Registry No. **1a**, 57695-04-2; **1b**, 75464-77-6; **1b**·HCl, 83547-43-7; **2a**, 83547-39-1; **2a**·3HCl, 83546-65-0; **2a**·3HBr, 83547-38-0; **2b**·3HCl, 83546-66-1; **2c**, 83546-67-2; **2d**, 83546-68-3; **2e**, 83546-69-4; **2f**·3HCl, 83546-70-7; **2g**, 83546-71-8; **2h**·HCl, 83546-72-9; **2i**·HCl, 83546-73-0; **2j**·4HCl, 83546-74-1; **2k**·HCl, 83560-59-2; **2l**·HCl, 83546-75-2; **2m**, 83546-76-3; **2n**, 83546-77-4; **2o**·HCl, 83546-78-5; **2p**, 83546-79-6; **2q**, 83546-80-9; **2r**, 83546-81-0; **2s**, 83546-82-1; 2t, 83546-83-2; **2u**, 83547-40-4; **2u**·2HCl, 83546-84-3; **2v**·2HCl, 83546-85-4; **2w**·2HCl, 83546-86-5; **2x**·2HCl, 83546-87-6; **2y**·2HCl, 83546-88-7; **2z**·2.5H_3PO_4, 83546-89-8; **2aa**·2HCl, 83546-91-2; **2bb**, 83546-92-3; **2cc**·HCl, 83546-93-4; **2dd**, 83546-94-5; **2ee**·$C_4H_4O_4$, 83546-96-7; **2ff**, 83546-97-8; **2gg**·2HCl, 83546-98-9; **2hh**·2HCl, 83546-99-0; **2ii**·2HCl, 83547-00-6; **2jj**, 83547-01-7; **2kk**·2HCl, 83547-02-8; **3a**·3HCl, 83547-17-5; **3b**·2HCl, 83547-18-6; **3c**·2HCl, 83547-19-7; **3d**·2HCl, 83547-20-0; **4a**·HCl, 83547-21-1; **4b**·2H_3PO_4, 83547-23-3; **4c**, 83547-24-4; **5**, 57514-21-3; **6**, 75464-75-4; **7a**, 83547-03-9; **7b**, 83547-04-0; **7c**, 83547-05-1; **7d**, 83547-06-2; **7e**, 83547-07-3; **7f**, 83547-08-4; **7g**, 83547-09-5; **7h**, 83547-10-8; **7i**, 83547-11-9; **7j**, 83547-12-0; **7k**, 83560-60-5; **8**, 83547-13-1; **9a**, 83547-14-2; **9b**, 83547-15-3; **9c**, 83547-16-4; **10**, 31166-44-6; **11**, 83547-25-5; **12**·3HCl, 83547-26-6; **13**·2HCl, 83547-27-7; **14**·2HCl, 83547-28-8; **15**·HCl, 83547-29-9; **16**, 83547-30-2; **17**·2HBr, 83547-41-5; $Cl(CH_2)_6OH$, 2009-83-8; $Br(CH_2)_5COCl$, 22809-37-6; P, 110-85-0; 1-Me-P, 109-01-3; 1-[$(CH_2)_{11}CH_3$]-P, 54722-40-6; 1-cyclohexyl-P, 17766-28-8; 1-($CH_2CH_2SO_2C_2H_5$)-P, 83547-31-3; 1-($CH_2CHOHCH_3$)-P, 1074-54-0; 1-($CH_2CH_2CH_2OH$)-P, 5317-32-8; 1-($CH_2CHOHCH_2OH$)-P, 7483-59-2; 1-[$CH_2CHOHCH_2N(C_2H_5)_2$]-P, 4232-58-0; 1-[$(CH_2)_3N(CH_3)_2$]-P, 877-96-3; 1-[$CH_2CH_2OCH(C_6H_5)_2$]-P, 60703-69-7; 1-[$CH(C_6H_5)(4\text{-}Cl\text{-}C_6H_4)$]-P, 303-26-4; 1-(3,4-$Cl_2$-$C_6H_3$)-P, 57260-67-0; 1-(2-pyridyl)-P, 34803-66-2; 3-CH_3-1-[$CH_2CH_2CH(CH_3)_2$]-P, 83547-32-4; 2,6-$(CH_3)_2$-P, 108-49-6; 1(2*H*)-3,4-H_2-Q, 3476-89-9; 1-($SO_2C_6H_5$)-1(2*H*)-3,4-H_2-Q, 6344-73-6; 1-($SO_2C_2H_5$)-P, 14172-55-5; 1-($CO_2C_2H_5$)-P, 120-43-4; 1-($CO_2CH_2C_6H_5$)-P, 31166-44-6; 1-[$COCH(C_2H_5)_2$]-P, 83547-33-5; 1-($CONHC_2H_5$)-P, 75529-72-5; 1-[$CON(CH_2CH_3)_2$]-P, 119-54-0; 1-[$CON(CH_2CH_2CH_3)_2$]-P, 41340-64-1; 1-[$CON(C_6H_5)_2$]-P, 1804-36-0; 1-($CSNHC_2H_5$)-P, 83547-34-6; 1-[C(=NCN)SCH_3]-P, 83547-35-7; 1-[$CON(CH_2)_4$]-P, 73331-93-8; 1-Me-D, 4318-37-0; *trans*-3,5-Me_2-TM, 83547-36-8; *cis*-3,5-Me_2-TM, 83547-37-9; TM, 123-90-0; TM *S*-oxide, 39213-13-3; TM *S*,*S*-dioxide, 39093-93-1; 3,5-Me_2-M, 123-57-9; 1-(CH_2CH_2OH)-P, 103-76-4; 2-(ethylsulfonyl)ethanol 4-methylbenzenesulfonate, 19387-92-9; phenylmethyl 4-[2-(ethylsulfonyl)ethyl]-1-piperazinecarboxylate, 83547-42-6.

Buspirone Analogues. 1. Structure–Activity Relationships in a Series of *N*-Aryl- and Heteroarylpiperazine Derivatives

J. P. Yevich,* D. L. Temple, Jr., J. S. New, Duncan P. Taylor, and L. A. Riblet

CNS Research–Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, Indiana 47721.
Received June 9, 1982

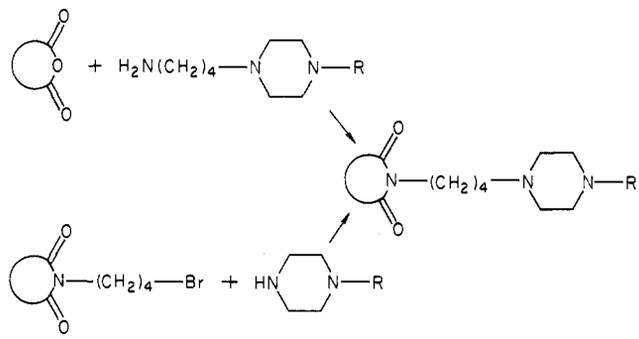
A series of analogues of buspirone was synthesized in which modifications were made in the aryl moiety, alkylene chain length, and cyclic imide portion of the molecule. These compounds were tested in vitro for their binding affinities to rat brain membrane sites labeled by either the dopamine antagonist [^{3}H]spiperone or the α_1-adrenergic antagonist [^{3}H]WB-4101. Compounds were also tested in vivo for tranquilizing properties and induction of catalepsy. Potency at the [^{3}H]spiperone binding site was affected by alkylene chain length and imide portion composition. Nonortho substituents on the aryl moiety had little effect on [^{3}H]spiperone binding affinity. Structure–activity relationships of ortho substituents demonstrated only modest correlations between the receptor binding data and physical parameters of the substituents. The complex nature of the drug–receptor interactions may be understood in terms of the fit of buspirone to a hypothetical model of the dopamine receptor.

Buspirone (1), a member of a series of previously reported *N*-(4-arylpiperazin-1-yl)alkyl cyclic imides,[1,2] has shown anxiolytic activity in several conflict behavioral paradigms[3] and calming effects in aggressive rhesus monkeys.[4] Clinical studies of buspirone have demonstrated it to have a unique anxioselective profile; i.e., its efficacy in the treatment of anxiety neuroses is comparable to that of diazepam but without benzodiazepine-related side effects.[5,6] Such side effects are well-known and documented.[7,8]

Earlier pharmacological studies of buspirone[9,10] led to its evaluation in schizophrenia; however, the drug exhibited only transient activity even at high doses.[11] Subsequent pharmacological investigation, directed at determining its mechanism of action, have demonstrated that while buspirone is without effect upon benzodiazepine binding and GABA binding or uptake, it may possess both agonist and antagonist activity at dopaminergic receptors.[12-19] This profile of mixed agonist and antagonist properties may be relevant to buspirone's mechanism of anxioselective action in which no sedation, anticonvulsant, or muscle relaxant properties are associated with the drug.

In addition to in vivo studies, we have employed the receptor-binding methodology used to characterize buspirone to both investigate a number of newer buspirone analogues and to reexamine previously described members of the series.

Scheme I



* Department of Chemical Research, Mead Johnson Pharmaceutical Division, Evansville, IN 47721.

(1) Y.-H. Wu, K. R. Smith, J. W. Rayburn, and J. W. Kissel, *J. Med. Chem.*, 12, 876 (1969).
(2) Y.-H. Wu, J. W. Rayburn, L. E. Allen, H. C. Ferguson, and J. W. Kissel, *J. Med. Chem.*, 15, 477 (1972).
(3) R. J. Hartmann and J. Geller, *Proc. West. Pharmacol Soc.*, 24, 179 (1981).
(4) E. C. Tompkins, A. J. Clemento, D. P. Taylor, and J. L. Perhach, Jr., *Res. Commun. Psychol. Psychiatry Behav.*, 5, 337 (1981).
(5) H. L. Goldberg and R. J. Finnerty, *Am. J. Psychiatry*, 136, 1184 (1979).
(6) K. Rickels, *J. Clin. Psychiatry*, 42(11), 40 (1981).

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Chemistry. Table I lists experimental and physical data for new compounds. The latter were prepared via the synthetic methodology[1,2,20,21] described for such earlier analogues as **1–5**, **10–17**, and **19–21**, which are included in Tables II and III. Scheme I summarizes the general methods of synthesis employed for all new compounds reported with representative experimental procedures listed below. Starting materials, such as the alkylated imide derivatives, anhydrides, and the substituted piperazine derivatives, if not commercially available were synthesized by known methodology.

8-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione (1). Method A. A mixture containing equimolar amounts of 3,3-tetramethyleneglutaric anhydride and 4-(2-pyrimidinyl)-1-piperazinebutanamine in toluene was refluxed for 10 h under a Dean-Stark trap. The volatiles were removed in vacuo, and the resulting solid was recrystallized from 2-propanol, affording a white product (78%), mp 201.5–202.5 °C (corr).

Method B. A mixture containing 2.5 g (8.2 mmol) of 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione, 1.3 g (8.2 mmol) of 1-(2-pyrimidinyl)piperazine, and 2.2 g (15.9 mmol) of anhydrous potassium carbonate in 150 mL of acetonitrile was refluxed for 12 h. The solution was filtered, and the volatiles were removed in vacuo, affording a solid that upon recrystallization from 2-propanol yielded 2.2 g (71.4%) of white product.

Biology. We determined the antipsychotic potential of buspirone analogues and reference compounds by measuring the ability of various concentrations of a compound to inhibit binding at sites in membranes from rat corpus striatum that had been labeled in vitro with [^{3}H]spiperone. We determined tranquilizer activity by measuring the ability of various doses of an orally administered compound to block the response of rats trained to avoid an electric shock (inhibition of the conditioned avoidance response, CAR). The CAR is a nonspecific test for tranquilizing activity; however, anxiolytics (including benzodiazepines at high doses), certain antidepressants, as well as antipsychotics exhibit activity in this test. Compounds that were shown to be active in the inhibition of the CAR (median effective dose less than 100 mg/kg) were tested for their ability to induce catalepsy after various orally administered doses. We determined the potential for α_1-adrenergic receptor blockade by measuring the ability of various concentrations of a compound to inhibit binding at sites in membranes from rat cerebral cortex that has been labeled with the α_1-adrenergic antagonist [[(2-(2,6-[^{3}H]dimethoxyphenoxy)ethyl]amino]-methyl]benzodioxane ([^{3}H]WB-4101).

Results and Discussion

Biological Results. The ability to inhibit [^{3}H]spiperone binding was affected by the length of the alkylene chain (see Table II). Compounds possessing a four-carbon chain (**1**, **4–7**, **11**, **14**, **15**, and **18–21**) displaced [^{3}H]spiperone at low concentrations (50–960 nM), especially when compared to their ethylene and propylene homologues. For example, in the case of the *N*-(3-chlorophenyl)-piperazines (**16–18**), both two- and three-carbon compounds were unable to displace [^{3}H]spiperone at concentrations as high as 1000 nM, while the four-carbon homologues had an IC_{50} of 236 nM. Similarly, the four-carbon *N*-(2-methoxyphenyl)piperazine (**14**) was very potent in the displacement of [^{3}H]spiperone (IC_{50} = 51 nM), whereas the two- and three-carbon homologues (**12** and **13**) were less potent (IC_{50} = 91 and 430 nM). Again, the four-carbon compound (**7**) of the homologous 3-cyano-2-pyridinyl compounds (**7–9**) was most potent at inhibiting binding at [^{3}H]spiperone-labeled sites (IC_{50} = 110 nM) when compared to the IC_{50}'s for the shorter-chain homologues (**8** and **9**).

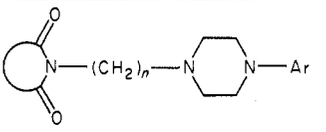
The composition of the imide portion of the molecule also played a role in determining the ability to inhibit [^{3}H]spiperone binding, as shown in Table III. The 2-azaspiro[4.5]decanedione and 2-azaspiro[4.6]undecanedione analogues (**22** and **23**) of the buspirone (**1**) 8-azaspiro[4.5]decanedione system showed some ability to inhibit [^{3}H]spiperone binding, while the 8-azaspiro[4.6]undecanedione analogue (**24**) displayed low potency. Various other nonspiro imide or bicyclo imide derivatives, shown in Table III, exhibited little or no ability to inhibit [^{3}H]-spiperone binding (IC_{50}'s greater than 1000 nM). Compound **28** was more potent than buspirone in displacing [^{3}H]spiperone from postsynaptic dopamine sites; however, it had a much greater interaction with α-adrenergic sites.

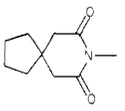
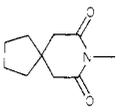
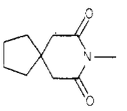
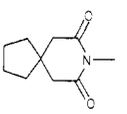
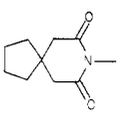
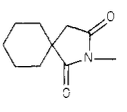
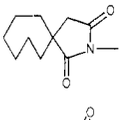
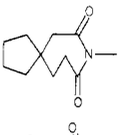
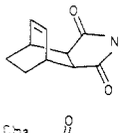
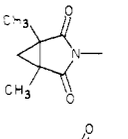
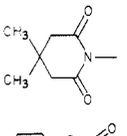
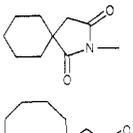
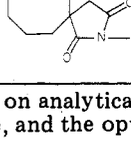
In previous studies,[1,2] inhibition of the CAR was determined in female rats (Harlan Laboratories) following intraperitoneal administration of test compounds. The data shown in Tables II and III were obtained following oral administration of test compounds to male rats (Charles River Laboratories). Generally, the results obtained in the new system paralleled those previously obtained. However, the ability to inhibit [^{3}H]spiperone binding did not parallel the CAR inhibitory activity in every case. For instance, **17** was a potent inhibitor of the CAR (ED_{50} = 28.2 mg/kg) but a weak inhibitor of [^{3}H]-spiperone binding (IC_{50} > 1000 nM). Similarly, **26**, **27**, **29**, and **32** inhibited the CAR (ED_{50} = 52.5–91.6 mg/kg) without displacing [^{3}H]spiperone from its binding sites.

Lack of α_1-adrenergic receptor blockade is considered advantageous for certain drug classes because this phenomenon is associated with undesirable side effects (hy-

(7) T. A. Ban, W. T. Brown, T. DaSilva, M. A. Gagnon, C. T. LaMont, H. E. Lehmann, F. H. Lowg, J. Ruedy, and E. M. Sellers, *Can. Med. Assoc. J.*, **124**, 1439 (1981).
(8) M. H. Lader and H. Peturson, *Biol. Psychiatry*, **16**, 1195 (1981).
(9) L. E. Allen, A. J. Mueller, and J. W. Kissel, *Pharmacologist*, **11**, 247 (1969).
(10) L. E. Allen, H. C. Ferguson, and R. H. Cox, Jr., *Arzneim.-Forsch.*, **24**, 917 (1974).
(11) G. L. Sathananthan, I. Sanghvi, N. Phillips, and S. Gershon, *Curr. Ther. Res.*, **18**, 701 (1975).
(12) D. P. Taylor, D. K. Hyslop, and L. A. Riblet, *Trans. Am. Soc. Neurochem.*, **11**, 193 (1980).
(13) L. A. Riblet, L. E. Allen, D. K. Hyslop, D. P. Taylor, and R. C. Wilderman, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **39**, 752 (1980).
(14) D. P. Taylor, D. K. Hyslop, and L. A. Riblet, *Soc. Neurosci. Abstr.*, **6**, 791 (1980).
(15) D. P. Taylor, E. M. Ashworth, J. A. Becker, and D. K. Hyslop, *Trans. Am. Soc. Neurochem.*, **12**, 280 (1981).
(16) H. C. Stanton, D. P. Taylor, and L. A. Riblet, in "The Neurobiology of the Nucleus Accumbens", R. B. Chronister and J. F. DeFrance, Eds., Haer Institute, Brunswick, ME, 1981, p 316.
(17) D. P. Taylor, H. C. Stanton, and L. A. Riblet, *Int. Congr. Pharmacol., 8th*, 593, abstr (1981).
(18) L. A. Riblet, D. P. Taylor, J. A. Becker, D. K. Hyslop, and R. C. Wilderman, *Soc. Neurosci. Abstr.*, **7**, 865 (1981).
(19) B. A. McMillen, M. K. Sanghera, R. T. Matthews, and D. C. German, *Soc. Neurosci. Abstr.*, **7**, 575 (1981).
(20) U.S. Patent 3 398 151 (1968).
(21) U.S. Patent 3 717 634 (1973).
(22) J. Clark and D. D. Perrin, *Q. Rev., Chem. Soc.*, 18, 295 (1964).
(23) M. T. Tribble and J. G. Traynham, *J. Am. Chem. Soc.*, **91**, 379 (1969).
(24) C. Hansch, A. Leu, S. H. Unger, R. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
(25) F. E. Norrington, R. M. Hyde, S. G. Williams, and R. Wooton, *J. Med. Chem.*, **18**, 604 (1975).

Table I. Buspirone Analogues: *N*-[(4-Substituted-1-piperazinyl)alkyl]-Substituted Cyclic Imides

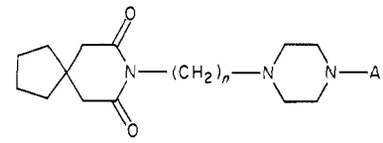


compd		*n*	Ar	recrystn solvent	mp, °C	yield,[a] %	formula
6		4	3-OCH_3-2-C_5H_3N	EtOH/Et_2O	194–196	45.6	$C_{23}H_{34}N_4O_3 \cdot 2HCl$
7		4	3-CN-2-C_5H_3N[b]	acetone/Et_2O	180–182	31.7	$C_{23}H_{31}N_5O_2 \cdot HCl$
8		3	3-CN-2-C_5H_3N	EtOH	196–198	43.7	$C_{22}H_{29}N_5O_2 \cdot HCl$
9		2	3-CN-2-C_5H_3N	EtOH	237–239	56.2	$C_{21}H_{27}N_5O_2 \cdot HCl$
18		4	3-Cl-C_6H_4	CH_3CN	208.5–210	68.4	$C_{23}H_{32}ClN_3O_2 \cdot 2HCl$
22		4	2-$C_4H_3N_2$[c]	EtOH	212–216	96.2	$C_{21}H_{31}N_5O_2 \cdot 2HCl$
23		4	2-$C_4H_3N_2$	EtOH	215–219	37.0	$C_{22}H_{33}N_5O_2 \cdot 2HCl$
24		4	2-$C_4H_3N_2$	*i*-PrOH	197–201	17.8	$C_{22}H_{33}N_5O_2 \cdot 2HCl$
25		4	2-$C_4H_3N_2$	EtOH	246–247	63.1	$C_{22}H_{29}N_5O_2 \cdot HCl$
26		4	2-$C_4H_3N_2$	EtOH/Et_2O	193–195	29.6	$C_{19}H_{27}N_5O_2 \cdot HCl$
27		4	2-$C_4H_3N_2$	*i*-PrOH	192.5–195	22.4	$C_{19}H_{29}N_5O_2 \cdot HCl \cdot 0.25H_2O$
28		2	2-OCH_3-C_6H_4	EtOH	214–215 dec	62.4	$C_{22}H_{31}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$
29		2	2-OCH_3-C_6H_4	xylene	190–196 dec	90.0	$C_{24}H_{35}N_3O_3 \cdot 2HCl \cdot H_2O$

[a] Based on analytically pure sample (within ±0.4% of the theoretical value). Many of these compounds were synthesized only once, and the optimal conditions were not established. [b] C_5H_3N represents pyridyl. [c] $C_4H_3N_2$ represents pyrimidinyl.

potension and sedation). The potential for α_1-adrenergic receptor blockade appears to be dependent on the electronic nature of the aryl ring. The weak affinity for [^{3}H]WB-4101-labeled sites exhibited by the π-deficient pyridine ring containing compound **5** was further decreased by the introduction of an electron-withdrawing 3-cyano group (**7**). However, considerable enhancement of the affinity for α_1-adrenergic receptor binding sites was ob-

Table II. Biological Activity of Substituted Piperazine Derivatives Related to Buspirone: Aryl and Alkylene Chain Modifications



compd	*n*	Ar	inhibn of [^{3}H]spiperone binding: IC_{50}, nM	inhibn of CAR: ED_{50}, mg/kg	inhibn of [^{3}H]WB-4101 binding: IC_{50},[c] nM	induction of catalepsy: ED_{50}, mg/kg
1	4	2-$C_4H_3N_2$[a]	120	47.9 (41.3-55.5)[b]	1400	>200
2	3	2-$C_4H_3N_2$	>1000	>100		
3	5	2-$C_4H_3N_2$	>1000	>100		
4	4	4-CH_3-2-$C_4H_2N_2$	350	52.4 (47.0-58.5)	>1000	>200
5	4	2-C_5H_4N	960	34.0 (30.9-37.5)	275	>120
6	4	3-OCH_3-2-C_5H_3N[d]	120	55.2 (44.4-68.6)	65	>200
7	4	3-CN-2-C_5H_3N	110	45.0 (37.1-54.6)	360	>200
8	3	3-CN-2-C_5H_3N	540	>100		
9	2	3-CN-2-C_5H_3N	640	>100		
10	2	C_6H_5	>1000	>100		
11	4	C_6H_5	430	30.7 (26.1-36.1)		180 (77.0-151.3)[b]
12	2	2-OCH_3-C_6H_4	91	52.1 (43.3-62.6)	>1000	>200
13	3	2-OCH_3-C_6H_4	430	24.1 (16.4-35.5)	19	
14	4	2-OCH_3-C_6H_4	51	20.5 (17.8-23.6)	13	>80
15	4	2-Cl-C_6H_4	290	77.8 (70.0-86.4)		109 (69.6-172.9)
16	2	3-Cl-C_6H_4	>1000	>100		
17	3	3-Cl-C_6H_4	>1000	28.2 (19.9-39.9)	150	>120
18	4	3-Cl-C_6H_4	236	27.6 (20.6-36.9)	23	
19	4	2-CH_3-C_6H_4	390	~100	44	
20	4	2-F-C_6H_4	110	37.1 (33.6-40.9)		91.1 (58.7-141.5)
21	4	2-NO_2-C_6H_4	50	48.8 (43.8-54.3)	110	>200
haloperidol			6.6	2.8 (2.3-3.5)		0.58 (0.34-1.02)
thioridazine			85	126.2 (102.2-156.0)		45.2 (27.5-74.3)
clozapine			890	24.1 (20.5-28.2)		>200
(+)-butaclamol			2.0			

[a] $C_4H_3N_2$ represents pyrimidinyl. [b] 95% fiducial limits are given in parentheses. [c] IC_{50} = 9 nM for phentolamine. [d] C_5H_3N represents pyridyl.

tained by the addition of an electron-donating 3-methoxy substituent (**6**). A number of the phenyl derivatives, particularly the o-methoxy compounds **13** and **14**, exhibited high affinity for [^{3}H]WB-4101-labeled sites with IC_{50}'s of 19 and 13 nM, respectively. Interestingly, the two-carbon homologue (**12**) does not displace the labeled ligand. Compounds possessing the more π-deficient 2-pyrimidinyl moiety displayed little affinity for [^{3}H]WB-4101 binding sites.

Of the various imide groups evaluated, the azaspirodecanedione moiety affords not only the strongest affinity for dopaminergic binding sites but also the most favorable selectivity relative to α_1-adrenergic blocking potential. For example, compounds **24** and **26** have a greater affinity for α_1-adrenergic receptor binding sites than buspirone. Moreover, the ability of the *N*-(2-methoxyphenyl)-piperazines **28**, **30**, and **32** to inhibit [^{3}H]WB-4101 binding is greater than that of the 8-azaspiro[4.5]decane-7,9-dione analogue (**12**).

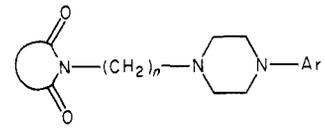
Structure–Activity Relationships. Nonortho substituents appeared to have little effect on activity in 8-azaspiro[4.5]decane-7,9-dione series. The 3-chlorophenyl derivatives **16** and **18** showed approximately the same level of activity in both the inhibition of the CAR and in the displacement of [^{3}H]spiperone compared to their respective phenyl analogues **10** and **11**. Also, the 4-methyl-2-pyrimidinyl compound **4** is quite similar to buspirone (**1**) in both in vitro and in vivo tests.

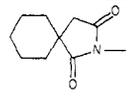
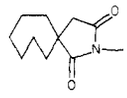
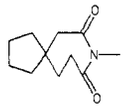
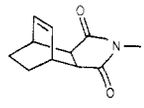
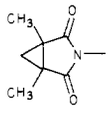
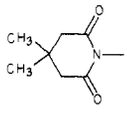
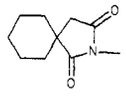
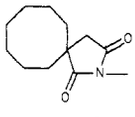
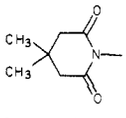
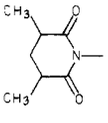
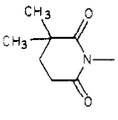
The addition of ortho substituents in both the *N*-phenyl- and *N*-(2-pyridyl)piperazine series resulted in an enhancement of affinity for [^{3}H]spiperone-labeled binding sites with little change in potency to inhibit the CAR. The ratios of the IC_{50}'s for [^{3}H]spiperone displacement for the unsubstituted phenyl (**11**) and 2-pyridyl (**5**) compounds to the IC_{50}'s of the corresponding ortho-substituted derivatives are shown in Table IV. These ratios reflect the relative increase in binding attributable to the various ortho moieties. Also listed in Table IV are electronic (σ), steric bulk (MR), and distributive (π) parameters for each substituent.

There is no apparent correlation of the displacement of spiperone binding with substituent electronic factors because such electronically disparate groups as OCH_3 and NO_2 in the *N*-phenylpiperazine series and OCH_3 and CN in the *N*-(2-pyridyl)piperazines elicit quite similar enhancement of binding. While we have not established whether the basic nitrogen atom bonded to the aromatic portion of the buspirone molecule is necessary for CNS activity, it is possible that structural features that affect the basicity of this nitrogen may consequently influence activity. For example, steric interaction between *o*-aryl substituents and the α-methylene protons of the piperazine ring might destabilize the rotatory conformation of the aryl ring, which permits maximal overlap between the aromatic π electrons and the nitrogen lone pair. This would decrease lone-pair delocalization and, thus, increase the basicity of the aryl-substituted nitrogen.

Even if such a steric effect were operative in the ortho-substituted buspirone analogues, there is no smooth correlation between the binding data and the steric bulk parameters of the various substituents. The groups with the largest MR values (OCH_3, NO_2, and CN) did cause the greatest increase in binding. However, while the steric parameters of these three groups are not appreciably greater than those of CH_3 and Cl, the latter two substituents were 5–6 times less effective at enhancing binding.

Table III. Biological Activity of Substituted Piperazine Derivatives Related to Buspirone Imide Modifications



compd		n	Ar	inhibn of [^{3}H]spiperone binding: IC_{50}, nM	inhibn of CAR: ED_{50}, mg/kg	inhibn of [^{3}H]WB-4101[b] binding: IC_{50}, nM	induction of catalepsy: ED_{50}, mg/kg
22		4	2-$C_4H_3N_2$	630	58.4 (50.1-68.0)[a]	>1000	>200
23		4	2-$C_4H_3N_2$	570	51.5 (43.3-61.2)	>1000	>200
24		4	2-$C_4H_3N_2$	930	>50	820	
25		4	2-$C_4H_3N_2$	>1000	>100		
26		4	2-$C_4H_3N_2$	>1000	91.6 (74.0-113.5)	680	>360
27		4	2-$C_4H_3N_2$	>1000	52.5 (41.2-66.9)	>1000	>200
28		2	2-OCH_3-C_6H_4	92	~50.0	240	
29		2	2-OCH_3-C_6H_4	>1000	69.9 (44.4-110.0)	>1000	>240
30		2	2-OCH_3-C_6H_4	660	91.2 (68.0-122.2)	895	>360
31		2	2-OCH_3-C_6H_4	>1000	>100		
32		2	2-OCH_3-C_6H_4	>1000	55.3 (43.1-71.1)	480	>200

[a] 95% fiducial limits are given in parentheses. [b] IC_{50} = 9 nM for phentolamine.

Furthermore, the *o*-fluoro compound **20** exhibited 3–4 times the binding affinity of the *o*-CH_3 (**19**) and *o*-Cl (**15**) analogues, although the MR value of F is smaller than that of CH_3 or Cl. On the basis of available data, we can only conclude that the steric effect of any ortho substituent will cause an increase in binding relative to ortho-unsubstituted compounds.

There is fairly good inverse correlation between the displacement of spiperone binding and the lipophilicity (π) of the various groups. The approximate order of receptor binding enhancement is $OCH_3 \cong NO_2 \cong CN > F > CH_3 = Cl$, while the order of lipophilicity is $CH_3 \cong Cl > F > OCH_3 = NO_2 = CN$.

In summation, the *N*-phenylpiperazine derivatives tend to exhibit a more antipsychotic-like profile than do their "aza" analogues, the *N*-(2-pyridinyl)- and *N*-(2-pyrimidinyl)piperazines. Phenyl derivatives (e.g., **11**, **13**, **14**, **17**, and **18**) were among the most potent compounds in the CAR, and ortho-substituted *N*-phenylpiperazines, such as **12**, **15**, and **21**, showed the greatest affinity for [^{3}H]spiperone-

Table IV. Correlation of Dopamine Receptor Binding Enhancement by Ortho Substituents with Their Electronic, Steric, and Distributive Parameters

ortho substit	dopamine receptor enhancement	σ_o	MR[e]	π
OCH_3	8.6,[a] 8.0[b]	0[c]	7.87	−0.33[f]
CN	8.7[b]	1.18[d]	6.33	−0.33[f]
NO_2	8.8[a]	1.72[c]	7.36	−0.28[g]
F	4.0[a]	0.47[c]	0.92	0.0[f]
Cl	1.5[a]	0.67[c]	6.03	0.76[f]
CH_3	1.1[a]	0.1[c]	5.65	0.84[f]

[a] Ratio of the IC_{50} for [^{3}H]spiperone displacement for the *N*-phenylpiperazine **11** to that of the appropriate ortho-substituted compound (i.e., o-OCH_3, **14**; o-NO_2, **21**; o-F, **20**; o-Cl, **15**; o-CH_3, **19**. [b] Ratio of the IC_{50} for [^{3}H]spiperone displacement for the *N*-(2-pyridyl)piperazine **5** to that of the appropriate 3-substituted *N*-(2-pyridyl)piperazine (i.e., 3-OCH_3, **6**; 3-CN, **7**). [c] Hammett σ (electronic constant) for ortho substituent obtained from ionization of 2-substituted anilinium ions.[22] [d] σ_o value calculated from hydroxy chemical shift of 2-substituted phenol.[23] [e] Group molar refractivity (steric bulk parameter); values taken from ref 24. [f] Distribution parameter for ortho substituent; values from ref 25. [g] Reference 24.

labeled dopamine receptors. Based on the dopamine theory of schizophrenia,[26] the antipsychotic activity of drugs has been well correlated with their in vitro binding at dopamine receptors.[27-29] Most of the phenyl compounds exhibited significant α-adrenergic activity, which, although not central to antipsychotic efficacy, is a pharmacological characteristic of such major tranquilizers as the butyrophenones, phenothiazines, and clozapine. Several of the *N*-phenylpiperazines also caused catalepsy.

Pharmacological Divergence in Buspirone Analogues. From the initial screening in this series of buspirone analogues, compounds **7** and **27** emerge as interesting leads most likely to be pharmacologically distinct from one another. In Table V the most salient difference between analogue **27** and compound **7** or buspirone is their ability to inhibit [^{3}H]spiperone binding. The lack of affinity of compound **27** for this binding site contrasts sharply with the values for either buspirone or compound **7**. This dichotomy is interesting, since all three compounds possess comparable values in the inhibition of the conditioned avoidance response. Yet the similarities between **27** and buspirone in the rat apomorphine stereotypy, catalepsy, and α-adrenergic receptor binding tests differ significantly from what is observed for **7**. Quantitative cortical EEG studies conducted in cats, a similar capacity to induce contralateral turning in rats with unilateral 6-hydroxydopamine-induced lesions of the substantia nigra, and the ability to reverse phenothiazine-induced catalepsy in rats further corroborate a commonality in pharmacological function between **27** and **1** (Riblet et al., manuscript in preparation).

The fact that **7** shares only a portion of the activity in these screens found in **27** and **1** but still potently inhibits [^{3}H]spiperone binding may originate in the realm of interactive, multisite dopamine receptors. On a continuum between agonist and antagonist properties, analogue **7** offers a biological profile appropriate for an antipsychotic that incorporates an anxiolytic function. In contrast, **27** presents a biological profile that suggests it would be anxioselective in clinical trials. Other analogues of buspirone may be hybrids of both of these.

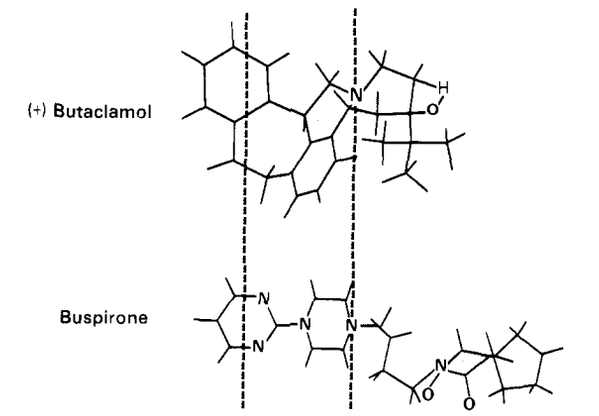


Figure 1. Comparisons of Dreiding models of D(+)-butaclamol and buspirone.

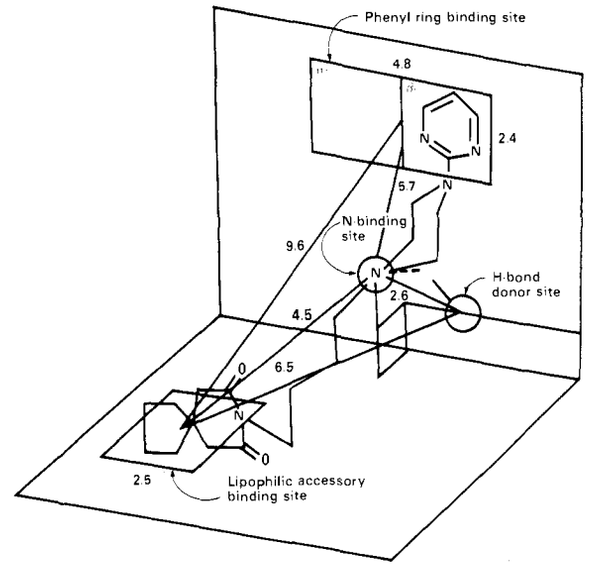
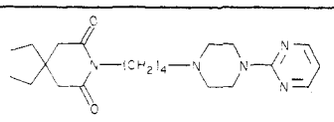
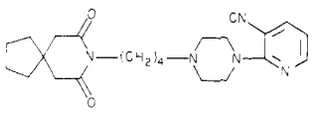
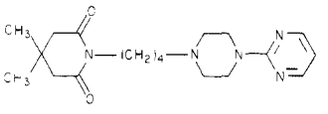


Figure 2. Buspirone binding at the dopamine receptor site (after Humber et al.[30]).

Buspirone Analogues and Dopamine Receptor Topography. Humber and his associates[30-32] have reported extensive studies with D(+)-butaclamol and other benzocycloheptapyridoisoquinolines directed at mapping the structural features of the dopamine receptor site. These studies have described the dopamine receptor site as consisting of an aromatic ring binding site, a nitrogen binding site, and a hydrogen bond donor site as primary binding sites. Importantly, a lipophilic accessory binding site was also described, which is located on the dopamine receptor macromolecule and effectively accommodates the *tert*-butyl groups of butaclamol and certain other of its analogues. The lipophilic accessory binding site apparently provides additional hydrophobic binding for appropriately

(26) S. H. Snyder, S. P. Banerjee, H. I. Yamamura, and D. Greenberg, *Science*, **184**, 1243 (1974).
(27) I. Creese, D. R. Burt, and S. H. Snyder, *Science*, **192**, 481 (1976).
(28) D. C. U'Prichard and S. H. Snyder in "Animal Models in Psychiatry and Neurology", I. Hanin and E. Usdin, Eds., Pergamon, New York, 1979, p 477.
(29) P. Seeman, M. Titeler, J. Tedesco, P. Weinrich, and D. Sinclair, *Adv. Biochem. Psychopharmacol.*, **19**, 167 (1978).
(30) L. G. Humber, F. T. Bruderlein, A. H. Philipp, M. G. Götz, and K. Voith, *J. Med. Chem.*, **22**, 761 (1979).
(31) A. H. Philipp and L. G. Humber, *J. Med. Chem.*, **22**, 768 (1979).
(32) L. G. Humber, A. H. Philipp, F. T. Bruderlein, M. Gotz, and K. Voit, in "Computer-Assisted Drug Design", R. C. Olson and R. E. Christofferson, Eds. (*ACS Symp. Ser.*, **no. 112**), 1979, p 227.

Table V. Selected Pharmacology of Buspirone and Two Analogues

compd	structure	inhibn of [^{3}H]spiperone binding: IC_{50}, nM	inhibn of CAR: ED_{50}, mg/kg	inhibn of apomorphine-induced stereotypy: ED_{50}, mg/kg	induction of catalepsy: ED_{50}, mg/kg	inhibn of [^{3}H]WB-4101 binding: IC_{50}, nM
1		120	48	28	>200	>1000
7		110	45	36.6	>200	360
27		300	53	inactive	>200	>1000

substituted antagonist drugs to provide enhanced blockade of the portion of the dopamine receptor normally occupied by dopamine.

Comparisons of Dreiding models of D(+)-butaclamol with the nonrigid molecule buspirone (Figure 1) lead to the following conclusions: (1) The pyrimidine nucleus of buspirone binds to one face of the phenyl ring binding site, which may also incorporate the piperazine nitrogen as part of the π system. (2) The side chain substituted nitrogen fits the dopamine nitrogen binding site, and the aryl nitrogen may not be necessary for binding. (3) The 8-azaspiro[4.5]decane-7,9-dione moiety fits the lipophilic accessory binding site, which is 4.5 Å from the nitrogen binding site. The quaternary spiro carbon (C-1) of the buspirone 8-azaspiro[4.5]decane-7,9-dione moiety corresponds directly with *tert*-butyl quaternary carbon of D-(+)-butaclamol. Buspirone fitted to the hypothetical dopamine receptor site is illustrated in Figure 2. (4) The 4.5-Å distance between the bound nitrogen and the quaternary center of the azaspirodecanedione is readily achieved with a bent side-chain conformation for the four-carbon alkylene chain. The two-carbon alkylene chain analogue fits more directly; however, this quasi-interaction is less tolerant of minor changes in either the lipophilic binding moiety of the molecule or modification in the aromatic portion of the drug. The fewer degrees of conformational freedom available to rotamers of the two-carbon relative to the four-carbon alkylene chain analogues dictates that more stringent lipophilic, electronic, and steric parameters are required by the former before a useful prototype is established in this series. The three-carbon analogues require a considerably more strained conformation in their methylene chain to achieve the 4.5-Å distance and appear to be the least favorable option in the two-, three-, or four-carbon series. The previously discussed binding data for homologous *N*-arylpiperazines supports the hypothesis that the four-carbon chain affords an optimal fit to the dopamine receptor site, although the relative binding affinity of two- and three-carbon homologues remains somewhat ambiguous.

The single-crystal X-ray structure of buspirone hydrochloride is shown in Figure 3, and relevant bond angle and distance data are listed in Table VI. The distance between the center of the pyrimidine ring and the protonated, piperazine nitrogen (N2) of buspirone is 5.51 Å, which is close to the 5.7-Å distance between the center of the aromatic binding site and nitrogen location site in the Humber receptor model.[29] Thus, the principal binding moieties of buspirone, the heteroaryl group, and the basic nitrogen

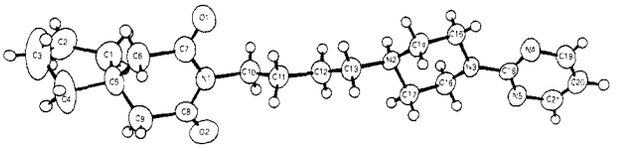


Figure 3. Drawing of a single molecule of buspirone hydrochloride showing 50% probability ellipsoids.

atom, have a mutual spatial relationship that is compatible with dopamine receptor fit. In the solid state, the extended conformation of buspirone's four-carbon chain results in a distance of 9.03 Å between the basic nitrogen (N2) and the spiro carbon (C5); this is considerably greater than the 4.5-Å distance between the nitrogen location site and lipophilic accessory site in the receptor model. However, in a physiological environment the butylene chain of buspirone would not be constrained to the extended conformation; its flexibility would permit the bent conformation optimal for receptor binding as depicted in Figure 2.

The superimposition of buspirone analogues upon the hypothetical receptor may explain the aforementioned effect of ortho substituents. Such substituents (at least those having π or lone-pair electrons) might interact with the α portion of the planar aromatic binding site. A dopamine receptor model recently proposed by Olson et al.[33] comprises a primary (π_1) aromatic binding site and a proximal auxiliary (π_2) site that can accommodate either a second aromatic ring or an electron-rich substituent. Binding to the π_2 site was suggested as being essential for antagonist but not agonist activity. Obviously, the ortho substituent effect that we have described may be attributable to interaction of the substituents with such an auxiliary site.

Structural fit and affinity are both required for receptor binding. Regardless of whether the agonist and antagonist subsites of central dopaminergic receptor complexes are independent entities[34] or are cooperatively linked,[35] it is likely that they have similar, though not identical, ligand structural requirements. It has been suggested that dopamine receptor mapping is not necessarily limited to the

(33) G. L. Olson, H. Cheung, K. D. Morgan, J. F. Blount, L. Todaro, and L. Berger, A. B. Davidson, and E. Boff, *J. Med. Chem.*, 24, 1026 (1981).
(34) I. Creese, T. Usdin, and S. H. Snyder, *Nature (London)*, 278, 577 (1979).
(35) J. E. Leyson, W. Gommeren, and P. M. Laduron, *Arch. Int. Pharmacodyn.*, 242, 312 (1979).

Table VI. X-ray Data for Buspirone Hydrochloride[a]

Bond Distances

atom 1	atom 2	distance, Å	atom 1	atom 2	distance, Å	atom 1	atom 2	distance, Å
O1	C7	1.201 (5)	C2	H3	0.89 (5)	C11	H16	0.97 (4)
O2	C8	1.204 (5)	C2	H4	0.99 (9)	C12	C13	1.504 (7)
N1	C7	1.403 (6)	C3	C4	1.525 (13)	C12	H17	1.01 (5)
N1	C8	1.390 (6)	C3	H5	0.84 (5)	C12	H18	0.97 (4)
N1	C10	1.481 (6)	C3	H6	1.13 (15)	C13	H19	0.97 (5)
N2	C13	1.497 (5)	C4	C5	1.530 (8)	C13	H20	0.96 (5)
N2	C14	1.495 (6)	C4	H7	0.82 (5)	C14	C15	1.491 (7)
N2	C17	1.501 (6)	C4	H8	1.04 (6)	C14	H22	0.94 (4)
N2	H21	0.91 (5)	C5	C6	1.512 (8)	C14	H23	0.95 (4)
N3	C15	1.455 (6)	C5	C9	1.517 (8)	C15	H24	0.96 (5)
N3	C16	1.446 (6)	C6	C7	1.501 (7)	C15	H25	0.96 (4)
N3	C18	1.374 (5)	C6	H9	0.94 (4)	C16	C17	1.497 (6)
N4	C18	1.327 (6)	C6	H10	0.96 (5)	C16	H26	0.98 (4)
N4	C19	1.327 (6)	C8	C9	1.508 (7)	C16	H27	0.99 (4)
N5	C18	1.351 (5)	C9	H11	0.96 (5)	C17	H28	0.94 (3)
N5	C21	1.330 (6)	C9	H12	0.87 (5)	C17	H29	1.00 (5)
C1	C2	1.470 (9)	C10	C11	1.501 (7)	C19	C20	1.365 (7)
C1	C5	1.536 (7)	C10	H13	0.99 (4)	C19	H30	0.91 (4)
C1	H1	0.96 (5)	C10	H14	0.93 (4)	C20	C21	1.358 (7)
C1	H2	0.98 (6)	C11	C12	1.513 (6)	C20	H31	0.96 (4)
C2	C3	1.408 (11)	C11	H15	1.04 (4)	C21	H32	0.99 (5)
Cl	N2	3.097 (4)	Cl	H21	2.19 (5)			
O2	O2	3.143 (7)						
N2	C5	9.03 (1)	N2 to pyrimidinyl ring centroid 5.51 (1)					

Bond Angles

atom 1	atom 2	atom 3	angle, deg	atom 1	atom 2	atom 3	angle, deg	atom 1	atom 2	atom 3	angle, deg
C7	N1	C8	125.5	C4	C5	C6	112.3 (6)	N2	C13	H20	105.0 (3)
C7	N1	C10	115.9 (5)	C4	C5	C9	110.8 (6)	C12	C13	H19	109.0 (3)
C8	N1	C10	118.5 (4)	C6	C5	C9	106.5 (5)	C12	C13	H20	116.0 (3)
C13	N2	C14	112.5 (4)	C5	C6	C7	113.7 (5)	H19	C13	H20	108.0 (4)
C13	N2	C17	113.2 (4)	C5	C6	H9	113.0 (3)	N2	C14	C15	112.0 (5)
C13	N2	H21	106.0 (3)	C5	C6	H10	107.0 (3)	N2	C14	H22	107.0 (3)
C14	N2	C17	107.9 (4)	C7	C6	H9	104.0 (3)	N2	C14	H23	104.0 (3)
C14	N2	H21	107.0 (3)	C7	C6	H10	102.0 (3)	C15	C14	H22	112.0 (3)
C17	N2	H21	110.0 (3)	H9	C6	H10	117.0 (4)	C15	C14	H23	110.0 (3)
C15	N3	C16	115.1 (4)	O1	C7	N1	120.2 (4)	H22	C14	H23	111.0 (4)
C15	N3	C18	120.3 (4)	O1	C7	C6	123.6 (5)	N3	C15	C14	110.4 (5)
C16	N3	C18	120.9 (4)	N1	C7	C6	116.2 (5)	N3	C15	H24	109.0 (3)
C18	N4	C19	114.4 (5)	O2	C8	N1	122.4 (5)	N3	C15	H25	106.0 (2)
C18	N5	C21	114.5 (5)	O2	C8	C9	121.8 (6)	C14	C15	H24	112.0 (3)
C2	C1	C5	107.3 (6)	N1	C8	C9	115.7 (6)	C14	C15	H25	109.0 (3)
C2	C1	H1	117.0 (3)	C5	C9	C8	115.3 (6)	H24	C15	H25	110.0 (4)
C2	C1	H2	112.0 (3)	C5	C9	H11	108.0 (4)	N3	C16	C17	110.9 (4)
C5	C1	H1	111.0 (4)	C5	C9	H12	105.0 (3)	N3	C16	H26	108.0 (3)
C5	C1	H2	108.0 (4)	C8	C9	H11	107.0 (4)	N3	C16	H27	110.0 (3)
H1	C1	H2	101.0 (5)	C8	C9	H12	105.0 (3)	C17	C16	H26	108.0 (3)
C1	C2	C3	107.9 (8)	H11	C9	H12	116.0 (6)	C17	C16	H27	109.0 (2)
C1	C2	H3	119.0 (4)	N1	C10	C11	113.0 (4)	H26	C16	H27	112.0 (4)
C1	C2	H4	106.0 (6)	N1	C10	H13	107.0 (2)	N2	C17	C16	111.2 (5)
C3	C2	H3	125.0 (4)	N1	C10	H14	107.0 (2)	N2	C17	H28	106.0 (2)
C3	C2	H4	100.0 (7)	C11	C10	H13	112.0 (2)	N2	C17	H29	108.0 (2)
H3	C2	H4	94.0 (7)	C11	C10	H14	112.0 (3)	C16	C17	H28	113.0 (2)
C2	C3	C4	107.2 (8)	H13	C10	H14	105.0 (3)	C16	C17	H29	110.0 (2)
C2	C3	H5	110.0 (4)	C10	C11	C12	110.5 (4)	H28	C17	H29	108.0 (4)
C2	C3	H6	122.0 (7)	C10	C11	H15	113.0 (2)	N3	C18	N4	117.0 (4)
C4	C3	H5	121.0 (4)	C10	C11	H16	110.0 (3)	N3	C18	N5	115.8 (5)
C4	C3	H6	83.0 (7)	C12	C11	H15	108.0 (2)	N4	C18	N5	127.2 (4)
H5	C3	H6	111.0 (7)	C12	C11	H16	112.0 (3)	N4	C19	C20	124.2 (6)
C3	C4	C5	102.1 (7)	H15	C11	H16	103.0 (3)	N4	C19	H30	115.0 (3)
C3	C4	H7	103.0 (5)	C11	C12	C13	111.1 (4)	C20	C19	H30	121.0 (3)
C3	C4	H8	78.0 (4)	C11	C12	H17	110.0 (3)	C19	C20	C21	116.0 (6)
C5	C4	H7	114.0 (4)	C11	C12	H18	110.0 (2)	C19	C20	H31	115.0 (3)
C5	C4	H8	118.0 (4)	C13	C12	H17	115.0 (3)	C21	C20	H31	129.0 (3)
H7	C4	H8	127.0 (6)	C13	C12	H18	106.0 (3)	N5	C21	C20	123.7 (5)
C1	C5	C4	103.9 (5)	H17	C12	H18	105.0 (4)	N5	C21	H32	109.0 (3)
C1	C5	C6	111.1 (6)	N2	C13	C12	112.7 (4)	C20	C21	H32	127.0 (3)
C1	C5	C9	112.3 (6)	N2	C13	H19	106.0 (3)				
Cl	H21	N2	173.0 (3)								

[a] Crystal data: Single-crystal diffractometry, graphite monochromatized Cu $K\alpha$, λ = 1.54184 Å. Monoclinical cell parameters and calculated volume: a = 14.699 (5), b = 7.107 (3), c = 21.455 (8) Å, β = 103.77 (3), v = 2176.9 Å^3. For z = 4 and M_r = 421.97, the calculated density is 1.29 g/cm^3. Space group $P2_1/n$. Numbers in parentheses are estimated standard deviations in the least significant digits.

site at which D(+)-butaclamol and other antipsychotics act as antagonists.[30] The rigid D(+)-butaclamol molecule may represent a near-optimal fit to the antagonist conformation of the dopamine receptor. While the flexible buspirone molecule can adopt a conformation compatible with the antagonist site, its much weaker binding relative to D-(+)-butaclamol may reflect the fact that buspirone is not structurally constrained to this conformation. Due to this conformational mobility, buspirone should also be capable of fitting the topography of agonist sites. Indeed, guanyl nucleotides decrease the ability of buspirone to inhibit [^{3}H]spiperone binding in a fashion qualitatively similar to dopamine agonists.[15,16] Moreover, administration of apomorphine to rats with unilateral lesions of the substantia nigra resulted in contralateral turning, just as did buspirone administration as mentioned earlier.[18]

The binding affinity of the π-deficient 2-pyrimidinyl moiety of buspirone to the planar aromatic binding site of the Humber model should not be as great as that of the relatively π-rich aromatic systems common to both neurleptic agents and dopamine agonists such as apomorphine. We have found that *N*-(2-pyrimidinyl)piperazine per se does not displace [^{3}H]spiperone at concentrations as high as 1000 nM. Thus, the additional affinity provided by interaction of the 8-azaspiro[4.5]decane-7,9-dione moiety at a lipophilic accessory binding site is essential to the dopaminergic activity of buspirone.

Catalepsy is considered to be an indicator of a compound's propensity to produce undesirable extrapyramidal side effects (EPS). The lack of catalepsy seen with many buspirone analogues that do bind to the dopamine receptor site probably indicates a selectivity among multiple dopamine receptor sites or selective activity in specific brain tissues. Such selectivity has been noted in the action of other drugs. For instance, classical neuroleptic agents, such as haloperidol, increase dopamine turnover to a greater extent in the corpus striatum than in the mesolimbic system, while the reverse is true of newer agents claimed not to produce extrapyramidal side effects (clozapine, sulpiride, thioridazine, and mezilamine).[36-38]

The mechanism of buspirone's anxiolytic action is not known at this time. It may be attributable to the ability of buspirone to interact pre- and/or postsynaptically with both agonist and antagonist conformations of central dopaminergic receptors. A subtle balance of these multisite interactions could result in anxiolytic action while suppressing the side effects associated with most dopamine agonists and antagonists. Dopamine has been implicated in the etiology and pharmacotherapy of anxiety.[39] Buspirone is also devoid of both the adrenolytic activity common to tricyclic and butyrophenone antipsychotics and the anticholinergic activity of the tricyclics.[14]

Experimental Section

Conditioned Avoidance Response (CAR). Fasted, male Sprague–Dawley rats were trained to climb or hurdle a barrier in a shuttle box within 30 s of being placed in the box. Training consisted of subjecting the animals to 11 trials at 3-min intervals on the 1st day, followed by one reinforcement (foot shock) and two trials daily for 9 days. On the 10th day, groups of 5–10 animals were administered drug or vehicle by oral gavage and tested at the time of maximal activity for suppression of the CAR. Responses were obtained over a 30-min interval (11 trials at 3-min intervals), pooled, and tabulated with responses from other dose levels in order to calculate the dose that suppressed the CAR in 50% of the animals (ED_{50}).[40]

Catalepsy. Groups of 10 nonfasted, male Sprague–Dawley rats were administered doses of drugs by oral gavage and placed in individual animal cages located in a quiet room. At 1-, 2-, and 3-h intervals, we checked the animals for catalepsy by carefully picking them up and placing their front feet on the top edge of the cage. Animals remaining motionless for 30 s were scored as having catalepsy present.[41] The dose of drug that produced catalepsy in 50% of the animals (ED_{50}) was determined according to the method of Berkson.[40]

Apomorphine-Induced Stereotypy. Groups of 10 nonfasted male Sprague–Dawley rats were administered doses of drugs by oral gavage and challenged with apomorphine, 0.5 mg/kg, sc, in a volume of 5 mL/kg, at the previously determined time of peak effect. The animals were placed in individual cages and observed for 30 min for stereotypic behavior: sniffing, licking, rearing, and occasional intermittant biting of the cage.[42] Blockade of all these activities for the full 30 min was considered inhibition. The dose of drug that inhibited stereotypies in 50% of the animals (the ED_{50}) was determined according to the method of Berkson.[40]

Receptor-Binding Assays. Dopamine Receptor Binding. The relative affinities of compounds for dopamine receptor binding sites were evaluated on the basis of their ability to displace [^{3}H]spiperone from washed membranes obtained from rat corpora striata.[43] Male Sprague–Dawley rats were decapitated, the brains were removed, and the corpora striata were dissected and stored at −80 °C until required. Pooled corpora striata were homogenized with a polytron homogenizer, and membranes were recovered and washed once by centrifugation at 39000*g* for 10 min in 40 mL of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)–KOH, pH 7.4 (20 °C). The washed membranes were resuspended in 100 vol of buffer containing 120 mM sodium chloride, 5 mM potassium chloride, 2 mM calcium chloride, 1 mM magnesium chloride, 0.1% (w/v) ascorbic acid, and 10 μM pargyline. This suspension was incubated at 37 °C for 10 min and held on ice for binding. Binding was measured following incubation of 20–50 μg of membrane protein in the presence of 100 pM [^{3}H]spiperone (New England Nuclear, sp act. = 25.64 Ci/mmol; less than K_D of 250 pM) and compound in duplicate in a final volume of 1 mL for 15 min at 37 °C. Specific binding amounted to 91% of total binding and was defined by the displacement of radioactivity in the presence of 10 μM D(+)-butaclamol. Filtration and counting procedures have been described.[41] The concentration of compound that inhibited specific binding by 50% (IC_{50}) was obtained from linear regression analysis of log-probit transforms of the data obtained with three to five concentrations of each compound.

α_1-Adrenergic Receptor Binding. The relative affinities of compounds for α_1-adrenergic receptor binding sites were evaluated on the basis of their ability to displace [^{3}H]WB-4101 from washed membranes obtained from rat cerebral cortices.[44] Male Sprague–Dawley rats were decapitated, the brains were removed, and the cerebral cortices were dissected and stored at −80 °C until required. Pooled cortices were homogenized and washed as described for corpora striata above. The washed membranes were resuspended in 50 vol of Hepes–KOH buffer and held on ice for binding. Binding was measured following incubation of 0.8–1.4 mg of membrane protein in the presence of 100 pM [^{3}H]WB-4101 (New England Nuclear, sp act. = 24.4–25.4 Ci/mmol; less than K_D of 300 pM) and compound in duplicate in a final volume of 2 mL for 15 min at 25 °C. Specific binding amounted to 88% of total binding and was defined by the displacement of radio-

(36) H. R. Bürki, W. Ruch, H. Asper, M. Baggiolini, and G. Stille, *Eur. J. Pharmacol.*, **27**, 180 (1974).
(37) M. B. Bowers, *Clin. Pharmacol. Ther.*, **17**, 74 (1975).
(38) A. Uzan, G. Le Fur, N. Mitrani, M. Kabouche, and A.-M. Donadieu, *Life Sci.*, **23**, 261 (1978).
(39) D. P. Taylor, L. A. Riblet, and H. C. Stanton in "Anxiolytics: Neurochemical, Behavioral, and Clinical Perspectives", J. B. Malick, S. J. Enna, and H. I. Yamamura, Eds., Raven Press, New York, 1982, in press.
(40) J. Berkson, *Am. Stat. Assoc. J.*, **48**, 565 (1953).
(41) B. Costall and R. J. Naylor, *Psychopharmacology*, **34**, 233 (1974).
(42) P. A. J. Janssen, C. J. E. Niemegeers, K. H. L. Schellekens, and F. M. Lenaerts, *Arzneim.-Forsch.*, **17**, 841 (1967).
(43) D. R. Burt, I. Creese, and S. H. Snyder, *Mol. Pharmacol.*, **12**, 800 (1976).
(44) J. E. Rosenblatt, C. B. Pert, J. F. Tallman, A. Pert, and W. E. Bunney, Jr., *Brain Res.*, **160**, 186 (1979).

activity in the presence of 1 μM phentolamine. Filtration and counting procedures have been described.[42] The concentration of compound that inhibited specific binding by 50% (IC_{50}) was obtained from linear regression analysis of log-probit transforms of the data obtained with three to five concentrations of each compound.

Acknowledgment. We are grateful to Drs. W. T. Comer and H. C. Stanton for quotidian discussion, D. J. Mann and M. Pohl for preparation of the manuscript, and L. E. Allen, E. M. Ashworth, J. A. Becker, D. K. Hyslop, W. Lobeck, R. A. Winnecke, and R. E. Yeager for technical assistance. The X-ray analysis of buspirone hydrochloride was performed by the Molecular Structure Corp., College Station, TX.

Registry No. 1, 36505-84-7; 2, 83928-56-7; 3, 57648-88-1; 4, 83928-57-8; 5, 57648-86-9; 6, 80827-69-6; 6 (base), 80827-68-5; 7, 80827-60-7; 7 (base), 80827-70-9; 8, 83928-58-9; 8 (base), 83928-70-5; 9, 83928-59-0; 9 (base), 83928-71-6; 10, 21090-07-3; 11, 21225-87-6; 12, 21102-94-3; 13, 21102-96-5; 14, 21103-03-7; 15, 21103-05-9; 16, 21103-20-8; 17, 21103-18-4; 18, 83928-60-3; 18 (base), 83947-21-1; 19, 21103-08-2; 20, 22684-83-9; 21, 21098-22-6; 22, 83928-61-4; 22 (base), 83928-72-7; 23, 83928-62-5; 23 (base), 83928-73-8; 24, 83928-63-6; 24 (base), 83947-20-0; 25, 83928-64-7; 25 (base), 83928-74-9; 26, 83928-65-8; 26 (base), 83928-75-0; 27, 83928-66-9; 27 (base), 83928-76-1; 28, 83928-67-0; 28 (base), 83928-77-2; 29, 83928-68-1; 29 (base), 83928-78-3; 30, 83928-69-2; 31, 25024-93-5; 32, 25024-94-6; 3,3-tetramethyleneglutaric anhydride, 5662-95-3; 4-(2-pyrimidinyl)-1-piperazinebutanamine, 33386-20-8; 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione, 80827-62-9; 1-(2-pyrimidinyl)piperazine, 20980-22-7.

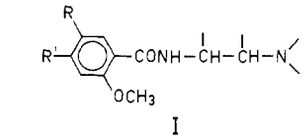
Theoretical Conformational Studies of Some Dopamine Antagonistic Benzamide Drugs: 3-Pyrrolidyl- and 4-Piperidyl Derivatives

Han van de Waterbeemd and Bernard Testa*

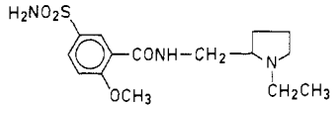
School of Pharmacy, University of Lausanne, CH-1005 Lausanne, Switzerland. *Received March 24, 1982*

Model derivatives of 3-pyrrolidyl- and 4-piperidyl-o-methoxybenzamides, as representatives of neuroleptic substituted benzamide drugs, have been investigated by theoretical conformational analysis. Folded conformers of 2-methoxy-*N*-(1-methyl-3-pyrrolidyl)benzamide have the lowest energy, but extended conformers are only a few kilocalories per mole less stable. As regards the piperidyl derivative, it has been found that folded conformers are of much higher energy than extended ones. These and previous results are discussed in terms of the pharmacologically active conformers of substituted benzamide drugs and of possible modes of interaction with the dopamine receptor.

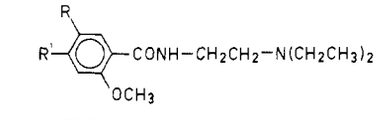
Substituted o-methoxybenzamide drugs (substituted o-anisamides, orthopramides) are a group of dopamine (DA) receptor antagonists having the general structure I.



I



II



III R = Cl, R' = NH_2

IV R = SO_2CH_3, R' = H

Representative drugs from this class are mainly centrally active and are used as neuroleptics [e.g., sulpiride (II)], antiemetics [e.g., metoclopramide (III)], or against various forms of dyskinesia [e.g., tiapride (IV)]. The mechanism of action of these compounds is not fully understood, but it is generally accepted that they act selectively as DA antagonists on a population of DA receptors not linked to adenylate cyclase.[1-6] Such a selectivity must be accounted for by molecular structural properties, including physicochemical properties and stereochemical features. As a result of this working hypothesis, topographical and conformational features of orthopramide drugs are of considerable interest for an understanding of their receptor selectivity and its rational improvement. Stereoselective activity has been observed for (-)-sulpiride and (-)-sultopride.[1]

Our laboratory has previously reported the conformational behavior of metoclopramide (III)[7] and sulpiride (II)[8] as examined by theoretical (PCILO) methods. Metoclopramide is thus believed to have only limited conformational freedom due to two intramolecular H bonds acting as conformational "locks" and favoring folded forms. The studies with sulpiride have also revealed that the minimum energy conformer is a folded, intramolecularly H-bonded form ($N^+/O=$ distance 2.56 Å), with extended conformers being only 3–4 kcal/mol less stable.

The distance between the basic nitrogen atom and the center of the aromatic ring, which is believed to be a crucial feature of dopamine agonistic and antagonistic activity, is very close to 5 Å in the fully extended dopamine molecule.[9] This is at least 1 Å shorter than the corresponding

(1) P. Jenner, A. Clow, C. Reavill, A. Theodorou, and C. D. Marsden, *J. Pharm. Pharmacol.*, **32**, 39 (1980).
(2) P. Jenner and C. D. Marsden, *Life Sci.*, **25**, 479 (1979).
(3) J. W. Kebabian and D. B. Calne, *Nature (London)*, **277**, 93 (1979).
(4) P. Laduron, *Trends Pharmacol. Sci.* 1, 471 (1980).
(5) J. W. Kebabian and T. E. Cote, *Trends Pharmacol. Sci.*, **2**, 69 (1981).
(6) P. Sokoloff, M. P. Martres, and J. C. Schwartz, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **315**, 89 (1980).
(7) A. Pannatier, L. Anker, B. Testa, and P. A. Carrupt, *J. Pharm. Pharmacol.*, **33**, 145 (1981).
(8) H. van de Waterbeemd and B. Testa, *Helv. Chim. Acta*, **64**, 2183 (1981).
(9) A. S. Horn and J. R. Rogers, *J. Pharm. Pharmacol.*, **32**, 521 (1980).

0022-2623/83/1826-0203$01.50/0