Registry No. 1, 1490-25-1; 2, 52787-46-9; 3, 102284-27-5; 4, 15761-39-4; 5a, 102284-28-6; 5b, 102284-29-7; 5c (diastereomer 1), 102284-30-0; 5c (diastereomer 2), 102284-64-0; 6a, 102284-31-1; 6b, 102284-32-2; 6c, 102284-33-3; 6d, 102284-34-4; 6e, 102284-35-5; 7a, 102284-36-6; 7b, 102284-37-7; 7c, 102284-38-8; 7d, 102284-39-9; 7e, 102284-40-2; 8a, 92279-27-1; 8b, 92279-28-2; 8c, 92279-29-3; 8d, 92279-30-6; 8e, 92279-31-7; 9, 101130-03-4; 10, 102284-41-3; 11, 102284-42-4; 12a, 102284-43-5; 12b, 102284-44-6; 12c, 102284-45-7; 12d, 102284-46-8; 12e, 102284-47-9; 12f, 102284-48-0; 12g, 102284-49-1; 12h, 102284-50-4; 13a, 102284-51-5; 13b, 102284-52-6; 13c, 102284-53-7; 13d, 102306-03-6; 13e, 102284-54-8; 13f, 102284-55-9; 13g, 102284-56-0; 13h, 102284-57-1; 14a, 92279-32-8; 14b, 92279-33-9; 14c, 102284-58-2; 14d, 102306-04-7; 14e, 102284-59-3; 14f, 102284-60-6; 14g, 102284-61-7; 14h, 102284-62-8; Ala-Ala, 1948-31-8; *p*-aminophenol, 123-30-8; DL-2-amino-3-methyl-1-butanol, 16369-05-4; phenyl isocyanate, 103-71-9; dimethylcarbamoyl chloride, 79-44-7; isopropyl isocyanate, 1795-48-8; isopropylamine, 75-31-0; *p*-nitrophenyl chloroformate, 7693-46-1; *S*-benzyl chlorothioformate, 37734-45-5; 1-methyl-5-tetrazolyl chlorothioformate, 102284-63-9; 1-methyl-5-mercaptotetrazole, 13183-79-4; phosgene, 75-44-5; *o*-aminophenol, 95-55-6; *p*-nitrophenyl isocyanate, 100-28-7; elastase, 9004-06-2.

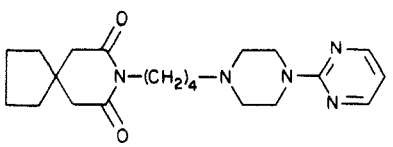
Buspirone Analogues. 2. Structure–Activity Relationships of Aromatic Imide Derivatives

James S. New,* Joseph P. Yevich, Michael S. Eison, Duncan P. Taylor, Arlene S. Eison, Leslie A. Riblet, Cam P. VanderMaelen, and Davis L. Temple, Jr.

Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Research Center, Wallingford, Connecticut 06492-7660. *Received September 20, 1985*

Several analogues of the novel anxiolytic buspirone were synthesized and evaluated in vivo for tranquilizing activity and their ability to reverse neuroleptic-induced catalepsy. The in vitro binding affinities of these compounds were also examined for both the α_1 and dopamine D_2 receptor systems. The general structure–activity relationships of this series highlight compounds 17, 21, and 32 as having anticonflict activity. Each of these structures contains the 1-(2-pyrimidinyl)piperazine moiety linked by a tetramethylene chain to a variable cyclic imide moiety. Compound 32 (4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedione) was found to be equipotent with buspirone in its anxiolytic activity and was therefore selected for extensive preclinical characterization. The pharmacology of buspirone and 32 is contrasted, and the potent serotonin agonist properties of 32 are discussed with reference to its potential contribution to the anxioselective mechanism of this compound.

Anxiolytic drugs that express their antianxiety activity without the anticonvulsant, sedative, or muscle relaxant effects normally associated with the use of the benzodiazepines are termed anxioselective. Buspirone (1) is unique in this class since it does not share a mechanism of anxiolysis mediated through the benzodiazepine–GABA–chloride ionophore receptor complex utilized by other anxioselective drugs. The diverse neuropharmacologic effects of buspirone, which include subtle involvement with the dopamine, GABA, serotonin, and adrenergic neurotransmitter systems, may collectively facilitate its mechanism of anxiolysis. Elucidation of this mechanism has been the subject of numerous pharmacologic investigations which have evolved a midbrain modulator role to define the anxiolytic specificity of this novel drug.[1,2] This working hypothesis postulates the anxioselective vector of buspirone alleviates anxiety through the summation of its various polysynaptic actions, which modify the diffuse assemblage of neurochemical inputs involved in the etiology of this disease.



1

The anxiolytic effects of buspirone are known to be dependent on two distinct substructural pharmacophores within the molecular framework: the azaspirodecanedione imide and the arylpiperazine moiety found in its pyrimidinylpiperazine fragment. Previous structure–activity studies have focused on carbocyclic imides as potential surrogates for the azaspirodecanedione imide present in 1.[3-5] This resulted in adaptation of a generalized topology for a potential receptor site that can accommodate the critical molecular features of this structural class. This current report extends the structure–activity relationships (SAR) of buspirone and explores replacement of both imide and pyrimidinylpiperazine fragments in this drug with new aromatic imide moieties and arylpiperazine groups.

Chemistry. The synthesis of target structures **10–24**, which incorporate a pyrrolidinedione ring, is accomplished with standard published procedures. Briefly, condensation of commercially available aldehydes or ketones (2) with ethyl cyanoacetate under either Cope or Knoevenagel reaction conditions yields a variety of α,β-unsaturated cyanoacetates, 3 (Scheme I). The reaction of 3 (R^2 = H, phenyl, or $(CH_2)_n$-Ar) with potassium cyanide in aqueous ethanol yields the dicyano intermediates 4. Either acid or base hydrolysis of 4 generates the succinic acid derivatives 7, which on conversion to their respective anhydrides react with ammonia and are cyclized to the imides represented by 8.

An alternative synthesis of 8 involves the Emmons–Horner condensation of triethyl phosphonoacetate with the appropriate ketone forming the α,β-unsaturated ethyl ester 5. Reaction of 5 with potassium cyanide in DMF (60 °C) yields 6 whose hydrolysis also affords 7. The subsequent elaboration of either the anhydrides or intermediary

(1) Taylor, D. P.; Allen, L. E.; Becker, J. A.; Crane, M.; Hyslop, D. K.; Riblet, L. A. *Drug Dev. Res.* **1984**, *4*, 95.
(2) Eison, M. S.; Eison, A. S. *Drug Dev. Res.* **1984**, *4*, 109.
(3) Yevich, J. P.; Temple, D. L.; New, J. S.; Taylor, D. P.; Riblet, L. A. *J. Med. Chem.* **1983**, *28*, 194.
(4) Wu, Y.-H.; Smith, R.; Rayburn, J. W.; Kissel, J. W. *J. Med. Chem.* **1969**, *12*, 876.
(5) Wu, Y.-H.; Rayburn, J. W.; Allen, L. E.; Ferguson, H. C.; Kissel, J. W. *J. Med. Chem.* **1972**, *15*, 477.

0022-2623/86/1829-1476$01.50/0

pyrrolidinediones to the target structures is accomplished with previously reported methodologies.[3-5] The various phthalimide derivatives used in the synthesis of compounds **25–31** are commercially available. Table I summarizes the experimental and physical data for new compounds. General procedures detailing the synthesis of target structures are reported in the Experimental Section.

Pharmacology. The tranquilizing activity of the target compounds is determined 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). Activity in this test is described by the dose inhibiting the CAR in half the animals tested. The potential for α_1-adrenergic receptor blockade is measured by the ability of a compound to protect mice against a lethal-dose injection of norepinephrine. Potential dopaminergic activity associated with these structures is measured by the ability of various concentrations of a compound to inhibit the in vitro binding of [^{3}H]spiperone at membrane sites isolated from rat corpus striatum; an IC_{50} value less than 1000 nM indicates significant binding of the ligand to the receptor (e.g., IC_{50} = 7 nM for haloperidol). The Vogel conflict paradigm is employed as a determinant of antianxiety activity for buspirone analogues and measures the ability of the compound to attenuate the shock-induced suppression of water consumption by rats. The ability of a compound to reverse trifluoperazine-induced catalepsy, while of unknown pharmacologic origin, suggests such structures will have little or no propensity to induce extrapyramidal symptoms frequently found with drugs possessing potent dopaminergic blocking characteristics. The pharmacological activity of the various compounds reviewed here is reported in Table II. Pertinent pharmacologic methodologies are presented in the Experimental Section.

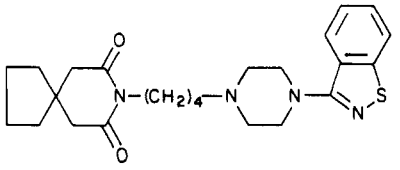
Results and Discussion

None of the compounds examined have potent postsynaptic dopamine (DA) D_2 receptor affinity although the tendency for this effect is greater in the pyrrolidinedione series (**10–24**) than for the phthalimide analogues (**25–31**). Compounds **10** and **15**, which incorporate a phenyl pyrrolidinedione, and **20**, **22**, and **24**, which have either a tetralin or indan ring spirally fused to a pyrrolidinedione, represent derivatives active in the CAR (Table II); only the nitro-substituted compound **28** and **29** have similar activity in the phthalimide class. As previously observed, contraction of the optimum four-carbon chain length, which links the imide and arylpiperazine substructures in **10**, attenuates the CAR activity in compounds **11** and **12**.[3]

Heteroaryl Ring Effects. Variation of the heteroaryl ring component in a series of molecules that retain the same imide substructure leads to a predictable pattern of biological activity. Compounds **13–15**, which combine a 4-fluorophenyl and methyl group at their quaternary center, and **20**, **22**, and **23**, which have a spirally fused 1,2,3,4-tetrahydronaphthalene ring at this center, were each coupled to the pyrimidine, benzisothiazole, and pyridine ring systems. Generally, the postsynaptic affinity for DA D_2 sites increases, and potency in the CAR decreases, as the heteroaryl groups alternate from the pyrimidine to the pyridine, and lastly to the benzisothiazole ring, in molecules which possess the same imide substructure. While compounds **17–19** present a diphenyl-substituted imide linked to these various heterocyclic systems, their lack of consistent biological activity precludes their inclusion in any cross-comparisons. None of the compounds reported herein demonstrated in vivo α_1-receptor blockade as assayed by their ability to block norepinephrine-induced lethality in mice.

Anxiolytic and Catalepsy Reversal Activity. Compounds **17**, **21**, and **32** have significant activity in the Vogel conflict paradigm at doses of 10, 5, and 1 mg/kg (MED, po), respectively; the antianxiety activity of buspirone or diazepam is equipotent with compound **32** in this paradigm. Fusion of the spiro linkage in a position α to the phenyl ring in **20** nullifies the anticonflict effects found in the structural isomer **21**. This is surprising based on the structural modifications allowed between buspirone and **32** without disruption of their anxiolytic effects, suggesting the SAR mediating this activity in the aromatic imide substructural class is more restrictive. Although there is no established biochemical nexus correlating the role of catalepsy reversal with anxiolytic activity, the presence of both activities in buspirone, **32**, and **21** suggests clinical use of these drugs would not induce extrapyramidal symptoms normally associated with dopamine antagonists. The lack of a mutually predictive quality existing between catalepsy reversal and anxiolytic activities is apparent in their lack of concordance in the profiles of compounds **13**, **15**, **17**, **24**, and **25**.

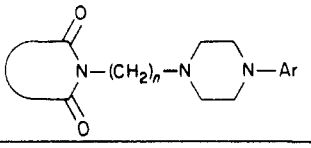
Pharmacology of 32 (BMY 13805) vs. Buspirone. The molecular components of buspirone and its related analogues represent a unique structural class of psychoactive agents unrelated to the tricyclics, butyrophenones, or benzodiazepines. The untraditional pharmacological profiles of this structural family have invoked a blend of neurochemical, behavioral, and electrophysiological methodologies endeavoring to elucidate the mechanism of their psychoactive properties. One member of the series, **33** (BMY 13859), has been the focus of an extensive SAR study due to its promising antipsychotic potential.[6] The complex pharmacological investigations of buspirone's anxioselective mechanism describe a vanguard member of a novel anxiolytic class, which engages a variety of neural substrates to elicit its therapeutic effects.[7-14] Recognition of a multimodulatory potential in its prototypes has stimulated the characterization of **32**, which



33

(6) Yevich, J. P.; New, J. S.; Smith, D. W.; Lobeck, W. G.; Catt, J. D.; Minielli, J. L.; Eison, M. S.; Taylor, D. P.; Riblet, L. A.; Temple, D. L. *J. Med. Chem.* **1986**, *29*, 359.
(7) Temple, D. L.; Yevich, J. P.; New, J. S. *J. Clin. Psychiatry, Sect. 2* **1982**, *43*, 4.
(8) Riblet, L. A.; Taylor, D. P.; Eison, M. S.; Stanton, H. C. *J. Clin. Psychiatry Sect. 2* **1982**, *43*, 11.
(9) Weissman, B. A.; Barrett, J. E.; Brady, L. S.; Witkin, J. M.; Mendelson, W. B.; Paul, S. M.; Skolnick, P. *Drug Dev. Res.* **1984**, *4*, 83.
(10) Gershon, S.; Eison, A. S. *J. Clin. Psychiatry, Sect. 2* **1983**, *44*, 11, 145.
(11) Skolnick, P.; Paul, S. M. *J. Clin. Psychiatry, Sect. 2* **1982**, *43*, 12, 40.
(12) Riblet, L. A.; Eison, A. S.; Eison, M. S.; Newton, R. E.; Taylor, D. P.; Temple, D. L. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1983**, *7*, 663.
(13) Taylor, D. P.; Riblet, L. A.; Stanton, H. C.; Eison, A. S.; Eison, M. S.; Temple, D. L. *Pharmacol. Biochem. Behav. Suppl. 1* **1982**, *17*, 25.
(14) Garattini, S.; Caccia, S.; Mennini, T. *J. Clin. Psychiatry, Sect. 2* **1982**, *12*, 19.

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



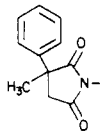
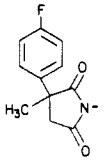
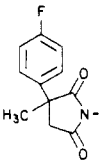
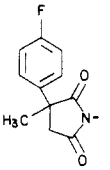
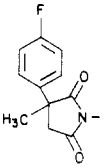
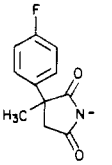
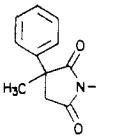
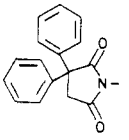
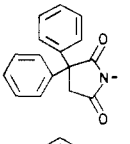
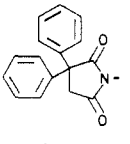
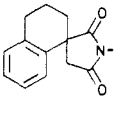
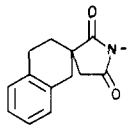
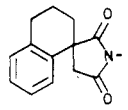
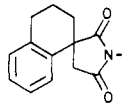
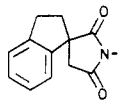
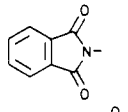
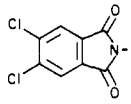
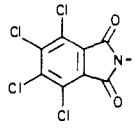
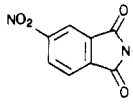
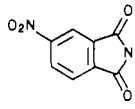
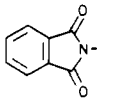
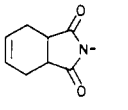
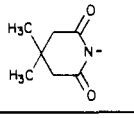
compd	structure	*n*	Ar	recrystn solvent	mp, °C	yield,[a] %	formula	anal.[b]
10		4	2-$C_4H_3N_2$[c]	CH_3CN	190–200	82	$C_{23}H_{29}N_5O_2 \cdot 1.8HCl$	C, H, N
11		2	2-$C_4H_3N_2$	EtOH	214–216	62	$C_{21}H_{24}FN_5O_2 \cdot HCl \cdot 0.2EtOH$	C, H, N
12		3	2-$C_4H_3N_2$	EtOAc/*i*-PrOH	185–189	94	$C_{22}H_{26}FN_5O_2 \cdot HCl$	C, H, N
13		4	2-$C_6H_3N_2$[d]	*i*-PrOH	168–170.5	75	$C_{25}H_{28}FN_5O_2 \cdot HCl$	C, H, N
14		4	3-C_7H_4NS[e]	*i*-PrOH	188–189.5	73	$C_{26}H_{29}FN_4O_2S \cdot HCl$	C, H, N
15		4	2-$C_4H_3N_2$	*i*-PrOH	163–169	69	$C_{23}H_{28}FN_5O_2 \cdot HCl$	C, H, N
16		4	2-$C_4H_3N_2$	EtOAc	185–186	96	$C_{23}H_{26}F_3N_5O_2 \cdot 1.8C_7H_8O_3S$[f]	C, H, N
17		4	2-$C_4H_3N_2$	*i*-PrOH	201.5–203.5	83	$C_{28}H_{31}N_5O_2 \cdot HCl$	C, H, N
18		4	3-C_7H_4NS	*i*-PrOH	188–189.5	72	$C_{31}H_{32}N_4O_2S \cdot HCl$	C, H, N
19		4	2-$C_6H_3N_2$	*i*-PrOH/EtOAc	179–182	84	$C_{30}H_{31}N_5O_2 \cdot HCl$	C, H, N
20		4	2-$C_4H_3N_2$	EtOH	241–247	99	$C_{25}H_{31}N_5O_2 \cdot 2HCl$	C, H, N

Table I (Continued)

compd	structure	n	Ar	recrystn solvent	mp, °C	yield,[a] %	formula	anal.[b]
21		4	2-$C_4H_3N_2$	EtOH/CH_3CN	241–243.5	69	$C_{25}H_{31}N_5O_2$·HCl	C, H, N
22		4	2-$C_6H_3N_2$	EtOH	196–198	24.3	$C_{27}H_{31}N_5O_2$·HCl	C, H, N
23		4	2-C_7H_4NS	EtOH	207–212	35	$C_{28}H_{32}N_4O_2S$·$C_7H_8O_3S$	C, H, N
24		4	2-$C_4H_3N_2$	CH_3CN	241–248	72	$C_{24}H_{29}N_5O_2$·2HCl	C, H, N
25		4	2-$C_4H_3N_2$	*i*-PrOH	137–139.5	62.2	$C_{20}H_{23}N_5O_2$	C, H, N
26		4	2-$C_4H_3N_2$	*i*-PrOH	145–146.5	86	$C_{20}H_{21}Cl_2N_5O_2$	C, H, N
27		4	2-$C_4H_3N_2$	CH_3CN	154–156	90	$C_{20}H_{19}Cl_4N_5O_2$	C, H, N
28		4	2-$C_4H_3N_2$	CH_3CN	130–133	56	$C_{20}H_{22}N_6O_4$	C, H, N
29		4	2-$C_6H_3N_2$	*i*-PrOH	205–208	20	$C_{22}H_{22}N_6O_4$·HCl	C, H, N
30		4	2-$C_5H_4FN_2S$[g]	EtOH	235–237	93	$C_{21}H_{24}FN_5O_2S$·HCl	C, H, N
31		4	2-$C_4H_3N_2$	CH_3CN	209–210	63	$C_{20}H_{27}N_5O_2$·HCl	C, H, N
32		4	2-$C_4H_3N_2$	*i*-PrOH	192.5–195	22	$C_{19}H_{29}N_5O_2$·HCl·0.25H_2O	C, H, N

[a] Based on analytically pure sample. [b] Values obtained agree with calculated values within 0.4%. [c] $C_4H_3N_2$ represents pyrimidine. [d] $C_6H_3N_2$ represents 3-pyridinecarbonitrile. [e] C_7H_4NS represents 1,2-benzisothiazole. [f] Formulated as the tosic acid salt. [g] $C_5H_4FN_2S$ represents 5-fluoro-4-(methylthio)pyrimidine.

incorporates the antianxiety activity of its progenitor, in addition to significant serotonin agonist effects which extend the role of this intervention present in buspirone.[15,16]

The numerous conformers available to both buspirone and **32** enhance the facility of these structures to accommodate, in part, diverse neurotransmitter receptors that have varying ligand requirements (the X-ray crystal structure of buspirone has been published[3]). The structural homology extant in these molecules, however, indicates their respective receptor interactions will be very similar. The lack of DA D_2 receptor binding affinity, and also the failure of **32** to block apomorphine-induced stereotypies in the rat, represents preliminary differences that challenge this supposition and contrasts this compound from the profile of buspirone.[17] The unique ability to reverse neuroleptic-induced catalepsy, and their activity in potentiating a cholinergically induced catalepsy in rats,

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Table II. Biological Activity of Buspirone Analogues[a]

compd	n	Ar	binding at dopamine D_2 receptor—rat corpus striatum (vs. [^{3}H]spiperone): IC_{50},[b] nM	rat conditioned avoidance response: ED_{50},[c] mg/kg, po	reversal of trifluoperazine-induced catalepsy: ED_{50},[d] mg/kg, po	vogel anticonflict effects: MED, mg/kg, po
10	4	2-$C_4H_3N_2$	>1000	60		
11	2	2-$C_4H_3N_2$	>1000	>100	>20[e]	
12	3	2-$C_4H_3N_2$	>1000	>100	>20	
13	4	2-$C_6H_3N_2$	710	100	20	I.A.[f]
14	4	3-C_7H_4NS	120	100	>20	
15	4	2-$C_4H_3N_2$	>1000	56	11	I.A.
16	4	2-$C_4H_3N_2$	>1000	>100	>20	I.A.
17	4	2-$C_4H_3N_2$	>1000	>100	>20	10.0
18	4	3-C_7H_4NS	230	>100	>20	
19	4	2-$C_6H_3N_2$	>1000	>100	>20	
20	4	2-$C_4H_3N_2$	>1000	50	>20	I.A.
21	4	2-$C_4H_3N_2$	540	58	13	5.0
22	4	2-$C_6H_3N_2$	310	100	>20	
23	4	3-C_7H_4NS	280	>100	>20	
24	4	2-$C_4H_3N_2$	1300	54	11.5	I.A.
25	4	2-$C_4H_3N_2$	>1000	65	20	I.A.
26	4	2-$C_4H_3N_2$	>1000	>100		
27	4	2-$C_4H_3N_2$	>1000	>100	>20	
28	4	2-$C_4H_3N_2$	>1000	75	>20	
29	4	2-$C_6H_3N_2$	>1000	78	>20	
30	4	2-$C_5H_4FN_2S$	>1000	>100	>20	
31	4	2-$C_4H_3N_2$	>1000	>100		
32	4	2-$C_4H_3N_2$	>1000	53	6.5	1.0

[a] See Experimental Section for discussion of pharmacologic methods and calculation of ED_{50} or IC_{50} values. Corresponding values for buspirone are given in footnotes b–d. [b] IC_{50} = 120 nM. [c] ED_{50} = 48 mg/kg, po. [d] ED_{50} = 3.6 mg/kg, po. [e] This represents the maximum dose examined. [f] I.A., inactive; the maximum dose examined was 20 mg/kg.

Table III. Pharmacology of Buspirone vs. BMY 13805 (**32**)

index	buspirone	BMY 13805
reversal of trifluoperazine-induced catalepsy (ED_{50}, mg/kg, po)	3.6	6.5
Vogel conflict paradigm (MED, mg/kg, po)	1.0	1.0
inhibition of dorsal raphe neuron firing (ED_{50}, mg/kg, iv)	0.01	0.025
induces serotonin syndrome (ED_{50}, mg/kg, po)	inactive	17.1
binding at serotonin $5HT_1$-type sites—cortex (IC_{50}, nm vs. [^{3}H]serotonin)	>1000	>1000
binding at serotonin $5HT_1$-type sites—Hippocampus (IC_{50}, nM vs. [^{3}H]serotonin)	95	670
binding at serotonin $5HT_2$-type sites (IC_{50}, nM vs. [^{3}H]spiperone)	740	inactive

is still subserved by the structures of these compounds (Table III). The anxioselective specificity found in both **32** and buspirone is reflected in their lack of anticonvulsant activity and absence of binding affinity at α- or β-adrenergic, cholinergic, GABA, or benzodiazepine receptors. In addition, neither compound induces catalepsy, potentiates hexobarbital or ethanol administration, or causes muscle weakness and motor incoordination. The equipotent anxiolytic effects of these compounds are manifested in both the Vogel conflict and foot shock-induced aggression paradigms in rats.

Neurochemical Profiles of Buspirone and 32. Buspirone and **32** elevate levels of the DA metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in the striatum of rats following acute administration.[18-20] Buspirone parallels these changes with increases in tyrosine hydroxylase activity and whole brain dopamine levels, while **32** is devoid of these dopaminergic effects.[21] Both compounds decrease striatal levels of norepinephrine and elevate levels of the major norepinephrine metabolite, 3-methoxy-4-hydroxyphenyl glycol sulfate (MOPEG-SO_4), following acute administration. Contrary to benzodiazepine effects, extracellular single-cell recording techniques indicate buspirone and **32** increase impulse flow in noradrenergic neurons of the locus coeruleus.[22] The neuronal uptake mechanisms involving serotonin, GABA, or norepinephrine are unaffected by **32** or buspirone, and the quantitative EEG profiles of these compounds in cats are similar.

Serotonergic Activity of Buspirone and 32. Both these drugs potently inhibit the firing of dorsal raphe neurons in electrophysiological experiments in the rat.[23] Neither buspirone or **32** binds to serotonin $5HT_1$-type sites isolated from the frontal cortex but, they display potency in the hippocampal section of rat brain enriched in $5HT_1$ receptors (Table III).[24] Chronic administration of either drug causes a significant decrease (B_{max} values) in serotonin $5HT_2$-type receptors despite their low in vitro affinity for this receptor.[25,26] Many clinically effective antidepressant agents also down-regulate central serotonin $5HT_2$ recep-

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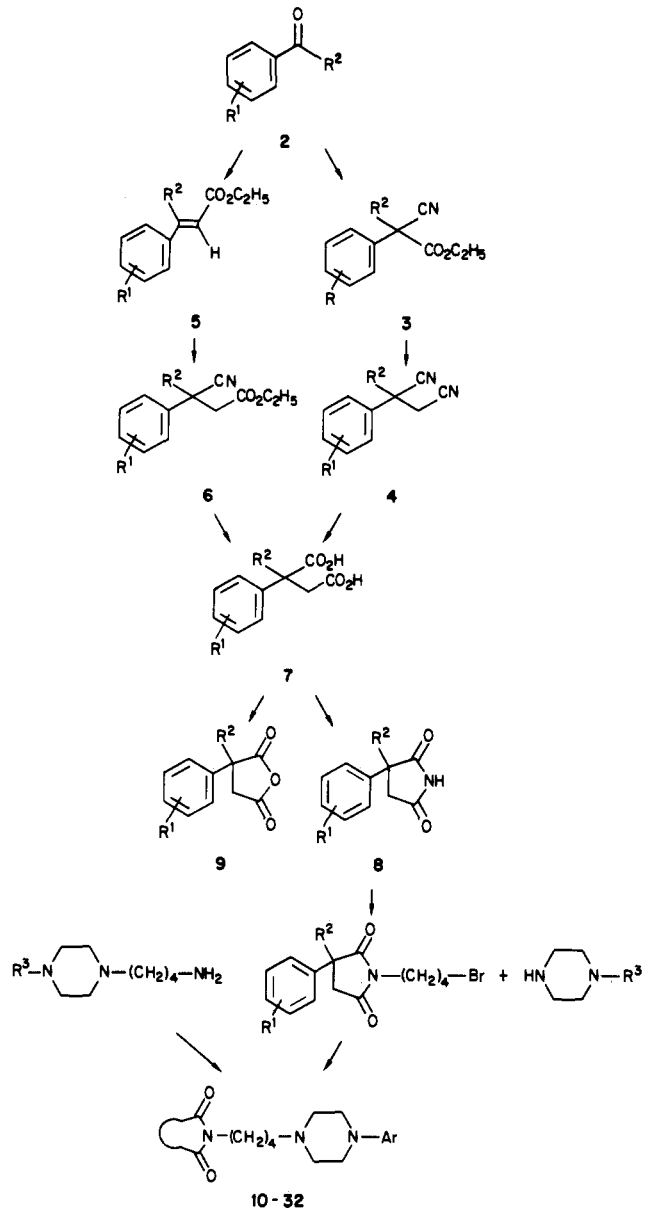
Scheme I



tors, suggesting that potential antidepressant indications may complement the anxiolytic efficacy of buspirone and **32**. In addition, both drugs stimulate 5HT-sensitive adenylate cyclase activity.[27] The anxiolytic activity of buspirone and **32** may in part involve serotonergic mediation, since 5,7-DHT serotonin lesions abrogate the anticonflict effects of these compounds. Compound **32** also induces behaviors in rats characteristic of the serotonin syndrome, an effect which is magnified in intraventricular serotonin-lesioned animals; unilateral serotonin-lesioned rats rotate contralaterally when treated with this drug. The inability of buspirone to elicit this response may not portray its lack of serotonin agonist activity, but rather may represent other aspects of its profile, perhaps its more dominant dopaminergic properties whose inhibitory inputs suppress development of the serotonin syndrome. Alternatively, the structural differences between **32** and buspirone, albeit minor, may facilitate the interaction of **32** with specific subpopulations of serotonin receptors that sterically exclude similar binding of the larger buspirone molecule. The more robust serotonin agonist activity of **32** is also evident with the attenuation of its normal hypergesic effects on the electric foot shock induced vocalization threshold and its ability to potentiate the acoustic startle reflex in rats, both of which are blocked by 5-HT lesions.[28]

Conclusions

Buspirone and its analogue **32** have equipotent anxiolytic effects but differ considerably in the extent of their dopaminergic and serotonergic interactions. Buspirone has more potent dopaminergic activity relative to **32**, while the latter has a more profound effect on the serotonergic system. The SAR of related analogues indicates the anxiolytic activity is dependent on inclusion of the pyrimidinylpiperazine substructure for the heteroaryl component in these molecules, but allows interchange of structurally diverse imide groups for these corresponding moieties in buspirone and **32**. The significant serotonin agonist effects of **32**, which are partly expressed by buspirone, suggest the serotonergic system may be contributing to the antianxiety activity of this unique class of compounds. The complete definition of this anxiolytic activity will undoubtedly encompass other factors present in the complex pharmacology of the *N*-[(4-heteroaryl)-(1-piperazinyl)alkyl]imide structural class. Elucidation of this mechanism is the subject of on-going research.

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}).[29]

Dopamine Receptor Binding Assay. 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.[30] Male Sprague–Dawley rats were decapitated, the brains removed, and the corpora striata 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, specific activity = 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.[31] 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 3–5 concentrations of each compound.

α_1-Adrenergic Receptor Blockade. Nonfasted, male mice

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were orally administered test compounds initially at a 100 mg/kg dose (20 animals/dose level). Thirty minutes later, the animals were injected intravenously with a lethal dose (1.5 mg/kg) of norepinephrine bitartrate (minimum dose to produce 100% lethality). Animals observed alive after 1 h indicated positive α-blocking action, and additional doses of 50 and 25 mg/kg were investigated to calculate a dose protecting 50% of the animals (ED_{50}) according to the method of Berkson.[29]

Vogel Conflict Test. Reverse day–night cycled rats were water deprived for 24 h, then given 15 min free access to water (i.e., no shocks) in the operant test cages (bottle training). Rats who are not successfully bottle trained are eliminated from the study, and the rats who learned the location of the drinking bottle are further water deprived for an additional 23.5 h prior to testing. Test compounds are orally administered prior to testing, and the animals are placed in the Vogel cages where a protocol quantifies the number of licks (water ingestion) at the water spout. After a predetermined number of licks, brief electric shocks are initiated across the cage floor grid. The number of licks during nonshock and shock periods, the total number of shocks, and the cumulative latency to lick following shocks can be recorded. Pharmacological activity in this test is reflected in drugged rats licking more than controls, by a Student's t test ($p < 0.05$).[32] Test drugs demonstrating activity with a MED value of 10 mg/kg (po) or less are considered to have antianxiety activity.

Chemistry. All IR spectra were recorded on a Nicolet MX-1 FT-IR spectrometer. The ^{1}H-NMR spectra were recorded on a Perkin-Elmer R-32 spectrometer, and the ^{13}C NMR spectra were recorded on a Varian FT-80 spectrometer in 5-mm-o.d. sample tubes in either $CDCl_3$ or Me_2SO-d_6, using 2% (v/v) tetramethylsilane or perdeuteriodimethylsulfoxide as the internal reference. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected.

1,2-Dicyano-2-(4-methylphenyl)propane (4; R^1, $R^2 = CH_3$). Typical Procedure. A solution of the α,β-unsaturated cyanoacetate **3** (R^1, $R^2 = CH_3$) (86 g, 0.38 mol), and potassium cyanide (40.9 g, 0.63 mol) is refluxed 4.5 h in 90% ethanol (500 mL). The resulting dark solution is evaporated, suspended in water (100 mL), and extracted with dichloromethane (2 × 150 mL). The organic layers are combined, dried ($MgSO_4$), and evaporated, and bulb-to-bulb distillation affords 54.8 g (78%) of a light-green syrup:[33] bp 124–126 °C (0.1 mm); NMR ($CDCl_3$) δ 1.88 (s, 3 H), 2.35 (s, 3 H), 2.94 (s, 3 H), 7.31 (m, 2 H). Anal. ($C_{12}H_{12}N_2$) C, H, N.

1,2-Dicarboxylic Acid Derivatives of General Structure 7. Typical Procedure. A solution of 1-cyano-1,2,3,4-tetrahydro-1-naphthaleneacetonitrile, 4 (R^1 = H, $R^2 = -(CH_2)_3-$) (36.0 g, 0.18 mol), and sodium hydroxide (108.0 g, 0.27 mol) is refluxed for 40 h in 30% ethanol (700 mL). The cooled solution is acidified with hydrochloric acid (concd), and the mixture is extracted with chloroform (3 × 250 mL). The organic washings are combined, dried ($MgSO_4$), filtered, and evaporated yielding 41.0 g (97.6%) of representative structure **7** as an off-white solid. This material is used crude in the next step without additional purification.

1′,2′,3,4,4′,5′-Hexahydrospiro[naphthalene-2(1*H*),3′-[3*H*]-pyrrole-2′,5′-dione. General Structure 8: Typical Procedure. A mixture of 2-carboxy-1,2,3,4-tetrahydro-2-naphthaleneacetic acid (35 g, 0.15 mol) and acetic anhydride (105 g) is refluxed 3 h; the solution is cooled, and the excess anhydride is evaporated yielding a dark solid which is recrystallized from $CHCl_3$–hexane (1:1) affording 32 g of a white solid: mp 98–100.5 °C. This solid is mixed with 30% NH_4OH (160 g) in acetonitrile (300 mL) and refluxed 2.5 h. The solvent is evaporated, and the collected dark residue is mixed with xylene and refluxed with a Dean–Stark trap until the evolution of water has ceased. Evaporation of this solution affords a dark solid which is recrystallized from 2-propanol yielding 24 g (75.5%) of a white solid corresponding to the general imide structure, **8**: mp 234–236 °C; NMR (Me_2SO-d_6) δ 1.89 (m, 2 H), 2.29 (d, J = 17.6 Hz, 1 H), 2.65 (d, J = 17.6 Hz, 1 H), 2.88 (m, 4 H), 7.08 (m, 4 H), 11.17 (br s, 1 H). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

1′-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]spiro-[1,2,3,4-tetrahydronaphthalene-2,3′-pyrrolidine]-2′,5′-dione Hydrochloride (21). Typical Procedure. A mixture of 1′-(4-bromobutyl)-1′,2′,3,4,4′,5′-hexahydrospiro[naphthalene-2(1*H*),3′-[3*H*]pyrrole]-2′,5′-dione (3.9 g, 0.011 mol), 1-(2-pyrimidinyl)piperazine (1.82 g, 0.011 mol), and potassium carbonate (2.76 g, 0.02 mol) is refluxed 24 h in acetonitrile (180 mL). The solution is filtered, washed with water (2 × 100 mL), dried ($MgSO_4$), and evaporated. The collected syrup is flash chromatographed (3% EtOH–$CHCl_3$). The appropriate fractions are combined, evaporated, and dissolved in acetonitrile before treatment with an equivalent amount of ethanolic hydrochloric acid. Cooling leads to crystallization of 3.5 g (68.6%) of the hydrochloride salt: mp 241–243.5 °C; NMR (Me_2SO-d_6) δ 1.65 (m, 4 H), 1.90 (m, 2 H), 2.35 (d, J = 17.8 Hz, 1 H), 2.73 (d, J = 17.8 Hz, 1 H), 2.95 (m, 8 H), 3.44 (m, 6 H), 4.67 (br d, J = 13.0 Hz), 6.72 (t, J = 4.6 Hz, 1 H), 7.09 (m, 4 H), 8.42 (d, J = 4.6 Hz, 2 H), 11.75 (br s, 1 H). Anal. ($C_{25}H_{31}N_5O_2$·HCl) C, H, N.

Registry No. **3** (R^1, $R^2 = CH_3$), 14505-28-3; **4** (R^1 = H, $R^2 = -(CH_2)_3-$), 73593-88-1; **4** (R^1, $R^2 = CH_3$), 86945-26-8; **7** (R^1 = H, $R^2 = -(CH_2)_3-$), 17516-09-5; **10**, 98054-87-6; **10**·2HCl, 102492-96-6; **11**, 98054-88-7; **11**·HCl, 102492-97-7; **12**, 98054-89-8; **12**·HCl, 102492-98-8; **13**, 102492-95-5; **13**·HCl, 102492-99-9; **14**, 98054-91-2; **14**·HCl, 102493-00-5; **15**, 98054-72-9; **15**·HCl, 98054-73-0; **16**, 98035-53-1; **16**·$2C_7H_8O_3S$, 102493-01-6; **17**, 98054-78-5; **17**·HCl, 98054-79-6; **18**, 98035-55-3; **18**·HCl, 102493-02-7; **19**, 102493-10-7; **19**·HCl, 102493-03-8; **20**, 98035-56-4; **20**·HCl, 102493-04-9; **21**, 98054-85-4; **21**·HCl, 98054-86-5; **22**, 102493-11-8; **22**·HCl, 102493-05-0; **23**, 98035-57-5; **23**·$C_7H_8O_3S$, 102493-06-1; **24**, 98035-58-6; **24**·2HCl, 102493-07-2; **25**, 95604-92-5; **26**, 98035-59-7; **27**, 98035-60-0; **28**, 98054-49-0; **29**, 102493-12-9; **29**·HCl, 102493-08-3; **30**, 98054-75-2; **30**·HCl, 98054-76-3; **31**, 98054-50-3; **31**·HCl, 102493-09-4; **32**, 83928-76-1; **32**·HCl, 83928-66-9; 1′,2′,4,4′,5′-hexahydro[naphthalene-2(1*H*),3′-[3*H*]pyrrole-2′,5′-dione, 98054-83-2; 1′-(4-bromobutyl)-1′,2′,3,4,4′,5′-hexahydrospiro[naphthalene-2(1*H*),3′-[3*H*]pyrrole]-2′,5′-dione, 98054-84-3; 1-(2-pyrimidinyl)piperazine, 20980-22-7; 2-carboxy-1,2,3,4-tetrahydro-2-naphthaleneacetic acid, 98054-81-0.

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