Supporting Data

Inhibition of 17β-HSD1: SAR of Bicyclic Substituted Hydroxyphenylmethanones and Discovery of New Potent Inhibitors with Thioether Linker

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1. **HPLC Purity Control of Final Compounds**

Purity of compounds 1 to 28 was determined using LC/MS as follows:

The Surveyor®-LC-system consisted of a lamp, an autosampler, and a PDA detector. Mass spectrometry was performed on a Surveyor MSQ plus (ThermoFisher, Dreieich, Germany). The single quadrupole mass spectrometer was equipped with an electrospray interface (ESI). The system was operated by the standard software Xcalibur®.

A RP C18 NUCLEODUR® 100-5 (3 mm) column (Macherey-Nagel GmbH, Düren, Germany) was used as stationary phase. All solvents were HPLC grade.

Solvent system:

In a gradient run the percentage of acetonitrile (containing 0.1% TFA) was increased from an initial concentration of 5% at 0 min to 100% for 5 min.

The injection volume was 10µL and flow rate was set to 800 µL/min. Ms analysis was carried out with a capillary temperature of 350 °C and CID of 75 V. Spectra were acquired in positive ionization mode from 100 to 1000 m/z and 254 nm UV-mode.

In both cases the injection volume was 5 µL and flow rate was set to 350 µL/min. MS analysis was carried out at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive ionization mode from 100 to 1000 m/z and full scan UV-mode.

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2. $^1$HNMR and $^{13}$CNMR data of target compounds

*(3-Chlorophenyl)(5-phenylthiophen-2-yl)methanone (3):* purified by CC (hexane/ethyl acetate 98:2); yield: 12 % (30 mg). $^1$H NMR (CD$_3$COCD$_3$) $\delta$ 7.89-7.82 (m, 4H), 7.76 (d, $J$=4.1 Hz, 1H), 7.73-7.69 (m, 1H), 7.65-7.62 (m, 2H), 7.54-7.49 (m, 2H), 7.48 -7.43 (m, 1H); $^{13}$C NMR (CD$_3$COCD$_3$) $\delta$ 186.61, 154.12, 142.57, 140.85, 137.61, 135.04, 134.03, 132.94, 131.31, 130.45, 129.41, 128.31, 127.24, 125.77; MS (ESI): 299.80 (M+H)$^+$. 

*Methyl 3-(5-phenylthiophene-2-yl-carbonyl)benzoate (4):* purified by CC (dichloromethane/methanol 95:5); yield: 18 % (108 mg; yellow oil). $^1$H NMR (CD$_3$COCD$_3$) $\delta$ 8.47 (dt, $J$= 2.0, 1.1 Hz, 1H), 8.30-8.26 (m, 1H), 8.17-8.13 (m, 1H), 7.85-7.81 (m, 2H), 7.78-7.73 (m, 2H), 7.64 (d, $J$= 3.8 Hz, 1H), 7.53-7.49 (m, 2H), 7.48-7.42 (m, 1H), 3.94 (s, 3H); $^{13}$C NMR (CD$_3$COCD$_3$) $\delta$ 187.12, 166.67, 154.02, 142.74, 139.32, 137.41, 134.07, 133.62, 131.63, 130.44, 130.26, 130.01, 127.17, 125.62, 52.76; MS (ESI): 323.42 (M+H)$^+$. 

*N-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-benzenesulfonamide (11):* purified by CC (hexane/ethyl acetate 90:10); yield: 39 % (150 mg; white solid; mp. 136 °C). $^1$H NMR (CDCl$_3$) $\delta$ 7.60 – 7.56 (m, 2H), 7.41 – 7.37 (m, 1H), 7.34 – 7.29 (m, 1H), 7.24 (d, $J$ = 0.9 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.18 – 7.15 (m, 2H), 7.10 (t, $J$ = 8.0 Hz, 1H), 7.07 (d, $J$ = 4.0 Hz, 1H), 7.01 (ddd, $J$ = 7.7, 1.7, 1.0 Hz, 1H), 6.96 – 6.94 (m, 1H), 6.69 (ddd, $J$ = 8.2, 2.5, 0.9 Hz, 1H), 3.86 (q, $J$ = 7.0 Hz, 2H), 1.22 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 186.93, 159.43, 153.77, 141.55, 139.04, 138.89, 136.75, 136.13, 134.35, 133.25, 130.19, 129.63, 129.21, 127.21, 125.94, 125.03, 124.11, 121.92, 118.74, 115.25, 112.57, 63.64, 14.78; MS (ESI): 463.92 (M+H)$^+$. 

*N-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-benzamide (12):* purified by CC (hexane/ethyl acetate 85:15); yield: 80 % (180 mg; white solid; mp. 145-6 °C). $^1$H NMR (CDCl$_3$) $\delta$ 8.15 (br. s, 1H, NH), 8.05 (t, $J$ = 1.8 Hz, 1H), 8.00 (ddd, $J$ = 8.1, 2.2, 1.0 Hz, 1H), 7.91 – 7.85 (m, 2H), 7.66 (d, $J$ = 4.0 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.56 – 7.52 (m, 1H), 7.48 (ddd, $J$ = 9.5, 5.2, 3.1 Hz, 3H), 7.31 (dd, $J$ = 10.7, 6.0 Hz, 2H), 7.24 – 7.21 (m, 1H), 7.18 – 7.15 (m, 1H), 6.89 (ddd, $J$ = 8.2, 2.5, 0.9 Hz, 1H), 4.07 (q, $J$ = 7.0 Hz, 2H), 1.43 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 187.73, 166.22, 159.67, 153.72, 142.17, 138.98, 138.45, 136.49, 134.81, 134.74, 132.34, 130.41, 129.53, 129.11, 127.36, 125.36, 124.45, 124.20, 121.07, 119.00, 115.48, 112.74, 63.87, 15.04; MS (ESI): 428.84 (M+H)$^+$. 

*N-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-4-methoxy-benzenesulfonamide (13):* purified by CC (hexane/ethyl acetate 85:15); yield: 75 % (179 mg; white solid; mp. 162-3 °C). $^1$H NMR (CD$_3$COCD$_3$) $\delta$ 7.81 – 7.76 (m, 2H), 7.72 (ddd, $J$ = 2.2, 1.7, 0.5 Hz, 1H), 7.60 – 7.57 (m, 2H), 7.54 (d, $J$ = 4.0 Hz, 1H), 7.52 (ddd, $J$ = 8.1, 2.2, 1.4 Hz, 1H), 7.50 – 7.46 (m, 1H),
7.42 – 7.37 (m, 1H), 7.37 – 7.33 (m, 1H), 7.32 – 7.28 (m, 1H), 7.08 – 7.03 (m, 2H), 7.00 (ddd, \( J = 8.0, 2.5, 1.2 \) Hz, 1H), 4.15 (q, \( J = 7.0 \) Hz, 2H), 3.85 (s, 3H), 1.40 (t, \( J = 7.0 \) Hz, 3H); \(^{13}\)C NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 187.16, 164.15, 160.65, 153.66, 142.81, 139.69, 139.31, 136.96, 135.31, 132.29, 131.30, 130.51, 130.21, 125.57, 125.55, 125.10, 121.71, 119.35, 116.29, 115.19, 112.98, 64.30, 56.13, 15.08; MS (ESI): 493.92 (M+H).°

3-Cyano-N-(3-(5-(3-ethoxyphenyl)-thiophene-2-yl-carbonyl)phenyl)-benzenesulfonamide (14): purified by CC (hexane/ethyl acetate 85:15); yield: 46 % (180 mg; white solid; mp. 130-1 °C). \(^{1}H\) NMR (CDCl\(_3\)) \( \delta \) 8.04 – 7.99 (m, 1H), 7.92 (ddd, \( J = 8.0, 1.9, 1.1 \) Hz, 1H), 7.74 – 7.69 (m, 1H), 7.52 – 7.48 (m, 1H), 7.47 – 7.45 (m, 2H), 7.40 (ddd, \( J = 8.1, 2.1, 1.5 \) Hz, 1H), 7.39 – 7.34 (m, 1H), 7.26 – 7.22 (m, 2H), 7.15 (ddd, \( J = 7.7, 1.7, 0.9 \) Hz, 1H), 7.10 – 7.07 (m, 1H), 6.84 (ddd, \( J = 8.0, 2.5, 0.9 \) Hz, 1H), 4.00 (q, \( J = 7.0 \) Hz, 2H), 1.36 (t, \( J = 7.0 \) Hz, 3H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 187.01, 159.44, 154.27, 141.21, 140.74, 139.20, 136.42, 136.34, 136.23, 134.20, 131.16, 130.79, 130.27, 129.82, 126.56, 125.24, 124.26, 122.17, 118.77, 116.98, 115.39, 113.75, 112.58, 63.66, 14.78; MS (ESI): 488.84 (M+H).°

N-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)phenyl)-isophthalamic acid methyl ester (15): purified by CC (hexane/ethyl acetate 85:15); yield: 78 % (370 mg; white solid; mp. 138-9 °C). \(^{1}H\) NMR (CDCl\(_3\)) \( \delta \) 8.45 (dd, \( J = 6.3, 4.7 \) Hz, 2H), 8.12 (d, \( J = 7.8 \) Hz, 1H), 8.08 (dd, \( J = 7.8, 1.5 \) Hz, 1H), 7.98 (dd, \( J = 8.1, 1.2 \) Hz, 1H), 7.58 (dd, \( J = 9.3, 5.9 \) Hz, 2H), 7.49 (t, \( J = 7.8 \) Hz, 1H), 7.43 (t, \( J = 7.8 \) Hz, 1H), 7.27 – 7.21 (m, 2H), 7.15 (d, \( J = 7.7 \) Hz, 1H), 7.12 – 7.07 (m, 1H), 6.83 (dd, \( J = 8.2, 1.8 \) Hz, 1H), 4.01 (q, \( J = 7.0 \) Hz, 2H), 3.84 (s, 3H), 1.37 (t, \( J = 7.0 \) Hz, 3H). \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 187.47, 166.17, 165.02, 159.40, 153.52, 141.82, 138.67, 138.08, 136.28, 134.91, 134.42, 132.88, 132.03, 130.65, 130.14, 129.27, 129.13, 127.78, 125.26, 124.18, 124.09, 120.98, 118.72, 115.23, 114.55, 63.60, 52.42, 14.78; MS (ESI): 485.93 (M).°

(3-Methoxyphenyl)(5-phenylthiophen-2-yl)methanone (17c): purified by CC (hexane/dichloromethane 6:4 to dichloromethane pure); yield: 66 % (1021 mg; yellow oil). \(^{1}H\) NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 7.84-7.79 (m, 2H), 7.75 (d, \( J = 3.9 \) Hz, 1H), 7.52-7.46 (m, \( J = 3.9 \) Hz, 1H), 7.29 (t, \( J = 7.9 \) Hz, 1H), 7.24-7.22 (m, 1H), 7.29 (s, 3H).

(3-Hydroxyphenyl)(5-phenylthiophen-2-yl)methanethione (18): purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane : ethyl acetate 8:2); yield 14 % (68 mg). \(^{1}H\) NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 9.82 (s, 1H), 7.87-7.84 (m, 2H), 7.76 (d, \( J = 4.1 \) Hz, 1H), 7.61 (d, \( J = 3.9 \) Hz, 1H), 7.52-7.41 (m, 5H), 7.40-7.38 (m, 1H), 7.24-7.22 (m, 1H), 3.90 (s, 3H).

(3-Hydroxyphenyl)(5-phenylthiophen-2-yl)methanethione (18): purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane : ethyl acetate 8:2); yield 14 % (68 mg). \(^{1}H\) NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 9.82 (s, 1H), 7.87-7.84 (m, 2H), 7.76 (d, \( J = 4.1 \) Hz, 1H), 7.52-7.46 (m, 4H), 7.29 (t, \( J = 7.9 \) Hz, 1H), 7.12-7.09 (m, 1H), 7.08 (t, \( J = 1.9 \) Hz, 1H), 7.01 (ddd, \( J = 1.1, 2.4, 8.1 \) Hz, 1H); \(^{13}\)C NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 157.45, 157.00, 152.95, 147.30, 133.85, 132.50, 129.90, 129.55, 129.50, 129.30, 126.40, 126.15, 126.00, 119.35, 118.65, 115.30; MS (ESI): 297.15 (M+H).°

3-((5-Phenylthiophen-2-yl)methyl)phenol (19): no further purification was required; yield 49 % (65 mg). \(^{1}H\) NMR (CD\(_3\)COCD\(_3\)) \( \delta \) 8.24 (s, 1H), 7.65-7.52 (m, 2H), 7.41-7.30 (m, 2H), 7.28-7.20 (m, 2H), 7.17-7.08 (m, 1H), 6.92-6.83 (m, 1H), 6.82-6.75 (m, 2H), 6.72-6.70 (m, 1H), 4.11 (s, 4H), 3.90 (s, 3H), 1.37 (t, \( J = 7.0 \) Hz, 3H).
2-(1-(3-Methoxyphenyl)vinyl)-5-phenylthiophene (20): purified by preparative TLC (hexane/ethyl acetate 8:2); yield 5% (4.3 mg; yellow oil). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.42 (s, 1H), 7.70 - 7.63 (m, 2H), 7.42 (d, \(J= 7.8\) Hz, 1H), 7.38 (d, \(J= 3.8\) Hz, 1H), 7.31 (d, \(J= 7.8\) Hz, 1H), 7.25 (d, \(J= 8.2\) Hz, 1H), 6.97 (d, \(J= 3.8\) Hz, 1H), 6.96 - 6.94 (m, 2H), 6.89 - 6.82 (m, 1H), 5.60 (d, \(J= 0.7\) Hz, 1H), 5.25 (d, \(J= 0.7\) Hz, 1H); MS (ESI): 266.66 (M+H).\(^+\)

(5-((3-Hydroxymethyl)phenyl)thiophen-2-yl)-(3-methoxyphenyl)methanone (21a): purified by CC (hexane/ethyl acetate 7:3); yield: 73% (120 mg; brown oil). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.70 (s, 1H), 7.64 (d, \(J= 4.0\) Hz, 1H), 7.62 - 7.59 (m, 1H), 7.46 (dt, \(J= 7.5, 1.3\) Hz, 1H), 7.44 - 7.37 (m, 4H), 7.36 (d, \(J= 4.0\) Hz, 1H), 7.14 (ddd, \(J= 8.1, 2.7, 1.1\) Hz, 1H), 4.76 (d, \(J= 4.3\) Hz, 2H), 3.87 (s, 3H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 187.77, 159.63, 153.06, 142.20, 141.93, 139.32, 135.95, 133.57, 129.40, 129.35, 127.54, 125.54, 125.55, 124.72, 124.00, 121.64, 118.54, 113.73, 64.90, 55.47; MS (ESI): 325.26 (M+H).\(^+\)

(5-(4-(Hydroxymethyl)phenyl)thiophen-2-yl)-(3-methoxyphenyl)methanone (22a): purified by CC (hexane/ethyl acetate 7:3); yield: 70% (118 mg; brown oil). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.57 (d, \(J= 1.8\) Hz, 1H), 7.57 - 7.55 (m, 1H), 7.53 (d, \(J= 4.0\) Hz, 1H), 7.53 (dt, \(J= 7.6, 1.4\) Hz, 1H), 7.49 (dd, \(J= 9.5, 5.8\) Hz, 1H), 7.41 (dd, \(J= 9.5, 5.8\) Hz, 1H), 7.38 (dt, \(J= 7.6, 1.4\) Hz, 1H), 7.36 - 7.33 (m, 1H), 7.14 (ddd, \(J= 7.7, 2.5, 1.4\) Hz, 1H), 4.73 (s, 2H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 187.74, 159.63, 152.97, 142.11, 141.93, 139.32, 135.98, 132.59, 129.40, 127.59, 126.47, 123.83, 121.63, 118.52, 113.72, 64.79, 55.47; MS (ESI): 311.56 (M+H).\(^+\)

(3-(Benzyloxy)phenyl)(5-(3-hydroxyphenyl)thiophen-2-yl)methanone (23b): purified by column chromatography (hexane/ethyl acetate 7:3); yield: 81% (2.7 g; yellow solid, mp. 142.0 °C). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.63 (s, 1H), 7.60 (t, \(J= 4.0\) Hz, 1H), 7.52 - 7.46 (m, 6H), 7.41 (t, \(J= 7.8\) Hz, 1H); MS (ESI): 311.24 (M+H).\(^+\).

\(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 158.50, 145.05, 143.40, 142.95, 135.50, 130.45, 129.85, 128.10, 126.10, 123.80, 120.65, 116.45, 116.35, 114.35, 36.70; MS (ESI): 278.56 (M+H).\(^+\).

\(^{13}\)C NMR (CD\(_3\)COCD\(_3\)) \(\delta\) 158.50, 145.05, 143.40, 142.95, 135.50, 130.45, 129.85, 128.10, 127.30, 126.10, 123.80, 126.65, 116.45, 116.35, 114.35, 36.70; MS (ESI): 278.56 (M+H).\(^+\).
(3-(Benzyloxy)phenyl)(5-(3-isopropoxyphenyl)thiophen-2-yl)methanone (23a): recrystallized from hexane/acetone (9:1); yield: 90 % (400 mg, white solid, mp. 98 °C). \(^1\)H NMR (CD\(_3\)COCD\(_3\)) \(\delta 7.61\) (d, \(J= 4.0\) Hz, 1H), 7.57 (d, \(J= 4.0\) Hz, 1H), 7.52-7.46 (m, 5H), 7.43-7.30 (m, 7H), 7.01 (dd, \(J= 1.2, 2.4, 7.9\) Hz, 1H), 5.25 (s, 2H), 4.74 (sept, \(J= 6.7\) Hz, 1H), 1.34 (d, \(J= 6.0\) Hz, 6H); \(^{13}\)C NMR (CD\(_3\)COCD\(_3\)) \(\delta 186.71, 160.94, 158.51, 153.32, 143.22, 140.35, 138.11, 136.92, 135.43, 131.37, 130.62, 129.45, 128.81, 128.50, 125.64, 122.33, 120.22, 119.47, 116.38, 115.55, 113.04, 75.10, 29.14, 19.52; MS (ESI): 442.70 (M+H)+.

(3-Hydroxyphenyl)(5-(3-isobutoxyphenyl)thiophen-2-yl)methanone (24a): recrystallized in hexane/acetone (9:1); yield: 44 % (152 mg, white solid, mp. 94 °C). \(^1\)H NMR (CD\(_3\)COCD\(_3\)) \(\delta 7.62\) (d, \(J= 4.0\) Hz, 1H), 7.58 (d, \(J= 4.0\) Hz, 1H), 7.52-7.46 (m, 5H), 7.43-7.30 (m, 7H), 7.01 (dd, \(J= 1.2, 2.4, 7.9\) Hz, 1H), 5.25 (s, 2H), 3.87 (d, \(J= 6.4\) Hz, 2H), 2.10 (m, 1H), 1.05 (d, \(J= 6.7\) Hz, 6H); \(^{13}\)C NMR (CD\(_3\)COCD\(_3\)) \(\delta 187.61, 160.94, 159.70, 153.42, 143.01, 140.34, 138.11, 136.92, 135.43, 131.37, 130.62, 129.45, 128.81, 128.50, 125.64, 122.33, 120.22, 119.47, 116.38, 115.55, 113.04, 75.10, 70.72, 29.13, 19.57; MS (ESI): 442.70 (M+H)+.

(3-Hydroxyphenyl)(5-(3-hydroxyphenyl)sulfanylthiophen-2-yl)methanone (25): purified by CC (Dichloromethane/methanol 99.5:0.5); yield 51 % (140 mg; yellow oil). \(^1\)H NMR (CD\(_3\)OD) \(\delta 7.60\) (d, \(J= 4.0\) Hz, 1H), 7.34 (t, \(J= 7.9\) Hz, 1H), 7.29 – 7.25 (m, 1H), 7.22 – 7.17 (m, 3H), 7.05 (ddd, \(J= 8.1, 2.5, 1.1\) Hz, 1H), 6.91 (ddd, \(J= 7.7, 1.7, 0.9\) Hz, 1H), 6.88 – 6.85 (m, 1H), 6.77 (ddd, \(J= 8.2, 2.4, 0.9\) Hz, 1H); \(^{13}\)C NMR (CD\(_3\)OD) \(\delta 188.97, 159.59, 158.92, 147.64, 146.28, 140.15, 137.06, 137.00, 133.34, 131.50, 130.78, 122.64, 121.32, 120.80, 118.28, 116.50, 116.49; MS (ESI): 328.92 (M)+.
(5-Bromothiophen-2-yl)(4-methoxyphenyl)methanone (27b): purified by CC (hexane/ethyl acetate 98:2); yield: 82% (1.5 g; yellow powder; mp. 103-104 °C). $^1$H NMR (CDCl$_3$) δ 8.13 – 8.09 (m, 2H), 7.75 (d, $J = 3.9$ Hz, 1H), 7.45 (d, $J = 3.9$ Hz, 1H), 7.22 – 7.20 (m, 2H), 4.14 (s, 3H); $^{13}$C NMR (CDCl$_3$) δ 185.80, 162.10, 144.02, 137.59, 133.23, 130.55, 125.10, 113.75, 55.40.

(4-Methoxyphenyl)-(5-(3-methoxyphenyl)sulfanylthiophen-2-yl)methanone (27a): purified by CC (hexane/ethyl acetate 99:1); yield 90% (322 mg; brown oil). $^1$H NMR (CDCl$_3$) δ 8.15 – 8.10 (m, 2H), 7.78 (d, $J = 3.9$ Hz, 1H), 7.53 – 7.49 (m, 1H), 7.41 (d, $J = 3.9$ Hz, 1H), 7.27 – 7.23 (m, 2H), 7.21 (dt, $J = 7.6$, 1.9 Hz, 2H), 7.08 (ddd, $J = 8.3$, 2.5, 0.8 Hz, 1H), 4.14 (s, 3H), 4.04 (s, 3H); $^{13}$C NMR (CDCl$_3$) δ 185.84, 163.18, 160.16, 146.00, 143.97, 136.55, 134.24, 132.43, 131.50, 130.28, 130.19, 122.40, 115.62, 113.75, 113.55, 55.48, 55.35.

(4-Hydroxyphenyl)-(5-(3-hydroxyphenyl)sulfanylthiophen-2-yl)methanone (27): purified by CC (dichloromethane/methanol 99:1); yield 69% (190 mg; yellow oil). $^1$H NMR (CD$_3$OD) δ 7.78 – 7.76 (m, 1H), 7.75 – 7.74 (m, 1H), 7.53 (d, $J = 3.9$ Hz, 1H), 7.17 – 7.12 (m, 2H), 6.91 – 6.89 (m, 1H), 6.89 – 6.87 (m, 1H), 6.86 (ddd, $J = 8.2$, 2.4, 0.9 Hz, 1H), 6.85 – 6.83 (m, 1H), 6.73 (ddd, $J = 8.2$, 2.4, 0.9 Hz, 1H); $^{13}$C NMR (CD$_3$OD) δ 187.86, 163.59, 159.47, 146.94, 145.93, 137.47, 136.20, 133.80, 133.03, 131.47, 129.96, 122.34, 117.94, 116.44, 116.28; MS (ESI): 329.78 (M+H)$^+$. 

(5-(3-Hydroxybenzenesulfonyl)-thiophen-2-yl)(4-hydroxyphenyl)methanone (28): purified by CC (dichloromethane/methanol 99:1); yield 53% (58 mg; yellow solid; mp. 88-90°C). $^1$H NMR (CD$_3$OD) δ 7.82 – 7.80 (m, 1H), 7.80 – 7.78 (m, 1H), 7.73 (d, $J = 4.0$ Hz, 1H), 7.61 (d, $J = 4.0$ Hz, 1H), 7.47 (ddd, $J = 7.8$, 1.7, 1.1 Hz, 1H), 7.43 (d, $J = 8.0$ Hz, 1H), 7.40 (t, $J = 2.0$ Hz, 1H), 7.08 (ddd, $J = 8.0$, 2.5, 1.1 Hz, 1H), 6.92 – 6.90 (m, 1H), 6.90 – 6.88 (m, 1H); $^{13}$C NMR (CD$_3$OD) δ 187.49, 164.27, 159.84, 151.51, 150.11, 143.49, 134.41, 134.28, 133.36, 132.01, 129.13, 122.31, 119.37, 116.57, 114.87; MS (ESI): 362.38 (M+H)$^+$. 

S7
3. Brief description of the biological assays

3.1. Preparation of human 17β-HSD1 and 17β-HSD2. Human 17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[1] Fresh human placenta was homogenized, and cytosolic fraction and microsomes were separated by fractional centrifugation. For the partial purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction.

3.2. Inhibition of human 17β-HSD1. Inhibitory activities were evaluated by an established method with minor modifications.[1] Briefly, the enzyme preparation was incubated with NADH (500 µM) in the presence of potential inhibitors at 37°C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA (1 mM). Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-3H]-E1 (final concentration: 500 nM, 0.15 µCi). After 10 min, the incubation was stopped with HgCl2 and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 µm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1200 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E2/(%E2 + %E1)]x100

Each value was calculated from at least three independent experiments.

3.3. Inhibition of human 17β-HSD2. The h17β-HSD2 inhibition assay was performed similarly to the h17β-HSD1 procedure. The microsomal fraction was incubated with NAD+ [1500 µM], test compound and a mixture of unlabelled- and [2,4,6,7-3H]-E2 (final concentration: 500 nM, 0.11 µCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E1/(%E1 + %E2)]x100

3.4. Preparation of murine 17β-HSD1. Recombinant mouse 17β-HSD1 enzyme was produced by transfection of HEK 293 cells with a mouse 17β-HSD1 expression plasmid (coding sequence of NM_010475 in pCMV6Entry vector, OriGene Technologies, Inc.). 48 hours after transfection cells were homogenized by sonication (3 x 10 s) in a buffer containing saccharose (40 mM Tris,
250 mM saccharose, 5 mM EDTA, 7 mM DTT, 1 mM PMSF, pH 7.5). Cell lysate was centrifuged (1000 g, 15 min, 4°C) and 20% glycerol was added to the supernatant before aliquots were frozen and stored at -70°C.[2]

3.5. *Inhibition of murine 17ß-HSD1.* Inhibitory activities of the compounds towards mouse 17ß-HSD1 were evaluated by an established method with minor modifications.[3] The enzyme preparation was incubated with inhibitors, NADPH (0.5 mM) and a mixture of unlabeled- and [3H]-E1 (final concentration: 10 nM, 0.15 μCi) for 10 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

\[
\text{%conversion} = \left( \frac{\text{%E2}}{\text{%E2} + \text{%E1}} \right) \times 100
\]

4. **References of the supporting data**


5. Representative $^1$HNMR and $^{13}$CNMR spectra
13C NMR of Compound 25

Molecular Weight = 328.41
Molecular Formula = C17H12O3S2
1H NMR of Compound 26

Molecular Weight: 360.41
Molecular Formula: C17H12O5S2
1H NMR of Compound 26

Molecular Weight = 360.41
Molecular Formula = C17H12O5S2
1H NMR of Compound 27

Molecular Weight = 328.41
Molecular Formula = C17H12O3S2
1H NMR of Compound 28

Molecular Weight = 360.41
Molecular Formula = C17H12O5S2