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Atypical steroid-based abiraterone analogues: synthesis and binding affinity to Cytochrome P450 17A1

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1. Position of abiraterone (1a) in its binding site of CYP17A1 (PDB ID: 3RUK).

UCSF Chimera 1.15 software was used for visualization.[S1]

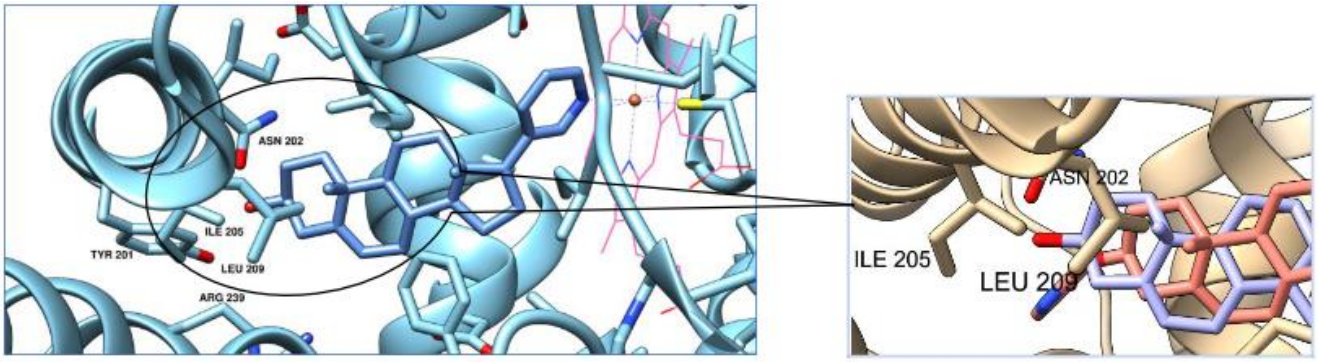


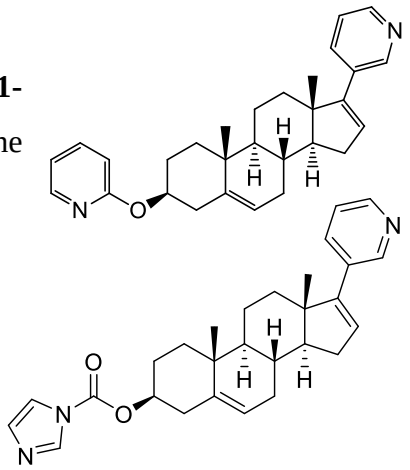
Figure S1 Complex of abiraterone **1a** (shown in blue) in its binding site according to the data from PDB (ID: 3RUK) visualized in UCSF Chimera 1.15 software (heme is displayed by pink lines, the heme iron is shown as an orange sphere). The tab displays a larger image of the C^3-hydroxyl binding region with molecule **1a** (shown in lilac) and molecule **1c** (shown in orange) as determined by molecular docking (performed using AutoDock Vina 1.1.2 software:[S2] water molecules and abiraterone were removed before the procedure, the protein atoms were charged according to the standard Kollman method using the AutoDock Tools 1.5.6 program, grid box size: 15.0 Å×28.5 Å×18.0 Å with grid center x=26.358 Å, y=–0.048 Å, z=33.783 Å, energy range=4, exhaustiveness=30, the complex with the best scoring function was chosen).

2. Chemistry and analytical data

All solvents were technical grade and distilled from standard drying agents. The starting materials and reagents – 5-androsten-3β-ol-17-one (androstenolone, **3**), 2-bromopyridine, 1,1′-carbonyldiimidazole (CDI), (3β)-17-iodoandrosta-5,16-dien-3-ol for the synthesis of compound **6**, *N*,*N*-diisopropylethylamine (DIPEA), imidazole et al. were purchased as high-grade commercial products (*Cayman Chemical, Sigma-Aldrich, Chemieliva Pharmaceutical*) and used without further purification. (3β)-Androst-5-en-3-ol (**6**) was obtained by H_2/Ranay nickel reduction of (3β)-17-iodoandrosta-5,16-dien-3-ol.[S3] Liquid column flash- or conventional chromatography was performed using silica gel «Macherey–Nagel» (0.063–0.2 mm). Thin-layer chromatography (TLC) was performed on ALUGRAM Xtra G/UV254 silica gel sheets. ^{1}H and ^{13}C NMR spectra were recorded on Agilent 400-MR spectrometer (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at 28 ºC. Chemical shifts (δ) are reported in ppm referenced to residual solvent peak ($CDCl_3$, δ_H=7.24 ppm, δ_C=77.0 ppm; methanol-d_4, δ_H=4.87 ppm, δ_C=49.15 ppm); spin-spin coupling constants (*J*) are reported in Hz. Liquid chromatography (LC) and ElectroSpray ionization mass spectrometry (ESI-MS) data were obtained on an Agilent 1100 LC/MSD with an Agilent 1100 SL quadrupole mass spectrometer, eluting with 0.05 % TFA in H_2O and 0.05 % TFA in MeCN (positive-ion monitoring mode). CHN elemental analysis was performed using a Carlo-Erba ER-20 analyzer. IR-spectra were registered on FT-*IR* Termo Nicolet IR200 Spectrometer with 4 cm^{-1} resolution, absorption bands are given in cm^{-1}. Melting points are determined on capillary melting point apparatus and are uncorrected.

(3β)-17-(Pyridin-3-yl)-3-(pyridin-2-yloxy)androsta-5,16-diene (1c) was obtained from abiraterone, 2-bromopyridine and potassium *tert*-butoxide by literature procedure.[S4] Spectral data coincided with the data reported.[S4]

(3β)-17-(Pyridin-3-yl)androst-5,16-dien-3-yl (1*H*-imidazol-1-yl)carboxylate (1d) was obtained as earlier described[S5] from abiraterone (0.100 g, 0.29 mmol) and CDI (0.050 g, 0.31 mmol), yield 95% (0.128 g), white crystals, m.p. 216 – 218ºC. NMR ^{1}H spectrum signals coincide with the data reported.[S5]



^{1}H NMR (δ, $CDCl_3$): 1.06 (s, 3 H, H18); 1.13 (s, 3 H, H19); 1.20 – 1.29 (m, 1 H); 1.47 – 1.82 (m, 8 H); 1.96 (dt, 1 H, *J* = 13.5, 3.2); 2.05 – 2.12 (m, 4 H); 2.25 – 2.31 (m, 1 H); 2.53 – 2.54 (m, 2 H); 4.81 – 4.89 (m, 1 H, H3); 5.49 (d, 1H, H6, *J* = 4.7); 6.0 – 6.01 (m, 1 H, H16); 7.07 (s, 1 H, H4Im); 7.23 (dd, 1 H, H5Py, *J* = 8.0, 4.8); 7.43 (s, 1 H, H5Im); 7.64 (dt, 1 H, H4Py, *J* = 8.0, 2.0); 8.14 (s, 1 H, H2Im); 7.47 (dd, 1 H, H6Py, *J* = 4.8, 1.4); 8.63 (d, 1 H, H2Py, *J* = 1.4).

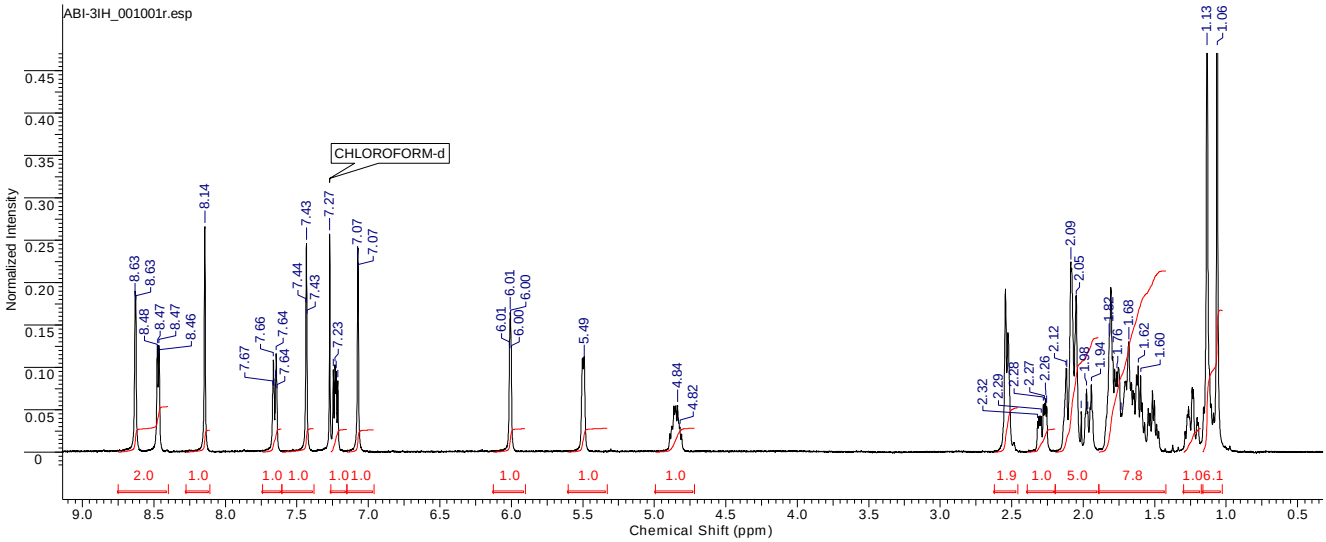


Figure S2 ^{1}H NMR spectrum of compound **1d**.

^{13}C NMR (δ, $CDCl_3$): 16.2 (C18), 18.8 (C19), 20.4, 27.2, 29.9, 31.1, 31.4, 34.7, 36.3, 36.3, 37.5, 46.9, 49.7, 57.0, 78.1 (C3), 116.7, 122.6, 122.9, 128.8, 130.1 (C6), 132.5, 133.3, 136.7, 138.6, 147.5 (C2,6-Py), 147.7 (COO), 151.2 (C17).

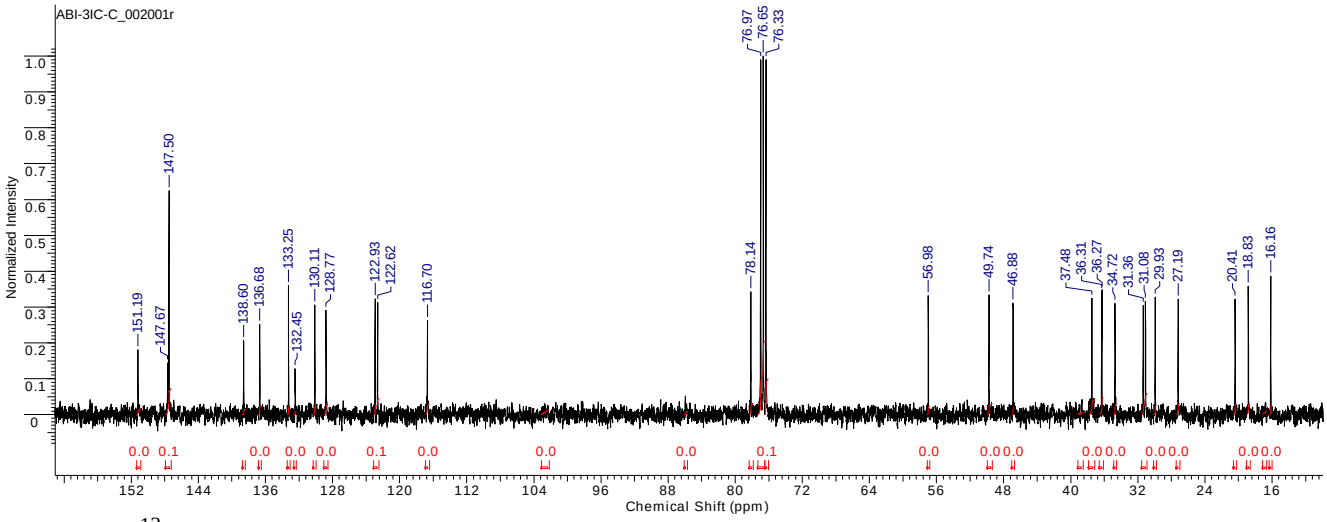
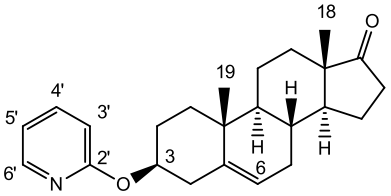


Figure S3 ^{13}C NMR spectrum of compound **1d**.

MS (ESI), m/z: 444.5 [M+H]. Calculated for $C_{28}H_{34}N_3O_2$: 444.3.

(3β)-3-(Pyridin-2-yloxy)andost-5-en-17-one (4). To a solution of androstenolone **3** (0.150 g, 0.58 mmol) and 2-bromopyridine (0.111 g, 0.70 mmol) in dioxane (10 ml) was added sodium *tert*-butoxide (0.97 g, 0.87 mmol) and the mixture was refluxed at 100°C 48 h. The solvent was evaporated under reduced pressure, mixed with distilled water (20 ml) and extracted with ethyl acetate (2×20 ml). Combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure and chromatographed (eluent: ethyl acetate – petroleum ether 40–70°C, 1:3) to give **4** as white crystals (0.128 g, yield 60%). M.p. 225 – 228°C.



^{1}H NMR (δ, $CDCl_3$): 0.89 (s, 3 H, CH_3); 1.09 (s, 3 H, CH_3); 1.01 – 1.16 (m, 1 H); 1.18 – 1.35 (m, 3

H); 1.43 – 1.60 (m, 3 H); 1.64 – 1.74 (m, 2 H); 1.83 – 1.98 (m, 4 H); 2.03 – 2.15 (m, 3H); 2.37 – 2.50 (m, 2 H); 2.55 (ddd, 1 H, *J* = 13.1, 4.8, 2.3); 4.86 – 4.95 (m, 1 H, H3); 5.44 (d, 1 H, H6, *J* = 5.1); 6.68 (d, 1 H, H3Py, *J* = 8.4); 6.82 (dd, 1 H, H5Py, *J* = 6.8, *J* = 5.1); 7.54 (m, 1 H, H4Py, *J* = 8.4, *J* = 6.8, *J* = 2.0); 8.12 (dd, 1 H, H6Py, *J* = 5.1, *J* = 1.5).

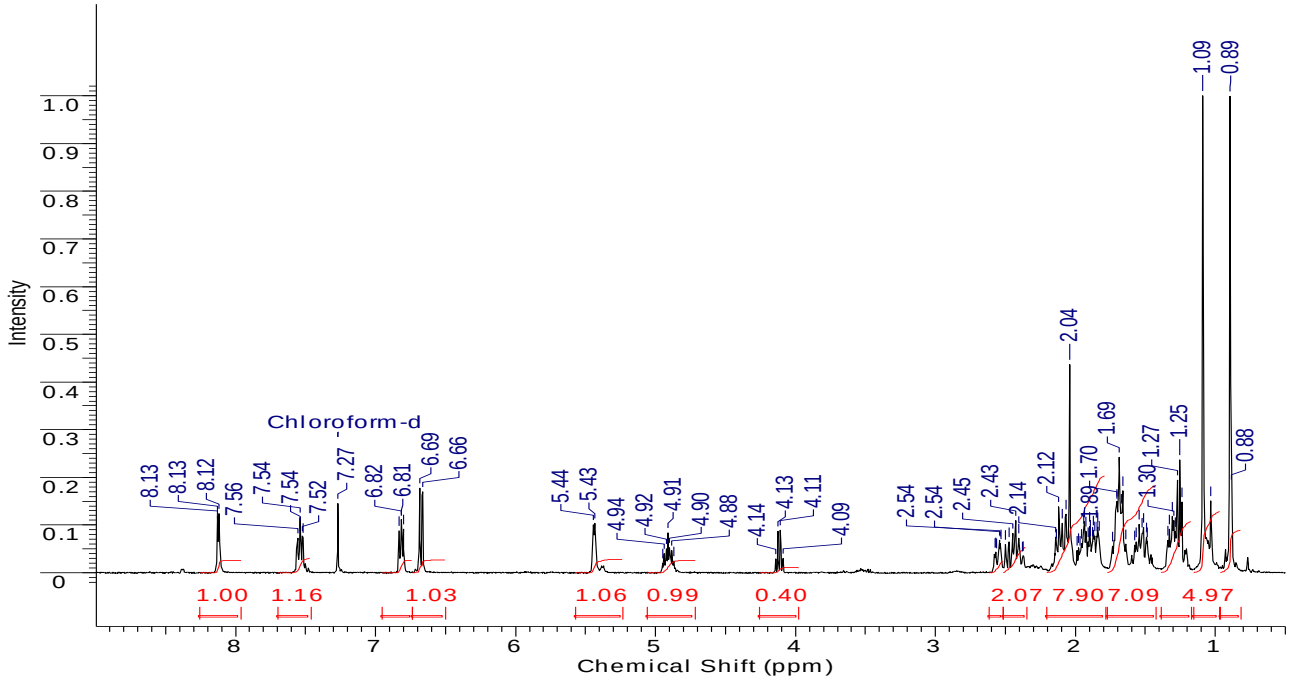


Figure S4 ^{1}H NMR spectrum of compound **4**.

^{13}C NMR (δ, $CDCl_3$): 13.1 (C18), 19.0 (C19), 19.9, 21.5, 27.6, 30.4, 31.0, 31.1, 35.5, 36.5, 36.7, 38.1, 47.1, 49.8, 51.3, 73.7 (C3), 111.0, 116.0, 121.0, 138.1, 140.2, 146.5, 162.9, 220.8 (C=O).

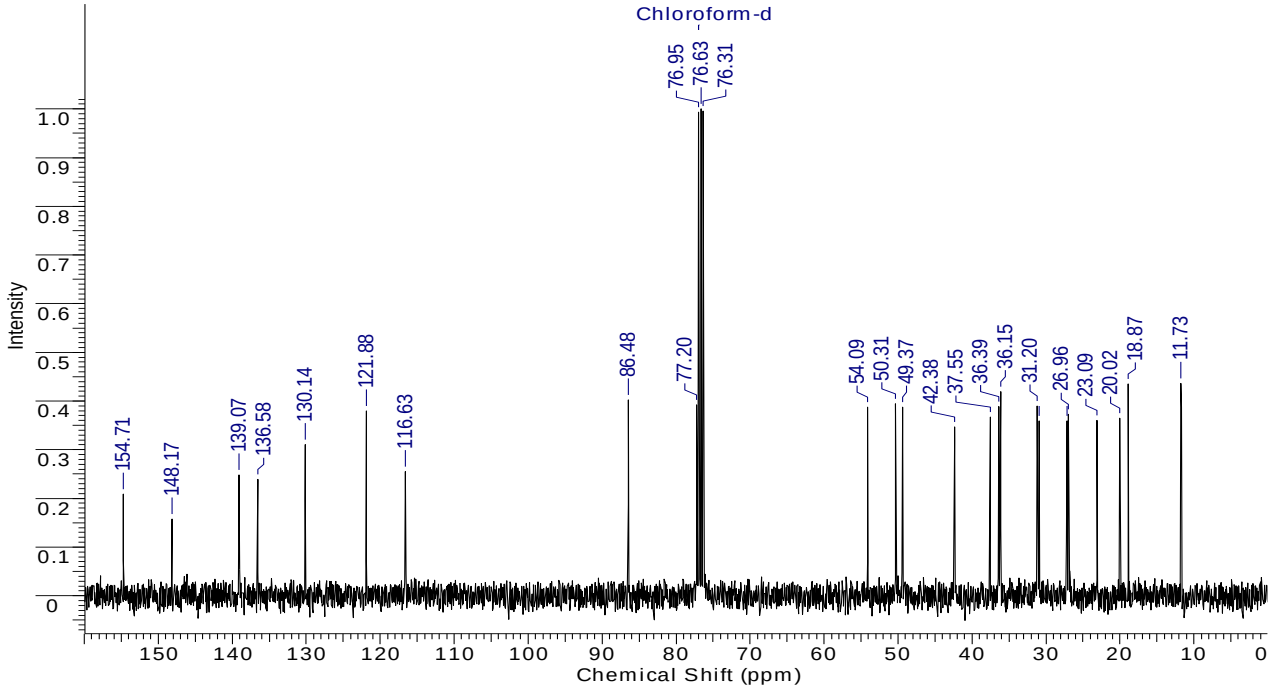


Figure S5 ^{13}C NMR spectrum of compound **4**.

IR spectrum (KBr, cm^{-1}): 846, 950, 988, 994, 1007, 1025, 1041, 1137, 1254, 1270, 1286, 1307, 1370, 1430, 1469, 1568 (C-H^{Py}), 1592 (C-H^{Py}), 1734 (C=O), 2842, 2866, 2896, 2939.

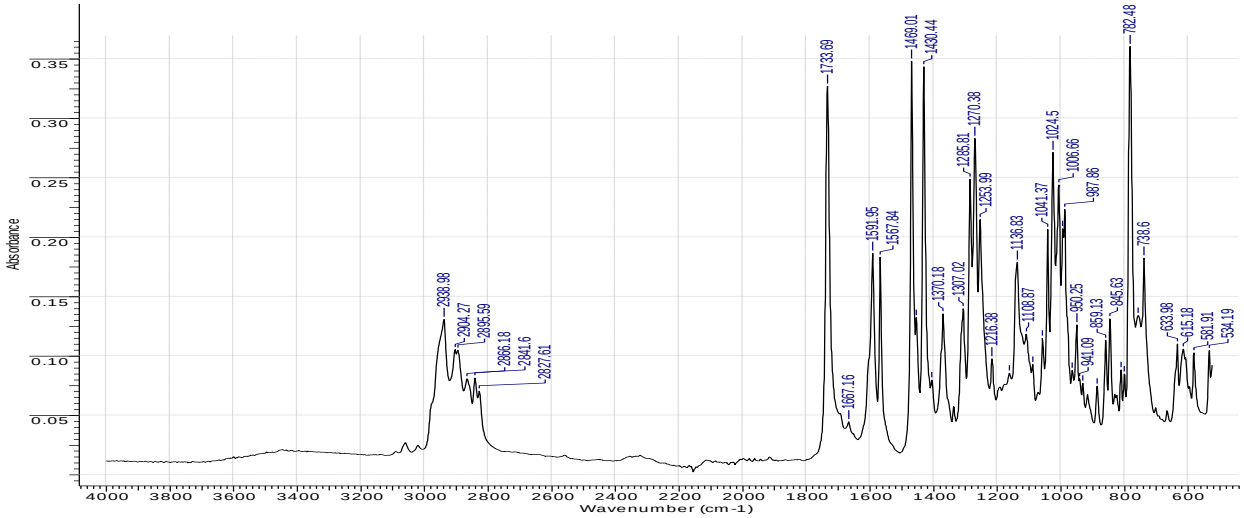
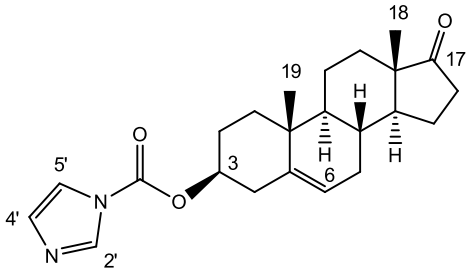


Figure S6 IR spectrum of compound **4**.

MS (ESI), m/z: 366.1 [M+H], 271.6, 253.6. Calculated for $C_{24}H_{32}NO_2$: 366.5.

(3β)-17-Oxoandrost-5-en-3-yl (1*H*-imidazol-1-yl)carboxylate (5). An acetonitrile (5 ml) solution of androstenolone **3** (0.120 g, 0.47 mmol) and CDI (0.101 g, 0.62 mmol) in a tightly closed ampule was heated at 100°C for 12 h. The reaction mass was cooled and diluted with distilled water (60 ml). The precipitate was filtered, washed with distilled water and air-dried to give **5** as white crystals (0.114 g, yield 63%). M.p. 230 – 233°C.



^{1}H NMR (δ, $CDCl_3$): 0.92 (s, 3 H, H18); 1.03 – 1.11 (m, 1 H); 1.12 (s, 3 H, H19); 1.19 – 1.36 (m, 3 H); 1.46 – 1.62 (m, 2 H); 1.65 – 1.75 (m, 3 H); 1.76 – 1.85 (m, 1 H); 1.84 – 1.92 (m, 2 H); 1.94 – 2.02 (m, 2 H); 2.05 – 2.18 (m, 3 H); 2.44 – 2.55 (m, 3 H); 4.80 – 4.88 (m, 1 H, H3); 5.50 (d, 1 H, H6, *J* = 5.1); 7.08 (s, 1 H, H4Im); 7.44 (s, 1 H, H5Im); 8.15 (s, 1 H, H2Im).

^{13}C NMR (δ, $CDCl_3$): 13.5, 19.3, 20.3, 21.8, 27.5, 30.7, 31.3, 31.4, 35.8, 36.6, 36.7, 37.8, 47.5, 50.0, 51.6, 76.7(C3), 117.0 (C5Im), 122.9 (C6), 130.5 (C4Im), 137.0, 138.9 (C2Im), 148.0 (COO), 220.8 (C17).

IR spectrum (KBr, cm^{-1}): 846, 890, 941, 967, 999, 1057, 1089, 1097, 1135, 1175, 1238, 1279, 1315, 1365, 1396, 1466, 1732 (C=O), 1751 (C=O ester), 2857, 2914, 2962.

MS (ESI), m/z: 383.6 [M+H], 339.1, 271.0. Calculated for $C_{23}H_{31}N_2O_3$: 383.5.

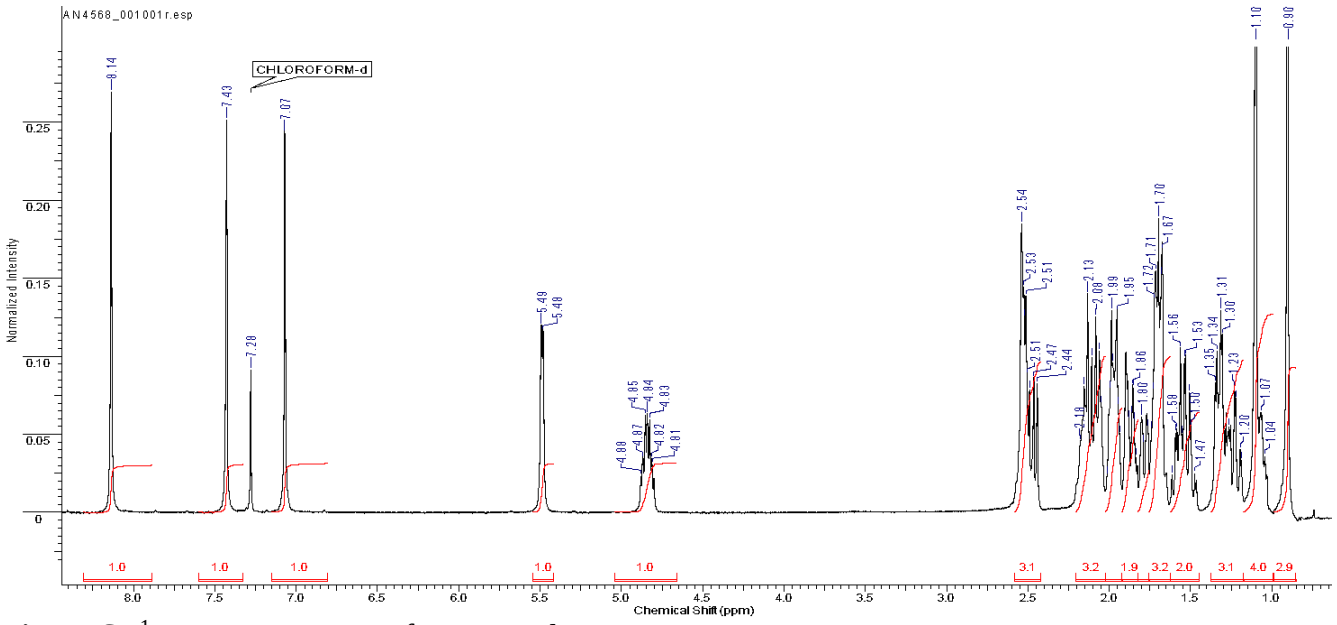


Figure S7 ^{1}H NMR spectrum of compound **5**.

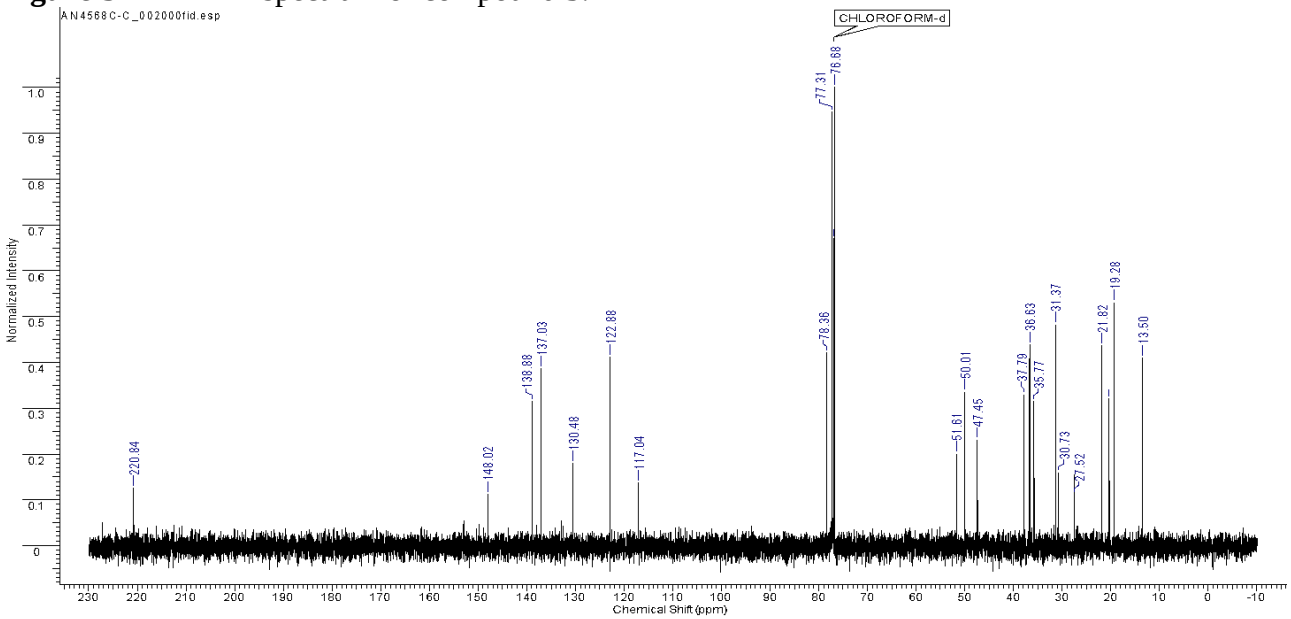


Figure S8 ^{13}C NMR spectrum of compound **5**.

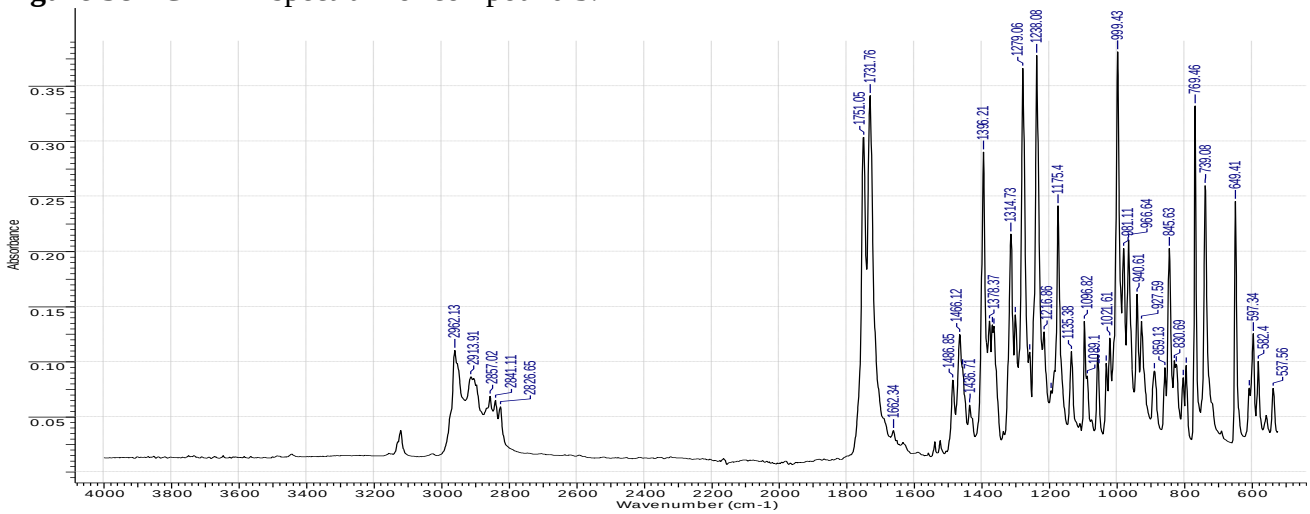
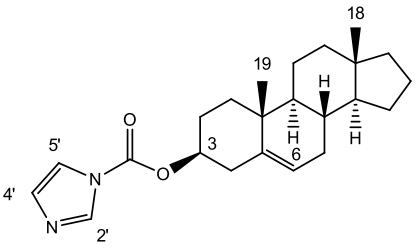


Figure S9 IR spectrum of compound **5**.

(3β)-Androst-5-en-3-yl (1*H*-imidazol-1-yl)carboxylate (7). Synthesized as compound **5** from (3β)-androst-5-en-3-ol **6** (0.116 g, 0.42 mmol) and CDI (0.182 g, 1.12 mmol). Yield of **7** 67% (0.104 g), white crystals, m.p. 145 – 147ºC.



^{1}H NMR (δ, $CDCl_3$): 0.73 (s, 3 H, H18); 0.91 – 1.05 (m, 2 H); 1.08 (s, 3 H, H19); 1.15 – 1.26 (m, 5 H); 1.42 – 1.52 (m, 3 H); 1.54 – 1.70 (m, 4 H); 1.74 – 1.79 (m, 2 H); 1.96 (dt, 1 H, *J* = 13.5, 3.4); 2.02 – 2.09 (m, 2 H); 2.50 – 2.52 (m, 2 H); 4.80 – 4.88 (m, 1H, H3); 5.46 (d, 1H, H6, *J* = 4.3); 7.07 (s, 1 H, H4Im); 7.43 (s, 1 H, H5Im); 8.14 (s, 1 H, H2Im).

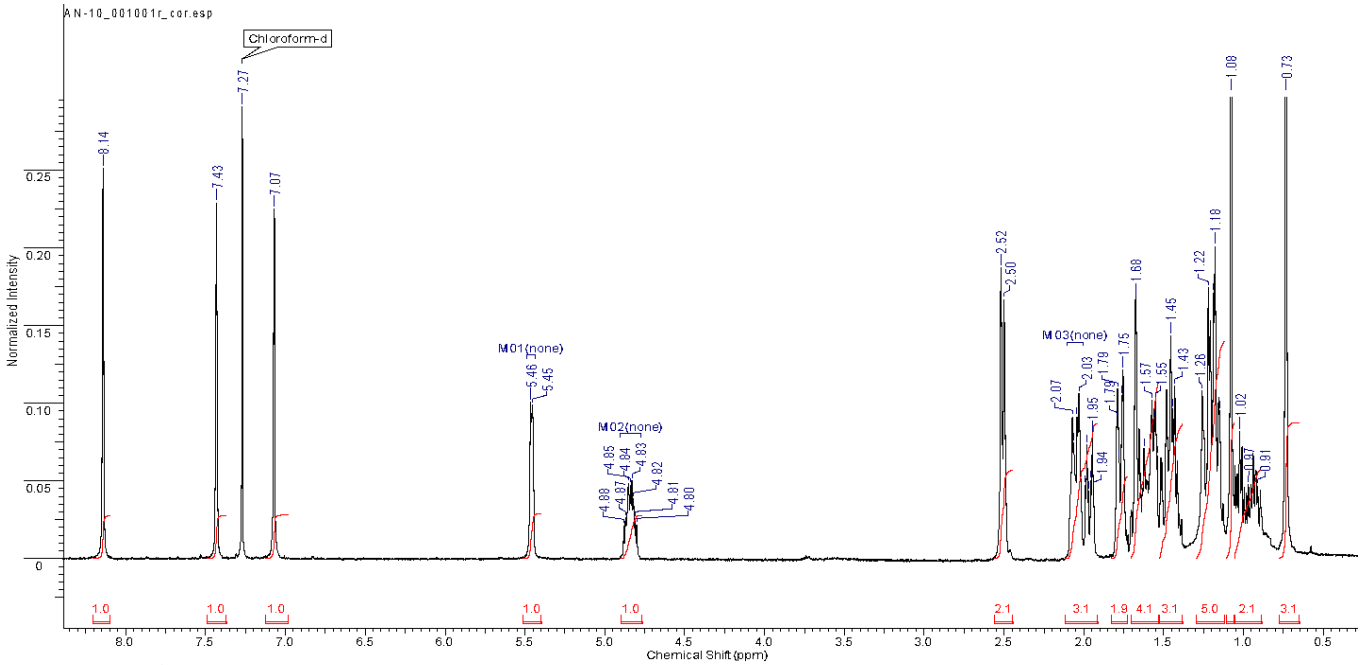


Figure S10 ^{1}H NMR spectrum of compound **7**.

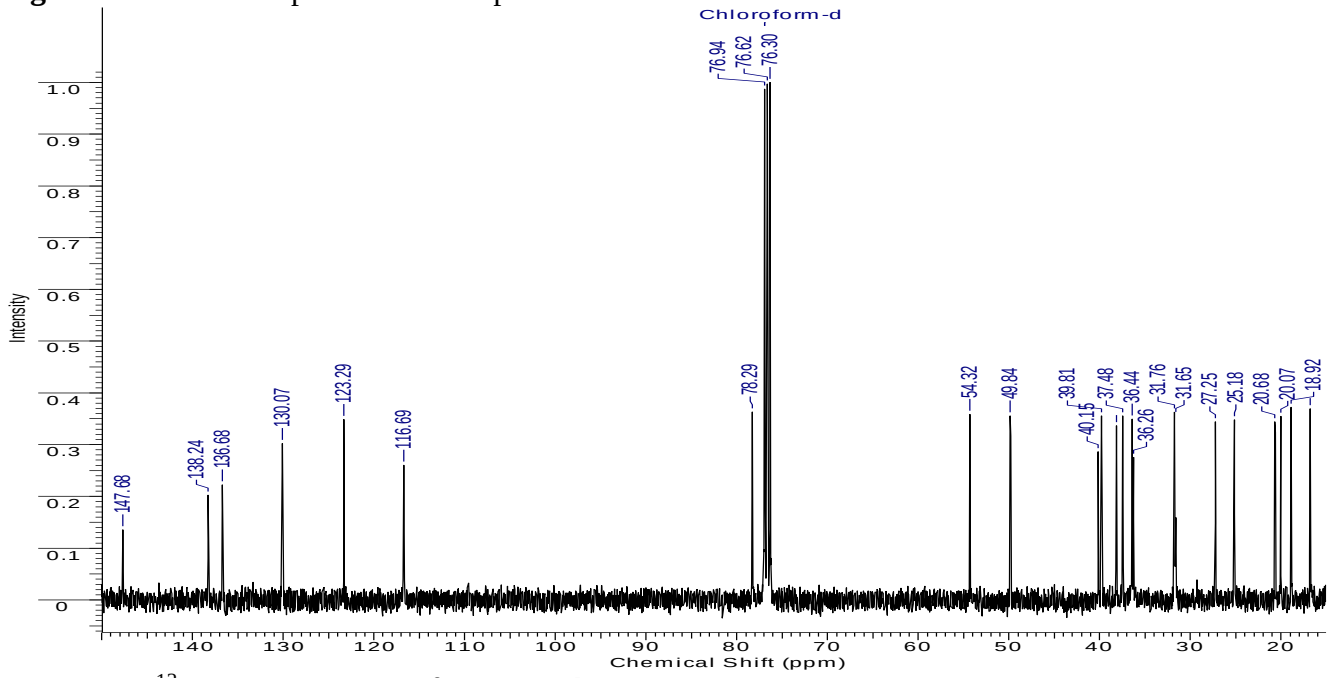
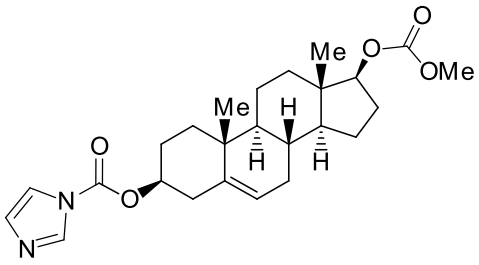


Figure S11 ^{13}C NMR spectrum of compound **7**.

^{13}C NMR (δ, $CDCl_3$): 16.8 (C18), 18.9 (C19), 20.1, 20.7, 25.2, 27.3, 31.7, 31.7, 36.3, 36.4, 37.5, 38.2, 39.8, 40.2, 49.8, 54.3, 78.3 (C3), 116.7 (C5Im), 123.3 (C6), 130.1 (C4Im), 136.7, 138.2 (C2Im), 147.7 (COO).

MS (ESI), m/z: 369.5 [M+H], 325.8, 257.9. Calculated for $C_{23}H_{33}N_2O_2$: 369.5.

(3β, 17β)-17-(Methoxycarbonyloxy)androst-5-en-3-yl (1*H*-imidazol-1-yl)carboxylate (8). To a solution of compound **5** (0.90 g, 0.24mmol) in methanol (7 ml) was added sodium borohydride (0.025 g, 0.65 mmol). The mixture was stirred at room temperature 5 h, then diluted with distilled water and extracted with CH_2Cl_2 (3×20 ml). Combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure and flash-chromatographed (eluent: methylene chloride) to give **8** as white crystals (0.050 g, yield 47%). M.p. 164 – 167ºC.



^{1}H NMR (δ, $CDCl_3$): 0.94 (s, 3 H, CH_3); 1.01 – 1.06 (m, 1 H); 1.06 (s, 3 H, CH_3); 1.12 – 1.21 (m, 2 H); 1.27 – 1.34 (m, 1 H); 1.41 – 1.50 (m, 2 H); 1.57 – 1.79 (m, 7 H); 1.86 – 2.08 (m, 4 H); 2.30 – 2.48 (m, 3 H); 3.79 (s, 3 H, CH_3O); 4.46 – 4.55 (m, 1 H, H3); 4.86 (t, 1 H, H17, *J* = 8.7); 5.43 (d, 1 H, H6, *J* = 4.8); 7.09 (s, 1 H, H4Im); 7.43 (s, 1 H, H5Im); 8.14 (s, 1 H, H2Im). The assignment of C^{17} configuration was made in accordance with X-ray analysis data for *bis*-carbamate **9** (see below).

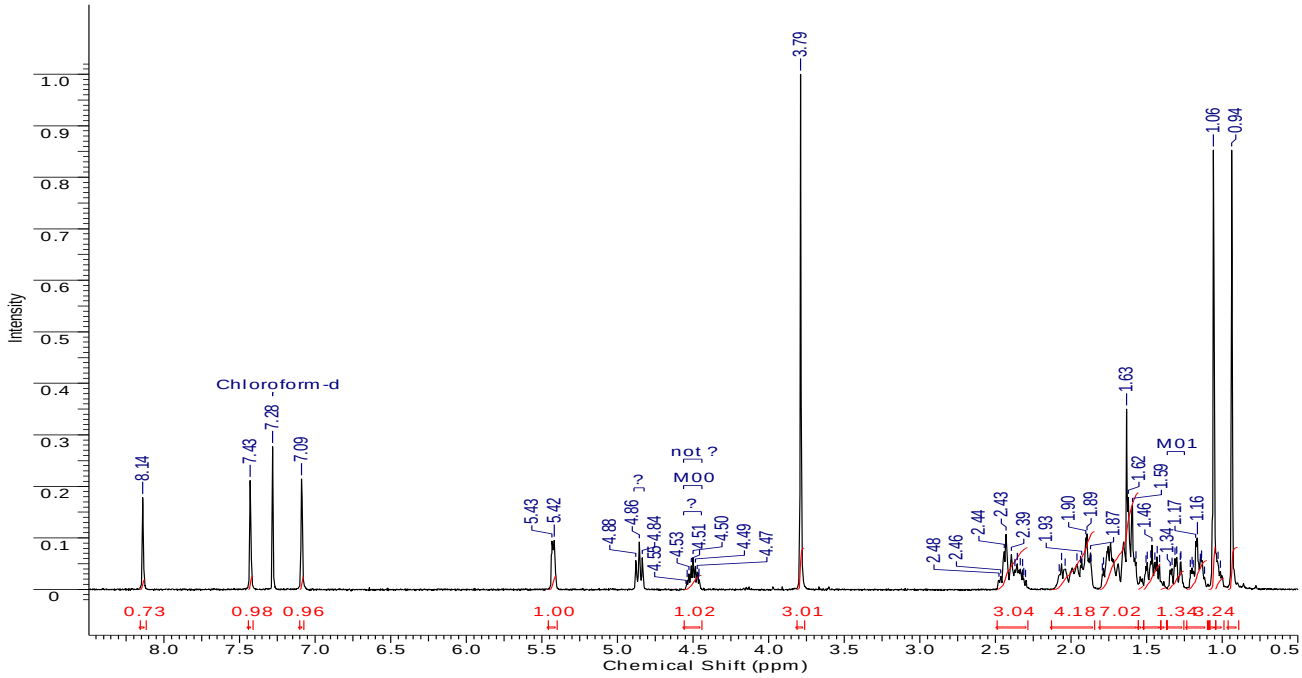


Figure S12 ^{1}H NMR spectrum of compound **8**.

^{13}C NMR (δ, $CDCl_3$): 12.1 (C18), 19.2 (C19), 20.4, 23.5, 27.3, 27.6, 31.3, 31.6, 36.5, 36.6, 36.8, 37.9, 42.8, 49.7, 50.7, 54.5 (CH_3O), 77.6 (C3), 86.8 (C17), 117.0 (C5Im), 122.3 (C6), 130.5 (C4Im), 137.0 (C5), 139.4 (C2Im), 148.5 (COO), 155.1 (CO_2Me).

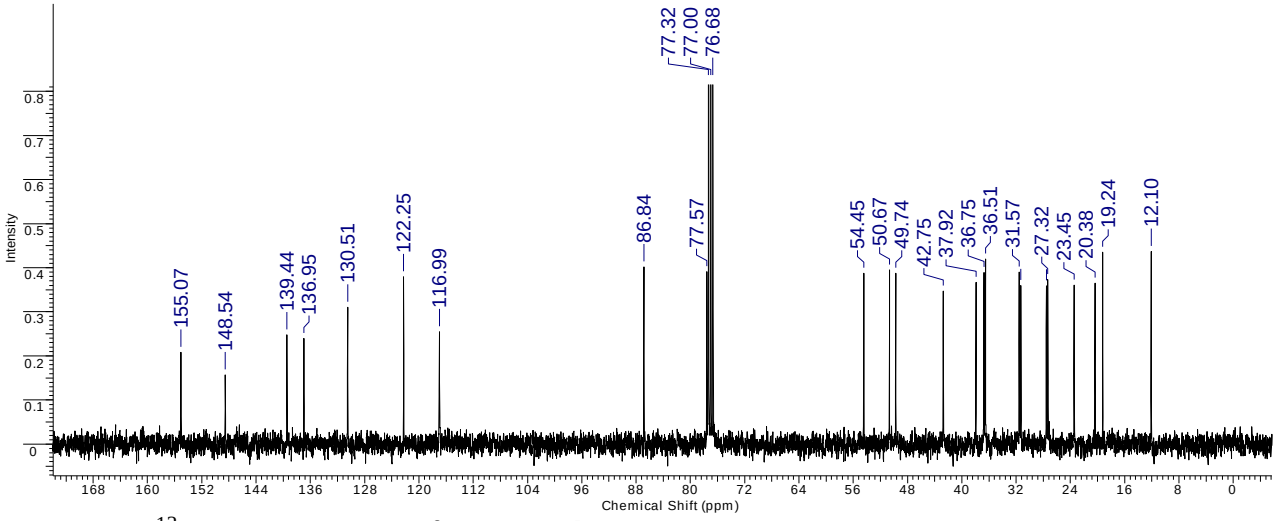


Figure S13 ^{13}C NMR spectrum of compound **8**.

MS (ESI), m/z: 399.5 [M+H], 444.1 [M+2H]. Calculated for $C_{25}H_{36}N_2O_4$: 444.5.

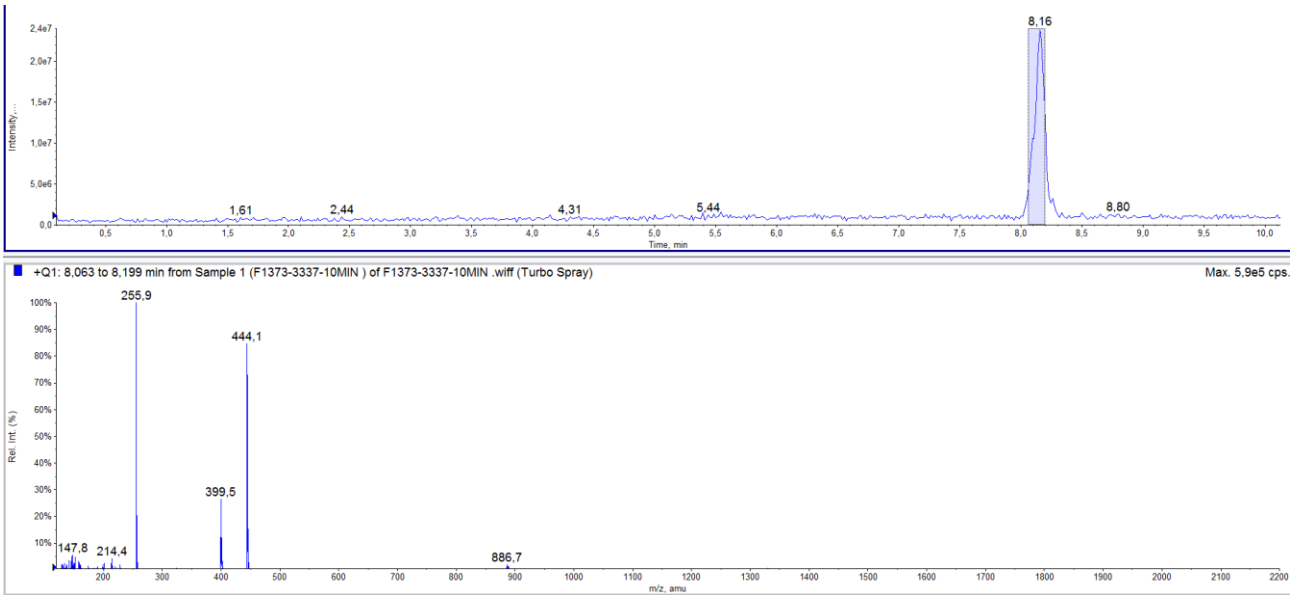
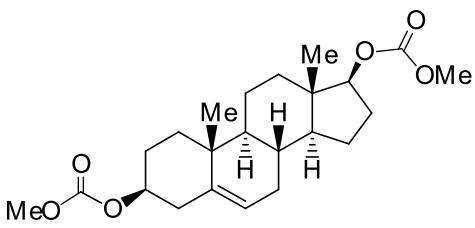


Figure S14 ESI-MS spectrum of compound **8**.

(3β, 17β)-17-(Methoxycarbonyloxy)androst-5-en-3-yl methoxycarboxylate (9) was obtained by slow crystallization of compound **8** from warmed CH_2Cl_2/MeOH (1:1) and subjected to X-ray analysis (see Figures 2, S15 and main text). ^{1}H NMR (δ) spectrum of the solution obtained by dissolving crystals in $CDCl_3$: 0.85 (s, 3 H, CH_3); 0.98 – 1.06 (m, 2 H); 1.04 (s, 3 H, CH_3); 1.12 – 1.25 (m, 2 H); 1.29 – 1.36 (m, 1 H); 1.39 – 1.70



(m, 7 H); 1.82 – 2.06 (m, 4 H); 2.17 – 2.32 (m, 1 H); 2.39 – 2.45 (m, 2 H); 3.78 (s, 6 H, CH_3O); 4.45 – 4.56 (m, 2 H, H3+17); 5.41 (d, 1 H, H6, *J* = 5.1).

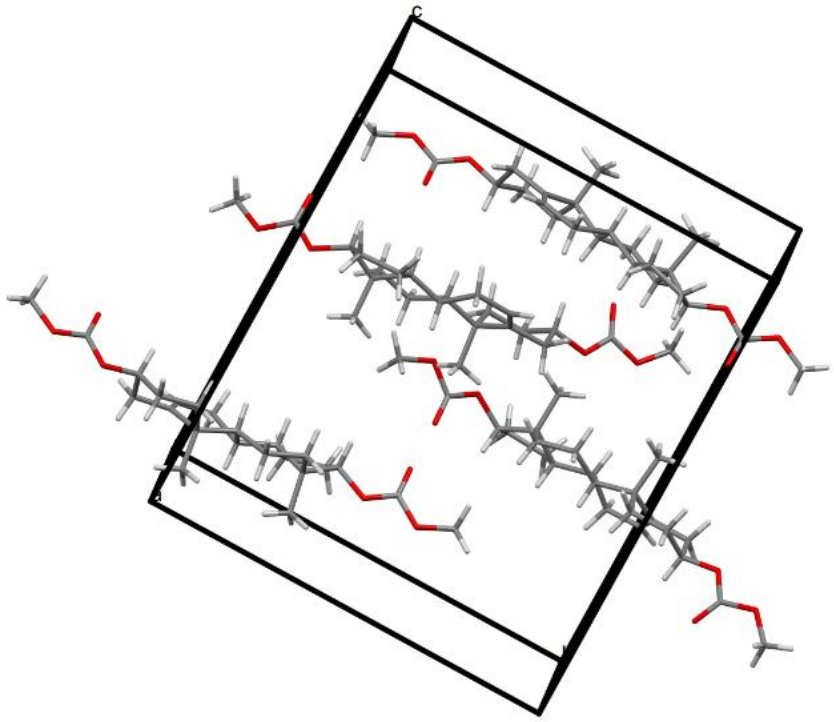


Figure S15 The unit cell for (3β, 17β)-17-(methoxycarbonyloxy)androst-5-en-3-yl methoxycarboxylate **9**.

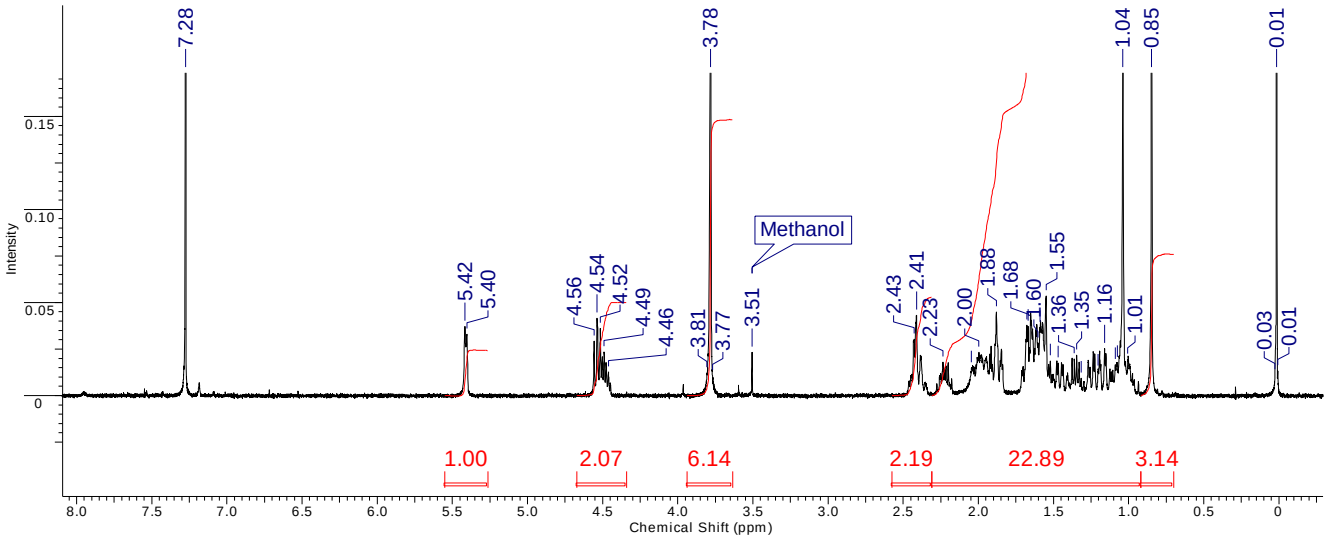


Figure S16 ^{1}H NMR spectrum of compound **9**.

3. Biochemical Assay

Recombinant protein expression and purification. Human CYP17A1 Δ2-19 variant[S6] with C-terminal four-residue histidine tag was obtained as previously described.[S3]

***In vitro* ligand binding assays**. The interaction of synthesized compounds and abiraterone (as a control) with recombinant human CYP17A1 was measured using differential spectrophotometric titration method.[S3] For the titration curves fitting, the tight binding equation (Morison equation) (S1) was used:

$$\Delta A = \Delta A_{max}\left\{([P_T] + [L_T] + K_D) - \left[([P_T] + [L_T] + K_D)^2 - 4[P_T][L_T]\right]^{\frac{1}{2}}\right\}/2[P_T] \quad \text{(S1)}.$$

where ΔA is the observed absorbance change, ΔA_{max} is the maximum absorbance change, K_D is the apparent equilibrium dissociation constant, $[P_T]$ and $[L_T]$ are the total concentrations of CYP17A1 and inhibitor, respectively.

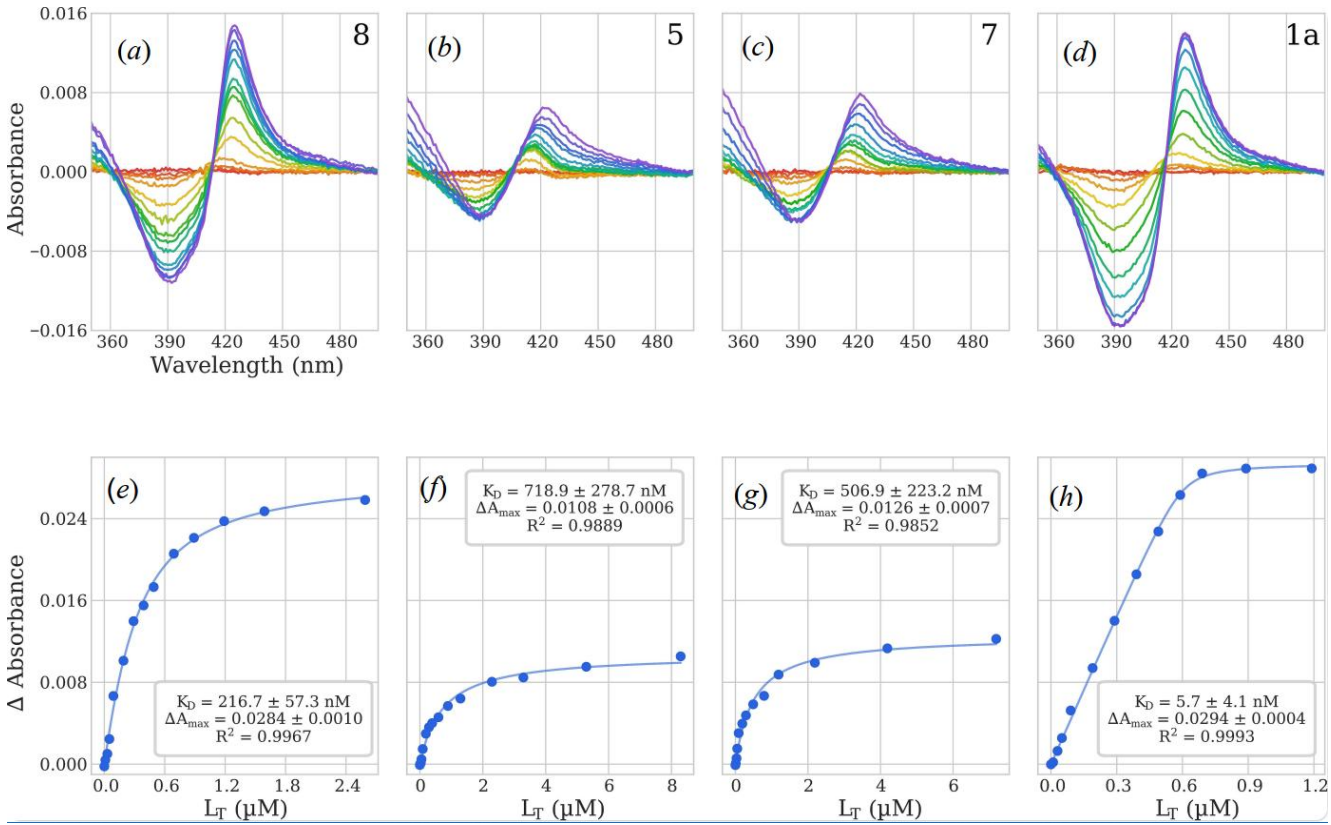


Figure S17 UV-visible differential spectrophotometric titration for equilibrium dissociation constant (K_D) determination. Absorbance change measured during the titration of CYP17A1 (1 μM) with compounds 8 (*a*), **5** (*b*), **7** (*c*) or abiraterone (**1a**) and corresponding binding isotherms (*e*–*h*). The solid line on binding isotherms represents the nonlinear fit for K_D determination

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