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Discovery of 4,5-Diphenyl-1,2,4-triazole Derivatives as a Novel Class of Selective Antagonists for the Human V_{1A} Receptor

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Abstract—In the search for a novel class of selective antagonists for the human V_{1A} receptor, high-throughput screening (HTS) of the Yamanouchi chemical library using CHO cells expressing the cloned human V_{1A} (hV_{1A}) receptor led to the discovery of 5-(4-biphenyl)-4-(2-methoxyphenyl)-3-methyl-1,2,4-triazole (**3**) which possessed the novel 4,5-diphenyl-1,2,4-triazole structure. Subsequent structure–activity relationships studies on a series of the 4,5-diphenyl-1,2,4-triazole derivatives related to **3** revealed that the 4,5-diphenyl-1,2,4-triazole structure played an essential role in exerting high affinity for the hV_{1A} receptor and that introduction of a basic amine moiety to the methoxy part of the 4-phenyl ring was effective in the improvement of both affinity for the hV_{1A} receptor and selectivity versus the hV_2 receptor. Compound **3** and the 2-(morpholino)ethoxy derivative (**11b**) were shown to be antagonists for the hV_{1A} receptor, from their effects on AVP-induced $[Ca^{2+}]_i$ response in CHO cells expressing the hV_{1A} receptor. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Arginine vasopressin (AVP), a peptide hormone secreted by the posterior pituitary, is an important regulator of cardiovascular homeostasis and fluid. The actions of AVP are mediated by specific receptors located in a variety of tissues and organs.^{1,2} These AVP receptors in periphery have been classified into two subtypes (V_{1A} and V_2). AVP activates phospholipase A_2 , C, and D through the V_{1A} receptors which results in the mobilization of intracellular calcium.³ It causes vasoconstriction, platelet aggregation, and proliferation of cells.⁴ In contrast, the V_2 receptors stimulates adenylate cyclase and plays an predominant role in controlling free water and urea reabsorption in the kidney.⁵ These two AVP receptor subtypes have recently been cloned and stably expressed.⁶

AVP is thought to be closely related to several diseases, including heart failure, hypertension, renal diseases, and hyponatremia. Consequently, the development of subtype selective AVP receptor antagonists appears essential for elucidating the pathophysiological roles of AVP and may be beneficial in treating these diseases.

Recently, two nonpeptide selective antagonists for the V_{1A} receptor, OPC-21268 (**1**)^{7,8} and SR49059 (**2**),⁹ have been reported for which clinical trials are underway.

In the course of the search for AVP antagonists at our laboratories, a series of the 1,3,4,5-tetrahydrobenzazepine derivatives such as YM087^{10,11} was discovered to be potent and orally active antagonist for both the V_{1A} and V_2 receptors. We moved into the next research program which addressed the development of a novel class of selective antagonists for the V_{1A} receptor. It has been shown that there are marked species differences in AVP receptor affinity and specificity of ligands.¹² Indeed, although **1** shows high affinity for the rat V_{1A} receptor ($K_i = 0.0235 \mu M$),¹³ it displays poor affinity for the cloned human V_{1A} receptor even at $10 \mu M$.¹⁴ On the other hand, **2** has extremely high affinity for the cloned human V_{1A} receptor ($K_i = 0.00053 \mu M$).¹⁴ Based on these facts, we decided to employ Chinese hamster ovary (CHO) cells stably expressing the cloned human V_{1A} and V_2 receptor subtypes (hV_{1A} and hV_2) for receptor binding assay, in order to discover a novel class of selective antagonists for the hV_{1A} receptor.

High-throughput screening (HTS) of the Yamanouchi chemical library based on the hV_{1A} receptor binding assay led to the identification of the 4,5-diphenyl-1,2,4-

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triazole derivative, **3**, which possessed a novel structure and showed hV_{1A} affinity ($K_i = 0.120 \mu\text{M}$) and moderate selectivity versus hV_2 ($hV_2/hV_{1A} = 21$). This identification prompted us to choose **3** as a lead for our program to investigate selective antagonists for the hV_{1A} receptor, which focused on improvement of both hV_{1A} affinity and selectivity versus hV_2 . In this report, the discovery, synthesis, and pharmacological evaluation of the novel 4,5-diphenyl-1,2,4-triazole derivatives are described (Fig. 1).

Chemistry

The 1,2,4-triazole structure of the novel compounds was constructed by two general methods, Method A and B

(Scheme 1). Method A shows the construction of the 1,2,4-triazole rings by substitution reaction with amines to the 1,3,4-oxadiazole rings (**5**). Biphenyl-4-carboxylic acid was converted to the *N,N'*-diacylhydrazines (**4**) by coupling with a hydrazine hydrate and subsequent acylation. Compounds **4** were cyclized to the 1,3,4-oxadiazoles (**5**) with P_2O_5 . Compounds **5** were finally subjected to a substitution reaction with an anisole or a benzylamine to afford the target compounds **3**, **6** and **7**. Method B illustrates the preparation of the 1,2,4-triazole ring by cyclization of the thioimides (**8**) with hydrazides. The 2-substituted anilines were transformed to the thioimides (**8**) in three steps, and subsequent cyclization of **8** with hydrazides gave the desired compounds **9**. The benzyloxy derivative (**9j**) was hydrogenated to afford

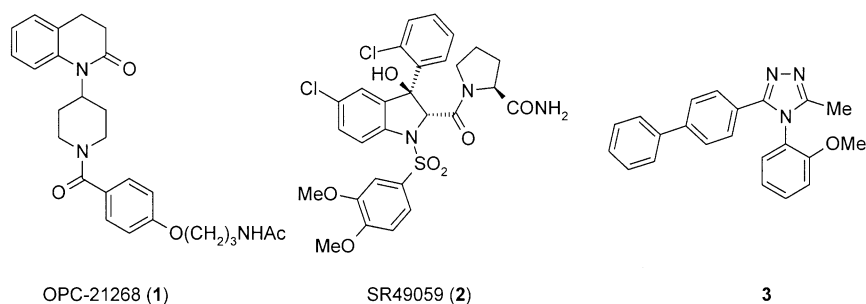
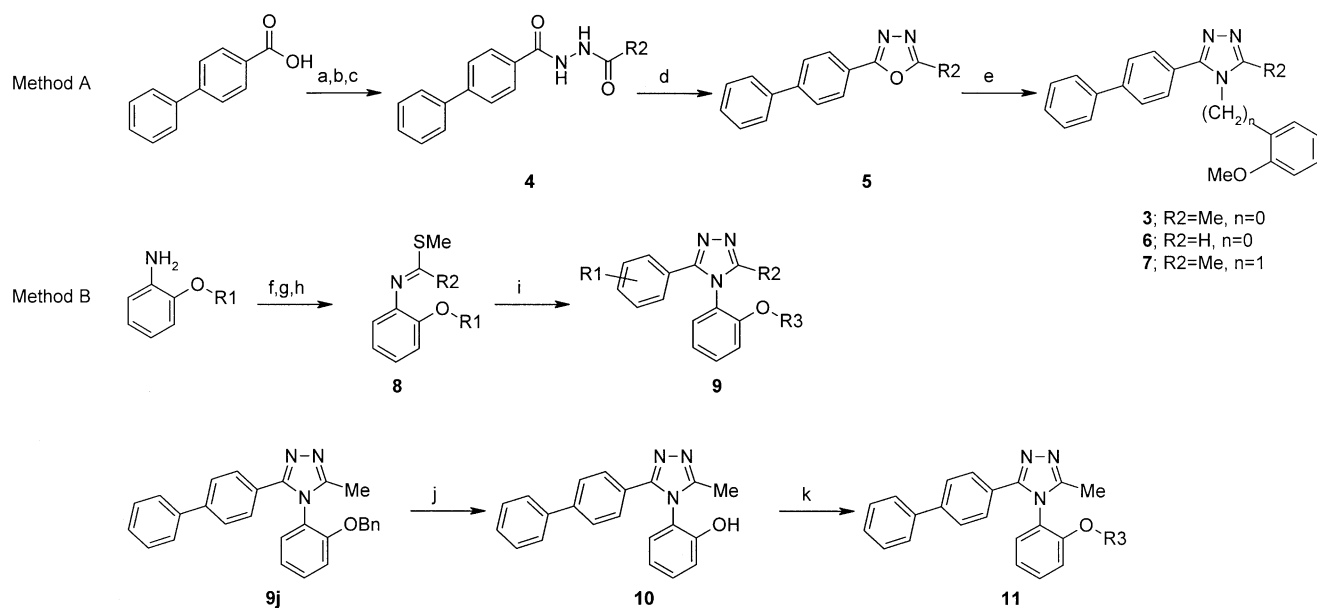
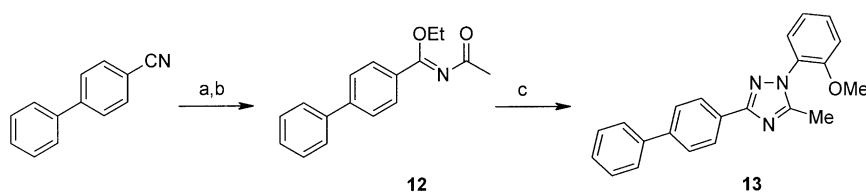


Figure 1.



Scheme 1. (a) $SOCl_2$, cat.DMF, THF; (b) $H_2NNH_2 \cdot H_2O$, THF; (c) R_2CO_2COMe , pyridine; (d) P_2O_5 , toluene, reflux; (e) *o*-anisole or *o*-methoxybenzylamine, 150 °C; (f) R_2COCl , pyridine; (g) P_2S_5 , toluene, 70 °C; (h) MeI, K_2CO_3 , CH_3CN , 50 °C; (i) $ArCONHNH_2$, DMF, 120 °C; (j) H_2 , Pd/C, MeOH; (k) R_3X , NaH, DMF, 60 °C.



Scheme 2. (a) 4 N HCl(g)/AcOEt, EtOH; (b) AcCl, Et₃N, toluene; (c) *o*-OMeC₆H₄NHNH₂, toluene, 60 °C.

the phenol (**10**). Compound **10** was then converted to **11** by *O*-alkylation. The 1,3-diphenyl derivative (**13**) was prepared as shown in Scheme 2. A 4-cyanobiphenyl was transformed to the acylimidate (**12**), and then compound **12** was cyclized with phenylhydrazine to afford **13**.

Pharmacology

Binding assay

Radioligand binding assays for the hV_{1A} and hV₂ receptors were performed according to the reported protocols¹⁴ using [³H]-AVP on CHO stably expressing the hV_{1A}, and the hV₂ receptor, respectively.

Antagonist activities for the hV_{1A} receptor

Antagonist activities of selected compounds for the hV_{1A} receptor were examined by measuring the inhibition of an AVP-induced increase in intracellular Ca²⁺ concentration ([Ca²⁺]_i) of CHO cells expressing the hV_{1A} receptor.¹⁴ IC₅₀ value, which means the concentration of the compounds required to inhibit 10^{−8} M AVP-induced response by 50%, was determined.

Results and Discussion

The binding affinities of the synthesized compounds for the hV_{1A} and hV₂ receptors were evaluated according to the reported procedure (Table 1).¹⁴

Table 1. Binding affinities of 1,2,4-triazole derivatives for the cloned human V_{1A} and V₂ receptors

Compd	R1	R2	R3	K _i , ^a μM		Selectivity K _i ratio hV ₂ /hV _{1A}
				hV _{1A}	hV ₂	
3	4'-Ph	Me	Me	0.120±0.027	2.46±0.18	21
13				> 10	N.T. ^b	
7				5.89 ^c	N.T. ^b	
9a	3'-Ph	Me	Me	8.39±0.88	19.1±0.40	2.3
9b	2'-Ph	Me	Me	> 10	N.T. ^b	
9c	4'-PhO	Me	Me	2.67±0.35	13.3±1.7	5.0
9d	4'-PhCH ₂ O	Me	Me	4.22±0.31	11.8±0.71	2.8
9e	4'-(pyrrol-1-yl)	Me	Me	0.281±0.015	18.3±1.5	65
9f	4'-(piperidino)	Me	Me	0.0972±0.019	14.8±1.3	150
9g	4'-(morpholino)	Me	Me	2.84±0.74	201±36	71
6	4'-Ph	H	Me	0.401±0.030	16.4±1.2	41
9h	4'-Ph	Et	Me	0.0876±0.0090	1.28±0.26	15
9i	4'-Ph	<i>n</i> -Pr	Me	0.0664±0.017	0.875±0.15	13
10	4'-Ph	Me	H	0.288±0.017	21.8±1.4	76
11a	4'-Ph	Me	Et	0.168±0.045	2.87±0.36	17
9j	4'-Ph	Me	CH ₂ Ph	6.66±1.4	3.04±0.23	0.46
11b	4'-Ph	Me		0.0348±0.010	3.03±0.37	87
11c	4'-Ph	Me		0.0551±0.0085	8.47±0.33	150
1				> 10 0.00053	> 10	0.00053
2				±0.000080	0.178±0.041	340

^aK_i values were obtained from 3–4 independent experiments. Each value indicates a mean ± SEM.

^bNot tested.

^cMean from two experiments.

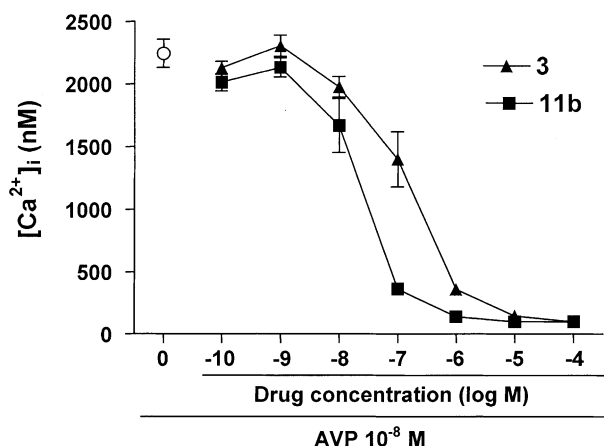


Figure 2. Effects of **3** and **11b** on AVP-induced $[Ca^{2+}]_i$ increases in the human V_{1A} receptor expressing CHO cells. Each point represents mean $\pm$ SEM from three independent experiments. The IC_{50} values [half-maximal concentration inhibiting 10^{-8} M AVP-induced response ($\bigcirc$)] for **3** and **11b** were 145 and 22.1 nM, respectively.

Initially, we examined the position of the substituents on the triazole ring of **3**. Translocation of the 2-methoxyphenyl moiety at the 4-position of the triazole ring into the 2-position afforded the 2,5-diphenyl derivative (**13**) which showed poor hV_{1A} affinity. Substitution of the 2-methoxyphenyl moiety with a 2-methoxybenzyl moiety gave the 4-benzyl derivative (**7**) which also had significantly decreased hV_{1A} affinity compared to **3**. These results indicated that the 4,5-diphenyl-1,2,4-triazole structure played an essential role in exerting high hV_{1A} affinity.

Next, we studied the effects of modifying the substituents on the 5-phenyl ring of the triazole ring. Translocation of the phenyl moiety at the 4'-position of **3** into the 3'- or 2'-position (**9a–b**) resulted in a considerable reduction of hV_{1A} affinity.

We then examined the effects of the substituents at the 4'-position on the 5-phenyl ring. The phenoxy- (**9c**), the benzyloxy derivatives (**9d**) showed even weaker hV_{1A} affinity than **3**. In contrast, the pyrrole- (**9e**) and the piperidine derivative (**9f**) had slightly weak or comparable hV_{1A} affinity ($K_i = 0.281$ and 0.0972 μ M, respectively) compared to **3**. Moreover, both **9e** and **9f** showed high selectivity versus hV_2 ($hV_2/hV_{1A} = 65$ and 150 , respectively) because of their weak hV_2 affinities. Surprisingly, the morpholine derivative (**9g**) also possessed high selectivity ($hV_2/hV_{1A} = 71$), in spite of its lower hV_{1A} affinity ($K_i = 2.84$ μ M). The nitrogen atom attached to the 4'-position of **9e–g** might contribute to achieve the receptor selectivity.

Subsequently, we investigated the substituents at the 3-position of the triazole ring. The unsubstituted derivative (**6**) had lower hV_{1A} affinity but improved selectivity compared to **3**. The ethyl- (**9h**) and the *n*-propyl derivative (**9i**) showed similar hV_{1A} affinity and selectivity to **3**, suggesting the tolerance against alkyl chain in this portion.

Finally, we modified the methoxy moiety on the 4-phenyl ring of the triazole ring. The phenol derivative (**10**) showed lower hV_{1A} affinity but higher selectivity compared to **3**. The ethoxy derivative (**11a**) had a similar binding profile to **3**. Meanwhile, the benzyloxy derivative (**9j**) exhibited significantly decreased hV_{1A} affinity and no selectivity. There might be a limitation on lipophilicity in this portion. Introduction of basic amine moieties to the methoxy part gave **11b–c** which displayed both improved hV_{1A} affinities and selectivity. Especially the 2-(morpholino)ethoxy derivative (**11b**) showed the excellent binding profile ($K_i = 0.0348$ μ M, $hV_2/hV_{1A} = 87$). These results suggested that the basic nitrogen atom of **11b** or **11c** might contribute to the potent binding of compounds to the hV_{1A} receptor.

On the basis of the binding studies, we examined **3** and **11b** for their antagonist activities for the hV_{1A} receptor (Fig. 2). Both compounds inhibited an AVP-induced $[Ca^{2+}]_i$ increase in a concentration-dependent manner with IC_{50} values of 0.145 and 0.0221 μ M, respectively. These IC_{50} values corresponded well with the K_i values obtained from receptor binding assay. In addition, the existence of **3** or **11b** alone did not affect the $[Ca^{2+}]_i$ level. These results demonstrated that both **3** and **11b** were antagonists for the hV_{1A} receptor.

Conclusion

The search for the development of selective antagonists for the hV_{1A} receptor by HTS using CHO cells expressing the hV_{1A} receptor led to the discovery of **3** which possessed the novel 4,5-diphenyl-1,2,4-triazole structure. Compound **3** was chosen as a lead and structural modifications have been conducted. Receptor binding assays for a series of the 4,5-diphenyl-1,2,4-triazole derivatives related to **3** revealed that the 4,5-diphenyl-1,2,4-triazole structure played an essential role in exerting high hV_{1A} affinity, and that the nitrogen atom attached to the 4'-position of **9e–g** might contribute to the improvement of selectivity versus hV_2 . It was also demonstrated that introduction of a basic amine moiety to the methoxy part of the 4-phenyl ring (**11b–c**) was effective in the improvement of both hV_{1A} affinity and selectivity. Compounds **3** and **11b** were shown to be antagonists for the hV_{1A} receptor, from their effects on AVP-induced $[Ca^{2+}]_i$ response in CHO cells expressing the hV_{1A} receptor. Further research of this series to discover potent, orally active selective antagonists for the hV_{1A} receptor is in progress and will be reported in the near future.

Experimental

Chemistry

Melting points were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. 1H NMR spectra were recorded on a JEOL JNM-LA300 or

a JNM-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 Hitachi M-80 or a JEOL JMS-LX2000 spectrometer. The elemental analyses were performed with a Yanaco MT-5 micro-analyzer (C, H, N) and were within $\pm 0.4\%$ of theoretical values. Drying of organic solutions during workup was done over anhydrous Na_2SO_4 .

***N'*-Acetyl-biphenyl-4-carboxylic hydrazide (4a).** A solution of biphenyl-4-carboxylic acid (10.0 g, 50.7 mmol) and *N,N*-dimethylformamide (DMF; 0.20 mL) in SOCl_2 (37.0 mL, 507 mmol) was stirred under reflux for 3 h. After cooling at room temperature, the mixture was concentrated in vacuo to give biphenyl-4-carbonyl chloride as a colorless solid. To a solution of $\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$ (123 mL, 2530 mmol) in tetrahydrofuran (THF; 120 mL) was added dropwise a solution of the biphenyl-4-carbonyl chloride obtained above in THF (150 mL) at 0°C , and the mixture was stirred at 0°C for 0.5 h. The mixture was concentrated in vacuo and to the residue was added H_2O (200 mL) to allow precipitation. The precipitate was collected by filtration and washed with $\text{NaHCO}_3\text{aq.}$ (300 mL) to give light yellow powder. The powder was recrystallized from EtOH to give biphenyl-4-carboxylic hydrazide (9.43 g, 88%) as a light yellow powder. To a solution of the carboxylic hydrazide obtained above (1.00 g, 4.71 mmol) in pyridine (20 mL) was added Ac_2O (0.889 mL, 9.42 mmol) at 0°C , and the mixture was stirred at room temperature for 3 h. The mixture was concentrated in vacuo and to the residue was added Et_2O (20 mL) to allow precipitation. The precipitate was collected by filtration to give **4a** (0.900 g, 75%) as a colorless powder: ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.94 (3H, s), 7.40–7.53 (3H, m), 7.73–7.88 (4H, m), 8.01 (2H, d, $J=8.1$ Hz), 9.93 (1H, br s), 10.39 (1H, br s); MS (FAB) m/z 255 (MH^+).

***N'*-Formyl-biphenyl-4-carboxylic hydrazide (4b).** This was prepared by a procedure similar to that described for **4a** by using HCO_2Ac instead of Ac_2O (71%) as a colorless powder; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 7.40–7.53 (3H, m), 7.74–7.86 (4H, m), 7.99 (2H, d, $J=8.1$ Hz), 8.15 (1H, s), 9.96 (1H, br s), 10.34 (1H, br s); MS (FAB) m/z 241 (MH^+).

2-(4-Biphenyl)-5-methyl-1,3,4-oxadiazole (5a). To a solution of **4a** (3.71 g, 14.6 mmol) in toluene (140 mL) was added P_2O_5 (10.4 g, 73.0 mmol), and the mixture was stirred under reflux for 6 h. After cooling at room temperature, the mixture was poured into H_2O (200 mL) at 0°C . The mixture was made alkaline with 5 N NaOHaq. and extracted with AcOEt (250 mL $\times$ 2). The combined extract was washed with saturated $\text{NaHCO}_3\text{aq.}$ (300 mL) and saturated NaClaq. (300 mL), and then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ AcOEt = 3/1) to give **5a** (2.01 g, 58%) as a colorless amorphous powder: ^1H NMR (300 MHz, CDCl_3) δ 2.20 (3H, s), 7.38–7.51 (3H, m), 7.63–7.66 (2H, m), 7.73–7.76 (2H, m), 8.14–8.17 (2H, m); MS (FAB) m/z 237 (MH^+).

2-(4-Biphenyl)-1,3,4-oxadiazole (5b). This was prepared from **4b** by a procedure similar to that described for **5a** (74%) as a light yellow amorphous powder; ^1H NMR (300 MHz, CDCl_3) δ 7.36–7.52 (3H, m), 7.62–7.67 (2H, m), 7.73–7.77 (2H, m), 8.15–8.19 (2H, m); MS (FAB) m/z 223 (MH^+).

5-(4-Biphenyl)-4-(2-methoxyphenyl)-3-methyl-1,2,4-triazole (3). The mixture of **5a** (0.422 g, 1.79 mmol) and *o*-anisidine (5 mL) was stirred at 150°C for 14 h. After cooling at room temperature, the mixture was purified by column chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}$ = 30/1) to give crude **3** as a brown solid. The solid was recrystallized from $\text{AcOEt}-\text{Et}_2\text{O}$ to give **3** (0.192 g, 31%) as a light brown powder: mp $199-200^\circ\text{C}$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 2.16 (3H, s), 3.72 (3H, s), 7.10–7.14 (1H, m), 7.29–7.47 (7H, m), 7.55–7.59 (1H, m), 7.64–7.67 (4H, m); MS (FAB) m/z 342 (MH^+). Anal. calcd for $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}$: C, 77.40; H, 5.61; N, 12.31. Found: C, 77.27; H, 5.68; N, 12.00.

3-(4-Biphenyl)-4-(2-methoxyphenyl)-1,2,4-triazole (6). This was prepared from **5b** by a procedure similar to that described for **3** (12%) as a brown powder; mp $205-207^\circ\text{C}$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 3.63 (3H, s), 7.11 (1H, t, $J=7.5$ Hz), 7.25 (1H, d, $J=8.4$ Hz), 7.35–7.57 (7H, m), 7.67–7.69 (4H, m), 8.72 (1H, s); MS (FAB) m/z 328 (MH^+). Anal. calcd for $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O} \cdot 0.1\text{H}_2\text{O}$: C, 76.62; H, 5.27; N, 12.77. Found: C, 76.68; H, 5.19; N, 12.66.

5-(4-Biphenyl)-4-(2-methoxybenzyl)-3-methyl-1,2,4-triazole (7). This was prepared from **5a** by a procedure similar to that described for **3** using *o*-methoxybenzylamine instead of *o*-anisidine (28%) as a light orange powder; mp $199-201^\circ\text{C}$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 2.31 (3H, s), 3.75 (3H, s), 5.21 (2H, s), 6.56 (1H, d, $J=7.5$ Hz), 6.89 (1H, t, $J=7.5$ Hz), 7.03 (1H, d, $J=8.1$ Hz), 7.27–7.67 (6H, m), 7.70–7.79 (4H, m); MS (FAB) m/z 356 (MH^+). Anal. calcd for $\text{C}_{23}\text{H}_{21}\text{N}_3\text{O} \cdot 0.2\text{C}_4\text{H}_{10}\text{O}$: C, 77.21; H, 6.26; N, 11.35. Found: C, 77.18; H, 6.11; N, 11.10.

5-(4-Biphenyl)-4-(2-methoxyphenyl)-3-propyl-1,2,4-triazole (9i). To a solution of *o*-anisidine (7.10 g, 57.7 mmol) in pyridine (70 mL) was added dropwise a solution of butyryl chloride (7.18 mL, 69.2 mmol) in THF (35 mL) at 0°C , and the mixture was stirred at 0°C for 3 h. The mixture was concentrated in vacuo, and the residue was partitioned between AcOEt (200 mL) and H_2O (150 mL). The organic layer was washed with 1 N HClaq. (150 mL), saturated $\text{NaHCO}_3\text{aq.}$ (150 mL), and saturated NaClaq. (150 mL), and then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ AcOEt = 5/1) to give *N*-(2-methoxyphenyl)butyramide (11.6 g, 99%) as a light yellow syrup. To a solution of the butyramide obtained above (2.50 g, 12.9 mmol) in toluene (60 mL) was added P_2S_5 (3.16 g, 14.2 mmol), and the mixture was stirred at 70°C for 3 h. After cooling 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 = 8/1) to give *N*-(2-methoxyphenyl)thiobutyramide (1.54 g, 57%) as a yellow syrup. A suspension of the thiobutyramide obtained above (1.53 g, 7.31 mmol), K_2CO_3 (3.03 g, 21.9 mmol), and MeI (1.23 mL, 19.7 mmol) in CH_3CN (25 mL) was stirred at 50 °C for 3 h. After cooling at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between AcOEt (200 mL) and H_2O (150 mL). The organic layer was washed with saturated NaCl aq. (150 mL), and dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 20/1) to give **8i** (1.33 g, 81%) as a yellow syrup. A solution of **8i** (0.818 g, 3.66 mmol) and 4-biphenylcarboxylic hydrazide (0.457 g, 2.15 mmol) in DMF (8 mL) was stirred at 120 °C for 26 h. After cooling 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 **9i** as a yellow syrup. The syrup was crystallized from AcOEt–hexane to give **9i** (0.168 g, 21%) as a colorless powder: mp 145–146 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ 0.86 (3H, t, J = 7.4 Hz), 1.51–1.63 (2H, m), 2.44 (3H, t, J = 7.4 Hz), 3.70 (3H, s), 7.12 (1H, dt, J = 7.6, 1.2 Hz), 7.29 (1H, dd, J = 8.4, 1.2 Hz), 7.34–7.39 (1H, m), 7.42–7.47 (4H, m), 7.54–7.60 (1H, m), 7.63–7.68 (4H, m); MS (FAB) m/z 370 (MH^+). Anal. calcd for $C_{24}H_{23}N_3O$: C, 78.02; H, 6.27; N, 11.37. Found: C, 77.98; H, 6.57; N, 11.40.

Compounds **9a–h** and **9j** were prepared by a procedure similar to that described for **9i**. Yields referred to the final cyclization step.

5-(3-Biphenyl)-4-(2-methoxyphenyl)-3-methyl-1,2,4-triazole (9a). (70%); mp 112–113 °C (Et_2O –hexane); 1H NMR (400 MHz, $DMSO-d_6$) δ 2.18 (3H, s), 3.69 (3H, s), 7.14 (1H, dt, J = 7.7, 1.2 Hz), 7.30–7.50 (10H, m), 7.56–7.69 (2H, m); MS (FAB) m/z 342 (MH^+). Anal. calcd for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31. Found: C, 77.69; H, 5.55; N, 12.26.

5-(2-Biphenyl)-4-(2-methoxyphenyl)-3-methyl-1,2,4-triazole (9b). (17%); mp 142–143 °C (Et_2O –hexane); 1H NMR (400 MHz, $DMSO-d_6$) δ 2.04 (3H, s), 3.44 (3H, s), 6.33 (1H, d, J = 7.8 Hz), 6.74 (1H, t, J = 7.7 Hz), 6.92–6.96 (3H, m), 7.23–7.33 (5H, m), 7.38–7.51 (3H, m); MS (FAB) m/z 342 (MH^+). Anal. calcd for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31. Found: C, 77.67; H, 5.53; N, 12.28.

4-(2-Methoxyphenyl)-3-methyl-5-(4-phenoxyphenyl)-1,2,4-triazole (9c). (72%); mp 149–150 °C (AcOEt–hexane); 1H NMR (400 MHz, $DMSO-d_6$) δ 2.13 (3H, s), 3.70 (3H, s), 6.89–6.93 (2H, m), 7.00–7.04 (2H, m), 7.09 (1H, dt, J = 7.7, 1.1 Hz), 7.16–7.21 (1H, m), 7.27 (1H, dd, J = 8.4, 0.9 Hz), 7.31–7.44 (5H, m), 7.54 (1H, dt, J = 7.4, 1.7 Hz); MS (FAB) m/z 358 (MH^+). Anal. calcd for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 73.80; H, 5.35; N, 11.77.

5-(4-Benzyloxyphenyl)-4-(2-methoxyphenyl)-3-methyl-1,2,4-triazole (9d). (64%); mp 157–158 °C (AcOEt–hexane); 1H NMR (400 MHz, $DMSO-d_6$) δ 2.12 (3H, s),

3.33 (3H, s), 5.07 (2H, s), 6.96 (2H, d, J = 8.7 Hz), 7.09 (1H, t, J = 7.2 Hz), 7.25–7.43 (9H, m), 7.51–7.57 (1H, m), 7.54 (1H, dt, J = 7.4, 1.7 Hz); MS (FAB) m/z 372 (MH^+). Anal. calcd for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.37; H, 5.73; N, 11.28.

4-(2-Methoxyphenyl)-3-methyl-5-[4-(pyrrol-1-yl)phenyl]-1,2,4-triazole (9e). (65%); mp 181–183 °C (AcOEt–hexane); 1H NMR (400 MHz, $CDCl_3$) δ 2.29 (3H, s), 3.73 (3H, s), 6.32 (1H, t, J = 2.0 Hz), 7.00–7.15 (5H, m), 7.28 (2H, d, J = 9.0 Hz), 7.45–7.55 (3H, m), 7.54 (1H, dt, J = 7.4, 1.7 Hz); MS (FAB) m/z 331 (MH^+). Anal. calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.70; H, 5.62; N, 16.83.

4-(2-Methoxyphenyl)-3-methyl-5-[4-(piperidino)phenyl]-1,2,4-triazole (9f). (42%); mp 125–126 °C (AcOEt–hexane); 1H NMR (400 MHz, $CDCl_3$) δ 1.50–1.65 (6H, m), 2.24 (3H, s), 3.05–3.15 (4H, m), 3.73 (3H, s), 6.75 (2H, d, J = 9.0 Hz), 6.95–7.10 (3H, m), 7.25–7.30 (2H, m), 7.45–7.50 (1H, m); MS (FAB) m/z 349 (MH^+). Anal. calcd for $C_{21}H_{24}N_4O$: C, 72.39; H, 6.94; N, 16.08. Found: C, 72.16; H, 6.94; N, 16.06.

4-(2-Methoxyphenyl)-3-methyl-5-[4-(morpholino)phenyl]-1,2,4-triazole (9g). (31%); mp 190–192 °C (AcOEt–hexane); 1H NMR (400 MHz, $CDCl_3$) δ 2.25 (3H, s), 3.14 (4H, t, J = 5.0 Hz), 3.73 (3H, s), 3.81 (4H, t, J = 5.0 Hz), 6.74 (2H, d, J = 9.0 Hz), 6.95–7.15 (3H, m), 7.32 (2H, d, J = 9.0 Hz), 7.45–7.52 (1H, m); MS (FAB) m/z 351 (MH^+). Anal. calcd for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.28; H, 6.51; N, 15.96.

5-(4-Biphenyl)-3-ethyl-4-(2-methoxyphenyl)-1,2,4-triazole (9h). (59%); mp 122–123 °C (AcOEt–hexane); 1H NMR (400 MHz, $DMSO-d_6$) δ 1.14 (3H, t, J = 7.5 Hz), 2.43–2.51 (2H, m), 3.71 (3H, s), 7.12 (1H, t, J = 7.7 Hz), 7.29 (1H, d, J = 8.4 Hz), 7.34–7.47 (6H, m), 7.54–7.67 (5H, m); MS (FAB) m/z 356 (MH^+). Anal. calcd for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82. Found: C, 77.78; H, 6.09; N, 11.82.

4-(2-Benzyloxyphenyl)-5-(4-biphenyl)-3-methyl-1,2,4-triazole (9j). (66%); mp 171–172 °C (AcOEt–hexane); 1H NMR (400 MHz, $CDCl_3$) δ 2.31 (3H, s), 4.95 (1H, d, J = 13.0 Hz), 5.06 (1H, d, J = 13.0 Hz), 6.95–7.15 (4H, m), 7.20–7.60 (14H, m); MS (FAB) m/z 418 (MH^+). Anal. calcd for $C_{28}H_{23}N_3O$: C, 80.55; H, 5.55; N, 10.06. Found: C, 80.25; H, 5.50; N, 9.91.

5-(4-Biphenyl)-4-(2-hydroxyphenyl)-3-methyl-1,2,4-triazole (10). To a solution of **9j** (0.100 g, 0.240 mmol) in MeOH (10 mL) was added 10% Pd/C (w/w, 0.080 g), and the mixture was stirred under hydrogen atmosphere at room temperature for 2 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3/MeOH$ = 10/1) to give crude **10** as a colorless solid. The solid was recrystallized from AcOEt–hexane to give **10** (0.055 g, 70%) as a colorless powder: mp > 300 °C; 1H 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⁺). Anal. calcd for C₂₁H₁₇N₃O: C, 77.04; H, 5.23; N, 12.84. Found: C, 76.88; H, 5.02; N, 12.70.

5-(4-Biphenyl)-3-methyl-4-[2-(2-morpholinoethoxy)phenyl]-1,2,4-triazole (11b). To a suspension of **10** (0.200 g, 0.610 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (0.186 g, 1.00 mmol) in DMF (3 mL) was added 60% NaH (0.150 g, 3.80 mmol), and the mixture was stirred at 60 °C for 2 h. After cooling at room temperature, the mixture was partitioned between AcOEt (20 mL) and H₂O (20 mL). The organic layer was washed with saturated NaCl aq. (20 mL), and then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CHCl₃/MeOH = 10/1) to give crude **11b** as a colorless solid. The solid was recrystallized from AcOEt–hexane to give **11b** (0.185 g, 69%) as a colorless powder: mp 137–139 °C; ¹H NMR (400 MHz, CDCl₃) δ 2.33 (3H, s), 2.25–2.45 (4H, m), 2.45–2.65 (2H, m), 3.50–3.65 (4H, m), 3.90–4.20 (2H, m), 7.00–7.60 (13H, m); MS (FAB) *m/z* 441 (MH⁺). Anal. calcd for C₂₇H₂₈N₄O₂: C, 73.61; H, 6.41; N, 12.72. Found: C, 73.57; H, 6.46; N, 12.65.

Compounds **11a** and **11c** were prepared by a procedure similar to that described for **11b**.

5-(4-Biphenyl)-4-(2-ethoxyphenyl)-3-methyl-1,2,4-triazole (11a). (61%); mp 153–155 °C (AcOEt–hexane); ¹H NMR (400 MHz, CDCl₃) δ 1.18 (3H, t, *J* = 7.0 Hz), 2.32 (3H, s), 3.85–4.05 (2H, m), 7.02 (1H, t, *J* = 7.0 Hz), 7.16 (1H, d, *J* = 7.0 Hz), 7.25–7.60 (11H, m); MS (FAB) *m/z* 356 (MH⁺). Anal. calcd for C₂₃H₂₁N₃O: C, 77.72; H, 5.96; N, 11.82. Found: C, 77.46; H, 6.26; N, 11.66.

5-(4-Biphenyl)-3-methyl-4-{2-[(4-methylpiperazin-1-yl)-carbonyl]methoxy}-1,2,4-triazole (11c). (47%); mp 153–155 °C (AcOEt–hexane); ¹H NMR (400 MHz, CDCl₃) δ 2.22 (3H, s), 2.34 (3H, s), 2.05–2.40 (4H, m), 3.25–3.40 (2H, m), 3.43–3.70 (2H, m), 4.66 (2H, s), 7.03–7.60 (13H, m); MS (FAB) *m/z* 468 (MH⁺). Anal. calcd for C₂₈H₂₉N₅O₂: C, 71.93; H, 6.25; N, 14.98. Found: C, 71.88; H, 6.23; N, 14.86.

3-(4-Biphenyl)-1-(2-methoxyphenyl)-5-methyl-1,2,4-triazole (13). To a suspension of 4-cyanobiphenyl (1.01 g, 5.66 mmol) in EtOH (0.458 mL, 11.4 mmol) was added 4N HCl(g)/AcOEt (11.3 mL, 45.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 24 h. To the resulting mixture was added Et₂O (50 mL) to allow precipitation. The precipitate was collected by filtration and washed with Et₂O to give ethyl biphenyl-4-carboximidate hydrochloride (1.07 g, 72%) as a colorless powder. To a suspension of the carboximidate obtained above (1.07 g, 4.08 mmol) and Et₃N (1.44 mL, 10.3 mmol) in toluene (16 mL) was added dropwise a solution of AcCl (0.337 mL, 4.74 mmol) in toluene (4 mL), and the mixture was stirred at room temperature for 20 h. The precipitate was collected by filtration and washed with EtOH to give **12** as a brown solid. To a suspension of **12** obtained above in toluene (40 mL) were added Et₃N (1.14 mL, 8.16 mmol) and 2-methoxyphenylhydrazine hydrochloride (1.07 g, 6.12 mmol), and the mixture was stirred at 60 °C for 20 h. After cooling

at room temperature, the mixture was poured into saturated NaHCO₃ aq. (50 mL) and the whole was extracted with CHCl₃ (25 mL × 3). The combined extract was washed with saturated NaCl aq. (40 mL), and then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 2/1) to give **13** as a brown foam: ¹H NMR (300 MHz, DMSO-*d*₆) δ 2.30 (3H, s), 3.84 (3H, s), 7.16 (1H, t, *J* = 7.8 Hz), 7.30–7.61 (6H, m), 7.73–7.80 (4H, m), 8.10 (2H, d, *J* = 8.1 Hz); MS (FAB) *m/z* 342 (MH⁺). Anal. calcd for C₂₂H₁₉N₃O · 0.5H₂O: C, 75.41; H, 5.75; N, 11.99. Found: C, 75.38; H, 5.60; N, 11.97.

Pharmacology

Binding assay. Human AVP receptor subtypes were stably expressed in CHO cells and plasma membranes prepared according to the reported protocols.¹⁴ [³H]-AVP (0.5–1.0 nM, 80 Ci mmol^{−1}, DuPont-New England Nuclear) was added to each membrane preparation, which were then incubated with various concentrations of compounds in 250 μM of assay buffer (50 mM Tris–HCl, pH 7.4, 10 mM MgCl₂, and 0.1% bovine serum albumin). After 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 MgCl₂) 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* = IC₅₀/(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}, 1.21 ± 0.37 nM for hV₂, respectively (*n* = 5).

Antagonist activities for the hV_{1A} receptor.¹⁴ CHO cells expressing hV_{1A} were plated on coverglasses (13.5 mm in diameter) and serum-starved for 24 h. Cell monolayers were loaded in MEM-α with Fura 2-acetoxymethyl ester (2 μM/coverglass) for 30 min at 37 °C. They were then washed with PBS, transferred to a Fura 2-free medium and incubated for an additional 30 min at 37 °C. The loaded monolayers were then stored in Krebs–Henseleit–HEPES buffer (NaCl 130 mM, KCl 5 mM, CaCl₂ 1.25 mM, MgSO₄ 0.8 mM, glucose 5.5 mM, HEPES 20 mM, and 0.1% bovine serum albumin, pH 7.4). The coverglass was placed into a quartz cuvette containing 2 mL Krebs–Henseleit–HEPES buffer and maintained at 37 °C with continuous stirring. When terminal equilibrium was reached, the fluorescence signal was recorded with a CAF-110 spectrofluorometer (Japan Spectrometer Co.) at both 340 and 380 nm excitation wavelengths, and 500 nm emission wavelength. After recording the baseline signal for 3 min, AVP was added to the cuvette to stimulate the mobilization of

intracellular calcium in the presence or absence of compounds. Fluorescence measurements were converted to $[Ca^{2+}]_i$ by determining maximal fluorescence (R_{max}) with the nonfluorescent Ca^{2+} ionophore, ionomycin (25 μ M), after which minimal fluorescence (R_{min}) was obtained by adding 3 nM EGTA. From the ratio of fluorescence at 340 and 380 nm, the $[Ca^{2+}]_i$ was determined using the following equation: $[Ca^{2+}]_i$ (nM) = $K_d \times [(R - R_{min}) / (R_{max} - R) \times b]$. The term b is the ratio of fluorescence of Fura 2 at 380 nm in zero and saturating Ca^{2+} , K_d is the dissociation constant of Fura 2 for Ca^{2+} , assumed to be 224 nM.¹⁵

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