N-[2-[(Substituted chroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamines: Synthesis and Wide Range of Antagonism at the Human 5-HT$_{1A}$ Receptor

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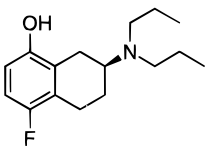
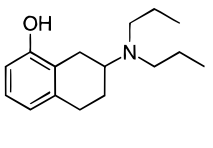
A series of *N*-[2-[(substituted chroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamines was prepared and examined for their 5-HT$_{1A}$ receptor antagonist activity. The parent compound **3a** and seven analogs bearing five kinds of substituents on the chroman ring were prepared from the corresponding 8-hydroxychroman intermediates. Radioligand binding assays proved the compounds **3a**−**h** to have high affinity for the rat hippocampal 5-HT$_{1A}$ receptor with varied selectivity for adrenaline α_1 and dopamine D_2 receptors. Their antagonism was evaluated in a forskolin-stimulated adenylate cyclase assay performed with CHO cells expressing the human 5-HT$_{1A}$ receptor. Among the series, the C6-fluoro analog **3c** showed both extremely potent affinity (K_i = 0.22 nM) and antagonism (EC_{50} = 13 nM) for the 5-HT$_{1A}$ receptor. Correlation analysis using substituent descriptors revealed a linear and negative correlation between molar refractivity of the C6-substituent and the binding affinity expressed in pK_i.

Introduction

The 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor is one of the 5-HT receptor subpopulations in which great interest has been taken. Numerous articles and reviews have dealt with structure−*affinity* relationships for various classes of 5-HT$_{1A}$ receptor ligands. However, only fragmentary information is available on the structural features or requirements for agonism or antagonism.[1−3] This is largely due to the lack of a standard assay procedure to quantify the agonist or antagonist property of the ligands. In vivo pharmacological models,[4] which are the most frequently used assays, may involve various difficulties arising from the diversity of the ligand pharmacokinetics, "cross talk" with other receptor systems, the coexistence of contrarily acting pre- and postsynaptic 5-HT$_{1A}$ receptors, etc. Therefore, it is dangerous to compare compounds of different classes in these assays. The forskolin-stimulated adenylate cyclase (FSC) assay in animal hippocampal cells is a potential in vitro measure of the activity of 5-HT$_{1A}$ receptor ligands, but the assay is difficult to conduct because its 5-HT$_{1A}$-sensitive fraction is generally small (usually less than 30% of total FSC).[5,6] Consequently, we can find very few examples of structure−*activity* relationship (SAR) studies based on this assay in the literature.[7,8] In such a study, (*S*)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin [(*S*)-UH-301, (*S*)-**1**] was reported as one of the analogs of the standard agonist 8-OH-DPAT (**2**).[9] This C5-fluoro analog antagonizes various effects of **2** itself including the reduction of FSC activity.[10] This is simple but significant information on SAR for the 5-HT$_{1A}$ receptor ligands.



(*S*)-**1**



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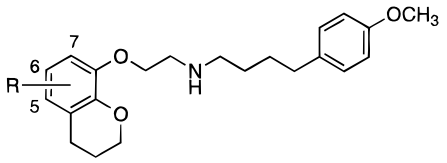
A series of 2-(aryloxy)ethylamine derivatives was found by us which showed equipotent affinity for both α_1 and 5-HT$_{1A}$ receptors. Further modifications of the structure led to the chroman derivative **3a** which had improved affinity and selectivity for the 5-HT$_{1A}$ receptor. We have now addressed our attention to substitution on the chroman ring and its effect on the antagonist activity. In order to quantify and compare the activity, we performed the FSC assay in Chinese hamster ovary (CHO) cells expressing the human 5-HT$_{1A}$ receptor. Thus a series of *N*-[2-[(substituted chroman-8-yl)oxy]-ethyl]-4-(4-methoxyphenyl)butylamines **3a**−**h** was synthesized and examined for 5-HT$_{1A}$ receptor antagonist activity.



3a–h

Chemistry

The synthesis of 8-hydroxychroman intermediates is summarized in Scheme 1. Both **5a**,**b** were prepared from the methoxy-substituted precursors. The C8-oxygen functionality of the C6-halo-substituted compounds (**5c**,**d**) was introduced via the Friedel−Crafts acylation, Baeyer−Villiger oxidation, and saponification. For the synthesis of **5e**,**g**,**h**, an alternative oxidative process was employed to avoid regioscrambling during the acylation step. For example, 6-methoxy-4-chromanone (**8e**) was initially oxidized to 1,5-benzodioxepine **11e** and subsequently treated with $AlCl_3$ to undergo Fries rearrangement, and then the resulting **12e** was reduced by heterogeneous catalytic hydrogenation to give **5e**.

The 8-hydroxychroman intermediates **5a**−**e**,**g**,**h** were converted to the desired products via *O*-bromoethylation followed by coupling with the amine **14** (Scheme

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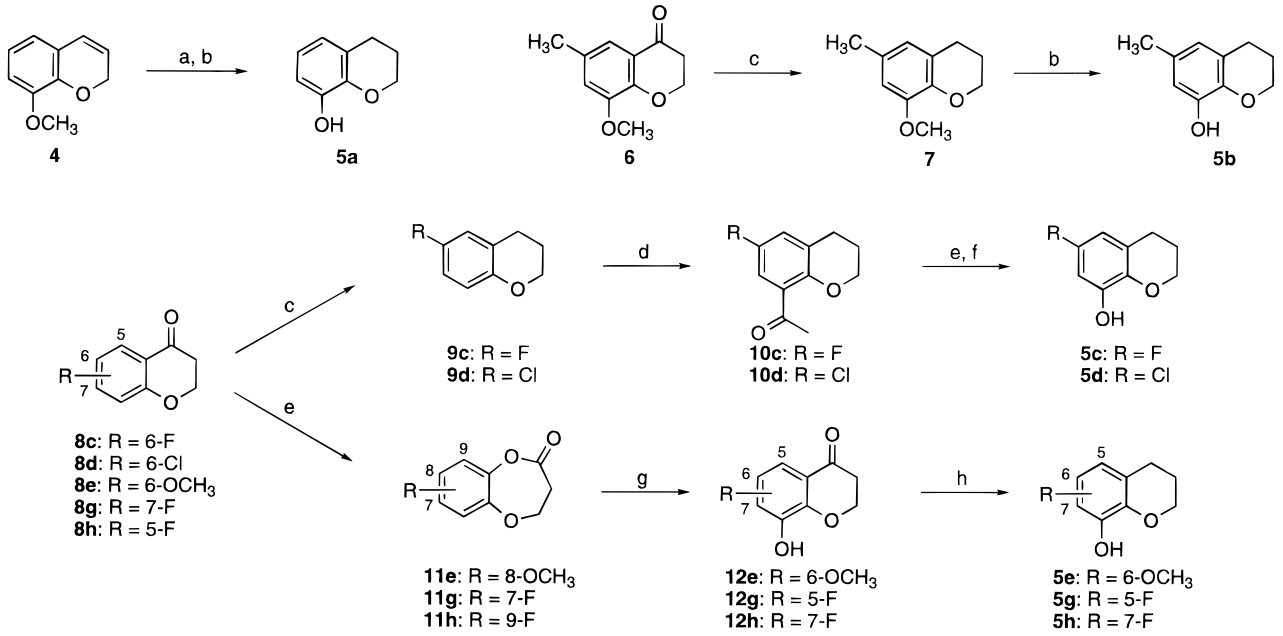
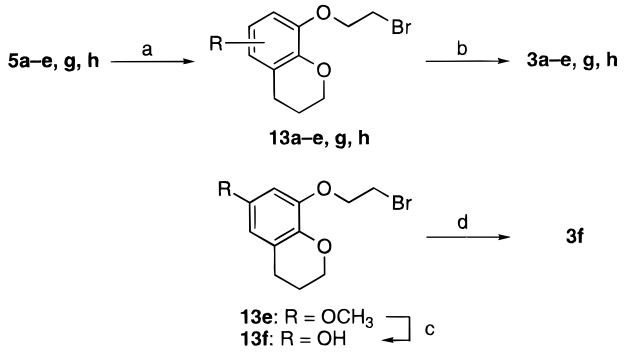
Scheme 1[a]



[a] Conditions: (a) 1 atm of H_2, Pd−C, EtOH, rt; (b) cHBr, reflux; (c) 1 atm of H_2, Pd−C, AcOH, rt; (d) CH_3COCl, $AlCl_3$, DCE, 0 °C; (e) mCPBA, TFA, CH_2Cl_2, rt; (f) $NaOH_{aq}$, MeOH; (g) $AlCl_3$, DCE, rt; (h) 4 atm of H_2, Pd−C, AcOH, rt.

Scheme 2[a]



[a] Conditions: (a) $BrCH_2CH_2Br$, $NaOH_{aq}$, n-$Bu_4N^+HSO_4^-$, 70 °C; (b) 4-$CH_3OC_6H_4(CH_2)_4NH_2$ (**14**), K_2CO_3, CH_3CN, reflux; (c) cHBr, reflux; (d) **14**, CH_3CN, reflux.

2). Phase-transfer conditions facilitated the bromoethylation process. Demethylation of **13e** under acidic conditions gave **13f**, which was then treated with an excess of **14** without additional base to lead to **3f**. The yields of the final coupling step, melting points, and formulae for the analytically pure materials are listed in Table 1.

Pharmacology

The compounds **3a**−**h** were evaluated for in vitro activity using radioligand binding assays and the FSC assay. Binding assays for 5-HT$_{1A}$, α_1, and D_2 receptors were performed according to standard protocols using rat brain tissues. The results are given in K_i[11] and listed in Table 2. The FSC assay was performed with CHO cells expressing the human 5-HT$_{1A}$ receptor. The antagonist activity was assessed in the presence of 0.1 μM **2**.

Results and Discussion

Radioligand binding studies demonstrated that **3a**−**h** possess potent affinity for the rat hippocampal 5-HT$_{1A}$ receptor and differ in their selectivity for α_1 and D_2 receptors (Table 2). The rank order of the substituent for 5-HT$_{1A}$ receptor affinity was 5-F > H > 6-F ≈ 6-OH ≈ 7-F > 6-CH_3 > 6-Cl > 6-OCH_3, generally the reverse of that for the relative steric bulk (*vide infra*). In contrast, binding to the other receptors was somewhat more complicated, and the steric factor was not dominant. For example, introduction of fluorine at the C5-position (**3g**) resulted in a substantial reduction of α_1 and D_2 receptor affinities along with a slight enhancement of 5-HT$_{1A}$ receptor affinity. Thus the compound **3g** displayed extraordinarily high affinity (K_i = 0.051 nM) and excellent selectivity (172-fold vs α_1, 69-fold vs D_2) for the 5-HT$_{1A}$ receptor.

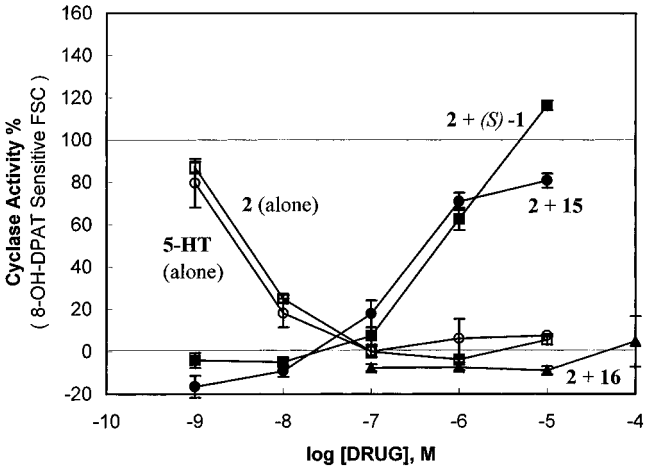


Figure 1. Effects of known compounds in the FSC assay with CHO cells expressing the human 5-HT$_{1A}$ receptor: inhibition of FSC by **2** (□) and 5-HT (○) and reversal by (*S*)-**1** (■), **15** (●), and **16** (▲), in the presence of 0.1 μM **2**. All experiments were performed in triplicate, and each point represents mean ± SEM.

The response of our FSC system was examined using the standard agonists (Figure 1). It is well established that the 5-HT$_{1A}$ receptor is negatively coupled with adenylate cyclase and that its agonists reduce the FSC activity.[12] Thus **2** and the intrinsic agonist 5-HT were

Table 1. *N*-[2-[(Substituted chroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamines

compd	substituent	formula[a]	yield, %[b]	mp, °C (solvent)	anal.
3a	H	$C_{22}H_{29}NO_3 \cdot 0.5C_4H_4O_4$	51	123−125 (*i*-PrOH)	C,H,N
3b	6-CH_3	$C_{23}H_{31}NO_3 \cdot 0.5C_4H_4O_4$	60	121−123 (*i*-PrOH−*i*-Pr_2O)	C,H,N
3c	6-F	$C_{22}H_{28}FNO_3 \cdot 0.5C_4H_4O_4$	60	138−141 (EtOH−Et_2O)	C,H,N,F
3d	6-Cl	$C_{22}H_{28}ClNO_3 \cdot 0.5C_4H_4O_4$	37	145−146 (EtOH−Et_2O)	C,H,N,Cl
3e	6-OCH_3	$C_{23}H_{31}NO_4 \cdot HCl$	56	111−112 (*i*-PrOH−*i*-Pr_2O)	C,H,N,Cl
3f	6-OH	$C_{22}H_{29}NO_4 \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$	44	175−176 (acetone)	C,H,N
3g	5-F	$C_{22}H_{28}FNO_3 \cdot 0.5C_4H_4O_4$	57	129−130 (EtOH)	C,H,N,F
3h	7-F	$C_{22}H_{28}FNO_3 \cdot C_2H_2O_4$	66	161−163 (EtOH)	C,H,N,F

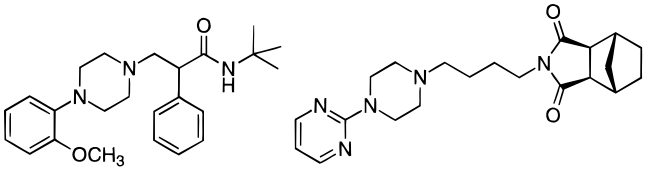
[a] $C_4H_4O_4$ and $C_2H_2O_4$ represent fumaric acid and oxalic acid, respectively. [b] Yield refers to analytically pure material obtained at the final coupling step (unoptimized).

Table 2. Receptor Binding Profile of **3a**−**h**, (*S*)-**1**, **15**, and **16**[a]

compd	receptor affinity (K_i, nM)[b]		
	5-HT_{1A}	α_1	D_2
3a	0.118 (0.116−0.120)	5.19 (5.10−5.29)	0.581 (0.564−0.599)
3b	0.779 (0.760−0.797)	35.1 (34.4−35.9)	1.79 (1.74−1.85)
3c	0.221 (0.219−0.223)	2.71 (2.66−2.76)	0.259 (0.250−0.268)
3d	1.29 (1.26−1.30)	14.3 (14.2−14.4)	0.489 (0.474−0.504)
3e	3.07 (2.99−3.16)	27.9 (26.3−29.7)	1.75 (1.72−1.77)
3f	0.271 (0.269−0.274)	98.4 (95.8−101.1)	1.76 (1.69−1.84)
3g	0.051 (0.050−0.052)	8.77 (8.54−9.01)	3.53 (3.38−3.68)
3h	0.350 (0.340−0.359)	71.5 (70.3−72.8)	21.3 (19.7−22.9)
(*S*)-**1**[c]	46.0 (45.4−46.6)	6080 (5980−6180)	614 (537−701)
15[d]	27.9 (27.4−28.4)	897 (882−913)	176 (172−181)
16[e]	21.5 (21.2−21.8)	1260 (1220−1300)	ND[f]

[a] Radioligands (tissues) used were [^{3}H]-8-OH-DPAT (rat hippocampus), [^{3}H]prazosin (rat cortex), and [^{3}H]spiperone (rat striatum) for 5-HT_{1A}, α_1, and D_2 receptors, respectively. [b] K_i values were obtained from two or three experiments each performed in triplicate. Values in parentheses indicate 95% confidence intervals. [c] Lit.: 5-HT_{1A}, K_i = 52 nM; D_2, K_i = 400 nM (ref 9). [d] Lit.: 5-HT_{1A}, IC_{50} = 33.9 ± 3.3 nM; α_1, IC_{50} = 1490 ± 404 nM (ref 13). [e] Lit.: 5-HT_{1A}, K_i = 25.0 nM (ref 14). [f] Not determined.

applied to the system, and each of them dose-dependently reduced the activity. The agonist-sensitive fraction, typically 40% of the total FSC, was about 1.5−2 times greater than that observed in animal hippocampal tissue preparations.[5,6] The effect of **2** (0.1 μM) was reversed by the well-established antagonists (*S*)-**1** and (±)-*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropanamide (WAY-100135, **15**)[13] in a dose-dependent manner. The EC_{50} values (refer to the ligand concentration causing 50% recovery of FSC) were 510 and 320 nM, respectively. In contrast, the partial agonist tandospirone (**16**)[14] (0.1−100 μM) did not affect the reduced FSC level. As reported by Bockaert et al.[6] for buspirone in the usual FSC assay, buspirone-type partial agonists may behave as agonists toward FSC; in other words, FSC assays may potentially discriminate antagonists from partial agonists. Based on these results, we concluded the assay to be appropriate for the assessment of **3a**−**h** and decided to carry out the reversal experiment for direct comparison of their antagonism.



15 **16**

Figure 2 shows the dose−response of **3a**−**f** toward the reduction of FSC activity induced by 0.1 μM **2**. Compounds **3c** (6-F), **3d** (6-Cl), and **3e** (6-OCH_3) fully reversed the reduction with EC_{50} values of 14, 25, and

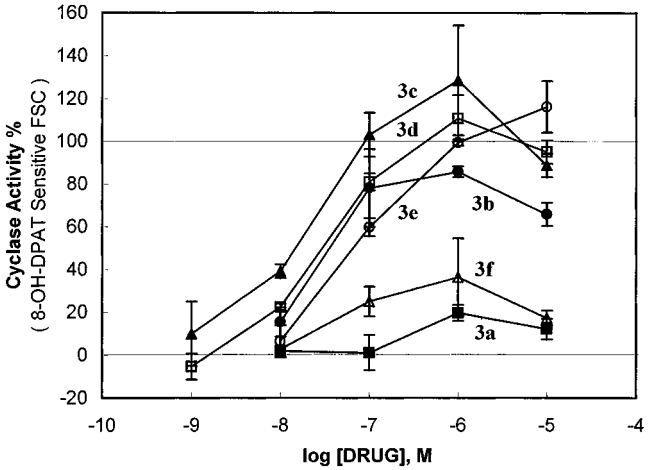


Figure 2. Effects of **3a**−**f** in the FSC assay with CHO cells expressing the human 5-HT_{1A} receptor: reversal of FSC by **3a** (■), **3b** (●), **3c** (▲), **3d** (□), **3e** (○), and **3f** (△), in the presence of 0.1 μM **2**. All experiments were performed in triplicate, and each point represents mean ± SEM.

63 nM, respectively. The 6-methyl derivative **3b** showed a reversal similar to **3d** at lower concentrations, whereas the maximum effect did not appear to reach the full level. The 6-hydroxy derivative **3f** was still less effective, and the unsubstituted molecule **3a** exhibited no obvious antagonism. Furthermore, compounds **3g** (5-F) and **3h** (7-F) were tested at a single concentration (1 μM) to show no significant recovery of the FSC level (**3g**, 16.2 ± 5.9%; **3h**, −9.0 ± 6.6%). Thus, not only the type of the substituent but also the position significantly affected the antagonism of the series. The compounds **3c**−**e** would be regarded as potent 5-HT_{1A} receptor antagonists, whereas the others might be weaker antagonists or (partial) agonists which widely vary in their antagonist activity.

We attempted some quantitative approaches to elucidate the relationships between the substituents and the biological activities. The highly flexible nature of the compounds discouraged us from energy minimization-based 3D modeling. We then performed correlation analyses using various substituent parameters. A linear and negative correlation was thus found between molar refractivity (MR)[15] of the C6-substituent and 5-HT_{1A} binding affinity, expressed in pK_i (−log K_i), of compounds **3a**−**f** (Figure 3). This may support our previously mentioned suggestion; that is, in other words, the smaller the C6-substituent, the higher affinity by the derivative. Another analysis demonstrated the correlation between pEC_{50} (−log EC_{50}) and pK_i of the compounds for which the EC_{50} values were available (Figure 4). The result of the regression analysis (R^2 = 0.979) indicates this correlation is well expandable to

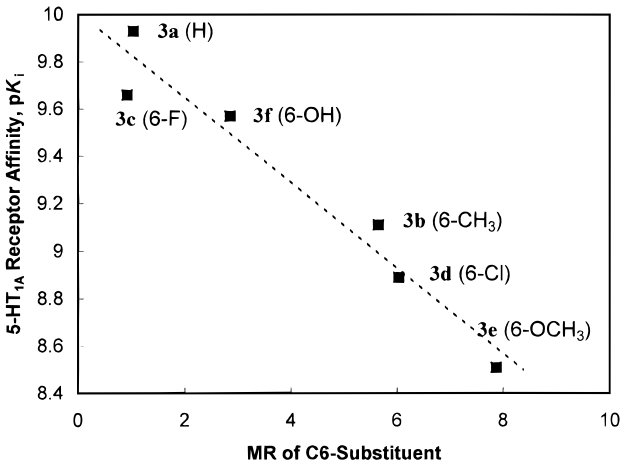


Figure 3. Plot of 5-HT$_{1A}$ receptor affinity in pK_i versus MR of C6-substituent, for **3a**−**f**. The broken line represents the linear regression (pK_i = −0.181MR + 10.0; R^2 = 0.949, n = 6).

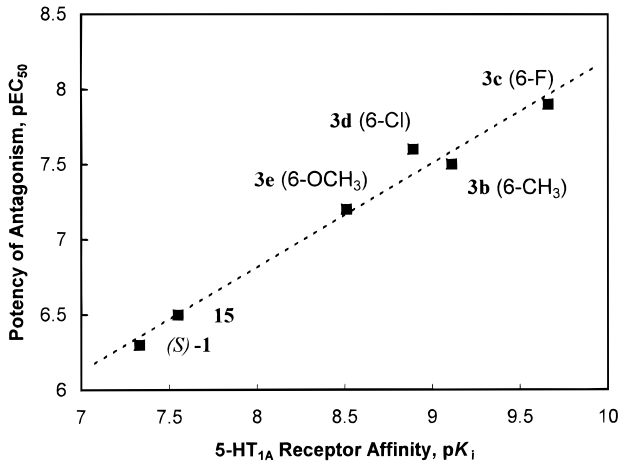


Figure 4. Plot of 5-HT$_{1A}$ receptor antagonism in pEC$_{50}$ versus the affinity in pK_i, for **3b**−**e**, (*S*)-**1**, and **15**. The broken line represents the linear regression (pEC$_{50}$ = 0.693pK_i + 1.27; R^2 = 0.979, n = 6).

the other classes of compounds [i.e., (*S*)-**1** and **15**]. Thus, the EC$_{50}$ value appears to reflect the binding affinity in the case of full or fairly potent antagonists. However, we could explain neither the lower antagonist activity of the compounds possessing higher affinity (**3a**,**g**) nor the essential difference between the compounds having higher (**3b**−**e**) and lower (**3a**,**f**−**h**) antagonist activity in terms of steric, electronic, or hydrophobic parameters.

Sylte et al. recently demonstrated molecular dynamics simulations of **1** and **2** with a refined version of a human 5-HT$_{1A}$ receptor model.[16] They reported that the fluorine atom induced alteration of the electrostatic potentials around the C8-hydroxy group to weaken the hydrogen bonding between the group and the receptor. Hillver et al., who originally reported **1**, stressed the difference in electronic distribution between **1** and **2**.[9] In the case of the present compounds, however, we cannot explain the wide range of antagonism simply by differences in the electronic distribution for the following reasons: (1) Although methoxy-substituted **3e** and hydroxy-substituted **3f** behave differently in the FSC assay, the electrostatic characteristics of these substituents are quite similar. (2) Both the moieties of the electron-donating methoxy (in **3e**) and the electron-withdrawing chloro (in **3d**) elicit similar antagonism when incorporated in **3a**.

The present data seem to indicate that the antagonism of the present compounds is a function of multiple factors partly or largely independent from those that determine the binding affinity. In order to fully characterize the compounds and to better understand the receptor−ligand interactions, further studies are required in areas of radiolabeled ligand binding, receptor mutagenesis, computational modeling, etc.

In conclusion, the FSC assay with CHO cells expressing the human 5-HT$_{1A}$ receptor revealed a wide range of antagonism within the series and provided some hints for their SAR. The striking effects of the substituents emphasize the importance of a detailed and comprehensive survey of the closely related analogs. The present study also provided compound **3c** which showed both extremely potent affinity and antagonism for the 5-HT$_{1A}$ receptor, though its selectivity was not satisfactory. It has been proposed that selective 5-HT$_{1A}$ receptor antagonists may have a therapeutic potential in the treatment of various central nervous system disorders.[17] Such pinpoint modifications as demonstrated here will be applicable to other classes of ligands and will serve as a powerful tool for the development of novel 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 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. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of theoretical values. Preparative column chromatography was performed with Wakogel C-200 (Wako; 100−200 mesh). Drying of organic solutions during workup was done over anhydrous $MgSO_4$.

Authentic Materials. Compound (*S*)-**1** [(*S*)-UH-301] was prepared as a hydrochloride salt according to the reported method:[9] mp 216−218 °C (lit. mp 215.5−217 °C); $[\alpha]^{22}_D$ (*c* 1.0, MeOH) −82.8° (lit. −83.7°). Anal. ($C_{16}H_{24}NOF\cdot HCl$) C, H, N, Cl, F. Compound **15** (WAY-100135) was prepared as a dihydrochloride salt according to the reported method:[13] mp 220−223 °C (lit. mp 230−231 °C). Anal. ($C_{24}H_{33}N_3O_2\cdot 2HCl$) C, H, N, Cl. Compound **16** (tandospirone) was prepared as a citrate salt according to the reported method:[14] mp 167.5−168.5 °C (lit. mp 169.5−170 °C). Anal. ($C_{21}H_{29}N_5O_2\cdot C_6H_8O_7$) C, H, N.

8-Acetyl-6-fluorochroman (10c). 6-Fluorochroman (**9c**) was previously obtained from 6-fluoro-4-chromanone (**8c**) by catalytic hydrogenation. To a cooled (ice−salt) suspension of $AlCl_3$ (3.86 g, 28.9 mmol) in 1,2-dichloroethane (DCE; 50 mL) was added a solution of acetyl chloride (2.48 g, 31.6 mmol) in DCE (4 mL), and the mixture was stirred below −5 °C for 15 min. A DCE solution (8 mL) of **9c** (4.0 g, 26.3 mmol) was then added dropwise to the mixture. The temperature was maintained below −10 °C during the addition. After the addition of the substrate, the reaction mixture was stirred at ice−salt temperature (ca. −10 °C) for 1 h; the mixture was then poured into ice−water and extracted with Et_2O. The organic phase was washed with saturated $NaHCO_3$ solution followed by brine, dried, and concentrated to give **10c** as an oil (4.94 g, 98%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.24 (1H, dd, J = 9.0, 3.2 Hz, C7-H), 6.90 (1H, dd, J = 8.1, 3.2 Hz, C5-H), 4.26 (2H, t, J = 5.2 Hz, C2-H_2), 2.83 (2H, t, J = 6.8 Hz, C4-H_2), 2.59 (3H, s, CH_3), 2.16−1.89 (2H, m, C3-H_2); MS(EI) *m/e* 169 ($M^+ - CH_3$), 194 (M^+).

6-Fluoro-8-chromanol (5c). Baeyer−Villiger oxidation was carried out under Canan Koch's conditions.[18] To a cooled (ice bath) solution of **10c** (4.90 g, 25.2 mmol) and *m*-chloroperbenzoic acid (mCPBA; 80% purity, 10.9 g, 50.5 mmol) in CH_2Cl_2 (100 mL) was added trifluoroacetic acid (TFA; 1.94 mL, 25.2 mmol), and the mixture was allowed to warm up to room temperature. After stirring overnight (14 h), the resulting suspension was diluted with CH_2Cl_2 (100 mL) and cooled to 0 °C. To the cooled suspension was added slowly chilled 5% aqueous Na_2SO_3 (100 mL), and the mixture was vigorously stirred until the exothermic reaction ceased. The precipitate formed was removed by filtration and washed with CH_2Cl_2, then the filtrate was separated, and the organic phase was washed with saturated $NaHCO_3$ solution followed by brine, dried, and concentrated (**caution**: prior to concentration the absence of oxidative species should be confirmed with a test strip). To a solution of the residual material in MeOH (60 mL) was added dropwise 1 N NaOH (55 mL), and the mixture was stirred at room temperature for 1 h. The mixture was then acidified with 1 N HCl to form a precipitate which was collected, washed with water, and then recrystallized from hexane−EtOAc (4:1) to give **5c** as a yellow solid (3.10 g, 73%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.48 (1H, dd, J = 9.7, 3.0 Hz, C5-H), 6.31 (1H, dd, J = 9.2, 3.0 Hz, C7-H), 5.61 (1H, s, OH), 4.21 (2H, t, J = 5.3 Hz, C2-H_2), 2.74 (2H, t, J = 6.6 Hz, C4-H_2), 2.14−1.88 (2H, m, C3-H_2); MS(EI) *m/e* 168 (M^+).

6-Chloro-8-chromanol (5d): prepared from 6-chloro-4-chromanone (**8d**) by a procedure similar to that described for **5c**; ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.73 (1H, d, J = 2.8 Hz, C5-H), 6.57 (1H, d, J = 2.8 Hz, C7-H), 5.23 (1H, s, OH), 4.22 (2H, t, J = 5.4 Hz, C2-H_2), 2.73 (2H, t, J = 6.3 Hz, C4-H_2), 2.13−1.87 (2H, m, C3-H_2); MS(EI) *m/e* 184 (M^+).

8-Methoxy-1,5-benzodioxepin-2-one (11e). To a solution of 6-methoxy-4-chromanone (**8e**; 4.45 g, 25.0 mmol) in CH_2Cl_2 (50 mL) were added mCPBA (80% purity, 7.01 g, 32.5 mmol) and TFA (285 mg, 2.50 mmol), and the mixture was stirred at room temperature for 15 h. Na_2HPO_4 (3.55 g, 25.0 mmol) and Na_2SO_3 (3.15 g, 25.0 mmol), both well ground, were added successively to the mixture, and the suspension was vigorously stirred for 2 h. Insoluble materials were filtered off, and the filtrate was quickly washed with chilled $NaHCO_3$ solution and brine, then dried, and concentrated. The residual solid material was recrystallized from *i*-Pr_2O to give **11e** as yellow granules (2.70 g, 55%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.12−7.01 (1H, m, C6-H), 6.76−6.62 (2H, m, C7-H, C9-H), 4.50 (2H, t, J = 6.8 Hz, C4-H_2), 3.79 (3H, s, OCH_3), 2.85 (2H, t, J = 6.8 Hz, C3-H_2); MS(EI) *m/e* 194 (M^+).

6-Methoxy-4-oxo-8-chromanol (12e). To a cooled (ice bath) suspension of $AlCl_3$ (3.43 g, 25.8 mmol) in DCE (25 mL) was added **11e** (2.50 g, 12.9 mmol), and the reaction vessel was shielded from light. The mixture was stirred at room temperature for 3 days, poured into ice−water, and then extracted with EtOAc. The organic phase was washed with water and brine, dried, and concentrated to give solid material, which was purified by column chromatography eluted with $CHCl_3$−MeOH (100:0 to 100:1) to give **12e** as a yellow solid (1.21 g, 48%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.88 (1H, d, J = 3.0 Hz, C5-H), 6.76 (1H, d, J = 3.0 Hz, C7-H), 5.52 (1H, br s, OH), 4.59 (2H, t, J = 6.4 Hz, C2-H_2), 3.78 (3H, s, OCH_3), 2.83 (2H, t, J = 6.4 Hz, C3-H_2); MS(FAB) *m/e* 194 (M^+), 195 (M^+ + 1).

6-Methoxy-8-chromanol (5e). A mixture of **12e** (970 mg, 5.0 mmol), 10% Pd−C (194 mg), and glacial acetic acid (20 mL) was stirred under 4 atm of H_2 at room temperature for 48 h. The mixture was filtered on a Celite pad, and the filtrate was concentrated. The residue was purified by column chromatography eluted with $CHCl_3$ to give **5e** as an oil (610 mg, 68%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.37 (1H, d, J = 3.1 Hz, C5-H), 6.14 (1H, d, J = 3.1 Hz, C7-H), 5.59 (1H, s, OH), 4.17 (2H, t, J = 5.2 Hz, C2-H_2), 3.71 (3H, s, OCH_3), 2.73 (2H, t, J = 6.5 Hz, C4-H_2), 2.12−1.86 (2H, m, C3-H_2); MS(EI) *m/e* 180 (M^+).

5-Fluoro-8-chromanol (5g): prepared as an oil from 7-fluoro-4-chromanone (**8g**) by a procedure similar to that described for **5e**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.67 (1H, dd, J = 8.5, 4.5 Hz, C7-H), 6.48 (1H, dd, J = 8.5, 8.1 Hz, C6-H), 5.21 (1H, s, OH), 4.24 (2H, t, J = 5.2 Hz, C2-H_2), 2.74 (2H, t, J = 6.7 Hz, C4-H_2), 2.04−1.99 (2H, m, C3-H_2); MS(EI) *m/e* 168 (M^+).

7-Fluoro-8-chromanol (5h): prepared as an oil from 5-fluoro-4-chromanone (**8h**) by a procedure similar to that described for **5e**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.60 (1H, dd, J = 9.9, 8.5 Hz, C6-H), 6.50 (1H, dd, J = 8.5, 6.1 Hz, C6-H), 5.25 (1H, s, OH), 4.25 (2H, t, J = 5.3 Hz, C2-H_2), 2.74 (2H, t, J = 6.4 Hz, C4-H_2), 2.04−1.99 (2H, m, C3-H_2); MS(EI) *m/e* 168 (M^+).

Synthesis of 3a−e,g,h: General Procedure. The synthesis of *N*-[2-[(6-fluorochroman-8-yl)oxy]ethyl]-4-(4-methoxyphenyl)butylamine fumarate (2:1) (**3c**) is typical. A mixture of **5c** (1.68 g, 10 mmol), 1,2-dibromoethane (17.2 mL, 200 mmol), 3 N NaOH (6.7 mL, 20.1 mmol), and $Bu_4N^+HSO_4^-$ (170 mg, 0.50 mmol) was vigorously stirred at 70 °C for 2 h. Another portion of 3 N NaOH (3.3 mL, 9.9 mmol) was added, and the reaction mixture was stirred for 1 h at the same temperature. After cooling, CH_2Cl_2 (50 mL) was added to the mixture and the organic phase was separated, washed successively with 1 N NaOH, 1 N HCl, water, and brine, then dried, and concentrated. The residual solid material was recrystallized from MeOH to give 8-(2-bromomethoxy)-6-fluorochroman (**13c**) as a brownish solid (2.22 g, 81%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.49 (1H, dd, J = 9.8, 3.0 Hz, C5-H), 6.42 (1H, dd, J = 8.5, 3.0 Hz, C7-H), 4.28 (2H, t, J = 6.7 Hz, OCH_2), 4.22 (2H, t, J = 5.3 Hz, C2-H_2), 3.64 (2H, t, J = 6.7 Hz, CH_2Br), 2.76 (2H, t, J = 6.7 Hz, C4-H_2), 2.02−1.97 (2H, m, C3-H_2); MS(EI) *m/e* 274 (M^+), 276 (M^+ + 2).

The bromide **13c** (550 mg, 2.0 mmol), 4-(4-methoxyphenyl)-butylamine (**14**; 1.08 g, 6.0 mmol), K_2CO_3 (415 mg, 3.0 mmol), and CH_3CN (20 mL) were mixed and refluxed for 5 h. Acetone (40 mL) was added to the mixture, and insoluble material was removed by filtration. The filtrate was concentrated and purified by column chromatography eluted with $CHCl_3$−MeOH (100:1) to give the free base of **3c** as a slightly yellow oil (553 mg, 1.48 mmol, 74%). This was treated with fumaric acid (83 mg, 0.72 mmol) in EtOH and recrystallized from EtOH−Et_2O to give **3c** as white crystals (520 mg, 60% from **13c**): mp 138−141 °C. Anal. ($C_{22}H_{28}FNO_3 \cdot 0.5C_4H_4O_4$) C, H, N, F.

8-[2-[[4-(4-Methoxyphenyl)butyl]amino]ethoxy]-6-chromanol Fumarate (2:1) (3f). 8-(2-Bromoethoxy)-6-methoxychroman (**13e**) was obtained from **5e** as described for **13c**. A mixture of **13e** (400 mg, 1.39 mmol) and 48% HBr (8 mL, 71 mmol) was heated to reflux for 30 min. After that period, the mixture was poured into ice−water and extracted with EtOAc. The organic phase was dried and concentrated; then the residue was separated by column chromatography eluted with $CHCl_3$. The first fraction was collected and purified by recrystallization from *i*-Pr_2O−hexane to give 8-(2-bromoethoxy)-6-chromanol (**13f**) as amber crystals (52 mg, 14%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.36−6.27 (1H, m, C5-H), 6.22−6.14 (1H, m, C7-H), 4.38−4.12 (4H, m, OCH_2, C2-H_2), 3.63 (2H, t, J = 6.8 Hz, CH_2Br), 2.73 (2H, t, J = 6.5 Hz, C4-H_2), 2.10−1.86 (2H, m, C3-H_2); MS(EI) *m/e* 272 (M^+), 274 (M^+ + 2).

The bromide **13f** (50 mg, 0.183 mmol) and the amine **14** (164 mg, 0.915 mmol) were dissolved in CH_3CN (1 mL), and the solution was stirred at 75 °C for 2.5 h. The mixture was concentrated and purified by preparative TLC (Kieselgel 60F_{254}; Merck) developed twice with $CHCl_3$−MeOH−ammonia (85:15:1.5) to give the free base of **3f** as an oil (34 mg, 0.092 mmol, 50%). This was treated with fumaric acid (5.3 mg, 0.046 mmol) in acetone and recrystallized from acetone to give **3f** as white crystals (35 mg, 44% from **13f**): mp 175−176 °C. Anal. ($C_{22}H_{29}NO_4 \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

Receptor Binding Assays. 5-HT_{1A} receptor binding assays were performed according to the method of Peroutka with [^{3}H]-8-OH-DPAT on rat hippocampus.[19] Adrenaline α_1 and dopamine D_2 receptor binding assays were performed according to the reported method with [^{3}H]prazosin on rat cortex and [^{3}H]spiperone on rat striatum, respectively.[20,21]

Cloning and Expression of Human 5-HT_{1A} Receptor. Human 5-HT_{1A} receptor genes[22] were amplified from human placenta genomic DNA by the PCR using oligonucleotides: 5′-TTAGATCTCGAATCTTCGCGCTGCTTTTTCTTCCCTCC-3′ and 5′-AAAGATCTAGTGAATGGGACGGATCCTGTAGCCT-

CGAC-3′. The fragments were inserted downstream of the SV40 early promoter in the expression vector pVY1 (a derivative of pSV2) containing DHFR gene as a selection marker. Resulting plasmids were transferred into CHO DHFR(−) cells with the DEAE-dextran method. Transfected cells were screened in MEM α medium, and high-expression clones were selected in a binding assay using [^{3}H]-8-OH-DPAT.

FSC Assay with CHO Cells Expressing Human 5-HT$_{1A}$ Receptor. Cell membranes were prepared using the method of Fargin et al.[22] and were used for the FSC assay. The FSC assay was performed according to the method of Salomon et al.[23] with modifications. Final composition of the reaction medium was 50 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, 1 mM EGTA, 2 mM dithiothreitol, 30 μg/mL BSA, 5 mM creatine phosphate, 400 units/mL creatine kinase, 0.5 mM 3-isobutyl-1-methylxanthine, 0.1 mM GTP, 0.1 mM ATP, 1 mM cyclic AMP, 10 μM forskolin, 2 μCi of [α-^{32}P]ATP, and 0.05 μCi of [^{3}H]cyclic AMP. After 11 min of incubation at 30 °C, the reaction was stopped by the addition of the stop solution (2% SDS, 40 mM ATP, and 1.4 mM cyclic AMP, in 50 mM Tris-HCl, pH 7.4). The [^{32}P]cyclic AMP formed and the [^{3}H]cyclic AMP as an internal standard were isolated using Dowex columns and alumina columns. Cyclic AMP production was determined by liquid scintillation spectroscopy and corrected for relative recovery. The FSC activity is expressed as the percentage of the response induced by 0.1 μM **2** (routinely 35−45% of total FSC level). Reversal experiments were conducted in the presence of 0.1 μM **2**. The EC_{50} values were calculated with RS/1 (BBN Software Products Corp.) running on a VAX computer.

Correlation Analyses. Seven descriptors for aromatic substituents were attempted in the correlation analysis for compounds **3a**−**f**, namely, π, MR, F, R, σ_m, σ_p, and E_s.[15,24] No correlation was observed between the 5-HT$_{1A}$ receptor binding affinity and the descriptors other than MR. The C5- and C7-substituted compounds **3g**,**h**, respectively, were excluded in order to simplify the analysis. The pEC_{50} (−log EC_{50}) values of the compounds **3b**−**e**, (*S*)-**1**, and **15** were used in the analysis for the potency of antagonism. Data analyses were carried out using Microsoft Excel version 5.0 (Microsoft Corp.) running on Windows 3.1.

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