rat PK data[a]
hUT K_i = 16 nM	$T_{1/2}$ = 3.8 h
CYP2D6 = 0.75 μM	Vdss = 23 L/kg
CYP3A4 = 1.4 μM	CL = 97 mL/min/kg
Rat aorta K_b = 28 nM	Oral F (%) = 0–3%

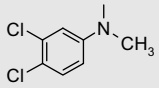
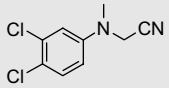
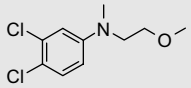
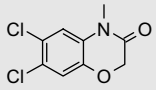
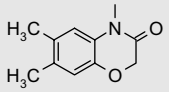
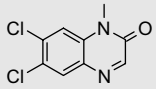
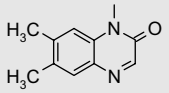
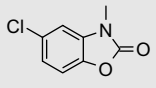
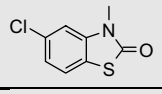
[a] Rat PK data based on 2 mg/kg iv dose and 4 mg/kg solution oral dose.

active in tissues, this compound was found to be a reversible and surmountable (competitive) antagonist with multi-species activity.[16] Despite the high affinity and potency of (**1**), the overall developability profile of this compound suffered from cytochrome P450 liabilities (mainly CYP2D6 and CYP3A4 inhibition) and its pharmacokinetic (PK) profile demonstrated poor oral bioavailability, as illustrated in Table 1. The data reveal the large volume of distribution, high clearance, and low oral bioavailability for this compound. However, compound **1** served as an acceptable starting point for further investigation.

One area that was only briefly examined in the initial hit-to-lead efforts was the amide group appended to the piperidine core. It was hypothesized that by hindering rotation or introducing additional conformational constraints in this amide 'side-chain' group, one might improve oral bioavailability[18a] and possibly bias the conformation towards one that is more favorable for hUT bind-

Table 2
SAR summary table



			In vitro data				
#	Side-chain (R)	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)
1		Pyrrolidine	16	3200	2500	0.75	1.4
2		Morpholine	79	1000	1600	2.3	0.77
3		Pyrrolidine	2	790	1600	2.3	1.1
4		Morpholine	6	5000	4000	3.7	0.49
5		Pyrrolidine	8	1000	960	0.82	1.2
6		Morpholine	130	10,000	3200	3.2	0.48
7		Pyrrolidine	0.4	500	680	1.6	1.8
8		Morpholine	2	7900	1300	4.8	0.73
9		Pyrrolidine	0.4	5000	390	5.1	3.9
–		Morpholine	–	–	–	–	–
10		Pyrrolidine	0.6	3200	930	2.9	1.2
11		Morpholine	3	15,000	18,000	21	1
12		Pyrrolidine	1	6300	1400	8.4	6.8
13		Morpholine	5	30,000	6900	6.7	2.7
14		Pyrrolidine	5	5000	150	2.2	2.1
15		Morpholine	40	1300	730	3.5	1.3
16		Pyrrolidine	1.2	1300	330	1.7	1
17		Morpholine	6	7900	3500	8.0	0.68

[a] Mean 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.

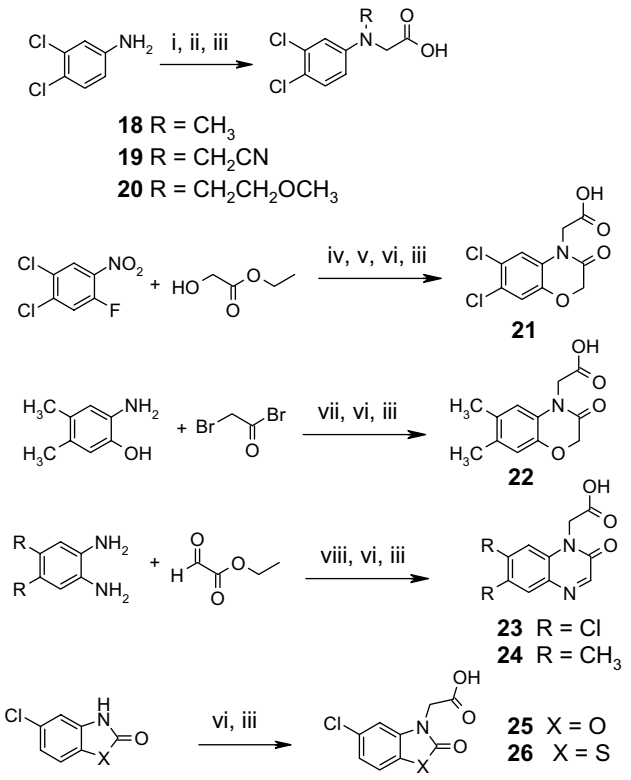
Table 3
Rat PK data for representative analogs

#	C_{max} (ng/mL)	$T_{1/2}$ (h)	CL (mL/min/kg)	Vdss (L/kg)	Oral F (%)
PK data[a]					
A1	100	3.8	97	23	0–3
3	120	3.2	100	27	12
4	370	2.6	60	8	7
5	95	2.8	120	27	30
17	160	5.0	130	26	<1

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

ing.[18b] The strategy was to hinder rotation of this flexible moiety by replacing the methyl group with larger substituents, or to eliminate entirely one rotatable bond by creating fused rings.

The initial set of compounds that were prepared employed pyrrolidine as the basic amino group, and were direct analogs of **1** varying only the amide side-chain moiety. While all compounds in Table 2 showed a measurably higher binding affinity for hUT than **1**, the sub-nanomolar compounds **7**, **9**, and **10** are especially noteworthy since they have 10-fold greater hUT binding affinity. Also, compounds **9** and **12** possess an improved P450 profile for both CYP2D6 and CYP3A4 (DEF) relative to **1**. The comparison of the P450 profile for **9** and **12** to **7** and **10**, respectively, demonstrates the reduction in P450 inhibition that can be obtained by replacing the chlorine substituents with methyl groups on the amide moiety. During the course of lead development, it was discovered that the pyrrolidine analogs described in Table 2 have activity at two other molecular targets. All of these compounds are kappa opioid agonists, and some, such as **3** and **7** have sub-micromolar activity at this receptor.[19]



Scheme 1. Reagents and conditions: (i) ethyl bromoacetate, diisopropylethylamine, NMP, 90 °C, 97%; (ii) alkyl halide, K_2CO_3 or diisopropylethylamine, NMP, 80–120 °C, 16–63%; (iii) LiOH, THF, H_2O, 99%; (iv) KF, dioxane, 100 °C, 96%; (v) $SnCl_2$·$2H_2O$, EtOH, HCl, 83%; (vi) NaH, ethyl bromoacetate, DMF, 50–90%; (vii) $NaHCO_3$, Cs_2CO_3, DMF, 80%; (viii) EtOH, 85 °C, 83%.

In addition, these compounds have affinity at both the $Na_V1.5$ cardiac sodium channel and the rat brain batrachotoxinin (BTX) sensitive sodium channel.[20] While all of these compounds have some level of affinity at the sodium channel, some such as **9** and **14**, have nanomolar affinity. Compounds with this level of sodium channel affinity could not progress through animal models such as rat pharmacokinetic studies or higher species in vivo pharmacodynamic studies due to potential cardiac toxicity. It was also unclear if kappa opioid agonism would interfere with in vivo studies and complicate the interpretation of results. Thus, attenuating activity at the kappa opioid receptor and the sodium channel became goals of equal importance to the improvement of the P450 and PK profiles. In order to ensure safety in the in vivo studies, compounds with off-target activities in the mid-to-high micromolar range at the sodium channel and kappa opioid receptor were targeted. One successful structural modification which demonstrated a definite attenuation in the off-target activity for both was the replacement of the pyrrolidine moiety with a less basic morpholine group (Table 2). Direct comparison of the corresponding pyrrolidine/morpholine analog pairs (e.g., **10** versus **11**) demonstrates that (with the exception of **1** versus **2**) a 2- to 20-fold reduction in sodium channel affinity was realized. Another benefit of the structural change from pyrrolidine to morpholine is a general weakening of the kappa agonist potency by 4- to 6-fold for most analogs. Unfortunately, the fold-selectivity for hUT binding affinity versus both the kappa opioid receptor and the sodium channel remained largely the same in both the pyrrolidine and morpholine sets of compounds due to the parallel loss in hUT binding affinity, which was 5- to 6-fold for most analogs. However, the morpholine compounds, unlike many of their pyrrolidine counterparts, could be evaluated in vivo because they have off-target activities in the mid-to-high micromolar range, which is above the safety thresholds for many in-house in vivo studies. Finally, the results of the transformation from pyrrolidine to morpholine on P450 inhibition were mixed, so that CYP2D6 inhibition declined while CYP3A4 inhibition increased.

For both the pyrrolidine and morpholine analogs, introducing conformational constraints appeared to be a successful strategy for preparing compounds with high hUT binding affinity which also translated into high functional potency in rats. Compounds **1**, **2**, **3**, **5**, and **14** (representing both the pyrrolidine and morpholine sub-series) were evaluated in the rat aortic ring contraction assay,[17] and all compounds were functionally potent at levels comparable to the in vitro rat binding affinity. For example, compound **3** has a rat aorta K_b of 5 nM. However, the conformational constraint strategy was less successful in addressing the poor oral bioavailability in this series. Only compounds **3** and **5** demonstrated any substantial improvement in oral bioavailability (Table 3). Although compound **5** does show a markedly improved oral bioavailability compared to compound **1**, the clearance and volume of distribution are similar to **1**, and **5** is a sub-micromolar CYP2D6 inhibitor.

The preparation of the *cis*, racemic 3-phenyl-2-(1-aminomethyl)piperidine core, was described in a prior publication.[16a] Preparation of the acid side-chains is described in Scheme 1. The acid side-chains were coupled to the piperidine core using standard amide bond-forming reaction conditions (e.g., BOP, diisopropylethylamine, DMF).

In summary, lead development of **1** has led to the identification of potent and selective hUT inhibitors. Compounds with restricted conformations in the amide side-chain have improved hUT binding affinity, which in some cases was more than 10-fold greater than **1**. Modulating the electronics of the aryl group by replacing the chloride with a methyl group provided a handle to improve the P450 profile. By replacing the pyrrolidine moiety

with the less basic morpholine substituent, off-target activity at the kappa opioid receptor and the sodium channel was attenuated to the point that the compounds could be safely evaluated in vivo. A few compounds, such as **3** and **5** had modestly improved oral bioavailability compared to **1**, but in general, the overall pharmacokinetic profiles for most compounds were similar to the lead. Further optimization to improve the pharmacokinetic profile while maintaining the improvements in potency and attenuated off-target activity discovered herein will be the subject of future publications.

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