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Novel Vasopressin V2 Receptor-selective Antagonists, Pyrrolo[2,1-*a*]quinoxaline and Pyrrolo[2,1-*c*][1,4]benzodiazepine Derivatives

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Abstract—The intent of the work was to study the structure–activity relationships of AVP receptor antagonists bearing a chiral ring as a partial structure since such studies had been reported for only achiral compounds. In the present paper, we deal with compounds consisting of the chiral tricyclic hetero ring (1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoxaline and 1,2,3,10,11,11a-hexahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine) and 2-phenylbenzanilide analogues. These compounds exhibited a highly selective affinity for V2 receptor, and their stereochemical configuration had a great influence on V2 receptor binding. **VP-343** (*N*-[4-[[(2*S*,3a*R*)-2-hydroxy-2,3,3a,4-tetrahydropyrrolo[1,2-*a*]quinoxalin-5(1*H*)-yl]carbonyl]phenyl]-4′-methyl[1,1′-biphenyl]-2-carboxamide), **VP-365** (*N*-[4-[[(11a*S*)-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-10(5*H*)-yl]carbonyl]phenyl][1,1′-biphenyl]-2-carboxamide) and **VP-339** (N-[4-[[(11aS)-5-oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-10(5*H*)-yl]carbonyl]phenyl]-[1,1′-biphenyl]-2-carboxamide) were the most potent compounds in vitro and in vivo. The IC_{50} values of **VP-343**, **VP-365** and **VP-339** against V2 receptor were 0.772, 1.18 and 0.216 nM, respectively. The ED_{300} values (dose required to increase three times the urine volume of the control rats; oral administration) of **VP-343**, **VP-365** and **VP-339** were 0.22, 0.31 and 0.78 mg/kg, respectively.

Introduction

Arginine vasopressin (AVP) is a well-known peptide hormone with a variety of biological actions on distinct tissues. Recently, three receptor subtypes (V1a, V1b and V2) have been cloned and found to belong to the seven transmembrane G protein-coupled receptors superfamily.[1–3] The V1a receptors are mainly located in vascular smooth muscle[4] and liver cells.[5] Manning et al.[6] reported that the vasoconstriction activity by AVP was due to the interaction between AVP and the V1a receptor. Jard et al.[7] reported that the V1b receptors were located in the anterior pituitary where they stimulated corticotropin release. AVP activates phospholipase C-mediated hydrolysis of polyphosphoinositides through the V1a and V1b receptors to generate two second messengers, inositol-1,4,5-triphosphate, which induces an increase of free intracellular calcium from the endoplasmic reticulum, and 1,2-diacylglycerol, which activates protein kinase C.[8] As for the V2 receptor, Butlen et al.[9] reported that they were located in the kidney where they controlled free water and urea reabsorption. The receptor stimulates adenylate cyclase, which results in the production of cyclic AMP.

AVP may play a role in several disease conditions, including heart failure, hypertension, hyponatremia, and syndrome of inappropriate antidiuretic hormone secretion. The study on selective AVP antagonists is essential for the elucidation of the pathophysiological role of AVP.

Until recently, all potent AVP receptor antagonists reported have been peptide analogues of AVP[10–14] and have not had good oral bioavailability, with some exhibiting partial agonistic activity.[15,16] Recently, orally effective nonpeptide V1a-selective (OPC-21268[17]) and V2-selective (OPC-31260[18]) antagonists have been reported (Fig. 1).

The study on structure–activity relationships (SAR) for OPC-31260 indicated several important facts. They can be summarized as follows: (1) the benzene-fused seven-membered ring system was significant for V2 (and V1a) antagonists; (2) the ortho substituents on the terminal

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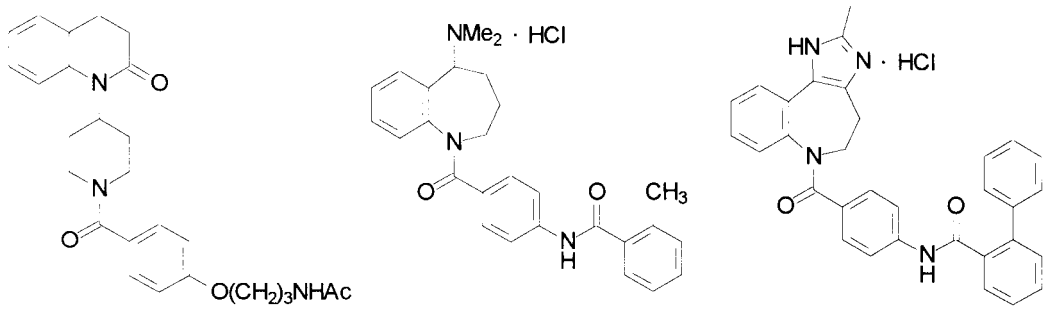


Figure 1.

benzoyl ring seemed to be important both for a high affinity for the V2 receptor and good oral activity.

Tahara et al.[19] reported the biochemical and pharmacological properties of YM-087, which was a nonpeptide antagonist of both V1a and V2 receptors. The study on the systematically modified compounds relevant to YM-087 clarified the structure features required for the binding affinity for vasopressin receptors. These studies revealed that the introduction of a lipophilic group such as 2-phenyl or 2-(4-substituted phenyl) on the terminal benzoyl ring exhibited more potent antagonistic activities than that of 2-methyl group in the in vivo tests towards both V1a and V2 receptors.[20]

The study on the SAR of the compound bearing chiral ring as a partial structure has not been reported on the AVP receptor antagonists. As for the several peptide hormones such as cholecystokinin,[21] substance P[22] and Progesterone,[23] the importance of the chirality of receptor antagonists has been elucidated.

We intended to perform the SAR studies on AVP receptor antagonists with chirality. Therefore, our study was focused on the SAR of the compounds consisting of the chiral tricyclic hetero ring and the benzanilide moiety bearing 2-phenyl or 2-(4-substituted phenyl) group. The two different types of compounds were studied with respect to the six and seven-membered ring system. One was 1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoxaline derivatives (Q type, compound **1**). The other was 1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine derivatives (B type, compounds **2** and **3**) (Fig. 2). These compounds were synthesized from the standpoint of introducing chirality into the six and seven-membered ring system such as OPC-21268, OPC-31260 and YM-087. In the present paper, we deal with the synthesis and biological properties of these novel AVP antagonists.

Results and Discussion

Chemistry

The synthesis of Q type compounds (**1a–1d**) is shown in Schemes 1 and 2. As the starting material, *trans*-4-hydroxy-L-proline (or *cis*-4-hydroxy-D-proline) methyl ester hydrochloride was used for the synthesis of **4a** and **4c**. The stereochemical inversion of the hydroxy group of **4a,c** that used the improved Mitsunobu condition,[24] gave **4b** and **4d**, then the resulting esters **5a** and **5c** were hydrolyzed with aqueous NaOH to give **4b,d**. Recrystallization in AcOEt/$CHCl_3$ gave optically pure **4b,d**. Because the amides **6a–d** were very labile, the two consecutive reductions of the nitro group (**4a–d**) and the resulting amide group (**6a–d**) were successively done without isolating **6a–d**. The amines **7a–d** were purified as the hydrochloride. The compounds **1a–d** were prepared by acylating **7a–d** with 4-(4′-methyl-2-biphenylcarboxamido)benzoyl chloride. Overall yields of **1a–d** were 21–37% in general.

The synthesis of B type compounds (**2**, **3**) is shown in Scheme 3. Reduction of the carbonyl groups in the dilactams **8a**[25] and **8b**[25] with lithium aluminium hydride gave the diamines **9a**[26] and **9b** in 87% and 90% yields,

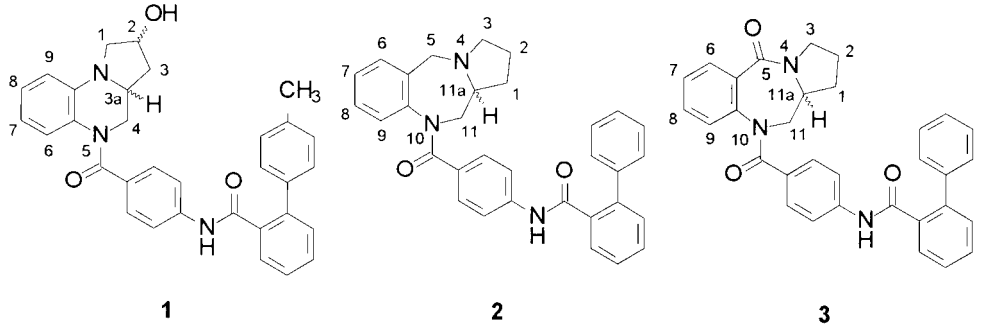
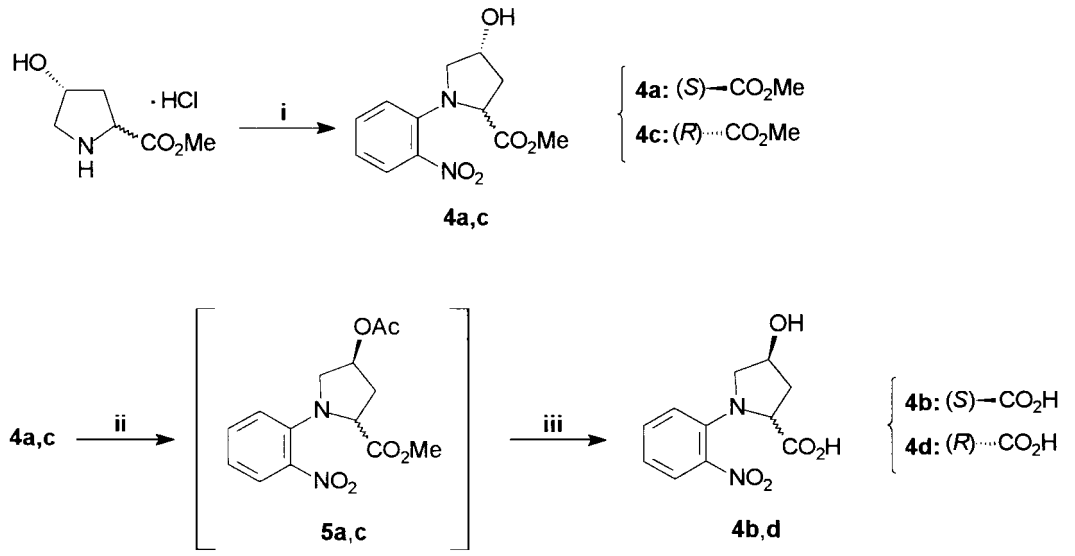
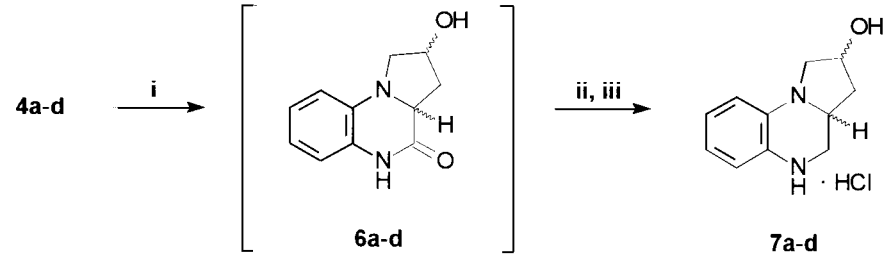
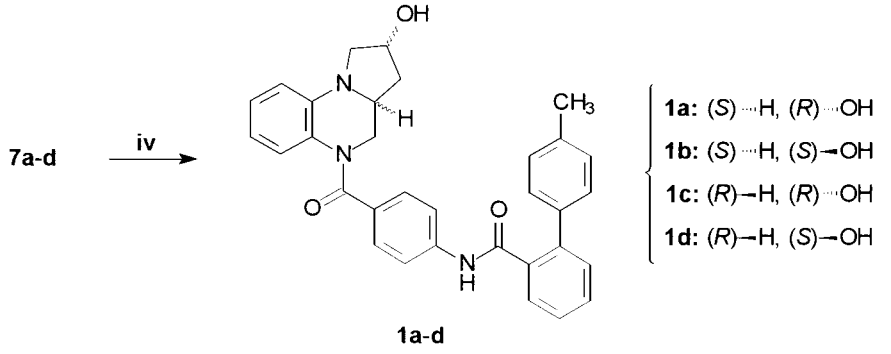


Figure 2.



Scheme 1. i) 1-fluoro-2-nitrobenzene, Et_3N, CH_3CN, 60 °C, 16 h; ii) Ph_3P, DIPAD, AcOH, toluene, rt, 2 h; iii) 1. aq. NaOH, rt, 20 h 2.HCl





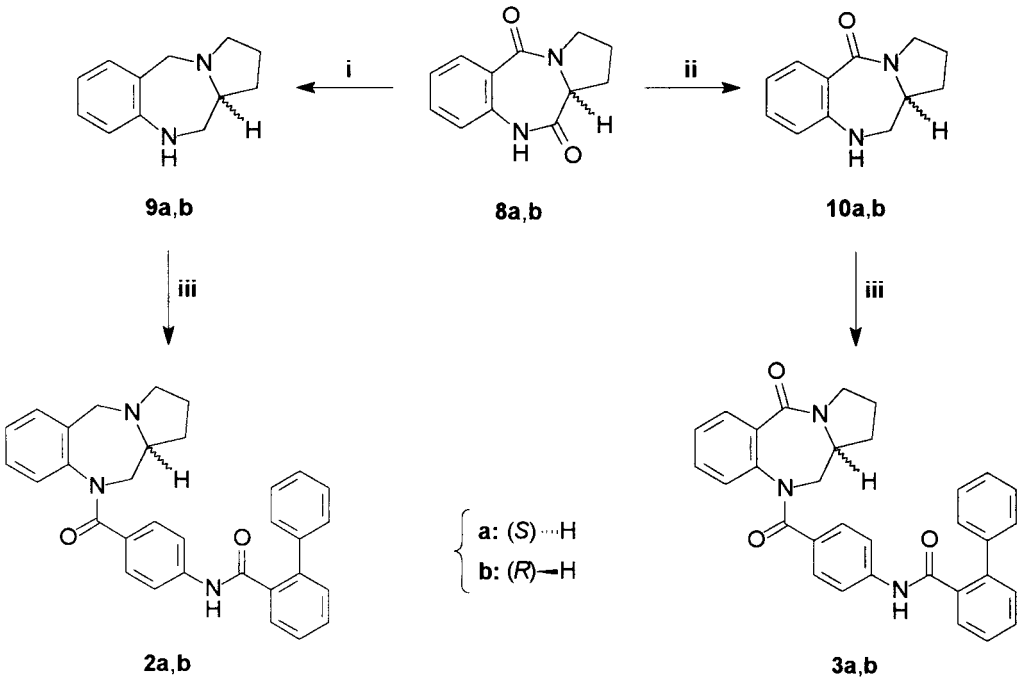
Scheme 2. i) 10% Pd-C, Et_3N, THF, 45 °C, 4 h; ii) $NaBH_4$, THF, ref., 18 h; iii) HCl; iv) 4-(4′-methyl-2-biphenylcarboxamido)benzoyl chloride, pyridine, CH_2Cl_2, rt, 18 h.

respectively. Selective reduction of the carbonyl group at C-11 position in **8a** and **8b** in refluxing glyme containing 2 equiv $NaBH_4$ and 1.3 equiv trifluoroacetic acid (TFA)[27] for 4 h gave quantitatively the monoamines **10a**[28] and **10b**. The yield, however, was generally poor when the acid instead of TFA (e.g. AcOH or MsOH) was used. To achieve the quantitative yield, the above experimental condition could be recommended. The compounds **2a** and **2b** were prepared by acylating **9a**, **9b** with 4-[(2-phenylbenzoyl)amino]benzoyl chloride[29] in 81% and 79% yields, respectively. The compounds **3a** and **3b** were prepared by acylating **10a** and **10b** with the above acid chloride in 73% and 70% yields, respectively.

Binding study

The binding studies were done by a slightly modified method of Thibonnier et al.[30] with rat liver (V1a receptor) and kidney (V2 receptor) plasma membranes. The affinity of compounds was determined by competition studies with [^{3}H]d(CH_2)$_5$Tyr(Me)AVP ([^{3}H]-V1 antagonists) and [^{3}H]desGly(NH_2)d(CH_2)$_5$[D-Ile^2,Ile^4]AVP ([^{3}H]-V2 antagonists) as a radioligand.

As shown in Tables 1 and 2, all the test compounds showed the strong affinity and the high selectivity for V2 receptor binding. The results of the binding study on



Scheme 3. i) $LiAlH_4$, THF, ref., 3 h; ii) $NaBH_4$, TFA, glyme, ref., 4 h; iii) 4-[(2-phenylbenzoyl)amino]benzoyl chloride, pyridine, CH_2Cl_2, rt, 18 h

Table 1. Vasopressin receptor binding affinities of Q type

Compound	C-3a position H	C-2 position X	IC_{50} (nM) V1a	IC_{50} (nM) V2	UV (mL/kg) i.v.	UV (mL/kg) p.o.
1a	(*S*) H	(*R*) OH	8130	27.6	15.3	N.T.
1b	(*S*) H	(*S*) OH	2600	7.30	28.3	N.T.
1c	(*R*) H	(*R*) OH	780	9.77	60.6	90.3
1d (VP-343)	(*R*) H	(*S*) OH	110	0.772	79.2	96.3

N.T., not tested.

Table 2. Vasopressin receptor binding affinities of B type

Compound	C-11a position H	IC_{50} (nM) V1a	IC_{50} (nM) V2	UV (mL/kg) i.v.	UV (mL/kg) p.o.
2a	(*S*) H	21.5%[a]	3.07	29.6	73.8
2b (VP-365)	(*R*) H	127	1.18	77.5	121.6
3a (VP-339)	(*S*) H	15.8%[a]	0.216	58.6	102.7
3b	(*R*) H	0%[a]	116	9.9	N.T.

N.T., not tested.
[a]The values are the inhibition percent at the concentration of 10^{-6} M.

Q type (**1** in Fig. 2) are shown in Table 1. It is of interest that the stereochemical configuration at C-3a position of 1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoxaline ring had an influential effect on the binding affinity for V2 (and V1a) receptor (**1a** versus **1c**, **1b** versus **1d**). It is also noted that the binding affinity for the V1a and V2 greatly changed depending on the stereochemical configuration of C-2 position, i.e. (*S*)-isomers were more potent than (*R*)-isomers (**1a** versus **1b**, **1c** versus **1d**).

The results of the binding study on B type (**2**, **3** in Fig. 2) are shown in Table 2. In the case of compound **2**, the stereochemical configuration at C-11a position of 1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine ring was not important, because the binding affinity for V2 receptor of **2a** and **2b** was similar. On the contrary, as for compound **3**, in which a lactam moiety is contained, the configuration at C-11a position strongly affected the binding affinity for V2 receptor (**3a** versus **3b**).

Diuretic activity

The diuretic activity was determined by measuring the urine volume (UV in Tables 1 and 2) collected for 4 h after administration. The dose of the test compounds was 3 mg/kg for intravenous administration (i.v.) and 30 mg/kg for oral administration (p.o.), and 4 rats were evaluated. The mean values of UV for control rats after i.v. ($n=21$) and p.o. ($n=8$) were 12.5 and 6.9 mL/kg, respectively. All the compounds, except for the compound **3b**, exhibited the antagonistic activities for V2 receptor. The compounds **1c**, **1d**, **2b** and **3a** remarkably increased the urine volume in comparison with that of the control rats. The ED_{300} values (oral dose required to increase three times the urine volume of the control rats) of **1d** (**VP-343**), **2b** (**VP-365**) and **3a** (**VP-339**) were 0.22, 0.31 and 0.78 mg/kg, respectively.

Conclusion

The data presented here revealed, for the first time, that the stereochemical configuration of the chiral compounds had a great influence on V2 receptor binding. These results have important implications for the development of V2 receptor antagonists and could be useful to develop more potent V2 receptor antagonists.

Since the compounds of pyrrolo[1,2-*a*]quinoxaline and pyrrolo[2,1-*c*][1,4]benzodiazepine with different chirality bind to V2 receptor in greatly different affinity, it seems that such chromophores could be the pharmacophores for V2 receptor. Through the present studies, **VP-339**, **VP-343** and **VP-365** could be candidates for diuretics and the more detailed biological activities of these compounds will be reported elsewhere.

Experimental

General procedures

Melting points were determined on a Yanoco MP-S3 apparatus and are uncorrected. IR spectra (cm^{-1}) were recorded on a JASCO IR-810 spectrometer. ^1H NMR spectra were measured with a JEOL JMN-GSX 270 spectrometer. Chemical shifts are recorded in the δ scale relative to tetramethylsilane as an internal standard. Mass spectra (MS) were measured on a JEOL JMS-AX 505W instrument. Optical rotations were determined with a JASCO DIP-140 polarimeter. In general, reactions were carried out in dry solvents under N_2, unless otherwise mentioned. All commercially available chemicals were used as supplied by the manufacturer. Column chromatography was performed on silica gel 60 (70–230 mesh) from Merck. The radioligands [^3H]d(CH_2)$_5$Tyr(Me)AVP ([^3H]-V1 antagonists; specific activity, 1813 GBq/mM) and [^3H]desGly(NH$_2$)d(CH_2)$_5$[D-Ile^2,Ile^4]AVP ([^3H]-V2 antagonists; specific activity, 2116.4 GBq/mM) were obtained from DuPont.

(2*S*,4*R*)-4-Hydroxy-1-(2-nitrophenyl)proline methyl ester (4a). A solution of *trans*-4-hydroxy-L-proline methyl ester hydrochloride (7.26 g, 40 mM), 1-fluoro-2-nitrobenzene (6.0 g, 42.5 mM) and triethylamine (7.7 mL, 54.7 mM) in acetonitrile (100 mL) was stirred for 16 h at 60 °C. The reaction mixture was cooled to room temperature, poured into 1 N HCl (100 mL) cooled in an ice-bath and then extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was chromatographed on silica gel column (mobile phase; $CHCl_3$, and then $CHCl_3$:MeOH = 99:1) to give **4a** as a red oil (9.9 g, 93%): ^1H NMR ($CDCl_3$) δ 1.74 (1H, d, J = 4.0 Hz), 2.20–2.35 (1H, m), 2.43–2.55 (1H, m) 2.82–2.92 (1H, m), 3.70 (3H, s), 3.86 (1H, dd, J = 11.0, 3.7 Hz), 4.55–4.77 (2H, m), 6.81–6.99 (2H, m), 7.34–7.47 (1H, m), 7.77 (1H, dd, J = 8.4, 1.8 Hz); IR (neat) 3480, 1735, 1605, 1510, 1360, 1275, 1205, 1175 cm^{-1}.

(2*R*,4*R*)-4-Hydroxy-1-(2-nitrophenyl)proline methyl ester (4c). *cis*-4-Hydroxy-L-proline methyl ester hydrochloride and 1-fluoro-2-nitrobenzene were subjected to the same reaction described for the synthesis of **4a**. The compound **4c** (97%) was obtained as a red oil: ^1H NMR ($CDCl_3$) δ 2.16–2.26 (1H, m), 2.50–2.62 (1H, m), 2.91 (1H, d, J = 9.0 Hz) 3.41–3.48 (1H, m), 3.57 (1H, dd, J = 10.5, 5.4 Hz), 3.75 (3H, s), 4.40–4.55 (2H, m), 6.81–6.89 (2H, m), 7.35–7.43 (1H, m), 7.64–7.79 (1H, m); IR (neat) 3450, 1740, 1605, 1510, 1350, 1280, 1210, 1180 cm^{-1}.

(2*S*,4*S*)-4-Hydroxy-1-(2-nitrophenyl)proline (4b). Diisopropyl azodicarboxylate (DIPAD, 3.27 mL, 16.62 mM) was added dropwise to a solution of **4a** (2.95 g, 11.08 mM), triphenylphosphine (4.36 g, 16.62 mM) and acetic acid (1.27 mL, 22.16 mM) in dry toluene (45 mL) in an ice-bath. After being stirred at room temperature for 2 h, 2 N NaOH (33.2 mL) was added to the reaction mixture, and then the reaction mixture was stirred at room temperature for 20 h. The water layer was separated, acidified with 4N HCl (25 mL) and then extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered and then concentrated under reduced pressure. Recrystallization from AcOEt/$CHCl_3$ gave **4b** (2.01 g, 63%) as light yellow crystals: mp 245–250 °C (dec.); ^1H NMR (DMSO-d_6) δ 2.06 (1H, dt, J = 11.7, 4.9 Hz), 2.42–2.55 (1H, m), 2.99 (1H, dd, J = 10.7, 2.9 Hz), 3.36 (1H, dd, J = 10.7, 4.9 Hz), 4.19 (1H, dd, J = 8.8, 4.9 Hz), 4.37 (1H, bs), 6.78–6.88 (2H, m), 7.22 (1H, d, J = 7.8 Hz), 7.47–7.55 (1H, m), 7.77–7.82 (1H, m); IR (KBr) 3440, 1700, 1605, 1565, 1505, 1485, 1440, 1275.

(2*R*,4*S*)-4-Hydroxy-1-(2-nitrophenyl)proline (4d). In the similar manner as described above, **4d** (63%) was prepared from **4c**. Recrystallization from AcOEt/$CHCl_3$ gave light yellow crystals: mp 225–230 °C (dec.); ^1H NMR (DMSO-d_6) δ 2.07–2.20 (1H, m), 2.39 (1H, dd, J = 12.7, 6.8 Hz), 2.57–2.64 (1H, m), 3.67 (1H, dd, J = 10.8, 3.4 Hz), 4.45 (1H, s), 4.59 (1H, dd, J = 10.3, 6.8 Hz), 5.13 (1H, bs), 6.86–6.94 (1H, m), 7.00 (1H, d, J = 8.1 Hz), 7.51–7.59 (1H, m), 7.83 (1H, dd, J = 8.1, 1.5 Hz); IR (KBr) 3380, 1700, 1605, 1575, 1515, 1445, 1350, 1280, 1080.

4b: 100% e.e., **4d**: 100% e.e. [The e.e. values were determined by HPLC with Mightysil® RP-18 (0.1% AcOH, 30% acetonitrile/water, 1 mL/min). The retention times were 10 and 7 min for **4b** and **4d**, respectively.]

(2*R*,3a*S*)-2-Hydroxy-1,2,3,3a,4,5-hexahydro-pyrrolo[1,2-*a*]quinoxaline hydrochloride (7a). A solution of **4a** (30.7 g, 115 mM) and Et_3N (16.1 mL, 115 mM) in THF (310 mL) was stirred at 45 °C in the presence of 10% palladium carbon (3.1 g) under H_2 at 1 atm pressure for 4 h. The catalyst was removed by filtration and washed with THF (200 mL). To the combined THF solution was added $NaBH_4$ (17.4 g, 461 mM), and the reaction mixture was stirred while refluxing for 18 h under nitrogen atmosphere. The reaction mixture was cooled in an ice bath and diluted with water (500 mL). The resulting mixture was carefully neutralized with 4N HCl (100 mL) in an ice bath, extracted with AcOEt (2×400 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to the half volume (ab. 600 mL). To the ice-cooled extract (ab. 600 mL) was added dropwise 4N HCl/AcOEt (57.7 mL, 0.231 M) and the mixture was stirred for 1 h in an ice bath. The precipitate was collected by filtration and recrystallized from MeOH to give **7a** (10.68 g, 41%) as pale red needles: mp 215–221 °C (dec.); ^1H NMR (DMSO-d_6) δ 1.70–1.80 (1H, m), 2.14 (1H, dd, J = 12.5, 5.3 Hz), 2.84 (1H, t, J = 11.5 Hz), 3.34–3.47 (2H, m), 3.81 (1H, dd, J = 11.5, 3.2 Hz), 4.01–4.12 (1H, m), 4.54 (1H, t, J = 3.9 Hz), 6.67–6.74 (2H, m), 7.23–7.30 (2H, m), 11.51 (2H, br); IR

(KBr) 3450, 2920, 2890, 2850, 2740, 2675, 2640, 1620, 1515, 1360, 1350, 1200, 755 cm^{-1}.

(2*S*,3a*S*)-2-Hydroxy-1,2,3,3a,4,5-hexahydro-pyrrolo[1,2-*a*]quinoxaline hydrochloride (7b). In a similar manner as described above, **7b** (48%) was prepared from **4b**. Recrystallization from MeOH gave pale red needles: mp 206–212 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 1.61 (1H, dt, J = 12.2, 7.6 Hz), 2.32 (1H, quint, J = 6.1 Hz), 2.88 (1H, t, J = 11.2 Hz), 3.04 (1H, dd, J = 9.8, 5.4 Hz), 3.54–3.78 (3H, m), 4.44 (1H, quint, J = 6.1 Hz), 6.62–6.67 (2H, m), 7.14–7.19 (2H, m), 11.28 (2H, br); IR (KBr) 3450, 2850, 2740, 2670, 2640, 1615, 1510, 1365, 1355, 750 cm^{-1}.

(2*R*,3a*R*)-2-Hydroxy-1,2,3,3a,4,5-hexahydro-pyrrolo[1,2-*a*]quinoxaline hydrochloride (7c). In a similar manner as described above, **7c** (49%) was prepared from **4c**. Recrystallization from MeOH gave pale red needles. ^{1}H NMR, mp and IR were identical to those of **7b**.

(2*S*,3a*R*)-2-Hydroxy-1,2,3,3a,4,5-hexahydro-pyrrolo[1,2-*a*]quinoxaline hydrochloride (7d). In a similar manner as described above, **7d** (76%) was prepared from **4d**. Recrystallization from MeOH gave pale red needles. ^{1}H NMR, mp and IR were identical to those of **7a**.

***N*-[4-[[(2*R*,3a*S*)-2-Hydroxy-2,3,3a,4-tetrahydro-pyrrolo-[1,2-*a*]quinoxalin-5(1*H*)-yl]carbonyl]-phenyl]-4′-methyl-[1,1′-biphenyl]-2-carboxamide (1a).** To an ice-cold solution of **7a** (1.50 g, 6.62 mM) and pyridine (1.1 mL, 13.23 mM) in CH_2Cl_2 (30 mL) was added dropwise while stirring a solution of 4-(4′-methyl-2-biphenylcarboxamido)benzoyl chloride (2.31 g, 6.62 mM) in CH_2Cl_2 (46 mL) over a period of 0.5 h. The reaction mixture was stirred at room temperature for 18 h and diluted with water (100 mL). The resulting solution was extracted with AcOEt (100 mL) and the organic layer was washed with 0.8 N HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The residue was chromatographed on silica gel (mobile phase; $CHCl_3$:MeOH = 49:1) to give **1a** (2.18 g, 65%) as a yellowish powder: $[\alpha]_D^{27} = -333.28$ (MeOH, c = 1.013); ^{1}H NMR (DMSO-d_6, 90 °C) δ 1.69 (1H, ddd, J = 12.5, 10.5, 4.4 Hz), 2.14 (1H, dd, J = 12.5, 5.4 Hz), 2.39 (3H, s), 2.66 (1H, dd, J = 12.2, 10.5 Hz), 3.46 (2H, d, J = 2.4 Hz), 3.88–3.99 (1H, m), 4.55 (1H, bs), 4.76 (1H, dd, J = 12.5, 3.9 Hz), 4.88 (1H, d, J = 3.7 Hz), 6.41–6.47 (1H, m), 6.63 (1H, dd, J = 8.1, 1.5 Hz), 6.81 (1H, dd, J = 8.1, 1.5 Hz), 7.02 (1H, ddd, J = 8.1, 7.3, 1.5 Hz), 7.25 (2H, d, J = 7.8 Hz), 7.37–7.44 (4H, m), 7.50–7.66 (6H, m), 10.12 (1H, s); IR (KBr) 3400, 2920, 2850, 1620, 1600, 1515, 1505, 1410, 1320 cm^{-1}; HRMS m/e 503.2177 ($C_{12}H_{16}N_2$ requires 503.2209).

***N*-[4-[[(2*S*,3a*S*)-2-Hydroxy-2,3,3a,4-tetrahydro-pyrrolo-[1,2-*a*]quinoxalin-5(1*H*)-yl]carbonyl]-phenyl]-4′-methyl-[1,1′-biphenyl]-2-carboxamide (1b).** In a similar manner as described above, **1b** (77%) was prepared from **7b** as a yellowish powder: $[\alpha]_D^{27} = -334.12$ (MeOH, c = 1.014); ^{1}H NMR (DMSO-d_6, 90 °C) δ 1.49–1.60 (1H, m), 2.26–2.35 (4H, m), 2.74 (1H, dd, J = 12.5, 10.3 Hz), 3.01–3.08 (1H, m), 3.56–3.69 (2H, m), 4.44–4.60 (2H, m), 4.89 (1H, d, J = 4.8 Hz), 6.33–6.39 (1H, m), 6.56 (1H, dd, J = 8.1, 1.5 Hz), 6.73 (1H, dd, J = 8.1, 1.5 Hz), 6.93 (1H, ddd, J = 8.1, 7.3, 1.5 Hz), 7.16 (2H, dd, J = 8.4, 0.7 Hz), 7.27–7.35 (4H, m), 7.41–7.57 (6H, m), 10.02 (1H, s); IR (KBr) 3400, 1630, 1605, 1515, 1505, 1410, 1375, 1320, 1250 cm^{-1}; HRMS m/e 503.2238 ($C_{12}H_{16}N_2$ requires 503.2209).

***N*-[4-[[(2*R*,3a*R*)-2-Hydroxy-2,3,3a,4-tetrahydro-pyrrolo-[1,2-*a*]quinoxalin-5(1*H*)-yl]carbonyl]-phenyl]-4′-methyl-[1,1′-biphenyl]-2-carboxamide (1c).** In a similar manner as described above, **1c** (60%) was prepared from **7c** as a yellowish powder. $[\alpha]_D^{27} = +341.55$ (MeOH, c = 1.042). ^{1}H NMR and IR were identical to those of **1b**; HRMS m/e 503.2189 ($C_{12}H_{16}N_2$ requires 503.2209).

***N*-[4-[[(2*S*,3a*R*)-2-Hydroxy-2,3,3a,4-tetrahydro-pyrrolo-[1,2-*a*]quinoxalin-5(1*H*)-yl]carbonyl]-phenyl]-4′-methyl-[1,1′-biphenyl]-2-carboxamide (1d).** In a similar manner as described above, **1d** (79%) was prepared from **7d** as a yellowish amorphous powder. $[\alpha]_D^{27} = +355.36$ (MeOH, c = 1.082). ^{1}H NMR and IR were identical to those of **1a**; HRMS m/e 503.2207 ($C_{12}H_{16}N_2$ requires 503.2209). **1a**: 99% e.e., **1b**: 99% e.e., **1c**: 99% e.e. and **1d**: 99 % e.e. [The e.e. values were determined by HPLC with Chiralcel OD-RH® (60% acetonitrile/water, 0.5 mL/min). The retention times were 35, 18, 12 and 11 min for **1a**, **1b**, **1c** and **1d**, respectively.

(11a*S*)-1,2,3,10,11,11a-Hexahydro-5*H*-pyrrolo-[2,1-*c*][1,4]-benzodiazepine (9a). A solution of **8a** (3 g, 13.9 mM) in dry THF (100 mL) was added dropwise to the suspension of $LiAlH_4$ (2.1 g, 55.2 mM) in dry THF (50 mL) at 0 °C. The mixture was refluxed for 3 h. It was cooled to room temperature, and the excess of $LiAlH_4$ was decomposed by adding AcOEt and then water. The mixture was diluted with ether. The organic layer was separated, and the aqueous layer was extracted with ether. The combined organic layer was washed with brine, dried over $MgSO_4$ and then concentrated under reduced pressure. Recrystallization from AcOEt gave **9a** (2.3 g, 87%) as colorless crystals: mp 108–109 °C; ^{1}H NMR ($CDCl_3$) δ 1.39–1.54 (1H, m), 1.69–1.98 (3H, m), 2.40–2.52 (2H, m), 2.74 (1H, dd, J = 13.1, 12.7 Hz), 3.12–3.19 (1H, m), 3.29–3.46 (1H, m), 3.50 (1H, d, J = 13.4 Hz), 3.81 (1H, d, J = 13.4 Hz), 6.71 (1H, d, J = 7.6 Hz), 6.81 (1H, dd, J = 7.6, 7.2 Hz), 7.04–7.13 (2H, m); IR (KBr) 2960, 1600, 1580, 1480, 1370, 1290, 1090 cm^{-1}; HRMS m/e 188.1291 ($C_{12}H_{16}N_2$ requires 188.1313).

(11a*R*)-1,2,3,10,11,11a-Hexahydro-5H-pyrrolo-[2,1-*c*][1,4]-benzodiazepine (9b). In a similar manner as described above, **9b** (90%) was prepared from **8b**. Recrystallization from AcOEt gave a yellowish powder. ^{1}H NMR, mp and IR were identical to those of **9a**. **9a**: 98% e.e. and **9b**: 99% e.e. (The e.e. values were determined by HPLC with Chiralcel OD-RH®, 25% acetonitrile/0.1M aqueous KPF_6, 0.5 mL/min. The retention times were 14 and 17 min for **9a** and **9b**, respectively.).

(11a*S*)-1,2,3,10,11,11a-Hexahydro-5*H*-pyrrolo-[2,1-*c*][1,4]-benzodiazepin-5-one (10a). To a solution of **8a** (5.05 g, 23.4 mM) in glyme (50 mL) was added $NaBH_4$ (1.76 g,

46.6 mM) at room temperature. Trifluoroacetic acid (3.43 g, 30.1 mM) in glyme (50 mL) was added dropwise to the reaction mixture over a period of 0.5 h at the room temperature. The mixture was refluxed for 4 h. The reaction mixture was cooled, carefully quenched with brine, dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Recrystallization from AcOEt gave **10a** (4.52 g, 96%) as colorless crystals: mp 181–182 °C; ^{1}H NMR ($CDCl_3$) δ 1.64–1.97 (3H, m), 2.17–2.32 (1H, m), 3.33 (1H, dd, J = 12.5, 8.5 Hz), 3.50–3.73 (2H, m), 3.77–3.94 (2H, m), 6.55 (1H, dd, J = 8.1, 1.1 Hz), 6.74–6.81 (1H, m), 7.15–7.22 (1H, m), 8.01 (1H, dd, J = 8.1, 1.5 Hz); IR (KBr) 3330, 2950, 1620, 1595, 1495, 1450, 1365, 1260 cm^{-1}; HRMS m/e 202.1057 ($C_{12}H_{14}N_2O$ requires 202.1106).

(11a*R*)-1,2,3,10,11,11a-Hexahydro-5*H*-pyrrolo-[2,1-*c*][1,4]-benzodiazepin-5-one (10b). In a similar manner as described above, **10b** (95%) was prepared from **8b**. Recrystallization from AcOEt gave a yellowish powder. ^{1}H NMR, mp and IR were identical to those of **10a**.

***N*-[4-[[(11a*S*)-2,3,11,11a-Tetrahydro-1*H*-pyrrolo-[2,1-*c*]-[1,4]benzodiazepin-10(5*H*)-yl]carbonyl]-phenyl][1,1′-biphenyl]-2-carboxamide (2a).** To an ice-cold solution of **9a** (1.50 g, 6.62 mM) and pyridine (1.1 mL, 13.23 mM) in CH_2Cl_2 (30 mL) was added dropwise while stirring a solution of 4-[(2-phenylbenzoyl)amino]benzoyl chloride (2.31 g, 6.62 mM) in CH_2Cl_2 (45 mL) over a period of 0.5 h. The reaction mixture was stirred at room temperature for 18 h and diluted with water (100 mL). The resulting mixture was extracted with CH_2Cl_2 and the organic layer was washed with 0.8 N HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The residue was chromatographed on silica gel (mobile phase; $CHCl_3$:MeOH = 49:1), followed by crystallization from AcOEt:hexane (1:3) to give **2a** (2.70 g, 81%) as a white powder: mp 178–179.5 °C; $[\alpha]_D^{27}$ = −297.18 (MeOH, c = 1.003); ^{1}H NMR ($CDCl_3$) δ 1.42–1.58 (1H, m), 1.71–1.93 (2H, m), 1.95–2.10 (1H, m), 2.48–2.71 (3H, m), 3.10–3.21 (1H, m), 3.72–3.93 (2H, m), 5.05–5.20 (1H, m), 6.61 (1H, d, J = 8.1 Hz), 6.80–6.99 (4H, m), 7.02–7.14 (3H, m), 7.26–7.29 (1H, m), 7.33–7.57 (8H, m), 7.85 (1H, d, J = 6.6 Hz); IR (KBr) 1640, 1600, 1520, 1400, 1320, 1270 cm^{-1}; HRMS m/e 487.2284 ($C_{32}H_{29}N_3O_2$ requires 487.2260).

***N*-[4-[[(11a*R*)-2,3,11,11a-Tetrahydro-1*H*-pyrrolo-[2,1-*c*]-[1,4]benzodiazepin-10(5*H*)-yl]carbonyl]-phenyl][1,1′-biphenyl]-2-carboxamide (2b).** In a similar manner as described above, **2b** (79.3%) was prepared from **9b**. Recrystallization from AcOEt:hexane gave a white powder. ^{1}H NMR, mp and IR were identical to those of **2a**: mp 178–179.5 °C; $[\alpha]_D^{27}$ = +298.40 (MeOH, c = 1.009); FABHRMS m/e 488.2301 $[M+H]^+$ ($C_{32}H_{29}N_3O_2$ requires 488.2338).

***N*-[4-[[(11a*S*)-5-Oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepin-10(5*H*)-yl]-carbonyl]phenyl][1,1′-biphenyl]-2-carboxamide (3a).** To an ice-cold solution of **10a** (0.81 g, 4.0 mM) and pyridine (0.66 mL, 8.0 mM) in CH_2Cl_2 (20 mL) was added dropwise while stirring, a solution of 4-[(2-phenylbenzoyl)amino]benzoyl chloride (1.53 g, 4.4 mM) in CH_2Cl_2 (15 mL) over a period of 0.5 h. The reaction mixture was stirred at room temperature for 18 h and diluted with water (100 mL). The resulting solution was extracted with CH_2Cl_2 and the organic layer was washed with 0.8 N HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The residue was chromatographed on silica gel (mobile phase; $CHCl_3$:MeOH = 49:1), followed by crystallization from AcOEt:hexane (1:3) to give **3a** (1.64 g, 73%) as a white powder: mp 233–235 °C; $[\alpha]_D^{27}$ = +531.50 (MeOH, c = 1.042); ^{1}H NMR ($CDCl_3$) δ 1.87–1.96 (1H, m), 2.03–2.19 (3H, m), 3.58–3.69 (2H, m), 3.79–3.93 (2H, m), 4.29 (1H, t, J = 12.8 Hz), 6.64 (1H, d, J = 6.7 Hz), 6.79–6.91 (3H, m), 7.07 (2H, d, J = 8.4 Hz), 7.13 (1H, td, J = 7.7, 1.8 Hz), 7.23–7.31 (1H, m), 7.32–7.57 (8H, m), 7.76 (1H, dd, J = 7.3, 1.5 Hz), 7.86 (1H, d, J = 7.3 Hz); IR (KBr) 1630, 1520, 1410, 1360, 1320 cm^{-1}; FABHRMS m/e 502.2101 $[M+H]^+$ ($C_{32}H_{27}N_3O_3$ requires 502.2131).

***N*-[4-[[(11a*R*)-5-Oxo-2,3,11,11a-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepin-10(5*H*)-yl]-carbonyl]phenyl][1,1′-biphenyl]-2-carboxamide (3b).** In a similar manner as described above, **3b** (70%) was prepared from **10b**. Recrystallization from AcOEt:hexane gave a white powder. ^{1}H NMR, mp and IR were identical to those of **3a**: mp 233–235 °C; $[\alpha]_D^{27}$ = −518.94 (MeOH, c = 1.018); FABHRMS m/e 502.2104 $[M+H]^+$ ($C_{32}H_{27}N_3O_3$ requires 502.2131).

3a: 100% e.e. and **3b**: 100% e.e. (The e.e. values were determined by HPLC with Chiralcel OD-RH®, 40% acetonitrile/water, 0.5 mL/min. The retention times were 36 and 29 min for **3a** and **3b**, respectively.).

Biological methods

1. Receptor binding studies. For the V1a receptor binding assay, the test compounds (3×10^{-12}–10^{-5} M) and 2.12 nM [^{3}H]-V1 antagonist were incubated with 5 mg (protein equivalent) of liver membranes in 1 mL of 50 mM Tris–HCl (pH 7.4) containing 10 mM KCl, 10 mM $MgCl_2$ and 0.1% bovine serum albumin (BSA). For the V2 receptor binding assay, the test compounds (3×10^{-12}–10^{-5} M) and 1.8 nM [^{3}H]-V2 antagonist were incubated with 5 mg (protein equivalent) of kidney membranes in 1 mL of 50 mM Tris–HCl (pH 7.4) containing 2 mM KCl, 1 mM $MgCl_2$ and 0.1% BSA. After incubation for 60 min at 25 °C, the free ligands were separated by filtration with a cell harvester, and then the filters were washed three times with 50 mM Tris–HCl (pH 7.4). The radioactivity trapped on the filters was counted with a liquid scintillation counter. Specific binding was calculated as a total binding minus non-specific binding, which was determined using 10^{-5} M unlabeled AVP. The concentration of test compounds that caused 50% inhibition (IC_{50}; nM) of the specific binding of [^{3}H]-V1 or [^{3}H]-V2 antagonist was determined by regression analysis of displacement curves. The values are the means obtained from 1–5 independent experiments performed in duplicate.

2. Diuretic activity. Rats (weighting 240–320 g) were fasted for 16–20 h. Immediately after the test compounds or vehicle were administered intravenously (3 mg/kg) or orally (30 mg/kg) to the rats, saline (25 mL/kg) was administered orally. As a vehicle, *N*,*N*-dimethylformamide and 5% gum arabic were used for intravenous and oral administration, respectively. Spontaneously voided urine was collected for 4 h. The mean values of the urine volume for control rats after intravenous ($n=21$) and oral ($n=8$) administration were 12.5 and 6.9 mL/kg, respectively. The oral dose (ED_{300}; mg/kg) required to increase three times the urine volume of the control rats, to which the vehicle was administered, was determined with respect to several compounds.

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