Design, Synthesis, and Evaluation of Chromen-2-ones as Potent and Selective Human Dopamine D4 Antagonists

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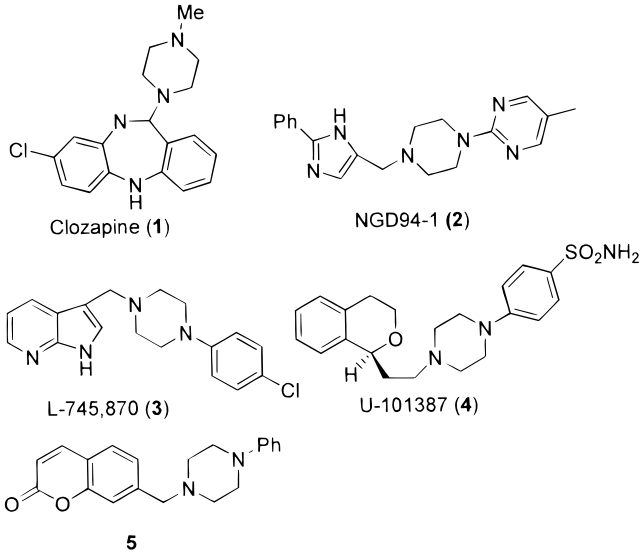
The discovery of a series of chromen-2-ones with selective affinity for the dopamine (DA) D4 receptor is described. Target compounds were tested for binding to cloned human DA D2L, D3, and D4.2 receptor subtypes expressed in Chinese hamster ovary K1 cells. Several compounds demonstrated high affinity (<20 nM, K_i) and greater than 100-fold selectivity for DA D4.2 versus DA D2L receptors. The results of a SAR study are discussed within. In a DA D4 functional assay measuring [^{3}H]thymidine uptake, target compounds showed antagonist activity at the D4.2 receptor. Compound **22**, 7-[(2-phenylaminoethylamino)methyl]chromen-2-one, increased DOPA (L-3,4-dihydroxyphenylalanine) accumulation 51% in the hippocampus and 23% in the striatum of rat brains when dosed orally at 20 mg/kg.

Introduction

Schizophrenia is a psychotic disorder characterized by positive symptoms, e.g., hallucinations and disorganized thought, and negative symptoms, e.g., apathy and social withdrawal. This socially and economically debilitating disease is fairly common striking approximately 1% of the population.[1] Past therapy, while effective in ameliorating the positive symptoms in some patients, did little to alleviate the negative symptoms. In addition, most classical agents are associated with side effects such as extrapyramidal syndrome (EPS) and tardive dyskinesia that limit compliance.

Dopamine (DA) neuronal hyperactivity has been implicated in the etiology of schizophrenia. Five DA receptor subtypes have been identified and cloned.[2] These are divided into two main families: the D-1 like, which include D1 and D5 receptor subtypes, and the D-2 like, which include D2, D3, and D4 receptor subtypes. The efficacy and neurological side effects of antipsychotic agents have been correlated with the blockade of DA D2-like receptors. DA D2 receptor subtypes are widespread in the limbic and striatal regions of the brain as well as in the prefrontal cortical areas.[3] There is a greater expression of messenger RNA for DA D4 receptors in the frontal cortical and mesolimbic areas, which are associated with antipsychotic efficacy, than in the striatal areas, which are associated with neurological side effects.[3]

Clozapine (**1**)[4,5] was the first marketed agent that is effective toward ameliorating the negative symptoms of schizophrenia and lacked significant EPS of previous antipsychotics. Its use, however, is limited by the prevalence of potentially fatal agranulocytosis in 1−2% of treated patients.[6] Clozapine binds with greater affinity to the DA D4 than to the DA D2 receptor subtypes.[4] Therefore a compound that binds preferentially to DA D4 receptors may have antipyschotic activity without the neurological side effects associated with classical DA D2-like antagonists.



Recently several selective DA D4 antagonists have been reported. These include NGD94-1[7] (**2**), L-745,870[8] (**3**), and U-101387[9] (**4**). However, these selective DA D4 antagonists have not proved so far to be efficacious in clinical trials.[10] Whether the activity of clozapine is due to some other mechanism than its DA D4 antagonism is still unknown.

High-volume screening of our compound library uncovered **5** with high affinity and selectivity (versus DA D2L and D3) for the DA D4.2 receptor. It shares the arylpiperazine moiety with compounds **2**−**4** and the methylene spacer with **2** and **3**, but it incorporates a chromen-2-one nucleus. Compound **5** was used as a starting point for our SAR studies. Exploration of this compound began by varying the position of the alkyl

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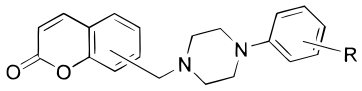
Table 1. Initial Optimization of Compound **5**

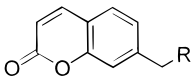


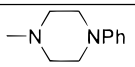
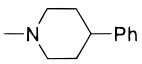
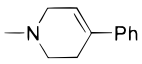
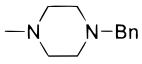
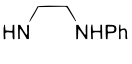
Receptor Binding (Ki, nM)[a]

Compound	Position	R	Binding (Ki, nM)[a] D4.2[b]	D2L[b]	D2/D4
5	7	H	27	5300	196
6	6	H	30	2170	72
7	7	4-Me	17	5700	336
8	6	4-Me	11	5836	530
9	7	2-Me	16	1940	121
10	6	2-Me	63	1212	19
11	7	3-Me	32	4400	137
12	6	3-Me	74	NT[c]	
13	7	3-Cl,4-Me	6.9	5882	852
14	7	3,4-diMe	14	433	31
15	7	4-F	59	NT	
16	7	4-Cl	93	NT	
17	7	4-OMe	75	NT	
18	7	t-butyl	227	NT	

[a] Ligand D2L and D4.2, [^{3}H]spiperone. [b] K_i values were obtained from six concentrations run in triplicate by a nonlinear regression analysis. [c] NT, not tested.

Table 2. Effect of Replacing the Phenylpiperazine Moiety on Receptor Binding



Compound	R	Receptor Binding (Ki, nM)[a] D4.2[b]	D2L[b]	D2/D4
5		27	5300	196
19		19	91	4.8
20		4.4	121	27.5
21		2700	NT[c]	
22		5.8	2920	503

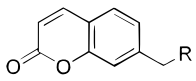
[a] Ligand D2L and D4.2, [^{3}H]spiperone. [b] K_i values were obtained from six concentrations run in triplicate by a nonlinear regression analysis. [c] NT, not tested.

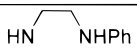
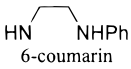
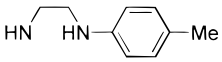
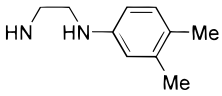
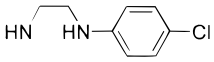
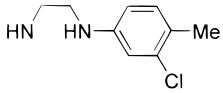
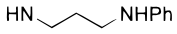
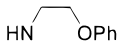
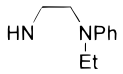
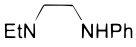
group on the chromen-2-one nucleus and adding substituents to the piperazinyl phenyl (Table 1). We next replaced the phenylpiperazine moiety with various alkyl- and arylamines. This led us to the most promising compound in this series, **22**, containing an aminoethylaniline moiety (Table 2). We further attempted to optimize the activity of **22** by varying chain length, phenyl substituents, and linkage atoms (Table 3).

Chemistry

The phenylpiperazine chromen-2-ones were readily prepared as outlined in Scheme 1. Commercially available methylchromen-2-ones were brominated[11] under free radical conditions. Subsequent coupling of the bromides with the requisite arylpiperazines in refluxing acetonitrile with potassium carbonate as the acid scav-

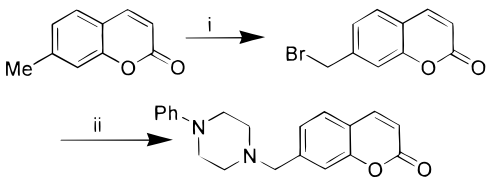
Table 3. Optimization of the Side Chain Amine



Compound	R	Receptor Binding (Ki, nM)[a] D4.2[b]	D2L[b]	D2/D4
22		5.8	2920	503
23	 6-coumarin	311	NT[c]	
24		10	2087	208
25		21	2189	104
26		14	696	50
27		16	5882	362
28		31	NT	
29		43	NT	
30		2664	NT	
31		410	NT	

[a] Ligand D2L and D4.2, [^{3}H]spiperone. [b] K_i values were obtained from six concentrations run in triplicate by a nonlinear regression analysis. [c] NT, not tested.

Scheme 1[a]

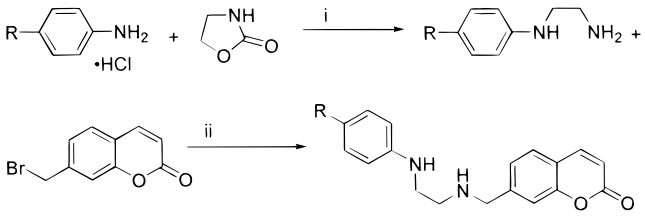


[a] Reagents and conditions: (i) *N*-bromosuccinimide, AlBN, high-intensity light, reflux, $CHCl_3$; (ii) phenylpiperazine, K_2CO_3, reflux, CH_3CN.

enger provided the target compounds. These reactions were high yielding (>80%).

Very few *N*-(2′-aminoethylene)anilines are commercially available. They were prepared as outlined in Scheme 2 from the condensation of the requisite aniline hydrochloride and 2-oxazolidone.[12] The resulting anilines were coupled with the bromomethylchromen-2-ones under analogous conditions employed for the phenylpiperazine chromen-2-ones. Excess amounts of the primary amines were used in order to minimize bis-adduct formation. The *N*-ethyl analogue **31** was made by selectively alkylating **5** with ethyl iodide. The *N*-ethyl

Scheme 2[a]



[a] Reagents and conditions: (i) 140 °C, neat, 18 h; (ii) excess amine, K_2CO_3, reflux, CH_3CN.

analogue **32** was made from condensing *N*-ethylaniline with 2-oxazolidone[12] and coupling the resulting product with the requisite bromomethylchromen-2-one.

Pharmacology

Cloned human dopamine D4.2, D3, and D2L receptors expressed in CHO-K1 cells were used to determine the DA receptor affinities of compounds using the radioligand [^{3}H]spiperone.[13] Compounds that had affinities for the DA D4.2 receptor of less than 20 nM (K_i) and selectivities for the DA D4.2 receptor versus the DA D2L receptor of greater than 100-fold were evaluated further

in vitro. Only the more potent and selective compounds were also screened for affinity for the DA D3 receptor. Intrinsic activities of the selective compounds were determined by measuring their abilities to block stimulation of mitogenesis as measured via [^{3}H]thymidine uptake caused by the full DA agonist quinpirole in CHO cells transfected with DA D4.2 or D2L receptors.[14] Compounds with 20% or less intrinsic activity in this model are considered antagonists. Antagonists with IC_{50}'s < 20 nM were evaluated further.

In vivo, DA antagonists are known to increase the rate of brain DA synthesis in rodents.[13] Specifically, the effect of a compound on catecholamine synthesis can be evaluated by measuring its ability to increase the accumulation of the catecholamine precursor DOPA (L-3,4-dihydroxyphenylalanine) after the inhibition of L-aromatic amino acid decarboxylase with NSD 1015.[13] A compound with the desired profile would increase dopamine synthesis in the DA D4-rich hippocampus preferentially to the DA D2-rich striatal regions.

Compounds that were orally active with the desired profile in the dopamine synthesis assay were further evaluated in tests predictive of antipsychotic activity: reversal of amphetamine-stimulated locomotor activity[15] and inhibition of spontaneous locomotor activity in rats.[16] Key compounds were also assayed for their ability to inhibit the apomorphine-induced disruption of prepulse inhibition of acoustic startle test in rats, a model predictive of antipsychotic activity in humans.[17,18] Active compounds were evaluated in the catalepsy test in rats, a test predictive of extrapyramidal side effects.[19] Finally, a compound was screened for its affinity to other receptors to ensure the observed effects were due to DA D4.

Results and Discussion

Compound **5** has fairly good affinity (IC_{50} = 27 nM) and selectivity (~200-fold) for the DA D4.2 receptor versus the DA D2L receptor. It was also 120-fold selective for DA D4.2 versus DA D3. We first evaluated the effect of the position of the linkage to the chromenone ring (Table 1). The 6- and 7-linked compounds were more synthetically accessible than the 5- or 8-linked compounds.

In general, the 7-linked analogues were slightly more potent (see compounds **9** versus **10** and **11** versus **12**) and selective for the DA D4.2 receptor than the comparable 6-linked analogues. Placing substituents on the phenyl ring increased potency and selectivity in some cases. The 4-methyl analogues **7** and **8** were more potent and selective than their parent compounds **5** and **6**. We placed the methyl group at different positions around the ring (compounds **9**−**12**) and found the 4-position optimal. While adding a 3-chloro to **7** increased potency and selectivity (**13**), adding an additional methyl (**14**) decreased selectivity. The methyl group was replaced with electron-withdrawing groups resulting in a decrease in potency (see compounds **15** and **16**). Replacing the methyl group with a more electron-rich group, the methoxy, significantly reduced potency (e.g. **17**). We studied steric effects by increasing bulk in the 4-position with a *tert*-butyl moiety. This compound (**18**) was much less active. Our explorations led us to the discovery of **13**, which is approximately 4-fold more potent and selective than the original lead. There was no clear relationship between DA D4.2 receptor potency and the electron-donating or -withdrawing abilities of the substituents. Only a certain amount of steric bulk could be tolerated, however.

Table 4. Functional Assays of Compounds with High Affinity and Selectivity for DA D4.2 Receptors

compd	inhibn of [^{3}H]thymidine uptake (% intrinsic activity; IC_{50}, nM)[a]	DOPA levels, 10 mg/kg ip[b] (% of control ± SEM)	
		striatal	hippocampal
8	0%; >1000	22 ± 1[d]	−16 ± 9
13	0%; 5.4	19 ± 6[d]	27 ± 9[d]
22	0%; 1.3	20± 6[d]	32± 9[d]
22 (oral)	0%; 1.3	23[c]± 10[d]	51[c] ± 18[d]
24	0%; 98	not tested	not tested

[a] Effects measured in CHO p-5 cells transfected with the h-D4.2 receptor. Intrinsic activity measured relative to the full agonist quinpirole. [b] Striatal and hippocampal DA syntheses were measured by HPLC with electrochemical detection in rats given drug 60 min previously and the L-aromatic amino acid decarboxylase inhibitor NSD 1015 30 min prior to sacrifice by decapitation. Data are expressed as the percentage increase of DA synthesis (as indicated by DOPA levels) relative to control animals. Each value is a mean of 4 animals. [c] Animals were dosed 20 mg/kg, po. [d] P < 0.05 versus control.

We substituted various arylamines for the phenylpiperazine moiety (see Table 2). Replacing the piperazine with a piperidine (**19** versus **5**) or a tetrahydropyridine (**20**) resulted in analogues that were more potent but not as selective for the DA D4.2 receptor. Adding a methylene spacer (the benzylpiperazine **21**) was not tolerated. Replacing the piperazine with a diaminoethylene moiety results in a compound (**22**) more potent and selective than the original lead.

We changed our focus to exploring the SAR of **22** (Table 3). The 6-substituted analogue **23** was much less potent. Phenyl substituents that increased the activity in the phenylpiperazine series were appended to give compounds **24**, **25**, and **27**. Although these compounds were fairly potent and selective and thus were chosen for further evaluation, they were not superior to the parent **22**. These compounds also had more affinity for DA D3 with K_i values ranging from 97 nM for compound **27** to 424 nM for compound **22**. An electron-withdrawing substituent was added to give compound **26**, which was potent but did not have the required 100-fold selectivity.

Increasing the chain length decreased activity (**28** versus **22**). Replacing the anilino nitrogen atom with an oxygen (**29**) also led to a decrease in DA D4.2 activity. Both secondary amine functionalities appear necessary for activity (**30** and **31**) as both *N*-ethyl analogues are weak.

Compounds **8**, **13**, **22**, and **24** were selected for additional testing in functional assays (Table 4) based on their high potency (<20 nM for DA D4.2) and high selectivity for D4.2 versus D2L (D2/D4 > 100). The intrinsic activities of these compounds were evaluated in the [^{3}H]thymidine uptake assay. Compounds **13**, **22**, and **24** had intrinsic activities of 0% with IC_{50}'s of 5.4, 1.3, and 98 nM, respectively, in inhibiting quinpirole-induced stimulation of [^{3}H]thymidine uptake. These data indicate that these compounds are antagonists. It is unclear why **24** is so weak in this functional assay. Compound **8** was inactive in this assay and not consid-

Table 5. Behavioral Effects of Compound **22**

test	result
reversal of prepulse inhibition of acoustic startle in mice, ip	active at 30 mg/kg
inhibition of amphetamine-stimulated locomotor activity in rats (ED_{50}, ip)	3 mg/kg
inhibition of spontaneous motor activity in rats, ip	not active at 30 mg/kg
catalepsy test in rats, po	not active at 10 mg/kg

Table 6. Receptor Binding Data for Compound **22**

receptor	binding (K_i, nM)
DA D4.2	5.8[a]
DA D3	424[a]
DA D2L	2920[a]
α1	2670[b]
α2	>4700[c]
5HT1a	3711[d]
5HT2	>4350[e]

[a] Ligand, [^{3}H]spiperone; K_i values were obtained from six concentrations run in triplicate by a nonlinear regression analysis. [b] Ligand, [^{3}H]prazosin; $N = 1$: screening value. [c] Ligand, [^{3}H]MK-912; $N = 1$: screening value. [d] Ligand, [^{3}H]8-OH-DPAT; $N = 1$: screening value. [e] Ligand, [^{3}H]ketanserin; $N = 1$: screening value.

ered further. All the above compounds were inactive (IC_{50} > 1000) in the DA D2L [^{3}H]thymidine uptake studies.

Compounds **13** and **22** were identified as the most promising candidates from the in vitro studies and were evaluated in a series of in vivo tests. Compound **13** produced only minimal increases in DOPA accumulation at 10 mg/kg, ip, in the hippocampus (HI) and in the striatum (ST) (27% and 19%, respectively). Compound **22** produced similar results (32% and 20% in the HI and ST, respectively) at the same dose (10 mg/kg, ip). However, when rats were dosed orally at 20 mg/kg, **22** increased DOPA accumulation by 51% in the DA D4-enriched hippocampus but only 23% in the predominantly DA D2-expressing striatum. Of all the compounds considered, only **22** had the desired profile. It was evaluated further in tests predictive of antipsychotic activity. Like nonselective classical DA D2 antagonists such as haloperidol, **22** reversed amphetamine-stimulated locomotor activity in rats with an ED_{50} of 3 mg/kg, ip (Table 5). Unlike classical DA D2 antagonists, however, **22** had no effect on spontaneous locomotor activity in rodents. It also did not cause catalepsy in rats at 10 mg/kg, po. In addition, **22** was active at 30 mg/kg, ip, in the prepulse inhibition of acoustic startle test in rodents. It was screened for its affinity for other receptors to ensure that its effects were due to its DA D4 affinity (Table 6). It did not bind significantly to serotonergic and adrenergic receptors. The only other significant binding observed was weak affinity (424 nM) for the DA D3 receptor.

In summary, a DA D4 antagonist **5** was identified from mass screening. Various structural aspects of the molecule were investigated to optimize its potency and selectivity. Compound **22** was identified from these studies, and it was found to be active in vivo in tests predictive of antipsychotic activity. Furthermore, it was inactive in a test predictive of extrapyramidal side effects, a liability of classical DA D2 antagonists. Administration orally of **22** led to greater increases of dopamine synthesis in the hippocampal region, a DA D4-enriched area, versus the striatal region, a DA D2-enriched area, that is consistent with the rationale of the desirability of a DA D4 antagonist. This compound will be a useful tool for exploring the significance of the role of blocking DA D4 receptors in the treatment of schizophrenia.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by the Analytical Chemistry staff at Parke-Davis. Where analyses are indicated with the elemental symbols, the results are within 0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on a Varian Unity 400 spectrometer with chemical shifts reported in ppm relative to internal tetramethylsilane. Mass spectra were recorded on a VG Masslab Trio-2A mass spectrometer. Column chromatography separations were carried out using Merck silica gel 60, 230−400 mesh ASTM, on a medium pressure setup. Reactions were usually carried out under a nitrogen atmosphere, and organic solutions were concentrated at aspirator pressure on a rotary evaporator.

7-(4-Phenylpiperazin-1-ylmethyl)chromen-2-one (5). A mixture of 7-bromomethylchromen-2-one[11] (1.0 g, 4.18 mmol), 1-phenylpiperazine (0.68 g, 4.18 mmol), and potassium carbonate (2.0 g, 14.5 mmol) was heated under reflux in acetonitrile (100 mL) 18 h. The reaction mixture was cooled and filtered over Celite and the filtrate concentrated under vacuum to provide a crude solid. Recrystallization from ethyl ether provided **5** (0.45 g, 33%): mp 156−158 °C; ^{1}H NMR ($CDCl_3$) δ = 2.62 (t, J = 4.64 Hz, 4H), 3.19 (t, J = 4.64 Hz, 4H), 3.63 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.86 (t, J = 7.33 Hz, 1H), 6.90 (d, J = 8.30 Hz, 2H), 7.22−7.29 (m, 3H), 7.35 (s, 1H), 7.42 (d, J = 7.81 Hz, 1H), 7.67 (d, J = 9.52 Hz, 1H); CIMS m/z 321 (MH^+). Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

6-(4-Phenylpiperazin-1-ylmethyl)chromen-2-one Hydrochloride (6). This was prepared similarly to **5** starting with 6-bromomethylchromen-2-one.[11] The compound was converted to the hydrochloride salt with ethereal hydrogen chloride: mp 195−201 °C; ^{1}H NMR (DMSO-d_6) δ = 3.10 (m, 4H), 3.35 (m, 2H), 3.89 (HDO), 6.53 (d, J = 9.52 Hz, 1H), 6.81 (t, J = 7.1 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H), 7.22 (t, J = 7.32 Hz, 2H), 7.48 (d, J = 8.3 Hz, 1H), 7.88 (dd, J = 2.2 and 8.6 Hz, 1H), 7.91 (d, J = 2.2 Hz, 1H), 8.04 (d, J = 9.52 Hz, 1H); CIMS m/z 321 (MH^+). Anal. ($C_{20}H_{20}N_2O_2 \cdot 1.7HCl \cdot 1.0H_2O$) C, H, N, Cl^-, H_2O.

7-(4-Tolylpiperazin-1-ylmethyl)chromen-2-one (7). This was prepared similarly to **5** starting with 1-(*p*-tolyl)piperazine. Recrystallization from ethyl acetate provided **7** in 22% yield: mp 151−152 °C; ^{1}H NMR ($CDCl_3$) δ = 2.25 (s, 3H), 2.61 (t, J = 4.64 Hz, 4H), 3.15 (t, J = 4.64 Hz, 4H), 3.62 (s, 2H), 6.38 (d, J = 9.52 Hz, 1H), 6.82 (d, J = 8.30 Hz, 2H), 7.05 (d, J = 8.30 Hz, 2H), 7.28 (br s, 1H), 7.34 (s, 1H), 7.42 (d, J = 7.33 Hz, 1H), 7.67(d, J = 9.7 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2 \cdot 0.1H_2O$) C, H, N, H_2O.

6-(4-Tolylpiperazin-1-ylmethyl)chromen-2-one (8). This was prepared similarly to **5** starting with the 6-bromomethylchromen-2-one and 1-(*p*-tolyl)piperazine. Recrystallization from ethyl acetate provided **8** in 53% yield: mp 154−155 °C; ^{1}H NMR ($CDCl_3$) δ = 2.23 (s, 3H), 2.57 (t, J = 4.64 Hz, 4H), 3.12 (t, J = 4.64 Hz, 4H), 3.58 (s, 2H), 6.40 (d, J = 9.52 Hz, 1H), 6.80 (d, J = 9.70 Hz, 2H), 7.05 (d, J = 9.70 Hz, 2H), 7.43 (s, 1H), 7.55 (d, J = 9.0 Hz, 1H), 7.85 (d, J = 9.7 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

7-(2-Tolylpiperazin-1-ylmethyl)chromen-2-one (9). This was prepared similarly to **5** starting with 1-(*o*-tolyl)piperazine. Recrystallization from ethyl acetate/heptane provided **9** in 51% yield: mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ = 2.25 (s, 3H), 2.60 (br s, 4H), 2.93 (t, J = 4.64 Hz, 4H), 3.62 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.95 (t, J = 9.50 Hz, 1H), 7.07 (d, J = 9.70 Hz, 1H), 7.15 (m, 2H), 7.25(d, J = 7.9 Hz, 1H), 7.38 (s, 1H), 7.41 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 9.7 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

6-(2-Tolylpiperazin-1-ylmethyl)chromen-2-one (10). This was prepared similarly to **9** starting with 6-bromomethylchromen-2-one. Recrystallization from ethyl ether provided **10** in 15% yield: mp 131−132 °C; ^{1}H NMR ($CDCl_3$) δ = 2.25 (s, 3H), 2.60 (br s, 4H), 2.95 (t, J = 4.64 Hz, 4H), 3.58 (s, 2H), 6.40 (d, J = 9.50 Hz, 1H), 6.90−6.98 (m, 2H), 7.07 (d, J = 9.70 Hz, 1H), 7.15 (m, 2H), 7.25 (d, J = 7.9 Hz, 1H), 7.45 (s, 1H), 7.51 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 9.7 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

7-(3-Tolylpiperazin-1-ylmethyl)chromen-2-one (11). This was prepared similarly to **5** starting with 1-(*m*-tolyl)piperazine. Recrystallization from ethyl acetate provided **11** in 76% yield: mp 105−106 °C; ^{1}H NMR ($CDCl_3$) δ = 2.25 (s, 3H), 2.60 (t, J = 4.6 Hz, 4H), 3.16 (t, J = 4.6 Hz, 4H), 3.62 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.65 (d, J = 7.80 Hz, 1H), 6.72 (m, 2H), 7.15 (t, J = 7.80 Hz, 1H), 7.25 (d, J = 8.7 Hz, 1H), 7.35 (s, 1H), 7.42 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 9.7 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

6-(3-Tolylpiperazin-1-ylmethyl)chromen-2-one (12). This was prepared similarly to **11** starting with the 6-bromomethylchromen-2-one. Recrystallization from ethyl acetate provided **12** in 65% yield: mp 149−150 °C; ^{1}H NMR ($CDCl_3$) δ = 2.25 (s, 3H), 2.58 (t, J = 4.6 Hz, 4H), 3.18 (t, J = 4.6 Hz, 4H), 3.57-(s, 2H), 6.40 (d, J = 9.5 Hz, 1H), 6.65 (d, J = 9.70 Hz, 1H), 6.70−6.75 (m, 2H), 7.10 (t, J = 7.80 Hz, 1H), 7.25 (d, J = 8.7 Hz, 1H), 7.45 (s, 1H), 7.52 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 9.7 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

7-(4-(3-Chloro-4-methylphenyl)piperazin-1-ylmethyl)-chromen-2-one (13). This was prepared similarly to **5** starting with 1-(3-chloro-4-methylphenyl)piperazine. Recrystallization in ethyl acetate provided **13** in 55% yield: mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ = 2.21 (s, 3H), 2.60 (br s, 4H), 3.10 (br s, 4H), 3.60 (s, 2H), 6.37(d, J = 9.52 Hz, 1H), 6.65 (dd, J = 2.7 and 7.8 Hz, 1H), 6.85 (d, J = 2.7 Hz, 1H) 7.05 (d, J = 7.8 Hz, 1H), 7.35 (s, 1H), 7.45 (d, J = 8.7 Hz, 1H) 7.68 (d, J = 9.52 Hz, 1H); CIMS m/z 369 (MH^+). Anal. ($C_{21}H_{23}N_2O_2Cl$) C, H, N, Cl.

7-(4-(3,4-Dimethylphenyl)piperazin-1-ylmethyl)-chromen-2-one (14). This was prepared similarly to **5** starting with 1-(3,4-dimethylphenyl)piperazine. Column chromatography (5% 2-propanol in chloroform) provided **14** in 52% yield: mp 175−176 °C; ^{1}H NMR ($CDCl_3$) δ = 2.16 (s, 3H), 2.21 (s, 3H), 2.65 (br s, 4H), 3.20 (br s, 4H), 3.62 (s, 2H), 6.38 (d, J = 9.8 Hz, 1H), 6.66 (d, J = 7.3 Hz, 1H), 6.72 (s, 1H), 6.95 (d, J = 8.3 Hz, 1H), 7.30 (m, 1H), 7.34 (s, 1H), 7.42 (d, J = 8.37 Hz, 1H) 7.68 (d, J = 9.8 Hz, 1H); CIMS m/z 349 (MH^+). Anal. ($C_{22}H_{24}N_2O_2 \cdot 0.1H_2O$) C, H, N, H_2O.

7-(4-(4-Fluorophenyl)piperazin-1-ylmethyl)chromen-2-one (15). This was prepared similarly to **5** starting with 1-(4-fluorophenyl)piperazine. Recrystallization in ethyl acetate provided **15** in 15% yield: mp 137−138 °C; ^{1}H NMR ($CDCl_3$) δ = 2.60 (t, J = 4.64 Hz, 4H), 3.12 (t, J = 4.64 Hz, 4H), 3.61 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.82 (m, 2H), 6.90(m, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.32 (s, 1H), 7.42 (d, J = 7.81 Hz), 7.67 (d, J = 9.52 Hz, 1H); CIMS m/z 339 (MH^+). Anal. ($C_{20}H_{19}N_2O_2F$) C, H, N, F.

7-(4-(4-Chlorophenyl)piperazin-1-ylmethyl)chromen-2-one (16). This was prepared similarly to **5** starting with 1-(4-chlorophenyl)piperazine. Recrystallization in ethyl acetate provided **16** in 55% yield: mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ = 2.60 (br s, 4H), 3.15 (br s, 4H), 3.62 (s, 2H), 6.38 (d, J = 9.52 Hz, 1H), 6.82 (d, J = 9.03 Hz, 2H), 7.17 (d, J = 9.03 Hz, 2H), 7.27 (d, J = 6.8 Hz, 1H), 7.34 (s, 1H), 7.42 (d, J = 7.81 Hz, 1H), 7.67 (d, J = 9.52 Hz, 1H); CIMS m/z 355 (MH^+). Anal. ($C_{20}H_{19}N_2O_2Cl$) C, H, N, Cl.

7-(4-(4-Methoxyphenyl)piperazin-1-ylmethyl)chromen-2-one (17). This was prepared similarly to **5** starting with 1-(4-methoxyphenyl)piperazine. Recrystallization in ethyl acetate provided **17** in 51% yield: mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ = 2.65 (br s, 4H), 3.12 (br s, 4H), 3.68 (s, 2H), 3.75 (s, 3H), 6.38 (d, J = 9.52 Hz, 1H), 6.82 (m, 2H), 6.90 (m, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.32 (s, 1H), 7.42 (d, J = 7.81 Hz), 7.67 (d, J = 9.52 Hz, 1H); CIMS m/z 351 (MH^+). Anal. ($C_{21}H_{22}N_2O_3$) C, H, N.

7-(4-(4-*tert*-Butylphenyl)piperazin-1-ylmethyl)chromen-2-one (18). This was prepared similarly to **5** starting with 1-(4-*tert*-butylphenyl)piperazine. Recrystallization in ethyl acetate provided **18** in 46% yield: mp 174−175 °C; ^{1}H NMR ($CDCl_3$) δ = 1.13 (s, 9H), 2.60 (t, J = 4.64 Hz, 4H), 3.17 (t, J = 4.64 Hz, 4H), 3.61 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.85 (d, J = 7.8 Hz, 2H), 6.90 (m, 2H), 7.25 (m, 3H), 7.35 (s, 1H), 7.42 (d, J = 7.81 Hz, 1H), 7.67 (d, J = 9.52 Hz, 1H); CIMS m/z 376 (MH^+). Anal. ($C_{24}H_{28}N_2O_2$) C, H, N.

7-(4-Phenylpiperidin-1-ylmethyl)chromen-2-one (19). This was prepared similarly to **5** starting with 4-phenylpiperdine. Recrystallization in ethyl acetate provided **19** in 25% yield: mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ = 1.78 (m, 4H), 2.12 (m, 2H), 2.5 (m, 1H), 2.95 (m, 2H), 3.55 (s, 2H), 6.37 (d, J = 9.77 Hz, 1H), 7.16−7.27 (m, 6H), 7.34 (s, 1H), 7.42 (d, J = 7.56 Hz, 1H), 7.68 (d, J = 9.52 Hz, 1H); CIMS m/z 320 (MH^+). Anal. ($C_{21}H_{21}NO_2$) C, H, N.

7-(4-Phenyl-3,6-dihydro-2*H*-pyridin-1-ylmethyl)-chromen-2-one (20). This was prepared similarly to **5** starting with 4-phenyltetrahydropyridine. Chromatography eluting with ethyl acetate/hexanes (3:1) provided **20** in 20% yield: mp 143−148 °C; ^{1}H NMR ($CDCl_3$) δ = 2.54 (s, 2H), 2.70 (s, 2H), 3.16 (s, 2H) 3.69 (s, 2H), 5.99 (s, 1H), 6.34 (d, J = 9.52 Hz, 1H), 7.18 (m, 2H), 7.3 (t, J = 8.05 Hz, 2H), 7.31−7.33 (m, 3H), 7.45 (m, 1H), 7.63 (d, J = 9.52 Hz, 1H); CIMS m/z 317 (MH^+). Anal. ($C_{21}H_{19}NO_2$) C, H, N.

7-(4-(4-Benzyl)piperazin-1-ylmethyl)chromen-2-one (21). This was prepared similarly to **5** starting with 1-benzylpiperazine. Recrystallization in ethyl acetate provided **21** in 36% yield: mp 138−140 °C; ^{1}H NMR ($CDCl_3$) δ = 2.50 (br s, 8H), 3.55 (s, 2H), 3.60 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.85 (d, J = 7.8 Hz, 2H), 6.90 (m, 2H), 7.25 (m, 3H), 7.35 (m, 5H), 7.42 (d, J = 7.81 Hz, 1H), 7.67 (d, J = 9.52 Hz, 1H); CIMS m/z 335 (MH^+). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

7-[(2-Phenylaminoethylamino)methyl]chromen-2-one (22). A mixture of 7-bromomethylchromen-2-one (1.5 g, 6.3 mmol), *N*-phenylethylenediamine (5.0 g, 37 mmol), and potassium carbonate (4.0 g, 29 mmol) in acetonitrile (200 mL) was heated under reflux for 18 h. The mixture was cooled and filtered and the filtrate concentrated to a residue that was purified by chromatography (elution with 10% 2-propanol in dichloromethane). Trituration of the product with ethyl ether provided **22** (0.95 g, 51%): mp 89−91 °C; ^{1}H NMR ($CDCl_3$) δ = 1.5 (s, 2H + HDO), 2.92 (m, 2H), 3.18 (m, 2H), 3.83 (s, 2H), 6.55 (d, J = 9.52 Hz, 1H), 6.85 (d, J = 7.81 Hz, 2H), 6.62 (t, J = 7.9 Hz, 1H), 7.10 (t, J = 7.8 Hz, 2H), 7.25 (m, 2H), 7.38 (d, J = 7.81 Hz, 1H), 7.62 (d, J = 9.52 Hz, 1H); CIMS m/z 295 (MH^+). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

6-[(2-Phenylaminoethylamino)methyl]chromen-2-one (23). This was prepared similarly to **22** except 6-bromomethylchromen-2-one was used. Trituration of the product, after chromatography with 10% 2-propanol in dichloromethane, with ethyl ether provided **23** in 60% yield: mp 84−86 °C; ^{1}H NMR (DMSO-d_6) δ = 2.65 (t, J = 6.34 Hz, 2H), 3.05 (q, J = 5.37 Hz, 2H), 3.3 (HDO), 3.74 (s, 2H), 5.45 (t, J = 5.37 Hz, 1H), 6.43−6.48 (m, 2H), 6.52 (d, J = 8.06 Hz, 2H), 7.02 (t, J = 8.06 Hz, 2H), 7.30 (d, J = 8.54 Hz, 1H), 7.55 (dd, J = 8.24 and 1.71 Hz, 1H), 7.63 (d, J = 1.7 Hz, 1H), 8.00 (d, J = 9.52 Hz, 1H); CIMS m/z 295 (MH^+). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

7-[(2-*p*-Tolylaminoethylamino)methyl]chromen-2-one (24). This was prepared similarly to **22** except *N*-(*p*-tolyl)-ethylenediamine[11] was used as the amine. After chromatography with 7% 2-propanol in dichloromethane and crystallization in ethyl ether, **24** was obtained in 40% yield: mp 92−95 °C; ^{1}H NMR ($CDCl_3$) δ = 1.55 (br s, 2H), 2.17 (s, 3H), 2.83 (t, J = 5.6 Hz, 2H), 3.16 (t, J = 5.6 Hz, 2H), 3.83 (s, 2H), 6.32 (d, J = 9.52 Hz, 1H), 6.50 (d, J = 8.3 Hz, 2H), 6.92 (d, J = 8.1 Hz, 2H), 7.22 (m, 2H), 7.37 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 9.52 Hz, 1H); CIMS m/z 309 (MH^+). Anal. ($C_{19}H_{20}N_2O_2 \cdot 0.1H_2O$) C, H, N, H_2O.

7-[(2-(3,4-Dimethylphenylamino)ethylamino)methyl]-chromen-2-one (25). This was prepared similarly to **22** except *N*-(3,4-dimethylphenyl)ethylenediamine[11] was used as the amine. After chromatography with 10% 2-propanol in dichlo-

romethane and crystallization in ethyl ether, **25** was obtained in 40% yield: mp 92−95 °C; ^{1}H NMR ($CDCl_3$) δ = 2.1 (s, 3H), 2.15 (s, 3H), 2.85 (t, J = 5.6 Hz, 2H), 3.20 (t, J = 5.6 Hz, 2H), 3.83 (s, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.39 (dd, J = 2.2 and 9.2 Hz, 1H), 6.42 (d, J = 2.28 Hz, 1H), 6.90 (d, J = 7.8 Hz, 1H), 7.22 (m, 2H), 7.4 (d, J = 7.82 Hz, 1H), 7.65 (d, J = 9.52 Hz, 1H); CIMS m/z 322 (MH^+). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

7-[(2-(3-Chlorophenylamino)ethylamino)methyl]-chromen-2-one (26). This was prepared similarly to **22** except *N*-(3-chlorophenyl)ethylenediamine[11] was used as the amine. After chromatography with 10% 2-propanol in dichloromethane and crystallization in ethyl ether, **26** was obtained in 45% yield: mp 99−100 °C; ^{1}H NMR (CDCl3) δ = 1.45 (br s, 1H), 2.85 (t, J = 5.62 Hz, 2H), 3.15 (t, J = 5.6 Hz, 2H), 3.83 (s, 2H), 4.15 (br s, 1H), 6.37 (d, J = 9.52 Hz, 1H), 6.50 (d, J = 7.8 Hz, 2H), 7.05 (d, J = 7.8 Hz, 2H), 7.10 (t, J = 7.8 Hz, 2H), 7.22 (m, 2H), 7.4 (d, J = 7.81 Hz, 1H), 7.65 (d, J = 9.52 Hz, 1H); CIMS m/z 329 (MH^+). Anal. (C18H17N2O2Cl) C, H, N, Cl.

7-[(2-(3-Chloro-4-methylphenylamino)ethylamino)-methyl]chromen-2-one (27). This was prepared similarly to **22** except *N*-(3-chloro-4-methylphenyl)ethylenediamine[11] was used as the amine. After chromatography with 10% 2-propanol in dichloromethane and crystallization in ethyl ether, **27** was obtained in 65% yield: mp 77−78 °C; ^{1}H NMR ($CDCl_3$) δ = 1.45 (br s, 1H), 2.20 (s, 3H), 2.85 (t, J = 5.6 Hz, 2H), 3.17 (t, J = 5.6 Hz, 2H), 3.86 (s, 2H), 4.15 (br s, 1H), 6.37 (d, J = 9.52 Hz, 1H), 6.42 (dd, J = 2.2 and 7.8 Hz), 6.58 (d, J = 2.2 Hz, 1H), 6.95 (d, J = 7.8 Hz, 1H), 7.20 (m, 2H), 7.4 (d, J = 7.81 Hz, 1H), 7.65 (d, J = 9.52 Hz, 1H); CIMS m/z 343 (MH^+). Anal. ($C_{19}H_{20}N_2O_2Cl$) C, H, N, Cl.

7-[(3-Phenylaminopropylamino)methyl]chromen-2-one (28). This was prepared similarly to **22** except *N*-phenylpropylenediamine was used. The *N*-phenylpropylenediamine was prepared in two steps by heating a mixture of aniline (5.0 g, 53.7 mmol), 3-bromophthalimide (14.3 g, 53.7 mmol), and potassium carbonate (8.3 g, 60 mmol) in MeCN under reflux for 48 h. Column chromatography of the concentrated reaction mixture with ethyl acetate:hexanes (1:1) provided 5.5 g of the intermediate. This was treated with hydrazine hydrate (2 g, 33 mmol) in refluxing ethanol (150 mL) for 1.5 h. The reaction mixture was cooled and concentrated to an oily residue. The residue was partitioned between 1 N NaOH (aq) and chloroform (200 mL of each). Concentration of the dried (potassium carbonate) organic layer provided 2.4 g of *N*-phenylpropylenediamine, which was 90% pure as judged by ^{1}H NMR. This was coupled with 7-bromomethylchromen-2-one as previously described. After chromatography with 10% 2-propanol in dichloromethane and crystallization in ethyl ether, **28** was obtained in 20% yield: mp 68−69 °C; ^{1}H NMR ($CDCl_3$) δ = 1.8 (m, 2H), 2.77 (t, J = 5.6 Hz, 2H), 3.10 (t, J = 5.6 Hz, 2H), 3.83 (s, 2H), 6.35 (d, J = 9.52 Hz, 1H), 6.57 (d, J = 7.8 Hz, 2H), 6.65 (t, J = 7.9 Hz, 1H), 7.10 (t, J = 7.8 Hz, 2H), 7.22 (m, 2H), 7.4 (d, J = 7.81 Hz, 1H), 7.65 (d, J = 9.52 Hz, 1H); CIMS m/z 309 (MH^+). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

7-[(2-Phenoxyethylamino)methyl]chromen-2-one (29). This was prepared similarly to **22** except 2-phenoxyethylamine was used as the base. After chromatography with 5% 2-propanol in dichloromethane and crystallization in ethyl ether, **29** was obtained in 55% yield: mp 62−64 °C; ^{1}H NMR ($CDCl_3$) δ = 3.0 (t, J = 5.6 Hz, 2H), 3.95 (s, 2H), 4.07 (t, J = 5.6 Hz, 2H), 6.37 (d, J = 9.52 Hz, 1H), 6.83 (d, J = 9.70 Hz, 2H), 6.90 (t, J = 6 Hz, 1H), 7.25 (m, 3H), 7.30 (s, 1H), 7.40 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 9.7 Hz, 1H); CIMS m/z 296 (MH^+). Anal. ($C_{18}H_{17}NO_3$) C, H, N.

7-[(2-(Ethylphenylamino)ethylamino)methyl]chromen-2-one Hydrochloride (30). This was prepared similarly to **22** except *N*,*N*-ethylphenylethylenediamine was used as the amine. The amine was prepared from the coupling of the *N*-ethylaniline with 2-oxazalidone.[11] After chromatography with 5% 2-propanol in dichloromethane, the compound was converted to the hydrochoride salt in ethyl ether by addition of 1 N hydrogen chloride in ether to provide **30** in 65% yield: mp 209−210 °C; ^{1}H NMR (DMSO-d_6) δ = 1.02 (t, J = 6.83 Hz, 2H), 3.02 (br s, 2H), 3.33 (q, J = 6.8 4 Hz, 2H), 3.63 (s, 2H), 3.77 (HDO), 4.24 (s, 2H), 6.50 (d, J = 9.72 Hz, 1H), 6.64 (br s, 1H), 6.8 (br s, 2H), 7.15 (m, 2H), 7.50 (d, J = 7.56 Hz, 1H), 7.63 (s, 1H), 7.73 (d, J = 7.81 Hz, 1H), 8.05 (d, J = 9.72 Hz, 1H), 9.6 (s, 2H); CIMS m/z 323 (MH^+). Anal. ($C_{20}H_{22}N_2O_2$·1.65HCl·0.35H_2O) C, H, N, Cl, H_2O.

7-{[Ethyl(2-phenylaminoethyl)amino]methyl}chromen-2-one Hydrochloride (31). A mixture of **22** (1.06 g, 3.6 mmol), iodoethane (0.29 mL, 3.6 mmol), and potassium carbonate (2 g, 14 mmol) was stirred at room temperature in acetonitrile (100 mL) for 4 days. The reaction mixture was filtered and the filtrate concentrated in vacuo. The residue was chromatographed eluting with 2% 2-propanol in chloroform. An ethereal solution of the product was converted to the hydrochloride salt by the addition of 1 N hydrogen chloride (ether) to provide **31** (430 mg, 37%): mp 225−230 °C; ^{1}H NMR (DMSO-d_6) δ = 1.26 (t, J = 7.1 Hz, 3H), 3.21 (m, 4H), 3.52 (t, J = 6.6 Hz, 2H), 3.6−4.15 (br s, ∼2H), 4.44 (s, 2H), 6.51 (d, J = 9.52 Hz, 1H), 6.57 (m, 3H), 7.02 (t, J = 8.0 Hz, 2H), 7.59 (d, J = 8.06 Hz, 1H), 7.71 (d, J = 8.05 Hz, 1H), 7.79 (s, 1H), 8.18 (d, J = 9.77 Hz, 1H); CIMS m/z 323 (MH^+). Anal. ($C_{20}H_{22}N_2O_2$·1.15HCl·0.3H_2O) C, H, N, Cl^-, H_2O.

Receptor Binding. The in vitro affinities of compounds for cloned human DA receptors versus [^{3}H]spiperone in CHO K1 cells were determined as previously described. [13]

Mitogenesis Assay. The effects of test compounds on [^{3}H]-thymidine uptake were determined as described by Chio[14] with minor modifications. In brief, CHO 10001 cells transfected with human D4.2 receptors were plated on 96-well plates in a minimum essential medium (αMEM, Gibco) with 10% fetal calf serum containing penicillin (100 units/mL) and streptomycin (100 μg/mL). Forty-eight hours later, cells were washed with serum-free media and maintained thereafter in serum-free media. After 24 h, test compounds were added. Eighteen hours later, [^{3}H]thymidine (0.25 μCi/well was added for 2 h, trypsin (100 μL of 0.25%) was added for 1 h, and the assay was terminated by filtration using a Brandel 96-well harvester. The filters were counted for radioactivity using the LKB β-plate counting system.

Effects on DA Synthesis.[13] Striatal and hippocampal DA synthesis were measured by HPLC with electrochemical detection in rats given drug 60 min previously and the L-aromatic amino acid decarboxylase inhibitor NSD 1015 30 min prior to sacrifice by decapitation. Data are expressed as the percentage increase of DA synthesis (as indicated by DOPA levels) relative to control animals.

Rat Spontaneous Locomotor Activity.[16] Harlan Sprague−Dawley rats were dosed ip 30 min before monitoring open field levels in the dark in 16 × 16 × 12 in. Plexiglas cubicles with 16 photobeams spaced 1 in. apart on all four sides of the monitors. Activity data (total distance traveled in cm) for six 5-min intervals were recorded automatically by computer. Data were expressed as percent of vehicle-treated control activity. Significant changes in activity levels, relative to controls, were determined by *t*-test. The dose that could be expected to decrease locomotion by 50% (ED_{50}) and the 95% confidence limits were estimated by regression analysis.

Rat Amphetamine-Stimulated Locomotor Activity Studies.[15] Rats were dosed ip with saline or 0.5 mg/kg *d*-amphetamine and placed in the Omnitech Digiscan activity monitors for a 20-min acclimation period, followed by either ip saline or the test compound. Rats were returned to their respective chambers and locomotor activity (cm traveled) was measured for 30 min. Data were expressed as total distance traveled (in cm). Statistical significance between groups was calculated using a *t*-test. Amphetamine reversal ED_{50}'s and 95% confidence limits were calculated using regression analysis.

Prepulse Inhibition of Acoustic Startle in Rats (PPI).[17,18] Rats were dosed ip with vehicle or the test compound 15 min prior to sc injection of saline or apomorphine (0.25 mg/kg), then placed in one of eight San Diego Instruments acoustic startle chambers for a 30-min test. The test session consisted of a total of 90 trials after a 5-min test acclimation period of 70 dB of white noise. The first and last

10 trials are 120-dB pulse-alone trials of each of the following 5 trial types in pseudo-random order: (1) no stimulus, (2) 72-dB prepulse 100 ms prior to a 120-dB startle pulse, (3) 74-dB prepulse + 120-dB pulse, (4) 78-dB prepulse + 120-dB pulse, and (5) 86-dB prepulse + 120-dB pulse. Intertrial interval was 7−23 s. The prepulses (2, 4, 8, and 16 dB over the 70-dB background noise) were 20 ms in duration, while the startle pulses were 40 ms in duration. Data are shown as %PPI (calculated for each individual rat using the 16-dB prepulse values), and statistical significance was calculated between treatment groups using a *t*-test. The minimal effective dose required to produce a significant reversal of apomorphine-disrupted PPI was used as the efficacy measure.

Catalepsy Test in Rats.[19] Rats were dosed orally prior to the catalepsy test. The front paws were placed on an elevated bar, and the time (s) that the paws remained on the bar was recorded for three consecutive trials of 1-min duration. Rats were tested again 2 h postdose. Data shown is the mean response time for the third trial.

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