Synthesis and Pharmacological Characterization of Novel 6-Fluorochroman Derivatives as Potential 5-HT_{1A} Receptor Antagonists

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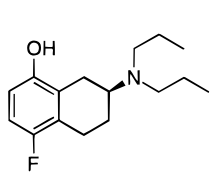
A series of novel 6-fluorochroman derivatives was prepared and evaluated as antagonists for the 5-HT_{1A} receptor. *N*-2-[[(6-Fluorochroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamine (**3**; *J. Med. Chem.* **1997**, *40*, 1252−1257) was chosen as a lead, and structural modifications were done on the aliphatic portion of the chroman ring, the tether linking the middle amine and the terminal aromatic ring, the aromatic ring, and lastly the amine. Radioligand binding assays proved that the majority of the novel compounds behaved as good to excellent ligands at the 5-HT_{1A} receptor, some of which were selective with respect to α_1-adrenergic and D_2-dopaminergic receptors. The antagonist activity of the compounds was assessed in the forskolin-stimulated adenylate cyclase assays in CHO cells expressing the human 5-HT_{1A} receptors. Among the modifications attempted, introduction of an oxo or an optically active hydroxy moiety at the chroman C-4 position was effective in ameliorating the receptor selectivity. Six analogues were selected through the in vitro screens and further evaluated for their in vivo activities. A 4-oxochroman derivative (**31n**), having a terminal 1,3-benzodioxole ring, demonstrated antagonist activities toward 8-OH-DPAT-induced behavioral and electrophysiological responses in rats.

Introduction

Selective antagonists for the 5-HT_{1A} receptor have been hypothesized to have potential in the treatment of various psychiatric disorders including anxiety, dementia, schizophrenia, etc.[1] More recently, it was proposed that the combination of a 5-HT_{1A} receptor antagonist and a selective serotonin reuptake inhibitor (SSRI) might facilitate the onset of the SSRI's antidepressant action.[2] However, considerable research efforts over years[3−5] have afforded only a few compounds which act as selective antagonists at 5-HT_{1A} receptors, for example, (*S*)-UH-301 (**1**)[6,7] and WAY-100635 (**2**).[8,9] The efforts have also revealed that most of the previously described "5-HT_{1A} receptor antagonists" are not true antagonists but (partial) agonists, in terms of their activities at the somatodendritic (presynaptic) 5-HT_{1A} autoreceptors which are located in the dorsal raphe nucleus.[3,10,11] The term "silent antagonist" has thus been employed to distinguish a true antagonist (which is devoid of any agonist activity) from partial agonists.[12] Moreover, even a slight modification of such selective antagonists often results in substantial reduction in their affinity, selectivity, or potency of antagonism for the 5-HT_{1A} receptor.[13−15] These factors have hampered the development of selective 5-HT_{1A} receptor antagonists, and no drugs of this class have been approved to date for clinical use.

A series of chroman derivatives was recently disclosed by us which show potent affinity and a wide range of antagonism for the 5-HT_{1A} receptor.[16] In particular, a 6-fluorochroman derivative (**3**) was shown to have an extremely potent affinity (K_i = 0.22 nM) and in vitro antagonism, though its selectivity for 5-HT_{1A} vs α_1-adrenergic and D_2-dopaminergic receptors was not satisfactory. Preliminary studies in our laboratory showed that **3** could act as a functional antagonist at both α_1 and D_2 receptors.[17] This discouraged us from further developing this compound, since blockade of these receptors might cause undesirable side effects such as orthostatic hypotension (α_1), prolactin stimulation (D_2), extrapyramidal symptoms (D_2), etc.[18,19] In addition, α_1 receptor blockade could oppose autoreceptor-mediated effects of 5-HT_{1A} antagonists.[20] Therefore, **3** was chosen as a lead for our research program on 5-HT_{1A} receptor antagonists, which addressed improvement of receptor selectivity. Another aim of the study was to elucidate the structure−activity relationships for the analogues of **3**. In the case of intrinsic ligands for these receptors (i.e., serotonin, epinephrine, and dopamine), the receptor recognition and differentiation should largely depend on their chemical features around the



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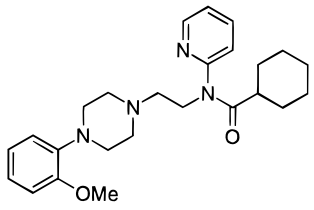

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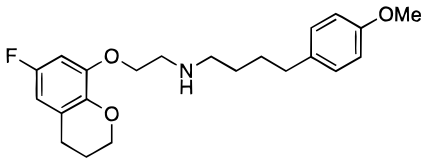

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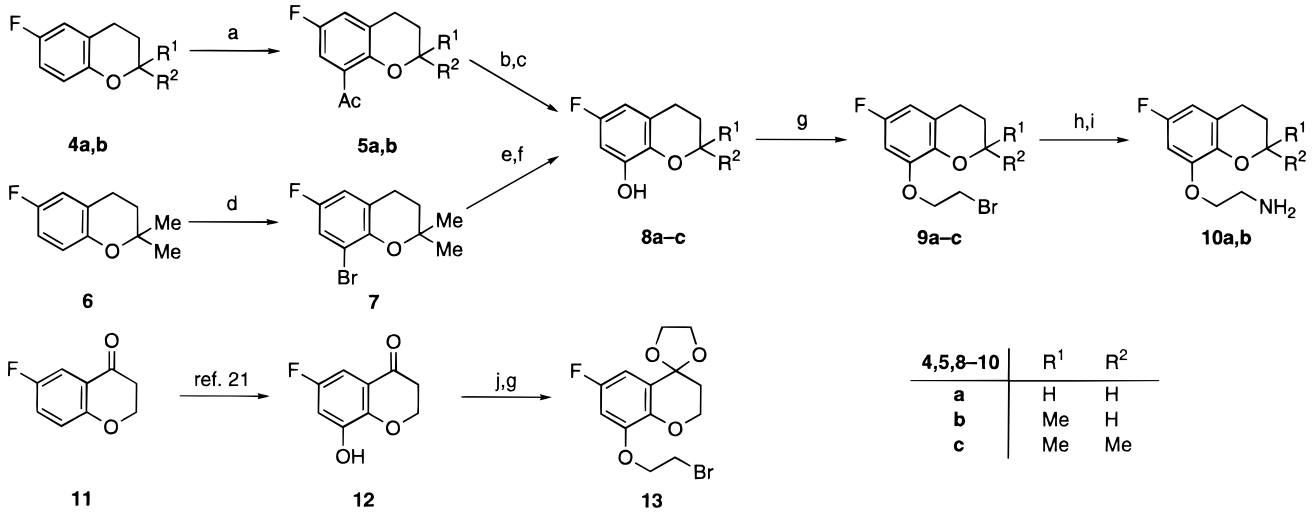
Scheme 1[a]



[a] Conditions: (a) AcCl, $AlCl_3$, DCE, 0 °C; (b) *m*-CPBA, TFA, CH_2Cl_2; (c) aqueous NaOH, MeOH; (d) $PhCH_2NMe_3{\cdot}Br_3$, $CaCO_3$, MeOH−CH_2Cl_2, reflux; (e) *n*-BuLi, THF, −70 °C, then $(n\text{-}BuO)_3B$, rt; (f) 30% aqueous H_2O_2, NaOH; (g) $BrCH_2CH_2Br$, aqueous NaOH, $n\text{-}Bu_4NHSO_4$, 70 °C; (h) $PhCH_2NH_2$ (excess), MeCN, reflux; (i) HCOOH, Pd−C, MeOH; (j) ethylene glycol, $CH(OEt)_3$, *p*-TsOH, benzene, azeotropic.

aromatic nuclei (the type of ring system, the number and the position of hydroxy moieties, etc.). Thus our working hypothesis is that the chroman ring of **3** mimics the indole ring of serotonin and that a structural modification around the chroman ring may change the receptor selectivity. However, as shown in the previous report, a fluoro moiety at the chroman C-6 position is essential for the binding and potent antagonism,[16] and hence the aromatic portion of the chroman ring does not seem amenable to modifications.

Accordingly, we made several attempts to modify the aliphatic portion of the chroman ring and examined the analogues for their binding affinities for 5-HT_{1A}, α_1, and D_2 receptors. Subsequently, we modified and optimized the tether linking the middle amine and the terminal aromatic ring, substitutions on the aromatic ring, and lastly the amine. All compounds throughout the series were also evaluated for their ability to antagonize the 8-OH-DPAT-induced suppression of the forskolin-stimulated adenylate cyclase (FSC) in Chinese hamster ovary (CHO) cells expressing the human 5-HT_{1A} receptors. Several compounds selected through the in vitro screens were studied for their in vivo activity toward 5-HT_{1A} receptor-mediated behavioral responses in rats. Furthermore, compound **31n**, a 6-fluoro-4-oxochroman derivative, was examined for its antagonism for the 8-OH-DPAT-induced suppression of 5-HT_{1A} neuronal cell firing at the rat dorsal raphe nucleus. Thus the synthesis, structure−activity relationships, and further pharmacological characterization of the novel analogues of **3** are described herein.

Chemistry

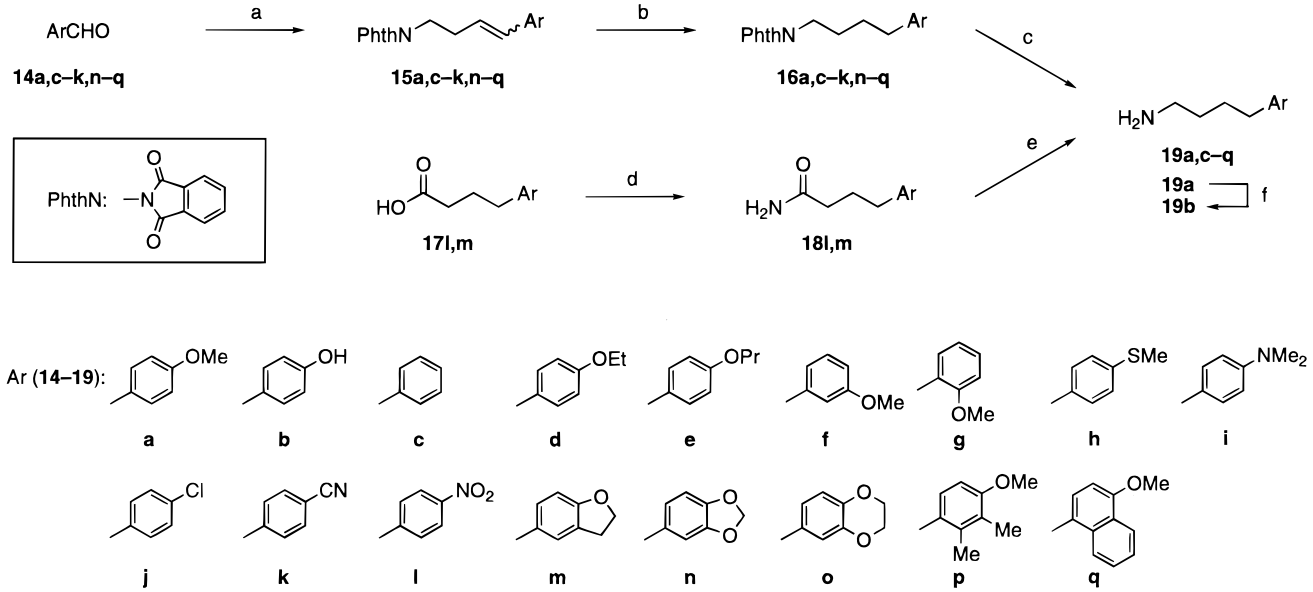
The basic structure of the target compounds was constructed by coupling of a chroman-containing component and an ω-arylalkyl component, for which preparations are illustrated in Schemes 1 and 2, respectively. For the chroman-containing component, intermediates **8a**,**b** were prepared according to the method that we reported previously.[16] Compound **8c** was prepared by in situ oxidation of an arylboronic ester generated from the corresponding bromide (**7**), thereby avoiding decomposition of **6** which is labile under Friedel−Crafts conditions. The 8-hydroxychroman intermediates, **8a**−**c** and **12**,[21] were transformed into appropriate precursors for the coupling with their counterparts (Scheme 1). In regard to the arylalkyl component, 4-arylbutylamines (**19a**,**c**−**k**,**n**−**q**) were prepared from the corresponding aryl aldehydes through Wittig reaction, hydrogenation, and deprotection; alternatively, **19l**,**m** were prepared from arylbutyric acids (**17l**,**m**) via amides (Scheme 2). Compound **19b** was prepared from **19a** by demethylation under acidic conditions.

Scheme 3 summarizes the general methods to couple these components: method A consists of the formation of an amide and subsequent reduction; methods B and C feature the coupling of a bromide and an amine, with the latter including a deprotection process for the carbonyl group. The chemical yields and other data for the analytically pure materials obtained through these methods are listed in Table 1. The 4-oxochroman derivatives **31a** and **32** were further converted into several other compounds using conventional synthetic techniques (Scheme 4). An oxime (**33**) and alcohols (**34**, **37**) were obtained respectively by usual oximation and borohydride reduction. The alcohol **34** exclusively afforded the methyl ether **35** by treatment with concentrated HCl in MeOH, whereas it dehydrated under azeotropic conditions to give the 2*H*-chromene **36**. The racemate of **34** was separated into the enantiomers using a chiral HPLC technique. The preparation of the tertiary amine derivatives is illustrated in Scheme 5. Compound **39** was obtained by the coupling of **13** and the *N*-methyl precursor **38**. Compound **40** was obtained by the N-alkylation of **31n**.

Pharmacology

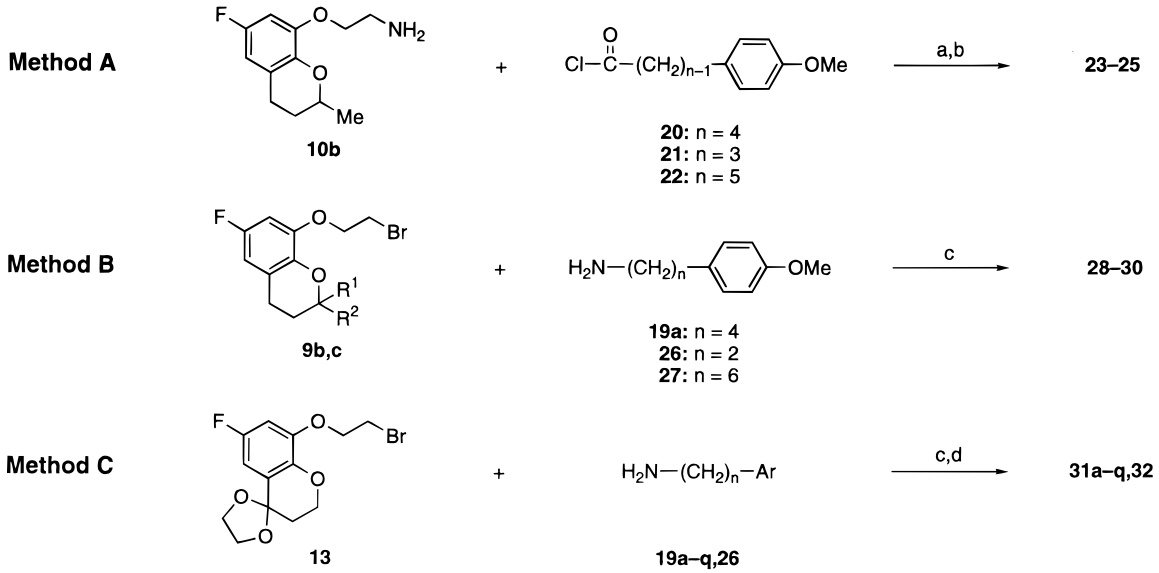
In Vitro Assays. Radioligand binding assays for 5-HT_{1A}, α_1, and D_2 receptors were performed according to the standard protocols using [^{3}H]-8-OH-DPAT on rat hippocampus,[22] [^{3}H]prazosin on rat cortex,[23] and [^{3}H]-spiperone on rat striatum,[24] respectively. The forskolin-stimulated adenylate cyclase (FSC) assays were performed with CHO cells expressing the human 5-HT_{1A}

Scheme 2[a]



[a] Conditions: (a) $C_6H_4(CO)_2N(CH_2)_3PPh_3^+Br^-$, NaOMe, 1,4-dioxane; (b) H_2, Pd−C, EtOH; (c) $MeNH_2$, MeOH, rt; (d) $(COCl)_2$, cat. DMF, CH_2Cl_2, then aqueous NH_3; (e) BH_3·SMe_2, THF, reflux; (f) conc HBr, reflux.

Scheme 3[a]



[a] Conditions: (a) Et_3N, CH_2Cl_2; (b) BH_3·SMe_2, THF, reflux; (c) K_2CO_3, MeCN, reflux; (d) aqueous HCl.

receptors. Preliminary one-point assessment was conducted using cell membranes of the CHO cells as described previously.[16] The antagonist activity was measured in the presence of 0.1 μM 8-OH-DPAT and expressed as percent of FSC recovery by 1 μM test compound (Tables 2 and 3). The full dose−response curves (both as agonist and as antagonist) were obtained using the whole cells instead of the cell membranes; compounds were examined for their effects on the FSC activity in the presence/absence of 0.3 μM 8-OH-DPAT (Figure 1).

Behavioral Responses. In vivo antagonist activity of selected compounds was examined in a behavioral response test in reserpinized rats. Administration of 5-HT$_{1A}$ agonists such as 8-OH-DPAT elicits characteristic behavioral responses in rats known as the serotonin syndrome.[25] These responses are believed to be caused by the stimulation of central postsynaptic 5-HT$_{1A}$ receptors.[26] Rats were pretreated with reserpine and then administered 8-OH-DPAT (0.25 mg/kg sc) to induce the responses. The magnitude of the typical responses, forepaw treading (FT) and flat body posture (FBP), were scored; compounds were evaluated for their ability to lower the score.

Dorsal Raphe Firing. To assess activity of compounds at the somatodendritic 5-HT$_{1A}$ autoreceptors, firing of the serotonergic neurons was recorded at the dorsal raphe nucleus in anesthetized rats. These receptors are inhibitory, and their activation attenuates the rate of the neuronal firing.[27,28] Compound **31n** was evaluated in this assay for its agonist and antagonist activities.

Results and Discussion

The novel compounds derived from **3** were primarily evaluated in vitro for their receptor binding and the FSC

Table 1. 6-Fluorochroman Derivatives Obtained by the General Methods

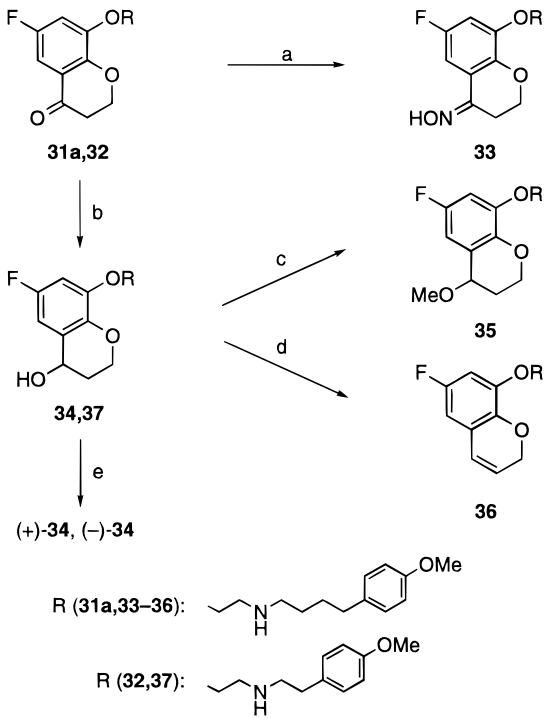
compd	materials[a]		method	formula[b]	solvent[c]	yield,%[d]	mp, °C	anal.
23	**10b**	**20**	A	$C_{23}H_{30}FNO_3 \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$	EE	73	104–106	C,H,N,F
24	**10b**	**21**	A	$C_{22}H_{28}FNO_3 \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$	E	61	124–127	C,H,N,F
25	**10b**	**22**	A	$C_{24}H_{32}FNO_3 \cdot 0.5C_4H_4O_4 \cdot H_2O$	EE	52	92–93	C,H,N,F
28	**9c**	**19a**	B	$C_{24}H_{32}FNO_3 \cdot 0.5C_4H_4O_4 \cdot H_2O$	E	60	98–102	C,H,N,F
29	**9b**	**26**	B	$C_{21}H_{26}FNO_3 \cdot HCl \cdot 0.25H_2O$	S	76	138–140	C,H,N,Cl,F
30	**9b**	**27**	B	$C_{25}H_{34}FNO_3 \cdot 0.5C_4H_4O_4 \cdot H_2O$	EN	71	72–73	C,H,N,F
31a	**13**	**19a**	C	$C_{22}H_{26}FNO_4 \cdot 0.5C_4H_4O_4$	A	71	137–139	C,H,N,F
31b	**13**	**19b**	C	$C_{21}H_{24}FNO_4 \cdot HCl$	T	21	123–125	C,H,N,Cl,F
31c	**13**	**19c**	C	$C_{21}H_{24}FNO_3 \cdot 0.5C_4H_4O_4$	IN	52	141–142	C,H,N,F
31d	**13**	**19d**	C	$C_{23}H_{28}FNO_4 \cdot 0.5C_4H_4O_4$	N	50	136–139	C,H,N,F
31e	**13**	**19e**	C	$C_{24}H_{30}FNO_4 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$	N	52	143–144	C,H,N,F
31f	**13**	**19f**	C	$C_{22}H_{26}FNO_4 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$	N	54	145–146	C,H,N,F
31g	**13**	**19g**	C	$C_{22}H_{26}FNO_4 \cdot C_4H_4O_4$	N	30	151–152	C,H,N,F
31h	**13**	**19h**	C	$C_{22}H_{26}FNO_3S \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$	N	37	145–146	C,H,N,F,S
31i	**13**	**19i**	C	$C_{23}H_{29}FN_2O_3 \cdot 2HCl$	N	25	172–173	C,H,N,Cl,F
31j	**13**	**19j**	C	$C_{21}H_{24}ClFNO_3 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$	N	71	142–143	C,H,N,Cl,F
31k	**13**	**19k**	C	$C_{22}H_{23}FN_2O_3 \cdot 0.5C_4H_4O_4$	N	45	169–170	C,H,N,F
31l	**13**	**19l**	C	$C_{21}H_{23}FN_2O_5 \cdot 0.5C_4H_4O_4$	I	23	140–141	C,H,N,F
31m	**13**	**19m**	C	$C_{23}H_{26}FNO_4 \cdot 0.5C_4H_4O_4$	N	63	148–149	C,H,N,F
31n	**13**	**19n**	C	$C_{22}H_{24}FNO_5 \cdot HCl$	N	74	154–155	C,H,N,Cl,F
31o	**13**	**19o**	C	$C_{23}H_{26}FNO_5 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$	N	67	187–188	C,H,N,F
31p	**13**	**19p**	C	$C_{24}H_{30}FNO_4 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$	N	69	139–140	C,H,N,F
31q	**13**	**19q**	C	$C_{26}H_{28}FNO_4 \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$	MN	30	157–158	C,H,N,F
32	**13**	**26**	C	$C_{20}H_{22}FNO_4 \cdot 0.5C_4H_4O_4$	N	81	177–178	C,H,N,F

[a] Starting materials used for the coupling. See text. [b] $C_4H_4O_4$ represents fumaric acid. [c] Recrystallization solvents: A, acetone; E, EtOH; EE, EtOH−Et_2O; EN, EtOH−MeCN; I, *i*-PrOH; IN, *i*-PrOH−MeCN; MN, MeOH−MeCN; N, MeCN; S, EtOAc; T, THF. [d] Yields refer to the analytically pure material based on the corresponding chroman-containing component (i.e., **9b**,**c**, **10b**, or **13**).

recovery (Tables 2 and 3). First, modifications of the chroman ring were attempted. Mono- and dimethylation at the chroman C-2 position in **23** and **28** had little effect on affinities for 5-HT_{1A}, α_1, and D_2 receptors but obviously decreased the FSC recovery. Introduction of an oxo moiety at the chroman C-4 position in **31a** led to a marked reduction in affinities for α_1 and D_2 receptors (respectively 75- and 250-fold, relative to **3**) while retaining a fair affinity (14-fold reduction) for the 5-HT_{1A} receptor. Thus **31a** has 66- and 20-fold selectivity for 5-HT_{1A} vs α_1 and D_2 receptors, respectively. A racemate of the 4-hydroxychroman derivative (**34**) appeared to have selectivity similar to that of **3**. The optical resolution, however, gave a pair of enantiomers which showed differing selectivity in the receptor binding; (+)-**34** preferred the D_2 receptor, whereas (−)-**34** favored the 5-HT_{1A} receptor. Both enantiomers fully recovered the FSC activity in the present conditions. Therefore, (−)-**34** was consequently recognized as a putative 5-HT_{1A} receptor antagonist with moderate selectivity (19-fold vs α_1 and 13-fold vs D_2). The oxime **33** and the C-4 methoxy analogue **35** showed somewhat low affinities for the 5-HT_{1A} receptor (18−28 nM) but still demonstrated relatively large recovery of FSC (>80%). Optical resolution of **35** was not attempted. The 2*H*-chromene derivative **36** exhibited the highest 5-HT_{1A} receptor affinity among the series (K_i = 0.159 nM) and better selectivity only vs the α_1 receptor (52-fold).

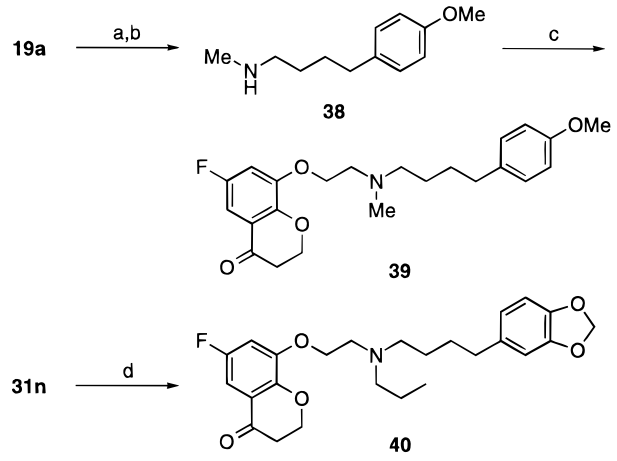
Next, the effect of modifying the tether length (*n*: the number of methylenes) was examined. Among the 6-fluoro-2-methylchroman series (**29**, **24**, **23**, **25**, **30**; *n* = 2−6), the K_i values for 5-HT_{1A} receptor differed in an inconsistent manner (0.19−0.27 nM at *n* = 2, 4; 7.3−

Scheme 4[a]



[a] Conditions: (a) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH; (b) $NaBH_4$, EtOH; (c) conc HCl, MeOH, reflux; (d) *p*-TsOH, toluene, azeotropic; (e) HPLC (CHIRALPAK AD, EtOH).

Scheme 5[a]



[a] Conditions: (a) ClCOOEt, aqueous $NaHCO_3$, CH_2Cl_2; (b) $LiAlH_4$, THF, reflux; (c) **13**, K_2CO_3, MeCN, reflux, then aqueous HCl; (d) *n*-PrBr, K_2CO_3, MeCN, reflux.

9.5 nM at $n = 3, 5, 6$), whereas the FSC recovery appeared to raise continuously with the tether length. The ethylene-tethered compounds (**29**, **32**, **37**) showed receptor affinities similar to, but less recovery of FSC than, the corresponding butylene homologues (**23**, **31a**, **34**, respectively). It should be noted that all the homologues examined here ($n = 2-6$) exhibited appreciable affinity for the 5-HT$_{1A}$ receptor.

These results allowed us to conclude that the original butylene group ($n = 4$) was optimum, since it furnished the analogues with a good balance of affinity and ability to recover FSC. We then chose the 4-oxochroman derivative **31a** as a new lead for further optimization because of its superior selectivity.

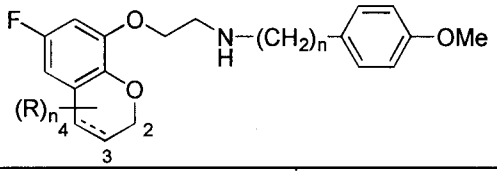
Modifications of the terminal aromatic ring, therefore, originated from **31a** (Table 3). Substitutions for the 4-methoxy moiety by a hydroxy group in **31b** or a hydrogen in **31c** or displacement of the moiety to the 3- or 2-position (in **31f**,**g**) caused considerable reduction in 5-HT$_{1A}$ receptor affinity. This indicates that the 4-methoxy moiety of **31a** plays an essential role in interactions with the 5-HT$_{1A}$ receptors. Homologation of the ethereal alkyl groups in **31d**,**e**, as well as 2,3-dimethylation in **31p**, also reduced the affinity. Furthermore, 2,3-benzo fusion in **31q** resulted in 220-fold reduction of the affinity (K_i = 680 nM). Thus the compounds with augmented bulk around the terminal aromatic ring exhibited lower affinities for the 5-HT$_{1A}$ receptor, accompanied by a decrease in FSC recovery. This suggests an existence of a size-limited space of the binding site accommodating the aromatic ring. In contrast, the binding to α_1 and D_2 receptors seems less sensitive to the bulkiness of the terminal ring. Other substitutions at the 4-position gave compounds **31h**−**l** showing similar K_i values for the 5-HT$_{1A}$ receptor (11−28 nM), but their ability to recover FSC ranged fairly widely (ca. 0−50% recovery by 1 μM each compound). Among them, compounds with an electron-donating group (**31h**,**i**) seemed to show larger recovery of FSC in comparison to those with an electron-withdrawing group (**31j**−**l**). The 3,4-cyclized analogues, **31m**−**o**, demonstrated striking resemblance to **31a** in the in vitro profiles. It is noteworthy that an aryl methyl ether is often subjected to oxidative metabolic demethylation; therefore the replacement of the moiety with oxygen-containing heterocycles as those in **31m**−**o** may afford resistance against metabolism. Lastly, two tertiary amine derivatives (**39**, **40**) were prepared and evaluated. However, they showed only moderate affinities and no apparent antagonist activity for the 5-HT$_{1A}$ receptor, indicating another limitation in modifications of **3**.

The in vitro agonist/antagonist activities of **1**, **31a**,**n**, and **34** were further examined in the FSC assay using the whole CHO cells (Figure 1). Neither of these compounds (up to 10^{-5} M) exhibited statistically significant changes of the FSC activity. On the other hand, each of them demonstrated full reversal of the 8-OH-DPAT-induced inhibition in a same concentration range. Therefore, the compounds **31a**,**n** and **34** are likely to be 5-HT$_{1A}$ receptor antagonists as well as **1**.

Thus, the structural modifications led us to several novel compounds that exhibit antagonist activity and improved selectivity for the 5-HT$_{1A}$ receptor. Modifications of the chroman ring (i.e., introduction of an oxo or an optically active hydroxy moiety at the C-4 position) afforded the greatest enhancement of selectivity. This agrees with our working hypothesis noted in the Introduction. Other structural changes, for example, the terminal ring modifications, generally resulted in a decrease in affinity and selectivity. The considerable tolerance of 5-HT$_{1A}$ receptor binding of the analogues toward the tether modifications suggests that the terminal ring may contribute to the binding as an auxiliary element. The arylalkyl component of the present compounds, however, plays a significant role in interactions with the receptor, especially in their effects upon antagonist activity.

On the basis of the in vitro assay results, we chose **31a**,**m**−**o** for in vivo evaluation in the rat behavioral

Table 2. Effect of Modifications of the Chroman Ring and the Tether Length

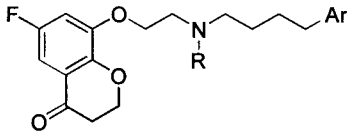


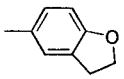
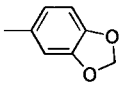
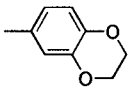
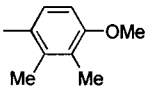
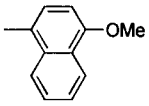
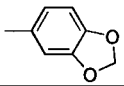
			receptor affinity (K_i), nM[a,b]			selectivity, K_i ratio		FSC
compd	$(R)_n$	n	5-HT_{1A}	α_1	D_2	α_1/5-HT_{1A}	D_2/5-HT_{1A}	recovery, %[c]
3[d]	H	4	0.221 (0.219–0.223)	2.71 (2.66–2.76)	0.259 (0.250–0.268)	12	1.2	121 ± 3
23	2-Me	4	0.270 (0.265–0.276)	4.07 (3.98–4.17)	0.312 (0.303–0.322)	15	1.2	53 ± 1
28	2,2-Me_2	4	0.435 (0.417–0.453)	3.57 (3.46–3.69)	0.149 (0.145–0.153)	8.2	0.34	44 ± 6
31a	4-=O	4	3.07 (3.02–3.11)	203 (199–208)	62.3 (61.2–63.4)	66	20	59 ± 1
33	4-=NOH	4	27.7 (27.0–28.4)	207 (204–210)	11.7 (11.6–11.8)	7.5	0.42	83 ± 3
34	4-OH	4	0.716 (0.704–0.727)	10.4 (10.1–10.8)	0.724 (0.706–0.742)	15	1.0	108 ± 2
(+)-**34**	(+)-4-OH[e]	4	2.10 (2.08–2.13)	73.3 (71.9–74.7)	0.482 (0.472–0.492)	35	0.23	109 ± 1
(–)-**34**	(–)-4-OH[e]	4	0.310 (0.304–0.317)	5.86 (5.69–6.04)	4.11 (3.74–4.51)	19	13	111 ± 4
35	4-OMe	4	18.3 (17.9–18.7)	175 (174–177)	9.07 (8.85–9.29)	9.6	0.50	111 ± 4
36	(3,4-dehydro)	4	0.159 (0.154–0.165)	8.31 (8.16–8.45)	0.288 (0.283–0.292)	52	1.8	109 ± 3
29	2-Me	2	0.188 (0.185–0.190)	2.73 (2.62–2.85)	8.13 (7.88–8.37)	15	43	12 ± 1
24	2-Me	3	9.53 (9.37–9.69)	19.9 (19.6–20.3)	4.98 (4.82–5.14)	2.1	0.52	40 ± 3
25	2-Me	5	7.33 (7.21–7.46)	19.1 (18.8–19.4)	3.02 (2.92–3.12)	2.6	0.41	59 ± 3
30	2-Me	6	8.99 (8.85–9.13)	30.8 (30.4–31.3)	2.31 (2.26–3.36)	3.4	0.26	76 ± 3
32	4-=O	2	4.04 (3.94–4.14)	317 (297–338)	38.0 (36.0–40.1)	78	9.4	9 ± 3
37	4-OH	2	2.34 (2.32–2.37)	13.5 (13.2–13.8)	2.99 (2.87–3.12)	5.8	1.3	68 ± 7
1[d]			46.0 (45.4–46.6)	6080 (5980–6180)	614 (537–701)	130	13	73 ± 1
prazosin			ND	0.097 (0.087–0.109)	ND			
haloperidol			ND	ND	0.590 (0.580–0.599)			

[a] K_i values were obtained from two or three experiments each performed in triplicate. Values in parentheses indicate 95% confidence intervals. [b] ND, not determined. [c] The recovery of FSC (suppressed with 0.1 μM 8-OH-DPAT) by 1 μM test compound. Values represent mean ± SEM (n = 3). [d] Data taken from ref 16. [e] The absolute configuration is unknown.

responses test and added **34** and **36** which were less selective but potent in their affinity and in vitro antagonist activity. Compounds **1** and **3** were also tested as references (Table 4). All the tested compounds significantly antagonized both the responses of forepaw treading and flat body posture at subcutaneous doses of 1−3 mg/kg (3−10 mg/kg for **31o**). The compounds given alone (up to 10 mg/kg sc) did not induce apparent

Table 3. Modifications of the Terminal Aromatic Ring and the Middle Amine



			receptor affinity (K_i), nM[a,b]			selectivity, K_i ratio		FSC
compd	Ar	R	5-HT$_{1A}$	α_1	D_2	α_1/5-HT$_{1A}$	D_2/5-HT$_{1A}$	recovery, %[c]
31a	4-C_6H_4OMe	H	3.07 (3.02–3.11)	203 (199–208)	62.3 (61.2–63.4)	66	20	59 ± 1
31b	4-C_6H_4OH	H	55.8 (55.3–56.5)	120 (117–122)	23.3 (22.1–24.7)	2.2	0.42	26 ± 1
31c	C_6H_5	H	41.8 (40.6–43.1)	91.5 (89.2–93.9)	43.9 (41.4–46.6)	2.2	1.1	16 ± 6
31d	4-C_6H_4OEt	H	18.0 (17.8–18.1)	663 (652–676)	55.1 (52.3–57.9)	37	3.1	52 ± 10
31e	4-C_6H_4OPr	H	48.8 (47.4–50.2)	856 (839–874)	ND	18		33 ± 7
31f	3-C_6H_4OMe	H	43.4 (41.8–45.1)	249 (242–255)	111 (107–115)	5.7	2.6	3 ± 11
31g	2-C_6H_4OMe	H	24.0 (23.7–24.2)	75.6 (74.6–76.6)	23.7 (21.8–25.8)	3.2	0.99	44 ± 3
31h	4-C_6H_4SMe	H	18.4 (18.3–18.6)	315 (301–329)	38.8 (38.2–39.4)	17	2.1	49 ± 7
31i	4-$C_6H_4NMe_2$	H	28.3 (27.8–28.8)	574 (563–586)	34.6 (34.0–35.2)	20	1.2	37 ± 5
31j	4-C_6H_4Cl	H	19.0 (18.7–19.2)	98.9 (97.2–100.5)	32.3 (31.0–33.7)	5.2	1.7	30 ± 8
31k	4-C_6H_4CN	H	11.0 (10.9–11.2)	355 (347–364)	70.4 (67.3–73.6)	32	6.4	17 ± 2
31l	4-$C_6H_4NO_2$	H	19.3 (19.1–19.5)	258 (244–273)	ND	13		3 ± 3
31m		H	4.19 (3.98–4.42)	169 (164–173)	49.6 (48.2–50.9)	40	12	68 ± 6
31n		H	2.67 (2.62–2.72)	209 (207–211)	42.1 (41.5–42.8)	78	16	55 ± 3
31o		H	2.60 (2.56–2.64)	303 (297–310)	25.1 (24.4–25.7)	120	9.7	65 ± 3
31p		H	77.0 (75.5–78.4)	152 (150–154)	ND	2.0		17 ± 5
31q		H	680 (634–730)	198 (189–206)	153 (148–158)	0.29	0.23	−15 ± 2
39	4-C_6H_4OMe	Me	52.7 (51.6–53.7)	1350 (1340–1370)	ND	26		10 ± 4
40		Pr	69.0 (67.2–70.8)	734 (690–781)	1540 (1490–1580)	11	22	6 ± 2

[a–c] See the corresponding footnotes of Table 2.

behavioral changes. These results suggest that the compound **3** and the selected analogues can permeate the blood−brain barrier and antagonize the effect of 8-OH-DPAT at central postsynaptic 5-HT$_{1A}$ receptors.

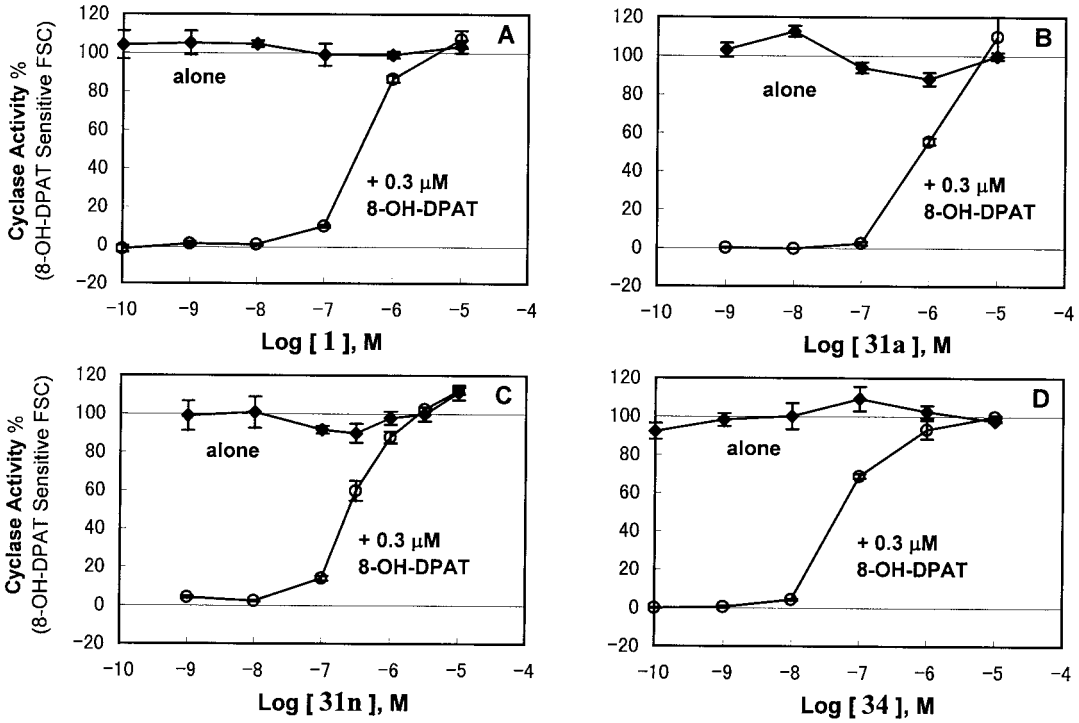


Figure 1. Dose−response curves in the FSC assay with whole CHO cells expressing the human 5-HT_{1A} receptors. Each panel shows the responses by the test compound alone (◆) and those in the presence of 0.3 μM 8-OH-DPAT (○). All experiments were performed in triplicate, and each point represents mean ± SEM. The EC_{50} values (refer to the ligand concentration causing 50% recovery of FSC) for **1**, **31a**,**n**, and **34** were 395 ± 31, 1020 ± 95, 321 ± 42, and 57.6 ± 5.5 nM, respectively.

The benzodioxine derivative (**31o**) was somewhat weaker in this assay, despite this compound having considerable similarity to **31a**,**m**,**n** in both chemical structure and in vitro profile.

Compound **31n** was further examined in the cell firing assay (Figure 2). A cumulative injection of 8-OH-DPAT (0.32−2.5 μg/kg iv) caused a dose-dependent decrease of the firing, in accordance with a previous report.[28] Pretreatment with **31n** (0.32 mg/kg iv) suppressed the effect of 8-OH-DPAT and caused a marked shift of the dose−response curve to the right. For instance, the effect of 2.5 μg/kg 8-OH-DPAT, causing 90% inhibition of the firing, was completely abolished by the pretreatment. In contrast, administration of **31n** alone did not affect the firing rate even at higher doses (0.32−2.5 mg/kg iv). Thus the recorded dorsal raphe firing illustrates the antagonism of **31n** at the presynaptic 5-HT_{1A} autoreceptors. Because of its lack of agonist activity in the FSC and cell firing assays, **31n** can be regarded as a putative 5-HT_{1A} antagonist.

In conclusion, the structural modifications of **3** brought varied changes to the analogues in their binding affinity, selectivity, and antagonist activity for the 5-HT_{1A} receptor. Introduction of an oxo or an optically active hydroxy moiety on the chroman C-4 position afforded **31a** and (−)-**34**, both of which show improved 5-HT_{1A} receptor selectivity. The tether modifications showed that the original butylene group provides a superior balance of the affinity, selectivity, and antagonist activity in comparison to ethylene and other alkylene groups. Modifications of the terminal aromatic ring revealed that the 4-methoxy moiety of **31a** plays essential roles in both 5-HT_{1A} receptor binding and antagonism. At the same time, the modifications afforded compounds **31m**−**o** demonstrating excellent binding profiles and in vitro antagonist activity as **31a**. N-Alkylations of the middle amine caused a decrease in affinity and antagonist activity. The in vivo evaluations, the behavioral and electrophysiological tests, showed antagonist activity of **31n** at both the pre- and postsynaptic 5-HT_{1A} receptors. Since **31n** still retains appreciable D_2 receptor affinity, its therapeutic utility should carefully be investigated. Also necessary are further studies to pursue more selective compounds as well as to confirm whether the present compounds including **31n** are "silent". Nevertheless, this study should provide a new approach to potent and selective 5-HT_{1A} receptor antagonists.

Experimental Section

Melting points were determined with a Yanaco MP-S3 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM EX-90, a JEOL JNM LA-400, or a JEOL JNM GX-500 spectrometer and were referenced to an internal standard, tetramethylsilane. Mass spectra were recorded on a HP 5890A-MSD or a JEOL JMS DX-300 mass spectrometer, and the ionization method was chosen from EI and FAB. For salts, assignments of ion peaks are based on the basic component. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens and S) and were within ±0.4% of theoretical values. The optical resolution by HPLC was performed with a Shimadzu LC-8A preparative liquid chromatograph equipped with a Daicel CHIRALPAK AD column. Optical rotation measurements were obtained with a Horiba SEPA-200 polarimeter. Preparative column chromatography was performed with Wakogel C-200 (Wako; 100−200 mesh). Drying of organic solutions during workup was done over anhydrous $MgSO_4$. In the pharmacological experiments, all drug doses are in terms of the free base.

6-Fluoro-2,2-dimethylchroman-8-ol (8c). To a mixture of 6-fluoro-2,2-dimethylchroman (**6**; 900 mg, 5.00 mmol), $CaCO_3$ (1.00 g, 10.0 mmol), MeOH (7.5 mL), and CH_2Cl_2 (7.5

Table 4. Effect of **1**, **3**, and Selected 6-Fluorochroman Derivatives on the 8-OH-DPAT-Induced Behavioral Responses in Reserpinized Rats

compd	dose,[a] mg/kg sc	n	behavior score[b] FT	FBP
1	0	8	9.9 ± 0.7	12.6 ± 0.2
	1	6	1.3 ± 0.3**	3.5 ± 1.0**
	3	6	0.5 ± 0.5**	1.8 ± 0.9**
3	0	6	8.8 ± 0.7	11.5 ± 0.4
	1	6	1.8 ± 0.2**	3.8 ± 0.3**
	3	6	0.5 ± 0.5**	3.0 ± 0.9**
31a	0	6	10.2 ± 0.7	11.3 ± 0.7
	1	6	3.8 ± 0.7**	6.0 ± 0.6**
	3	6	1.3 ± 0.3**	2.3 ± 0.6**
31m	0	8	10.1 ± 0.5	12.3 ± 0.4
	1	6	4.0 ± 0.8**	6.8 ± 1.4**
	3	6	1.2 ± 0.4**	3.8 ± 1.8**
31n	0	12	7.8 ± 0.4	9.7 ± 0.4
	1	6	3.0 ± 0.7**	5.3 ± 0.7**
	3	6	1.5 ± 0.6**	3.0 ± 0.6**
31o	0	7	7.7 ± 0.8	11.0 ± 0.7
	3	6	5.8 ± 0.8	8.5 ± 0.6*
	10	6	3.9 ± 0.4**	6.5 ± 0.7**
34	0	10	8.6 ± 0.5	12.4 ± 0.3
	1	6	3.3 ± 0.8**	6.8 ± 1.3**
	3	6	1.8 ± 0.6**	4.0 ± 1.1**
36	0	6	11.8 ± 0.7	11.8 ± 0.5
	1	6	1.3 ± 0.5**	3.7 ± 0.5**
	3	6	0.0 ± 0.0**	2.8 ± 0.9**

[a] Doses are in terms of the free base. [b] Values represent mean ± SEM. Statistics by Mann−Whitney U-test: **$p < 0.01$; *$p < 0.05$; versus control (dose = 0).

mL) was added portionwise trimethylbenzylammonium tribromide (1.95 g, 5.00 mmol), and the mixture was heated to reflux for 16 h. The resulting suspension was diluted with Et_2O and washed successively with 1 N HCl, aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$, and brine. The organic phase was dried, concentrated, and chromatographed (hexane) to give 8-bromo-6-fluoro-2,2-dimethylchroman (**7**) as a colorless oil (830 mg, 64%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.10 (br dd, J = 8.0, 3.1 Hz, 1H, C7−H), 6.75 (app ddt, J = 8.6, 3.0, 0.9 Hz, 1H, C5−H), 2.77 (br t, J = 6.8 Hz, 2H, C4−H_2), 1.79 (t, J = 6.8 Hz, 2H, C3−H_2), 1.35 (s, 6H, CH_3); MS (EI) m/z 258 (M^+), 260 ($M^+ + 2$).

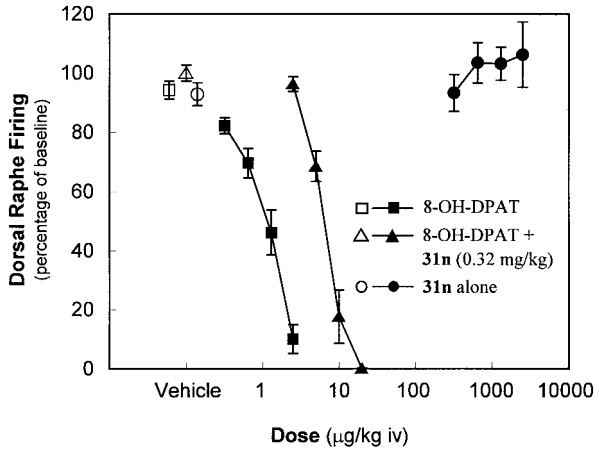


Figure 2. Effects of 8-OH-DPAT and **31n** on the serotonergic neuronal cell firing and antagonism of **31n** for the effect of 8-OH-DPAT, recorded at the dorsal raphe nucleus in anesthetized rats. Each point represents mean ± SEM (n = 6−9/point). The baseline firing rate was 1.53 ± 0.09 spikes/s (n = 27).

To a cooled (−70 °C) solution of **7** (780 mg, 3.01 mmol) in anhydrous THF (15 mL) was added dropwise a solution of butyllithium (1.42 M in hexane; 2.23 mL, 3.17 mmol) over a period of 2 min. After the mixture stirred at the same temperature for 3 min, $B(OBu)_3$ (1.39 g, 6.03 mmol) was added, and the reaction mixture was allowed to warm and stir for 20 min at room temperature. To the resulting mixture were added 3 N NaOH (3.0 mL) and aqueous H_2O_2 (30%, 1.0 mL), and the mixture was vigorously stirred for 20 min. The mixture was then acidified with 1 N HCl and extracted with Et_2O, and the organic phase was dried, passed through a short column of silica gel, and concentrated to give **8c** as a colorless oil (550 mg, 93%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.49 (dd, J = 9.6, 3.0 Hz, 1H, C7−H), 6.33 (dd, J = 9.2, 3.0 Hz, 1H, C5−H), 2.72 (t, J = 6.8 Hz, 2H, C4−H_2), 1.81 (t, J = 6.8 Hz, 2H, C3−H_2), 1.34 (s, 6H, CH_3); MS (EI) m/z 196 (M^+).

8-(2-Bromoethoxy)-6-fluoro-2-methylchroman (9b) and 8-(2-Bromoethoxy)-6-fluoro-2,2-dimethylchroman (9c). The title compounds were prepared by a procedure similar to that reported for 8-(2-bromoethoxy)-6-fluorochroman (**9a**).[16] **9b**: ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.6−6.4 (m, 2H, C5−H and C7−H), 4.29 (app t, J = 6.0 Hz, 2H, OCH_2), 4.3−4.1 (m, 1H, C2−H), 3.63 (app t, J = 6.0 Hz, 2H, CH_2Br), 2.9−2.7 (m, 2H, C4−H_2), 2.1−1.7 (m, 2H, C3−H_2), 1.43 (d, J = 6.5 Hz, 3H, CH_3); MS (EI) m/z 288 (M^+), 290 (M^+ + 2). **9c**: ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.6−6.4 (m, 2H, C5−H and C7−H), 4.28 (app t, J = 6.0 Hz, 2H, OCH_2), 3.61 (app t, J = 6.0 Hz, 2H, CH_2Br), 2.75 (t, J = 6.8 Hz, 2H, C4−H_2), 1.79 (t, J = 6.8 Hz, 2H, C3−H_2), 1.35 (s, 6H, CH_3); MS (EI) m/z 302 (M^+), 304 (M^+ + 2).

2-[(6-Fluorochroman-8-yl)oxy]ethylamine (10a). A mixture of **9a** (3.73 g, 13.6 mmol) and benzylamine (14.9 mL, 136 mmol) in MeCN (40 mL) was heated to reflux for 3 h. The solvent and a large part of the remaining benzylamine were distilled out under reduced pressure, and the residue was chromatographed (hexane−EtOAc, 4:1 to 1:1) to give *N*-benzyl-2-[(6-fluorochroman-8-yl)oxy]ethylamine as an oil (3.64 g, 89%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.4−7.2 (m, 5H, benzyl aromatic), 6.6−6.3 (m, 2H, C5′−H and C7′−H), 4.18 (t, J = 5.2 Hz, 2H, C2′−H_2), 4.09 (t, J = 5.4 Hz, 2H, C2−H_2), 3.86 (s, 2H, benzylic), 3.04 (t, J = 5.4 Hz, 2H, C1−H_2), 2.74 (t, J = 6.5 Hz, 2H, C4′−H_2), 2.2−1.8 (m, 3H, C3′−H_2 and NH); MS (FAB) m/z 302 (M^+ + 1).

The *N*-benzylamine (2.62 g, 8.70 mmol) was mixed with 10% Pd−C (0.80 g) in MeOH (30 mL); to this suspension was added carefully HCOOH (3.0 mL), and the mixture was stirred at room temperature for 1 h. The mixture was then filtered on a Celite pad, and the filtrate was concentrated to dryness. The residual solid was dissolved in 1 N NaOH and extracted with $CHCl_3$, and the organic phase was washed with brine, dried, and concentrated to give **10a** as a colorless oil (1.84 g, 100%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.6−6.3 (m, 2H, C5′−H and C7′−H), 4.22 (t, J = 5.2 Hz, 2H, C2′−H_2), 4.00 (t, J = 5.3 Hz, 2H, C2−H_2), 3.11 (t, J = 5.3 Hz, 2H, C1−H_2), 2.76 (t, J = 6.5 Hz, 2H, C4′−H_2), 2.1−1.8 (m, 2H, C3′−H_2); MS (EI) m/z 211 (M^+).

2-[(6-Fluoro-2-methylchroman-8-yl)oxy]ethylamine (10b): prepared from **9b** by a procedure similar to that described for **10a**; ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.6−6.4 (m, 2H, C5′−H and C7′−H), 4.3−4.1 (m, 1H, C2′−H), 3.99 (t, J = 5.2 Hz, 2H, C2−H_2), 3.08 (t, J = 5.2 Hz, 2H, C1−H_2), 2.9−2.7 (m, 2H, C4′−H_2), 2.1−1.7 (m, 2H, C3′−H_2), 1.42 (d, J = 6.4 Hz, 3H, CH_3); MS (EI) m/z 225 (M^+).

8-(2-Bromoethoxy)-6-fluorochroman-4-one Ethylene Ketal (13). A mixture of 6-fluoro-8-hydroxychroman-4-one (**12**)[21] (7.28 g, 40.0 mmol), ethylene glycol (4.96 g, 80.0 mmol), $CH(OEt)_3$ (11.84 g, 80.0 mmol), and *p*-TsOH·H_2O (1.52 g, 8.00 mmol) in benzene (200 mL) was heated to reflux for 2 h. The emerging water was trapped with a Dean−Stark apparatus. After cooling below 10 °C, the solution was washed quickly with a chilled solution of $NaHCO_3$−NaCl, followed successively by water and brine, and concentrated. The residual oil was mixed with 1,2-dibromoethane (34.5 mL, 400 mmol), 3 N NaOH (53 mL, 160 mmol), and $Bu_4N^+HSO_4^-$ (0.68 g, 2.0 mmol); then the mixture was vigorously stirred at 70 °C for 2 h. The reaction mixture was partitioned, and the organic phase was washed with water and brine, dried, and concentrated. The resulting viscous oil was dissolved in hot MeOH (28 mL) and cooled to allow precipitation of **13** as a white crystalline solid (11.1 g, 83%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.75 (dd, J = 9.1, 3.1 Hz, 1H, aromatic), 6.64 (dd, J = 9.6, 3.1 Hz, 1H, aromatic), 4.40 (t, J = 5.7 Hz, 2H, C2−H_2), 4.3−4.1 (m, 4H, OCH_2CH_2O), 4.29 (app t, J = 6.6 Hz, 2H, C8−OCH_2), 3.63 (app t, J = 6.6 Hz, 2H, CH_2Br), 2.13 (t, J = 5.7 Hz, 2H, C3−H_2); MS (EI) m/z 332 (M^+), 334 (M^+ + 2).

4-Arylbutylamines 19a,c−q: General Procedure. The synthesis of 4-(1,3-benzodioxol-5-yl)butylamine (**19n**) is typical. To a mixture of piperonal (**14n**; 1.50 g, 10.0 mmol) and triphenyl(3-phthalimidopropyl)phosphonium bromide (5.30 g, 10.0 mmol) in 1,4-dioxane (40 mL) was added NaOMe (28 wt % in MeOH, 2.1 mL, 10 mmol), and the mixture was stirred at room temperature for 12 h. The resulting suspension was mixed with 5% aqueous NaCl and extracted with EtOAc; then the organic phase was washed with water and brine, dried, and concentrated. The residue was treated with MeOH (50 mL) to allow precipitation of (*E*/*Z*)-*N*-[4-(1,3-benzodioxol-5-yl)but-3-enyl]phthalimide (**15n**). The precipitate was collected and mixed with 10% Pd−C (20 mg), 1,4-dioxane (40 mL), and EtOH (40 mL); then the mixture was stirred under 1 atm of H_2 for 3 h. The reaction mixture was filtered on a Celite pad, and the filtrate was concentrated to give *N*-[4-(1,3-benzodioxol-5-yl)butyl]phthalimide (**16n**) as a white crystalline solid (2.14 g, 66% from **14n**): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.9−7.6 (m, 4H, phthalyl), 6.8−6.5 (m, 3H, benzodioxole aromatic), 5.91 (s, 2H, OCH_2O), 3.70 (t, J = 7.2 Hz, 2H, NCH_2), 2.60 (t, J = 7.4 Hz, 2H, benzylic), 1.8−1.6 (m, 4H); MS (EI) m/z 323 (M^+).

Compound **16n** (1.90 g, 5.88 mmol) was mixed with $MeNH_2$ (40 wt % in MeOH, 19 mL), and the mixture was stirred at room temperature for 14 h. The reaction mixture was roughly concentrated, and the wet residue was immediately mixed with 1 N HCl (40 mL) and washed with $CHCl_3$. The acidic solution was neutralized with 5 N NaOH and extracted with $CHCl_3$, and the organic phase was washed with brine, dried, and concentrated to give 4-(1,3-benzodioxol-5-yl)butylamine (**19n**) as an oil (0.95 g, 84%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.8−6.5 (m, 3H, aromatic), 5.90 (s, 2H, OCH_2O), 2.69 (t, J = 6.7 Hz, 2H, benzylic), 2.54 (t, J = 7.0 Hz, 2H, CH_2N), 1.8−1.4 (m, 4H), 1.34 (br s, 2H, NH_2); MS (EI) m/z 193 (M^+). The 4-arylbutylamines **19a**,**c**−**q** obtained by this procedure were used for the coupling without further purification.

4-(4-Aminobutyl)phenol (19b). 4-(4-Methoxyphenyl)butylamine (**19a**) was prepared according to the general procedure described above: ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.07 (app d, J = 9.0 Hz, 2H, meta to OMe), 6.82 (app d, J = 9.0 Hz, 2H, ortho to OMe), 3.77 (s, 3H, OCH_3), 2.70 (t, J = 6.7 Hz, 2H,

benzylic), 2.56 (t, J = 7.0 Hz, 2H, CH_2N), 1.8−1.4 (m, 4H), 1.73 (br s, 2H, NH_2); MS (EI) m/z 179 (M^+).

Compound **19a** (1.79 g, 10.0 mmol) was mixed with concentrated HBr (20 mL), and the mixture was heated to reflux for 1 h. The reaction mixture was concentrated to dryness, and the residue was dissolved in water (5 mL); the solution was then neutralized with powdery $NaHCO_3$, followed by addition of NaCl to salt out a precipitate. The precipitate was collected and washed with a minimum amount of chilled water to give **19b** as a solid (0.84 g, 51%): ^{1}H NMR (90 MHz, DMSO-d_6) δ 8.30 (br s, 3H), 6.97 (d, J = 8.6 Hz, 2H, meta to OH), 6.70 (d, J = 8.6 Hz, 2H, ortho to OH), 2.9−2.7 (m, 2H, benzylic), 2.6−2.4 (m, 2H, CH_2N), 1.7−1.5 (m, 4H); MS (EI) m/z 165 (M^+).

4-Arylbutylamines 19l,m from 4-Arylbutyric Acids. The synthesis of 4-(2,3-dihydrobenzofuran-5-yl)butylamine (**19m**) is described. 4-(2,3-Dihydrobenzofuran-5-yl)butyric acid (**17m**) was prepared from 2,3-dihydrobenzofuran by Friedel−Crafts acylation (succinic anhydride, $AlCl_3$, 1,2-dichloroethane) and subsequent catalytic hydrogenation (1 atm of H_2, Pd−C, AcOH): ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.00 (br s, 1H, COOH), 7.00 (br s, 1H, C4′−H), 6.90 (br d, J = 7.9 Hz, 1H, C6′−H), 6.70 (d, J = 7.9 Hz, 1H, C7′−H), 4.54 (t, J = 9.0 Hz, 2H, C2′−H_2), 3.17 (t, J = 9.0 Hz, 2H, C3′−H_2), 2.60 (t, J = 7.2 Hz, 2H, C4−H_2), 2.36 (t, J = 6.9 Hz, 2H, C2−H_2), 2.2−1.7 (m, 2H, C3−H_2); MS (EI) m/z 206 (M^+).

To a solution of **17m** (1.65 g, 8.00 mmol) in CH_2Cl_2 (20 mL) were added $(COCl)_2$ (1.12 g, 8.80 mmol) and DMF (0.1 mL), and the mixture was stirred for 2 h. The mixture was concentrated to dryness and dissolved again in CH_2Cl_2 (20 mL). To this solution was added dropwise concentrated NH_4OH (10 mL) with vigorous stirring, and the mixture was further stirred for 1 h at room temperature. The formed precipitate was dissolved by adding $CHCl_3$, and the organic phase was separated, washed with aqueous $NaHCO_3$, dried, and concentrated to give crude 4-(2,3-dihydrobenzofuran-5-yl)butyramide (**18m**) as a white solid (1.41 g). To a solution of crude **18m** (1.41 g) in THF (21 mL) was added BH_3·SMe_2 (2.1 mL, 21 mmol), and the mixture was heated to reflux for 4 h. After the mixture cooled, to the reaction mixture were added dropwise MeOH (2.5 mL) and concentrated HCl (1.8 mL), and the mixture was further refluxed for 30 min. The reaction mixture was made basic with 1 N NaOH and extracted with EtOAc. The organic phase was washed with 1 N NaOH and brine, dried, passed through a short column of silica gel, and concentrated to give **19m** as an oil (1.15 g, 75% from **17m**): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.00 (br s, 1H, C4′−H), 6.89 (br d, J = 8.1 Hz, 1H, C6′−H), 6.69 (d, J = 8.1 Hz, 1H, C7′−H), 4.54 (t, J = 8.6 Hz, 2H, C2′−H_2), 3.17 (t, J = 8.6 Hz, 2H, C3′−H_2), 2.70 (t, J = 6.5 Hz, 2H, $ArCH_2$), 2.55 (t, J = 7.2 Hz, 2H, CH_2N), 1.9−1.2 (m, 6H, C2−H_2, C3−H_2, and NH_2); MS (EI) m/z 191 (M^+).

General Procedures for the Coupling of the Chroman-Containing Component and the Arylalkyl Component (Table 1): Method A. The synthesis of *N*-[2-[(6-fluoro-2-methylchroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamine fumarate (2:1) (**23**) is described. To a solution of **10b** (225 mg, 1.00 mmol) and Et_3N (121 mg, 1.20 mmol) in CH_2Cl_2 (2 mL) was added dropwise a CH_2Cl_2 (2 mL) solution of 4-(4-methoxyphenyl)butyryl chloride (**20**; 1.10 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was washed successively with 1 N HCl, 1 N NaOH, and brine, then dried, and concentrated. To a solution of the residual solid (a crude amide) in THF (5 mL) was added BH_3·SMe_2 (0.30 mL, 3.0 mmol), and the mixture was heated to reflux for 3 h. After cooling, the reaction was quenched by a careful addition of MeOH (0.3 mL) and concentrated HCl (0.2 mL), and the mixture was further refluxed for 30 min and cooled to room temperature. The mixture was diluted with Et_2O (50 mL), and the solution was washed with 1 N NaOH and brine, dried, and concentrated. The residue was treated with fumaric acid (52 mg, 0.45 mmol), and the crude salt was recrystallized from EtOH−Et_2O to give **23** as white crystals (0.25 hydrate, 328 mg, 73% from **10b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (br s, 2H), 7.05 (d, J = 9.0 Hz, 2H), 6.79 (d, J = 9.0 Hz, 2H), 6.71 (s, 1H), 6.48 (dd, J = 9.6, 2.8 Hz, 1H), 6.40 (dd, J = 8.4, 2.8 Hz, 1H), 4.20−4.05 (m, 1H), 4.18 (t, J = 4.7 Hz, 2H), 3.76 (s, 3H), 3.25 (t, J = 4.7 Hz, 2H), 2.97 (t, J = 7.4 Hz, 2H), 2.9−2.6 (m, 2H), 2.55 (t, J = 7.4 Hz, 2H), 2.0−1.9 (m, 2H), 1.8−1.6 (m, 4H), 1.36 (d, J = 6.4 Hz, 3H); MS (FAB) m/z 388 (M^+ + 1).

Method B. The synthesis of *N*-[2-[(6-fluoro-2,2-dimethylchroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamine fumarate (2:1) (**28**) is described. A mixture of **9c** (273 mg, 0.900 mmol), **19a** (538 mg, 2.70 mmol), and K_2CO_3 (187 mg, 1.35 mmol) in MeCN (5 mL) was heated to reflux for 4 h. Insoluble matter was filtered off, and the filtrate was concentrated and chromatographed ($CHCl_3$−MeOH, 99:1) to give the free base of **28** as a slightly yellow oil (271 mg, 0.675 mmol, 75%). This was treated with fumaric acid (37 mg, 0.32 mmol), and the crude salt was recrystallized from EtOH to give **28** as white crystals (monohydrate, 260 mg, 60% from **9c**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.09 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 6.71 (dd, J = 9.4, 3.1 Hz, 1H), 6.53 (dd, J = 9.2, 3.1 Hz, 1H), 6.45 (s, 1H), 4.07 (t, J = 5.5 Hz, 2H), 3.71 (s, 3H), 2.99 (t, J = 5.5 Hz, 2H), 2.76 (t, J = 7.2 Hz, 2H), 2.69 (t, J = 6.7 Hz, 2H), 2.52 (t, J = 7.4 Hz, 2H), 1.72 (t, J = 6.7 Hz, 2H), 1.6−1.5 (m, 4H), 1.24 (s, 6H); MS (FAB) m/z 402 (M^+ + 1).

Method C. The synthesis of 8-[2-[4-(1,3-benzodioxol-5-yl)-butylamino]ethoxy]-6-fluorochroman-4-one hydrochloride (**31n**) is described. A mixture of **13** (500 mg, 1.50 mmol), **19n** (869 mg, 4.50 mmol), and K_2CO_3 (311 mg, 2.25 mmol) in MeCN (10 mL) was heated to reflux for 4 h. After cooling, the resulting suspension was mixed with 2 N HCl (10 mL) and stirred at room temperature for 1 h. Then the mixture was made basic with aqueous NaOH and extracted with EtOAc. The organic phase was dried, concentrated, and chromatographed ($CHCl_3$−MeOH, 98:2) to give the free base of **31n** as an oil (478 mg, 79%). This was treated with excess of a 1,4-dioxane solution of HCl(g), and the crude salt was recrystallized from MeCN to give **31n** as a white solid (485 mg, 74% from **13**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (br s, 2H), 7.34 (dd, J = 10.3, 2.9 Hz, 1H), 7.07 (dd, J = 8.3, 2.9 Hz, 1H), 6.82 (s, 1H), 6.80 (d, J = 7.8 Hz, 1H), 6.67 (d, J = 7.8 Hz, 1H), 5.96 (s, 2H), 4.56 (t, J = 6.3 Hz, 2H), 4.40 (t, J = 5.2 Hz, 2H), 3.33 (t, J = 5.2 Hz, 2H), 3.02 (t, J = 7.2 Hz, 2H), 2.82 (t, J = 6.3 Hz, 2H), 2.53 (t, J = 7.3 Hz, 2H), 1.7−1.5 (m, 4H); MS (FAB) m/z 402 (M^+ + 1).

6-Fluoro-8-[2-[4-(4-methoxyphenyl)butylamino]ethoxy]chroman-4-one Oxime Fumarate (2:1) (33). 6-Fluoro-8-[2-[4-(4-methoxyphenyl)butylamino]ethoxy]chroman-4-one (the free base of **31a**) was prepared according to method C. A mixture of the free base of **31a** (194 mg, 0.500 mmol), K_2CO_3 (276 mg, 2.00 mmol), NH_2OH·HCl (104 mg, 1.50 mmol), EtOH (4 mL), and H_2O (1 mL) was stirred at 85 °C for 2 h. The reaction mixture was diluted with 5% aqueous NaCl and extracted with CH_2Cl_2. The organic phase was dried, concentrated, and chromatographed ($CHCl_3$−MeOH, 98:2) to give the free base of **33** as a viscous oil (106 mg, 0.263 mmol, 53%). This was treated with fumaric acid (15.0 mg, 0.129 mmol), and the crude salt was recrystallized from EtOH−Et_2O to give **33** as colorless crystals (0.25 hydrate, 70 mg, 30% from the free base of **31a**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.48 (br s, 1H), 7.10 (d, J = 8.5 Hz, 2H), 7.05 (dd, J = 9.8, 3.0 Hz, 1H), 6.94 (dd, J = 10.4, 3.0 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 6.46 (s, 1H), 4.15 (t, J = 6.1 Hz, 2H), 4.13 (t, J = 5.5 Hz, 2H), 3.71 (s, 3H), 3.04 (t, J = 5.5 Hz, 2H), 2.80 (t, J = 6.1 Hz, 2H), 2.76 (t, J = 7.4 Hz, 2H), 2.53−2.50 (m, 2H), 1.6−1.5 (m, 4H); MS (EI) m/z 402 (M^+); mp 183−185 °C. Anal. ($C_{22}H_{27}FN_2O_4$·$0.5C_4H_4O_4$·$0.25H_2O$) C, H, N, F.

6-Fluoro-8-[2-[4-(4-methoxyphenyl)butylamino]ethoxy]chroman-4-ol Fumarate (2:1) (34). To an ice-cooled solution of the free base of **31a** (1.66 g, 4.30 mmol) in EtOH (20 mL) was added $NaBH_4$ (163 mg, 4.30 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with aqueous NH_4Cl, and the resulting mixture was extracted with EtOAc. The organic phase was dried, passed through a short column of silica gel, and concentrated to give the free base of **34** as a white solid (1.65 g, 99%). The free base (390 mg, 1.00 mmol) was treated with fumaric acid

(57 mg, 0.48 mmol), and the crude salt was recrystallized from EtOH−Et_2O to give **34** as white crystals (378 mg, 84%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.10 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.5 Hz, 2H), 6.80 (dd, J = 10.8, 2.9 Hz, 1H), 6.71 (dd, J = 8.8, 2.9 Hz, 1H), 6.45 (s, 1H), 4.57 (t, J = 4.8 Hz, 1H), 4.15 (t, J = 5.2 Hz, 2H), 4.11 (t, J = 5.6 Hz, 2H), 3.71 (s, 3H), 3.04 (t, J = 5.6 Hz, 2H), 2.78 (t, J = 6.8 Hz, 2H), 2.51 (t, J = 6.8 Hz, 2H), 2.0−1.8 (m, 2H), 1.6−1.5 (m, 4H); MS (FAB) m/z 390 (M^+ + 1); mp 139−141 °C. Anal. ($C_{22}H_{28}FNO_4$·0.5$C_4H_4O_4$) C, H, N, F.

(+)-6-Fluoro-8-[2-[4-(4-methoxyphenyl)butylamino]ethoxy]chroman-4-ol [(+)-34] and (−)-6-Fluoro-8-[2-[4-(4-methoxyphenyl)butylamino]ethoxy]chroman-4-ol [(−)-34]. A CHIRALPAK AD column (i.d. = 10 mm, l = 250 mm), packed with amylose tris(3,5-dimethylphenylcarbamate) stationary phase,[29] was treated with 0.1% Et_3N in EtOH prior to use. The free base of **34** (100 mg) was dissolved in EtOH (10 mL), and the solution (0.2 mL) was injected to the column and eluted with EtOH (1.2 mL/min) at ambient temperature. The eluates were detected by UV absorption at λ = 285 nm and pooled into fractions; the fractions from 40 runs were thus collected automatically. First was eluted (+)-**34** (t_R 16.1 min, total amount 31.0 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J = 8.8 Hz, 2H, meta to OMe), 6.81 (d, J = 8.8 Hz, 2H, ortho to OMe), 6.66 (dd, J = 8.8, 2.9 Hz, 1H, chroman aromatic), 6.59 (dd, J = 9.8, 2.9 Hz, 1H, chroman aromatic), 4.73 (t, J = 4.3 Hz, 1H, chroman C4−H), 4.3−4.2 (m, 2H, chroman C2−H_2), 4.06 (t, J = 5.4 Hz, 2H, OC$H_2$$CH_2$N), 3.78 (s, 3H, OCH_3), 3.01 (t, J = 5.4 Hz, 2H, OCH_2CH_2N), 2.68 (t, J = 7.1 Hz, 2H, ArCH_2), 2.57 (t, J = 7.3 Hz, 2H, NCH_2), 2.2−2.0 (m, 2H, chroman C3−H_2), 1.7−1.5 (m, 4H); MS (FAB) m/z 390 (M^+ + 1); $[\alpha]^{20}_D$ (c 0.15, $CHCl_3$) +39°; optical and chemical purity in the same chromatography conditions, respectively 100.0% and 99.5% (peak area). Subsequently was eluted (−)-**34** (t_R 18.5 min, total amount 30.1 mg): $[\alpha]^{20}_D$ (c 0.15, $CHCl_3$) −40°; optical and chemical purity, respectively 99.6% and 99.4%. The ^{1}H NMR and mass spectra of (−)-**34** were identical to those observed for (+)-**34**.

***N*-[2-[(6-Fluoro-4-methoxychroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamine Oxalate (1:1) (35).** The free base of **34** (200 mg, 0.510 mmol) was mixed with MeOH (2 mL) and concentrated HCl (0.08 mL), and the mixture was heated to reflux for 14 h. The mixture was made basic with 1 N NaOH and extracted with CH_2Cl_2. The organic phase was dried, concentrated, and chromatographed ($CHCl_3$−MeOH, 99:1) to give the free base of **35** (124 mg, 0.310 mmol, 60%). This was treated with oxalic acid (27 mg, 0.30 mmol) in MeOH to give **35** as white crystals (140 mg, 54% from the free base of **34**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.0 (2−3H, br s), 7.11 (d, J = 8.5 Hz, 2H), 6.91 (dd, J = 10.3, 3.0 Hz, 1H), 6.84 (d, J = 8.5 Hz, 2H), 6.75 (dd, J = 9.1, 3.0 Hz, 1H), 4.28−4.20 (m, 4H), 4.06 (td, J = 11.0, 2.4 Hz, 1H), 3.71 (s, 3H), 3.35 (s, 3H), 3.29 (t, J = 5.2 Hz, 2H), 3.02 (t, J = 7.6 Hz, 2H), 2.53 (t, J = 7.0 Hz, 2H), 2.06−1.91 (m, 2H), 1.7−1.6 (m, 4H); MS (EI) m/z 403 (M^+); mp 188 °C. Anal. ($C_{23}H_{30}FNO_4$·$C_2H_2O_4$) C, H, N, F.

***N*-[2-[(6-Fluoro-2*H*-chromen-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamine Fumarate (2:1) (36).** A mixture of the free base of **34** (625 mg, 1.60 mmol), p-TsOH·H_2O (366 mg, 1.93 mmol), 1,4-dioxane (10 mL), and toluene (5 mL) was heated to reflux for 1 h under azeotropic dehydration conditions. The mixture was diluted with EtOAc and washed successively with aqueous $NaHCO_3$, water, and brine. The organic phase was dried, concentrated, and chromatographed ($CHCl_3$−MeOH, 99:1) to give the free base of **36** (450 mg, 1.21 mmol, 76%). This was treated with fumaric acid (66 mg, 0.59 mmol), and the crude salt was recrystallized from EtOH−Et_2O to give **36** as crystals (384 mg, 56% from the free base of **34**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.10 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 6.82 (dd, J = 10.8, 3.1 Hz, 1H), 6.58 (dd, J = 8.5, 3.1 Hz, 1H), 6.46 (dt, J = 9.8, 1.8 Hz, 1H), 6.45 (s, 1H), 5.98 (dt, J = 9.8, 3.6 Hz, 1H), 4.71 (dd, J = 3.6, 1.8 Hz, 2H), 4.10 (t, J = 5.5 Hz), 3.71 (s, 3H), 3.00 (t, J = 5.5 Hz, 2H), 2.74 (t, J = 7.0 Hz, 2H), 2.51 (t, J = 7.3 Hz, 2H), 1.6−1.5 (m, 4H); MS (EI) m/z 371 (M^+); mp 139−141 °C. Anal. ($C_{22}H_{26}FNO_3$·0.5$C_4H_4O_4$) C, H, N, F.

6-Fluoro-8-[2-[2-(4-methoxyphenyl)ethylamino]ethoxy]chroman-4-ol fumarate (2:1) (37): prepared from **32** by a procedure similar to that described for **34** (72%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.0 Hz, 2H), 6.80 (dd, J = 10.0, 2.8 Hz, 1H), 6.70 (dd, J = 8.8, 2.8 Hz, 1H), 6.49 (s, 1H), 4.57 (t, J = 4.8 Hz, 1H), 4.2−4.1 (m, 2H), 4.09 (t, J = 5.6 Hz, 2H), 3.72 (s, 3H), 3.05 (t, J = 5.6 Hz, 2H), 2.94 (t, J = 7.6 Hz, 2H), 2.74 (t, J = 7.6 Hz, 2H), 2.0−1.8 (m, 2H); MS (FAB) m/z 362 (M^+ + 1); mp 153−155 °C (from EtOH). Anal. ($C_{20}H_{24}FNO_4$·0.5$C_4H_4O_4$) C, H, N, F.

6-Fluoro-8-[2-[*N*-methyl-4-(4-methoxyphenyl)butylamino]ethoxy]chroman-4-one Hydrochloride (39). *N*-Methyl-4-(4-methoxyphenyl)butylamine (**38**) was prepared by reduction of an *N*-ethoxycarbonyl derivative of **19a** ($LiAlH_4$, THF, reflux): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.11 (d, J = 8.8 Hz, 2H, meta to OMe), 6.82 (d, J = 8.8 Hz, 2H, ortho to OMe), 3.77 (s, 3H, OCH_3), 2.7−2.4 (m, 4H, benzylic and NCH_2), 2.41 (s, 3H, NCH_3), 1.84 (br s, 1H, NH), 1.8−1.4 (m, 4H); MS (EI) m/z 193 (M^+).

This was coupled with **13** by a procedure similar to method C to give **39** (48%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.70 (br s, 1H), 7.23−7.21 (m, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.92−6.89 (m, 1H), 6.82 (d, J = 8.5 Hz, 2H), 4.61 (br s, 1H), 4.54 (br s, 1H), 4.53 (t, J = 6.1 Hz, 2H), 3.78 (s, 3H), 3.56 (br s, 1H), 3.47 (br s, 1H), 3.28 (br s, 1H), 3.12 (br s, 1H), 2.92 (s, 3H), 2.80 (t, J = 6.1 Hz, 2H), 2.63 (t, J = 7.3 Hz, 2H), 1.93 (br s, 2H), 1.70 (br s, 2H); MS (FAB) m/z 402 (M^+ + 1); mp 126−128 °C (from THF−Et_2O). Anal. ($C_{23}H_{28}FNO_4$·HCl) C, H, N, Cl, F.

6-Fluoro-8-[2-[*N*-propyl-4-(1,3-benzodioxol-5-yl)butylamino]ethoxy]chroman-4-one Oxalate (1:1) (40). A mixture of the free base of **31n** (600 mg, 1.50 mmol), n-C_3H_7-Br (185 mg, 1.50 mmol), and K_2CO_3 (207 mg, 1.50 mmol) in MeCN (15 mL) was heated to reflux for 2 h. Insoluble matter was filtered off, and the filtrate was concentrated and chromatographed ($CHCl_3$−MeOH, 98:2) to give the free base of **40** as a solid (600 mg, 1.35 mmol, 90%). This was treated with oxalic acid (121 mg, 1.35 mmol), and the crude salt was recrystallized from MeCN to give **40** as a white solid (420 mg, 53% from the free base of **31n**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (dd, J = 9.8, 2.9 Hz, 1H), 7.05 (dd, J = 8.3, 2.9 Hz, 1H), 6.80 (d, J = 7.8 Hz, 1H), 6.79 (s, 1H), 6.65 (d, J = 7.8 Hz, 1H), 5.95 (s, 2H), 4.53 (t, J = 6.3 Hz, 2H), 4.34 (t, J = 4.8 Hz, 2H), 3.40 (br s, 2H), 3.10−2.95 (m, 4H), 2.80 (t, J = 6.3 Hz, 2H), 2.53 (t, J = 7.8 Hz, 2H), 1.7−1.5 (m, 6H), 0.90 (t, J = 7.3 Hz, 3H); MS (FAB) m/z 444 (M^+ + 1); mp 148−149 °C. Anal. ($C_{25}H_{30}FNO_5$·$C_2H_2O_4$) C, H, N, F.

Pharmacology. FSC Assay with Whole CHO Cells Expressing the Human 5-HT$_{1A}$ Receptors. CHO cells (stable transfectant of the human 5-HT$_{1A}$ receptor genomic gene,[30] 1.5 × 10^5 cells/well)[16] were plated in a 24-well plate 48 h before the experiment. The cells were preincubated with "reaction buffer" (1 mM 3-isobutyl-1-methylxanthine, 1 mM $MgCl_2$, and 0.85 mM $CaCl_2$ in PBS; 250 μL) for 20 min at 37 °C. After aspiration was added the reaction buffer (250 μL) containing 10 μM forskolin and the test drug; for the reversal experiments (to assess antagonist activity), 0.3 μM 8-OH-DPAT was also included in the reaction buffer. Cyclic AMP was then allowed to accumulate for 8 min at 37 °C. The reaction was terminated by the addition of 0.2 N aqueous HCl (250 μL), and the mixture was incubated on ice for 1 h. Cyclic AMP production was measured with a radioimmunoassay kit (Yamasa Shoyu, Chiba, Japan). The FSC activity was expressed as percentage of the control level based on the response that was induced by 0.3 μM 8-OH-DPAT.

Behavioral Responses. Male Wistar rats (Japan SLC) were treated with reserpine 18 h prior to use. Fifteen minutes after the administration of a test compound (or a vehicle), 8-OH-DPAT (0.25 mg/kg) was subcutaneously injected to rats to induce the behavioral responses. Forepaw treading (FT) and flat body posture (FBP)[25] were scored every 3 min for 15 min beginning 3 min after the injection of 8-OH-DPAT, according to a ranked intensity scale where 0 = absent, 1 =

occasional, 2 = frequent, 3 = continuous. Scores for the first 1 min of each 3-min period were summed for each animal.

Recording of Dorsal Raphe Cell Firing. Male Wistar rats (Japan SLC) were cannulated in their femoral vein after being anesthetized. The rats were placed in a stereotaxic apparatus; then a tungsten electrode (10 MΩ at 100 Hz) was lowered into the dorsal raphe nucleus (A, 1.2; L, 0.0; H, 3.5; from the interaural line).[31] The serotonergic neurons were identified by broad duration (2−3 ms) and slow firing rate (0.5−2.5 Hz) with regular rhythm.[27] Baseline activity of these neurons was recorded for 10 min, and then saline was infused. Five minutes later, a test compound was injected cumulatively at 5-min intervals in a volume of 0.3 mL/kg. The spontaneous cell firing was calculated for 2 min starting 30 s after the injection and expressed as percentage of the baseline firing rate. For the evaluation of its antagonist activity, **31n** was infused 5 min before the cumulative injection of 8-OH-DPAT.

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