(5-Amino-1,3-dimethyl-1*H*-pyrazol-4-yl)(2-fluorophenyl)methanones. A Series of Novel Potential Antipsychotic Agents

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(5-Amino-1,3-dimethyl-1*H*-pyrazol-4-yl)(2-fluorophenyl)methanone (1) was found to have an antipsychotic-like profile in behavioral tests predictive of antipsychotic efficacy but, unlike available antipsychotic agents, did not bind in vitro to dopamine receptors. Upon further evaluation, 1 was found to cause clonic seizures in aged rodents. An examination of related structures revealed that 5-(substituted aminoacetamide) analogues of 1 shared this novel pharmacology and did not cause seizures. The synthesis and pharmacological evaluation of this series of compounds are described. Two compounds, 2-(diethylamino)acetamide (**25**) and 2-[[3-(2-methyl-1-piperidinyl)propyl]-amino]acetamide (**38**), were selected for examination in secondary tests. Like known antipsychotics both compounds reduced spontaneous locomotion in mice at doses that did not cause ataxia and inhibited conditioned avoidance selectively in both rats and monkeys. Unlike known antipsychotics neither **25** nor **38** elicited dystonic movements in haloperidol-sensitized cebus monkeys, a primate model of antipsychotic-induced extrapyramidal side effects. Biochemical studies indicated that these compounds act via a nondopaminergic mechanism. Neither **25** nor **38** bound to dopamine receptors in vitro or caused changes in striatal dopamine metabolism in vivo. In addition, they did not raise serum prolactin levels as do known antipsychotics. Although adverse animal toxicological findings have precluded clinical evaluation of these agents, the present results indicate that it is possible to identify at the preclinical level nondopaminergic compounds with antipsychotic-like properties.

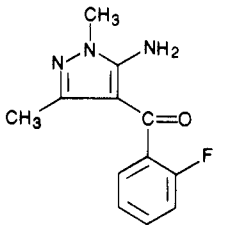
The discovery of the antipsychotic effects of chlorpromazine led to a therapeutic revolution in the treatment of schizophrenia.[1] Since then, numerous other antipsychotic agents have been developed. These have included, in addition to the tricyclic drugs of which chlorpromazine is representative, a variety of butyrophenones, diarylbutylamines, and benzamides; examples of each of these chemical classes are in clinical use.[2]

The preclinical pharmacological actions and clinical efficacy of all of these drugs are commonly attributed to their blockade of brain dopamine receptors.[3] However, these agents have incomplete antipsychotic efficacy in man and produce neurological side effects including the extrapyramidal syndrome (EPS) and tardive dyskinesia (TD).[4]

There is considerable interest in discovering improved drugs for the treatment of psychosis having little or no potential for these neurological side effects. Since both the efficacy and side effects of available drugs are thought to be due to their antidopaminergic actions, our efforts have been directed toward identifying potential antipsychotic agents that do not have such actions. One approach has been to identify compounds that have antipsychotic-like profiles in recognized preclinical behavioral tests but that do not act via the brain dopaminergic system.

As part of our CNS research program, a series of (1,3-dialkyl-5-amino-1*H*-pyrazol-4-yl)arylmethanones was prepared as intermediates for the synthesis of potential anxiolytic compounds.[5] Evaluation of these compounds in a general CNS survey test in mice revealed CNS depressant activity and in some cases potential anticonvulsant properties. In addition, one compound from this series, (5-amino-1,3-dimethyl-1*H*-pyrazol-4-yl)(2-fluorophenyl)methanone (**1**), decreased locomotion in mice without producing ataxia, a profile characteristic of antipsychotic drugs.[6]

Interestingly this activity appeared to be specific for the 2-fluorophenyl analogue. The unsubstituted phenyl analogue and compounds containing a variety of other phenyl substituents had little activity in this test. Increasing the size of the alkyl substituents at the 1- and 3-positions on the pyrazole ring increased the amount of nonspecific CNS depression. Several compounds in which the phenyl ring was replaced with heteroaromatic moieties were also examined. With the exception of the 2-thienyl analogue, none had significant activity. Since antipsychotic-like activity appeared to be unique for such compounds, we became interested in exploring their structure–activity relationships in more detail in order to identify novel compounds that could be developed as potential antipsychotic drugs.



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Chemistry

Several general synthetic routes to (1,3-dialkyl-5-amino-1*H*-pyrazol-4-yl)arylmethanones have been described in the literature including those outlined by Butler, Wise, and DeWald.[6] The reaction of 4-aroyl-5-chloropyrazoles with ammonia has been used to synthesize a variety of phenyl-substituted analogues of **1**. However, in the case of the 2-fluoro analogue **1** (Scheme I), only a very

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(1) Delay, J.; Deniker, P. *Le Congres des Al. et Neurol. de Langue Fr.; Compte Rendue du Congres*; Masson et Cie: Paris, 1952.
(2) Enna, S. J.; Coyle, J. T. *Neurochemical, Behavioral, and Clinical Perspectives*; Coyle, J. T., Enna, S. J., Eds.; Raven: New York, 1983; p 1.
(3) Creese, I.; Burt, D. R.; Snyder, S. H. *Science (Washington, D.C.)* **1976**, *192*, 481.
(4) Crow, T. J. *Br. Med. J.* **1980**, *2801*, 1. Andreasen, N. C.; Olsen, S. *Arch. Gen. Psychiatry* **1982**, *39*, 789. Ayd, F. J., Jr. *Neurochemical, Behavioral, and Clinical Perspectives*; Coyle, J. T., Enna, S. J., Eds.; Raven: New York, 1983; p 75. Jeste, D. V.; Wyatt, R. J. *Am. J. Psychiatry* **1981**, *138*, 297.
(5) DeWald, H. A.; Lobbestael, S. J.; Butler, D. E. *J. Med. Chem.* **1977**, *20*, 1562.
(6) Butler, D. E.; Wise, L. D.; DeWald, H. A. *J. Med. Chem.* **1984**, *27*, 1396.

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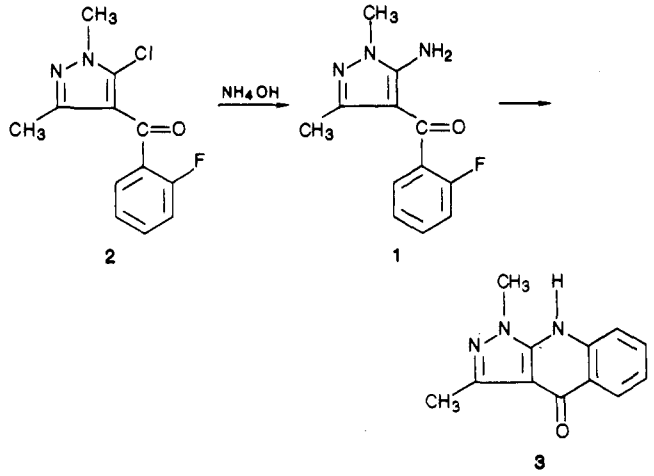
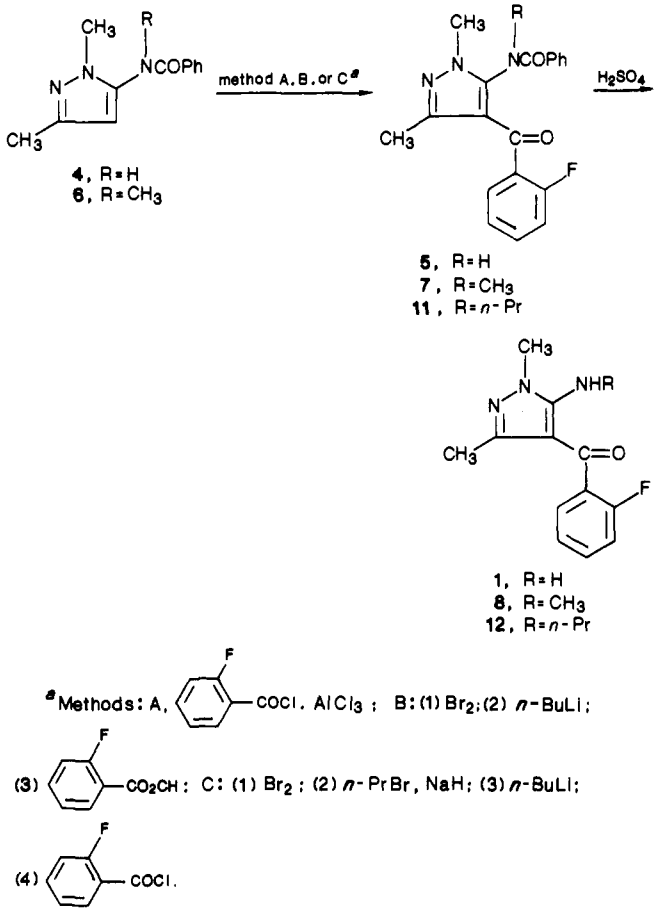
Scheme I



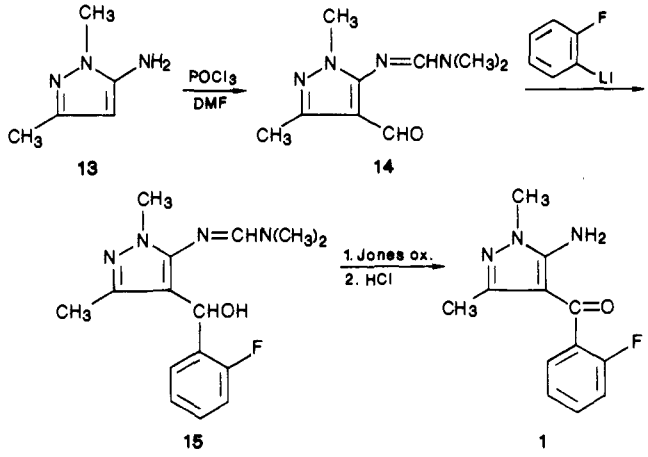
Scheme II



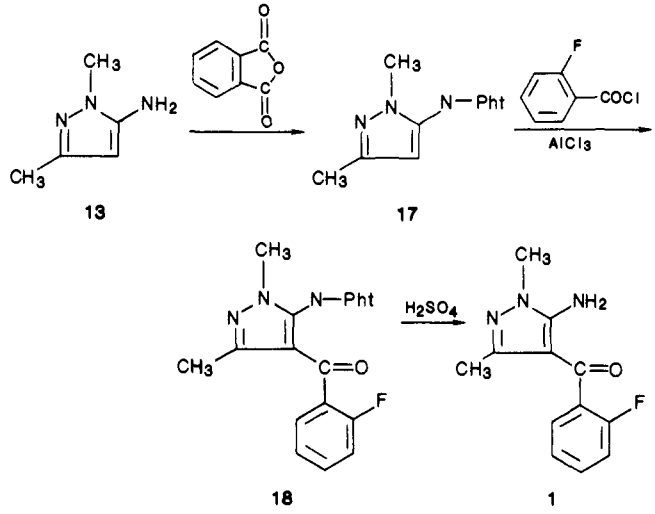
small amount of product could be isolated from the reaction of the 5-chloro intermediate **2**[7] with ammonium hydroxide at high temperature under pressure. The major product isolated from this reaction was the cyclized product **3**.[8]

In order to synthesize significant quantities of this compound for further evaluation and also for use as a chemical intermediate, alternate routes were explored. While the *N*-methyl analogue **8** could be prepared in approximately 70% by Friedel–Crafts benzoylation of amide **6** followed by hydrolysis of the intermediate benzamide **7** (Scheme IIA), only about a 25% yield of **1** (R = H) could

Scheme III



Scheme IV

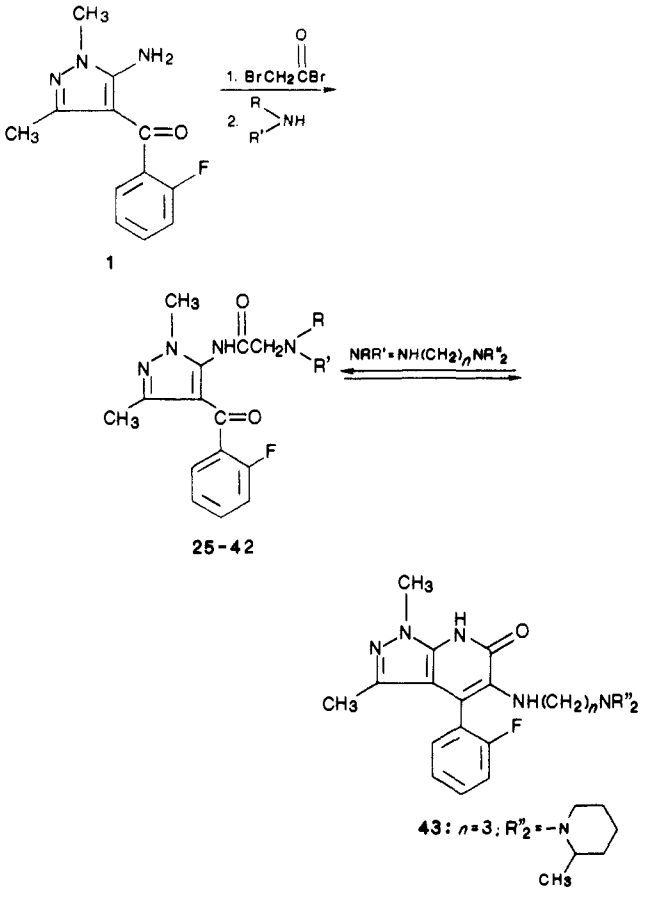


be obtained by this sequence.[6] A somewhat better route described recently by DeWald (Scheme IIB) involved bromination of **4**, followed by lithium exchange and reaction of the lithium compound with methyl 2-fluorobenzoate to give **5**, which was hydrolyzed to **1**.[9] In a U.S. patent Swett and associates described the reaction of 1,3-dimethyl-1*H*-pyrazol-5-amine **13**[10] with the Vilsmeier complex to give carboxaldehyde **14** in which the 5-amino moiety was conveniently protected as an amidine.[11] Reaction of **14** with (2-fluorophenyl)lithium at −60 °C gave alcohol **15**, which was subsequently oxidized with Jones reagent and deprotected with hydrochloric acid to yield **1** (Scheme III). The overall yield for this sequence was about 35%. By far the most useful route involved protection of **13** as phthalimide **17** (Scheme IV). Reaction of **17** with 2-fluorobenzoyl chloride under Friedel–Crafts conditions gave the intermediate ketone **18**. Deprotection of the amine with 60% sulfuric acid afforded **1** in a 58% overall yield from **13**.

The 5-amino group of **1** was found to be completely unreactive toward alkylation. The *N*-methyl analogue of **1**, compound **8**, was prepared by Scheme IIA while the *N*-propyl analogue, **12**, was synthesized by reaction of *N*-(4-bromo-1,3-dimethyl-1*H*-pyrazol-5-yl)benzamide[9] with bromopropane followed by lithium exchange and reaction of the lithium intermediate with 2-fluorobenzoyl chloride

(7) Butler, D. E.; DeWald, H. A. *J. Org. Chem.* **1971**, *36*, 2542.
(8) DeWald, H. A. *J. Heterocycl. Chem.* **1974**, *11*, 1061.
(9) DeWald, H. A. *J. Heterocycl. Chem.* **1984**, *21*, 1175.
(10) Taylor, E. C.; Hartke, K. S. *J. Am. Chem. Soc.* **1959**, *81*, 2456.
(11) Swett, L. R.; Ratajczyk, J. D.; Young, P. R. U.S. Patent 3 544 585, 1970.

Scheme V



to yield 11. Hydrolysis of 11 with sulfuric acid furnished compound 12 (Scheme II-C).

Acetamides **19–21** were prepared by refluxing 1 with the appropriate acid halide while **22** was obtained in a similar manner from 8. Sulfamide **24** was synthesized in two steps by reaction of 5-(methylamino)-1,3-dimethyl-1*H*-pyrazole with methanesulfonyl chloride/pyridine followed by Friedel–Crafts benzoylation. Acetamides **25–42** were synthesized in two steps (Scheme V). Treatment of 1 with bromoacetyl bromide gave the bromoacetamide.[6] In cases where the subsequent alkylation step was carried out with a propylenediamine, mixtures of the desired products and ring-closed byproducts were obtained (e.g., **43** with **38**). The products were separated by column chromatography. By control of the reaction temperatures and workup conditions, the amounts of this cyclized material could be minimized.

Results and Discussion

As shown in Table I, compound 1 inhibited spontaneous locomotion in mice (ED_{50} = 8.0 mg/kg ip) at doses that did not cause ataxia. This profile of activity has been shown to be characteristic of antipsychotic drugs.[12] However, unlike known antipsychotic agents, 1 did not bind appreciably to dopamine receptors in vitro as reflected by its lack of ability to displace [^{3}H]haloperidol from rat striatal membranes.[13] In keeping with an antipsychotic-like profile, 1 also showed oral activity in rats; it inhibited both one-way shuttle box conditioned avoidance (ED_{50} = 18 mg/kg) and Sidman avoidance responding (ED_{50} = 20 mg/kg).[14,15] However, 1 caused clonic seizures at minimally effective dose levels in some of the more aged rodents (>1 yr old) initially used in the Sidman avoidance test.

Table I. Pharmacological Profile of 1

inhibn of locomotion/ataxia in mice: ED_{50},[a] mg/kg ip	8.0/>30
inhibn of cond avoid. in rats:[b] ED_{50}, mg/kg po	18
inhibn of Sidman avoid. in rats:[c] ED_{50}, mg/kg po	20
displacement of [^{3}H]haloperidol binding:[d] IC_{50}, nM	>10000

[a] At least three doses of each drug were tested in nine animals at each dose. [b] Ten rats were tested at each dose level. Avoidance performance was measured during 6 h in 19 consecutive trials. [c] At least three doses of each drug were tested in four animals at each dose. [d] IC_{50}s were calculated from four or more concentrations done in triplicate.

In order to identify compounds with pharmacological profiles similar to 1, but without the propensity for causing seizures, a variety of structures related to 1 were synthesized and examined in aged rats. An important structural modification involved substitution on the 5-amino moiety of 1 (Table II). The *N*-methyl and *N*-propyl analogues, **8** and **12** respectively, had activities similar to 1 in the locomotor and ataxia tests; however, they also produced signs of clonic seizures in aged rodents. The same profile was seen with acetamides **19–21**. Compounds **22** and **24** in which the amide nitrogen atom is also substituted with a methyl group were completely inactive in the locomotor test.

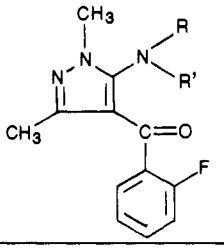
Interestingly, 2-(diethylamino)acetamide **25** was active in the locomotor and ataxia test and did not show any signs of clonic seizures in aged rodents. Analogues of **25** were examined in order to identify those with the best overall chemical and pharmacological profiles. After the initial ip evaluation in the locomotor and ataxia test in mice, the target compounds were tested po for suppression of shuttle box conditioned avoidance in rats, and when appropriate ED_{50} values were determined. As shown in Table III, activity was retained in the mouse model with cyclic amines **26–28**. The triazaspirodecanone analogue, **29**, was more active in the oral conditioned avoidance test than in the ip locomotor and ataxia test. Activity in the avoidance procedure was retained if the number of methylene groups was increased by one, **30** vs. **26**. Increasing the steric bulk adjacent to the amino group, **31** and **32**, resulted in loss of activity in both models.

Compound **33**, a substituted propylenediamine analogue, showed activity in both mouse and rat tests. This led to the examination of additional derivatives with related structural features (**34–42**, Table IV). The compounds displaying the most interesting pharmacological profiles in both behavioral models were ethylenediamine **35** and propylenediamines **38** and **42**. None of the compounds in Table III and IV bound significantly to dopamine receptors as measured by their ability to displace [^{3}H]haloperidol (IC_{50} > 10 μM) or caused clonic seizures in aged rodents.

Two compounds, the 2-(diethylamino)acetamide **25** and the 2-[3-(2-methylpiperidin-1-yl)propyl]acetamide **38**, were selected for further studies. The behavioral profiles of these compounds are compared with those of thioridazine and clozapine (Table V). In addition to their efficacy in the two behavioral tests noted above, **25** and **38** inhibited Sidman avoidance when administered orally to rats with ED_{50}'s of 25.6 and 21.3 mg/kg, respectively. Both compounds also displayed oral activity in a Sidman avoidance test in squirrel monkeys with ED_{50}'s of 15.6 and 12.7 mg/kg, respectively.

(12) Coughenour, L. L.; McLean, J. R.; Parker, R. B. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351.
(13) Burt, D. R.; Creese, I.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 800.
(14) Davidson, A.; Weidley, E. *Life Sci.* **1976**, *18* 1279.
(15) Sidman, M. *J. Comp. Physiol. Psychol.* **1953**, *46*, 253.

Table II. [1,3-Dimethyl-5-(substituted amino)-1*H*-pyrazol-4-yl](2-fluorophenyl)methanones



no.	R	R′	recryst solvent	yield,[a] %	mp, °C	formula[b]	inhibn of LMA/ataxia test: MED,[c] mg/kg ip	clonic seizures in aged rats[d]
1[e]	H	H					<10	+
8[f]	CH_3	H					30	+
12	n-C_3H_7	H	CH_3CN	45		$C_{15}H_{18}FN_3O$[g]	<10	+
19[h]	$COCH_3$	H	EtOH	30	185–187	$C_{14}H_{14}FN_3O_2$	30	+
20	$COCHCl_2$	H	EtOAc	72	169–171	$C_{14}H_{12}Cl_2N_3O_2$	30	+
21[h]	$COCF_3$	H	EtOH	30	212–214	$C_{14}H_{11}F_4N_3O_2$	<10	+
22[h]	$COCF_3$	CH_3	ether	86	91–93	$C_{15}H_{13}F_4N_3O_2$	>100	+
24	SO_2CH_3	CH_3	EtOAc	60	77–79	$C_{14}H_{16}FN_3O_3S$	>90	+
25	$COCH_2N(C_2H_5)$	H	iPrOH	80	170–173	$C_{18}H_{23}FN_4O_2 \cdot 2HCl$	30	-

[a] No attempts were made to maximize yields. [b] All compounds were analyzed for C, H, N. Except where noted, values agreed with calculated values with in ±0.4%. Compounds were characterized as free bases except where salts are noted below. [c] Compounds were evaluated for inhibition of locomotor activity (LMA) and ataxia at 10, 30, and 100 mg/kg (except for **24**, which was tested at 10, 30, and 90 mg/kg). The minimally effective dose (MED) is the lowest dose tested that reduced locomotion by 60% or greater while producing ataxia in less than 60% of the mice. [d] Compounds were examined for their potential to produce seizures in groups of four Long–Evans rats (>1 year old). If any sign of clonic seizures was seen for a particular compound, it was designated as causing seizures. No attempts were made to quantify the results. [e] Synthesized as described in ref 6. [f] Synthesized as described in ref 5. [g] N: calcd, 15.27; found, 14.78. [h] Synthesized by the method used for **20**.

Compounds **25** and **38** were examined for their potential for causing extrapyramidal side effects (EPS). Briefly, the model involved the use of haloperidol-sensitized cebus monkeys.[16] These monkeys, when tested with behaviorally active doses of antipsychotics that produce EPS in man (e.g., chlorpromazine, thioridazine, or haloperidol), showed dystonias and dyskinesias. Clozapine, an antipsychotic that does not produce EPS in man did not produce dystonic signs in these sensitized monkeys. Neither **25** nor **38** produced any dystonic signs in this model when tested at and above behaviorally active dose levels. Absence of activity in this highly predictive model is strongly suggestive of reduced liability for EPS in man.

Known antipsychotic drugs inhibit compulsive cage climbing produced by the dopamine receptor agonist, apomorphine, at doses comparable to those that inhibit locomotor activity in mice.[17] At 100 mg/kg neither **25** nor **38** altered the climbing induced by apomorphine. Unlike known antipsychotic drugs that are antiemetic, both **25** and **38** caused emesis in dogs at high doses (56 mg/kg).[18] These emetic effects were not antagonized by the dopamine antagonist haloperidol.

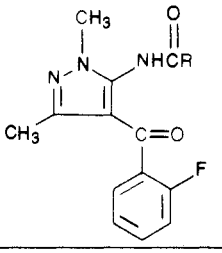
As shown in Table VI, **25** and **38** did not exhibit any significant in vitro affinity for dopamine, muscarinic, adrenergic, serotonergic, adenosine-A_1, benzodiazepine, or GABA receptors.[13,19–25] In addition to their lack of affinity for dopamine D_2 receptors, neither compound appeared to have any affinity for dopamine D_1 receptors as judged by their lack of effect on both basal and dopamine-stimulated striatal adenylate cyclase activity (Table VII).[26]

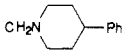
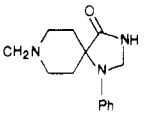
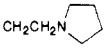
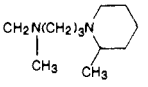
Unlike known antipsychotic agents, neither **25** nor **38** altered brain dopamine turnover. Neither compound affected the accumulation of the dopamine metabolite homovanillic acid (HVA) in the rat striatum.[27] In addition (Table VIII), **38** did not alter the disappearance of rat brain dopamine after blockade of synthesis with α-methyl-*p*-tyrosine (pT).[28] Compound **38** also did not alter the turnover of norepinephrine as judged by the lack effect on the pT-induced depletion of brain norepinephrine. Interestingly, both **25** and **38** lowered serum prolactin levels in vivo, but neither was effective in attenuating the haloperidol-induced elevation of prolactin.[29] Finally, neither compound antagonized the accumulation of brain dopamine induced by γ-butyrolactone (GBL).[30]

The behavioral and neurochemical profile of **25** and **38** can be clearly distinguished from that of clinically available antipsychotic drugs, all of which appear to act by blockade of brain dopamine receptors. For example, neither compound displayed affinity for D_1 or D_2 dopamine receptors, elevated dopamine metabolite or prolactin levels, or inhibited apomorphine-induced cage climbing as would be

(16) Barany, S.; Ingvast, A.; Gunne, L. M. *Res. Commun. Pathol. Pharmacol.* **1979**, *25*, 269.
(17) Constentin, J.; Protein, P.; Schwartz, J. *Nature* **1975**, *257*, 405; *Psychopharmacol.* **1976**, *50*, 1.
(18) Janssen, P. A. J.; Niemegeers, C. J. E.; Schellekens, K. H. L. *Arzneim.-Forsch.* **1965**, *15*, 1196.
(19) Ellis, J.; Hoss, W. *Brain Res.* **1980**, *193*, 189.
(20) U'Prichard, D. C.; Bechtel, W. D.; Rouot, B. M.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 47.
(21) Bylund, D. B.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 568.
(22) Peroutka, S. J.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 687.
(23) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547.
(24) Supavilai, P.; Karobath, M. *Eur. J. Pharmacol.* **1981**, *70*, 183.
(25) Enna, S. J.; Snyder, S. H. *Brain Res.* **1975**, *100*, 81.
(26) Kebabian, J. W.; Petzold, G. L.; Greengard, P. *Proc. Natl. Acad. Sci., U.S.A.* **1972**, *69*, 2145.
(27) Wilk, S.; Watson, E.; Travis, B. *Eur. J. Pharmacol.* **1975**, *30*, 238.
(28) Anden, N. E.; Butcher, G. S.; Corrodi, H.; Fuxe, K.; Ungerstedt, U. *Eur. J. Pharmacol.* **1970**, *11*, 303.
(29) Frantz, A. G. *Prog. Brain Res.* **1973**, *39*, 311.
(30) Walters, J. R.; Roth, R. H. *Biochem. Pharmacol.* **1976**, *25*, 649.

Table III. 2-(Substituted amino)-*N*-[4-(2-fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]acetamides



no.[a]	R	recryst-solvent	yield,[b] %	mp, °C	formula[c]	inhibn of LMA/ataxia test: MED,[d] mg/kg ip	suppressn of cond avoid. in rats: ED_{50},[e] mg/kg po	inhibn of [^{3}H]-haloperidol binding: IC_{50},[f] μM
25	$CH_2(C_2H_5)_2$	*i*-PrOH	66	189–190	$C_{18}H_{23}FN_4O_2 \cdot HCl$	30	18	>10
26		ether	29	115–117	$C_{18}H_{21}FN_4O_2$	30	48	>10
27		EtOAc	73	138–140	$C_{19}H_{23}FN_4O_2$	30	>100	>10
28		CH_3CN	93	105–106	$C_{25}H_{27}FN_4O_2 \cdot C_4H_4O$	30	100	>10
29		EtOAc-Et_2O	82	177–179	$C_{27}H_{29}FN_6O_3$	100	15.5	>10
30		EtOAc	70	108–110	$C_{19}H_{23}FN_4O_2$	100	15	>10
31		hexane	45	102–104	$C_{19}H_{23}FN_4O_2$	100	>100	>10
32		ether	30	117–119	$C_{20}H_{25}N_4O_2$	>100	>56	>10
33		CH_3CN		150–151	$C_{24}H_{34}FN_5O_2 \cdot 2C_4H_4O \cdot 0.5H_2O$	30	21	>10

[a] Compounds **26–33** were synthesized by the route described for compound **25**. [b–d] See footnotes *a–c*, respectively, in Table II. [e] See footnote *b* in Table I. [f] See footnote *d* in Table I.

expected of dopamine antagonists.

The decrease in serum prolactin levels and induction of emesis are traits of dopamine autoreceptor agonists. However, unlike dopamine agonists, neither **25** nor **38** was able to attenuate the elevation of prolactin caused by haloperidol. Additionally, since haloperidol did not antagonize the emesis caused by these agents, these effects were probably due to a nondopaminergic mechanism. Added to this, their lack of affinity to D_2 dopamine receptors, their inability to reduce dopamine synthesis in the GBL model, and their failure to decrease dopamine metabolite levels argue against a dopamine autoreceptor agonist mechanism of action for either compound.

The lack of adrenergic receptor binding and lack of effect on norepinephrine metabolism suggest that these compounds would not produce the adrenergic-mediated cardiovascular effects seen with many clinically available antipsychotic drugs.

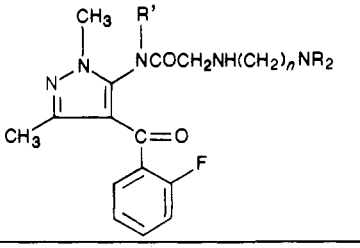
Since amino amides **25–42** are all derivatives of **1**, it is possible that these compounds are prodrugs of **1**. This possibility does not diminish the novelty of the activity profiles of **25** and **38** since **1**, like **25** and **38**, is a novel compound that is not a brain dopamine antagonist. Compound **1** did not have appreciable affinity for dopamine or other neurotransmitter receptors as shown in Table VI. In addition, in vivo tests in which **25** or **38** may be converted to **1** clearly distinguish the activity of these agents from known antipsychotics. Although the fact that the amino amides did not cause clonic seizures as did **1** may argue against their role as prodrugs, it remains possible that pharmacokinetics factors could mediate the reduced seizure liability.

As part of their preclinical evaluation, both **25** and **38** were examined in the Ames assay and were found to be free of bacterial mutagenic activity. However, in a 13-week toxicological evaluation in Wistar rats, both compounds produced mammary carcinomas.[31] This result necessitated the termination of the development of these compounds.

In summary, the present results demonstrate that compounds can be identified that produce behavioral effects similar to known antipsychotic drugs in preclinical tests that do not appear to interact with the brain dopamine system. We were also able to demonstrate that compounds with this behavioral profile could be identified that did not produce signs of EPS in a highly predictive primate model and that did not raise serum prolactin levels as do all clinically available antipsychotic drugs. The novel behavioral pharmacology seen in this series was supported biochemically in the in vitro receptor binding assays and in in vivo studies of brain catecholamine metabolism.

(31) Aust, A. E.; Andrews, L. K.; de la Iglesia, F. A. *Proc. Am. Assoc. Cancer Res.* **1985**, *26*, 92.

Table IV. 2-[Substituted (aminoalkyl)amino]-*N*-[4-(2-fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]acetamides



no.[a]	*n*	R′	NR_2	recryst solvent	yield,[b] %	mp, °C	formula[c]	inhibn of LMA/ataxia, test: MED,[d] mg/kg ip	suppressn of cond avoid. in rats: ED_{50},[e] mg/kg po	inhibn of [^{3}H]-haloperidol binding: IC_{50},[f] μM
34	2	H	$N[CH(CH_3)_2]_2$	CH_3CN	42	117–120	$C_{21}H_{32}FN_5O_2 \cdot 2C_4H_4O_2$[g]	100	71	>10
35	2	H		CH_3CN	32	152–155	$C_{21}H_{28}FN_5O_2 \cdot 2C_4H_4O_4 \cdot 0.5H_2O$	30	38	>10
36	3	H	$N(CH_3)_2$	CH_3CN–EtOAc	28	136–139	$C_{19}H_{26}FN_5O_2 \cdot 2C_4H_4O_4$	80	56	>10
37	3	H		CH_3CN	33	161–164	$C_{22}H_{30}FN_5O_2 \cdot 2C_4H_4O_4$	100	16	>10
38	3	H		CH_3CN–EtOAc	38	148–150	$C_{23}H_{32}FN_5O_2 \cdot 2C_4H_4O_4$	30	43	>10
39	3	CH_3		CH_3CN–ether	20	150–152	$C_{24}H_{34}FN_5O_2 \cdot 2C_4H_4O_4$[h]	>100	>100	>10
40	3	H		CH_3CN	20	167–168	$C_{23}H_{32}FN_5O_2 \cdot 2C_4H_4O_4$	100	100	>10
41	3	H		CH_3CN	20	168–170	$C_{23}H_{32}FN_5O_2 \cdot 2C_4H_4O_4$	100		>10
42	3	H	$NHC(CH_3)_3$	MeOH	46	187–188	$C_{21}H_{30}FN_5O_2 \cdot 2C_4H_4O_4 \cdot 0.5H_2O$[i]	30	46	>10

[a] Compounds **34–42** were synthesized by the route described for compound **38**. [b–d] See footnotes *a–c*, respectively, in Table II. [e] See footnote *b* in Table I. [f] See footnote *d* in Table I. [g] N: calcd, 10.98; found, 11.40. [h] C: calcd, 56.88; found, 56.02. [i] C: calcd, 67.13; found, 66.69.

Table V. Behavioral Profiles of **25** and **38**

test	**25**	**38**	thioridazine	clozapine
inhibn of locomotion/ataxia in mice: ED_{50},[a] mg/kg ip	17.5/>100	21.4/>100	2.9/>10	5.65/10
inhibn of cond avoid. in rats: ED_{50},[b] mg/kg po	18.0	43.5	5.8	22.0
inhibn of Sidman avoid. in rats: ED_{50},[c] mg/kg po	25.6	21.3	19.7	14.4
inhibn of Sidman avoid. in squirrel monkeys: ED_{50},[c] mg/kg po	15.6	12.7	4.3	4.1
inhibn of apomorphine-induced climbing in mice: ED_{50}[d], mg/kg ip	>100	>100	3.4	10.0
EPS signs in cebus monkeys: MED,[e] mg/kg po	>100	>200	2.5	>30
emesis in dogs: MED,[e] mg/kg po	56	56	antiemetic	antiemetic

[a–c] See footnotes *a–c*, respectively, in Table I. [d] Ten mice were used for each drug dose. ED_{50}'s were generated from at least three doses. [e] Minimal effective doses (MED) were measured in at least three animals. The emetic effects were not antagonized by 1 mg/kg doses of haloperidol.

Table VI. Affinities for Various Neurotransmitter Receptors in Rat Brain[a]

receptor	[^{3}H] ligand	1	**25**	**38**	haloperidol	clozapine
dopamine	haloperidol	>10 000	>10 000	>10 000	0.4	30
muscarinic	QNB	>10 000	>10 000	>10 000	6600	20
α_1-adrenergic	WB4101	>10 000	>10 000	>10 000	54	40
α_2-adrenergic	clonidine	>10 000	>10 000	>10 000	6000	40
β_1-adrenergic	DHA		>10 000	>10 000	>10 000	>10 000
β_2-adrenergic	DHA		>10 000	>10 000	>10 000	>10 000
serotonin-1	5-HT		>10 000	>10 000	~20,000	8700
serotonin-2	spiroperidol		>10 000	>10 000	33	20
adenosine A-1	CHA	>10 000	>10 000	>10 000		
GABA	GABA	>10 000	>10 000	>10 000		
benzodiazepine	flunitrazepam	>10 000	>10 000	>10 000		

[a] Reported as IC_{50}s (nM) to displace the following ligands. These values were determined from four or five concentrations by a nonlinear regression analysis.[32]

Table VII. Additional Biochemical Effects of 25 and 38

compd	adenylate cyclase[a] IC_{50}, μM	serum prolactin[b] mg/kg po	serum prolactin[b] % control ± SEM	striatal HVA[c] dose, mg/kg po	striatal HVA[c] % control ± SEM	GBL-induced DA accumuln[d] dose, mg/kg ip	GBL-induced DA accumuln[d] % control ± SEM
25	>10	1	87.4 ± 14.3	8	131.9 ± 6.4	25	85.5 ± 5.0
		10	52.9 ± 6.7[e]	25	114.9 ± 6.4		
		100	21.8 ± 6.7[e,f]	83	102.1 ± 14.9		
38	>10	100	51.0 ± 20.4[e,f]	25	95.8 ± 8.3	25	91.2 ± 3.1
haloperidol		1	224 ± 161[e]	1	348.9 ± 44.7[e]		
bromocriptine		1	32.7 ± 2.0[e,f]			10	82.4 ± 3.1[g]
apomorphine						5	75.0 ± 4.5[g]
fluphenazine	0.08						

[a] Data in these experiments were obtained by varying the concentration of the test substance while the concentration of dopamine (20 μM) was held constant. Basal and dopamine stimulated formation was (mean ± SEM) 50.7 ± 3.2 and 79 ± 4.2 pmol of cyclic AMP/mg of protein per min. None of the test compounds significantly affected basal levels of cyclic AMP. [b] Rats were sacrificed at 1 h after administration of compounds. Each value is a mean from groups of five animals. Control values: 4.9 ± 2.0 to 11.9 ± 3.5 ng/mL (mean ± SEM). [c] Rats were sacrificed 2 h after dosing with compounds. No effects of compound were observed on striatal dopamine levels. HVA control: 0.47 ± 0.07 μg/g (mean ± SEM). [d] Test compounds were injected 30 min before administration of γ-butyrolactone (GBL) (750 mg/kg ip), and rats were killed 1 h after GBL dosing. Each value is a mean from brains (minus brain stem and cerebellum) of four to six animals. Average control values were 1.14 ± 0.05 and 2.22 ± 0.09 μg/g (mean ± SEM) for saline- and GBL-treated animals, respectively. Each value is expressed as percentage of the GBL-treated group. [e] $p < 0.05$ vs. control-treated group. [f] The prolactin increase induced by 1 mg/kg po of haloperidol was antagonized by 0.5 mg/kg po of bromocriptine but not by either 100 mg/kg po of **25** or **38**. [g] $p < 0.05$ vs. control GBL treated group.

Table VIII. Effects of 38 on Norepinephrine (NE) and Dopamine (DA) Turnover

compd	dose, mg/kg ip	NE (% control ± SEM)	DA (% control ± SEM)
vehicle		100.1 ± 3.45	100.0 ± 3.05
38	64	102.8 ± 1.22	96.6 ± 3.76
clozapine	10	108.7 ± 4.24	114.6 ± 2.18[b]
vehicle + pT	250	54.2 ± 1.62[c]	41.7 ± 2.22[c]
38 + pT	64 ± 250	53.7 ± 2.49	45.6 ± 1.83
clozapine + pT	10 ± 250	37.2 ± 1.83[d]	41.3 ± 2.17

[a] Vehicle or agents were injected 30 min before α-methyl-*p*-tyrosine (pT). Rats were killed 4 h after pT. At least five determinations were made for each value. NE and DA values are expressed as a percent of vehicle treated values (μg/g ± SEM): NE, 0.58 ± 0.02; DA, 0.74 ± 0.02. [b] $p < 0.01$ vs. vehicle-treated group. [c] $p < 0.001$ vs. vehicle-treated group. [d] $p < 0.001$ vs. pT-treated group.

Unfortunately because of the toxicity of these agents, clinical verification for this type of an antipsychotic profile will have to await another species of compounds.

Experimental Section

Melting points were determined in a Thomas-Hoover melting point apparatus in open capillary tubes and are uncorrected. The structures of the compounds were confirmed by elemental analysis, infrared spectrometer, and NMR spectrometry. Infrared spectra were recorded on a Digilab FTP-14 infrared spectrometer, and NMR spectra were obtained on a Varian EM 390 90 MHz or Brucker 90 spectrometer and were consistent with the proposed structures. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values. TLC was carried out with 0.25-mm silica gel F254 (E. Merck) glass plates. GLC was carried out with a Shimadzu GC Mini 2 gas chromatograph equiped with fid.

***N*-(4-Bromo-1,3-dimethyl-1*H*-pyrazol-5-yl)-*N*-propylbenzamide (10).** To a solution of 29 g (0.1 mol) of *N*-(4-bromo-1,3-dimethyl-1*H*-pyrazol-5-yl)benzamide (**9**)[9] and 18.5 g (0.15 mol) of 3-bromopropane in 200 mL of DMF under nitrogen was added portionwise 2.5 g (0.11 mol) of sodium hydride. After addition was completed, the mixture was warmed to 50 °C for 3 h. The solvent was removed in vacuo, and the residue was dissolved in dichloromethane. The organic extracts were washed with water, dried over magnesium sulfate, and evaporated. The residue was slurried with petroleum ether and collected to yield 20.9 g (62%) of **10**, mp 108–110 °C. Anal. ($C_{15}H_{18}BrN_3O$) C, H, N.

***N*-[4-(2-Fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]-*N*-propylbenzamide (11).** A solution of 17 g (50 mmol) of **10** in 170 mL of THF was cooled to −60 °C, and 40 mL of *n*-butyllithium in heptane (53 mmol) was added dropwise with stirring under nitrogen. Stirring was continued at −60 °C for 1.5 h after which a solution of 8.5 g (53 mmol) of 2-fluorobenzoyl chloride in 20 mL of THF was added dropwise. The mixture was allowed to warm to 0 °C, and 75 mL of saturated ammonium chloride was added. The product was extracted into dichloromethane. The organic extracts were dried over anhydrous magnesium sulfate and evaporated. The residual oil was crystallized from petroleum ether to afford 7.1 g (39%) of **11**, mp 100–105 °C. Anal. ($C_{22}H_{22}FN_3O_2$) C, H, N.

[1,3-Dimethyl-5-(propylamino)-1*H*-pyrazol-4-yl](2-fluorophenyl)methanone (12). A mixture of 6.1 g (16 mmol) of **11** in 55 mL of 75% (v/v) sulfuric acid was stirred at 80–90 °C for 5 h and poured into 250 mL of ice water. The resulting mixture was filtered. The filtrate was made alkaline by the addition of concentrated ammonium hydroxide and extracted with dichloromethane. The organic extracts were dried over magnesium sulfate and evaporated. The residue was chromatographed on silica gel (acetonitrile). There was obtained 2.0 g (45%) of **12** as a colorless oil. Anal. ($C_{15}H_{18}FN_3O$) C, H, N.

5-[[(Dimethylamino)methylene]amino]-α-(2-fluorophenyl)-1,3-dimethyl-1*H*-pyrazole-4-methanol (15). To a solution of 140 mL (0.20 mol) of *n*-butyllithium in hexane and 140 mL of THF at −70 °C was added 35 g (0.2 mol) of 1-bromo-2-fluorobenzene in 20 mL of THF under nitrogen. The mixture was stirred at −70 °C for about 15 min after which a solution of 37 g (0.19 mol) of 5-[[(dimethylamino)methylene]-amino]-1,3-dimethyl-1*H*-pyrazole-4-carboxaldehyde (**14**)[11] in 50 mL of THF was added dropwise. The resulting mixture was stirred at −70 °C for an additional 2 h and alllowed to warm to room temperature over 18 h. To the viscous suspension was added 100 mL of saturated ammonium chloride. The layers were separated, and the organic extracts were dried over magnesium sulfate. Evaporation of the solvent gave a yellow oil that was crystallized from ether. There was obtained 36.0 g (65%) of colorless solid, mp 109–113 °C. Recrystallization from ethyl acetate–petroleum ether gave an analytical **15**, mp 111–113 °C. Anal. ($C_{15}H_{19}FN_4O$) C, H, N.

[5-[[(Dimethylamino)methylene]amino]-1,3-dimethyl-1*H*-pyrazol-4-yl](2-fluorophenyl)methanone (16). To 53 g (0.183 mol) of **15** in 1 L of dichloromethane at 0 °C was added 50 mL (~0.24 mol) of Jones reagent. The solution was stirred for an additional 20 min during which the reaction was allowed to warm to room temperature. A solution of 50 g of sodium carbonate in 400 mL of water was added. The layers were separated, and the organic layer was washed with saturated sodium bicarbonate solution. The organic extracts were dried over anhydrous magnesium sulfate and evaporated. The residue was recrystallized from ether to afford 22.7 g (43%) of **16**, mp 145 °C. Recrystal-

lization from ethyl acetate yielded an analytical sample, mp 152–154 °C. Anal. ($C_{15}H_{17}FN_4O$), C, H, N.

Hydrolysis of 16. (5-Amino-1,3-dimethyl-1*H*-pyrazol-4-yl)(2-fluorophenyl)methanone (1). A solution of 22 g (0.76 mol) of **16** in 200 mL of 3 N HCl was warmed on a steam bath for 18 h. The solvent was evaporated, and the solid was partitioned between dichloromethane and dilute sodium hydroxide solution. The layers were separated. The organic layer was washed with dilute sodium bicarbonate solution and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded crude oil that was crystallized from ether to afford 13.0 g of solid, mp 103–106 °C. The product was chromatographed over silica gel (acetonitrile) to give 10.0 g of **1**, mp 105–108 °C.

2-(1,3-Dimethyl-1*H*-pyrazol-5-yl)-1*H*-isoindole-1,3(2*H*)-dione (17). To a stirred suspension of 96 g (0.65 mol) of 5-amino-1,3-dimethylpyrazole hydrochloride[10] in 2 L of toluene was added 96 g (0.65 mol) of phthalic anhydride and 160 mL of triethylamine. The resulting mixture was heated to reflux, and the evolved water was collected on a Dean–Stark apparatus. When the evolution of water had ceased (after 2 h), the toluene was evaporated. The residual solid was slurried with 100 mL of water and filtered; the product was washed with a further 20 mL of water. Upon drying, there was obtained 131 g (84%) of **17** as a white crystalline solid, mp 199–202 °C. Anal. ($C_{13}H_{11}N_3O_2$) C, H, N.

2-[4-(2-Fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]-1*H*-isoindole-1,3(2*H*)-dione (18). To a stirred suspension of 61 g (0.45 mol) of aluminum chloride in 260 mL of *s*-tetrachloroethane at 10 °C under nitrogen was added 70.8 g (0.44 mol) of 2-fluorobenzoyl chloride dropwise over 30 min. During addition, the reaction temperature was maintained at 5–10 °C. After stirring for an additional 30 min, an almost clear solution had resulted, and a fine suspension of 109 g (0.45 mol) of **17** in 112 mL of *s*-tetrachloroethane was slowly added over 20 min at 10–15 °C. The reaction mixture was stirred for an additional 1 h during which it was allowed to warm to room temperature. The mixture was heated to reflux, slowly increasing the temperature from 85 to 130 °C. After 4 h the reaction was allowed to slowly cool and stand at room temperature for 18 h. The excess reactants were decomposed by pouring the reaction mixture onto a solution of 37 mL of hydrochloric acid and ice. The product was extracted into dichloromethane. The combined organic extracts were washed with water and dried over magnesium sulfate. The solvent was evaporated, and the residue was slurried with petroleum ether. There was isolated 133 g (81%) of **18**, mp 215–217 °C. Recrystallization from 2-propanol afforded an analytical sample, mp 226–227 °C. Anal. ($C_{20}H_{14}FN_3O_3$) C, H, N.

Hydrolysis of 18. (5-Amino-1,3-dimethyl-1*H*-pyrazol-4-yl)(2-fluorophenyl)methanone (1). A mixture of 66.5 g (0.183 mol) of **18** and 470 g of 75% (v/v) sulfuric acid was stirred at 75 °C for 4 h. The reaction was poured with stirring onto ice and filtered to remove the precipitated phthalic acid. The filtrate was cooled in an ice bath and made alkaline by slow addition of concentrated ammonium hydroxide at 25–30 °C. The mixture was extracted with dichloromethane. The organic extracts were dried over magnesium sulfate and evaporated. The residual oil was crystallized from toluene to give 36 g (85%) of **1**, mp 107–109 °C.

2,2-Dichloro-*N*-[4-(2-fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]acetamide (20). To 4.7 g (20 mmol) of **1** in 50 mL of warm benzene was added 5.0 g (20 mmol) of dichloroacetic anhydride. The solution was refluxed for 5 h, diluted with benzene, and washed with a standard sodium bicarbonate solution. A white precipitate that separated during the sodium bicarbonate extraction was collected, washed with water, and dried. There was obtained 5.0 g (72%) of colorless solid, mp 168–170 °C. Recrystallization from ethyl acetate gave an analytical sample of **20**, mp 169–171 °C. Anal. ($C_{14}H_{12}FCl_2N_3O_2$) C, H, N.

***N*-(1,3-Dimethyl-1*H*-pyrazol-5-yl)-*N*-methylmethanesulfonamide (23).** A solution of 20 g (0.16 mol) of 5-(methylamino)-1,3-dimethyl-1*H*-pyrazole in 50 mL of pyridine was treated with 19 g (0.16 mol) of methanesulfonyl chloride. The mixture was warmed on a steam bath for 2 h and poured into ice water. The aqueous solution was made alkaline with concentrated ammonium hydroxide and extracted repeatedly with dichloromethane. The combined organic extracts were washed with water and dried over magnesium sulfate. The solvent was evaporated, and the residue was recrystallized from ethyl acetate–petroleum ether to afford 22 g (68%) of **23**, mp 105–107 °C. Anal. ($C_7H_{13}N_3O_2S$) C, H, N.

***N*-[4-(2-Fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]-*N*-methylmethanesulfonamide (24).** To a suspension of 14.5 g (0.107 mol) of aluminum chloride in 200 mL of tetrachloroethane was slowly added 17 g (0.107 mol) of 2-fluorobenzoyl chloride followed by 21 g (0.103 mol) of **23**. The resulting mixture was refluxed for 6 h, cooled, and poured into dilute hydrochloric acid. The layers were separated. The organic extracts were washed with 200 mL of 6 N ammonium hydroxide and dried over magnesium sulfate. The solvent was evaporated, and the residue was distilled to yield 19 g (60%) of **24**, bp 195–200 °C (0.3 mm). Upon standing, the product crystallized and was recrystallized from ethyl acetate–petroleum ether to afford an analytical sample, mp 77–79 °C. Anal. ($C_{14}H_{16}FN_3O_3S$) C, H, N.

General Synthetic Route for 25–32. 2-(Diethylamino)-*N*-[4-(2-fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]acetamide Hydrochloride (25). To a suspension of 66.4 g (0.188 mol) of 2-bromo-*N*-[4-(2-fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]acetamide (**44**)[6] in 750 mL of dichloromethane cooled to −10 °C was added 32 mL (0.23 mol) of triethylamine followed by 21.0 g (0.30 mol) of diethylamine. The mixture was stirred at room temperature for 72 h. The resulting orange solution was washed with 500 mL of saturated aqueous sodium bicarbonate solution and dried over anhydrous magnesium sulfate. Evaporation of the solvent in vacuo gave 70.5 g of orange oil. The oil was dissolved in 300 mL of THF and treated with excess anhydrous hydrogen chloride in 2-propanol. The solution was cooled in an ice bath, and precipitation of the product was induced. The product was collected and washed with ethyl acetate. Recrystallization from acetonitrile afforded 61.0 g (77%) of **25** as a dihydrochloride, mp 170–173 °C.

A solution of 55.8 g (0.133 mol) of dihydrochloride **25** in 600 mL of 1-butanol was refluxed until no evolution of hydrogen chloride could be detected with prewetted Alkacid Test Ribbon (approximately 1 h). After an additional 1 h at reflux, the volume of the solution was adjusted to 250 mL. The solution was partially cooled and diluted with anhydrous ether to a volume of 500 mL. A crystalline solid slowly formed. After 18 h at room temperature, the product was collected, washed with 1-butanol–ether (1:1) and finally with ether. There was isolated 47.4 g (66% based on **44**) of white crystalline monohydrochloride **25**, mp 189–190 °C. Anal. ($C_{18}H_{23}FN_4O_2 \cdot HCl$) C, H, N.

General Synthetic Procedure for 33–42. *N*-[4-(2-Fluorobenzoyl)-1,3-dimethyl-1*H*-pyrazol-5-yl]-2-[[3-(2-methyl-1-piperidinyl)propyl]amino]acetamide (38). A solution of 18.7 g (0.053 mol) of **44** in 1 L of dichloromethane was treated in one portion with 16.5 g (0.106 mol) of 2-methyl-1-piperidinylpropylamine was refluxed for 17 h. The mixture was cooled and washed with two 50-mL portions of 5% sodium bicarbonate solution. The dichloromethane solution was dried over magnesium sulfate and evaporated to dryness. The residue was taken up in 1 L of ether and filtered. The solution was treated with ethereal maleic acid. The precipitate was collected and recrystallized from acetonitrile–ethyl acetate to give 9.2 g (38%) of **38** as the dimaleate salt, mp 148–150 °C. Anal. ($C_{23}H_{32}FN_5O_2 \cdot 2C_4H_4O_4$) C, H, N.

The filtrate from the above recrystallization was chromatographed on a silica gel column (50 g) (ethyl acetate). After recrystallization from ethyl acetate there was isolated 1.20 g (6%) of **4-(2-fluorophenyl)-1,7-dihydro-1,3-dimethyl-5-[[3-(2-methyl-1-piperidinyl)propyl]amino]-6*H*-pyrazolo[3,4-*b*]pyridin-6-one (43)**, mp 146–149 °C. Anal. ($C_{23}H_{30}FN_5O$), C, N, N.

Pharmacological Methods. Locomotor Activity and Ataxia Test.[12] Nine unfasted Swiss–Webster male mice (Buckberg Laboratories) weighing 20–30 g were tested in groups in three with each drug dose. Treatments were administered intraperitoneally 1 h prior to testing. All dosages were calculated as parent compound and given in volumes of 10 mL/kg. Compounds were dissolved or suspended in 0.2% methocel. A two-part testing procedure was started 1 h postinjection. First, ataxia was assessed with an inverted screen test. This test consisted of placing mice on individual wire screens that were rotated 180° at the start of a 60-s observation period. The number of mice falling off the

inverted screen was recorded. Immediately following the screen test, the animals were tested for inhibition of locomotion. Each group of three mice was placed in an actophotometer consisting of a cylindrical chamber the center of which contained the illumination for six photocells located on the perimeter. Six light beam interruptions produced one activity count. Locomotion activity was recorded by computer at 10-min intervals for 60 min. Data obtained from the screen test were expressed as percent of mice falling off the screen. Data derived from the locomotor activity of the drug-treated mice were compared to the activity of vehicle-treated control animals and were expressed as percent inhibition of spontaneous locomotion.

Conditioned Avoidance in Rats.[14] Fasted male Wistar rats (150–250 g) were placed individually in test chambers consisting of an electrified grid and an escape platform. Rats were trained during 20 consecutive trials to jump to the platform at the sound of a buzzer to avoid electric shock (1.1 mA/s) delivered through the grid floor. After drug administration, rats were tested for the avoidance response every 20 min for 6 h. Responses were recorded as avoidance (rats leave the grid before shocks), escapes (rats leave the grid during shocks), or failures (rats fail to get off the grid during a trial). Data were calculated from the latency in seconds to emit a response. Groups of 10 rats were tested with each dose level. Avoidance inhibition was determined in each of 19 trials. Data from the four consecutive trials during which the largest drug effects were seen were averaged to yield the peak drug effects. The percent blockade of avoidance was determined by the formula percent = (average peak latency to avoid shock × 100)/(latency to shock). Rats were used only once for this test. Estimation of ED_{50} for blockade of avoidance was made from a graphical plot of the data employing at least three dose levels.

Sidman Avoidance Procedures.[15] Mature male Long–Evans rats or mature squirrel monkeys were trained on a modified Sidman avoidance schedule using standard operant conditioning chambers equipped with a single wall-mounted response lever. Depression of the lever postponed the delivery of an electric shock through the grid floor (2.4 mA/0.5 s for rats and 4.0 mA/0.5 s for monkeys) for 20 s (R–S interval = 20 s). Failure to depress the lever resulted in the delivery of a shock every 10 s (S–S interval = 10 s). Depression of the lever during the S–S interval terminated shock and returned the animal to the R–S interval. The duration of each test was 6 h. Drug effects were expressed as percentage inhibition of nondrug avoidance responding with each animal serving as its own control. At least three doses of each drug were tested in four animals with test sessions separated by at least 1 week. ED_{50}'s for avoidance inhibition were calculated from nonlinear regression analysis of the dose–effect functions.[32]

Extrapyramidal Side Effect Test in Cebus Monkeys.[16] Cebus monkeys were treated with 2 mg/kg of haloperidol po once weekly. After 6–12 weeks of treatment the monkeys became sensitized to haloperidol and related drugs whose effects are mediated by dopaminergic blockade. When subsequently tested with haloperidol or other available antipsychotics, these monkeys developed a syndrome of dystonias and dyskinesias including involuntary twisting of the neck or torso, tongue protrusion, compulsive biting, oral dyskinesias, flailing about the cage, tonic extension of the limbs, and bizarre hand postures. The test procedure consisted of dosing (by gavage) groups of three to six monkeys with test compound and observing them at hourly intervals for 6–8 h for signs of the dystonic syndrome. Monkeys showing any number or degree of unequivocal signs of dystonias were identified as positive responders at that dose. Minimal effective doses for dystonic effects were determined.

Blockade of Apomorphine-Induced Cage Climbing.[17] Ten male Swiss–Webster mice (25–35 g) were tested with each drug dose. Groups of five mice were placed in cylindrical cages for 30 min to permit acclimation. The mice were dosed with test agent or vehicle and returned to the cages. One hour later the mice were dosed with 3 mg/kg sc of apomorphine and returned to the cages. Beginning 15 min after apomorphine administration, climbing was rated during repeated 30-s tests conducted every 5 min for 30 min. During each test, each of the mice observed was given a score, as follows: 0 = no paws on the sides of the cage; 1 = one, two, or three paws on the sides of the cage; 2 = four paws grasping the sides of the cage (climbing). The sum of the climbing scores during all tests was obtained for each treatment. Drug effects on climbing were expressed as percentage inhibition relative to the incidence of climbing in the control (vehicle + apomorphine) group. ED_{50}'s were calculated by nonlinear regression analysis.[32]

Emesis Test.[18] Fasted dogs (7–12 kg) were dosed with the test compounds. Groups of three animals were normally tested with each dose. After dosing, the animals were observed once every hour for up to 6 h for evidence of emesis as well as other autonomic or behavioral signs. The minimal active dose was defined as the lowest dose producing emesis in any animal tested.

Receptor Binding Assays. The relative affinities of compounds for a variety of receptors were evaluated. The receptors assayed, radioligands (final concentrations), brain area, reference standard (final concentrations), and method used, respectively, were as follows: dopamine D_2, [^{3}H]haloperidol (0.1 nM), rat striatum, (+)-butaclamol (0.1 μM), by the method of Burt et al.;[13] muscarinic, [^{3}H]quinuclidinyl benzilate (0.03 nM), rat brain stem, oxotremorine (100 μM), according to the method of Ellis and Hoss;[19] α_1-adrenergic, [^{3}H]-2-[[[2-(2,6-dimethoxyphenoxy)-ethyl]amino]methyl]-2,3-dihydro-1,4-benzodioxin ([^{3}H]WB4101) (0.5 nM), rat frontal cerebral cortex, (−)-norepinephrine (100 μM), according to the method of U'Prichard et al.;[20] α_2-adrenergic [^{3}H]clonidine (0.4 nM), rat cerebral cortex, (−)-norepinephrine (10 μM), according to the method of U'Prichard et al.;[20] β-adrenergic, [^{3}H]dihydroalprenolol ([^{3}H]-DHA) (0.5 nM), rat cerebral cortex (β_1) and rat cerebellum (β_2), (−)-alprenolol (1 μM), according to Bylund and Snyder;[21] serotonin-1, [^{3}H]-5-hydroxytryptamine creatinine sulfate ([^{3}H]-5HT) (2.0 nM), rat cortex, (+)-butaclamol (1 μM), according to the method of Peroutka and Snyder;[22] serotonin-2, [^{3}H]spiroperidol (0.2 nM), rat cerebral cortex, (+)-butaclamol (1.0 μM), by the method of Peroutka and Snyder;[22] adenosine-A_1, *N*-6-cyclohexyl[^{3}H]adenosine [^{3}H]-CHA (1.0 nM), rat whole brain minus brain stem and cerebellum, theophylline (1 mM), by the method of Bruns et al.;[23] GABA, [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) (25 nM), rat whole brain minus cerebellum and brain stem membranes, GABA (1 mM), according to Enna and Snyder.[25] The test compounds were examined at two or more concentrations in each assay. The IC_{50} values were determined from four or five concentrations by a computer nonlinear curve fit program.[32] All radioligands were obtained from New England Nuclear. Membranes were prepared from male Long–Evans hooded rats (150–250 g).

Adenylate Cyclase.[26] Adenylate cyclase activity in homogenates of rat corpus striatum was determined according to the method of Kebabian et al., except that the cyclic AMP generated was determined by a radioimmunoassay method.[33] The brain homogenates were obtained from male Long–Evans rats (200–300 g). Assays were performed in triplicate.

Effects on Catecholamine Metabolism. Male Long–Evans rats weighing 160–225 mg/kg were administered test agent. At the appropriate time, the animals were killed by decapitation. The brains were removed, dissected,[34] and frozen until the catecholamine and metabolite contents were extracted and isolated on a Sephadex G-10 column.[35] These concentrations of NE and DA were measured spectrofluorometrically by modifications of the methods of Shellenberger and Gordon[36] and Earley and Leonard.[35] The HVA content of rat striatum was measured spectrofluorometrically by the method of Anden et al.[37] as modified by Earley and Leonard.[35]

Prolactin Assay.[29] Seven-week-old male Sprague–Dawley rats were used. Following decapitation of the rats, trunk blood was obtained at 1, 2, 3, and 4 h after oral administration of the test

(32) Parker, R. B.; Waud, D. R. *J. Pharmacol. Exp. Ther.* **1973**, *183*, 1.

(33) Steiner, A. L. *Assay of Cyclic Neurotides by Radioimmunoassay Methods*; Hardman, J. G., O'Malley, B. W., Eds.; Academic: New York, 1974; Vol. 38, p 96.

(34) Glowinski, J.; Iversen, L. L. *J. Neurochem.* **1966**, *13*, 665.

(35) Earley, C. J.; Leonard, B. E. *J. Pharmacol. Methods* **1978**, *1*, 67.

(36) Shellenberger, M. K.; Gordon, S. H. *Anal. Biochem.* **1971**, *39*, 356.

(37) Anden, N. E.; Roos, B. E.; Werdinus, B. *Life Sci.* **1963**, *2*, 448.

compounds. Serum prolactin levels were determined by the method and reagents for radioimmunoassay provided by the Hormone Distribution Program of NIAMDD, using PRL-RP-1 and PRL-S-7, and [^{125}I]-PRL purchased from New England Nuclear Co.

Registry No. 1, 31272-21-6; 8, 56877-15-7; 9, 85723-93-9; 10, 103094-23-1; 11, 103068-59-3; 12, 103068-60-6; 13 (5-*N*-Me), 103068-68-4; 13·HCl, 103068-64-0; 14, 103068-61-7; 15, 103068-62-8; 16, 103068-63-9; 17, 103068-65-1; 18, 103068-66-2; 19, 103068-97-9; 20, 103068-67-3; 21, 103068-98-0; 22, 103068-99-1; 23, 103068-69-5; 24, 103068-70-8; 25, 85747-92-8; 25·2HCl, 85723-66-6; 25·HCl, 103068-82-2; 26, 85723-00-8; 26·2HCl, 103068-74-2; 26·HCl, 85723-65-5; 27, 85723-04-2; 27·2HCl, 103068-75-3; 27·HCl, 103068-83-3; 28, 103068-71-9; 28·2HCl, 103068-76-4; 28·HCl, 103068-84-4; 29, 85723-12-2; 29·2HCl, 103068-77-5; 29·HCl, 103094-24-2; 30, 85723-17-7; 30·2HCl, 103068-78-6; 30·HCl, 103068-85-5; 31, 103068-72-0; 31·2HCl, 103068-79-7; 31·HCl, 103068-86-6; 32, 103068-73-1; 32·2HCl, 103068-80-0; 32·HCl, 103068-87-7; 33, 85723-38-2; 33·$C_4H_4O_4$, 85723-39-3; 34, 103068-88-8; 34·2$C_4H_4O_4$, 103068-90-2; 35, 85723-48-4; 35·2$C_4H_4O_4$, 85723-49-5; 36, 85723-22-4; 36·2$C_4H_4O_4$, 85723-23-5; 37, 85723-44-0; 37·2$C_4H_4O_4$, 85723-45-1; 38, 85723-20-2; 38·2$C_4H_4O_4$, 85723-21-3; 38 (bromo deriv), 63960-69-0; 39, 103068-89-9; 39·2$C_4H_4O_4$, 103068-91-3; 40, 85723-34-8; 40·2$C_4H_4O_4$, 85723-35-9; 41, 85723-32-6; 41·2$C_4H_4O_4$, 85723-33-7; 42, 85723-42-8; 42·2$C_4H_4O_4$, 85723-43-9; 43 (n = 2, R″$_2$ = N(CH(CH$_3$)$_2$)$_2$), 103068-92-4; 43 (n = 2, R″$_2$ = c-N(CH$_2$)$_5$), 103094-25-3; 43 (n = 3, R″$_2$ = N(CH$_3$)$_2$), 103068-93-5; 43 (n = 3, R″$_2$ = c-N(CH$_2$)$_5$), 103068-94-6; 43 (n = 3, R″$_2$ = c-N(CH(CH$_3$)(CH$_2$)$_4$), 85723-84-8; 43 (n = 3, N-CH$_3$, R″$_2$ = c-N(CH(CH$_3$)(CH$_2$)$_4$), 103068-95-7; 43 (n = 3, c-N(CH$_2$CH(CH$_3$)(CH$_2$)$_3$) = R″$_2$), 103068-96-8; 43 (n = 3, c-N((CH$_2$)$_2$CH(CH$_3$)(CH$_2$)$_2$) = R″$_2$), 103094-26-4; 43 (n = 3, R″$_2$ = NHC(CH$_3$)$_3$), 103094-27-5; 43 (n = 3, N-CH$_3$, R″$_2$ = c-N(CH(CH$_3$)(CH$_2$)$_4$), 103068-81-1; 2-FC$_6$H$_4$COCl, 393-52-2; 2-BrC$_6$H$_4$F, 1072-85-1; H$_2$N(CH$_2$)$_2$N(CH(CH$_3$)$_2$), 121-05-1; H$_2$N(CH$_2$)$_3$N(CH$_3$)$_2$, 109-55-7; H$_2$N(CH$_2$)$_3$NC(CH$_3$)$_3$, 52198-64-8; pyrrolidine, 123-75-1; piperidine, 110-89-4; 4-phenylpiperidine, 771-99-3; 4-(1-phenyl-1,2,3,4,5-pentahydro-4-oxopyrimid-5-yl)piperidine, 1021-25-6; 1-piperidineethanamine, 27578-60-5; 2-methyl-1-piperidinepropanamine, 25560-00-3; 3-methyl-1-piperdinepropanamine, 14156-91-3; 4-methyl-1-piperidinepropanamine, 6241-30-1; 1-piperidinepropanamine, 3529-08-6; *N*-methyl-2-methyl-1-piperidinepropanamine, 85723-73-5.

Selective Thromboxane Synthetase Inhibitors. 3. 1*H*-Imidazol-1-yl-Substituted Benzo[*b*]furan-, Benzo[*b*]thiophene-, and Indole-2- and -3-carboxylic Acids

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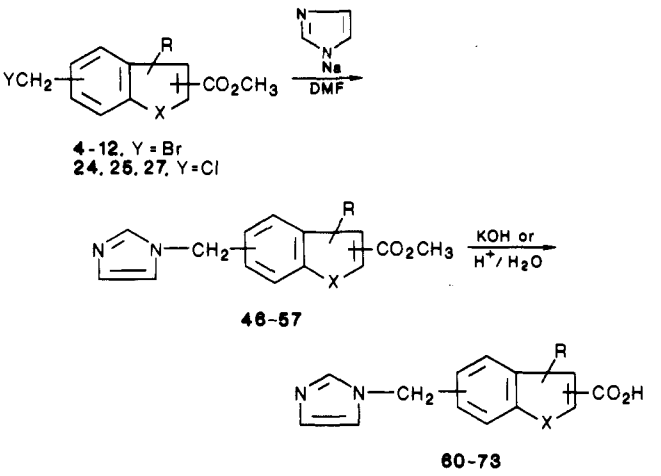
The preparation of a series of 1*H*-imidazol-1-yl-substituted benzo[*b*]furan-, benzo[*b*]thiophene-, and indolecarboxylic acids is described. Most of the compounds were potent inhibitors of TxA_2 synthetase in vitro, and the distance between the imidazole and carboxylic acid groups was found to be important for optimal potency. The most potent compound in vivo was 6-(1*H*-imidazol-1-ylmethyl)-3-methylbenzo[*b*]thiophene-2-carboxylic acid (**71**), which, in conscious dogs, showed a similar profile of activity to that of dazoxiben (**1**).

1-Substituted imidazoles are known to inhibit thromboxane synthetase, the enzyme that converts prostaglandin H_2 (PGH_2) to the potent vasoconstrictor and platelet-aggregating agent thromboxane A_2 (TxA_2).[1-7] Thus, they are potentially useful for the treatment or prevention of cardiovascular conditions where vasospasm or thrombosis may be a contributing factor.[8-11] It has been shown that, in many cases, introduction of a carboxylic acid group into the imidazole 1-substituent can increase potency against TxA_2 synthetase.[2-4,6,7] The presence of a carboxyl function has the additional advantage of reducing activity against other enzymes that are susceptible to inhibition by 1-substituted imidazoles such as liver microsomal cyto-

Scheme I



(1) Tai, H.-H.; Yuan, B. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 236.
(2) Yoshimoto, Y.; Yamamoto, S.; Hayaishi, O. *Prostaglandins* **1978**, *16*, 529.
(3) Kayama, N.; Sakaguchi, K.; Kaneko, S.; Kubota, T.; Fukuzawa, T.; Kawamura, S.; Yoshimoto, T.; Yamamoto, S. *Prostaglandins* **1981**, *21*, 543.
(4) Iizuka, K.; Akahane, K.; Momose, D.; Nakazawa, M.; Tanouchi, T.; Kawamura, M.; Ohyama, I.; Kajiwara, I.; Iguchi, Y.; Okada, T.; Taniguchi, K.; Miyamoto, T.; Hayashi, M. *J. Med. Chem.* **1981**, *24*, 1139.
(5) Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *Agents Actions* **1981**, *11*, 274.
(6) Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *J. Med. Chem.* **1985**, *28*, 1427.
(7) Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *J. Med. Chem.* **1986**, *29*, 342.
(8) Moncada, S.; Gryglewski, R. J.; Bunting, S.; Vane, J. R. *Prostaglandins* **1976**, *12*, 715.
(9) Moncada, S.; Bunting, S.; Mullane, K.; Thorogood, P.; Vane, J. R. *Prostaglandins* **1977**, *13*, 611.
(10) Moncada, S.; Higgs, E. A.; Vane, J. R. *Lancet* **1977**, *1*, 18.
(11) Nijkamp, F. P.; Moncada, S.; White, H. L.; Vane, J. R. *Eur. J. Pharmacol.* **1979**, *44*, 179.

0022-2623/86/1829-1637$01.50/0