1-Naphthylpiperazine Derivatives as Potential Atypical Antipsychotic Agents

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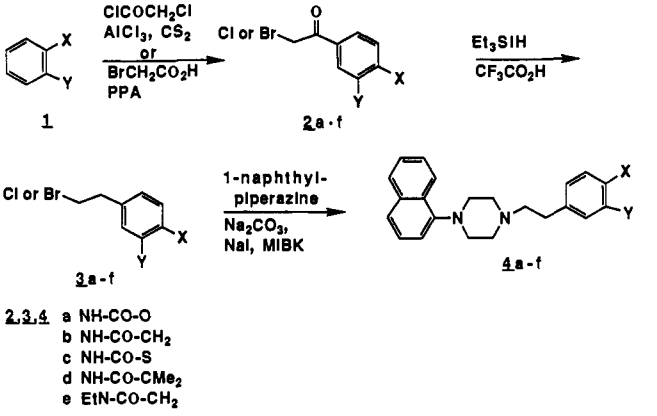
The design and synthesis of a series of potential atypical antipsychotic agents based on the structure of 1-naphthylpiperazine are described. The incorporation of dopamine antagonist activity into the parent structure was achieved with heterocyclic surrogates for the catechol moiety of dopamine. Compound 4b from this series showed a biochemical profile that translated to behavioral activity in the rat predictive of an antipsychotic agent with a low propensity to cause extrapyramidal side effects in man.

Many strategies have been attempted in the search for so-called "atypical" antipsychotic agents, operationally defined as those that do not possess the liability for causing extrapyramidal side effects (EPS) in the clinic.[1] In devising one such strategy, we were attracted to a report describing the ability of buspirone, a novel anxiolytic agent, to reverse catalepsy induced in rats by haloperidol.[2] Induction of catalepsy in rats is generally viewed as indicative of a compound's propensity to cause EPS in man;[3] the combination of dopamine blockade necessary to achieve antipsychotic efficacy with this catalepsy-reversal activity, which should protect against EPS, seemed to offer the prospect of a novel "atypical" antipsychotic agent. The chemical starting point chosen for this effort was 1-naphthylpiperazine, a compound with a pharmacological activity profile at serotonergic receptors, 5-HT_{1a} agonist activity coupled with 5-HT_2 antagonism,[4] similar to that of buspirone. While it does not possess intrinsic dopaminergic antagonism, 1-naphthylpiperazine is more amenable than buspirone to the structural variation necessary for incorporation of this activity. Our implementation of this strategy was aided by recent advances in the structural characterization of dopaminergic[5] and serotonergic[6] receptors showing that they are both members of the G protein coupled receptor superfamily. This structural homology was expected to help in design of ligands that would bind to both these receptors. As described below, our approach produced 5-[2-[4-(1-naphthyl)-piperazinyl]ethyl]-2,3-dihydroindol-2-one (**4b**; Figure 1) which possesses pharmacological activity suggestive of an "atypical" antipsychotic agent.

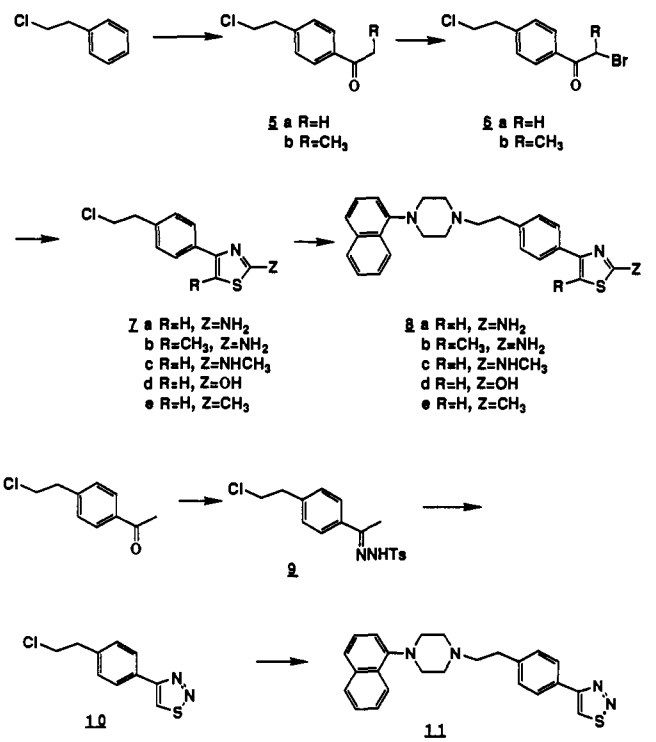
Chemistry

The synthetic methodology used for preparing the desired derivatives of 1-naphthylpiperazine is shown in Schemes I and II. Compounds **2** and **3**, the requisite precursors for N-alkylation of 1-naphthylpiperazine, were prepared by the literature method or a suitable modification thereof.[7] The reduction of **2** to **3** via triethylsilane in trifluoroacetic acid is notable for its toleration of a wide variety of delicate functionality. Compounds **6**, which were not isolated but used directly in the following step, and **7** were prepared from the appropriate ketone **5**. A similar alkylation of 1-naphthylpiperazine with compounds **7** then produced derivatives **8**. Compounds **9** and **10** were prepared on the basis of literature methods;[8] reaction of 1-naphthylpiperazine with **10** then provided **11**. Physical properties for test compounds are listed in Table III.

Scheme I



Scheme II



(1) Lowe, J. A., III; Seeger, T. F.; Vinick, F. J. *Med. Res. Rev.* **1988**, *8*, 475.
(2) McMillen, B. A.; Mattiace, L. A. *J. Neural Transm.* **1983**, *57*, 255.
(3) See ref 1 for supporting examples.
(4) Glennon, R. A.; Slusher, R. M.; Lyon, R. A.; Titeler, M.; McKenney, J. D. *J. Med. Chem.* **1986**, *29*, 2375.
(5) Bunzow, J. R.; Van Tol, H. H. M.; Grandy, D. K.; Albert, P.; Salon, J.; Christie, M.; Machida, C. A.; Neve, K.; Civelli, O. *Nature* **1988**, *336*, 783.
(6) Julius, D.; Huang, K. N.; Livelli, T. J.; Axel, R.; Jessell, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 928.
(7) Calgnard, D. H.; Couquelet, J.; Lesieur, D.; Lespagnol, C.; Lamar, J. C.; Beaughard, M. *Farmaco Ed. Sci.* **1985**, *40*, 854.
(8) Brown, H. P.; Meier, H. *Tetrahedron* **1975**, *31*, 637.

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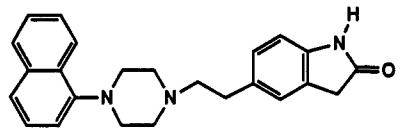
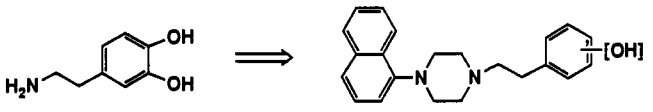


4b

Figure 1.

Scheme III



Dopamine, agonist

Dopamine antagonist

Biology

Compounds were tested for affinity at D_2 and 5-HT_2 receptors by autoradiographic binding to rat brain sections. Competitive inhibition of [^{3}H]-*N*-propylapomorphine in rat striatum and [^{3}H]ketanserin (5-HT_2) in frontal cortex was quantified and used to generate an IC_{50} for each compound of interest. Standard rat brain homogenate binding assays were used to measure affinity for α noradrenergic and serotonin 5-HT_{1a} receptors, using [^{3}H]prazosin and [^{3}H]-8-OH-DPAT, respectively. In vivo measurement of potential antipsychotic efficacy was determined by use of inhibition of *d*-amphetamine-induced hyperlocomotion in rats and inhibition of apomorphine-induced stereotypy in rats, both of which arise by stimulation of dopaminergic mechanisms.

The 1-naphthylpiperazine derivatives described herein do not induce catalepsy, but most have the novel property of reversing catalepsy induced by the typical neuroleptic haloperidol. Relative potency in this regard was determined by calculating the minimal effective dose (MED) for reversal of haloperidol catalepsy at 1 h after subcutaneous administration. In the buspirone-type anxiolytic drug class, a correlation has been noted between catalepsy reversal and the ability to turn off 5-HT cell firing in the rat dorsal raphe.[9] This was assessed in the present series by testing compounds **4a**, **4b**, and **8a** for inhibition of 5-HT cell firing.

Discussion

The strategy selected to achieve incorporation of the requisite dopamine-blocking activity into the 1-naphthylpiperazine nucleus is based on ideas promulgated some time ago by Ariens.[10] Briefly, his approach involves modification of the structure of a receptor agonist, in this case dopamine, with a large lipophilic group on the amino position, which binds to the *accessory binding site* adjacent to the agonist binding site and transforms the agonist into an antagonist. With 1-naphthylpiperazine as the lipophilic group connected to a basic amine, the modification shown in Scheme III can be envisaged.

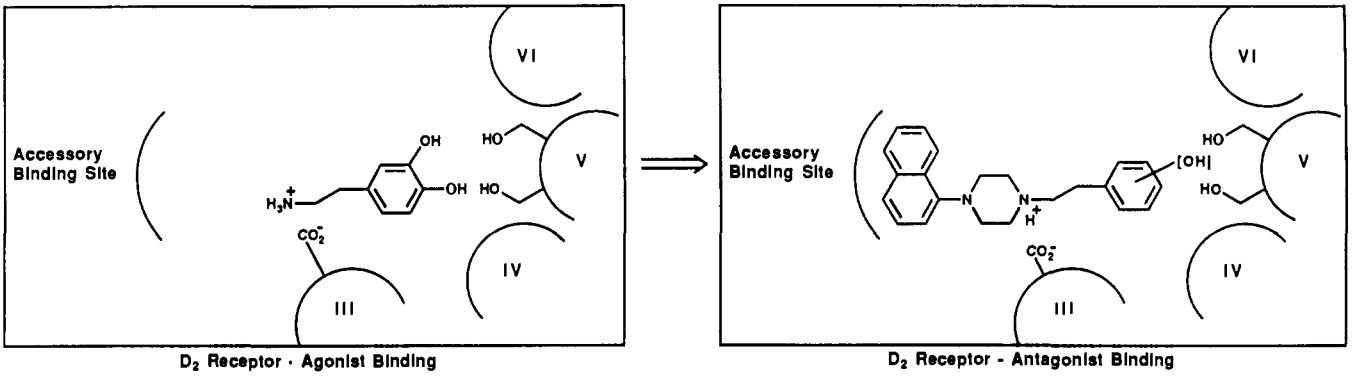
We were especially eager to test the hypothesis that a hydrogen-bonding (donating or receiving) group is necessary to mimic the catechol group of dopamine. This hypothesis is based on studies of the β_2 adrenergic receptor, which show a pair of serine residues that coordinates with the catechol group of noradrenaline.[11] A similar pair of serine residues is present in the analogous position in the sequence determined for the cloned dopamine (D_2) receptor gene.[5] With the naphthylpiperazine moiety bound at the accessory binding site, an appropriate hydrogen-bonding group could be held by a phenethyl side chain so as to reach these serine residues, as shown in the depiction of the D_2 receptor in Scheme IV. In this scheme, which is based on a published model of the β-adrenergic receptor,[12] the trans-membrane helices which comprise the ligand binding domain of the receptor are viewed end-on from above the plane of the membrane and are arranged in analogy to the structure found from low-resolution X-ray studies of rhodopsin.[13] The residues that interact with dopamine are then depicted as projecting from their respective helices, and the proposed accessory binding site is depicted at one end of the ligand binding site where only antagonists can reach it. We chose heterocyclic groups, both fused and appended to the phenethyl side chain, which would offer various modes of hydrogen-bond interactions with the D_2 receptor serine residues. These molecules are shown in Schemes I and II. There is considerable literature precedent for molecules of type **4**[14] and type **8**[15] as ligands for the dopamine D_2 receptor, which suggests that the interactions we envisaged are reasonable.

The in vitro structure–activity relationship (SAR) of compounds **4**, **8**, and **11** is shown in Table I. The initial trial compound, **4a**, which mimics both the position and acidity of one of the phenol groups in dopamine, gratifyingly possesses the desired in vitro affinity for the D_2 receptor. That position rather than acidity is the determining factor is shown by the activity of compound **4b**, an oxindole, which is far less acidic than a phenol. A larger atom, such as sulfur in **4c**, or steric bulk, such as the *gem*-dimethyl group in **4d**, in the heterocyclic ring causes a loss of D_2 binding potency, as does substitution on the atom simulating the phenol, as in **4e**. Hence a proton-bearing heteroatom at this position affords a high affinity for the D_2 receptor, provided it is not encumbered by steric size or bulk. These SAR observations are reinforced in compounds **8** and **11**, which show weaker D_2 receptor affinity than compounds **4a** and **4b**, presumably by virtue of their greater steric bulk. Compounds **8b** and **8c** are particularly illustrative of this trend. It is nonetheless encouraging that a variety of potential hydrogen-bonding groups are capable of interactions with the dopamine receptor.

Of equal importance to the achievement of dopaminergic activity was the potent activity of compounds **4** at the 5-HT_{1a} and 5-HT_2 receptors (Table I). In fact, the most potent affinity in this series of compounds was found at the 5-HT_{1a} receptor followed by that at the 5-HT_2 and D_2 receptors. It is especially intriguing that the introduction of D_2 receptor affinity into the 1-naphthylpiperazine structure via compounds **4** also improves affinity for 5-HT_2 receptors, suggesting that the strategy of mimicking a

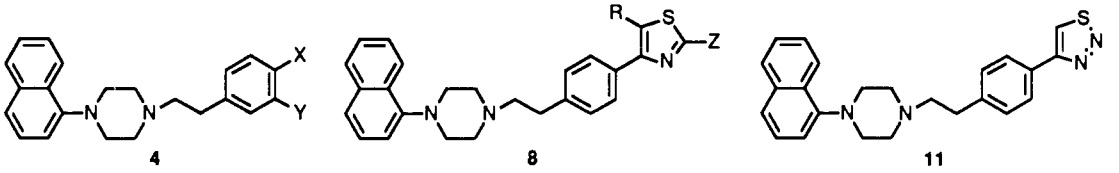
(9) McMillen, B. A.; Scott, S. M.; Davanzo, E. A. *J. Pharm. Pharmacol.* **1988**, *40*, 885.
(10) Ariens, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. In *The Receptors: A Comprehensive Treatise*; O'Brien, R. D., Ed.; Plenum: New York, 1979; Vol. 1, pp 33–91.
(11) Strader, C. D.; Candelore, M. R.; Hill, W. S.; Sigal, I. S.; Dixon, R. A. F. *J. Biol. Chem.* **1989**, *264*, 13572.
(12) Strader, C. D.; Sigal, I. S.; Dixon, R. A. F. *FASEB J.* **1989**, *3*, 1825.
(13) Blanck, A.; Oesterhelt, D.; Ferrando, E.; Schegk, E. S.; Lottspeich, F. *EMBO J.* **1989**, *8*, 3963.
(14) Weinstock, J.; Gaitanopoulos, D. E.; Stringer, O. D.; Franz, R. G.; Hieble, J. P.; Kinter, L. B.; Mann, W. A.; Flaim, K. E.; Gessner, G. J. *J. Med. Chem.* **1987**, *30*, 1166.
(15) Caprathe, B. W.; Jaen, J. C.; Wise, L. D.; Heffner, T. G.; Pugsley, T. A.; Meltzer, L. T. *Abstracts of Papers*, 199th National Meeting of the American Chemical Society Boston, MA; American Chemical Society: Washington, D.C., 1990; MEDI 96.

Scheme IV. Model of D_2 Receptor, Based on Studies of the β-Adrenergic Receptor, Showing Proposed Agonist and Antagonist Binding Used in Receptor Antagonist Design



D_2 Receptor · Agonist Binding D_2 Receptor - Antagonist Binding

Table I. In Vitro Characterization of 1-Naphthylpiperazine Derivatives



4 8 11

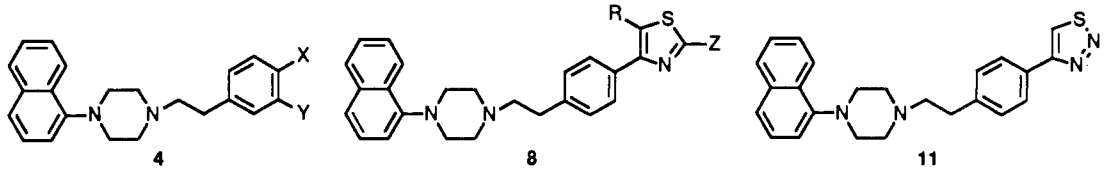
CPD	X	Y	D_2[a]	α[b]	5-HT_{1a}[c]	5-HT_2[d]
4a	-HNCOO-		44	45 ± 8	6.2	10
4b	$-HNCOCH_2-$		38 ± 11	24 ± 18	4.87 ± 0.6	20 ± 2
4c	-HNCOS-		112	24	2.2	50
4d	$-HNCOC(CH_3)_2-$		86	40	6.9	11.8
4e	$-(C_2H_5)NCOCH_2-$		81.2	248	2.9	ND[e]
	R	Z				
8a	H	NH_2	72 ± 13	135	11	385
8b	CH_3	NH_2	159	234	ND	ND
8c	H	$NHCH_3$	155	252	ND	ND
8d	H	OH	80 ± 37	65 ± 10	ND	249
8e	H	CH_3	108	166	ND	ND
11			83	105 ± 26	ND	237
1-naphthylpiperazine			>1000	ND	ND	62
haloperidol			3.1 ± 0.6	19	>1000	56 ± 10
clozapine			172 ± 31	17 ± 1	600	24 ± 2

[a] Binding to the D_2 receptor in rat brain, using [^{3}H]NPA as ligand, given in nM units. IC_{50} values were determined from dose–response curves of three log concentrations of the test compounds, each concentration in triplicate. Mean ± standard deviation for three separate determinations was obtained for **4b**, **8a**, **8d**, haloperidol, and clozapine. [b] Binding to the α receptor in rat brain, using [^{3}H]prazosin as ligand, given in nM units. IC_{50} values were determined from dose–response curves of three log concentrations of the test compounds, each concentration in triplicate. Mean ± standard deviation for three separate determinations was obtained for **4a**, **4b**, **8d**, 11, and clozapine. [c] Binding to the $5HT_{1a}$ receptor in rat brain, using [^{3}H]-8-hydroxy-2-(di-*n*-propylamine)tetralin as ligand, given in nM units, or as a percent inhibition of binding at 100 nM concentration of test compound. IC_{50} values were determined from dose–response curves of three log concentrations of the test compounds, each concentration in triplicate. Mean ± standard deviation for three separate determinations was obtained for **4b**. [d] Binding to the 5-HT_2 receptor in rat brain, using [^{3}H]ketanserin as ligand, given in nM units, or as a percent inhibition of binding at 100 nM concentration of test compound. IC_{50} values were determined from dose–response curves of three log concentrations of the test compounds, each concentration in triplicate. Mean ± standard deviation for three separate determinations was obtained for **4b**, haloperidol, and clozapine. [e] ND, not determined.

phenolic group, present in both dopamine and serotonin, can succeed at more than one receptor simultaneously. The considerable reduction in 5-HT_2 affinity in compounds 8 and 11 relative to compounds 4 suggests that the 5-HT_2 receptor is more sensitive than the 5-HT_{1a} receptor to steric bulk in antagonists of this structural type.

Compounds 4, 8, and 11 also afforded behavioral activity in animal models of antipsychotic efficacy consistent with blockade of dopamine activity, as shown in Table II. While the translation from subcutaneous (ED_{50} = 1.6 mg/kg) to oral (ED_{50} = 40.5 mg/kg) activity against amphetamine-induced hypermotility was somewhat unimpressive in compound **4a**, it was better for **4b**, which gave a roughly 10-fold separation between ED_{50} doses by the subcutaneous and oral routes in this test. Complete in vivo characterization of **4b** was then carried out, as shown in Table II, confirming its impressive "atypical" character. Compound **4b** blocks behaviors, such as amphetamine-induced hypermotility and discrete-trial conditioned avoidance, thought to be mediated by the mesolimbic dopaminergic system, which is believed to be the primary locus of pathology in psychotic disorders. At similar doses, **4b** does not block the stereotypic behavior induced by apomorphine, a behavior thought to reflect activation of striatal dopamine receptors and alteration of extrapyramidal motor function. Even more intriguing is the activity of **4b** in reversing haloperidol-induced catalepsy combined with its inability to induce catalepsy on its own at oral doses up to 75 mg/kg. Since the ability to inhibit cell firing in the dorsal raphe nucleus has been suggested as the basis for reversal of haloperidol-induced catalepsy by anxiolytic drugs such as buspirone,[9] we studied **4b** in the dorsal raphe cell firing assay. Figure 2 shows a representative experiment demonstrating the ability of **4b** to inhibit the firing rate of serotonergic neurons in the dorsal raphe nucleus of rats when given by iv injection. Compounds **4a** and **8a** also showed this inhibitory activity in similar experiments. The serotonergic neurons of the dorsal raphe nucleus

Table II. In Vivo Characterization of 1-Naphthylpiperazine Derivatives



4 8 11

CPD	X	Y	AMPH Loc[a]	95% CL[b]	APO ster[c]	cat. rev[d]
4a		-HNCOO-	1.6 (sc)/ 40.5 (po)	0.35–7.27 (48) 18.58–88.29 (56)	10 (sc)	>10 (sc)
4b		$-HNCOCH_2-$	1.21 (sc)/ 13.27 (po)	0.55–2.67 (24) 10.27–17.14 (112)	3.2 (sc) 17.8 (po)	>10 (sc) 10 (po)
4c		-HNCOS-	ND[f]		>10 (sc)	ND
4d		$-HNCOC(CH_3)_2-$				
4e		$-(C_2H_5)NCOCH_2-$				
	R	Z				
8a	H	NH_2	25.34 (po)	19.35–33.19 (112)	10 (ip)/ 32 (po)	3.2 (po)
8b	CH_3	NH_2	>10 (sc)		>3.2 (sc)	>3.2 (sc)
8c	H	$NHCH_3$	ND		>10 (sc)	ND
8d	H	OH	>10 (sc)		>17.8 (sc)	10 (sc)
8e	H	CH_3	ND		>10 (sc)	ND
11			ND		>10 (sc)	ND
1-naphthylpiperazine			ND		ND	ND
haloperidol			0.12 (po)	0.09–0.17 (64)	0.032 (sc)	IA[e]
clozapine			6.9 (sc)	3.48–13.7 (40)	>32 (po)	IA

[a] ED_{50} value, given in milligrams/kilogram, for inhibition of amphetamine-induced locomotor behavior in rats: sc, subcutaneous administration; ip, intraperitoneal administration; po, oral administration. [b] 95% confidence limits for AMPH Loc values, with the number of animals used for the determination in parentheses. The ED_{50} and 95% confidence limit values for AMPH Loc were determined by probit analysis of means converted to percent inhibition. [c] MED for inhibition of apomorphine-induced stereotypy behavior in rats. Statistical significance at a given dose was determined by ANOVA followed by Dunnett's *t* test. Multiples of half-log doses were used, and the minimal effective dose (MED) was the lowest dose level showing statistically significant activity. [d] MED for reversal of haloperidol-induced catalepsy behavior in rats. Statistical significance at a given dose was determined by ANOVA followed by Dunnett's *t* test. Multiples of half-log doses were used, and the minimal effective dose (MED) was the lowest dose level showing statistically significant activity. [e] IA, inactive. [f] ND, not determined.

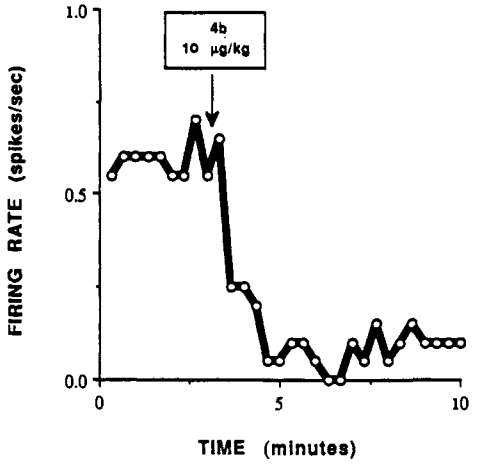


Figure 2. Representative ratemeter tracing demonstrating inhibition of spontaneous serotonergic neuron firing in the dorsal raphe nucleus by a single 10 µg/kg intravenous injection of **4b**. Data points were obtained from consecutive 20 s surplus periods.

provide inhibitory inputs to the striatal dopamine system which controls motor function. By inhibiting firing of these serotonergic neurons, **4b** may produce a disinhibition of the dopaminergic motor function control system, alleviating some of the inhibitory effects of haloperidol responsible for producing catalepsy. A recent report that both 5-HT_{1a} agonists and 5-HT_2 antagonists are capable of blocking haloperidol-induced catalepsy[16] suggests that the serotonergic profile of **4b** may suit it especially well to reverse catalepsy. Further biological profiling that substantiates the "atypical" properties of **4b** will be published separately.[17]

Conclusion

Compound **4b** represents a successful implementation of the Ariens strategy of receptor antagonist design via appendage of a lipophilic group to the amino portion of a receptor agonist structure. The SAR of the heterocyclic phenol mimics described here is consistent with the initial hypothesis that the dopamine D_2 receptor recognizes dopamine via at least one of its phenol groups. Given the increase in potency of compounds **4** over 1-naphthylpiperazine at the 5-HT_2 receptor, these heterocyclic groups are particularly versatile phenol mimics. In retaining the serotonergic profile of the parent compound, 1-naphthylpiperazine, while adding potent D_2 receptor blockade, **4b** has achieved in vivo activity consistent with antipsychotic efficacy and, at the same time, a low probability of causing EPS. Confirmation of the validity of these results for man can only come through clinical study. In the meantime, **4b** affords an example of the successful implementation of a number of medicinal chemistry strategies that may see application in other systems in the future.

Experimental Section

Melting points were obtained on a Hoover melting point apparatus and are uncorrected. NMR spectra were obtained on a Varian XL-300 or a Bruker AM-300 spectrometer, with tetramethylsilane as internal standard. IR spectra were obtained on Perkin-Elmer 283B and 1420 spectrometers. Mass spectra were obtained on a Finnegan 4510 mass spectrometer, and high-resolution mass spectra were obtained on an AE-9 instrument. TLC analysis was carried out on EM Kieselgel 60 F_{254} 5 × 20 cm plates. Elemental analyses were carried out by the Analytical Laboratory of Pfizer Central Research, and are within ±0.4% of theory unless otherwise noted.

(16) Hicks, P. B. *Life Sci.* **1990**, *47*, 1609.

(17) Seeger, T. F. Manuscript in preparation.

Table III. Physical Properties of Compounds 4, 8, and 11

CPD	formula	mp, °C	yield, %	anal.
4a	$C_{23}H_{23}N_3O_2 \cdot 2HCl \cdot {}^5/_4H_2O$	282–285	23	C,H,N
4b	$C_{24}H_{25}N_3O \cdot 2HCl$	310–315	84	C,H,N
4c	$C_{23}H_{23}N_3OS \cdot H_2O$	198–220	36	C,H,N[a]
4d	$C_{26}H_{29}N_3O \cdot {}^3/_2HCl$	185–190	56	C,H,N
4e	$C_{26}H_{29}N_3O \cdot 2HCl \cdot H_2O$	>305	21	C,H,N
8a	$C_{25}H_{26}N_4S \cdot 3HCl$	274–277	31	C,H,N
8b	$C_{26}H_{28}N_4S \cdot 2HCl \cdot {}^3/_2H_2O$	160–165	54	C,H,N
8c	$C_{26}H_{28}N_4S \cdot 2HCl \cdot {}^7/_4H_2O$	272–273	63	C,H,N
8d	$C_{25}H_{25}N_3OS \cdot HCl \cdot {}^1/_2H_2O$	307–309	18	C,H,N
8e	$C_{26}H_{27}N_3S \cdot 2HCl \cdot H_2O$	305–307	48	C,H,N
11	$C_{24}H_{24}N_4S \cdot HCl \cdot {}^1/_3H_2O$	286–289	50	C,H,N

[a] H: calcd, 6.18; found, 5.75.

Syntheses. The examples presented below illustrate the method for preparation of the compounds listed in Table III; physical data for the compounds are listed there. Compound **5a** was prepared by a literature method.[18]

Method A. General Method for the Preparation of Bromoacetyl Compounds 2a and 2c: 6-(2-Bromoacetyl)benzoxazol-2(3*H*)-one (2a). Prepared according to the literature procedure[7] as follows: To a 500-mL three-necked round-bottomed flask equipped with mechanical stirrer and N_2 inlet were added 200 g of PPA, 13.51 g (0.1 mol) of benzoxazolone, and 13.89 g (0.1 mol) of bromoacetic acid. The reaction was heated with stirring at 115 °C for 2.5 h and poured into 1 kg of ice. The mixture was stirred mechanically for 1 h to form a purple solid, which was then filtered off and washed with water. The solid was slurried with acetone for 30 min, a small amount of purple solid filtered off, and the brown filtrate evaporated. The resulting dark brown gum was slurried with 150 mL of ethanol for 30 min, and the brown solid was filtered off and washed with ethanol. This solid had mp 192–194 °C (lit.[19] mp 228 °C) and was used directly without further purification.

6-(2-Bromoacetyl)benzothiazol-2(3*H*)-one (2c): prepared according to method A in 23% yield; mp 220–222 °C; NMR (δ, DMSO-d_6) 4.95 (s, 2 H), 7.10 (d, J = 8, 1 H), 7.92 (dd, J = 2, 8, 1 H), 8.15 (d, J = 2, 1 H); MS (M^+) 271/272 (10/11, parent, Br^{79}/Br^{81}), 178 (100), 150 (16).

Method B. General Method for the Preparation of Chloroacetyl Compounds 2b, 2d, and 2e. 3,3-Dimethyl-5-(chloroacetyl)-2,3-dihydroindol-2-one Hemihydrate (2d). To a 250-mL round-bottomed flask equipped with condenser and N_2 inlet were added 15.4 g (115 mmol) of aluminum chloride, 100 mL of carbon disulfide, 1.93 mL (24.2 mmol) of chloroacetyl chloride, and 3.0 g (18.6 mmol) of 3,3-dimethyl-2,3-dihydroindol-2-one.[20] The reaction was stirred to a thick, gummy deposit and then refluxed 3 h. The solvent was then decanted, and the gummy residue was stirred in ice/water until it became a solid suspension. The solid was filtered, washed with water, and dried to give 4.15 g (94%): mp 229–231 °C; ^{1}H NMR (δ, $CDCl_3$) 1.38 (s, 6 H), 3.72 (s, 2 H), 6.96 (d, J = 8, 1 H), 7.78 (s, 1 H), 7.81 (d, J = 8, 1 H); IR (cm^{-1}, KBr) 1728, 1677 (C=O); MS (M^+) 237/239 (12/4.2, parent, Cl^{35}/Cl^{37}), 189 (11), 188 (100), 160 (14). Anal. Calcd for $C_{12}H_{12}NO_2Cl \cdot {}^1/_2H_2O$: C, 58.43; H, 5.31; N, 5.68. Found: C, 58.26; H, 4.74; N, 5.62.

5-(Chloroacetyl)oxindole (2b): prepared according to method B in 98% yield; mp 228–230 °C; NMR (δ, DMSO-d_6) 3.40 (s, 2 H), 5.05 (s, 2 H), 6.8–7.9 (m, 3 H).

5-(Chloroacetyl)-*N*-ethyloxindole (2e): prepared according to method B in 81% yield; mp 157–159 °C; NMR (δ, $CDCl_3$) 1.30 (t, 3 H), 3.60 (s, 2 H), 3.85 (q, 2 H), 4.70 (s, 2 H), 6.85–8.15 (m, 2 H).

Method C. General Method for the Preparation of Chloroethyl and Bromoethyl Compounds 3. 3,3-Dimethyl-5-(2-chloroethyl)-2,3-dihydroindol-2-one Quarterhydrate (3d). The literature procedure[19] was utilized as follows: To a 125-mL round-bottomed flask equipped with an addition funnel and N_2 inlet were added 4.15 g (17.47 mmol) of 3,3-dimethyl-5-(chloroacetyl)-2,3-dihydroindol-2-one (**2d**) and 13.45 mL (174.7 mmol) of trifluoroacetic acid. The solution was cooled to 0 °C and 6.41 mL (40.13 mmol) of triethylsilane was added dropwise over 2 min. The reaction was stirred and warmed to 45 °C for 20 min and then allowed to stir at room temperature for 40 h. It was then poured into ice/water, layered with hexane, stirred vigorously, filtered, washed with hexane, and dried to give a tan solid: 3.2 g (82%), mp 161–165 °C; ^{1}H NMR (δ, $CDCl_3$) 1.38 (s, 6 H), 3.02 (t, J = 7, 2 H), 3.66 (t, J = 7, 2 H), 6.83 (d, J = 8, 1 H), 7.0 (m, 2 H), 8.29 (bs, 1 H); IR (cm^{-1}, KBr) 1711 (C=O); MS (M^+) 223/225 (39/13, parent, Cl^{35}/Cl^{37}), 174 (100), 146 (50). Anal. Calcd for $C_{12}H_{14}NOCl \cdot {}^1/_4H_2O$: C, 63.16; H, 6.40; N, 6.14. Found: C, 63.22; H, 6.24; N, 6.06.

5-(2-Bromoethyl)benzoxazol-2(3*H*)-one (3a): prepared according to method C in 11% yield; mp 148–151 °C (lit.[5] mp 152–154 °C); NMR (δ, $CDCl_3$) 3.16 (t, 2 H), 3.54 (t, 2 H), 7.0–7.3 (m, 3 H), 9.3 (bs, 1 H); IR (cm^{-1}, KBr) 1767 (C=O); MS (M^+) 241/243 (23.5/17.4, parent Br^{79}/Br^{81}), 162 (15.9), 148 (100), 104 (9.4).

5-(2-Chloroethyl)oxindole (3b): prepared according to method C in 64% yield; mp 168–179 °C; NMR (δ, DMSO-d_6) 3.00 (t, 2 H), 3.48 (s, 2 H), 3.82 (t, 2 H), 6.7–7.2 (m, 3 H); MS (M^+) 195/197 (30/11, parent, Cl^{35}/Cl^{37}), 147 (18), 146 (100), 118 (48), 91 (13), 77 (11).

5-(2-Bromoethyl)benzothiazol-2(3*H*)-one (3c): prepared according to method C in 91% yield; mp 176–178 °C; NMR (δ, DMSO-d_6) 2.15 (m, 2 H), 3.03 (m, 2 H), 6.50 (d, J = 9, 1 H), 6.97 (dd, J = 2, 9, 1 H), 7.35 (d, J = 2, 1 H); MS (M^+) 257/259 (11/15, parent, Br^{79}/Br^{81}), 164 (26), 91 (100), 65 (26), 55 (28).

5-(2-Chloroethyl)-*N*-ethyloxindole (3e): prepared according to method C in 93% yield; mp 120–122 °C; NMR (δ, $CDCl_3$) 1.30 (t, 3 H), 3.10 (t, 2 H), 3.55 (t, 2 H), 3.65–4.0 (m, 4 H), 6.8–7.3 (m, 3 H); MS (M^+) 223/225 (47/13, parent, Cl^{35}/Cl^{37}), 174 (100), 146 (45), 118 (28), 91 (19).

Method D. General Method for the Preparation of Compounds 7 via Compounds 6. 4-[4-(2-Chloroethyl)phenyl]-2-aminothiazole Hydrobromide (7a). To a 50-mL round-bottomed flask equipped with N_2 inlet were added 0.91 g (5 mmol) of 4-(2-chloroethyl)acetophenone and 5 mL of acetic acid. To the stirring solution was added 0.26 mL (5 mmol) of bromine dropwise over 2 min. The solution was stirred at room temperature for 1 h, taken up in ethyl acetate, and washed with water, saturated aqueous sodium bicarbonate solution, and brine, dried, and evaporated to an oil (**6a**). The oil was taken up in 25 mL of acetone and treated with 0.38 g (5 mmol) of thiourea, and the reaction heated at reflux for 3 h. The reaction was cooled to room temperature and allowed to stand for 2.5 h and then the precipitate was collected, washed with acetone, and dried to give 0.81 g (51%) of a white solid: mp 193–195 °C; NMR (δ, DMSO-d_6) 3.03 (t, 2 H), 3.84 (t, 2 H), 7.21 (s, 1 H), 7.37 (d, 2 H), 7.63 (d, 2 H), 8.2–9.2 (bs, 3 H); IR (cm^{-1}, DMSO) 1615 (C=O).

4-[4-(2-Chloroethyl)phenyl]-2-amino-5-methylthiazole Hydrobromide (7b): prepared according to method D in 46% yield; mp 209–211 °C; NMR (δ, DMSO-d_6) 2.26 (s, 3 H), 3.08 (t, 2 H), 3.90 (t, 2 H), 7.45 (m, 4 H); IR (cm^{-1}, KBr) 1620; MS (M^+) 252/254 (92/41, parent, Cl^{35}/Cl^{37}), 203 (100), 161 (41), 147 (12), 128 (26), 115 (21), 102 (18), 101 (15), 89 (11), 82 (42), 81 (19), 80 (35), 79 (16), 77 (17).

4-[4-(2-Chloroethyl)phenyl]-2-(methylamino)thiazole Hydrobromide (7c): prepared according to method D in 16% yield: mp 103–107 °C; NMR (δ, DMSO-d_6) 3.10 (t, 2 H), 3.48 (s, 3 H), 3.93 (t, 2 H), 7.20 (s, 1 H), 7.3–7.8 (m, 4 H); IR (cm^{-1}, DMSO) 1630; MS (M^+) 252/254 (82/36, parent, Cl^{35}/Cl^{37}), 224 (10), 204 (15), 203 (100), 174 (14), 147 (36), 115 (21), 103 (12), 102 (16), 101 (13), 89 (11).

4-[4-(2-Chloroethyl)phenyl]-2-methylthiazole Hydrobromide (7e): prepared according to method D in 33% yield; mp 85–89 °C; NMR (δ, DMSO-d_6) 2.71 (s, 3 H), 3.12 (t, 2 H), 3.85 (t, 2 H), 7.91 (s, 1 H), 7.3–7.8 (m, 4 H).

Modification of Method D for the Preparation of 4-[4-(2-Chloroethyl)phenyl]thiazol-2-one (7d).[21] To a 125-mL round-bottomed flask equipped with N_2 inlet were added 9.1 g

(18) Greber, G.; Egle, G. *Makromol. Chem.* **1962**, *54*, 119.
(19) Vaccher-Ledein, M. P.; Barbry, D.; Bante, J. P. *Bull. Soc. Pharm. Lille* **1984**, *37*, 89.
(20) Domanig, R. *Arch. Pharm.* **1981**, *314*, 271.
(21) deStevens, G.; Frutchey, A.; Halamandaris, A.; Luts, H. A. *J. Am. Chem. Soc.* **1957**, *79*, 5263.

(50 mmol) of 4-(2-chloroethyl)acetophenone and 25 mL of acetic acid. To the stirred solution was added 2.58 mL (50 mmol) of bromine dropwise over 2 min. The reaction was stirred at room temperature for 30 min, taken up in ethyl acetate, washed with water, saturated aqueous sodium bicarbonate solution, and brine, dried over sodium sulfate, and evaporated to an oil. The oil was taken up in 250 mL of acetone, treated with 4.9 g (50 mmol) of potassium thiocyanate, and stirred at room temperature for 3 h. The precipitate was filtered and the filtrate evaporated. The residue was taken up in ethyl acetate, washed with water and brine, dried over sodium sulfate, and evaporated to a solid. The solid was taken up in 100 mL of boiling ethanol and treated slowly with 83 mL of 1 N HCl and then refluxed for 14 h. The reaction was cooled and the precipitate filtered, washed with water, and dried to give 8.2 g (68%) of a white solid: mp 226–229 °C; NMR (δ, DMSO-d_6) 3.02 (t, 2 H), 3.83 (t, 2 H), 6.70 (m, 1 H), 7.2–7.7 (m, 4 H), 11.7 (m, 1 H); IR (cm^{-1}, DMSO) 1670 (C=O); MS (M$^+$) 239/241 (50/21, parent, Cl^{35}/Cl^{37}), 190 (100), 147 (28), 130 (15), 117 (21), 115 (11), 103 (12), 90 (14), 89 (13), 77 (14).

Method E. General Method for the Preparation of Naphthylpiperazine Derivatives 4, 8, and 11. 3,3-Dimethyl-5-[2-[4-(1-naphthyl)piperazinyl]ethyl]-2,3-dihydroindol-2-one Hydrochloride (4d). To a 125-mL round-bottomed flask equipped with condenser and N_2 inlet were added 1.05 g (4.72 mmol) of 3,3-dimethyl-5-(2-chloroethyl)-2,3-dihydroindol-2-one (**3d**), 1.00 g (4.72 mmol) of 1-naphthylpiperazine,[4] 1.00 g (9.44 mmol) of sodium carbonate, 2 mg of sodium iodide, 0.82 mL (4.72 mmol) of diisopropylethylamine, and 35 mL of methyl isobutyl ketone. The mixture was refluxed 5 days, cooled, and filtered, and the filtrate was evaporated. The residue was chromatographed on silica gel with methylene chloride/ethyl acetate as eluant to give an oil, which was taken up in methylene chloride/ether and precipitated as its hydrochloride salt by ether saturated with dry HCl. The resulting white solid, after drying, gave 1.15 g (56%): mp 185–190 °C; ^{1}H NMR (δ, DMSO-d_6) 1.26 (s, 6 H), 3.10 (m, 2 H), 3.2–3.5 (m, 8 H), 3.69 (m, 2 H), 6.82 (d, J = 8, 1 H), 7.0–8.1 (series of multiplets, 9 H); IR (cm^{-1}, KBr) 1708 (C=O); MS (M$^+$) 399 (1.4, parent), 226 (21), 225 (100), 182 (11), 154 (11), 70 (44). Anal. ($C_{26}H_{29}N_3O\cdot{}^3/_2$HCl) C, H, N.

6-[2-[4-(1-Naphthyl)piperazinyl]ethyl]benzoxazol-2(3*H*)-one Hydrochloride Hydrate (4a): prepared according to method E in 23% yield: mp 282–285 °C; NMR (δ, DMSO-d_6) 3.1–3.8 (m, 13 H) and 7.1–8.2 (m, 10 H); IR (cm^{-1}, KBr) 1767 (C=O); MS (M$^+$) 373 (2.6, parent), 225 (100), 182 (15.3), 154 (14.4), 70 (27.4). Anal. ($C_{23}H_{23}N_3O_2\cdot2$HCl$\cdot{}^5/_4H_2O$) C, H, N.

5-[2-[4-(1-Naphthyl)piperazinyl]ethyl]-2,3-dihydroindol-2-one Dihydrochloride (4b): prepared according to method E in 84% yield; mp 310–315 °C; NMR (δ, DMSO-d_6) 3.0–3.9 (m, 12 H), 3.48 (s, 2 H), 6.8–8.2 (m, 10 H), 10.42 (s, 1 H); IR (cm^{-1}, KBr) 1735, 1699 (C=O); MS (M$^+$) 373 (17), 372 (45), 371 (19, parent), 225 (100), 182 (13), 159 (28), 157 (93), 146 (11), 141 (19), 93 (30), 81 (24), 79 (77), 78 (37), 70 (16). Anal. ($C_{24}H_{25}N_3O\cdot2$HCl) C, H, N.

6-[2-[4-(1-Naphthyl)piperazinyl]ethyl]benzothiazol-2(3*H*)-one Hydrate (4c): prepared according to method E in 36% yield; mp 198–220 °C; NMR (δ, DMSO-d_6) 2.7–3.5 (m, 12 H), 7.0–8.2 (m, 10 H); IR (cm^{-1}, KBr) 1661 (C=O); MS (M$^+$) 389 (5, parent), 226 (16), 225 (98), 182 (12), 154 (21), 136 (13), 127 (15), 70 (100). Anal. ($C_{23}H_{23}N_3SO\cdot H_2O$) C, H, N.

1-Ethyl-5-[2-[4-(1-Naphthyl)piperazinyl]ethyl]-2,3-dihydroindol-2-one Hydrochloride Hydrate (4e): prepared according to method E in 22% yield; mp >305 °C; NMR (δ, DMSO-d_6) 1.14 (t, 3 H), 3.03–3.72 (m, 10 H), 7.05 (m, 3 H), 7.45–7.72 (m, 4 H), 7.95 (m, 1 H), 8.16 (m, 1 H); IR (cm^{-1}, KBr) 1726, 1718 (C=O); MS (M$^+$) 399 (1, parent), 226 (21), 225 (96), 182 (30), 174 (17), 154 (29), 141 (18), 123 (24), 70 (100). Anal. ($C_{26}H_{29}N_3O\cdot$HCl$\cdot H_2O$) C, H, N.

4-[4-[2-[4-(1-Naphthyl)piperazinyl]ethyl]phenyl]-2-aminothiazole Trihydrochloride (8a): prepared according to method E in 31% yield; mp 274–277 °C; NMR (δ, DMSO-d_6) 3.2–3.6 (m, 10 H), 3.7 (m, 2 H), 7.32 (s, 1 H), 7.2–8.2 (m, 11 H); IR (cm^{-1}, KBr) 1626. MS (M$^+$) 414 (7, parent), 226 (13), 225 (100), 154 (9), 70 (4). Anal. ($C_{25}H_{26}H_4S\cdot3$HCl) C, H, N.

4-[4-[2-[4-(1-Naphthyl)piperazinyl]ethyl]phenyl]-2-amino-5-methylthiazole Dihydrochloride Hydrate (8b): prepared according to method E in 54% yield: mp 160–165 °C; NMR (δ, DMSO-d_6) 2.32 (s, 3 H), 3.2–3.6 (m, 10 H), 3.7 (m, 2 H), 7.2–8.2 (m, 11 H); IR (cm^{-1}, KBr) 1620; MS (M$^+$) 428 (3, parent), 226 (17), 225 (100), 182 (11), 154 (11), 78 (42), 70 (20), 63 (37). Anal. ($C_{26}H_{28}N_4S\cdot2$HCl$\cdot{}^3/_2H_2O$) C, H, N.

4-[4-[2-[4-(1-Naphthyl)piperazinyl]ethyl]phenyl]-2-(methylamino)thiazole Dihydrochloride Dihydrate (8c): prepared according to method E in 63% yield; mp 272–273 °C; NMR (δ, DMSO-d_6) 3.07 (s, 3 H), 3.2–3.6 (m, 10 H), 3.7–3.8 (m, 2 H), 7.26 (s, 1 H), 7.2–8.2 (m, 11 H); IR (cm^{-1}, KBr) 1628; MS (M$^+$) 428 (28, parent), 226 (42), 225 (100), 203 (22), 182 (18), 154 (26), 127 (15), 91 (25), 70 (33). Anal. ($C_{26}H_{28}N_4S\cdot2$HCl$\cdot2H_2O$) C, H, N.

4-[4-[2-[4-(1-Naphthyl)piperazinyl]ethyl]phenyl]thiazol-2(3*H*)-one Hydrochloride Hemihydrate (8d): prepared according to method E in 18% yield: mp 307–309 °C; NMR (δ, DMSO-d_6) 3.1–3.5 (m, 10 H), 3.65 (d, 2 H), 6.85 (s, 1 H), 7.2–8.2 (m, 8 H), 11.55 (m, 1 H), 11.85 (s, 1 H); IR (cm^{-1}, KBr) 1668 (C=O); MS (M$^+$) 415 (5, parent), 226 (13), 225 (100), 182 (7), 154 (6), 70 (5). Anal. ($C_{25}H_{25}N_3OS\cdot$HCl$\cdot{}^1/_2H_2O$) C, H, N.

4-[4-[2-[4-(1-Naphthyl)piperazinyl]ethyl]phenyl]-2-methylthiazole Dihydrochloride Hydrate (8e): prepared according to method E in 48% yield; mp 305–307 °C; NMR (δ, DMSO-d_6) 2.73 (s, 3 H), 3.2–3.8 (m, 12 H), 7.98 (s, 1 H), 7.2–8.2 (m, 11 H); IR (cm^{-1}, KBr) 1592. MS (M$^+$) 413 (13, parent), 226 (37), 225 (100), 210 (13), 188 (29), 182 (32), 168 (11), 155 (14), 154 (43), 147 (23), 141 (19), 128 (19), 127 (31), 115 (17), 70 (68). Anal. ($C_{26}H_{27}N_3S\cdot2$HCl$\cdot H_2O$) C, H, N.

4-[4-[2-[4-(1-Naphthyl)piperazinyl]ethyl]phenyl]-1,2,3-thiadiazole Hydrochloride Quarterhydrate (11): prepared according to method E in 50% yield: mp 286–289 °C; NMR (δ, DMSO-d_6 and TFA) 3.6 (m, 2 H), 3.9 (m, 2 H), 4.4–4.7 (m, 8 H), 7.5–8.2 (m, 11 H), 9.42 (s, 1 H); IR (cm^{-1}, KBr) 1593; MS (M$^+$) 400 (parent, 11), 226 (18), 225 (100), 182 (12), 154 (14), 127 (10), 70 (42). Anal. ($C_{24}H_{24}N_4S\cdot$HCl$\cdot{}^1/_4H_2O$) C, H, N.

4-(2-Chloroethyl)acetophenone (*p*-Tolylsulfonyl)hydrazone (9). To a 500-mL round-bottomed flask equipped with condenser and N_2 inlet were added 32 g (176 mmol) of 4-(2-chloroethyl)acetophenone (**5a**), 32.7 g (176 mmol) of tosyl hydrazide, and 250 mL of ethanol. The reaction was refluxed for 3 h, cooled, and evaporated. The product crystallized on standing in ether to give a solid: mp 122–125 °C; 20.3 g (33%); NMR (δ, $CDCl_3$) 2.12 (s, 3 H), 2.34 (s, 3 H), 3.0 (t, 2 H), 3.64 (t, 2 H), 7.1–7.9 (2 para substituted aromatic patterns, 8 H), 8.53 (s, 1 H).

4-[4-(2-Chloroethyl)phenyl]-1,2,3-thiadiazole (10). To a 100 mL-round-bottomed flask equipped with N_2 inlet were added 1.3 g (3.71 mmol) of the tosyl hydrazone **9** and 3.0 mL (41.1 mmol) of thionyl chloride. The reaction gave a crystalline precipitate on standing at room temperature for 1 h, which was collected with hexane to give a solid, mp 80–81 °C, 0.33 g (39%). The remainder of the reaction was chromatographed with methylene chloride to afford an additional 0.33 g of product: NMR (δ, $CDCl_3$) 3.18 (t, J = 6, 2 H), 3.80 (t, J = 6, 2 H), 7.2 and 8.0 (para-substituted aromatic pattern, 4 H), 8.65 (s, 1 H); MS (M$^+$) 224 (6, parent), 196 (15), 147 (100), 111 (10).

4-(2-Chloroethyl)propiophenone (5b). A solution was prepared by adding 8.69 mL (50 mmol) of propionyl chloride to a suspension of 7.34 g (55 mmol) of aluminum chloride in 35 mL of ethylene dichloride. This solution was added at room temperature to a solution of 6.58 mL (100 mmol) of phenethyl chloride in 10 mL of ethylene dichloride. The solution began to darken and give off HCl and was stirred at room temperature for 25 min and then poured into ice/water. The layers were separated, and the organic layer was washed with 1 N HCl, saturated aqueous sodium bicarbonate solution, and brine, dried over sodium sulfate, and evaporated to an oil, 2.8 g (100%), which was used without further purification: NMR (δ, $CDCl_3$) 1.16 (t, 3 H), 2.6–3.1 (m, 4 H), 3.68 (m, 2 H), 7.2 (d, 2 H), 7.9 (d, 2 H); IR (cm^{-1}, neat): 1690 (C=O).

Biological Methods. [^{3}H]NPA Autoradiographic Binding. Adult, male Sprague-Dawley rats were decapitated, and the brains were quickly removed and frozen in isopentane (−30 °C) for 30 s and stored at −65 °C. The brains were subsequently sectioned (20-μm thickness) on a cryostat, and the sections were mounted on subbed slides and dried overnight under vacuum in a refrigerator. The sections were incubated in 50 mM Tris buffer (pH 7.5) containing 0.1% ascorbic acid and 1 mM EDTA with 0.6 nM

[^{3}H]NPA (*N*-propylnorapomorphine) and the test drug at room temperature for 1 h. Butaclamol (2 μM) was added to some sections to determine nonspecific binding. The slides were washed twice for 10 min each with fresh ice-cold 50 mM Tris buffer and dipped in ice-cold water to remove excess salts. The slides were then dried in a stream of compressed air and exposed to tritium sensitive film for 6 weeks. Analysis of optical density and extrapolation to levels from known ^{3}H standards (Amersham) were made with a Cambridge Instrument Quantimet 920 image analysis system.

[^{3}H]Prazosin Binding Assay. Fresh whole rat brain with the cerebellum removed was homogenized in 20 vol ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) with a Polytron. It was then centrifuged at 20000*g* for 10 min. The supernatant was discarded and the pellet resuspended in fresh Tris-HCl buffer (20 vol) with a Polytron, and the centrifugation was repeated. The supernatant was again discarded and the pellet resuspended in 50 vol ice-cold buffer (Tris, pH 8.0). In the assay, tubes (in triplicate) were charged with 800 μL of [^{3}H]prazosin (final concentration 0.22 nM) and 200 μL of drug solution and then 1 mL of tissue suspension. The tubes were incubated at 25 °C for 30 min and then rapidly filtered under vacuum through glass fiber filters and rinsed with 10 mL of ice-cold buffer. The blank was 50 μM norepinephrine. Filters were counted in a scintillation counter with 10–15 mL of aquasol.

[^{3}H]Ketanserin Binding Assay. Adult, male Sprague-Dawley rats were decapitated and the brains were quickly removed and frozen in isopentane (−30 °C) for 30 s. They were subsequently sectioned (20-μm sections) on a cryostat, and the sections were mounted on subbed slides, which were dried overnight under vacuum in a refrigerator. The sections were incubated in 50 mM Tris buffer (pH 7.5) with 1 nM [^{3}H]ketanserin and the test drug at room temperature for 30 min. Nonspecific binding was determined with 2 μM pipamperone. The slides were then washed twice, 10 min each with fresh ice-cold 50 mM Tris buffer, and dipped in ice-cold water to remove excess salt. The slides were then dried under a stream of compressed air and exposed to tritium-sensitive film for 6 weeks to allow analysis by computer densitometry as described above.

[^{3}H]-8-Hydroxy-2-(di-*n*-propylamino)tetralin Binding Assay. The tissue used was the P2 fraction from forebrains of male rats, which had been homogenized in 0.32 M sucrose, brought up to 100 mL volume, and centrifuged at 1000*g* for 8 min. The combined supernatants from this step and one more repeated on the P1 pellet were combined and centrifuged at 17000*g* for 25 min. The resulting P2 pellet was resuspended in 100 mL of 5 mM Tris acetate pH 7.4 buffer, left on ice for at least 10 min to lyse cells, and then washed twice with Tris buffer. It was then resuspended in the same buffer, placed in a water bath at 30 °C for 30 min, and then kept on ice. For the assay, it was diluted to 1 mg/mL in 50 mM Tris-HCl pH 7.4, 10 μM pargyline, 4 mM $CaCl_2$, 0.1% ascorbic acid pH 7.4. The incubation was carried out with test drug and 40 mL of a 50 nM solution of [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin at 30 °C for 20 min. A cell harvester (Brandel) was used to collect bound material, and the filter disks were rinsed with cold Tris-HCl buffer, placed in vials with scintillation fluid, and counted on a scintillation counter.

Serotonergic Cell-Firing Assay. Male Sprague-Dawley rats (250–350 mg) were anesthetized with chloral hydrate (400 mg/kg, ip), prepared with a femoral vein catheter, and fixed in a Kopf stereotaxic frame. Single unit activity was recorded from the dorsal raphe nucleus (DRN) using glass-coated, tungsten microelectrodes (impedance = 2-4 MΩ at kHz) and conventional extracellular signal processing. Spontaneously active serotonergic neurons were identified on line by their characteristic slow, regular firing rate, long duration, and notched action potentials.[22] Single unit activity was continuously plotted on line as a firing-rate histogram. The test compound was dissolved in a mixture of 5% DMSO and 95% physiological saline and administered by intravenous injection into the femoral vein in a volume of 0.1 mL.

Reversal of Apomorphine-Induced Stereotypy in Rats. Adult, male Sprague-Dawley rats (five animals per group) weighing from 250 to 300 g were placed two to a cage and injected with the test drug, made up at an injection volume of 2 mL/kg body weight, or saline. At time intervals of 1, 3, and 5 h, each animal received an sc injection of apomorphine, made up in distilled water and 0.1% ascorbic acid, at a dose of 0.75 mg/kg in a volume of 1 mL/kg body weight. The animals were observed for stereotypic behavior at 10-min intervals up to 50 min and rated according to the following scale: 0, no movement; 1, moving around cage or discontinuous sniffing; 2, continuous sniffing; 3, discontinuous oral movements (licking, chewing, or biting); 4, continuous chewing and biting. The rating scores were analyzed by one-way variance to determine difference among the groups of test animals; Dunnett's multiple range test was used to determine whether each test drug group was significantly different from control.

Reversal of Haloperidol-Induced Catalepsy in Rats. Adult, male Sprague-Dawley rats (five animals per group), weighing between 280 and 325 g, were injected with haloperidol (1 mg/kg sc, dissolved in glacial acetic acid and water, pH adjusted to 4.5 with sodium bicarbonate). After 3 h, the animals were tested for cataleptic behavior by standing them upright and placing their front paws on a horizontal metal rod set 4 in. above the table surface. The latency time for the animal to drop either both forelimbs or its head below the bar was recorded, with a cutoff at 90 s as the maximum time permitted for the test. The animals were then assigned to one of three groups to achieve an equal distribution of base-line latency times. Test drug or vehicle was then administered, and the animals were tested for catalepsy duration at 30-min intervals for 2.5 h thereafter. The scores were analyzed by one-way variance to determine differences among the groups of test animals; Dunnett's multiple range test was used to determine whether each test drug group was significantly different from control.

Reversal of Amphetamine-Induced Locomotor Behavior in Rats. Adult, male Sprague-Dawley rats, eight animals per group, weighing from 275 to 325 g were placed in automatic activity measuring boxes and allowed to habituate overnight. The next day, the animals were weighed and dosed with test compound suspended in 90% saline/5% ethanol/5% Emulphor. Amphetamine sulfate was administered at a dose of 1.36 mg/kg 1 h later. Horizontal locomotor activity was measured as the number of floor quadrants crossed in the 3-h period following *d*-amphetamine administration. Treatment groups were compared with ANOVA followed by Dunnett's multiple comparisons, and ED_{50} and 95% confidence limit values were determined by probit analysis of means converted to percent inhibition.

(22) Aghajanian, G. K.; Kaigler, H. J. *Brain Res.* **1974**, *81*, 364.