Journal of Medicinal Chemistry

Brief Article

pubs.acs.org/jmc

N-Benzoyl-1,5-benzothiazepine and Its *S*-Oxide as Vasopressin Receptor Ligands: Insight into the Active Stereochemistry around the Seven-Membered Ring

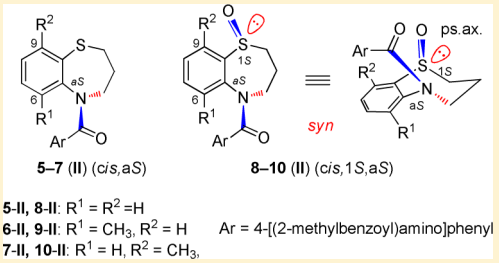
Tetsuya Yoneda,[†] Hidetsugu Tabata,[†] Tomohiko Tasaka,[‡] Tetsuta Oshitari,[†] Hideyo Takahashi,[†] and Hideaki Natsugari*[,†]

[†]Faculty of Pharma Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

[‡]Affinity Science Corporation, 4-1-1 Akasaka, Minato-ku, Tokyo 107-0052, Japan

Supporting Information

ABSTRACT: The stereochemistry of *N*-benzoyl-1,5-benzothiazepine and its *S*-oxide derivatives as vasopressin receptor ligands was examined in detail by freezing the conformation with a methyl group at the C6 or C9 of 1,5-benzothiazepine. It was revealed that the active forms recognized by the receptors are (*cis*,a*S*) for 1,5-benzothiazepine (**5**−**7**)-**II** and (*cis*,1*S*,a*S*) (*syn*) for its *S*-oxide (**8**−**10**)-**II**. The C9-methyl derivative of 1,5-benzothiazepine *S*-oxide (**10-II**) was designed and synthesized, achieving the putative active *syn*-isomer.



INTRODUCTION

Since the first discovery of a non-peptide arginine vasopressin (AVP) V_2 receptor antagonist (mozavaptan[1]) (Figure 1),

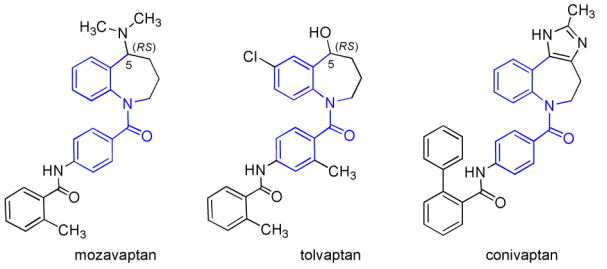


Figure 1. Non-peptide VP receptor antagonists.

extensive research to find new ligands (antagonists and agonists) has been carried out.[2] To date, the "vaptan" class of ligands (e.g., tolvaptan,[3] conivaptan,[4] and lixivaptan[5]) (Figure 1) has been developed as agents for the treatment of hyponatremia, congestive heart failure, etc. Interest is still growing in the search for new ligands, their new indications, and the biological role of the V_{1a} and V_{1b} subtype receptors. A topic of recent interest is the approval of tolvaptan as the first drug for autosomal-dominant polycystic kidney disease (ADPKD).[6] Many of the vaptan class of drugs contain a preserved scaffold, i.e., a benzo-fused seven-membered-ring nitrogen heterocycle (e.g., 1-benzazepine, 1,4-benzodiazepine) linked through N-1 to a substituted *p*-amidobenzoyl group (Figure 1). Thus far, we have investigated the conformation of several benzo-fused seven-membered-ring nitrogen heterocycles (e.g., 1-benzazepine, 1,5-benzodiazepine, and 1,5-benzothiazepine) to reveal the atropisomeric properties in relation to the biological activity for receptors (e.g., NK_1,[7a] $GABA_A$,[7b] VP[7c]) or enzymes (γ-secretase,[7d] ACAT[7e]). In our previous communication,[7c] atropisomerism in *N*-benzoyl-1,5-benzodiazepines (for **1-I**/**II**) as VP receptor ligands was described (Figure 2), in which the *cis*/*trans*-amide rotamers[8]

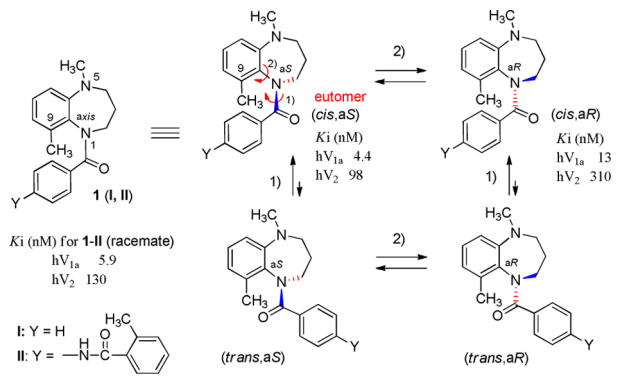


Figure 2. Four stereoisomers in **1** (**I**, **II**) and affinity at the VP receptors (K_i) for **1-II**.[7c]

around the N−C(=O) bond and (a*S*)/(a*R*)-axial isomers[9] based on the Ar−N(CO) (sp^2−sp^2) axis were considered (Figure 2). The molecules with a substituent at the ortho-position (C9) on the benzene ring (e.g., CH_3, **1-I**/**II**) were conformationally "frozen" and could be separated into the (a*S*)- and (a*R*)-axial isomers using chiral HPLC, although the conformational change in the molecules without the substituent

Received: February 18, 2015

DOI: 10.1021/acs.jmedchem.5b00289
J. Med. Chem. XXXX, XXX, XXX−XXX

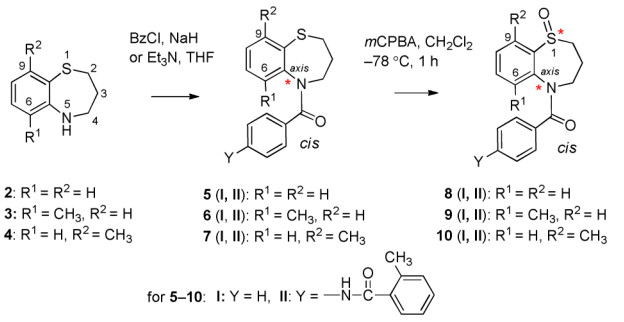
was too rapid for isolation of the isomers (i.e., achiral form) at room temperature (rt). That study led to the finding of the importance of axial chirality in exerting the biological activity of the vaptan class of AVP receptor ligands, exhibiting the active structure with the (*cis*,aS)-form of the type **1-II** (Figure 2).[7c] Recently, we have also become interested in the 1,5-benzothiazepin-4-one *S*-oxide,[10] which may exist as the major metabolite of the biologically active sulfide derivatives, to reveal the unique stereochemical properties originating in the stereogenic elements at the sulfur atom and axis.

In this context, we synthesized the *N*-benzoyl-1,5-benzothiazepine[11a,b] and its *S*-oxide derivatives as VP receptor ligands and examined the stereochemistry (conformation and configuration) in detail by freezing the conformation with a methyl group at the C6 or C9 of 1,5-benzothiazepine to identify the active molecular form recognized by the receptor. The results are presented here.

CHEMISTRY

Preparation of *N*-Benzoyl-1,5-benzothiazepine and Its *S*-Oxide Derivatives. *N*-Benzoyl-1,5-benzothiazepine (**5**–**7**) and its *S*-oxide derivatives (**8**–**10**) were prepared using conventional methods starting from 1,5-benzothiazepines (**2**–**4**)[12] (Scheme 1). N-Benzoylation of **2**–**4** using benzoyl

Scheme 1. Preparation of *N*-Benzoyl-1,5-benzothiazepine and Its *S*-Oxide



chloride and *p*-(2-methylbenzamido)benzoyl chloride gave **5-I**–**7-I** and **5-II**–**7-II**, respectively. The **I** series of compounds (Y = H) were prepared to examine the basic stereochemistry and the **II** series of compounds [Y = *p*-(2-methylbenzamido)-amino] were used for evaluation of the biological activity, both of which were shown to have similar structural features from the analytical data. As for the *cis*/*trans*-amide rotamers around the N–C(=O) bond, **5**–**10** all existed predominantly in the *cis*-form, and the *trans*-isomer was negligible in the ^{1}H NMR spectrum (Figure 3).[13] In the ^{1}H NMR spectrum ($CDCl_3$) of **5** (**I**, **II**), all the methylene protons (6H) of the thiazepine ring were observed as separated peaks, which indicates that the protons are diastereotopic and suggests the presence of axial chirality caused by the Ar–N(CO) axis. The atropisomers, however, could not be separated at rt using chiral HPLC, which implies that the energy barrier between the atropisomers of **5** (**I**, **II**) is low, and thus **5** exists as an achiral compound at rt. On the other hand, in **6** (**I**, **II**), which has a CH_3 group at C6 (R^1 = CH_3), the three CH_2 protons of **6** appeared as six sharp separated peaks, suggesting that the rotation around the Ar–N(CO) axis is restricted to form stable atropisomers, and **6** exists as the racemates of separable (a*S*)- and (a*R*)-atropisomers at rt. 7 (**I**, **II**) with a methyl group at C9 (R^2 =

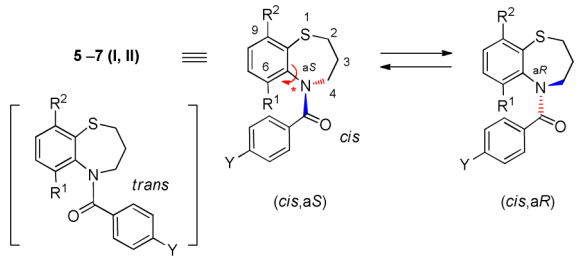


Figure 3. (a*S*)/(a*R*) axial chirality in *cis*-*N*-benzoyl-1,5-benzothiazepines (**5**–**7**) (**I**, **II**). As for the *cis*/*trans* amide rotamer, the *trans*-isomer shown in brackets was negligible in the ^{1}H NMR spectrum.

CH_3) was prepared for freezing the ring conformation of the *S*-oxide derivatives **10** (**I**, **II**) (described below). S-Oxidation of the 1,5-benzothiazepines (**5**–**7**) (**I**, **II**) was performed with *m*-chloroperoxybenzoic acid (*m*CPBA) (1.05 equiv) at −78 °C for 1 h in CH_2Cl_2 to afford the sulfoxides **8**–**10** (**I**, **II**) (61–81%).[14] Theoretically, the sulfoxides **8**–**10** exist as a mixture of diastereomers (*anti*/*syn*-isomers) originating in the two stereogenic elements at the sulfur atom and axis; the description "*anti*/*syn*" is used for the relative arrangement of the *S*-oxygen atom and the *N*-benzoyl group, i.e., *anti* and *syn* denote the opposite (1S*,aR*) and the same side (1S*,aS*) arrangement, respectively (Figure 4). The *anti* and *syn* forms possess the *S*-

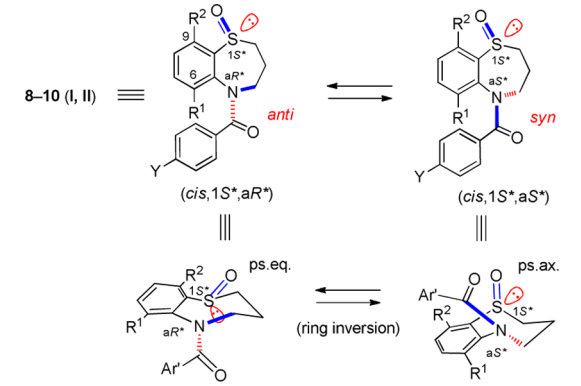


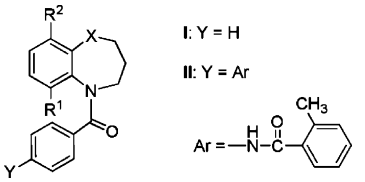
Figure 4. The *anti*/*syn* diastereomers in *cis*-*N*-benzoyl-1,5-benzothiazepine *S*-oxides (**8**–**10**) (**I**, **II**).

oxygen disposed in a ps.eq. and a ps.ax. orientation, respectively, in a chairlike conformation. Compounds **8** and **10** each existed as single isomer as analyzed by nonchiral HPLC, whereas **9** existed as the *anti*/*syn* mixture (~97:3), and only the major isomer was isolated. Further stereochemical characterization of the sulfoxides **8**–**10** in relationship with the activity is described in detail in Results and Discussion.

Separation of the Racemate into the Enantiomers [6, 8–10] and Their Physicochemical Properties (Table 1). Separation of the *N*-benzoyl derivatives in the racemic form into the enantiomers with HPLC using a chiral column was attempted for the sulfide (**6-I**/**II**) and the *S*-oxides (**8-I**/**II**, **9-I**, and **10-I**/**II**). The (a*R*)/(a*S*)-atropisomers of **6-I**/**II**, the (1*R*)/(1*S*)-enatiomers of **8-I**/**II** and **10-I**/**II**, and the enantiomers of **9-I** (only for the major diastereomer) were separated and successfully isolated using preparative HPLC. The physicochemical properties ($[\alpha]_D$ and $\Delta G^{\ddagger}$ values) are shown in Table 1. The absolute stereochemistry of the separated enantiomers was unambiguously determined by the X-ray structure analysis of (−)-**6-I**, (−)- and (+)-**8-I**, (−)-**9-I** (major isomer), and

DOI: 10.1021/acs.jmedchem.5b00289
J. Med. Chem. XXXX, XXX, XXX–XXX

Table 1. Physicochemical Properties of the Separated Enantiomers of *N*-Benzoyl-1,5-benzothiazepines (6-I/II) and the *S*-Oxides (8-I/II, 9-I, 10-I/II)



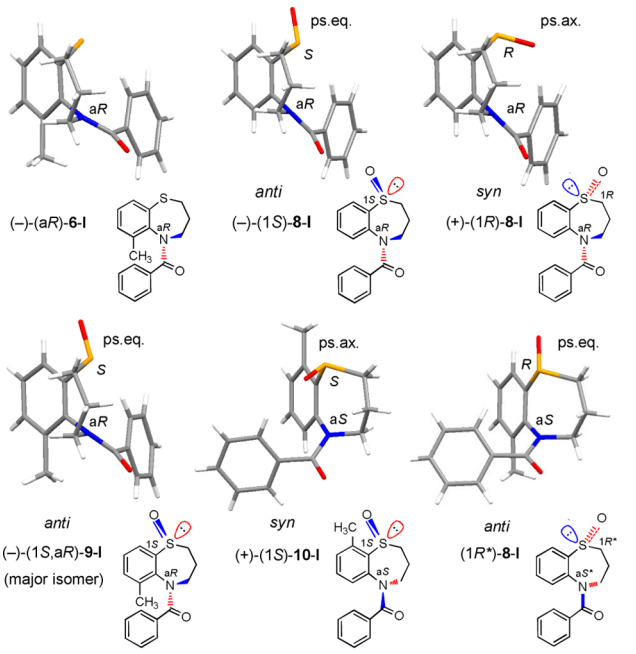
	X	R^1	R^2	Y	$[\alpha]_D^{23}$ (CH_3OH)	$\Delta G^{\ddagger}$,[a] kJ/mol
6-I	S	CH_3	H	H	(aS): +214.0 (aR): −220.7	99[b]
6-II	S	CH_3	H	Ar	(aS): +226.1 (aR): −246.7	99[b]
8-I	S=O	H	H	H	(1*S*): −184.1 (1*R*): +184.0	64[c]
8-II	S=O	H	H	Ar	(1*S*): −188.5 (1*R*): +187.0	NT[d]
9-I[e]	S=O	CH_3	H	H	(1*S*,a*R*): −202.8 (1*R*,a*S*): +164.5[f]	NT[d]
10-I	S=O	H	CH_3	H	(1*S*): +424.8 (1*R*): −425.4	NT[d]
10-II	S=O	H	CH_3	Ar	(1*S*): +456.5 (1*R*): −455.5	NT[d]

[a]Activation free energy barrier to rotation of axis. [b]Racemized in toluene at 37 °C after 3 h. [c]Estimated by VT NMR. [d]Not tested. [e]Major diastereomer [(1*S**,a*R**)(=*anti*)] of **9-I**; the minor isomer [(1*S**,a*S**)(= *syn*)] could not be isolated because of the instability. [f]~90% ee as analyzed by chiral HPLC.

(+)-**10-I** as shown in Figure 5.[15] The X-ray structures also showed that all the compounds possess the *cis* and chair forms and that the *anti* and *syn* diastereomers of **8**−**10** have the *S*-oxygen disposed in a ps.eq. and a ps.ax. orientation, respectively. The absolute stereochemistry of the **II**-series of compounds (**6**, **8**, **10**) was determined from the (+)/(−) angle of the optical rotation α of the corresponding **I**-series of compounds. The stereochemical stability of the separated atropisomers of **6-I/II** and conformational (diastereomeric) isomers of **8-I** was next examined. The activation free energy barrier to rotation ($\Delta G^{\ddagger}$)[16] and the conditions required for racemization of the enantiomers of **6-I/II** are shown in Table 1. The enantiomers of **6-I/II** had the $\Delta G^{\ddagger}$ value of 99 kJ/mol (24 kcal/mol) and racemized after ~3 h in toluene at 37 °C. The stability of the conformational (diastereomeric) isomers of **8-I** was next estimated using variable-temperature ^{1}H NMR (VT NMR)[17] in DMSO-d_6 (Table 1), in which the signals of one of the diastereomeric C4-methylene protons (δ 4.7 and 4.9 ppm with a ratio of ~10:1) of the thiazepine ring coalesced at around 323 K (50 °C) with a calculated $\Delta G^{\ddagger}$ value of 64 kJ/mol (15 kcal/mol).[18]

RESULTS AND DISCUSSION

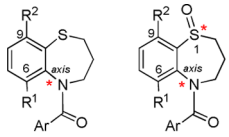
Affinity at the V_{1a} and V_2 Receptors (Table 2). The in vitro affinities at the human VP V_{1a} and V_2 receptors of the **II** series of compounds [Y = (2-methylbenzoyl)amino] are shown in Table 2. First, the achiral compounds (**5-II**, **7-II**) and compounds in the racemic form (**6-II**, **8-II**, **9-II**, **10-II**) were evaluated. The benzothiazepines (**5-II**−**7-II**) exhibited good potency (K_i) at the 10 nanomolar level for the V_{1a} receptor. It is interesting to note that the compounds bearing a methyl group



ps.ax. = pseudoaxial, ps.eq. = pseudoequatorial

Figure 5. X-ray crystal structures of the optically active compounds (−)-(a*R*)-**6-I**, (−)-(1*S*)-**8-I**, (+)-(1*R*)-**8-I**, (−)-(1*S*,a*R*)-**9-I**, and (+)-(1*S*)-**10-I** and the racemate (1*R**)-**8-I**. The crystal structure of (1*R**)-**8-I** was extracted from the CIF data of X-ray analysis of the racemate **8-I**.

Table 2. In Vitro Affinity for Human VP V_{1a} and V_2 Receptors of 5−10 (II)



5, 8 (II): $R^1 = R^2 = H$
6, 9 (II): $R^1 = CH_3$, $R^2 = H$
7,10 (II): $R^1 = H$, $R^2 = CH_3$

Ar = 4-[(2-methylbenzoyl)-amino]phenyl

		K_i (μM)[a]	
		hV_{1a}	hV_2
5-II	(achiral)	0.068	0.021
6-II	racemate	0.037	0.360
	(+)-(a*S*)	0.019	0.184
	(−)-(a*R*)	0.147	1.800
7-II	(achiral)	0.048	0.880
8-II	racemate	0.910	0.870
	(−)-(1*S*)	0.550	0.820
	(+)-(1*R*)	57% inh[b]	1.240
9-II[c]	racemate (1*S**,a*R**)	55% inh[b]	22% inh[b]
10-II	racemate	0.870	34% inh[b]
	(+)-(1*S*)[d]	0.660	48% inh[b]
	(−)-(1*R*)[e]	53% inh[b]	0% inh[b]

[a]K_i values shown are the mean of duplicate measurements. [b]Inhibition (%) at 10 μM. [c]Major diastereomer. [d]Axial chirality is confined to (a*S*). [e]Axial chirality is confined to (a*R*).

on the benzene ring (**6-II** and **7-II**) have a tendency to bind selectively to V_{1a} rather than to V_2 receptors. Among the *S*-oxide derivatives (**8-II**−**10-II**), which could be potential metabolites of the sulfides (**5-II**−**7-II**), **8-II**, and **10-II** showed almost equal, moderate activity for the V_{1a} receptor. However,

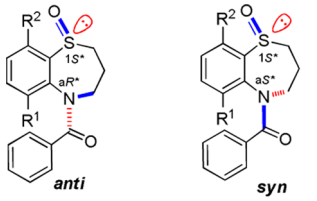
DOI: 10.1021/acs.jmedchem.5b00289
J. Med. Chem. XXXX, XXX, XXX−XXX

the potency of the *S*-oxide **9-II** (the major diastereomer) was considerably diminished.

Next, the separated enantiomers of **6-II**, **8-II**, and **10-II** were subjected to the binding assay to examine the difference in potency between the enantiomers. As shown in Table 2, the enantiomers exhibited clear differences in the affinities between the isomers with median potency in the corresponding racemate. As for the (a*S*)/(a*R*)-atropisomers of **6-II**, the (a*S*)-isomer exhibited higher potency than the (a*R*)-isomer. As for the (1*S*)/(1*R*)-enantiomer of **8-II** and **10-II**, both of the (1*S*)-isomers had greater potency than the (1*R*)-isomers.

Active Molecular Forms Recognized by the VP Receptors. The clear difference in potency between the enantiomers of **6-II**, **8-II**, and **10-II** indicates that the stereochemistry (conformation and configuration) at the scaffold region is recognized by the receptors. As for the thiazepine **6-II**, the VP receptors recognize the (*cis*,a*S*)-form, which is in accordance with the VP ligands of the 1,5-benzodiazepine series **1-II** reported in our previous communication.[7c] This may hold true for the achiral thiazepines **5-II** and **7-II**; the binding form is assumed to have the (*cis*,a*S*)-form, although a pair of the (a*S*)/(a*R*)-axial isomers is rapidly interconverting and inseparable. For the *S*-oxides **8-II**–**10-II**, the situation is rather complex because the *anti*/*syn* diastereomers due to the two stereogenic centers at the sulfur atom and axis as described in Figure 4 are present in **8**–**10** (**I**, **II**). Table 3 shows the ratio of the diastereomers as analyzed by ^{1}H NMR.

Table 3. Equilibrium Ratio for Stereoisomers (*anti*, *syn*) in *cis*-*N*-Benzoyl-1,5-benzothiazepine *S*-Oxides (8-I, 9-I, and 10-I) Estimated by ^{1}H NMR



	R^1	R^2	*anti*:*syn*
8-I[a]	H	H	100:0 in $CDCl_3$
			87:13 in CD_3OD
			91:9 in DMSO-d_6
9-I[b]	CH_3	H	97:3 (by HPLC)
10-I[a]	H	CH_3	0:100 in $CDCl_3$, CD_3OD

[a]For **8-I** and **10-I**, the ratio (*anti*/*syn*) at the equilibrium state in solution at rt was estimated by ^{1}H NMR. Both compounds each existed as single isomer as analyzed by nonchiral HPLC. [b]For **9-I**, a mixture of *anti*/*syn* isomers was obtained in the S-oxidation. The ratio was estimated by nonchiral HPLC and ^{1}H NMR.

Compound **8-I** (R^1 = R^2 = H) exhibited an interesting conformational property in the ^{1}H NMR spectrum; i.e., although at 295 K (22 °C) in $CDCl_3$, only a single isomer (presumed to be *anti*) was observed, in polar solvents another minor isomer (presumed to be *syn*) appeared with a ratio of ~91:9 (in DMSO-d_6) or ~87:13 (in CD_3OD) (Table 3). The sulfoxide **9-I** (R^1 = CH_3, R^2 = H) was revealed to be a mixture of diastereomers with a ratio of ~97:3 by HPLC and ^{1}H NMR analyses, from which only the major isomer could be isolated. Attempted isolation of the minor isomer in the pure form failed because the minor isomer readily isomerized to the major one, resulting in a mixture of isomers. The structure of the major isomer was determined to be *anti*[=(1*S**,a*R**)] form by the X-ray structure analysis of the separated optically active (−)-**9-I**. Although determination of the relative *anti*/*syn* stereochemistry of the *S*-oxides **8-I** and **9-I** in solution was not easy, it could be finally assigned after obtaining the pure *syn*[=(1*S**,a*S**)] isomer (**10-I**) (described below): i.e., the ^{1}H NMR spectrum of **10-I** accorded with that of the minor isomer of **8-I** and **9-I** (see Figure S4 in the Supporting Information).

Since the *S*-oxide **9-II** (the major diastereomer) exhibited diminished activity, the presumption of the active form of the *S*-oxide derivatives led to confusion. Considering that (−)-(1*S*)-**8-II** was the active enantiomer, the relative stereochemistry of the active form of **8-II** was tentatively presumed to be (1*S*,a*R*), which is the major *anti*-diastereomer observed in the ^{1}H NMR spectrum (Table 3). The (a*R*)-stereochemistry at the axis, however, is inconsistent with the active (a*S*)-form of the thiazepines **5-II**–**7-II**. Thus, we speculated that the actual active form of (−)-(1*S*)-**8-II** is the minor form [(1*S*,a*S*)(=*syn*)] in the equilibrium state (Table 3). The energy barrier between the two conformers (diastereomers) seems fairly surmountable under ambient conditions as suggested by the X-ray structure analysis of **8-I** (Figure 5). As shown in Figure 5, the X-ray structure analysis of the chiral (−)-(1*S*)-**8-I** and (+)-(1*R*)-**8-I** exhibited diastereomeric structures with a chairlike form, i.e., (−)-(1*S*)-**8-I** has an *anti*-form, and (+)-(1*R*)-**8-I** a *syn*-form, whereas interestingly the racemate **8-I** (1*R**) exhibited *anti*-stereochemistry. The appearance of the diastereomeric *syn* and *anti*structures in the crystalline enantiomers and racemate of **8-I** could be a result of a fortuitous crystal packing arrangement and implies that the energy barrier between the two conformers (diastereomers) is fairly low.[18] Thus, it may not be discrepant to assume that the active stereochemistry of the *S*-oxide derivatives is that of the minor isomer [(1*S*,a*S*)(=*syn*)] (Figure 6). The weak potency of the *S*-oxide **9-II** (major isomer)

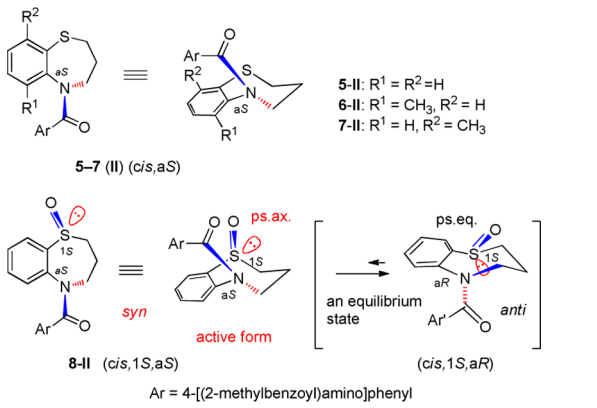


Figure 6. Active stereochemistry of the thiazepines (**5**–**7**)-**II** and the *S*-oxide derivative (**8-II**).

(Table 2) may also be ascribed to the (1*S**,a*R**)(=*anti*)-stereochemistry, and the active isomer would be the minor diastereomer with (1*S**,a*S**)(=*syn*)-stereochemistry, which could not be isolated.

After obtaining various information on the active stereochemistry of the *S*-oxide derivatives, we designed the benzothiazepine *S*-oxide with a C9-methyl group (**10**) to obtain the putative active *syn*[=(1*S**,a*S**)]-stereochemistry (Figure 7). Because of the steric effect of the C9 methyl

DOI: 10.1021/acs.jmedchem.5b00289
J. Med. Chem. XXXX, XXX, XXX−XXX

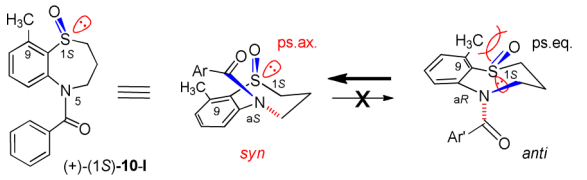


Figure 7. (1*S*)-5-Benzoyl-9-methyl-1,5-benzothiazepine *S*-oxide [(+)-(1*S*)-**10-I**)] with the putative active stereochemistry [(1*S*,a*S*) = *syn*] at the scaffold region in which the *S*-oxygen occupies a pseudoaxial position in a chairlike form.

group, the *S*-oxide group would be confined to adopt a sterically less hindered ps.ax. orientation in a chairlike form. Gratifyingly, as expected, **10** existed only in the desired *syn*[=(1*S**,a*S**)]-form in the solid [X-ray crystal analysis for (+)-(1*S*)-**10**] (Figure 5) and solution (^{1}H NMR) states, and the activity of **10-II** resided in the (1*S*)-enantiomer with the (a*S*)-axis as shown in Table 2. These results are consistent with our presumption of the active stereochemistry.

These prospects for the active molecular forms further suggest the active stereochemistry of the vaptan class of VP ligands (e.g., mozavaptan, tolvaptan) developed as racemates. Considering that the eutomer (more potent enantiomer) of these drugs has (5*S*)-stereochemistry,[19] although rotation around the Ar–N(CO) axis is rapidly occurring and the diastereomers are inseparable, the receptors in the body are presumed to recognize the stereochemistry of the (*cis*,5*S*,a*S*)-(*syn*)-form with C5-substituent in the ps.ax. orientation as described in this study (Figure 8).

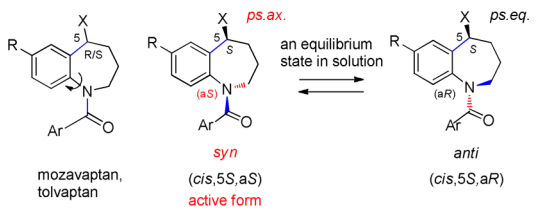


Figure 8. Plausible active form of vaptan class of VP ligands with a C5-substituent (e.g., mozavaptan, tolvaptan). For the exact structures of mozavaptan and tolvaptan, see Figure 1.

CONCLUSION

By freezing the conformation with a methyl group at the C6 or C9 of 1,5-benzothiazepine, we elucidated the stereochemistry (conformation and configuration) of *N*-benzoyl-1,5-benzothiazepine and its *S*-oxide and gained insight into the actual active stereochemistry of the scaffold region of the VP ligands. On the basis of these structural insights, the C9-methyl derivative of 1,5-benzothiazepine *S*-oxide (**10**) was designed and synthesized, achieving stereoselective formation of the putative active *syn*-isomer. When exerting biological activity by binding with ligands, the target receptors or enzymes must recognize the actual active structure caused by the labile conformational change of the seven-membered ring, where chirality may also exist in the latent form. We hope that this study will contribute to future drug design and development.

EXPERIMENTAL SECTION[20]

N-Benzoylation of 2,3,4,5-Tetrahydro-1,5-benzothiazepines (2–4) Using Benzoyl Chloride and *p*-(2-Methylbenzamido)-benzoyl Chloride: Typical Example Described for Preparation of 5-I. To a stirred solution of **2**[12] (113 mg, 0.68 mmol) in THF (7 mL) at 0 °C under argon was added sodium hydride (60% in oil) (41 mg, 1.03 mmol). The mixture was stirred at 25 °C for 30 min, cooled to 0 °C, and treated with benzoyl chloride (0.24 mL, 2.05 mmol). After it was stirred at 25 °C for 1 h, the mixture was treated with H_2O and extracted with ethyl acetate. The extract was washed with brine, dried, and concentrated. The concentrate was purified by column chromatography (silica gel, ethyl acetate/hexane = 1:5) to afford **5-I**[21] as colorless crystals (170 mg, 0.63 mmol, 93%).

Similarly, 6-methyl (**6-I**) and 9-methyl (**7-I**) derivatives were prepared from the corresponding 1,5-benzothiazepines (**3**, **4**)[12] according to a similar procedure described for the preparation of **5-I** from **2**. Also, the **II**-series of compounds (**5-II**, **6-II**, and **7-II**) were similarly prepared from the corresponding 1,5-benzothiazepines by reaction with *p*-(2-methylbenzamido)benzoyl chloride. The physicochemical data of **5**–**7** (**I**, **II**) are shown in the Supporting Information.

S-Oxidation of *N*-Benzoyl-2,3,4,5-tetrahydro-1,5-benzothiazepines (5–7/I, II): Typical Example Described for Preparation of 8-I. To a solution of **5-I** (50 mg, 0.19 mmol) in CH_2Cl_2 (1 mL) at −78 °C under argon was added dropwise a solution of *m*CPBA (49 mg, 0.19 mmol) in CH_2Cl_2 (1.7 mL) with stirring. After being stirred at −78 °C for 1 h, the mixture was treated with sat. aq $NaHCO_3$ and extracted with ethyl acetate. The extract was washed with brine, dried, and concentrated. The concentrate was purified by column chromatography (silica gel, ethyl acetate/hexane = 2:1) to afford sulfoxide as colorless crystals **8-I** (39 mg, 0.14 mmol, 72%) and sulfone (12 mg, 0.04 mmol, 21%).

Compounds **8-II**, **9** (**I**, **II**), and **10** (**I**, **II**) were prepared from the corresponding 1,5-benzothiazepines according to a similar procedure described for the preparation of **8-I** from **5-I**. The physicochemical data of **8**–**10** (**I**, **II**) are shown in the Supporting Information.

Separation of the *N*-Benzoyl-1,5-benzothiazepines (6-I/II) and Their *S*-Oxides (8-I/II, 9-I, and 10-I/II) into the Enantiomers. The racemic compounds, **6-I**/**II**, **8-I**/**II**, **9-I**, and **10-I**/**II** were separated using chiral HPLC (CHIRALPAK IA for **6-I**, **10-I**, and **10-II**, CHIRALPAK IB for **6-II**, CHIRALPAK OJ-H for **8-I** and **9-I**, CHIRALPAK IC for **8-II**). The separation conditions and physicochemical properties of the enantiomers are shown in the Supporting Information.

Single-Crystal X-ray Analysis. The crystal structures of the optically active compounds (−)-(a*R*)-**6-I**, (−)-(1*S*)-**8-I**, (+)-(1*R*)-**8-I**, (−)-(1*S*,a*R*)-**9-I**, and (+)-(1*S*)-**10-I** and the racemate (1*R**)-**8-I** were obtained by single-crystal X-ray analysis (Figure 5). Typical crystal data and CIF files are in the Supporting Information.

Stereochemical Stability of Atropisomers of 6 (I, II) and Diastereomer of 8-I. The stereochemical (thermodynamic) stability of (a*R*/a*S*)-atropisomers of **6** (**I**, **II**) and diastereomer of **8-I** was determined as previously described.[7c,e] The details including the figures of conversion profiles are shown in the Supporting Information.

In Vitro Binding Assay to hV_{1a} and hV_2. Binding of [^{3}H](Arg^8)-vasopressin to human VP V_{1a} and V_2 receptors was measured by conventional methods. The procedure is shown in the Supporting Information. All test compounds (shown in Table 2) had a purity of ≥95% as determined by HPLC analyses.

ASSOCIATED CONTENT

Supporting Information

General experimental procedure, ^{1}H, ^{13}C, and 2D NMR spectra and physicochemical properties for new compounds, VT NMR for **8-I**, figures of thermal isomerization rate of atropisomers for (a*R*)/(a*S*)-**6-I** and (a*R*)/(a*S*)-**6-II**, X-ray crystal data (CIF) for (a*R*)-**6-I**, (1*R*)-**8-I**, (1*S*)-**8-I**, (1*R**)-**8-I**, (1*S*,a*R*)-**9-I**, and (1*S*)-**10-I**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: natsu@pharm.teikyo-u.ac.jp. Phone: +81-3-3964-8096.

DOI: 10.1021/acs.jmedchem.5b00289
J. Med. Chem. XXXX, XXX, XXX–XXX

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (25460154) and a Grant-in-Aid for Young Scientists (B) (25860091) from the Japan Society for the Promotion of Science. H.T. extends thanks for financial support by Astellas Foundation for Research on Metabolic Disorders (2013) and MEXT-Supported Program for the Strategic Research Foundation at Private Universities (2013−2017).

■ ABBREVIATIONS USED

ADPKD, autosomal-dominant polycystic kidney disease; AVP, arginine vasopressin; VP, vasopressin; NK, neurokinin; ACAT, acyl-coenzyme A/cholesterol acyltransferase; GABA, γ-aminobutyric acid; *m*CPBA, *m*-chloroperoxybenzoic acid; VT NMR, variable-temperature ^{1}H NMR; ps.ax., pseudoaxial; ps.eq., pseudoequatorial

■ REFERENCES

(1) Ogawa, H.; Yamashita, H.; Kondo, K.; Yamamura, Y.; Miyamoto, H.; Kan, K.; Kitano, K.; Tanaka, M.; Nakaya, K.; Nakamura, S.; Mori, T.; Tominaga, M.; Yabuuchi, Y. Orally active, nonpeptide vasopressin V_2 receptor antagonists: a novel series of 1-[4-(benzoylamino)-benzoyl]-2,3,4,5-tetrahydro-1*H*-benzazepines and related compounds. *J. Med. Chem.* **1996**, *39*, 3547−3555.

(2) Reviews on recent advances in VP receptor ligands: (a) Ryckmans, T. Advances in vasopressin receptor agonists and antagonists. *Annu. Rep. Med. Chem.* **2009**, *44*, 129−147. (b) Veeraveedu, P. T.; Palaniyandi, S. S.; Yamaguchi, K.; Komai, Y.; Thandavarayan, R. A.; Sukumaran, V.; Watanabe, K. Arginine vasopressin receptor antagonists (vaptans): pharmacological tools and potential therapeutic agents. *Drug Discovery Today* **2010**, *15*, 826−841.

(3) Kondo, K.; Ogawa, H.; Yamashita, H.; Miyamoto, H.; Tanaka, M.; Nakaya, K.; Kitano, K.; Yamamura, Y.; Nakamura, S.; Onogawa, T.; Mori, T.; Tominaga, M. 7-Chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-1-benzazepine (OPC-41061): a potent, orally active nonpeptide arginine vasopressin V_2 receptor antagonist. *Bioorg. Med. Chem.* **1999**, *7*, 1743−1754.

(4) Tahara, A.; Tomura, Y.; Wada, K.; Kusayama, T.; Tsukada, J.; Takanashi, M.; Yatsu, T.; Uchida, W.; Tanaka, A. Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V_{1A} and V_2 receptor antagonist, in vitro and in vivo. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 301−308.

(5) 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. 5-Fluoro-2-methyl-*N*-[4-(5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-10(11*H*)-ylcarbonyl)-3-chlorophenyl]benzamide (VPA-985): an orally active arginine vasopressin antagonist with selectivity for V_2 receptors. *J. Med. Chem.* **1998**, *41*, 2442−2444.

(6) Tolvaptan (Samsca) was approved as the first drug for ADPKD in Japan in March 2014.

(7) (a) Natsugari, H.; Ikeura, Y.; Kamo, I.; Ishimaru, T.; Ishichi, Y.; Fujishima, A.; Tanaka, T.; Kasahara, F.; Kawada, M.; Doi, T. Axially chiral 1,7-naphthyridine-6-carboxamide derivatives as orally active tachykinin NK_1 receptor antagonists: synthesis, antagonistic activity, and effects on bladder functions. *J. Med. Chem.* **1999**, *42*, 3982−3993. (b) Lee, S.; Kamide, T.; Tabata, H.; Takahashi, H.; Shiro, M.; Natsugari, H. Axial chirality and affinity at the $GABA_A$ receptor of pyrimido[1,2-*a*][1,4]benzodiazepines and related compounds. *Bioorg. Med. Chem.* **2008**, *16*, 9519−9523. (c) Tabata, H.; Nakagomi, J.; Morizono, D.; Oshitari, T.; Takahashi, H.; Natsugari, H. Atropisomerism in the vaptan class of vasopressin receptor ligands: the active conformation recognized by the receptor. *Angew. Chem., Int. Ed.* **2011**, *50*, 3075−3079. (d) Tabata, H.; Akiba, K.; Lee, S.; Takahashi, H.; Natsugari, H. Atropisomeric properties of the dibenzo[*b*,*d*]azepin-6-one nucleus. *Org. Lett.* **2008**, *10*, 4871−4874. (e) Tabata, H.; Wada, N.; Takada, Y.; Nakagomi, J.; Miike, T.; Shirahase, H.; Oshitari, T.; Takahashi, H.; Natsugari, H. Active conformation of seven-membered-ring benzolactams as new ACAT inhibitors: latent chirality at N5 in the 1,5-benzodiazepin-2-one nucleus. *Chem.—Eur. J.* **2012**, *18*, 1572−1576.

(8) The description "cis/trans" is used for the relative arrangement of the two benzene rings.

(9) The terms a*S* and a*R* (chiral axis nomenclature) correspond to *P* and *M* (helix nomenclature), respectively.

(10) Tabata, H.; Yoneda, T.; Oshitari, T.; Takahashi, H.; Natsugari, H. Stereochemistry of 1,5-benzothiazepin-4-one *S*-oxide: insight into the stereogenic elements at the sulfur atom and axis. *J. Org. Chem.* **2013**, *78*, 6264−6270.

(11) (a) *N*-Benzoyl-1,5-benzothiazepines with several substituents at the 2 position as VP receptor antagonists have been reported: Urbanski, M. J.; Chen, R. H.; Demarest, K. T.; Gunnet, J.; Look, R.; Ericson, E.; Murray, W. V.; Rybczynski, P. J.; Zhang, X. 2,5-Disubstituted 3,4-dihydro-2*H*-benzo[*b*][1,5]thiazepines as potent and selective V_2 arginine vasopressin receptor antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4031−4034. (b) *N*-Benzoyl-1,5-benzothiazepine (**5-II**) and the *S*-oxide (**8-II**) (racemate) were briefly described in a patent application: Ogawa, H.; Miyamoto, H.; Kondo, K.; Yamashita, H.; Nakaya, K.; Komatsu, H.; Tanaka, M.; Takara, S.; Tominaga, M.; Yabuchi, Y. Preparation of *N*-benzoyl benzo-fused heterocyclic compounds as vasopressin antagonists. Jpn. Kokai Tokkyo Koho, JP 04321669, 1992.

(12) Ito, S.; Tomiyama, R. Reduction of 4-thiochromanone oximes with lithium aluminum hydride and related reactions. *Sci. Rep. Hirosaki Univ.* **1990**, *27*, 16−22.

(13) Although several very small peaks that may originate in the trans-isomer were observed in the ^{1}H NMR spectrum, the peaks were not sufficient to assign the structure. The cis-stereochemistry was determined by the NOESY experiment on **6-I** and **9-I**, in which the 6-CH_3 group showed a correlation with the protons of the benzoyl ring. Formation of the cis-structure in **5**−**10** is consistent with the report that *N*-benzoyl-*N*-methylanilines exist in a cis-structure: Azumaya, I.; Kagechika, H.; Fujiwara, Y.; Itoh, M.; Yamaguchi, K.; Shudo, K. *J. Am. Chem. Soc.* **1991**, *113*, 2833−2838.

(14) The sulfones were formed as minor products (15−21%).

(15) The absolute stereochemistry was determined based on the Flack parameter.

(16) Determination of $\Delta G^{\ddagger}$ values: Petit, M.; Lapierre, A. J. B.; Curran, D. P. Relaying asymmetry of transient atropisomers of *o*-iodoanilides by radical cyclizations. *J. Am. Chem. Soc.* **2005**, *127*, 14994−14995.

(17) Determination of $\Delta G^{\ddagger}$ values by VT NMR: Boiadjiev, S. E.; Lightner, D. A. Atropisomerism in linear tetrapyrroles. *Tetrahedron* **2002**, *58*, 7411−7421.

(18) Preliminary molecular mechanics calculations of **8-I** using B3LYP/6-311+G(2df,2p) indicated that the total energy of the chairlike conformation with the *S*-oxide in a ps.eq. orientation [(1*R**,a*S**)(=*anti*)-form] is slightly more stable than that of the ps.ax. orientation [(1*R**,a*R**)(=*syn*)-form] by 6.0 kJ/mol (1.4 kcal/mol).

(19) In the case of mozavaptan and a tolvaptan-like compound, the eutomer is reported to be the (−)-(5*S*)-isomer and the distomer to be the (+)-(5*R*)-isomer: Ootsubo, K.; Yamashita, S.; Uchida, M.; Morita, K. Preparation of optically active 5-hydroxybenzoazepines as vasopressin antagonists. Jpn. Kokai Tokkyo Koho, JP 0680641, 1994.

(20) For general experimental methods, see the Supporting Information.

(21) Mushkalo, L. K.; Fedorova, I. P. Synthesis of 2,3,4,5-tetrahydro-1,5-benzothiazepine. *Ukr. Khem. Zh.* **1954**, *20*, 305−307.

DOI: 10.1021/acs.jmedchem.5b00289
J. Med. Chem. XXXX, XXX, XXX−XXX