1,3,4-Thiadiazol-2-amine Derivatives as Urotensin-II Receptor (UT) Antagonists

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Because urotensin-II (U-II), the most potent of all vasoconstrictors, is involved in the regulation of cardiovascular functions, it has received great attention as a potential therapeutic target.[1] U-II, a cysteine-linked cyclic peptide, is expressed in a variety of tissues, including blood vessels, heart, liver, kidney, skeletal muscle, and lung.[2] The effects of this peptide are mediated through its interaction with a G protein-coupled receptor known as GPR14 or the urotensin-II receptor (UT).[3] Upon binding of U-II to UT, complex signal transduction pathways are activated to control a wide range of physiological effects associated with cardiovascular activities including vasoconstriction, vasodilation, cell proliferation, and hypertrophy.[4] Furthermore, the results of several studies demonstrate that elevated U-II plasma levels and increased levels of UT expression are associated with numerous cardiorenal and metabolic diseases, including hypertension,[5] heart failure,[6] atherosclerosis,[7] diabetes,[8] and renal failure.[9] Therefore, UT has become one of the most promising therapeutic targets for the treatment of heart failure as well as a broad range of other cardiovascular maladies.[10]

Although scientists in a number of pharmaceutical companies have expended a great of effort to develop a variety of pharmacophore derivatives of UT antagonists,[11] a significant need still exists for potent and selective UT antagonists. In our continuing efforts,[12] we utilized a virtual screening approach using LigandScout 3.0 (inte:ligand) to uncover new chemical scaffolds that could serve as UT antagonists. This study led to the identification of 1,3,4-thiadiazole ureas possessing the aryloxymethyl group at C-5 as hit compounds. In an experimental investigation, we observed that the 2,4-dimethylphenoxymethyl derivative **1** exhibits a low UT binding affinity, which corresponds to 6.6% inhibition at 10 μM (Figure 1). With the aim of enhancing the binding activity, several substances were tested in which the phenylurea group attached to 1,3,4-thiadiazole moiety of **1** is altered. In particular, replacement of the phenylurea group with aminobenzyl group and further introduction of piperidin-4-yloxy group, which was expected to provide an additional interaction with U-II receptor, at the *para* position of aminobenzyl group resulted in a large increase in binding affinity. Based on these preliminary observations, we carried out the synthesis, biological evaluation, and structure–activity relationship (SAR) study of several 1,3,4-thiadiazol-2-amine derivatives. The results of this effort, in which a highly potent UT antagonist with a 0.13 μM of IC_{50} value was uncovered, are described below.

The general routes employed for the preparation of 1,3,4-thiadiazol-2-amine derivatives **2** are outlined in Scheme 1. Commercially available phenols **3** were treated with ethyl bromoacetate in the presence of potassium carbonate to produce the corresponding 2-aryloxy ethyl acetates **4**, which were then subjected to hydrolysis with 3N NaOH in methanol to yield the respective 2-aryloxy acetic acids **5**. Condensation reactions of **5** with thiosemicarbazide in phosphorus oxychloride gave 1,3,4-thiadiazol-2-amines **6**,[13] which then were protected with Boc (*tert*-butoxycarbonyl) group to afford **7** as key intermediates. In parallel sequences, 2-substituted phenols **9** were coupled with 1-Boc-4-hydroxypiperidine under Mitsunobu conditions to form the corresponding ethers **10**, which upon treatment with NBS (*N*-bromosuccinimide) and AIBN (2,2′-azobis(2-methylpropionitrile)) in carbon tetrachloride generated benzylic bromides **11**. The target compounds **2** were then produced by simple *N*-alkylation reactions of **7** with **11** in the presence of potassium carbonate followed by deprotection of *N*-Boc groups of the formed products **8** with 4M HCl in dioxane.

The binding affinities of the 1,3,4-thiadiazol-2-amine derivatives **2** to membranes of HEK293 cells expressing the human UT receptor were determined by conducting a competitive binding assay with Eu-labeled U-II and a time-resolved fluorometric (TRF) assay.[14] The initial SAR study focused on an exploration of substances containing a variety of substituents on the aryloxymethyl group at C-5 of the 1,3,4-thiadiazol-2-amine moiety. The results, summarized in Table 1, show that, while the unsubstituted member of this group **2a** has a moderate binding affinity toward UT (IC_{50} = 1.44 μM), an analog possessing a *m*-chloro group (**2c**) has a 1.3-fold increased UT binding activity compared to **2a**. However, repositioning the chloro substituent from the *meta* (**2c**) to the *ortho*

(**2b**) or *para* (**2d**) positions resulted in a slight reduction in the UT binding activity. In addition, binding affinities of fluoro substituted analogs **2e** and **2f** also displayed similar trends. The results of additional studies showed that, in general, the disubstituted analogs **2g–j** and **2n–p** had higher binding affinities than monosubstituted derivatives **2a–f**, except for the 2,4- (**2k**), 3,5- (**2l**), and 2,6- (**2m**) dichloro derivatives. The 3,4-dimethoxy derivative **2g** displayed an enhanced UT binding activity (IC_{50} = 0.87 μM) compared to that of **2a**. While the 3,4-difluoro analog **2i** exhibited a similar UT binding activity as that of **2g**, incorporation of two methyl groups at the C-3 and C-4 position (**2h**) led to a 2.1-fold increase in binding affinity. Importantly, the 3,4-dichloro analog **2j** was observed to have the most potent UT binding activity (IC_{50} = 0.13 μM) among all of the substances tested. In addition, other disubstituted derivatives such as 3-Me–4-Cl (**2n**), 3-Cl–4-F (**2o**), and 3-CF_3–4-F (**2p**) were found to have comparatively low binding affinities than **2j**. Finally, studies were carried out to probe the effects of substituents (*Z*) in place of chloro group at the *meta* position of 4-(piperidin-4-yloxy)benzyl moiety. The results demonstrate that removal (Z = H, **2q**) or replacing it with fluoro (**2r**) or methyl (**2s**) leads to large decreases in binding affinities compared to that of the chloro analog **2j**. These

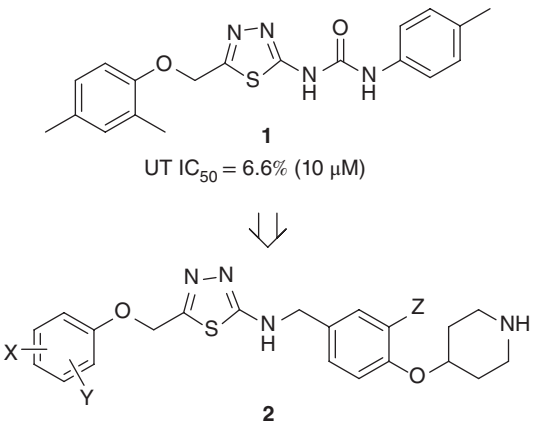
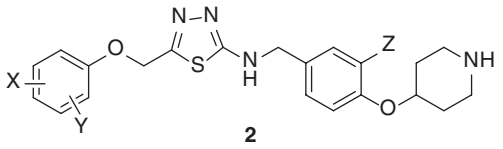


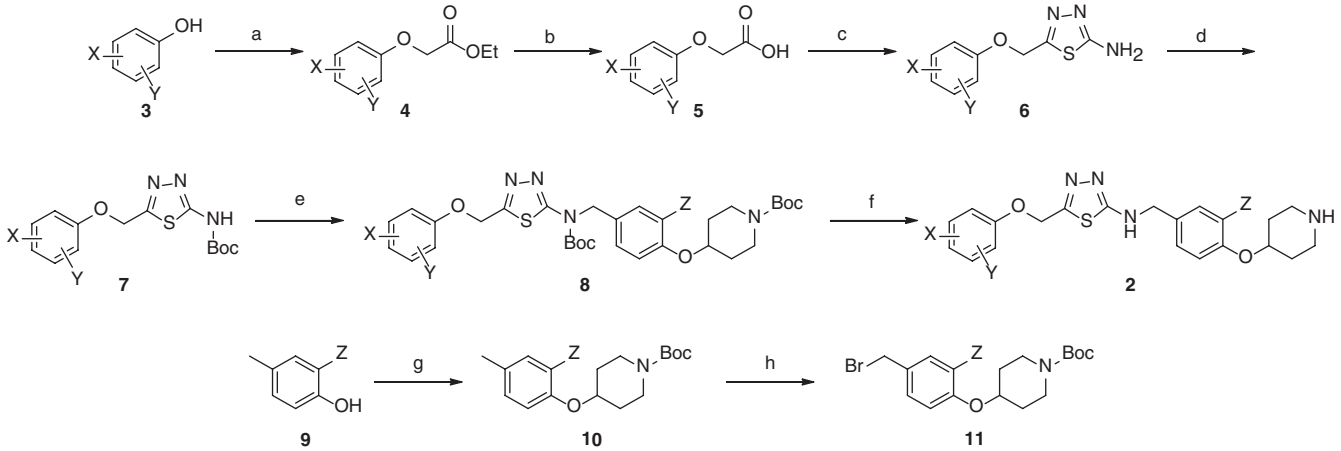
Figure 1. Structural modification of 1,3,4-thiadiazole based UT antagonists.

Table 1. Substituents effects on the UT binding affinity of 1,3,4-thiadiazol-2-amines.



Compound	X	Y	Z	UT IC_{50}[a,b] (μM)
2a	H	H	Cl	1.44
2b	2-Cl	H	Cl	1.12
2c	3-Cl	H	Cl	1.09
2d	4-Cl	H	Cl	1.31
2e	3-F	H	Cl	1.21
2f	4-F	H	Cl	1.49
2g	3-OMe	4-OMe	Cl	0.87
2h	3-Me	4-Me	Cl	0.41
2i	3-F	4-F	Cl	0.78
2j	3-Cl	4-Cl	Cl	0.13
2k	2-Cl	4-Cl	Cl	1.46
2l	3-Cl	5-Cl	Cl	2.17
2m	2-Cl	6-Cl	Cl	3.26
2n	3-Me	4-Cl	Cl	0.67
2o	3-Cl	4-F	Cl	0.64
2p	3-CF3	4-F	Cl	0.55
2q	3-Cl	4-Cl	H	2.53
2r	3-Cl	4-Cl	F	3.57
2s	3-Cl	4-Cl	Me	4.64

[a] UT binding affinities of compounds were determined by using a competitive binding with Eu-U-II and a TRF assay.
[b] Values are means of at least two measurements.



Scheme 1. (a) Ethyl bromoacetate, K_2CO_3, DMF, 100 °C, 2 h; (b) 3N NaOH, MeOH, rt, 1 h; (c) $POCl_3$, $NH_2CSNHNH_2$, reflux, 4 h; (d) $(Boc)_2O$, DMAP, THF, 50 °C, 20 h; (e) **11**, K_2CO_3, DMF, rt, 5 h; (f) 4 M HCl in 1,4-dioxane, rt, 1 h; (g) 1-Boc-4-hydroxypiperidine, DEAD, PPh_3, THF, rt, 5 h; (h) NBS, AIBN, CCl_4, reflux, 3 h.

results indicate that the property of substituents at the *meta* position of 4-(piperidin-4-yloxy)benzyl moiety might play a critical role in determining binding to UT.

In summary, the SAR studies described above led to the finding that of 1,3,4-thiadiazol-2-amine derivatives, containing *N*-(3-chloro-4-(piperidin-4-yloxy)benzyl) group, serve as potent UT antagonists. The results of this extensive optimization effort, probing the effects of aryloxymethyl group at C-5 on the 1,3,4-thiadiazol-2-amine moiety, led to the identification of the 3,4-dichloro analog **2j**, a highly potent UT antagonist with an IC_{50} value of 0.13 μM. Further studies are being carried out with the aim of improving the pharmacokinetic properties of the 1,3,4-thiadiazol-2-amine derivatives.

Experimental

Synthesis of ethyl 2-(3,4-dichlorophenoxy)acetate (4j). To a stirred solution of 3,4-dichlorophenol (**3j**, 2.0 g, 12.3 mmol) in *N*,*N*-dimethylformamide (50 mL) were added ethyl bromoacetate (2.0 mL, 18.4 mmol) and K_2CO_3 (3.4 g, 24.5 mmol). The reaction mixture was stirred at 100 °C for 2 h. After cooling, water (100 mL) was added, and the mixture was extracted with ethyl acetate (100 mL × 2). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (ethyl acetate/*n*-hexane = 1/20, v/v) to obtain the title compound (2.7 g, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, J = 9.1 Hz, 1H), 7.00 (s, 1H), 6.76 (d, J = 9.1 Hz, 1H), 4.59 (s, 2H), 4.24 (q, J = 7.2 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H).

Synthesis of 2-(3,4-dichlorophenoxy)acetic acid (5j). To a stirred solution of **4j** (2.5 g, 9.8 mmol) in MeOH (30 mL) was added 3N NaOH solution (13.1 mL, 39.3 mmol). After being stirred at room temperature for 1 h, the mixture was diluted with water (100 mL), acidified with 3N HCl, and extracted with dichloromethane (100 mL × 2). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give the title compound (2.12 g, 97% yield) . ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.49 (d, J = 8.8 Hz, 1H), 7.21 (s, 1H), 6.92 (d, J = 8.8 Hz, 1H), 4.73 (s, 2H).

Synthesis of 5-((3,4-dichlorophenoxy)methyl)-1,3,4-thiadiazol-2-amine (6j). A solution of **5j** (2.4 g, 11.0 mmol) and thiosemicarbazide (2.0 g, 22.0 mmol) in phosphorus oxychloride (30 mL) was stirred at reflux for 4 h. After cooling, water (50 mL) was added. After being stirred at reflux for 12 h, the mixture was cooled to room temperature and neutralized with 5N NaOH. The resulting solid was separated by filtration, washed with water, and dried *in vacuo* to give the title compound (2.9 g, 95% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.52 (d, J = 9.0 Hz, 1H), 7.36 (s, 1H), 7.03 (d, J = 9.0 Hz, 1H), 5.31 (s, 2H).

Synthesis of *tert*-butyl (5-((3,4-dichlorophenoxy) methyl)-1,3,4-thiadiazol-2-yl) carbamate (7j). To a stirred solution of **6j** (200 mg, 0.72 mmol) in THF (6.0 mL) were added di-*tert*-butyl dicarbonate (550 mg, 2.52 mmol) and DMAP (17 mg, 0.14 mmol). The mixture was stirred at 50 °C for 20 h. After cooling, water (50 mL) was added, and the mixture was extracted with ethyl acetate (50 mL × 2). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (ethyl acetate/*n*-hexane = 1/2, v/v) to obtain the title compound (168 mg, 63% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, J = 8.8 Hz, 1H), 7.10 (s, 1H), 6.85 (d, J = 8.8 Hz, 1H), 5.36 (s, 2H), 1.56 (s, 9H).

Synthesis of *tert*-butyl 4-(4-(((*tert*-butoxycarbonyl)(5-((3,4-dichlorophenoxy)methyl)-1,3,4-thiadiazol-2-yl) amino)methyl)-2-chlorophenoxy)piperidine-1-carboxylate (8j). To a stirred solution of **7j** (100 mg, 0.27 mmol) in *N*,*N*-dimethylformamide (1.0 mL) were added **11** (130 mg, 0.32 mmol) and K_2CO_3 (75 mg, 0.54 mmol). The mixture was stirred at room temperature for 5 h. After cooling, water (50 mL) was added, and the mixture was extracted with ethyl acetate (50 mL × 2). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (ethyl acetate/*n*-hexane = 1/3, v/v) to obtain the title compound (98 mg, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.29–7.36 (m, 2H), 7.11 (s, 1H), 6.86 (d, J = 8.6 Hz, 2H), 5.36 (s, 2H), 5.25 (s, 2H), 4.46–4.53 (m, 1H), 3.58–3.69 (m, 2H), 3.37–3.48 (m, 2H), 1.78–1.89 (m, 4H), 1.54 (s, 9H), 1.46 (s, 9H).

Synthesis of *N*-(3-chloro-4-(piperidin-4-yloxy)benzyl)-5-((3,4-dichlorophenoxy)methyl)-1,3,4-thiadiazol-2-amine (2j). A solution of **8j** (50 mg, 0.08 mmol) in 1.0 mL of HCl solution (4M in 1,4-dioxane) was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo* to give the title compound (34 mg, 94% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 (s, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 6.97 (s, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 5.13 (s, 2H), 4.62 (s, 2H), 4.54 (m, 1H), 3.60 (m, 2H), 3.44 (m, 2H), 1.84–1.87 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.7, 157.2, 154.0, 151.6, 132.6, 132.1, 131.6, 129.9, 128.2, 123.9, 123.0, 117.5, 116.7, 116.5, 71.0, 65.2, 63.5, 47.5, 27.3; HR-MS (EI): Calcd. for $C_{21}H_{21}Cl_3N_4O_2S$ $[M]^+$ 498.0451, Found 498.0451.

Synthesis of *tert*-butyl 4-(2-chloro-4-methylphenoxy) piperidine-1-carboxylate (10, Z = Cl). To a solution of 2-chloro-4-methylphenol **9** (Z = Cl) (500 mg, 3.50 mmol) and 1-Boc-4-hydroxypiperidine (1.1 g, 5.25 mmol) in THF (20 mL) were added diethyl azodicarboxylate (DEAD) (2.39 mL, 5.25 mmol, 40 wt % in toluene), and triphenylphosphine (1.38 g, 5.25 mmol). After being stirred at room temperature for 5 h, the mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL × 2). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The resulting residue was subjected to silica gel column chromatography (ethyl acetate/*n*-

hexane = 1/20, v/v) to give the title compound (1.0 g, 88% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (s, 1H), 7.00 (d, J = 8.3 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 4.43–4.51 (m, 1H), 3.63–3.75 (m, 2H), 3.36–3.48 (m, 2H), 2.29 (s, 3H), 1.76–1.95 (m, 4H), 1.49 (s, 9H).

Synthesis of *tert*-butyl 4-(4-(bromomethyl)-2-chlorophenoxy)piperidine-1-carboxylate (11, Z = Cl). A carbon tetrachloride (10 mL) solution of **10** (Z = Cl) (420 mg, 1.28 mmol), *N*-bromosuccinimide (251 mg, 1.41 mmol, NBS), and azobisisobutyronitrile (21 mg, 0.13 mmol, AIBN) was stirred at reflux for 3 h. The mixture was concentrated under reduced pressure, giving a residue that was subjected to silica gel column chromatography (ethyl acetate/*n*-hexane = 1/20, v/v) to afford the title compound (350 mg, 67% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.25 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 4.58 (m, 1H), 4.45 (s, 2H), 3.68 (m, 2H), 3.50 (m, 2H), 1.89–1.91 (m, 4H), 1.49 (s, 9H).

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Supporting Information. Experimental procedures and spectral data of compounds **2a–2s.** This material can be found in the online version.

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