Bioorganic & Medicinal Chemistry 17 (2009) 3130–3141

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and structure–activity relationships of amide derivatives of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetic acid as selective arginine vasopressin V_2 receptor agonists

Issei Tsukamoto [a,*], Hiroyuki Koshio [a], Takahiro Kuramochi [a], Chikashi Saitoh [a], Hiroko Yanai-Inamura [a], Chika Kitada-Nozawa [a], Eisaku Yamamoto [a], Takeyuki Yatsu [a], Yoshiaki Shimada [a], Shuichi Sakamoto [b], Shin-ichi Tsukamoto [a]

[a] Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan
[b] Technology, Astellas Pharma Inc., 3-17-1 Hasune, Itabashi, Tokyo 174-8612, Japan

ARTICLE INFO

Article history:
Received 24 December 2008
Revised 2 March 2009
Accepted 3 March 2009
Available online 9 March 2009

Keywords:
(4,4-Difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetamide derivatives
Vasopressin V_2 receptor
Vasopressin V_{1a} receptor
CYP inhibitory activity

ABSTRACT

A series of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetamide derivatives was synthesized, and their structure–activity relationships were examined in order to identify potent and selective arginine vasopressin V_2 receptor agonists. Attempts to substitute other chemical groups in place of the 2-pyridilmethyl moiety of **1a** led to the discovery that potent V_2 binding affinity could be obtained with a wide range of functional groups. This structural tolerance allowed for the manipulation of other attributes, such as selectivity against V_{1a} receptor affinity or avoidance of the undesirable inhibition of cytochrome P450 (CYP), without losing potent affinity for the V_2 receptor. Some representative compounds obtained in this study were also found to decrease urine volume in awake rats.

1. Introduction

Arginine vasopressin (AVP) is a cyclic nonapeptide that is produced and secreted by the hypothalamo-neurohypophysical system. The vasopressin receptor family consists of three subtypes, V_{1a}, V_{1b}, and V_2.[1–3] The V_{1a} receptor, which exists mainly in vascular smooth muscle and platelets, is involved in blood pressure control. The V_{1b} receptor, which is found in the hypophysis pituitary gland, regulates adrenocorticotropic hormone (ACTH) secretion. The V_2 receptor, which has been identified in the kidney, stimulates coupled adenylate cyclase, increases cyclic adenosine monophosphate (cAMP), and plays an important role in water reabsorption. In other words, stimulation of the V_2 receptor with AVP causes water reabsorption in the kidney by increasing cAMP with subsequent activation of the aquaporin-2 water channel, which results in reducing urine volume. This implies that a V_2 receptor agonist can be used to treat diseases such as central diabetes insipidus and nocturia.[4] Desmopressin (dDAVP),[5] **OPC-51803**,[6] and **VNA932**[7] are established in the literature as compounds with this mechanism of action (Fig. 1). In addition, we recently reported that (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetamide derivatives are also novel V_2 receptor agonists (Fig. 2).[8]

As we reported previously, the chemical structure of the 'tail'-moiety (Fig. 2) in our compound strictly regulates agonist-antagonist switching for the vasopressin V_2 receptor.[8] In this paper, we describe the synthesis, biological activity, and structure–activity relationships (SARs) of the 'head'-moiety of **1a** as investigated by replacing the 2-pyridilmethyl group with various other chemical groups. In addition to the binding affinities for the V_2 and V_{1a} receptors, the cAMP accumulation activity, in vivo action, and CYP inhibitory activity of some representative compounds are also shown.

2. Chemistry

The syntheses of test compounds (**1a–1w**) are shown in Scheme 1–8. Carboxylic acid (**1q**), which is the common intermediate for preparation of various amide derivatives, was synthesized from **2** in the same manner as reported in the previous paper.[8] Condensation of **1q** with amines in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD) and 1-hydroxybenzotriazole (HOBt) yielded the target compounds

* Corresponding author. Tel.: +81 29 863 6682; fax: +81 29 852 5387.
E-mail address: issei.tsukamoto@jp.astellas.com (I. Tsukamoto).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.03.001

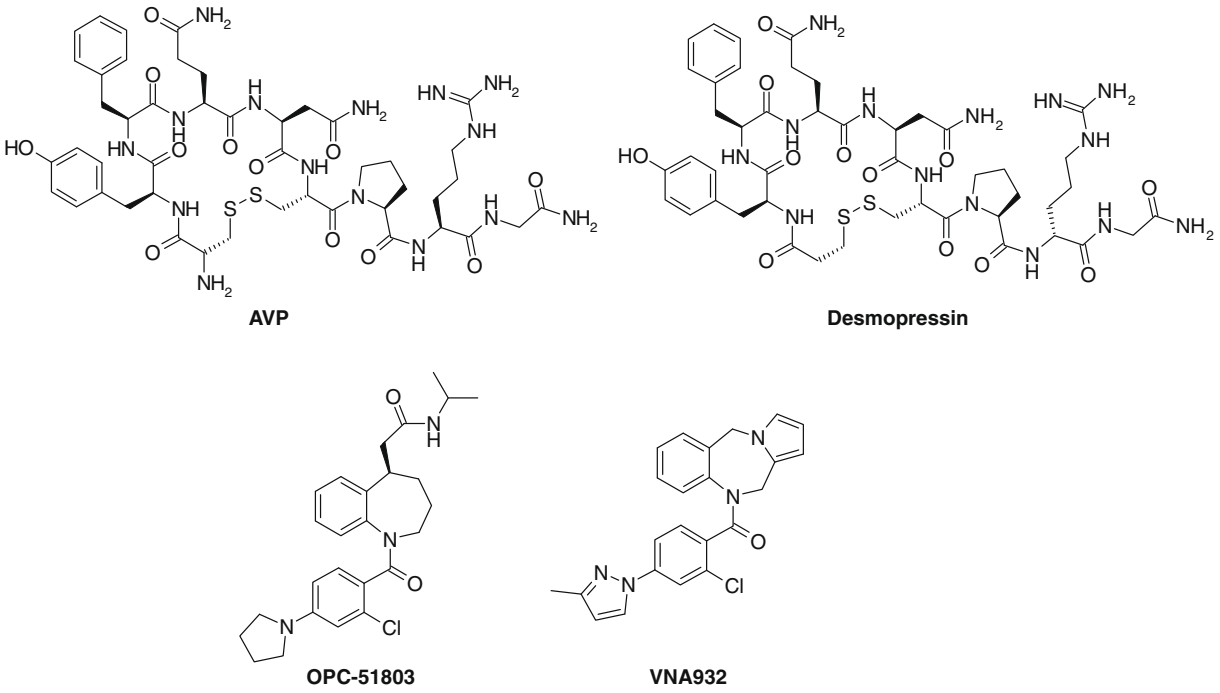


Figure 1. Chemical structure of AVP and well-known arginine vasopressin V_2 receptor agonists.

(**1a–1p**, **1s–1u**, and **1w**;Scheme 1). The carboxamide form (**1r**) was prepared from **1q** and aqueous ammonia via activation by thionyl chloride (Scheme 2). Compound **1g**, which is an amino pyridine derivative, and **1v**, which has a glycine unit, were synthesized in a stepwise manner as shown in Schemes 3 and 4, respectively.

Schemes 5–8 show the preparation of the benzylamine parts, which are used for the amidation shown in Scheme 1. Methoxy-substituted **9a** and dimethylamino-substituted **9b** were synthesized from 2,6-dibromopyridine (**6**). *ipso*-Substitution reactions with **6** and corresponding nucleophiles afforded **7a** and **7b**. The other bromo group was converted to a formyl group via lithiation by *n*-BuLi and quenching by dimethylformamide. The resulting aldehydes (**8a** and **8b**) were treated with hydroxylamine and sub-

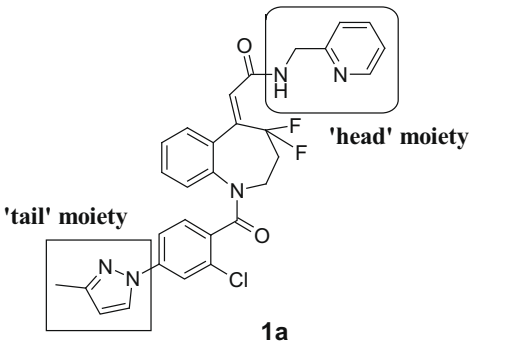
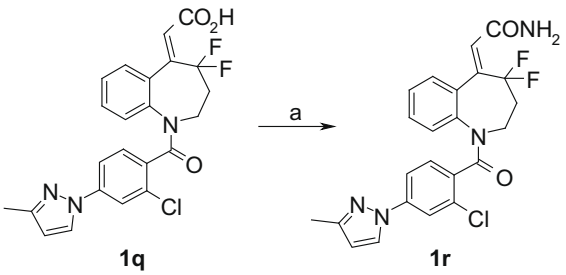
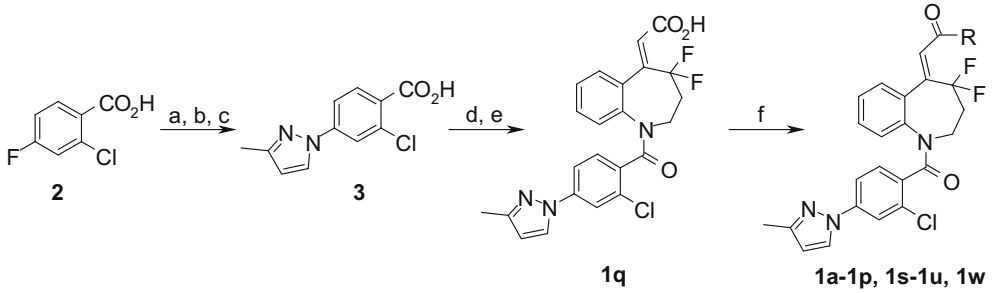


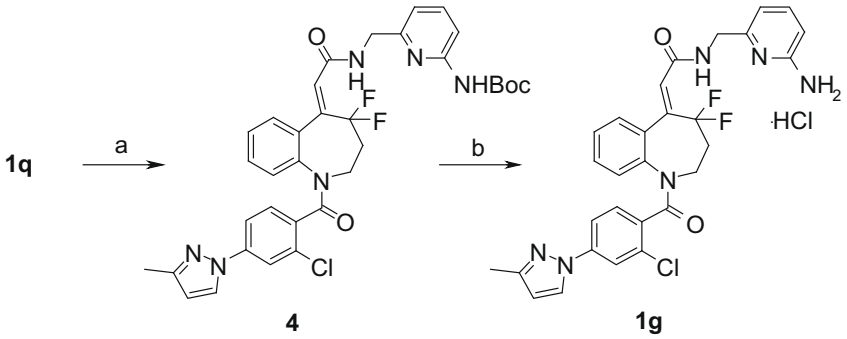
Figure 2. Chemical structure of arginine vasopressin V_2 agonist recently reported.



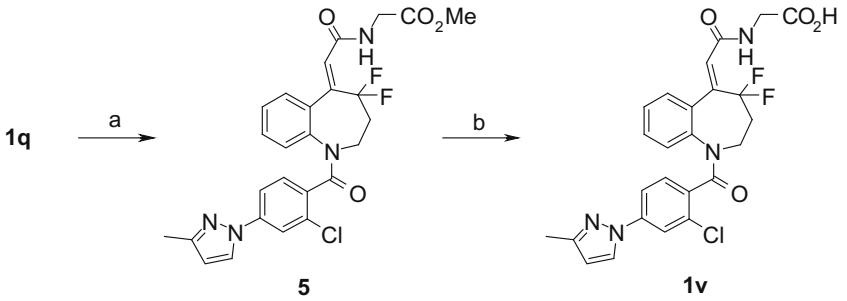
Scheme 2. Reagents and conditions: (a) $SOCl_2$, cat. DMF, THF then aq NH_3.



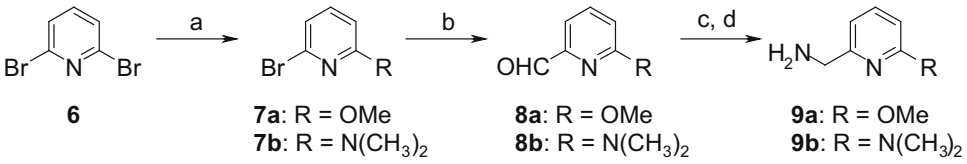
Scheme 1. Reagents and conditions: (a) H_2SO_4, MeOH; (b) 3-methylpyrazole, K_2CO_3, NMP; (c) aq HCl, acetic acid; (d) $SOCl_2$, cat. DMF, THF then methyl (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetate[9], pyridine; (e) aq NaOH, MeOH; (f) amine, WSCD, HOBt, DMF, (then HCl).



Scheme 3. Reagents and conditions: (a) **11**, WSCD, HOBt, DMF; (b) HCl, ethyl acetate.

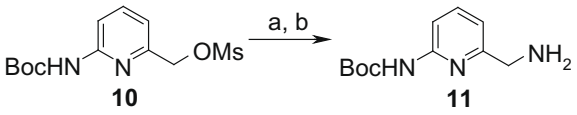


Scheme 4. Reagents and conditions: (a) glycine methyl ester, WSCD, HOBt, DMF; (b) aq NaOH, MeOH.

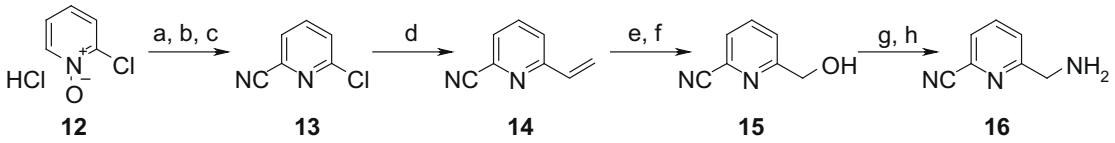


Scheme 5. Reagents and conditions: (a) NaOMe, MeOH (for **7a**) or dimethylamine, DMF, H_2O (for **7b**); (b) *n*-BuLi, THF then DMF; (c) hydroxylamine hydrochloride, K_2CO_3, EtOH; (d) H_2, Raney-Ni, EtOH.

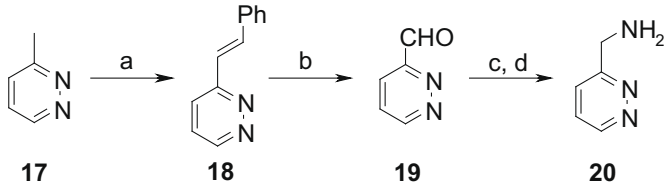
sequently hydrogenated in the presence of Raney-Ni to yield the (aminomethyl)pyridine derivatives **9a** and **9b** (Scheme 5). *tert*-Butyl [6-(aminomethyl)pyridin-2-yl]carbamate (**11**) was synthesized from a mesylate intermediate already reported (**10**)[10,11] via an azidation and a reduction (Scheme 6). 6-(Aminomethyl)pyridine-2-carbonitrile (**16**) was prepared as shown in Scheme 7. A cyano group was introduced at the 6-position of the 2-chloropyridine in accordance with an established method[12] using a commercially available N-oxide material (**12**). The chloro-substituted compound (**13**) underwent a Stille-coupling[13] reaction to afford **14**. The vinyl group in **14** was transformed into a hydroxymethyl group via ozonolysis, and subsequently reduced by $NaBH_4$ to yield an alcohol (**15**). Lastly, **15** was converted into the targeted amine (**16**) via a phthalimide intermediate. Scheme 8 illustrates the synthesis of 3-(aminomethyl)pyridazine (**20**). Compound **18** was synthesized by condensation of 3-methylpyridazine (**17**) with benzaldehyde.[14] The olefin compound (**18**) was oxidatively cleaved to afford the aldehyde (**19**), which was then converted into the amine compound (**20**) in a manner similar to that described in Scheme 5.



Scheme 6. Reagents and conditions: (a) NaN_3, DMF; (b) H_2, Pd–C, EtOH.



Scheme 7. Reagents and conditions: (a) KOMe, MeOH; (b) dimethyl sulfate; (c) NaCN, H_2O; (d) tributyl(vinyl)tin, $Pd(PPh_3)_4$, toluene; (e) O_3, MeOH then Me_2S; (f) $NaBH_4$, MeOH; (g) phthalimide, DEAD, PPh_3, THF; (h) hydrazine hydrate, MeOH, $CHCl_3$.



Scheme 8. Reagents and conditions: (a) benzaldehyde, $ZnCl_2$; (b) $NaIO_4$, cat. OsO_4, *t*-BuOH, acetone, H_2O; (c) hydroxylamine hydrochloride, K_2CO_3, EtOH; (d) H_2, Pd–C, EtOH.

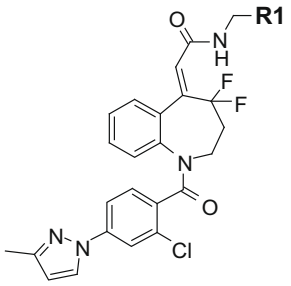
3. Results and discussion

Synthesized compounds **1a–1u** were evaluated for their human V_2 and V_{1a} receptor binding affinity, which was quantified in terms of K_i in a radioligand binding assay.

Compound **1a**, which was reported previously, showed V_2[8] and V1a receptor affinity with K_i values of 4.8 nM and 26 nM, respectively. The replacement of the pyridin-2-yl group in **1a** with a pyridin-3-yl group (**1b**), a pyridin-4-yl group (**1c**), and a phenyl group (**1d**) were examined first. As shown in Table 1, both **1b** and **1c** had a binding affinity for the V_2 receptor about fivefold lower than **1a**. Phenyl substitution (**1d**) also caused affinity for the V_2 receptor to decrease. In contrast, even with all the structural conversions described above, no obvious fluctuation in the affinity for the V_{1a} receptor was observed. This indicates that polarity at some positions, such as the pyridin-2-yl group, is preferable because it increases affinity for V_2 receptor binding. Therefore, such a modification is expected to generate better selectivity against V_{1a} binding affinity, which is not affected by the polar atom.

Table 2 shows the binding affinity values for compounds **1e** to **1i**, which have various functional groups near the nitrogen atom on the pyridine ring of **1a** (Table 2). Compound **1e** which possesses an electronically neutral methyl group, and each compound in Table 2, including **1f** to **1h**, to which an electron donating substituent was introduced, and **1i**, which has electron deficient cyano group, maintained both a binding affinity for V_2 and selectivity against the V_{1a} receptor comparable to those for compound **1a**. Sterically hindered substitution of a dimethylamino group (**1h**) did not cause obvious changes in the binding affinity profile either. These results suggest that the region near the pyridin-2-yl group gives wide latitude to structural conversion, both electronically and sterically, while maintaining high affinity for the V_2 receptor.

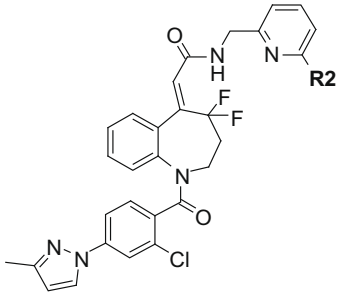
Table 1
Binding affinity of pyridyl and benzyl-substituted derivatives for V_2 and V_{1a} receptors



Compound	R^1	Binding affinity: K_i (nM)		Selectivity
		V_2 [a]	V_{1a} [b]	V_{1a}/V_2
1a	Pyridin-2-yl	4.8	26	5.4
1b	Pyridin-3-yl	21	40	1.9
1c	Pyridin-4-yl	20	15	0.75
1d	Phenyl	19	43	2.3

[a] Binding affinity for human V_2 receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.
[b] Binding affinity for human V_{1a} receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.

Table 2
Binding affinity of substituted-pyridyl derivatives for V_2 and V_{1a} receptors



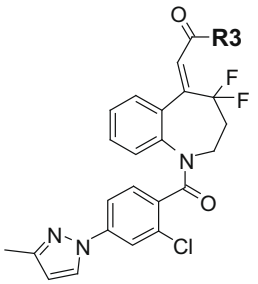
Compound	R^2	Binding affinity: K_i (nM)		Selectivity
		V_2 [a]	V_{1a} [b]	V_{1a}/V_2
1a	H	4.8	26	5.4
1e	CH_3	5.6	30	5.4
1f	OCH_3	16	47	2.9
1g	NH_2	10	38	3.8
1h	$N(CH_3)_2$	11	48	4.4
1i	CN	7.4	22	3.0

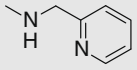
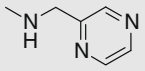
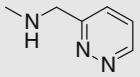
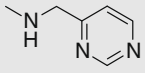
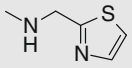
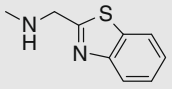
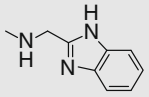
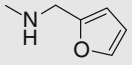
[a] Binding affinity for human V_2 receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.
[b] Binding affinity for human V_{1a} receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.

The SARs of compounds in which various hetero aromatic rings were introduced instead of a pyridin-2-yl group are summarized in Table 3. All compounds from **1j** to **1p** possess a hetero atom at a position similar to that of the nitrogen atom on the pyridine ring of **1a**. Among those compounds, the pyridazin-3-yl substituted **1k** showed higher V_2 binding affinity than **1a,** with a K_i value of 3.4 nM. The binding affinity of **1k** for the V_{1a} receptor was slightly lower than that of **1a**, and the V_2/V_{1a} ratio was 13:1, which was significantly better than that for **1a**. Other compounds with nitrogen-containing hetero aromatic rings (**1j**, **1l**, **1m**, **1n**, and **1o**) maintained moderate affinity to the V_2 receptor. Meanwhile the introduction of a furan ring (**1p**), which is less polar than pyridine or pyridazine, resulted in reduced binding affinity for the V_2 receptor. This tendency agrees with the SARs in Table 1.

Table 4 exhibits the SARs of compounds containing aliphatic groups in the 'head' moiety. Interestingly, the carboxylic acid derivative (**1q**) and carboxamide form (**1r**), each of which have a structurally simple 'head' moiety, had modest binding affinity for the V_2 receptor, although the values were lower than that for **1a**. More noteworthy is that the binding affinity of **1q** for the V_{1a} receptor almost disappeared (K_i = 6300 nM), and the V_2/V_{1a} selectivity ratio increased to 252:1. The effect of the introduction of several alkyl chains were also examined (**1s–1w**). While compound **1s**, which has a hydrophobic *n*-propyl group, had lower affinity for the V_2 receptor than **1a**, hydroxyethyl-substituted **1t** recovered its comparably high binding affinity to **1a**. This observation is similar the case of aromatic substituents, where a polar functional group at the appropriate position is preferable to V_2 binding affinity (Table 1 and Table 3). However, (dimethylamino)ethylamide (**1u**) had about 10-fold lower affinity to the V_2 receptor. The introduction of a glycine unit maintained moderate binding affinity for the V_2 receptor, with a K_i value of 22 nM, and showed low affinity for the V_{1a} receptor, with a K_i value of 230 nM (**1v**). The results for **1q** and **1v** clearly indicate that an acidic function could decrease binding affinity for the V_{1a} receptor. An amide derivative of **1v** had high affinity for the V_2 receptor (**1w**, K_i = 11 nM), but high selectivity was not retained.

Table 3
Binding affinity of hetero aromatic ring-substituted derivatives for V_2 and V_{1a} receptors



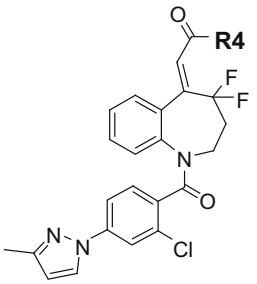
Compound	R^3	Binding affinity: K_i (nM)		Selectivity
		V_2 [a]	V_{1a} [b]	V_{1a}/V_2
1a		4.8	26	5.4
1j		14	57	4.1
1k		3.4	43	13
1l		11	44	4.0
1m		11	43	3.9
1n		11	55	5.0
1o		12	72	6.0
1p		27	72	2.7

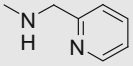
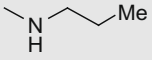
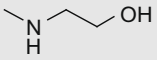
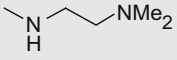
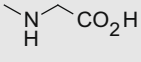
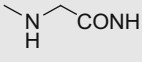
[a] Binding affinity for human V_2 receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.
[b] Binding affinity for human V_{1a} receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.

Some representative compounds identified in the binding assays were evaluated for their agonistic activity to V_2 receptor and in vivo activity. Their agonistic activity was assessed using the cAMP accumulation test to determine EC_{50} value and maximal production. Pyridazine-substituted **1k** exhibited potent cAMP accumulation activity (EC_{50} = 16 nM, E_{max} = 86%), although it was slightly less intense than that of **1a**. The agonistic activity of **1q** and **1t**, in which an aliphatic group is substituted in the 'head' moiety, was found to be extremely potent and comparable to that of **1a** (Table 5).

In vivo activity was assessed as total antidiuretic effect after 4-h in water-loaded rats (Fig. 3). Compounds **1k** and **1t** suppressed the urine excretion rate considerably within the 4 h immediately following intravenous administration. However, the carboxylic acid derivative **1q** did not decrease urine volume. The particularly high polarity of its carboxyl group was considered to have a negative impact on pharmacokinetic parameters such as metabolism and elimination.

Table 4
Binding affinity of aliphatic group-substituted derivatives for V_2 and V_{1a} receptors



Compound	R^4	Binding affinity: K_i (nM)		Selectivity
		V_2 [a]	V_{1a} [b]	V_{1a}/V_2
1a		4.8	26	5.4
1q		25	6300	252
1r		33	190	5.8
1s		18	13	0.72
1t		9.7	28	2.9
1u		53	59	1.1
1v		22	230	10
1w		11	40	3.6

[a] Binding affinity for human V_2 receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.
[b] Binding affinity for human V_{1a} receptors. The receptors expressed on CHO cells were used. All assays were performed in triplicate.

Lastly, the CYP inhibitory activity of **1a**, **1k**, **1q,** and **1t** were examined (Table 6). While compounds with a nitrogen-containing hetero aromatic ring (**1a**, **1k**) did not have a preferable overall CYP inhibitory activity profile, compounds with an aliphatic group substitution (**1q**, **1t**) were found to avoid potent inhibitory activity against almost all of the CYP species described in Table 6.

4. Conclusion

A series of amide derivatives of (4,4-difluoro-1,2,3,4-tetrahydro-5*H*-1-benzazepin-5-ylidene)acetic acid were synthesized and evaluated with respect to following biological profiles: binding affinity for the V_2 and V_{1a} receptors, cAMP production activity, in vivo antidiuretic effect, and CYP inhibitory activity. As a result of this exploration and the SAR study, the new insights described below were gained.

(1) The 'head' moiety (Fig. 2) of this derivative tolerates a wide range of structural conversions, including aromatic and aliphatic parts with various functional groups, which maintain high affinity for the V_2 receptor. In particular, the presence of a polar hetero atom at a certain position often increases the affinity for V_2 receptor.

Table 5
cAMP accumulation activity of compound **1a**, **1k**, **1q** and **1t**

Compound	cAMP accumulation	
	EC_{50}[a] (nM)	E_{max}[b] (%)
1a	1.8	95.9
1k	16	86.4
1q	4.2	92.4
1t	6.3	93.9

[a] 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.
[b] 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.

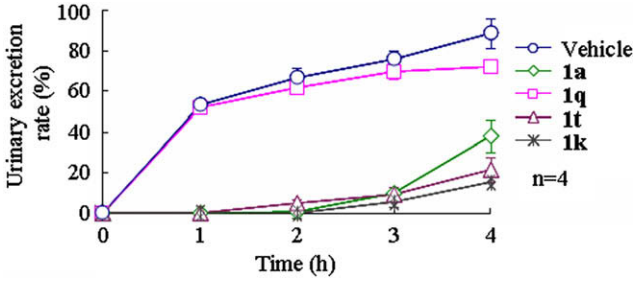


Figure 3. Effects of the iv administration of compounds **1a**, **1k**, **1q** and **1t** on urinary excretion rate in water-loaded rats. The urinary excretion rate is the ratio of urine volume to the volume of water loaded. All assays were performed in quadruplicate.

Table 6
Inhibitory activity against cytochrome P450

Compound	IC_{50} (μM)			
	CYP 2C9[a]	CYP 2C19[b]	CYP 2D6[c]	CYP 3A4[d]
1a	9.1	5.1	14	11
1k	13	7.0	8.9	7.9
1q	>20	>20	>20	>100
1t	>20	5.5	>20	47

[a] IC_{50} values against CYP2C9 were determined in fluorescent P450 inhibition assays. All assays were performed in duplicate.
[b] IC_{50} values against CYP2C19 were determined in fluorescent P450 inhibition assays. All assays were performed in duplicate.
[c] IC_{50} values against CYP2D6 were determined in fluorescent P450 inhibition assays. All assays were performed in duplicate.
[d] IC_{50} values against CYP3A4 were determined in competitive inhibition assays using Midazolam. All assays were performed in duplicate.

(2) The introduction of an acidic functional group in the 'head' region effectively decreases the affinity for the V_{1a} receptor.
(3) Some compounds with a substituted aliphatic group avoided the undesirable inhibition of CYP without losing potent affinity for the V_2 receptor.

These findings eventually led to the representative compound **1t**, which is a practical vasopressin V_2 receptor agonist that could be used for the treatment of disorders such as central diabetes insipidus and nocturia.

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-Chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoic acid (**3**), (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 (**1q**) and (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 (**1a**) were prepared from 2-chloro-4-fluorobenzoic acid (**2**) according to the procedure described in our previous paper.[8]

5.1.1. 2-Bromo-6-methoxypyridine (7a)

To a mixture of 2,6-dibromopyridine (3.00 g) in methanol (30 ml) was added sodium methoxide (28% in methanol, 25.3 ml), and the mixture was stirred overnight at 85 °C. The solvent was evaporated, and the residue was partitioned between water and ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 2/1) to give the title compound **7a** (2.10 g, 88%) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (3H, s), 6.87 (1H, d, J = 10.0 Hz), 7.23 (1H, d, J = 10.0 Hz), 7.66 (1H, t, J = 10.0 Hz). MS (GC) *m/z* 188 [M].

5.1.2. 2-Bromo-6-dimethylaminopyridine (7b)

A solution of 2,6-dibromopyridine (3.00 g) and aqueous dimethylamine (50%, 40 ml) in DMF (40 ml) was refluxed for 3 h. The reaction mixture was partitioned between water and ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 10/1) to give the title compound **7b** (2.37 g, 93%) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.99 (6H, s), 6.60 (1H, d, J = 8.4 Hz), 6.70 (1H, d, J = 7.6 Hz), 7.39 (1H, dd, J = 8.4, 7.6 Hz). MS (EI) *m/z* 200 [M-1], 202 [M+1].

5.1.3. 2-Methoxypyridine-6-carboxaldehyde (8a)

To a solution of **7a** (2.00 g) in THF (20 ml) was added *n*-butyllithium (1.52 mol/l in *n*-hexane, 8.40 ml) at −78 °C. After stirring for 1 h, DMF (949 mg) was slowly added, and the reaction mixture was stirred for 30 min with the maintenance of −78 °C for the whole process. The reaction was quenched by aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 20/1) to give the title compound **8a** (1.02 g, 70%) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96 (3H, s), 7.16 (1H, dd, J = 11.2, 1.2 Hz), 7.59 (1H, dd, J = 9.6, 1.2 Hz), 7.95 (1H, ddd, J = 11.2, 9.6, 0.8 Hz), 9.90 (1H, d, J = 0.8 Hz). MS (FAB) *m/z* 138 $[M+1]^+$.

5.1.4. 2-Dimethylaminopyridin-6-carboxaldehyde (8b)

Compound **8b** was prepared according to the procedure described for **8a** from **7b**. The title compound **8b** (1.55 g, 87%) was obtained as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.10 (6H, s), 6.95 (1H, d, J = 8.8 Hz), 7.14 (1H, d, J = 6.8 Hz), 7.71 (1H, dd, J = 8.8, 6.8 Hz), 9.81 (1H, s). MS (FAB) *m/z* 151 $[M+1]^+$.

5.1.5. 1-(6-Methoxypyridin-2-yl)methanamine (9a)

A mixture of **8a** (1.00 g), hydroxylamine hydrochloride (1.04 g) and potassium carbonate (2.05 g) in ethanol (20 ml) was stirred at 80 °C for 2 h. After evaporation, the residue was partitioned between water and ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give 6-methoxypyridine-2-carbaldehyde oxime (1.02 g, 91%) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (3H, s), 6.81 (1H, d, J = 10.0 Hz), 7.35 (1H, d, J = 10.0 Hz), 7.73 (1H, t, J = 10.0 Hz), 7.99 (1H, s), 11.62 (1H, s). MS(FAB) m/z 153 $[M+1]^+$. 6-Methoxypyridine-2-carbaldehyde oxime (720 mg) was hydrogenated at the pressure of 2.0 kgf/cm^2 in the presence of Raney-Ni in ethanol (20 ml) for 3.5 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. This residue was purified by silica gel column chromatography (chloroform/methanol = 25/1) to give the title compound **9a** (101 mg, 15%) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.25–3.45 (2H, br), 3.70 (2H, s), 3.83 (3H, s), 6.62 (1H, d, J = 10.8 Hz), 7.00 (1H, d, J = 10.8 Hz), 7.64 (1H, t, J = 10.8 Hz). MS (EI) m/z 138 [M].

5.1.6. 6-(Aminomethyl)-*N*,*N*-dimethylpyridin-2-amine (9b)

Compound **9b** was prepared according to the procedure described for **9a** from **8b**. The title compound **9b** (994 mg, 64% in two steps) was obtained as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65–1.85 (2H, br), 3.00 (6H, s), 3,60 (2H, s), 6.44 (1H, d, J = 8.4 Hz), 6,57 (1H, d, J = 7.2 Hz), 7.41–7.45 (1H, m). MS (FAB) m/z 152 $[M+1]^+$.

5.1.7. *tert*-Butyl [6-(aminomethyl)pyridin-2-yl]carbamate (11)

To a mixture of {6-[(*tert*-butoxycarbonyl)amino]pyridin-2-yl}-methyl methanesulfonate[11] (2.82 g) in DMF (30 ml) was added sodium azide (910 mg) at ice-chilled temperature, and the mixture was stirred at room temperature for 4 h. The reaction mixture was partitioned between water and ethyl acetate, and the organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 5/1) to give *tert*-butyl [6-(azidomethyl)pyridin-2-yl]carbamate (2.29 g, 98%) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (9H, s), 4.41 (2H, s), 7.06 (1H, dd, J = 6.0, 2.4 Hz), 7.75–7.78 (2H, m), 9.77 (1H, s). MS (FAB) 250 $[M+1]^+$. *tert*-Butyl [6-(azidomethyl)pyridin-2-yl]carbamate (1.55 g) was hydrogenated in the presence of Pd–C (10%, 300 mg) in ethanol (20 ml) for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give the title compound **11** (1.38 g, quantitative yield) as colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (9H, s), 3.69 (2H, s), 4.30–4.40 (2H, br), 7.06 (1H, d, J = 7.2 Hz), 7.58–7.69 (2H, m), 9.57 (1H, s) MS (FAB) m/z 224 $[M+1]^+$.

5.1.8. 6-Chloropyridine-2-carbonitrile (13)[12]

To a solution of 2-chloropyridin-N-oxide (11.2 g) hydrochloride in methanol (100 ml) was added potassium methoxide (4.59 g) at ice-chilled temperature, and the mixture was stirred for 10 min. After evaporating the solvent, ethanol was added to the residue and insoluble matter was removed by filtration. The filtrate was concentrated in vacuo, and to the residue was added dimethyl sulfate (6.20 ml), then the mixture was stirred at room temperature for 3 h. The mixture was washed by diethyl ether using decantation manner, and to the residue were added water (140 ml) and sodium cyanide (12.8 g). The mixture was stirred at 0 °C, and resulting brown solid was collected by filtration. The crude solid was purified by silica gel column chromatography (chloroform/methanol = 50/1) to give the title compound **13** (4.08 g, 45%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (1H, dd, J = 8.4, 0.8 Hz), 7.65 (1H, dd, J = 7.6, 0.8 Hz), 7.82 (1H, dd, J = 8.4, 7.6 Hz). MS (EI) m/z 138 [M].

5.1.9. 6-Vinylpyridine-2-carbonitrile (14)

A mixture of **13** (3.00 g), tributyl(vinyl)tin (6.54 ml) and tetrakis(triphenylphosphine)palladium (627 mg) in toluene (30 ml) was refluxed for 5 h. After the reaction was cooled to room temperature, aqueous potassium fluoride was added, then resulting insoluble matter was removed by filtration. The filtrate was extracted with ethyl acetate, and the organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 10/1) to give the title compound **14** (2.63 g, 93%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (1H, d, J = 10.8 Hz), 6.33 (1H, d, J = 17.6 Hz), 6.81 (1H, dd, J = 17.6, 10.8 Hz), 7.53 (1H, d, J = 7.6 Hz), 7.55 (1H, d, J = 6.8 Hz), 7.78 (1H, t, J = 8.0 Hz). MS (EI) m/z 130 [M].

5.1.10. 6-Hydroxymethylpyridine-2-carbonitrile (15)

A mixture of **14** (2.11 g) in methanol (20 ml) was stirred at −78 °C for 30 min with ozone-bubbling. Dimethyl sulfide (5.95 ml) was added to the mixture at −78 °C, and the reaction was then allowed to be warmed to room temperature. The solvent was evaporated, and the residue was partitioned between water and chloroform. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 10/1) to give 6-formylpyridine-2-carbonitrile (1.72 g, 80%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (1H, dd, J = 7.8, 1.5 Hz), 8.07 (1H, t, J = 7.8 Hz), 8.17 (1H, dd, J = 7.8, 1.5 Hz), 10.07 (1H, d, J = 1.0 Hz). MS (GC) 132 [M]. To a solution of 6-formylpyridine-2-carbonitrile (600 mg) in methanol (12 ml) was added sodium borohydride (172 mg), and the mixture was stirred at room temperature for 30 min. The solvent was evaporated, and the residue was purified by silica gel column chromatography (chloroform/methanol = 50/1) to give the title compound **15** (460 mg, 76%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 (1H, t, J = 5.2 Hz), 4.83 (2H, d, J = 5.2 Hz), 7.56 (1H, dd, J = 8.0, 0.4 Hz), 7.63 (1H, dd, J = 8.0, 0.4 Hz), 7.86 (1H, t, J = 8.0 Hz). MS (FAB) 135 m/z $[M+1]^+$.

5.1.11. 6-Aminomethylpyridine-2-carbonitrile (16)

To a solution of **15** (460 mg), phthalimide (505 mg) and triphenylphosphine (900 mg) in THF (10 ml) was added DEAD (40% in toluene, 1.58 ml), and the mixture was stirred overnight at room temperature. Resulting precipitate was collected by filtration, washed by THF, and dried in vacuo to give 6-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]pyridine-2-carbonitrile (366 mg, 41%) as pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.05 (2H, s), 7.50 (1H, d, J = 7.6 Hz), 7.59 (1H, d, J = 7.6 Hz), 7.76–7.82 (3H, m), 7.89–7.92 (2H, m). MS (FAB) m/z 264 $[M+1]^+$. A mixture of 6-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]pyridine-2-carbonitrile (365 mg) and hydrazine hydrate (0.074 ml) in methanol (5 ml) and chloroform (10 ml) was stirred overnight at room temperature. The solvent was evaporated, and the residue was purified by silica gel column chromatography (chloroform/methanol = 10/1) to give the title compound **16** (40 mg, 22%) as colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85–1.95 (2H, br), 3.96 (2H, s), 7.49–7.55 (2H, m), 7.75 (1H, t, J = 8.0 Hz). MS (GC) m/z 133 [M].

5.1.12. 3-(2-Phenylvinyl)pyridazine (18)[14]

A mixture of 3-methylpyridazine (1.83 ml), benzaldehyde (4.07 ml) and zinc chloride (545 mg) was heated at 150 °C for 2 h. After the reaction mixture was cooled to room temperature, that was partitioned between aqueous sodium hydroxide and chloroform. The organic phase was washed with water and brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (chloroform/metha-

nol = 20/1) to give the title compound **18** (2.19 g, 60%) as pale brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.47 (5H, m), 7.59–7.73 (4H, m), 9.06 (1H, dd, J = 4.8, 1.6 Hz). MS (FAB) m/z 183 $[M+1]^+$.

5.1.13. Pyridazin-3-carboxaldehyde (19)

A mixture of **18** (1.57 g), sodium periodate (3.57 g), osmium tetraoxide (2.5 wt.% in *t*-BuOH, 11.0 ml), acetone (15 ml), *t*-BuOH (15 ml) and water (15 ml) was stirred at room temperature for 2 days. Insoluble matter was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (chloroform/methanol = 50/1) to give the title compound **19** (730 mg, 81%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (1H, dd, J = 8.4, 5.2 Hz), 8.08 (1H, dd, J = 8.4, 2.0 Hz), 9.40 (1H, dd, J = 5.2, 2.0 Hz), 10.41–10.42 (1H, m). MS (GC) m/z 108 [M].

5.1.14. Pyridazin-3-ylmethylamine (20)

Pyridazin-3-carbaldehyde oxime (64 mg, 52%), which is the intermediate for the title compound **20**, was prepared according to the procedure described for **8a** from **19** (108 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.50 (1H, s), 7.50 (1H, dd, J = 8.8, 4.8 Hz), 8.01 (1H, dd, J = 8.8, 2.0 Hz), 8.52 (1H, s), 9.16 (1H, dd, J = 4.8, 2.0 Hz). MS (EI) 123 [M]. Pyridazin-3-carbaldehyde oxime (60 mg) was hydrogenated in the presence of Pd–C (10%, 60 mg) in ethanol (10 ml) for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give the title compound **20** (55 mg, quantitative yield) as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60–1.80 (2H, br), 4.21 (2H, s), 7.46 (1H, dd, J = 8.4, 4.8 Hz), 7.53 (1H, dd, J = 8.4, 1.6 Hz), 9.10 (1H, dd, J = 4.8, 1.6 Hz). MS (EI) m/z 109 [M].

5.1.15. (*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-3-ylmethyl)acetamide hydrochloride (1b)

A mixture of **1q** (300 mg), 3-(aminomethyl)pyridine (71 mg), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD) (192 mg) and 1-hydroxy-1*H*-benzotriazole (HOBt) (135 mg) in DMF (15 ml) was stirred at room temperature for 3 h. The reaction mixture was partitioned between aqueous sodium hydrogen carbonate and ethyl acetate, then the organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (chloroform/methanol = 50/1) to yield the salt-free form of **1b**, which was dissolved in chloroform and subsequently treated with hydrochloride (4 M in ethyl acetate). Resulting precipitate was collected by filtration and dried in vacuo to give the title compound **1b** (221 mg, 57%) as colorless amorphous solid. ^{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), 4.57 (2H, s), 4.76–4.90 (1H, br), 6.34 (1H, d, J = 2.4 Hz), 6.52 (1H, s), 6.98 (1H, d, J = 7.3 Hz), 6.96–7.14 (1H, br), 7.18 (1H, t, J = 7.3 Hz), 7.25 (1H, t, J = 7.3 Hz), 7.32 (1H, d, J = 7.4 Hz), 7.58 (1H, d, J = 8.8 Hz), 7.83 (1H, s), 7.95–8.02 (1H, m), 8.38 (1H, d, J = 2.4 Hz), 8.40 (1H, s), 8.81 (1H, s), 8.82 (1H, s), 9.15 (1H, s). MS (FAB) m/z 548 $[M+1]^+$. Anal. Calcd for $C_{29}H_{24}ClF_2N_5O_2 \cdot 0.3C_4H_8O \cdot 0.2C_2H_6O \cdot 1.3HCl \cdot 1.3H_2O$: C, 56.57; H, 4.89; N, 10.78; Cl, 12.55; F, 5.85. Found: C, 56.37; H, 4.64; N, 10.91; Cl, 12.65; F, 5.66.

5.1.16. (*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-4-ylmethyl)acetamide hydrochloride (1c)

Compound **1c** was prepared according to the procedure described for **1b** from **1q** and 4-(aminomethyl)pyridine. The title compound **1c** (247 mg, 64%) was obtained as colorless crystals. Mp: 272–274 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.30–2.55 (1H, br), 2.57–2.90 (1H, br), 3.10–3.31 (1H, br), 4.43 (2H, d, J = 6.0 Hz), 4.74–4.90 (1H, br), 6.33 (1H, d, J = 2.4 Hz), 6.50 (1H, s), 6.98 (1H, d, J = 7.8 Hz), 7.00–7.08 (1H, br), 7.18 (1H, td, J = 7.8, 1.4 Hz), 7.26 (1H, t, J = 7.8 Hz), 7.30–7.36 (3H, m), 7.58 (1H, d, J = 6.9 Hz), 7.84 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 8.53–8.56 (2H, m), 8.99 (1H, s). MS (FAB) m/z 548 $[M+1]^+$. Anal. Calcd for $C_{29}H_{24}ClF_2N_5O_2 \cdot HCl \cdot 0.5H_2O$: C, 58.69; H, 4.42; N, 11.80; Cl, 11.95; F, 6.40. Found: C, 58.48; H, 4.27; N, 11.71; Cl, 11.92; F, 6.19.

5.1.17. (*Z*)-*N*-Benzyl-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}acetamide (1d)

A mixture of **1q** (220 mg), benzylamine (56 mg), WSCD (150 mg) and HOBt (104 mg) in DMF (20 ml) was stirred overnight at room temperature. The reaction mixture was partitioned between aqueous sodium hydrogen carbonate and ethyl acetate, then the organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was crystallized and recrystallized (ethyl acetate/diethyl ether) to give the title compound **1d** (229 mg, 87%) as colorless crystals. Mp: 168–169 °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), 4.40 (2H, d, J = 5.9 Hz), 4.76–4.90 (1H, br), 6.33 (1H, d, J = 2.5 Hz), 6.41 (1H, s), 6.97 (1H, d, J = 7.8 Hz), 6.97–7.07 (1H, br), 7.17 (1H, t, J = 7.8 Hz), 7.25 (1H, t, J = 7.8 Hz), 7.32 (1H, d, J = 7.3 Hz), 7.35–7.37 (5H, m), 7.55 (1H, d, J = 8.3 Hz), 7.83 (1H, s), 8.37 (1H, d, J = 2.5 Hz),8.89 (1H, s). MS (FAB) m/z 547 $[M+1]^+$. Anal. Calcd for $C_{30}H_{25}ClF_2N_4O_2$: C, 65.87; H, 4.61; N, 10.24; Cl, 6.48; F, 6.95. Found: C, 65.75; H, 4.52; N, 10.23; Cl, 6.42; F, 6.90.

5.1.18. (*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*-[(6-methyl-2-pyridyl)methyl]acetamide hydrochloride (1e)

Compound **1e** was prepared according to the procedure described for **1b** from **1q** and 1-(6-methylpyridin-2-yl)methanamine.[15] The title compound **1e** (343 mg, 87%) was obtained as colorless crystals. Mp: 185–188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.35–2.55 (1H, br), 2.70 (3H, s), 2.70–2.85 (1H, br), 3.12–3.30 (1H, br), 4.67 (2H, br s), 4.75–4.90 (1H, br), 6.34 (1H, d, J = 2.5 Hz), 6.53 (1H, s), 6.99 (1H, d, J = 7.8 Hz), 7.00–7.12 (1H, br), 7.19 (1H, td, J = 7.8, 1.5 Hz), 7.26 (1H, d, J = 7.8 Hz), 7.33 (1H, d, J = 7.8 Hz), 7.55–7.70 (3H, m), 7.84 (1H, s), 8.23–8.33 (1H, br), 8.39 (1H, d, J = 2.5 Hz), 9.23 (1H, br s). MS (FAB) m/z 562 $[M]^+$. Anal. Calcd for $C_{30}H_{26}ClF_2N_5O_2 \cdot HCl \cdot 1.3H_2O$: C, 57.94; H, 4.80; N, 11.26; Cl, 11.40; F, 6.11. Found: C, 58.02; H, 4.41; N, 11.27; Cl, 11.07; F, 6.23.

5.1.19. (*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*-[(6-methoxy-2-pyridyl)methyl]acetamide hydrochloride (1f)

Compound **1f** was prepared according to the procedure described for **1b** from **1q** and **9a**. The title compound **1f** (289 mg, 71%) was obtained as colorless crystals. Mp: 165–167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.35–2.55 (1H, br), 2.70–2.90 (1H, br), 3.05–3.30 (1H, br), 3.87 (3H, s), 4.40 (2H, d, J = 4.9 Hz), 4.75–5.00 (1H, br), 6.34 (1H, d, J = 2.4 Hz), 6.48 (1H, s), 6.72 (1H, d, J = 8.3 Hz), 6.95–6.97 (2H, m), 7.00–7.10 (1H, br), 7.18 (1H, td, J = 7.8, 1.5 Hz), 7.26 (1H, td, J = 7.8, 1.5 Hz), 7.33 (1H, dd, J = 7.8, 1.5 Hz), 7.57 (1H, d, J = 8.3 Hz), 7.72 (1H, dd, J = 8.3, 7.3 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 8.98 (1H, br s). MS (FAB) m/z 578 $[M]^+$. Anal. Calcd for $C_{30}H_{26}ClF_2N_5O_3 \cdot HCl \cdot 0.2H_2O$: C, 58.30; H, 4.47; N, 11.33; Cl, 11.47; F, 6.15. Found: C, 58.15; H, 4.46; N, 11.28; Cl, 11.14; F, 6.26.

5.1.20. (*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*-[(6-dimethylamino-2-pyridyl)methyl]acetamide hydrochloride (1h)

Compound **1h** was prepared according to the procedure described for **1b** from **1q** and **9b**. The title compound **1h** (199 mg, 73%) was obtained as white solid. Mp: 113–116 °C (decomposed); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.35–2.55 (1H, br), 2.70–2.85 (1H, br), 3.10–3.35 (1H, br), 3.24 (6H, s), 4.55 (2H, br s), 4.73–4.90 (1H, br), 6.34 (1H, d, J = 2.4 Hz), 6.53 (1H, s), 6.80 (1H, d, J = 6.3 Hz), 6.99 (1H, d, J = 7.3 Hz), 6.99–7.12 (1H, br), 7.19 (1H, t, J = 7.3 Hz), 7.27 (1H, t, J = 7.3 Hz), 7.34 (1H, d, J = 7.3 Hz), 7.58 (1H, d, J = 8.3 Hz), 7.84(1H, s), 7.86–7.94 (1H, br), 7.96 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 9.08–9.30 (1H, br). MS (FAB) *m/z* 591 $[M+1]^+$. Anal. Calcd for $C_{31}H_{29}ClF_2N_6O_2\cdot HCl\cdot 2H_2O$: C, 56.11; H, 5.16; N, 12.67; Cl, 10.69; F, 5.73. Found: C, 56.06; H, 5.28; N, 12.62; Cl, 10.67; F, 5.46.

5.1.21. (*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*-[(6-cyano-2-pyridyl)methyl]acetamide (1i)

Compound **1i** was prepared according to the procedure described for **1d** from **1q** and **16**. The title compound **1i** (31 mg, 18%) was obtained as colorless crystals. Mp: 197–199 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.25–2.55 (1H, br), 2.60–2.90 (1H, br), 3.05–3.30 (1H, br), 4.53 (2H, br s), 4.47–4.57 (1H, br), 6.34 (1H, d, J = 2.4 Hz), 6.50 (1H, s), 6.98 (1H, d, J = 7.8 Hz), 6.99–7.08 (1H, br), 7.18 (1H, td, J = 7.8, 1.5 Hz), 7.25 (1H, t, J = 7.8 Hz), 7.32 (1H, dd, J = 7.8, 1.5 Hz), 7.57 (1H, d, J = 8.8 Hz), 7.73 (1H, d, J = 8.3 Hz), 7.84(1H, s), 7.97 (1H, d, J = 8.3 Hz), 8.10 (1H, d, J = 8.3 Hz), 8.38 (1H, d, J = 2.4 Hz), 9.14 (1H, br s). MS (FAB) *m/z* 573 $[M+1]^+$. Anal. Calcd for $C_{30}H_{23}ClF_2N_6O_2\cdot 0.25C_3H_8O\cdot 0.1HCl\cdot 0.5H_2O$: C, 61.49; H, 4.38; N, 13.99; Cl, 6.49; F, 6.33. Found: C, 61.69; H, 4.49; N, 13.67; Cl, 6.59; F, 6.15.

5.1.22. (*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*-(pyrazin-2-ylmethyl)acetamide hydrochloride (1j)

Compound **1j** was prepared according to the procedure described for **1b** from **1q** and 1-pyrazin-2-ylmethanamine.[16] The title compound **1j** (324 mg, 84%) was obtained as white amorphous solid. ^{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), 4.61 (2H, d, J = 5.8 Hz), 4.76–4.90 (1H, br), 6.33 (1H, d, J = 2.4 Hz), 6.49 (1H, s), 6.97 (1H, d, J = 7.3 Hz), 6.97–7.10 (1H, br), 7.17 (1H, t, J = 7.3 Hz), 7.25 (1H, t, J = 7.3 Hz), 7.32 (1H, d, J = 7.2 Hz), 7.57 (1H, d, J = 8.3 Hz), 7.83 (1H, s), 8.37 (1H, d, J = 2.4 Hz), 8.57 (1H, d, J = 2.4 Hz), 8.61–8.63 (1H, m), 8.68 (1H, s), 9.13 (1H, s). MS (FAB) *m/z* 549 $[M+1]^+$. Anal. Calcd for $C_{28}H_{23}ClF_2N_6O_2\cdot 0.9HCl\cdot 1.5H_2O$: C, 55.49; H, 4.42; N, 13.87; Cl, 11.11; F, 6.27. Found: C, 55.54; H, 4.28; N, 13.45; Cl, 10.97; F, 5.73.

5.1.23. (*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*-(pyridazin-3-ylmethyl)acetamide (1k)

Compound **1k** was prepared according to the procedure described for **1d** from **1q** and **20**. The title compound **1k** (105 mg, 38%) was obtained as pale yellow crystals. Mp: 211–213 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.27–2.55 (1H, br), 2.65–2.85 (1H, br), 3.10–3.30 (1H, br), 4.63–4.73 (2H, br), 4.74–4.88 (1H, br), 6.33 (1H, d, J = 2.4 Hz), 6.49 (1H, s), 6.98 (1H, d, J = 7.3 Hz), 6.96–7.12 (1H, br), 7.18 (1H, t, J = 7.3 Hz), 7.25 (1H, t, J = 7.3 Hz), 7.32 (1H, d, J = 7.3 Hz), 7.57 (1H, d, J = 8.3 Hz), 7.66 (1H, d, J = 8.3 Hz), 7.74 (1H, dd, J = 4.9 Hz, 8.3 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 9.12–9.19 (1H, br), 9.17 (1H, d, J = 4.9 Hz). MS (FAB) *m/z* 549 $[M+1]^+$. Anal. Calcd for $C_{28}H_{23}ClF_2N_6O_2\cdot 0.2H_2O$: C, 60.86; H, 4.27; N, 15.21; Cl, 6.42; F, 6.88. Found: C, 60.75; H, 4.26; N, 15.09; Cl, 6.30; F, 6.89.

5.1.24. (*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*-(pyrimidin-4-ylmethyl)acetamide (1l)

Compound **1l** was prepared according to the procedure described for **1d** from **1q** and 1-(pyrimidin-4-yl)methanamine. The title compound **1l** (246 mg, 22%) was obtained as colorless crystals. Mp: 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.35–2.55 (1H, br), 2.65–2.90 (1H, br), 3.15–3.30 (1H, br), 4.48 (2H, d, J = 5.8 Hz), 4.75–4.92 (1H, br), 6.34 (1H, d, J = 2.4 Hz), 6.52 (1H, s), 6.98 (1H, d, J = 7.8 Hz), 7.00–7.12 (1H, br), 7.18 (1H, td, J = 7.8, 1.5 Hz), 7.26 (1H, t, J = 7.8 Hz), 7.34 (1H, d, J = 7.8 Hz), 7.50 (1H, d, J = 4.9 Hz), 7.58 (1H, d, J = 6.8 Hz), 7.84 (1H, s), 8.38 (1H, d, J = 2.5 Hz), 8.81 (1H, d, J = 4.9 Hz), 9.08–9.16 (2H, m). MS (FAB) *m/z* 549 $[M+1]^+$. Anal. Calcd for $C_{28}H_{23}ClF_2N_6O_2$: C, 61.26; H, 4.22; N, 15.31; Cl, 6.46; F, 6.92. Found: C, 61.22; H, 4.21; N, 14.99; Cl, 6.37; F, 6.97.

5.1.25. (*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*-(thiazol-2-ylmethyl)acetamide (1m)

Compound **1m** was prepared according to the procedure described for **1d** from **1q** and 1-(1,3-thiazol-2-yl)methanamine.[17] The title compound **1m** (271 mg, 74%) was obtained as colorless crystals. Mp: 218–219 °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), 4.69 (2H, d, J = 4.3 Hz), 4.74–4.90 (1H, br), 6.33 (1H, d, J = 2.5 Hz), 6.45 (1H, s), 6.98 (1H, d, J = 7.5 Hz), 6.98–7.10 (1H, br), 7.18 (1H, t, J = 7.5 Hz), 7.25 (1H, t, J = 7.5 Hz), 7.32 (1H, d, J = 7.5 Hz), 7.56 (1H, d, J = 8.6 Hz), 7.68 (1H, d, J = 3.3 Hz), 7.76 (1H, d, J = 3.3 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.5 Hz), 9.28 (1H, s). MS (FAB) *m/z* 554 $[M]^+$. Anal. Calcd for $C_{27}H_{22}ClF_2N_5O_2S\cdot 0.5H_2O$: C, 57.60; H, 4.12; N, 12.44; S, 5.70; Cl, 6.30; F, 6.75. Found: C, 57.78; H, 4.02; N, 12.33; S, 6.10; Cl, 6.01; F, 6.43.

5.1.26. (*Z*)-*N*-(1,3-Benzothiazol-2-ylmethyl)-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}acetamide (1n)

Compound **1n** was prepared according to the procedure described for **1d** from **1q** and 1-(1,3-benzothiazol-2-yl)methanamine.[18] The title compound **1n** (281 mg, 70%) was obtained as colorless crystals. Mp: 204–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.30–2.55 (1H, br), 2.67–2.90 (1H, br), 3.10–3.24 (1H, br), 4.76–4.94 (3H, m), 6.33 (1H, d, J = 2.5 Hz), 6.48 (1H, s), 6.99 (1H, d, J = 7.8 Hz), 7.00–7.12 (1H, br), 7.18 (1H, t, J = 7.6 Hz), 7.26 (1H, t, J = 7.3 Hz), 7.34 (1H, d, J = 7.8 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.49–7.58 (2H, m),7.84 (1H, s), 7.98 (1H, d, J = 7.9 Hz), 8.12 (1H, d, J = 7.9 Hz), 8.38 (1H, d, J = 2.4 Hz), 9.39 (1H, s). MS (FAB) *m/z* 604 $[M]^+$. Anal. Calcd for $C_{31}H_{24}ClF_2N_5O_2S$: C, 61.64; H, 4.00; N, 11.59; S, 5.31; Cl, 5.87; F, 6.29. Found: C, 61.46; H, 4.09; N, 11.50; S, 5.24; Cl, 5.87; F, 6.31.

5.1.27. (*Z*)-*N*-(1*H*-Benzoimidazol-2-ylmethyl)-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}acetamide hydrochloride (1o)

Compound **1o** was prepared according to the procedure described for **1b** from **1q** and 1-(1*H*-benzimidazol-2-yl)methanamine. The title compound **1o** (67 mg, 16%) was obtained as white solid. Mp: 199–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.23 (3H, s), 2.40–2.50 (1H, br), 2.67–2.89 (1H, br), 3.11–3.23 (1H, br), 4.75–4.90 (1H, br), 4.83 (2H, s), 6.35 (1H, d, J = 2.4 Hz), 6.53 (1H, s), 7.00 (1H, d, J = 7.8 Hz), 7.02–7.15 (1H, br), 7.20 (1H,

t, J = 7.8 Hz), 7.27 (1H, t, J = 7.8 Hz), 7.32 (1H, d, J = 7.8 Hz), 7.50 (2H, dd, J = 6.1,3.2 Hz), 7.61 (1H, d, J = 8.7 Hz), 7.79 (2H, dd, J = 6.1,3.2 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 9.39 (1H, s). MS (FAB) m/z 587 $[M]^+$. Anal. Calcd for $C_{31}H_{25}ClF_2N_6O_2 \cdot HCl \cdot 1.5H_2O$: C, 57.24; H, 4.49; N, 12.92; Cl, 10.90; F, 5.84. Found: C, 57.45; H, 4.58; N, 12.86; Cl, 10.71; F, 5.63.

5.1.28. (*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-furylmethyl)acetamide (1p)

Compound **1p** was prepared according to the procedure described for **1d** from **1q** and 1-(2-furyl)methanamine. The title compound **1p** (158 mg, 45%) was obtained as colorless crystals. Mp: 187–188 °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), 4.38 (2H, d, J = 5.4 Hz), 4.75–4.90 (1H, br), 6.30–6.35 (2H, m), 6.41 (1H, s), 6.41–6.45 (1H, m), 6.96 (1H, d, J = 7.8 Hz), 6.96–7.10 (1H, br), 7.17 (1H, t, J = 7.3 Hz), 7.24 (1H, t, J = 7.3 Hz), 7.30 (1H, d, J = 7.3 Hz), 7.55 (1H, d, J = 6.9 Hz), 7.61 (1H, s), 7.83 (1H, s), 8.36 (1H, d, J = 2.4 Hz), 8.89 (1H, s). MS (FAB) m/z 537 $[M+1]^+$. Anal. Calcd for $C_{28}H_{23}ClF_2N_4O_3$: C, 62.63; H, 4.32; N, 10.43; Cl, 6.60; F, 7.08. Found: C, 62.59; H, 4.40; N, 10.39; Cl, 6.56; F, 6.93.

5.1.29. (*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}acetamide (1r)

To a mixture of **1q** (210 mg) in 1,2-dichloroethane (20 ml) was added thionyl chloride (2.0 ml), and the mixture was stirred at room temperature for 30 min. Reaction mixture was evaporated and subsequently azeotroped with toluene. A solution of the residue in acetonitrile was poured into aqueous ammonia, then resulting precipitate was collected by filtration and dried in vacuo to give the title compound **1r** (259 mg, quantitative yield) as white solid. Mp: 235–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20 (3H, s), 2.30–2.55 (1H, br), 2.60–2.85 (1H, br), 3.11–3.23 (1H, br), 4.75–4.90 (1H, br), 6.33 (1H, d, J = 2.4 Hz), 6.34 (1H, s), 6.95 (1H, d, J = 7.3 Hz), 6.94–7.10 (1H, br), 7.17 (1H, t, J = 7.3 Hz), 7.21–7.31 (2H, m), 7.34 (1H, s), 7.57 (1H, d, J = 8.1 Hz), 7.83 (1H, s), 7.85 (1H, s), 8.37 (1H, d, J = 2.4 Hz). MS (FAB) m/z 457 $[M+1]^+$. Anal. Calcd for $C_{23}H_{19}ClF_2N_4O_2 \cdot 0.4NH_3$: C, 59.58; H, 4.39; N, 13.29; Cl, 7.65; F, 8.19. Found: C, 59.80; H, 4.31; N, 13.55; Cl, 7.58; F, 8.08.

5.1.30. (*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*-propylacetamide (1s)

Compound **1s** was prepared according to the procedure described for **1d** from **1q** and propan-1-amine. The title compound **1s** (20 mg, 8%) was obtained as colorless crystals. Mp: 196–198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (3H, t, J = 7.3 Hz), 1.50 (2H, sextet, J = 7.3 Hz), 2.22 (3H, s), 2.30–2.50 (1H, br), 2.65–2.85 (1H, br), 3.00–3.25 (3H, br), 4.75–4.90 (1H, br), 6.33 (1H, d, J = 2.0 Hz), 6.36 (1H, s), 6.97 (1H, d, J = 7.8 Hz), 6.98–7.08 (1H, br), 7.16 (1H, td, J = 7.8, 1.5 Hz), 7.24 (1H, t, J = 7.8 Hz), 7.30 (1H, d, J = 7.8 Hz), 7.56 (1H, d, J = 8.8 Hz), 7.83 (1H, s), 8.33–8.39 (1H, br), 8.37 (1H, d, J = 2.0 Hz). MS (FAB) m/z 499 $[M+1]^+$. Anal. Calcd for $C_{26}H_{25}ClF_2N_4O_2 \cdot 0.2H_2O$: C, 62.14; H, 5.09; N, 11.15; Cl, 7.05; F, 7.56. Found: C, 62.07; H, 5.26; N, 11.01; Cl, 6.98; F, 7.39.

5.1.31. (*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-hydroxyethyl)acetamide (1t)

Compound **1t** was prepared according to the procedure described for **1d** from **1q** and 2-aminoethanol. The title compound **1t** (425 mg, 85%) was obtained as colorless crystals. Mp: 181–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.25–2.52 (1H, br), 2.65–2.90 (1H, br), 3.15–3.35 (1H, br), 3.23 (1H, q, J = 5.8 Hz), 3.48 (1H, m), 4.73 (1H, t, J = 5.3 Hz), 4.76–4.88 (1H, br), 6.33 (1H, d, J = 2.5 Hz), 6.36 (1H, s), 6.97 (1H, d, J = 7.3 Hz), 6.98–7.07 (1H, br), 7.16 (1H, dt, J = 1.5, 7.3 Hz), 7.24 (1H, t, J = 7.3 Hz), 7.31 (1H, dd, J = 1.5, 7.3 Hz), 7.55 (1H, d, J = 8.3 Hz), 7.83 (1H, s), 8.37 (1H, d, J = 2.5 Hz), 8.39–8.47 (1H, br). MS (FAB) m/z 501 $[M+1]^+$. Anal. Calcd for $C_{25}H_{23}ClF_2N_4O_3$: C, 59.94; H, 4.63; N, 11.18; Cl, 7.08; F, 7.59. Found: C, 59.68; H, 4.68; N, 11.09; Cl, 6.89; F, 7.63.

5.1.32. (*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-dimethylaminoethyl)acetamide hydrochloride (1u)

Compound **1u** was prepared according to the procedure described for **1b** from **1q** and *N*,*N*-dimethylethane-1,2-diamine. The title compound **1u** (50 mg, 19%) was obtained as white solid. Mp: 172–174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.30–2.55 (1H, br), 2.70–2.90 (7H, br), 3.05–3.20 (3H, br), 3.48–3.60 (2H, br), 4.75–4.90 (1H, br), 6.34 (1H, d, J = 2.5 Hz), 6.44 (1H, s), 6.99 (1H, d, J = 7.3 Hz), 6.99–7.11 (1H, br), 7.19 (1H, td, J = 7.3, 2.0 Hz), 7.25 (1H, td, J = 7.3, 2.0 Hz), 7.30 (1H, dd, J = 7.3, 2.0 Hz), 7.57 (1H, d, J = 8.3 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.5 Hz) 8.58–8.67 (1H, br),. MS (FAB) m/z 528 $[M+1]^+$. Anal. Calcd for $C_{27}H_{28}ClF_2N_5O_2 \cdot HCl \cdot 3H_2O$: C, 52.43; H, 5.70; N, 11.32; Cl, 11.46; F, 6.14. Found: C, 52.08; H, 5.70; N, 11.17; Cl, 11.36; F, 6.00.

5.1.33. (*Z*)-*N*-Carbamoylmethyl-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}acetamide (1w)

Compound **1w** was prepared according to the procedure described for **1d** from **1q** and glycinamide. The title compound **1w** (158 mg, 61%) was obtained as colorless crystals. Mp: 157–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.35–2.52 (1H, br), 2.68–2.85 (1H, br), 3.10–3.30 (1H, br), 3.71–3.81 (2H, m), 4.60–5.00 (1H, br), 6.33 (1H, d, J = 2.5 Hz), 6.42 (1H, s), 6.98 (1H, d, J = 7.3 Hz), 6.95–7.13 (1H, br), 7.10–7.17 (1H, br), 7.17 (1H, t, J = 7.3 Hz), 7.25 (1H, t, J = 7.3 Hz), 7.25–7.33 (1H, br), 7.34 (1H, d, J = 7.3 Hz), 7.57 (1H, d, J = 6.8 Hz), 7.84 (1H, s), 8.38 (1H, d, J = 2.5 Hz), 8.59–8.70 (1H, br). MS (FAB) m/z 513 $[M]^+$. Anal. Calcd for $C_{25}H_{22}ClF_2N_5O_3 \cdot 0.2H_2O$: C, 58.02; H, 4.36; N, 13.53; Cl, 6.85; F, 7.34. Found: C, 57.98; H, 4.31; N, 13.46; Cl, 6.84; F, 7.61.

5.1.34. (*Z*)-*N*-[(6-Aminopyridin-2-yl)methyl]-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}acetamide hydrochloride (1g)

Compound **4** (270 mg, 62%) was prepared according to the procedure described for **1d** from **1q** and **11** as colorless oil. MS (FAB) m/z 663 $[M]^+$. Compound **4** (270 mg) was treated with 4 M hydrochloride in ethyl acetate (20 ml) at room temperature for 12 h. Ethanol (0.5 ml) and ethyl acetate (20 ml) were added to the mixture, then the resulting precipitate was collected and dried in vacuo to give the title compound **1g** (74 mg, 30%) as white solid. Mp: 185–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.30–2.50 (1H, br), 2.65–2.85 (1H, br), 3.10–3.30 (1H, br), 4.47 (2H, d, J = 7.3 Hz), 4.65–5.05 (1H, br), 6.34 (1H, d, J = 2.5 Hz), 6.57 (1H, s), 6.78 (1H, d, J = 7.3 Hz), 6.90 (1H, d, J = 9.3 Hz), 6.99 (1H, d, J = 7.8 Hz), 7.03–7.14 (1H, br), 7.19 (1H, dt, J = 1.9, 7.8 Hz), 7.26 (1H, t, J = 7.8 Hz), 7.32 (1H, d, J = 2.0 Hz), 7.62 (1H, d, J = 7.3 Hz), 7.83 (1H, s), 7.92 (1H, t, J = 7.8 Hz), 8.03–8.16 (2H, br), 8.37 (1H, d, J = 2.4 Hz), 9.15 (1H, s), 13.79–14.20 (1H, br). MS (FAB) m/z 563 $[M]^+$. Anal. Calcd for $C_{29}H_{25}ClF_2N_6O_2 \cdot HCl \cdot 1.3H_2O$: C, 55.92; H, 4.63; N, 13.49; Cl, 11.38; F, 6.10. Found: C, 56.01; H, 4.57; N, 13.11; Cl, 11.47; F, 6.08.

5.1.35. Methyl (*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}acetylamino)acetate (5)

Compound **5** was prepared according to the procedure described for **1d** from **1q** and glycine methyl ester. The title compound **5** (420 mg, 53%) was obtained as colorless crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.30–2.55 (1H, br), 2.65–2.80 (1H, br), 3.15–3.30 (1H, br), 3.67 (3H, s), 3.98 (2H, d, J = 5.9 Hz), 4.75–4.90 (1H, br), 6.33 (1H, d, J = 2.5 Hz), 6.41 (1H, s), 6.98 (1H, d, J = 7.8 Hz), 6.99–7.09 (1H, br), 7.17 (1H, td, J = 7.8, 1.4 Hz), 7.25 (1H, t, J = 7.6 Hz), 7.32 (1H, d, J = 7.3 Hz), 7.57 (1H, d, J = 8.3 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 8.87 (1H, br s). MS (FAB) m/z 529 $[M+1]^+$.

5.1.36. (*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}acetylamino)acetic acid (1v)

A mixture of **5**, 1 M aqueous sodium hydroxide in methanol was stirred overnight at room temperature. The mixture was neutralized with 1 M aqueous hydrochloride, and that was extracted with chloroform. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was crystallized and recrystallized (*n*-hexane/ethyl acetate = 3/2) to give the title compound **1v** (116 mg, 48%) as colorless crystals. Mp: 167–169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (3H, s), 2.30–2.55 (1H, br), 2.60–2.85 (1H, br), 3.10–3.25 (1H, br), 3.89 (2H, br s), 4.75–4.95 (1H, br), 6.33 (1H, d, J = 2.5 Hz), 6.39 (1H, s), 6.97 (1H, d, J = 7.9 Hz), 6.95–7.11 (1H, br), 7.17 (1H, td, J = 7.9, 1.4 Hz), 7.25 (1H, t, J = 7.9 Hz), 7.33 (1H, d, J = 7.9 Hz), 7.57 (1H, d, J = 8.3 Hz), 7.83 (1H, s), 8.38 (1H, d, J = 2.5 Hz), 8.76 (1H, br s), 12.64 (1H, br s). MS (FAB) m/z 515 $[M+1]^+$. Anal. Calcd for $C_{25}H_{21}ClF_2N_4O_4$: C, 58.31; H, 4.11; N, 10.88; Cl, 6.89; F, 7.38. Found: C, 58.02; H, 4.10; N, 10.81; Cl, 6.73; F, 7.13.

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.[19] 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 (PerkinElmer) 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 (PerkinElmer) using the scintillation cocktail (MicroScinti-40™, PerkinElmer). 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 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 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,[20] using the K_d values yielded from each separate experiment. $K_i = IC_{50}/(1 + [[^3H]\text{vasopressin concentration}]/K_d)$.

5.2.2. 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.

5.2.3. 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.[21] 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.).[22]

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 mean ± standard error. The activities of test compounds for cAMP production were calculated by logistic regression as EC_{50} values.

5.2.4. 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 mean ± standard error.

5.3. CYP inhibitory activity

5.3.1. Fluorescent P450 inhibition assay (CYP2C9)[23]

Using a 96-well plate and 7-methoxy-4-trifluoromethylcoumarin (75 μM) as a substrate, each test compound (from 0.31 to 20 μM) was incubated with rCYP2C9 bactsome (3.1 pmol, purchased from Cypex Ltd). These incubations were conducted for 30 min at 37 °C in 200 μL (total volume) of 25 mM phosphate buffer (pH 7.4) and in the presence of a NADPH-generating system (8.2 μM $NADP^+$, 0.41 mM glucose-6-phosphate, 0.41 mM $MgCl_2$, and 0.4 units/mL glucose-6-phosphate dehydrogenase). Sulfaphenazole, a potent and specific CYP2C9 inhibitor, was used as the positive control in each study. The reaction was terminated by adding 0.5 M 2-amino-2-hydroxymethyl-1,3-propanediol aqueous solution containing 80% acetonitrile, and the fluorescence intensity (excitation wavelength: 409 nm, fluorescence wavelength: 530 nm) was measured using a fluorescence plate reader.

5.3.2. Fluorescent P450 inhibition assays (CYP2C19)[23]

The CYP2C19 inhibition assay was performed in a manner similar to that for CYP2C9. The positive control (tranylcypromine) was incubated for 20 min with substrate (3-cyano-7-ethoxycoumarin, 25 μM), enzyme (2.4 pmol), and phosphate buffer (50 mM). The excitation and fluorescence wavelengths used were 409 nm and 460 nm, respectively.

5.3.3. Fluorescent P450 inhibition assays (CYP2D6)[23]

The CYP2D6 inhibition assay was performed in a manner similar to that for CYP2C9. The positive control (quinidine) was incubated for 15 min with substrate (3-[2-(*N*,*N*-diethyl-*N*-methylamino)-ethyl]-7-methoxy-4-methylcoumarin, 1.5 μM), enzyme (5.0 pmol), and phosphate buffer (100 mM). The excitation and fluorescence wavelengths used were 390 nm and 460 nm, respectively.

5.3.4. Competitive inhibition of CYP3A4 in human liver microsomes

Midazolam 1′-hydroxylation was used to monitor CYP3A4 activity. Each test compound (0.3−100 μM) was co-incubated with midazolam (2 μM) in the presence of human liver microsomes (0.1 mg/mL) and NADPH (1.0 mM) at 37 °C for 20 min. At the end of the incubation, the reaction was terminated by adding an aqueous solution containing 80% acetonitrile. The concentration of 1′-hydroxymidazolam was determined using LC–MS/MS analysis. The inhibition of CYP3A4 activity was assessed by comparing the amount of 1′-hydroxymidazolam formed in the presence of varying concentrations of inhibitor to the amount of 1′-hydroxymidazolam formed in the solvent control. In each study, verapamil, a potent and specific CYP3A4 inhibitor, was used as the positive control.

Acknowledgments

The authors deeply acknowledge Dr. Mitsuaki Ohta, Dr. Minoru Okada and Dr. Seijiro Akamatsu for their helpful support to this research. The authors are also grateful to the staff of the Division of Analytical Science Laboratories for elemental analysis and spectral measurement.

References and notes

1. (a) Hardman, J. G.; Limbird, L. E. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 10th ed.; McGraw-Hill: New York, 2001; (b) Guyton, A. C.; Hall, J. E. *Textbook of Medical Physiology*, 9th ed.; W.B. Saunders: Medina, 1999. pp 933–944; (c) Michel, R. H.; Kirk, C. J.; Billah, M. M. *Biochem. Soc. Trans.* **1979**, *7*, 861.
2. Thibonnier, M.; Roberts, J. M. *J. Clin. Invest.* **1985**, *76*, 1857.
3. (a) Laszlo, F. A.; Laszlo, F.; De Weid, D. *Pharmacol. Rev.* **1991**, *43*, 73; (b) Bouby, N.; Bachmann, S.; Bichet, D.; Bankir, L. *Am. J. Physiol.* **1990**, *258*, F973.
4. (a) Asplund, R.; Sundberg, B.; Bengtsson, P. *BJU Int.* **1999**, *83*, 591; (b) Weiss, J. P.; Blaivas, J. G. *J. Urol.* **2000**, *163*, 5.
5. (a) Dimson, S. B. *Lancet* **1977**, *1*, 1260; (b) Tuvemo, T. *Acta Pediatr. Scand.* **1978**, *67*, 753.
6. (a) Kondo, K.; Ogawa, H.; Shinohara, T.; Kurimura, M.; Tanada, Y.; Kan, K.; Yamashita, H.; Nakamura, S.; Hirano, T.; Yamamura, Y.; Mori, T.; Tominaga, M.; Itai, A. *J. Med. Chem.* **2000**, *43*, 4388; (b) Kondo, K.; Kan, K.; Tanada, Y.; Bando, M.; Shinohara, T.; Kurimura, M.; Ogawa, H.; Nakamura, S.; Hirano, T.; Yamamura, Y.; Kido, M.; Mori, T.; Tominaga, M. *J. Med. Chem.* **2002**, *45*, 3805.
7. (a) Venkatesan, A. M.; Grosu, G. T.; Failli, A. A.; Chan, P. S.; Coupet, J.; Saunders, T.; Mazandarani, H.; Ru, X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5003; (b) Failli, A. A.; Shumsky, J. S.; Steffan, R. J.; Caggiano, T. J.; Williams, D. K.; Trybulsky, E. J.; Ning, X.; Lock, Y.; Tanikella, T.; Hartmann, D.; Chan, P. S.; Park, C. H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 954.
8. Tsukamoto, I.; Koshio, H.; Kuramochi, T.; Akamatsu, S.; Saitoh, C.; Yanai-Inamura, H.; Kitada, C.; Yamamoto, E.; Yatsu, T.; Sakamoto, S.; Tsukamoto, S. *Bioorg. Med. Chem.* **2008**, *16*, 9524.
9. Shimada, Y.; Akane, H.; Taniguchi, N.; Matsuhisa, A.; Kawano, N.; Tanaka, A. *Chem. Pharm. Bull.* **2005**, *53*, 589.
10. Ager, D. J.; Prakash, I. *Synth. Commun.* **1995**, *25*, 739.
11. Mitsuya, M.; Kobayashi, K.; Kawakami, K.; Satoh, A.; Ogino, Y.; Kakikawa, T.; Ohtake, N.; Kimura, T.; Hirose, H.; Sato, A.; Numazawa, T.; Hasegawa, T.; Noguchi, K.; Mase, T. *J. Med. Chem.* **2000**, *43*, 5017.
12. Elman, B. *Tetrahedron* **1985**, *41*, 4941.
13. Stille, J. K. *Angew. Chem.* **1986**, *98*, 504.
14. Bunnage, M.E.; Mathias, J.P.; Street, S.D.A.; Wood, A. Patent U.S. 6,251,904.
15. Catherine, B.; Catherine Bougault; Marie-Therese, A.; Andre, D.; Jean-Louis, P.; Jean-Marc, L.; Laurent Le, P. *J. Am. Chem. Soc.* **2001**, *123*, 8053.
16. Abushanab, E.; Bindra, A. P.; Goodman, L.; Petersen, H. *J. Org. Chem.* **1973**, *38*, 2049.
17. Alessandro, D.; Francisxo, M.; Pedro, M.; Isabel, R.; Tomas, T. *Synthesis* **1996**, *5*, 641.
18. Ozawa, T.; Kitagawa, H.; Yamamoto, Y.; Takahata, S.; Iida, M.; Osaki, Y.; Yamada, K. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 7325.
19. Tahara, A.; Saito, M.; Sugimoto, T.; Tomura, Y.; Wada, K.; Kusayama, T. *Br. J. Pharmacol.* **1998**, *125*, 1463.
20. Lazareno, S.; Bridsall, N. *Br. J. Pharmacol.* **1993**, *109*, 1110.
21. Tahara, A.; Saito, M.; Sugimoto, T.; Tomura, Y.; Wada, K.; Kusayama, T.; Tsukada, J.; Ishii, N.; Yatsu, T.; Uchida, W.; Tanaka, A. *Br. J. Pharmacol.* **1998**, *125*, 1463.
22. Takasaki, J.; Kamohara, M.; Saito, T.; Matsumoto, M.; Matsumoto, S.; Ohishi, T.; Soga, T.; Matsushime, H.; Furuichi, K. *Mol. Pharmacol.* **2001**, *60*, 432.
23. Crespi, C. L.; Miller, V. P.; Penman, B. W. *Analyt. Biochem.* **1997**, *248*, 188.