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Synthesis and preliminary screening for the biological activity of some steroidal Δ⁴-unsaturated semicarbazone derivatives

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Abstract

Eleven new steroidal mono- and bis(semicarbazones) **2a–e, 4d** and **3a–e** have been prepared starting from various 3-oxo-α,β-unsaturated steroids. Mono-semicarbazones **2a–e** were further subjected to ethyl chloroacetate in boiling absolute ethanol but, instead of expected intramolecular cyclocondensation reaction products, the new carbazate esters **5a–e** were obtained. The structures of all synthesized compounds and identification of each *E/Z* isomer were deduced by elemental analysis, HRMS, NMR, and IR spectroscopy. Preliminary screening for the cytotoxic activity *in vitro* of the new compounds has been conducted against three cancer cell lines, K562, Jurkat and HeLa cells. HeLa cells were the most sensitive while K562 cells were the least sensitive to the cytotoxic action of the novel steroid derivatives. Compounds **2e, 3c** and **5e** were found to have the best but still moderate cytotoxic effects. All tested compounds showed very weak antimicrobial activities. These results demonstrate that the replacement of thioxo group with carbonyl group in steroidal hydrazone derivatives resulted in decrease in their biological activity.

Highlights

- The synthesis of new steroidal mono- and bis(semicarbazones) was performed
- The new carbazate esters were obtained
- The structures were determined by IR, NMR, HRMS and elemental analysis
- The antimicrobial and cytotoxic activities of the compounds were tested
- The replacement of thioxo group with carbonyl group in steroidal hydrazone derivatives resulted in decrease in their biological activity

1. Introduction

In recent years transformations of steroidal molecules have become one of the main area of interest in steroidal chemistry research. As a very important group of natural compounds, steroids play an essential role in some of the most fundamental biological functions in the human body [1]. Also, steroid based chemotherapeutics are widely used in the pharmacological industry. Therefore, certain functional and structural modifications of the steroid core can be very useful, giving compounds with new and more pronounced biological activity. It has been found so far that many of such modified steroidal derivatives, showing significant antibacterial, antiviral, antitumor or other biological activities, have been developed as drugs and anticancer agents [2]. For example, modifications involving changes in A- and A- and D-rings of steroidal molecule by the addition of heteroatom (N, S, O) or new functional group, have been reported to enhance the biological activities of steroidal compounds [3-8]. Within these studies, synthesis of new steroidal thiosemicarbazones, semicarbazones and hydrazones have received extensive attention of scientists [9-16].

The transformations of A- and A- and D-ring in series of 3-oxo-α,β-unsaturated steroids has also become the main topic of our research lately. Thus, earlier we reported a few papers on synthesis and biological activity of several sulfur-, and sulfur and phosphorus-containing steroidal compounds, including 3-thiones and 3,17-dithiones [17], new acyclic steroidal dimers where two identical steroid molecules were joined via ring A − ring A connection through various spacer groups (sulphur, 1,2,4-trithiolanes, phosphorotrithioates), as well as 17-spiro-, and 17-substituted-1,3,2-oxathiaphospholane-2-sulfides [18-20]. Recently we reported preparation and configuration analysis of new steroidal mono- and bis(thiosemicarbazones), and corresponding derivatives with heterocyclic rings, 3-spiro and 3,17-dispiro thiadiazolines (mono- and bis(1,3,4-thiadiazolines)) [21] and 3- and 3,17-substituted mono- and bis(thiazolidin-4-ones) [1] and their *in vitro* cytotoxic activity. It was found that 3-thiosemicarbazones and almost all examined thiazolidinone derivatives (both mono and bis) exerted selective concentration-dependent cytotoxic activities on six tested malignant cell lines. Among them HeLa and K562 cells were the most sensitive to the cytotoxic effects of these compounds. Additionally, these compounds showed a strong anti-angiogenic acitivity.

Based on these studies and in the context of the importance of developing new cancer drugs, the present work has been designed to synthesize new derivatives of 3-oxo-α,β-

unsaturated steroids, to evaluate and compare their *in vitro* cytotoxic activity with those reported earlier. Also, the aim of this paper was to examine the effect of heteroatom, S and O, incorporated in our investigated steroidal derivatives on their biological activity. For that purpose and as a continuation of our previous reserarch, in our current work we describe synthesis of new steroidal mono- and bis(semicarbazones) and corresponding derivatives, together with preliminary screening for their antimicrobial and cytotoxic activity.

2. Experimental

2.1. Chemistry

All synthetic reagents were analytically pure and solvents were prepared according to the standard procedures before being used. The starting steroid derivatives were purchased from Galenika AD (Belgrade, Serbia) and were recrystallized from a suitable solvent. Flash column chromatography (FCC) was carried out with Merck silica gel 0.040–0.063 mm. Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} plates. Melting points were determined on a Digital melting point WRS-1B apparatus and are uncorrected. IR spectra were recorded with Perkin-Elmer FT-IR 1725X spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 and/or CDCl$_3$ with Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125 MHz) and Varian Gemini-200 spectrometers (^{1}H at 200 MHz, ^{13}C at 50 MHz) and TMS was used as an internal reference. Chemical shifts (δ) are measured in ppm and coupling constants (*J*) in Hz. The homonuclear 2D (NOESY, COSY) and the heteronuclear 2D ^{1}H-^{13}C spectra (HSQC, HMBC) were recorded with the usual settings. The high-resolution mass spectra (HRMS) were taken with Agilent 6210 TOF LC/MS or Thermo Scientific HESI-LTQ Orbitrap XL spectrometers. Elemental analyses were performed on Vario EL *III*.

2.1.1. General procedure for the synthesis of semicarbazones and bis(semicarbazones)

To a solution of steroid (**1a–e**) (3 mmol) in dried ethanol (150 mL) semicarbazide (4 mmol) was added. The solution was then allowed to reflux for 1–2 h under stirring. After completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure. The residue was chromatographed by FCC using the indicated solvent system. In most of the cases the products were obtained as inseparable mixtures of *E* and *Z* diastereoisomers.

2.1.1.1. 19-Norandrost-4-ene-3,17-dione 3-semicarbazone (2a). The title compound was obtained starting from 19-norandrost-4-ene-3,17-dione (**1a**) (815 mg). Elution with CH_2Cl_2/MeOH (100/2.5) gave **2a** (466 mg, 47.1%). R_f = 0.28 (CH_2Cl_2/MeOH = 20:1). Mp > 200 °C (decomp.). IR (ATR/cm^{-1}): 3422 and 3146 (NH), 1732 (C=O), 1678 (C=O), 1566, 1427 (C=N), 1087. ESI-TOF-MS: *m/z* for $C_{19}H_{27}N_3O_2$ [M+H]$^+$: calcd 330.21760, found 330.21713; *m/z* for $C_{19}H_{27}N_3O_2$ [M+Na]$^+$: calcd 352.19955, found 352.20030; *m/z* for $C_{19}H_{27}N_3O_2$ [M+K]$^+$: calcd 368.17349 found 368.17497.

(2a-E) from the mixture *E/Z* = 55:45. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.71 (t, *J* = 10.5 Hz, 1H, H-9), 0.84 (s, 3H, H$_3$C-18), 0.97 (br.t, *J* = 9.5 Hz, 1H, Hα-7), 1.14–1.30 (m, 4H, Hα-1, Hβ-11, Hα-12, H-14), 1.40–1,54 (m, 2H, H-8, Hβ-15), 1.63 (br.d, *J* = 11 Hz, 1H, Hβ-12), 1.75–1.88 (m, 3H, Hβ-7, Hα-11, Hα-15), 1.90 (m, 1H, Hβ-2), 1.94–2.29 (m, 4H, Hβ-1, Hα-6, H-10, Hα-16), 2.34–2.43 (m, 2H, Hβ-6, Hβ-16), 2.68 (dt, *J* = 16.5, 4 Hz, 1H, Hα-2), 5.83 (s, 1H, H-4), 6.20 (br.s, 2H, NH$_2$), 9.11 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 219.7 (s, C-17), 157.3 (s, C=O), 148.5 (s, C-5), 147.2 (s, C-3), 121.9 (d, C-4), 49.5 (d, C-14), 49.0 (d, C-9), 47.1 (s, C-13), 40.7 (d, C-10), 39.2 (d, C-8), 35.3 (t, C-16), 34.1 (t, C-6), 31.2 (t, C-12), 29.8 (t, C-7), 25.8 (t, C-1), 25.3 (t, C-11), 22.6 (t, C-2), 21.2 (t, C-15), 13.4 (q, C-18).

(2a-Z) from the mixture *E/Z* = 55:45. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.71 (t, *J* = 10.5 Hz, 1H, H-9), 0.84 (s, 3H, H$_3$C-18), 0.97 (br.t, *J* = 9.5 Hz, 1H, Hα-7), 1.14–1.30 (m, 4H, Hα-1, Hβ-11, Hα-12, H-14), 1.40–1,54 (m, 2H, H-8, Hβ-15), 1.63 (br.d, *J* = 11 Hz, 1H, Hβ-12), 1.75–1.88 (m, 3H, Hβ-7, Hα-11, Hα-15), 1.94–2.29 (m, 6H, Hβ-1, Hα-2, Hβ-2, Hα-6, H-10, Hα-16), 2.34–2.43 (m, 2H, Hβ-6, Hβ-16), 6.52 (s, 1H, H-4), 6.15 (br.s, 2H, NH$_2$), 9.33 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 219.6 (s, C-17), 157.3 (s, C=O), 153.7 (s, C-5), 144.5 (s, C-3), 112.9 (d, C-4), 49.4 (d, C-14), 49.3 (d, C-9), 47.1 (s, C-13), 42.1 (d, C-10), 39.3 (d, C-8), 35.3 (t, C-16), 34.9 (t, C-6), 31.1 (t, C-12), 30.0 (t, C-2), 30.0 (t, C-7), 27.3 (t, C-1), 25.1 (t, C-11), 21.2 (t, C-15), 13.4 (q, C-18).

Further elution with CH_2Cl_2/MeOH (100/12) gave

2.1.1.2. 19-Norandrost-4-ene-3,17-dione bis(semicarbazone) (3a) (280 mg, 24.2%). R_f = 0.15 (CH_2Cl_2/MeOH = 20:1). Mp > 200 °C (decomp.). IR (ATR/cm^{-1}): 3461 and 3199 (NH), 2925, 1688 (C=O), 1572, 1467, 1420 (C=N), 1088. ESI-TOF-MS: *m/z* for $C_{20}H_{30}N_6O_2$ [M+H]$^+$: calcd 387.25030, found 387.24978; *m/z* for $C_{20}H_{30}N_6O_2$ [M+2H]$^{2+}$: calcd 194.12879, found 194.12894.

(*3a-E*) from the mixture *E*/*Z* = 1:1. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.75 (m, 1H, H-9), 0.85 (s, 3H, H$_3$C-18), 0.97 (td, *J* = 12.5, 3 Hz, 1H, Hα-7), 1.09, (m, 1H, H-14), 1.20–1.29 (m, 3H, Hα-1, Hβ-11, Hα-12), 1.31–1.43 (m, 2H, H-8, Hβ-15), 1.73–1.88 (m, 5H, Hβ-1, Hβ-7, Hα-11, Hβ-12, Hα-15), 1.92 (m, 1H, Hβ-2), 1.96 (m, 1H, H-10), 2.11–2.19 (m, 2H, Hα-6, Hα-16), 2.33–2.42 (m, 2H, Hβ-6, Hβ-16), 2.69 (dt, *J* = 16.5, 4 Hz, 1H, Hα-2), 5.83 (s, 1H, H-4), 6.09 (br.s, 2H, NH$_2$), 6.14 (br.s, 2H, NH$_2$), 8.71 (s, 1H, NH), 9.06 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 163.0 (s, C-17), 157.4 (s, C=O), 157.3 (s, C=O), 148.7 (s, C-5), 147.3 (s, C-3), 121.8 (d, C-4), 51.9 (d, C-14), 49.3 (d, C-9), 43.9 (s, C-13), 40.9 (d, C-10), 39.3 (d, C-8), 34.2 (t, C-6), 33.9 (t, C-12), 30.6 (t, C-7), 26.2 (t, C-16), 25.9 (t, C-1), 25.7 (t, C-11), 22.9 (t, C-15), 22.6 (t, C-2), 17.0 (q, C-18).

(*3a-Z*) from the mixture *E*/*Z* = 1:1. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.75 (m, 1H, H-9), 0.85 (s, 3H, H$_3$C-18), 0.97 (td, *J* = 12.5, 3 Hz, 1H, Hα-7), 1.09, (m, 1H, H-14), 1.20–1.29 (m, 3H, Hα-1, Hβ-11, Hα-12), 1.31–1.43 (m, 2H, H-8, Hβ-15), 1.73–1.88 (m, 5H, Hβ-1, Hβ-7, Hα-11, Hβ-12, Hα-15), 2.02 (m, 1H, H-10), 2.11–2.19 (m, 2H, Hα-6, Hα-16), 2.21 (m, 1H, Hβ-2), 2.33–2.42 (m, 2H, Hβ-6, Hβ-16), 2.29 (dt, *J* = 14.5, 4 Hz, 1H, Hα-2), 6.09 (br.s, 2H, NH$_2$), 6.20 (br.s, 2H, NH$_2$), 6.51 (s, 1H, H-4), 8.71 (s, 1H, NH), 9.29 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 162.9 (s, C-17), 157.4 (s, C=O), 157.2 (s, C=O), 153.9 (s, C-5), 144.6 (s, C-3), 112.8 (d, C-4), 51.8 (d, C-14), 49.5 (d, C-9), 43.9 (s, C-13), 42.3 (d, C-10), 40.4 (d, C-8), 35.0 (t, C-6), 33.8 (t, C-12), 30.4 (t, C-7), 30.1 (t, C-2), 27.3 (t, C-1), 26.2 (t, C-16), 25.5 (t, C-11), 22.9 (t, C-15), 17.0 (q, C-18).

2.1.1.3. Androst-4-ene-3,17-dione 3-semicarbazone (2b). The title compound was obtained starting from androst-4-ene-3,17-dione (**1b**) (860 mg). Elution with CH$_2$Cl$_2$/MeOH (100/2.5) gave **2b** (520 mg, 50.5%). R_f = 0.30 (CH$_2$Cl$_2$/MeOH = 20:1). Mp > 205 °C (decomp.). IR (ATR/cm^{-1}): 3448 and 3199 (NH), 2937, 1739 (C=O), 1682 (C=O), 1577, 1428 (C=N), 1088. ESI-TOF-MS: *m/z* for C$_{20}$H$_{29}$N$_3$O$_2$ [M+H]$^+$: calcd 344.23325, found 344.23283.

(*2b-E*) from the mixture *E*/*Z* = 8:2. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.81 (s, 3H, H$_3$C-18), 0.82 (m, 1H, H-9, overlapped with H$_3$C-18), 0.93 (qd, *J* = 13, 3.5 Hz, 1H, Hα-7), 1.01 (s, 3H, H$_3$C-19), 1.15 (td, *J* = 12.5, 3.5 Hz, 1H, Hα-12), 1.21–1.30 (m, 2H, Hα-1, H-14), 1.35 (m, 1H, Hβ-11), 1.49 (dtd, *J* = 3, 3.5, 3 Hz, 1H, Hβ-15), 1.57 (m, 1H, Hα-11), 1.61–1.68 (m, 2H, Hβ-1, Hβ-12), 1.79–1.89 (m, 3H, Hβ-7, H-8, Hα-15), 1.92–2.03 (m, 2H, Hβ-2 , Hα-16), 2.18 (dt,

J = 12, 3.5 Hz, 1H, Hα-6), 2.31 (td, J = 14, 4.5 Hz, 1H, Hβ-6), 2.38 (dd, J = 19, 8.5 Hz, 1H, Hβ-16), 2.65 (dt, J = 17, 3 Hz, 1H, Hα-2), 5.73 (s, 1H, H-4), 6.19 (br.s, 2H, NH$_2$), 9.06 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 219.6 (s, C-17), 157.2 (s, C=O), 152.8 (s, C-5), 146.8 (s, C-3), 121.2 (d, C-4), 53.2 (d, C-9), 50.2 (d, C-14), 46.9 (s, C-13), 37.2 (s, C-10), 35.3 (d, C-16), 34.7 (d, C-8), 34.5 (t, C-1), 31.4 (t, C-6), 31.2 (t, C-12), 30.7 (t, C-7), 21.4 (t, C-15), 20.4 (t, C-11), 20.3 (t, C-2), 17.5 (q, C-19), 13.4 (q, C-18).

(**2b-Z**) from the mixture E/Z = 8:2**.** ^{1}H-NMR (500 MHz, DMSO-d_6): 0.81 (s, 3H, H$_3$C-18), 0.82 (m, 1H, H-9, overlapped with H$_3$C-18), 0.93 (qd, J = 13, 3.5 Hz, 1H, Hα-7), 1.07 (s, 3H, H$_3$C-19), 1.15 (td, J = 12.5, 3.5 Hz, 1H, Hα-12), 1.25 (m, 1H, H-14), 1.31 (m, 1H, Hα-1), 1.49 (dtd, J = 3, 3.5, 3 Hz, 1H, Hβ-15), 1.57 (m, 1H, Hβ-11), 1.61–1.68 (m, 2H, Hβ-1, Hβ-12), 1.79–1.89 (m, 3H, Hβ-7, H-8, Hα-15), 1.92–2.03 (m, 2H, Hα-11, Hα-16), 2.18 (dt, J = 12, 3.5 Hz, 1H, Hα-6), 2.19 (m, 1H, Hβ-2), 2.33 (m, 1H, Hβ-6), 2.38 (dd, J = 19, 8.5 Hz, 1H, Hβ-16), 2.29 (m, 1H, Hα-2), 6.12 (br.s, 2H, NH$_2$), 6.43 (s, 1H, H-4), 9.30 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 219.6 (s, C-17), 157.8 (s, C-5), 157.2 (s, C=O), 143.9 (s, C-3), 112.0 (d, C-4), 53.5 (d, C-9), 50.1 (d, C-14), 46.9 (s, C-13), 38.3 (s, C-10), 36.1 (t, C-1), 35.3 (d, C-16), 34.6 (d, C-8), 32.1 (t, C-6), 31.2 (t, C-12), 31.1 (t, C-7), 27.3 (t, C-2), 21.4 (t, C-15), 20.1 (t, C-11), 17.8 (q, C-19), 13.4 (q, C-18).

Further elution with CH$_2$Cl$_2$/MeOH (100/12) gave

*2.1.1.4. Androst-4-ene-3,17-dione bis(semicarbazone) (**3b**)* (162 mg, 13.5%). R_f = 0.15 (CH$_2$Cl$_2$/MeOH = 20:1). Mp > 200 °C (decomp.). IR (ATR/cm^{-1}): 3458 and 3320 (NH), 2937, 1666 (C=O), 1573, 1475, 1426 (C=N), 1122, 1088. ESI-TOF-MS: *m/z* for C$_{21}$H$_{32}$N$_6$O$_2$ [M+H]$^+$: calcd 401.26595, found 401.26542; *m/z* for C$_{21}$H$_{32}$N$_6$O$_2$ [M+2H]$^{2+}$: calcd 201.13661, found 201.13684.

(**3b-E**) from the mixture E/Z = 55:45. ^{1}H-NMR (500 MHz, CDCl$_3$/CD$_3$OD): 0.94 (s, 3H, H$_3$C-18), 0.95 (m, 1H, H-9, overlapped with H$_3$C-18), 0.95 (td, J = 12.5, 4 Hz, 1H, Hα-7), 1.13 (s, 3H, H$_3$C-19), 1.18, (m, 1H, H-14, overlapped with H$_3$C-19 from *Z*-isomer), 1.37 (m, 1H, Hα-12), 1.43–1.58 (m, 3H, Hα-1, Hβ-11, Hβ-15), 1.61–1.75 (m, 2H, H-8, Hα-11), 1.85–2.05 (m, 4H, Hβ-1, Hβ-7, Hβ-12, Hα-15), 2.15 (m, 1H, Hβ-2), 2.22 (dd, J = 18.5, 9 Hz, 1H, Hα-16), 2.29 (br.t, J = 2 Hz, 1H, Hα-6) 2.36 (br.t, J = 3 Hz, 1H, Hβ-6), 2.42 (dd, J = 18.5, 8.5 Hz, 1H, Hβ-16), 2.60 (dt, J = 16.5, 4 Hz, 1H, Hα-2), 5.83 (s, 1H, H-4). ^{13}C NMR (125 MHz, CDCl$_3$/CD$_3$OD): 165.0 (s, C-17), 158.5 (s, C=O), 158.3 (s, C=O), 154.9 (s, C-5), 149.1 (s, C-

3), 120.3 (d, C-4), 53.5 (d, C-9), 52.7 (d, C-14), 43.8 (s, C-13), 37.3 (s, C-10), 34.8 (d, C-8), 34.3 (t, C-1), 33.5 (t, C-12), 31.6 (t, C-6), 31.1 (t, C-7), 25.2 (t, C-16), 22.9 (t, C-15), 20.4 (t, C-11), 19.8 (t, C-2), 17.0 (q, C-19), 16.2 (q, C-18).

(*3b-Z*) from the mixture E/Z = 55:45. ^{1}H-NMR (500 MHz, CDCl$_3$/CD$_3$OD): 0.94 (s, 3H, H$_3$C-18), 0.95 (m, 1H, H-9, overlapped with H$_3$C-18), 0.95 (td, J = 12.5, 4 Hz, 1H, Hα-7), 1.18 (s, 3H, H$_3$C-19), 1.18, (m, 1H, H-14, overlapped with H$_3$C-19), 1.37 (m, 1H, Hα-12), 1.43–1.58 (m, 3H, Hα-1, Hβ-11, Hβ-15), 1.61–1.75 (m, 2H, H-8, Hα-11), 1.85–2.05 (m, 4H, Hβ-1, Hβ-7, Hβ-12, Hα-15), 2.22 (dd, J = 18.5, 9 Hz, 1H, Hα-16), 2.32 (m, 1H, Hα-6), 2.36 (m, 1H, Hβ-2, overlapped with Hβ-6 from *E*-isomer), 2.42 (dd, J = 18.5, 8.5 Hz, 1H, Hβ-16), 2.43 (m, 1H, Hα-2), 2.48 (m, 1H, Hβ-6), 6.15 (s, 1H, H-4). ^{13}C NMR (125 MHz, CDCl$_3$/ CD$_3$OD): 164.8 (s, C-17), 162.2 (s, C=O), 160.6 (s, C-5), 158.3 (s, C=O), 147.5 (s, C-3), 110.2 (d, C-4), 53.9 (d, C-9), 52.6 (d, C-14), 43.9 (s, C-13), 38.6 (s, C-10), 36.0 (t, C-1), 34.7 (d, C-8), 33.4 (t, C-12), 32.5 (t, C-6), 31.6 (t, C-7), 27.2 (t, C-2), 25.2 (t, C-16), 22.9 (t, C-15), 20.2 (t, C-11), 17.5 (q, C-19), 16.3 (q, C-18).

2.1.1.5. (E)-Androsta-4,9(11)-diene-3,17-dione 3-semicarbazone (2c). The title compound was obtained starting from androsta-4,9(11)-diene-3,17-dione (**1c**) (850 mg). Elution with CH$_2$Cl$_2$/MeOH (100/5) gave **2c** as a pure *E*-isomer (780 mg, 76%). R_f = 0.30 (CH$_2$Cl$_2$/MeOH = 20:1). Mp > 205 °C (decomp.). IR (ATR/cm^{-1}): 3427 and 3211 (NH), 2927, 1734 (C=O), 1686 (C=O), 1592, 1473 (C=N), 1089. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.78 (s, 3H, H$_3$C-18), 0.96 (qd, J = 13, 3.5 Hz, 1H, Hα-7), 1.18 (s, 3H, H$_3$C-19), 1.50 (m, H-14), 1.56 (dtd, J = 3, 3.5, 3 Hz, 1H, Hβ-15), 1.68 (td, J = 13.5, 4.5 Hz, 1H, Hα-12), 1.90 (m, 1H, Hα-1), 1.98–2.05 (m, 4H, Hβ-1, Hβ-7, Hβ-12, Hα-15), 2.06–2.17 (m, 2H, Hβ-2, Hα-16), 2.26 (dt, J = 11.5, 3 Hz, 1H, Hα-6), 2.34 (br.t, J = 11 Hz, 1H, H-8), 2.40 (dd, J = 18.5, 8.5 Hz, 1H, Hβ-16), 2.50 (m, 1H, Hβ-6), 2.73 (dt, J = 17, 3.5 Hz, 1H, Hα-2), 5.48 (d, J = 6 Hz, 1H, H-11), 5.74 (s, 1H, H-4), 6.24 (bs, 2H, NH$_2$), 9.18 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 220.3 (s, C-17), 157.2 (s, C=O), 151.1 (s, C-5), 146.6 (s, C-3), 146.3 (s, C-9), 121.3 (d, C-4), 116.3 (d, C-11), 47.3 (d, C-14), 45.2 (s, C-13), 39.8 (s, C-10), 36.6 (d, C-8), 35.8 (d, C-16), 33.1 (t, C-1), 32.7 (t, C-12), 31.4 (t, C-6), 31.1 (t, C-7), 26.3 (q, C-19), 22.3 (t, C-15), 20.8 (t, C-2), 13.6 (q, C-18). ESI-TOF-MS: m/z for C$_{20}$H$_{27}$N$_3$O$_2$ [M+H]$^+$: calcd 342.21760, found 342.21731; m/z for C$_{20}$H$_{27}$N$_3$O$_2$ [M+Na]$^+$: calcd 364.19955, found 364.20002.

Further elution with CH$_2$Cl$_2$/MeOH (100/20) gave

2.1.1.6. Androsta-4,9(11)-diene-3,17-dione bis(semicarbazone) (3c). Yield (120 mg, 10%). R_f = 0.15 (CH$_2$Cl$_2$/MeOH = 20:1). Mp > 200 °C (decomp.). IR (ATR/cm^{-1}): 3463 and 3202 (NH), 1685(C=O), 1581, 1473, 1428 (C=N), 1089. ESI-TOF-MS: *m/z* for C$_{21}$H$_{30}$N$_6$O$_2$ [M+H]$^+$: calcd 399.25030, found 399.24961; *m/z* for C$_{21}$H$_{30}$N$_6$O$_2$ [M+2H]$^{2+}$: calcd 200.12879, found 200.12900; *m/z* for C$_{21}$H$_{30}$N$_6$O$_2$ [M+Na]$^+$: calcd 421.23225, found 421.23209.

(3c-E) from the mixture E/Z = 85:15. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.79 (s, 3H, H$_3$C-18), 0.96 (qd, *J* = 13, 3.5 Hz, 1H, Hα-7), 1.17 (s, 3H, H$_3$C-19), 1.26 (m, H-14), 1.43 (dtd, *J* = 3, 3, 3 Hz, 1H, Hβ-15), 1.68 (td, *J* = 13.5, 5 Hz, 1H, Hα-1), 1.88–2.02 (m, 3H, Hβ-1, Hβ-7, Hα-15), 2.06–2.13 (m, 3H, Hβ-2, Hα-12, Hβ-12), 2.18 (dd, *J* = 19, 9.5 Hz, 1H, Hα-16), 2.21 (m, 1H, H-8), 2.24 (dt, *J* = 18, 3 Hz, 1H, Hα-6), 2.44 (dd, *J* = 18.5, 9 Hz, 1H, Hβ-16), 2.48 (m, 1H, Hβ-6), 2.74 (dt, *J* = 17, 3.5 Hz, 1H, Hα-2), 5.48 (d, *J* = 5 Hz, 1H, H-11), 5.75 (s, 1H, H-4), 6.14 (br.s, 2H, NH$_2$), 6.24 (s, 2H, NH$_2$), 8.81 (s, 1H, NH), 9.15 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 162.6 (s, C-17), 157.4 (s, C=O), 157.3 (s, C=O), 151.4 (s, C-5), 146.6 (s, C-3), 146.1 (s, C-9), 121.3 (d, C-4), 117.0 (d, C-11), 49.7 (d, C-14), 42.2 (s, C-13), 39.7 (s, C-10, overlapped with DMSO), 36.4 (t, C-8), 36.2 (d, C-12), 32.6 (t, C-1), 31.7 (t, C-6), 31.5(t, C-7), 26.6 (t, C-16), 26.2 (q, C-19), 24.0 (t, C-15), 20.8 (t, C-2), 17.1 (q, C-18).

(3c-Z) from the mixture E/Z = 85:15. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.79 (s, 3H, H$_3$C-18), 0.96 (qd, *J* = 13, 3.5 Hz, 1H, Hα-7), 1.23 (s, 3H, H$_3$C-19), 1.26 (m, H-14), 1.43 (dtd, *J* = 3, 3, 3 Hz, 1H, Hβ-15), 1.79 (td, *J* = 13.5, 3.5 Hz, 1H, Hα-1), 1.88–2.02 (m, 3H, Hβ-1, Hβ-7, Hα-15), 2.06–2.10 (m, 1H, Hα-12, Hβ-12), 2.18 (dd, *J* = 19, 9.5 Hz, 1H, Hα-16), 2.21 (m, 1H, H-8), 2.24 (dt, *J* = 18, 3 Hz, 1H, Hα-6), 2.32 (t, *J* = 3.5 Hz, 1H, Hβ-2), 2.38 (m, 1H, Hα-2), 2.44 (dd, *J* = 18.5, 9 Hz, 1H, Hβ-16), 2.48 (m, 1H, Hβ-6), 5.48 (d, *J* = 5 Hz, 1H, H-11), 6.14 (br.s, 2H, NH$_2$), 6.18 (s, 2H, NH$_2$), 6.45 (s, 1H, H-4), 8.81 (s, 1H, NH), 9.35 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 162.6 (s, C-17), 157.4 (s, C=O), 157.3 (s, C=O), 156.4 (s, C-5), 146.1 (s, C-9), 144.2 (s, C-3), 117.0 (d, C-11), 112.3 (d, C-4), 49.7 (d, C-14), 42.2 (s, C-13), 41.0 (s, C-10, overlapped with DMSO), 36.4 (t, C-8), 36.2 (d, C-12), 34.4 (t, C-1), 32.1 (t, C-6), 31.5 (t, C-7), 27.6 (t, C-2), 26.6 (t, C-16), 26.6 (q, C-19), 24.0 (t, C-15), 17.1 (q, C-18).

2.1.1.7. 11α-Hydroxyandrost-4-ene-3,17-dione 3-semicarbazone (2d). The title compound was obtained starting from androst-4-ene-11α-ol-3,17-dione (**1d**) (910 mg). Elution with CH$_2$Cl$_2$/MeOH (100/5) gave **2d** (163 mg, 15.1%). R_f = 0.53 (CH$_2$Cl$_2$/MeOH = 20:2). Mp > 218 °C (decomp.). IR (ATR/cm^{-1}): 3456 (OH), 3209 (NH), 2929, 1731 (C(17)=O), 1689

(C=O), 1572 and 1458, (C=N), 1087, 730. HESI-Orbitrap MS: *m/z* for $C_{20}H_{29}N_3O_3$ [M+H]$^+$: calcd 360.2282, found 360.2264

(**2d-E**) from the mixture *E/Z* = 80:20. ^{1}H-NMR (200 MHz, CDCl$_3$): 0.93 (s, 3H, H$_3$C-18), 1.00–1.15 (m, 2H, Hα-7, H-9), 1.21 (s, 3H, H$_3$C-19), 1.26–1.49 (m, 2H), 1.52–1.75 (m, 3H), 1.81–2.36 (m, 9H), 2.40–2.59 (m, 2H) 2.66 (dt, *J* = 14, 3.2 Hz, 1H, Hα-1), 4.01 (td, *J* = 10.8, 4.6 Hz, 1H, Hβ-11), 5.83 (s, 1H, H-4), 8.31 (s, 1H, NH). ^{13}C NMR (50 MHz, CDCl$_3$): 219.0 (s, C-17), 157.9 (s, C=O), 155.0 (s, C-5), 148.8 (s, C-3), 121.7 (d, C-4), 68.7 (d, C-11), 59.1 (d, C-9), 50.1 (d, C-14), 48.0 (s, C-13), 48.0 (t, C-12), 39.0 (s, C-10), 35.8 (t, C-16), 34.7 (d, C-8), 34.5 (t, C-1), 32.8 (t, C-6), 30.3 (t, C-7), 21.6 (t, C-15), 20.6 (t, C-2), 18.5 (q, C-19), 14.6 (q, C-18).

(**2d-Z**) from the mixture *E/Z* = 80:20. ^{1}H-NMR (200 MHz, CDCl$_3$): 0.93 (s, 3H, H$_3$C-18), 1.00–1.15 (m, 2H, Hα-7, H-9), 1.26 (s, 3H, H$_3$C-19), 1.26–1.49 (m, 2H), 1.52–1.75 (m, 3H), 1.81–2.36 (m,9H), 2.40–2.59 (m, 3H), 4.01 (td, *J* = 10.8, 4.6 Hz, 1H, Hβ-11), 6.15 (s, 1H, H-C(4)), 8.09 (s, 1H, NH). ^{13}C NMR (50 MHz, CDCl$_3$): 219.0 (s, C-17), 157.9 (s, C=O), 155.0 (s, C-5), 148.8 (s, C-3), 111.8 (d, C-4), 68.7 (d, C-11), 59.1 (d, C-9), 50.1 (d, C-14), 48.0 (s, C-13), 48.0 (t, C-12), 39.0 (s, C-10), 35.8 (t, C-16), 34.7 (d, C-8), 34.5 (t, C-1), 32.8 (t, C-6), 30.3 (t, C-7), 27.7 (t, C-2), 21.6 (t, C-15), 18.5 (q, C-19), 14.6 (q, C-18).

Further elution with the same eluent gave

*2.1.1.8. (E)-11α-Hydroxyandrost-4-ene-3,17-dione 17-semicarbazone (**4d**)* as a pure *E*-isomer (147 mg, 13.6%). R_f = 0.44 (CH$_2$Cl$_2$/MeOH = 20:2). Mp > 215 °C (decomp.). IR (ATR/cm^{-1}): 3456 (OH), 3196 (NH), 2940, 1695 (C=O), 1638 (C(3)=O), 1576 and 1415, (C=N), 1083. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.83 (s, 3H, H$_3$C-18), 1.01 (qd, *J* = 13, 3 Hz, 1H, Hα-7), 1.06 (t, *J* = 11.5 Hz, 1H, H-9), 1.13 (m, 1H, H-14), 1.25 (s, 3H, H$_3$C-19), 1.26 (m, 1H, Hα-12, overlapped with H$_3$C-19), 1.35 (dtd, *J* = 3, 2.5, 3 Hz, 1H, Hβ-15), 1.60 (qd, *J* = 11, 3 Hz, 1H, H-8), 1.75 (m, 1H, Hα-15), 1.82 (br.d, *J* = 12.5 Hz, 1H, Hβ-7), 1.93 (td, *J* = 14, 4.5 Hz, 1H, Hβ-2), 2.09 (dd, *J* = 12, 4 Hz, 1H, Hβ-12), 2.14 (t, *J* = 4 Hz, 1H, Hα-1), 2.17–2.26 (m, 2H, Hα-6, Hα-16), 2.28–2.44 (m, 3H, Hβ-6, Hβ-16, Hβ-1), 2.58 (dt, *J* = 14.5, 4 Hz, 1H, Hα-2), 3.85 (m, 1H, Hβ-11), 4.35 (d, *J* = 7.5 Hz, 1H,OH-11), 5.61 (s, 1H, H-4), 6.08 (bs, 2H, NH$_2$), 8.73 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 198.6 (s, C-3), 171.5 (s, C-5), 162.1 (s, C-17), 157.3 (s, C=O), 123.6 (d, C-4), 67.2 (d, C-11), 58.6 (d, C-9), 51.9 (d, C-14), 45.1 (t, C-12), 44.2 (s, C-13), 39.5 (s, C-10), 36.9 (t, C-2), 33.9 (t, C-1), 33.8 (d, C-8), 32.8 (t, C-6), 30.9

(t, C-7), 26.3 (t, C-16), 22.9 (t, C-15), 18.0 (q, C-19), 17.9 (q, C-18). HESI-Orbitrap MS: *m/z* for $C_{10}H_{29}N_3O_3$ [M+H]$^+$: calcd 360.2282, found 360.2264.

Further elution with CH_2Cl_2/MeOH (100/30) gave

2.1.1.9. (E)-11α-Hydroxyandrost-4-ene-3,17-dione bis(semicarbazone) (3d) as a pure *E*-isomer (215 mg, 17.2%). R_f = 0.21 (CH_2Cl_2/MeOH = 20:2). Mp > 223 °C (decomp.). IR (ATR/cm^{-1}): 3457 (OH), 3284 (NH), 2927, 1681 (C=O), 1571 and 1420, (C=N), 1082. ^{1}H-NMR (200 MHz, CDCl$_3$/CD$_3$OD): 0.76 (s, 3H, H$_3$C-18), 0.85–0.99 (m, 2H, Hα-7, H-9), 1.05 (s, 3H, H$_3$C-19), 1.16–1.29 (m, 2H), 1.35–1.55 (m, 2H), 1.60–1.86 (m, 3H), 1.93–2.29 (m, 6H), 2.32–2.56 (m, 3H), 3.83 (m, 1H, Hβ-11), 5.65 (s, 1H, H-4), 8.10 (s, 1H, NH), 8.39 (s, 1H, NH). ^{13}C NMR (50 MHz, CDCl$_3$/CD$_3$OD): 163.9 (s, C-17), 158.6 (s, C=O), 158.5 (s, C=O), 155.7 (s, C-5), 149.6 (s, C-3), 121.1 (d, C-4), 68.1 (d, C-11), 58.6 (d, C-9), 51.8 (d, C-14), 44.6 (t, C-12), 44.3 (s, C-13), 38.7 (s, C-10), 35.6 (d, C-8), 34.4 (t, C-1), 32.7 (t, C-6), 30.9 (t, C-7), 25.6 (t, C-16), 23.0 (t, C-15), 20.3 (t, C-2), 18.1 (q, C-19), 17.4 (q, C-18). HESI-Orbitrap MS: *m/z* for $C_{21}H_{32}N_6O_6$ [M+H]$^+$: calcd 417.2609, found 417.2626.

2.1.1.10. Progesterone 3-semicarbazone (2e). The title compound was obtained starting from progesterone (**1e**) (940 mg). Elution with CH_2Cl_2/MeOH (100/4) gave **2e** (480 mg, 43.1%). R_f = 0.30 (CH_2Cl_2/MeOH = 20:1). Mp > 200 °C (decomp.). IR (ATR/cm^{-1}): 3473 and 3310 (NH), 2930, 1696 (C=O), 1676 (C=O), 1577, 1428 (C=N), 1358, 757. ESI-TOF-MS: *m/z* for $C_{22}H_{33}N_3O_2$ [M+H]$^+$: calcd 372.26455, found 372.26441; *m/z* for $C_{22}H_{33}N_3O_2$ [M+Na]$^+$: calcd 394.24650, found 394.24726.

(2e-E) from the mixture *E/Z* = 90:10. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.56 (s, 3H, H$_3$C-18), 0.83 (td, *J* = 12, 3.5 Hz, 1H, H-9), 0.90 (qd, *J* = 13.5, 3.5 Hz, 1H, Hα-7), 1.01 (s, 3H, H$_3$C-19), 1.11–1.20 (m, 2H, H-14, Hα-16), 1.25–1.51 (m, 4H, Hα-1, H-8, Hβ-11, Hα-12), 1.53–1.59 (m, 2H, Hα-11, Hβ-15), 1.63 (m, 1H, Hβ-16), 1.72 (br.d, *J* = 12.5 Hz, 1H, Hβ-7), 1.88 (m, 1H, Hβ-1), 1.94–2.05 (m, 3H, Hβ-2, Hβ-12, Hα-15), 2.06 (s, 3H, H$_3$C-21), 2.16 (dt, *J* = 13.5, 3 Hz, 1H, Hα-6), 2.29 (td, *J* = 12.5, 3 Hz, 1H, Hβ-6), 2.57 (t, *J* = 9 Hz, 1H, H-17), 2.68 (dt, *J* = 17, 3 Hz, 1H, Hα-2), 5.73 (s, 1H, H-4), 6.21 (m, 2H, NH$_2$), 9.08 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): 208.6 (s, C-20), 157.2 (s, C=O), 153.0 (s, C-5), 146.8 (s, C-3), 121.1 (d, C-4), 62.6 (d, C-17), 55.5 (d, C-14), 53.0 (d, C-9), 43.3 (s, C-13), 38.0 (t, C-12), 37.1 (s, C-10), 35.2 (d, C-8), 34.5 (t, C-1), 31.9 (t, C-7), 31.6 (t, C-6), 31.2 (q, C-21), 24.0 (t, C-16), 22.2 (t, C-15), 21.0 (t, C-11), 20.4 (t, C-2), 17.5 (q, C-19), 13.1 (q, C-18).

(*2e-Z*) from the mixture *E/Z* = 90:10. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.56 (s, 3H, H$_3$C-18), 0.83 (td, *J* = 12, 3.5 Hz, 1H, H-9), 0.90 (qd, *J* = 13.5, 3.5 Hz, 1H, Hα-7), 1.06 (s, 3H, H$_3$C-19), 1.11–1.20 (m, 2H, H-14, Hα-16), 1.25–1.51 (m, 4H, Hα-1, H-8, Hβ-11, Hα-12), 1.53–1.59 (m, 2H, Hα-11, Hβ-15), 1.63 (m, 1H, Hβ-16), 1.72 (br.d, *J* = 12.5 Hz, 1H, Hβ-7), 1.88 (m, 1H, Hβ-1), 1.94–2.05 (m, 2H, Hβ-12, Hα-15), 2.06 (s, 3H, H$_3$C-21), 2.16 (dt, *J* = 13.5, 3 Hz, 1H, Hα-6), 2.23 (m, 1H, Hβ-2), 2.29 (td, *J* = 12.5, 3 Hz, 1H, Hβ-6), 2.57 (t, *J* = 9 Hz, 1H, H-17), 2.35 (m, 1H, Hα-2), 6.14 (m, 2H, NH$_2$), 6.43 (s, 1H, H-4), 9.32 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): 208.6 (s, C-20), 158.1 (s, C-5), 157.2 (s, C=O), 144.2 (s, C-3), 111.9 (d, C-4), 62.6 (d, C-17), 56.1 (d, C-14), 53.2 (d, C-9), 43.3 (s, C-13), 38.2 (s, C-10), 38.0 (t, C-12), 36.2 (t, C-1), 35.1 (d, C-8), 32.3 (t, C-7), 32.2 (t, C-6), 31.2 (q, C-21), 27.4 (t, C-2), 24.0 (t, C-16), 22.2 (t, C-15), 20.8 (t, C-11), 17.8 (q, C-19), 13.1 (q, C-18).

Further elution with CH$_2$Cl$_2$/MeOH (100/15) gave

2.1.1.11. (E)-Pregn-4-ene-3,20-dione bis(semicarbazone) **3e** as a pure *E*-isomer (128 mg, 10.2%). R_f = 0.18 (CH$_2$Cl$_2$/MeOH = 20:2). Mp > 220 °C (decomp.). IR (ATR/cm^{-1}): 3453, 3189 (NH), 2937, 1687 (C=O), 1570, 1437 (C=N), 1116, 766. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.55 (s, 3H, H$_3$C-18), 0.81 (m, 1H, H-9), 0.89 (qd, *J* = 13, 3.5 Hz, 1H, Hα-7), 1.00 (s, 3H, H$_3$C-19), 1.08–1.21 (m, 2H, H-14, Hα-15), 1.23–1.36 (m, 3H, Hα-1, Hβ-11, Hβ-12), 1.44 (qd, *J* = 10.5, 2 Hz, 1H, H-8), 1.50–1.58 (m, 2H, Hα-11, Hα-16), 1.61 (m, 1H, Hβ-15), 1.73 (m, 1H, Hβ-7), 1.76 (s, 3H, H$_3$C-21), 1.80 (m, 1H, Hα-12), 1.87 (br.d, *J* = 13 Hz, 1H, Hβ-1), 1.97 (dtd, *J* = 5, 5, 4.5 Hz, 1H, Hβ-2), 2.15 (dt, *J* = 13.5, 2.5 Hz, 1H, Hα-6), 2.19–2.25 (m, 2H, Hβ-16, Hα-17), 2.29 (td, *J* = 13.5, 4 Hz, 1H, Hβ-6), 2.67 (dt, *J* = 17, 3 Hz, 1H, Hα-2), 5.72 (s, 1H, H-4), 6.14 (br.s, 2H, NH$_2$), 6.20 (s, 2H, NH$_2$), 8.78 (s, 1H, NH), 9.06 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 157.4 (s, C=O), 157.1 (s, C=O), 153.0 (s, C-5), 148.8 (s, C-20), 146.8 (s, C-3), 120.9 (d, C-4), 58.2 (d, C-17), 55.2 (d, C-14), 53.3 (d, C-9), 43.3 (s, C-13), 38.2 (t, C-12), 37.0 (s, C-10), 35.4 (d, C-8), 34.5 (t, C-1), 31.9 (t, C-6), 31.6 (t, C-7), 23.8 (t, C-15), 22.7 (t, C-16), 21.0 (t, C-11), 20.4 (t, C-2), 17.6 (q, C-21), 17.5 (q, C-19), 13.1 (q, C-18). ESI-TOF-MS: *m/z* for C$_{23}$H$_{36}$N$_6$O$_2$ [M+H]$^+$: calcd 429.29725, found 429.29678; *m/z* for C$_{22}$H$_{33}$N$_3$O$_2$ [M+Na]$^+$: calcd 451.27920, found 451.27953.

2.1.2. Synthesis of ethyl hydrazinecarboxilates

Into a solution of steroidal semicarbazones (**2a–e**, 1 mmol) and anhydrous sodium acetate (123 mg, 1.5 mmol) in absolute ethanol (30 mL), ethyl chloroacetate (244 mg, 2 mmol) was slowly added. The mixture was heated under reflux until the reaction was completed (for 48–96 h, monitored by TLC), allowed to attain room temperature and then

poured into ice water. The resulting solution was extracted with chloroform (three times), and dried over sodium sulfate. Evaporation of the solvent afforded a solid which was purified by column chromatography, using toluene/EtOAc as eluent (yield, and the ratio of eluents mentioned in each experiment).

*2.1.2.1. Ethyl (17-oxo-19-norandrost-4-en-3-ylidene)hydrazinecarboxylate (**5a**).* The title compound was obtained starting from (**E**)-19-norandrost-4-ene-3,17-dione 3-semicarbazone **2a** (330 mg). Elution with PhMe/EtOAc (7/3) gave compound **5a** (*E*/*Z* = 55:45) (114 mg, 31.7%). R_f = 0.67 (CH_2Cl_2/MeOH = 20:1). Mp > 160 °C (decomp.). IR (ATR/cm^{-1}): 3232 and 2928 (NH), 1737 (C=O), 1686 (C=O), 1424 (C=N), 1300, 1057. Anal. calcd for $C_{21}H_{30}N_2O_3$: C 70.36; H 8.44; N 7.81; Found: C 69.87; H 8.10; N 7.44.

*(**5a-E**)* from the mixture *E*/Z = 6:4. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.80 (m, 1H, H-9), 0.92 (s, 3H, H$_3$C-18), 1.07 (td, *J* = 12.5, 3.5 Hz, 1H, Hα-7), 1.22–1.36 (m, 7H, Hα-1, Hβ-11, Hβ-12, H-14, <u>CH$_3$</u>CH$_2$), 1.45 (m, 1H, H-8), 1.56 (td, *J* = 9, 3.5 Hz, 1H, Hβ-15), 1.83 (m, 1H, Hα-12), 1.86–2.00 (m, 5H, Hβ-2, Hβ-7, H-10, Hα-11, Hα-15), 2.10 (dd, *J* = 19, 9 Hz, 1H, Hα-16), 2.14–2.22 (m, 2H, Hβ-1, Hα-6), 2.38–2.50 (m, 2H, Hβ-6, Hβ-16), 2.56 (dt, *J* = 15.5, 3.5 Hz, 1H, Hα-2), 4.28 (br.d, *J* = 5 Hz, 2H, CH$_3$<u>CH$_2$</u>), 6.09 (s, 1H, H-4), 7.86 (s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 220.9 (s, C-17), 154.2 (s, C=O), 151.5 (s, C-3), 150.3 (s, C-5), 122.2 (d, C-4), 61.9 (t, CH$_3$<u>CH$_2$</u>), 50.3 (d, C-14), 49.5 (d, C-9), 47.8 (s, C-13), 41.5 (d, C-10), 40.0 (d, C-8), 35.8 (t, C-16), 34.7 (t, C-6), 31.4 (t, C-12), 30.1 (t, C-7), 26.0 (t, C-1), 25.9 (t, C-11), 22.2 (t, C-2), 21.7 (t, C-15), 14.6 (q, <u>CH$_3$</u>CH$_2$), 13.8 (q, C-18).

*(**5a-E**)* from the mixture *E*/Z = 6:4. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.71 (td, *J* = 13, 2.5 Hz, 1H, H-9), 0.84 (s, 3H, H$_3$C-18), 0.96 (td, *J* = 11.5, 2.5 Hz, 1H, Hα-7), 1.14 (br.s, 1H, Hα-1), 1.17 (m, 1H, Hβ-12), 1.18–1.23 (m, 4H, Hβ-11, <u>CH$_3$</u>CH$_2$), 1.28 (m, 1H, H-14), 1.44 (br.d, *J* = 11.5 Hz,1H, H-8), 1.50 (dtd, *J* = 2.5 Hz, 1H, Hβ-15), 1.63 (d, *J* = 11 Hz, 1H, Hα-12), 1.75–1.88 (m, 3H, Hβ-7, Hα-11, Hα-15), 1.92 (dt, J = 17.5, 3.5 Hz, 1H, Hβ-2), 1.95–2.09 (m, 3H, Hβ-1, H-10, Hα-16), 2.18 (m, 1H, Hα-6), 2.34–2.44 (m, 2H, Hβ-6, Hβ-16), 2.71 (dt, *J* = 17, 4 Hz, 1H, Hα-2), 4.09 (q, *J* = 7Hz, 2H, CH$_3$<u>CH$_2$</u>), 5.86 (s, 1H, H-4), 9.78 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 220.4 (s, C-17), 154.1(s, C=O), 151.8 (s, C-3), 149.9 (s, C-5), 121.7 (d, C-4), 60.2 (t, CH$_3$<u>CH$_2$</u>), 49.5 (d, C-14), 49.0 (d, C-9), 47.1 (s, C-13), 40.8 (d, C-10), 39.2 (d, C-8), 35.3 (t, C-16), 34.1 (t, C-6), 31.2 (t, C-12), 29.7 (t, C-7), 27.1 (t, C-1), 25.9 (t, C-11), 23.0 (t, C-2), 21.3 (t, C-15), 14.6 (q, <u>CH$_3$</u>CH$_2$), 13.4 (q, C-18).

(*5a-Z*) from the mixture *E*/Z = 6:4. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.80 (m, 1H, H-9), 0.92 (s, 3H, H$_3$C-18), 1.07 (td, *J* = 12.5, 3.5 Hz, 1H, Hα-7), 1.22–1.36 (m, 8H, Hα-1, H-8, Hβ-11, Hβ-12, H-14, *CH$_3$*CH$_2$), 1.57 (dtd, *J* = 3.5 Hz, 1H, Hβ-15), 1.83 (m, 1H, Hα-12), 1.86–2.00 (m, 3H, Hβ-7, Hα-11, Hα-15), 2.03 (m, 1H, H-10), 2.10 (dd, *J* = 19, 9 Hz, 1H, Hα-16), 2.16 (m, 1H, Hβ-1), 2.27 (td, *J* = 14, 4 Hz, 2H, Hβ-2, Hα-6), 2.38–2.50 (m, 2H, Hβ-6, Hβ-16), 2.56 (dt, *J* = 15.5, 3.5 Hz, 1H, Hα-2), 4.28 (br.d, *J* = 5 Hz, 2H, CH$_3$*CH$_2$*), 6.12 (s, 1H, H-4), 8.05 (br.s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 220.6 (s, C-17), 156.7 (s, C-3), 154.2 (s, C=O), 150.5 (s, C-5), 111.1 (d, C-4), 61.9 (t, CH$_3$*CH$_2$*), 50.2 (d, C-14), 50.0 (d, C-9), 47.8 (s, C-13), 43.3 (d, C-10), 40.2 (d, C-8), 35.9 (t, C-6), 35.8 (t, C-16), 31.3 (t, C-12), 30.5 (t, C-2), 30.5 (t, C-7), 27.4 (t, C-1), 25.6 (t, C-11), 21.7 (t, C-15), 14.6 (q, *CH$_3$*CH$_2$), 13.8 (q, C-18).

(*5a-Z*) from the mixture *E*/Z = 6:4. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.71 (td, *J* = 13, 2.5 Hz, 1H, H-9), 0.84 (s, 3H, H$_3$C-18), 0.96 (td, *J* = 11.5, 2.5 Hz, 1H, Hα-7), 1.17 (m, 1H, Hβ-12), 1.18–1.23 (m, 6H, Hα-1, H-8, Hβ-11, *CH$_3$*CH$_2$), 1.49 (m, 1H, H-14), 1.50 (dtd, *J* = 2.5 Hz, 1H, Hβ-15), 1.63 (d, *J* = 11 Hz, 1H, Hα-12), 1.75–1.88 (m, 3H, Hβ-7, Hα-11, Hα-15), 1.95–2.09 (m, 3H, Hβ-1, H-10, Hα-16), 2.18 (m, 2H, Hβ-2, Hα-6), 2.29 (dt, *J* = 14.5, 4 Hz, 1H, Hα-2), 2.34–2.44 (m, 2H, Hβ-6, Hβ-16), 4.09 (q, *J* = 7 Hz, 2H, CH$_3$*CH$_2$*), 6.51 (s, 1H, H-4), 9.98 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 220.3 (s, C-17), 154.1 (s, C=O), 154.4 (s, C-3), 148.9 (s, C-5), 112.9 (d, C-4), 60.1 (t, CH$_3$*CH$_2$*), 49.4 (d, C-14), 49.3 (d, C-9), 47.1 (s, C-13), 42.1 (d, C-10), 39.2 (d, C-8), 35.3 (t, C-16), 34.9 (t, C-6), 31.1 (t, C-12), 30.0 (t, C-2), 29.9 (t, C-7), 27.1 (t, C-1), 25.1 (t, C-11), 21.3 (t, C-15), 14.6 (q, *CH$_3$*CH$_2$), 13.4 (q, C-18).

2.1.2.2. Ethyl (17-oxoandrost-4-en-3-ylidene-hydrazinecarboxylate (5b). The title compound was obtained starting from androst-4-ene-3,17-dione 3-semicarbazone (**2b**) (*E*/Z = 8:2) (340 mg). Elution with PhMe/EtOAc (6/4) gave **5b** (*E*/Z = 9:1), (185.5 mg, 49.8%). R_f = 0.39 (PhMe:EtOAc = 6:4, double devolopment). Crystallization from the mixture CH$_2$Cl$_2$/CH$_3$OH did not yield crystals suitable for X-ray analysis. Mp = 121.6–123.9 °C. IR (ATR/cm^{-1}): 3232 (NH), 2928, 2864, 1737 (C=O), 1687, 1424, 1300 (C=N), 1057. Anal. calcd for C$_{22}$H$_{32}$N$_2$O$_3$ × 0.5 CH$_2$Cl$_2$ × 0.2 C$_7$H$_8$: C 66.45; H 8.06; N 6.40; Found: C 66.11; H 8.04; N 6.23.

(*5b-E*) from the mixture *E*/Z = 8:2. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.90 (s, 3H, H$_3$C-18), 0.91 (m, 1H, H-9, overlapped with H$_3$C-18), 1.03 (qd, *J* = 13, 3.5 Hz, 1H, Hα-7), 1.08 (s, 3H, H$_3$C-19), 1.24–1.29 (m, 2H, Hα-12, H-14), 1.31 (t, *J* = 7.5 Hz, 3H, *CH$_3$*CH$_2$), 1.40–1.48 (m, 2H,

Hα-1, Hα-11), 1.54 (dtd, J = 2, 2, 2 Hz, 1H, Hβ-15), 1,65–1.70 (m, 2H, H-8, Hβ-11), 1.84 (d, J = 12.5 Hz, 1H, Hβ-12), 1.89 (m, 1H, Hβ-7), 1.94–2.02 (m, 2H, Hβ-1, Hα-15), 2.04–2.12 (m, 2H, Hβ-2, Hα-16), 2.20–2.33 (m, 2H, Hα-6, Hβ-6), 2.46 (dd, J = 19.5, 9 Hz, 1H, Hβ-16), 2.54 (br.d, J = 16 Hz, 1H, Hα-2), 4.28 (br.d, J = 5 Hz, 2H, CH$_3$$\underline{CH_2}$), 6.00 (s, 1H, H-4), 7.83 (br.s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 220.8 (s, C-17), 154.9 (s, C-5), 154.3 (s, C=O), 150.9 (s, C-3), 121.3 (d, C-4), 61.9 (t, CH$_3$$\underline{CH_2}$), 53.6 (d, C-9), 51.0 (d, C-14), 47.5 (s, C-13), 37.7 (s, C-10), 35.8 (d, C-16), 35.4 (t, C-8), 34.7 (t, C-1), 32.0 (t, C-6), 31.4 (t, C-12), 30.9 (t, C-7), 21.8 (t, C-15), 20.7 (t, C-11), 20.1 (t, C-2), 17.8 (q, C-19), 14.6 (q, $\underline{CH_3}$CH$_2$), 13.7 (q, C-18).

(**5b-Z**) from the mixture E/Z = 8:2. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.90 (s, 3H, H$_3$C-18), 0.91 (m, 1H, H-9, overlapped with H$_3$C-18), 1.03 (qd, J = 13, 3.5 Hz, 1H, Hα-7), 1.14 (s, 3H, H$_3$C-19), 1.24–1.29 (m, 2H, Hα-12, H-14), 1.31 (t, J = 7.5 Hz, 3H, $\underline{CH_3}$CH$_2$), 1.40–1.48 (m, 2H, Hα-1, Hα-11), 1.54 (dtd, J = 2, 2, 2 Hz, 1H, Hβ-15), 1.65–1.70 (m, 2H, H-8, Hβ-11), 1.84 (d, J = 12.5 Hz, 1H, Hβ-12), 1.88–1.93 (m, 2H, Hβ-1, Hβ-7), 1.98 (m, 1H, Hα-15), 2.09 (m, 1H, Hα-16), 2.20–2.33 (m, 2H, Hα-6, Hβ-6), 2.40 (m, 1H, Hβ-2), 2.46 (dd, J = 19.5, 9 Hz, 1H, Hβ-16), 2.49 (m, 1H, Hα-2), 4.28 (br.d, J = 5 Hz, 2H, CH$_3$$\underline{CH_2}$), 6.05 (s, 1H, H-4), 8.03 (br.s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 220.7 (s, C-17), 161.1 (s, C-5), 154.4 (s, C=O), 154.3 (s, C-3), 110.2 (d, C-4), 61.9 (t, CH$_3$$\underline{CH_2}$), 54.1 (d, C-9), 50.9 (d, C-14), 47.6 (s, C-13), 39.2 (s, C-10), 36.5 (t, C-1), 35.8 (d, C-16), 35.3 (t, C-8), 33.1 (t, C-6), 31.4 (t, C-12), 31.2 (t, C-7), 27.8 (t, C-2), 21.8 (t, C-15), 20.4 (t, C-11), 18.2 (q, C-19), 14.6 (q, $\underline{CH_3}$CH$_2$), 13.7 (q, C-18).

2.1.2.3. (E)-Ethyl (17-oxoandrosta-4,9(11)-dien-3-ylidene)hydrazinecarboxylate (**5c**). The title compound was obtained starting from (*E*)**-**androsta-4,9(11)-diene-3,17-dione 3-semicarbazone (**2c-E**) (340 mg). Elution with PhMe/EtOAc (65/35) gave **5c** (198 mg, 53.5%). R_f = 0.40 (PhMe:EtOAc = 6:4, double development). Crystallization from the mixture CHCl$_3$/CH$_3$OH did not afford crystals suitable for X-ray analysis. Mp > 77 °C (decomp.). IR (ATR/cm^{-1}): 3258, 2964 and 2928 (NH), 1735 (C=O), 1517, 1449 (C=N), 1231, 1057. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.87 (s, 3H, H$_3$C-18), 1.11 (qd, J = 12.5, 3.5 Hz, 1H, Hα-7), 1.24 (s, 3H, H$_3$C-19), 1.32 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$CH$_2$), 1.50 (m, H-14), 1.61 (m, 1H, Hβ-15), 1.89 (td, J = 13, 5 Hz, 1H, Hα-12), 2.03–2.09 (m, 3H, Hα-1, Hβ-7, Hβ-12), 2.11 (m, 1H, Hβ-1), 2.13 (m, 1H, Hα-15), 2.15 (m, 1H, Hα-16), 2.24 (m, 1H, Hβ-2), 2.34 (m, 1H, H-8), 2.27 (t, J = 3 Hz, 1H, Hα-6), 2.48 (m, 1H, Hβ-16), 2.51 (m, 1H, Hβ-6), 2.58 (dt, J = 16.5, 3.5 Hz, 1H, Hα-2), 4.29 (br.d, J = 6 Hz, 2H, CH$_3$$\underline{CH_2}$), 5.52 (d, J = 5 Hz, 1H, H-11), 6.02 (s, 1H, H-4), 7.72 (s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 221.4 (s, C-17), 153.5 (s, C-5), 150.8 (s,

C=O), 150.7 (s, C-3), 146.0 (s, C-9), 121.5 (d, C-4), 117.2 (d, C-11), 61.9 (t, CH$_3$$\underline{CH_2}$), 48.2 (d, C-14), 46.0 (s, C-13), 40.3 (s, C-10), 37.3 (t, C-8), 36.4 (d, C-16), 33.6 (t, C-1), 32.8 (t, C-12), 32.1 (t, C-6), 31.4 (t, C-7), 26.8 (q, C-19), 22.8 (t, C-15), 20.5 (t, C-2), 14.7 (q, $\underline{CH_3}$CH$_2$), 14.0 (q, C-18). Anal. calcd for C$_{22}$H$_{30}$N$_2$O$_3$ × 0.3 CHCl$_3$ × 0.3 C$_7$H$_8$: C 67.18; H 7.54; N 6.35; Found: C 67.35; H 7.66; N 6.50.

2.1.2.4. (E)-Ethyl (11α-hydroxy-17-oxoandrost-4-en-3-ylidene)-hydrazinecarboxylate (5d). The title compound was obtained starting from 11α-hydroxyandrost-4-ene-3,17-dione 3-semicarbazone (**2d**) (*E*/*Z* = 8:2) (360 mg). Elution with PhMe/EtOAc (5/5) gave **5d** (*E*/*Z* = 8:2), (161.8 mg, 41.7%). R_f = 0.49 (PhMe:EtOAc = 6:4). Mp > 120 °C (decomp.). IR (ATR/cm^{-1}): 3450 (OH), 3219 (NH), 2935, 1733 (C=O), 1527 and 1458, (C=N), 1236, 1026, 736. Anal. calcd for C$_{22}$H$_{32}$N$_2$O$_4$ × 0.9 CHCl$_3$: C 55.46; H 6.69; N 5.65; Found: C 55.36; H 6.21; N 5.21.

(**5d-E**) from the mixture *E*/*Z* = 8:2. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.93 (s, 3H, H$_3$C-18), 1.03–1.13 (m, 2H, Hα-7, H-9), 1.21 (s, 3H, H$_3$C-19), 1.28–1.35 (m, 4H, $\underline{CH_3}$CH$_2$, Hβ-12), 1.39 (m, 1H, H-14), 1.54 (t, *J* = 12 Hz, Hβ-15), 1.64 (br.d, *J* = 11Hz, H-8), 1.715 (td, *J* = 13.5, 4.5 Hz, Hβ-1), 1.88 (br.d, *J* = 14 Hz, Hβ-7), 1.96 (m, 1H, Hα-15), 2.13 (m, 3H, Hβ-2, Hα-12, Hα-16), 2.26–2.35 (m, 2H, Hα-6, Hβ-6), 2.39–2.51 (m, 2H, Hα-2, Hβ-16), 2.67 (br.d, *J* = 13.5 Hz, Hα-1), 4.02 (br.s, 1H, Hβ-11), 4.27 (br.s, 2H, CH$_3$$\underline{CH_2}$), 6.00 (s, 1H, H-4), 7.83 (s, 1H, NH). ^{13}C NMR (50 MHz, CDCl$_3$): 219.1 (s, C-17), 155.2 (s, C-5), 154.3 (s, C=O),151.2 (s, C-3), 122.1 (d, C-4), 68.9 (d, C-11), 61.9 (t, CH$_3$$\underline{CH_2}$), 59.1 (d, C-9), 50.2 (d, C-14), 48.1 (s, C-13), 42.9 (t, C-12), 39.1 (s, C-10), 36.0 (t, C-1), 35.8 (t, C-16), 34.8 (d, C-8), 32.9 (t, C-6), 30.5 (t, C-7), 21.8 (t, C-15), 20.5 (t, C-2), 18.6 (q, C-19), 14.7 (q, $\underline{CH_3}$CH$_2$), 14.7 (q, C-18).

(**5d-Z**) from the mixture *E*/*Z* = 8:2. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.93 (s, 3H, H$_3$C-18), 1.03–1.13 (m, 2H, Hα-7, H-9), 1.27 (s, 3H, H$_3$C-19), 1.28–1.35 (m, 4H, $\underline{CH_3}$CH$_2$, Hβ-12), 1.51 (m, 1H, H-14), 1.54 (t, *J* = 12 Hz, Hβ-15), 1.64 (br.d, *J* = 11Hz, H-8), 1.82 (m, 1H, Hβ-1), 1.93 (m, 1H, Hβ-7), 1.96 (m, 1H, Hα-15), 2.13 (m, 3H, Hβ-2, Hα-12, Hα-16), 2.35–2.39 (m, 2H, Hα-6, Hβ-6), 2.39–2.47 (m, 2H, Hα-2, Hβ-16), 2.49 (m, Hα-1), 4.02 (br.s, 1H, Hβ-11), 4.27 (br.s, 2H, CH$_3$$\underline{CH_2}$), 6.05 (s, 1H, H-4), 8.03 (s, 1H, NH). ^{13}C NMR (50 MHz, CDCl$_3$): 218.9 (s, C-17), 161.0 (s, C-5), 154.3 (s, C=O), 150.3 (s, C-3), 111.1 (d, C-4), 68.7 (d, C-11), 61.9 (t, CH$_3$$\underline{CH_2}$), 59.5 (d, C-9), 50.1 (d, C-14), 48.1 (s, C-13), 42.8 (t, C-12), 40.7

(s, C-10), 38.1 (t, C-1), 35.8 (t, C-16), 34.6 (d, C-8), 33.9 (t, C-6), 31.0 (t, C-7), 27.8 (t, C-2), 21.8 (t, C-15), 19.0 (q, C-19), 14.7 (q, $\underline{CH_3}$CH$_2$), 14.7 (q, C-18).

2.1.2.5. (E)-Ethyl (20-oxopregn-4-en-3-ylidene)hydrazinecarboxylate (5e). The title compound was obtained starting from (*E*)-progesterone 3-semicarbazone (**2e-E**) (370 mg). Elution with PhMe/EtOAc (7/3) gave **5e** (194.8mg, 48.7%). R_f = 0.48 (PhMe:EtOAc = 6:4, double development). Crystallization from diisopropyl ether did not afford crystals suitable for X-ray analysis. Mp = 83.7 °C. IR (ATR/cm^{-1}): 3275, 2937 (NH), 1701 (C=O), 1518, 1449 (C=N), 1267, 1047. ^{1}H-NMR (500 MHz, DMSO-d_6): 0.56 (s, 3H, H$_3$C-18), 0.82 (td, *J* = 11.5, 2.5 Hz, 1H, H-9), 0.90 (br.q, *J* = 12 Hz, 1H, Hα-7), 1.00 (s, 3H, H$_3$C-19), 1.09–1.17 (m, 2H, H-14, Hα-16), 1.21 (t, *J* = 7 Hz, 3H, $\underline{CH_3}$CH$_2$), 1.25–1.48 (m, 4H, Hα-1, H-8, Hβ-11, Hα-12), 1.50–1.60 (m, 2H, Hα-11, Hβ-15), 1.62 (m, 1H, Hβ-16), 1.72 (br.d, *J* = 11.5 Hz, 1H, Hβ-7), 1.86 (br.d, *J* = 13 Hz, 1H, Hβ-1), 1.94–2.03 (m, 3H, Hβ-2, Hβ-12, Hα-15), 2.06 (s, 3H, H$_3$C-21), 2.20 (t, *J* = 4 Hz, 1H, Hα-6), 2.29 (m, 1H, Hβ-6), 2.56 (t, *J* = 9 Hz, 1H, H-17), 2.70 (d, *J* = 17 Hz, 1H, Hα-2), 4.10 (q, *J* = 7 Hz, 2H, CH$_3$$\underline{CH_2}$), 5.75 (s, 1H, H-4), 9.78 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): 208.6 (s, C-20), 154.2 (s, C-5 and C=O), 151.3 (s, C-3), 120.9 (d, C-4), 62.6 (d, C-17), 60.2 (t, CH$_3$$\underline{CH_2}$), 55.4 (d, C-14), 53.0 (d, C-9), 43.3 (s, C-13), 38.0 (t, C-12), 37.0 (s, C-10), 35.2 (d, C-8), 34.5 (t, C-1), 31.8 (t, C-7), 31.6 (t, C-6), 31.1 (q, C-21), 24.0 (t, C-16), 22.2 (t, C-15), 20.9 (t, C-11), 20.7 (t, C-2), 17.4 (q, C-19), 14.6 (q, $\underline{CH_3}$CH$_2$), 13.0 (q, C-18). Anal. calcd for C$_{24}$H$_{38}$N$_2$O$_3$: C 71.96; H 9.06; N 6.99; Found: C 72.33; H 9.26, N 6.44.

2.1.3. Synthesis of ethyl (17-oxo-19-norandrost-4-en-3-ylidene)hydrazinecarboxylate (5a) from 19-norandrost-4-ene-3,17-dione 3-semicarbazone (2a) without ethyl chloroacetate, using ethanol as nucleophile

Solution of 19-norandrost-4-ene-3,17-dione 3-semicarbazone (**2a**) (330 mg, 1 mmol) in absolute ethanol (30 mL, 0.5 mol) was heated under reflux (80 °C) for 48 h, until the reaction was completed, monitored by TLC. Evaporation of the solvent afforded desired product (170 mg, 47.3 %) as a pale yellow solid which was purified by flash column chromatography. After several consecutive chromatographies (elution with PhMe/EtOAc (65/35)) diastereomerically pure major isomer **5a-E** was obtained (98.6 mg, 27.4%). The rest was *E*/*Z* mixture (2:8) of **5a** (49.7 mg, 13.8 %).

(5a-E): ^{1}H-NMR (500 MHz, CDCl$_3$): 0.78 (br.d, J = 11 Hz, 1H, H-9), 0.90 (s, 3H, H$_3$C-18), 1.04 (qd, J = 13, 3.5 Hz, 1H, Hα-7), 1.23–1.35 (m, 7H, Hα-1, Hβ-11, Hβ-12, H-14, _CH$_3$_CH$_2$), 1.42 (q, J = 10.5 Hz, 1H, H-8), 1.54 (dbr.sd, J = 3 Hz, 1H, Hβ-15), 1.78–1.90 (m, 3H, Hβ-7, Hα-11, Hα-12), 1.92–2.00 (m, 2H, H-10, Hα-15), 2.02 (t, J = 5 Hz, 1H, Hβ-2), 2.08 (dd, J = 18.5, 9 Hz, 1H, Hα-16), 2.11–2.22 (m, 2H, Hβ-1, Hα-6), 2.38–2.47 (m, 2H, Hβ-6, Hβ-16), 2.53 (dt, J = 16.5, 4 Hz, 1H, Hα-2), 4.26 (br.d, J = 5.5 Hz, 2H, CH$_3$_CH$_2$_), 6.08 (s, 1H, H-4), 7.69 (s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 220.9 (s, C-17), 154.0 (s, C=O), 151.4 (s, C-3), 150.5 (s, C-5), 122.2 (d, C-4), 61.9 (t, CH$_3$_CH$_2$_), 50.4 (d, C-14), 49.6 (d, C-9), 47.8 (s, C-13), 41.6 (d, C-10), 40.1 (d, C-8), 35.8 (t, C-16), 34.8 (t, C-6), 31.5 (t, C-12), 30.1 (t, C-7), 26.1 (t, C-1), 26.0 (t, C-11), 22.2 (t, C-2), 21.8 (t, C-15), 14.7 (q, _CH$_3$_CH$_2$), 13.9 (q, C-18).

(5a-Z) from the mixture E/Z = 2:8. ^{1}H-NMR (500 MHz, CDCl$_3$): 0.81 (br.d, J = 10.5 Hz, 1H, H-9), 0.92 (s, 3H, H$_3$C-18), 1.08 (qd, J = 12.5, 3.5 Hz, 1H, Hα-7), 1.24 (m, 2H, Hβ-11, Hβ-12), 1.28 (m, 1H, H-14), 1.29–1.36 (m, 4H, Hα-1, _CH$_3$_CH$_2$), 1.48 (qd, J = 11, 2.5 Hz, 1H, H-8), 1.57 (dtd, J = 3.5 Hz, 1H, Hβ-15), 1.83 (dd, J = 8.5, 3 Hz, 1H, Hα-12), 1.87–2.00 (m, 3H, Hβ-7, Hα-11, Hα-15), 2.04 (m, 1H, H-10,), 2.10 (dd, J = 19.5, 9 Hz, 1H, Hα-16), 2.18 (m, 1H, Hβ-1), 2.27 (td, J = 12, 3.5 Hz, 2H, Hβ-2, Hα-6), 2.42–2.51 (m, 2H, Hβ-6, Hβ-16), 2.54 (dt, J = 14, 3.5 Hz, 1H, Hα-2), 4.275 (br.d, J = 5 Hz, 2H, CH$_3$_CH$_2$_), 6.14 (s, 1H, H-4), 8.05 (br.s, 1H, NH). ^{13}C NMR (125 MHz, CDCl$_3$): 220.6 (s, C-17), 156.7 (s, C-3), 154.6 (s, C=O), 150.5 (s, C-5), 111.1 (d, C-4), 61.9 (t, CH$_3$_CH$_2$_), 50.2 (d, C-14), 50.0 (d, C-9), 47.8 (s, C-13), 43.3 (d, C-10), 40.1 (d, C-8), 35.9 (t, C-6), 35.8 (t, C-16), 31.3 (t, C-12), 30.5 (t, C-2), 30.5 (t, C-7), 27.4 (t, C-1), 25.5 (t, C-11), 21.7 (t, C-15), 14.7 (q, _CH$_3$_CH$_2$), 13.8 (q, C-18).

2.2. Biology

2.2.1. Cytotoxicity assay

The cytotoxic activity of the compounds was evaluated against three human malignant cell lines: cervical adenocarcinoma (HeLa), chronic myelogenous leukemia (K562) and acute T-cell leukemia Jurkat cell line. HeLa (2,000 cells per well) were seeded into 96-well microtiter plates and 20 h later, after the cell adherence, five different concentrations of tested compounds were added to the cells, except for the control cells to which only nutrient medium was added. K562 cells (5,000 cells per well) and Jurkat cells (15,000 cells per well) were seeded 2 h before addition of compounds. The cytotoxicity assay procedure has been described elsewhere [20,21]. Stock solutions of compounds were prepared in DMSO at a

concentration of 10 mM. For each experiment, the solutions were diluted with nutrient medium and applied to cells to various final concentrations ranging from 6.25 μM to 100 μM or 12.50 μM to 200 μM. The final concentration of DMSO solvent never exceeded 1%, which was non-toxic to the cells. The positive control was chemotherapy drug cisplatin. Survival of cells was determined by MTT assay after 72 h of continuous action, according to the method of Mosmann [22], which was modified by Ohno and Abe [23] and described in detail in our previous studies [18-21].

All tested cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA).

2.2.2. Antimicrobial activity

The antibacterial activity was evaluated using two different strains of bacteria, one Gram-positive bacteria: *Clostridium sporogenes* (ATCC 19404) and one Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 9027). Antibacterial activity was determined by well diffusion method [24]. In each Petri dish (90 mm diameter) 22 mL of Nutrient agar (Hi Media, Mumbai, India) and 100 μL of bacterial suspension (10^6 cells/dish) were added. Eight millimeter diameter well was then punched carefully using a sterile cork borer and 100 μL of test substance (1 mg/100 μL DMSO) were added into each labeled well. Amikacin (30 μg/100 μL H_2O) was used as positive control, while 100 μL of water and DMSO served as negative controls. After the inoculation of the organisms, compounds and controls, the plates were incubated for 24 h at 37 °C. Zones of inhibition were recorded in millimeters.

The fungus tested was *Aspergillus brasiliensis* (ATCC 16404). Sabouraud dextrose agar (Torlak, Belgrade, Serbia) was prepared according to the manufacturer's instruction. In each sterile Petri dish (90 mm diameter) 22 mL of previously prepared agar suspension was poured and 100 μL of fungus (10^5 spores/dish) was added. Eight millimeter diameter well was punched using a sterile cork borer. Into each well 100 μL of test substance (1 mg/100 μL DMSO) were added. Nystatin (30 μg/100 μL DMSO) was used as a positive control, while 100 μL of DMSO served as a negative control. The plates were incubated for 48 h at 24 °C. Antifungal activity was determined by measuring the diameter of inhibition zone in millimeters.

2.2.3. The brine shrimp test

The brine shrimp test of toxicity was performed against freshly hatched nauplii of *Artemia salina* [25]. The method was slightly modified by our team and described earlier [19]. The compounds were dissolved in DMSO and diluted by artificial seawater until range of concentrations (0.01–0.50 mg/mL) was obtained. The final concentration of DMSO was 1% and did not cause changes of viability of nauplii. The number of nauplii was approximately 20. Surviving nauplii were counted after 24 h, and LC_{50} (concentration lethal to 50% of the nauplii) were determined after statistical analysis. All the tests were performed in triplicate.

3. Results and Discussion

3.1. Chemistry

The target compounds, new steroidal mono- and bis(semicarbazones), were synthesized by a general procedure starting from steroids (**1a–e**) and semicarbazide in dried ethanol (Scheme 1).
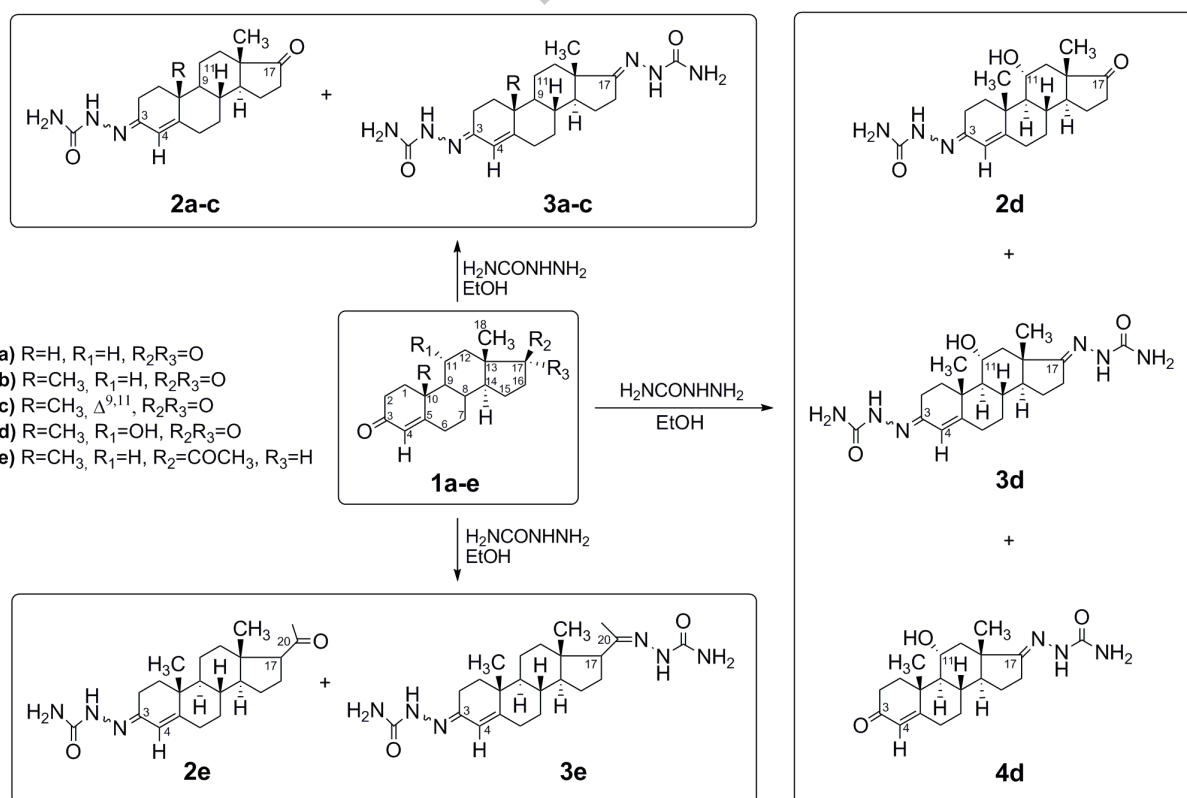
Scheme 1

In our previous work [21], we found that regardless of the molar ratio of steroids and thiosemicarbazide (equimolar or 1:2, respectively), a mixture of mono- and bis(thiosemicarbazones) was always obtained. Therefore, this time, the reaction was conducted with a small excess of reagent until complete consumption of steroids **1a–e**, as monitored by thin layer chromatography (TLC), giving 3-semicarbazones **2a–e** in the yields of 15.1–76% and 3,17- or 3,20-bis(semicarbazones) **3a–e** in the yields of 10.2–24.2% (see Experimental). Additionally, in the reaction of 11α-hydroxyandrost-4-ene-3,17-dione (**2d**), 17-mono-semicarbazone **4d** was obtained in the yield of 13.6%. The structures of the synthesized compounds were confirmed by elemental analysis, HRMS, ^{1}H NMR, ^{13}C NMR and IR spectroscopy. For the detailed characterization and complete ^{1}H and ^{13}C assignments of each compound and isomers (where they were obtained), the 2D NMR (HSQC, HMBC, NOESY, COSY) experiments were performed (Supporting Material).

The NMR analysis for the obtained compounds revealed the presence of two diastereoisomers (*Z* and *E*) with different configurations in the hydrazone moiety at the C-3 position, determined on the basis of the NOESY correlations, while at C-17 and C-20 (for progesterone derivative) only one isomer was obtained. The ratio of the isomers was deduced by comparing the peak areas of the H-4 and H–N–N=C(3) in the corresponding ^{1}H NMR spectra.

The ^{1}H NMR spectrum of **2a** showed two singlets for olefinic H-4 proton. In (*Z*)-isomer H-4 appears at lower field (s, 6.52 ppm), than that in the (*E*)-isomer (s, 5.83 ppm) due to the orientation of the of semicarbazone moiety at C-3. For the same reason Hα-2 proton in (*E*)-isomer resonates well-separated downfield from the other ring protons as dt at 2.68 ppm. Also, there were broad singlets for the H$_2$N protons, at δ 6.20 ppm and 6.15 ppm and two singlets for amide H–N proton at δ 9.11 ppm and 9.33 ppm for (*E*)- and (*Z*)-isomer, respectively. The characteristic signals in the ^{13}C NMR spectrum were: at δ 22.6 ppm and 30.0 ppm (C-2), 121.9 ppm and 112.9 ppm (C-4), 147.2 ppm and 144.5 ppm (C-3) and 148.5 ppm and 153.7 ppm (C-5) for (*E*)- and (*Z*)-isomer, respectively**.** The presence of the NOESY correlations between amide proton at 9.11 ppm and Hα-2 at 2.68 ppm, Hβ-2 at 1.90 ppm, and, although weak, correlations of the same proton with H-4 at 5.82 ppm and H$_2$N at 6.20 ppm provided evidence for the *E*-configuration, while in the (*Z*)-isomer N-H proton at δ 9.33 ppm showed NOESY cross-peaks with H-4 at 6.52 ppm and H$_2$N at 6.15 ppm and no correlations were observed with the protons at C-2 (Fig. 1).

Figure 1.

Almost the same ^{1}H and ^{13}C NMR signal arrangements were observed for all synthesized semicarbazones **2a–e.**

3,17-Bis(semicarbazone) **3a** was also obtained as a mixture of the two isomers differing only in the configuration at C(3)=N double bond. The ^{1}H NMR spectrum of **3a** contained two singlets for olefinic H-4 proton at δ 5.83 ppm and 6.51 ppm, two broad singlets for the H_2N protons from the semicarbazone moiety at C-3 at δ 6.20 ppm and 6.14 ppm and two singlets for N-H proton also from the semicarbazone moiety at C-3 confirmed by the HMBC correlations of the H–N protons with C-3 (9.06 ppm/147.3 ppm for 3*E*, and 9.29 ppm/144.6 ppm for 3*Z*)**.** One broad singlet for H_2N from semicarbazone moiety at C-17 appeared at δ 6.09 ppm for both 3*E*- and 3*Z*-isomer, as well as the singlet for H–N proton at δ 8.71 ppm with HMBC correlation with C-17 (8.71 ppm/163.0 ppm) indicating that in C-17 position only one isomer was obtained (Fig. 1) which was also confirmed by comparing the peak areas of these signals. The *E* configuration of the C(17)=N double bond was evidenced on the basis of the NOESY correlation of NH at 8.71 ppm with Hα-16 at 2.14 ppm and the absence of any correlation with the CH_3-18. This was expected because the strong steric repulsion between CH_3-18 from sterane skeleton and semicarbazone moiety at C-17 prevents the formation of (*Z*)-isomer (Fig. 1) [21].

This can be also applied to all synthesized 3,17-bis(semicarbazones) **3a–d**.

In the case of compound **3e,** pregn-4-ene-3,20-dione bis(semicarbazone), possible rotation around C(17)–C(20) axis and presence of CH_3-21 methyl group must be considered which is why it could be assumed that due to the strong steric hindrance the formation of (*Z*)-isomer in semicarbazone moiety at C-20 is impossible. Anyhow, the NOESY correlation between amide proton from hydrazone moiety at 8.78 ppm and CH_3-21 at 1.76 ppm and the absence of any correlation with CH_3-18 or H_2C-16 protons provided unambiguous evidence for *E*-configuration (Fig. 2). In addition, inspection of the Dreiding models suggests that this correlation, regardless of the rotation around C(17)–C(20) axis, is possible only when the geometry of C(20)=N double bond is *E*.

Figure 2.

When thus obtained semicarbazones **2a–e** as a mixture of both isomers (*E* and *Z*) were subjected to ethyl chloroacetate in boiling absolute ethanol containing anhydrous sodium acetate, which usually acts as the cyclizing agent, the expected intramolecular cyclocondensation reaction leading to formation of 1,3-oxazolidin-4-one did not occur. Instead, ethyl hydrazinecarboxilates **5a–e** were obtained (Scheme 2).
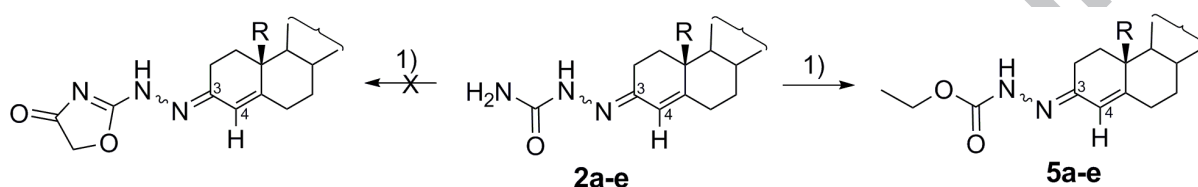
1) ClCH$_2$CO$_2$C$_2$H$_5$, CH$_3$COONa, EtOH, 80 °C, 24 h

Scheme 2

The structure of compounds **5a–e** was deduced from their NMR spectral data where two sets of NMR resonances suggest that these new derivatives were, as expected, obtained as a mixture of isomers. Most of the signals assigned to the carbon atoms forming sterane sceleton in compounds **2a–e** (*E* and *Z*) and **5a–e** (*E* and *Z*), did not differ significantly. Thus, ^{1}H/^{13}C NMR spectra of **5a** showed two signals for HC-4 at *δ* 5.86/121.7 ppm and 6.51/112.9 ppm, two signals for Hα-2 proton at *δ* 2.71/22.9 ppm and 2.29/30.0 ppm as well as two signals for NH in ^{1}H NMR spectra at *δ* 9.78 ppm and 9.98 ppm for (*E*)- and (*Z*)-isomer, respectively, confirming the presence of two diastereoisomers with different configurations at C(3)=N double bond. However, significant difference was observed for the amino group from semicarbazone moiety in compounds **2a–e** (at *δ* 6.20 ppm and 6.15 ppm for (*E*)- and (*Z*)-isomer, respectively), the absence of which was evident in ^{1}H NMR spectra of compounds **5a–e**. Instead, new ^{1}H triplets/^{13}C quartets at *δ* 1.31/14.6 ppm and ^{1}H quartets/^{13}C triplets at 4.28/61.9 ppm appeared indicating the indroduction of the new CH$_3$CH$_2$OCO moiety. Also, in the HMBC NMR spectrum of **5a** compound (recorded in DMSO) the presence of cross peaks between carbonyl C-atom at *δ* 154.1 ppm and NH proton at *δ* 9.78 ppm as well as methylene protons at *δ* 4.09 ppm confirmed the proposed structure (Fig. 3). In addition, in the IR spectra of **5a–e** the absorption bands for the primary amino group from semicarbazone moiety at about 3450 cm^{-1} were missing. Finally, the molecular formulas of **5a–e** were established on the basis of microanalyses data.

Distinguishing between *Z*- and *E*-configuration in the stereoisomeric pairs of **5a-e** was possible on the basis of the NMR chemical shifts of HC-4, H_2C-2 and NH and by comparison of the spectral data with those of the parent steroidal semicarbazones **2a–e** as well as by the presence (for *E*) and the absence (for *Z*) of the NOESY correlations between amide proton and protons at C-2. In the (*Z*)-isomer N-H proton showed NOESY cross-peak with H-4 (Figure 3).

Figure 3.

Regarding compounds **5b–e**, almost the same patterns of ^1H and ^{13}C NMR spectra were observed as in the spectra for **5a** (Experimental and Supplementary).

The mechanism of the formation of these carbazate derivatives has already been described as a simple exchange reaction using semihydrazone and alcohols upon heating at 60–150 °C [26].

In order to prove this, we prepared the experiment with 19-norandrost-4-ene-3,17-dione 3-semicarbazone (**2a**) in boiling absolute ethanol without presence of ethyl chloroacetate and sodium acetate. After 48 h of reflux and evaporation of the solvent compound **5a** was obtained in the yield of 47% (Scheme 3).
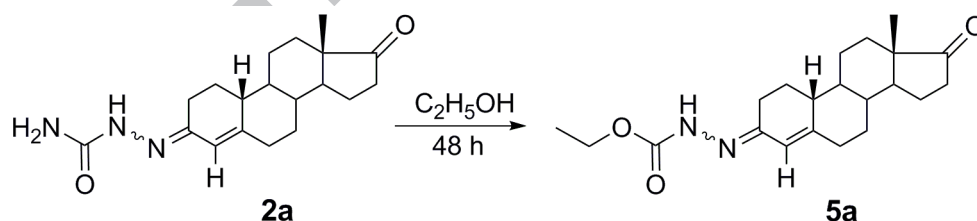
Scheme 3

3.2. Biological evaluation

3.2.1. Cytotoxic activity

The *in vitro* cytotoxic activity of the new steroidal semicarbazone derivatives was tested against two human malignant cell lines which were found to be the most sensitive to the cytotoxic action of our previously synthesized, corresponding steroidal thiosemicarbazones [1,21], chronic myelogenous leukemia K562 and human cervical adenocarcinoma HeLa cells. Since our previously investigated compounds [1,21] showed the

best activity against K562 cells, in this study the activity towards another leukemia cell line was also investigated. For that purpose a human acute T-cell leukemia Jurkat cell line was selected. Cisplatin (CDDP) was employed as the positive control. The results from MTT assays are presented in Table 1. It should be noted that compounds **3e** and **5d** were poorly soluble in DMSO, and their activity was not possible to examine.

Table 1.

Unexpectedly, almost all investigated compounds exhibited a very poor cytotoxic action against K562 cells. Compounds **2d**, **3d** and **4d** were practically inactive, while the other compounds tested exerted very weak cytotoxic activity with IC_{50} values from 71.6–163.7 μM. The exception was compound **5e** which showed moderate activity against this cell line with IC_{50} of 40.5 μM. The same compound exhibited the best cytotoxic action towards Jurkat cells with IC_{50} of 31.8 μM. Again, compounds **2d**, **3d** and **4d** were the least active with IC_{50} values from 126.3–180.1 μM. Also, all other investigated compounds exerted more pronounced cytotoxic activity against this leukemia cell line (IC_{50} values from 51.2–119.6 μM) but still very low compared to cisplatin.

Of all examined malignant cell lines, HeLa cells were the most sensitive to the cytotoxic action of the six newly synthesized steroid derivatives **2c**, **2e**, **3b**, **3c**, **5c**, and **5e**. The best cytotoxic activity was exhibited by compounds **2e, 3c** and **5e** with IC_{50} values of 27.7, 21.4 and 22.4 μM, respectively, while compounds **2c, 3b** and **5c** exerted moderate activity with IC_{50} of 42.7, 57.2 and 62.5 μM, respectively. Compounds **2a**, **2b**, **3a**, **5a** and **5b** showed very weak activity against HeLa cells with IC_{50} from 85.6–152.4 μM while compounds **2d**, **3d** and **4d** again proved to be almost inactive.

The results mentioned above indicate that of examined compounds progesterone derivatives **2e** and **5e** exhibited the highest cytotoxic actions against tested malignant cell lines. Compounds **2c**, **3c** and **5c**, derived from androsta-4,9(11)-diene-3,17-dione showed moderate cytotoxic activity, while androstene and 19-nor-androstene derivatives were the least active. Compounds **2d**, **3d** and **4d**, 11α-hydroxy semicarbazones, were practically inactive against all tested malignant cell lines and these findings are in accordance with our previous results obtained for other 11α-hydroxy derivatives [1,21], and corrooborates the hypothesis that the substitution of a hydrogen atom

by proton-donating OH-group in 11α-position led to a dramatic decrease in activity [1,21]. However, these results demonstrate that new steroidal mono- and bis(semicarbazones) and their derivatives were significantly less active than corresponding steroidal thiosemicarbazones.

3.2.2. Antimicrobial activity

The *in vitro* antimicrobial activity of all new compounds was tested against Gram-positive bacteria *Clostridium sporogenes* and Gram-negative bacteria *Pseudomonas aeruginosa* and against one fungal species *Aspergillus brasiliensis* by agar well diffusion method.

All synthesized ethyl hydrazinecarboxilates (exception is **5d** which was completely insoluble in DMSO) as well as compounds **2d**, **3a**, **3d** and **4d** exhibited a weak inhibitory activity against both tested bacteria. In all cases the inhibition zones were much smaller than those for amikacin (Table 2). Also, these compounds were found to have weak antifungal activity against *A. brasiliensis*. However, it should be noted that compounds **5b** and **5c** showed a slightly better activity with approximately the same inhibition zone as nystatin. Here, it should be taken into account that the concentration of nystatin, which was used as positive control was much lower.

Table 2.

3.2.3. The brine shrimp test

In the brine shrimp test, which has been established as a safe, practical, and economic method for the determination of the *in vivo* bioactivity of synthetic compounds, general toxicity against nauplii of the brine shrimp *Artemia salina* was evaluated. The LC_{50} values obtained for the newly synthesized compounds are shown in Table 3. All examined compounds were found to be less toxic when compared to the cisplatin. However, compounds **5b** and **5c** as well as **3d** with the best antimicrobial activity, were also found to be the most active in the brine shrimp assay.

Table 3.

4. Conclusion

Using various 3-oxo-α,β-unsaturated steroids as starting materials, 6 new steroidal mono-semicarbazones **2a–e** and **4d** and 5 bis(semicarbazones) **3a–e** were prepared. Mono-semicarbazones **2a–e** were further subjected to ethyl chloroacetate in boiling absolute ethanol

containing anhydrous sodium acetate, which did not lead to the expected intramolecular cyclocondensation reaction. Instead, carbazate esters **5a–e** were obtained. The structures of the synthesized compounds were confirmed by elemental analysis, HRMS and IR, ^{1}H NMR, ^{13}C NMR spectroscopy. The thorough NMR analysis indicated the presence of two diastereoisomers (*Z* and *E*) with different configurations in the hydrazone moiety at the C-3 position, determined on the basis of the NOESY correlations, while at C-17 and C-20 (for progesterone derivatives) only one isomer was obtained.

Preliminary screening for the *in vitro* cytotoxic activity of the new compounds has been conducted. Unexpectedly, almost all investigated compounds exhibited a very poor cytotoxic action against K562 cells. Towards Jurkat cells tested compounds exerted more pronounced cytotoxic activity, while HeLa cells were the most sensitive to the cytotoxic action of the six newly synthesized steroid derivatives. Among these compounds **2e, 3c** and **5e** were found to have the best but still moderate cytotoxic effects against all malignant cells tested.

These results demonstrate that new steroidal mono- and bis(semicarbazones) and their derivatives were significantly less active than corresponding steroids with thiosemicarbazone moiety. Obviously, the replacement of thioxo group with carbonyl group in steroidal hydrazone derivatives resulted in dramatic decrease in their cytotoxic activity.

Similarly, all tested compounds showed weak antimicrobial activities as well as *in vivo* toxicity against brine shrimp *Artemia salina*.

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Figure captions

Scheme 1. Synthesis of new steroidal mono- and bis(semicarbazones)

Scheme 2. Synthesis of new steroidal ethyl hydrazinecarboxilates

Scheme 3. Synthesis of ethyl (17-oxo-19-norandrost-4-en-3-ylidene)hydrazinecarboxylate (**5a**) from 19-norandrost-4-ene-3,17-dione 3-semicarbazone (**2a**) without ethyl chloroacetate, using ethanol as nucleophile
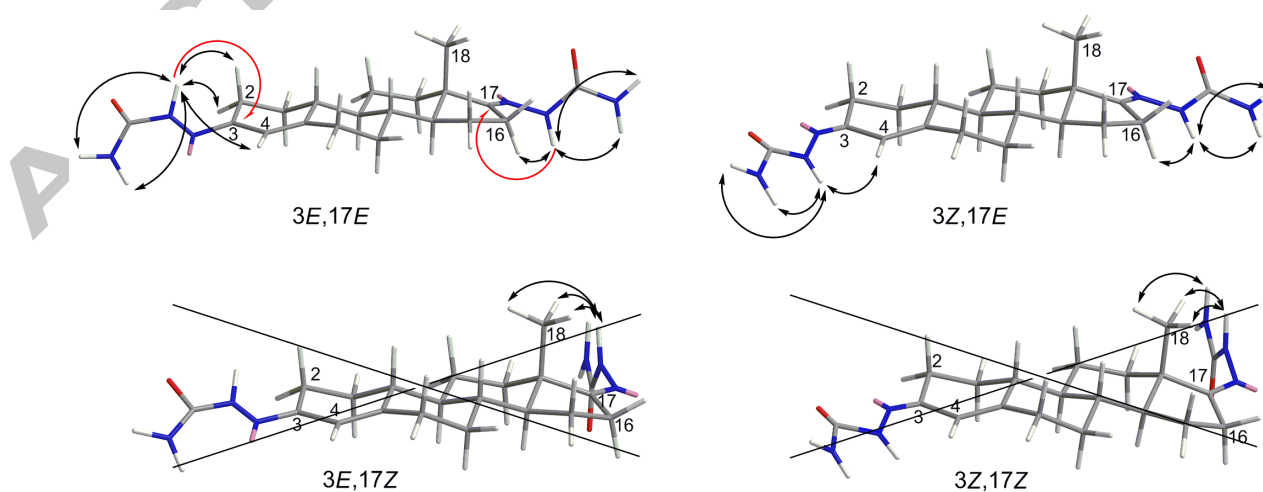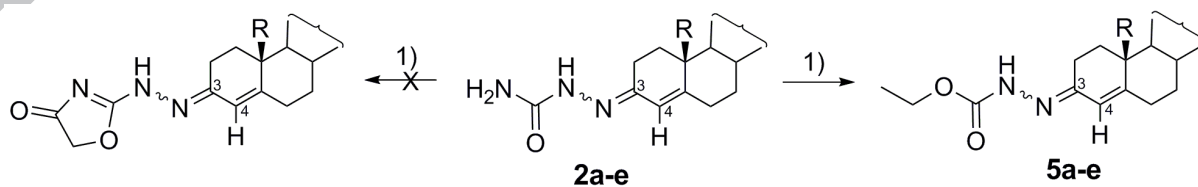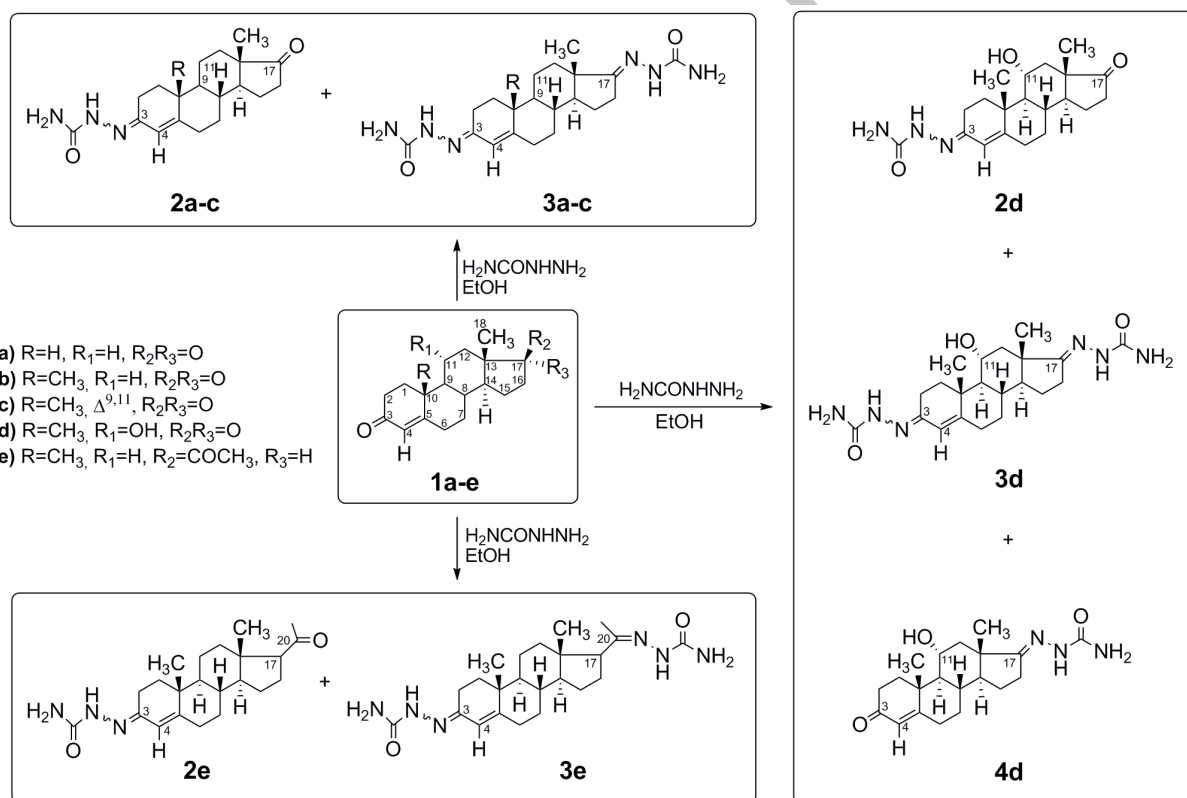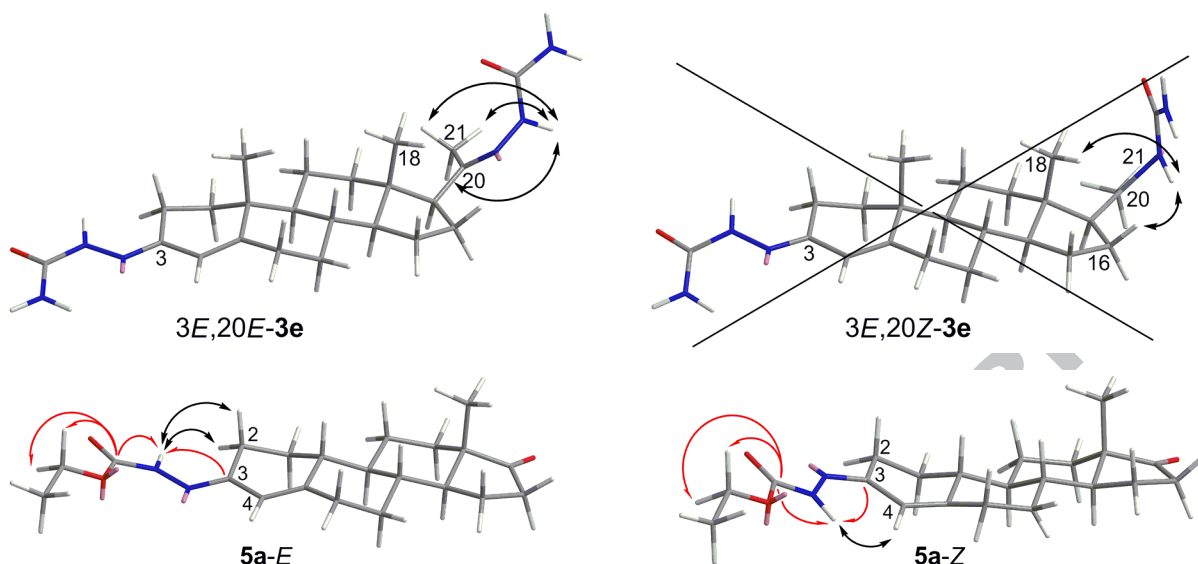
Fig. 1. 3D representation of the possible and proposed configuration at C(3)=N and C(17)=N double bonds with key HMBC (H/C ⌒) and NOESY (⌒) correlations

Fig. 2. 3D representation of the absolute configuration on C(20)=N double bond in **3e** with the key NOESY correlations

Fig. 3. 3D representation of the proposed configuration at C(3)=N double bond in compound **5a** with key HMBC (H/C ⌒) and NOESY (⌒) correlations

3E,20E-**3e**

3E,20Z-**3e**

5a-E

5a-Z

2a-c + **3a-c**

2d + **3d** + **4d**

a) R=H, R₁=H, R₂R₃=O
b) R=CH₃, R₁=H, R₂R₃=O
c) R=CH₃, Δ⁹,¹¹, R₂R₃=O
d) R=CH₃, R₁=OH, R₂R₃=O
e) R=CH₃, R₁=H, R₂=COCH₃, R₃=H

1a-e

H₂NCONHNH₂
EtOH

H₂NCONHNH₂
EtOH

H₂NCONHNH₂
EtOH

2e + **3e**

2a-e
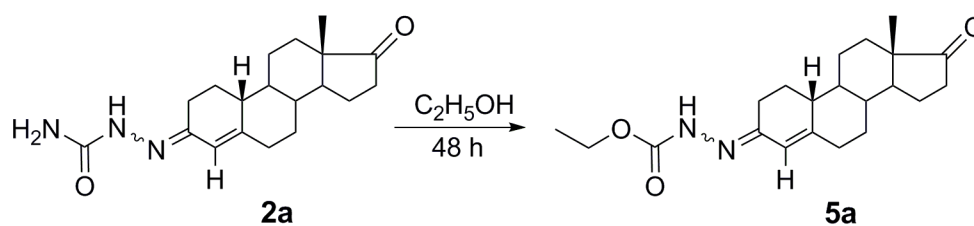
5a-e

1) ClCH₂CO₂C₂H₅, CH₃COONa, EtOH, 80 °C, 24 h

Table 1. The *in vitro* cytotoxic activity of compounds **2a–e, 3a–e, 4d** and **5a–e** (contrentacion which induced 50% decrease (IC_{50}) in malignant cell survival)

Comp.	$IC_{50} \pm SD$ (µM)		
	HeLa	K562	Jurkat
2a	152.4 ± 7.8	143.8 ± 4.9	74.5 ± 8.0
2b	100.6 ± 7.8	158.4 ± 4.7	80.1 ± 2.4
2c	42.7 ± 7.0	95.5 ± 5.1	155.7 ± 4.8
2d	177.3 ± 19.9	191.5 ± 7.5	126.3 ± 10.3
2e	27.7 ± 6.3	81.5 ± 4.7	51.2 ± 5.7
3a	85.6 ± 15.7	91.7 ± 0.1	76.9 ± 2.5
3b	57.2 ± 16.0	163.7 ± 19.3	119.6 ± 14.6
3c	21.4 ± 3.2	71.6 ± 4.7	87.4 ± 3.5
3d	189.9 ± 17.5	199.2 ± 1.4	169.0 ± 2.7
3e	/	/	/
4d	192.4 ± 6.6	191.3 ± 7.9	180.1 ± 18.1
5a	144.0 ± 15.2	112.7 ± 7.1	69.8 ± 2.2
5b	132.5 ± 17.5	100.0 ± 8.6	75.4 ± 4.4
5c	62.5 ± 4.3	113.0 ± 12.8	75.8 ± 9.6
5d	/	/	/
5e	22.4 ± 1.9	40.5 ± 4.1	31.8 ± 0.1
SC*	>200	>200	>200
CDDP	4.6 ± 0.1	6.0 ± 0.1	3.4 ± 0.2

*Semicarbazide

Table 2. Antimicrobial activity of the investigated compounds tested by agar well diffusion method

Comp.	Inhibition zone (mm)		
	C. sporogenes	*P. aeruginosa*	*A. brasiliensis*
2a	/	/	/
2b	/	/	/
2c	/	/	/
2d	12	10	10
2e	/	/	/
3a	10	10	10
3b	/	/	/
3c	/	/	/

3d	16	14	10
3e	/	/	/
4d	10	10	10
5a	10	10	12
5b	18	16	30
5c	12	14	28
5d	/	/	/
5e	12	10	10
Amikacin	22	20	/
Nystatin	/	/	30

Table 3. Brine shrimp lethality test results (LC_{50} values of the cytotoxic activity for the investigated compounds against *A. salina*)

Comp.	$LC_{50}(mM)$
2a	0.124
2b	0.172
2c	0.196
2d	0.134
2e	0.135
3a	0.179
3b	0.120
3c	0.158
3d	0.094
3e	/
4d	0.147
5a	0.122
5b	0.027
5c	0.032
5d	/
5e	0.151
CDDP	0.006

Highlights

- The synthesis of new steroidal mono- and bis(semicarbazones) was performed
- The new carbazate esters were obtained
- The structures were determined by IR, NMR, HRMS and elemental analysis
- The antimicrobial and cytotoxic activities of the compounds were tested

- The replacement of thioxo group with carbonyl group in steroidal hydrazone derivatives resulted in decrease in their biological activity