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Phenylpiperidine-benzoxazinones as urotensin-II receptor antagonists: Synthesis, SAR, and in vivo assessment

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Abstract—Various 4-phenylpiperidine-benzoxazin-3-ones were synthesized and biologically evaluated as urotensin-II (U-II) receptor antagonists. Compound **12i** was identified from in vitro evaluation as a low nanomolar antagonist against both rat and human U-II receptors. This compound showed in vivo efficacy in reversing the ear-flush response induced by U-II in rats.

Urotensin-II (U-II) is a cysteine-linked cyclic peptide that activates the G-protein coupled receptor (GPCR) known as the urotensin-II receptor (UT receptor; GPR14), which is expressed in a variety of tissues, including blood vessels, heart, liver, kidney, skeletal muscle, and lung.[1] U-II was originally isolated from the urophysis of the goby fish, but it has subsequently been found in all classes of vertebrates.[1a,b] The composition of the peptide ranges from 11 amino acids in humans to 14 amino acids in mice, with a highly conserved region of six residues, CFWKYC, in the ring, of which WKY was shown to be essential for bioactivity.[2] Although U-II exhibits potent vasoactive properties, its role and the role of the UT receptor, in mammalian physiology and pathophysiology, are not completely understood.[1c,3] Nonetheless, U-II and the UT receptor have been implicated in a number disease states including hypertension, congestive heart failure, and chronic renal failure.[4] Therefore, a U-II receptor antagonist may afford potential therapeutic agents against these diseases, and some antagonists have been reported.[5]

Our objective was to identify a compound with low nanomolar potency against both rat and human UTs to provide a research tool for evaluating the role of U-II and its receptor in disease models. Our peptide structure–function study, combined with construction of a U-II receptor homology model, yielded the ligand Ac-CFWK(2-Nal)C–NH_2 (**1**) as a potent U-II agonist.[2b] Utilizing this ligand as a structural probe, a FLIPR-based high-throughput screen (HTS)[6] was undertaken on a 40,000 member diversity library to generate a lead antagonist. Thus, we identified a moderately potent compound, **3a** (Table 1, IC_{50} = 7.1 μM), which contained a 4-(4-chlorophenyl)piperidine subunit, a dimethylcarboxamide group, a two-carbon linker, and a benzoxazin-3-one heterocycle. Compound **3a** and its close derivatives (**3b**–**e**) were synthesized according to the chemistry in Scheme 1. Coupling of carboxylic acid **2**[7] with the corresponding primary or secondary amine using HBTU gave the intermediate amides. The BOC-protecting group was removed with HCl in dioxane, followed by reductive amination with (3-oxo-2,3-dihydrobenzo[1,4]oxazin-4-yl)acetaldehyde to give target compounds **3a**–**e**.

Compound **3a** contains a chlorine atom in the 4-position of the phenyl ring attached to the piperidine. Removing this chlorine, as in **3b**, resulted in a threefold loss in potency at the rat UT receptor. Our reported ligand–receptor model was used to optimize **3b**.[2b] The WKY ligand pharmacophore and tyrosine-binding pocket were identified as prominent features of this computational model. A qualitative comparison of the WKY pharmacophore to **3a** suggested that adding an aryl group at the

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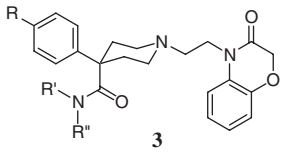
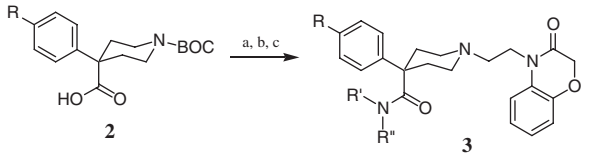
Table 1. In vitro biological data for compounds **3**



3

Compound	R	R′	R″	Rat U-II IC_{50} (μM)[a]
3a	Cl	Me	Me	7.1
3b	H	Me	Me	24
3c	H	H	CH_2Ph	43
3d	H	H	CH_2CH_2Ph	12
3e	H	H	$CH_2CH_2CH_2Ph$	9.1

[a] Functional inhibition of the rat UT receptor transfected in CHOK1 cells ($N = 2$). See Ref. 6.



Scheme 1. Reagents and conditions: (a) 1.0 mol equiv of R′R″NH, 1.5 mol equiv of HBTU, cat. HOBt, 3.0 mol equiv of NMM, DMF, 0 °C, 3 h, 21–42%; (b) 4 M HCl, 1,4-dioxane, 1 h, 95%; (c) 1.0 equiv of (3-oxo-2,3-dihydrobenzo[1,4]oxazin-4-yl)acetaldehyde (**6**, Scheme 2), 1.1 mol equiv of $Me_4NBH(OAc)_3$, 1.1 mol equiv of Et_3N, $(ClCH_2)_2$, 4 h, 70–80%. Abbreviations: HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine.

amide position might be advantageous for reaching the putative tyrosine-binding pocket. The initial compound, benzyl amide **3c**, did not show an increase in potency, but the related phenylethyl (**3d**) and phenylpropyl (**2e**) analogues did, although the potency was still at the single-digit micromolar level. This interaction compensated for the loss in potency on removing the chlorine atom from the phenyl group on the piperidine ring.

An interesting result was obtained by transposing the amide group from the piperidine ring (**3e**) to the phenyl ring (**4**) (Fig. 1). Since **4** gave a slight increase in potency, further SAR development was directed to the series in which **4** is the prototype.

Aryl substituted piperidines **12a**–**i** were constructed (Scheme 2) by allylation of 4H-benzo[1,4]oxazin-3-one followed by dihydroxylation and oxidative cleavage to provide intermediate acetaldehyde **6**. The phenylpi-

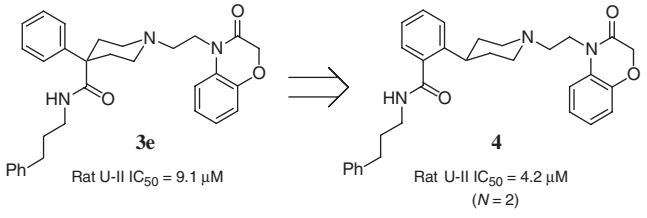
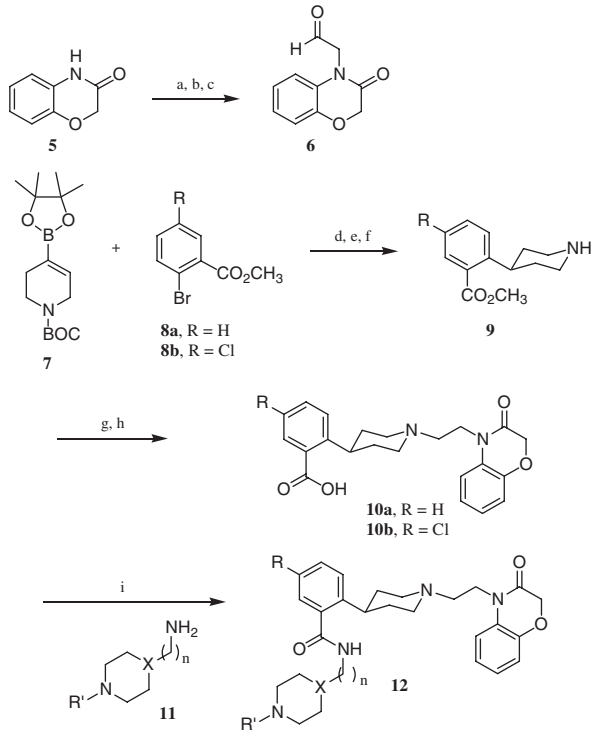


Figure 1. Change in connectivity leading to **4**.

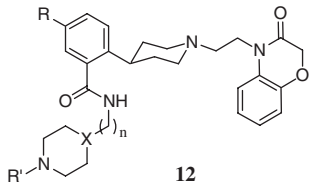


Scheme 2. Reagents and conditions: (a) 1.1 mol equiv of NaH, 1.1 mol equiv of allyl bromide, DMF, 4 °C—rt, 20 h, 82%; (b) 2.9 mol equiv of $K_3Fe(CN)_6$, 0.05 mol equiv of $K_2OsO_4{\cdot}H_2O$, 2.9 mol equiv of K_2CO_3, $^tBuOH/H_2O$ (1:1), 4 °C—rt, 24 h, 96%; (c) 3.0 mol equiv of $NaIO_4$, $MeOH/H_2O$ (5:1), 2 h, 83%; (d) 7 mol% $PdCl_2$ dppf, 3.0 mol equiv of K_2CO_3, DMF, 90 °C, 5 h, 57–65%; (e) 0.1 mol equiv of PtO_2, H_2, 15 psig, EtOH/AcOH (1:1), 10 h, 90–95%; (f) 4 M HCl, 1,4-dioxane, 2 h, 93%; (g) 1.0 mol equiv of (3-oxo-2,3-dihydrobenzo[1,4]oxazin-4-yl)acetaldehyde (**6**), 1.1 mol equiv of $Me_4NBH(OAc)_3$, $(ClCH_2)_2$, 1.1 mol equiv of Et_3N, 4 h, 70–80%; (h) 3 N NaOH, MeOH, 4 h, 61–66%; (i) 1.0 mol equiv of **9**, 1.5 mol equiv of HBTU, cat. HOBt, 3.0 mol equiv of NMM, DMF, 0 °C, 3 h, 30–70%. Abbreviations: see Scheme 1; dppf, 1,1′-bis(diphenylphosphino)ferrocene.

peridine core was synthesized by a palladium-catalyzed coupling of boronic ester **7** with aryl bromide **8**,[8] followed by hydrogenation of the double bond using platinum oxide and removal of the BOC-protecting group with HCl in dioxane provided intermediate **9** in good overall yield. Reductive amination of intermediate **9** with acetaldehyde **6**, followed by hydrolysis of the methyl ester provided acids **10a** and **10b**, which could be used to explore a variety of amide substituents. HBTU-mediated coupling of **10** with the appropriate amines **11** provided derivatives **12a**–**i**. Compound **12j** was obtained by acid cleavage of the BOC-group within **12i**.

Replacing the phenylpropyl group in **4** with an *N*-BOC-piperidinylmethyl group, as in **12a**, retained the potency (Table 2). Reintroduction of a chlorine atom into the 4-position of the aryl ring gave a robust increase in potency, as exemplified by **12b**. Other hydrophobic carbonyl-containing amine-substituents were also tolerated, such as CBZ (**12c**), amides (**12d** and **e**), and a benzyl urea (**12f**). These derivatives were also very potent against human UT with a K_i value in a binding

Table 2. In vitro biological data for compounds **12a–j**



12

Compound	*n*	X	R	R′	Rat UT IC_{50} (μM)[a]	Hu UT K_i (μM)[b]
12a	1	CH_2	H	BOC	2.5	0.43
12b	1	CH_2	Cl	BOC	0.60	0.14
12c	1	CH_2	Cl	CBZ	0.40	0.054
12d	1	CH_2	Cl	C(O)Ph	0.52	0.16
12e	1	CH_2	Cl	$C(O)CH_2CMe_3$	0.33	0.027
12f	1	CH_2	Cl	$C(O)NCH_2Ph$	0.21	0.030[c]
12g	1	CH_2	Cl	SO_2CH_2Ph	0.053	0.030[c]
12h	2	CH_2	Cl	BOC	0.10	0.077
12i[11]	2	N	Cl	BOC	0.010	0.065
12j	2	N	Cl	H	1.9	NT

[a] Inhibition of rat UT receptor transfected into CHOK1 cells ($N = 2$, Ref. 6).
[b] Inhibition of human UT receptor in whole-cell binding ($N = 2$, Refs. 9,10).
[c] $N = 1$, NT = not tested.

assay of 30–54 nM.[9,10] Interestingly, benzylsulfonamide derivative **12g** provided a 4- to 10-fold increase in potency against rat UT as compared to the carboxamide derivatives **12b–e**, although there was no increase in affinity against human U-II. Increasing the chain length between the amide nitrogen and the piperidine ring from one carbon (**12b**) to two carbons (**12h**) led to a sixfold improvement in potency against rat UT. A threefold increase in affinity was also noted against human UT. Replacing the piperidine ring of **12h** with a piperazine ring, as in **12i**, yielded the most dramatic effect with a 10-fold increase in potency against rat UT. Removal of the BOC-group in **12j** led to a dramatic decrease in potency, with an IC_{50} of 1.9 μM. These results support the original hypothesis that the amide substituent may be occupying a hydrophobic tyrosine-binding pocket.

When **12i** was docked into a rat U-II receptor homology model[12] using the piperidine ring as the restriction point (to Asp130), the BOC-piperazine group moved into the tyrosine-binding pocket (Fig. 2) without bias.

Compound **12i** was selected for in vivo evaluation because it met the criteria of excellent potency at both rat and human UT receptors, while the rat iv pk (2 mg/kg) showed $t_{1/2} = 127$ min and $C_{max} = 553$ ng/ml. This compound was examined in the U-II-induced ear-flush model in rats to demonstrate that it could block a U-II-mediated effect (Fig. 3).[13] Subcutaneous administration of U-II showed an increase in ear pinna temperature with a maximum effect at 30 min. Compound **12i** showed no effect on ear pinna temperature when administered alone; however, it did diminish the increase in ear pinna temperature when administered 15 min prior to the administration of U-II.

In summary, a novel series of phenylpiperidine-benzoxazinone U-II receptor antagonists are described. The original HTS hit was successfully optimized to provide a low-nanomolar inhibitor against both rat and human UT. A Suzuki–Miyaura coupling allowed for the change in connectivity, which led to the discovery of **12i**. In vivo efficacy of **12i** was shown in the U-II-induced ear-flush model in rats. This model offers a convenient means to measure a pharmacodynamic response of a UT antagonist and thereby guage a compound's efficacy prior to advancement into time-consuming chronic disease models.

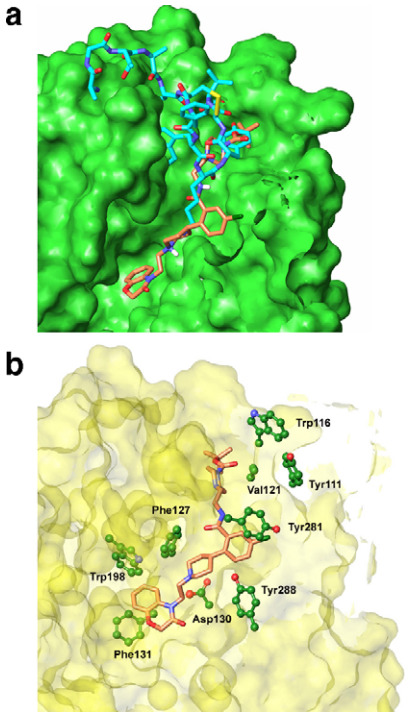


Figure 2. (a) Overlay of **12i** (orange) and goby U-II (blue) in a rat UT homology model. (b) Key residues in the UT receptor-binding pocket.

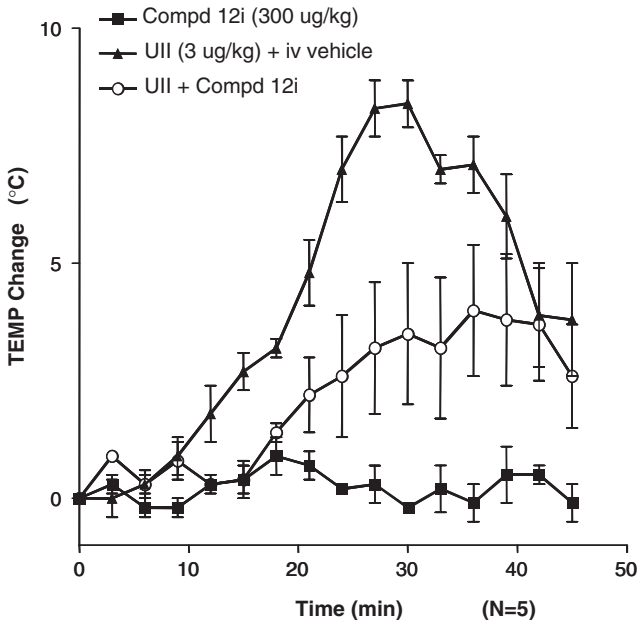


Figure 3. Effects of **12i** on ear pinna temperature changes. Compound **12i** was administered intravenously 15 min prior to the administration of U-II at a dose of 300 μg/kg. Data are mean temperature changes from baseline ± SEM ($n = 5$ per group).

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6. *Calcium mobilization*. Calcium mobilization was measured on the Molecular Devices FLIPR (Fluorescence Imaging Plate Reader) instrument in CHOK1 cells transfected with the rat UT receptor and loaded with Calcium Assay Kit (Molecular Devices). The test compound was added followed by 5 nM of U-II agonist peptide **1** and inhibition of response seen in the absence of compound was measured. IC_{50}s were calculated by using a log-logit plot of percent inhibition. See Ref. 2b for further details.
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9. *Whole cell binding*. I-125 U-II binding was determined in RMS13 cells. Compounds and I-125 U-II were co-incubated for 2.5 h. Following washing, the samples were solubilized with 1% SDS in 0.5 N NaOH and radioactivity determined. GraphPad Prism was used to analyze the binding data and K_i calculated via the Cheung–Prussoff equation. See Ref. 10 for further details.
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11. Purified by RP HPLC (gradient elution with 10–60% acetonitrile in water, each with 0.1% TFA) and lyophilized to yield Compound **12i** as white solid (trifluroacetate salt, 0.26 g, 72%). ^{1}H NMR (300 MHz, DMSO) δ 7.58–7.53 (m, 2H), 7.37 (d, $J = 8.5$ Hz, 1H), 7.30 (m, 1H), 7.12–7.05 (m, 3H), 4.70 (s, 2H), 4.3–3.1 (m, 21H), 2.0–1.8 (m, 4H), 1.42 (s, 9H); MS (ES^+) *m/z* 626.1 (M+1); Anal. Calcd for $C_{33}H_{44}ClN_5O_5$–3.6CF_3CO_2H: C, 46.58; H, 4.63; N, 6.76. Found: C, 46.25, H, 4.48; N, 6.73.
12. An optimized rat U-II homology model was constructed by using bovine rhodopsin X-ray crystal structure (PDB code 1L9H) as a template and following the same procedure described in Ref. 2b Molecular docking into the homology model was done using Glide (Schrodinger LLC, Portland, OR) with OPLS_2005 force field. Feature constraint was employed in docking, using Asp-130 as an anchor point. A basic nitrogen feature has to be fitted into the proximity of the carboxylic acid oxygen atoms.
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