17α-*O*-(Aminoalkyl)oxime Derivatives of 3*β*,14*β*-Dihydroxy-5*β*-androstane and 3*β*-Hydroxy-14-oxoseco-D-5*β*-androstane as Inhibitors of Na^+,K^+-ATPase at the Digitalis Receptor

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The synthesis and binding affinities to the digitalis Na^+,K^+-ATPase receptor of a series of 3*β*,14*β*-dihydroxy-5*β*-androstane and 3*β*-hydroxy-14-oxoseco-D-5*β*-androstane derivatives bearing a 17α-(aminoalkoxy)imino chain are reported; some derivatives were also studied for their inotropic activity. Our recently proposed model of interaction of molecules with the digitalis receptor was used to design these compounds. On that basis, the possibility to design novel potent inhibitors of Na^+,K^+-ATPase without being constrained by the stereochemistry of the classical digitalis skeleton in the D-ring region was predicted. The binding affinities of the most potent compounds in the two series, (*EZ*)-17α-{2-[(2-aminoethoxy)imino]ethyl}-5*β*-androstane-3*β*,14*β*-diol (**6f**) and (*EZ*)-3*β*-hydroxy-17α-{2-[(2-aminoethoxy)imino]ethyl}-14,15-seco-5*β*-androstan-14-one (**24c**) are higher than that of the potent natural compound digitoxigenin, despite the unusual α-exit of the substituent in position 17 of **6f** or the disruption of the D-ring in **24c**. These results further support the validity of our recently proposed model of binding at the digitalis receptor. Results of the inotropic tests on guinea pig atrium deserve further investigation on the pharmacological profile of these derivatives.

Introduction

Digitalis cardiac glycosides are well-known drugs that are used clinically to improve myocardial contractility in the treatment of congestive heart failure.[1] Their action is mainly due to inhibition of Na^+,K^+-ATPase, an enzyme located in the cell membrane that promotes the outward transport of Na^+ and the inward transport of K^+;[2] the most potent inhibitors of Na^+,K^+-ATPase are cardenolides such as digoxin, digitoxin, and digitoxigenin (Figure 1). Life-threatening cardiac arrhythmias are the major problem with these compounds; the search for novel inotropic agents with a more favorable therapeutic index has prompted a lot of work on digitalis-like compounds[3] and a recent NIH-sponsored trial,[4] demonstrating a neutral effect of digoxin treatment on mortality, has renewed interest in the search for a safer positive inotropic agent acting through the inhibition of Na^+,K^+-ATPase.

Some authors suggest that the possibility of separating the toxic from positive inotropic effects may reside in compounds able to discriminate among the different isoforms of the target enzyme;[3,5] studies on compounds with high binding affinities on Na^+,K^+-ATPase and with high structural diversity in comparison with the classical digitalis compounds may be a tool to achieve this goal.

In a preceding paper we showed that it was possible to obtain high binding affinities to the digitalis receptor of Na^+,K^+-ATPase with seco-D steroids, i.e., compounds in which the D ring of the steroid skeleton is broken;[6] these seco-D derivatives share some features with cassaine (Figure 1), an *Erythrophleum* alkaloid with digitalis-like behavior. More recently, on the basis of the structural and stereochemical parallels among cassaine, digitoxigenin, and 14,15-secodigitoxigenin analogues, we have drawn a new model for the relative alignment of cassaine at the digitalis receptor; as a consequence, (dimethylamino)ethyl 17α-acrylate analogues of digi-

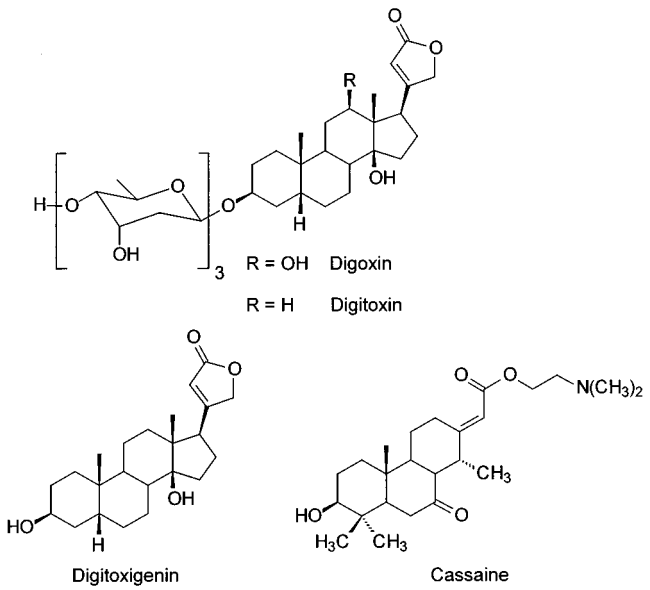

Figure 1. Structures of natural compounds with digitalis activity.

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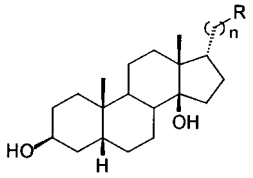
Table 1. Binding Affinity on Na^+,K^+-ATPase

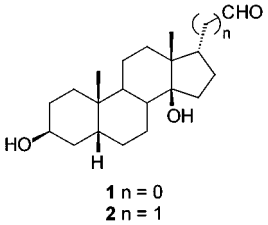


compd	n	R	*E/Z*	yield (%)	binding[a] (IC_{50}, μM)
digitoxigenin					0.063
compd A					6.3
6a	0	$CH{=}NO(CH_2)_2N(CH_3)_2$	80/20	67	1.6
6b	0	$CH{=}NO(CH_2)_2NH_2$	90/10	61	0.4
6c	0	$CH{=}NO(CH_2)_3NH_2$	90/10	64	0.4
6d	0	$CH{=}NO(CH_2)_4NH_2$	80/20	49	1.0
6e	1	$CH{=}NO(CH_2)_2N(CH_3)_2$	75/25	62	0.25
6f	1	$CH{=}NO(CH_2)_2NH_2$	90/10	40	0.05
6g	1	$CH{=}NO(CH_2)_3NH_2$	70/30	61	0.2
6h	1	$CH{=}NO(CH_2)_4NH_2$	70/30	61	0.8

[a] Means of values determined in two to three separate experiments in duplicate. The affinity for the receptor site of Na^+,K^+-ATPase was evaluated by the displacement of the specific [^{3}H]ouabain binding from Na^+,K^+-ATPase receptor[18a] isolated from dog kidney and purified according to Jørghensen.[18b]

toxigenin were predicted and found to be good inhibitors of Na^+,K^+-ATPase. The high affinity showed by the corresponding 14,15-seco derivatives confirmed the hypothesis that seco-D digitoxigenin analogues could be considered cassaine mimics. These results demonstrate that it is possible to design Na^+,K^+-ATPase inhibitors without being constrained by the stereochemistry of the classic digitalis skeleton in the D-ring region.[7]

Following these findings we designed two series of derivatives with basic substituents, already known to produce high affinities as 17β-substituents in the digitalis series:[8] (i) 17α-[(aminoalkoxy)imino]alkyl analogues of digitoxigenin (Table 1) and (ii) seco-D compounds bearing an (aminoethoxy)imino chain at position 17 (Table 2).



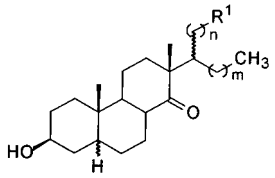
1 n = 0
2 n = 1

Figure 2. Structures of digitalis-like 17α-aldehydes.

These compounds allow us to further explore the requirements for a strong interaction with the Na^+,K^+-ATPase and are a step forward toward new very active non-digitalis structures, hopefully having a more favorable therapeutic index, i.e., good inotropic activity with lower proarrhythmogenic effects, of classical digitalis compounds.

Synthesis of 17α-[(Aminoalkoxy)imino]alkyl Analogues of Digitoxigenin. Variations of the 17α-[(aminoalkoxy)imino]alkyl substituents were as follows: (i) the length of the iminic chain, (ii) the length of the alkoxy chain, and (iii) the amino group (primary or tertiary). The starting compounds for the synthesis of the 17α-[(aminoalkoxy)imino]methyl and 17α-[(aminoalkoxy)imino]ethyl analogues of digitoxigenin in Table 1 were the known 17α-carbaldehyde **1**[9] and the unknown 17α-acetaldehyde **2** of Figure 2. In Scheme 1 the known 17-keto derivative **3**[10] gave, by treatment with diethyl cyanomethylphosphonate in the presence of sodium hydride, the unsaturated nitrile **4**[11] as a *E/Z* mixture (about 7/3) in 73% yield; **4** was then reduced with magnesium in MeOH to give the 17α-cyanomethyl derivative **5** (49% yield), which was finally reacted with DIBAL-H to yield the desired aldehyde **2** (93% yield). From **1** and **2** the oximic derivatives **6a**−**h** of Table 1 were obtained by reaction with the corresponding hydroxylamine dihydrochlorides[8] in dioxane/water at room temperature (Scheme 2); the oximes were obtained as *E/Z* mixtures (see Table 1).

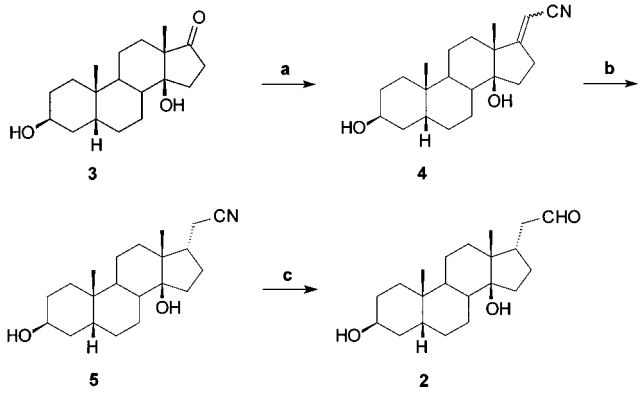
Table 2. Binding Affinity on Na^+,K^+-ATPase



compd	5	17	n	m	R^1	*E/Z*	yield (%)	binding[a] (IC_{50}, μM)
digitoxigenin								0.063
compd B								0.2
compd C								0.13
compd D								0.25
24a	β	α	0	1	$CH{=}NO(CH_2)_2N(CH_3)_2$	65/35	16	0.5
24b	β	α	1	1	$CH{=}NO(CH_2)_2N(CH_3)_2$	75/25	87	0.1
24c	β	α	1	1	$CH{=}NO(CH_2)_2NH_2$	85/15	62	0.04
24d	β	β	0	1	$CH{=}NO(CH_2)_2N(CH_3)_2$	80/20	34	4.0
24e	β	β	1	1	$CH{=}NO(CH_2)_2N(CH_3)_2$	60/40	38	0.8
24f	β	α	1	0	$CH{=}NO(CH_2)_2N(CH_3)_2$	80/20	77	0.13
24g	β	α	1	0	$CH{=}NO(CH_2)_2NH_2$	90/10	59	0.1
24h	β	α	1	2	$CH{=}NO(CH_2)_2NH_2$	55/45	75	0.2
24i	α	α	1	1	$CH{=}NO(CH_2)_2N(CH_3)_2$	80/20	70	0.25
24j	α	α	1	1	$CH{=}NO(CH_2)_2NH_2$	70/30	80	0.16

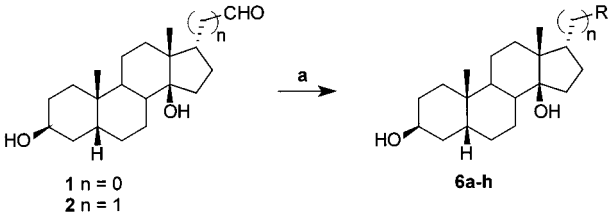
[a] Means of values determined in two to three separate experiments in duplicate. The affinity for the receptor site of Na^+,K^+-ATPase was evaluated by the displacement of the specific [^{3}H]-ouabain binding from Na^+,K^+-ATPase receptor[18a] isolated from dog kidney and purified according to Jørghensen.[18b]

Scheme 1[a]

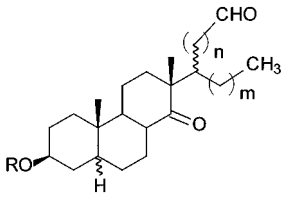


[a] Reagents and conditions: (a) NaH (60% oily dispersion, washed with *n*-hexane), diethyl cyanomethylphosphonate, THF, room temperature; (b) Mg, MeOH, reflux; (c) DIBAL-H (1 M in *n*-hexane), CH_2Cl_2, −5 °C.

Scheme 2[a]



[a] Reagents and conditions: (a) hydroxylamines dihydrochlorides, NaOAc, dioxane/water, room temperature. For the nature of R see Table 1.



7 = 5β; 17α; n = 0; m = 1
8 = 5β; 17α; n = 1; m = 1
9 = 5β; 17β; n = 0; m = 1
10 = 5β; 17β; n = 1; m = 1
11 = 5β; 17α; n = 1; m = 0
12 = 5β; 17α; n = 1; m = 2
13 = 5α; 17α; n = 1; m = 1

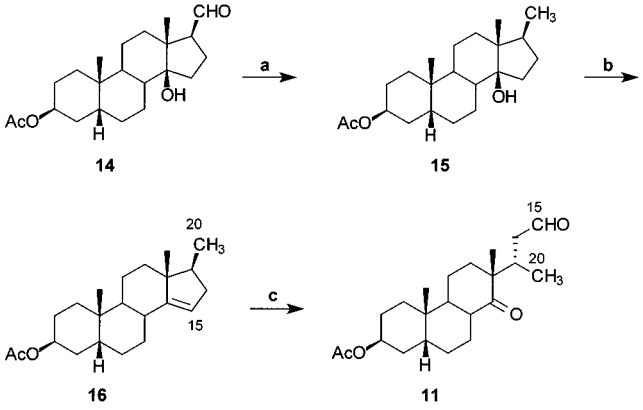
R = protecting group

Figure 3. Structures of seco-D aldehydes.

Synthesis of Seco-D Compounds. The use of the ethoxy chain is a constant in this series as a consequence of the results obtained with the 17α derivatives described above; the structural variations introduced in this series, beyond the length of the iminic chain and the nature of the aminic group already seen in the first series, were as follows: (i) the stereochemistry of the substituent at position 17, (ii) the stereochemistry at position 5, and (iii) the length of the alkyl chain at position 17.

The starting compounds for the syntheses of seco-D derivatives of Table 2 were the corresponding aldehydes **7**−**13** shown in Figure 3; the syntheses of aldehydes **7**−**10** were previously reported,[7] and the remaining compounds **11**, **12**, and **13** could be analogously obtained starting from 3*β*-acetoxy-14*β*-hydroxy-5*β*-androstane-17*β*-carbaldehyde **14**[12] (Scheme 3), 3*β*,14*β*-dihydroxy-5*β*-androstane-17*β*-carbaldehyde **17**[13] (Scheme 4), and

Scheme 3[a]



[a] Reagents and conditions: (a) *p*-toluenesulfonhydrazide, AcOH, room temperature, then $NaBH_3CN$, ZnI_2 cat., MeOH, reflux; (b) $SOCl_2$, pyridine, 0 °C; (c) O_3, CH_2Cl_2, −78 °C, then Zn, AcOH, room temperature.

3*β*-acetoxy-5α-pregn-14-en-20-one **22**[14] (Scheme 5), respectively. It must be noted that the alkyl chains in compounds **7**−**13** originate from 17*β*-oxo substituents in the starting materials (i.e., from aldehydic or acetyl groups), while the aldehydic groups originate from an ozonolytic cleavage of the 14,15-double bond. As a consequence the C-17 stereogenic centers in the final aldehydes have inverted stereochemistry in comparison with the starting compound.

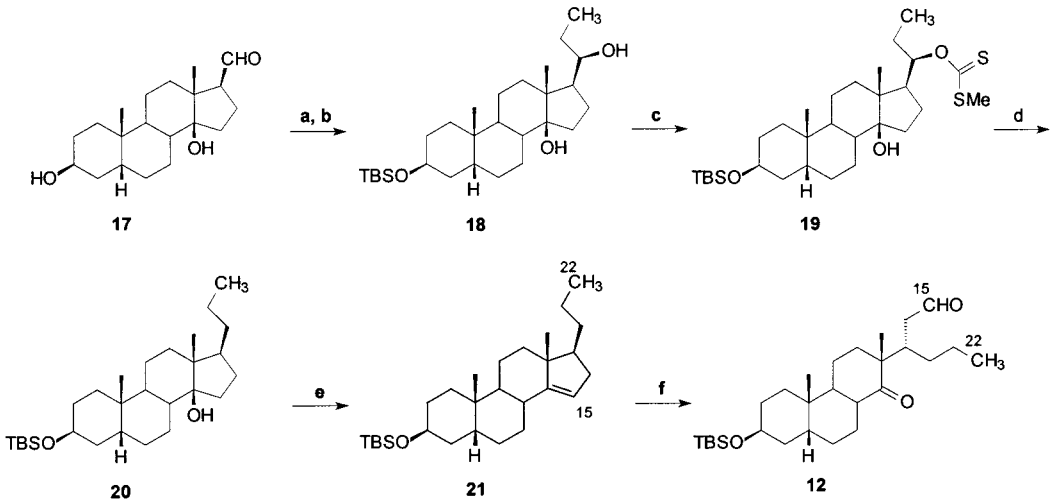
The biological results (Table 2) of the derivatives **24a**−**24e** synthesized from the aldehydes **7**−**10** directed our efforts to the synthesis of compounds **24f**−**24j** with $n = 1$ and the α configuration at position 17, from the starting aldehydes **11**, **12**, and **13**; it should be noted that these features were also those that better fit our model of superposition with cassaine.

Aldehyde **11** (Scheme 3) was obtained starting from compound **14** by reaction with *p*-toluenesulfonhydrazide in AcOH at room temperature to give the corresponding tosylhydrazone, which was reduced with $NaBH_3CN$ in the presence of a catalytic amount of ZnI_2[15] in methanol at reflux temperature to give the 17*β*-methyl derivative **15** (65% yield); dehydration of the tertiary hydroxy group at position 14 with $SOCl_2$ in pyridine (0 °C to room temperature) gave compound **16** in 98% yield. The Δ^{14} derivative **16** was reacted with ozone in CH_2Cl_2 at −78 °C and then with zinc and acetic acid at room temperature, to give the desired keto aldehyde **11** (95% yield).

The keto aldehyde **12** (Scheme 4) was obtained by means of a Grignard reaction with ethylmagnesium bromide on the crude 3*β*-(*tert*-butyldimethylsilyl)oxy derivative of **17,** to give the 20*β*-hydroxy compound **18** as the unique isomer in a 72% yield from **17**; **18** was transformed into the xanthate **19** by sequential treatment with NaH, CS_2, and MeI; the crude xanthate was reduced with Bu_3SnH/AIBN[16] to give the 17*β*-propyl derivative **20** (83% yield from **18**). The procedure followed to pass from intermediate **20** to the aldehyde **12** was the same as that described in Scheme 3; yield from **20** to **12** was 39%.

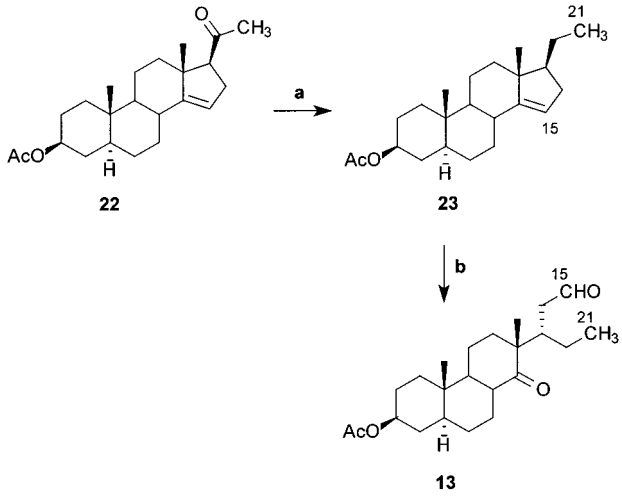
Finally, the keto aldehyde **13** (Scheme 5) was obtained in 67% overall yield starting from **22** by a deoxygenation step and ozonolysis analogously to those described in Scheme 3.

Scheme 4[a]



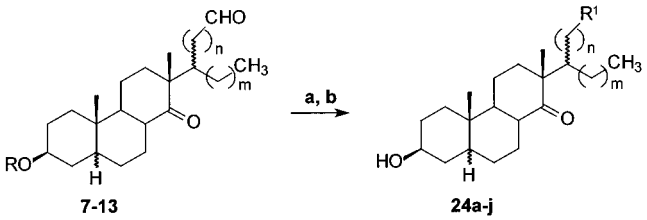
[a] Reagents and conditions: (a) *tert*-butyldimethylsilyl chloride, imidazole, DMF, room temperature; (b) 3 M EtMgBr in Et_2O, toluene, room temperature; (c) NaH (65% oily dispersion), THF, reflux, then CS_2, MeI, reflux; (d) Bu_3SnH, AIBN cat., toluene, reflux; (e) $SOCl_2$, pyridine, 0 °C; (f) O_3, CH_2Cl_2, −78 °C, then Zn, AcOH, room temperature.

Scheme 5[a]



[a] Reagents and conditions: (a) *p*-toluenesulfonhydrazide, AcOH, room temperature, then $NaBH_3CN$, ZnI_2 cat., MeOH, reflux; (b) O_3, CH_2Cl_2, −78 °C, then Zn, AcOH, room temperature.

Scheme 6[a]

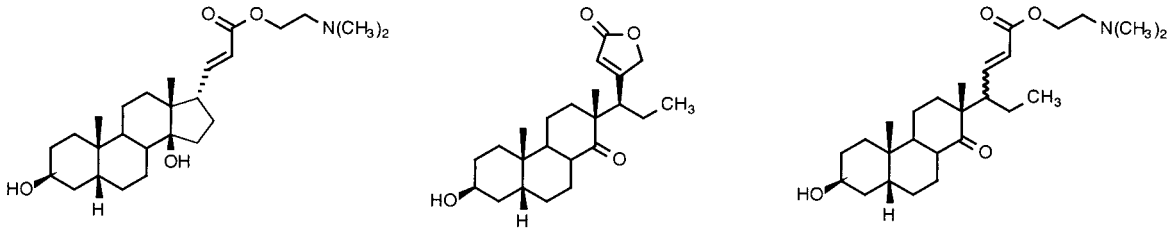


[a] Reagents and conditions: (a) [(dimethylamino)ethyl]hydroxylamine dihydrochloride or (aminoethyl)hydroxylamine dihydrochloride, NaOAc, dioxane/water, room temperature, or for **24a**, [(dimethylamino)ethyl]hydroxylamine, pyridine/THF, room temperature; (b) 1 M NaOH, MeOH, room temperature, or for **24h**, 1% HCl in EtOH, room temperature. For the nature of R^1 see Table 2.

All the aldehydes synthesized were used as crude materials as soon as they were obtained from the ozonolytic step, since they were found to be relatively unstable during the purification procedures. The [(dimethylamino)ethyl]- and (aminoethyl)oximes **24a**−**j** (Scheme 6) were synthesized from the above-described aldehydes **7**−**13** by reaction with the appropriate *O*-[2-(dimethylamino)ethyl]- or *O*-(2-aminoethyl)hydroxylamine dihydrochlorides[8] in dioxane/water at room temperature, except for **24a**, where basic conditions (see Experimental Section) were used to avoid the formation of a cyclic compound[17] instead of the desired oxime, and subsequent cleavage of the protecting groups at position 3 was by basic hydrolysis for acetates and acidic hydrolysis for TBS ether. Again, as for the 17α-derivatives of digitoxigenin reported in Table 1, the oximes of Table 2 were obtained as *E*/*Z* mixtures.

Results and Discussion

The 17α-substituited digitalis-like derivatives **6a**−**h** (Table 1) were evaluated in vitro for displacement of the specific [^{3}H]ouabain binding from the Na^+,K^+-ATPase receptor[18a] isolated from dog kidney and purified according to Jørghensen.[18b] As reference bindings, the affinities of digitoxigenin (Figure 1) and [2-(*N*,*N*-dimethylamino)ethyl]-(*E*)-(3β,14β-dihydroxy-5β-androstane)-17α-acrylate (Figure 4, compound A) are reported. The compounds of general formula **6** can be divided into two series with $n = 0$ and $n = 1$: all compounds derived from the aldehyde with $n = 1$ gave stronger binding values than the corresponding derivatives from the aldehyde with $n = 0$, but all compounds **6a**−**h** showed better binding affinities than reference compound A. In both series, compounds with a primary amine group showed better binding values than those of the tertiary amine analogues (**6b** and **6f** vs **6a** and **6e**, respectively). The alkoxy chain that allowed the best interaction with the receptor was, in both series, an ethoxy chain (**6b** and **6f**). However, with $n = 0$, compound **6c** with the propoxy chain showed a similar value and the butoxy derivative **6d** had a 2.5-fold decrease in the binding affinity; on the contrary, with $n = 1$, the decrease was already pronounced in the propoxy chain derivative **6g** and the butoxy derivative **6h** showed a 16-fold decrease. The best value was reached with compound **6f**, which showed a binding affinity slightly higher than that of digitoxigenin and very near that of the most active seco-D derivative of Table 2, compound **24c**.



[2-(*N*,*N*-Dimethylamino)ethyl]-(*E*)-(3β,14β-dihydroxy-5β-androstane)-17α-acrylate (**Compound A**)[7]

3β-Hydroxy-14-oxo-14,15-seco-5β-card-20(22)-enolide (**Compound B**)[6]

[2-(*N*,*N*-Dimethylamino)ethyl]-(*E*)-3β-hydroxy-14-oxo-14,15-seco-5β-androstane-17α-acrylate (**Compound C**)[7]

[2-(*N*,*N*-Dimethylamino)ethyl]-(*E*)-3β-hydroxy-14-oxo-14,15-seco-5β-androstane-17β-acrylate (**Compound D**)[7]

Figure 4. Structures of reference compounds.

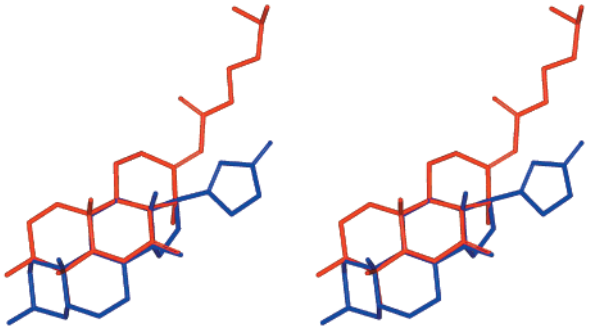


Figure 5. Stereo 3D stick model of the superposition between cassaine (red) and digitoxigenin (blue).

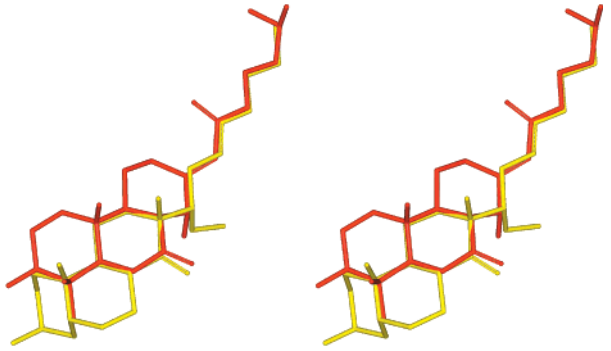


Figure 6. Stereo 3D stick model of the superposition between cassaine (red) and compound **24c** (yellow).

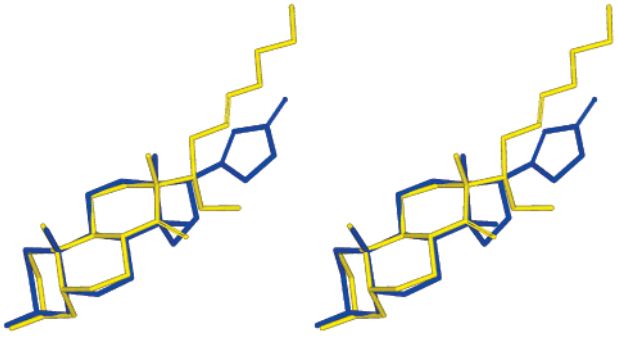


Figure 7. Stereo 3D stick model of the superposition between digitoxigenin (blue) and compound **24c** (yellow).

These results can be rationalized on the basis of our model of binding mode at the digitalis receptor site on Na^+,K^+-ATPase;[7] with this model we showed the possibility of obtaining good binding interactions with digitalis-like compounds having aminoethyl ester substituents originating from the 17α position of the steroid skeleton: as expected, the substitution of the basic ester of the relatively weak compound A with the extraordinarily efficacious (aminoalkyl)oxime group[8] gave a more than 100-fold affinity increase (**6f**).

The basic chain seco-D derivatives (Table 2) were evaluated in vitro as described above for compounds **6a**−**h**; as reference, binding affinities of digitoxigenin (Figure 1), 3*β*-hydroxy-14-oxo-14,15-seco-5*β*-card-20(22)-enolide,[6] [2-(*N*,*N*-dimethylamino)ethyl]-(*E*)-3*β*-hydroxy-14-oxo-14,15-seco-5*β*-androstane-17α-acrylate[7] and [2-(*N*,*N*-dimethylamino)ethyl]-(*E*)-3*β*-hydroxy-14-oxo-14,15-seco-5*β*-androstane-17*β*-acrylate[7] (Figure 4, compounds B, C, and D, respectively) are reported. The most striking peculiarity of the novel superposition proposed by us is that the unsaturated ester of cassaine stretches out in the 17α region of digitoxigenin (Figure 5). In the case of 14,15-seco compounds, for example, compound **24c**, there is a good superposition with cassaine (Figure 6), and the 17*β*-ethyl group allows a strong interaction, possibly through van der Waals forces, with the hydrophobic pocket in the receptor, which accommodates also the 14α-methyl group of cassaine or the C16 methylene of digitoxigenin (Figure 7).

As anticipated,[7] seco-D derivatives with a basic chain in the 17α configuration showed better binding affinities than the analogous 17*β* derivatives (see **24a** and **24b** *vs* **24d** and **24e,** respectively). The introduction of a methylene group in the iminic chain allowed a better interaction with the receptor as shown by higher affinity of compound **24b** vs **24a**. A slightly higher affinity of the primary (aminoalkyl)oximes compared to the dimethylamino analogues is a common feature in this series as well as in the digitalis derivatives reported in the previous paper.[8] The primary amino derivative **24c** showed the highest affinity value in the seco-D series, a value higher than that of digitoxigenin and of the three reference compounds. Derivatives **24g** and **24h** with the methyl ($m = 0$) or propyl ($m = 2$) aliphatic chains, respectively, showed a slightly lower affinity than the corresponding ethyl ($m = 1$) derivative **24c**; from these data it was inferred that the ethyl group is the best fitting group for the hydrophobic pocket where the 14C-methyl group of cassaine is also supposed to be located.[19]

Change in configuration at the A/B ring junction, from 5*β* to 5α, caused a reduced affinity for the receptor for both 5α derivatives **24i** and **24j** compared to the 5*β* counterparts **24b** and **24c**. It must be underlined that **24j**, which has none of the structural requirements of

Table 3. Inotropic Activity on Electrically Driven Guinea Pig Left Atrium

compd	E_{max}[a] (% increase from basal force)	concn to obtain E_{max} (μM)	EC_{50}[b] (μM)
6c	86	100	18.0
6f	134	10	3.5
24b	109	10	2.0
24c	87	10	1.5
24f	130	30	3.0
24h	196	30	5.6
digitoxigenin	200	3	0.57

[a] Maximal increase in force of contraction. [b] Concentrations producing 50% of the maximal increase in force of contraction were calculated from concentration−response curves.

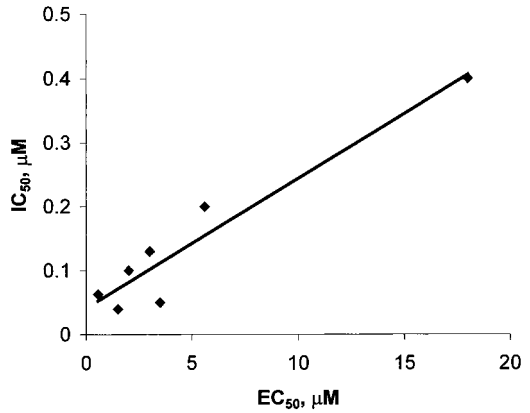


Figure 8. Correlation between binding affinity (IC_{50}, micromoles per liter) and inotropic activity (EC_{50}, micromoles per liter).

the classical digitalis derivatives, still shows very high affinity for the Na^+,K^+-ATPase receptor, close to 0.1 μM.

Some representative compounds were chosen for inotropic tests on electrically driven guinea pig left atrium (Table 3); results show a good correlation with the binding potency (Figure 8; $r = 0.957$, $r^2 = 0.916$, $n = 7$, $p < 0.001$), supporting the hypothesis that high affinity for the Na^+,K^+-ATPase pump site is associated with cardiac inotropic efficacy.

Conclusions

The series of 17α-[(aminoalkoxy)imino]alkyl analogues of digitoxigenin and seco-D compounds bearing an (aminoethoxy)imino chain at position 17 described here were designed on the basis of our recently reported model of interaction of cassaine analogues at the digitalis receptor site at Na^+,K^+-ATPase. Affinities higher than that of digitoxigenin have been found in both series (**6f** and **24c**), and almost all compounds showed IC_{50} lower than 1 μM. These results are a validation of our model and demonstrate that it is possible to obtain compounds with high binding affinities at Na^+,K^+-ATPase without being forced to maintain the stereochemistry of the classical digitalis skeleton. Results of the inotropic tests on guinea pig atrium deserve further investigation on the pharmacological profile of these derivatives.

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 parts per million (ppm) downfield from tetramethylsilane as internal standard and coupling constants (J values) are in hertz. ^{1}H NMR assignments were drawn from classical arguments on chemical shift and coupling constant behavior. Mass spectral data were obtained with the electron impact ionization technique at 70 eV from a Finnigan INCOS-50 mass spectrometer by use of the direct exposure probe (DEP). Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates with fluorescent indicator (Merck). Flash column chromatography (FCC) was performed on silica gel (Merck, 40−63 mesh). Solutions were dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. Solvents and reagents were used as purchased from Aldrich.

(3*β*,14*β*-Dihydroxy-5*β*-androstane)-17α-acetaldehyde (2). To a solution of (*EZ*)-17-cyanomethylen-5*β*-androstane-3*β*,14*β*-diol **4**[11] (1.6 g, 4.86 mmol) in MeOH (50 mL), magnesium (turnings, 5.2 g, 0.214 mol) was added portionwise over 30 min, together with a single crystal of iodine. After 5 min the reaction mixture spontaneously reached the reflux temperature and was then maintained at such temperature for 2 h. The reaction mixture was cooled to 0 °C and a 3 N HCl solution was added until a pH of about 1 was reached. The solution was extracted with EtOAc and the organic layer was washed with a saturated solution of Na_2HPO_4 and then brine, dried, and evaporated to give a residue that was crystallized from EtOAc to give 17α-cyanomethyl-5*β*-androstane-3*β*,14*β*-diol **5** (0.79 g, 49%). ^{1}H NMR ($CDCl_3$) δ 0.96 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.19 (dd, 1H, J = 16.7, 9.5, C*H*HCN), 2.34 (dd, 1H, J = 16.7, 5.7, C*H*HCN), 2.47 (m, 1H, 17-H), 4.13 (m, 1H, 3-H). MS *m*/*z* 331 (11%, M^+), 203 (40), 176 (100); mp 195−198 °C.

To a solution of **5** (1.79 g, 5.38 mmol) in CH_2Cl_2 (80 mL) kept at −5 °C, a 1 M solution of DIBAL-H in *n*-hexane (25 mL, 25.0 mmol) was added dropwise in 30 min; after a further 30 min at the same temperature, the reaction mixture was diluted with an equal volume of EtOAc and then poured into a 20% citric acid solution (200 mL). The two layers were separated and the aqueous layer was extracted with EtOAc; the organic layers were combined, washed with a saturated solution of Na_2HPO_4 and then brine, dried, and evaporated to give **2** (1.66 g, 93%) as a solid sufficiently pure by TLC and ^{1}H NMR for use in the reaction with hydroxylamines. ^{1}H NMR ($CDCl_3$) δ 0.97 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 4.15 (m, 1H, 3-H), 9.78 (t, 1H, J = 3.0, CHO).

(*EZ*)-17α-[[2-(*N*,*N*-Dimethylamino)ethoxy]imino]methyl-5*β*-androstane-3*β*,14*β*-diol Oxalate (6a). To a solution of 3*β*,14*β*-dihydroxy-5*β*-androstane-17α-carbaldehyde **1**[9] (0.35 g, 1.09 mmol) in dioxane/water (8.0/2.5 mL) were added NaOAc (0.21 g, 2.56 mmol) and 2-(dimethylamino)ethoxyamine dihydrochloride[8] (0.23 g, 1.28 mmol), and the reaction mixture was stirred at room temperature for 1 h. The organic solvent was evaporated, the aqueous suspension was treated with $NaHCO_3$ and extracted with EtOAc, and the organic layer was dried and evaporated. The aqueous suspension was extracted with EtOAc and the organic layer was dried and evaporated. The crude product was purified by FCC with $CHCl_3$/MeOH/NH_4-OH (98.5:2.5:0.25) as eluant to yield **6a** (0.3 g, 67%). The product was then isolated as oxalate from EtOH, white solid. ^{1}H NMR (CD_3OD) δ 0.96 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.84 (m, 0.7H, 17-H *E* isomer), 2.93 (m, 6H, $N(CH_3)_2$), 3.43 (m, 2H, NCH_2), 3.61 (m, 0.3H, 17-H *Z* isomer), 4.05 (m, 1H, 3-H), 4.33 (m, 2H, OCH_2), 6.79 (d, 0.3H, J = 8.1, CH=N *Z* isomer), 7.47 (d, 0.7H, J = 7. 8, CH=N *E* isomer). MS *m*/*z* 406 (4%, M^+ base), 203 (2), 58 (100); mp 157−160 °C. Anal. ($C_{24}H_{42}N_2O_3 \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N, H_2O.

(*EZ*)-17α-[(2-Aminoethoxy)imino]methyl-5*β*-androstane-3*β*,14*β*-diol (6b). Prepared in 61% yield from aldehyde **1** and 2-aminoethoxyamine dihydrochloride[8] by the procedure described above for the preparation of **6a**. The product was isolated as free base from Et_2O, white solid. ^{1}H NMR (CD_3OD) δ 0.96 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.83 (m, 0.9H, 17-H *E* isomer), 2.86 (t, 2H, J = 6.5, NCH_2), 3.68 (m, 0.1H, 17-H *Z* isomer), 4.05 (m, 3H, 3-H + OCH_2), 6.68 (d, 0.1H, J = 8.1, CH=N *Z* isomer), 7.41 (d, 0.9H, J = 7. 8, CH=N *E* isomer). MS *m*/*z* 378 (2%, M^+ base), 318 (100), 203 (2); mp 134−138 °C. Anal. ($C_{22}H_{38}N_2O_3 \cdot 0.25H_2O$) C, H, N, H_2O.

(*EZ*)-17α-[(3-Aminopropoxy)imino]methyl-5*β*-androstane-3*β*,14*β*-diol (6c). Prepared in 64% yield from aldehyde **1** and 3-aminopropoxyamine dihydrochloride[8] by the procedure described above for the preparation of **6a**. The product was then isolated as free base from EtOH, white solid. ^{1}H NMR (DMSO-d_6) 0.83 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.71 (q, 0.9H, J = 8.0, 17-H *E* isomer), 2.56 (t, 2H, J = 6.5, NCH_2), 3.47 (q, 0.1H, J = 8.4,17-H *Z* isomer), 3.87 (m, 1H, 3-H), 3.96 (t, 2H, J = 6.5, OCH_2), 6.60 (d, 0.1H, J = 7.9, CH=N *Z* isomer), 7.30 (d, 0.9H, J = 8.0, CH=N *E* isomer). MS *m*/*z* 392 (2, M^+ base), 302 (100), 203 (5), 74 (75); mp 159−167 °C. Anal. ($C_{23}H_{40}N_2O_3\cdot 0.25H_2O$) C, H, N, H_2O.

(*EZ*)-17α-[(4-Aminobutoxy)imino]methyl-5*β*-androstane-3*β*,14*β*-diol (6d). Prepared in 49% yield from aldehyde **1** and 4-aminobutoxyamine dihydrochloride[8] by the procedure described above for the preparation of **6a**. The product was then isolated as free base from EtOH, white solid. ^{1}H NMR (DMSO-d_6) 0.83 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.51 (t, 2H, J = 6.9, NCH_2), 2.71 (0.8H, q, J = 8.0, 17-H *E* isomer), 3.48 (m, 0.2H, q, J 8.4, 17-H *Z* isomer), 3.90 (m, 3H, 3-H + OCH_2), 6.61 (d, 0.2H, J = 7.9, CH=N *Z* isomer), 7.30 (d, 0.8H, J = 8.0, CH=N *E* isomer). MS *m*/*z* 406 (1%, M^+ base), 302 (40), 203 (3), 88 (100); mp 146−149 °C. Anal. ($C_{24}H_{42}N_2O_3$) C, H, N.

(*EZ*)-17α-{2-[[2-(*N*,*N*-Dimethylamino)ethoxy]imino]-ethyl}-5*β*-androstane-3*β*,14*β*-diol (6e). Prepared in 62% yield from aldehyde **2** and 2-(dimethylamino)ethoxyamine dihydrochloride by the procedure described above for the preparation of **6a**. The product was then isolated as free base from Et_2O, white solid. ^{1}H NMR (CD_3OD) δ 0.94 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.28 (s, 2.25H, $N(CH_3)_2$ *E* isomer), 2.30 (s, 0.75H, $N(CH_3)_2$ *Z* isomer), 2.61 (t, 1.5H, J = 5.6, NCH_2 *E* isomer), 2.65 (t, 0.5H, J = 5.6, NCH_2 *Z* isomer), 4.05 (m, 1H, 3-H), 4.10 (t, 1.5H, J = 5.6, OCH_2 *E* isomer), 4.17 (t, 0.5H, J = 5.6, OCH_2 *Z* isomer), 6.71 (t, 0.25H, J = 5.6, CH=N *Z* isomer), 7.41 (t, 0.75H, J = 6.2, CH=N *E* isomer**.** MS *m*/*z* 420 (4, M^+ base), 203 (1), 58 (100); mp (123) 169 °C. Anal. ($C_{25}H_{44}N_2O_3\cdot 0.25H_2O$) C, H, N, H_2O.

(*EZ*)-17α-{2-[(2-Aminoethoxy)imino]ethyl}-5*β*-androstane-3*β*,14*β*-diol (6f). Prepared in 40% yield from aldehyde **2** and 2-aminoethoxyamine dihydrochloride by the procedure described above for the preparation of **6a**. The product was then isolated as free base from MeOH, white solid. ^{1}H NMR (CD_3OD) δ 0.93 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.85 (t, 2H, J = 5.6, NCH_2), 4.00 (t, 2H, J = 5.6, OCH_2), 4.04 (m, 1H, 3-H), 6.72 (t, 0.1H, J = 5.6, CH=N *Z* isomer), 7.44 (t, 0.1H, J = 6.2, CH=N *E* isomer. MS *m*/*z* 392 (7%, M^+ base), 272 (100), 203 (9); mp 192−195 °C. Anal. ($C_{23}H_{40}N_2O_3\cdot 0.25H_2O$) C, H, N, H_2O.

(*EZ*)-17α-{2-[(3-Aminopropoxy)imino]ethyl}-5*β*-androstane-3*β*,14*β*-diol (6 g).

Prepared in 61% yield from aldehyde **2** and 3-aminopropoxyamine dihydrochloride by the procedure described above for the preparation of **6a**. The product was then isolated as free base from Et_2O, white solid. ^{1}H NMR (CD_3OD) δ 0.94 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.72 (m, 2H, NCH_2), 4.06 (m, 3H, 3-H + OCH_2), 6.70 (t, 0.3H, J = 5.6, CH=N *Z* isomer), 7.38 (t, 0.7H, J = 6.2, CH=N *E* isomer. MS *m*/*z* 406 (2%, M^+ base), 334 (100), 203 (2), 74 (25); mp 51−62 °C. Anal. ($C_{24}H_{42}N_2O_3\cdot 0.33H_2O$) C, H, N, H_2O.

(*EZ*)-17α-{2-[(4-Aminobutoxy)imino]ethyl}-5*β*-androstane-3*β*,14*β*-diol (6h). Prepared in 60% yield from aldehyde **2** and 4-aminobutoxyamine dihydrochloride by the procedure described above for the preparation of **6a**. The product was then isolated as free base from Et_2O, white solid. ^{1}H NMR (CD_3OD) δ 0.94 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.65 (m, 2H, NCH_2), 3.9−4.1 (m, 3H, 3-H + OCH_2), 6.69 (t, 0.3H, J = 5.6, CH=N *Z* isomer), 7.37 (t, 0.7H, J = 6.2, CH=N *E* isomer. MS *m*/*z* 420 (2, M^+ base), 334 (100), 203 (2), 88 (80); mp 115−119 °C. Anal. ($C_{25}H_{44}N_2O_3$) C, H, N.

3*β*-Acetoxy-17*β*-methyl-14,15-seco-5*β*-androstane-14,15-dione (11). A solution of 3*β*-acetoxy-17*β*-methyl-5*β*-androst-14-ene **16** (0.75 g, 2.28 mmol) in CH_2Cl_2 (40 mL) was cooled at −78 °C and a stream of ozone was passed through until the reaction was complete (ca. 1 h). The excess of ozone was removed by a stream of nitrogen, and then zinc (3.0 g, 45.88 mmol) and AcOH (4.5 mL) were slowly added and the temperature was allowed to rise to room temperature. After 3 h of stirring the mixture was filtered, the solid was washed with CH_2Cl_2, and the solution was evaporated. The residue was dissolved in EtOAc and washed with a saturated solution of Na_2HPO_4; the organic layer was dried and evaporated to give **11** (0.78 g, 95%) as a white solid. This compound is scarcely stable and was immediately used without any further purification in the reaction with hydroxylamines. ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3H, $CHCH_3$), 1.06 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 2.07 (s, 3H, CH_3CO), 5.06 (m, 1H, 3-H), 9.79 (d, 1H, J = 3.0, CHO).

3*β*-(*tert*-Butyldimethylsilyl)oxy-17*β*-propyl-14,15-seco-5*β*-androstane-14,15-dione (12). Prepared in 91% yield from 3*β*-(*tert*-butyldimethylsilyl)oxy-17*β*-propyl-5*β*-androst-14-ene **21** by the procedure described above for the preparation of **11**. Thick oil; this compound is scarcely stable and was immediately used without any further purification in the reaction with hydroxylamines. ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.89 (s, 9H, t-BuSi), 1.00 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 2.15 (1H, m, C*H*CHO), 2.40 (m, 1H, 17-H), 2.54 (1H, m, C*H*HCHO), 4.03 (m, 1H, 3-H), 9.79 (br s, 1H, CHO).

3*β*-Acetoxy-14,15-secopregnane-14,15-dione (13). Prepared in 91% yield from 3*β*-acetoxypregn-14-ene **23** by the procedure described above for the preparation of **11**. Thick oil; this compound is scarcely stable and was immediately used without any further purification in the reaction with hydroxylamines. ^{1}H NMR ($CDCl_3$) δ 0.88 (s, 3H, CH_3), 0.89 (t, 3H, CH_2CH_3), 1.10 (s, 3H, CH_3), 2.18 (1H, m, C*H*CHO), 2.33 (m, 1H, 17-H), 2.52 (1H, m, C*H*HCHO), 4.70 (m, 1H, 3-H), 9.81 (t, 1H, J = 3.0, CHO).

3*β*-Acetoxy-17*β*-methyl-5*β*-androst-14-ene (16). To a solution of 3*β*-acetoxy-14*β*-hydroxy-5*β*-androstane-17*β*-carbaldehyde **14**[12] (0.91 g, 2.51 mmol) in AcOH (6 mL) was added *p*-toluenesulfonhydrazide (0.56 g, 3.02 mmol), and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was then poured in a saturated solution of Na_2HPO_4 and extracted with EtOAc, and the organic layer was dried and evaporated. The residue was dissolved in MeOH (20 mL), and ZnI_2 (0.12 g, 0.376 mmol) was added. To this solution $NaBH_3CN$ (0.26 g, 4.19 mmol) was added portionwise, and the temperature was brought to reflux. After 3 h the solution was cooled and evaporated, the crude product was dissolved in EtOAc and neutralized with 0.1 N NaOH; the organic layer was dried and evaporated. The residue was purified by FCC with *n*-hexane/Et_2O (75:25) as eluant to give 3*β*-acetoxy-14*β*-hydroxy-17*β*-methyl-5*β*-androstane **15** (0.57 g, 65%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.93 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.03 (d, 3H, J = 6.5, 17-CH_3), 2.05 (s, 3H, CH_3CO), 5.08 (m, 3H, 3-H).

To a solution of **15** (0.55 g, 1.58 mmol) in pyridine (10 mL) kept at 0 °C was added $SOCl_2$ (0.31 mL, 4.25 mmol). The solution was stirred at the same temperature for 2.5 h and then poured into 10 mL of 1 N HCl and crushed ice; the mixture was extracted with Et_2O, and the organic layer was washed with a saturated solution of Na_2HPO_4, dried, and evaporated to give **16** (0.51 g, 98%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H, CH_3), 0.97 (d, 3H, J = 6.5, 17-CH_3), 0.99 (s, 3H, CH_3), 2.07 (s, 3H, CH_3CO), 5.07 (m, 1H, 15-H), 5.17 (m, 1H, 15-H).

3*β*-(*tert*-Butyldimethylsilyl)oxy-17*β*-propyl-5*β*-androst-14-ene (21). To a solution of ethylmagnesium bromide in Et_2O (3 M, 10 mL, 30 mmol) kept at room temperature was added dropwise a solution of 3*β*-(*tert*-butyldimethylsilyl)oxy-14*β*-hydroxy-5*β*-androstane-17*β*-carbaldehyde (1.0 g, 2.3 mmol) in 40 mL of toluene (prepared from the known 3*β*,14*β*-dihydroxy-5*β*-androstane-17*β*-carbaldehyde **17**[13]), and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then poured into a saturated, chilled solution of NH_4Cl and the suspension was extracted with EtOAc. The organic layer was dried and evaporated to give 3*β*-(*tert*-butyldimethylsilyl)oxy-14*β*-hydroxy-17*β*-(1*R*-hydroxypropyl)-5*β*-androstane **18** (1.05 g, 72%) as a solid sufficiently pure by TLC and ^{1}H NMR for use in the subsequent reaction. ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.89 (s, 9H, t-BuSi), 0.92 (t,

3H, CHCH_3), 0.93 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 3.66 (dd, 1H, J = 6.5, 7.5, CHOH), 4.03 (m, 1H, 3-H).

To a solution of **18** (1.0 g, 2.15 mmol) in THF (40 mL) was added portionwise NaH (0.47 g of 65% oily dispersion, 10.75 mmol), and the reaction mixture was refluxed for 2 h. To this mixture CS_2 (1.3 mL, 21.5 mmol) was added dropwise at the same temperature, and after 0.5 h, MeI (2.7 mL, 43.0 mmol) was added dropwise. After 0.5 h at reflux, the reaction mixture was cooled to 0 °C and a saturated solution of NH_4Cl (20 mL) was added. The suspension was extracted with EtOAc and the organic layer was dried and evaporated to give 3β-(*tert*-butyldimethylsilyl)oxy-14β-hydroxy-17β-{1*R*-[[(methylthio)-thiocarbonyl]oxy]propyl}-5β-androstane **19** (1.2 g) as a thick oil that was used in the subsequent reaction without any further purification. A solution of **19** (1.2 g, 2.15 mmol), tris-(trimethylsilyl)silane (1.2 mL, 3.87 mmol), and AIBN (0.05 g, 0.3 mmol) in toluene (40 mL) was refluxed for 2 h; then tris-(trimethylsilyl)silane (0.5 mL, 1.62 mmol) and AIBN (0.05 g, 0.3 mmol) were added and the mixture was refluxed for 1 h. The reaction mixture was cooled to 0 °C and a saturated solution of NaCl (20 mL) was added. The suspension was extracted with EtOAc; the organic layer was dried and evaporated to dryness. The residue was purified by FCC with *n*-hexane/EtOAc (95:5) as eluant to give 3β-(*tert*-butyldimethylsilyl)oxy-14β-hydroxy-17β-propyl-5β-androstane **20** (0.8 g, 83% from **18**) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.88 (t, 3H, CH_3 CH_2), 0.89 (s, 12H, t-BuSi + CH_3), 0.92 (s, 3H, CH_3), 4.04 (m, 1H, 3-H).

To a solution of **20** (0.8 g, 1.8 mmol) in pyridine (11 mL) kept at 0 °C was added $SOCl_2$ (0.24 mL, 3.34 mmol). The solution was stirred at 0 °C for 2.5 h and then poured in 10 mL of 1 N HCl and crushed ice. The mixture was extracted with EtOAc; the organic layer was washed with a saturated solution of Na_2HPO_4, dried, and evaporated to dryness. The residue was purified by FCC with *n*-hexane as eluant to give **21** (0.33 g, 43%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.81 (s, 3H, CH_3), 0.89 (s, 9H, t-BuSi), 0.91 (t, 3H, CH_3CH_2), 0.96 (s, 3H, CH_3), 2.32 (m, 1H, 16-H), 4.01 (m, 1H, 3-H), 5.16 (m, 1H, 15-H).

3β-Acetoxypregn-14-ene (23). To a solution of 3β-acetoxy-pregn-14-en-20-one **22**[14] (0.52 g, 1.45 mmol) in AcOH (5 mL) was added *p*-toluensulfonhydrazide (0.35 g, 1.88 mmol), and the mixture was stirred at room temperature for 3 h. The solvent was evaporated to dryness; the residue obtained was dissolved in MeOH (20 mL), and ZnI_2 (0.062 g, 0.196 mmol) was added. To this solution was added portionwise $NaBH_3$-CN (0.15 g, 2.45 mmol), and the temperature was raised to the boiling point of the reaction mixture. After 2 h the solvent was evaporated, and the crude product was dissolved in EtOAc and neutralized with 0.1 N NaOH; the organic layer was dried and evaporated. The crude product was purified by FCC with *n*-hexane/EtOAc (97:3) as eluant to give **23** (0.37 g, 74%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.91 (t, 3H, J = 6.5, CH_3CH_2), 2.03 (s, 3H, CH_3CO), 2.37 (m, 1H, 16-H), 4.70 (m, 1H, 3-Hax), 5.17 (m, 1H, 15-H).

(*EZ*)-3β-Hydroxy-17α-[[2-(*N*,*N*-dimethylamino)ethoxy]-imino]methyl-14,15-seco-5β-androstan-14-one Oxalate (24a). To a solution of 3β-acetoxy-14-oxo-14,15-seco-5β-androstane-17α-carbaldehyde **7**[7] (0.29 g, 0.81 mmol) in THF (15 mL) and pyridine (1.4 mL) was added dropwise a 1.4 M solution of 2-(dimethylamino)ethoxyamine in THF (3.0 mL, 4.2 mmol), and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then poured into a saturated solution of NaCl and extracted with EtOAc. The organic layer was dried and evaporated to give a crude residue that was purified by FCC with CH_2Cl_2/MeOH (90:10) as eluant to give **24a** as the 3β-acetoxy derivative (81 mg). This compound was dissolved in MeOH (3.0 mL) and a 1 M solution of NaOH (1.0 mL, 1.0 mmol) was added; the reaction mixture was stirred at room temperature for 16 h and the organic solvent was evaporated. The aqueous suspension was extracted with EtOAc and the organic layer was dried and evaporated to yield **24a** (66 mg, 16% from **7**). The product was then isolated as oxalate from EtOAc, white solid. ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 3H, CH_2CH_3), 1.04 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 2.38 (m, 0.65H, 17-H *E* isomer), 2.51 (m, 1H, 8-H), 2.91 (m, 6H, $N(CH_3)_2$), 3.20 (m, 0.35H, 17-H *Z* isomer),3.42 (m, 2H, NCH_2), 4.12 (m, 1H, 3-H), 4.42 (m, 2H, OCH_2), 6.67 (d, 0.35H, J = 8.0, CH=N *Z* isomer), 7.34 (d, 0.65H, J = 7.9, CH=N *E* isomer). MS *m*/*z* 406 (5%, M^+ base), 58 (100); mp 66−82 °C (decomp). Anal. ($C_{24}H_{42}N_2O_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N, H_2O.

(*EZ*)-3β-Hydroxy-17α-{2-[[2-(*N*,*N*-dimethylamino)ethoxy]imino]ethyl}-14,15-seco-5β-androstan-14-one Oxalate (24b). To a solution of 3β-acetoxy-14,15-seco-14,15-dioxo-5β-pregnane **8**[7] (0.27 g, 0.80 mmol) in dioxane/water (5.4/2.0 mL) were added NaOAc (0.32 g, 3.90 mmol) and 2-(dimethylamino)ethoxyamine dihydrochloride (0.15 g, 0.82 mmol), and the reaction mixture was stirred at room temperature for 1 h. The organic solvent was evaporated, the aqueous suspension was neutralized with $NaHCO_3$ and extracted with EtOAc, and the organic layer was dried and evaporated. The crude residue was dissolved in MeOH (12 mL) and a 1 M solution of NaOH (2.7 mL, 2.7 mmol) was added; the reaction mixture was stirred at room temperature for 24 h and the organic solvent was evaporated. The aqueous suspension was extracted with EtOAc and the organic layer was dried and evaporated. The crude product was purified by FCC with $CHCl_3$/MeOH/NH_4-OH (98.5:2.5:0.25) as eluant to yield **24b** (0.29 g, 87% from **8**). The product was then isolated as oxalate from Et_2O, white solid. ^{1}H NMR ($CDCl_3$) δ 0.93 (m, 3H, CH_2CH_3), 1.04 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 2.28 (m, 1H, 17-H), 2.56 (m, 1H, 8-H), 2.89 (m, 6H, $N(CH_3)_2$), 3.38 (m, 2H, NCH_2), 4.12 (m, 1H, 3-H), 4.39 (m, 1.5H, OCH_2 *E* isomer), 4.44 (m, 0.5H, OCH_2 *Z* isomer), 6.87 (t, 0.25H, J = 5.3, CH=N *Z* isomer), 7.34 (dd, 0.75H, J = 5.7, 6.6, CH=N *E* isomer). MS *m*/*z* 420 (4%, M^+ base), 315 (3), 171 (3), 58 (100); mp 80−123 °C (decomp). Anal. ($C_{25}H_{44}N_2O_3 \cdot C_2H_2O_4$) C, H, N.

(*EZ*)-3β-Hydroxy-17α-{2-[(2-aminoethoxy)imino]ethyl}-14,15-seco-5β-androstan-14-one Oxalate (24c). Prepared in 62% yield from aldehyde **8** and 2-aminoethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate from Et_2O, white solid. ^{1}H NMR (DMSO-d_6) δ 0.84 (m, 3H, CH_2CH_3), 0.94 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 2.24 (1H, m, 17-H), 2.55 (1H, m, 8-H), 3.01 (m, 2H, NCH_2), 3.84 (m, 1H, 3-H), 4.06 (m, 2H, OCH_2), 6.90 (t, 0.15H, J = 5.0, CH=N *Z* isomer), 7.52 (d, 0.85H, J = 6.0, CH=N *E* isomer). MS *m*/*z* 392 (5%, M^+ base), 143 (100); mp 164−167 °C (decomp). Anal. ($C_{23}H_{40}N_2O_3 \cdot C_2H_2O_4$) C, H, N.

(*EZ*)-3β-Hydroxy-17β-[[2-(*N*,*N*-dimethylamino)ethoxy]-imino]methyl-14,15-seco-5β-androstan-14-one Oxalate (24d). Prepared in 34% yield from 3β-acetoxy-14-oxo-14,15-seco-5β-androstane-17β-carbaldehyde **9**[7] and 2-(dimethylamino)ethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate from Et_2O, white solid. ^{1}H NMR (CD_3OD) δ 0.90 (m, 3H, CH_2CH_3), 1.05 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 2.21 (m, 0.8H, 17-H *E* isomer), 2.65 (1H, m, 8-H), 2.94 (s, 3H, $N(CH_3)_2$), 3.21 (m, 0.2H, 17-H *Z* isomer), 3.45 (m, 2H, NCH_2), 4.02 (m, 1H, 3-H), 4.34 (m, 2H, OCH_2), 6.81 (d, 0.2H, J = 9.3, CH=N *Z* isomer), 7.51 (d, 0.8H, J = 8.7, CH=N *E* isomer). MS *m*/*z* 406 (4%, M^+ base), 58 (100); mp 65−67 °C. Anal. ($C_{24}H_{42}N_2O_3 \cdot C_2H_2O_4 \cdot 1.5H_2O$) C, H, N, H_2O.

(*EZ*)-3β-Hydroxy-17β-{2-[[2-(*N*,*N*-dimethylamino)ethoxy]imino]ethyl}-14,15-seco-5β-androstan-14-one Oxalate (24e). Prepared in 38% yield from 3β-acetoxy-17α-ethyl-14,15-seco-5β-androstane-14,15-dione **10**[7] and 2-(dimethylamino)ethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate from Et_2O, white solid. ^{1}H NMR (CD_3OD) δ 0.96 (m, 3H, CH_2CH_3), 1.03 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 2.35 (0.6H, m, 17-H *E* isomer), 2.63 (1H, m, 8-H), 2.95 (s, 3H, $N(CH_3)_2$), 3.48 (m, 2.4H, 17-H *Z* isomer + NCH_2), 4.02 (m, 1H, 3-H), 4.34 (m, 2H, OCH_2), 6.94 (t, 0.4H, J = 5.6, CH=N *Z* isomer), 7.57 (d, 0.6H, J = 6.8, CH=N *E* isomer). MS *m*/*z* 420 (10%, M^+ base), 315 (13), 171 (10), 58 (100); mp 90−93 °C. Anal. ($C_{25}H_{44}N_2O_3 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N, H_2O.

(*EZ*)-3β-Hydroxy-15-[*2-(N,N*-dimethylamino)ethoxy]-imino-17β-methyl-14,15-seco-5β-androstan-14-one Oxalate (24f). Prepared in 77% yield from aldehyde **11** and 2-dimethylaminoethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate from Et_2O, white solid. ^1H NMR (CD_3OD) δ 0.84 (d, 3H, J = 6.9, CHCH_3), 1.05 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 2.07 (m, 1H, 17-H), 2.36 (m, 1H, 16-H), 2.69 (1H, m, 8-H), 2.94 (s, 3H, $N(CH_3)_2$), 3.21 (m, 0.2H, 17-H *Z* isomer), 3.45 (m, 2H, NCH_2), 4.02 (m, 1H, 3-H), 4.32 (m, 2H, OCH_2), 6.88 (d, 0.2H, J = 5.6, CH=N *Z* isomer), 7.54 (dd, 0.8H, J = 5.0, 7.5, CH=N *E* isomer). MS *m/z* 406 (4%, M^+ base), 301 (6), 157 (2), 58 (100); mp 60−65 °C. Anal. ($C_{24}H_{42}N_2O_3\cdot C_2H_2O_4\cdot H_2O$) C, H, N, H_2O.

(*EZ*)-3β-Hydroxy-15-(2-aminoethoxy)imino-17β-methyl-14,15-seco-5β-androstan-14-one Oxalate (24g). Prepared in 59% yield from aldehyde **11** and 2-aminoethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate from Et_2O, white solid. ^1H NMR (CD_3OD) δ 0.84 (d, 3H, J = 6.9, CHCH_3), 1.05 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 2.06 (m, 1H, 17-H), 2.36 (m, 1H, 16-H), 2.68 (1H, m, 8-H), 3.21 (m, 2H, NCH_2), 4.02 (m, 1H, 3-H), 4.20 (m, 2H, OCH_2), 6.85 (d, 0.1H, J = 5.0, CH=N *Z* isomer), 7.53 (dd, 0.9H, J = 5.0, 7.1, CH=N *E* isomer). MS *m/z* 378 (20%, M^+ base), 301 (95), 129 (100); mp 177−178 °C (decomp). Anal. ($C_{22}H_{38}N_2O_3\cdot C_2H_2O_4$) C, H, N.

(*EZ*)-3β-Hydroxy-15-(2-aminoethoxy)imino-17β-propyl-14,15-seco-5β-androstan-14-one Oxalate (24h). Prepared in 75% yield from aldehyde **12** and 2-aminoethoxyamine dihydrochloride by the procedure described above for the preparation of **24b** but using an acidic procedure (1% HCl in EtOH, 20 h, room temperature) for the cleavage of the 3β-OTBS group. The product was then isolated as oxalate monohydrate from Et_2O, white solid. ^1H NMR (CD_3OD) δ 0.88 (t, 1.65H, CH_2CH_3 *E* isomer), 0.91 (t, 1.35H, CH_2CH_3 *Z* isomer), 1.04 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 3.22 (m, 2H, NCH_2), 4.02 (m, 1H, 3-H), 4.2 (m, 2H, NCH_2), 6.90 (t, 0.45H, J = 5.0, CH=N *Z* isomer), 7.57 (d, 0.55H, J = 6.8, CH=N *E* isomer). MS *m/z* 406 (1%, M^+ base), 329 (70), 157 (100); mp 153−157 °C. Anal. ($C_{24}H_{42}N_2O_3\cdot C_2H_2O_4\cdot H_2O$) C, H, N, H_2O.

(*EZ*)-3β-Hydroxy-17α-{2-[[2-(*N,N*-dimethylamino)ethoxy]imino]ethyl}-14,15-secoandrostan-14-one Oxalate (24i). Prepared in 70% yield from aldehyde **13** and 2-(dimethylamino)ethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate monohydrate from EtOAc, white solid. ^1H NMR (CD_3OD) δ 0.92 (s, 3H, CH_3), 0.93 (m, 3H, CH_2CH_3), 1.16 (s, 3H, CH_3), 2.62 (m, 1H, 8-H), 2.94 (s, 6H, $N(CH_3)_2$), 3.44 (m, 2H, NCH_2), 3.52 (m, 1H, 3-Hax), 4.31 (m, 1.6H, OCH_2 *E* isomer), 4.37 (m, 0.4H, OCH_2 *Z* isomer), 6.95 (t, 0.2H, J = 5.0, CH=N *Z* isomer), 7.60 (dd, 0.8H, J = 5.5, 7.1, CH=N *E* isomer). MS *m/z* 420 (2%, M^+ base), 315 (6), 58 (100); mp 160−163 °C. Anal. ($C_{25}H_{44}N_2O_3\cdot C_2H_2O_4\cdot 0.5H_2O$) C, H, N, H_2O.

(*EZ*)-3β-Hydroxy-17α-{2-[(2-aminoethoxy)imino]ethyl}-14,15-secoandrostan-14-one Oxalate (24j). Prepared in 80% yield from aldehyde **13** and 2-aminoethoxyamine dihydrochloride by the procedure described above for the preparation of **24b**. The product was then isolated as oxalate from EtOAc, white solid. ^1H NMR (CD_3OD) δ 0.91 (s, 3H, CH_3), 0.92 (t, 2.1H, CH_2CH_3 *E* isomer), 0.95 (t, 3H, CH_2CH_3 *Z* isomer), 1.15 (s, 3H, CH_3), 2.61 (m, 1H, 8-H), 3.22 (m, 2H, NCH_2), 3.52 (m, 1H, 3-Hax), 4.19 (m, 1.4H, OCH_2 *E* isomer), 4.25 (m, 0.6H, OCH_2 *Z* isomer), 6.91 (t, 0.3H, J = 5.0, CH=N *Z* isomer), 7.52 (dd, 0.7H, J = 5.6, 7.5, CH=N *E* isomer). MS *m/z* 359 (2), 315 (100), 143 (25); mp 108−110 °C. Anal. ($C_{23}H_{40}N_2O_3\cdot 1.5C_2H_2O_4$) C, H, N.

Biological Tests: Na^+,K^+-ATPase Binding. Binding affinity for dog kidney Na^+,K^+-ATPase receptor was determined in a competitive binding assay, employing [^{3}H]ouabain as displaced ligand. The IC_{50} values (concentration that inhibits ouabain binding by 50%) represent the means of values determined in two to three separate experiments in duplicate and were calculated by a nonlinear least-squares fitting algorithm.

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 (millimolar): 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 of 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 twice the threshold). After a 60 min equilibration period, cumulative concentrations of the compounds were added, each concentration being left in contact until the maximal response or arrhythmias were observed.

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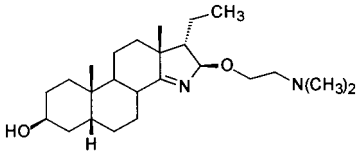

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