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# Communications to the Editor

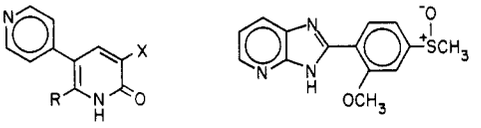
## Cardiotonic Agents. 1. 4,5-Dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones: Novel Positive Inotropic Agents for the Treatment of Congestive Heart Failure

*Sir:*

The pathophysiology of heart failure consists of (1) myocardial failure and (2) the consequent systemic response to the failing myocardium that ultimately leads to peripheral congestion and edema.[1,2] The fundamental defect is an impairment of ventricular function that results in an inadequate output to meet the metabolic and circulatory demands of the body. Effective therapy of heart failure is directed to enhancement of the contractile state of the myocardium with positive inotropic agents, adjustment of the peripheral circulatory state with peripheral vasodilators, reduction in volume with diuretics, or by a combination of these therapeutic approaches.[3,4]

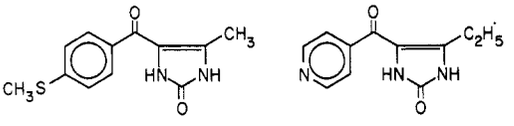
In the U.S., there are currently two classes of drugs available to treat congestive heart failure whose principal component of action is to provide positive inotropic support for the heart: the cardiac glycosides (digitoxin and digoxin) and the sympathomimetic agents (dobutamine and dopamine).[5,6] Of these, the cardiac glycosides are the only orally effective agents; however, their use is limited mainly by arrhythmogenic liability.[7] Dobutamine and dopamine are limited by chronotropic liability and oral ineffectiveness. The absence of safe, orally effective, positive inotropic agents for the treatment of congestive heart failure has stimulated the development of several new nonsympathomimetic cardiotonic agents:[3] amrinone,[8] milrinone,[9,10] sulmazole,[11,12] fenoximone (MDL-17043),[13,14]

Chart I. New Cardiotonic Agents



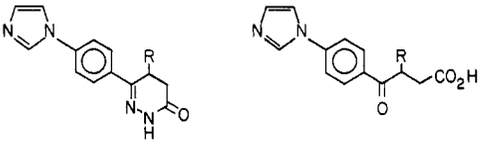
amrinone: X = $NH_2$; R = H
milrinone: X = CN; R = $CH_3$

sulmazole



fenoximone MDL-19205

Chart II. 4,5-Dihydro-6-[4-(1*H*-imidazo-1-yl)phenyl]-3(2*H*)-pyridazinones and Chemical Intermediates



1: R = H (CI-914)
2: R = $CH_3$ (CI-930)

3: R = H
4: R = $CH_3$

and MDL-19205[15-17] (Chart I). In this paper we report two new potent positive inotropic agents that have been developed for the treatment of congestive heart failure: 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinone (**1**, CI-914) and 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-5-methyl-3(2*H*)-pyridazinone (**2**, CI-930) Chart II).

**Chemistry.** The 4,5-dihydro-3(2*H*)-pyridazinones **1** and **2** were prepared by reaction of the requisite 4-(1*H*-imidazol-1-yl)-γ-oxobenzenebutanoic acids, **3** and **4**, with hydrazine in ethanol followed by treatment with HCl to afford **1**·HCl (80%, mp 290–291 °C) and **2**·HCl (80%, mp 296–298 °C). The acid **3** was prepared by reaction of fluorobenzene with succinic anhydride in the presence of $AlCl_3$ in $CH_2Cl_2$, followed by reaction of the resulting 4-fluoro-γ-oxobenzenebutanoic acid with imidazole (NaH/$Me_2SO$ at 100 °C for 17 h) to give **3** (80%, mp 272–273 °C dec). 4-(1*H*-Imidazol-1-yl)-β-methyl-γ-oxobenzenebutanoic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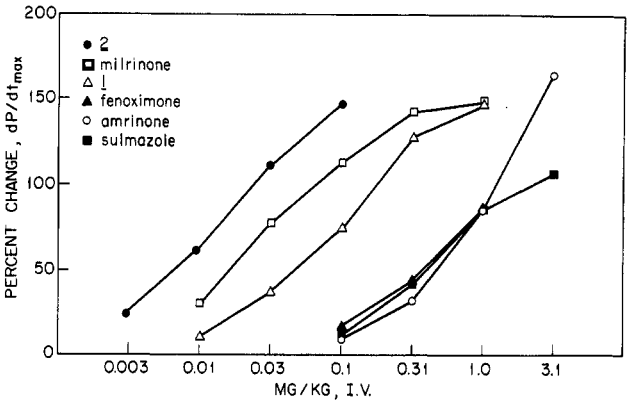


**Figure 1.** Effects of 1, 2, amrinone, milrinone, sulmazole, and fenoximone on myocardial contractility ($d_p/dt_{max}$) in anesthetized dogs.

acid, **4**, was prepared from 4-(1*H*-imidazol-1-yl)benzaldehyde by reaction of the aldehyde with morpholine and KCN,[18] followed by Michael addition of the resultant $\alpha$-aryl-4-morpholinylacetonitrile to 2-butenenitrile in the presence of a catalytic amount of KOH. Hydrolysis afforded **4** (41% from 4-(1*H*-imidazol-1-yl)benzaldehyde), mp 180–181 °C. Elemental analyses for all new compounds were within 0.4% of theoretical values. $^1$H NMR, IR, and mass spectra were consistent with the assigned structures.

**Pharmacology.** The positive inotropic activities of **1** and **2** were determined in acutely instrumented anesthetized and chronically instrumented conscious dogs,[19] by monitoring percentage increases in maximum $dP/dt$ ($dP/dt_{max}$) of left ventricular pressure. Heart rate and aortic blood pressure also were monitored simultaneously. Administration of increasing intravenous $^1/_2$ log doses of 1 (0.01–1.0 mg/kg) to anesthetized dogs produced increases in myocardial contractility ($dP/dt_{max}$) of from 10.2 ± 1.3% to 146.7 ± 25% ($p \leq 0.05$ vs. control), which were accompanied by heart rate changes of 0 ± 1.2% to 33.8 ± 17.0%. Aortic blood pressure was reduced from 0.7 ± 0.4% to 22.4 ± 2.8%. Intravenous administration of increasing $^1/_2$ log doses of **2** (0.001–0.10 mg/kg) to anesthetized dogs produced increases in $dP/dt_{max}$ of 10.5 ± 9.0% to 148.5 ± 22.5% with attendant increases in heart rate of 2.5 ± 2.1% to 43.8 ± 12.0%. Reductions of mean aortic blood pressure of 7.4 ± 1.3% and 19.0 ± 1.3% were recorded at 0.03 and 0.10 mg/kg, with no effects at lower doses. Figure 1 depicts the complete dose–response relationships for myocardial contractility in the anesthetized dog for **1** and **2**. For comparison, the dose–response relationships for amrinone, milrinone, sulmazole, and fenoximone are also presented in Figure 1. In conscious instrumented dogs, oral administration of 1.0 mg/kg of **1** produced a 42.6 ± 4.1% ($n$ = 5) increase in $dP/dt_{max}$ with an attendant increase in heart rate of 48.1 ± 6.7%. Similarly, **2** produced a 50.2 ± 7.1% ($n$ = 5) increase in $dP/dt_{max}$ with a 41.6 ± 11.3% increase in heart rate at 0.1 mg/kg. For each compound, the duration of positive inotropic action was up to 6 h.

**Mechanism of Action.** The mechanism of inotropic action of **1** and **2** has been extensively investigated. Initial studies indicated that **1** had no direct effect on calcium transport, uptake, or release in isolated sarcoplasmic reticulum vesicles, no effect on $Na^+$, $K^+$-ATPase or adenylate cyclase activity, and no effect on mitochondrial respiration or calcium uptake;[20] however, **1** inhibited a crude bovine cardiac phosphodiesterase by 50% at 190 $\mu$M. Following the method of Thompson et al.,[21] three distinct molecular forms of phosphodiesterase were isolated from guinea pig ventricular muscle, and the effects of both **1** and **2** on each were examined. The 4,5-dihydro-3(2*H*)-pyridazinones **1** and **2** are specific inhibitors of guinea pig cardiac phosphodiesterase fraction III—the low $K_m$, low $V_{max}$, calmodulin-independent fraction that hydrolyzes cAMP but not cGMP. For fraction III, the enzyme inhibition $IC_{50}$ values for **1** and **2** are 8.0 and 0.6 $\mu$M, respectively. The $IC_{50}$ values for **1** and **2** on fraction I are 3400 $\mu$M and on fraction II are 800 and 500 $\mu$M, respectively. The substrate concentration for all studies was 1 $\mu$M.

**Table I.** Positive Inotropic $ED_{50}$ Values in Anesthetized Dogs and $IC_{50}$ Values on Fraction III of Guinea Pig Phosphodiesterase for Cardiotonic Agents

| cardiotonic agent | mean $ED_{50}$ ± SEM, mg/kg | $n$ | PDE (III) $IC_{50}$, $\mu$M (95% CL)[a] |
|---|---|---|---|
| 1 (CI-914) | 0.045 ± 0.006[b] | 6 | 8.0 (4.0–10) |
| 2 (CI-930) | 0.013 ± 0.006 | 8 | 0.6 (0.2–1.0) |
| amrinone | 0.389 ± 0.028 | 7 | 50 (40–54) |
| milrinone | 0.037 ± 0.014 | 5 | 2.5 (2.0–3.0) |
| sulmazole | 0.435 ± 0.085 | 4 | 500 (400–600) |
| fenoximone | 0.283 ± 0.020 | 2 | 14 (11–16) |

[a] $IC_{50}$ values were determined by measuring the inhibitory effects of each agent over a concentration range of $1.0 \times 10^{-7}$ to $1.0 \times 10^{-4}$ M or $1.0 \times 10^{-6}$ to $1.0 \times 10^{-3}$ M for the less potent agents. Each value represents the mean three to five experiments using different preparations of phosphodiesterases. [b] The $ED_{50}$ values were calculated from the linear portion of the dose–response curve for each animal via least-squares analysis. The $ED_{50}$ values were then averaged, providing the means and SEM.

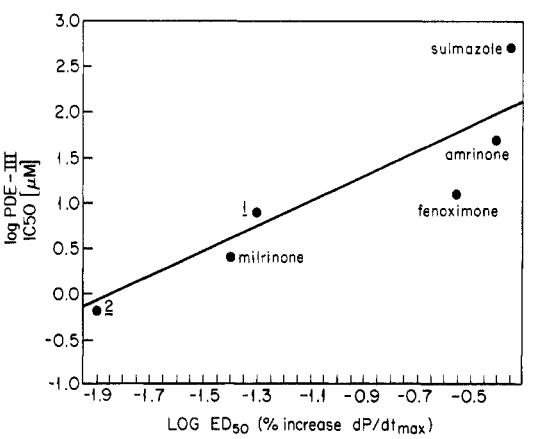


**Figure 2.** Correlation of in vivo positive inotropic potency with fraction III cardiac phosphodiesterase inhibition.

The biochemical evaluation suggests that selective inhibition of a specific molecular form of cardiac phosphodiesterase represents the principal component the positive inotropic action of **1** and **2**. In further support of this concept, **1** ($1 \times 10^{-4}$ M) potentiates the positive inotropic response to isoproterenol in isolated rabbit papillary muscle and significantly increases intracellular cAMP levels in rabbit isolated ventricular papillary muscle.[20]

Table I lists the experimentally determined $ED_{50}$ values for positive inotropic activity in the anesthetized dog (AD-$ED_{50}$) and the $IC_{50}$ values vs. guinea pig phosphodiesterase fraction III (PDE) for **1**, **2**, amrinone, milrinone,

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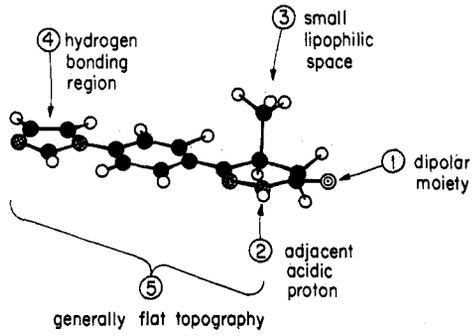


**Figure 3.** Five-point model illustrated with CAMSEQ-II generated structure of the 4,5-dihydro-3(2*H*)-pyridazinone **2**.

sulmazole, and fenoximone. By applying a linear regression analysis to log (AD-$ED_{50}$) vs. log (PDE), an equation describing the positive inotropic potency and enzyme inhibition was determined:

$$\log \text{AD-ED}_{50} = 0.56 \log (\text{PDE}) - 1.60$$

$$r = 0.89 \qquad s = 0.32 \qquad n = 6$$

The graphical relationship of the correlation is depicted in Figure 2. This correlation strongly suggests that the principal component of the positive inotropic action of these agents is inhibition of fraction III cardiac phosphodiesterase. This finding is consistent with recently reported studies with amrinone[22,23] and milrinone[9] on crude cardiac phosphodiesterase and with fenoximone on purified cardiac phosphodiesterase.[24] Initial studies with amrinone[25] suggested that inhibition of cAMP–PDE was not a component of its mechanism. It is of considerable significance that the present report is the first to correlate, in a quantitative manner, the in vivo positive inotropic potencies of several different chemical classes with inhibition of a specific molecular fraction of cardiac phosphodiesterase. Sulmazole has been reported to have a direct action on intracellular calcium transport that may also contribute to its positive inotropic action.[26]

**Molecular Modeling.** Analysis of several new cardiotonic agents by molecular modeling techniques suggests spatial and electronic similarities among cardiotonics of diverse structural classes such as **1**, **2**, amrinone, milrinone, and fenoximone. This observation has resulted in a hypothetical five-point model for positive inotropic activity. Figure 3 denotes the conformational structure of **2** as determined by CAMSEQ-II.[27] The salient features of the five-point model are as follows: (1) the presence of a strong dipole (carbonyl) at one end of the molecule, (2) an adjacent acidic proton, (3) a methyl-sized lipophilic space, (4) a relatively flat overall topography, and (5) a basic or hydrogen bond acceptor site opposite the dipole.[28]

In conclusion, we are reporting two members of a new class of potent positive inotropic agents: 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones, **1** and **2**. Compound **2** is the most potent nonsympathomimetic, noncardiac glycoside cardiotonic agent reported to date. The principal component of positive inotropic mechanism is consistent with inhibition of fraction III cardiac phosphodiesterase, and through the use of molecular modeling techniques, a five-point model has been constructed to rationalize structural features that are necessary for positive inotropic activity in this class of selective cAMP–PDE inhibitors.

**Acknowledgment.** The technical assistance of G. Bobowski, R. Potoczak, R. McNish, and M. Quade is gratefully acknowledged. Helpful discussions regarding the molecular modeling studies and statistical analyses were provided by J. Topliss, J. Vinson, D. Ortwine, W. Ewy, Y. C. Park, and D. Worth.

**Registry No.** **1**, 84243-58-3; **1**·HCl, 89198-09-4; **2**, 86798-59-6; **2**·HCl, 90791-23-4; **3**, 84243-57-2; **4**, 88427-81-0; fluorobenzene, 462-06-6; succinic anhydride, 108-30-5; 4-fluoro-γ-oxobenzenebutanoic acid, 366-77-8; imidazole, 288-32-4; 4-(1*H*-imidazol-1-yl)benzaldehyde, 10040-98-9; hydrazine, 302-01-2.

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## Oxidation of Sparteines by Cytochrome P-450: Evidence against the Formation of *N*-Oxides

*Sir:*

(−)-Sparteine (**1**) is an alkaloid with antiarrhythmic properties, which is oxidized by cytochrome P-450 to yield $\Delta^2$- and $\Delta^5$-dehydrosparteine as major products.[1,2] Our interests in the genetic polymorphism of sparteine metabolism[3] led to further investigation of the reported obligate intermediacy of *N*-oxides in the metabolism of sparteine and its derivatives.[1,4] The $\Delta^5$-oxidation of **1** is catalyzed by purified P-$450_{UT-H}$ and anti-P-$450_{UT-H}$ inhibited the formation of >95% of this activity in microsomes,[3] and this reaction was examined as a model for N-oxidation vs. dealkylation with sparteine as well as other substrates. The formation of these and a number of other *N*-oxides, as intermediates in dealkylation reactions or as stable metabolites,[5] is not in accord with either several earlier dealkylation studies[6] or our current view of the mechanism of oxidation in cytochrome P-450 catalyzed reactions.[7]

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