Synthesis and Biological Evaluation of 1-(1,2-Benzisothiazol-3-yl)- and (1,2-Benzisoxazol-3-yl)piperazine Derivatives as Potential Antipsychotic Agents

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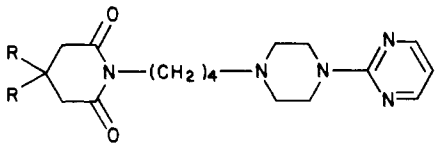
Members of the series of title compounds were tested for potential antipsychotic activity in relevant receptor binding assays and behavioral screens. Structure–activity relationships within the series are discussed. Compound **24** (BMY 13859-1), a (1,2-benzisothiazol-3-yl)piperazine derivative, was selected for further study because of its potent and selective profile in primary CNS tests. It was active in the Sidman avoidance paradigm and blocked amphetamine-induced stereotyped behavior in dogs for up to 7 h. The compound's lack of typical neuroleptic-like effects in the rat catalepsy test and its failure to produce dopamine receptor supersensitivity following chronic administration indicate that it should not cause the movement disorders commonly associated with antipsychotic therapy. Although **24** has potent affinity for dopaminergic binding sites, its even greater affinity for serotonin receptors suggests that a serotonergic component may be relevant to its atypical profile. Compound **24** is currently undergoing clinical evaluation in schizophrenic patients.

Since the introduction of chlorpromazine in the early 1950s, the drug therapy of schizophrenia has involved the use of neuroleptic agents which pharmacologically act as dopamine (DA) antagonists. According to the DA hypothesis of schizophrenia, the symptoms of the disorder arise from dysfunction of central dopaminergic neurotransmission and the therapeutic effect of antipsychotic drugs is attributable to their blockade of DA receptors.[1-4]

While existing antipsychotic drugs are effective in alleviating the symptoms of schizophrenia, their use is frequently attended by the development of extrapyramidal side effects (EPS).[5] Chronic administration of antipsychotics can cause the serious and often irreversible syndrome of tardive dyskinesia.[6] Thus there is a compelling need to develop newer atypical agents which would retain efficacy but have a much lower incidence of debilitating side effects.

On the basis of tranquilizing activity observed for it and related compounds,[7-10] the 1-(pyrimidin-2-yl)piperazine derivative buspirone (**1a**) was initially investigated as a potential antipsychotic but was found not to show useful antipsychotic activity at high doses in schizophrenic patients.[11] Subsequently buspirone has been shown to be



1a, R,R = $-(CH_2)_4-$
b, R = CH_3

a nonsedating, non-benzodiazepine anxiolytic agent with clinical efficacy comparable to that of diazepam.[12-16] The drug has been characterized by preclinical studies as having dopaminergic activity including modest DA D_2 receptor binding[17-19] and recent evidence suggests that it may act as a selective presynaptic DA receptor antagonist.[20] Its dopaminergic component may not be essential to buspirone's anxiolytic effects since the analogue gepirone (**1b**) shows potent antianxiety activity in animal models but lacks affinity for DA receptors.[19,21] Neither drug causes catalepsy in rats, a model predictive of EPS in man, and in fact potently reverse neuroleptic-induced catalepsy.[22,23]

Despite the failure of buspirone as an antipsychotic agent, we felt that appropriately designed structural analogues of the drug might well exhibit efficacy in the treatment of schizophrenia while retaining buspirone's lack of EPS liability. Hypothetical models of the DA receptor have been proposed by Humber et al.[24,25] and Olson et al.;[26] we have previously observed that buspirone satisfies the ligand structure requirements of the Humber model.[19] One

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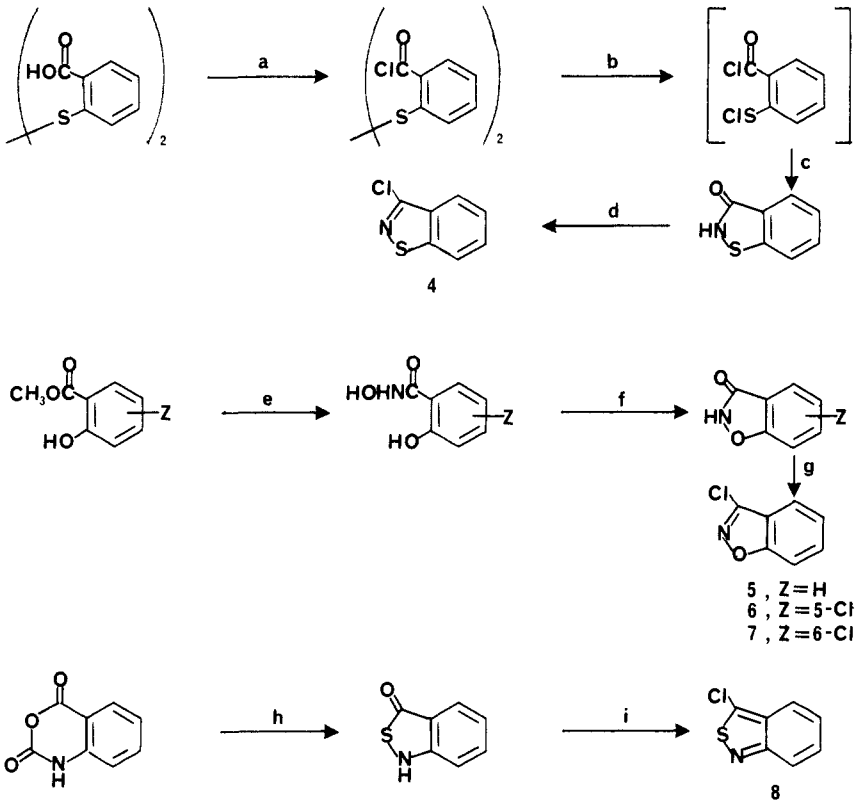
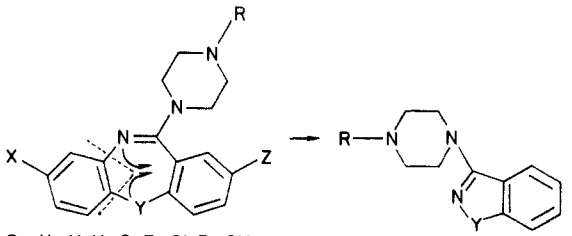
Scheme I[a]



[a] (a) $SOCl_2$, DMF, toluene, 75 °C; (b) Cl_2, CH_2Cl_2; (c) NH_4OH; (d) $POCl_3$, 120 °C; (e) $NH_2OH \cdot HCl$, aqueous NaOH; (f) carbonyldiimidazole, THF, reflux, (g) $POCl_3$, Et_3N, reflux; (h) 1, H_2S, aqueous NaOH, room temperature; 2, 15% H_2O_2; (i) $POCl_3$, pyridine, reflux.

of the key elements of both models is a planar binding site for an aromatic group, and in the case of buspirone, this site weakly accommodates the π-deficient pyrimidine ring. Thus replacement of the pyrimidine ring by a more π-electron-rich aromatic group could result in compounds which, unlike buspirone, have useful affinity for postsynaptic DA receptors. Our prior structure–activity studies on a series of buspirone analogues revealed that heteroarylpiperazine derivatives exhibited a more selective profile of biological activity with fewer undesirable side effects than did the corresponding phenyl compounds. Hence, we chose to focus our quest for atypical antipsychotic agents on buspirone-related structures bearing heteroarylpiperazine moieties, and, in particular, on moieties having some structural relationship to known antipsychotics.

The general structure **2** represents a series of tricyclic antipsychotic drugs including clothiapine (**2a**), loxapine (**2b**), and clozapine (**2c**), the latter being regarded as an atypical agent with a low propensity for causing EPS.[27] As depicted, one may conceptualize a hypothetical cleavage of such tricyclic compounds followed by bond formation between the heteroatoms to afford bicyclic structures **3**. In essence **3** is "derived" from **2** by extrusion of the elements of a fused benzene ring. We conjectured that compounds of type **3** bearing appropriate side chains (R) might exhibit significant DA receptor affinity and possess favorable selectivity with respect to tranquilizing activity vs. side effects. This paper describes the synthesis and biology of a series of compounds of general structure **3** in which the heteroaromatic group is a 1,2-benzisothiazole (Y = S), 1,2-benzisoxazole (Y = O), or a heterocycle isomeric with these systems.



Chemistry. Literature methods were adapted for the preparation of the 3-chloro derivatives of the 1,2-benzisothiazole[28] (**4**), 1,2-benzisoxazole[29-31] (**5–7**), and 2,1-benzisothiazole[32] (**8**) systems as shown in Scheme I. Neat reaction of **4–8** with excess piperazine in a sealed vessel (method A, Scheme II) afforded the heteroarylpiperazines **9–13** (Table I). Compounds **14** and **15** were similarly obtained from the reaction of **4** and **5**, respectively, with homopiperazine. The use of excess piperazine essentially eliminated the formation of bis-substituted piperazines as side products.

Compounds **23–37** and **40** (Table II) were prepared by alkylation of the piperazine intermediates with the appropriate *N*-haloalkyl imides (**16**)[33-36] in an aprotic solvent

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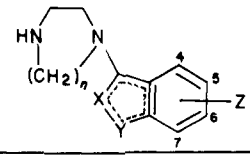
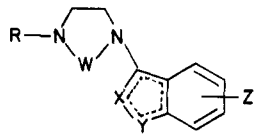
Table I. Bicyclic Heteroarylpiperazines



compd	*n*	X	Y	Z	recryst solvent	yield, %	mp, °C	formula[a]
9	2	N	S	H	MeOH–EtOH	60	280 dec	$C_{11}H_{13}N_3S\cdot HCl$[b]
10	2	N	O	H	MeOH–EtOH	81	326 dec	$C_{11}H_{13}N_3O\cdot HCl$
11	2	N	O	5-Cl	*c*	63	*d*	$C_{11}H_{12}ClN_3O$
12	2	N	O	6-Cl	*c*	79	*d*	$C_{11}H_{12}ClN_3O$
13	2	S	N	H	EtOH	90	274–276	$C_{11}H_{13}N_3S\cdot 2HCl$
14	3	N	S	H	EtOH	65	215–220	$C_{12}H_{15}N_3S\cdot 2HCl$
15	3	N	O	H	*c*	90	*d*	$C_{12}H_{15}N_3O$

[a] C, H, N analyses were within the ±0.4% of calculated values unless otherwise indicated. [b] While a number of these intermediates were characterized as HCl salts, the free bases were employed in subsequent reactions. [c] Compound not purified; crude material used in subsequent step. [d] Not determined.

Table II. Bicyclic Heteroarylpiperazine Derivatives



compd	R	W	X	Y	Z	recrystn solvent	yield,[a] %	mp, °C	formula[b]
23	*A*	$-(CH_2)_2-$	N	S	H	EtOAc	25	124–126	$C_{23}H_{30}N_4O_2S\cdot 2C_7SO_3H\cdot 2H_2O$
24	*B*	$-(CH_2)_2-$	N	S	H	*i*-PrOH	56[c] 88[d]	219–220	$C_{24}H_{32}N_4O_2S\cdot HCl$
25	*C*	$-(CH_2)_2-$	N	S	H	MeCN	45	200–202	$C_{18}H_{22}N_4O_2S_2\cdot 2HCl$[e]
26	*D*	$-(CH_2)_2-$	N	S	H	EtOH	84	214	$C_{22}H_{28}N_4O_2S_2\cdot HCl\cdot 0.1H_2O$[f]
27	*E* (R^1,R^2 = CH_3)	$-(CH_2)_2-$	N	S	H	Et_2O	64	146–147	$C_{22}H_{30}N_4O_2S$
28	*E* (R^1,R^2 = C_2H_5)	$-(CH_2)_2-$	N	S	H	MeCN	29	179–183	$C_{24}H_{34}N_4O_2S\cdot HCl\cdot 0.25H_2O$[f]
29	*E* (R^1 = CH_3, R^2 = *n*-C_3H_7)	$-(CH_2)_2-$	N	S	H	*i*-PrOH	44	163–165	$C_{24}H_{34}N_4O_2S\cdot HCl$
30	*B*	$-(CH_2)_2-$	N	O	H	*i*-PrOH	40	96–98	$C_{24}H_{32}N_4O_3\cdot 0.5H_2O$[f]
31	*B*	$-(CH_2)_2-$	N	O	5-Cl	*i*-PrOH	49	120.5–122	$C_{24}H_{31}ClN_4O_3$
32	*B*	$-(CH_2)_2-$	N	O	6-Cl	*i*-PrOH	10	127.5–128.5	$C_{24}H_{31}ClN_4O_3$
33	*C*	$-(CH_2)_2-$	N	O	H	MeOH	34	104.5–106.5	$C_{18}H_{22}N_4O_3S\cdot 0.25H_2O$[f]
34	*C*	$-(CH_2)_2-$	N	O	5-Cl	*i*-PrOH	29	127–129	$C_{18}H_{21}ClN_4O_3S$
35	*D*	$-(CH_2)_2-$	N	O	H	*i*-PrOH	88	212–214	$C_{22}H_{28}N_4O_3S\cdot HCl\cdot 0.75H_2O$[d]
36	*B*	$-(CH_2)_3-$	N	S	H	*i*-PrOH	8	178–181	$C_{25}H_{34}N_4O_2S\cdot HCl$
37	*D*	$-(CH_2)_3-$	N	O	H	*i*-PrOH	34	163–166	$C_{23}H_{30}N_4O_3S\cdot HCl\cdot 0.25H_2O$[f]
38	*B*	$-CH_2C(CH_3)H-$	N	S	H	EtOH	8	102–104	$C_{25}H_{34}N_4O_2S\cdot 0.25H_2O$[f]
39	*B*	$-C(CH_3)HCH_2-$	N	S	H	EtOH	22	221–223	$C_{25}H_{34}N_4O_2S\cdot HCl$
40	*B*	$-(CH_2)_2-$	S	N	H	EtOH	57	225–227	$C_{24}H_{32}N_4O_2S\cdot 2HCl\cdot 0.5H_2O$[f]
41	*B*	$-(CH_2)_2-$	*F*			MeCN	73	126.5–127.5	$C_{24}H_{32}N_4O_2S$
42	4-$FC_6H_4CO(CH_2)_3-$	$-(CH_2)_2-$	N	S	H	MeOH	45	251–254	$C_{21}H_{22}FN_3OS\cdot HCl$
43	4-$FC_6H_4CO(CH_2)_3-$	$-(CH_2)_2-$	N	O	H	MeOH	25	260–262	$C_{21}H_{22}FN_3O_2\cdot HCl$
44	4-$FC_6H_4CHOH(CH_2)_3-$	$-(CH_2)_2-$	N	S	H	EtOH	69	200–202	$C_{21}H_{24}FN_3OS\cdot HCl$
45	4-$FC_6H_4CHOH(CH_2)_3-$	$-(CH_2)_2-$	N	O	H	EtOH	35	142.5–143.5	$C_{21}H_{24}FN_3O_2$

[a] Yields were not optimized in most cases. [b] C, H, N analyses were within ±0.4% of calculated values unless otherwise indicated. [c] Via method B. [d] Via intermediate 17. [e] N: calcd, 12.09%; found, 13.10%. [f] Level of hydration determined by Karl Fisher water analysis.

such as acetonitrile in the presence of potassium carbonate (method B). An alternate method, employed in the large-scale prepararation of compound **24**, entailed the reaction of the spiro-fused quaternary salt **17** with the appropriate imide. The alkylation of piperazines **9** and **10** with the halide **18** followed by aqueous acidic workup to hydrolyze the ketal protecting group gave the butyrophenones **42** and **43**. Sodium borohydride reduction of the butyrophenones gave carbinols **44** and **45**.

Compound **41** was obtained from the piperazine **19**[37] and 2-chlorobenzothiazole while the neat reaction of **4** with the isomeric α-methylpiperazines **21** and **22** gave **38** and **39**, respectively. Intermediate **21** was the sole product isolated from treatment of halide **20** with 2-methylpiperazine, alkylation occurring exclusively on the less hindered nitrogen. Reaction of **20** with 1-benzyl-3-methylpiperazine followed by catalytic debenzylation afforded **22**.

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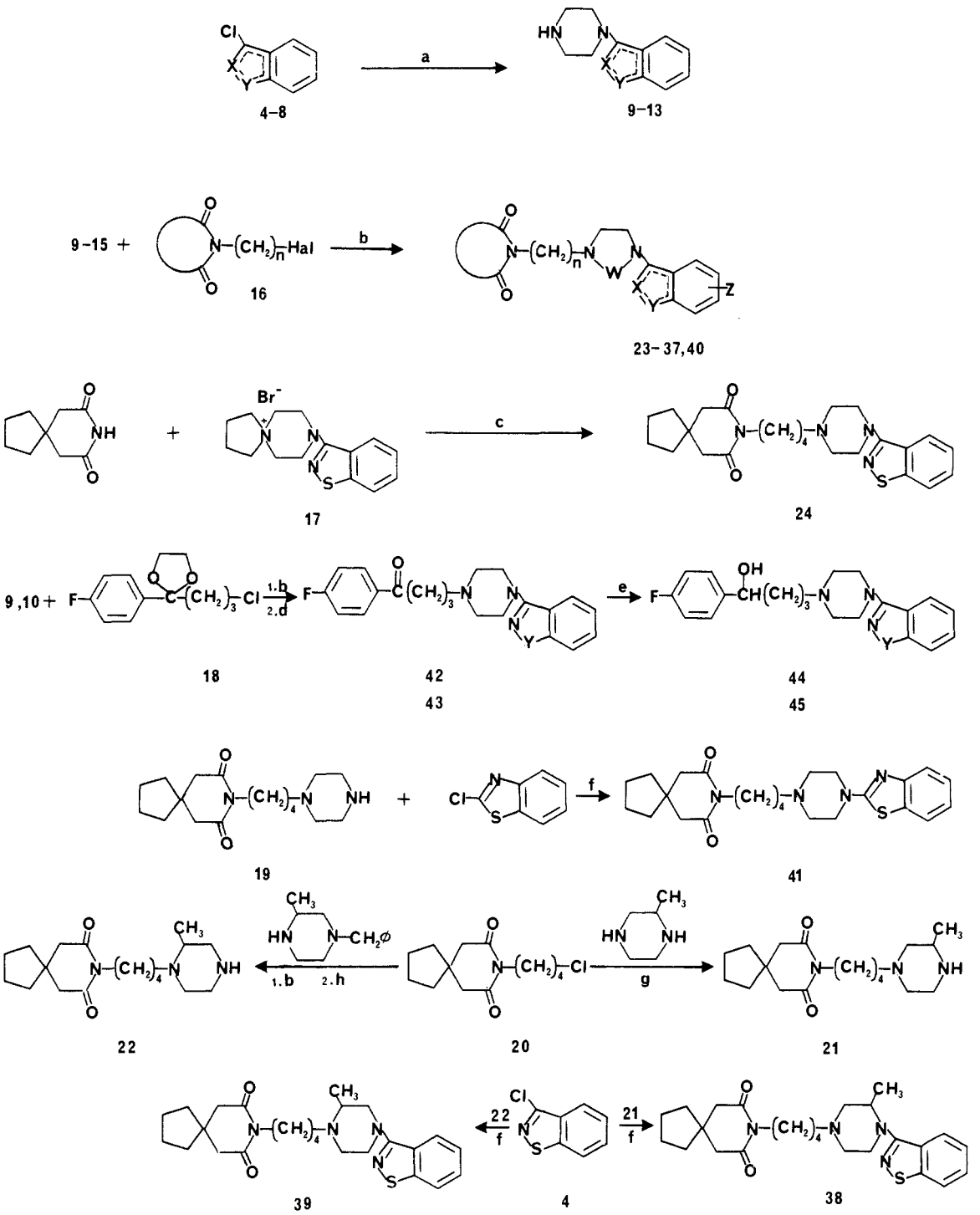
Scheme II[a]



[a] (a) 5–6 equiv of piperazine, 125 °C (method A); (b) K_2CO_3, KI, MeCN, reflux (method B); (c) K_2CO_3, 18-crown-6, xylene, reflux; (d) EtOH, 3 N HCl, reflux; (e) $NaBH_4$, EtOH, room temperature; (f) neat, 120 °C; (g) toluene, reflux; (h) H_2, 10% Pd/C, EtOH.

Biology. All compounds were tested for their in vitro affinity for rat striatal DA D_2 receptors labeled with [^{3}H]spiperone and most were also tested for their binding to cortical α_1-adrenergic receptors vs. [^{3}H]WB-4101. Tranquilizer activity was assessed by measuring the ability of compounds to block the response of rats trained to avoid an electric shock (inhibition of conditioned avoidance response, CAR). Various CAR-active compounds were also screened in rats for their inhibition of apomorphine (APO) induced stereotypy, an in vivo measure of interactions with the DA system, and for their ability to cause catalepsy in rats, a test predictive of potential extrapyramidal effects. Compounds were administered orally in all in vivo tests. We regard compounds having values greater than 1000 nM in the binding assays or values greater than 100 mg/kg in the behavioral paradigms as being inactive in these respective tests.

Results and Discussion

The biological test results in a number of primary screens for both the title compounds and various standard reference agents are shown in Table III.

Structure–Activity Relationships. 1. Side-Chain Modifications. The N-unsubstituted piperazines **9**, **10**,

Table III. Biological Activity of Heteroarylpiperazine Derivatives and Reference Agents in Primary CNS Screens

compd	inhibn of [^{3}H]spiperone binding: IC_{50}, nM	inhibn of [^{3}H]WB-4101 binding: IC_{50}, nM	inhibn of CAR: ED_{50}, mg/kg, po	induction of catalepsy: ED_{50} mg/kg, po	inhibn of apomorphine-induced stereotypy: ED_{50}, mg/kg, po
9	>1000	510	>100		
10	>1000	165	>100		
13	>1000	>1000	13.5 (9.2–19.9)[a]		
23	145	3	>100		
24	8	47	10.5 (5.0–22.1)	84.4 (55.6–128.2)	13.1 (9.3–18.4)
25	53	0.9	27.8 (19.2–40.4)		
26	42	9	11.6 (8.2–16.5)	45.0 (32.1–63.1)	
27	23	4	4.1 (3.0–5.5)	15.2 (10.5–22.0)	4.8 (3.6–6.5)
28	49		35.2 (21.9–56.6)		
29	55	9	24.3 (17.2–34.3)		
30	25	7	10.6 (7.4–15.2)	68.2 (42.8–108.6)	20.2 (14.5–28.1)
31	360	46	>100		
32	59	5	26.0 (18.4–36.8)		
33	240	1	8.3 (6.0–11.7)	11.7 (7.7–17.8)	
34	440	33	>100		
35	78		8.1 (6.6–9.9)	21.5 (15.9–29.2)	
36	235		>100		
37	>1000		>100		
38	64		>100		
39	203		>100		
40	870	33	23.2 (18.3–29.4)	50.0 (39.0–64.1)	65.9 (45.5–95.6)
41	145	454	>100		
42	10	8	7.4 (4.2–13.2)	16.4 (10.8–24.9)	
43	69	5	5.8 (4.1–8.4)	39.9 (29.1–54.6)	
44	2	2	2.4 (1.9–2.9)	5.0 (3.7–6.7)	
45	30	2	1.0 (0.74–1.4)	2.2 (1.4–3.3)	2.3 (1.4–3.8)
haloperidol	7	130	2.8 (2.3–3.5)	0.58 (0.34–1.02)	0.5 (0.4–0.7)
chlorpromazine	40	31	38.7 (32.6–46.0)	4.1 (2.4–7.1)	9.6 (7.1–13.0)
thioridazine	60	65	126.0 (102.2–156.0)	45.2 (27.5–74.3)	280.0 (212–369)
trifluoperazine	16	350	6.9 (6.2–7.7)	3.0 (2.2–4.8)	6.5 (4.6–9.2)
clozapine	440	62	24.1 (20.5–28.2)	>200	49.2 (33.4–72.3)

[a] In this and subsequent tables, 95% fiducial limits are given in parentheses.

and **13** failed to bind to DA receptors, and while **13** exhibited significant CAR activity, both **9** and **10** were inactive. Compound **23**, the only compound bearing an imide moiety appended to an alkylene chain of less than four carbons, was inactive in vivo, whereas its four-carbon homologue **24** was potent in both the CAR and stereotypy tests. While the DA receptor affinity of **23** was at least an order of magnitude less than that of **24**, its α_1 affinity was more than tenfold greater. The attenuation of dopaminergic binding in going from the four- to the three-carbon chain homologue parallels that observed in a series of buspirone analogues.[19]

Among the imide group variations evaluated for the four-carbon chain compounds the azaspirodecanedione moiety as in **24** and **30** appears optimal. Thiazolidinedione derivatives such as **25**, **26**, **33**, and **35** retained good tranquilizing activity but showed a greater tendency to induce catalepsy than did the azaspirodecanediones. Relative to **24** and **30**, the thiazolidinediones had diminished DA binding. Compounds **25** and **33** were quite potent at α_1 receptors which may portend their having a greater liability of associated side effects such as hypotension and sedation. The *gem*-dialkyl imides **27–29** also presented less favorable biological profiles than did **24**. Although the *gem*-dimethyl compound **27** was about twice as potent as **24** in inhibition of both the CAR and APO stereotypy, it was 5–6 times more potent in the catalepsy test. Again, as with the thiazolidinediones, the *gem*-dialkyl derivatives showed less D_2 affinity and greater α_1 affinity than did the corresponding azaspirodecanedione. Comparing the data of **28** and **29** with that of **27** indicates that increasing the bulk of one or both of the alkyl groups causes a reduction in both tranquilizing activity and DA binding. Among the butyrophenones **42** and **43** and corresponding carbinols **44** and **45** were several quite potent compounds. Carbinol **44** had the lowest DA receptor IC_{50} (2 nM) of any compound tested accompanied by very good CAR activity. The ED_{50} values of **45** in the CAR and stereotypy tests were 1.0 and 2.3 mg/kg, respectively, making it the most potent agent in the entire series. Unfortunately, these compounds were again compromised by their enhanced cataleptic activity and α_1 binding.

2. Piperazine Ring Modifications. Permutations of the piperazine ring resulted in marked diminution of both in vivo and in vitro activities. The homopiperazines **36** and **37** as well as the α-methylpiperazines **38** and **39** were inactive in the CAR paradigm and showed DA binding IC_{50} values ranging from 8- to greater than 100-fold higher than that of compound **24**. Since it is unlikely that the structural modifications of the piperazine ring would have rendered **36–39** incapable of permeating the blood–brain barrier, perhaps the lack of tranquilizing activity associated with these analogues is attributable to their weakened interaction with DA receptors.

3. Heteroaryl Group Modifications. Although both the 1,2-benzisothiazole and 1,2-benzisoxazole rings impart desirable biological properties, comparison of the data for compounds **24** and **30** clearly shows the benzisothiazole **24** to be the preferred compound. It is more potent than **30** in several efficacy tests and is less potent in side-effect tests. Introduction of chlorine substituents in the benzisoxazole system as in **31**, **32**, and **34** caused significant attenuation of activity relative to the deschloro prototypes **30** and **33**. Comparing compounds **31** (5-chloro) and **32** (6-chloro) suggests that position 6 of the benzisoxazole ring is more tolerant to substitution than is position 5 (study of substituent effects in the 1,2-benzisothiazole system is under current investigation).

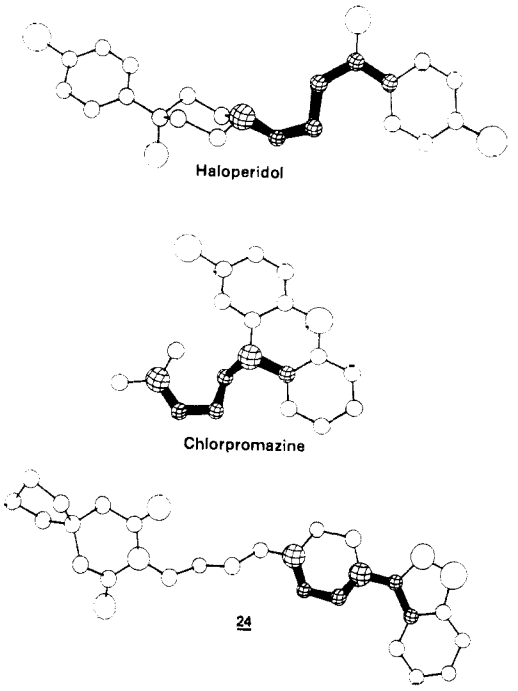


Figure 1. Computer-generated, energy-minimized structures of haloperidol, chlorpromazine, and **24**.[41] S-shaped conformations denoted by bold lines and cross-hatched atoms.

Compound **24** also presents a more favorable biological profile than does the isomeric 2,1-benzisothiazole derivative **40**. However, it is interesting that, while **40** is essentially inactive in the DA binding assay (IC_{50} = 870 nM), it does exhibit at least a modicum of in vivo activity. Since the structural difference between **24** and **40** is simply the transposition of two heteroatoms in the bicyclic moiety, the reason for the considerable discrepancy in DA receptor affinity of the two compounds is ambiguous. Ab initio calculations of the molecular energies of various heteroaromatic molecules have shown that Kekulé structures such as 1,2-benzisothiazole and benzothiazole are somewhat more stable than aza-quinonoid structures like 2,1-benzisothiazole.[38,39] This could be attributable to greater resonance stabilization for the Kekulé structures reflecting enhanced aromatic character relative to the aza-quinonoid systems. It is a reasonable supposition that ligand binding to DA receptors should be roughly proportional to factors influencing the aromaticity of ligand moieties interacting with the planar aromatic binding site of the receptor as depicted in the Humber model.

Even though the aromaticity of benzothiazoles is comparable to that of 1,2-benzisothiazoles, the introduction of a benzothiazole moiety was found to be an unsuitable modification as compound **41** failed to block the CAR response and was nearly 20-fold less potent than **24** in binding to DA receptors.

The more active members of the series (**24**, **27**, **30**, **42–45**), while generally less potent than haloperidol in efficacy tests (CAR, APO stereotypy, DA binding), showed in most cases significantly greater activity than did chlorpromazine, thioridazine, or clozapine. Several of these compounds, in particular **24**, exhibited a markedly lower propensity to produce "cataleptoid" effects than any of the reference agents except clozapine (see subsequent discussion).

On the basis of empirical and structural considerations of classical neuroleptics of the phenothiazine and butyrophenone types, Janssen has suggested that antipsychotic activity is optimized in molecules which can adopt an S-shaped conformation of a six-atom unit, the termini of which are a basic nitrogen atom and a ring carbon of an aromatic ring.[40] As shown in Figure 1, compounds such as **24** can, like chlorpromazine and haloperidol, accommodate the criterion of an S-shaped conformation.

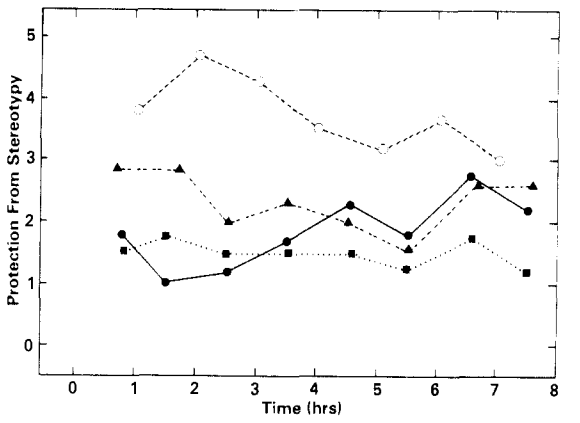


Figure 2. Time-related protection from amphetamine-induced stereotypy in dogs by various oral doses of compound **24** [(●) 1.25 mg/kg, (▲) 2.5 mg/kg, (■) 5.0 mg/kg] vs. control [(○) no drug]. The following ratings, based on intensity of repetititve motor behavior, were assigned on the basis of predominant behavior observed during time-sample periods: 0 = inactive, lying prone; 1 = sitting, looking about, alert; 2 = intermittent locomotion, normal pace; 3 = repetitive path, normal pace; 4 = repetitive path, hyperactive, exaggerated pace; 5 = fixed-pace, repetitive behavior to the exclusion of locomotion.

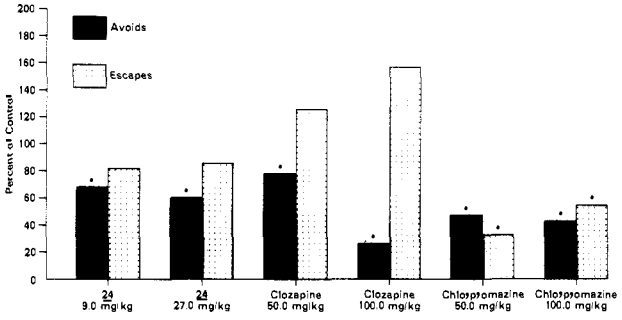


Figure 3. Sidman avoidance activity in rats of oral doses of **24**, clozapine, and chlorpromazine. Statistically significant difference from control denoted by an asterisk.

Compound 24 (BMY 13859-1). Because of its attractive profile of activity in the primary pharmacological tests, compound **24** (BMY 13859-1) was selected for further study. As shown in Figure 2, **24** at oral doses of 1.25–5.0 mg/kg attenuated amphetamine-induced stereotyped behavior in dogs with a duration of action of 5–7 h. Certain amphetamine effects upon nonstereotyped behaviors (orientation, staring, yawning, and grooming) were also antagonized by **24**. Further details of this study have been reported.[42]

In the Sidman avoidance paradigm, **24** was evaluated vs. chlorpromazine and clozapine at oral doses which were approximate multiples of the CAR ED_{50}'s of each drug. The data represented in Figure 3 shows that **24**, like chlorpromazine, tended to reduce both avoids and escapes

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Table IV. Receptor Binding Activities of 24 and Reference Antipsychotic Agents[a]

binding site	24	haloperidol	thioridazine	clozapine
histamine$_1$[b] vs. [^{3}H]pyrilamine	142		99	18
muscarinic[c] cholinergic vs. [^{3}H]QNB	>1000		106	91
serotonin$_1$ (5-HT$_1$)[c] vs. [^{3}H]5-HT	13	>1000		587
serotonin$_2$ (5-HT$_2$)[d] vs. [^{3}H]spiperone	0.4	61	18	21
GABA[e] vs. [^{3}H]muscimol	>1000		>1000	>1000
glycine[f] vs. [^{3}H]strychnine	>1000			
glutamate[d] vs. [^{3}H]kainic acid	>1000			
α_2-adrenergic[d] vs. [^{3}H]clonidine	>1000			
imipramine[c,g] vs. [^{3}H]imipramine	>1000			
high-affinity[d,h] desipramine vs. [^{3}H]desipramine	>1000			

[a] All data reported as IC_{50} values (nM). [b] Rat whole forebrain tissue. [c] Rat hippocampal tissue. [d] Rat cortical tissue. [e] Rat cerebellar tissue. [f] Rat medulla pons tissue. [g] Serotonin uptake site. [h] Norepinephrine uptake site.

from aversive stimulus (electric footshock) although the attenuation of escape response by **24** was not statistically significant. Under our experimental conditions, **24** qualitatively resembles clozapine in that it caused a greater reduction in the avoidance response at both the doses evaluated (clozapine caused nonstatistically significant increases in escapes).

In addition to being evaluated for its binding to DA and α_1-adrenergic receptors, **24** was tested in a number of other receptor binding assays in comparison to reference agents (Table IV). While it was inactive at amino acid and α_2-adrenergic recognition sites and at norepinephrine and serotonin uptake sites, **24** exhibited modest affinity for histamine sites and quite high affinity for the serotonin 5-HT$_1$ and 5-HT$_2$ receptor subtypes (IC_{50} = 13 and 0.4 nM, respectively). The drug was in fact 20 times more potent at 5-HT$_2$ than at DA receptors and its 5-HT$_2$ binding activity was approximately 50–150 times that of the reference agents. It was recently found that **24** potently blocks both amphetamine and LSD discriminative cues.[43] Block of the LSD cue indicates that the drug may have a significant serotonin antagonist component. It has previously been suggested that the mechanism of action and therapeutic efficacy of various antipsychotic drugs may involve interactions with the serotonergic system[44,45] and that the lower incidence of EPS associated with certain atypical antipsychotics may be due to their serotonin antagonist activity.[46,47] The atypical agent, clozapine, has been shown to exhibit serotonin antagonist properties[46,48] and chronic administration of the drug caused marked reduction in cortical serotonin receptor density without enhancement of striatal DA receptor density.[49] Current studies in our laboratories are aimed at elucidating the serotonergic involvement of **24**.

As shown in Table V, prototypical agents like haloperidol, chlorpromazine, and thioridazine should have high potential for causing EPS on the basis of their ratios of activity in efficacy vs. catalepsy tests. Clozapine does not cause catalepsy which is consistent with its low incidence of EPS in man. Although **24** is active in the catalepsy test, its safety ratios indicate that it should have a much lower risk of causing EPS than do marketed antipsychotics of the butyrophenone and phenothiazine types. Furthermore, it was observed that the effects of **24** in the catalepsy test qualitatively differed from those caused by classical neuroleptics. In contrast to neuroleptic (trifluoperazine) treated rats which were quite rigid and which had a high level of muscle tone, the **24** animals appeared flaccid with reduced muscle tone. They thus resembled animals treated with sedating doses of benzodiazepines. Also, buspirone, which has been shown to potently reverse trifluoperazine-induced catalepsy, failed to reverse the response to **24**, suggesting that the effect of the latter in the catalepsy test pharmacologically differs from that of typical neuroleptic drugs. As shown by its inhibition of spontaneous motor activity and potentiation of CNS depressants such as hexobarbital and ethanol (Table V), **24** has significant sedative action. The drug's sedation may be associated with α_1-antagonist activity as evidenced by its α_1-receptor binding and inhibition of norepinephrine-induced lethality. Thus it is possible that the "cataleptoid" behavior caused by **24** following oral administration is attributable to its sedative component rather than to extrapyramidal effects. Recently, McMillen reported that **24** was somewhat less potent than trifluoperazine in inducing a cataleptic effect in female rats when administered subcutaneously; its effect in male animals was significantly weaker.[50]

As depicted in Tables IV and V, respectively, **24** has no affinity for cholinergic receptors and its in vivo anticholinergic action is insignificant. Although the lower incidence of EPS associated with some antipsychotic drugs may be attributable to their anticholinergic activity, this issue is not clearly resolved.[51] Since anticholinergic agents can cause a variety of unpleasant side effects (dry mouth, urine retention, memory impairment), an antipsychotic drug, such as **24**, which has no cholinergic involvement would have a distinct therapeutic advantage provided that it also produced little or no EPS.

The possibility that the long-term usage of an antipsychotic can lead to tardive dyskinesia in man may be predicted by the drug's ability to cause DA receptor proliferation following its chronic administration to laboratory animals; the increase in receptor number after chronic neuroleptic treatment is well documented.[52,53] Table VI shows the effects upon both DA and 5-HT$_2$ receptor binding resulting from 29-day administration of **24** and trifluoperazine to rats at doses comparable to their ED_{50} values for the inhibition of APO-induced stereotypy.

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Table V. Side-Effect Profile of 24 and Reference Antipsychotic Agents

test[a]	24	haloperidol	chlorpromazine	thioridazine	clozapine
CAR ED_{50}/catalepsy ED_{50}	0.12	4.7	9.4	2.8	nil
APO stereotypy ED_{50}/catalepsy ED_{50}	0.16	0.8	2.3	6.2	nil
inhibn of norepinephrine lethality (mouse)	12.5 (8.3–18.8)	26.0 (17.0–39.8)	2.72 (2.12–3.48)	2.2 (1.5–3.1)	3.5 (1.8–6.9)
inhibn of physostigmine lethality (mouse)	294 (176–491)	>80		45.0 (28–71)	5.4 (4.0–7.3)
inhibn[b] of spontaneous motor activity (rat)	8.4	0.73	17.2[c]	33.0	17.5
inhibn[b] of spontaneous motor activity (mouse)	17.3	0.45	3.6[c]	12.8	1.9
hexobarbital potentiation (rat)	6.7 (3.9–11.4)			27.3 (16.3–45.6)	1.69 (1.06–2.70)
ethanol potentiation (rat)	9.3 (6.5–13.1)	0.28 (0.17–0.45)		56.0 (37.2–84.3)	3.23 (2.15–4.85)
ALD_{50}[b] (mouse)	1530	500	250	250	219
ALD_{50} (rat)	1850				

[a] Activities reported as ED_{50} values (mg/kg, po) unless otherwise indicated. [b] Fiducial limits not determined. [c] Administered intraperitonealy.

Table VI. Binding Constants Obtained through Saturation Analyses[a]

	dopamine saturation		5-HT_2 saturation	
treatment	B_{max}, fmol/mg of protein	K_D, pM	B_{max}, fmol/mg of protein	K_D, pM
trifluoperazine	207.0 ± 7.2 (2)[b]	532.5 ± 45.3 (2)	53.43 ± 4.48 (10)[c]	1043 ± 104 (10)
24	160.9 ± 8.6 (2)	333.7 ± 7.6 (2)	70.98 ± 3.52 (9)	963 ± 45 (9)
vehicle	164.8 ± 3.4 (2)	343.9 ± 12.3 (2)	68.17 ± 3.25 (10)	986 ± 89 (10)

[a] Values are mean ± SEM for the number of preparations in parentheses. [b] $p < 0.05$ vs. vehicle (Student's t test). [c] $p < 0.02$ vs. vehicle (Student's t test).

Whereas **24** did not cause a statistically significant alteration in any of the binding parameters, trifluoperazine treatment increased receptor number (B_{max}) for DA binding and decreased both DA and 5-HT_2 receptor affinity. The observed diminution of affinity may be due to the fact that trifluoperazine is not completely eliminated within 24 h of its final dose, thus diluting the receptor radiolabel concentrations; this has been observed previously.[53] Our findings that **24** does not produce DA receptor supersensitivity are consistent with those of McMillen who reported that while the acutely administered drug maximally increased striatal and frontal cortex DA metabolism, its minipump infusion over 2 weeks failed to alter either D_2 receptor B_{max} or the acute response of DA metabolism to haloperidol.[50] We conclude that **24** should not be likely to cause tardive dyskinesia.

In summary, the preclinical evaluation of **24**, a 3-(1-piperazinyl)-1,2-benzisothiazole derivative, indicates that it should exhibit efficacy in the clinical treatment of schizophrenia while accompanied by minimal side effects. Since the initiation of our studies, HRP 913, a 3-substituted 1,2-benzisoxazole has been reported as also having a pharmacological profile suggestive of antipsychotic activity.[54]

Compound **24** is currently undergoing clinical study in schizophrenic patients.

Experimental Section

Chemistry. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The spectra of all reported compounds were consistent with the assigned structures. The IR spectra were recorded on either a Beckman IR-9 or a Nicolet MX-1 FT-IR spectrometer using KBr pellets. All ^1H NMR spectra were recorded on a Perkin-Elmer R-32 spectrometer in either deuteriochloroform with 2% (v/v) tetramethylsilane as the internal reference or perdeuteriodimethyl sulfoxide. Mass spectra were obtained on a Finnegan 4023 GC/MS instrument. Elemental C, H, N analyses were run on a Perkin-Elmer 240B analyzer and Karl Fischer water determinations were made with an Aquatest II apparatus. Analytically pure compounds showed a single spot on Analatech silica gel plates of 0.25-mm thickness and were visualized with UV or I_2. Preparative-scale chromatographic separations were performed via flash chromatography using Aldrich flash chromatography columns packed with Universal Scientific silica 32–63 and eluted with mixtures of $CHCl_3$ or CH_2Cl_2 and MeOH (2–10% v/v). The syntheses of compounds **9** and **24**, which exemplify the general preparative methods A and B, along with that of intermediates **4** and **17** have been conducted on scales of up to 40–50 mol. All other target compounds were prepared on scales of no greater than 0.02 mol. In most cases no efforts were made to optimize yields.

3-Chloro-1,2-benzisothiazole (4). A slurry of 2,2′-dithiosalicyclic acid (2017 g, 6.58 mol) and thionyl chloride (1654 g, 13.83 mol) in toluene (10 L) and DMF (40 mL) was heated at 75 °C for 18 h and the dark solution then cooled to 5–10 °C. The crystalline precipitate was collected by filtration, washed with cold Skelly F, and air-dried to afford 1619 g (71%) of 2,2′-dithiobisbenzoyl chloride, mp 154–156 °C (lit.[55] mp 155–156 °C). Chlorine (239 g, 3.37 mol) was bubbled into a stirred suspension of the acid chloride (1157 g, 3.37 mol) in CH_2Cl_2 (8.5 L) and the resulting solution was added to concentrated NH_4OH (2.9 L) with vigorous stirring. After stirring for 1 h the mixture was filtered, and the solid was collected by filtration, washed with water, and dried at 60 °C in vacuum, providing 902 g of 1,2-benzisothiazol-3(2*H*)-one, mp 155–157 °C (lit.[56] mp 158 °C). A mixture of the benzisothiazolone (818.5 g, 5.41 mol) and phosphorus oxychloride (1114 g, 7.26 mol) was gradually heated over 2 h to 120 °C and then maintained at this temperature for 1.5 h. The hot solution was poured into water (8 L), ice being added to hold the temperature at 45–50 °C. After cooling to room temperature the mixture was extracted with CH_2Cl_2 (4 L) and the extract evaporated to a dark oil. The oil was extracted with Skelly B (4 × 1 L) and the extract treated with Darco (30 g) and Celite (20 g), filtered, and evaporated to provide 743.9 g of a yellow oil. Distillation under reduced pressure gave 707 g (77%) of compound **4** as a colorless oil, bp 80–85 °C (0.75 mm), which readily crystallized, mp 39–41 °C (lit.[57] mp 40 °C).

3-Chloro-1,2-benzisoxazole (5). According to literature methods,[29,31] methyl salicylate was converted to 1,2-benzisoxazol-3(2*H*)-one in 11% overall yield. Heating the latter (21.4 g, 0.158 mol) with phosphorus oxychloride (52.9 g, 0.348 mol) and triethylamine (15.9 g, 0.158 mol) under reflux for 16 h gave upon workup 19.6 g (81%) of **5**[30] as a yellow oil which was used without further purification.

3-Chloro-2,1-benzisothiazole (8). According to literature methods,[32] isatoic anhydride (36.7 g, 0.225 mol) was converted to 2,1-benzisothiazol-3(1*H*)-one (7.45 g, 22%). Treatment of the latter (7.19 g, 0.048 mol) with phosphorus oxychloride (7.2 mL)

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and pyridine (3.6 mL) at 130–140 °C gave upon workup and distillation under reduced pressure 5.45 g (67%) of **8** as a yellow oil, bp 50–57 °C (0.01 mm).

Method A. 1-(1,2-Benzisothiazol-3-yl)piperazine Hydrochloride. A mixture of anhydrous piperazine (1582 g, 18.36 mol) and compound **4** (622 g, 3.67 mol) in a sealed, evacuated 4-L suction flask was heated in a oven at 125 °C for 24 h. The orange melt was then quenched in 4.8 L of ice water and 50% NaOH (293 g, 3.67 mol) was added in one portion. The mixture was extracted with CH_2Cl_2 (3 × 1 L), and the combined extracts were washed with water and evaporated to give 743 g of crude product. Recrystallization from EtOAc gave 548 g (68%) of **9** free base, mp 90–92 °C. A sample of the free base in ether treated with ethanolic HCl and recrystallized from MeOH–EtOH gave analytically pure hydrochloride salt, mp 280 °C dec.

Method B. 8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione (24). A mixture of compound **9** (24.3 g, 0.11 mol), 8-(4-chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione[33] (28.3 g, 0.11 mol), anhydrous K_2CO_3 (32.4 g, 0.23 mol), and KI (3.9 g, 0.023 mol) in MeCN (1 L) was stirred and heated under reflux for 20 h. The reaction mixture was filtered and concentrated in vacuo, and the residue was triturated with ether and refigerated. The resulting solid was collected by filtration and recrystallized from MeCN to afford 31.1 g of **24** free base, mp 124–126 °C. A suspension of the free base in *i*-PrOH was treated with ethanolic HCl to give 29.6 g (56%) of the hydrochloride salt **24**, mp 219–220 °C.

8-(1,2-Benzisothiazol-3-yl)-8-aza-5-azoniaspiro[4.5]decane Bromide (17). A mixture of the piperazine **9** (541 g, 2.47 mol), 1,4-dibromobutane (586 g, 2.71 mol), anhydrous K_2CO_3 (818 g, 5.92 mol), and 18-crown-6 (0.75 g) in 95% EtOH (5.8 L) was heated under reflux with stirring for 24 h. The hot reaction mixture was filtered, and the filtrate was concentrated in vacuo to about one-third the initial volume, refiltered, and cooled. The crystalline precipitate was collected and dried in vacuo at 100 °C to provide 798 g (91%) of **17**, mp 258–259 °C dec. Anal. ($C_{15}H_{20}BrN_3S \cdot 0.33H_2O$) C, H, N.

Preparation of 24 via Intermediate 17. A mixture of the quaternary salt **17** (802.6 g, 2.27 mol), 8-azaspiro[4.5]decane-7,9-dione (378.8 g, 2.27 mol), anhydrous K_2CO_3 (360.0 g, 2.6 mol), and 18-crown-6 (0.75 g) in xylene (4.8 L) was stirred and heated under reflux for 22 h. The hot mixture was filtered, the filtrate concentrated in vacuo, and the crude product recrystallized from MeCN. The free base (900.4 g) was converted to the hydrochloride salt **24** as described above, affording 950 g (88%).

4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-1-(4-fluorophenyl)-1-butanone Hydrochloride (42). A mixture of piperazine **9** (3.86 g, 0.0176 mol), 2-(3-chloropropyl)-2-(4-fluorophenyl)-1,3-dioxolane[58] (4.31 g, 0.0176 mol), anhydrous K_2CO_3 (2.43 g, 0.0176 mol), and KI (0.88 g, 0.0053 mol) in MeCN (180 mL) was heated under reflux for 20 h. The reaction mixture was filtered and the filtrate concentrated in vacuo. The residual oil was dissolved in $CHCl_3$ and filtered and the filtrate concentrated to an oily residue which was taken up in 100 mL of EtOH and 10 mL of 3 N HCl. The mixture was heated under reflux for 15 min, cooled, and diluted with MeCN. The resulting solid was collected and recrystallized from EtOH to afford 3.3 g (45%) of product, mp 251–254 °C.

4-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-1-(4-fluorophenyl)-1-butanone Hydrochloride (43). According to the procedure for compound **42**, the reaction of 2-(3-chlorophenyl)-2-(4-fluorophenyl)-1,3-dioxane (3.62 g, 0.0148 mol) and piperazine **10** (3.24 g, 0.0148 mol) gave 1.5 g (25%) of **43**, mp 260–262 °C.

α-[3-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]propyl]-4-fluorobenzenemethanol Hydrochloride (44). Sodium borohydride (1.0 g, 0.026 mol) was added portionwise to a stirred suspension of **42** (3.34 g, 0.008 mol) in absolute EtOH (150 mL). After stirring for 20 h the mixture was acidified with ethanolic HCl, stirred an additional 2 h, and concentrated in vacuo. The residue was partitioned between $CHCl_3$ and 1 N NaOH and the $CHCl_3$ phase dried ($MgSO_4$) and freed of solvent to give 2.3 g (69%) of **44** free base. A portion of the free base in EtOH treated with ethanolic HCl gave the hydrochloride salt, mp 200–202 °C.

α-[3-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]propyl]-4-fluorobenzenemethanol (45). According to the procedure for compound **44**, sodium borohydride reduction of **43** (2.0 g, 0.005 mol) gave after recrystallization from EtOH 0.64 g (35%) of **45**, mp 142.5–143.5 °C.

8-[4-(3-Methyl-1-piperazinyl)butyl]-8-azaspiro[4.5]decane-7,9-dione (21). A mixture of 8-(4-chlorobutyl]-8-azaspiro[4.5]decane-7,9-dione (**20**; 8.14 g, 0.027 mol) and 2-methylpiperazine (5.41 g, 0.054 mol) in toluene (40 mL) was heated under reflux for 16 h, filtered, and concentrated in vacuo. The residue was redissolved in $CHCl_3$, the solution extracted with 1 N HCl, and the extract shaken with ether. The acid phase was basified with 3 N NaOH and extracted with $CHCl_3$, and the extract was dried ($MgSO_4$) and evaporated to an oil. Distillation under reduced pressure afforded 6.22 g (72%) of a pale yellow oil, bp 180–185 °C (0.01 mm). A sample of the free base treated with ethanolic HCl gave the hydrochloride salt, mp 196–198 °C. Anal. ($C_{18}H_{31}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-3-methyl-1-piperazinyl]-butyl]-8-azaspiro[4.5]decane-7,9-dione Hydrate (38). The piperazine **21** (5.0 g, 0.0155 mol) and 3-chloro-1,2-benzisothiazole (**4**; 2.63 g, 0.0155 mol) were heated for 16 h at 120 °C in a sealed stainless steel bomb. The reaction mixture was partitioned betwen 1 N HCl and ether and the aqueous acidic phase was basified with 3 N NaOH and extracted with $CHCl_3$. The dried ($MgSO_4$) extract was concentrated in vacuo and the residue flash chromatographed on silica gel using $CHCl_3$–MeOH (2% v/v) as eluant. Fractions containing a single component of R_f 0.45 (silica gel, $CHCl_3$–EtOH, 10% v/v) were combined and evaporated. The residue was recrystallized from EtOH to provide 0.55 g (8%) of **38**, mp 102–104 °C.

8-[4-(2-Methyl-1-piperazinyl)butyl]-8-azaspiro[4.5]decane-7,9-dione (22). A mixture of 1-benzyl-3-methylpiperazine[59] (8.45 g, 0.02 mol), 8-[4-chlorobutyl]-8-azaspiro[4.5]decane-7,9-dione (**20**; 6.04 g, 0.02 mol), anhydrous K_2CO_3 (8.3 g, 0.06 mol), and KI (0.5 g, 0.003 mol) in MeCN (200 mL) was stirred and heated under reflux for 18 h. The hot reaction mixture was filtered, the filtrate concentrated in vacuo, and the residue dissolved in $CHCl_3$ and washed with water. The organic layer was dried ($MgSO_4$) and evaporated to provide 7.45 g (91%) of the *N*-benzyl derivative of **22**. Treatment of the free base in EtOH with ethanolic HCl gave the dihydrochloride salt, mp 238–240 °C. Anal. ($C_{25}H_{37}N_3O_2 \cdot 2HCl \cdot 0.25H_2O$) C, H, N. The salt (5.85 g, 0.012 mol) in EtOH (100 mL) was shaken on a parr apparatus in the pressure of 10% palladium on charcoal (0.5 g) until the theoretical consumption of H_2 had occurred. The reaction mixture was filtered, the filtrate concentrated in vacuo, and the residue triturated with EtOH (30 mL). Upon standing, a crystalline precipitate formed, was collected and dried to give 3.85 g (81%) of **22** dihydrochloride, mp 215–217 °C. Anal. ($C_{18}H_{31}N_3O_2 \cdot 2HCl$) C, H, N. An aqueous solution of the salt was made basic with 1 N NaOH and extracted with $CHCl_3$ and the dried ($MgSO_4$) extract concentrated in vacuo to provide the free base in quantitative yield.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-2-methyl-1-piperazinyl]-butyl]-8-azaspiro[4.5]decane-7,9-dione Hydrochloride (39). A mixture of **22** free base (4.76 g, 0.0178 mol) and 3-chloro-1,2-benzisothiazole (**4**; 2.51 g, 0.0178 mol) was heated in a sealed stainless steel bomb at 120 °C for 16 h. The reaction mixture was partitioned betwen 1 N HCl and ether and the aqueous phase basified with 3 N NaOH and extracted with $CHCl_3$. The dried ($MgSO_4$) extract was concentrated in vacuo and the residue dissolved in EtOH and treated with ethanolic HCl. The filtered crude salt was recrystallized from EtOH to afford 1.6 g (22%) of **39**, mp 221–223 °C.

In Vivo Tests. Procedures for the CAR, inhibition of APO stereotypy, and catalepsy tests have been previously described[19] as has the protocol for attenuation of amphetamine stereotypy in dogs.[42] Spontaneous motor activity was assessed by the annular cage method.[60] The following procedures were followed for other in vivo tests.

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Sidman Avoidance Test. This test was performed by modification of published methodolgoy.[61] Rats were placed in an operant situation in which they received electric footshock (0.6 mA) every 30 s unless they "avoided" the shock by pressing the appropriate lever. If rats failed to avoid shock, they were still able to "escape" on-going shock by pressing the appropriate lever. The number of avoids, escapes, shocks taken, and incorrect lever presses were computer monitored. Groups of rats were trained in the paradigm in once-daily sessions until they were performing stably. Rats with established performance stability were orally dosed 30 min prior to a test session. Data were analyzed by comparing each rat's number of avoids and escapes after administration of test compounds to the mean of its previous 3 drug-free days, in a paired-comparison t test. Thus, each rat served as its own control.

Potentiation of Ethanol or Hexobarbital Hypnosis. Five groups of nonfasted male rats were dosed, four groups with test drug and one with vehicle (distilled H_2O or 0.5% methocel). Each of the test-drug-treated groups was dosed intraperitoneally with either hexobarbital sodium (40 mg/kg) or EtOH (10 mL of 20% solution/kg) at different postdrug time intervals (15, 30, 60, or 120 min) and the control group was dosed 15 min after vehicle. Animals were observed for about 10–15 min for loss of righting reflex of 1-min duration. If activity was present, a dose response was run at the determined time of peak effect with use of 10 animals per dose. The determination of ED_{50} values and fiducial limits in this and other behavioral tests was done by using the method of Berkson.[62]

Protection against Norepinephrine Lethality. Two groups of 10 nonfasted male mice were dosed orally with either test compound (at an initial dose of 100 mg/kg) or vehicle (distilled H_2O or 0.5% methocel). At 30-min posttest drug, both groups were injected with norepinephrine at a dose equivalent to an LD_{99} (1.5 mg/kg, iv) and animals were observed for lethality for up to 1 h. Test drugs showing protection against lethality were evaluated at lower dose levels in groups of 10 animals per dose to establish a dose response.

Protection against Physostigmine Lethality. The procedure used is the same as described for the norepinephrine lethality test except that lethality was induced by intraperitoneal dosing of animals with physostigmine (1.25 mg/kg) at 30-min posttest drug.

Receptor-Binding Assays. DA and α_1-adrenergic receptor binding tests were performed as previously described.[19] Binding assays at other receptor sites followed the procedures given in the indicated references: histamine$_1$,[63] muscarinic cholinergic,[64] serotonin$_1$,[65] serotonin$_2$,[66] GABA,[67] glycine,[68] glutamate,[69] α_2-adrenergic,[70] imipramine,[71] desipramine.[72]

Determination of Effects of Chronically Administered 24 on DA and Serotonin Binding. Adult, male Sprague–Dawley rats were treated orally with **24** (11 mg/kg), trifluoperazine (6.5 mg/kg), or vehicle (distilled water, 10 mL/kg), once daily for 29 consecutive days, between 8 and 9 a.m. Doses chosen were comparable to the ED_{50} for inhibition of apomorphine-induced stereotypy. Rats were sacrificed 24 h after the last dose, and brain parts were quickly dissected and frozen for receptor binding studies. The dopaminergic binding determination was made with use of the right striatum from each animal.[73] Tissues were homogenized in ice-cold 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid–KOH, pH 7.4 (20 °C) (Hepes·KOH), and washed twice by centrifugation. The washed membranes were resuspended in exactly 4 mL (approximately 100 volumes/g of original tissue) of 50 mM Hepes·KOH, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 μM pargyline, and 0.1 (w/v) ascorbic acid. The membranes were incubated for 10 min at 37 °C and then held on ice for the assay. Membranes (662–1244 μg of protein) were incubated in quadruplicate for 15 min at 37 °C in the presence of 0.08 nM [^{3}H]spiperone (New England Nuclear, 26.7 Ci/mmol; the dissociation constant had been previously found to be 0.5 nM). One tube contained 10 μM D-(+)butaclamol, which displaced 88.4% of the radioactivity bound to membranes in the other three tubes for control animals. Filtration and counting procedures have been described.[74] For the 5-HT$_2$ assays, cerebral cortices from the right half of the brain were homogenized and washed once by centrifugation in ice-cold Hepes·KOH, pH 7.4. The washed membranes were resuspended in exactly 20 mL of Hepes·KOH, pH 7.4; 10 mL of this homogenate was resuspended in 50 volumes of 50 mM Hepes·KOH, pH 7.4, containing 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% (w/v) ascorbic acid, incubated at 37 °C for 10 min, and then held on ice for the 5-HT$_2$ assay. Membranes (1033–1227 μg of protein) were incubated in the presence of 0.15 nM [^{3}H]spiperone (New England Nuclear, 26.7 Ci/mmol) in quadruplicate for 15 min at 37 °C. One tube contained 1 μM d-lysergide, which displaced 60.7% of the radioactivity bound to membranes in the other three tubes for control animals. In each of the above assays, all tissue samples were analyzed on the same day. Because a significant difference was found between trifluoperazine and control animals in the DA and 5-HT$_2$ assays, saturation analysis assays were run. For the DA assay, left striata were pooled (five animals/pool, with two pools for each treatment) and washed as described. The washed membranes were resuspended in 100 volumes/g of original tissue of 50 mM Hepes·KOH, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 μM pargyline, and 0.1% (w/v) ascorbic acid, incubated at 37 °C for 10 min, and then held on ice. Membranes (1246–1425 μg of protein) were incubated in quadruplicate for 15 min at 37 °C in the presence of 0.02, 0.04, 0.1, 0.4, and 1.0 nM [^{3}H]spiperone (New England Nuclear, 26.7 Ci/mmol). Two tubes contained 10 μM D-(+)butaclamol which displaced 71.5, 77.7, 84.6, 90.7, and 88.1% (respectively, for the concentration given) of the radioactivity bound to membranes in the other two tubes for control animals. For the 5-HT$_2$ assay, left cerebral cortices were washed as previously described. Membranes were then resuspended in 100 volumes/g of original tissue of 50 mM Hepes·KOH, pH 7.4, containing 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% (w/v) ascorbic acid, incubated for 10 min at 37 °C, and then held on ice. Membranes (1073–1351 μg of protein) were incubated in the presence of 0.2, 0.4, 0.8, 1.6, and 3.2 nM [^{3}H]spiperone (New England Nuclear, 26.7 Ci/mmol) in quadruplicate for 15 min at 37 °C. Two tubes contained 1 μM d-lysergide which displaced 63.6, 60.9, 56.9, 47.5, and 34.4% (respectively, for the concentrations given) of radioactivity bound to membranes in the other two tubes for control animals. Protein values for all of the above assays were determined by the method of Lowry et al.[75] using bovine serum albumin as standard.

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Registry No. 4, 7716-66-7; **5**, 16263-52-8; **6**, 16263-53-9; **7**, 16263-54-0; 8, 34231-96-4; **9**, 87691-87-0; **9**·HCl, 87691-88-1; **10**, 87691-89-2; **10**·HCl, 87691-90-5; 11, 99748-43-3; **12**, 87757-07-1; **13**, 87757-03-7; **13**·2HCl, 87757-04-8; 14, 99748-44-4; 14·2HCl,

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Synthesis and Activity of 6-Aryl-3-(hydroxypolymethyleneamino)pyridazines in Animal Models of Epilepsy

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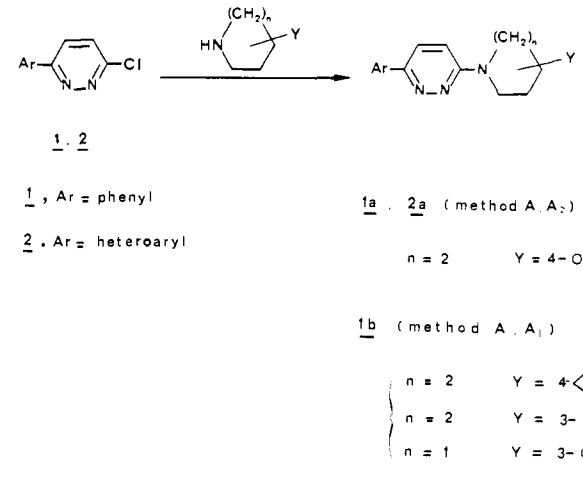
A series of 6-aryl-3-(hydroxypolymethyleneamino)pyridazines derivatives was synthesized and evaluated for anticonvulsant activity. The compounds were screened in mice for their ability to antagonize maximal electroshock- and bicuculline-induced seizures; neurotoxicity was evaluated in the rotorod test. The anticonvulsant activity of the most potent compounds in this series was also examined in kindled amygdaloid rats and in photoepileptic *Papio papio* baboons. Phenobarbital, diphenylhydantoin, carbamazepine, and sodium valproate were used as standard antiepileptic drugs. The structure–activity relationships in this series were examined by either varying the aryl ring in the 6-position of the pyridazine ring or by modifying the 3-amino side chain. Only the compounds with a phenyl ring in the 6-position of the pyridazine ring exhibited appreciable anticonvulsant activity. Furthermore, a 4-hydroxypiperidine side chain in the 3-position of the pyridazine ring appeared essential for anticonvulsant activity. Substituting the phenyl ring with a Cl in the 2-position led to a substantial increase of activity; disubstituting the phenyl ring with a Cl in the 2- and 4-positions yielded the most potent compounds in this series, some of which were as potent or more potent than phenobarbital. Two compounds, 6-(2-chlorophenyl)-3-(4-hydroxypiperidino)pyridazine (**2**) and 6-(2,4-dichlorophenyl)-3-(4-hydroxypiperidino)pyridazine (**3**), were selected for further studies. Clinical evaluation of these compounds is in progress.

In recent years, few new compounds have been developed as antiepileptic drugs,[1] yet the therapeutic efficacy of available antiepileptic drugs cannot be defined as totally satisfactory since 20–30% of patients still experience inadequate seizure control.[2-4] Moreover antiepileptic drugs may cause burdening adverse effects, such as drowsiness, ataxia, gastrointestinal disturbances, hepatotoxicity, gingival hyperplasia, and hirsutism.[4-6] This warrants the continuing search for antiepileptic drugs with more selective anticonvulsant activity and lower toxicity. The precise mechanisms by which clinically useful antiepileptic drugs exert their anticonvulsant activity is yet poorly understood.[7] Several authors[8-12] have emphasized the potential usefulness of γ-aminobutyric acid (GABA) mimetics for the treatment of epilepsy. Phenobarbital, sodium valproate, diphenylhydantoin, and benzodiazepines have

Scheme I



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