Synthesis and Biological Evaluation of Novel Compounds within a Class of 3-Aminochroman Derivatives with Dual 5-HT$_{1A}$ Receptor and Serotonin Transporter Affinity

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Compounds containing a 5-carbamoyl-8-fluoro-3-amino-3,4-dihydro-2*H*-1-benzopyran and a 3-alkylindole moiety linked through a common basic nitrogen were prepared and evaluated for 5-HT$_{1A}$ affinity, serotonin rat transporter affinity, and functional antagonist activity in vitro. **26a** was found to be the most potent and selective compound in this series and was shown to possess neurochemical activity in vivo by producing acute and rapid increases in 5-HT in the rat frontal cortex.

Introduction

The discovery and development of new antidepressants remain an active area of research. Selective serotonin (5-HT) reuptake inhibitors (SSRIs) have become a primary mode of therapy because of fewer side effects compared to the traditional tricyclic antidepressants (TCAs),[1,2] but they suffer from a 2−6 week delay in the onset of therapeutic efficacy.[3] It is thought that this activity onset delay is the result of activation of somatodendritic 5-HT$_{1A}$ autoreceptors, which reduce cell-firing activity, limiting the amount of synaptic 5-HT. Following chronic antidepressant treatment, desensitization of the 5-HT$_{1A}$ autoreceptors occurs, resulting in a more pronounced increase in serotonergic activity compared to acute treatment.[4] Antagonism of the 5-HT$_{1A}$ autoreceptor should block the reduced rate of neuronal firing, thus allowing the effects of the SSRI to be seen more rapidly.[5] Several studies have shown that antidepressant effects of an SSRI can be accelerated by the coadministration of a 5-HT$_{1A}$ antagonist.[6,7] For example, in animal models, the 5-HT$_{1A}$ antagonist WAY-100635 was found to potentiate the antidepressant effects of several SSRIs when given in combination.[7,8] In some clinical studies,[9] but not all,[6] the mixed 5-HT$_{1A}$/β-adrenoceptor antagonist pindolol in combination with SSRIs has shown acceleration of antidepressant effects. Significant amounts of work in our laboratories[10] and others[11,12] have focused on creating a single molecular entity possessing 5-HT$_{1A}$ antagonism and 5-HT reuptake inhibition through utilization of a common basic nitrogen linking the 5-HT$_{1A}$ and SSRI moieties. This generic strategy was previously termed "the overlapping type approach".[11]

Several years ago, 5-methoxy-3-(di-*n*-propylamino)chroman (5-OMe-DPAC) (Figure 1) was discovered as a selective 5-HT$_{1A}$ ligand vs other 5-HT sites and D_2 sites in rat brain membranes.[13] Subsequently, different 3-amino-3,4-dihydro-2*H*-1-benzopyran derivatives were explored and developed as 5-HT$_{1A}$ agonists.[14] The combination of 5-carboxamide, 8-fluoro substitutions with *R* stereochemistry at the 3-amino position generated potent 5-HT$_{1A}$ antagonists[15] such as robalzotan (NAD-299) (Figure 1),

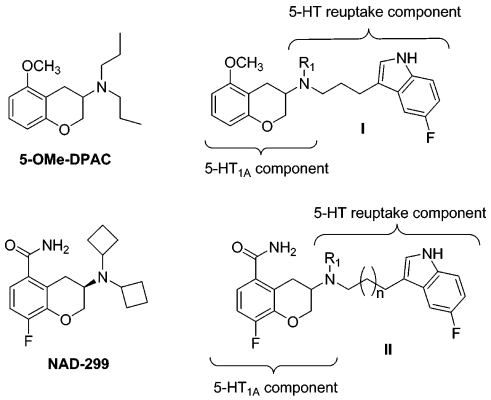

Figure 1. Structures of 5-OMe-DPAC and NAD-299 and design strategy.

which was developed for the potential treatment of depression and anxiety.[16]

Using the "overlapping type approach", we found in preliminary studies that the combination of 5-methoxychroman with a straight chain aminoalkylindole moiety, known to be a SSRI pharmacophore,[17] generated compounds possessing dual affinity for the 5-HT$_{1A}$ receptor and the 5-HT reuptake site (Figure 1, **I**). In light of these results, we then chose to combine 5-carbamoyl-8-fluoro-3-amino-3,4-dihydro-2*H*-1-benzopyran with the straight chain alkylindole moiety (Figure 1, **II**). In this report, we discuss the synthesis and structure−activity relationships of two new series of chroman derivatives **I** and **II** and outline their dual activities.

Chemistry

The 5-HT$_{1A}$ template **3** was synthesized from the benzaldehyde **1** according to literature procedures[18] (Scheme 1). The synthesis of moiety **8** is shown in Scheme 2 following a slightly modified synthetic route previously published by other researchers.[19] A key improvement was the nitration of **6** carried out using a phase transfer reagent, 18-crown-6, in the presence of potassium nitrite and iodine under sonication conditions,[20] resulting in an improved yield (60%) of the nitro derivative **7**. Reduction of the double bond of **7** followed by phase transfer hydrogenation generated **8** as a racemate at the 3-position of the chroman ring. The enantiomers were separated by chiral

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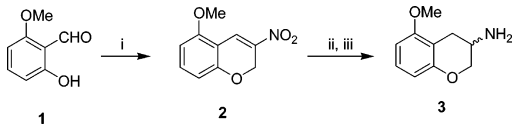
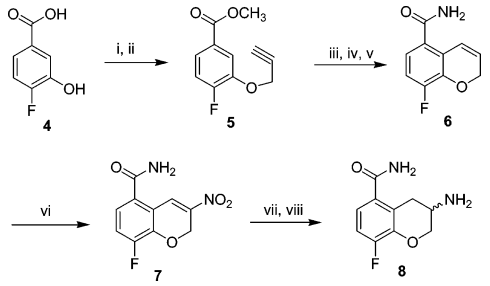
Scheme 1[a]



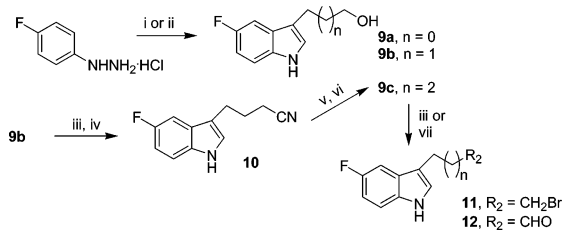
[a] Reagents: (i) nitroethanol, $Bu_2NH-HCl$, isoamyl acetate; (ii) $NaBH_4$, SiO_2, $CHCl_3$, ^iPrOH; (iii) $NH_2NH_2-H_2O$, Raney Ni, EtOH.

Scheme 2[a]



[a] Reagents: (i) trimethylorthoformate, H_2SO_4, MeOH; (ii) propargyl bromide, K_2CO_3, acetone; (iii) *N*,*N*-diethylaniline; (iv) NaOH, EtOH−H_2O; (v) CDI, THF followed by NH_3(g), THF; (vi) 18-crown-6, KNO_2, I_2, THF−pyridine, sonication; (vii) $NaBH_4$, SiO_2, $CHCl_3-^iPrOH$; (viii) $NH_2NH_2 \cdot H_2O$, Raney Ni, EtOH−THF.

Scheme 3[a]



[a] Reagents: (i) 2,3-dihydrofuran, THF−H_2O followed by ethylene glycol, $ZnCl_2$; (ii) 2,3-dihydropyran, dioxane−H_2O; (iii) CBr_4, PPh_3, CH_2Cl_2; (iv) NaCN, DMF; (v) KOH, EtOH−H_2O; (vi) LAH, THF; (vii) TFA, pyridine, DMSO−benzene, DCC.

resolution with tartaric acid or by chiral HPLC of the final target molecule. The stereochemistry of moiety **8** was determined by NMR studies and later confirmed by X-ray crystallography of one of the final targets.

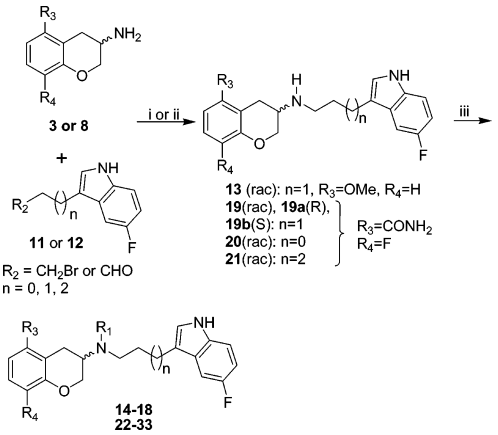
The synthesis of the 5-HT reuptake moieties **11** and **12** is shown in Scheme 3. The two- and three-carbon linker analogues **9a** and **9b** were generated through a Fischer indole synthesis and converted to the bromo (**11**) or aldehyde (**12**) derivative. The four-carbon linker derivative **9c** was prepared by homologation of **9b** using straightforward chemistry.

Novel compounds **13**−**33** were synthesized using the route shown in Scheme 4. After coupling of the chroman amine moiety **3** or **8** to the alkylindole moiety **11** or **12** through alkylation or reductive amination to generate the secondary amine derivatives, the final products (R_1 = Me, Et, Pr, methylcyclopropyl, cyclobutyl) were obtained by reductive amination with the desired aldehyde or cyclobutanone.

Results and Discussion

Here, we have explored the combination of two well-known 5-HT_{1A} templates and a known serotonin transporter moiety through a common basic nitrogen, which resulted in two series of novel compounds, which possess affinity for the 5-HT_{1A} and serotonin rat transporter receptors. Serotonin transporter affinity, functional activity at the h-5HT transporter, 5-HT_{1A} receptor affinity, 5-HT_{1A} intrinsic activity (cAMP), and α_1 receptor affinity are summarized in Table 1.

Scheme 4[a]

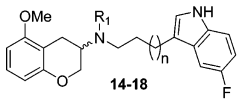


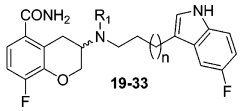
[a] Reagents: (i) Br-alkylindole, TEA, DMSO; (ii) aldehyde alkylindole, $NaBH_3CN$, HOAc, MeOH; (iii) aldehyde or cyclobutanone, $NaBH_3CN$, HOAc, MeOH.

In **14**−**19** and **22**−**26**, we first investigated the effect of substitution on the basic nitrogen and stereochemistry at C3 of the chroman ring and found, as summarized in Table 1, that tertiary amines were preferred for 5-HT_{1A} affinity. In **II**, propyl (**24a**,**b**), methylcyclopropyl (**25a**,**b**), and cyclobutyl (**26a**) were found to be the best substituents while a secondary amine (**19a**) and ethyl (**23a**,**b**) resulted in a 5- to 10-fold decrease in activity. In contrast, methyl substitution (**22**) generated a less potent compound. Similarly, in **I**, ethyl (**15**) and methylcyclopropyl (**17**) were best while methyl (**14**), propyl (**16**), and cyclobutyl (**18**) showed moderate affinity. These results suggest that the steric environment of the basic nitrogen plays a significant role within the binding pocket of the 5-HT_{1A} receptor. Interestingly, serotonin transporter affinity was minimally affected by substitution on the basic nitrogen in both series and most compounds had excellent (**14**−**18**, **23a**,**b**, **24a**,**b**, **25a**,**b**, **26a**,**b**) to good affinity (**18a**, **19a**, **22**) for the r-5HT reuptake site. Additionally, most of these derivatives had good functional activity at the human transporter. The *R* stereochemistry at C3 of the chroman ring was very important for 5-HT_{1A} antagonist activity and desired in vitro properties for a dual-acting new molecular entity. In the carboxamide series **II**, only the cyclobutyl derivatives (**26a**,**b**) showed full antagonism for both enantiomers, as measured by a cAMP assay, but with weaker affinity of the *S* enantiomer (**26b**) at the 5-HT_{1A} receptor. In contrast, **23b**, **24b**, and **25b** (*S* stereoisomers) had excellent 5-HT_{1A} affinities, but they were found to be full or partial agonists functionally. Interestingly analogues **23a** and **25a** (*R* enantiomers) showed full antagonism at the 5-HT_{1A} receptor, but **24a** was a partial agonist suggesting that substitution on the basic nitrogen may have some effect on the agonist/antagonist properties of these compounds. Surprisingly, in the methoxy series **I**, partial agonism was seen with the racemic cyclobutyl derivative **18** and its *R* enantiomer (**18a**) was a full antagonist with moderate affinities at the 5-HT_{1A} receptor and 5-HT reuptake site. In general, more antagonism at the 5-HT_{1A} receptor was observed for the carboxamide derivatives (**II**) than the methoxychroman analogues (**I**). All of these compounds showed acceptable binding selectivity over the α_1 receptor.

Compounds **24**−**33** explored the effect of different chain lengths between the carboxamide chroman headpiece and the 3-alkyl-5-fluoroindole moiety, with differing substitutions on the basic nitrogen as summarized in Table 1. Linkers of three (**24**−**26**) or four (**30**, **32**, **33**) carbons were preferred for desirable in vitro properties with comparable affinities at the 5-HT_{1A}

Table 1. Affinities for h-5-HT_{1A} Receptor, r-5HT Transporter, and α_1 Receptor, Functional Activity at the h-5HT Transporter, and in Vitro 5-HT_{1A} Antagonist Activity in CHO Cells for **14**−**19** and **22**−**33**[a]





				5-HT transporter		5-HT_{1A} receptor		
compd	R_1	*n*	stereo	affinity[b] K_i (SEM), nM	function[c] IC_{50} (SEM), nM	affinity[d] K_i (SEM), nM	cAMP[e] E_{max} IC_{50}, nM	α_1 receptor[f] K_1, nM
14	Me	1	rac	6.8	15	40(0.07)	91	610
15	Et	1	rac	2.2(1.3)	64(5.0)	2.3(0.07)	96	207
16	Pr	1	rac	9.0(3.0)	386(153)	15.8(1.5)	80	820
17	MecPr	1	rac	4.3	42.6	1.68(0.2)	91	44%
18	cBu	1	rac	13(4.0)	400(169)	15.6(0.2)	37	15%
18a	cBu	1	*R*	41	732	2.14(1.1)	0(23)	15%
19a	H	1	*R*	20.4(3.8)	197(54)	45.2(2.0)	85	21%
22	Me	1	*R*	14.8(4.8)	116(7)	266(65)	nd	37%
23a	Et	1	*R*	3.4(1.1)	42.7(9.6)	34.9(2.8)	0(135)	640
23b	Et	1	*S*	3.6(0.7)	37.3(2.5)	18.6(3.2)	91	545
24	Pr	1	rac	7.1(1.7)	128(44)	3.5(0.9)	84	nd
24a	Pr	1	*R*	8.0(0.9)	181(22)	1.5(0.1)	78	1150
24b	Pr	1	*S*	4.7(0.7)	59.9(14.3)	3.2(1.0)	97	46%
25	MecPr	1	rac	3.5(0.5)	53.5(27.5)	2.3(0.8)	0(66)	32%
25a	MecPr	1	*R*	3.9(0.7)	59.7(35.3)	2.1(0.09)	0(70)	2023
25b	MecPr	1	*S*	4.2(1.3)	36.3(3.6)	0.1(0.03)	69	26%
26	cBu	1	rac	3.0(0.5)	25.2(8.3)	9.3(2.1)	0(61)	16%
26a	cBu	1	*R*	1.5(0.04)	18.7(1.8)	1.2(0.35)	0(27)	21%
26b	cBu	1	*S*	5.3(0.3)	17.4(10)	253(31)	0(926)	nd
27	Et	0	rac	60.2(5.0)	883(71)	33.2(5.5)	0(68)	416
28	Et	2	rac	0.4(0.2)	24.1(10.5)	330(33)	0(>1000)	328
29	Pr	0	rac	88(17)	>1000	17.6(0.5)	0(68)	484
30	Pr	2	rac	12.2(3.4)	217(43)	4.4(0.3)	0(125)	494
31	MecPr	0	rac	97.8(9.6)	>1000	7.3(0.7)	0(97)	636
32	MecPr	2	rac	3.3(0.5)	82.4(42.3)	2.1(0.5)	0(51)	506
33	cBu	2	rac	10.4(4.6)	300(110)	4.8(0.02)	0(24)	645
fluoxetine				3.9(0.35)	39.4(3.1)			
WAY-100635						0.96(0.21)	0(7.06)	

[a] K_i and IC_{50} are the mean of at least two experiments ± SEM (performed in triplicate, determined from nine concentrations). Values without SEM are for a single determination only: nd, not determined. Percentages represent inhibition of binding at 1 μM. [b] Binding affinity for the 5-HT transporter by displacement of [^{3}H]paroxetine from rat cortical membranes.[21] K_i values were calculated from IC_{50} values using the method of Cheng and Prusoff.[22] [c] Inhibition of [^{3}H]-5-HT uptake by human 5-HT transporter in Jar cells.[23] [d] Binding affinity at human 5-HT_{1A} receptor in CHO cells labeled with [^{3}H]-8-OH-DPAT.[24] [e] Maximal agonist effect relative to 5-HT in inhibiting forskolin-stimulated adenylate cyclase activity.[25] [f] Binding affinity at rat cortical α_1 adrenergic receptor labeled with [^{3}H]prazosin.[26]

receptor, the r-5-HT transporter, and good functional activity at the human transporter. Although the two-carbon linker derivatives (**27**, **29**, **31**) showed reduced affinity at the 5-HT reuptake site, they retained moderate 5-HT_{1A} affinity regardless of the substitution on the basic nitrogen. Interestingly, two- and four-carbon linker analogues were in general full antagonists at 5-HT_{1A} as racemates in contrast to the three-carbon derivatives (**24** vs **29** and **30**). Regardless of the chain length, propyl, methylcyclopropyl, and cyclobutyl were preferred substituents on the basic nitrogen. In keeping with the SAR of this series, these compounds again demonstrated acceptable binding selectivity over the α_1 receptor.

Conclusion

The combination of a known 5-HT_{1A} antagonist (5-carbamoyl-8-fluoro-3-amino-3,4-dihydro-2*H*-1-benzopyran) template and a transporter moiety (aminoalkylindole) sharing a common basic nitrogen resulted in compounds with desirable affinities at the serotonin reuptake site and 5-HT_{1A} receptor. A structure−activity relationship study of this series has shown that (1) the activity for the 5-HT_{1A} receptor was dependent on the substitution on the basic nitrogen, with cyclobutyl and methylcyclopropyl being preferred for 5-HT_{1A} affinity (the chain length had minimal effect except for ethyl substitution on the four-carbon linker derivative resulting in diminished 5-HT_{1A} binding affinity), (2) the transporter affinity was primarily dependent on the length of the alkyl chain linking the 5-HT_{1A} and indole moieties with three- and four-carbon linkers as most desirable and secondarily on the substitution on the basic nitrogen, and (3) the stereochemistry at the 3-position of the chroman ring played an important role in the 5-HT_{1A} receptor agonist or antagonist properties of these compounds with the R stereochemistry usually favored for antagonism. One of the best compounds having dual activities was **26a**, which demonstrated excellent 5-HT_{1A} and serotonin transporter binding affinities and full antagonism at the 5-HT_{1A} receptor. Additionally, **26a** showed greater than 100-fold selectivity when tested against appropriate biogenic amine receptors and was selected for further evaluation in vivo. Indeed, an oral microdialysis study (30 mg/kg) has shown that **26a** *acutely* elevates serotonin levels in the rat frontal cortex (Figure 2) to a similar extent of chronic (14 day) SSRI treatment.[4] These results are consistent with the in vitro profile of this compound and suggest that **26a** readily antagonizes the 5-HT_{1A} receptor and inhibits the 5-HT transporter. These neurochemical results, with studies outlined in the Introduction, suggest that this compound may exhibit a pharmacological profile consistent with "rapid-onset" antidepressant activity. Additional SAR studies on both series and a more complete in vivo profiling of **26a** will be the subjects of future publications.

Experimental Section

Chemistry. Melting points were determined on a MEL-TEMP apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity Plus 400 spectrometer or a Unity INOVA Varian

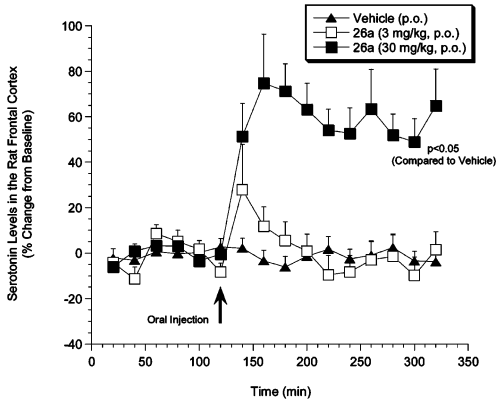


Figure 2. Acute in vivo microdialysis study of **26a**. Oral treatment with 30 mg/kg significantly ($p < 0.05$) increased 5-HT levels compared to the lower dose (3 mg/kg) or vehicle-treated rats, suggesting that this compound crosses the blood−brain barrier to activate the 5-HT_{1A} receptor and 5-HT transporter. These results are consistent with published literature showing that combining SSRI treatment with a 5-HT_{1A} antagonist increases 5-HT levels in this brain region.[8]

500 MHz spectrometer. Chemical shifts δ are reported in ppm relative to DMSO-d_6 at 2.49 ppm or $CHCl_3$-d at 7.27 ppm as an internal standard. Mass spectra were recorded on a Micromass LCT spectrometer. CHN combustion analyses were determined on a Perkin-Elmer 2400 analyzer or were performed by Robertson Microlit (Madison, NJ). All analyzed compounds are within ±0.4% of the theoretical value unless otherwise indicated. Optical rotations were measured using a Jasco P-1020 polarimeter. Solvents and reagents were used as purchased. All final targets were converted to the HCl salt by dissolution in ethyl acetate and addition of 1 M HCl/Et_2O, followed by filtration unless otherwise indicated.

General Procedure A (Alkylation Reaction): 8-Fluoro-3-{[3-(5-fluoro-1*H*-indol-3-yl)propyl]amino}chromane-5-carboxamide (19). A solution of **8** (0.81 g, 3.8 mmol), **11** ($n = 1$) (0.55 g, 2.1 mmol), and triethylamine (0.60 mL, 4.2 mmol) in anhydrous DMSO (20 mL) was stirred at 90 °C for 9.5 h. The mixture was cooled to room temperature, diluted with EtOAc, and extracted with H_2O (2×). The organic layer was treated with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. Chromatography (5:4:1 EtOAc/hexane/MeOH (1% NH_4OH)) afforded 0.48 g (60%) of **19** as a peach solid. Conversion to the mono-HCl salt generated an off-white solid: mp 122 °C, dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.90−2.08 (m, 2H), 2.68−2.82 (m, 2H), 2.98−3.25 (m, 3H), 3.27−3.45 (m, 2H), 3.71−3.88 (m, 1H), 4.27−4.46 (m, 2H), 6.82−6.98 (m, 1H), 7.08−7.21 (m, 2H), 7.22−7.27 (m, 1H), 7.27−7.37 (m, 2H), 7.43 (s, 1H), 7.81 (s, 1H), 9.00 (s, 1H), 10.95 (s, 1H); >99.9% ee by chiral HPLC; MS (ESI) *m*/*z* 384([M − H]$^-$). Anal. ($C_{21}H_{21}F_2N_3O_2$·1.20HCl) C, H, N. Chiral separation of **19** was carried out by SFC on a Chiralcel AS column (2 cm × 25 cm) using 40% MeOH in CO_2 (100 bar). The enantiomers were isolated and characterized as mono-HCl salts. **19a** (white solid): mp 89 °C, dec; $[\alpha]^{25}_D$ +19.8° (*c* 1%, DMSO); MS (ES) *m*/*z* 384.2 ([M − H]$^-$). Anal. ($C_{21}H_{21}F_2N_3O_2$·1HCl·1.20H_2O) C, H. N: calcd 9.47, found 10.06. **19b** (white solid): mp 87°C, dec; $[\alpha]^{25}_D$ −16.0° (*c* 1%, DMSO); >99.9% ee by chiral HPLC; MS (ES) *m*/*z* 386.1 ([M + H]$^+$).

General Procedure B (Reductive Amination): 8-Fluoro-3-{[2-(5-fluoro-1*H*-indol-3-yl)ethyl]amino}chromane-5-carboxamide (20). To **8** (0.38 g, 1.8 mmol) in anhydrous MeOH (29 mL) under nitrogen at room temperature were added **12** ($n = 0$) (0.33 g, 1.89 mmol), acetic acid (0.23 mL, 4.32 mmol), and sodium cyanoborohydride (0.23 g, 3.6 mmol). The mixture was stirred at room temperature overnight, quenched with 1 N NaOH/H_2O, and concentrated. The residue was taken up in CH_2Cl_2/H_2O and extracted with CH_2Cl_2 (3×). The organic layer was treated with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. Chromatography (5:4:1 EtOAc/hexane/MeOH (1% NH_4OH)) afforded 0.53 g (79%) of **20**. Conversion to the mono-HCl salt generated a white solid: mp 134 °C, dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.93−3.16 (m, 2H), 3.18−3.53 (m, 5H), 3.78−3.99 (m, 1H), 4.24−4.55 (m, 2H), 6.84−7.01 (m, 1H), 7.09−7.26 (m, 2H), 7.30−7.49 (m, 4H), 7.82 (s, 1H), 9.18 (s, 1H), 11.08 (s, 1H); MS (ES) *m*/*z* 370.2 ([M − H]$^-$). Anal. ($C_{20}H_{19}F_2N_3O_2$·1HCl·0.25H_2O) C, H, N.

General Procedure C (Introduction of Cyclobutyl on Basic Nitrogen): 3-{Cyclobutyl[3-(5-fluoro-1*H*-indol-3-yl)propyl]-amino}-8-fluorochromane-5-carboxamide (26). To **19** (0.14 g, 0.35 mmol) in anhydrous MeOH (6 mL) under nitrogen at room temperature were added cyclobutanone (0.070 mL, 0.876 mmol), acetic acid (0.050 mL, 0.84 mmol), and sodium cyanoborohydride (0.044 g, 0.70 mmol). The mixture was stirred at room temperature overnight. More cyclobutanone (0.026 mL), acetic acid (0.21 mL), and sodium cyanoborohydride (0.22 g) were added after 24 and 48 h, at which time the reaction went to completion. The workup was the same as for Procedure B. Chromatography (5:4:1 EtOAc/hexane/MeOH (1% NH_4OH)) afforded 0.12 g (78%) of **26** as a sticky gum. Conversion to the mono-HCl salt generated an off-white solid: mp 109 °C, dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51−1.75 (m, 2H), 1.85−2.57 (m, 7H), 2.59−2.76 (m, 2H), 3.06−3.48 (m, 3H), 3.88−4.14 (m, 2H), 4.31−4.43 (m, 1H), 4.45−4.59 (m, 1H), 6.84−6.95 (m, 1H), 7.10−7.25 (m, 3H), 7.26−7.35 (m, 2H), 7.38−7.49 (m, 1H), 7.72−7.89 (m, 1H), 10.11−10.42 (m, 1H), 10.93 (s, 1H); MS (ES) *m*/*z* 438.2 ([M − H]$^-$). Anal. ($C_{25}H_{27}F_2N_3O_2$·1.10HCl·0.50H_2O) C, H, N. The enantiomers of **26** were separated by chiral HPLC using a Chiralcel AD column (2 cm × 25 cm) and 16% IPA in hexane/DEA as the mobile phase. They were isolated and characterized as mono-HCl salts. **26a** (white solid): mp 129 °C, dec; $[\alpha]^{25}_D$ −26.76° (*c* 1%, DMSO); 99.5% ee by chiral HPLC; MS (ES) *m*/*z* 440.1 ([M + H]$^+$). Anal. ($C_{25}H_{27}F_2N_3O_2$·1HCl·0.40H_2O) C, H, N. **26b** (white solid): mp 129 °C, dec; $[\alpha]^{25}_D$ +27.56° (*c* 1%, DMSO); 95% ee by chiral HPLC; MS (ES) *m*/*z* 438.2([M − H]$^-$). Anal. ($C_{25}H_{27}F_2N_3O_2$·1HCl·0.50H_2O) C, H, N.

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Supporting Information Available: Details of synthesis and analytical data for all final targets, biological assays, and in vivo microdialysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Blackwell, B. Adverse effects of antidepressant drugs. Part 1: Monoamine oxidase inhibitors and tricyclics. *Drugs* **1981**, *21*, 201−219.

(2) Evrard, D. A.; Harrison, B. L. Recent approaches to novel antidepressant therapy. *Annu. Rep. Med. Chem.* **1999**, *34*, 1−9.

(3) Katz, M. M.; Tekell, J. L.; Bowden, C. L.; Brannan, S.; Houston, J. P.; Berman, N.; Frazer, A. Onset and early behavioral effects of pharmacologically different antidepressants and placebo in depression. *Neuropsychopharmacology* **2004**, *29*, 566−579 and references therein.

(4) Kreiss, D. S.; Lucki, I. Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 866−876.

(5) (a) Briner, K.; Dodel, R. C. New approaches to rapid onset antidepressants. *Curr. Pharm. Des.* **1998**, *4*, 291−302. (b) Schechter, L. E.; Kelly, M. G. An overview of 5-HT_{1A} receptor antagonists: historical perspective and therapeutic targets. *Serotonin* **1997**, *2*, 299−309.

(6) Ballesteros, J.; Callado, L. F. Effectiveness of pindolol plus serotonin uptake inhibitors in depression, a meta-analysis of early and late outcomes from randomized controlled trials *J. Affective Disord.* **2004**, *79*, 137−147 and references therein.

(7) Romero, L.; Artigas, F. Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT_{1A} receptor antagonists in the dorsal Raphe pathway: role of somatodendritic autoreceptors. *J. Neurochem.* **1997**, *68*, 2593−2603.

(8) Beyer, C. E.; Boikess, S.; Luo, B.; Dawson, L. A. Comparison of the effects of antidepressants on norepinephrine and serotonin concentrations in the rat frontal cortex: an in-vivo microdialysis study. *J. Psychopharmacol.* **2002**, *16*, 297−304.
(9) Blier, P.; Bergeron, R. J. The use of pindolol to potentiate antidepressant medication. *J. Clin. Psychiatry* **1998**, *59* (Suppl. 5), 16−23.
(10) Evrard, D. A.; Zhou, P.; Yi, S. Y.; Zhou, D.; Smith, D. L.; Sullivan, K. M.; Hornby, G. A.; Schechter, L. E.; Andree, T. H.; Mewshaw, R. E. Studies towards the next generation of antidepressants. Part 4: Derivatives of 4-(5-fluro-1*H*-indol-3-yl)cyclohexylamine with affinity for the serotonin transporter and the 5-HT_{1A} receptor. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 911−914 and references therein.
(11) Perez, M.; Pauwels, P. J.; Pallard-Sigogneau, I,; Fourrier, C.; Chopin, P.; Palmier, C.; Colovray, V.; Halazy, S. Design and synthesis of new potent, silent 5-HT_{1A} antagonists by covalent coupling of aminopropanol derivatives with selective serotonin reuptake inhibitors. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3423−3428.
(12) Rocco, V. P.; Spinazze, P. G.; Kohn, T. J.; Honigschmidt, N. A.; Nelson, D. L.; Wainscott, D. B.; Ahmad, L. J.; Shaw, J.; Threlkeld, P. G.; Wong, D. T. Advances toward new antidepressants beyond SSRIs: 1-aryloxy-3-piperidinylpropan-2-ols with dual 5-HT_{1A} receptor antagonism/SSRI activities. Part 4. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3939−3942 and references therein.
(13) Podona, T.; Guardiola-Lemaitre, B.; Caignard, D.-H.; Adam, G.; Pfeiffer, B.; Renard, P.; Guillaumet, G. 3,4-Dihydro-3-amino-2*H*-1-benzopyran derivatives as 5-HT_{1A} receptor ligands and potential anxiolytic agents. 1. Synthesis and structure−activity relationship studies. *J. Med. Chem.* **1994**, *37*, 1779−1793.
(14) Hammarberg, E.; Nordvall, G.; Leideborg, R.; Nylof, M.; Hanson, S.; Johansson, L.; Thorberg, S.-O.; Tolf, B.-R.; Jerning, E.; Svantesson, G. T.; Mohell, N.; Ahlgren, C.; Westlind-Danielsson, A.; Csoregh, I.; Johansson, R. Synthesis of novel 5-substituted 3-amino-3,4-dihydro-2*h*-1-benzopyran derivatives and their interactions with the 5-HT_{1A} receptor. *J. Med. Chem.* **2000**, *43*, 2837−2850.
(15) Evenden, J. L.; Hammarberg, E. M.; Hansson, H. S.; Hellberg, S. E.; Johansson, L. G.; Lundkvist, J. R. M.; Ross, S. B.; Sohn, D. D.; Thorberg, S. O. (*R*)-5-Carbamoyl-8-fluoro-3-*N*,*N*-disubstituted-amino-3,4-dihydro-2*H*-1-benzopyrans. U.S. 5,616,610, April 1, 1997.
(16) Ross, S. B.; Thorberg, S.-O.; Jerning, E.; Mohell, N.; Stenfors, C.; Wallsten, C.; Milchert, I. G.; Ojteg, G. A novel selective 5-HT_{1A} receptor antagonist. *CNS Drug Rev.* **1999**, *5*, 213−232.
(17) Mewshaw, R. E.; Zhou, D.; Zhou, P.; Shi, X.; Hornby, G.; Spangler, T.; Scerni, R.; Smith, D.; Schechter, L. R.; Andree, T. H. Studies toward the discovery of the next generation of antidepressants. 3. Dual 5-HT_{1A} and serotonin transporter affinity within a class of *N*-aryloxyethylindolylalkylamines. *J. Med. Chem.* **2004**, *47*, 3823−3842.
(18) Al Neirabeyeh, M.; Reynaud, D.; Podona, T.; Ou, L.; Perdicakis, C.; Coudert, G.; Guillaumet, G.; Pichat, L.; Gharib, A.; Sarda, N. Methoxy and hydroxyl derivatives of 3,4-dihydro-3-(di-*n*-propylamino)-2*H*-1-benzopyrans: new synthesis and dopaminergic activity. *Eur. J. Med. Chem.* **1991**, *26*, 497−504.
(19) Hanson, S.; Johansson, L.; Sohn, D. D. Process for the Manufacture of 3-*N*,*N*-Dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide. U.S. 6,197,978 B1, March 6, 2001.
(20) Ghosh, D.; Nichols, D. E. An improved method for the preparation of cyclic conjugated nitroolefins. *Synthesis* **1996**, 195−197.
(21) Cheetham, S. C.; Viggers, J. A.; Slater, N. A.; Heal, D. J.; Buckett, W. R. [^{3}H]-Paroxetine binding in rat frontal cortex strongly correlates with [^{3}H]5-HT uptake: effect of administration of various antidepressant treatments. *Neuropharmacology* **1993**, *32*, 737−743.
(22) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(23) Ramamoorthy, J. D.; Ramamoorthy, S.; Papapetropoulos, A.; Catravas, J. D.; Leibach, F. H.; Ganapathy, V. Cyclic AMP-independent up-regulation of the human serotonin transporter by staurosporine in chloriocarcinoma cells. *J. Biol. Chem.* **1995**, *270*, 17189−17195.
(24) Hall, M. D.; El Mestikawy, S.; Emerit, M. B.; Pichat, L.; Hamon, M.; Gozlan, H. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino) tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J. Neurochem.* **1985**, *44*, 1685−1696.
(25) Dunlop, J.; Zhang, Y.; Smith, D. L.; Schechter, L. E. Characterization of 5-HT_{1A} receptor functional coupling in cells expressing the human 5-HT_{1A} receptor as assessed with the cytosensor microphysiometer. *J. Pharmacol. Toxicol. Methods* **1998**, *40*, 47−55.
(26) Morrow, A. L.; Creese, I. Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB4104 and [^{3}H]prazosin binding. *Mol. Pharmacol.* **1986**, *29*, 321−330.

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