Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3081–3084

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Bridged Bicyclic Vasopressin Receptor Antagonists with V_2-Selective or Dual V_{1a}/V_2 Activity

Alexey B. Dyatkin,[a,*] William J. Hoekstra,[a] Dennis J. Hlasta,[a] Patricia Andrade-Gordon,[a] Lawrence de Garavilla,[a] Keith T. Demarest,[b] Joseph W. Gunnet,[b] William Hageman,[a] Richard Look[b] and Bruce E. Maryanoff[a,*]

[a]*Drug Discovery, Johnson and Johnson Pharmaceutical Research and Development, Spring House, PA 19477-0776, USA*
[b]*Drug Discovery, Johnson and Johnson Pharmaceutical Research and Development, Raritan, NJ 08869-0602, USA*

Received 5 June 2002; accepted 2 August 2002

Abstract—The synthesis and biological testing of a novel series of nonpeptide vasopressin receptor antagonists, containing a bridged bicyclic nucleus, are reported. Variation of substituents (R_1–R_3) in general formula **3**, and the configuration of the stereocenter, resulted in potent V_2-selective (e.g., **4**) and balanced dual V_{1a}/V_2 (e.g., **10**) compounds. Data from receptor binding, cell-based functional, and in vivo assays are presented.
© 2002 Elsevier Science Ltd. All rights reserved.

Arginine vasopressin (AVP), a nonapeptide hormone that is secreted mainly from the posterior pituitary gland, exerts its biological action through two major G-protein-coupled receptors, V_{1a} and V_2.[1,2] The V_{1a} receptor subtype, which exists predominantly in vascular smooth muscle cells, platelets, the liver, adrenal glands, and the uterus, is responsible for the cardiovascular effects of AVP, such as vasoconstriction. The V_2 receptor subtype, which exists in the kidneys, is responsible for controlling water reabsorption and salt (NaCl) balance. Thus, there is therapeutic potential for vasopressin V_2-selective and dual V_{1a}/V_2 receptor antagonists, such as in treating congestive heart failure, hypertension, renal disease, edema, and hyponatremia.[3] The goal for this work was the discovery of both types of agents.

Benzodiazepine heterocycles have been employed successfully as a scaffold in several nonpeptide vasopressin receptor antagonists. Representative compounds are **1** (VPA-985; lixivaptan)[4] and **2** (VP-365)[5] (Fig. 1), as well as OPC-31260 (not shown),[6] which exhibit high selectivity for V_2 receptors in vitro and good in vivo efficacy in rat aquaresis studies. We supposed that the introduction of a basic amino group might improve physicochemical properties of the target compounds. Saturation of the pyrrole portion of the molecule, as in **2**, also changes the steric aspects of the tricyclic template to modulate potency and selectivity. Although both the *R* and *S* isomers of **2** are selective V_2 receptor antagonists, the enantiomer derived from (*S*)-proline exhibits lower affinity. We envisioned the replacement of proline in the synthesis of **2** by bridged bicyclic amino acids to afford compounds of type **3**, which might exhibit good potency and in vivo efficacy as V_2-selective and dual V_{1a}/V_2 agents. Indeed, this approach was successful as exemplified by derivatives **4** and **10**. Compound **4**, with the *R* absolute configuration, shows potent V_2-selective activity and compound **10**, with the *S* configuration, shows potent, balanced V_{1a}/V_2 activity.

The synthetic methodology for preparation of the requisite bridged bicyclic amino acids in enantiomerically enriched form is well developed.[7,8] The general approach to these intermediates is presented in Scheme 1. Diels–Alder cycloaddition of a cyclic diene to an activated imine, formed from α-methyl-benzylamine and ethyl glyoxalate, was catalyzed by CF_3CO_2H (TFA) for cyclopentadiene or TFA-BF_3 for 1,3-cyclohexadiene. The cycloadducts were obtained with high diastereoselectivity,

*Corresponding authors. Tel.: +1-215-628-5008; email: adyatkin@prdus.ini.com (A. B. Dyatkin); tel.: +1-215-628-5530; fax: +1-215-628-4985; e-mail: bmaryano@prdus.jnj.com (B. E. Maryanoff);

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00649-2

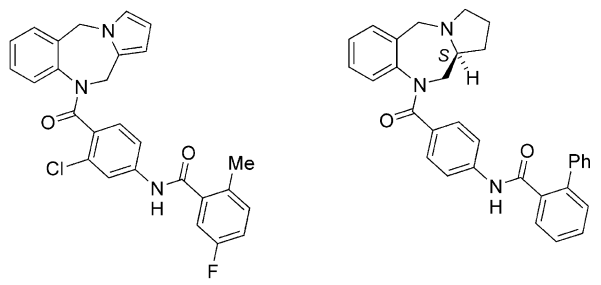
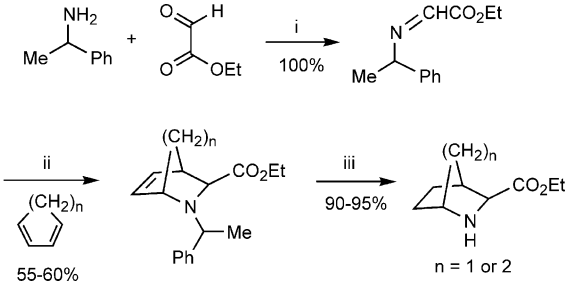


Figure 1. Nonpeptide vasopressin receptor antagonists.

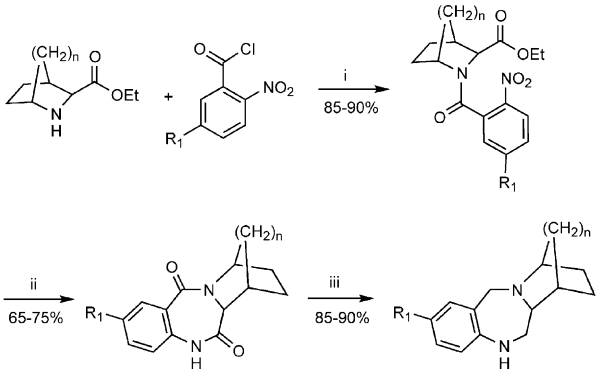


Scheme 1. (i) Toluene, reflux ($-H_2O$). (ii) TFA ($n=1$) or TFA/$BF_3{\cdot}Et_2O$ ($n=2$), CH_2Cl_2 or DMF, $-20\,^{\circ}C$. (iii) H_2, Pd/C, EtOH.

with the (*R*)-(+)-amine providing the (*S*)-cycloadduct. The diastereomers were separated by flash-column chromatography and hydrogenated to reduce the alkene. During the hydrogenation step, the nitrogen was deprotected, as well, to yield the bicyclic amino esters in good yields.

For the preparation of the benzodiazepines, we employed the method of Carabateas.[9] Acylation of the bicyclic amino esters with 2-nitrobenzoyl chloride, followed by tandem reduction-cyclization, provided benzodiazepinediones, which were reduced by $LiAlH_4$ in THF (Scheme 2). These reactions occurred without loss of the stereochemical integrity at the original stereogenic center. The commercial availability of substituted 2-nitrobenzoic allowed us to prepare 3-substituted benzodiazepines in this way.

Target compounds were prepared in 45–60% overall yield based on the amino acid component.



Scheme 2. (i) CH_2Cl_2, Et_3N, 0 °C; (ii) Fe, AcOH, reflux; (iii) LAH, THF, 0 °C.

Further chemical modification of the benzodiazepine, involving acylation with 4-nitrobenzoyl chloride, reduction of the nitro group, and acylation of the aniline, provided the desired target molecules (Scheme 3).

In Vitro Activity

We evaluated a series of bridged benzodiazepine targets for binding to human V_{1a} and V_2 receptors and for cell-based functional activity (Table 1). In general, the (*S*)-enantiomers demonstrated high V_2 activity along with V_{1a} activity. In contrast, **4** (the *R*-enantiomer of **5**) was V_2-selective. Introduction of Cl atoms in the 2-position of benzodiazepine ring also increased affinity of compounds toward V_{1a} receptors.

We introduced substituted 4-aminobenzoic acids that have been favorable with other benzodiazepine-based vasopressin receptor antagonists.[1] Variations of R_2 and R_3 groups did not significantly affect the potency or V_{1a}/V_2 selectivity of the compounds.

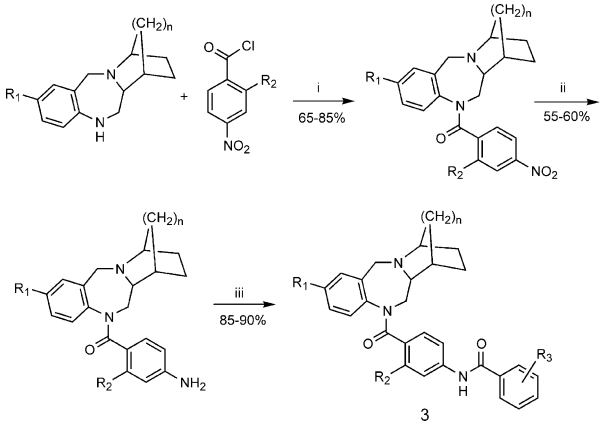
Bioavailability and Metabolism

In rats, **4**–**6** exhibited modest bioavailability and plasma clearance rates. Selected pharmacokinetic properties are given in the Table 2.

Compounds **4** and **6** were tested in a cytochrome P450 inhibition screen involving recombinant enzymes. Compound **4** was A potently inhibitor of CYP3A4, whereas **6** had a small effect on CYP3A4 and no effect on CYP1A2, CYP2C19 or CYP2D6; **6** was most active on CYP2C9 ($IC_{50}=7.4\ \mu M$).[10]

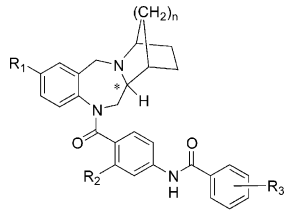
Aquaretic Activity in Rats

We investigated some potent derivatives for in vivo aquaretic activity in conscious hydrated male Sprague–Dawley rats. Urine volume and osmolality were measured 4 h after oral administration of the test compounds. The results are presented in Table 3 for **4**



Scheme 3. (i) CH_2Cl_2, Et_3N, 0 °C. (ii) Zn dust, NH_4Cl, MeOH, reflux. (iii) CH_2Cl_2, Et_3N, ArCOCl, 0 °C.

Table 1. Vasopressin V_2 and V_{1a} binding and functional data



Compd[a]	R_1	R_2	R_3	n	Config[b]	V_2 IC_{50}, μM[c]	V_{1a} IC_{50}, μM[c]	hV_2funct., K_i, μM[d]	hV_{1a} funct., K_i, μM[d]
4	H	Cl	2-Ph	1	*S*[e]	0.004	0.024	0.009	0.13
5	H	Cl	2-Ph	1	*R*[f]	0.015	7%[g]	0.03	—
6	Cl	H	2-(4′-MeC_6H_4)	1	*S*	0.005	0.024	0.013	0.023
7	Cl	Cl	2-(4′-MeC_6H_4)	1	*S*	0.003	0.035	—	—
8	Cl	H	2-F	1	*S*	0.026	0.068	0.180	2.6
9	Cl	H	2-Cl	1	*S*	0.010	0.005	0.032	0.13
10	Cl	H	2-CF_3	1	*S*	0.007	0.004	0.009	0.04
11	H	H	2-Ph	2	*R*/*S*	0.006	~0.1	—	—
12	H	Cl	2-Ph	2	*R*/*S*	0.004	30%[g]	—	—
13	H	Cl	3-F-5-Me	2	*S*	0.019	15%[g]	—	—
14	Cl	H	2-Cl	2	*S*	0.013	0.035	0.52	0.62
15	Cl	H	2-(4′-MeC_6H_4)	2	*S*	0.072	0.059	—	—
VPA-985[h]						0.005	0.15	0.09	—
OPC-31260[h]						0.028	0.25	0.16	0.89

[a]Target compounds were purified by flash-column chromatography and crystallized as HCl salt hydrates (EtOAc). Purities were judged by reverse-phase HPLC/MS at 215 nm and 254 nm; all compounds were analyzed by 300-MHz ^1H NMR and MS; compounds tested in vivo gave satisfactory elemental analyses. Enantiomeric purity (>99% ee) was confirmed by chiral HPLC.
[b]Absolute configuration for the stereocenter designated by an asterisk (*).
[c]Inhibition of [^3H]-Arg-vasopressin binding to recombinant human vasopressin V_{1a} and V_2 receptors. IC_{50} values unless noted otherwise.
[d]Inhibition of vasopressin receptor activation caused by AVP was determined in HEK-293 cells expressing human V_{1a} or V_2 receptors; changes in intracellular Ca^{2+} (V_{1a}) or cAMP (V_2) concentra-tions were measured.
[e]$[\alpha]_D^{20}$ +188° (*c* 0.175, MeOH).
[f]$[\alpha]_D^{20}$ −206° (*c* 0.193, MeOH).
[g]Percentage inhibition value, determined at 0.1 μM.
[h]Reference standards with in-house data.

Table 2. Oral pharmacokinetic properties of **4**–**6**[a]

Compd	Oral $t_{1/2}$ (h)	*F* (%)	Clearance, iv (mg/kg/μM-h)	Oral C_{max} (μM)
4	5	14	1.2	0.65
5	2.5	10	3.3	0.23
6	2.5	7	2.1	0.32

[a]Mature male rats (250–300 g) were used. Each compound was administered at a dose of 30 mg/kg, p.o. ($N=3$) and 3 mg/kg, iv ($N=3$). The plasma levels for the compounds were determined by LC-MS.

(10-fold V_2-selective), **5** (V_2-selective), and **6** (balanced V_{1a}/V_2).

It is evident that **4**–**6** have significant aquaretic activity, as they strongly increased urine volume and decreased urine osmolality in a dose-dependent manner. The salt-sparing activity of **6** and OPC-31260 were shown by the small amount of sodium and potassium lost through the urine (Table 4), as compared to the large volume of urine produced.

Table 3. In vivo diuretic effect in rats

Dose, po (mg/kg)	No. of animals	Urine volume (mL)[a]	Urine osmolality (mOsm/kg)[a]
4			
Vehicle	10	1.1±0.2	663±62
0.3	9	2.5±0.3	444±79
1	10	6.0±0.7	299±34
3	10	15.1±1.4	180±9
10	10	29.9±2.0	138±11
5			
Vehicle	10	0.6±0.1	892±70
0.3	9	1.4±0.1	408±37
1	10	3.4±0.4	303±29
3	10	9.4±1.1	238±19
10	10	19.5±1.4	172±9
6			
Vehicle	10	2.3±0.3	441±30
1	10	2.9±0.2	387±15
3	10	5.8±0.5	226±9
10	10	17.8±1.3	159±9
OPC-31260			
Vehicle	16	2.1±0.4	608±50
1	8	2.3±0.5	530±42
3	10	5.4±0.7	372±39
10	9	12.2±1.0	238±18
30	8	21.5±3.0	165±17

[a]Mean±SE.

Reversal of AVP-Induced Hypertension in Rats

Vasopressin plays an important role in regulating blood pressure by means of the V_{1a} receptors in the cardiovascular system. Activation of V_{1a} receptors on arterial smooth muscle cells results in vasoconstriction and an increase in blood pressure. In this assay, we assessed the ability of VPA-985, **4**, **6**, and **10** to reverse exogenous AVP-induced hypertension in pentobarbital-anesthetized male Long–Evans rats. AVP was diluted in saline

Table 4. Na, K, and creatinine excretion

Dose, po (mg/kg)	Na (mEq/4 h)[a]	K (mEq/4 h)[a]	Creatinine (mg/dL) [a]
6			
Vehicle	0.05±0.01	0.28±0.03	36.6±2.9
1	0.07±0.01	0.30±0.02	31.4±1.5
3	0.16±0.02	0.34±0.02	16.2±1.0
10	0.52±0.04	0.64±0.03	7.7±0.6
OPC-31260			
Vehicle	0.05±0.01	0.35±0.02	56.1±8.6
3	0.17±0.04	0.52±0.04	30.5±5.4
10	0.42±0.08	0.62±0.04	14.1±2.2
30	0.50±0.09	0.74±0.03	9.2±0.9

[a]Mean±SE; $N=8$–10.

and infused at 30 ng/kg/min, iv (estimated to be an ED_{90}) to produce a stable 50–60 mm Hg increase in mean arterial blood pressure. From the linear portion of the cumulative dose–response curves, ED_{50} values (dose to elicit a 50% return to baseline blood pressure) were interpolated. VPA-985 has an ED_{50} value of 1121±516 μg/kg (mean±SE, $N=5$); **4**, **6**, and **10** are more potent with ED_{50} values of 217±43 μg/kg ($N=6$), 172±119 μg/kg ($N=8$), and 380±286 μg/kg ($N=5$), respectively. Note that **6** tended to be the most potent of the bicyclic compounds and that this correlates with the relative potency in the V_{1a} functional assay. Although **10** was more potent than **4** in the functional assay, this difference could not be detected in the blood pressure assay in rats. Because of specific differences in the vasopressin receptors, it may be difficult to draw a direct comparison between the blood pressure assay in rats and the functional assay in cells expressing the human receptor, especially with the limited number of compounds involved.

Summary

We have identified a novel series of nonpeptide vasopressin receptor antagonists comprised of a benzodiazepine ring that is fused to a bridged bicyclic amine. The variation of substituents and absolute configuration resulted in V_2-selective (e.g., **4**) and balanced V_{1a}/V_2 (e.g., **10**) vasopressin receptor antagonists, some of which have good efficacy in vitro and in vivo.

Acknowledgements

We thank Eric Ericson, Gail Thompson, Barbara Haertlein, and Pat Sasso for technical assistance; Dr. Zhengyin Yan for cytochrome P450 inhibition data; and Mary Evangelisto for determinations of enantiomeric purity.

References and Notes

1. (a) Albright, J. D.; Chan, P. S. *Curr. Pharm. Des.* **1997**, *3*, 615. (b) Thibonnier, M.; Conarty, D. M.; Preston, J. A.; Wilkins, P. L.; Berti-Mattera, L. N.; Mattera, R. *Adv. Exp. Med. Biol.* **1998**, *449*, 251.
2. Trybulski, E. J. *Annu. Rep. Med. Chem.* **2001**, *36*, 159.
3. Yasu, T.; Tomura, Y.; Tahara, A.; Wada, K.; Tsukada, J.; Uchida, W.; Tanaka, A.; Takenaka, T. *Eur. J. Pharmacol.* **1997**, *321*, 225.
4. (a) Albright, J. D.; Reich, M. F.; Delos Santos, E. G.; 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) Martinez-Castelao, A. *Curr. Op. Invest. Drugs* **2001**, *2*, 525. (c) Ashwell, M. A.; Bagli, J. F.; Caggiano, T. J.; Chan, P. S.; Molinari, A. J.; Palka, C.; Park, C. H.; Rogers, J. F.; Sherman, M.; Trybulski, E. J.; Williams, D. K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 783.
5. Ohtake, Y.; Naito, A.; Hasegawa, H.; Kawano, K.; Morizono, D.; Taniguchi, M.; Tanaka, Y.; Matsukawa, H.; Naito, K.; Oguma, T.; Ezure, Y.; Tsuriya, Y. *Bioorg. Med. Chem.* **1999**, *7*, 1247.
6. Matsuhisa, A.; Tanaka, A.; Kikuchi, K.; Shimada, Y.; Yatsu, T.; Yanagisawa, I. *Chem. Pharm. Bull.* **1997**, *45*, 1870.
7. Abraham, H.; Stella, L. *Tetrahedron* **1992**, *48*, 9707.
8. Bertilsson, S. K.; Ekegren, J. K.; Modin, S. A.; Andersson, P. G. *Tetrahedron* **2001**, *57*, 6399.
9. Carabateas, P. M. U.S. Patent 3,860,600. *Chem. Abstr.* **1975**, *83*, 58892.
10. Yan, Z.; Caldwell, G. W. *Curr. Top. Med. Chem.* **2001**, *1*, 403.