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Preparation of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene) acetamide derivatives as novel arginine vasopressin V_2 receptor agonists

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Urine volume

ABSTRACT

The present work describes the discovery of novel series of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepine-5-ylidene)acetamide derivatives as arginine vasopressin (AVP) V_2 receptor agonists. By replacing the amide juncture in **YM-35278** with a direct ring connection gave compound **10a**, which acts as a V_2 receptor agonist. These studies provided the potent, orally active non-peptidic V_2 receptor agonists **10a** and **10j**.

1. Introduction

Arginine vasopressin (AVP) is a cyclic nonapeptide that is produced and secreted by the hypothalamo-neurohypophysial system. Vasopressin receptors are classified by three subtypes, V_{1a}, V_{1b}, and V_2. The V_{1a} receptor, which exists in vascular smooth muscle and platelets, is involved in blood pressure control, and V_{1b}, which is distributed mainly in the hypophysis pituitary gland, regulates adrenocorticotropic hormone (ACTH) secretion. The presence of the V_2 receptor, which is involved in water reabsorption, has been identified in the kidney.[1] Stimulation of the V_2 receptor with AVP, for which production is triggered by increased blood pressure, causes water reabsorption in the kidney by the process of increasing cyclic adenosine monophosphate (cAMP) with subsequent activation of aquaporin-2 water channel. This implies that a V_2 receptor agonist could reduce urine volume, and, thus potentially treat diseases such as central diabetes insipidus and nocturia.

Currently, desmopressin (1-desamino-8-D-Arg vasopressin), a peptide analogue of AVP, is the only V_2 receptor agonist on the market, and is used for the treatment of central diabetes insipidus. Several other non-peptidic V_2 receptor agonists, such as **OPC-51803** and **VNA932**, have been reported[2–5]; however, none of them have been launched into market so far (Fig. 1).

Here, we describe the preparation of the novel V_2 receptor agonist **10a** via structural conversion from **YM-35278**,[6] a V_2 receptor antagonist found in a previous study (Fig. 2). Further research of structural requirements for agonist activity to provide another potent, orally active, non-peptidic V_2 receptor agonist, (2*Z*)-2-{1-[2-chloro-4-(3-methylpyrrolidin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (**10j**), is also reported. Compounds **10a** and **10j** were both found to decrease urine volume in water-loaded rats.

2. Chemistry

All compounds evaluated for V_2 agonist activity were prepared as described in Schemes 1–3. Commercially available 2-chloro-4-fluorobenzoic acid (**1**) was esterified under acid conditions to afford **2**. Treatment of **2** with cyclic amines following hydrolysis provided 4-substituted 2-chlorobenzoic acid-derived intermediates (**4a–4f**, **4h–4o**). 2-Chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzoic acid (**4g**) was synthesized from 2-chloro-4-fluorobenzonitrile (**5**) and 3-methylpyrazole, which was then hydrolyzed[7,8] via a benzamide derivative (**7**), as shown in Scheme 1.

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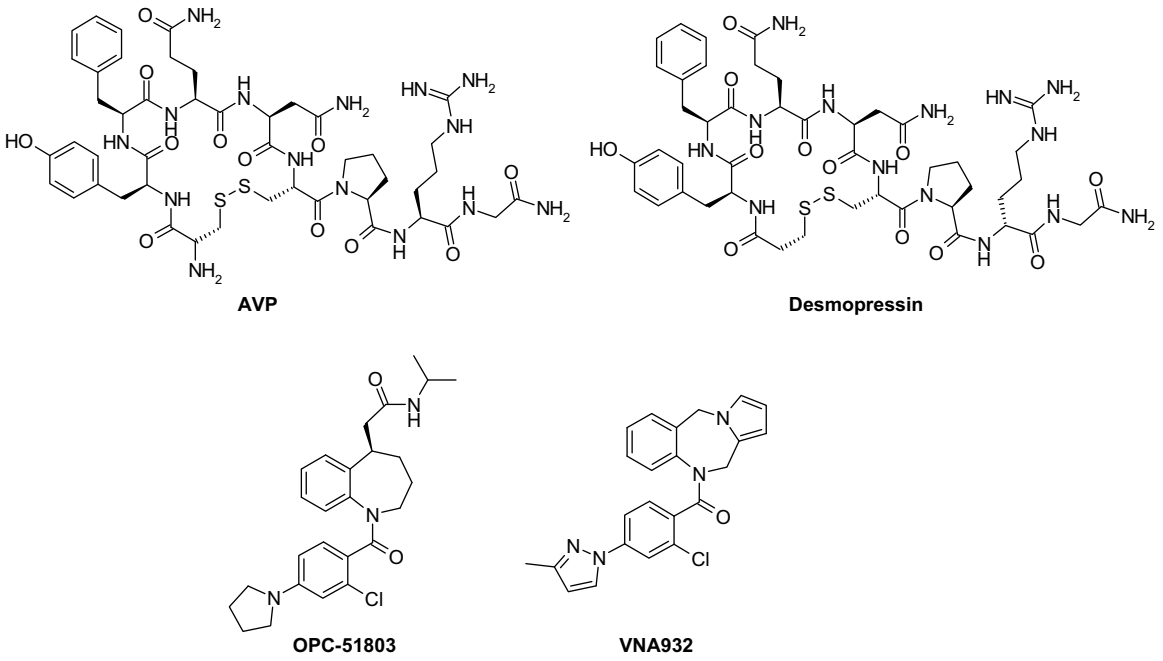


Figure 1. Chemical structures of AVP and well-known arginine vasopressin V_2 receptor agonists.

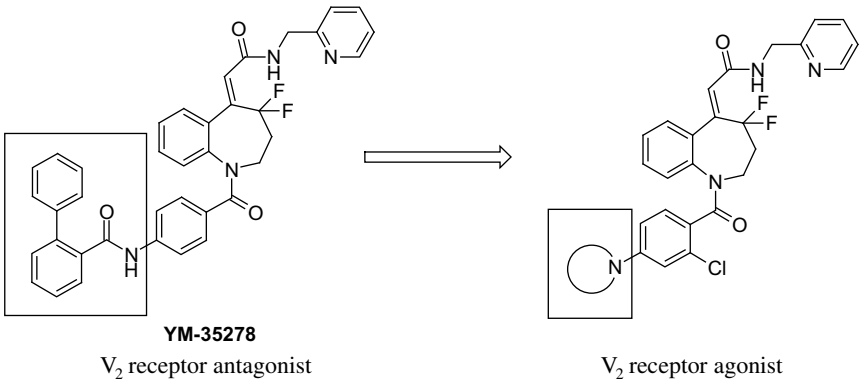


Figure 2. Chemical structures of **YM-35278** and designs for V_2 receptor agonists.

Transformation of **4a–4k**, **4n**, and **4o** into the corresponding benzoyl chlorides, and subsequent amidation with methyl (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzoazepin-5-ylidene)acetate[9] yielded compounds **8a–8k**, **8n**, and **8o**, which were treated with aqueous sodium hydroxide to afford **9a–9k**, **9n**, and **9o**. Condensation of those carboxylic acids with 2-aminomethylpyridine in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole yielded the target derivatives **10a–10k**, **10n**, and **10o** (Scheme 2).

Compounds **10l** and **10m** were synthesized from **4l** and **4m** via condensation with another benzazepin derivative (**13**), as shown in Scheme 3.

3. Result and discussion

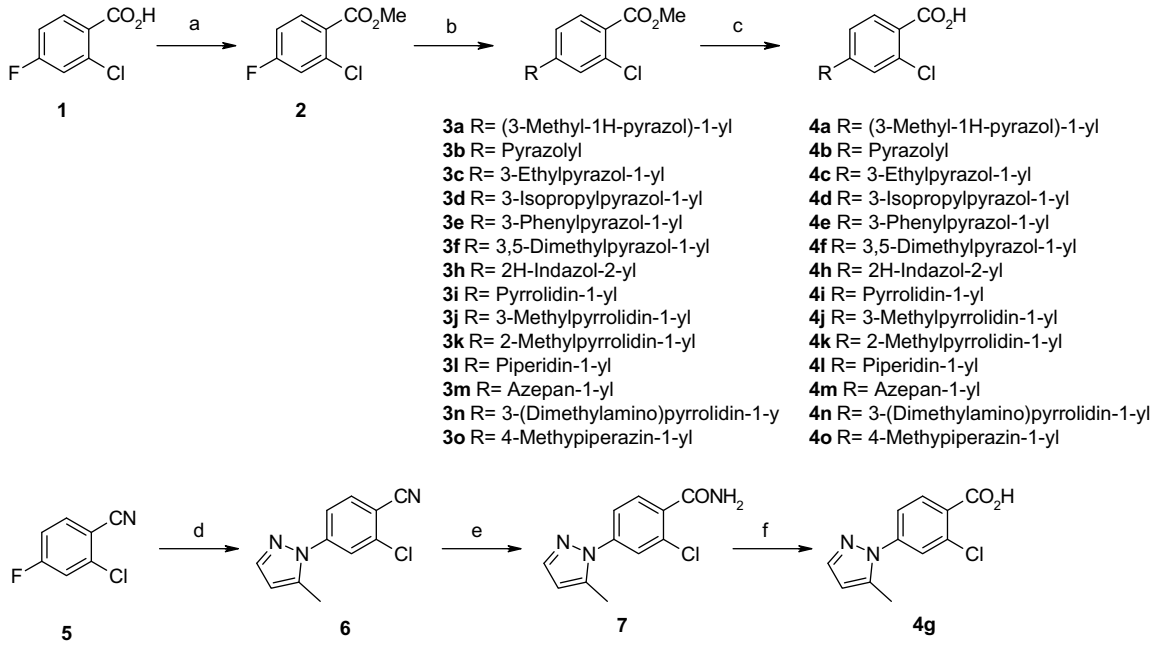
The K_i values of compounds **10a–10o** for human V_2 receptor were evaluated in a radioligand binding study. The activity of compounds **10a–10o** for cAMP production was also determined as EC_{50} value and intrinsic activity.

As shown in Table 1, replacing the biphenylamide moiety of **YM-35278**, a V_2 receptor antagonist found previously, with the 3-methylpyrazol-1-yl group yielded compound **10a**, which has V_2 agonist activity. Compound **10a** displayed high binding affinity for the human V_2 receptor with a K_i value of 4.8 nM and potent cAMP accumulation activity (EC_{50} = 1.8 nM).

This observation agrees with the reports by Kondo et al.[2] and Venkatesan et al.,[4] which show that compounds that link to V_2 antagonists via an amino group instead of an amide-linkage exhibit V_2 agonist activity.

Table 2 illustrates the exploration of substitutions in the pyrazole ring of compound **10a**. Compound **10b**, in which the 3-methyl group in the pyrazole ring is absent, showed intense affinity for the V_2 receptor, with a K_i value of 9.2 nM. However, the cAMP accumulation activity of this compound was moderate, with an ED_{50} value of 192 nM. More bulky substitutions, such as replacing the methyl group of **10a** with an ethyl group (**10c**), isopropyl group (**10d**), or phenyl group (**10e**), also resulted in decreased agonist potency, while binding affinity was maintained. Compounds **10f**, **10g**, and **10h**, which have a 3,5-dimethyl group, 5-methyl group, and an indazole ring, respectively, showed a similar tendency to decrease cAMP production.

As described above, although all compounds substituted into the pyrazole ring exhibited good V_2 receptor binding affinity, most of them showed less potent cAMP accumulation activity. This indi-

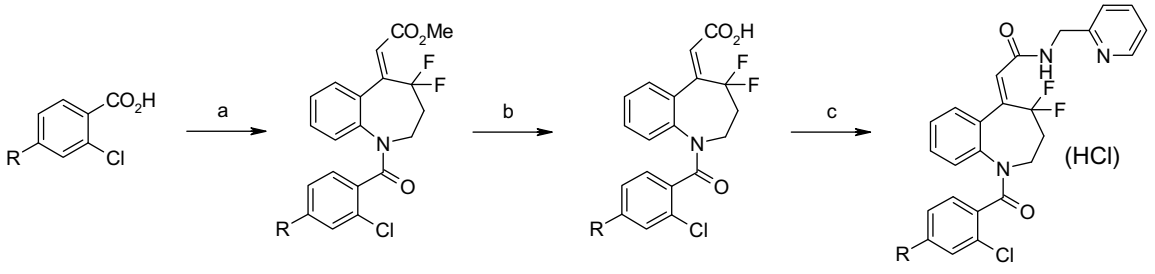


Scheme 1. Reagents and conditions: (a) H_2SO_4, MeOH; (b) amine, K_2CO_3, NMP; (c) aq HCl or aq NaOH; (d) NaH, 3-methylpyrazole, DMF; (e) H_2O_2, K_2CO_3, DMSO; (f) $NaNO_2$, H_2SO_4, H_2O.

cates that the steric requirement for producing intrinsic activity is extremely strict around this region. Among these compounds, the original 3-methylpyrazole derivative **10a** was the only one possessing both good binding affinity and potent agonist activity. In the cAMP accumulation assay, the maximum response to **10a** was quite comparable to that of AVP itself.

Table 3 shows the activity of compounds that have aliphatic rings in place of the pyrazole ring in compound **10a**. Compounds substituted with five- to seven-membered hydrophobic rings (**10i**–**10m**) maintained binding affinity to the V_2 receptor. With regard to intrinsic activity, pyrrolidine-substituted **10i** and 3-methylpyrrolidine-substituted **10j** exhibited activity comparable to that of **10a** in the cAMP production assay, with ED_{50} values of 3.13 nM and 1.25 nM, respectively. However, the 2-methyl pyrrolidine derivative **10k** showed a substantial decrease in cAMP accumulation. Substitution with piperidine (**10l**) was less effective, yielding an ED_{50} value of 104 nM, which is 33-fold less potent than compound **10i**. Substitution of a seven-membered ring (**10m**) also caused a decrease (206-fold), with an ED_{50} value of 646 nM. Amine functional groups, such as the 3-(dimethylamino)pyrrolidine (**10n**) and 4-methylpiperazine-1-yl groups (**10o**), led to a decrease in V_2 receptor binding affinity. Compounds **10n** and **10o** also showed little cAMP production activity.

These results indicate that steric bulkiness does not hinder V_2 receptor binding, but it has a severely negative impact on agonist activity, as is also observed in Table 2. Sterically-unhindered nearly



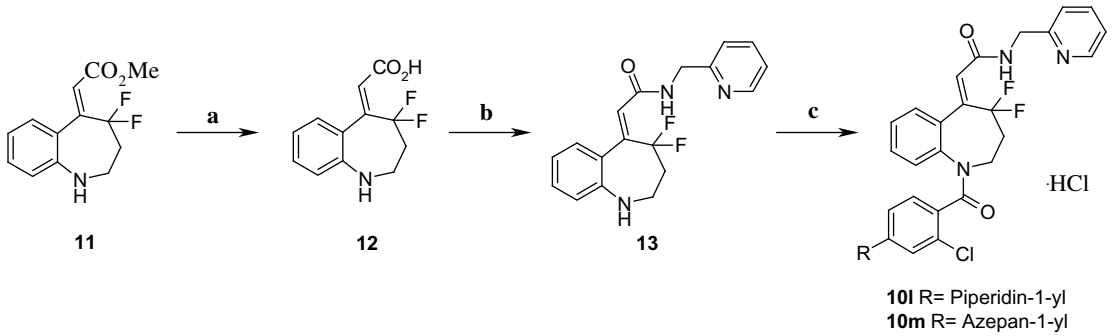
4a-4k, 4n, 4o

8a R= (3-Methyl-1H-pyrazol)-1-yl
8b R= Pyrazolyl
8c R= 3-Ethylpyrazol-1-yl
8d R= 3-Isopropylpyrazol-1-yl
8e R= 3-Phenylpyrazol-1-yl
8f R= 3,5-Dimethylpyrazol-1-yl
8g R= (5-Methyl-1H-pyrazol)-1-yl
8h R= 2H-Indazol-2-yl
8i R= Pyrrolidin-1-yl
8j R= 3-Methylpyrrolidin-1-yl
8k R= 2-Methylpyrrolidin-1-yl
8n R= 3-(Dimethylamino)pyrrolidin-1-y
8o R= 4-Methypiperazin-1-yl

9a R= (3-Methyl-1H-pyrazol)-1-yl
9b R= Pyrazolyl
9c R= 3-Ethylpyrazol-1-yl
9d R= 3-Isopropylpyrazol-1-yl
9e R= 3-Phenylpyrazol-1-yl
9f R= 3,5-Dimethylpyrazol-1-yl
9g R= (5-Methyl-1H-pyrazol)-1-yl
9h R= 2H-Indazol-2-yl
9i R= Pyrrolidin-1-yl
9j R= 3-Methylpyrrolidin-1-yl
9k R= 2-Methylpyrrolidin-1-yl
9n R= 3-(Dimethylamino)pyrrolidin-1-y
9o R= 4-Methypiperazin-1-yl

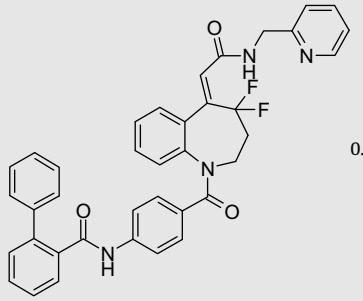
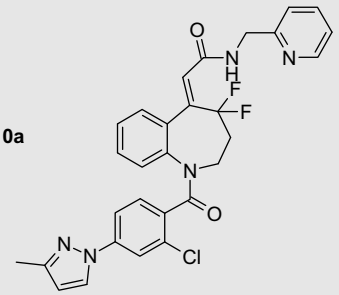
10a R= (3-Methyl-1H-pyrazol)-1-yl
10b R= Pyrazolyl
10c R= 3-Ethylpyrazol-1-yl
10d R= 3-Isopropylpyrazol-1-yl
10e R= 3-Phenylpyrazol-1-yl
10f R= 3,5-Dimethylpyrazol-1-yl
10g R= (5-Methyl-1H-pyrazol)-1-yl
10h R= 2H-Indazol-2-yl
10i R= Pyrrolidin-1-yl
10j R= 3-Methylpyrrolidin-1-yl
10k R= 2-Methylpyrrolidin-1-yl
10n R= 3-(Dimethylamino)pyrrolidin-1-yl
10o R= 4-Methypiperazin-1-yl

Scheme 2. Reagents and conditions: (a) $SOCl_2$, cat. DMF, THF then methyl (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate[9] (**11**), pyridine; (b) aq NaOH; (c) 2-(aminomethyl)pyridine, WSCD, HOBt, DMF, then HCl.



Scheme 3. Reagents and conditions: (a) aq NaOH; (b) 2-(aminomethyl)pyridine, WSCD, HOBt, DMF; (c) **4l** or **4m**, $SOCl_2$, cat. DMF, THF, then HCl.

Table 1
Binding affinity and cAMP accumulation activity of **YM-35278** and **10a**

	Binding affinity for V_2 K_i[a] (nM)	cAMP accumulation EC_{50}[b] (nM)
YM-35278 	0.40 (rat)	Not effective
10a 	19 (rat) 4.8 (human)	1.8

[a] Human or rat V_2 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.

neutral structures, such as pyrrolidine-1-yl and 3-methylpyrrolidine-1-yl group, maintain effective V_2 binding and intrinsic activity here as observed in Table 3.

The oral antidiuretic effects of compounds **10a** and **10j**, which possess good binding affinity and excellent cAMP production activity, as mentioned above, were evaluated. As shown in Figure 3, both **10a** and **10j** reduced urine volume in water-loaded rats with ED_{50} values of 0.26 mg/kg po and 0.31 mg/kg po, respectively.

4. Conclusion

In an attempt to discover novel V_2 receptor agonists, a series of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetamide derivatives were designed and synthesized via structural modification of the previously found V_2 receptor antagonist **YM-35278**. The novel V_2 agonist **10a** was discovered as a result of replacing the amide linkage of **YM-35278** with a direct pyrazolyl group. Further exploration of structure–activity relationships indicated that the structural requirements for agonist activity are highly specific, while receptor binding tolerates steric bulkiness. Among the compounds reported here, **10a** and **10j** were identified as potent V_2 receptor agonists with high binding affinity. Furthermore, oral administration of **10a** or **10j** was found to significantly decrease urine volume in water-loaded rats.

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, and N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens), and were within ±0.4% of theoretical values.

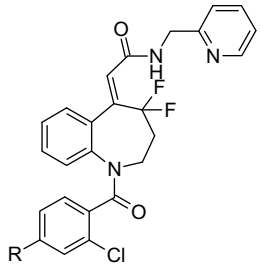
5.1.1. Methyl 2-chloro-4-fluorobenzoate (2)

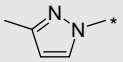
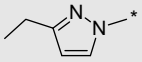
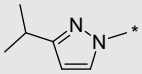
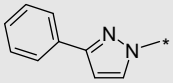
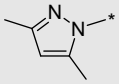
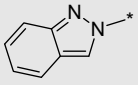
To a mixture of 2-chloro-4-fluorobenzoic acid (20.0 g, 115 mmol) in methanol (200 ml) was added sulfuric acid (10 ml), and the mixture was heated at 60 °C for 17 h. The reaction was then allowed to cool to room temperature, the solvent was reduced by evaporation, the residue was diluted in cold water and neutralized with potassium carbonate. The aqueous solution was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give the title compound **2** (20.9 g, 96%) as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (3H, s), 7.03 (1H, td, J = 8.8, 2.4 Hz), 7.20 (1H, dd, J = 8.4, 2.4 Hz), 7.90 (1H, dd, J = 8.8, 6.0 Hz). MS (EI) m/z = 188 [M].

5.1.2. Methyl 2-chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoate (3a)

A mixture of methyl 2-chloro-4-fluorobenzoate (**2**; 20.9 g, 111 mmol), 3-methylpyrazole (9.38 ml, 117 mmol) and potassium carbonate (30.7 g, 222 mmol) in 1-methyl-2-pyrrolidinone (NMP) (150 ml) was heated at 120 °C for 3 h. The reaction was then allowed to cool to room temperature and partitioned between water

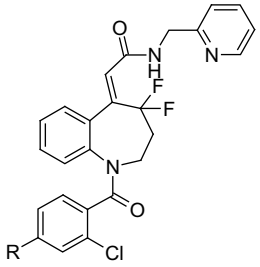
Table 2
Binding affinity and cAMP accumulation activity of substituted pyrazole derivatives

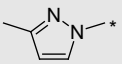
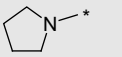
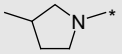
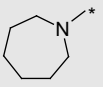
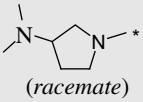
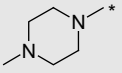


Compound	R	Binding affinity for hV_2 K_i[a] (nM)	cAMP accumulation EC_{50}[b] (nM)	AMP accumulation Max activity[c]
10a		4.8	192	95.9
10b		9.2	61.9	73.4
10c		21	>10,000	86.8
10d		18	>10,000	4.9
10e		11	>10,000	4.9
10f		9.1	>10,000	15.9
10g		6.0	>10,000	5.7
10h		20	>10,000	8.7

[a] Human V_2 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 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.

Table 3
Binding affinity and cAMP accumulation activity of aliphatic ring-substituted derivatives



Compound	R	Binding affinity for hV_2 K_i[a] (nM)	cAMP accumulation EC_{50}[b] (nM)	AMP accumulation Max activity[c]
10a		4.8	1.8	95.9
10I		22	3.13	107.1
10J	 (*racemate*)	14	1.25	90.4
10K	 (*racemate*)	16	1100	59.3
10l		14	104	75.3
10m		11	646	62.8
10n	 (*racemate*)	200	>10,000	15.9
10o		170	>10,000	35.5

[a] Human V_2, 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 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.

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 = 20/1–10/1) to give the title compound **3a** (9.25 g, 33%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (3H, s), 3.94 (3H, s), 6.30 (1H, d, J = 2.4 Hz), 7.60 (1H, dd, J = 8.8, 2.4 Hz), 7.82 (1H, d, J = 2.4 Hz), 7.85 (1H, d, J = 2.4 Hz), 7.96 (1H, d, J = 8.8 Hz). MS (FAB) m/z 251 $[M+H]^+$.

5.1.3. Methyl 2-chloro-4-(1*H*-pyrazol-1-yl)benzoate (3b)

Compound **3b** was prepared according to the procedure described for **3a** from **2** and pyrazole. The title compound **3b** (830 mg, 55%) was obtained as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95 (3H, s), 6.52 (1H, dd, J = 2.6, 1.9 Hz), 7.66 (1H, dd, J = 8.4, 2.2 Hz), 7.76 (1H, d, J = 1.5 Hz), 7.87 (1H, d, J = 2.2 Hz), 7.98 (1H, d, J = 1.5 Hz) 7.99 (1H, d, J = 8.6 Hz). MS (FAB) m/z 237 $[M+H]^+$.

5.1.4. Methyl 2-chloro-4-(3-ethyl-1*H*-pyrazol-1-yl)benzoate (3c)

Compound **3c** was prepared according to the procedure described for **3a** from **2** and 3-ethylpyrazole. The title compound **3c** (692 mg, 32%) was obtained as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, t, J = 7.7 Hz), 2.75 (2H, q, J = 7.7 Hz), 3.94 (3H, s), 6.33 (1H, d, J = 2.6 Hz), 7.60 (1H, dd, J = 8.6, 2.2 Hz), 7.83 (1H, d, J = 2.2 Hz), 7.86 (1H, d, J = 2.6 Hz), 7.96 (1H, d, J = 8.6 Hz). MS (FAB) m/z 265 $[M+H]^+$.

5.1.5. Methyl 2-chloro-4-(3-isopropyl-1*H*-pyrazol-1-yl)benzoate (3d)

Compound **3d** was prepared according to the procedure described for **3a** from **2** and 3-isopropylpyrazole. The title compound

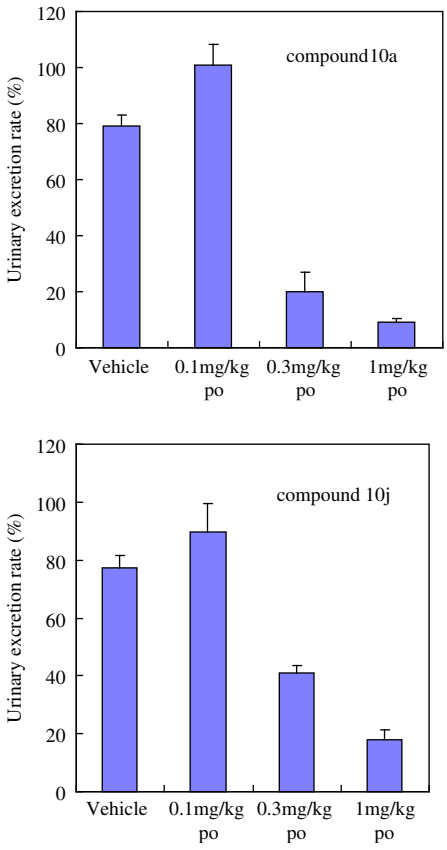


Figure 3. Effects of the oral administration of compounds **10a** and **10j** on urinary excretion rate in water-loaded rats. The urinary excretion rate indicates the percentage of urine volume to loaded water volume. All assays were performed in quadruplicate.

3d (982 mg, 35%) was obtained as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (6H, d, J = 6.9 Hz), 3.00–3.13 (1H, m), 3.94 (3H, s), 6.34 (1H, d, J = 2.6 Hz), 7.60 (1H, dd, J = 8.6, 2.2 Hz), 7.84 (1H, d, J = 2.2 Hz), 7.86 (1H, d, J = 2.6 Hz), 7.96 (1H, d, J = 8.6 Hz). MS (FAB) m/z 279 $[M+H]^+$.

5.1.6. Methyl 2-chloro-4-(3-phenyl-1*H*-pyrazol-1-yl)benzoate (3e)

Compound **3e** was prepared according to the procedure described for **3a** from **2** (1.90 g, 10.1 mmol) and 3-phenylpyrazole (1.40 g, 10.0 mmol). The title compound **3e** (2.00 g, 64%) was obtained as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (3H, s), 7.15 (1H, d, J = 2.7 Hz), 7.37–7.42 (1H, m), 7.45–7.51 (2H, m), 7.95–8.07 (4H, m), 8.18 (1H, d, J = 1.9 Hz), 8.77 (1H, d, J = 2.7 Hz). MS (FAB) m/z 313 $[M+H]^+$.

5.1.7. Methyl 2-chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzoate (3f)

Compound **3f** was prepared according to the procedure described for **3a** from **2** (1.90 g, 10.1 mmol) and 3,5-dimethylpyrazole (960 mg, 10.0 mmol). The title compound **3f** (500 mg, 19%) was obtained as colorless oil. ^{1}H NMR (300 MHz, DMSO- d_6) δ 2.19 (3H, s), 2.40 (3H, s), 3.88 (3H, s), 6.15 (1H, s), 7.65 (1H, dd, J = 8.4, 2.0 Hz), 7.76 (1H, d, J = 2.0 Hz), 7.95 (1H, d, J = 8.4 Hz). MS (FAB) m/z 265 $[M+H]^+$.

5.1.8. Methyl 2-chloro-4-(2*H*-indazol-2-yl)benzoate (3h)

Compound **3h** was prepared according to the procedure described for **3a** from **2** (1.90 g, 10.1 mmol) and indazole (1.20 g, 10.0 mmol). The title compound **3h** (600 mg, 21%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO- d_6) δ 3.90 (3H, s), 7.13 (1H, t, J = 7.3 Hz), 7.36 (1H, t, J = 8.8 Hz), 7.72 (1H, d, J = 8.8 Hz), 7.78 (1H, d, J = 8.4 Hz), 8.06 (1H, dd, J = 8.4, 1.1 Hz), 8.23 (1H, d, J = 8.6 Hz), 8.37 (1H, s), 9.29 (1H, s). MS (FAB) m/z 287 $[M+H]^+$.

5.1.9. Methyl 2-chloro-4-(pyrrolidin-1-yl)benzoate (3i)

Compound **3i** was prepared according to the procedure described for **3a** from **2** and pyrrolidine. The title compound **3i** (6.04 g, 88%) was obtained as pink solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00–2.06 (4H, m), 3.29–3.35 (4H, m), 3.85 (3H, s), 6.39 (1H, dd, J = 8.8, 2.4 Hz), 6.54 (1H, d, J = 2.4 Hz), 7.84 (1H, d, J = 8.8 Hz). MS (FAB) m/z 240 $[M+H]^+$.

5.1.10. Methyl 2-chloro-4-(3-methylpyrrolidin-1-yl)benzoate (3j)

Compound **3j** was prepared according to the procedure described for **3a** from **2** and 3-methylpyrrolidine. The title compound **3j** (1.69 g, 84%) was obtained as cream solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (3H, d, J = 6.6 Hz), 1.58–1.66 (1H, m), 2.10–2.21 (1H, m), 2.32–2.48 (1H, m), 2.89 (1H, dd, J = 9.5, 7.9 Hz), 3.27–3.51 (3H, m), 3.85 (3H, s), 6.36 (1H, dd, J = 8.8, 2.4 Hz), 6.51 (1H, d, J = 2.4 Hz), 7.84 (1H, d, J = 8.8 Hz). MS (FAB) m/z 254 $[M+H]^+$.

5.1.11. Methyl 2-chloro-4-(2-methylpyrrolidin-1-yl)benzoate (3k)

Compound **3k** was prepared according to the procedure described for **3a** from **2** and 2-methylpyrrolidine. The title compound **3k** (2.08 g, 78%) was obtained as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (3H, d, J = 6.2 Hz), 1.69–1.80 (1H, m), 1.98–2.16 (3H, m), 3.16–3.27 (1H, m), 3.40–3.48 (1H, m), 3.85 (3H, s), 3.87–3.97 (1H, m), 6.41 (1H, dd, J = 8.8, 2.4 Hz), 6.55 (1H, d, J = 2.6 Hz), 7.83 (1H, d, J = 8.8 Hz). MS (FAB) m/z 254 $[M+H]^+$.

5.1.12. Methyl 2-chloro-4-(piperidin-1-yl)benzoate (3l)

Compound **3l** was prepared according to the procedure described for **3a** from **2** and piperidine. The title compound **3l** (1.27 g, 94%) was obtained as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63–1.70 (6H, m), 3.28–3.35 (4H, m), 3.86 (3H, s), 6.71 (1H, dd, J = 9.0, 2.6 Hz), 6.85 (1H, d, J = 2.6 Hz), 7.82 (1H, d, J = 9.0 Hz). MS (APCI) m/z 254 $[M+H]^+$.

5.1.13. Methyl 4-(azepan-1-yl)-2-chlorobenzoate (3m)

Compound **3m** was prepared according to the procedure described for **3a** from **2** and azepane. The title compound **3m** (794 mg, 56%) was obtained as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51–1.60 (4H, m), 1.74–1.84 (4H, m), 3.47 (4H, t, J = 6.0 Hz), 3.85 (3H, s), 6.53 (1H, dd, J = 9.0, 2.8 Hz), 6.66 (1H, d, J = 2.6 Hz), 7.82 (1H, d, J = 9.0 Hz). MS (EI) m/z 267 [M].

5.1.14. 2-Chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoic acid (4a)

A mixture of methyl 2-chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoate (**3a**; 9.25 g, 36.9 mmol) and aqueous hydrochloric acid (6 M, 10 ml, 60 mmol) in acetic acid (10 ml) was refluxed for 13 h. The reaction was then allowed to cool to room temperature and subsequently poured into cold water. Resulting precipitates were collected by filtration and dried in vacuo to give the title compound **4a** (8.56 g, 98%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (3H, s), 6.32 (1H, d, J = 2.4 Hz), 7.63 (1H, dd, J = 8.8, 2.4 Hz), 7.86 (1H, d, J = 2.4 Hz), 7.88 (1H, d, J = 2.8 Hz), 8.12 (1H, d, J = 8.8 Hz). MS (FAB) m/z 235 $[M-H]^-$.

5.1.15. 2-Chloro-4-(1*H*-pyrazol-1-yl)benzoic acid (4b)

A mixture of methyl 2-chloro-4-(1*H*-pyrazol-1-yl)benzoate (**3b**; 1.75 g, 7.39 mmol) and aqueous sodium hydroxide (5 M, 5 ml, 25 mmol) in methanol (20 ml) was refluxed for 45 min. The reaction was then allowed to cool to room temperature and subse-

quently poured into aqueous hydrochloride. Resulting precipitates were collected by filtration and dried in vacuo to give the title compound **4b** (1.59 g, 96%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.61–6.64 (1H, m), 7.84 (1H, d, J = 1.5 Hz), 7.93–7.97 (2H, m), 8.06 (1H, d, J = 1.8 Hz), 8.68 (1H, d, J = 2.8 Hz). MS (FAB) m/z 223 $[M+H]^+$.

5.1.16. 2-Chloro-4-(3-ethyl-1*H*-pyrazol-1-yl)benzoic acid (4c)

Compound **4c** was prepared according to the procedure described for **4b** from **3c**. The title compound **4c** (589 mg, 90%) was obtained as cream solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (3H, t, J = 7.7 Hz), 2.66 (2H, q, J = 7.7 Hz), 6.46 (1H, d, J = 2.2 Hz), 7.87 (1H, dd, J = 8.8, 1.5 Hz), 7.94 (1H, d, J = 8.6 Hz), 7.99 (1H, d, J = 1.7 Hz), 8.55 (1H, d, J = 2.4 Hz). MS (FAB) m/z 251 $[M+H]^+$.

5.1.17. 2-Chloro-4-(3-isopropyl-1*H*-pyrazol-1-yl)benzoic acid (4d)

Compound **4d** was prepared according to the procedure described for **4b** from **3d**. The title compound **4d** (873 mg, 94%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (6H, d, J = 6.8 Hz), 2.93–3.04 (1H, m), 6.48 (1H, s), 7.87 (1H, d, J = 8.6 Hz), 7.92–8.01 (2H, m), 8.54 (1H, s), 13.20–13.40 (1H, br). MS (FAB) m/z 265 $[M+H]^+$.

5.1.18. 2-Chloro-4-(3-phenyl-1*H*-pyrazol-1-yl)benzoic acid (4e)

Compound **4e** was prepared according to the procedure described for **4b** from **3e** (1.90 g, 6.08 mmol). The title compound **4e** (1.80 g, 99%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.14 (1H, d, J = 2.8 Hz), 7.36–7.52 (3H, m), 7.94–8.03 (4H, m), 8.14 (1H, s), 8.76 (1H, d, J = 2.8 Hz), 13.30–13.44 (1H, br). MS (FAB) m/z 297 $[M-H]^-$.

5.1.19. 2-Chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzoic acid (4f)

Compound **4f** was prepared according to the procedure described for **4b** from **3f** (500 mg, 1.89 mmol). The title compound **4f** (440 g, 93%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.19 (3H, s), 2.39 (3H, s), 6.14 (1H, s), 7.43 (1H, dd, J = 8.4, 2.2 Hz), 7.72 (1H, d, J = 2.0 Hz), 7.93 (1H, d, J = 8.4 Hz), 13.36–13.54 (1H, br). MS (FAB) m/z 249 $[M-H]^-$.

5.1.20. 2-Chloro-4-(2*H*-indazol-2-yl)benzoic acid (4h)

Compound **4h** was prepared according to the procedure described for **4b** from **3h** (600 mg, 2.09 mmol). The title compound 4 h (580 mg, quantitative yield) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.13 (1H, ddd, J = 8.4, 6.6, 0.8 Hz), 7.35 (1H, ddd, J = 8.8, 6.6, 1.1 Hz), 7.72 (1H, dd, J = 8.8, 1.1 Hz), 7.78 (1H, d, J = 8.4 Hz), 8.03 (1H, d, J = 8.6 Hz), 8.20 (1H, dd, J = 8.6, 2.2 Hz), 8.33 (1H, d, J = 2.2 Hz) 9.28 (1H, d, J = 0.9 Hz), 13.50–13.62 (1H, br). MS (FAB) m/z 271 $[M-H]^-$.

5.1.21. 2-Chloro-4-(pyrrolidin-1-yl)benzoic acid (4i)

Compound **4i** was prepared according to the procedure described for **4b** from **3i**. The title compound **4i** (3.39 g, 60%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.92–1.99 (4H, m), 3.25–3.31 (4H, m), 6.50 (1H, dd, J = 8.6, 2.4 Hz), 6.53 (1H, d, J = 2.4 Hz), 7.76 (1H, d, J = 8.6 Hz). MS (FAB) m/z 226 $[M+H]^+$.

5.1.22. 2-Chloro-4-(3-methylpyrrolidin-1-yl)benzoic acid (4j)

Compound **4j** was prepared according to the procedure described for **4b** from **3j**. The title compound **4j** (1.53 g, quantitative yield) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (3H, d, J = 6.6 Hz), 1.51–1.66 (1H, m), 2.04–2.15 (1H, m), 2.28–2.42 (1H, m), 2.84 (1H, dd, J = 9.7, 7.7 Hz), 3.22–3.50 (3H, m), 6.47 (1H, dd, J = 8.6, 2.4 Hz), 6.51 (1H, d, J = 2.2 Hz), 7.76 (1H, d, J = 8.8 Hz), 12.16–12.54 (1H, br). MS (FAB) m/z 240 $[M+H]^+$.

5.1.23. 2-Chloro-4-(2-methylpyrrolidin-1-yl)benzoic acid (4k)

Compound **4k** was prepared according to the procedure described for **4b** from **3k**. The title compound **4k** (1.69 g, 89%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, d, J = 6.2 Hz), 1.63–1.75 (1H, m), 1.91–2.11 (3H, m), 3.11–3.21 (1H, m), 3.35–3.44 (1H, m), 3.92–4.02 (1H, m), 6.50–6.56 (2H, m), 7.73–7.79 (1H, m), 12.24–12.40 (1H, br). MS (FAB) m/z 240 $[M+H]^+$.

5.1.24. 2-Chloro-4-(piperidin-1-yl)benzoic acid (4l)

Compound **4l** was prepared according to the procedure described for **4b** from **3l**. The title compound **4l** (997 mg, 88%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53–1.62 (6H, m), 3.30–3.36 (4H, m), 6.84–6.94 (2H, m), 7.74 (1H, d, J = 8.8 Hz), 12.45–12.52 (1H, br).

5.1.25. 4-(Azepan-1-yl)-2-chlorobenzoic acid (4m)

Compound **4m** was prepared according to the procedure described for **4b** from **3m**. The title compound **4m** (676 mg, 90%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42–1.51 (4H, m), 1.64–1.75 (4H, m), 3.49 (4H, t, J = 5.9 Hz), 6.64–6.70 (2H, m), 7.74 (1H, d, J = 9.3 Hz), 12.27–12.38 (1H, br).

5.1.26. 2-Chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzonitrile (6)

To a suspension of sodium hydride (60% dispersion in mineral oil, 1.54 g, 38.5 mmol) in *N*,*N*-dimethylformamide (DMF) (30 ml) was added 3-methylpyrazole (3.06 ml, 38.0 mmol), and the mixture was stirred at -20 °C for 1 h. Then, a solution of 2-chloro-4-fluorobenzonitrile (**5**; 5.00 g, 32.1 mmol) in DMF (5 ml) was slowly added to the reaction mixture, and that was stirred overnight at room temperature. Water was added to the mixture, and resulting precipitates were collected by filtration. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 10/1–9/1) and subsequent recrystallization from Et_2O/*n*-hexane to give the title compound **6** (690 mg, 10%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.46 (3H, s), 6.38 (1H, s), 7.69 (1H, s), 7.78 (1H, dd, J = 8.4, 2.0 Hz), 7.99 (1H, d, J = 1.8 Hz), 8.12 (1H, d, J = 8.4 Hz). MS (FAB) m/z 218 $[M+H]^+$.

5.1.27. 2-Chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzamide (7)

To a mixture of 2-chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzonitrile (**6**; 650 mg, 2.99 mmol) and potassium carbonate (69 mg, 0.50 mmol) in DMSO (5 ml) was added hydrogen peroxide (30%, 0.42 ml, 3.75 mmol), and the mixture was stirred at room temperature for 30 min. Ice-cold water was added to the mixture, and resulting precipitates were collected by filtration, and subsequently dried in vacuo to give the title compound **7** (520 mg, 74%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.39 (3H, s), 6.32 (1H, s), 7.58 (2H, s), 7.61 (1H, d, J = 1.5 Hz), 7.65–7.70 (2H, m), 7.94–8.00 (1H, br). MS (FAB) m/z 236 $[M+H]^+$.

5.1.28. 2-Chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzoic acid (4g)

To a flask with water (4 ml) at 0 °C were added sulfuric acid (10 ml), 2-chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzamide (**7**; 500 mg, 2.12 mmol), and sodium nitrite (732 mg, 10.6 mmol) sequentially, and the mixture was stirred at room temperature for 3 h. Ice-cold water was added to the mixture, and resulting precipitates were collected by filtration, and subsequently dried in vacuo to give the title compound **4g** (450 mg, 90%) as white solid. ^{1}H

NMR (300 MHz, DMSO-d_6) δ 2.43 (3H, s), 6.34 (1H, s), 7.62–7.67 (2H, m), 7.76 (1H, d, J = 2.0 Hz), 7.95 (1H, d, J = 8.4 Hz). MS (FAB) m/z 235 $[M-H]^-$.

5.1.29. Methyl (2*Z*)-{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}acetate (8a)

A mixture of 2-chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoic acid (**4a**; 8.56 g, 36.2 mmol), DMF (several drops), and thionyl chloride (3.98 ml, 54.6 mmol) in tetrahydrofuran (THF) (100 ml) was stirred at room temperature for 1 h. The reaction mixture was evaporated and azeotroped with toluene. To a solution of this residue in pyridine (50 ml) was added a solution of methyl (2*Z*)-(4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate[9] (9.17 g, 36.2 mmol) in THF (25 ml), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated, and the residue was 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 ($CHCl_3$/methanol = 20:1) to give the title compound **8a** (10.0 g, 59%) as pale yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.40–2.60 (2H, m), 3.20–3.30 (1H, br), 3.78 (3H, s), 4.75–4.85 (1H, br), 6.33 (1H, d, J = 2.4 Hz), 6.59 (1H, s), 6.90–7.10 (1H, br), 7.06 (1H, d, J = 7.3 Hz), 7.22 (1H, t, J = 7.3 Hz), 7.27 (1H, t, J = 7.3 Hz), 7.35 (1H, d, J = 7.3 Hz), 7.59 (1H, d, J = 8.3 Hz), 7.81 (1H, s), 8.38 (1H, d, J = 1.9 Hz). MS (FAB) m/z 472 $[M+H]^+$.

5.1.30. Methyl (2*Z*)-{1-[2-chloro-4-(1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8b)

Compound **8b** was prepared according to the procedure described for **8a** from **4b**. The title compound **8b** (570 mg, 63%) was obtained as pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20–2.80 (2H, m), 3.20–3.40 (1H, br), 3.86 (3H, s), 4.90–5.15 (1H, br), 6.13 (1H, s), 6.45 (1H, t, J = 2.8 Hz), 6.95–7.01 (1H, m), 7.04 (1H, d, J = 7.6 Hz), 7.12 (1H, td, J = 7.6, 1.6 Hz), 7.20 (1H, t, J = 7.6 Hz), 7.30 (1H, dd, J = 7.6, 1.6 Hz), 7.32–7.37 (1H, m), 7.66–7.70 (2H, m), 7.82 (1H, d, J = 2.8 Hz). MS (FAB) m/z 458 $[M+H]^+$.

5.1.31. Methyl (2*Z*)-{1-[2-chloro-4-(3-ethyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8c)

Compound **8c** was prepared according to the procedure described for **8a** from **4c**. The title compound **8c** (785 mg, 83%) was obtained as colorless amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.26 (3H, t, J = 7.6 Hz), 2.25–2.80 (2H, m), 2.69 (2H, q, J = 7.6 Hz), 3.20–3.50 (1H, br), 3.86 (3H, s), 4.80–5.20 (1H, br), 6.12 (1H, s), 6.26 (1H, d, J = 2.6 Hz), 6.92–6.97 (1H, m), 7.03 (1H, d, J = 7.5 Hz), 7.11 (1H, dt, J = 1.5, 7.6 Hz), 7.19 (1H, dt, J = 1.3, 7.4 Hz), 7.27–7.33 (2H, m), 7.63 (1H, d, J = 2.0 Hz), 7.72 (1H, d, J = 2.6 Hz). MS (FAB) m/z 486 $[M+H]^+$.

5.1.32. Methyl (2*Z*)-{1-[2-chloro-4-(3-isopropyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8d)

Compound **8d** was prepared according to the procedure described for **8a** from **4d**. The title compound **8d** (971 mg, 77%) was obtained as colorless amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.28 (6H, d, J = 7.0 Hz), 2.30–2.80 (2H, m), 2.95–3.10 (1H, m), 3.20–3.50 (1H, br), 3.86 (3H, s), 4.80–5.15 (1H, br), 6.13 (1H, s), 6.27 (1H, d, J = 2.6 Hz), 6.9–6.97 (1H, m), 7.04 (1H, d, J = 7.7 Hz), 7.11 (1H, dt, J = 1.5 ,7.7 Hz), 7.19 (1H, dt, J = 1.3 ,7.3 Hz), 7.27–7.34 (2H, m), 7.64 (1H, d, J = 2.0 Hz), 7.71 (1H, d, J = 2.6 Hz). MS (FAB) m/z 500 $[M+H]^+$.

5.1.33. Methyl (2*Z*)-{1-[2-chloro-4-(3-phenyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8e)

Compound **8e** was prepared according to the procedure described for **8a** from **4e**. The title compound **8e** (200 mg, 37%) was obtained as white solid. ^{1}H NMR (300 MHz, DMSO-d_6): 3.34 (3H, s), 6.62 (1H, s), 7.04–7.11 (2H, m), 7.19–7.3 (2H, m), 7.33–7.48 (5H, m), 7.75 (1H, d, J = 7.5 Hz), 7.87–7.93 (2H, m), 7.96 (1H, s), 8.54–8.62 (1H, m). MS (FAB) m/z 534 $[M+H]^+$.

5.1.34. Methyl (2*Z*)-{1-[2-chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8f)

Compound **8f** was prepared according to the procedure described for **8a** from **4f**. The title compound **8f** (400 mg, 47%) was obtained as white solid. ^{1}H NMR (300 MHz, $CDCl_3$): 2.24 (3H, s), 2.25 (3H, s), 2.10–2.80 (2H, m), 3.25–3.45 (1H, br), 3.85 (3H, s), 4.85–5.15 (1H, br), 5.97 (1H, s), 6.12 (1H, s), 6.90–7.31 (6H, m), 7.43 (1H, s). MS (FAB) m/z 486 $[M+H]^+$.

5.1.35. Methyl (2*Z*)-{1-[2-chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8g)

Compound **8g** was prepared according to the procedure described for **8a** from **4g**. The title compound **8g** (683 mg, 85%) was obtained as colorless amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6): 2.29 (3H, s), 2.45–2.65 (2H, m), 3.30–3.40 (1H, m), 3.77 (3H, s), 4.70–4.90 (1H, m), 6.27 (1H, s), 6.58 (1H, s), 7.06–7.13 (1H, m), 7.20–7.39 (5H, m), 7.54–7.61 (2H, m). MS (FAB) m/z 472 $[M+H]^+$.

5.1.36. Methyl (2*Z*)-{1-[2-chloro-4-(2*H*-indazol-2-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8h)

Compound **8h** was prepared according to the procedure described for **8a** from **4h**. The title compound **8h** (300 mg, 29%) was obtained as pale yellow amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): 2.30–2.80 (2H, m), 3.20–3.45 (1H, m), 3.87 (3H, s), 4.85–5.20 (1H, m), 6.15 (1H, s), 7.03–7.23 (5H, m), 7.28–7.34 (2H, m), 7.59 (1H, d, J = 8.3 Hz), 7.65 (1H, d, J = 8.4 Hz), 7.70 (1H, d, J = 9.0 Hz), 7.91 (1H, d, J = 1.8 Hz), 8.31 (1H, s). MS (FAB) m/z 508 $[M+H]^+$.

5.1.37. Methyl (2*Z*)-{1-[2-chloro-4-(pyrrolidin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8i)

Compound **8i** was prepared according to the procedure described for **8a** from **4i**. The title compound **8i** (920 mg, 92%) was obtained as yellow amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.93–2.07 (6H, m), 2.54–2.61 (2H, br), 3.13–3.23 (4H, br), 3.84 (3H, s), 6.10–6.16 (2H, m), 6.36–6.44 (2H, m), 6.62–6.71 (1H, m), 6.93–7.02 (1H, m), 7.12–7.23 (2H, m),. MS (FAB) m/z 461 $[M+H]^+$.

5.1.38. Methyl (2*Z*)-{1-[2-chloro-4-(3-methylpyrrolidin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8j)

Compound **8j** was prepared according to the procedure described for **8a** from **4j**. The title compound **8j** (414 mg, 44%) was obtained as yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 1.09 (3H, d, J = 6.8 Hz), 1.50–1.70 (3H, m), 2.02–2.15 (1H, m), 2.28–2.80 (4H, m), 3.20–3.35 (3H, m), 3.84 (3H, s), 6.00–6.08 (1H, m), 6.12 (1H, s), 6.33–6.39 (1H, m), 6.61–6.68 (1H, m), 6.94–7.01 (1H, m), 7.10–7.21 (2H, m), 7.26–7.31 (1H, m). MS (FAB) m/z 475 $[M+H]^+$.

5.1.39. Methyl (2*Z*)-{1-[2-chloro-4-(2-methylpyrrolidin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8k)

Compound **8k** was prepared according to the procedure described for **8a** from **4k**. The title compound **8k** (413 mg, 40%) was obtained as yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 1.06–1.12 (3H, m), 1.53–1.72 (3H, m), 1.91–2.09 (4H, m), 2.40–2.58 (1H, m), 3.00–3.11 (1H, m), 3.23–3.34 (1H, m), 3.71–3.81 (1H, m), 3.84 (3H, s), 6.08–6.17 (2H, m), 6.37–6.45 (1H, m), 6.58–6.69 (1H, m), 6.94–7.03 (1H, m), 7.09–7.23 (2H, m), 7.26–7.33 (1H, m). MS (FAB) *m*/*z* 475 $[M+H]^+$.

5.1.40. Methyl (2*Z*)-(1-{2-chloro-4-[3-(dimethylamino)pyrrolidin-1-yl]benzoyl}-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate (8n)

A mixture of methyl 2-chloro-4-fluorobenzoate (**2**; 1.20 g, 6.36 mmol), 3-(dimethylamino)pyrrolidine (880 mg, 7.68 mmol), and potassium carbonate (1.30 g, 9.24 mmol) in NMP (8 ml) was heated overnight at 120 °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 sodium sulfate, filtered, and concentrated in vacuo. A mixture of this residue and aqueous sodium hydroxide (5 M, 4 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 hydrochloride. Resulting precipitates were collected by filtration and dried in vacuo to give a hydrochloride salt of compound **4n** (1.95 g, quantitative yield) as brown solid. A mixture of **4n** (730 mg, 2.40 mmol) and thionyl chloride (5 ml) was stirred at 80 °C for 45 min. 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[9] (500 mg, 1.97 mmol) and the mixture was stirred at 60 °C for 3 h. The solvent was evaporated, and the residue was partitioned between aqueous citric acid and $CHCl_3$. 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 ($CHCl_3$/methanol = 30:1) to give the title compound **8n** (791 mg, 80%) as brown amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 1.83–1.89 (1H, m), 2.12–2.29 (9H, m), 2.35–2.65 (1H, m), 2.72–2.83 (1H, m), 2.98–3.07 (1H, m), 3.16–3.27 (1H, m), 3.28–3.41 (2H, m), 3.80–3.86 (4H, m), 6.02–6.13 (2H, m), 6.35–6.39 (1H, m), 6.63–6.70 (1H, m), 6.92–6.70 (1H, m), 7.11–7.32 (3H, m). MS (FAB) *m*/*z* 504 $[M+H]^+$.

5.1.41. Methyl (2*Z*)-{1-[2-chloro-4-(4-methylpiperazin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (8o)

Compound **8o** was prepared according to the procedure described for **8n**. The title compound **8o** (614 mg, 57%) was obtained as pale brown amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 2.28–2.33 (4H, m), 2.45–2.51 (5H, m), 3.10–3.30 (5H, m), 3.81–3.87 (4H, m), 6.10 (1H, s), 6.46 (1H, d, *J* = 7.2 Hz), 6.68–6.75 (2H, m), 6.98 (1H, d, *J* = 8.0 Hz), 7.12 (1H, t, *J* = 7.2 Hz), 7.18 (1H, t, *J* = 7.8 Hz), 7.26–7.29 (1H, m). MS (FAB) *m*/*z* 490 $[M+H]^+$.

5.1.42. (2*Z*)-{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}acetic acid (9a)

Methyl (2*Z*)-{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}-acetate (**8a**; 9.37 g, 19.9 mmol) in THF (50 ml) and methanol (20 ml) was treated with sodium hydroxide (1.0 M, 20.0 ml, 20.0 mmol) at 50 °C for 12 h. The reaction was then allowed to cool to room temperature and partitioned between water and chloroform. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was crystallized (*n*-hexane/ethyl acetate) and subsequently recrystallized (*n*-hexane/ethyl acetate) to give the title compound **9a** (9.00 g, 99%) as colorless crystals. Mp: 252–254 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.40–2.65 (2H, br), 3.10–3.25 (1H, br), 4.73–4.91 (1H, br), 6.33 (1H, d, *J* = 2.4 Hz), 6.50 (1H, s), 7.04 (1H, d, *J* = 7.3 Hz), 6.93–7.10 (1H, br), 7.20 (1H, dt, *J* = 1.5, 7.3 Hz), 7.26 (1H, t, *J* = 7.3 Hz), 7.32 (1H, d, *J* = 7.3 Hz), 7.60 (1H, d, *J* = 8.3 Hz), 7.81 (1H, s), 8.38 (1H, d, *J* = 2.4 Hz), 13.15–13.30 (1H, br). MS (FAB) *m*/*z* 458 $[M+H]^+$.

5.1.43. (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*-(pyridin-2-ylmethyl)acetamide hydrochloride (10a)

A mixture of (2*Z*)-{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}acetic acid (**9a**; 770 mg, 1.68 mmol), 2-(aminomethyl)pyridine (0.19 ml, 1.85 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD) (490 mg, 2.52 mmol), and 1-hydroxy-1*H*-benzotriazole (HOBt) (310 mg, 2.52 mmol) in DMF 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 $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography ($CHCl_3$/methanol = 25:1) and subsequently treated with hydrochloride in ethyl acetate, then evaporated. The residue was crystallized from ethanol and collected by filtration to give the title compound **10a** (687 mg, 70%) as colorless crystals. Mp: 214–217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.40–2.50 (1H, br), 2.67–2.89 (1H, br), 3.11–3.23 (1H, br), 4.73 (2H, d, *J* = 5.4 Hz), 4.76–4.90 (1H, br), 6.34 (1H, d, *J* = 2.5 Hz), 6.41(1H, s), 6.99 (1H, d, *J* = 7.8 Hz), 7.19(1H, t, *J* = 7.8 Hz), 7.26(1H, t, *J* = 7.8 Hz), 7.33(1H, d, *J* = 6.8 Hz), 7.61 (1H, d, *J* = 8.3 Hz), 7.79–7.88 (3H, m) ,8.35–8.45 (2H, m) ,8.79 (1H, d, *J* = 4.8 Hz), 9.30 (1H, s). MS (FAB) *m*/*z* 548 $[M+H]^+$. Anal. Calcd for $C_{29}H_{24}ClF_2N_5O_2 \cdot HCl \cdot 0.1H_2O$: C, 59.41; H, 4.33; N, 11.95; Cl, 12.09; F, 6.48. Found: C, 59.19; H, 4.40; N, 11.85; Cl, 12.06; F, 6.40.

5.1.44. (2*Z*)-2-{1-[2-Chloro-4-(1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10b)

Methyl (2*Z*)-{1-[2-chloro-4-(1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}acetate (**8b**; 560 mg, 1.22 mmol) in THF and methanol was treated with sodium hydroxide (1.0 M, 3.0 ml, 3.0 mmol) at room temperature for 4 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 crude product of **9b**. A mixture of foregoing crude product of **9b**, 2-(aminomethyl)pyridine (0.18 ml, 1.93 mmol), WSCD (390 mg, 1.93 mmol), and HOBt (240 mg, 1.93 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 $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/methanol = 60:1) and subsequently treated with hydrochloride in ethyl acetate, then evaporated. Resulting solid was crystallized from ethanol and collected by filtration to give the title compound **10b** (547 mg, 79%) as colorless crystals. Mp: 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31–2.48 (1H, br), 2.64–2.90 (1H, br), 3.05–3.24 (1H, br), 4.73 (2H, d, *J* = 5.3 Hz), 4.76–4.90 (1H, br), 6.55 (1H, t, *J* = 1.5 Hz), 6.56 (1H, s), 7.01 (1H, d, *J* = 7.3 Hz), 7.05–7.17 (1H, br), 7.20 (1H, dt, *J* = 1.5, 7.3 Hz), 7.26 (1H, t, *J* = 7.3 Hz), 7.32–7.37 (1H, m), 7.68 (1H, dd, *J* = 1.5, 8.3 Hz), 7.74–7.77 (1H, m), 7.79–7.87 (2H, m),

7.91 (1H, s), 8.43 (1H, t, J = 7.9 Hz), 8.52 (1H, d, J = 2.4 Hz), 8.80 (1H, d, J = 5.4 Hz), 9.31 (1H, s). MS (FAB) m/z 534 $[M+H]^+$. Anal. Calcd for $C_{28}H_{22}ClF_2N_5O_2{\cdot}HCl$: C, 58.96; H, 4.06; N, 12.28; Cl, 12.43; F, 6.66. Found: C, 58.95; H, 4.02; N, 12.28; Cl, 12.28; F, 6.85.

5.1.45. (2*Z*)-2-{1-[2-Chloro-4-(3-ethyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10c)

Compound **10c** was prepared according to the procedure described for **10b**. The title compound **10c** (363 mg, 71%) was obtained as colorless crystals. Mp: 199–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19 (3H, t, J = 7.6 Hz), 2.25–2.40 (1H, br), 2.60 (2H, q, J = 7.6 Hz), 2.70–2.85 (1H, br), 3.10–3.25 (1H, br), 4.74 (2H, d, J = 5.6 Hz), 4.75–4.90 (1H, br), 6.38 (1H, d, J = 2.4 Hz), 6.57 (1H, s), 6.99 (1H, d, J = 7.6 Hz), 7.01–7.15 (1H, br), 7.19 (1H, dt, J = 1.3, 8.0 Hz), 7.26 (1H, t, J = 7.4 Hz), 7.33 (1H, dd, J = 1.2, 8.0 Hz), 7.62 (1H, d, J = 8.8 Hz), 7.79 - 7.88 (3H, m), 8.39 (1H, d, J = 2.4 Hz), 8.43 (1H, t, J = 7.8 Hz), 8.80 (1H, d, J = 5.6 Hz), 9.30 (1H, s). MS (FAB) m/z 562 $[M]^+$. Anal. Calcd for $C_{30}H_{26}ClF_2N_5O_2$.HCl: C, 60.21; H, 4.55; N, 11.70; Cl, 11.85; F, 6.35. Found: C, 60.07; H, 4.41; N, 11.67; Cl, 11.77; F, 6.31.

5.1.46. (2*Z*)-2-{1-[2-Chloro-4-(3-isopropyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10d)

Compound **10d** was prepared according to the procedure described for **10b**. The title compound **10d** (424 mg, 74%) was obtained as colorless crystals. Mp: 205–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.21 (6H, d, J = 6.8 Hz), 2.25–2.40 (1H, br), 2.65–2.80 (1H, br), 2.85–3.02 (1H, m), 3.10–3.25 (1H, br), 4.74 (2H, d, J = 5.2 Hz), 4.75–4.90 (1H, br), 6.41 (1H, d, J = 2.4 Hz), 6.58 (1H, s), 6.99 (1H, d, J = 7.6 Hz), 7.00–7.15 (1H, br), 7.19 (1H, t, J = 7.6 Hz), 7.26 (1H, t, J = 7.4 Hz), 7.34 (1H, d, J = 8.0 Hz), 7.62 (1H, d, J = 8.4 Hz), 7.79–7.88 (3H, m), 8.39 (1H, d, J = 2.4 Hz), 8.44 (1H, t, J = 7.6 Hz), 8.81 (1H, d, J = 5.2 Hz), 9.31 (1H, s). MS (FAB) m/z 576 $[M]^+$. Anal. Calcd for $C_{31}H_{28}ClF_2N_5O_2{\cdot}HCl$: C, 60.79; H, 4.77; N, 11.43; Cl, 11.58; F, 6.20. Found: C, 60.50; H, 4.61; N, 11.73; Cl, 11.60; F, 6.04.

5.1.47. (2*Z*)-2-{1-[2-Chloro-4-(3-phenyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide (10e)

Compound **10e** was prepared according to the procedure described for **10b**. The title compound **10e** (160 mg, 70%) was obtained as colorless crystals. Mp: 164–166 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25–2.60 (1H, br), 2.65–2.95 (1H, br), 3.10–3.35 (1H, br), 4.50 (2H, d, J = 5.7 Hz), 4.75–4.95 (1H, br), 6.51 (1H, s), 6.99–7.47 (11H, m), 7.71–7.99 (5H, m), 8.53–8.56 (1H, m) ,8.59 (1H, d, J = 2.8 Hz), 9.05 (1H, s). MS (FAB) m/z 610 $[M]^+$. Anal. Calcd for $C_{34}H_{26}ClF_2N_5O_2$: C, 66.94; H, 4.30; N, 11.48; Cl, 5.81; F, 6.23. Found: C, 66.61; H, 4.53; N, 11.19; Cl, 5.68; F, 6.13.

5.1.48. (2*Z*)-2-{1-[2-Chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10f)

Compound **10f** was prepared according to the procedure described for **10b**. The title compound **10f** (180 mg, 59%) was obtained as colorless crystals. Mp: 221–222 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.13 (3H, s), 2.25 (3H, s), 2.30–2.55 (1H, br), 2.65–2.90 (1H, br), 3.15–3.30 (1H, br), 4.67 (2H, d, J = 5.7 Hz), 4.75–4.85 (1H, br), 6.07 (1H, s), 6.54 (1H, s), 6.98–7.35 (6H, m), 7.55 (1H, s), 7.70–7.79 (2H, m), 8.32 (1H, d, J = 7.9 Hz), 8.75 (1H, d, J = 5.1 Hz), 9.23 (1H, s). MS (FAB) m/z 562 $[M]^+$. Anal. Calcd for $C_{30}H_{26}ClF_2N_5O_2{\cdot}HCl{\cdot}0.7H_2O$: C, 58.96; H, 4.68; N, 11.46; Cl, 11.60; F, 6.22. Found: C, 59.23; H, 4.73; N, 11.18; Cl, 11.24; F, 6.17.

5.1.49. (2*Z*)-2-{1-[2-Chloro-4-(5-methyl-1*H*-pyrazol-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10g)

Compound **10g** was prepared according to the procedure described for **10b**. The title compound **10g** (153 mg, 79%) was obtained as colorless crystals. Mp: 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (3H, s), 2.20–2.42 (1H, br), 2.69–2.89 (1H, br), 3.08–3.24 (1H, br), 4.64 (2H, d, J = 4.9 Hz), 4.75–4.97 (1H, br), 6.27 (1H, d, J = 1.2 Hz), 6.34 (1H, s), 7.02 (1H, d, J = 7.3 Hz), 7.05–7.16 (1H, br), 7.20 (1H, t, J = 7.3 Hz), 7.28 (1H, t, J = 7.3 Hz), 7.31–7.37 (2H, m), 7.56 (1H, d, J = 1.2 Hz), 7.60 (1H, s), 7.63–7.74 (2H, m), 8.19–8.31 (1H, m), 7.73 (1H, d, J = 4.9 Hz), 9.19 (1H, s). MS (FAB) m/z 548 $[M+H]^+$. Anal. Calcd for $C_{29}H_{24}ClF_2N_5O_2{\cdot}HCl{\cdot}0.2H_2O$: C, 59.23; H, 4.35; N, 11.91; Cl, 12.06; F, 6.46. Found: C, 59.19; H, 4.36; N, 11.86; Cl, 12.03; F, 6.20.

5.1.50. (2*Z*)-2-{1-[2-Chloro-4-(2*H*-indazol-2-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10h)

Compound **10h** was prepared according to the procedure described for **10b**. The title compound **10h** (220 mg, 75%) was obtained as colorless crystals. Mp: 158–160 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25–2.55 (1H, m), 3.10–3.30 (1H, m), 3.95–4.05 (1H, m), 4.69 (2H, d, J = 5.5 Hz), 4.70–4.95 (1H, m), 6.58 (1H, s), 7.02–7.38 (7H, m), 7.57–7.81 (4H, m), 7.94 (1H, d, J = 8.6 Hz), 8.19 (1H, s), 8.33 (1H, t, J = 7.8 Hz), 8.76 (1H, d, J = 5.5 Hz), 9.12 (1H, s), 9.20–9.31 (1H, m). MS (FAB) m/z 584 $[M]^+$. Anal. Calcd for $C_{32}H_{24}ClF_2N_5O_2{\cdot}0.85HCl{\cdot}H_2O$: C, 60.72; H, 4.28; N, 11.06; Cl, 10.36; F, 6.00. Found: C, 60.62; H, 4.25; N, 10.78; Cl, 10.20; F, 5.75.

5.1.51. (2*Z*)-2-[1-(2-Chloro-4-pyrrolidin-1-ylbenzoyl)-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene]-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10i)

Compound **10i** was prepared according to the procedure described for **10b**. The title compound **10i** (456 mg, 40%) was obtained as pale yellow crystals. Mp: 194–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.82–1.69 (4H, br), 2.41–2.48 (1H, br), 2.53–2.60 (1H, br), 3.08–3.17 (5H, br), 4.72 (2H, d, J = 5.4 Hz), 4.75–4.81 (1H, br), 6.17–6.25 (1H, br), 6.38–6.44 (1H, br), 6.48 (1H, s), 6.67–6.75 (1H, br), 6.84–6.91 (1H, br), 7.15–7.35 (3H, m), 7.79–7.86 (2H, m), 8.43 (1H, t, J = 7.8 Hz), 8.79 (1H, d, J = 4.9 Hz), 9.25 (1H, s). MS (FAB) m/z 537 $[M]^+$. Anal. Calcd for $C_{29}H_{27}ClF_2N_4O_2{\cdot}HCl$: C, 60.74; H, 4.92; N, 9.77; Cl, 12.36; F, 6.63. Found: C, 60.42; H, 4.78; N, 9.81; Cl, 12.34; F, 6.36.

5.1.52. *rac*-(2*Z*)-2-{1-[2-Chloro-4-(3-methylpyrrolidin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10j)

Compound **10j** was prepared according to the procedure described for **10b**. The title compound **10j** (298 mg, 59%) was obtained as colorless crystals. Mp: 200–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (3H, d, J = 6.9 Hz), 1.45–1.59 (1H, m), 1.94–2.08 (1H, m), 2.23–2.33 (1H, m), 2.35–2.47 (1H, br), 2.57–2.64 (1H, br), 2.67–2.78 (1H, m), 2.92–3.05 (1H, br), 3.08–3.17 (1H, m), 3.19–3.25 (1H, m), 3.28–3.37 (1H, m), 4.74 (2H, d, J = 5.3 Hz), 4.76–4.93 (1H, br), 6.17–6.26 (1H, br), 6.35–6.43 (1H, br), 6.49 (1H, s), 6.65–6.77 (1H, br), 6.84–6.93 (1H, br), 7.16–7.34 (3H, m), 7.82–7.90 (2H, m), 8.45 (1H, t, J = 7.8 Hz), 8.80 (1H, d, J = 5.3 Hz), 9.27 (1H, t, J = 5.3 Hz). MS (FAB) m/z 551 $[M]^+$. Anal. Calcd for $C_{30}H_{29}ClF_2N_4O_2{\cdot}HCl{\cdot}0.3H_2O$: C, 60.77; H, 5.20; N, 9.45; Cl, 11.96; F, 6.41. Found: C, 60.87; H, 5.10; N, 9.50; Cl, 12.15; F, 6.55.

5.1.53. *rac*-(2*Z*)-2-{1-[2-Chloro-4-(2-methylpyrrolidin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10k)

Compound **10k** was prepared according to the procedure described for **10b**. The title compound **10k** (220 mg, 44%) was obtained as colorless crystals. Mp: 182–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00 (3H, d, J = 5.4 Hz), 1.56–1.68 (1H, m), 1.83–2.04 (3H, m), 2.22–2.47 (1H, br), 2.54–2.78 (1H, br), 2.96–3.05 (1H, br), 3.12–3.35 (2H, br), 3.75–3.84 (1H, br), 4.73 (2H, d, J = 5.3 Hz), 4.78–4.87 (1H, br), 6.23–6.32 (1H, br), 6.38–6.46 (1H, br), 6.50 (1H, s), 6.66–6.75 (1H, br), 6.84–6.93 (1H, br),7.15–7.29 (2H, m), 7.30–7.38 (1H, m), 7.83–7.89 (2H, m), 8.45 (1H, t, J = 7.8 Hz), 8.80 (1H, d, J = 5.3 Hz), 9.27 (1H, t, J = 5.3 Hz). MS (FAB) m/z 551 [M]$^+$. Anal. Calcd for $C_{30}H_{29}ClF_2N_4O_2\cdot HCl\cdot 0.3H_2O$: C, 60.77; H, 5.20; N, 9.45; Cl, 11.96; F, 6.41. Found: C, 60.77; H, 5.00; N, 9.57; Cl, 12.08; F, 6.28.

5.1.54. *rac*-(2*Z*)-2-(1-{2-Chloro-4-[3-(dimethylamino)pyrrolidin-1-yl]benzoyl}-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10n)

Compound **10n** was prepared according to the procedure described for **10b**. The title compound **10n** (659 mg, 66%) was obtained as colorless crystals. Mp: >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.21–2.49 (4H, m), 2.75 (6H, s), 3.00–3.22 (2H, m), 3.35–3.64 (3H, m), 3.83–3.97 (1H, m), 4.69 (2H, d, J = 6.0 Hz), 4.70–5.00 (1H, br), 6.31 (1H, d, J = 7.2 Hz), 6.46 (1H, s), 6.53 (1 H, s), 6.75–6.77 (1H, m), 6.88 (1H, d, J = 6.8 Hz), 7.15–7.34 (3H, m), 7.71–7.79 (2H, m), 8.33 (1H, t, J = 7.8 Hz), 8.75 (1H, d, J = 5.2 Hz), 9.27 (1H, s), 11.61 (1H, s). MS (FAB) m/z 580 [M]$^+$. Anal. Calcd for $C_{31}H_{32}ClF_2N_5O_2\cdot 1.8HCl\cdot 2H_2O$: C, 54.62; H, 5.59; N, 10.27; Cl, 14.56; F, 5.57. Found: C, 54.77; H, 5.51; N, 10.24; Cl, 14.43; F, 5.34.

5.1.55. (2*Z*)-2-{1-[2-Chloro-4-(4-methylpiperazin-1-yl)benzoyl]-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene}-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10o)

Compound **10o** was prepared according to the procedure described for **10b**. The title compound **10o** (497 mg, 62%) was obtained as colorless crystals. Mp: 206–208 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.25–2.60 (1H, m), 2.65–2.80 (4H, m), 2.95–3.15 (5H, m), 3.37–3.42 (2H, m), 3.77–3.83 (2H, m), 4.75 (2H, d, J = 5.2 Hz), 4.76–4.90 (1H, br), 6.48 (1H, s), 6.72–6.77 (1H, m), 6.81–6.98 (3H, m), 7.19 (1H, t, J = 7.2 Hz), 7.26 (1H, t, J = 7.4 Hz), 7.32 (1H, d, J = 6.8 Hz), 7.80–7.88 (2H, m), 8.45 (1H, t, J = 7.8 Hz), 8.80 (1H, d, J = 5.2 Hz), 9.33 (1H, s), 11.39 (1H, s),. MS (FAB) m/z 566 [M]$^+$. Anal. Calcd for $C_{30}H_{30}ClF_2N_5O_2\cdot 2HCl\cdot 4H_2O$: C, 50.68; H, 5.67; N, 9.85; Cl, 14.96; F, 5.34. Found: C, 50.29; H, 5.50; N, 9.82; Cl, 14.78; F, 5.18.

5.1.56. (2*Z*)-(4,4-Difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetic acid (12)

Methyl (2*Z*)-(4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate (**11**; 8.00 g, 31.6 mmol) in THF (20 ml) and methanol (20 ml) was treated with sodium hydroxide (1.0 M, 45.0 ml, 45.0 mmol) overnight at room temperature. After the pH value was adjusted around 5.0–6.0 by addition of aqueous hydrochloride, the mixture was partitioned between water and chloroform. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the title compound **12** (4.57 g, 60%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.54–2.69 (2H, m), 3.42 (2H, t, J = 6.0 Hz), 6.20 (1H, s), 6.61 (1H, dd, J = 0.9, 8.1 Hz), 6.82 (1H, dt, J = 1.0, 7.4 Hz), 7.16 (1H, dt, J = 1.3, 8.0 Hz), 7.22 (1H, dd, J = 1.5, 7.7 Hz). MS (FAB) m/z 240 [M+H]$^+$.

5.1.57. (2*Z*)-2-(4,4-Difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(pyridin-2-ylmethyl)acetamide (13)

Methyl (2*Z*)-2-(4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(pyridin-2-ylmethyl)acetate (**12**; 4.57 g, 19.1 mmol), 2-(aminomethyl)pyridine (2.22 ml, 21.0 mmol), WSCD (5.60 g, 29.0 mmol), and HOBt (3.60 mg, 29.0 mmol) in DMF (45 ml) were 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 crude product was purified by silica gel column chromatography ($CHCl_3$/methanol = 25:1) to give the title compound **13** (6.29 g, quantitative yield) as green amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40–2.65 (2H, m), 3.41 (2H, t, J = 5.9 Hz), 4.66 (2H, d, J = 5.3 Hz), 4.75–5.20 (1H, br), 6.30 (1H, s), 6.59 (1H, dd, J = 0.8, 8.1 Hz), 6.79 (1H, dt, J = 0.9, 7.4 Hz), 7.09–7.26 (4H, m), 7.37 (1H, d, J = 7.9 Hz), 7.71 (1H, dt, J = 1.6, 7.8 Hz), 8.52 (1H, d, J = 4.6 Hz). MS (FAB) m/z 330 [M+H]$^+$.

5.1.58. (2*Z*)-2-[1-(2-Chloro-4-piperidin-1-ylbenzoyl)-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene]-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10l)

A mixture of 2-chloro-4-(piperidin-1-yl)benzoic acid (**4l**; 350 mg, 1.46 mmol), DMF (several drops), and thionyl chloride (0.22 ml, 2.20 mmol) in THF (10 ml) was stirred at room temperature for 2.5 h. The reaction mixture was evaporated and azeotroped with toluene. To a solution of this residue in acetonitrile (20 ml) were added (2*Z*)-2-(4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)-*N*-(pyridin-2-ylmethyl)acetamide (**13**, 400 mg, 1.21 mmol) and pyridine (0.40 ml, 4.90 mmol), and the mixture was stirred overnight at 80 °C. The reaction was then allowed to cool to room temperature and evaporated. The residue was partitioned between aqueous citric acid and chloroform. The organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography ($CHCl_3$/methanol/aq NH_3 = 25:1:0.1) and subsequently treated with hydrochloride in ethyl acetate, then evaporated. Resulting solid was recrystallized from ethanol and collected by filtration to give the title compound **10l** (176 mg, 24%) as colorless crystals. Mp: 196–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46–1.60 (6H, br), 2.44–2.49 (1H, br), 2.55–2.61 (1H, br), 3.11–3.17 (4H, br), 3.22–3.25 (1H, br), 4.68 (2H, d, J = 5.4 Hz), 4.74–4.88 (1H, br), 6.46 (1H, s), 6.64–6.73 (1H, br), 6.75–6.81 (1H, br), 6.83–6.93 (2H, m), 7.15–7.36 (3H, m), 7.73–7.82 (2H, m), 8.34 (1H, t, J = 7.4 Hz), 8.77 (1H, d, J = 4.9 Hz), 9.21 (1H, s). MS (FAB) m/z 551 [M]$^+$. Anal. Calcd for $C_{30}H_{29}ClF_2N_4O_2\cdot HCl\cdot 0.5H_2O$: C, 60.41; H, 5.24; N, 9.39; Cl, 11.89; F, 6.37. Found: C, 60.71; H, 5.17; N, 9.38; Cl, 11.96; F, 6.31.

5.1.59. (2*Z*)-2-[1-(4-Azepan-1-yl-2-chlorobenzoyl)-4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene]-*N*-(pyridin-2-ylmethyl)acetamide hydrochloride (10m)

Compound **10m** was prepared according to the procedure described for **10l** from **4m** and **13**. The title compound **10m** (165 mg, 22%) was obtained as colorless crystals. Mp: 194–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.33–1.45 (4H, br), 1.55–1.67 (4H, br), 2.41–2.47 (1H, br), 2.54–2.59 (1H, br), 3.02–3.21 (1H, br), 3.28–3.44 (4H, br), 4.67 (2H, d, J = 5.4 Hz), 4.72–4.93 (1H, br), 6.33–6.42 (1H, br), 6.46 (1H, s), 6.53–6.58 (1H, br), 6.62–6.71 (1H, br), 6.83–6.90 (1H, br), 7.13–7.34 (3H, m), 7.71–7.80 (2H, m), 8.34 (1H, t, J = 7.3 Hz), 8.76 (1H, d, J = 4.9 Hz), 9.18 (1H, s). MS (FAB) m/z 565 [M]$^+$. Anal. Calcd for $C_{31}H_{31}ClF_2N_4O_2\cdot HCl\cdot 0.5H_2O$: C, 60.99; H, 5.45; N, 9.18; Cl, 11.61; F, 6.22. Found: C, 61.18; H, 5.34; N, 9.19; Cl, 11.68; F, 6.17.

5.2. Biology

5.2.1. Binding assay for human V_2 receptor

Chinese hamster ovary (CHO) cells stably expressing human V2 receptors, which were established by Tahara et al.,[10] 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 was 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 3. 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 means ± 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 nonlinear 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,[12] using the K_d values yielded from each separate experiment, $K_i = IC_{50}/(1 + [[^3H]\text{vasopressin concentration}]/K_d)$.

5.2.2. 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.[11] 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 phosphate-buffered 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.).[13]

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 results are expressed as the means ± standard error. The activities of test compounds for cAMP production were calculated by logistic regression as EC_{50} values.

5.2.3. Antidiuretic 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 every hour up to 4 h after water loading. 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 (version 8.2, SAS Institute Japan). The results are expressed as the mean (95% confidence interval) or the means ± standard error.

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