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Optimization of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepine-5-ylidene) acetamide derivatives as arginine vasopressin V_2 receptor agonists and discussion of their binding modes

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ABSTRACT

A series of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepine-5-ylidene)acetamide derivatives were optimized to achieve potent agonistic activity, both in vitro and in vivo, for the arginine vasopressin V_2 receptor, resulting in the eventual discovery of compound **1g**. Molecular modeling of compound **1g** with V_2 receptor was also examined to evaluate the binding mode of this series of compounds.

1. Introduction

Arginine vasopressin (AVP) is a cyclic nonapeptide that is produced and secreted by the hypothalamo-neurohypophysial system. Stimulation of the V_2 receptor with AVP induces water reabsorption in the kidneys by increasing cAMP with subsequent activation of the aquaporin-2 water channel, resulting in a reduction in urine volume.[1] This implies that a V_2 receptor agonist may be used to treat diseases such as central diabetes insipidus and nocturia.[2] Well-known compounds which exhibit this mechanism of action include desmopressin (dDAVP),[3] **OPC-51803**,[4] and **VNA932**[5] (Fig. 1). More recently, the utility of novel benzylurea derivatives has been reported.[6] In a previous paper, we reported on the primary exploration of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetamide derivatives as novel V_2 receptor agonists (Fig. 2).[7]

In our previous study, we showed that the structure of the tail moiety was crucial in achieving expression of agonistic activity. Specifically, the tail had to be a less sterically hindered hydrophobic moiety, such as 3-methylpyrrazolyl or 3-methylpyrrolidinyl. In contrast, the head moiety was found to have wide latitude for V_2 binding and agonistic activity. In particular, the presence of a polar hetero atom at a certain position often increased the compound's affinity for the V_2 receptor (Fig. 2). For example, 2-hydroxyethyl group in the head position is preferable.

Here, in order to develop a derivative with potent in vitro and in vivo V_2 agonistic activity, we describe the structural optimization of this series of compounds by combining above-mentioned head and tail structures to discover (2*Z*)-2-(4,4-difluoro-1-{4-[(3*S*)-3-methylpyrrolidin-1-yl]-2-(trifluoromethyl)benzoyl}-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(2-hydroxyethyl)acetamide (**1g**), which showed potent V_2 agonistic activity both in vitro and in vivo. We also discuss the binding of compound **1g** with the V_2 receptor, using the molecular modeling method.

2. Chemistry

The synthetic route for the compounds evaluated for their V_2 activity is shown in Scheme 1. Treatment of methyl 2-chloro-4-fluorobenzoate (**2**) or methyl 4-fluoro-2-(trifluoromethyl)benzoate (**3**) with cyclic amines gave **4b–4g**. Hydrolysis of the ester groups and subsequent amidations with methyl (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate[8] using corresponding acid chlorides resulted in compounds **5b–5g**. The esters in the head moiety were converted to the carboxylic acids and then reacted

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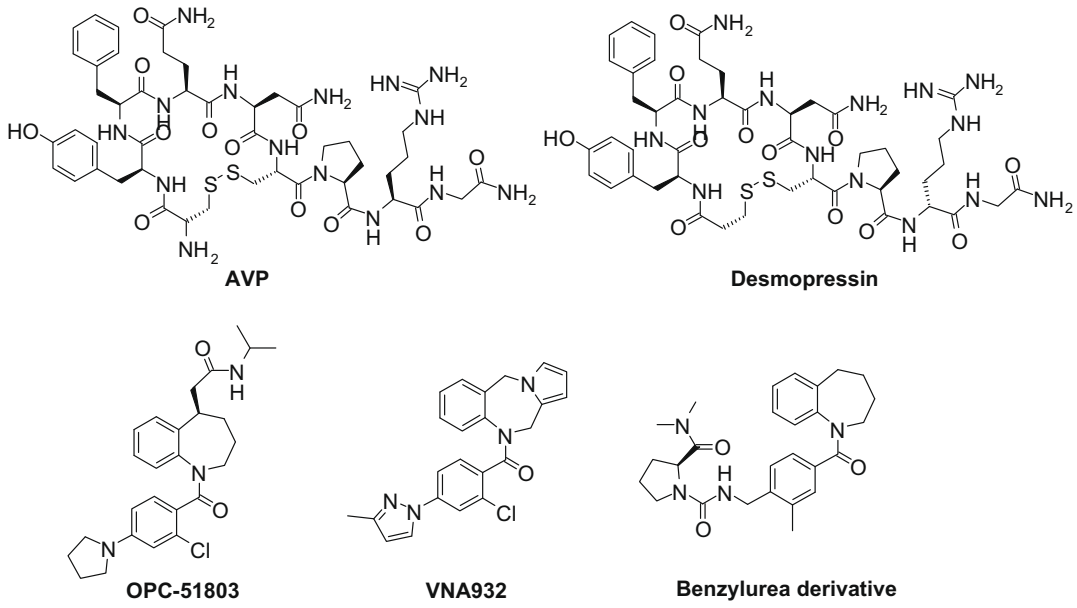


Figure 1. Chemical structures of AVP and well-known arginine vasopressin V_2 receptor agonists.

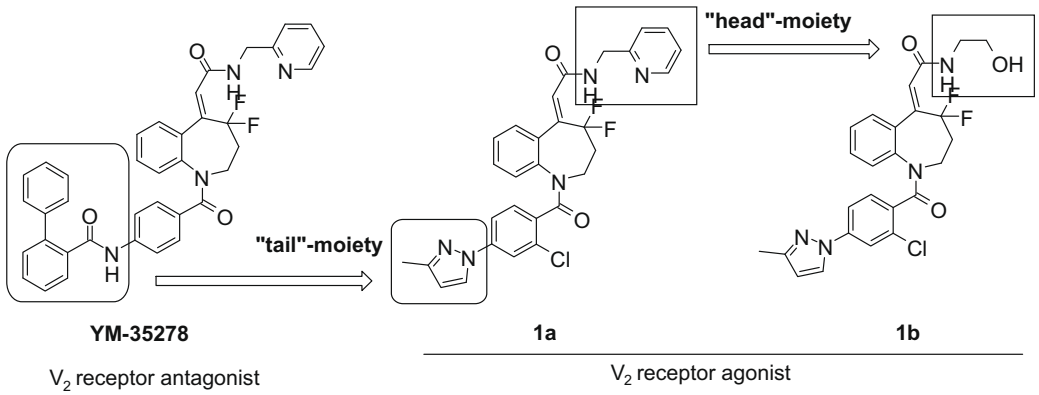


Figure 2. An outline of our previous study regarding (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepine-5-ylidene)acetamide derivatives as arginine vasopressin V_2 receptor agonists.

with 2-hydroxyethylamine in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole to yield the target derivatives **1b–1g** (Scheme 1).

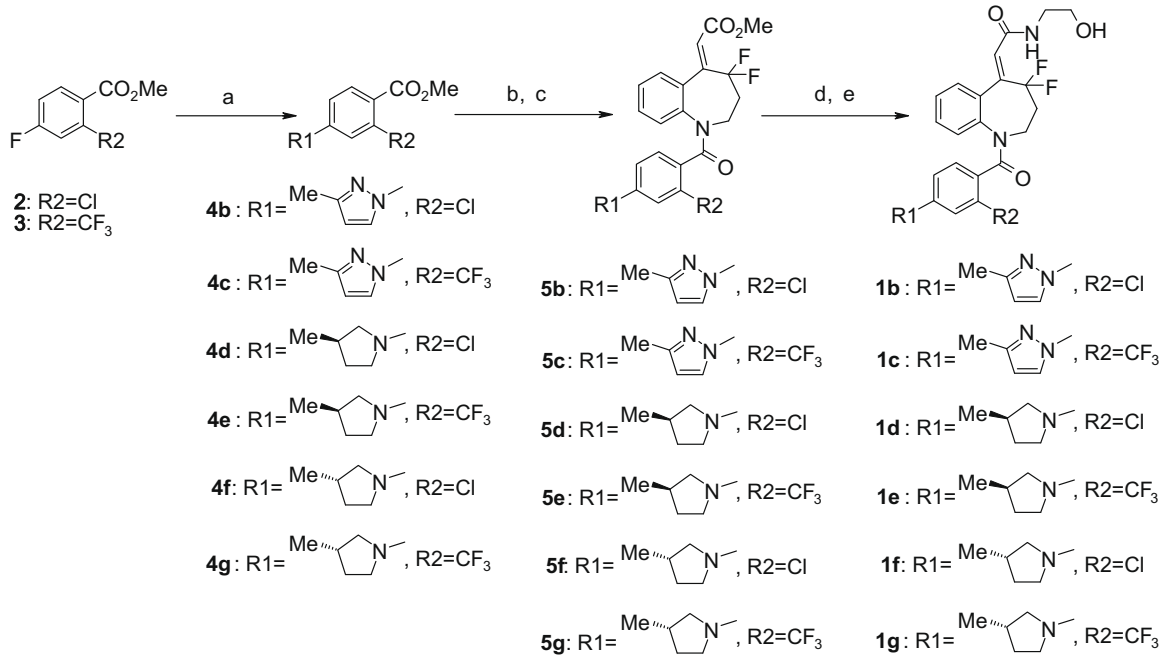
3. Results and discussion

To optimize the derivatives, 3-methylpyrazole, (*R*)-3-methylpyrrolidine, and (*S*)-3-methylpyrrolidine were introduced into the tail moiety, while the head moiety was fixed with 2-hydroxyethyl amide. At the same time, introduction of either chloro- or trifluoromethyl group as R_2 substitution was also examined.

Most compounds (**1b**, **1d**–**1g**) showed potent binding affinity to the V_2 receptor, with K_i values of approximately 10 nM, though only **1c** was found to reduce the affinity. With regard to the substitution of the benzene ring, replacing the chloro group with a trifluoromethyl group resulted in decreased binding affinity to the V_{1a} receptor in every case. Attaching 3-methylpyrrolidine ring as tail moiety led to significantly more potent intrinsic efficacy compared to the 3-methylpyrazole derivative. Further, **1e** and **1g** exhibited excellent in vivo anti-diuretic activity, with respective ED_{50} values of 0.040 mg/kg po and 0.012 mg/kg po. In the present study, the (*S*)-3-methylpyrrolidine derivative exhibited more potent anti-diuretic activity in rats, while both of the (*R*)-3-methylpyrrolidine and (*S*)-3-methylpyrrolidine had nearly identical in vitro profiles, possibly due to the fact that the (*S*)-3-methylpyrrolidine derivative is more stably metabolized in rats. As a collateral evidence, the pharmacodynamics of compound **1e** and **1g** are illustrated in Figure 3, in which compound **1g** showed more consistent suppression effect of urinary excretion rate than **1e**. Thus we successfully obtained the selective, potent, orally active V_2 receptor agonist **1g** after optimizing several moieties (see Table 1).

A structural model of the human V_2 receptor-**1g** complex is shown in Figure 4. A homology model for the human V_2 receptor was constructed using the recently identified bovine rhodopsin crystal structure[9] as a template, and a docking study of **1g** was performed using GOLD ver. 3.1.1 (Cambridge Crystallographic Data Center, Cambridge, UK).[10]

Results from the docking study indicated that the binding site for **1g** is located in a pocket surrounded by the human V_2 receptor transmembrane helices III, V, VI, and VII. The tail moiety of **1g** occupies the bottom of the pocket where hydrophobic structures



Scheme 1. Reagents and conditions: (a) amine, K_2CO_3, NMP; (b) aq NaOH, MeOH; (c) $SOCl_2$, catalytic DMF, THF then methyl (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate,[8] pyridine; (d) aq NaOH, MeOH; (e) 2-hydroxyethylamine, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF.

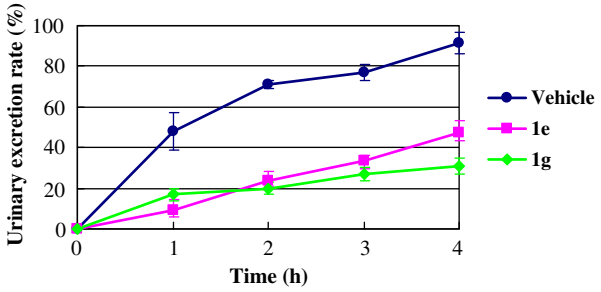


Figure 3. Pharmacodynamics of compounds **1e** and **1g**, orally-administered 0.03 mg/kg, on urinary excretion rate in water-loaded rats. The urinary excretion rate is the ratio of urine volume to the volume of loaded water. All assays were performed in $n = 3–6$.

are preferred, and the head moiety extends into the solvent, as this moiety is able to easily tolerate a range of chemical conversion. These observations may help explain our previous findings. Observations that substituting polar groups such as *N*-methyl piperazine and dimethylamino pyrrolidine in the tail moiety led to markedly reduced binding affinity than hydrophobic substitutions[7a] were well supported by the structure model of the tail moiety. Further, with regard to the head moiety, structure of the binding pocket revealed in the docking study showed that this region had wide latitude for structural conversion and was thus a good place to tune physiochemical properties as well as pharmacological activities. The interaction between the 2-hydroxyethyl group in the head moiety and Gln180 was also considered helpful in achieving more effective binding between this compound and the V_2 receptor, indicating that the introduction of a polar atom into the appropriate position in the head moiety (such as a 2-hydroxyethyl group or pyridin-2-yl-methyl group[7b]) often increases binding potency.

4. Conclusion

Here, to develop a derivative with potent in vitro and in vivo V_2 agonistic activity, a series of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepine-5-ylidene)acetamide derivatives were optimized by structural combination of head and tail moiety in addition to benzene ring substitutions. These attempts led to the eventual discovery of (2*Z*)-2-(4, 4-difluoro-1-{4-[(3*S*)-3-methylpyrrolidin-1-yl]-2-(trifluoromethyl)benzoyl}-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(2-hydroxyethyl)acetamide (**1g**), which showed extremely potent in vivo anti-diuretic activity, with an ED_{50} value of 0.012 mg/kg po in rats. Further, the binding mode of this series of derivatives was determined in a molecular modeling study between compound **1g** and the human V_2 receptor. The docking study well agreed to the actual SARs of this series of derivatives.

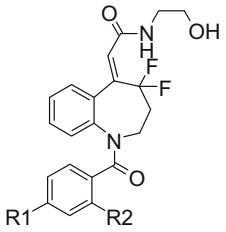
5. Experiment

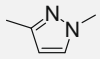
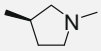
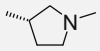
5.1. Chemistry

In general, reagents and solvents were used as purchased without further purification. Melting points were determined with a Yanaco MP-500D melting point apparatus and left uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer. Chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal standard (NMR descriptions; s, singlet; d, doublet; t, triplet; q, quartet; dt, double triplet; m, multiplet, and br, broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of theoretical values.

(2*Z*)-2-{1-[2-Chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(2-hydroxy-

Table 1
Binding affinity, cAMP accumulation activity and in vivo activity of compounds **1b**–**1g**



	R_1 =	R_2 =	Binding affinity: K_i[a] (nM)			cAMP accumulation		Anti-diuretic activity ED_{50}[d] (mg/kg *po*)
			V_2 (nM)	V_{1a} (nM)	V_{1a}/V_2	EC_{50}[b] (nM)	IA[c] (%)	
1b		Cl	9.7	28	2.9	6.3	93.9	0.14
1c		CF_3	78	170	2.2	2.4	115	0.20
1d		Cl	13	13	1.0	0.99	118	—
1e		CF_3	16	39	2.4	1.0	114	0.040
1f		Cl	14	17	1.2	0.98	109	—
1g		CF_3	14	43	3.1	1.0	111	0.012

[a] Binding affinity for human V_2 and V_{1a} receptors. Receptors expressed on CHO cells were used. All assays were performed in triplicate.
[b] EC_{50} values were determined as the concentration of the test compound required to increase the cAMP level to 50% of the maximum response to AVP. All assays were performed in triplicate.
[c] Intrinsic activity (IA) was calculated as the percentage (%) of the maximum response to the test compound compared to the maximum response (100%) to AVP. All assays were performed in triplicate.
[d] Effects of oral administration of test compounds on urinary excretion rate in water-loaded rats. The ED_{50} value represents the dose of the test compounds required to decrease the urinary excretion rate by 50%.

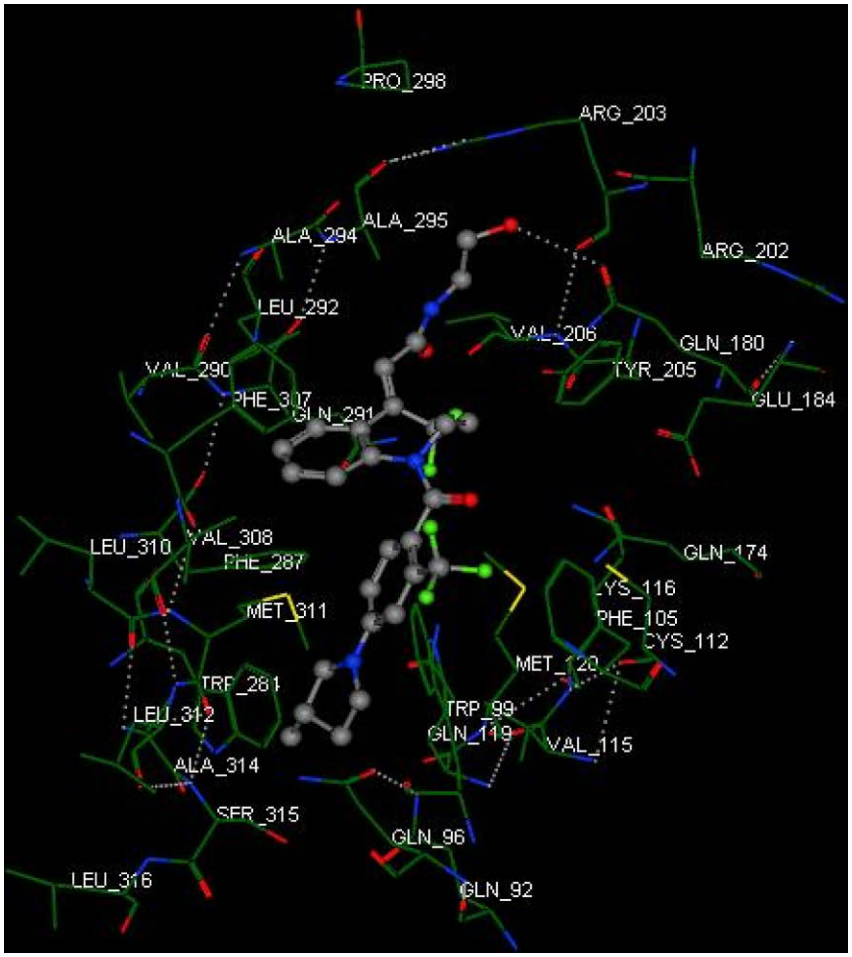


Figure 4. Structural model of the human V_2 receptor-**1g** complex. The stick-and-ball representation shows the compound **1g**.

ethyl)acetamide (**1b**) was prepared from 2-chloro-4-fluorobenzoic acid according to the procedure described in our previous paper.[7]

5.1.1. Methyl 4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl) benzoate (4c)

A mixture of methyl 4-fluoro-2-(trifluoromethyl)benzoate (**3**; 20.8 g, 93.5 mmol), 3-methylpyrazole (10.8 g, 131 mmol) and potassium carbonate (25.8 g, 187 mmol) in dimethylformamide (DMF) (150 mL) was heated overnight at 100 °C. The reaction was then allowed to cool to room temperature and partitioned between water and ethyl acetate. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 15:1) to give the title compound **4c** (15.2 g, 57%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (3H, s), 3.88 (3H, s), 6.45 (1H, d, J = 2.4 Hz), 8.00 (1H, d, J = 8.4 Hz), 8.20 (1H, d, J = 9.6 Hz), 8.25 (1H, s), 8.64 (1H, d, J = 2.0 Hz).

5.1.2. (*R*)-Methyl 2-chloro-4-(3-methylpyrrolidin-1-yl)benzoate (4d)

Compound **4d** was prepared according to the procedure described for **4c**. The title compound **4d** (830 mg, 33%) was obtained as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (3H, d, J = 6.9 Hz), 1.53–1.64 (1H, m), 2.05–2.14 (1H, m), 2.29–2.40 (1H, m), 2.82–2.89 (1H, m), 3.25–3.50 (3H, m), 3.75 (3H, s), 6.50 (1H, dd, J = 8.8, 2.5 Hz), 6.54 (1H, d, J = 2.5 Hz), 7.75 (1H, d, J = 8.8 Hz). MS (FAB) m/z 254 $[M+1]^+$.

5.1.3. (*R*)-Methyl 4-(3-methylpyrrolidin-1-yl)-2-(trifluoro methyl)benzoate (4e)

Compound **4e** was prepared according to the procedure described for **4c**. The title compound **4e** (790 mg, 42%) was obtained as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (3H, d, J = 6.6 Hz), 1.61–1.73 (1H, m), 2.12–2.23 (1H, m), 2.36–2.50 (1H, m), 2.89–2.97 (1H, m), 3.31–3.55 (3H, m), 3.86 (3H, s), 6.56 (1H, dd, J = 8.8, 2.6 Hz), 6.81 (1H, d, J = 2.4 Hz), 7.85 (1H, d, J = 8.8 Hz). MS (FAB) m/z 288 $[M+1]^+$.

5.1.4. (*S*)-Methyl 2-chloro-4-(3-methylpyrrolidin-1-yl)benzoate (4f)

Compound **4f** was prepared according to the procedure described for **4c**. The title compound **4f** (546 mg, 58%) was obtained as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (3H, d, J = 6.4 Hz), 1.53–1.64 (1H, m), 2.05–2.14 (1H, m), 2.29–2.40 (1H, m), 2.82–2.89 (1H, m), 3.24–3.49 (3H, m), 3.75 (3H, s), 6.49 (1H, dd, J = 8.8, 2.5 Hz), 6.53 (1H, d, J = 2.4 Hz), 7.75 (1H, d, J = 8.8 Hz). MS (FAB) m/z 254 $[M+1]^+$.

5.1.5. (*S*)-Methyl 4-(3-methylpyrrolidin-1-yl)-2-(trifluoro methyl)benzoate (4g)

Compound **4g** was prepared according to the procedure described for **4c**. The title compound **4g** (710 mg, 60%) was obtained as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.08 (3H, d, J = 6.4 Hz), 1.55–1.66 (1H, m), 2.07–2.16 (1H, m), 2.31–2.42 (1H, m), 2.88–2.93 (1H, m), 3.28–3.55 (3H, m), 3.77 (3H, s), 6.74 (1H, dd, J = 8.8, 2.4 Hz), 6.80 (1H, d, J = 2.5 Hz), 7.80 (1H, d, J = 8.8 Hz). MS (FAB) m/z 288 $[M+1]^+$.

5.1.6. Methyl (2*Z*)-2-{4,4-difluoro-1-[4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl)benzoyl]-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (5c)

A mixture of methyl 4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl)benzoate (**4c**; 1.09 g, 3.83 mmol) and aqueous sodium hydroxide (5 M, 4.0 mL, 20 mmol) in methanol (20 mL) was refluxed for 1 h. The reaction was then allowed to cool to room temperature and subsequently poured into aqueous hydrochloric acid. Resulting precipitates were collected by filtration and dried in vacuo to give 4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl) benzoic acid (1.00 g, 97%) as white solid. A mixture of 4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl) benzoic acid (1.00 g, 3.70 mmol), DMF (several drops) and oxalyl chloride (0.80 mL) in tetrahydrofuran (10 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated and azeotroped with toluene. To a solution of this residue in pyridine (10 mL) was added methyl (2*Z*)-(4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate[8] (850 mg, 3.36 mmol), and the mixture was stirred overnight at room temperature. The solvent was evaporated, and the residue was partitioned between water and chloroform. The organic phase was washed with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 4:1) to give the title compound **5c** (1.70 g, quantitative yield) as colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.23 (3H, s), 2.40–2.60 (1H, m), 3.15–3.30 (1H, m), 3.50–3.75 (1H, m), 3.79 (3H, s), 4.60–4.96 (1H, br), 6.37 (1H, d, J = 2.4 Hz), 6.74 (1H, s), 6.84 (1H, d, J = 7.8 Hz), 6.86–6.97 (1H, m), 7.21 (1H, dt, J = 1.4, 7.8 Hz), 7.28 (1H, t, J = 7.8 Hz), 7.40 (1H, d, J = 7.3 Hz), 7.87 (1H, d, J = 8.8 Hz), 8.09 (1H, s), 8.48 (1H, d, J = 2.5 Hz). MS (FAB) m/z 506 $[M+H]^+$.

5.1.7. Methyl (2*Z*)-2-(1-{2-chloro-4-[(3*R*)-3-methylpyrrolidin-1-yl]benzoyl}-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate (5d)

Compound **5d** was prepared according to the procedure described for **5c**. The title compound **5d** (330 mg, 22% from **4d**) was obtained as colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (3H, d, J = 6.4 Hz), 1.48–1.58 (1H, m), 1.97–2.09 (1H, m), 2.25–2.55 (3H, m), 2.64–2.77 (1H, m), 3.10–3.40 (4H, m), 3.76 (3H, s), 4.40–5.05 (1H, br), 6.13–6.25 (1H, m), 6.34–6.40 (1H, m), 6.51 (1H, s), 6.60–6.67 (1H, m), 6.88–6.98 (1H, m), 7.18–7.36 (3H, m). MS (FAB) m/z 475 $[M+H]^+$.

5.1.8. Methyl (2*Z*)-2-(4,4-difluoro-1-{4-[(3*R*)-3-methyl pyrroli din-1-yl]-2-(trifluoromethyl)benzoyl}-1,2,3,4-tetrahydro -5*H*-1-benzazepin-5-ylidene)acetate (5e)

Compound **5e** was prepared according to the procedure described for **5c**. The title compound **5e** (1.07 g, 77% from **4e**) was obtained as colorless amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (3H, d, J = 6.2 Hz), 1.53–1.62 (3H, m), 2.05–2.83 (5H, m), 3.15–3.44 (3H, m), 3.84 (3H, s), 6.14–6.21 (2H, m), 6.60–6.68 (2H, m), 6.82–6.89 (1H, m), 7.06–7.33 (3H, m). MS (FAB) m/z 509 $[M+H]^+$.

5.1.9. Methyl (2*Z*)-2-(1-{2-chloro-4-[(3*S*)-3-methylpyrrolidin-1-yl]benzoyl}-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate (5f)

Compound **5f** was prepared according to the procedure described for **5c**. The title compound **5f** (433 mg, 45% from **4f**) was obtained as colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (3H, d, J = 6.4 Hz), 1.47–1.58 (1H, m), 1.98–2.09 (1H, m), 2.23–2.55 (3H, m), 2.65–2.76 (1H, m), 3.10–3.40 (4H, m), 3.76 (3H, s), 4.50–5.05 (1H, br), 6.13–6.25 (1H, m), 6.33–6.41 (1H, m), 6.50 (1H, s), 6.60–6.69 (1H, m), 6.88–6.98 (1H, m), 7.18–7.36 (3H, m). MS (FAB) m/z 475 $[M+H]^+$.

5.1.10. Methyl (2*Z*)-2-(4,4-difluoro-1-{4-[(3*S*)-3-methylpyrroli din-1-yl]-2-(trifluoromethyl)benzoyl}-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate (5g)

Compound **5g** was prepared according to the procedure described for **5c**. The title compound **5g** (840 mg, 68% from **4g**) was obtained as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (3H,

d, J = 6.3 Hz), 1.48–1.62 (1H, m), 2.00–2.09 (1H, m), 2.27–2.50 (3H, m), 2.71–2.79 (1H, m), 3.13–3.39 (4H, m), 3.77 (3H, s), 4.58–4.95 (1H, br), 6.38–6.81 (5H, m), 7.15–7.40 (3H, m). MS (FAB) m/z 509 $[M+H]^+$.

5.1.11. (2*Z*)-2-{4,4-Difluoro-1-[4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl)benzoyl]-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(2-hydroxyethyl)acetamide (1c)

Methyl (2*Z*)-2-{4,4-difluoro-1-[4-(3-methyl-1*H*-pyrazol-1-yl)-2-(trifluoromethyl)benzoyl]-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (**5c**; 1.69 g, 3.36 mmol) in methanol (20 mL) was treated with sodium hydroxide (1.0 M, 6.0 mL, 6.0 mmol) at room temperature for 14 h. The reaction was partitioned between aqueous hydrochloride and chloroform. The organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the corresponding carboxylic acid (1.78 g, quantitative yield). A mixture of the foregoing carboxylic acid (350 mg, 0.71 mmol), 2-aminoethanol (0.060 mL, 1.07 mmol), WSCD (274 mg, 1.07 mmol), and HOBt (106 mg, 1.07 mmol) in DMF (10 mL) was stirred overnight at room temperature. The reaction mixture was partitioned between water and ethyl acetate, then the organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/methanol = 35:1) and subsequently crystallized from *n*-hexane/2-propanol = 4:1 to give the title compound **1c** (295 mg, 78%) as colorless crystals. Mp: 125–128 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (3H, s), 2.37–2.45 (1H, m), 2.71–2.87 (1H, m), 3.08–3.29 (4H, m), 3.49 (2H, t, J = 6.4 Hz), 4.70–4.92 (1H, br), 6.36 (1H, d, J = 2.5 Hz), 6.48 (1H, s), 6.77 (1H, d, J = 7.8 Hz), 7.03 (1H, d, J = 8.8 Hz), 7.15 (1H, dt, J = 1.5, 7.8 Hz), 7.25 (1H, dt, J = 1.5, 7.8 Hz), 7.34 (1H, dd, J = 1.5, 7.8 Hz), 7.84 (1H, dd, J = 1.5, 8.8 Hz), 8.09 (1H, d, J = 1.5 Hz), 8.47 (1H, d, J = 2.5 Hz), 8.51 (1H, t, J = 5.3 Hz). MS (FAB) m/z 535 $[M+1]^+$. Anal. Calcd for $C_{26}H_{23}F_5N_4O_3{\cdot}0.5H_2O$: C, 57.46; H, 4.45; N, 10.31; F, 17.48. Found: C, 57.53; H, 4.35; N, 10.10; F, 17.69.

5.1.12. (2*Z*)-2-(1-{2-Chloro-4-[(3*R*)-3-methylpyrrolidin-1-yl]benzoyl}-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(2-hydroxyethyl)acetamide (1d)

Compound **1d** was prepared according to the procedure described for **1c**. The title compound **1d** (126 mg, 94% in hydrolysis step, 83% in amidation) was obtained as colorless crystals. Mp: 127–129 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (3H, d, J = 5.4 Hz), 1.45–1.55 (1H, m), 1.98–2.05 (1H, m), 2.20–2.35 (1H, m), 2.65–2.75 (1H, m), 3.08–3.31 (6H, m), 3.43–3.49 (2H, m), 4.33 (1H, d, J = 3.9 Hz), 4.71 (1H, t, J = 5.4 Hz), 4.75–4.90 (1H, br), 6.13–6.18 (1H, m), 6.27 (1H, s), 6.36–6.40 (1H, m), 6.62–6.69 (1H, m), 6.81–6.90 (1H, m), 7.13–7.32 (3H, m), 8.32–8.41 (1H, m). MS (FAB) m/z 504 $[M+1]^+$. Anal. Calcd for $C_{26}H_{28}ClF_2N_3O_3{\cdot}C_3H_8O$: C, 61.75; H, 6.43; N, 7.45; Cl, 6.29; F, 6.74. Found: C, 61.39; H, 6.28; N, 7.54; Cl, 6.13; F, 6.62.

5.1.13. (2*Z*)-2-(4,4-Difluoro-1-{4-[(3*R*)-3-methylpyrrolidin-1-yl]-2-(trifluoromethyl)benzoyl}-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(2-hydroxyethyl)acetamide (1e)

Compound **1e** was prepared according to the procedure described for **1c**. The title compound **1e** (167 mg, quantitative yield in hydrolysis step, 74% in amidation) was obtained as colorless crystals. Mp: 174–177 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (3H, d, J = 6.8 Hz), 1.47–1.60 (1H, m), 2.00–2.10 (1H, m), 2.25–2.40 (2H, m), 2.65–2.81 (2H, m), 3.14–3.50 (8H, m), 4.72 (1H, t, J = 5.4 Hz), 4.76–4.91 (1H, br), 6.35–6.43 (2H, m), 6.62 (1H, s), 6.65–6.74 (2H, m), 7.16 (1H, t, J = 7.3 Hz), 7.22 (1H, t, J = 7.3 Hz), 7.31 (1H, d, J = 6.8 Hz), 8.43 (1H, s). MS (FAB) m/z 538 $[M+1]^+$. Anal. Calcd for $C_{27}H_{28}F_5N_3O_3$: C, 60.33; H, 5.25; N, 7.82; F, 17.67. Found: C, 60.12; H, 5.10; N, 7.93; F, 17.86.

5.1.14. (2*Z*)-2-(1-{2-Chloro-4-[(3*S*)-3-methylpyrrolidin-1-yl]benzoyl}-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(2-hydroxyethyl)acetamide (1f)

Compound **1f** was prepared according to the procedure described for **1c**. The title compound **1f** (103 mg, 92% in hydrolysis step, 79% in amidation) was obtained as colorless crystals. Mp: 124–126 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (3H, d, J = 5.9 Hz), 1.45–1.57 (1H, m), 1.98–2.07 (1H, m), 2.20–2.34 (1H, m), 2.65–2.75 (1H, m), 3.08–3.31 (6H, m), 3.43–3.49 (2H, m), 4.34 (1H, d, J = 3.9 Hz), 4.72 (1H, t, J = 5.4 Hz), 4.75–4.90 (1H, br), 6.13–6.18 (1H, m), 6.27 (1H, s), 6.36–6.40 (1H, m), 6.62–6.70 (1H, m), 6.81–6.90 (1H, m), 7.13–7.32 (3H, m), 8.32–8.41 (1H, m). MS (FAB) m/z 504 $[M+1]^+$. Anal. Calcd for $C_{26}H_{28}ClF_2N_3O_3{\cdot}C_3H_8O$: C, 61.75; H, 6.43; N, 7.45; Cl, 6.29; F, 6.74. Found: C, 62.04; H, 6.39; N, 7.51; Cl, 6.20; F, 6.76.

5.1.15. (2*Z*)-2-(4,4-Difluoro-1-{4-[(3*S*)-3-methylpyrrolidin-1-yl]-2-(trifluoromethyl)benzoyl}-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(2-hydroxyethyl)acetamide (1g)

Compound **1g** was prepared according to the procedure described for **1c**. The title compound **1g** (157 mg, 93% in hydrolysis step, 60% in amidation) was obtained as colorless crystals. Mp: 178–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (3H, d, J = 6.3 Hz), 1.47–1.59 (1H, m), 2.00–2.10 (1H, m), 2.25–2.40 (2H, m), 2.65–2.81 (2H, m), 3.14–3.50 (8H, m), 4.72 (1H, t, J = 5.4 Hz), 4.76–4.90 (1H, br), 6.35–6.42 (2H, m), 6.62 (1H, s), 6.65–6.75 (2H, m), 7.16 (1H, t, J = 7.3 Hz), 7.22 (1H, t, J = 7.3 Hz), 7.31 (1H, d, J = 6.8 Hz), 8.43 (1H, s). MS (FAB) m/z 538 $[M+1]^+$. Anal. Calcd for $C_{27}H_{28}F_5N_3O_3$: C, 60.33; H, 5.25; N, 7.82; F, 17.67. Found: C, 60.32; H, 5.01; N, 7.84; F, 17.38.

5.2. Docking study

The program, MOE (CCG, Montreal, Canada)[11] was used to build and geometry optimize the ligand structure of **1g**. The energy minimizations were carried out with the MMFF94x force field. A homology model for the human V_2 receptor was constructed using the recently determined the bovine rhodopsin crystal structure[9] as a template, using the program, MOE. Docking calculation of **1g** was performed with the program, GOLD ver.3.1.1 (CCDC, Cambridge, UK).[10]

5.3. Biology

5.3.1. Binding assay for human V_2 receptor

Chinese hamster ovary (CHO) cells stably expressing human V2 receptors, which were established by Tahara et al.[12] were used. Cells were washed with phosphate buffered saline, and then collected in ice-cold hypotonic buffer (10 mmol/L Tris–HCl, 5 mmol/L EDTA, pH 7.4). Subsequently, cells were collected using a cell scraper and then homogenized using POLYTRON® followed by centrifugation (1000*g*, 10 min) at 4 °C. The supernatant was centrifuged (35,000*g*, 30 min) at 4 °C, and the pellet was suspended in Tris buffer. Membrane fractions were stored at −80 °C until used for binding assay. The concentration of membrane protein was determined by the Coomassie blue method using BSA as a standard.

The affinities of test compounds for human V_2 receptor were evaluated by the radioligand binding study. For the competitive binding study, 50 μL of drug solution and 50 μL of [^{3}H]vasopressin (final concentration of 0.91 nmol/L) were mixed with 150 μL of membrane suspension in 50 mmol/L Tris–HCl (pH 7.4) buffer containing 10 mmol/L $MgCl_2$ and 0.1% bovine serum albumin in a final

volume of 250 μL. This mixture was incubated at room temperature for 60 min. Reactions were terminated by filtration through UniFilter® GF/B (Perkin–Elmer) using a MicroMate Cell Harvester (Packard Instrument Company, Meriden, CT, USA) and the filter was washed with ice-cold Tris buffer. The radioactivity retained on the filter was counted by TopCount™ microplate scintillation counter (Perkin–Elmer) using the scintillation cocktail (MicroScinti-40™, Perkin–Elmer). Nonspecific binding or total binding were determined by including 1 μmol/L AVP or without test compounds in the reaction mixture, respectively. The number of concentrations of compounds was 11, appropriately chosen from 1×10^{-11} to 1×10^{-5} mol/L, using a common ratio of approximately three. We also performed the saturation binding study to yield the dissociation constants (K_d values) of [^{3}H]vasopressin for each human V_2 receptors. A membrane suspension was incubated with various concentrations of [^{3}H]vasopressin (0.1–3.2 nmol/L) in the absence or presence of 1 μmol/L AVP. Assay conditions were the same as those described for the competitive binding assay.

All values were determined by four separate experiments performed in triplicate and represented as the mean ± SEM. Statistical analysis was performed using a SAS software (SAS Institute, USA). Specific binding was calculated as total binding minus nonspecific binding. The concentration of each compound required to reduce specific binding of [^{3}H]vasopressin by 50% (IC50 value) was obtained by non-linear regression analysis. A K_d value of [^{3}H]vasopressin for each vasopressin receptor was yielded by Scatchard plot analysis. The affinity constants (K_i values) were calculated from the following equation,[13] using the K_d values yielded from each separate experiment. $K_i = IC_{50}/(1 + [[^3H]\text{vasopressin concentration}]/K_d)$.

5.3.2. Binding assay for human V_{1a} receptor

The binding assay for human V_{1a} receptor was performed in a manner similar to that for human V_2 receptor.

5.3.3. Stimulatory effect on the production of intracellular cAMP in human vasopressin V_2 receptor

CHO cells stably expressing human V_2 receptors, prepared by Tahara et al. were used.[12] The cells were incubated in α-MEM, containing 10% fetal bovine serum (FBS, Invitrogen Japan K.K.), 1% penicillin/streptomycin (Invitrogen Japan K.K.), and 0.1% amethopterin (dihydrofolate reductase inhibitor), in the absence of nucleic acid, at 37 °C, in an atmosphere of 95% air/5% CO_2.

CHO cells expressing human V_2 receptors were grown to subconfluence on a 96-well plate, and then incubated in serum-free medium for 24 h before assay. The medium was replaced with α-MEM containing 1 mmol/L 3-isobutyl-1-methylxantine (IBMX, Sigma) and 0.1% bovine serum albumin (BSA, Sigma) then the test compound was added and incubated at 37 °C for 10 min in order to induce a reaction. The cells were then dissolved in phosphatebuffered saline (PBS, Invitrogen Japan K.K.) containing 0.2% triton X-100. The cAMP level in the cell lysate was determined using the homogenous time resolved fluorescence (HTRF) assay with a cyclic AMP kit (Nihon Schering K.K.).[14]

Intrinsic activity was calculated as the percentage (%) of the maximum response to the test compound compared to the maximum response (100%) to AVP. All data analyses were performed using SAS. The activities of test compounds for cAMP production were calculated by logistic regression as EC_{50} values.

5.3.4. Anti-diuretic effect in water-loaded rats

A test to determine which rats would be selected for use was performed at least a week before the beginning of the study. In this test, male Wistar rats (SLC, 200–300 g) were given distilled water (30 mL/kg) orally. Afterwards, the animals were kept in metabolic cages, and urine was collected for 4 h after water loading. Animals whose urinary excretion rate was at least 70% of the volume of water-loaded (which was regarded as 100%) were used. While the animals were deprived of feed and water, they orally received the test compounds without anesthesia. Distilled water (30 mL/kg, po) was loaded 15 min after administration, and the animals were kept in a metabolic cage. Urine was collected for 4 h after water loading. (n = 3–6) The urinary excretion rate (%) was calculated by regarding the volume of loaded water as 100%. Linear regression was performed to obtain the doses of the test compounds required to decrease the urinary excretion rate to 50% (ED_{50}). All analyses were performed using SAS.

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