Dihydropyridazinone Cardiotonics: Synthesis and Inotropic Activity of 5'-(1,4,5,6-Tetrahydro-6-oxo-3-pyridazinyl)spiro[cycloalkane-1,3'-[3*H*]indol]-2'(1'*H*)-ones

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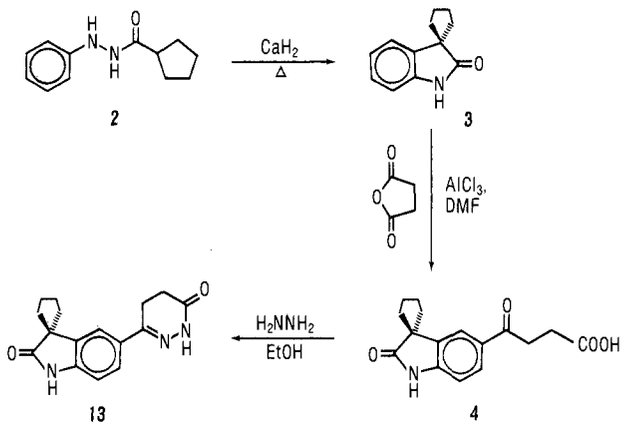
In the 1,3-dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one series of cardiotonics, we found that a spirocycloalkyl ring may be annealed to the 3-position of the indolone moiety while retaining inotropic activity. An inverse relationship was found between spirocycloalkyl ring size and inotropic potency. ED_{50} values of the spirocyclopropane **10**, spirocyclobutane **12**, and spirocyclopentane **13** were 2.7, 35, and 133 μg/kg, respectively, following iv administration to pentobarbital-anesthetized dogs. The most potent compound prepared was **11** (5'-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)spiro[cyclopropane-1,3'-[3*H*]indol]-2'(1'*H*)-one), the 4-methyl analogue of **10**. This compound had an iv ED_{50} of 1.5 μg/kg. Oral activity was evaluated by administering 50 μg/kg of **10** to conscious, chronically instrumented dogs. A 39% increase in LV dP/dt_{60} was observed, and an inotropic effect was demonstrable in excess of 7 h. Thus, the spirocyclic dihydropyridazinone inotropes are potent, long-acting, orally effective cardiotonics. Compound **11** was a potent inhibitor (IC_{50} = 13 nM) of cAMP phosphodiesterase derived from canine cardiac sarcoplasmic reticulum (SR-PDE). Importantly, -log IC_{50} values for inhibition of SR-PDE for this entire series of compounds were highly correlated ($r = 0.949$, $p < 0.02$) with their inotropic -log ED_{50} values, supporting the hypothesis that inhibition of this enzyme contributes to the mechanism of action of the spirocyclic dihydropyridazinones.

Several noncatecholamine, nonglycoside cardiotonics, including milrinone,[1,2] piroximone,[3] and isomazole,[4-6] are being studied clinically for the chronic management of congestive heart failure. This new class of cardiotonics, which simultaneously displays inotropic and vasodilator activities, incontrovertibly produces salutary hemodynamic effects in patients with severe congestive heart failure following either intravenous or short-term oral administration.[7] However, the role of these agents in long-term management of congestive heart failure remains controversial. There is concern that the reduced inotropic state of the failing heart may represent an energy-conserving, self-protective adaptation and that chronic stimulation of the failing heart may increase mortality by hastening progression of the disease state.[8] However, in the case of this new class of cardiotonics, potentially deleterious myocardial effects arising from the energy-consuming inotropic activities may be counterpoised by the salubrious energy-sparing effects of afterload reduction.[9]

Scheme I



Another point of controversy regarding these agents is their possible arrhythmogenicity. Mechanistically, these cardiotonics appear to derive their inotropic and vasodilator effects, at least in part, from an isozyme-specific inhibition of phosphodiesterase, resulting in an increase in intracellular cyclic AMP.[10,11] Because one of the most common reasons for mortality in heart failure patients is sudden death,[12] use of an agent that increases contractility by a potentially arrhythmogenic increase in myocardial cyclic AMP is of obvious concern. However, in several clinical studies, no statistically significant effect of milrinone on cardiac electrophysiology or ventricular dysrhythmias was demonstrable.[13-15] Because the chronic efficacy and safety of digitalis are still being debated[16,17]

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(7) For a recent review, see: Colucci, W. S.; Wright, R. F.; Braunwald, E. *N. Engl. J. Med.* **1986**, *314*, 349.
(8) For a discussion of the theoretical concerns regarding long-term inotropic therapy, see: Katz, A. M. *Circulation, Suppl. III* **1986**, *73*, 184.
(9) In the case of milrinone, direct vasodilator and direct inotropic activities are both manifest at pharmacologically relevant plasma concentrations and contribute to the drug's overall hemodynamic effects in most patients. For definitive publications, see: Cody, R. J.; Muller, F. B.; Kubo, S. H.; Rutman, H.; Leonard, D. *Circulation* **1986**, *73*, 124. Ludmer, P. L.; Wright, R. F.; Arnold, J. M. O.; Ganz, P.; Braunwald, E.; Colucci, W. S. *Circulation* **1986**, *73*, 130.
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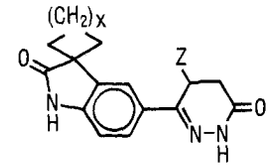
Table I. Structures and Properties of Spirocyclic Dihydropyridazinone Cardiotonics



no.	X	Z	formula	mp, °C	recryst solvent	anal.
10	0	H	$C_{14}H_{13}N_3O_2 \cdot {}^1/_2H_2O$	>300	EtOH	C, H, N
11	0	CH_3	$C_{15}H_{15}N_3O_2$	291–293	EtOH	C, H, N
12	1	H	$C_{15}H_{15}N_3O_2$	>300	EtOH	C, H, N
13	2	H	$C_{16}H_{17}N_3O_2$	>300	*a*	C, H, N

[a] Isolated by flash chromatography.

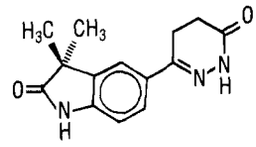
Table II. Biological Activities of Dihydropyridazinone Cardiotonics and Related Inotropes

	anesthetized dog[a]				
no.	ED_{50} for contractility, μg/kg iv	% increase in HR	% decrease in MAP	*n*	SR-PDE[b] inhibition: IC_{50}, μM
1 (LY195115)	6.8 ± 0.7	11.8 ± 1.1	15.2 ± 1.5	4	0.13 (0.11–0.15)
10	2.7 ± 1.6	16 ± 4	16 ± 12	2	0.073 (0.044–0.13)
11	1.5 ± 1.8	17 ± 8.5	35 ± 18	2	0.013 (0.008–0.022)
12	34.8 ± 17	13 ± 5	17 ± 6	3	0.29 (0.21–0.39)
13	133 ± 56	14 ± 3	17 ± 1	2	0.93 (0.72–1.2)
14 (amrinone)[c]	389 ± 28				4.8 (3.5–6.4)
15 (milrinone)[c]	37 ± 14				1.1 (0.89–1.3)
16 (enoximone)[c]	283 ± 20				2.7 (1.6–4.6)
17 (CI-914)[c]	45 ± 6				0.46 (0.38–0.55)
18 (CI-930)[c]	13 ± 6				0.12 (0.095–0.15)

[a] ED_{50} values were determined by linear regression analysis and are reported as the mean ± SEM ($n > 2$) or mean ± range ($n = 2$) of experimental values. Heart rate and mean arterial blood pressure values are the percent changes recorded at the inotropic ED_{50} values. Control values were contractility, 50 g tension; heart rate (HR), 127 ± 3 beats/min; mean arterial blood pressure (MAP), 99 ± 3 mmHg. [b] SR-PDE = sarcoplasmic reticulum derived phosphodiesterase. Values in parentheses are 95% confidence limits derived from regression analysis of the logit transformation as described in ref 34. [c] Anesthetized dog ED_{50} values for these literature compounds were taken from ref 24.

200 years after its introduction for the treatment of cardiac disorders, it is not anticipated that the controversy surrounding this new class of cardiotonics will be resolved until several well-designed, carefully controlled clinical trials are completed.

We recently described the synthesis, inotropic activity, and structure-activity relationships (SAR) of 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one (**1**, LY195115).[18] This compound is a long-acting, potent cardiotonic; oral administration of 25 μg/kg to conscious, chronically instrumented dogs results in a 50% increase in LV dP/dt_{60} that is sustained in excess of 23 h.[19] We have continued our SAR studies of dihydropyridazinone cardiotonics and in this paper we describe the synthesis and inotropic activity of a series of 5′-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)spiro[cycloalkane-1,3′-[3*H*]indol]-2′(1′*H*)-ones.



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Results and Discussion

Chemistry. The first member of the series investigated, the spirocyclopentane congener **13** (Table I), was synthesized as depicted in Scheme I. Base-induced rearrangement of cyclopentanecarboxylic acid 2-phenylhydrazide (**2**) resulted in formation of the spirocyclopentaneindol-2-one **3** in 75% yield.[20,21] The dihydropyridazinone ring was then constructed by using standard synthetic methodology: Reaction of **3** with succinic anhydride in an aluminum chloride/DMF melt according to the general method of Thyes et al.,[22] followed by hydrazine cyclization, produced **13**. The cyclobutane congener **12** (Table I) was prepared in an analogous fashion, but the base-catalyzed rearrangement leading to formation of the spirocyclic indolone proceeded in poor yield (14%).

Because rearrangement of cyclopropanecarboxylic acid 2-phenylhydrazide did not produce the desired spirocyclopropane indolone **7**, this material was synthesized as depicted in Scheme II. Reaction of indol-2-one (**5**) with an excess of sodium hydride and 1,2-dibromoethane, followed by magnesium-induced debromoethylation afforded

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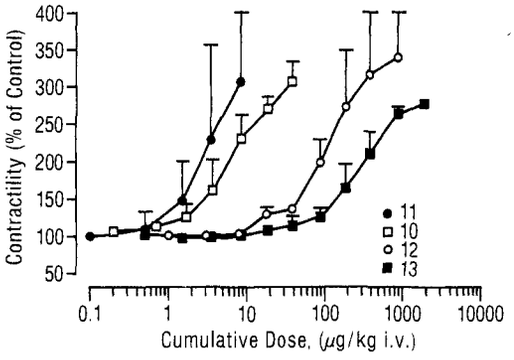
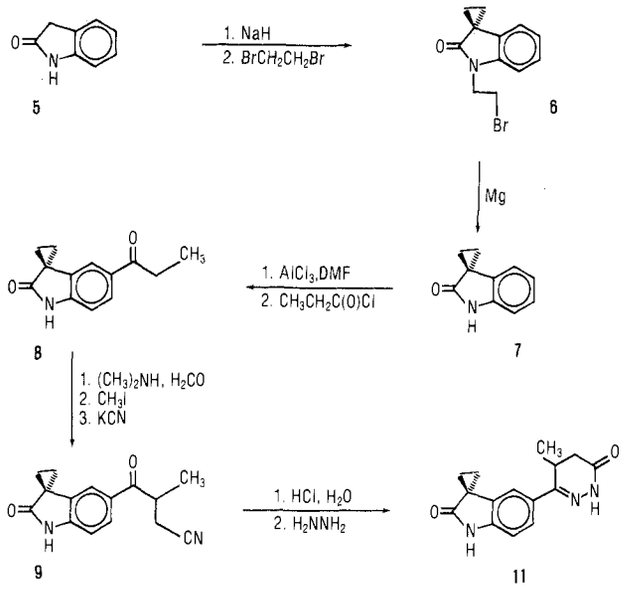


Figure 1. Dose-dependent effects of **10–13** on myocardial contractility in pentobarbital-anesthetized dogs. Increasing doses of drug were administered iv at 5-min intervals and peak responses recorded. Each point is the mean ± SEM (n = 3) or mean ± range (n = 2) of experimental values. Symbols without 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.

Scheme II



7. Friedel-Crafts acylation with succinic anhydride, followed by hydrazine cyclization, produced **10** (Table I). Synthesis of the 4-methyl analogue **11** was also straightforward (Scheme II). Friedel-Crafts reaction of **7** with propionyl chloride yielded **8**. To our surprise, no acid-induced cleavage of the cyclopropane ring was observed in either this reaction or the previously described Friedel-Crafts acylation involving succinic anhydride. Formation of the Mannich base, quaternarization with iodomethane, followed by reaction with potassium cyanide, produced γ-keto nitrile **9**. Sequential acid-catalyzed hydrolysis and hydrazine cyclization completed the synthesis of **11**.

Inotropic Activity of Spirocyclic Dihydropyridazinones. We examined the inotropic activity of these compounds after intravenous administration to open-chested, pentobarbital-anesthetized dogs; a Walton-Brodie strain-guage arch was used to monitor right ventricular contractility. ED_{50} values were determined by linear regression analysis, and data are summarized in Table II and Figure 1. The spirocyclopentane analogue **13** increased contractility in a dose-dependent fashion, with an ED_{50} of 133 μg/kg. It should be noted that the analogous nonspirocyclic 3,3-diethyl analogue was less potent, with an ED_{50} of 741 μg/kg.[18] Thus, reducing the degrees of conformational freedom of the ethyl substituents by formation of the spirocyclopentane ring led to more than a 5-fold increase in inotropic potency. Investigation of the homologues **10** and **12** indicated that spirocycloalkane ring size and inotropic potency are inversely related. ED_{50} values of the spirocyclobutane and spirocyclopropane were 34.8 and 2.7 μg/kg, respectively. When compared to the parent, unsubstituted compound 1,3-dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one (ED_{50} = 24 μg/kg),[18] incorporation of the spirocyclobutane ring maintained potency, whereas the spirocyclopropane ring increased potency 10-fold. This confirms work described in our publication on **1** which revealed that sterically undemanding, lipophilic substituents were advantageous at the 3-position of the indolone ring in these dihydropyridazinone cardiotonics.[18]

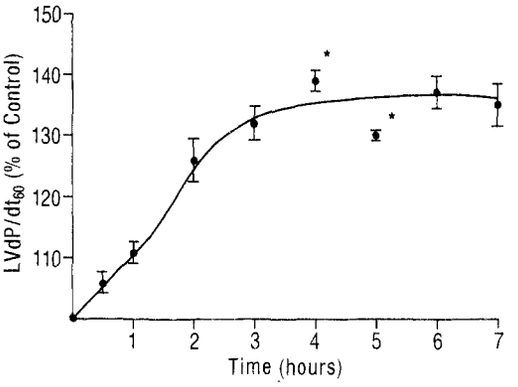


Figure 2. Effects of **10** on LV dP/dt_{60} after oral administration of 50 μg/kg to conscious, chronically instrumented dogs. Values are mean ± SEM of three experiments. Control (base line) values were as follows: LV dP/dt_{60}, 38 ± 2 s^{-1}; heart rate, 55 ± 4 beats/min; LVSBP, 102 ± 2 mmHg. *, $p < 0.05$ compared to control.

Work conducted in our laboratory and by others has demonstrated that a 4-methyl substituent in the dihydropyridazinone ring (β to the dihydropyridazinone carbonyl) leads to an increase in inotropic potency of this class of cardiotonics.[18,23] Consequently, we synthesized **11**, the 4-methyl analogue of the spirocyclopropane congener **10**. After iv administration to anesthetized dogs, compound **11** had an inotropic ED_{50} of 1.5 μg/kg (Table II), a 44% enhancement of potency relative to **10**. In fact, **11** is the most potent noncatecholamine, nonglycoside positive inotrope we have examined to date.

Effects of these compounds on heart rate and mean arterial blood pressure in anesthetized dogs were also monitored, and the magnitude of the changes at inotropic ED_{50} values are shown in Table II. Effects were similar for the compounds except that **11**, the 4-methyl analogue, tended to produce a greater decrease in mean arterial blood pressure than the other congeners.

For any drug to have potential utility in the chronic management of congestive heart failure, oral activity is an important property. Consequently, inotropic activity of **10** was examined after oral administration to conscious, chronically instrumented dogs (Figure 2). LV dP/dt_{60}, the first derivative of left ventricular pressure development at 60 mmHg, was monitored as an index of contractility. After oral administration of 50 μg/kg, a 39% increase in LV dP/dt_{60} was observed 4.0 h postadministration, and

(23) Sircar, I.; Duell, B. L.; Bobowski, G.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1985**, *28*, 1405.

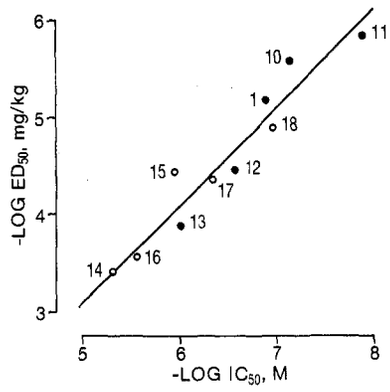


Figure 3. Relationship between $-\log IC_{50}$ values for inhibition of cAMP PDE of sarcoplasmic reticulum vesicles, and $-\log ED_{50}$ values for stimulation of cardiac contractility in anesthetized dogs; $-\log IC_{50}$ values were determined at 1 μM cAMP with free sarcoplasmic reticulum vesicles as described in the Experimental Section. $-\log ED_{50}$ values for spirocyclic dihydropyridazinones (closed circles) were calculated from data in Table II, whereas $-\log ED_{50}$ values for compounds 14–18 (open circles) were taken from published data.[19,24]

this inotropic effect was maintained throughout the 7-h experiment. These data indicate that **10** is a potent, long-acting, and orally effective cardiotonic.

Mechanistic Studies: Inhibition of Sarcoplasmic Reticulum Phosphodiesterase. A number of compounds from this new class of cardiotonics, including milrinone, piroximone, isomazole, and **1**, have been shown to inhibit a specific isozyme of myocardial cAMP phosphodiesterase, generally referred to as PDE III on the basis of its order of elution from anion-exchange chromatography columns. Inhibition of this enzyme appears to play a role in the ability of these agents to elicit inotropic and vasodilator effects.[10,11,24] PDE III is the solubilized form of a particulate enzyme,[25] and we have recently provided evidence that the subcellular origin of this enzyme is the sarcoplasmic reticulum.[26,27] Compound **1** was shown to be a linear competitive inhibitor of highly purified canine sarcoplasmic reticulum derived phosphodiesterase (SR-PDE), with a K_i of 80 nM.[26,27] Because of the potent inotropic activity of the spirocyclic dihydropyridazinones, and their structural relationship to **1**, we examined these compounds for their ability to inhibit this enzyme.

All spirocyclic compounds were inhibitors of SR-PDE. For example, the most potent inotrope of this study, **11**, proved to be the most potent inhibitor of SR-PDE that we have examined to date, with an IC_{50} of 13 nM. As an inhibitor of SR-PDE, **11** is 35-, 85-, and 369-fold more potent than CI-914, milrinone, or amrinone, respectively (vide infra). To examine the relationship, if any, between inhibition of SR-PDE and inotropic actions of this series, SR-PDE IC_{50} values were compared with inotropic ED_{50} values (Table II), and results are shown in Figure 3. For the spirocyclic dihydropyridazinones (closed circles), there was a highly significant correlation between in vitro SR-PDE inhibition and in vivo inotropic effects ($r = 0.949$, $p < 0.02$). When pharmacologically related but structurally diverse inotropes such as amrinone (**14**), milrinone (**15**), enoximone (**16**), CI-914 (**17**), and CI-930 (**18**) were included in the study (Figure 3, open circles), the correlation was even more significant ($r = 0.960$, $p < 0.001$). These data, in conjunction with previous studies involving **1**,[26,27] support the hypothesis that the pharmacological effects of the spirocyclic dihydropyridazinones result from competitive inhibition of SR-PDE.

Conclusions. We have demonstrated that in the 1,3-dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one series of of cardiotonics, a spirocycloalkyl ring may be annealed to the 3-position of the indolone moiety while retaining inotropic activity. When cycloalkyl ring size is four or five, inotropic potency is maintained or decreased relative to the unsubstituted compound, whereas a ring size of three results in a 10-fold increase in potency. Potency can be further increased by incorporation of a 4-methyl substituent into the dihydropyridazinone moiety. Spirocyclopropane analogue **10** was found to be both potent and long-acting after oral administration to dogs. Finally, $-\log IC_{50}$ values for inhibition of SR-PDE for this series of compounds were highly correlated with their inotropic $-\log ED_{50}$ values, supporting the hypothesis that inhibition of this enzyme contributes to the mechanism of action of the spirocyclic dihydropyridazinones.

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 with 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 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 reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation".

Spiro[cyclopentane-1,3′-[3*H*]indol]-2′(1′*H*)-one (3). A mixture of **2**[28] (5 g, 24.5 mmol) and calcium hydride (1.64 g, 39.2 mmol) was slowly heated over a 2.5-h period to 240 °C and maintained at this temperature for 30 min. The reaction mixture was cooled to room temperature and a solution of 20 mL water and 50 mL methanol was slowly added. After hydrogen evolution ceased, concentrated hydrochloric acid was added until the mixture was pH 1. The mixture was warmed at 100 °C for 1 h and 5 N sodium hydroxide was used to adjust the pH to 3. The precipitate was filtered and dried to afford 3.37 g (74%) of homogeneous material. The analytical sample was obtained by recrystallization from ethyl acetate/hexane: mp 110–111 °C (lit.[28] mp 113 °C). Anal. ($C_{12}H_{13}NO$) C, H, N.

5′-(1,4,5,6-Tetrahydro-6-oxo-3-pyridazinyl)spiro[cyclopentane-1,3′-[3*H*]indol]-2′(1′*H*)-one (13). DMF (3.9 mL, 49.4 mmol) was added in a dropwise fashion to anhydrous aluminum chloride (23.5 g, 176 mmol), and the exothermic reaction was allowed to cool to room temperature. An intimate mixture of succinic anhydride (1.77 g, 17.6 mmol) and **3** (3.3 g, 17.6 mmol) was slowly added to the $AlCl_3$/DMF melt. The reaction mixture was stirred 1 h at 80 °C, slowly poured onto ice, and acidified with concentrated hydrochloric acid, and the product was isolated by filtration. Recrystallization from DMF/water provided 1.3 g (25%) of **4** (Scheme I) with mp 160 °C dec.

A mixture of **4** (1.2 g, 4.2 mmol) and 85% hydrazine hydrate (0.54 mL, 9.2 mmol) in 250 mL of absolute ethanol was refluxed 4 h and cooled to room temperature. Removal of solvent in vacuo followed by flash chromatography (0–5% methanol in methylene chloride gradient, silica gel) provided 440 mg (37%) of homoge-

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(27) Kauffman, R. F.; Crowe, V. G.; Utterback, B. G.; Robertson, D. W. *Mol. Pharmacol.* **1986**, *30*, 609.
(28) Moore, R. F.; Plant, S. G. P. *J. Chem. Soc.* **1951**, 3475.

neous **13** as an off-white solid with mp >300 °C. Anal. ($C_{16}H_{17}N_3O_2$) C, H, N.

Cyclobutanecarboxylic Acid 2-Phenylhydrazide (19). A solution of cyclobutanecarboxylic acid chloride (40.0 g, 333 mmol) in 200 mL of DMF was added dropwise to a solution of phenylhydrazine (36.5 mL, 371 mmol) and pyridine (32.7 mL, 405 mmol) in 300 mL of DMF at room temperature. After the mixture was stirred overnight at room temperature, product isolation (1 N hydrochloric acid, ethyl acetate, water, brine, Na_2SO_4) and recrystallization from ethyl acetate provided 46.46 g (72%) of **19** as white crystals with mp 160–161 °C. Anal. ($C_{11}H_{14}N_2O$) C, H, N.

5′-(1,4,5,6-Tetrahydro-6-oxo-3-pyridazinyl)spiro[cyclobutane-1,3′-[3*H*]indol]-2′(1′*H*)-one (12). Spiro[cyclobutane-1,3′-[3*H*]indol]-2′(1′*H*)-one (**20**) was prepared as previously described for **3**, with use of **19** (10 g, 52.0 mmol) and calcium hydride (3.5 g, 83.2 mmol). Product isolation (ethyl acetate, water, brine, Na_2SO_4) and flash chromatography (silica gel, 0–50% ethyl acetate in hexane gradient) provided 1.27 g (14%) of homogeneous **20** as a light yellow solid. ^1H NMR and mass spectra were consistent with the assigned structure.

Via the procedure previously described for **4**, 1′,2′-dihydro-γ,2-dioxospiro[cyclobutane-1,3′-[3*H*]indole]-5′-butanoic acid (**21**) was prepared with use of the following reagents: DMF (2.61 mL, 33.2 mmol), aluminum chloride (15.8 g, 118.5 mmol), succinic anhydride (1.19 g, 11.85 mmol), and **20** (2.05 g, 11.85 mmol). Product isolation afforded 1.1 g (34%) of **21** as a yellow solid that was used without additional purification. The mass spectrum was consistent with the assigned structure.

A mixture of **21** (1.1 g, 4.03 mmol) and 85% hydrazine hydrate (0.52 mL, 8.9 mmol) in 200 mL of absolute ethanol was refluxed 1.5 h and cooled slowly to room temperature. Filtration afforded 560 mg (52%) of **12** as light yellow crystals with mp >300 °C. Anal. ($C_{15}H_{15}N_3O_2$) C, H, N.

1′-(2-Bromoethyl)spiro[cyclopropane-1,3′-[3*H*]indol]-2′(1′*H*)-one (6). Sodium hydride (4.6 g of a 60% suspension in oil, 115 mmol) was added in portions to a solution of indol-2-one (**5**) (5 g, 38 mmol) in 250 mL of DMF. The reaction mixture was stirred 15 min at room temperature, cooled to 0 °C, and stirred for an additional 15 min. A solution of 1,2-dibromoethane (6.64 mL, 77 mmol) in 125 mL of DMF was added in one portion, and the reaction mixture was warmed to room temperature and stirred for 2 h. Product isolation (water, ethyl acetate, water, brine, Na_2SO_4) and flash chromatography (5–20% ethyl acetate in hexane gradient, silica gel) provided 1.46 g (15%) of homogeneous **6** as a light-red oil. Anal. ($C_{12}H_{12}BrNO$) C, H, N.

Spiro[cyclopropane-1,3′-[3*H*]indol]-2′(1′*H*)-one (7). Approximately one-fourth of a solution of **6** (14.0 g, 52.6 mmol) in 150 mL of anhydrous THF was added in a dropwise fashion to magnesium (12.8 g, 526 mg-atom). Several microliters of 1,2-dibromoethane was added to initiate the reaction, and gas evolution became apparent. The remainder of the solution of **6** was added in a dropwise fashion and the reaction mixture was refluxed 18 h. The solution was cooled to room temperature, filtered, and evaporated in vacuo. The residue was treated with 1 N hydrochloric acid and extracted first with methylene chloride and then with ethyl acetate. Removal of solvents in vacuo followed by flash chromatography provided 3.64 g (44%) of **7** as a light-yellow solid with mp 182–184 °C (lit.[29] mp 185–187 °C). Anal. ($C_{10}H_9NO$) C, H, N.

1′,2′-Dihydro-γ,2-dioxospiro[cyclopropane-1,3′-[3*H*]-indole]-5′-butanoic Acid (22). Via the procedure previously described for **4**, **22** was prepared with use of the following reagents: DMF (0.69 mL, 8.8 mmol), aluminum chloride (4.2 g, 31.4 mmol), succinic anhydride (315 mg, 3.1 mmol), and **7** (0.500 g, 3.1 mmol). Product isolation and recrystallization from DMF/water afforded 467 mg (58%) of **22** as light tan crystals with mp 210 °C dec. Anal. ($C_{14}H_{13}NO_4$) C, H, N.

5′-(1,4,5,6-Tetrahydro-6-oxo-3-pyridazinyl)spiro[cyclopropane-1,3′-[3*H*]indol]-2′(1′*H*)-one (10). A mixture of **22** (350 mg, 1.34 mmol) and 85% hydrazine hydrate (0.17 mL, 2.95 mmol) in 25 mL of absolute ethanol was refluxed 4 h and cooled slowly to room temperature. Filtration afforded 224 mg (65%) of **10** as light yellow crystals with mp >300 °C. Anal. ($C_{14}H_{13}N_3O_2 \cdot {}^1/_2H_2O$) C, H, N.

5′-(1-Oxopropyl)spiro[cyclopropane-1,3′-[3*H*]indol]-2′(1′*H*)-one (8). DMF (7.5 mL, 95.6 mmol) was added in a dropwise fashion to anhydrous aluminum chloride (45.5 g, 342 mmol) to form a melt. After the exothermic reaction had cooled to 40 °C, a mixture of proprionyl chloride (2.97 mL, 34.1 mmol) and **7** (5.43 g, 34.1 mmol) was added in portions. The reaction was heated to 70 °C for 2 h and poured onto ice. Concentrated hydrochloric acid (50 mL) was added, the mixture was cooled, and the precipitate was filtered and dried to provide 7.0 g (95%) of homogeneous product as a solid. Recrystallization from ethyl acetate gave 4.9 g of **8** with mp 183.5–185 °C. Anal. ($C_{13}H_{13}NO_2$) C, H, N.

1′,2′-Dihydro-*N*,*N*,*N*,β-tetramethyl-γ,2′-dioxospiro[cyclopropane-1,3′-[3*H*]indole]-5′-propanaminium Iodide (23). Dimethylamine hydrochloride (3.56 g, 43.6 mmol) and formaldehyde (2.84 mL of a 37% aqueous solution, 34.9 mmol) were stirred for 15 min at room temperature. Acetic anhydride (17.6 mL, 186 mmol) was added to this mixture, and the reaction mixture was heated to approximately 40 °C. An exothermic reaction ensued, and the temperature increased to 105 °C. The reaction was allowed to cool to 90 °C, and **8** (6.25 g, 29.1 mmol) was added in one portion. The reaction mixture was maintained at 90 °C for 2 h, cooled to room temperature, and stirred overnight. The reaction was concentrated in vacuo, 200 mL of acetone was added, and the mixture was refluxed 10 min. Solvent was removed under reduced pressure, and the residue was dissolved in water and extracted with ethyl acetate (discarded). The pH of the aqueous solution was adjusted to 8 with 5 N sodium hydroxide. Product isolation (ethyl acetate, brine, Na_2SO_4) afforded 4.2 g of Mannich base as an oil.

Iodomethane (3.44 mL, 55.2 mmol) was added to a solution of the Mannich base (4.2 g) in 100 mL of acetone. The reaction was stirred overnight at room temperature. The precipitate was filtered and dried to provide 4.44 g (37% over two steps) of **23** as a white solid with mp 163–170 °C. Anal. ($C_{17}H_{23}IN_2O_2$) C, H, N.

1′,2′-Dihydro-β-methyl-γ,2′-dioxospiro[cyclopropane-1,3′-[3*H*]indole]-5′-butanenitrile (9). A solution of potassium cyanide (1.7 g, 25.7 mmol) in 50 mL of water was added to a solution of **23** (4.44 g, 10.7 mmol) in 100 mL of methanol. The reaction mixture was stirred overnight at room temperature. Product isolation (water, ethyl acetate, water, brine, Na_2SO_4) followed by flash chromatography (0–55% ethyl acetate in hexane gradient, silica gel) yielded 2.40 g (88.2%) of homogeneous **9** as a white solid with mp 143–146 °C. Anal. ($C_{15}H_{14}N_2O_2$) C, H, N.

5′-(1,4,5,6-Tetrahydro-4-methyl-6-oxo-3-pyridazinyl)spiro[cyclopropane-1,3′-[3*H*]indol]-2′(1′*H*)-one (11). A mixture of nitrile **9** (2.40 g, 9.4 mmol) and 30 mL of 6 N hydrochloric acid was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and diluted with 200 mL of water. The precipitate was filtered and dried to obtain 1.68 g of the carboxylic acid as a light-yellow powder with mp 161–162 °C. ^1H NMR and mass spectra were consistent with the assigned structure.

Hydrazine hydrate (0.78 mL of an 85% solution, 13.3 mmol) was added to a suspension of the unpurified acid (1.65 g, 6.0 mmol) in 25 mL of ethanol, and the reaction mixture was heated to reflux 3.5 h. The reaction mixture was slowly cooled to 0 °C, the the precipitate was filtered. This provided 1.05 g (42% over two steps) of **11** as a white solid with mp 291–293 °C. Anal. ($C_{15}H_{15}N_3O_2$) C, H, N.

Pharmacological Methods. 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 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 units/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

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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 on changes in preload and afterload. Subcutaneous pin electrodes provided a lead II ECG. Increasing 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} values were determined by linear regression analysis and are reported as the mean ± SEM of experimental values. A different set of animals was used for each test compound.

Conscious Dog Studies. Male mongrel dogs weighing 15–36 kg were chronically instrumented to monitor $LVdP/dt_{60}$ (the first derivative of left ventricular pressure at 60 mmHg), peak systolic blood 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 2 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. Dogs were fasted 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.

Preparation of Sarcoplasmic Reticulum Vesicles. Subfractions of sarcoplasmic reticulum (SR) vesicles, A–E, were prepared from ventricles of pentobarbital-anesthetized dogs as described by Jones and co-workers.[30,31] Fraction E, which originates from free SR vesicles,[32] was used in all inhibition studies. Aliquots of free SR vesicles were stored frozen at −80 °C until used. Under these conditions no loss of PDE activity was detected after 6 months of storage. Vesicle protein was determined by the method of Lowry.[33]

Enzyme Assays. Cyclic nucleotide phosphodiesterase was assayed by the two-step technique of Thompson et al.[25] PDE reactions were initiated by adding sufficient enzyme to hydrolyze less than 20% of the substrate (1 μM cAMP) in 60 min at room temperature (22 ± 2 °C). Reactions were terminated by placing tubes in boiling water for 45 s. PDE activity was linear vs. time and protein concentration; test compounds had no effect upon the snake venom (*Ophiophagus hannah*) used to convert [^{3}H]-AMP to [^{3}H]adenosine in the second step of the assay (data not shown). Dimethyl sulfoxide was utilized as solvent for PDE inhibitors. Solutions were prepared on the day of an experiment, and controls were run to ensure that carryover solvent (2.5%, v/v) did not affect assay results. IC_{50} values and 95% confidence limits were calculated as previously described.[34]

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Registry No. **2**, 103490-49-9; **3**, 41058-67-7; **4**, 103490-50-2; **5**, 59-48-3; **6**, 103490-47-7; **7**, 13861-75-1; **8**, 83419-51-6; **9**, 103490-56-8; **10**, 103490-44-4; **11**, 103515-48-6; **12**, 103490-46-6; **13**, 107081-83-4; **19**, 103490-51-3; **20**, 103490-52-4; **21**, 103490-53-5; **22**, 103490-48-8; **23**, 103490-54-6; CH_3CH_2COCl, 79-03-8; HCHO, 50-00-0; $\overline{CH_2CH_2CH_2}CHCOCl$, 5006-22-4; succinic anhydride, 108-30-5; 2′,8-dioxo-β-methylspiro[cyclopropane-1,3′-indol]-5′-butanoic acid, 103490-57-9.

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Estrogenic Affinity Labels: Synthesis, Irreversible Receptor Binding, and Bioactivity of Aziridine-Substituted Hexestrol Derivatives

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To develop an affinity label for the estrogen receptor that would be an estrogen agonist, rather than antagonist, we prepared several aziridine derivatives of the potent nonsteroidal estrogen hexestrol ((3*R**,4*S**)-3,4-bis(4-hydroxyphenyl)hexane) bearing an aziridine function on the side chain. Three functional groups link the hexestrol ligand and the aziridine: a carbonyl group (ketone or ester), a thioether, or a methylene chain. The apparent competitive binding affinity of these derivatives for the estrogen receptor ranges from 1.8% to 25% that of estradiol, and most of them bind in a time-dependent, irreversible manner with the receptor, although the rate and efficiency of this binding vary widely, often with relatively small changes in structure. This is consistent with the irreversible attachment requiring a precise alignment of activating and reacting residues in the binding site of the receptor. The estrogenic and antiestrogenic activity of these aziridine derivatives was investigated in MCF-7 human breast cancer cells. Most of the compounds are agonists, with one being an antagonist. The derivative (6*R**,7*S**)-1-*N*-aziridinyl-6,7-bis(4-hydroxyphenyl)-5-nonanone (keto-nonestrol aziridine **3**) appears to have the most ideal behavior of the estrogenic affinity labeling agents prepared: It is an agonist, and it binds to receptor irreversibly, efficiently, and quite rapidly.

The affinity labeling concept, whereby reversibly binding ligands are elaborated into chemically reactive derivatives capable of covalently labeling binding proteins, has been applied effectively to develop covalent labeling agents for steroid receptors.[1] Both photochemically reactive and electrophilic derivatives have been prepared, and labeling studies have been conducted with estrogen, androgen, progestin, and corticosteroid receptors.[1] In general, the affinity labeling agents have high covalent labeling selectivity, since they retain a good deal of the binding selectivity of the reversibly binding ligands.

We have found that an aziridine analogue of tamoxifen, tamoxifen aziridine (**1b**), is an effective affinity label for the estrogen receptor.[2] It is capable of covalent labeling

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