Synthesis and Pharmacological Evaluation of 5-(4-Biphenyl)-3-methyl-4-phenyl-1,2,4-triazole Derivatives as a Novel Class of Selective Antagonists for the Human Vasopressin V_{1A} Receptor

Akio Kakefuda,* Takeshi Suzuki, Takahiko Tobe, Junko Tsukada,† Atsuo Tahara, Shuichi Sakamoto, and Shin-ichi Tsukamoto

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

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A series of 5-(4-biphenyl)-3-methyl-4-phenyl-1,2,4-triazole derivatives were prepared and evaluated as selective antagonists for the human vasopressin V_{1A} receptor. The compounds were examined for their affinity to the cloned human V_{1A} receptor (hV_{1A}) and selectivity vs the cloned human V_2 receptor (hV_2). By utilizing the structure−activity relationship on 4,4-difluoro-5-methylidene-2,3,4,5-tetrahydrobenzazepine derivatives as dual antagonists for the V_{1A} and V_2 receptors in our previous study, we found that substituting the methoxy group at the 2-position of the 4-phenyl ring with (4-methylpiperazin-1-yl)alkoxy moieties brought about marked improvement of both affinity to hV_{1A} and selectivity vs hV_2. Further introduction of a methyl group into the 6-position of the 4-phenyl ring resulted in additional improvement of selectivity. One particular compound, 5-(4-biphenyl)-3-methyl-4-{2-[6-(4-methyl-1-piperazinyl)-hexyloxy]phenyl}-1,2,4-triazole (**19**) showed potent affinity to hV_{1A} with a K_i value of 1.04 nM and high selectivity with a 1700-fold selectivity vs hV_2. We also found marked differences in the affinity of compounds in this series between the human and the rat receptors. Compound **19** was further examined for its V_{1A} receptor antagonist activity in rats. As a result, **19** demonstrated antagonist activities toward an arginine vasopressin-induced increase in diastolic blood pressure after intravenous or oral administration and long-lasting oral activity.

Introduction

Arginine vasopressin (AVP) is a peptide hormone secreted by the posterior pituitary and has been known to play important physiological roles in vasoconstriction and antidiuresis. AVP exerts its effects through binding to specific receptors coupled to distinct second messengers. To date, two peripheral AVP receptor subtypes, the V_{1A} and V_2 receptors, have been identified.[1,2] Through the V_{1A} receptors, AVP activates phospholipase A_2, C, and D,[3] which results in the mobilization of intracellular calcium and protein phosphorylation.[4] The V_{1A} receptors are present in vascular smooth muscle cells, hepatocytes, platelets, and mesangial cells. These receptors serve to mediate the contraction, proliferation, and hypertrophy of cells.[5−9] In contrast, the V_2 receptors stimulate adenylate cyclase resulting in the production of cyclic adenosine 3′,5′-phosphate (AMP) and are present in renal epithelial cell lines (LLC-PK_1) where they control free water and urea reabsorption.[10−11]

Abnormal secretion of AVP is thought to be involved in the pathology of several diseases and disorders including heart failure, hypertension, renal diseases, and hypernatremia.[12] Consequently, antagonists for the AVP receptors are considered to be beneficial in treating the above diseases.[13]

Investigations into the development of nonpeptide antagonists for the AVP receptors afforded (*Z*)-4′-({4,4-difluoro-5-[(4-dimethylamino-1-piperidyl)carbonylmethylene]-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl}carbonyl)-2-phenylbenzanilide (**3**), which is a dual antagonist for the V_{1A} and V_2 receptors.[14] A subsequent research program was aimed at the development of subtype selective antagonists for the AVP receptor, which appears essential in elucidating the pathophysiological roles of AVP.

So far, two nonpeptide selective antagonists for the V_{1A} receptor, OPC-21268 (**1**)[15,16] and SR49059 (**2**),[17] have been reported (Chart 1). Clinical studies for **2** are underway, and its therapeutic effects have been found.[18,19] These facts prompted us to investigate a novel class of selective antagonists for the V_{1A} receptor.

It has been reported that there are marked differences in the AVP receptor specificity between different species.[17,20−21] For instance, **1** showed poor affinity to the cloned human V_{1A} receptor even at 10 μM,[22] despite its high affinity to the rat V_{1A} receptor (K_i = 23.5 nM).[23] Therefore, we employed Chinese hamster ovary (CHO) cells, which stably express the cloned human V_{1A} or V_2 receptor subtypes (hV_{1A} and hV_2) for a receptor binding assay.[23] High-throughput screening (HTS) of the Yamanouchi chemical library based on a hV_{1A} binding assay led to the identification of 5-(4-biphenyl)-4-(2-methoxyphenyl)-3-methyl-1,2,4-triazole (**4**). Compound **4** was found to possess a novel structure and show moderate hV_{1A} affinity and selectivity vs hV_2 (K_i = 120 nM, hV_2/hV_{1A} = 21). Blockade of the V_2 receptor is known to cause an increase in urine volume,[23] which may complicate the pharmacological evaluation of compounds. Therefore, the aim of our research was focused

* To whom correspondence should be addressed. Tel: +81-298-54-1545. Fax: +81-298-52-5387. E-mail: kakefuda@yamanouchi.co.jp.
† Clinical Development Department.

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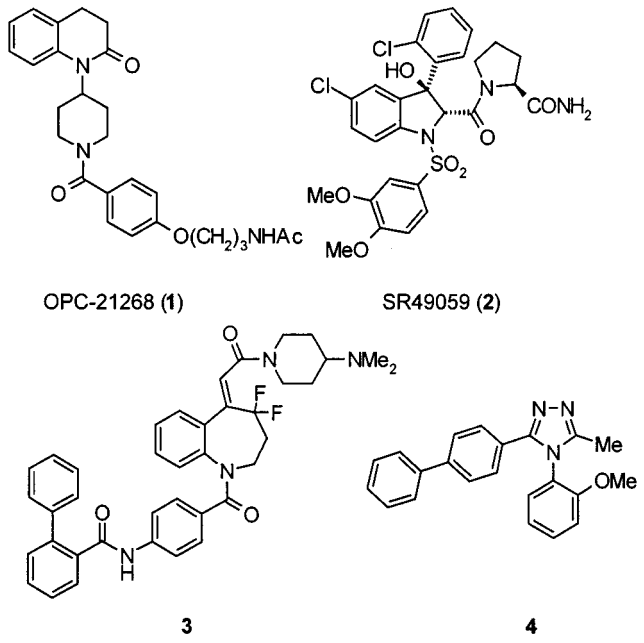
Chart 1



on not only enhancement of hV_{1A} affinity but also improvement of selectivity vs hV_2. Our previous study on the structure−activity relationships (SAR) of **3** and its derivatives revealed that the terminal basic moiety of **3** was important in exerting potent binding affinity, especially for the V_{1A} receptor.[14] Thus, on the basis of our hypothetical superimposition of **4** and the 1-benzoyl-2,3,4,5-tetrahydrobenzazepine part of **3** as shown in Figure 1, we examined the effect of the basic moiety on hV_{1A}, linked with the 4-phenyl ring of **4** using an appropriate tether. Subsequently, we modified the terminal basic part and the tether. Finally, we introduced a methyl moiety into the 6-position of the 4-phenyl ring to investigate its steric effect. Representative compounds were studied for their binding affinity to the rat V_{1A} and V_2 receptors (rV_{1A} and rV_2) in order to determine the species differences in the affinity of compounds. Furthermore, 5-(4-Biphenyl)-3-methyl-4-{2-[6-(4-methyl-1-piperazinyl)hexyloxy]phenyl}-1,2,4-triazole (**19**), which showed high affinity and selectivity to hV_{1A}, was examined for its V_{1A} antagonist activity in rats.

Chemistry

The synthesis of the novel 5-(4-biphenyl)-3-methyl-4-phenyl-1,2,4-triazole derivatives is summarized in Schemes 1 and 2. Compounds **16**−**32** were prepared from aminophenols as shown in Scheme 1. The aminophenols were converted to the thioimidates (**5**−**8**) in four steps, and then, compounds **5**−**8** were subjected to a cyclization reaction with an acyl hydrazide to give the 1,2,4-triazole derivatives (**9**−**12**). Compounds **9**−**12** were finally transformed to the target compounds **16**−**27** and **30** by bromoalkylation and substitution with amines. The hydrochloride salts of versatile intermediates (**13**−**15**) were prepared by condensation of acyl chlorides with diamines, which were then subjected to a reaction with **9** or **12** to afford **28**, **29**, **31**, and **32**.

The synthesis of 4-piperidyl derivatives (**37**−**39**) is illustrated in Scheme 2. The ethylene and butylene derivatives (**37** and **38**) were prepared from the commercially available alcohols (**33** and **34**) by successive protection, tosylation, substitution, and deprotection. The propylene derivative (**39**) was synthesized from **9** by condensation with alcohol and hydrogenation of the pyridine ring.

Pharmacology

Binding Assay. Radioligand binding assays for hV_{1A} and hV_2 were performed according to the reported protocols[22] using [^{3}H]AVP on CHO cells stably expressing hV_{1A} or hV_2, respectively (Table 1). Binding assays for rV_{1A} and rV_2 were also performed using [^{3}H]AVP on rat liver or [^{3}H]AVP on rat kidney, respectively (Table 2).[23]

In Vivo Assay. In vivo activities of **19** were examined by measuring the inhibition of the AVP-induced increase

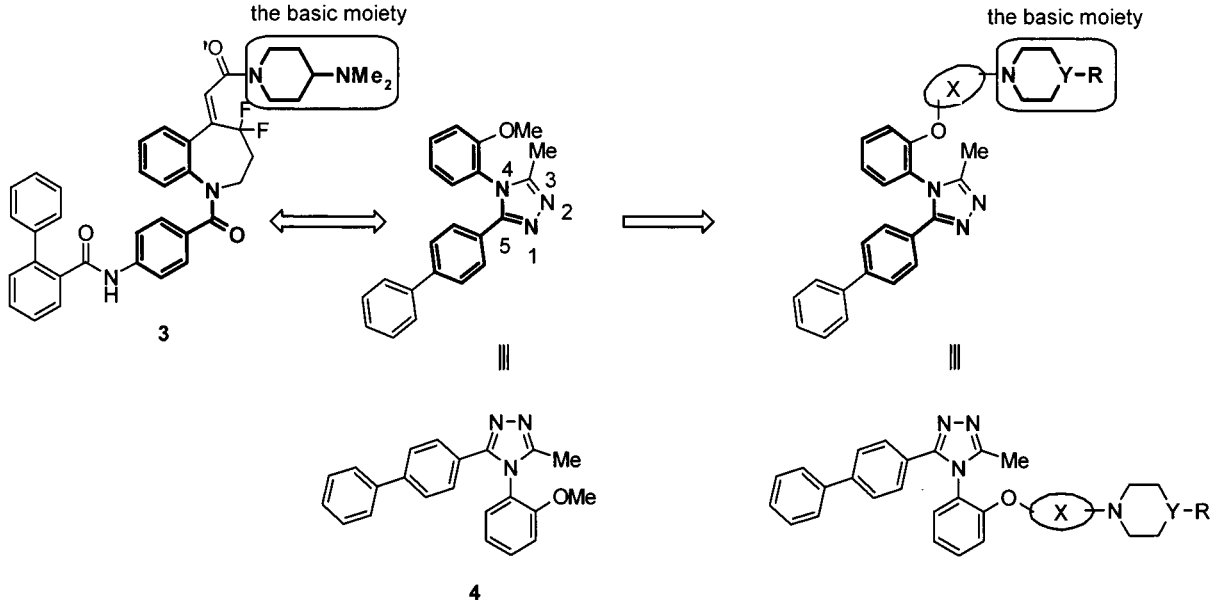
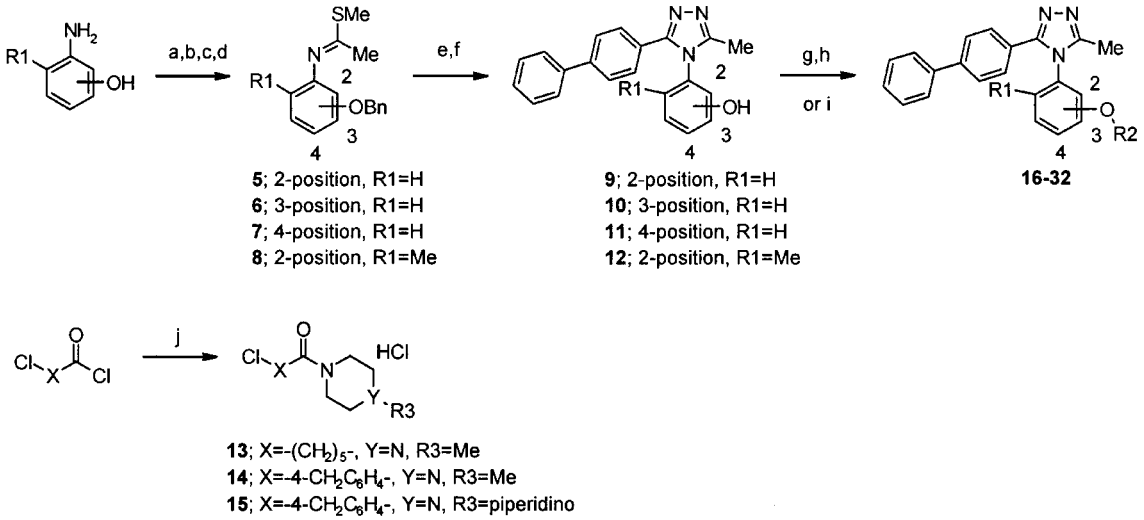


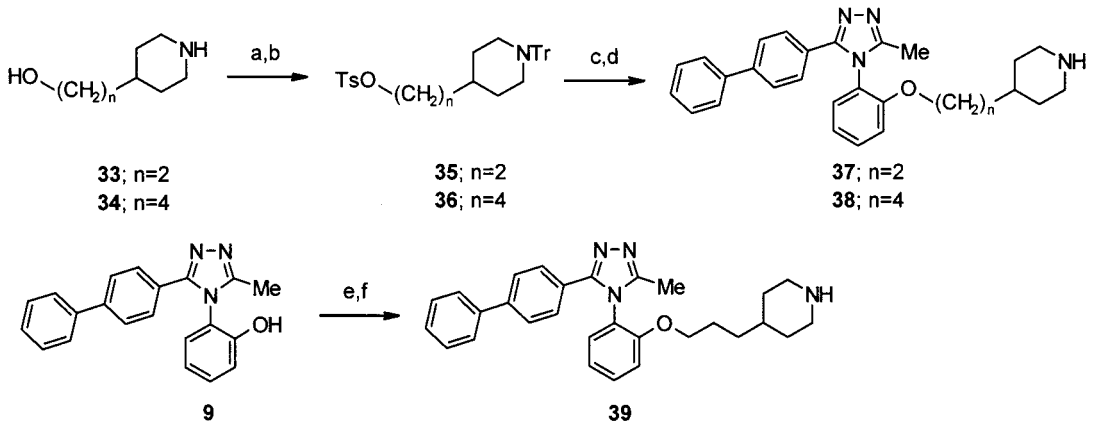
Figure 1. Hypothetical superimposition of **3** and **4**.

Scheme 1. Synthesis of 5-(4-Biphenyl)-3-methyl-4-phenyl-1,2,4-triazole Derivatives[a]



[a] Reagents and conditions: (a) Ac_2O, AcOEt; (b) BnBr, K_2CO_3, CH_3CN, reflux; (c) P_2S_5, toluene, 70 °C; (d) MeI, K_2CO_3, CH_3CN, 50 °C; (e) $ArCONHNH_2$, DMF, 120 °C; (f) H_2, Pd/C, DMF; (g) $Br(CH_2)_nBr$, K_2CO_3, CH_3CN, reflux; (h) amine, K_2CO_3, CH_3CN, reflux; (i) **13**−**15**, K_2CO_3, CH_3CN, reflux; (j) amine, CH_3CN.

Scheme 2. Synthesis of 4-Piperidyl Derivatives[a]



[a] Reagents and conditions: (a) TrCl, Et_3N, DMF; (b) TsCl, pyridine; (c) **9**, K_2CO_3, CH_3CN, reflux; (d) 4 N HCl(g)/AcOEt; (e) 4-(3-hydroxypropyl)pyridine, PPh_3, DEAD, THF; (f) H_2, PtO_2, AcOH.

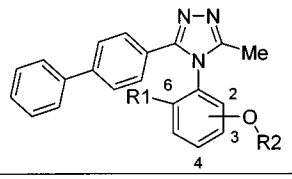
in diastolic blood pressure (DBP) in pithed rats after intravenous (iv) or in conscious rats after oral (po) administration (Figures 3 and 4).[23] The dose of **19** required to inhibit AVP-induced DBP response by 50%, the ID_{50} value, was determined.

Results and Discussion

The synthesized compounds were primarily evaluated for their binding affinity to hV_{1A} and hV_2. The results were expressed as K_i values, and the selectivity for hV_{1A} vs hV_2 (K_i ratio, hV_2/hV_{1A}) is presented in Table 1.

Initially, we examined the effects of the tether between the terminal basic moiety and the 4-phenyl ring. Although the 4-(dimethylamino)-1-piperidyl moiety of **3** has been shown to be effective in exerting high affinity to the V_{1A} receptor,[14] a variety of metabolism at the 4-dimethylamino part has been observed in our previous study. In addition, replacement of the 4-dimethylaminopiperidine moiety with other basic moieties has been found to retain the affinity to the V_{1A} receptor.[14] We chose a 4-methyl-1-piperazinyl moiety as the terminal basic part. The propoxy derivative (**16**) showed improved hV_{1A} affinity (K_i = 38.3 nM) and selectivity vs hV_2 (hV_2/hV_{1A} = 270), by 3-fold and 13-fold as compared to **4**, respectively. We then examined the effects of changing the length of the alkyl chain between the 4-methylpiperazine moiety and the 4-phenyl ring of **16**. Figure 2A shows the relation between the hV_{1A} affinity and the alkyl chain number (n) of compounds **16**−**22**. The more the alkyl chain number from propylene (n = 3, **16**) was, the higher hV_{1A} affinity of derivatives was until a peak for hV_{1A} affinity appeared at hexylene (n = 6, **19**). Further elongation of the alkyl chain from **19** caused a decrease in the hV_{1A} affinity of compounds (**20**−**22**). A similar tendency was observed between the hV_2 affinity and the alkyl chain number; however, the increase in range for hV_2 affinity was lower than that for hV_{1A} affinity. These results revealed that a peak for selectivity also appeared at hexylene (n = 6, **19**) as shown in Figure 2B. Of the compounds **16**−**22**, **19** had the highest hV_{1A} affinity (K_i = 1.04 nM) and selectivity (hV_2/hV_{1A} = 1700), which were 120-fold and 81-fold higher

Table 1. Binding Affinities of 5-(4-Biphenyl)-3-methyl-4-phenyl-1,2,4-triazole Derivatives to the Cloned Human V_{1A} and V_2 Receptors



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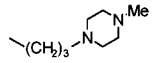
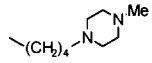
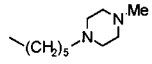
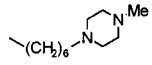
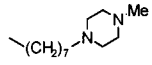
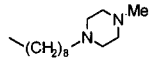
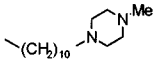
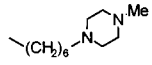
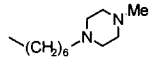
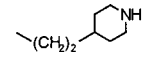
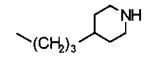
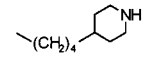
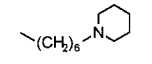
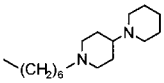
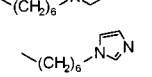
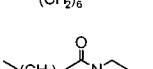
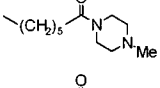
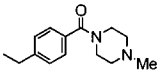
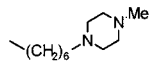
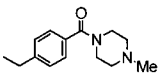
Compd.	R1	position	R2	K_i,[a] nM		Selectivity K_i ratio
				hV_{1A}	hV_2	hV_2/hV_{1A}
4	H	2	Me	120±27	2460±180	21
16	H	2		38.3±7.1	10200±790	270
17	H	2		29.1±4.3	10200±990	360
18	H	2		4.09±0.14	4850±230	1200
19	H	2		1.04±0.15	1780±160	1700
20	H	2		4.50±1.0	2470±230	550
21	H	2		13.2±3.6	8890±1800	670
22	H	2		39.4±10	14300±1200	360
23	H	3		214[b]	N.T.[c]	
24	H	4		>10000[b]	N.T.[c]	
37	H	2		55.5±13	8170±810	150
39	H	2		7.84±1.9	7370±360	940
38	H	2		2.12±0.21	4430±500	2100
25	H	2		13.9±2.4	6930±450	500
26	H	2		1.05±0.11	979±70	930
27	H	2		9.06±2.3	11100±650	1200
28	H	2		9.16±0.98	6650±240	730
29	H	2		3.03±0.31	585±32	190
30	Me	2		1.46±0.13	14400±1300	9900
31	Me	2		8.09±0.68	8520±900	1100
32	Me	2		1.01±0.15	1920±130	1900
1				>10000	>10000	
2				0.530±0.080	178±41	340
3				0.620±0.23	1.19±0.02	1.9

[a] K_i values were obtained from three to four independent experiments performed in duplicate. Each value indicates a mean ± SEM. [b] Mean from two experiments. [c] Not tested.

Table 2. Binding Affinities of Representative Compounds to the Rat V_{1A} and V_2 Receptors and the Cloned Human V_{1A} and V_2 Receptors

	K_i (nM)[a]				selectivity K_i ratio	
compd	rV_{1A}	hV_{1A}	rV_2	hV_2	rV_2/rV_{1A}	hV_2/hV_{1A}
19	17.3 ± 2.3	1.04 ± 0.15	1240 ± 110	1780 ± 160	72	1700
26	9.69 ± 2.5	1.05 ± 0.11	1010 ± 220	979 ± 70	100	930
29	62.7 ± 12	3.03 ± 0.31	364 ± 65	585 ± 32	5.8	190
30	102 ± 8.8	1.46 ± 0.13	11 700 ± 1700	14 400 ± 1300	110	9900
32	32.4 ± 5.7	1.01 ± 0.15	1840 ± 330	1920 ± 130	57	1900
1	23.5 ± 4.4	>10 000	>10 000	>10 000	>430	
2	1.43 ± 0.17	0.530 ± 0.080	285 ± 36	178 ± 41	200	340
3	0.160 ± 0.040	0.620 ± 0.23	0.770 ± 0.16	1.19 ± 0.02	4.8	1.9

[a] K_i values were obtained from four to six independent experiments performed in duplicate. Each value indicates a mean ± SEM.

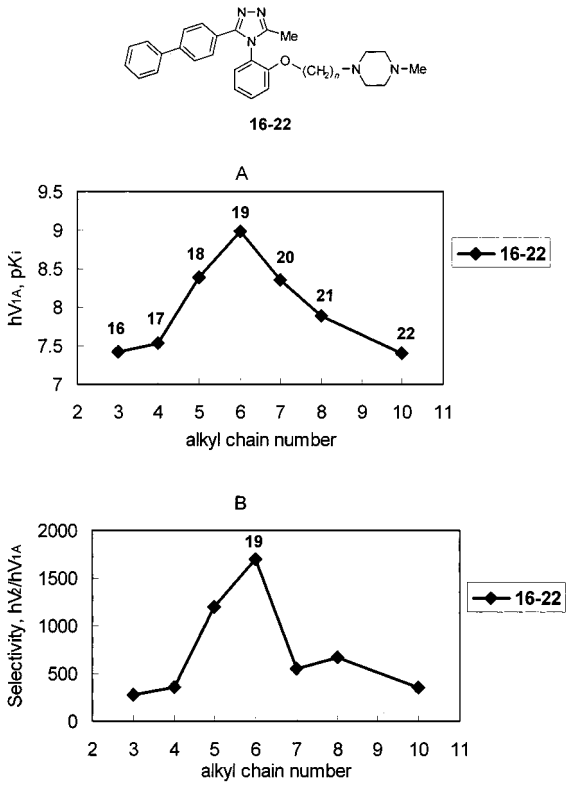


Figure 2. Relation between hV_{1A} affinities of the alkyl chain number (A) and selectivities and the alkyl chain number (B) in **16**−**22**.

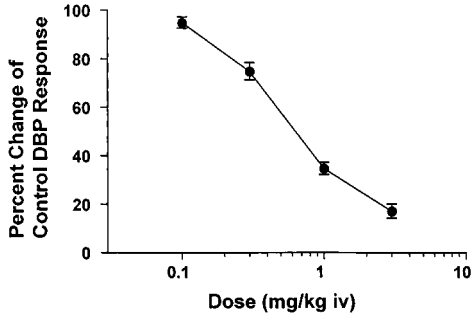


Figure 3. Inhibitory effects of intravenous administration of **19** on AVP (30 mU/kg)-induced increases in DBP in pithed rats. The inhibition was expressed as a percent change of control response. Each value indicates a mean ± SEM from four independent experiments. The ID_{50} value (half-maximal dose inhibiting an AVP-induced response) was 0.695 ± 0.044 mg/kg iv.

than those of **4**, respectively. Moreover, **19** was better than **2** with respect to selectivity (hV_2/hV_{1A} = 1700 for **19** and 340 for **2**, respectively).

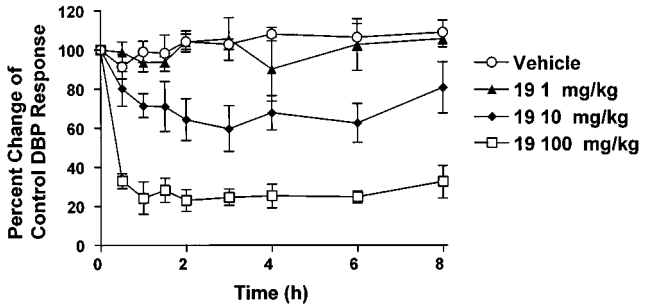


Figure 4. Time course of inhibitory effects of oral administration of **19** on AVP (30 mg/kg)-induced increases in DBP in conscious rats. The inhibition was expressed as a percent change of control response. Each value indicates a mean ± SEM from five independent experiments.

Subsequently, we studied the effect of substituting the position of the 6-(4-methyl-1-piperazinyl)hexyloxy moiety on the 4-phenyl ring. The *m*-substituted (**23**) and the *p*-substituted (**24**) derivatives showed significantly decreased or no hV_{1A} affinity. These results suggested that ortho substitution on the 4-phenyl ring might be suitable for high hV_{1A} affinity.

The 4-piperidyl derivatives (**37**−**39**) were also evaluated. The butylene derivative (**38**) exhibited high hV_{1A} affinity and selectivity (K_i = 2.12 nM, hV_2/hV_{1A} = 2100), which were comparable to those of **19**. This result suggested that the 4-(4-piperidyl)butoxy moiety of **38** might contribute to high hV_{1A} affinity, as in the case with the 6-(4-methyl-1-piperazinyl)hexyloxy moiety of **19**.

Next, we examined the effects of modifying the piperazine part of **19** to investigate the role of the nitrogen atoms in affinity. The 1-piperidyl derivative (**25**), the 1-imidazolyl derivative (**27**), and the amide derivative (**28**) showed approximately 1/10 times the hV_{1A} affinity of **19**. However, the 4-(1-piperidyl)-1-piperidyl derivative (**26**) exhibited comparable hV_{1A} affinity (K_i = 1.05 nM) to **19**. It appered that compounds with two basic nitrogens were superior to compounds with one basic nitrogen or aromatic nitrogens with respect to the hV_{1A} affinity. On the other hand, **29** with the 4-carbonylbenzyloxy moiety as the tether possessed high hV_{1A} affinity (K_i = 3.03 nM), despite the fact that it has only one basic nitrogen. The 4-carbonylbenzyloxy moiety was found to be one of the preferable tethers.

Finally, we introduced a methyl group into the 6-position of the 4-phenyl ring to investigate the steric effect of the six substituent. With respect to hV_{1A} affinity, the 6-hexyloxy derivative (**30**) and the 4-carbonylbenzyloxy derivative (**31**) were comparable to or

slightly less than the corresponding unsubstituted derivatives (**19** and **29**, respectively). For hV_2 affinity, however, both **30** and **31** were much less than the unsubstituted derivatives. These resulted in approximately 6-fold improvement of selectivity in both **30** and **31**. In particular, **30** showed the highest selectivity (hV_2/hV_{1A} = 9900) in this series. Furthermore, the 4-(1-piperidyl)-1-piperidyl derivative (**32**) of **31** displayed high hV_{1A} affinity (K_i = 1.01 nM) and selectivity (hV_2/hV_{1A} = 1900), which were comparable to those of **19**. These results indicated that further introduction of a methyl group into the 6-position of the 4-phenyl ring afforded an additional improvement in selectivity. This methyl group may contribute to the improvement in selectivity by maintaining the 4-phenyl ring in a more preferable conformation. From these results described above, we concluded that introduction of a basic moiety linked with the 4-phenyl ring by an appropriate tether (i.e., hexyloxy) brought about marked improvement of both hV_{1A} affinity and selectivity vs hV_2.

To investigate differences in the affinity of compounds to the AVP receptors of different species, we examined the binding affinity of our representative compounds together with known AVP antagonists to the rat AVP receptors, as shown in Table 2. All selected compounds in this series (**19**, **26**, **29**, **30**, and **32**) showed lower affinity to rV_{1A} than to hV_{1A} by 9−70-fold. Interestingly, the series of compounds exhibited similar affinity to both rV_2 and hV_2, which resulted in a lower selectivity of compounds for the rat receptors than those for the human receptors. For example, **19** showed relatively low rV_{1A} affinity (K_i = 17.3 nM) and selectivity (rV_2/rV_{1A} = 72), which were 17- and 24-fold lower than those to the human receptors, respectively. In contrast, **2** and **3** exhibited similar binding affinity to both the rat and the human receptors. Therefore, differences in the affinity of compounds between the human and the rat receptors should depend on their structures.

Compounds in this series had good rV_{1A} affinity and selectivity vs rV_2, although their potencies were less than those for the human receptors. Compound **19** was chosen and examined further to evaluate its V_{1A} receptor antagonist activity in rats. Intravenous administration of **19** to pithed rats dose dependently antagonized an AVP (30 mU/kg)-induced increase in DBP via the V_{1A} receptor with an ID_{50} value of 0.695 mg/kg (Figure 3). Furthermore, after oral administration of **19** (1, 10, and 100 mg/kg) to conscious rats, the time courses of antagonism effects against AVP-induced increase in DBP were monitored (Figure 4). Compound **19** dose dependently antagonized an AVP-induced increase in DBP, and its effect lasted for at least 8 h at doses of more than 10 mg/kg. In contrast, **19** did not affect the urine volume even at a dose of 100 mg/kg. These results demonstrated that **19** was a selective and orally active antagonist for the V_{1A} receptor in rats.

Conclusion

A series of 5-(4-biphenyl)-3-methyl-4-phenyl-1,2,4-triazole derivatives were prepared and evaluated for their affinity to hV_{1A} and selectivity vs hV_2. By utilizing the SAR on 4,4-difluoro-5-methylidene-2,3,4,5-tetrahydrobenzazepine derivatives, we found that substitution of the methoxy group at the 2-position of the 4-phenyl ring with (4-methyl-1-piperazinyl)alkoxy moieties brought about marked improvement of both affinity and selectivity for hV_{1A}. Alteration of the length of the alkyl chain between the 4-methylpiperazine moiety and the 4-phenyl ring revealed that peaks appeared at hexylene for both hV_{1A} affinity and selectivity. It was found that further introduction of a methyl group into the 6-position of the 4-phenyl ring afforded an additional improvement of selectivity. One of the synthesized compounds, **19**, showed high affinity and selectivity for hV_{1A} with a K_i value of 1.04 nM and a 1700-fold selectivity vs hV_2, respectively. We also found marked differences in both the affinity for V_{1A} and the selectivity vs hV_2 of compounds between the human and the rat AVP receptors. In vivo evaluation in rats demonstrated that compound **19** was a selective and orally active antagonist for the V_{1A} receptor and that its activity was long-lasting. We believe that this study would provide a novel approach to selective antagonists for the human V_{1A} receptor.

Experimental Section

Chemistry. Melting points were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. ^1H nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JMN-LA300 or JEOL JMN-EX400 spectrometer, and the chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. For salts, assignments of ion peaks are based on the basic component. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of theoretical values. High-performance liquid chromatography (HPLC) analyses were performed on a Hitachi L-7400 (UV detector) with a Hitachi L-7100 (pump); a TSKgel ODS-80TM column was used; eluent CH_3CN/0.01 M KH_2PO_4(aq) system at a flow rate of 1.0 mL/min. Drying of organic solutions during workup was done over anhydrous Na_2SO_4.

5-(4-Biphenyl)-4-(2-hydroxyphenyl)-3-methyl-1,2,4-triazole (9). To a solution of 2-aminophenol (8.12 g, 74.4 mmol) in AcOEt (80 mL) was added dropwise Ac_2O (16.3 mL, 168 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The mixture was concentrated in vacuo, and the residual solid was recrystallized from AcOEt−hexane to give 2-hydroxyacetanilide (10.9 g, 97%) as a light yellow powder. To a suspension of the acetanilide obtained above (10.9 g, 72.0 mmol) and K_2CO_3 (19.9 g, 144 mmol) in CH_3CN (200 mL) was added benzyl bromide (9.72 mL, 79.2 mmol), and the mixture was stirred at 70 °C for 8 h. After it was cooled at room temperature, the precipitate was removed by filtration and the filtrate was concentrated in vacuo. The residual solid was recrystallized from AcOEt−hexane to give 2-(benzyloxy)acetanilide (15.2 g, 86%) as a colorless powder. To a solution of the anilide obtained above (22.6 g, 93.5 mmol) in toluene (300 mL) was added P_2S_5 (23.0 g, 104 mmol), and the mixture was stirred at 70 °C for 2 h. After it was cooled at room temperature, the precipitate was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 2/1) to give 2-(benzyloxy)thioacetanilide (19.5 g, 81%) as a brown oil. A suspension of the thioacetanilide obtained above (19.4 g, 75.4 mmol), K_2CO_3 (30.0 g, 217 mmol), and MeI (20.0 g, 140 mmol) in CH_3CN (300 mL) were stirred at 50 °C for 3 h. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between AcOEt (500 mL) and H_2O (300 mL). The organic layer was washed with saturated NaCl(aq) (300 mL), dried, and concentrated in vacuo. The residue was purified by column chromatography on silica

gel (hexane/AcOEt = 2/1) to give **5** (16.2 g, 79%) as a yellow oil. A solution of **5** (5.06 g, 18.7 mmol) and 4-biphenylcarboxylic hydrazide (3.60 g, 17.0 mmol) in *N*,*N*-dimethylformamide (DMF; 50 mL) was stirred at 120 °C for 23 h. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 50/1) to give crude 4-(2-benzyloxy)-5-(4-biphenyl)-3-methyl-1,2,4-triazole (5.60 g, 79%) as a light yellow solid. To a solution of the triazole obtained above (4.62 g, 11.0 mmol) in DMF (75 mL) was added 10% Pd/C (w/w, 0.900 g), and the mixture was stirred under hydrogen pressure (4 kg/cm^3) at room temperature for 19 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residual solid was recrystallized from AcOEt−hexane to give 2-hydroxyacetanilide (10.9 g, 97%) as a light yellow powder The syrup was crystallized from AcOEt−hexane to give **9** (3.45 g, 95%) as a beige powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.17 (3H, s), 6.95 (1H, t, J = 8.0 Hz), 7.07 (1H, t, J = 8.0 Hz), 7.30−7.53 (7H, m), 7.60−7.65 (4H, m), 10.33 (1H, s). MS (FAB): m/z 328 (MH^+).

Compounds **10**−**12** were prepared by a procedure similar to that described for **9**. Yields referr to the cyclization step to construct the 1,2,4-triazole ring.

5-(4-Biphenyl)-4-(3-hydroxyphenyl)-3-methyl-1,2,4-triazole (10). 68%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25 (3H, s), 6.76 (1H, d, J = 1.8 Hz), 6.80 (1H, d, J = 7.8 Hz), 6.95 (1H, dd, J = 8.4, 2.4 Hz), 7.33−7.49 (7H, m), 7.66−7.68 (4H, m). MS (FAB): m/z 328 (MH^+).

5-(4-Biphenyl)-4-(4-hydroxyphenyl)-3-methyl-1,2,4-triazole (11). 66%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.22 (3H, s), 6.90 (2H, d, J = 8.7 Hz), 7.23 (2H, d, J = 8.7 Hz), 7.34−7.46 (7H, m), 7.65−7.68 (4H, m). MS (FAB): m/z 328 (MH^+).

5-(4-Biphenyl)-4-(2-hydroxy-6-methylphenyl)-3-methyl-1,2,4-triazole (12). 41%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80 (3H, s), 2.13 (3H, s), 6.82 (1H, d, J = 7.5 Hz), 6.95 (1H, d, J = 8.1 Hz), 7.25−7.52 (7H, m), 7.64−7.68 (4H, m), 10.46 (1H, br s). MS (FAB): m/z 328 (MH^+).

5-(4-Biphenyl)-3-methyl-4-{2-[6-(4-methyl-1-piperazinyl)-hexyloxy]phenyl}-1,2,4-triazole (19). To a suspension of **9** (1.04 g, 3.18 mmol) and K_2CO_3 (1.76 g, 12.7 mmol) in CH_3CN (30 mL) was added 1,6-dibromohexane (1.52 mL, 9.54 mmol), and the mixture was stirred at 70 °C for 3 h. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ (30 mL × 3) and H_2O (50 mL). The organic layer was concentrated in vacuo, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 40/1) to give 5-(4-biphenyl)-4-[2-(6-bromohexyloxy)phenyl]-3-methyl-1,2,4-triazole (0.924 g, 59%) as a light yellow solid. To a suspension of the bromide obtained above (0.333 g, 0.679 mmol) and K_2CO_3 (0.282 g, 2.04 mmol) in CH_3CN (8 mL) was added 1-methylpiperazine (0.452 mL, 4.07 mmol), and the mixture was stirred at 70 °C for 14 h. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ (15 mL × 3) and H_2O (30 mL). The organic layer was concentrated in vacuo, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10/1) to give crude **19** (0.252 g) as a light yellow foam. The foam was crystallized from Et_2O−hexane to give **19** (0.201 g, 58%) as a light yellow powder; mp 96−97 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18−1.23 (4H, m), 1.35−1.44 (2H, m), 1.51−1.60 (2H, m), 2.22−2.30 (4H, m), 2.27 (3H, s), 2.29 (3H, s), 2.42 (6H, br s), 3.80−3.87 (1H, m), 3.91−3.98 (1H, m), 7.02−7.07 (2H, m), 7.17 (1H, dd, J = 7.7, 1.7 Hz), 7.31−7.56 (10H, m). MS (FAB): m/z 510 (MH^+). Anal. ($C_{32}H_{39}N_5O$) C, H, N.

Compounds **16**−**18**, **20**−**22**, and **25**−**27** were prepared from **9** by a procedure similar to that described for **19**. Yields referr to the final substitution step.

5-(4-Biphenyl)-3-methyl-4-{2-[3-(4-methyl-1-piperazinyl)-propoxy]phenyl}-1,2,4-triazole (16). 41%; mp 127−128 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67−1.76 (2H, m), 2.14−2.34 (10H, m), 2.23 (3H, s), 2.29 (3H, s), 3.86−3.94 (1H, m), 3.97−4.04 (1H, m), 7.04−7.10 (1H, m), 7.19 (1H, dd, J = 7.5, 1.5 Hz), 7.31−7.56 (11H, m). MS (FAB): m/z 468 (MH^+). Anal. ($C_{29}H_{33}N_5O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[4-(4-methyl-1-piperazinyl)-butoxy]phenyl}-1,2,4-triazole (17). 60%; mp 103−104 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35−1.45 (2H, m), 1.54−1.63 (2H, m), 2.21−2.40 (10H, m), 2.27 (3H, s), 2.30 (3H, s), 3.82−3.89 (1H, m), 3.93−4.00 (1H, m), 7.03−7.08 (1H, m), 7.18 (1H, dd, J = 8.1, 1.8 Hz), 7.31−7.56 (11H, m). MS (FAB): m/z 482 (MH^+). Anal. ($C_{30}H_{35}N_5O\cdot0.5H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[5-(4-methyl-1-piperazinyl)-pentyloxy]phenyl}-1,2,4-triazole (18). 59%; mp 115−116 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.01−1.12 (2H, m), 1.16−1.29 (2H, m), 1.37−1.48 (2H, m), 2.04−2.21 (10H, m), 2.10 (3H, s), 2.16 (3H, s), 3.81−4.01 (2H, m), 7.12 (1H, t, J = 7.7 Hz), 7.27 (1H, d, J = 8.1 Hz), 7.34−7.56 (7H, m), 7.63−7.67 (4H, m). MS (FAB): m/z 496 (MH^+). Anal. ($C_{31}H_{37}N_5O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[7-(4-methyl-1-piperazinyl)-heptyloxy]phenyl}-1,2,4-triazole (20). 67%; mp 67−68 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (6H, br s), 1.36−1.44 (2H, m), 1.50−1.57 (2H, m), 2.24−2.30 (4H, m), 2.28 (3H, s), 2.29 (3H, s), 2.43 (6H, br s), 3.80−3.87 (1H, m), 3.90−3.98 (1H, m), 7.02−7.06 (2H, m), 7.16 (1H, dd, J = 8.1, 1.8 Hz), 7.31−7.56 (10H, m). MS (FAB): m/z 524 (MH^+). Anal. ($C_{33}H_{41}N_5O\cdot0.15H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[8-(4-methyl-1-piperazinyl)-octyloxy]phenyl}-1,2,4-triazole (21). 64%; mp 70−71 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19 (8H, br s), 1.39−1.46 (2H, m), 1.52−1.57 (2H, m), 2.25−2.30 (6H, m), 2.28 (3H, s), 2.29 (3H, s), 2.45 (4H, br s), 3.79−3.87 (1H, m), 3.90−3.98 (1H, m), 7.02−7.06 (2H, m), 7.16 (1H, dd, J = 8.0, 1.7 Hz), 7.30−7.56 (10H, m). MS (FAB): m/z 538 (MH^+). Anal. ($C_{34}H_{43}N_5O\cdot0.25H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[10-(4-methyl-1-piperazinyl)decyloxy]phenyl}-1,2,4-triazole (22). 50%; mp 87−88 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22−1.78 (12H, m), 1.40−1.57 (4H, m), 2.27−2.32 (4H, m), 2.28 (3H, s), 2.29 (3H, s), 2.45 (6H, br s), 3.80−3.87 (1H, m), 3.90−3.98 (1H, m), 7.02−7.06 (2H, m), 7.16 (1H, dd, J = 8.1, 1.7 Hz), 7.30−7.56 (10H, m). MS (FAB): m/z 566 (MH^+). Anal. ($C_{36}H_{47}N_5O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[6-(piperidino)hexyloxy]-phenyl}-1,2,4-triazole (25). 49%; mp 79−81 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16−1.26 (4H, m), 1.48−1.60 (8H, m), 1.73 (4H, br s), 2.29 (3H, s), 2.39−2.65 (4H, m), 3.83−3.90 (1H, m), 3.94−4.01 (1H, m), 7.03−7.08 (2H, m), 7.17 (1H, dd, J = 8.1, 2.1 Hz), 7.31−7.56 (10H, m). MS (FAB): m/z 495 (MH^+). Anal. ($C_{32}H_{38}N_4O\cdot2.0H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-(2-{6-[(4-piperidino)piperidino]hexyloxy}phenyl)-1,2,4-triazole (26). 51%; mp 90−91 °C (Et_2O−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16−1.22 (4H, m), 1.36−1.46 (4H, m), 1.52−1.60 (8H, m), 1.71−1.85 (4H, m), 2.16−2.21 (3H, m), 2.29 (3H, s), 2.48 (4H, br s), 2.85−2.95 (2H, m), 3.80−3.87 (1H, m), 3.91−3.98 (1H, m), 7.02−7.06 (2H, m), 7.16 (1H, dd, J = 8.0, 1.7 Hz), 7.31−7.56 (10H, m). MS (FAB): m/z 578 (MH^+). Anal. ($C_{37}H_{47}N_5O$) C, H, N.

5-(4-Biphenyl)-4-{2-[6-(1-imidazolyl)hexyloxy]phenyl}-3-methyl-1,2,4-triazole Dihydrochloride (27). 69%; mp 80−82 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, DMS-d_6): δ 1.09−1.20 (4H, m), 1.41−1.50 (2H, m), 1.65−1.74 (2H, m), 2.36 (3H, s), 3.86−3.94 (1H, m), 3.96−4.04 (1H, m), 4.11 (2H, t, J = 7.2 Hz), 7.20 (1H, t, J = 7.7 Hz), 7.33 (1H, d, J = 8.4 Hz), 7.37−7.77 (15H, m), 9.21 (1H, s). MS (FAB): m/z 478 (MH^+). Anal. ($C_{30}H_{31}N_5O\cdot2HCl\cdot2.25H_2O$) C, H, N, Cl.

5-(4-Biphenyl)-3-methyl-4-{3-[6-(4-methyl-1-piperazinyl)-hexyloxy]phenyl}-1,2,4-triazole (23). Compound **23** was prepared from **10** by a procedure similar to that described for **19**. Yields refer to the final substitution step; 43%; mp 85−87 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31−1.55 (4H, m), 1.72−1.81 (2H, m), 2.27−2.45 (12H, m), 2.28 (3H, s), 2.38 (3H, s), 3.92 (2H, t. J = 6.6 Hz), 6.73 (1H, t, J = 2.0 Hz),

6.79−6.81 (1H, m), 7.01−7.05 (1H, m), 7.31−7.57 (10H, m). MS (FAB): *m/z* 510 (MH^+). Anal. ($C_{32}H_{39}N_5O\cdot0.25H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{4-[6-(4-methyl-1-piperazinyl)-hexyloxy]phenyl}-1,2,4-triazole (24). Compound **24** was prepared from **11** by a procedure similar to that described for **19**. Yields refer to the final substitution step; 76%; mp 124−125 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34−1.56 (6H, m), 1.78−1.87 (2H, m), 2.21−2.45 (10H, m), 2.29 (3H, s), 2.35 (3H, s), 4.00 (2H, t. J = 6.3 Hz), 6.97−7.15 (4H, m), 7.23−7.57 (9H, m). MS (FAB): *m/z* 510 (MH^+). Anal. ($C_{32}H_{39}N_5O\cdot0.25H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{6-methyl-2-[6-(4-methyl-1-piperazinyl)hexyloxy]phenyl}-1,2,4-triazole Trihydrochloride (30). Compound **30** was prepared from **12** by a procedure similar to that described for **19**. Yields refer to the final substitution step; 25%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.15−1.20 (6H, m), 1.47−1.67 (2H, m), 1.91−2.00 (2H, m), 1.99 (3H, s), 2.25 (3H, s), 2.83 (3H, s), 3.03 (1H, br s), 3.40−3.70 (4H, m), 3.98−4.07 (3H, m), 7.06 (1H, d, J = 7.8 Hz), 7.19 (1H, d, J = 8.7 Hz), 7.37−7.54 (6H, m), 7.69−7.75 (4H, m). MS (FAB): *m/z* 524 (MH^+). Anal. ($C_{32}H_{39}N_5O\cdot2.75HCl\cdot4.0H_2O$) C, H, N, Cl.

1-(6-Bromohexanoyl)-4-methylpiperazine Hydrochloride (13). To a solution of 1-methylpiperazine (0.399 g, 3.98 mmol) in CH_3CN (4 mL) was added dropwise a solution of 6-bromohexanoyl chloride (1.02 g, 4.78 mmol) in CH_3CN (10 mL) at 0 °C, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated in vacuo, and the residual solid was suspended with Et_2O and filtered to give **13** (1.22 g, 98%) as a light yellow powder. This compound was used in the next step without further purification.

5-(4-Biphenyl)-3-methyl-4-(2-{5-[(4-methyl-1-piperazinyl)carbonyl]pentyloxy}phenyl)-1,2,4-triazole Dihydrochloride (28). To a suspension of **9** (0.249 g, 0.770 mmol) and K_2CO_3 (0.426 g, 3.08 mmol) in CH_3CN (10 mL) was added **13** (0.362 g, 1.16 mmol), and the mixture was stirred at 70 °C for 23 h. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ (15 mL × 3) and H_2O (30 mL). The organic layer was concentrated in vacuo, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10/1) to give the free base of **28** (0.287 g) as a colorless syrup. This material was converted to its hydrochloride salt by treating it with 4 N HCl(g)/AcOEt (3.0 mL, 12.0 mmol). The crude salt was suspended with Et_2O and filtered to give **28** (0.282 g, 56%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.07−1.11 (3H, m), 1.32−1.54 (4H, m), 2.20−2.25 (2H, m), 2.36 (3H, s), 2.73 (3H, s), 2.91−3.02 (3H, m), 3.30−3.45 (2H, m), 3.86−4.00 (3H, m), 4.38−4.42 (1H, m), 7.19 (1H, t, J = 7.8 Hz), 7.33 (1H, d, J = 8.1 Hz), 7.37−7.52 (5H, m), 7.60−7.75 (7H, m), 11.43 (1H, br s). MS (FAB): *m/z* 524 (MH^+). Anal. ($C_{32}H_{37}N_5O_2\cdot1.75HCl\cdot4.0H_2O$) C, H, N, Cl.

1-(4-Chloromethylbenzoyl)-4-methylpiperazine Hydrochloride (14). Compound **14** was prepared from 4-chloromethylbenzoyl chloride by a procedure similar to that described for **13** (95%). This compound was used in the next step without further purification.

5-(4-Biphenyl)-3-methyl-4-(2-{4-[(4-methyl-1-piperazinyl)carbonyl]benzyloxy}phenyl)-1,2,4-triazole (29). Compound **29** was prepared from **9** and **14** by a procedure similar to that described for **28** (60%); mp 179−180 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27−2.43 (4H, m), 2.29 (3H, s), 2.32 (3H, s), 3.35 (2H, br s), 3.75 (2H, br s), 4.95 (1H, d. J = 12.5 Hz), 5.06 (1H, d. J = 12.5 Hz), 7.02−7.14 (4H, m), 7.24−7.57 (13H, m). MS (FAB): *m/z* 544 (MH^+). Anal. ($C_{34}H_{33}N_5O_2$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-(6-methyl-2-{4-[(4-methyl-1-piperazinyl)carbonyl]benzyloxy}phenyl)-1,2,4-triazole (31). Compound **31** was prepared from **12** and **14** by a procedure similar to that described for **28** (60%); mp 161−164 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 (3H, s), 2.22−2.32 (4H, m), 2.26 (3H, s), 2.29 (3H, s), 3.36 (2H, br s), 3.74 (2H, br s), 5.02 (1H, d. J = 12.8 Hz), 5.09 (1H, d. J = 12.8 Hz), 6.92 (1H, d, J = 8.1 Hz), 6.97 (1H, d, J = 7.8 Hz), 7.08 (2H, d, J = 8.1 Hz), 7.26−7.57 (12H, m). MS (FAB): *m/z* 558 (MH^+). Anal. ($C_{35}H_{35}N_5O_2$) C, H, N.

1-(4-Chloromethylbenzoyl)-4-piperidinopiperidine Hydrochloride (15). Compound **15** was prepared from 4-chloromethylbenzoyl chloride and 4-piperidinopiperidine by a procedure similar to that described for **13** (96%). This compound was used in the next step without further purification.

5-(4-Biphenyl)-3-methyl-4-(6-methyl-2-{4-[(4-piperidinopiperidino)carbonyl]benzyloxy}phenyl)-1,2,4-triazole (32). Compound **32** was prepared from **12** and **15** by a procedure similar to that described for **28** (60%); mp 147−150 °C (AcOEt−hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44−1.60 (8H, m), 1.75−1.97 (2H, m), 1.99 (3H, s), 2.26 (3H, s), 2.51 (5H, br s), 2.66−2.91 (2H, m), 3.70 (1H, br s), 4.71 (1H, br s), 5.02 (1H, d. J = 12.6 Hz), 5.09 (1H, d. J = 12.6 Hz), 6.92 (1H, d, J = 8.1 Hz), 6.97 (1H, d, J = 7.2 Hz), 7.08 (2H, d, J = 8.1 Hz), 7.25−7.57 (12H, m). MS (FAB): *m/z* 626 (MH^+). Anal. ($C_{40}H_{43}N_5O_2\cdot0.25H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[2-(4-piperidyl)ethoxy]-phenyl}-1,2,4-triazole (37). To a suspension of **9** (0.220 g, 0.670 mmol) and K_2CO_3 (1.00 g, 7.24 mmol) in CH_3CN (10 mL) was added **35** (n = 2, 0.370 g, 0.700 mmol), and the mixture was stirred at 80 °C for 4 h. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ (15 mL × 3) and H_2O (30 mL). The organic layer was concentrated in vacuo, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 50/1) to give 5-(4-biphenyl)-3-methyl-4-{2-[2-(1-trityl-4-piperidyl)ethoxy]phenyl}-1,2,4-triazole (0.250 g, 55%) as a colorless foam. To a solution of the piperidine obtained above (0.250 g, 0.370 mmol) in EtOH (3 mL) was added 4 N HCl(g)/1,4-dioxane (2 mL), and the mixture was stirred at 70 °C for 20 min. After it was cooled at room temperature, the mixture was concentrated in vacuo. The residue was made alkaline with K_2CO_3(aq) and extracted with $CHCl_3$ (15 mL × 3). The combined extract was concentrated in vacuo, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10/1) to give crude **37** as a light yellow solid. The solid was recrystallized from AcOEt−hexane to give **37** (0.092 g, 57%) as a colorless powder; mp 120−122 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95−1.55 (7H, m), 2.29 (3H, s), 2.35−2.55 (2H, m), 2.90−3.00 (2H, m), 3.85−4.05 (2H, m), 7.07 (1H, t, J = 7.2 Hz), 7.10−7.60 (12H, m). MS (FAB): *m/z* 439 (MH^+). Anal. ($C_{28}H_{30}N_4O\cdot0.5H_2O$) C, H, N.

5-(4-Biphenyl)-3-methyl-4-{2-[4-(4-piperidyl)butoxy]-phenyl}-1,2,4-triazole Dihydrochloride (38). Compound **38** was prepared from **9** and **36** (n = 4) by a procedure similar to that described for **37** (47%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95−1.75 (11H, m), 2.30 (3H, s), 2.55−2.85 (2H, m), 3.00−3.25 (2H, m), 3.80−4.05 (2H, m), 7.10−7.80 (13H, m), 8.81 (1H, br s), 9.05 (1H, br s). MS (FAB): *m/z* 467 (MH^+). Anal. ($C_{29}H_{32}N_4O\cdot2HCl\cdot2.5H_2O$) C, H, N, Cl.

5-(4-Biphenyl)-3-methyl-4-{2-[3-(4-piperidyl)propoxy]-phenyl}-1,2,4-triazole Dihydrochloride (39). To a solution of **9** (0.220 g, 0.670 mmol), 4-(3-hydroxypropyl)pyridine (0.274 g, 2.00 mmol), and Ph_3P (0.520 g, 2.00 mmol) in tetrahydrofuran (THF, 10 mL) was added dropwise diethyl azodicarboxylate (0.350 g, 2.00 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated in vacuo, and the residue was made alkaline with K_2CO_3(aq) and extracted with $CHCl_3$ (15 mL × 3). The combined extract was concentrated in vacuo, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 40/1) to give 5-(4-biphenyl)-3-methyl-4-{2-[3-(4-pyridyl)propoxy]phenyl}-1,2,4-triazole (0.251 g, 84%) as a colorless solid. To a solution of the pyridine obtained above (0.195 g, 0.430 mmol) in AcOH (10 mL) was added PtO_2 (0.090 g), and the mixture was stirred under hydrogen atmosphere at room temperature for 7 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was made alkaline with K_2CO_3(aq) and extracted with $CHCl_3$ (15 mL × 3). The combined extract was concentrated in vacuo, and the residue

was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10/1) to give the free base of **39** (0.180 g) as a colorless syrup. This material was converted to its hydrochloride salt by treating it with 4 N HCl(g)/AcOEt (1.0 mL, 4.0 mmol). The crude salt was suspended with AcOEt and filtered to give **39** (0.144 g, 64%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95−1.75 (11H, m), 2.30 (3H, s), 2.50−2.75 (2H, m), 3.00−3.15 (2H, m), 3.80−4.05 (2H, m), 7.10−7.80 (13H, m), 8.79 (1H, br s), 9.02 (1H, br s). MS (FAB): *m*/*z* 453 (MH^+). HPLC analysis (CH_3CN/0.01 M KH_2PO_4(aq) (8:2)) t_R = 4.85 min (97.2%).

Pharmacology. Binding Assay: For the Cloned Human Receptors. The cloned human AVP receptor subtypes were stably expressed in CHO cells and plasma membranes prepared according to the reported protocols.[22] [^{3}H]AVP (0.5−1.0 nM, 80 Ci mmol^{-1}, DuPont-New England Nuclear) was added to each membrane preparation, which was then incubated with various concentrations of compounds in 250 μM assay buffer (50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 0.1% bovine serum albumin). After an incubation period (60 min, 25 °C), the reaction was terminated by the addition of 3 mL of ice-cold Tris buffer (50 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$) followed immediately by rapid filtration through 96 well GF/B UniFilter Plates using a MicroMate Cell Harvester (Packard Instrument Company). The filters were rinsed twice, and the radioactivity retained on the filters was counted with a TopCount Microplate Scintillation Counter (Packard Instrument Company). Nonspecific binding was determined using 1 μM unlabeled AVP. The radioligand binding data were analyzed by GraphPad PRISM (GraphPAD Software, Inc.). The inhibitory dissociation constant (K_i) was calculated from the following formula: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of radioligand present in the tube and K_d is the dissociation constant of radioligand obtained from Scatchard plot analysis. The K_d values were 0.39 ± 0.13 nM for hV_{1A} and 1.21 ± 0.37 nM for hV_2, respectively (n = 5).

For the Rat Receptors. Binding assays were performed using [^{3}H]AVP on plasma membranes prepared from rat liver or kidney according to the reported protocols.[23]

In Vivo Assay: In Pithed Rats (iv).[23] Male Wistar rats were anesthetized with ether and pithed by inserting a steel rod via the orbit and foramen magnum down into the whole length of the spinal canal. Immediately after pithing, the rats were bilaterally vagotomized at the neck and artificial ventilation with room air was started with a rodent respirator at a frequency of 50 cycles/min and a volume of 1 mL/100 g body weight. A catheter was inserted into the carotid artery and femoral vein for recording of artery blood pressure and intravenous administration, respectively. Rats were kept warm at 37 °C by means of a thermostat-controlled heating board. For intravenous injection, **19** was dissolved in DMF. After blood pressure was stabilized, the DMF solution of **19** or the vehicle (DMF) was given (0.5 mL/kg iv) 5 min before the injection of AVP (30 mU/kg iv). The dose of **7d** causing 50% inhibition of the pressor response to AVP (ID_{50}) was calculated.

In Conscious Rats (po). Male Wister rats were anesthetized with pentobarbital (50 mg/kg ip). The left carotid artery and ipsilateral jugular vein were cannulated with a polyethylene tube (PE−50) for determination of blood pressure and heart rate and for intravenous administration of AVP (30 mU/kg). The animals were allowed to recover for 2 days after oreration, during which time they were housed in individual cages with free access to rat chow and water. Blood pressure was measured with a pressure transducer (AP-200T) coupled to the carotid arterial cannula and continuously recorded via a polygraph system. Heart rate was measured from blood pressure pulse waves. After stabilization of both parameters was achieved, AVP (30 mU/kg) was administered through the venous cannula. The injection of AVP was repeated at intervals of about 15 min, each injection being given as soon as blood pressure returned to a preinjection level. Responses to two consecutive doses of AVP showing approximately constant amplitudes of blood pressure elevation were averaged to be taken as a control response. Each rat was then treated orally with a single dose of the 0.5% methylcellulose aqueous solution of **19** or the vehicle (0.5% methylcellulose aqueous solution), and any changes in blood pressure were noted.

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