Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6623–6628
Bioorganic & Medicinal Chemistry Letters

Next-generation spirobenzazepines: Identification of RWJ-676070 as a balanced vasopressin V_{1a}/V_2 receptor antagonist for human clinical studies

Min Amy Xiang,[a] Philip J. Rybczynski,[a] Mona Patel,[a] Robert H. Chen,[a] David F. McComsey,[b] Han-Cheng Zhang,[b] Joseph W. Gunnet,[a] Richard Look,[a] Yuanping Wang,[b] Lisa K. Minor,[b] H. Marlon Zhong,[b] Frank J. Villani,[b] Keith T. Demarest,[b] Bruce P. Damiano[b] and Bruce E. Maryanoff[b,*]

[a]*Research and Early Development, Johnson & Johnson Pharmaceutical Research & Development, 8 Clarke Drive, Cranbury, NJ 08512, USA*
[b]*Research and Early Development, Johnson & Johnson Pharmaceutical Research & Development, Welsh & McKean Roads, Spring House, PA 19477-0776, USA*

Received 27 June 2007; revised 14 September 2007; accepted 14 September 2007
Available online 19 September 2007

Abstract—We have continued to explore spirobenzazepines as vasopressin receptor antagonists to follow up on RWJ-339489 (**2**), which had advanced into preclinical development. Further structural modifications were pursued to find a suitable backup compound for human clinical studies. Thus, we identified carboxylic acid derivative **3** (RWJ-676070; JNJ-17158063) as a potent, balanced vasopressin V_{1a}/V_2 receptor antagonist with favorable properties for clinical development. Compound **3** is currently undergoing human clinical investigation.

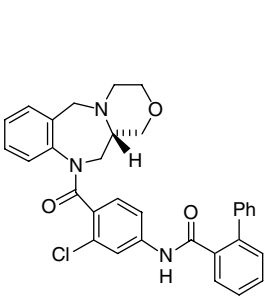
For several years, we have prospected for nonpeptide vasopressin receptor antagonists to advance compounds into human clinical trials.[1] We managed to identify different series of potent V_2-selective antagonists,[2a–f] from which three compounds were selected for preclinical development. One of these, RWJ-351647 (**1**), was investigated in humans and found to be well tolerated and efficacious as an aquaretic agent.[1,3] Potent V_{1a} receptor antagonism proved to be more elusive, until we discovered a chemical series based on spirocyclic benzazepines.[4] This novel series also enabled us to surface dual vasopressin V_{1a}/V_2 receptor antagonists, which have potential for wider clinical utility, especially for treating congestive heart failure and renal disorders.[5]

Our initial reports on spirobenzazepines[4a,b] disclosed RWJ-339489 (**2**; *R*-enantiomer),[4b] which is notable for its excellent V_{1a} and V_2 affinity, good potency, and balanced ratio in V_{1a} and V_2 functional assays. In rats, **2** had useful oral bioavailability ($F = 22\%$; $t_{1/2} = 6.5$ h) and produced dose-dependent aquaresis (at 10 mg/kg, p.o., urine output was elevated by 1100% and urine osmolality was reduced by 75%),[1] the latter being a gauge of V_2 receptor antagonism in vivo. On the basis of the many drugworthy properties of **2**, we advanced it into preclinical development as a dual vasopressin V_{1a}/V_2 antagonist. However, because of unexpected toxicology findings at suprapharmacological doses, **2** was abandoned as a clinical candidate. In this paper, we describe our effort to identify a suitable, next-generation backup compound, which was realized in the form of RWJ-676070 (**3**; JNJ-17158063).[4c]

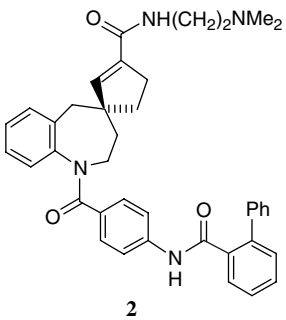
In seeking a backup compound for **2**, we were interested in reducing its relatively high molecular weight (MW = 612.8 Da), which would increase the probability of success.[6] One approach was to decrease the size of the substituent on the 5-membered ring. However, it was also important to retain some polar character at that

Keywords: Vasopressin receptor; Antagonist; Spirobenzazepine.
* Corresponding author. Tel.: +1 215 6285530; fax: +1 215 6284985; e-mail: bmaryano@prdus.jnj.com

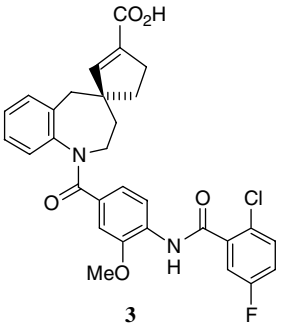
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.059



1

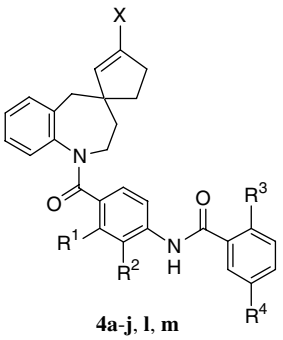


2

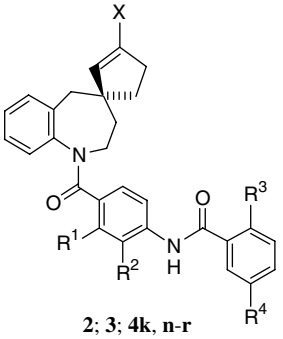


3

Table 1. Vasopressin binding and functional data for spirobenzazepine derivatives[a]



4a-j, l, m



2; 3; 4k, n-r

Compound	X	R^1	R^2	R^3	R^4	V_{1a} bndg[b] IC_{50}, nM	V_2 bndg[b] IC_{50}, nM	V_{1a} funct.[c] IC_{50}, nM	V_2 funct.[c] IC_{50}, nM
4a	CO_2H	H	H	Ph	H	37	14	20	360
4b	CH_2OH	H	H	Ph	H	9	18	9	190
4c[d]	CH_2NHMe	H	H	Ph	H	18	44	630	1800
4d	CO_2H	H	H	F	H	~100	15%	ND	ND
4e	CO_2Me	H	H	F	H	15	23%	130	>10,000
4f	CO_2H	Cl	H	Me	F	10%	67%	ND	ND
4g	CH_2OH	Cl	H	Me	F	~100	66%	ND	ND
4h[d]	CH_2NHMe	Cl	H	Me	F	0%	17%	ND	ND
4i	CO_2H	MeO	H	Me	F	15%	110	11,600	140
4j	CO_2H	H	H	Cl	F	32	76	780	410
4k[e]	CO_2H	H	H	Cl	F	13	53	380	100
4l	CO_2Me	H	H	Cl	F	40	>200	190	>10,000
4m	CO_2H	H	MeO	Cl	F	19	92	89	120
3[f]	CO_2H	H	MeO	Cl	F	5	34	38	52
4n[e]	CO_2H	H	EtO	Cl	F	8	71	50	150
4o[g,h]	Tetrazol-5-yl	H	MeO	Cl	F	ND	ND	63	40
4p[g,i]	$C(O)NHSO_2NH_2$	H	MeO	Cl	F	ND	ND	27	11
4q[g,j]	Imidazol-2-yl	H	MeO	Cl	F	ND	ND	95	730
4r[g,k]	Imidazolin-2-yl	H	MeO	Cl	F	ND	ND	320	1050
2[l]	C(O)NHR[m]	H	H	Ph	H	2	8	45	36
VPA-985[n]						150	5	>5000	91

[a] Target compounds were purified by silica gel flash-column chromatography. All compounds were characterized by ESI-MS and 300-MHz ^1H NMR. Compounds are racemates unless noted otherwise. ND, not determined.

[b] Inhibition of [^3H]-Arg-vasopressin binding to recombinant human vasopressin V_{1a} or V_2 receptors ($N = 2–6$). IC_{50} values are given unless noted otherwise as % inhibition at 100 nM.

[c] Inhibition of Arg-vasopressin-induced effects on cells expressing either human V_{1a} or V_2 receptors. IC_{50} values represent the concentration of compound that antagonizes 50% of the cellular calcium mobilization for V_{1a} receptors or cellular cAMP accumulation for V_2 receptors caused by 1 nM Arg-vasopressin ($N = 1–4$).

[d] Isolated as a free base.

[e] Prepared from (*R*)-(+)-**I** (>99% ee).

[f] (*R*)-(+) enantiomer; $[\alpha]_D^{25} + 19.5°$ (*c* 0.14, $CHCl_3$); >99% enantiomeric purity by chiral HPLC (ChiralPak AD-H column).

[g] (*R*) enantiomer; prepared from **3**.

[h] $[\alpha]_D^{25} + 193°$ (*c* 0.84, $CHCl_3$).

[i] $[\alpha]_D^{25} + 103.9°$ (*c* 1.32, $CHCl_3$).

[j] $[\alpha]_D^{25} + 71.2°$ (*c* 1.23, $CHCl_3$).

[k] $[\alpha]_D^{25} + 13.1°$ (*c* 1.23, $CHCl_3$).

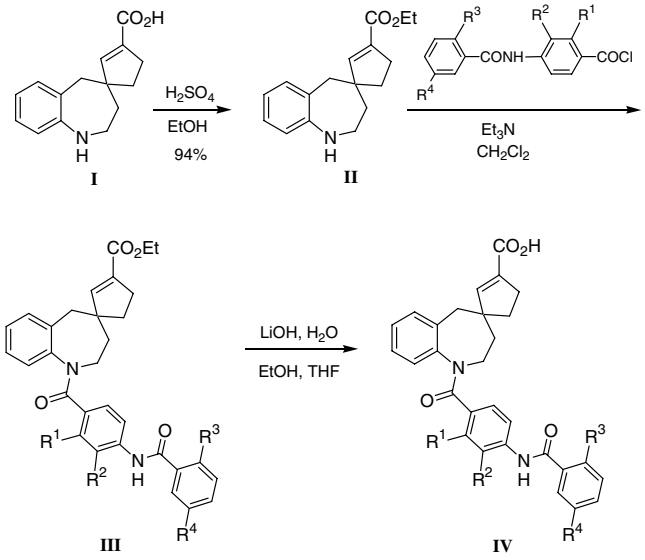
[l] (*R*)-(+) enantiomer; >99% enantiomeric purity by chiral HPLC. The biodata reported here for **2** are taken from Ref. 4b.

[m] R = $CH_2CH_2NMe_2$.

[n] Reference standard lixivaptan (Ref. 7a).

site, such as a carboxylic acid or amine, to confer reasonable aqueous solubility to the target molecules. Thus, analogues of general structure **4** were considered (Table 1). Our prior, intensive studies on vasopressin receptor antagonists,[2,4] and the published results of others,[7] have provided a good foundation for appreciating the structure–activity relationships (SARs) associated with aromatic ring substitution. Therefore, we did not revisit this particular facet. Rather, we decided to begin with certain *most-favored arrangements for the 4-(benzamido)benzoyl subunit*, especially those that would enhance V_{1a} receptor antagonist action.[2,4,7] In general, these subunits are: 4-(2-phenylbenzamido)benzoyl, 4-(2-methyl-5-fluorobenzamido)-2-chlorobenzoyl, and 4-(2-chloro-5-fluorobenzamido)benzoyl.

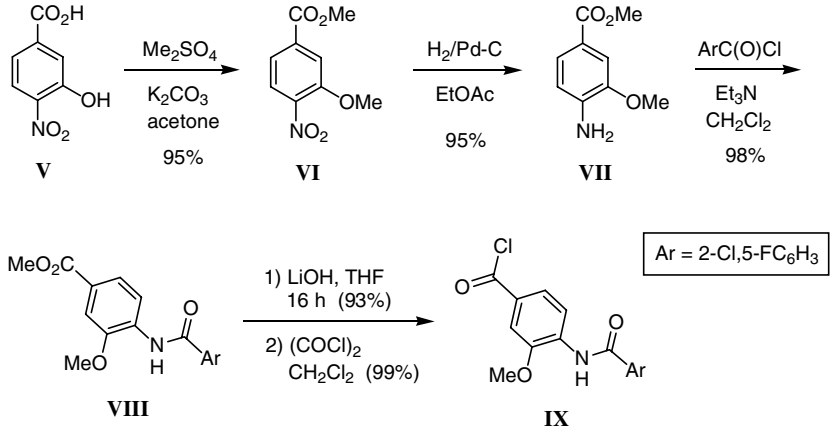
The synthesis of **3** and its analogues departs from the synthesis described earlier for **2** and its analogues,[4b] in that we now employed spirobenzazepine **I** as a key intermediate.[8] Compound **3** was separated into its individual enantiomers, and a single-crystal X-ray determination on the (−)-10-camphorsulfonate salt of (*R*)-**I** established the absolute stereochemistry (vide infra), which was conveyed into the relevant target compounds (Table 1). The chemistry starting with **I** is shown in Scheme 1. For the second step, the 4-(benzamido)benzoyl subunits were generated in a standard manner, except for the 3-alkoxy versions. Illustrating for the 3-methoxy case, we synthesized (2-chloro-5-fluorobenzamido)-3-methoxybenzoyl chloride (**IX**) to assemble **3**, **4m**, and **4o–r** (Scheme 2). The alcohol (**4b**, **4g**) and amine (**4c**, **4h**) target compounds were prepared starting with known spirobenzazepine **X**,[4b] according to the chemistry outlined in Scheme 3. The tetrazole analogue **4o** of carboxylic acid **3** was prepared by forming the primary carboxamide, dehydrating it to the nitrile with cyanuric chloride (DMF, 3 h), and reacting the nitrile with sodium azide (DMF, 125 °C, 15 h). Acylsulfamide **4p** was obtained by reacting the acid chloride of **3** with sulfamide (100 °C, 1 h). Imidazoline **4r** was prepared by converting the primary carboxamide to a methyl imidate ($Me_3O^+BF_4^-$, CH_2Cl_2), followed by reaction with ethylene diamine (CH_2Cl_2, 24 h). Imidazole **4q** was obtained by oxidizing **4r** with MnO_2 (CH_2Cl_2, 72 h).



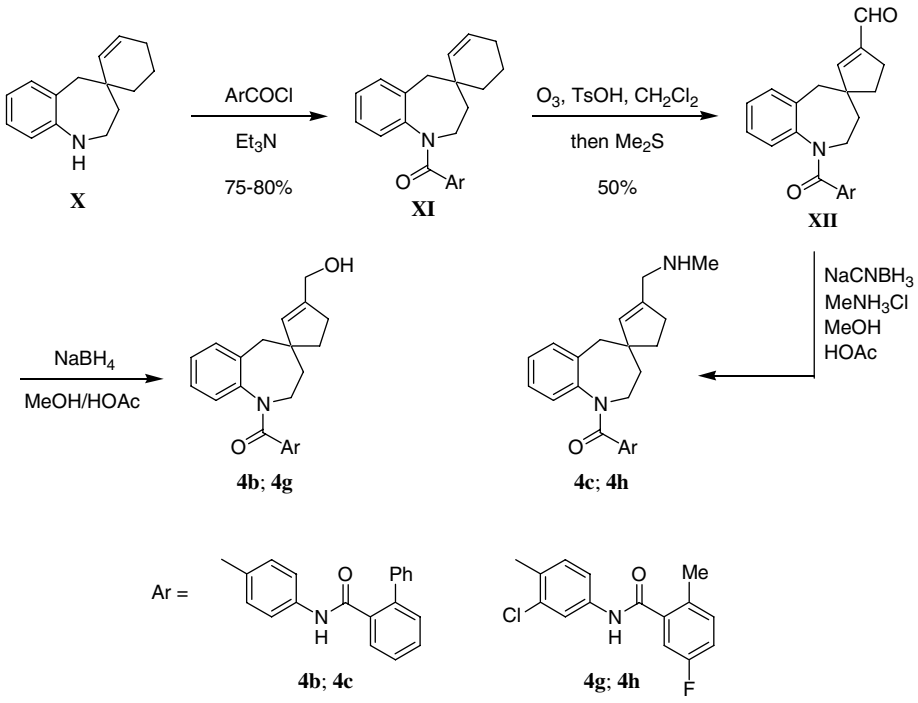
Scheme 1.

Intermediate **I** formed a crystalline salt with (−)-10-camphorsulfonic acid, as one diastereomer. A single crystal of the salt (from MeOH) was subjected to X-ray diffraction to furnish the structure and absolute stereochemistry of the spirobenzazepine unit, which turned out to be *R*.[9] The structure of the salt is depicted in Figure 1.

Since our goal was a potent, next-generation, dual V_{1a}/V_2 antagonist, we directed our sights on obtaining candidate compounds expeditiously by capitalizing on already-published structure–activity correlations.[2,4,7] Use of the 4-(benzamido)benzoyl subunits mentioned above would increase the chances of obtaining the more elusive V_{1a} receptor antagonist activity. At first, the analogues were evaluated for affinity to V_{1a} and V_2 receptors in a binding assay;[10] then, compounds of interest were further evaluated for cellular V_{1a} and V_2 functional activity (Table 1).[11] It was not sufficient just to have high potency in receptor binding, since high functional antagonism is crucial. Suitably potent dual antagonists in the



Scheme 2.



Scheme 3.

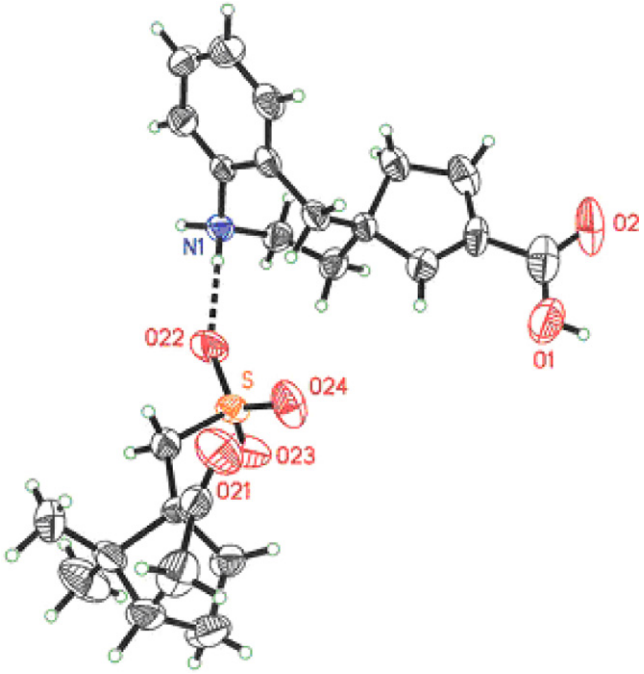


Figure 1. An ORTEP plot of the molecular structure of (*R*)-(+)-**I**, (4*R*)-1,2,3,5-tetrahydrospiro[4*H*-1-benzazepine-4,1′-[2]cyclopentene]-3′-carboxylic acid, as a salt with (1*R*)-(−)10-camphorsulfonic acid, showing the atom-numbering scheme for the heteroatoms.

functional assays were assessed for oral bioavailability in rats, metabolic stability in vitro, and inhibition of cytochrome P450 isozymes in vitro.

The carboxylic acid parent of **2**, **4a**, exhibited activity of interest, as did the carbinol (**4b**) and amine (**4c**) congeners, although **4c** was less potent in the functional assays. Replacement of the phenyl group of the acid with a fluoro (**4d**) or a methyl (**4f–i**) was not auspicious. 2-Chloro-5-fluoro substitution (**4j**) led to positive results in binding affinity, but not in functional activity. However, in exploring some new SAR territory, we found that addition of a 3-methoxy (**4m**, **3**) or 3-ethoxy (**4n**) group to this chloro/fluoro motif can yield a desirable profile (cf. **2**). The methoxy derivative as the *R*-enantiomer, **3**, was potent in the functional V_{1a} and V_2 assays with a favorable V_{1a}/V_2 ratio of nearly 1. Compound **3** is a white crystalline solid with an acceptable molecular weight (M_w = 549.0 Da) for a nonpeptide vasopressin antagonist[2,4,7] and good aqueous solubility at pH 7.4 (>1 mg/mL). Given the favorable attributes of **3**, we sought some close-in derivatives with acidic or basic replacements for the CO_2H group, represented by **4o–r**. The functional V_{1a} and V_2 potencies, and V_{1a}/V_2 ratios, for **4o** and **4p** (IC_{50} values of 63/40 and 27/11, respectively; cf. **2** and **3**) are particularly noteworthy. Clearly, it is possible to obtain potent V_{1a} and V_2 receptor antagonist activity with either a basic or acidic moiety attached to the spirocyclopentene ring.

Additional studies were conducted with **3**. With rat V_{1a} and V_2 vasopressin receptors, its functional IC_{50} values were 640 and 30 nM.[12] The pharmacokinetics of **3** were assessed in rats, dogs, and cynomolgus monkeys. Thus, **3** was found to have excellent oral bioavailability (F = 68%, 45%, and 23%, respectively) with good oral plasma half-lives ($t_{1/2}$ = 8.7, 5.1, and 10.9 h).

Oral administration of **3** to hydrated conscious rats produced a dose-dependent aquaretic effect (Table 2). For example, at an oral dose of 30 mg/kg, it caused a

Table 2. Effect of **3** on urine volume and osmolality in rats[a]

Dose, p.o. (mg/kg)	Urine volume (mL)	Urine volume % vehicle	Urine osmolality (mOsm/kg)	Urine osmolality % vehicle
Vehicle	2.3 ± 0.4		1070 ± 150	
1	3.0 ± 0.2	130	920 ± 89	87
3	3.8 ± 0.5[b]	165	510 ± 110[b]	48
10	7.2 ± 1.5[b]	310	560 ± 110[b]	52
30	15.9 ± 2.5[b]	690	180 ± 26[b]	17

[a] Compound **3** was administered orally to conscious hydrated male rats at the specified dose level. Each value represents the mean ± SE ($N = 6$–10).

[b] $P < 0.05$ versus the vehicle values (Dunnett's multiple comparison test).

690% increase of urine output over untreated controls, with an 83% reduction of urine osmolality. By way of comparison, a 10 mg/kg oral dose of lixivaptan (VPA-985) is reported to alter urine output/osmolality by +450%/−70%.[7a] Compound **3** was also an efficacious aquaretic agent in female beagle dogs (at 30 mg/kg, p.o., urine output and osmolality were altered by +1130% and −78%) and cynomolgus monkeys.[13]

To assess the V_{1a} activity of **3** in vivo, we evaluated its ability to reverse hypertension in rats induced by Arg-vasopressin. We infused a saline solution of Arg-vasopressin intravenously (30 ng/kg/min) to male Long–Evans rats to produce a stable increase in mean arterial pressure of 40–60 mm Hg. The test compound was administered i.v. in cumulative doses and the reduction in blood pressure was monitored. The ED_{50} for **3** (from a linear section of the dose–response curve) was 644 ± 123 μg/kg ($N = 6$).[14]

In conclusion, we manipulated the structure of dual vasopressin V_{1a}/V_2 receptor antagonist **2** to devise a suitable backup compound for clinical development. The amide group was changed to a carboxylic acid; the pendant phenyl group was changed to a chloro; and fluoro and methoxy substituents were added. The resultant analogue, **3**, is a potent, dual V_{1a}/V_2 antagonist with functional activity in vitro and in vivo. In addition, **3** possesses favorable attributes for advancement as a clinical candidate.[15] Consequently, **3** (RWJ-676070; JNJ-17158063)[1a,4c] is now undergoing clinical studies in humans.[16]

Acknowledgments

We thank Michael J. Costanzo for helpful discussions and advice. We thank Victor Day of the Crystalytics Company for the single-crystal X-ray diffraction study on intermediate **I**.

References and notes

1. (a) Maryanoff, B. E. *Acc. Chem. Res.* **2006**, *39*, 831; (b) Maryanoff, B. E. In *Drug Discovery and Development*; Chorghade, M. S., Ed.; Wiley: Hoboken, NJ, 2006; Vol. 1, pp. 313–337.
2. (a) Matthews, J. M.; Greco, M. N.; Hecker, L. R.; Hoekstra, W. J.; Andrade-Gordon, P.; de Garavilla, L.; Demarest, K. T.; Ericson, E.; Gunnet, J. W.; Hageman, W.; Look, R.; Moore, J. B.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 753; (b) Dyatkin, A. B.; Hoekstra, W. J.; Hlasta, D. J.; Andrade-Gordon, P.; de Garavilla, L.; Demarest, K. T.; Gunnet, J. W.; Hageman, W.; Look, R.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3081; (c) Matthews, J. M.; Hoekstra, W. J.; Dyatkin, A. B.; Hecker, L. R.; Hlasta, D. J.; Poulter, B. L.; Andrade-Gordon, P.; de Garavilla, L.; Demarest, K. T.; Ericson, E.; Gunnet, J. W.; Hageman, W.; Look, R.; Moore, J. B.; Reynolds, C. H.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2747; (d) Matthews, J. M.; Hlasta, D. J.; Andrade-Gordon, P.; Demarest, K. T.; Ericson, E.; Gunnet, J. W.; Hageman, W.; Look, R.; Moore, J. B.; Maryanoff, B. E. *Lett. Drug Des. Discov.* **2005**, *2*, 601; (e) Hoekstra, W. J.; Dyatkin, A. B.; Maryanoff, B. E.; Matthews, J. M. U.S. Patent 6,713,475, 2004; (f) Urbanski, M. J.; Chen, R. H.; Demarest, K. T.; Gunnet, J.; Look, R.; Ericson, E.; Murray, W. V.; Rybczynski, P. J.; Zhang, X. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4031.
3. (a) Gunnet, J. W.; Matthews, J. M.; Maryanoff, B. E.; de Garavilla, L.; Andrade-Gordon, P.; Damiano, B.; Hageman, W.; Look, R.; Stahle, P.; Streeter, A.; Wines, P. G.; Demarest, K. T. *Clin. Exp. Pharmacol. Physiol.* **2006**, *33*, 320; (b) Ros, J.; Fernandez-Varo, G.; Munoz-Luque, J.; Arroyo, V.; Rodes, J.; Gunnet, J. W.; Demarest, K. T.; Jimenez, W. *Br. J. Pharmacol.* **2005**, *146*, 654.
4. (a) Xiang, M. A.; Chen, R. H.; Demarest, K. T.; Gunnet, J.; Look, R.; Hageman, W.; Murray, W. V.; Combs, D. W.; Patel, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2987; (b) Xiang, M. A.; Chen, R. H.; Demarest, K. T.; Gunnet, J.; Look, R.; Hageman, W.; Murray, W. V.; Combs, D. W.; Rybczynski, P. J.; Patel, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3143; (c) Patel, M.; Rybczynski, P. J.; Xiang, M. A. U.S. Pat. Appl., US2004266752, 2004; (d) Chen, R. H.; Xiang, M. A. PCT Int. Appl., WO2002002531, 2002.
5. (a) Francis, G. S.; Tang, W. H. W. *JAMA* **2004**, *291*, 2017; (b) Goldsmith, S. R.; Gheorghiade, M. *J. Am. Coll. Cardiol.* **2005**, *46*, 1785.
6. (a) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3; (b) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
7. (a) Albright, J. D.; Reich, M. F.; Santos, E. G. D.; Dusza, J. P.; Sum, F. W.; Venkatesan, A. M.; Coupet, J.; Chan, P. S.; Ru, X.; Mazandarani, H.; Bailey, T. *J. Med. Chem.* **1998**, *41*, 2442; (b) Matsuhisa, A.; Taniguchi, N.; Koshio, H.; Yatsu, T.; Tanaka, A. *Chem. Pharm. Bull.* **2000**, *48*, 21.
8. (a) Zhong, H. M. et al., Unpublished results; presented at the 228th ACS National Meeting, Philadelphia, PA, Aug 22–26, 2004, ORGN-524; (b) See: Deng, X.; Kenny, B.; Liang, J. T.; Mani, N.; Villani, F. J.; Zhang-Plasket, F.; Zhong, H. U.S. Pat. Appl., US2004259857, 2004.
9. Single crystals of $[(R)\text{-}C_{15}H_{18}NO_2][(1R)\text{-}(-)\text{-}C_{10}H_{15}SO_4]$ (from MeOH, mp 289–291 °C) are orthorhombic [space group $P2_12_12_1$–D_2^4 (No. 19)] with $a = 7.095(3)$ Å, $b = 15.592(5)$ Å, $c = 21.478(7)$ Å, $V = 2376(1)$ Å^3, and $Z = 4$ cation/anion formula units [$d_{calcd} = 1.330$ g cm^{-3}; $\mu_a(MoK_\alpha) = 0.178$ mm^{-1}]. A total of 3285 reflections having 2θ (Mo K_α) < 50.7° were collected on a Bruker P4 diffractometer using 1.20°-wide ω scans and graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å); 3081 of these reflections were unique. Lattice constants were determined with the Bruker XSCANS software package using 35 centered reflections. 'Direct methods' were used to solve the structure and the resulting structural parameters were refined with F^2 data to convergence by using

counter-weighted, full-matrix, least-squares techniques and a structural model that incorporated anisotropic thermal parameters for all nonhydrogen atoms and isotropic thermal parameters for all hydrogen atoms. The final agreement factors are: R_1 (unweighted, based on F) = 0.056 for 2194 independent reflections having 2θ(Mo K_α) < 50.7° and $I > 2\sigma(I)$; R_1 (unweighted, based on F) = 0.091 and wR_2 (weighted, based on F^2) = 0.131 for all 3081 independent reflections having 2θ(Mo K_α) < 50.7°. The absolute configuration assigned to the chiral cation was confirmed experimentally by the refined value of 0.10(7) for the 'Flack' absolute structure parameter. The carboxyl (H_{1O}) and amine (H_{1N1} and H_{1N2}) hydrogen atoms were located from a difference Fourier map and refined as independent isotropic atoms. The positional parameters of the amine hydrogens were allowed to vary in least-squares cycles, but the carboxyl hydrogen was placed at an idealized tetrahedral position, with free rotation about the C–O bond in least-squares cycles. The remaining hydrogen atoms were included in the structure-factor calculations as idealized atoms (assuming sp^2- or sp^3-hybridized carbon atoms and C–H bond lengths of 0.94–0.99 Å) 'riding' on their respective carbon atoms. The isotropic thermal parameters for H_{1O}, H_{1N1}, and H_{1N2} refined to final U_{iso} values of 0.18(5), 0.04(1), and 0.07(2) $Å^2$, respectively; those for the remaining hydrogens were fixed at values of 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameters of the carbon atoms to which they are bonded. Atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre (Accession No.: CCDC 660933).

10. Receptor binding was performed with recombinant human V_{1a} or V_2 receptor preparations derived from the membranes of transfected HEK-293 cells. Compounds were evaluated for their ability to displace [^{3}H]-Arg-vasopressin.
11. Inhibition of receptor activation induced by Arg-vasopressin was quantitated by measuring intracellular Ca^{2+} concentration in HEK-293 cells expressing human V_{1a} receptors and by measuring cAMP levels in HEK-293 cells expressing human V_2 receptors.
12. Inhibition of AVP-induced activation of rat V_{1a} (Ca^{2+}) and V_2 (cAMP) receptors in transfected HEK-293 cells.
13. Details on the aquaresis studies with **3** in different animal species will be published separately.
14. Details on the V_{1a} antagonist activity of **3** in in vivo assays will be published separately.
15. Compound **3** has good pharmacokinetics in multiple species, does not display hERG binding, does not inhibit cytochrome P450 enzymes, does not develop tolerance on chronic administration, and shows no adverse CNS, cardiovascular, metabolic, or endocrine effects in vivo at elevated doses. At suprapharmacological doses, **3** did not display the unexpected toxicology that was observed with **2**.
16. (a) Conivaptan[16b,c] is a dual V_{1a}/V_2 receptor antagonist that has received marketing approval in the USA for treating hyponatremia, albeit by intravenous administration only (http://www.fda.gov/Cder/drug/InfoSheets/patient/conivaptanPIS.htm; accessed Aug 2007). For background on conivaptan (YM-087), see: (b) Matsuhisa, A.; Taniguchi, N.; Koshio, H.; Yatsu, T.; Tanaka, A. *Chem. Pharm. Bull.* **2000**, *48*, 21; (c) Tahara, A. et al. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 301.