effect was evaluated by the method of Randall and Baruth.[19] Charles River male CD_1 mice weighing 20-22 g were fasted for 24 h before the test. The mice, 10 animals per dose, were given orally logarithmically graded doses of each prostaglandin. Immediately after administration, the animals were put into individual cages with blotting paper in the bottom for 1 h, and diarrhea was scored from the size of the spots of dampness produced by stools, with use of the arbitrary scale from 0 to 4 as described.[19] The ED_{50} values were calculated by the method of Miller and Tainter.[20]

Acknowledgment. We thank Dr. P. Ferrari and G. Tuan for IR and mass spectra and Dr. M. Nebuloni and his staff for elemental analyses. We are indebted to Dr. G. Tarzia and Prof. G. G. Nathansohn for helpful discussion and criticisms.

Registry No. **1a**, 61408-40-0; **1b**, 76817-33-9; (±)-**2a**, 61408-83-1; **2b**, 76831-72-6; **4a**, 61408-57-9; **4b**, 61408-58-0; **4c**, 76817-34-0; **4d**, 76817-35-1; **5e**, 103456-75-3; **5e** (dipyranyl deriv.), 103530-63-8; **5f**, 103456-76-4; **5f** (dipyranyl deriv.), 103530-64-9; **5g**, 103456-77-5; **5g** (dipyranyl deriv.), 103530-65-0; **5h**, 103456-78-6; **5h** (dipyranyl deriv.), 103530-66-1; **6e**, 103456-79-7; **6e** (dipyranyl deriv.), 76817-44-2; **6e** (9-hydroxy; 11,15-dipyranyl), 76817-48-6; **6f**, 103456-80-0; **6f** (dipyranyl deriv.), 76817-42-0; **6f** (9-hydroxy-11,15-dipyranyl deriv.), 76817-46-4; **6g**, 103456-81-1; **6g** (dipyranyl deriv.), 76817-45-3; **6g** (9-hydroxy-11,15-dipyranyl deriv.), 76817-49-7; **6h**, 103456-82-2; **6h** (dipyranyl deriv.), 76817-43-1; 6h (9-hydroxy-11,15-dipyranyl deriv.), 76817-47-5; **7e**, 61408-62-6; **7e** (11,15-dipyranyl deriv.), 103530-67-2; **7f**, 61408-61-5; **7f** (11,15-dipyranyl deriv.), 103530-68-3; **7g**, 61408-59-1; **7g** (11,15-dipyranyl deriv.), 103530-69-4; **7h**, 61408-60-4; **7h** (11,15-dipyranyl deriv.), 103530-70-7; **8e**, 61408-67-1; **8e** (dipyranyl deriv.), 103530-71-8; **8f**, 61408-68-2; **8f** (dipyranyl deriv.), 103530-72-9; **8g**, 61408-65-9; **8g** (dipyranyl deriv.), 103530-73-0; **8h**, 61408-66-0; **8h** (dipyranyl deriv.), 103530-74-1; **9e**, 76817-54-4; **9e** (dipyranyl deriv.), 76817-52-2; **9f**, 76822-56-5; **9f** (dipyranyl deriv.), 76817-50-0; **9g**, 76817-55-5; **9g** (dipyranyl deriv.), 76817-53-3; **9h**, 76817-56-6; **9h** (dipyranyl deriv.), 76817-51-1; (±)-**10**, 70908-63-3; **11a**, 103456-74-2; **11a** (*S*-amphetamine salt), 103456-83-3; **11b**, 103456-84-4; **11b** (*S*-amphetamine salt), 103456-85-5; **12a**, 103530-62-7; 2-hexanone, 591-78-6; (±)-2-hydroxy-2-methylhexanenitrile, 103456-72-0; (±)-2-hydroxy-2-methylhexanoic acid methyl ester, 103530-61-6; (±)-2-methoxy-2-methylhexanoic acid methyl ester, 103456-73-1; dimethyl methylphosphonate, 756-79-6.

(19) Randall, L. O.; Baruth, M. *Arch. Int. Pharmacodyn. Ther.* **1976**, *220*, 94.
(20) Miller, R. C.; Tainter, N. L. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261.

Dihydropyridazinone Cardiotonics: The Discovery and Inotropic Activity of 1,3-Dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one[1,2]

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The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received November 7, 1985*

We discovered that **6** (*N*-[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)phenyl]acetamide) is a potent positive inotrope in dogs, and we have prepared several lactam analogues of this agent. These included **16** (1,3-dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one), **32** (the analogous quinolin-2-one), and **37** (the analogous benzazepin-2-one). The inotropic ED_{50}'s of these compounds were 24, 3.3, and 5.2 μg/kg, respectively, after iv administration to pentobarbital-anesthetized dogs. Compound **20** (LY195115, 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one), the geminal dimethyl analogue of **16**, was 3.5-fold more potent than **16** when administered iv (ED_{50} = 6.8 μg/kg). However, the most profound effect of the geminal alkyl substitution was on oral activity. The approximate ED_{50}'s of **20** and **16** after oral administration to conscious dogs were 25 and 400 μg/kg, respectively. The increase in contractility produced by 25 μg/kg of **20** was maximally sustained in excess of 8 h. Thus, **20** is one of the most potent and long-acting oral inotropes described to date.

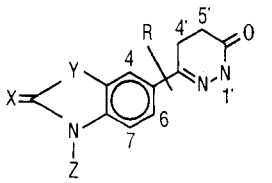
Although several new drugs for the treatment of congestive heart failure (CHF) have been introduced in recent years, many patients remain symptomatically compromised and mortality continues to be high.[3] The most salient pathophysiological features of CHF are a diminution of ventricular contractility and profound, sympathetically mediated vasoconstriction. Thus, both peripheral vasodilators[4] and positive inotropes[5] have salubrious effects in CHF patients; the hemodynamic effects of these two classes of drugs are similar.[6]

Development of a new generation of cardiotonics with combined inotropic and vasodilator activities for the chronic management of CHF has engendered considerable interest. Such agents with dual activities ameliorate the symptoms of CHF by simultaneously exerting a direct positive inotropic effect on the failing myocardium and reducing impedance to ventricular ejection. Several of these dual-activity cardiotonics, from diverse chemical

(1) Portions of this work have been presented previously: Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Pollock, G. D.; Wilson, H.; Hayes, J. S. *Abstracts of Papers*, 190th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1985. Kauffman, R. F.; Crowe, V. G.; Robertson, D. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 1049. Krushinski, J. H.; Hayes, J. S.; Beedle, E. E.; Pollock, G. D.; Wilson, H.; Robertson, D. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 810. Hayes, J. S.; Pollock, G. D.; Bowling, N.; Wilson, H.; Fletcher, M.; Robertson, D. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 1049. Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Pollock, G. D.; Wilson, H.; Hayes, J. S. 9th International Symposium on Medicinal Chemistry, Berlin (West), September 1986.
(2) A note on nomenclaure: For most of the compounds described in this paper (e.g., **6**, **7**, and **20**) the dihydropyridazinone moiety is systematically named as a 1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl substituent. Hence, a methyl substituent β to the dihydropyridazinone carbonyl is termed a 4-methyl substituent. However, some of the compounds mentioned in this paper are systematically named as 6-aryl-4,5-dihydro-3(2*H*)-pyridazinones (e.g., **38** and **39**). For these compounds a methyl substituent β to the dihydropyridazinone carbonyl is termed a 5-methyl substituent (note ref 40).
(3) *Drug Treatment of Heart Failure*; Cohn, J. N., Ed.; Yorke Medical Books: New York, 1983.
(4) Massie, B.; Ports, T.; Chatterjee, K.; Parmley, W.; Ostland, J.; O'Young, J.; Haugham, F. *Circulation* **1981**, *63*, 269.
(5) Weber, K. T. *Am. J. Med.* **1982**, *72*, 665.
(6) Taylor, S. H.; Silke, B.; Nelson, G. I. C. *Eur. Heart J.* **1982**, *3*, 19. Sonnenblick, E. H.; Mancini, D. M.; LeJemtel, T. H. *Am. J. Cardiol.* **1985**, *55*, 41A.

0022-2623/86/1829-1832$01.50/0

Table I. Structures and Properties of Dihydropyridazinone Cardiotonics

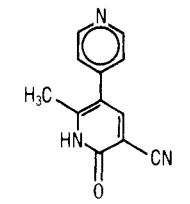


no.	X	Y	Z	R	formula	mp, °C	recrystn solvent	anal.
16	O	CH_2	H	H	$C_{12}H_{11}N_3O_2$	>300	DMF	C, H, N
17	O	CH_2	H	1′-CH_3	$C_{13}H_{13}N_3O_2$	276 dec	EtOH	C, H, N
18	O	$CH(CH_3)$	H	H	$C_{13}H_{13}N_3O_2$	>300	EtOH	C, H, N
19	O	$CH(C_2H_5)$	H	H	$C_{14}H_{15}N_3O_2$	271–2	EtOH	C, H, N
20	O	$C(CH_3)_2$	H	H	$C_{14}H_{15}N_3O_2$	>300	DMF/H_2O	C, H, N
21	O	$C(CH_3)_2$	H	4′-CH_3	$C_{15}H_{17}N_3O_2$	273–276.5	$EtOH/CHCl_3$	C, H, N
22	O	$C(CH_3)_2$	H	5′-CH_3	$C_{15}H_{17}N_3O_2$	273–4	EtOH	C, H, N
23	O	$C(CH_3)_2$	H	6-CH_3	$C_{15}H_{17}N_3O_2$	285 dec	EtOH	C, H, N
24	O	$C(CH_3)_2$	H	7-CH_3	$C_{15}H_{17}N_3O_2$	>300	EtOH	C, H, N
25	O	$C(CH_3)_2$	CH_3	H	$C_{15}H_{17}N_3O_2$	247–248	EtOH	C, H, N
26	O	$C(CH_3)_2$	CH_3	4′-CH_3	$C_{16}H_{19}N_3O_2$	225–226	EtOH	C, H, N
27	O	$C(CH_3)(C_2H_5)$	H	H	$C_{15}H_{17}N_3O_2$	260–261	EtOH	C, H, N
28	O	$C(CH_3)(C_2H_5)$	H	4′-CH_3	$C_{16}H_{19}N_3O_2$	222–224	EtOAc/MeOH/hexane	C, H, N
29	O	$C(C_2H_5)_2$	H	H	$C_{16}H_{19}N_3O_2$	221–223	EtOH	C, H, N
30	O	$C(CH_3)(n\text{-}C_3H_7)$	H	H	$C_{16}H_{19}N_3O_2$	148–150	THF/hexane	C, H, N
31	H, H	CH_2CH_2	H	H	$C_{13}H_{15}N_3O$	154–157	Et_2O/MeOH	C, H, N
32	O	CH_2CH_2	H	H	$C_{13}H_{13}N_3O_2$	>300	DMF/H_2O	C, H, N
33	O	CH_2CH_2	CH_3	H	$C_{14}H_{15}N_3O_2$	209–210	EtOH	C, H, N
34	O	CH_2CH_2	CH_3	4′-CH_3	$C_{15}H_{17}N_3O_2$	144–146	EtOAc	C, H, N
35	O	$C(CH_3)_2CH_2$[a]	H	H	$C_{15}H_{17}N_3O_2$	>300	EtOH	C, H, N
36	O	$CH_2C(CH_3)_2$[b]	H	H	$C_{15}H_{17}N_3O_2$	>300	DMF/H_2O	C, H, N
37	O	$CH_2CH_2CH_2$	H	H	$C_{14}H_{15}N_3O_2$	298–300	EtOH	C, H, N

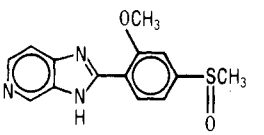
[a] Geminal dimethyl substituents are at benzylic carbon. [b] Geminal dimethyl substituents are α to carbonyl.

classes, have been described. These include milrinone (**1**),[7-9] isomazole (**2**, LY175326),[10-12] piroximone (**3**),[13-17] enoximone (**4**),[18-21] and Ro 13-6438 (**5**)[22-25] (Chart I). In addition to their well-characterized inotropic and vasodilator activities, compounds from this class of cardiotonics also inhibit platelet aggregation.[26]

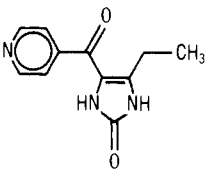
Chart I. Some Cardiotonics with Inotropic and Vasodilator Activities



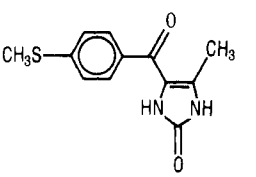
1, milrinone



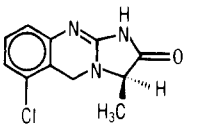
2, LY175326



3, piroximone



4, enoximone

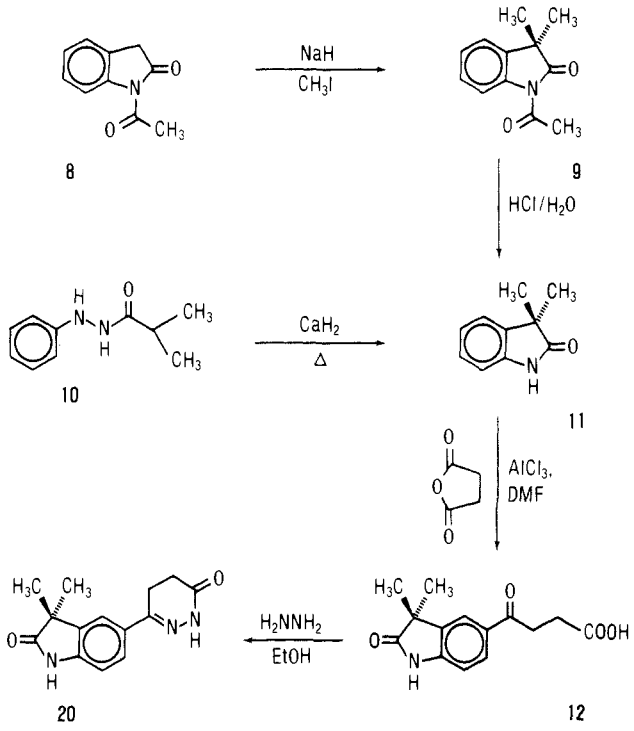


5, Ro 13-6438

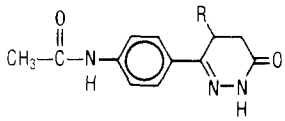
Pharmacological activities of the 4,5-dihydro-6-phenyl-3(2*H*)-pyridazinones have been actively studied

(7) Chemical name: 1,6-dihydro-2-methyl-6-oxo[3,4′-bipyridine]-5-carbonitrile.
(8) Alousi, A. A.; Stankus, G. P.; Stuart, J. C.; Walton, L. H. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 804.
(9) Jaski, B. E.; Fifer, M. A.; Wright, R. F.; Braunwald, E.; Colucci, W. S. *J. Clin. Invest.* **1985**, *75*, 643.
(10) Chemical name: 2-[2-methoxy-4-(methylsulfinyl)phenyl]-1*H*-imidazo[4,5-*c*]pyridine hydrochloride.
(11) Robertson, D. W.; Beedle, E. E.; Krushinski, J. H.; Pollock, G. D.; Wilson, H.; Wyss, V. L.; Hayes, J. S. *J. Med. Chem.* **1985**, *28*, 717.
(12) Hayes, J. S.; Pollock, G. D.; Wilson, H.; Bowling, N.; Robertson, D. W. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 318.
(13) Chemical name: 4-ethyl-1,3-dihydro-5-(4-pyridinylcarbonyl)-2*H*-imidazol-2-one.
(14) Dage, R. C.; Roebel, L. E.; Hsieh, C. P.; Woodward, J. K. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 35.
(15) Roebel, L. E.; Dage, R. C.; Cheng, H. C.; Woodward, J. K. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 43.
(16) Kariya, T.; Wille, L. J.; Dage, R. C. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 50.
(17) Petein, M.; Levine, B.; Cohn, J. N. *J. Am. Coll. Cardiol.* **1984**, *4*, 364.
(18) Chemical name: 1,3-dihydro-4-[4-(methylthio)benzoyl]-5-methyl-2*H*-imidazol-2-one.
(19) Schnettler, R. A.; Dage, R. C.; Grisar, J. M. *J. Med. Chem.* **1982**, *25*, 1477.
(20) Strain, J.; Grose, R.; Maskin, C. S.; LeJemtel, T. H. *Am. Heart J.* **1985**, *110*, 91.
(21) Shah, P.; Amin, D.; Hulse, S. *Circulation* **1985**, *71*, 326.
(22) Chemical name: (*R*)-6-chloro-3,5-dihydro-3-methylimidazo-[2,1-*b*]quinazolin-2(1*H*)-one.
(23) Eigenmann, R.; Gerold, M.; Holck, M. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 511.
(24) Holck, M.; Thorens, S.; Muggli, R.; Eigenmann, R. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 520.
(25) Belz, G. G.; Stern, H. C.; Butzer, R. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 86.
(26) Kauffman, R. F., unpublished observations. See also: Lipptton, H. L.; Horwitz, P. M.; McNamara, D. B.; Ignarro, L. J.; Landry, A. Z.; Hyman, A. L.; Kadowitz, P. J. *Prostaglandins, Leukotrienes Med.* **1985**, *18*, 193.

Scheme I



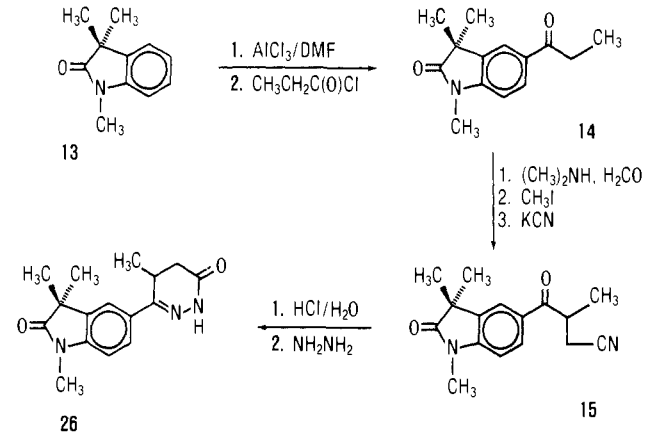
since 1967.[27] These investigations were focused primarily on hypotensive and platelet aggregation effects, the most salient cardiovascular activities of this series in rodents.[28,29] Extensive structure–activity relationship (SAR) studies[30-32] revealed that a 4-acetamido substituent on the phenyl ring (e.g., *N*-[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-phenyl]acetamide, **6**) was optimal for inhibiting platelet aggregation and reducing blood pressure. Moreover, inclusion of a 4-methyl substituent[2] (e.g., **7**) in the dihydropyridazinone moiety led to an increase in potency.



6. R=H
7. R=CH_3

Milrinone and **6** have considerable structural homology. We viewed **6** as an azadihydro milrinone analogue in which the pyridine ring of milrinone has been replaced with the acetamidophenyl moiety of **6**. This structural analogy led us to question whether **6** and **7** possess positive inotropic activity, and in this paper we conclusively demonstrate that they do. Moreover, we have prepared lactam analogues of these dihydropyridazinones, and these efforts have resulted in the discovery of several potent and long-acting oral inotropes. We now detail the synthesis and inotropic structure–activity relationships of these lactam analogues.

Scheme II



Results and Discussion

Chemistry. Compounds **6** and **7** were prepared according to reported procedures.[30-32] Synthesis of lactam analogues of **6** and **7** is illustrated by the synthesis of **20** (LY195115, 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one, Table I). The key intermediate in the synthesis of **20** was 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (**11**). This material could be prepared on a large scale by a variety of methods (Scheme I). For example, exhaustive methylation of 1-acetyl-1,3-dihydro-2*H*-indol-2-one (**8**) with iodomethane and sodium hydride, followed by acid-catalyzed removal of the protecting group, afforded **11** (59% yield for two steps). Alternatively this intermediate could be prepared in 98% yield by base-induced rearrangement[33] of 2-methylpropanoic acid, 2-phenylhydrazide (**10**). Standard synthetic procedures were then used to construct the dihydropyridazinone portion of **20**. Friedel–Crafts acylation of **11** with succinic anhydride in an aluminum chloride/DMF melt according to the general procedure of Thyes et al.[32] provided the 4-oxobutanoic acid **12** in 74% yield. Cyclization of **12** with hydrazine in refluxing ethanol resulted in the precipitation of analytically pure **20** in 88% yield. When desired, this material could be recrystallized in DMF/water or acetic acid/water.

Variation of commercially available starting materials enabled the synthesis of most congeners of **20** described in this paper (Table I). The majority of the benzo-fused lactams used as substrates in the Friedel–Crafts reactions are known in the literature (see Experimental Section). 3,4-Dihydro-3,3-dimethyl-2(1*H*)-quinolinone was prepared by methylation of the dianion of its monomethyl analogue, which was obtained by methylation of the dianion of 3,4-dihydro-2(1*H*)-quinolinone.

Congeners bearing a methyl substituent in the 4-position[2] of the dihydropyridazinone ring were more potent than their desmethyl analogues (vide infra). The synthesis of **26** (Scheme II) illustrates their preparation. Reaction of **13** and propionyl chloride in the presence of aluminum chloride/DMF afforded ketone **14** in 80% yield. Formation of the Mannich base and quaternarization with methyl iodide (79% yield over 2 steps), followed by reaction with potassium cyanide, provided γ-keto nitrile **15**. Hydrolysis to the corresponding acid followed by hydrazine cyclization afforded **26** in 64% yield (three steps).

Compound **22** was prepared by Friedel–Crafts acylation of **11** with methylsuccinic anhydride, as previously de-

(27) Bachmann, G.; Amann, A. French Patent 1507475, 1967; *Chem. Abstr.* **1969**, *70*, 37824t.
(28) Griffett, E. M.; Kinnon, S. M.; Kumar, A.; Lecker, D.; Smith, G. M.; Tomich, E. G. *Br. J. Pharmacol.* **1981**, *72*, 697.
(29) Fredholm, B. B.; Hedquist, P.; Vernet, L. *Biochem. Pharmacol.* **1978**, *27*, 2845.
(30) Curran, W. V.; Ross, A. *J. Med. Chem.* **1974**, *17*, 273.
(31) McEvoy, F. J.; Allen, G. R.; Jr. *J. Med. Chem.* **1974**, *17*, 281.
(32) Thyes, M.; Lehmann, H. D.; Gries, J.; Konig, H.; Kretzschmar, R.; Kunze, J.; Lebkucher, R.; Lenke, D. *J. Med. Chem.* **1983**, *26*, 800.
(33) For a general procedure, see: Endler, A. S.; Becker, E. I. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 657.

Table II. Inotropic Activity of Selected Dihydropyridazinone Cardiotonics in Cat Papillary Muscles

no.	cat papillary muscle contractility, % of control (response ratio[a])			
	10^{-6}	10^{-5}	10^{-4}	n
1 (milrinone)		148 ± 0.5 (0.75)	158 ± 14 (0.91)	2
6		128 ± 2 (0.57)	141 ± 2 (0.85)	2
7	167 ± 17 (0.67)	183 ± 30 (0.83)	216 ± 57 (1.16)	4
16	116 ± 8 (0.24)	147 ± 13 (0.70)	152 ± 15 (0.78)	4
20	134 ± 5 (0.35)	164 ± 17 (0.67)	169 ± 22 (0.72)	5
32	150 ± 5 (0.71)	156 ± 6 (0.80)	154 ± 8 (0.77)	4
37	121 ± 4 (0.26)	137 ± 7 (0.45)	139 ± 5 (0.48)	5
38 (CI-914)		147 ± 17 (0.38)	173 ± 40 (0.59)	4

[a] Response ratio = (percent increase to drug)/(percent increase to 10^{-6} M isoproterenol).

scribed,[30] followed by hydrazine cyclization. Compound **31**, which lacks the carbonyl group in the bicyclic moiety, was prepared from 1-acetyl-1,2,3,4-tetrahydroquinoline by the standard sequence: Friedel–Crafts acylation with succinic anhydride, hydrolysis of the *N*-acetyl protecting group, and finally, hydrazine cyclization (see Experimental Section).[34]

Inotropic Activity of 6 and 7. Hypotensive and platelet aggregation inhibition activities of the 4,5-dihydro-6-phenyl-3(2*H*)-pyridazinones have been actively investigated for two decades.[27] However, these studies, for the most part, were conducted in rodents. Compounds from the new generation of non-catecholamine, non-glycoside positive inotropes, which function at least in part by inhibition of phosphodiesterase III, do not display potent positive inotropic activity in rodents,[35] but rather exhibit hypotensive and platelet aggregation inhibition activities. Because of these pharmacological similarities and the previously discussed structural similarities between milrinone and the dihydropyridazinones, we synthesized **6** and **7** and tested them for inotropic activity in cat papillary muscles (Table II). Both dihydropyridazinones, as well as milrinone, produced significant, concentration-dependent increases in cat papillary muscle contractility. Effects were maximal within 5 min and were maintained throughout the experiment (30 min). Compound **7** was the most potent, and a 10^{-6} M concentration produced a 67% increase in contractility. Studies with other dihydropyridazinone cardiotonics (e.g., **20**) indicated that these in vitro positive inotropic responses were not blocked by a variety of adrenergic or histaminergic antagonists.

We next probed the compounds for inotropic activity following intravenous administration of increasing log doses to pentobarbital-anesthetized dogs. A Walton-Brodie strain-gauge arch was used to monitor right ventricular contractility, and ED_{50}'s were determined by linear regression analysis; the data are summarized in Table III and Figure 1. Both dihydropyridazinones, as well as the comparison drug milrinone, were potent positive inotropes. The ED_{50}'s for **6**, **7**, and milrinone were 11.6, 2.0, and 37 μg/kg, respectively. Moreover, both dihydropyridazinones were more efficacious than milrinone; that is, the former compounds increased contractility to values 250–300% of control, whereas milrinone provided a maximum increase of 200% of control. With all three compounds, the increases in contractility were rapid in onset (less than 15 s), maximal in less than 1 min, and associated with only modest tachycardia and decreases in mean arterial blood pressure (Table III). Compounds **6** and **7** are among the most potent non-catecholamine, non-glycoside positive inotropes we have examined. Compound **7** is 19-, 90-, and 150-fold more potent than either milrinone, piroximone, or sulmazole,[11,36] respectively. It is of interest that the 4-methyl[2] analogue **7** is approximately 6-fold more potent than its congener **6**. A similar methyl-induced increase in the potency of **7** as a platelet aggregation inhibitor was

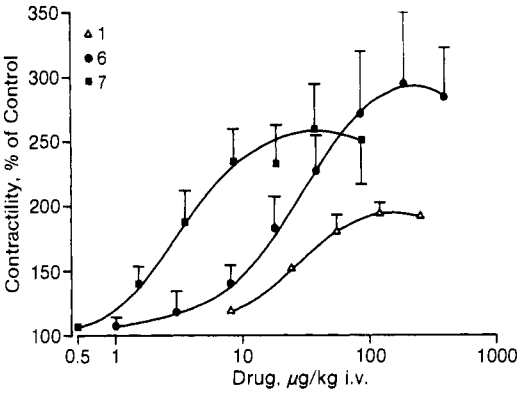


Figure 1. Dose-dependent effects of 1 (milrinone), **6**, and **7** on myocardial contractility in pentobarbital-anesthetized dogs. Drugs were administered at 5-min intervals and peak responses recorded. Each drug was studied in a separate group of animals. Each point is the mean ± SEM of experimental values. Symbols without error bars indicate that errors fell within the area of the symbols. Control (base-line) values were as follows: contractility, 50 g tension; heart rate (HR), 127 ± 3 beats/min; mean arterial blood pressure (MAP), 99 ± 3 mmHg.

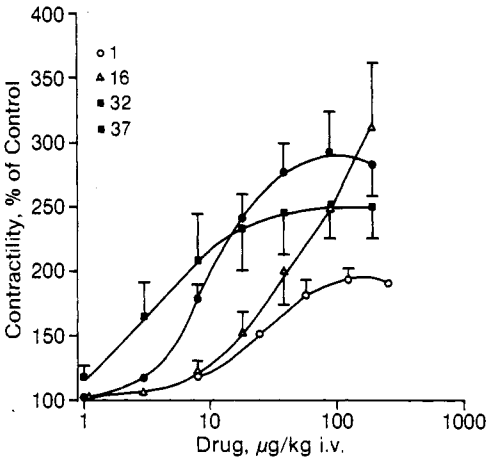


Figure 2. Dose-dependent effects of 1 (milrinone), **16**, **32**, and **37** on myocardial contractility in pentobarbital-anesthetized dogs. Refer to Figure 1 caption for experimental details.

(34) Several of these compounds have been prepared previously and studied as antithrombotic and antihypertensive agents. See: Nakao, T.; Setoguchi, S.; Yaoka, O. U.S. Patent 4 258 185, 1981. Extensive studies on the antithrombotic activity of a close analogue of **32**, Y-590, have been published: Mikashima, H.; Nakao, T.; Goto, K.; Ochi, H.; Yasuda, H.; Tsumagari, T. *Thromb. Res.* **1984**, *35*, 589. Mikashima, H.; Nakao, T.; Goto, K. *Thromb. Res.* **1983**, *31*, 599.

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Table III. Cardiovascular Profile of Dihydropyridazinone Cardiotonics in Anesthetized and Conscious Dogs

	anesthetized dog[a]				conscious dog[b]		
no.	ED_{50} for contractility, μg/kg iv	% increase in HR	% decrease in MAP	n	dose, μg/kg po	peak inotropic response,[c] % of control	n
1 (milrinone)	37 ± 9	9 ± 3	8 ± 2	4	500	162 ± 10	4
16	24 ± 8	5.6 ± 2.1	4.3 ± 1.9	6	500	164 ± 4	4
17	271 ± 53	11 ± 3	7 ± 3	2			
18	9.6 ± 1.8	8.0 ± 1.6	21.8 ± 4.3	4	25	141 ± 9	3
19	71 ± 4	18 ± 6	12 ± 2	2			
20	6.8 ± 0.7	11.8 ± 1.1	15.2 ± 1.5	4	25	151 ± 4[d]	4
21	3.2 ± 0.4	16 ± 2	26 ± 4	4	50	150 ± 2	2
22	15 ± 4	13 ± 1	20 ± 1	2			
23	40 ± 14	7.9 ± 2.6	0.5 ± 4.4	2			
24	9.5 ± 3.9	8.5 ± 0.7	17 ± 5.5	2			
25	52 ± 34	8 ± 4	13 ± 4	3			
26	4.4 ± 1.5	21.4 ± 2.7	26 ± 3	3			
27	22 ± 4	2.0 ± 2.5	3.1 ± 2.1	4	400	149 ± 6	4
28	14.8 ± 7	11 ± 4	20 ± 6	3			
29	741 ± 476	8.9 ± 12	7.5 ± 1.7	2			
30	19.8 ± 0.1	9.3 ± 6	9.6 ± 3.8	2			
31	257 ± 108	37 ± 12	12 ± 4	2			
32	3.3 ± 1.0	18.5 ± 11	8.7 ± 2.7	4	50	157 ± 10	4
33	12.4 ± 4.2	26 ± 13	21 ± 6	2	100	134	1
34	3.0 ± 1.2	16 ± 0.8	26 ± 7	4	25	168 ± 12	3
35	86 ± 9	5.9 ± 3.4	15 ± 7	2	200	116 ± 4	4
36	21 ± 9	8.5 ± 4.5	3 ± 7	2	400	133	1
37	5.2 ± 0.6	12 ± 1	18 ± 1	3	50	131	1
38 (CI-914)	46 ± 29	9.5 ± 6.5	18 ± 3.5	2			
6	11.6 ± 5.8	5.0 ± 1.0	6.0 ± 2.5	3	50	132 ± 17[e]	2
7	2.0 ± 0.5	11.7 ± 2.5	16.6 ± 8.6	3	25	170 ± 15[e]	2

[a] ED_{50}'s were determined by linear regression analysis and are reported as the mean ± SEM of experimental values. Heart rate and mean arterial blood pressure values are the % changes recorded at the inotropic ED_{50}'s. Control values were: contractility, 50 g tension; heart rate (HR), 127 ± 3 beats/min; mean arterial blood pressure (MAP), 99 ± 3 mmHg. [b] Drugs were administered in 000 gelatin capsules, and $LV\,dP/dt_{60}$ was used as an index of contractility. [c] Unless indicated otherwise, the peak response was measured 150-min postadministration. Values reported are the mean ± SEM of experimental values. [d] Peak response measured at 300 min. [e] Peak response measured at 120 min.

noted by Thyes and co-workers.[32]

Structure–Activity Relationships of Lactam Analogues. We prepared **16** because it is a direct analogue of **6** in which the *p*-acetamido substituent has been incorporated into a fused five-membered ring. Positive inotropic activity was retained, and the iv ED_{50} following administration to anesthetized dogs was 24 μg/kg; the compound was also active in vitro (Tables II and III). We then synthesized and examined congeners **32** and **37**. The homologous quinolin-2-one, **32**, and benzazepin-2-one, **37**, were both more potent than indol-2-one **16** (ED_{50} = 3.3, 5.2, and 24 μg/kg, respectively). The entire set of dose–response curves for these three congeners is displayed in Figure 2. As had been observed with **6** and **7**, all three dihydropyridazinones were more efficacious than milrinone. The molecular basis for increased efficacy is unknown; however, this is of little therapeutic importance because contractility would probably not be increased above milrinone's potential in a clinical setting.

When methylhydrazine replaced hydrazine in the cyclization step, *N*-methylpyridazinone **17** was produced. This compound was only one-tenth as potent as **16**, indicating either the importance of a relatively acidic proton or the lack of steric tolerance in this portion of the molecule. However, either methyl or geminal dimethyl substitutions (**18** and **20**, respectively) were well-tolerated in the 3-position of the indol-2-one portion of the molecule. In fact, both **18** and **20** were significantly ($p < 0.05$) more active than **16** (ED_{50} = 9.6, 6.8, and 24 μg/kg, respectively). Homologation of this 3-alkyl substitution to ethyl (**19**) or 3,3-diethyl (**29**) resulted in drastically diminished activity (ED_{50} = 71 and 741 μg/kg, respectively). In the geminal dialkyl analogues, however, if one substituent was maintained as methyl, then either ethyl (**27**) or propyl (**30**) substituents at the other 3-position were well-tolerated (iv ED_{50} = 22 and 19.8 μg/kg, respectively).

Because of the beneficial effects of 3,3-dimethyl substituents, these were maintained, and effects of methyl substitution on other portions of the molecule were examined. As expected (vide supra), methyl substitution at the 4-position[2] of the dihydropyridazinone (**21**) resulted in increased potency; in this instance, approximately a 2-fold enhancement of inotropic activity (iv ED_{50} = 3.2 μg/kg) was obtained relative to **20**. A methyl substituent at the 4-position[2] of the dihydropyridazinone invariably increased potency, but the magnitude of the increase was variable. As has been noted previously, **7** was approximately 6-fold more potent than its desmethyl analogue **6**, whereas **28**, the 4-methyl[2] congener of **27**, was 1.4-fold more potent than **27** (ED_{50} = 14.8 and 22 μg/kg, respectively). Inclusion of a methyl substituent at either the 5-position of the dihydropyridazinone (**22**) or the 6-position of the indolone ring (**23**) resulted in diminished potency (ED_{50} = 15 and 40 μg/kg, respectively), whereas the 7-methyl analogue **24** (ED_{50} = 9.5 μg/kg) was approximately as potent as **20**. A methyl substituent on the nitrogen of the indolone moiety decreased potency (**25**, ED_{50} = 52 μg/kg), but this diminution was abolished by inclusion of a 4-methyl[2] substituent in the dihydropyridazinone moiety (**26**, ED_{50} = 4.4 μg/kg).

In the quinolin-2-one series, the importance of the carbonyl group of the lactam moiety was probed by the synthesis of **31**. Remarkably, this compound was approximately 2 orders of magnitude less potent than its carbonyl-containing counterpart, **32** (ED_{50} = 257 and 3.3 μg/kg, respectively). A significant effect of the carbonyl group is on σ_p of the nitrogen substituent. The σ_p's for the nitrogen substituents of **31** and **32** are approximately −0.84 and 0.00, respectively. These electronic differences may be responsible for the dramatic carbonyl-induced potency

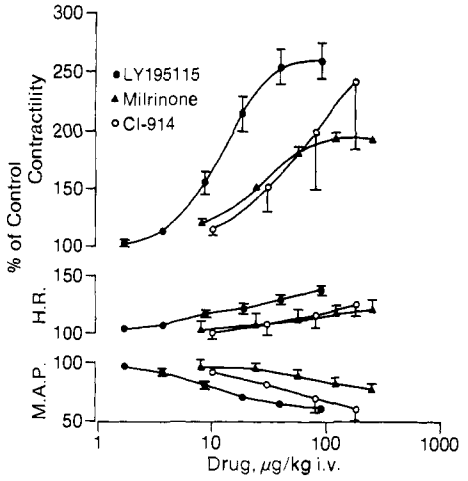
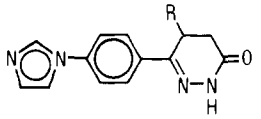


Figure 3. Dose-dependent effects of milrinone (1), LY195115 (**20**), and CI-914 (**38**) on myocardial contractility, heart rate, and mean arterial blood pressure in pentobarbital-anesthetized dogs. Refer to Figure 1 caption for experimental details.

differences between **31** and **32**. However, the carbonyl group may also serve as a hydrogen-bond acceptor site. As had been previously discovered in the indolone series, a lactam *N*-methyl substituent on the quinolin-2-one resulted in decreased activity (**33**, ED_{50} = 12.4 μg/kg), but a dihydropyridazinone 4-methyl[2] substituent restored potency (**34**, ED_{50} = 3.0 μg/kg) to that of the parent structure, **32**. Whereas geminal dimethyl substitution at the 3-position of the indol-2-one series resulted in increased potency, inclusion of geminal dimethyl substituents either α or β to the quinolin-2-one lactam carbonyl resulted in decreased potency (**36** and **35**, respectively). The molecular basis for this disparity is unknown.

Bristol and co-workers have independently discovered that 4,5-dihydro-6-phenyl-3(2*H*)-pyridazinones possess positive inotropic activity.[37] Whereas we examined lactam analogues of **6** and **7**, they found that an imidazole substituent could serve as an acetamido surrogate at the 4-position of the phenyl ring.[37,38] Two resulting compounds, **38** (CI-914)[39] and **39** (CI-930),[40] are subjects of clinical investigations.[38,41]



38. R=H, CI-914
39. R=CH_3, CI-930

We compared the cardiovascular profiles of **20**, milrinone, and **38** in anesthetized dogs, and the data are displayed in Table III and Figure 3. All three agents were

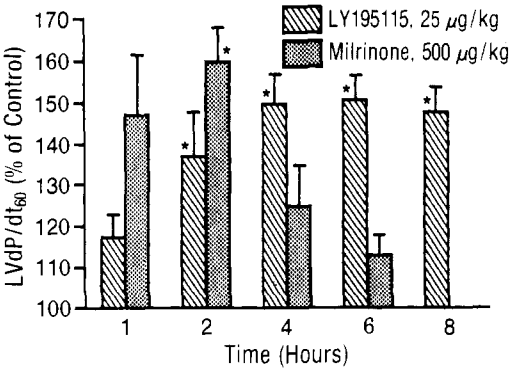


Figure 4. Effects of LY195115 (**20**) and milrinone (1) on LV$dP/dt_{60}$ after oral administration to conscious dogs. Chronically instrumented dogs received either drug or placebo (lactose) in 000 gelatin capsules. Values are the mean ± SEM of four experiments. Control (base-line) values were as follows: LV$dP/dt_{60}$, 38 ± 2 s^{-1}; heart rate, 55 ± 4 beats/min; LVSBP, 102 ± 2 mmHg. *, $p < 0.05$ compared to placebo.

potent positive inotropes; the ED_{50}'s of **20**, milrinone, and **38** were 6.8, 37, and 46 μg/kg, respectively. Moreover, all three compounds were selective inotropes; that is, they produced substantial increases in contractility before significant alterations in either heart rate or mean arterial blood pressure occurred.

Assessment of Oral Efficacy in Conscious Dogs. Because oral efficacy is of paramount importance in any agent designed for the chronic management of CHF, the inotropic activities of these compounds were studied after oral administration to conscious, chronically instrumented dogs. LV dP/dt_{60}, the first derivative of left ventricular pressure at 60 mmHg, was monitored as an index of contractility. All compounds examined were orally effective, but there were wide variations in potency (Table III). Administration of 500 μg/kg of indolone **16** produced a 64% increase in LV dP/dt_{60}, whereas homologues **32** and **37** were approximately 10-fold more potent. A cardinal discovery, however, was the effect of geminal dimethyl substitution on oral activity of the indol-2-one series. Compound **20** was an extremely potent oral inotrope, and 25 μg/kg increased contractility by 51 ± 4%. This level of oral potency relative to **16** was unanticipated in view of the fact that **20** is only 3.5 times more potent than **16** after iv administration. A possible explanation for the much larger differences in potency after oral administration would be a geminal-dimethyl-induced enhancement of oral bioavailability. Preliminary drug disposition studies with **20** in dogs indicate that oral bioavailability approaches 100%.[42] The effect on contractility of 25 μg/kg of **20** was of long duration (>8 h, Figure 4); the half-life of **20** in dogs has been determined to be approximately 14 h.[42] Doses that caused approximately 50% increases in LV dP/dt_{60} produced only minor increases in heart rate or peak left ventricular systolic blood pressure (data not shown). In this conscious dog model, administration of 500 μg/kg of milrinone produced a 62% increase in contractility, but the inotropic effect was of relatively short duration (Figure 4). Clinically, milrinone has a short half-life and must be administered 4–6 times a day;[43] in our canine studies, the inotropic responses to a 500 μg/kg dose were maximal in 2 h and were near base line at 6 h. Bristol et al.[37] reported a 42% increase in LV dP/dt_{max} after a dose of 1000 μg/kg

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of CI-914 to conscious, chronically instrumented dogs.

In summary, we have shown that **6** and **7** are potent positive inotropes. Synthesis of lactam analogues of **6** and **7** produced several highly potent cardiotonics. In the indol-2-one series, 3,3-dimethyl substitution resulted in an increase in iv potency and, especially, an increase in oral potency. In conscious dogs, the increase in contractility produced by 25 μg/kg of **20** was maximally sustained in excess of 8 h, indicating that this agent is one of the most potent and long-acting orally effective inotropes reported to date. On the basis of extensive pharmacological and long-term toxicological evaluations, **20** has been selected for development for the chronic management of CHF. Further reports regarding SAR, pharmacological, and mechanistic studies will be forthcoming.

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance (^{1}H NMR) spectra were obtained by use of a Bruker WM-270 spectrometer. Mass spectra were recorded from a Varian MAT CH-5 spectrometer. Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories; only symbols of elements analyzed are given, and they were within 0.4% of theoretical values unless indicated otherwise.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation".

The majority of the benzo-fused lactams used as substrates in the Friedel–Crafts reactions were prepared by the general reaction conditions outlined below and are known in the literature: 1,3-dihydro-3-methyl-2*H*-indol-2-one;[44] 1,3-dihydro-3-ethyl-2*H*-indol-2-one;[45] 1,3-dihydro-3,3,7-trimethyl-2*H*-indol-2-one;[46] 1,3-dihydro-3-ethyl-3-methyl-2*H*-indol-2-one;[47] 1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-one;[48] 1,3-dihydro-3,3-diethyl-2*H*-indol-2-one;[49] 3,4-dihydro-2(1*H*)-quinolinone;[50] 3,4-dihydro-4,4-dimethyl-2(1*H*)-quinolinone;[51] 3,4-dihydro-1-methyl-2(1*H*)-quinolinone;[52] and 1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one.[53] Experimental details are given below for the preparation of newly described benzo-fused lactams.

Syntheses of Benzo-Fused Lactams. The following procedures illustrate the synthetic methods used to prepare these lactams.

1-Acetyl-1,3-dihydro-2*H*-indol-2-one (8). A mixture of 1,3-dihydro-2*H*-indol-2-one (89.59 g, 673 mmol) and acetic anhydride (69.4 mL, 740 mmol) was heated at reflux overnight. The homogeneous reaction was cooled to room temperature. Recrystallization of the resulting solid from ethyl acetate provided 99.1 g (84.2%) of **8** as white crystals: mp 127.5–128 °C (lit.[54] mp 126 °C). Anal. ($C_{10}H_9NO_2$) C, H, N.

1,3-Dihydro-3,3-dimethyl-2*H*-indol-2-one (11). A solution of **8** (33.63 g, 192 mmol) in 500 mL of DMF was added over a 1-h period to a mixture of sodium hydride (16.1 g of a 60% dispersion in oil, 404 mmol; oil removed by hexane trituration) in 100 mL of DMF at 0 °C. Ten minutes following cessation of hydrogen evolution, iodomethane (29.9 mL, 480 mmol) was added in a dropwise fashion, and then the reaction mixture was allowed to warm to room temperature. After overnight stirring of the reaction mixture at room temperature, product isolation (water, ethyl acetate, water, brine, $MgSO_4$) yielded 44 g of **9** as a light red solid. The material was used in the following reaction without purification.

A mixture of **9** (40.5 g) and 6 N hydrochloric acid (420 mL) was refluxed for 1.5 h. The mixture was then poured into 1 L of water. Filtration and recrystallization from ethyl acetate/hexane provided 18.6 g (59% yield for 2 steps) of **11** as white crystals: mp 149–151 °C (lit.[55] mp 151–152 °C). Anal. ($C_{10}H_{11}NO$) C, H, N.

1,3-Dihydro-3,3,6-trimethyl-2*H*-indol-2-one. A mixture of 2-methylpropanoic acid 2-(3-methylphenyl)hydrazide (22.0 g, 114.6 mmol) and calcium hydride (7.2 g, 172 mmol) was slowly heated over a 2.5-h period to 230 °C and then maintained at this temperature for 30 min. The reaction was cooled to room temperature, and then a solution of 50 mL of methanol and 20 mL of water was slowly added. After hydrogen evolution ceased, concentrated hydrochloric acid was added until the mixture had a pH of 1. The mixture was warmed at 100 °C for 1 h, and then 5 N sodium hydroxide was used to adjust the pH to 4. The precipitate was filtered, dried, and recrystallized from THF/hexane to afford 14.56 g (73%) of product as white crystals: mp 172–173 °C. Anal. ($C_{11}H_{13}NO$) C, H, N.

1,3-Dihydro-3-methyl-3-propyl-2*H*-indol-2-one. This material was prepared as described above, with use of 2-methylpentanoic acid 2-phenylhydrazide (30 g, 145.6 mmol) and calcium hydride (10.1 g, 240 mmol). After the pH was adjusted to 4, the oily product was isolated (ethyl acetate, water, brine, Na_2SO_4) and chromatographed (silica gel, 0–2.5% methanol in methylene chloride gradient) to afford 18.6 g (67%) of product as a colorless oil. ^{1}H NMR and mass spectra were consistent with the assigned structure.

3,4-Dihydro-3,3-dimethyl-2(1*H*)-quinolinone. *n*-Butyllithium (219.4 mL of a 1.55 M solution in hexane, 340 mmol) was added over a 30-min period to a solution of diisopropylamine (48.1 mL, 349 mmol) in 100 mL of THF at 0 °C. After the reaction mixture had been stirred at 0 °C for an additional 30 min, a solution of 3,4-dihydro-2(1*H*)-quinolinone (25 g, 170 mmol) was added over 15 min. The reaction mixture was warmed to room temperature, stirred for 1.75 h, and then cooled to -78 °C. Iodomethane (10.6 mL, 170 mmol) was added in one portion, and the reaction mixture was allowed to warm to room temperature overnight. Product isolation (ethyl acetate, water, brine, $MgSO_4$) and flash chromatography (silica gel, 0–25% ethyl acetate in hexane) afforded 11.0 g (40%) of homogeneous 3,4-dihydro-3-methyl-2(1*H*)-quinolinone as an oil. ^{1}H NMR and mass spectra were consistent with the assigned structure.

A second methylation was effected in exactly the same manner, with use of the following reagent quantities: 3,4-dihydro-3-methyl-2(1*H*)-quinolinone (10.4 g, 64.6 mmol); diisopropylamine (19.46 mL, 139.1 mmol); *n*-butyllithium (87.4 mL of a 1.55 M solution in hexane, 135.4 mmol); and iodomethane (4.11 mL, 66 mmol). After chromatography, the material was recrystallized from THF/hexane to afford 5.9 g (52%) of product as white crystals: mp 155–156 °C. Anal. ($C_{11}H_{13}NO$) C, H, N.

Syntheses of Dihydropyridazinone Cardiotonics. The following procedures illustrate the synthetic methods used to prepare the dihydropyridazinone cardiotonics and their 4-methyl[2] analogues.

2,3-Dihydro-3,3-dimethyl-γ,2-dioxo-1*H*-indole-5-butanoic Acid (12). Dimethylformamide (45.9 mL, 584 mmol) was added in a dropwise fashion to anhydrous aluminum chloride (278.2 g,

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2.09 mol), and the exothermic reaction mixture was then allowed to cool to room temperature. An intimate mixture of succinic anhydride (20.9 g, 209 mmol) and 11 (33.6 g, 209 mmol) was slowly added to the $AlCl_3$/DMF melt. The reaction mixture was then stirred 2.5 h at 70 °C. The reaction mixture was slowly poured onto 2 L of ice, and the product was isolated by filtration. Recrystallization from DMF/water provided 40.17 g (74% yield) of **12** as a light tan solid: mp 223–225 °C. Anal. ($C_{14}H_{15}NO_4$) C, H, N.

1,3-Dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one (LY195115, 20). A mixture of **12** (56.33 g, 216 mmol) and 85% hydrazine hydrate (28.0 mL, 475 mmol) in 700 mL of absolute ethanol was refluxed for 4 h and then cooled slowly to room temperature. The precipitate was filtered and dried to afford 48.93 g (88% yield) of **20** as a light-tan solid: mp >300 °C. Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

1,3-Dihydro-1,3,3-trimethyl-5-(1-oxopropyl)-2*H*-indol-2-one (14). Dimethylformamide (30.5 mL, 389 mmol) was added in a dropwise fashion to anhydrous aluminum chloride (185.2 g, 1.39 mol) to form a melt. After the exothermic reaction mixture had cooled to 40 °C, a mixture of propionyl chloride (12.1 mL, 139 mmol) and **13** (24.3, 139 mmol) was added in portions. The reaction mixture was then heated at 70 °C for 1 h and poured onto ice. Concentrated hydrochloric acid (100 mL) was added, the mixture was cooled, and the precipitate was filtered and dried to afford 32.4 g of product. Recrystallization from THF/hexane gave 25.75 g (80% yield) of **14**: mp 123–124 °C. ^1H NMR and mass spectra were consistent with the assigned structure. Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70. Found: C, 71.35.

2,3-Dihydro-*N*,*N*,*N*,β,1,3,3-heptamethyl-γ,2-dioxo-1*H*-indole-5-propanaminium Iodide. Dimethylamine hydrochloride (13.3 g, 162 mmol) and formaldehyde (10.6 mL of a 37% aqueous solution) were stirred for 15 min at room temperature. Acetic anhydride (65.3 mL, 693 mmol) was added to this mixture, and the reaction mixture was heated to 40 °C. An exothermic reaction ensued, and the temperature increased to 110 °C. The reaction mixture was allowed to cool to 90 °C, and **14** (25.0 g, 108 mmol) was added in one portion. The reaction mixture was maintained at 90 °C for 2 h and then concentrated under reduced pressure. Acetone (500 mL) was added, and the mixture was refluxed for 10 min. Solvent was removed under reduced pressure; the residue was diluted with water and extracted with ethyl acetate (discarded). The aqueous solution was adjusted to pH 9 with 6 N sodium hydroxide. Product isolation (ethyl acetate, brine, Na_2SO_4) afforded 29.7 g of Mannich base as an oil.

Iodomethane (12.8 mL, 206 mmol) was added to a solution of the Mannich base (29.7 g) in 500 mL of acetone; the reaction mixture was stirred overnight at room temperature and then cooled to 0 °C. The precipitate was filtered and dried to provide 36.7 g (79% yield over two steps) of product as a white solid: mp 223–224 °C. Anal. ($C_{18}H_{27}IN_2O_2$) C, H, N.

1,3-Dihydro-1,3,3-trimethyl-5-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)-2*H*-indol-2-one (26). A solution of potassium cyanide (12.8 g, 196 mmol) in 100 mL of water was added to a solution of 2,3-dihydro-*N*,*N*,*N*,β,1,3,3-heptamethyl-γ,2-dioxo-1*H*-indole-5-propanaminium iodide (35.2 g, 81.9 mmol) in 100 mL of methanol, and the reaction mixture was stirred overnight at room temperature. Product isolation (water, ethyl acetate, water, brine, Na_2SO_4) gave 25 g of nitrile **15** as a clear oil. ^1H NMR and mass spectra were consistent with the assigned structure.

A mixture of **15** (25 g) and 300 mL of 6 N hydrochloric acid were heated at reflux for 2 h. The reaction mixture was cooled to room temperature and diluted with water. Product isolation (ethyl acetate, water, brine, Na_2SO_4) provided 24 g of the carboxylic acid as an amber oil. ^1H NMR and mass spectra were consistent with the assigned structure.

Hydrazine (10.6 mL, 85%, 180 mmol) was added to a solution of the unpurified acid (24 g) in 250 mL of absolute ethanol, and the reaction mixture was heated at reflux for 3.5 h. The reaction mixture was slowly cooled to 0 °C, and the precipitate was filtered. This provided 15.02 g (64% yield over three steps) of **26** as white crystals: mp 225–226 °C. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

1-Acetyl-γ-oxo-1,2,3,4-tetrahydroquinoline-6-butanoic Acid. This reaction was conducted exactly as described for the preparation of **12**, with use of the following reagents: dimethylformamide (34.3 mL, 436 mmol), aluminum chloride (207.6 g, 1.56 mol), succinic anhydride (15.6 g, 155.7 mmol), and 1-acetyl-1,2,3,4-tetrahydroquinoline (27.25 g, 155.7 mmol). Product isolation (ice, ethyl acetate, water, brine, Na_2SO_4) and flash chromatography (silica gel, 0–8% methanol in methylene chloride gradient) provided 27 g (63%) of homogeneous product as an oil. The ^1H NMR and mass spectra were consistent with the assigned structure.

γ-Oxo-1,2,3,4-tetrahydroquinoline-6-butanoic Acid. A mixture of 1-acetyl-γ-oxo-1,2,3,4-tetrahydroquinoline-6-butanoic acid (3.48 g, 12.7 mmol) and 25 mL of 12 N hydrochloric acid was heated to reflux. After 4 h at reflux the reaction mixture was poured onto ice, and the pH was adjusted to 5 with dilute sodium hydroxide. The resulting crystalline suspension was filtered and dried to afford 2.03 g (69%) of homogeneous product: mp 124–129 °C dec. Anal. ($C_{13}H_{15}NO_3$) C, H, N.

4,5-Dihydro-6-(1,2,3,4-tetrahydro-6-quinolinyl)-3(2*H*)-pyridazinone (31). A mixture of γ-oxo-1,2,3,4-tetrahydroquinoline-6-butanoic acid (1.94 g, 8.3 mmol) and 85% hydrazine hydrate (1.08 mL, 18.3 mmol) in 250 mL of absolute ethanol was refluxed for 1 h and then cooled to room temperature. Removal of solvent under reduced pressure and recrystallization from ether/methanol provided 860 mg (45%) of product as white crystals: mp 154–157 °C. Anal. ($C_{13}H_{15}N_3O$) C, H, N.

Pharmacological Methods. Isolated Cat Papillary Muscles. Cats of either sex were anesthetized with methoxyflurane, their hearts immediately removed, and papillary muscles dissected and suspended in individual muscle baths. A 27-gauge hook secured the muscle to an electrode mounted in the bottom of the bath, and a silk thread attached the tendon to a Statham isometric transducer. Baths contained Krebs–Henseleit solution (37.5 °C, bubbled with 95% O_2/5% CO_2) of the following millimolar composition: NaCl 118, KCl 4.5, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 11. A base-line tension of 1.0 g was applied to each tissue. Muscles were stimulated to contract by administration of square-wave pulses (2.0 ms in duration, 12 times/min, 20% above threshold voltage) delivered through the hook electrode and a second electrode positioned near the top of the muscle; contractions were recorded on a Beckman polygraph.

Muscles were equilibrated for 60 min prior to drug treatment. In order to assure that muscles were functioning properly, each was exposed to 10^{-6} M isoproterenol for 3 min. If the inotropic response was less than 120% of control, the muscle was rejected. This short-acting standard was washed out of the baths, and the muscles were allowed 30 min to stabilize. Inotropic responses to test compounds were examined by cumulative dosing (10^{-7}–10^{-4} M) at 30-min intervals.

Experiments in Anesthetized Dogs. Mongrel dogs of either sex (7–14 kg) were anesthetized with sodium pentobarbital (35 mg/kg iv). A positive-pressure pump was used to ventilate the dogs through an endotracheal tube (18 strokes/min, 20 mL/kg per stroke), and a heating pad maintained body temperature at 37–38 °C. Femoral arterial blood pressure was measured through a polyethylene catheter filled with heparin solution (16 U/mL) and connected to a Statham pressure transducer. The femoral vein was cannulated for iv drug administration. Heart rate was derived by means of a cardiotachometer that was triggered by the arterial pressure pulse. A Walton-Brodie strain-gauge arch sutured to the right ventricle of the heart measured cardiac contractility. Tension on the gauge was adjusted to 50 g, which corresponded to 10 mm of recorder pen deflection. Rapid iv injection of 50 mL of 5% dextran and mechanical compression of the aorta demonstrated that contractility measurements were independent of changes in preload and afterload. Subcutaneous pin electrodes provided a lead II ECG. Increasing log doses of test compounds were administered iv in volumes of 0.25–4.0 mL at 5-min intervals; no responses occurred with appropriate vehicle injections. ED_{50}'s were determined by linear regression analysis and are reported as the mean ± SEM of experimental values. Each drug was studied in a separate group of animals.

Conscious Dog Studies. Male mongrel dogs weighing 15–36 kg were chronically instrumented to monitor LV dP/dt_{60} (the first derivative of left ventricular pressure at 60 mmHg), peak systolic pressure, and heart rate. Under halothane/nitrous oxide anesthesia, a precalibrated Konigsberg P22 pressure transducer was

implanted into the left ventricle through a stab wound at the apex. Dogs were allowed to recover from surgery a minimum of two weeks before use in a study. Animals were conditioned to the test laboratory and trained to lie quietly for 4-h periods. This conditioning was necessary to obtain stable, reproducible results from day to day. Dogs were fasted for 18 h before an experiment, and gross behavioral observations of animals were made throughout each study. Drugs or placebo (lactose) were administered in 000 gelatin capsules.

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Inactivation of γ-Aminobutyric Acid Aminotransferase by (*S*,*E*)-4-Amino-5-fluoropent-2-enoic Acid and Effect on the Enzyme of (*E*)-3-(1-Aminocyclopropyl)-2-propenoic Acid

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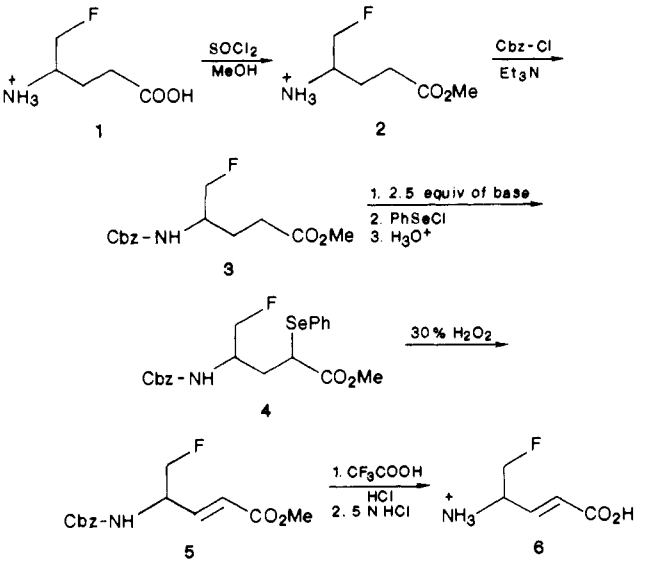
(*S*,*E*)-4-Amino-5-fluoropent-2-enoic acid (**6**) is synthesized in six steps starting from the known γ-aminobutyric acid aminotransferase (γ-Abu-T) inactivator, (*S*)-4-amino-5-fluoropentanoic acid (**1**). Compound **6** is a mechanism-based inactivator of γ-Abu-T: time-dependent inactivation is saturatable and protected by substrate; thiols do not protect the enzyme from inactivation; no enzyme activity returns upon dialysis. This compound (**6**) binds 50 times more tightly to γ-Abu-T than does the saturated analogue (**1**). No transamination of **6** occurs prior to inactivation. However, five molecules of **6** are required to inactivate the enzyme with concomitant release of five fluoride ions. Therefore, four molecules are being converted to product for each inactivation event. (*E*)-3-(1-Aminocyclopropyl)-2-propenoic acid is synthesized in seven steps from 1-aminocyclopropanecarboxylic acid. It is prepared as a cyclopropyl derivative of the proposed intermediate in the inactivation of γ-Abu-T by **6**. The cyclopropyl derivative, however, is a noncompetitive inhibitor and does not inactivate the enzyme. This study shows the usefulness and hazards of incorporation of a trans double bond into potential γ-Abu-T inactivators.

γ-Aminobutyric acid aminotransferase (γ-Abu-T)[1] is an important target in the design of anticonvulsant drugs.[2] Compounds that cross the blood–brain barrier and specifically inactivate this enzyme produce an increase in the concentration of an inhibitory neurotransmitter, γ-Abu, in the brain. This can result in an anticonvulsant effect.[3] Currently, 4-aminohex-5-enoic acid (vigabatrin), a mechanism-based inactivator[4-7] of γ-Abu-T, is in the latter phases of clinical trials and has been shown to be a potent drug for the treatment of epilepsy[8] and, to a lesser extent, tardive dyskinesia.[9] Previously, (*S*)-4-amino-5-fluoropentanoic acid was synthesized in our laboratories.[10] It

Scheme I. Synthetic Route to 6



(1) Abbreviations used throughout this paper include the following: γ-Abu-T, γ-aminobutyric acid aminotransferase; THF, tetrahydrofuran; PLP, pyridoxal 5′-phosphate.
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