

Supporting Information

Synthesis and biological evaluation of cyclicsulfamide derivatives as 11 β -hydroxysteroid dehydrogenase 1 inhibitors

Se Hoan Kim^{a,b}, Ju Han, Bok^a, Jae Hong Lee^{a,b}, Il Hyang Kim^{a,b}, Sung Wook Kwon^{a,b}, Gui Bin Lee^a, Seung Kyu Kang^a, Ji Seon Park^a, Won Hoon Jung^a, Hee Yeon Kim^a, Sang Dal Rhee^a, Sung Hoon Ahn^a, Myung Ae Bae^a, Deok Chan Ha^b, Ki Young Kim^a, Jin Hee Ahn^{a,*}

^a*Bio-Organic Science Division, Korea Research Institute of Chemical Technology,
Yuseong-Gu, Daejeon, 305-600, Korea*

^b*Department of Chemistry, Korea University, Sungbuk-gu, Seoul 136-701, Korea*

** To whom correspondence should be addressed. e-mail: jhahn@kRICT.re.kr*

General. All reported yields are isolated yields after column chromatography or crystallization. ¹H-NMR spectra were obtained on Bruker AVANCE-300 with TMS as internal reference. High-resolution mass spectra were obtained on the Autospec magnetic sector mass spectrometer (Micromass, Manchester, UK).

Synthetic Procedures

Compound 5

To a solution of chlorosulfonyl isocyanate (4 g, 28.26 mmol) in CH₂Cl₂ (80 mL), was added tert-butyl alcohol (2.45 g, 33 mmol) at 0 °C. The mixture was stirred for 30 min and then triethylamine (19.7 mL) and 3-chloropropylamine HCl (4.29 g, 33 mmol) were added at 5 °C. The reaction mixture was stirred for 2 h at room temperature, and then, extracted with CH₂Cl₂ and brine. The organic layer was dried over anhydrous MgSO₄, and the residue was purified by silica gel column chromatography to give tert-butyl *N*-(3-chloropropyl)sulfamoylcarbamate (**2**, 4.17 g, 54 %). ¹H NMR (300 MHz, CDCl₃): δ 5.25 (brs, 1H), 3.67 - 3.62 (m, 2H), 3.28 - 3.26 (m, 2H), 2.09 - 2.01 (m, 2H), 1.51 (s, 9H). To a solution of tert-butyl *N*-(3-chloropropyl)sulfamoylcarbamate (**2**, 2 g, 7.33 mmol) in DMSO (10 mL), was added K₂CO₃ (5.068 g, 36.665 mmol) at room temperature. The mixture was stirred for 4 h and extracted with ethyl acetate and NH₄Cl solution, and organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give tert-butyl 1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (**3**, 689 mg, 40 %, white solid). ¹H NMR (300 MHz, CDCl₃): δ 4.47 (brs, 1H), 3.95 - 3.91 (m, 2H), 3.55 - 3.49 (m, 2H), 1.89 - 1.82 (m, 2H), 1.54 (s, 9H). To a solution of tert-butyl 1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (**3**, 2 g, 8.464 mmol) in DMF (10 mL) were added K₂CO₃ (2.34 g, 16.93 mmol) and ethyl bromoacetate (1.7 g, 10.16 mmol). The mixture was stirred for 4 h at room temperature, extracted with ethyl acetate and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give tert-butyl 6-(2-ethoxy-2-oxoethyl)-1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (**4**, 2.2 g, 80 %, white solid). ¹H NMR (500 MHz, CDCl₃): δ 4.21 (q, *J* = 7.1 Hz, 2H), 3.98 - 3.96 (m, 4H), 3.68 - 3.66 (m, 2H), 1.89 - 1.85 (m, 2H), 1.51 (s, 9H), 1.28 (t, *J* = 7.1 Hz, 3H). To a solution of tert-butyl 6-(2-ethoxy-2-oxoethyl)-1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (100 mg, 0.310 mmol) in THF (5 mL) and MeOH (5 mL) was added LiOH (39.1 mg, 0.93 mmol) in H₂O (5 mL) at 0 °C. The mixture was stirred for 3 h, and then evaporated. The residue was extracted with ethyl acetate and pH 4 buffer solution. The organic layer was dried over MgSO₄ to give 2-(6-(tert-butoxycarbonyl)-1,1-dioxido-1,2,6-thiadiazinane-2-yl)acetic acid (70 mg, 77 %, yellow solid). ¹H NMR (300 MHz, DMSO-*d*₆): δ 12.75 (s, 1H), 3.93 (s, 2H), 3.81 - 3.77 (m, 2H), 3.59 - 3.55 (m, 2H), 1.82 - 1.78 (m, 2H), 1.44 (s, 9H). To a mixture of 2-(6-(tert-butoxycarbonyl)-1,1-dioxido-1,2,6-thiadiazinane-2-yl)acetic acid (60 mg, 0.204 mmol),

2-adamantan amine HCl (57.5 mg, 0.306 mmol), and TEA (41.3 mg, 0.408 mmol) in CH₂Cl₂ (5 mL) was added EDCI (117.3 mg, 0.612 mmol). The mixture was stirred for 5 h at room temperature, and extracted with CH₂Cl₂ and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give tert-butyl 6-(2-(adamantan-2-ylamino)-2-oxoethyl)-1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (**5**, 30 mg, 35 %). ¹H NMR (300 MHz, CDCl₃): δ 6.90 – 6.91 (m, 1H), 4.06 - 4.07 (m, 1H), 4.00 (t, *J* = 5.8 Hz, 2H), 3.83 (s, 2H), 3.66 (t, *J* = 5.8 Hz, 2H), 1.92 - 1.63 (m, 16H), 1.52 (s, 9H). HRMS (C₂₀H₃₃N₃O₅S): calcd, 427.2141 found, 427.2136.

Compound 6a

To a solution of tert-butyl 6-(2-(adamantan-2-ylamino)-2-oxoethyl)-1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (**5**, 150 mg, 0.351 mmol) in CH₂Cl₂, was added 4M HCl/in 1,4-dioxane(excess). The mixture was stirred for 4 h at room temperature and then evaporated organic solvent to give *N*-(adamantan-2-yl)-2-(1,1-dioxido-1,2,6-thiadiazinan-2-yl)acetamide hydrochloride (120 mg, 92 %, white solid). ¹H NMR (300 MHz, DMSO-d₆) : δ 7.06 - 7.57 (m, 1H), 7.00 (t, *J* = 6.9 Hz, 1H), 3.85 - 3.82 (m, 1H), 3.59 (s, 2H), 3.33 - 3.23 (m, 4H), 1.90 - 1.48 (m, 16H). To a solution of *N*-(adamantan-2-yl)-2-(1,1-dioxido-1,2,6-thiadiazinan-2-yl)acetamide hydrochloride (50 mg, 0.135 mmol) in DMF (1 mL), were added K₂CO₃ (93.3 mg, 0.675 mmol) and (bromomethyl)benzene (46.2 mg, 0.27 mmol). The mixture was stirred for 4 h at room temperature and extracted with ethyl acetate and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give *N*-(adamantan-2-yl)-2-(6-benzyl-1,1-dioxido-1,2,6-thiadiazinan-2-yl)acetamide (**6a**, 53 mg, 94 %, white solid). ¹H NMR (300 MHz, DMSO-d₆): δ 7.72 - 7.69 (m, 1H), 7.39 - 7.28 (m, 5H), 4.17 (s, 2H), 3.86 - 3.83 (m, 1H), 3.79 (s, 2H), 3.46 - 3.42 (m, 2H), 3.17 - 3.14 (m, 2H), 1.93 - 1.89 (m, 2H), 1.80 - 1.67 (m, 12H), 1.52 - 1.48 (m, 2H). HRMS (C₂₂H₃₁N₃O₃S): calcd, 417.2086 found, 417.2076.

Compound 6b

To a solution of *N*-((adamantan-2-yl)-2-(1,1-dioxido-1,2,6-thiadiazinan-2-yl)acetamide hydrochloride (50 mg, 0.135 mmol) in DMF (1 mL) were added K₂CO₃ (93.3 mg, 0.675 mmol) and (2-bromoethyl)benzene (50 mg, 0.27 mmol). The mixture was stirred for 4 h at room temperature and extracted with ethyl acetate and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give *N*-(adamantan-2-yl)-2-(1,1-dioxido-6-phenethyl-1,2,6-thiadiazinan-2-yl)acetamide (**6b**, 8 mg, 13 %, white solid). ¹H NMR (300 MHz, DMSO-d₆): δ 7.33 - 7.19 (m, 5H), 7.01 - 6.98 (m, 1H), 4.06 - 4.05 (m, 1H), 3.71 (s, 2H), 3.53 - 3.49 (m, 2H), 3.38 - 3.33 (m, 4H), 2.94 - 2.87 (m, 2H), 1.92 - 1.60 (m, 16H). HRMS (C₂₃H₃₃N₃O₃S): calcd, 431.2243 found, 431.2265.

Compound 13a (including 12)

To a solution of sulfonyl dichloride (5 g, 30.05 mmol) in acetonitrile (30 mL) was added 3-chloropropyl amine hydrochloride (486 mg, 5.00 mmol). The mixture was stirred for 18 h at 75~80 °C. The mixture was evaporated and extracted with diethyl ether and H₂O. The organic layer was evaporated and dried under vacuo to give 3-chloropropylsulfamoyl chloride (**8**, compound was used without further purification). To a solution of 3-chloropropylsulfamoyl chloride (**8**, 1.5 g, 7.81 mmol) in ether (20 mL) at -78 °C, was slowly added aniline (436.4 mg, 4.686 mmol) and triethylamine (1.58 g, 15.62 mmol). The mixture was stirred for 4 h, and extracted with ether and H₂O. The organic layer was evaporated and dried under vacuo to give **9**. (compound was used without further purification). To a solution of compound **9** (1.625 g, 6.53 mmol) in DMSO (15 mL), was added K₂CO₃ (903 mg, 6.53 mmol). The mixture was stirred for 2 h at room temperature, and extracted with ethyl acetate and NH₄Cl solution. The organic layer was dried over MgSO₄. The residue was crystallized with ether and n-hexane to give 2-phenyl-1,2,6-thiadiazinane 1,1-dioxide (**10**, 490 mg, 2.31 mmol, white solid). ¹H NMR (300 MHz, DMSO-d₆): δ 7.42 - 7.24 (m, 5H), 3.64 - 3.61 (m, 2H), 3.40 - 3.34 (m, 2H), 2.51 - 2.49 (m, 2H). To a solution of 2-phenyl-1,2,6-thiadiazinane 1,1-dioxide (**10**, 400 mg, 1.89 mmol) in DMF (2 mL), were added K₂CO₃ (522.4 mg, 3.78 mmol) and ethyl bromoacetate (379.1 mg, 2.27 mmol). The mixture was stirred for 4 h at room temperature, and extracted with ethyl acetate and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give ethyl 2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetate (**11**, 468 mg, 1.56 mmol, 83 %, white solid). ¹H NMR (300 MHz, CDCl₃): δ 7.45 - 7.34 (m, 3H), 7.30-7.25 (m, 2H), 4.25 (q, *J* = 7.1Hz, 2H), 4.03 (s, 2H), 3.82 - 3.73 (m, 4H), 1.99 - 1.91 (m, 2H), 1.30 (t, *J* = 7.1Hz, 3H). To a solution of ethyl 2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetate (**11**, 260 mg, 0.872 mmol) in THF (5 mL) and MeOH (5 mL) was added LiOH (182.9 mg, 4.36 mmol) in H₂O (5 mL). The mixture was stirred for 3 h room temperature and evaporated solvents. The residue was poured into ice water, acidified by 2N-HCl to pH 1 and extracted with ethyl acetate. The organic layer was dried over MgSO₄ to give 2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetic acid (**12**, 230 mg, 97 %, yellow solid). ¹H NMR (300 MHz, DMSO-d₆): δ 12.81 (s, 1H), 7.43 - 7.26 (m, 5H), 3.96 (s, 2H), 3.68 - 3.62 (m, 4H), 1.92 - 1.85 (m, 2H). To a solution of 2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetic acid (**12**, 100 mg, 0.37 mmol), 2-adamantan amine HCl (105 mg, 0.56 mmol) and TEA (74.9 mg, 0.74 mmol) in DCM (5 mL) was added EDCI (212.8 mg, 1.11 mmol). The mixture was stirred for 5 h at room temperature, and extracted with CH₂Cl₂ and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give *N*-(adamantan-2-yl)-2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetamide (**13a**, 80 mg, 54 %). ¹H NMR

(300 MHz, CDCl₃) : δ 7.41 - 7.26 (m, 5H), 7.02 - 6.99 (m, 1H), 4.07 (m, 1H), 3.94 (s, 2H), 3.81 - 3.74 (m, 4H), 2.03 - 1.94 (m, 3H), 1.85 - 1.74 (m, 10H), 1.66 - 1.60 (m, 3H). HRMS (C₂₁H₂₉N₃O₃S): calcd, 403.1930 found, 403.1934.

Compound 13b

To a solution of 2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetic acid (**12**, 50 mg, 0.19 mmol), EDCI (109.3 mg, 0.57 mmol), DMAP (cat) in DCM (5 mL) was added 1-adamantanamine (43 mg, 0.285 mmol). The mixture was stirred for 5 h at room temperature, extracted with CH₂Cl₂ and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give *N*-(adamantan-1-yl)-2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetamide (**13b**, 36 mg, 47 %). ¹H NMR (300 MHz, CDCl₃): δ 7.41 - 7.25 (m, 5H), 6.24 (brs, 1H), 3.83 (s, 2H), 3.78 - 3.70 (m, 4H), 2.08 - 1.99 (m, 11H), 1.69 - 1.67 (m, 6H). HRMS (C₂₁H₂₉N₃O₃S): calcd, 403.1930 found, 403.1936.

Compound 13c

A solution of 2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetic acid (**12**, 60 mg, 0.222 mmol) in oxalyl dichloride (2 mL) was stirred for 2 h at 50 °C, and then excess oxalyl chloride was evaporated. This crude acid chloride was dissolved in THF (2 mL). This solution was slowly added to a solution of 4-amino-tricyclo[3,3,1,1,3,7]decane-1-carboxamide (50 mg, 0.264 mmol) in NaHCO₃ solution (2 mL). The mixture was stirred for 30 min at room temperature, and extracted with ethyl acetate and brine. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give (*E*)-4-(2-(1,1-dioxido-6-phenyl-1,2,6-thiadiazinan-2-yl)acetamido)adamantane-1-carboxamide (**13c**, 16 mg, 11 %). ¹H NMR (300 MHz, CDCl₃): δ 7.41 - 7.30 (m, 5H), 6.96 - 6.97 (m, 1H), 5.69 (brs, 1H), 5.25 (brs, 1H), 4.00 - 3.98 (m, 1H), 3.94 (s, 2H), 3.81 - 3.74 (m, 4H), 2.17 - 1.68 (m, 15H). HRMS (C₂₂H₃₀N₄O₄S): calcd, 446.1988 found, 446.1958.

Compound 13d

¹H NMR (300 MHz, CDCl₃) : δ 7.46 - 7.41 (m, 1H), 7.34 - 7.26 (m, 2H), 7.18 - 7.12 (m, 2H), 6.96 (brs, 1H), 5.68 (brs, 1H), 5.34 - 5.26 (m, 1H), 4.07 - 3.96 (m, 1H), 3.95 (s, 2H), 3.81 - 3.77 (m, 4H), 2.17 - 1.58 (m, 15H). HRMS (C₂₂H₂₉FN₄O₄S): calcd, 464.1894 found, 464.1878.

Compound 13e

¹H NMR (300 MHz, CDCl₃) : δ 7.38 - 7.31 (m, 1H), 7.16 - 6.99 (m, 4H), 6.94 - 6.95 (m, 1H), 5.58 (brs, 1H), 5.35 (brs, 1H), 4.07 - 3.99 (m, 1H), 3.93 (s, 2H), 3.80 - 3.75 (m, 4H), 2.15 - 1.58

(m, 15H). HRMS (C₂₂H₂₉FN₄O₄S): calcd, 464.1894 found, 464.1886.

Compound 13f

¹H NMR (300 MHz, CDCl₃) : δ 7.43 - 7.32 (m, 2H), 7.10 - 7.01 (m, 2H), 6.90 - 9.95 (m, 1H), 4.04 - 4.03(m, 1H), 3.82 - 3.64 (m, 6H), 2.36 - 1.12 (m, 15H). HRMS (C₂₂H₂₉FN₄O₄S): calcd, 464.1894 found, 464.1896.

Compound 13g

¹H NMR (300 MHz, CDCl₃) : δ 6.95 (d, *J* = 8.0 Hz, 1H), 6.78 - 6.72 (m, 2H), 5.57 (brs, 1H), 5.28 (brs, 1H), 4.07 - 4.04 (m, 1H), 3.93 (s, 2H), 3.87 - 3.81 (m, 4H), 2.09 - 1.61 (m, 15H). HRMS (C₂₂H₂₇F₃N₄O₄S): calcd, 500.1705 found, 500.1697.

Compound 13 h

¹H NMR (300 MHz, CDCl₃) : δ 7.41 (s, 2H), 6.96 (d, *J* = 8.0 Hz, 1H), 5.57 (brs, 1H), 5.25 (brs, 1H), 4.15 - 4.03 (m, 3H), 3.87 - 3.83 (m, 4H), 2.09 - 1.42 (m, 15H). HRMS (C₂₂H₂₇F₃N₄O₄S): calcd, 500.1705 found, 500.1692.

Compound 18 a

To a solution of 3-Chloropropylamine hydrochloride (10.0 g, 76,9 mmol) in acetonitrile (150 mL) was slowly added sulfonyl chloride (62.2 g, 461.4 mmol). The reaction mixture was refluxed for 18 h, and then evaporated to give methyl 2-((chlorosulfonyl)amino)acetate (12.5 g, 85 %). To a solution of methyl 2-((chlorosulfonyl)amino)acetate (2.5 g, 12.1 mmol) in dichloromethane (80 mL) were added trichloroaniline (1.5 g, 7.6 mmol) and TEA (3.7 ml, 22.8 mmol) at room temperature. The mixture was stirred for 5 h at room temperature, and then extracted with EtOAc and brine. The organic layer was dried with Na₂SO₄. The residue was purified by silica gel column chromatography to give methyl 2-((*N*-(2,4,6-trichlorophenyl)sulfamoyl)amino)acetate. (1.0 g, 38 %). ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.37 (s, 1H), 7.82 (t, *J* = 6 Hz, 1H), 3.90 (d, *J* = 6 Hz, 2H), 3.72 (s, 3H). To a solution of methyl 2-((*N*-(2,4,6-trichlorophenyl)sulfamoyl)amino)acetate (300 mg, 0.863 mmol) in CH₃CN (10 mL) were added K₂CO₃ (239 mg, 1.726 mmol) and 1,2-dibromoethane (325 mg, 1.726 mmol). The mixture was refluxed for 12 h, and then extracted with ethyl acetate and H₂O. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give methyl 2-(1,1-dioxido-5-(2,4,6-trichlorophenyl)-1,2,5-thiadiazolidin-2-yl)acetate (210 mg, 65 %). ¹H NMR (300 MHz, CDCl₃): δ 7.44 (s, 2H), 3.93 (s, 2H), 3.93 - 3.90 (m, 2H), 3.87 - 3.81 (m, 2H), 3.80 (s, 3H). To a solution of methyl 2-(1,1-dioxido-5-(2,4,6-trichlorophenyl)-

1,2,5-thiadiazolidin-2-yl)acetate (200 mg, 0.535 mmol) in THF (5 mL) and MeOH (5 mL) was added LiOH (112.3 mg, 2.68 mmol) in H₂O (5 mL). The mixture was stirred for 3 h room temperature and evaporated solvents. The residue was poured into ice water, acidified by 2N-HCl to pH 1 and extracted with ethyl acetate. The organic layer was dried over MgSO₄ to give 2-(1,1-dioxido-5-(2,4,6-trichlorophenyl)-1,2,5-thiadiazolidin-2-yl)acetic acid (170 mg, 88 %). ¹H NMR (300 MHz, DMSO-d₆) : δ 12.93 (brs, 1H), 7.85 (s, 2H), 3.87 (s, 2H), 3.82 - 3.80 (m, 2H), 3.77 - 3.75 (m, 2H). To a solution of 4-aminoadamantane-1-carboxamide hydrochloride (70 mg, 0.303 mmol) in DMSO(1 mL) and *i*-PrOH (5 mL) were added DIPEA (0.25 g, 1.95 mmol), 2-(1,1-dioxido-5-(2,4,6-trichlorophenyl)-1,2,5-thiadiazolidin-2-yl)acetic acid (109 mg, 0.303 mmol), EDCI (1.2 eq), and HOBT (1.2 eq). The reaction mixture was stirred for 5 h at room temperature, and then extracted with EtOAc and H₂O. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give 4-(2-(1,1-dioxido-5-(2,4,6-trichlorophenyl)-1,2,5-thiadiazolidin-2-yl)acetamido)adamantane-1-carboxamide (60 mg, 37 %). ¹H NMR (300 MHz, CDCl₃) : δ 7.46 (s, 2H), 7.16 (d, *J* = 8.0 Hz, 1H), 5.56 (brs, 1H), 5.22 (brs, 1H), 4.10 - 4.07 (m, 1H), 3.98 - 3.89 (m, 2H), 3.87 (s, 2H), 3.79 - 3.74 (m, 2H), 2.10 - 1.57 (m, 13H). HRMS (C₂₁H₂₅Cl₃N₄O₄S): calcd, 534.0662 found, 534.0627.

Compound 18b

¹H NMR (300 MHz, CDCl₃) : δ 7.43(s, 2H), 6.94 (d, *J* = 8.0 Hz, 1H), 5.57 (brs, 1H), 5.30 (brs, 1H), 4.04 - 4.01(m, 1H), 3.95 (s, 2H), 3.55 - 3.52 (m, 2H), 3.42 - 3.38 (m, 2H), 2.23 - 2.17 (m, 2H), 2.04 - 1.55 (m, 15H). HRMS (C₂₃H₂₉Cl₃N₄O₄S): calcd, 562.0975 found, 562.0971.

Compound 18c

¹H NMR (300 MHz, CDCl₃) : δ 7.43(s, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 5.57 (brs, 1H), 5.27 (brs, 1H), 4.05 - 4.02 (m, 1H), 3.92 (s, 2H), 3.22 (s, 3H), 3.09 (s, 3H), 2.16 - 1.57 (m, 13H). HRMS (C₂₁H₂₇Cl₃N₄O₄S): calcd, 536.0819 found, 536.0821.

Compound 18d

¹H NMR (300 MHz, CDCl₃) : δ 7.42-7.39 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 1H), 5.56 (brs, 1H), 5.22 (brs, 1H), 4.61 - 4.50 (m, 1H), 4.28 - 4.18 (m, 2H), 4.05 - 4.02 (m, 1H), 3.65 - 3.59 (m, 1H), 3.31 - 3.24 (m, 1H), 2.14 - 1.54 (m, 15H), 1.33 (d, *J* = 6.9 Hz, 3H). HRMS (C₂₃H₂₉Cl₃N₄O₄S): calcd, 562.0975 found, 562.0973.

Compound 18e

To a solution of ethyl 2-((*N*-(2,4,6-trichlorophenyl)sulfamoyl)amino)acetate (3.0 g, 8.3 mmol),

2-methylpropane-1,3-diol (1.18 ml, 8.3 mmol) and PPh₃ (5.4 g, 20.7 mmol) in THF was added DIAD (4 ml, 20.7 mmol) at 0 °C. The mixture was stirred for 3 h, and then extracted with EtOAc and H₂O. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give ethyl 2-(4-methyl-1,1-dioxido-6-(2,4,6-trichlorophenyl)-1,2,6-thiadiazinan-2-yl)acetate (2.3 g, 65 %). ¹H NMR (300 MHz, CDCl₃) δ 7.41 (d, *J* = 2.4 Hz, 1H), 7.37 (d, *J* = 2.4 Hz, 1H), 4.44 (d, *J* = 17 Hz, 1H), 4.37 - 3.86 (m, 5H), 3.59 - 3.41 (m, 1H), 3.38 - 3.13 (m, 1H), 2.75 - 2.53 (m, 1H), 1.32 (t, *J* = 7.1 Hz, 3H), 0.94 (d, *J* = 7.1 Hz, 3H). To a solution of methyl 2-(4-methyl-1,1-dioxido-6-(2,4,6-trichlorophenyl)-1,2,6-thiadiazinan-2-yl)acetate (2.3 g, 5.4 mmol) in THF (12 mL) and MeOH (12 mL) was added LiOH (1.1 g, 27.0 mmol) in H₂O. The mixture was stirred for 3 h room temperature and evaporated solvents. The residue was poured into ice water, acidified by 2N-HCl to pH 3 and extracted with ethyl acetate. The organic layer was dried over MgSO₄ to give 2-(4-Methyl-1,1-dioxido-6-(2,4,6-trichlorophenyl)-1,2,6-thiadiazinan-2-yl)acetic acid (2.0 g, 83 %). ¹H NMR (300 MHz, CDCl₃) δ 7.42 (d, *J* = 2.4 Hz, 1H), 7.38 (d, *J* = 2.4 Hz, 1H), 4.53 (d, *J* = 17 Hz, 1H), 4.16 - 3.88 (m, 4H), 3.40 - 3.38 (m, 1H), 3.20 - 3.15 (m, 1H), 2.57 - 2.11 (m, 1H), 0.93 (d, *J* = 7.1 Hz, 3H). To a solution of 4-aminoadamantane-1-carboxamide hydrochloride (900 mg, 3.9 mmol) in DMSO (40 mL) and *i*-PrOH (50 mL) were added DIPEA (2.5 g, 19.5 mmol), 2-(4-Methyl-1,1-dioxido-6-(2,4,6-trichlorophenyl)-1,2,6-thiadiazinan-2-yl)acetic acid (1.8 g, 4.7 mmol), EDCI (894 mg, 4.7 mmol), and HOBT (632 mg, 4.7 mmol). The reaction mixture was stirred for 5 h at room temperature, and then extracted with EtOAc and H₂O. The organic layer was dried over MgSO₄. The residue was purified by silica gel column chromatography to give 4-(2-(4-Methyl-1,1-dioxido-6-(2,4,6-trichlorophenyl)-1,2,6-thiadiazinan-2-yl)acetamido)adamantane-1-carboxamide (980 mg, 64 %). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.42 (d, *J* = 2.4 Hz, 1H), 7.39 (d, *J* = 2.4 Hz, 1H), 6.96 (d, *J* = 7.3 Hz, 1H), 5.56 (brs, 1H), 5.25 (brs, 1H), 4.33 (d, *J* = 17 Hz, 1H), 4.09 - 3.87 (m, 3H), 3.33 - 3.17 (m, 2H), 2.63 - 2.54 (m, 1H), 2.17 - 1.57 (m, 13H), 0.95 (d, *J* = 6.6 Hz, 3H). HRMS (C₂₃H₂₉Cl₃N₄O₄S): calcd, 562.0950 found, 562.0950.

Compound 18f

¹H NMR (300 MHz, CDCl₃): δ 7.42 (s, 2H), 6.93 (d, *J* = 8.0 Hz, 1H), 5.58 (s, 1H), 5.29 (m, 2H), 5.21 (s, 1H), 4.29 (s, 2H), 4.26 (s, 2H), 4.06 (m, 1H), 3.97 (s, 2H), 2.17 - 1.58 (m, 13H). HRMS (C₂₃H₂₇Cl₃N₄O₄S): calcd, 560.0819 found, 560.0818.

Compound 18g

¹H NMR (300 MHz, CDCl₃): δ 7.40 (s, 2H), 6.91 (d, *J* = 8.1 Hz, 1H), 5.55 (s, 1H), 5.23 (s, 1H), 4.23 (s, 2H), 4.04 (m, 1H), 3.62 (s, 2H), 3.59 (s, 2H), 2.08 - 1.25 (m, 13H), 0.71 (m, 4H). HRMS (C₂₄H₂₉Cl₃N₄O₄S): calcd, 574.0975 found, 574.0974.

Compound 18h

¹H NMR (300 MHz, CDCl₃) : δ 7.40 (s, 2H), 7.00 (d, *J* = 8.0 Hz, 1H), 5.55 (s, 1H), 5.23 (s, 1H), 4.07 (s, 2H), 4.05 (m, 1H), 3.68 (s, 2H), 3.46 (s, 2H), 2.09-1.25 (m, 13H), 1.24 (s, 6H). HRMS (C₂₄H₃₁Cl₃N₄O₄S): calcd, 576.1132 found, 576.1121.

Biological evaluation

Cell culture

Human and mouse 11β-HSD1 overexpressed CHO-K1 cells and human 11β-HSD2 overexpressed HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco/Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), 100 μg/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO₂.

In vitro assay for 11β-HSD1& 2 activity

To assay cellular 11β-HSD1 enzyme activity, human and mouse 11β-HSD1 overexpressed CHO-K1 cells were seeded at 2 × 10⁴ cells/well onto 96-well plates and were incubated in a medium containing 160 nM cortisone in the presence or absence of compounds for 3 h. To assay cellular 11β-HSD2 enzyme activity, human 11β-HSD1 overexpressed HEK293 cells were seeded at 2 × 10⁴ cells/well onto 96-well plates and were incubated in a medium containing 160 nM cortisone in the presence or absence of compounds for 24 h.

Small aliquots (10 μl) of the reaction mixtures were removed and subjected to homogeneous time resolved fluorescence (HTRF) cortisol assay according to the manufacturer's instructions (Nihon Schering, Tokyo, Japan). The HTRF assay is based on a competition between free cortisol and XL665-conjugated cortisol for the binding to an anticortisol antibody labeled with europium (Eu³⁺) cryptate. Eu³⁺ cryptate and XL665 act as donor and acceptor, respectively. If the two fluorophores will be in close proximity, fluorescence resonance energy transfer (FRET) occurs on excitation. A specific signal is expressed as percentage of Delta F, which is a value calculated from the ratio of 665 nm/615 nm [$(R_{\text{sample}} - R_{\text{negative}})/R_{\text{negative}} \times 100$], and is inversely proportional to the concentration of cortisol in the sample or the calibrator. The cortisol concentration was calculated from the calibration curve obtained from Delta F versus standard solution. IC₅₀ values of compounds were determined from concentration-dependent inhibition curves by GraphPad Prism software (GraphPad Software Inc., La Jolla, CA). Carbenoxolone (CBX) was used the reference compounds.

In vivo 11β-HSD1 inhibition (Ex vivo) assay for 11β-HSD1 activity in mice and monkey

Male C57Bl/6 lean mice, 12 weeks old, were orally gavaged with vehicle (0.5% carboxymethylcellulose sodium (CMC)/0.1% Tween 80 in H₂O) or compound at 20 mg/kg and sacrificed 2 h post dose, n = 4 per group. For monkey ex vivo assay, Male Cynomolgus monkeys (*Macaca fascicularis*), 2-4 years old, were orally gavaged with vehicle (0.5% CMC/0.1% Tween 80 in H₂O) or compound at 10 mg/kg and sacrificed 2 h post dose. Liver and fat pads were removed and sectioned into three 30–40 mg samples and placed into 24-well Falcon plates containing prewarmed assay media that consisted of 1 μM cortisone and 100 nM NADPH in DMEM. Plates were then transferred to a 37 °C/CO₂ incubator and incubated for 3 h. Then cortisol product in the media was quantitated using a cortisol ELISA kit (Assay Designs Inc., Ann Arbor, MI). Enzyme activity is expressed as pg/mL of product formed per mg wet tissue weight.

Statistics

The results are expressed as means ± S.E.M. The statistical significance was determined by Student's t test or one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. $P < 0.05$ was considered to be statistically significant.

CYP assay

The CYP450 enzymes (1A2, 2C19, 2D6 and 3A4) assay were carried out using fluorometric enzyme assays with Vivid CYP enzymes assay kit (PanVera, CA) in a 96-well microtiter plate following the manufacturer's instruction with some modification. Test compounds including the ketoconazole, □-naphthoflavone, sulfaphenazole and quinidine as known as CYP3A4, 1A2, and 2D6 inhibitors, respectively, were prepared in acetonitrile to give final concentrations of 10 μM. Briefly, to each well of the microtiter plate was added NADP generating solution (1.0 mM NADP⁺, 3.3 mM glucose 6-phosphate, 3.3 mM MgCl₂ • 6 H₂O, and 0.4 U/mL glucose 6-phosphate dehydrogenase in 10 mM K₃PO₄, pH 8.0) followed by the vehicle acetonitrile (control) and the test samples. Typically, for each P450 study, each plate containing one standard inhibitor was constructed. Plates were covered and then incubated at 37 °C for 20 min. The enzyme reaction was initiated by the addition of an enzyme/substrate (E/S) mixture (each CYP450 enzymes for 0.5 pmol and fluorogenic substrates for 5 μM substrate CYP3A4 Green, CYP1A2 Blue, CYP2C19 Blue and CYP2D6 Blue). The plate was further incubated for 20 min, followed by the addition of the stop solution to terminate the enzyme activity. Background reading was measured in a similar manner except for the E/S mixture which was added after the enzyme reaction was terminated. The fluorescence of substrate metabolite fluorescein was measured on a fluorescence plate reader with an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The effect of test compounds on CYP450 enzymes were

calculated as a percentage of the enzyme activity.

Pharmacokinetic study

Male Sprague-Dawley rats were cannulated with polyethylene tubing (PE-50, Intramedic, BD Bioscience) in the femoral vein under ketamin-administered anesthesia. **18e** was dissolved in a mixture of DMSO/ PEG400/ distilled water (0.5: 4: 5.5) and administered to rats by a bolus injection via the femoral vein at dose of 10 mg/kg. Blood samples were collected via the femoral vein at pre-dose and 2, 10, 30 min and 1, 2, 4, 6, 8, 24hr for the case of intravenous administration, or 15, 30 min and 1, 2, 4, 6, 8, 24hr for the oral administration case after **18e** administration. In case of cynomologous monkey, blood samples were directly collected from vein at 15, 30 min and 1, 2, 4, 6, 8, 24hr after the oral administration of 10 mg/kg dose. After centrifugation of blood samples, 100 μ l aliquots of plasma samples were collected and stored at -70°C before LC/MS/MS analysis. At the end of the experiment, pooled urine samples collected and measured exact volume. 1ml aliquots of each sample were taken and kept at -70°C . The concentrations of **18e** were determined by an LC/MS/MS (4000 QTRAP, AB SCIEX, Foster City, CA) with multiple reaction monitoring (MRM) mode. The plasma concentration vs time data were analysed by a non-compartmental method using the nonlinear least squares regression program WinNolin (Pharsight, Mountain View, CA). The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule extrapolated to infinity. The terminal elimination half-life ($t_{1/2}$), and the systemic clearance (CL) were obtained. The extent of absolute oral bioavailability (F) was estimated by comparing the AUC values after intravenous and oral administration of the same dose of **18e**. The peak plasma concentration (C_{max}) after oral administration was obtained by visual inspection from each rat's plasma concentration-time plot.