Supporting Information

Structure-Based Design and Synthesis of an Isozyme-Selective MTHFD2 Inhibitor with Tricyclic Coumarin Scaffold

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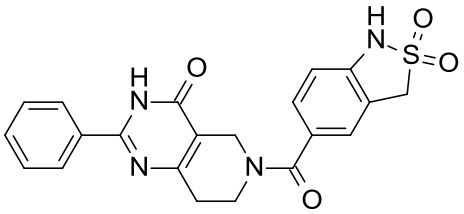
Contents

1. Synthesis

General

Unless otherwise noted, commercial reagents and solvents were obtained from suppliers and used as purchased. Normal-phase column chromatography was performed on silica gel (SiO_2) using prepackaged cartridges. Preparative reverse-phase high performance liquid chromatography (HPLC) was performed with a GILSON prepHPLC system. Conditions [column: Develosil Combi-RP-5 28 mm × 100 mm, gradient elution: 0.1% HCO_2H–H_2O / 0.1% HCO_2H–MeCN, flow rate: 25 mL/min, UV detection: 254 nm]. Analytical thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with silica gel 60 F_{254}. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-EX400 or Bruker AVANCE III 500 spectrometers, and chemical shifts are given in ppm from tetramethylsilane as an internal standard. Infrared spectra were recorded on KBr discs with a Jasco FT/IR-6100 typeA and are reported in wavenumbers (cm^{-1}). ESI/APCI mass spectra were recorded on Agilent Infinity 1260 series LC/MS. Purities of ≥90% were confirmed by the LC/MS for all test compounds. Conditions [column: Develosil Combi-RP-5 2.0 mm × 50 mm, gradient elution: 0.1% HCO_2H–H_2O / 0.1% HCO_2H–MeCN = 98/2 – 0/100 (v/v), flow rate: 1.2 mL/min, UV detection: 254 nm, column temperature: 40 °C, ionization: APCI/ESI]. High resolution mass spectra (HRMS) were obtained on LC/MS system composed of Waters Xevo Q-Tof MS system and Acuity UPLC system. Elemental analyses are indicated only by the symbols of the elements; analytical results were within 0.4% of the theoretical values. Ligand efficiency (LE) was calculated using the reported IC_{50} values[1,2] by the following equation: LE = 1.4 × pIC_{50}/heavy atom count (HAC).

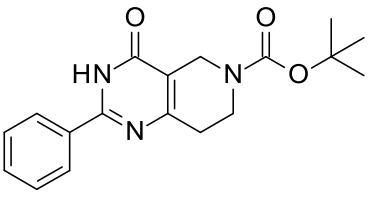
Synthetic information for HTS hit (1)



6-[(2,2-dioxido-1,3-dihydro-2,1-benzothiazol-5-yl)carbonyl]-2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one (1)

A DMF solution of 1,3-dihydro-2,1-benzothiazole-5-carboxylic acid 2,2-dioxide (21 mg, 0.10 mmol), 2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one (27 mg, 0.12 mmol), WSCI-HCl (19 mg, 0.10 mmol), HOBt (14 mg, 0.10 mmol), and *N*,*N*-diisopropylethylamine (17 μL, 0.10 mmol) was stirred at rt for 18 h. Concentration of the crude mixture and purification by preparative reverse-phase HPLC afforded **1** (3.91 mg, 9% yield) as a powder. ^1H-NMR (DMSO-D_6) δ: 12.81 (1H, br s), 10.91 (1H, br s), 8.08 (2H, d, J = 7.3 Hz), 7.62-7.38 (5H, m), 6.87 (1H, d, J = 7.9 Hz), 4.59 (2H, s), 4.47-4.34 (2H, br m), 3.87-3.68 (2H, br m), 2.83-2.73 (2H, br m). MS (ESI/APCI) m/z: 423.2 (calcd for $C_{21}H_{19}N_4O_4S$ $(M+H)^+$: 423.1).

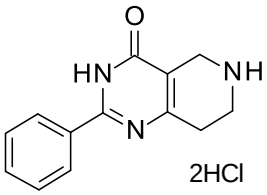
Synthetic information for MTHFD2 inhibitors



***tert*-butyl 4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6(4*H*)-carboxylate (S1)**

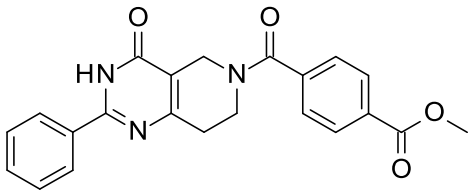
To a suspension of *N*-Boc-3-carboethoxy-4-piperidone (2.0 g, 7.37 mmol) and benzamidine hydrochloride (1.27 g, 8.11 mmol) in ethanol (20 mL) was added potassium carbonate (2.24 g, 16.2 mmol). The mixture was stirred at rt for 10 h. Insoluble solid was removed by filtration, and the filtrate was concentrated *in vacuo*. Purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) afforded **S1** (2.51 g, quant.) as a colorless solid. ^1H-NMR ($CDCl_3$) δ: 8.05 (2H, d, J = 6.1

Hz), 7.59-7.50 (3H, m), 4.45 (2H, s), 3.77-3.71 (2H, m), 2.83 (2H, s), 1.51 (9H, s). MS (ESI/APCI) m/z: 328.2 (calcd for $C_{18}H_{22}N_3O_3$ $(M+H)^+$: 328.2).



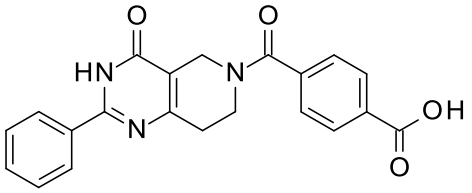
2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3H)-one 2HCl (S2)

To a suspension of **S1** (2.50 g, 7.6 mmol) in THF (20 mL) and MeOH (10 mL) was added 4N HCl 1,4-dioxane solution (20 mL). After stirring at 40 °C for 3 h, the solvent was removed *in vacuo*. The resulting residue was washed with Et_2O, filtrated, and dried to give **S2** (2.167 g, 95% yield) as a white solid. ^{1}H-NMR (DMSO-D_6, 80 °C) δ: 9.47 (2H, br s), 8.10 (2H, d, J = 6.7 Hz), 7.50-7.60 (3H, m), 3.96 (2H, s), 3.37-3.43 (2H, m), 2.90-2.94 (2H, m). MS (ESI/APCI) m/z: 228.2 (calcd for $C_{13}H_{14}N_3O$ $(M+H)^+$: 228.1).



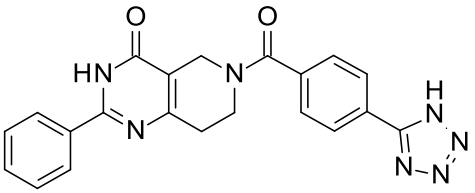
methyl 4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (15)

To a suspension of **S2** (200 mg, 0.67 mmol) and 4-methoxycarbonylbenzoic acid (133 mg, 0.73 mmol) in DMF were added WSCI-HCl (154 mg, 0.80 mmol), HOBt (91 mg, 0.67 mmol) and triethylamine (0.22 mL, 1.60 mmol) at 0 °C. After stirring for 6 h, the solution was concentrated *in vacuo* and diluted with CH_2Cl_2. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) to give **15** (219 mg, 84% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.14 (2H, d, J = 7.9 Hz), 8.04 (2H, br s), 7.60-7.52 (3H, m), 7.49-7.44 (2H, m), 4.85-4.35 (2H, m), 4.13-3.62 (2H, m), 3.96 (3H, s), 3.00-2.79 (2H, m). MS (ESI/APCI) m/z: 390.2 (calcd for $C_{22}H_{20}N_3O_4$ $(M+H)^+$: 390.1).



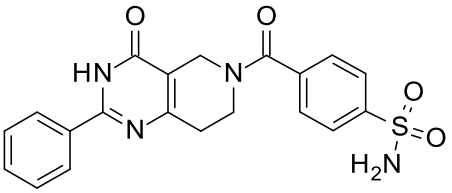
4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (3)

To a suspension of **15** (121 mg, 0.31 mmol) in MeOH (5 mL) was added 1M NaOH aq. (5 mL), and the mixture was stirred overnight at rt. 1M aq. HCl (5 mL) was added to it, and the resulting precipitate was filtrated and dried at 60 °C under reduced pressure to afford **3** (101 mg, 87% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 13.19 (1H, s), 12.86 (1H, s), 8.14-7.99 (4H, m), 7.63-7.46 (5H, m), 4.55-4.19 (2H, m), 3.98-3.52 (2H, m), 2.77 (2H, s). MS (ESI/APCI) m/z: 376.2 (calcd for $C_{21}H_{18}N_3O_4$ $(M+H)^+$: 376.1). HRMS (ESI): m/z calcd for $C_{21}H_{18}N_3O_4$ $(M+H)^+$ 376.1292. Found 376.1288. IR (KBr) 3435, 3081, 2899, 2498, 1898, 1682, 1644, 1605, 1557, 1509, 1444, 1324, 1239 cm^{-1}. Anal. Calcd for $C_{21}H_{17}N_3O_4$: C, 67.19; H, 4.56; N, 11.19. Found: C, 66.81; H, 4.67; N, 11.16.



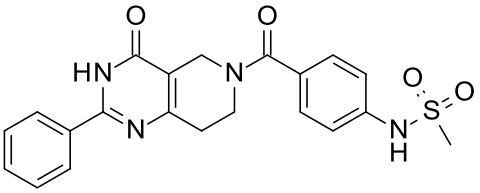
2-phenyl-6-[4-(1*H*-tetrazol-5-yl)benzoyl]-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one (2)

To a DMF (2 mL) solution of 4-(1*H*-tetrazol-5-yl)benzoic acid (77 mg, 0.40 mmol) were added WSCI-HCl (77 mg, 0.40 mmol), HOBt (46 mg, 0.34 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.092 mL, 0.67 mmol). The mixture was stirred at rt overnight. After concentration *in vacuo*, MeOH was added to the residue. The resulting solid was collected by filteration, washed with water, MeOH, and EtOAc to give **2** (90 mg, 68% yield) as a pale yellow solid. ^{1}H-NMR (DMSO-D_6) δ: 12.83 (1H, s), 8.17-8.03 (4H, m), 7.72 (2H, d, J = 7.9 Hz), 7.62-7.49 (3H, m), 4.58-4.24 (2H, m), 4.01-3.56 (2H, m), 2.80 (2H, s). MS (ESI/APCI) m/z: 400.2 (calcd for $C_{21}H_{18}N_7O_2$ $(M+H)^+$: 400.1).



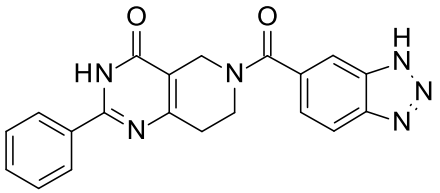
4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzenesulfonamide (4)

To a DMF (2 mL) solution of 4-sulfamoylbenzoic acid (74 mg, 0.37 mmol) were added WSCI-HCl (77 mg, 0.40 mmol), HOBt (46 mg, 0.34 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.092 mL, 0.67 mmol). The mixture was stirred at rt for 3 days. After concentration *in vacuo*, MeOH/CH_2Cl_2 (1:9) was added to the residue. The resulting solid was collected by filtration, washed with water and CH_2Cl_2, and dried at 60 ºC under reduced pressure to give **4** (95 mg, 69% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.85 (1H, s), 8.08 (2H, s), 7.92 (2H, d, J = 7.6 Hz), 7.69 (2H, d, J = 7.6 Hz), 7.61-7.48 (5H, m), 4.57-4.21 (2H, m), 3.98-3.51 (2H, m), 2.79 (2H, s). MS (ESI/APCI) m/z: 411.3 (calcd for $C_{20}H_{19}N_4O_4S$ $(M+H)^+$: 411.1).



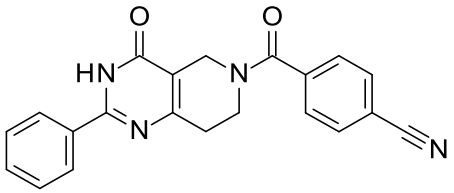
***N*-{4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]phenyl}methanesulfonamide (5)**

To a DMF (2 mL) solution of *p*-(methanesulfonamido)benzoic acid (86 mg, 0.40 mmol) were added WSCI-HCl (77 mg, 0.40 mmol), HOBt (46 mg, 0.34 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.092 mL, 0.67 mmol). The mixture was stirred at rt for 2 days. After concentration *in vacuo*, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 10/90 (v/v)). The obtained solid was washed with EtOAc and dried at 60 ºC under reduced pressure to give **5** (120 mg, 85% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.84 (1H, s), 10.11 (1H, s), 8.07 (2H, d, J = 6.7 Hz), 7.61-7.47 (5H, m), 7.28 (2H, d, J = 8.5 Hz), 4.52-4.29 (2H, m), 3.98-3.58 (2H, m), 3.09 (3H, s), 2.78 (2H, s). MS (ESI/APCI) m/z: 425.0 (calcd for $C_{21}H_{21}N_4O_4S$ $(M+H)^+$: 425.1).



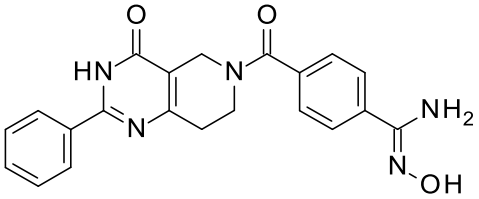
6-(1*H*-benzotriazol-6-ylcarbonyl)-2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one (6)

To a DMF (2 mL) solution of benzotriazole-5-carboxylic acid (60 mg, 0.37 mmol) were added WSCI-HCl (77 mg, 0.40 mmol), HOBt (46 mg, 0.34 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.092 mL, 0.67 mmol). The mixture was stirred at rt overnight. After concentration *in vacuo*, the residue was diluted with MeOH/CH_2Cl_2, washed with water, and dried over anhydrous Na_2SO_4. Filtration, removal of solvents by evaporation, and purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 10/90 (v/v)) afforded a colorless solid. The obtained solid was washed with CH_2Cl_2 and dried at 60 °C under reduced pressure to give **6** (50 mg, 40% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.82 (1H, s), 8.28-7.84 (4H, m), 7.67-7.42 (4H, m), 4.59-4.25 (2H, m), 4.01-3.54 (2H, m), 2.80 (2H, s). MS (ESI/APCI) m/z: 373.1 (calcd for $C_{20}H_{17}N_6O_2$ $(M+H)^+$: 373.1).



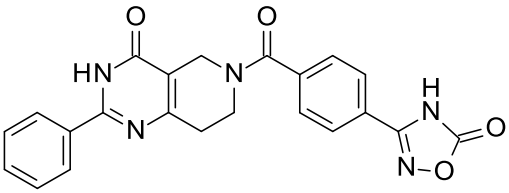
4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzonitrile (S3)

To a DMF (5 mL) solution of 4-cyanobenzoic acid (165 mg, 1.12 mmol), S2 (313 mg, 1.04 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (451 mg, 1.19 mmol) was added *N*,*N*-diisopropylethylamine (0.9 mL, 5 mmol). After stirring at rt for 3 days, the mixture was diluted with MeOH/$CHCl_3$ (1:9). The solution was washed with water (3 times), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Trituration with EtOAc/*n*-hexane (1:1) gave **S3** (318.5 mg, 86% yield) as a white powder. ^{1}H-NMR (DMSO-D_6) δ: 12.90-12.81 (1H, m), 8.13-8.03 (2H, m), 7.97 (2H, d, J = 7.9 Hz), 7.69 (2H, d, J = 7.9 Hz), 7.61-7.49 (3H, m), 4.56-4.16 (2H, m), 3.98-3.50 (2H, m), 2.77 (2H, br s). MS (ESI/APCI) m/z: 357.1 (calcd for $C_{21}H_{17}N_4O_2$ $(M+H)^+$: 357.1).



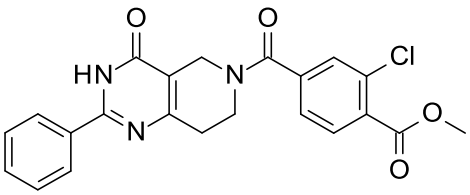
***N*′-hydroxy-4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzenecarboximidamide (S4)**

50% aq. hydroxylamine (0.8 mL) was added to the suspension of **S3** (80 mg, 0.22 mmol) in dimethylsulfoxide (1.5 mL) at rt. The mixture was stirred at 90 °C for 13 h. After cooling down to rt, water (5 mL) was added to the reaction mixture, and the resulting precipitate was collected by filtration. The solid was washed with water and EtOAc/*n*-hexane (1:1), and dried at 60 °C to give **S4** (68.2 mg, 78% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 12.74 (1H, s), 9.80 (1H, br s), 8.08 (2H, br s), 7.77 (2H, d, J = 7.9 Hz), 7.60-7.48 (5H, m), 5.92 (2H, s), 4.57-4.23 (2H, m), 4.00-3.55 (2H, m), 2.78 (2H, br s). MS (ESI/APCI) m/z: 390.1 (calcd for $C_{21}H_{20}N_5O_3$ $(M+H)^+$: 390.1).



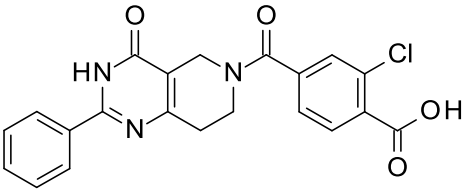
6-[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]-2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one (7)

To a DMF (1.5 mL) suspension of **S4** (63.2 mg, 0.162 mmol) was added 1,1'-carbonyldiimidazole (35 mg, 0.22 mmol) and the mixture was stirred at 65 °C for 9 days. During the reaction, the same amount of reagent (1,1'-carbonyldiimidazole) was added to the mixture for another three times until most of the starting material was consumed. Preparative reverse-phase HPLC, evaporation of volatile solvents in the obtained fractions, and following lyophilization afforded **7** (7.2 mg, 11% yield) as a pale brown powder. ^{1}H-NMR (DMSO-D_6) δ: 12.83 (1H, br s), 8.12-8.01 (2H, m), 7.91-7.81 (2H, m), 7.69-7.43 (5H, m), 4.58-4.22 (2H, m), 3.98-3.54 (2H, m), 2.77 (2H, s). MS (ESI/APCI) m/z: 416.2 (calcd for $C_{22}H_{18}N_5O_4$ $(M+H)^+$: 416.1).



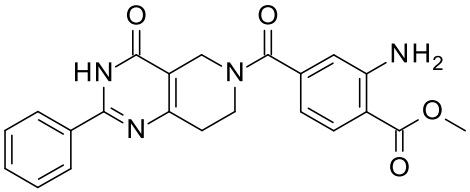
methyl 2-chloro-4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (S5)

To a DMF (3 mL) suspension of 3-chloro-4-(methoxycarbonyl)benzoic acid (72 mg, 0.33 mmol) were added WSCI-HCl (76 mg, 0.40 mmol), HOBt (45 mg, 0.33 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.11 mL, 0.80 mmol) at 0 °C. The mixture was stirred at rt for 2 days. Concentration *in vacuo* and purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) afforded **S5** (119 mg, 84% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.08 (2H, d, J = 7.3 Hz), 7.87 (1H, d, J = 7.9 Hz), 7.65 (1H, s), 7.56-7.47 (4H, m), 4.36 (2H, br s), 3.89 (3H, s), 3.73 (2H, br s), 2.80-2.74 (2H, m). MS (ESI/APCI) m/z: 424.3 (calcd for $C_{22}H_{19}ClN_3O_4$ $(M+H)^+$: 424.1).



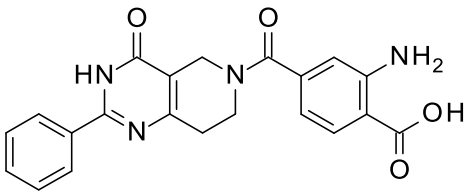
2-chloro-4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (8)

To a MeOH (10 mL) solution of **S5** (117 mg, 0.28 mmol) was added 1M NaOH aq. (5 mL). The mixture was stirred at rt for 2 h. 1M aq. HCl (5 mL) was added to it, and the resulting precipitate was filtrated and dried at 60 °C under reduced pressure to afford **8** (94 mg, 83% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.08 (2H, d, J = 7.9 Hz), 7.84 (1H, d, J = 7.9 Hz), 7.62-7.47 (5H, m), 4.37 (2H, s), 3.74 (2H, br s), 2.77 (2H, t, J = 5.5 Hz). MS (ESI/APCI) m/z: 410.1 (calcd for $C_{21}H_{17}ClN_3O_4$ $(M+H)^+$: 410.1).



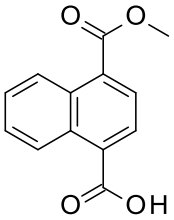
methyl 2-amino-4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (S6)

To a DMF (3 mL) suspension of 2-aminoterephthalic acid 1-methyl ester (66 mg, 0.33 mmol) were added WSCI-HCl (76 mg, 0.40 mmol), HOBt (45 mg, 0.33 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.11 mL, 0.80 mmol) at 0 °C. The mixture was stirred at rt for 2 days. Concentration *in vacuo* and purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) afforded **S6** (125 mg, 93% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.08 (2H, d, J = 7.3 Hz), 7.77 (1H, d, J = 8.5 Hz), 7.57-7.47 (3H, m), 6.83 (1H, s), 6.70-6.54 (3H, m), 4.36 (2H, s), 3.82 (3H, s), 3.74 (2H, br s), 2.79-2.73 (2H, m). MS (ESI/APCI) m/z: 405.2 (calcd for $C_{22}H_{21}N_4O_4$ $(M+H)^+$: 405.1).



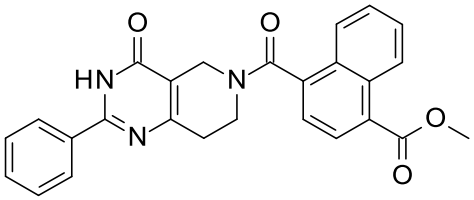
2-amino-4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (9)

To a MeOH (6 mL) solution of **S6** (48 mg, 0.12 mmol) was added 1M NaOH aq. (6 mL). The mixture was stirred overnight at rt. 1M aq. HCl (6 mL) was added to it, and the resulting precipitate was filtrated, washed with water and MeOH, and dried at 60 °C under reduced pressure to afford **9** (36 mg, 78% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.08 (2H, d, J = 7.3 Hz), 7.76 (1H, d, J = 7.9 Hz), 7.59-7.45 (3H, m), 6.80 (1H, d, J = 1.8 Hz), 6.54 (1H, dd, J = 7.9, 1.8 Hz), 4.36 (2H, s), 3.74 (2H, br s), 2.75 (2H, t, J = 5.5 Hz). MS (ESI/APCI) m/z: 391.2 (calcd for $C_{21}H_{19}N_4O_4$ $(M+H)^+$: 391.1).



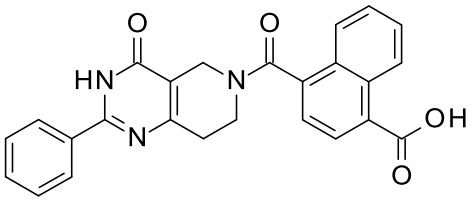
4-(methoxycarbonyl)naphthalene-1-carboxylic acid (S7)

To a mixture of dimethyl naphthalene-1,4-dicarboxylate (100 mg, 0.41 mmol), MeOH (3 mL) and THF (3 mL) was added 1M NaOH aq. (0.6 mL). The mixture was stirred overnight at rt. 1M aq. HCl (3 mL) was added to it, and the resulting precipitate was collected by filtration and dried at 60 °C under reduced pressure to afford crude **S7** (78 mg, 83% yield) as a colorless solid, which was used for the next step without further purification. MS (ESI/APCI) m/z: 229.1 (calcd for $C_{13}H_9O_4$ $(M–H)^–$: 229.1).



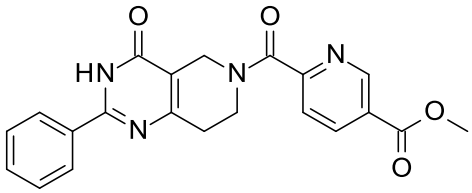
methyl 4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]naphthalene-1-carboxylate (S8)

To a DMF (3 mL) solution of crude **S7** (67 mg, 0.23 mmol) were added WSCI-HCl (55 mg, 0.28 mmol), HOBt (32 mg, 0.23 mmol), **S2** (70 mg, 0.23 mmol) and triethylamine (0.08 mL, 0.56 mmol). The mixture was stirred at rt overnight. Concentration *in vacuo* and purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) afforded **S8** (87 mg, 85% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.76 (1H, d, J = 8.5 Hz), 8.18-8.01 (3H, m), 7.94-7.44 (7H, m), 4.75-3.95 (2H, m), 3.98 (3H, s), 3.45-2.60 (4H, m). MS (ESI/APCI) m/z: 440.3 (calcd for $C_{26}H_{22}N_3O_4$ $(M+H)^+$: 440.2).



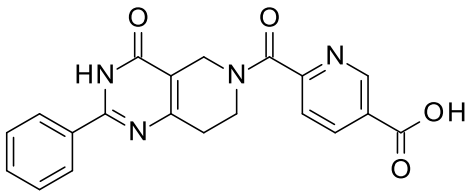
4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]naphthalene-1-carboxylic acid (10)

To a MeOH (10 mL) solution of **S8** (85 mg, 0.19 mmol) was added 1M NaOH aq. (3 mL). The mixture was stirred overnight at rt. 1M aq. HCl (3 mL) was added to it, and the resulting precipitate was collected by filtration, washed with water and MeOH, and dried at 60 ºC under reduced pressure to afford **10** (68 mg, 83% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.78 (1H, s), 8.87 (1H, d, J = 8.5 Hz), 8.17-8.03 (3H, m), 7.90-7.78 (1H, m), 7.71-7.45 (6H, m), 4.77-3.86 (2H, m), 3.45-2.46 (4H, m). MS (ESI/APCI) m/z: 426.2 (calcd for $C_{25}H_{20}N_3O_4$ $(M+H)^+$: 426.1).



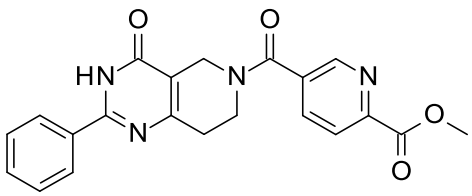
methyl 6-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]pyridine-3-carboxylate (S9)

To a DMF (5 mL) solution of 5-methoxycarbonylpyridine-2-carboxylic acid (50 mg, 0.28 mmol) were added WSCI-HCl (107 mg, 0.55 mmol), HOBt (38 mg, 0.28 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.09 mL, 0.61 mmol). The mixture was stirred at rt overnight. Then it was concentrated *in vacuo*, dissolved with EtOAc, and washed with 10% aq. citric acid, sat. aq. NaHCO3, and brine, respectively. Filtration, concentration *in vacuo* and tritulation with EtOAc/*n*-hexane afforded **S9** (107 mg, 99% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 9.24 (1H, d, J = 8.5 Hz), 8.45-8.42 (1H, m), 8.12-8.03 (2H, m), 7.86-7.78 (1H, m), 7.60-7.49 (3H, m), 4.83-4.58 (2H, m), 4.14-3.82 (2H, m), 4.01-4.00 (3H, m), 3.02-2.96 (2H, m). MS (ESI) m/z: 391.1 (calcd for $C_{21}H_{19}N_4O_4$ $(M+H)^+$: 391.1).



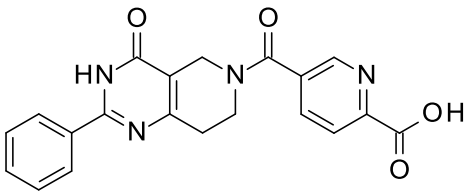
6-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]pyridine-3-carboxylic acid (11)

To a MeOH (4 mL) solution of **S9** (105 mg, 0.27 mmol) was added 1M NaOH aq. (2.5 mL). The mixture was stirred overnight at rt. 10% aq. citric acid was added to it and MeOH was removed under reduced pressure. The resulting precipitate was collected by filtration, washed with water, and dried under reduced pressure to afford **11** (82 mg, 81% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 13.59 (1H, br s), 12.93-12.78 (1H, br m), 9.10 (1H, br s), 8.43-8.39 (1H, m), 8.08 (2H, t, J = 8.8 Hz), 7.78 (1H, t, J = 7.6 Hz), 7.61-7.49 (3H, m), 4.58-4.26 (2H, m), 4.01-3.60 (2H, m), 2.84-2.73 (2H, m). MS (ESI) m/z: 377.2 (calcd for $C_{20}H_{17}N_4O_4$ $(M+H)^+$: 377.1).



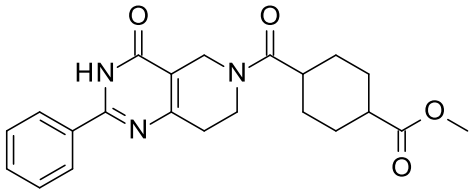
methyl 5-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]pyridine-2-carboxylate (S10)

To a DMF (3 mL) solution of 6-methoxycarbonylpyridine-3-carboxylic acid (47 mg, 0.26 mmol) were added WSCI-HCl (55 mg, 0.28 mmol), HOBt (32 mg, 0.23 mmol), **S2** (70 mg, 0.23 mmol) and triethylamine (0.08 mL, 0.56 mmol). The mixture was stirred at rt for 2 days. Concentration *in vacuo* and purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) afforded **S10** (21 mg, 23% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.80 (1H, s), 8.13-8.05 (4H, m), 7.57-7.48 (3H, m), 4.49-4.32 (2H, m), 3.92 (3H, s), 3.85-3.73 (2H, m), 2.79 (2H, t, J = 6.1 Hz). MS (ESI/APCI) m/z: 391.2 (calcd for $C_{21}H_{19}N_4O_4$ $(M+H)^+$: 391.1).



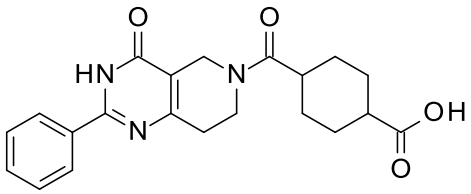
5-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]pyridine-2-carboxylic acid (12)

To a mixture of **S10** (21 mg, 0.054 mmol) and MeOH (5 mL) was added 1M NaOH aq. (2 mL). The mixture was stirred overnight at rt. 1M aq. HCl (2 mL) was added to it, and the resulting precipitate was collected by filtration, washed with water and MeOH, and dried at 60 ºC under reduced pressure to afford **12** (16 mg, 79% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.78 (1H, d, J = 1.2 Hz), 8.13-8.04 (4H, m), 7.59-7.47 (3H, m), 4.41 (2H, br s), 3.77 (2H, br s), 2.80 (2H, t, J = 5.5 Hz). MS (ESI/APCI) m/z: 377.2 (calcd for $C_{20}H_{17}N_4O_4$ $(M+H)^+$: 377.1).



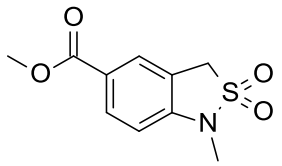
methyl 4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]cyclohexanecarboxylate (S11)

To a solution of **S2** (199 mg, 0.67 mmol) and 4-(methoxycarbonyl)cyclohexane-1-carboxylic acid (136 mg, 0.73 mmol) in CH_2Cl_2 (4 mL) were added WSCI-HCl (153 mg, 0.80 mmol), HOBt-H_2O (103 mg, 0.67 mmol) and triethylamine (0.28 mL, 2.0 mmol). After stirring for 3 days at rt, the solution was diluted with MeOH-$CHCl_3$. The organic layer was washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 1/99 – 8/92 (v/v)) to give *cis*/*trans* mixture of **S11** (270 mg, quant.) as a white solid (*d.r.* = *ca.* 3:2). MS (ESI/APCI) m/z: 396.3 (calcd for $C_{22}H_{26}N_3O_4$ $(M+H)^+$: 396.2).



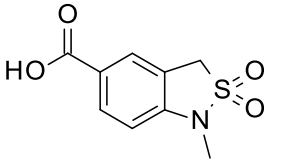
4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]cyclohexanecarboxylic acid (13)

To a MeOH (6 mL) suspension of *cis*/*trans* mixture of **S11** (270 mg, 0.68 mmol) was added 1M NaOH aq. (6 mL). The mixture was stirred at rt for 4 h. 1M aq. HCl (6 mL) was added to it, and the resulting precipitate was collected by filtration. The solid was washed with water and dried at 60 ºC under reduced pressure to afford *cis*/*trans* mixture of **13** (184 mg, 71% yield) as a white solid (*d.r.* = *ca.* 3:2). ^{1}H-NMR (DMSO-D_6) δ: 12.09 (2H, br s), 8.08 (2H, d, J = 7.3 Hz), 7.57-7.46 (3H, m), 4.35 (2H, s), 3.78-3.72 (2H, m), 2.78-2.64 (3H, m), 2.23-1.35 (9H, m). MS (ESI/APCI) m/z: 382.3 (calcd for $C_{21}H_{24}N_3O_4$ $(M+H)^+$: 382.2).



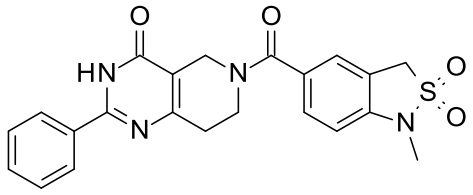
methyl 1-methyl-1,3-dihydro-2,1-benzothiazole-5-carboxylate 2,2-dioxide (S12)

To a DMF (1.5 mL) solution of methyl 1,3-dihydro-2,1-benzothiazole-5-carboxylate 2,2-dioxide (100 mg, 0.44 mmol) and potassium carbonate (60.8 mg, 0.44 mmol) was added iodomethane (0.110 mL, 1.76 mmol) and the mixture was stirred at rt for 3.5 h. Sat. aq. NH4Cl was added to the reaction mixture and it was extracted with EtOAc/*n*-hexane (3:1). The organic layer was washed with water (twice) and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford **S12** (100 mg, 94% yield) as a brown solid. ^{1}H-NMR ($CDCl_3$) δ: 8.08-8.05 (1H, m), 7.96-7.94 (1H, m), 6.75 (1H, d, J = 8.5 Hz), 4.39 (2H, s), 3.91 (3H, s), 3.20 (3H, s).



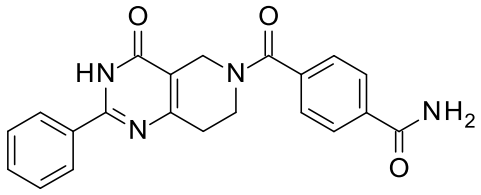
1-methyl-1,3-dihydro-2,1-benzothiazole-5-carboxylic acid 2,2-dioxide (S13)

To a mixture of **S12** (98 mg, 0.41 mmol), MeOH (5 mL) and THF (5 mL) was added 1M NaOH aq. (5 mL). After stirring for 1 h at rt, 1M aq. HCl (6 mL) was added to the mixture, and it was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford **S13** (61 mg, 66% yield) as a brown solid. ^{1}H-NMR (DMSO-D_6) δ: 7.97-7.93 (1H, m), 7.89-7.88 (1H, m), 7.03 (1H, d, J = 8.5 Hz), 4.77 (2H, s), 3.11 (3H, s).



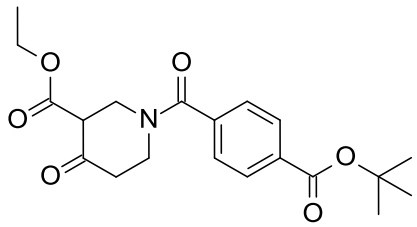
6-[(1-methyl-2,2-dioxido-1,3-dihydro-2,1-benzothiazol-5-yl)carbonyl]-2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one (14)

To a suspension of **S2** (27 mg, 0.090 mmol) and **S13** (21 mg, 0.092 mmol) in CH_2Cl_2 (2 mL) were added WSCI-HCl (22.1 mg, 0.108 mmol), HOBt-H_2O (13.7 mg, 0.090 mmol) and triethylamine (0.0374 mL, 0.270 mmol). After stirring overnight at rt, the solution was diluted with $CHCl_3$, washed with 10% aq. citric acid, sat. aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating the solvent, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 1/99 – 8/92 (v/v)) to give **14** (24.4 mg, 62% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 12.84 (1H, s), 8.10-8.04 (2H, m), 7.60-7.48 (5H, m), 7.03 (1H, d, J = 8.5 Hz), 4.74 (2H, s), 4.46-4.33 (2H, m), 3.94-3.60 (2H, m), 3.10 (3H, s), 2.81-2.75 (2H, m). MS (ESI/APCI) m/z: 437.2 (calcd for $C_{22}H_{21}N_4O_4S$ $(M+H)^+$: 437.1).



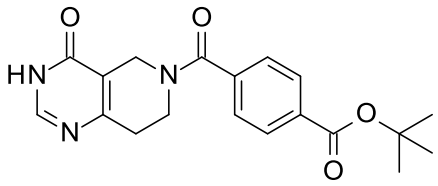
4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzamide (16)

To a DMF (3 mL) suspension of terephthalamic acid (61 mg, 0.37 mmol) were added WSCI-HCl (76 mg, 0.40 mmol), HOBt (45 mg, 0.33 mmol), **S2** (100 mg, 0.33 mmol) and triethylamine (0.11 mL, 0.80 mmol). The mixture was stirred at rt for 2 days. After concentration *in vacuo*, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 5/95 – 15/85 (v/v)). The obtained solid was washed with EtOAc to afford **16** (52 mg, 42% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.90-12.80 (1H, br m), 8.13-7.93 (5H, m), 7.61-7.48 (6H, m), 4.53-4.22 (2H, m), 3.96-3.56 (2H, m), 2.78 (2H, s). MS (ESI/APCI) m/z: 375.2 (calcd for $C_{21}H_{19}N_4O_3$ $(M+H)^+$: 375.1).



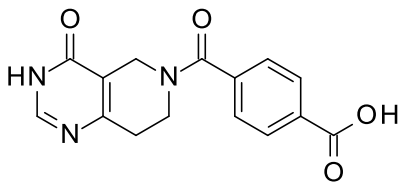
ethyl 1-[4-(*tert*-butoxycarbonyl)benzoyl]-4-oxopiperidine-3-carboxylate (22)

To a DMF (30 mL) solution of ethyl 4-oxo-3-piperidinecarboxylate hydrochloride (3.0 g, 14.4 mmol) and 4-(*tert*-butoxycarbonyl)benzoic acid (3.2 g, 14.4 mmol) were added WSCI-HCl (5.4 g, 28.9 mmol), HOBt-H_2O (2.22 g, 14.4 mmol) and triethylamine (3.0 mL, 21.7 mmol). The mixture was stirred at rt for 2 days. After concentration *in vacuo*, water (100 mL) was added to the mixture, and it was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by column chromatography (SiO_2, EtOAc/*n*-hexane = 21/79 – 42/58 (v/v)) to give **22** (3.5 g, 65% yield) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 8.07-8.02 (2H, m), 7.53-7.43 (2H, m), 4.43-3.44 (6H, m), 2.58-2.34 (2H, m), 1.61 (9H, s), 1.39-1.15 (4H, m). MS (ESI/APCI) m/z: 376.3 (calcd for $C_{20}H_{26}NO_6$ $(M+H)^+$: 376.2).



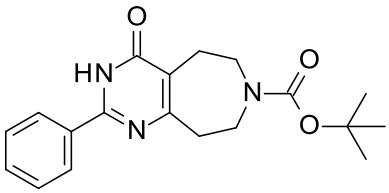
***tert*-butyl 4-[(4-oxo-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (23a)**

To a solution of **22** (58 mg, 0.15 mmol) in ethanol (1.5 mL) were added formamidine hydrochloride (14 mg, 0.17 mmol) and potassium carbonate (47 mg, 0.34 mmol). The mixture was stirred at rt for 3 days. After concentration *in vacuo*, 10% aq. citric acid was added to the mixture. The resulting precipitate was filtered, washed with water, dissolved with ethanol, and concentrated under reduced pressure to give **23a** (19 mg, 35% yield) as a colorless solid. MS (ESI/APCI) m/z: 356.2 (calcd for $C_{19}H_{22}N_3O_4$ $(M+H)^+$: 356.2).



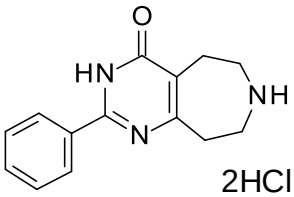
4-[(4-oxo-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (24)

To a CH_2Cl_2 (5 mL) solution of **23a** (19 mg, 0.053 mmol) was added trifluoroacetic acid (5 mL) at rt. The mixture was stirred at rt overnight. Concentration *in vacuo* and tritulation with EtOAc/*n*-hexane afforded **24** (13 mg, 81% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.54 (1H, br s), 8.11-8.05 (1H, m), 8.03-8.00 (2H, m), 7.61-7.54 (2H, m), 4.45-4.13 (2H, m), 3.91-3.48 (2H, m), 2.71-2.64 (2H, m). MS (ESI/APCI) m/z: 300.2 (calcd for $C_{15}H_{14}N_3O_4$ $(M+H)^+$: 300.1).



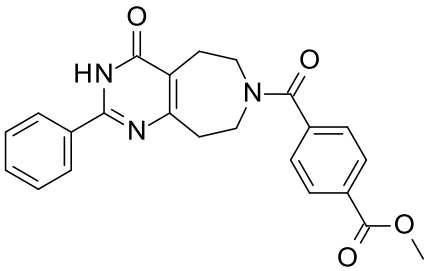
***tert*-butyl 4-oxo-2-phenyl-3,4,5,6,8,9-hexahydro-7*H*-pyrimido[4,5-*d*]azepine-7-carboxylate (19a)**

To a suspension of ethyl 1-Boc-5-oxoazepane-4-carboxylate (500 mg, 1.75 mmol) and benzamidine hydrochloride (302 mg, 1.93 mmol) in ethanol (10 mL) was added potassium carbonate (533 mg, 3.86 mmol) at rt. The mixture was stirred at rt overnight. Insoluble materials were removed by filteration, and the filtrate was concentrated *in vacuo*. Purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) afforded **19a** (433 mg, 72% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.06 (2H, d, J = 6.7 Hz), 7.60-7.50 (3H, m), 3.72-3.55 (4H, m), 3.07 (2H, br s), 2.97 (2H, br s), 1.50 (9H, s). MS (ESI/APCI) m/z: 342.3 (calcd for $C_{19}H_{24}N_3O_3$ $(M+H)^+$: 342.2).



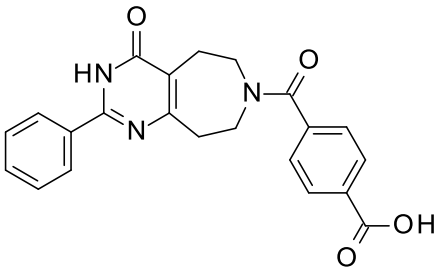
2-phenyl-3,5,6,7,8,9-hexahydro-4*H*-pyrimido[4,5-*d*]azepin-4-one 2HCl (S14)

To a MeOH (10 mL) suspension of **19a** (432 mg, 1.27 mmol) was added 4M HCl in 1,4-dioxane (5 mL) at rt. The mixture was stirred at rt for 2 h. Concentration *in vacuo* and trituration with EtOAc afforded **S14** (398 mg, quant.) as a colorless solid. MS (ESI/APCI) m/z: 242.2 (calcd for $C_{14}H_{16}N_3O$ $(M+H)^+$: 242.1).



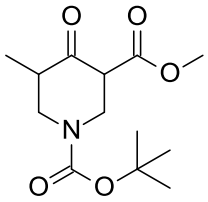
methyl 4-[(4-oxo-2-phenyl-3,4,5,6,8,9-hexahydro-7*H*-pyrimido[4,5-*d*]azepin-7-yl)carbonyl]benzoate (20a)

To a DMF (6 mL) solution of **S14** (100 mg, 0.32 mmol) and 4-methoxycarbonylbenzoic acid (64 mg, 0.35 mmol) were added WSCI-HCl (74 mg, 0.38 mmol), HOBt (44 mg, 0.32 mmol) and triethylamine (0.11 mL, 0.77 mmol) at 0 °C. The mixture was stirred at rt for 2 days. After evaporating solvents, the residue was dissolved with CH_2Cl_2, and the organic layer was washed with water and dried over anhydrous Na_2SO_4. Filtration, concentration *in vacuo*, and purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 3/97 (v/v)) afforded **20a** (110 mg, 86% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.15-8.04 (4H, m), 7.61-7.48 (5H, m), 4.02-3.90 (5H, m), 3.63-3.50 (2H, m), 3.26-3.09 (2H, m), 3.02-2.87 (2H, m). MS (ESI/APCI) m/z: 404.3 (calcd for $C_{23}H_{22}N_3O_4$ $(M+H)^+$: 404.2).



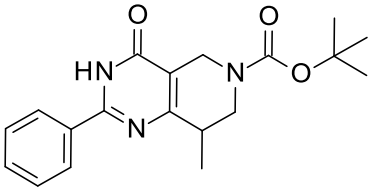
4-[(4-oxo-2-phenyl-3,4,5,6,8,9-hexahydro-7*H*-pyrimido[4,5-*d*]azepin-7-yl)carbonyl]benzoic acid (25)

To a MeOH (10 mL) suspension of **20a** (108 mg, 0.27 mmol) was added 1M NaOH aq. (5 mL). The mixture was stirred at rt for 2 h. 1M aq. HCl (5 mL) was added to it, and the resulting precipitate was collected by filtration. The solid was washed with water and MeOH, and dried at 60 °C under reduced pressure to afford **25** (85 mg, 82% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 13.16 (1H, br s), 12.79 (1H, br s), 8.16-7.98 (4H, m), 7.61-7.44 (5H, m), 3.91-3.73 (2H, m), 3.53-3.39 (2H, m), 3.14-2.76 (4H, m). MS (ESI/APCI) m/z: 390.1 (calcd for $C_{22}H_{20}N_3O_4$ $(M+H)^+$: 390.1).



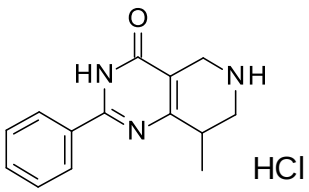
1-*tert*-butyl 3-methyl 5-methyl-4-oxopiperidine-1,3-dicarboxylate (17b)

To a solution of Boc_2O (378 mg, 1.73 mmol) in CH_2Cl_2 (5 mL) were added 3-methyl-5-methoxycarbonyl-4-piperidone hydrochloride (300 mg, 1.44 mmol) and triethylamine (0.208 mL, 2.02 mmol), and the mixture was stirred at rt until the starting material was consumed. The solution was washed with water, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (SiO_2, EtOAc/*n*-hexane = 1/4 (v/v)) to afford **17b** (392 mg, quant.) as an oil. MS (ESI/APCI) m/z: 172.3, 216.2 (calcd for $C_8H_{14}NO_3$ $(M–Boc+H)^+$: 172.1, for $C_9H_{14}NO_5$ $(M–t\text{Bu}+H)^+$: 216.1).



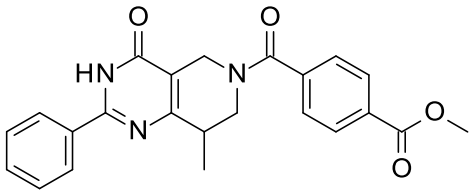
***tert*-butyl 8-methyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6(4*H*)-carboxylate (19b)**

To a suspension of **17b** (387 mg, 1.43 mmol) and benzamidine hydrochloride (246 mg, 1.57 mmol) in ethanol (6 mL) was added potassium carbonate (435 mg, 3.14 mmol) at rt. The mixture was stirred at rt overnight. Insoluble materials were removed by filteration, and the filtrate was concentrated *in vacuo*. Purification by column chromatography (SiO_2, EtOAc/CH_2Cl_2 = 1/9 – 1/1 (v/v)) afforded **19b** (303 mg, 62% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 12.80 (1H, s), 8.12-8.06 (2H, m), 7.60-7.49 (3H, m), 4.44-4.06 (2H, m), 3.60-3.49 (2H, m), 2.82-2.73 (1H, m), 1.44 (9H, s), 1.22 (3H, d, J = 6.7 Hz). MS (ESI/APCI) m/z: 342.2 (calcd for $C_{19}H_{24}N_3O_3$ $(M+H)^+$: 342.2).



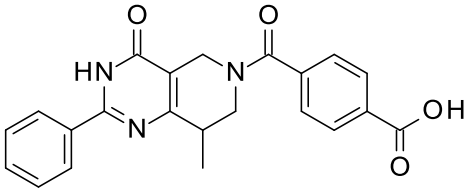
8-methyl-2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one HCl (S15)

To a MeOH (1.5 mL) suspension of **19b** (157 mg, 0.46 mmol) was added 4M HCl in 1,4-dioxane (1.5 mL) at rt. The mixture was stirred at rt for 1.5 h. Concentration under reduced pressure gave crude **S15** (128 mg, quant.) as a white solid, which was used for the next step without further purification. MS (ESI/APCI) m/z: 242.1 (calcd for $C_{14}H_{16}N_3O$ $(M+H)^+$: 242.1).



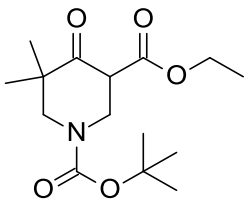
methyl 4-[(8-methyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (20b)

To a mixture of **S15** (128 mg, 0.46 mmol) and 4-methoxycarbonylbenzoic acid (91.4 mg, 0.51 mmol) in CH_2Cl_2 (4 mL) were added WSCI-HCl (107.5 mg, 0.55 mmol), HOBt-H_2O (70.6 mg, 0.46 mmol) and triethylamine (0.192 mL, 1.38 mmol). After stirring at rt overnight, the solution was diluted with CH_2Cl_2. The organic layer was washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating solvents, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 5/95 (v/v)) to give **20b** (180 mg, 97% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 12.92-12.74 (1H, m), 8.14-8.04 (4H, m), 7.65-7.49 (5H, m), 4.64-4.41 (1H, m), 4.25-4.19 (1H, m), 3.94-3.60 (5H, m), 2.96-2.81 (1H, m), 1.36-1.10 (3H, m). MS (ESI/APCI) m/z: 404.3 (calcd for $C_{23}H_{22}N_3O_4$ $(M+H)^+$: 404.2).



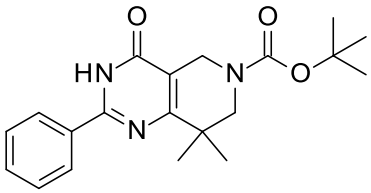
4-[(8-methyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (26)

To a MeOH (5 mL) suspension of **20b** (144 mg, 0.36 mmol) was added 1M NaOH aq. (5 mL). The mixture was stirred at rt for 2 h. 1M aq. HCl (5 mL) was added to it, and the resulting precipitate was collected by filtration, washed with water, and dried at 60 °C under reduced pressure to afford **26** (109 mg, 78% yield) as a white solid. ^{1}H-NMR (DMSO-D_6, 80 °C) δ: 12.63 (2H, br s), 8.13-7.99 (4H, m), 7.58-7.47 (5H, m), 4.50-4.28 (2H, m), 3.82-3.51 (2H, m), 2.89 (1H, br s), 1.25 (3H, s). MS (ESI/APCI) m/z: 390.3 (calcd for $C_{22}H_{20}N_3O_4$ $(M+H)^+$: 390.1).



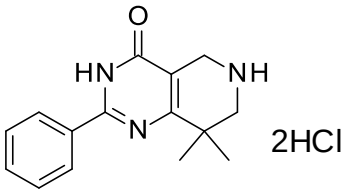
1-*tert*-butyl 3-ethyl 5,5-dimethyl-4-oxopiperidine-1,3-dicarboxylate (17c)

To a THF (5 mL) solution of 1-Boc-3,3-dimethyl-4-oxopiperidine (200 mg, 0.88 mmol) at –78 °C was added lithium bis(trimethylsilyl)amide (1.0 M in THF, 1.06 mL), and the mixture was stirred for 1 h at the same temperature. Ethyl cyanoformate (0.104 mL, 1.06 mmol) was added to the solution and the mixture was stirred for 1 h at –78 °C. Water was added to the mixture, and it was extracted with EtOAc (3 times). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude **17c** (263 mg, quant.), which was used for the next step without further purification. MS (ESI/APCI) m/z: 200.3, 244.2 (calcd for $C_{10}H_{18}NO_3$ $(M–Boc+H)^+$: 200.1, for $C_{11}H_{18}NO_5$ $(M–t\text{Bu}+H)^+$: 244.1).



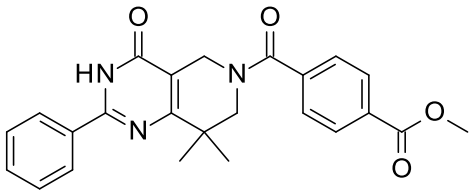
***tert*-butyl 8,8-dimethyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6(4*H*)-carboxylate (19c)**

To a suspension of **17c** (263 mg, 0.88 mmol) and benzamidine hydrochloride (152 mg, 0.97 mmol) in ethanol (10 mL) was added potassium carbonate (267 mg, 1.93 mmol) at rt. The mixture was stirred at rt overnight. Insoluble materials were removed by filteration, and the filtrate was concentrated *in vacuo*. Purification by column chromatography (SiO_2, $MeOH/CH_2Cl_2$ = 0/100 – 5/95 (v/v)) afforded **19c** (233 mg, 75% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.79 (1H, s), 8.11 (2H, d, J = 7.3 Hz), 7.58-7.51 (3H, m), 4.27 (2H, s), 3.42 (2H, s), 1.44 (9H, s), 1.22 (6H, s). MS (ESI/APCI) m/z: 356.3 (calcd for $C_{20}H_{26}N_3O_3$ $(M+H)^+$: 356.2).



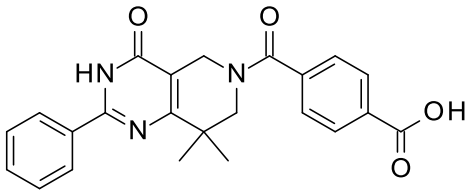
8,8-dimethyl-2-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one 2HCl (S16)

To a MeOH (10 mL) suspension of **19c** (232 mg, 0.65 mmol) was added 4M HCl in 1,4-dioxane (5 mL) at rt. The mixture was stirred at rt for 2 h. Concentration *in vacuo* and trituration with EtOAc gave **S16** (190 mg, 89% yield) as a colorless solid. MS (ESI/APCI) m/z: 256.3 (calcd for $C_{15}H_{18}N_3O$ $(M+H)^+$: 256.1).



methyl 4-[(8,8-dimethyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (20c)

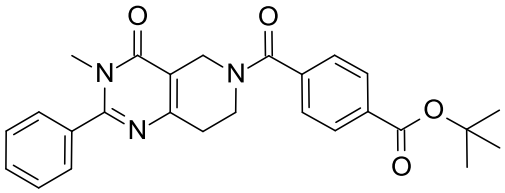
To a DMF (6 mL) solution of **S16** (100 mg, 0.30 mmol) and 4-methoxycarbonylbenzoic acid (61 mg, 0.34 mmol) were added WSCI-HCl (71 mg, 0.37 mmol), HOBt (41 mg, 0.30 mmol) and triethylamine (0.11 mL, 0.73 mmol). The mixture was stirred at rt for 2 days. Concentration *in vacuo* and purification by column chromatography (SiO_2, $MeOH/CH_2Cl_2$ = 0/100 – 3/97 (v/v)) afforded **20c** (95 mg, 75% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.13 (2H, d, J = 8.5 Hz), 8.11-8.05 (2H, m), 7.57-7.51 (3H, m), 7.48-7.43 (2H, m), 4.87-4.39 (2H, m), 3.96 (3H, s), 3.89-3.41 (2H, m), 1.48-1.18 (6H, m). MS (ESI/APCI) m/z: 418.2 (calcd for $C_{24}H_{24}N_3O_4$ $(M+H)^+$: 418.2).



4-[(8,8-dimethyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (27)

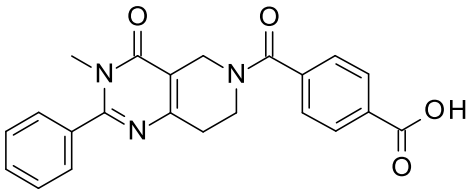
To a MeOH (10 mL) suspension of **20c** (95 mg, 0.23 mmol) was added 1M NaOH aq. (5 mL). The mixture was stirred at rt for 2 h. 1M aq. HCl (5 mL) was added to it, and the resulting precipitate was collected by filtration. The solid was washed with

water and MeOH, and dried at 60 °C under reduced pressure to afford **27** (87 mg, 95% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 12.63 (1H, br s), 8.11 (2H, d, J = 7.9 Hz), 8.02 (2H, d, J = 7.9 Hz), 7.59-7.48 (5H, m), 4.39 (2H, s), 3.61 (2H, s), 1.25 (6H, s). MS (ESI/APCI) m/z: 404.2 (calcd for $C_{23}H_{22}N_3O_4$ $(M+H)^+$: 404.2).



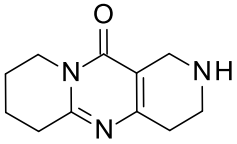
***tert*-butyl 4-[(3-methyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoate (23b)**

To a solution of **22** (201 mg, 0.53 mmol) and *N*-methylbenzenecarboximidamide hydrochloride (100 mg, 0.59 mmol) in ethanol (3.2 mL) was added potassium carbonate (163 mg, 1.17 mmol). The reaction mixture was stirred at 70 °C for 6 h. The mixture was diluted with ethanol, and insoluble materials were removed by filtration. The filtrate was concentrated *in vacuo* and purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 = 1/9 – 4/6 (v/v)) to afford **23b** (82.5mg, 35% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.02-7.97 (2H, m), 7.63-7.58 (4H, m), 7.56-7.51 (3H, m), 4.54-4.22 (2H, m), 3.96-3.51 (2H, m), 3.36-3.23 (3H, m), 2.76-2.68 (2H, m), 1.57 (9H, s). MS (ESI/APCI) m/z: 446.2 (calcd for $C_{26}H_{28}N_3O_4$ $(M+H)^+$: 446.2).



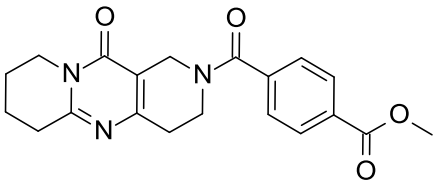
4-[(3-methyl-4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)carbonyl]benzoic acid (28)

To a CH_2Cl_2 (1.5 mL) solution of **23b** (38.5 mg, 0.086 mmol) was added trifluoroacetic acid (0.5 mL) at rt. The mixture was stirred at rt for 6 h. Concentration *in vacuo* and tritulation with Et_2O afforded **28** (19.4 mg, 58% yield) as a white solid. ^{1}H-NMR (DMSO-D_6, 80 °C) δ: 8.03-8.00 (2H, m), 7.59-7.50 (7H, m), 4.43-4.37 (2H, m), 3.82-3.66 (2H, m), 3.30 (3H, s), 2.73-2.69 (2H, m). MS (ESI/APCI) m/z: 390.3 (calcd for $C_{22}H_{20}N_3O_4$ $(M+H)^+$: 390.1).



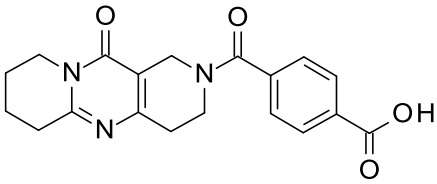
1,2,3,4,6,7,8,9-octahydro-11*H*-dipyrido[1,2-*a*:4',3'-*d*]pyrimidin-11-one (S17)

To a suspension of ethyl 4-oxo-3-piperidinecarboxylate hydrochloride (1.00 g, 4.82 mmol) and 2-iminopiperidine hydrochloride (650 mg, 4.82 mmol) in ethanol (9 mL) was added sodium ethoxide solution (6 mL, *ca*. 20% in ethanol, 15.5 mmol) at rt. Insoluble salt was removed by filtration, and the filtrate was stirred at 85–90 °C for 6 h. The resulting precipitate was collected by filtration, washed with CH_2Cl_2, dried under reduced pressure, and purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 20/80 (v/v)) to afford **S17** (384 mg, 39% yield) as a yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 3.95 (2H, t, J = 6.1 Hz), 3.81 (2H, s), 3.14-3.10 (2H, m), 2.91-2.87 (2H, m), 2.63-2.59 (2H, m), 2.00-1.85 (4H, m). MS (ESI/APCI) m/z: 206.2 (calcd for $C_{11}H_{16}N_3O$ $(M+H)^+$: 206.1).



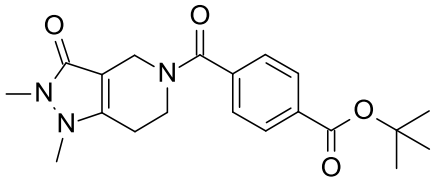
methyl 4-[(11-oxo-4,6,7,8,9,11-hexahydro-1*H*-dipyrido[1,2-*a*:4',3'-*d*]pyrimidin-2(3*H*)-yl)carbonyl]benzoate (20d)

To a solution of **S17** (78.3 mg, 0.38 mmol) and 4-methoxycarbonylbenzoic acid (76 mg, 0.42 mmol) in CH_2Cl_2 (4 mL) were added WSCI-HCl (88.2 mg, 0.46 mmol), HOBt-H_2O (58.2 mg, 0.38 mmol) and triethylamine (0.159 mL, 1.14 mmol). After stirring overnight at rt, the solution was diluted with CH_2Cl_2. The organic layer was washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and filtrated. After evaporating solvents, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 4/96 – 12/88 (v/v)) to give **20d** (126 mg, 90% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 8.04 (2H, d, J = 7.9 Hz), 7.62-7.56 (2H, m), 4.45-4.12 (2H, m), 3.93-3.81 (5H, m), 3.77-3.46 (2H, m), 2.84-2.76 (2H, m), 2.69-2.60 (2H, m), 1.93-1.71 (4H, m). MS (ESI/APCI) m/z: 368.3 (calcd for $C_{20}H_{22}N_3O_4$ $(M+H)^+$: 368.2).



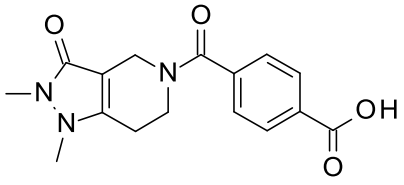
4-[(11-oxo-4,6,7,8,9,11-hexahydro-1*H*-dipyrido[1,2-*a*:4',3'-*d*]pyrimidin-2(3*H*)-yl)carbonyl]benzoic acid (29)

To a MeOH (5 mL) solution of **20d** (107 mg, 0.29 mmol) was added 1M NaOH aq. (5 mL). The mixture was stirred at rt for 2 h. After 1M aq. HCl (6 mL) was added to it until pH 2, the mixture was extracted with $CHCl_3$ (3 times) and MeOH-$CHCl_3$ (twice), respectively. The combined organic layers were dried over anhydrous Na_2SO_4, filtrated, concentrated *in vacuo*, and triturated with EtOAc to afford **29** (69 mg, 67% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 13.19 (1H, s), 8.01 (2H, d, J = 8.5 Hz), 7.60-7.52 (2H, m), 4.45-4.13 (2H, m), 3.91-3.81 (2H, m), 3.76-3.47 (2H, m), 2.83-2.77 (2H, m), 2.68-2.59 (2H, m), 1.95-1.72 (4H, m). MS (ESI/APCI) m/z: 354.2 (calcd for $C_{19}H_{20}N_3O_4$ $(M+H)^+$: 354.1).



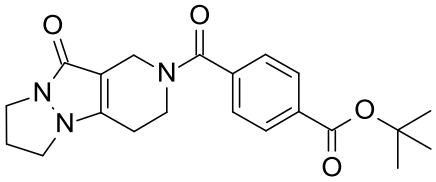
***tert*-butyl 4-[(1,2-dimethyl-3-oxo-1,2,3,4,6,7-hexahydro-5*H*-pyrazolo[4,3-*c*]pyridin-5-yl)carbonyl]benzoate (31a)**

A mixture of **22** (91 mg, 0.24 mmol), 1,2-dimethylhydrazine dihydrochloride (39 mg, 0.29 mmol) and triethylamine (0.17 mL, 1.2 mmol) in ethanol (5 mL) was refluxed overnight. After evaporating solvents, water was added to the residue and it was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and purified by column chromatography (SiO_2, MeOH/CH_2Cl_2) to afford **31a** (55 mg, 61% yield) as a colorless solid. MS (ESI/APCI) m/z: 372.4 (calcd for $C_{20}H_{26}N_3O_4$ $(M+H)^+$: 372.2).



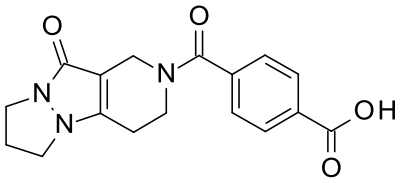
4-[(1,2-dimethyl-3-oxo-1,2,3,4,6,7-hexahydro-5*H*-pyrazolo[4,3-*c*]pyridin-5-yl)carbonyl]benzoic acid (32)

To a CH_2Cl_2 (2 mL) solution of **31a** (55 mg, 0.15 mmol) was added trifluoroacetic acid (2 mL) at rt. The mixture was stirred at rt for 6 h. Concentration *in vacuo* and trituration with Et_2O afforded **32** (18 mg, 39% yield) as a pale brown solid. ^{1}H-NMR (DMSO-D_6) δ: 8.01 (2H, d, J = 7.9 Hz), 7.61-7.51 (2H, m), 4.34-3.45 (4H, m), 3.29-3.19 (6H, m), 2.71-2.58 (2H, m). MS (ESI/APCI) m/z: 316.3 (calcd for $C_{16}H_{18}N_3O_4$ $(M+H)^+$: 316.1).



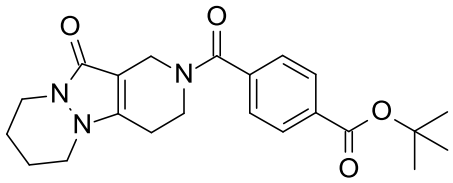
***tert*-butyl 4-[(10-oxo-4,7,8,10-tetrahydro-1*H*,6*H*-pyrazolo[1',2':1,2]pyrazolo[4,3-*c*]pyridin-2(3*H*)-yl)carbonyl]benzoate (31b)**

A mixture of **22** (84 mg, 0.22 mmol), pyrazolidine dihydrochloride (39 mg, 0.27 mmol) and triethylamine (0.155 mL, 1.11 mmol) in ethanol (5 mL) was refluxed overnight. After evaporating solvents, water was added to the residue and it was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and purified by column chromatography (SiO_2, EtOAc/*n*-hexane) to afford **31b** (86 mg, quant.) as a pale yellow oil, which was used for the next step without further purification. MS (ESI/APCI) m/z: 384.3 (calcd for $C_{21}H_{26}N_3O_4$ $(M+H)^+$: 384.2).



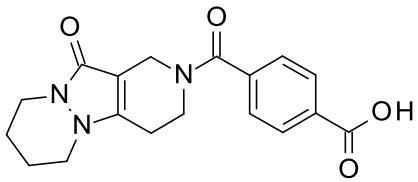
4-[(10-oxo-4,7,8,10-tetrahydro-1*H*,6*H*-pyrazolo[1',2':1,2]pyrazolo[4,3-*c*]pyridin-2(3*H*)-yl)carbonyl]benzoic acid (33)

To a CH_2Cl_2 (2 mL) solution of **31b** (86 mg, 0.22 mmol) was added trifluoroacetic acid (2 mL) at rt. The mixture was stirred at rt for 6 h. Concentration *in vacuo* and trituration with Et_2O afforded **33** (22 mg, 24% yield) as a pale brown solid. ^{1}H-NMR (DMSO-D_6) δ: 8.06-7.95 (2H, m), 7.62-7.47 (2H, m), 4.35-3.45 (10H, m), 2.69-2.52 (2H, m). MS (ESI/APCI) m/z: 328.3 (calcd for $C_{17}H_{18}N_3O_4$ $(M+H)^+$: 328.1).



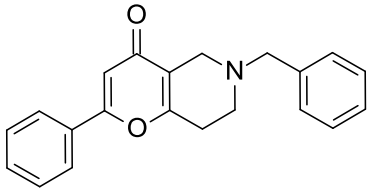
***tert*-butyl 4-[(11-oxo-4,6,7,8,9,11-hexahydro-1*H*-pyrido[3',4':4,5]pyrazolo[1,2-*a*]pyridazin-2(3*H*)-yl)carbonyl]benzoate (31c)**

A mixture of **22** (72 mg, 0.19 mmol), hexahydropyridazine dihydrochloride (40 mg, 0.23 mmol) and triethylamine (0.140 mL, 1.01 mmol) in ethanol (5 mL) was refluxed overnight. After evaporating solvents, water was added to the residue and it was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and purified by column chromatography (SiO_2, EtOAc/*n*-hexane) to afford **31c** (57 mg, 75% yield) as a pale yellow oil. MS (ESI/APCI) m/z: 398.2 (calcd for $C_{22}H_{28}N_3O_4$ $(M+H)^+$: 398.2).



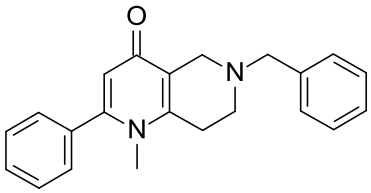
4-[(11-oxo-4,6,7,8,9,11-hexahydro-1*H*-pyrido[3',4':4,5]pyrazolo[1,2-*a*]pyridazin-2(3*H*)-yl)carbonyl]benzoic acid (34)

To a CH_2Cl_2 (2 mL) solution of **31c** (57 mg, 0.14 mmol) was added trifluoroacetic acid (2 mL) at rt. The mixture was stirred at rt for 6 h. Concentration *in vacuo* and trituration with Et_2O afforded **34** (25 mg, 51% yield) as a pale brown solid. ^{1}H-NMR (DMSO-D_6) δ: 8.07-8.00 (2H, m), 7.59-7.52 (2H, m), 4.31-3.99 (2H, m), 3.92-3.38 (6H, m), 2.67-2.56 (2H, m), 1.87-1.69 (4H, m). MS (ESI/APCI) m/z: 342.1 (calcd for $C_{18}H_{20}N_3O_4$ $(M+H)^+$: 342.1).



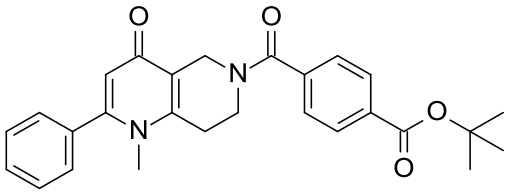
6-benzyl-2-phenyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridin-4-one (36)

A toluene (25 mL) solution of 1-benzyl-4-piperidone (1.89 mL, 10.6 mmol) and morpholine (0.921 mL, 10.6 mmol) was refluxed for 9 h under Dean-Stark apparatus. After the removal of the solvent, the residue and morpholine (0.46 mL, 5.28 mmol) were dissolved in toluene (35 mL) again and refluxed for 8 h under the same conditions. After concentrated under reduced pressure, crude 4-(1-benzyl-3,6-dihydro-2*H*-pyridin-4-yl)morpholine (2.71 g, quant.) was obtained as a yellow oil. This crude material (943.6 mg, 3.65 mmol) and ethyl benzoylacetate (1.27 mL, 7.31 mmol) were dissolved in xylenes (10 mL) and refluxed for 5 h under Dean-Stark apparatus. The mixture was poured into 1M aq. HCl, and the resulting precipitate was collected by filtration, washed with a small portion of water and CH_2Cl_2, dried at 60 ºC to afford HCl salt of **37** (215 mg, 17% yield) as a yellow solid. The aqueous phase was washed with CH_2Cl_2, and NaOH pellets were added into it until the aqueous phase became basic indicated by pH test paper. It was extracted with CH_2Cl_2 (three times), and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, EtOAc/*n*-hexane = 1/3 – 1/1 (v/v)) afforded a free form of **36** (104 mg, 9% yield) as a yellow solid. ^{1}H-NMR ($CDCl_3$, for free form) δ: 7.78-7.72 (2H, m), 7.51-7.44 (3H, m), 7.39-7.27 (5H, m), 6.72 (1H, s), 3.74 (2H, s), 3.51 (2H, s), 2.79 (4H, s). MS (ESI/APCI) m/z: 318.2 (calcd for $C_{21}H_{20}NO_2$ $(M+H)^+$: 318.1).



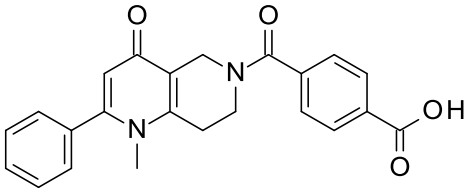
6-benzyl-1-methyl-2-phenyl-5,6,7,8-tetrahydro-1,6-naphthyridin-4(1*H*)-one (37)

A mixture of **36** (103 mg, 0.32 mmol) and methylamine solution (40% in water, 11 mL) was refluxed for 12 h. After evaporating the solvent, the residue was dissolved in 40% MeOH solution of methylamine (4 mL) and stirred at 100 ºC for 1 h under microwave irradiation. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 1/99 – 10/90 (v/v)) to afford **37** (93.4 mg, 87% yield) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 7.50-7.25 (10H, m), 6.29 (1H, s), 3.75 (2H, s), 3.65 (2H, s), 3.32 (3H, s), 2.81-2.72 (4H, m). MS (ESI/APCI) m/z: 331.4 (calcd for $C_{22}H_{23}N_2O$ $(M+H)^+$: 331.2).



***tert*-butyl 4-[(1-methyl-4-oxo-2-phenyl-1,5,7,8-tetrahydro-1,6-naphthyridin-6(4*H*)-yl)carbonyl]benzoate (S18)**

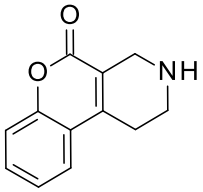
To a MeOH (8 mL) solution of **37** (93.4 mg, 0.28 mmol) and 1M aq. HCl (0.311 mL) was added 20% $Pd(OH)_2/C$ (44.7 mg) under N_2 atmosphere. The mixture was vigorously stirred at rt under H_2 atmosphere (balloon) for 50 min. Under N_2, the mixture was diluted with MeOH, and filtered over a glass-fiber filter (twice). The filtrate was concentrated at 35 °C until the liquid volume became *ca*. 5 mL. To this solution were added 4-(*tert*-butoxycarbonyl)benzoic acid (114 mg, 0.51 mmol), WSCI-HCl (135 mg, 0.71 mmol), HOBt-H_2O (78.3 mg, 0.51 mmol) and triethylamine (0.157 mL, 1.13 mmol). The mixture was stirred at rt for 2 h. After evaporating solvents, the residue was diluted with CH_2Cl_2, washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and filtrated. Concentration under reduced pressure followed by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 10/90 (v/v)) afforded **S18** (113 mg, 90% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6, 80 °C) δ: 8.00-7.95 (2H, m), 7.58-7.54 (2H, m), 7.53-7.49 (3H, m), 7.43-7.38 (2H, m), 5.94-5.91 (1H, m), 4.36-4.28 (2H, m), 3.90-3.64 (2H, m), 3.30 (3H, s), 2.93-2.86 (2H, m), 1.57 (9H, s). MS (ESI/APCI) m/z: 445.2 (calcd for $C_{27}H_{29}N_2O_4$ $(M+H)^+$: 445.2).



4-[(1-methyl-4-oxo-2-phenyl-1,5,7,8-tetrahydro-1,6-naphthyridin-6(4*H*)-yl)carbonyl]benzoic acid (38)

To a CH_2Cl_2 (3 mL) solution of **S18** (92.7 mg, 0.21 mmol) was added trifluoroacetic acid (1 mL) at rt. The mixture was stirred at rt for 2 h. Concentration *in vacuo* and trituration with Et_2O afforded **38** (81 mg, quant.) as a white solid. ^{1}H-NMR (DMSO-D_6, 80 °C) δ: 8.05-8.01 (2H, m), 7.60-7.53 (5H, m), 7.47-7.42 (2H, m), 6.34 (1H, s), 4.51-4.44 (2H, m), 3.83-3.76 (2H, m), 3.46 (3H, s), 3.04-2.98 (2H, m). MS (ESI/APCI) m/z: 389.2 (calcd for $C_{23}H_{21}N_2O_4$ $(M+H)^+$: 389.1).

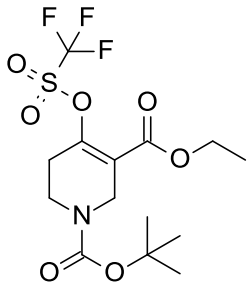
Synthesis of 39 (1st generation)



1,2,3,4-tetrahydro-5*H*-chromeno[3,4-*c*]pyridin-5-one (39)

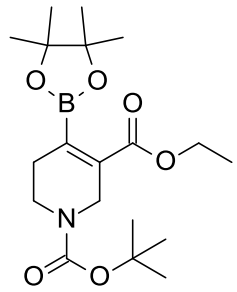
The procedure was followed by the reported one.[3]

Synthesis of 39 (2nd generation)



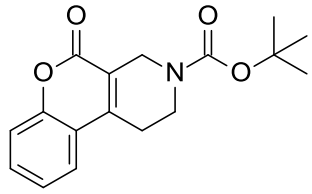
1-*tert*-butyl 3-ethyl 4-{[(trifluoromethyl)sulfonyl]oxy}-5,6-dihydropyridine-1,3(2*H*)-dicarboxylate (S19)

To a CH_2Cl_2 (200 mL) solution of *N*-Boc-4-oxo-3-piperidinecarboxylic acid ethyl ester (7.22 g, 26.6 mmol) and *N*,*N*-diisopropylethylamine (6.83 mL, 39.9 mmol) was added trifluoromethanesulfonic anhydride (5.37 mL, 31.9 mmol) in CH_2Cl_2 (25 mL) dropwise for 1 h at 0 °C. After stirring for 3 h at the same temperature, sat. aq. $NaHCO_3$ solution was added to the reaction mixture and it was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, EtOAc/*n*-hexane = 3/97 – 20/80 (v/v)) to give **S19** (7.80 g, 73% yield) as a yellow oil. The ^{1}H-NMR spectrum was matched with the reported one.[4]



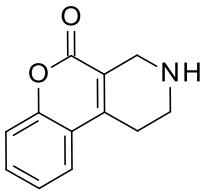
1-*tert*-butyl 3-ethyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1,3(2*H*)-dicarboxylate (42)

A mixture of **S19** (4.59 g, 11.4 mmol), bis(pinacolato)diboron (3.47 g, 13.7 mmol), potassium acetate (3.37 g, 34.3 mmol) and Pd(dppf)Cl_2 CH_2Cl_2 adduct (930 mg, 1.14 mmol) in 1,4-dioxane (50 mL) was stirred at 110 °C for 3 h under N_2 atmosphere. After cooling down to rt, the mixture was filtered through Celite and concentrated *in vacuo*. Purification by column chromatography (SiO_2, EtOAc/*n*-hexane = 1/9 – 1/3 (v/v)) afforded **42** (3.43 g, 79% yield) as a pale yellow oil. The ^{1}H-NMR spectrum was matched with the reported one.[5]



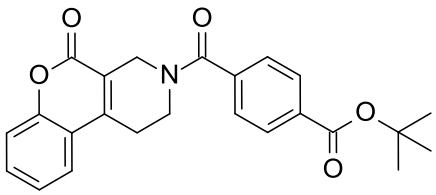
***tert*-butyl 5-oxo-1,5-dihydro-2*H*-chromeno[3,4-*c*]pyridine-3(4*H*)-carboxylate (S20)**

A mixture of **42** (500 mg, 1.31 mmol), 2-bromophenol (0.145 mL, 1.38 mmol), Pd(dppf)Cl_2 CH_2Cl_2 adduct (53.5 mg, 0.066 mmol), $NaHCO_3$ (275 mg, 3.28 mmol), THF (10 mL) and water (2.5 mL) was refluxed for 5.5 h. After cooling down to rt, the mixture was diluted with EtOAc, and filtered over Celite. The filtrate was concentrated *in vacuo*, and purified by column chromatography (SiO_2, EtOAc/*n*-hexane = 1/9 – 4/6 (v/v)) to afford **S20** (177 mg, 45% yield) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 7.54 (1H, d, J = 7.3 Hz), 7.52-7.47 (1H, m), 7.33 (1H, d, J = 8.3 Hz), 7.32-7.28 (1H, m), 4.40 (2H, s), 3.73 (2H, t, J = 5.9 Hz), 2.90-2.85 (2H, m), 1.47 (9H, s).



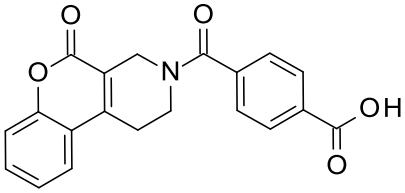
1,2,3,4-tetrahydro-5*H*-chromeno[3,4-*c*]pyridin-5-one (39)

To a solution of **S20** (174 mg, 0.58 mmol) in CH_2Cl_2 (3 mL) was added trifluoroacetic acid (1 mL) at 0 ºC. The mixture was stirred at rt for 3.5 h. After evaporating volatile materials, sat. aq. $NaHCO_3$ was added to the residue, and it was extracted with CH_2Cl_2 (twice). The combined organic layer was dried over anhydrous Na_2SO_4, filtrated, and concentrated under reduced pressure to give **39** (123 mg, quant.) as a pale yellow solid. The analytical spectra were confirmed to be the same as the product **39** obtained from the 1st generation route. ^{1}H-NMR (DMSO-D_6) δ: 7.69 (1H, d, J = 7.8 Hz), 7.55 (1H, t, J = 7.1 Hz), 7.38-7.33 (2H, m), 3.54 (2H, s), 2.96 (2H, t, J = 5.6 Hz), 2.72 (2H, t, J = 5.6 Hz). MS (ESI/APCI) m/z: 202.2 (calcd for $C_{12}H_{12}NO_2$ $(M+H)^+$: 202.1).



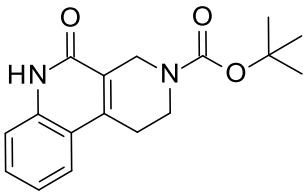
***tert*-butyl 4-[(5-oxo-1,5-dihydro-2*H*-chromeno[3,4-*c*]pyridin-3(4*H*)-yl)carbonyl]benzoate (40)**

To a suspension of **39** (120 mg, 0.596 mmol), 4-(*tert*-butoxycarbonyl)benzoic acid (146 mg, 0.656 mmol), HOBt-H_2O (92 mg, 0.596 mmol) and WSCI-HCl (138 mg, 0.716 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (0.248 mL, 1.79 mmol) at rt. After stirring at rt for 4.5 h, the reaction mixture was diluted with CH_2Cl_2, and washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine, respectively. The organic layer was dried over anhydrous Na_2SO_4, and purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 = 4/96 – 20/80 (v/v)) to give **40** (227 mg, 94% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 8.02-7.97 (2H, m), 7.82-7.71 (1H, m), 7.66-7.58 (3H, m), 7.49-7.39 (2H, m), 4.56-4.24 (2H, m), 3.99-3.56 (2H, m), 3.06-2.97 (2H, m), 1.57 (9H, s). MS (ESI/APCI) m/z: 350.3, 406.1 (calcd for $C_{20}H_{16}NO_5$ (M–*t*Bu+H)$^+$: 350.1, for $C_{24}H_{24}NO_5$ $(M+H)^+$: 406.2).



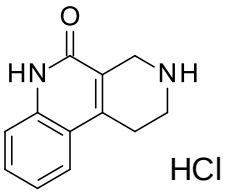
4-[(5-oxo-1,5-dihydro-2*H*-chromeno[3,4-*c*]pyridin-3(4*H*)-yl)carbonyl]benzoic acid (41, DS44960156)

To a CH_2Cl_2 (3 mL) solution of **40** (184 mg, 0.45 mmol) was added trifluoroacetic acid (1 mL) dropwise at rt. After stirring for 1.5 h at rt, the resulting mixture was concentrated *in vacuo*, and triturated with EtOAc. The solid was collected by filtration, and washed with EtOAc to afford **41** (DS44960156, 125.7 mg, 79% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 13.21 (1H, br s), 8.06-8.01 (2H, m), 7.83-7.72 (1H, m), 7.67-7.58 (3H, m), 7.49-7.39 (2H, m), 4.56-4.26 (2H, m), 3.99-3.57 (2H, m), 3.05-2.98 (2H, m). ^{13}C-NMR (DMSO-D_6, as a mixture of rotamers) δ: 168.8, 168.4, 166.7, 158.9, 151.5, 146.6, 146.3, 139.6, 131.8, 131.4, 129.5, 127.3, 127.0, 124.6, 124.2, 119.3, 118.8, 116.4, 45.1, 42.6, 40.2, 37.5, 24.9, 23.9. MS (ESI/APCI) m/z: 350.3 (calcd for $C_{20}H_{16}NO_5$ $(M+H)^+$: 350.1). HRMS (ESI): m/z calcd for $C_{20}H_{14}NO_5$ $(M–H)^-$ 348.0878. Found 348.0895. IR (KBr) 3408, 2895, 2673, 2554, 1709, 1648, 1607, 1573, 1511, 1456, 1433, 1397, 1320, 1299, 1258, 1142, 1089, 1042, 749 cm^{-1}. Anal. Calcd for $C_{20}H_{15}NO_5 \cdot 0.2H_2O$: C, 68.06; H, 4.40; N, 3.97. Found: C, 67.94; H, 4.48; N, 3.97.



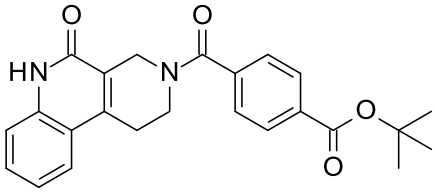
***tert*-butyl 5-oxo-1,4,5,6-tetrahydrobenzo[*c*][2,7]naphthyridine-3(2*H*)-carboxylate (S21)**

A mixture of **42** (100 mg, 0.26 mmol), 2-bromoaniline (46.4 mg, 0.27 mmol), Pd(dppf)Cl_2 CH_2Cl_2 adduct (10.6 mg, 0.013 mmol), $NaHCO_3$ (56.7 mg, 0.67 mmol), THF (2 mL) and water (0.5 mL) was stirred at 100 ºC for 1.5 h under microwave irradiation. After cooling down to rt, the mixture was diluted with EtOAc, washed with water (twice) and brine, and dried over anhydrous Na_2SO_4. Filtration, concentration under reduced pressure, and trituration with ethanol afforded **S21** (45 mg, 57% yield) as a white solid. ^{1}H-NMR (DMSO-D_6) δ: 11.85 (1H, s), 7.71 (1H, d, J = 7.3 Hz), 7.49 (1H, t, J = 7.3 Hz), 7.33 (1H, d, J = 7.9 Hz), 7.22 (1H, t, J = 7.3 Hz), 4.26 (2H, s), 3.66-3.62 (2H, m), 2.94-2.88 (2H, m), 1.44 (9H, s). MS (ESI/APCI) m/z: 245.1, 301.2 (calcd for $C_{13}H_{13}N_2O_3$ (M–*t*Bu+H)$^+$: 245.1, for $C_{17}H_{21}N_2O_3$ (M+H)$^+$: 301.1).



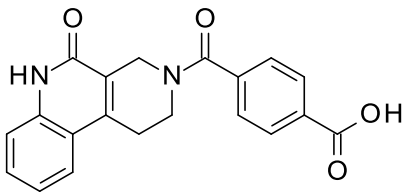
2,3,4,6-tetrahydrobenzo[*c*][2,7]naphthyridin-5(1*H*)-one HCl (43)

To a suspension of **S21** (42.7 mg, 0.14 mmol) in MeOH (2 mL) was added 4M HCl in 1,4-dioxane (1 mL) at rt. The mixture was stirred at rt for 1 h. Concentration under reduced pressure gave crude **43** (33.7 mg, quant.) as a white solid, which was used for the next step without further purification.



***tert*-butyl 4-[(5-oxo-1,4,5,6-tetrahydrobenzo[*c*][2,7]naphthyridin-3(2*H*)-yl)carbonyl]benzoate (44)**

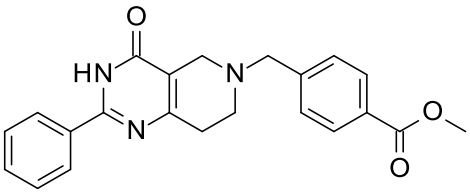
To a solution of **43** (33.7 mg, 0.14 mmol), 4-(*tert*-butoxycarbonyl)benzoic acid (146 mg, 0.656 mmol) and triethylamine (0.0197 mL, 0.14 mmol) in MeOH (3 mL) was added DMT-MM hydrate (46.2 mg, 0.16 mmol) at rt. After stirring at rt for 2 h, the reaction mixture was concentrated *in vacuo*, and the residue was diluted with water. It was extracted with $CHCl_3$, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, and filtrated. Purification by column chromatography (SiO_2, MeOH/CH_2Cl_2 = 0/100 – 10/90 (v/v)) to give **44** (49.7 mg, 86% yield) as a white solid. ^{1}H-NMR (DMSO-D_6, 80 ºC) δ: 11.58 (1H, s), 7.99-7.96 (2H, m), 7.70-7.66 (1H, m), 7.57-7.54 (2H, m), 7.49-7.45 (1H, m), 7.35-7.32 (1H, m), 7.23-7.19 (1H, m), 4.45-4.39 (2H, m), 3.84-3.69 (2H, m), 3.01-2.98 (2H, m), 1.57 (9H, s). MS (ESI/APCI) m/z: 405.2 (calcd for $C_{24}H_{25}N_2O_4$ (M+H)$^+$: 405.2).



4-[(5-oxo-1,4,5,6-tetrahydrobenzo[*c*][2,7]naphthyridin-3(2*H*)-yl)carbonyl]benzoic acid (45)

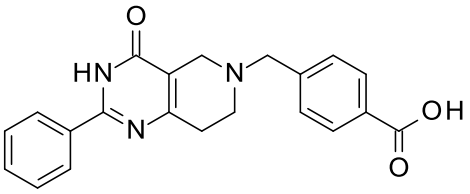
A mixture of **44** (38.6 mg, 0.095 mmol), MeOH (1 mL), and 4M HCl in 1,4-dioxane (1 mL) was stirred at rt overnight. Concentration of the reaction mixture under reduced pressure gave methyl ester of **45**. The residue was dissolved in MeOH (1 mL) and 1M NaOH aq. (1 mL) was added to it. The mixture was stirred at rt for 1.5 h. 1M aq. HCl (1 mL) and MeOH (1 mL) was added to the mixture, and the resulting precipitate was collected by filtration, washed with water, and dried. Preparative reverse-phase HPLC and following trituration with ethanol at 50 °C afforded **45** (10.3 mg, 31% yield) as a white solid. ^{1}H-NMR (DMSO-D_6, 80 °C) δ: 11.59 (1H, s), 8.03-8.00 (2H, m), 7.69 (1H, d, J = 7.9 Hz), 7.56 (2H, d, J = 7.9 Hz), 7.50-7.45 (1H, m), 7.34 (1H, d, J = 7.9 Hz), 7.23-7.19 (1H, m), 4.47-4.39 (2H, m), 3.84-3.73 (2H, m), 3.11-2.96 (2H, m). MS (ESI/APCI) m/z: 349.1 (calcd for $C_{20}H_{17}N_2O_4$ $(M+H)^+$: 349.1).

Synthetic information for the compound without the linker carbonyl group



methyl 4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)methyl]benzoate (S22)

Sodium triacetoxyborohydride (159 mg, 0.75 mmol) was added to the mixture of **S2** (150 mg, 0.50 mmol), methyl 4-formylbenzoate (91 mg, 0.55 mmol) and THF (10 mL). After stirring at rt overnight, sat. aq. NaHCO3 was added to the mixture and it was extracted with CH_2Cl_2. The organic layer was washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, EtOAc/CH_2Cl_2 = 1/1 (v/v)) afforded **S22** (50 mg, 27% yield) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 11.83 (1H, s), 8.07-8.01 (4H, m), 7.55-7.39 (5H, m), 3.93 (3H, s), 3.80 (2H, s), 3.52 (2H, s), 2.91-2.78 (4H, m). MS (ESI/APCI) m/z: 376.1 (calcd for $C_{22}H_{22}N_3O_3$ $(M+H)^+$: 376.2).



4-[(4-oxo-2-phenyl-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-6(4*H*)-yl)methyl]benzoic acid (S23)

To a MeOH (10 mL) solution of **S22** (50 mg, 0.13 mmol) was added 1M NaOH aq. (3 mL). The mixture was stirred at rt for 3 days. 1M aq. HCl (3 mL) was added to it, and the resulting precipitate was filtrated and dried at 60 °C under reduced pressure to afford **S23** (42 mg, 87% yield) as a colorless solid. ^{1}H-NMR (DMSO-D_6) δ: 12.87 (1H, br s), 12.67 (1H, br s), 8.07 (2H, d, J = 7.3 Hz), 7.94 (2H, d, J = 8.5 Hz), 7.60-7.47 (5H, m), 3.78 (2H, s), 3.27 (2H, s), 2.80-2.70 (4H, m). MS (ESI/APCI) m/z: 362.2 (calcd for $C_{21}H_{20}N_3O_3$ $(M+H)^+$: 362.1).

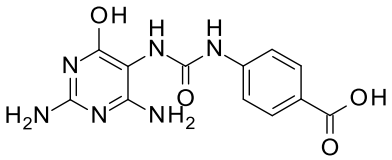
2. X-ray crystallography

Protein production of X-ray crystallography

A DNA fragment encoding human MTHFD2 (residues 36-338) was amplified by PCR and inserted into pET15b vector (Novagen) to produce an N-terminal 6xHis-tagged MTHFD2. The expression was performed in E. coli strain ArcticExpress (DE3) RIL (Agilent). After sonication and centrifugation of the cells, the supernatant was applied to a HisTrap FF crude column (GE Healthcare), and the protein was eluted with a gradient of from 20 mM to 500 mM imidazole. A subsequent gel filtration was carried out using a HiLoad 16/600 Superdex 200 pg column (GE Healthcare) with buffer consisting of 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM DTT. The MTHFD2 were collected and concentrated to 16 mg/mL.

Crystal preparation

Co-crystals of 4-[(2,4-diamino-6-hydroxy-pyrimidin-5-yl)carbamoylamino]benzoic acid[6] (**S24**, a weak binder, cell-free IC_{50} > 30 μM in our MTHFD2 enzymatic assay) and MTHFD2 were prepared using the sitting-drop vapor diffusion method at 293K in 28% *i*-PrOH/ 0.1M bis-Tris, pH 6.5/ 3% PEG200/ 10 mM spermidine. Bound compound was removed from co-crystals by incubation in 10% *i*-PrOH/ 0.1M bis-Tris HCl, pH6.5/ 3% PEG200/ 25% glycerol/ 10 mM spermidine/ 10 mM K phosphate buffer, pH 8.5 for 1 day at 293K. Then, the test compounds were introduced to MTHFD2 crystals by soaking in the same solution containing 1 to 10 mM test compound and 2.5 mM $MgCl_2$ at 293K for 1 to 4 days. Obtained crystals were flash-frozen and stored in liquid nitrogen until use.



S24

Crystal structure determination

Diffraction data were collected at the beam-lines of Tsukuba Photon factory as shown in Supplementary Table S1. After data processing, initial phase were determined by *PHASER*[7] using human MTHFD1 structure (PDB ID = 1DIA[8]) as a search model for molecular replacement. After that, phase refinement and model building were carried out using *REFMAC5*[9] and *COOT*[10]. Statistics of data processing and phase refinement are summarized in Supplementary Table S1. Figures describing crystal structures are drawn by *pymol*[11].

Table S1. Statistics for data collection and phase refinement.

[a]R_{merge} = ΣhΣj |<I(h)> - I(h)j | / ΣhΣj <I(h)>, where <I(h)> is the mean intensity of symmetry-related reflections. [b]*R*-value = Σ | |Fobs| - |Fcalc| | / Σ |Fobs|. R_{free} for 5.2% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

	compound **1**	DS44960156
Data Collection		
X-ray source	PF BL-17A	PF AR NW-12A
Wavelength (Å)	0.98	1.00
Space group	$P6_5$	$P6_5$
Unit cell dimensions	*a*=*b*=116.5 Å, *c*=113.2 Å	*a*=*b*=116.4, *c*=113.2
	α=*β*=90 °, *γ*=120 °	*α*=*β*=90 °, *γ*=120 °
Resolution (Å)	49.36-2.50	46.05-2.25
	(2.57-2.50)	(2.31-2.25)
Total No. of observations	153418 (11519)	354192 (13351)
Unique reflections	30155 (2214)	41331 (3207)
Redundancy	5.1 (5.2)	8.6 (4.2)
Completeness (%)	99.9 (99.9)	99.4 (97.1)
I/σ(I)	20.6 (2.9)	13.8 (1.0)
R_{merge}[a]	0.040 (0.470)	0.092 (0.937)
Refinement		
Resolution (Å)	25-2.5	25-2.25
No. of reflections	30124	41260
RMS Bonds (Å)	0.006	0.006
RMS Angles (°)	1.645	1.096
No. of atoms		
protein	4280	4395
water and solvent	66	181
ligand	60	104
Average B value (Å^2)		
protein	78.4	51.5
water and solvent	76.0	54.7
ligand	81.0	53.9
R-value[b]	0.2032	0.2034
R_{free}[b]	0.2542	0.2434

Figure S1. Blue mesh represents Fo-Fc map contoured at 3.0σ which is calculated without model-bias of bound compound. (A) MTHFD2-compound **1** complex (PDB ID: 6JID). (B) MTHFD2-DS44960156 complex (PDB ID: 6JIB).

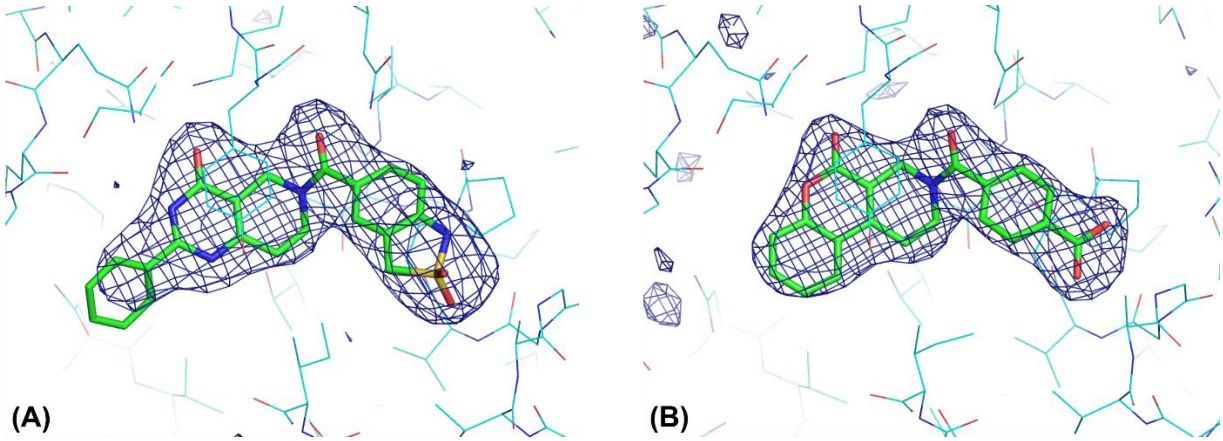
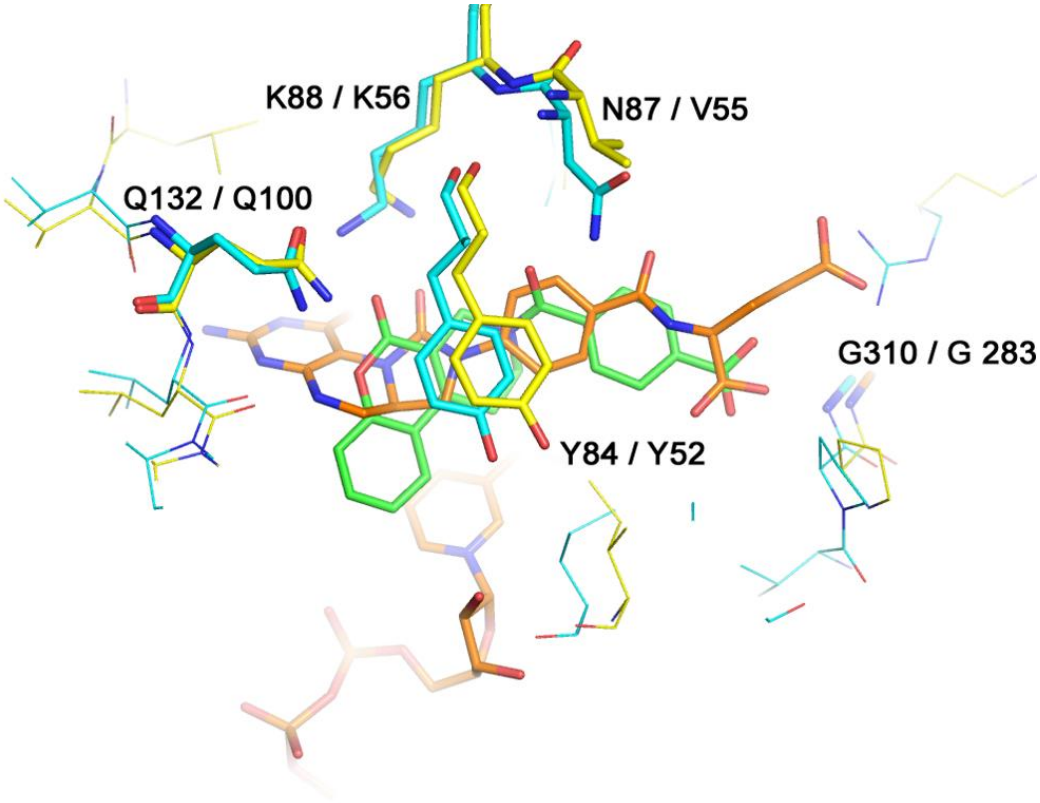


Figure S2. Superposition of MTHFD2-DS44960156 (PDB ID: 6JIB, green) and MTHFD1-LY345899-NADP (PDB ID: 6ECQ, orange). Amino acid residues within 5 Å from the ligand are displayed as line. The four residues described in the manuscript are highlighted with a caption of residue name for MTHFD2 (blue) / MTHFD1 (yellow).



3. Enzymatic assay procedure

For MTHFD2 NAD-dependent dehydrogenase assay, 0.125 μg/mL MTHFD2 recombinant protein, 100 μM NAD, 0.2 mg/mL tetrahydrofolate (THF), 2.5 mM formaldehyde, 5 mM $MgCl_2$, and 10% DMSO or compounds were mixed in 384-well plate (Greiner, 781801, UV transparent). The amount of mixture was 40 μL/well. After incubation for 30 min at room temperature, the reaction was stopped by adding HCl.

For MTHFD1 NADP-dependent dehydrogenase assay, 0.125 μg/mL MTHFD1 recombinant protein, 82.7 μM NADP, 0.3 mg/mL tetrahydrofolate (THF) , 2.5 mM formaldehyde, 5 mM $MgCl_2$, and 10% DMSO or compounds were mixed in 384-well plate (Greiner, 781801, UV transparent). The amount of mixture was 40 μL/well. After incubation for 30 min at room temperature, the reaction was stopped by adding HCl.

The product methenyl-THF was detected by the absorbance at 355 nm. IC_{50} values were calculated from quadruplicate experiments using GraphPad Prism as shown in Table S2. The revised structure of LY374571 was used for the standard for the assays.[6] MTHFD2 pIC_{50} = 6.99 ± 0.047 (*N* = 12), MTHFD1 pIC_{50} = 6.70 ± 0.043 (*N* = 6).

Table S2. List of IC_{50} values for MTHFD2 inhibitors.

[a]95% confidence intervals (95% CI) are shown in parentheses. ND: No data.

compound	MTHFD2 IC_{50} (μM)[a]		MTHFD1 IC_{50} (μM)[a]
1	8.3	(7.640 – 8.994)	>100
2	10	(8.647 – 12.25)	ND
3	2.7	(2.198 – 3.395)	>30
4	>30		ND
5	>30		ND
6	>30		ND
7	>30		ND
8	0.94	(0.8529 – 1.028)	ND
9	3.1	(2.791 – 3.384)	ND
10	1.4	(1.223 – 1.528)	ND
11	15	(11.71 – 18.85)	ND
12	10	(9.010 – 11.45)	ND
13	>30		ND
14	>30		ND
15	>30		ND
16	>30		ND
24	>30		ND
25	>30		ND
26	>30		>30
27	>30		ND
28	24	(21.21 – 27.33)	>30
29	8.2	(6.418 – 10.45)	>30
32	11	(9.841 – 12.90)	>30
33	6.6	(5.967 – 7.360)	>30
34	1.9	(1.674 – 2.108)	>30
38	2.3	(1.992 – 2.724)	>30
41	1.6	(1.395 – 1.858)	>30
45	4.6	(4.066 – 5.109)	>30
S23	>30		ND

4. References

(1) Gustafsson, R.; Jemth, A. S.; Gustafsson, N. M. S.; Farnegardh, K.; Loseva, O.; Wiita, E.; Bonagas, N.; Dahllund, L.; Llona-Minguez, S.; Haggblad, M.; et al. Crystal Structure of the Emerging Cancer Target MTHFD2 in Complex with a Substrate-Based Inhibitor. *Cancer Res.* **2017**, *77*, 937–948.

(2) Fu, C.; Sikandar, A.; Donner, J.; Zaburannyi, N.; Herrmann, J.; Reck, M.; Wagner-Döbler, I.; Koehnke, J.; Müller, R. The Natural Product Carolacton Inhibits Folate-Dependent C1 Metabolism by Targeting FolD/MTHFD. *Nat. Commun.* **2017**, *8*, 1529.

(3) Brown, R. E.; Shavel, J. J. US3946008.

(4) Zheng, G.; Smith, A. M.; Huang, X.; Subramanian, K. L.; Siripurapu, K. B.; Deaciuc, A.; Zhan, C. G.; Dwoskin, L. P. Structural Modifications to Tetrahydropyridine-3-Carboxylate Esters En Route to the Discovery of M5-Preferring Muscarinic Receptor Orthosteric Antagonists. *J. Med. Chem.* **2013**, *56*, 1693–1703.

(5) Campeau, L. C.; Dolman, S. J.; Gauvreau, D.; Corley, E.; Liu, J.; Guidry, E. N.; Ouellet, S. G.; Steinhuebel, D.; Weisel, M.; O'Shea, P. D. Convergent Kilo-Scale Synthesis of a Potent Renin Inhibitor for the Treatment of Hypertension. *Org. Process Res. Dev.* **2011**, *15*, 1138–1148.

(6) Eadsforth, T. C.; Pinto, A.; Luciani, R.; Tamborini, L.; Cullia, G.; Micheli, C. D.; Marinelli, L.; Cosconati, S.; Novellino, E.; Presti, L. L.; Cordeiro da Silva, A.; Conti, P.; Hunter, W. N.; Costi, M. P. Characterization of 2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl Ureido Based Inhibitors of *Trypanosoma brucei* FolD and Testing for Antiparasitic Activity. *J. Med. Chem.* **2015**, *58*, 7938−7948.

(7) McCoy, A. J.; Grosse-Kunstleve R. W.; Adams P. D.; Winn M. D.; Storoni L. C.; Read R. J. Phaser crystallographic software. *J. Appl. Crystallogr.* **2007**, *40*, 658–674.

(8) Schmidt, A.; Wu, H.; Mackenzie, R. E.; Chen, V. J.; Bewly, J. R.; Ray, J. E.; Toth, J. E.; Cygler, M. Structures of Three Inhibitor Complexes Provide Insight into the Reaction Mechanism of the Human Methylenetetrahydrofolate Dehydrogenase/Cyclohydrolase. *Biochemistry* **2000**, *39*, 6325–6335.

(9) Murshudov, G. N.; Skubák, P.; Lebedev, A. A.; Pannu, N. S.; Steiner, R. A.; Nicholls, R. A.; Winn, M. D.; Long, F.; Vagin A. A. REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallogr. D Biol. Crystallogr.* **2011**, *67*, 355–367.

(10) Emsley, P.; Cowtan, K. *Coot*: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2126–2132.

(11) The PyMOL Molecular Graphics System, Version 1.7.2.1 Schrödinger, LLC.