Synthesis, Cardiotonic Activity, and Structure−Activity Relationships of 17β-Guanylhydrazone Derivatives of 5β-Androstane-3β,14β-diol Acting on the Na^+,K^+-ATPase Receptor

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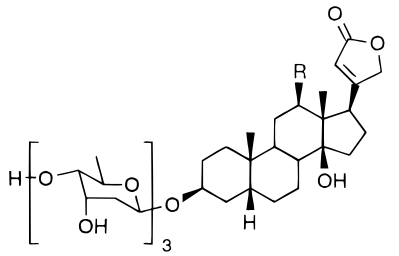
A series of digitalis-like compounds, with the lactone ring shifted from the original position through a spacer or replaced by a series of guanylhydrazone substituent-bearing chains, was synthesized and evaluated for inhibition of Na^+,K^+-ATPase and for inotropic activity. The highest Na^+,K^+-ATPase inhibition (IC_{50}) and inotropic activity (EC_{50}) were reached with the vinylogous guanylhydrazone **5** where a cardenolide-like polarized α,β-unsaturated system and a basic guanidino group were both present at the 17β-position; for this compound IC_{50} and EC_{50} values were comparable to or higher than those of Thomas' parent guanylhydrazone **1**, digitoxigenin, and digoxin. A substantial improvement of the desired positive inotropic activity versus the toxic arrhythmogenic concentration was not reached within this series; only a slightly better therapeutic index can be envisaged for compounds **5** and **4**, even though, for the latter, to the detriment of potency, presumably because of a weaker interaction with the receptor, due to the lack of a cardenolide-like polarized system.

Introduction

Digitalis cardiac glycosides, such as digoxin and digitoxin (Chart 1), are drugs clinically used to improve cardiac contractility in the treatment of congestive heart failure (CHF); they act through inhibition of Na^+,K^+-ATPase, a cell membrane enzyme which promotes the outward transport of Na^+ ions and the inward transport of K^+ ions.[1] The major problem with these compounds, and specifically digoxin, is the low therapeutic index due to life-threatening cardiac arrhythmias.[2] Many other drugs with different mechanisms of action were tried, but the most promising alternatives to glycosides in ameliorating cardiac performance, the β-adrenergic agonists, were found to develop tolerance and the PDE inhibitors were found to accelerate the progression of the disease and to increase mortality.[3] Whether digoxin increases mortality or not has long been disputed. A recent NIH-sponsored trial showed a neutral effect on mortality (risk ratio of 1.0),[4] and that has renewed interest in the search for novel digitalic inotropic agents with a more favorable therapeutic index.

In naturally occurring digitalic compounds the unsaturated γ- and δ-lactones present in position 17β of the steroidal skeleton are associated with high affinity for the Na^+,K^+-ATPase receptor, although other substituents can substitute for the lactones without a (dramatic) loss of affinity.[1,5] Recently,[6] the importance of basicity in 17β-amidinohydrazonomethyl digitalis-like derivatives confirmed Thomas' previous hypothesis[7] that an ion-pair interaction between one of the carboxylate residues present in the α-subunit of the Na^+,K^+-ATPase and the hydrazone group in the cationic form is relevant for the interaction with the receptor. An alternative two-point attachment model of interaction[7] considers the partially positive electron poor β-carbon atom and the partially negative electron rich carbonyl oxygen of the 17β-(α,β-unsaturated)lactone substituent: these two points are thought to interact with anionic and hydrogen donor sites of the receptor, respectively.

Chart 1



R = OH **Digoxin**

R = H **Digitoxin**

With the aim to further explore the requirements for an optimum interaction with the Na^+,K^+-ATPase and possibly for a novel, safer inotropic agent, we synthesized the guanylhydrazone derivatives and the vinylogous cardenolide-like compound **9** listed in Table 1.

Chemistry

The synthesis of compounds **1**−**3** (Table 1) has been previously reported.[6] The guanidinoamino compound **4** was obtained by catalytic hydrogenation of the corresponding hydrazono derivative **1** (Scheme 1). Compounds **5**−**7** and **8** were prepared from the aldehydes **14** and **15**, respectively, and the appropriate hydrazino derivative in dioxane/water solution at pH 3 (Scheme 2); the two isomeric derivatives **6** and **7** were obtained in the same reaction and separated by flash chromatography. Aldehydes **14** and **15** were obtained in two steps by reduction with DIBAH of the known esters **10**[8] and **11**[8] to the allylic alcohols **12** and **13** respectively, and subsequent allylic oxidation with MnO_2. The

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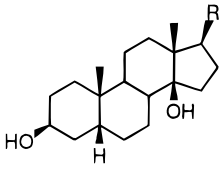
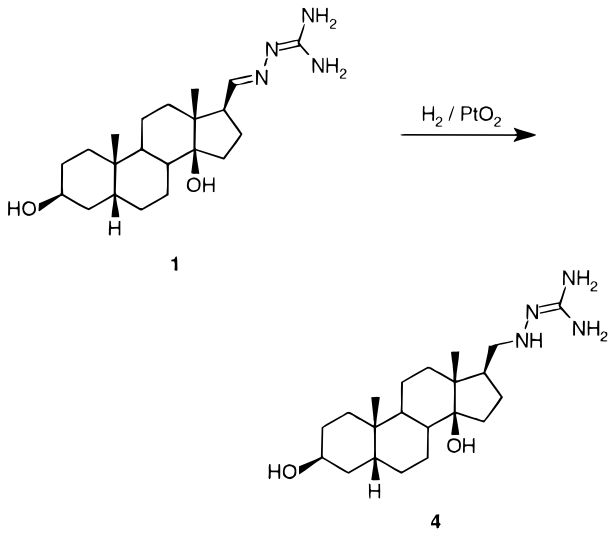
Table 1. Structure and Physical Data for Compounds **1**−**9**



compd	R	mp, °C	yield, %	mol formula	anal.[a]
1	(*E*)-CH=N−N=C(NH_2)$_2$[b]				
2	(*E*)-CH=N−NH(2-imidazolinyl)[b]				
3	(*E*)-CH=N−NH[2-(1,4,5,6-tetrahydropyrimidinyl)][b]				
4	CH_2−NH−N=C(NH_2)$_2$	226−229	40	$C_{21}H_{38}N_4O_2$·HCl	C, H, N, Cl
5	(*E*,*E*)-CH=CH−CH=N−N=C(NH_2)$_2$	197−200 dec	56	$C_{23}H_{38}N_4O_2$·0.8H_2O	C, H, N, H_2O
6	(*E*,*Z*)-CH=CH−CH=N−NH(2-imidazolinyl)	210−211	5	$C_{25}H_{40}N_4O_2$·H_2O	C, H, N, H_2O
7	(*E*,*E*)-CH=CH−CH=N−NH(2-imidazolinyl)	172−177	60	$C_{25}H_{40}N_4O_2$·H_2O	C, H, N, H_2O
8	(*E*,*E*,*E*)-(CH=CH)$_2$−CH=N−N=C(NH_2)$_2$	257−260	70	$C_{25}H_{40}N_4O_2$·0.4H_2O	C, H, N, H_2O
9	(*E*)-CH=CH(2,5-dihydro-5-oxo-3-furyl)	218−223	55	$C_{25}H_{36}O_4$·0.25H_2O	C, H, H_2O
digitoxigenin	2,5-dihydro-5-oxo-3-furyl				
digoxin					

[a] All compounds gave satisfactory results (±0.4%). [b] The syntheses of compounds **1**−**3** have been reported in ref 6.

Scheme 1



digitoxigenin vinylogous butenolide derivative **9** was obtained from the aldehyde **16**[9] by Horner−Emmons reaction with phosphonate **17**[10] (Scheme 3).

All the synthesized compounds were obtained in the *E*, *E*,*E*, or *E*,*E*,*E* configuration, while compound **6**, obtained as a byproduct in the preparation of compound **7**, was obtained in the *E*,*Z* configuration. Compounds in the *E*, *E*,*E*, or *E*,*E*,*E* configuration exhibited good stability in H_2O/DMSO solution at pH 7.4 (phosphate buffer) and 37 °C, while the *E*,*Z* compound **6** tended to isomerize to the more stable *E*,*E* configuration (*E*,*E*/*E*,*Z* = 25/75 after 4 h, 40/60 after 24 h; ^{1}H NMR analysis); for this reason compound **6** was not evaluated in the atrium test.

Biological Activity

All compounds were evaluated *in vitro* for inhibition of dog kidney Na^+,K^+-ATPase activity,[11] measured as percent hydrolysis of [^{32}P]ATP.[12] The inotropic activity was investigated *in vitro* by determining the effects on the contractile force of the electrically driven guinea pig left atrium. The arrhythmogenic activity was determined in the same preparation from the onset of the first series of ectopic beats. Biological data are shown in Table 2. The following compounds were chosen as reference compounds: Thomas' guanylhydrazone **1** for structure similarity, digitoxigenin for the same steroidal aglyconic skeleton, and digoxin as the most commonly prescribed cardiac glycoside.

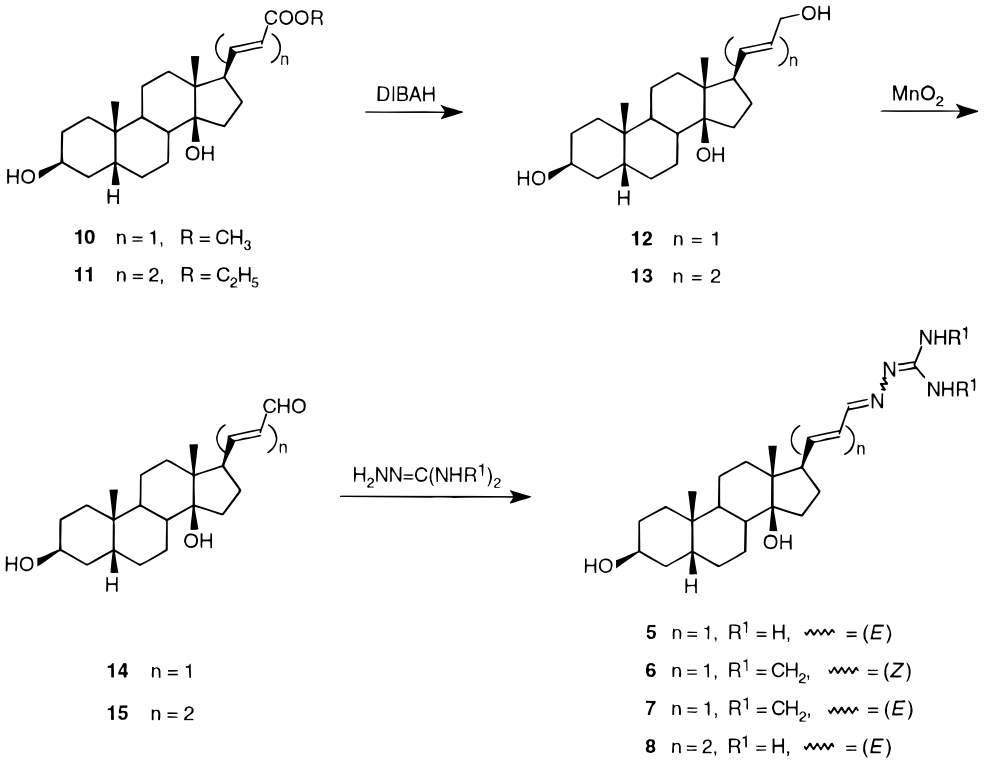
Derivative **5** was the most potent of the synthesized compounds, showing 10 times higher inhibitory potency (IC_{50}) and inotropic potency (EC_{50}) than parent compound **1**. With respect to both digitoxigenin and digoxin, compound **5** was 3 times more active in inhibiting the isolated enzyme and displayed comparable inotropic activity; the arrhythmias also occurred at similar concentrations, approximately 10 times greater than the EC_{50}; however, with compound **5** the percentage of preparations showing arrhythmias was lower. Compound **4** exhibited comparable inotropic activity as **1** and a better therapeutic index than both **5** and digoxin, since no arrhythmias were observed up to a concentration over 10 times the EC_{50} (i.e., 100 μM, the maximum obtainable concentration). Compound **3** showed an average inotropic potency but a poor efficacy (low E_{max}). Compounds **2** and **7**−**9** were the least potent inotropic agents in this series ($EC_{50} \geq 9$ μM); however, **2** and **9** did not induce arrhythmias at the highest obtainable concentration (300 μM). None of the synthesized compounds displayed a greater efficacy (E_{max}) than the three reference compounds.

Discussion

The ability of the synthesized compounds to inhibit Na^+,K^+-ATPase confirms and extends the finding of a recent report[6] that basicity, i.e., a strong ionic interaction, is relevant for the activity on the Na^+,K^+-ATPase receptor. This can be inferred from the biological data of compounds **1**−**4** and particularly from those of **4**, whose fairly good activities, and possibly its therapeutic ratio, the more interesting of the series, are accounted for by the presence of the strongly basic (substituted) guanidino group only. The higher activity (both IC_{50} and EC_{50}) of compound **1** versus its saturated analogue **4** might be ascribed to an additional partial positive charge at C-20, induced by the nitrogen atom through the iminic double bond.

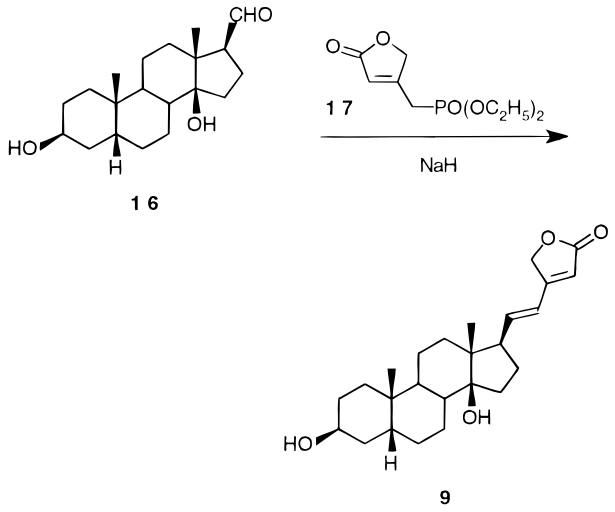
From the IC_{50} and EC_{50} of compound **5**, it can be inferred that the association of a basic guanidino group and a polarized system, which closely resembles the α,β-unsaturated lactone system present in digitoxigenin and digoxin, permits a very strong interaction with the

Scheme 2



10 n = 1, R = CH_3
11 n = 2, R = C_2H_5
12 n = 1
13 n = 2
14 n = 1
15 n = 2
5 n = 1, R^1 = H, ~ = (*E*)
6 n = 1, R^1 = CH_2, ~ = (*Z*)
7 n = 1, R^1 = CH_2, ~ = (*E*)
8 n = 2, R^1 = H, ~ = (*E*)

Scheme 3



16
17
9

receptor and is productive of very high Na^+,K^+-ATPase inhibition: also in this case some degree of improvement of the therapeutic ratio in comparison with digoxin, even though not relevant, can be observed.

The lower activity of **7** with respect to the analogue **5** might possibly be due to the presence of a more bulky imidazolino group preventing a closer interaction of the protonated imidazoline group with a carboxylate of the enzyme; the same holds for the parent compounds **2** versus **1**.[6]

A further shift of the iminic nitrogen atom is detrimental for the activity, as is demonstrated by compound **8**, vinylogous derivative of compound **5**, and is confirmed by the parallel low activity of the lactone **9**, vinylogous derivative of digitoxigenin. The lower inhibitory potency of the *syn* isomer **6** versus its *anti* isomer **7** might be ascribed to the different steric arrangement preventing the proper orientation of the electron lone pair of the iminic nitrogen atom toward the hydrogen donor group of the enzyme and/or the ion-pair interaction.

A highly significant correlation was found between inhibitory potency on the Na^+,K^+-ATPase and inotropic potency in isolated atria ($r^2 = 0.73$, $n = 10$, $p < 0.01$). The correlation was found also for the structurally homogeneous set of the hydrazone derivatives **1**−**5**, **7**, and **8** ($r^2 = 0.70$, $n = 7$, $p < 0.05$). Disappointingly, a strong correlation was also found between inhibitory potency on the Na^+,K^+-ATPase and the arrhythmogenic concentration ($r^2 = 0.99$, $n = 6$, $p < 0.01$), even though only six compounds could be evaluated due to insolubility at higher concentrations of the other three.

The efficacy (E_{max}) of the tested compounds could not be related to Na^+,K^+-ATPase inhibition, inotropic potency, or arrhythmogenic activity. Similar lack of correlation between Na^+,K^+-ATPase inhibition and efficacy was already reported by Thomas[7] and supposed to be a result of different transport or distribution for the two biological systems and/or of the effects on different isoforms of the cell's total Na^+,K^+-ATPase responsible of the myocardial contractility. Anyway, high potency and/or maximal efficacy may not have great importance for a good therapeutic agent, provided that a reasonable potency is present, the most important feature being a better therapeutic index with respect to digoxin.

Conclusions

In this series both inotropic and arrhythmogenic properties are linked to the inhibition of Na^+,K^+-ATPase in such a way that it was not possible to discriminate effectively between the two biological effects, although a certain improvement in the therapeutic ratio was reached with compounds **4** and **5**, even though not of such a degree to be proposed as an alternative to such an established and known drug as digoxin.

Table 2. Biological Data for Compounds **1**−**9**

compd	Na^+,K^+-ATPase inhibition IC_{50},[a] μM	inotropic activity, contractile force			no. of preps	arrhythmias	
		E_{max},[b] % increase from basal force	conctn to obtain E_{max}, μM	EC_{50},[c] μM		arrhythmogenic conctn, μM	% of preps with arrhythmias
1	1.58	265	30	4	7	30	57
2	6.31	129	300	17	3	300	0
3	5.00	62	30	5	6	30	0
4	2.00	166	30	8	3	100	0
5	0.16	140	3	0.4	5	3	20
6	6.31	nt	nt	nt	nt	nt	nt
7	2.50	136	100	9	4	100	50
8	7.94	98	100	15	3	300	100
9	3.98	110	100	14	4	300	0
digitoxigenin	0.50	200	3	0.6	5	10	60
digoxin	0.50	184	1	0.4	6	3	50

[a] Concentrations able to inhibit 50% of enzyme activity. Mean of two or three experiments. [b] Maximal increase in force of contraction. [c] Concentrations producing 50% of the maximal increase in force of contraction were calculated from concentration−response curves. nt: not tested.

As far as the structure−activity relationship is concerned, the contemporary presence of a basic (guanidino) group at a proper distance and of a polarized α,β-unsaturated system in the 17β-substituent can be considered important for Na^+,K^+-ATPase inhibitory potency and inotropic activity. These two requirements are both present in compound **5** and may account for its biological activity being comparable to or higher than that of digitoxigenin and digoxin.

Some new features of the 17β-substituent have been thus discovered and are important for strong binding to the receptor; this opens the route for trying different, new, and unexploited functional groups at position 17β bearing either a basic group only (like in compound **4**) or a 1,4-polarized system together with a basic center (like in compound **5**), hopefully providing compounds with a greater therapeutic ratio and safer therapeutic agents.

Experimental Section

Chemistry. Elemental analyses were performed by Redox, Cologno Monzese, Italy. ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer at 300.13 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard, and coupling constants (*J* values) are in Hz. ^{1}H NMR assignments were drawn from classical arguments on chemical shift and coupling constant behavior. Mass spectral data were obtained with electron impact ionization technique at 70 eV from a Finnigan INCOS-50 mass spectrometer using the direct exposure probe (DEP). Chromatographies were carried out on silica gel (Baker 7024-02) in all instances. Solvents and reagents were used as purchased; reagents were purchased from Aldrich.

17β-[(Guanidinoamino)methyl]-5β-androstane-3β,14β-diol Hydrochloride (4). A mixture of 17β-[(guanidinoimino)-methyl]-5β-androstane-3β,14β-diol hydrochloride (**1**)[6] (0.50 g, 1.2 mmol) and PtO_2 (0.50 g) in EtOH (25 mL) was hydrogenated in a Parr shaker at 60 psi at room temperature for 4 days. After filtration the solution was evaporated under reduced pressure. The residue was crystallized successively from EtOH/EtOAc and EtOH to give **4** (0.20 g, 40%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 0.81 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 2.66 (dd, 1H, *J* = 8.0, 3.0, C*H*HN), 2.77 (dd, 1H, *J* = 8.0, 8.0, CH*H*N), 3.85 (m, 1H, 3-H); MS *m*/*z* 378 (2, M^+ base), 360 (10), 91 (10), 87 (100).

(*E*,*E*)-21-[(Guanidinoimino)methyl]-5β-pregn-20-ene-3β,14β-diol (5). A suspension of aminoguanidine hydrogencarbonate (1.05 g, 7.7 mmol) in H_2O (25 mL) and dioxane (70 mL) was acidified to pH 3 with 3 N HCl until dissolution. A solution of (*E*)-3β,14β-dihydroxy-5β-pregn-20-ene-21-carboxaldehyde (**14**) (2.42 g, 7.0 mmol) in dioxane (20 mL) was added dropwise. Additional 0.1 N HCl was added sometimes to keep the pH around 3. After stirring for 3 days, the mixture was evaporated under reduced pressure. The residue was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH, 80:20:3) to afford an off-white solid which was triturated with Et_2O/EtOH to give **5** (1.62 g, 56%) as a hydrate, white solid: ^{1}H NMR (CD_3OD) δ 0.85 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.28 (m, 1H, 17-H), 4.05 (m, 1H, 3-H), 5.98 (dd, 1H, *J* = 9.5, 15.5, 21-H), 6.20 (dd, 1H, *J* = 9.5, 15.5, 20-H), 7.65 (d, *J* = 9.5, CH=N); MS *m*/*z* 402 (2, M^+), 384 (2), 246 (6), 203 (7), 137 (5), 111 (100).

21-[(2-Imidazolin-2-yl-(*Z*)-hydrazono)methyl]-5β-pregn-20(*E*)-ene-3β,14β-diol (6). Compound **6** was synthesized as in the above-described reaction using 2-hydrazinoimidazoline hydrobromide (1.19 g, 6.6 mmol). The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH, 90:10:1). The fractions containing the product with R_f 0.50 were collected and evaporated under reduced pressure to afford an off-white solid which was triturated with EtOAc/EtOH to give **6** (0.13 g, 5%) as a monohydrate, white solid: ^{1}H NMR (CD_3OD) δ 0.85 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.30 (m, 1H, 17-H), 3.51 (s, 4H, NCH_2CH_2N), 4.05 (m, 1H, 3-H), 6.28 (dd, 1H, *J* = 10.3, 15.5, 20-H), 6.60 (dd, 1H, *J* = 9.4, 15.5, 21-H), 6.97 (d, *J* = 9.4, CH=N); MS *m*/*z* 428 (1, M^+), 163 (4), 137 (100).

(*E*,*E*)-21-[(2-Imidazolin-2-ylhydrazono)methyl]-5β-pregn-20-ene-3β,14β-diol (7). Compound **7** was isolated as in the above-described reaction collecting the fractions containing the product with R_f 0.40. After trituration with EtOAc/EtOH, **7** was obtained (1.60 g, 60%) as a monohydrate, white solid: ^{1}H NMR (CD_3OD) δ 0.85 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.30 (m, 1H, 17-H), 3.51 (s, 4H, NCH_2CH_2N), 4.05 (m, 1H, 3-H), 5.97 (dd, 1H, *J* = 9.5, 15.5, 21-H), 6.20 (dd, 1H, *J* = 9.5, 15.5, 20-H), 7.65 (d, *J* = 9.5, CH=N); MS *m*/*z* 428 (1, M^+), 163 (7), 137 (100).

(*E*,*E*,*E*)-17β-[5-(Guanidinoimino)-1,3-pentadienyl]-5β-androstane-3β,14β-diol (8). Compound **8** was synthesized as in the above-described reaction for compound **5** using (*E*,*E*)-17β-(4-formyl-1,3-butadienyl)-5β-androstane-3β,14β-diol (**15**) (0.48 g, 1.3 mmol). After 20 days the crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH, 85:15:1.5) to afford an off-white solid which was triturated with Et_2O/EtOH to give **8** (0.40 g, 70%) as a hydrate, white solid: ^{1}H NMR (DMSO-d_6) δ 0.72 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 2.11 (m, 1H, 17-H), 3.80 (s, 1H, 14-OH), 3.87 (m, 1H, 3-H), 4.17 (d, 1H, *J* = 3.0, 3-OH), 5.39 (bb, 2H, NH_2), 5.62 (bb, 2H, NH_2), 5.92 (m, 2H, C*H*=C*H*CH=CHCH=N), 6.16 (dd, 1H, *J* = 9.4, 15.4, C*H*CH=N), 6.32 (dd, 1H, *J* = 9.4, 15.4, C*H*=CHCH=N), 7.64 (d, *J* = 9.4, CH=N); MS *m*/*z* 428 (59, M^+), 370 (17), 351 (23), 219 (21), 203 (40), 137 (100).

(*E*)-21-(2,5-Dihydro-5-oxo-3-furyl)-5β-pregn-20-ene-3β,-14β-diol (9). To a suspension of NaH (55% dispersion in oil, 0.35 g, 8.0 mmol) in dry THF (200 mL) was added dropwise a solution of the phosphonate **17**[10] (1.90 g, 8.0 mmol) in THF (25 mL). After 15 min, a solution of the aldehyde **16**[9] (1.50 g, 4.7 mmol) in THF (25 mL) was added dropwise. After 20 h the solution was poured into 5% aqueous NaH_2PO_4 (100 mL) and the resulting mixture extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine, dried

over Na_2SO_4, and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 1:1) to afford **9** (1.05 g, 55%) as a hydrate, white solid: ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 2.23 (m, 1H, 17-H), 4.13 (m, 1H, 3-H), 4.93 (s, 2H, CH_2O), 5.79 (s, 1H, C=CHCO), 6.20 (d, 1H, J = 15.0, 21-H), 6.41 (dd, 1H, J = 15.0, 9.0, 20-H); MS m/z 382 (21), 246 (33), 204 (21), 203 (100), 147 (21), 137 (37), 136 (34).

(*E*)-3β,14β-Dihydroxy-5β-pregn-20-ene-21-carboxaldehyde (14). To a solution of (*E*)-methyl 3β,14β-dihydroxy-5β-pregn-20-ene-21-carboxylate[8] (**10**) (3.31 g, 8.8 mmol) in dry THF (75 mL) at −78 °C was added dropwise a 1 M solution of DIBAH in THF (88 mL, 88 mmol). The solution was allowed to warm to room temperature overnight. To the solution cooled to −35 °C was added dropwise 1 N H_2SO_4 (200 mL) followed by NaCl (5 g). After dissolution of NaCl the organic layer was separated and the aqueous phase extracted with EtOAc (2 × 100 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to dryness to afford (*E*)-17β-(3-hydroxyprop-1-en-1-yl)-5β-androstane-3β,14β-diol (**12**) (2.95 g, 96%): ^{1}H NMR ($CDCl_3$) δ 0.85 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 4.08 (d, 2H, CH_2OH), 4.12 (m, 1H, 3-H), 5.47 (dt, 1H, =CHCH$_2$OH), 5.88 (dd, 1H, CH=CHCH$_2$OH). This material showed a single spot on TLC and was used in the next step without further purification.

A mixture of crude **12** (2.90 g, 8.3 mmol) and MnO_2 (17.4 g) in dioxane (70 mL) was stirred at room temperature for 3 h. The mixture was filtered through a Celite pad and the solution evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 6:4) to afford **14** (2.45 g, 85%) as a white solid: mp 192−193 °C (lit.[13] mp 98−99 °C, prepared from the aldehyde **16** and diethyl 2-(cyclohexylamino)vinylphosphonate in the presence of NaH followed by acid hydrolysis of the adduct); ^{1}H NMR (CD_3OD) δ 0.88 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.48 (m, 1H, 17-H), 4.05 (m, 1H, 3-H), 5.90 (dd, 1H, J = 8.1, 15.5, 21-H), 7.19 (dd, 1H, J = 10.6, 15.5, 20-H), 9.43 (d, J = 8.1, CHO). Anal. ($C_{22}H_{34}O_3$) C, H.

(*E*,*E*)-17β-(4-Formyl-1,3-butadienyl)-5β-androstane-3β,14β-diol (15). Compound **15** was prepared as in the above-described sequence for compound **14** starting from (*E*,*E*)-17β-[4-(ethoxycarbonyl)-1,3-butadienyl]-5β-androstane-3β,14β-diol[8] (**11**) (1.10 g). The intermediate (*E*,*E*)-17β-(5-hydroxy-1,3-pentadienyl)-5β-androstane-3β,14β-diol (**13**) [^{1}H NMR (DMSO-d_6) δ 0.73 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 3.79 (s, 1H, OH), 3.87 (m, 1H, 3-H), 3.91 (m, 2H, CH_2O), 4.19 (d, 1H, 3-OH), 4.62 (m, 1H, CH$_2$OH), 5.58 (m, 1H), 5.79 (m, 2H), 6.08 (m, 1H)] was obtained in 92% yield and used without further purification to give the crude compound **15** which was crystallized from Me_2CO/EtOAc to afford pure **15** (50%) as a white solid: mp 231−235 °C; ^{1}H NMR (DMSO-d_6) δ 0.75 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.23 (m, 1H, 17-H), 3.87 (m, 1H, 3-H), 3.97 (s, 1H, 14-OH), 4.19 (d, 1H, J = 2.6, 3-OH), 6.03 (dd, 1H, J = 8.2, 15.1, CHCHO), 6.16 (dd, 1H, J = 11.0, 15.1, CHCH=CHCHO), 6.54 (dd, 1H, J = 10.2, 15.1, CH=CHCH=CHCHO), 7.30 (dd, 1H, J = 11.0, 15.1, CH=CHCHO), 9.47 (d, J = 8.2, CHO). Anal. ($C_{24}H_{36}O_3$) C, H.

Biology. 1. Na$^+$,K$^+$-ATPase Inhibition. Na$^+$,K$^+$-ATPase was isolated and purified from dog kidney according to Jørghensen.[11] The inhibition of the enzyme activity was measured as percent of hydrolysis of [^{32}P]ATP in the presence and absence of the tested compound.[12] The concentrations able to inhibit 50% (IC_{50}) of enzyme activity were calculated by a nonlinear least-squares curve-fitting computer program.

2. Guinea Pig Atria. Isolated guinea pig left atria (from 300−500 g male animals) were placed in 20 mL organ baths containing a solution of the following composition (mM): NaCl, 131.6; KCl, 5.6; $CaCl_2$, 1.8; NaH_2PO_4, 1.036; $NaHCO_3$, 24.99; glucose, 11; sucrose, 13; under 500 mg resting tension, at 32 °C. The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The preparations were stimulated by platinum electrodes by square-wave pulses at a frequency of 1 Hz (1 ms duration, voltage 2 times the treshold). After a 60 min equilibration period, cumulative concentrations of the compounds were added, each concentration being left in contact until a maximal response or arrhythmias were observed.

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References

(1) Thomas, R.; Gray, P.; Andrews, J. Digitalis: Its Mode of Action, Receptor, and Structure-Activity Relationships. *Adv. Drug Res.* **1990**, *19*, 312−562.
(2) Hoffmann, B. F.; Bigger, J. T., Jr. Digitalis and Allied Cardiac Glycosides. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press, Inc.: New York, 1990; pp 814−839.
(3) Packer, M. A Novel Approach to the Development of Positive Inotropic Agents for Chronic Heart Failure. *J. Cardiovasc. Pharmacol.* **1995**, *26* (Suppl. 1), S52−S56.
(4) The Digitalis Investigation Group. The Effect of Digoxin on Mortality and Morbidity in Patients with Heart Failure. *N. Engl. J. Med.* **1997**, *336*, 525−533.
(5) 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.
(6) 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.
(7) Thomas, R.; Boutagy, J.; Gelbart, A. Cardenolides Analogs. V. Cardiotonic Activity of Semisynthetic Analogs of Digitoxigenin. *J. Pharmacol. Exp. Ther.* **1974**, *191*, 219−231.
(8) Boutagy, J.; Thomas, R. Cardenolide Analogues II. Synthesis of C17β-Unsaturated Esters and Derivatives. *Aust. J. Pharm. Sci.* **1972**, *NS1*, 67−95.
(9) Gobbini, M.; Torri, M. An Expeditious Route to 3β,14β-Dihydroxy-5β-androstane-17β-carboxaldehyde. *Synth. Commun.* **1997**, *27*, 1115−1122.
(10) Boeckman, R. K., Jr.; Soo Sung, K. Stereochemical Control in the Intramolecular Diels-Alder Reaction. 2. Structural and Electronic Effects on Reactivity and Selectivity. *J. Am. Chem. Soc.* **1982**, *104*, 1033−1041.
(11) Jørghensen, P. L. Purification and Characterization of Na$^+$,K$^+$-ATPase. III. Purification from the Outer Medulla of Mammalian Kidney after Selective Removal of Membrane Components by Sodium Dodecylsulphate. *Biochim. Biophys. Acta* **1974**, *356*, 36−52.
(12) Noel, F.; Godfraind, T. Heterogeneity of Ouabain Specific Binding Sites and Sodium-Potassium ATPase Inhibition in Microsomes from Rat Heart. *Biochem. Pharmacol.* **1984**, *33*, 47−53.
(13) Fullerton, D. S.; Pankaskie, M. C.; Ahmed, K.; From, A. H. L. Cardenolide Analogues. 1. A 17β-Unsaturated Aldehyde. *J. Med. Chem.* **1976**, *19*, 1330−1333.

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