Articles

Novel Compounds Possessing Potent cAMP and cGMP Phosphodiesterase Inhibitory Activity. Synthesis and Cardiovascular Effects of a Series of Imidazo[1,2-*a*]quinoxalinones and Imidazo[1,5-*a*]quinoxalinones and Their Aza Analogues

D. D. Davey,* P. W. Erhardt, E. H. Cantor, S. S. Greenberg, W. R. Ingebretsen, and J. Wiggins

Departments of Medicinal Chemistry and Pharmacology, Berlex Laboratories, Inc., 110 E. Hanover Avenue, Cedar Knolls, New Jersey 07927. *Received January 18, 1991*

A series of novel imidazoquinoxalinones and their aza analogues were prepared by the cyclization of *o*-amino(1*H*-imidazol-1-yl)aryls and heteroaryls with carbonyldiimidazole. The compounds were screened for inhibition of Type I and Type IV phosphodiesterases (PDE's) and evaluated for their vasorelaxant and positive inotropic activities in vitro. In general, compounds having potent PDE inhibitory activity also possessed good inotropic and vasodilator activity, although linear correlations between these activities could not be established.

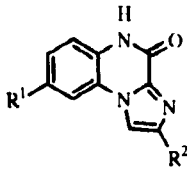
The use of nitrates during treatment of congestive heart failure (CHF) has become well established.[1,2] This class of compounds, which includes nitroglycerin, sodium nitroprusside, and ISDN, produces vascular relaxation after increasing intracellular cGMP levels through stimulation of guanylate cyclase.[3,4] The initial profile, obtained when high doses of the nitrates are employed, is that of balanced venous and arterial dilation, resulting in both decreased preload and afterload pressures on the heart.[5] Unfortunately, the nitrates may not prove to be suitable for long-term therapy of CHF[6] due mainly to the development of tolerance.[7,8] Since the tachyphylaxis observed for these compounds is thought to involve diminished biochemical activation of the nitrates by sulfhydryl compounds[9,10] and/or other receptor-related events,[11,12] we felt that a compound that could increase cGMP levels through inhibition of cGMP (Type I) phosphodiesterase (PDE) might circumvent the problem of tolerance. Furthermore, when coupled with positive inotropic activity achieved through inhibition of cardiac cAMP (Type IV) PDE,[13] such balanced inodilators might have added benefit for the chronic treatment of CHF.[14,15]

As an initial structural approach, we considered the xanthines as a reasonable starting point for the development of a new series of inodilators. One compound from this class, 3-isobutyl-1-methylxanthine (IBMX), is a moderately potent nonselective inhibitor of cAMP and cGMP PDE's,[16,17] with activity in both our in vitro positive inotropic and vascular relaxant screens (Table II). Like many other xanthines, however, IBMX possesses CNS activity,[18-21] which precludes its use as a cardiovascular

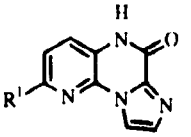
(1) Geltman, E. M. Mild Heart Failure: Diagnosis and Treatment. *Am. Heart J.* **1989**, *118*, 1277.
(2) Cohn, J. N. Nitrates Are Effective in the Treatment of Chronic Congestive Heart Failure: The Protagonist's View. *Am. J. Cardiol.* **1990**, *66*, 444.
(3) Ignarro, L. J.; Lippton, H.; Edwards, J. C.; Baricos, W. H.; Hyman, A. L.; Kadowitz, P. J.; Gruetter, C. A. Mechanism of Vascular Smooth Muscle Relaxation by Organic Nitrates, Nitrites, Nitroprusside and Nitric Oxide: Evidence for the Involvement of *S*-Nitrosothiols as Active Intermediates. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 739.
(4) Lincoln, T. M. Cyclic GMP and Mechanisms of Vasodilation. *Pharmac. Ther.* **1989**, *41*, 479.
(5) Abrams, J. Nitrate Use in Congestive Heart Failure. *Drug Therapy* **1989**, 109.
(6) Packer, M. Are Nitrates Effective in the Treatment of Chronic Heart Failure? Antagonist's Viewpoint. *Am. J. Cardiol.* **1990**, *66*, 458.
(7) Parker, J. O. Nitrate Tolerance. *Am. J. Cardiol.* **1987**, *60*, 44H.
(8) Zeller, F. P. Tolerance to Nitrates in Ischemic Heart Disease. *Drug Intell. Clin. Pharm.* **1987**, *21*, 857.
(9) Brien, J. F.,; McLaughlin, B. E.; Breedon, T. H.; Bennett, B. M.; Nakatsu, K.; Marks, G. S. Biotransformation of Glycerol Trinitrate Occurs Concurrently with Relaxation of Rabbit Aorta. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 608.
(10) Henry, P. J.; Drummer, O. H.; Horowitz, J. D. *S*-Nitrosothiols as Vasodilators: Implications Regarding Tolerance to Nitric Oxide-containing Vasodilators. *Br. J. Pharmacol.* **1989**, *98*, 757.
(11) Ahlner, J.; Andersson, R. G. G.; Axelsson, K. L.; Ekstam Ljusegren, M. Development of Tolerance Towards Glyceryl Trinitrate In Vitro. *Arzneim.-Forsch.* **1987**, *37*, 914.
(12) Bennett, B. M.; Schröder, H.; Hayward, L. D.; Waldman, S. A.; Murad, F. Effect of In Vitro Organic Nitrate Tolerance on Relaxation, Cyclic GMP Accumulation, and Guanylate Cyclase Activation by Glyceryl Trinitrate and the Enantiomers of Isoidide Dinitrate. *Circ. Res.* **1988**, *63*, 693.
(13) Erhardt, P. W. In Search of the Digitalis Replacement. *J. Med. Chem.* **1987**, *30*, 231.
(14) Pang, D. C. Cyclic AMP and Cyclic GMP Phosphodiesterases: Target for Drug Development. *Drug Dev. Res.* **1988**, *12*, 85.
(15) Cargnelli, C.; Piovan, D.; Bova, S.; Padrini, R.; Ferrari, M. Present and Future Trends in Research and Clinical Applications of Inodilators. *J. Cardiovasc. Pharmacol.* **1989**, *14*(Suppl. 8), S124.
(16) Weiss, R.; Hardman, J.; Wells, J. Resistance of a Separated Form of Canine Ureteral Phosphodiesterase Activity to Inhibition by Xanthines and Papaverine. *Biochem. Pharmacol.* **1981**, *30*, 2371.
(17) Schoeffter, P.; Lugnier, C.; Demesy-Waeldele, F.; Stoclet, J. C. Role of Cyclic AMP- and Cyclic GMP-Phosphodiesterases in the Control of Cyclic Nucleotide Levels and Smooth Muscle Tone in Rat Isolated Aorta. *Biochem. Pharmacol.* **1987**, *36*, 3965.
(18) Wu, P. H.; Phillis, J. W.; Nye, M. J. Alkylxanthines as Adenosine Receptor Antagonists and Membrane Phosphodiesterase Inhibitors in Central Nervous Tissue: Evaluation of Structure–Activity Relationships. *Life Sci.* **1982**, *31*(25), 2857-67.
(19) Grant, S. J.; Redmond, D. E., Jr. Methylxanthine Activation of Noradrenergic Unit Activity and Reversal by Clonidine. *Eur. J. Pharmacol.* **1982**, *85*, 105.
(20) Galloway, M. P.; Roth, R. H. Neuropharmacology of 3-Isobutylmethylxanthine: Effects on Central Noradrenergic Systems in Vivo. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 1.

0022-2623/91/1834-2671$02.50/0 © 1991 American Chemical Society

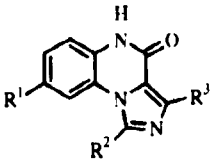
Table I. Subetituted Imidazoquinoxalines and Aza Analogues



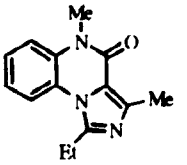
1,3,4



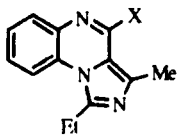
2,5



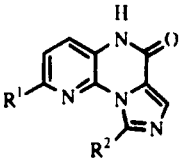
6,7,12,14-18



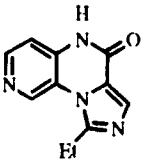
8



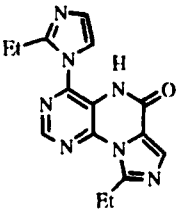
9,20



10,13



11

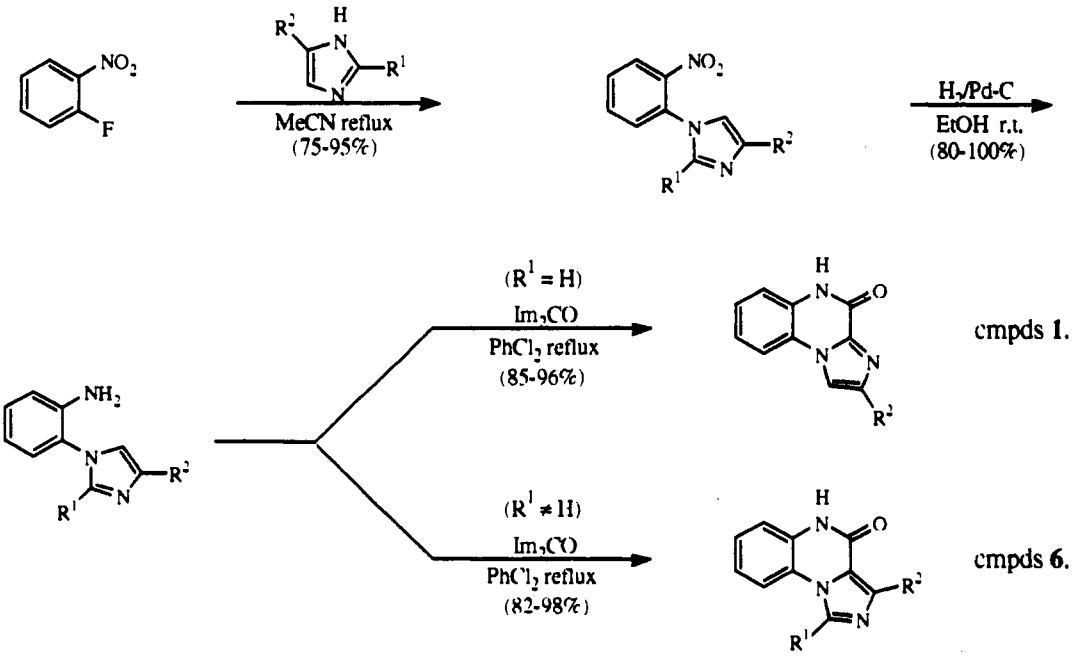


19

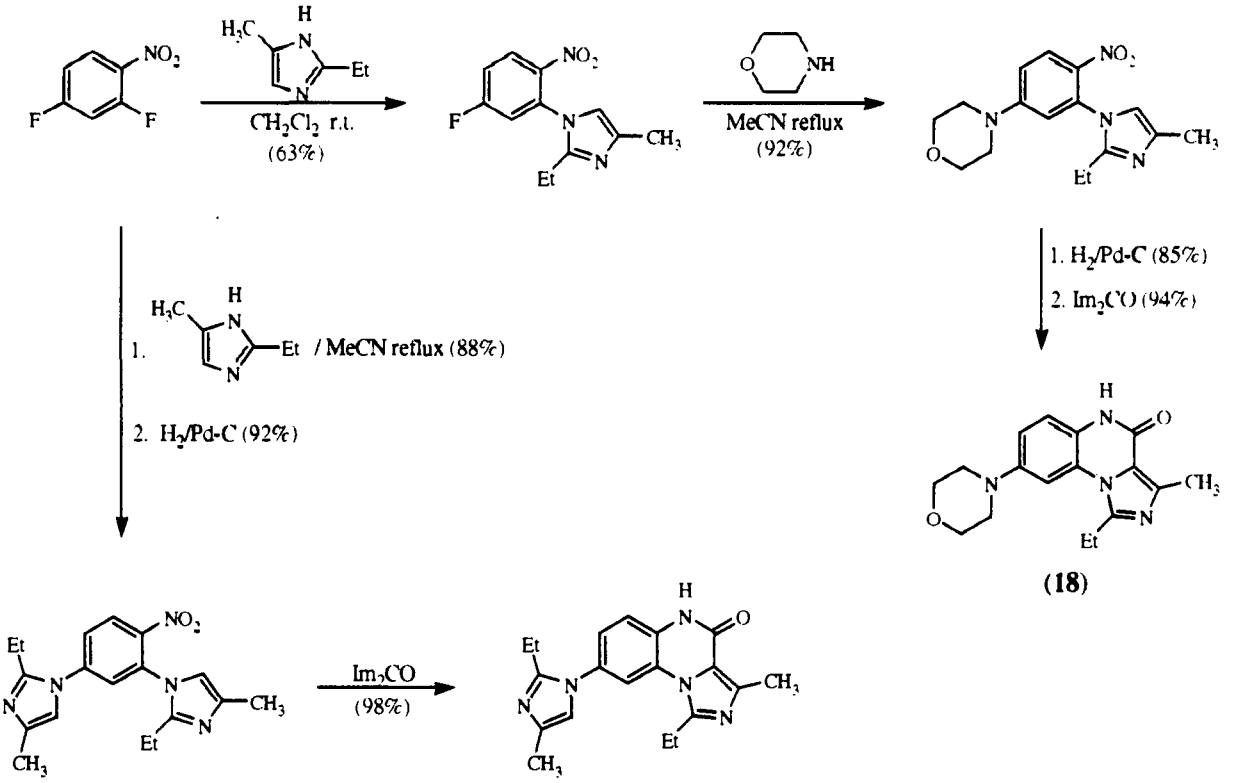
compd	R^1	R^2	R^3	X	mp, °C	cryst solvent[a]	formula	anal.[b]
1	H	–	H	–	>300	1,2-Cl_2Ph	$C_{10}H_7N_3O$	C, H, N
2	H	–	–	–	>300	H_2O	$C_9H_6N_4O$	C, H, N
3	H	–	Me	–	>300	1,2-Cl_2Ph	$C_{11}H_9N_3O$	C, H, N
4	Im[c]	–	H	–	>350	H_2O	$C_{13}H_9N_5O\cdot H_2O$	C, H, N
5	Im	–	–	–	>350	H_2O	$C_{12}H_6N_6O$	C, H, N
6	H	Me	–	–	>300	1,2-Cl_2Ph	$C_{11}H_9N_3O$	C, H, N
7	H	Et	Me	–	275–6	H_2O	$C_{13}H_{13}N_3O$	C, H, N
8	–	–	–	–	222–3	DMF	$C_{14}H_{15}N_3O$	C, H, N
9	–	–	–	NH_2	192–4	EtOH	$C_{12}H_{14}N_4$	C, H, N
10	H	Et	–	–	251–3	H_2O	$C_{11}H_{10}N_4O$	C, H, N
11	–	–	–	–	>300	H_2O	$C_{11}H_{10}N_4O$	C, H, N
12	2-MeIm	Me	–	–	>300	1,2-Cl_2Ph	$C_{15}H_{13}N_5O$	C, H, N
13	2-MeIm	Me	–	–	>300	H_2O	$C_{14}H_{12}N_6O$	C, H, N
14	Im	Me	–	–	>350	H_2O	$C_{14}H_{11}N_5O$	C, H, N
15	Im	Et	–	–	>300	1,2-Cl_2Ph	$C_{15}H_{13}N_5O$	C, H, N
16	Im	Et	Me	–	>300	H_2O	$C_{16}H_{15}N_5O\cdot 0.2H_2O$	C, H, N
17	2-Et-4-MeIm	Et	Me	–	>300	H_2O	$C_{16}H_{21}N_5O\cdot 0.2H_2O$	C, H, N
18	morpholin-4-yl	Et	Me	–	>300	H_2O	$C_{17}H_{20}N_4O_2$	C, H, N
19	–	–	–	–	233–5	EtOAc	$C_{15}H_{16}N_7O\cdot 0.4H_2O$	C, H, N
20	–	–	–	Cl	125–6	pet ether	$C_{13}H_{12}ClN_3$	C, H, N

[a] H_2O indicates the product was precipitated by neutralization of an aqueous acid solution. [b] All C, H, and N data agreed within ±0.4% of theory. [c] Im = –NCH=NCH=CH.

Scheme I



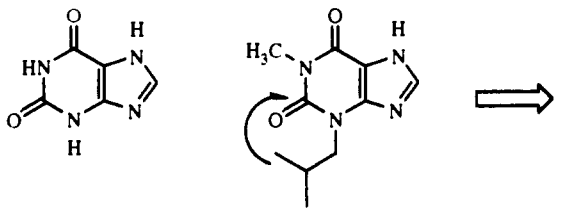
Scheme II



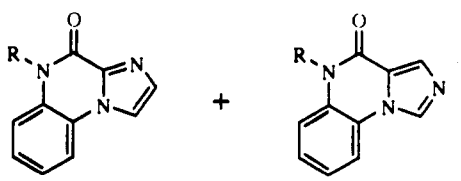
(18)

(17)

drug. Therefore, non-xanthine compounds, with similar structural and functional features, became the initial targets for our studies. The design of these targets involved "tieing back" the isobutyl group present in IBMX onto the pyrimidine ring to form a tricyclic ring system and changing the positions of certain of the ring nitrogens. Such changes should reduce the hydrophobic character compared to IBMX while perhaps retaining high affinity for the PDE's. This proposal led to a series of imidazo[1,2-*a*]quinoxalinones, imidazo[1,5-*a*]quinoxalinones, and related compounds, whose preparation and biological activities are reported herein.



Xanthine **IBMX**



1,2-*a* **1,5-*a***

Chemistry

Our prior experience with condensation reactions of 1-substituted and 1,2-disubstituted imidazoles to form six-membered rings[22,23] led us to propose a new method for the convenient preparation of imidazo[1,2-*a*]- and -[1,5-*a*]quinoxalinones and related compounds. This process is illustrated in Scheme I. Treatment of 2-(1*H*-imidazol-1-yl)benzenamine with 1.2 equiv of carbonyldiimidazole in refluxing 1,2-dichlorobenzene for 1 h provided imidazo[1,2-*a*]quinoxalin-5(6*H*)-one (1) in 92% yield. In a similar fashion, cyclization of 2-(2-methyl-1*H*-imidazol-1-yl)benzenamine with 1.2 equiv of carbonyldiimidazole afforded 1-methylimidazo[1,5-*a*]quinoxalin-5(6*H*)-one (6) in 90% yield.

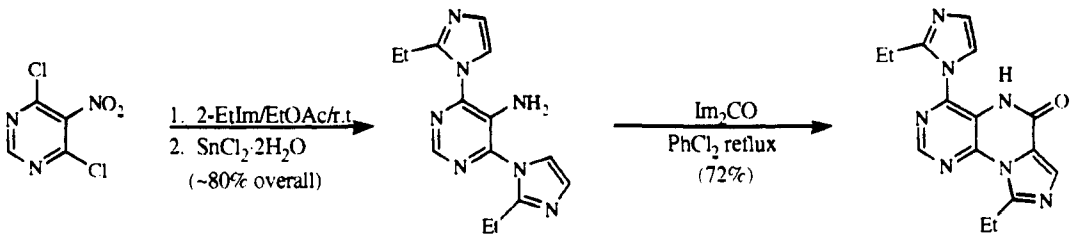
For the preparation of 8-substituted imidazoquinoxalinones, the procedure outlined in Scheme II was employed. In general, treatment of 2,4-difluoronitrobenzene with 1 equiv of an imidazole and excess potassium carbonate for 72–96 h at room temperature, results in ~60% isolated yield (after column chromatography) of the ortho-displaced product, together with approximately equal amounts of the bis-substituted compound and unreacted 2,4-difluoronitrobenzene. None of the mono para-displaced material was detected by ^{1}H NMR. Treatment of the monosubstituted compounds with 2 equiv of an imidazole or secondary amine in refluxing acetonitrile afforded the disubstituted nitrobenzenes, which were then reduced and cyclized to provide imidazoquinoxalinones 14, 15, 16, and 18. The 2,4-disubstituted(1*H*-imidazol-1-yl)nitrobenzenes, obtained by reaction of 2,4-difluoronitrobenzene with 4–5 equiv of an imidazole

(21) Ortmann, R.; Meisburger, J. G. Rolipram Forms a Potent Discrimitive Stimulus in Drug Discrimination Experiments in Rats. *Psychopharmacology (Berlin)* **1986**, *89*, 273.

(22) Davey, D. D. Synthesis of Novel 8-Arylimidazo[1,2-*a*]pyridines and 8-Arylimidazo[1,5-*a*]pyridines. *J. Org. Chem.* **1987**, *52*, 1863.

(23) Davey, D.; Erhardt, P. W.; Lumma, W. C., Jr.; Wiggins, J.; Sullivan, M.; Pang, D.; Cantor, E. Cardiotonic Agents. 1. Novel 8-Aryl-Substituted Imidazo[1,2-*a*]- and -[1,5-*a*]pyridines and Imidazo[1,5-*a*]pyridinones as Potential Positive Inotropic Agents. *J. Med. Chem.* **1987**, *30*, 1337.

Scheme III



(19)

Table II. In Vitro Biological Activity and Phosphodiesterase Inhibition

compd	FPM[a] CFC_{20}	VR[b] EC_{50} MV	VR[b] EC_{50} CA	PDE[c] IC_{50} cAMP	PDE[c] IC_{50} cGMP
1	10	NR	NR	49	44
2	2.2	10	11	44	27
3	11	0.4	5.1	42	36
4	3.1	3.1	28	6	13
5	0.5	9.3	14	5	39
6	2.1	2.8	49	16	27
7	0.2	0.8	1.7	2	0.4
8	2.3	3.8	12	18	17
9	NR	3.4	11	22	38
10	0.1	0.5	0.3	1	7
11	0.3	2.2	5.2	5	5
12	1.2	1.1	5.5	6	24
13	0.4	2.0	24	1	3
14	2.0	2.8	30	8	19
15	1.2	0.3	5.3	2	3
16	0.6	0.4	1.7	2	4
17	1.0	0.1	0.5	0.4	0.3
18	0.5	0.05	0.7	0.8	0.9
19	NR	23	59	NR	14
IBMX	1.5	1.0	5.5	20	28
nitroglycerin	NR	0.007	0.01	NR	NR
milrinone	0.5	0.7	1.6	6	NR

[a] Drug concentration (μM) causing a 20% increase in contractile force (CF) of ferret papillary muscle (FPM). Data represent the mean from at least two determinations where the ranges for all data were within ±10%. For active compounds the inotropic effect was not altered by coadministration of 1 μM propranolol. NR indicates C_{20} was not reached at 100 μM. [b] Drug concentration (μM) causing 50% vascular relaxation (VR) in canine mesenteric veins (MV) and coronary arteries (CA) precontracted with phenylephrine and prostaglandin F2 α, respectively. Data represent the mean from at least three determinations where the ranges for all data were within ±20%. NR indicates an EC_{50} was not reached at 100 μM. [c] Drug concentrations (μM) causing 50% inhibition of crude phosphodiesterases (PDE) obtained from canine cardiac (cAMP) and thoracic aorta (cGMP) homogenates. Data represent the mean from at least three determinations where the ranges for all data were within ±10%. NR indicates an IC_{50} was not reached at 100 μM.

in refluxing acetonitrile, were reduced and cyclized to give compounds **4**, **12**, and **17**.

The aza compounds (**2**, **5**, **10**, **13**, and **19**) were similarly prepared in good yield from the corresponding chloro- and dichloronitropyridines and 4,6-dichloro-5-nitropyrimidine, with the exception of compound **11**, which was synthesized from 3-fluoro-4-nitropyridine *N*-oxide. For the preparation of compound **19** (Scheme III), both the nitro and amino intermediates were light- and/or air-sensitive, and were used without purification. Early attempts at hydrogenation of 4,6-bis(2-ethyl-1*H*-imidazol-1-yl)-5-nitropyrimidine with a variety of catalysts resulted in the formation of complex reaction mixtures. Attempted reduction with $SnCl_2$ in ethanol gave 4-ethoxy-6-(2-ethyl-1*H*-imidazol-1-yl)pyrimidin-5-amine as the major isolated product. The amino compound was eventually prepared in ~80% overall yield by the reaction of 4,6-dichloro-5-nitropyrimidine with 5 equiv of 2-ethylimidazole in ethyl acetate at room temperature for 24 h, followed by the addition of 6 equiv of $SnCl_2$. Standard cyclization of the crude amine afforded **19** in 72% yield.

Compounds **8** and **9** were prepared from compound **7** (Scheme IV). Treatment of the sodium salt of **7** with iodomethane in DMF at room temperature gave the N-methylated compound **8** in 81% yield. Chlorination of **7** with phosphorus oxychloride *N*,*N*-diethylaniline provided compound **20** in 81% yield. Reaction of **20** with ammonia in isopropanol at 100 °C afforded **9** in 87% yield.

Results and Discussion

All of the compounds were tested in vitro in an inotropic screen and in a vascular relaxation screen. Potent and moderately active compounds were also tested for the ability to inhibit crude preparations of cAMP and cGMP PDE's. IBMX, nitroglycerin, and milrinone were included for comparison. The results are summarized in Table II.

For the 1,2-*a* series, addition of a methyl group at the R^2 position (**1** → **3**) dramatically improved vasorelaxant activity without increasing PDE inhibitory potency. Improved cAMP PDE inhibitory potency could be achieved by placement of an imidazole substituent at the 8-position (**1** → **4**; **2** → **5**). This effect was less pronounced in the 1,5-*a* series (**7** → **16**; **17**, **18**).

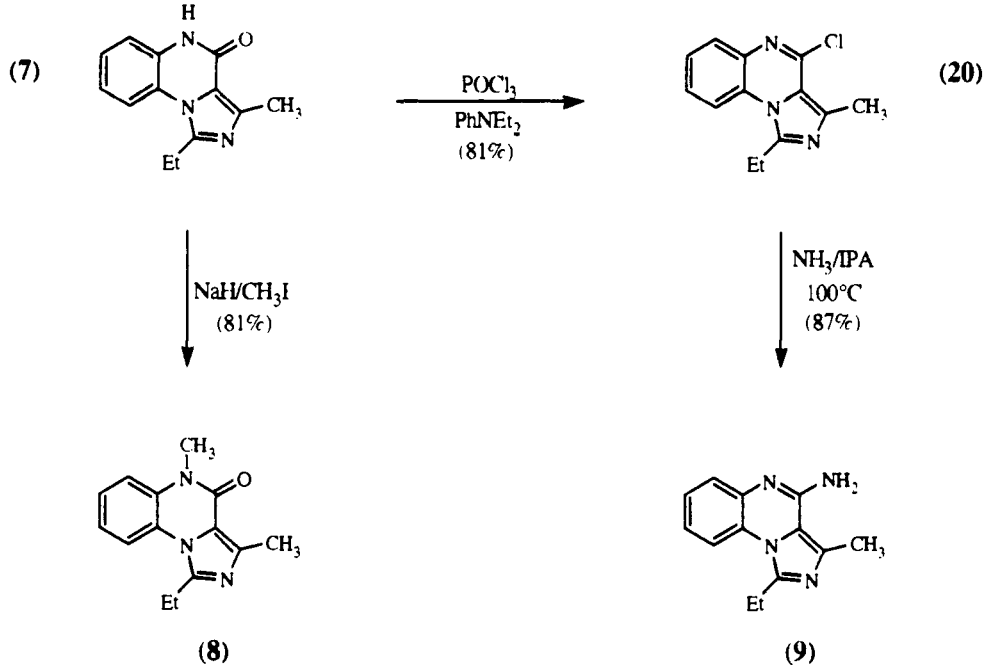
For the 1,5-*a* series, replacement of the R^2 methyl substituent by an ethyl group greatly enhanced activity in all screens (**14** → **15**). The addition of a methyl substituent at R^3 moderately increased in vitro potency without changes in PDE inhibitory activity (**15** → **16**). Potency in all screens decreased with methylation of the amide nitrogen (**7** → **8**) and by replacement of the carbonyl oxygen with an amino group (**7** → **9**). The amino compound was also devoid of positive inotropic activity.

In some instances minor variations within these series produced unexpected results. For example, when the phenyl ring in compound **4** was changed to a pyridine ring, the resulting compound **5** was 3 times less potent as a cGMP PDE inhibitor and mesenteric vein relaxant, but 6 times more potent as a positive inotrope with no corresponding increase in cAMP PDE inhibitory activity. A similar modification of compound **12** to give **13** resulted in increased positive inotropic and cAMP and cGMP inhibitory potency but decreased vasorelaxant activity.

Overall, the 1,5-*a* series of compounds possessed more potent cardiovascular and PDE inhibitory activities in comparison to the 1,2-*a* series. While this may likely be due to the positions of substituents on the imidazole ring of the tricyclic system, the limited availability of 4-substituted imidazoles, together with the difficulty in preparing 1-substituted imidazo[1,2-*a*]quinoxalinones by our method, caused us to focus our efforts on the 1,5-*a* series.

Many of the compounds prepared were more potent than IBMX as cAMP and cGMP PDE inhibitors, and to a lesser extent, more potent vasorelaxants and positive inotropes in vitro. However, as shown in Table II, PDE

Scheme IV



inhibitory potency did not correlate with the in vitro vasorelaxant and inotropic activities. Some possible explanations for this discrepancy may be differences in solubility and differences in intracellular and extracellular distribution. Another possibility is that these compounds possess additional undefined mechanisms. The latter possibility is currently under investigation.

During the early course of this work, several compounds representing various structural types were administered iv to anesthetized dogs. These results are summarized in Table III. As previously indicated for the in vitro results, the observed hemodynamic effects for the test compounds did not correlate well with PDE inhibitory activity. Compound **2**, a weak PDE inhibitor, was equally active as a vasodilator and only moderately less potent as a positive inotrope in this model when compared to compound **7**, which was a potent PDE inhibitor. Compound **10** was the most balanced inodilator (LV dP/dt vs MAP) in this model, with approximately 3-fold greater vasodilating potency than nitroglycerin. IBMX was equiactive to **10** as a positive inotrope, but was less active as a vasodilator and PDE inhibitor.

In order to assess potential CNS activity, several of the compounds (including **2**, **7**, and **10**) were examined in a rodent neuropharmacologic profile model.[24] At doses up to 30 mg ip and 300 mg po, none of the compounds tested produced any significant neuropharmacological effects, with the exception of compound **7**, which produced mild CNS depression at doses of 3, 10, and 30 mg/kg ip. Vasodilation (characterized by reddening of the skin) was noted for several of the compounds. The series also appeared to be devoid of anticonvulsant and antipyretic or hypothermic activity.

In conclusion, the novel imidazoquinoxalinones and related analogues presented herein represent a new class of inodilators that display varying effects on the vasculature. In addition, a new and efficient route for the preparation of these types of compounds has been presented.

(24) Details for the rodent neuropharmacological profile model can be obtained by contacting Pharmakon Laboratories, Waverly, PA.

Table III. Biological Activity of Selected Compounds in Anesthetized Dogs[a]

compd	dose (mg/kg)	LV dP/dt(%Δ)	HR (%Δ)	MAP (%Δ)
2	0.3	NC	NC	NC
2	1.0	42	NC	NC
2	3.0	81	24	−40
7	0.3	39	22	NC
7	1.0	85	42	NC
7	3.0	135	65	−40
10	0.1	31	NC	−17
10	0.3	40	30	−25
10	1.0	76	50	−40
IBMX	0.1	25	17	NC
IBMX	0.3	41	32	NC
IBMX	1.0	69	45	−18
nitroglycerin	0.3	−9	NC	−20
nitroglycerin	1.0	−14	NC	−30
nitroglycerin	3.0	−17	NC	−39
milrinone	0.03	31	12	NC
milrinone	0.1	58	20	−15
milrinone	0.3	82	34	−33

[a] Percent change in force of contraction of the left ventricle (LV dP/dt), heart rate (HR) and mean arterial blood pressure (MAP) after intraveneous administration of each drug at the indicated dose. Data represent the mean from at least four determinations where the ranges for data within each column were within the following limits: LV dP/dt ± 10%; HR ± 5%; and MAP ± 15%. NC indicates <10% change from control.

Although we were unable to establish linear correlations between the in vitro activities and inhibition of the PDE's for the compounds overall, compound **19** deserves additional comment. While this compound was weakly active as a vasorelaxant and cGMP PDE inhibitor, it was completely devoid of positive inotropic activity and was the only compound prepared which did not reach an IC_{50} for inhibition of cAMP PDE. We concluded that substitution at the 4-position of the imidazopteridine ring system could be used to develop a series of selective inhibitors of cGMP PDE. The latter compounds would more closely match the profile of the nitrates and would not be complicated by positive inotropic activity while still potentially being devoid of tolerance. This proposal led to a series of novel imidazopteridinones, the results of which will be the subject of a future publication.

Experimental Section

Chemistry. Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were obtained on either a Sargent/Welch 3-300 spectrophotometer or a Beckman Aculab 2 spectrophotometer. ^1H NMR spectra were recorded on a Varian XL-300 spectrometer. Elemental analyses were performed by the Berlex Analytical Department or Galbraith Laboratories, Inc. Column chromatography was carried out on Merck silica gel 60, 230–400 mesh.

General Procedure for the Preparation of 2-(1*H*-Imidazol-1-yl)-4-fluoronitrobenzenes. 2-Ethyl-1-(5-fluoro-2-nitrophenyl)-4-methyl-1*H*-imidazole. A mixture of 50 g (0.31 mol) of 2,4-difluoronitrobenzene, 34 g (0.31 mol) of 2-ethyl-4-methylimidazole, and 100 g of K_2CO_3 in 600 mL of CH_2Cl_2 was stirred at room temperature for 4 days. The reaction mixture was washed with 1 L of water, dried over $MgSO_4$, and chromatographed on 700 g of silica gel with 10% EtOAc/CH_2Cl_2 as eluent. Concentration of the pure fractions, followed by crystallization from petroleum ether, gave 48.5 g (63%) of a yellow solid: mp 55–57 °C; NMR ($CDCl_3$) δ 1.19 (t, 3), 2.22 (s, 3), 2.41 (q, 2), 6.60 (s, 1), 7.12 (d, 1), 7.28 (m, 1), 8.07 (m, 1).

General Procedure for the Preparation of Imidazo[1,2-*a*]quinoxalin-4(5*H*)-ones and Imidazo[1,5-*a*]quinoxalin-4(5*H*)-ones. 1-Methyl-8-(2-methyl-1*H*-imidazol-1-yl)-imidazo[1,5-*a*]quinoxalin-4(5*H*)-one (12). A mixture of 50 g (0.31 mol) of 2,4-difluoronitrobenzene, 60 g (0.73 mol) of 2-methylimidazole, and 100 g of K_2CO_3 in 500 mL of MeCN was heated at reflux for 24 h. The solvent was removed under vacuum, and the residue was slurried in 1 L of CH_2Cl_2 and washed twice with 2 L of water. The CH_2Cl_2 portion was dried over $MgSO_4$ and charcoal treated, and the solvent was removed under vacuum. Crystallization of the residue with EtOAc afforded 70 g (80%) of 1,1'-(1-nitrobenzene-2,4-diyl)bis(2-methyl-1*H*-imidazole) as a yellow solid: mp 153–4 °C; IR (CH_2Cl_2) 1620, 1605, 1540, 1505, 1415 cm^{-1}; NMR ($CDCl_3$) δ 2.31 (s, 3), 2.52 (s, 3), 6.98 (d, 1), 7.12 (m, 3), 7.45 (d, 1), 7.66 (q, 1), 8.26 (d, 1).

The above compound (58 g, 0.20 mol) was dissolved in 1 L of ethanol with 5 g of 10% palladium on carbon and hydrogenated at 50 psi for 2 h. The catalyst was removed by filtration, and the residue concentrated under vacuum and crystallized from EtOAc to provide 43 g (83%) of 2,4-bis(2-methyl-1*H*-imidazol-1-yl)-benzenamine as a white solid: mp 203–5 °C; IR (Nujol) 3420, 3330, 1630, 1510 cm^{-1}; NMR (DMSO) δ 2.15 (s, 3), 2.25 (s, 3), 5.18 (s, 2), 6.84 (d, 1), 6.93 (m, 2), 7.11 (s, 2), 7.18 (m, 2).

The above amino compound (10 g, 39.5 mmol) and carbonyldiimidazole (7 g, 43.2 mmol) were dissolved in 300 mL of 1,2-dichlorobenzene and heated at reflux under N_2 for 1.5 h. After cooling to room temperature, the solids were filtered and washed with acetone to give 9.8 g (89%) of 12 as an off-white solid: IR (Nujol) 1675, 1510, 1460 cm^{-1}; NMR (DMSO) δ 2.35 (s, 3), 2.95 (s, 3), 6.94 (s, 1), 7.40 (m, 3), 7.77 (s, 1), 7.97 (s, 1), 11.5 (s, 1).

The following compounds were prepared in an analagous manner. **Imidazo[1,2-*a*]quinoxalin-4(5*H*)-one** (1): IR (Nujol) 1675, 1630, 1440, 1400 cm^{-1}; NMR (DMSO) δ 7.2 (m, 3), 7.61 (s, 1), 8.11 (d, 1), 8.55 (s, 1), 11.85 (s, 1).

Imidazo[1,2-*a*]pyrido[2,3-*e*]pyrazin-6(5*H*)-one (2) (prepared from 2-chloro-3-nitropyridine): IR (Nujol) 1725, 1685, 1590 cm^{-1}; NMR (CF_3CO_2D) δ 7.87 (m, 1), 8.23 (m, 2), 8.78 (d, 1), 8.91 (s, 1).

2-Methylimidazo[1,2-*a*]quinoxalin-4(5*H*)-one (3): IR (Nujol) 1680, 1620, 1515 cm^{-1}; NMR (DMSO) δ 2.55 (s, 3), 7.10 (m, 3), 8.08 (d, 1), 8.67 (s, 1), 11.18 (s, 1).

8-(1*H*-Imidazol-1-yl)imidazo[1,2-*a*]quinoxalin-4(5*H*)-one (4): IR (Nujol) 1700, 1540, 1515 cm^{-1}; NMR (DMSO) δ 7.17 (s, 1), 7.45 (d, 1), 7.65 (s, 1), 7.71 (d, 1), 7.84 (s, 1), 8.35 (s, 1), 8.45 (s, 1) 8.64 (s, 1).

2-(1*H*-Imidazol-1-yl)imidazo[1,2-*a*]pyrido[2,3-*e*]pyrazin-6(5*H*)-one (5) (prepared from 2,6-dichloro-3-nitropyridine): IR (Nujol) 1700, 1600, 1495 cm^{-1}; NMR (D_2O/DCl) δ 7.80 (s, 1), 8.21–8.45 (m, 4), 8.90 (s, 1), 9.80 (s, 1).

1-Methylimidazo[1,5-*a*]quinoxalin-4(5*H*)-one (6): IR (Nujol) 1680, 1610, 1560, 1490 cm^{-1}; NMR (DMSO) δ 2.92 (s, 3), 7.20 (m, 3), 7.73 (s, 1), 8.06 (d, 1), 11.38 (s. 1).

1-Ethyl-3-methylimidazo[1,5-*a*]quinoxalin-4(5*H*)-one (7): IR (Nujol) 1680, 1610, 1580, 1510 cm^{-1}; NMR (DMSO) δ 1.32 (t, 3), 2.54 (s, 3), (q, 2), 7.17 (m, 3), 7.92 (d, 1), 11.11 (s, 1).

9-Ethylimidazo[1,5-*a*]pyrido[2,3-*e*]pyrazin-6(5*H*)-one (10) (prepared from 2-chloro-3-nitropyridine): IR (Nujol) 1675, 1600, 1550 cm^{-1}; NMR (DMSO) δ 1.33 (t, 3), 3.42 (q, 2), 7.41 (m, 1), 7.65 (m, 2), 8.24 (d, 1).

1-Ethylimidazo[1,5-*a*]pyrido[4,3-*e*]pyrazin-4(5*H*)-one (11) (prepared from 3-fluoro-4-nitropyridine *N*-oxide): IR (Nujol) 1670, 1655, 1605, 1500 cm^{-1}; NMR (DMSO) δ 1.39 (t, 3), 3.28 (q, 2), 7.26 (d, 1), 7.85 (s, 1), 8.24 (d, 1), 9.16 (s, 1).

9-Methyl-2-(2-methyl-1*H*-imidazol-1-yl)imidazo[1,5-*a*]-pyrido[2,3-*e*]pyrazin-6(5*H*)-one (13) (prepared from 2,6-dichloro-3-nitropyridine): IR (Nujol) 1680, 1610, cm^{-1}; NMR (DMSO) δ 2.51 (s, 3), 2.96 (s, 3), 6.96 (s, 1), 7.56 (s, 1), 7.61 (d, 1), 7.79 (s, 1), 7.82 (d, 1).

1-Methyl-8-(1*H*-imidazol-1-yl)imidazo[1,5-*a*]quinoxalin-4(5*H*)-one (14): IR (Nujol 1675, 1515, 1400 cm^{-1}; NMR (DMSO) δ 3.04 (s, 3), 7.15 (s, 1), 7.41 (m, 1), 7.63 (d, 1), 7.76 (m, 2), 8.08 (d, 1), 8.30 (s, 1).

1-Ethyl-8-(1*H*-imidazol-1-yl)imidazo[1,5-*a*]quinoxalin-4-(5*H*)-one (15): IR (Nujol) 1680, 1520, 1415 cm^{-1}; NMR (DMSO) δ 1.41 (t, 3), 3.41 (q, 2), 7.19 (s, 1), 7.44 (m, 1), 7.62 (d, 1), 7.75 (m, 2), 8.04 (d, 1), 8.28 (s, 1).

1-Ethyl-8-(1*H*-imidazol-1-yl)-3-methylimidazo[1,5-*a*]-quinoxalin-4(5*H*)-one (16): IR (Nujol) 1670, 1520, 1445 cm^{-1}; NMR (CF_3CO_2D) δ 1.68 (t, 3), 2.94 (s, 3), 3.69 (q, 2), 7.76 (m, 4), 8.30 (s, 1), 9.19 (s, 1).

1-Ethyl-8-(2-ethyl-4-methyl-1*H*-imidazol-1-yl)-3-methyl-imidazo[1,5-*a*]quinoxalin-4(5*H*)-one (17): IR (Nujol) 1670, 1590, 1525 cm^{-1}; NMR (DMSO) δ 1.12 (t, 3), 1.35 (t, 3), 2.15 (s, 3), 2.51 (s, 3), 2.55 (q, 2), 3.20 (q, 2), 7.09 (s, 1), 7.38 (s, 2), 7.83 (s, 1).

1-Ethyl-3-methyl-8-(morpholin-4-yl)imidazo[1,5-*a*]-quinoxalin-4(5*H*)-one (18): IR (Nujol) 1670, 1520, 1445 cm^{-1}; NMR (DMSO) δ 1.36 (t, 3), 2.51 (s, 3), 3.11 (m, 4), 3.23 (q, 2), 3.76 (m, 4), 7.00 (d, 1), 7.15 (d, 1), 7.35 (s, 1).

9-Ethyl-4-(2-ethyl-1*H*-imidazol-1-yl)imidazo[5,1-*b*]pteridin-6(5*H*)-one (19). A mixture of 25 g (0.13 mol) for 4,6-dichloro-5-nitropyridimine and 75 g (0.78 mol) of 2-ethylimidazole in 600 mL of EtOAc was stirred at room temperature for 24 h. Tin(II) chloride dihydrate (176 g, 0.78 mol) was then added, and the mixture stirred rapidly at room temperature for 2 h. The reaction mixture was made basic with aqueous K_2CO_3, the EtOAc portion was dried over $MgSO_4$ and charcoal treated, and the solvent was removed under vacuum to afford 38 g of the pyrimidinamine as a brown oil.

The above amine (5.6 g, 19.8 mmol) and carbonyldiimidazole (4.8 g, 29.6 mmol) were dissolved in 200 mL of 1,2-dichlorobenzene and heated at reflux under N_2 for 2 h. The product was isolated by chromatograph (500 g silica gel, 10% MeOH/CH_2Cl_2) and crystallized from EtOAc to give 4.5 g (72%) of a shite solid: IR (Nujol) 1690, 1585 cm^{-1}; NMR (DMSO) δ 1.13 (t, 3), 1.36 (t, 3), 2.58 (q, 2), 3.44 (q, 2), 7.01 (d, 1), 7.31 (d, 1), 7.87 (s, 1), 8.84 (s, 1).

3,5-Dimethyl-1-ethylimidzo[1,5-*a*]quinoxalin-4(5*H*)-one (8). To a slurry of 5 g (22 mmol) of 7 in 200 mL of DMF was added 1 g (25 mmol) of NaH (60% in oil), and the mixture stirred for 2 h at room temperature under N_2. Iodomethane (1.5 mL, 24 mmol) was added, and the mixture was stirred for an additional 1 h. The resulting precipitate was filtered and washed with 10% KOH, water, and then acetone to provide 4.3 g (81%) of a white solid: IR (Nujol) 1660, 1580, 1510 cm^{-1}; NMR (DMSO) δ 1.36 (t, 3), 2.55 (s, 3), 3.22 (q, 2), 3.50 (s, 3), 7.44 (t, 1), 7.47 (m, 2), 8.00 (d, 1).

4-Chloro-1-ethyl-3-methylimidazo[1,5-*a*]quinoxaline (20). A mixture of 40 g (0.18 mol) of 7, 45 mL of *N,N*-diethylaniline, and 600 mL of phosphorus oxychloride was heated at reflux under N_2 for 5 h. The excess $POCl_3$ was removed under vacuum, and the residue dissolved in 500 mL of CH_2Cl_2, washed with 1 L of water, dried over $MgSO_4$, and chromatographed on 500 g of silica gel using CH_2Cl_2. Concentration of the pure fractions, followed by crystallization from petroleum ether, provided 35 g (81%) of a white solid: IR (CH_2Cl_2) 1410, 1245 cm^{-1}; NMR ($CDCl_3$) δ 1.52 (t, 3), 2.80 (s, 3), 3.32 (q, 2), 7.45 (m, 2), 7.81 (d, 1), 8.00 (d, 1).

1-Ethyl-3-methylimidazo[1,5-*a*]quinoxalin-4-amine (9). A solution of 5.5 g (22.4 mmol) of 20 in 60 mL of 2-propanol saturated with NH_3 was heated at 110 °C in a pressure tube for 48

h. The cooled reaction mixture was poured into 500 mL water, and the resulting solids were filtered, washed with water, and recrystallized from EtOH to afford 4.4 g (87%) of a white solid: IR (CH_2Cl_2) 3520, 3410, 1625, 1605, 1590, 1485 cm^{-1}; NMR ($CDCl_3$) δ 1.36 (t, 3), 2.60 (s, 3), 3.23 (q, 2), 6.54 (s, 2), 7.17 (t, 1), 7.27 (t, 1), 7.41 (d, 1), 7.96 (d, 1).

Biology. Papillary Muscle Studies. The in vitro determination of positive inotropic activity in ferret papillary muscle strips was conducted according to published procedures.[25]

Vascular Relaxation Studies. Canine heart and mesenteric tissues were removed and placed in oxygenated (95% O_2, 5% CO_2) physiological salt solution (PSS) maintained at 37 °C. The circumflex coronary artery and mesenteric vein was separated from the adventia, cut into approximately 2-mm lengths, and then placed onto standard holders for use in an aerated muscle bath maintained at 37 °C. Coronary arteries were stretched to a tension of 2 g and mesenteric veins were stretched to a tension of 1 g, following which both vessels were allowed to relax. Final tensions were then equilibrated at 2.5 and 1 g, respectively. To determine the optimum preload for each tissue, repetitive challenges with 20 mM KCl were followed by washing and adjustment of the tension for each tissue until there was no longer an improvement in response to KCl. Tissues were then relaxed for 30 min. Functional criteria were established by testing coronary arteries with KCl and mesenteric veins with phenylephrine. When contractions reached a plateau, arteries were challenged with acetylcholine and veins with 20 μL (100 units/mL) thrombin. Tissues that did not relax at least 65% were not used for the screen. The tissues were then washed and allowed to relax for another 30 min.

One of each tissue was used for each test compound. Arteries were precontracted with prostaglandin F2 α and veins with phenylephrine. When tissues were at a steady state of contraction, compounds were added to the bath cumulatively, beginning at 0.01 μM and increasing to as high as 100 μM for weakly active compounds. After each drug challenge, relaxation was allowed to reach its new plateau before adding the next drug concentration. After the last drug challenge, the tissues were rinsed with warm PSS and the process repeated every 10 min until the tissues were completely relaxed. Arteries were then again constricted with prostaglandin F2 α and veins with phenylephrine followed by challenges with acetylcholine and thrombin, respectively. Tissues that did not respond similarly (within ±10% relaxations) to their predrug treatments were not used in the data analysis.

Raw data was digitized and collected with both computer and strip chart recorder. Total force was calculated by computer for basal, agonist standards and each dose of relaxant and test compound. Actual force (total force − basal force) was also calculated for all compounds. Delta force was calculated for each dose of test compound. A negative delta force represents relaxation. Delta force as a percent of control was calculated by dividing delta force by the actual force of the agonist and multiplying by 100. Delta force as a percentage of maximum relaxation was calculated by dividing the delta force for each dose of test compound by the delta force for the dose of compound which produced the maximum relaxation. EC_{50}'s for delta force as a percent of control and delta force as a percent of maximum relaxation were calculated by linear interpolation between points on the dose response curve which bracketed the point of 50% relaxation.

Phosphodiesterase Studies. The biochemical determinations of inhibition of crude cAMP and crude cGMP phosphodiesterases obtained from canine cardiac and thoracic aorta tissues, respectively, were conducted according to published procedures.[23,26]

Hemodynamic Studies. The in vivo measurements of heart rate and contractility (LV dP/dt) and of effects on mean arterial blood pressure were conducted according to published procedures.[23]

Acknowledgment. We thank Dr. Walton Caldwell and members of the Medicinal Chemistry Analytical Section for their help in the physical characterizations of the compounds in this study. We also thank Elena Ho, Thomas Lasser, Anne Smart, Michael Walega, and Patrice Wiggins of our Pharmacology Department for their technical assistance in the pharmacological evaluations of these compounds. Special appreciation is given to Dr. William C. Lumma, Jr., for his support of this work.

Registry No. 1, 133307-45-6; 2, 134485-88-4; 3, 133307-46-7; 4, 133307-26-3; 5, 133307-49-0; 6, 134485-89-5; 7, 134485-90-8; 8, 134485-91-9; 9, 134485-92-0; 10, 134485-93-1; 11, 133307-32-1; 12, 133307-25-2; 13, 133307-47-8; 14, 133307-40-1; 15, 133307-41-2; 16, 133307-39-8; 17, 133307-36-5; 18, 133307-38-7; 19, 134485-94-2; 20, 134485-95-3; 2,4-difluoronitrobenzene, 446-35-5; 2-ethyl-4-methylimidazole, 931-36-2; 2-ethyl-1-(5-fluoro-2-nitrophenyl)-4-methyl-1*H*-imidazole, 133307-22-9; 2-methylimidazole, 693-98-1; 1,1′-(1-nitrobenzene-2,4-diyl)bis(2-methyl-1*H*-imidazole), 134485-96-4; 2,4-bis(2-methyl-1*H*-imidazol-1-yl)benzenamine, 134485-97-5; carbonyldiimidazole, 530-62-1; 2,6-dichloro-3-nitropyridine, 16013-85-7; 2-chloro-3-nitropyridine, 5470-18-8; 3-fluoro-4-nitropyridine *N*-oxide, 769-54-0; 4,6-dichloro-5-nitropyrimidine, 4316-93-2; 2-ethylimidazole, 1072-62-4; 4,6-bis(2-ethyl-1*H*-imidazol-1-yl)-5-pyrimidinamine, 134485-98-6; phosphodiesterase, 9025-82-5.

(25) Hagedorn, A. A.; Erhardt, P. W.; Lumma, W. C., Jr.; Wohl, R. A.; Cantor, E.; Chou, Y.-L.; Ingebretsen, W. R.; Lampe, J. W.; Pang, D. C.; Pease, C. A.; Wiggins, J. Cardiotonic Agents. 2. (Imidazoyl)aroylimidazolones, Highly Potent and Selective Positive Inotropic Agents. *J. Med. Chem.* **1987**, *30*, 1342.

(26) Pang, D.; Cantor, E.; Hagedorn, A.; Erhardt, P.; Wiggins, J. Tissue Specificity of cAMP-Phosphodiesterase Inhibitors: Rolipram, Amrinone, Milrinone, Enoximone, Piroximone, and Imazodan. *Drug Dev. Res.* **1988**, *14*, 141.