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Novel analogues of Istaroxime, a potent inhibitor of Na^+,K^+-ATPase: Synthesis, structure–activity relationship and 3D-quantitative structure–activity relationship of derivatives at position 6 on the androstane scaffold

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ABSTRACT

We report the synthesis and biological properties of novel analogues of Istaroxime acting as positive inotropic compounds through the inhibition of the Na^+,K^+-ATPase. We explored the chemical space around the position 6 of the steroidal scaffold by changing the functional groups at that position and maintaining a basic oximic chain in position 3. Some compounds showed inhibitory potencies of the Na^+,K^+-ATPase higher than Istaroxime and many of the compounds tested in vivo were safer than digoxin, the classic digitalis compound currently used for the treatment of congestive heart failure as inotropic agent. The 3D-QSAR analyses using comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) methods have been successfully applied to a set of 63 androstane derivatives as Na^+,K^+-ATPase inhibitors. The contour plots provide many useful insights into relationships between structural features and inhibitory potency.

1. Introduction

Heart failure is the pathophysiological state in which the pumping action of the heart is impaired, with a loss of cardiac contractile function (cardiac dysfunction), to varying degrees whereby the delivery of blood (cardiac output) becomes inadequate to satisfy the oxygen and nutritional needs of the body. Decreased cardiac output leads to excessive fluid accumulation in the body (congestion); the result is congestive heart failure (CHF). Fatigue, breathlessness, and peripheral edema are the symptoms in patients with this syndrome and severely limit their normal activities. Therapy of CHF is aimed at improving the pumping performance of the heart and reducing cardiac load by reducing vasoconstriction. According to treatment guidelines and depending on the stage of the illness, different classes of drugs are available to limit and contrast the decline of cardiac pump efficiency: angiotensin-converting enzyme inhibitors; angiotensin II receptor blockers; inotropic agents; diuretics; beta-blockers.[1]

The digitalis cardiac glycoside digoxin (Chart 1) is the only inotropic drug currently prescribed for chronic treatment of CHF. Cardiac glycosides improve the pumping performance of the heart via inhibition of the Na^+,K^+-ATPase, an ubiquitous membrane protein that promotes the outward transport of Na^+ and the inward transport of K^+ against their concentration gradients. Na^+,K^+-ATPase inhibition increases intracellular Na^+, which is then extruded by the Na^+,Ca^{2+} exchanger, leading to an increase of intracellular Ca^{2+} levels. The availability of higher Ca^{2+} concentrations increases cardiac myocytes contractile force (positive inotropic effect).[2] Despite its proven efficacy,[3] a risk related to digoxin therapy is the proarrhythmogenicity which emerges at a two to three times higher serum concentration than the therapeutic with disturbances of conduction and cardiac arrhythmias.[4]

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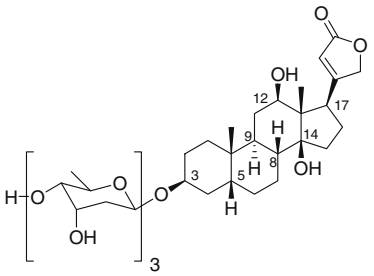
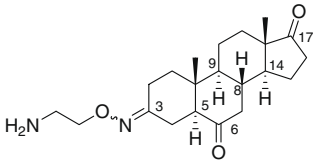


Digoxin



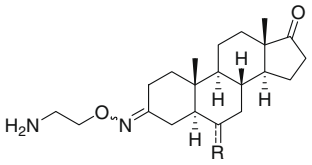
Istaroxime

Chart 1. Structures of digoxin and Istaroxime.

In one of our past works on digitalis-like compounds, the bent digitalis skeleton was replaced by the flat 5α,14α-androstane scaffold properly substituted in position 3 with an aminoalkyloxime and in position 6 with hydroxy or oxo groups.[5] Istaroxime (referred to as PST 2744; Chart 1), emerged as a very promising positive inotropic compound which is now in phase II clinical trials for the treatment of CHF.[6,7]

In the previous paper, the androstane skeleton was used as a scaffold to study the space around the basic chain in position 3 of our lead compound, Istaroxime; some compounds demonstrated higher potencies than Istaroxime on the receptor and all tested compounds resulted less proarrhythmogenic than digoxin.[8] In this paper, we describe new analogues of Istaroxime in which the effects of substitution at position 6 of the androstane scaffold on the activity at the Na^+,K^+-ATPase were studied. We performed a wide differentiation of the carbonyl group present at position 6 of Istaroxime, with the purpose of obtaining compounds with a higher potency and better therapeutic index than those of the parent compound.

Table 1
Structure and Na^+,K^+-ATPase inhibition for compounds bearing the 2-aminoethoxyimino chain (**1**–**33**)



Compounds	R	Na^+,K^+-ATPase inhibition, IC_{50}[a] (μM)
Digoxin		0.31
Istaroxime	Oxo	0.11
Compound **A**	α-OH	0.52
Compound **B**	β-OH	2.1
1	$=CH_2$	0.042
2	$=CF_2$	0.19
3	(*E*) =CHMe	1.1
4	Spirocyclopropane	0.85
5	β-Me	6.0
6	α-Me	0.30
7	α-$CH=CH_2$	0.53
8	α-C≡CH	0.20
9	(*E*) =NOH	0.024
10	(*E*) =NOMe	0.017
11	(*E*) =NOEt	1.7
12	(*E*) =NOAllyl	43
13	(*E*) =CHCN	1.7
14	(*E*) =CHCOOMe	64
15	(*E*) $=CHCH_2OH$	0.77
16	α-CH_2CH_2OH	0.32
17	α-CH_2OH	0.18
18	β-CH_2OH	1.6
19	α-CH_2OMe	0.50
20	β-CH_2OMe	3.0
21	*R*-Spirooxirane	0.91
22	*S*-Spirooxirane	0.87
23	α-OMe	0.71
24	β-OMe	1.5
25	α-OEt	3.1
26	α-ONO_2	0.33
27	β-ONO_2	2.9
28	α-COOH	16
29	α-CN	1.1
30	α-COOMe	0.41
31	α-$CONH_2$	0.63
32	α-NH–CHO	0.16
33	α-NH–$COCH_3$	45

[a] Concentrations able to inhibit 50% of Na^+,K^+-ATPase enzyme activity; mean of two or three experiments.

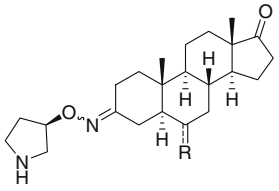
2. Chemistry

The oximes **1**–**58** listed in Tables 1–3, were synthesized from the appropriate 3,17-diketones and the corresponding *O*-substituted hydroxylamine dihydrochlorides in a THF/water solution at room temperature as mixture of *E* and *Z* isomers, in an approximate 1:1 ratio (Scheme 1, see Section 5).

The required starting 3,17-diketones of Scheme 1 were obtained as described in Schemes 2–7. As shown in Scheme 2, 6-methyleneandrostane-3,17-dione **61** was obtained by reaction of 3,3:17,17-bis(ethylendioxy)androstane-6-one[5] **59** with methyltriphenylphosphonium bromide in THF in the presence of potassium *tert*-butoxide followed by deprotection of the ethylene ketals groups of **60** with PTSA in acetone at room temperature, in 84% overall yield. From **61**, the epimeric mixture of 6-spiro-(2′-oxirane) derivatives was obtained by reaction with MCPBA in CH_2Cl_2 at 0 °C; a rather difficult separation by flash chromatography gave the pure (6*S*)-6-spiro-(2′-oxirane)androstane-3,17-dione **62** (30% yield) and (6*R*)-6-spiro-(2′-oxirane)androstane-3,17-dione **63** (15%). 6β-Hydroxymethylandrostane-3,17-dione **65** was obtained with a very good stereoselectivity from **60** by reaction with 1 M BH_3·THF complex in THF at 0 °C followed by addition of water, NaOH and H_2O_2 (95% yield) and subsequent acidic deprotection of the ethylene ketals of **64** (85% yield). Etherification of the alcohol **64** with NaH and CH_3I in THF at 0 °C followed by acidic deprotection of the bis(ethylendioxy) derivative **66** gave 6β-methoxymethylandrostane-3,17-dione **67** (76% yield). 6-(Spirocyclopropane)androstane-3,17-dione **68** was obtained in 48% yield from 6-methylene derivative **60** by reaction with CH_2I_2 in the presence of Et_2Zn in toluene at 60 °C followed by acidic deprotection of the intermediate bis(ethylendioxy) derivative. Even though a small amount of 6β-methyl derivative was present in the hydroboration reaction of **60**, 6β-methylandrostane-3,17-dione **71** was more easily obtained by radical deoxygenation of the imidazole-1-carbothioate derivative **69** (obtained from **64** by reaction with TCDI in CH_2Cl_2 at 40 °C in the presence of DMAP in 83% yield) with Ph_3SnH and AIBN in toluene (42% yield) and following acidic deprotection of the ethylene ketals of **70**.

The compounds obtained from the key intermediate 6α-carboxaldehyde **73** are shown in Scheme 3. The aldehyde **73** was obtained

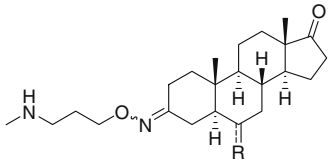
Table 2
Structure and Na^+,K^+-ATPase inhibition for compounds bearing the 3-[(3*R*)-3-pyrrolidinyloxyimino] chain (**34**–**47**)



Compound	R	Na^+,K^+-ATPase inhibition, IC_{50}[a] (μM)
Compound **C**	Oxo	0.026
34	$=CH_2$	0.046
35	$=CF_2$	0.021
36	Spirocyclopropane	0.22
37	α-Me	0.34
38	α-C≡CH	0.11
39	(*E*) =NOH	0.016
40	(*E*) =NOMe	0.018
41	α-CH_2CH_2OH	0.058
42	α-CH_2OH	0.026
43	α-CH_2OMe	0.32
44	α-ONO_2	0.38
45	α-NH–CHO	0.041
46	α-$CONH_2$	0.14
47	α-COOMe	0.16

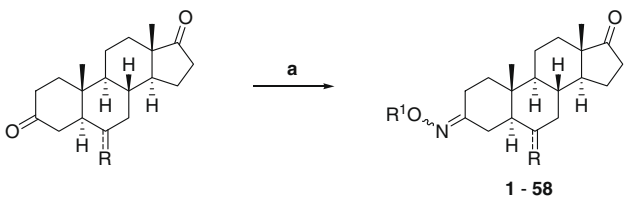
[a] Concentrations able to inhibit 50% of Na^+,K^+-ATPase enzyme activity; mean of two or three experiments.

Table 3
Structure and Na^+,K^+-ATPase inhibition for compounds bearing the 3-*N*-methylaminopropoxyimino chain (**48**–**58**)



Compounds	R	Na^+,K^+-ATPase inhibition, IC_{50}[a] (μM)
Compound **D**	Oxo	0.25
48	$=CH_2$	0.57
49	Spirocyclopropane	1.3
50	α-Me	0.91
51	α-C≡CH	1.2
52	(*E*) =NOH	0.20
53	(*E*) =NOMe	0.33
54	α-CH_2OH	0.69
55	α-CH_2OMe	2.0
56	α-NH–CHO	1.1
57	α-$CONH_2$	1.9
58	α-COOMe	4.0

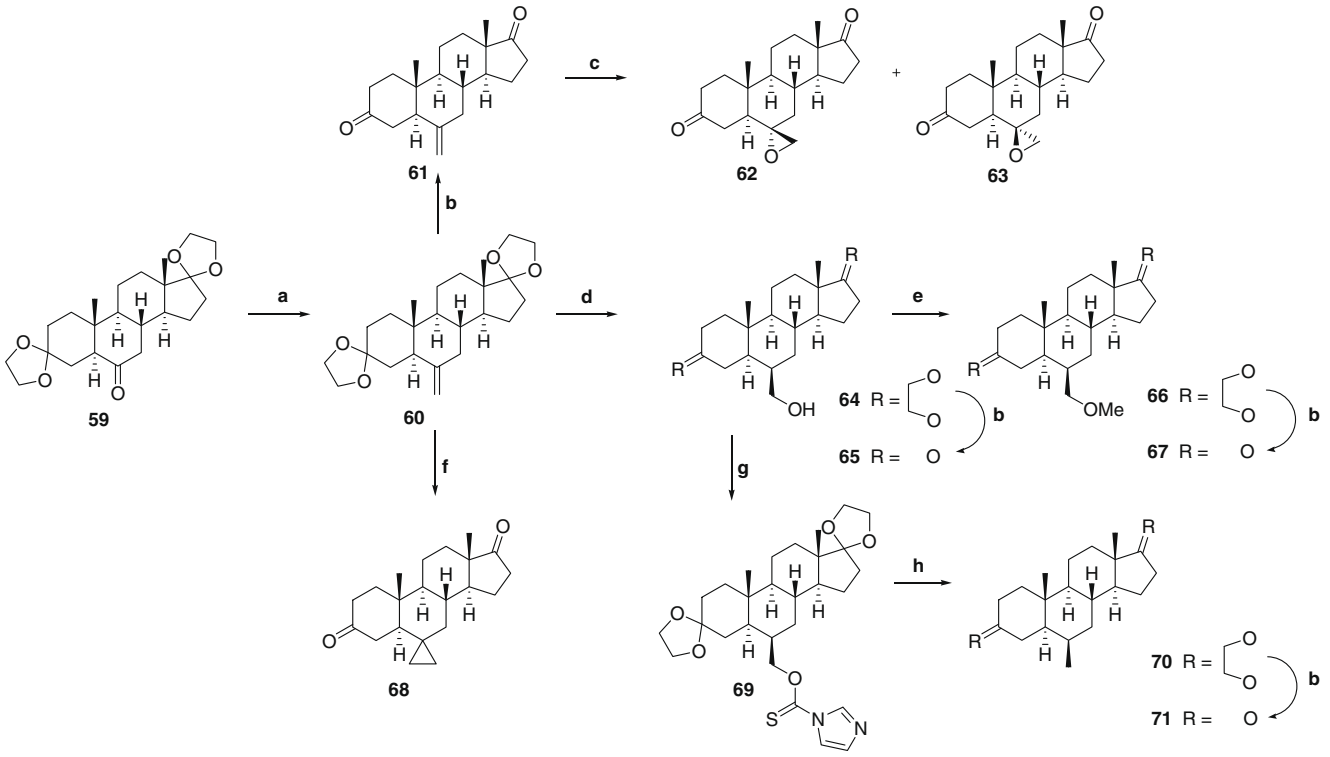
[a] Concentrations able to inhibit 50% of Na^+,K^+-ATPase enzyme activity; mean of two or three experiments.



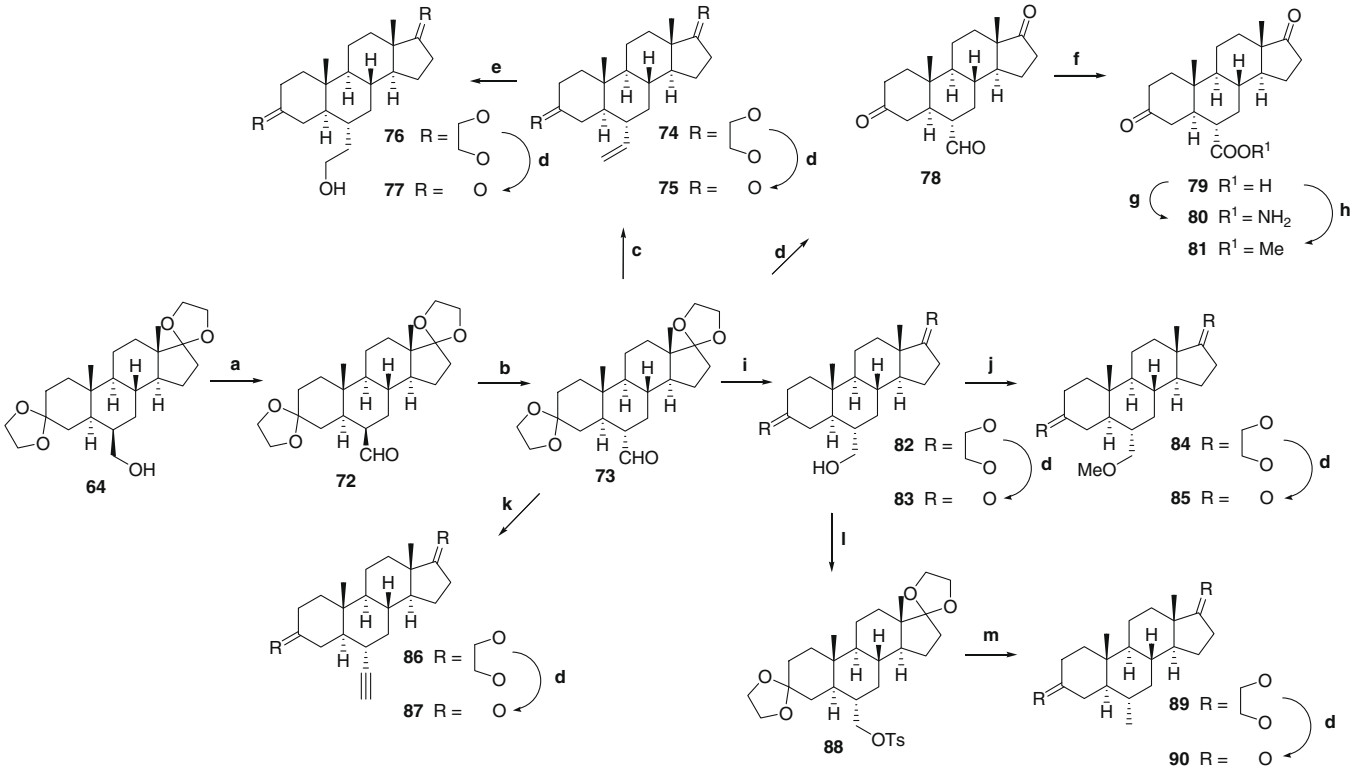
Scheme 1. Reagents and condition: (a) hydroxylamines, THF/water, room temperature.

with an efficient method starting from the known 6-keto derivative **59** (shown in Scheme 2) in 72% overall yield over four steps. Oxidation of the 6β-hydroxymethylandrostane **64** with IBX[9] in DMSO at room temperature gave the 6β-formylandrostane **72** in 83% yield; isomerization of the axial formyl group of **72** with K_2CO_3 in MeOH at room temperature yielded the equatorial epimeric aldehyde **73** in 94% yield. 6α-Vinylandrostane-3,17-dione **75** was obtained in 63% yield by reaction of the aldehyde **73** with methyltriphenylphosphonium bromide in THF at 0 °C in the presence of potassium *tert*-butoxide, to give **74**, followed by acidic deprotection of the bis(ethylendioxy) derivative. 6α-(2-Hydroxyethyl)androstane-3,17-dione **77** was prepared from bis(ethylendioxy)-6α-vinyl derivative **74** in 96% yield by oxidative hydroboration followed by acidic deprotection of the ethylene ketals **76**. Acidic deprotection of 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** gave **78** in 85% yield**;** 6α-carboxyandrostane-3,17-dione **79** was obtained by oxidation of the aldehyde group of **78** with 1 N aqueous $KMnO_4$ in *tert*-butanol and 5% aqueous Na_2HPO_4 solution in 96% yield. 6α-Carbamoylandrostane-3,17-dione **80** and 6α-methoxycarbonylandrostane-3,17-dione **81** were obtained from the acid **79**, by reaction with $SOCl_2$ in toluene at 85 °C, followed by treatment of the intermediate with 2 M NH_3 solution in THF at 0 °C, (60% yield) or by reaction with DMAP, EDAC and methanol in CH_2Cl_2 at 0 °C (70% yield), respectively. 6α-Hydroxymethylandrostane-3,17-dione **83** was obtained by reduction of the aldehyde **73** with $NaBH_4$ followed by acidic deprotection of the ethylene ketals (73% yield). The intermediate **82** was reacted with NaH in THF at 0 °C and then with CH_3I to give, after deprotection, 6α-methoxymethylandrostane-3,17-dione **85** (74% yield). The ethynyl derivative **87** was prepared from 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** by reaction with (chloromethyl)triphenylphosphonium chloride in THF and *n*-butyllithium at −78 °C followed by heating at 70 °C, and reaction of the crude product thus obtained with *n*-butyllithium in THF at −78 °C again; final deprotection gave 6α-ethynylandrostane-3,17-dione **87** in 22% overall yield. Finally, 6α-methylandrostane-3,17-dione **90** was obtained in 68% yield by the following sequence: reaction of the 6α-hydroxymethyl derivative **82** with *p*TSCl in the presence of DABCO in CH_2Cl_2 at 0 °C to give the corresponding 4-methylbenzenesulfonate **88**; reduction of the sulfonate with $NaBH_4$ in DMSO at 80 °C to the 6α-methylandrostane derivative **89** and final deprotection of the ethylene ketals.

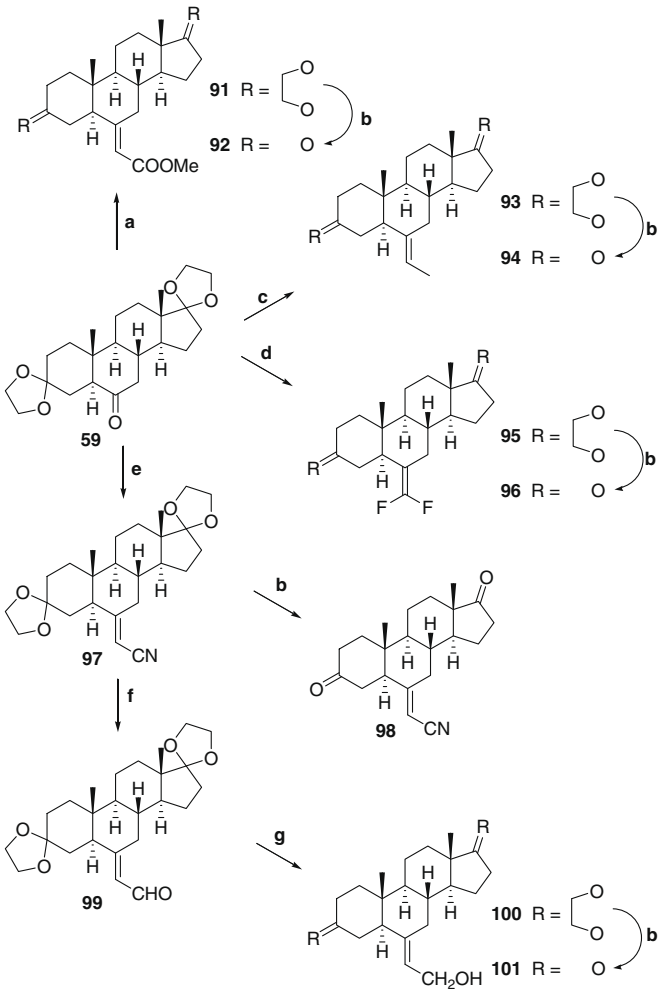
In Scheme 4, the preparations of the exomethylene derivatives are shown starting from the common intermediate 3,3:17,17-bis(ethylendioxy)androstane-6-one **59**.[5] Methyl {3,17-dioxoandrostane-6-[(*E*)-ylidene]}acetate **92** was obtained by reaction of **59** with trimethylphosphonoacetate in DME in the presence of *tert*-butyllithium and heating at 110 °C to give the unsaturated ester **91** in 35% yield, followed by deprotection of the latter compound with PTSA in acetone in 70% yield. 6-[(*E*)-Ethyliden]androstane-3,17-dione **94** was obtained in 92% yield by reaction of **59** with (ethyl)triphenylphosphonium bromide in THF at 0 °C in the presence of potassium *tert*-butoxide followed by deprotection of the ethylene ketals of **93**. 6-Difluoromethyleneandrostane-3,17-dione **96** was obtained in 85% yield by reaction of **59** and the anion generated from diethyl difluoromethylenephosphonate and *tert*-butyllithium in DME at −78 °C; the intermediate 3,3:17,17-bis(ethylendioxy) derivative **95** was finally deprotected. 3,17-Dioxoandrostane-6-[(*E*)-ylidene]acetonitrile **98** was obtained by reaction of **59** and diethyl cyanomethylphosphonate in the presence of NaH (60% dispersion in mineral oil) in THF at room temperature to give **97** (71% yield) that in turn was deprotected to afford **98** in 62% overall yield. The nitrile **97** was transformed into the corresponding unsaturated aldehyde **99** in 55% yield by reaction with 1 M DIBAH in CH_2Cl_2 at −78 °C and following hydrolysis in the presence of silicagel; the aldehyde **99** was reduced with $NaBH_4$



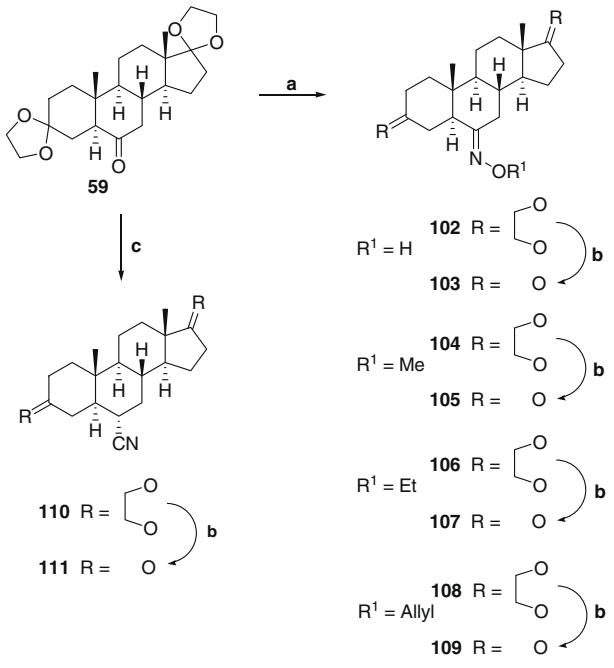
Scheme 2. Reagents and conditions: (a) methyltriphenylphosphonium bromide, potassium *tert*-butoxide, THF, room temperature; (b) PTSA, acetone, room temperature; (c) MCPBA, CH_2Cl_2, 0 °C; (d) BH_3·THF complex, THF, 0 °C, then NaOH, H_2O_2, water, room temperature; (e) NaH, CH_3I, THF, 0 °C; (f) CH_2I_2, Et_2Zn, toluene, 60 °C; (g) TCDI, DMAP, CH_2Cl_2, 40 °C; (h) Ph_3SnH, AIBN, toluene, 110 °C.



Scheme 3. Reagents and conditions: (a) IBX, DMSO, room temperature; (b) K_2CO_3, MeOH, room temperature; (c) methyltriphenylphosphonium bromide, potassium *tert*-butoxide, THF, 0 °C; (d) PTSA, acetone, room temperature; (e) BH_3·THF complex, THF, 0 °C, then NaOH, H_2O_2, water, room temperature; (f) 1 N aqueous $KMnO_4$, *tert*-butanol, 5% aqueous Na_2HPO_4, room temperature; (g) $SOCl_2$, toluene, 85 °C, then 2 M NH_3 THF solution, 0 °C; (h) DMAP, EDAC, MeOH, CH_2Cl_2, 0 °C; (i) $NaBH_4$, dioxane/H_2O 9/1, room temperature; (j) NaH, CH_3I, THF, 0 °C; (k) (chloromethyl)triphenylphosphonium chloride, *n*-butyllithium, THF, −78 °C, then heating at 70 °C, then *n*-butyllithium, −78 °C; (l) *p*TSCl, DABCO, CH_2Cl_2, 0 °C; (m) $NaBH_4$, DMSO, 80 °C.



Scheme 4. Reagents and conditions: (a) trimethylphosphonoacetate, *tert*-butyllithium, DME, 110 °C; (b) PTSA, acetone, room temperature; (c) (ethyl)triphenylphosphonium bromide, potassium *tert*-butoxide, THF, 0 °C; (d) diethyl difluoromethylenephosphonate, *tert*-butyllithium, DME, −78 °C; (e) diethyl cyanomethylphosphonate, NaH (60% dispersion in mineral oil), THF, room temperature; (f) 1 M DIBAH, CH_2Cl_2, −78 °C, then water, THF, silicagel, room temperature; (g) $NaBH_4$, MeOH, 0 °C.



Scheme 5. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$ or the appropriate *O*-alkyl hydroxylamine hydrochloride, Na_2HPO_4, THF/water, room temperature; (b) PTSA, acetone, room temperature; (c) toluene-4-sulfonylmethyl isocyanide, potassium *tert*-butoxide, DMSO, room temperature.

in MeOH at 0 °C to give the allyl alcohol **100** (90% yield); final deprotection gave **101** in 60% yield.

In Scheme 5, and 6-[(*E*)-hydroxyimino]androstane-3,17-dione **103** was obtained by reaction of 3,3:17,17-bis(ethylendioxy)androstane-6-one **59**[4] and $NH_2OH{\cdot}HCl$ in the presence of Na_2HPO_4 in THF/water at room temperature to give the (*E*)-oxime **102** (93% yield) that was deprotected with PTSA in acetone at room temperature (70% yield). In the same manner by using the appropriate *O*-alkyl hydroxylamine hydrochloride, 6(*E*)-methoxyiminoandrostane-3,17-dione **105** (68%, two steps yield), 6-[(*E*)-ethoxyimino]androstane-3,17-dione **107** (90%, two steps yield), and 6-[(*E*)-allyloxyimino]androstane-3,17-dione **109** (64%, two steps yield), were obtained. 6α-Cyanoandrostane-3,17-dione **111** was obtained by reaction of the 6-oxo derivative **59** and toluene-4-sulfonylmethyl isocyanide in the presence of potassium *tert*-butoxide in DMSO at room temperature to give 6α-cyano-3,3:17,17-bis(ethylendioxy)androstane **110** (31% yield) that in turn was deprotected (75% yield).

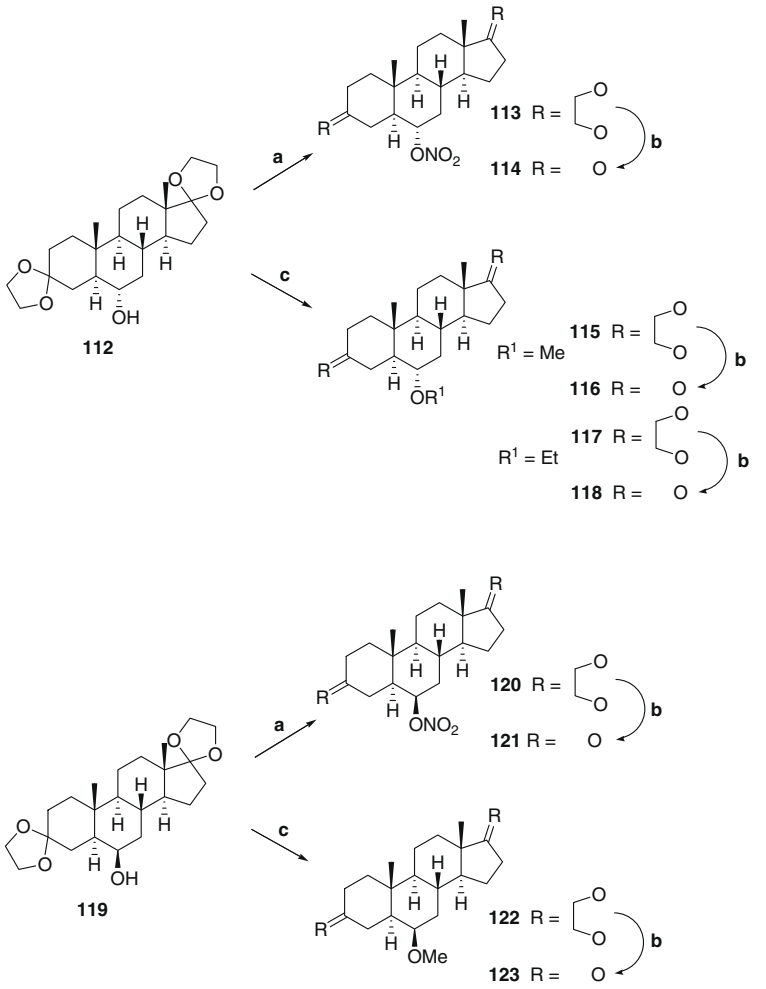
In Scheme 6, 3,17-dioxoandrostane-6α-yl nitrate **114** was obtained by esterification of 3,3:17,17-bis(ethylendioxy)androstane-6α-ol[5] **112** with 65% HNO_3 in acetic anhydride at 0 °C to give the nitrate **113** in 89% yield, which, after deprotection of the ethylene ketals, gave **114** in 75% yield; analogously, 3,17-dioxoandrostane-6β-yl nitrate **121** was obtained starting from 3,3:17,17-bis(ethylendioxy)androstane-6β-ol[5] **119**. 6α-Methoxy derivative **116** was obtained from 3,3:17,17-bis(ethylendioxy)androstane-6α-ol[5] **112** by reaction with CH_3I in the presence of KH in THF at 0 °C (79% yield) followed by deprotection; the 6β-methoxy derivative **123** was prepared from the epimeric 6β-alcohol **119** by reaction with CH_3I in the presence of NaH in THF at 0 °C in a lower yield (52%), while the 6α-ethoxy derivative **118** was obtained from **112** by reaction with EtI and NaH in THF at reflux (49% yield); the deprotection of the ethylene ketals gave yields in the 84–95% range.

The preparations of the amides **126** and **128** are shown in Scheme 7. The key intermediate 3,3:17,17-bis(ethylendioxy)-6α-aminoandrostane **124** was obtained by reduction of the corresponding 6-(*E*)-hydroxyiminoandrostane **102** with sodium in *n*-propanol at reflux in 53% yield. 6α-Formamidoandrostane-3,17-dione **126** was obtained in 91% yield by reaction of **124** with formic acid in $CHCl_3$ and pyridine in the presence of DCC at 0 °C to give **125** followed by deprotection with PTSA in acetone. 6α-Acetamidoandrostane-3,17-dione **128** was obtained in 90% yield by reaction of **124** with acetic anhydride in pyridine to give **127** which was then deprotected.

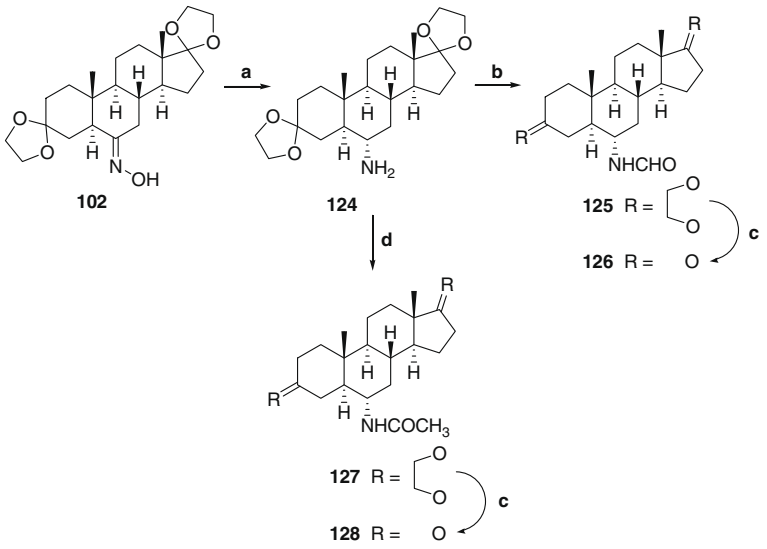
3. Results and discussion

All target compounds were tested in vitro for their inhibitory activity on purified dog kidney Na^+,K^+-ATPase, as measured by the ^{32}P-ATP hydrolysis method (see data in Tables 1–3).[10,11] Some compounds, showing high inhibitory potency in vitro, were investigated in vivo for their inotropic activity and lethal effect by slow intravenous infusion in anesthetized guinea pigs (results reported in Table 4). Digoxin was chosen as reference compound because it is the most commonly prescribed cardiac glycoside in the treatment of CHF; Istaroxime is the lead compound in this series of androstane derivatives and compounds **A**,[5] **B**,[5] **C**,[8] and **D**[8] are reported here for comparison.

The following structure–activity relationships (SARs) are based on the in vitro data shown in Tables 1–3. For clarity of discussion,



Scheme 6. Reagents and conditions: (a) 65% HNO_3, acetic anhydride, 0 °C; (b) PTSA, acetone, room temperature; (c) CH_3I, NaH, THF, 0 °C or EtI, NaH, THF, reflux.



Scheme 7. Reagents and conditions: (a) sodium, *n*-propanol, reflux; (b) formic acid, DCC, $CHCl_3$ and pyridine, 0 °C; (c) PTSA, acetone, room temperature; (d) acetic anhydride, pyridine, room temperature.

the different substitutions at position 6 will be treated considering the three aminoalkyl oxime chains at position 3 separately. The choice of the three different amines was based on their potencies and different structures, as reported in a recent study about the systematic variation of the oximic chain present in Istaroxime.[8] The 2-aminoethyl oxime was chosen as reference chain since it is present in Istaroxime and as example of primary amine with high potency. The (3*R*)-3-pyrrolidinyloxyimino chain was used because

Table 4
Inotropic and toxic effects in anesthetized guinea pig

Compound	E_{max}[a]% increase in dP/dt_{max}	ED_{max}[b] (μmol/kg)	ED_{80}[c] (μmol/kg)	Deads/ treated	Lethal dose/ED_{80}
Digoxin	127	0.97	0.41	10/10	3.2
Istaroxime	182	5.7	1.8	7/8	26
6	203	5.8	3.1	0/3	—
8	163	9.9	4.6	0/3	—
9	180	4.3	0.84	2/5	11
10	183	5.8	2.4	0/5	—
16	238	17	2.5	2/3	23
17	166	7.4	1.8	0/3	—
23	167	18	4.3	1/5	9.0
26	172	9.8	3.6	1/5	8.0
32	174	27	2.0	1/5	33
35	244	18	6.7	0/3	—
38	147	6.0	3.7	0/3	—
39	144	4.3	1.5	0/3	—
41	183	5.1	1.9	0/3	—
43	201	12	6.8	0/3	—
45	142	6.0	1.6	2/5	11
46	109	8.8	2.2	0/4	—
47	164	23	11	0/4	—
50	213	18	8.1	0/3	—
52	158	15	7.1	0/3	—
54	263	9.0	1.5	2/2	28
56	177	2.8	0.93	2/2	47

[a] Maximal increase in dP/dt_{max}.
[b] Dose inducing maximum positive inotropic effect.
[c] Inotropic potency: dose increasing $+dP/dt_{max}$ by 80%, calculated from dose–response curves.

some cyclic amines on the oximic group at position 3 gave compounds with a higher inhibitory potency than the linear amine analogues (in particular (*E*,*Z*)-3-[(3*R*)-3-pyrrolidinyloxyimino]androstane-6,17-dione, compound **C**, was found to be about four times more potent than Istaroxime) and as example of a secondary cyclic amine. To complete the series we chose a secondary linear amine chain on the oximic group in position 3, namely the 3-*N*-methylaminopropoxyimino; compound **D** that carries this chain is reported with potency about half the value of Istaroxime.

We demonstrated in the preceding papers[5,8] that the substituent at position 6 in Istaroxime plays an important role in the interaction with the receptor; referring to the model in which the rotated *E* isomer of Istaroxime is superimposed on cassaine[12] and digitoxigenin (Fig. 1a and 1b), the keto group in that position corresponds to the keto group at position 7 in cassaine and to the 14β-hydroxy in digitoxigenin. We have also shown that the reduction of the 6-keto group in Istaroxime (IC_{50}, 0.11 μM) brings to a lower potency and that the decrease is more pronounced for the 6β-hydroxy group (compound **B**, 2.1 μM) than for the 6α-hydroxy (compound **A**, 0.52 μM); an explanation could be that a 6α-hydroxy group places the OH in the same spatial position of the 14β-hydroxy in digitoxigenin (Fig. 1d), while the β-hydroxy places the OH in a spatial position very close to the nonpolar methylene 15 of digitoxigenin (Fig. 1e). Therefore, it seems that a polar 6-keto or a 6α-hydroxy substituent are of great importance in Istaroxime while we can assume that there is a space around the 6β position probably available for nonpolar substituents; the latter space in digitoxigenin is occupied by the 15 and 16 methylene groups and

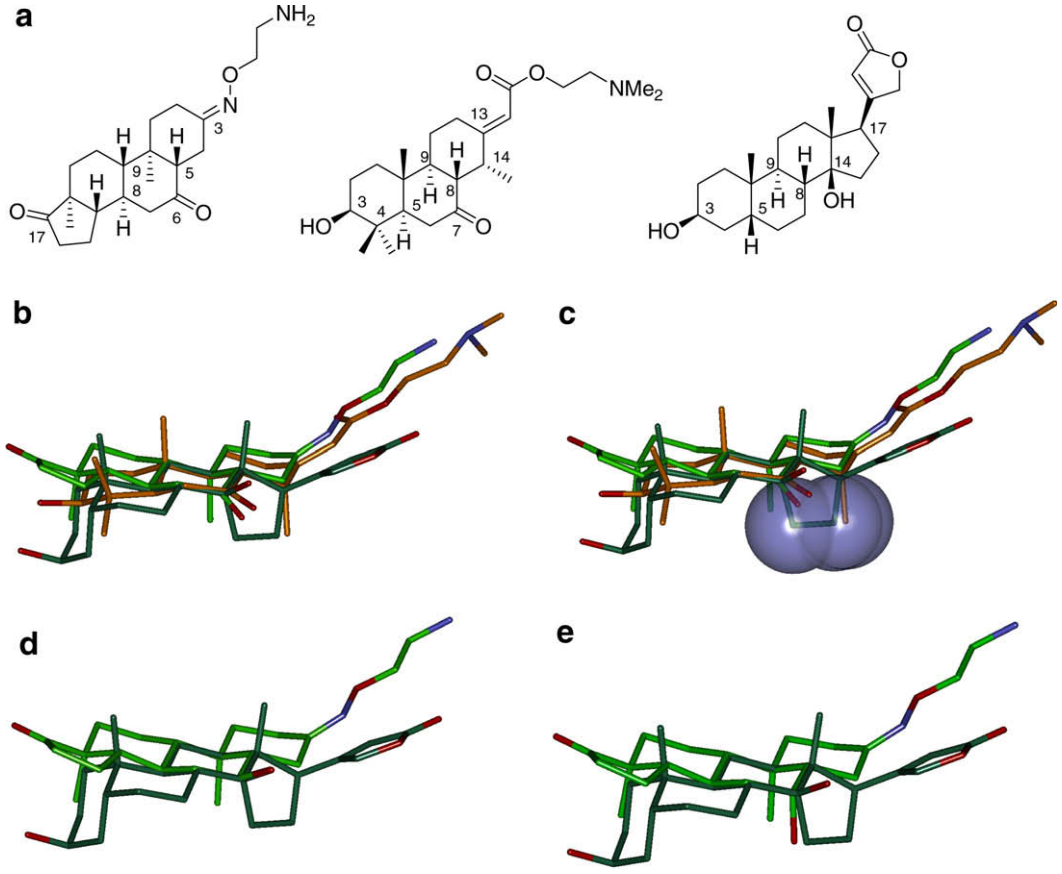


Figure 1. (a) Structures of rotated *E* isomer of Istaroxime, cassaine and digitoxigenin. (b) Superposition of rotated *E* isomer of Istaroxime (light green), cassaine (orange) and digitoxigenin (dark green). (c) Superposition of rotated *E* isomer of Istaroxime, cassaine and digitoxigenin with the VDW volumes corresponding to C15 and C16 of digitoxigenin and C14 of cassaine. (d) Superposition of rotated *E* isomer of compound **A** (light green) and digitoxigenin (dark green). (e) Superposition of rotated *E* isomer of compound **B** (light green) and digitoxigenin (dark green).

in cassaine by the 14α-methyl (see Fig. 1c, in which the cited nonpolar groups are surrounded by VDW surface).

To explore the two hypotheses we synthesized derivatives with nonpolar and polar substituents at position 6.

3.1. 3-(2-Aminoethoxyimino) derivatives with nonpolar substituents at position 6

The change of the carbonyl group with an exomethylene increased the inhibitory potency on the pump (**1**, 0.042 μM vs 0.11 μM for Istaroxime); a difluoroexomethylene (**2**, 0.19 μM) slightly reduced the activity while the *E*-ethyliden substituent sharply reduced it (**3**, 1.1 μM) as well as the β-methyl (**5**, 6.0 μM) and, to a lesser extent, the spirocyclopropane (**4**, 0.85 μM). α-Vinyl (**7**, 0.53 μM) and α-methyl (**6**, 0.30 μM) showed higher potencies than the β-methyl and the α-ethynyl group brought up the activity at a level similar to that of Istaroxime (**8**, 0.20 μM). An exomethylene group has some similarities with a ketone, both in term of shape and in term of electronic distribution; an important difference is that there is not the possibility to act as a hydrogen-bond acceptor. In the superposition of the exomethylene derivative **1** and Istaroxime, the new $=CH_2$ group could assume the same position of the 6-ketone, but it shows a better interaction with the receptor notwithstanding its apolarity in comparison with the ketone. This fact could be explained by a slight shift of the position of the molecule in the receptor (e.g., by rotating around the major axis of the plane that contains the androstane scaffold; Fig. 2), to better accommodate the nonpolar substituent in the available nonpolar space described above without losing the other favorable interactions, the most important being the one due to the basic chain. The difluoroexomethylene derivative **2** is similar to **1**. The reduced potencies of 6β-methyl, 6α-methyl and the spirocyclopropane derivative (that can be seen as a 'merge' of the latter two) seem to point toward the importance of the presence of an unsaturated bond. The same feature is present also in **3**, but the extra methyl group on the double bond is oriented in the opposite direction with respect to the proposed nonpolar favorable region; this fact could explain its 25-fold reduced activity compared to that of **1**. The other two compounds carry a double (**7**) or a triple (**8**) bond roughly on the same plane that contains the androstane scaffold; as the more straight alkynyl group gave a higher inhibitory activity, we can suppose that this zone of the receptor is very selective in respect to the position of the substituent; again, as for **1**, the molecule could rotate on the major axis of the androstane's plane, thus permitting the alkynyl group to reach the nonpolar zone occupied by the D ring of digitoxigenin.

3.2. 3-(2-Aminoethoxyimino) derivatives with polar substituents at position 6

The oxime group at position 6 showed a very interesting increment of inhibitory activity (**9**, 0.024 μM); the double bond C=O was substituted with a similar C=N, both in term of geometry and electronic distribution; of course the new hydroxyl group points in a well defined direction (the new oxime is in the *E* form). This portion of space in the binding site on the receptor could accommodate a polar group such as the OH, while the presence of a methyl group in **3** negatively impacted on activity (1.1 μM). The introduction of a methyl (**10**, 0.017 μM), ethyl (**11**, 1.7 μM), and allyl (**12**, 43 μM) on the oxime group gave the following information: a methyl group is well tolerated as the potency is almost the same as that of the unsubstituted hydroxyl; hence there is no necessity of a donating hydrogen-bond group, but a receiving one could be important; the available space fits for a methyl while an ethyl group and, especially, an allyl group are too bulky (for the latter the decrease in activity is more than 1700 times when compared with **9**). Maintaining a double bond in the substituent, the introduction of a yliden-(*E*)-acetonitrile (**13**, 1.7 μM) reduced 70 times the potency in comparison with **9**; a very low activity was obtained with the introduction of a yliden-(*E*)-methylacetate (**14**, 64 μM); a slightly higher activity than that of **13** was shown by the 2-hydroxy-(*E*)-ethyliden derivative (**15**, 0.77 μM). These results indicate that a polarized double bond in position 6 is important and the space around the chain is rather limited as the severe fall of potency of **12** and **14** demonstrates.

Continuing our exploration of the space around the position 6, we prepared the saturated hydroxy derivatives. The 6α-hydroxymethyl derivative **17**, (0.18 μM) showed the highest inhibitory potency in this close series; its homologous 6α-(2-hydroxyethyl) derivative (**16**, 0.32 μM) was slightly more potent than the simple 6α-hydroxy (compound **A**, 0.52 μM). All these hydroxy derivatives are more potent than the allylic alcohol **15** (0.77 μM); it seems as though the saturated chains could better fit the space occupied by the 14β-hydroxy in digitoxigenin. The 6α-methoxymethyl derivative **19** (0.50 μM) decreased the activity: this could be due to the increasing space requirement or to the impossibility to act as a hydrogen-bond donor (moreover, also 14β-OMe digitoxigenin showed a binding affinity for the Na^+,K^+-ATPase receptor about 90 times lower than digitoxigenin itself).[13] The 6β-epimer of **17** (**18**, 1.6 μM) confirms the diminished potency already reported for a polar group in the β-face of the androstane scaffold; its *O*-methyl ether (**20**, 3.0 μM) further reduced the activity. A similar decrease in potency was observed for the 6α-methoxy derivative **23** (0.71 μM) in comparison to its corresponding hydroxy derivative compound **A**; a bulkier ethyl ether (**25**, 3.1 μM) was more detrimental. The formal cyclization of the two hydroxymethyl derivatives to give the corresponding epimeric spirooxiranes gave practically the same potency (**21**, 0.91 μM and **22**, 0.87 μM); there is no difference in the α- or β-face for the two diastereoisomeric oxiranes, probably because the strained ring reduces the α and β-characteristic of the oxygen; the electronic effect of the oxygen is negligible since the spirocyclopropane analogue **4** showed a comparable potency (0.85 μM). A more favorable effect of a polar group in the α-face was confirmed by the two epimeric nitrosyl compounds, 6α-ONO_2 (**26**, 0.33) and 6β-ONO_2 (**27**, 2.9 μM).

The introduction of a 6α-carboxylic group gave a very low inhibitory potency (**28**, 16 μM); the potency increased from the corresponding 6α-cyano derivative (**29**, 1.1 μM) to the 6α-methoxycarbonyl analogue (**30**, 0.41 μM) and 6α-carboxamide (**31**, 0.63 μM). As the space occupied by these carboxylic derivatives is similar, we can argue that the broad range of activity (about 40 times between **28** and **29**) could be explained by the electronic environment of the substituents, where the negatively charged carboxylate is hardly recognized by the receptor. The high inhibition obtained with the carboxamide induced us to prepare the 'reverse' derivative 6α-formamido (**32**, 0.16 μM) obtaining very high inhibitory potency; on the other hand the 6α-acetamido derivative (**33**, 45 μM) fell down of about 280 times.

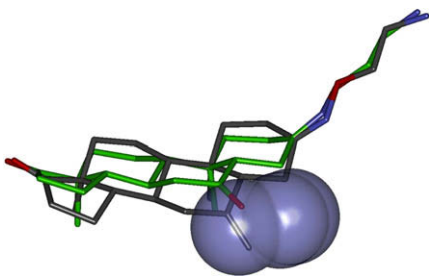


Figure 2. Superposition of *E* isomers of Istaroxime (light green) and **1** (gray), the latter rotated along the major axis of the androstane ring to reach the VDW volumes. Istaroxime is oriented as in Figure 1.

3.3. 3-[(3*R*)-3-Pyrrolidinyloxyimino]androstane derivatives

Fourteen 3-[(3*R*)-3-pyrrolidinyloxyimino]androstane derivatives (see Table 2) were synthesized from the scaffold with the substituents at position 6 that gave the highest inhibitions when coupled with the Istaroxime's oximic chain. Comparing the couples of compounds with the pyrrolidinyloxy versus aminoethoxy chain, in 10 of 14 cases the potencies were increased for the cyclic members, ranging from 1.5 to about 9 times; in the remaining four cases there was no change or a very slight decrease. These results are in agreement with what was observed in the case of Istaroxime and its cyclic analogue.

3.4. 3-(3-*N*-Methylaminopropoxyimino) androstane derivatives

As explained above, 11 3-(3-*N*-methylaminopropoxyimino) androstane derivatives were synthesized (see Table 3) to complete the series. A comparison of the couples *N*-methylaminopropoxy- versus aminoethoxy-chain shows that in 8 of 11 cases a diminished potency of more than threefold was obtained, with a maximum of about 19 times when **53** (0.33 μM) is compared with **10** (0.017 μM). These results are in agreement with what was seen in the case of Istaroxime and its *N*-methylaminopropoxy analogue (the former was twice as potent as the secondary linear amine), even though the reduction is sometimes greater than that found for the parent couple.

3.5. 3D-Quantitative structure–activity relationship

3D-Quantitative structure–activity relationship (3D-QSAR) studies have been performed on this series of digitalis-like Na^+,K^+-ATPase inhibitors to develop models that can bring together the different classes of molecules, explain and predict the inhibitory activity found on the Na^+,K^+-ATPase. Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were performed on a total of 63 molecules from which a training set of 44 compounds and a test set of 19 compounds were selected using the chemical descriptors obtained with the software DRAGON and applying a multivariate analysis on them with the aid of software SIMCA-P.

We performed the 3D-QSAR on the *E* isomers of the molecules, by assigning the experimental data of the *E*/*Z* mixture as if they were the values of the *E* isomers, based on the consideration that the experimental inhibitory value of a mixture is closer to the value hypothetically obtainable with the pure *E* isomer (the *E* isomer is the one with the highest activity in a couple of *E* and *Z* isomers, as predicted by our model and experimentally demonstrated).[5,8] This assertion is supported by comparison of the few available experimental inhibitory data of the correlated *E*/*Z* mixture, the pure *E* and the *Z* isomers. In the following examples, both the inhibition value of the *E* isomer and the one of the mixture are always higher than the value of the *Z* isomer: (i) *E*/*Z* Istaroxime (0.11 μM),[8] *E* isomer of Istaroxime (0.056 μM),[8] *Z* isomer of Istaroxime (0.63 μM);[8] (ii) *E*/*Z* compound **C** (0.026 μM),[8] *E* isomer of compound **C** (0.016 μM),[8] *Z* isomer of compound **C** (0.25 μM);[8] (iii) *E*/*Z* 3-(2-aminoethoxyimino)androstane-6α,17β-diol (1.6 μM),[5] its *E* isomer (2.0 μM)[4] and its *Z* isomer (12.0 μM).[5] The inhibitory values used in the 3D-QSAR analyses are those reported in Tables 1–3, in their −log form (pIC_{50}).

Molecules for 3D-QSAR were prepared with the following procedure. The 3D model of each compound in its *E* form was constructed in MACROMODEL 9.5[14] and submitted to a cycle of minimization, conformational analysis and again minimization with GB/SA continuum model to simulate water solvation (see Section 5); a series of conformers for each molecule (all the conformers in an energy range of 3.0 kcal mol^{-1} or the first 30 conformers for the more flexible molecules) was saved for the subsequent step of alignment and superposition. The molecules were obviously aligned on the rigid steroidal skeleton, but the concern regarded the oxime chain in position 3. The *E* isomer of compound **C** was considered as the driving molecule in this alignment because it is one of the derivatives of this series with the highest inhibitory potency and has a cyclic amine on the oximic chain, with a smaller number of conformers in comparison with a linear amino alkyl chain; the conformer of compound **C** used was that with the best superposition with the conformers of Istaroxime (rms). The other molecules of Tables 1 and 2 were aligned with the respective conformer that gave the best superposition with the said conformer of compound **C** (Fig. 3a). compound **D** was used as a reference to align the compounds of Table 3, with a longer aminoalkyl chain. In order to decide the conformer to be used, compound **D** was aligned with cassaine (superimposed with Istaroxime in Fig. 1) to choose its conformer that placed the amino head closer to that of cassaine while its alkylene chain was better superposed on the portion $CH–CH_2–N$ of the 3-(*R*)-pyrrolidinyl ring of compound **C** (Fig. 3b). Again, the other molecules of Table 3 were aligned with the respective conformer that gave the best superposition with compound **D**.

In SYBYL 8.0 software suite[15] the selected conformers were superposed using the 'align database' routine with the *E* isomer of compound **C** as 'template molecule' and the androstane scaffold depleted of the hydrogen atoms, with C=O in position 17, C=N in 3 and without substituents in 6 as 'common substructure' (the 63 compounds aligned are reported in Fig. 4); the atom charge for the aligned molecules were recalculated applying the Gasteiger-Huckel method and the molecules were imported in the DRAGON 5.0[16] environment for the calculation of more than 1600 molecular descriptors.[17] Descriptors with constant and near-constant values and with a pair correlation of 0.95 were discarded; the remaining 494 descriptors were imported in SIMCA-P 11.5[18] for statistical analysis. Principal component analysis (PCA) technique was applied obtaining a matrix in which the descriptors are expressed in term of scores (t) and loadings (p). Scores t1, t2, etc. are new

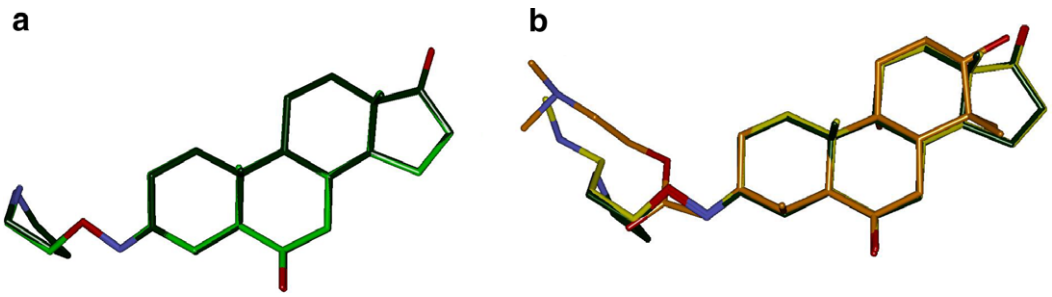


Figure 3. (a) Superposition of *E* isomers of Istaroxime (light green) and compound **C** (dark green). (b) Superposition of cassaine (orange) and *E* isomers of compound **C** (dark green) and compound **D** (yellow).

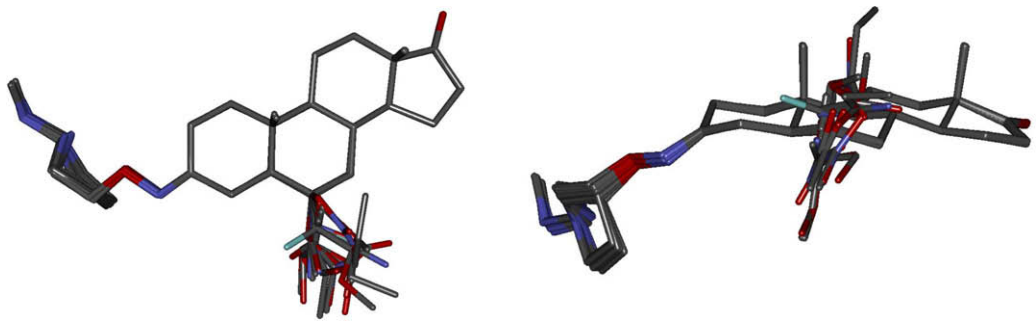


Figure 4. The 63 molecules of training and test sets superposed as for the 3D-QSAR analyses.

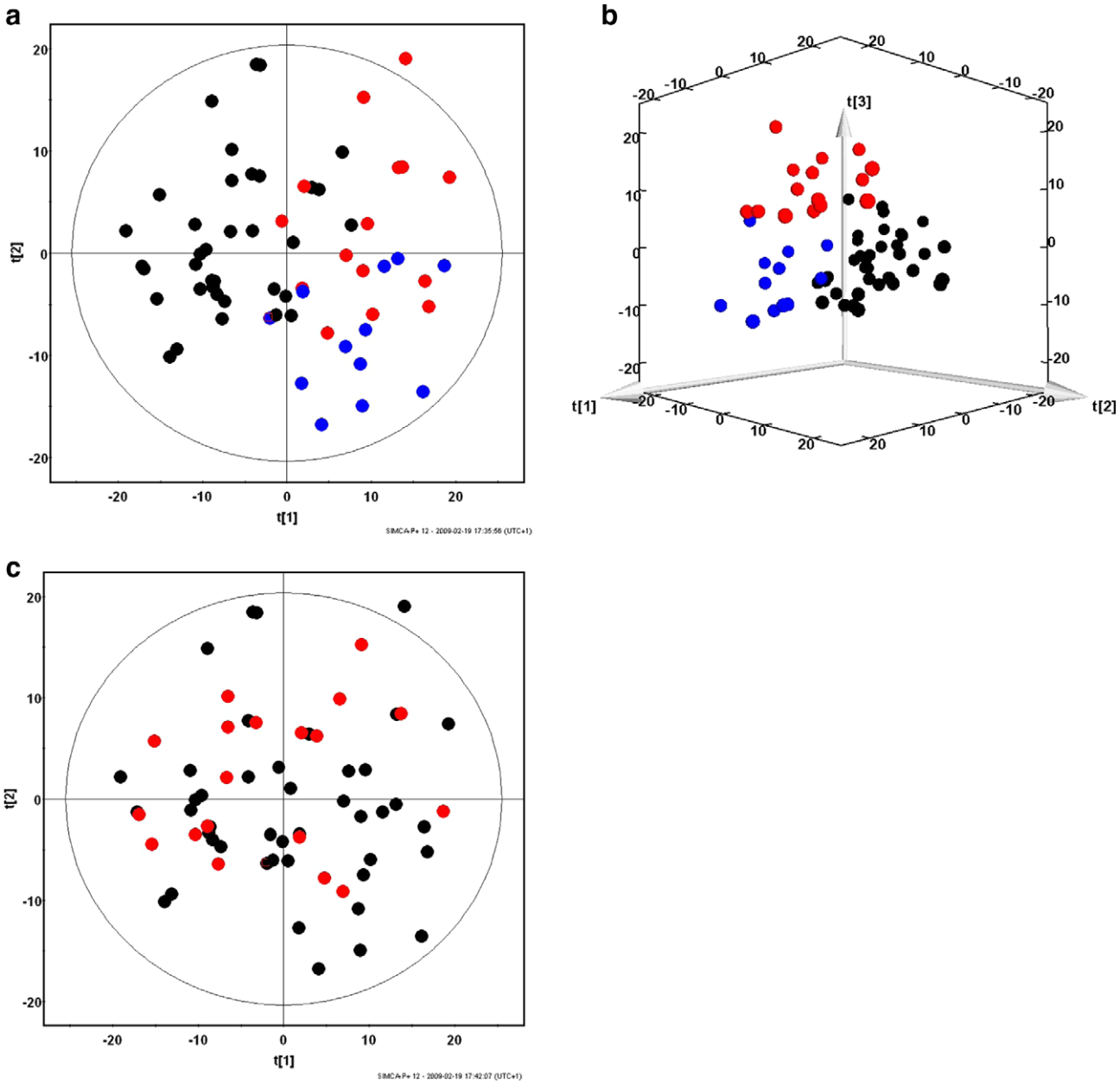


Figure 5. (a) PCA score plot of first component (t1) versus second component (t2). The dots represented the molecules are colored according to Table 1 (black), Table 2 (red) and Table 3 (blue). (b) PCA 3D score plot of first component (t1), second component (t2) and third component (t3); dots colored as previously. (c) PCA score plot of first component (t1) versus second component (t2). The dots are colored according to training (black) and test (red) sets.

variables summarizing the X variables (the descriptors); they are orthogonal, that is, completely independent of each other. The score t1 (first component) explain the largest variation of the X space, followed by t2, etc. Hence, the scatters plot of t1 versus t2

displays how the molecules are situated with respect to each other in the chemical space. Molecules that are close to each other are similar; molecules far away from each other are dissimilar. By means of PCA the scores t1 and t2 were able to separate to some extent the three classes of compounds, that is, the compounds belonging to the three tables (Fig. 5a), and even a better and almost complete separation among the three classes was obtained in the 3D graphic in which also the t3 component was added (Fig. 5b). This partition among the different classes of compounds indicates that the molecular descriptors obtained with DRAGON and elaborated with SIMCA-P were able to extract the 'chemical nature' of the molecules. Only one molecule (Table 2, **44**, 6α-ONO_2) is a weak outlier, being outside the Hotelling T^2 circle describing the 95% confidence region of the scores' graph. With the aid of this graph we manually selected a test set of 19 compounds (about 30% of the entire dataset), taking care to choose representative compounds in term of position in the X space, activity on Na^+,K^+-ATPase (expressed as pIC_{50}) and presence of the three different oximic chains; the remaining 44 compounds constituted the training set (Fig. 5c). The training set was used in the CoMFA and CoMSIA analyses to build a predictive model of the inhibition of the Na^+,K^+-ATPase; the test set was used to validate the model.

3D-QSAR analyses were performed with the tools included in SYBYL 8.0. In the CoMFA method, a relationship is seeked among the biological activities of a set of compounds and their electrostatic and steric properties.[19,20] The molecules under study are superposed in their putative bioactive conformations and then placed in a box of a regularly spaced grid of 2.0 Å. The lattice was extended to 4 Å beyond the van der Waals volume of each molecule in the *X*, *Y*, and *Z* directions. A sp^3 carbon atom of radius 1.52 Å and charge +1.0 was used as a probe to calculate both the steric and electrostatic fields at each node of the grid. A 3D-QSAR model has been derived from partial least-squares (PLS)[21] linear regression that permits to correlate the inhibitory activities (pIC_{50}; the values span over more than three orders of magnitude) with CoMFA values. The optimum number (*N*) of PLS components, corresponding to the smallest standard error of prediction (SEP), was determined by the leave-one-out (LOO) cross-validation procedure, applying a column filter of 2.0 kcal mol^{-1} so that only the steric and electrostatic energies with values greater than 2.0 kcal mol^{-1} were considered in the PLS analysis. The optimal *N* obtained was used in a non-cross-validated PLS analysis to get the model parameters such as correlation coefficient (r^2), SEE, and *F* value. Good statistical results were obtained from the training set of 44 molecules as reported in Table 5. From this analysis the predictions of the Na^+,K^+-ATPase inhibitory values of the training set and of the test set are reported in Figure 6 and, in tabulated form, in Supplementary data. Referring to the results for the test set, 16 compounds of 19 are predicted within a range less than one order of magnitude (and 9 of these 16 are less than 0.5) giving an adequate result taking into consideration the compelled assumption of assigning the experimental data of the *E*/*Z* mixture to the *E* isomers, thus probably underestimating the 'real' inhibitory potencies of the *E* isomers. The three compounds with an error in the prediction greater than one (in the oval in Fig. 6) are all predicted more potent than the experimental value and all belong to the first class of compounds (Table 1; bearing the 2-aminoethoxyimino chain); the substituents in position 6 present in these three derivatives, for the low activity that they convey to the compounds, are not represented in the other two classes and for that reason they are not well characterized in the 3D-QSAR model.

The CoMFA steric and electrostatic contour plots for all 63 molecules are displayed in Figure 7. Significant green contours sur-

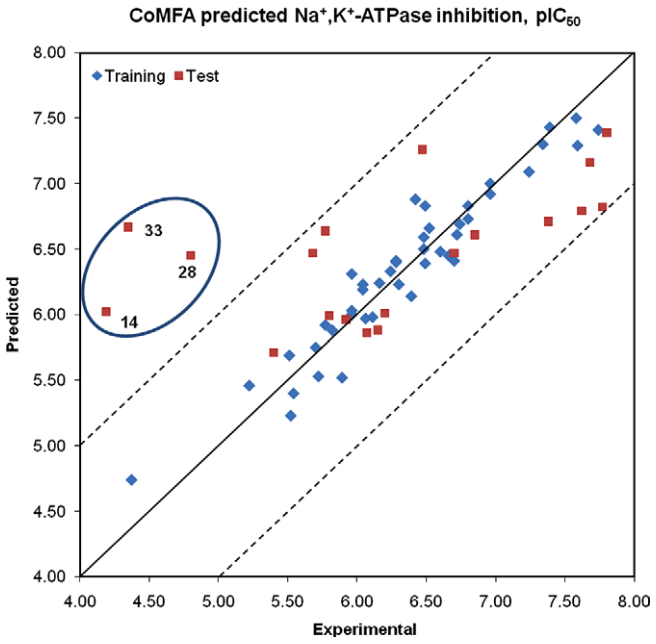


Figure 6. CoMFA predicted Na^+,K^+-ATPase inhibition of training (cyan) and test (red) sets. In addition to the line of ideal correlation, dotted lines are given, indicating deviations of ±1 logarithmic unit. Outliers are labeled by their compound numbers.

Table 5
CoMFA and CoMSIA statistical results

Statistical parameters	CoMFA	CoMSIA							
	SE	SEHDA	SEDA	SEHA	SEHD	SEA	SED	SEH	SE
q^2	0.68	0.43	0.44	0.53	0.55	0.52	0.57	0.58	0.57
SEP	0.39	0.53	0.53	0.48	0.48	0.49	0.46	0.45	0.45
r^2	0.93	0.85	0.83	0.84	0.85	0.82	0.85	0.86	0.78
SEE	0.18	0.28	0.29	0.29	0.27	0.30	0.27	0.26	0.32
F	98.20	33.62	30.20	33.19	35.63	28.61	34.9	45.30	34.84
N	5	6	6	6	6	6	6	5	4
Contributions									
S	0.64	0.09	0.09	0.15	0.10	0.20	0.13	0.22	0.33
E	0.36	0.25	0.29	0.36	0.31	0.46	0.35	0.48	0.67
H		0.12		0.21	0.15			0.30	
D		0.36	0.40		0.44		0.52		
A		0.18	0.22	0.28		0.34			

[a] q^2, Leave-one-out (LOO) cross-validated correlation coefficient; SEP, standard error of prediction; r^2, non-cross-validated correlation coefficient; SEE, standard error of estimate; *F*, *F*-test value; *N*, optimum number of components; S, steric field; E, electrostatic field; H, hydrophobic field; D, hydrogen-bond donor field; A, hydrogen-bond acceptor field.

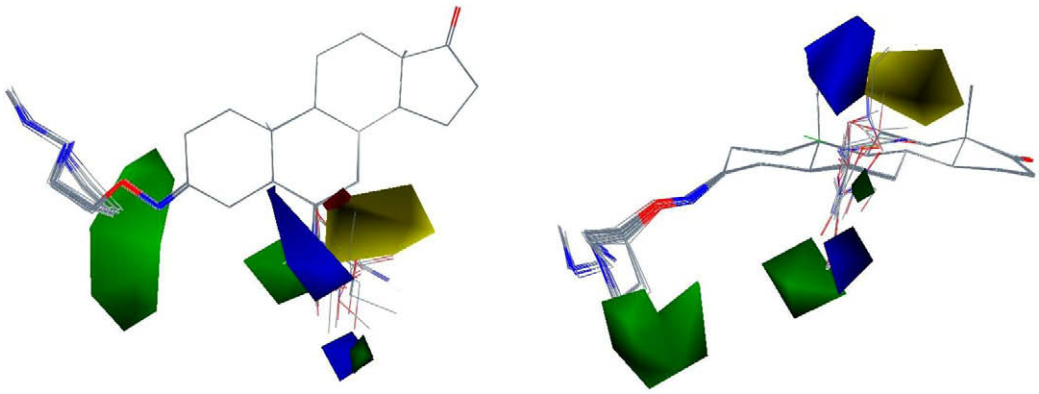


Figure 7. CoMFA standard deviation coefficient steric and electrostatic contour plots; all the 63 molecules of training and test sets are displayed for reference. Sterically favored areas (contribution level 80%) are represented by green polyhedral. Sterically disfavored areas (contribution level 20%) are represented by yellow polyhedral. Positive charge favored areas (contribution level 80%) are represented by blue polyhedral. Negative charge favored areas (contribution level 20%) are represented by red polyhedral.

rounding the heterocyclic rings of the oximic chain and the space under the substituent in position 6 (i.e., toward the α-face of the steroid scaffold), represent favored steric areas to increase inhibition of the Na^+,K^+-ATPase; the small green area located in front of the position 6 is less significant. The green area around the heterocyclic rings supports the evidence that most of the compounds of Table 2 with this feature showed enhanced Na^+,K^+-ATPase inhibitory potency than the corresponding compounds with a linear oximic chain. Examples of compounds that locate the substituents in 6 in the green area below that position are **42** (α-CH_2OH, 0.026 μM), **17** (α-CH_2OH, 0.18 μM), and **47** (α-COOMe, 0.16 μM). A yellow contour above the substituent in position 6 (i.e., toward the β-face of the steroid scaffold) suggests that bulkier substituents in 6β or spanning towards the β-face, are close to the sterically unfavorable yellow region and therefore are detrimental for the inhibitory activity as demonstrated by compounds like **12** (NOAllyl, 43 μM), **20** (β-CH_2OMe, 3.0 μM), and **27** (β-ONO_2, 2.9 μM) that exhibit lower inhibitory activities than the corresponding analogues **10** (=NOMe, 0.017 μM), **19** (α-CH_2OMe, 0.50 μM), and **26** (α-ONO_2, 0.33 μM) which have groups at position 6 that do not span in the yellow region. Taking into account that electrostatic characteristics report for about one third of the CoMFA model, significant blue contours can be found above the position 6 and on the axis of the 6α position of the androstane scaffold and represent regions where positively charged substituents favor the Na^+,K^+-ATPase inhibitory activity. The blue region above the position 6 receives the alkyl part of the 6-substituents of some compounds (**20**, β-CH_2OMe, 3.0 μM; **5**, β-Me, 6.0 μM; **11**, =NOEt, 1.7 μM) while the blue region below the position 6 accepts compounds like **38** (α-C≡CH, 0.11 μM) and **47** (α-COOMe, 0.16 μM). A single small red contour, where negatively charged substituents favor activity, is located above position 6 close to the disfavored steric yellow region; the red region accounts for only a little contribution on the activity; in fact compounds that put substituents with a partial negative charge in this region showed relatively low potencies because they are not able to counteract the negative steric effect pointed out by the yellow region: **24** (β-OMe, 1.5 μM); **27** (β-ONO_2, 2.9 μM); and **12** (=NOAllyl, 43 μM).

CoMSIA[22] is an extension of the CoMFA method and both are based on the assumption that changes in binding affinities of ligands are related to changes in molecular properties, represented by fields. They differ only in the implementation of the fields; in CoMSIA Gaussian-type distance dependence was applied to measure the relative attenuation of the field position of each atom in the grid leading to a smoother sampling of the fields around the molecules when compared to CoMFA. CoMSIA analysis was performed with the same lattice box as that used for the CoMFA calculations on the aligned molecules of the training set and validated with the 19 molecules of the test set. Five different similarity fields are calculated: steric, electrostatic, hydrogen-bond donor, hydrogen-bond acceptor, and hydrophobic. Maintaining the steric and electrostatic fields, we systematically explored the combination with the other three fields: the PLS statistical values obtained for each model are reported in Table 5. Compared to CoMFA, no one of the CoMSIA's models performed better, consequently we do not use these results to predict the inhibitory activities of the molecules, but simply to obtain a representation of the fields around the molecules, especially for those not present in CoMFA. The CoMSIA's steric and electrostatic contours were found to be comparable to the corresponding CoMFA contours even if in reverse percentage; since these contours were discussed above, the following section will deal with the CoMSIA H-bond donor and acceptor contours as well as the CoMSIA hydrophobic contours of the three different models: SEA, SED, and SEH (Fig. 8). In SEA and SED models the accepting and donating contours describe regions of space where hydrogen-bond donors or acceptors on the putative Na^+,K^+-ATPase receptor will enhance binding affinity.[23] These fields are created using extension points from acceptors and donors from the ligands into space where supposed corresponding groups would lie on the receptor: the acceptor field contains information about where hydrogen-bond donating groups should be on the receptor; the donor field describes where hydrogen-bond acceptor groups should be located on the receptor. These results can be seen as an indication of an existing correlation between the activities of the molecules of the training set and the features accepting or donating H bonds present on the compounds. In Figure 8a the contour maps of hydrogen-bond acceptor field in the CoMSIA model SEA are shown; the magenta contour, acceptor favored, encloses areas where acceptor hydrogen-bonds on the ligands are expected to enhance activity; it lies primarily in the 6α region reflecting the high potency of compounds having this kind of substituents as for those of **30** and **47** (α-COOMe; 0.41 and 0.16 μM, respectively), **19** and **43** (α-CH_2OMe; 0.50 and 0.32 μM, respectively). The two red contours, acceptor disfavored, are located in the 6β-face and on the axis of the 4α position; in the 6β region hydrogen-bond acceptors on ligands are expected to reduce activity as demonstrated by relatively low potency of compounds like **20** (β-CH_2OMe, 3.0 μM) or **27** (β-ONO_2, 2.9 μM). The smaller red region close to 4α probably accounts for the very high potency of compounds having a 6-oxo substituent (Istaroxime, 0.11 μM and compound **C**, 0.026 μM) from which an acceptor extension point in this direction originates; therefore lack of this feature reduces the potency. In Figure 8b the contour maps of hydrogen-bond donor field in the CoMSIA model SED are shown; the cyan contours, do-

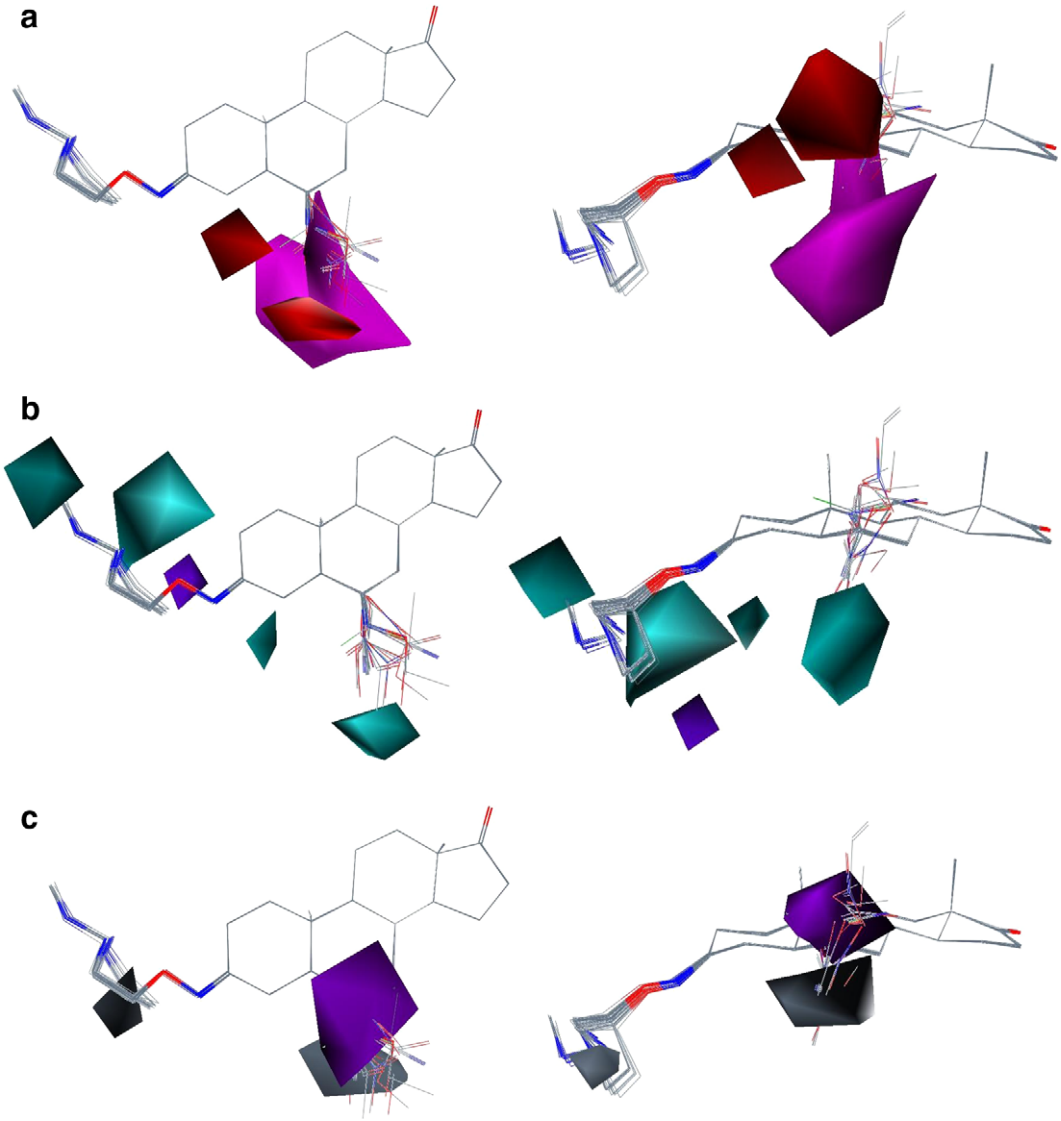


Figure 8. (a) CoMSIA standard deviation coefficient hydrogen-bond acceptor contour plots; all the 63 molecules of training and test sets are displayed for reference. Favored areas (contribution level 80%) are represented by magenta polyhedral. Disfavored areas (contribution level 20%) are represented by red polyhedral. (b) CoMSIA standard deviation coefficient hydrogen-bond donor contour plots; all the 63 molecules of training and test sets are displayed for reference. Favored areas (contribution level 80%) are represented by cyan polyhedral. Disfavored areas (contribution level 20%) are represented by purple polyhedral. (c) CoMSIA standard deviation coefficient hydrophobic contour plots; all the 63 molecules of training and test sets are displayed for reference. Favored areas (contribution level 80%) are represented by violet polyhedral. Disfavored areas (contribution level 20%) are represented by gray polyhedral.

nor favored (H-bond acceptor on the receptor), cover four regions: the two close to the oximic chains explain the high activity of molecules with a 2-aminoethoxyimino or (3*R*)-3-pyrrolidinyloxyimino chains (**9**, **10**, **39**, and **40**), that in these regions project the donor extension points originated from the amine groups; the small region near position 4 is expression of the 6α-CH_2OH substituents present in **17**, **42**, and **54**; the region in 6α position receives the donor extension points of compounds like **16** and **41** (6α-CH_2CH_2OH), **17** and **42** (6α-CH_2OH). The single small purple contour, donor disfavored, is hardly explained, but probably can be attributable to the compound of the 2-aminoethoxyimino class in the training set with very low activity and that has a donor extension point in the vicinity, namely **12**. In Figure 8c the contour maps of hydrophobic fields in the CoMSIA model SEH are shown. The violet contour, hydrophobes favored (or hydrophiles disfavored), lies on the axis of a double bonded substituent in position 6 and can be rationalized by contribute of high potency compounds like **2** (=CF_2, 0.19 μM), **34** (=CH_2, 0.046 μM), and **36** (spirocyclopropane, 0.22 μM). The gray contours, hydrophobes disfavored (or hydrophiles favored), are located in the 6α region and in the proximity of the pyrrolidinyl ring on the oximic chain: the former is explained by the hydrophilic effect of substituents like those of compounds with high potency as for **16** and **41** (α-CH_2CH_2OH; 0.32 and 0.058 μM, respectively), **17** and **42** (α-CH_2OH; 0.18 and 0.026 μM, respectively), **26** and **44** (α-ONO_2; 0.33 and 0.38 μM, respectively); the latter is entirely due to the compounds of Table 3 present in the training set, that, bearing the 3-*N*-methylaminopropoxyimino chain, do not have a nitrogen atom in this 'gray region' and therefore account for a hydrophobic environment negatively correlated to the potency.

3.6. In vivo activity in guinea pigs

Some compounds showing high inhibitory potency on the Na^+,K^+-ATPase were investigated in vivo in the anesthetized guinea pig (Table 4). Even though none of the tested compounds showed inotropic potencies (ED_{80}) comparable to digoxin, all displayed safety ratios higher than digoxin, and even higher than Istaroxime,

as for **32**, **54**, and **56**. Further, many compounds showed not determinable lethal dose/ED_{80} ratios, since no animals died (although a small number of animals was used). The reported ability of low digoxin concentrations to stimulate Ca^{2+} release from the sarcoplasmic reticulum[24] may partly account for its proarrhythmic properties. The contribution of other mechanisms, such as Sarco/Endoplasmic Reticulum Calcium ATPase Isoform 2a (SERCA2a) involved in the lower proarrhythmogenicity of Istaroxime,[25] was not investigated on these compounds.

It was not possible to find particular substituents conferring higher safety to the compounds: only a trend might be attributable to the 6α-NHCHO group, even though with a high variability (compounds **32**, **45**, and **56**, with lethal dose/ED_{80} ratios 33, 11, and 47, respectively).

4. Conclusions

The compounds reported in this paper confirm the interest in the androstane skeleton, with a proper basic substituent at position 3 as a pharmacophore for positive inotropic activity, and different groups at position 6. Such compounds have the desirable advantage of a higher safety as compared to digoxin, the classic digitalis compound currently used for the treatment of congestive heart failure as inotropic agent.

The 3D-QSAR analysis using CoMFA and CoMSIA methods have been successfully applied to a set of 63 androstane derivatives as Na^+,K^+-ATPase inhibitors. The contour plots provide many useful insights into relationships between structural features and inhibitory activity. These features could be used to expand the class exploring different positions of the androstane scaffold, such as 5 and 7.

5. Experimental section

5.1. General details

General experimental details regarding chemistry are reported elsewhere.[26] Compound purity was assessed by ^{1}H NMR and elemental analysis (reported as Supplementary data).

5.2. Chemistry

5.2.1. General procedure for the reaction of Scheme 1

To a stirred solution of the appropriate ketone (1 equiv) in THF (0.15 M), a solution of the appropriate hydroxylamine dihydrochloride (1 equiv) in H_2O (0.30 M) was rapidly added dropwise. After 1.5 h, NaCl (7.5 equiv) was added and the mixture stirred for 10 min. The phases were separated and the aqueous phase extracted with THF (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness.

5.2.2. Fumarate salts

The crude product was purified by flash chromatography (SiO_2, $CHCl_3$/MeOH/26% NH_4OH 90/10/1). To the concentrated fractions containing the pure compound a stoichiometric amount of fumaric acid in MeOH was added. After addition of a 1/1 mixture of EtOAc/Et_2O, the precipitate was filtered.

5.2.3. Hydrochloride salts

The crude product was triturated with EtOAc/Et_2O and filtered.

5.2.4. General procedure for the hydrolysis of the 3,3:17,17-bis(ethylendioxy) groups to give the 3,17-Diketo derivatives

A solution of 3,3:17,17-bis(ethylendioxy) derivative (1.0 mmol) and PTSA·H_2O (4.8 mmol) in acetone (40 mL) was stirred at room temperature for 3 h. The solution was neutralized by addition of 5% aqueous $NaHCO_3$ and acetone was evaporated. The aqueous suspension was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4 and evaporated to dryness.

5.3. Target compounds 1–58

5.3.1. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-methyleneandrostane-17-one hydrochloride (1)

Prepared in 90% yield from 6-methyleneandrostane-3,17-dione **61** and 2-aminoethoxyamine dihydrochloride.[26] ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.08 (br b, 3H, NH_3^+), 4.83 (br s, 0.5H, C=CH_2), 4.80 (br s, 0.5H, C=CH_2), 4.52 (br s, 0.5H, C=CH_2), 4.49 (br s, 0.5H, C=CH_2), 4.09 (m, 2H, CH_2–O), 0.77 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{34}N_2O_2$·HCl) C, H, Cl, N.

5.3.2. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-difluoromethylene-androstane-17-one hydrochloride (2)

Prepared in 61% yield from 6-difluoromethyleneandrostane-3,17-dione **96** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.62 (br b, 3H, NH_3^+), 4.07 (m, 2H, CH_2–O), 3.25 (m, 0.5H, H-4eq *Z* isomer), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.01 (m, 2H, CH_2–N), 0.89 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{32}F_2N_2O_2$·HCl) C, H, Cl, N.

5.3.3. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-[(*E*)-ethyliden] androstane-17-one hydrochloride (3)

Prepared in 71% yield from 6-[(*E*)-ethyliden]androstane-3,17-dione **94** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.01 (br b, 3H, NH_3^+), 5.01 (q, 0.5H, C=CH), 4.97 (q, 0.5H, C=CH), 4.08 (m, 2H, CH_2–O), 2.69 (m, 1H, H-7eq), 0.77 (s, 3H, CH_3), 0.72 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{36}N_2O_2$·HCl) C, H, Cl, N.

5.3.4. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6β-methylandrostane-17-one hydrochloride (4)

Prepared in 64% yield from 6β-methylandrostane-3,17-dione **71** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.89 (br b, 3H, NH_3^+), 4.06 (m, 2H, CH_2–O), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.02 (m, 2H, CH_2–N), 2.81 (m, 0.5H, H-4eq *Z* isomer), 0.96 (s, 1.5H, CH_3), 0.95 (s, 1.5H, CH_3), 0.91 (d, 1.5H, CH_3), 0.90 (d, 1.5H, CH_3), 0.81 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{36}N_2O_2$·HCl) C, H, Cl, N.

5.3.5. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-(spirocyclopropane) androstane-17-one hydrochloride (5)

Prepared in 92% yield from 6-(spirocyclopropane)androstane-3,17-dione **68** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.88 (br b, 3H, NH_3^+) 4.06 (m, 2H, CH_2–O), 3.06 (m, 0.5H, H-4eq *Z* isomer), 3.00 (m, 2H, CH_2–N), 2.70 (m, 0.5H, H-2eq *E* isomer), 0.96 (s, 3H, CH_3), 0.79 (s, 3H, CH_3), 0.57–0.16 (m, 4H, cyclopropane); white solid. Anal. ($C_{22}H_{36}N_2O_2$·HCl) C, H, Cl, N.

5.3.6. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-methylandrostane-17-one hydrochloride (6)

Prepared in 83% yield from 6α-methylandrostane-3,17-dione **90** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.83 (br b, 3H, NH_3^+), 4.07 (m, 2H, CH_2–O), 3.16 (m, 0.5H, H-4eq *Z* isomer), 3.06 (m, 0.5H, H-2eq *E* isomer), 3.00 (m, 2H, CH_2–N), 0.89 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.84 (d, 1.5H, CH_3), 0.81 (d, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{36}N_2O_2$·HCl) C, H, Cl, N.

5.3.7. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-vinylandrostane-17-one hydrocloride (7)

Prepared in 90% yield from 6α-vinylandrostane-3,17-dione **75** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.95 (br b, 3H, NH_3^+), 5.51 (m, 1H, C=CH), 4.98 (m, 2H, C=CH_2), 4.05 (m, 2H, CH_2–O), 3.06 (m, 0.5H, H-2eq *E* isomer), 3.01 (m, 2H, CH_2–N), 2.97 (m, 0.5H, H-4eq *Z* isomer), 0.91 (s, 1.5H, CH_3), 0.90 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{36}N_2O_2$·HCl) C, H, Cl, N.

5.3.8. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-ethynylandrostane-17-one hydrochloride (8)

Prepared in 76% yield from 6α-ethynylandrostane-3,17-dione **87** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 7.90 (br b, 3H, NH_3^+), 4.06 (m, 2H, CH_2–O), 2.98 (d, 0.5H, C≡CH), 2.97 (d, 0.5H, C≡CH), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{34}N_2O_2$·HCl) C, H, Cl, N.

5.3.9. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-[(*E*)-hydroxyimino] androstane-17-one hydrochloride (9)

Prepared in 70% yield from 6-[(*E*)-hydroxyimino]androstane-3,17-dione **103** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 10.58 (s, 0.5H, N–OH), 10.51 (s, 0.5H, N–OH), 7.98 (m, 3H, NH_3^+), 4.08 (m, 2H, CH_2–O), 3.29 (m, 1H, H-7eq), 3.13 (m, 0.5H, H-4eq *Z* isomer), 3.10 (m, 0.5H, H-2eq *E* isomer), 3.02 (m, 2H, CH_2–N), 0.79 (s, 6H, CH_3, CH_3); white solid. Anal. ($C_{21}H_{33}N_3O_3$·HCl) C, H, Cl, N.

5.3.10. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-[(*E*)-methoxyimino] androstane-17-one hydrochloride (10)

Prepared in 60% yield from 6-[(*E*)-methoxyimino]androstane-3,17-dione **105** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.03 (br b, 3H, NH_3^+), 4.09 (m, 2H, CH_2–O), 3.75 (s, 1.5H, CH_3–O), 3.73 (s, 1.5H, CH_3–O), 3.19 (m, 1H, H-7eq), 3.11 (m, 0.5H, H-4eq *Z* isomer), 3.08 (m, 0.5H, H-2eq *E* isomer), 3.02 (m, 2H, CH_2–N), 0.78 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{35}N_3O_3$·HCl) C, H, Cl, N.

5.3.11. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-[(*E*)-ethoxyimino] androstane-17-one hydrochloride (11)

Prepared in 80% yield from 6-[(*E*)-ethoxyimino]androstane-3,17-dione **107** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO, ppm from TMS): δ 7.85 (br b, 3H, NH_3^+), 4.07 (m, 2H, CH_2–O), 4.00 (q, 1H, CH_2–O), 3.98 (q, 1H, CH_2–O), 3.20 (m, 1H, H-7eq), 3.11 (m, 0.5H, H-4eq *Z* isomer), 3.08 (m, 0.5H, H-2eq *E* isomer), 3.03 (m, 2H, CH_2–N), 1.17 (t, 1.5H, CH_3), 1.16 (t, 1.5H, CH_3), 0.78 (s, 6H, CH_3, CH_3); white solid. Anal. ($C_{23}H_{37}N_3O_3$·HCl) C, H, Cl, N.

5.3.12. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-[(*E*)-allyloxyimino] androstane-17-one fumarate (12)

Prepared in 75% yield from 6-[(*E*)-allyloxyimino]androstane-3,17-dione **109** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO, ppm from TMS): δ 9.01 (br b, 4H, COOH, NH_3^+), 6.40 (s, 2H, CH=CH), 5.93 (m, 1H, C=CH), 5.18 (m, 2H, C=CH_2), 4.49 (m, 2H, C=C–CH_2–O), 4.05 (m, 2H, CH_2–O), 3.22 (m, 1H, H-7eq), 3.09 (m, 0.5H, H-4eq *Z* isomer), 3.07 (m, 0.5H, H-2eq *E* isomer), 2.98 (m, 2H, CH_2–N), 0.78 (s, 6H, CH_3, CH_3); white solid. Anal. ($C_{24}H_{37}N_3O_3$·$C_4H_4O_4$) C, H, N.

5.3.13. (*E*,*Z*)-3-(2-Aminoethoxyimino)-17-oxoandrostane-6-[(*E*)-ylidene]acetonitrile hydrochloride (13)

Prepared in 61% yield from 3,17-dioxoandrostane-6-[(*E*)-ylidene]acetonitrile **98** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.07 (br b, 3H, NH_3^+), 5.26 (br s, 1H, C=CH-CN), 4.08 (m, 2H, CH_2–O), 3.11 (m, 0.5H, H-2eq *E* isomer), 3.07 (m, 0.5H, H-4eq *Z* isomer), 3.02 (m, 2H, CH_2–N), 2.82 (m, 1H, H-7eq), 0.79 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{33}N_3O_2$·HCl) C, H, Cl, N.

5.3.14. Methyl {3-{(*E*,*Z*)-[2-aminoethoxyimino]}-17-oxoandrostane-6-[(*E*)-ylidene]}acetate hydrochloride (14)

Prepared in 87% yield from methyl {3,17-dioxoandrostane-6-[(*E*)-ylidene]}acetate **92** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.09 (br b, 3H, NH_3^+), 5.45 (s, 0.5H, C=CH), 5.41 (s, 0.5H, C=CH), 4.10 (m, 2H, CH_2–O), 3.94 (m, 1H, H-7eq), 3.62 (s, 3H, CH_3–O), 0.77 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{36}N_2O_4$·HCl) C, H, Cl, N.

5.3.15. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6-{(*E*)-[2-hydroxyethylidene]}androstane-17-one hydrochloride (15)

Prepared in 70% yield from 6-[(*E*)-2-hydroxyethylidene]androstane-3,17-dione **101** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.75 (br b, 3H, NH_3^+), 5.08 (br t, 0.5H, C=CH), 5.05 (br t, 0.5H, C=CH), 4.56 (t, 0.5H, OH), 4.53 (t, 0.5H, OH), 4.12–3.92 (m, 4H, CH_2–O, CH_2–O), 3.09 (m, 0.5H, H-2eq *E* isomer), 3.05 (m, 2H, CH_2–N), 3.02 (m, 0.5H, H-4eq *Z* isomer), 2.65 (m, 1H, H-7eq), 0.77 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{36}N_2O_3$·HCl) C, H, Cl, N.

5.3.16. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-(2-hydroxyethyl) androstane-17-one hydrochloride (16)

Prepared in 85% yield from 6α-(2-hydroxyethyl)androstane-3,17-dione **77** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.95 (br b, 3H, NH_3^+), 4.35 (br, 1H, OH), 4.08 (m, 2H, CH_2–ON), 3.42 (m, 2H, CH_2–O), 3.22 (m, 0.5H, H-4eq *Z* isomer), 3.06 (m, 0.5H, H-2eq *E* isomer), 3.02 (m, 2H, CH_2–N), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{38}N_2O_3$·HCl) C, H, Cl, N.

5.3.17. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-hydroxymethylandrostane-17-one hydrochloride (17)

Prepared in 60% yield from 6α-hydroxymethylandrostane-3,17-dione **83** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.73 (br b, 3H, NH_3^+), 4.37 (t, 1H, OH), 4.06 (m, 2H, CH_2–ON), 3.37 (m, 2H, CH_2–O), 3.16 (m, 0.5H, H-4eq *Z* isomer), 3.06 (m, 0.5H, H-2eq *E* isomer), 3.02 (m, 2H, CH_2–N), 0.89 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{36}N_2O_3$·HCl) C, H, Cl, N.

5.3.18. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6β-hydroxymethylandrostane-17-one hydrochloride (18)

Prepared in 85% yield from 6β-hydroxymethylandrostane-3,17-dione **65** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.14 (br b, 3H, NH_3^+), 4.42 (t, 0.5H, OH), 4.40 (t, 0.5H, OH), 4.08 (m, 2H, CH_2–ON), 3.35 (m, 2H, CH_2–O), 3.05 (m, 0.5H, H-2eq *E* isomer), 3.00 (m, 2H, CH_2–N), 2.91 (m, 0.5H, H-4eq *Z* isomer), 0.84 (s, 1.5H, CH_3), 0.82 (s, 1.5H, CH_3), 0.80 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{36}N_2O_3$·HCl) C, H, Cl, N.

5.3.19. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-methoxymethylandrostane-17-one hydrochloride (19)

Prepared in 33% yield from 6α-methoxymethylandrostane-3,17-dione **85** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.82 (br b, 3H, NH_3^+), 4.06 (m, 2H, CH_2–ON), 3.22 (m, 2H, CH_2–O), 3.20 (s, 3H, CH_3–O), 3.11 (m, 0.5H, H-4eq *Z* isomer), 3.05 (m, 0.5H, H-2eq *E*

isomer), 3.02 (m, 2H, CH_2–N), 0.89 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{38}N_2O_3$·HCl) C, H, Cl, N.

5.3.20. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6β-methoxymethylandrostane-17-one hydrochloride (20)

Prepared in 60% yield from 6β-methoxymethylandrostane-3,17-dione **67** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.06 (br b, 3H, NH_3^+), 4.07 (m, 2H, CH_2–ON), 3.35 (m, 2H, CH_2–O), 3.23 (s, 1.5H, CH_3), 3.22 (s, 3H, CH_3), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.02 (m, 2H, CH_2–N), 2.92 (m, 0.5H, H-4eq *Z* isomer), 0.86 (s, 1.5H, CH_3), 0.85 (s, 1.5H, CH_3), 0.81 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{38}N_2O_3$·HCl) C, H, Cl, N.

5.3.21. (*E*,*Z*)-3-(2-Aminoethoxyimino)-(6*R*)-6-(spiro-2′-oxirane) androstane-17-one hydrochloride (21)

Prepared in 50% yield from (6*R*)-6-spiro-(2′-oxirane)androstane-3,17-one **63** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.75 (br b, 3H, NH_3^+), 4.06 (m, 2H, CH_2–ON), 3.07 (m, 0.5H, H-2eq *E* isomer), 2.99 (m, 2H, CH_2–N), 2.83 (m, 0.5H, H-4eq *Z* isomer), 2.75 (d, 0.5H, CH_2–O), 2.72 (d, 0.5H, CH_2–O), 2.30 (d, 0.5H, CH_2–O), 2.27 (d, 0.5H, CH_2–O), 0.96 (s, 1.5H, CH_3), 0.94 (s, 1.5H, CH_3), 0.80 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{34}N_2O_3$·HCl) C, H, Cl, N.

5.3.22. (*E*,*Z*)-3-(2-Aminoethoxyimino)-(6*S*)-6-(spiro-2′-oxirane) androstane-17-one hydrochloride (22)

Prepared in 40% yield from (6*S*)-6-spiro-(2′-oxirane)androstane-3,17-one **62** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.80 (br b, 3H, NH_3^+), 4.06 (m, 2H, CH_2–ON), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.01 (m, 2H, CH_2–N), 2.90 (m, 0.5H, H-4eq *Z* isomer), 2.76 (d, 1H, CH_2–O), 2.57 (d, 1H, CH_2–O), 0.92 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{34}N_2O_3$·HCl) C, H, Cl, N.

5.3.23. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-methoxyandrostane-17-one hydrochloride (23)

Prepared in 62% yield from 6α-methoxyandrostane-3,17-dione **116** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.98 (br b, 2H, NH_3^+), 4.07 (m, 2H, CH_2–O), 3.40 (dd, 1H, CH–O), 3.24 (s, 1.5H, CH_3–O), 3.23 (s, 1.5H, CH_3–O), 3.05 (m, 0.5H, H-4eq *Z* isomer), 2.97 (m, 0.5H, H-2eq *E* isomer), 0.89 (s, 1.5H, CH_3), 0.88 (m, 1.5H, CH_3), 0.78 (m, 3H, CH_3); white foam. Anal. ($C_{22}H_{36}N_2O_3$·HCl) C, H, Cl, N.

5.3.24. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6β-methoxyandrostane-17-one hydrochloride (24)

Prepared in 34% yield from 6β-methoxyandrostane-3,17-dione **123** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.98 (br b, 3H, NH_3^+), 4.08 (m, 2H, CH_2–O), 3.23 (m, 1H, CH–O), 3.21 (s, 1.5H, CH_3), 3.20 (s, 1.5H, CH_3), 3.06 (m, 0.5H, H-4eq *Z* isomer), 3.02 (m, 2H, CH_2–N), 2.88 (m, 0.5H, H-2eq *E* isomer), 0.97 (s, 1.5H, CH_3), 0.96 (s, 1.5H, CH_3), 0.79 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{36}N_2O_3$·HCl) C, H, Cl, N.

5.3.25. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-ethoxyandrostane-17-one fumarate (25)

Prepared in 88% yield from 6α-ethoxyandrostane-3,17-dione **118** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.50 (m, 3H, NH_3^+), 4.02 (m, 2H, CH_2–O), 1.08 (t, 1.5H, CH_3), 1.07 (t, 1.5H, CH_3), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{38}N_2O_3$·$C_4H_4O_4$) C, H, N.

5.3.26. (*E*,*Z*)-3-(2-Aminoethoxyimino)-17-oxoandrostane-6α-yl nitrate fumarate (26)

Prepared in 33% yield from 3,17-dioxoandrostane-6α-yl nitrate **114** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.76 (br b, 4H, NH_3^+, COOH), 6.41 (s, 2H, CH=CH), 4.98 (m, 1H, CH–O), 4.04 (m, 2H, CH_2–ON), 3.16 (m, 0.5H, H-4eq *Z* isomer), 3.06 (m, 0.5H, H-2eq *E* isomer), 2.98 (m, 2H, CH_2–N), 0.98 (s, 1.5H, CH_3), 0.97 (s, 1.5H, CH_3), 0.80 (s, 3H, CH_3); white solid. Anal. ($C_{21}H_{33}N_3O_5$·$C_4H_4O_4$) C, H, N.

5.3.27. (*E*,*Z*)-3-(2-Aminoethoxyimino)-17-oxoandrostane-6β-yl nitrate fumarate (27)

Prepared in 60% yield from 3,17-dioxoandrostane-6β-yl nitrate **121** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.41 (br b, 4H, NH_3^+, COOH), 6.40 (s, 2H, CH=CH), 5.23 (m, 0.5H, CH–O), 5.19 (m, 0.5H, CH–O), 4.03 (m, 2H, CH_2–O), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.05 (m, 0.5H, H-4eq *Z* isomer), 2.96 (m, 2H, CH_2–N), 1.00 (s, 1.5H, CH_3), 0.99 (s, 1.5H, CH_3), 0.80 (s, 3H, CH_3); white solid. Anal. ($C_{21}H_{33}N_3O_5$·$C_4H_4O_4$) C, H, N.

5.3.28. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-carboxyandrostane-17-one hydrochloride (28)

Prepared in 80% yield from 6α-carboxyandrostane-3,17-dione **79** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.24 (br b, 4H, NH_3^+, COOH), 4.07 (m, 2H, CH_2–O), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.01 (m, 2H, CH_2–N), 2.93 (m, 0.5H, H-4eq *Z* isomer), 0.90 (s, 1.5H, CH_3), 0.89 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{34}N_2O_4$·HCl) C, H, Cl, N.

5.3.29. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-cyanoandrostane-17-one fumarate (29)

Prepared in 65% yield from 6α-cyanoandrostane-3,17-dione **111** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 9.07 (br b, 4H, NH_3^+, COOH), 6.40 (s, 2H, CH=CH), 4.07 (m, 2H, CH_2–ON), 3.25 (m, 0.5H, H-4eq *Z* isomer), 3.07 (m, 0.5H, H-2eq *E* isomer), 2.99 (m, 2H, CH_2–N), 2.77 (m, 1H, CH-CN), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{33}N_3O_2$·–$C_4H_4O_4$) C, H, N.

5.3.30. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-methoxycarbonylandrostane-17-one hydrochloride (30)

Prepared in 62% yield from 6α-methoxycarbonylandrostane-3,17-dione **81** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.75 (br b, 3H, NH_3^+), 4.06 (m, 2H, CH_2–ON), 3.60 (s, 3H, CH_3–O), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.01 (m, 2H, CH_2–N), 2.79 (m, 0.5H, H-4eq *Z* isomer), 0.91 (s, 1.5H, CH_3), 0.90 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{36}N_2O_4$·HCl) C, H, Cl, N.

5.3.31. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-carbamoylandrostane-17-one fumarate (31)

Prepared in 40% yield from 6α-carbamoylandrostane-3,17-dione **80** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.00 (br b, 4H, NH_3^+, COOH), 7.38 (s, 0.5H, $CONH_2$), 7.32 (s, 0.5H, $CONH_2$), 6.80 (s, 0.5H, $CONH_2$), 6.78 (s, 0.5H, $CONH_2$), 6.40 (s, 2H, CH=CH), 4.05 (m, 2H, CH_2–ON), 3.06 (m, 0.5H, H-2eq *E* isomer), 2.99 (m, 2H, CH_2–N), 2.91 (m, 0.5H, H-4eq *Z* isomer), 0.89 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{33}N_3O_2$·1.5$C_4H_4O_4$) C, H, N.

5.3.32. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-formamidoandrostane-17-one fumarate (32)

Prepared in 59% yield from 6α-formamidoandrostane-3,17-dione **126** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR

(300 MHz, DMSO-d_6, ppm from TMS): δ 8.20 (m, 4H, NH_3^+, COOH), 8.10–7.60 (m, 2H, NHCH–O), 6.44 (s, 2H, CH=CH), 4.05 (m, 2H, CH_2–ON), 3.72 (m, 1H, CH–N), 3.16 (m, 0.5H, H-4eq *Z* isomer), 3.06 (m, 0.5H, H-2eq *E* isomer), 2.97 (m, 2H, CH_2–N), 0.93 (s, 1.5H, CH_3), 0.92 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{22}H_{35}N_3O_3 \cdot C_4H_4O_4$) C, H, N.

5.3.33. (*E*,*Z*)-3-(2-Aminoethoxyimino)-6α-acetamidoandrostane-17-one hydrochloride (33)

Prepared in 84% yield from 6α-acetamidoandrostane-3,17-dione **128** and 2-aminoethoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.87 (br b, 3H, NH_3^+), 7.83 (d, 0.5H, NH-CO), 7.67 (d, 0.5H, NH-CO), 4.07 (m, 2H, CH_2–ON), 3.65 (m, 1H, CH–N), 3.15 (m, 0.5H, H-4eq *Z* isomer), 3.07 (m, 0.5H, H-2eq *E* isomer), 3.03 (m, 2H, CH_2–N), 1.81 (s, 1.5H, CH_3–CO), 1.79 (s, 1.5H, CH_3–CO), 0.93 (s, 1.5H, CH_3), 0.91 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{37}N_3O_3 \cdot HCl$) C, H, Cl, N.

5.3.34. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6-methyleneandrostane-17-one hydrochloride (34)

Prepared in 75% yield from 6-methyleneandrostane-3,17-dione **61** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride.[8] ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 9.01 (br b, 2H, NH_2^+), 4.82 (m, 1H, C=CH), 4.74 (m, 1H, CH–O), 4.50 (m, 1H, C=CH), 0.77 (s, 3H, CH_3), 0.76 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{36}N_2O_2 \cdot HCl$) C, H, Cl, N.

5.3.35. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6-difluoromethyleneandrostane-17-one hydrochloride (35)

Prepared in 71% yield from 6-difluoromethyleneandrostane-3,17-dione **96** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 9.10 (br b, 2H, NH_2^+), 4.70 (m, 1H, CH–O), 0.89 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{34}F_2N_2O_2 \cdot HCl$) C, H, Cl, N.

5.3.36. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6-(spirocyclopropane)androstane-17-one hydrochloride (36)

Prepared in 91% yield from 6-(spirocyclopropane)androstane-3,17-dione **68** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 9.02 (br b, 2H, NH_2^+), 4.72 (m, 1H, CH–O), 2.98 (m, 0.5H, H-2eq *E* isomer), 2.63 (m, 0.5H, H-4eq *Z* isomer), 0.96 (s, 1.5H, CH_3), 0.95 (s, 1.5H, CH_3), 0.79 (s, 3H, CH_3), 0.55-(-0.15) (m, 4H, cyclopropane); white solid. Anal. ($C_{25}H_{38}N_2O_2 \cdot HCl$) C, H, Cl, N.

5.3.37. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-methylandrostane-17-one fumarate (37)

Prepared in 84% yield from 6α-methylandrostane-3,17-dione **90** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.50 (br b, 3H, NH_2^+, COOH), 6.41 (m, 2H, CH=CH), 4.73 (m, 0.5H, CH–O), 4.70 (m, 0.5H, CH–O), 3.12 (m, 0.5H, H-4eq *Z* isomer), 2.98 (m, 0.5H, H-2eq *E* isomer), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.84 (d, 1.5H, CH_3), 0.81 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{38}N_2O_2 \cdot C_4H_4O_4$) C, H, N.

5.3.38. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-ethynylandrostane-17-one hydrochloride (38)

Prepared in 90% yield from 6α-ethynylandrostane-3,17-dione **87** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.98 (br b, 2H, NH_2^+), 4.75 (m, 1H, CH–O), 2.98 (d, 0.5H, C≡CH), 2.94 (m, 0.5H, C≡CH), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.79 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{36}N_2O_2 \cdot HCl$) C, H, Cl, N.

5.3.39. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6-[(*E*)-hydroxyimino]androstane-17-one hydrochloride (39)

Prepared in 77% yield from 6-[(*E*)-hydroxyimino]androstane-3,17-dione **103** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 10.56 (s, 0.5H, N–OH), 10.52 (s, 0.5H, N–OH), 9.25 (br b, 2H, NH_2^+), 4.74 (m, 1H, CH–O), 0.78 (s, 6H, CH_3, CH_3); white solid. Anal. ($C_{23}H_{35}N_3O_3 \cdot HCl$) C, H, Cl, N.

5.3.40. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6-[(*E*)-methoxyimino]androstane-17-one hydrochloride (40)

Prepared in 70% yield from 6-[(*E*)-methoxyimino]androstane-3,17-dione **105** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 9.05 (br b, 2H, NH_2^+), 4.74 (br s, 1H, CH–O), 3.75 (s, 1.5H, CH_3–O), 3.73 (s, 1.5H, CH_3–O), 0.81 (s, 3H, CH_3), 0.76 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{37}N_3O_3 \cdot HCl$) C, H, Cl, N.

5.3.41. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-(2-hydroxyethyl)androstane-17-one hydrochloride (41)

Prepared in 78% yield from 6α-(2-hydroxyethyl)androstane-3,17-dione **77** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.95 (br b, 2H, NH_2^+), 4,74 (m, 1H, CH–O), 4.34 (t, 1H, OH), 0.89 (s, 1.5H, CH_3), 0.88 (s, 1.5H, CH_3), 0.76 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{40}N_2O_3 \cdot HCl$) C, H, Cl, N.

5.3.42. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-hydroxymethylandrostane-17-one hydrochloride (42)

Prepared in 57% yield from 6α-hydroxymethylandrostane-3,17-dione **83** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 9.23 (br b, 2H, NH_2^+), 4.72 (m, 1H, CH–O), 4.37 (t, 1H, OH), 0.88 (s, 1.5H, CH_3), 0.86 (s, 1.5H, CH_3), 0.86 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{38}N_2O_3 \cdot HCl$) C, H, Cl, N.

5.3.43. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-methoxymethylandrostane-17-one fumarate (43)

Prepared in 60% yield from 6α-methoxymethylandrostane-3,17-dione **85** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 9.00 (br b, 3H, NH_2^+, COOH), 6.40 (s, 2H, CH=CH), 4.71 (m, 1H, CH–O), 3.22 (s, 1.5H, CH_3–O), 3.21 (s, 1.5H, CH_3–O), 0.88 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{40}N_2O_3 \cdot C_4H_4O_4$) C, H, N.

5.3.44. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-17-oxoandrostane-6α-yl nitrate hydrochloride (44)

Prepared in 41% yield from 3,17-dioxoandrostane-6α-yl nitrate **114** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.96 (br b, 2H, NH_2^+), 4.99 (m, 1H, CH–ONO_2), 4.74 (m, 1H, CH–O), 0.99 (s, 1.5H, CH_3), 0.98 (s, 1.5H, CH_3), 0.80 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{35}N_3O_5 \cdot HCl$) C, H, Cl, N.

5.3.45. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-formamidoandrostane-17-one hydrochloride (45)

Prepared in 70% yield from 6α-formamidoandrostane-3,17-dione **126** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 9.38 (br b, 3H, NH_2^+), 8.42–7.50 (m, 2H, NH-CH–O), 4.76 (m, 0.5H, CH–O), 4.71 (m, 0.5H, CH–

O), 3.72 (m, 1H, CH–N), 0.93 (s, 1.5H, CH_3), 0.92 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{37}N_3O_3$·HCl) C, H, Cl, N.

5.3.46. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-carbamoylandrostane-17-one hydrochloride (46)

Prepared in 65% yield from 6α-carbamoylandrostane-3,17-dione **80** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 8.80 (br b, 2H, NH_2^+), 7.38 (br b, 0.5H, NH-CO), 7.31 (br b, 0.5H, NH-CO), 6.92 (br b, 0.5H, NH-CO), 6.78 (br b, 0.5H, NH-CO), 4.62 (m, 1H, CH–O), 0.89 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{23}H_{37}N_3O_3$·HCl) C, H, Cl, N.

5.3.47. (*E*,*Z*)-3-[(3*R*)-3-Pyrrolidinyloxyimino]-6α-methoxycarbonylandrostane-17-one hydrochloride (47)

Prepared in 74% yield from 6α-methoxycarbonylandrostane-3,17-dione **81** and 3-(*R*)-pyrrolidinyloxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.88 (br b, 2H, NH_2^+), 4.72 (m, 1H, CH–O), 3.61 (s, 1.5H, CH_3–O), 3.60 (s, 1.5H, CH_3–O), 2.99 (m, 0.5H, H-2eq *E* isomer), 2.74 (m, 0.5H, H-4eq *Z* isomer), 0.91 (s, 1.5H, CH_3), 0.90 (s, 1,5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{38}N_2O_4$·HCl) C, H, Cl, N.

5.3.48. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6-methyleneandrostane-17-one fumarate (48)

Prepared in 70% yield from 6-methyleneandrostane-3,17-dione **61** and 3-*N*-methylaminopropoxyamine dihydrochloride.[7] ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 9.10 (br b, 3H, NH_2^+, COOH), 6.41 (s, 2H, CH=CH), 4.82 (m, 0.5H, C=CH), 4.80 (m, 0.5H, C=CH), 4.52 (m, 0.5H, C=CH), 4.49 (m, 0.5H, C=CH), 3.96 (t, 2H, CH_2–O), 3.01 (m, 0.5H, H-2eq *E* isomer), 2.96 (m, 0.5H, H-4eq *Z* isomer), 2.80 (m, 2H, CH_2–N), 2.46 (s, 1.5H, CH_3–N), 2.45 (s, 1.5H, CH_3–N), 0.77 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{40}N_2O_3$·$C_4H_4O_4$) C, H, N.

5.3.49. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6-(spirocyclopropane)androstane-17-one hydrochloride (49)

Prepared in 93% yield from 6-(spirocyclopropane)androstane-3,17-dione **68** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.55 (br b, 2H, NH_2^+), 3.95 (m, 2H, CH_2–O), 2.96 (m, 0.5H, H-2eq *E* isomer), 2.88 (m, 2H, CH_2–N), 2.62 (m, 0.5H, H-4eq *Z* isomer), 2.52 (s, 3H, CH_3–N), 0.96 (s, 1.5H, CH_3), 0.95 (s, 1.5H, CH_3), 0.79 (s, 3H, CH_3), 0.60–0.05 (m, 4H, cyclopropane); white solid. Anal. ($C_{25}H_{40}N_2O_2$·HCl) C, H, Cl, N.

5.3.50. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-methylandrostane-17-one hydrochloride (50)

Prepared in 79% yield from 6α-methylandrostane-3,17-dione **90** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.54 (br b, 2H, NH_2^+), 3.95 (t, 2H, CH_2–O), 2.96 (m, 0.5H, H-2eq *E* isomer), 2.88 (t, 2H, CH_2–N), 2.61 (m, 0.5H, H-4eq *Z* isomer), 2.52 (s, 3H, CH_3–N), 0.95 (s, 1.5H, CH_3), 0.94 (s, 1.5H, CH_3), 0.79 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{40}N_2O_2$·HCl) C, H, Cl, N.

5.3.51. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-ethynylandrostane-17-one hydrochloride (51)

Prepared in 70% yield from 6α-ethynylandrostane-3,17-dione **87** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.90 (br b, 2H, NH_2^+), 3.98 (m, 2H, CH_2–O), 3.44 (m, 0.5H, H-4eq *Z* isomer), 3.00 (m, 0.5H, H-2eq *E* isomer), 3.01 (d, 0.5H, C≡CH), 2.97 (d, 0.5H, C≡CH), 2.86 (m, 2H, CH_2–N), 2.49 (s, 1.5H, CH_3–N), 2.48 (s, 1.5H, CH_3–N), 0.87 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{38}N_2O_2$·HCl) C, H, Cl, N.

5.3.52. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6-[(*E*)-hydroxyimino]androstane-17-one hydrochloride (52)

Prepared in 72% yield from 6-[(*E*)-hydroxyimino]androstane-3,17-dione **103** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): δ 10.57 (s, 0.5H, N–OH), 10.53 (s, 0.5H, N–OH), 8.70 (br b, 2H, NH_2^+), 3.96 (m, 2H, CH_2–O), 2.51 (s, 1.5H, CH_3–N), 2.50 (s, 1.5H, CH_3–N), 0.77 (s, 6H, CH_3, CH_3); white solid. Anal. ($C_{23}H_{37}N_3O_3$·HCl) C, H, Cl, N.

5.3.53. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6-[(*E*)-methoxyimino]androstane-17-one hydrochloride (53)

Prepared in 60% yield from 6-[(*E*)-methoxyimino]androstane-3,17-dione **105** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.60 (br b, 2H, NH_2^+), 3.97 (m, 2H, CH_2–O), 3.75 (s, 1.5H, CH_3–O), 3.73 (s, 1.5H, CH_3–O), 3.19 (dd, 1H, H-7eq), 3.05 (m, 0.5H, H-4eq *Z* isomer), 2.99 (m, 0.5H, H-2eq *E* isomer), 2.90 (m, 2H, CH_2–N), 2.52 (s, 3H, CH_3–N), 0.78 (s, 1.5H, CH_3), 0.78 (s, 1.5H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{39}N_3O_3$·HCl) C, H, Cl, N.

5.3.54. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-hydroxymethylandrostane-17-one hydrochloride (54)

Prepared in 67% yield from 6α-hydroxymethylandrostane-3,17-dione **83** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.64 (br b, 2H, NH_2^+), 4.36 (t, 1H, OH), 3.96 (m, 2H, CH_2–ON), 3.33 (m, 2H, CH_2–O), 3.16 (m, 0.5H, H-4eq *Z* isomer), 2.97 (m, 0.5H, H-2eq *E* isomer), 2.89 (m, 2H, CH_2–N), 2.51 (s, 3H, CH_3-N), 0.88 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{40}N_2O_3$·HCl) C, H, Cl, N.

5.3.55. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-methoxymethylandrostane-17-one fumarate (55)

Prepared in 67% yield from 6α-methoxymethylandrostane-3,17-dione **85** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 10.00 (br b, 3H, NH_2^+, COOH); 6.44 (s, 2H, CH=CH), 3.95 (t, 2H, CH_2–O), 3.21 (s, 1.5H, CH_3–O), 3.20 (s, 1.5H, CH_3–O), 3.08 (m, 0.5H, H-4eq *Z* isomer), 2.96 (m, 0.5H, H-2eq *E* isomer), 2.85 (t, 2H, CH_2–N), 2.50 (s, 1.5H, CH_3-N), 2.48 (s, 1.5H, CH_3-N), 0.87 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{42}N_2O_3$·HCl) C, H, Cl, N.

5.3.56. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-formamidoandrostane-17-one hydrochloride (56)

Prepared in 70% yield from 6α-formamidoandrostane-3,17-dione **126** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.57 (br b, 2H, NH_2^+), 8.06–7.57 (m, 2H, NH-CH–O), 3.96 (m, 2H, CH_2–O), 3.72 (m, 1H, CH–N), 3.07 (m, 0.5H, H-4eq *Z* isomer), 2.97 (m, 0.5H, H-2eq *E* isomer), 2.88 (m, 2H, CH_2–N), 2.52 (s, 3H, CH_3-N), 0.93 (s, 1.5H, CH_3), 0.92 (s, 1.5H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{39}N_3O_3$·HCl) C, H, Cl, N.

5.3.57. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-carbamoylandrostane-17-one hydrochloride (57)

Prepared in 84% yield from 6α-carbamoylandrostane-3,17-dione **80** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS; the specific *E* and *Z* signal attributions were not assigned): 8.53 (br b, 2H, NH_2^+), 7.36 (br b, 0.5H, NH_2-CO), 7.32 (br b 0.5H, NH_2-CO), 6.79 (br b, 1H, NH_2-CO), 3.95 (m, 2H, CH_2–O), 2.54 (s, 1.5H, CH_3-N), 2.51 (s,

1.5H, CH_3-N), 0.89 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{24}H_{39}N_3O_3$·HCl) C, H, Cl, N.

5.3.58. (*E*,*Z*)-3-(3-*N*-Methylaminopropoxyimino)-6α-methoxycarbonylandrostane-17-one hydrochloride (58)

Prepared in 65% yield from 6α-methoxycarbonylandrostane-3,17-dione **81** and 3-*N*-methylaminopropoxyamine dihydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.36 (br b, 2H, NH_2^+), 3.95 (m, 2H, CH_2–O), 3.61 (s, 1.5H, CH_3–O), 3.60 (s, 1.5H, CH_3–O), 2.98 (m, 0.5H, H-2eq *E* isomer), 2.87 (m, 2H, CH_2–N), 2.77 (m, 0.5H, H-4eq *Z* isomer), 2.53 (s, 1.5H, CH_3-N), 2.52 (s, 1.5H, CH_3-N), 0.91 (s, 3H, CH_3), 0.78 (s, 3H, CH_3); white solid. Anal. ($C_{25}H_{40}N_2O_4$·HCl) C, H, Cl, N.

5.4. 3,17-Diketo intermediates

5.4.1. 6-Methyleneandrostane-3,17-dione (61)

To a stirred suspension of methyltriphenylphosphonium bromide (9.50 g, 26.6 mmol) in dry THF (77 mL) cooled at 0 °C under N_2, potassium *tert*-butoxide (2.91 g, 25.9 mmol) was added. After stirring for 10 min, a solution of 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (2.60 g, 6.6 mmol) in dry THF (77 mL) was added dropwise at room temperature over 0.5 h. After 0.5 h at room temperature, the mixture was quenched by addition of 5% NaH_2PO_4 aqueous solution and extracted with Et_2O (2 × 60 mL). The combined organic extracts were washed with 5% NaH_2PO_4 aqueous solution, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, cyclohexane/EtOAc 85/15) to give 3,3:17,17-bis(ethylendioxy)-6-methyleneandrostane **60** (2.66 g, 97%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.68 (m, 1H, C=CH_2), 4.36 (m, 1H, C=CH_2), 3.88–3.71 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.74 (s, 3H, CH_3), 0.62 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **60** (1.05 g, 2.7 mmol) was hydrolyzed according to the general method to give **61** in 87% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.85 (m, 1H, C=CH_2), 4.50 (m, 1H, C=CH_2), 0.92 (s, 3H, CH_3), 0.86 (s, 3H, CH_3).

5.4.2. (6*S*)-6-Spiro-(2′-oxirane)androstane-3,17-dione (62) and (6*R*)-6-spiro-(2′-oxirane)androstane-3,17-dione (63)

To a solution of 6-methyleneandrostane-3,17-dione **61** (0.76 g, 2.5 mmol) in CH_2Cl_2 (44 mL) stirred at 0 °C, MCPBA (0.933 mg, 5.4 mmol) was added in three portions over 0.5 h. After stirring at room temperature for 3 h the mixture was washed with 5% aqueous $NaHCO_3$ solution, 40% aqueous $NaHSO_3$ solution, 5% aqueous Na_2HPO_4 solution and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, toluene/acetone 95/5) to give the title compounds **62** (30%) and **63** (15%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): **62**: δ 2.79 (d, 1H, C–CH_2–O), 2.59 (d, 1H, C–CH_2–O), 1.16 (s, 3H, CH_3), 0.88 (s, 3H, CH_3); **63**: δ 1.17 (s, 3H, CH_3), 0.90 (s, 3H, CH_3).

5.4.3. 6β-Hydroxymethylandrostane-3,17-dione (65)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6-methylene-androstane **60** (2.89 g, 7.4 mmol) in dry THF (29 mL) at 0 °C under N_2, 1 M BH_3·THF complex in THF (5.21 mL, 5.21 mmol) was added. After completing the addition, the mixture was stirred at 0 °C for 3 h. H_2O (2.3 mL) was cautiously added dropwise followed by 3 N NaOH (3 mL) and 9.8 M H_2O_2 (0.91 mL). After stirring at room temperature overnight, H_2O (20 mL) was added. The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 45/55) to give 3,3:17,17-bis(ethylendioxy)-6β-hydroxymethylandrostane **64** (2.86 g, 95%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.94–3.75 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.52 (m, 2H, CH_2–O), 3.36 (t, 1H, OH), 0.84 (s, 3H, CH_3), 0.81 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **64** was hydrolyzed according to the general method to give **65** in 85% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.71–3.47 (m, 3H, CH_2–OH), 1.08 (s, 3H, CH_3), 0.89 (s, 3H, CH_3).

5.4.4. 6β-Methoxymethylandrostane-3,17-dione (67)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6β-hydroxymethylandrostane **64** (0.80 g, 1.9 mmol) in dry THF (11 mL) at 0 °C, under N_2, NaH (60% dispersion, 96 mg, 2.4 mmol) was added. After stirring the mixture at 0 °C for 1 h, CH_3I (144 μL, 0.33 g, 1.7 mmol) was added. After stirring overnight at room temperature, H_2O (10 mL) was added. The mixture extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/acetone 90/10) to give 3,3:17,17-bis(ethylendioxy)-6β-methoxymethylandrostane **66** (0.70 g, 84%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.32 (m, 2H, CH_2–O), 3.24 (s, 3H, CH_3–O), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **66** was hydrolyzed according to the general method to give **67** in 90% yield. The crude was purified by flash chromatography (SiO_2, *n*-hexane/CH_2Cl_2/acetone 70/10/20). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.45 (m, 2H, CH_2–O), 3.27 (s, 3H, CH_3–O), 1.10 (s, 3H, CH_3), 0.89 (s, 3H, CH_3).

5.4.5. 6-(Spirocyclopropane)androstane-3,17-dione (68)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6-methyleneandrostane **60** (200 mg, 0.51 mmol) in dry toluene (10 mL) under N_2, 1 M Et_2Zn in *n*-hexane (2.5 mL) was added. After heating at 60 °C, CH_2I_2 (0.42 mL, 1.4 g, 5.21 mmol) was added in portions over 15 min. After 26 h the mixture was cooled and quenched by careful addition of 1 N HCl. The suspension was extracted with Et_2O. The combined organic extracts were washed with 5% aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was dissolved in acetone (20 mL) and PTSA·H_2O (39 mg, 0.20 mmol) was added and the solution stirred at room temperature for 1 h. The solution was neutralized by addition of 5% aqueous $NaHCO_3$ and acetone was evaporated. The aqueous suspension was extracted with EtOAc. The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/CH_2Cl_2/EtOAc 90/5/5) to give the title compound **68** (78 mg, 48%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 1.17 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.60-(-0.15) (m, 4H, cyclopropane).

5.4.6. 6β-Methylandrostane-3,17-dione (71)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6β-hydroxymethylandrostane **64** (90 mg, 0.22 mmol) and DMAP (5 mg, 0.041 mmol) in CH_2Cl_2 (3 mL) under N_2, TCDI (78 mg, 0.44 mmol) was added. After stirring 2 h at 40 °C H_2O was added and the mixture was extracted with CH_2Cl_2 (2×). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness. The mixture was purified by flash chromatography (SiO_2, *n*-hexane/CH_2Cl_2/acetone 70/15/15) to give *O*-[3,3:17,17-bis(ethylendioxy)androstane-6β-ylmethyl] imidazole-1-carbothioate **69** (95 mg, 83%). ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.43 (dd, 1H, imidazole), 7.76 (dd, 1H, imidazole), 7.08 (dd, 1H, imidazole), 4.82 (dd, 1H, CH_2–O), 4.68 (dd, 1H, CH_2–O), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.84 (s, 3H, CH_3), 0.79 (s, 3H, CH_3).

To a stirred solution of Ph_3SnH (193 mg, 0.55 mmol) in dry toluene (2 mL) under Ar, AIBN (5 mg, 0.030 mmol) was added. After

stirring 20 min at 90 °C, a solution of **69** (95 mg, 0.18 mmol) in dry toluene (2 mL) was added dropwise. After stirring for 2 h at 110 °C, the mixture was evaporated to dryness and the residue purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 98/2) to give 3,3:17,17-bis(ethylendioxy)-6β-methylandrostane **70** (30 mg, 42%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.92 (s, 3H, CH_3), 0.88 (d, 3H, CH_3), 0.85 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **70** was hydrolyzed according to the general method to give **71** in 94% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 1.18 (s, 3H, CH_3), 0.98 (d, 3H, CH_3), 0.90 (s, 3H, CH_3).

5.4.7. 6α-Vinylandrostane-3,17-dione (75)

To a solution of 3,3:17,17-bis(ethylendioxy)-6β-hydroxymethylandrostane **64** (0.63 g, 1.5 mmol) in DMSO (6 mL), IBX (0.87 g, 3.1 mmol) was added and stirred at room temperature for 1 h. The mixture was quenched by addition of H_2O (30 mL) and Et_2O (30 mL). After stirring for 15 min, the mixture was filtered and the cake was washed with Et_2O. The layers were separated and the aqueous phase was extracted with Et_2O (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 75/35) to give 3,3:17,17-bis(ethylendioxy)-6β-formylandrostane **72** (0.52 g, 83%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 9.92 (d, 1H, CH–O), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.81 (s, 3H, CH_3), 0.77 (s, 3H, CH_3).

A mixture of 3,3:17,17-bis(ethylendioxy)-6β-formylandrostane **72** (0.61 g, 1.5 mmol), K_2CO_3 (0.90 g, 6.5 mmol) in MeOH (57 mL) was stirred overnight at room temperature. After evaporation, the residue was treated with H_2O (20 mL) and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine (3 × 20 mL), dried over Na_2SO_4 and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** (0.57 g, 94%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 9.41 (d, 1H, CH–O), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.90 (s, 3H, CH_3), 0.84 (s, 3H, CH_3).

3,3:17,17-Bis(ethylendioxy)-6α-vinylandrostane **74** was prepared in 70% yield from 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** according to the procedure described above for the preparation of 3,3:17,17-bis(ethylendioxy)-6-methyleneandrostane **60**. The crude was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 88/12). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.47 (m, 1H, C=CH), 4.91 (m, 2H, C=CH_2), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.88 (s, 3H, CH_3), 0.83 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **74** was hydrolyzed according to the general method to give **75** in 92% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.51 (m, 1H, C=CH), 4.97 (m, 2H, C=CH_2), 1.14 (s, 3H, CH_3), 0.98 (s, 3H, CH_3).

5.4.8. 6α-(2-Hydroxyethyl)androstane-3,17-dione (77)

3,3:17,17-Bis(ethylendioxy)-6α-(2-hydroxyethyl)androstane **76** was prepared in 96% yield from 3,3:17,17-bis(ethylendioxy)-6α-vinylandrostane **74** by the oxidative hydroboration according to the procedure described above for the preparation of 3,3:17,17-bis(ethylendioxy)-6β-hydroxymethylandrostane **64**. The crude was purified by flash chromatography (SiO_2, *n*-hexane/acetone 80/20). ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 4.25 (t, 1H, OH), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.35 (m, 2H, CH_2–O), 0.75 (s, 3H, CH_3), 0.74 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **76** was hydrolyzed according to the general method to give **77** in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 4.32 (t, 1H, OH), 3.39 (m, 2H, CH_2–O), 0.98 (s, 3H, CH_3), 0.79 (s, 3H, CH_3).

5.4.9. 6α-Carboxyandrostane-3,17-dione (79) and 6α-Carbamoylandrostane-3,17-dione (80)

6α-Formylandrostane-3,17-dione **78** was prepared in 85% yield from 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** by the Wittig reaction according to the procedure described above for the preparation of 6-methyleneandrostane-3,17-dione **61**. The combined organic extracts were washed with H_2O, dried over Na_2SO_4 and evaporated to dryness to give 6α-formylandrostane-3,17-dione **78**. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 9.50 (d, 1H, CH–O), 1.16 (s, 3H, CH_3), 0.88 (s, 3H, CH_3).

To a stirred suspension of 6α-formylandrostane-3,17-dione **78** (1.77 g, 5.6 mmol) in *t*-ButOH (35 mL) and 5% aqueous Na_2HPO_4 solution (21.5 mL), 1 N aqueous $KMnO_4$ (35 mL) was added. After five minutes at room temperature, the mixture was quenched by addition of 40% aqueous $NaHSO_3$ solution. The suspension was filtered, washed with H_2O and the filtrate was freeze-dried. The residue was taken up with H_2O (50 mL) and extracted with EtOAc (4 × 70 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness to give 6α-carboxyandrostane-3,17-dione **79** (1.80 g, 96%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 11.99 (br b, 1H, COOH), 1.01 (s, 3H, CH_3), 0.79 (s, 3H, CH_3).

To a stirred suspension of 6α-carboxyandrostane-3,17-dione **79** (1.20 g, 3.9 mmol) in dry toluene (12 mL), $SOCl_2$ (1.2 mL, 1.9 g, 118.97 mmol) was added. After stirring 5.5 h at 85 °C the solution was cooled at 0 °C and 2 M NH_3 solution in THF (6 mL) was added. After stirring overnight at room temperature, the mixture was evaporated to dryness. The residue was treated with CH_2Cl_2 and H_2O and extracted with CH_2Cl_2. The combined organic extracts were washed with 10% K_2CO_3 solution, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/acetone 50/50) to give the title compound **80** (720 mg, 60%). ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.27 (br s, 1H, NH-CO), 6.78 (br s, 1H, NH-CO), 1.00 (s, 3H, CH_3), 0.80 (s, 3H, CH_3).

5.4.10. 6α-Methoxycarbonylandrostane-3,17-dione (81)

To a stirred solution of 6α-carboxyandrostane-3,17-dione **79** (680 mg, 2.0 mmol) in CH_2Cl_2 (30 mL) at 0 °C, MeOH (160 μL), DMAP (20 mg, 0.16 mmol), and EDAC (800 mg, 4.1 mmol) were added. After stirring overnight at room temperature, H_2O was added and the mixture was extracted with CH_2Cl_2 (2×). The combined organic extracts were washed with H_2O, brine, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 60/40) to give the title compound **81** (500 mg, 70%). ^{1}H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 3.59 (s, 3H, CH_3–O), 1.02 (s, 3H, CH_3), 0.79 (s, 3H, CH_3).

5.4.11. 6α-Hydroxymethylandrostane-3,17-dione (83)

To a stirred suspension of 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** (0.52 g, 1.3 mmol) in dioxane/H_2O 9/1 (25 mL), $NaBH_4$ (49.0 mg, 1.3 mmol) was added and the mixture was stirred overnight at room temperature. To the solution NaCl was added and the layers were separated. The aqueous phase was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6α-hydroxymethylandrostane **82** (0.45 g, 86%) ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.57–3.25 (m, 3H, CH_2–OH), 0.86 (s, 3H, CH_3), 0.83 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **82** was hydrolyzed according to the general method to give **83** in 85% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.50 (m, 3H, CH_2–OH), 1.11 (s, 3H, CH_3), 0.88 (s, 3H, CH_3).

5.4.12. 6α-Methoxymethylandrostane-3,17-dione (85)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6α-hydroxymethylandrostane **82** (14, 0.80 g, 1,9 mmol) in dry THF (11 mL) at 0 °C, under N_2, NaH (60% dispersion, 96 mg, 2,4 mmol) was added. After stirring the mixture at 0 °C for 1 h, CH_3I (144 μL, 0.33 g, 1.7 mmol) was added. After stirring overnight at room temperature, H_2O (10 mL) was added and the mixture extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/acetone 90/10) to give 3,3:17,17-bis(ethylendioxy)-6α-methoxymethylandrostane **84** (0.70 g, 84%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.25 (dd, 1H, CH_2–O), 3.23 (s, 3H, CH_3–O), 3.14 (dd, 1H, CH_2–O), 0.85 (s, 3H, CH_3), 0.82 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **84** was hydrolyzed according to the general method to give **85** in 88% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.25 (s, 3H, CH_3–O), 3.24 (m, 2H, CH_2–O), 1.11 (s, 3H, CH_3), 0.87 (s, 3H, CH_3).

5.4.13. 6α-Ethynylandrostane-3,17-dione (87)

To a stirred solution of (chloromethyl)triphenylphosphonium chloride (1.20 g, 3.4 mmol) in dry THF (20 mL) at −78 °C under argon, 1.6 M *n*-butyllithium in *n*-hexane (1.5 mL) was added dropwise. After 30 min at room temperature, a solution of 3,3:17,17-bis(ethylendioxy)-6α-formylandrostane **73** (0.28 g, 0.7 mmol) in dry THF (7 mL) was added dropwise. The mixture was heated at 70 °C for 1 h and then cooled to room temperature. The mixture was quenched by addition of brine and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, and evaporated to dryness. The crude product was dissolved in dry THF (20 mL) and stirred at −78 °C. To the resulting solution 1.6 M *n*-butyllithium in *n*-hexane (2.24 mL) under argon was added dropwise. After 1 h at room temperature the mixture was quenched by addition of brine and extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6α-ethynylandrostane **86** (160 mg, 46%), sufficiently pure to be used in the next step without further purification. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.85 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 2.46 (d, 1H, C≡CH), 0.82 (s, 3H, CH_3), 0.86 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **86** was hydrolyzed according to the general method to give **87** in 46% yield. The residue was purified by flash chromatography (SiO_2, cyclohexane/CH_2Cl_2/acetone 80/10/10). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 2.56 (d, 1H, C≡CH), 1.12 (s, 3H, CH_3), 0.87 (s, 3H, CH_3).

5.4.14. 6α-Methylandrostane-3,17-dione (90)

To a stirred solution of DABCO (0.55 g, 4.9 mmol) and 3,3:17,17-bis(ethylendioxy)-6α-hydroxymethylandrostane **82** (1.00 g, 2.4 mmol) in dry CH_2Cl_2 (20 mL), under N_2 at 0 °C, *p*TSCl (0.703 g, 3.4 mmol) was added. After stirring 2 h at room temperature, the mixture was filtered and the cake was washed with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was triturated with *n*-hexane/EtOAc (60/40) and filtered. After drying under vacuum at 40 °C, 3,3:17,17-bis(ethylendioxy)-6α-[4-methyl(benzenesulfonyloxy)methyl]androstane **88** (1.11 g, 80%) was obtained. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.00–3.74 (m, 10H, O–CH_2CH_2–O, O–CH_2CH_2–O, CH_2–O), 0.82 (s, 3H, CH_3), 0.80 (s, 3H, CH_3).

To a stirred solution of $NaBH_4$ (0.15 g, 3.9 mmol) in dry DMSO (90 mL), under N_2, 3,3:17,17-bis(ethylendioxy)-6α-[4-methyl(benzenesulfonyloxy)methyl]androstane **88** (1.11 g, 1.9 mmol) was added in portions over 15 min. After stirring for 3 h at 80 °C, the mixture was quenched at room temperature by careful addition of H_2O (200 mL). The suspension was extracted with Et_2O. The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The mixture was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 90/10) to give 3,3:17,17-bis(ethylendioxy)-6α-methylandrostane **89** (0.70 g, 90%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.85 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.79 (d, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **89** was hydrolyzed according to the general method to give **90** in 94% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 1.18 (s, 3H, CH_3), 0.98 (d, 3H, CH_3), 0.90 (s, 3H, CH_3).

5.4.15. Methyl {3,17-dioxoandrostane-6-[(*E*)-ylidene]}acetate (92)

To a stirred solution of trimethylphosphonoacetate (5.17 mL, 5.8 g, 31.9 mmol) in DME (5.75 mL) at 0 °C under N_2 a 1.5 M solution of *tert*-butyllithium in *n*-pentane (18.5 mL) was added dropwise. After stirring 15 min at the same temperature a solution of 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (1.00 g, 2.5 mmol) in DME (15 mL) was added dropwise. The mixture was heated at 110 °C for 8 h and, after cooling, quenched by addition of H_2O and extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/CH_2Cl_2/acetone 80/10/10) to give {3,3:17,17-bis(ethylendioxy)androstane-6-[(*E*)-ylidene]}acetic acid methyl ester **91** (400 mg, 35%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS: δ 5.34 (s, 1H, C=CH), 4.05–3.75 (m, 9H, O–CH_2CH_2–O, O–CH_2CH_2–O, H-7eq), 3.62 (s, 3H, CH_3–O), 0.82 (s, 3H, CH_3), 0.72 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **91** was hydrolyzed according to the general method to give **92** in 70% yield. The residue was purified by flash chromatography (n-hexane/CH_2Cl_2/acetone 75/15/15). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS: δ 5.42 (br s, 1H, C=CH), 4.16 (dd, 1H, H-7eq), 3.66 (s, 3H, CH_3–O), 0.99 (s, 3H, CH_3), 0.87 (s, 3H, CH_3).

5.4.16. 6-[(*E*)-Ethyliden]androstane-3,17-dione (94)

3,3:17,17-Bis(ethylendioxy)-6-[(*E*)-ethyliden]androstane **93** was prepared in 96% yield from 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** and (ethyl)triphenylphosphonium bromide by the Wittig reaction according to the procedure described above for the preparation of 3,3:17,17-bis(ethylendioxy)-6-methyleneandrostane **60**. The mixture was purified by flash chromatography (SiO_2, cyclohexane/EtOAc 85/15). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.91 (m, 1H, C=CH), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 2.69 (m, 1H, H-7eq), 0.81 (s, 3H, CH_3), 0.66 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **93** was hydrolyzed according to the general method to give **94** in 96% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.99 (m, 1H, C=CH), 2.86 (dd, 1H, H-7eq), 0.93 (s, 3H, CH_3), 0.86 (s, 3H, CH_3).

5.4.17. 6-Difluoromethyleneandrostane-3,17-dione (96)

To a stirred solution of diethyl difluoromethylene phosphonate (0.67 μL) in DME (5.75 mL) in *n*-pentane (1.1 mL) at −78 °C, 1.5 M pentane solution of *tert*-butyllithium (2.75 mL) was added dropwise under argon. After 15 min at the same temperature, a solution of 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (0.50 g, 1.3 mmol) in DME (4.5 mL) and *n*-pentane (1.25 mL) was added dropwise. The mixture was stirred at −78 °C for further 30 min and warmed up to room temperature. *n*-Pentane was distilled off and after heating at 80 °C for 4 h the mixture was quenched with H_2O and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic

extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, cyclohexane/Et_2O 70/30) to give 3,3:17,17-bis(ethylendioxy)-6-difluoromethyleneandrostane **95** (0.47 g, 85%). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.85 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.83 (s, 3H, CH_3), 0.84 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **95** was hydrolyzed according to the general method to give **96** in 99% yield. ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 1.12 (s, 3H, CH_3), 0.88 (s, 3H, CH_3).

5.4.18. 3,17-Dioxoandrostane-6-[(*E*)-ylidene]acetonitrile (98)

To a stirred suspension of NaH (60% dispersion in mineral oil, 204 mg, 5.1 mmol) in THF (6 mL) at room temperature, diethyl cyanomethylphosphonate (895 μL, 980 mg, 5.5 mmol) was added. After stirring for 0.5 h 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (200 mg, 0.5 mmol) was added to the yellow mixture. After stirring at reflux for 2 h the mixture was quenched by addition of brine and extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/CH_2Cl_2/acetone 70/20/20) to give 3,3:17,17-bis(ethylendioxy)androstane-6-[(*E*)-ylidene]acetonitrile **97** (150 mg, 71%). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.03 (t, 1H, C=CH), 3.85 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 2.90 (dd, 1H, H-7eq), 0.84 (s, 3H, CH_3), 0.73 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **97** was hydrolyzed according to the general method to give **98** in 87% yield. ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.15 (s, 1H, C=CH), 3.04 (dd, 1H, H-7eq), 1.01 (s, 3H, CH_3), 0.90 (s, 3H, CH_3).

5.4.19. 6-[(*E*)-2-Hydroxyethylidene]androstane-3,17-dione (101)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)androstane-6-[(*E*)-ylidene]acetonitrile **97** (214 mg, 0.5 mmol) in dry CH_2Cl_2 (10 mL) at −78 °C under N_2, 1 M DIBAH in CH_2Cl_2 (1.56 mL) was added dropwise. The mixture was stirred at −78 °C for 1 h and then quenched by careful addition of a 2 M solution of isopropyl alcohol in toluene (0.80 mL). After 1 h, H_2O (70 μL) and THF (2.8 mL) were added and after an additional hour SiO_2 (0.76 g) and Na_2SO_4 (1.52 g) were added. The mixture was stirred for 1 h, filtered through a Celite pad and the filter cake washed with EtOAc. The filtrate was dried over Na_2SO_4, evaporated to give 3,3:17,17-bis(ethylendioxy)androstane-6-[(*E*)-ylidene]acetaldehyde **99** (0.18 g, 55%). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 9.98 (dd, 1H, CH–O), 5.46 (d, 1H, C=CH), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.35 (dd, 1H, H-7eq), 0.75 (s, 3H, CH_3), 0.64 (s, 3H, CH_3).

To a stirred suspension of 3,3:17,17-bis(ethylendioxy)androstane-6-[(*E*)-ylidene]acetaldehyde **99** (110 mg, 0.26 mmol) in MeOH (2.5 mL) at 0 °C, $NaBH_4$ (5 mg, 0.13 mmol) was added and the mixture was stirred for 1 h. Acetone (100 μL) was added and the mixture evaporated. The residue was treated with H_2O and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6-[(*E*)-2-hydroxyethylidene]androstane **100** (100 mg, 90%). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.07 (m, 1H, C=CH), 4.11 (m, 2H, CH_2–O), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.41 (t, 1H, OH), 2.67 (m, 1H, H-7eq), 0.80 (s, 3H, CH_3), 0.69 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **100** was hydrolyzed according to the general method to give **101** in 60% yield. The crude was purified by flash chromatography (SiO_2, *n*-hexane/acetone 70/30). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS: δ 5.14 (m, 1H, C=CH), 4.17 (m, 2H, CH_2–O), 3.53 (t, 1H, OH), 2.84 (dd, 1H, H-7eq), 0.97 (s, 3H, CH_3), 0.85 (s, 3H, CH_3).

5.4.20. 6-[(*E*)-Hydroxyimino]androstane-3,17-dione (103)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (1.10 g, 2.8 mmol) in THF (22 mL) a solution of $NH_2OH{\cdot}HCl$ (0.33 g, 4.7 mmol), $Na_2HPO_4{\cdot}12H_2O$ (1.71 g, 4.7 mmol) in H_2O (7.2 mL) was added. After stirring overnight at room temperature, NaCl was added and the mixture was extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6-[(*E*)-hydroxyimino]androstane **102** (1.08 g, 93%). ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 10.34 (s, 1H, N–OH), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.16 (dd, 1H, H-7eq), 0.74 (s, 3H, CH_3), 0.64 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **102** was hydrolyzed according to the general method to give **103** in 70% yield. The residue was purified by flash chromatography (SiO_2, *n*-hexane/acetone 70/30). ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 10.61 (s, 1H, N–OH), 3.29 (dd, 1H, H-7eq), 0.88 (s, 3H, CH_3), 0.79 (s, 3H, CH_3).

5.4.21. 6-[(*E*)-Methoxyimino]androstane-3,17-dione (105)

3,3:17,17-Bis(ethylendioxy)-6-[(*E*)-methoxyimino]androstane **104** was prepared from 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (1.00 g, 2.5 mmol) and $NH_2OCH_3{\cdot}HCl$ by the procedure described above for the preparation of 3,3:17,17-bis(ethylendioxy)-6-[(*E*)-hydroxyimino]androstane **103**. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6-[(*E*)-methoxyimino]androstane **104** (1.04 g, 97%). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.73 (s, 3H, CH_3–O), 3.22 (dd, 1H, H-7eq), 0.82 (s, 3H, CH_3), 0.75 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **104** was hydrolyzed according to the general method to give **105** in 70% yield. ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.78 (s, 3H, CH_3–O), 3.37 (dd, 1H, H-7eq), 1.01 (s, 3H, CH_3), 0.98 (s, 3H, CH_3).

5.4.22. 6-[(*E*)-Ethoxyimino]androstane-3,17-dione (107)

3,3:17,17-Bis(ethylendioxy)-6-[(*E*)-ethoxyimino]androstane **106** was prepared in 90% yield starting from 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** and $NH_2OCH_2CH_3{\cdot}HCl$ by the procedure described above for the preparation of 3,3:17,17-bis(ethylendioxy)-6-[(*E*)-hydroxyimino]androstane **103**. ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.99 (q, 2H, CH_2–O), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.25 (dd, 1H, H-7eq), 1.17 (t, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.75 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **106** was hydrolyzed according to the general method to give **107** in quantitative yield. ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.03 (q, 2H, CH_2–O), 3.40 (dd, 1H, H-7eq), 1.20 (t, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.87 (s, 3H, CH_3).

5.4.23. 6-[(*E*)-Allyloxyimino]androstane-3,17-dione (109)

Using the same reaction conditions described for the preparation of **103** and starting from 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (250 mg, 0.6 mmol) and *O*-allylhydroxylamine hydrochloride (140 mg, 1.27 mmol), 3,3:17,17-bis(ethylen-dioxy)-6-[(*E*)-allyloxyimino]androstane **108** was obtained (260 mg, 91%). ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.97 (m, 1H, C=CH), 5.22 (m, 1H, C=CH_2), 5.11 (m, 1H, C=CH_2), 4.47 (m, 2H, CH_2–O), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.28 (dd, 1H, H-7eq), 0.82 (s, 3H, CH_3), 0.75 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **108** was hydrolyzed according to the general method to give **109** in 70% yield. ^1H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.99 (m, 1H, C=CH), 5.25 (m, 1H, C=CH_2), 5.14 (m, 1H, C=CH_2), 4.52 (m, 2H, CH_2–O), 1.01 (s, 3H, CH_3), 0.88 (s, 3H, CH_3).

5.4.24. 6α-Cyanoandrostane-3,17-dione (111)

To a solution of toluene-4-sulfonylmethyl isocyanide (2.23 g, 11.4 mmol) in anhydrous DMSO (13 mL), stirred under N_2, potassium *tert*-butoxide (3.55 g, 31.5 mmol) was added. After stirring for 5 min, anhydrous MeOH (0.40 mL) was added dropwise, followed after 10 min by 3,3:17,17-bis(ethylendioxy)androstane-6-one **59** (3.27 g, 8.4 mmol). After 72 h at room temperature, the reaction was quenched by addition of H_2O and the mixture was neutralized by addition of 1 N HCl and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with H_2O, 5% $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 70/30) to give 6α-cyano-3,3:17,17-bis(ethylendioxy)androstane **110** (1.05 g, 31%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 2.60 (m, 1H, CH-CN), 0.89 (s, 3H, CH_3), 0.82 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **110** was hydrolyzed according to the general method to give **111** in 75% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 2.82 (ddd, 1H, CH-CN), 1.16 (s, 3H, CH_3), 0.87 (s, 3H, CH_3).

5.4.25. 3,17-Dioxoandrostane-6α-yl nitrate (114)

To a solution of acetic anhydride (2.53 mL, 2.7 g, 26.4 mmol) and 65% HNO_3 (0.592 mL) cooled at 0 °C, 3,3:17,17-bis(ethylendioxy)androstane-6α-ol[5] **112** (2.5 g, 6.4 mmol) was added in one portion. After 2 h the mixture was quenched by careful addition of ice and 5% aqueous $NaHCO_3$ solution and was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)androstane-6α-yl nitrate **113** as a white solid (2.50 g, 89%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 4.94 (m, 1H, CH–ON), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 0.98 (s, 3H, CH_3), 0.85 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **113** was hydrolyzed according to the general method to give **114** in 75% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.09 (ddd, 1H, CH–ON), 1.25 (s, 3H, CH_3), 0.90 (s, 3H, CH_3).

5.4.26. 6α-Methoxyandrostane-3,17-dione (116)

20% KH in oil (0.59 mg, 3.5 mmol) were washed with dry Et_2O (2 × 5 mL) in the reaction flask under nitrogen. The remaining solid was suspended in dry THF (5 mL) and 3,3:17,17-bis(ethylendioxy)androstane-6α-ol[5] **112** (1.15 g, 2.9 mmol) in THF (11 mL) was added dropwise. After 30 min CH_3I (220 μL, 3.5 mmol) was added in one portion. After 1 h the mixture was quenched with water and extracted with EtOAc. The organic extracts were dried over Na_2SO_4, and evaporated to dryness. The crude was purified by flash chromatography (SiO_2, cyclohexane/acetone 80/20) to give 6α-methoxy-3,3:17,17-bis(ethylendioxy)androstane **115** as a white solid (0.93 g, 79%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.78 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.18 (s, 3H, CH_3–O), 2.85 (m, 1H, CH–O), 0.76 (s, 3H, CH_3), 0.75 (s, 3H, CH_3).

A solution of 3,3:17,17-bis(ethylendioxy) derivative **115** (0.80 g, 1.96 mmol) and PTSA·H_2O (1.86 g) in acetone (8 mL) was stirred at room temperature for 1 h. The solution was added with water (7 L) and after 1 h acetone was evaporated. The aqueous suspension was extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness. The crude was purified by flash chromatography (SiO_2, cyclohexane/acetone 80/20) to give 6α-methoxyandrostane-3,17-dione **116** as a white solid (0.54 g, 86%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.22 (s, 3H, CH_3–O), 3.02 (ddd, 1H, CH–O), 0.99 (s, 3H, CH_3), 0.79 (s, 3H, CH_3).

5.4.27. 6α-Ethoxyandrostane-3,17-dione (118)

To a solution of 3,3:17,17-bis(ethylendioxy)androstane-6α-ol **112** (1.40 g, 3.56 mmol) in dry THF (35 mL) at 0 °C, 60% NaH in oil (0.45 mg, 11.0 mmol) was added. After 30 min C_2H_5I (0.86 mL, 10.8 mmol) was added and the mixture was heated at reflux for 5 h. After cooling the reaction was quenched with water and extracted with EtOAc. The organic extracts were dried over Na_2SO_4, and evaporated to dryness. The crude was purified by flash chromatography (SiO_2, cyclohexane/acetone 95/5) to give 6α-ethoxy-3,3:17,17-bis(ethylendioxy)androstane **117** as a white foam (0.78 g, 52%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.85 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.60–3.20 (m, 2H, CH_2–O), 3.01 (ddd, 1H, CH–O), 1.08 (t, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.82 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **117** was hydrolyzed according to the general method. The crude was purified by flash chromatography (SiO_2, cyclohexane/acetone 80/20) to give 6α-ethoxyandrostane-3,17-dione **118** as a white solid (0.50 g, 84%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.70–3.30 (m, 2H, CH_2–O), 3.20 (m, 1H, CH–O), 1.12 (s, 3H, CH_3), 1.11 (t, 3H, CH_3), 0.87 (s, 3H, CH_3).

5.4.28. 3,17-Dioxoandrostane-6β-yl nitrate (121)

3,3:17,17-Bis(ethylendioxy)androstane-6β-yl nitrate **120** was prepared in 50% yield from 3,3:17,17-bis(ethylendioxy)androstane-6β-ol[5] **119** following the procedure described above for the preparation of 3,3:17,17-bis(ethylendioxy)androstane-6α-yl nitrate **113**. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.16 (m, 1H, CH–ON), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 1.00 (s, 3H, CH_3), 0.85 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **120** was hydrolyzed according to the general method to give **121** in 75% yield. The crude product was purified by flash chromatography (SiO_2, cyclohexane/acetone/CH_2Cl_2 70/15/15) to give II-ab. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 5.24 (ddd, 1H), 2.72 (dd, 1H, CH–ON), 1.25 (s, 3H, CH_3), 0.90 (s, 3H, CH_3).

5.4.29. 6β-Methoxyandrostane-3,17-dione (123)

To a solution of 3,3:17,17-bis(ethylendioxy)androstane-6β-ol **119** (1.10 g, 2.8 mmol) in dry THF (16 mL) at 0 °C, 60% NaH in oil (0.45 mg, 3.3 mmol) was added. After 30 min CH_3I (0.29 μL, 4.5 mmol) was added. After stirring at room temperature for 4 days, the reaction was quenched with water and extracted with EtOAc. The organic extracts were dried over Na_2SO_4, and evaporated to dryness. The crude was purified by flash chromatography (SiO_2, cyclohexane/acetone 80/20) to give 6β-methoxy-3,3:17,17-bis(ethylendioxy)androstane **122** as a white foam (0.56 g, 49%). ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.85 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.21 (s, 3H, CH_3–O), 3.16 (m, 1H, CH–O), 0.95 (s, 3H, CH_3), 0.84 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **122** was hydrolyzed according to the general method to give **123** as a white foam in 95% yield. ^{1}H NMR (300 MHz, acetone-d_6, ppm from TMS): δ 3.27 (s, 3H, CH_3–O), 3.24 (m, 1H, CH–O), 1.18 (s, 3H, CH_3), 0.88 (s, 3H, CH_3).

5.4.30. 6α-Formamidoandrostane-3,17-dione (126)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6-(*E*)-hydroxyiminoandrostane **102** (0.88 g, 2.1 mmol) in *n*-PrOH (26 mL), Na (2.0 g) was added in small pieces over 20 min. The mixture was stirred at reflux for 2 h. After cooling to room temperature, the mixture was quenched by careful addition of MeOH. To the solution H_2O was added carefully and the organic solvent was evaporated. The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness. The mixture was puri-

fied by flash chromatography (SiO_2, $CHCl_3$/MeOH/26% NH_4OH 90/10/1) to give 3,3:17,17-bis(ethylendioxy)-6α-aminoandrostane **124** (0.45 g, 53%). ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 2.29 (m, 1H, CH–N), 0.75 (s, 3H, CH_3), 0.74 (s, 3H, CH_3).

A 2 M solution of formic acid in $CHCl_3$ (0.67 mL) was added dropwise to a solution of DCC (106 mg, 0.5 mmol) in $CHCl_3$ at 0 °C. The mixture was stirred for further 5 min and then added to an ice-cooled solution of 3,3:17,17-bis(ethylendioxy)-6α-aminoandrostane **124** (100 mg, 0.2 mmol) in pyridine (0.70 mL) over 30 min. The mixture was then stirred in an ice bath for 4 h. Evaporation of the solvent was followed by addition of Et_2O. The precipitate was removed by filtration and washed with Et_2O. The combined organic extracts were evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6α-formamidoandrostane **125** (100 mg, 95%). ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.98–7.43 (m, 2H, NH–CHO), 3.89–3.00 (m, 9H, O–CH_2CH_2–O, O–CH_2CH_2–O, CH–N), 0.81 (s, 3H, CH_3), 0.77 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **125** was hydrolyzed according to the general method to give **126** in 96% yield. ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 8.02–7.56 (m, 2H, NH–CHO), 3.74 (m, 1H, CH–N), 1.04 (s, 3H, CH_3), 0.80 (s, 3H, CH_3).

5.4.31. 6α-Acetamidoandrostane-3,17-dione (128)

To a stirred solution of 3,3:17,17-bis(ethylendioxy)-6α-aminoandrostane **124** (100 mg, 0.2 mmol) in dry pyridine (0.5 mL) at 0 °C, under N_2, $(CH_3CO)_2O$ (48 μL, 51.8 mg, 0.5 mmol) was added dropwise. The mixture was stirred at room temperature for 1.5 h and the solution was evaporated to dryness. The residue was taken up with H_2O and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness to give 3,3:17,17-bis(ethylendioxy)-6α-acetamidoandrostane **127** (103 mg, 94%). ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.55 (d, 1H, NH), 3.80 (m, 8H, O–CH_2CH_2–O, O–CH_2CH_2–O), 3.53 (m, 1H, CH–N), 1.75 (s, 3H, CH_3–CO), 0.80 (s, 3H, CH_3), 0.75 (s, 3H, CH_3).

The 3,3:17,17-bis(ethylendioxy) derivative **128** was hydrolyzed according to the general method to give **127** in 96% yield. ^1H NMR (300 MHz, DMSO-d_6, ppm from TMS): δ 7.61 (d, 1H, NH), 3.67 (m, 1H, CH–N), 1.78 (s, 3H, CH_3–CO), 1.04 (s, 3H, CH_3), 0.80 (s, 3H, CH_3).

5.5. Conformational energy calculations

The conformational analyses of the target compounds **1**–**58** in their *E* isomer form were performed using the OPLS2005 all-atom force field as implemented in the MACROMODEL 9.5 program; PR conjugate gradient was used in all the minimization steps, with the derivative convergence set to 0.05 (kJ/mol)/Å, with a maximum of 5000 iterations. The Monte Carlo multiple minimum method was used in the conformational search (OPLS2005, 5000 steps, torsion rotations allowed on the amino chain and, where appropriate, on the substituent in position 6 of the androstane scaffold). All conformations within 50 kJ/mol of the identified lowest energy conformer were minimized again (Multiple Minimization routine in the program) using OPLS2005 in conjunction with the GB/SA continuum model to simulate water solvation.

5.6. Biology

5.6.1. Na^+,K^+-ATPase inhibition

Na^+,K^+-ATPase was isolated and purified from dog kidney according to Jørghensen.[10] The inhibition of the enzyme activity was measured as % of hydrolysis of ^{32}P-ATP in the presence and in the absence of the tested compound.[11] The concentrations able to inhibit 50% (IC_{50}) of enzyme activity were calculated by a non-linear least-squares curve fitting computer program.

5.6.2. Inotropic and Toxic Effects in Anesthetized Guinea pigs

Male guinea pigs weighing 400-450 g were anesthetized with urethane (1.5 g/kg ip). Body temperature was maintained at 37°C by a homeothermic blanket system (Harward Apparatus). A microtip pressure transducer (Millar SPR-407, Houston TX) was introduced into the left ventricle through the right carotid artery to measure ventricular pressure (LVP). Recordings were fed to a Gould (RS 3800) polygraph and to an AST 486 computer and analyzed by IDAS software (Mangoni, Pisa, Italy). A polythene cannula (PE50) was inserted into a jugular vein for drug infusion and the trachea was intubated to facilitate spontaneous respiration. After a stabilization period, the test substance was injected at the rate of 0.16 mL/min by means of a Harvard P22 pump (Harward Apparatus) until the animal died or up to a maximum of 90 min. The doses inducing the maximum inotropic effect and death were determined. The compounds were dissolved in DMSO and the resulting solution diluted with physiological solution to obtain final solutions containing 1% DMSO. The starting concentration of the infused solution was based on the EC50 found in the electrically driven guinea pig atria. If no effect was found, the concentration was raised until satisfactory inotropic effect was found or death occurred.

Supplementary data

Supplementary data (elemental analysis results for target compounds and Table of CoMFA predictions of the Na^+,K^+-ATPase inhibitory values of the training set and of the test set) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.095.

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