3-Benzisothiazolylpiperazine Derivatives as Potential Atypical Antipsychotic Agents

Harry R. Howard,* John A. Lowe, III,* Thomas F. Seeger, Patricia A. Seymour, Stevin H. Zorn, Patrick R. Maloney, Frank E. Ewing, Michael E. Newman, Anne W. Schmidt, Jerome S. Furman, Gwendolyn L. Robinson, Elisa Jackson, Celeste Johnson, and Jean Morrone

Central Research Division, Pfizer Inc., Eastern Point Road, Groton, Connecticut 06340

Received August 22, 1995[⊗]

A series of substituted phenethyl derivatives of 3-benzisothiazolylpiperazine incorporating potent D_2 and 5-HT_{2A} antagonist activity was investigated as an approach to a novel atypical antipsychotic agent. The *in vitro* profile of **8e** from this series is a combination of D_2 receptor affinity comparable to the typical antipsychotic agent haloperidol and a 5-HT_{2A}/D_2 ratio comparable to the atypical agent clozapine. *In vivo* **8e** possesses activity consistent with an efficacious antipsychotic agent with less tendency to induce extrapyramidal side effects in man.

The atypical antipsychotic agents, exemplified by clozapine, **1**, offer improved treatment of schizophrenia by combining efficacy with less propensity to cause harmful central nervous system (CNS) side effects.[1] Recent attention in this field has been focused on determining which of clozapine's many *in vitro* attributes is responsible for its atypical profile. One hypothesis is based on the observation that clozapine, in common with other atypical antipsychotics, possesses a higher antagonist affinity at the serotonin 5-HT_{2A} as compared with the dopamine D_2 receptor.[2] Risperidone, **2**, which also has a favorable 5-HT_{2A}/D_2 ratio,[3] has demonstrated clinical efficacy with a reduced propensity to induce extrapyramidal side effects (EPS),[4] supporting the importance of this ratio. Two other extensively studied agents based on the clozapine structure, olanzapine, **3**,[5] and tiospirone, **4**,[6] are also reported to have favorable 5-HT_{2A}/D_2 ratios. (The ratio of affinities for the 5-HT_{2A} and D_2 receptors is reported as 5-HT_{2A}/D_2, calculated as $(1/K_i$ value for the 5-HT_{2A} binding$)/(1/K_i$ value for the D_2 binding).)

Previous publications from this laboratory have described the design and synthesis of a series of potential atypical antipsychotic agents based on the structure of 1-naphthylpiperazine.[7] In seeking to improve the dopaminergic antagonist potency of these compounds, and hence their potential clinical efficacy, we were attracted to the structure and biochemical profile of **4**. The heteroatom-substituted phenethyl groups used in the 1-naphthylpiperazine series proved especially effective in maintaining a favorable 5-HT_{2A}/D_2 ratio and were expected to afford similarly promising properties when appended to the 3-benzisothiazolylpiperazine nucleus of **4**. The following report chronicles the implementation of this strategy, which led to the discovery of the putative atypical antipsychotic agent **8e**.

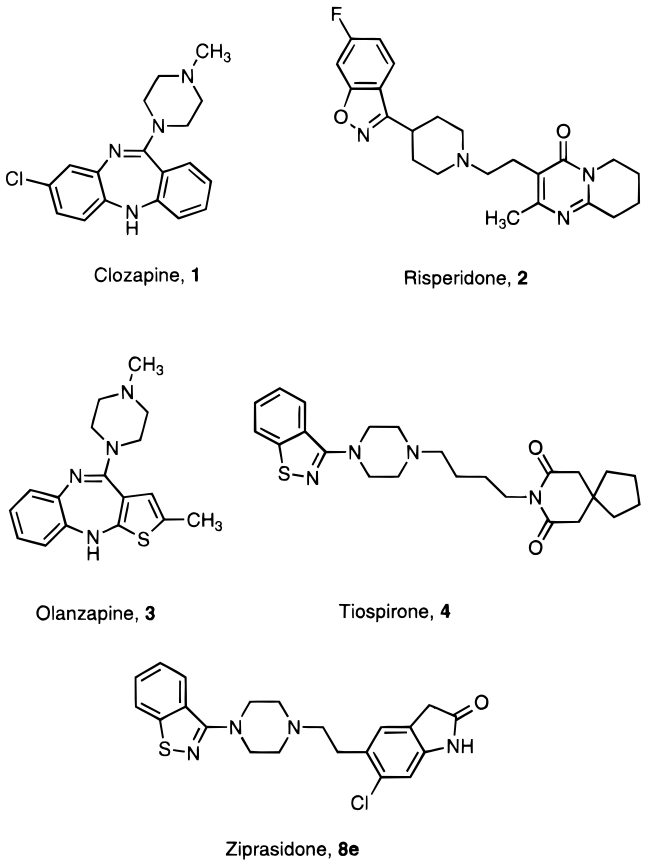


Figure 1. Selected 'atypical' and putative 'atypical' antipsychotic agents.

Chemistry

The preparation of the benzisothiazolylpiperazine derivatives is outlined in Scheme 1 and is based on our earlier work in the 1-naphthylpiperazine series.[7] The synthesis of the oxindoles **5d**,**e** began with Wolff−Kishner reduction of the corresponding isatin. After Friedel−Crafts acylation with the appropriate chloroacetyl chloride followed by triethylsilane/trifluoroacetic acid reduction of the resulting aryl ketone, the side chain **7** was appended to 3-benzisothiazolylpiperazine *via* base-catalyzed condensation in methyl isobutyl ketone (MIBK) or DMF. A troublesome side reaction, aldol condensation of the oxindole side chain with MIBK, led to a reduced yield of product in compounds **8d**−**8f**. Physical properties of final products are listed in Table 3.

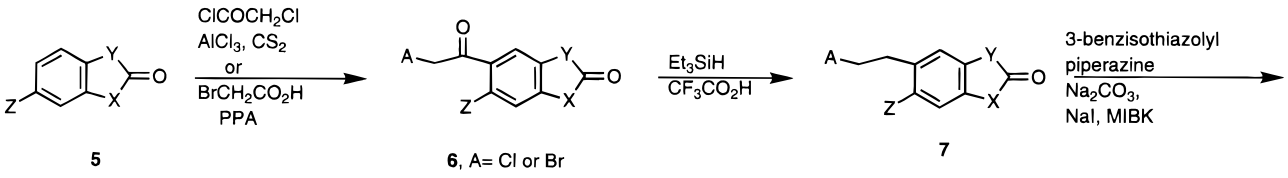
Biology

Compounds were tested for affinity at D_2 and 5-HT_{2A} receptors by homogenate binding in rat brain. Competitive inhibition of [^{3}H]spiperone (D_2) in rat striatum

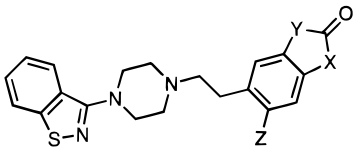
[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1995.

0022-2623/96/1839-0143$12.00/0

Scheme 1. Synthesis of 3-Benzisothiazolylpiperazine Derivatives



5 **6**, A= Cl or Br **7**



8

5-8	X	Y	Z
a	NH	O	H
b	NH	CH_2	H
c	NH	S	H
d	NH	CH_2	F
e	NH	CH_2	Cl
f	NEt	CH_2	H
g	NH	$C(CH_3)_2$	H
h	NH	$C((CH_2)_4)$	H

and [^{3}H]ketanserin (5-HT_{2A}) in frontal cortex was quantified and used to generate a pK_i value for each compound of interest. Inhibition of serotonin-induced phosphatidylinositol turnover in rat brain cerebral cortical slices was used as a functional measure of 5-HT_2 receptor antagonism. A standard rat brain homogenate binding assay was used to measure affinity for α_1 noradrenergic receptors using [^{3}H]prazosin. *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. Another behavioral test associated with antipsychotic efficacy, the conditioned avoidance paradigm, measures the response of rats trained to avoid footshock signaled by an auditory cue. Antipsychotic agents have been shown to disrupt conditioned avoidance responding without impairment of escape responding. The tendency to induce catalepsy in rats was used as an indication of the propensity to cause EPS.

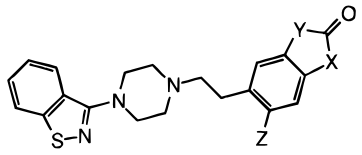
Results and Discussion

The first antipsychotic agent to qualify for the category of 'atypical', that is, an agent showing efficacy without causing EPS in patients, was clozapine (Figure 1). Given the unfortunate incidence of agranulocytosis with clozapine, there has been considerable effort to reproduce its clinical profile in a novel structure. Figure 1 outlines some of the agents resulting from these studies, which utilize a variety of strategies in an attempt to reach this goal. Our strategy was based on our previous observation,[8] as well as on related studies,[9] that the putative atypical antipsychotic agents possess greater affinity for the 5-HT_{2A} receptor than the D_2 receptor. A 92% correlation between an atypical profile and a 5-HT_{2A}/D_2 ratio of $\geq$3.2 or greater has also been demonstrated.[10] In addition, the potent 5-HT_2 receptor antagonist ritanserin has been reported to reduce the incidence of EPS produced by neuroleptic treatment[11] and improve negative symptoms in schizophrenia.[12] Thus a favorable 5-HT_{2A}/D_2 ratio was selected for optimization *in vitro*. Two ratios were selected for *in vivo* optimization. The first, catalepsy induction compared with blockade of amphetamine-induced hypermotility, was used to confirm the atypical profile predicted *in vitro*. The second ratio, blockade of spontaneous as compared with amphetamine-induced hypermotility, predicts relative sedative liability.

The chemical strategy designed to afford the desired 5-HT_{2A}/D_2 ratio was based on our previously reported work using heterocyclic surrogates to mimic the catechol and indole portions of dopamine and serotonin, respectively.[7] These oxindole-related heterocyclic groups, when appended to an arylpiperazine such as 1-naphthylpiperazine, afford high-affinity antagonists at both D_2 and 5-HT_{2A} receptors. In order to improve their D_2 receptor affinity, these groups were appended to 3-benzisothiazolylpiperazine, a derivative of which, the putative atypical antipsychotic agent tiospirone, **4**, was previously shown to possess potent D_2 receptor affinity.[6] Thus compounds **8a**−**c** were found to possess both high D_2 receptor affinity and selectivity for the 5-HT_{2A} receptor, as shown in Table 1. Compound **8b** seemed especially attractive for further modification due to its superior D_2 receptor affinity. This greater affinity translates to better activity *in vivo* in blocking amphetamine-induced locomotor behavior in the rat, thought to be mediated by the mesolimbic dopamine system and thus predictive of antipsychotic efficacy, as shown in Table 2. In addition, the 5-HT_{2A}/D_2 ratio of **8b** (Table 1) surpasses that of clozapine, risperidone, and tiospirone. The D_2/α_1 ratio of **8b**, when compared with that of clozapine, suggests **8b** might have a lower propensity to cause orthostatic hypotension, an effect generally thought to involve α adrenergic blockade. The superior 5-HT_{2A}/D_2 ratio of **8b** is confirmed by its favorable ratio for blockade of amphetamine-induced locomotor activity, an efficacy model, compared with its induction of catalepsy, thought to be an indicator of the potential to induce EPS.

As shown in Table 1, further modifications of **8b** afforded compounds with similar affinity for the D_2 receptor, with compounds **8d**,**e**,**g** maintaining **8b**'s favorable 5-HT_{2A}/D_2 ratio. To demonstrate 5-HT_{2A} receptor antagonism, blockade of 5-HT stimulation of phosphatidylinositol turnover in rat brain cerebral cortical slices was employed.[13] In this system **8e** is a potent antagonist of the 5-HT-mediated response (K_i = 2.3 $\pm$ 0.9 nM, Figure 2). It showed the best ratio of D_2 to α_1 receptor blockade and preserved the favorable *in vivo* ratio of amphetamine-induced to spontaneous hy-

Table 1. *In Vitro* Characterization of 3-Benzisothiazolylpiperazine Derivatives



				pK_i values			K_i values			affinity ratios	
compd	X	Y	Z	D_2[a]	5-HT_{2A}[b]	α[c]	D_2[a]	5-HT_{2A}[b]	α[c]	D_2/5-HT_{2A}	D_2/α
8a	NH	O	H	8.20 ± 0.03 (4)	9.81 ± 0.06 (4)	8.38 ± 0.06 (4)	6.3	0.15	4.2	42	1.5
8b	NH	CH_2	H	8.42 ± 0.03 (5)	9.52 ± 0.07 (6)	8.52 ± 0.07 (3)	3.8	0.30	3.0	13	1.3
8c	NH	S	H	8.29 ± 0.11 (3)	9.57 ± 0.08 (3)	8.63 ± 0.11 (3)	5.1	0.27	2.3	19	2.2
8d	NH	CH_2	F	8.38 ± 0.06 (3)	9.45 ± 0.05 (3)	8.19 ± 0.12 (5)	4.2	0.35	6.5	12	0.65
8e	NH	CH_2	Cl	8.32 ± 0.04 (6)	9.38 ± 0.04 (5)	7.98 ± 0.04 (3)	4.8	0.42	11	11	0.44
8f	NEt	CH_2	H	8.53 ± 0.08 (3)	8.84 ± 0.08 (3)	8.35 ± 0.13 (5)	3.0	1.4	4.5	2.1	0.67
8g	NH	$C(CH_3)_2$	H	8.51 ± 0.11 (3)	9.48 ± 0.05 (3)	8.68 ± 0.14 (4)	3.1	0.33	2.1	9.4	1.5
8h	NH	$C(CH_2)_4$	H	8.38 ± 0.07 (3)	8.84 ± 0.05 (3)	8.29 ± 0.06 (3)	4.2	1.4	5.1	3.0	0.82
haloperidol				9.15 ± 0.04 (3)	7.35 ± 0.13 (5)	8.20 ± 0.11 (4)	0.71	45	6.3	0.016	0.11
clozapine				7.08 ± 0.08 (6)	7.82 ± 0.05 (3)	8.25 ± 0.11 (4)	83	15	5.6	5.5	15
risperidone				8.43 ± 0.03 (4)	9.27 ± 0.04 (4)	9.13 ± 0.15 (3)	3.7	0.54	0.74	6.8	5.0
tiospirone				8.67 ± 0.08 (6)	9.24 ± 0.03 (6)	8.83 ± 0.13 (4)	2.1	0.58	1.5	3.6	1.4

[a] Binding to the D_2 dopamine receptor in rat brain, using [^{3}H]NPA as ligand, given as the pK_i value followed by SEM and number of determinations in parentheses, with the K_i value in the adjacent column in nM units for comparison and generation of ratios. pK_i values were determined from dose−response curves of three log concentrations of the test compounds, each concentration in triplicate. [b] Binding to the 5-HT_2 serotonin receptor in rat brain, using [^{3}H]ketanserin as ligand, given as the pK_i value followed by SEM and number of determinations in parentheses, with the K_i value in the adjacent column in nM units for comparison and generation of ratios. pK_i values were determined from dose−response curves of three log concentrations of the test compounds, each concentration in triplicate. [c] Binding to the α adrenergic receptor in rat brain, using [^{3}H]prazosin as ligand, given as the pK_i value followed by SEM and number of determinations in parentheses, with the K_i value in the adjacent column in nM units for comparison and generation of ratios. pK_i values were determined from dose−response curves of three log concentrations of the test compounds, each concentration in triplicate.

Table 2. *In Vivo* Characterization of 3-Benzisothiazolylpiperazine Derivatives

	ED_{50} (po) values, mg/kg					ratios	
compd	amph[a]	spon[b]	apom[c]	cata[d]	cond[e]	spon/amph	cata/amph
8a	4.2 (2.7−5.9)	9.8 (7.0−14.6)	21.6 (6.3−74)	>32	17.8	2.3	7.6
8b	1.4 (0.35−3.6)	8.5 (3.7−12.6)	8.4 (5.7−12.3)	18.8 (15.1−23.4)	6.7 (5.1−8.7)	6.1	21
8c	2.7 (2.3−3.2)	2.7 (1.1−5.5)	23.8	>56	12.3 (6.9−22)	1.0	>20
8d	1.0 (0.65−1.4)	3.1 (1.8−5.1)	9.1	17.8	9.0	3.1	16
8e	1.5 (1.1−2.5)	8.9 (5.3−19.1)	2.4 (1.5−3.9)	12.1 (9.7−15.1)	2.6 (0.42−5.0)	5.9	8.1
8f	1.1 (N = 1)	2.1 (0.95−3.2)	4.7	10	1.8 (0.13−4.13)	1.9	6.2
8g	2.3 (1.1−3.6)	10.7 (7.6−20.5)	10	17.8	2.6	4.7	8.1
8h	5.8 (1.2−16.8)	NT	9.0	32	NT		4.8
haloperidol	0.14 (0.10−0.20)	0.68 (0.27−1.4)	0.25 (0.19−0.33)	0.79 (0.43−1.5)	0.93 (0.49−1.7)	4.9	5.6
clozapine	12.8 (7.1−25.7)	8.18 (4.7−18.3)	>32.0	>32.0	>17.8	0.64	>32
risperidone	0.44 (0.23−0.72)	1.2 (0.85−1.74)	1.8 (1.2−2.9)	5.7 (3.7−8.8)	1.6 (0.57−4.4)	2.7	13
tiospirone	4.8 (N = 1)	15.5 (11.1−24.0)	18.8 (6.6−54)	20.1 (7.8−51.9)	11.7 (5.0−27)	3.2	6.7

[a] Inhibition of amphetamine-induced locomotor behavior in rats, via oral administration. The 95% confidence limits are given in parentheses beneath, except for those compounds tested once, as indicated. [b] Inhibition of spontaneous locomotor behavior in rats, via oral administration. The 95% confidence limits are given in parentheses beneath, except for those compounds tested once, as indicated. [c] Inhibition of apomorphine-induced stereotypy in rats, via oral administration. The 95% confidence limits are given in parentheses beneath, except for those compounds tested once, as indicated. For these latter compounds, the value indicated is an MED_{50} value. [d] Induction of catalepsy in rats, via oral administration. The 95% confidence limits are given in parentheses beneath, except for those compounds tested once, as indicated. [e] Inhibition of conditioned avoidance response in rats, via oral administration. The 95% confidence limits are given in parentheses beneath, except for those compounds tested once, as indicated.

permotility blockade found in **8b**. Compound **8e** also blocked conditioned avoidance responding in rats without impairing escape responding, indicative of efficacy with minimum liability to produce sedative side effects. On the basis of these results, **8e**, ziprasidone, has been selected for further evaluation in clinical trials in man.

Conclusion

The series of 3-benzisothiazolylpiperazine derivatives described in this report provides support for the hypothesis that a favorable ratio of 5-HT_{2A}/D_2 receptor binding affinity translates to less induction of catalepsy as compared with blockade of amphetamine-induced

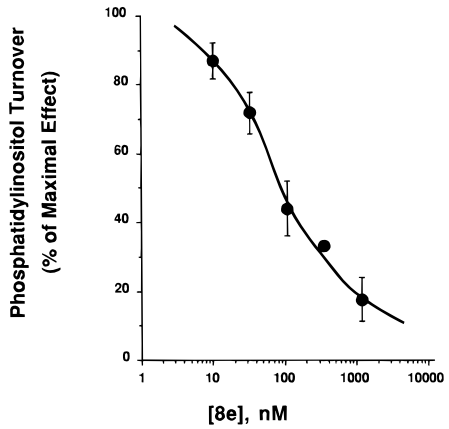


Figure 2. Effect of **8e** on 5-HT_2 receptor-stimulated phosphatidylinositol turnover in rat brain cerebral cortical slices. Each point is the mean inhibition of the 5-HT (100 mM)-induced effect and represents the mean of 3−5 data points ± SEM derived from separate experiments each performed in triplicate.

Table 3. Physical Properties of 3-Benzisothiazolylpiperazine Derivatives

compd	yield, %	mp, °C	formula
8a	50	185−187	$C_{20}H_{20}N_4O_2S\cdot 0.5H_2O$
8b	59	288−288.5	$C_{21}H_{22}N_4OS\cdot 1.25HCl$
8c	77	288−290	$C_{20}H_{20}N_4OS_2\cdot HCl\cdot H_2O$
8d	19	291−293	$C_{21}H_{21}FN_4OS\cdot HCl\cdot H_2O$
8e	20	>300	$C_{21}H_{21}ClN_4OH\cdot HCl\cdot 0.5H_2O$
8f	25	278−279	$C_{23}H_{26}N_4OS\cdot HCl\cdot CH_2Cl_2$
8g	40	289−291	$C_{23}H_{26}N_4OS\cdot HCl\cdot 0.5H_2O$
8h	50	291−293 dec	$C_{25}H_{28}N_4OS\cdot HCl\cdot 0.5H_2O$

locomotor activity. In particular, compound **8e** optimizes these properties and has modest α_1 receptor affinity and a minimal tendency to block spontaneous locomotor activity, indicating a reduced propensity to cause hypotension and sedation in the clinic, respectively. The *in vitro* profile of **8e** is thus a combination of D_2 receptor affinity comparable to the typical antipsychotic agent haloperidol and a 5-HT_{2A}/D_2 ratio comparable to the atypical agent clozapine. The ultimate confirmation of the hypothesis that this *in vitro* profile combined with its favorable *in vivo* activity will result in an effective antipsychotic with low EPS liability awaits the outcome of clinical evaluation of **8e**.

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 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. Radioisotopic ligands were purchased from New England Nuclear, Boston, MA, or Amersham, Arlington Heights, IL. Apomorphine hydrochloride and *d*-amphetamine were purchased from Research Biochemicals Inc. (Natick, MA). Risperidone was a gift of Janssen, Inc., tiospirone was a gift of Bristol-Myers Squibb, Inc., and clozapine was a gift of Sandoz, Inc. Other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Syntheses. The examples presented below illustrate the method for preparation of the compounds listed in Table 3; physical data for the compounds are listed there. The following compounds were prepared by literature methods: 3-benzisothiazolylpiperazine,[6] **6a**−**c**,[7] **7a**−**c**,[7] **5d**,[14] **5e**,[14] and **5g**.[15] Compounds **5d**−**h** were converted to **7d**−**h** by the method used for **7a**−**c**.[7]

General Method for the Preparation of 4-Substituted (1,2-Benzisothiazol-3-yl)piperazines 8. 6-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)benzoxazolone (8a). To a solution of 1.22 g (5.02 mmol) of 6-(2-bromoethyl)benzoxazolone and 1.10 g (5.02 mmol) of 3-(1,2-benzisothiazolyl)-piperazine in 40 mL MIBK were added 0.53 g (5.02 mmol) of sodium carbonate and 2 mg of sodium iodide. The mixture was refluxed for 3 days, cooled, and evaporated. The residue was taken up in ethyl acetate and chromatographed on silica gel using ethyl acetate as eluent. The product fractions were concentrated and triturated with isopropyl ether to afford a white solid, mp 183−186 °C, 962 mg (50%). $R_f = 0.2$ in ethyl acetate. ^{1}H-NMR (δ, DMSO-d_6): 2.6−2.9 (m, 8H), 3.5 (m, 4H), 7.05 (m, 2H), 7.23 (s, 1H), 7.46 (t, 1H), 7.57 (t, 1H), 8.07 (d, 2H), 11.5 (bs, 1H). IR (cm^{-1}, KBr): 1762 (C═O). MS (*m*/*e*, %): 380 (parent, 1), 232 (22), 93 (21), 81 (20), 80 (26), 78 (100), 65 (39), 63 (67), 61 (47). Anal. ($C_{20}H_{20}N_4O_2S\cdot{}^1/_2\ H_2O$) C, H, N.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)-1,3-dihydro-2(1*H*)-indol-2-one (8b): prepared in 59% yield as the hydrochloride salt, mp 288−288.5 °C. ^{1}H-NMR (δ, DMSO-d_6): 3.1 (m, 2H), 3.4 (m, 4H), 3.51 (s, 2H), 3.5−3.8 (m, 4H), 4.1 (m, 2H), 6.81 (d, 1H), 7.1−7.2 (m, 2H), 7.49 (t, 1H), 7.62 (t, 1H), 8.14 (t, 2H). ^{13}C-NMR (δ, DMSO-d_6): 176.3, 162.2, 152.2, 142.5, 129.6, 128.2, 127.7, 127.0, 126.3, 124.9, 124.7, 124.1, 121.3, 19.2, 56.6, 50.5, 46.6, 35.8, 29.0. IR (cm^{-1}, KBr): 1697 (C═O). MS (*m*/*e*, %): 378 (parent, 1), 233 (14), 232 (100), 177 (16), 91 (11), 56 (11). Anal. ($C_{21}H_{22}N_4OS\cdot 1.25HCl$) C, H, N.

6-(4-(2-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)benzothiazolone (8c): prepared in 77% yield as the hydrochloride hydrate, mp 288−290 °C. ^{1}H-NMR (δ, DMSO-d_6): 3.1−3.7 (m, 10H), 4.1 (m, 2H), 7.0−8.1 (m, 7H). IR (cm^{-1}, KBr): 1680 (C═O). MS (*m*/*e*, %): 396 (parent, 1), 233 (16), 232 (100), 177 (19), 98 (4), 97 (4), 56 (7). Anal. ($C_{20}H_{20}N_4OS_2\cdot HCl\cdot H_2O$) C, H, N.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)-6-fluoro-1,3-dihydro-2(1*H*)-indol-2-one (8d): prepared in 19% yield as the hydrochloride hydrate, mp 291−293 °C. ^{1}H-NMR (δ, CD_3OD): 3.15 (m, 2H), 3.4−3.6 (m, 8H), 3.80 (m, 2H), 4.20 (d, 2H), 6.7 (d, 1H), 7.22 (d, 1H), 7.47 (t, 1H), 7.57 (t, 1H), 7.95 (d, 1H), 8.05 (d, 1H). MS (*m*/*e*, %): 396 (M^+, 1), 232 (100), 177 (53), 91. Anal. ($C_{21}H_{21}FN_4OS\cdot HCl\cdot H_2O$) C, H, N.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)-6-chloro-1,3-dihydro-2(1*H*)-indol-2-one (8e): prepared in 20% yield using isoamyl alcohol instead of MIBK as the hydrochloride hemihydrate, mp >300 °C. ^{1}H-NMR (δ, DMSO-d_6): 3.15−3.60 (m, 10H), 3.72 (d, 2H), 4.10 (d, 2H), 6.88 (s, 1H), 7.28 (s, 1H), 7.48 (t, 1H), 7.60 (t, 1H), 8.14 (dd, 2H), 10.6 (s, 1H), 11.4 (bs, 1H). MS (*m*/*e*, %): 412 (M^+, 0.4), 233 (18), 232 (100), 177 (19). IR (KBr, cm^{-1}) 1708, 1628, 1489. Anal. ($C_{21}H_{21}ClN_4OS\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)-1-ethyl-1,3-dihydro-2(1*H*)-indol-2-one (8f): prepared in 25% yield as the hydrochloride salt, mp 278−279 °C. ^{1}H-NMR (δ, CD_3OD): 1.25 (t, 3H), 3.18 (m, 2H), 3.50 (m, 8H), 3.75 (m, 4H), 4.20 (d, 2H), 5.24 (s, 2H, CH_2Cl_2 - one mole), 7.0 (d, 1H), 7.30 (s+d, 2H), 7.48 (t, 1H), 7.55 (t, 1H), 7.98 (d, 1H), 8.08 (d, 1H). MS (*m*/*e*, %): 406 (M^+, 1), 232 (100), 177, 163. Anal. ($C_{23}H_{26}N_4OS\cdot HCl\cdot CH_2Cl_2$) C, H, N.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)-3,3-dimethyl-1,3-dihydro-2(1*H*)-indol-2-one (8g): prepared in 40% yield as the hydrochloride hemihydrate, mp 289−291 °C. ^{1}H-NMR (δ, CD_3OD): 1.35 (s, 6H), 3.10 (m, 2H), 3.4−3.6 (m, 10H), 6.92 (d, 1H), 7.15 (d, 1H), 7.22 (s, 1H), 7.45 (t, 1H), 7.55 (t, 1H), 7.95 (d, 1H), 8.05 (d, 1H). MS (*m*/*e*, %): 406 (M^+, 1), 324, 232 (100), 177, 146. Anal. ($C_{23}H_{26}N_4OS\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

5′-(2-(4-(1,2-Benzoisothiazol-3-yl)piperazinyl)ethyl)-3,3-spirocyclopentane-1,3-dihydro-2(1*H*)-indol-2-one (8h): prepared in 50% yield as the hydrochloride hemihydrate, mp 291−293 °C. ^{1}H-NMR (δ, $CDCl_3$): 1.6−2.1 (m, 8H), 3.0−

3.25 (bs + m, 6H), 3.45 (d, 2H), 4.0 (d, 4H), 6.70 (d, 1H), 6.92 (d + s, 2H), 7.25 (t, 1H), 7.40 (t, 1H), 7.75 (dd, 2H), 9.3 (s, 1H). MS (*m/e*, %): 432 (M^+, <1), 233, 232 (100), 200 (11), 177 (36). Anal. ($C_{25}H_{28}N_4OS \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

1′,3-Dihydrospiro[cyclopentane-1,3′-2′(1*H*)-indol-2′-one (5h). To a suspension of oil-free NaH (4.2 g of 60% NaH in mineral oil, 0.105 mol, washed with hexane) in 25 mL of DMF, under N_2 with ice bath cooling, was added a solution of *N*-acetyloxindole[16] (8.76 g, 0.05 mol) in 125 mL of DMF over a 1 h period. Twenty minutes later, 1,4-diiodobutane (6.6 mL, 0.05 mol) was added dropwise, and after a further 1 h, the reaction mixture was allowed to warm to room temperature. After stirring overnight, the reaction mixture was poured over 200 mL of ice/H_2O, stirred for 30 min, and extracted with Et_2O. The Et_2O layer was washed (H_2O, saturated NaCl), dried ($MgSO_4$), and evaporated *in vacuo* to an oil. Flash chromatography (230−400 mesh silica gel, EtOAc:Hex, 5:95) provided a white solid, 7.15 g (62.4%), mp 94−96 °C. MS (*m/e*, %): 229 (M^+, 15), 187, 158, 146 (100).

A solution of the above solid (7.0 g, 0.03 mol) in 200 mL of MeOH and 200 mL of 4% aqueous NaOH was heated on a steam bath for 30 min. The cooled solution was acidified to ca. pH 1 and concentrated *in vacuo*. The solid residue was triturated with H_2O, filtered, dried, and recrystallized from hexanes as a white solid, 5.24 g (92%), mp 87−89 °C (lit.[9] mp 95−96 °C). MS (*m/e*, %): 187 (M^+, 146) (100).

Biological Methods. [^{3}H]Spiperone Receptor-Binding Assay. Standard receptor-binding methods were used to label dopamine D_2 receptors using [^{3}H]spiperone as previously described.[17] Briefly, rats were sacrificed by decapitation and the brains rapidly removed. The corpus striatum was dissected and homogenized in 20 vol at 50 mM Tris HCl (pH 7.2) buffer using a Brinkman Polytron homogenizer (setting 7 for 15 s). The homogenate was centrifuged for 10 min at 40000*g*. The pellet was resuspended in fresh ice-cold 50 mM Tris (pH 7.2) buffer with the Polytron homogenizer and recentrifuged. The final pellet was resuspended in 50 mM Tris HCl buffer (pH 7.2) containing 1 mM $MgCl_2$ and 100 mM NaCl. Assays were initiated by the addition of tissue to tubes containing [^{3}H]spiperone (0.2 nM) and drug or buffer in a final volume of 1 mL. Blocking agents (500 nM cinanserin and 1 mM prazosin) were present to prevent [^{3}H]spiperone from labeling 5-HT_2 and α_1 noradrenergic receptors. Nonspecific binding was defined as the radioligand bound in the presence of 10 μM (+)-butaclamol. After a 15 min incubation at 37 °C, tissue samples were filtered onto Whatman GF/B glass fiber filters using a Brandel harvester and rinsed two times with 5 mL of ice-cold 50 mM Tris HCl (pH 7.4) buffer. Filters were soaked in 10 mL of Ready-Safe (Beckman Instruments), and the radioactivity was quantified using liquid scintillation counting.

[^{3}H]Ketanserin Receptor-Binding Assay. Standard receptor-binding methods were used to label 5-HT_2 receptors using [^{3}H]ketanserin as previously described.[17] Briefly, rats were sacrificed by decapitation and the brains rapidly removed. The frontal cortex was dissected and homogenized in 20 vol of 50 mM Tris HCl (pH 7.7) buffer using a Brinkman Polytron homogenizer (setting 7 for 15 s). The homogenate was centrifuged for 10 min at 40000*g*. The pellet was resuspended in fresh ice-cold 50 mM Tris (pH 7.7) buffer with the Polytron homogenizer and recentrifuged. The final pellet was resuspended in 50 mM Tris HCl buffer (pH 7.7). Assays were initiated by the addition of tissue to tubes containing [^{3}H]ketanserin (0.7 nM) and drug or buffer in a final volume of 1 mL. Blocking agents (500 nM pyrilamine and 1 mM prazosin) were present to prevent [^{3}H]ketanserin from labeling histamine-1 and α_1 noradrenergic receptors. Nonspecific binding was defined as the radioligand bound in the presence of 10 μM methysergide. After a 15 min incubation at 37 °C, tissue samples were filtered onto Whatman GF/B glass fiber filters using a Brandel harvester and rinsed two times with 5 mL of ice-cold 50 mM Tris HCl (pH 7.4) buffer. Filters were soaked in 10 mL of Ready-Safe (Beckman Instruments), and the radioactivity was quantified using liquid scintillation counting.

[^{3}H]Prazosin Receptor-Binding Assay. Standard receptor-binding methods were used to label α_1 noradrenergic receptors using [^{3}H]prazosin as previously described.[17] Briefly, rats were sacrificed by decapitation and the brains rapidly removed. The cortex was dissected and homogenized in 20 vol of 50 mM Tris HCl (pH 8.0) buffer using a Brinkman Polytron homogenizer (setting 7 for 15 s). The homogenate was centrifuged for 10 min at 40000*g*. The pellet was resuspended in fresh ice-cold 50 mM Tris (pH 8.0) buffer with the Polytron homogenizer and recentrifuged. The final pellet was resuspended in 50 mM Tris HCl buffer (pH 8.0). Assays were initiated by the addition of tissue to tubes containing [^{3}H]prazosin (0.2 nM) and drug or buffer in a final volume of 1 mL. Nonspecific binding was defined as the radioligand bound in the presence of 10 μM phentolamine. After a 30 min incubation at 25 °C, tissue samples were filtered onto Whatman GF/B glass fiber filters using a Brandel harvester and rinsed two times with 5 mL of ice-cold 50 mM Tris HCl (pH 7.4) buffer. Filters were soaked in 10 mL of Ready-Safe (Beckman Instruments), and the radioactivity was quantified using liquid scintillation counting.

Antagonism of Apomorphine-Induced Stereotypy. Rats were administered test compounds or vehicle and individually placed into clear Plexiglas cages for observation. Three hours later, apomorphine hydrochloride (0.75 mg/kg sc) was administered to all subjects. Beginning 10 min later, rats were rated for stereotypy by an observer who was blind with respect to treatment, and the rating was repeated at 10 min intervals for a total of five ratings, using the following scale:[18] 0 = no stereotyped behavior, 1 = discontinuous sniffing, 2 = continuous sniffing, 3 = continuous sniffing and discontinuous biting, gnawing, or licking, and 4 = continuous biting, gnawing, or licking and no locomotor activity. Ratings for each subject were combined to produce a total stereotypy score (rating). Treatment groups were compared with Kruskal−Wallis ANOVA followed by Kruskal−Wallis multiple comparisons (RS-1 program, BBN Software Products, Cambridge, MA), and ID_{50} values were calculated by linear regression analysis of the dose−response data.

Antagonism of *d*-Amphetamine-Induced Hyperactivity. Locomotor activity was measured in custom-made Plexiglas chambers (30 cm × 30 cm) housed in sound-attenuating wooden cabinets. Horizontal locomotor activity was monitored and recorded by a PDP/11 computer and measured as the number of floor quadrants crossed in a given time period. After an overnight habituation period to the chambers, rats were administered vehicle or test compounds followed 60 min later by *d*-amphetamine sulfate (1.4 mg/kg sc) or vehicle. Data were recorded for the 3 h duration of the *d*-amphetamine hyperactivity effect. Treatment group means were compared with ANOVA followed by Dunnett's *t*-test, and ID_{50} values, defined as the dose that produced a 50% inhibition of the *d*-amphetamine response, were calculated by linear regression analysis of the dose−response data.

Inhibition of Conditioned Avoidance Behavior. Rats were trained in an active avoidance paradigm in Colbourn shuttleboxes housed in standard Colbourn isolation chambers. Training consisted of repeated pairings of a tone and light cue (20 s intertrial interval × 30 trials) followed 5 s later by a 1 mA shock, until a shock avoidance criterion of 80% avoidance was achieved on three successive daily runs. On test days, animals were first given 30 predrug avoidance trials in a morning session. In the afternoon session, animals were administered test compounds or vehicle and presented with 30 additional avoidance trials beginning 120 min later. Mean predrug and postdrug avoidances were compared with repeated measures ANOVA followed by Newman−Keuls test. ID_{50} values were calculated by linear regression analysis of the dose−response data.

Induction of Catalepsy. Rats were administered test compounds or vehicle and tested for catalepsy 120 and 180 min later. The catalepsy score for each subject was taken as the average of two trials. Testing was accomplished by placing each rat in an upright position with its forepaws resting on a horizontal bar (9 mm diameter) suspended at a height of 10 cm and recording the latency to remove the forepaws and climb down to a normal posture. The maximum time limit was set at 90 s. A mean immobility score of 20 s was used as the

criterion for the presence of catalepsy. An extrapolated ED_{20s} dose was calculated by linear regression analysis of the dose−response data.

Inhibition of Spontaneous Locomotor Activity. The effect of test compounds on spontaneous locomotor activity was examined under conditions associated with high base-line activity to assess its locomotor activity suppressant properties and to evaluate the possible contribution of general behavioral depression to the results obtained in the *d*-amphetamine antagonism test. At the beginning of the 12 h dark cycle, rats were administered vehicle or various doses of test compounds and immediately placed into activity chambers which were unfamiliar to the animals, for the measurement of locomotor activity. Horizontal locomotor activity was measured over a 4 h period in order to assess drug effects during the time period which corresponded to measurement of *d*-amphetamine antagonism. Treatment group means were compared with ANOVA followed by Dunnett's *t*-test, and ID_{50} values were calculated by linear regression analysis of the dose−response data.

Measurement of Inositol Phosphate Accumulation in Rat Brain Slices. [^{3}H]Inositol phosphate ([^{3}H]IP) accumulation in rat brain slices was examined using a modification[19] of an earlier method.[20] In brief, rat brain cerebral cortical slices (0.35 × 0.35 mm) were prepared using a McIlwain tissue chopper and then incubated for 45 min at 37 °C in a modified Krebs−Ringer buffer (NaCl, 118 mM; KCl, 5 mM; $CaCl_2$, 1.3 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; and dextrose, 11.1 mM) oxygenated with 95% O_2−5% CO_2. During this time the buffer was changed at 15 min intervals, with 10 mM LiCl added before the final preincubation period. The slices were then preincubated for an additional 30 min at 37 °C with [^{3}H]-*myo*-inositol (1 μCi/tube) in the presence of LiCl and 10 μM pargyline. After the prelabeling period, slices were exposed to the various test agents for 45 min under the same experimental conditions. Brain slices were exposed to antagonists 5 min prior to agonist exposure. The reaction was terminated by the addition of a chloroform:methanol (1:2, v/v) solution, and the water soluble [^{3}H]IPs were isolated by a batch technique employing a Dowex AG 1-X8 anion exchange resin.[20]

References

(1) Lowe, J. A., III; Seeger, T. F.; Vinick, F. J. Atypical Antipsychotics − Recent Findings and New Perspectives. *Med. Res. Rev.* **1988**, *8*, 475−485.

(2) Deutch, A. Y.; Moghaddam, B.; Innis, R. B.; Krystal, J. H.; Aghajanian, G. K.; Bunney, B. S.; Charney, D. S. Mechanisms of Action of Atypical Antipsychotic Drugs. *Schizophr. Res.* **1991**, *4*, 121−156.

(3) Leysen, J. E.; Gommeren, W.; Eens, A.; de Chaffoy de Courcelles, D.; Stoof, J. C.; Janssen, P. A. J. Biochemical Profile of Risperidone, a New Antipsychotic *J. Pharmacol. Exp. Ther.* **1988**, *247*, 661−670.

(4) For example, see: Claus, A.; Bollen, J.; De Cuyper, H.; Eneman, M.; Malfroid, M.; Peuskens, J.; Heylen, S. Risperidone versus Haloperidol in the Treatment of Chronic Schizophrenic In-Patients: a Multicentre Double-Blind Comparative Study. *Acta Psychiatr. Scand.* **1992**, *85*, 295−305. Ceskova, E.; Svestka, J. Double-Blind Comparison of Risperidone and Haloperidol in Schizophrenic and Schizoaffective Psychoses. *Pharmacopsychiatry* **1993**, *26*, 121−124.

(5) Fuller, R. W.; Snoddy, H. D. Neuroendocrine Evidence for Antagonism of Serotonin and Dopamine Receptors by Olanzapine (LY-170,053), an Antipsychotic Drug Candidate. *Res. Commun. Chem. Pathol. Pharmacol.* **1992**, *77*, 87−93.

(6) Yevich, J. P.; New, J. S.; Smith, D. W.; Lobeck, W. G.; Catt, J. D.; Minielli, J. L.; Eison, M. S.; Taylor, D. P.; Riblet, L. A.; Temple, D. L., Jr. Synthesis and Biological Evaluation of 1-(1,2-Benzisothiazol-3-yl) and (1,2-Benzisoxazol-3-yl)Piperazine Derivatives as Potential Antipsychotic Agents. *J. Med. Chem.* **1986**, *29*, 359−369.

(7) Lowe, J. A., III; Seeger, T. F.; Nagel, A. A.; Howard, H. R.; Seymour, P. A.; Heym, J. H.; Ewing, F. E.; Newman, M. E.; Schmidt, A. W.; Furman, J. S.; Vincent, L. A.; Maloney, P. R.; Robinson, G. L.; Reynolds, L. S.; Vinick, F. J. 1-Naphthylpiperazine Derivatives as Potential Atypical Antipsychotic Agents. *J. Med. Chem.* **1991**, *34*, 1860−1864.

(8) Schmidt, A. W.; Seeger, T. F. Serotonin 5-Hydroxytryptamine-2 Potency as a Predictor of Antipsychotic Side Effect Liability. *Neurosci. Abstr.* **1986**, *12*, 479.

(9) Altar, C. A.; Wasley, A. M.; Neale, R. F.; Stone, G. A. Typical and Atypical Antipsychotic Occupancy of D2 and S2 Receptors: An Autoradiographic Analysis in Rat Brain. *Brain Res. Bull.* **1986**, *16*, 517.

(10) Meltzer, H. Y.; Matsubara, S.; Lee, J.-C. Classification of Typical and Atypical Antipsychotic Drugs on the Basis of Dopamine D-1 and D-2 and Serotonin$_2$ pK_i Values. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238−246.

(11) Bersani, G.; Grispini, A.; Marini, S.; Pasini, A.; Valducci, M.; Ciani, N. Neuroleptic Induced Extrapyramidal Side Effects: Clinical Perspectives with Ritanserin (R-55,667), a New Selective 5-HT_2 Receptor Blocking Agent. *Curr. Ther. Res.* **1986**, *40*, 492−499.

(12) Niemegeers, C. J.; Awouters, F.; Janssen, P. A. Serotonin Antagonism Involved in the Antipsychotic Effect. Confirmation With Ritanserin and Risperidone. *Encephale* **1990**, *16*, 147−151.

(13) Conn, P. J.; Sanders-Bush, E. Regulation of Serotonin Stimulated Phosphoinositide Hydrolysis: Relation to the Serotonin 5-HT_2 Binding Site. *J. Neurosci.* **1986**, *6*, 3669−3675.

(14) Molloy, B. B. Pharmaceutical compositions containing substituted 2-oxo-indolines and the use thereof to treat anxiety and tension. U.S. Patent 3,882,236; *Chem. Abstr.* **1975**, *83*, 79075y.

(15) Robertson, D. W.; Krushinski, H. J.; Beedle, E. E.; Wyss, V.; Pollock, G. D.; Wilson, H.; Kauffman, R. F.; Hayes, J. S. Dihydropyrazinone Cardiotonics: The Discovery and Inotropic Activity of 1,3-Dihydro-3,3-Dimethyl-5-(1,4,5,6-Tetrahydro-6-oxo-3-pyrazinyl)-2H-indol-2-one. *J. Med. Chem.* **1986**, *29*, 1832.

(16) Owellen, R. Some Reactions of Tetrahydrocarbazolechloroindolenine. *J. Org. Chem.* **1974**, *39*, 69.

(17) Seeger, T. F.; Seymour, P. A.; Schmidt, A. W.; Zorn, S. H.; Schulz, D. W.; Lebel, L. A.; McLean, S.; Guanowsky, V. L.; Howard, H. R.; Lowe, J. A., III; Heym, J. Ziprasidone (CP-88,059): A New Antipsychotic with Mixed D2 and 5-HT2 Receptor Antagonist Activity. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 101−113.

(18) Costall, B.; Naylor, R. J. Stereotyped and Circling Behavior Induced by Dopaminergic Agonists after Lesions of the Midbrain Raphe Nuclei. *Eur. J. Pharmacol.* **1974**, *29*, 206−222.

(19) Zorn, S. H.; Duman, R. S.; Krogsgaard-Larsen, P.; Micheletti, R.; Giraldo, E.; Giachetti, A.; Enna, S. J. (R)-Nipecotic Acid Ethyl Ester: a Direct Acting Cholinergic Agonist that Displays Greater Efficacy at M2 than at M1 Muscarinic Receptors. *J. Pharmacol. Exp. Ther.* **1987**, *242* (1), 173−178.

(20) Brown, E. B.; Kendall, D. A.; Nahorski, S. R. Inositol Phospholipid Hydrolysis in Rat Brain Cerebral Cortical Slices: I. Receptor Characterization. *J. Neurochem.* **1984**, *42*, 1379−1387.

JM950625L