Supporting Information

Discovery of a Highly Potent and Selective MEK inhibitor: *N*-{3-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2*H*-pyrido[4,3-*d*]pyrimidin-1-yl]phenyl}acetamide dimethylsulfoxide solvate (GSK1120212, JTP-74057 DMSO solvate)

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Biological Assays

Evaluation of Cell Growth Inhibitory Activity by ^{3}H-thymidine Uptake Assay

ACHN cells (DS Pharma Biomedical, Osaka, Japan) and HT-29 cells (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI 1640 medium and McCoy's 5A medium, respectively, both of which were supplemented with 10% fetal bovine serum and antibiotics. ACHN cells or HT-29 cells were plated into 96-well plates at 1.0×10^4/90 μL/well and incubated in 5% CO_2 incubator at 37 °C for 24 h. Test compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and stored frozen at −20 °C. Half-log serial dilutions of the test compounds were prepared with DMSO at 1,000-fold higher concentrations than those to be tested and subsequently 100-fold diluted with medium to prepare test compound solutions (i.e. 10-fold higher concentrations than those evaluated). Ten microliters of the test compound solution was then added to the wells of pre-cultured cells, and incubation was continued. After 18 h, [^{3}H]thymidine (Perkin Elmer, Wellesley, MA, USA) was added at 0.25 μCi/well and the plates were further incubated at 37 °C for 6 h in 5% CO_2 incubator. The cells were then collected onto UniFilter-96 GF/B microplates (Perkin Elmer) using a cell harvester (Perkin Elmer), and the radioactivity of the ^{3}H incorporated by the cells was measured with a scintillation counter (Top Count, Perkin Elmer). The compounds were evaluated using 4 arbitrary concentrations in triplicate. IC_{50} was calculated from the equation for the line between two points on either side of 50% inhibition, with the radioactivity of the DMSO control considered 100%.

Cell growth assays and Cellular ERK1/2 phosphorylation assay

SK-MEL-28, and HCT116, cell lines were obtained from ATCC and grown in the recommended media at 37 ºC, 5% CO_2 in a humidified incubator. Cells were plated in triplicate 96 well microtitre plates at

5000 cells per well in culture media. GSK1120212 dissolved in DMSO or negative control (0.1% DMSO) were added the following day and one plate was harvested with 50 µl of CellTiter-Glo (Promega) for a time 0 (T = 0) measurement. Remaining duplicate cell plates were typically incubated for 72 h. Cells were then lysed with 50 µl CellTiter-Glo, and chemiluminescent signal was read on the Wallac EnVision 2100 plate reader. For measurement of cellular ERK1/2 phosphorylation, cells were seeded and treated with GSK1120212 as above, and lysed after 72 h in Tris lysis buffer (Meso Scale Discovery) supplemented with phosphatase and protease inhibitors (Sigma). All samples were analyzed with a phospho-ERK1/2 ELISA (Meso Scale Discovery) according to manufacturer's protocol. Plates were read on MSD.SI6000 and curves were analyzed using the XLfit (IDBS Ltd.) curve-fitting tool. For comparison of the growth assay curve and pERK1/2 assay curve, data were background subtracted and normalized to the vehicle treatment control.

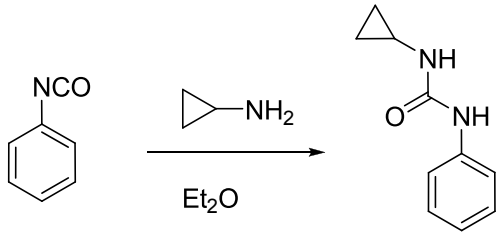
Tumor xenograft models

A549 (human non-small cell lung carcinoma) model was established from cells grown in tissue culture and harvested aseptically using a trypsin digest. Female athymic mice (strain *nu/nu*) were injected subcutaneously with between 5×10^6 and 10^7 cells in 50% martigel. Tumors were allowed to establish for one to four weeks before use. GSK1120212 was administered orally at the indicated doses in 0.2 ml/20 gram by weight in 0.5% HPMC ((Hydroxypropyl)methyl cellulose, Sigma cat # H7509-250 g) and 0.1% Tween 80 (Sigma cat # P1754-500 ml) in distilled water pH 8.0. Tumors were measured twice weekly using Vernier calipers. Antitumor activity was defined as tumor growth inhibition representing the % volume differential in tumor growth between the treated and control tumors at the time vehicle tumors exceeded a volume of 1000 mm^3. All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

Experimental Procedures

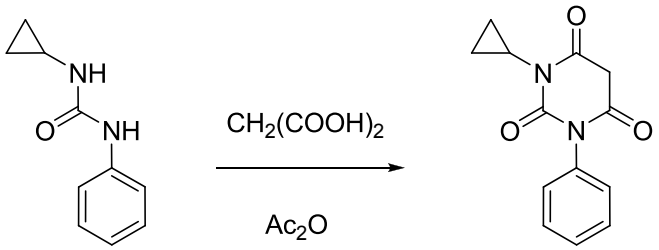
Reagents and solvents were purchased from commercially available sources and used without further purification. Flash chromatography was performed using silica gel (Kanto Chemical, 40–100 μm) with standard techniques. Melting points were determined using a Yanagimoto micro melting point apparatus or BÜCHI B-540 melting point instrument and were uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a JEOL JNM-A300W, Bruker AMX-300 or JEOL JNM-AL400 spectrometer in a solvent indicated. Chemical shifts (δ) are reported in parts per million relative to internal standard tetramethylsilane. Elemental analysis was performed with a Perkin-Elmer 2400 Series II CHNS/O analyzer. Mass spectra (ESI+) were recorded on a ThermoQuest LCQ mass spectrometer. High-resolution mass spectra were obtained with a JEOL SX 102A spectrometer. All compounds that appear in SAR tables in the manuscript were determined to be ≥90% pure by NMR.

Preparations of Compounds 3, 4a, 4b, 4e, 4j–4m:



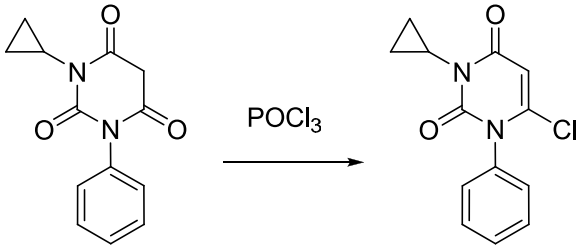
3.1 **3.2**

To an ice-water bath cooled solution of phenyl isocyanate **3.1** (2.17 mL, 20 mmol) in Et_2O (20 mL) was added a solution of cyclopropylamine (1.52 mL, 22 mmol) in Et_2O (10 mL) over 10 min. This mixture was stirred at room temperature for 1.5 h. Et_2O/hexane (1/1, 30 mL) was added, and the stirring was continued at room temperature for 30 min. The precipitate was collected by filtration, rinsed with Et_2O/hexane (1/1, 10 mL), and dried under vacuum to afford **3.2** (3.45 g, 98%) as a white solid.



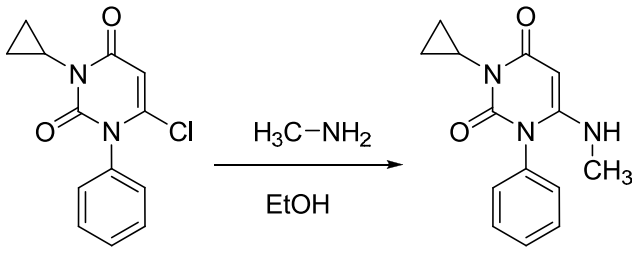
3.2 **3.3**

To a suspension of **3.2** (3.44 g, 19.5 mmol) in acetic anhydride (6.9 mL) was added malonic acid (2.23 g, 21.5 mmol). The mixture was heated in a 100 °C bath for 6 h. The mixture was concentrated at reduced pressure to a small volume and was added Et_2O/hexane (1/1, 30 mL). Resultant precipitate was collected by filtration, washed with Et_2O, and dried under vacuum to afford **3.3** (2.36 g, 49%) as a solid.



3.3 **3.4**

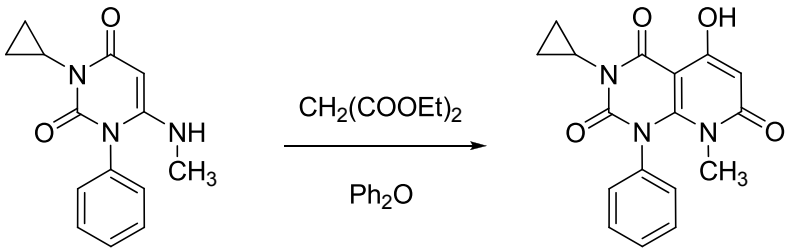
The mixture of **3.3** (2.35 g, 9.62 mmol), phosphorus oxychloride (13.5 mL, 144 mmol) and water (0.26 mL, 14.4 mmol) was heated in a 90 °C bath for 3 h. The reaction mixture was poured onto water, and extracted with ethyl acetate. The combined organic layers were washed with water and brine successively, dried over magnesium sulfate, and concentrated at reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate, 4/1) to afford **3.4** (198 mg, 8%) as a white solid.



3.4 **3.5**

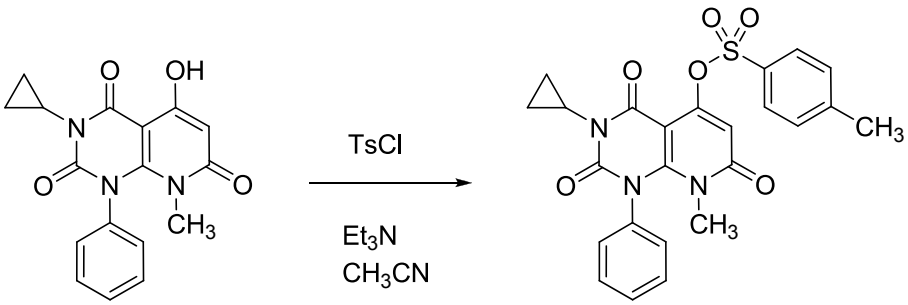
To a suspension of **3.4** (194 mg, 0.74 mmol) in ethanol (0.74 mL) was added methyl amine in methanol

(1.13 mL, 11.1 mmol, 40 wt %). The mixture was heated in an 80 °C bath for 2 h and concentrated at reduced pressure. The crude residue was diluted with chloroform, washed with water and brine successively, dried over sodium sulfate, and concentrated at reduced pressure to afford **3.5** (183 mg, 96%) as a white solid.



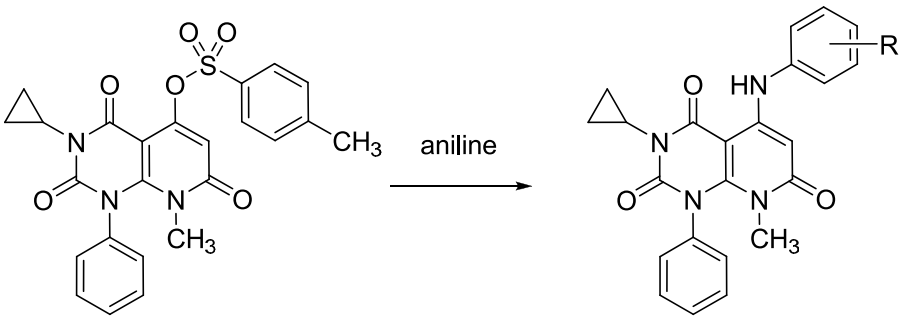
3.5 **3.6**

To a suspension of **3.5** (179 mg, 0.70 mmol) in diphenyl ether (0.35 mL) was added diethylmalonate (0.53 mL, 3.48 mmol). The mixture was heated in a 220 °C bath for 5 h and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 15/1) to afford **3.6** (191 mg, 84%) as a white solid.



3.6 **3.7**

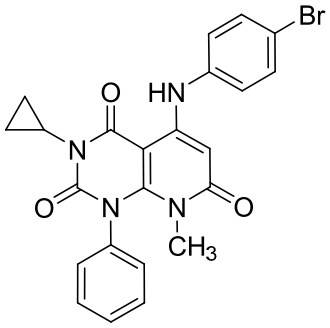
To a suspension of **3.6** (187 mg, 0.58 mmol) in acetonitrile (5.8 mL) was added *p*-toluenesulfonyl chloride (165 mg, 0.86 mmol) and triethylamine (0.12 mL, 0.86 mmol). The mixture was heated in an 80 °C bath for 3 h and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 15/1) to afford **3.7** (261 mg, 95%) as a white solid.



3.7 **3, 4a, 4b, 4e, 4j-4m**

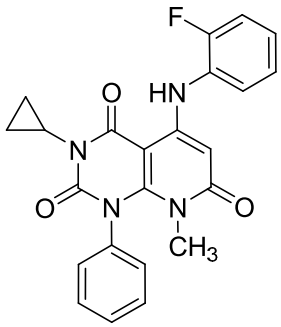
The mixture of **3.7** and aniline (10–20 equiv) was heated in a 150 °C bath for 6 h. The mixture was purified by flash chromatography to afford **3, 4a, 4b, 4e, 4j**–**4m** (56–91%) as a solid.

5-(4-Bromophenylamino)-3-cyclopropyl-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (3)



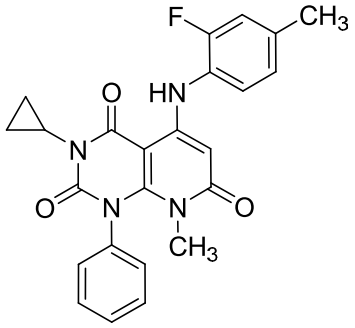
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (m, 2H), 1.19 (m, 2H), 2.76 (tt, *J* = 7.0, 4.0 Hz, 1H), 2.82 (s, 3H), 5.80 (s, 1H), 7.17 (dt, *J* = 9.3, 2.4 Hz, 2H), 7.31 (dt, *J* = 6.0, 1.8 Hz, 2H), 7.39–7.56 (m, 5H), 10.40 (s, 1H); MS (ESI) *m*/*z* 479, 481 $(M+H)^+$.

3-Cyclopropyl-5-(2-fluorophenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (4a)



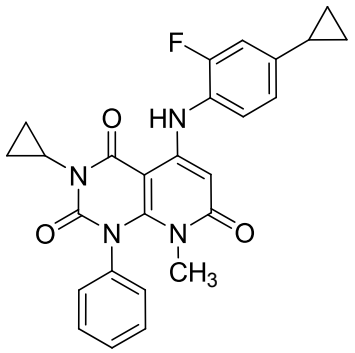
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76 (m, 2H), 1.04 (m, 2H), 2.60 (s, 3H), 2.68 (tt, J = 7.0, 4.0 Hz, 1H), 5.34 (d, J = 1.1 Hz, 1H), 7.25–7.56 (m, 9H), 10.53 (s, 1H); MS (ESI) m/z 419 $(M+H)^+$.

3-Cyclopropyl-5-(2-fluoro-4-methylphenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (4b)



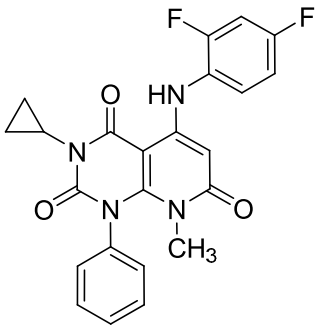
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (m, 2H), 1.18 (m, 2H), 2.37 (s, 3H), 2.77 (m, 1H), 2.81 (s, 3H), 5.59 (s, 1H), 6.98 (m, 2H), 7.25 (t, J = 8.3 Hz, 1H), 7.33 (m, 2H), 7.40–7.50 (m, 3H), 10.16 (s, 1H); MS (ESI) m/z 433 $(M+H)^+$.

3-Cyclopropyl-5-(4-cyclopropyl-2-fluorophenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (4e)



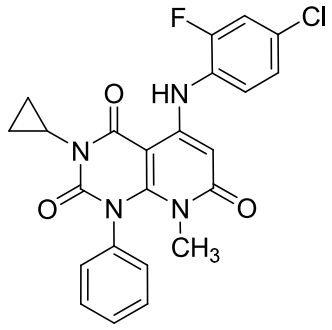
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70 (m, 2H), 0.87 (m, 2H), 1.02 (m, 2H), 1.19 (m, 2H), 1.90 (tt, J = 8.4, 5.1 Hz, 1H), 2.77 (tt, J = 7.3, 4.0 Hz, 1H), 2.81 (s, 3H), 5.58 (d, J = 1.1 Hz, 1H), 6.82–6.92 (m, 2H), 7.24 (t, J = 8.1 Hz, 1H), 7.32 (dt, J = 6.6, 2.0 Hz, 2H), 7.39–7.53 (m, 3H), 10.16 (s, 1H); MS (ESI) m/z 459 $(M+H)^+$.

3-Cyclopropyl-5-(2,4-difluorophenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (4j)



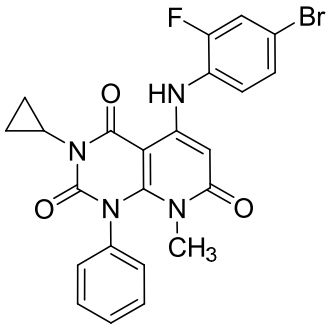
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (m, 2H), 1.19 (m, 2H), 2.77 (tt, J = 7.2, 4.0 Hz, 1H), 2.81 (s, 3H), 5.49 (s, 1H), 6.88–6.98 (m, 2H), 7.28–7.38 (m, 3H), 7.39–7.52 (m, 3H), 10.15 (s, 1H); MS (ESI) *m*/*z* 437 $(M+H)^+$.

5-(4-Chloro-2-fluorophenylamino)-3-cyclopropyl-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (4k)



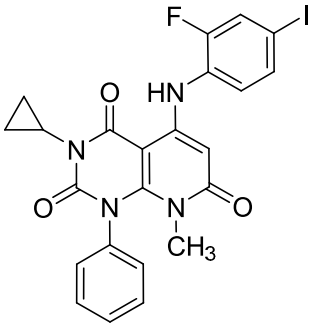
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75 (m, 2H), 1.04 (m, 2H), 2.60 (s, 3H), 2.68 (tt, J = 7.2, 3.8 Hz, 1H), 5.34 (d, J = 1.1 Hz, 1H), 7.37 (dt, J = 8.5, 1.1 Hz, 1H), 7.40–7.55 (m, 5H), 7.56 (t, J = 8.7 Hz, 1H), 7.63 (dd, J = 10.6, 2.3 Hz, 1H), 10.54 (s, 1H); MS (ESI) *m*/*z* 453 $(M+H)^+$.

5-(4-Bromo-2-fluorophenylamino)-3-cyclopropyl-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione (4l)



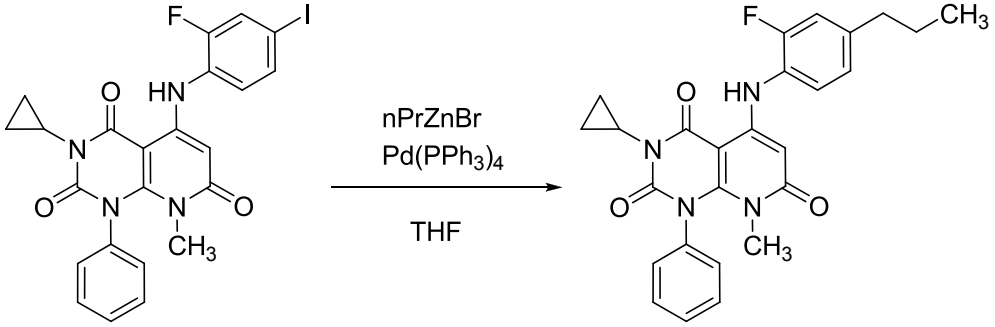
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76 (m, 2H), 1.05 (m, 2H), 2.60 (s, 3H), 2.67 (m, 1H), 5.37 (s, 1H), 7.42–7.52 (m, 7H), 7.73 (d, J = 12.0 Hz, 1H), 10.55 (s, 1H); MS (ESI) m/z 497, 499 $(M+H)^+$.

3-Cyclopropyl-5-(2-fluoro-4-iodophenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine -2,4,7-trione (4m)



^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (m, 2H), 1.19 (m, 2H), 2.77 (tt, J = 7.0, 4.0 Hz, 1H), 2.82 (s, 3H), 5.69 (d, J = 1.1 Hz, 1H), 7.11 (dd, J = 8.8, 8.3 Hz, 1H), 7.31 (dt, J = 7.3, 3.8 Hz, 2H), 7.40–7.59 (m, 5H), 10.36 (s, 1H); MS (ESI) m/z 545 $(M+H)^+$.

Preparation of Compound 4d:

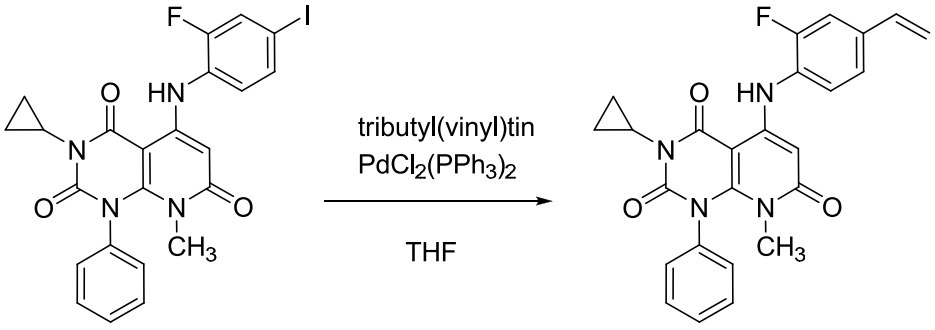


4m **4d**

To a suspension of **4m** (50 mg, 0.092 mmol) and $Pd(PPh_3)_4$ (21 mg, 0.0184 mmol) in THF (2.5 mL) was

added *n*-propylzinc bromide (0.23 mL, 0.11 mmol, 0.5 M in THF). The mixture was heated in a 50 °C bath for 9 h. After cooling to room temperature, the reaction was quenched with sat. ammonium chloride, and extracted with chloroform. The extracts were washed with brine, dried over magnesium sulfate, and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 40/1) to afford 3-cyclopropyl-5-(2-fluoro-4-propylphenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione **4d** (8 mg, 18%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H), 1.19 (m, 2H), 1.66 (m, 2H), 2.60 (t, *J* = 7.7 Hz, 2H), 2.77 (tt, *J* = 7.3, 4.0 Hz, 1H), 2.82 (s, 3H), 5.61 (d, *J* = 1.1 Hz, 1H), 6.94–7.04 (m, 2H), 7.27 (t, *J* = 8.3 Hz, 1H), 7.33 (m, 2H), 7.41–7.53 (m, 3H), 10.18 (s, 1H); MS (ESI) *m*/*z* 461 $(M+H)^+$.

Preparation of Compound 4f:

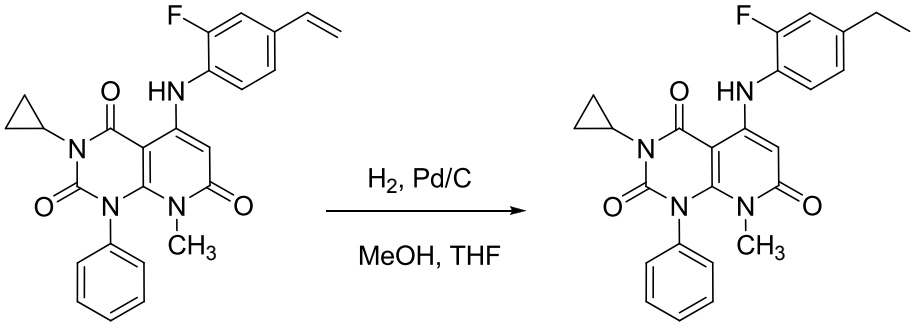


4m **4f**

To a suspension of **4m** (50 mg, 0.092 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.018 mmol) was added tributyl(vinyl)tin (0.040 mL, 0.138 mmol). The mixture was heated in a 60 °C bath for 4 h. The mixture was evaporated and the crude residue was purified by flash chromatography (chloroform/acetone, 40/1–20/1) to afford 3-cyclopropyl-5-(2-fluoro-4-vinylphenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione **4f** (22 mg, 53%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (m, 2H), 1.20 (m, 2H), 2.77 (tt, *J* = 7.2, 4.2 Hz, 1H), 2.82 (s, 3H), 5.31 (d, *J* = 10.8 Hz, 1H), 5.72 (s, 1H), 5.74 (d, *J* = 17.6 Hz,

1H), 6.66 (dd, *J* = 17.6, 10.8 Hz, 1H), 7.19 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.24 (dd, *J* = 11.6, 1.9 Hz, 1H), 7.31 (dt, *J* = 7.9, 2.0 Hz, 2H), 7.36 (t, *J* = 8.1 Hz, 1H), 7.40–7.51 (m, 3H), 10.34 (s, 1H); MS (ESI) *m*/*z* 445 $(M+H)^+$.

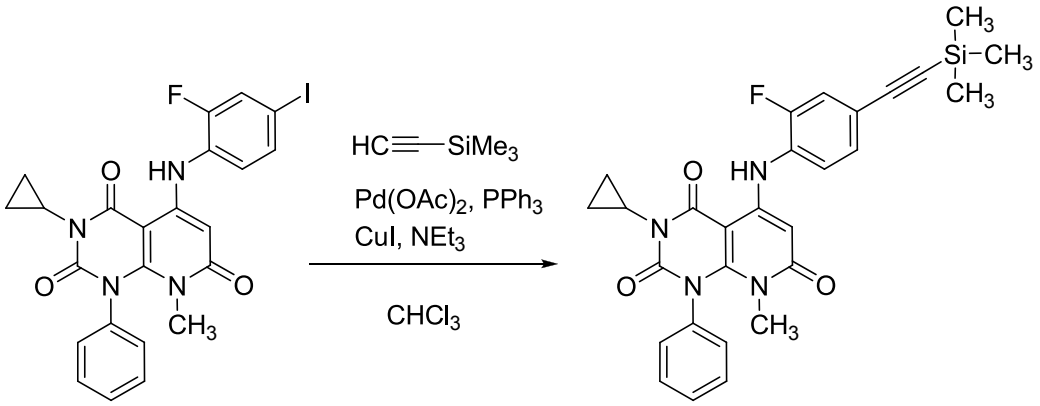
Preparation of Compound 4c:



4f **4c**

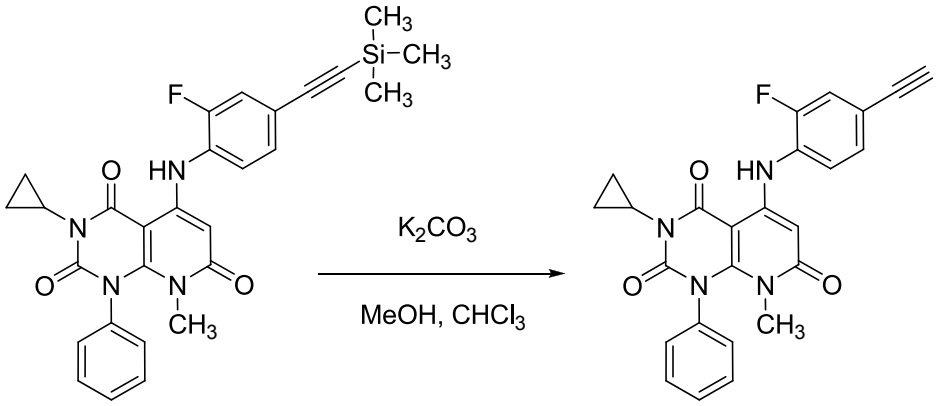
To a solution of **4f** (15 mg, 0.034 mmol) in methanol (1.0 mL) and THF (1.0 mL) was added Pd/C (50 mg, 10 wt %). Under a hydrogen atmosphere (1 atm), the mixture was stirred at room temperature for 5 h. The reaction mixture was filtered with the aid of Celite and concentrated to afford 3-cyclopropyl-5-(4-ethyl-2-fluorophenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione **4c** (12 mg, 79%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (m, 2H), 1.19 (m, 2H), 1.25 (t, *J* = 7.6 Hz, 3H), 2.66 (q, *J* = 7.6 Hz, 2H), 2.77 (tt, *J* = 7.2, 4.0 Hz, 1H), 2.81 (s, 3H), 5.59 (d, *J* = 1.2 Hz, 1H), 6.96–7.04 (m, 2H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.31 (m, 2H), 7.39–7.51 (m, 3H), 10.16 (s, 1H); MS (ESI) *m*/*z* 447 $(M+H)^+$.

Preparation of Compound 4g:



4m **4.15**

To a solution of **4m** (50 mg, 0.092 mmol) in chloroform (3.0 mL) were added trimethylsilylacetylene (0.016 mL, 0.11 mmol), triethylamine (0.026 mL, 0.184 mmol), triphenylphosphine (2.4 mg, 0.0092 mmol), $Pd(OAc)_2$ (1.0 mg, 0.0046 mmol), and CuI (0.9 mg, 0.0046 mmol). The mixture was stirred at room temperature for 4 h. The same reagents as above were added twice in 3 h interval while stirring at room temperature. The mixture was heated in a 50 °C bath for 1.5 h. The mixture was filtered with the aid of Celite and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 50/1) to afford **4.15** (44 mg, 93%) as a gray solid.

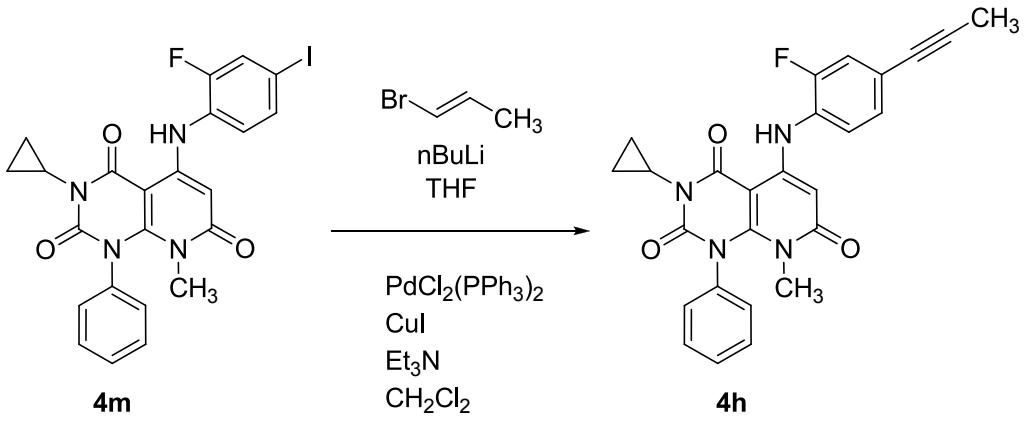


4.15 **4g**

To a solution of **4.15** (20 mg, 0.039 mmol) in MeOH (0.3 mL) and chloroform (0.6 mL) was added potassium carbonate (2.7 mg, 0.092 mmol). After 3 h stirring, the same amount of reagent as above was added to the reaction mixture. After 3 h, the reaction mixture was diluted with chloroform, washed with water, dried over sodium sulfate, and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 60/1–15/1) to afford

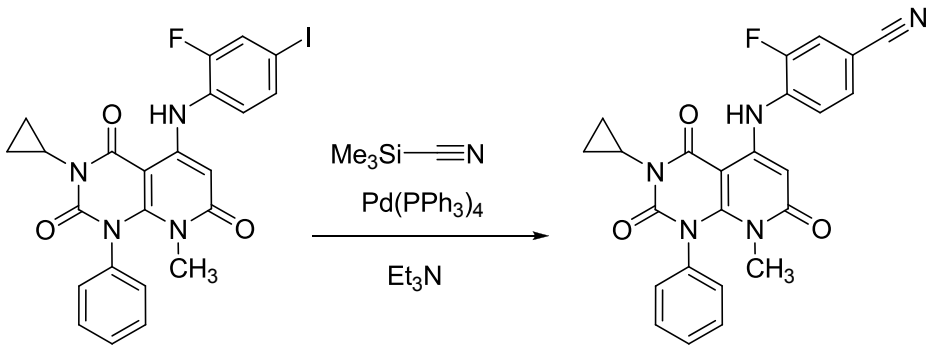
3-cyclopropyl-5-(4-ethynyl-2-fluorophenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione **4g** (4 mg, 21%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (m, 2H), 1.20 (m, 2H), 2.77 (tt, *J* = 7.2, 3.9 Hz, 1H), 2.82 (s, 3H), 3.12 (s, 1H), 5.80 (s, 1H), 7.26–7.33 (m, 3H), 7.35–7.52 (m, 5H), 10.50 (s, 1H); MS (ESI) *m*/*z* 443 $(M+H)^+$.

Preparation of Compound 4h:



To a cooled (−78 °C) solution of 1-bromo-1-propene (0.079 mL, 0.92 mmol) in THF (1.0 mL) was added *n*BuLi (0.85 mL, 1.29 mmol, 1.52 M in hexane). The mixture was stirred at that temperature for 2 h. Water (0.023 mL, 1.29 mmol) was added, and the mixture was allowed to warm to 0 °C. To this mixture was added **4m** (50 mg, 0.092 mmol), triethylamine (0.10 mL, 0.74 mmol), $PdCl_2(PPh_3)_2$ (14 mg, 0.018 mmol), and CuI (3.5 mg, 0.018 mmol) in CH_2Cl_2 (1.0 mL). The mixture was stirred at room temperature for 17 h, filtered with the aid of Celite and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 50/1–30/1) to afford 3-cyclopropyl-5-(2-fluoro-4-prop-1-ynylphenylamino)-8-methyl-1-phenyl-1*H*,8*H*-pyrido[2,3-*d*]pyrimidine-2,4,7-trione **4h** (21 mg, 49%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (m, 2H), 1.19 (m, 2H), 2.06 (s, 3H), 2.77 (tt, *J* = 7.2, 3.9 Hz, 1H), 2.82 (s, 3H), 5.74 (s, 1H), 7.14–7.21 (m, 2H), 7.28–7.36 (m, 3H), 7.39–7.51 (m, 3H), 10.38 (s, 1H); MS (ESI) *m*/*z* 457 $(M+H)^+$.

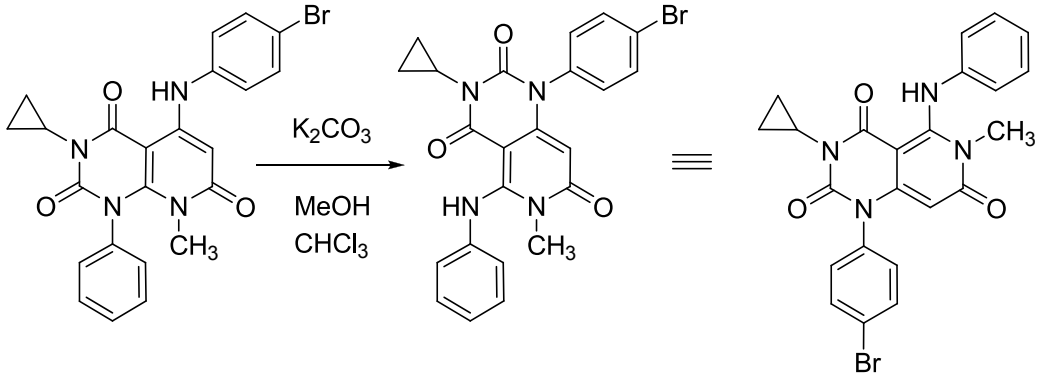
Preparation of Compound 4i:



4m **4i**

To a suspension of **4m** (50 mg, 0.092 mmol) in triethylamine (2.0 mL) were added trimethylsilyl cyanide (0.018 mL, 0.138 mmol) and $Pd(PPh_3)_4$ (21 mg, 0.018 mmol). The mixture was heated in a 90 °C bath for 8 h. The same amount of reagents above was added, and the mixture was heated in a 90 °C bath for 9 h and concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 40/1–30/1) to afford 4-(3-cyclopropyl-8-methyl-2,4,7-trioxo-1-phenyl-1,2,3,4,7,8-hexahydropyrido[2,3-*d*]pyrimidin-5-ylamino)-3-fluorobenzonitrile **4i** (16 mg, 38%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (m, 2H), 1.21 (m, 2H), 2.78 (tt, J = 7.3, 3.7 Hz, 1H), 2.85 (s, 3H), 6.01 (s, 1H), 7.31 (dt, J = 6.6, 1.7 Hz, 2H), 7.43–7.56 (m, 5H), 7.64 (t, J = 8.3 Hz, 1H), 10.93 (s, 1H); MS (ESI) m/z 444 $(M+H)^+$.

Preparation of Compound 5:



3 **5**

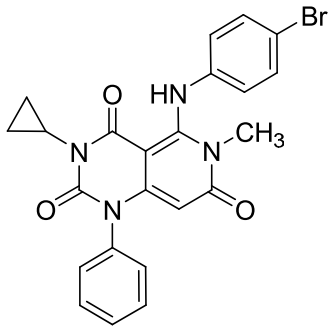
To a solution of **3** (50 mg, 0.10 mmol) in methanol (1.5 mL) and chloroform (1.5 mL) was added potassium carbonate (15 mg, 0.10 mmol). The mixture was stirred at room temperature for 17 h and

concentrated at reduced pressure. The crude residue was purified by flash chromatography (chloroform/acetone, 30/1) to afford 1-(4-bromophenyl)-3-cyclopropyl-6-methyl-5-phenylamino-1*H*,6*H*-pyrido[4,3-*d*]pyrimidine-2,4,7-trione **5** (48 mg, 96%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (m, 2H), 1.14 (m, 2H), 2.71 (tt, *J* = 7.2, 4.0 Hz, 1H), 3.11 (s, 3H), 5.00 (s, 1H), 7.02 (d, *J* = 7.7 Hz, 2H), 7.12 (dt, *J* = 9.1, 2.4 Hz, 2H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.37 (dd, *J* = 7.7, 7.4 Hz, 2H), 7.67 (dt, *J* = 9.1, 2.4 Hz, 2H), 11.71 (s, 1H); MS (ESI) *m*/*z* 479, 481 $(M+H)^+$.

Preparation of Compound 6:

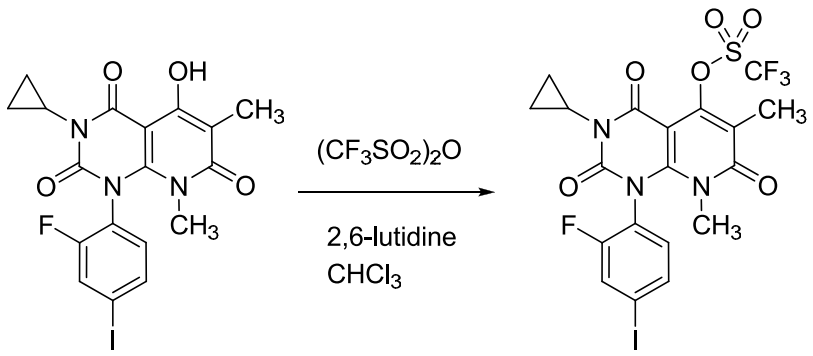
Compounds **6** was similarly prepared as described for **5**.

5-(4-Bromophenylamino)-3-cyclopropyl-6-methyl-1-phenyl-1*H*,6*H*-pyrido[4,3-*d*]pyrimidine-2,4,7-trione (6)



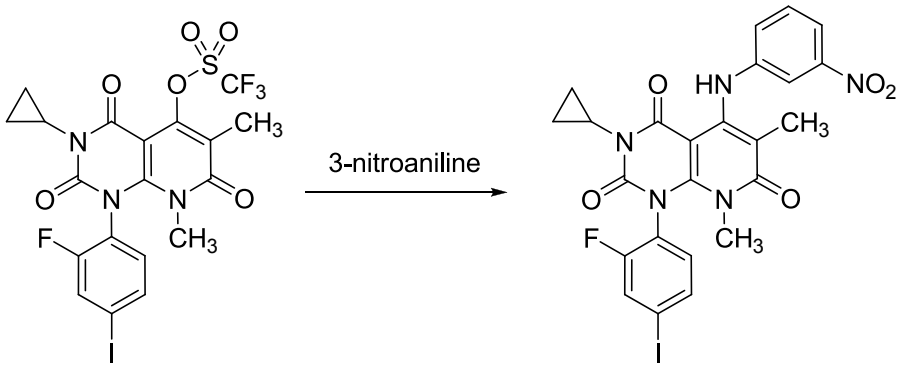
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (m, 2H), 1.14 (m, 2H), 2.72 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.13 (s, 3H), 5.06 (s, 1H), 6.90 (d, *J* = 8.8 Hz, 2H), 7.24 (dt, *J* = 7.0, 1.8 Hz, 2H), 7.45–7.60 (m, 5H), 11.68 (s, 1H); MS (ESI) *m*/*z* 479, 481 $(M+H)^+$.

Preparation of Compound 8b (JTP-74057):



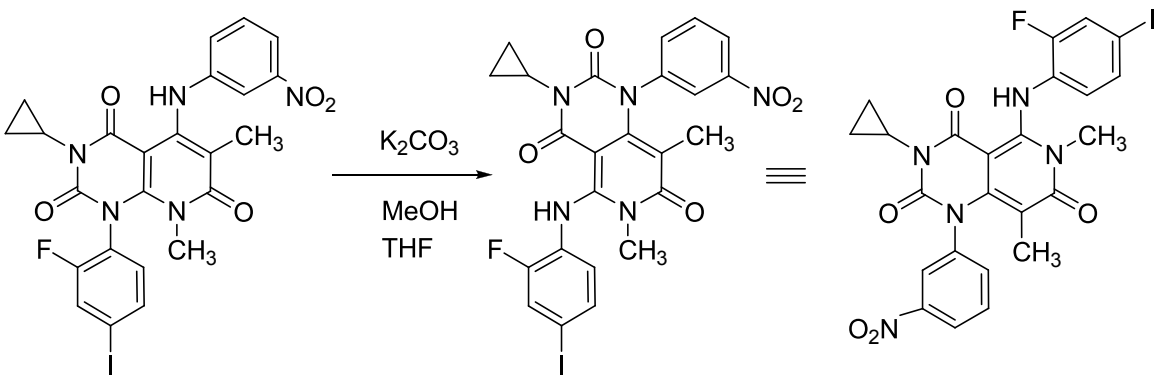
8.1 **8.2**

8.1 was similarly prepared as described for **3.6**. To an ice-cooled mixture of **8.1** (6.4 g, 13.2 mmol) and 2,6-lutidine (2.0 mL, 17.4 mmol) in chloroform (33.6 mL) was added trifluoromethanesulfonic anhydride (2.92 mL, 17.4 mmol) in 5 min. The mixture was stirred at room temperature for 1 h. An additional amount of 2,6-lutidine (0.50 mL, 4.35 mmol) and trifluoromethanesulfonic anhydride (0.73 mL, 4.35 mmol) was added to the reaction mixture at 0 °C. The stirring was continued at room temperature for 30 min. The reaction was quenched with water, and extracted with chloroform. The extracts were washed with brine, dried over sodium sulfate, and concentrated at reduced pressure. The crude residue was taken up to ethanol and crystallized. The precipitate was collected by filtration and dried under vacuum to afford **8.2** (6.89 g, 85%) as a pale yellow solid.



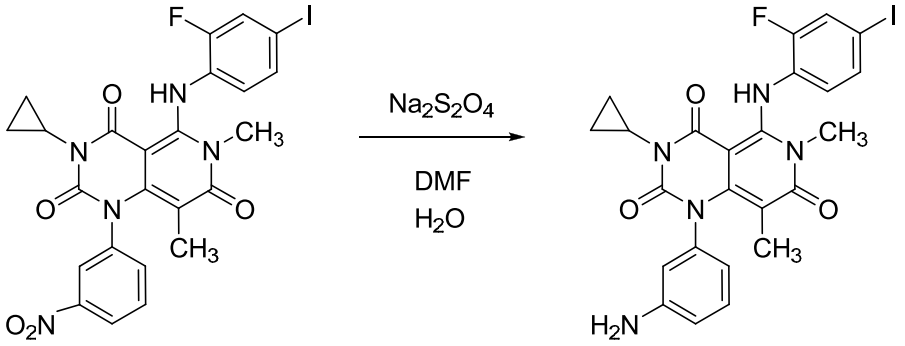
8.2 **8.3**

The mixture of **8.2** (6.5 g, 10.6 mmol) and 3-nitroaniline (7.3 g, 52.8 mmol) was heated in a 130 °C bath for 2 h. After cooling to room temperature, the mixture was taken up to acetone/ether and crystallized. The precipitate was collected by filtration and dried under vacuum to afford **8.3** (5.86 g, 90%) as a solid.



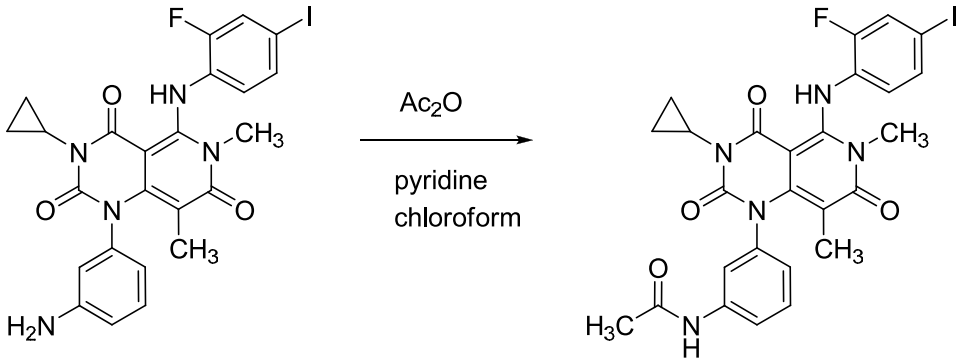
8.3 **8.4**

To a suspension of **8.3** (5.86 g, 9.7 mmol) in methanol (18 mL) and THF (41 mL) was added potassium carbonate (1.34 g, 9.7 mmol). The mixture was heated in an 80 °C bath for 3 h. After cooling to room temperature, water (120 mL) was added. The precipitate was collected by filtration, washed with water, and dried under vacuum to afford **8.4** (5.09 g, 87%) as a solid.



8.4 **8.5**

To a suspension of **8.4** (3.78 g, 6.27 mmol) in DMF (40 mL) was added sodium hydrosulfite (3.27 g, 18.8 mmol) and water (2.0 mL). The mixture was heated in a 90 °C bath for 1 h. After cooling to room temperature, the reaction mixture was diluted with water (120 mL). The precipitate was collected by filtration, washed with water, and dried under vacuum to afford **8.5** (3.10 g, 86%) as a solid.

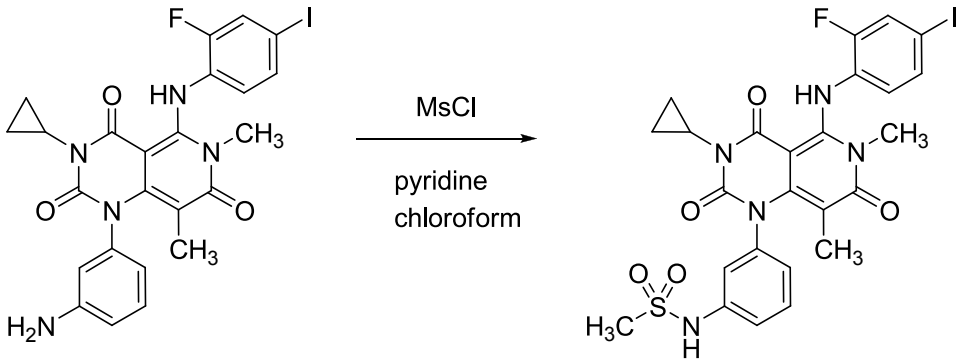


8.5 **8b (JTP-74057)**

To an ice-cooled suspension of **8.5** (100 mg, 0.174 mmol) in pyridine (1.0 mL) and chloroform (2.0 mL) was added acetic anhydride (0.020 mL, 0.209 mmol). The mixture was stirred at room temperature for 2 h. The mixture was poured onto 1 M HCl and extracted with chloroform. The extracts were washed with brine, dried over sodium sulfate, and concentrated at reduced pressure. The crude residue was taken up to ethyl acetate and crystallized. The precipitate was collected by filtration and dried under vacuum to afford

N-{3-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2*H*-pyrido[4,3-*d*]pyrimidin-1-yl]phenyl}acetamide **8b (JTP-74057)** (45 mg, 42%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.67 (m, 2H), 0.95 (m, 2H), 1.25 (s, 3H), 2.04 (s, 3H), 2.62 (tt, J = 7.0, 4.2 Hz, 1H), 3.07 (s, 3H), 6.92 (dd, J = 8.7, 8.5 Hz, 1H), 7.03 (m, 1H), 7.36 (dd, J = 8.2, 7.3 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.57–7.63 (m, 2H), 7.79 (dd, J = 10.4, 1.9 Hz, 1H), 10.10 (s, 1H), 11.08 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 8.1, 12.9, 23.9, 24.8, 33.8, 88.1 (d, J = 6.9 Hz), 90.2, 101.9, 118.2, 120.0, 123.9, 124.8 (d, J = 21.5 Hz), 124.9, 128.1 (d, J = 10.8 Hz), 128.6, 133.9 (d, J = 3.1 Hz), 139.5, 140.2, 144.8, 150.8, 150.9, 154.1 (d, J = 250.3 Hz), 162.7, 164.1, 168.4. HRMS (ESI) Calcd for $C_{26}H_{24}N_5O_4FI$: 616.08515. Found: 616.08483 $(M+H)^+$. mp 300–301 °C.

Preparation of Compound 8e:



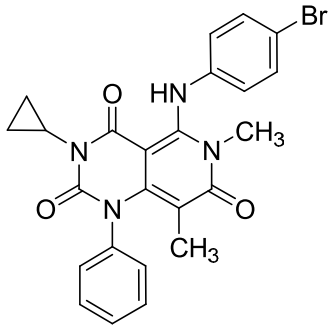
8.5 **8e**

To an ice-cooled suspension of **8.5** (127 mg, 0.222 mmol) in pyridine (1.3 mL) and chloroform (1.3 mL) was added methanesulfonyl chloride (0.021 mL, 0.267 mmol). The mixture was stirred at room temperature for 2 h. An additional amount of methanesulfonyl chloride (0.0035 mL, 0.044 mmol) was added, and the mixture was stirred at room temperature for 30 min. The mixture was poured onto 1 M HCl and extracted with chloroform. The extracts were washed with brine, dried over sodium sulfate, and concentrated at reduced pressure. The crude residue was taken up to chloroform/acetonitrile and crystallized. The precipitate was collected by filtration and dried under vacuum to afford *N*-{3-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2*H*-pyrido[4,3-*d*]pyrimidin-1-yl]-phenyl}methanesulfonamide **8e** (114 mg, 79%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.67 (m, 2H), 0.95 (m, 2H), 1.25 (s, 3H), 2.62 (m, 1H), 3.01 (s, 3H), 3.08 (s, 3H), 6.92 (dd, J = 8.7, 8.3 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.20–7.26 (m, 2H), 7.41 (dd, J = 8.1, 7.5 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.79 (dd, J = 10.6, 1.9 Hz, 1H), 9.89 (s, 1H), 11.08 (s, 1H); MS (ESI) m/z 652 $(M+H)^+$.

Preparation of Compounds 7, 8a, 8c, 8d, 8f:

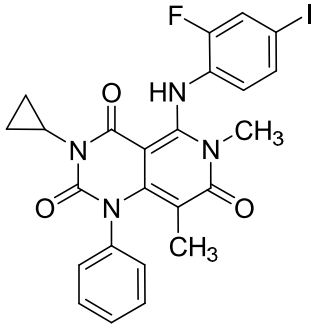
Compounds **7**, **8a**, **8c**, **8d**, **8f** were similarly prepared as described for **8b** and **8e**.

5-(4-Bromophenylamino)-3-cyclopropyl-6,8-dimethyl-1-phenyl-1*H*,6*H*-pyrido[4,3-*d*]pyrimidine-2, 4,7-trione (7)



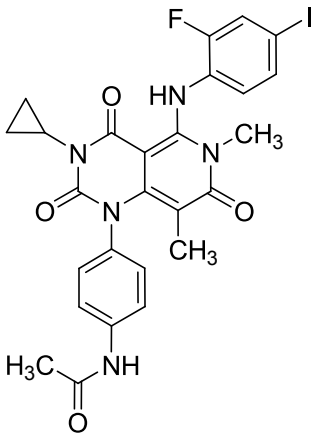
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (m, 2H), 1.12 (m, 2H), 1.36 (s, 3H), 2.73 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.20 (s, 3H), 6.86 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.4, 7.3 Hz, 2H), 7.48 (d, *J* = 8.5 Hz, 2H), 11.36 (s, 1H); MS (ESI) *m*/*z* 493, 495 $(M+H)^+$.

3-Cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-1-phenyl-1*H*,6*H*-pyrido[4,3-*d*]pyrimidine-2,4,7-trione (8a)



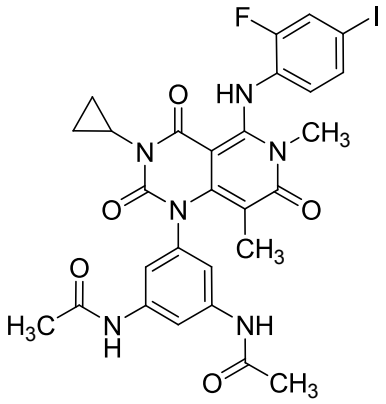
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.67 (m, 2H), 0.96 (m, 2H), 1.18 (s, 3H), 2.62 (m, 1H), 3.07 (s, 3H), 6.93 (dd, *J* = 8.7, 8.3 Hz, 1H), 7.34–7.50 (m, 5H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.79 (dd, *J* = 10.4, 1.7 Hz, 1H), 11.06 (s, 1H); MS (ESI) *m*/*z* 559 $(M+H)^+$.

***N*-{4-[3-Cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2*H*-pyrido[4,3-*d*]pyrimidin-1-yl]-phenyl}acetamide (8c)**



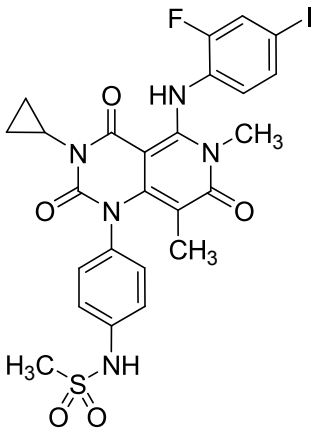
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.66 (m, 2H), 0.95 (m, 2H), 1.23 (s, 3H), 2.07 (s, 3H), 2.62 (m, 1H), 3.07 (s, 3H), 6.92 (dd, J = 8.3, 7.9 Hz, 1H), 7.28 (d, J = 9.0 Hz, 2H), 7.55 (d, J = 7.9 Hz, 1H), 7.63 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 10.6 Hz, 1H), 10.10 (s, 1H), 11.06 (s, 1H); MS (ESI) m/z 616 $(M+H)^+$.

_N_-{3-Acetylamino-5-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2_H_-pyrido[4,3-_d_]pyrimidin-1-yl]-phenyl}acetamide (8d)



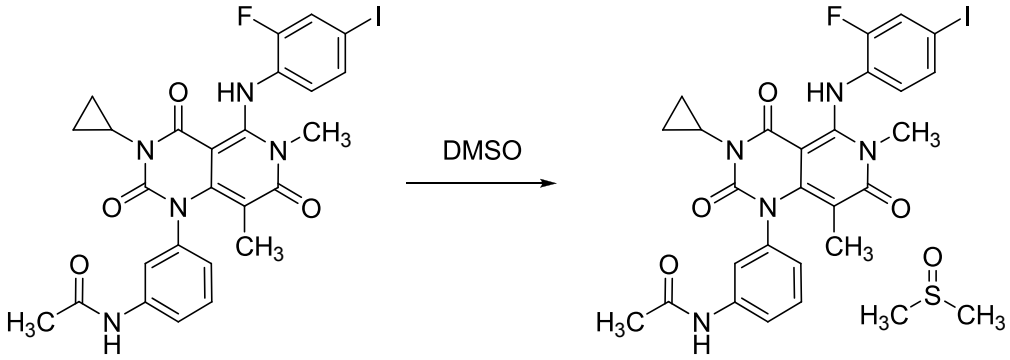
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.66 (m, 2H), 0.94 (m, 2H), 1.33 (s, 3H), 2.03 (s, 6H), 2.62 (m, 1H), 3.08 (s, 3H), 6.90 (dd, J = 8.9, 8.7 Hz, 1H), 7.28 (s, 2H), 7.55 (d, J = 8.7 Hz, 1H), 7.78 (d, J = 10.2 Hz, 1H), 7.92 (s, 1H), 10.08 (s, 2H), 11.09 (s, 1H).; MS (ESI) m/z 673 $(M+H)^+$.

_N_-{4-[3-Cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2_H_-pyrido[4,3-_d_]pyrimidin-1-yl]-phenyl}methanesulfonamide (8f)



^{1}H NMR (400 MHz, DMSO-d_6) δ 0.66 (m, 2H), 0.95 (m, 2H), 1.23 (s, 3H), 2.62 (tt, J = 6.7, 4.2 Hz, 1H), 3.04 (s, 3H), 3.07 (s, 3H), 6.93 (dd, J = 8.8, 8.6 Hz, 1H), 7.25 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.6 Hz, 1H), 7.79 (dd, J = 10.2, 1.9 Hz, 1H), 9.95 (s, 1H), 11.06 (s, 1H); MS (ESI) m/z 652 $(M+H)^+$.

Preparation of Compound 1 (GSK1120212, JTP-74057 DMSO solvate):



8b **1 (GSK1120212, JTP-74057 DMSO Solvate)**

8b (8.0 g) was suspended in DMSO (40.0 mL, 5 v/w). The mixture was heated in an 80 °C bath. The suspension turned to a yellow solution after ca. 15 min. After stirring 1–2 h at that temperature, a white precipitate was observed. The suspension was stirred at that temperature for additional 3 h, and then stirred at room temperature overnight. The precipitate was collected by filtration, washed with DMSO (4 mL × 3), and dried under vacuum at 60 °C to afford *N*-{3-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2*H*-pyrido[4,3-*d*]pyrimidin-1-yl]phenyl}acetamide dimethylsulfoxide solvate **1 (GSK1120212, JTP-74057**

DMSO solvate) (8.3 g, 92%) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (m, 2H), 1.12 (m, 2H), 1.41 (s, 3H), 2.14 (s, 3H), 2.62 (s, 6H), 2.74 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.20 (s, 3H), 6.70 (dd, *J* = 8.4, 8.4 Hz, 1H), 6.99 (dt, *J* = 6.3, 2.3 Hz, 1H), 7.28–7.35 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.52 (dd, *J* = 9.6, 1.9 Hz, 1H), 7.64 (brs, 1H), 7.71 (brs, 1H), 11.29 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 8.6, 13.6, 24.8, 25.5, 34.8, 41.2, 88.3 (d, *J* = 6.9 Hz), 90.1, 103.9, 119.1, 120.5, 124.5, 125.3, 126.0 (d, *J* = 21.5 Hz), 128.4 (d, *J* = 11.5 Hz), 129.4, 134.2 (d, *J* = 3.8 Hz), 139.1, 140.5, 145.0, 151.9, 152.2, 155.3 (d, *J* = 254.1 Hz), 164.0, 165.0, 168.5. MS (ESI) *m*/*z* 616 $(M+H)^+$. Anal. Calcd for $C_{26}H_{23}N_5O_4FI \cdot 1.0C_2H_6OS$: C, 48.49; H, 4.21; N, 10.10. Found: C, 48.32; H, 4.17; N, 9.94.