the binding of the drug to susceptible targets such as DNA and membrane.

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Registry No. NADH dehydrogenase, 9079-67-8.

Cardiotonic Agents. 9. Synthesis and Biological Evaluation of a Series of (*E*)-4,5-Dihydro-6-[2-[4-(1*H*-imidazol-1-yl)phenyl]ethenyl]-3(2*H*)-pyridazinones: A Novel Class of Compounds with Positive Inotropic, Antithrombotic, and Vasodilatory Activities for the Treatment of Congestive Heart Failure

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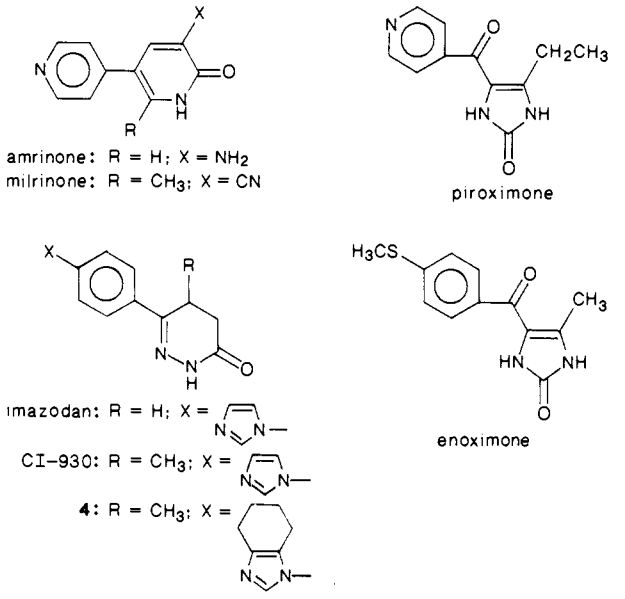
A novel series of analogues of (*E*)-4,5-dihydro-6-[2-[4-(1*H*-imidazol-1-yl)phenyl]ethenyl]-3(2*H*)-pyridazinone was synthesized as a variation on the imazodan series. The compounds were evaluated for (i) hemodynamic activity, (ii) cyclic AMP-phosphodiesterase inhibitory activity (human platelets and guinea pig heart tissue), and (iii) platelet aggregation inhibitory activity. The insertion of the ethenyl moiety between the phenyl and dihydropyridazinone rings produced novel compounds that retained the potent inotropic/vasodilator activity of the parent imazodan series and enhanced the platelet aggregation inhibitory potency. Compound **3d**, the most potent in this series, demonstrated in vivo antithrombotic activity. The synthesis and the biological activity of these new pyridazinone analogues are reported.

The extensive search to find a nonglycoside, noncatecholamine digitalis replacement led to the discovery of several new cardiotonic drugs,[1-6] including amrinone,[7,8] milrinone,[9,10] enoximone,[11,12] piroximone,[13,14] and imazodan (Chart I). The positive inotropic and vascular relaxant actions of these agents are apparently due to the selective inhibitory effects on the low k_m cyclic AMP-specific, cyclic GMP-inhibited form of phosphodiesterase (PDE IIIC) present in cardiac and vascular muscle.[15] Although clinical studies have shown that these agents provide both acute and sustained hemodynamic improvement,[16] their influence on the natural history of heart failure is unclear, and the precise role of these agents in the long-term management of heart failure remains controversial.[17] We have previously reported on imazodan and CI-930, two selective PDE IIIC inhibitors for the treatment of congestive heart failure.[18,19] The favorable clinical results seen with imazodan[20] and CI-930[21] encouraged the development of a second generation cardiotonic that would possess a greater balance of inotropic and vasodilator activity, as well as additional actions that might influence the underlying pathology and progression of congestive heart failure. From a therapeutic perspective, it may be advantageous to administer a cardiotonic agent with platelet aggregation inhibitory activity to those patients with a history of myocardial infarction and an increased risk of coronary or pulmonary thrombosis.[22-27]

Recently, the synthesis and the biological activity of lixazinone (RS-82856), a potent and selective inhibitor of the type IIIC phosphodiesterase, has been reported.[28,29] This agent, which contains major structural elements of two structurally dissimilar compounds, cilostamide and anagrelide (Chart II), exhibits potent inotropic and antithrombotic properties. In this compound the *N*-cyclohexyl-*N*-methyl-4-oxybutyramide side chain was of significant value as a steric and/or lipophilic pharmacophore

Chart I



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‡ Department of Pharmacology.

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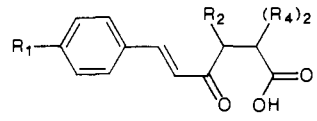
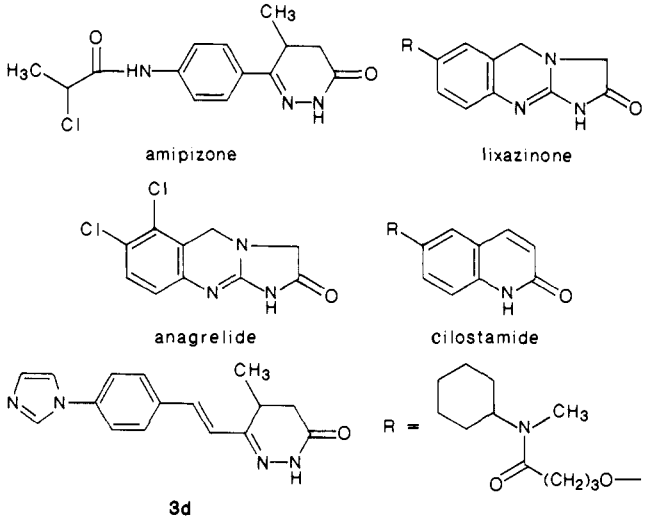
Table I. (*E*)-6-[4-(1*H*-Azol-1-yl)phenyl]-4-oxo-5-hexenoic Acids



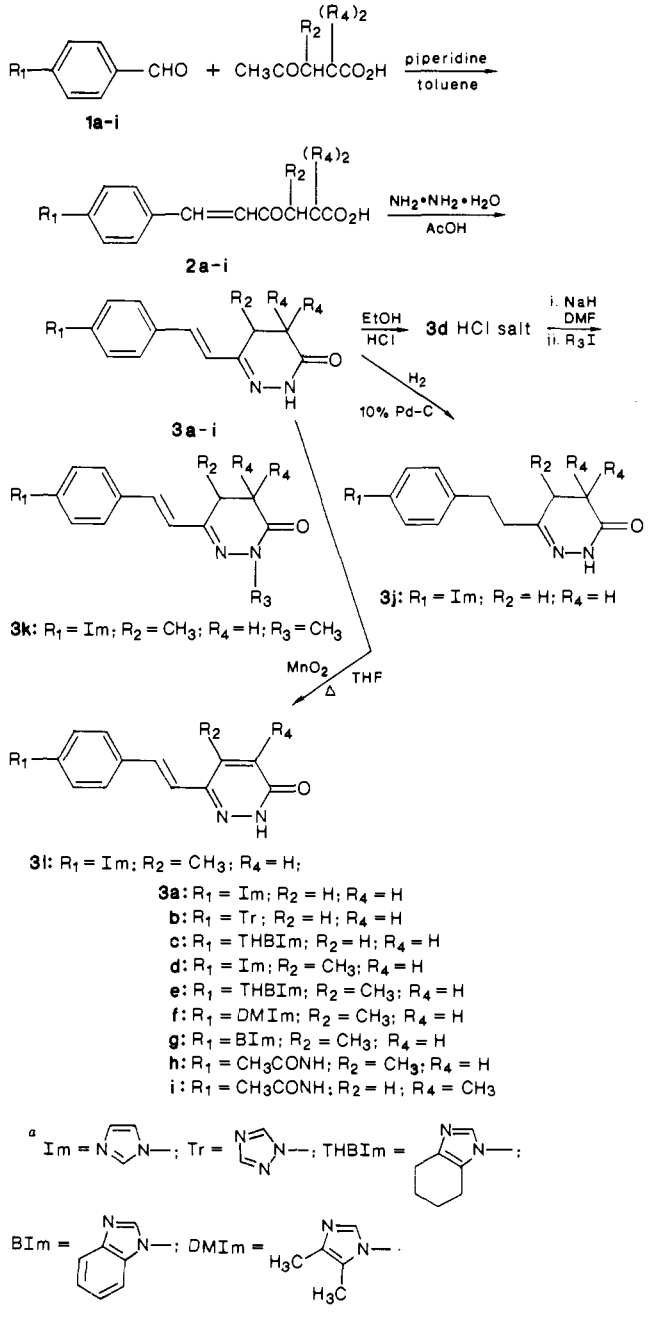
cmpd no.	R_1	R_2	R_4	mp, °C	recrystallization solvent	yield,[a] %	empirical formula
2a	Im	H	H	274–275	ethanol	55	$C_{15}H_{14}N_2O_3$
2b	Tr	H	H	215–217	methanol	20	$C_{14}H_{13}N_3O_3$
2c	THBIm	H	H	235–236	ethanol	5	$C_{19}H_{20}N_2O_3$
2d	Im	CH_3	H	199–200	2-propanol	48	$C_{16}H_{16}N_2O_3$
2e	THBIm	CH_3	H	233–234	water	7	$C_{20}H_{22}N_2O_3 \cdot 0.5H_2O$
2f	DMIm	CH_3	H	210–211	EtOAc	33	$C_{18}H_{20}N_2O_3$
2g	BIm	CH_3	H	180–181	ethanol	19	$C_{20}H_{18}N_2O_3$
2h	CH_3CONH	CH_3	H	189–190	2-propanol	52	$C_{15}H_{17}NO_4$
2i	CH_3CONH	H	CH_3	175–177	CH_3CN	33	$C_{16}H_{19}NO_4 \cdot C_5H_{11}N$[b]

[a] Yield was not optimized. [b] Isolated and analyzed as piperidine salt.

Chart II



Scheme I[a]



3a: R_1 = Im; R_2 = H; R_4 = H
b: R_1 = Tr; R_2 = H; R_4 = H
c: R_1 = THBIm; R_2 = H; R_4 = H
d: R_1 = Im; R_2 = CH_3; R_4 = H
e: R_1 = THBIm; R_2 = CH_3; R_4 = H
f: R_1 = DMIm; R_2 = CH_3; R_4 = H
g: R_1 = BIm; R_2 = CH_3; R_4 = H
h: R_1 = CH_3CONH; R_2 = CH_3; R_4 = H
i: R_1 = CH_3CONH; R_2 = H; R_4 = CH_3

[a] Im = ; Tr = ; THBIm = ;
BIm = ; DMIm = .

responsible for both inotropic and antiplatelet activity. However, no in vivo studies regarding inhibition of thrombus formation by RS-82856 have been reported to date.

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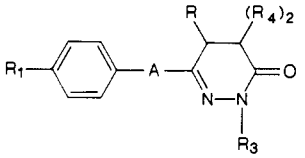
Table II. (*E*)-4,5-Dihydro-6-[2-[4-(1*H*-azol-1-yl)phenyl]ethenyl]-3(2*H*)-pyridazinones 3a–n



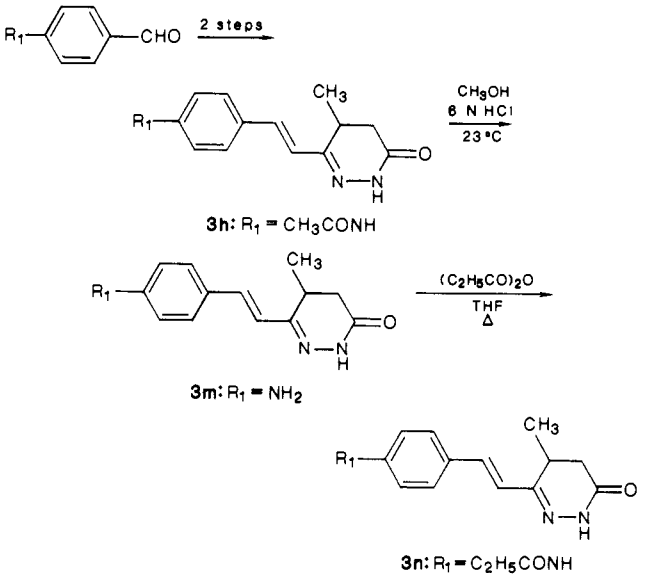
cmpd no.	R_1	A	R_2	R_3	R_4	mp, °C	recrystallization solvent	yield,[a] %	mol. form.	myocardial contractility dose (mg/kg) to cause 50% inc. in dP/dt_{max} (*n*)
3a	Im	CH=CH	H	H	H	233–234	methanol	30	$C_{15}H_{14}N_4O$	0.02 (2)
3b[b]	Tr	CH=CH	H	H	H	255–256	methanol	41	$C_{14}H_{13}N_5O$	
3c	THBIm	CH=CH	H	H	H	235–236	ethanol	45	$C_{19}H_{20}N_4O$	0.1 (2)
3d	Im	CH=CH	CH_3	H	H	179–180	2-propanol	44	$C_{16}H_{16}N_4O$	0.008 (6)
3d	Im	CH=CH	CH_3	H	H	267–269	ethanol	90	$C_{16}H_{16}N_4O$·HCl	0.008 ± 0.006 (6)
3e	THBIm	CH=CH	CH_3	H	H	246–248	2-propanol	43	$C_{20}H_{22}N_4O$	0.05 (2)
3f	DMIm	CH=CH	CH_3	H	H	227–228	ethanol	59	$C_{18}H_{20}N_4O$	0.05 (2)
3g	BIm	CH=CH	CH_3	H	H	219–220	THF-CH_3CN	50	$C_{20}H_{18}N_4O$	0.09 (3)
3h	CH_3CONH	CH=CH	CH_3	H	H	206–207	ethanol	40	$C_{15}H_{17}N_3O_2$	0.02 (2)
3i	CH_3CONH	CH=CH	H	H	$(CH_3)_2$	255–257	ethanol	42	$C_{16}H_{19}N_3O_2$	1.0 (1)
3j	Im	CH_2CH_2	H	H	H	195–196	ethanol	77	$C_{15}H_{16}N_4O$	1.0 (2)
3k	Im	CH=CH	CH_3	CH_3	H	126–127	ether/EtOAc	50	$C_{17}H_{18}N_4O$	1.0 (3)
3l[c]	Im	CH=CH	CH_3	H	H	239–240	ethanol	15	$C_{16}H_{14}N_4O$	0.2 (2)
3m	H_2N	CH=CH	CH_3	H	H	202–203	ethanol	46	$C_{13}H_{15}N_3O$	1.0 (2)
3n	C_2H_5CONH	CH=CH	CH_3	H	H	235–236	ethanol	53	$C_{16}H_{19}N_3O_2$	0.2

[a] Yield was not optimized. [b] Insoluble for iv administration. [c] 3l is the pyridazinone derivative.

Increase in lipophilicity in the imazodan series was achieved by insertion of an ethenyl bridge spacer between the aryl (B) and pyridazinone (A) rings. This modification also altered the distance between the two important binding sites, namely, the carbonyl of the A ring and the hydrogen accepting moiety of the heterocycle (C) ring. In the present study we report on this new series of positive inotropic/vasodilatory compounds with potent in vitro antiplatelet activity. Additionally, the results of the in-depth pharmacological studies including the in vivo antithrombotic activity of 4,5-dihydro-6-[2-[4-(1*H*-imidazol-1-yl)phenyl]ethenyl]-5-methyl-3(2*H*)-pyridazinone (**3d**), the best compound in the series, are also the subject of this article.

Chemistry. The 4,5-dihydro-3(2*H*)-pyridazinones (**3a–i**, Table II) were prepared by reaction of the requisite acids (**2a–i**, Table I) with hydrazine hydrate in acetic acid. The acids, in turn, were prepared by condensation of the corresponding aldehydes (**1a–i**)[30] with suitably substituted levulinic acids[31] in the presence of piperidine in refluxing toluene[32] (Scheme I). Oxidation of **3d** with MnO_2 provided the corresponding pyridazinone **3l**. Alkylation of **3d** was affected with NaH and methyl iodide to give **3k**.

Scheme II



The syntheses of **3m** and **3n** were accomplished from **3h** as shown in Scheme II. The acylamino moiety in **3h** was hydrolyzed with aqueous methanolic hydrochloride acid at room temperature to provide the corresponding amine **3m**, which was converted to the propionamide analogue **3n** upon treatment with propionic anhydride under reflux.

Biological Results and Discussions

Positive Inotropic Activity. The hemodynamic responses to compounds in Table II were evaluated following intravenous administration in acutely instrumented anesthetized dogs and following oral administration in the conscious dog as described in the Experimental Section. Heart rate, myocardial contractility (dP/dt_{max}), and aortic blood pressure were recorded. Intravenous dose–response measurements were determined with at least four doses of each compound.

The dose of each compound required to increase myocardial contractility by 50% (ED_{50}) is shown in Table II. Compound **3a** produced a substantial increase in myo-

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Table III. log P Values of 4,5-Dihydro-6-[4-(1H-azol-1-yl)phenyl]-3(2H)-pyridazinones[a]

cmpd	log P	cmpd	log P
imazodan	0.96	**3d**	1.71
CI-930	1.29	**3e**	3.10
4	2.52	**3h**	1.27
3a	1.21	amipizone	1.40
3c	2.47		

[a] Determined by high-pressure liquid chromatography (pH 7.4).[40]

cardial contractility and was twice as potent as imazodan (ED_{50} values of 0.02 versus 0.045, respectively). Addition of a methyl group at the 5-position of the 4,5-dihydro-3-(2H)-pyridazinone (DHPZ) ring (**3d**) enhanced the inotropic potency, but the effect was less significant than that in the imazodan and amrinone series. As suggested earlier,[33] hydrophobic and topological effects of the methyl substituent seem to play a major role in altering inotropic potency. In the imazodan series the 5-pseudoaxial methyl substituent was accommodated to reinforce binding without disturbing the interaction of other groups. By contrast, in the present series the methyl group would be expected to force rotation of the pyridazinone ring about its main axis, and in the absence of any compensating bond rotations in the molecule, the purine-mimicking group would be displaced from its optimal binding position, resulting in a loss of potency compared to the imazodan series.

The structure–activity relationships of this series seem to retain several key features of the parent series (e.g., imazodan), although several differences exist. Most of the modifications reduced inotropic potency, however. Reduction of the ethenyl group to the ethyl (**3j**) as well as oxidation of the DHPZ ring to the pyridazinone (**3l**) caused a 10-fold reduction in potency. Replacement of the acidic hydrogen from the amide moiety (e.g., N*H*CO) produced compound **3k** with substantial loss in inotropic potency. Substitution on the imidazole ring decreased inotropic potency 10-fold; for example, compare IC_{50} of **3d** (0.008) with **3e–q** (0.05–0.09). It is worth mentioning, however, that the 4,5,6,7-tetrahydrobenzimidazole analogue of CI-930 (**4**) was the most potent inotrope found in the series.[34] It was reported earlier that the imidazole ring could be replaced successfully with an acetylamino moiety (CH_3CONH) with the retention or enhancement of activity.[35] A reversal in the SAR was noted in this series, however (ED_{50}s of 0.02 mg/kg for **3h** and 0.008 mg/kg for **3d**). Increasing the size of the acyl group from acetyl (**3h**) to propionyl (**3n**) caused a further reduction of potency. Lipophilicity was also found to play a role in distinguishing among analogues in these two pyridazinone series. In the imazodan series the most lipophilic compound was the most potent. In contrast, in the ethenyl series analogues of increasing lipophilicity (**3a**, **3h**, **3d**, **3c**, and **3e**), an actual maximum of potency was observed with **3d**, with compounds both more and less polar being less potent (Table III). This correlation is significant since it defines an optimal lipophilic window for access and/or binding to the enzyme.

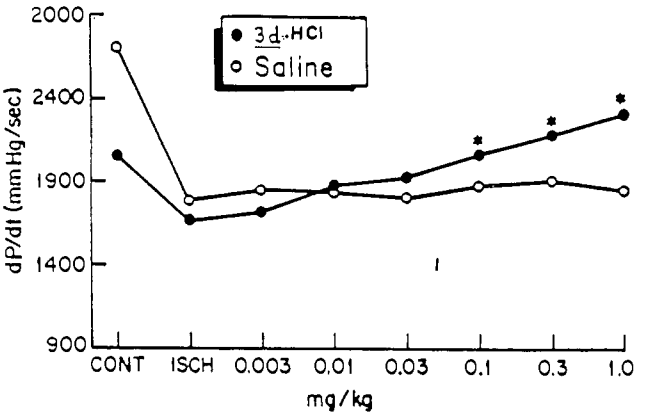

Figure 1. Effect of **3d**·HCl and saline on left ventricular contractility (dP/dt) following acute ischemia-induced heart failure in anesthetized dogs (n = 6). Asterisk indicates $p < 0.05$.

On the basis of the initial evaluation compound **3d** was selected for in-depth study. The hemodynamic activity of the hydrochloride salt of **3d** was evaluated in acutely instrumented anesthetized dogs, by measuring dP/dt_{max} of left ventricular pressure, heart rate (HR), and aortic blood pressure (BP). Compound **3d** infused over 1 min in doses ranging from 1 to 300 μg/kg produced increases in contractility of 2 ± 3% to 125 ± 37%. These increases were accompanied by increases in HR of 0.2 ± 2.0% to 33 ± 7% (Table IV). Minor changes in BP were noted at doses of 1 and 3 μg/kg, while decreases of 13 ± 2% to 40 ± 2% occurred at doses of 10 to 300 μg/kg. Dose-related decreases in forelimb vascular resistance (FVR) ranging from 0.3 ± 4% to −54 ± 2% were also observed at doses between 1 and 300 μg/kg. Table V summarizes the comparative inotropic dose–response relationships for **3d**, CI-930, imazodan, amrinone, milrinone, enoximone, and piroximone. These data indicate that the inotropic profile of **3d** is very similar to other reference agents although slight enhancement of potency was achieved in comparison to CI-930.

The hemodynamic response to compound **3d** was also evaluated before and 15 min after β-adrenoceptor blockade with nadolol. The effects of **3d** (0.01 mg/kg) on contractility, HR, BP, and FVR were not significantly reduced by nadolol (Table VI). These results indicate a nonadrenergic inotropic mechanism of action for **3d**.

The efficacy of **3d** for its ability to reverse heart failure was studied in a model of acute ischemia-induced myocardial depression. Cardiac depression was produced in anesthetized dogs by total occlusion of the left anterior descending coronary artery and partial occlusion of the circumflex coronary artery. Indices of failure were an increase in left ventricular end diastolic pressure (LVEDP) of approximately 10 mmHg and a decrease in cardiac output (CO) of approximately 30%. Animals received **3d** (3–1000 μg/kg) or saline (time and volume control) as iv boluses. The effects of **3d** and saline on myocardial contractility are summarized in Figure 1. Compound **3d** produced dose-dependent increases in left ventricular contractility at doses of 0.1, 0.3, and 1.0 mg/kg ($p < 0.05$). Inotropic activity at the 1.0 mg/kg dose exceeded the preischemic level by 13%. Saline treatment had no effect on myocardial contractility. This study illustrates that **3d** reversed acute myocardial depression induced by ischemia.

The hemodynamic profile of orally administered drug **3d** was evaluated in chronically instrumented conscious dogs. A dose of 0.3 mg/kg increased myocardial contractility by 104 ± 23% (n = 5) 45 min after dosing. At this time HR was increased by 75 ± 22%, while BP was

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Table IV. Effect of **3d**·HCl on Hemodynamic Parameters in Anesthetized Dogs ($n = 6$)[a]

dose, mg/kg	LV myocardial contractility	heart rate	blood pressure	forelimb resistance
		base line		
	2271 ± 160 mmHg/s	152 ± 7 bpm	123 ± 3 mmHg	2.77 ± 0.52 mmHg/mL per min
		% change from base line		
0.001	2 ± 3	0.2 ± 2	−2 ± 2	0.3 ± 4
0.003	19 ± 3[b]	5 ± 3	−5 ± 1[b]	−15 ± 9
0.01	65 ± 9[b]	12 ± 3[b]	−13 ± 2[b]	−36 ± 3[b]
0.03	117 ± 23[b]	23 ± 4[b]	−24 ± 3[b]	−49 ± 2[b]
0.10	134 ± 27[b]	28 ± 4[b]	−35 ± 2[b]	−58 ± 3[b]
0.30	125 ± 37[b]	33 ± 7[b]	−40 ± 2[b]	−54 ± 2[b]

[a] Values are maximum response from control average ± SEM. [b] Significant at 0.05 compared to control.

Table V. Effects of **3d**·HCl, CI-930, Imazodan, Milrinone, Piroximone, Enoximone, and Amrinone on Myocardial Contractility in Anesthetized Dogs

	% change, contractility (dP/dt_{max})[a] dose, mg/kg, iv							
cmpd (n)	0.001	0.003	0.01	0.03	0.1	0.3	1.0	3.1
3d·HCl (6)	2 ± 3	19 ± 3	65 ± 9[b]	117 ± 23[b]	134 ± 27[b]			
CI-930 (6)	11 ± 9	25 ± 4[b]	51 ± 11[b]	124 ± 30[b]	149 ± 23[b]			
imazodan (6)			10 ± 1[b]	37 ± 8[b]	74 ± 13[b]	127 ± 14[b]	147 ± 25[b]	
milrinone (6)			31 ± 10	78 ± 23[b]	113 ± 29[b]	143 ± 24[b]	149 ± 20[b]	
piroximone (4)			8 ± 1	16 ± 4	41 ± 6	93 ± 15	144 ± 20[b]	
enoximmone (4)			5 ± 1	7 ± 1	19 ± 1	47 ± 4	83 ± 6	
amrinone (6)					9 ± 1	32 ± 4	85 ± 8	165 ± 18

[a] Values are maximum response from control average ± SEM. [b] Significant at 0.05 compared to control.

Table VI. Cardiovascular Activity of **3d**·HCl in the Presence of β-Adrenoceptor Blockade in Anesthetized Dogs ($n = 4$)[a]

	LV dP/dt_{max}	heart rate	blood pressure, S/D[b]	forelimb vascular resistance
		base line		
	2327 ± 123 mmHg/s	136 ± 14 bpm	116 ± 6/94 ± 5 mmHg	4.71 ± 0.62 mmHg/mL per min
		% change from base line		
3d·HCl (0.01 mg/kg)	56 ± 16[d]	18 ± 8	−9 ± 2/−11 ± 2[d]	−19 ± 3[d]
		base line		
nadolol (1 mg/kg iv)	1869 ± 59 mmHg/s	121 ± 8 bpm	109 ± 4/88 ± 4 mmHg	5.23 ± 1.13 mmHg/mL per min
		% change from base line		
3d·HCl[c] (0.01 mg/kg)	25 ± 6[d]	6 ± 2	−10 ± 4/−13 ± 4	−13 ± 3[d]

[a] Values are mean ± SEM. [b] S/D = systolic/diastolic. [c] 15 min following nadolol administration. [d] Significantly different from base line, $p < 0.05$.

reduced by 22 ± 4%. The duration of inotropic and vasodilator activity was greater than 4 h, which is very similar to that of CI-930 reported earlier.[19]

Vasodilator Activity. The direct vasodilator activity of **3d** was quantitated in the anesthetized dog by using an isolated forelimb perfusion technique. Compound **3d**, as well as imazodan, CI-930, amrinone, milrinone, and enoximone, was administered locally in the bronchial artery perfused at constant flow, and forelimb perfusion pressure was recorded continuously. At constant blood flow, changes in perfusion pressure are directly proportional to changes in vascular resistance. Compound **3d**, imazodan, CI-930, amrinone, milrinone, and enoximone all produced dose-dependent decreases in forelimb perfusion pressure, with a maximum reduction of about 35–40%. Doses that produced a 20% reduction (ED_{20}) in forelimb perfusion pressure for **3d**, CI-930, imazodan, milrinone, enoximone, and amrinone were 2.3, 2.24, 14.8, 76.8, 86, and 748 μg, respectively. Thus, **3d** was the most potent vasodilator of the group, being roughly 44 times more potent than milrinone on a molar basis. Early reports indicate RS 82856 was only 18 times more potent than milrinone.[28]

As pointed out above, **3d** is also one of the most potent inotropic agents in this class. Table VII summarizes the ratio of positive inotropic activity (ED_{20}, left ventricular dP/dt_{max}) to direct vasodilator activity (ED_{20}, forelimb perfusion pressure) for several of the agents studied. The ratio of 1.29 for **3d** indicates that this agent has a greater balance of vasodilator and inotropic activities than does imazodan (1.05), CI-930 (0.88), amrinone (0.23), milrinone (0.05), or enoximone (0.58). Thus, the ethenyl modification in the imazodan series resulted in a significant enhancement in vasodilator potency.

Table VII. Ratio of Positive Inotropic ED_{20}[a] (PI) to Peripheral Vasodilation ED_{20}[b] (PV) of **3d** and Reference Cardiotonic Agents

cmpd	PI/PV
3d	1.29
imazodan	1.05
CI-930	0.88
enoximone	0.58
amrinone	0.23
milrinone	0.05

[a] Doses produced a 20% increase in $dPdt_{max}$ were calculated from an iv bolus dose–response curve in anesthetized dogs. [b] Doses produced a 20% decrease in forelimb perfusion pressure calculated from dose–response curve.

Antithrombotic Activity: In Vitro Human Platelet Aggregation. Compounds **3d** and several analogues were evaluated for platelet aggregation inhibitory activity in vitro using human thrombocyte-enriched plasma and hu-

Table VIII. Effects of 3d and Reference Cardiotonic Agents on Platelet Aggregation Inhibition in Vitro

cmpd	IC_{50}, M[a] human platelet-rich plasma ADP	human platelet-rich plasma collagen	human whole blood ADP	human whole blood collagen
3a	6×10^{-6}	2×10^{-7}		
3c	5×10^{-6}	4.5×10^{-7}	1.7×10^{-6}	1.0×10^{-6}
3d	3×10^{-7}	3×10^{-8}	9×10^{-8}	1.5×10^{-9}
3e	6×10^{-6}	6×10^{-7}		
3i	4×10^{-6}	2.5×10^{-7}		
3f	3×10^{-5}	1.5×10^{-6}		
4	4×10^{-6}	1.5×10^{-8}	5.0×10^{-7}	7.5×10^{-7}
CI-930	4×10^{-6}	1×10^{-8}	4×10^{-7}	4.5×10^{-5}
amipizone	3.5×10^{-7}	1.5×10^{-7}	1×10^{-8}	4×10^{-7}
amrinone	2.5×10^{-4}	1.5×10^{-5}	5×10^{-5}	5×10^{-4}
enoximone	1.2×10^{-4}	1.5×10^{-5}		
imazodan	2×10^{-5}	3×10^{-6}	8×10^{-6}	1.7×10^{-5}
milrinone	1×10^{-5}	1×10^{-7}	9×10^{-6}	3.5×10^{-7}
piroximone	6×10^{-5}	1.5×10^{-5}		

[a] Values were obtained from concentration curves generated by using 2–4 replications at 10^{-4} to 10^{-9} M of drug. See Experimental Section for details.

Table IX. Inotropic and Antithrombotic Activity of 3d·HCl and Amipizone in Anesthetized Dogs

cmpd	dose, μg/kg per min (n)	thrombus wt, mg	% inhibn thrombus wt	% increase dP/dt
3d-HCl	1 (6)	10.7[b]	40 ± 13	19 ± 6
	3 (6)	6.3[b]	65 ± 6	77 ± 11
	10 (6)	10.0	44 ± 11	103 ± 12
amipizone	0.3 (4)	8.3[b]	54 ± 8	3 ± 1
	1 (2)	4.8[b]	73 ± 10	10 ± 4
	3 (4)	4.1[b]	77 ± 15	23 ± 6
saline	(8)	17.9	0	0

[a] Mean ± SEM. [b] $p < 0.05$ vs saline.

Table X. Inhibition of Phosphodiesterase, Fraction III, by 3d and Analogues and Reference Agents

cmpd	IC_{50},[a] μM (lower and upper limit) guinea pig hearts	IC_{50},[a] μM (lower and upper limit) human platelets	selectivity index, IC_{50}, cardiac/platelets
3a	1.1	0.4	2.75
3d	0.25 (0.20–0.33)	0.077 (0.05–0.10)	3.24
3e	0.1 (0.08–0.22)	0.018 (0.014–0.022)	1.55
3h	2.8 (2.12–3.48)	0.87 (0.78–0.99)	3.21
3f[b]	(53)	(87)	
imazodan	8	3.4	2.3
CI-930	0.6	0.4	1.5
4	0.15	0.11	1.4
amrinone	50	16	3.1
milrinone	2.5	1.1	2.2

[a] IC_{50} values were determined by measuring the inhibitory effects of each agent over a concentration range of 1.0×10^{-7} to 1.0×10^{-4} M or 1.0×10^{-6} to 1.0×10^{-3} M for the less potent agents. Each value represents the mean of 2 to 4 experiments by using different preparations of phosphodiesterases and were calculated from the dose–response curve.[34] [b] Values indicate the percent inhibition at 10^{-5} M.

man whole blood. Summarized in Table VIII are the in vitro platelet aggregation IC_{50} values for **3d** and several other phosphodiesterase inhibitory cardiotonics, imazodan, CI-930, milrinone, amrinone, enoximone, and piroximone. Amipizone was included in the study because of its structural similarity with these agents and also its reported antiplatelet activity.[36,37] As Table VIII shows **3d** is a potent platelet aggregation inhibitory agent, with in vitro IC_{50} values of 0.03 μM and 0.3 μM against collagen-stimulated and ADP-stimulated aggregation, respectively. These results show that **3d** is 100 to 1000 times more potent as an inhibitor of platelet aggregation than are the reference PDE inhibitor cardiotonics amrinone and milrinone and roughly equipotent to RS 82856 (IC_{50} = 0.11 μM).[29]

Antithrombotic Activity: In Vivo Activity in Anesthetized Dogs. The in vivo antithrombotic activity of **3d** was evaluated in an anesthetized dog coronary artery thrombosis model using decreases in thrombus weight as an indicator of efficacy.[24] Circumflex coronary artery thrombosis was induced by endothelial denudation by an intraluminal silver-coated copper electrode; 50 μA of current applied to the electrode resulted in endothelial damage. Martorana et al.[24] has suggested that in this model collagen acts as a primary proaggregatory stimulus.

Each animal received a single dose of **3d** (1.0, 3.0, or 10.0 μg/kg/min) or saline. Neither amrinone or milrinone was tested in vivo due to poor in vitro platelet aggregation inhibitory activity. Each dose of **3d** was administered for 60 min, after which the animals were euthanized and the thrombus dissected from the vessel and weighed. Table IX summarizes the effects of **3d**, amipizone, and saline on thrombus weight and left ventricular contractility. These studies illustrate that **3d** possesss a potent antithrombotic activity at doses which exert substantial positive inotropic effects. In contrast, amipizone, while having antithrombotic activity, had little inotropic effect. The combination of platelet antiaggregatory activity of **3d** in addition to inotropic and vasodilatory activities suggests that **3d** may be potentially beneficial in patients with congestive heart failure in danger of coronary thrombosis.

Mechanism of Action

Selective Inhibition of Cardiac and Platelet Phosphodiesterase (PDE, Type III). The effects of **3d** and several analogues on the different molecular forms of phosphodiesterase were studied by using guinea pig cardiac tissue, and IC_{50} values are reported in Table X. Compound **3d** was a potent inhibitor of the cyclic AMP-specific, cyclic GMP-inhibited subclass of phosphodiesterase exhibiting an IC_{50} value of 0.25 μM (Table X). Compound **3d** exerted minimal inhibitory effects on the other subclasses of phosphodiesterase (IC_{50}, > 1000 μM). The tetrahydrobenzimidazole analogue **3e** was the most potent inhibitor in this series with an IC_{50} value of 0.11 μM. Similar structure–activity relationships were observed in the imazodan series.[35] These results show that the insertion of the ethenyl moiety in the imazodan series en-

(36) Thyes, M.; Legmann, H. D.; Gries, J.; Konig.; Kretzschmar, R.; Kunze, J.; Lebkucher, R.; Lenke, D. *J. Med. Chem.* **1983**, *26*, 800.
(37) Original monograph, *Drugs Future* **1982**, *7*, 714.

hanced the PDE inhibitory potency.

Compound **3d** was also a potent selective inhibitor of the type III phosphodiesterase in human platelets, with an IC_{50} value of 0.077 μM (Table X), which is 70-fold higher than the reported IC_{50} value of RS-82856 (0.001 μM).[29] These results indicate a stronger correlation between inhibition of cAMP-PDE and platelet aggregation for **3d** than RS-82856. As Table X shows, compound **3d** and the other agents examined exerted a more potent inhibitory effect on the type III phosphodiesterase in platelets than in cardiac muscle.

Inhibition of the type III phosphodiesterase in cardiac muscle and platelets, and a resultant increase in intracellular cyclic AMP, is presumed to be the primary mechanism responsible for the inotropic and platelet antiaggregatory activity of **3d** and its analogues. This agent inhibited platelet aggregation and type III phosphodiesterase activity at roughly the same concentration, suggesting that compound **3d** penetrates the membrane very well.[38]

Conclusion. In conclusion, a new series of novel cardiotonic agents that was designed and synthesized as a minor modification of our earlier leads and which possesses direct inotropic, peripheral vasodilatory actions and in vitro antiplatelet actions has been described. The attachment of the lipophilic pharmacophore in the imazodan series resulted in a significant enhancement in the vasodilator and antiplatelet activity potency although the inotropic potency enhancement was not observed across the series. More importantly, however, is the fact that in general this modification improved both cardiac and platelet cAMP-PDE inhibitory potency. In addition, one of these agents, **3d**, also possesses potent in vivo platelet aggregation inhibitory activity. This agent demonstrated an overall potency advantage over CI-930 and other reference agents although the hemodynamic profile was very similar. The in vitro cAMP-PDE profile, both cardiac and platelet, and inhibition of ADP-induced platelet aggregation activity of **3d** and RS-82856 were comparable. Studies indicate that the compound **3d** acts by increasing the cAMP levels in cardiac and platelet tissue via selective inhibition of the cyclic AMP-specific cyclic GMP-inhibited form of phosphodiesterase (type IIIC PDE). The added feature of platelet antiaggregatory activity is considered to be beneficial for patients with congestive heart failure who are at risk of coronary thrombosis.

Experimental Section

Melting points were 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 with methylene chloride and methanol (9:1) as eluants. Elemental analyses were within 0.4% of theoretical values unless otherwise stated. 4,5-Dimethylimidazole was prepared by following the procedure of Brederick et al.[39]

General Procedure for the Synthesis of (*E*)-6-[4-(1*H*-Azol-1-yl)phenyl]-4-oxo-5-hexenoic Acids (Table I). (*E*)-6-[4-(1*H*-Imidazol-1-yl)phenyl]-3-methyl-4-oxo-5-hexenoic Acid (2d). A mixture of 9.5 g (55 mmol) of 4-(1*H*-imidazol-1-yl)benzaldehyde, 6.4 g (55 mmol) of β-methyllevulinic acid and 3 mL of piperidine in 150 mL of toluene was heated at reflux with a Dean–Stark apparatus until the theoretical amount of water had collected (ca. 6 h). The reaction mixture was cooled and the supernatant toluene was decanted, leaving behind a red oil. The oil was triturated with an excess of hot 2-propanol and the solution was concentrated to ca. 25 mL and allowed to cool. The solid was filtered, washed successively with cold 2-propanol followed by ether, and air-dried to give 8 g of the title compound, mp 199–200 °C. Recrystallization from 2-propanol provided 6.3 g of analytically pure material.

General Procedure for the Synthesis of 4,5-Dihydro-6-[2-[4-(1*H*-azol-1-yl)phenyl]ethenyl]-3(2*H*)-pyridazinone (3a–i, Table II). (*E*)-4,5-Dihydro-6-[2-[4-(1*H*-imidazol-1-yl)phenyl]ethenyl]-5-methyl-3(2*H*)-pyridazinone (3d). A solution of 1.2 g of **2d** in 20 mL of glacial acetic acid containing 0.3 g of hydrazine hydrate was heated at reflux for 30 h when TLC (SiO_2, $CHCl_3$/EtOH 4:1) indicated nearly complete conversion of the acid **2d** to the product. Acetic acid was removed by distillation under vacuum, the residue was taken up with cold water, and the solution was adjusted to pH 6.0. The precipitate was filtered, washed with cold water, and air-dried to give 0.6 g of the title compound, mp 178–179 °C. Recrystallization from 2-propanol gave 0.4 of the analytically pure material, mp 179–180 °C.

Ethanolic HCl was added to a hot solution of the base (**3d**, 6 g) in EtOH (75 mL) and the solution was allowed to stand overnight at room temperature. The crystallized material was filtered, and the residue was washed successively with cold ethanol followed by ether and air-dried to give 5.2 g of the monohydrochloride **3d**·HCl, mp 267–269 °C.

4,5-Dihydro-6-[2-[4-(1*H*-imidazol-1-yl)phenyl]ethyl]-3-(2*H*)-pyridazinone (3j). A solution of 2.6 g of **3a** in 100 mL of 2-methoxyethanol containing 0.5 g of 10% Pd/C was shaken under a hydrogen atmosphere for 13 h. The catalyst was filtered, the filtrate was evaporated to dryness, and the residue was crystallized from ethanol to give 2 g of the above product, mp 195–196 °C.

(*E*)-6-[2-[4-(1*H*-Imidazol-1-yl)phenyl]ethenyl]-5-methyl-3(2*H*)-pyridazinone (3l). A mixture of 1.3 g of **3d** and 4.8 g (MnO_2) in 75 mL of dry THF was heated at reflux with stirring for 18 h. The reaction mixture was filtered and the residue was washed thoroughly with hot THF. The filtrate and the washings were combined and evaporated to a light brown gum that was crystallized from ethanol to provide 0.22 g of the desired product **3l**, mp 239–240 °C.

(*E*)-4,5-Dihydro-6-[2-[4-(1*H*-imidazol-1-yl)phenyl]-ethenyl]-2,5-dimethyl-3(2*H*)-pyridazinone (3k). To a slurry of 0.2 g NaH (60% oil suspensions, 4.5 mmol) in 10 mL of DMF was added a solution of 1.15 g (4.1 mmol) of **3d** in 10 mL of DMF, and the reaction mixture was stirred at 23 °C until the evolution of hydrogen ceased (ca. 15 min). A solution of iodomethane (0.7 g, 4.5 mmol) in 5 mL of DMF was added, and the reaction mixture was allowed to stir for 1 h at room temperature. DMF was distilled under vacuum, the residue was treated with water, and the solution was extracted with EtOAc. The organic extract was washed with brine, dried ($MgSO_4$), and evaporated to yield a gum, which failed to crystallize. This material was purified by chromatography (SiO_2, ether) to yield 0.8 g of the product, which was recrystallized from ether/EtOAc (2:1) to provide 0.55 g of the title compound **3k**, mp 126–127 °C.

(*E*)-4,5-Dihydro-6-[2-(4-aminophenyl)ethenyl]-5-methyl-3(2*H*)-pyridazinone (3m). A solution of 1.5 g of **3h** in a mixture of 20 mL of CH_3OH and 20 mL of 6 N HCl was allowed to stand 48 h at room temperature when TLC (SiO_2, $CHCl_3$/EtOH 10:1) showed the absence of the starting material. The solution was concentrated, the residue was diluted with water, and the neutral organic material, if any, was extracted with ether. The aqueous layer was made basic with $KHCO_3$ and extracted with EtOAc. The organic extract was washed with brine, dried (Na_2SO_4), and stripped, and the residue was purified by crystallization from EtOH to give 0.6 g of the title product **3m**, mp 202–203 °C.

***N*-[4-[2-(1,4,5,6-Tetrahydro-4-methyl-6-oxo-3-pyridazinyl)ethenyl]phenyl]propionamide (3n).** A mixture of 0.3 g of **3m** and 0.2 g of propionic anhydride in 20 mL of dry THF was heated at reflux for 3 h. The solution was evaporated, and the residue was crystallized from EtOH to yield 0.2 g of the desired product **3n**, mp 235–236 °C.

Pharmacological Methods. Anesthetized Dog Model. Six adult mongrel dogs of either sex were anesthetized with pentobarbital, 35 mg/kg, iv, and were subsequently maintained under

(38) Mikashima, H.; Nakao, T.; Goto, K.; Ochi, H.; Yasuda, H.; Tsumagari, T. *Thrombosis Res.* **1984**, *35*, 589–594.
(39) Bredereck, H.; Theilig, G. *Chem. Ber.* **1953**, *86*, 88.
(40) Haky, J. E.; Young, A. M. *J. Liq. Chromatogr.* **1984**, *7*, 675.

anesthesia with a continuous infusion of pentobarbital, 3.5 mg/kg/h. The trachea was intubated, and the animals were permitted to breathe spontaneously. A cannula was inserted into the femoral vein for administering test agents. A Miller catheter tip pressure transducer (Model PC-350) 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).

Left ventricular and aortic blood pressures were recorded on a strip chart recorder. Heart rate, 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 (0.1 or 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 mL/kg over a period of 1 min unless otherwise designated. The test agents were administered in an ascending dose manner. Usually, half-log intervals were maintained between doses, with typical dosing consisting of four to six doses (e.g., 0.01, 0.03, 0.1, 0.3, 1.0 mg/kg). A 10-min interval was used between doses. 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.

Separate groups of dogs, prepared as above, were used for evaluating the effect of β-adrenoceptor blockade on the cardiovascular actions of test agents. Compound **3d** at 0.01 μg/kg was administered intravenously before and after nadolol, 1 mg/kg, iv. Isoproterenol, 0.31 μg/kg, was used to test for functional β-blockade.

All injections were given in a volume equal to 0.1 mL/kg. Each injection was made over a 1-min period except isoproterenol, which was injected as a bolus.

Conscious Dog Model. Five adult male mongrel dogs were prepared by surgically implanting devices for measuring ECG, aortic blood pressure, aortic blood flow, and left ventricular blood pressure. These animals were allowed to recover from surgery for at least 2 weeks prior to undergoing testing. On the day of the test, the dogs were caged and connected to appropriate interfacing for recording the indicated cardiovascular parameters on a strip chart recorder. Heart rate, aortic blood pressure, left ventricular blood pressure, and aortic blood flow were measured directly; myocardial contractility was determined by obtaining dP/dt_{max} of left ventricular pressure. Cardiac output and total peripheral resistance were derived from heart rate, aortic flow, and aortic blood pressure. The test agent was then administered by gavage to the fasted dog either as a solution or as a suspension in a single-dose or multiple-dose fashion.

Data are expressed as means ± SEM. Statistical analysis of the data was performed by using a student's t test for paired or unpaired data. The probability value, $p < 0.05$, was accepted as level of significance.

Anesthetized Dogs, Innervated Forelimb Perfusion. Ten adult mongrel dogs of either sex were anesthetized and maintained with sodium pentobarbital, 30 mg/kg iv and 3.0 mg/kg/h, and ventilated with a positive pressure respirator. Arterial blood pressure, heart rate, and left ventricular pressure were measured as described above. Each animal was heparinized with 40000 units of sodium heparin 30 min prior to right brachial artery cannulation. The brachial artery was isolated and cannulated proximal to the deep brachial artery with a polyethylene catheter that was then connected via a Sigma perfusion lamp to a catheter from the femoral artery. The forelimb was considered essentially isolated from the circulation when perfusion pressure dropped below 5 mmHg when the pump was turned off. The right forelimb was perfused at a constant flow of 80 mL/min via the pressure-independent blood pump interposed between the femoral and brachial arteries. Forelimb perfusion pressure was recorded continuously from the brachial artery cannula proximal to its entry into the brachial artery. Each dog was allowed 30 min to stabilize following surgical preparation. **3d**, 0.2–100 μg, and milrinone, 1–1000 μg, were injected as bolus doses in volumes of 0.1, 0.2, 0.4, or 0.8 mL into the brachial cannula upstream from the blood pump to assure adequate mixing.

Relationship of Positive Inotropic and Peripheral Vasodilator Activities. The ratio of the positive inotropic to vasodilator activities of **3d** and other reference agents was determined from data generated in the above experiments. For this determination, a selection of 20% change in myocardial contractility and forelimb perfusion pressure was chosen (in this case, arbitrarily defined as ED_{20}s). The linear portion of the dose–response curves for changes in myocardial contractility (dP/dt_{max}) and in perfusion pressure were analyzed by using logarithmic curve-fit analysis to determine ED_{20}s. The correlation coefficients, ED_{20}, and the ratio of positive inotropic to peripheral vasodilator activities are summarized in Table VII.

Ischemia-Induced Model of Heart Failure. Ten adult mongrel dogs of either sex were anesthetized with sodium pentobarbital (32 mg/kg, iv) with anesthesia maintained by a continuous pentobarbital infusion (3.5 mg/kg/h). The trachea was intubated and positive pressure ventilation was applied. Systolic, diastolic, and mean arterial blood pressure (MBP) were recorded continuously from a Millar Mikro-Tip transducer positioned at the arch of the aorta. Left ventricular end diastolic pressure (LVEDP), an index of contractility (dP/dt), and heart rate (HR) were derived from another Millar transducer positioned in the left ventricular chamber and recorded continuously. The lead II electrocardiogram was recorded continuously. Cardiac output (CO) was determined by the thermodilution technique. A catheter was inserted in a femoral vein for intravenous bolus drug dosing. An incision was made in the left fifth intercostal space to expose the heart. The pericardium was incised and the left anterior descending coronary artery (LAD) and the circumflex coronary arteries isolated. Two sutures (size 00) were placed loosely around the LAD. A Hartman occluder was positioned around the circumflex artery and secured to a ring stand. Saline-moistened gauze was placed over the heart and the chest incision was covered with plastic wrap to minimize heat and moisture loss. Following a 30-min stabilization period, and a 30-min control period, myocardial depression was begun with the first step of a three-stage occlusion. One of the sutures around the LAD was tied around a 22-gauge needle. The needle was immediately removed after the ligature was securely knotted to produce a partial occlusion of the LAD. Thirty minutes were allowed before the LAD was totally occluded with the remaining ligature. Thirty minutes were again allowed before starting the gradual occlusion of the circumflex coronary artery. The Hartman occluder was gradually adjusted to constrict the circumflex artery to achieve a 30% reduction in CO and an increase in LVEDP of approximately 10 mmHg. Once stable cardiac depression was obtained, an additional 30 min were allowed before dosing was started. Hemodynamic parameters were determined as the average of minutes 25–30 for each perturbation to this point. Compound **3d** (0.003–1.0 mg/kg, $n = 6$) was given in rising half-log iv bolus doses in 3 mL of saline at terminate intervals. Saline, in equal volumes, was given as the control treatment ($n = 4$) at 10-min intervals. Hemodynamic parameters were taken as the peak response in dP/dt to the bolus injections.

Isolation of Phosphodiesterases and Assay of Activity. The three molecular forms of phosphodiesterase (PDE; Type I, Type II, and Type III) present in guinea pig left ventricular tissue were discretely eluted from a DEAE column by using a sodium acetate gradient.[15] Cross contamination was eliminated by chromatography of pooled fractions of each peak. Following complete separation, the combined PDE 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 hydrolyic activity was observed with storage of up to 6 weeks).

In evaluating the inhibiting effect of the different agents examined on Type I, Type II, and Type III cardiac PDE, the enzyme concentration in the assay was adjusted to ensure that reaction velocity was linear for 30 min at 30 °C and that hydrolysis of substrate ([^{3}H]cyclic AMP or [^{3}H]cyclic GMP) did not exceed 10–20% of the available substrate in the absence of any inhibitor. The concentration of substrate was 1.0 μM for these studies. All agents examined were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the reaction medium was 2.5%. This concentration of DMSO inhibited enzyme activity

by approximately 10%. IC_{50} values (the concentration that produces 50% inhibition of substrate hydrolysis) were determined from concentration–response curves that 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 using different enzyme preparations for each concentration–response curve.

In Vitro Platelet Aggregation Using Human Platelet-Rich Plasma. Blood was collected from volunteers who had not ingested aspirin or other nonsteroidal antiinflammatory drugs within the preceding 2 weeks and had not eaten with 9 h before blood draw. Blood was collected in 4.5-mL portions in Vacutainer 6462S silicone-coated tubes containing 0.5 mL of 3.8% trisodium citrate. Usually six portions of 4.5 mL are drawn from each volunteer. The blood collected from three or four volunteers was pooled prior to centrifugation. The pooled blood was put in 50-mL polyethylene tubes and centrifuged at 80*g* (ca. 600 rpm) in an International Model K centrifuge wit 240 rotor for 20 min at room temperature. A portion (approximately two-thirds) of the supernatant platelet-rich plasma (PRP) was removed and set aside, and the remaining blood sample was recentrifuged at 1400*g* (ca. 2800 rpm) for 15 min to prepare platelet-poor plasma (PPP). The platelet content of the PRP was determined with a Coulter Thrombocounter. The PRP was adjusted to a count of 250 000 platelets per microliter using the PPP.

Test drugs were dissolved in small amounts of DMSO followed by dilution with saline (final concentration of DMSO was 1%). Other lower concentrations were prepared by serial dilution in saline.

Platelet-rich plasma adjusted to 250 000 platelets per microliter was distributed in 0.36-mL aliquots into silicone-coated cuvettes of 0.312-in. diameter. Addition of a drug solution or saline (0.02 mL) was followed by addition of aggregating agents (ADP or collagen suspension, 0.02 mL). Extent of aggregation (ADP stimulus) or rate of aggregation (collagen stimulus) was determined by using a Payton Scientific Dual Channel Aggregation Module, Model 300B. Appropriate concentrations of aggregating agents were determined by an initial brief titration.

A concentration curve was generated by using 2 to 4 replications at 10^{-4} to 10^{-9} M of drug addition in vitro to determine each IC_{50} value. For ADP-induced aggregation, height in millimeters of aggregation curves for the control (saline only) were compared to heights of curves after drug addition. For collagen-induced aggregation the major slope (longest straight line portion) of the aggregation curves for the control (saline only) and the drug-treated group were compared. In both aggregations, the heights of the curves (ADP) or the major slopes of the curves (collagen) were expressed as "percent of control". Estimate of IC_{50} values were then made after plotting these values against drug concentration on semilog paper.

Platelet Aggregation Using Human Whole Blood. Human blood samples were obtained from volunteers who had not ingested aspirin or other nonsteroidal antiinflammatory drugs within the preceding 2 weeks and had fasted for at least 9 h prior to blood draw. The blood from each volunteer was collected in either portions of 4.5-mL each Vacutainer tubes containing 0.5 mL of citrate anticoagulant. Usually bloods from three volunteers were collected and pooled.

Aggregation tests were run by using the Chronolog Whole Blood Aggregometer, Model 560VS, with 0.456-in. diameter cuvettes, P/N 367. Each test cuvette contained 0.5 mL of blood, 0.05 mL of drug solution, 0.05 mL of aggregating agent (either ADP or collagen at appropriate concentrations), and saline solution to bring the total volume to 1 mL.

The operating principle of the whole blood aggregometer involves measurement of the electrical impedance between two platinum electrodes immersed in the whole blood sample. When platelets aggregate in the whole blood aggregometer, they coat the electrodes to a greater or lesser degree, thereby impeding the current between the electrodes to an extent proportional to the amount of aggregation that has occurred 5 min after the addition of the aggregating agent. The extent of aggregation of test samples (2–6 replications) was compared to the extent of aggregation of control samples and is expressed as "percent of control". The test values as percent of control values were plotted versus the logs of the drug concentrations and the IC_{50} value was obtained by extrapolation.

In Vivo Model of Thrombosis in the Anesthetized Dog. Adult dogs were anesthetized with sodium pentobarbital (32 mg/kg, iv), the trachea intubated and respirated artificially. Anesthesia was maintained by a continuous infusion of sodium pentobarbital (3.5 mg/kg/h, iv) via a catheter in the femoral vein. The heart was expossd through a left lateral thoracotomy. The circumflex coronary artery was isolated close to its origin for a distance of approximately 5 cm. This allowed for the placement of an electromagnetic flow probe around the vessel and placement of a silver-coated copper wire between the probe and origin of the vessel.

Compound **3d** (1, 3, or 10 μg/kg per min) and amipizone (0.3, 1, or 3 μg/kg/min) were dissolved in saline and infused at a fixed rate of 0.39 mL/min. Drug concentrations (free base) of infusate were adjusted for each experiment on the basis of the animal weight. A third group of animals received saline at 0.39 mL/min. Animals received one treatment.

Hemodynamic parameters monitored include mean arterial blood pressure (BP), heart rate (HR), left ventricular dP/dt, left circumflex coronary artery blood flow (LCX flow), and cardiac output (CO). Following surgical preparation, animals were allowed 30 min to stabilize. Continuous drug infusion was started 15 min prior to the delivery of 50 μA of current to the circumflex artery to initiate intimal injury and thrombus formation. Current and drug were continuously delivered for the duration of the experiment. Animals were euthanatized by an overdose of KCl after 60 min of electrical stimulation. The heart was removed, the circumflex artery dissected, and the thrombus removed and weighed.

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