Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3143–3146
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation of nonpeptide substituted spirobenzazepines as potent vasopressin antagonists

Min Amy Xiang, Robert H. Chen,*,† Keith T. Demarest, Joseph Gunnet, Richard Look, William Hageman, William V. Murray, Donald W. Combs, Philip J. Rybczynski and Mona Patel*

Endocrine Therapeutics and Metabolic Disorders, Johnson and Johnson Pharmaceutical Research and Development, L.L.C., 1000 Route 202, Bldg: PCC, Room: PC110, Raritan, NJ 08869, USA

Received 17 March 2004; accepted 7 April 2004

Abstract—A series of substituted spirobenzazepines was prepared and evaluated as V_{1a} and V_2 dual vasopressin receptor antagonists. Compounds **7p** and **7q** have been shown to be not only potent inhibitors of vasopressin receptors, but also have exhibited an excellent overall pharmaceutical suitability profile.

Arginine vasopressin (AVP), a cyclic peptide hormone released from the posterior pituitary, plays an important role in the homeostasis of fluid osmolality and volume status.[1] The hormone exerts its actions by interacting with three well characterized G-protein mediated receptor sub-types: vascular V_{1a}, hormone releasing V_{1b} (V_3) and renal V_2 receptors. V_1 receptors antagonists are potentially useful for the treatment of arterial hypertension, congestive heart failure and peripheral arterial disease.[2] V_2 receptor antagonists would be useful for the treatment of congestive heart failure, liver cirrhosis, nephritic syndrome and any state of excessive retention of water.[2]

Conivaptan and Lixivaptan (Fig. 1) are known AVP antagonists amongst others that are currently undergoing clinical trials. Both clinical candidates are based on the benzazepine scaffold, with Conivaptan being a dual V_{1a}/V_2 receptor antagonist and Lixivaptan being a selective V_2 receptor antagonist.[3] During the course of our research efforts directed towards the discovery of a dual V_{1a}/V_2 vasopressin receptor antagonist, a series of spirobenzazepines was identified as potent V_{1a} selective and V_{1a}/V_2 dual antagonists.[4] Although the initial data for spirocyclohexenes was promising, a poor solubility profile as well as a poor metabolic stability profile precluded additional preclinical studies. Further

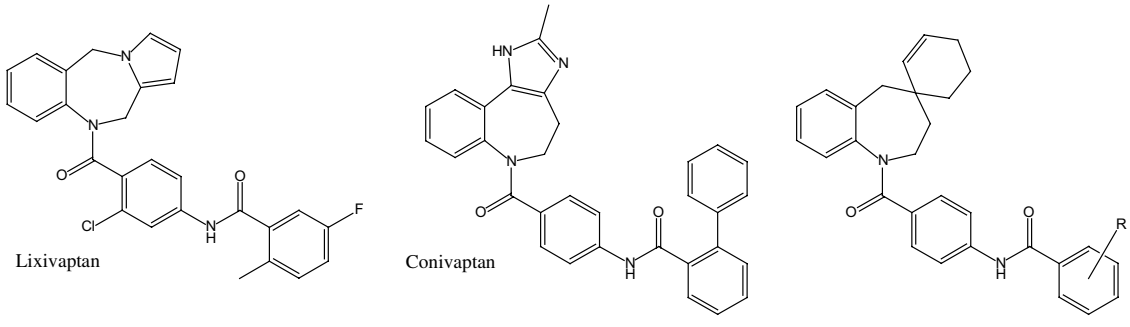


Figure 1. V_{1a}/V_2 antagonists.

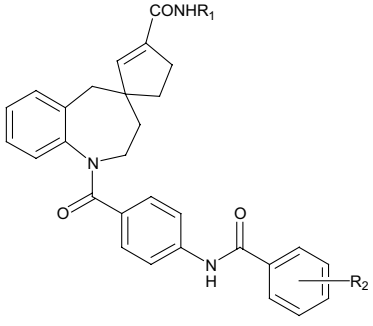
* Corresponding authors. Tel.: +1-908-707-3558; fax: +1-908-203-8109; e-mail: mpatel5@prdus.jnj.com
† Current address: Ace Chemicals and Pharmaceutical, M. L. King, Jr. Boulevard, Newark, NJ 07102, USA.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.016

development of the spirobenzazepine scaffold led to the discovery of the spiropentene framework. The preparation and evaluation of this series of compounds is described herein. The preparation of compound **1** has been previously described.[5] Compound **1** was acylated with *para* nitrobenzoyl chloride in dichloromethane to provide **2** in good yield. Ring contraction of the six membered cyclohexyl ring (**2**) to the five membered cyclopentene ring with a pendant carboxaldehyde group (**3**) was accomplished by ozonolysis of the double bond followed by recyclization.[6] The conversion of the formyl group in compound **3** to the corresponding ester was accomplished in a two step process involving first, an oxidation of the aldehyde to the acid using sodium chlorite, followed by Fischer esterification to provide the ester. The nitro group was reduced by treatment with tin(II) chloride in refluxing ethanol to provide amino ester **4**. Acylation of the aniline moiety with an appropriately substituted benzoyl chloride resulted in compound **5**. Base hydrolysis of the ester provided acid (**6**). Coupling of acid (**6**) with a variety of amines using EDC and HOBt in dichloromethane afforded **7a**–**o** (Table 1).[7] Compounds **7a** and **7e** required the use of a *t*BDMS protected alcohol, which resulted in an additional final step of deprotection with TBAF in tetrahydrofuran in quantitative yields. Furthermore, for the preparation of compound **7c**, coupling was carried with a methyl ester protected glycine, which was deprotected under basic conditions to give the carboxylic acid in good yield (Scheme 1).

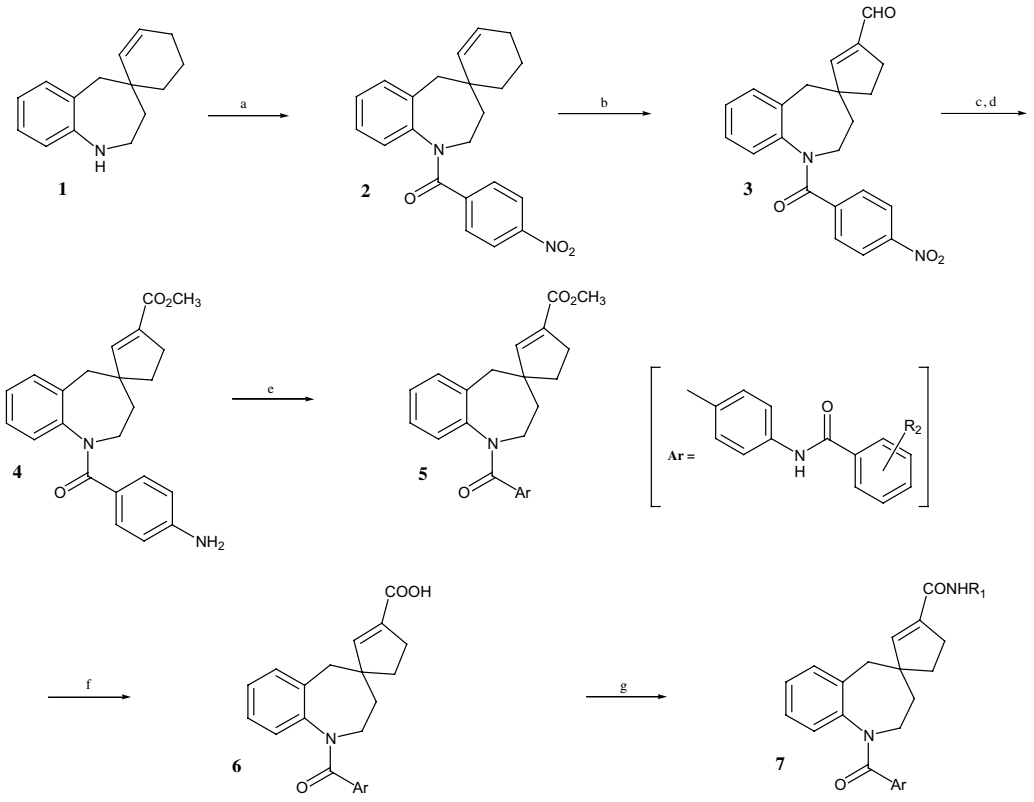
Table 1 details the in vitro potency of the spirobenzazepines. The development of SAR on the benzazepine scaffold involved variations at both the alkyl amido side chain (R_1) and substitution on the phenyl ring (R_2). The first set of compounds prepared were **7a**–**d**, wherein a phenyl substituent was introduced as R_2 and a variety of side chains were explored at the R_1 position.[3d,e] These compounds were observed to be dual V_{1a}/V_2 receptor antagonists. Next, we sought to introduce a 2-fluoro substituent at the R_2 position and retained previously explored R_1 groups. Compounds **7e** and **7f** demonstrate selectivity for the V_{1a} receptor as compared to compounds **7a** and **7b**. It seems that a 2-fluoro substituent imparts V_{1a} selectivity. In vitro activity observed for compounds **7g**–**i**, wherein variation at R_1 was introduced while retaining a 2-fluoro substituent at R_2 validates this conclusion.

Table 1. In vitro potency of the spirobenzazepines



Compound #	R_1	R_2	V_{1a} binding[8] IC_{50} (nM)	V_{1a} functional[8] IC_{50} (nM)	V_2 binding[8] IC_{50} (nM)	V_2 functional[8] IC_{50} (nM)
7a	$(CH_2)_2OH$	2-Ph	6	5	11	170
7b	$(CH_2)_2N(CH_3)_2$	2-Ph	5	4	11	100
7c	CH_2COOH	2-Ph	24	78	18	350
7d	$(CH_2)_3N(CH_3)_2$	2-Ph	5	29	16	20
7e	$(CH_2)_2OH$	2-F	4	99	>1000	>1000
7f	$(CH_2)_2N(CH_3)_2$	2-F	8	126	>1000	>1000
7g	$(CH_2)_2$N⟨O⟩	2-F	4	227	>1000	nd[b]
7h	$(CH_2)_2$N⟨⟩	2-F	7	950	>1000	>1000
7i	$(CH_2)_2$N⟨⟩	2-F	8	155	>1000	>1000
7j	$(CH_2)_2N(CH_3)_2$	2-CH_3, 5-F	5	65	20	105
7k	$(CH_2)_3N(CH_3)_2$	2-CH_3, 5-F	5	4	23	31
7l	CH_2COOCH_3	2-CH_3, 5-F	7	135	10	440
7m	$(CH_2)_2$N⟨⟩	2-CH_3, 5-F	10	48	39	48
7n	$(CH_2)_2$N⟨⟩	2-CH_3, 5-F	19	29	39	48
7o	$(CH_2)_2$N⟨⟩	2-CH_3, 5-F	16	71	8	9
7p[a]	$(CH_2)_2N(CH_3)_2$	2-Ph	2	45	8	36
7q[a]	$(CH_2)_2N(CH_3)_2$	2-F	4	78	341	646

[a] Compound is a single enantiomer (*R*).
[b] nd = not determined.



Scheme 1. Reagents and conditions: (a) 4-nitrobenzoyl chloride, TEA, DCM, 70–90%; (b) ozone, DMS; TsOH–H_2O, DCM, 60–75%; (c) $NaClO_2$, DMSO, NaH_2PO_4, H_2O; concd H_2SO_4, ethanol, 80–90% over two steps. (d) $SnCl_2$, ethanol, 60–90%. (e) ArCOCl, TEA, DCM, 60–70%. (f) LiOH, THF–H_2O, 60–70%; (g) HOBt, EDC, RNH_2, DCM, 60–65%.

Next, we sought to introduce a known substitution pattern in a 2-CH_3-5-F moiety at the R_2 position.[3a,b,c] Compounds **7j–o** appear to be dual V_{1a}/V_2 antagonists. Again, we observe in compounds **7j**, **7k**, **7m**, **7n** and **7o** that selectivity is determined by substituents at R_2 rather than by R_1 substituents. Therefore, we concluded R_1 substituents may be utilized to adjust the pharmaceutical suitability profile of this series of compounds. Compounds bearing a three carbon linker in R_1 such as compounds **7d** and **7k** as well as those bearing a morpholine (**7g**), pyrrolidine (**7i**, **7n** and **7o**) and piperidine (**7h** and **7m**) moiety had observed pK_a values in the range of 9–10 and were deemed to have poor absorption potential as they would likely be protonated at physiological pH. As compounds **7b** and **7f** were of interest, the racemic mixtures of each were chromatographically resolved and active enantiomer (*R*) identified as compounds **7p** and **7q**, respectively.[9] Compound **7p** was recognized as a dual V_{1a}/V_2 antagonist and **7q** as a V_{1a} selective antagonist, and as a result were subjected to additional assays (Table 2). The solubility profiles of both compounds were satisfactory as were the pK_a and log*P* values. Human liver microsome stability assays were carried out to determine half-life values for possible Phase I oxidative metabolism. The $t_{1/2}$ values for both **7p** and **7q** were higher than our acceptable minimum of 30 min. Encouraged by this data, we embarked on additional key assays to determine Caco-2 permeability potential, cytochrome P450 inhibition profile as well as the Ames mutagenicity tests.

Both compound **7p** and **7q** subjected to Caco-2 permeation assay and were considered to have high absorption potential (Papp[A to B] $\geqslant 1.0\times10^{-6}$ cm/s). As drug–drug interactions are often the result of cytochrome P450 inhibition, we sought to profile **7p** and **7q** through a series of cytochrome P450 isozymes. Compounds **7p** and **7q** had IC_{50} values significantly higher than 10 μM and were therefore not considered potential inhibitors. In the Ames test both compounds were negative. Due to good intrinsic activity and a good pharmaceutical suitability profile, compounds **7p** and **7q** were selected for pharmacokinetic studies in rats (Table 3). To explore possible species differences, the antagonistic activities of

Table 2. Pharmaceutical suitability properties of **7p** and **7q**

Compound #	Solubility (mg/mL) @ pH 2	Solubility (mg/mL) @ pH 7.4	pK_a	Log*P*	HLM stability ($t_{1/2}$) minutes[a]
7p	0.16	0.04	8.36	3.63	65
7q	0.32	0.07	8.18	2.45	43

[a] Human liver microsomal stability assay was run at a concentration of 5 μM for the compounds. Studies were conducted by Absorption Systems, Exton, PA.

Table 3. Pharmacokinetic profiles for **7p** and **7q** in rats

Compound #	Route	Dose (mg/kg)	AUC (μM h)	C_{max} (μM)	T_{max} (h)	$t_{1/2}$ (h)	F (%)
7p	Oral	30	4.57	0.79	4	6.47	22
	IV	3	2.06	2.66	NA[a]	1.68	NA[a]
7q	Oral	30	7.04	1.1	4.67	4.15	42
	IV	3	1.68	1.8	NA[a]	4.35	NA[a]

[a] NA = not applicable.

7p and **7q** were evaluated in cells transfected with rat V_{1a} and V_2 cells. **7p** had IC_{50} values of 33 and 5 nM in rat V_{1a} and V_2 binding assay, respectively. **7q** had IC_{50} values of 433 and 444 nM in rat V_{1a} and V_2 binding assay, respectively.

Following a single oral dose of **7p** (30 mg/kg) in rats, a C_{max} of 0.79 μM and a T_{max} of 4 h was observed. The compound was moderately eliminated as evidenced by a terminal half-life of 6.47 h and the absolute oral bioavailability was determined to be 22%. Following an intravenous administration of **7p** (3 mg/kg), the apparent volume of distribution (6836 mL/kg) was greater than the total body water for the species, indicating extensive distribution of **7p** outside of the plasma. Following a single oral dose of **7q** (30 mg/kg) in rats, a mean T_{max} value of 4.67 h was observed and was moderately eliminated based on a terminal half-life of 4.15 h. The absolute oral bioavailability was determined to be 42%. Following an intravenous dose of **7q** (3 mg/kg), the apparent volume of distribution (6675 mL/kg) exceeded total body water indicating distribution of **7q** outside of the plasma. Compounds **7p** and **7q** have demonstrated an overall balance of good permeability, oral bioavailability and a long terminal half-life.

Optimization of our spirobenzazepine template led to the discovery of highly potent dual V_{1a}/V_2 (**7p**) and selective V_{1a} (**7q**) vasopressin receptor antagonists. Both compounds have not only shown good in vitro and in vivo activity, but have also shown good pharmaceutical suitability profiles.

References and notes

1. (a) Laszlo, F. A.; Laszlo, F., Jr. *Drug News Perspect.* **1993**, *6*, 591; (b) Michel, R. H.; Kirk, C. J.; Billah, M. M. *Biochem. Soc. Trans.* **1979**, *7*, 861; (c) Jard, S. *Kidney Int., Supp.* **1988**, *26*, 38; (d) Thibonnier, M. *Regul. Pep.* **1992**, *38*, 1.
2. (a) Laszlo, F. A.; Laszlo, F., Jr.; De Wied, D. *Pharmacol. Rev.* **1991**, *43*, 73; (b) Mah, S. C.; Hofbauer, K. G. *Drugs Fut.* **1987**, *12*, 1055; (c) Paranjape, S. B.; Thibonnier, M. *Exp. Opin. Invest. Drugs* **2001**, *10*, 825; (d) Trybulski, E. J. In *Annual Reports in Medicinal Chemistry*; Doherty, A. H., Ed.; Academic: San Diego, 2001; Vol. 36, pp 159–168; (e) Albright, J. D.; Chan, P. S. *Curr. Pharm. Des.* **1997**, *3*, 615.
3. Lixivaptan: (a) Martinez-Castelao, A. *Curr. Opin. Investig. Drugs* **2001**, *2*, 525; (b) 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; (c) Albright, J. D.; Delos Santos, E. G.; Dusza, J. P.; Chan, P. S.; Coupet, J.; Ru, X.; Mazandarani, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 695; (d) Conivaptan: Norman, P.; Leeson, P. A.; Rabasseda, X.; Castaner, J.; Castaner, R. M. *Drugs Fut.* **2000**, *25*, 1121; (e) Matsushita, A.; Taniguchi, N.; Koshio, H.; Yatsu, T.; Tanaka, A. *Chem. Pharm. Bull.* **2000**, *48*, 21.
4. 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.
5. Chen, R. H.; Xiang, M. A. WO 02/02531 A1, 2002.
6. Chen, R. H; Xiang, M.; Moore, J. B., Jr.; Beavers, M. P. U.S. Patent 5,972,939, 1999.
7. All compounds provided satisfactory spectral data (^{1}H NMR, CI-MS/ESI/MS and were homogeneous by TLC).
8. Rigas, A.; Levine, L. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 230. Binding affinities were determined by measuring the inhibition of ^{3}[H] AVP binding to cloned human V_{1a} and V_2 receptors. Functional activity was determined by (a) the accumulation of cAMP was measured in transfected HEK-293 cells expressing human V_2 receptors and (b) the intracellular calcium mobilization was measured in HEK-293 cells transfected to express human V_{1a} receptors.
9. The absolute stereochemistry (*R* enantiomer) of compounds **7p** and **7q** was assigned via crystal structure analysis of an intermediate prepared by H. Marlon Zhong and co-workers of the Chemical Development Group, Johnson and Johnson Pharmaceutical Research and Development, L.L.C.