Interaction of 16-hydroxymethylidene derivatives of androstane and estrone with thiohydrazides of oxamic acids

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Reaction of thiohydrazides of oxamic acids with 16-hydroxymethylidene derivatives of androstane and estrone involves the hydroxymethylidene group and leads to thiohydrazones, which undergo heterocyclization to give 16-(1,3,4-thiadiazol-2-yl)-substituted steroids.

Key words: androstane, estrone, thiohydrazides of oxamic acids, thiohydrazones, 1,3,4-thiadiazols.

Reaction of steroids, which have active groups in the ring D, with thiohydrazides of oxamic acids is different from the reaction with hydrazines and carboxhydrazides.[1—5] For example, reactions of dehydropregnenolone with hydrazines and hydrazides usually result in formation of pyrazoles.[3—5] In similar conditions thiohydrazides of oxamic acids give 2′-(*N*-arylcarbamoyl)-5′-methyl-16α-hydroxyspiro(androst-4-ene-17,6′-[1,3,4]-thiadiazin-3-ones).[4,5]

Proceeding our study[6—8] of steroids with heterocyclic substituents, we studied the reaction of 16-hydroxymethylidene derivatives of androstane (**1**) and estrone (**2**) with thiohydrazides of oxamic acids **3a**,**b**.

Compounds **1** and **2** exist in dynamic equilibrium of keto-aldehyde and keto-enol tautomers[9] (see Scheme 1). As known,[9—12] 16-hydroxymethylidene derivatives of androstane (**1**) and estrone (**2**) react with hydrazines and hydrazides to give corresponding [17,16-*c*] pyrazoles.

We have found, that the presence of the thioxo group in compounds **3a**,**b** changes the direction of the reaction, and the cyclization takes place in the side chain giving the 1,3,4-thiadiazole fragment. For example, heating of compounds **1** and **2** with equimolar amount of thiohydrazides **3a**,**b** in ethanol in the presence of the catalytic amount of TsOH gave the corresponding steroid thiohydrazones **4a**,**b** and **5a**,**b** with 80—90% yields (see Scheme 1). When the twofold excess of the thiohydrazides was used, compounds **4** and **5** were obtained as only products evidencing the 17-positioned keto group left intact.

In the solution, compounds **4** and **5** exist in three basic tautomeric forms, which are the thioxo (**A**), the thiol (B) and the cyclic form (**C**) (see Scheme 2).

Initially, the dissolved compounds **4** and **5** take the form **A**, which is readily tautomerized to the forms **B** and **C**. This type of tautomerism is characteristic of hydrazones of thiohydrazides of oxamic acid and was described in detail.[13] The ^{1}H NMR spectrum of the form **A** had characteristic signals at δ 8.58 and 11.1 attributed to the protons of the groups —N=CH— and NH—C=S, correspondingly. The ^{13}C NMR spectrum of the form **B** has a signal at δ 167.2, which is assigned to the CSH moiety. The ^{1}H NMR spectrum of the form **C** has the signal of the heterocyclic proton at δ 5.6.

The cyclization of the thiohydrazone fragment in dioxane under reflux in the presence of TsOH occurs in the side chain of the steroid thiohydrazones **4** and **5** to give the corresponding thiadiazoles **6** and **7** in 60—64% yields (Scheme 3). The cyclization is oxidative and may be air promoted.

Synthesis of hydrazones **4** and **5** and the following cyclization can be combined into a one-pot procedure, which is refluxing of the mixture of compounds **1** or **2** with the corresponding thiohydrazides in acetic acid. In this case 3-OH acetylation of the steroid part takes place along with the cyclization, and the acetates **8** and **9** are obtained (Scheme 4).

In contrast to the transformations of the steroids **1** and **2**, interaction of the related 2-hydroxymethylidene-1-indanone (**10**) with the thiohydrazide **3a** leads to the cleavage of the molecule and removal of indanone along with the formation of the 1,3,4-thiadiazole cycle (Scheme 5). For example, heating of compound **10** with the equimolar quantity of the thiohydrazide **3a** in ethanol in the presence of the catalytic amount of TsOH leads to *N*-(4-methoxyphenyl)-1,3,4-thiadiazole-2-carboxamide **11** in a yield of 69%. Also, 1-indanone (**12**) was isolated from the reaction mixture.

Transformations of this type, which include the removal of the 1,3,4-thiadiazole fragment, have been described earlier[14] for the derivatives of 3-formyl-4-hydroxycoumarin.

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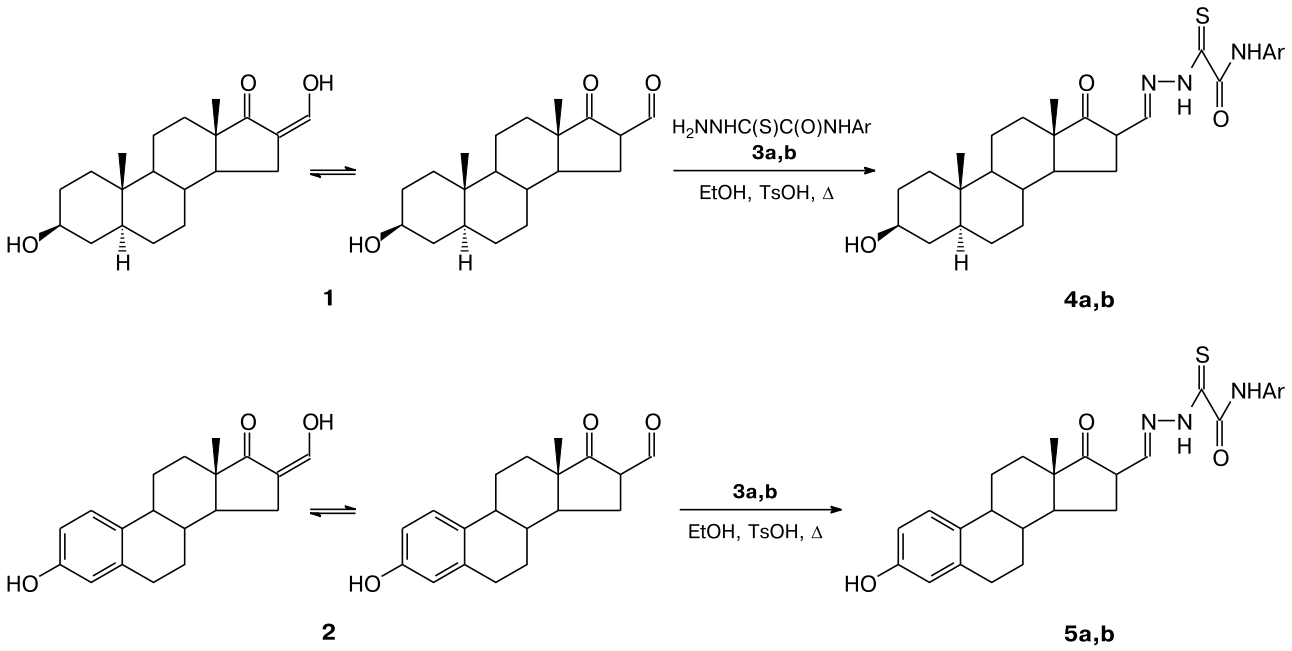
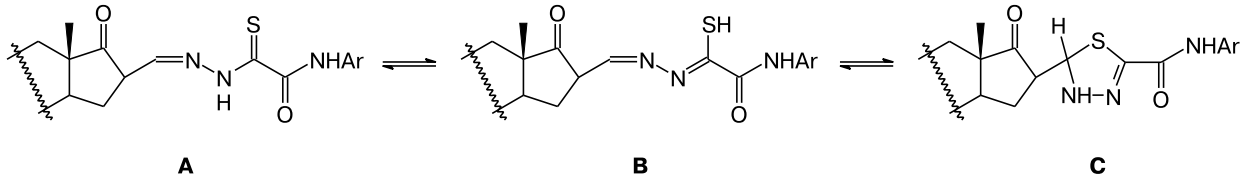
Scheme 1

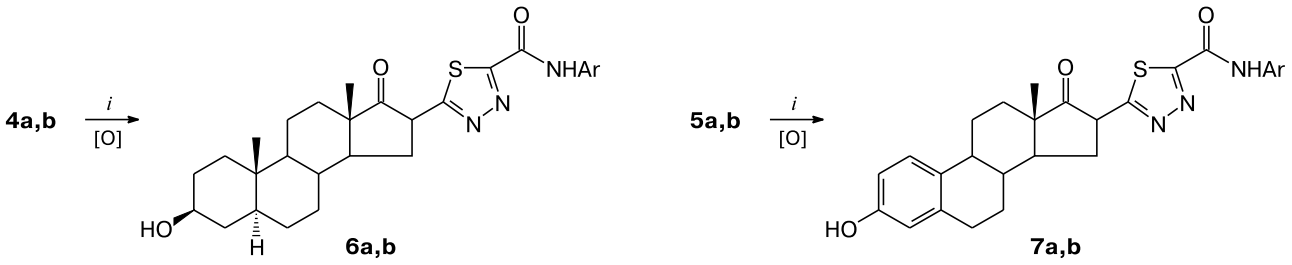


3—5: Ar = 4-$MeOC_6H_4$ (**a**), 4-FC_6H_4 (**b**)

Scheme 2



Scheme 3



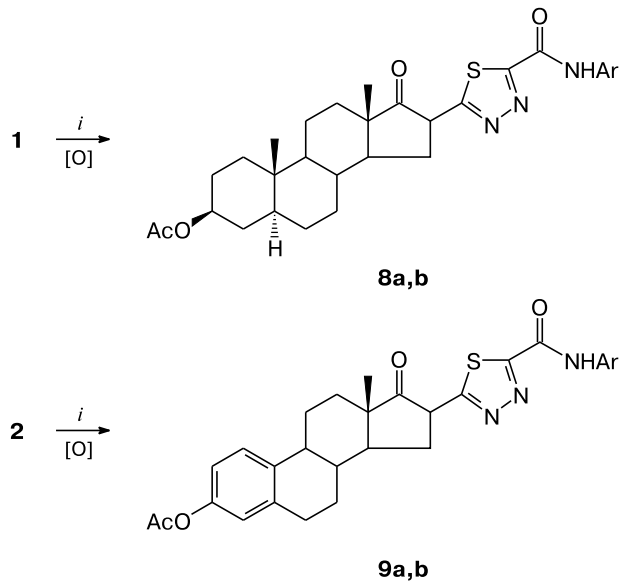
Reagents and conditions: *i.* TsOH, dioxane, reflux.

In the opinion of the authors,[14] the oxidation is accomplished by DMSO, which is used as a solvent. In our case, in the absence of DMSO the air oxygen can be an oxidant.

On the other hand, we can conjecture, that losing of the thiadiazole fragment during the cyclization can result from six-membered ring sigmatropic rearrangement. The detailed mechanism is beyond the scope of this study.

Interaction of compounds **1**, **2**, **10** with thiohydrazides **3** in different conditions, in particular in toluene in the presence of the catalytic amount of AcOH, or in benzene in the presence the catalytic amount of $BF_3 \cdot Et_2O$, leads

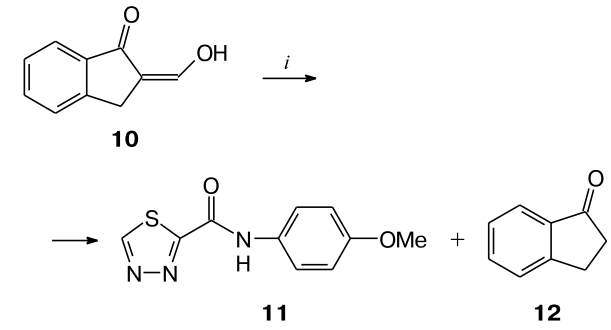
Scheme 4



8, **9:** Ar = 4-$MeOC_6H_4$ (**a**), 4-FC_6H_4 (**b**)

Reagents and conditions: *i*. **3a**,**b**, AcOH, reflux.

Scheme 5



Reagents and conditions: *i*. **3a**, TsOH, EtOH, reflux.

to complex mixtures, which were resolved to give 12—20% of isolated products **6**, **7** and **11**.

Thus, we have shown that compounds **3a,b** with the thioxo group interact with β-keto aldehydes **1**, **2**, **10** in a special way which includes the cyclization of the thiooxamidic fragment into the 1,3,4-thiadiazole group.

Experimental

Melting points were measured on a Boetius hot stage with a heating rate of 4 °C min^{-1} and are uncorrected. High resolution mass spectra were recorded on a Bruker micrOTOF II instrument using the electrospray ionization (ESI) technique.[15] The measurements were done in the positive-ion mode (capillary voltage was 4500 V) and in the ion-negative mode (capillary voltage was 3200 V); the mass scan range was *m/z*: 50—3000 D; the external and internal calibration was performed (Electrospray Calibrant Solution, Fluka); solutions of samples in acetonitrile, methanol or water were syringed; the flow rate was 3 μL min^{-1}, nitrogen was used as the nebulizing gas (4 L min^{-1}); the temperature of the interface 180 °C. ^{1}H NMR spectra were recorded on NMR spectrometers Bruker WM-300 (300 MHz) and Bruker DRX-500 (500 MHz) at 303 K, and ^{13}C NMR spectra were measured on a Bruker WM-300 (75 MHz). The signals of the solvents were used as the references. The course of the reactions and purity of products was monitored by TLC on Kieselgel-G (Merck Si 254F) using a AcOEt—hexane mixture as an eluent, the spots were visualised with $Ce(SO_4)_2$ solution in 10% H_2SO_4. Column chromatography was carried out on silica gel Acros 60A (0.060—0.200). Solvents were purified and dried using standard procedures. The reagents were purchased from Acros. The starting thiohydrazides **3a**,**b** were obtained as described.[16] 16-Hydroxymethylidene derivatives of androstane (**1**) and estrone (**2**) were obtained by Claisen condensation of ethyl formate with 3β-hydroxy-5α-androstan-17-one and estrone.[10]

3β-Hydroxy-16-hydroxymethylidene-5α-androstan-17-one (1). To a stirred solution of 3β-hydroxy-5α-androstan-17-one (1.8 mmol) in pyridine (20 mL) ethyl formate (11.9 mmol) and sodium methoxide (27 mmol) were added slowly under N_2 atmosphere at room temperature. The reaction mixture was stirred for 18 h at room temperature; the course of the reaction was monitored by TLC. After that the reaction mixture was poured to the solution of AcOH (9 mL) in water (80 mL), the mixture was extracted with dichloromethane (3×20 mL). The combined organic fractions were washed with water (3×70 mL) and extracted with 2% KOH in water (3×100 mL). The basic fractions were combined and washed with diethyl ether (3×15 mL). The basic layer was separated and AcOH was added until the pH 5. The precipitate was separated, washed with heptane (3×30 mL), dried *in vacuo* and recrystallized from acetone. The yield of compound **1** was 96%, m.p. 167—169 °C (acetone). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 0.68 (m, 1 H, H(9)); 0.77 (s, 3 H, C(13)Me); 0.79 (s, 3 H, C(10)Me); 0.91, 1.61 (m, 2 H, H(1)); 0.95, 1.72 (m, 2 H, H(7)); 1.07 (m, 1 H, H(5)); 1.13, 1.43 (m, 2 H, H(4)); 1.15 (s, 1 H, H(14)); 1.15, 1.65 (m, 2 H, H(2)); 1.24, 1.26 (m, 2 H, H(6)); 1.26, 1.65 (m, 2 H, H(12)); 1.28, 1.58 (m, 2 H, H(11)); 1.52 (s, 1 H, H(8)); 1.88, 2.46 (m, 2 H, H(15)); 3.35 (m, 1 H, H(3)); 7.34 (s, 1 H, H(1′)). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 11.9 (C(19)); 14.1 (C(18)); 20.0 (C(11)); 24.1 (C(15)); 28.1 (C(6)); 30.4 (C(7)); 31.2 (C(12)); 31.3 (C(2)); 34.0 (C(8)); 35.2 (C(10)); 36.4 (C(1)); 38.0 (C(4)); 44.3 (C(5)); 47.4 (C(13)); 49.3 (C(14)); 53.9 (C(9)); 69.1 (C(3)); 113.1 (C(16)); 149.8 (C(1′)); 208.4 (C(17)). Found (%): C, 74.63; H, 9.39. $C_{20}H_{30}O_3$. Calculated (%): C, 75.43; H, 9.50. MS (ESI), *m/z*: 319.

Reaction of compound 1 with thiohydrazides 3a,b in ethanol. To a stirred and heated (70 °C) solution of compound **1** (1.32 mmol) in ethanol (40 mL) thiohydrazide **3a**,**b** (1.58 mmol) and a catalytic amount of TsOH were added. The mixture was stirred at this temperature for 7 h, kept for 12 h at 20 °C and poured onto the ice. The precipitate was filtered off, washed with water (3×50 mL), hexane (3×30 mL), air-dried and the residue was subjected to column chromatography on silica gel (the eluent was AcOEt—hexane, 1 : 1) and products **4a**,**b** were obtained.

3β-Hydroxy-16-{2-[2-(4-methoxyphenylamino)-2-oxo-1-thioxoethyl]hydrazonomethyl}androstan-17-one (4a). Yield 86%,

m.p. 239—241 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.67 (s, 1 H, H(9)); 0.76 (s, 3 H, C(13)Me); 0.78 (s, 3 H, C(10)Me); 0.89, 1.59 (m, 2 H, H(1)); 0.92, 1.71 (m, 2 H, H(7)); 1.04 (s, 1 H, H(5)); 1.14, 1.41 (m, 2 H, H(4)); 1.21, 1.70 (m, 2 H, H(2)); 1.23, 1.25 (m, 2 H, H(6)); 1.25, 1.61 (m, 2 H, H(12)); 1.27, 1.58 (m, 2 H, H(11)); 1.29 (s, 1 H, H(14)); 1.49 (s, 1 H, H(8)); 1.74, 2.03 (m, 2 H, H(15)); 3.08 (m, 1 H, H(16)); 3.44 (s, 1 H, H(3)); 3.72 (s, 3 H, OMe); 5.60 (s, 1 H, H(1′) (**C**)); 6.87 (d, 2 H, H_{Ar}, J = 8.7 Hz); 7.60 (d, 2 H, H_{Ar}, J = 8.7 Hz); 8.58 (s, 1 H, H(1′) (**A**, **B**)); 10.00 (s, 1 H, NHCO); 11.10 (s, 1 H, NHCS (**A**)). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.0 (C(18)), 12.9 (C(19)), 20.1 (C(11)), 23.9 (C(15)), 28.1 (C(6)), 30.7 (C(7)), 31.3 (C(12)), 32.9 (C(2)), 33.7 (C(8)), 35.3 (C(10)), 36.5 (C(1)), 38.0 (C(4)), 44.2 (C(13)), 44.4 (C(5)), 48.3 (C(14)), 49.4 (C(16)), 53.9 (C(9)), 55.1 (OMe), 69.2 (C(3)), 113.8 (C_{Ar}), 121.6 (C_{Ar}), 131.3 (C_{Ar}), 155.6 (C_{Ar}), 157.4 (C(3′)), 167.2 (C(2′) (**B**)), 190.3 (C(2′) (**A**)), 217.9 (C(17)). Found (%): C, 66.08; H, 7.62; N, 7.78; S, 6.23. $C_{29}H_{39}N_3O_4S$. Calculated (%): C, 66.26; H, 7.48; N, 7.99; S, 6.10. MS (ESI), *m/z*: 526.

3β-Hydroxy-16-{2-[2-(4-fluorophenylamino)-2-oxo-1-thioxoethyl]hydrazonomethyl}androstan-17-one (4b). Yield 79%, m.p. 228—232 °C (ethyl acetate—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.78 (s, 3 H, C(13)Me); 0.81 (s, 3 H, C(10)Me); 3.44 (s, 1 H, H(3)); 5.60 (s, 1 H, H(1′) (**C**)); 7.42 (m, 2 H, H_{Ar}); 7.69 (m, 2 H, H_{Ar}); 8.61 (s, 1 H, H(1′) (**A**, **B**)); 10.13 (s, 1 H, NHCO); 10.95 (s, 1 H, NHCS (**A**)). ^{13}C NMR (DMSO-d_6), δ: 12.2 (C(18)), 12.9 (C(19)), 20.0 (C(11)), 23.9 (C(15)), 28.3 (C(6)), 30.6 (C(7)), 31.1 (C(12)), 32.9 (C(2)), 33.6 (C(8)), 35.3 (C(10)), 36.5 (C(1)), 38.0 (C(4)), 44.0 (C(13)), 44.4 (C(5)), 48.5 (C(14)), 49.2 (C(16)), 53.9 (C(9)), 69.2 (C(3)), 122.9 (C_{Ar}), 127.1 (C_{Ar}), 129.5 (C_{Ar}), 141.3 (C_{Ar}), 163.4 (C(3′)), 167.4 (C(2′) (**B**)), 190.5 (C(2′) (**A**)), 218.1 (C(17)). Found (%): C, 65.65; H, 7.22; F, 3.52; N, 7.99; S, 6.45. $C_{28}H_{36}FN_3O_3S$. Calculated (%): C, 65.47; H, 7.06; F, 3.70; N, 8.18; S, 6.24. MS (ESI), *m/z*: 514.

Cyclization of thiohydrazones 4a,b. The solution of thiohydrazones **4a**,**b** (1.32 mmol) and the catalytic amount of TsOH was kept in refluxing dioxane for 8 h in contact with atmosphere and the course of the reaction was monitored by TLC. Then the reaction mixture was poured onto ice, the precipitate was filtered off, washed with water (3×20 mL), heptane (3×20 mL) and air-dried. The residue was subjected to column chromatography on silica gel (the eluent was AcOEt—hexane, 1 : 1) and products **6a**,**b** were obtained.

5-(3β-Hydroxy-17-oxoandrostan-16-yl)-*N*-(4-methoxyphenyl)-1,3,4-thiadiazole-2-carboxamide (6a). Yield 76%, m.p. 176—179 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.74 (m, 1 H, H(9)); 0.81 (s, 3 H, C(10)Me); 0.84, 1.05 (s, 3 H, C(13)Me); 0.93, 1.61 (m, 2 H, H(1)); 1.05, 1.73 (m, 2 H, H(7)); 1.09 (m, 1 H, H(5)); 1.16, 1.44 (m, 2 H, H(4)); 1.23, 1.70 (m, 2 H, H(2)); 1.25, 1.65 (m, 2 H, H(12)); 1.27, 1.28 (m, 2 H, H(6)); 1.32, 1.62 (m, 2 H, H(11)); 1.53 (s, 1 H, H(14)); 1.63 (s, 1 H, H(8)); 2.28, 2.53 (m, 2 H, H(15)); 3.30 (s, 1 H, H(3)); 3.75 (s, 3 H, OMe); 4.41 (m, 1 H, H(16)); 6.94 (m, 2 H, H_{Ar}); 7.72 (m, 2 H, H_{Ar}); 10.93, 10.95 (s, 1 H, NHCO). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.2 (C(19)), 14.3 (C(18)), 20.1 (C(11)), 28.1 (C(6)), 30.9 (C(7)), 31.0 (C(12)), 33.0 (C(2)), 34.0 (C(8)), 35.4 (C(10)), 36.7 (C(1)), 38.0 (C(4)), 38.3 (C(15)), 44.1 (C(13)), 44.4 (C(5)), 48.1 (C(14)), 49.9 (C(16)), 54.1 (C(9)), 55.1 (OMe), 69.3 (C(3)), 114.0 (C_{Ar}), 122.1 (C_{Ar}), 130.4 (C_{Ar}), 156.1 (C_{Ar}), 156.2 (C(3′)), 164.3 (C(2′)), 164.7 (C(1′)), 217.5, 217.8 (C(17)). Found (%): C, 66.38; H, 7.32; N, 7.88; S, 6.33. $C_{29}H_{37}N_3O_4S$. Calculated (%): C, 66.51; H, 7.12; N, 8.02; S, 6.12. MS (ESI), *m/z*: 524.

5-(3β-Hydroxy-17-oxoandrostan-16-yl)-*N*-(4-fluorophenyl)-1,3,4-thiadiazole-2-carboxamide (6b). Yield 79%, m.p. 186—188 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.83 (s, 3 H, C(10)Me); 1.06 (s, 3 H, C(13)Me); 3.32 (s, 1 H, H(3)); 4.35 (m, 1 H, H(16)); 7.42 (m, 2 H, H_{Ar}); 7.81 (m, 2 H, H_{Ar}); 10.19 (s, 1 H, NHCO). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.2 (C(19)), 14.6 (C(18)), 20.1 (C(11)), 28.4 (C(6)), 30.9 (C(7)), 31.3 (C(12)), 33.0 (C(2)), 34.0 (C(8)), 35.5 (C(10)), 36.7 (C(1)), 38.0 (C(4)), 38.6 (C(15)), 44.1 (C(13)), 44.4 (C(5)), 48.5 (C(14)), 49.2 (C(16)), 54.1 (C(9)), 69.3 (C(3)), 121.2 (C_{Ar}), 125.5 (C_{Ar}), 133.7 (C_{Ar}), 141.7 (C_{Ar}), 159.6 (C(3′)), 164.5 (C(2′)), 164.7 (C(1′)), 217.3, 217.8 (C(17)). Found (%): C, 65.61; H, 6.87; F, 3.54; N, 8.09; S, 6.46. $C_{28}H_{34}FN_3O_3S$. Calculated (%): C, 65.73; H, 6.70; F, 3.71; N, 8.21; S, 6.27. MS (ESI), *m/z*: 512.

Reaction of compound 1 with thiohydrazides 3a,b in acetic acid. To the stirred solution of compound **1** (1.0 mmol) in acetic acid (25 mL) thiohydrazide **3a**,**b** (1.1 mmol) was added, the mixture was refluxed for 1—2 h and the course of the reaction was monitored by TLC. Then the reaction mixture was poured onto the ice, the precipitate was filtered off, washed with water (3×50 mL), heptane (3×25 mL) and air-dried. The residue was subjected to column chromatography on silica gel (the eluent was AcOEt—hexane, 1 : 1) and products **8a**,**b** were obtained.

5-(3β-Acetoxy-17-oxoandrostan-16-yl)-*N*-(4-methoxyphenyl)-1,3,4-thiadiazole-2-carboxamide (8a). Yield 76%, m.p. 185—188 °C (AcOEt—hexane, 1 : 1). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.74 (m, 1 H, H(9)); 0.81 (s, 3 H, C(10)Me); 0.84, 1.05 (s, 3 H, C(13)Me); 0.93, 1.61 (m, 2 H, H(1)); 1.05, 1.73 (m, 2 H, H(7)); 1.09 (m, 1 H, H(5)); 1.16, 1.44 (m, 2 H, H(4)); 1.23, 1.70 (m, 2 H, H(2)); 1.25, 1.65 (m, 2 H, H(12)); 1.27, 1.28 (m, 2 H, H(6)); 1.32, 1.62 (m, 2 H, H(11)); 1.53 (s, 1 H, H(14)); 1.63 (s, 1 H, H(8)); 1.96 (s, 3 H, CH_3 (OAc)); 2.28, 2.53 (m, 2 H, H(15)); 3.30 (s, 1 H, H(3)); 3.75 (s, 3 H, OMe); 4.41 (m, 1 H, H(16)); 4.69 (s, 1 H, H(3)); 6.94 (m, 2 H, H_{Ar}); 7.72 (m, 2 H, H_{Ar}); 10.93, 10.95 (s, 1 H, NHCO).^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.2 (C(19)), 14.3 (C(18)), 20.1 (C(11)), 21.2 (CH_3 (AcO)); 28.1 (C(6)), 30.9 (C(7)), 31.0 (C(12)), 33.0 (C(2)), 34.0 (C(8)), 35.4 (C(10)), 36.7 (C(1)), 38.0 (C(4)), 38.3 (C(15)), 44.1 (C(13)), 44.4 (C(5)), 48.1 (C(14)), 49.9 (C(16)), 54.1 (C(9)), 55.1 (OMe), 73.3 (C(3)), 114.0 (C_{Ar}), 122.1 (C_{Ar}), 130.4 (C_{Ar}), 156.1 (C_{Ar}), 156.2 (C(3′)), 164.3 (C(2′)), 164.7 (C(1′)), 170.0 (C=O (AcO)), 217.5, 217.8 (C(17)). Found (%): C, 65.94; H, 6.86; N, 7.54; S, 5.56. $C_{31}H_{39}N_3O_5S$. Calculated (%): C, 65.82; H, 6.95; N, 7.43; S, 5.67. MS (ESI), *m/z*: 566.

5-(3β-Acetoxy-17-oxoandrostan-16-yl)-*N*-(4-fluorophenyl)-1,3,4-thiadiazole-2-carboxamide (8b). Yield 79%, m.p. 194—197 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.83 (s, 3 H, C(10)Me); 1.06 (s, 3 H, C(13)Me); 4.71 (s, 1 H, H(3)); 4.35 (m, 1 H, H(16)); 7.42 (m, 2 H, H_{Ar}); 7.81 (m, 2 H, H_{Ar}); 10.19 (s, 1 H, NHCO). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.2 (C(19)), 14.6 (C(18)), 20.1 (C(11)), 21.4 (CH_3 (AcO)), 28.4 (C(6)), 30.9 (C(7)), 31.3 (C(12)), 33.0 (C(2)), 34.0 (C(8)), 35.5 (C(10)), 36.7 (C(1)), 38.0 (C(4)), 38.6 (C(15)), 44.1 (C(13)), 44.4 (C(5)), 48.5 (C(14)), 49.2 (C(16)), 54.1 (C(9)), 73.3 (C(3)), 121.2 (C_{Ar}), 125.5 (C_{Ar}), 133.7 (C_{Ar}), 141.7 (C_{Ar}), 159.6 (C(3′)), 164.5 (C(2′)), 164.7 (C(1′)), 170.0 (C=O (AcO)), 217.3, 217.8 (C(17)). Found (%): C, 65.19; H, 6.43; F, 3.54; N, 7.46; S, 5.67. $C_{30}H_{36}FN_3O_4S$. Calculated (%): C, 65.08; H, 6.55; F, 3.43; N, 7.59; S, 5.79. MS (ESI), *m/z*: 554.

3-Hydroxy-16-hydroxymethylidene-1,3,5(10)-estratrien-17-one (2). Ethyl formate (14.5 mmol) and sodium methoxide (33 mmol) were slowly added to the stirred solution of estrone (2.2 mmol) in pyridine (20 mL) under argon atmosphere at room temperature, the reaction mixture was stirred for 18 h, and the course of the reaction was monitored by TLC. Then the mixture was poured to the solution of AcOH (9 mL) in water (80 mL), the mixture was extracted with dichloromethane (3×20 mL). The combined organic fractions were washed with water (3×70 mL) and extracted with 2% KOH in water (3×100 mL). The basic water fractions were combined and washed with diethyl ether (3×15 mL). The basic layer was separated and AcOH was added until the pH 5. The precipitate was separated, washed with heptane (3×30 mL), dried *in vacuo* and recrystallized from acetone. The yield of compound **2** was 78%, m.p. 229—231 °C (acetone). ^{1}H NMR (300 MHz, DMSO-d_6), δ: 0.81 (s, 3 H, C(13)Me); 1.34, 1.91 (m, 2 H, H(7)); 1.39, 2.30 (m, 2 H, H(11)); 1.39, 1.77 (m, 2 H, H(12)); 1.39 (m, 1 H, H(14)); 1.49 (m, 1 H, H(8)); 1.98, 2.59 (m, 2 H, H(15)); 2.16 (m, 1 H, H(9)); 2.77 (m, 2 H, H(6)); 6.47 (s, 1 H, H(4)); 6.52 (m, 1 H, H(2)); 7.03 (m, 1 H, H(1)); 7.40 (s, 1 H, H(1′)). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 14.2 (C(18)), 23.8 (C(15)), 25.5 (C(11)), 26.1 (C(7)), 28.8 (C(6)), 31.3 (C(12)), 37.4 (C(8)), 43.3 (C(9)), 47.6 (C(13)), 48.3 (C(14)), 112.6 (C(2)), 113.0 (C(16)), 114.8 (C(4)), 125.6 (C(1)), 130.0 (C(10)), 136.9 (C(5)), 150.1 (C(1′)), 154.8 (C(3)), 208.3 (C(17)). Found (%): C, 76.65; H, 7.39. $C_{19}H_{22}O_3$. Calculated (%): C, 76.48; H, 7.43. MS (ESI), *m/z*: 299.

Reaction of compound 2 with thiohydrazides 3a,b in ethanol. To the stirred and heated (70 °C) solution of compound **2** (1.67 mmol) in ethanol (40 mL) thiohydrazide **3a**,**b** (1.84 mmol) and the catalytic amount of TsOH were added. The reaction mixture was stirred at this temperature for 7 h and kept for 12 h at 20 °C, and the course of the reaction was monitored by TLC. Then the reaction mixture was poured onto ice, the precipitate was filtered off, washed with water (3×50 mL), and air-dried. The residue was subjected to column chromatography on silica gel (the eluent was AcOEt—hexane, 1 : 1) and products **5a**,**b** were obtained.

3-Hydroxy-16-{2-[2-(4-methoxyphenylamino)-2-oxo-1-thioxoethyl]hydrazonomethyl}-1,3,5(10)-estratrien-17-one (5a). Yield 61%, m.p. 153—156 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.83 (s, 3 H, C(13)Me); 1.44, 1.76 (m, 2 H, H(12)); 1.34, 1.91 (m, 2 H, H(7)); 1.40, 2.31 (m, 2 H, H(11)); 1.47 (s, 1 H, H(8)); 1.57 (m, 1 H, H(14)); 1.72, 2.08 (m, 2 H, H(15)); 2.16 (s, 1 H, H(9)); 2.74 (m, 2 H, H(6)); 3.15 (m, 1 H, H(16)); 3.75 (s, 3 H, OMe); 5.60 (s, 1 H, H(1′) (**C**)); 6.45 (s, 1 H, H(4)); 6.52 (s, 1 H, H(2)); 6.94 (m, 2 H, H_{Ar}); 7.04 (s, 1 H, H(1)); 7.73 (m, 2 H, H_{Ar}); 8.51 (s, 1 H, H(1′) (**A**, **B**)); 9.98 (s, 1 H, NHCO); 10.91 (s, 1 H, NHCS (**A**)). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.8 (C(18)), 24.0 (C(15)), 25.5 (C(11)), 26.5 (C(7)), 29.0 (C(6)), 31.5 (C(12)), 37.1 (C(8)), 43.5 (C(9)), 44.3 (C(13)), 47.4 (C(14)), 49.3 (C(16)), 55.2 (OMe), 112.8 (C(2)), 113.9 (C_{Ar}), 115.0 (C(4)), 122.3 (C_{Ar}), 125.9 (C(1)), 129.9 (C(10)), 130.7 (C_{Ar}), 137.1 (C(5)), 155.0 (C(3)), 156.2 (C_{Ar}), 157.6 (C(3′)), 167.1 (C(2′) (**B**)), 190.1 (C(2′) (**A**)), 217.3 (C(17)). Found (%): C, 66.45; H, 6.27; S, 6.42. $C_{28}H_{31}N_3O_4S$. Calculated (%): C, 66.51; H, 6.18; S, 6.34. MS (ESI), *m/z*: 506.

3-Hydroxy-16-{2-[2-(4-fluorophenylamino)-2-oxo-1-thioxoethyl]hydrazonomethyl}-1,3,5(10)-estratrien-17-one (5b). Yield 58%, m.p. 141—143 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.80 (s, 3 H, C(13)Me); 2.75 (m, 2 H, H(6)); 5.62 (s, 1 H, H(1′) (**C**)); 6.44 (m, 1 H, H(4)); 6.52 (s, 1 H, H(2)); 7.04 (s, 1 H, H(1)); 7.40 (m, 2 H, H_{Ar}); 7.72 (m, 2 H, H_{Ar}); 8.52 (s, 1 H, H(1′) (**A**, **B**)); 10.21 (s, 1 H, NHCO). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.8 (C(18)), 23.9 (C(15)), 25.7 (C(11)), 26.9 (C(7)), 29.2 (C(6)), 31.3 (C(12)), 37.5 (C(8)), 43.7 (C(9)), 44.1 (C(13)), 47.6 (C(14)), 49.2 (C(16)), 112.8 (C(2)), 115.0 (C(4)), 122.4 (C_{Ar}), 125.9 (C(1)), 126.8 (C_{Ar}), 129.5 (C_{Ar}), 129.9 (C(10)), 137.2 (C(5)), 141.3 (C_{Ar}), 155.0 (C(3)), 163.1 (C(3′)), 167.2 (C(2′)), 190.1 (C(2′) (**A**)); 217.3, 217.7 (C(17)). Found (%): C, 65.82; H, 5.67; F, 3.93; S, 6.40. $C_{27}H_{28}FN_3O_3S$. Calculated (%): C, 65.70; H, 5.72; F, 3.85; S, 6.50. MS (ESI), *m/z*: 494.

Cyclization of compounds 5a,b. The solution of thiohydrazone **5a**,**b** (0.132 mmol) in dioxane (10 mL) was refluxed in the presence of the catalytic amount of TsOH, and the course of the reaction was monitored by TLC. The reaction mixture was poured onto ice, the residue was filtered off, washed with water (3×50 mL), hexane (3×25 mL), air-dried and subjected to column chromatography on silica gel (the eluent was ethyl acetate—hexane, 1 : 1) and products **7a**,**b** were obtained.

5-[3-Hydroxy-17-oxoestra-1,3,5(10)-trien-16-yl]-*N*-(4-methoxyphenyl)-1,3,4-thiadiazole-2-carboxamide (7a). Yield 72%, m.p. 162—166 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.82 (s, 3 H, C(13)Me); 1.37, 1.92 (m, 2 H, H(7)); 1.42, 1.75 (m, 2 H, H(12)); 1.42, 2.31 (m, 2 H, H(11)); 1.47 (s, 1 H, H(14)); 1.56 (s, 1 H, H(8)); 2.19 (m, 1 H, H(9)); 2.76 (m, 2 H, H(6)); 3.75 (s, 3 H, OMe); 4.33 (m, 1 H, H(16)); 6.47 (m, 1 H, H(4)); 6.53 (s, 1 H, H(2)); 6.95 (m, 2 H, H_{Ar}); 7.05 (s, 1 H, H(1)); 7.73 (m, 2 H, H_{Ar}); 10.96 (s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 13.5 (C(18)), 25.5 (C(11)), 26.3 (C(7)), 38.5 (C(15)), 29.0 (C(6)), 31.8 (C(12)), 37.2 (C(8)), 47.3 (C(13)), 43.7 (C(9)), 49.3 (C(14)), 49.8 (C(16)), 55.2 (OMe), 112.8 (C(2)), 113.9 (C_{Ar}), 114.9 (C(4)), 120.9 (C_{Ar}), 122.2 (C_{Ar}), 125.9 (C(1)), 129.9 (C(10)), 137.2 (C(5)), 155.0 (C(3)), 156.0 (C_{Ar}), 156.2 (C(3′)), 164.3 (C(2′)), 164.5 (C(1′)), 219.8 (C(17)). Found (%): C, 66.67; H, 5.88; N, 8.48; S, 6.29. $C_{28}H_{29}N_3O_4S$. Calculated (%): C, 66.78; H, 5.80; N, 8.34; S, 6.37. MS (ESI), *m/z*: 504.

5-[3-Hydroxy-17-oxoestra-1,3,5(10)-trien-16-yl]-*N*-(4-fluorophenyl)-1,3,4-thiadiazole-2-carboxamide (7b). Yield 69%, m.p. 174—176 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.81 (s, 3 H, C(13)Me); 2.76 (m, 2 H, H(6)); 3.75 (s, 3 H, OMe); 4.38 (m, 1 H, H(16)); 6.47 (m, 1 H, H(4)); 6.57 (s, 1 H, H(2)); 7.42 (m, 2 H, H_{Ar}); 7.05 (s, 1 H, H(1)); 7.84 (m, 2 H, H_{Ar}); 10.89 (s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 13.1 (C(18)), 25.7 (C(11)), 26.3 (C(7)), 29.0 (C(6)), 31.5 (C(12)), 37.4 (C(8)), 38.5 (C(15)), 43.7 (C(9)), 47.3 (C(13)), 49.3 (C(14)), 49.8 (C(16)), 112.8 (C(2)), 114.9 (C(4)), 121.3 (C_{Ar}), 125.9 (C(1)), 126.5 (C_{Ar}), 129.6 (C_{Ar}), 130.0 (C(10)), 137.2 (C(5)), 141.3 (C_{Ar}), 155.0 (C(3)), 156.6 (C(3′)), 164.5 (C(2′)), 164.8 (C(1′)), 219.8 (C(17)). Found (%): C, 66.11; H, 5.27; F, 3.97; N, 8.43; S, 6.42. $C_{27}H_{26}FN_3O_3S$. Calculated(%): C, 65.97; H, 5.33; F, 3.86; N, 8.55; S, 6.52. MS (ESI), *m/z*: 492.

Reaction of compound 2 with thiohydrazides 3a,b in acetic acid. To the stirred solution of ketone **2** (1.2 mmol) in acetic acid (25 mL), thiohydrazide **3a**,**b** (1.3 mmol) was added, the reaction mixture was refluxed for 1—2 h, and the course of the reaction was monitored by TLC. The reaction mixture was poured onto ice, the residue was filtered off, washed with water (3×50 mL), hexane (3×25 mL), air-dried and subjected to column chromato-

graphy on silica gel (the eluent was AcOEt—hexane, 1 : 1) and the products **9a**,**b** were obtained.

5-[3-Acetoxy-17-oxoestra-1,3,5(10)-trien-16-yl]-*N*-(4-methoxyphenyl)-1,3,4-thiadiazole-2-carboxamide (9a). Yield 72%, m.p. 170—173 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.82 (s, 3 H, C(13)Me); 1.37, 1.92 (m, 2 H, H(7)); 1.42, 1.75 (m, 2 H, H(12)); 1.42, 2.31 (m, 2 H, H(11)); 1.47 (s, 1 H, H(14)); 1.56 (s, 1 H, H(8)); 1.96 (s, 3 H, CH_3 (OAc)); 2.19 (m, 1 H, H(9)); 2.76 (m, 2 H, H(6)); 3.75 (s, 3 H, OMe); 4.33 (m, 1 H, H(16)); 6.47 (m, 1 H, H(4)); 6.53 (s, 1 H, H(2)); 6.95 (m, 2 H, H_{Ar}); 7.05 (s, 1 H, H(1)); 7.73 (m, 2 H, H_{Ar}); 10.96 (s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 13.5 (C(18)), 21.2 (CH_3 (AcO)), 25.5 (C(11)), 26.3 (C(7)), 29.0 (C(6)), 31.8 (C(12)), 37.2 (C(8)), 38.2 (C(15)), 47.3 (C(13)), 43.7 (C(9)), 49.3 (C(14)), 49.8 (C(16)), 55.2 (OMe), 112.8 (C(2)), 113.9 (C_{Ar}), 114.9 (C(4)), 120.9 (C_{Ar}), 122.2 (C_{Ar}), 125.9 (C(1)), 129.9 (C(10)), 137.2 (C(5)), 149.0 (C(3)), 156.0 (C_{Ar}), 156.2 (C(3´)), 164.3 (C(2´)), 164.5 (C(1´)), 170.2 (C=O (AcO)), 219.8 (C(17)). Found (%): C, 66.13; H, 5.65; N, 7.61; S, 5.99. $C_{30}H_{31}N_3O_5S$. Calculated (%): C, 66.04; H, 5.73; N, 7.70; S, 5.88. MS (ESI), *m/z*: 546.

5-[3-Acetoxy-17-oxoestra-1,3,5(10)-trien-16-yl]-*N*-(4-fluorophenyl)-1,3,4-thiadiazole-2-carboxamide (9b). Yield 69%, m.p. 182—185 °C (AcOEt—hexane). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.81 (s, 3 H, C(13)Me); 2.76 (m, 2 H, H(6)); 3.75 (s, 3 H, OMe); 4.38 (m, 1 H, H(16)); 6.47 (m, 1 H, H(4)); 6.57 (s, 1 H, H(2)); 7.42 (m, 2 H, H_{Ar}); 7.05 (s, 1 H, H(1)); 7.84 (m, 2 H, H_{Ar}); 10.89 (s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 13.1 (C(18)), 21.4 (CH_3 (AcO)), 25.7 (C(11)), 26.3 (C(7)), 29.0 (C(6)), 31.5 (C(12)), 37.4 (C(8)), 38.2 (C(15)), 43.7 (C(9)), 47.3 (C(13)), 49.3 (C(14)), 49.8 (C(16)), 112.8 (C(2)), 114.9 (C(4)), 121.3 (C_{Ar}), 125.9 (C(1)), 126.5 (C_{Ar}), 129.6 (C_{Ar}), 130.0 (C(10)), 137.2 (C(5)), 141.3 (C_{Ar}), 149.0 (C(3)), 156.6 (C(3´)), 164.5 (C(2´)), 164.8 (C(1´)), 170.1 (C=O (AcO)), 219.8 (C(17)). Found (%): C, 65.39; H, 5.18; F, 3.67; N, 7.74; S, 6.14. $C_{29}H_{28}FN_3O_4S$. Calculated (%): C, 65.27; H, 5.29; F, 3.56; N, 7.87; S, 6.01. MS (ESI), *m/z*: 534.

2-Hydroxymethylidene-1-indanone (10) was obtained by the reaction of 1-indanone with ethyl formate in the presence of sodium methoxide.[17]

***N*-(4-Methoxyphenyl)-1,3,4-thiadiazole-2-carboxamide (11).** To the stirred solution of 2-hydroxymethylidene-1-indanone (**10**) (1.25 mmol) in ethanol (35 mL), thiohydrazide **3a** (1.5 mmol) and catalytic amount of TsOH were added at room temperature and the mixture was stirred and refluxed for 1—2 h (TLC monitoring). Then the reaction mixture was poured into water (70 mL), extracted with ethyl acetate (3×30 mL), combined organic fractions were washed with water (3×35 mL), dried over $MgSO_4$ and the solvent was evaporated. The residue was subjected to column chromatography on silica gel (the eluent was AcOEt—hexane, 1 : 1). Product **11** was obtained with the yield 69%, m.p. 192—194 °C (AcOEt—hexane). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.86 (s, 3 H, OMe); 6.95 (d, 2 H, H_{Ar}, J = 8.7 Hz); 7.62 (d, 2 H, H_{Ar}, J = 8.7 Hz); 9.11 (s, 1 H, NH); 9.33 (s, 1 H, H(1)). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 56.8 (OMe), 112.6 (C_{Ar}), 122.3 (C_{Ar}), 135.1 (C_{Ar}), 155.5 (C_{Ar}), 156.2 (C(3)), 159.3 (C(1)), 164.3 (C(2)). Found (%): C, 51.13; H, 3.74; N, 17.78; S, 13.74. $C_{10}H_9N_3O_2S$. Calculated (%): C, 51.05; H, 3.86; N, 17.86; S, 13.63. MS (ESI), *m/z*: 236.

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