Synthesis and Structure−Activity Relationships of 5,6,7,8-Tetrahydro-4*H*-thieno[3,2-*b*]azepine Derivatives: Novel Arginine Vasopressin Antagonists

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A variety of novel heterocyclic compounds having thienoazepine, pyrroloazepine, furoazepine, and thienodiazepine skeletons were synthesized, most of which exhibited potent antagonism of [^{3}H]-AVP specific binding in assays using rat liver (V1), rat kidney (V2), human platelet plasma membranes, and recombinant human CHO cells (V2), as well as antagonizing AVP-induced hypertension in rats (V1, intravenous) and showing a diuretic effect in rats (V2, oral). By detailed studies of the structure−activity relationships of these compounds, the thienoazepine derivative **1** was found to be a very potent combined V1 and V2 antagonist. After further pharmacological and toxicological evaluation as well as physical properties, the hydrochloride **2** (JTV-605) of compound **1** was selected for clinical studies as a potent AVP antagonist with a long duration of action.

Introduction

Arginine vasopressin (AVP) is a neurohypophyseal nonapeptide that has a wide variety of physiological activities in mammals. It plays an important role in regulating water and solute excretion by the kidneys and also participates in other physiological functions such as blood pressure control, regulation of platelet aggregation, and ACTH secretion. Therefore, AVP may be involved in various conditions such as heart failure, hypertension, angina, hyponatremia, and dysmenorrhea.[1] After development of the early peptide AVP antagonists that lacked oral bioavailability and had a short half-life,[2] the orally effective nonpeptide antagonists OPC-21268 (a selective V1a antagonist), OPC-31260 (compound **3**) (a selective V2 antagonist),[3] SR-49059 (a selective V1a antagonist), SR-121463 (a V2 antagonist),[4] YM-087 (V1 and V2 dual antagonists),[5] and VP-343 (a V2 antagonist)[6] were synthesized. We began synthetic studies searching for more potent combined V1 and V2 antagonists as well as selective V2 antagonists. We constructed several basic heterocyclic skeletons, including thienoazepine,[7] pyrroloazepine, furoazepine, and thienodiazepine[8] and discovered derivatives that exhibited potent AVP antagonism.[9] Compound **4**, one of the compounds we were studying, was independently patented by American Cyanamid.[10] However, this compound did not show good oral absorption in rats. Therefore, we continued investigations to improve oral absorption as well as potency and duration. Using molecular modeling, we found compound **1** among a set of compounds which showed satisfactory bioavailability and extremely potent activities as AVP antagonists. The discovery of a series of potent AVP antagonist analogues containing a central benzene ring substituted with a nitrogen-containing side chain was new to AVP research.

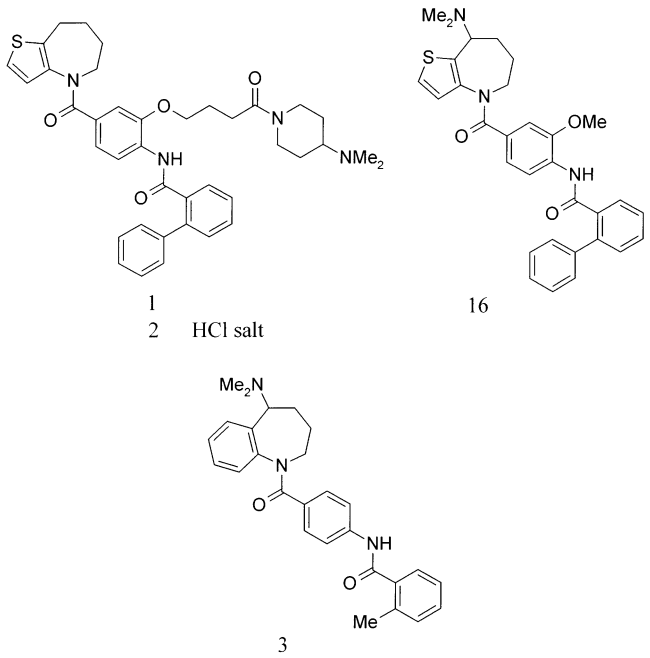

Figure 1. Novel thienoazepines and AVP antagonist **3**.

Evaluation of the pharmacological and toxicological properties of compound **1** led us to choose its hydrochloride **2** for clinical evaluation. In this report we disclose synthetic methods and bioactivity data of compound **1** and analogues.

Chemistry

We developed new heterocyclic fused azepines and diazepines for the basic skeleton. As we reported previously,[7] DIBAH (diisobutylaluminum hydride) reduction of heterocyclic fused cyclohexanone oxime **a** gave regioselectively heterocyclic fused azepine **b** as the sole compound. Benzoylation of compound **b** afforded compound **c** which was successively treated with hydrogen

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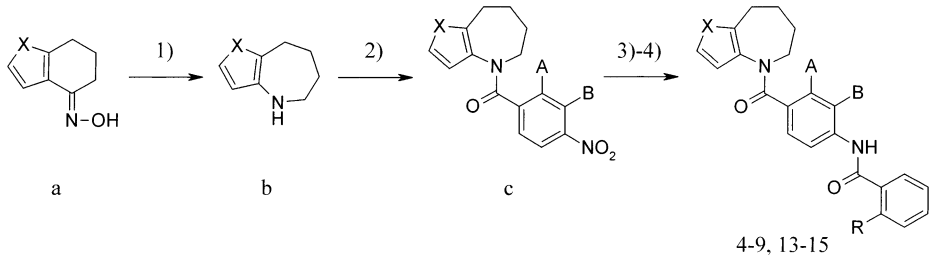
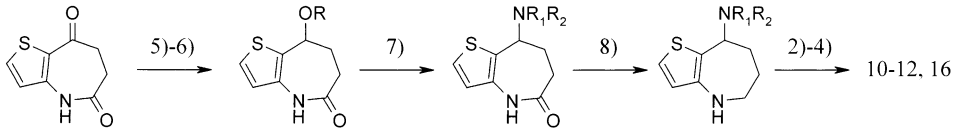
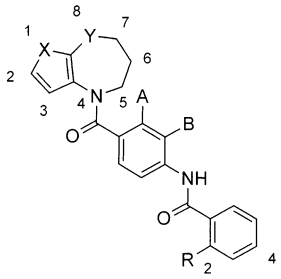
Scheme 1. Synthesis of Compounds **4**−**9**, **13**−**15**



Scheme 2. Synthesis of Compounds **10**−**12**, **16**[a]



[a] Reaction conditions: (1) DIBAH, (2) 4-nitrobenzoyl chloride, 3-methoxy-4-nitrobenzoyl chloride, or 2-methoxy-4-nitrobenzoyl chloride/Et_3N, (3) H_2/Pd−C, (4) 2-phenylbenzoyl chloride, 2-methylbenzoyl chloride, or 2-chlorobenzoyl chloride/Et_3N, (5) $NaBH_4$, (6) MsCl/pyridine, (7) amine derivatives, (8) $LiAlH_4$.

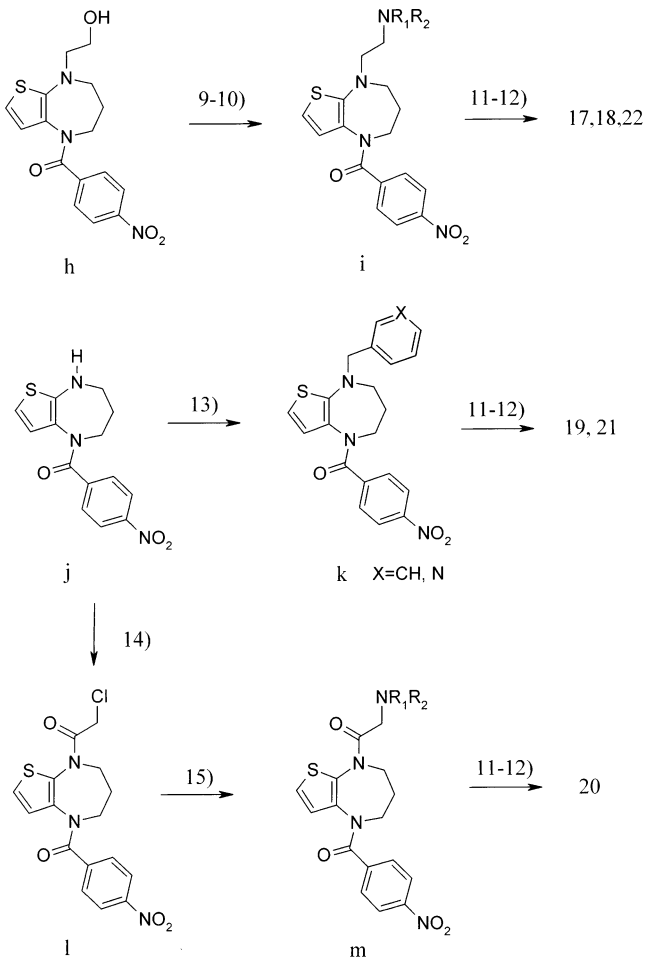


#	X	Y	R	A	B
4	S	CH_2	Ph	H	H
5	O	CH_2	Ph	H	H
6	NH	CH_2	Ph	H	H
7	NMe	CH_2	Ph	H	H
8	S	CH_2	Me	H	H
9	S	CH_2	Cl	H	H
10	S	$CHNMe_2$	Ph	H	H
11	S	$CHNMe_2$	Me	H	H
12	S	$CHN(Me)CH_2CH_2$-(N)	Ph	H	H
13	S	CH_2	Ph	OMe	H
14	S	CH_2	Ph	H	OMe
15	S	CH_2	Ph	H	OH
16	S	$CHNMe_2$	Ph	H	OMe
17	S	$N(CH_2)_2$-(N)	Ph	H	H
18	S	$N(CH_2)_2NMe_2$	Ph	H	H
19	S	NCH_2-(N)	Ph	H	H
20	S	$NCOCH_2$-(N-Me)	Ph	H	H
21	S	NCH_2-(Ph)	Ph	H	H
22	S	$N(CH_2)_2$-(N-Me)	Ph	H	H

Figure 2. A list of thienoazepines, furoazepines, pyrroloazepines, and thienodiazepines.

on Pd−C and substituted benzoyl chloride to give 5,6,7,8-tetrahydrothieno[3,2-*b*]azepines **4**−**9** and **13**−**15** (see Scheme 1 and Figure 2). On the other hand, azepines having a dialkylamino group were synthesized as shown in Scheme 2. Namely, reduction of 5,6,7,8-tetrahydro-4*H*-thieno[3, 2-*b*]azepine-5, 8-dione **d**[11] with $NaBH_4$ yielded the alcohol, which was treated with mesyl chloride to give compound **e**. Nucleophilic sub-

Scheme 3[a]



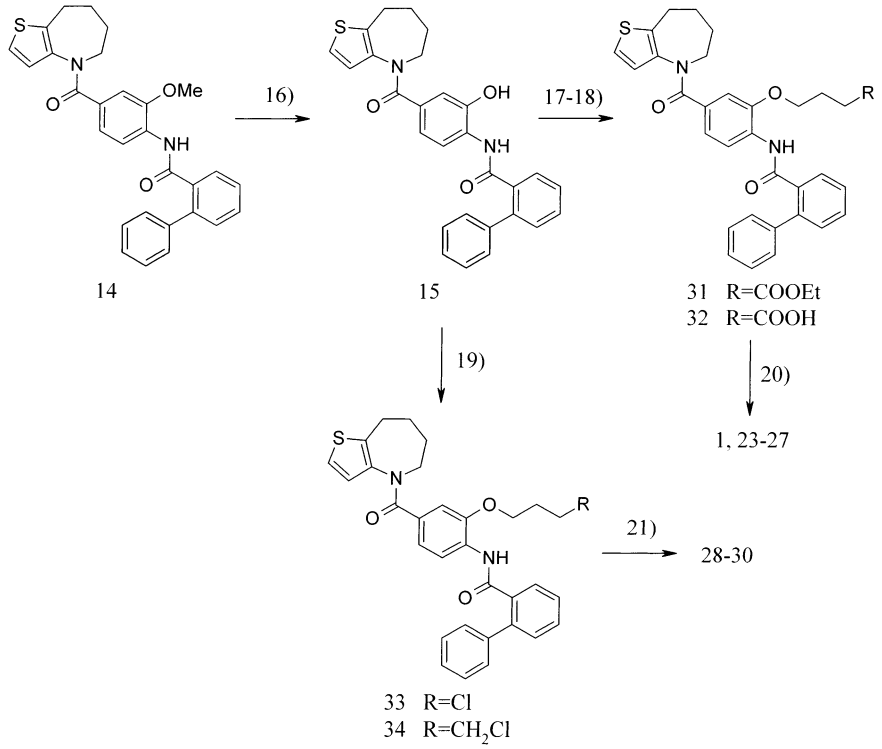
[a] Reaction conditions: (9) MsCl, pyridine, (10) amine derivatives, K_2CO_3, KI, (11) H_2, PtO_2, (12) 2-phenylbenzoyl chloride, Et_3N, (13) 3-pyridinylmethyl chloride or benzyl chloride, K_2CO_3, KI, (14) chloroacetyl chloride, Et_3N, (15) amine derivative, KI.

Table 1. Data of Specific Binding of Thienoazepines **4**, **8**−**16**, Furoazepine **5**, Pyrroloazepines **6** and **7**, and Thienodiazepines **17**−**22** to AVP Receptor, Antagonistic Effect on AVP-Induced Hypertension, and Diuretic Effect

compound no.	[^{3}H]-AVP binding IC_{50} (nM)[a]				blood pressure:[b] ID_{50} (mg/kg, iv)	urine volume:[b] (mL/4 h) [dose mg/kg, po]
	V1 rat	V2 rat	V1 human	V2 human		
3	5000 ± 140	300 ± 30	2,000 ± 90	500 ± 15	>0.3	9.3 ± 0.7 [30], 2.9 ± 0.1 [10]
4	290 ± 20	310 ± 25	300 ± 20	5.0 ± 0.4	0.11 ± 0.05	0.4 ± 0.2 [30]
5	2100 ± 150	300 ± 30	2900 ± 280	>10000	n.t.[c]	n.t.
6	390 ± 28	200 ± 15	2100 ± 120	>10000	0.07 ± 0.01	n.t.
7	300 ± 22	380 ± 36	210 ± 16	60 ± 6	0.15 ± 0.07	0.8 ± 0.4 [30]
8	>10000	5100 ± 450	4000 ± 250	>10000	>0.3	n.t.
9	1900 ± 160	200 ± 15	290 ± 25	>10000	>0.3	n.t.
10	480 ± 14	20 ± 2	2000 ± 90	30 ± 3	>0.3	9.9 ± 0.8 [30], 7.6 ± 1.3 [10]
11	2000 ± 30	100 ± 15	780 ± 100	<10	n.t.	2.9 ± 0.4 [10]
12	3200 ± 300	50 ± 5	2200 ± 160	50 ± 3	n.t.	3.8 ± 0.4 [10]
13	960 ± 100	30 ± 2	490 ± 35	30 ± 3	>0.3	3.5 ± 0.2 [30]
14	110 ± 10	<10	50 ± 3	<10	0.11 ± 0.02	4.5 ± 0.5 [30], 2.5 ± 0.2 [10]
15	200 ± 20	<10	60 ± 4	<10	0.04 ± 0.01	2.3 ± 0.1 [10]
16	2000 ± 140	10 ± 3	3000 ± 250	20 ± 2	>0.3	12.0 ± 1.1 [30], 9.3 ± 1.6 [10]
17	200 ± 15	10 ± 3	800 ± 55	200 ± 20	>0.3	6.3 ± 0.4 [10]
18	300 ± 27	20 ± 2	400 ± 35	80 ± 10	0.31 ± 0.06	6.7 ± 0.5 [10]
19	500 ± 45	30 ± 3	700 ± 50	60 ± 5	0.14 ± 0.05	1.2 ± 0.2 [10]
20	>10000	100 ± 10	>10000	>10000	n.t.	n.t.
21	500 ± 50	70 ± 5	400 ± 35	80 ± 15	>0.3	1.2 ± 0.4 [10]
22	200 ± 20	10 ± 3	1000 ± 90	50 ± 3	>0.3	7.4 ± 0.7 [10]

[a] V1 rat; liver, V1 human; platelet, V2 rat; adrenal medulla, V2 human; recombinant, CHO cells, $n = 3$, mean ± SEM (standard errors). [b] Rat; $n = 3$, mean ± SEM. [c] Not tested.

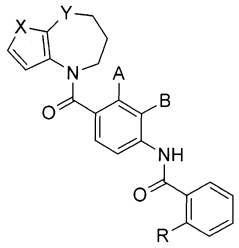
Scheme 4[a]



[a] Reaction conditions: (16) BBr_3 or $AlCl_3$/NaI, (17) $BrCH_2COOEt$ or $Br(CH_2)_3COOEt$/KI, K_2CO_3, (18) KOH−H_2O, (19) $Br(CH_2)_3Cl$ or $Br(CH_2)_4Cl$, K_2CO_3, (20) ClCOOiBu, Et_3N, amine derivatives, (21) amine derivatives, KI.

stitution of compound **e** with several amines and successive reduction of the carbonyl group with $LiAlH_4$ yielded compound **g**, which was converted into compounds **10**−**12**, and **16** by the same procedures as shown in Scheme 1. As for 5,6,7,8-tetrahydro-4*H*-thieno[2,3-*b*][1,4]diazepine derivatives, compounds **17**−**22** were prepared by the procedure reported previously (see Scheme 3 and Figure 2).[8]

Next, introduction of new substituents at position A or B on the benzene ring was performed in order to find more potent thienoazepine derivatives. As described later (Table 1), by addition of a suitable nitrogen-containing chain, good bioavailability, and very potent V1 and V2 antagonistic activity were obtained. Thus, compound **14** was converted into compound **15** using BBr_3 or $AlCl_3$/NaI (see Scheme 4). Treatment of compound **15** with ethyl 4-bromobutyrate in the presence of KI and K_2CO_3, followed by hydrolysis with KOH, gave a carboxylic acid **35**, which was converted to compound **1** by successive treatment with isobutyl chloroformate (IBCF) and 4-dimethylaminopiperidine/triethylamine. Successively, compounds **23**−**27** were obtained using either ethyl bromoacetate or ethyl 4-bromobutyrate and the procedure mentioned above. Compounds **28**−**30**



#	X	Y	R	A	B
1	S	CH_2	Ph	H	$O(CH_2)_3CON$ ⟨⟩ NMe_2
23	S	CH_2	Ph	H	$O(CH_2)_3CON$ ⟨⟩ NMe
24	S	CH_2	Ph	H	$O(CH_2)_3CON$ ⟨⟩ NMe
25	S	CH_2	Ph	H	$O(CH_2)_3CON$ ⟨⟩ NEt_2
26	S	CH_2	Ph	H	$O(CH_2)_3CON$ ⟨⟩ N ⟨⟩
27	S	CH_2	Ph	H	OCH_2CON ⟨⟩ NMe_2
28	S	CH_2	Ph	H	$O(CH_2)_4N(Me)(CH_2)_2NMe_2$
29	S	CH_2	Ph	H	$O(CH_2)_3N$ ⟨⟩ NMe
30	S	CH_2	Ph	H	$O(CH_2)_4N$ ⟨⟩ NMe

Figure 3. A list of thienoazepines with a nitrogen-containing side chain.

were synthesized by alkylation of **15** with ω-haloalkyl bromide and substitution of the resulting alkyl halide with the corresponding amines (see Scheme 4 and Figure 3).

Results and Discussion

Initially, we performed [^{3}H]-AVP binding assays using rat liver membranes (for V1), rat kidney membranes (for V2), human platelet plasma membranes (for V1), and recombinant human CHO cell membranes (for V2) and successively studied the hypotensive effect (V1, iv) in rats with AVP-induced hypertension and the diuretic effect (V2, po) in rats (see Experimental Section). First, we compared three types of heterocyclic azepines, thienoazepine, furoazepine, and pyrroloazepine derivatives **4**−**7**. Among them, thienoazepine **4** was very potent in the human V2 binding assay (IC_{50} = 5 nM) as *N*-methyl pyrroloazepine **7** (IC_{50} = 60 nM). Compounds **4** and **7** were more potent than compound **3**, except in the rat V2 binding assay, but they did not increase 4 h urine output even at 30 mg/kg. Presumably, this was due to the nonpolarity of these compounds. Since furoazepine **5** and pyrroloazepine **6** were very weak in the human V2 binding assay, we selected the thienoazepine skeleton for synthesis of other derivatives. A variety of R group substituents on the benzene ring were studied and it was found that the 2-phenyl group was essential for potent antagonistic activity by comparing this group with the 2-chloro and 2-methyl groups (see Figure 2). Substitution at the 2-position was found to be crucial, because a compound with 4-phenyl substitution on the benzene ring showed weaker activity (IC_{50} = >10 μM) in the V1 and V2 binding assays. Introduction of a methoxy group to the middle benzene ring increased the inhibition of [^{3}H]-AVP binding. Thus, compounds **14** and **15** exhibited potent V2 (rat and human) as well as V1 (human) antagonistic activity. As for the position of methoxy group substitution, it was found that the 2-position (B) was preferable to the 3-position (A) for stronger activity by comparing compounds **14** and **13**. Despite improved potency in these studies, compounds **13**−**15** had an unsatisfactory effect on urine output in vivo, compared to compound **3**. To achieve potent V2 antagonistic activity in vivo, several substituted or unsubstituted dialkylamino groups were introduced at position-8 of the azepine ring. Compounds **10** and **12** were thus obtained, which showed increased oral absorption and strong V2 antagonism. In particular, compound **10** exhibited satisfactory V2 selectivity. Introduction of a substitution at position-8 seemed to be crucial for V2 selectivity. When a methoxy group was added to position-2 on the benzene ring, compound **16** was obtained, which was superior to compound **3** in both potency and V2 selectivity in vitro and in vivo (effect on urine output) (see Table 1).

Next, we studied the AVP antagonistic activity of compounds with a thienodiazepine skeleton. Compounds **17**−**22**, synthesized as in our previous report,[8] showed fairly potent V2 receptor binding, but did not always have good V1 binding. The diuretic effect in vivo depended upon substitution of the alkyl group on a nitrogen atom at position-8; a 2-(pyrrolidin-1-yl)ethyl, a 2-(dimethylamino)ethyl, and a 2-(4-methylpiperazin-1-yl)ethyl group on the nitrogen atom increased the diuretic effect, while a benzyl or a pyridin-3-ylmethyl group reduced it. Thus, compounds **17**, **18**, and **22** were comparable in activity to compound **3**, but were unfortunately chemically unstable.

Based on the structure−activity studies mentioned above, thienoazepines **10** and **16**, which were more potent and more V2-selective than compound **3**, were obtained by introduction of a suitable group at position-8 of the tetrahydrothieno[3,2-*b*]azepine ring. The finding that compounds **14** and **15**, with a methoxy or a hydroxy group on the benzene ring and without any substitution at position-8 of the thienoazepine ring, showed combined V1 and V2 antagonism prompted us to search for more potent combined antagonists. In our overlap studies using the Discover program from Accelrys (see molecular modeling experiments.), suitable overlaps were observed between the thiophene moiety and the phenyl group of tyrosine, between the 2-phenyl group on the biphenyl group and the phenyl group of phenylalanine, between the carbonyl group near the azepine ring and that of cysteine-1, and between the carbonyl group near the biphenyl group and that of phenylalanine (see Figure 4a and 4b). The N-Me nitrogen of the side chain of compound **1** seemed to fit the arginine nitrogen of the AVP molecule. Thus, we supposed that the nitrogen containing side chain on the middle benzene ring might contribute more potent activity. Therefore, we first synthesized compound **1** designed in this way. Addition of a side chain at position-B on the middle benzene ring resulted in good bioavailability and extremely strong specific binding to the AVP receptor (V1; human platelets and V2; recombinant human CHO cells), as well as

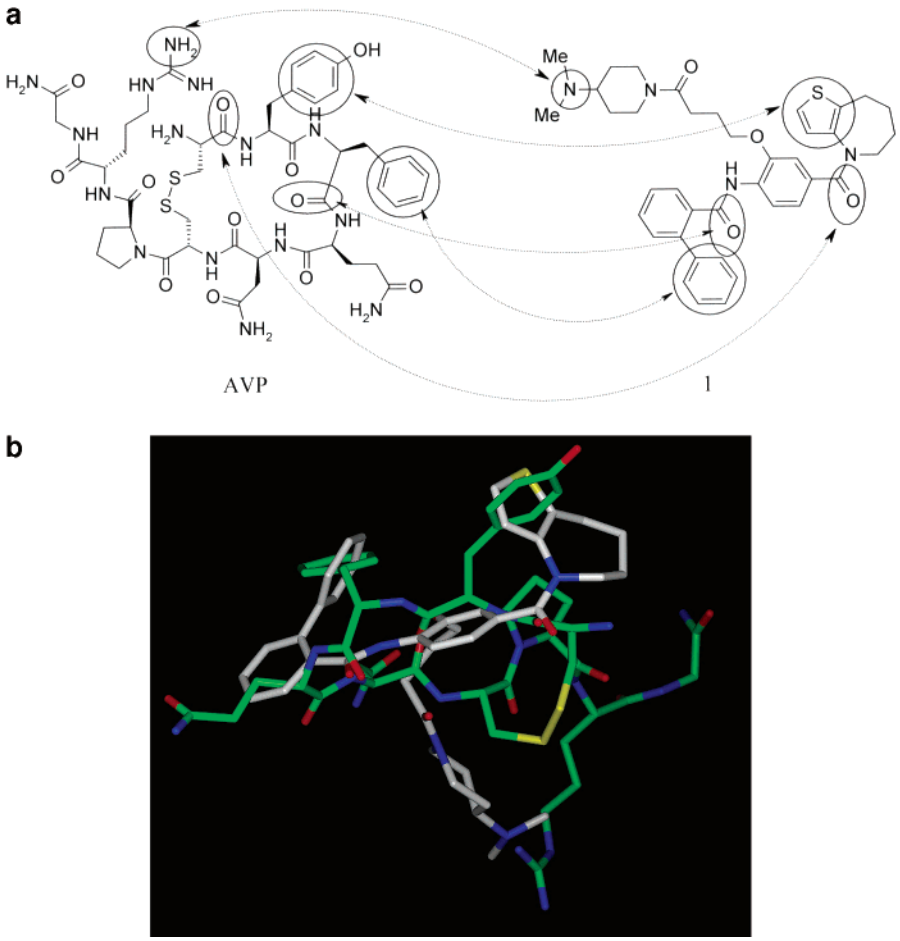


Figure 4. (a) Correlation of functional groups between AVP and **1**. (b) Overlay of **1** (white) and AVP (green).

Table 2. Data of Specific Binding of Thienoazepines **1**, **23**−**30** to AVP Receptor, Antagonistic Effect on AVP-induced Hypertension, and Diuretic Effect

compound no.	[^{3}H]-AVP binding IC_{50} (nM)[a]				blood pressure:[b] ID_{50} (mg/kg, iv)	urine volume:[b] (mL/4 h) [dose mg/kg, po]
	V1 rat	V2 rat	V1 human	V2 human		
1	6.0 ± 0.4	3.0 ± 0.2	3.0 ± 0.3	20 ± 1.0	0.005 ± 0.002	22.0 ± 1.1 [10]
23	20 ± 1.2	3.0 ± 0.3	50 ± 4.0	10 ± 1.0	0.008 ± 0.001	19.1 ± 2.4 [10]
24	20 ± 1.1	1.0 ± 0.1	10 ± 0.9	6.0 ± 0.3	0.03 ± 0.01	17.6 ± 1.2 [10]
25	4.0 ± 0.3	6.0 ± 0.5	4.0 ± 0.3	9.0 ± 0.8	0.01 ± 0.005	18.0 ± 1.0 [10]
26	20 ± 1.6	20 ± 1.8	10 ± 0.1	30 ± 2.8	0.03 ± 0.01	8.0 ± 0.6 [10]
27	20 ± 1.9	4.0 ± 0.3	50 ± 4.5	8.0 ± 0.7	0.02 ± 0.01	15.0 ± 1.8 [10]
28	20 ± 1.5	8.0 ± 0.8	5.0 ± 0.1	80 ± 4.3	0.02 ± 0.01	10.2 ± 1.0 [10]
29	2.0 ± 0.1	<1	<1	2.0 ± 0.1	0.03± 0.01	18.3 ± 2.2 [10]
30	3.0 ± 0.2	7.0 ± 0.5	5.0 ± 0.1	1.0 ± 0.1	<0.01	16.9 ± 1.4 [10]

[a] V1 rat; liver, V1 human; platelet, V2 rat; adrenal medulla, V2 human; recombinant, CHO cells, $n = 3$, mean ± SEM (standard errors). [b] Rat; $n = 3$, mean ± SEM.

potent antagonism of AVP-induced hypertension and a marked diuretic (Table 2). Compound **1** showed antagonism of AVP-induced hypertension and had a potent diuretic effect as well as showing very strong V1 and V2 receptor binding. We then modified the side chain in various ways. The piperidine ring of compound **1** was replaced with an *N*-methylhomopiperazine ring (compound **23**) or a 4-*N*-methylpiperazine ring (compound **24**), and the N-Me_2 group of **1** was replaced with an N-Et_2 group (compound **25**) or a piperidine ring (compound **26**). The carbonyl group of **1** was changed into a methylene group and the piperidine ring of **1** was converted to a 4-*N*-methylpiperazine group to give compound **30**. Compound **28** with a cleaved piperazine ring was also prepared, as well as compound **27** with a shortened methylene chain and compound **29** without the carbonyl group of compound **23**. Although compounds **1**, **23**−**25**, **29**−**30** showed the desired potency in every assay, compound **1** exhibited the most potent diuretic effect and hypotensive effect as well as the strong specific binding to V1 and V2 receptors. In toxicological evaluations in rats, compounds **24** and **30** were excluded because of hepatic toxicity. The mechanism of action of hydrochloride salt **2** of compound **1** proved to be antagonistic on V2 receptor of human platelet. Compound **2** was obtained as a fine crystalline form, while the salts of **23**−**25** and **29**−**30** were not obtained as crystals but as amorphous powders. Compound **2** was found to be the most desirable compound because of its favorable physical properties (crystallization, pharmaceutical stability, etc.), oral absorption, toxicological profile, and ease of production. Therefore,

compound **1** (**2**) was chosen for further pharmacological evaluations.

In conclusion, by detailed studies of the structure−activity relationships of heterocyclic compounds having thienoazepine, pyrroloazepine, furoazepine, and thienodiazepine skeletons, compound **1** (**2**) was found to be a very potent combined V1 and V2 antagonist, while compound **16** was superior to compound **3** (selective V2 antagonist) with respect to potency and V2 selectivity both in vitro and in vivo. After further pharmacological and toxicological evaluation (data will be reported elsewhere) as well as the physical properties (crystallization and stability), the hydrochloride **2** of compound **1** was finally selected for clinical studies as a potent dual V1 and V2 AVP antagonist.

Experimental Section

General Methods. Chemistry. Melting points were determined with a Yanagimoto (Yanako) micro melting point apparatus (HK-10D) and are uncorrected. ^{1}H NMR spectra (δ, ppm) were recorded using a JEOL JNMA 300 (300 MHz) spectrometer with tetramethylsilane as the internal standard, and structure analysis of AVP was carried out using a Bruker AMX-500 (500 MHz) spectrometer. IR spectra (cm^{-1}) were obtained with a Perkin-Elmer FT 1650 infrared spectrometer. Mass spectra (FAB^+) were recorded with a Finnigan TSQ 700 instrument. HPLC was performed with a Shimazu LC-6A instrument, and thin-layer chromatography (TLC) was carried out on Merck silica gel plates (60F-254). Column chromatography was performed with Merck silica gel (70−230 mesh).

8-Hydroxy-4,6,7,8-tetrahydrothieno[3,2-*b*]azepin-5-one (35) (see Scheme 2)**.** To a stirred suspension of 6,7-dihydrothieno[3,2-*b*]azepin-5,8-dione (1.58 g, 8.78 mmol) in MeOH (30 mL) was added $NaBH_4$ (330 mg, 8.68 mmol) at room temperature and stirring was continued for 3 h. The reaction was quenched with water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give compound **35** (996 mg, 61.9%). NMR (DMSO-d_6) δ ppm. 1.95−2. 25 (2H, m), 2.30−2.45 (1H, m), 2.50−2.65 (1H, m), 4.60 (1H, t, J = 4.5 Hz), 6.70 (1H, d, J = 5.4 Hz), 7.39 (1H, d, J = 5.4 Hz), 9.84 (1H, brs). MS (FAB, m/z) = 184 ($M^+ + 1$). Anal. ($C_8H_9NO_2S$) C, H, N.

8-Dimethylamino-4,6,7,8-tetrahydrothieno[3,2-*b*]azepin-5-one (36). To a stirred solution of compound **35** (500 mg, 2.75 mmol) in pyridine (20 mL) was added methanesulfonyl chloride (473 mg, 4.13 mmol) at 0 °C. After 4 h, the solvent was removed in vacuo and the residue was dissolved in DMF (30 mL). To the solution were added K_2CO_3 (3.8 g, 27.5 mmol) and dimethylamine hydrochloride (2.2 g, 27.0 mmol). Stirring was continued for 18 h, and the solvent was removed in vacuo. To the residue was added a saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated to give compound **36** (270 mg, 47.0%). NMR (DMSO-d_6) δ ppm. 1.60−1.85 (1H, m), 1.95−2.15 (1H, m), 2.20 (6H, s), 2.30−2.60 (2H, m), 3.92 (1H, dd, J = 12.0, 6.0 Hz), 6.65 (1H, d, J = 6.0 Hz), 7.29 (1H, d, J = 6.0 Hz), 9.69 (1H, brs). MS (FAB, m/z) = 211 ($M^+ + 1$). Anal. ($C_{10}H_{14}N_2OS$) C, H, N.

8-Dimethylamino-4-(3-methoxy-4-nitrobenzoyl)-5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepine (37). To a stirred suspension of $LiAlH_4$ (184 mg, 4.84 mmol) in anhydrous THF (40 mL) was added compound **36** (510 mg, 2.44 mmol), and the mixture was refluxed for 3 h. After cooling, the reaction was quenched with Et_2O (80 mL), and the excess reagent was carefully inactivated with the minimum amount of water. The organic layer was decanted, dried over $MgSO_4$, and evaporated to give a residue, which was dissolved in $CHCl_3$. To the resulting solution were added Et_3N (1.0 mL) and 3-methoxy-4-nitrobenzoyl chloride [prepared from 3-methoxy-4-nitrobenzoic acid (575 mg, 2.92 mmol) and thionyl chloride (3.0 mL, 41 mmol)[9]]. The mixture was stirred 14 h at room temperature. The mixture was washed with water and brine, dried over $MgSO_4$, and evaporated to give a residue, which was purified by SiO_2 column chromatography (*n*-hexane:EtOAc = 2:1) to afford compound **37** (884 mg, 96.6%). NMR ($CDCl_3$) δ ppm. 1.80−2.05 (4H, m), 2.17−2.40 (1H, m), 2.37 (6H, s), 3.50−3.60 (1H, m), 3.86 (3H, s), 6.17 (1H, d, J = 3.7 Hz), 6.87 (1H, d, J = 3.7 Hz), 6.98 (1H, d, J = 6.8 Hz), 7.13 (1H, brs), 7.65 (1H, d, J = 6.8 Hz). MS (FAB, m/z) = 376 ($M^+ + 1$). Anal. ($C_{18}H_{21}N_3O_4S$) C, H, N.

***N*-[4-[[8-Dimethylamino-5,6,7,8-tetrahydro-4*H*-thieno-[3,2-*b*]azepin-4-yl]carbonyl]-2-methoxyphenyl]-1, 1′-biphenyl-2-carboxamide (16).** To a stirred solution of compound **37** (417 mg, 1.11 mmol) in acetic acid (4.0 mL) were added Fe powder (248 mg, 4.43 mmol) and water (0.06 mL). After stirring at 50 °C for 4 h, the reaction was quenched with $CHCl_3$ (20 mL) and the mixture was filtered through Celite. The filtrate was made basic with an aqueous solution of 1 N NaOH (150 mL) and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give a residue, which was dissolved in $CHCl_3$ (15 mL). To the resulting solution were added 2-phenylbenzoyl chloride and Et_3N (0.46 mL, 3.31 mmol), and the mixture was stirred for 3 h at room temperature. [Preparation[8,9] of 2-phenylbenzoyl chloride: To 2-phenylbenzoic acid (264 mg, 1.33 mmol) in $CHCl_3$ (3 mL) was added thionyl chloride (1.75 mL, 24.0 mmol). The mixture was refluxed for 4 h. The mixture was evaporated under reduced pressure to give the residue, which was dissolved in toluene. Evaporation in vacuo gave 2-phenylbenzoyl chloride. Without purification, the chloride was used in the reaction.] The mixture was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and evaporated to give a residue, which was purified by SiO_2 column chromatography (*n*-hexane:AcOEt = 1:1) to afford compound **16** (374 mg, 64.2%), mp 168.2−168.8 °C (from Et_2O). IR (KBr) 1665, 1635 cm^{-1}, NMR ($CDCl_3$) δ ppm. 1.70−2.03 (4H, m), 2.14−2.30 (1H, m), 2.37 (6H, s), 3.44 (3H, s), 3.64 (1H, s), 4.05 (1H, m), 6.15 (1H, brs), 6.78 (1H, d, J = 5.0 Hz), 6.82 (1H, s), 6.90 (1H, d, J = 8.3 Hz), 7.29−7.53 (8H, m), 7.69 (1H, s), 7.81 (1H, d, J = 7.6 Hz), 8.26 (1H, d, J = 8.3 Hz). MS (FAB, m/z) = 526 ($M^+ + 1$). Anal. ($C_{31}H_{31}N_3O_3S$) C, H, N.

Ethyl 4-[2-[[[1,1′-Biphenyl]-2-carbonyl]amino]-5-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenoxy]-butyrate (31). To a stirred solution of compound **15** (1.0 g, 2.14 mmol) in acetone (85 mL) were added ethyl 4-bromobutyrate (1.2 g, 6.15 mmol), anhydrous K_2CO_3 (0.87 g, 6.28 mmol), and KI (71 mg, 0.43 mmol). The mixture was refluxed for 12 h, diluted with water, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give a residue, which was purified by SiO_2 column chromatography (AcOEt:*n*-hexane = 1:3) to give compound **31** (921 mg, 73.8%). NMR ($CDCl_3$) δ ppm. 1.24 (3H, t, J = 7.2 Hz), 1.70−2.05 (6H, m), 2.27 (2H, t, J = 7.2 Hz), 2.90−3.00 (2H, m), 3.60−4.00 (4H, m), 4.08 (2H, q, J = 7.2 Hz), 6.20 (1H, brs), 6.68 (1H, d, J = 5.4 Hz), 6.75−6.80 (1H, m), 6.82 (1H, d, J = 9.0 Hz), 7.25−7.60 (8H, m), 7.65 (1H, s), 7.75−7.80 (1H, m), 8.30 (1H, d, J = 8.0 Hz). MS (FAB, m/z) = 583 ($M^+ + 1$). Anal. ($C_{34}H_{34}N_2O_5S$) C, H, N.

4-[2-[[[1,1′-Biphenyl]-2-carbonyl]amino]-5-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenoxy]-butyric Acid (32). To a stirred solution of compound **31** (820 mg, 1.41 mmol) in MeOH (100 mL) was added KOH (140 mg, 2.5 mmol). The reaction mixture was refluxed for 2 h, and the solvent was removed under reduced pressure to give a residue, which was dissolved in $CHCl_3$ and an aqueous solution of 1 N HCl (10 mL). The organic layer was separated and washed with brine, dried over anhydrous Na_2SO_4, and evaporated to yield the solid (**32**) (614 mg, 78.6%). IR(KBr): 1718, 1643, 1528 cm^{-1}, NMR (DMSO-d_6) δ ppm. 1.50−2.00 (6H, m), 2.25−2.40 (2H, m), 2.75−3.00 (2H, m), 3.50−4.00 (2H, m), 6.30 (1H, brs), 6.70−7.00 (3H, m), 7.25−7.70 (9H, m), 7.70−7.85 (1H, m), 9.03 (1H, s), 12.2 (1H, s). MS (FAB, m/z) = 555 ($M^+ + 1$). Anal. ($C_{32}H_{30}N_2O_5S$) C, H, N.

***N*-[2-[4-(4-Dimethylaminopiperidino)-4-oxobutoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (1).** To a stirred

solution of compound **32** (100 mg, 0.18 mmol) in DMF (1.5 mL) was added Et_3N (96 μL, 0.69 mmol) under argon gas. Then isobutyl chloroformate (29 μL, 0.22 mmol) was added at 0 °C. After stirring for 30 min, a suspension of 4-(dimethylamino)-piperidine dihydrochloride (46 mg, 0.23 mmol) and Et_3N (64 μL, 0.46 mmol) in DMF (1.5 mL) was added. Stirring was continued at 0 °C for 2 h and then at room temperature for 1 h. DMF was removed in vacuo (50 °C, 4−6 mmHg) to give a residue, which was dissolved in AcOEt, and the resulting mixture was washed with saturated aqueous solution of $NaHCO_3$. The organic layer was separated, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to give the solid (**1**) (120 mg). IR (KBr): 1640 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.20−1.50 (2H, m), 1.50−2.10 (11H, m), 2.20−2.40 (8H, m), 2.45−2.60 (1H, m), 2.80−3.00 (2H, m), 3.60−4.20 (4H, m), 4.50 (1H, d, J = 12.0 Hz), 6.20 (1H, brs), 6.67 (1H, d, J = 5.0 Hz), 6.70−6.85 (2H, m), 7.25−7.60 (8H, m), 7.70−7.80 (2H, m), 8.26 (1H, d, J = 8.0 Hz). MS (FAB, m/z) = 665 ($M^+ + 1$). Anal. ($C_{39}H_{44}N_4O_4S$) C, H, N.

***N*-[2-[4-(4-Dimethylaminopiperidino)-4-oxobutoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide Hydrochloride (2) (JTV-605).** To a stirred solution of compound **1** (120 mg, 0.18 mmol) in AcOEt (1 mL) was added dropwise 4 N HCl in AcOEt (0.25 mL). After 30 min, the precipitate was collected, and recrystallization from acetone (2 mL) yielded colorless crystals, which were dried at 40 °C and 0.3−0.5 mmHg for 4 h (**2**) (JTV-605) (102 mg, 82%). mp 211.5−213.5 °C. IR (KBr) 1635, 1525 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.39−1.59 (2H, m), 1.67−1.87 (4H, brs), 1.90−2.05 (5H, m), 2.25−2.30 (3H, m), 2.46 (1H, t, J = 12.3 Hz), 2.63 (3H, s), 2.73 (3H, s), 2.89−3.00 (3H, m), 3.24 (1H, t, J = 12.2 Hz), 3.85 (2H, d, J = 13.2 Hz), 3.87 (2H, brs), 4.67 (2H, d, J = 13.2 Hz), 6.18 (1H, brs), 6.72 (1H, d, J = 5.7 Hz), 7.24−7.35 (4H, m), 7.42−7.58 (4H, m), 7.74−7.78 (2H, m), 8.24 (1H, d, J = 7.8 Hz), 12.70 (1H, brs). MS (FAB, m/z) = 665 ($M^+ + 1$). Anal. ($C_{39}H_{45}ClN_4O_4S$) C, H, N.

***N*-[2-[4-(4-Methylperhydro-1,4-diazepin-1-yl)-4-oxobutoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)-carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (23).** Compound **23** (171 mg, 81%) was prepared using compound **32** (180 mg, 0.33 mmol) and 1-methylhomopiperazine (48 μL, 0.39 mmol) as described for compound **1** (see Figure 3). IR (KBr) 1638 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.70−2.00 (8H, m), 2.20−2.40 (5H, m), 2.40−2.60 (4H, m), 2.80−3.00 (2H, m), 3.30−3.50 (2H, m), 3.50−4.10 (6H, m), 6.20 (1H, brs), 6.60−6.70 (1H, m), 6.70−6.90 (2H, m), 7.20−7.60 (8H, m), 7.70−7.80 (2H, m), 8.27 (1H, d, J = 9.0 Hz). MS (FAB, m/z) = 651 ($M^+ + 1$). Anal. ($C_{38}H_{42}N_4O_4S$) C, H, N.

***N*-[2-[4-(4-Methylpiperazin-1-yl)-4-oxobutoxy]-4-[5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl]carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (24).** Compound **24** (131 mg, 63.4%) was prepared using compound **32** (180 mg, 0.33 mmol) and 1-methylpiperazine (43 μL, 0.39 mmol) as described for compound **1**. mp 188−190°C (from 2-propanol). IR (KBr) 1641 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.70−1.85 (2H, m), 1.85−2.05 (4H, m), 2.20−2.40 (9H, m), 2.85−3.00 (2H, m), 3.35 (2H, t, J = 5.0 Hz), 3.55 (2H, t, J = 5.0 Hz), 3.69 (2H, t, J = 6.0 Hz), 3.70−4.10 (2H, m), 6.20 (1H, brs), 6.70−6.90 (4H, m), 7.25−7.40 (3H, m), 7.40−7.60 (5H, m), 7.70−7.80 (2H, m), 8.26 (1H, d, J = 8.0 Hz). MS (FAB, m/z) = 637 ($M^+ + 1$). Anal. ($C_{37}H_{40}N_4O_4S$) C, H, N

***N*-[2-[4-(4-Diethylaminopiperidino)-4-oxobutoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (25).** Compound **25** (741 mg, 63.0%) was prepared using compound **32** (941 mg, 1.70 mmol) and 4-diethylaminopiperidine dihydrochloride (428 mg, 1.87 mmol) as described for compound **1**. IR (KBr) 1640, 1522 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.00 (6H, t, J = 7.2 Hz), 1.20−1.40 (2H, m), 1.65−2.00 (8H, m), 2.21 (2H, t, J = 7.1 Hz), 2.35−2.60 (5H, m), 2.60−2.90 (4H, m), 3.55−3.95 (5H, m), 4.48 (1H, d, J = 12.6 Hz), 6.13 (1H, brs), 6.60 (1H, J = 4.8 Hz), 6.65−6.80 (2H, m), 7.20−7.50 (8H, m), 7.65−7.70 (2H, m), 8.19 (1H, d, J = 8.1 Hz). MS (FAB, m/z) = 693 ($M^+ + 1$). Anal. ($C_{41}H_{48}N_4O_4S$) C, H, N.

***N*-[2-[4-(4-Piperidinopiperidino)-4-oxobutoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (26).** Compound **26** (612 mg, 86.9%) was prepared using compound **32** (554 mg, 1.00 mmol) and 4-piperidinopiperidine (336 mg, 2.00 mmol) as described for compound **1**. IR (KBr) 1638, 1522 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.25−1.60 (7H, m), 1.77 (4H, m), 1.91 (2H, t, J = 5.9 Hz), 1.95−2.05 (2H, m), 2.27 (2H, t, J = 6.2 Hz), 2.40−2.60 (6H, m), 2.80−2.85 (3H, m), 3.60−3.80 (6H, m), 4.53 (1H, d, J = 10.8 Hz), 6.19 (1H, brs), 6.67 (1H, d, J = 4.6 Hz), 6.78 (1H, brs), 6.80 (1H, brs), 7.26−7.60 (7H, m), 7.75−7.77 (2H, m), 8.26 (1H, d, J = 7.0 Hz). MS (FAB, m/z) 705 ($M^+ + 1$). Anal. ($C_{42}H_{48}N_4O_4S$) C, H, N.

***N*-[2-[2-(4-Dimethylaminopiperidino)-2-oxoethoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin 4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (27).** To a stirred solution of compound **15** (374 mg, 0.80 mmol) in acetone (10 mL) and DMF (10 mL) were added ethyl bromoacetate (200 mg, 1.20 mmol), K_2CO_3 (112 mg, 0.81 mmol), and KI (120 mg, 0.73 mmol). The mixture was refluxed for 5 h, diluted with water, and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated to leave the residue (480 mg). Without purification, the residue was dissolved in a solution of KOH (121 mg, 2.1 mmol) in MeOH. The mixture was refluxed for 2 h, diluted with water, and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated to leave the residue (300 mg), which was dissolved in $CHCl_3$ (8 mL). To the stirred solution were added 4-dimethylaminopiperidine (150 mg, 1.17 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) (216 mg, 0.85 mmol), and Et_3N (0.24 mL, 1.72 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with $CHCl_3$, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and evaporated to leave the residue, which was purified by SiO_2 column chromatography ($CHCl_3$:MeOH = 19:1) to provide compound **27** (280 mg, 55.0% from compound **15**).

IR (KBr) 1654 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.40−1.50 (4H, m), 1.72−1.82 (2H, m), 1.90−2.00 (2H, m), 2.49 (6H, s), 2.50−2.60 (2H, m), 2.90−3.00 (4H, m), 3.70−3.90 (2H, m), 4.20−4.30 (2H, m), 4.54 (1H, m), 6.19 (1H, brs), 6.66 (1H, brs), 6.80 (1H, brs), 6.90 (1H, m), 7.25−7.60 (7H, m), 7.76−7.90 (2H, m), 8.20 (1H, m), 8.41 (1H, m). MS (FAB, m/z) = 637 ($M^+ + 1$). Anal. ($C_{37}H_{40}N_4O_4S$) C, H, N.

***N*-[2-(3-Chloropropoxy)-4-[(5,6,7,8-tetrahydro-4*H*-thieno-[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (33).** To a stirred solution of compound **15** (600 mg, 1.28 mmol) in acetone were added K_2CO_3 (531 mg, 3.85 mmol), KI (43 mg, 0.26 mmol), and 1-bromo-3-chloropropane (0.19 mL, 1.92 mmol). The mixture was stirred with refluxing for 15 h, concentrated under reduced pressure, and dissolved in AcOEt. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated to give a residue, which was purified by SiO_2 column chromatography (AcOEt:*n*-hexane = 1:3) to yield compound **33** (581 mg, 83.4%) as colorless crystals, mp 178.6−179.2 °C (from Et_2O). IR (KBr) 1621, 1523 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.69−1.81 (2H, m), 1.90−2.02 (4H, m), 2.87−2.91 (2H, m), 3.45 (2H, t, J = 6.8 Hz), 3.73−3.77 (2H, m), 3.92 (2H, brs), 6.18 (1H, brs), 6.65 (1H, d, J = 5.1 Hz), 6.75 (1H, s), 6.83 (1H, d, J = 8.4 Hz), 7.27−7.57 (9H, m), 7.75 (1H, d, J = 7.5 Hz), 8.30 (1H, d, J = 8.4 Hz). MS (FAB, m/z) = 545 ($M^+ + 1$). Anal. ($C_{31}H_{29}ClN_2O_3S$) C, H, N.

***N*-[2-(4-Chlorobutoxy)-4-[(5,6,7,8-tetrahydro-4*H*-thieno-[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (34).** Compound **34** (320 mg, 89.4%) was synthesized using compound **15** (300 mg, 0.64 mmol) and 1-bromo-4-chlorobutane (0.11 mL, 0.96 mmol) as described for compound **33**. IR (KBr) 1671, 1637 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.65−1.85 (6H, m), 1.90−2.10 (2H, m), 2.85−2.95 (2H, m), 3.45−4.15 (6H, m), 6.10−6.25 (1H, m), 6.60−6.85 (3H, m), 7.25−7.80 (10H, m), 8.25−8.40 (1H, m). MS (FAB, m/z) = 559 ($M^+ + 1$). Anal. ($C_{32}H_{31}ClN_2O_3S$) C, H, N.

***N*-[2-[3-(4-Methylperhydro-1,4-diazepin-1-yl)propoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (29).** To a solution of compound **33** (280 mg, 0.51 mmol) in CH_3CN (1.5 mL) and DMF (1.5 mL) were added KI (8.5 mg, 0.051 mmol) and 1-methylhomopiperazine (95.7 μL, 0.77 mmol). The mixture was refluxed for 4 h and concentrated under reduced pressure to give a residue, which was dissolved in AcOEt. Then the mixture was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and evaporated to give a residue, which was purified by SiO_2 column chromatography ($CHCl_3$:MeOH = 40:1 then 10:1) to afford compound **29** (244 mg, 76.3%). IR (neat) 1639, 1522 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.69−2.02 (8H, m), 2.37−2.50 (2H, m), 2.42 (3H, s), 2.65−2.74 (8H, m), 2.89−2.93 (2H, m), 3.65−3.71 (2H, m), 3.82 (2H, brs), 6.20 (1H, brs), 6.67 (1H, d, J = 5.1 Hz), 6.75−6.82 (2H, m), 7.30−7.58 (8H, m), 7.66 (1H, s), 7.75 (1H, d, J = 7.8 Hz), 8.26 (1H, d, J = 7.5 Hz). MS (FAB, m/z) = 623 (M^+ + 1). Anal. ($C_{37}H_{42}N_4O_3S$) C, H, N.

***N*-[2-[4-[*N*′-(2-Dimethylaminoethyl)-*N*′-methylamino]-butoxy]-4-[5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl]carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (28).** Compound **28** (103 mg, 30.7%) was obtained from compound **34** (300 mg, 0.54 mmol) and *N*, *N*, *N*′-trimethylethylenediamine (110 μL, 0.87 mmol) as described for compound **29**. IR ($CHCl_3$) 1672, 1640 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.35−1.65 (4H, m), 1.80−2.10 (4H, m), 2.15−2.55 (15H, m), 2.80−2.95 (2H, m), 3.50−4.10 (4H, m), 6.10−6.30 (1H, m), 6.60−6.90 (3H, m), 7.20−7.60 (8H, m), 7.67 (1H, brs), 7.70−7.80 (1H, m), 8.20−8.35 (1H, m). MS (FAB, m/z) = 625 (M^+ + 1). Anal. ($C_{37}H_{44}N_4O_3S$) C, H, N.

***N*-[2-[4-(4-Methylpiperazin-1-yl)butoxy]-4-[(5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepin-4-yl)carbonyl]phenyl]-(1,1′-biphenyl)-2-carboxamide (30).** Compound **30** (150 mg, 44.9%) was obtained as crystals from compound **34** (300 mg, 0.54 mmol) and 1-methylpiperazine (80 μL, 0.72 mmol) as described for compound **29**. mp 134.7−135.4°C (from AcOEt). IR ($CHCl_3$) 1672, 1639 cm^{-1}. NMR ($CDCl_3$) δ ppm. 1.40−2.05 (10H, m), 2.20−2.65 (11H, m), 2.85−3.00(2H, m), 3.50−4.10 (4H, m) 6.10−6.30 (1H, m), 6.60−6.90 (3H, m), 7.20−7.60 (8H, m), 7.65 (1H, brs), 7.70−7.80 (1H, m), 8.20−8.35 (1H, m). MS (FAB, m/z) = 623 (M^+ + 1). Anal. ($C_{37}H_{42}N_4O_3S$) C, H, N.

Structural Analysis of AVP. Structural analysis of AVP in solution was performed following the methodology by Wuthrich and co-workers.[12] All NMR (500 MHz) experiments were performed with a 5 mM sample of AVP dissolved in DMSO-d_6. ^{1}H NMR signals of amino acids were assigned at 30 °C using 2D DQF−COSY,[13] TOCSY,[14] and NOESY[15] experiments. Interproton distances were derived from estimates of NOE cross-peak volumes in NOESY experiments acquired with mixing-times ranging from 50 to 300 ms. Coupling constants were measured from a E-COSY experiment.[16] Amide protection from DMSO-d_6 was deduced from chemical shift temperature coefficients measured using NOESY experiments. The set of restraints derived from these data included 73 interproton distances and one hydrogen bond between Asn^5 NH and Tyr^2 CO.

Molecular Modeling Experiments. The overlaid structures of AVP and **1** were created by Discover program from Accelrys using CVFF force fields. Molecular dynamics and minimization were carried out with consensus restrains between the corresponding groups of AVP and **1** shown by Figure 4a, taking no account of intermolecular interactions between two molecules. The distances between centroids were forced to zero for overlapping the aromatic rings. The distances of the corresponding atoms of the carbonyl groups or the amino groups were forced to zero for the studies. Hydrogen atom protonated to the amino group was also used for the studies as well as the nitrogen atom of the amino group. The final minimization was continued until the maximum derivative was less than 0.01 kcal/Å with 1.0 kcal/mol/Å^2 as force constants for each overlapping. During this calculation, NOE and hydrogen bond restraints of AVP by proton NMR study mentioned above were also used. Graphical displays were given from the Insight II molecular modeling system from Accelrys (see Figure 4b).

Pharmacological Experiments. Materials. The radioligand [^{3}H]AVP with a specific activity of 80 Ci/mmol was obtained from DuPont-New England Nuclear (Boston, MA), and AVP was obtained from Peptide Institute Inc. (Suita, Japan). Synthesized compounds were initially dissolved in dimethyl sulfoxide (DMSO) at 10^{-2} M and then diluted to the desired concentration with the assay buffer. The final concentration of DMSO in the buffer did not exceed 1% at which level [^{3}H]AVP binding was not affected. BSA was purchased from Sigma Chemical Co. (St. Louis, MO). Kits for the protein assay were purchased from Bio-Rad Laboratories (Richmond, CA).

Animals. Male Sprague Dawley (S.D.) rats weighing 250−350 g were used. All animals were maintained under a 12 h light/dark cycle with food and water available ad libitum.

Radioligand Binding Studies. Rats were sacrificed by decapitation, and their liver and kidneys were quickly removed. All subsequent steps were carried out at 4 °C. The hepatic plasma membrane fraction was prepared by a modification of the method of Nakamura et al.[17] The renal medullary plasma membrane fraction was prepared by the method of Cambell et al.[18] Human platelet plasma membranes were obtained from healthy volunteers by the method of Vittet et al.[19] Human V2 receptor plasma membranes were prepared from human V2 receptor-expressing CHO cells by a modification of the method of Morel et al.[20] Protein concentrations were determined with a DC protein assay kit (Bio-Rad Laboratories) using BSA as the standard. For competition studies, the radioligand (1−2 nM) was added to each membrane preparation, and the mixture was incubated with various concentrations of test compounds in 250 μL of assay buffer containing 100 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, and 0.1% BSA. Binding was initiated by addition of the plasma membrane preparations, and incubation was done for 20 min at 37 °C to allow equilibrium to be established. After incubation, the reaction was terminated by addition of 2.5 mL of ice-cold Tris buffer (100 mM Tris-HCl, pH 8.0), followed immediately by rapid filtration through a GF/B UniFilter. After rinsing three times, the radioactivity retained on the filters was counted with a liquid-scintillation counter (Packard Instrument Company). Nonspecific binding was determined with 1 μM unlabeled AVP, and specific binding was calculated as the total binding minus nonspecific binding. The concentration of each test compound that caused 50% inhibition (IC_{50}) of specific binding of [^{3}H]AVP was determined by regression analysis of displacement curves.

Assessment of the Effect on Blood Pressure Male Sprague Dawley rats weighing 250−350 g were anesthetized with urethane (1.3 g/kg ip). Catheters were inserted into the femoral artery and vein for recording of arterial blood pressure and intravenous drug administration, respectively. The animals were kept at 37 °C using a thermostat-controlled heating board. After stabilization of the blood pressure, each test compound or vehicle was given (1 mL/kg. iv) at 2 min before injection of AVP (30 mU/kg. iv). The dose of a test compound causing 50% inhibition of the maximal pressor response to AVP (ID_{50}) was calculated.

Assessment of the Effect on Urine Output. Rats were fasted for 16−20 h before the experiment, and some animals were also deprived of drinking water for 16 to 20 h to stimulate endogenous AVP secretion. Test compounds or vehicle were administered orally and spontaneously voided urine was collected for 4 h using a metabolic cage.

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