Adult female beagles, 6–11 kg body weight, were surgically prepared with innervated (Pavlov) gastric pouches that were drained by Thomas-type gastric cannulae.[20] Following surgical recovery, each dog was trained to stand quietly in a dog restraining sling and was conscious for all studies. Experiments began at least 3 weeks after surgery, and no dog was used more than once per week.

Dogs were food-deprived with access to water for 24 h prior to experiments. Following a 30-min basal collection period, the prostaglandins or vehicle were administered into the gastric pouch in a 2–3-mL volume. Thirty minutes later the gastric pouch was emptied and gastric secretion was stimulated by feeding 10–12 oz of dog food (Fromm All Beef, Federal Foods Inc., Thiensville, WI). Gastric juice samples were collected from the pouch by gravity drainage over a 4-h period at 30-min intervals. The volume of secretion was measured (mL/30 min), and the acidity (mequiv/L) was determined by electrometric titration to pH 7.0 with 0.1 N NaOH. These two parameters were multiplied to obtain the total acid output (mequiv/30 min) for each collection period.

Percent reduction of total acid output from control was calculated over each 4-h experiment for doses of prostaglandin. ED_{50} values and 95% confidence limits were determined from inhibition of secretion curves.[21]

Ethanol-Induced Gastric Lesion Studies.[17] Male Charles River rats weighing 180–210 g were food-deprived for 24 h before the experiment but had access to drinking water. Doses of prostaglandin from 0.5 to 500 μg/kg were administered intragastrically to groups of six rats in a volume of 10 mL/kg. A separate control group of animals received only phosphate buffer vehicle. Thirty minutes following administration of the prostaglandins, each rat received an intragastric 1-mL dose of absolute ethanol. One hour after ethanol administration, the animals were sacrificed by CO_2 asphyxiation, and the stomachs were removed, opened, and gently rinsed with tap water. The gastric mucosa was visually inspected for lesions and scored on a severity scale of 1 to 7 with 7 indicating the complete absence of lesions. Statistically significant reduction in the formation of ethanol lesions compared to control animals was determined by a χ^2 test using the method of maximum likelihood.[22]

Acknowledgment. We thank C. Anglin and J. Casler for technical assistance in the antisecretory studies, R. Bianchi and his group for performing the mucosal protection studies, the group of E. Hajdu for spectral data, L. Swenton for assistance in interpretation of spectral data, the group of E. Zielinski for microanalyses, L. Householder for artwork, and P. Polin for typing the manuscript.

Registry No. **1a**, 81026-63-3; **1b** (*R* isomer), 109976-44-5; **1b** (*S* isomer), 109976-45-6; **3**, 2356-16-3; **5**, 109976-39-8; **6**, 109976-40-1; **7a**, 109976-41-2; **7a** (methylsulfonyl deriv.), 109996-15-8; **7b**, 109976-42-3; **8**, 109976-43-4; **9**, 33884-43-4; **10**, 109976-36-5; **10** (hemiacetal), 38299-92-2; **11**, 109976-37-6; **13**, 109976-38-7; **2**-furaldehyde, 98-01-1; **2**-[1-hydroxy-3-(1,3-dioxan-2-yl)propyl]furan, 109976-35-4; (*E*)-(tri-*n*-butylstannyl)-4-methyl-4α-[(trimethylsilyl)oxy]-1-octene, 69442-81-5; methyl acetate, 79-20-9.

(20) Burrows, L. *J. Surg. Res.* **1964**, *4*, 147.
(21) Kendall, M.; Stuart, A. *The Advanced Theory of Statistics*, 4th ed.; MacMillan: New York, 1977; Vol. I, p 246.
(22) Fisher, R. A. *Statistical Methods for Research Workers*, 14th ed.; Hafner: New York, 1970; Chapter 9.

Cardiotonic Agents. 7. Inhibition of Separated Forms of Cyclic Nucleotide Phosphodiesterase from Guinea Pig Cardiac Muscle by 4,5-Dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones and Related Compounds. Structure–Activity Relationships and Correlation with in Vivo Positive Inotropic Activity

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The structure–activity relationships of a series of 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones and related compounds were investigated for the in vivo inhibition of different forms of cyclic nucleotide phosphodiesterase (PDE) isolated from guinea pig ventricular muscle. With few exceptions, these 4,5-dihydropyridazinones were potent inhibitors of cardiac type III phosphodiesterase, which is a low K_m, cyclic AMP specific form of the enzyme. The inhibitory effects on cardiac type I and type II phosphodiesterase, both of which hydrolyze cyclic AMP as well as cyclic GMP, were minimal. The most selective PDE III inhibitor was CI-930 (**10**), the 5-methyl analogue of imazodan (CI-914, **1**), with an IC_{50} of 0.6 μM. The most potent inhibitor of PDE III was the 4,5,6,7-tetrahydrobenzimidazole analogue of **10** (**31**), with an IC_{50} of 0.15 μM. This paper describes the structural features that impart both selectivity for inhibiting type III phosphodiesterase and potency of inhibition. In addition, correlations between in vitro PDE inhibitory potency, in vivo positive inotropic potency, and physicochemical properties are discussed.

Within the last decade a number of novel non-glycoside, non-catechol cardiotonic agents have been identified as potential replacements for digitalis in the treatment of congestive heart failure. These agents include amrinone, milrinone, carbazeran, sulmazole, enoximone, piroximone, imazodan (CI-914), and CI-930.[1] Until recently the mechanism responsible for the increase in cardiac contractility produced by these agents was not known. Early studies suggested that the inotropic response was unrelated to direct effects on cardiac β receptors, sarcoplasmic reticulum, or mitochondria or to the modulation of adenylate cyclase or Na^+,K^+-ATPase.[2,3]

Recently, however, it has been demonstrated that all of these novel cardiotonic agents exert an inhibitory effect on cardiac phosphodiesterase (PDE) activity.[2] Subsequent studies in which the inhibitor effects on the different molecular forms of phosphodiesterase present in cardiac

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(1) Wetzel, B.; Hanel, N. *Annu. Rep. Med. Chem.* **1984**, *19*, 71.
(2) Weishaar, R. E.; Quade, M.; Boyd, D.; Schenden, J.; Kaplan, H. R. *Drug Dev. Res.* **1983**, *3*, 517.
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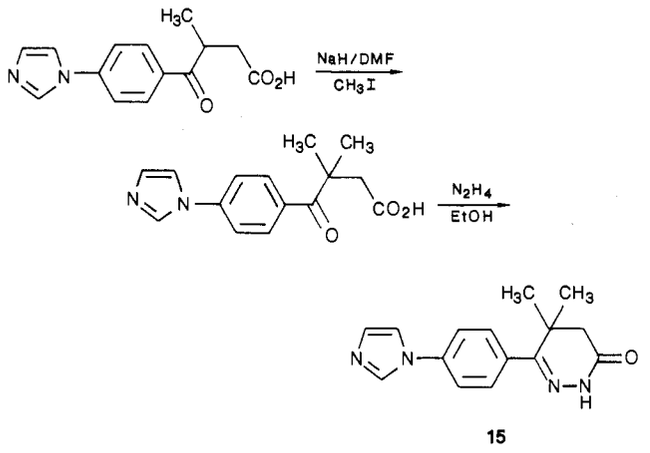
0022-2623/87/1830-1955$01.50/0

Scheme I



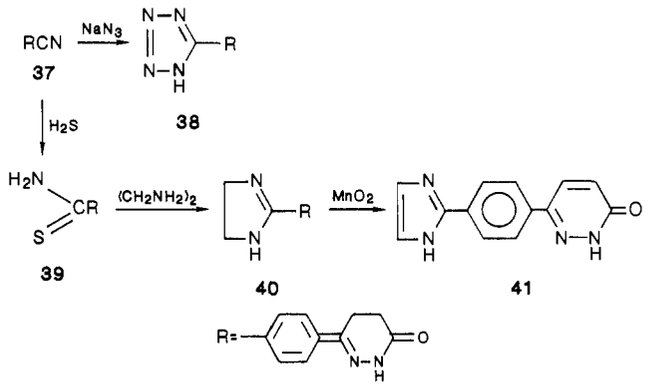
muscle were evaluated have shown that several of these agents, most notably amrinone, enoximone, piroximone, imazodan, and CI-930, are selective inhibitors of type III phosphodiesterase, a low K_m, cyclic AMP specific form of the enzyme.[4,5] Supportive evidence that inhibition of type III phosphodiesterase represents the cardiotonic mechanism of action for these agents is provided by the observation that these agents (i) produce selective concentration-dependent increases in tissue levels of cyclic AMP but not cyclic GMP, (ii) potentiate the positive inotropic response to isoproterenol, and (iii) restore contractility to isolated potassium-depolarized cardiac muscle.[6-10]

Several of the other novel cardiotonics, including milrinone and carbazeran, also potently inhibit the activity of type III phosphodiesterase from cardiac muscle. These agents, however, also exert significant inhibitory effects on the two other molecular forms of phosphodiesterase present in cardiac muscle.[11] As yet, no study has demonstrated which structural features of these agents are responsible for selective PDE III inhibitory activity. In the present investigation, the inhibitory effects of a number of analogues of CI-914 (1) and CI-930 (10) on the different molecular forms of phosphodiesterase present in cardiac muscle were examined. In addition, those structural elements that are associated with selective inhibition of type III phosphodiesterase and those that produce a nonselective inhibitory effect were determined. Finally, the relationship between in vitro phosphodiesterase inhibitory activity and in vivo positive inotropic activity was evaluated.

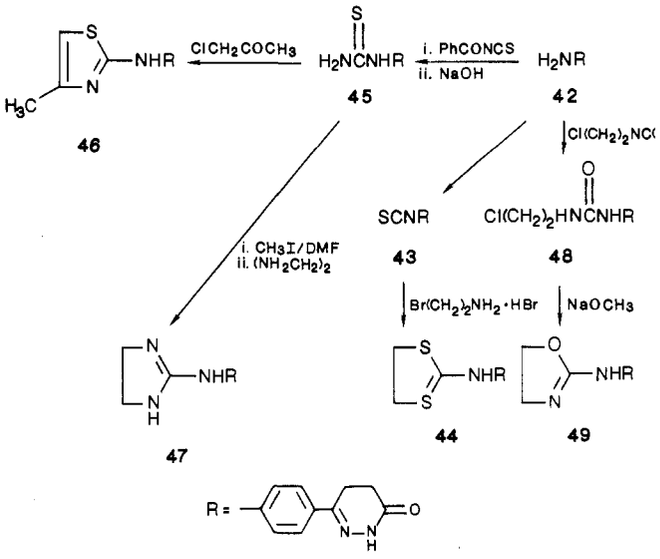
Chemistry

The synthesis of compounds 1–36 (Tables I and II) except 15 was reported earlier.[12] The synthesis of 15 was

Scheme II



Scheme III



accomplished from 4-(1*H*-imidazol-1-yl)-β-methyl-γ-oxobenzenebutanoic acid[12] by alkylation with iodomethane to give the requisite β,β-dimethyl compound, which was cyclized with hydrazine (Scheme I).

The synthesis of 4,5-dihydro-6-[4-(1*H*-azolyl)phenyl]-3(2*H*)-pyridazinones in which the azole is attached via carbon instead of nitrogen (38 and 41, Table II) is illustrated in Scheme II. The tetrazole 38 was obtained from benzonitrile 37[13] by reaction with sodium azide. The nitrile 37 was also treated with hydrogen sulfide to give the corresponding thioamide 39, which was condensed with ethylenediamine to give the imidazoline 40. Manganese dioxide oxidation of 40 afforded 41.

The 4-amino heterocyclic analogues 44, 46, 47, and 49 (Table II) were synthesized from the corresponding 4-amino compound 42 as illustrated in Scheme III. The amine 42[13] was converted to the isothiocyanate 43[12] in good yield by the reaction of 1,1′-thiocarbonyldiimidazole. The thiazoline 44 was obtained from the isothiocyanate 43 by treatment with 2-bromoethylamine hydrobromide. Reaction of 42 with benzoyl isothiocyanate followed by base hydrolysis gave the thiourea 45, which, when treated with chloroacetone, afforded the thiazole 46. The thiourea 45 was converted to the isothiouronium salt by treatment with iodomethane/*N*,*N*-dimethylformamide, followed by treatment wth ethylenediamine to give the imidazoline 47. Compound 42 was treated with 2-chloroethylisocyanate to give the (chloroethyl)urea 48, which was cyclized with base to give the aminooxazoline 49.

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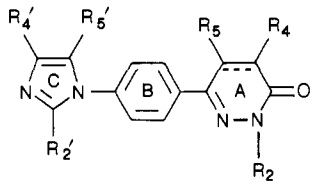
Table I. Phosphodiesterase Inhibitory Activity of 4,5-Dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones and Related Compounds

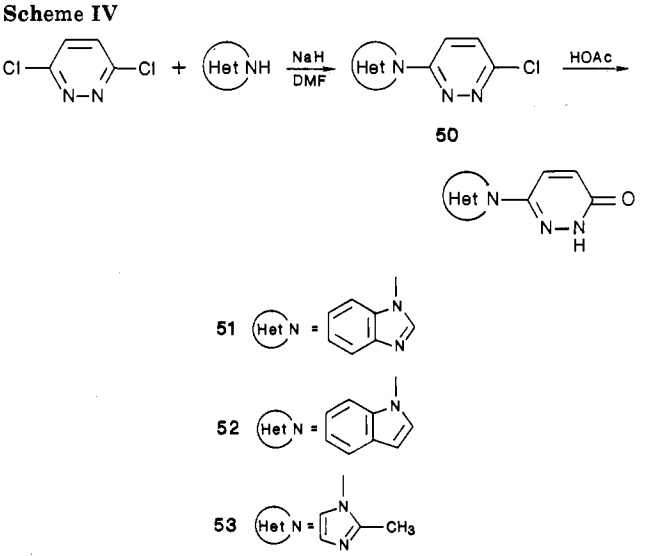


no.	R_2	R_4	R_5	R_2'	R_4'	R_5'	DHPZ (DP) or PZ (P)	% inhibition[a,b] at 10^{-5} M (10^{-4} M) PDE I cAMP	PDE I cGMP	PDE II cAMP	PDE II cGMP	PDE III cAMP
1	H	H	H	H	H	H	DP	3 (8)	3 (10)	4 (17)	7 (20)	57 (76)
2	H	H	H	H	H	H	P	4 (17)	6 (15)	15 (16)	11 (27)	56 (80)
3	CH_3	H	H	H	H	H	DP	1 (22)	3 (26)	5 (33)	1 (32)	13 (47)
4	CH_3	H	H	H	H	H	P	1	9	16	12	20
5	CH_2Ph	H	H	H	H	H	DP	1 (39)	13 (33)	22 (70)	18 (70)	47 (79)
6	CH_2CH_2OAc	H	H	H	H	H	DP	1	5	23	11	16
7	CH_2CH_2OH	H	H	H	H	H	P	(21)	(37)	(17)	(28)	(33)
8	H	CH_3	H	H	H	H	DP	0 (8)	2 (8)	15 (27)	7 (26)	25 (63)
9	H	CH_3	H	H	H	H	P	(48)	(49)	(55)	(62)	(81)
10	H	H	CH_3	H	H	H	DP	3 (9)	9 (12)	5 (18)	7 (23)	69
11	H	H	CH_3	H	H	H	P	0 (24)	4 (14)	4 (34)	8 (39)	26 (64)
12	H	H	CH_2CH_3	H	H	H	DP	1 (2)	1 (8)	5 (35)	13 (40)	18 (54)
13	H	$-(CH_2)_4-$		H	H	H	DP	1 (22)	2 (16)	14 (38)	8 (44)	8 (34)
14	H	$(CH_3)_2$	H	H	H	H	DP	7	2	22	13	27
15	H	H	$(CH_3)_2$	H	H	H	DP	2	5	17	6	47
16	H	$CONHNH_2$	H	H	H	H	P	6	4	20	12	21
17	H	$CONH_2$	H	H	H	H	P	11	16	18	15	24
18	H	NH_2	H	H	H	H	P	6 (20)	7 (28)	13 (44)	10 (51)	41 (64)
19	H	H	H	CH_3	H	H	DP	1 (16)	0 (18)	8 (19)	4 (22)	57 (64)
20	H	H	H	CH_3	H	H	P	(20)	(15)	(19)	(27)	(66)
21	H	H	H	SH	H	H	DP	(2)	(1)	(6)	(3)	(57)
22	H	H	H	SCH_3	H	H	DP	7 (22)	13 (23)	10 (28)	12 (34)	57 (67)
23	H	H	H	$SOCH_3$	H	H	DP	(7)	(2)	(6)	(3)	(57)
24	H	H	H	H	CH_2OH	H	DP	1 (9)	3 (9)	6 (18)	5 (23)	59 (68)
25	H	H	H	CH_2CH_3	CH_3	H	DP	9 (51)	7 (53)	14 (37)	9 (44)	59 (76)
26	H	H	H	CH_2CH_3	CH_3	H	P	(33)	(41)	(41)	(47)	(79)
27	H	H	H	CH_2CH_3	CH_3	Br	P	20 (64)	16 (63)	20 (79)	26 (80)	63 (90)
28	H	H	H	H	$-CH=CHCH=CH-$		DP	9 (25)	9 (36)	24 (67)	24 (70)	66 (88)
29	H	H	H	H	$-(CH_2)_4-$		DP	8 (38)	7 (37)	20 (62)	22 (69)	60 (87)
30	H	H	H	H	$-(CH_2)_4-$		P	30 (52)	27 (56)	(67)	(72)	(91)
31	H	H	CH_3	H	$-(CH_2)_4-$		DP	13 (57)	17 (54)	21 (65)	21 (68)	64 (90)

[a] Substrate concentration was 1.0 μM. [b] Values represent the average of two to four separate experiments.

The synthesis of 6-azolyl-3(2*H*)-pyridazinones **51–53** lacking the phenyl group is illustrated in Scheme IV. 3,6-Dichloropyridazine was condensed with the anion of benzimidazole to give mono- and bisalkylated products, which were separated by chromatography to give the chloride **50**. The chloride **50** was subsequently hydrolyzed with glacial acetic acid to give **51**. Compounds **52** and **53** were similarly obtained from indole and 2-methylimidazole.[14] The physical properties of these compounds are provided in Table III.

Scheme IV



Results and Discussion

The target compounds of this study were evaluated for their ability to inhibit guinea pig cardiac phosphodiesterase. Theophylline and papaverine were chosen as standards for comparison in these studies because of their reported activity as relatively nonselective phosphodiesterase inhibitors.[5,15]

The isolation of different forms of cardiac phosphodiesterase and their characterization were done by the method of Thompson et al.[16] Briefly, guinea pig left ventricular tissue was found to contain three distinct molecular forms of phosphodiesterases, which vary with regard to substrate specificity (cyclic AMP or cyclic GMP), kinetic characteristics (K_m and V_{max}), and their ability to be stimulated by calmodulin.[5] Type I PDE is a low K_m enzyme that has comparable affinity for cyclic AMP and

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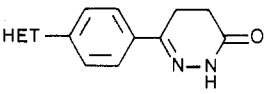
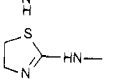
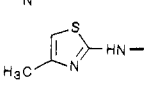
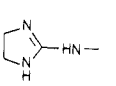
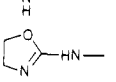
(15) Wells, J. N.; Wu, Y. J.; Baird, C. E.; Hardman, H. A. *Mol. Pharmacol.* **1975**, *11*, 775.
(16) Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 69.

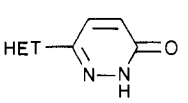
Table II. Phosphodiesterase Inhibitory Activity of 4,5-Dihydro-6-(substituted phenyl)-3(2*H*)-pyridazinones

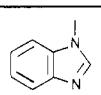
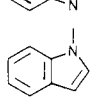
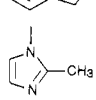


no.[c]	HET	DHPZ (DP) or PZ (P)	% inhibition[a,b] at 10^{-5} M (10^{-4} M) PDE I cAMP	PDE I cGMP	PDE II cAMP	PDE II cGMP	PDE III cAMP
32[d]		DP	6 (9)	3 (8)	10 (21)	10 (28)	28 (65)
33		DP	12	15	27	27	(73)
34		DP	7	6	6 (20)	7 (24)	33 (51)
35		DP	29	20	25	21	70
36		DP	0	13	20	21	68
38		DP	21	20	37	29	86
41		P	8 (18)	10 (23)	10 (34)	7 (41)	51 (69)
44		DP	18	20	31	33	58
46		DP	7	2	23	23	58
47		DP	33	33	28	16	58
49		DP	3 (26)	6 (12)	9 (36)	4 (31)	54 (68)

[a] Substrate concentration was 1.0 μM. [b] Values represent the average of two to four separate experiments. [c] The intermediates **37**, **39**, **40**, **42**, **43**, **45**, and **48** were not evaluated for PDE inhibitory activity. [d] Imidazole ring is attached to the 3-position of the phenyl ring instead of the 4-position.

Table III. Phosphodiesterase Inhibitory Activity of 6-Substituted-3(2*H*)-pyridazinones



no.	HET	DHPZ (DP) or PZ (P)	% inhibition[a,b] at 10^{-4} M PDE I cAMP	PDE I cGMP	PDE II cAMP	PDE II cGMP	PDE III cAMP	formula	mp, °C (crystn solv)	yield, %
51		P	26	25	31	32	68	$C_{11}H_8N_4O$	248–249 (ethanol)	30
52		P	17	35	63	60	68	$C_{12}H_9N_3O$	260–261 (ethanol)	50
53		P	4	15	1	0	42	$C_8H_8N_4O$	250–251 (DMF)	60

[a] Substrate concentration was 1.0 μM. [b] Values represent the average of two to four separate experiments.

cyclic GMP and is stimulated by calmodulin. Type II is a high K_m enzyme that is not stimulated by calmodulin and has comparable affinity for cyclic AMP and cyclic GMP. Type III is a low K_m cyclic AMP specific enzyme that is not stimulated by calmodulin. Detailed descriptions are included in the Experimental Section.

Tables I–III summarize the phosphodiesterase (PDE) inhibitory effects of the test agents, expressed as percent inhibition. IC_{50} values on PDE I, PDE II, and PDE III of a selected group of compounds are listed in Table IV. In addition, ratios of PDE III/PDE II and PDE III/PDE I are also included in Table IV. In general, most of the compounds studied were potent and relatively selective inhibitors of PDE III. Slight modifications of the parent structure **1** altered both potency and selectivity.

Modification of the 4,5-Dihydro-3(2*H*)-pyridazinone Ring (Ring A). No change in the PDE inhibitory activity of unsubstituted 4,5-dihydropyridazinone (DHPZ) was apparent when compared with the corresponding pyridazinones (PZ); for example, com-

Table IV. Compounds Chosen for In-Depth Studies

compd	ED_{50}, mg/kg obsd[a] (n)	eq 1	eq 2	log k′	IC_{50},[b] μM PDE I cAMP	PDE I cGMP	PDE II cAMP	PDE II cGMP	PDE III cAMP	IC_{50} ratios PDE III/PDE I (×10³) cAMP	PDE III/PDE II (×10³) cAMP
1 (CI-914)	0.045 ± 0.006 (6)	0.15	0.092	0.94	>1000	>1000	760 (630–880)[c]	500 (400–600)	8.0 (6.0–10.0)	<8.0	10.5
2	0.11 ± 0.21 (3)	0.14	0.12	1.3	660 (350–970)	580 (410–750)	270 (210–330)	190 (150–230)	7.2 (6.1–8.3)	10.9	46.6
3	1.0	0.72	0.83	1.9	>1000	>1000	370 (290–450)	280 (200–350)	110 (92–120)	<110.0	297.3
8	1.0	0.41	0.39	1.3	>1000	>1000	260 (200–320)	280 (250–310)	43 (37–49)	<43.0	165.4
10 (CI-930)	0.013 ± 0.006 (8)	0.031	0.022	1.3	>1000	>1000	460 (400–510)	380 (320–430)	0.6 (0.2–1.0)	<0.6	1.3
11	1.1	0.45	0.32	0.96	660 (370–960)	>1000	210 (180–240)	170 (170–210)	50 (34–65)	75.7	238.1
12	1.0	0.57	0.66	1.5	>1000	>1000	100 (64–140)	71 (45–98)	74 (57–90)	<74.0	740.0
13	1.0	0.91	0.48	2.0	380 (330–430)	550 (440–670)	150 (130–170)	130 (120–140)	220 (170–270)	578.9	>1000
18	0.3	0.27	0.25	1.3	>500	>500	110 (89–130)	88 (75–100)	22 (14–30)	<44.0	200.0
19	0.3	0.069	0.059	1.4	790 (450–1100)	530 (360–710)	420 (330–510)	380 (350–440)	2.3 (1.5–3.2)	2.9	5.4
24	0.034 ± 0.012 (4)	0.098	0.036	0.35	>1000	>1000	480 (430–540)	350 (290–410)	4.1 (2.8–5.4)	<4.1	8.5
25	0.1	0.089	0.12	1.9	86 (81–92)	80 (75–87)	170 (150–180)	92 (73–110)	3.5 (2.2–4.8)	40.7	20.6
28	0.1	0.047	0.069	2.1	280 (190–380)	220 (76–370)	42 (34–51)	34 (28–41)	1.2 (0.63–1.7)	4.3	28.5
29	0.064 ± 0.016 (3)	0.044	0.077	2.3	180 (110–240)	140 (76–210)	49 (40–57)	39 (31–47)	1.1 (0.67–1.5)	6.1	22.4
31	0.025 ± 0.006 (6)	0.013	0.024	2.5	75 (65–86)	85 (64–110)	46 (41–51)	43 (33–54)	0.15 (0.13–0.9)[d]	2.0	3.2
32	0.3 ± 0.061 (3)	0.39	0.34	1.2	930 (620–1200)	>1000	330 (260–390)	340 (270–400)	40 (27–53)	43.0	121.2
41	0.017 ± 0.003 (3)	0.19	0.14	1.1	460 (230–700)	330 (180–380)	190 (170–220)	180 (140–210)	12 (–3.1–27)	26.1	63.1
amrinone[e]	0.389 ± 0.028				>1000	>1000	720 (560–880)	580 (400–770)	50 (40–54)	<50.0	63.8
milrinone[e]	0.037 ± 0.014				310 (190–430)	340 (170–510)	220 (140–300)	200 (120–290)	2.5 (2.0–3.0)	8.0	11.3

[a] Values are doses producing 50% increases in dP/dt_{max} from control levels calculated from the dose–response curves and are expressed as means ± SEM for compounds 1, 2, 10, 24, 29, 31, 32, 41, amrinone, and milrinone (significant at $p < 0.05$). For the remaining compounds, values are the arithmetic means of two separate experiments. n represents number of dogs. [b] IC_{50} value is the concentration required to inhibit enzyme activity by 50%. Values were obtained from two to four separate concentration–response curves. Substrate concentration was 1.0 μM. [c] Values in parentheses represent 95% confidence limits as determined by the method of Hubert.[21] [d] Biphasic dose–response curve. [e] Compounds not included in the regressions.

pare **1**, **3**, **19**, **25**, and **29** with **2**, **4**, **20**, **26**, and **30**. Substitution in the 2-position of the DHPZ ring (**1**, CI-914, IC_{50} = 8 μM) with alkyl (**3**, IC_{50} = 110 μM) or substituted alkyl, such as **6** and **7**, caused a significant reduction in PDE III inhibitory potency. The potency to inhibit PDE I or PDE II remained unaltered. An acidic hydrogen appears to be necessary for potent PDE III inhibition. Substitution with a methyl group in the 4-position reduced PDE III inhibitory potency about fivefold (**8**, IC_{50} = 43 μM vs. **1**, IC_{50} = 8 μM) whereas the 5-methyl analogue of **1** (**10**, CI-930, IC_{50} = 0.6 μM) was 13 times more potent. Compound **10** still maintained a high degree of selectivity for type III PDE (Table IV). Further increase in the size of the alkyl group in the 5-position caused a dramatic reduction in

Table V. Correlation Matrix

	log ED_{50}	log (cAMP PDE I)	log (cAMP PDE II)	log (cAMP PDE III)	log (cGMP PDE I)	log (cGMP PDE II)	log k'
log ED_{50}	1.0						
log (cAMP PDE I)	0.51	1.0					
log (cAMP PDE II)	−0.0010	0.71	1.0				
log (cAMP PDE III)	0.79	0.63	0.28	1.0			
log (cGMP PDE I)	0.53	0.96	0.63	0.66	1.0		
log (cGMP PDE II)	0.022	0.78	0.98	0.29	0.71	1.0	
log k'	−0.056	−0.81	−0.73	−0.28	−0.67	−0.73	1.0

PDE III inhibitory potency (**12**, IC_{50} = 74 μM). Dimethyl substitution in the 5-position (**15**) resulted in slightly reduced inhibitory potency for PDE III with no significant changes for PDE I and II. Compound **13**, in which the 4- and 5-positions are connected by a six-membered ring, was inactive. These data strongly suggest a PDE III binding region that accommodates a small lipophilic group in the 5-position of the DHPZ ring, thus resulting in optimal inhibitory potency.[3,18]

The effect of substitution in the PZ ring on PDE III inhibitory potency is, however, quite different. An alkyl substitution at the 4-position (**9**) retained the PDE III inhibitory potency of the parent compound **2**, but selectivity was substantially reduced compared to that of **2**. By contrast, the potency and the selectivity of the 5-methyl analogue of **2** were reduced sevenfold (**11**, IC_{50} = 50 μM vs. **2**, IC_{50} = 7.2 μM). The difference in the inhibitory potency of the alkyl DHPZ and PZ compounds may be explained on the basis of molecular planarity.[3,18] The very potent DHPZ **10** is relatively planar (rings A, B, and C) in its minimum energy conformation whereas the less potent PZ **11** is not.[3,17] A complete report of the molecular modeling, X-ray crystallographic, and spectroscopic studies of these compounds is the subject of another paper.[18] A threefold reduction in PDE III inhibitory potency was observed with compound **8** (IC_{50} = 22 μM), in which the 4-position of the pyridazinone ring is substituted by an amino group.

Modification of the Imidazole Ring (Ring C). Substitution on the imidazole ring altered activity appreciably. Substitution at the 2′-position with alkyl (**19**), mercapto (**21**), methylthio (**22**), and methylsulfinyl (**23**) caused a slight reduction in potency but retained selectivity for PDE III (see Table I). Substitution of the 4′-hydrogen with hydroxymethyl (**24**, IC_{50} = 4.1 μM) caused a twofold increase in the inhibitory potency (**1**, IC_{50} = 8 μM). Dialkyl substitution (**25**, IC_{50} = 3.5 μM) retained potency for PDE III although it was less selective (see Table IV). The trisubstituted imidazole **27** is the least selective. Compounds in which 4′- and 5′-positions of the imidazole ring are substituted with a benzene ring (**28**, IC_{50} = 1.2 μM) and a tetrahydrobenzene ring (**29**, IC_{50} = 1.1 μM) were significantly less selective although the inhibitory potencies were increased eightfold. The presence of a methyl group in the 5-position of the 4,5-dihydropyridazinone ring (DHPZ) **31** caused a further increase in the inhibitory potency for PDE III (IC_{50} = 0.15 μM), but the compounds also had significant inhibitory effects for PDE I and PDE II. The following general trend appears for the rank order of selectivity: no substitution > monosubstitution > disubstitution > trisubstitution.

Since the selectivity for the PDE III inhibitory effect was dependent upon slight modifications of the imidazole ring, several compounds were evaluated in which the imidazole was replaced with other heterocycles (see Table II). Compounds **38** and **41** retained potency but reduced selectivity of the parent compound **1**. Compounds **44**, **46**, **47**, and **49**, in which the heterocycle and the phenyl DHPZ moiety are separated by an amino function, were also evaluated. Amino heterocycle substitution in place of imidazole reduced potency to inhibit PDE III activity but did not affect PDE I or PDE II to a great extent. An exception was the aminothiazoline **44**, in which the inhibitory potency for PDE III was maintained. Although some of the compounds obtained by heterocyclic replacement of imidazole are potent inhibitors of cardiac PDE III, those compounds were quite insoluble in aqueous buffers and were very poorly soluble in strong acid. Their usefulness was, therefore, limited, and no further work was conducted.

Modification of the Phenyl Group (Ring B). Several compounds were evaluated in which ring A was directly linked to ring C (Table III). These results indicate that lack of the phenyl group decreases potency and increases selectivity (for example, compare **20** with **53**).

Intercorrelations between Biological Data. In order to explore further structure–activity relationships, a series of compounds were selected for in-depth study (Table IV). Selection was based on structural variation, solubility in different buffers, and a range of biological potencies and physicochemical properties. The inhibitory effects these compounds exerted on each molecular form of cardiac phosphodiesterase are expressed as IC_{50} values, the concentration that produces a 50% inhibition of substrate hydrolysis. The positive inotropic effects were evaluated intravenously in an acutely instrumented dog model as described in the Experimental Section.[12] The doses that produce a 50% increase in myocardial contractility (ED_{50}) derived by measuring changes in the first derivative of left ventricular pressure (dP/dt_{max}) were calculated from the dose–response curve and are expressed as ED_{50} values.

Interestingly, several high correlations exist among phosphodiesterase types I and II and lipophilicity (log k') but not with type III (Table V).[19,20] Thus, while selectivity for PDE III was easily attained in this series, selectivity for an individual PDE I or II fraction may be difficult to achieve.

Inhibition of cAMP PDE III was best correlated with ED_{50} (eq 1).[3,12]

$$\log ED_{50} = [0.61\ (\pm 0.12)]\ \log (\text{cAMP PDE III}) - [1.4\ (\pm 0.15)] \quad (1)$$

$$r^2 = 0.62,\ F = 25,\ s = 0.43,\ n = 17$$

(17) Moos, W. H.; Sircar, I.; Bristol, J. A. Presented in part at the 3rd Joint Meeting of the American Chemical Society Great Lakes and Central Regions, Kalamazoo, MI, May 24, 1984; MEDI-247.

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(20) Haky, J. E.; Young, A. M. *J. Liq. Chromatogr.* **1984**, *7*, 675.

Consideration of possible drug distribution phenomena in comparing intrinsic (i.e., in vitro) enzyme activity with pharmacological (i.e., in vivo) responses often improves correlations. In fact, slight improvement was observed when lipophilicity (log k') was incorporated into these analyses (eq 2), but distribution parameters may not be particularly important here due to intravenous administration.

$$\log ED_{50} = [0.67\ (\pm 0.12)] \log (\text{cAMP PDE III}) + [0.37\ (\pm 0.18)] \log k' - [2.0\ (\pm 0.33)] \quad (2)$$

$$r^2 = 0.71,\ F = 17,\ s = 0.39,\ n = 17$$

Conclusions

Within the reported series of 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones, (1) the imidazole moiety (ring C) is primarily responsible for PDE isozyme specificity, whereas (2) the 4,5-dihydro-3(2*H*)-pyridazinone ring (ring A) is responsible for inhibitory potency; (3) the B ring acts mainly as a spacer; (4) the phosphodiesterase type I andd II isozymes are intercorrelated, suggesting that selectivity for these individual fractions may be difficult to achieve; (5) positive inotropic potency is best correlated with inhibition of cAMP PDE type III.

Experimental Section

Melting points are uncorrected and were taken on a Thomas-Hoover capillary melting point apparatus. IR and ^1H NMR spectra of all new compounds were consistent with the proposed structures. Each analytical sample was homogeneous by TLC performed on silica gel plates using methylene chloride and methanol (9:1) as eluants. Elemental analyses were within 0.4% of theoretical values unless otherwise stated.

4,5-Dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-5,5-dimethyl-3(2*H*)-pyridazinone (15). To a slurry of sodium hydride (60% oil suspension, 0.8 g, 0.02 mol) in 5 mL of DMF was added a solution of 2.58 g (0.01 mol) of 4-(1*H*-imidazol-1-yl)-β-methyl-γ-oxobenzenebutanoic acid[12] in 20 mL of DMF at room temperature with stirring. A deep red solution resulted. Iodomethane (1 mL, 0.02 mol) in 5 mL of DMF was added dropwise, and an exotherm was observed with the precipitation of sodium iodide. After the mixture was stirred overnight at room temperature, the DMF was distilled under reduced pressure, and the residue was partitioned between water and chloroform. The chloroform solution was washed successively with saturated aqueous $NaHCO_3$ and water, dried, and distilled, leaving a residual oil (1.1 g). This oil was used for cyclization without further purification. It was dissolved in 15 mL of ethanol containing hydrazine hydrate (80%, 0.5 g) and refluxed for 4 h. The residue obtained after removal of ethanol was purified by chromatography over silica gel using chloroform/methanol (10:1). The product was obtained as a solid, which was recrystallized from ethyl acetate/chloroform to give analytically pure **15**, mp 234–235 °C. Anal. ($C_{15}H_{16}N_4O$) C, H, N.

4,5-Dihydro-6-[4-(1*H*-tetrazol-5-yl)phenyl]-3(2*H*)-pyridazinone (38). A stirred mixture of 4.0 g (0.02 mol) of **37**, 2.6 g (0.04 mol) of NaN_3, and 2.14 g (0.04 mol) of NH_4Cl in 100 mL of DMF was heated at 100 °C for 10 h and then at 130 °C for 4 h. After cooling to 23 °C, the inorganic salts were filtered and washed with DMF. The filtrate was concentrated in vacuo to ca. 15 mL and adjusted to pH 1.5 with 6 N HCl. The resulting solid hydrochloride was filtered, dissolved in NH_4OH (pH 8.5), and precipitated by the addition of acetic acid to pH 6.5. The above process was repeated once to obtain a nearly white solid, which was collected and crystallized from ethanol to give 1.8 g (37%) of **38**, mp 281–282 °C. Anal. ($C_{11}H_{10}N_6O$) C, H, N.

4-(1,4,5,6-Tetrahydro-6-oxo-3-pyridazinyl)benzenecarbothioamide (39). Hydrogen sulfide was bubbled slowly for 8 min through a solution of **37** in 75 mL of DMF containing 1.8 g of ethylenediamine, and the reaction mixture was allowed to stand for 1 h at room temperature when a white precipitate began to form. The solid was filtered, and the filtrate was poured into 300 mL of cold water. Filtration afforded 3.8 g of analytically pure product (**39**), mp 255–256 °C. Anal. ($C_{11}H_{11}N_3OS$) C, H, N, S.

6-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (40). A slurry of 3 g of **39** in 5 mL of ethylenediamine was heated at 100 °C for 0.5 h, giving a clear solution. The solution was poured into 50 mL of ice/water, and the solid was filtered, washed with a small volume of ice/water, and air-dried to give 2.5 g (20%) of **40**, mp 230 °C. Anal. ($C_{13}H_{14}N_4O_2 \cdot 0.5H_2O$) C, H, N.

6-[4-(1*H*-Imidazol-2-yl)phenyl]-3(2*H*)-pyridazinone (41). A mixture of 2.5 g of **40** and 15 g of manganese dioxide in 15 mL of DMF was stirred vigorously and heated to 100 °C for 48 h. The suspension was filtered, and the residue was washed with hot DMF. The filtrate and washings were combined and evaporated under reduced pressure, leaving a residue, which was crystallized from ethanol to give 0.6 g (49%) of **41**, mp 236–238 °C. Anal. ($C_{13}H_{10}N_4O$) C, H, N.

6-[4-[(4,5-Dihydro-2-thiazolyl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (44). A mixture of 0.57 g (0.002 mol) of **43**, 0.51 g (0.002 mol) of 2-bromoethylamine hydrobromide, and 0.34 g of anhydrous K_2CO_3 in 12 mL of DMF was heated at 80 °C for 4 h. The reaction mixture was cooled and filtered, and the filtrate was concentrated under reduced pressure. The residue was treated with water, neutralized, and filtered to give 0.5 g (73%) of **44**, mp 217–218 °C. Anal. ($C_{13}H_{14}N_4OS$) C, H, N.

***N*-[4-(1,4,5,6-Tetrahydro-6-oxo-3-pyridazinyl)phenyl]-thiourea (45).** Benzoyl chloride (1.4 g, 0.01 mol) was added dropwise to a solution of 0.8 g (0.01 mol) of ammonium thiocyanate in 5 mL of hot acetone. After the initial reaction subsided, the reaction mixture was heated at reflux for 0.25 h. A solution of 1.89 g (0.01 mol) of **42** in 20 mL of hot DMF was added to the reaction mixture with stirring followed by heating at 100 °C for 6 h. The reaction mixture was cooled, poured into water, and filtered to yield 2 g of *N*-[[[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)phenyl]amino]thioxomethyl]benzamide, mp 232–233 °C dec.

To a suspension of 0.7 g of the above benzamide in 7 mL of ethanol was added 1.6 mL of 10% ethanolic sodium hydroxide followed by heating for 2 h. The mixture was cooled, diluted with water, and filtered to give 0.4 g of **45**, mp 236–237 °C. Anal. ($C_{11}H_{12}N_4OS$) C, H, N, S.

4,5-Dihydro-6-[4-[(4-methyl-2-thiazolyl)amino]phenyl]-3-(2*H*)-pyridazinone (46). A mixture of 1.2 g (5 mmol) of **45** and 0.5 g (5.4 mmol) of chloroacetone in 20 mL of DMF was heated on a steam bath for 4 h. The DMF was distilled under reduced pressure, and the residue was treated with water. The pH was adjusted to 8, the mixture was filtered, and the solid was crystallized from DMF to yield 0.8 g (24%) of **46**, mp 306–307 °C. Anal. ($C_{14}H_{14}N_4OS$) C, H, N.

6-[4-[(4,5-Dihydro-1*H*-imidazol-2-yl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (47). A solution of 2.8 g of **45** in 20 mL of DMF at 80 °C was treated with a solution of 3.0 g of iodomethane in 10 mL of DMF, and the reaction mixture was stirred for 2 h. The DMF was distilled under reduced pressure, and the residue was treated with ethanol and filtered, giving 2.4 g of the isothiouronium salt as an off-white solid, which was used for cyclization without further purification.

A mixture of 2.3 g of the above isothiouronium salt and 1.0 g of ethylenediamine in 20 mL of ethanol was heated at reflux for 2 h. The solution was concentrated to a small volume, and the solid was filtered, washed with ethanol, and air-dried to yield 0.7 g (40%) of **47**, mp 235–236 °C. Anal. ($C_{13}H_{15}N_5O$) C, H, N.

6-[4-[(4,5-Dihydro-2-oxazolyl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (49). A mixture of 1.89 g (0.01 mol) of **42** and 1.1 g (0.15 mol) of 2-chloroethyl isocyanate in 20 mL of DMF was heated at 80 °C for 4 h. The DMF was distilled under reduced pressure, and the residue was treated with water. The solid was filtered, washed with water, and air-dried to give 2.5 g of *N*-(2-chloroethyl)-*N'*-[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-phenyl]urea (**48**), mp 212–213 °C. Anal. ($C_{13}H_{15}ClN_4O_2$) C, H, N, Cl.

A mixture of 1.8 g (6 mmol) of the above urea **48** and 0.7 g (12 mmol) of NaOMe in 20 mL of DMF was heated at 100 °C for 5 h. The DMF was distilled under reduced pressure, the residue was treated with water, and the pH of the solution was adjusted to 6. The solid was filtered, washed with water, and crystallized

from DMF to yield 1.2 g (62%) of **49**, mp 332–333 °C. Anal. ($C_{13}H_{14}N_4O_2$) C, H, N.

6-(1*H*-Benzimidazol-1-yl)-3(2*H*)-pyridazinone (51). A solution of 11.8 g (0.1 mol) of benzimidazole in 70 mL of DMF was added dropwise to a slurry of 5 g (0.1 mol) of sodium hydride (60% oil suspension) in 50 mL of DMF. The reaction mixture was stirred at ambient temperature for an additional hour. A solution of 16.5 g (0.11 mol) of 3,6-dichloropyridazine in 25 mL of DMF was added dropwise, and the reaction mixture was stirred for 18 h at room temperature. The DMF was distilled, and the residue was poured into ice water and filtered. The solid was washed with water and air-dried to give 16 g of the monoalkylated product **50**, which was used directly for hydrolysis.

A solution of 14 g of the above chloride in 20 mL of glacial acetic acid was refluxed for 6 h. The acetic acid was removed under reduced pressure, and the solid was treated with water, filtered, and recrystallized from ethanol to give 4 g of the desired amide **51**, mp 248–249 °C. Via the procedure described above, one additional compound was prepared: 6-(1*H*-indol-1-yl)-3(2*H*)-pyridazinone (**52**).

6-(2-Methyl-1*H*-imidazol-1-yl)-3(2*H*)-pyridazinone (53). A solution of 4.5 g of 3-chloro-6-(2-methyl-1*H*-imidazol-1-yl)-pyridazine[14] in 20 mL of acetic acid was refluxed for 4 h. The reaction mixture was cooled and filtered to obtain 3.7 g of **54** as the hydrochloride salt. The latter was dissolved in water and neutralized with ammonium hydroxide to give 2.5 g of the corresponding free base, which was crystallized from DMF to give **53**, mp 250–251 °C dec.

Isolation of Cardiac Phosphodiesterase Isozymes and Assay of Activity. The method of Thompson et al. was used to isolate cardiac phosphodiesterases.[16] Briefly, male guinea pigs were anesthetized with diethyl ether and the hearts removed and rinsed with ice-cold saline. Left ventricular and septal muscle from three to four hearts was used for each isolation. The tissue was minced, homogenized by using a Polytron, and sonicated. The homogenate was then centrifuged for 20 min at 30000*g* and the resulting supernatant filtered through gauze and applied to a DEAE-cellulose column. The phosphodiesterases were eluted from the column by using a continuous 70–1000 mM sodium acetate gradient (pH 6.5, containing 2-mercaptoethanol). Fractions were collected and assayed for cyclic AMP and cyclic GMP phosphodiesterase activity.[16] Substrate concentration for these assays was 1.0 μM. Fractions containing high levels of type I, type II, or type III phosphodiesterase activity were pooled and dialyzed overnight against a large excess of 70 mM sodium acetate/5 mM 2-mercaptoethanol. Cross-contamination was removed by rechromatography.[16] Following complete separation, the combined phosphodiesterase fractions were concentrated to 14% of the original volume, diluted to 65% with ethylene glycol monoethyl ether, and stored at −20 °C (no significant change in hydrolytic activity was observed after storage of up to 6 weeks).

In evaluation of the inhibitory effects of the different agents examined in type I, type II, and type III cardiac phosphodiesterase, the protein concentration in the assay was adjusted to ensure that reaction velocity was linear for 30 min at 30 °C and that hydrlysis of substrate ([^{3}H]cAMP or [^{3}H]cGMP) did not exceed 10–20% of the available substrate in the absence of inhibitor. The concentration of substrate was 1.0 μM for these studies. All agents examined were dissolved in dimethyl sulfoxide (Me_2SO). The final concentration of Me_2SO in the reaction medium was 2.5%. In control experiments this concentration of Me_2SO inhibited enzyme activity by approximately 10%. IC_{50} values (concentration that produced 50% inhibition of substrate hydrolysis) were determined from concentration–response curves which ranged from 10^{-7} to 10^{-4} M for the more potent inhibitors and from 10^{-5} to 10^{-3} M for the less potent inhibitors (half-log increments). Two to four such concentration–response curves were generated for each agent, typically by using different enzyme preparations for each concentration–response curve.

Pharmacological Methods. Anesthetized Dog Model. Adult mongrel dogs of either sex were anesthetized with pentobarbital, 35 mg/kg, iv, and were subsequently maintained under anesthesia with a continuous infusion of pentobarbital, 5 mg kg^{-1} h^{-1}. A cannula was inserted into the femoral vein for administering test agents. A Millar catheter tip pressure transducer was inserted into the ascending aorta via the femoral artery for measuring aortic blood pressure. Another similar transducer was passed into the left ventricle via the left carotid artery for measuring left ventricular blood pressure. Needle electrodes were placed subcutaneously for recording a lead II electrocardiogram (ECG). Heart rate, determined by using a biotachometer triggered from the R wave of the ECG, and the first derivative of left ventricular blood pressure (dP/dt), obtained with a differentiator amplifier coupled to the corresponding pressure amplifier, were also recorded. A period of 30 min was utilized to obtain control data prior to administration of test agent. Depending on solubility of the agent, compounds were dissolved in 0.9% saline solution or in dilute HCl or NaOH (0.1–1.0 N) and were diluted to volume with normal saline. Each dose of the test agent was administered in a volume of 0.1 mg/kg over a period of 1 min in a cumulative manner. Usually, half-log intervals were maintained between doses with typical dosing consisting of four to six doses (for example, 0.01, 0.03, 0.1, 0.3, 1.0 mg/kg) in order to establish any dose–response relationships. A 10–30-min interval was used between doses for the variables to reach a steady state. Only one compound was administered to any one animal. The inotropic activity of a compound was determined by measuring changes in dP/dt_{max} of left ventricular pressure from preceding base line. Data are expressed as means ± SEM or arithmetic means of two experiments. Statistical analysis of the data was performed with use of a Student's test for paired or unpaired data. The probability value, $p < 0.05$, was accepted as level of significance.

Data Processing. Regression analyses were run on an IBM 3083 mainframe using the SAS program package.[19] High-pressure liquid chromatography (pH 7.4) was used to determine log k' values, a measure of lipophilicity.[20]

In eq 1–2, the figures in parentheses are the standard errors of the regression coefficients. For a given equation, the value r^2 is the square of the correlation coefficient, F relates the variance of the null hypothesis to the correlation variance, s is the root mean square error, and n represents the number of compounds included in the analysis. For comparison, the error of the biological data (s'), estimated as the square root of the mean of the squared errors from replicate analyses, is approximately 0.30 for dP/dt_{max} and 0.20 for PDE inhibition.

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Registry No. **1**, 109890-30-4; **2**, 84243-59-4; **3**, 86798-81-4; **4**, 86798-94-9; **5**, 97150-60-2; **6**, 97150-56-6; **7**, 97150-63-5; **8**, 88427-72-9; **9**, 97150-64-6; **10**, 86798-59-6; **11**, 86798-76-7; **12**, 88427-75-2; **13**, 97150-59-9; **14**, 104271-56-9; **15**, 109890-13-3; **16**, 97150-66-8; **17**, 97150-67-9; **18**, 97150-65-7; **19**, 86798-78-9; **20**, 86798-87-0; **21**, 86798-61-0; **22**, 86798-62-1; **23**, 86798-63-2; **24**, 86798-54-1; **25**, 86798-79-0; **26**, 86798-88-1; **27**, 97150-62-4; **28**, 86798-56-3; **29**, 86798-69-8; **30**, 86798-77-8; **31**, 97150-57-7; **32**, 84243-58-3; **33**, 97150-61-3; **34**, 86798-71-2; **35**, 86798-72-3; **36**, 86798-73-4; **37**, 36725-21-0; **37**·HCD, 109890-14-4; **38**, 109890-15-5; **39**, 109890-16-6; **40**, 109890-17-7; **41**, 109764-50-3; **42**, 21282-90-6; **43**, 86798-60-9; **44**, 109890-18-8; **45**, 87693-29-6; **45** (isothiouronium salt), 109890-21-3; **46**, 109890-20-2; **47**, 109890-22-4; **48**, 109890-23-5; **49**, 109890-24-6; **50**, 109890-25-7; **51**, 109890-26-8; **52**, 109890-29-1; **53**, 109890-28-0; **53**·HtCP, 109890-27-9; 4-(1*H*-imidazol-1-yl)-β-methyl-γ-oxobenzenebutanoic acid, 88427-81-0; 4-(1*H*-imidazol-1-yl)-β,β-dimethyl-γ-oxobutanoic acid, 109890-12-2; *N*-[[[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)phenyl]amino]-thioxomethyl]benzamide, 109890-19-9; 2-chloroethyl isocyanate, 1943-83-5; 3,6-dichloropyridizine, 141-30-0; 3-chloro-6-(2-methyl-1*H*-imidazol-1-yl)pyridazine, 75792-66-4; ethylenediamine, 107-15-3; 2-bromoethylamine hydrobromide, 2576-47-8; benzoyl chloride, 98-88-4; chloroacetone, 78-95-5; benzimidazole, 51-17-2.

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