Benzisoxazole- and Benzisothiazole-3-carboxamides as Potential Atypical Antipsychotic Agents

Nicholas J. Hrib,*,† John G. Jurcak,† Kendra L. Burgher,‡ Paul G. Conway,‡ Harold B. Hartman,‡ Lisa L. Kerman,‡ Joachim E. Roehr,‡ and Ann T. Woods‡

Neuroscience Strategic Business Unit, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey 08876

Received December 13, 1993®

A series of benzisoxazole- and benzisothiazole-3-carboxamides has been prepared and tested for potential antipsychotic activity. In general, the compounds showed an affinity for dopamine D_2 and serotonin $5HT_{2A}$ and $5HT_{1A}$ receptors. Several members of this series have demonstrated activity in animal models predictive of potential antipsychotic activity. In addition, compounds **18**, **19**, **22**, **27**, **28**, **43**, and **44** have also shown a potential for reduced EPS liability as suggested by the ratio of activity seen in mesolimbic-mediated vs nigrostriatal-mediated behavioral assays.

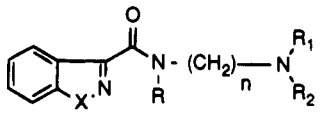
Introduction

All clinically-effective antipsychotic agents have been shown to inhibit postsynaptic dopaminergic neurotransmission.[1,2] However, most of these agents are typical, *i.e.*, show some propensity for the development of extrapyramidal side effects (EPS), either acutely (dystonia, pseudo-Parkinsonism) or on chronic administration (Tardive Dyskinesia).[3] Clozapine, an atypical antipsychotic, is almost totally devoid of EPS liability. However, a small percentage of clozapine patients are at risk for the development of agranulocytosis, a potentially fatal blood disorder. In the United States, treatment with clozapine is restricted to a small, closely-monitored population of treatment-refractory patients.[4]

Many theories have been advanced to explain the atypical pharmacological profile of clozapine. One hypothesis, that a combination of serotonin $5HT_{2A}$/dopamine D_2 receptor antagonism in a proper ratio is necessary for atypicality, has received much attention of late. Antipsychotic drugs which show a reduced propensity for the development of EPS have demonstrated a higher affinity for the $5HT_{2A}$ receptor than the D_2 receptor.[5] The ratio of activities at these receptors has been advanced as an explanation for the atypical profile of clozapine.[6] Clozapine also shows a higher affinity for the $5HT_{1A}$ receptor than for the D_2 receptor. The $5HT_{1A}$ receptor has also been implicated in the activity of atypical antipsychotic agents,[7] and $5HT_{1A}$ agonists 8-OH-DPAT, buspirone, and ipsaspirone have been found to reverse haloperidol-induced catalepsy,[8] and in fact a combination of $5HT_{1A}$ agonism and D_2 antagonism was the basis for the design of a series of potential atypical antipsychotic agents.[9]

With these considerations in mind, we prepared a series of benzisothiazole- and benzisoxazole-3-carboxamides of the general structure **1**. These compounds were designed to interact with serotonin $5HT_{1A}$ and $5HT_{2A}$ receptors and, to a lesser extent, with dopamine D_2 receptors. We began with the expectation that compounds which possessed an aryl- or aroylpiperidine or -piperazine moiety would demonstrate the dopamine D_2 antagonist activity required of an antipsychotic agent. We then needed to incorporate some structural feature of serotonin itself and felt that a 6,5-fused heterocyclic ring such as benzisoxazole or benzisothiazole would serve well as a mimic for the tryptamine indole ring. The carboxamide linkage would serve as a versatile functional system for generating a variety of structural analogues.

Many of these compounds have shown activity in an animal model predictive of potential antipsychotic activity. In addition, several members of this series have shown a potential for reduced extrapyramidal side effect liability. The synthesis of these compounds and details of their structure–activity relationships are presented below.



1a (X = S)
1b (X = O)

Chemistry

The synthesis of the target compounds **1a**,**b** (Table 1) proceeds through the intermediacy of the key benzisothiazole- and benzisoxazole-3-carboxylic acid chlorides **2a** (X = S) and **2b** (X = 0). These compounds are prepared by variations of literature procedures, as illustrated in Schemes 1 and 2.

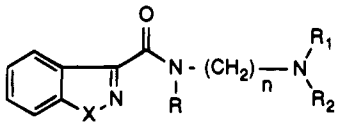
Preparation of the benzisothiazole-3-carboxylic acid chlorides is outlined in Scheme 1. Thiophenol is acylated with oxalyl chloride to provide the intermediate aid chloride **3**. This intermediate is isolated and, without further purification, treated with $AlCl_3$ in an intramolecular Friedel–Crafts reaction to give the thioisatin **4**, previously prepared by Papa, Schwenk, and Ginsburg.[10] Compound **4** is dissolved in aqueous ammonium hydroxide and treated dropwise with aqueous hydrogen peroxide to provide in one step the known 1,2-benzisothiazole-3-carboxamide **5**.[11] Saponification of **5** with aqueous hydroxide gives carboxylic acid **6a** which is heated with thionyl chloride to give acid chloride **2a**[11] (X = S) (Scheme 1).

Synthesis of the benzisoxazole moieties begins with nitrophenylacetic acid **7** which is esterified to provide **8**. Nitrosation of **8** with isoamyl nitrite provides the oxime ester **9**; this compound is cyclized upon treatment

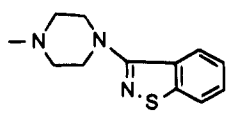
* Author to whom correspondance should be addressed.
† Department of Chemical Research.
‡ Department of Biological Research.
® Abstract published in *Advance ACS Abstracts*, June 15, 1994.

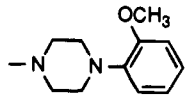
0022-2623/94/1837-2308$04.50/0

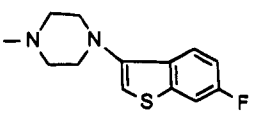
Table 1. Benzisoxazole- and Benzisothiazole-3-carboxamides

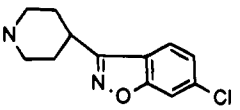


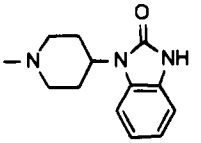
compd no.	X	R	*n*	NR^1R^2 [a]	formula[b]	mp, °C	method of preparation
17	S	H	2	BIT	$C_{21}H_{21}N_5OS_2$	160–163	A
18	S	H	4	BIT	$C_{23}H_{25}N_5OS_2 \cdot HCl \cdot 0.5H_2O$	203–205	B
19	S	CH_3	4	BIT	$C_{24}H_{27}N_5OS_2 \cdot HCl$	210–211	C
20	S	H	4	OMP	$C_{23}H_{28}N_4O_2S \cdot 2HCl$	175–178	B
21	S	CH_3	4	OMP	$C_{24}H_{30}N_4O_2S \cdot 2HCl \cdot 0.5H_2O$	169–171	C
22	S	H	2	OMP	$C_{21}H_{24}N_4O_2S \cdot 2HCl$	205–208	A
23	O	H	2	OMP	$C_{21}H_{24}N_4O_3$	107–110	A
24	S	CH_3	3	OMP	$C_{23}H_{28}N_4O_2S \cdot 2HCl$	166–169	C
25	S	H	3	OMP	$C_{22}H_{28}N_4O_2S \cdot 2HCl$	191–194	A
26	S	CH_3	4	FBT	$C_{25}H_{27}FN_4OS_2 \cdot HCl$	183–185	C
27	O	H	2	FBT	$C_{22}H_{21}FN_4O_2S \cdot HCl$	225–228	A
28	O	CH_3	4	FBT	$C_{25}H_{27}FN_4O_2S \cdot HCl$	145–147	C
29	S	H	3	CBI	$C_{23}H_{23}ClN_4O_2S \cdot HCl$	221–223	A
30	S	H	2	CBI	$C_{22}H_{21}ClN_4O_2S$	132–134	A
31	S	H	2	OBI	$C_{22}H_{23}N_5O_2S$	174–177	A
32	S	H	2	BNP	$C_{21}H_{24}N_4OS \cdot 2HCl \cdot 0.5H_2O$	207–210	A
33	S	H	2	MCP	$C_{20}H_{21}ClN_4OS$	115–117	A
34	S	H	2	MEP	$C_{15}H_{20}N_4OS \cdot 2HCl$	227–230	A
35	S	H	2	FBP	$C_{22}H_{22}FN_3O_2 \cdot HCl \cdot 0.5H_2O$	188–190	A
36	S	CH_3	4	FBP	$C_{25}H_{28}FN_3O_2S \cdot HCl$	148–150	C
37	O	$CH(CH_3)_2$	3	FBP	$C_{26}H_{30}FN_3O_3 \cdot HCl \cdot 0.5H_2O$	177–179	D
38	O	CH_3	3	FBP	$C_{24}H_{26}FN_3O_3$	85–87	C
39	O	CH_3	2	FBP	$C_{23}H_{24}FN_3O_3 \cdot HCl$	218–221	D
40	S	CH_3	3	FBP	$C_{24}H_{26}FN_3O_2S \cdot HCl$	189–191	C
41	S	CH_3	2	FBP	$C_{23}H_{24}FN_3O_2S \cdot HCl \cdot 0.5H_2O$	214–217	D
42	O	$CH(CH_3)_2$	2	FBI	$C_{25}H_{27}FN_4O_3$	118–120	D
43	S	CH_3	2	FBI	$C_{23}H_{28}N_4O_2S \cdot HCl \cdot 0.5H_2O$	211–214	D
44	O	CH_3	2	FBI	$C_{23}H_{23}FN_4O_3$	94–97	D
45	S	CH_3	4	FBI	$C_{25}H_{27}FN_4O_2S \cdot HCl$	204–205	C

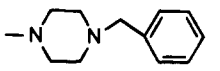
BIT = 

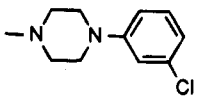
OMP = 

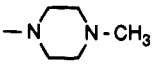
FBT = 

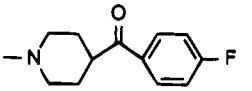
CBI = 

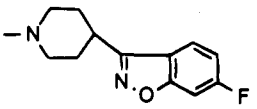
OBI = 

BNP = 

MCP = 

MEP = 

FBP = 

FBI = 

[a] All compounds gave satisfactory elemental analyses (±0.4%) for C, H, and N.

with base to yield 1,2-benzisoxazole-3-carboxylic acid ester **10b**. Hydrolysis of **10b** with strong acid provides the known carboxylic acid **11b**,[12] which on heating with thionyl chloride provides acid chloride **2b** (X = O) (Scheme 2).

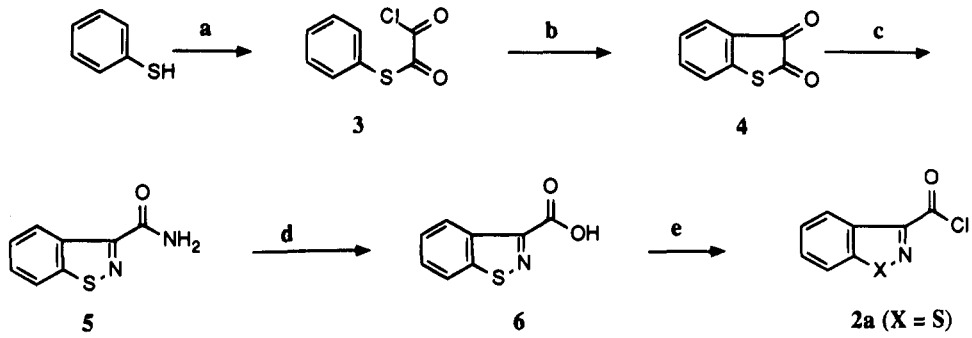
The target compounds **1a,b** were prepared from intermediates **2a,b** by one of the four general methods illustrated in Scheme 3. Some targets were prepared by utilizing the procedure described by Amoretti *et al.*,[11] namely by treatment of acid chlorides **2a,b** with primary (haloalkyl)amines to provide (haloalkyl)amides **12a,b**. Alkylation of **12a,b** with various secondary amines provides secondary amide targets **1a,b** (Method A).

Other targets were prepared by direct treatment of acid chlorides **2a,b** with primary alkylamines **13** to give targets **1a,b** (method B).

A number of tertiary amide targets were prepared via a procedure which began with treatment of acid chlorides **2a,b** with simple alkylamines to give secondary amides **14a,b**. Formation of the amide anions with base followed by alkylation with dihaloalkanes provides the (haloalkyl)amides **15a,b**. Alkylation with various amides provides tertiary amide targets **1a,b** (method C).

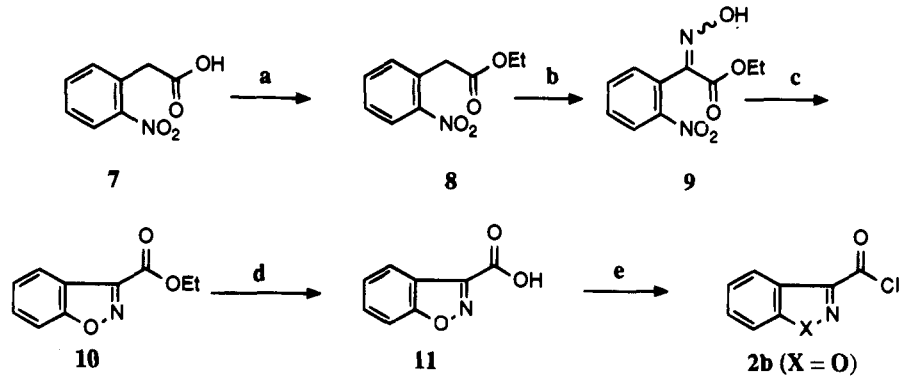
Finally, some tertiary amide targets could not be obtained by alkylation of secondary amides as in method C. These were obtained by acylation of the appropriate

Scheme 1. Synthesis of Benzisothiazole-3-carboxylic Acid Chlorides (**2a**, X = S)[a]



[a] Reagents: (a) oxalyl chloride, CH_2Cl_2; (b) $AlCl_3$, CH_2Cl_2; (c) NH_4OH, aqueous H_2O_2; (d) aqueous NaOH; (e) $SOCl_2$.

Scheme 2. Synthesis of Benzisoxazole-3-carboxylic Acid Chlorides (**2b**, X = O)[a]



[a] Reagents: (a) EtOH, H_2SO_4, toluene reflux; (b) isoamyl nitrite, NaOEt, EtOH; (c) NaH, diglyme; (d) 70% H_2SO_4; (e) $SOCl_2$.

amino alcohols to provide (hydroxyalkyl)amides **16a,b**. These compounds were converted to the mesylates *in situ* by treatment with methanesulfonyl chloride; displacement of the methanesulfonyl group with secondary amines provided tertiary amide targets **1a,b** (method D).

Results and Discussion

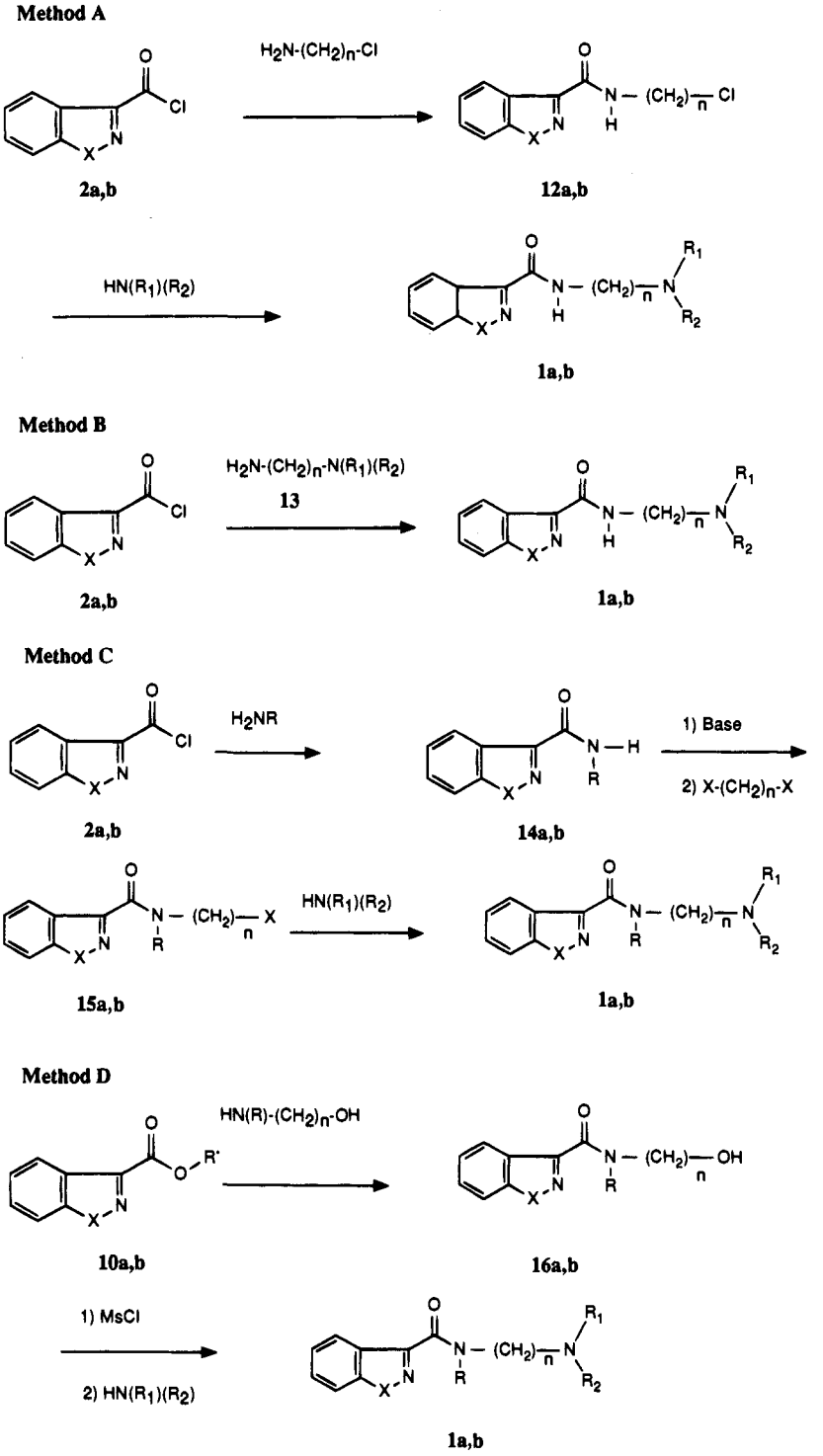
All compounds in this series were evaluated *in vitro* for affinity at the serotonin $5HT_{1A}$, $5HT_{2A}$, dopamine D_2, and α_1 receptors (Table 2). Concurrently, targets were screened for potential antipsychotic activity in a behavioral model, the inhibition of apomorphine-induced climbing in mice (CMA)[13,14] (Table 3). This behavior is mediated by the limbic dopaminergic pathway, which has been associated with the therapeutic effects of antipsychotic agents. Compounds which proved most potent in this assay were then examined for their ability to inhibit apomorphine-induced stereotypy in rats (APO-S).[15] This is a single-dose assay which has been recognized as a rapid and efficient method for detecting potential EPS liability. The potential for extrapyramidal side effects has been linked to activation of the nitrostriatal dopamine system.[16] The inhibition of apomorphine-induced stereotypy in rats is a behavioral assay mediated by this dopaminergic pathway.[17] Therefore, an agent which demonstrates potential atypical antipsychotic activity should be active in the climbing-mouse assay (mediated by the limbic dopaminergic pathway) and weak or inactive in inhibiting agonist-induced rat stereotypy (Table 4). Indeed, while haloperidol is potently active in both these assays, clozapine displays only weak activity in the stereotypy model even at high doses.

Structure–Activity Considerations. The majority of targets in this series show greater *in vitro* affinity for the dopamine D_2 receptor than clozapine. Compound **45**, which showed the highest D_2 affinity (72.6 nM) was a member of the subgroup of compounds which showed the greatest potency *in vivo*. Conversely, those compounds with extremely weak D_2 affinity were only weakly active in the CMA assay.

Like clozapine, many members of the series possessed higher affinity for the serotonin $5HT_{2A}$ and/or $5HT_{1A}$ receptors than for the D_2 receptor. A ratio of $D_2/5HT_{2A}$ affinity greater than 1 has been correlated with reduced liability for EPS in a series of antipsychotic agents.[5] Of the compounds with reasonable dopamine D_2 affinity, ratios of $D_2/5HT_{2A}$ affinity were highest with compounds **43** and **44**. The substitution of a benzisothiazolecarboxamide for a benzisoxazolecarboxamide did not drastically affect the *in vitro* affinities of direct analogues except in one case, namely compounds **38** *vs* **40**, where the change reduced dopamine D_2 receptor affinity 3-fold, with a concomitant reduction in *in vivo* activity.

Most of the compounds also showed potent affinity for the α_1 receptor. There have appeared in the literature reports linking a combination of dopamine D_2 and α_1 receptor antagonism to potential atypical antipsychotic activity; for example the concurrent administration of haloperidol with prazosin has been reported to produce electrophysiological effects on dopamine neuron subpopulations identical to those observed with repeated clozapine administration alone.[18] However, in this series of compounds the observed potential for atypicality was found to be more consistent with affinity for $5HT_{1A}$ or $5HT_{2A}$ receptors along with dopamine D_2 affinity. Since the compounds do possess potent α_1

Scheme 3. Synthesis of Target Compounds



receptor affinity, they will be evaluated for cardiovascular liability. The lead compounds tested to date, **18**, **19**, and **22**, show no toxicity in rats at doses up to 80 mg/kg ip.

For good *in vivo* activity in this series, the presence of an aroyl or aryl moiety directly bonded to position 4 of the substituent piperidine or piperazine was required. However, three exceptions to this observation were chlorinated compounds **29**, **30**, and **33**. The binding of **33** to the D_2 receptor was exceptionally weak and could acccount for the reduced activity. However, D_2 binding of **29** and **30** is almost identical to that of compound **40**, which remains potent in the CMA. In comparing direct analogues, a two- or four-carbon chain length was found to be slightly superior to a three-carbon chain, *cf.* compounds **20** and **22** *vs* **25**, and **21** *vs* **24**.

By far the most critical factor to the activity in this series was the choice of substituent on the piperidine or piperazine ring. Compounds with substituted phenyl moieties (compounds **20**–**25** and **33**) as a class were less potent than those substituted with aroyl systems (compounds **35**–**40**); these in turn were generally less potent than targets with a bicyclic heteroaryl substituent. The greatest potency in the CMA was observed with com-

Table 2. *In Vitro* Activity

compd no.	displacement of ligand binding (IC_{50}, μM)			
	D_2 receptor[a]	$5HT_{2A}$ receptor[b]	$5HT_{1A}$ receptor[c]	α_1 receptor[d]
17	0.169 (0.069–0.415)	0.475 (0.137–1.64)	0.098 (0.048–0.2)	0.019 (0.016–0.023)
18	0.36 (0.195–0.656)	0.201 (0.107–0.378)	0.018 (0.013–0.024)	0.005 (0.0021–0.0106)
19	0.208 (0.16–0.27)	0.013 (0.01–0.173)	0.0186 (0.0147–0.0234)	0.0058 (0.0012–0.0288)
20	0.271 (0.160–0.4598)	0.724 (0.0826–6.34)	0.0023 (0.0018–0.0031)	0.0064 (0.0055–0.0074)
21	0.136 (0.1056–0.1746)	0.125 (0.037–0.421)	0.004 (0.0033–0.0057)	0.0033 (0.0014–0.0079)
22	0.283 (0.225–0.356)	0.55 (0.183–1.658)	0.0039 (0.0019–0.0081)	0.0041 (0.0012–0.014)
23	0.473 (0.362–0.617)	0.391 (0.059–2.57)	0.021 (0.0157–0.0284)	0.0021 (0.0005–0.0081)
24	0.541 (0.409–0.718)	1.65 (0.797–3.41)	0.149 (0.113–0.198)	0.0107 (0.0086–0.0134)
25	0.193 (0.105–0.357)	3.11 (0.562–17.2)	0.0847 (0.0609–0.118)	0.0183 (0.0141–0.0238)
26	0.671 (0.369–1.22)	0.232 (0.0469–1.15)	0.3662 (0.1834–0.7312)	0.0063 (0.0041–0.0097)
27	1.3687 (0.7091–2.642)	0.3089 (0.09–1.06)	1.0376 (0.4265–2.5244)	>10
28	0.9259 (0.7155–1.1984)	0.1879 (0.0777–0.454)	0.1278 (0.101–0.161)	0.0032 (0.00097–0.0107)
29	0.7391 (0.2082–2.6239)	0.115 (0.055–0.241)	3.7153 (3.005–4.5935)	0.0938 (0.0587–0.1498)
30	0.7116 (0.376–1.346)	0.215 (0.073–0.6298)	3.4521 (2.8288–4.2126)	0.239 (0.034–1.68)
31	5.589 (2.021–15.45)	0.641 (0.136–3.02)	4.7336 (3.6167–6.1955)	0.0398 (0.0309–0.0511)
32	>10	>10	1.8776 (1.4128–2.4954)	2.57 (1.76–3.76)
33	3.191 (0.8779–11.59)	0.726 (0.264–1.99)	0.0516 (0.0406–0.0722)	0.0815 (0.0297–0.223)
34	>10	>10	>10	>10
35	0.2856 (0.1273–0.641)	0.017 (0.013–0.023)	0.92 (0.7185–1.1781)	0.0157 (0.0081–0.0304)
36	0.2456 (0.1002–0.6018)	0.0302 (0.0223–0.041)	0.3389 (0.1726–0.666)	0.0032 (0.001–0.0102)
37	0.8042 (0.6172–1.0478)	0.29 (0.0717–1.17)	2.3 (0.86–6.3)	0.124 (0.0953–0.162)
38	2.3779 (1.9077–2.9641)	0.1234 (0.0963–0.158)	6.65 (3.06–14.4)	0.043 (0.0314–0.0584)
39	0.584 (0.3524–0.9679)	0.027 (0.02–0.037)	1.79 (0.549–5.84)	0.1082 (0.0851–0.1376)
40	0.7166 (0.5347–0.9603)	0.068 (0.052–0.09)	4.67 (3.11–7.01)	0.00611 (0.002–0.0183)
41	0.5489 (0.1237–2.4349)	0.051 (0.383–0.0676)	2.5 (1.9–3.2)	0.0753 (0.0572–0.099)
42	0.2034 (0.1137–0.3641)	0.0297 (0.225–0.039)	0.21 (0.16–0.27)	0.0055 (0.0026–0.0116)
43	0.264 (0.19–0.3668)	0.0328(0.00487–0.22)	0.82 (0.42–1.6)	0.0094 (0.0095–0.0194)
44	0.197 (0.147–0.264)	0.0176 (0.013–0.024)	0.44 (0.35–0.56)	0.104 (0.0326–0.333)
45	0.0726 (0.0567–0.093)	0.013 (0.0096–0.0165)	0.1596 (0.1214–0.2099)	0.174 (0.127–0.237)
cloz	1.61 (1.24–2.07)	0.072 (0.055–0.0944)	1.0155 (0.7418–1.3901)	0.032 (0.025–0.042)
hal	0.0327 (0.0236–0.0454)	0.129 (0.07–0.239)	7.079 (4.94–10.113)	0.0825 (0.0769–0.089)
risp	0.037 (0.024–0.047)	0.0026 (0.0019–0.004)	0.95 (0.75–1.22)	0.0029 (0.0016–0.0052)

[a] Versus [^{3}H]spiroperidol in striatum. [b] Versus [^{3}H]spiroperidol in cortex. [c] Versus [^{3}H]-8-OH-DPAT in hippocampus. [d] Versus [^{3}H]WB-4101 in whole brain minus cerebellum.

Table 3. *In Vivo* Actvitiy: Inhibition of apomorphine-induced climbing (mouse)

compd no.	ED_{50} mg/kg ip [po][a]	compd no.	ED_{50} mg/kg ip [po]
17	>20[b]	**32**	>20
18	8.3 (7.6–9.0)	**33**	>20
19	2.49 (2.05–3.01)	**34**	>20
20	7.24 (6.7–67.8)		
21	9.61 (9.19–10.06)	**35**	3.64 (3.21–4.18)
22	6.3 (4.7–9.0) [28.7 (26.5–31.2)])	**36**	8.2 (7.8–8.7)
23	>20	**37**	20.2 (16.9–24.2)
24	>20	**38**	>20
25	13.0 (12.05–14.04)	**39**	13.4 (12.6–14.3)
26	4.5 (4.28–4.73) [19.3 (17.5–21.1)]		
27	4.9 (4.4–5.6) [26.4 (23.5–30.3)]	**40**	7.7 (3.3–18.0)
28	5.0 (4.7–5.4) [21.6 (19.9–23.9)]	41	8.0 (3.7–17.0)
29	>20	**42**	1.3 (0.63–2.90)
30	>20	**43**	1.7 (1.5–1.9)
31	>20	**44**	1.3 (0.98–1.60)
		45	1.5 (1.30–1.60)
clozapine	9.10 ± 1.77 (6) [23.2 (21.1–25.9)]		
haloperidol	0.194 ± 0.056 (2) [0.28 (0.27–0.29)]		
risperidone	0.062 (0.047–0.077) [0.28 (0.25–0.30)]		

[a] Values are mean ED_{50} ± SEM (n) or ED_{50} and 95% confidence limits. [b] ED_{50} not determined but greater than screening dose of 20 mg/kg ip.

Table 4. Potential for Atypical Antipsychotic Activity *in vivo*.

compd no.	ED_{50}, mg/kg, or % inhibition at dose administered ip	
	inhibition of apomorphine-induced climbing (mouse)	inhibition of apomorphine-induced stereotypy (rat)
18	8.3 (7.6–9.0)[a]	17% at 40
19	2.49 (2.05–3.01)	39.2 (32.1–47.8)
20	7.24 (6.7–67.8)	83% at 40
21	9.61 (9.19–10.06)	100% at 40
22	6.3 (4.7–9.0)	51.8 (41.7–64.3)
25	13.0 (12.05–14.04)	17% at 40
26	4.5 (4.28–4.73)	33.0 (27.4–39.9)
27	4.9 (4.4–5.6)	17% at 40
28	5.0 (4.7–5.4)	52.0 (39.8–67.8)
35	3.64 (3.21–4.18)	25.7 (19.9–33.1)
36	8.2 (7.8–8.7)	60% at 40
43	1.7 (1.5–1.9)	50% at 13
44	1.3 (0.98–1.60)	33% at 13
45	1.5 (1.30–1.60)	67% at 10
clozapine	9.10 ± 1.77 (6)	33% at 40
haloperidol	0.194 ± 0.056 (2)	0.576 ± 0.148 (2)
risperidone	0.06 (0.047–0.077)	3.2 (2.1–4.8)

[a] Values are mean ED_{50} ± SEM (n), ED_{50} and 95% confidence limits, or percent inhibition at dose.

pounds bearing a (6-fluorobenzisoxazol-3-yl)piperidine moiety, *i.e.*, compounds **42**, **43**, **44**, and **45**. Compound **45** in fact showed a 10-fold greater affinity for the D_2 receptor and a 3-fold increase in potency in the CMA assay over its benzo[*b*]thiophene analogue, compound **26**.

With regard to the potential for atypicality, good-to-excellent ratios of CMA/APO-S activity (and thus potential for atypicality) were found for compounds **18**, **19**, **22**, **27**, **28**, **43**, and **44**. These represent a diverse set of chemical entities which vary in chain length, amide substituent, and aryl-piperidine or -piperazine moiety.

Therefore, the structural requirements for potential atypical antipsychotic activity in this series are not obvious. Interestingly, of the compounds demonstrating potential atypicality, all showed a higher affinity for the $5HT_{2A}$ or the $5HT_{1A}$ receptor, or both, than for the D_2 receptor. This observation would appear to add support to the involvement of serotonin in the mechanism of action of atypical antipsychotics.

Conclusions

A series of benzisothiazole- and benzisoxazole-3-carboxamides has been prepared and submitted for biological evaluation. Many members of this series displayed a higher affinity for serotonin $5HT_{2A}$ and/or $5HT_{1A}$ receptors than for dopamine D_2 receptors, a biochemical profile consistent with potential atypical antipsychotic activity. *In vivo*, a number of target compounds showed potential antipsychotic activity in an animal model, namely the inhibition of apomorphine-induced mouse climbing.

In addition, some compounds (**18**, **19**, **22**, **27**, **28**, **43**, and **44**) also displayed a potential for atypicality in animal models *in vivo*. These compounds were more active in behavioral assays mediated by the limbic dopaminergic system (inhibition of apomorphine-induced mouse climbing) than in assays mediated by the nigrostriatal dopaminergic system (inhibition of apomorphine-induced stereotypy in rats). Further preclinical evaluation of the leading compounds in this series, including evaluation in chronic-dosing regimens, is in progress.

Experimental Section

All structures are supported by their IR (Perkin-Elmer 547) and ^1H NMR (Varian XL-200) spectra. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Mass spectra were determined on a Finnigan 4000 GC–MS equipped with an INCOS data system. Elemental analyses were performed by Oneida Research Services, Inc., Whitesboro NY, or Robertson Microlit Laboratories, Inc., Madison, NJ.

The following examples are illustrative of the methods used to prepare the target compounds.

Method A: ***N*-[2-[1-(2-Methoxyphenyl)-4-piperazinyl]-ethyl]-1,2-benzisothiazole-3-carboxamide Dihydrochloride (22).** A mixture of 1,2-benzisothiazole-3-[*N*-(2-chloroethyl)-carboxamide] (**15a**, **R** = **H**, **X** = **Cl**, ***n*** = 2) (2.24 g, 9.3 mmol), prepared according to the method of Amoretti *et al.*,[11] and 1-(*o*-methoxyphenyl)piperazine (1.8 g, 9.3 mmol) in 100 mL of dry 1-methyl-2-pyrrolidinone was heated with stirring to 120 °C under N_2. After 24 h the mixture was cooled to room temperature and poured into saturated aqueous Na_2CO_3. This aqueous phase was extracted with Et_2O, and the combined organic extracts were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica using Et_2O as eluent. The fractions containing desired product were combined and concentrated, and the residual oil was taken up in Et_2O. The bis-HCl salt of the product was precipitated by the addition of HCl in Et_2O and recrystallized from Et_2O/CH_2Cl_2 to provide 1.3 g (2.8 mmol, 31%), mp 205–208 °C, homogeneous by TLC (silica, EtOAc, R_f = 0.58). The IR ($CHCl_3$), NMR ($CDCl_3$), and mass spectrum (M^+ = 423, EI, 70 eV) were in agreement with the structure. ^1H NMR (200 MHz, $CDCl_3$): 3.40–3.62 (m, 4 H), 3.64–3.98 (m, 4 H), 3.98 (s, 3 H), 4.12 (dd, J = 5.6 and 11.2, 2 H), 4.42 (br s, 2 H), 6.98 (d, J = 8.3, 1 H), 7.02 (d, J = 7.0 Hz, 1 H), 7.27 (m, 2 H), 7.50–7.68 (m, 3 H), 7.98 (d, J = 8.6 Hz, 1 H), 8.63 (m, 1 H), 8.90 (d, J = 8.6 Hz, 1 H), 13.35 (br s, 1 H).

Method B: ***N*-[4-[1-(1,2-Benzisothiazol-3-yl)-4-piperazinyl]butyl]-1,2-benzisothiazole-3-carboxamide Hydrochloride Hemihydrate (18).** A mixture of 1,2-benzisothiazole-3-carboxylic acid chloride (2.6 g, 13.2 mmol), 1-(1,2-benzisothiazol-3-yl)-4-(4-aminobutyl)piperazine (3.45 g, 11.9 mmol), and triethylamine (5 mL, 35.9 mmol) in 100 mL of sieve-dried toluene was heated to 80 °C with stirring overnight. After 24 h the mixture was cooled to room temperature and added to water. The organic phase was drawn off, and the aqueous phase was extracted with EtOAc. The EtOAc extracts and the toluene phase were combined and dried over $MgSO_4$. The organic phase was then filtered and concentrated *in vacuo* and the residue chromatographed on silica using EtOAc eluent. The fractions containing the desired product were combined and concentrated to provide an oil which was taken up in Et_2O. The HCl salt of this amine was precipitated by the addition of ethereal HCl, recrystallized from CH_2Cl_2/Et_2O, and dried (0.1 mmHg, refluxing iPrOH temperature) to provide the product as a hemihydrate, mp 203–205 °C, homogeneous by TLC (silica, 10:90 CH_3OH:EtOAc, R_f = 0.55). The IR ($CHCl_3$), NMR ($CDCl_3$), and mass spectrum (M^+ = 451, EI, 70 eV) were consistent with the structure. The yield was 1.6 g (3.2 mmol, 27%). ^1H NMR (200 MHz, $CDCl_3$): 1.80 (m, 2 H), 2.04 (m, 2 H), 3.18 (m, 4 H), 3.56 (m, 4 H), 4.10 (m, 4 H), 7.30–7.62 (m, 4 H), 7.70 (t, J = 6.3 Hz, 1 H), 7.82 (m, 2 H), 7.98 (d, J = 7.9 Hz, 1 H), 8.92 (d, J = 8.1 Hz, 1 H), 12.9 (br s, 2 H).

Method C: ***N*-Methyl-*N*-(4-bromobutyl)-1,2-benzisoxazole-3-carboxamide (15b1, R = CH₃, X = Br, *n* = 4).** To a suspension of sodium hydride (1.09 g, 60% dispersion in oil) and dimethylformamide (10 mL), cooled in an ice bath, was added a solution of *N*-methyl-1,2-benzisoxazole-3-carboxamide (prepared analogously to the benzisothiazolecarboxamides described previously)[11] (4.38 g) and dimethylformamide (10 mL) with stirring at a rate such as to maintain gentle evolution of hydrogen. After the addition was complete, the reaction mixture was stirred at ice bath temperature for 10 min. The ice bath was removed, and the mixture was added dropwise to a solution of 1,4-dibromobutane (7.8 mL) and dimethylformamide (10 mL) with stirring. This mixture was stirred at ambient temperature overnight; water (250 mL) was then added. The mixture was extracted with ether, and the combined extracts were washed with water and saturated sodium chloride, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated, and the residue was chromatographed on silica gel using 5% ethyl acetate:dichloromethane eluent. The appropriate fractions were collected and evaporated to give 4.28 g (55%) of *N*-methyl-*N*-(4-bromobutyl)-1,2-benzisoxazole-3-carboxamide as an oil. ^1H NMR (200 MHz, $CDCl_3$, mixture of amide rotamers): δ 1.73–2.1 (m, 4H), 3.22 (s, 1.5H), 3.33 (s, 1.5H), 3.35–3.46 (m, 1H), 3.52 (t, J = 6.7 Hz, 1H), 3.60–3.80 (m, 2H), 7.33–7.47 (m, 1H), 7.53–7.72 (m, 2H), 8.00 (d, J = 8.7 Hz, 1H).

N*-Methyl-*N*-[4-(1-(6-fluorobenzo[*b*]thiophene-3-yl)-4-piperazinyl)-butyl]-1,2-benzisoxazole-3-carboxamide Hydrochloride (28).** A mixture of *N*-methyl-*N*-(4-bromobutyl)-1,2-benzisoxazole-3-carboxamide (**15b1**, **R** = **CH₃**, **X** = **Br**, ***n = 4) (4.22 g, 0.0136 mol), 1-(6-fluorobenzo[*b*]thiophene-3-yl)-piperazine (3.89 g, 0.0165 mol), potassium carbonate (5.00 g, 0.0362 mol), sodium iodide (0.80 g), and acetonitrile (200 mL) was heated at 75 °C for 17 h under nitrogen. The reaction was filtered, the insolubles were washed with dichloromethane, and the filtrate was concentrated under reduced pressure. The residue was taken up in dichloromethane, washed with 5% sodium hydroxide (100 mL) and water (100 mL), and dried (sodium sulfate), and the solvent was evaporated under reduced pressure. The crude product was purified via chromatography on silica gel, using 7.5% methanol in ethyl acetate as eluant, to give 3.58 g of a viscous liquid (R_f = 0.43, 7.5% methanol in ethyl acetate, silica gel). The hydrochloride salt of the amine was prepared. Recrystallization from ethanol/ethyl acetate gave 2.1 g (31%) of a beige powder, mp 145–147 °C. The IR ($CHCl_3$), NMR ($CDCl_3$, 200 MHz), and mass spectrum (M^+ = 466, EI, 70 eV) were consistent for the assigned structure. ^1H NMR (200 MHz, $CDCl_3$; mixture of amide rotamers): δ 1.80–2.76 (m, 4H), 3.00–3.83 (m, 15 H),

6.76 (s, 1 H), 7.06–7.23 (m, 1 H), 7.37–7.73 (m, 5 H), 7.93–8.06 (m, 1 H), 12.9 (br s, 1 H).

Method D: *N*-(1-Methylethyl)-*N*-(2-hydroxyethyl)-1,2-benzisoxazole-3-carboxamide (16b1, R = iPr, *n* = 2). A mixture of ethyl 1,2-benzisoxazol-3-carboxylate (10 g, 0.052 mol), 2-[(1-methylethyl)amino]ethanol (16.1 g, 0.16 mol), and toluene (80 mL) was heated to 140 °C in a stainless steel bomb for 4 h. The resulting solution was diluted with ether (50 mL), washed with 5% $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL), dried (sodium sulfate), and concentrated under reduced pressure. The resulting brown liquid was chromatographed on silica gel (elution with 60% ethyl acetate in hexanes) to give 10.6 g (81%) of an off-white solid (R_f = 0.37, silica gel, 75% ethyl acetate in hexanes). Recrystallization of the solid from dichloromethane/hexanes afforded white crystals, mp 92–94 °C. The IR ($CHCl_3$), ^1H NMR ($CDCl_3$, 200 MHz), and MS (M + 1 = 249, CI, 70 eV) were consistent for the assigned structure. ^1H NMR (200 MHz, $CDCl_3$; mixture of amide rotamers, ratio 3.2:1): δ 1.26 and 1.38 (2 d, *J* = 6.7 Hz, 6 H), 2.96 (t, 5.3 Hz, O*H*), 3.60–4.00 (m, 4H), 4.51–4.81 (m, 1H), 7.31–7.49 (m, 1H), 7.53–7.70 (m, 2H), 7.93 and 8.00 (2 d, *J* = 7.3 Hz, 1H).

***N*-(1-Methylethyl)-*N*-[2-[1-(6-fluoro-1,2-benzisoxazol-3-yl)-4-piperadinyl]ethyl]-1,2-benzisoxazole-3-carboxamide (42).** Under a nitrogen atmosphere methanesulfonyl chloride (3.7 g, 0.032 mol) was rapidly added to a 0 °C solution of *N*-(1-methylethyl)-*N*-(2-hydroxyethyl)-1,2-benzisoxazole-3-carboxamide (**16b1, R = iPr, *n* = 2**) (8.00 g, 0.0322 mol), triethylamine (3.27 g, 0.32 mol), and tetrahydrofuran (150 mL), and the resulting mixture was stirred at 0 °C for 30 min. To the mixture was added a suspension of 4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidine (7.8 g, 0.035 mol), triethylamine (6.5 g, 0.065 mol), and tetrahydrofuran (60 mL) rapidly, and the mixture was heated at reflux for 12 h. Water was added to dissolve the insolubles and the solution concentrated under reduced pressure. The residue was taken up in dichloromethane (150 mL), washed with 10% NaOH (100 mL) and water (100 mL), dried (sodium sulfate), and concentrated under reduced pressure. The residue was chromatographed on silica gel (elution with 80% ethyl acetate in hexanes) to give 5.92 g of a beige solid. Recrystallization of the solid from ether afforded 2.3 g (16%) of fine white needles, mp 118–120 °C (R_f = 0.33, ethyl acetate, silica gel). The IR ($CHCl_3$), ^1H NMR ($CDCl_3$, 200 MHz), and MS (M^+ = 450, EI, 70 eV) were consistent with the assigned structure. ^1H NMR (200 MHz, $CDCl_3$; mixture of amide rotamers): δ 1.28 and 1.40 (2 d, *J* = 7.4 Hz, 6 H), 1.73–3.33 (m, 11 H), 3.66–3.83 (m, 2H), 4.58 and 4.77 (2 m, 1 H), 6.93–7.13 (m, 1 H), 7.16–7.30 (m, 1 H), 7.33–7.46 (m, 1 H), 7.53–7.77 (m, 3 H), 7.87–8.03 (m, 1H).

***In Vitro* Studies.** Receptor binding assays were performed according to previously reported procedures.[19]

Apomorphine-Induced Climbing in Mice. This method is a modification of Protais *et al.*[13] and Costall *et al.*[14] Male CD-1 mice (18–30 g) were individually placed in wire-mesh stick cages (4 × 4 × 10 in.) and were allowed 1 h for adaptation. Animals (8/dose group) received either distilled water or test drugs ip 30 or 60 min prior to apomorphine challenge (1.5 mg/kg sc). Animals were then observed for climbing behavior for 30 min. ED_{50} values were calculated by linear regression analysis.

Apomorphine-Induced Stereotypy in Rats. The procedure is a modification of Janssen *et al.*[15] Male Wistar rats (150–250 g) were dosed ip with distilled water or test compounds (6–10/dose group). After 50 min, apomorphine (1.5 mg/kg sc) was administered and the rats were placed in individual opaque plastic cages (40 × 22 × 18 cm). After 10 min, the rats were observed for the presence of continuous stereotyped licking or sniffing behavior.

Acknowledgment. We wish to thank Anastasia Linville of the Physical Methods Department for spectroscopic data and Toni Ann Wettlaufer, Roy Corbett, and Sharon Hardiman for additional biological assays. We also wish to thank Hoechst-Roussel Scholars Crystal Williams and Alrich Gray of Howard University for preparing two of the target compounds.

References

(1) Marder, S. R.; Wirshing, W. C.; Van Putten, T. Drug treatment of schizophrenia-overview of recent research. *Schizophrenia Res.* **1991**, *4*, 81–90.
(2) Davis, K. L.; Kahn, R. S.; Ko, G.; Davidson, M. Dopamine in Schizophrenia: A Review and Reconceptualization. *Am. J. Psychiatry* **1991**, *148*, 1474–1486.
(3) Remington, G. Pharmacotherapy of Schizophrenia. *Can. J. Psychiatry* **1989**, *34*, 211–220.
(4) Fitton, A.; Heel, R. C. Clozapine A Review of its Pharmacological Properties, and Therapeutic Use in Schizophrenia. *Drugs* **1990**, *40*, 722.
(5) Meltzer, H. Y.; Matsubara, S.; Lee, J.-C. Classification of Typical and Atypical Antipsychotic Drugs on the basis of Dopamine D-1, D-2, and serotonin$_2$ pK_i values. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238–246.
(6) Meltzer, H. Y.; Bastani, B.; Ramirez, L.; Matsubara, S. Clozapine: New Research on Efficacy and Mechanism of Action. *Eur. Arch. Psychiatr. Neurol. Sci.* **1989**, *238*, 332–339.
(7) Ahlenius, S.; Antipsychotic-like Properties of the 5HT$_{1A}$ Agonist 8-OH-DPAT in the Rat. *Pharmacol. Toxicol.* **1989**, *64*, 3–5.
(8) Hicks, P. B. The Effect of Serotonergic Agents on Haloperidol-induced Catalepsy. *Life Sci.* **1990**, *47*, 1609–1615.
(9) 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*, 1860–1866.
(10) Papa, D.; Schwenk, E.; Ginsburg, H. F. Reductions with Nickel-Aluminum Alloy and Aqueous Alkali. Part VII. Hydrogenolysis of Sulfur Compounds. *J. Org. Chem.* **1949**, *14*, 723–731.
(11) Amoretti, L.; Catellani, P. L.; Impicciatore, M.; Cavaggioni, A. Proprieta Biologiche di Composti 1,2-Benzisotiazolici. (Biological Properties of 1,2-Benzisothiazole Compounds.) *Il Farmaco-Ed. Sci.* **1972**, *27*, 855–869.
(12) Kemp, D. S.; Paul, K. G.; The Physical Organic Chemistry of Benzisoxazoles. III. The Mechanism and the Effects of Solvents on Rates of Decarboxylation of Benzisoxazole-3-carboxylic acids. *J. Am. Chem. Soc.* **1975**, *97*, 7305–7312.
(13) Costall, B.; Naylor, R. J.; Nohira, V. Climbing behavior induced by apomorphine in mice: a potent model for the detection of neuroleptic activity. *Eur. J. Pharmacol.* **1978**, *50*, 39.
(14) Protais, P.; Constentin, J.; Schwartz, J. Climbing Behavior Induced by Apomorphine in Mice: A Simple Test for the Study of Dopamine Receptors in Striatum. *Psychopharmacology* **1976**, *50*, 1–6.
(15) Janssen, P. A. J.; Niemegeers, C. J. C.; Jageneau, A. H. M. Apomorphine-antagonism in Rats. *Arzneim-Forsch.* **1960**, *10*, 1003–1005.
(16) Lowe, J. A., III; Seeger, T. F.; Vinick, F. J.; Atypical Antipsychotics-Recent Findings and New Perspectives. *Med. Res. Rev.* **1988**, *8*, 475–497.
(17) Moore, N. C.; Gershon, S. Which Atypical Antipsychotics are Identified by Screening Tests? *Clin. Neuropharmacol.* **1989**, *12*, 167–184.
(18) Chiodo, L. A.; Bunney, B. S. Possible Mechanisms by which Repeated Clozapine Administration Differentially Affects the Activity of Two Subpopulations of Midbrain Dopamine Neurons. *J. Neurosci.* **1985**, *5*, 2539–2544.
(19) Huger, F. P.; Smith, C. P.; Chiang, Y.; Glamkowski, E. G.; Ellis, D. B. Pharmacological Evaluation of HP 370, A Potential Atypical Antipsychotic Agent: 2. In Vitro Profile. *Drug Dev. Res.* **1987**, *11*, 169.
(20) Presented in part in poster format at the 203rd American Chemical Society National Meeting, San Francisco, CA, April 1992; Abstract MEDI 92.