

## HYDROINDENIC-GUANYLHYDRAZONES. SYNTHESIS AND EVALUATION AS INOTROPIC AGENTS

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**Abstract:** The synthesis and inotropic activity of two families of hydroindenic compounds are described. Among them, a *bis*-guanylhyazone derivative has demonstrated to produce an interesting positive inotropic effect on guinea pig atria, displaying at higher dosis a similar effect to that elicited by digoxin. © 1998 Elsevier

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Congestive heart failure is one of the most prevalent diseases in the western countries<sup>1</sup>, with a high incidence in the elderly, which accounts for the increment in the number of deaths, hospitalizations and medical costs at a time when the morbidity and mortality from other common cardiovascular diseases are on the decline<sup>2</sup>. The therapy for its treatment consists of three major classes of drugs: 1) positive inotropics, to improve heart function, 2) diuretics, to facilitate the loss of sodium and water, and 3) vasodilators, to reduce periferal vascular resistance<sup>3</sup>. In spite of the great research effort, the overall mortality rate from congestive heart failure over a five years period is 50%. New drugs have been designed for the management of congestive heart failure, such as carvedilol<sup>4</sup>, an unique agent acting through several pharmacological actions, which produces a decrease of heart loading.

Due to their well established inotropic effect, the long time known cardiac glycosides are still in the clinical use for the treatment of congestive heart failure, despite their low therapeutic index. Many derivatives and semisynthetic analogues have been prepared in the last decades<sup>5</sup>, in order to obtain new inotropic agents devoid of the toxic effect produced by the cardiac glycosides.

As a part of a programme aimed at the obtention of new compounds containing the C,D-rings system of digitalis glycosides, we published the synthetic methodology developed for this purpose<sup>6-7</sup>. By this procedure, we prepared octahydro- and perhydroindene (hydrindane) derivatives introducing differently substituted chains at C<sub>1</sub> (equivalent to the C<sub>17</sub> of the steroid) and functionalities at C<sub>5</sub><sup>8</sup>. The rationale to prepare these compounds is based on the lack of definite structural requirements of steroidal derivatives for the inotropic activity, that has

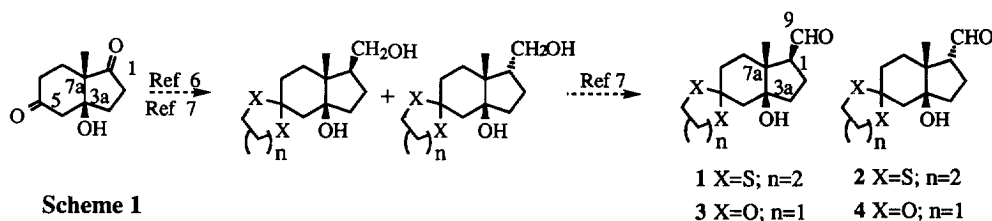
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even suggested that the digitalis pharmacophore is the steroid skeleton itself<sup>9</sup>. In this research, we decided to investigate the ability of small molecules carrying structural parts of the steroid as positive inotropic agents. By introducing different chains as replacement of the butenolide on the C1 position, we could assess the contribution of the C,D-ring-C17 substituent of the steroid skeleton to their cardiotonic activity. The non steroidal structure of these molecules could also contribute to decrease the toxicity of these analogues in comparison to the highly toxic natural models.

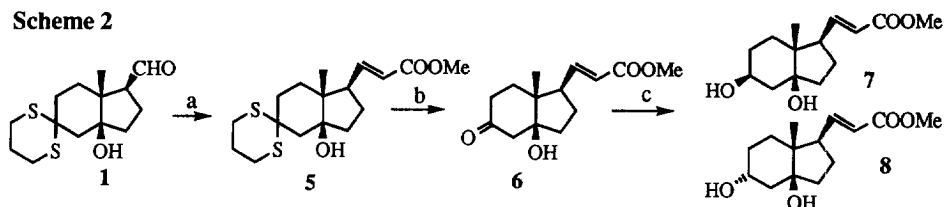
In this communication we report the synthesis and inotropic activity of two new families of hydroindenenic compounds. The first one maintains the methyl and hydroxyl groups on the *cis*-junction of C,D-rings of the steroid. The second family are hexahydroindenenes, with higher modification of the geometry, prepared to evaluate the influence of a double bond in C<sub>3a</sub>-C<sub>4</sub>, equivalent to positions C<sub>14</sub>-C<sub>8</sub> of the steroid. Up to our knowledge there is not reference so far of compounds with similar type of structural variation possessing inotropic activity.

## Chemistry

The synthesis of both families of compounds has been brought about following the procedure described in previous papers for the synthesis of key intermediate aldehydes 1-4 (Scheme 1), starting from the readily available enantiomerically pure Hajos-Parrish ketone<sup>10</sup>. These aldehydes allowed us to convert the major  $\alpha$  isomer 2 into the isomer 1 of required  $\beta$  stereochemistry at C1<sup>7</sup> (epimerization under basic conditions produced a 1:2 mixture in 9:1 ratio), when they were protected as the dithiane derivatives. It has been now proved that it is also the case with the aldehyde protected as dioxolane 4, which is converted into the  $\beta$ -isomer 3 under the same conditions, although the ratio 3:4 in the epimerization mixture is 6:4.

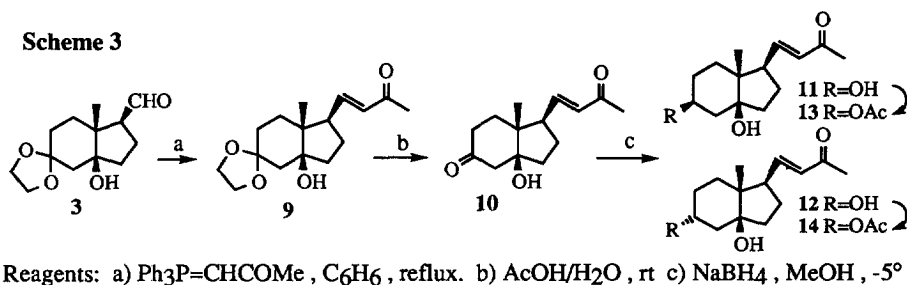


Aldehydes 1 and 3 were transformed into the target molecules through different condensation reactions, leading to the preparation of unsaturated chain analogues at C1. One of the groupings that can replace the butenolide ring of cardenolides is the methyl acrylate moiety<sup>5</sup>, which was introduced (Scheme 2) by a Wittig reaction with methoxycarbonylmethylenephosphorane to yield compound 5. By deprotection<sup>11</sup> to 6 followed by reduction, two very simple derivatives 7 and 8, with C,D-rings partial steroid structure and a lactone replacing moiety, were synthesized.



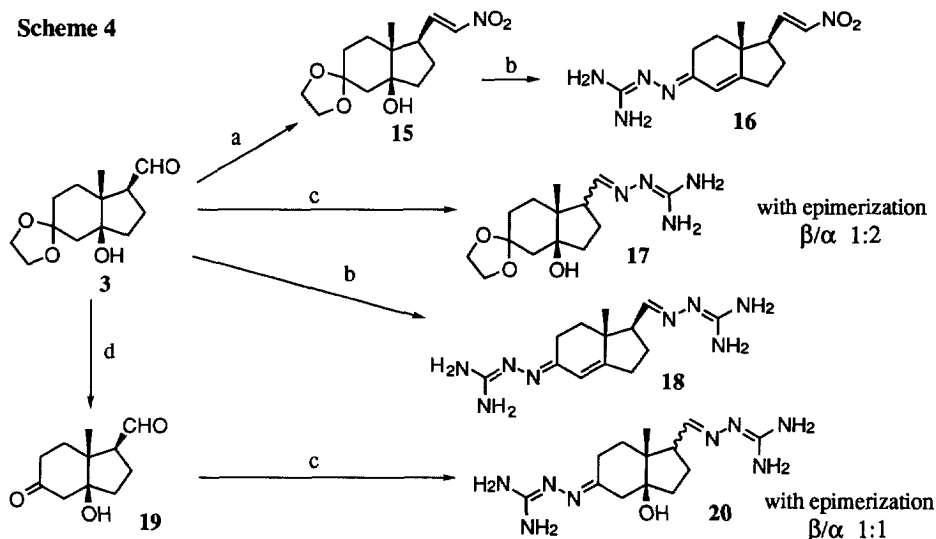
Reagents: a)  $\text{Ph}_3\text{P}=\text{CHCOOMe}$ ,  $\text{C}_6\text{H}_6$ , reflux. b)  $\text{HgO}$ ,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , THF, rt c)  $\text{NaBH}_4$ , MeOH,  $-5^\circ\text{C}$

By a similar methodology, starting from the aldehyde **3** with the keto group at C5 protected as dioxolane, the butenone moiety was introduced in **9**. Deprotection to **10** and reduction yielded dihydroxyketones **11** and **12**, that were respectively acetylated to **13** and **14** (Scheme 3). Compounds **7-8** and **11-14** were fully characterized as the *E*-isomers at the double bond.

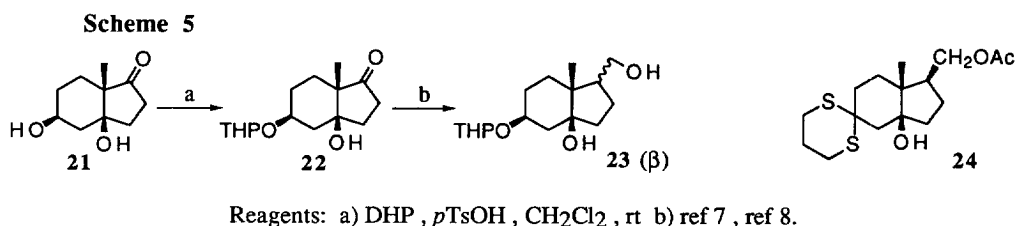


A wide variety of molecules can be designed from starting materials **1** and **3** as hydrindane based analogues of cardenolides. In order to have a general view of the effect of different substituents at C1 we decided to prepare the simpler hydroxymethyl molecules, the unsaturated nitro derivatives and the guanylhhydrazones<sup>12</sup>, as representatives of small chain, highly electron withdrawing and positively charged (protonated) residues at that position.

The synthesized molecules of these classes are presented in Schemes 4 and 5 below.



Unsaturated nitro moiety was introduced by condensation of aldehyde **3** with nitromethane, to afford protected derivative **15**, which gave the 9*E*-nitro-guanylhydrazone-hexahydroindene product **16**. Direct introduction of the 9*E*-guanylhydrazone residues on this aldehyde gave the monoguanylhydrazones **17** or the bis-guanylhydrazone **18** derivatives, depending on the reaction conditions. It was also possible to synthesize the bis-guanylhydrazone **20** maintaining the hydroxyl group at C3a from the ketoaldehyde **19**, obtained by deprotection of the starting aldehyde **3**. As representative of small C<sub>1</sub> chain analogues, hydroxymethyl protected compound **23** was synthesized by the standard methodology from the hydroxyketone **22**, obtained by selective reduction of the Hajos-Parrish ketone followed by treatment with DHP. Compound **24**, obtained in intermediate steps of Scheme 1, was also assayed.



## Pharmacology

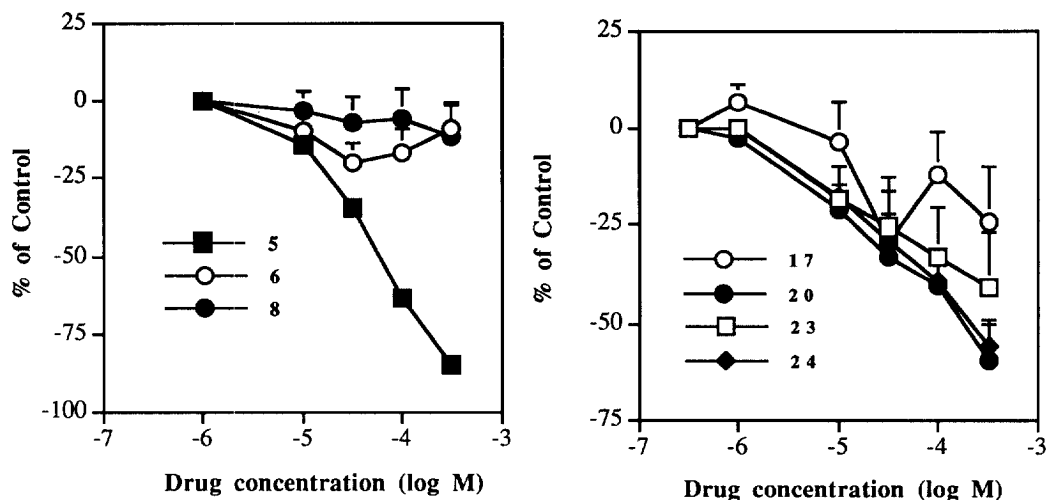
The inotropic effect of representative target molecules and synthetic intermediates was evaluated on isolated guinea pig atria, following the described methodology<sup>13</sup>. The spontaneously beating right atrium was used to evaluate the effect on the cardiac frequency, while the left atrium was maintained under constant electrical stimulation and was used to measure the effect on the force of contraction.

The assays were carried out by incorporating increasing cumulative concentrations ( $10^{-7}$  to  $5 \times 10^{-4}$  M) in a 20 minutes intervals. The results are expressed in % respect to the basal conditions before the incorporation of the compounds, as shown in figures 1 and 2. The activities are compared to the effect of digoxin, that was used as reference.

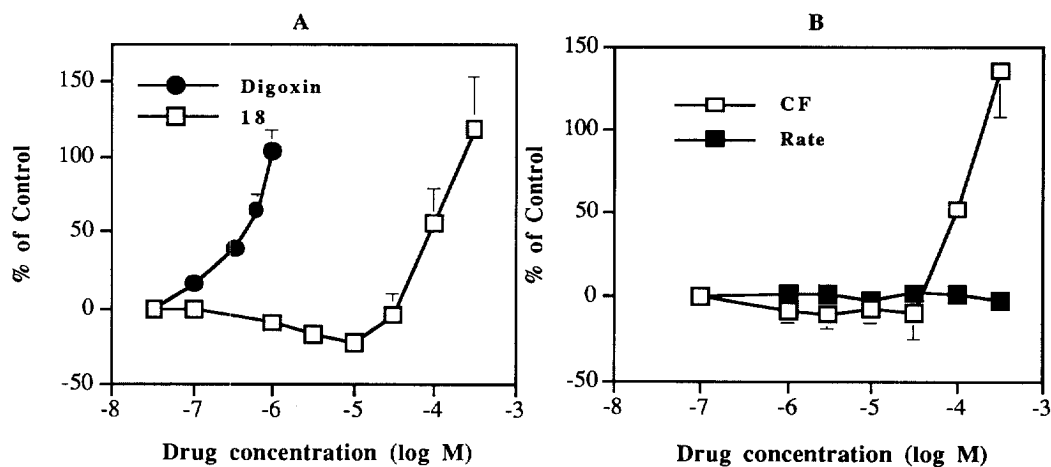
Most of the selected compounds belong to family I, with an hydroxyl group at C3a, having a selection of structural moieties at C<sub>1</sub> and C<sub>5</sub>. Compounds of family II, with a double bond at C3a-C<sub>4</sub>, are formed by dehydration during the introduction of the guanylhydrazone. The tested compounds have very different effects under the assayed conditions (figures 1 and 2).

The unsaturated esters **6**, **8** and ketones **13**, **14** displayed no effect on the rate or on the force of contraction. Other compounds of family I, unsaturated ester **5** and C<sub>1</sub> small chain derivatives **23**, **24** and guanylhydrazones **17** and **20**, demonstrated (as epimeric mixtures) negative inotropic activity, with a reduction of the force of contraction up to 56% for **24** and 85% for **5** at concentrations of  $3 \times 10^{-4}$  M.

Both guanylhydrazones also diminish the contractile force of right atria without modifying its spontaneous contraction rate (data not shown).



**Figure 1.** Effects of compounds 5, 6, 8, 17, 20, 23, and 24 on the peak contractile force in isolated guinea-pig electrically driven left atria. Ordinate: % of control values. Abscissa: drug concentration (log M). Each point represents the mean of at least five experiments; vertical bars represent the S.E.M.



**Figure 2.** Panel A: Effect of digoxin and compound 18 on the peak contractile force in isolated guinea-pig electrically driven left atria. Panel B: Effect of compound 18 on the rate (Rate) and peak contractile force (CF) of spontaneous contractions in isolated guinea-pig right atria. Ordinate: % of control values. Abscissa: drug concentration (log M). Each point represents the mean of at least five experiments; vertical bars represent the S.E.M.

In family II, compound 18 showed a very interesting effect on the contraction, displaying a concentration-dependent positive inotropic effect on both atria, with the same % increase than the control digoxin, although at a much higher concentration ( $3 \times 10^{-4}$  M). Interestingly, the cardiac frequency was not affected under any of the assayed concentrations (figure 2). Similar results (not shown) were observed for compound 16 in preliminary assays.

## Conclusion

These assays demonstrated a very good inotropic effect of the new *bis*-guanylhyazone derivative **18**, belonging to family II of the synthesized hydrindane based derivatives. The absence of alterations on the cardiac frequency associated with its positive inotropic effect is an outstanding characteristic of this compound. The compounds carrying a carbon chain at C<sub>1</sub> do not display positive inotropic effect, but some of them have a negative effect. The same happens with guanylhyazone derivatives of family I, carrying the hydroxyl group with the same configuration at C<sub>3a</sub> than the C<sub>14</sub> of natural agents.

In comparison to cardenolides, the geometry of the positive inotropic agents **16** and **18** is very different, with a displacement of the C<sub>1</sub> chain respect to the more planar bicyclic system than the observed for the butenolide in relation to the bent C,D-ring system of the steroid. In consequence, the activity can be attributed either to a different interaction with the digitalic receptor or to an alternative mechanism of action.

After those promising results, we are currently investigating the activity of other guanyl-hydrindanic molecules and their mode of action.

## Acknowledgements

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