Atropisomerism

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Atropisomerism in the Vaptan Class of Vasopressin Receptor Ligands: The Active Conformation Recognized by the Receptor**

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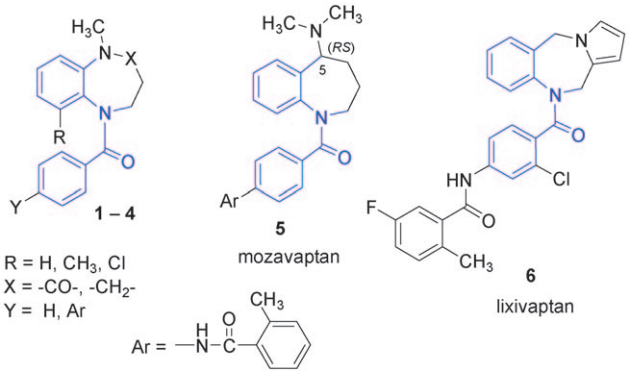
Chiral compounds are generally thought of as compounds with classical chiral centers (stereogenic elements, mostly asymmetric carbon atoms). However, a nonplanar compound may be chiral because it incorporates other (stereogenic) elements, which comprise axes and planes. Axial chirality (atropisomerism)[1] is caused by restricted rotation about a single bond (axis), of which the most extensively studied is the sp^2–sp^2 axial chirality of biaryl compounds, as exemplified by 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP). Aside from biaryls, aryl amides and anilides, which exist in many biologically active compounds as a part of the pharmacophore, also possess sp^2–sp^2 atropisomerism based on the aryl–amide axis.[2] Although often overlooked, such atropisomerisms are latent in many organic molecules. It should be noted that, even if the conformational change is too rapid for the enantiomers to be isolated, target molecules such as receptors and enzymes will recognize the active enantiomeric form to exert biological activity.

Since the first discovery of a non-peptide arginine vasopressin (AVP) V_2 receptor antagonist (**5**; mozavaptan[3]) (Scheme 1), extensive research to find new ligands (antagonists and agonists) has been carried out.[4] To date, the "vaptan" class of ligands (e.g., lixivaptan (**6**),[5] tolvaptan,[6] and conivaptan[7]) has been developed as agents for the treatment of hyponatremia, congestive heart failure, and so forth. Interest is still growing in the search for new ligands and their new indications as well as in determining the biological role of the subtype V_{1a} and V_{1b} receptors. Many of the vaptan class of drugs contain a preserved scaffold, that is, a benzo-fused seven-membered-ring nitrogen heterocycle (e.g., benzazepine, 1,4-benzodiazepine) linked through N-1 to a substituted *p*-amidobenzoyl group (e.g., **5**, **6**; Scheme 1). To the best of our knowledge, however, regardless of the vast number of studies performed in this field, there has been no previous discussion of the atropisomeric structure around the scaffold region (blue lines in the structures in Scheme 1), which should play an important role in regard to its activity.



Scheme 1. Vaptan class of vasopressin receptor ligands with the *N*-benzoyl benzo-fused seven-membered-ring nitrogen heterocycles as the scaffold structure: *N*-benzoyl-1,5-benzodiazepine derivatives (**1**–**4**), mozavaptan (**5**), and lixivaptan (**6**).

Herein, we report on the atropisomerism of the scaffold region of the newly discovered AVP receptor ligands *N*-benzoyl-1,5-benzodiazepines (**1**–**4**; Scheme 1), and the actual active conformation recognized by the receptor, which was clarified by freezing the conformation in the molecules.

At first sight, the presence of chirality may often be overlooked, although the *cis* and *trans* rotamers[8] around the N–C(=O) bond can be envisioned. However, a careful survey of the chemical structure reveals that the region contains the a*S* and a*R* atropisomeric structure[9] based on the Ar–N(C=O) (sp^2–sp^2) axis as well as the *cis* and *trans* rotamers. Thus, theoretically the four stereoisomers (conformers) shown in Scheme 2 may exist in the scaffold region. When the AVP receptor binds to the ligand (e.g., **5**, **6**), it may recognize the conformation of the region as suitable for binding. To gain an insight into the actual active structure recognized by the receptor we planned to separate the isomers by freezing the conformation with a substituent at the *ortho* position (R) and to examine the biological activities.

The 1,5-benzodiazepine nucleus was selected in this study as the benzo-fused seven-membered-ring nitrogen heterocycle. A few studies on AVP receptor ligand binding have already been reported with this heterocycle. *N*-Benzoyl derivatives of 1,5-benzodiazepin-2-ones (**1**–**3**) and the re-

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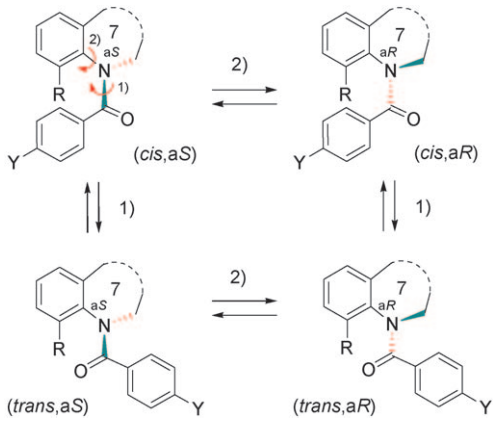
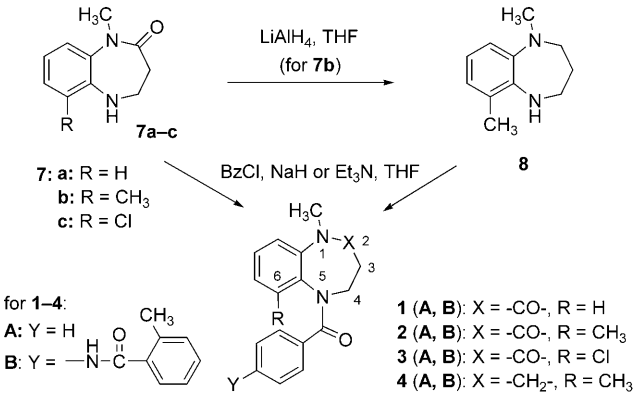


Scheme 2. Conformation of the scaffold region of vasopressin ligands with benzo-fused seven-membered-ring nitrogen heterocycles: 1) rotation around the N–(C=O) bond to form *cis* and *trans* rotamers, and 2) rotation around the Ar–N axis to form a*S* and a*R* atropisomers.

duced-type 1,5-benzodiazepine (**4**) were prepared by *N*-benzoylation of the parent 1,5-benzodiazepines (**7a–c** and **8**) (Scheme 3):[10] *N*-Benzoylation using benzoyl chloride and *p*-(2-methylbenzamido)benzoyl chloride gave **1A**–**4A** (Y = H) and **1B**–**4B** (Y = (2-methylbenzoyl)amino), respectively. Compounds **2** (**A**, **B**) and **4** (**A**, **B**) have a methyl group at the *ortho* position (at C6) of the benzene ring (R = CH_3), and **3** (**A**, **B**) has a chloro group at the same position (R = Cl), and both groups provide a rotation barrier for the axis.



Scheme 3. Preparation of *N*-benzoyl-1,5-benzodiazepin-2-ones (**1**–**3**) and -1,5-benzodiazepines (**4**).

First, the conformations of the *N*-benzoyl-1,5-benzodiazepines (**1**–**4**) were examined in detail using **1A** (Y = R = H) and **2A** (Y = H, R = CH_3). Compound **1A** showed only one stereoisomer in the ^{1}H NMR ($CDCl_3$) spectrum, which was presumed to have a *cis* arrangement around the N–C(=O) bond by comparison with the data of **2A**. Interestingly, in the ^{1}H NMR spectrum of **1A**, each of the four methylene protons of the diazepine ring were observed as separated signals at δ = 2.62, 2.71, 3.82, and 4.78 ppm (each 1H, broad). This observation indicates that the protons are diastereotopic and suggests the presence of axial chirality caused by the Ar–N(C=O) (sp^2–sp^2) axis. The atropisomers, however, could not be separated at room temperature by HPLC on a chiral stationary phase, which implies that the energy barrier between the atropisomers of **1A** is low. A similar structural feature was also obtained for **1B** (Y = (2-methylbenzoyl)amino, R = H). On the other hand, compound **2A**, which has a CH_3 group at the C6-position, showed two sets of signals in a ratio of approximately 10:1 in the ^{1}H NMR spectrum. The signals of the aryl–CH_3 group and a proton of the C4-methylene group in the different isomers could be clearly distinguished: the major isomer appeared at δ = 1.97 (3H) and 4.95 ppm (1H), and the minor isomer at δ = 2.38 (0.3H) and 4.32 ppm (0.1H). Since the protons located over a benzene ring are observed at higher field in the ^{1}H NMR spectrum and the protons located within the deshielding cone of a carbonyl group are observed at lower field, the major and minor isomers are confirmed to have *cis* and *trans* conformations, respectively.[11] The two methylene protons of **2A** appeared as separated four sharp signals in the ^{1}H NMR spectrum, which suggests that the rotation around the Ar–N(C=O) axis is restricted to form stable atropisomers. Compound **2A** was actually separated into the respective enantiomers [(+)-**2A** and (−)-**2A**] by preparative HPLC on a chiral stationary phase. Each enantiomer exists as an equilibrium mixture of *cis* and *trans* conformers in solution, as observed in the racemate **2A** (a 10:1 ratio in the ^{1}H NMR ($CDCl_3$) spectrum), thus indicating that the rotation around the amide bond is too rapid for isolation of the conformers at room temperature. Fortunately, both enantiomers could be subjected to X-ray structure analysis,[12] which revealed that the stereochemistry of the (+) isomer is *cis*,a*S* and that of the (−) isomer *cis*,a*R* in the crystal (Figure 1). Thus, the major

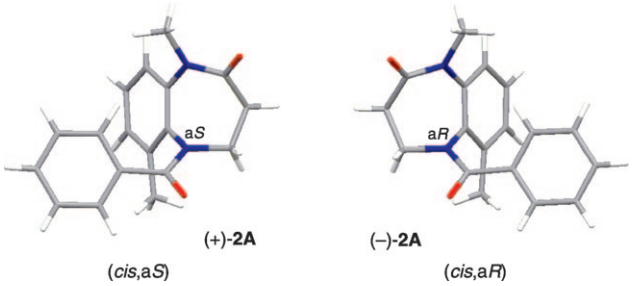
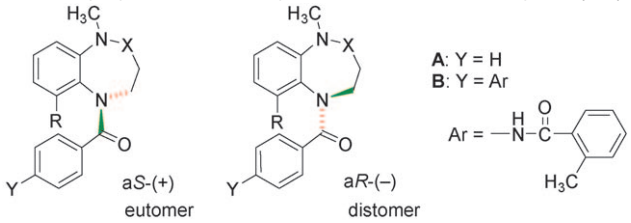


Figure 1. X-ray crystal structures of the enantiomers, (+)-**2A** (=*cis*,a*S*) and (−)-**2A** (=*cis*,a*R*), generated from the CIF files.

isomer of **2A** (racemate and enantiomers) exists in a *cis* arrangement in solution as well as in the solid state. This is consistent with the report[13] that *N*-benzoyl-*N*-methylanilines exist in a *cis* structure. Similar structural features were observed for **2B**, **3A**, **3B**, **4A**, and **4B** as for **2A**. The results are summarized in Table 1.

To compare the stereochemistry of the diazepine ring systems of **2** and **4**, the (+) enantiomer of **4A** was subjected to X-ray crystal analysis. The configuration of (+)-**4A** was revealed to be *cis*,a*S*, as shown in Figure 2.[12] Overall, the stereochemistry of **2** and **4** in the crystal state is similar, except

Table 1: Physicochemical properties of the atropisomers of 1,5-benzodiazepines (**1**–**4**).



	R	X	Y	$[\alpha]_D^{23}$ (CH_3OH)[a] (a*S*)[c]-(+)	(a*R*)[c]-(−)	$\Delta G^{\ddagger}$ [kJ mol^{-1}]	Racemization[b]	*cis*:*trans* ratio
1A	H	C=O	H		–[d]			10:–[e]
1B	H	C=O	Ar		–[d]			10:–[e]
2A	CH_3	C=O	H	+113.1	−115.0	104	50 °C, 5 h[f]	10:1
2B	CH_3	C=O	Ar	+211.4	−209.0	104	50 °C, 5 h[f]	10:0.7
3A	Cl	C=O	H	+75.7	−78.9	99	37 °C, 4 h	10:1
3B	Cl	C=O	Ar	+192.4	−191.7	100	37 °C, 6 h	10:0.7
4A	CH_3	CH_2	H	+151.0	−146.7	96	37 °C, 2 h	10:–[e]
4B	CH_3	CH_2	Ar	+185.3	−180.0	96	37 °C, 2 h	10:–[e]

[a] For each concentration (c = 0.08–0.185 g ml^{-1}), see the Supporting Information. [b] Conditions required for racemization in toluene. [c] For description of a*S* and a*R*, see ref. [9]. [d] Not separable at room temperature. [e] The *trans* isomer could not be observed in the ^{1}H NMR spectrum. [f] Isomerized to 50% *ee* at 37 °C for 6 h in toluene.

Figure 2. X-ray crystal structure of (+)-**4A** (=*cis*,a*S*) generated from the CIF file.

for the orientation of the C3-methylene group (Figure 1 vs. Figure 2). Thus, the (+)/(−) angle of optical rotation α of the enantiomers is diagnostic in determining the absolute configuration of **2**–**4**; the a*S* and a*R* isomers have (+) and (−) angles, respectively (Table 1).

The stereochemical stability of these separated enantiomers of **2**–**4** was examined next. The activation free-energy barrier to rotation ($\Delta G^{\ddagger}$)[14] and the conditions required for racemization of the enantiomers are shown in Table 1. The enantiomers of **2A** and **2B** (R = CH_3, X = C=O) showed stereochemical stability with a $\Delta G^{\ddagger}$ value of 104 kJ mol^{-1}. **3A** and **3B** (R = Cl, X = C=O) were less stable ($\Delta G^{\ddagger}$ = 99–100 kcal mol^{-1}). The higher stereochemical stability of **2** than of **3** may be explained largely by the greater steric bulk (van der Waals radius) of the methyl group (2.0 Å) compared with that of the Cl atom (1.75 Å). Also the electronic (inductive) effect of the Cl atom may affect ring flipping by reducing the electron density on the amide nitrogen atom, and so lowering the barrier. In the case of the enantiomers of **4A** and **4B** (R = CH_3, X = CH_2), the barrier to rotation ($\Delta G^{\ddagger}$ = 96 kJ mol^{-1}) is lower than that of **2A** and **2B**, which reflects the flexibility of the fully reduced diazepine ring of **4** (Table 1).

After obtaining diverse information on the atropisomeric properties of **1**–**4**, the in vitro affinities at the human vasopressin V_{1a} and V_2 receptors were evaluated using the **B** series of compounds (**1B**–**4B**; Y = (2-methylbenzoyl)amino) including the atropisomers (Table 2). Fortunately, all the compounds showed good potency in the binding experiment. Compound **1B**, which could not be separated into its atropisomers, exhibited affinity (K_i) at the 10-nanomolar level for both V_{1a} and V_2 receptors. Compounds **2B**, **3B**, and **4B** were first examined for their potency in binding using the racemate. It is interesting to note that these compounds bearing an *ortho* substituent have a

Table 2: In vitro affinity for human vasopressin V_{1a} and V_2 receptors of **1B**, **2B**, **3B**, and **4B,** including the atropisomers.

		K_i [nM][a] hV_{1a}	hV_2
1B	(achiral)	55	23
2B	racemate	88	680
	a*S*-(+)	70	640
	a*R*-(−)	620	4700
3B	racemate	80% Inh.[b]	70% Inh.[b]
4B	racemate	5.9	130
	a*S*-(+)	4.4	98
	a*R*-(−)	13	310
Arg^8-vasopressin		0.16	2.2

[a] K_i values shown are the means of triplicate measurements. For 95% confidence limits, see the Supporting Information. [b] Inhibition (Inh.) [%] at 10 μM.

tendency to bind with higher selectivity to V_{1a} rather than to V_2 receptors (Table 2). Among them, compound **4B**, which showed excellent V_{1a}-selective affinity (K_i: 5.9 nM for V_{1a}, 130 nM for V_2), is a worthy candidate for further biological studies. Next, the a*S* and a*R* atropisomers of **2B** and **4B** were subjected to the assay, which revealed the importance of the stereochemistry at the scaffold region for biological activity. The atropisomers a*S*/a*R* of **2B** exhibited an about 9–13-fold difference in the affinities between the isomers; the a*S* isomer is the eutomer with greater potency and the a*R* isomer is the distomer [K_i [nM] a*S*/a*R* = 70:620 for V_{1a}, 640:4700 for V_2]. The atropisomers (a*S*/a*R*) of **4B** exhibited an about threefold difference in affinities; the a*S* isomer again had greater potency [K_i [nM] a*S*/a*R* = 4.4:13 for V_{1a}, 98:310 for V_2].

The results clearly indicate that the scaffold region of these AVP receptor ligands plays an important role in terms

of the activity. The receptor recognizes the stereochemistry by binding with the *cis*,a*S* form of the ligands. This may hold true for the general vaptan class of AVP receptor ligands (e.g., **1B**, **5**, and **6**), which do not possess the *ortho* substituent (R) in the benzene ring. When binding to the ligands, the receptor must recognize their *cis*,a*S* conformation.

In this regard, mozavaptan (**5**), which was developed as a racemate at the C5-position, has interesting implications. A related study on **5** revealed that the 5*S* enantiomer is the eutomer, and the 5*R* isomer is the distomer.[15,16] An important observation about the structure of 1-benzoyl-5-methylbenzazepine (in the racemic form; **11**), which constitutes the basic structure of **5**, has been reported,[17] indicates that two conformers exist in a 4:1 ratio in solution (by ^{1}H NMR spectroscopy); the major isomer was shown to have the methyl substituent in the axial orientation (Figure 3). The

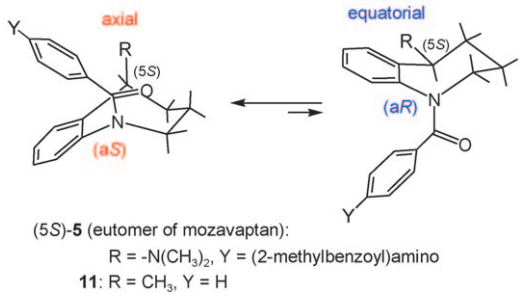


Figure 3. Conformation of *N*-benzoyl-5-substituted-benzazepines [(5*S*)-**5** and **11**]: the 5-substituent predominantly occupies the axial position in solution, thereby determining the conformation of the ring including the axial chirality.

stereochemistry around the benzoylamide moiety was not mentioned in that report. However, considering that the azepine ring has a chairlike conformation in the stable form,[18] the conformation (configuration) around the *N*-benzoylamide (scaffold) region is presumed to be inevitably determined by the C5 configuration, as shown in Figure 3; that is, in the case of the eutomer of **5**, the 5*S* configuration controls the conformation around the scaffold region so that it possesses a*S* configuration, which is in good agreement with our assumption for the eutomers.

In conclusion, by freezing the atropisomerism at the scaffold region in the AVP ligands we succeeded in gaining an insight into the actual active structure with the *cis*,a*S* form. Thus far, the chirality caused by the conformational change has not been given attention in the vast number of studies on the AVP ligands. Such axial chiralities may exist in a latent form in many biologically active molecules. We should bear in mind that the target receptors or enzymes must recognize the actual active structure of the molecules. We hope that this study will contribute to future drug design and development.

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[8] The description "*cis*/*trans*" is used for the relative arrangement of the two benzene rings.

[9] Herein, for convenience, the description of the axial chirality (a*S* and a*R*) is used so that the substituent R is lower in priority than the seven-membered ring chain [formally, the definition is reversed in the case of **3** (**A** and **B**; R = Cl)]. The terms a*S* and a*R* (chiral axis nomenclature) correspond to *P* and *M* (helix nomenclature), respectively.

[10] For the preparation of the parent 1,5-benzodiazepines (**7a–c**, **8**), see the Supporting Information.

[11] The *cis*- and *trans*-stereochemical assignment is further supported by the NOESY experiment using 5-*p*-chlorobenzoyl-1,6-dimethyl-2*H*-1,5-benzodiazepin-2-one at −30 °C, in which the aryl *ortho*-CH_3 group in the major *cis* isomer showed a correlation with the protons of the benzoyl ring (see the Supporting Information).

[12] CCDC 803349 ((−)-**2A**), 803350 ((+)-**2A**), and 803351 ((+)-**4A**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. The absolute stereochemistry was determined based on the Flack parameter ($Cu_{K\alpha}$ radiation was used for the measurement).

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[15] IC_{50} values [nM] for the rat V_{1a} and V_2 receptors: mozavaptan (**5**) (racemate), 1400 for V_{1a} and 12 for V_2;[3,16] the eutomer of **5** (5*S*), 540 for V_{1a} and 15 for V_2);[16] the distomer of **5** (5*R*), 8500 for V_{1a} and 90 for V_2.[16]
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