A Novel Class of Cardiotonic Agents: Synthesis and Biological Evaluation of 5-Substituted 3,6-Dihydrothiadiazin-2-ones with Cyclic AMP Phosphodiesterase Inhibiting and Myofibrillar Calcium Sensitizing Properties

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As part of a search for new cardiotonic agents significantly sensitising the myocardial contractile proteins to calcium, together with cardiac cyclic AMP-PDE inhibitory activity, we have discovered that novel 5-substituted 3,6-dihydrothiadiazin-2-ones may fulfill both properties. The sensitising effect of the contractile proteins to calcium, assessed by the shift in the calcium sensitivity of canine cardiac myofibrillar magnesium-dependent ATPase, is determined by steric and electronic requirements. The requirements for phosphodiesterase inhibition, especially that of a near-planar arrangement for the phenyl and thiadiazin-2-one ring are consistent with those already described for analogous pyridazinones. The synthesis and structure–activity relationships are discussed.

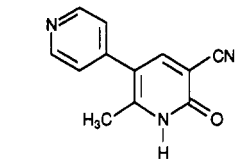
Congestive heart failure (CHF) is a common syndrome which has been recently suggested to be the most prevalent cause of death in hospitalized patients.[1] Digitalis and related cardiac glycosides have been employed in the treatment of CHF for nearly two centuries.[2] However these agents are limited in their therapeutic use by arrhythmogenic liability and consequently narrow therapeutic range. Sympathomimetic agents such as dobutamine and dopamine have also been successfully used in the acute treatment of CHF. However most of the existing sympathomimetic agents display both a lack of oral efficacy and tachyphylaxis resulting from β receptor down regulation.[3] This lack of safe, orally-active positive inotropic agents has initiated considerable efforts devoted to the search for novel cardiotonics.

Several noncatecholamine, nonglycoside cardiotonics which simultaneously display inotropic and vasodilator activities have been clinically studied, e.g. milrinone (1),[4] enoximone (2),[5] imazodan (3),[6] and indolidan (4).[7,8] (Chart I). The major mechanism of action of these compounds appears to be linked with the inhibition of the cardiac low-Km, cAMP-specific phosphodiesterase, cGMP inhibited and rolipram insensitive,[4,9] and, for some of them, to a particulate or membrane-bound isoform of this enzyme[10] which was demonstrated to be located on the sarcoplasmic reticulum.[8,11]

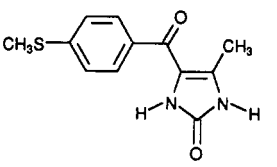
This class of drugs incontrovertibly produces salutary haemodynamic effects, resulting in an improvement in quality of life of patients with severe CHF, following either intravenous or oral administration.[5] Despite this positive aspect, the use of these drugs remains debatable as a beneficial effect on patient survival has not been established.[12] Moreover, the use of an agent that may increase contractility by an increase in myocardial cyclic AMP is of obvious concern, as such a mechanism may favor arrhythmias. With this class of "inodilators" there is also a risk that the beneficial energy-sparing effects of afterload reduction may be counterpoised by the energy-consuming inotropic activity.

For all these reasons, we focused our efforts on the discovery of compounds which also act by an enhancement of the sensitivity of myofilaments to calcium, a mechanism first described by Herzig and Rüegg[13] and developed by Solaro and co-workers.[14] This "calcium-sensitizing" effect has first been described as contributing to the positive inotropic effect of sulmazole (5),[13-15] and later also to that of isomazole (6),[16,17] pimobendan (7),[18-20] adibendan (8),[21,22] and 3-amino-6-methyl-5-phenyl-2(1*H*)-pyridinone (APP 201-533) (9)[23] (Chart II).

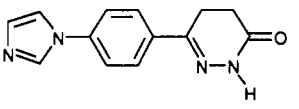
Chart I



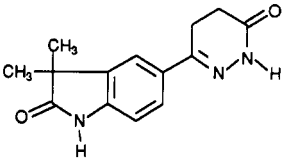
1 milrinone



2 enoximone



3 imazodan



4 indolidan

Table I. Biochemical Activities of Compound 10 Compared to Standards Claimed To Combine PDE Inhibition with Ca^{2+} Sensitization of Cardiac Myofibrils

no.	SR-PDE inhibition IC_{50},[a] μM	myofibrillar ATPase ΔpCa_{50}[b]	[μM]	n[c]	pCa_{50} control[d]
5 (sulmazole)	140.00	0.05 ± 0.02	200	6	6.64 ± 0.03
7 (pimobendan)	0.56	0.09 ± 0.03	200	6	6.71 ± 0.04
8 (adibendan)	0.52	0.16 ± 0.03	200	6	6.82 ± 0.03
9 (APP 201-533)	18.00	0.10 ± 0.04	200	6	6.75 ± 0.05
10	1.20	0.39 ± 0.03	200	5	6.77 ± 0.08

[a] IC_{50} was determined according to procedure described in "biochemistry" from the mean inhibition curve obtained from three to four different SR-PDE preparations. [b] ΔpCa_{50} is the shift, induced by each compound, of the Ca^{2+} concentration (expressed as its negative logarithm) required for half-maximal activation of the cardiac myofibrillar ATPase. Values, determined as described in "biochemistry" are expressed as mean ± SEM. [c] n is the number of experiments performed on different cardiac myofibrillar preparations. [d] pCa_{50} control is the Ca^{2+} concentration (expressed as its negative logarithm) required for half-maximal activation of the cardiac myofibrillar ATPase, in the absence of the drug.

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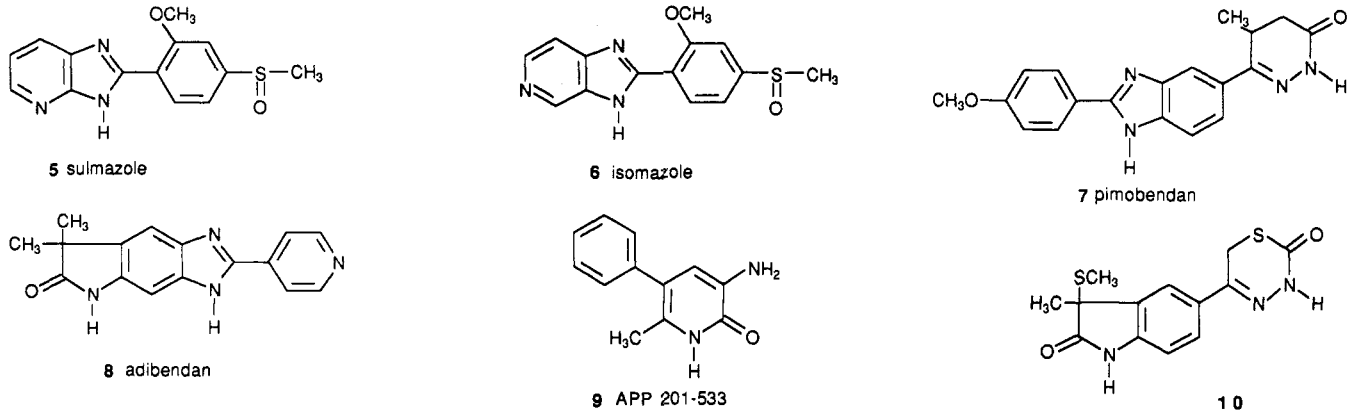
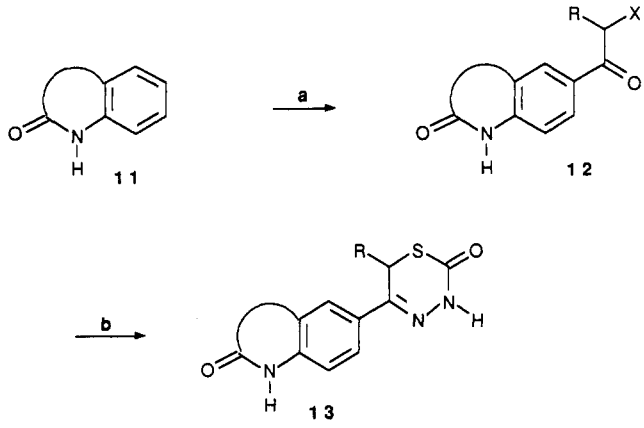
(4) For a review, see: Erhardt, P. W. *J. Med. Chem.* **1987**, *30*, 231-237.

0022-2623/92/1835-0163$03.00/0

Chart II



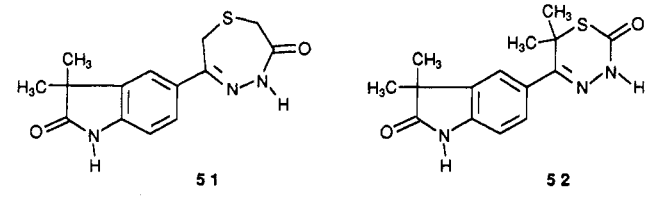
Scheme I[a]



[a] (a) RCHXCOCl, $AlCl_3$, DMF, 80 °C; (b) $H_2NNHC(S)OCH_3$, acetonitrile, reflux.

The potential interest of this mechanism is that it does not require an increase in the cytosolic free calcium concentration to augment tension development, and thus it avoids the risk of calcium overload and associated arrhythmias. In addition, experiments measuring heat production in rat papillary muscle[24] demonstrated that the contraction evoked by calcium-sensitizing agents are produced more economically than those evoked by adrenergic drugs or pure phosphodiesterase inhibitors, suggesting that this mode of action might be of benefit for the failing heart.

Chart III



In this study we have shown that 5-(3,6-dihydro-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-1,3-dihydro-3-methyl-3-methylthio)-2*H*-indol-2-one (**10**) at 200 μM was more active in shifting the calcium sensitivity of canine myofibrillar magnesium-dependent ATPase, an enzymatic activity closely related to calcium binding proteins,[14] than all the lead compounds of Chart II (Table I). However, the drug concentration of **10** required to observe significant activity on myofibrillar ATPase was at least 2 orders of magnitude higher than that required for a significant inhibition of the phosphodiesterase activity.

In order to fully evaluate the effects of calcium sensitisation we focused our efforts on the design of compounds that were as active as possible on the myofibrillar ATPase activity and as weak as possible on the inhibition of the phosphodiesterase activity.

Results and Discussion

Chemistry. The 3,6-dihydrothiadiazin-2-ones have been obtained through the general route illustrated in Scheme I.

Starting from an heterocycle containing an unsubstituted nitrogen atom, we could prepare the para-positioned α-haloacyl intermediate by a simple Friedel–Crafts reaction using aluminium chloride in DMF. This intermediate was condensed with *O*-methyl thiocarbazate[25,26] by refluxing for a few hours in acetonitrile or ethanol. In some cases,

(5) For a review of the clinical evalution, see the supplements of the August issue of *Am. J. Cardiol.* **1987**, *60*(5), whole issue.
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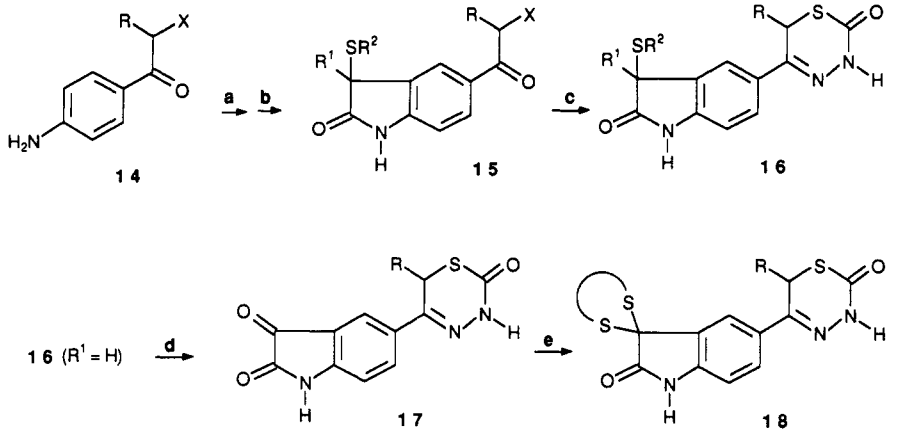
Scheme II[a]



[a] (a) (1) t-BuOCl, CH_2Cl_2, −65 °C; (2) alkyl α-alkylthiocarboxylate; (3) NEt_3; (b) HCl 2 N, room temperature; (c) $H_2NNHC(S)OCH_3$, acetonitrile, reflux; (d) (1) NCS, CH_2Cl_2, room temperature, (2) HgO, BF_3·Et_2O, THF, H_2O; (e) alkanedithiol, BF_3·Et_2O, AcOH, room temperature.

the yield of this condensation has been improved by adding either triethylamine or trifluoroacetic acid as catalyst. Compounds **35**, **41–46** and **53** to **55** have been prepared by the same sequence, using bromopropanoyl bromide and the appropriate heterocycle.

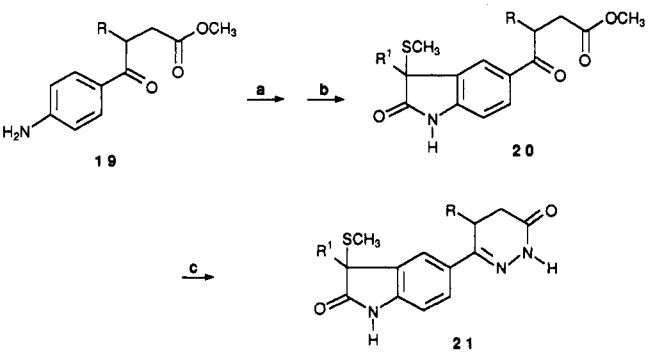
The same route afforded **31**, **32**, **33**, and **40** using bromoacetyl chloride and 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one, 1,3-dihydro-2*H*-indol-2-one, 1,3-dihydro-3-methyl-2*H*-indol-2-one, and 3,4-dihydro-2(1*H*)-quinolinone, respectively. In the same manner 2-bromo-2-methylpropanoyl chloride condensed with 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one, followed by a cyclization with *O*-methyl thiocarbazate, led to the 6,6-dimethylthiadiazinone analogue **52** (Chart III).

This sequence could not be used in the case of 3-alkylthio-substituted indolones, due to the poor stability of thioethers towards Friedel–Crafts conditions. A more general procedure reported elsewhere[27,28] starting from 1-(4-aminophenyl)-2-chloropropanone[29] afforded these compounds (Scheme II) via a Gassman reaction.[30]

This reaction applied on the substituted aniline **14** afforded the 3-substituted indole **15**. This procedure can be used with linear α-alkylthio carboxylate esters like methyl (methylthio)propanoates or ethyl (methylthio)acetate[31] but also with cyclic thio ethers like methyl 2-thiolanecarboxylates[32] to afford, for example, the starting nucleus for the synthesis of **48**.

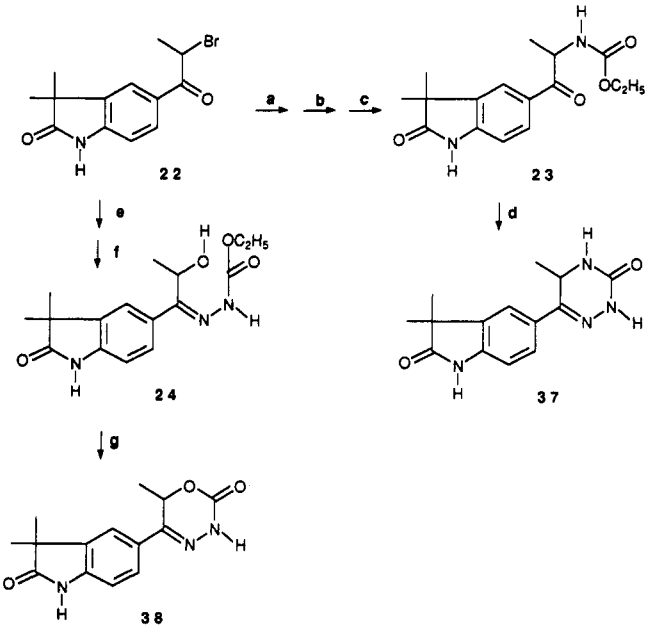
The same procedure, starting from methyl 3-(4-aminophenyl)-3-oxobutanoate (**19**) afforded easily, after cyclization with hydrazine, compounds of type **21** (Scheme III). Gassman described an interesting transformation of 3-(methylthio)indolone into isatine.[33] The method, applied to structure like **16**, afforded the key intermediate **17**[27] for compounds of type **18** (Scheme II). Other heterocycles, replacing the thiadiazinone ring, have also been prepared. The compound **23** (Scheme IV) was obtained by successive substitution of the bromide **22** by an azide (NaN_3, dioxane), reduction of the resulting functionality into an amino group (H_2, Pd/C, EtOH, HCl, 1 atm, room temperature),

Scheme III[a]



[a] (a) (1) t-BuOCl, CH_2Cl_2, −65 °C; (2) alkyl α-alkylthiocarboxylate; (3) NEt_3; (b) HCl 2 N, room temperature; (c) H_2NNH_2, EtOH, reflux.

Scheme IV[a]



[a] (a) NaN_3, dioxane; (b) H_2, Pd/C, EtOH, HCl, 1 atm, room temperature; (c) EtOCOCl, pyridine, EtOH, room temperature; (d) H_2NNH_2, EtOH, reflux; (e) H_2O, DMF, 100 °C; (f) ethyl carbazate, EtOH, HCl, reflux; (g) EtONa, EtOH, room temperature.

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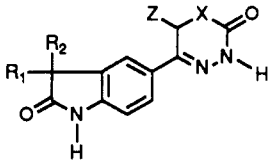
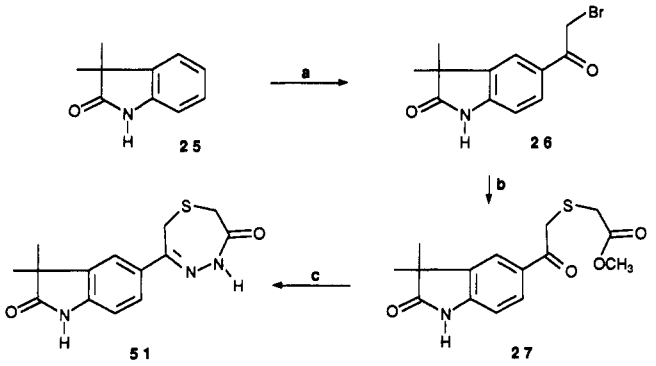
Table II. Structure and Ca^{2+} Sensitizing Properties of Substituted Indolones



no.	R_1	R_2	X	Z	myofibrillar ATPase ΔpCa_{50}[a]	[μM]	n[b]	pCa_{50} control[c]
10	CH_3	SCH_3	S	H	0.39 ± 0.03	200	5	6.77 ± 0.08
28	CH_3	SCH_3	CH_2	H	0.07 ± 0.06	200	3	6.90 ± 0.08
29	CH_3	SCH_3	S	CH_3	0.33 ± 0.08	200	3	6.83 ± 0.11
30	CH_3	SCH_3	CH_2	CH_3	0.11 ± 0.06	200	4	6.60 ± 0.10
31	CH_3	CH_3	S	H	0.16 ± 0.06	200	4	6.82 ± 0.10
32	H	H	S	H	0.08 ± 0.02	200	3	6.72 ± 0.12
33	H	CH_3	S	H	0.15 ± 0.02	200	3	6.74 ± 0.02
34	H	SCH_3	S	H	0.28 ± 0.09	200	3	6.84 ± 0.07
35	CH_3	CH_3	S	CH_3	0.31 ± 0.06	200	4	6.83 ± 0.10
4 (indolidan)	CH_3	CH_3	CH_2	H	−0.04 ± 0.04	200	6	6.73 ± 0.06

[a] See footnote *b* in Table I. [b] See footnote *c* in Table I. [c] See footnote *d* in Table I.

Scheme V[a]



[a] (a) $BrCH_2COCl$, $AlCl_3$, DMF, 80 °C; (b) $HSCH_2CO_2CH_3$, K_2CO_3, CH_3CN, reflux; (c) H_2NNH_2, CH_3CN, reflux.

and condensation with ethyl chloroformate (pyridine, EtOH, room temperature). Subsequent cyclization with hydrazine (EtOH, reflux) afforded the triazinone **37**. The oxadiazinone **38** was obtained after hydrolysis of the bromide **22** (H_2O/DMF, 100 °C), condensation with ethyl carbazate (EtOH, HCl, reflux), affording **24**, and cyclization under basic conditions (EtONa, EtOH, room temperature). The synthesis of the selenodiazinone **39** was complex and has been described elsewhere.[34]

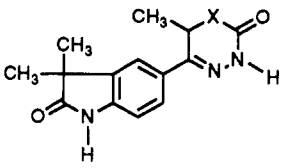
The compound **51** was obtained in a two-step sequence via condensation of the α-bromo ketone **26** with methyl thioacetate (K_2CO_3, CH_3CN, reflux), followed by a cyclisation with hydrazine (EtOH, reflux) (Scheme V).

Mechanistic Studies. The sensitization of myocardial contractile proteins to calcium was assessed on the canine cardiac myofibrillar Ca^{2+}-dependent Mg^{2+} ATPase (MF-ATPase) by measuring the shift of the pCa–MF-ATPase curve at a given compound concentration. The data were expressed as the shift in the negative logarithm of Ca^{2+} concentration required for half-maximal activation (ΔpCa_{50}).

The inhibition of cardiac phosphodiesterase was assessed on the canine cardiac sarcoplasmic reticulum bound, cGMP inhibited, high affinity cAMP-PDE (SR-PDE).

Inotropic Activity. The positive inotropic activities were determined in acutely instrumented anaesthetized and in chronically instrumented conscious dogs by monitoring percentage increases in maximum dP/dt (dP/dt max) of left ventricular pressure. Heart rate was monitored simultaneously.

Table III. Structure and Biochemical Properties of Some Heterocyclic Indolones



no.	X	SR-PDE inhibition IC_{50},[a] μM	myofibrillar ATPase ΔpCa_{50}[b]	[μM]	n[c]	pCa_{50} control[d]
35	S	0.33	0.24 ± 0.05	100	4	6.91 ± 0.09
36	CH_2	0.24	−0.09 ± 0.02	100	3	6.88 ± 0.10
37	NH	0.17	−0.04 ± 0.02	100	3	6.96 ± 0.08
38	O	0.96	0.04 ± 0.02	100	3	6.96 ± 0.08
39	Se	0.54	0.30 ± 0.07	100	3	6.88 ± 0.10

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] See footnote *c*, Table I. [d] See footnote *d*, Table I.

Structure–Activity Relationship in Vitro and in Vivo. Our initial target was compound **10**. Table II clearly shows that compound **10**, unlike indolidan (**4**), possesses calcium-sensitizing properties. In order to know which structural features, present in compound **10** but not in **4**, might be responsible for conferring this new activity, we initially prepared the remaining compounds of Table II. The biological data obtained from these new compounds clearly suggested that the introduction of a sulfur atom into the pyridazine ring favored the desired activity. Furthermore, substitution of the indolone moiety also appeared essential, with compound **32** being only marginally active.

Wishing to check whether sulfur is the only atom bearing the desired activity, we synthesized a short series of heterodiazines. Data of Table III clearly established that (compared to a pyridazinone **36**, a triazinone **37**, or an oxadiazinone **38**) the thiadiazinone **35** and the obviously similar selenodiazinone **39** displayed a significant superiority in calcium-sensitizing activity. Despite a similar activity of the selenium derivative, we chose to develop the thiadiazinones, the synthesis being easier and the selenium atom introducing a potential risk of toxicity.

The next step in our SAR study was to check whether the indole moiety was a definite requirement for our target

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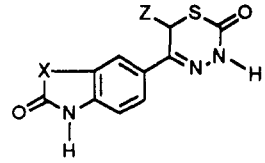
Table IV. Structure and Ca^{2+}-Sensitizing Properties of Heteroaryldihydrothiadiazinones



			myofibrillar ATPase			
no.	X	Z	ΔpCa_{50}[a]	[μM]	n[b]	pCa_{50} control[c]
40	CH_2CH_2	H	0.00 ± 0.01	30	3	6.88 ± 0.10
41	$CH_2C(CH_3)_2$	CH_3	0.45 ± 0.14	30	3	6.94 ± 0.10
42	$C(CH_3)_2CH_2$	CH_3	0.33 ± 0.07	30	3	6.71 ± 0.07
43	$OC(CH_3)_2$	CH_3	0.15 ● 0.03	30	3	6.99 ± 0.22
44	$NHC(CH_3)_2$	CH_3	0.24 ± 0.02	30	3	6.84 ± 0.04
35	$C(CH_3)_2$	CH_3	0.09 ● 0.03	30	4	6.91 ± 0.09

[a] See footnote *b*, Table I. [b] See footnote *c*, Table I. [c] See footnote *d*, Table I.

Table V. Cardiovascular Profile of Dihydrothiadiazinone Cardiotonics in Conscious Dog

		peak responses: % changes of control		
no.	dose, μg/kg po[a]	dP/dt max[b]	HR	n[c]
35	30	73 ± 9	8 ± 4	3
41	30	21 ± 4	5 ± 5	4
42	30	26 ± 6	24 ± 5	2
43	30	67 ± 5	13 ± 4	4
44	30	38 ± 9	9 ± 4	3

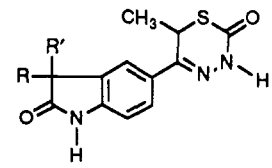
[a] Drugs were solubilized in DMI + Tween 80 + H_2O (1:2:2 mL) and administered in gelatine capsules. [b] LV dP/dt max was used as an index of contractility. [c] Reported values are the mean ± SEM of n values.

activity. The data of Table IV demonstrated again that substitution of the heteroaromatic ring dramatically improved the activity of the compounds (e.g., **41** and **42** compared to **40**; the additional methyl on the thiadiazine ring of **41** and **42** is unlikely to account for such a change, as can be seen from the comparison between **10** and **29**).

From a biochemical point of view several of the heterocycles of Table IV seemed superior to the indolone. Nevertheless, further in vivo oral testing (Table V) demonstrated a higher inotropic potency and better selectivity (vs chronotropy) for the indole compounds, confirming an observation already made by Robertson and co-workers[7] within a series of pyridazinone compounds. While these differences in the inotropic potency may likely be explained by differences in bioavailability when given po, the dissociation of inotropic vs chronotropic effects may more adequately be explained by differences in isozymic forms of PDE in sino-atrial vs ventricular tissue. Recent investigations with inodilators and other PDE inhibitors would support this.[35]

With the continuing aim of maximizing the calcium-sensitizing properties and of minimizing the PDE inhibitory activity of these compounds, we designed a set of spiro derivatives of increasing bulkiness (Table VI) based upon the results of Tables II and IV and on previous data[36] obtained for a short series of spirocyclic dihydropyridazinone cardiotonics. These latter results suggested that the SR-PDE inhibitory potency in these related

Table VI. Structure and Biochemical Activities of Spirocyclic Dihydrothiadiazinone Cardiotonics



		SR-PDE inhibition	myofibrillar ATPase			
no.	R, R′	IC_{50},[a] μM	ΔpCa_{50}[b]	[μM]	n[c]	pCa_{50} control[d]
45	$-(CH_2)_2-$	0.036	0.14 ● 0.07	30	4	7.07 ± 0.03
46	$-(CH_2)_4-$	1.100	0.35 ± 0.03	30	4	6.80 ± 0.06
47	$-(CH_2)_5-$	1.300	0.39 ± 0.05	30	4	6.88 ± 0.06
48	$-(CH_2)_3S-$	1.400	0.37 ± 0.06	30	4	6.77 ± 0.06
49	$-S(CH_2)_2S-$	0.350	0.57 ● 0.03	30	4	6.82 ● 0.06
50	$-S(CH_2)_3S-$	0.260	0.76 ± 0.12	30	4	6.86 ± 0.08
50	$-S(CH_2)_3S-$		0.65 ± 0.06	10	4	6.81 ± 0.07
50	$-S(CH_2)_3S-$		0.48 ● 0.03	3	4	6.88 ± 0.06
50	$-S(CH_2)_3S-$		0.12 ± 0.01	1	4	6.78 ● 0.05

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I.

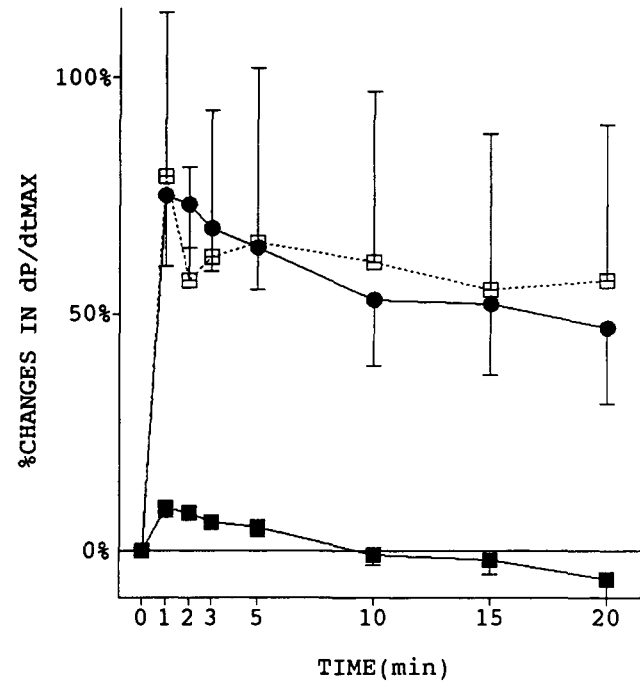


Figure 1. Effects of compounds **35** (filled circles) and **50** on myocardial contractility (as assessed by changes in dP/dt max) in chloralose-anaesthetized dogs. Each drug was studied in a separate group of animals. Compound **35** was given at 0.01 $mg \cdot kg^{-1}$. In the case of compound **50**, two doses are shown, these being 0.01 $mg \cdot kg^{-1}$ (■) and 0.3 $mg \cdot kg^{-1}$ (□). Each point is the mean ± SEM of three values.

cardiotonic molecules tends to be negatively correlated with the size of the spirocycle.

The most interesting member of this new spirocyclic series was **50**. This compound showed a similar level of PDE inhibitory activity to that displayed by **35** but was approximately **30** times more potent than this latter structure as a calcium-sensitizing agent.

Although not a pure calcium-sensitizing agent, compound **50** constituted our most interesting lead and we decided to evaluate it in vivo. Surprisingly, after iv administration to the anaesthetized dog, this compound was 30 times less potent in producing an inotropic effect than **35** (Figure 1). At the doses leading to similar inotropic activities, both compounds behave quite similarly as va-

(35) Komas, N.; Lugnier, C.; Le Bec, A.; Serradeil-Le Gal, C.; Barthélémy, G.; Stoclet, J. C. *J. Cardiovas. Pharm.* **1989**, *14*, 213–220.

(36) Robertson, D. W.; Krushinski, J. H.; Pollock, G. D.; Wilson, H.; Kauffman, R. F.; Hayes, J. S. *J. Med. Chem.* **1987**, *30*, 824–827.

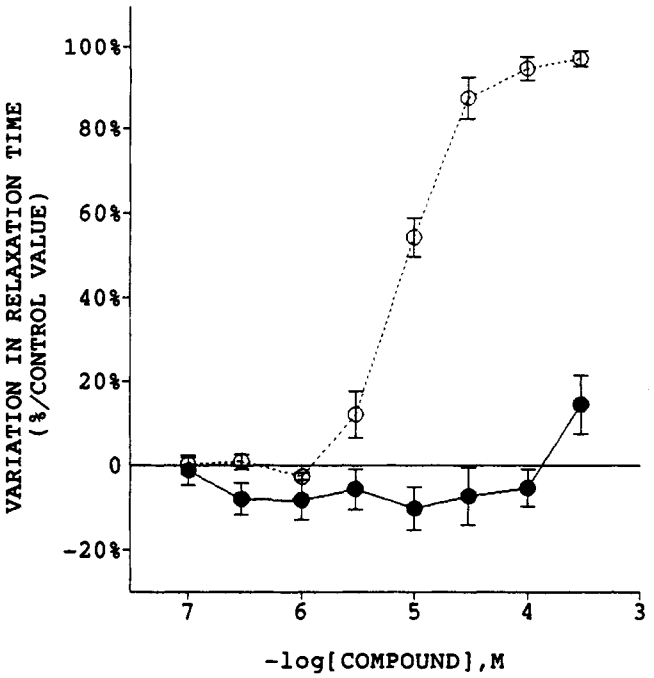


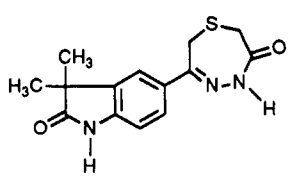
Figure 2. Lusitropic effects in vitro of compounds **35** (filled circles) and **50** (open circles) (as assessed by changes in relaxation time) on the guinea pig left atrium. Each drug was studied in a separate group of animals. Each point is the mean ± SEM of four values.

sodilating agents and increase the heart rate similarly. This poor inotropic activity of compound **50** is in apparent contradiction with several literature references in this area[14,15,20,22,37] which argue for a positive contribution of calcium sensitization to positive inotropic activity. One report,[38] however, suggests that if myofibrillar proteins are made more sensitive to calcium at the lower concentrations of calcium which occur during the diastolic phase, an increased wall stiffness may impair relaxation and ventricular filling. A comparison of **50** with **35** on in vitro relaxation (Figure 2) was in line with this concern.

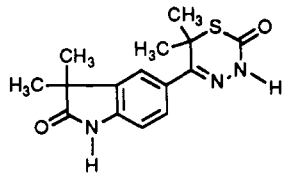
In order to further clarify these results, efforts were directed toward the design of "pure" calcium-sensitizing agents through the complete removal of the PDE inhibitory properties of these compounds. The literature[6,39,40,41] highlights, at least for structures related to dihydropyridazinones, the need for a near-planar arrangement of the aromatic and the pyridazinone rings to give rise to highly active PDE inhibitors. We therefore decided to prepare a number of structures that would force our compounds into a twisted configuration (**51–54**).

Unfortunately, although the PDE inhibitory effects of these derivatives dropped dramatically, the calcium-sensitizing properties also disappeared (Tables VII and VIII) (Compare for example **53**, **54** with **35** and **55**, Table VIII).

Table VII. Biochemical Activities of "Nonplanar" Dihydrothiadiazinone Analogues



51

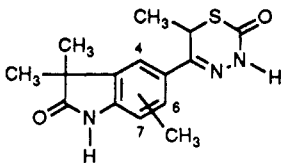


52

no.	SR-PDE inhibition IC_{50},[a] μM	myofibrillar ATPase ΔpCa_{50}[b]	[μM]	n[c]	pCa_{50} control[d]
51	28.0	−0.04 ± 0.03	200	3	6.72 ± 0.02
52	66.0	−0.02 ± 0.02	200	4	6.71 ± 0.07
31 (for comparison)	0.64	0.16 ± 0.06	200	3	6.82 ± 0.10
35 (for comparison)	0.33	0.31 ± 0.06	200	4	6.83 ± 0.10

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I.

Table VIII. Structure and Biochemical Activities of Isomers of a Cardiotonic Dihydrothiadiazinone Derivative



no.	methyl position	SR-PDE inhibition IC_{50},[a] μM	myofibrillar ATPase ΔpCa_{50}[b]	[μM]	n[c]	pCa_{50} control[d]
53	4	9.20	−0.07 ± 0.00	30	3	6.88 ± 0.10
54	6	3.30	−0.06 ± 0.01	30	3	6.88 ± 0.10
55	7	0.24	0.26 ± 0.03	30	3	7.01 ± 0.03
35	none	0.32	0.09 ● 0.03	30	4	6.84 ± 0.04

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I.

These results emphasize the need for a near-planar topography not only for the PDE inhibitory actions but also for the calcium-sensitizing effects of these compounds.

Table IX summarizes the structures and the biochemical properties of the compounds cited.

Conclusion/Summary

A series of novel 5-substituted 3,6-dihydrothiazin-2-ones have been prepared. Two potential positive inotropic mechanisms have been assessed by measuring (i) inhibition of the sarcoplasmic reticulum bound isoform of the low-Km cAMP phosphodiesterase (SR-PDE) and (ii) the calcium sensitization of myofibrillar ATPase activity considered as an index of myofibrillar protein sensitisation to calcium.

The SAR rules for PDE inhibition are widely consistent with those already observed for the analogous pyridazinones. The corresponding requirements for achieving calcium-sensitizing effects are as follows: (i) the indolone and heterocyclic parts of the molecule must be able to achieve a relative coplanarity, (ii) the heterocyclic part must contain a sulfur (or selenium) atom, and (iii) substituents (preferably bulky groups) are required at the 3-position of the indole moiety.

In vivo data suggested that these compounds could be very potent inotropic drugs (e.g. **35**), but unexpectedly and in opposition to some current concepts, it appears that strong calcium-sensitizing properties may impair both inotropism and lusitropism. Clearly such findings need to be supported by further experimental data. These studies are currently under investigation and will be reported later.

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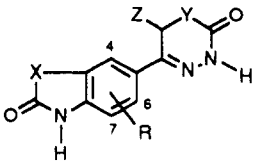
(39) Robertson, D. W.; Jones, N. D.; Krushinski, J. H.; Pollock, G. D.; Swartzendruber, J. K.; Hayes, J. S. *J. Med. Chem.* **1987**, *30*, 623–627.
(40) (a) Erhardt, P. W.; Hagedorn, A. A. III; Sabio, M. *Mol. Pharmacol.* **1988**, *33*, 1–13. (b) Erhardt, P. W.; Hagedorn, A. A. III; Davey, D.; Pease, C. A.; Venepalli, B. R.; Griffin, C. W.; Gomez, R. P.; Wiggins, J. R.; Ingebretsen, W. R.; Pang, D.; Cantor, E. *J. Med. Chem.* **1989**, *32*, 1173–1176.
(41) Toma, L.; Cignarella, G.; Barlocco, D.; Ronchetti, F. *J. Med. Chem.* **1990**, *33*, 1591–1594.

Table IX. Structures and Biochemical Properties of the Cited Heterodiazinone Cardiotonics



no.	X	Y	Z	R	(method)	mp, °C	formulas[f]	SR-PDE[a] IC_{50}, μM	myofibrillar ATPase ΔpCa_{50}[b]	[μM]
10	$C(CH_3)SCH_3$	S	H	H	(C)[27]	213	$C_{13}H_{13}N_3O_2S_2$	1.20	0.39 ± 0.03	200
28	$C(CH_3)SCH_3$	CH_2	H	H	*c*	272	$C_{14}H_{15}N_3O_2S{\cdot}0.75H_2O$[g]	NT[m]	0.07 ± 0.06	200
29	$C(CH_3)SCH_3$	S	CH_3	H	(C)[27]	255	$C_{14}H_{15}N_3O_2S_2{\cdot}0.25H_2O$	NT[n]	0.33 ± 0.08	200
30	$C(CH_3)SCH_3$	CH_2	CH_3	H	*c*	249	$C_{15}H_{17}N_3O_2S{\cdot}0.25H_2O$	NT[m]	0.11 ± 0.06	200
31	$C(CH_3)_2$	S	H	H	(A)	280	$C_{13}H_{13}N_3O_2S{\cdot}0.25H_2O$	NT[n]	0.16 ± 0.06	200
32	CH_2	S	H	H	(A)[42]	>320 dec	$C_{11}H_9N_3O_2S{\cdot}0.4H_2O$	NT[m]	0.08 ± 0.02	200
33	$CH(CH_3)$	S	H	H	(A)	267	$C_{12}H_{11}N_3O_2S$[h]	NT[n]	0.15 ± 0.02	200
34	$CH(SCH_3)$	S	H	H	(C)[27]	204	$C_{12}H_{11}N_3O_2S_2{\cdot}0.25H_2O$	NT[n]	0.28 ± 0.09	200
35	$C(CH_3)_2$	S	CH_3	H	(A)	270	$C_{14}H_{15}N_3O_2S{\cdot}0.25H_2O$	0.33	0.24 ± 0.05	100
36	$C(CH_3)_2$	CH_2	CH_3	H	*d*[7]	280	ref 7	0.24	−0.09 ± 0.02	100
37	$C(CH_3)_2$	NH	CH_3	H	*c*	292	$C_{14}H_{16}N_4O_2{\cdot}0.25H_2O$	0.17	−0.04 ± 0.02	100
38	$C(CH_3)_2$	O	CH_3	H	*c*	239	$C_{14}H_{15}N_3O_2{\cdot}0.25H_2O$[i]	0.96	0.04 ± 0.02	100
39	$C(CH_3)_2$	Se	CH_3	H	*e*	235	$C_{14}H_{15}N_3O_2Se{\cdot}0.5H_2O$	0.54	0.30 ± 0.07	100
40	CH_2CH_2	S	H	H	(A)[43]	282	ref 43	NT[m]	0.00 ± 0.01	30
41	$CH_2C(CH_3)_2$	S	CH_3	H	(A)	288	$C_{15}H_{17}N_3O_2S{\cdot}0.4H_2O$	NT[n]	0.45 ± 0.14	30
42	$C(CH_3)_2CH_2$	S	CH_3	H	(A)	245	$C_{15}H_{17}N_3O_2S{\cdot}0.2CH_2Cl_2$	NT[n]	0.33 ± 0.07	30
43	$OC(CH_3)_2$	S	CH_3	H	(A)	265	$C_{14}H_{15}N_3O_3S{\cdot}0.5H_2O$	NT[n]	0.15 ± 0.03	30
44	$NHC(CH_3)_2$	S	CH_3	H	(A)	312	$C_{14}H_{16}N_4O_2S{\cdot}0.2H_2O$	NT[m]	0.24 ± 0.02	30
45	$C(CH_2)_2$	S	CH_3	H	(A)	281	$C_{14}H_{13}N_3O_2S$	0.04	0.14 ± 0.07	30
46	$C(CH_2)_4$	S	CH_3	H	(A)	275	$C_{16}H_{17}N_3O_2S{\cdot}0.3H_2O$[j]	1.10	0.35 ± 0.03	30
47	$C(CH_2)_5$	S	CH_3	H	(A)	235	$C_{17}H_{19}N_3O_2S$	1.30	0.39 ± 0.05	30
48	$C[(CH_2)_3S]$	S	CH_3	H	(C)	205	$C_{15}H_{15}N_3O_2S_2$	1.40	0.33 ± 0.06	30
49	$C[S(CH_2)_2S]$	S	CH_3	H	(B)	157	$C_{14}H_{13}N_3O_2S_3{\cdot}0.4H_2O$	0.35	0.57 ± 0.03	30
50	$C[S(CH_2)_3S]$	S	CH_3	H	(B)	268	$C_{15}H_{15}N_3O_2S_3$	0.26	0.76 ± 0.12	30
51	$C(CH_3)_2$	SCH_2	H	H	*c*	145	$C_{14}H_{15}N_3O_2S{\cdot}H_2NNH_2$[k]	28.0	−0.04 ± 0.03	200
52	$C(CH_3)_2$	S	$(CH_3)_2$	H	(A)	275	$C_{15}H_{17}N_3O_2S{\cdot}0.25H_2O$	66.0	−0.02 ± 0.02	200
53	$C(CH_3)_2$	S	CH_3	$4\text{-}CH_3$	(A)	279	$C_{15}H_{17}N_3O_2S$	9.20	−0.07 ± 0.00	30
54	$C(CH_3)_2$	S	CH_3	$6\text{-}CH_3$	(A)	240	$C_{15}H_{17}N_3O_2S{\cdot}0.3H_2O$[l]	3.30	−0.06 ± 0.01	30
55	$C(CH_3)_2$	S	CH_3	$7\text{-}CH_3$	(A)	250	$C_{15}H_{17}N_3O_2S$	0.24	0.26 ± 0.03	30

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] See Experimental Section. [d] See Experimental Section, example 28. [e] See ref 34. [f] All compounds exhibited satisfactory C, H, and N analyses except where indicated otherwise. [g] H: calcd, 4.48; found, 4.93. [h] C: calcd, 55.16; found, 54.70. [i] N: calcd, 15.12; found, 14.67. [j] C: calcd, 59.90; found, 59.49. [k] N: calcd, 21.79; found, 21.16. [l] N: calcd, 13.61; found, 13.15. [m] NT: not tested. [n] PDE inhibition has only been evaluated on an earlier test on soluble CGI-PDE.

Experimental Section

Methods. Melting points were taken in open capillary tubes on a Gallenkamp melting point apparatus and are uncorrected. Proton magnetic resonance (^1H NMR) spectra were recorded in deuteriochloroform or dimethyl sulfoxide-d_6 on a Brucker ACE 200 MHz spectrometer. Chemical shifts are recorded in units (ppm relative to tetramethylsilane as the internal standard). The infrared spectra were of samples in potassium bromide, measured using a Shimadzu IR 408 model spectrometer.

Microanalytical data were provided by the Physical and Analytical Service Unit of the SmithKline Beecham Pharmaceutics Research Laboratories at Harlow, Great Britain. Only symbols of elements analyzed are given, and they were within 0.4% of theoretical values unless indicated.

Unless it is noted, a standard procedure was used for isolation of the product. This involved filtration or extensive extraction with a solvent (washing of extract with aqueous solutions, when mentioned), drying over magnesium sulfate, and evaporation under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agent are mentioned in parentheses after the words "product isolation".

The synthesis of 1,3-dihydro-5-(3,6-dihydro-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-3-methyl-3-(methylthio)-2*H*-indol-2-one[27] (**10**); 1,3-dihydro-5-(3,6-dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-3-methyl-3-(methylthio)-2*H*-indol-2-one[27] (**29**); 1,3-dihydro-5-(3,6-dihydro-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-2*H*-indol-2-one[42] (**32**); 1,3-dihydro-5-(3,6-dihydro-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-3-(methylthio)-2*H*-indol-2-one[27] (**34**); 1,3-dihydro-5-(3,6-dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-3,3-dimethyl-2*H*-indol-2-one[34] (**35**); 1,3-dihydro-5-(1,4,5,6-tetrahydro-4-methyl-6-oxopyridazin-3-yl)-3,3-dimethyl-2*H*-indol-2-one[7] (**36**); 1,3-dihydro-5-(3,6-dihydro-6-methyl-2-oxo-2*H*-1,3,4-selenodiazin-5-yl)-3,3-dimethyl-2*H*-indol-2-one[34] (**39**); and 3,4-dihydro-6-(3,6-dihydro-2-oxo-1,3,4-thiadiazin-5-yl)-2(1*H*)-quinolinone[43] (**40**) are described in the reference cited therein.

The following benzofused lactams used as substrate in the Friedel–Crafts reactions were prepared following literature procedures as indicated: 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one;[7] 1,3-dihydro-3,3,4-trimethyl-2*H*-indol-2-one;[28] 1,3-dihydro-3,3,6-trimethyl-2*H*-indol-2-one;[7] 1,3-dihydro-3,3,7-trimethyl-2*H*-indol-2-one;[44] 3,4-dihydro-3,3-dimethyl-2(1*H*)-quinolinones;[7] 3,4-dihydro-4,4-dimethyl-2(1*H*)-quinolinone;[45] 1,4-dihydro-4,4-dimethyl-2*H*-3,1-benzoxazine-2-one;[46] 3,4-dihydro-4,4-dimethyl-2-(1*H*)-quinazolinone;[47] spiro[cyclopropane-1,3′-[3*H*]indol]-2′-(1′*H*)-one;[36,48] spiro[cyclopentane-1,3′-[3*H*]indol]-2(1′*H*)-one;[36,49]

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(49) Moore, R. F.; Plant, S. G. P. *J. Chem. Soc.* **1951**, 3475–3478.

spiro[cyclohexane-1,3'-[3*H*]indol]-2'(1'*H*)-one;[28,49] 1,3-dihydro-3-methyl-2*H*-indol-2-one;[49] 3,4-dihydro-2(1*H*)-quinolinone.[51]

Synthesis of Dihydrothiadiazinones and Related Cardiotonics. The following procedures illustrate the synthetic methods used to prepare the 1,3-dihydro-1,3,4-thiadiazinones listed in Table IX.

Method A. 5-(2-Chloro-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one. Aluminium chloride (13.3 g, 0.1 mol) and 2.2 mL of DMF were mixed and heated with stirring at 70 °C for 15 min. After cooling to 40 °C, 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (1.5 g, 9.3 mmol) and 2-chloropropionyl chloride (1.25 g, 9.8 mmol) were added and then stirred at 70 °C for 1 h. The reaction mixture was poured onto 100 g of crushed ice and 10 mL of 10 N HCl. The product was isolated using the standard workup (EtOAc, H_2O, brine, $MgSO_4$) and triturated with Et_2O to afford 1.25 g of compound used directly in the next step without further purification.

1,3-Dihydro-5-(3,6-dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-3,3-dimethyl-2*H*-indol-2-one (35). Crude 5-(2-chloro-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (0.125 g, 4.9 mmol), *O*-methyl thiocarbazate[25,26] (0.53 g, 5 mmol), and CH_3CN (10 mL) were refluxed for 2 h. After evaporation to dryness, the residual oil was purified by chromatography on silica gel (hexane/EtOAc, 1/1) to yield 0.4 g (28%) of a crystalline compound: mp 270 °C; IR (KBr) ν 3170, 1725, 1680 cm^{-1}; NMR (DMSO-d_6) δ 1.29 (s, 6 H, CH_3), 1.50 (d, 3 H, CH_3), 4.70 (q, 1 H, CH), 6.92 (d, 1 H, Ar), 7.70 (m, 2 H, Ar), 10.51 (s, 1 H, exch. D_2O, NH), 11.51 (s, 1 H, exch. D_2O, NH). Anal. ($C_{14}H_{15}N_3O_2S\cdot 0.25H_2O$): C, H, N.

Method B. 5'-(3,6-Dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)spiro[1,3-dithiolane-2,3'-[3*H*]indol]-2'(1'*H*)-one (49). To a suspension of 5-(3,6-dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-1*H*-indole-2,3-dione[27] (17) (0.5 g, 1.8 mmol) in AcOH (80 mL) were added boron trifluoride etherate (1 mL) and ethanedithiol (500 mg). The suspension was stirred overnight and then poured in H_2O (350 mL). The white precipitate was filtered, washed with H_2O and then with Et_2O, and dried under vacuum: yield 260 mg (40%); NMR (DMSO-d_6) δ 1.46 (d, 3 H, J = 7 Hz, CH_3), 3.76 (m, 4 H, CH_2), 4.74 (q, 1 H, J = 7 Hz, CH), 6.94 (d, 1 H, J = 8 Hz, Ar), 7.72 (d, 1 H, J = 8 Hz, Ar), 7.91 (s, 1 H, Ar), 10.91 (s, 1 H, exch. D_2O, NH), 11.65 (s, 1 H, exch. D_2O, NH).

5'-(3,6-Dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)spiro[1,3-dithiane-2,3'-[3*H*]indol]-2'(1'*H*)-one (50). Starting from 5-(3,6-dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-1*H*-indol-2,3-dione (17) the method B, using propanedithiol instead of ethanedithiol, afforded the desired compound: yield 15%; NMR (DMSO-d_6) δ 1.46 (d, 3 H, J = 7 Hz, CH_3), 1.90 (m, 1 H, CH_2), 2.28 (m, 1 H, CH_2), 2.74 (m, 2 H, CH_2), 3.81 (m, 2 H, CH_2), 4.74 (q, 1 H, J = 7 Hz, CH), 6.97 (d, 1 H, J = 8 Hz, Ar), 7.75 (d, 1 H, J = 8 Hz, Ar), 7.78 (s, 1 H, Ar), 10.96 (s, 1 H, exch. D_2O, NH), 11.65 (s, 1 H, exch. D_2O, NH).

5'-(3,6-Dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)spiro[thiolane-2,3'-[3*H*]indol]-2'(1'*H*)-one (48). 5'-(2-Chloro-1-oxopropyl)spiro[thiolane-2,3'-[3*H*]indol]-2'(1'*H*)-one (Method C). A solution of 1-(4-aminophenyl)-2-chloropropanone[29] (20 g, 0.11 mol) in CH_2Cl_2 (290 mL) was cooled to −65 °C. *tert*-Butyl hypochlorite (11.8 g, 0.11 mol) in CH_2Cl_2 (40 mL) was added dropwise, and stirring was continued for 15 min. Methyl 2-thiolanecarboxylate[32] (16.1 g, 0.11 mol) in CH_2Cl_2 (40 mL) was then added dropwise at −65 °C, and stirring was continued for 1.5 h at this temperature. Triethylamine (11 g, 0.11 mol) in CH_2Cl_2 (40 mL) was then added, and the reaction temperature slowly raised to ambient. Water (100 mL) was added, and the reaction mixture extracted with CH_2Cl_2. The organic layer was washed with H_2O and concentrated. The oily residue was taken up in Et_2O (100 mL), 2 N HCl (50 mL) was added, and the resulting mixture was stirred overnight. The aqueous layer was extracted with EtOAc, and the organic phases were washed with H_2O, decolorized with charcoal, and dried over $MgSO_4$. Removing of the solvent afforded 16.0 g of an oil used in the next step without further purification. Yield: 57%. ^1H NMR was consistent with the assigned structure.

5'-(3,6-Dihydro-6-methyl-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)spiro[thiolane-2,3'-[3*H*]indol]-2'(1'*H*)-one (48). Cyclization of the above obtained compound with *O*-methyl thiocarbazate as in method A, afforded the desired compound: yield 46%; NMR (DMSO-d_6) δ 1.46 (d, 3 H, J = 7 Hz, CH_3), 2.14–2.43 (m, 4 H, CH_2), 3.20 (m, 2 H, CH_2), 4.76 (q, 1 H, J = 7 Hz, CH), 6.90 (d, 1 H, J = 8 Hz, Ar), 7.67 (d, 1 H, J = 8 Hz, Ar), 10.70 (s, 1 H, exch. D_2O, NH), 11.60 (s, 1 H, exch. D_2O, NH).

1,3-Dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-3-methyl-3-(methylthio)-2*H*-indol-2-one (28). 4-(2,3-Dihydro-3-methyl-3-(methylthio)-2-oxo-1*H*-indol-5-yl)-4-oxobutanoic Acid. Starting from 4-(4-aminophenyl)-4-oxobutanoic acid,[52] and ethyl 2-(methylthio)propionate,[31] the method C led to a crude oily product used in the next step without further purification.

1,3-Dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-3-methyl-3-(methylthio)-2*H*-indol-2-one (28). The crude product obtained in the previous reaction was refluxed for 3 h in EtOH in presence of 1.5 equiv of hydrazine hydrate. After concentration to dryness, the residue was recrystallized in hot EtOH: overall yield 5%; mp 272 °C; IR (KBr) ν 3150, 1710, 1640, 1612 cm^{-1}; NMR (DMSO-d_6) δ 1.55 (s, 3 H, CH_3), 1.90 (s, 3 H, CH_3), 2.40 (t, 2 H, J = 8 Hz, CH_2), 2.92 (t, 2 H, J = 8 Hz, CH_2), 6.90 (d, 1 H, J = 9 Hz, Ar), 7.60 (d, 1 H, J = 9 Hz, Ar), 7.68 (s, 1 H, Ar), 10.63 (s, 1 H, exch. D_2O, NH), 10.76 (s, 1 H, exch. D_2O, NH). Anal. ($C_{14}H_{15}N_3O_2S$, 0.75 H_2O): C, N; H: calcd, 4.48; found, 4.93.

1,3-Dihydro-5-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)-3-methyl-3-(methylthio)-2*H*-indol-2-one (30). The compound was obtained in the same manner as for **28**, starting from 4-(4-aminophenyl)-3-methyl-4-oxobutanoic acid:[53] overall yield 13%; mp 249 °C; IR (KBr) ν 3200, 1730, 1680, 1620 cm^{-1}; NMR (DMSO-d_6) δ 1.06 (d, 3 H, J = 8 Hz, CH_3), 1.55 (s, 3 H, CH_3), 1.9 (s, 3 H, CH_3), 2.1–2.9 (m, 3 H, CH_2CH), 6.9 (d, J = 8 Hz, 1 H, Ar), 7.6 (d, J = 8 Hz, 1 H, Ar), 7.72 (s, 1 H, Ar), 10.63 (s, 1 H, exch. D_2O, NH), 10.8 (s, 1 H, exch. D_2O, NH). Anal. ($C_{15}H_{17}N_3O_2S\cdot 0.25H_2O$): C, H, N.

1,3-Dihydro-5-(2,3,4,7-tetrahydro-3-oxo-1,4,5-thiadiazepin-6-yl)-3,3-dimethyl-2*H*-indol-2-one (51). Methyl [[2-(2,3-Dihydro-3,3-dimethyl-2-oxo-1*H*-indol-5-yl)-2-oxoethyl]thio]acetate (27). A mixture of 2-bromo-1-(2,3-dihydro-3,3-dimethyl-2-oxo-1*H*-indol-5-yl)ethanone[28] (3.2 g, 11.3 mmol) in CH_3CN (32 mL), K_2CO_3 (1.56 g), and methyl α-mercaptoacetate (1.36 g, 12 mmol) was refluxed for 1 h. After concentration, trituration in isopropyl alcohol afforded 1.6 g (46%) of a solid used in the next step without further purification.

1,3-Dihydro-5-(2,3,4,7-tetrahydro-3-oxo-1,4,5-thiadiazepin-6-yl)-3,3-dimethyl-2*H*-indol-2-one (51). Crude methyl [[2-(2,3-dihydro-3,3-dimethyl-2-oxo-1*H*-indol-5-yl)-2-oxoethyl]thio]acetate (27) (1.6 g, 5 mmol) and hydrazine hydrate (1 g) were refluxed for 4 h in CH_3CN (20 mL). Concentration and trituration in isopropyl alcohol afforded 65% of pure material: mp 145 °C; IR (KBr) ν 3100, 1718, 1680, 1630 cm^{-1}; NMR (DMSO-d_6) δ 1.28 (s, 6 H, CH_3), 3.09 (s, 2 H, CH_2), 3.80 (s, 2 H, CH_2), 6.80 (s, 1 H, J = 8 Hz, Ar), 7.40 (dd, 1 H, J = 8 Hz, J'' = 1.0 Hz, Ar), 7.55 (d, 1 H, J'' = 1 Hz, Ar), 9.20 (s, 1 H, exch. D_2O, NH), 10.18 (s, 1 H, exch. D_2O, NH). Anal. ($C_{14}H_{15}N_3O_2S\cdot H_2NNH_2$): C, H; N: calcd 21.79; found, 21.16.

1,3-Dihydro-5-(1,2,3,6-tetrahydro-6-methyl-2-oxo-1,3,4-triazin-5-yl)-3,3-dimethyl-2*H*-indol-2-one (37). 5-(2-Azido-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one. A solution of NaN_3 (3.2 g) in H_2O (20 mL) was added to a solution of 5-(2-bromo-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (5 g, 17 mmol) in dioxane (45 mL) at room temperature. The reaction mixture was stirred for 1.5 h, and then H_2O (120 mL) was added. Product isolation ($CHCl_3$, H_2O, $MgSO_4$) afforded 4.19 g of crystals used in the next step without further purification.

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^{1}H NMR was consistent with the assigned structure.

5-(2-Amino-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one Hydrochloride. Crude 5-(2-azido-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-ones (4.13 g, 16 mmol) and 10% palladium on charcoal (0.81 g) in a mixture of 12 N aqueous HCl (1.85 mL), EtOH (30 mL), and $CHCl_3$ (30 mL) were vigorously stirred at room temperature under hydrogen atmosphere (1 bar) for 20 h. Water (250 mL) and $CHCl_3$ (250 mL) were added, and the resulting mixture was filtered. The organic phase was extracted with H_2O, and the combined aqueous phases were evaporated to dryness. The residue was triturated in petroleum ether to yield 4.23 g of crystals used in the next step without further purification. ^{1}H NMR was consistent with the assigned structure.

Ethyl 2-[2-(3,3-Dimethyl-2-oxo-1*H*-indol-5-yl)-1-methyl-2-oxoethyl]carbamate (23). To a solution of 5-(2-amino-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one hydrochloride (16.5 g, 61 mmol) in a mixture of H_2O (35 mL), pyridine (10.4 mL), and EtOH (150 mL) was added dropwise ethyl chloroformate (7.3 mL) in EtOH (15 mL) at room temperature with stirring. The stirring was maintained for 17 h, and then the solvent was evaporated. The residue was taken up in H_2O (300 mL). Product isolation (CH_2Cl_2, H_2O, $MgSO_4$) afforded 6.3 g of compound used in the next step without further purification: mp 61–62 °C; ^{1}H NMR was consistent with the assigned structure. Anal. ($C_{16}H_{20}N_2O_4$): C, H, N.

1,3-Dihydro-5-(1,2,3,6-tetrahydro-6-methyl-2-oxo-1,3,4-triazin-5-yl)-3,3-dimethyl-2*H*-indol-2-one (37). A mixture of crude ethyl 2-[2-(3,3-dimethyl-2-oxo-1*H*-indol-5-yl)-1-methyl-2-oxoethyl]carbamate (23) (6.3 g, 21 mmol), hydrazine hydrate (10.5 mL), H_2O (50 mL), and EtOH (5 mL) was refluxed with stirring for 16 h. After cooling to room temperature a precipitate was filtered off, washed with H_2O, and dried. The resulting crystals were washed with a diisopropyl ether–acetone mixture (15:1) and dried, yielding 3.3 g of white crystals: mp 292 °C; NMR (DMSO-d_6) δ 1.18 (d, 3 H, J = 6.6 Hz, CH_3), 1.27 (s, 6 H, CH_3), 4.61 (m, 1 H, CH), 6.87 (d, 1 H, J = 8 Hz, Ar), 7.44 (s, 1 H, exch. D_2O, NH), 7.53 (dd, 1 H, J = 8 Hz, J' = 1.2 Hz, Ar), 7.55 (d, 1 H, J = 1.2 Hz, Ar), 9.92 (s, 1 H, exch. D_2O, NH), 10.51 (s, 1 H, exch. D_2O, NH). Anal. ($C_{14}H_{16}N_4O_2 \cdot 0.25H_2O$): C, H, N.

5-(3,6-Dihydro-6-methyl-2-oxo-2*H*-1,3,4-oxadiazin-5-yl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (38). 1,3-Dihydro-5-(2-hydroxy-1-oxopropyl)-3,3-dimethyl-2*H*-indol-2-one. A mixture of 5-(2-bromo-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (10 g, 33 mmol), DMF (30 mL), and H_2O (20 mL) was stirred at 100 °C for 4 h. The solvent was evaporated under vacuum, and the residue was purified by chromatography on silica gel (CH_2Cl_2–acetone, 8:2), affording 2.0 g of crystals: mp 173 °C; ^{1}H NMR was consistent with the assigned structure.

Ethyl 2-[1-(3,3-Dimethyl-2-oxo-1*H*-indol-5-yl)-2-hydroxypropylidene]hydrazinecarboxylate (24). A mixture of 1,3-dihydro-5-(2-hydroxy-1-oxopropyl)-3,3-dimethyl-2*H*-indol-2-one (2.5 g, 11 mmol), ethyl carbazate (1.29 g, 12 mmol), EtOH (6.25 mL), and 6 drops of 0.1 N aqueous HCl was stirred at reflux for 10 min. The reaction mixture was evaporated to dryness, and the residue was triturated in Et_2O to yield 2.55 g of crystals: mp 213 °C; ^{1}H NMR was consistent with the assigned structure. Anal. ($C_{16}H_{21}N_3O_4 \cdot 0.2H_2O$): C, H, N.

5-(3,6-Dihydro-6-methyl-2-oxo-2*H*-1,3,4-oxadiazin-5-yl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (38). An alcoholic sodium ethoxide solution prepared from 1.72 g of Na and 50 mL of EtOH was added dropwise to a suspension of ethyl 2-[1-(3,3-dimethyl-2-oxo-1*H*-indol-5-yl)-2-hydroxypropylidene]hydrazinecarboxylate (24) (2.55 g, 8 mmol) in EtOH (50 mL). At the end of the addition, the clear solution was stirred at room temperature for 3 h. The solvent was evaporated, and the residue was taken up in H_2O (50 mL) acidified with 0.1 N aqueous HCl. The precipitate was filtered off, washed with H_2O, and dried, yielding 1.8 g of crystals: mp 239 °C; IR (KBr) ν 3200, 3100, 2970, 2930, 1700, 1635 cm^{-1}; NMR (DMSO-d_6) δ 1.28 (s, 6 H, CH_3), 1.43 (d, 3 H, CH_3), 5.76 (q, 1 H, CH), 6.91 (d, 1 H, J = 8.2 Hz, Ar), 7.57 (d, 1 H, J = 8.2 Hz, Ar), 7.72 (s, 1 H, Ar), 10.60 (s, 1 H, exch. D_2O, NH), 11.01 (s, 1 H, exch. D_2O, NH). Anal. ($C_{14}H_{15}N_3O_3 \cdot 0.25H_2O$): C, H; N: calcd, 15.12; found, 14.67.

Pharmacological Methods. Biochemistry. Influence of Compounds on Ca^{2+}-Dependent ATPase Activity of Cardiac Myofibrils: pCa–ATPase Activity Curves on Canine Cardiac Myofibrils. Canine cardiac myofibrils free of membrane contaminants were prepared using the method of Solaro et al.[54] Ca^{2+}-dependent myofibrillar ATPase activity was determined at 21 °C by measuring the rate of release of inorganic phosphate (P_i). Assays were performed using the method described by Solaro and Ruegg[14] in reaction mixtures containing 0.6–0.7 mg/mL myofibrillar protein, 80 mM KCl, 20 mM imidazole, 3 mM $MgCl_2$, 2 mM Na_2ATP, 1 mM EGTA, and the desired amount of $CaCl_2$. The amount of $CaCl_2$ was varied between 0 and 0.9 mM and pCa (–log of concentration of free Ca^{2+}) was computed using 2.514×10^6 M^{-1} as the apparent affinity of Ca^{2+} for EGTA at pH 7.0. Myofibrils were preincubated for 5 min in the presence of the studied compound, reaction was initiated by the addition of Na_2ATP, and after an incubation period of 12 min, reaction was quenched by the addition of an equal volume of ice-cold 10% aqueous trichloroacetic acid. Protein was pelleted by centrifugation and P_i was determined colorimetrically using a malachite green method.[55]

Inhibition of the Sarcoplasmic Reticulum Bound Low-Km cAMP Phosphodiesterase (SR-PDE). The inhibition of the SR-PDE[8] was performed using sarcoplasmic reticulum vesicles prepared from dog left ventricle as previously described by Jones and co-workers.[56] Microsomes were prepared from canine ventricular tissue which is subjected to vigorous initial homogenization and to several centrifugation steps to remove nuclei, cell debris, and mitochondria. Free SR vesicles were isolated by sucrose density gradient centrifugation of microsomes after selective Ca^{2+} oxalate loading.

The activity of cAMP-PDE was assayed by radiochemical procedure at 30 °C in a medium containing 12 mM Tris-HCl, pH 7.7, 0.5 mM $MgCl_2$, 137 mM NaCl, 20 mM glucose, and 1 μM [^{3}H]cAMP. This assay is a modification of the two-step technique of Thompson and co-workers[57] in which the substrate and products were separated using Dowex 1-x8 resin after AMP was fully converted to adenosine by 5′-nucleotidase. PDE reactions were initiated by adding sufficient enzyme to hydrolyze less than 25% of the substrate, and PDE activity was linear vs time during all the assay. Test compounds had no significant effect upon the snake venom (*Crotalus atrox*) used to convert [^{3}H]AMP to [^{3}H]adenosine in the second step of the assay (data not shown). Moreover, neither the recovery of [^{3}H]adenosine, nor that of unreacted [^{3}H]cAMP were significantly affected by the test compounds (data not shown). PDE activity was determined in triplicate at 10 inhibitor concentrations (10^{-9}–10^{-4} M) in order to generate inhibition curves. DMSO was utilized as solvent for PDE inhibitors, and controls were run to ensure that carryover solvent (1%, v/v) did not affect assay results. Results are expressed as IC_{50} values which were determined after linearization (Hill plot) of the mean inhibition curve obtained from, at least, three different SR-PDE preparations.

Measurement of Inotropic Activity in Anaesthetized Dogs. Dogs were anaesthetized with intravenous chloralose (100 mg·kg^{-1}). The saphenous vein and the femoral and carotid arteries were cannulated for compound injection and for the recording of blood pressure and left ventricular pressure (LVP), respectively. LVP was recorded by means of a Millar-tip catheter, introduced to the left ventricule via the carotid artery. The signal was differentiated to give dP/dt max, which was used as the index of cardiac contractility.

Following surgery, an equilibration period of 1 h, at least, was allowed. The compounds were administered intravenously in DMSO. Changes in dP/dt max (mmHg·sec^{-1}) were recorded and expressed as percent changes from control values (without compound under study).

Measurement of in Vitro Relaxation in Guinea Pig Left Atrium. Guinea pigs of either sex (300–450 g) were anaesthetized with sodium pentobarbitol, 60 mg·kg^{-1}, before opening the chest

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cavity. The left atrium was suspended in Krebs-Henseleit solution (KH) containing the following (mM): NaCl (118), KCl (4.7), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25), and glucose (11). The KH solution was maintained at 32 °C and gassed with 95% O_2, 5% CO_2 leading to a pH of 7.3–7.4. The preparation was allowed to stabilize for 1 h before starting the experiment. The effect of the compounds on electrically evoked atrial contractions (2 Hz, 2 ms, twice the threshold) was examined after cumulative addition to the organ bath. The mechanical performance was recorded isometrically.

A high-speed recording (200 mm/s) at each concentration allows calculation of relaxation time (RT). The variations in RT were expressed as percent changes from control values.

Cardiotonic Activity in the Conscious Instrumented Dog. Male beagle dogs, weighing 10–14 kg, were chronically instrumented to monitor LV dP/dt max (the first derivative of left ventricular pressure) and heart rate. Under fluothane anesthesia a Koenigsberg P_5 tip micromanometer 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. Dogs were placed in a quiet room, parameters being recorded outside. To reduce the uncertainty due to solubility, drugs (and placebo) were solubilized in dimethylimidazolidinone–Tween 80–H_2O (1:2:2) and administered in 000 gelatin capsules. Drugs were administered after a control period of 90 min and parameters recorded for 22 h.

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Registry No. **10**, 122280-58-4; **17** (R = Me), 122280-68-6; **22**, 122281-20-3; **23**, 122281-23-6; **24**, 122281-24-7; **25**, 19155-24-9; **26**, 137516-11-1; **27**, 137516-12-2; **28**, 137516-13-3; **29**, 122280-62-0; **30**, 137516-14-4; **31**, 122280-59-5; **32**, 137516-15-5; **33**, 122280-61-9; **34**, 122280-67-5; **35**, 122280-60-8; **36**, 100644-04-0; **37**, 122280-91-5; **38**, 122297-45-4; **39**, 137516-16-6; **40**, 103969-58-0; **41**, 120223-10-1; **42**, 137516-17-7; **43**, 137516-18-8; **44**, 137516-19-9; **45**, 122280-72-2; **46**, 122280-63-1; **47**, 122280-73-3; **48**, 122280-77-7; **49**, 122280-69-7; **50**, 122280-70-0; **51**, 137516-20-2; **52**, 122280-88-0; **53**, 122280-85-7; **54**, 122280-86-8; **55**, 122280-87-9; 2-chloropropionyl chloride, 7623-09-8; 5-(2-chloro-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one, 122281-03-2; 1-(4-aminophenyl)-2-chloropropanone, 25021-66-3; methyl 2-thiolanecarboxylate, 113990-87-7; 5′-(2-chloro-1-oxopropyl)spiro[thiolane-2,3′-[3*H*-indol]-2′(1′*H*)-one, 122281-10-1; 4-(4-aminophenyl)-4-oxobutanoic acid, 6945-94-4; ethyl 2-(methylthio)propionate, 40800-76-8; 4-(2,3-dihydro-3-methyl-3-(methylthio)-2-oxo-1*H*-indol-5-yl)-4-oxobutanoic acid, 137516-21-3; methyl α-mercaptoacetate, 2365-48-2; 5-(2-azido-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one, 122281-21-4; 5-(2-amino-1-oxopropyl)-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one hydrochloride, 122281-22-5; 1,3-dihydro-5-(2-hydroxy-1-oxopropyl)-3,3-dimethyl-2*H*-indol-2-one, 122297-47-6; phosphodiesterase, 9025-82-5.

Heteroatom Analogues of Bemoradan: Chemistry and Cardiotonic Activity of 1,4-Benzothiazinylpyridazinones

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A series of close analogues of the potent, long-acting cardiotonic bemoradan (**2a**) was synthesized and examined in both in vitro and in vivo test systems. Changing the oxygen heteroatom at the 1-position of the benzoxazine ring of bemoradan to sulfur gave **4a**, a more potent enzyme inhibitor and in vivo cardiotonic compound by the iv route. Intraduodenal administration of bemoradan, however, showed a superior response compared to its sulfur analogue, possibly due to oxidation of sulfur followed by a facile Pummerer rearrangement. Model studies were performed to examine the effect of the oxidation state of sulfur. Lack of a heteroatom at the 1-position, **3a** (Y-590), afforded a compound with activity and potency very similar to those of bemoradan while the 1-selena compound gave a much less potent analogue **5**. Analogues having a methyl group on the 4-nitrogen (**2b**, **3b**, and **4b**) were less potent than the desmethyl compounds, but all of these compounds have potent PDE III inhibiting activity and the ability to increase cardiac force in an anesthetized dog preparation when given iv.

Introduction

In recent years, a number of highly potent positive inotropes have been described in the literature.[1] Many of these compounds, acting through the inhibition of cyclic nucleotide phosphodiesterase III isozyme (PDE III)[2] isolated from heart muscle, consist of a pyridazinone ring attached to a substituted aromatic nucleus. In a subset of these compounds, typified by indolidan[3] (**1**) and bemoradan[4] (**2a**), the pyridazinone ring is attached to a benzo-fused heterocycle. The nature of this benzo-fused heterocyclic fragment of the molecule would seem to be important to the physical properties as well as the biological effects, metabolism, and distribution of the compounds. We have reported on the synthesis and biological properties of bemoradan,[5] and now report on the synthesis and biological properties of a series of compounds differing by the substitution of one atom in the 1-position of the benzoxazine ring of bemoradan (see Table I). Although

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