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Synthesis and structure–activity relationships of new carbonyl guanidine derivatives as novel dual 5-HT$_{2B}$ and 5-HT$_7$ receptor antagonists

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ABSTRACT

To identify potent dual 5-HT$_{2B}$ and 5-HT$_7$ receptor antagonists, we synthesized a series of novel carbonyl guanidine derivatives and examined their structure–activity relationships. Among these compounds, *N*-(9-hydroxy-9*H*-fluorene-2-carbonyl)guanidine (**10**) had a good in vitro profile, that is, potent affinity for human 5-HT$_{2B}$ and 5-HT$_7$ receptor subtypes (K_i = 1.8 nM and K_i = 17.6 nM, respectively) and high selectivity over 5-HT$_{2A}$, 5-HT$_{2C}$, α_1, D_2 and M_1 receptors. Compound **10** also showed a suppressing effect on 5-HT-induced dural protein extravasation in guinea pigs when orally administered.

1. Introduction

Migraine is a complex paroxysmal disorder resulting in a periodically occurring pulsating headache in which a strong pain occurs in one side or both sides of the head and continues from several hours to 3 days. Although migraine affects a substantial proportion of the population, the exact pathophysiology is not fully understood. Recently, it was suggested that the morbid state of migraine is advanced by the following hypothetical mechanism.[1] Firstly, dural blood vessel is contracted by a rapid increase in blood 5-hydroxytryptamine (5-HT, serotonin) levels, followed by the metabolism and depletion of 5-HT and the 5-HT depletion cause vasodilatation of dural blood vessels. During this time, the trigeminal nerve is activated and releases plasma proteins, such as substance P (SubP), and vasoactive peptides, such as calcitonin gene-related peptide (CGRP). The release of SubP and CGRP promote inflammation around the dural blood vessels, which results in migraine. The above findings therefore suggest that 5-HT plays an important roles in the onset of migraine.[2]

Medicines for migraine are divided into two categories; preventive medicines and treatment medicines. Agents exhibiting 5-HT receptor antagonism have been reported effective in preventing anti-migraine.[3] Among the anti-migraine 5-HT receptor antagonists, Pizotifen (**1**; Fig. 1) was launched in Europe, but a high frequency of adverse effects at its effective dose were reported, including fatigue, sleepiness, giddiness and weight gain.[4] These side effects might be due to Pizotifen (**1**) having a high affinity for most 5-HT receptor subtypes and adrenergic α_1, muscarine M_1, dopamine D_2 and other receptors.[5]

In recent years, a number of pharmacological studies on 5-HT receptor subtypes have been conducted. One study found that 5-HT$_{2B}$ receptor antagonist inhibited *m*-chlorophenylpiperazine (mCPP)-induced leakage of protein outside of the dural blood vessel in guinea pigs,[6] and that 5-HT$_{2B}$ receptor localized on vascular smooth muscle caused nitric oxide (NO) release, which accelerated the release of CGRP and SubP from trigeminal nerves.[7] The selective 5-HT$_{2B}$ receptor antagonist RS-127445 (**2**)[8] also showed a suppressing effect on mCPP-induced plasma extravasations in the dural blood vessels of anesthetized rats.[9] 5-HT$_7$ receptor was also reported to be distributed in the trigeminal nerve,[10] contribute to vasodilatation of the cerebrovascular smooth muscle,[11] and accelerate protein leakage in the dural matar blood vessel.[12] Administration of the selective 5-HT$_7$ antagonist SB-269970 (**3**)[13] was recently shown to partially inhibit CGRP release evoked by the trigeminal ganglion (TEGS) in a rat model,[14] and 5-HT$_{1D}$, 5-HT$_{2B}$ and 5-HT$_7$ receptors have been shown to be expressed in the dural matar blood vessels.[15] Taking these previous findings into account, we

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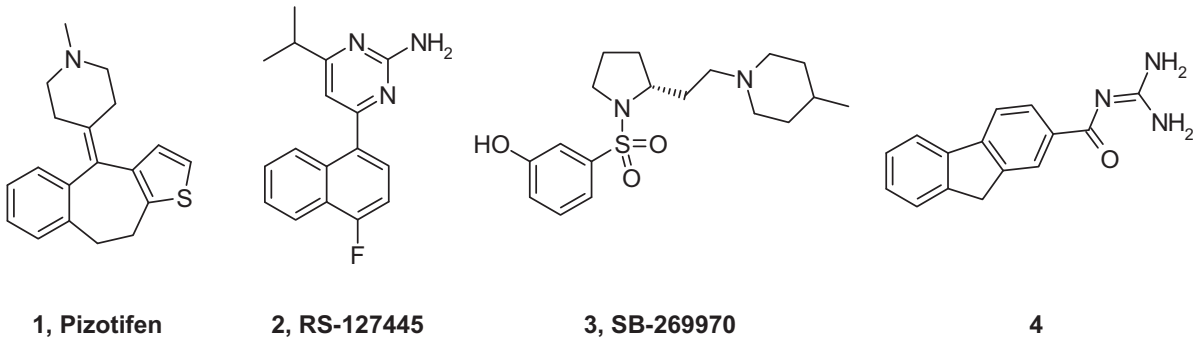


Figure 1. Structures of Pizotifen (**1**), RS-127445 (**2**, 5-HT_{2B} antagonist), SB-269970 (**3**, 5-HT_7 antagonist), and HTS hit (**4**).

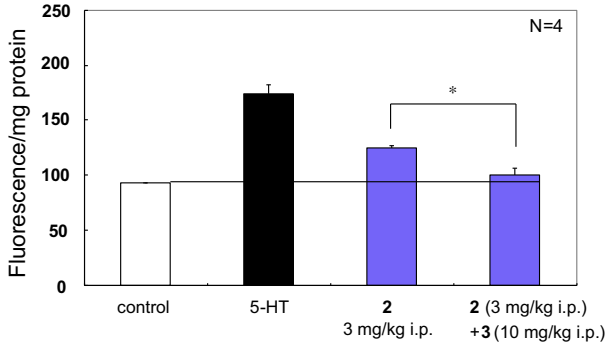
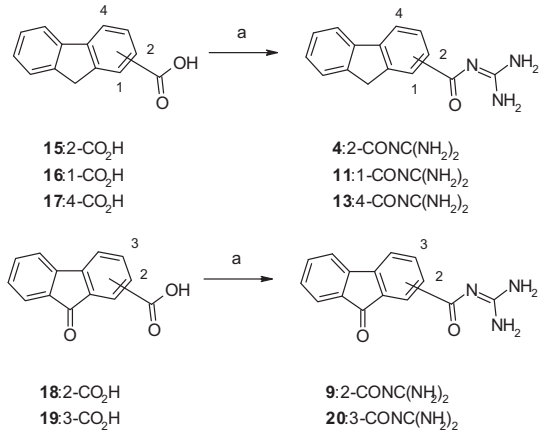


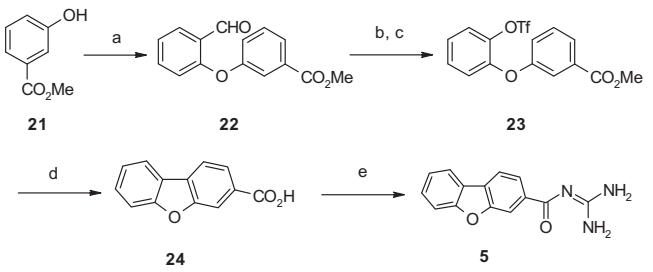
Figure 2. Suppressing effects of single dosings of compounds on the 5-HT induced dural plasma protein extravasation in guinea pigs. Values are mean or ±SEM for four animals in each group. * <0.05: significant difference. Student's *t*-test.



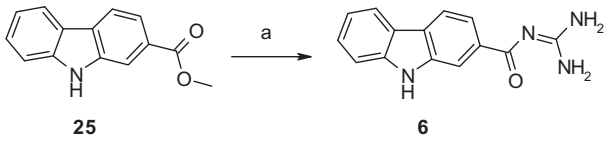
Scheme 1. Reagent and condition: (a) CDI, DMF, rt then guanidine hydrochloride, NaH, DMF, rt.

hypothesized that dual 5-HT_{2B} and 5-HT_7 receptor antagonists may represent a new class of medicines that prevent migraine with a reduction in the number and severity of side effects.

We previously reported a synergistic effect using the 5-HT_{2B} receptor selective antagonist RS-127445 (**2**) and the 5-HT_7 receptor selective antagonist SB-269970 (**3**).[16] RS-127445 (**2**) showed an effect to reduce amount of leaked protein from dural blood vessel in guinea pigs evoked by 5-HT at 3 mg/kg intraperitoneal administration (ip), but did not reduce to the normal level when the dose was increased from 3 mg/kg to 10 mg/kg. SB-269970 (**3**) also showed a similar effect at 10 mg/kg ip in the same model, but did not reduce the amount of leaked protein to the reference value when the dose was increased from 10 mg/kg to 30 mg/kg. When both compounds



Scheme 2. Reagents and conditions: (a) 2-fluorobenzaldehyde, K_2CO_3, DMF, 70 °C; (b) *m*-chloroperoxybenzoic acid, $CHCl_3$, 30 °C then concd HCl (cat), MeOH; (c) trifluoromethanesulfonic anhydride, pyridine, CH_2Cl_2; (d) $Pd(PPh_3)_2Cl_2$ (cat), DBU, LiCl, DMF, 145 °C; (e) CDI, DMF, rt then guanidine carbonate, rt.



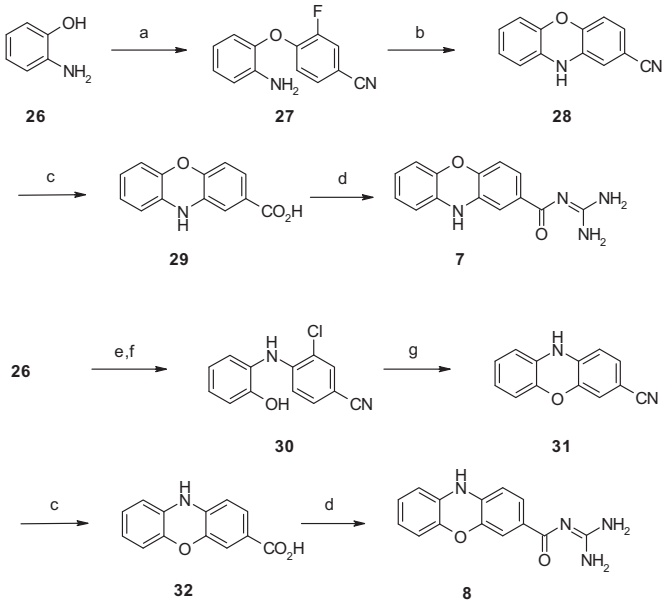
Scheme 3. Reagent and condition: (a) guanidine hydrochloride, NaH, DMF, 70 °C.

(RS-127445 (**2**): 3 mg/kg ip, SB-269970 (**3**): 10 mg/kg ip, respectively) were simultaneously administered, the amount of leaked protein was suppressed nearly completely to the normal level in the same model (Fig. 2). This result shows that when 5-HT_{2B} and 5-HT_7 receptors are simultaneously inhibited, a cumulative effect is observed that cannot be attained by selective inhibition of only one receptor.

We next focused our attention on creating novel dual 5-HT_{2B} and 5-HT_7 receptor antagonists as a potential new class of preventive medicines for anti-migraines. Here, to identify novel dual 5-HT_{2B} and 5-HT_7 receptor antagonists, we conducted high-throughput screening (HTS) of the Astellas chemical library, which led to the discovery of *N*-(diaminomethylene)-9*H*-fluorene-2-carboxamide (**4**). We now describe the synthesis and structure–activity relationships (SAR) of carbonyl guanidine derivatives as novel dual 5-HT_{2B} and 5-HT_7 receptor antagonists.

2. Chemistry

Synthesis of fluorene carbonyl guanidine derivatives **4**, **11**, **13**, **9** and **20** is depicted in Scheme 1. Fluorene carboxylic acids **15**–**18** were commercially available and fluorene carboxylic acid **19** was prepared based on a protocol reported by Weisburger et al.[17] Condensation with guanidine and fluorene carboxylic acids **15**–**19** in the presence of 1,1′-carbonyldiimidazole (CDI) gave fluorene carbonyl guanidine derivatives **4**, **11**, **13**, **9** and **20**.

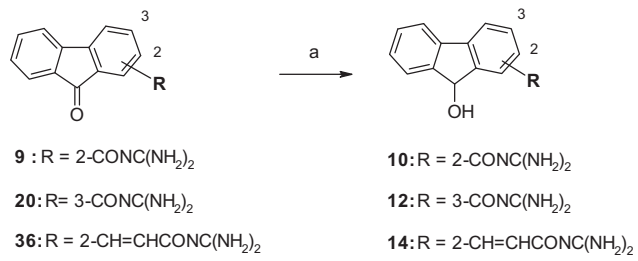


Scheme 4. Reagents and conditions: (a) 3,4-difluorobenzonitrile, NaH, DMF, 0 °C; (b) DMF, 165 °C; (c) concd HCl, AcOH, 120 °C; (d) CDI, DMF, 50 °C then guanidine hydrochloride, NaH, DMF, rt; (e) 3-chloro-4-fluorobenzonitrile, NaH, DMF, 0 °C; (f) NaH, DMF, 100 °C; (g) K_2CO_3, DMF, 150 °C.

Preparation of dibenzo[*b*,*d*]furan carbonyl guanidine derivative **5** is outlined in Scheme 2. A base promoted reaction of 2-fluorobenzaldehyde with phenol **21** yielded the aryloxy benzaldehyde derivative **22**, followed by Bayer–Villiger oxidation with *m*-chloroperbenzoic acid (*m*-CPBA) to furnished phenol formate. The formate was hydrolyzed to free phenol, which was converted to triflate **23** by treatment with triflic anhydride. Palladium-catalyzed cyclization of the triflate **23** provided caroboxylic acid **24**. Condensation with **24** and guanidine in the presence of CDI gave compound **5**.

Scheme 3 shows the synthesis of carbazole carbonyl guanidine derivative **6**. Condensation with **25**[18] and guanidine under thermal condition gave target compound **6**.

10*H*-Phenoxazine carbonyl guanidine derivative **7**, **8** were synthesized as shown in Scheme 4. 10*H*-Phenoxazine carboxylic acids **29** and **32** were prepared by cyano-activated fluoro displacement reactions based on a protocol by Eastmond et al.[19] A fluoro displacement reaction between 3,4-difluorobenzonitrile and 2-aminophenol **26** gave aminoether **27**, followed by thermal reaction in the absence of base yielded 2-cyanophenoxazine **28**. On the other hand, a reaction between 3-chloro-4-fluorobenzonitrile and 2-aminophenol **26** gave aminoether, followed by Smiles rearrangement with base to furnish phenol **30**. Cyclization of the phenol **30** gave 3-cyanophenoxazine **31**. Hydrolysis of the cyanophenoxazines **28** and **31** under acidic conditions generated corresponding carboxylic acids **29** and **32**. Condensation of the carboxylic acids **29** and **32** with guanidine in the presence of CDI gave compounds **7** and **8**, respectively.



Scheme 6. Reagent and condition: (a) $NaBH_4$, MeOH, rt.

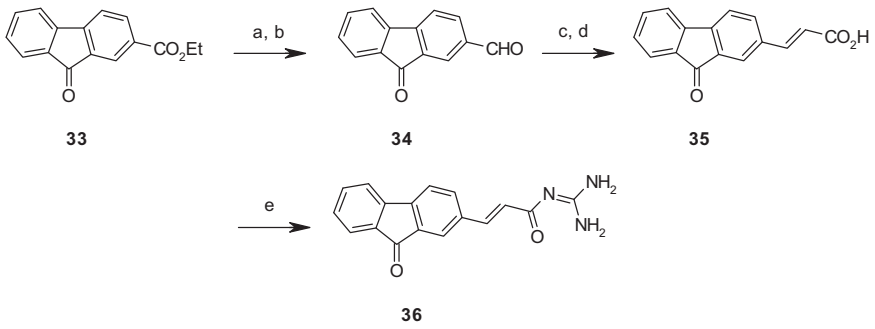
Scheme 5 shows the synthesis of (2*E*)-*N*-(diaminomethylene)-3-(9-oxo-9*H*-fluoren-2-yl)acrylamide (**36**). Ethyl 9-oxo-9*H*-fluorene-2-carboxylate **33** was converted into aldehyde **34** by reduction of the ester with lithium aluminum hydride ($LiAlH_4$) followed by oxidatation of the diol with manganese(IV) oxide (MnO_2). Wittig reaction of the resulting aldehyde **34** with methyl (triphenylphosphoranylidene)acetate gave acrylic acid ester, which was converted to acrylic acid **35** by treatment with NaOH. Condensation of the acrylic acid **35** with guanidine in the presence of CDI gave compound **36**.

Conversion of 9-oxo-fluorene derivatives **9**, **20** and **36** to 9-hydroxy-9*H*-fluorene derivatives **10**, **12** and **14** is shown in Scheme 6. Reduction of the 9-oxo-fluorenone derivatives **9**, **20** and **36** with sodium borohydride ($NaBH_4$) gave the target compounds **10**, **12** and **14**.

3. Results and discussion

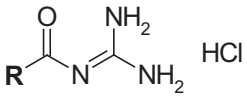
5-HT_{2B} and 5-HT_7 receptor binding affinities of the synthesized compounds **4**–**14** were determined using the displacement of antagonist ([^{3}H]Mesulergine) radioligand binding to human 5-HT_{2B} receptor sites in HEK293 cell membranes, and using agonist ([^{3}H]5-HT) radioligand binding to human 5-HT_7 receptor sites in CHO cell membranes, respectively.

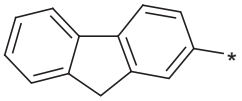
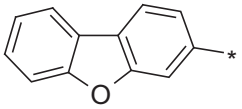
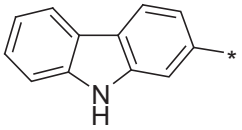
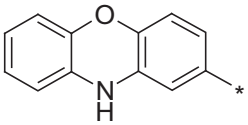
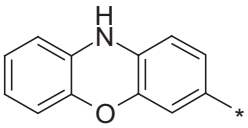
Compound **4** was found to have extremely potent affinity for 5-HT_{2B} and 5-HT_7 receptors (K_i = 1.8 nM and K_i = 12.4 nM, respectively), whereas its aqueous solubility was poor (solubility at pH 6.8 <1 μg/mL). We carried out an SAR study with compound **4** as the lead compound to improve both affinity and aqueous solubility. To improve aqueous solubility, reducing lipophilicity[20] or increasing topographical polar surface area (TPSA)[21] have been proven effective strategies. Lipophilicities were estimated by



Scheme 5. Reagents and conditions: (a) $LiAlH_4$, THF, rt; (b) MnO_2, $CHCl_3$, rt; (c) $Ph_3P{=}CHCO_2Me$, toluene, 90 °C; (d) 1 M NaOH aq, MeOH–THF, rt; (e) CDI, DMF, rt then guanidine hydrochloride, NaH, DMF, rt.

Table 1
5-HT_{2B} and 5-HT_7 receptor binding affinities of tricyclic ring derivatives **4**–**8**



Compound	–R	5-HT_{2B} K_i[a,b] (nM)	5-HT_7 K_i[a,c] (nM)	Solubility pH6.8 (μg/mL)	*C*Log*P*[f]	TPSA[g]
4[d]		1.8	12.4	<1	2.61	81.5
5		2.1	>100	NT[e]	2.65	94.6
6		10	42	NT[e]	2.13	97.3
7		3.6	144	NT[e]	2.22	102.7
8		2.4	>1000	NT[e]	2.22	102.7

[a] The data were obtained from at least two independent experiments.
[b] K_i for [^{3}H]Mesulergine binding; human 5-HT_{2B} receptor expressed in HEK293 cells.
[c] K_i for [^{3}H]5-HT binding; human 5-HT_7 receptor expressed in CHO cells.
[d] Free form.
[e] Not tested.
[f] *C*Log*P* value was calculated by ACD/Labs Software, version 12.01.
[g] TPSA value was calculated by MOE2011.10.

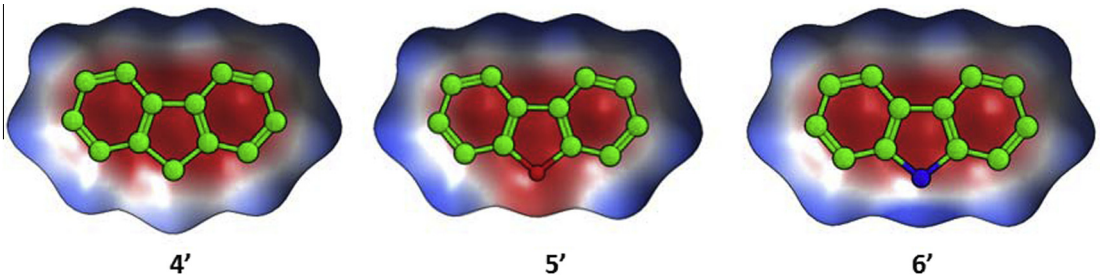


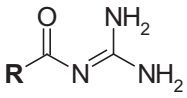
Figure 3. Atomic charge distributions of fluorene ring (**4**′), dibenzofuran ring (**5**′), and carbazole ring (**6**′). Compounds are colored blue for nitrogen, red for oxygen and green for carbon. Connolly surfaces[24] around the core scaffolds are colored red for negative charge and blue for positive charge.

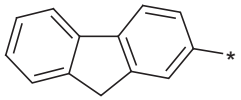
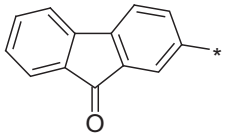
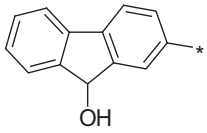
*C*Log*P* values, calculated using ACD Log*P* prediction software[22] and TPSA values were calculated using Molecular Operating Environment (MOE[23]) software.

At the initiation of our SAR studies, we hypothesized that the carbonyl guanidine moiety of compound **4** would be a structure mimicking a basic amino moiety in 5-HT. We subsequently synthesized derivatives containing modifications of the fluorene ring. We first studied the SAR of compounds containing with a dibenzofuran, carbazole, and phenoxazine (Table 1). The *C*Log*P* values (2.13–2.65) of compounds **5**–**8** were equal to or lower than that of compound **4** (2.61), and compounds **5**–**8** exhibited higher TPSA values (94.6–102.7) than that of compound **4** (81.5). Replacement of the fluorene moiety with a dibenzofuran moiety (**5**) resulted in a loss of affinity for 5-HT_7 receptor, although almost equal affinity for 5-HT_{2B} receptor (K_i = 1.8 nM). Replacement of the fluorene moiety with a carbazole moiety (**6**) caused a slight decrease in affinity for 5-HT_{2B} and 5-HT_7 receptors (K_i = 10 nM and K_i = 42 nM, respectively). Conversion of the fluorene moiety to 2-phenoxazine moiety (**7**) and 3-phenoxazine moiety (**8**) resulted in significantly decreased affinity for 5-HT_7 receptor, although almost equally potent to 5-HT_{2B} receptor (K_i = 3.6 nM and K_i = 2.4 nM, respectively). These results suggested that the 6,5,6-membered ring systems were preferable to the 6,6,6-membered ring systems.

We calculated the atomic charge distributions of the fluorene moiety of compound **4**, the dibenzofuran moiety of compound **5** and the carbazole moiety of compound **6** to examine these results

Table 2
5-HT_{2B} and 5-HT_7 receptor binding affinities of 9-position substituent fluorene derivatives **4**, **9** and **10**



Compound	–R	5-HT_{2B} K_i[a,b] (nM)	5-HT_7 K_i[a,c] (nM)	*C*Log*P*[e]	TPSA[f]
4		1.8	12.4	2.61	81.5
9		0.4	185	2.09	98.5
10[d]		1.8	17.6	0.98	101.7

[a] The data were obtained from at least two independent experiments.
[b] K_i for [^{3}H]Mesulergine binding; human 5-HT_{2B} receptor expressed in HEK293 cells.
[c] K_i for [^{3}H]5-HT binding; human 5-HT_7 receptor expressed in CHO cells.
[d] HCl salt.
[e] *C*Log*P* value was calculated by ACD/Labs Software, version 12.01.
[f] TPSA value was calculated by MOE2011.10.

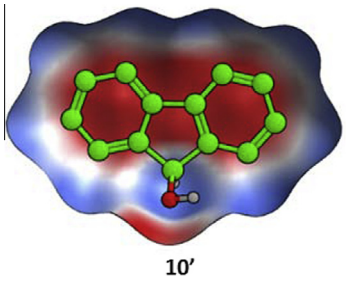


Figure 4. Atomic charge distributions of 9-hydoxy fluorene (**10′**). Compound is colored gray for polar-hydrogen, red for oxygen and green for carbon. Connolly surfaces[24] around the core scaffolds are colored red for negative charge and blue for positive charge.

further (Fig. 3). For the fluorene ring 4′ and the carbazole ring 6′, the positive charge (blue) was distributed on the center-bottom side of the 6,5,6-membered ring, whereas the dibenzofuran ring 5′ had a negative charge (red) in the corresponding region. These results suggested that a positive charge was localized at the center–bottom side of the 6,5,6-membered ring, resulting in affinity for 5-HT_7 receptor. On the other hands, the atomic charge distributions were not related to 5-HT_{2B} receptor affinity. On the basis of these results, we selected compound **4** as the lead compound for further optimizations.

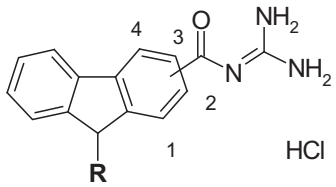
Next, we investigated the effects of substitution on the 9-position of fluorene ring of compound **4** (Table 2). The *C*log*P* values of compounds **9** (2.09) and **10** (0.98) were lower than that of compound **4** (2.61), and the TPSA values of compounds **9** (98.5) and **10** (101.7) were higher than that of compound **4** (81.5). The introduction of a carbonyl group (**9**) to the 9-position of fluorene ring resulted in an increased affinity for 5-HT_{2B} receptor (K_i = 0.4 nM), while a significantly decreased affinity for 5-HT_7 receptor was observed (K_i = 185 nM). On the other hands, the introduction of a hydroxyl group (**10**) to the 9-position of fluorene ring maintained the affinity towards 5-HT_{2B} and 5-HT_7 receptors (K_i = 1.8 nM and K_i = 17.6 nM, respectively), and indicated a possible improvement in both affinity and aqueous solubility. Additionally, as shown in Figure 3, the calculated atomic charge distributions of the fluorene moiety of compound **10** were consistent with the results shown in Figure 4.

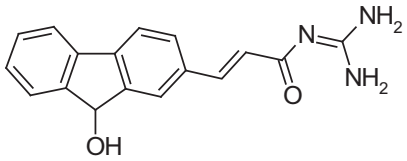
We then examined the effects of substitution patterns of a carbonyl guanidine moiety on the fluorene ring (Table 3). Compound **11** showed a 20-fold loss of affinity for 5-HT_7 receptor (K_i = 239 nM) compared to compound **4**, while affinities of both compounds for 5-HT_{2B} receptor were maintained. Compounds **12** and **13** exhibited a loss of affinity for 5-HT_7 receptor, and a significantly decreased affinity for 5-HT_{2B} receptor was observed (K_i = 86.8 nM and K_i = 27.4 nM, respectively). Moreover, insertion of an olefin linker between the fluorene ring and a carbonyl guanidine (**14**) resulted in a decrease in affinity for 5-HT_{2B} and 5-HT_7 receptors (K_i = 52 nM and K_i = >100 nM, respectively). These results indicated that direct substitution of the carbonyl guanidine moiety at the 2-position on fluorene ring was an essential structure.

Having identified **10** as a promising compound, we investigated other off-target activities (5-HT_{2A}, 5-HT_{2C}, adrenergic α_1, dopamine D_2 and muscarine M_1 receptors) and aqueous solubility. The results are summarized in Table 4. While Pizotifen (**1**) has no selectivity to other off-target receptors, both **4** and **10** showed low affinity for 5-HT_{2A}, D_2 and M_1 receptors. The HTS hit compound **4** showed moderate affinities for 5-HT_{2c} and α_1 receptors (K_i = 200 nM and K_i = 790 nM, respectively), however, compound **10** showed considerably weaker affinities for 5-HT_{2c} and α_1 receptors (K_i = >1000 nM) than compound **4**. Furthermore, as predicted with the *C*log*P* value or the TPSA value, compound **10** showed higher solubility than compound **4** in a pH 6.8 aqueous solution. These results demonstrated that compound **10** had an excellent 5-$HT_{2B/7}$ selectivity versus other off-target receptors, with significantly-improved aqueous solubility over compound **4**.

The functional activities of compounds **4** and **10** on human 5-HT_{2B} and 5-HT_7 receptors were determined by measuring inhibi-

Table 3
5-HT_{2B} and 5-HT_7 receptor binding affinities of carbonyl guanidine derivatives **4**, **10**–**14**



Compound	–R	Carbonyl guanidine	5-HT_{2B} K_i[a,b] (nM)	5-HT_7 K_i[a,c] (nM)
4	H	2	1.8	12.4
10	–OH	2	1.8	17.6
11	H	1	1.1	239
12	–OH	3	86.8	>1000
13	–H	4	27.4	>1000
14			52	>100

[a] The data were obtained from at least two independent experiments.
[b] K_i for [^{3}H]Mesulergine binding; human 5-HT_{2B} receptor expressed in HEK293 cells.
[c] K_i for [^{3}H]5-HT binding; human 5-HT_7 receptor expressed in CHO cells.

Table 4
Affinity for other receptors, and aqueous solubility of compounds **4** and **10**

Compound	K_i (nM)							Solubility
	5-HT_{2B}[a,b]	5-HT_7[a,c]	5-HT_{2A}[d]	5-HT_{2C}[d]	α_1[d]	D_2[d]	M_1[d]	pH 6.8 (μg/mL)
Pizotifen (**1**)	2.0	25	7.5	1.4	75	2.4	2.0	NT[e]
4	1.8	12.4	1000	200	790	1400	>1000	<1
10	1.8	17.6	>1000	>1000	>1000	>1000	>1000	10<

[a] The data were obtained from at least two independent experiments.
[b] K_i for [^{3}H]Mesulergine binding; human 5-HT_{2B} receptor expressed in HEK293 cells.
[c] K_i for [^{3}H]5-HT binding; human 5-HT_7 receptor expressed in CHO cells.
[d] K_i value was measured in accordance with the established method.[28–31]
[e] Not tested.

Table 5
Human 5-HT_{2B} and 5-HT_7 receptor antagonistic activity of **4** and **10**

Compound	5-HT_{2B} IC_{50}[a,b] (nM)	5-HT_7 % inhibition[a,c] (%)
4	36.4	98
10	19.6	97

[a] The data were obtained from at least three independent experiments.
[b] IC_{50} against [^{3}H]PI metabolism; human 5-HT_{2B} receptor expressed in HEK293 cells.
[c] % Inhibition at 10 μM against cAMP production; human 5-HT_7 receptor expressed in CHO cells.

tory activity of PI metabolism in HEK 293 cells or cAMP production in CHO cells. Compounds **4** and **10** both showed antagonistic activity towards human 5-HT_{2B} and 5-HT_7 receptors (Table 5).

Given its potent inhibitory activity against 5-$HT_{2B/7}$ receptors and high selectivity against other off-target receptors, compound **10** was submitted for in vivo studies. The results are presented with % change values [that is, suppressing effects compared with control and how the compounds suppressed the protein extravasation] in Table 6. As shown in Figure 5A, single intraperitoneal doses (0.3–3 mg/kg) of compound **10** suppressed 5-HT-induced dural protein extravasation in guinea pigs in a dose-dependent manner. When compound **10** was administered at 3 mg/kg ip, the amount of leaked protein was suppressed nearly completely to the normal level (% change = 93%). This was a comparable effect when 5-HT_{2B} receptor selective antagonist RS-127445 (**2**) and 5-HT_7 receptor selective antagonist SB-269970 (**3**) were simultaneously administered in the same model (% change = 91%). When only RS-127445 (**2**) was administered, the% change value was 60%. That is, a dual 5-HT_{2B} and 5-HT_7 receptor antagonist compound **10** can inhibit leakage of the inflammatory protein nearly complete by its cumulative effect that cannot be attained by selective inhibition of only one receptor. In addition, compound **10** showed a suppressive effect in the same model when orally administered (3–30 mg/kg) in a dose-dependent manner (Fig. 5B). Taken together, these

Table 6
Suppressing effects of **10**, RS-127445 (**2**, 5-HT_{2B} receptor antagonist) and SB-269970 (**3**, 5-HT_7 receptor antagonist) on 5-HT induced dural plasma protein extravasation in guinea pigs

Compound	% Change[a] (%)
RS-127445 (**2**)[b]	60
RS-127445 (**2**) and SB-269970 (**3**)[c]	91
10[d]	93

[a] % Change from 5-HT group. The following formula was used in this calculation: % change = [compound group − control]/[5-HT group − control] ×100%. See Section 5.
[b] At 3 mg/kg ip.
[c] Simulutaneously administration of **2** (3 mg/kg ip) and **3** (10 mg/kg ip).
[d] At 3 mg/kg ip.

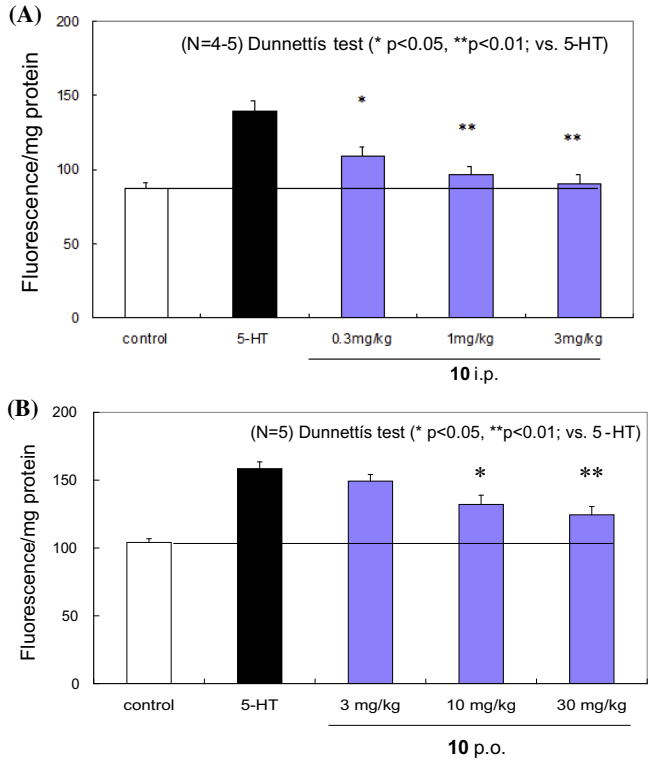


Figure 5. Suppressing effects of single dosings of compounds on the 5-HT induced dural plasma protein extravasation in guinea pigs. (A) intraperitoneal dosing of **10**. (B) oral dosings of **10**. Values are mean or ±SEM for five animals in each group. $^{*}p$ <0.05, $^{**}p$ <0.01 versus 5-HT. Dunnett's multiple comparison test.

findings strongly supported the potential of dual 5-HT_{2B} and 5-HT_7 receptor antagonists in preventive medicine for anti-migraines.

4. Conclusion

In this investigation of novel dual 5-HT_{2B} and 5-HT_7 receptor antagonists, a series of carbonyl guanidines with various tricyclic aromatic rings were synthesized and evaluated. Structure–activity relationship (SAR) studies of this novel class of compounds revealed that fluorene 2-carbonyl guanidine played an important structures in showing potent inhibitory activity against both 5-HT_{2B} and 5-HT_7 receptors. The atomic charge distributions of 6,5,6-membered rings suggested that a positive charge is localized at the 9-position of fluorene ring, endowing affinity for 5-HT_7 receptor. Of this series, *N*-(9-hydroxy-9*H*-fluorene-2-carbonyl)guanidine (**10**) exerts potent affinity for human 5-HT_{2B} and 5-HT_7 receptor subtype (K_i = 1.8 nM and K_i = 17.6 nM, respectively) with high selectivity over 5-HT_{2A}, 5-HT_{2C}, α_1, D_2 and M_1 receptors. Compound **10** also showed a suppressing effect on 5-HT-induced dural protein extravasation in gunea pigs when orally administered. Further investigation will be carried out to improve the potency of this series of compounds and the results will be reported in due course.

5. Experimental

5.1. Chemistry

Uncorrected melting points (Mps) were determined using BÜCHI B-545 or Yanaco MP-500D micro melting apparatuses. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300, JEOL JNM-EX400 or JEOL JNM-A500 spectrometer and were referenced to an internal standard, tetramethylsilane. The abbreviations used for the signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; dd, double doublet; dt, double triplet; td, triple doublet; m, multiplet. Mass spectra were recorded on a Hitachi M-80 or JEOL JMS-DX300 mass spectrometer, and the ionization method was chosen from ESI and FAB. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (Cl). Preparative column chromatography was performed with Wakogel C-200 or Merck silica gel 60 or Fuji silisia NH_2 Chromatorex.

5.1.1. *N*-(Diaminomethylene)-9*H*-fluorene-2-carboxamide (4)

To a solution of 9*H*-fluorene-2-carboxylic acid (**15**, 520 mg, 2.47 mmol) in DMF (6 mL) was added 1,1′-carbonyldiimidazole (CDI) (440 mg, 2.71 mmol) at room temperature, the reaction mixture was stirred at 50 °C for 1 h, then allowed to cool. This mixture was added under ice-cooling to a solution which had been prepared by addition of NaH (55% dispersion in mineral oil; 500 mg, 12.5 mmol) to DMF (6 mL) solution of guanidine hydrochloride (1.18 g, 12.3 mmol) and stirring at room temperature for 1 h, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and added $CHCl_3$ and water. The aqueous layer was extracted with $CHCl_3$, and the combined organic layer was washed with 1 M NaOH aq, dried over $MgSO_4$. After evaporation in vacuo, the resultant residue was purified by silica gel column chromatography (EtOAc), recrystalization from MeOH to give the title compound **4** (140 mg, 23%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6) δ: 3.96 (2H, s), 7.31–7.42 (2H, m), 7.60 (1H, d, J = 6.9 Hz), 7.88 (1H, d, J = 8.6 Hz), 7.93 (1H, d, J = 8.3 Hz), 8.12 (1H, dd, J = 1.4, 8.3 Hz), 8.31 (1H, s). MS (FAB) m/z: 252 (M^++H). Mp: 210–212 °C. Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.61; H, 5.18; N, 16.63.

5.1.2. Methyl 3-(2-formylphenoxy)benzoate (22)

To a solution of methyl 3-hydroxybenzoate (2 g, 13.1 mmol) and 2-fluorobenzaldehyde (1.38 mL, 13.1 mmol) in DMF (15 mL) was added potassium carbonate (2.72 g, 19.6 mmol) and the mixture was stirred at 70 °C for 18 h, and then allowed to cool. The reaction mixture was concentrated in vacuo and added EtOAc and water. The aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried over $MgSO_4$. After evaporation in vacuo, the resultant residue was purified by silica gel column chromatography (EtOAc–hexane) to give the title compound **22** (2.54 g, 76%) as a white solid.

^{1}H NMR ($CDCl_3$) δ: 3.91 (3H, s), 6.90 (1H, d, J = 8.4 Hz), 7.21–7.30 (2H, m), 7.47 (1H, t, J = 8.0 Hz), 7.53–7.56 (1H, m), 7.71–7.72 (1H, m), 7.86 (1H, dt, J = 1.2, 8.0 Hz), 7.96 (1H, dd, J = 1.2, 8.4 Hz). MS (ESI) m/z: 257 (M^++H).

5.1.3. Methyl 3-(2-tifluoromethanesulofonate)benzoate (23)

To a solution of **22** (2.54 g, 9.9 mmol) in chlololform (40 mL) was add *m*-chloroperoxybenzoic acid (4.28 g, 24.7 mmol) and the mixture was stirred at 30 °C for 3 h, then cooled down to room temperature. The reaction mixture was quenched by addition of dilute aqueous sodium hydrogen sulfate solution. The organic layer was washed with saturated aqueous sodium bicarbonate solution and water, dried over Na_2SO_4 and evaporated in vacuo to give the corresponding formate as a pale yellow oil, which was used for the next step without further purification. To a solution of the formate obtained above in MeOH (50 mL) was added 2 drops conc. HCl aq, and the solution was stirred at room temperature for 1.5 h. After addition of sodium carbonate (1 g), the solution was filtered and evaporated in vacuo, the resultant residue was purified by silica gel column chromatography (MeOH–$CHCl_3$) to give the phenol (2.25 g, 93%) as a white solid. To a cold (0 °C) solution of the phenol obtained above (2.18 g,

8.92 mmol) and pyridine (3.60 mL, 44.6 mmol) in CH_2Cl_2 (25 mL) was added triflic anhydride (2.0 mL, 11.5 mmol), then the mixture was warmed up to room temperature and stirred for 30 min. After addition of water (100 mL), the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with 1 M HCl aq, saturated aqueous sodium bicarbonate solution and brine, dried over $MgSO_4$. After evaporation in vacuo, the resultant residue was purified by silica gel column chromatography ($CHCl_3$) to give the title compound **23** (3.35 g, quantitative) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ: 3.90 (3H, s), 6.98 (1H, dd, J = 1.2, 8.0 Hz), 7.17 (1H, dt, J = 1.6, 8.0 Hz), 7.26–7.33 (2H, m), 7.36 (1H, dd, J = 1.2, 8.0 Hz), 7.45 (1H, t, J = 8.0 Hz), 7.70–7.72 (1H, m), 7.86 (1H, dt, J = 1.2, 8.0 Hz). MS (ESI) m/z: 377 (M^++H).

5.1.4. Dibenzo[*b*,*d*]furan-3-carboxylic acid (24)

A solution of **23** (1.01 g, 2.7 mmol), $Pd(PPh_3)_2Cl_2$ (192 mg, 0.27 mmol), LiCl (347 mg, 8.2 mmol), and DBU (0.5 mL, 3.3 mmol) in DMF (30 mL) was stirred at 145 °C for 22.5 h. After cooling, Et_2O and water were added and the aqueous layer was extracted with Et_2O. The aqueous layer was acidified (pH 2.0) with 1 M HCl aq and the resulting solid was filtered. The combined organic layer was washed with brine, dry over $MgSO_4$ and evaporated in vacuo and the resulting residue was added water and acidified (pH2.0) with 1 M HCl aq and the resulting solid was filtered. These two solids were combined and purified by silica gel column chromatography ($CHCl_3$–MeOH) and recrystallization from EtOH to give the title compound **24** (102 mg, 17.8%) as a white solid.

^{1}H NMR (DMSO-d_6) δ: 7.46 (1H, t, J = 7.6 Hz), 7.61 (1H, td, J = 0.8, 7.6 Hz), 7.77 (1H, d, J = 8.0 Hz), 8.01 (1H, dd, J = 0.8, 8.0 Hz), 8.20 (1H, s), 8.24 (1H, d, J = 8.4 Hz), 8.27 (1H, d, J = 8.4 Hz), 13.15 (1H, br s). MS (FAB) m/z: 211 (M^+−H).

5.1.5. *N*-(Diaminomethylene)dibenzo[*b*,*d*]furan-3-carboxamide monohydrochloride (5)

To a solution of dibenzo[*b*,*d*]furan-3-carboxylic acid (**24**, 100 mg, 0.47 mmol) in DMF (3 mL) was added CDI (115 mg, 0.70 mmol) and the mixture was stirred at room temperature for 1 h. To the mixture was added guanidine carbonate (203 mg, 1.12 mmol) and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo. To the resultant residue was added water and stirred at room temperature for 30 min. The precipitate was collected by filtration, washed with water, dried in vacuo at 50 °C to give *N*-(diaminomethylene)dibenzo[*b*,*d*]furan-3-carboxamide (102 mg) as a white solid. The compound was converted to its monohydrochloride salt by treating it with 4 M HCl–EtOAc (0.2 mL, 0.8 mmol) in EtOH (3.5 mL). The crude salt was suspended with EtOH and filtered to give the title compound **5** (102 mg, 75%) as a white solid.

^{1}H NMR (DMSO-d_6) δ: 7.50 (1H, t, J = 7.8 Hz), 7.66 (1H, t, J = 7.8 Hz), 7.81 (1H, d, J = 8.3 Hz), 8.21 (1H, d, J = 7.8 Hz), 8.29 (1H, d, J = 7.3 Hz), 8.37 (1H, d, J = 7.8 Hz), 8.59 (1H, s), 8.62 (2H, br s), 8.85 (2H, br s), 12.27 (1H, s). MS (FAB) m/z: 254 (M^++H). Mp: 299–300 °C. Anal. Calcd for $C_{14}H_{11}N_3O_2{\cdot}0.95HCl{\cdot}0.3H_2O$: C, 57.33; H, 4.31; N, 14.33; Cl, 11.48. Found: C, 57.14; H, 4.15; N, 14.23; Cl, 11.68.

5.1.6. Methyl 9*H*-carbazole-2-carboxylate (25)

The title compound was prepared according to a literature protocol.[18]

^{1}H NMR (DMSO-d_6) δ: 3.30 (3H, s), 7.21 (1H, td, J = 1.2, 8.0 Hz), 7.47 (1H, td, J = 8.0, 1.2 Hz), 7.56 (1H, d, J = 8.0 Hz), 7.78 (1H, dd, J = 1.2, 8.0 Hz), 8.11 (1H, s), 8.20 (1H, d, J = 8.0 Hz), 8.23 (1H, d, J = 8.0 Hz), 11.53 (1H, s). MS (FAB) m/z: 226 (M^++H).

5.1.7. *N*-(Diaminomethylene)-9*H*-carbazole-2-carboxamide monohydrochloride (6)

To a cold (0 °C) solution of guanidine hydrochloride (573 mg, 6 mmol) in DMF (6.5 mL) was added portion wise NaH (60% dispersion in mineral oil; 192 mg, 4.8 mmol) and the mixture was stirred at room temperature for 1 h. To the mixture was added dropwise a solution of **25** (271 mg, 1.2 mmol) in DMF (6.5 mL) and the mixture was stirred at 70 °C for 2 days. After cooling, the reaction mixture was concentrated in vacuo. To the resultant residue was added water and stirred at room temperature for 3 h. The precipitate was collected by filtration, washed with water, dried in vacuo at 50 °C. The resultant residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to give *N*-(diaminomethylene)-9*H*-carbazole-2-carboxamide (236 mg) as a pale yellow solid. The compound was converted to its monohydrochloride salt by treating it with 4 M HCl–EtOAc (0.45 mL, 1.8 mmol) in EtOH (9.0 mL). The crude salt was suspended with EtOH and filtered to give the title compound **6** (266 mg, 77%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6) δ: 7.24 (1H, td, J = 1.0, 7.3 Hz), 7.51 (1H, td, J = 1.0, 7.3 Hz), 7.58 (1H, d, J = 8.3 Hz), 7.97 (1H, dd, J = 1.5, 8.3 Hz), 8.24 (1H, d, J = 7.3 Hz), 8.32 (1H, d, J = 6.4 Hz), 8.33 (1H, s), 8.56 (2H, br s), 8.83 (2H, br s), 11.80 (1H, s), 12.04 (1H, s). MS (FAB) m/z: 253 (M^++H). Mp: >250 °C decompose. Anal. Calcd for $C_{14}H_{12}N_4O{\cdot}HCl{\cdot}0.3C_2H_6O{\cdot}0.1H_2O$: C, 57.62; H, 4.97; N, 18.41; Cl, 11.65. Found; C, 57.67; H, 4.97; N, 18.21; Cl, 11.81.

5.1.8. 4-(2-Aminophenoxy)-3-fluorobenzonitrile (27)

To a cold (0 °C) solution of 2-aminophenol (3.0 g, 27.4 mmol) in DMF (30 mL) was added portion wise NaH (55% dispersion in mineral oil; 1.26 g, 28.7 mmol) and the mixture was stirred at 0 °C for 30 min. To this cold mixture was added 3,4-difluorobenzonitrile (4.02 g, 28.7 mmol) and the mixture was stirred at 0 °C for 3 days. Reaction was quenched by addition of water, and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4. After evaporation in vacuo, the resultant residue was purified by silica gel column chromatography (EtOAc–hexane) to give the title compound **27** (4.88 g, 78%) as an orange oil.

^{1}H NMR (DMSO-d_6) δ: 5.12 (2H, s), 6.58 (1H, td, J = 1.5, 7.4 Hz), 6.75 (1H, t, J = 7.3 Hz), 6.85 (1H, dd, J = 1.4, 8.3 Hz), 6.90 (1H, dd, J = 1.0, 7.8 Hz), 7.01 (1H, td, J = 1.4, 7.9 Hz), 7.58 (1H, dt, J = 1.4, 8.3 Hz), 7.99 (1H, dd, J = 2.0, 11.3 Hz). MS (FAB) m/z: 227 (M^+−H).

5.1.9. 10*H*-Phenoxazine-2-carbonitrile (28)

A solution of **27** (4.88 g, 21.3 mmol) in DMF (25 mL) was stirred at 145 °C for 4 days. After cooling, water was added to the mixture and the aqueous layer was extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4. After evaporation in vacuo, the resultant residue was purified by silica gel column chromatography (EtOAc–hexane) to give the title compound **28** (494 mg, 11%) as a brown solid.

^{1}H NMR (DMSO-d_6) δ: 6.47 (1H, dd, J = 0.9, 7.8 Hz), 6.58–6.65 (2H, m), 6.68 (1H, d, J = 2.0 Hz), 6.72 (1H, d, J = 8.3 Hz), 6.77 (1H, td, J = 1.9, 7.8 Hz), 7.02 (1H, dd, J = 1.9, 8.3 Hz), 8.55 (1H, s). MS (FAB) m/z: 207 (M^+−H).

5.1.10. 10*H*-Phenoxazine-2-carboxylic acid (29)

A solution of **28** (287 mg, 1.37 mmol) and conc. HCl (14 mL) in AcOH (14 mL) was stirred at 120 °C for 18 h, then allowed to cool. The reaction mixture was concentrated in vacuo. To the resultant residue was added water and stirred at room temperature for 30 min. The precipitate was collected by filtration, washed with water, dried in vacuo at 50 °C to give the title compound **29** (297 mg, 95%) as a gray solid.

^{1}H NMR (DMSO-d_6) δ: 6.44 (1H, dd, J = 1.4, 7.8 Hz), 6.56–6.66 (3H, m), 6.75 (1H, td, J = 1.4, 7.3 Hz), 7.03 (1H, d, J = 2.0 Hz), 7.17

(1H, dd, J = 1.9, 8.3 Hz), 8.36 (1H, s), 12.63 (1H, br s). MS (FAB) m/z: 226 (M^+−H).

5.1.11. *N*-(Diaminomethylene)-10*H*-phenoxazine-2-carboxamide monohydrochloride (7)

The title compound was prepared in the same manner as described for **4** using **29** instead of **15** in 53% yield.

^{1}H NMR (DMSO-d_6) δ: 6.49 (1H, dd, J = 1.5, 7.9 Hz), 6.58–6.66 (2H, m), 6.73–6.79 (2H, m), 7.04 (1H, d, J = 2.4 Hz), 7.48 (1H, dd, J = 2.4, 8.3 Hz), 8.45 (2H, br s), 8.59 (1H, s), 8.64 (2H, br s), 11.69 (1H, s). MS (FAB) m/z: 269 (M^++H) Mp: 264–269 °C Anal. Calcd for $C_{14}H_{12}N_4O_2$·HCl·1.2H_2O: C, 51.52; H, 4.76; N, 17.17; Cl, 10.86. Found: C, 51.42, H, 4.84; N, 17.10; Cl, 10.95.

5.1.12. 3-Chloro-4-[(2-hydroxyphenyl)amino]benzonitrile (30)

4-(2-Aminophenoxy)-3-chlorobenzonitrile was prepared in the same manner as described for **27** using 3-chloro-4-fluorobenzonitrile instead of 3,4-difluorobenzonitrile in 75% yield as a pale orange solid. To a hot (100 °C) solution of 4-(2-aminophenoxy)-3-chlorobenzonitrile (5.0 g, 20.5 mmol) in DMF (25 mL) was added portion wise NaH (55% dispersion in mineral oil; 938 mg, 21.5 mmol) and the mixture was stirred at 100 °C for 2 h. After cooling, water was added to the mixture and the aqueous layer was extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4. After evaporation in vacuo, the resultant residue was purified by silica gel column chromatography (EtOAc–hexane) to give the title compound **30** (3.52 g, 70%) as a beige solid.

^{1}H NMR (DMSO-d_6) δ: 6.58 (1H, d, J = 8.8 Hz), 6.85 (1H, td, J = 1.0, 7.3 Hz), 6.96 (1H, dd, J = 1.5, 7.8 Hz), 7.10 (1H, td, J = 1.5, 7.3 Hz), 7.18 (1H, dd, J = 2.0, 7.8 Hz), 7.48 (1H, dd, J = 2.0, 8.8 Hz), 7.74 (1H, s), 7.85 (1H, d, J = 2.0 Hz), 9.72 (1H, br s). MS (FAB) m/z: 243 (M^+−H).

5.1.13. 10*H*-Phenoxazine-3-carbonitrile (31)

A solution of **30** (3.0 g, 12.3 mmol), K_2CO_3 (5.1 g, 36.9 mmol) in DMF (50 mL) was stirred at 150 °C for 4 h. After cooling, water was added and the precipitate was collected by filtration, washed with water, dried in vacuo at 50 °C. The resultant residue was purified by silica gel column chromatography (EtOAc–hexane) to give the title compound **31** (1.49 g, 58%) as a yellow solid.

^{1}H NMR (DMSO-d_6) δ: 6.48 (1H, d, J = 8.3 Hz), 6.60–6.66 (2H, m), 6.73–6.78 (1H, m), 6.97 (1H, d, J = 2.0 Hz), 7.16 (1H, dd, J = 1.9, 8.3 Hz), 8.90 (1H, s). MS (FAB) m/z: 207 (M^+−H).

5.1.14. 10*H*-Phenoxazine-3-carboxylic acid (32)

The title compound was prepared in the same manner as described for **29** using **31** instead of **28** in 14% yield.

^{1}H NMR (DMSO-d_6) δ: 6.45–6.49 (2H, m), 6.60–6.64 (2H, m), 6.72–6.76 (1H, m), 7.02 (1H, d, J = 1.9 Hz), 7.34 (1H, dd, J = 1.5, 8.3 Hz), 8.73 (1H, s), 12.43 (1H, s). MS (FAB) m/z: 226 (M^+−H).

5.1.15. *N*-(Diaminomethylene)-10*H*-phenoxazine-3-carboxamide monohydrochloride (8)

The title compound was prepared in the same manner as described for **4** using **32** instead of **15** in 77% yield.

^{1}H NMR (DMSO-d_6) δ: 6.51–6.54 (2H, m), 6.64–6.69 (2H, m), 6.75–6.79 (1H, m), 7.29 (1H, d, J = 1.9 Hz), 7.59 (1H, dd, J = 2.0, 8.3 Hz), 8.35 (2H, br s), 8.57 (2H, br s), 9.06 (1H, s), 11.43 (1H, s). MS (FAB) m/z: 269 (M^++H) Mp: 286–293 °C Anal. Calcd for $C_{14}H_{12}N_4O_2$·HCl·0.4H_2O: C, 53.90; H, 4.46; N, 17.96; Cl, 11.37. Found: C, 53.92; H, 4.48; N, 17.86; Cl, 11.44.

5.1.16. *N*-(Diaminomethylene)-9-oxo-9*H*-fluorene-2-carboxamide (9)

The title compound was prepared in the same manner as described for **4** using 9-oxo-9*H*-fluorene-2-carboxylic acid (**18**) instead of **15** in 58% yield.

^{1}H NMR (DMSO-d_6) δ: 7.42 (1H, t, J = 8.3 Hz), 7.62–7.67 (2H, m), 7.80–7.85 (2H, m), 8.28 (1H, dd, J = 1.4, 7.8 Hz), 8.31 (1H, s). MS (FAB) m/z: 266 (M^++H). Anal. Calcd for $C_{15}H_{11}N_3O_2$·0.1H_2O: C, 67.46; H, 4.23; N, 15.73. Found: C, 67.38; H, 4.17; N, 15.77.

5.1.17. *N*-(9-Hydroxy-9*H*-fluorene-2-carbonyl)guanidine monohydrochloride (10)

To a solution of **9** (400 mg, 1.51 mmol) in MeOH (10 mL) was added sodium borohydride ($NaBH_4$) (110 mg, 3.02 mmol) and the mixture was stirred at room temperature for 1 h. Reaction was quenched by addition of water, and the solution was evaporated in vacuo. To the resultant residue was added $CHCl_3$ and 1 M NaOH aq and the precipitate was collected by filtration to give *N*-(9-hydroxy-9*H*-fluorene-2-carbonyl)guanidine. The compound was converted to its monohydrochloride salt by treating it with 4 M HCl–EtOAc (0.2 mL, 0.8 mmol) in EtOH (30 mL). The crude salt was suspended with EtOH and filtered to give the title compound **10** (380 mg, 83%) as a colorless solid.

^{1}H NMR (DMSO-d_6) δ: 5.59 (1H, s), 6.07 (1H, br s), 7.40–7.49 (2H, m), 7.66 (1H, dd, J = 1.5, 8.3 Hz), 7.93–7.95 (1H, m), 8.01 (1H, d, J = 8.8 Hz), 8.23–8.28 (2H, m), 8.55 (2H, br s), 8.70 (2H, br s), 11.87 (1H, s). MS (FAB) m/z: 268 (M^++H) Mp: 236–238 °C Anal. Calcd for $C_{15}H_{13}N_3O_2$·HCl·H_2O: C, 55.99; H, 5.01; N, 13.06; Cl, 11.02. Found: C, 55.65; H, 4.87; N, 12.99; Cl, 11.30.

5.1.18. *N*-(9*H*-Fluorene-1-carbonyl)guanidine monohydrochloride (11)

The title compound was prepared in the same manner as described for **4** using 9*H*-fluorene-1-carboxylic acid (**16**) instead of **15** in 51% yield.

^{1}H NMR (DMSO-d_6) δ: 4.26 (2H, s), 7.37–7.46 (2H, m), 7.62–7.68 (2H, m), 8.00 (1H, d, J = 6.8 Hz), 8.03 (1H, d, J = 7.8 Hz), 8.25 (1H, d, J = 7.8 Hz), 8.54 (2H, br s), 8.67 (2H, br s), 11.87 (1H, s). MS (ESI) m/z: 252 (M^++H). Mp: 261–263 °C Anal. Calcd for $C_{15}H_{11}N_3O_2$·HCl: C, 62.61; H, 4.90; N, 14.60; Cl, 12.32. Found: C, 62.59; H, 4.85; N, 14.60; Cl, 12.36.

5.1.19. 9-Oxo-9*H*-fluorene-3-carboxylic acid (19)

The title compound was prepared according to a literature protocol.[17]

^{1}H NMR (DMSO-d_6) δ: 7.42 (1H, t, J = 7.6 Hz), 7.64–7.67 (2H, m), 7.70 (1H, d, J = 7.6 Hz), 7.94 (1H, dd, J = 2.4, 7.6 Hz), 8.29 (1H, s), 13.43 (1H, br s). MS (FAB) m/z: 225 (M^++H).

5.1.20. *N*-(9-Oxo-9*H*-fluorene-3-carbonyl) guanidine monohydrochloride (20)

The title compound was prepared in the same manner as described for **4** using **19** instead of **15** in 97% yield.

^{1}H NMR (DMSO-d_6) δ: 7.47 (1H, t, J = 7.4 Hz), 7.68–7.73 (2H, m), 7.80 (1H, d, J = 7.6 Hz), 7.91 (1H, d, J = 7.6 Hz), 8.06 (1H, d, J = 7.6 Hz), 8.57 (2H, br s), 8.67 (1H, s), 8.72 (2H, br s), 12.22 (1H, s). MS (FAB) m/z: 266 (M^++H). Anal. Calcd for $C_{15}H_{11}N_3O_2$·HCl: C, 59.31; H, 4.65; N, 13.83; Cl, 11.67. Found: C, 58.95; H, 4.59; N, 13.54; Cl, 11.32.

5.1.21. *N*-(9-Hydroxy-9*H*-fluorene-3-carbonyl)guanidine monohydrochloride (12)

The title compound was prepared in the same manner as described for **10** using **20** instead of **9** in 78% yield.

^{1}H NMR (DMSO-d_6) δ: 5.59 (1H, s), 6.06 (1H, br s), 7.39 (1H, t, J = 7.4 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.63 (1H, d, J = 8.0 Hz), 7.78 (1H, d, J = 8.0 Hz), 7.90 (1H, d, J = 7.2 Hz), 8.07 (1H, dd, J = 1.2, 8.0 Hz), 8.55 (2H, br s), 8.74 (1H, s), 8.82 (2H, br s), 12.14 (1H, s). MS (FAB) m/z: 268 (M^++H). Anal. Calcd for $C_{15}H_{13}N_3O_2$·HCl: C, 59.31, H, 4.65; N, 13.83; Cl, 11.67. Found: C, 59.15; H, 4.69; N, 13.66; Cl, 11.62.

5.1.22. *N*-(9*H*-Fluorene-4-carbonyl)guanidine monohydrochloride (13)

The title compound was prepared in the same manner as described for **4** using 9*H*-fluorene-4-carboxylic acid (**17**) instead of **15** in 3% yield.

^{1}H NMR (DMSO-d_6) δ: 4.01 (2H, s), 7.36–7.44(2H, m), 7.47 (1H, t, J = 7.6 Hz), 7.64–7.66 (1H, m), 7.69 (1H, d, J = 7.6 Hz), 7.84 (1H, d, J = 7.2 Hz), 7.87–7.91 (1H, m), 8.57 (2H, br s), 8.74 (2H, br s), 12.18 (1H, s). MS (FAB) m/z: 252 (M^++H). Anal. Calcd for $C_{15}H_{13}N_3O$·HCl: C, 62.41; H, 4.90; N, 14.60; Cl, 12.32. Found: C, 62.44; H, 4.79; N, 14.53; Cl, 12.37.

5.1.23. 9-Oxo-9*H*-fluorene-2-carbaldehyde (34)

To a cold (0 °C) solution of ethyl 9-oxo-9*H*-fluorene-2-carboxylate (1.50 g, 5.95 mmol) in THF (15 mL) was added portion wise $LiAlH_4$ (451 mg, 11.9 mmol), and the mixture was warmed up to room temperature and stirred for 30 min at that temperature. Reaction was quenched with Na_2SO_4·10H_2O, NaCl and brine. The mixture was filtered and the residue was washed with THF. After evaporated in vacuo to give 2-(hydroxymethyl)-9*H*-fluoren-9-ol as a yellow solid, which was used for the next step without further purification. A solution of the alcohol compound obtained above, MnO_2 (12.8 g, 147 mmol) in $CHCl_3$ (130 mL) was stirred at room temperature overnight. Celite was added to the reaction mixture, then filtered and washed with $CHCl_3$. The filtrate was evaporated in vacuo and the resulting residue was purified by silica gel column chromatography ($CHCl_3$–hexane) to give the title compound **34** (871 mg, 70% in two steps) as a yellow solid.

^{1}H NMR (DMSO-d_6) δ: 7.50 (1H, t, J = 7.6 Hz), 7.68–7.72 (2H, m), 7.96 (1H, d, J = 7.6 Hz), 8.06 (2H, s), 8.18 (1H, dd, J = 1.4, 7.6 Hz), 10.04 (1H, s). MS (EI) m/z: 208 (M^++H).

5.1.24. (2*E*)-3-(9-Oxo-9*H*-fluoren-2-yl)acrylic acid (35)

A solution of **34** (871 mg, 4.18 mmol), methyl (triphenylphosphoranylidene)acetate (1.40 g, 4.18 mmol) in toluene (15 mL) was stirred at 90 °C for 5 h, then allowed to cool. The reaction mixture was concentrated in vacuo, the resultant residue was purified by silica gel column chromatography ($CHCl_3$–EtOAc–hexane) to give methyl (2*E*)-3-(9-oxo-9*H*-fluoren-2-yl)acrylate (931 mg, 84%) as a yellow solid. To a mixture of the ester compound obtained above in MeOH (5 mL) and THF (5 mL) was added 1 M NaOH aq (1.7 mL) and the mixture was stirred at room temperature overnight. The reaction mixture was acidified (pH 1.0) with 1 M HCl aq. The precipitate was collected by filtration, washed with water, dried in vacuo to give the title compound **35** (357 mg, 94%) as a yellow solid.

^{1}H NMR (DMSO-d_6) δ: 6.65 (1H, d, J = 16.1 Hz), 7.42 (1H, t, J = 7.3 Hz), 7.63–7.67 (3H, m), 7.86 (1H, d, J = 7.8 Hz), 7.93–7.96 (2H, m), 12.46 (1H, br s). MS (FAB) m/z: 250 (M^+−H).

5.1.25. (2*E*)-*N*-(Diaminomethylene)-3-(9-oxo-9*H*-fluoren-2-yl)acrylamide (36)

The title compound was prepared in the same manner as described for **4** using **35** instead of **15** in 78% yield.

^{1}H NMR (DMSO-d_6) δ: 6.60 (2H, d, J = 15.6 Hz), 7.39 (1H, t, J = 7.3 Hz), 7.45 (1H, s), 7.49 (1H, s), 7.61–7.65 (3H, m), 7.76 (1H, s), 7.82–7.84 (4H, m). MS (FAB) m/z: 292 (M^++H). Mp: 217–220 °C Anal. Calcd for $C_{17}H_{13}N_3O_2$·H_2O: C, 66.01; H, 4.89; N, 13.58. Found: C, 66.20; H, 4.92; N, 13.52.

5.1.26. (2*E*)-*N*-(Diaminomethylene)-3-(9-hydroxy-9*H*-fluoren-2-yl)acrylamide monohydrochloride (14)

The title compound was prepared in the same manner as described for **10** using **36** instead of **9** in 82% yield.

^{1}H NMR (DMSO-d_6) δ: 5.55 (1H, d, J = 5.9 Hz), 5.99 (1H, d, J = 7.3 Hz), 6.79 (1H, d, J = 15.6 Hz), 7.35–7.44 (2H, m), 7.62 (1H, d, J = 7.4 Hz), 7.72 (1H, d, J = 8.7 Hz), 7.85 (1H, d, J = 6.8 Hz), 7.88–7.95 (3H, m), 8.41 (4H, br s), 12.21 (1H, s). MS (FAB) m/z: 294 (M^++H) Mp: 280–283 °C Anal. Calcd for $C_{17}H_{15}N_3O_2$·HCl·0.1H_2O: C, 61.58; H, 4.92; N, 12.67; Cl, 10.69. Found: C, 61.40; H, 5.00; N, 12.41; Cl, 10.40.

5.2. Molecular modeling

5.2.1. Calculation of atomic charge distribution

This caluculation was performed using the 6,5,6-membered ring structures for the sake of shorthand. The structures of **4′**, **5′**, **6′** and **10′** were sketched in MOE.[23] The molecular conformations were optimized to the semiempirical AM1 level and electrostatic potential-fitted atomic partial charges were calculated using MOPAC 7.1 module in MOE. Analytic Connolly surfaces[24] that surrounded the van der Waals surfaces of the core scaffold are generated and colored with red for negative charge and blue for positive charge, respectively, using the program MOE.

5.3. Pharmacology

5.3.1. Binding assay of 5-HT$_{2B}$ receptor

5.3.1.1. Membrane preparation. A human 5-HT$_{2B}$ receptor expressing cell was prepared in accordance with a reference.[25] HEK293-EBNA cell was used as the gene transferring cell. Cultured HEK293-EBNA cells expressing human 5-HT$_{2B}$ receptor were washed with PBS(−). The cells were scraped in the presence of PBS(−), and the cells were recovered by centrifugation (1000 rpm, 10 min, 4 °C). They were homogenized using Polytron (PTA 10-TS) in the presence of 5 mM Tris-HCl (pH 7.4) buffer and centrifuged (40,000 xg. 10 min, 4 °C). They were suspended using a homogenizer in the presence of 50 mM Tris–HCl (pH 7.4) buffer. They were subjected to centrifugation (40,000 xg, 10 min, 4 °C), suspended in 50 mM Tris–HCl (pH 7.4) and stored at −80 °C.

5.3.1.2. Receptor binding assay. A total volume of 500 μL containing 50 mM Tris–HCl–4 mM $CaCl_2$ (pH 7.4) buffer, the human 5-HT$_{2B}$ receptor expressing HEK293-EBNA cell membrane preparation and a radio ligand [^{3}H] Mesulergine (3.1 TBq/mmol) was incubated at 25 °C for 1 h. The compound was dissolved in 100% DMSO and diluted to respective concentrations. Nonspecific binding was defined as the binding quantity in the presence of 1 μM ritanserin, and the result of subtracting the nonspecific binding quantity from the total binding quantity was defined as the specific binding quantity. This was mixed with 4 mL of 50 mM Tris–HCl buffer (pH 7.4) and filtered under a reduced pressure using a GF/B glass filter, and the filter was washed (4 mL × 3) with the same buffer. The glass filter was soaked in 5 mL of a liquid scintillator (Aquasol-2) and the radioactivity was measured using a liquid scintillation counter. Concentration of the compound which inhibits 50% of the receptor binding, IC_{50} value, was obtained by nonlinear regression analysis using SAS (ver. 6.11), and the K_i value which represents its affinity for the receptor was calculated using the formula of Cheng & Prussoff;[26] $K_i = IC_{50}/(1 + [L]/[K_d])$ ([L]: ligand concentration, [K_d]: dissociation constant).

5.3.2. Binding assay of 5-HT$_7$ receptor

5.3.2.1. Membrane preparation. A human 5-HT$_7$ receptor expressing cell was prepared in accordance with references.[27] CHO cell was used as the gene transferring cell.

Cultured CHO cells expressing human 5-HT$_7$ receptor were washed with PBS(−). The cells were scraped in the presence of PBS(−), and the cells were recovered by centrifugation (1000 rpm, 10 min, 4 °C). They were homogenized using Polytron (PTA 10-TS) in the presence of 5 mM Tris–HCl (pH 7.4) buffer and centrifuged (40,000 xg 10 min, 4 °C). They were suspended using a homogenizer in the presence of 50 mM Tris–HCl (pH 7.4)

buffer. They were subjected to centrifugation (40,000 xg 10 min, 4 °C), suspended in 50 mM Tris–HCl (pH 7.4) and stored at −80 °C.

5.3.2.2. Receptor binding assay. A total volume of 500 μL containing 50 mM Tris–HCl–4 mM $CaCl_2$ (pH 7.4) buffer, the human 5-HT_7 receptor expressing CHO cell membrane preparation and a radio ligand [^{3}H]5-HT (3.40 TBq/mmol) was incubated at 25 °C for 1 h. The compound was dissolved in 100% DMSO and diluted to respective concentrations. Nonspecific binding was defined as the binding quantity in the presence of 10 μM metergoline, and the result of subtracting the nonspecific binding quantity from the total binding quantity was defined as the specific binding quantity. This was mixed with 4 mL of 50 mM Tris–HCl buffer (pH 7.4) and filtered under at educed pressure using a GF/B glass filter, and the filter was washed (4 mL × 3) with the same buffer. The glass filter was soaked in 5 mL of a liquid scintillator (Aquasol-2) and the radioactivity was measured using a liquid scintillation counter. Concentration of the compound which inhibits 50% of the receptor binding, IC_{50} value, was obtained by nonlinear regression analysis using SAS (ver. 6.11), and the K_i value which represents its affinity for the receptor was calculated using the formula of Cheng & Prussoff;[26] $K_i = IC_{50}/(1 + [L]/[K_d])$ ([L]: ligand concentration, [K_d]:dissociation constant).

5.3.3. Binding test of 5-HT_{2A}, 5-HT_{2C}, α_1, M_1 and D_2 receptors

Affinity of the compounds for 5-HT_{2A}, 5-HT_{2C}, α_1, M_1 and D_2 receptors were verified using conventionally known techniques.[28–31]

5.3.4. PI metabolism assay

HEK293-EBNA cells expressing human $5HT_{2B}$ receptors were seeded on collagen I-coated 24-well plates at the density of 1 × 10^5 cells/well and cultured overnight at CO_2 incubator (37 °C, 5% CO_2). At the next day, wells were labeled with [^{3}H]-myo-inositol (3 μCi/mL) and cultured for overnight. At the third day, labeled cells were washed with PBS (137 mM NaCl, 2.68 mM KCl, 1.05 mM $MgCl_2$, 0.9 mM $CaCl_2$, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4) and incubated for 20 min at 37 °C. After incubation, cells were further incubated with test compounds (0.3–10,000 nM, geometric ratio: approximately 3) in PBS including 10 mM LiCl (PBS-Li) for 20 min at 37 °C. After incubation, reaction was started by exchanging to 5-HT and test compound including PBS-Li. After incubation (20 min at 37 °C), Reaction was terminated by exchanging reaction mixture to 1 mL of ice-cold 0.2 M perchlolic acid. After standing (1–3 h at 4 °C), 100 μL of 2 N KOH and 50 μL of 100 mM EDTA-Na were added into each well and mix. One mL of reaction extract was added to AG1 8 columns (BIO-RAD, CA, USA), and washed with 7 mL of wash solution (5 mM sodium tetraborate, 60 mM sodium formate). IPs were extracted by addition of 4 mL of elution solution (0.1 M formate, 1 M ammonium formate), and whole elute were collected into liquid scintillation vials. Radioactivity of the elute were counted using liquid scintillation counter. IC_{50} values against 5-HT-induced IPs accumulation were calculated from the non-linear regression analysis using SAS ver.8.2. Final values were represented as the means of three separate experiments.

5.3.5. cAMP production assay

CHO cells expressing human $5HT_7$ receptors were seeded at the density of 5 × 10^4 cells/well on 96-well plates and cultured overnight in a CO_2 incubator (37 °C, 5% CO_2). At the next day, wells were washed twice with PBS-IBMX-ascorbate (137 mM NaCl, 2.68 mM KCl, 1 mM $MgCl_2$, 0.85 mM $CaCl_2$, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 1 mM isobutylmethylxantine and 1 mM L-(+)-ascorbic acid) and incubated for 20 min at 37 °C. After incubation, reaction was started by exchanging to PBS-IBMX-ascorbate including agonists and antagonists (1–10,000 nM, geometric ratio: approximately 3). After incubation (10 min at 37 °C), reaction was terminated by exchanging the reaction mixture to 50 μL of ice-cold 0.1 M HCl. After standing for 1 h at 4 °C, 5 μL of 1 M NaOH was added into wells and mix. Cyclic AMP level was determined using Biotrak cAMP EIA System. The IC_{50} and maximal inhibition (I_{max}) values were calculated from the non-linear regression analysis using SAS ver.8.2. Final values were represented as the means of three separate experiments.

5.3.6. Suppressing effect on 5-HT-induced dural plasma protein extravasation in guinea pigs

In this test system, suppressing effect was evaluated by measuring leaked protein in the presence of a compound to be tested, and this was carried out by partially modifying the method reported by Rachel et al.[32]

The male Hartley guinea pigs (250–350 g) were anesthetized by peritoneal administration (ip) of urethane (1.5 g/kg). By applying simple canulation to femoral vein, 50 mg/kg of a fluorescent protein (FITC-BSA) was intravenously administered (iv) and 5 min thereafter, physiological saline or 1 μM of 5-HT was intravenously administered. By carrying out perfusion with physiological saline 15 min thereafter, blood was washed out.

Compound **2**, **3** and **10** were intraperitoneally administered, or compound **10** was orally administered, 30 min before administration of the fluorescent protein. By detaching the skull, dura mater was took out and incubated at 37 °C for 16 h in a tube in the presence of physiological saline of pH 11. Centrifugation was carried out, and the supernatant was dispensed into a plate. Fluorescence intensity was measured using an fluorescence plate reader (excitation wavelength 485 nm, absorption wavelength 530 nm). By measuring dura mater weight, fluorescence intensity per mg dural protein was calculated.

5.3.7. Aqueous solubility

To 13 μL of a 10 mM DMSO solution of a test compound that had been prepared in advance was added exactly 1 mL of a second liquid (pH 6.8) for a disintegration test of Japanese Pharmacopoeia, followed by shaking at 25 °C for 20 h, thereby giving a sample stock solution. Next, using a filter impregnated with 200 μL of the sample stock solution, 200 μL of a fresh sample stock solution was added for filtration to obtain a liquid, which was taken as a sample solution. Separately to this, to 10 μL of the 10 mM DMSO solution of the test compound was added accurately 1 mL of methanol, followed by stirring, thereby giving a standard solution. 10 μL portions each of the sample solution and standard solution were tested by liquid chromatography, and the ratio of the peak area of the sample solution to the peak area of the standard solution was determined, thereby calculating the solubility.

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References and notes

1. (a) Moskowitz, M. A. *Ann. Neurol.* **1984**, *16*, 157; (b) Hirose, G. *Nihon Naika Gakkai Zasshi* **2001**, *90*, 595.
2. (a) Stephen, D. S. *Headache* **1994**, *34*, 408; (b) Mehrotra, S.; Gupta, S.; Chan, K. Y.; Villalón, C. M.; Centurión, D.; Saxena, P. R.; MaassenVanDenBrink, A. *Naunyn Schmiedebergs Arch. Pharmacol.* **2008**, *378*, 371.
3. Johnson, K. W.; Phebus, L. A.; Cohen, M. L.; Jucker, E. *Prog. Drug Res.* **1998**, *51*, 219.

4. Mylecharane, E. J. *J. Neurol.* **1991**, *238*, S45.
5. (a) Arnt, J.; Hyttel, J.; Larsen, J. J. *Acta Pharm. Toxicol.* **1984**, *55*, 363; (b) Leysen, J. E.; Awouters, F.; Kennis, L.; Laduron, P. M.; Vandenberk, J.; Janssen, P. A. J. *Life Sci.* **1981**, *28*, 1015; (c) Newman-Tancredi, A.; Conte, C.; Chaput, C.; Verriele, L.; Audinot-Bouchez, V.; Lochon, S.; Lavielle, G.; Millan, M. J. *Naunyn Schmiedebergs Arch. Pharmacol.* **1997**, *355*, 682.
6. Johnson, K. W.; Nelson, D. L.; Dieckman, D. K.; Wainscott, D. B.; Lucaites, V. L.; Audia, J. E.; Owton, W. M.; Phebus, L. A. *Cephalalgia* **2003**, *23*, 117.
7. (a) Manivet, P.; Mouillet-Richard, S.; Callebert, J.; Nebigil, C. G.; Maroteaux, L.; Hosoda, S.; Kellermann, O.; Launay, J.-M. *J. Biol. Chem.* **2000**, *275*, 9324; (b) Wei, E. P.; Moskowitz, M. A.; Boccalini, P.; Kontos, H. A. *Circ. Res.* **1992**, *70*, 1313.
8. Bonhaus, D. W.; Flippin, L. A.; Greenhouse, R. J.; Jaime, S.; Rocha, C.; Dawson, M.; Natta, K. V.; Chang, L. K.; Pulido-Rios, T.; Webber, A.; Leung, E.; Eglen, R. M.; Martin, G. R. *Br. J. Pharmacol.* **1999**, *127*, 1075.
9. Poissonnet, G.; Parmentier, J. G.; Boutin, J. A.; Goldstein, S. Mini Rev *Mini-Rev. Med. Chem.* **2004**, *4*, 325.
10. Terrón, J. A.; Bouchelet, I.; Hamel, E. *Neurosci. Lett.* **2001**, *302*, 9.
11. Terrón, J. A. *Eur. J. Pharmacol.* **2002**, *439*, 1.
12. Pierce, P. A.; Xie, G.-X.; Peroutka, S. J.; Levine, J. D. *Region. Anesth.* **1996**, *21*, 219.
13. Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. M.; Forbes, I. T.; Jennings, A. J.; Kings, F. D.; Middlemiss, D. N.; Rahman, S. K.; Saunder, D. V.; Collin, L. L.; Hagan, J. J.; Riley, G. J.; Thomans, D. R. *J. Med. Chem.* **2000**, *43*, 342.
14. Wang, X.; Fang, Y.; Liang, J.; Yin, Z.; Mian, J.; Luo, N. *Headache* **2010**, *50*, 579.
15. Ullmer, C.; Schmuck, K.; Kalkman, H. O.; Lübbert, H. *FEBS Lett.* **1995**, *370*, 215.
16. (a) Akuzawa, S.; Watanabe, T. WO 2,005,079,845.; (b) Yamada, H.; Itahana, H.; Moritomo, A.; Matsuzawa, T.; Harada, K.; Fujiyasu, J.; Koga, Y.; Oku, M.; Okazaki, T.; Akuzawa, S. WO 2,005,080,322.
17. (a) Weisburger, J. H. *J. Org. Chem.* **1958**, *23*, 1193; (b) Koshel, G. N.; Magagnini, P.; Koshel, S. G.; Lebedeva, N. V.; Postnova, M. V.; Kulichikhin, V. G.; Bilibin, A. Y. *Russ. J. Org. Chem.* **2001**, *37*, 830.
18. Holzapfel, C. W.; Dwyer, C. *Heterocycles* **1998**, *48*, 1513.
19. Eastmond, G. C.; Glichrist, T. L.; Paprotny, J.; Steiner, A. *New J. Chem.* **2001**, *25*, 385.
20. (a) Hansch, C.; Quinlan, J. E.; Lawrence, G. L. *J. Org. Chem.* **1968**, *33*, 347; (b) Valvani, S. C.; Yalkowsky, S. H.; Roseman, T. J. *J. Pharm. Sci.* **1981**, *70*, 502.
21. Ali, J.; Camilleri, P.; Brown, M. B.; Hutt, A. J.; Kirton, S. B. *J. Chem. Inf. Model.* **2012**, *52*, 2950.
22. ACD/Labs version 12.01; Advanced Chemistry Development: Toronto, Canada.
23. Molecular Operating Environment (MOE), 2011.10; Chemical Computing Group: 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2011.
24. Connolly, M. L. *J. Appl. Crystallogr.* **1983**, *16*, 548.
25. Schmuck, K.; Ullmer, C.; Engels, P.; Lübbert, H. *FEBS Lett.* **1994**, *342*, 85.
26. Cheng, Y. C.; Prussoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
27. (a) Bard, J. A.; Zgombick, J.; Adham, N.; Vaysse, P.; Branchek, T. A.; Weinshank, R. L. *J. Biol. Chem.* **1993**, *268*, 23422; (b) Jasper, J. R.; Kosaka, A.; To, Z. P.; Chang, D. J.; Eglen, R. M. *Br. J. Pharmacol.* **1997**, *122*, 126.
28. Leysen, J. E.; Niemegeers, C. J. E.; Nueten, J. M. V.; Laduron, P. M. *Mol. Pharmacol.* **1982**, *21*, 301.
29. Michel, M. C.; Brodde, O.-E.; Schnepel, B.; Behrendt, J.; Tschada, R.; Motulsky, H. J.; Insel, P. A. *Mol. Pharmacol.* **1989**, *35*, 324.
30. Potter, L. T.; Ferrendelli, C. A.; Hanchett, H. E. *Cell. Mol. Neurol.* **1988**, *8*, 181.
31. Terai, M.; Hidaka, K.; Nakamura, Y. *Eur. J. Pharmacol.* **1989**, *173*, 177.
32. Spokes, R. A.; Middlefell, V. C. *Eur. J. Phamacol.* **1995**, *281*, 75.