Synthesis and Biological Evaluation of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) Inhibitors Based on a Thieno[2,3-d]pyrimidin-4(3*H*)-one Core

Annamaria Lilienkampf, [†] *Sampo Karkola,* [†] *Sari Alho-Richmond,* [†] *Pasi Koskimies,* [‡] *Nina Johansson,* [‡] *Kaisa Huhtinen,* [‡] *Kimmo Vihko,* [‡] *and Kristiina Wähälä*[†,]***

[†]Laboratory of Organic Chemistry, Department of Chemistry, P.O. Box 55, FIN-00014 University of Helsinki, Finland, [‡]Hormos Medical Ltd., PharmaCity, FIN-20520 Turku, Finland

Supporting Information

1. Synthesis of compounds 3a–3e and 15a–15b

General procedure[1] for the synthesis of 3a–3e and 15a–15b: $POCl_3$ (2.9 mL, 31 mmol) was added dropwise to **2a** (5.0 g, 21 mmol) and ε-caprolactam (3.5 g, 31 mmol) in anhydrous 1,2-dichloroethane (100 mL). The mixture was heated under reflux until no starting material was detected on TLC (1-2 h, 10% EtOAc–CH_2Cl_2), after which 4/5 of the solvent was evaporated. Water (100 mL) was added and the solution was made basic with 20% KOH. The solution was extracted with of CH_2Cl_2 (3 x 20 mL), washed with of brine (20 mL) and water (20 mL), and dried with Na_2SO_4. After filtration the solvent was evaporated. Recrystallization from EtOH afforded **3c** in 90% yield. The analytical data of compounds **3a–3d** and **15b** corresponded to the data published previously.[1]

1,2,3,4,5,8,9,10,11,12-Decahydro-14H-cyclohepta[4',5']thieno[2',3':4,5]pyrimido[1,2-a]azepin-14-one (3e). Was synthesized using **2b** and ε-caprolactam as starting materials. Yield 85%, white crystals; mp 154 °C (EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.69 (m, 4H), 1.77 (m, 2H), 1.83 (m, 4H), 1.88 (m, 2H), 2.82 (m, 2H), 3.01 (m, 2H), 3.34 (m, 2H), 4.35 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 25.3, 27.3, 27.74, 27.78, 27.79, 29.6, 29.9, 32.6, 37.4, 42.1, 120.8, 136.9, 137.1, 158.8, 159.0, 160.4; HRMS (ESI) calculated for $C_{16}H_{21}N_2OS$ $[M + H]^+$ 289.1369, found 289.1357

5,6,7,8-Tetrahydro-3-methyl-[1]benzothieno[2,3-d]pyrimidin-4(*3H*)-one (15a). Was synthesized using **2a** and acetamide as starting materials. The crude product was purified by flash chromatography using CH_2Cl_2–EtOAc (1:1) as an eluent. Yield 74%; mp 72 °C (EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.86 (m, 4H), 2.78 (m, 2H), 3.02 (m, 2H), 3.55 (s, 3H), 7.90 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.3, 22.9, 25.3, 25.7, 33.7, 122.7, 131.5, 134.2, 145.7, 158.4, 162.3; MS (EI) *m/z* 220

[1] Lilienkampf, A.; Heikkinen, S.; Mutikainen, I.; Wähälä, K. Synthesis of isomeric enamine derivatives of fused cycloalkeno thieno[2,3-d]pyrimidin-4(3*H*)-ones. Stereoelectronic effect on the regioselectivity. *Synthesis,* **2007**, *17*, 2699-2705

2. Synthesis of compounds 11–14

4(-Chloroacetyl)morpholine (11). Chloroacetyl chloride (7.5 mL, 100 mmol), in 50 mL of dry ether, was added dropwise to morpholine (22 mL, 200 mmol) in 250 mL of dry ether at -20 °C. The reaction mixture was allowed to reach room temperature and stirred for 30 minutes. The mixture was filtered and the filtrate was evaporated. The crude product, which was isolated as light brown oil, was purified by distillation in vacuo (~ 1 mbar) to give compound **11** as viscose oil in 67% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49-3.53 (m, 2H), 3.60-3.73 (m, 6H), 4.04 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 40.6, 42.5, 46.8. 66.5, 66.7, 165.3; EI-MS (*m/z*) 163

Carbonodithioic Acid *O*-ethyl S-[2-(4-morpholinyl)-2-oxoethyl] Ester (12). 4(-Chloroacetyl)morpholine **11** (1.0 g, 6.1 mmol) and potassium ethyldithiocarbonate (1.5 g, 9.4 mmol) in dry DMF (15 mL) were stirred under argon in room temperature for two hours. The reaction was quenched with water (30 mL) and the solution was extracted with CH_2Cl_2 (3 x 10 mL). The organic phase was washed exhaustively with water and dried with Na_2SO_4. Filtration and evaporation of the solvent gave compound **12** as white solid in 90 % yield. Compound **12** was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, 3H, *J* = 7.1 Hz), 3.56-3.74 (m, 6H), 4.09 (s, 2H), 4.65 (q, 2H, *J* = 7.1 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) 13.9, 39.2, 42.8, 46.8, 66.8, 66.9, 70.9, 165.5, 213.8; EI-MS (*m/z*) 259

4(-Mercaptoacetyl)morpholine (13). 1,3-Diaminopropane (0.17 mL, 2.07 mmol) was added dropwise under argon at 0 °C to **12** (0.43 g, 1.72 mmol) in dry THF (20 mL). The reaction mixture was stirred for 30 min and $CHCl_3$ (40 mL) was added. The solution was washed successively with 20 mL of 0.5 M H_2SO_4, brine and water, and dried with Na_2SO_4. After filtration the solvent was evaporated and the crude product was purified by flash chromatography using 10% MeOH-CH_2Cl_2 as an eluent. Compound **13** was obtained as colorless oil in 67% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09 (t, 1H, *J* = 7.8 Hz), 3.33 (d, 2H, *J* = 7.6 Hz), 3.47-3.50 (m, 2H), 3.61-3.73 (m, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 25.9, 42.5, 46.7, 66.6, 66.8, 168.7; ESI-MS $[M + H]^+$ 162

4-[(Ethylthio)acetyl]morpholine (14). 4(-Chloroacetyl)morpholine **11** (2.95 g, 0.02 mmol) and potassium ethyldithiocarbonate (4 g, 0.025 mmol) in abs. EtOH (35 mL) under argon were refluxed for 6 hours. After cooling to room temperature, 35 mL of NH_3 (aq.) was added and the reaction mixture was stirred overnight. The solution was extracted with CH_2Cl_2 (3 x 20 mL) and the organic phase was dried with Na_2SO_4. The solution was filtered and the solvent was evaporated. Distillation under reduced pressure (~ 1 mbar) gave compound **14** as colorless viscose oil in 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H, *J* = 7.3Hz), 2.65 (q, 2H, *J* = 7.5Hz), 3.31 (s, 2H), 3.49-3.52 (m, 2H), 3.61-3.72 (m, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.4, 26.3, 32.7, 42.1, 46.8, 66.6, 66.8, 168.0; EI-MS (*m/z*) 189

3. Synthesis of compounds 21, 22, and 24–26

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(propylthio)-[1]Benzothieno[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde 3-oxime (21). NaOAc (44 mg, 0.53 mmol) was added at 0 °C to $NH_2OH{\cdot}HCl$ (37 mg, 0.53 mmol) in EtOH (3 mL). After 5 minutes compound **6b** (100 mg, 0.27 mmol) in THF (16 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. Water (150 mL) was added and the formed precipitate was filtered. Recrystallization from EtOH afforded **21** in 83% yield as a pale yellow powder; mp 227 °C (dec.); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, 3H, *J* = 7.3 Hz), 1.59 (apparent sext, 2H, *J* = 7.3 Hz), 1.82 (m, 6H), 2.74 (t, 2H, *J* = 7.3 Hz), 2.84 (m, 2H), 3.05 (m, 2H), 3.22 (m, 2H), 4.36 (m, 2H), 7.80 (broad s, 1H), 8.77 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.4, 22.2, 23.2, 24.4, 25.3, 27.8, 29.7, 37.6, 38.2, 42.4, 121.1, 129.7, 133.9, 135.7, 136.9, 150.5, 158.8, 160.2, 163.5; HRMS (ESI) calculated for $C_{19}H_{24}N_3O_2S_2$ $[M+H]^+$ 390.1304, found 390.1312.

4-Chloro-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde 3-oxime (22). NaOAc (240 mg, 2.9 mmol) was added at 0 °C to $NH_2OH{\cdot}HCl$ (200 mg, 2.9 mmol) in EtOH (20 mL). After 5 minutes, compound **5b** in THF (30 mL) was added and the reaction mixture was stirred

at room temperature for 2 hours. Water (350 mL) was added and the formed precipitate was filtered. Recrystallization from EtOH afforded **22** in 75% yield as a pale yellow powder; ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.61-1.80 (m, 6H), 2.82 (m, 2H), 3.02 (m, 2H), 3.13 (m, 2H), 4.29 (m, 2H), 8.15 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (300 MHz, d_6-DMSO) δ 22.3, 24.5, 25.1, 27.5, 29.4, 37.2, 42.3, 120.6, 124,1, 126,7, 130.7, 135.4, 146.0, 158.4, 162.3, 163.1; HRMS (ESI) calculated for $C_{16}H_{17}N_3O_2S$ $[M+H]^+$ 350.0725, found 350.0718.

4-Chloro-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxylic acid (24). Compound **5b** (0.34 g, 1.0 mmol) and 2-methyl-2-butene (10.0 mmol, 1.1 mL) were dissolved in THF (5 mL). $NaClO_2$ (grade ~80%, 0.27 g. 0.54 mmol) and $NaH_2PO_4 \cdot H_2O$ (0.74g, 0.54 mmol) in 10 mL of *t*-BuOH-H_2O (5:1) were added to the solution and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into H_2O (100 mL), extracted with CH_2Cl_2 (3 x 30 mL), washed with brine (30 mL), and dried with Na_2SO_4. After filtration the solvent was evaporated. Recrystallization from EtOH afforded **24** in 72% yield as a beige powder. ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.71 (m, 6H), 2.79 (m, 2H), 3.06 (m, 2H), 3.14 (m, 2H), 4.31 (m, 2H), 13.07 (broad s, 1H); ^{13}C NMR (300 MHz, d_6-DMSO) δ 21.8, 24.3, 26.3, 26.6, 28.6, 36.5, 41.5, 119.6, 123.6, 127.6, 130.3, 136.1, 157.7, 162.0, 163.6, 166.2; HRMS (ESI) calculated for $C_{16}H_{16}ClN_2O_3S$ $[M+H]^+$ 351.0565, found 351.0561.

2,7,8,9,10,11-Hexahydro-3-(3-oxo-1-butenyl)-4-(propylthio)-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azepin-13(1*H*)-one (25). NaOH (1M aq. solution, 0.75 mL) was added dropwise into 1-thiopropane (33 μL, 0.36 mmol) in acetone (1 mL) at 0 °C. The solution was stirred for 5 min after which **5b** in acetone (25 mL) was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 40 min. Reaction mixture was poured into a large volume of water (~500 mL), neutralized with 0.1 M HCl and kept overnight at 5 °C. The product was filtered and purified by flash chromatography using EtOAc–CH_2Cl_2 2:8 as an eluent. Yield 65%, yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, 3H, *J* = 7.3 Hz), 1.61 (m, 2H), 1.82 (m, 6H), 2.38 (s, 3H), 2.72 (m, 2H), 2.79 (t, 2H, *J* = 7.1 Hz), 3.05 (m, 2H), 3.26 (m, 2H), 4.37 (m, 2H), 6.34 (d, 1H, *J* = 16.1 Hz), 8.28 (d, 1H, *J* =

16.1 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.3, 22.4, 23.1, 25.2, 25.4, 27.2, 27.7, 29.6, 37.6, 38.3, 42.3, 120.9, 127.9, 133.1, 134.3, 137.1, 137.7, 140.9, 158.7, 160.4, 164.0, 198.9; HRMS (ESI) calculated for $C_{22}H_{27}N_2O_2S_2$ $[M+H]^+$ 415.1508, found 415.1509.

2-[(2,2-Dimethyl-1-oxopropyl)amino]-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic Acid Ethyl Ester (26). Pivaloyl chloride (3.83 mL, 31 mmol) was added dropwise to **2a** (5.0 g, 22 mmol) and Et_3N (4.3 mL, 31 mmol) in CH_2Cl_2 (50 mL). After stirring at room temperature for 3 hours, the reaction mixture was quenched with water, extracted with CH_2Cl_2 (3 x 20 mL), and washed consecutively with 20 mL of 0.1 M HCl, 1 M NaOH and water. The organic phase was dried with Na_2SO_4, filtrated and evaporated. Crystallization from EtOH afforded **26** as pale yellow crystals in 92% yield, mp 99°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 1.38 (t, 3H, *J* = 7.2 Hz), 1.79 (m, 4H), 2.64 (m, 2H), 2.77 (m, 2H), 4.34 (q, 2H, *J* = 7.2 Hz), 11.63 (broad s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.5, 23.1, 23.2, 24.5, 26.6, 27.5, 39.4, 60.5, 111.6, 126.7, 130.9, 148.3, 166.97, 176.0; HRMS (ESI) calculated for $C_{16}H_{24}NO_3S$ $[M + H]^+$ 310.1471, found 310.1481

4. Characterization of compounds 23 and 27–29

4-Chloro-2,7,8,9,10,11-hexahydro-3-(hydroxymethyl)-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azepin-13(1*H*)-one (23). Synthesized from **5b** and recrystallisized from EtOAc. Yield 78%; mp 160 °C; ^{1}H NMR (500 MHz, d_6-DMSO) δ 1.67 (m, 2H), 1.75 (m, 4H), 2.63 (m, 2H), 3.03 (m, 2H), 3.10 (m, 2H), 4.22 (s, 2H), 4.30 (m, 2H), -*OH* exchanged; ^{13}C NMR (300 MHz, d_6-DMSO) δ 21.8, 24.5, 26.1, 26.8, 28.7, 36.5, 41.5, 60.1, 117.1, 119.8, 130.1, 131.6, 135.1, 157.7, 160.8, 161.6; HRMS (ESI) calculated for $C_{16}H_{18}ClN_2O_2S$ $[M+H]^+$ 337.0772, found 337. 0788.

2-[(2,2-Dimethyl-1-oxopropyl)amino]-4,5,6,7-tetrahydro-7-oxo-benzo[b]thiophene-3-carboxylic acid Ethyl Ester (27). Yield 80%, white crystals; mp 121 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 9H), 1.43 (t, 3H, *J* =

7.2 Hz), 2.16 (m, 2H), 2.56 (m, 2H), 3.08 (m, 2H), 4.40 (q, 2H, *J* = 7.2 Hz), 11.92 (broad s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.4, 23.9, 26.6, 27.2, 37.7, 39.6, 61.2, 112.0, 127.5, 150.0, 156.7, 166.4, 176.6, 192.4; HRMS (ESI) calculated for $C_{16}H_{22}NO_4S$ $[M+H]^+$ 324.1264, found 324.1268.

7-Chloro-2-[(2,2-dimethyl-1-oxopropyl)amino]-6-formyl-4,5-dihydro-benzo[b]thiophene-3-carboxylic acid ethyl ester (28). Purified by recrystallization from EtOH. Yield 92%, yellow crystals; mp 184 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.43 (t, 3H, *J* = 7.2 Hz), 2.74 (m, 2H), 3.09 (m, 2H), 4.41 (quin, 2H, *J* = 7.2 Hz), 10.15 (s, 1H), 11.85 (broad s, 1H)**;** ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.8, 21.5, 23.0, 26.7, 38.9, 60.9, 112.2, 125.5, 125.6, 140.4, 140.5, 153.0, 165.7, 176.1, 187.9**;** HRMS (ESI) calculated for $C_{17}H_{21}ClNO_4S_1$ $[M+H]^+$ 370.0874, found 370.0861.

2-[(2,2-Dimethyl-1-oxopropyl)amino]-6-formyl-4,5-dihydro-7-(thiopropyl)-benzo[b]thiophene-3-carboxylic acid ethyl ester (29). Yield 73%, yellow powder; mp 104 °C (EtOH)**;** ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, 3H, *J* = 7.3 Hz), 1.37 (s, 9H), 1.42 (t, 3H, *J* = 7.2 Hz), 1.61 (m, 2H), 2.69 (m, 2H), 2.85 (triplet like m, 2H), 3.02 (m, 2H), 4.40 (q, 2H, *J* = 7.2 Hz), 10.43 (s, 1H), 11.83 (broad s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.2, 14.4, 22.2, 23.2, 23.5, 27.3, 38.7, 39.4, 61.2, 112.9, 130.0, 135.6, 139.0, 144.3, 153.2, 166.4, 176.4, 190.7; HRMS (ESI) calculated for $C_{20}H_{28}NO_4S_2$ $[M+H]^+$ 410.1454, found 410.1453.

5. ^{13}C NMR data for 1, 6a–6i, 7a–7j, 8–10, and 18

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(phenylthio)-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (1). ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.26, 22.36, 25.0, 27.6, 29.5, 37.6, 42.3, 120.2, 127.4, 129.4, 129.5, 133.8, 134.4, 138.7, 139.2, 141.3, 158.5, 161.3, 165.5, 190.7

4-(Ethylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]-pyrimido[1,2-a]azepine-3-carboxaldehyde (6a). ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.9, 22.1, 22.3, 25.1, 27.6, 29.5, 30.6, 37.6, 42.4, 120.8, 135.4, 138.3, 138.7, 142.6, 158.6, 161.3, 165.3, 191.0

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(propylthio)-[1]benzothieno[2',3':4,5]-pyrimido[1,2-a]azepine-3-carboxaldehyde (6b). Yield ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.3, 22.3, 22.4, 23.3, 25.2, 27.7, 29.7, 37.8, 38.7, 42.5, 121.0, 135.5, 138.46, 138.48, 143.4, 158.7, 161.4, 165.4, 191.1

4-(Butylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]-pyrimido[1,2-a]azepine-3-carboxaldehyde (6c). Yield ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.8, 21.9, 22.4, 22.5, 25.4, 27.9, 29.8, 32.0, 36.6, 37.9, 42.6, 121.1, 135.7, 138.5, 138.6, 143.6, 158.8, 161.5, 165.5, 191.2

4-[(1-Methylethyl)thio)]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido-[1,2-a]azepine-3-carboxaldehyde (6d). ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.0, 22.3, 23.1, 25.1, 27.6, 29.5, 37.6, 40.9, 42.3, 120.8, 136.1, 138.1, 138.8, 142.8, 158.5, 161.2, 165.3, 191.2

4-[(1,1-Dimethylethyl)thio)]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (6e). ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.0, 22.1, 25.1, 27.6, 29.5, 31.5, 37.6, 42.3, 50.0, 120.7, 137.3, 139.2, 140.9, 141.3, 158.6, 161.1, 165.0, 192.3

4-(Cyclopentylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]-pyrimido[1,2-a]azepine-3-carboxaldehyde (6f). ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.0, 22.3, 24.3, 25.1, 27.6, 29.5, 33.3, 37.6, 42.3, 49.3, 120.8, 136.0, 138.1, 138.4, 143.6, 158.5, 161.2, 165.3, 191.1

4-(Cyclohexylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]-pyrimido[1,2-a]azepine-3-carboxaldehyde (6g). ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.0, 22.3, 25.1, 25.5, 25.9, 27.6, 29.5, 33.4, 37.6, 42.3, 49.0, 120.9, 136.4, 138.0, 138.8, 142.4, 158.6, 161.2, 165.2, 191.3

4-[(3-Formyl)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azepinyl)thio]-propionic acid (6h). ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.16, 22.18, 25.0, 27.5, 29.5, 30.7, 34.1, 37.5, 42.4, 120.9, 134.8, 138.7, 139.0, 141.9, 158.5, 161.5, 165.2, 174.8, 190.7

4-[2-(4-Morpholinyl)-2-oxoethylthio]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (6i). ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.17, 22.19, 25.1, 27.6, 29.5, 37.2, 37.6, 42.2, 42.4, 46.7, 66.5, 66.7, 121.0, 134.4, 138.9, 139.6, 140.6, 158.5, 161.5, 165.3, 165.8, 190.35, 190.37

4-(4-Methylphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7a). ^{13}C NMR (500 MHz, $CDCl_3$) δ 21.1, 22.2, 22.3, 25.0, 27.6, 29.5, 37.6, 42.3, 120.1, 129.9, 130.1, 130.3, 134.5, 137.7, 138.3, 139.2, 142.0, 158.5, 161.2, 165.5, 190.8

4-(4-Fluorophenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7b). ^{13}C NMR ($CDCl_3$) δ 22.2, 22.3, 25.0, 27.5, 29.5, 37.6, 42.4, 116.8 (d, $^{o}J_{CF}$ = 22.6 Hz), 120.1, 128.7, 131.9 (d, $^{m}J_{CF}$ = 8.6 Hz), 134.0, 138.5, 139.4, 141.5, 158.4, 161.4, 162.4 (d, $^{p}J_{CF}$ = 248.4 Hz), 165.4, 190.5

4-[(3-Formyl-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azepin-4-yl)thio]benzoic acid (7c). ^{13}C NMR (300 MHz, d_6-DMSO) δ 21.7, 22.0, 24.2, 26.6, 28.7, 36.6, 41.6, 119.6, 127.9, 129.1, 130.3, 132.3, 138.0, 139.1, 139.2, 139.7, 157.6, 162.4, 164.7, 166.6, 189.7

4-[4-Methoxyphenylthio]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7d). ^{3}C NMR (300 MHz, $CDCl_3$) δ 22.2, 22.3, 25.0, 27.6, 29.5, 37.6, 42.3, 55.3, 115.2, 119.9, 123.7, 132.5, 134.2, 137.7, 139.3, 142.8, 158.5, 159.6, 161.2, 165.5, 190.7

4-(4-Hydroxyphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7e). ^{13}C NMR (300 MHz, d_6-DMSO) δ 21.7, 21.9, 24.3, 26.7, 28.7, 36.6, 41.5, 116.7, 119.1, 120.8, 132.4, 132.7, 136.8, 138.9, 141.7, 157.6, 157.8, 162.2, 164.7, 189.7

4-(3-Hydroxyphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7f). ^{13}C NMR (300MHz, d_6-DMSO) δ 21.8, 21.9, 24.3, 26.6, 28.7, 36.6, 41.5, 114.6, 115.2, 119.38, 119.43, 130.5, 132.9, 134.3, 138.2, 138.8, 139.6, 157.6, 158.1, 162.3, 164.7, 189.8

4-(2-Hydroxyphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7g). ^{13}C NMR (300 MHz, d_6-DMSO) δ 21.81, 21.83, 24.3, 26.7, 28.7, 36.6, 41.5, 115.5, 119.0, 119.4, 120.1, 128.8, 130.5, 132.9, 137.4, 138.5, 140.4, 155.7, 157.6, 162.2, 164.6, 189.9

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(4-pyridylthio)-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7h). ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.3, 22.5, 25.1, 27.6, 29.6, 37.7, 42.5, 120.6, 121.7, 133.8, 137.3, 139.6, 140.4, 146.0, 150.1, 158.5, 161.8, 165.8, 190.0

4-(Benzylthio)-1,2,7,8,9,10,11,13-Octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7i). ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.8, 22.2, 25.1, 27.6, 29.5, 37.6, 40.4, 42.4, 120.9, 127.8, 128.6, 128.9, 135.5, 136.3, 138.3, 140.0, 140.7, 158.8, 161.3, 165.3, 190.5

4-(2-Naphtylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2',3':4,5]pyrimido[1,2-a]azepine-3-carboxaldehyde (7j). ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.3, 22.4, 25.0, 27.5, 29.5, 37.5, 42.3, 120.1, 126.5, 126.7, 126.9, 127.4, 127.8, 128.7, 129.3, 131.0, 132.3, 133.7, 134.4, 138.7, 139.3, 141.4, 158.5, 161.2, 165.5, 190.7

1,2,3,8,9-Hexahydro-10-oxo-6-(propylthio)-[1]benzothieno[2,3-d]pyrrolo[1,2-a]pyrimidine-7-carboxaldehyde (8). ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.3, 19.7, 22.2, 22.4, 23.2, 32.6, 38.8, 46.6, 121.3, 135.3, 137.8, 138.4, 143.3, 157.8, 161.1, 167.3, 191.0

2,3,8,9,10,11,12,14-Octatahydro-14-oxo-5-(propylthio)-1H-cyclohepta-[4',5']thieno[2',3':4,5]pyrimido[1,2-a]azepine-4-carboxaldehyde (9). ^{13}C NMR (500 MHz, $CDCl_3$) δ 13.2, 23.5, 25.1, 25.3, 26.2, 27.6, 29.6, 37.1, 37.2, 37.6, 42.4, 121.1, 135.1, 144.1, 145.2, 148.6, 158.7, 161.2, 165.5, 189.7

1,2,7,9,10,11,12,14-Octahydro-14-oxo-4-(propylthio)-8H-[1]benzothieno[2',3':4,5]pyrimido[1,2-a]azocine-3-carboxaldehyde (10). ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.2, 22.1, 22.3, 23.1, 24.2, 26.1, 28.7, 30.6, 35.8, 38.5, 42.8, 121.0, 135.2, 138.2, 138.3, 143.3, 158.5, 160.8, 165.8, 191.0

3,4,5,6-Tetrahydro-3-methyl-4-oxo-8-(propylthio)-[1]benzothieno[2,3-d]pyrimidine-7-carboxaldehyde (18). ^{13}C NMR (500 MHz, $CDCl_3$) δ 13.2, 22.1, 22.3, 23.2, 33.9, 38.7, 123.1, 136.9, 137.7, 138.9, 142.8, 147.2, 158.2, 165.6, 191.0

6. HPLC purity determinations

HPLC Method A. Purity analysis was performed using Waters 990 HPLC instrument. Isocratic eluation with 30% CH_2Cl_2 and 70% EtOAc; column Merck Lichrospher Si-60, 5 μm, 250-4, with corresponding precolumn; flow rate 1 ml/min, run time 20 min; detection: Waters 996 diode array detector, UV 254 nm.

HPLC Method B. Purity analysis was performed using Hewlett Packard 1100 HPLC instrument. Isocratic eluation with 30% CH_2Cl_2, 65% EtOAc, and 5% MeOH; column Supelco Lichrospher Si-60, 5 μm, 250mm x 4.6 mm, with corresponding precolumn; flow rate 1 ml/min, run time 20 min. detection: diode array detector, UV 254 nm.

HPLC Method C. Agilent 1100 HPLC system with a Synergi 4 μ Hydro-RP 80A column, with detection at 254 nm on a variable wavelength detector G1314A; flow rate = 1.4 mL/min; gradient eluation over 20 minutes, from 10% MeOH–H_2O to 100% MeOH with 0.05% TFA. Sample dissolved into DMF.

HPLC Method D. As method C, except gradient eluation from 30% MeOH–H_2O to 100% MeOH with 0.05% TFA.

Table 1. The HPLC purity of the key target compounds.

Compound	Purity (%)	Retention time	Method
1	99.2	3.97	A
6a	99.5	4.88	B
6b	100.0	4.31	B
6c	98.3	4.11	B
6d	98.6	4.40	B
6e	99.5	4.60	B
6f	98.0	3.99	A
6g	99.0	3.80	A
6h	95.9	11.37	D
6i	95.9	13.92	C
7a	97.7	4.01	B
7b	99.3	4.16	B
7c	95.5	12.48	D
7d	98.9	4.79	B
7e	99.1	3.90	B
7f	97.0	16.86	C
7g	100.0	3.87	B
7h	98.9	11.97	C
7i	98.3	4.00	A
7j	99.3	3.91	B
8	100.0	15.16	A
9	97.8	13.44	D
10	99.0	4.13	A
18	98.6	7.05	B
19a	100.0	3.81	A
19b	100.0	3.76	A
20a	99.3	6.27	A

20b	99.6	6.07	A
21	98.4	3.70	B
25	99.1	3.95	B

7. ERα and ERβ binding analysis

Estrogen Receptor (ER) α and β Competitor Assay kits were used, according to the manufacturer's protocol (Invitrogen, Carlsbad, CA), to analyze *in vitro* binding of 17β-HSD1 active compounds to ERα or ERβ. Briefly, recombinant human ERβ (30 nM) or ERα (10 nM) were incubated with 1 nM of a fluorescent ligand (Fluormone™ ES2) to form an ER/ Fluormone™ ES2 complex with high fluorescence polarization. The complex was then added to eight different concentrations of test compound in 384-well microplate and incubated in the dark at room temperature for 2 hours. The polarization values were measured using an Ultra multiwell plate reader (Tecan, Switzerland) and results were calculated with the Prism software (GraphPad). EC_{50} average values of estradiol, as a positive control, were 7 nM and 8 nM for ERα or ERβ, respectively. The concentration of the test compound that results in a half-maximal shift in polarization value equals the IC_{50} of the test compound, which is a measure of the relative affinity of the test compound for the ERα or ERβ.

Table 2. ERα and ERβ binding analysis

Compound	**ERα EC_{50} (µM)**	**EC_{50} ERβ (µM)**
1	>10	>10
5d	>10	-
6b	>1	>1
6e	>10	>1
6f	>10	>1
6g	>10	>1
7f	>1	>1
10	>1	>1
19b	>1	>1
Estrone (E2)	0.007*	0.008*

*Average values