Structure−Activity Relationships of a Series of Novel (Piperazinylbutyl)thiazolidinone Antipsychotic Agents Related to 3-[4-[4-(6-Fluorobenzo[*b*]thien-3-yl)-1-piperazinyl]butyl]-2,5,5-trimethyl-4-thiazolidinone Maleate

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HP-236 (3-[4-[4-(6-Fluorobenzo[*b*]thien-3-yl)-1-piperazinyl]butyl]-2,5,5-trimethyl-4-thiazolidinone maleate; P-9236) (**54**) displayed a pharmacological profile indicative of potential atypical antipsychotic activity. A series of piperazinyl butyl thiazolidinones structurally related to this compound were prepared and evaluated *in vitro* for dopamine D_2 and serotonin $5HT_2$ and $5HT_{1A}$ receptor affinity. The compounds were examined *in vivo* in animal models of potential antipsychotic activity and screened in models predictive of extrapyramidal side effect (EPS) liability. The synthesis of these compounds, details of their structure−activity relationships, and discovery of a new lead, compound **50**, as well as further development of the profiles of compounds **50** and **54** are described.

Introduction

Schizophrenia is a complex and devastating disease, with a worldwide lifetime prevalence of almost 1% of the general population.[1] The disease exhibits a variety of symptoms, and in fact it is still not known whether the many symptoms of schizophrenia are the product of a single or a multiple disease entity.[2] Traditionally, the symptoms have been loosely grouped into positive and negative syndromes.[3] The positive symptoms of schizophrenia include psychotic symptoms such as hallucinations and delusions. The negative symptoms, for example apathy, lack of motivation, and social withdrawal, can persist after positive symptoms have been allieviated and hinder the patient's complete return to society. Recently, models of the disease have appeared which distinguish dissociative thought processes and cognitive impairment as a distinct third cluster of symptoms whose therapeutic response parallels that of the positive symptoms.[4]

Compounds which inhibit postsynaptic dopaminergic neurotransmission have been effective in the treatment of schizophrenia.[5,6] However, most of these agents are typical, *i.e.*, they show some propensity for the development of extrapyramidal side effects (EPS), either acutely (dystonia, pseudo-Parkinsonism) or on chronic administration (Tardive Dyskinesia).[7] In addition, few of these agents have shown efficacy against the negative symptom cluster. Clozapine, an atypical antipsychotic, is almost totally devoid of EPS liability and was reported to be effective in the treatment of both positive and negative symptoms. 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.[8]

It has been observed that clozapine and other antipsychotic drugs which show a reduced propensity for the development of EPS have demonstrated a higher affinity for the $5HT_2$ receptor than the D_2 receptor in rat membrane preparations.[9] This has resulted in the hypothesis that a combination of serotonin $5HT_2$ and dopamine D_2 receptor antagonism in a proper ratio is one way to achieve atypical antipsychotic activity. The ratio of activities at these receptors has been advanced as one explanation for the atypical profile of clozapine.[10]

Clozapine shows affinity for a number of other receptors as well. For example, its affinity for the serotonin $5HT_{1A}$ receptor is higher than that for the D_2 receptor. The $5HT_{1A}$ receptor has been implicated in the activity of atypical antipsychotic agents.[11] The $5HT_{1A}$ agonists 8-OH-DPAT, buspirone, and ipsapirone have been found to reverse haloperidol-induced catalepsy,[12] 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.[13]

A number of new antipsychotic agents which have been reported to show a profile predictive of atypical antipsychotic activity in animal models act *via* the mehanism of combined dopamine D_2 and serotonin $5HT_2$ antagonism. These compounds include risperidone,[14] sertindole,[15] and iloperidone (HP 873).[16] Recently, we reported the synthesis and pharmacological profile of a new potential atypical antipsychotic agent, (3-[4-[4-(6-fluorobenzo[*b*]thien-3-yl)-1-piperazinyl]butyl]-2,5,5-trimethyl-4-thiazolidinone maleate (P-9236; HP-236) (compound **54**). This compound demonstrated a profile of potential atypical antipsychotic activity in a number of animal models.[17] The electrophysiological profile of **54** was also strongly indicative of atypicality.[17,18]

A series of compounds structurally related to **54**, designed for both D_2 and $5HT_2$ receptor antagonism, was prepared by varying both the arylpiperazine moiety and the substituents on the thiazolidinone ring (Table 1). We expected that the arylpiperazine moiety would impart the dopamine D_2 antagonist activity

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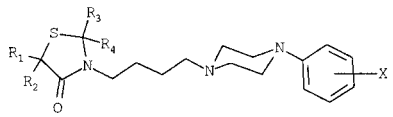
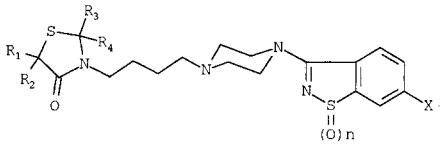
Table 1

3-[4-[4-PHENYL-1-PIPERAZINYL]BUTYL]-4-THIAZOLIDINONES.



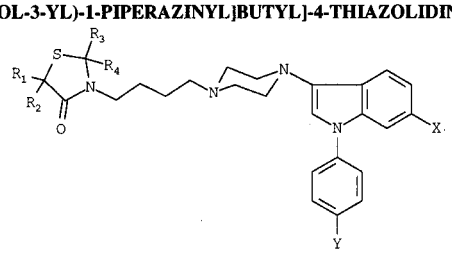
Cmpd	R_1	R_2	R_3	R_4	X	Formula[a]	mp	Rec. solvent
1	H	H	CH_3	CH_3	2-OCH_3	C20 H31 N3 O2 S-HCl-H2O	189-192	Et_2O
2	CH_3	H	H	H	2-OCH_3	C19 H29 N3 O2 S-C2H2O4[b]	129-131	EtOAc
3	CH_3	CH_3	H	H	2-OCH_3	C20 H31 N3 O2 S-2HCl	213-218	EtOH
4	CH_3	CH_3	CH_3	CH_3	2-OCH_3	C22 H37 Cl2 N3 O2 S	138 (dec)	EtOH
5	-$(CH_2)_4$-		H	H	2-OCH_3	C22 H35 Cl2 N3 O2 S	144 (dec)	EtOH/Et_2O
6	H	H	H	H	3-OCH_3	C18 H27 N3 O2 S-HCl	161-162	Et_2O
7	H	H	CH_3	CH_3	3-SCH_3	C20 H31 N3 O S2-2HCl	202 (dec)	EtOH
8	H	H	H	H	3-CF_3	C18 H24 F3 N3 O S-HCl-0.5H2O	138-140	Et_2O
9	H	H	CH_3	CH_3	3-CF_3	C20 H28 F3 N3 O S-2HCl	184 (dec.)	iPrOH/EtOH
10	CH_3	CH_3	H	H	3-CF_3	C20 H28 F3 N3 O S-HCl	169-171	EtOH/EtOAc
11	H	H	H	H	2-CH_3	C18 H27 N3 O S-HCl	207-209	EtOH/EtOAc
12	CH_3	CH_3	H	H	2-CH_3	C20 H31 N3 O S-HCl	230-235	EtOH/EtOAc
13	H	H	H	H	3-CH_3	C18 H27 N3 O S-HCl	201-203	EtOH/EtOAc
14	H	H	CH_3	CH_3	3-CH_3	C20 H31 N3 O S-2HCl	204 (dec)	EtOH
15	CH_3	CH_3	H	H	3-CH_3	C20 H33 Cl2 N3 O S	145 (dec)	EtOH
16	CH_3	CH_3	CH_3	CH_3	3-CH_3	C22 H37 Cl2 N3 O S	182 (dec)	EtOH
17	H	H	H	H	2,3-di-CH_3	C19 H29 N3 O S-HCl	228-230	Et_2O
18	CH_3	CH_3	H	H	2,3-di-CH_3	C21 H33 N3 O S-HCl	248 (dec)	CH_2Cl_2/EtOAc
19	H	H	H	H	2-Cl	C17 H24 Cl N3 O S-HCl	185-187	CH_2Cl_2/EtOAc
20	H	H	H	H	3-Cl	C17 H24 Cl N3 O S-HCl	157-159	Et_2O
21	H	H	CH_3	H	3-Cl	C18 H26 Cl N3 O S-HCl	180-183	Et_2O
22	H	H	CH_3	CH_3	3-Cl	C19 H28 N3 Cl O S-2HCl	205-207	EtOH
23	H	H	H	H	4-Cl	C17 H24 Cl N3 O S-HCl	186-188	Et_2O
24	H	H	H	H	4-F	C17 H24 N3 O S F	84-85	hexane/CH_2Cl_2

3-[4-[4-(1,2-BENZISOTHIAZOL-3-YL)-1-PIPERAZINYL]BUTYL]-4-THIAZOLIDINONES.



Cmpd	R1	R2	R3	R4	X	n	Formula	mp	Rec. solvent
25	H	H	H	H	H	0	C18 H24 N4 O S2-HCl	220-225	EtOH
26	H	H	CH_3	CH_3	H	0	C20 H28 N4 O S2-HCl	213-216	EtOH/EtOAc
27	CH_3	H	H	H	H	0	C19 H26 N4 O S2	113-115	EtOAc/hexane
28	CH_3	CH_3	H	H	H	0	C20 H28 N4 O S2-HCl	222-227	EtOH/EtOAc
29	CH_3	CH_3	CH_3	H	H	0	C21 H30 N4 O S2-HCl	209-214	EtOH/EtOAc
30	CH_3	CH_3	CH_3	H	Cl	0	C21 H29 Cl N4 O S2-HCl	204-206	Et_2O
31	CH_3	CH_3	CH_3	CH_3	H	0	C22H32N4OS2-HCl-0.5H2O	218-221	EtOH/EtOAc
32	CH_2CF_3	H	H	H	H	0	C20 H25 F3 N4 O S2-HCl	188-200	EtOH
33	$C(CH_3)_2OH$	H	H	H	H	0	C21 H30 N4 O2 S2-HCl	147-150	EtOH/EtOAc
34		H	H	H	H	0	C26 H30 N4 O S2-HCl	192-195	EtOH/EtOAc
35	-$(CH_2)_4$-		H	H	H	0	C22 H30 N4 O S2-HCl	200-203	CH_2Cl_2
36	-$(CH_2)_4$-		H	H	H	2	C22 H30 N4 O3 S2	137-140	EtOAc
37	-$(CH_2)_4$-		CH_3	H	H	0	C23 H32 N4 O S2-HCl	210-215	CH_2Cl_2
38	-$(CH_2)_5$-		H	H	H	0	C23 H32 N4 O S2-HCl	209 (dec.)	EtOH
39	-$(CH_2)_5$-		H	H	Cl	0	C23 H31 Cl N4 O S2-HCl	202-205	CH_2Cl_2/Et_2O

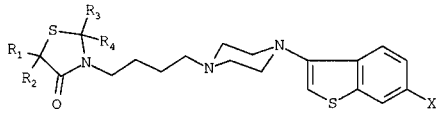
3-[4-[4-(1-PHENYL-1H-INDOL-3-YL)-1-PIPERAZINYL]BUTYL]-4-THIAZOLIDINONES.



Cmpd	R1	R2	R3	R_4	X	Y	Formula	mp	Rec. solvent
40	CH_3	CH_3	H	H	H	H	C27 H34 N4 O S-2HCl	209-211	CH_2Cl_2/Et_2O
41	CH_3	CH_3	H	H	H	F	C27 H33 F N4 O S-2HCl	195-198	hexane/CH_2Cl_2/Et_2O
42	CH_3	CH_3	CH_3	H	H	H	C28 H36N4 O S-2HCl	217 (dec.)	EtOH
43	CH_3	CH_3	CH_3	H	H	F	C28 H35 F N4 O S-2HCl-0.5H2O	182-185	CH_2Cl_2/Et_2O
44	CH_3	CH_3	CH_3	H	F	H	C28 H35 F N4 O S-2HCl	207-210	EtOH
45	-$(CH_2)_4$-		CH_3	H	H	H	C30 H38 N4 O S-2HCl	214-217	EtOH
46	-$(CH_2)_5$-		H	H	H	H	C30 H38 N4 O S-2HCl	158 (dec.)	EtOH
47	-$(CH_2)_5$-		H	H	H	F	C30 H37 F N4 O S	134-136	Et_2O

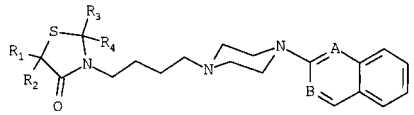
Table 1 (Continued)

3-[4-[4-(BENZO[*b*]THIEN-3-YL)-1-PIPERAZINYL]BUTYL]-4-THIAZOLIDINONES.



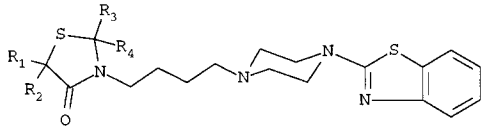
Cmpd	R1	R2	R3	R4	X	Formula	mp	Rec. solvent
48	-$(CH_2)_4$-		H	H	H	C23 H31 N3 O S2-C4H4O4[c]	177-180	EtOH
49	-$(CH_2)_4$-		CH_3	H	Cl	C24 H32 N3 O S2-HCl	211-214	CH_2Cl_2
50	-$(CH_2)_4$-		CH_3	H	F	C24 H 32 F N3 O S2-C4H4O4[c]	174-176	iPrOH
51	-$(CH_2)_5$-		H	H	H	C24 H33 N3 O S2-C4H4O4[c]	191-192	EtOH
52	$C(CH_3)_2OH$	H	H	H	F	C22 H30 F N3 O2 S2-C4H4O4[c]	159-161	EtOH/iPrOH
53	CH_3	CH_3	H	H	F	C21 H28 F N3 O S2-C4H4O4[c]	188-190	MeOH/EtOAc
54	CH_3	CH_3	CH_3	H	F	C22 H30 F N3 O S2-C4H4O4[c]	169-170	EtOH
55	CH_3	CH_3	CH_3	H	Cl	C22 H30 Cl2 N3 O S2-HCl[d]	199-202	$CHCl_3$/hexane

3-[4-[4-(QUINOLYL)-1-PIPERAZINYL]BUTYL]-4-THIAZOLIDINONES.



Cmpd	R1	R2	R3	R4	A	B	Formula	mp	Rec. solvent
56	H	H	H	H	N	C	C20 H26 N4 O S	106-107.5	EtOAc/cyclohexane
57	CH_3	CH_3	H	H	N	C	C22 H30 N4 O S	110.5-111.5	EtOAc/hexane
58	CH_3	CH_3	H	H	C	N	C22 H30 N4 O S	145-146.5	CH_2Cl_2/hexane

3-[4-[4-(2-BENZOTHIAZOLYL)-1-PIPERAZINYL]BUTYL]-4-THIAZOLIDINONES.



Cmpd	R1	R2	R3	R4	Formula	mp	Rec. solvent
59	H	H	H	H	C18 H24 N4 O S2	111-112	CH_2Cl_2/hexane
60	CH_3	CH_3	H	H	C20 H28 N4 O S	101-102	CH_2Cl_2/hexane

a) All analysis values were within 0.4% except where indicated. b) oxalate salt. c) maleate salt d) calculated for $C_{22}H_{30}ClN_3OS_2$-HCl: C 54.09%, H 6.40%, N 8.60%; found: C 53.67%, H 6.31%, N 8.60%.

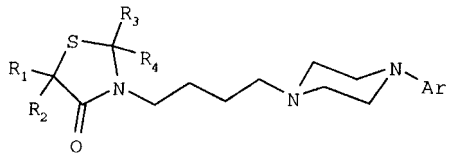


Figure 1. (Piperazinylbutyl)thiazolidinones.

required of an antipsychotic agent and confer serotonin $5HT_2$ antagonist activity as well. This property of arylpiperazines and -piperidines had been shown in a number of antipsychotic series investigated in these and other laboratories.[16,32] Variations on the thiazolidinone ring, which were expected to impart subtle changes in activity, in fact sometimes produced dramatic differences in otherwise similar molecules. Many of these compounds demonstrate a potent ability to inhibit apomorphine-induced climbing in mice (CMA), an animal model predictive of potential antipsychotic activity. Also, some compounds have shown more potent activity in this limbically-mediated behavioral assay (CMA) vs a striatally-mediated assay, namely the inhibition of apomorphine-induced stereotypies in the rat (APO-S). Such a ratio of activities in these two *in vivo* assays may be indicative of a reduced propensity for a compound to cause extrapyramidal side effects.[9,10]

In addition, some compounds were tested in an animal model predictive for the potential efficacy of an antipsychotic agent against the negative symptom of social withdrawal.[19] Typical antipsychotic agents such as haloperidol decrease social interaction behavior in this model, while atypical agents such as clozapine increase this behavior.

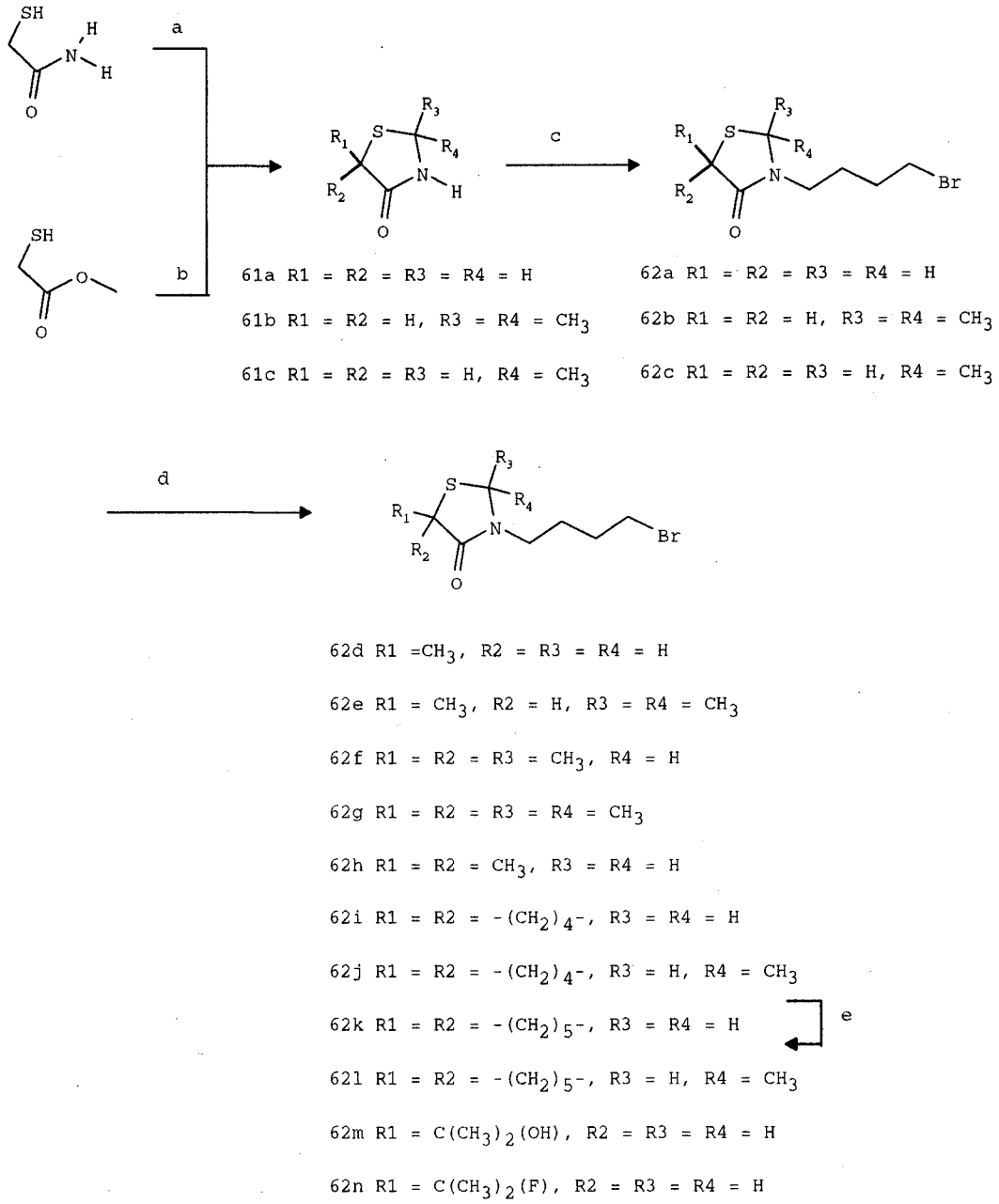
We present here the synthesis, *in vitro*, and *in vivo* evaluation of these compounds, and also the identification and profiling of a new lead in this series, compound **50**, as well as further evaluation of compound **54**.

Chemistry

The synthesis of the target compounds **1**−**60** can be divided into three parts: (A) the synthesis of a substituted 4-thiazolidinone, (B) the preparation of an arylpiperazine, and (C) the coupling of these components.

A. Preparation of 3-(4-Halobutyl)-4-thiazolidinones. The condensation of thioglycolamide with either formaldehyde or 2,2-dimethoxypropane provided the 4-thiazolidinones **61a** and **61b**,[20] respectively. The intermediate 2-methyl-4-thiazolidinone **61c**, previously prepared by another method,[21] was synthesized by treating methyl thioglycolate with acetaldehyde−ammonia trimer. The 4-thiazolidinones **61a**−**c** were alkylated with 1,4-dibromobutane to give the 2-substituted 3-(4-bromobutyl)-4-thiazolidinones **62a**−**c**. The 5-monosubstituted intermediates were prepared by reacting compounds **62a** or **62b** with 1 equiv of lithium bis(trimethylsilyl)amide followed by an alkylating agent to provide compounds **62d**, **62e**, and **62m**. The 5,5-disubstituted and spiro-substituted intermediates were synthesized by adding 2 equiv of lithium bis(trimeth-

Scheme 1[a]



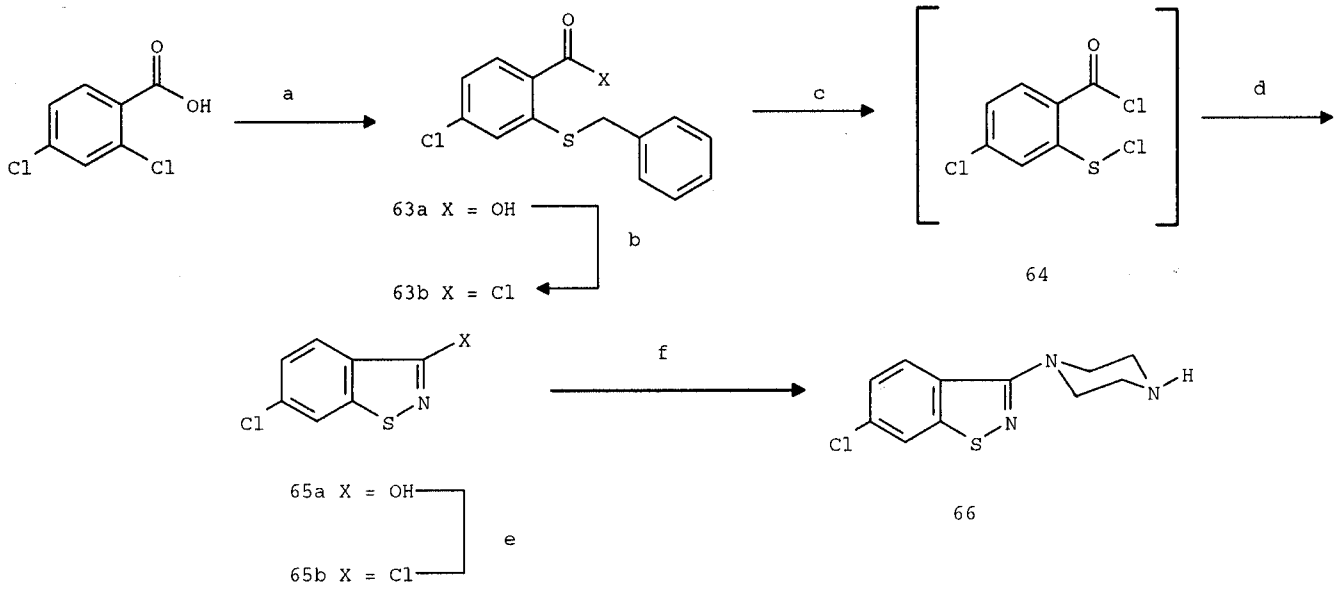
[a] Reagents: (a) formalin (**61a**) or 2,2-dimethoxypropane (**61b**); (b) acetaldehyde−ammonia trimer (**61c**); (c) NaH, $Br(CH_2)_4Br$; (d) $LiN(TMS)_2$, alkylating agents; (e) DAST (see Experimental Section for details).

ylsilyl)amide to a solution of compounds **62a**−**c** and an alkylating agent to yield compounds **62f**−**l**. Treatment of **62m** with (dimethylamido)sulfur trifluoride (DAST) provided compound **62n** (Scheme 1).

B. Preparation of Bicyclic Heteroarylpiperazines. Literature methods were adopted for the preparation of the 3-piperazinyl derivatives of the 1,2-benzisothiazole[22] and *N*-phenylindole[23−25] systems, with the following exceptions. For the preparation of 1-(6-chloro-1,2-benzisothiazol-3-yl)piperazine, the requisite 3-hydroxy-6-chloro-1,2-benzisothiazole was prepared as follows. Ullman coupling of benzyl mercaptan and 2,4-dichlorobenzoic acid generated the 2-(benzylthio)-4-chlorobenzoic acid, **63a**. This compound was converted to the corresponding acid chloride **63b** which was then treated with chlorine gas, oxidatively cleaving the *S*-benzyl group to provide an intermediate sulfuryl chloride **64**. This solution was then added dropwise to ammonium hydroxide, stirred, and then acidified analogously to the literature procedure[22] to provide 3-hydroxy-6-chloro-1,2-benzisothiazole, **65a**. Conversion of this compound to the 3,6-dichloro-1,2-benzisothiazole **65b** followed by displacement of the 3-chloride with piperazine provided the desired intermediate heteroarylpiperazine **66** (Scheme 2).

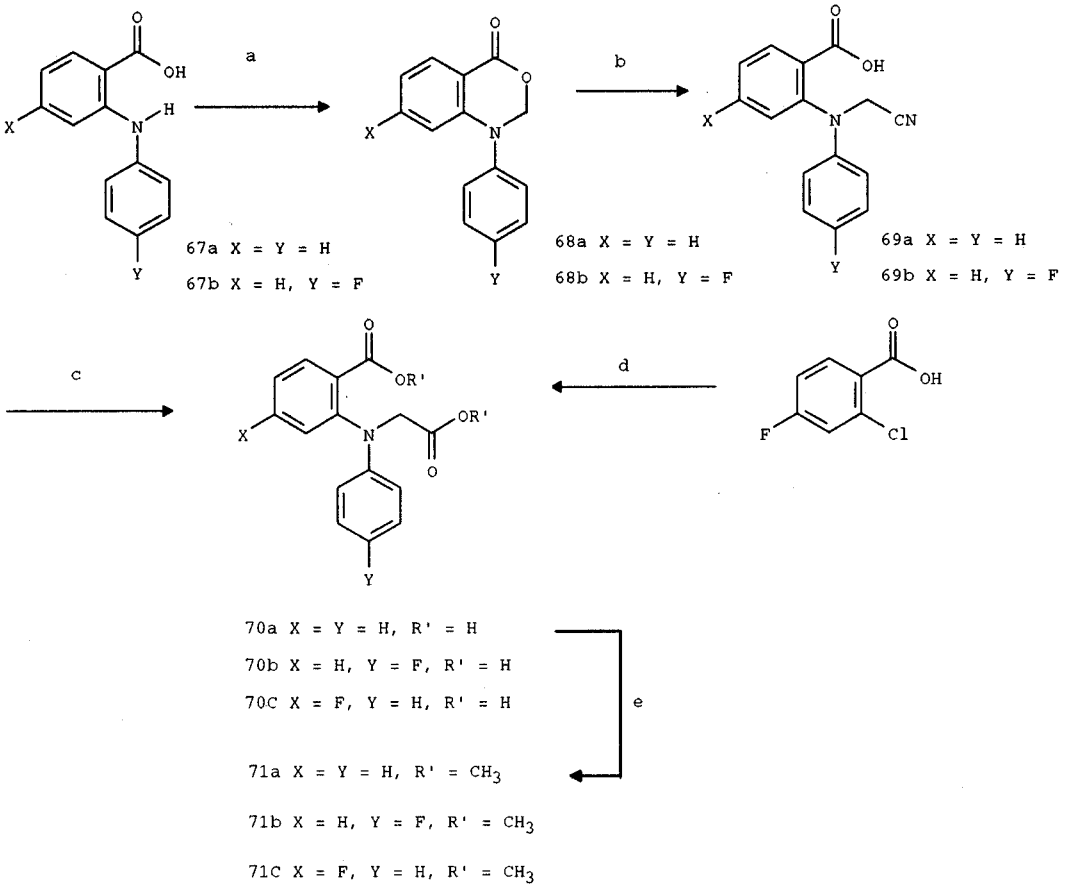
Two routes to the 1-aryl-2-carbalkoxy-3-hydroxyindole intermediates which are required for the preparation of the 3-piperazinyl-1-phenylindole systems are shown in Schemes 3 and 4. The first method proceeds via treatment of benzoic acids **67** with formaldehyde to form the benzoxazines **68**, which are opened by treatment with potassium cyanide to provide cyano acids **69**. These intermediates are converted to diacids **70a**,**b**.[23] For the preparation of 1-(6-fluoro-1-phenyl-1*H*-indol-3-yl)piperazine, we utilized an alternative route which avoided the use of potassium cyanide. The Ullmann reaction of *N*-phenylglycine and 2-chloro-4-fluorobenzoic acid provided the intermediate diacid **70c** in one step.[24]

Scheme 2[a]



[a] Reagents: (a) benzyl mercaptan, K_2CO_3, CuCl; (b) $SOCl_2$; (c) Cl_2(g), CCl_4; (d) (1) NH_4OH, (2) 6 N HCl; (e) $POCl_3$, PCl_5; (f) piperazine.

Scheme 3[a]



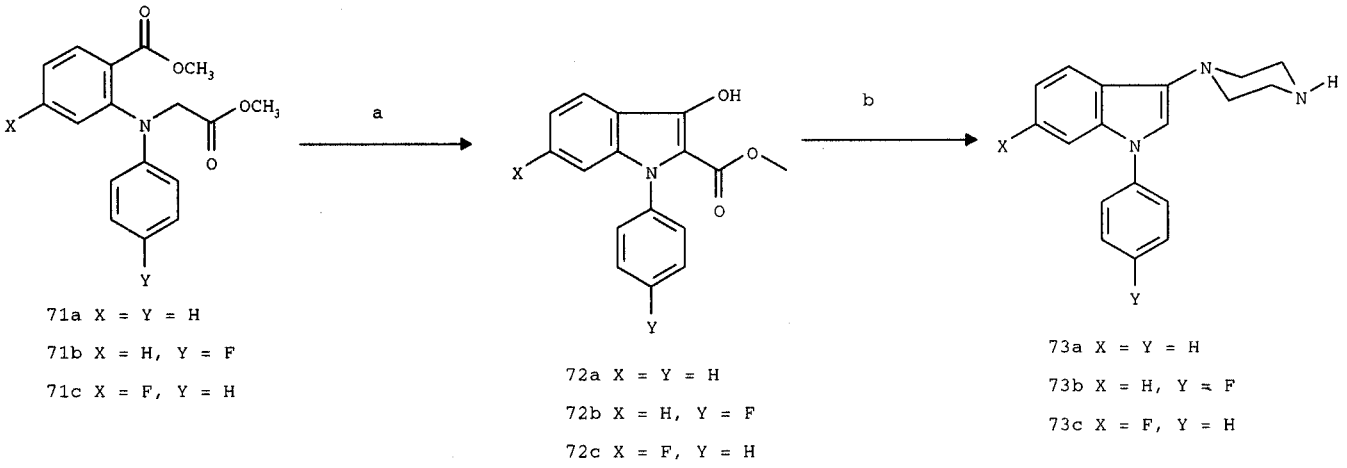
[a] Reagents: (a) formalin, ethanol; (b) KCN, H_2O; (c) aqueous NaOH; (d) *N*-phenylglycine; (e) NaOH, MeI (see ref 23 for experimental details).

These compounds were converted to diesters **71**. Dieckmann cyclization of diesters **71** as described in the literature provided the 2-hydroxy-3-carbomethoxyindoles **72**.[23] These compounds were converted to the 3-piperiazinyl-1-phenylindoles **73** following literature procedures as illustrated in Scheme 4.[25]

The 1-benzo[*b*]thien-3-ylpiperazine systems were prepared using literature methods to prepare the intermediate 3-aminobenzo[*b*]thiophene-2-carboxylates as illustrated for the preparation of the 6-fluoro derivative **74a**.[26] In a variation of the literature procedure, decarbomethoxylation was carried out using *N*-methylpiperazine to provide **75a**. Displacement of the amino group with piperazine provided the desired intermediate benzo[*b*]thienylpiperazine **76a** (Scheme 5).

The piperazine derivatives of quinoline, isoquinoline, and benzothiazole were prepared by displacement of the corresponding chlorides, which were commercially avail-

Scheme 4[a]



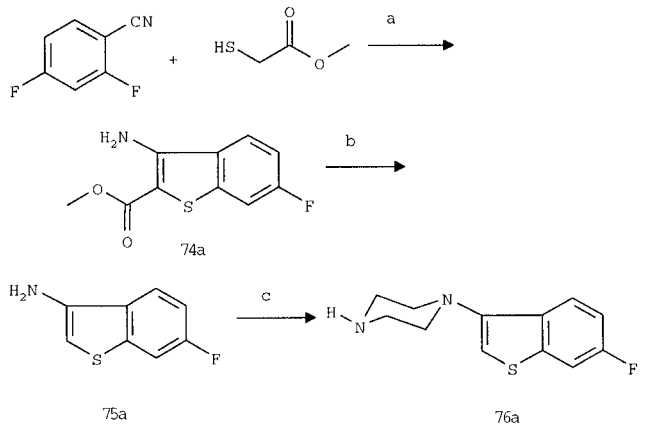
71a X = Y = H
71b X = H, Y = F
71c X = F, Y = H

72a X = Y = H
72b X = H, Y = F
72c X = F, Y = H

73a X = Y = H
73b X = H, Y = F
73c X = F, Y = H

[a] Reagents: (a) NaOMe, MeOH; (b) $MgCl_2-6H_2O$, then piperazine (see ref 25 for details).

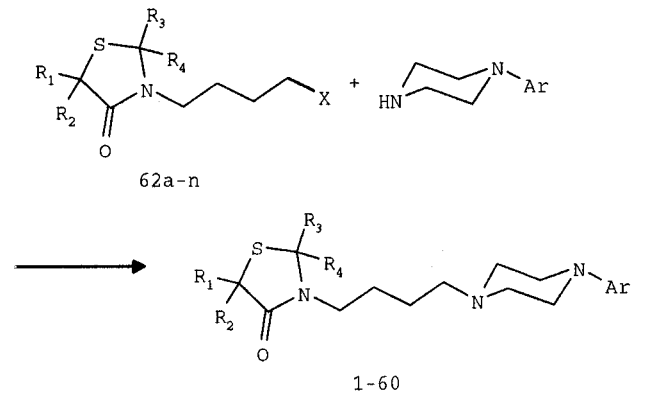
Scheme 5[a]



74a
75a
76a

[a] Reagents: (a) methyl thioglycolate, $LiOH-H_2O$; (b) *N*-methylpiperazine; (c) piperazine, *N*-methylpiperidone.

Scheme 6[a]



62a-n
1-60

[a] Reagents: (a) K_2CO_3, CH_3CN.

able or were prepared via literature methods. The monocyclic arylpiperazines were commercially available.

C. Coupling. The arylpiperazines were alkylated (Scheme 6) with the substituted 3-(4-bromobutyl)-4-thiazolidinones **62a−n** to give the target compounds **1−60** (Table 1).

Results and Discussion

Compounds in this series were evaluated *in vitro* for affinity at the dopamine D_2 receptor (Table 2). Concurrently, targets were screened for potential antipsychotic activity in a behavioral model, the inhibition of apomorphine-induced climbing in mice (CMA)[27,28] (Table 3). This behavior is mediated by the limbic dopaminergic pathway, which has been associated with the therapeutic effects of antipsychotic agents. Compounds which showed potency in this assay using intraperitoneal (ip) administration were then evaluated orally (po) in the CMA assay to assess oral bioavailability. Additionally, compounds which demonstrated activity in CMA were examined for their ability to inhibit apomorphine-induced stereotypy in rats (APO-S).[29] This is a behavioral assay mediated by activation of the nigrostriatal dopamine system, a pathway which has been linked to potential EPS liability.[30,31] Therefore, an agent which demonstrates potential atypical antipsychotic activity should be active in the climbing-mouse assay and weak or inactive in inhibiting agonist-induced rat stereotypy (Table 4). Indeed, while haloperidol is potently active in both these assays, clozapine and risperidone display only weak activity in the stereotypy model even at high doses relative to their effective dose in the CMA assay.

Structure−Activity Considerations

In the CMA assay, compounds with a simple monocyclic arylpiperazine moiety were in general less potent than compounds which possessed a bicyclic [5,6]-fused heteroaryl-3-piperazine system. (This has been observed in other series of antipsychotic agents from these laboratories.)[32] Quinolyl, isoquinolyl, and 2-benzothiazolyl substituents also provided less potent targets. Therefore, much of our overall effort was devoted to discovering which bicyclic [5,6]-fused system would produce the optimal pharmacological profile. It was discovered that the most potent dopamine D_2 affinity was found in compounds possessing the 1,2-benzisothiazol-3-ylpiperazine substituent (compound **35**, 15.7 nM) and the (6-fluorobenzo[*b*]thien-3-yl)piperazine substituent (compound **53**, 14 nM). The former substituent was also found in the compound with the highest $5HT_2$ affinity (**28**, 1.34 nM). Like clozapine, many members of the series possessed higher affinity for the serotonin $5HT_2$ and/or $5HT_{1A}$ receptors than for the D_2 receptor, possibly predictive of potential atypical antipsychotic activity.[9] Of the compounds demonstrating good activity in the CMA assay, ratios of $D_2/5HT_2$ affinity were highest with compounds **28** (ratio 41), **27** (ratio 16), **54** (ratio 19), and **50** (ratio 7.3). We were aware that the serotonin $5HT_2$ receptor binding studies using rat membrane do not distinguish between the recently characterized $5HT_2$ subtypes. Thus, we also evaluated

Table 2. *In Vitro* Receptor Affinity (IC_{50}, μmol, and 95% Confidence Limits)

compd no.	D_2[a]	IC_{50}, μmol	$5HT_2$[b]	IC_{50}, μmol	$5HT_{1A}$[c]	IC_{50}, μmol
1	0.514	(0.295−0.895)			0.010	(0.008−0.013)
2	0.786	(0.583−1.059)			0.022	(0.016−0.030)
3	0.548	(0.309−0.972)	0.588	(0.354−0.979)	0.011	(0.008−0.014)
4	0.304	(0.233−0.397)			0.018	(0.011−0.028)
5	0.15	(0.09−0.252)	0.492	(0.272−0.887)	0.002	(0.001−0.003)
6	7.49	(5.69−9.86)			0.139	(0.106−0.183)
7	2.43	(1.46−4.05)	0.503	(0.31−0.817)	0.009	(0.005−0.017)
8	1.96	(1.52−2.53)			0.034	(0.025−0.044)
9	1.13	(0.86−1.49)				
10	1.64	(1.28−2.11)	0.090	(0.043−0.188)	0.007	(0.005−0.008)
11	1.61	(0.84−3.09)			0.061	(0.048−0.078)
12	1.77	(1.03−3.06)	0.569	(0.456−0.711)	0.013	(0.010−0.017)
13	12.6	(6.8−23.2)			0.140	(0.073−0.269)
14	3.75	(1.92−7.32)			0.026	(0.015−0.045)
15	2.64	(1.44−4.85)			0.012	(0.009−0.016)
16	1.84	(1.0−3.38)	0.439	(0.247−0.781)	0.038	(0.028−0.051)
17	0.778	(0.449−1.349)			0.083	(0.053−0.131)
18	0.916	(0.69−1.217)	0.414	(0.244−0.701)	0.006	(0.005−0.007)
19	0.652	(0.376−1.131)			0.023	(0.017−0.032)
20	2.21	(1.24−3.93)			0.057	(0.035−0.092)
21	2.23	(1.33−3.75)			0.010	(0.008−0.012)
22	0.835	(0.613−1.136)				
23	8.76	(4.08−18.82)			1.28	(0.56−2.94)
24	12.9	(5.5−30)			0.140	(0.106−0.183)
25	0.224	(0.133−0.38)	0.007	(0.003−0.014)	0.011	(0.006−0.019)
26	0.193	(0.113−0.332)	0.012	(0.006−0.022)	0.007	(0.004−0.013)
27	0.086	(0.068−0.110)	0.005	(0.004−0.007)	0.011	(0.008−0.014)
28	0.055	(0.043−0.071)	0.001	(0.0007−0.002)	0.008	(0.004−0.014)
29			0.002	(0.001−0.004)	0.007	(0.006−0.010)
30	0.286	(0.068−1.21)	0.271	(0.066−1.12)	0.324	(0.137−0.764)
31	0.201	(0.124−0.326)	0.0035	(0.0018−0.0066)	0.017	(0.011−0.029)
32	0.136	(0.103−0.181)			0.003	(0.001−0.005)
33	0.029	(0.022−0.038)	0.0189	(0.0151−0.0236)	0.012	(0.010−0.016)
34	0.697	(0.432−1.125)	1.11	(0.36−3.42)	0.052	(0.029−0.094)
35	0.016	(0.009−0.026)	0.003	(0.001−0.008)	0.002	(0.001−0.003)
36			>20			
37	0.154	(0.09−0.239)	0.0096	(0.0053−0.018)	0.003	(0.002−0.004)
38	0.029	(0.021−0.039)	0.018	(0.009−0.036)	0.002	(0.001−0.003)
39	0.671	0.341−1.322)	1.06	(0.22−5.18)	0.969	(0.529−1.78)
40	0.191	0.142−0.258)	0.294	(0.154−0.561)	1.73	(1.27−2.35)
41	0.131	(0.112−0.153)	0.053	(0.023−0.119)	2.33	(1.29−4.20)
42	0.20	(0.168−0.239)	0.186	(0.045−0.766)	2.55	(1.97−3.31)
43	0.059	(0.0001−24.9)	0.126	(0.03−0.53)	3.05	(0.22−7.63)
44	1.09	(0.431−2.76)	0.119	(0.010−1.28)	8.08	(0.068−9.58)
45	0.356	(0.204−0.622)	0.0981	(0.0494−0.1949)	11.7	(9.20−15.0)
46	0.467	(0.279−0.781)	0.045	(0.020−0.098)		
47	0.108	(0.067−0.174)	0.142	(0.116−0.173)	9.03	(7.06−11.6)
48	0.244	(0.158−0.377)	0.016	(0.009−0.028)	0.002	(0.001−0.002)
49	0.611	(0.154−2.42)	0.58	(0.392−0.858)	0.776	(0.322−1.86)
50	0.954	(0.285−3.191)	0.131	(0.101−0.168)	0.189	(0.145−0.245)
51	0.218	(0.131−0.36)	0.196	(0.093−0.411)	0.102	(0.067−0.156)
52	0.453	(0.206−0.993)	0.105	(0.041−0.267)	0.091	(0.041−0.203)
53	0.014	(0.007−0.3)	0.01	(0.008−0.013)		
54	1.21	(0.38−3.82)	0.056	(0.026−0.12)	0.061	(0.031−0.119)
55	4.73	(2.18−10.27)	1.54	(0.62−3.79)	0.855	(0.362−2.019)
56			0.641	(0.394−1.044)	0.332	(0.176−0.624)
57			0.446	(0.248−0.803)	0.153	(0.121−0.193)
58	8.43	(6.46−11)	0.085	(0.046−0.158)	0.629	(0.479−0.826)
59			8.42	(4.13−17.15)	0.367	(0.225−0.601)
60			2.31	(1.28−4.14)	1.37	(1.05−1.80)
clozapine	1.61	(1.24−2.07)	0.072	(0.055−0.094)	1.01	(0.74−1.39)
haloperidol	0.033	(0.024−0.045)	0.129	(0.07−0.239)	7.079	(4.94−10.13)
risperidone	0.037	(0.024−0.047)	0.0026	(0.0019−0.0042)	0.95	(0.75−1.22)

[a] Dopamine D_2 binding determined in rat striatum using [^{3}H]spiperone as ligand. IC_{50} values determined from seven-point concentration curves done in duplicate. [b] Serotonin $5HT_2$ binding determined in rat cortex using [^{3}H]spiperone as ligand. IC_{50} values determined from seven-point concentration curves done in duplicate. [c] Serotonin $5HT_{1A}$ binding determined in rat hippocampus using [^{3}H]-8-OH-DPAT as ligand. IC_{50} values determined from seven-point concentration curves done in duplicate.

the affinity of lead compounds **50** and **54** for the cloned human $5HT_{2A}$ receptor (Table 7).

The two most potent compounds in the CMA assay, **33** (ED_{50} = 0.127 mg/kg ip) and **52** (ED_{50} = 0.2 mg/kg ip), shared as a common structural feature a 5-(1-hydroxy-1-methylethyl) substituent on the 4-thiazolidinone moiety. With regard to CMA potency, the thiazolidinone system was tolerant to up to three substituents; however, tetrasubstitution at the 2- and 5-positions decreased the CMA potency.

In the benzisothiazolyl series, chlorine substitution at the 6-position decreased D_2 and, in particular, $5HT_2$ receptor affinity and also reduced potency in the CMA assay (*cf.* compound **30** vs **29** and **39** vs **38**). Oxidation of the sulfur of the benzisothiazole moiety also reduced D_2 affinity and CMA potency (*cf.* compound **36** vs **35**).

Table 3. Potential for Antipsychotic Activity: Inhibition of Apomorphine-Induced Climbing in Mice

compd no.	ED_{50}, mg/kg ip[a]	ED_{50}, mg/kg po	compd no.	ED_{50}, mg/kg ip	ED_{50}, mg/kg po
1	>20[b]		33	0.127 (0.116−0.14)	0.99 (0.8−1.24)
2	12 (10.7−13.2)		34	4.5 (4.0−5.0)	−44% at 10
3	10.7 (9.0−12.5)		35	0.6 (0.52−0.69)	4.4 (3.9−4.9)
4	>20		36	>20	
5	10.3 (9.0−12.0)		37	5.1 (4.4−6.6)	30.3 (28.6−32.1)
6	>20		38	0.99 (0.88−1.1)	6.8 (2.2−11.4)
7	>20		39	2.946 (2.566−3.313)	−38% at 14.5
8	19.3 (18.4−20.3)		40	1.349 (1.203−1.506)	2.3 (1.4−3.8)
9	>20		41	1.55 (1.33−1.80)	2.22 (2.09−2.36)
10	16.7 (15.7−17.7)		42	4.617 (4.094−5.145)	4. (4.1−5.6)
11	14.3 (13.2−15.6)		43	1.03 (0.95−1.12)	2.7 (2.4−3.1)
12	>20		44	10.21 (9.213−11.391)	
13	>20		45	4.191 (3.692−4.698)	13.8 (12.9−14.8)
14	17.1 (14.9−20.3)		46	8.8 (7.9−10.0)	
15	>20		47	>20	
16	>20		48	1.44 (1.24−1.63)	−17% at 7
17	>20		49	>20	
18	15.4 (13.8−17.3)		50	3.5 (3.066−3.956)	6.1 (1.1−34.3)
19	>20		51	5.04 (4.415−5.669)	−27% at 25
20	15.5 (12.8−19.0)		52	0.2 (0.182−0.218)	1.3 (1.1−1.4)
21	>10		53	0.92 (0.07−11.7)	2.6 (1.2−5.6)
22	>20		54	2.8 (2.5−3.1)	15.43 (14.4−16.6)
23	>20		55	>20	
24	>20		56	>20	
25	>20		57	>20	
26	>20		58	>20	
27	2.94 (2.75−3.15)	23.2 (20.8−25.4)	59	>20	
28	1.43 (1.23−1.63)		60	>20	
29		30.5 (27.9−33.6)	clozapine	9.10 ± 1.77 (6)[c]	23.2 (21.1−25.9)
30	12.69 (11.32−14.46)		haloperidol	0.194 ± 0.056 (2)[c]	0.28 (0.27−0.29)
31	>20		risperidone	0.062 (0.047−0.077)	0.28 (0.25−0.3)
32	2.91 (2.59−3.29)	14.9 (12.5−17.8)			

[a] ED_{50} and 95% confidence limits. [b] ED_{50} was not determined but is greater than screening dose reported. [c] ED_{50} ± SEM (*n*).

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

compd no.	inhibition of apomorphine-induced climbing (mouse) (ED_{50}, mg/kg ip)[a]	inhibition of apomorphine-induced stereotypy (rat) (ED_{50}, mg/kg, or % activity at dose, ip)
27	2.94 (2.75−3.15)	0% at 20, 17% at 40[b]
28	1.43 (1.23−1.63)	8.9 (7.0−11.3)
29	(30.5 (27.9−33.6)) (po)	0% at 20, 40% at 40
32	2.91 (2.59−3.29)	3.3 (2.2−5.0)
33	0.127 (0.116−0.14)	2.3 (1.6−3.1)
34	4.5 (4.0−5.0)	0% at 40,50% at 80
35	0.6 (0.52−0.69)	90% at 10
37	(30.3 (28.6−32.1))(po)	10% at 20, 100% at 30
38	0.99 (0.88−1.1)	100% at 10
39	2.946 (2.566−3.313)	0% at 20, 80% at 40
40	1.349 (1.203−1.506)	19.6 (19.6−22.5)
41	1.55 (1.33−1.80)	7.1 (5.6−9.0)
42	4.617 (4.094−5.145)	0% at 40, 67% at 80
43	1.03 (0.95−1.12)	16.4 (10.0−26.7)
45	4.191 (3.692−4.698)	41.7 (32.1−54.3)
46	8.8 (7.9−10.0)	27.4 (17.2−43.8)
50	3.5 (3.066−3.956)	20% at 50, 50% at 60
51	5.04 (4.415−5.669)	33% at 40, 50% at 80
52	0.2 (0.182−0.218)	10% at 2.5, 40% at 5
53	0.92 (0.07−11.7)	0% at 10
54	2.8 (2.5−3.1)	61.4 (54.2−69.6)
clozapine	9.10 ± 1.77 (6)[c]	33% at 40
haloperidol	0.194 ± 0.056 (2)[c]	0.6 (0.40−0.80)
risperidone	0.062 (0.047−0.077)	3.2 (2.1−4.8)

[a] ED_{50} and 95% confidence limits. [b] ED_{50} was not determined but is greater than screening dose reported. [c] ED_{50} ± SEM (*n*).

The series of compounds bearing an *N*-phenylindole piperazine moiety did not demonstrate high affinity for the $5HT_{1A}$ receptor. In this series, the presence of a fluorine at the 6-position of the indole ring reduced both D_2 affinity and CMA potency (*cf.* compound **44** vs **42**). However, no correlation was observed between the presence of a 4-fluorine on the pendant phenyl ring and CMA potency.

In the benzo[*b*]thien-3-yl series, again, the presence of a 6-chloro substituent produced compounds with reduced D_2 affinity and CMA potency (*cf.* compounds **55** vs **54** and **49** vs **50**). With regard to the desired in vivo profile, the best ratios of CMA/APO-S activity (and thus potential for atypicality) were found in this series, in compounds **50**, **52**, **53**, and **54**. Compound **54** (HP 236) demonstrated one of the higher ratios of $D_2/5HT_2$ affinity (21.7) and an almost equally greater preference for the $5HT_{1A}$ receptor over the D_2 receptor (17.5). Compound **50** showed a ratio of 7.28. However, compound **52** showed only a modest preference for either $5HT_2$ (4.31) or $5HT_{1A}$ (4.9) over D_2, and compound **53** had an almost equal affinty for D_2 and $5HT_2$. Because of the structural resemblance of compound **50** to compound **54**, and their similar pharmacological profiles, as well as the good $D_2/5HT_2$ ratio observed, we examined the electrophysiological profile of compound **50** in the chronic single-unit assay.[33] On chronic administration, atypical antipsychotic agents have been shown to reduce the number of active dopamine neurons only in the A 10 (ventral tegmental) dopaminergic pathway, associated with mood and behavior and thus with the therapeutic effects of antipsychotics. Typical agents such as haloperidol depress dopamine activity in both the A 10 and the A 9 (nigrostriatal) pathway, the latter being associated with motor control and therefore EPS liability. Like clozapine and compound **54**, compound **50** also displayed an atypical profile in this assay, as shown in Table 5.

For optimal effectiveness, an antipsychotic agent should have efficacy for both the positive and negative symptomatology of schizophrenia. One setback in the development of these agents has been the lack of reliable preclinical assays to predict a compound's effectiveness for treating negative symptoms. To address this issue,

Table 5. Chronic Single Unit Sampling of Compounds **54** and **50**[a]

compd no.	dose, mg/kg ip	n	no. of A 10 neurons	% change	no. of A 9 neurons	% change
vehicle		10	10.4 ± 0.8		9.0 ± 0.8	
haloperidol	0.5	10	6.8 ± 0.7	−35**	6.2 ± 0.9	−30*
vehicle		11	8.8 ± 0.4		10.0 ± 0.4	
clozapine	20	6	1.8 ± 0.3	−79**	13.7 ± 0.6	37*
vehicle		10	10.4 ± 0.73		12.3 ± 0.56	
54	10	12	6.8 ± 0.67	−34**	14 ± 0.77	14
vehicle		10	9.2 ± 0.85		11.1 ± 0.57	
54	20	10	4.0 ± 0.3	−57**	14.7 ± 1.09	32
vehicle		10	10.3 ± 0.62		10.8 ± 0.51	
50	10	10	7.5 ± 0.72	−27**	9.5 ± 0.83	−12
vehicle		10	10.3 ± 0.62		10.8 ± 0.51	
50	20	10	6.3 ± 0.68	−39**	9.9 ± 0.99	-8

[a] *p < 0.05 vs vehicle. **p < 0.01 vs vehicle. Duration was 21 days. Number of units/12 tracks (±SEM).

Table 6. Effects of Antipsychotic Agents and Test Compounds on Social Interaction and on Total Activity in Rats[a]

drug	dose, mg/kg ip	social interaction X + SEM	% change	total activity X + SEM	% change
vehicle		95.8 ± 2.0		144.8 ± 3.9	
haloperidol	0.05	77.2 ± 7.7*	−19	129.2 ± 9.0	−11
haloperidol	0.125	65.6 ± 6.1*	−32	112.3 ± 4.4*	−22
vehicle		108.3 ± 6.8		172.0 ± 3.9	
clozapine	5.0	123.3 ± 7.9	+14	140.3 ± 7.5*	−18
clozapine	10.0	151.3 ± 9.0*	+40	146.3 ± 7.5*	−15
vehicle		96.0 ± 3.4		145.4 ± 4.5	
50	1.25	108.0 ± 6.4	+13	146.4 ± 3.6	+1
vehicle		93.4 ± 4.4		130.4 ± 4.6	
50	2.5	106.5 ± 4.3	+14	106.3 ± 3.7	−18
50	5.0	97.3 ± 6.1	+5	82.1 ± 4.7*	−36
vehicle		100.0 ± 3.4		142.0 ± 5.4	
54	1.25	114.0 ± 3.7	+14	144.3 ± 5.4	+1
54	2.5	109.1 ± 4.6	+9	96.0 ± 4.8*	−32

[a] *p < 0.05 compared with vehicle control.

we developed a preclinical assay for the negative symptoms of social withdrawal in schizophrenic patients, namely, the social interaction test in rodents.[19] The atypical antipsychotic agent clozapine increased social interaction behavior, while the typical antipsychotic agent haloperidol decreased this behavior in rodents. The present results show that both compounds **50** and **54** had a pharmacological profile similar to clozapine in this animal model for negative symptoms (Table 6). Compound **50** at 1.25 and 2.5 mg/kg increased social interaction behavior by 13% and 14%, respectively, while compound **54** at 1.25 mg/kg increased social interaction behavior by 14%. These compounds increased social behavior at doses similar to those that produced efficacy in other preclinical antipsychotic screening assays such as the CMA assay. The mechanism of action responsible for these effects of compounds **50** and **54** remains uncertain; however, the present studies show that these compounds have high affinity for the $5HT_{1A}$ receptor. Previous studies have demonstrated that clozapine also has affinity for this receptor subtype.[19] In addition, the selective $5HT_{1A}$ agonist 8-OH-DPAT also increased social interaction behavior in rats. Finally, the clinical observation that the $5HT_{1A}$ agonist buspirone was effective in reducing stress-induced psychosocial deficits in chronic schizophrenic patients[34,35] supports these preclinical findings that antipsychotic agents with affinity for the $5HT_{1A}$ receptor subtype would be beneficial in treating the negative symptom of social withdrawal. Therefore the *in vitro* binding profile and *in vivo* activity of compounds **50** and **54** suggest a pharmacological profile with efficacy for both the positive and negative symptoms of schizophrenia. While we have not carried out experiments to determine the functional activity of compounds **50** and **54** at this receptor, the previously mentioned observations lead us to expect that these compounds would also be agonists at the $5HT_{1A}$ receptor.

Recently it has been shown that clozapine shows a high affinity for the human dopamine D_4 receptor subtype.[36] The atypical profile of clozapine has been attributed to its affinity for this receptor, since plasma levels of clozapine at therapeutic doses have been associated with the level of D_4 affinity, not D_2 affinity.[37,38] Very recently olanzapine, an antipsychotic currently under clinical investigation which has shown an atypical profile preclinically, has also demonstrated an affinity for the dopamine D_4 receptor subtype.[39] These observations led us to investigate the dopamine D_4 affinity of selected compounds in this series vs standard antipsychotic agents clozapine and haloperidol and the clinical compound olanzapine (Table 7). We discovered that compounds **50** and **54** also show high affinity for the human D_4 receptor subtype. Thus the atypical profile of compounds **50** and **54** in behavioral and electrophysiological models might also be attributed to a higher affinity for dopamine D_4 receptors than for dopamine D_2 receptors.

Conclusions

A series of (piperazinylbutyl)thiazolidinones was prepared and submitted for biological evaluation. Many members of this series displayed a higher affinity for the serotonin $5HT_2$ receptor than for dopamine D_2 receptor. This biochemical profile is consistent with potential atypical antipsychotic activity.

In vivo, many compounds in this series showed potential antipsychotic activity in an animal model, namely the inhibition of apomorphine-induced mouse climbing. In addition, some compounds also displayed a potential for atypicality in animal models *in vivo*.

Table 7. Affinity for Cloned Human Dopamine D 4.2 Receptor[a,b] and Cloned Human 5HT2A Receptor[c]

compd no.	rat D_2 (IC_{50}, μM)	rat $5HT_2$ (IC_{50}, μM)	human $D_{4.2}$ (IC_{50}, μM)	human $D_{4.2}$ (K_i, μM)	human $5HT_{2A}$ (IC_{50}, μM)	human $5HT_{2A}$ (K_i, μM)
clozapine	1.61 (1.24−2.07)	0.072 (0.055−0.094)	0.058 (0.024−0.144)	0.01	0.012 (0.008−0.018)	0.005
haloperidol	0.033 (0.024−0.045)	0.129 (0.07−0.239)	0.006 (0.004−0.01)	0.001	0.288 (0.205−0.404)	0.108
olanzapine	0.158 (0.115−0.219)	0.047 (0.036−0.062)	0.173 (0.116−0.260)	0.029	0.009 (0.004−0.019)	0.004
54	1.212 (0.384−3.826)	0.056 (0.026−0.12)	0.029 (0.023−0.043)	0.005	0.017 (0.012−0.026)	0.008
50	0.954 (0.285−3.191)	0.131 (0.101−0.168)	0.713 (0.341−1.492)	0.117	0.008 (0.006−0.012)	0.004

[a] IC_{50} and 95% confidence limits. [b] Human D2 receptor in CHO cells. [c] Serotonin 5HT2A receptor in BEK cells.

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). The lead compounds in this series, **50** and **54**, also showed an atypical profile in electrophysiological assays and increased social interaction in rats, an indication of potential efficacy against the negative symptoms of schizophrenia. Leading compounds from this series were selected for toxicological evaluation and additional preclinical studies.

Experimental Section

All structures are supported by their IR (Perkin-Elmer 547) and ^{1}H 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. Analyses were performed routinely only on target compounds and selected intermediates.

3-[4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]-1-thia-3-azaspiro[4.4]nonan-4-one Dihydrochloride (5). A mixture of 3-(4-bromobutyl)-1-thia-3-azaspiro[4.4]nonan-4-one (**62i**)-(4.60 g, 15.7 mmol), 1-(2-methoxyphenyl)piperazine (3.33 g, 17.3 mmol), K_2CO_3 (5.42 g, 39.3 mmol), NaI (310 mg), and CH_3-CN (200 mL) was heated at 65 °C (bath temperature) under nitrogen. After 6 h, TLC analysis (silica gel, 40% ethyl acetate/hexanes) showed the starting bromide, R_f = 0.46, to be consumed. The mixture was cooled to room temperature, ethyl acetate (150 mL) was added, and the inorganics were filtered. The filtrate was concentrated *in vacuo* to a residue which was taken up into methylene chloride (200 mL), washed with H_2O (100 mL) and brine (100 mL), and dried (Na_2SO_4). Concentration *in vacuo* gave an amber liquid which was purified by chromatography on silica gel, eluting with 5% methanol in methylene chloride, yielding 5.78 g of a liquid. The liquid was dissolved in hot ether/ethanol and the solution made acidic with ethereal HCl, yielding, after slow cooling, 3.43 g of fine white crystals: mp 144 °C (begin decomposition); ^{1}H NMR ($CDCl_3$) δ 13.6 (br s, 1 H), 8.24 (d, J = 7.6 Hz, 1 H), 7.47 (t, J = 8.0 Hz, 1 H), 7.10−7.01 (m, 2 H), 5.18−5.06 (m, 2 H), 4.56−4.28 (m with a s at 4.33, 4 H), 4.08 (s, 3 H), 3.67−3.52 (m, 4 H), 3.45 (t, J = 6.7 Hz, 2 H), 3.33−3.14 (m, 2 H), 2.37−2.15 (m, 2 H), 2.07−1.59 (m, 10 H); mass spectrum (EI, 70 eV) M^+ 403. Anal. ($C_{22}H_{33}N_3O_2S$·2HCl) C, H, N.

3-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-5-(1-hydroxy-1-methylethyl)-4-thiazolidinone Hydrochloride (33). A mixture of 3-(4-bromobutyl)-5-(2-hydroxyisopropyl)-4-thiazolidinone (**62m**) (4.00 g, 13.5 mmol), 3-piperazinyl-1,2-benzisothiazole hydrochloride (3.80 g, 14.9 mmol), K_2CO_3 (8.00 g, 57.9 mmol), NaI (450 mg), and acetonitrile (200 mL) was heated at 80 °C under nitrogen. After 17 h the mixture was filtered, the insolubles were washed with dichloromethane, and the filtrate was concentrated *in vacuo*. The residue was taken up in dichloromethane (200 mL), washed with 5% NaOH (100 mL) and H_2O (100 mL), and dried. Evaporation of the solvent at reduced pressure gave a viscous brown liquid. The crude product was chromatographed on silica gel. Elution with 8% methanol in dichloromethane gave 5.06 g of an amber liquid. The HCl salt of the amine was prepared. Recrystallization from ethanol/ethyl acetate afforded 2.26 g (35.6%) of a fine crystalline solid: mp 147−150 °C; ^{1}H NMR (DMSO-d_6) δ 11.3 (br s, 1 H), 8.20−8.05 (m, 2 H), 7.65−7.40 (m, 2 H), 4.78 (br s, 1 H), 4.40−4.25 (m, 2 H), 4.17−3.93 (m, 2 H), 3.73 (s, 1 H), 3.70−3.07 (m, 12 H), 1.83−1.43 (m, 4 H), 1.32 (s, 3 H), 1.19 (s, 3 H); mass spectrum (EI, 70 eV) M^+ 434. Anal. ($C_{21}H_{29}N_4O_2S_2$·HCl) C, H, N.

3-[4-[4-(6-Chloro-1,2-benzisothiazol-3-yl)-1-piperazinyl]-butyl]-1-thia-3-azaspiro[4.5]decan-4-one Hydrochloride (39). A mixture of 3-(4-bromobutyl)-1-thia-3-azaspiro[4.5]-decan-4-one (**62k**) (2.7 g, 7.9 mmol), 1-(6-chloro-1,2-benzisothiazol-3-yl)piperazine (**66**) (2.0 g, 7.9 mmol), K_2CO_3 (2.2 g, 15.8 mmol), and NaI (200 mg) in 100 mL of dry CH_3CN was heated to 80 °C with stirring under N_2. After 8 h the mixture was cooled to room temperature, the CH_3CN was removed *in vacuo*, and the residue was partitioned between Et_2O/H_2O. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica using EtOAc as eluent to provide 2.64 g of a clear oil. This product was taken up in Et_2O/CH_2Cl_2 and the HCl salt precipitated out by the addition of HCl in Et_2O. The salt was recrystallized from Et_2O/CH_2Cl_2 to provide 1.56 g (3.02 mmol, 38.2%) of product as a white solid: mp 202−205 °C; homogeneous by TLC (silica, 0.1:9.9:90 NH_4OH:CH_3OH:EtOAc, R_f = 0.55); ^{1}H NMR ($CDCl_3$) δ 12.8 (br s, 1 H), 7.82 (d, J = 1.7 Hz, 1 H), 7.72 (d, J = 8.8 Hz, 1 H), 7.36 (dd, J = 1.8 and 8.7 Hz, 1 H), 4.27 (s, 2 H), 4.17−3.93 (m, 4 H), 3.61−3.34 (m, 4 H), 3.24−3.00 (m, 4 H), 2.17−1.12 (m, 14 H); mass spectrum (EI, 70 eV) M^+ = 478. Anal. ($C_{23}H_{31}N_4OS_2Cl$·HCl) C, H, N.

3-[4-[4-(6-Fluoro-1-phenyl-1*H*-indol-3-yl)-1-piperazinyl]-butyl]-2,5,5-trimethyl-4-thiazolidinone Dihydrochloride (44). A mixture of 3-(4-bromobutyl)-2,5,5-trimethyl-4-thiazolidinone (**62f**) (1.66 g, 5.92 mmol), 1-(6-fluoro-1-phenyl-1*H*-indol-3-yl)piperazine (1.75 g, 5.92 mmol), K_2CO_3 (2.50 g, 18.1 mol), NaI (150 mg), and acetonitrile (100 mL) was heated at 60 °C under nitrogen. After 22.5 h, the dark mixture was filtered, the inorganics were washed with dichloromethane (50 mL), and the filtrate was concentrated under reduced pressure. The residue was taken up in dichloromethane (100 mL), washed with 5% NaOH (60 mL) and H_2O (60 mL), and dried (Na_2SO_4). Concentration *in vacuo* gave a dark viscous liquid. Chromatography on silica gel (7.5% methanol in dichloromethane eluent) afforded a viscous liquid (R_f = 0.37, 10% methanol in dichloromethane). The dihydrochloride salt of this amine was prepared and recrystallized from ethanol to yield 1.46 g (43.5%) of a beige powder: mp 207−210 °C; ^{1}H NMR (DMSO-d_6) δ 11.4 (br s, 1 H), 7.75 (dd, J = 5.5 and 8.8 Hz, 1 H), 7.70−7.50 (m, 4 H), 7.49−7.23 (m with a dd at 7.31), 7.00 (ddd, J = 2.2, 9.1, and 9.1 Hz, 1 H), 6.52 (br s), 4.82 (q, J = 6.0 Hz), 3.77−3.07 (m, 12 H), 1.90−1.33 (m with a d at 1.49 and two s at 1.47 and 1.44, 13 H); mass spectrum (EI, 70 eV) M^+ 494. Anal. ($C_{22}H_{30}FN_4OS$·2HCl) C, H, N.

3-(4-(4-(6-Fluorobenzo[*b*]thiophene-3-yl)-1-piperazinyl)-butyl)-2-methyl-3-azaspiro[4.4]nonan-4-one Maleate (50). A mixture of 3-(4-bromobutyl)-2-methyl-3-azaspiro[4.4]nonan-4-one (**62j**) (5.00 g, 16.3 mmol), 1-(6-fluorobenzo[*b*]thien-3-yl)-piperazine (4.55 g, 19.3 mmol), K_2CO_3 (8.00 g, 57.9 mmol), NaI (0.400 g), and acetonitrile was heated at 80 °C under nitrogen for 2.5 h and then at room temperature for an additional 64 h. The mixture was filtered, the insolubles were washed with dichloromethane, and the filtrate was concentrated under reduced pressure. The residue was taken up in dichloromethane (250 mL), washed successively with 5% NaOH (125 mL), H_2O (125 mL), and then brine (125 mL), dried (Na_2SO_4), and concentrated under reduced pressure to a viscous brown liquid. The liquid was chromatographed on silica gel, eluting with 5% methanol in dichloromethane, to afford 5.90 g (78.4%)

of a beige solid, mp 85−89 °C. The maleic acid salt of this amine was prepared and recrystallized from 2-propanol to yield 4.38 g of an off-white solid: mp 174−176 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.57 (dd, J = 5.0 and 8.9 Hz, 1 H), 7.49 (dd, J = 2.3 and 8.8 Hz, 1 H), 6.73 (s, 1 H), 6.29 (s, 2 H), 4.65 (q, J = 6.1 Hz, 1 H), 3.80−3.00 (m, 12 H), 2.43−2.16 (m, 2 H), 2.03−1.58 (m, 10 H), 1.54 (d, J = 6.1 Hz, 3 H); mass spectrum (70 eV, EI) M^+ 401. Anal. ($C_{24}H_{32}FN_3OS_2 \cdot C_4H_4O_4$) C, H, N.

3-[4-[4-(6-Fluorobenzo[*b*]thien-3-yl)-1-piperazinyl]butyl]-2,5,5-trimethyl-4-thiazolidinone Maleate (54). A mixture of 3-(4-bromobutyl)-2,5,5-trimethyl-4-thiazolidinone (**62f**) (4.00 g, 14.3 mmol), 1-(6-fluorobenzo[*b*]thien-3-yl)piperazine (3.71 g, 15.7 mmol), K_2CO_3 (6.00 g, 43.4 mmol), NaI (400 mg), and acetonitrile was heated at 50 °C under nitrogen. After 16 h, the mixture was filtered, the insolubles were washed with dichloromethane (100 mL), and the filtrate was concentrated *in vacuo*. The residue was taken up in dichloromethane (200 mL), washed with 5% NaOH (100 mL), H_2O (100 mL), and dried over Na_2SO_4. The dichloromethane was removed *in vacuo* and the resulting viscous liquid chromatographed on silica gel, eluting with 5% methanol in dichloromethane, to give 3.40 g of a viscous amber liquid (R_f = 0.58, 10% methanol in dichloromethane). The maleic acid salt of the amine was prepared and recrystallized from ethanol to afford 2.09 g (26.5%) of a white solid: mp 170−171 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.60 (dd, 1 H, J = 5.0, 8.8 Hz), 7.50 (dd, 1 H, J = 2.3, 8.7 Hz), 7.15 (ddd, 1 H, J = 2.3, 8.8, and 8.8 Hz), 6.73 (s, 1 H), 6.30 (s, 2 H), 4.66 (q, 1 H, J = 6.1 Hz), 3.73−3.16 (m, 12 H), 1.87−1.65 (m, 4 H), 1.57−1.52 (two s superimposed on d, 9 H); mass spectrum (EI, 70 eV) M^+ 435. Anal. ($C_{22}H_{30}N_3FOS_2 \cdot C_4H_4O_4$) C, H, N.

3-(4-Bromobutyl)-2-methyl-4-thiazolidinone (62c). To a stirred suspension of 2-methyl-4-thiazolidinone (**61c**) (20 g, 171 mmol) in 500 mL of anhydrous dimethylformamide under N_2 was added in one portion KOH (19.1 g, 342 mmol). Stirring was continued for 30 min, providing a yellow solution. At this time, 1,4-dibromobutane (61 mL, 513 mmol) was added in one portion. After 1 h, TLC (silica, EtOAc) showed no remaining starting material. The mixture was quenched in 600 mL water, and the aqueous phase was extracted exhaustively with EtOAc. The organic extracts were combined and dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Chromatography of the residue using a 3:1 hexanes/EtOAc eluent provided 16 g (63.6 mmol, 37%) of the product as an oil, homogeneous by TLC (silica, 2:1 hexanes/EtOAc, R_f = 0.27): ^{1}H NMR ($CDCl_3$) δ 4.75 (q, J = 6 Hz, 1 H), 3.85−3.40 (m, 5 H), 3.20−3.01 (m, 1 H), 1.91−1.57 (m, 4 H), 1.54 (d, J = 6 Hz, 3 H); mass spectrum (Br^{79} and Br^{81}, EI, 70 eV) M^+ 251 and 253. Anal. ($C_{18}H_{14}BrNOS$) C, H, N.

3-(4-Bromobutyl)-2,5,5-trimethyl-4-thiazolidinone (62f). To a −73 °C solution of 2-methyl-3-(4-bromobutyl)-4-thiazolidinone (**62c**) (6.00 g, 23.8 mmol), methyl iodide (11.0 g, 77.4 mmol), and THF (50 mL) under nitrogen was added lithium bis(trimethylsilyl)amide (50.0 mmol) in THF (50 mL) at a rate to maintain the internal temperature <−55 °C. The resulting amber solution was stirred at <−55 °C for 10 min and then allowed to warm to −40 °C, at which temperature 1 N HCl (250 mL) was added. The aqueous mixture was extracted with 25% benzene/ether (3 × 125 mL). The combined extracts were washed with brine (200 mL), dried (Na_2SO_4), and concentrated to a brown liquid which was chromatographed on silica gel, eluting with a 35−65% gradient of ethyl acetate in hexanes, yielding 5.07 g (76%) of a liquid, R_f = 0.37 (30% ethyl acetate in hexanes). The liquid was distilled to give 3.80 g of a clear liquid: bp 109−114 °C at 0.20 mmHg; ^{1}H NMR ($CDCl_3$) δ 4.68 (q, J = 6 Hz, 1 H), 3.77−3.60 (m, 1 H), 3.45 (t, J = 6 Hz, 2 H), 3.28−3.03 (m, 1 H), 1.93−1.60 (m, 4 H), 1.55−1.51 (two singlets superimposed on a doublet, 9 H); mass spectrum (Br^{79} and Br^{81}, 70 eV, EI) M^+ 279 and 281. Anal. ($C_{10}H_{18}BrNOS$) C, H, N.

3-(4-Bromobutyl)-1-thia-3-azaspiro[4.4]nonan-4-one (62i). To a −76 °C solution of 3-(4-bromobutyl)-4-thiazolidinone (**62a**) (4.75 g, 19.9 mmol) and THF (120 mL) under nitrogen was added lithium bis(trimethylsilyl)amide (20.3 mmol) in THF (20.3 mL) rapidly, immediately followed by 1,4-diiodobutane (15.5 g, 50.0 mmol). After 12 min, a solution of lithium bis(trimethylsilyl)amide (62.0 mmol) in THF (62 mL) was added over a period of 0.5 h. The resulting cloudy yellow reaction was allowed to warm to −45 °C, at which temperature 1 N HCl (250 mL) was added. The resulting aqueous mixture was extracted with ether (4 × 110 mL). The combined extracts were washed with brine (250 mL), dried (Na_2SO_4), and concentrated to a dark brown liquid. The liquid was chromatographed on silica gel (elution with 40% ethyl acetate in hexanes) to give 3.34 g (57.5%) of a faint yellow liquid (R_f = 0.49). The liquid was distilled using a short-path distillation apparatus at 0.20 mmHg (bath temperature 120−140 °C) to give 2.35 g (40.4%) of a clear oil: ^{1}H NMR ($CDCl_3$) δ 4.32 (s, 2 H), 3.54−3.36 (m, 4 H), 2.40−2.16 (m, 2 H), 2.00−1.60 (m, 10 H). Mass spectrum (Br^{79} and Br^{81}, EI, 70 eV) M^+ 291 and 293. Anal. ($C_{11}H_{18}BrNOS$) C, H, N.

3-(4-Bromobutyl)-5-(1-hydroxy-1-methylethyl)-4-thiazolidinone (62m). To a solution of 3-(4-bromobutyl)-4-thiazolidinone (**62a**) (10.0 g, 42 mmol) and tetrahydrofuran (200 mL) cooled to −78 °C under nitrogen was added rapidly a solution of lithium bis(trimethylsilyl)amide (44 mmol) and tetrahydrofuran (44 mL) followed immediately by acetone (10 mL, 0.137 mol). The resulting solution was stirred at −78 °C for 20 min and removed from the cold bath, and 0.5 N HCl (250 mL) was added. The aqueous mixture was placed under reduced pressure to remove some of the tetrahydrofuran. The resulting mixture was extracted with ether (3 × 125 mL). The combined extracts were washed with brine (200 mL), dried (Na_2SO_4), and concentrated to a viscous brown liquid. The liquid was chromatographed on silica gel, eluting with 50−100% ethyl acetate in hexanes, to afford 8.96 g (72.0%) of an amber oil (R_f = 0.37, silica gel, 50% ethyl acetate/hexanes). The oil was dried at 0.2 mmHg for 48 h: ^{1}H NMR ($CDCl_3$) δ 4.40−4.27 (m, 2 H), 3.85 (br s, 1 H), 3.60−3.28 (m, 4 H), 2.00−1.65 (m, 4 H), 1.29 (s, 3 H), 1.27 (s, 3 H); mass spectrum (Br^{79} and Br^{81}, EI, 70 eV) M^+ 296 and 298. Anal. ($C_{10}H_{18}BrNO_2S$) C, H, N.

3-(4-Bromobutyl)-5-(1-fluoro-1-methylethyl)-4-thiazolidinone (62n). To a −67 °C solution of (dimethylamido)-sulfur trifluoride (3.15 g, 23.7 mmol) and dichloromethane (100 mL) under nitrogen was added a solution of 3-(4-bromobutyl)-5-(1-hydroxy-1-methylethyl)-4-thiazolidinone (**62m**) (7.01 g, 23.7 mmol) and dichloromethane (30 mL) dropwise over a period of 35 min. The resulting solution was allowed to warm to ambient temperature over a period of 80 min. Cold H_2O (75 mL) was added, and the layers were separated. The organic layer was washed successively with H_2O (75 mL) and then brine (100 mL), dried ($NaSO_4$), and concentrated to a yellow-orange liquid. The liquid was purified by chromatography on silica gel, eluting with 40−60% ethyl acetate in hexanes, to give 6.01 g (85.1%) of a yellow oil. A 2.07 g sample of oil was distilled using a molecular still (120 °C/0.20 mmHg) to give 1.75 g of a clear liquid (R_f = 0.46, silica gel, 40% ethyl acetate/hexanes): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.42 (d, J = 7 Hz, 1 H), 4.23 (d, J = 7 Hz, 1 H), 3.88 (d, J = 15 Hz, 1 H), 3.60−3.27 (m, 4 H), 1.98−1.40 (m with two d (each 21 Hz), 10 H); mass spectrum (Br^{79} and Br^{81}, CI, 70 eV) M^+ 298 and 300. Anal. ($C_{10}H_{17}BrFNOS$) C, H, N.

2-(Benzylthio)-4-chlorobenzoic Acid (63a). A mixture of 2,4-dichlorobenzoic acid (91.0 g, 476 mmol), benzyl mercaptan (58.6 mL, 500 mmol), K_2CO_3 (131 g, 829 mmol), and CuCl (1.0 g) in 700 mL of sieve-dried DMF was heated with stirring to 120 °C under N_2. After 18 h the mixture was allowed to cool to room temperature and filtered, and the residual solids were triturated with DMF, decanted, and combined with the filtrate. This DMF phase was diluted with 800 mL of H_2O, and the pH was adjusted with 6 N HCl to pH 1 with precipitation of a white solid. The solid was collected, washed with H_2O, and recrystallized from acetone/H_2O to provide 84.42 g (303.6 mmol, 63.8%) of a white solid: mp 205−208 °C; homogeneous by TLC (silica, EtOAc, R_f = 0.34); ^{1}H NMR (DMSO-d_6) δ 13.2 (br s, 1 H), 7.89 (d, J = 8.4 Hz, 1 H), 7.53−7.20 (m, 7 H), 4.27 (s, 2 H); mass spectrum (EI, 70 eV) M^+ 278. Anal. ($C_{14}H_{11}ClO_2S$) C, H.

2-(Benzylthio)-4-chlorobenzoic Acid Chloride (63b). A mixture of 2-(benzylthio)-4-chlorobenzoic acid (**3a**) (42.08 g, 150 mmol) and thionyl chloride (13.24 mL, 180 mmol) in 200 mL of toluene and 2 mL of DMF was heated to reflux with stirring. After 3 h, no starting material remained by TLC, and the

mixture was allowed to cool to room temperature. Addition of 700 mL of hexane caused the precipitation of a yellow solid which was collected and air-dried. This provided the desired acid chloride which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 8.00−7.77 (m, 1 H), 7.57−7.19 (m, 7 H), 4.26 (s, 2 H).

3-Hydroxy-6-chloro-1,2-benzisothiazole (65a). 2-(Benzylthio)-4-chlorobenzoic acid chloride (20 g, 67.6 mmol) was stirred in 250 mL of CCl_4. Chlorine gas was bubbled into the stirred mixture until all acid chloride was gone by TLC (approximately 1 h). This solution was added dropwise to a stirred solution of ammonium hydroxide (250 mL), which caused the precipitation of a gummy solid. Stirring was continued for a further 1 h, and then the mixture was carefully acidified to pH 3 with 6 N HCl. The solid precipitate was collected and dried to provide 12.3 g (66.7 mmol) of product, which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 8.19 (s, 1 H), 8.00−7.77 (m, 2 H), 7.48 (d, J = 8 Hz, 1 H).

3,6-Dichloro-1,2-benzisothiazole (65b). A mixture of 3-hydroxy-6-chloro-1,2-benzisoxazole (**65a**) (3.6 g, 19.4 mmol) and PCl_5 (4.4 g) in $POCl_3$ (5 mL) was heated to 120 °C with stirring. After 2 h no starting material remained. The mixture was cooled to room temperature, poured into ice water, and extracted with CH_2Cl_2. The organic phase was drawn off, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by being washed through a short pad of silica gel using hexane as eluent. The fractions containing desired product were combined and concentrated *in vacuo* to provide the product as a fluffy, yellow-white solid. The yield was 3.8 g (18.7 mmol): ^{1}H NMR ($CDCl_3$) δ 8.03−7.87 (m, 2 H), 7.49 (d, J = 9 Hz, 1 H); mass spectrum (EI, 70 eV) M^+ 203.

1-(6-Chloro-1,2-benzisothiazol-3-yl)-3-piperazine (66). A mixture of 3,6-dichloro-1,2-benzisothiazole (2.96 g, 14.6 mmol) and piperazine (12.54 g, 146 mmol) in 150 mL of chlorobenzene was heated to reflux with stirring for 18 h. The mixture was allowed to cool to room temperature, diluted with Et_2O, and partitioned between organics and water. The organic phase was separated, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide 2.0 g of the desired product as a waxy solid: ^{1}H NMR ($CDCl_3$) δ 7.83−7.78 (d superimposed on s, 3 H), 7.31 (d, J = 8.3 Hz, 1 H), 3.54−3.40 (m, 4 H), 3.17−3.03 (m, 4 H), 1.84 (br s, 1 H). Anal. ($C_{15}H_{12}NO_4$) C, H, N.

2-[(Carboxymethyl)phenylamino]-4-fluorobenzoic Acid (70c). A mixture of 2-chloro-4-fluorobenzoic acid (11.5 g, 66.1 mmol), *N*-phenylglycine (12.0 g, 79.4 mmol), K_2CO_3 (41.5 g, 300 mmol), CuCl (0.82 g, 9.1 mmol), and *N*-methylpyrrolidinone (130 mL) was heated under nitrogen between 135 and 152 °C. After 0.5 h the mixture was diluted with H_2O (600 mL), made basic, and extracted with ether (2 × 300 mL). The aqueous layer was acidified with concentrated HCl and extracted with ether (3 × 300 mL). The combined extracts were washed successively with H_2O (350 mL) and then brine (350 mL), dried (Na_2SO_4), and concentrated to a brown foam. Trituration of the foam with dichloromethane/hexane gave 7.24 g of a beige solid. Recrystallization from dichloromethane gave 2.83 g (14.8%) of a white fibrous solid: mp 120−122 °C dec; ^{1}H NMR (DMSO-d_6) δ 12.8 (br s, 2 H), 7.98 (dd, J = 6.8 and 9.4 Hz, 1 H), 7.27−7.05 (m, 4 H), 6.72 (t, J = 7.3 Hz, 1 H), 6.48 (d, J = 7.8 Hz, 2 H), 4.36 (s, 2 H); mass spectrum (EI, 70 eV) M^+ 289. Anal. ($C_{15}H_{12}NO_4$) C, H, N.

Methyl 3-Amino-6-fluorobenzo[*b*]thiophene-2-carboxylate (74a). To a mixture of 2,4-difluorobenzonitrile (229 g, 1650 mmol), lithium hydroxide monohydrate (104 g, 2480 mmol), and dimethylformamide (802 mL) at −10 °C was added methyl thioglycolate (210 g, 1980 mmol) over 7 h via a syringe pump. The reaction was diluted with water (4.0 L), and 73.5 g of crude methyl 3-amino-6-fluorobenzo[*b*]thiophene-2-carboxylate, prepared by a similar procedure, was added to the mixture. The pH of the slurry was raised from 8.5 to 9.4 by the addition of lithium hydroxide monohydrate over 3 h. The white solid was collected by filtration, washed with water (2.0 L), and dried to afford 286 g (64.4%) of a white solid. A 20.0 g sample of crude product was purified by filtration through silica gel with methanol/dichloromethane followed by chromatography on silica gel, eluting with 50% dichloromethane/hexanes, to yield 12.9 g of a white solid, TLC (silica gel, dichloromethane) R_f = 0.31. Recrystallization of the solid from ether/hexanes gave 8.65 g of a fluffy white solid: mp 157−158 °C; ^{1}H NMR ($CDCl_3$) δ 7.57 (dd, J = 5.0 and 8.9 Hz, 1 H), 7.39 (dd, J = 2.3 and 8.7 Hz, 1 H), 7.09 (ddd, J = 2.3, 8.8, and 8.8 Hz, 1 H), 5.89 (br s, 2 H), 3.87 (s, 3 H); mass spectrum (EI, 70 eV) M^+ 225. Anal. ($C_{10}H_8FNO_2S$) C, H, N.

1-(6-Fluorobenzo[*b*]thien-3-yl)-piperazine Maleate (76a). A mixture of methyl-6-fluoro-3-aminobenzo[*b*]thiophene-2-carboxylate **(74a)** (20.11 g, 89.3 mmol), 1-methylpiperazine (13.10 g, 180 mmol), and 1-methyl-2-pyrrolidinone (100 mL) was heated at 176 °C under nitrogen for 2 h. The resulting dark solution was diluted with H_2O (400 mL) and extracted with ether (3 × 200 mL). The combined extracts were washed with H_2O and brine and dried (sodium sulfate). Concentration under reduced pressure gave 9.32 g of crude 3-amino-6-fluorobenzo[*b*]thiophene **(75a)** as a brown liquid (R_f = 0.39, 50% ethyl acetate in hexanes, silica gel). A mixture of the crude intermediate (**75a**, 9.32 g), piperazine (15.0 g, 174 mmol), and 1-methyl-2-pyrrolidinone (100 mL) was heated between 186 and 192 °C under nitrogen for 14 h. The black mixture was cooled, diluted with H_2O (500 mL), and extracted with ether (3 × 200 mL). The combined extracts were washed successively with H_2O (250 mL) and then brine (250 mL) and dried (potassium carbonate). Concentration under reduced pressure gave a brown liquid. Chromatography of the crude product on silica gel (eluting with 30% methanol in dichloromethane) gave 3.03 g of a yellow viscous liquid (R_f = 0.22, 30% methanol in dichloromethane, silica gel). The free amine was taken up in 2-propanol (20 mL), and a solution of maleic acid (1.49 g, 12.8 mmol) in 2-propanol (20 mL) was added. Concentration under reduced pressure of the resulting mixture gave a brown solid (4.52 g). Recrystallization from methanol/ethyl acetate afforded 2.72 g (8.80%) of off-white crystals: mp 173−175 °C; ^{1}H NMR (DMSO-d_6) δ 20.1 (br s, 1 H), 8.80 (br s, 2 H), 7.90−7.80 (m, 2 H), 7.28 (ddd, J = 2.4, 9.0, and 9.0 Hz, 1 H), 7.07 (s, 1 H), 6.04 (s, 2 H), 3.43−3.13 (m, 8 H); mass spectrum (EI, 70 eV) M^+ 236. Anal. ($C_{12}H_{13}FN_2S\cdot C_4H_4O_4$) C, H, N.

***In Vitro* Studies.** Receptor binding assays in rat membrane preparations were performed according to previously reported procedures.[40]

(A) [^{3}H]Spiperone Binding to Human Dopamine D_4 Sites Transfected into CHO Cells ($D_{4.7}$ Variant from Receptor Biology, Inc.). A competitive receptor binding assay was conducted using membranes expressing human dopamine D_4 receptors (the $D_{4.7}$ isoform) obtained from Receptor Biology Inc. (Baltimore, MD) and [^{3}H]spiperone (0.5 nM; specific activity 113 Ci/mmol) as the labeled ligand. Specific binding is defined using 10 μM haloperidol. The incubation buffer contained 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2 mM $CaCl_2$ (pH 7.7). Compounds of interest were incubated with D_4 membranes and [^{3}H]spiperone at 37 °C for 25 min; incubation was terminated by vacuum filtration through GF/B glass fiber filters and washed with 50 mM Tris buffer containing no salts (pH adjusted to 7.7 with HCl). Filters were presoaked in 50 mM Tris buffer containing 0.05% polyethylenimine. K_i values were calculated from two to four displacement curves (nine concentrations) in duplicate.

(B) [^{3}H]Methylspiperone Binding to Human $5HT_{2A}$ Sites Expressed in BHK Cells. Competitive receptor binding assays are run using membranes obtained from BHK cells expressing the human serotonin $5HT_{2A}$ receptor. This cell line was created by the Molecular Neurobiology Group of Hoechst Marion Roussel. Briefly 0.8 nM [^{3}H]methylspiperone (NEN no. 856, 82 Ci/mmol) is used to label the sites and 10 μM methysergide maleate (RBI M-137) is used to define nonspecific binding. The incubation buffer is 50 mM Tris-HCl (pH 7.7) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Compounds of interest (100 μL/eight concentrations 1.0−0.0003 μM) are incubated with $5HT_{2A}$ membranes (750 μL/50−80 μg) and 0.8 nM [^{3}H]methylspiperone (150 μL) at 37 °C for 30 min in 96 cube-tube styrene plates. Incubation is terminated by vacuum filtration (Tomtech Harvester Mach III) through presoaked Packard GF/B Unifilters treated with 0.05% polyethylenimine. Filters are washed with ice-cold 50 mM Tris-HCl containing no salts. Lastly, filter plates are counted in a Packard TopCount MicroPlate Scintillator.

***In Vivo* Studies. (A) Apomorphine-Induced Climbing in Mice.** This method is a modification of Costall *et al.*[27] and Protais *et al.*[28] 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 (eight per 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.

(B) Apomorphine-Induced Stereotypy in Rats. The procedure is a modification of Janssen *et al.*[29] 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.

(C) Social Interaction in Rats. The procedure is a modification of that used by File[41] and Gardner and Guy.[42] Pairs of male Wistar rats (200−275 g) were placed in an arena (45 × 45 × 40 cm) and allowed to acclimate for 8 min on two consecutive days. On the third day, rats naive to one another were assigned to treatment groups, six pairs per treatment group, and the rats received test drug or vehicle. After 30 or 60 min, the appropriate rats were paired and placed in the test arena for observation of social interaction behavior (time spent sniffing partner, climbing over partner, following partner, mutual grooming, etc.) for 5 min. Social interaction time (in seconds) and total activity (counts per body leingth of movement) for the test groups were compared to control, and statistical significance was determined by a one-way ANOVA and Duncan's multiple range test.

Dopamine Neuron Sampling. Single unit studies were performed according to previously published procedures.[16]

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