A Series of 6- and 7-Piperazinyl- and -Piperidinylmethylbenzoxazinones with Dopamine D4 Antagonist Activity: Discovery of a Potential Atypical Antipsychotic Agent

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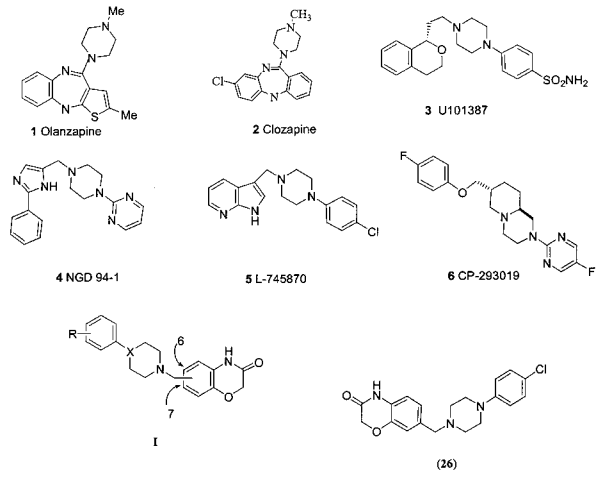
As part of a program to develop dopamine D4 antagonists for the treatment of schizophrenia, we discovered a series of 6- and 7-(phenylpiperazinyl)- and -(phenylpiperidinyl)methylbenzoxazinones through mass screening of our compound library. A structure−activity relationship SAR study was carried out involving substituents on the phenyl ring, and several selective D4 antagonists were identified. The 7-substituted benzoxazinones showed more activity in neurochemical and behavioral tests than the 6-substituted series. One of the most potent and selective compounds (**26**) was found to have potent activity in animal tests predictive of antipsychotic activity in humans after oral administration. This paper describes the SAR of the benzoxazinone series and the preclinical characterization of **26**.

Introduction

Schizophrenia is a complex psychiatric disorder affecting up to 50 million people worldwide.[1] The primary therapy for the disease is administration of dopamine (DA) antagonists such as olanzapine (**1**), which is an antagonist at DA D2 receptors. The blockade of postsynaptic DA D2 receptors in the mesolimbic and prefrontal cortex regions of the brain is thought to be responsible for decreasing the positive symptoms of schizophrenia such as hallucinations and delusions.[2] Unfortunately, blockade of DA D2 receptors in the striatum causes extrapyramidal side effects (EPS) and tardive dyskinesia.[3] In addition, DA D2 receptor blockade can lead to increased prolactin levels resulting in galactorrhea.[4] Cloning studies showed that the DA D2 receptor family consists of the DA D2, D3,[5] and D4[6] receptor subtypes.

The discrete localization of DA D4 receptor mRNA in cortical and mesolimbic regions of the brain (regions believed to be involved in thought and emotion) and its relative absence from nigral−striatal regions controlling motor function suggest the DA D4 receptor is a good target for the drug treatment of schizophrenia.[6−8] Most of the typical antipsychotic agents were found to have similar affinities for DA D2 and DA D4 receptors, but the atypical antipsychotic agent clozapine (**2**) was found to have a 7−14-fold greater affinity for DA D4 receptors compared to DA D2 receptors.[6−9]

Clozapine has been found to be effective in relieving the positive symptoms of schizophrenia in a portion of the patients resistant to treatment by other DA antagonists. Since it also has activity against negative symptoms (i.e., withdrawal, loss of drive, flattened effect) and it has a decreased incidence of EPS,[10] clozapine is an atypical antipsychotic agent.[11] Correlation of DA D4 (but not D2 or D3) receptor binding affinity with concentrations of clozapine in plasma of patients after clinically efficacious doses also suggests that DA D4 receptors are a potential target in mediating the drug's antipsychotic effects.[12] Clinical use of clozapine requires careful monitoring due to a 1−2% occurrence of the potentially fatal blood disorder agranulocytosis[13] and it is limited primarily to patients refractory to other treatments. Therefore, improved agents are still needed. The preferential binding of clozapine at the DA D4 receptor along with the localization of the receptor suggests that antagonists selective for the DA D4 receptor might offer advantages over existing antipsychotic agents.



Several laboratories have reported success in generating DA D4 receptor antagonists with greater than 100-fold selectivity in affinity for the DA D4 receptor subtype compared to the DA D2 receptor subtype as exemplified by compounds **3**,[14] **4**,[15] **5**,[16] **6**,[17] and others.[18−26] Clinical trials with the DA D4 antagonist L-745,870 (**5**) have been disappointing.[27] This result is not surprising in retrospect given the lack of activity of the compounds in animal behavioral tests predictive of antipsychotic efficacy.[16] A recent report[28] suggests that **5** acts as a

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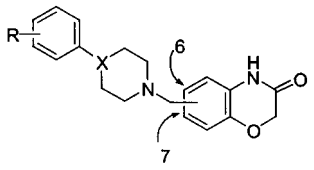
Table 1. Receptor Binding of Target Compounds[a]



compd	R	X	6/7 subs	D4 K_i (nM)	D3 K_i (nM)	D2 K_i (nM)
16	3,4-di Me	CH	6	1.8	>3030	572
17	4-Me	CH	6	2.8	>3030	52.5
18	4-OMe	CH	6	3.8	>3030	698
19	4-Me	N	6	4.3	2490	493
20	3,4-di Me	N	6	5.1	864	2340
21	4-OMe	N	6	12.3	>3030	5590
22	H	N	6	18.3	839	2200
23	4-Cl	N	6	62.4	>3030	>5880
24	4-Me	CH	7	2.6	232	287
25	3,4-di Me	CH	7	2.6	859	610
26	4-Cl	N	7	4.3	679	413
27	4-OMe	N	7	5	1790	>5880
28	H	N	7	6.1	10.4	129
29	4-OMe	CH	7	6.7	428	1130
30	3,4-di Me	N	7	6.9	78.5	346
31	4-Me	N	7	10.7	208	2980
	L-754870			0.91	>3030	908

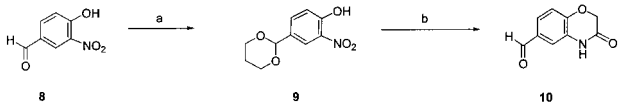
[a] Measured as ability of test compound to displace radioligand in CHO cells transfected with receptor cDNA. Tests were run in triplicate and results did not vary by more than 25%.

partial agonist at the DA D4 receptor and may explain its lack of effect in animal tests predictive of antipsychotic activity. Our research efforts have focused on finding compounds that not only are potent and selective antagonists at the DA D4 receptor, but also have neurochemical and behavioral effects in vivo that are predictive of antipsychotic efficacy. Desirable compounds would also be inactive in tests predictive of side effects associated with the standard neuroleptics. In this article we outline the discovery of a series of piperazinylmethylbenzoxazinones of the general structure I having this desired profile.

Chemistry

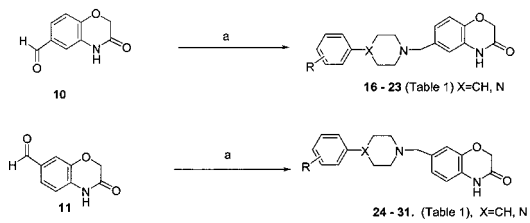
A general route for the preparation of 3-oxobenzoxazine carboxaldehydes was developed as outlined in Scheme 1. The 4-carboxaldehyde derivative of 2-nitrophenol **8** was reacted with propanediol forming the corresponding propylidine acetal **9**. In a multistep procedure the nitro group of **9** was reduced, the resulting 2-aminophenol was treated directly with chloroacetyl chloride, and the acetal was hydrolyzed. Treatment of the resulting product with potassium carbonate in acetonitrile gave the 3-oxobenzoxazine-6-carboxaldehyde **10**. The 3-oxobenzoxazine-7-carboxaldehyde **11** was prepared in a similar manner from the 3-carboxaldehyde derivative of 2-nitrophenol. The target compounds in Table 1 were made by reacting aldehydes **10** and **11** with a variety of phenylpiperazines or phenylpiperidines in the presence of sodium cyanoborohydride (method A, Scheme 2). Alternatively, aldehydes **10** and **11** could be reduced to give the benzylic alcohol, which was activated with tosyl chloride and displaced with various phenylpiperazines or phenylpiperidines (method B, Scheme 3).

Scheme 1. Synthesis of Benzoxazinone Aldehydes[a]



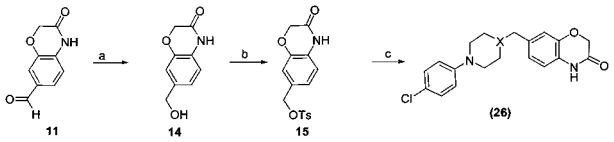
[a] Reagents and conditions: (a) 1,3-propanediol, pTsOH, toluene, reflux. (b) (1) RaNi, THF, (2) chloroacetyl chloride, $NaHCO_3$, THF, (3) HCl, MeOH, (4) K_2CO_3, CH_3CN.

Scheme 2. Preparation of Target Compounds by Method A[a]



[a] Reagents and conditions: (a) substituted phenylpiperazine or phenylpiperidine, $NaCNBH_3$, 1,2-dichloroethane.

Scheme 3. Synthesis of **26** by Method B[a]



[a] Reagents and conditions: (a) MeOH, $NaBH_4$. (b) TsCl, pyridine, 0 °C. (c) 4-chlorophenylpiperazine, K_2CO_3, CH_3CN, reflux.

Results and Discussion

Table 1 shows the binding data for the target compounds at the DA D2, D3, and D4 receptors. Earlier work in our laboratories and others showed that substituents in the 3 or 4 position of the phenyl ring attached to the piperazine or piperidine led to compounds with increased selectivity and affinity at the DA D4 receptor.[29] In the series with 6-substituted benzoxazinones (**16**−**23**), the affinity for the DA D4 receptor ranges from 2 to 62 nM. In general, phenylpiperidines (**16**−**18**) have slightly higher DA D4 affinity than the corresponding phenylpiperazines (**19**−**21**), although they are less selective for DA D4 vs D2 receptors.

In the 7-substituted benzoxazinones (**24**−**31**) there is not such a wide range of binding affinities. This series, with the exception of compound **28**, all have about 100-fold selectivity for the DA D4 receptor over the DA D2 receptor. The most noticeable difference between the 6- and 7-benzoxazinones is that the binding affinity of the 4-chloro compound improves from 62 nM in the 6-substituted series (**23**) to 4.6 nM in the 7-substituted series (**26**). Because of its potency and selectivity at the DA D4 receptor, **26** was tested in a [^{3}H]thymidine functional assay.[30] It blocked the quinpirole-stimulated uptake of thymidine with an IC_{50} of 1.5 nM, while having no effect on [^{3}H]thymidine uptake on its own (intrinsic activity = 0). These results indicate that **26** is an antagonist at the DA D4 receptor. At the DA D2 receptor, **26** is a weak partial agonist (intrinsic activity = 41%) with an IC_{50} of 932 nM. In this functional assay **26** shows greater than 600-fold selectivity for the DA D4 vs the DA D2 receptor.

To test for in vivo functional effects, those compounds that showed the highest affinity and selectivity for the DA D4 receptor were tested for their ability to increase

Table 2. Effects of Compounds on DA Synthesis in Rat Hippocampus (HI) and Striatum (ST) Measured as Accumulation of DOPA[a]

						% change in DA synthesis	
compd	R	X	6/7 subs	dose (mg/kg)	route	HI	ST
16	3,4-diMe	CH	6	10	po	128 ± 10	104 ± 4
18	4-OMe	CH	6	30	po	93 ± 5	96 ± 8
19	4-Me	N	6	20	po	89 ± 5	111 ± 9
20	3,4-diMe	N	6	10	ip	122 ± 8	126 ± 7
21	4-OMe	N	6	30	po	91 ± 8	110 ± 9
25	3,4-diMe	CH	7	10	po	103 ± 7	99 ± 7
26	4-Cl	N	7	10	po	146 ± 5	110 ± 7
27	4-OMe	N	7	10	po	122 ± 9	104 ± 9
29	4-OMe	CH	7	20	po	132 ± 4	126 ± 2
31	4-Me	N	7	10	po	133 ± 7	140 ± 5

[a] Compounds and NSD1015 (100 mg/kg, ip) were administered 60 and 30 min, respectively, before animal termination. Striatum and mesolimbic brain regions were removed and homogenized. The supernatant was analyzed by HPLC with electrochemical detection for the presence of DOPA. Each value is the mean of 4−8 animals and is expressed as percent of control values (ng/g ± SEM) that were 119 ± 8 and 1378 ± 96 for hippocampus and striatum, respectively. Data were analyzed by one-way analysis of variance and a Newman-Keuls test was used to determine significantly different group means, with $P < 0.05$ being considered significant.

dopamine synthesis (measured as increased levels of DOPAC) in the rat hippocampus (HI) and striatum (ST) (Table 2). Desirable compounds would selectively increase DA synthesis in the hippocampus, a brain area rich in DA D4 receptors, and not in the striatum, a brain area with low DA D4 receptor concentration.[6−8] We also looked for oral activity in this assay because this is the preferred route of administration for antipsychotic agents. As can be seen from Table 2, the 6-substituted benzoxazinones had weak activity in this assay. The most active 6-benzoxazinone (**16**) increases DA synthesis in the HI by only 28% after oral administration. The 7-substituted series had more oral activity. The most active compound (**26**) increases DA synthesis by 46% in the HI while having no significant effect in the ST after oral administration. This is consistent with earlier findings that the DA D4 receptor is localized in the mesolimbic regions of the brain.[7] Because of this compound's selectivity and potency it was carried on to further in vitro and in vivo studies.

To see if the observed effects are selectively mediated by the DA D4 receptor, compound **26** was tested for

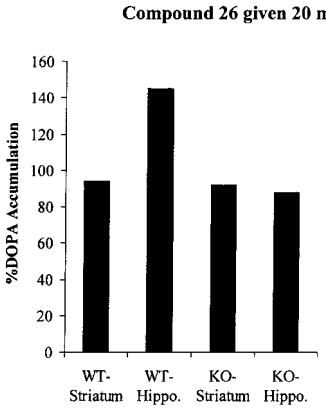


Figure 1. Effects of **26** on dopamine synthesis in wild type (WT) versus D4 knockout (KO) mice.

binding to other major central nervous system (CNS) receptors and ion channels (Table 3). It had only modest affinity (K_i = 200−800 nM) for adrenergic, 5-HT-1A, and σ receptors. Only micromolar activity at muscarinic M5 and 5-HT2 receptors was observed, and **26** was found to be inactive at a large number of other ion channels and receptors.

Another indication that the observed effects are D4-mediated is the fact that **26** increased DOPAC levels in the hippocampus of wild-type mice while it had no effects on DOPAC levels in D4 knockout mice in our assay (Figure 1). It is also interesting to note that there was no effect on the DA synthesis in the striatum of either type of mice. This is to be expected since there are few D4 receptors in the striatum.[7]

The oral activity of **26** in tests predictive of antipsychotic activity suggests that it may be efficacious in drug treatment of the symptoms of schizophrenia. Figure 2 shows that the compound blocked amphetamine-stimulated locomotor activity after oral administration in the rat. As can be seen in the figure, administration of amphetamine (0.5 mg/kg ip) increased locomotor activity by a factor of 3−4-fold. As the oral dose of **26** was increased, the locomotor activity dose dependently returned to near control levels. The ED_{50} for **26** in this test is 2.2 mg/kg (po). Activity in this paradigm suggests that **26** will have antipsychotic activity in humans.[31]

Another test predictive of antipsychotic activity is the restoration of prepulse inhibition of the acoustic startle

Table 3. In Vitro Profile of **26**: *Estimated* Binding Affinities (Panlabs) of **26** for Various Receptor Binding Sites Where Affinity Was Less Than 50% at 10 μM[a]

receptor	ligand	tissue	~IC_{50} (nM)	~K_i (nM)	ref cmpd. K_i(nM)
adrenergic α_{1A}	[^{3}H]prazosin	rat submaxillary gland	500	203	prazosin, 0.07
adrenergic α_{1B}	[^{3}H]prazosin	rat liver	1425	787	prazosin, 0.15
adrenergic-α_{2A}	[^{3}H]MK-912	human recombinant	1100	411	yohimbine, 3.1
adrenergic-α_{2B}	[^{3}H]yohimbine	rat kidney cortex	630	368	yohimbine, 2.0
5-HT-1A	[^{3}H]-8-OH-DPAT	human recombinant	1400	792	metergoline, 13
5-HT-2	[^{3}H]ketanserin	rat brain	2700	1678	ketanserin, 1.3
σ-1	[^{3}H]pentazocine	guinea pig brain	285	232	haloperidol, 1.8
σ-2	[^{3}H]Ifenprodil	rat brain	375	231	ifenprodil, 4.9
muscarinic M5	[^{3}H]NMS	human recombinant	1340	964	4-DAMP, 0.86

[a] The Panlabs screen indicated that **26** had no significant affinity for a large number of other receptors or ion channels (IC_{50} values > 10 μM) including the following: adenosine A1, A2A, and A3, adrenergic β-1, β-2, and β-3, angiotensin ATI and AT2, bombesin, bradykinin B1 and B2, calcium L channels (three different sites), calcium N channels, cannabinoid CB1 and CB2, cholecystokinin CCKA and CCKB, dopamine D1 and D5, endothelin ETA and ETB, estrogen, GABA-A (agonist site, benzodiazepine and chloride channel), glucocorticoid, glutamate, kainate and NMDA, glycine, strychnine-sensitive, histamine H1, H2, and H3, interleukin-1α, leukotriene D4, muscarinic M1, M2, M3, and M4, neurokinin NK1 and NK2, neuropeptide Y1 and Y2, nicotinic acetylcholine (central), opiate δ, κ and μ, K+ channel [KATP], [KV], [SKCa], serotonin 5-HT3, serotonin 5-HT4, testosterone, thromboxane A2, TNF-α, vasoactive intestinal peptide-VIP1, and vasopressin V1.

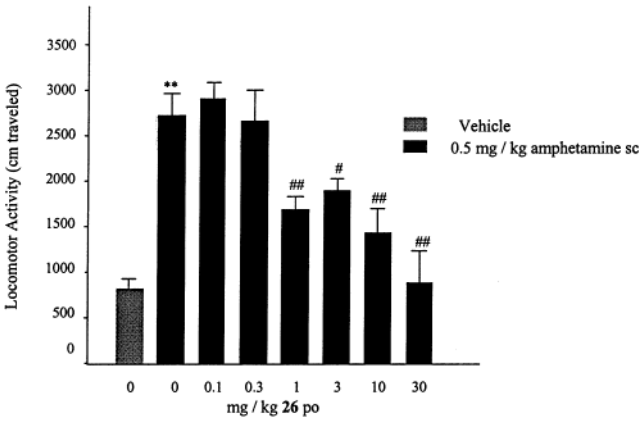


Figure 2. Effects of **26** on the amphetamine-stimulated locomotor activity in rats. Control rats were given only vehicle; the rest were given 0.5 mg/kg amphetamine sc before testing. $**p < 0.01$ from vehicle. $\#p < 0.05$ from amphetamine. $\#\#p < 0.01$ from amphetamine.

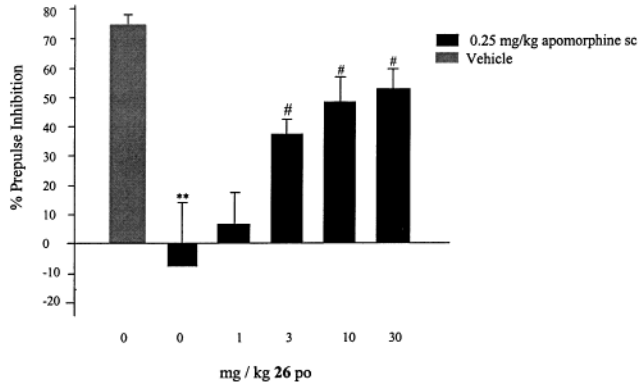


Figure 3. Compound **26** reverses the apomorphine disruption of prepulse inhibition of acoustic startle. Control rats were given only vehicle while the rest were given 0.25 mg/kg apomorphine sc. $**p < 0.01$ from vehicle, $\#p < 0.05$ from apomorphine.

reflex in rats. It is known that schizophrenic patients exhibit diminished prepulse inhibition of acoustic startle (PPI), and this phenomenon can be mimicked in rats by treatment with apomorphine, a known dopamine agonist.[32,33] The antipsychotics currently on the market will restore PPI in these apomorphine-treated rats. In this test, rats were placed in a chamber and exposed to a startle stimulus. The rats exhibited a startle response and the magnitude of the response was measured. If the same rat was exposed to a "prepulse" immediately before the startle stimulus, the startle response was diminished. This is prepulse inhibition of acoustic startle. Figure 3 shows that rats treated with only vehicle exhibited about 75% prepulse inhibition (PPI) while those rats treated with apomorphine exhibited no significant PPI. Compound **26** dose-dependently restored PPI to near control levels with an oral minimum effective dose (MED) of 3 mg/kg, indicating that it may be efficacious as an antipsychotic agent.

In tests predictive of side effects associated with the standard neuroleptics, **26** is inactive. Figure 4 shows that **26** did not cause catalepsy[34] in rats at 22 mg/kg po (7 times ED_{50}), indicating little or no potential for causing the EPS associated with other neuroleptics. Figure 5 shows that **26** did not increase the prolactin levels of

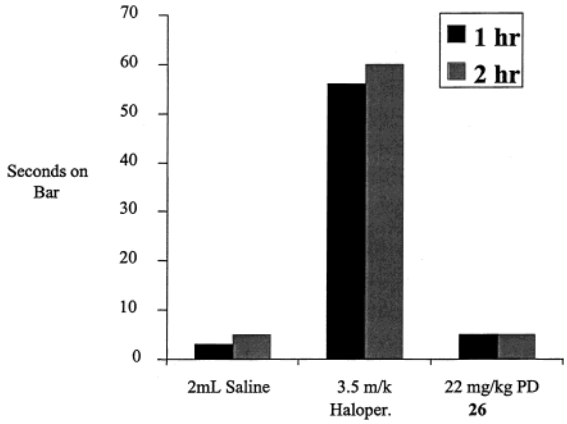


Figure 4. Catalepsy profile of **26**. At 22 mg/kg, it has no significant effects.

mice after ip administration of 30 mg/kg (10 times ED_{50}), indicating that it does not act at DA D2 receptors.

Conclusion

Unlike recent DA D4 antagonists reported in the literature such as **5**[16] and **3**,[14] **26** is orally active in animal models predictive of antipsychotic activity. It restores prepulse inhibition of acoustic startle in apomorphine-treated rats and it reverses amphetamine-stimulated locomotor activity after oral administration in the rat. Both of these results suggest that **26** (identified as CI-1030) will be an efficacious antipsychotic., It is not likely to have the side effects associated with typical antipsychotics because it does not increase prolactin secretion or cause catalepsy in the rat.

Experimental Section

Chemistry. Phenylpiperazines were purchased from Aldrich Chemical Co. or Janssen. Phenylpiperidines were prepared as described in the literature.[35] All reactions were monitored by thin-layer chromatography (TLC) on Merck glass plates precoated with 0.25 mm of silica gel. Chromatography for purification was done with Merck silica gel (230−400 mesh) or Elution Solution Flashelute cartridges packed with 32−63 μM silica gel. Solvents are reported as v/v solutions. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were recorded on a Finnigan 4500 mass spectrometer or on a Micromass Platform LC mass spectrometer and are chemical ionization (CI) spectra. The spectra are reported as M^+ peaks and their intensity relative to the base peak (100%). Proton NMR spectra were recorded on either a Varian 300 or a Varian 400 spectrometer with tetramethylsilane (TMS) as an internal standard. Multiplicity is indicated as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, and m = multiplet. Combustion analyses were performed by either Robertson Microlit Laboratories or QTI Laboratories, and the results were within 0.4% of the theoretical values for the elements indicated.

3-Oxo-3,4-dihydro-2*H*-benz[1,4]oxazine-6-carboxaldehyde (10). 4-Hydroxy-3-nitrobenzaldehyde (25 g, 149.6 mmol) was added to a solution of 1,3-propanediol (17.1 g, 224.4 mmol) in 250 mL of toluene. A catalytic amount of *p*-toluenesulfonic acid was added, and the mixture was warmed to reflux with removal of water by a Dean-Stark trap. After 12 h, the mixture was cooled to room temperature and extracted with saturated $NaHCO_3$ (3 × 150 mL) followed by 100 mL of brine. Drying over Na_2SO_4 followed by evaporation of the solvent gave 31.5 g of an oil, which was hydrogenated over Ra Ni in THF. When the theoretical amount of H_2 was taken up, the catalyst was removed by filtration, and the solution was immediately treated with chloroacetyl chloride (16.6 g, 146.8 mmol) and

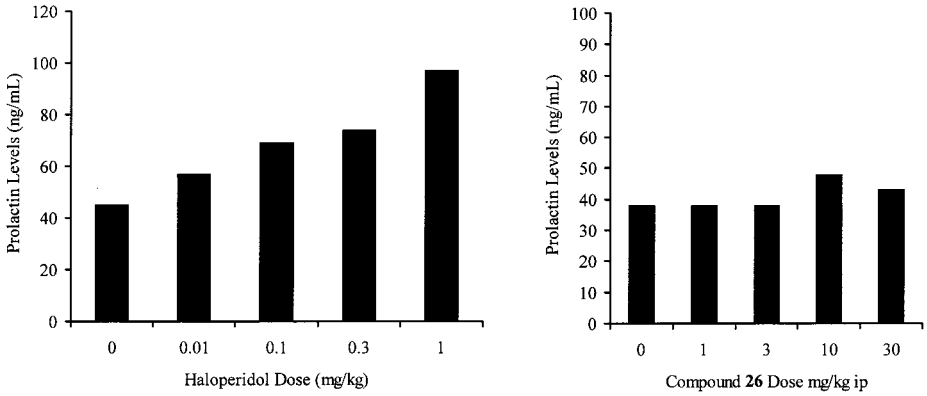


Figure 5. Effects of **26** on prolactin levels in mice. At 30 mg/kg, **26** does not increase prolactin levels, indicating that it does not act at D2 receptor.

$NaHCO_3$ (23.5 g, 279.7 mmol) at room temperature. After 1.5 h, the THF was evaporated and the residue was taken up in a 50/50 mixture of MeOH/H_2O and acidified to pH = 1 with concentrated HCl. The resulting solution was stirred at room temperature overnight. The MeOH was evaporated and the solid that formed was collected by filtration. It was treated with K_2CO_3 (57.9 g, 419.6 mmol) in 200 mL of CH_3CN at room-temperature overnight. The solvent was evaporated, and the solid remaining was triturated with 200 mL of H_2O. The remaining solid was dried at room temperature under vacuum to give 19.7 g (79% yield) of **10**. An aliquot was recrystallized from EtOAc. Mp = 219−221 °C. MS 178 (M^+, 10%) 219 (M + CH_3CN, 100%). ^1H NMR (DMSO-d_6) δ 4.67 (s, 2H), 7.10 (d, 1H, J = 8 Hz), 7.33 (d, 1H, J = 2 Hz), 7.49 (dd, 1H, J = 8 and 2 Hz), 9.80 (s, 1H), 10.96 (s, 1H). Analysis for $C_9H_7NO_3$: C, H, N.

3-Oxo-3,4-dihydro-2*H*-benz[1,4]oxazine-7-carboxaldehyde (11) was prepared by the same method as **10** starting from 3-hydroxy-4-nitrobenzaldehyde. Mp = 220−222 °C. MS 178 (M^+, 100%). ^1H NMR (DMSO-d_6) δ 4.65 (s, 2H), 7.08 (d, 1H, J = 8.0 Hz), 7.32 (d, 1H, J = 1.9 Hz), 7.47 (dd, 1H, J = 8.3 and 2.0 Hz), 9.78 (s, 1H), 10.9 (s, 1H). Analysis for $C_9H_7NO_3$: C, H, N.

6-[[4-(4-Methylphenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (19). Acetic acid (0.34 g, 5.6 mmol) was added to a solution of 3-oxo-3,4-dihydro-2*H*-benz[1,4]oxazine-6-carboxaldehyde **(10)** (1.0 g, 5.6 mmol) and (4-methylphenyl)-piperazine (0.98 g, 5.6 mmol) in 50 mL of dichloroethane. $NaBH(OAc)_3$ (2.5 g, 11.9 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The reaction was quenched by addition of 50 mL of H_2O, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were dried over Na_2SO_4. Evaporation of the solvent followed by recrystallization of the solid from EtOAc gave 0.81 g (43% yield) of **19**. Mp = 212−214 °C. MS 338 (M^+, 100%). ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H), 2.56 (t, 4H, J = 4.9 Hz), 3.09 (t, 4H, J = 4.9 Hz), 3.44 (s, 2H), 4.57 (s, 2H), 6.79 (s, 2H), 6.81 (d, 2H, J = 8.5 Hz), 7.03 (d, 2H, J = 8.5 Hz), 7.9 (s, 1H). Analysis for $C_{21}H_{25}N_3O_2$: C, H, N.

7-(Hydroxymethyl)-4*H*-benz[1,4]oxazin-3-one (14). 3-Oxo-3,4-dihydro-2*H*-benzo[1,4]oxazine-7-carboxaldehyde (**11**) (1.0 g, 5.6 mmol) was added to a solution of $NaBH_4$ (0.11 g, 2.8 mmol) in 25 mL of MeOH at 0 °C. After being stirred at 0 °C for 1 h, the reaction was quenched with 10 mL of 1.0 N HCl, and the MeOH was evaporated. The precipitate was collected and air-dried overnight to give 0.7 g (76% yield) of 7-(hydroxymethyl)-4*H*-benz[1,4]oxazin-3-one (**14**). An analytical sample was obtained by recrystallization from EtOAc. Mp = 206−208 °C. MS 180 (M^+, 100%). ^1H NMR (DMSO-d_6) δ 4.33 (d, 2H, J = 5 Hz), 4.48 (s, 2H), 5.07 (t, 1H, J = 5.6 Hz), 6.79 (d, 1H, J = 14.7 Hz), 6.82−6.83 (m, 2H), 10.6 (s, 1H). Analysis for $C_9H_9NO_3$: C, H, N.

7-[[[(4-Methylphenyl)sulfonyl]oxy]methyl]-4*H*-benz[1,4]oxazin-3-one (15). 7-(Hydroxymethyl)-4*H*-benz[1,4]oxazin-3-one (**14**) (0.6 g, 3.7 mmol) was added to a solution of 4-toluenesulfonyl chloride (0.74 g, 3.9 mmol) in 10 mL of pyridine at 0 °C. The mixture was stirred at 0 °C for 5 h and warmed to room temperature. The pyridine was evaporated and the solid remaining was dried under vacuum overnight to give 1.2 g of 7-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-4*H*-benz[1,4]oxazin-3-one (**15**), which was used without further purification.

7-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (26). A suspension of 7-[[[(4-methylphenyl)-sulfonyl]oxy]methyl]-4*H*-benz[1,4]oxazin-3-one (**15**) (12.7 g, 38.1 mmol), 1-(4-chlorophenyl)piperazine (7.5 g, 38.1 mmol), and K_2CO_3 (26.4 g, 190.5 mmol) in 300 mL of acetonitrile was warmed to reflux under Ar overnight. The mixture was cooled to room temperature, and the solvent was evaporated. The solid remaining was taken up in 200 mL of H_2O and stirred at room temperature. The solid was collected and air-dried. The dry solid was recrystallized twice from EtOAc to give 6.8 g (50% yield) of 7-[[4-(4-chlorophenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (**26**). Mp = 234−236 °C MS 358 (M^+, 100%). ^1H NMR (DMSO-d_6) δ 2.45 (m, 4H), 3.10 (m, 4H), 3.40 (s, 2H), 6.85 (m, 3H), 6.89 (d, 2H, J = 9.2 Hz), 7.10 (d, 2H, J = 9.2 Hz). Analysis for $C_{19}H_{20}ClN_3O_2$: C, H, N, Cl.

6-[[4-(3,4-Dimethylphenyl)piperidin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (16) was prepared by method A. Mp = 175−177 °C. MS 351 (M^+, 100%). ^1H NMR ($CDCl_3$) δ 1.75 (m, 4H), 2.05 (m, 2H), 2.20 (s, 3H), 2.21 (s, 3H), 2.40 (m, 1H), 2.93 (d, 2H, J = 12 Hz), 3.40 (s, 2H), 4.59 (s, 2H), 6.79 (s, 1H), 6.90 (s, 2H), 6.99 (d, 1H, J = 8 Hz), 6.97 (s, 1H), 7.02 (d, 1H, J = 8 Hz). Analysis for $C_{22}H_{26}N_2O_2$: C, H, N.

6-[[4-(*p*-Tolyl)piperidin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (17) was prepared by method A. Mp = 178−180 °C. MS 337 (M^+, 100%). ^1H NMR ($CDCl_3$) δ 1.75 (m, 4H), 2.05 (m, 2H), 2.30 (s, 3H), 2.45 (m, 1H), 2.93 (d, 2H, J = 12 Hz), 3.40 (s, 2H), 4.60 (s, 2H), 6.83 (s, 1H), 6.95 (s, 2h), 7.10 (s, 4H). Analysis for $C_{21}H_{24}N_2O_2$: C, H, N.

6-[[4-(4-Methoxyphenyl)piperidin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (18) was prepared by method A. Mp = 177−178 °C. MS 353 (M^+, 100%). ^1H NMR ($CDCl_3$) δ 1.75 (m, 4H), 2.05 (m, 2H), 2.40 (m, 1H), 2.93 (d, 2H, J = 12 Hz), 3.40 (s, 2H), 3.75 (s, 3H), 4.60 (s, 2H), 6.80 (m, 3H), 6.90 (m, 2H), 7.14 (d, 2H, J = 7 Hz). Analysis for $C_{21}H_{24}N_2O_3$: C, H, N.

6-[[4-(3,4-Dimethylphenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (20) was prepared by method A. Mp = 148−153 °C. MS 352 (M^+, 100%). ^1H NMR ($CDCl_3$) δ 2.19 (s, 3H), 2.20 (s, 3H), 2.58 (s, 4H), 3.10 (s, 4H), 3.24 (s, 2H), 4.60 (s, 2H), 6.62 (dd, 1H, J = 8.3 and 2.5 Hz), 6.70 (d, 1H, J = 2.9 Hz), 6.80 (s, 1H), 6.89 (s, 2H), 6.98 (d, 1H, J = 8.5 Hz), 8.10 (s, 1H). Analysis for $C_{21}H_{25}N_3O_2$: C, H, N.

6-[[4-(4-Methoxyphenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (21) was prepared by method A. Mp = 202−203 °C. MS 353 (M^+, 95%). ^1H NMR ($CDCl_3$) δ 2.58 (m, 4H), 3.08 (m, 4H), 3.40 (s, 2H), 3.75 (s, 3H), 4.60 (s, 2H), 6.78 (m, 3H), 6.80−6.95 (m, 4H), 8.05 (s, 1H). Analysis for $C_{20}H_{23}N_3O_3$: C, H, N.

6-[4-Phenylpiperazin-1-ylmethyl]-4*H*-benz[1,4]oxazin-3-one (22) was prepared by method A. Mp = 207−209 °C. MS

323 (M^+, 100%). ^{1}H NMR (DMSO-d_6) δ 2.48 (m, 4H), 3.15 (m, 4H), 3.45 (s, 2H), 4.30 (s, 2H), 6.70−6.98 (m, 6H), 7.14 (m, 2H), 10.80 (s, 1H). Analysis for $C_{19}H_{21}N_3O_2 \cdot 0.5H_2O$: C, H, N.

6-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-4*H*-benz-[1,4]oxazin-3-one (23) was prepared by method A. Mp = 250−252 °C. MS 357 (M^+, 80%), 162 ($C_7H_8NO_2$, 100%). ^{1}H NMR (DMSO-d_6) δ 2.48 (m, 4H), 3.15 (m, 4H), 3.19 (s, 2H), 4.30 (s, 2H), 6.80 (m, 3H), 6.90 (d, 2H, J = 9.0 Hz), 7.15 (d, 2H, J = 9.0 Hz), 10.60 s, 1H). Analysis for $C_{19}H_{20}N_3O_2Cl \cdot 0.5H_2O$: C, H, N.

7-[[4-(*p*-Tolyl)piperidin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (24) was prepared by method A. Mp = 186−188 °C. MS 337 (M^+, 100%). ^{1}H NMR ($CDCl_3$) δ 1.75 (m, 4H), 2.05 (m, 2H), 2.35 (s, 3H), 2.40 (m, 1H), 2.93 (d, 2H, J = 12 Hz), 3.40 (s, 2H), 4.60 (s, 2H), 6.72 (d, 1H, J = 8 Hz), 6.93 (d, 1H, J = 7.4 Hz), 7.00 (s, 1H), 7.11 (s, 4H). Analysis for $C_{21}H_{24}N_2O_2$: C, H, N.

7-[[4-(3,4-Dimethylphenyl)piperidin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (25) was prepared by method A. Mp = 192−194 °C. MS 351 (M^+, 100%). ^{1}H NMR (DMSO-d_6) δ 1.60 (m, 4H), 1.95 (m, 2H), 2.09 (s, 3H), 2.11 (s, 3H), 2.35 (m, 1H), 2.79 (d, 2H, J = 11.2 Hz), 3.35 (s, 2H), 4.70 (s, 2H), 6.75−6.90 (m, 4H), 6.92−6.98 (m, 3H). Analysis for $C_{22}H_{26}N_2O_2$: C, H, N.

7-[[4-(4-Methoxyphenyl)piperazin-1-yl]methyl]-4*H*-benz-[1,4]oxazin-3-one (27) was prepared by method A. Mp = 235 °C. MS 353 (M^+, 100%). ^{1}H NMR ($CDCl_3$) δ 2.58 (m, 4H), 3.08 (m, 4H), 3.40 (s, 2H), 3.85 (s, 3H), 4.60 (s, 2H), 6.69 (d, 1H, J = 7.8 Hz), 6.78−6.85 (m, 3H), 6.88−6.90 (m, 3H), 6.99 (s, 1H). Analysis for $C_{20}H_{23}N_3O_3$: C, H, N.

7-[[4-Phenylpiperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (28) was prepared by method A. Mp = 191−193 °C. MS 324 (M^+, 100%). ^{1}H NMR ($CDCl_3$) δ 2.55 (m, 4H), 3.10 (m, 4H), 3.45 (s, 2H), 4.58 (s, 2H), 6.64 (dd, 1H, J = 8 and 2.6 Hz), 6.82 (m, 1H), 6.93 (m, 3H), 6.99 (s, 1H), 7.24 (d, 2H, J = 12.4 Hz), 7.90 (s, 1H). Analysis for $C_{19}H_{21}N_3O_2$: C, H, N.

7-[[4-(4-Methoxyphenyl)piperidin]-1-ylmethyl]-4*H*-benz-[1,4]oxazin-3-one (29) was prepared by method A. Mp = 198−199 °C. MS 353 (M^+, 100%). ^{1}H NMR ($CDCl_3$) δ 1.75 (m, 4H), 2.05 (m, 2H), 2.45 (m, 1H), 2.93 (d, 2H, J = 12 Hz), 3.40 (s, 2H), 3.79 (s, 3H), 4.60 (s, 2H), 6.64 (dd, 1H, J = 8 and 2.6 Hz), 6.80 (d, 2H, J = 8.2 Hz), 6.90 (dd, 1H, J = 8.3 and 2.0 Hz), 6.98 (s, 1H), 7.10 (d, 2H, J = 8.6 Hz), 8.0 (s, 1H). Analysis for $C_{21}H_{24}N_2O_3$: C, H, N.

7-[[4-(3,4-Dimethylphenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (30) was prepared by method A. Mp = 195−197 °C. MS 352 (M^+, 100%). ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H), 2.20 (s, 3H), 2.55 (m, 4H), 3.10 (m, 4H), 3.45 (s, 2H), 4.58 (s, 2H), 6.64 (dd, 1H, J = 8.2 and 2.4 Hz), 6.68 (d, 2H, J = 7.8 Hz), 6.91 (dd, 1H, J = 8.5 and 1.8 Hz), 6.97 (d, 2H, J = 6.4 Hz), 7.60 (s, 1H). Analysis for $C_{21}H_{25}N_3O_2$: C, H, N.

7-[[4-(*p*-Tolyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one (31) was prepared by method A. Mp = 208−210 °C. MS 338 (M^+, 100%). ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H), 2.55 (m, 4H), 3.10 (m, 4H), 3.45 (s, 2H), 4.58 (s, 2H), 6.70 (d, 1H, J = 7.8 Hz), 6.80 (d, 2H, J = 8.6 Hz), 6.91 (d, 1H, J = 7.8 Hz), 6.97 (s, 1H), 7.02 (d, 2H, J = 8.8 Hz), 7.72 (s, 1H). Analysis for $C_{20}H_{23}N_3O_2$: C, H, N.

Pharmacology: Receptor Binding Assays for DA D2L, D3, and D4.2 Receptors. CHO cells (provided by Dr. James Granneman, Wayne State University, Detroit, MI) transfected with human D2L, D3, and D4.2 cDNA were grown and harvested as previously described.[30] Confluent cultures were harvested by replacing the media with cold phosphate-buffered saline (PBS) containing 0.05% EDTA followed by centrifugation at 1000*g* for 2 min. The pellets obtained were suspended in appropriate volume of ice-cold buffer (25 mM Tris-HCl and 1 mM EDTA, pH 7.4; TE buffer) and centrifuged at 20000*g* for 15 min at 4 °C. The final pellets obtained were suspended in TE buffer and homogenized with a Polytron (Brinkman Instruments, Westbury, NY) at setting 6 for 5 s for use in radioligand binding assays or stored at −80 °C. CHO-K1 cell membranes for use in the assay were homogenized with a Polytron at setting 6 for 5 s and stored at −80 °C in 1 mL aliquots until used in the assay.

Competition binding assays were conducted as follows: CHO-K1 cell membranes (400 μL) in appropriate ice-cold buffer (25 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM KCl, 1.5 mM $CaCl_2$, 1 mM EDTA, and 120 mM NaCl, pH 7.4), 50 μL of either drug (0.1−10 000 nM or 0.01−1000 nM for a total of six concentrations) or buffer, and 50 μL of [^{3}H]ligand (0.2 nM, final concentration for D2 and D4, and 0.5 nM, final concentration for D3) were added to polypropylene microtubes (Marsh Biomedical Products, Inc., Rochester, NY) to give a total volume of 500 μL. Incubation proceeded for 2 h at room temperature and was terminated by rapid filtration followed by four washes with 1 mL of buffer on Whatman GF/B filters on a Brandel MLR96 cell harvester (Biomedical Research and Development, Inc., Gaithersburg, MD). Following the addition of liquid scintillation cocktail, the radioactivity remaining was counted with a Wallac 1205 Betaplate liquid scintillation counter (50% efficiency). Specific binding was defined as total binding minus binding in the presence of 1 μM haloperidol and this ranged from 90% to 95%. All assays were performed in triplicate.

Biochemical Experiments. The effects of compounds on DA synthesis were measured by monitoring DOPA (L-3,4-dihydroxyphenylalanine) accumulation after inhibiting L-aromatic amino acid decarboxylase with NSD-1015 in animals treated with GBL to reduce DA neuronal firing.[30,36] Test compounds were administered 1 h and NSD-1015 was administered 30 min before animals were sacrificed by decapitation. The brain was rapidly removed and placed on an ice-cooled plate for dissection of striatum, nucleus accumbens plus olfactory tubercle (mesolimbic region), and brain stem. Tissue samples were frozen on dry ice and stored at −70 °C until assayed.

On the day of assay samples were thawed, homogenized in 0.5−1.0 mL of 0.1 M phosphate−citrate buffer (pH 2.5) containing 15% methanol, and centrifuged for 1 min in a Beckman Microfuge B. Samples were analyzed on a C_{18} reverse-phase analytical column (3 μm spheres, 150 × 4.6 mm; IB-SIL, Phenomenex, Torrance, CA) coupled to an electrochemical detector (Waters 464 pulsed electrochemical detector) equipped with a glassy carbon electrode set at a potential of +0.75 V relative to an Ag/AgCl reference. The HPLC mobile phase consisted of 0.05 mM sodium phosphate, 0.03 M citrate buffer (pH 2.7), 0.1 mM disodium EDTA, 0.035% sodium octanesulfate, and 25% methanol. Data were analyzed by one-way analysis of variance, and a Newman-Keuls test was used to determine significantly different group means, with $P < 0.05$ being considered significant.

Mitogenesis Assay. The effects of test compounds on [^{3}H]-thymidine uptake were measured as previously described.[30] CHO p-5 cells transfected with human D2 or D4.2 cDNA were seeded into 96-well plates at a density of about 5000 cells/well and grown at 37 °C in a minimum essential medium. (αMEM, Gibco) with 10% calf serum for 2 days. The wells were rinsed with serum-free medium three times and fresh medium was added along with either test compound in water or vehicle alone. Eight wells of each plate received 100 μL of αMEM with 10% fetal calf serum. After culture for 16−17 h, [^{3}H]thymidine (1 μCi/well) was added for 4 h. The cells were trypsinized and harvested onto filter mats with a 96-well Brandel cell harvester. The filters were counted in a Beta-Plate scintillation counter.

Rat Amphetamine-Stimulated Locomotor Activity Studies. Rats were dosed po with water or test compound and placed in Omnitech motion detection chambers for a 15 min acclimation period. They were dosed ip with saline or D-amphetamine and returned to the chamber for an additional 15 min. Locomotor activity was then recorded for 30 min, and data were expressed as total distanced traveled in centimeters. Statistical significance between groups was calculated by a *t*-test, and amphetamine reversal ED_{50}s and 95% confidence limits were calculated by regression analysis.

Prepulse Inhibition of Acoustic Startle in Rats. Rats were dosed po with vehicle or with compound **26** 30 min prior to a sc injection of saline or apomorphine (0.25 mg/kg) and

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 white noise. The fires and last 10 trials were 120 dB pulse-alone trials (not included in the PPI calculation). The middle 70 trials consisted of 20 120-dB pulse-alone trials and 10 trials of each of the following five trial types in pseudorandom 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 were 20 ms in duration, while the startle pulses were 40 ms in duration. Data were shown as percent PPI (calculated for each individual rat by using the 16 dB prepulse values), and statistical significance was calculated between treatment groups by a *t*-test. The minimal effective dose (MED) required to produce a significant reversal of apomorphine-disrupted PPI was used as the efficacy measure.

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