Supplementary Information

Access to Steroidal Pyridazines via Modified Thiohydrazides

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General Information

NMR spectra were acquired on Bruker Avance 600 and 300 spectrometers at room temperature; the chemical shifts δ were measured in ppm relative to the solvent (\(^1^H\): CDCl\(_3\), δ = 7.27 ppm, DMSO-d\(_6\), δ = 2.50 ppm; \(^1^C\): CDCl\(_3\), δ = 77.00 ppm, DMSO-d\(_6\), δ = 39.50 ppm). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet. The coupling constants (J) are in Hertz. The structures of all compounds were established using 1D NMR (\(^1^H\), \(^1^C\), JMOD) and 2D NMR (\(^1^H\)-\(^1^H\) COSY, \(^1^H\)-\(^1^H\) TOCSY, \(^1^H\)-\(^1^H\) ROESY, \(^1^C\)-\(^1^H\) HMBC, \(^1^C\)-\(^1^H\) HSQC, \(^1^H\)-\(^1^H\) NOESY) spectroscopy. Infrared spectra were measured on a FT-IR spectrometer in KBr pellets. High-resolution and accurate mass spectra were obtained on Bruker micrOTOF-QTM ESI-TOF (Electrospray Ionization/Time of Flight) and Thermo Scientific* LTQ Orbitrap mass spectrometers. Melting points (mp) are uncorrected and were measured on a Boetius capillary melting point apparatus. Analytical thin layer chromatography (TLC) was carried out on silica gel plates (silica gel 60 F254 aluminum supported plates); the visualization was accomplished with an UV lamp (365 nm) and using chemical staining with \([\text{Ce(SO}_4]_2\)/H\(_2\)SO\(_4\). Column chromatography was performed on silica gel 60 (60-120 mesh, Merck). 3β-Acetate androst-5-en-3β-ol-17-one, 17β-hydroxy-17α-methyl-5α-androstan-3-one, 17β-hydroxy-5α-androstan-3-one, estrone, phosphorus oxychloride, morpholine and hydrazine hydrate were commercially available and were used without additional purification. All reactions were carried out using freshly distilled and dry solvents. Parent chloroacetanilides were prepared according to published procedures.¹

Typical experimental procedures

I. Preparation of oxamic acids thiohydrazides

General procedure for the preparation of oxamic acids thiohydrazides: Various oxamic acid thiohydrazides substrates were prepared from the corresponding chloroacetanilides in high yields by the treatment with hydrazine according to the literature procedure.\(^2\)

Representative compound: 2-Hydrayzil-N-(4-methoxyphenyl)-2-thioxoacetamide (3a).

![Diagram of the synthesis of 2-Hydrayzil-N-(4-methoxyphenyl)-2-thioxoacetamide (3a)](image)

To a solution of sulfur (2.0 g, 62.5 mmol) in morpholine (7 mL) and DMF (7 mL), chloroacetanilide (2.0 g, 10.0 mmol) was added with stirring. The reaction mixture was kept overnight at room temperature (TLC monitoring). The mixture was poured into water (70 mL). The precipitate that formed was filtered off, dried, and dissolved in acetone to remove the sulfur excess. The organic fraction was separated, and the solvent was evaporated in vacuo. The solid residue was recrystallized from ethanol to give monothiooxamide as a white solid in 95% yield (2.7 g, 9.5 mmol).

To a solution of monothiooxamide (2.7 g, 9.5 mmol) in DMF (10 mL), hydrazine hydrate (2.7 mL, 50 mmol) was added, and the mixture was allowed to stand at room temperature for 10-12 h. After completion of the reaction (TLC monitoring), the reaction mixture was poured into water (40 mL) and the solution was acidified with hydrochloric acid to pH 6. The precipitate was filtered off and recrystallized from ethanol to give thiohydrazides in 62% yield (1.3 g, 5.9 mmol). Mp 159-160°C (mp lit 161-163°C). \(^1\)H NMR (300 MHz, CDCl₃): δ 3.75 (s, 3H, OMe); 6.91 (d, \(J = 8.83\) Hz, 2H, Ar); 7.70 (d, \(J = 8.83\) Hz, 2H, Ar); 10.12 (br.s, 1H, NH).

3-Hydrazinyl-N-(4-chlorophenyl)-2-thioxoacetamide (3b). The general procedure was followed using chloroacetanilide (2.04 g, 10.0 mmol), sulfur (2.0 g, 62.5 mmol) in morpholine (7 mL) and DMF (7 mL). Workup afforded analytically pure compound as a pale yellow solid (1.84 g, 85%), mp 169-171°C (mp lit 172-174°C). The spectral data matched that reported by Krayushkin and coworkers.\(^2\)\(^1\)H NMR (CDCl₃, 300 MHz): δ 7.42 (d, 2H, \(J = 8.73\) Hz, Ar); 7.86 (d, 2H, \(J = 8.72\) Hz, Ar); 10.28 (s, 1H, NH).

3-Hydrazinyl-N-(4-(trifluoromethyl)phenyl)-2-thioxoacetamide (3c). The general procedure was followed using chloroacetanilide (2.37 g, 10.0 mmol), sulfur (2.0 g, 62.5 mmol) in morpholine (7 mL) and DMF (7 mL). Workup afforded analytically pure compound as a pale yellow solid (1.58 g, 60%), mp 169-170°C. The spectral data matched that reported by Krayushkin and coworkers.\(^2\)\(^1\)H NMR (DMSO-\(d_6\), 300 MHz): δ 7.70 (d, 2H, \(J = 8.07\) Hz, Ar); 8.00 (d, 2H, \(J = 8.07\) Hz, Ar); 10.50 (s, 1H, NH).

3-Hydrazinyl-N-phenyl-2-thioxoacetamide (3d). The general procedure was followed using chloroacetanilide (1.7 g, 10.0 mmol), sulfur (2.0 g, 62.5 mmol) in morpholine (7 mL) and DMF (7 mL). The spectral data matched that reported by Krayushkin and coworkers.\(^2\) Workup afforded analytically pure compound as a white solid (1.53 g, 83%), mp 145-146°C (mp lit 144-147°C).\(^1\)H NMR


\(^3\) The signals of the NH₂NH groups were not observed in \(^1\)H NMR.
2-Hydrazinyl-N-(3-methoxyphenyl)-2-thiooxoacetamide (3e). The general procedure was followed using chloroacetanilide (2.00 g, 10.0 mmol), sulfur (2.0 g, 62.5 mmol) in morpholine (7 mL) and DMF (7 mL). Workup afforded analytically pure compound as a pale yellow solid (1.62 g, 71%), mp 165-168°C. The spectral data matched that reported by Krayushkin and coworkers. \( ^{1} \)H NMR (DMSO-\( d_{6} \), 300 MHz): \( \delta \) 7.15-7.16 (m, 1H, Ph); 7.40-7.43 (m, 2H, Ph); 7.64 (d, 2H, \( J = 7.92 \) Hz, Ph); 10.20 (s, 1H, NH).

2-Hydrazinyl-N-(4-fluorophenyl)-2-thiooxoacetamide (3f). The general procedure was followed using chloroacetanilide (1.90 g, 10.0 mmol), sulfur (2.0 g, 62.5 mmol) in morpholine (7 mL) and DMF (7 mL). Workup afforded analytically pure compound as a pale yellow solid (1.81 g, 85%), mp 170-172°C (mp\(_{ \text{lit} } \) 157-160°C). The spectral data matched that reported by Krayushkin and coworkers. \( ^{1} \)H NMR (DMSO-\( d_{6} \), 300 MHz): \( \delta \) 7.22 (dd, 2H, \( J = 8.79 \) Hz, Ar); 7.80 (dd, 2H, \( J = 4.40, 8.79 \) Hz, Ar); 10.29 (s, 1H, NH).
II. Preparation of chlorovinyl aldehydes

General procedure for the preparation of 17-chloro-16-formyl steroids: Various chloroformyl steroid substrates were prepared from the corresponding ketosteroids in high yield by the treatment with phosphorus oxychloride according to a modified literature procedure.¹

17α,17β-Dimethyl-2-formyl-3-chloro-18-norandroste-2,13-diene (2).

A flame-dried round bottom flask containing a stir bar and DMF (0.70 mL, 8.9 mmol) in CH₂Cl₂ (8 mL) was cooled to 0°C and 0.75 mL of POCl₃ (8.1 mmol) was added dropwise while stirring. The mixture was stirred for 90 minutes, before a dropwise addition of 17β-hydroxy-17α-methyl-5α-androstan-3-one (0.35 g, 1.2 mmol) solution in CH₂Cl₂ (10 mL). The mixture was warmed to ambient temperature and stirred for 18 hours. The reaction was delutted with CH₂Cl₂ (20 mL) poured over ice and quenched with NaHCO₃. Once neutralized, the organic fraction was washed with water (3×25 mL) and brine (1×20 mL). The organic phase was dried over Na₂SO₄, filtered, and concentrated to afford the product. The residue was purified by silica gel (60-120 mesh) column chromatography with a petroleum ether – benzene (6:1) as eluent, to give product as white solid (0.23 g, yield 61 %). Mp 116-117°C.

¹HNMR (600 MHz, CDCl₃): δ 0.70 (s, 3H, 19-CH₃); 0.97 (s, 6H, 17'-CH₃, 17''-CH₃); 0.97-1.13 (m, 2H, 9-CH, 7-CH₂); 1.21-1.33 (m, 2H, 6-CH₂, 11-CH₂); 1.54-1.67 (m, 4H, 5-CH, 6-CH₂, 16-CH₂); 1.78 (d, 1H, J = 17.40 Hz, 1-CH₂); 1.82-1.89 (m, 1H, 12-CH₂); 1.90-1.97 (m, 2H, 7-CH₂, 11-CH₂); 1.98-2.04 (m, 2H, 8-CH, 12-CH₂); 2.04-2.10 (m, 1H, 15-CH₂); 2.21-2.27 (m, 1H, 15-CH₂); 2.35 (dd, 1H, J = 3.00, 11.40 Hz, 4-CH₂); 2.45 (dd, 1H, J = 4.20, 11.40 Hz, 4-CH₂); 2.63 (d, 1H, 17.40 Hz, 1-CH₂); 10.22 (s, 1H, CHO).

¹³CNMR (125 MHz, CDCl₃): δ 11.4 (19-CH₃); 22.1 (12-CH₂); 22.7 (11-CH₂); 26.4 (17'-CH₃); 26.7 (17''-CH₃); 28.1 (6-CH₂); 29.7 (15-CH₂); 30.6 (7-CH₂); 34.3 (10-C); 36.4 (8-CH); 37.8 (1-CH₂); 39.5 (16-CH₂); 40.3 (4-CH₂); 42.5 (5-CH); 45.4 (17-C); 51.1 (9-CH); 132.2 (2-C); 135.3 (14-C); 141.1 (13-C); 149.9 (3-C); 191.5 (CHO). IR(KBr): 2928, 2858 (CH), 1675(CO), 1623 (C=C). HRMS (ESI) for C₂₁H₂⁹ClONa ([M+Na]⁺): calcd 355.1799, found ([M+Na]⁺) 355.1797.

17β-Formyloxy-2-formyl-3-chloro-androste-2-ene (6).

A flame-dried round bottom flask containing a stir bar and DMF (0.87 mL, 11 mmol) in CH₂Cl₂ (10 mL) was cooled to 0°C and 0.91 mL of POCl₃ (9.8 mmol) was added dropwise while stirring. The mixture was stirred for 90 minutes, before a dropwise addition of androstane (0.41 g, 1.4 mmol) solution in CH₂Cl₂ (15 mL). The mixture was warmed to ambient temperature and stirred for 22 hours. The reaction was delutted with CH₂Cl₂ (20 mL) poured over ice and quenched with NaHCO₃. Once neutralized, the organic fraction was washed with water (3×20 mL) and brine (1×20 mL). The organic phase was dried over Na₂SO₄, filtered, and concentrated to afford the product. The residue was purified by silica gel (60-120 mesh) column chromatography with a benzene as eluent, to give product as white solid (0.31 g, yield 61 %).

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recipitate was then filtered, washed with water (3×50 mL), and dried in air. The product was isolated by crystallization from aqueous methanol as a white solid in 65% yield (3.7 g, 10 mmol). Mp 178-183°C. The spectral data matched that reported by Lane and coworkers. 

3β-Acetoxy-16-formyl-17-chloro-androsta-5,16-diene (9).

Phosphorus oxychloride (15 mL, 60 mmol) was added dropwise with stirring to a solution of androstan-5(0 g, 15 mmol) in dimethylformamide (40 mL) at 55-60°C. The reaction mixture was allowed to attain 65°C and then heated for 3h. The mixture was poured onto ice-water (100 mL). The crude precipitate was filtered, washed with water (3×50 mL), and dried in air. The product was isolated by crystallization from aqueous methanol as a white solid in 70% yield (3.3 g, 11 mmol). Mp 218-220°C. The spectral data matched previously reported. 

3-Hydroxy-16-formyl-17-chloro-1,3,5,(10),16-estratetraene (13).

Phosphorus oxychloride (15 mL, 60 mmol) was added dropwise with stirring to a solution of estrone (4.0 g, 15 mmol) in dimethylformamide (40 mL) at 55-60°C. The reaction mixture was allowed to attain 65°C and then heated for 3h. The mixture was poured onto ice-water (100 mL). The crude precipitate was filtered, washed with water (3×50 mL), and dried in air. The product was isolated by crystallization from aqueous methanol as a white solid in 70% yield (3.3 g, 11 mmol). Mp 218-220°C. The spectral data matched previously reported. 


The signal of the OH group was not observed in 1H NMR of 3β-hydroxy-17-chloro-16-formyl-estrone-16-ene.
III. Preparation of pyridazines

**Synthesis of 17α,17β-Dimethyl-18-nor-5α-androsta-2,13-diene[3,2-d]-3'-{(N-arylcarbamoyl)pyridazines 4.**

![Chemical Structure](image)

Oxamic acid thiohydrazide (0.17mmol) was added to a solution of the 17α,17β-dimethyl-2-formyl-3-chloro-18-norandrostane-2,13-diene (56 mg, 0.17mmol) and p-toluenesulfonic acid monohydrate (3.1 mg, 10 mol%) in ethanol (30 mL). The reaction mixture was refluxed for 2h until the complete conversion of the intermediate hydrozone (TLC monitoring). The resulted mixture was cooled to room temperature and solvent removed under reduced pressure. The crude product was purified by column chromatography using silica gel (60-120 mesh) column chromatography with chloroform/MeOH (150:1) as eluent.

**17α,17β-Dimethyl-18-nor-5α-androsta-2,13-diene[3,2-d]-3'{-[N-(4-methoxyphenyl)carbamoyl]pyridazine 4a.**

Yield 68% (54 mg). Pale yellow solid, Mp 111-112°C.

1H NMR (600 MHz, CDCl3): δ 0.75 (s, 3H, 19-CH3); 0.98 (s, 6H, 17′-CH3, 17″-CH3); 1.02-1.15 (m, 2H, 7-CH2, 9-CH); 1.29-1.35 (m, 1H, 11-CH2); 1.38-1.45 (m, 1H, 6-CH2); 1.53-1.60 (m, 1H, 5-CH); 1.60-1.68 (m, 2H, 16-CH2); 1.72-1.78 (m, 1H, 6-CH2); 1.87-1.97 (m, 2H, 11-CH2,12-CH2); 1.98-2.12 (m, 4H, 7-CH2, 8-CH, 12-CH2, 15-CH2); 2.23-2.30 (m, 1H, 15-CH2); 2.53 (d, 1H, J = 17.40 Hz, 1-CH2); 2.88 (dd, 1H, J = 12.00, 18.00 Hz, 4-CH2); 2.94 (d, 1H, J = 17.40 Hz, 1-CH2); 3.54 (dd,1H, J = 4.80, 18.00 Hz, 4-CH2); 3.82 (s, 3H, OMe); 6.92 (d, 2H, J = 8.40 Hz,2CH_arom); 7.67 (d, 2H, J = 8.40 Hz,2CH_arom); 8.98 (s, 1H, CH_pyridazine); 10.15 (1H, NH).13C NMR (125 MHz, CDCl3): δ 11.6 (19-CH3); 22.2 (12-CH2); 22.7 (11-CH2); 26.4 (17′-CH3); 26.7 (17″-CH3); 28.8 (6-CH2); 29.7 (15-CH2); 30.3 (4-CH2); 30.7 (7-CH2); 34.1 (10-C); 36.5 (8-CH); 39.5 (16-CH2); 41.0 (1-CH2); 41.1 (5-CH); 45.4 (17-C); 51.3(9-CH); 55.0 (OMe); 114.3 (2CH_arom); 121.6 (2CH_arom); 130.8 (C_arom); 135.6 (14-C); 140.2 (3-C); 140.6 (13-C); 141.4 (2-C); 150.1 (DSPyridazine); 153.1 (CH_pyridazine); 156.7 (C_arom); 161.6 (CO). IR(KBr): 3316 (NH), 2949, 2830, 1761, 1639, 1576, 1490, 1435, 1360, 1276, 1171, 1094, 978, 752 (cm-1). HRMS (ESI) for C23H33N5O2 ([M+H]+): calcld 472.2959, found 472.2949.

**17α,17β-Dimethyl-18-nor-5α-androsta-2,13-diene[3,2-d]-3’{-[N-(4-chlorophenyl)carbamoyl]pyridazine 4b.**

Yield 82% (66 mg). Pale yellow solid, Mp 96-97°C.

1H NMR (600 MHz, CDCl3): δ 0.76 (s, 3H, 19-CH3); 0.99 (s, 6H, 17′-CH3, 17″-CH3); 1.02-1.13 (m, 2H, 7-CH2, 9-CH); 1.29-1.36 (m, 1H, 11-CH2); 1.38-1.47 (m, 1H, 6-CH2); 1.53-1.59 (m, 1H, 5-CH); 1.60-1.68 (m, 2H, 16-CH2); 1.74-1.79 (m, 1H, 6-CH2); 1.88-1.97 (m, 2H, 11-CH2, 12-CH2); 1.99-2.12 (m, 4H, 7-CH2, 8-CH, 12-CH2, 15-CH2); 2.23-2.30 (m, 1H, 15-CH2); 2.54 (d,1H,J = 17.40 Hz, 1-CH2); 2.87 (dd, 1H,J = 12.00, 18.00 Hz, 4-CH2); 2.95 (d, 1H,J = 17.40 Hz, 1-CH2); 3.52 (dd, 1H,J = 4.80, 18.00 Hz, 4-CH2); 7.35 (d, 2H,J = 8.40 Hz,2CH_arom); 7.73 (d, 2H, J = 8.40 Hz,2CH_arom); 8.99 (s, 1H, CH_pyridazine); 10.30 (s, 1H, NH).13C NMR (125 MHz, CDCl3): δ 11.6 (19-CH3); 22.2 (12-CH2); 22.7 (11-CH2); 26.4 (17′-CH3); 28.8 (6-CH2); 29.7 (15-CH2); 30.3 (4-CH2); 30.7 (7-CH2); 34.1 (10-C); 36.5 (8-CH); 39.5 (16-CH2); 41.0 (1-CH2); 41.1 (5-CH); 45.4 (17-C); 51.3(9-CH); 121.2 (2CH_arom); 129.1 (2CH_arom); 129.7 (C_arom); 135.5 (14-C); 136.2 (C_arom); 140.6 (3-C); 141.0 (13-C); 141.4(2-C); 149.8 (C_pyridazine); 153.0 (CH_pyridazine); 161.8 (CO). IR(KBr): 3325 (NH), 2928, 2857 (CH), 1689(CO), 1590, 1520 cm⁻¹. HRMS (ESI) for C29H35ClN3O ([M+H]+): calcld 476.2463, found 476.2460.

1H NMR (600 MHz, CDCl3): δ 0.76 (s, 3H, 19-CH3); 1.00 (s, 6H, 17'-CH3, 17''-CH3); 1.02-1.13 (m, 2H, 7-CH2, 9-CH); 1.30-1.38 (m, 1H, 11-CH2); 1.40-1.49 (m, 1H, 6-CH2); 1.55-1.62 (m, 1H, 5-CH); 1.62-1.71 (m, 2H, 16-CH2); 1.76-1.81 (m, 1H, 6-CH2); 1.90-1.97 (m, 2H, 11-CH2, 12-CH2); 2.01-2.13 (m, 4H, 7-CH2, 8-CH,12-CH2, 15-CH2); 2.25-2.33 (m, 1H, 15-CH2); 2.53 (d, 1H, J = 17.40 Hz, 1-CH2); 2.88 (dd, 1H, J = 12.60, 19.80 Hz, 4-CH2); 2.94 (d, 1H, J = 17.40 Hz, 1-CH2); 3.55 (dd, 1H, J = 4.80, 19.80 Hz, 4-CH2); 7.65 (d, 2H, J = 8.40 Hz, 2CHarom); 7.89 (d, 2H, J = 8.40 Hz, 2CHarom); 8.98 (s, 1H, CHpyridazine); 10.47 (s, 1H, NH). 13C NMR (125 MHz, CDCl3): δ 11.6 (19-CH3); 22.2 (12-CH2); 22.8 (11-CH2); 26.4 (17''-CH3); 26.7 (17'-CH3); 28.9 (6-CH2); 29.8 (15-CH2); 30.3 (4-CH2); 30.8 (7-CH2); 34.1 (10-C); 36.6 (8-CH); 39.6 (16-CH2); 41.0 (1-CH2); 41.3 (5-CH); 45.5 (17-C); 51.4 (9-CH); 119.6 (2CHarom); 126.4 (q, JCF = 3.0 Hz, 2CHarom); 135.6 (14-C); 140.0 (3-C); 140.2 (13-C); 140.7 (2-C); 141.5 (Caram); 153.9 (CHpyridazine); 162.4 (CO).8IR(KBr): 3326 (NH), 2931, 2859 (CH), 1695(CO), 1616, 1597, 1529, 1324 (CF3) cm⁻¹. HRMS (ESI) for C30H35F3N3O ([M+H]+): calc 510.2727, found 510.2720.


Oxamic acid thiobenzamide (0.17mmol) was added to a solution of 17β-formyloxy-2-formyl-3-chloroandrost-2-ene(62 mg, 0.17mmol) and p-toluenesulfonic acid monohydrate (3.1 mg, 10 mol%) in ethanol (30 mL). The reaction mixture was refluxed for 3h until the complete conversion of the intermediate hydrozone (TLC monitoring). The resulted mixture was cooled to room temperature and solvent removed under reduced pressure. The crude product was purified by column chromatography using chloroform/MeOH(60:1) as eluent.


Yield 73% (58 mg). Pale yellow solid, Mp237-238°C. The spectral data matched previously reported.5

1H NMR (600 MHz, CDCl3): δ 0.84 (s, 3H, 19-CH3); 0.85 (s, 3H, 18-CH3); 0.86-0.89 (m, 1H, 9-CH); 0.96-0.98 (m, 1H, 7-CH2); 1.00-1.02 (m, 1H, 14-CH); 1.14 (t, 1H, J = 13.00 Hz, 12-CH2); 1.30 (m, 1H, 15-CH2); 1.38 (m, 1H, 6-CH2); 1.42-1.44 (m, 1H, 8-CH); 1.45-1.47 (m, 1H, 16-CH2); 1.48-1.50 (m, 1H, 11-CH2); 1.52-1.55 (m, 1H, 5-CH); 1.62-1.64 (m, 1H, 15-CH2); 1.68-1.70 (m, 1H, 11-CH2); 1.70-1.72 (m, 1H, 6-CH2); 1.78-1.80 (m, 1H, 7-CH2); 1.90 (d, 1H, J = 12.20 Hz, 12-CH2); 2.08-2.10 (m, 1H, 16-CH2); 2.48 (d, 1H, J = 16.70 Hz, 1-CH2); 2.83 (d, 1H, J = 16.70 Hz, 1-CH2); 2.89 (ddd, 1H, J = 12.60, 18.50 Hz, 4-CH2); 3.54 (d, 1H, J = 18.50 Hz, 4-CH2); 3.68 (t, 1H, J = 8.50 Hz, 17-CH); 3.82 (s, 3H, OMe); 6.92 (d, 2H, J = 8.40 Hz, 2CHarom); 7.67 (d, 2H, J = 8.40 Hz, 2CHarom); 8.93 (s, 1H, CHpyridazine); 10.11 (s, 1H, NH).913CNMR (125MHz, CDCl3): δ 11.1 (18-CH3); 11.8 (19-CH3); 20.7 (11-CH2); 23.3 (15-CH2); 28.3 (6-CH2); 30.4 (4-CH2); 30.5 (16-CH2); 31.7 (7-CH2); 34.1 (10-C); 35.5 (8-CH); 36.6 (12-CH2); 41.0 (1-CH2); 41.3 (5-CH); 42.9 (13-C); 50.9 (14-CH); 53.7 (9-CH); 55.5 (OMe); 81.8 (17-CH); 114.3 (2CHarom); 121.6 (2CHarom); 130.8 (Caram); 139.6 (C-C); 139.8 (2-C); 149.7 (Cpyridazine); 153.6 (Cpyridazine); 156.6 (Caram); 161.8 (CO). IR(KBr): 3423 (NH), 2929, 2862 (CH), 1688(CO), 1523, 1514.

8 The signals of the CF3 group and C were not observed in 13C NMR spectra.
9 The signal of the OH group was not observed in 1H NMR.

Yield 67% (54 mg). Pale yellow solid, Mp148-149°C.

1H NMR (600 MHz, DMSO-d6): δ 0.65 (s, 3H, 18-CH3); 0.69 (s, 3H, 19-CH3); 0.80-0.92 (m, 3H, 7-CH2, 9-CH, 14-CH); 1.00 (t, 1HJ = 9.00 Hz, 12-CH2); 1.16 (dq, 1HJ = 6.00, 12.00 Hz, 15-CH2); 1.21-1.42 (m, 4H, 6-CH2, 8-CH, 11-CH2, 16-CH2); 1.45-1.57 (m, 3H, 5-CH, 6-CH2, 15-CH2); 1.59-1.68 (m, 2H, 7-CH2, 11-CH2); 1.76-1.87 (m, 2H, 12-CH2, 16-CH2); 2.43 (d, 1HJ = 17.40 Hz, 1H, 1-CH2); 2.55 (dd, 1H, J = 12.00, 19.20 Hz, 4-CH2); 2.83 (d, 1HJ = 17.40 Hz, 1-CH2); 2.96 (dd, 1H, J = 4.80, 19.20 Hz, 4-CH2); 3.44 (t, 1HJ = 6.00 Hz, 17-CH); 4.42 (s, 1H, OH); 7.41 (d, 2HJ = 9.00 Hz, 2CHaryl); 7.87(d, 2HJ = 9.00 Hz, 2CHaryl); 9.04 (s, 1H, CHpyridazine); 10.93 (s, 1H, NH).13C NMR (125 MHz, DMSO-d6): δ 11.2 (18-CH3); 11.4 (19-CH3); 20.3 (11-CH2); 23.0 (15-CH2); 27.7 (6-CH2); 28.4 (4-CH2); 29.8 (16-CH2); 30.6 (7-CH2); 33.7 (10-C); 35.0 (8-CH2); 36.4 (12-CH2); 39.8 (1-CH2); 40.2 (5-CH); 42.4 (13-C); 50.4 (14-CH); 52.9 (9-CH); 80.0 (17-CH); 121.6 (2CHaryl); 127.6 (Caryl); 137.5 (Caryl); 138.1 (2-C); 152.8 (Cpyridazine); 153.7 (CHpyridazine); 163.6 (CO).IR(KBr): 3328 (NH); 2928, 2870 (CH), 1689(CO), 1590, 1519, 1493 cm⁻¹. HRMS (ESI) for C28H35ClN4O2 ([M+H]⁺): calc 480.2412, found 480.2403.


Oxamic acid thiohydrazide (0.17mmol) was added to a solution of the 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (65 mg, 0.17mmol) and p-toluensulfonic acid monohydrate (3.1 mg, 10 mol%) in ethanol (30 mL). The reaction mixture was stored at rt for 30 min, diluted with water (30 mL) and extracted with ethyl acetate (3×50 mL). Organic fraction was dried over Na₂SO₄, solvent was removed under reduced pressure and resulting solid hydrazone was resolved in ethanol (10 mL). Solution was refluxed for 1h and cooled to rt. Precipitate formed was filtered for 10d, in the over cases the crude product was purified by column chromatography using benzene/chloroform (1:1) as eluent.


Yield 73% (65 mg). Pale yellow solid, Mp269-270°C.

1H NMR (300 MHz, CDCl₃): δ 1.03-1.15 (m, 2H, 1-CH₂, 9-CH); 1.10 (s, 3H, 19-CH₃); 1.27 (s, 3H, 18-CH₃); 1.40-1.98 (m, 9H, 1-CH₂, 2-CH₂, 7-CH₂, 8-CH₁, 11-CH₂, 12-CH₂, 14-CH); 2.03 (s, 3H, Ac); 2.10-2.20 (m, 1H, 7-CH₂); 2.30-2.43 (m, 2H, 4-CH₂); 2.65 (dd, 1HJ = 16.00 Hz, 15-CH₂); 2.89 (dd, 1H, J= 6.00, 16.00 Hz, 15-CH₂); 3.10-3.14 (m, 1H, 12-CH₂); 3.82 (s, 3H, OMe); 4.60-4.63 (m, 1H, 3-CH); 5.41-5.44 (m, 1H, 6-CH); 6.92 (d, 2H, J= 8.00 Hz, 2CH₂aryl); 7.68 (d, 2H, J= 8.00 Hz, 2CH₂aryl); 9.20 (s, 1H, CHpyridazine); 9.98 (s, 1H, NH).13C NMR (125 MHz, DMSO-d₆): δ 16.3 (18-CH₃); 19.0 (19-CH₃); 20.1 (11-CH₂); 21.2 (COMe); 27.3 (2-CH₂); 29.9 (15-CH₂); 30.3 (8-CH₂); 31.0 (7-CH₂); 32.7 (12-CH₂); 36.6 (1-CH₂),37.1 (10-C); 37.8 (4-CH₂); 46.4 (13-C); 49.4 (9-CH); 55.4 (OMe); 56.4 (14-CH); 73.0 (3-CH); 113.9 (2CH₂aryl); 121.7 (2CH₂aryl); 121.7 (6-CH); 131.7 (Caryl); 140.0 (5-C); 146.1 (16-C); 150.0 (CHpyridazine); 151.7 (17-C); 152.2 (Cpyridazine); 156.0 (Caryl); 163.2 (CO); 170.0 (CO).IR(KBr): 3234 (NH); 2958, 2932, 2905, 2874 (CH), 1727(COO), 1680 (CON), 1597, 1518 cm⁻¹. HRMS (ESI) for C₃₁H₃₈N₃O₄ ([M+H]⁺): calc 516.2857, found 516.2841.
6'-[N-(4-Chlorophenyl)carbamoyl]-3β-acetoxyandrostan-5-eno[16,17-d]pyridazine 10b.

Yield 67% (59 mg). Pale yellow solid, Mp230-231°C.

1H NMR (600 MHz, DMSO-d6): δ 1.01-1.11 (m, 1H, 1-CH2); 1.05 (s, 3H, 19-CH3); 1.15 (s, 3H, 18-CH3); 1.42-1.48 (m, 1H, 12-CH2); 1.52-1.59 (m, 1H, 2-CH2); 1.55-1.63 (m, 1H, 14-CH); 1.58-1.67 (m, 2H, 11-CH2); 1.67-1.75 (m, 1H, 7-CH2); 1.75-1.82 (m, 1H, 2-CH2); 1.77-1.83 (m, 1H, 8-CH); 1.79-1.87 (m, 1H, 1-CH2); 1.98 (s, 3H, Ac); 2.07-2.13 (m, 1H, 7-CH2); 2.24-2.34 (m, 2H, 4-CH2); 2.34-2.40 (m, 1H, 12-CH2); 2.69 (dd, 1H, J = 15.60 Hz, 15-CH2); 2.86 (dd, 1H, J = 6.00, 15.60 Hz, 15-CH2); 4.42-4.46 (m, 1H, 3-CH); 5.39-5.41 (m, 1H, 6-CH); 7.42 (d, 2H, J = 7.80 Hz, 2CH(aram); 7.86 (d, 2H, J = 7.80 Hz, 2CH(aram); 9.34 (s, 1H, CH(pyridazine)); 10.95 (s, 1H, NH).13CNMR (125 MHz, DMSO-d6): δ 16.0 (18-CH3); 18.7 (19-CH3); 19.9 (11-CH2); 20.9 (CO(Me)); 27.2 (2-CH2); 29.5 (15-CH2); 30.0 (8-CH); 30.8 (7-CH2); 32.5 (12-CH2); 36.1 (11-CH2, 10-C); 37.5 (4-CH2); 46.2 (13-C); 49.2 (9-CH); 56.2 (14-CH); 73.0 (3-CH); 121.4 (6-CH); 121.5 (2CH(aram); 127.6(Carom); 128.6 (2CH(aram); 137.4(Carom); 139.8 (5-C); 145.1 (16-C); 150.3 (CH(pyridazine); 150.8 (17-C); 151.4 (Cpyridazine); 163.7 (CO); 169.5 (CO).IR(KBr): 3324 (NH), 2942, 2908, 2870 (CH), 1732(COO), 1697 (CON).

6'-[N-(4-Trifluoromethylphenyl)carbamoyl]-3β-acetoxyandrostan-5-eno[16,17-d]pyridazine 10c.

Yield 75% (71 mg). Pale yellowsolid, Mp 214-215°C.

1H NMR (600 MHz, DMSO-d6): δ 1.03-1.15 (m, 2H, 1-CH2, 9-CH); 1.03 (s, 3H, 19-CH3); 1.17 (s, 3H, 18-CH3); 1.44-1.52 (m, 1H, 12-CH2); 1.55-1.62 (m, 1H, 2-CH2); 1.60-1.67 (m, 1H, 14-CH); 1.60-1.70 (m, 2H, 11-CH2); 1.73-1.79 (m, 1H, 7-CH2); 1.77-1.82 (m, 1H, 2-CH2); 1.80-1.87 (m, 2H, 8-CH, 1-CH2); 1.98 (s, 3H, Ac); 2.10-2.17 (m, 1H, 7-CH2); 2.26-2.38 (m, 2H, 4-CH2); 2.40-2.46 (m, 1H, 12-CH2); 2.71 (dd, 1H, J = 16.20 Hz, 15-CH2); 2.87 (dd, 1H, J = 6.60, 16.20 Hz, 15-CH2); 4.45-4.48 (m, 1H, 3-CH); 5.40-5.42 (m, 1H, 6-CH); 7.73 (d, 2H, J = 8.40Hz, 2CH(aram); 8.05 (d, 2H, J = 8.40 Hz, 2CH(aram); 9.52 (s, 1H, CH(pyridazine); 11.08 (s, 1H, NH).13CNMR (125 MHz, DMSO-d6): δ 15.9 (18-CH3); 18.6 (19-CH3); 19.8 (11-CH2); 20.6 (COMe); 27.1 (2-CH2); 29.4 (15-CH2); 29.9 (8-CH); 30.6 (7-CH2); 32.4 (12-CH2); 36.0 (1-CH2, 10-C); 37.4 (4-CH2); 46.1 (13-C); 49.2 (9-CH); 57.0 (14-CH); 72.9 (3-CH); 119.8 (2CH(aram); 120.8 (q, J(C-F) = 33.1 Hz, Carom); 121.2 (6-CH); 122.5 (q, J(C-F) = 395.0 Hz, CF3); 125.7 (2CH(aram); 139.7 (5-C); 141.8(Carom); 145.0 (16-C); 150.2 (CH(pyridazine); 150.8 (17-C); 151.0 (Cpyridazine); 163.9 (CO); 169.3 (CO).IR(KBr): 3324 (NH), 2942, 2908, 2870 (CH), 1732(CO); 1697 (CON), 1616, 1599, 1529,1510 cm⁻¹. HRMS (ESI) for C30H35ClN3O3 [(M+H)+]: calcld 554.2625, found 554.2615.

6'-[N-(N-Phenylcarbamoyl)-3β-acetoxyandrostan-5-eno[16,17-d]pyridazine 10d.

Yield 82% (68 mg). Colorless solid, Mp282-283°C. The spectral data matched previously reported.5

1H NMR (600 MHz, DMSO-d6): δ 1.03 (s, 3H, 19-CH3); 1.07-1.09 (m, 1H, 9-CH); 1.16 (s, 3H, 18-CH3); 1.47-1.49 (m, 1H, 12-CH2); 1.56-1.58 (m, 1H, 2-CH2); 1.61-1.63 (m, 1H, 14-CH); 1.64-1.66 (m, 2H, 11-CH2); 1.74-1.78 (m, 1H, 7-CH2); 1.79-1.80 (m, 1H, 2-CH2); 1.81-1.83 (m, 1H, 8-CH); 1.82-1.84 (m, 1H, 1-CH2); 1.98 (s, 3H,Ac); 2.10-2.13 (m, 1H, 7-CH2); 2.31-2.33 (m, 2H, 4-CH2); 2.41-2.43 (m, 1H, 12-CH2); 2.69 (dd, 1H, J = 15.60 Hz, 15-CH2); 2.87 (dd, 1H, J = 6.00, 15.60 Hz, 15-CH2); 4.43-4.46 (m, 1H, 3-CH); 5.39-5.41 (m, 1H, 6-CH); 7.14 (t, 1H, J = 7.80 Hz, CH(aram); 7.37 (t, 2H, J = 7.80 Hz, 2CH(aram); 7.81 (d, 2H, J = 7.80 Hz, 2CH(aram); 9.32 (s, 1H, CH); 10.70 (s, 1H, NH).13CNMR (125 MHz, DMSO-d6): δ 15.4 (18-CH3); 18.1 (19-CH3); 19.3 (11-CH2); 20.2 (COMe); 26.6(2-CH2); 28.9 (15-CH2); 29.4 (8-CH); 30.2 (7-CH2); 32.0 (12-CH2); 35.5 (1-CH2, 10-C); 36.9 (4-CH2); 45.6(13-C); 48.7 (9-CH); 55.6(14-CH); 72.4 (3-CH); 119.3(2CH(aram); 120.7 (6-CH); 123.3 (CH(aram); 127.9(2CH(aram); 137.8
Oxamic acid thiohydrazide (0.17 mmol) was added to a solution of the 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (64 mg, 0.17 mmol) and p-toluenesulfonyl acid monohydrate (3.1 mg, 10 mol%) in ethanol (30 mL). The reaction mixture was refluxed for 3–5 h until the complete conversion of the intermediate hydrazide (TLC monitoring). The resulted mixture was cooled to room temperature and poured onto ice-water (40 mL). The resulting off-white precipitate was filtered, washed with water (3×15 mL) and heptane (3×15 mL), and dried in air. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (1:1) as eluent.

6′-[N-(4-Methoxyphenyl)carbamoyl]-3β-hydroxyandrost-5-eno[16,17-d]pyridazines 10α,δ.

Yield 85% (68 mg). Pale yellow solid, Mp220–224°C.

1H NMR (300 MHz, CDCl3): δ 0.93–1.09 (m, 2H, 1-CH2, 9-CH); 1.11 (s, 3H, 19-CH3); 1.26 (s, 3H, 18-CH3); 1.40–1.92 (m, 9H, 1-CH2, 2-CH2, 7-CH2, 8-CH, 11-CH2, 12-CH2, 14-CH); 2.08–2.12 (m, 1H, 1H, 6-CH); 6.92 (d, 2H, J = 9.16 Hz, 2CHarom); 7.66 (d, 2H, J = 9.16 Hz, 2CHarom); 9.19 (s, 1H, CHpyridazine); 9.98 (s, 1H, NH).1H NMR (75 MHz, DMSO-d6): δ 15.5 (18-CH3); 19.5 (19-CH3); 20.2 (11-CH2); 30.6 (15-CH2); 31.0 (8-CH); 31.7 (2-CH2); 31.7 (12-CH2); 33.9 (7-CH2); 36.8 (1-CH2); 37.1 (10-C); 42.3 (4-CH2); 48.2 (13-C); 50.2 (9-CH); 55.6 (14-CH); 57.1 (OMe); 71.7 (3-CH); 114.4 (2CHarom); 120.7 (6-CH); 121.7 (2CHarom); 131.0 (Carom); 141.6 (5-C); 146.4 (16-C); 149.7 (Cpyridazine); 150.5 (CHpyridazine); 154.0 (17-C); 156.7 (Carom); 161.4 (CO).IR (KBr): 3130 (OH); 2955, 2922, 2894 (CH), 1670 (CON), 166, 1598, 1527, 1515, 1457, 1437, 1415, 1236 cm⁻¹. HRMS (ESI) for C29H36N2O3 ([M+H]+): calcd 474.2751, found 474.2759.

6′-[N-(4-Chlorophenyl)carbamoyl]-3β-hydroxyandrost-5-eno[16,17-d]pyridazined 10β.

Yield 92% (74 mg). Pale yellow solid, Mp279–282°C.

1H NMR (300 MHz, CDCl3): δ 0.91–1.12 (m, 2H, 1-CH2, 9-CH); 1.10 (s, 3H, 19-CH3); 1.25 (s, 3H, 18-CH3); 1.40–1.93 (m, 9H, 1-CH2, 2-CH2, 7-CH2, 8-CH, 11-CH2, 12-CH2, 14-CH); 2.10–2.18 (m, 1H, 1H, 6-CH); 2.27–2.33 (m, 2H, 4-CH2); 2.65 (dd, 1H, J = 14.02, 16.48 Hz, 15-CH2); 2.87 (dd, 1H, J = 6.41, 16.48 Hz, 15-CH2); 3.09–3.14 (m, 1H, 12-CH2); 3.50–3.57 (m, 1H, 3-CH); 5.38–5.40 (m, 1H, 6-CH); 7.35 (d, 2H, J = 8.24 Hz, 2CHarom); 7.75 (d, 2H, J = 8.24 Hz, 2CHarom); 9.20 (s, 1H, CHpyridazine); 10.13 (s, 1H, NH).1H NMR (75 MHz, DMSO-d6): δ 15.4 (18-CH3); 19.3 (19-CH3); 20.7 (11-CH2); 30.6 (15-CH2); 31.0 (8-CH); 31.8 (2-CH2); 32.0 (12-CH2); 33.8 (7-CH2); 36.7 (10-C); 37.1 (1-CH2); 42.2 (4-CH2); 48.2 (13-C); 50.1 (9-CH); 57.0 (14-CH); 71.6 (3-)

10 The signal of the OH group was not observed in 1H NMR.
6'-[N-(3-Methoxyphenyl)carbamoyl]-3β-hydroxyandrost-5-eno[16,17-d]pyridazine 10e'.
Yield 86% (69 mg). Pale yellow solid, Mp179-180°C.

1H NMR (300 MHz, CDCl₃): δ 0.93-1.13 (m, 2H, 1-CH₂, 9-CH); 1.12 (s, 3H, 19-CH₃); 1.26 (s, 3H, 18-CH₃); 1.44-1.95 (m, 9H, 1-CH₂, 2-CH₂, 7-CH₂, 8-CH, 12-CH₂, 11-CH₂, 14-CH); 2.28-2.32 (m, 1H, 7-CH₂); 2.25-2.39 (m, 2H, 4-CH₂); 2.64 (dd, 1H, J = 13.19, 15.39 Hz, 15-CH₂); 2.86 (dd, 1H, J = 6.60, 15.39 Hz, 15-CH₂); 3.13-3.17 (m, 1H, 12-CH₂); 3.51-3.58 (m, 1H, 3-CH); 3.86 (s, 3H, OMe); 5.39-5.41 (m, 1H, 6-CH); 6.70-6.73 (m, 2H, 2CH₃); 7.25-7.30 (m, 1H, CH₃); 7.44 (m, 1H, CH₃); 9.19 (s, 1H, CH₂pyridazine); 10.05 (s, 1H, NH).

IR (KBr): 3316 (NH), 2961, 2936, 2904, 2819 (CH), 1696 (CON), 1535, 1522 cm⁻¹.

Synthesis of 6’-(N-arylcarbamoyl)-androst-4-ene-3-on-[16,17-d]pyridazines 11a,e.

Aluminium isopropoxide (0.12 mL, 0.6 mmol) was added to a solution of the 3β-hydroxyandrost-5-ene [16,17-d]pyridazines 10a’,f (0.1 mmol) and cyclohexanone (2.4 mL, 26 mmol) in dry toluene (4 mL). The reaction mixture was refluxed for 4h until the complete conversion of the starting material (TLC...
The resulted mixture was cooled to room temperature and poured onto AcOH(30% water solution, 8 mL). Organic fraction was washed with saturated solution of NaHCO$_3$, water fraction was extracted with CHCl$_3$ (3x7 mL). Combined organic fraction was washed with water till pH 7. Solvent was removed under reduced pressure. An excess of cyclohexanone was removed by steam distillation. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (1:1) as eluent.

6'-[N-(4-Methoxyphenyl)carbamoyl]-androst-4-ene-3-on[16,17-d]pyridazine11a.
Yield 58% (27 mg). Pale yellow solid, Mp208-210°C.

$^1$H NMR (300 MHz, CDCl$_3$): δ 1.24 (s, 3H, 19-CH$_3$); 1.27 (s, 3H, 18-CH$_3$); 0.93-3.17 (m, 17H); 3.82 (s, 3H, OMe); 5.77 (s, 1H, 4-CH); 6.92 (d, 2H, $J = 9.05$ Hz, 2CH$_{arom}$); 7.67 (d, 2H, $J = 9.05$ Hz, 2CH$_{arom}$); 9.20 (s, 1H, CH$_{pyridazine}$); 9.99 (s, 1H, NH).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 15.6 (19-CH$_3$); 17.3 (11-CH$_2$); 20.8 (18-CH$_3$); 29.7 (8-CH); 30.9 (7-CH$_2$); 31.5 (6-CH$_2$); 32.6 (2-CH$_2$); 33.7 (1-CH$_2$); 33.9 (15-CH$_2$); 35.5 (12-CH$_2$); 38.2 (10-C); 48.2 (13-C); 53.7 (9-CH$_2$); 55.6 (OMe); 56.3 (14-CH); 114.4 (2CH$_{arom}$); 121.7 (2CH$_{arom}$); 124.2 (4-CH); 130.9 (C$_{arom}$); 145.9 (16-C); 149.4 (CH$_{pyridazine}$); 150.4 (17-C); 153.6 (C$_{pyridazine}$); 156.7 (C$_{arom}$); 161.3 (CO); 170.1 (5-C); 199.4 (3-C). IR (KBr): 3356, 3423, 3326 (NH), 2930, 2862 (CH), 1673 (CON), 1664 (CO), 1531, 1513, 1266, 1233, 1184 cm$^{-1}$. HRMS (ESI) for C$_{29}$H$_{34}$N$_3$O$_3$ ([M+H]$^+$): calcd 472.2595, found 474.2598.

6'-[N-(4-Fluorophenyl)carbamoyl]-androst-4-ene-3-on[16,17-d]pyridazine11f.
Yield 60% (28 mg). Pale yellow solid, Mp253°C.

$^1$H NMR (300 MHz, CDCl$_3$): δ 1.26 (s, 3H, 19-CH$_3$); 1.30 (s, 3H, 18-CH$_3$); 0.86-3.18 (m, 17H); 5.77 (s, 1H, 4-CH); 7.06-7.12 (m, 2H, 2CH$_{arom}$); 7.70-7.75 (m, 2H, 2CH$_{arom}$); 9.21 (s, 1H, CH$_{pyridazine}$); 10.11 (s, 1H, NH).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 17.3 (19-CH$_3$); 19.4 (11-CH$_2$); 20.8 (18-CH$_3$); 29.8 (8-CH); 31.0 (7-CH$_2$); 31.6 (6-CH$_2$); 32.6 (2-CH$_2$); 33.7 (1-CH$_2$); 34.0 (15-CH$_2$); 35.6 (12-CH$_2$); 37.1 (10-C); 50.1 (13-C); 53.7 (9-CH$_2$); 56.3 (14-CH); 115.6 (d, $J_{CF} = 21.0$ Hz, 2CH$_{arom}$); 120.7 (4-CH); 121.7 (d, $J_{CF} = 7.9$ Hz, 2CH$_{arom}$); 133.8 (C$_{arom}$); 146.1 (16-C); 149.2 (CH$_{pyridazine}$); 150.6 (17-C); 153.7 (C$_{pyridazine}$); 161.5 (CO); 158.7 (d, $J_{CF} = 240.6$ Hz, C$_{arom}$); 170.0 (5-C); 199.3 (3-C).IR (KBr): 3312 (NH), 2930, 2862 (CH), 1673 (CON), 1647 (CO), 1538, 1527, 1508, 1407, 1231, 1207 cm$^{-1}$. HRMS (ESI) for C$_{28}$H$_{33}$FN$_3$O$_2$ ([M+H]$^+$): calcd 460.2395, found 460.2393.


Oxamic acid thiohydrazide (0.17 mmol) was added to a solution of 3-hydroxy-16-formyl-17-chloro-1,3,5,(10),16-estratriaene (53 mg, 0.17 mmol) and p-toluenesulfonic acid monohydrate (3.1 mg, 10 mol%) in ethanol (30 mL). The reaction mixture was refluxed for 2 h until the complete conversion of the intermediate hydrazone (TLC monitoring). The resulted mixture was cooled to room temperature and

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poured onto ice-water (40 mL). The resulting off-white precipitate was filtered, washed with water (3×15 mL) and heptane (3×15 mL), and dried in air. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (1:1) as eluent.

6'-[N-(4-Methoxyphenyl)carbamoyl]-3β-hydroxyestra1,3,5(10)trieno[16, 17-d]pyridazine 14a.

Yield 79% (61 mg). Pale yellow solid, Mp260°C. The spectral data matched previously reported.5

1H NMR (600 MHz, DMSO-d6): δ 1.12 (s, 3H, 18-CH3); 1.41-1.43 (m, 1H, 7-CH2); 1.50-1.54 (m, 1H, 11-CH2); 1.62-1.64 (m, 1H, 12-CH2); 1.65-1.67 (m, 1H, 8-CH); 1.77-1.79 (m, 1H, 14-CH); 1.91-1.93 (m, 1H, 7-CH2); 2.22-2.24 (m, 1H, 9-CH); 2.32-2.35 (m, 1H, 11-CH2); 2.40-2.42 (m, 1H, 12-CH2); 2.74-2.76 (m, 1H, 6-CH2); 2.75-2.78 (m, 1H, 15-CH2); 2.79-2.81 (m, 1H, 6-CH2); 2.97-2.99 (m, 1H, 15-CH2); 3.74 (s, 3H, OMe); 6.44 (d, 1H, J = 1.86 Hz, 4-CH); 6.46 (dd, 1H, J = 1.86, 8.80 Hz, 2-CH); 6.93 (d, 2H, J = 8.80 Hz, 2CHarom); 7.05 (d, 1H, J = 8.80 Hz, 1-CH); 7.72 (d, 2H, J = 8.80 Hz, 2CHarom); 9.32 (s, 1H, CHpyridazine); 10.59 (s, 1H, NH).1013CNMR (125 MHz, DMSO-d6): δ 16.4 (18-CH3); 25.8 (11-CH2); 27.3 (7-CH2); 29.0 (6-CH2); 29.6 (15-CH2); 36.9 (8-CH); 32.8 (12-CH2); 43.3 (9-CH); 46.7 (13-C); 55.2 (OMe); 55.6 (14-CH); 112.8 (2-CH); 114.0 (2CHarom); 115.0 (4-CH); 122.0 (2CHarom); 125.9 (1-CH); 130.0 (10-C); 131.8 (Carom); 137.0 (5-C); 146.0 (16-C); 150.3 (CHpyridazine); 150.9 (Cpyridazine); 151.9 (17-C); 155.1 (3-C); 155.8 (Carom); 163.4 (CO).

6'-[N-(3-Methoxyphenyl)carbamoyl]-3β-hydroxyestra1,3,5(10)trieno[16,17-d]pyridazine 14e.

Yield 83% (64 mg). Pale yellow solid, Mp178-180°C.

1H NMR (300 MHz, DMSO-d6): δ 1.15 (s, 3H, 18-CH3); 1.41-1.43 (m, 1H, 7-CH2); 1.40-1.54 (m, 1H, 11-CH2); 1.62-1.65 (m, 1H, 12-CH2); 1.65-1.69 (m, 1H, 8-CH); 1.77-1.80 (m, 1H, 14-CH); 1.91-1.99 (m, 1H, 7-CH2); 2.23-2.26 (m, 1H, 9-CH); 2.32-2.36 (m, 1H, 11-CH2); 2.40-2.43 (m, 1H, 12-CH2); 2.70-2.72 (m, 1H, 15-CH2); 2.75-2.78 (m, 1H, 6-CH2); 2.93-2.98 (m, 1H, 15-CH2); 3.77 (s, 3H, OMe); 6.48 (d, 1H, J = 1.95 Hz, 4-CH); 6.50 (dd, 1H, J = 1.95, 8.65 Hz, 2-CH); 6.75 (dd, 1H, J = 1.86, 8.37 Hz, CHarom); 7.03 (d, 1H, J = 8.65 Hz, 1-CH); 7.28 (dd, 1H, J = 8.37 Hz, CHarom); 7.42 (d, 1H, J = 8.37 Hz, CHarom); 7.51 (d, 1H, J = 1.86 Hz, CHarom); 9.02 (br.s, 1H, OH); 9.36 (s, 1H, CHpyridazine); 10.83 (s, 1H, NH).13C NMR (75 MHz, DMSO-d6): δ 16.4 (18-CH3); 25.7 (11-CH2); 27.2 (7-CH2); 28.9 (6-CH2); 29.3 (15-CH2); 37.0 (8-CH); 32.7 (12-CH2); 43.2 (9-CH); 46.6 (13-C); 55.0 (OMe); 55.6 (14-CH); 105.7 (Charom); 109.5 (Charom); 112.2 (Charom); 112.7 (2-CH); 114.9 (4-CH); 125.8 (1-CH); 129.6 (Charom); 129.9 (10-C); 136.9 (5-C); 139.8 (Carom); 145.1 (16-C); 150.4 (CHpyridazine); 151.0 (Cpyridazine); 151.7 (17-C); 155.0 (3-C); 159.5 (Carom); 163.8 (CO).IR (KBr): 3323 (NH), 3136, 2935, 2869 (CH), 1694 (CON), 1606, 1535, 1524, 1495, 1464, 1287, 1252, 1154 cm⁻¹. HRMS (ESI) for C28H36N3O3 ([M+H]+): calc 456.2282, found 456.2288.

6'-[N-(4-Fluorophenyl)carbamoyl]-3β-hydroxyestra1,3,5(10)trieno[16,17-d]pyridazine 14f.

Yield 91% (68 mg). Colorless solid, Mp232°C.

1H NMR (300 MHz, DMSO-d6): δ 1.15 (s, 3H, 18-CH3); 1.41-1.43 (m, 1H, 7-CH2); 1.52-1.57 (m, 1H, 11-CH2); 1.62-1.67 (m, 1H, 12-CH2); 1.67-1.70 (m, 1H, 8-CH); 1.77-1.79 (m, 1H, 14-CH); 1.93-1.96 (m, 1H, 7-CH2); 2.22-2.27 (m, 1H, 9-CH); 2.32-2.35 (m, 1H, 11-CH2); 2.40-2.44 (m, 1H, 12-CH2); 2.73-2.76 (m, 1H, 6-CH2); 2.75-2.78 (m, 1H, 15-CH2);
2.80-2.86 (m, 1H, 6-CH₂); 2.95-2.99 (m, 1H, 15-CH₂); 6.48 (d, 1H, J = 1.86 Hz, 4-CH); 6.53 (dd, 1H, J = 1.86, 8.80 Hz, 2-CH); 7.04 (d, 1H, J = 8.80 Hz, 1-CH); 7.24 (dd, 2H, J = 8.80, 8.87 Hz, 2CH₂arom); 7.89 (dd, 2H, J = 5.08, 8.80 Hz, 2CH₂arom); 9.05 (br.s, 1H, OH); 9.38 (s, 1H, CH₃pyridazine); 10.95 (s, 1H, NH).¹³CNMR (75 MHz, DMSO-d₆): δ 16.3 (18-CH₃); 26.0 (11-CH₂); 27.3 (7-CH₂); 29.0 (6-CH₂); 29.8 (15-CH₂); 36.9 (8-CH); 32.8 (12-CH₂); 43.3 (9-CH); 46.7 (13-C); 55.6 (14-CH); 112.8 (2-CH); 115.0 (4-CH); 115.2 (d, Jₐ₋₈F = 22.5 Hz, 2CH₂arom); 122.0 (d, Jₖ₋₈F = 7.6 Hz, 2CH₂arom); 125.9 (1-CH); 129.9 (10-C); 135.0 (C-arom); 136.9 (5-C); 145.2 (16-C); 150.5 (CHpyridazine); 151.2 (17-C); 151.6 (Cpyridazine); 155.0 (3-C); 159.3 (d, Jₐ₋₈F = 239.3 Hz, C-arom); 163.7 (CO).HRMS (ESI) for C₂₃H₂₇FN₃O₂ ([M+H]⁺): calc 444.2082, found 444.2071.

**Procedure for the preparation of hydrazone 15.**

![Diagram](image)

Oxamic acid thiohydrazide (25 mg, 0.11 mmol) was added to a solution of the 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (38 mg, 0.11 mmol) and p-toluenesulfonic acid monohydrate (1.9 mg, 10 mol%) in ethanol (30 mL). The reaction mixture was stored at rt for 30-40 min. The precipitate formed was filtered, washed with ethanol, and dried *in vacuo* to obtain product 15 as a yellow solid, mp 247-250°C, in 91% yield (58 mg, 0.091 mmol).

¹H NMR (600 MHz, DMSO-d₆, for the mixture of E-14-thione/14-thiadiazoline, 1:1)¹¹ δ 0.83-2.28 (m, 34H, E-14-thione+14-thiadiazoline); 0.94 (s, 6H, 19-CH₃, E-14-thione+14-thiadiazoline); 1.01 (s, 6H, 18-CH₃, E-14-thione+14-thiadiazoline); 1.96 (s, 6H, OAc, E-14-thione+14-thiadiazoline); 3.72 (s, 6H, OCH₃, E-14-thione+14-thiadiazoline); 4.44 (m, 2H, 3-CH, E-14-thione+14-thiadiazoline); 5.35-5.38 (m, 2H, 6-CH, E-14-thione+14-thiadiazoline); 6.31 (br.s, 1H, CH, 14-thiadiazoline); 6.86 (d, J = 8.6 Hz, 4H, 4-MeOCS₂H₄); 14-thione+14-thiadiazoline); 7.64 (d, 4H, J = 8.6 Hz, 4H, 4-MeOCS₂H₄); 14-thione+14-thiadiazoline); 8.81 (s, 1H, CH, 14-thione); 8.87 (br.s., 1H, NH, 14-thiadiazoline); 10.09 (s, 1H, NHCO, 14-thione); 10.36 (s, 1H, NHCO, 14-thiadiazoline); 13.74 (s, 1H, NHCS₂H₄, 14-thione).¹³C NMR (125 MHz, DMSO-d₆, for the mixture of E-14-thione/14-thiadiazoline/Z-14-thiol) δ 14.7, 14.7, 16.0, 18.7, 19.8, 19.9, 20.9, 27.2, 28.8, 29.2, 29.6, 29.7, 29.8, 30.0, 30.2, 30.3, 30.8, 32.5, 32.8, 33.0, 36.2, 37.5, 39.1, 39.2, 39.4, 39.5, 39.6, 39.8, 49.2, 49.4, 49.5, 53.0, 53.2, 55.1, 56.2, 67.9, 73.0, 113.6, 113.8, 113.9, 114.1, 121.3, 121.4, 121.5, 121.6, 130.5, 131.2, 132.9, 139.7, 143.2, 149.9, 150.4, 155.4, 155.6, 157.3, 157.6, 169.5, 184.8, 193.1. HRMS (ESI) for C₃₁H₃₈ClN₃NaO₅S ([M+Na]⁺): calc 606.2164, found 606.2161.

¹¹ In ¹H NMR of compound 15, the characteristic signals of Z-15-thione form are observed at δ 13.58 (CONH), 10.22 (NHCS) and 8.22 (CH=) ppm.
Biology

Cell cultures and evaluation of the inhibitory activity

The human breast cells, hormone dependent MCF-7 and hormone independent MDA-MB-231, were purchased from ATCC collection (USA) and stored in the cryobank of N.N. Blokhin Cancer Research Center (Moscow, Russia) until the present analysis. Cells were cultured in standard high glucose DMEM medium (PanEco, Russia) supplemented with 10% fetal calf serum (FCS) (HyClone, USA) and 0.1 mg/ml sodium pyruvate (Santa Cruz, USA) at 37°C, 5% CO₂ and 80-85% humidity (NuAir CO₂ incubator). The cell growth was evaluated by the MTT-test based on the accumulation of a MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Applichem, USA) by living cells. Briefly, the cells were seeded with 10⁴ cells per well in 24-well plates (Corning, USA) in 900 μL of medium. The tested compounds were dissolved in DMSO (Applichem, USA) to 5 mM before experiments and then were diluted in medium to required concentrations. Compounds with different concentrations in 100 μL of medium were added in 24 h after seeding and the cells were grown for 72 hours. After incubation with compounds medium was removed and MTT reagent dissolved in medium was added at a final concentration of 0.2 mg/ml to each well and incubated for 3 h. Then the cell supernatants were removed and the MTT formazan purple crystals were dissolved in 100% DMSO (350 μL per well). Plates were gently shaken and the density of absorbance was measured at 571 nm on MultiScan reader (ThermoFisher, USA). Viability of the cells was expressed after subtraction of blank value (the density of absorbance in the well w/o cells) from all wells. Dose-response curves were analyzed by regression analysis using sigmoid curves (Log(concentration) vs normalized density of absorbance). In this study, concentrations that inhibited half cell growth (IC₅₀ values) were provided using GraphPad Prism (USA).
Monitoring of Chemical Reaction 9 with 3a in Real Time with $^1$H NMR Spectroscopy in DMSO-$d_6$.
Monitoring of Chemical Reaction 9 with 3a in Real Time with $^1$H NMR Spectroscopy in CDCl$_3$

Table 1. Heat of reactions and Activation energy of disrotatory reaction (Kcal/Mol) calculated by semi-empirical quantum chemistry method PM6$^{12}$

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<th>Starting ketone (A)</th>
<th>Step 1 6π-electrocyclization (Δ$H_{\text{react}}$)</th>
<th>Step 2 aromatization (E$^\circ_{\text{act}}$)</th>
<th>Δ$H_{\text{react}}$</th>
<th>Step 1+ Step 2 (Δ$H_{\text{react}}$)</th>
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</tbody>
</table>

\(^1\)H NMR (CDCl\(_3\), 600 MHz) spectrum of compound 2

\(^13\)C NMR (CDCl\(_3\), 125 MHz) spectrum of compound 2
$^1\text{H}-^1\text{H}$ COSY NMR (CDCl$_3$, 600 MHz) spectrum of compound 2

$^1\text{H}-^{13}\text{C}$ HMBC NMR (CDCl$_3$, 600 MHz) spectrum of compound 2
$^1$H NMR (CDCl$_3$, 600 MHz) spectrum of compound 4a

$^{13}$C NMR (CDCl$_3$, 125 MHz) spectrum of compound 4a
$^1$H-$^{13}$C HSQC NMR (CDCl$_3$, 600 MHz) spectrum of compound 4a

$^1$H-$^{13}$C HMBC NMR (CDCl$_3$, 600 MHz) spectrum of compound 4a
$^1$H NMR (CDCl$_3$, 600 MHz) spectrum of compound 4b

$^{13}$C NMR (CDCl$_3$, 125 MHz) spectrum of compound 4b
$^1$H--$^1$H COSY NMR (CDCl$_3$, 600 MHz) spectrum of compound 4b

$^1$H--$^{13}$C HSQC NMR (CDCl$_3$, 600 MHz) spectrum of compound 4b
$^1$H NMR (CDCl$_3$, 600 MHz) spectrum of compound 4c

$^{13}$C NMR (CDCl$_3$, 125 MHz) spectrum of compound 4c
$^1$H-$^{13}$C HSQC NMR (CDCl₃, 600 MHz) spectrum of compound 4c
$^1$H NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 7b

$^{13}$C NMR (DMSO-$d_6$, 125 MHz) spectrum of compound 7b
$^{1}H^{13}C$ HMBC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 7b

$^{1}H^{13}C$ HSQC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 7b
$^1$H NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10a

$^{13}$C NMR (DMSO-$d_6$, 125 MHz) spectrum of compound 10a
$^{1}$$\text{H-}^{13}\text{C}$ HMBC NMR (DMSO-d$_6$, 600 MHz) spectrum of compound 10a

$^{1}$$\text{H-}^{13}\text{C}$ HSQC NMR (DMSO-d$_6$, 600 MHz) spectrum of compound 10a
$^1$H NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10b

$^{13}$C NMR (DMSO-$d_6$, 125 MHz) spectrum of compound 10b
$^1$H-$^1$H COSY NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10b

$^1$H-$^{13}$C HMBC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10b
$^{1}H$ NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10c

$^{13}C$ NMR (DMSO-$d_6$, 125 MHz) spectrum of compound 10c
$^1$H-$^{13}$C HSQC NMR (DMSO-d$_6$, 600 MHz) spectrum of compound 10c
$^1$H NMR (CDCl$_3$, 300 MHz) spectrum of compound 10a’

$^{13}$C NMR (CDCl$_3$, 75 MHz) spectrum of compound 10a’
$^3$H NMR (CDCl$_3$, 300 MHz) spectrum of compound 10b'.

$^{13}$C NMR (CDCl$_3$, 75 MHz) spectrum of compound 10b'.
$^1$H-$^1$H COSY NMR (CDCl$_3$, 600 MHz) spectrum of compound 10b'

$^1$H-$^1$H CHMBC NMR (CDCl$_3$, 600 MHz) spectrum of compound 10b'
$^1\text{H}-^{13}\text{C}HSQC$ NMR (CDCl$_3$, 600 MHz) spectrum of compound 10b'
$^1$H NMR (CDCl$_3$, 300 MHz) spectrum of compound 10e'

$^{13}$C NMR (CDCl$_3$, 75 MHz) spectrum of compound 10e'
$^1$H NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10f

$^{13}$C NMR (DMSO-$d_6$, 150 MHz) spectrum of compound 10f
$^1$H-$^{13}$CHSQC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10f'

$^1$H-$^{13}$CHMBC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 10f'
$^1$H NMR (CDCl$_3$, 300 MHz) spectrum of compound 11a'

$^{13}$C NMR (CDCl$_3$, 75 MHz) spectrum of compound 11a'
$^1$H NMR (CDCl$_3$, 300 MHz) spectrum of compound 11f$'$

$^{13}$C NMR (CDCl$_3$, 75 MHz) spectrum of compound 11f$'$
$^1$H NMR (DMSO-$d_6$, 300 MHz) spectrum of compound 14e

$^{13}$C NMR (DMSO-$d_6$, 75 MHz) spectrum of compound 14e
$^1$H NMR (DMSO-d$_6$, 300 MHz) spectrum of compound 14f

$^{13}$C NMR (DMSO-d$_6$, 75 MHz) spectrum of compound 14f
$^1$H NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 15

$^{13}$C NMR (DMSO-$d_6$, 125 MHz) spectrum of compound 15
$^1$H-$^{13}$CHMBC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 15

$^1$H-$^{13}$CHSQC NMR (DMSO-$d_6$, 600 MHz) spectrum of compound 15