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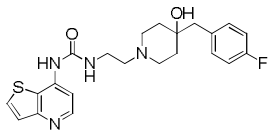

6n IC_{50} = 13 nM

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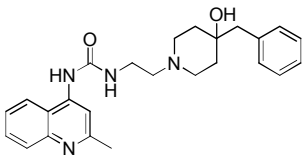
ABSTRACT

The preparation and SAR profile of thieno[3,2-*b*]pyridinyl urea derivatives as novel and potent urotensin-II receptor antagonists are described. An activity optimization study, probing the effects of substituents on thieno[3,2-*b*]pyridinyl core and benzyl group of the piperidinyl moiety, led to the identification of *p*-fluorobenzyl substituted thieno[3,2-*b*]pyridinyl urea **6n** as a highly potent UT antagonist with an IC_{50} value of 13 nM. Although **6n** displays good metabolic stability and low hERG binding activity, it has an unacceptable oral bioavailability.

Urotensin-II (U-II) is a cysteine-linked cyclic peptide that is expressed in a variety of tissues, including blood vessels, heart, liver, kidney, skeletal muscle, and lung.[1] U-II is known to be the most potent vasoconstrictor, displaying a 10 times greater potency than that of endothelin.[2] U-II is an endogenous ligand of the G protein-coupled receptor known as a GPR14 or the urotensin-II receptor (UT).[3] Activation of UT by binding of U-II promotes complex signal transduction pathways that control a wide range of physiological effects including vasoconstriction, vasodilatation, cell proliferation and hypertrophy.[4] Particularly interesting are UT's roles in cardiovascular functions that also include modulation of cardiac contractility, cardiomyocyte hypertrophy and fibrosis. These effects suggest that U-II and its receptors are involved in the pathogenesis of cardiovascular disease.[5] Furthermore, several studies have demonstrated that elevated plasma levels of U-II and increased levels of UT expression are associated with numerous cardiorenal and metabolic diseases, including hypertension,[6] heart failure,[7] atherosclerosis,[8] diabetes,[9] and renal failure.[10] Therefore, UT has emerged as one of the most promising therapeutic targets for treating heart failure as well as a broad range of other cardiovascular maladies. Since the discovery at Actelion that Palosuran[11,12] (**Fig. 1**) is a small molecule UT antagonist for cardiovascular indications, which reached phase II clinical stage for diabetic nephropathy in 2004 but ceased due to lack of efficacy, a variety of pharmacophore derivatives of UT antagonists have been developed.[13] In particular, urea based UT antagonists have been described by several research groups.[14]

As part of a continuing program aimed at the development of novel and potent UT antagonists,[15] we carried out a structure activity relationship (SAR) study of newly prepared 5-membered heteroaryl fused pyridinyl urea derivatives (see for example Table 1) that have the basic backbone structure of Palosuran. An initial effort focused on substances containing a modified pyridine core and explored the effects of 5-membered heteroaryl rings to the pyridine core (**1-7** in **Table 1**). The results show that the pyrrolo[2,3-*b*]pyridinyl derivative **1** exhibits moderate UT



UT hIC_{50} = 3.6 nM

Figure 1. Structure of Palosuran

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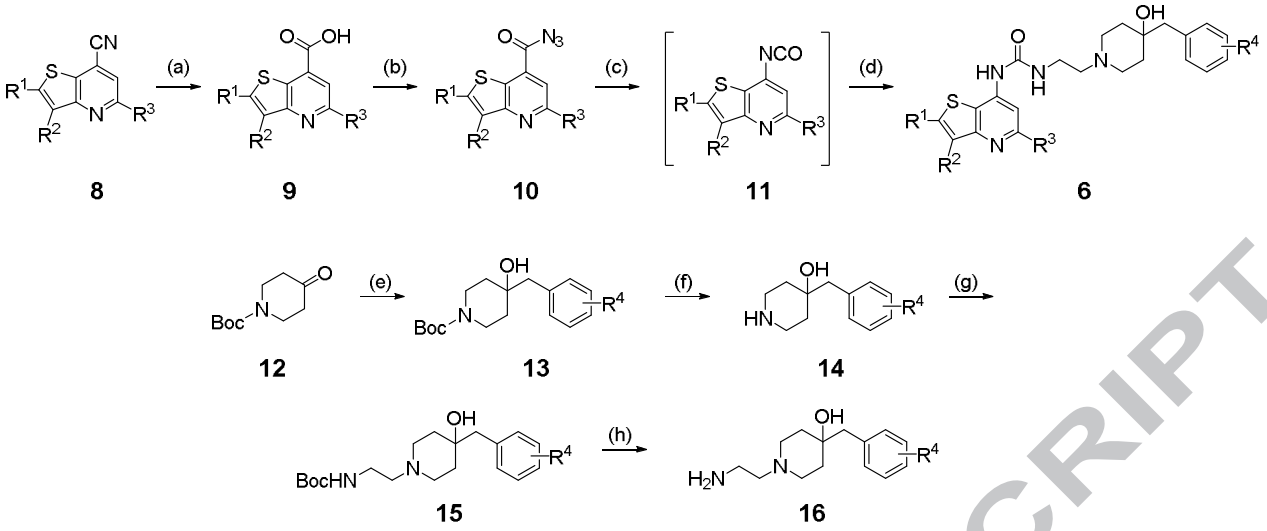
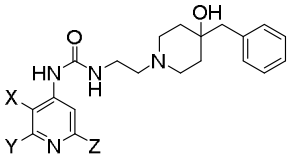


Scheme 1. Reagents and conditions: (a) 5N NaOH, EtOH, 90 °C; (b) diphenylphosphoryl azide (DPPA), Et_3N, DMF; (c) toluene, reflux; (d) **16**, Et_3N, dichloromethane; (e) R^4-benzylmagnesium chloride, THF; (f) conc HCl, 1,4-dioxane; (g) *tert*-butyl (2-bromoethyl)carbamate, K_2CO_3, NaI, DMF, 80 °C. (h) conc HCl, 1,4-dioxane.

Table 1. UT binding affinities of 5-membered heteroaryl fused pyridinyl urea derivatives



Compound	X	Y	Z	UT IC_{50} (nM)[a,b]
1			H	160
2			H	600
3			Me	220
4			H	2,140
5			H	450
6			H	36
7			H	930

[a] UT binding affinities were determined by using a competitive binding with Eu-U-II and a TRF assay.

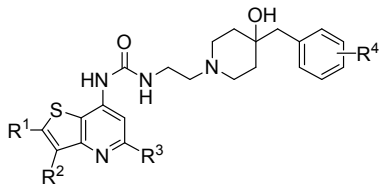
[b] Values are means of at least two measurements.

binding activity, whereas the pyrrolo[3,2-*b*] analog **2** has a 3.8-fold lower binding affinity compared to **1**. Futhermore, the related 1-methyl-1*H*-pyrazolo[3,4-*b*] (**3**), 3-methyl-3*H*-imidazo[4,5-*b*] (**4**) and furo[3,2-*b*] (**5**) fused pyridinyl analogs display largely decreased UT binding activities. Among the substances tested, thieno[3,2-*b*]pyridinyl analog **6** has the most highly potent UT binding activity (IC_{50} = 36 nM) which is 4.4-fold greater than that of **1**. In contrast, movement of the thiophene group from the [3,2-*b*] pyridine position in **6** to the [2,3-*b*] position in **7** results in a large loss in binding activity. Based on the preliminary results, the thieno[3,2-*b*]pyridinyl urea system was selected as the structural scaffold for the design, synthesis and SAR evaluation of novel UT antagonists.[15a]

The general synthetic route employed for the preparation of thieno[3,2-*b*]pyridinyl ureas **6** is outlined in **Scheme 1**. Nitriles **8**, serving as key intermediates in the pathways, were prepared starting with commercially available thieno[3,2-*b*]pyridine-7-ol using previously reported methods.[16] Treatment of the substituted thieno[3,2-*b*]pyridinyl carbonitriles **8** with 5N NaOH formed the corresponding carboxylic acids **9**, which upon reaction with diphenylphosphoryl azide/triethylamine gave the corresponding acyl azides **10**. In parallel sequences, 4-benzyl substituted piperidin-4-ols **13** were prepared by reaction of 1-boc-4-piperidone **12** with various benzyl Grignard reagents. Removal of the *N*-boc groups in **13** using conc HCl produced the corresponding piperidines **14**, which underwent alkylation with *tert*-butyl (2-bromoethyl)carbamate/ potassium carbonate to form the *tert*-butyl [2-(4-benzyl-4-hydroxypiperidin-1-yl)ethyl]carbamates **15**. Subsequent *N*-boc removal with conc HCl generated the 1-(2-aminoethyl)-4-benzyl piperidin-4-ols **16**. Completion of the routes to the target ureas **6** was accomplished by reaction of the thieno[3,2-*b*]pyridinyl isocyanates **11**, generated *in situ* by Curtius rearrangement of **10,** with amines **16**.

The binding affinities of the thieno[3,2-*b*]pyridinyl ureas to membranes of HEK293 cells expressing human UT receptor were determined by using a competitive binding assay with Eu-labeled U-II and a time-resolved fluorometric (TRF) assay.[17,18]

Table 2. Effects of substituents of thieno[3,2-*b*]pyridinyl urea derivatives on UT binding affinity



6

Compound	R^1	R^2	R^3	R^4	UT IC_{50}[a,b] (nM)
6	H	H	H	H	36
6a	Me	H	H	H	78
6b	Ph	H	H	H	47.3%[c]
6c	H	Br	H	H	3,220
6d	H	Me	H	H	1,230
6e	H	H	Cl	H	25.6%[c]
6f	H	H	Me	H	26
6g	H	H	Et	H	60
6h	H	H	Ph	H	25.4%[c]
6i	H	H	H	2-Me	20
6j	H	H	H	3-Me	140
6k	H	H	H	4-Me	190
6l	H	H	H	2-Br	20
6m	H	H	H	3-Br	42
6n	H	H	H	4-F	13

[a] UT binding affinities were determined by using a competitive binding with Eu-U-II and a TRF assay.

[b] Values are means of at least two measurements.

[c] Values are means of inhibition at 10 μM.

The SAR investigation began by exploring the effects on binding affinities of substituents (R^1, R^2 and R^3) on the thieno[3,2-b]pyridine structure. As the results in **Table 2** show, **6a** with a methyl group at the 2-position exhibits a 2-fold reduced UT binding affinity compared to that of the unsubstituted analog **6**. Additionally, introduction of a bulky phenyl group (**6b**) at this position results in a large decrease in binding affinity. 3-Bromo (**6c**) and 3-methyl (**6d**) substituted analogs also do not display improved binding activities. While the urea derivative containing chlorine (**6e**) at the 7-position displays low UT binding, introduction of a methyl group (**6f**) results in a highly potent UT binding activity (IC_{50} = 26 nM). As the size of alkyl group increases from methyl (**6f**) to ethyl (**6g**) and phenyl (**6h**), a decrease in UT binding activity occurs. A further exploration was conducted to evaluate the effects of substituents R^4 on the benzyl group of piperidinyl moiety. The *o*-methyl (**6i**) analog was found to have a 1.8-fold more potent UT binding affinity (IC_{50} = 20 nM) compared to **6**. Repositioning the methyl substituent from the *ortho* (**6i**) to the *meta* (**6j**) and para-(**6k**) positions leads to a large decrease in UT binding. Additionally, the bromine containing analogs **6l** and **6m** have high binding affinities and display similar trends. In comparison with *p*-methyl analog **6k**, the *p*-fluoro analog **6n** was found to have the most potent UT binding activity (IC_{50} = 13 nM) among all substances tested.

The properties of the *p*-fluorobenzyl substituted thieno[3,2-*b*]pyridinyl urea **6n** were further evaluated. This substance was observed to have good metabolic stability in human and rat liver microsomes (52% and 100% for 30 min, respectively). In addition, **6n** does not inhibit cytochrome P450 enzymes 3A4 (>10% at 10 μM) and it has a low hERG binding activity determined utilizing a patch clamp assay (IC_{50} = 5 μM). Furthermore, the results of a iv/po pharmacokinetic study (10 mg/kg) show that **6n** displays an acceptable clearance (Cl = 16 mL/min/kg) and half-life ($t_{1/2}$ = 5.1 h). However, **6n** exhibits low oral bioavailability (F = 5%), probably resulting from poor permeability (2.7 x 10^{-8}cm/s, PAMPA).

In summary, the results of the initial SAR studies have led to the identification of novel thieno[3,2-*b*]pyridinyl urea derivatives as UT antagonists. A systematic binding optimization study, in which substituents on the thieno[3,2-*b*]pyridine core and the benzyl group of the piperidine moiety, resulted in the identification of the *p*-fluorobenzyl substituted thieno[3,2-*b*]pyridine urea **6n** as a highly potent UT antagonist. This substance also displays good metabolic stability, low CYP450 3A4 enzyme and acceptable hERG activity. Further investigations of thieno[3,2-*b*]pyridinyl urea derivatives, concentrating on the improvement of PK profile, are now in progress.

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Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:

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18. Filtration-based TRF receptor binding assays with europium-labeled Urotensin-II (Eu-U-II) were performed in 96-well AcroWell™ plates with GHP membrane (PALL Life Sciences, Ann Arbor, MI, USA) by incubating 10 µg/well HEK293-hUT membranes with 2 nM of Eu-U-II in a total assay volume of 100 µL. U-II was labeled with europium (Eu) at N1 position by the PerkinElmer labeling service (Waltham, MA, USA) and hUT membranes were prepared from human embryonic kidney (HEK293) cells, stably expressing human urotensin-II receptor (hUT2R). The assay buffer contained 25 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% bovine serum albumin pH 7.4. Non-specific Eu–labeled hUT binding was determined experimentally by the presence of 1 µM unlabeled U-II. After incubation at room temperature for 90 min, the mixtures were filtered and washed in the automatic vacuum filtration system for filter plates. Europium was dissociated from the bound ligand by the addition of 150 µL of DELFIA enhancement solution (PerkinElmer Oy) and incubated for 15 min with shaking. Dissociated europium created highly fluorescent complexes, which were analyzed in a multilabel counter with a TRF option (Envision, PerkinElmer). The counter setting was excitation at 340 nm, 200 µs delay, and emission collection for 400 µs at 615 nm. The extent of antagonism was expressed as % displacement. The IC_{50} value was characterized in an 8-dose response study to generate the compound concentration required to yield 50% displacement.