Discovery of the First Nonpeptide Agonist of the GPR14/Urotensin-II Receptor: 3-(4-Chlorophenyl)-3-(2-(dimethylamino)ethyl)isochroman-1-one (AC-7954)

Glenn E. Croston,[†] Roger Olsson,[†] Erika A. Currier,[†] Ethan S. Burstein,[†] David Weiner,[†] Norman Nash,[†] Daniel Severance,[†] Stig G. Allenmark,[‡] Linda Thunberg,[‡] Jian-Nong Ma,[†] Nina Mohell,[†] Brian O'Dowd,[§] Mark R. Brann,[†,||] and Uli Hacksell*[,†]

ACADIA Pharmaceuticals Inc., 3911 Sorrento Valley Boulevard, San Diego, California 92121, Department of Chemistry, Göteborg University, SE-41296 Göteborg, Sweden, Department of Pharmacology, University of Toronto, Medical Science Building, Room 4353, Toronto, Ontario M5S 1A8, and Department of Pharmacology, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

Received June 6, 2002

Abstract: A functional cell-based screen identified 3-(4-chlorophenyl)-3-(2-(dimethylamino)ethyl)isochroman-1-one hydrochloride (AC-7954, **1**) as a nonpeptidic agonist of the urotensin-II receptor. Racemic **1** had an EC50 of 300 nM at the human UII receptor and was highly selective. Testing of the enantiopure (+)- and (−)- **1** revealed that the UII receptor activity of racemic **1** resides primarily in (+)-**1**. Being a selective nonpeptidic druglike UII receptor agonist, (+)-**1** will be useful as a pharmacological research tool and a potential drug lead.

The GPR14 gene was originally cloned as an orphan G-protein-coupled receptor (GPCR) based on its homology with known genes for GPCRs.[1] GPR14/urotensin-II receptor (UII receptor) mRNA was detected in a variety of tissues, including heart, vascular tissues, kidney, skeletal muscle, pancreas, and CNS.[2−4] Recently the urotensin-II (UII) neuropeptide, which provokes potent cardiovascular responses,[3,5] was identified as an agonist of the UII receptor.[3−4,6−7] The absence of specifically acting small-molecule UII agonists, however, hampers attempts to fully explore the function of the UII receptor.

To identify small-molecule UII receptor agonists, a functional mammalian cell-based R-SAT assay[8,9] was developed for high-throughput screening (HTS). For UII receptor HTS, NIH-3T3 cells were transiently transfected with urotensin-II receptor expression vector and reporter plasmid and frozen. Subsequently, cells were thawed, plated, and exposed to drug. Human UII produces a potent agonist response in this assay, with an EC50 of 3×10^{-12} and a 6.9-fold increase in signal, providing a sensitive assay for the identification of small-molecule UII receptor agonists. To further increase the throughput of uHTS, the UII receptor was multiplexed with vectors for the expression of six additional receptor targets: the muscarinic m3 receptor, and five orphan GPCRs: RBS11, GPR1, GPR21, EB12, and RE2. The inclusion of these additional receptor targets in the assay did not significantly alter the response characteristics of urotensin in this assay or the sensitivity of the assay while increasing significantly the number of drug−target interactions tested. We used this multiplexed R-SAT assay to screen a library of 180 000 small diverse organic molecules. This compound library had a significant overlap (67%) in chemical space with the MDDR library of known drugs. The library was heavily weighted to compounds within a molecular weight range suggested by standard druglikeness rules,[10] with an average molecular weight of 372 Da and an average of eight rotatable bonds. This allows for the rapid development of bioavailable drug leads from HTS hits in the library while maintaining druglike properties. Compounds with reactive functional groups were deselected for inclusion in retests.

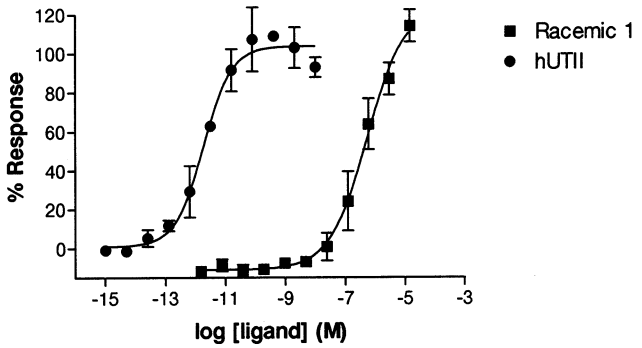


Figure 1. UII receptor activation by racemic **1**. Racemic **1** and control ligands were tested for agonist activity on the UII receptor in the functional cell-based R-SAT assay. UII receptor activation is determined through amplification of cotransfected β-galactosidase vector. Data were normalized as a percentage of the maximal control response (100% UII) and the solvent control response (0%). Data are the average of triplicate data points at each dose and error bars are the standard deviation.

In the high-throughput UII receptor assay, the maximal control response to human UII peptide on each test plate averaged 5.1-fold relative to the solvent control. Statistically, an assay with these response characteristics should detect compounds with 50% efficacy or higher with >99% confidence, providing a sensitive high-throughput screen for the detection of UIIR agonist compounds. Six hits were confirmed as UII receptor agonists from this screen. Of the hits confirmed, AC-7954, 3-(4-chlorophenyl)-3-(2-(dimethylamino)ethyl)isochroman-1-one hydrochloride (**1**) was identified as a novel nonpeptide agonist of the UII receptor with potency in R-SAT of 300 nM (Figure 1).

Compound **1** did not activate the muscarinic m3 receptor or the other receptors in the multiplex screening assay. To determine the selectivity of **1**, the compound was tested for activation of additional GPCRs in R-SAT assays: **1** produced no significant agonist response when tested up to 15 μM on receptors tested other than the UII receptor, including dopamine (D1,

* To whom correspondence should be addressed: 3911 Sorrento Valley Blvd., San Diego, CA 92121. Phone: (858) 558-2871. Fax: (858) 558-2872. E-mail: uhacksell@acadia-pharm.com.
[†] ACADIA Pharmaceuticals Inc.
[‡] Göteborg University.
[§] University of Toronto.
[||] University of California, San Diego.

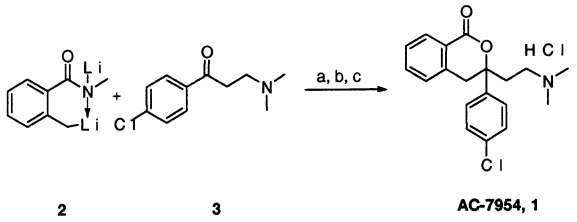
10.1021/jm025551+ CCC: $22.00
Published on Web 10/09/2002

Table 1. Comparison of the Potency of **1** and UII Peptide Agonists on the Human and Rat UII Receptors[a]

	human UII receptor		rat UII receptor	
	avg pEC50	std dev	avg pEC50	std dev
rat UII	11.7	0.0	10.8	0.1
mouse UII	11.7	0.1	11.1	0.2
human UII	11.3	0.1	11.0	0.4
racemic **1**	6.5	0.2	6.7	0.1
(+)-**1**	6.6	0.15	–	–

[a] Results were determined in R-SAT assays and are expressed as pEC50, the negative of the log EC50 in molarity. Results are the average ± standard deviation of three determinations of the EC50 where each compound was tested in eight doses in triplicate. The EC50 of (−)-**1** could not be accurately determined.

Scheme 1[a]



[a] Reagents and conditions: (a) THF/hexane, −60 °C, 1 h; (b) 1,2-dichlorobenzene, reflux; (d) HCl, dioxane.

D2, D5), muscarinic (m1, m3, m5), serotonin (5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT2A, 5-HT2B, 5-HT2C), histamine (H2), β-adrenergic (β-1, β-2) somatostatin (sst-2, sst-3, sst-5), CRF-1, CRF-2a, CRF-2b, κ-opioid, adrenomedullin, and CCK-a receptors. In addition, no significant response was observed on five receptors tested in inverse agonist mode (5-HT2A, 5-HT2B, 5-HT2C, 5-HT6a, and 5-HT7a). The receptor targets tested include the somatostatin and opioid receptor classes closest to the UII receptor in genetic sequence and no significant activity was observed at these targets. The UII peptide from human, rat, and mouse and **1** activated the human and rat UII receptors similarly (Table 1). These results confirm that compound **1** is a highly selective nonpeptidic agonist of the UII receptor.

Compound **1** was synthesized in 26% yield (Scheme 1) by addition of the dilithio salt of *N*-methyl-*O*-toluamide[11] (**2**) to 4-chloro-3-(*N*,*N*-dimethylamino)propiophenone (**3**) followed by heating of the resulting adduct to accomplish the desired intramolecular cyclization.

Resolution of racemic **1** into the enantiomers was achieved with preparative liquid chromatography using a Chiralpak AD column. This provided the (+)- and (−)-enantiomers of **1** in 99.3% ee and 98.7% ee, respectively (see Figure 2A). Testing of the resolved enantiomers in R-SAT revealed that the UII receptor agonist activity of **1** resided primarily in the (+)-**1** enantiomer, indicating that this activity is highly stereoselective (Figure 2B): the difference in EC50 values is about 100-fold.

Compound **1** is a highly selective nonpeptide agonist of the UII receptor. Nonpeptide small organic agonists of the UII receptor have not been previously reported, although nonpeptidic *antagonists* of the UII receptor were recently reported.[12] Compound **1** has a low molecular weight (MW = 329), druglike[10] lipophilicity (log D = 2.1), a basic amino function, pK_a 8.7, and exhibits limited conformation flexibility. The bicyclic scaffold of **1** is not present in other nonpeptide agonists of peptide

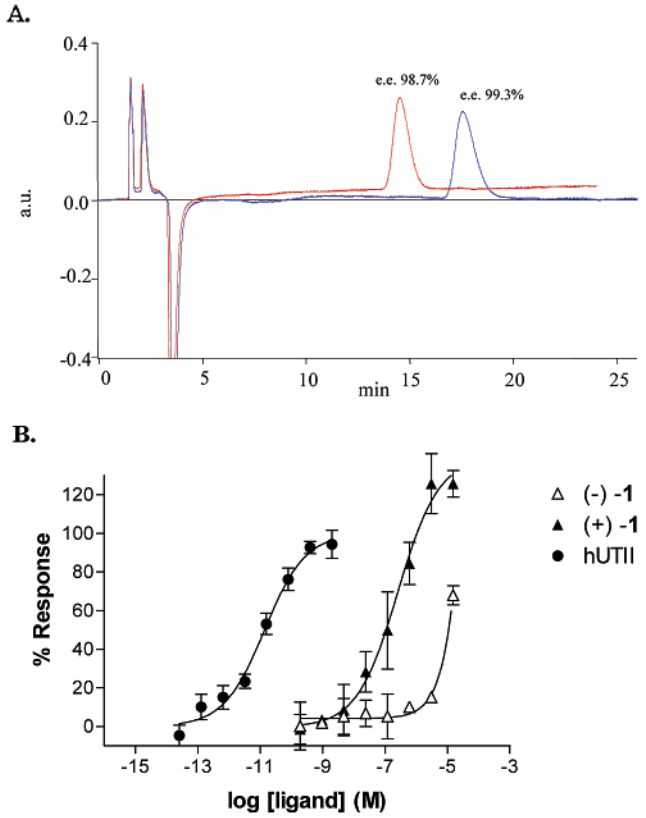


Figure 2. Liquid chromatographic resolution and biological activity of the enantiomers of **1. A.** Liquid chromatographic resolution. Baseline enantiomer resolution was achieved by the use of a Chiralpak AD 4.6 × 250 mm column (Daicel Chemical Co., Tokyo, Japan) and a mobile phase consisting of hexane/2-propanol/triethylamine (TEA)/acetic acid (94.8/5.0/0.1/0.1) at a flow rate of 2.0 mL/min. Detection was carried out at 225 nm. A 20 μL volume of a 1 mg/mL solution of the racemate in 2-propanol with a trace of triethylamine was injected. This yielded $k_1' = 5.9$ and $\alpha = 1.24$. *Preparative scale.* The preparative runs were performed with a Shimadzu LC-8A system, equipped with a Chiralpak AD 25 × 250 mm column and an injector loop of a total volume of 1 mL. The mobile phase composition and the solvent used for the racemate were the same as in the analytical scale separation. The flow rate, 20 mL/min, permitted repeated injections every 35 min. The injection volume was 750 μL of a 14 mg/mL solution of the racemate, that is 10.5 mg/run. Repeated manual fraction collection with omission of the middle part of the chromatogram gave the first (E1) and second (E2) eluted enantiomers with 98.7% ee and 99.3% ee, respectively, as determined by analytical chiral LC. **B.** Biological activity of **1** enantiomers. Resolved (+)- and (−)-**1** were tested in an R-SAT urotensin-II receptor assay for agonist activity. Data were normalized as a percentage of the maximal control response (100% UII) and the solvent control response (0%). Data are the average of triplicate data points at each dose and error bars are the standard deviation.

receptors and is amenable to parallel synthetic optimization. As such, (+)-**1** provides a research tool for the investigation of the role of the UII receptor in physiology and disease.

Compound **1** may also allow dissection of potential additional receptor subtypes that may transduce UII peptide signals.

A druglike molecule has certain advantages over use of the endogenous peptide ligand to elucidate UII receptor function. Small-molecule agonists such as **1** that are receptor selective are likely to be valuable

research tools to pharmacologically elucidate receptor function. Nonpeptide agonists of GPCRs with peptide ligands remain relatively unusual outside of the opioid receptors. Other peptide GPCRs for which nonpeptide agonists have been reported include CCK-a,[13] bradykinin-2,[14] angiotensin-1,[15] and the somatostatin receptors.[16] Some of these nonpeptide agonists are not peptides per se, but are peptidomimetics or peptoids that clearly resemble peptides. The structure of **1** is amenable to chemical modification and its predicted physical properties are druglike. In fact, **1** contains a molecular scaffold that has not previously been observed in peptide−receptor agonists or antagonists.

The bicyclic isochromane-based ring system contains a nonaromatic six-membered ring that adopts an envelope conformation, the C-3 carbon being positioned above or below the plane of the rest of the ring-system. According to molecular mechanics (MacroModel) calculations, both 3-substituents in compound **1** can adopt pseudoequatorial and pseudoaxial positions but conformations with the *p*-chlorophenyl group adopting an equatorial position are energetically favored by around 1 kcal/mol.

The mode of interaction of (+)-**1** with the UII receptor is not yet known and may involve interaction with the UII receptor at the same site as the UII peptide. Alternatively, (+)-**1** may modulate the UII receptor through a distinct ectopic binding site.[17] Homology modeling of the UII receptor indicates that the acidic Asp 130 is located on TM3, about two helix turns from the extracellular loop. Because of disulfide formation between two cysteines, UII forms an 18-membered ring. The cyclic part of UII is responsible for the biological activity,[18] and it contains a lysine which is essential for efficient UII receptor activation.[12] According to NMR experiments,[19] the lysine is not obscured by the other residues in UII and should be able to interact with Asp 130. It is likely that the basic amino function of compound (+)-**1** also interacts with Asp 130 if this small molecule modulates UII receptor activity at the same site as the UII peptide.

The identification of (+)-**1** as a nonpeptidic UII receptor agonist suggests that functional assays such as R-SAT may be valuable in the discovery of peptide receptor agonists or other modulators. Further research should explore the structural features of (+)-**1** as a unique type of peptidomimetic not previously encountered. The great degree of receptor selectivity and the high stereoselectivity of (+)-**1** as a UII receptor agonist will make it useful as a pharmacological tool in future studies to discern the function of the UII receptor in physiology and disease.[20]

Acknowledgment. We thank Dr. Tracy Spalding for valuable contributions.

Supporting Information Available: Experimental details for the synthesis of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Marchese, A.; Heiber, M.; Nguyen, T.; Heng, H. H.; Saldivia, V. R.; Cheng, R.; Murphy, P. M.; Tsui, L. C.; Shi, X.; Gregor, P.; et al. Cloning and chromosomal mapping of three novel genes, GPR9, GPR10, and GPR14, encoding receptors related to interleukin 8, neuropeptide Y, and somatostatin receptors. *Genomics* **1995**, *29*, 335−344.

(2) Totsune, K.; Takahashi, K.; Arihara, Z.; Sone, M.; Satoh, F.; Ito, S.; Kimura, Y.; Sasano, H.; Murakami, O. Role of urotensin II in patients on dialysis. *Lancet* **2001**, *358*. 810−811.

(3) 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. Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature* **1999**, *401*, 282−286.

(4) Liu, Q.; Pong, S. S.; Zeng, Z.; Zhang, Q.; Howard, A. D.; Williams, D. L., Jr.; Davidoff, M.; Wang, R.; Austin, C. P.; McDonald, T. P.; Bai, C.; George, S. R.; Evans, J. F.; Caskey, C. T. Identification of urotensin II as the endogenous ligand for the orphan G-protein-coupled receptor GPR14. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 174−178.

(5) Douglas, S. A.; Ohlstein, E. H. Human urotensin-II, the most potent mammalian vasoconstrictor identified to date, as a therapeutic target for the management of cardiovascular disease. *Trends Cardiovasc. Med.* **2000**, *10*, 229−237.

(6) Mori, M.; Sugo, T.; Abe, M.; Shimomura, Y.; Kurihara, M.; Kitada, C.; Kikuchi, K.; Shintani, Y.; Kurokawa, T.; Onda, H.; Nishimura, O.; Fujino, M. Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14). *Biochem. Biophys. Res. Commun.* **1999**, *265*, 123−129.

(7) Nothacker, H. P.; Wang, Z.; McNeill, AM.; Saito, Y.; Merten, S.; O'Dowd, B.; Duckles, S. P.; Civelli, O. Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. *Nat. Cell. Biol.* **1999**, *1*, 383−385.

(8) Messier, T. L.; Dorman, C. M.; Brauner-Osborne, H.; Eubanks, D.; Brann, M. R. High throughput assays of cloned adrenergic, muscarinic, neurokinin, and neurotrophin receptors in living mammalian cells. *Pharmacol. Toxicol.* **1995**, *76*, 308−311.

(9) Weiner, D. M.; Burstein, E. S.; Nash, N.; Croston, G. E.; Currier, E. A.; Vanover, K. E.; Harvey, S. C.; Donohue, E.; Hansen, H. C.; Andersson, C. M.; Spalding, T. A.; Gibson, D. F.; Krebs-Thomson, K.; Powell, S. B.; Geyer, M. A.; Hacksell, U.; Brann, M. R. 5-Hydroxytryptamine 2A receptor inverse agonists as antipsychotics. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 268−276.

(10) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3−26.

(11) Vaulx, R. N.; Puterbaugh, W. H.; Hauser, C. R. Metalation of *N*-methyl-o-toluamine with excess *n*-butyllithium. Condensations with ketones and aldehydes. Cyclizations. *J. Org. Chem.* **1964**, *29*, 3514−3517.

(12) Flohr, S.; Kurz, M.; Kostenis, E.; Brkovich, A.; Fournier, A.; Klabunde, T. Identification of Nonpeptidic Urotensin II Receptor Antagonists by Virtual Screening Based on a Pharmacophore Model Derived from Structure-Activity Relationships and Nuclear Magnetic Resonance Studies on Urotensin II. *J. Med. Chem.* **2002**, *45*, 1799−1805.

(13) de Tullio, P.; Delarge, J.; Pirotte, B. Therapeutic and chemical developments of cholecystokinin receptor ligands. *Expert Opin. Investig. Drugs* **2000**, *9*, 129−146.

(14) Ueno, A.; Naraba, H.; Kojima, F.; Morita, E.; Oh-ishi, S. FR190997, a novel bradykinin B2 agonist, expresses longer action than bradykinin in paw edema formation and hypotensive response. *Immunopharmacology* **1999**, *45*, 89−93.

(15) Perlman, S.; Schambye, H. T.; Rivero, R. A.; Greenlee, W. J.; Hjorth, S. A.; Schwartz, T. W. Non-peptide angiotensin agonist. Functional and molecular interaction with the AT1 receptor. *J. Biol. Chem.* **1995**, *270*, 1493−1496.

(16) Rohrer, S. P.; Birzin, E. T.; Mosley, R. T.; Berk, S. C.; Hutchins, S. M.; Shen, D. M.; Xiong, Y.; Hayes, E. C.; Parmar, R. M.; Foor, F.; Mitra, S. W.; Degrado, S. J.; Shu, M.; Klopp, J. M.; Cai, S. J.; Blake, A.; Chan, W. W.; Pasternak, A.; Yang, L.; Patchett, A. A.; Smith, R. G.; Chapman, K. T.; Schaeffer, J. M. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* **1998**, *282*, 737−740.

(17) For a discussion about ectopic binding, see: Spalding, T. A., Trotter, C.; Skjærbæk, N.; Messier, T. L.; Currier, E. A.; Burstein, E. S.; Li, D.; Hacksell, U.; Brann, M. R. Discovery of an Ectopic Activation Site on the m1 Musacarinic receptor. *Mol. Pharmacol.* **2002**, *61*, 1297−1302.

(18) Itoh, H.; Itoh, Y.; Rivier, J.; Lederis, K. Contraction of major artery segments of rat by fish neuropeptide urotensin II. *Am. J. Physiol.* **1987**, *252* (2 Pt 2), R361−R366.

(19) Bhaskaran, R.; Arunkumar, A. I.; Yu C. NMR and dynamical simulated annealing studies on the solution conformation of urotensin II. *Biochim. Biophys. Acta* **1994**, *1199*, 115−122.

(20) Structures related to 1 have been patented for compounds that elicited hypotension, hypertension, or diuresis. A U. S. patent involving related compounds from Houlihan, W. J, and Nadelson, J. in 1975 (US 3880885) relates diuretic and antihypertensive activity. The biological responses observed with these compounds may have been related to modulation of the UII receptor. Hence, the present study suggests a molecular mechanism for these earlier pharmacological observations.

JM025551+