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Synthesis and SAR of 5-aryl-furan-2-carboxamide derivatives as potent urotensin-II receptor antagonists

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ABSTRACT

The synthesis and biological evaluation as potential urotensin-II receptor antagonists of a series of 5-arylfuran-2-carboxamide derivatives **1**, bearing a 4-(3-chloro-4-(piperidin-4-yloxy)benzyl)piperazin-1-yl group, are described. The results of a systematic SAR investigation of furan-2-carboxamides with C-5 aryl groups possessing a variety of aryl ring substituents led to identification of the 3,4-difluorophenyl analog **1y** as a highly potent UT antagonist with an IC_{50} value of 6 nM. In addition, this substance was found to display high metabolic stability, and low hERG inhibition and cytotoxicity, and to have an acceptable PK profile.

Heart failure, in which abnormalities exist in both heart contraction and relaxation, has become a global pandemic. Incidences of this disease are continuously growing owing to the increase in the average age of the world population and the number of cardiovascular risk factors.[1] Among many others, the urotensin-II (U-II) and urotensin-II receptor (UT) system has received great attention as a therapeutic target for treatment of heart failure because of the pivotal role it plays in the regulation of cardiovascular functions.[2] U-II, composed of a cyclic neuropeptide bridged by cysteine, is also known to be one of the most potent vasoconstrictor.[3] This peptide is expressed in a variety of tissues, including blood vessels, heart, liver and kidney.[4] The effects of U-II are regulated by its binding to UT,[5] upon which it exerts complex signal transduction that induces a variety of physiologically cardiovascular responses including vasoconstriction, vasodilation, cell proliferation and hypertrophy.[6] In addition, observations made in a number of previous basic pharmacological and clinical studies demonstrate that expression of UT is low or undetectable in normal myocardium. In contrast, both the plasma concentration of U-II and amount of tissue expression of U-II and UT are greatly increased in numerous cardiorenal and metabolic diseases, including hypertension,[7] heart failure,[8] atherosclerosis,[9] diabetes[10] and renal failure.[11] The results suggest that the U-II and UT system could be implicated in the pathogenesis of cardiovascular disease.[12] Additional investigations have demonstrated that several UT antagonists improve cardiac hypertrophy and cardiac dysfunction in various animal models.[13] Therefore, antagonism of UT is considered to be one of the most promising therapeutic strategies for treatment of heart failure as well as a wide range of other cardiovascular diseases.[14] As a result, a significant effort has been made by a number of pharmaceutical companies to develop diverse UT antagonists.[15] To date, only few candidates uncovered in these investigations, including ACT-058362,[16] GSK-1440115[17] and SAR-101099 (structure undisclosed),[18] have reached the clinical trial stage for treatment for asthma and diabetic nephrophathy. However, all of these trials were discontinued owing to lack of efficacy of the substances in humans (Fig. 1). Consequently, a great need exists for the development of potent and selective UT antagonists.

In a previous study, we discovered that members of the benzo[*b*]thiophene and indole-2-carboxamide bearing *N*-(1-(3-bromo-4-(piperidin-4-yloxy)benzyl)piperidin-4-yl) families serve as potent UT antagonists.[19] In addition, an extensive structure–activity relationships (SAR) study led to the finding that a 5-cyano benzo[*b*]thiophene (IC_{50} = 25 nM) and 1-phenyl indole (IC_{50} = 20 nM) analog exhibit high UT binding affinities. In a continuation of this effort aimed at developing novel and potent UT antagonists as antihypertrophic agent,[20] we observed that 5-arylfuran-2-carboxamides **1** (Fig. 2), containing *N*-benzyl substituted piperazine moieties, have potent UT binding affinities. The results of an extensive investigation focused on the synthesis, biological evaluation and SAR study of substances in this family are described below.

The general route employed for preparation of the substituted 5-

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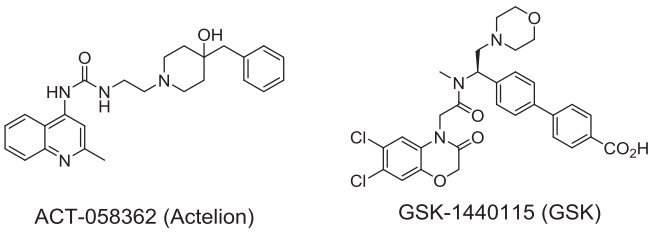
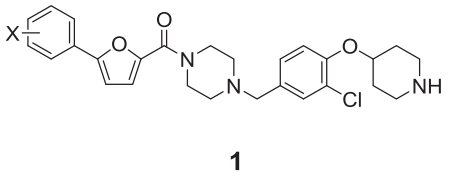


Fig. 1. Representative UT antagonists.



1

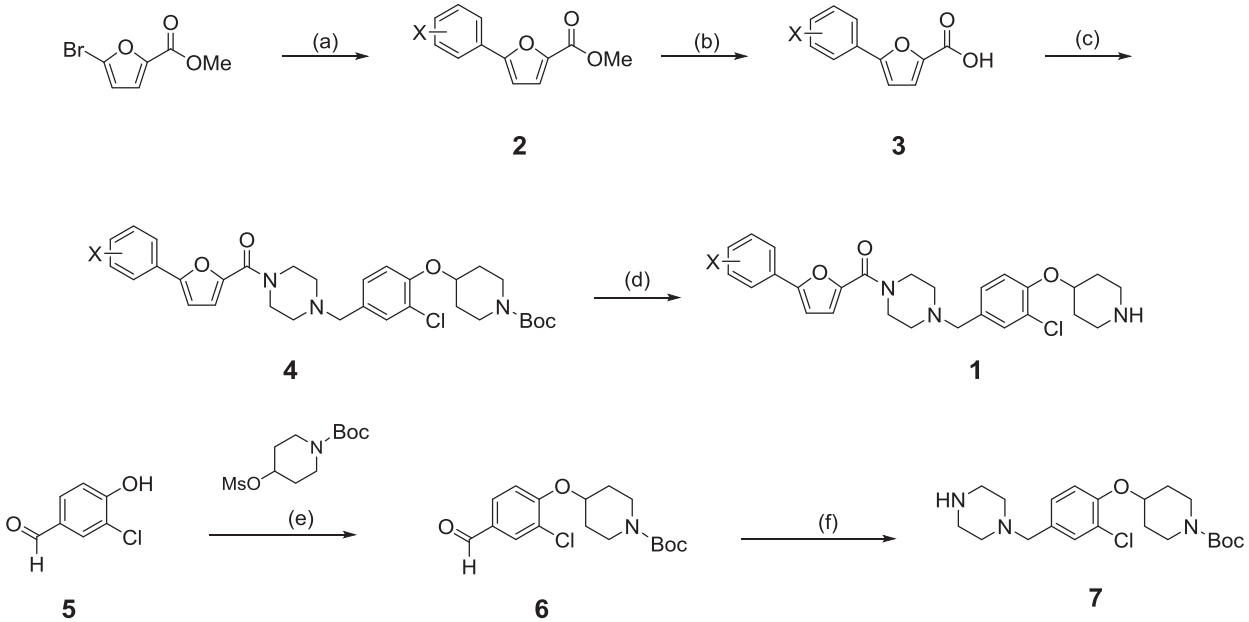
Fig. 2. 5-Arylfuran-2-carboxamide derivatives.

arylfuran-2-carboxamide derivatives **1** is outlined in Scheme 1. The sequence began from reactions of commercially available methyl 5-bromofuran-2-carboxylate with a variety of aryl boronic acids using Suzuki coupling conditions to form the corresponding methyl 5-aryl-substituted furan-2-carboxylates **2**. Hydrolysis of the esters **2** using 3 M NaOH in MeOH formed the respective carboxylic acids **3** in high yields. In a parallel sequence employed to synthesize the coupling partner for introduction of the piperazine moiety in the targets, 3-chloro-4-hydroxybenzaldehyde **5** was reacted with *tert*-butyl 4-((methylsulfonyl) oxy)piperidine-1-carboxylate in the presence of potassium carbonate to generate *tert*-butyl 4-(2-chloro-4-formylphenoxy)piperidine-1-carboxaldehyde **6**. Reductive amination of **6** with piperazine using sodium triacetoxyborohydride in tetrahydrofuran produced 1-(3-chloro-4-(piperidin-4-yloxy)benzyl)piperazine **7**. Carboxylic acids **3** were converted to the corresponding chlorides *in situ* using thionyl chloride, which then underwent amide coupling reactions with the piperazine derivative **7** in the presence of triethylamine to produce amides **4**. Subsequent removal of the *N*-Boc groups in **4** by treatment with conc. HCl in 1,4-dioxane generated the targets **1**.

An SAR investigation was conducted to assess the effects of substituents on the aryl moiety of 5-arylfuran-2-carboxamide derivatives **1** on UT binding affinities. Binding affinities of these substances to membranes of HEK293 cells expressing the human UT receptor were determined by using a competitive binding with Eu-labeled U-II and a time-resolved fluorometric (TRF) assay.[21] As can be seen by viewing the data in Table 1, the unsubstituted 5-arylfuran-2-carboxamide derivative **1a** has a moderate UT binding affinity (IC_{50} = 48 nM). Introduction of a fluoro group at the *meta*-position (**1c**) leads to a 5.3-fold increase in the UT binding affinity (IC_{50} = 9 nM). In contrast, substances having a fluoro group at *ortho*- (**1b**) and *para*- (**1d**) positions display relatively low UT binding affinities as reflected in their respective IC_{50} values of 93 and 68 nM. In addition, similar trends are observed for the chloro (**1e–g**) and cyano (**1h–j**) substituted analogs with the *meta*-substituted derivatives having the highest binding affinities (*m*-chloro (**1f**) IC_{50} = 20 nM, *m*-nitrile (**1i**) IC_{50} = 40 nM). Other substances, including those bearing *m*-nitro (**1k**), *m*-phenyl (**1m**) and *m*-methoxy (**1o**) substituted aryl groups, have higher UT binding affinities than their respective *para*-substituted counterparts **1l**, **1n**, and **1p**. Interestingly, 5-arylfuran-2-carboxamide derivatives containing *ortho*-alkyl and -alkoxy groups (**1q–1u**) have high UT binding affinities with IC_{50} values falling between 14 and 18 nM.

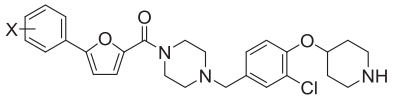
The effects on UT binding of multiple aryl ring substituents on the 5-aryl group of the furan-2-carboxamides were also assessed. The results show that the 3,4-dimethoxy (**1aa**) and 3,4-dichloro (**1ab**) aryl derivatives have UT binding affinities that are close to that of the parent **1a**. Likewise, analogs bearing 2,3- (**1v**), 2,4- (**1w**), 2,5- (**1x**) and 3,5- (**1z**) difluoro phenyl groups, and one containing a 3,4,5-trifluorophneyl group (**1ac**) display binding affinities in the range of IC_{50} value between 15 and 61 nM. In stark contrast, the 3,4-difluoro analog **1y** was found to exhibit the most potent UT binding affinity (IC_{50} = 6 nM) among all of the substances tested.[22]

Furthermore, antagonistic activities of 5-arylfuran-2-carboxamide derivatives **1** were also evaluated by measuring the change of U-II-induced intracellular calcium concentration in HEK293-aeq/UT cells.[23] As shown in Table 1, all of the derivatives showed a typical concentration-dependent antagonist response with IC_{50} ranging from 50 to 470 nM except for **1n**. Among those tested, **1e** has the most potent inhibition with IC_{50} value of 50 nM. In addition, **1y** also exhibited



Scheme 1. Reagents and conditions: (a) $ArB(OH)_2$, $Pd(PPh_3)_4$, 3 N Na_2CO_3, 1,4-dioxane, 100 °C, 5 h; (b) 3 N NaOH, MeOH, rt, 30 min.; (c) (i) $SOCl_2$, 1,2-dichloroethane, 120 °C, 2 h. (ii) **7**, Et_3N, dichloromethane, rt, 30 min.; (d) 4 M HCl, 1,4-dioxane, rt, 2 h.; (e) K_2CO_3, DMF, 80 °C, 19 h.; (f) (i) piperazine, Na_2SO_4, THF, rt; (ii) $NaBH(OAc)_3$, THF, 60 °C, 1 h.

Table 1
Binding affinities and antagonistic activity of 5-arylfuran-2-carboxamide derivatives **1** to membranes of HEK293 cells expressing the human UT receptor.



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Compound	X	Urotensin-II receptor binding affinity IC_{50}[a,b] (nM)	Antagonistic activity IC_{50}[b,c] (nM)
1a	H	48	230
1b	2-F	93	80
1c	3-F	9	110
1d	4-F	68	90
1e	2-Cl	33	50
1f	3-Cl	20	110
1g	4-Cl	66	370
1h	2-CN	48	90
1i	3-CN	43	280
1 j	4-CN	120	130
1k	3-NO_2	26	70
11	4-NO_2	31	70
1m	3-Ph	24	470
1n	4-Ph	60	1210
1o	3-OMe	15	80
1p	4-OMe	27	80
1q	2-Me	18	150
1r	2-CF_3	15	100
1s	2-Et	14	320
1t	2-OEt	18	120
1u	2-O/Pr	15	130
1v	2,3-di-F	15	90
1w	2,4-di-F	27	120
1x	2,5-di-F	24	260
1y	3,4-di-F	6	110
1z	3,5-di-F	61	260
1aa	3,4-di-OMe	34	160
1ab	3,4-di-Cl	38	240
1ac	3,4,5-tri-F	37	330

[a] Urotensin-II receptor binding affinities of derivatives were determined by using competitive binding with Eu-U-II and a TRF assay.
[b] Values are means of at least two measurements.
[c] The antagonistic activities of the derivatives to the urotensin-II receptor (UT) were determined by change of calcium mobilization in HEK293-aeq/UT cells. Detailed procedure of assay was described in Ref. 23.

moderate UT inhibition with IC_{50} value of 110 nM. This different potency between IC_{50} values in UT binding assay and calcium mobilization assay in HEK293-aeq/UT cells could be explained by physicochemical properties of derivatives such as cell permeability, solubility and stability in culture media.

Owing to its remarkably high binding affinity, the 5-(3,4-difluoropheny)furan-2-carboxamide **1y** was subjected to an evaluation of its metabolic stability, hERG, cytotoxicity and in vivo pharmacokinetic profile. As the results in Table 2 demonstrate, **1y** displays high metabolic stability in both human and rat liver microsomes. Additionally, **1y** has a low a hERG binding activity and it does not display cytotoxicity toward VERO, L929, NIH 3T3 and CHO-K1 cell lines. Finally, the results of an iv/po pharmacokinetic study (10 mg/kg) (Table 2) show that **1y** has an acceptable half-life and clearance, and it displays moderate oral bioavailability.

In summary, the study described above demonstrated that 5-arylfuran-2-carboxamide derivatives serve as novel UT antagonists. The results of a systematic optimization study, probing the effects of a variety of substituents on the 5-aryl group of 5-arylfuran-2-carboxamide derivatives, led to the finding that the 3,4-difluorophenyl analog

Table 2
Stability, hERG, cytotoxicity and in vivo pharmacokinetic profile of **1y**.

Assay	Results **1y**
Liver microsomal stability (human)[a]	98.2
Liver microsomal stability (rat)[a]	95.8
hERG[b]	4.0
Cytotoxicity[c]	VERO: 22.5 L929: 26.9 NIH 3T3: 34.0 CHO-K1: 29.7
In vivo PK[d]	
$t_{1/2}$ (h)	4.7
Oral AUC (pg h/mL)	0.6
iv CL (mL/kg min)	3.6
F (%)	19

[a] % original compound remained after 30 min incubation.
[b] IC_{50} (μM) values (binding assay).
[c] IC_{50} (μM) values in various mammalian cell lines. Cell information. VERO: African green monkey kidney cell line, L929: mouse fibroblast cell line, NIH 3T3: mouse embryonic fibroblast cell line, CHO-K1: Chinese hamster ovary cell line.
[d] Determined in rats by administration of 10 mg/kg, iv and po (n = 3).

1y is a highly potent UT antagonist with an IC_{50} value of 6 nM. In addition, this substance was observed to display good metabolic stability, hERG, cytotoxicity and an acceptable PK profile. Further studies evaluating the in vivo efficacy of **1y** in animal models will be performed in due course.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.12.058.

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22. Characterization data for compound **1y**: ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.70–7.78 (m, 2H), 7.61–7.67 (m, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.26 (d, J = 3.7 Hz, 1H), 7.03 (d, J = 3.7 Hz, 1H), 4.92–4.98 (m, 1H), 4.74–4.83 (m, 2H), 4.40 (s, 2H), 3.50–3.66 (m, 4H), 3.39–3.49 (m, 2H), 3.23–3.32 (m, 4H), 2.18–2.27 (m, 2H), 2.08–2.17 (m, 2H).
23. *Calcium mobilization assay in HEK293-aeq/UT cells*: A functional assay based on the luminescence of mitochondrial aequorin. Before the day of experiment, HEK293-aeq/UT cells at optimum growth were transferred onto black bottom 96 well plates at a density of 1×10^6 cells/ml with 100 μl of growth medium. After overnight incubation at room temperature in the dark with 5 μM of coelenterazine with constant agitation according to the manufacturer's instructions, the cells were diluted with assay buffer (1×10^5 cells·mL^{-1}) and incubated for 60 min. To measure antagonistic activity, cells were pretreated with derivatives for 15 min and 50 μl of U-II (0.1 μM) was injected. The light emission was recorded to determine cell activation using a Mithras LB 940 Multilabel Reader (Berthold Technologies, Bad Wildbad, Germany) and results were expressed in relative luminescence units.