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Supplementary material for

Reduction of Acyl Glucuronidation in a Series of Acidic 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) Inhibitors: The Discovery of AZD6925.

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Synthesis of Compounds:

All solvents and chemicals used were reagent grade. Anhydrous solvents tetrahydrofuran (THF), benzene, dimethoxyethane (DME) were purchased from Aldrich. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep^{TM}, Biotage or Crawford and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography–mass spectroscopy (LC-MS), gas chromatography–mass spectroscopy (GC-MS) and NMR analytical techniques and was >95% for all test compounds. ^{1}H NMR were recorded on a Varian Gemini 2000 (300 MHz) or a Bruker Avance DPX400 (400 MHz) and were determined in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference and coupling constant (J) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for TLC analysis. Solutions were dried over anhydrous magnesium sulfate and the solvent was removed by rotary evaporation under reduced pressure.

***N*-cyclohexyl-6-[(3*S*)-3-[2-(methanesulfonamido)-2-oxo-ethyl]-1-piperidyl]-2-propylsulfanyl-pyridine-3-carboxamide (2)**

To a solution of 2-[(3*S*)-1-[5-(cyclohexylcarbamoyl)-6-propylsulfanyl-2-pyridyl]-3-piperidyl]acetic acid **1** (185 mg, 0.44 mmol) in CH_2Cl_2 was added methane sulphonamide (84 mg, 0.44 mmol), 4-dimethylaminopyridine (54 mg, 0.44 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC.HCl), (85 mg, 0.44 mmol). The reaction was stirred at room temperature for 2 hours. CH_2Cl_2 (10 mL) and saturated $NaHCO_3$ (10 mL) were added and the mixture was vigorously stirred and then separated. The organic phase was then evaporated under reduced pressure and purified by flash silica chromatography, elution gradient 0 to 90% MeOH in CH_2Cl_2 to give a colourless oil that was triturated with Et_2O to give a white solid (122 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95 (3H, t, J = 7.4), 1.04 - 1.49 (7H, m), 1.53 - 1.98 (10H, m), 2.18 - 2.31 (2H, m), 2.73 - 2.79 (1H, m), 2.88 - 2.93 (1H, m), 2.95 - 3.01 (2H, m), 3.25 (3H, s), 3.57 – 3.71 (1H, m), 4.13 - 4.29 (2H, m), 6.47 (1H, d, J = 8.7), 7.63 (1H, d, J = 8.7), 7.80 (1H, d, J = 7.9), 11.76 (1H, s); HRMS (EI) for $C_{23}H_{37}O_4N_4S_2$ (MH^+); calcd, 497.2250; found, 497.2246.

***N*-cyclohexyl-2-propylsulfanyl-6-[(3*S*)-3-(1H-tetrazol-5-ylmethyl)-1-piperidyl]pyridine-3-carboxamide (3)**

6-[(3*S*)-3-(cyanomethyl)-1-piperidyl]-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **2** (400 mg, 1.00 mmol) dissolved in toluene (10 mL) under nitrogen, then sodium azide (195 mg, 3.00 mmol) and triethylamine hydrochloride (413 mg, 3.00 mmol) were added and the mixture heated to 80 °C for 24 hours. The mixture was cooled then water (10 mL) was added and extracted with ethyl acetate. The organic layer was separated and the aqueous layer acidified with 2N HCl to pH ~ 2. Extracted with ethyl acetate, combined organic extracts washed with water and brine, dried ($MgSO_4$), filtered, evaporated and dried to give a white solid (250 mg, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.4), 1.20 - 1.80 (13H, m), 1.83 - 1.93 (1H, m), 1.94 - 2.11 (3H, m), 2.58 - 2.68 (1H, m), 2.79 - 3.00 (5H, m), 3.90 - 4.13 (3H, m), 6.07 (1H, d, J = 8.7), 6.8 (1H, d, J = 8.7), 7.65 (1H, d, J = 7.8), 15.8 (1H, s); HRMS (EI) for $C_{22}H_{34}ON_7S$ (MH^+); calcd, 444.2540; found, 444.2536.

1-[1-[5-(cyclohexylcarbamoyl)-6-propylsulfanyl-pyridin-2-yl]-3-piperidyl]cyclopropane-1-carboxylic acid (4)

Prepared according to the procedure of **11** from 6-chloro-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **16** and methyl 1-(3-piperidyl)cyclopropanecarboxylate in 9% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.79 - 0.83 (2H, m), 0.96 (3H, t, J = 7.4), 1.04 - 1.20 (3H, m), 1.25 - 1.50 (6H, m), 1.53 - 1.78 (11H, m), 2.73 - 3.04 (4H, m), 3.32 (2H, t, J = 10.4), 3.65

(1H, s), 4.39 - 4.45 (2H, m), 6.50 (1H, d, *J* = 8.7), 7.61 (1H, d, *J* = 8.7), 7.79 (1H, d, *J* = 7.9), 12.21 (1H, s); HRMS (EI) for $C_{24}H_{36}O_3N_3S$ (MH^+); calcd, 446.2472; found, 446.2468.

2-[1-[5-(cyclohexylcarbamoyl)-6-propylsulfanyl-pyridin-2-yl]-3-piperidyl]-2-methyl-propanoic acid (5)

Prepared according to the procedure of **11** from 6-chloro-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **16** and methyl 2-methyl-2-(3-piperidyl)propanoate in 24% yield. ^{1}H NMR (300 MHz, DMSO-d_6) 0.95 (3H, t, *J* = 7.3), 1.09 (3H, s), 1.14 (3H, s), 1.24 - 1.53 (6H, m), 1.55 - 1.63 (5H, m), 1.71 - 1.79 (6H, m), 2.64 - 2.87 (3H, m), 3.05 - 3.12 (1H, m), 3.55 - 3.72 (1H, m), 4.26 - 4.50 (2H, m), 6.42 (1H, d, *J* = 8.7), 7.58 (1H, d, *J* = 8.7), 7.74 (1H, d, *J* = 7.9), 12.20 (1H, s); HRMS (EI) for $C_{24}H_{38}O_3N_3S$ (MH^+); calcd, 448.2628; found, 448.2624.

2-[(3*R*)-1-[5-(cyclohexylc arbamoyl)-6-propylsulfanyl-pyridin-2-yl]pyrrolidin-3-yl]acetic acid (6)

Prepared according to the procedure of **11** from 6-chloro-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **16** and methyl 2-[(3R)-pyrrolidin-3-yl]acetate in 19% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94 (3H, t, *J* = 7.3), 1.06 - 1.34 (5H, m), 1.55 - 1.78 (8H, m), 2.12 - 2.19 (1H, m), 2.36 - 2.44 (2H, m), 2.55 - 2.62 (1H, m), 2.96 (2H, t, *J* = 7.2), 3.01 – 3.13 (1H, m), 3.39 - 3.43 (1H, m), 3.53 - 3.68 (3H, m), 6.09 (1H, d, *J* = 8.6), 7.61 (1H, d, *J* = 8.6), 7.68 (1H, d, *J* = 7.8); HRMS (EI) for $C_{21}H_{32}O_3N_3S$ (MH^+); calcd, 406.2159; found, 406.2156.

(1*S*,5*R*)-3-[5-(cyclohexylcarbamoyl)-6-propylsulfanyl-pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (7)

Prepared according to the procedure of **11** from 6-chloro-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **16** and (1*R*,5*S*,6*r*)-methyl 3-azabicyclo[3.1.0]hexane-6-carboxylate in 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (3H, t, *J* = 7.4), 1.10 - 1.17 (1H, m), 1.24 - 1.28 (4H, m), 1.58 - 1.78 (8H, m), 2.15 – 2.22 (2H, m), 2.97 (2H, t, *J* = 7.1), 3.46 – 3.54 (2H, m), 3.59 – 3.72 (1H, m), 3.74 – 3.84 (2H, m), 6.13 (1H, d, *J* = 8.6), 7.62 (1H, d, *J* = 8.6), 7.76 (1H, d, *J* = 7.7); HRMS (EI) for $C_{21}H_{30}O_3N_3S$ (MH^+); calcd, 404.2002; found, 404.1999.

(1*S*,5*R*)-3-[5-(2-adamantylcarbamoyl)-6-propylsulfanyl-2-pyridyl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (8)

Prepared according to the procedure of **12** from 6-chloro-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **16** and butyl pyrrolidine-3-carboxylate in 14% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95 (3H, t, *J* = 7.4), 1.08 - 1.42 (5H, m), 1.46 - 1.73 (4H, m), 1.85 – 1.99 (2H, m), 2.16 – 2.33 (2H, m), 3.04 – 3.22 (3H, m), 3.38 – 3.62 (2H, m), 3.66 – 3.73 (2H, m), 3.85 – 3.98 (1H, m), 5.98 (1H, d, *J* = 8.6), 6.54 (1H, d, *J* = 7.6), 7.72 (1H, d, *J* = 8.6); HRMS (EI) for $C_{20}H_{30}O_3N_3S$ (MH^+); calcd, 392.2002; found, 392.2001.

(1*S*,5*R*)-3-[5-(2-adamantylcarbamoyl)-6-propylsulfanyl-pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (9)

Prepared according to the procedure of **11** from *N*-(2-adamantyl)-6-chloro-2-propylsulfanyl-pyridine-3-carboxamide **21a** and methyl (1*R*,5*S*)-3-azabicyclo[3.1.0]hexane-6-carboxylate in 69% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (t, 3H, *J* = 7.3), 1.36 – 1.41 (m, 1H), 1.44 - 1.91 (m, 14H), 2.00 – 2.12 (m, 2H), 2.15 - 2.21 (m, 2H), 3.01 (t, 2H, *J* = 7.2), 3.44 – 3.56 (m, 2H), 3.72 – 3.84 (m, 2H), 3.89 - 3.99 (m, 1H), 6.14 (d, 1H, *J* = 8.6), 7.53 - 7.70 (m, 2H), 12.24 (s, 1H); HRMS (EI) for $C_{25}H_{34}O_3N_3S$ (MH^+); calcd, 456.2315; found, 456.2311.

(1*S*,5*R*)-3-[5-(2-adamantylcarbamoyl)-6-ethylsulfanyl-pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (10)

Prepared according to the procedure of **11** from *N*-(2-adamantyl)-6-chloro-2-ethylsulfanyl-pyridine-3-carboxamide **21b** and methyl (1*R*,5*S*)-3-azabicyclo[3.1.0]hexane-6-carboxylate in 77% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, 3H, *J* = 7.3), 1.56 - 2.09 (m, 15H), 2.26 -

2.38 (m, 2H), 3.21 (q, 2H, *J* = 7.3), 3.50 -3.64 (m, 2H), 3.80 – 3.94 (m, 2H), 4.22 - 4.28 (m, 1H), 6.06 (d, 1H, *J* = 8.6), 7.01 (d, 1H, *J* = 7.5), 7.87 (d, 1H, *J* = 8.6); HRMS (EI) for $C_{24}H_{32}O_3N_3S$ (MH^+); calcd, 442.2159; found, 442.2158.

(1S,5R)-3-[5-(2-adamantylcarbamoyl)-6-methylsulfanyl-pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (11)
[General procedure for SNAr and ester hydrolysis]
Potassium carbonate (0.415 g, 3.00 mmol) was added to *N*-(2-adamantyl)-6-chloro-2-methylsulfanyl-pyridine-3-carboxamide **21c** (0.337 g, 1 mmol) and (1*R*,5*S*,6*r*)-methyl 3-azabicyclo[3.1.0]hexane-6-carboxylate hydrochloride (0.222 g, 1.25 mmol) in butyronitrile (5 mL) under nitrogen. The resulting suspension was stirred at 120 °C for 70 hours. The reaction mixture was diluted with EtOAc (25 mL), and washed sequentially with water (10 mL) and saturated brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford the intermediate ester (0.335 g, 76%) as a colourless gum. This was dissolved in methanol (2 mL) and THF (6 mL) then a solution of lithium hydroxide (1M, 1.517 mL, 1.52 mmol) was added. Water was added drop-wise until the solution went cloudy and the resulting solution was stirred at 20 °C for 16 hours. The organic solvents were removed by evaporation and the reaction mixture was adjusted to pH4 with 2M HCl. The precipitate was collected by filtration, washed with water (10 mL) and dried under vacuum to afford a white solid (315 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 - 2.10 (m, 15H), 2.27 - 2.37 (m, 2H), 2.50 – 2.61 (m, 3H), 3.51 – 3.65 (m, 2H), 3.82 - 3.97 (m, 2H), 4.18 - 4.31 (m, 1H), 6.05 (d, 1H, *J* = 8.6), 6.90 (d, 1H, *J* = 7.2), 7.84 (d, 1H, *J* = 8.6); HRMS (EI) for $C_{23}H_{30}O_3N_3S$ (MH^+); calcd, 428.2002; found, 428.1999.

(1*R*,5*S*)-3-[5-[[(2*r*,5*s*)-5-hydroxy-2-adamantyl]carbamoyl]-6-propylsulfanyl-pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (12)
Prepared according to the procedure of **11** from 6-chloro-N-((2*r*,5*s*)-5-hydroxy-2-adamantyl)-2-propylsulfanyl-pyridine-3-carboxamide **22a** and methyl (1*R*,5*S*)-3-azabicyclo[3.1.0]hexane-6-carboxylate in 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (3H, t, *J* = 7.3), 1.29 - 1.40 (3H, m), 1.58 - 1.74 (8H, m), 1.92 - 2.06 (5H, m), 2.18 - 2.21 (2H, m), 3.01 (2H, t, *J* = 7.2), 3.44 - 3.56 (2H, m), 3.74 - 3.88 (3H, m), 4.43 (1H, s), 6.15 (1H, d, *J* = 8.6), 7.61 (1H, d, *J* = 8.6), 7.64 (1H, d, *J* = 6.7), 12.28 (1H, s); HRMS (EI) for $C_{25}H_{34}O_4N_3S$ (MH^+); calcd, 472.2264; found, 472.2264.

(1*R*,5*S*)-3-[6-cyclopentylsulfanyl-5-[((2*r*,5*s*)-5-hydroxy-2-adamantyl)carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (13)
Prepared according to the procedure of **11** from 6-chloro-2-cyclopentylsulfanyl-N-((2*r*,5*s*)-5-hydroxy-2-adamantyl)pyridine-3-carboxamide **22b** and methyl (1*R*,5*S*)-3-azabicyclo[3.1.0]hexane-6-carboxylate in 39% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 - 1.39 (3H, m), 1.49 - 1.72 (12H, m), 1.93 - 2.04 (5H, m), 2.09 - 2.21 (4H, m), 3.48 - 3.53 (2H, m), 3.78 - 3.88 (3H, m), 3.95 - 4.02 (1H, m), 4.36 (1H, s), 6.14 (1H, d, *J* = 8.6), 7.56 (1H, d, *J* = 6.6), 7.61 (1H, d, *J* = 8.6), 12.19 (1H, s); HRMS (EI) for $C_{27}H_{36}O_4N_3S$ (MH^+); calcd, 498.2421; found, 498.2420.

(1*S*,5*R*)-3-[6-cyclopentylsulfanyl-5-[[5-(difluoromethoxy)-2-adamantyl]carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (14)
To a solution of methyl (1*S*,5*R*)-3-[6-cyclopentylsulfanyl-5-[[5-(difluoromethoxy)-2-adamantyl]carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate **24** (140 mg, 0.25 mmol) in MeOH (5 mL) was added 2M sodium hydroxide (1 mL, 2.00 mmol). The resulting solution was stirred at ambient temperature for 6 hours then evaporated to approximately quarter volume. Water (5 mL) was added then the mixture was acidified with 1M citric acid (2 mL) giving a white precipitate. This was stirred for 30 minutes then filtered,

washed with water and dried in vacuo at 50 ºC to afford a white solid (120 mg, 88%). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.32 - 1.44 (2H, m), 1.45 - 1.74 (7H, m), 1.83 - 1.90 (4H, m), 1.92 - 2.01 (4H, m), 2.05 - 2.21 (7H, m), 3.45 - .55 (2H, m), 3.72 - 3.85 (2H, m), 3.88 - 4.02 (2H, m), 6.14 (1H, d, *J* = 8.6), 6.89 (1H, t, *J* = 76.5), 7.60 (1H, d, *J* = 8.6), 7.69 (1H, d, *J* = 6.4), 12.3 (1H, s); HRMS (EI) for $C_{28}H_{36}O_4N_3F_2S$ (MH^+); calcd, 548.2389; found, 548.2385.

(1*R*,5*S*,6*r*)-3-(6-(cyclopentylthio)-5-(3-(pyridin-3-yl)pyrrolidine-1-carbonyl)pyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (15)

Lithium hydroxide monohydrate (102 mg, 2.44 mmol) was added to a stirred solution of (1R,5S,6r)-methyl 3-(6-(cyclopentylthio)-5-(3-(pyridin-3-yl)pyrrolidine-1-carbonyl)pyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate **29** (400 mg, 0.81 mmol) in methanol (5 mL) and water (2 mL). The resulting solution was stirred at ambient temperature for 24 hours. The bulk of the organic solvent was removed *in vacuo* and the resulting solution was acidified with 1N citric acid. The resulting suspension was extracted with EtOAc (3 x 30 ml). The combined organic layers were washed with HCl solution (pH3, 30 mL), sat brine (30 mL) then dried ($MgSO_4$), filtered and evaporated to yield a white solid (195 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 1.22 - 1.72 (8H, m), 1.90 - 2.38 (5H, m), 3.30 - 3.55 (5H, m), 3.64 (1H, s), 3.72 – 3.95 (3H, m), 4.01 - 4.07 (1H, m), 6.16 (1H, d, *J* = 8.0), 7.30 – 7.80 (2H, m), 7.71 (1H, s), 8.40 – 8.60 (2H, m), 12.18 (1H, s); HRMS (EI) for $C_{26}H_{31}O_3N_4S$ (MH^+); calcd, 479.2111; found, 479.2108.

6-[(3*S*)-3-(cyanomethyl)-1-piperidyl]-N-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide (17)

6-Chloro-*N*-cyclohexyl-2-propylsulfanyl-pyridine-3-carboxamide **16** (5.065 g, 16.18 mmol) and 2-[(3*S*)-3-piperidyl]acetonitrile hydrochloride (2.60 g, 16.18 mmol) slurried in butyronitrile (60 mL) then potassium carbonate (6.71 g, 48.54 mmol) added and the mixture heated to 120 °C for 3 days. The reaction was cooled to room temperature, ethyl acetate (75 mL) was added then the mixture washed with water and brine, dried ($MgSO_4$), filtered and evaporated. Purified by flash silica chromatography with 4:1 to 1:1 hexane:ethyl acetate as eluent to give title compound (3.5 g, 54%). ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, t), 1.17 - 1.54 (6H, m), 1.58 - 1.88 (7H, m), 1.96 - 2.17 (4H, m), 2.39 (2H, d), 2.90 - 3.25 (4H, m), 3.91 - 4.19 (2H, m), 4.32 - 4.43 (1H, m), 6.39 - 6.54 (1H, m), 6.45 (1H, d), 7.81 (1H, d); LRMS m/z (M^+ + H) 401.

***N*-(2-adamantyl)-2,6-dichloro-pyridine-3-carboxamide (19)**

Oxalyl chloride (8.72 ml, 100.00 mmol) was added dropwise to 2,6-dichloronicotinic acid **18** (9.60 g, 50 mmol) and *N*,*N*-dimethylformamide (0.039 ml, 0.50 mmol) in CH_2Cl_2 at 20 ºC over a period of 10 minutes under nitrogen. The resulting suspension was stirred at 20 °C for 2 hours. The resulting mixture was evaporated to dryness and the residue was azeotroped with toluene to afford the crude acid chloride, which was dissolved in CH_2Cl_2 (25 mL) and added portion wise to a stirred solution of 2-adamantanamine hydrochloride (9.39 g, 50.00 mmol) and *N*-Ethyldiisopropylamine (26.1 ml, 150 mmol) in CH_2Cl_2 cooled to 0 ºC, over a period of 15 minutes under nitrogen. The resulting suspension was stirred at 20 °C for 2 hours.
The reaction mixture was evaporated to dryness, stirred with water (50 mL)
for 10 mins and the precipitate was collected by filtration, washed with water (2 x 25 mL) and dried under vacuum to afford a cream solid (16.01 g, 98 %) which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69 - 1.76 (2H, m), 1.79 (2H, s), 1.82 - 1.96 (8H, m), 2.07 (2H, s), 4.27 (1H, d), 6.92 - 7.01 (1H, m), 7.39 (1H, d), 8.19 (1H, d); LRMS m/z (M^+ + H) 325

2,6-dichloro-*N*-((2*r*,5*s*)-5-hydroxyadamantan-2-yl)nicotinamide (20)

Oxalyl chloride (45.4 ml, 520.83 mmol) was added to a suspension of 2,6-dichloronicotinic acid **18** (50 g, 260.42 mmol) in CH_2Cl_2 (1000 ml, 20 vol) and *N*,*N*-Dimethylformamide (0.202 ml, 2.60 mmol). The resulting mixture was filtered, evaporated to dryness and the

residue was azeotroped with toluene (3 x 500 mL, 10 vol) to afford the crude acid chloride as a brown oil. A solution of 2,6-dichloronicotinoyl chloride (25.2 g, 119.60 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a stirred suspension of 4-aminoadamantan-1-ol (20.00 g, 119.6 mmol) and *N*-Ethyldiisopropylamine (24.83 mL, 143.52 mmol) in THF (400 mL) at 20 ºC, over a period of 30 minutes under nitrogen. The resulting suspension was stirred at room temperature for 18 hours. The reaction mixture was diluted with EtOAc (500 mL), and washed sequentially with water (100 mL) and saturated brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford the crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 100% EtOAc in CH_2Cl_2. Pure fractions were evaporated to dryness to afford a white solid (20.65 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 - 1.38 (2H, m), 1.60 - 1.67 (4H, d), 1.69 - 1.76 (2H, m), 1.87 - 1.94 (2H, m), 1.99 - 2.00 (1H, m), 2.04 - 2.09 (2H, m), 3.91 - 3.96 (1H, m), 4.47 (1H, s), 7.64 (1H, d), 7.95 (1H, d), 8.49 (1H, d); LRMS m/z (M^- - H) 339.

***N*-(2-adamantyl)-6-chloro-2-propylsulfanyl-pyridine-3-carboxamide (21a)**

Propanethiol (1.45 mL, 16 mmol) was added to *N*-(2-adamantyl)-2,6-dichloro-pyridine-3-carboxamide **19** (5.2 g, 16 mmol) and sodium carbonate (5.09 g, 48 mmol) in *N*,*N*-dimethylformamide (50 mL) at 20 ºC under nitrogen. The resulting suspension was stirred at 60 °C for 3 hours. The reaction mixture was diluted with EtOAc (400 mL) and washed with water (3 x 50mL), and saturated brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product which was recrystallised from 15% ethyl acetate/hexane to give N-(2-adamantyl)-6-chloro-2-propylsulfanyl-pyridine-3-carboxamide (4.9 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (t, 3H), 1.63 - 1.84 (m, 6H), 1.85 - 1.99 (m, 8H), 2.02 - 2.14 (m, 2H), 3.24 (t, 2H), 4.22 - 4.31 (m, 1H), 6.85 - 6.96 (m, 1H), 7.06 (d, 1H), 7.90 (d, 1H); LRMS m/z (M^+ + H) 365.

***N*-(2-adamantyl)-6-chloro-2-ethylsulfanyl-pyridine-3-carboxamide (21b)**

Ethanethiol (0.433 mL, 5.84 mmol) was added to *N*-(2-adamantyl)-2,6-dichloro-pyridine-3-carboxamide **19** (2.00 g, 6.15 mmol), and sodium carbonate (1.955 g, 18.45 mmol) in *N*,*N*-dimethylformamide (12 mL) at 20 ºC under nitrogen. The resulting suspension was stirred at 60 °C for 3 hours. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (3 x 20 mL), and saturated brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product which was recrystallised from 15% ethyl acetate/hexane to give *N*-(2-adamantyl)-6-chloro-2-ethylsulfanyl-pyridine-3-carboxamide (1.489 g, 69 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (t, 3H), 1.66 - 1.81 (m, 4H), 1.85 - 1.96 (m, 8H), 2.03 - 2.12 (m, 2H), 3.26 (q, 2H), 4.22 - 4.31 (m, 1H), 6.78 - 6.89 (m, 1H), 7.06 (d, 1H), 7.89 (d, 1H); LRMS m/z (M^+ + H) 351.

***N*-(2-adamantyl)-6-chloro-2-methylsulfanyl-pyridine-3-carboxamide (21c)**

Sodium thiomethoxide (0.409 g, 5.84 mmol) was added to *N*-(2-adamantyl)-2,6-dichloro-pyridine-3-carboxamide **19** (2.00 g, 6.15 mmol) in DMA (10 mL) at 20 ºC under nitrogen. The resulting suspension was stirred at 60 °C for 3 hours. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (3 x 20mL), and saturated brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product which was purified by crystallisation from EtOAc/isohexane to afford a white solid (1.150 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65 - 1.82 (m, 4H), 1.85 - 1.98 (m, 8H), 2.01 - 2.12 (m, 2H), 2.61 (s, 3H), 4.22 - 4.33 (m, 1H), 6.73 - 6.82 (m, 1H), 7.07 (d, 1H), 7.87 (d, 1H); LRMS m/z (M^+ + H) 337.

6-chloro-*N*-((2*r*,5*s*)-5-hydroxy-2-adamantyl)-2-propylsulfanyl-pyridine-3-carboxamide (22a)

1-Propanethiol (1.327 mL, 14.65 mmol) was added in one portion to 2,6-dichloro-*N*-(5-hydroxy-2-adamantyl)pyridine-3-carboxamide **20** (5.00 g, 14.65 mmol) and sodium carbonate (4.66 g, 43.96 mmol) in *N*,*N*-dimethylformamide (50 mL). The resulting suspension was

stirred at 60 °C for 3 hours. The mixture was cooled, evaporated, CH_2Cl_2 (250 mL) was added and the mixture was washed with water (3 x 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated to a sticky pale yellow solid. This was triturated with 4:1 hexane:ethyl acetate, filtered and dried to give the desired product as a white solid (5.0 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (3H, t), 1.22 - 1.3 (2H, m), 1.5 - 1.7 (8H, m), 1.85 - 1.96 (3H, m), 1.97-2.03 (2H, s), 3.0 (2H, t), 3.83-3.9 (1H, m), 4.34 (1H, s), 7.2 (1H, d), 7.65 (1H, d), 8.18 (1H, d); LRMS m/z (M^+ + H) 381.

6-chloro-2-cyclopentylsulfanyl-*N*-((2*r*,5*s*)-5-hydroxy-2-adamantyl)pyridine-3-carboxamide (22b)

Anhydrous sodium carbonate (1.104 mL, 26.37 mmol) was added in one portion to 2,6-dichloro-*N*-((2*r*,5*s*)-5-hydroxy-2-adamantyl)pyridine-3-carboxamide **20** (3.00 g, 8.79 mmol) and cyclopentyl mercaptan (0.946 mL, 8.79 mmol) in DMF (50 mL) under nitrogen. The resulting suspension was stirred at 60 ºC for 6 hours. The reaction mixture was concentrated and diluted with CH_2Cl_2 (150 mL), and washed sequentially with water (2 x 75 mL) and saturated brine (75 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product that was triturated with 4:1 isohexane:EtOAc to give the desired product (3.2 g, 89%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (2H, d), 1.47 - 1.6 (2H, m), 1.64-1.85 (10H, m), 1.94-2.08 (3H, m), 2.1-2.15 (2H, m), 2.16 - 2.28 (2H, m), 3.90 - 3.97 (2H, m), 4.45 (1H, s), 7.3 (1H, d), 7.70 - 7.75 (1H, m), 8.28 (1H, d); LRMS m/z (M^+ + H) 407.

methyl (1R,5S)-3-[6-cyclopentylsulfanyl-5-[(5-hydroxy-2-adamantyl)carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate (23)

A solution of trimethylsilyldiazomethane (2M solution in ether) (0.362 mL, 0.72 mmol) was added dropwise to a stirred solution of (1R,5S)-3-[6-cyclopentylsulfanyl-5-[(5-hydroxy-2-adamantyl)carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylic acid (180 mg, 0.36 mmol) in 3:2 toluene:methanol (5 mL) at 22 ºC, over a period of 1 minute. The resulting solution was stirred at ambient temperature for 1 hour. The mixture was evaporated and purified by flash silica chromatography with EtOAc. Pure fractions were evaporated to dryness to afford methyl (1R,5S)-3-[6-cyclopentylsulfanyl-5-[(5-hydroxy-2-adamantyl)carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate (180 mg, 97%) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 – 1.36 (2H, m), 1.44 – 1.77 (13H, m), 1.90 – 2.19 (7H, m), 2.23 – 2.26 (2H, m), 3.48 – 3.54 (2H, m), 3.62 (3H, s), 3.78 – 3.89 (3H, m), 3.92 – 4.02 (1H, m), 4.37 (1H, s), 6.15 (1H, d), 7.56 (1H, d), 7.62 (1H, d); LRMS m/z (M^+ + H) 512.

methyl (1S,5R)-3-[6-cyclopentylsulfanyl-5-[[5-(difluoromethoxy)-2-adamantyl]carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate (24)

A solution of 2-(fluorosulhonyl)difluoroacetic acid (0.106 mL, 1.03 mmol) in anhydrous acetonitrile (1 mL) was added dropwise to a solution of methyl (1R,5S)-3-[6-cyclopentylsulfanyl-5-[(5-hydroxy-2-adamantyl)carbamoyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate (175 mg, 0.34 mmol) and copper(I)iodide (13.03 mg, 0.07 mmol) in anhydrous acetonitrile (5 mL) warmed to 45 ºC, over a period of 1 hour under nitrogen. The resulting solution was stirred at 45 °C for 30 minutes. The reaction mixture was concentrated and diluted with EtOAc (25 mL), and washed sequentially with water (2 x 10 mL) and saturated brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product that was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford the desired product (140 mg, 73%) as a pale, yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 – 2.31 (24H, m), 3.53 – 3.60 (2H, m), 3.70 (3H, s), 3.81 – 3.90 (2H, m), 4.11 – 4.26 (2H, m), 6.06 (1H, d), 6.35 (1H, t), 7.02 (1H, d), 7.88 (1H, d); LRMS m/z (M^+ + H) 562.

tert-butyl 2,6-dichloropyridine-3-carboxylate (25)

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A suspension of 2,6-dichloronicotinic acid **18** (15.00 g, 78.13 mmol) in toluene (170 mL) was warmed to 90 ºC under nitrogen. To this suspension was added *N*,*N*-dimethylformamide di-*tert*-butyl acetal (74.9 mL, 312.50 mmol) dropwise. The resulting solution was stirred at 90 ºC for 5 hours. The reaction mixture was evaporated to dryness and redissolved in EtOAc (200 mL), and washed sequentially with saturated $NaHCO_3$ (100 mL) and saturated brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 30% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford *tert*-butyl 2,6-dichloronicotinate (17.13 g, 88%) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54 (9H, s), 7.67 (1H, d), 8.23 (1H, d)

methyl (1R,5S)-3-[6-chloro-5-[(2-methylpropan-2-yl)oxycarbonyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate (26)

A suspension of (1R,5S,6r)-methyl 3-azabicyclo[3.1.0]hexane-6-carboxylate (2.99 g, 21.16 mmol), *tert*-butyl 2,6-dichloronicotinate (5.00 g, 20.15 mmol) and triethylamine (3.37 mL, 24.18 mmol) in DMA (50 mL) was stirred at ambient temperature overnight. The reaction mixture was evaporated to dryness and redissolved in EtOAc (150 mL), and washed sequentially with 1N citric acid (50 mL), water (50 mL), and saturated brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 30% EtOAc in isohexane. Pure fractions were evaporated to afford (1R,5S,6r)-methyl 3-(5-(tert-butoxycarbonyl)-6-chloropyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate (2.18g, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (1H, t), 1.50 (9H, s), 2.19 - 2.21 (2H, m), 3.50 - 3.53 (2H, m), 3.62 (3H, s), 3.77 (2H, d), 6.12 (1H, d), 7.86 (1H, d); LRMS m/z (M^+ + H) 353.

methyl (1R,5S)-3-[6-cyclopentylsulfanyl-5-[(2-methylpropan-2-yl)oxycarbonyl]pyridin-2-yl]-3-azabicyclo[3.1.0]hexane-6-carboxylate (27)

Cyclopentanethiol (1.157 mL, 10.81 mmol) was added to a stirred suspension of potassium *tert*-butoxide (0.416 g, 3.71 mmol) in DMA (4 ml). The resulting suspension was stirred at ambient temperature for 10 minutes under nitrogen then treated with a solution of (1R,5S,6r)-methyl 3-(5-(tert-butoxycarbonyl)-6-chloropyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate (1.09 g, 3.09 mmol) in DMA (5 ml). The resulting reaction was stirred at ambient temperature for 2 hours then treated with sat NH_4Cl solution (15 ml). The reaction mixture was diluted with EtOAc (75 mL), and washed sequentially with saturated NH_4Cl (25 mL), water (50 mL), and saturated brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 10 to 30% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford (1R,5S,6r)-methyl 3-(5-(tert-butoxycarbonyl)-6-(cyclopentylthio)pyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate (0.968 g, 75%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 - 1.82 (16H, m), 2.10 – 2.22 (2H, m), 2.27 (2H, t), 3.60 (2H, d), 3.70 (3H, s), 3.87 (2H, d), 4.00 - 4.08 (1H, m), 5.95 (1H, d), 7.90 (1H, d); LRMS m/z (M^+ + H) 419.

2-(cyclopentylthio)-6-((1R,5S,6r)-6-(methoxycarbonyl)-3-azabicyclo[3.1.0]hexan-3-yl)nicotinic acid (28)

A solution of (1R,5S,6r)-methyl 3-(5-(tert-butoxycarbonyl)-6-(cyclopentylthio)pyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate (968 mg, 2.31 mmol) in hydrogen chloride (4M in dioxane) (30 ml, 120.00 mmol) was stirred at ambient temperature for 5 hours. The solvent was removed *in vacuo* to yield 2-(cyclopentylthio)-6-((1R,5S,6r)-6-(methoxycarbonyl)-3-azabicyclo[3.1.0]hexan-3-yl)nicotinic acid (760 mg, 91%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 - 1.80 (7H, m), 2.10 – 2.25 (2H, m), 2.29 (2H, s), 3.61 - 3.71 (5H, m), 3.80 – 4.00 (2H, m), 4.02 - 4.11 (1H, m), 6.00 (1H, d), 8.03 (1H, d); LRMS m/z (M^+ + H) 363.

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(1R,5S,6r)-methyl 3-(6-(cyclopentylthio)-5-(3-(pyridin-3-yl)pyrrolidine-1-carbonyl)pyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate (29)
Oxalyl chloride (0.274 mL, 3.15 mmol) was added dropwise to a stirred solution of 2-(cyclopentylthio)-6-((1R,5S,6r)-6-(methoxycarbonyl)-3-azabicyclo[3.1.0]hexan-3-yl)nicotinic acid (380 mg, 1.05 mmol) in CH_2Cl_2 (5 mL) with a drop of *N*,*N*-dimethylformamide at 0 ºC, under nitrogen. The resulting solution was allowed to warm to ambient temperature and stirred for 2 hours. The reaction mixture was evaporated to dryness and redissolved in CH_2Cl_2 (8 mL). The solution was cooled to 0 ºC then treated with a solution of 3-pyrrolidin-3-ylpyridine (171 mg, 1.15 mmol) and triethylamine (0.438 mL, 3.15 mmol) in CH_2Cl_2 (5 mL). The resulting reaction was allowed to warm to ambient temperature and stirred at this temperature for 2 hours. The reaction mixture was diluted with CH_2Cl_2 (50 mL), and washed sequentially with 1N citric acid (30 mL), water (30 mL), and saturated brine (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% MeOH in CH_2Cl_2. Pure fractions were evaporated to dryness to afford (1R,5S,6r)-methyl 3-(6-(cyclopentylthio)-5-(3-(pyridin-3-yl)pyrrolidine-1-carbonyl)pyridin-2-yl)-3-azabicyclo[3.1.0]hexane-6-carboxylate (400 mg, 77%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 – 1.70 (4H, m), 1.71 - 1.80 (3H, m), 2.11 - 2.20 (3H, m), 2.25 (2H, s), 2.36 (1H, s), 3.40 - 3.60 (4H, m), 3.69 (4H, s), 3.74 - 3.87 (4H, m), 4.05 - 4.12 (2H, m), 5.99 (1H, d), 7.20 – 7.30 (2H, m), 7.57 (1H, s), 8.40 – 8.60 (1H, m); LRMS m/z (M^+ + H) 493.

Biological Protocols:
Measurement of 11β-HSD1 activity: The conversion of cortisone to the active steroid cortisol by 11β-HSD1 oxo-reductase can be measured using a cortisol competitive homogeneous time resolved fluorescence assay (HTRF) assay (CisBio International, R&D, Administration and Europe Office, In Vitro Technologies - HTRF® / Bioassays BP 84175, 30204 Bagnols/Cèze Cedex, France). The evaluation of compounds was carried out using a baculovirus expressed N terminal 6-His tagged full length human, rat, dog or cynomolgous monkey 11β-HSD1 enzyme. The enzyme was purified from a detergent solublised cell lysate, using a copper chelate column. Inhibitors of 11β-HSD1 reduce the conversion of cortisone to cortisol, which is identified by an increase in signal, in the above assay. The assay incubation was carried out in black 384 well plates (Matrix, Hudson NH, USA). consisting of cortisone (Sigma, Poole, Dorset, UK, 160 nM), glucose-6-phosphate (Roche Diagnostics, 1 mM), NADPH (Roche Diagnostics, 100 μM), glucose-6-phosphate dehydrogenase (Roche Diagnostics, 12.5 μg/ml), EDTA (Sigma, Poole, Dorset, UK, 1 mM), assay buffer (K_2HPO_4/KH_2PO_4, 100 mM) pH 7.5, recombinant 11β-HSD1 (1.5 μg/ml) plus test compound in a total volume of 20 μl. The assay plates were incubated for 25 minutes at 37 °C and the reaction stopped by the addition of 10 μl of 0.5 mM glycerrhetinic acid (Sigma) plus cortisol-XL665. 10 μl of anti-cortisol Cryptate was then added and the plates and incubated for 2h at room temperature. Fluorescence at 665 nm and 620 nm was measured and the 665 nm:620 nm ratio calculated using an Envision plate reader. This data was then used to calculate IC_{50} values for each compound (Origin 7.5, Microcal software, Northampton MA, USA).
Measurement of 11-βHSD1 activity in isolated human adipocytes
Subcutaneous (sc) adipose tissue was obtained through a needle biopsy from the lower part of the abdomen after dermal local anesthesia with lidocaine (Xylocain; AstraZeneca, Södertälje, Sweden) from non-diabetic volunteers. Adipocytes were isolated from sc adipose tissue by collagenase digestion. In brief, adipocytes were isolated from the adipose tissue following shaking in medium 199 (Invitrogen, UK) supplemented with 5.6 mmol/l glucose, 4% BSA (Sigma-Aldrich, Poole, UK) and 0.6 mg/ml collagenase (Roche, Burgess Hill, UK) at 37 °C for ~60 minutes. After filtration through a 250 μm nylon mesh the adipocytes were washed 4

times with medium 199 supplemented with 5.6 mmol/l glucose and 1% BSA. Isolated adipocytes were diluted to approximate 3-5% lipocrit in containing DMEM (6 mmol/l glucose, 10% FCS (Invitrogen, UK), 1% Penicillin/Streptomycin (Invitrogen, UK)) and incubated in 6-well plates in duplicate for 6 h with ^{3}H-Cortisone (20 nmol/l, 1 μCi/ml, Amersham, Chalfont St. Giles, UK) containing DMEM (6 mmol/l glucose, cortisone 100 nmol/l (Sigma-Aldrich, Poole, UK), 10% FCS, 1% PEST), at 37°C, 5% CO_2. Following incubations, media samples were collected and cortisone to cortisol conversion analysed. Radiolabelled steroids were extracted using ethyl acetate, samples were evaporated to dryness under nitrogen and re-suspended in mobile phase for HPLC analysis (Methanol:H_2O, 50:50), adapted from Napolitano *et al.*[1] Radiolabelled steroids were separated using reversed phase HPLC, Agilent 1200 HPLC using a Kromasil C18 5μm column, 4.6 mm x 25 0mm (Crawford Scientific, Lanarkshire, UK) with methanol:H_2O (50:50) at flow rate of 1.5 ml/min. Radioactivity measured using a flow scintillation analyser (Radiomatic series 500TR, Perkin Elmer Analytical Instruments) with FLO-ONE software.

11β-HSD1 activity in liver and adipose tissue

Male C57Bl6J mice were maintained on a chow diet were dosed by oral gavage with compound suspended in HPLC/Tween. Animals were euthanased 1 h post dose using a rising concentration of CO_2 at a time post-dose as determined by PK. Blood samples were taken *via* cardiac puncture for compound determination. Activity of the 11β-HSD1 enzyme in liver and epididymal adipose tissue was measured as the conversion of ^{3}H-cortisone to ^{3}H-cortisol. Epididymal adipose tissue and liver pieces from mice were removed and snap frozen in liquid N_2. Tissues were weighed (approximately 100-200 mg), cut into 2-3 mm^3 pieces using scissors and incubated for 10 min (mouse liver) or 60 min (mouse adipose tissue) in the presence of ^{3}H-cortisone (20 nmol/l, 1 μCi/ml, specific activity 1.97GBq/mmol, Perkin Elmer) containing DMEM Ham F12 media (Sigma Aldrich, Poole, UK) supplemented with 10% FCS and 1% penicillin–streptomycin (Sigma Aldrich, Poole, UK) in 37 ˚C at 5% CO_2. After tissue incubations, medium samples were collected for analysis of ^{3}H-cortisone to ^{3}H-cortisol conversion. Radio-labelled steroids were extracted using ethyl acetate, samples were evaporated to dryness under nitrogen and re-suspended in mobile phase for HPLC analysis (Methanol:H_2O, 50:50), adapted from Napolitano *et al.*[1] Radiolabelled steroids were separated using reversed phase HPLC, Agilent 1200 HPLC using a Kromasil C18 5 μm column, 4.6 mm x 250 mm (Crawford Scientific, Lanarkshire, UK) with methanol:H_2O (50:50) at flow rate of 1.5 ml/min. Radioactivity measured using a flow scintillation analyser (Radiomatic series 500TR, Perkin Elmer Analytical Instruments) with FLO-ONE software.

Measurement of 11β-HSD2 activity: 11β-HSD2 catalyses the conversion of cortisol to cortisone. The compounds were incubated with a mixture consisting of 11β-HSD2 recombinant enzyme in 1 mM DTT, NAD (Roche Diagnostics, 2.5 mM) and cortisol (Sigma, Poole, Dorset, UK, 1 mM, 0.625 μM) in a total volume of 50 μl in 384 well plates. Assay plates were read 40 min post cortisol addition on a fluorescent plate reader (Envision) with signal excitation 340 nm (25 nm band width) and emission 460 nm.

Measurement of 17β-HSD1 activity: 17β-HSD1 catalyses the conversion of estrone to estradiol. The assay incubation was carried out in borosilicate glass tubes consisting of estradiol (Sigma, Poole, Dorset, UK, 160 nM), glucose-6-phosphate (Roche Diagnostics, 1 mM), NADPH (Roche Diagnostics, 100 μM), glucose-6-phosphate dehydrogenase (Roche Diagnostics, 12.5 μg/ml), EDTA (Sigma, Poole, Dorset, UK, 1 mM), assay buffer (K_2HPO_4/KH_2PO_4, 100 mM) pH 7.5, recombinant 17β-HSD1 (1.5 μg/ml) plus test compound containing 1 μCi 3H estrone (Perkin Elmer). The compounds were incubated for 20 minutes at room temperature and the reaction stopped by the addition of ethyl acetate. Radiolabelled steroids were separated using reversed phase HPLC, Agilent 1200 HPLC using a Zorbax Eclipse XDB-C18 5 μm column, 150 x 4.6 mm (Crawford Scientific, Lanarkshire, UK) with

acetonitrile:H_2O (50:50) at flow rate of 1 ml/min. Radioactivity measured using a flow scintillation analyser (Radiomatic series 500TR, Perkin Elmer Analytical Instruments) with FLO-ONE software.

Measurement of 17β-HSD3 activity: 17β-HSD3 catalyses the conversion of androstenedione to testosterone. The assay incubation was carried out in deep well plates tubes consisting of glucose-6-phosphate (Roche Diagnostics, 1mM), NADPH (Roche Diagnostics, 100 μM), glucose-6-phosphate dehydrogenase (Roche Diagnostics, 12.5 μg/ml), EDTA (Sigma, Poole, Dorset, UK, 1 mM), assay buffer (K_2HPO_4/KH_2PO_4, 100 mM) pH 7.5, recombinant 17β-HSD3 (1.5 μg/ml) plus test compound containing 1 μCi ^{3}H androstenedione (Perkin Elmer). The compounds were incubated for 90 minutes at 37 °C and the reaction stopped by the addition of 1 mM glycerrhetinic acid. Radiolabelled steroids were separated using reversed phase HPLC, Agilent 1200 HPLC using a Zorbax Eclipse XDB-C18 5 μm column, 150 x 4.6 mm (Crawford Scientific, Lanarkshire, UK) with acetonitrile:H_2O (35:65) at flow rate of 2 ml/min. Radioactivity measured using a flow scintillation analyser (Radiomatic series 500TR, Perkin Elmer Analytical Instruments) with FLO-ONE software.

References:

1 Napolitano, A.; Voice, M. W.; Edwards, C. R. W.; Seckl, J. R.; Chapman, K. E. 11β-hydroxysteroid dehydrogenase 1 in adipocytes: Expression is differentiation-dependent and hormonally regulated. *J. Steroid Biochem. Mol. Biol.* **1998**, *64,* 251-260.

Analysis protocol for acyl-glucuronide quantification

All animal experiments were performed in compliance with the relevant laws and institutional guidelines.

Blood samples were stored at -20 °C prior to analysis. Blood samples (50 μL) were protein precipitated with ice cold acetonitrile (Sigma-Aldrich) containing an internal standard. The precipitation solvent was acidified with 0.1% formic acid (Sigma-Aldrich) to enhance the stability of any acyl-glucuronide metabolites that may be present in the sample. The precipitated samples were then mixed and centrifuged at 4500 g for 10 mins. 50 μL of the resulting supernatant was removed and diluted with 300 μL of water prior to injection (50 μL) onto the LC-MS/MS system for analyte and acyl-glucuronide quantification.

Extracted samples were analysed on a TSQ Quantum Vantage mass spectrometer (ThermoFisher Scientific, Hemel Hempstead, UK). Parent and product masses were auto-tuned into the mass spectrometer using QuickQuan™ (version 2.4) and Xcalibur (verison 2.1) software (ThermoFisher Scientific, San Jose, California, USA). The capillary temperature was 270 °C, the vaporizer temperature was 300 °C, the sheath gas and auxillary gas flows were set at 60 and 20 arbitary units repectively.

A Surveyor MS Pump Plus HPLC pump, (Thermo Fisher Scientific, Hemel Hempstead, UK) and a CTC Analytics HTS PAL autosampler (Presearch Ltd, Basingstoke, UK) were used to introduce the samples to the mass spectrometer. Chromatography was performed on a Max-RP (50 mm x 2.1 mm ID, 5μ) HPLC column (Phenomenex, Macclesfield, UK) with mobile phase consisting of eluent (A) water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid. The linear gradient used was (T=minutes): at T=0.0, 95%A:5%B, T=3.0, 5%A:95%B, T=4.0, 5%A:95%B, T=4.1, 95%A:5%B, T=5.0, 95%A:5%B. The flow rate used was 750 μL/min.

Subsequent to analysis the data was quantified using QuickCalc™ software (Gubbs Inc, Alpharetta, Georgia, USA) by back calculation against the relevant calibration curve. A 1/x weighting was applied to the calibration curves which were constructed using a quadratic fit.

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Procedures for determination of physicochemical properties:

$logD_{7.4}$, plasma-protein binding and solubility measurements were made as described in; Buttar, D.; Colclough, N.; Gerhardt, S.; MacFaul, P. A.; Phillips, S. D.; Plowright, A.; Whittamore, P.; Tam, K.; Maskos, K.; Steinbacher, S.; Steuber, H. A. Combined spectroscopic and crystallographic approach to probing drug–human serum albumin interactions. *Bioorg. Med. Chem.* **2010**, *18,* 7486-7496.

$logD_{7.4}$:

$LogD_{7.4}$ measurements were made using a shake-flask method where the extent of partitioning between pH 7.4 buffer and octanol was measured. Compounds were dissolved in a known volume buffer, and following the addition of a known amount of octanol, the solutions were shaken for 30 min. Following centrifugation, analysis of the aqueous layer was performed by LC–UV to quantify the amount of compound in solution and then compared to analysis of the compound in solution before the addition of octanol to calculate the partitioning coefficient, $D_{7.4}$.

Solubility:

Assessments of aqueous solubility were made after an incubation of 24 h in pH 7.4 phosphate buffer. After centrifugation, analysis of the supernatant liquid was performed by LC–UV to quantify the amount of compound in solution.

Protein binding strength *via* equilibrium dialysis:

Dialysis membranes (Spectra/Por 2, 12–14 kDa molecular weight cut-off, 47 mm diameter, Spectrum Laboratories) were prepared for use by washing with distiled water and subsequent soaking in phosphate buffer (pH 7.4). Membranes were then blotted dry and placed between two 1 mL Teflon dialysis half-cells (Braun ScienceTec, Les Ulis, France). Each half-cell was filled individually with 1 mL of protein solution containing the compound of interest, while the corresponding half-cell was filled with 1 mL of isotonic phosphate buffer. Dialysis units were immersed in a 37 °C temperature-controlled water bath and rotated at 30 rpm for 18–19 h using a Dianorm apparatus (Braun ScienceTec). After this period, samples from both the half-cell containing buffer (protein free) and the half-cell containing protein were submitted for HPLC analysis using an Agilent 1100 series HPLC with a 110 binary pump and a UV diode ray detector. Acquisition and integration were carried out using Chemstation software (Agilent Technologies) version A.06.03 with relevant customised macro software. Integration of the subsequent chromatograms, are used to calculate the concentration of drug in the protein containing solution (Dp) and in the protein-free solutions (Df), which are then used to derive the binding constant for the test compound (K_1) assuming a 1:1 binding model as shown in Eq. 1 where the compound can only bind to a single site on the protein molecule. This is expressed mathematically in Eq. 2 where D and Df are the total and free drug concentrations, respectively, and Pr is the total protein concentration.

$$\mathrm{D} + \mathrm{P} \overset{K_1}{\rightleftharpoons} \mathrm{DP} \qquad \text{Eq. 1}$$

$$D = (D_\mathrm{f} + D_\mathrm{p}) = \frac{K_1 \cdot D_\mathrm{f} \cdot \mathrm{Pr}}{1 + K_1 \cdot D_\mathrm{f}} + D_\mathrm{f} \qquad \text{Eq. 2}$$