Brief Articles

Characterization of Orally Active Nonpeptide Vasopressin V_2 Receptor Agonist. Synthesis and Biological Evaluation of Both the (5*R*)- and (5*S*)-Enantioisomers of 2-[1-(2-Chloro-4-pyrrolidin-1-yl-benzoyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepin-5-yl]-*N*-isopropylacetamide

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The synthesis and evaluation of both the (*R*)- and (*S*)-enantioisomers about the 5-position on a tetrahydro-1*H*-1-benzazepine derivative were described. The absolute configuration of the (*R*,*R*)-isomer (**10**) was determined by X-ray crystallographic analysis. After evaluation of both enantiomers (compounds *R*-**2**, *S*-**2**) for binding affinity, cAMP accumulation, and an in vivo study using Brattleboro rats, *R*-**2** showed more potent activity as a V_2 receptor agonist than *S*-**2**.

Introduction

The cyclic nonapeptide arginine vasopressin (AVP) is a well-known hormone that exerts its major actions through three well-defined receptor subtypes: the V_{1a}, V_{1b}, and V_2 receptors.[1] The V_{1a} receptor subtype, which mainly exists in vascular smooth muscle cells, platelets, liver, the adrenal gland, and uterus, causes contraction and proliferation of the vascular smooth muscle, platelet aggregation, coagulation factor (factor VIII) release, and glycogenolysis. The V_{1b} receptor is found in the pituitary that is involved in the corticotropic response to stress through regulation of ACTH secretion and the potentiating effect on the corticotropin-releasing hormone (CRH). The V_2 receptor is present in the collecting duct of the kidney. Vasopressin-induced antidiuresis, mediated by the renal epithelial V_2 receptors, helps to maintain normal plasma osmolality, blood volume, and blood pressure.

Desmopressin (1-desamino-8-D-Arg vasopressin, DDAVP) is a peptide analogue of AVP, which is an agonist of the AVP V_2 receptor. It is used for the treatment of central diabetes insipidus and the control of nocturnal enuresis and may also be of use for the controlling of nocturia. However, DDAVP is a peptide analogue with variable bioavailability even via the intranasal route or oral formulation. Therefore, the discovery and the development of an orally effective nonpeptide V_2 agonist may be particularly useful in all respects.

As we have reported for nonpeptide V_2 agonists, 1-(2-chloro-4-pyrrolidin-1-ylbenzoyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepine (**1**) showed a high affinity for the human V_2 receptor and potent activity as an agonist (IC_{50} = 9 nM, 64.5% to maximal cAMP accumulation at a concentration of 10^{-6} M).[2] The antidiuretic effect of compound **1** was also confirmed by po administration using Brattleboro rats for reduced urine volume (7.3% to control, 1 mg/kg, 0−2 h) and increased urine osmolality (4.76-fold to control, 1 mg/kg), though the binding affinity of **1** for the rat V_2 receptor was less potent than for the human receptor (IC_{50} rat V_2 = 0.15 μM). After optimization and evaluation of the functional groups on the structure of **1**, the *N*-isopropylacetamide-substituted analogue **2** showed more potent activity by po administration than compound **1**. Further characterization of both the (5*R*)- and (5*S*)-enantiomers of **2** gave us some interesting results for the binding affinity and agonist activity. The syntheses of both enantiomers were achieved by optical resolution to form esters with optically active alcohols.

Chemistry

The synthesis of compound **2** (racemate), the (5*R*)-enantioisomer, and the 5*S*-enantioisomer are shown in Scheme 1. An α,β-unsaturated ester group was introduced by the Horner−Emmons reaction to give compound **4**. The reduction of the olefin and removal of the 1-tosyl group were simultaneously done by reacting with Mg in methanol to afford **5**. After condensing benzazepine **5** with 2-chloro-4-pyrrolidin-1-yl benzoic acid (**6**),[2] the ester group was hydrolyzed to give the carboxylic acid **8**. The *N*-isopropylacetamide derivative (**2**) was obtained by the condensation of **8** with isopropylamine using BOP-Cl.

A mixture of diastereoisomers **10** and **11** was obtained by the condensation of **8** with (*R*)-(−)-2-heptanol (**9**) using WSC and DMAP. After a brief separation of both

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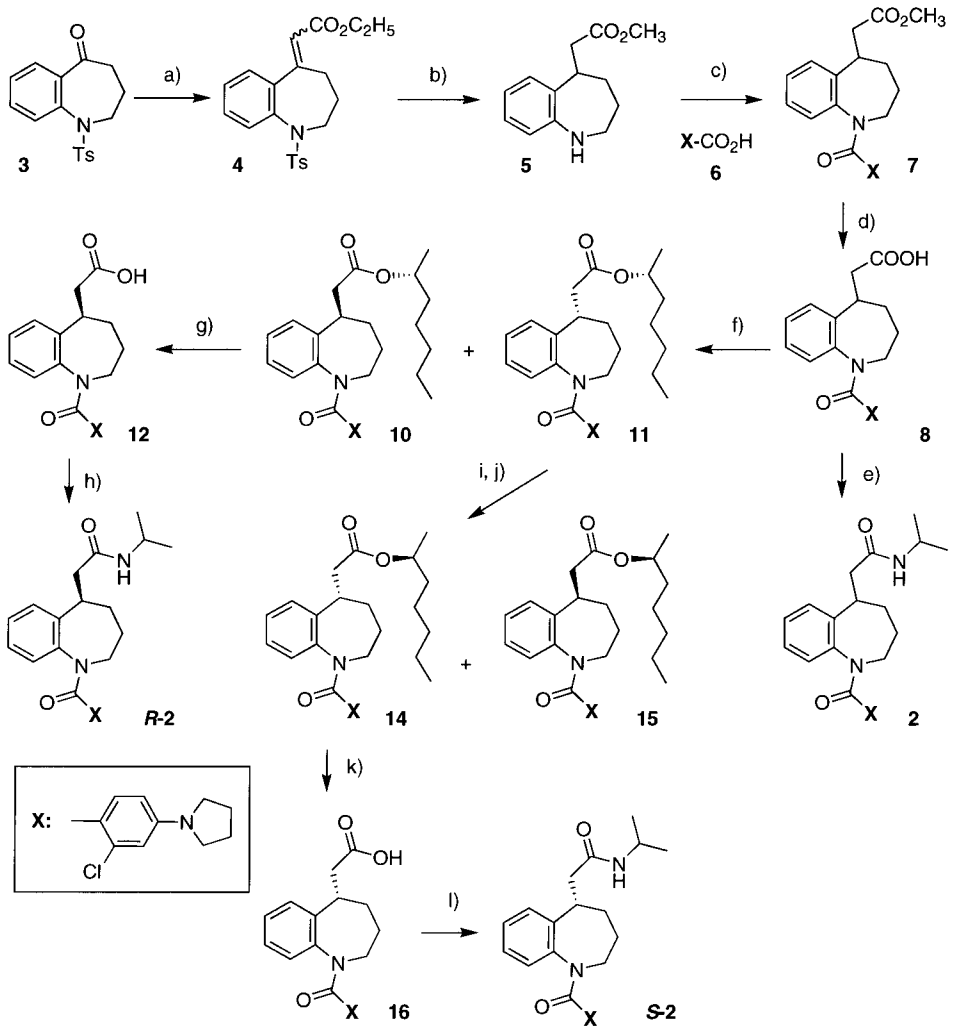
Scheme 1[a]



[a] Reagents: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (b) Mg, MeOH; (c) $SOCl_2$, NMP, CH_2Cl_2; (d) 6 N NaOH, MeOH; (e) isopropylamine, BOP-Cl, Et_3N, CH_2Cl_2; (f) (*R*)-2-heptanol (**9**), WSC, DMAP; (g) 5 N NaOH, MeOH; (h) isopropylamine, diethyl cyanophosphonate, DMF; (i) 6 N NaOH, MeOH; (j) WSC, DMAP, (*S*)-2-heptanol (**13**), DMF; (k) 5 N NaOH, MeOH; (l) isopropylamine, diethyl cyanophosphonate, DMF.

isomers by silica gel column chromatography, two fractions of both isomer-rich mixtures were obtained. Crystals were formed in a mixture of the polar substrate-enriched fraction. After several recrystallizations and analysis by X-ray crystallography, the absolute configuration of the polar substrate was determined on the basis of the configuration of the alcohol (**9**). The chiral centers of this compound (**10**) were assigned to have the (*R*,*R*)-configuration. The hydrolysis of the ester group (**10**) was achieved by heating with 5 N NaOH for 5 h at 50 °C to provide the carboxylic acid (**12**). The condensation of **12** with isopropylamine afforded the compound *R*-**2**. The absolute configuration at the 5-position of the resulting compound is suggested to be *R*.

The less polar substrate-enriched fraction, which was obtained as a mixture of **10** and **11**, was hydrolyzed, and the subsequent condensation with (*S*)-(+)-2-heptanol then gave a mixture of **14** and **15**. The formation of crystals was definitely expected for the isomer having the (*S*,*S*)-configuration. Actually, the (*S*,*S*)-isomer, compound **14**, was easily isolated after purification by several recrystallizations. After the hydrolysis of the ester group, the condensation of **16** with isopropylamine provided *S*-**2**.

Results and Discussion

The binding affinity for the human V_2 receptors of *R*-**2** is 0.33 ± 0.03 (IC_{50}, μM) and that of *S*-**2** is 0.98 ± 0.09. The binding affinity for the rat V_2 receptors of *R*-**2** is 0.23 ± 0.07 and that of *S*-**2** is 0.39 ± 0.04. Although the substitution of *N*-isopropylacetamide at the 5-position did not affect the binding affinity for the rat receptor, it caused a detrimental effect on the binding affinity for the human receptor. The binding affinity of *R*-**2** was 36.7-fold less potent than compound **1** for the human receptor, and *S*-**2** was 108.9-fold less potent than compound **1**. The binding affinity of *R*-**2** was 3 times more potent than *S*-**2** for the human receptor. This discrepancy in the binding affinity of the (*R*)- and (*S*)-isomers was small for the rat receptor (1.7-fold). Figure 1 shows the concentration-dependent percentage of the maximal cAMP accumulation for **2,** *R*-**2**, and *S*-**2** in the human receptor. *R*-**2** showed the accumulation of cAMP to be 52% at a concentration of 10^{-6} M. *S*-**2** showed a cAMP accumulation of 22% at a concentration of 10^{-4} M. Both isomers did not reach 100% of the maximal response with AVP; therefore, these compounds were determined to be partial agonists. Although the binding

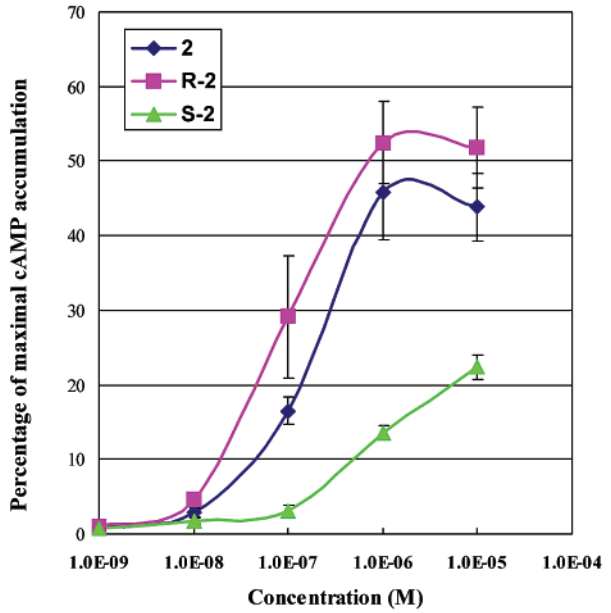


Figure 1. cAMP accumulation in response to nonpeptide compounds at the given concentration expressed as percent with standard error of the response obtained by stimulation with AVP (1 nM) in HeLa cells expressing human V_2 receptors.

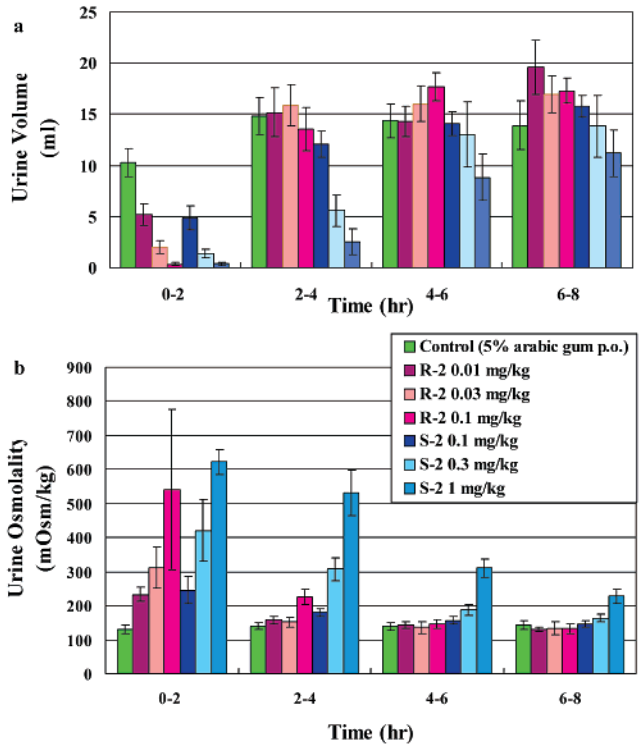


Figure 2. Antidiuretic action of compounds *R*-**2** and *S*-**2** administered orally to female Brattleboro rats: (a) urine volume; (b) urine osmolality. Values are expressed as mean ± SEM of five rats.

affinity of *R*-**2** is 3 times more potent than *S*-**2** for the human receptor, the accumulation of cAMP was observed at a very low concentration when *R*-**2** was used as the substrate. *S*-**2** showed poor agonist activity even at a high concentration. Figure 2 shows the antidiuretic action of compounds *R*-**2** and *S*-**2** orally administered to female Brattleboro rats.[3] A reduction of urine volume was observed after po administration of compound *R*-**2** at a dose of 0.01 mg/kg. An increase in the urine osmolarity was also seen under the same conditions. The same magnitude of antidiuretic action was observed using compound *S*-**2** by po administration at a dose of 0.1 mg/kg. Both compounds had an antidiuretic action in a dose-dependent manner.

Conclusion

We have prepared a series of benzazepine derivatives as AVP V_2 receptor agonists.[2] Although additional functional groups on the 5-position enhanced the oral activity, the binding affinity for the human receptor was dramatically affected by the difference in configuration at this position. *R*-**2** (OPC-51803) showed a more potent agonist activity both in vitro and in vivo than *S*-**2.**[4] This compound is the first reported example of an orally potent nonpeptide agonist for the AVP V_2 receptor and is expected to be an orally effective drug for treating patients with low circulating levels of AVP.[5]

Experimental Section

The radioligand binding assay and measurement of the cyclic AMP production using HeLa cells expressing the human AVP V_2 receptors were performed according to the reported method.[4,6]

Animals. Homozygous Brattleboro rats (weighing 180−300 g) were bred in our animal house (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The rats were housed in a temperature-, humidity-, and light-controlled room and given free access to food (MF; Oriental Yeast, Osaka, Japan) and water. The care and handling of these animals were in accordance with The Guidelines for Animal Experimentation at Otsuka Pharmaceutical Co., Ltd., October 1, 1994.

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Supporting Information Available: General experimental procedure for syntheses of compounds, single-crystal X-ray analysis of **10**, physical and spectral characterizations (^{1}H NMR, mass spectrometry) and physicochemical parameters, and tables listing crystal data and structure refinement, atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, and isotropic displacement parameters of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Altura, B. M.; Altura, B. T. Vascular smooth muscle and neurohypophysical hormones. *Fed. Proc.* **1977**, *36*, 1853−1860. (b) Howl, J.; Ismail, T.; Strain, A. J.; Kirk, C. J.; Anderson, D.; Wheatley, M. Characterization of human liver vasopressin receptor. *Biochem. J.* **1991**, *276*, 189−195. (c) Jard, S.; Lombard, C.; Marie, J.; Devilliers, G. Vasopressin receptors from cultured mesangial cells resemble V_{1a} type. *Am. J. Physiol.* **1987**, *253*, F41−F49. (d) Penit, J.; Faure, M.; Jard, S. Vasopressin and angiotensin II receptors in rat aortic smooth muscle cells in culture. *Am. J. Physiol.* **1983**, *244*, E72−E82. (e) Thibonnier, M.; Roberts, J. M. Characterization of human platelet vasopressin receptors. *J. Clin. Invest.* **1985**, *76*, 1857−1964. (f) Dorsa, D. M.; Majumdar, L. A.; Petracca, F. M.; Baskin, D. G.; Cornett, L. E. Characterization and localization of ^{3}H-arginine^8-vasopressin binding to rat kidney and brain tissue. *Peptides* **1983**, *4*, 699−706. (g) Birnbaumer, M. Vasopressin Receptors. *Trends Endocrinol. Metab.* **2000**, *10*, 406−410.

(2) Kondo, K.; Ogawa, H.; Shinohara, T.; Kurimura, M.; Tanada, Y.; Kan, K.; Yamashita, H.; Nakamura, S.; Hirano, T.; Yamamura, Y.; Mori, T.; Tominaga, M.; Itai, A. Novel design of nonpeptide AVP V2 receptor agonists: Structural requirements for an agonist having 1-(4-aminobenzoyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepine as a template. *J. Med. Chem.* **2000**, *43*, 4388−4397.

(3) (a) Schmale, H.; Richter, D. Single base deletion in the vasopressin gene is the cause of diabetes insipidus in Brattleboro rats. *Nature (London)* **1984**, *308*, 705−709. (b) Valtin, H.; Schroeder, H. A. Familial hypothalamic diabetes insipidus in rats (Brattleboro strain). 1964. *J. Am. Soc. Nephrol.* **1997**, *8*, 1333−1341.

(4) Nakamura, S.; Yamamura, Y.; Itoh, S.; Hirano, T.; Tsujimae, K.; Aoyama, M.; Kondo, K.; Ogawa, H.; Shinohara, T.; Kan, K.; Tanada, Y.; Teramoto, S.; Sumida, T.; Nakayama, S.; Sekiguchi, K.; Kambe, T.; Tsujimoto, G.; Mori, T.; Tominaga, M. Characterization of a novel nonpeptide vasopressin V_2-agonist, OPC-51803, in cells transfected human vasopressin receptor subtypes. *Br. J. Pharmacol.* **2000**, *129*, 1700−1706. (b) Nakamura, S.; Hirano, T.; Tsujimae, K.; Aoyama, M.; Kondo, K.; Yamamura, Y.; Mori, T.; Tominaga, M. Antidiuretic effects of a nonpeptide vasopressin V_2-receptor agonist, OPC-51803, administered orally to rats. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1005−1011.

(5) (a) Newsome, H. H., Jr. Vasopressin: Deficiency, excess and the syndrome of inappropriate antidiuretic hormone secretion. *Nephron* **1979**, *23*, 125−129. (b) Pullan, P. T.; Clappison, B. H.; Johnston, C. I. Plasma vasopressin and human neurophysins in physiological and pathological states associated with changes in vasopressin secretion. *J. Clin. Endocrinol. Metab.* **1979**, *49*, 580−587. (c) Norgaard, J. P.; Pedersen, E. B.; Djurhuus, J. C. Diurnal anti-diuretic-hormone levels in enuretics. *J. Urol.* **1985**, *134*, 1029−1031. (d) Rittig, S.; Knudsen, U. B.; Jonler, M.; Norgaard, J. P.; Pedersen, E. B.; Djurhuus, J. C. Adult enuresis. The role of vasopressin and atrial natriuretic peptide. *Scand. J. Urol. Nephrol., Suppl.* **1989**, *125*, 79−86. (e) Rittig, S.; Knudsen, U. B.; Norgaard, J. P.; Pedersen, E. B.; Djurhuus, J. C. Abnormal diurnal rhythm of plasma vasopressin and urinary output in patients with enuresis. *Am. J. Physiol.* **1989**, *256* (4, Part 2), F664−F671. (f) Steffens, J.; Netzer, M.; Isenberg, E.; Alloussi, S.; Ziegler, M. Vasopressin deficiency in primary nocturnal enuresis. Results of a controlled prospective study. *Eur. Urol.* **1993**, *24*, 366−370.

(6) Yamamura, Y.; Nakamura, S.; Itoh, S.; Hirano, T.; Onogawa, T.; Yamashita, T.; Yamada, Y.; Tsujimae, K.; Aoyama, M.; Kotosai, K.; Ogawa, H.; Yamashita, H.; Kondo, K.; Tominaga, M.; Tsujimoto, G.; Mori, T. OPC-41061, a highly potent human vasopressin V_2-receptor antagonist: pharmacological profile and aquaretic effect by single and multiple oral dosing in rats. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 860−867.

(7) North, A. C. T.; Phillips, D. C.; Mathews, F. S. A semi-empirical method of absorption correction. *Acta Crystallogr.* **1968**, *A24*, 351−359.

(8) *TEXSAN crystallographic software package*, version 1.11.; Molecular Structure Corporation: The Woodlands, TX, 1995 and 1999.

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