Bioorganic & Medicinal Chemistry Letters 18 (2008) 4936–4939

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

N-Alkyl-5*H*-pyrido[4,3-*b*]indol-1-amines and derivatives as novel urotensin-II receptor antagonists

Yonghui Wang [a,*], Zining Wu [c], Brian F. Guida [c], Sarah K. Lawrence [c], Michael J. Neeb [b], Ralph A. Rivero [a], Stephen A. Douglas [b], Jian Jin [b,*]

[a] *Oncology CEDD, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[b] *Cardiovascular and Urogenital CEDD, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA*
[c] *Molecular Discovery Research, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*

ARTICLE INFO

Article history:
Received 11 July 2008
Revised 13 August 2008
Accepted 15 August 2008
Available online 22 August 2008

Keywords:
Urotensin-II receptor antagonist
UT antagonist
Pyridoindole amine
Competitive
Reversible
Functional antagonist

ABSTRACT

High throughput screening of our compound collection led to the discovery of a novel series of *N*-alkyl-5*H*-pyrido[4,3-*b*]indol-1-amines as urotensin-II receptor antagonists. Synthesis, initial structure and activity relationships, functional and animal ortholog activities of the series are described.

Human urotensin-II (hU-II), the most potent mammalian vasoconstrictor identified to date,[1] and its cognate receptor hUT (formerly known as human GPR-14) are proposed to be involved in the (dys)regulation of cardiorenal function.[2] Together, they have been implicated in the etiology of numerous cardiorenal and metabolic diseases including hypertension,[3] heart failure,[4,5] atherosclerosis,[6] renal failure,[7] and diabetes.[8] The impressive pharmacological activity of U-II has stimulated a great deal of interest in developing small molecule UT modulators. Several non-peptidic UT ligands (**1–3**, Fig. 1) have recently been reported.[9,10] Herein, we describe the discovery of a novel series of *N*-alkyl-5*H*-pyrido[4,3-*b*]indol-1-amines as UT antagonists.

High throughput screening (HTS) of our compound collection using a fluorometric imaging plate reader (FLIPR) assay (measuring inhibition of hU-II induced $[Ca^{2+}]_i$-mobilization in HEK293 cells expressing human recombinant UT receptor)[11] identified compound **4** as a submicromolar antagonist ($pIC_{50} = 6.3$). The tricyclic compound **4** also showed good hUT binding affinity ($pK_i = 8.1$) in a [^{125}I]hU-II radioligand binding assay using HEK293 cell membranes stably expressing human recombinant UT receptors.[11] The hUT binding assay was used as the primary assay for SAR exploration.

The synthesis of *N*-alkyl-5*H*-pyrido[4,3-*b*]indol-1-amines started with commercially available phenylhydrazine **5** and 4-hydroxy-2(1*H*)-pyridinone **6** (Scheme 1). Heating **5** and **6** in diphenyl ether (DPE) gave 4-(2-phenylhydrazino)-2(1*H*)-pyridinone, which underwent Fischer indole cyclization under reflux condition to afford 2,5-dihydro-1*H*-pyrido[4,3-*b*]indol-1-one **7**.[12] Refluxing **7** in neat $POCl_3$, followed by HCl treatment produced the key intermediate, 1-chloro-5*H*-pyrido[4,3-*b*]indole **8**. Nucleophilic aromatic substitution of **8** with a variety of amines under conventional or microwave heating led to 5*H*-pyrido[4,3-*b*]indol-1-amines **9**.[12] *N*-Alkylation of **8** with alkyl halides under ultrasonic condition formed 1-chloro-5-alkyl-5*H*-pyrido[4,3-*b*]indoles **10**,[13] which upon nucleophilic aromatic substitution with different amines afforded 5-alkyl-5*H*-pyrido[4,3-*b*]indol-1-amines **11**.

N-Alkyl-1*H*-pyrido[2,3-*b*]indol-4-amines **14** were prepared from 4-chloro-9*H*-pyrido[2,3-*b*]indoles **13** via nucleophilic aromatic substitution with *N*-alkylamines (Scheme 2). The 4-chloro compounds **13** can be synthesized from 9*H*-pyrido[2,3-*b*]indole 1-oxides which can be made from the corresponding 9*H*-pyrido[2,3-*b*]indoles or α-carbolines **12**.[14]

We first explored the amino side-chain moiety by preparing analogs containing a variety of *N*-alkyl or aromatic amines. For straight chain alkylamines (**4, 9a–9d**), hUT binding affinity increases with the length of alkyl side-chain, e.g., Et < Pr < Bu, Pent, Hex (Table 1). Branched alkylamines (**9e–9g**) are also allowed

* Corresponding author. Tel.: +1 610 917 6678; fax: +1 610 917 7391.
E-mail address: yonghui.2.wang@gsk.com (Y. Wang).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.08.054

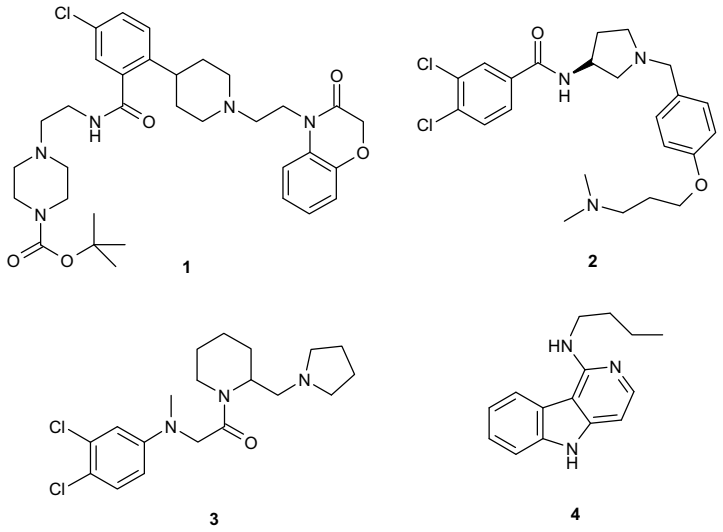
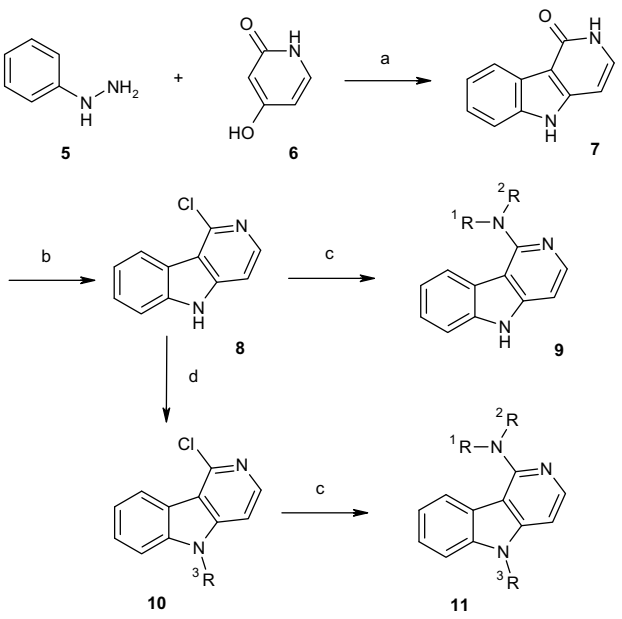


Figure 1. Examples of non-peptidic UT ligands (**1**–**3**) and HTS hit **4**.

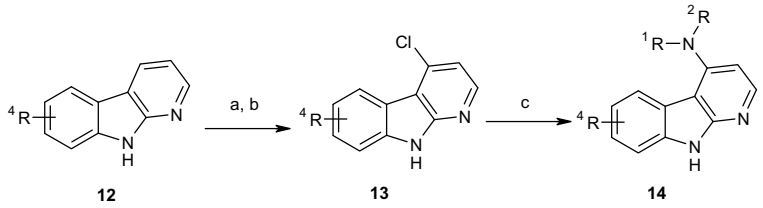


Scheme 1. Reagents and conditions: (a) i—DPE, heating (Dean-Stark); ii—DPE, reflux; (b) i—$POCl_3$, reflux; ii—HCl, heating (33% yield for two steps); (c) $^1R^2RNH$, heating; (d) ^3RX, KOH, TBAI, toluene, ultrasound.

but hUT binding affinity decreases dramatically when a branch is located at α-position of alkylamines. The trend was also observed for cycloalkyl amines (**9h–9k**). For example, both cyclopropyl amine and cyclohexyl amine were found to have little or no binding affinity while the corresponding cyclopropylmethyl amine and cyclohexylmethyl amine showed high hUT binding affinity. A similar trend was observed with aromatic amines (**9l–9o**), e.g., phenyl ≪ phenethyl < thienylmethyl, benzyl. In general, less sterically hindered amines exhibited good hUT binding affinity. For heteroalkyl amines, oxygen-containing analogs (**9p–9r**) showed good hUT binding affinities while nitrogen-containing compounds (**9s** and **9t**) were found to have no binding affinity. Furthermore, amide containing side chains (**9u** and **9v**) completely abolished binding affinity. Also notable is that compared to secondary amino groups, the tertiary amino groups (**9w** and **9x**) showed much less hUT binding affinity.

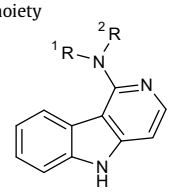
The SAR of other parts of the tricyclic molecule was subsequently explored (Table 2). While 5-methyl (**11a**) and 5-isopropyl (**11b**) had good hUT binding affinity, 5-benzyl (**11c**) showed poor binding affinity. All three compounds had less binding affinity to hUT compared to **4** (where ^3R = H). Tricyclic analogs with the pyrido-nitrogen at 4-position (or *N*-alkyl-1*H*-pyrido[2,3-*b*]indol-4-amines) were tested in the hUT binding assay. Compared to the corresponding analogs with pyrido-nitrogen at 2-position, these 4-pyrido tricyclic compounds were found to have less hUT binding affinity (comparing **4** to **14d**, **9p** to **14e**, **9i** to **14f**, and **9k** to **14g**). For 4-pyrido tricyclic compounds, substituents on indole phenyl ring (**14h–14j**) were well-tolerated with hUT binding pK_i's of 7.2, 7.3, and 7.5, respectively.

In addition to high hUT binding affinity, the tricyclic compounds showed good functional activities in a hUT FLIPR assay (Table 3). For example, compound **4** was found to be a competitive and reversible antagonist with a pA_2 of 7.0.[15] The tricyclic compounds were also evaluated for their cross-species activities and found to be active in a cat UT binding assay[16] (Table 3). For example, compound **4** showed cat UT binding pK_i of 6.2. However, these tricyclic compounds were inactive or much less active in a rat UT binding assay.[17]



Scheme 2. Reagents and conditions: (a) 30% H_2O_2, HOAc, heating; (b) $POCl_3$, DMF, rt; (c) $^1R^2RNH$, heating.

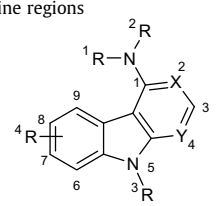
Table 1
SAR of the amino side-chain moiety



Compound	^{1}R-N-^{2}R	hUT binding (pK_i)[a]
9a	Et-NH	5.8
9b	Pr-NH	7.6
4	Bu-NH	8.4
9c	Pent-NH	8.4
9d	Hex-NH	8.2
9e	(1-Me)Bu-NH	6.1
9f	(2-Me)Bu-NH	8.0
9g	(3-Me)Bu-NH	7.9
9h	c-Pr-NH	5.6
9i	c-Pr-CH_2-NH	8.2
9j	c-Hex-NH	<5.1
9k	c-Hex-CH_2-NH	8.0
9l	Ph-NH	<5.1
9m	Bn-NH	7.9
9n	Ph-$(CH2)_2$-NH	7.0
9o	thienyl-CH_2-NH	7.6
9p	(2-OMe)Et-NH	7.7
9q	(2-OEt)Et-NH	7.9
9r	(tetrahydro-2-furanyl)-CH_2-NH	7.1
9s	[2-N$(Me)_2$]Et-NH	<5.1
9t	[2-(4-morpholinyl)]Et-NH	<5.1
9u	(2-acetylamino)Et-NH	<5.1
9v	(2-aminocarbonyl)Et-NH	<5.1
9w	(2-OMe)Et-NMe	<5.1
9x	$(Bu)_2$N	6.1

[a] Mean of at least two determinations; pK_i was calculated from K_i using formula, pK_i = −logK_i; for K_i determination, see Ref. 9e.

Table 2
SAR of the ^{3}R, ^{4}R, and pyridine regions



Compound	X	Y	^{1}R-N-^{2}R	^{3}R	^{4}R	hUT binding (pK_i)[a]
4	N	C	Bu-NH	H	H	8.4
11a	N	C	Bu-NH	Me	H	7.3
11b	N	C	Bu-NH	^{i}Pr	H	7.2
11c	N	C	Bu-NH	Bn	H	5.4
14d	C	N	Bu-NH	H	H	7.1
14e	C	N	(2-MeO)Et-NH	H	H	6.5
14f	C	N	c-Pr-CH_2-NH	H	H	7.0
14g	C	N	c-Hex-CH_2-NH	H	H	6.1
14h	C	N	Bu-NH	H	8-Me	7.2
14i	C	N	Bu-NH	H	8-Cl	7.3
14j	C	N	Bu-NH	H	6-OMe	7.5

[a] Mean of at least two determinations; pK_i was calculated from K_i using formula, pK_i = −logK_i; for K_i determination, see Ref. 9e.

Exemplars in the series exhibited good physicochemical properties. For example, compound **4** had good aqueous solubility (195 μM) and artificial membrane permeability (470 nm/s).[18] For selectivity, compound **4** showed at least 100-fold selectivity vs a number of 7TM receptors (5HT1A, 5HT1B, 5HT1D, 5HT2A, 5HT2C, 5HT3, 5H4A, 5HT6, 5HT7, Histamine H1, Histamine H3, Dopamine D2, Dopamine D3, Adrenergic alpha 1A, Adrenergic alpha 1B, Adrenergic beta 2, Adenosine A1, Adenosine A2a, Adenosine A2b,

Table 3
cat UT binding and hUT FLIPR data

Compound	cat UT binding pK_i[a]	hUT FLIPR pA_2[15]
4	6.2	7.0
9c	6.4	—
9d	6.7	—
9f	5.9	7.0
9g	5.8	—
9i	5.6	6.9
9m	6.0	7.7
9o	5.5	6.8
11a	6.0	—

[a] Mean of at least two determinations; pK_i was calculated from K_i using formula, pK_i = −logK_i; for K_i determination, see Ref. 9e.

M1, M2, M3, M4, M5, CXCR2) and about 400-fold selective for hUT over hERG (binding pIC_{50} = 5.8). However, compound **4** showed potent cytochrome P450 inhibition of the 2D6 (pIC_{50} = 8.1) and 3A4 (pIC_{50} = 7.0) isoforms. In an in vivo rat iv/po pharmacokinetic (PK) study (0.49 mg/kg iv, 0.97 mg/kg po), compound **4** showed high clearance, short half life, and low oral bioavailability (Clb = 110 mL/min/kg, $T_{1/2}$ = 1.3 h, F = 11%).

In summary, we discovered a novel series of *N*-alkyl-5*H*-pyrido[4,3-*b*]indol-1-amines and derivatives as urotensin-II receptor antagonists. Tractable SAR was demonstrated through analog synthesis. The compounds in the series exhibited high hUT binding affinity and functional and ortholog activities. The preliminary data suggests that this series constitutes a reasonable starting point for further lead optimization.

References and notes

1. Ames, R. S.; Sarau, H. M.; Chambers, J. K.; Willette, R. N.; Aiyar, N. V.; Romanic, A. M.; Louden, C. S.; Foley, J. J.; Sauermelch, C. F.; Coatney, R. W.; Ao, Z.; Disa, J.; Holmes, S. D.; Stadel, J. M.; Martin, J. D.; Liu, W. S.; Glover, G. I.; Wilson, S.; McNulty, D. E.; Ellis, C. E.; Elshourbagy, N. A.; Shabon, U.; Trill, J. J.; Hay, D. W.; Ohlstein, E. H.; Bergsma, D. J.; Douglas, S. A. *Nature* **1999**, *401*, 282.
2. Douglas, S. A.; Dhanak, D.; Johns, D. G. *Trends Pharmacol. Sci.* **2004**, *25*, 76.
3. (a) Matsushita, M.; Shichiri, M.; Imai, T.; Iwashina, M.; Tanaka, H.; Takasu, N.; Hirata, Y. *J. Hypertens.* **2001**, *19*, 2185; (b) Cheung, B. M.; Leung, R.; Man, Y. B.; Wong, L. Y. *J. Hypertens.* **2004**, *22*, 1341.
4. Russell, F. D.; Meyers, D.; Galbraith, A. J.; Bett, N.; Toth, I.; Kearns, P.; Molenaar, P. *Am. J. Physiol.* **2003**, *285*, H1576.
5. (a) Douglas, S. A.; Tayara, L.; Ohlstein, E. H.; Halawa, N.; Giaid, A. *Lancet* **2002**, *359*, 1990; (b) Ng, L. L.; Loke, I.; O'Brien, R. J.; Squire, I. B.; Davies, J. E. *Circulation* **2002**, *106*, 2877;; (c) Richards, A. M.; Nicholls, M. G.; Lainchbury, J. G.; Fisher, S.; Yandle, T. G. *Lancet* **2002**, *360*, 545;; (d) Lapp, H.; Boerrigter, G.; Costello-Boerrigter, L. C.; Jaekel, K.; Scheffold, T.; Krakau, I.; Schramm, M.; Guelker, H.; Stasch, J. P. *Int. J. Cardiol.* **2004**, *94*, 93.
6. (a) Bousette, N.; Patel, L.; Douglas, S. A.; Ohlstein, E. H.; Giaid, A. *Atherosclerosis* **2004**, *176*, 117; (b) Maguire, J. J.; Kuc, R. E.; Wiley, K. E.; Kleinz, M. J.; Davenport, A. P. *Peptides* **2004**, *25*, 1767.
7. (a) Totsune, K.; Takahashi, K.; Arihara, Z.; Sone, M.; Satoh, F.; Ito, S.; Kimura, Y.; Sasano, H.; Murakami, O. *Lancet* **2001**, *358*, 810; (b) Shenouda, A.; Douglas, S. A.; Ohlstein, E. H.; Giaid, A. *J. Histochem. Cytochem.* **2002**, *50*, 885; (c) Langham, R. G.; Gow, R.; Thomson, N. M.; Dowling, J. K.; Gilbert, R. E. *Am. J. Kidney Dis.* **2004**, *44*, 826.
8. (a) Totsune, K.; Takahashi, K.; Arihara, Z.; Sone, M.; Satoh, F.; Ito, S.; Murakami, O. *Clin. Sci.* **2003**, *104*, 1; (b) Wenyi, Z.; Suzuki, S.; Hirai, M.; Hinokio, Y.; Tanizawa, Y.; Matsutani, A.; Satoh, J.; Oka, Y. *Diabetologi* **2003**, *46*, 972.
9. (a) Flohr, S.; Kurz, M.; Kostenis, E.; Brkovich, A.; Fournier, A.; Klabunde, T. *J. Med. Chem.* **2002**, *45*, 1799; (b) Croston, G. E.; Olsson, R.; Currier, E. A.; Burstein, E. S.; Weiner, D.; Nash, N.; Severance, D.; Allenmark, S. G.; Thunberg, L.; Ma, J.; Mohell, N.; O'Dowd, B.; Brann, M. R.; Hacksell, U. *J. Med. Chem.* **2002**, *45*, 4950; (c) Clozel, M.; Binkert, C.; Birker-Robaczewska, M.; Boukhadra, C.; Ding, S.; Fischli, W.; Hess, P.; Mathys, B.; Morrison, K.; Muller, C.; Muller, C.; Nayler, O.; Qiu, C.; Rey, M.; Scherz, M. W.; Velker, J.; Weller, T.; Xi, J.; Ziltener, P. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 204; (d) Jin, J.; Dhanak, D.; Knight, S. D.; Widdowson, K.; Aiyar, N.; Naselsky, D.; Sarau, H. M.; Foley, J. J.; Schmidt, D. B.; Bennett, C. D.; Wang, B.; Warren, G. L.; Moore, M. L.; Keenan, R. M.; Rivero, R. A.; Douglas, S. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3229; (e) Douglas, S. A.; Behm, D. J.; Aiyar, N. V.; Naselsky, D.; Disa, J.; Brooks, D. P.; Ohlstein, E. H.; Gleason, J. G.; Sarau, H. M.; Foley, J. J.; Buckley, P. T.; Schmidt, D. B.; Wixted, W. E.; Widdowson, K.; Riley, G.; Jin, J.; Gallagher, T. F.; Schmidt, S. J.; Ridgers, L.; Christmann, L. T.; Keenan, R. M.; Knight, S. D.; Dhanak, D. *Br. J. Pharmacol.* **2005**, *145*, 620; (f) Lehmann, F.; Pettersen, A.; Currier, E. A.; Sherbukhin, V.; Olsson, R.; Hacksell, U.; Luthman, K. *J. Med. Chem.* **2006**, *49*, 2232; (g) Luci, D. K.; Ghosh, S.;

Smith, C. E.; Qi, J.; Wang, Y.; Haertlein, B.; Parry, T. J.; Li, J.; Almond, H. R.; Minor, L. K.; Damiano, B. P.; Kinney, W. A.; Maryanoff, B. E.; Lawson, E. C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6489; (h) Jin, J.; Wang, Y.; Wang, F.; Shi, D.; Erhard, K. F.; Wu, Z.; Guida, B. F.; Lawrence, S. K.; Behm, D. J.; Disa, J.; Vaidya, K. S.; Evans, C.; McMillan, L. J.; Rivero, R. A.; Neeb, M. J.; Douglas, S. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2860; (i) McAtee, J. J.; Dodson, J. W.; Dowdell, S. E.; Girard, G. R.; Goodman, K. B.; Hilfiker, M. A.; Neeb, M. J.; Sehon, C. A.; Sha, D.; Wang, G.; Wang, N.; Viet, A.; Zhang, D.; Aiyar, N.; Behm, D. J.; Carballo, L. H.; Evans, C. A.; Fries, H. E.; Nagilla, R.; Roethke, T. J.; Xu, X.; Yuan, C. K.; Douglas, S. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3500; (j) McAtee, J. J.; Dodson, J. W.; Dowdell, S. E.; Erhard, K.; Girard, G.; Goodman, K. B.; Hilfiker, M. A.; Jin, J.; Sehon, C. A.; Sha, D.; Shi, D.; Wang, F.; Wang, G. Z.; Wang, N.; Wang, Y.; Viet, A. Q.; Yuan, C. K.; Zhang, D.; Aiyar, N.; Behm, D. J.; Carballo, L. H.; Evans, C. A.; Fries, H. E.; Nagilla, R.; Roethke, T. J.; Xu, X.; Douglas, S. A.; Neeb, M. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3716; (k) Jin, J.; An, M.; Sapienza, A.; Aiyar, N. V.; Naselsky, D.; Sarau, H. M.; Foley, J. J.; Salyers, K. L.; Knight, S. D.; Keenan, R. M.; Rivero, R. A.; Dhanak, D.; Douglas, S. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3950.

10. For recent reviews, see: (a) Dhanak, D.; Neeb, M. J.; Douglas, S. A. *Ann. Reports Med. Chem.* **2003**, *38*, 99; (b) Jin, J.; Douglas, S. A. *Expert Opin. Ther. Patents* **2006**, *16*, 467; (c) Carotenuto, A.; Grieco, P.; Rovero, P.; Novellino, E. *Curr. Med. Chem.* **2006**, *13*, 267.
11. (a) For radioligand binding and $[Ca^{2+}]_i$-mobilization assay details, see: Dhanak, D.; Knight, S. D.; Jin, J.; Keenan, R. M. WO Patent 2001045711-A1, 2001; *Chem. Abstr.* **2001**, *135*, 76785.; (b) Ref. 9e.
12. (a) Ducrocq, C.; Civier, A.; Andre-Louisfert, J.; Bisagni, E. *J. Heterocycl. Chem.* **1975**, *12*, 963; (b) Nguyen, C. H.; Bisagni, E. *Tetrahedron* **1987**, *43*, 527; (c) Bisagni, E.; Nguyen, C. H.; Pierre, A.; Pepin, O.; De Cointet, P.; Gros, P. *J. Med. Chem.* **1988**, *31*, 398.
13. Davidson, R. S.; Patel, A. M.; Safdar, A. *Tetrahedron Lett.* **1983**, *24*, 5907.
14. (a) Stephenson, L.; Warburton, W. K. *J. Chem. Soc.* **1970**, 1355; (b) Elks, J.; Webb, G. B.; Gregory, G. I.; Cocker, J. D. Brit. 1972, Patent GB1268773.
15. FLIPR pA_2 value determination: the experiments were performed with various concentrations of agonist (urotensin-II) added to the cells in the presence and absence of antagonist compounds. Eleven concentrations of each test compounds were applied to the studies. EC_{50} values of the agonist were measured for all conditions and dose-ratios calculated for each concentration of antagonist. The antagonist potency of test compounds (pA_2 value) were calculated using Schild plot analysis using dose-ratios. See: Kenakin, T. *Pharmacologic Analysis of Drug–Receptor Interaction*, 3rd ed.; Lippincott-Raven Press, 1997.
16. For cat and rat UT receptor radioligand binding assay details, see: Ref. 9e.
17. The reason that the binding affinity was different across species for the tricyclic series was not clear. This phenomenon was also observed for the other UT ligand. See, Ref. 9c.
18. The artificial phospholipid membrane technique is similar to the widely used Caco-2 cell monolayer permeation technique. In short, egg phosphatidyl choline (1.8%) and cholesterol (1%) are dissolved in *n*-decane. A small amount of the volatile mixture is applied to the bottom of the microfiltration filter inserts. Phosphate buffer (0.05 M, pH 7.05) is quickly added to the donors and receivers, and the lipids are allowed to form self-assembled lipid bilayers across the small holes in the filter. Permeation experiment is initiated by spiking the compounds of interest to the donor sides, and the experiment is stopped at a pre-determined elapsed time. The samples are withdrawn and transferred to appropriate vials for analysis by HPLC with UV detection (215 nm).