Inotropic Activity of Hydroindene Amidinohydrazones

Luis G. Sevillano,[†] Concepción P. Melero,[†] Esther Caballero,[†] Fernando Tomé,[†] Lionel G. Lelièvre,[‡] Käthi Geering,[§] Gilles Crambert,[§] Rosalía Carrón,[||] Manuel Medarde,*[,†] and Arturo San Feliciano[†]

Facultad de Farmacia, Laboratorio de Química Orgánica y Farmacéutica, Campus Miguel de Unamuno, E-37007 Salamanca, Spain, Laboratoire de Pharmacologie des Transports Ioniques Membranaires, Université Paris 7, EA 2381, 75251 Paris, France, Institut de Pharmacologie et Toxicologie, Université de Lausanne, Bugnon 27, 1005 Lausanne, Switzerland, and Facultad de Farmacia, Laboratorio de Farmacología, Campus Miguel de Unamuno, E-37007 Salamanca, Spain

Received May 16, 2001

Several hydroindenic derivatives (7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-indenes), bearing an amidinohydrazone at C-5 and different moieties at C-1, have been synthesized and evaluated for their inotropic and chronotropic effects on right- and left-guinea-pig-atria activity. Three of them showed the same profile as digoxin, although with lower potency. The effect on Na^+,K^+-ATPase (NKA) was also evaluated for these three compounds, observing that two of them, with the same absolute configuration as natural cardenolides, are also NKA inhibitors, while the compound with the opposite configuration lacks such an effect. More interestingly, both active compounds act without affecting the cardiac rhythm. This could be related to the selective inhibition of the human $\alpha_2\beta_1$ isozyme (associated with the inotropic effect) with respect to the $\alpha_1\beta_1$ isozyme (associated with the maintenance of basal ionic levels in the cell and the toxic effect of cardenolides).

Introduction

Congestive heart failure (CHF) is a common syndrome in which a dysfunction of the heart causes a mismatch between the blood supply and its demand by the organs.[1] Currently, CHF represents a major and escalating public health problem.[2] Treatment strategies for CHF[3] are addressed at preventing and delaying progression of the disease and improving survival. Diuretics[4] are currently the drugs of first choice for patients with fluid retention, while therapies based on the use of angiotensin-converting enzyme inhibitors[5] have been shown to decrease mortality and the progression of CHF.

Cardiac glycosides (CG) have played a prominent role in the therapy of CHF since Withering (1785) first described the efficacy of the leaves of the common foxglove (*Digitalis purpurea*) in the treatment of dropsy. Today CG such as digoxin and methyldigoxin continue to be the choice for the treatment of chronic CHF.[6] Digoxin therapy[7] is believed to induce a decrease in the risk of CHF worsening irrespective of its effects on cardiac rhythm, systolic function, the severity of CHF, or therapy with ACE inhibitors. Nevertheless, a major problem in their clinical use is the low therapeutic index of CG, mainly due to their cardiac proarrhythmogenic activity. The search for less toxic agents has prompted much research into natural cardiotonic and synthetic compounds.[8] Recently, the identification of endogenous digitalis-like factors, which may be responsible for essential hypertension,[9] has opened a new field in the study of digitalic compounds.

The genins of the CG are steroids with three peculiar structural features, all of them initially assumed to be of major relevance for their pharmacological activity: the 17β-butenolide moiety, the C/D cis ring junction, and the 14β-hydroxyl function. Currently, it is known that the butenolide can be replaced by other groups such as aminoalkyl, nitroalkyl, methylacrylate, and amidinohydrazonoalkyl residues, which still retain affinity[10] for the digitalis receptor. Digitalis-like activity is also shown by a number of steroids and steroid-like substances that differ significantly in structure from the classic cardiotonic steroids. Alkaloids from *Erythrophleum* species, such as cassaine and its analogues,[11] and a series of other synthetic and semisynthetic digitalic analogues show significant structural differences from the classic cardenolides but are positive inotropics, and most appear to exert their action through the same mechanism.

The inotropic action of cardiac glycosides is mainly due to the inhibition of the Na^+,K^+-ATPase (NKA). This enzyme plays a crucial role in cellular ion homeostasis and constitutes the pharmacological receptor for digitalis CG in humans. NKA is a heteromeric enzyme with a 112 kDa α subunit and a 53 kDa glycosylated β subunit.[12] Four isoforms (α_1, α_2, α_3, and α_4) of the catalytic α subunit and three of the β subunit (β_1, β_2 and β_3) have been identified. In the rat heart, $\alpha_1\beta_1$ isozymes and $\alpha_2\beta_1$ isozymes are present, the former exhibiting a very low and the latter a high affinity for cardiac glycosides. In the human heart, the CG receptors[13] are $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$. These isozymes have similar intrinsic ouabain sensitivities but differ in their K^+ antagonism for ouabain binding.[14] At physiological K^+ concentrations, digitalis may predominantly bind to

* To whom correspondence should be addressed. Facultad de Farmacia. Phone: 34-923-294528. Fax: 34-923-294515. E-mail: medarde@gugu.usal.es.
[†] Laboratorio de Química Orgánica y Farmacéutica, Campus Miguel de Unamuno.
[‡] Université Paris 7.
[§] Université de Lausanne.
[||] Laboratorio de Farmacología, Campus Miguel de Unamuno.

10.1021/jm0109309 CCC: $22.00
Published on Web 12/08/2001

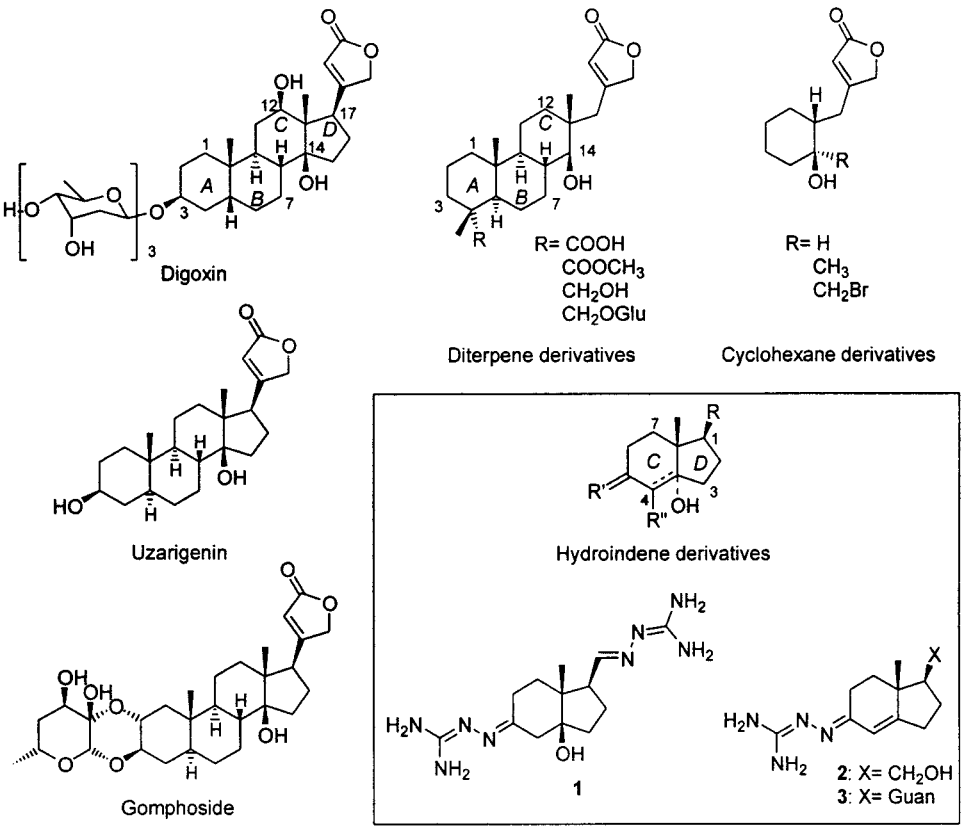


Figure 1. Structures of digoxin, uzarigenin, and gomphoside compared to diterpenic, cyclohexanic, and hydroindenic derivatives.

α_2 and α_3 isozymes and, although to a lesser extent, to α_1 isozymes. According to the results obtained in heterozygtous knockout mice, the α_2 isoforms may play a particular role in the regulation of calcium in the heart.[15] Special situations, such as digitalis overdose-due hypokaliemia, can lead to toxic effects. In light of this, it would be desirable to synthesize compounds that could selectively inhibit some of the "inotropic isozymes" without affecting the $\alpha_1\beta_1$ dimer responsible for the maintenance of the basal ionic gradients in the cell.

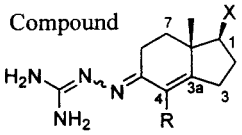
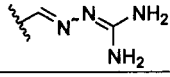
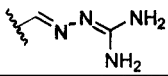
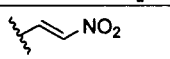
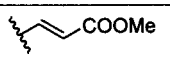
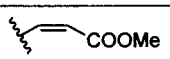
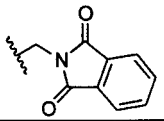
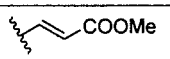
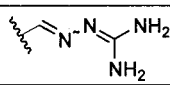
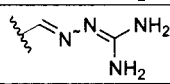
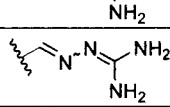
Because of the expected high contribution of the steroid moiety and the importance of the lactone ring, or an equivalent fragment, for the positive interaction with the digitalis binding site, we decided to investigate the influence of different skeletons on inotropic activity. Thus, we started with the synthesis of diterpenic cardenolide analogues[16] from sandaracopimaric acid (Figure 1). Like the cardiac glycosides, these synthetic compounds maintained the butenolide and the OH group at the 14β position. The most significant differences were the absence of the D ring, the A/B trans junction, and a different substitution pattern of the A ring. The trans junction of rings A and B should not produce any loss of cardiotonic activity because some compounds are potent cardioactive compounds with the A/B trans junction, such as uzarigenin and gomphoside.[17] However, these diterpenic analogues showed neither positive nor negative appreciable inotropic effects. We attributed this to the lack of the D ring of the steroid system, thus imparting excessive mobility to the lactone ring, which loses the possibility of attaining the proper arrangement in relation to the other structural moieties of the molecule.

More simplified cardenolide analogues, with only a cyclohexane equivalent to the C ring of the steroid, the OH group (14β of the steroid), and the lactone ring, were also devoid of any inotropic activity.[18] This was attributed to both the absence of the major part of the steroid skeleton and also to the excessive mobility of the lactone ring (Figure 1).

Consequently, we selected the hydroindene skeleton, representing the C, D rings of the steroid system, as the skeletal base for the synthesis of new compounds carrying a 3a-βOH (equivalent to the 14-βOH of cardenolides). An α-methyl group at C4-α was also introduced in some of them in order to fix the hydrindane system in a chair conformation of the cyclohexane ring having the angular methyl group at C7a in an axial disposition, identical to the C,D rings of natural cardenolides. The bis(amidinohydrazone) **1**, with the hydroxyl group equivalent to the 14β-OH function of the cardenolides and the expected configuration of the indane fragment, showed a negative inotropic effect as did the Δ^{3a} unsaturated hydroxymethyl derivative **2**. However, the Δ^{3a}-unsaturated analogue **3**, readily obtained by dehydration of the 3a-βOH, produced a fairly positive inotropic effect, although at a much higher concentration than digoxin[19] (Table 1). Interestingly, cardiac frequency was not substantially affected under any of the concentrations assayed.

Intrigued by these observations and with the aim of improving this positive inotropic effect while maintaining the absence of any effect on the heart rate, here we synthesized new bis amidinohydrazono hydroindene derivatives from the previously synthesized intermediates bearing, or not bearing, the methyl group at C4. We also decided to prepare compounds with the amidinohydrazono moiety at C_5 in order to maintain the molecular size and solubility of the complete cardenolide and to also prepare compounds carrying small groups

Table 1. Inotropic Activity of Amidinohydrazones on Electrically Driven Guinea Pig Left Atrium and Effect on Cardiac Frequency on Spontaneously Beating Guinea Pig Right Atria

Compound 	R =	X =	Emax (% from basal force)	Emax (% from basal frequency)	Emax (mM)
1	3a = βOH 3a,4 diH		- 59 ± 10.6	-15±4.4	0.3
2	H		- 47 ± 10.1	22±3.4	0.3
3	H		132 ± 46.8	-5.4±4.1	0.3
7	H		128 ± 34.2	-1.7±0.1	0.3
10	H		- 67 ± 17.4	13.2±2	0.3
11	H		- 76 ± 7.5	-54±15.3	0.3
15	H		86 ± 22.3	-18±8.7	0.1
16	H		46 ± 16.7	-16±1.7	0.3
19	Me		-41 ± 7.1	-0.2±3.8	0.3
20 (1β)	Me		108 ± 8.7	4.2±7.4	0.3
22 (1α)	Me		129 ± 6.5	33.7±6.3	0.3
30 (enantiomeric series)	H		170 ± 66.5	-5.1±7.4	0.3
Digoxin			103 ± 14.3	-28.3±3.7	10^{-3}
Strophanthidin			122 ± 58.4	80.4±39.4	10^{-3}

(−CH_2OH, −CH_2NH_2) or α,β-unsaturated chains (methoxycarbonylvinyl, nitrovinyl), which can replace the lactone ring in their binding to the receptor[8] at the C_1 position.

The inotropic effect of representative target molecules was evaluated on isolated guinea pig atria. To contribute to the knowledge of their mode of action, the compounds with the best pharmacological activity as positive inotropics were evaluated in vitro as Na^+,K^+-ATPase inhibitors. Our interest lay in knowing whether these compounds, which produce positive inotropic effects with no modification in the cardiac frequency, are also NKA inhibitors and might selectively inhibit one of the NKA isozymes.

Chemistry

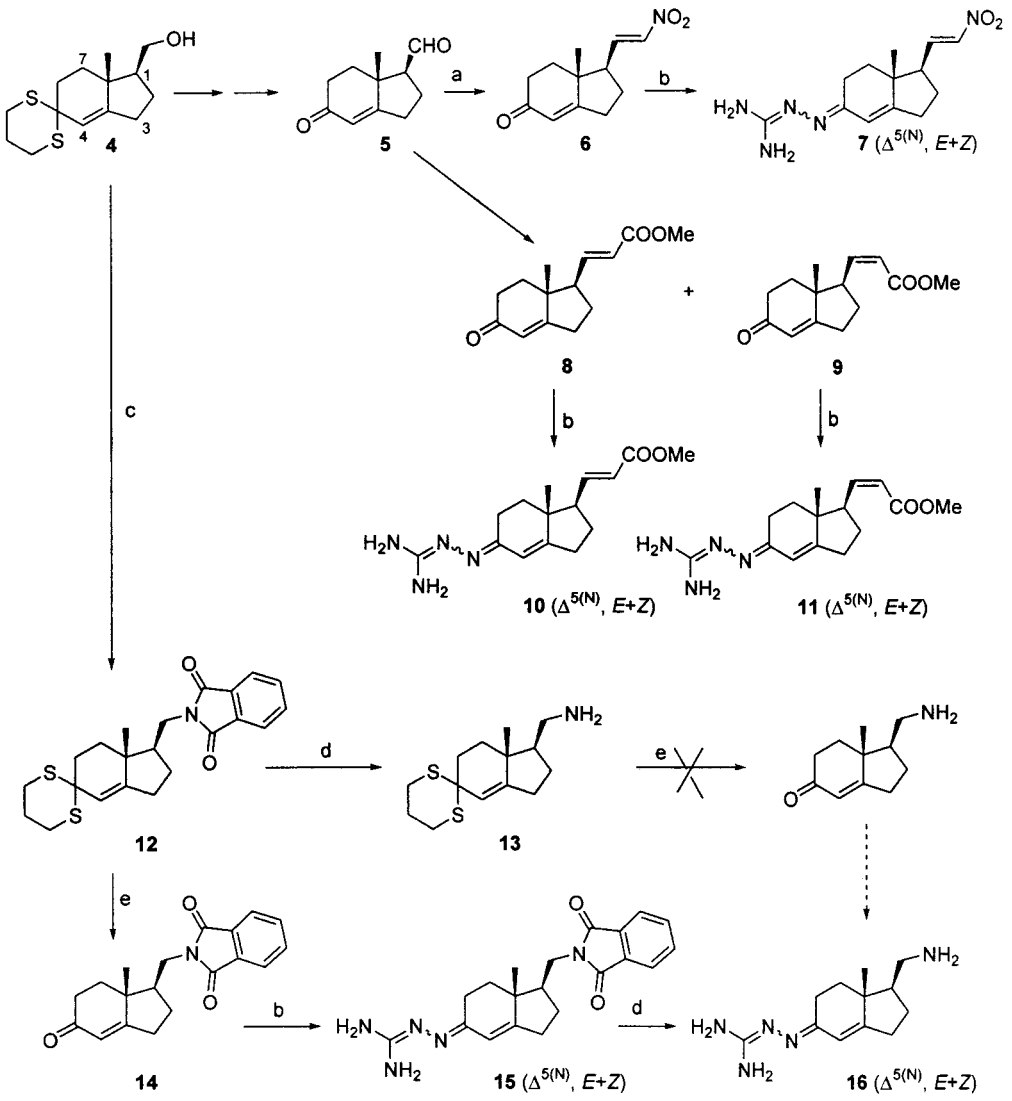
For the preparation of derivatives with the hydroindene skeleton (7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-indene), we decided to start from the readily available enantiopure compounds **4** and **5** and to carry out their transformation into the target molecules.

The $C_1\beta$ formyl derivative **5** was obtained as previously described by our group,[20] by PCC oxidation of the primary alcohol **4** (Scheme 1). Compound **5** contains several adequate structural features for the preparation of final products: (a) the same β disposition of the formyl group at C_1 and the methyl group at C_{7a}, similar to those of the C_{17} and C_{13} positions of model cardenolides, (b) a C_1 formyl group and a C_5 keto groups, useful for the introduction of the planned moieties, and (c) a higher stability than that of its $C_{1\alpha}$ epimer, thus avoiding its undesired epimerization at this position.

Starting with the preparation of unsaturated derivatives at C_1, the nitrovinyl derivative **6** was obtained via selective nitromethane condensation[21] using ammonium acetate as base. Because of the basic conditions, the carbon at C_1 position was epimerized to the equilibrium 10:1 $C_1\beta/C_1\alpha$ mixture, from which the condensation product **6** was isolated in 42% yield and the starting material recovered in 20% yield.

Transformation of ketone/formyl derivatives into the amidinohydrazone compounds can be completed by two procedures. One of them uses aminoguanidine hydrochloride and 2 N HCl (method A) and the other, aminoguanidine bicarbonate and 2 N HCl, added until an acidic pH is reached (method B).[22] Following method

Scheme 1[a]



[a] Reagents: (a) CH_3NO_2, NH_4OAc, reflux (1.5 h); (b) $H_2NNHC(NH)NH_2 \cdot HCl$, EtOH, reflux (45 min); (c) phthalimide, Ph_3P, DEAD, THF, room temp (19 h); (d) $N_2H_4 \cdot H_2O$, EtOH absolute, reflux (7 h); (e) HgO, $BF_3 \cdot OEt_2$, THF/H_2O, room temp (1 h).

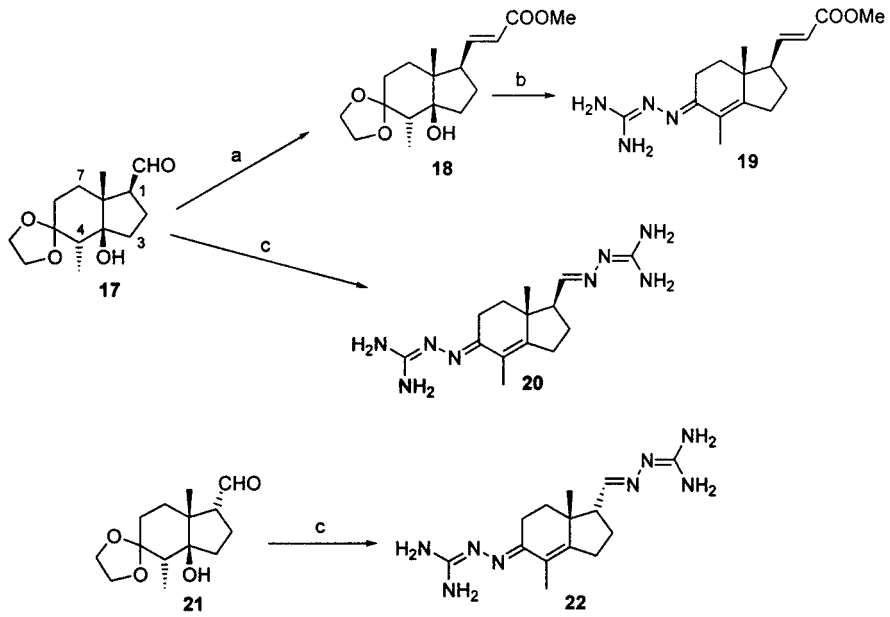
A, a 3:1 ($\Delta^{5(N)}$, *E*/*Z*) mixture of the C_1 nitrovinyl-C_5 amidinohydrazono derivative **7** was obtained.

Another of the groupings that can replace the butenolide ring of the cardenolides is the methoxycarbonylvinyl residue.[23] Compounds **8** (major) and **9** were prepared from the aldehyde **5** by the Wittig reaction with methoxycarbonylmethylentriphenylphosphorane and separated by flash chromatography. Both compounds were transformed into the amidinohydrazones **10** and **11** (6:1 $\Delta^{5(N)}$, *E*/*Z* mixtures).

Once **7**, **10**, and **11** had been prepared as representatives of compounds with unsaturated moieties at C_1 and carrying the amidinohydrazone at C_5, we decided to obtain the nitrogen derivative at C_{10}. The hydroxy derivative **4** was converted into the aminomethyl derivative via PCC oxidation followed by reductive amination,[24] although this methodology produces the desired compound in low yields, as described in the literature. Another possibility for introducing a nitrogenated function replacing the hydroxyl groups is the Mitsunobu reaction.[25,26] This procedure allows the transformation of the hydroxyl group into the amino group in good overall yield.[27] Treatment of **4** with phthalimide, Ph_3P, and diethyl azodicarboxylate gave the phthalimido derivative **12**, which was converted into the amine **13** by the Ing−Manske exchange reaction in which the phthalimide is heated with hydrazine.[28] The attempts to prepare the amino−enone directly from **13**, via deprotection of the dithiane with HgO and a Lewis acid, failed, and we were unable to isolate the desired compound. Because of the difficulties involved in preparing the intermediate amino−enone derivative, a new approach was attempted, starting from compound **12** and maintaining the phthalimido moiety until the last step. In this way compound **14** was obtained via HgO/$BF_3 \cdot OEt_2$ deprotection,[29] followed by condensation of the enone with aminoguanidine hydrochloride to produce the intermediate amidinohydrazone derivative **15**. By the exchange procedure with hydrazine, the final product **16** was obtained in an appropriate overall yield from **12**.

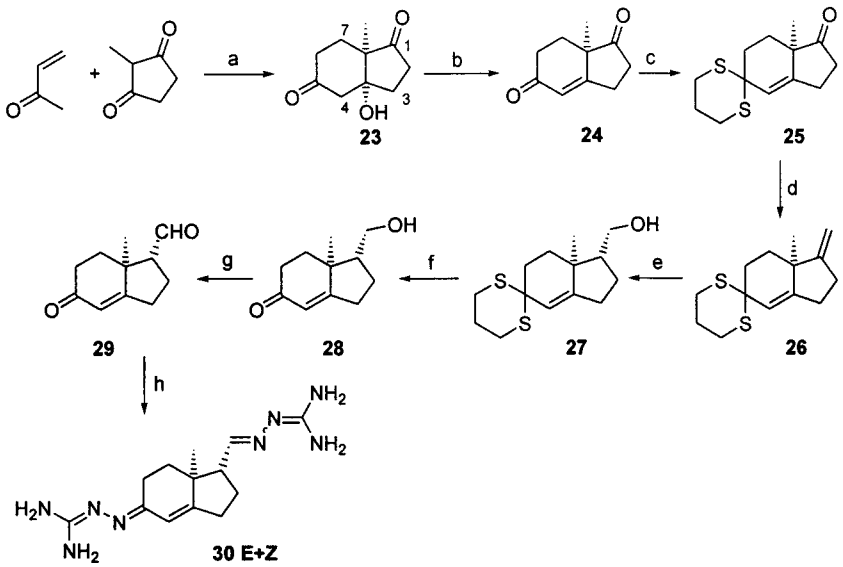
Already synthesized compounds carrying an αMe-C_4, such as the aldehydes **17** and **21**, were used to prepare C4-substituted analogues. In the same way (Scheme 2) as previously described and optimized, **19** was prepared by olefination of **17**, followed by aminoguanidine treat-

Scheme 2[a]



[a] Reagents: (a) $Ph_3P{=}CHCOOMe$, benzene, reflux (5.5 h); (b) $H_2NNHC(NH)NH_2{\cdot}H_2CO_3$, 2 N HCl, EtOH, reflux (2 h).

Scheme 3[a]



[a] Reagents: (a) (i) AcOH, H_2O, 70 °C (2 h), (ii) *R*-(+)-proline, DMF, room temp (24h); (b) pTsOH, benzene, reflux (1 h); (c) $HS(CH_2)_3SH$, TMSCl, $CHCl_3$, room temp (3 h); (d) $Ph_3P^+CH_3I^-$, NaO^tAm, benzene, reflux, (45 min); (e) (i) BBN, THF, room temp, (1.5 h), (ii) H_2O_2/OH^-, EtOH, 50 °C (1 h); (f) HgO, $BF_3{\cdot}OEt_2$, THF/H_2O, room temp (1 h); (g) CCP, molecular sieves 4 Å, CH_2Cl_2, room temp (2 h); (h) $H_2NNHC(NH)NH_2{\cdot}HCl$, EtOH, reflux (1 h).

ment of **18** by method B. To gain information about the influence of the stereochemistry at the C_1 position, both amidinohydrazono derivatives **20** and **22** with α and *β* configurations at this position were obtained. By use of the standard procedure, **20** was prepared directly from aldehyde **17** and **22** from the epimeric aldehyde **21**.[20]

We also decided to investigate the influence of the absolute stereochemistry in the inotropic and ATPase inhibition activities. The enantiomer of the most potent compound **3** was synthesized using the suitable enantiopure starting material and following a similar synthetic sequence as in the normal series (Scheme 3).

Compound **23** was prepared via enantioselective cyclization of the corresponding triketone, readily obtained from methylvinyl ketone and 2-methyl-1,3-cyclopentanedione, using *R*-(+) proline as a chiral catalyst. The dehydration of **23** was carried out under acidic conditions (*p*-TsOH). Transformation of **24** into the dithiane derivative by standard procedures[29] allowed us to obtain compound **25** with no detectable amounts of the C_1-protected products.[30] The one-carbon elongation at C_1 was carried out[31] following the Conia variation[32] of the Wittig reaction, thus producing the methylenation of **25**, which was transformed into **26** in high yield (67%). Hydroboration−oxidation of **26** led almost stereospecifically to **27**, the $^{\alpha}/_{\beta}$ ratio in the crude reaction product being $^{21}/_{1}$. Deprotection of dithiane **27** as described in standard procedures, followed by PCC oxidation of **28**, afforded aldehyde **29**. Compound **30** was then obtained by double condensation with aminoguanidine hydrochloride with acidic catalyst. By use of this optimized sequence, the enantiomeric final product **30**

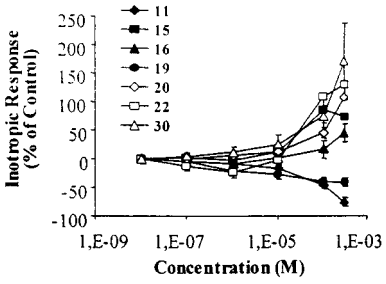


Figure 2. Cumulative concentration−response curves of compounds **11**, **15**, **16**, **19**, **20**, **22**, and **30** on isolated guinea pig left atrial contractile force. Each value represents the mean of at least five experiments. Vertical lines show ±SEM.

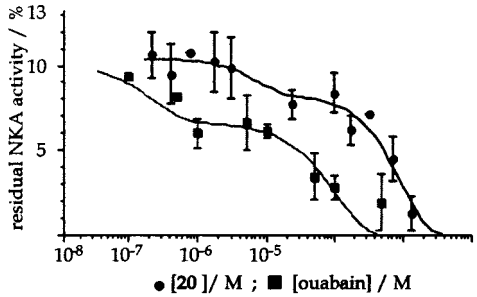


Figure 3. Inhibition of Na^+,K^+-ATPase activity in rat heart microsomes by ouabain and compound **20**. Each point represents the mean ± SEM of five to six measurements.

was obtained in 15% overall yield (seven steps) from the readily available diketone **23**.

Biological Activity

Inotropic Activity. The inotropic effect of representative target molecules was evaluated on isolated guinea pig atria following the described methodology.[33] The left atrium was maintained under constant electric stimulation and was used to measure the effect of samples on the contraction force, while the spontaneously beating right atrium was used to evaluate the effect on cardiac frequency.

The assays were carried out by increasing cumulative concentrations of drug (from 10^{-7} to 5×10^{-4} M) at 30 min intervals. The results are expressed as a percentage of control values obtained before incorporation of the compounds. The activities were compared to the effect of digoxin and strophantigenin, which were used as references.

The effects of several compounds on the left atrial contractile force are summarized in Table 1. The effect of compounds **1**−**3**, **7**, and **10** as inotropic agents has been discussed in previous papers.[19,20] Compounds **3**, **7**, **15**, **16**, **20**, **22**, and **30** showed positive inotropic effects at concentrations above 10^{-5} M (Figure 2). The most potent compounds were **3** and **30**. The maximum effects of these compounds mimicked those obtained with 10^{-6} M digoxin. Compounds **1**, **2**, **10**, **11**, and **19** showed a negative inotropic effect at concentrations higher than 10^{-6} M. Compounds **20** and **30** produced a concentration-dependent positive inotropic effect, starting at 10^{-6} M and attaining a maximum effect similar to that of digoxin, without affecting cardiac frequency (Table 1). Compound **22** induced a negative inotropic effect at low concentrations and a positive inotropic effect at higher concentrations, comparable to the reference inotropic compounds, and increased cardiac frequency at higher doses. Compounds **11** and **19** showed a concentration-dependent negative inotropic effect, and compounds **15** and **16** showed a slight positive inotropic effect at concentrations higher than 10^{-6} M (Figure 2).

Effect of Compound 20 on Rat Heart NKA Activity. The inhibitory effect of compound **20** on Na^+,K^+-ATPase activity from rat heart microsomes was compared to that of ouabain. As previously reported,[34] ouabain produced a biphasic inhibition curve (Figure 3), reflecting the presence of high-affinity $\alpha_2\beta$ ($K_i = 3 \times 10^{-7}$ M, 45% of total activity) and low-affinity $\alpha_1\beta$ ($K_i = 5 \times 10^{-5}$ M, 55% of total activity) Na^+,K^+-ATPase isozymes in rat heart. Compound **20** also produced a biphasic inhibition curve, but compared to ouabain, the K_i values were about 1 order of magnitude higher for both isozyme components ($K_i = 2 \times 10^{-6}$ and 5×10^{-4} M, respectively). This result is consistent with the positive inotropic effect observed for this compound at high concentrations (Table 1).

Effect of Compounds 7 and 30 on the Transport Activity of Human NKA Isozymes. Compounds **7** and **30** were tested for their ability to inhibit the transport activity of the three Na^+,K^+-ATPase isozymes present in the human heart. For this purpose, we expressed human $\alpha_1\beta_1$, $\alpha_2\beta_1$, or $\alpha_3\beta_1$ isozymes in *Xenopus* oocytes by cRNA injection and measured the transport activity by means of ^{86}Rb uptake, in the absence or presence of compounds **7** or **30** (1 mM). In the absence of the test compounds, NKA transport activity was 3−15 times higher in cRNA-injected oocytes than in noninjected oocytes. By deduction of the endogenous oocyte Na,K-pump activity measured in noninjected oocytes, in the absence or presence of compound **7** or **30**, from the transport activity measured in cRNA-injected oocytes, it was possible to assess the transport activity of the exogenous, expressed human NKA isozymes. At a concentration of 10^{-3} M, **30** exhibited no inhibitory effects on the transport activity of either isozyme (Figure 4), in contrast with ouabain, which completely inhibited all three human NKA isozymes.[14] This result suggests that the inotropic effect produced by **30** (Table 1) is mediated by a mechanism other than NKA inhibition. The differential effects of compound **30**, which does not inhibit NKA, and compound **20**, which is an inhibitor of NKA (Figure 3), may be explained in terms of different binding affinities due to the enantiomeric spatial distribution of the substituents on the hydroindene skeleton in the two compounds.

Similar to **30**, compound **7** (10^{-3} M) did not affect the transport activity of human $\alpha_1\beta_1$ isozymes but slightly inhibited ^{86}Rb uptake in oocytes expressing $\alpha_3\beta_1$ isozymes and by more than 50% in oocytes expressing $\alpha_2\beta_1$ isozymes. This latter effect might be responsible for the positive inotropic effect of compound **7** (Table 1).

Conclusions

The amidinohydrazono moiety at C_5 confers inotropic activity on several of the hydroindene derivatives synthesized by us. Nitrovinyl or the amidinohydrazono groupings at C1, which can replace the α,β-unsaturated lactone of digoxin in cardenolide analogues, also produce hydroindene derivatives displaying an inotropic effect.

As suggested by in vitro inhibition assays of rat NKA and human ^{86}Rb transport with selected compounds **7**

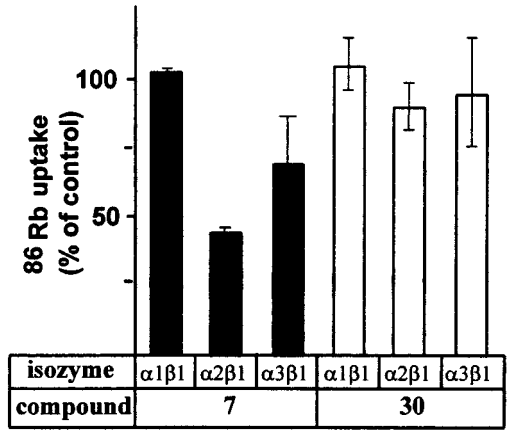


Figure 4. Effect of compounds **7** and **30** on the transport activity of human NKA isozymes. Human $\alpha_1\beta_1$, $\alpha_2\beta_1$, or $\alpha_3\beta_1$ Na^+,K^+-ATPase isozymes were expressed in *Xenopus* oocytes by cRNA injection, and ^{86}Rb uptake was measured in the absence or presence of 1 mM compound **7** or **30** as described in Experimental Section. Results represent ^{86}Rb uptake measured in the presence of 1 mM compound and are expressed as percentages of control measurements performed in the absence of the compounds. ^{86}Rb uptake measured in noninjected oocytes was deduced from that measured in cRNA-injected oocytes. Means ± SE from 15 to 25 oocytes from two different Xenopus females are shown. In the absence of compounds, ^{86}Rb uptake in cRNA-injected oocytes ranged between 30 and 190 pmol min^{-1} $oocyte^{-1}$ and was 3−15 times higher than in noninjected oocytes.

and **20** having the same absolute stereochemistry as natural cardenolides (C and D rings), the inotropic activity is associated with the inhibition of NKA. However, the enantiomeric bis(amidinohydrazone) **30**, which also displays a positive inotropic effect, lacks this ability.

The interest in the hydroindene derivatives **3**, **7**, and **20** lies in that they produce the same positive inotropic effect as digoxin, although at higher concentrations and without affecting heart rate. In the case of compound **7** a selective inhibition of the $\alpha_2\beta_1$ NKA isozyme is produced.

Experimental Section

Chemistry. General. We had previously synthesized **4**, **5**, **8**, **9**, **17**, and **21**.[20] Reagents were used as purchased without further purification. Solvents (THF, DMF, CH_2Cl_2, benzene) were dried and freshly distilled before use according to literature procedures. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040−0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063−0.200 mm; Merck) chromatography. TLC was performed on precoated silica gel polyester plates (0.25 mm thickness) with fluorescent indicator UV 254 (Polychrom SI F_{254}). Melting points were determined on a Buchi 510 apparatus and are uncorrected. Optical rotations were measured on a digital polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker WP 200-SY spectrometer at 200/50 MHz or on a Bruker DRX spectrometer at 400/100 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as an internal standard, and coupling constants (J values) are in hertz. GC−MS analyses were carried out with a Hewlett-Packard 5890 series II apparatus (70 eV). For FABHRMS analyses, a VG TS-250 apparatus (70 eV) was used. Elemental analyses were obtained with a LECO CHNS-932.

Preparation of Amidinohydrazones. Method A. To a 0.05 M solution of the ketone/aldehyde in EtOH, 1.05/2.1 equiv of the aminoguanidine hydrochloride and 1 equiv of 2 N HCl were added. The mixture was heated to reflux for 45 min, then cooled to room temperature, concentrated to dryness, and crystallized to obtain the corresponding amidinohydrazones.

Method B. To a suspension of aminoguanidine bicarbonate (2 equiv) in EtOH (5 mL), 2 N HCl until acidic pH and 1 equiv of the ketone/aldehyde (0.4 M) in EtOH were added. The mixture was refluxed for 2 h, cooled to room temperature, and evaporated, and the residue was crystallized from MeOH/ether.

(1*R*,7a*R*)-7a-Methyl-1-[(*E*)-2-nitrovinyl]-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one (6). A solution of aldehyde **5** (110 mg, 0.62 mmol) and ammonium acetate (62 mg, 0.80 mmol) in refluxing nitromethane (2.5 mL) was stirred for 1.5 h. The crude product was diluted with CH_2Cl_2, washed with brine, and dried (Na_2SO_4), yielding a mixture of nitro derivatives $C_{1\beta}$ and $C_{1\alpha}$ (10:1 ratio). The mixture was chromatographed (hexane/ethyl acetate 14:1), providing **6** (58 mg, 42%, white solid), 10 mg of mixture of both epimers, and recovering 23 mg of starting material. **6**: $[\alpha]^{23}_D$ +84.5° (*c* 0.36, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.13 (s, 3H); 5.83 (dd, 1H, J = 2.2, 1.8); 7.03 (d, 1H, J = 13.5); 7.29 (dd, 1H, J = 13.2, 8.0). MS *m*/*z* (%): 221 (M^+, 6).

(1*R*,7a*R*)-7a-Methyl-1-[(*E*)-2-nitrovinyl]-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one Amidinohydrazone (7). Following method A, from **6** (45 mg,0.16 mmol), **7** was obtained (3:1 $\Delta^{5(N)}$ *E*/*Z*, 30 mg, 62%) as a white solid after crystallization from MeOH/ether. $[\alpha]^{23}_D$ +34.6° (*c* 0.45, MeOH). ^1H NMR (CD_3OD), δ: 0.91 (s, 3H) (major); 0.99 (s, 3H) (minor); 5.91 (s, 1H) (major); 6.33 (s, 1H) (minor); 7.11 (d, 1H, J = 13.2); 7.23 (dd, 1H, J = 13.2, 7.3). HRMS (FAB^+) *m*/*z*: calcd, 278.1617 (MH^+); found, 278.1628. Anal. ($C_{13}H_{19}N_5O_2$·HCl) C, H, N.

Methyl (*E*)-[(1*R*,7a*R*)-5-Amidinohydrazono-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-yl]acrylate (10). Compound **10** was prepared from ketoester **8** following method A, as previously described.

Methyl (*Z*)-[(1*R*,7a*R*)-5-Amidinohydrazono-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-yl]acrylate (11). Following method A, from the enone **9** (46 mg, 0.20 mmol) after crystallization of the reaction mixture from CH_2Cl_2/diethyl ether, **11** was obtained (6:1 $\Delta^{5(N)}$ *E*/*Z*, 51 mg, 88%) as a white solid. $[\alpha]^{23}_D$ −117.6° (*c* 0.33, MeOH). ^1H NMR (CD_3OD), δ: 0.96 (s, 3H) (major); 1.09 (s, 3H) (minor); 3.70 (s, 3H); 5.90 (d, 1H, J = 10.9); 5.95 (s, 1H) (major); 6.17 (dd, 1H, J = 10.9, 10.9); 6.68 (s, 1H) (minor). HRMS (FAB^+) *m*/*z*: calcd, 291.1821 (MH^+); found, 291.1803. Anal. ($C_{15}H_{22}N_4O_2$·HCl) C, H, N.

***N*-[(1*S*,7a*R*)-7a-Methyl-5,5-propylendithio-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-ylmethyl]phthalimide (12).** To an ice-cooled solution of alcohol **4** (500 mg, 1.85 mmol), phthalimide (417 mg, 2.78 mmol) and triphenylphosphine (728 mg, 2.78 mmol) in THF (20 mL) and DEAD (0.44 mL, 2.78 mmol) was added dropwise under argon. The mixture was stirred at room temperature for 19 h. Then, THF was evaporated and the residue was dissolved in ethyl acetate and was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was chromatographed (hexane/ethyl acetate 7:1 as eluent) to yield phthalimide derivative **12** as an uncolored foaming oil (425 mg, 58%). $[\alpha]^{23}_D$ −7.6° (*c* 0.95, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.05 (s, 3H); 3.64 (dd, 1H, J = 13.9, 8.0); 3.82 (dd, 1H, J = 13.9, 6.2); 5.42 (s,1H); 7.72 (dd, 2H, J = 5.5, 2.9); 7.84 (dd, 2H, J = 5.5, 2.9). MS *m*/*z* (%): 399 (M^+, 22).

(1*S*,7a*R*)-[7a-Methyl-5,5-propylendithio-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-yl]methylamine (13). A solution of the phthalimido derivative **12** (240 mg, 0.60 mmol) and hydrazine hydrate (0.034 mL, 0.7 mmol) in 7 mL of absolute ethanol was heated to reflux for 7 h, producing a white precipitate. The reaction mixture was cooled to room temperature, and 0.4 mL of concentrated HCl was added. The precipitate was removed by filtration, the filtrate was neutralized with $NaHCO_3$, and NaOH was added until pH 10 was attained. The mixture was then extracted three times with ether and once with CH_2Cl_2 to obtain **13** (100 mg, 62%) as a white oil. $[\alpha]^{23}_D$ +62.5° (*c* 0.79, $CHCl_3$); ^1H NMR ($CDCl_3$), δ: 0.88 (s, 3H); 2.2−2.5(m, 2H, overlapped); 5.43 (s, 1H). MS *m*/*z* (%): 269 (M^+, 28).

***N*-[(1*S*,7a*R*)-7a-Methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-ylmethyl]phthalimide (14).** To a suspension of red HgO (325 mg, 1.50 mmol) in THF/H_2O 85:15 (9,2 mL), BF_3·OEt_2 (0.49 mL, 4.00 mmol) was added under argon, followed by a 0.02 M solution of compound **12** (400 mg, 1.00 mmol) in THF. The resulting mixture was stirred at room temperature for 1 h. Then, it was diluted with CH_2Cl_2 and filtered, and the filtrate was washed with aqueous Na_2CO_3 (saturated solution) and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed (hexane/ethyl acetate 2:1) to provide deprotected ketone **14** (130 mg, 42%) as a white foaming oil. $[\alpha]^{23}_D$ −31.8° (*c* 0.62, $CHCl_3$). ^{1}H NMR ($CDCl_3$), *δ*: 1.22 (s, 3H); 3.71 (dd, 1H, *J* = 13.9, 8.0); 3.85 (dd, 1H; *J* = 13.9, 6.6); 5.76 (s, 1H); 7.75 (dd, 2H, *J* = 5.5, 3.3); 7.87 (dd, 2H, *J* = 5.5, 3.9). MS *m*/*z* (%): 309 (M^+, 22).

***N*-[(1*S*,7a*R*)-5-Amidinohydrazono-7a-methyl-2,3,5,6,7,-7a-hexahydro-1*H*-inden-1-yl-methyl]phthalimide (15).** Following method A, compound **15**, a white foaming solid, was obtained (6:1 $\Delta^{5(N)}$ *E*/*Z*, 98%) and crystallized from MeOH/ether. $[\alpha]^{23}_D$ −61.5° (*c* 0.47, MeOH). ^{1}H NMR (CD_3OD), *δ*: 0.98 (s, 3H) (major); 1.05 (s, 3H) (minor); 3.57 (dd, 1H, *J* = 13.7, 8.0); 3.73 (dd, 1H, *J* = 13.7, 6.5); 5.84 (s, 1H) (major); 6.73 (s, 1H) (minor); 7.50−7.70 (m, 4H). HRMS (FAB^+) *m*/*z*: calcd, 366.1930 (MH^+); found, 366.1902. Anal. ($C_{20}H_{23}N_5O_2$·HCl) C, H, N.

(1*S*,7a*R*)-1-Aminomethyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one Amidinohydrazone (16). 16 was obtained from **15** as described for compound **13** (96 mg, 0.26 mmol). For purification, the precipitate was removed by filtration and the filtrate was dried with anhydrous Na_2CO_3 to obtain 48 mg (78%) of **16** (6:1 $\Delta^{5(N)}$ *E*/*Z*) as a white foaming solid. ^{1}H NMR (CD_3OD), *δ*: 0.50 (s, 3H) (major); 0,57 (s, 3H) (minor); 2.52 (dd, 1H, *J* = 12.5, 10.0); 2.79 (dd, 1H, *J* = 12.5, 3.7); 5.44 (d, 1H, *J* = 2.0) (major); 5.55 (s, 1H) (minor). HRMS (FAB^+) *m*/*z*: calcd, 238.2034 ($M3H^+$); found, 238.2053. Anal. ($C_{12}H_{21}N_5$·2HCl) C, H, N.

(1*S*,3a*S*,4*S*,7a*R*)-3a-Hydroxy-4,7a-dimethyl-5,5-ethylendioxyperhydroindene-1-carbaldehyde (17) and (1*R*,-3a*S*,4*S*,7a*R*)-3a-Hydroxy-4,7a-dimethyl-5,5-ethylendioxyperhydroindene-1-carbaldehyde (21). Aldehydes **17** (88%) and **21** (72%) were obtained by pyridinium chlorochromate (PCC) oxidation of the corresponding alcohols, which were prepared in 63% overall yield (1:1 mixture) from (3a*S*,4*S*,-7a*S*)-3a-hydroxy-4,7a-dimethylperhydroindene-1,5-dione[35] following the procedure described in previous papers.[19,20,30,31]

17: ^{1}H NMR ($CDCl_3$), *δ*: 0.99 (d, 3H, *J* = 6.9); 1.10 (s, 3H); 3.82−4.04 (m, 4H); 9.73 (d, 1H, *J* = 4.0). MS *m*/*z* (%): 254 (M^+, 35). **21**: ^{1}H NMR ($CDCl_3$), *δ*: 0.98 (d, 3H, *J* = 6.8); 1.29 (s, 3H); 1.96 (c, 1H, *J* = 6.8); 2.99 (dt, 1H, *J* = 8.5, 2.1); 3.83−4.03 (m, 4H); 9.80 (d, 1H, *J* = 2.1). MS *m*/*z* (%): 254 (M^+, 25).

Methyl (*E*)-[(1*R*,3a*S*,4*S*,7a*R*)-3-(3a-Hydroxy-4,7a-dimethyl-5,5-ethylendioxyperhydroinden-1-yl)]acrylate (18). A solution of **17** (187 mg, 0.74 mmol) in dry benzene (3.0 mL) was added to a suspension of methoxycarbonylmethylentriphenylphosphorane (379 mg, 1.11 mmol) in dry benzene (2.0 mL). The mixture was stirred at reflux under Ar for 7 h. The solvent was then evaporated, and the residue was chromatographed through a silica gel column (hexane/ethyl acetate 6:4) to yield ester **18** (215 mg, 94%) as a colorless oil, which was purified by crystallization from CH_2Cl_2/hexane. Mp: 95 °C (CH_2Cl_2/hexane). $[\alpha]^{23}_D$ +1.8° (*c* 0.60, $CHCl_3$). ^{1}H NMR ($CDCl_3$), *δ*: 0.92 (s, 3H); 0.99 (d, 3H, *J* = 6.9); 1.92 (q, 1H, *J* = 6.9); 3.71 (s, 3H); 3.82−4.04 (m, 4H); 5.64 (d, 1H, *J* = 15.7); 7.17 (dd, 1H, *J* = 15.7, 10.6). MS *m*/*z* (%): 292 (M^+-H_2O, 1).

Methyl (*E*)-[(1*R*,7a*R*)-5-Amidinohydrazono-4,7a-dimethyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-1-yl)]acrylate (19). Following method B, **19** (28%) was obtained as a white solid after recrystallization from MeOH/ether.^{1}H NMR (CD_3OD), *δ*: 0.80 (s, 3H); 1.72 (s, 3H); 3.62 (s, 3H); 5.82 (d, 1H, *J* = 15.7); 6.88 (dd, 1H, *J* = 15.7, 8.0). HRMS (FAB^+) *m*/*z*: calcd, 305.1977 (MH^+); found, 305.1987. Anal. ($C_{16}H_{24}N_4O_2$.HCl) C, H, N.

(1*S*,7a*R*)-4,7a-Dimethyl-5-oxo-2,3,5,6,7,7a-hexahydro-1*H*-indene-1-carbaldehyde Bis(amidinohydrazone) (20). Following method B, **20** (54%) was obtained as a white solid after crystallization from MeOH. Mp 220−221 °C. $[\alpha]^{23}_D$ +10.2° (*c* 0.47, H_2O); ^{1}H NMR (D_2O), *δ*: 0.73 (s, 3H); 1.58 (s, 3H); 7.35 (d, 1H, *J* = 6.0). MS *m*/*z* (%): 304 (M^+, 70). HRMS (FAB^+) *m*/*z*: calcd, 305.2203 (MH^+); found, 305.2247. Anal. ($C_{14}H_{24}N_8$·2HCl·H_2O) C, H, N.

(1*R*,7a*R*)-4,7a-Dimethyl-5-oxo-2,3,5,6,7,7a-hexahydro-1*H*-indene-1-carbaldehyde Bis(amidinohydrazone) (22). Following method B, **22** (39%) was obtained as a white solid after crystallization from MeOH. Mp 256 °C. $[\alpha]^{23}_D$ +4.8° (*c* 0.40, H_2O). ^{1}H NMR (CD_3OD), *δ*: 0.50 (s, 3H); 1.36 (s, 3H); 7.15 (d, 1H, *J* = 6.0). HRMS (FAB^+) *m*/*z*: calcd, 305.2203 (MH^+); found, 305.2176. Anal. ($C_{14}H_{24}N_8$.2HCl·H_2O) C, H, N.

(3a*R*,7a*R*)-3a-Hydroxy-7a-methylperhydroindene-1,5-dione (23). To a solution of the triketone (6.0 g, 33 mmol) obtained from methylvinyl ketone and 2-methylcyclopentane-1,3-dione in 33 mL of dried DMF, a total of 113.5 mg of *R*-(+)-proline was added. The mixture was stirred at room temperature for 24 h under Ar. Then it was filtered and evaporated, and the residue was chromatographed (hexane/EtOAc 1:1), yielding the diketone **23** (5.46 g, 91%). Mp 108−110 °C (CH_2Cl_2). $[\alpha]^{23}_D$ −57.6° (*c* 0.80, $CHCl_3$). ^{1}H NMR ($CDCl_3$), *δ*: 1.25 (s, 3H). MS *m*/*z* (%): 182 (M^+, 53).

(7a*R*)-7a-Methyl-2,3,5,6,7,7a-hexahydro-1*H*-indene-1,5-dione (24). To a solution of diketone **23** (6.6 g, 36.3 mmol) in 66 mL of dry benzene, 100 mg (0.5 mmol) of *p*-TsOH were added. The mixture was heated to reflux for 1 h under Ar. It was then diluted with EtOAc and washed with sodium bicarbonate and brine, and the organic solvent was dried and evaporated to dryness under reduced pressure, yielding **24** (5.4 g, 91%). Mp 61−63 °C (CH_2Cl_2). $[\alpha]^{23}_D$ −315.4° (*c* 1.05, toluene). ^{1}H NMR ($CDCl_3$), *δ*: 1.33 (s, 3H); 5.98 (d, 1H, *J* = 2.2). MS *m*/*z* (%): 164 (M^+, 100).

[(7a*R*)-7a-Methyl-1-oxo-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one]propylenic Thioacetal (25). To a solution of **24** (2.9 g, 17.7 mmol) in 9 mL of $CHCl_3$, 2.7 mL (26.8 mmol) of propane-1,3-dithiol and 0.5 mL of TMSCl (5.4 mmol) were added, and then the mixture was stirred under Ar at room temperature for 3 h. NaOH (4%) aqueous solution was then added. The mixture was diluted with EtOAc, extracted, and washed with brine. The organic layer was dried and evaporated to give a residue that was purified by precipitation with hexane, yielding 4.9 g (90%) of **25**. $[\alpha]^{23}_D$ −299.6° (*c* 0.71, $CHCl_3$). ^{1}H NMR ($CDCl_3$), *δ*: 1.16 (s, 3H); 5.75 (s, 1H). MS *m*/*z* (%): 254 (M^+, 100).

[(7a*S*)-7a-Methyl-1-methylene-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one]propylenic Thioacetal (26). To a suspension of 15.9 g (39.4 mmol) of methyltriphenylphosphonium iodide in 265 mL of dry benzene under Ar, a diluted solution of 4.5 N NaO^{t}Am (1.3 equiv, 8.2 g, 39.4 mmol) in benzene was added at room temperature; the bright-yellow phosphorane was immediately formed. The mixture was heated to reflux, and a solution of **25** (7.7 g, 30.3 mmol) in 55 mL of benzene was added and the mixture was gently refluxed for 45 min. After cooling and filtration, the solution was diluted with EtOAc and washed with brine. The residue was purified by chromatography (hexane/EtOAc 9:1), yielding **26** (5.2 g, 67%) as a white solid. Mp 113 °C (CH_2Cl_2). $[\alpha]^{23}_D$ −186.4° (*c* 0.96, $CHCl_3$). ^{1}H NMR ($CDCl_3$), *δ*: 1.11 (s, 3H); 4.77 (s, 2H); 5.51 (s, 1H). MS *m*/*z* (%): 252 (M^+, 81).

[(1*R*,7a*S*)-1-Hydroxymethyl-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one]propylenic Thioacetal (27). To a solution of 9-BBN (4.9 g, 40.4 mmol) in 40 mL of dry THF, a total of 5.1 g (20.2 mmol) of **26** was added at room temperature under Ar. The mixture was stirred for 1 h 10 min. The solution was then cooled to 0 °C, and 6 N NaOH (15 equiv) and 30% H_2O_2 (10 equiv) were added sequentially. The reaction mixture was heated to 50 °C for 1 h. Then, it was allowed to reach room temperature, diluted with EtOAc, and washed with brine. The residue was purified by column chromatography (hexane/EtOAc 3:1), yielding a mixture (88%) of alcohol **27** and its epimer $^{21}/_{1}$ ratio. $[\alpha]^{23}_D$ −90.9° (*c* 0.92, $CHCl_3$). ^{1}H NMR ($CDCl_3$ 400 MHz), *δ*: 0.92 (s, 3H); 3.63 (dd, 1H, *J* = 10.7, 7.1); 3.73 (dd, 1H, *J* = 10.7, 7.1); 5.45 (d, 1H, *J* = 1.9). MS *m*/*z* (%): 270 (M^+, 94).

(1*R*,7a*S*)-1-Hydroxymethyl-7a-methyl-2,3,5,6,7,7a-hexahydro-1*H*-inden-5-one (28). To a suspension of 2.4 g (11.1 mmol) of HgO in 47.4 mL of $^{85}/_{15}$ THF/H_2O stirred vigorously, 3.64 mL (29.6 mmol) of $BF_3{\cdot}OEt_2$ and a solution of 0.02 M **27** (2 g, 7.4 mmol) were added. The mixture was stirred at room temperature for 1 h and then diluted with CH_2Cl_2 and filtrated, and the filtrate was washed with saturated aqueous $NaHCO_3$ and NaCl solutions. The combined organic layers were dried (Na_2SO_4) and filtered, and the solvent was evaporated. The residue was passed through a silica gel column (hexane/EtOAc 2:8), yielding **28** (500 mg, 38%). $[\alpha]^{23}_D$ −81.0° (*c* 1.07, $CHCl_3$). ^{1}H NMR ($CDCl_3$ 400 MHz), δ: 1.05 (s, 3H); 3.67 (dd, 1H, J = 10.7, 7.0); 3.74 (dd, 1H, J = 10.7, 7.0); 5.72 (s, 1H). MS *m*/*z* (%): 180 (M^+, 17).

(1*R*,7a*S*)-7a-Methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1*H*-indene-1-carbaldehyde (29). To a suspension of pyridinium chlorochromate (880 mg, 4.08 mmol) and 4 Å molecular sieves (490 mg) in 136 mL of dry CH_2Cl_2, a solution of **28** (490 mg, 2.72 mmol) in 57 mL of dry CH_2Cl_2 was added under Ar. After being stirred at room temperature for 2 h, the mixture was passed through a silica gel column (hexane/EtOAc 1:2) to obtain **29** (387 mg, 80%) as a white solid. Mp 203 °C (CH_2Cl_2). $[\alpha]^{23}_D$ −172.8° (*c* 0.55, $CHCl_3$). ^{1}H NMR ($CDCl_3$), δ: 1.19 (s, 3H); 5.79 (d, 1H, J = 1.8); 9.87 (d, 1H, J = 1.8). MS *m*/*z* (%): 178 (M^+, 7).

(1*S*,7a*S*)-7a-Methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1*H*-indene-1-carbaldehyde Bis(amidinohydrazone) (30). Following method A, **30** was crystallized from MeOH (95%) as a white solid, mp 243−245 °C. $[\alpha]^{23}_D$ −4.8° (*c* 0.37, MeOH). ^{1}H NMR (CD_3OD), δ: 0.77 (s, 3H); 5.78 (s, 1H); 7.35 (d, 1H, J = 6.2). HRMS (FAB^+) *m*/*z*: calcd, 291.2045 (MH^+); found, 291.2036. Anal. ($C_{13}H_{22}N_8{\cdot}2HCl{\cdot}H_2O$) C, H, N.

In Vitro Tests. 1. Guinea-Pig-Atria Methods. Guinea pigs of either sex weighing 250−350 g were stunned by a sharp blow on the head, and their hearts were rapidly removed. Right and left atria were dissected and mounted vertically in 5 mL organ baths containing Tyrode solution of the following composition (mM): NaCl, 125; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 24; NaH_2PO_4, 0.42; glucose, 11. The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 and maintained at 34 °C. Under these conditions, right atria beated spontaneously while the left atria were electrically driven at a basal rate of 1 Hz through bipolar platinum electrodes with rectangular pulses (1 ms duration, voltage twice the threshold) delivered from a multipurpose programmable stimulator (Cibertec CS.220, Cibertec, S. A., Madrid, Spain). Rate and amplitude of contractions were measured isometrically by a force−displacement transducer and recorded on a Grass model 7B polygraph (Grass Instrument Co. Quincy, MA). Resting tension was adjusted to 1 g, and a 60 min equilibration period was allowed to elapse before control measurements were taken. After control values for each parameter were obtained, incremental drug concentrations were added to the bath at 30 min intervals to obtain a complete concentration−response curve. The values for different parameters obtained in the absence of drug were used as controls and compared with those obtained after each increment in drug concentration. The compounds were dissolved in DMSO, and the resulting solution was diluted with Tyrode solution to obtain a final concentration containing 1% DMSO in the organ bath.

2. Rat NKA Inhibition Assays. The enzymatic activities were measured at 37 °C and carried out with permeabilized microsomes (0.4 mg of DOC/mg of protein for 15 min at 20 °C) prepared from rat heart.[36] NKA activity was determined by the coupled assay method that measures the disappearance of NADH[37] and maintains a constant ATP level. Assays were carried out in a six-cell set: one cell for basal activity measurement (1 mM ouabain-insensitive ATPase activity), one cell for total activity, and four cells for four different inhibitor doses. The enzymatic reaction was initiated by the addition of permeabilized microsomal proteins to the assay medium, previously incubated at 37 °C for 10 min. The corresponding NADH oxidation was then continuously monitored for up to 30 min. Inhibition was calculated by comparing the activities in the presence of different concentrations of drug, after correcting the digitalis-insensitive ATPase activity, measured in the presence of $(1-5) \times 10^{-3}$ M ouabain.

3. Measurement of the Transport Activity of Human NKA Isozymes by ^{86}Rb Uptake. Oocytes were obtained from *Xenopus laevis* females[38] and were either injected or not with 10 ng of human α_1, α_2, or α_3 isoform cRNAs and 1 ng of human β_1 isoform cRNA.[14] Three days after cRNA injection, ^{86}Rb uptake was measured as previously described.[36] Briefly, oocytes were loaded with Na^+ for 2 h at 19 °C in a K^+-free solution I (90 mM NaCl, 1 mM Tris/HCl, pH 7.5) before incubation in solution II containing 2 mM $CaCl_2$, 5 mM $BaCl_2$, 5 mM MOPS, and 90 mM NaCl, pH 7.4 for 30 min at 19 °C. Oocytes were then incubated in solution II containing ^{86}RbCl (5 μCi/mL) and 5 mM KCl for 12 min at room temperature in the presence or absence of 1 mM test compound **7** or **30**. Oocytes were rinsed four times with ice-cold solution II, and individual oocytes were solubilized in 100 μL of 5% SDS before radioactivity was counted in a scintillation counter (2250CA TRICARB).

Acknowledgment. Financial support came from Junta de Castilla y León (Grant SA-02/99). L.G.S. and C.P.M. thank the Spanish Ministry of Education for a predoctoral grant. This work was supported in part by grants from Université Paris 7 (Grant 3409 R10/11), the "Réseau INSERM Inotropie" (L.G.L.).

Supporting Information Available: Table of GC−MS retention times and conditions for different compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Betkowski, A. S.; Hauptman, P. J. Update on recent clinical trials in congestive heart failure. *Curr. Opin. Cardiol.* **2000**, *15*, 293−303.
(2) McMurray, J. J.; Stewart, S. Epidemiology, aetiology, and prognosis of heart failure. *Heart* **2000**, *83*, 596−602.
(3) Ryden, L.; Remme, W. J. Treatment of congestive heart failure. Has the time come for decreased complexity? *Eur. Heart J.* **1999**, *20*, 867−871.
(4) Hampton, J. R. Results of Clinical Trials with Diuretics in Congestive Heart Failure. *Br. Heart J.* **1994**, *72* (Suppl. l2), 568−572.
(5) Francis, G. S. ACE Inhibition in Cardiovascular Disease. *N. Engl. J. Med.* **2000**, *342*, 145−153.
(6) Packer, M.; Cohn, J. N. Consensus recommendations for the management of chronic heart failure. *Am. J. Cardiol.* **1999**, *83A* (2), 1A−38A.
(7) Hauptman, P. J.; Kelly, R. A. Digitalis. *Circulation* **1999**, *99*, 1265−1270.
(8) Thomas, R.; Gray, P.; Andrews, J. Digitalis: Its Mode of Action, Receptor, and Structure−Activity Relationships. *Adv. Drug Res.* **1990**, *19*, 312−562.
(9) Ferrandi, M.; Manunta, P. Ouabain-like factor: is this the natriuretic hormone? *Curr. Opin. Nephrol. Hypertens.* **2000**, *9*, 165−171.
(10) Templeton, J. F.; Ling, Y.; Zeglam, T. H.; Marat, K.; LaBella, F. S. Synthesis of 20α- and 20β-Acetamido, Amino, Nitro and Hydroxy Derivatives of 14-Hydroxy-5β,14β-pregnane-3β-Glycosides: Pregnanes That Bind to the Digitalis Receptor. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2503−2517.
(11) De Munari, S.; Barassi, P.; Cerri, A.; Fedrizzi, G.; Gobbini, M.; Mabilia, M.; Melloni, P. A. New Approach to the Design of Novel Inhibitors of Na^+, K^+-ATPase: 17β-Substituted Seco-D-5β-Androstane as Cassaine Analogues. *J. Med. Chem.* **1998**, *41*, 3033−3040.
(12) Blanco, G.; Mercer, R. W. Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am. J. Physiol.* **1998**, *275*, F633−F650.
(13) McDonough, A. A.; Wang, J. N.; Farley, R. A. Significance of sodium pump isoforms in digitalis therapy. *J. Mol. Cell Cardiol.* **1995**, *27*, 1001−1009.
(14) Crambert, G.; Hasler, U.; Beggah, A. T.; Yu, C.; Modyanov, N. N.; Horisberger, J.-D.; Lelièvre, L.; Geering, K. Transport and Pharmacological Properties of Nine Different Human Na,K-ATPase Isozymes. *J. Biol. Chem.* **2000**, *275*, 1976−1986.

(15) James, P. F.; Grupp, I. L.; Grupp, G.; Woo, A. L.; Askew, G. R.; Croyle, M. L.; Walsh, R. A.; Lingrel, J. B. Identification of a specific role for the Na,K-ATPase alpha 2 isoform as a regulator of calcium in the heart. *Mol. Cell.* **1999**, *3*, 555−563.
(16) San Feliciano, A.; Medarde, M.; Caballero, E.; Hebrero, B.; Tomé, F.; Prieto, P.; Montero, M. J. Synthesis and evaluation of cardiotonic activity of diterpenic butenolides. *Eur. J. Med. Chem.* **1991**, *26*, 799−805.
(17) Chiu, F. C. K.; Watson, R. T. Conformational Factors in Cardiac Glycoside Activity. *J. Med. Chem.* **1985**, *28*, 509−515.
(18) Medarde, M.; Caballero, E.; Tomé, F.; García, A.; Montero, M. J.; Carrón, R.; San Feliciano, A. Synthesis and evaluation of cardiotonic activity of simple butenolides II. *Eur. J. Med. Chem.* **1993**, *28*, 887−892.
(19) Melero, C. P.; Sevillano, L. G.; Caballero, E.; Tomé, F.; Carrón, R.; Montero, M. J.; San Feliciano, A.; Medarde, M. Hydroindenic-guanylhydrazones. Synthesis and evaluation as inotropic agents. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3217−3222.
(20) Sevillano, L. G.; Melero, C. P.; Boya, M.; López, J. L.; Tomé, F.; Caballero, E.; Carrón, R.; Montero, M. J.; Medarde, M.; San Feliciano, A. Synthesis and Inotropic Activity of Hydroindene Derivatives. *Bioorg. Med. Chem.* **1999**, *7*, 2991−3001.
(21) Markgraf, J. H.; Finkelstein, M.; Cort, J. R. Canthine Analogs via Intramolecular Diels−Alder Reactions. *Tetrahedron* **1996**, *52*, 461−470.
(22) Quadri, L.; Cerri, A.; Ferrari, P.; Folpini, E.; Mabilia, M.; Melloni, P. Synthesis and Quantitative Structure−Activity Relationships of 17β-(Hydrazonomethyl)-5β-androstane-3β,14β-diol Derivatives That Bind to Na^+, K^+-ATPase Receptor. *J. Med. Chem.* **1996**, *39*, 3385−3393.
(23) Thomas, R. E. Cardiac drugs. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; John Wiley: New York, 1996; Vol. 2, pp 153−261.
(24) Mehta, G.; Prabhakar, Ch. A short, General Access to Folded *all-cis*-Azatetraquinane Ring Systems. *J. Org. Chem.* **1995**, *60*, 4638−4640.
(25) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1−28.
(26) Hughes, D. L. Progress in the Mitsunobu reaction. A review. *Org. Prep. Proc. Int.* **1996**, *28*, 127−164.
(27) Roush, W. R.; Straub, J. A.; Brown, J. R. Stereochemistry of the Epoxidations of Acyclic Allylic Amides. Applications toward the Synthesis of 2,3,6-Trideoxy-3-aminohexoses. *J. Org. Chem.* **1987**, *52*, 5127−5136.
(28) March, J. *Advanced Organic Chemistry. Reactions, mechanisms and structure*, 3rd ed.; Wiley & Sons: New York, 1985; p 378.
(29) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley & Sons: New York, 1999; pp 333−343.
(30) Medarde, M.; Caballero, E.; Tomé, F.; Melero, C. P.; Boya, M.; San Feliciano, A. Análogos sintéticos de cardenolidas con estructura simplificada. Derivados perhidroindénicos (Cardenolide synthetic analogues with simplified structure. Perhydroindenic Derivatives). *An. Quím.* **1995**, *91*, 89−94.
(31) Medarde M.; Caballero, E.; Tomé, F.; Gracia, P. G.; Boya, M.; San Feliciano, A. One carbon elongation of the Hajos−Parrish ketone. Synthesis of (+)-(3a*S*,7a*R*)-octahydro-3a-hydroxy-7a-methyl-1-methylene-5*H*-inden-5-one. *Synth. Commun.* **1995**, *25*, 1377−1382 and references therein.
(32) Conia, J. M.; Limasset, J. C. L'utilisation du *t*-amylate de sodium dans les réactions de Wittig (Use of sodium *t*-amylate in Wittig reactions). *Bull. Soc. Chim. Fr.* **1967**, 1936−1938.
(33) Pérez-Vizcaíno, F.; Carrón, R.; Delpón, E.; Tamargo, J. Effects of (*S*)-nafenodone, a new antidepressant, in isolated guinea-pig atrial and ventricular muscle fibres. *Eur. J. Pharmacol.* **1991**, *199*, 43−50.
(34) Mansier, P.; Lelievre, L. G. Ca^{2+}-free perfusion of rat heart reveals a Na^+/K^+-ATPase activity highly sensitive to ouabain. *Nature* **1982**, *300*, 535−537.
(35) Medarde, M.; Caballero, E.; Melero, C. P.; Tomé, F.; San Feliciano, A. Asymmetric synthesis of (+)-(3a*S*,4*S*,7a*S*)-3a-hydroxy-4,7a-dimethylperhydroindane-1,5-dione. *Tetrahedron: Asymmetry* **1997**, *8*, 2075−2077.
(36) Mansier, P.; Charlemagne, D.; Rossi, B.; Preteseille, M.; Swynghedauw, G.; Lelièvre, L. G. Isolation of impermeable inside-out vesicles from an enriched sarcolemma fraction of rat heart. *J. Biol. Chem.* **1983**, *258*, 6628−6635.
(37) Thomas, R.; Allen, J. C.; Pitts, B. J. R.; Schwartz, A. Cardenolide analogs. An explanation for the usual properties of AY 22241. *Eur. J. Pharmacol.* **1979**, *53*, 227−237.
(38) Geering, K.; Beggah, A.; Good, P.; Girardet, S.; Roy, S.; Schaer, D.; Jaunin, P. Oligomerization and maturation of Na,K-ATPase: functional interaction of the cytoplasmic NH_2 terminus of the beta subunit with the alpha subunit. *J. Cell Biol.* **1996**, *133*, 1193−1204.

JM0109309