Supporting Information

Discovery of Potent and Selective MTH1 Inhibitors for Oncology: Enabling Rapid Target (In)Validation

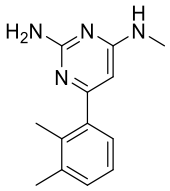
Julie Farand,*,† Jeffrey E. Kropf,‡ Peter Blomgren,‡ Jianjun Xu,‡ Aaron C. Schmitt,‡ Zachary E. Newby,† Ting Wang,† Eisuke Murakami,† Ona Barauskas,† Jawahar Sudhamsu,† Joy Y. Feng,† Anita Niedziela-Majka,† Brian E. Schultz,† Karen Schwartz,† Serge Viatchenko-Karpinski,† Dmytro Kornyeyev,† Adam Kashishian,‡ Peidong Fan,† Xiaowu Chen,† Eric B. Lansdon,† Michael O. Ports,‡ Kevin S. Currie,‡ William J. Watkins,† Gregory T. Notte†

† Gilead Sciences, Inc. 333 Lakeside Drive, Foster City, California 94404, United States
‡ Gilead Sciences, Inc. 199 East Blaine Street, Seattle, Washington 98102, United States

Table of Contents

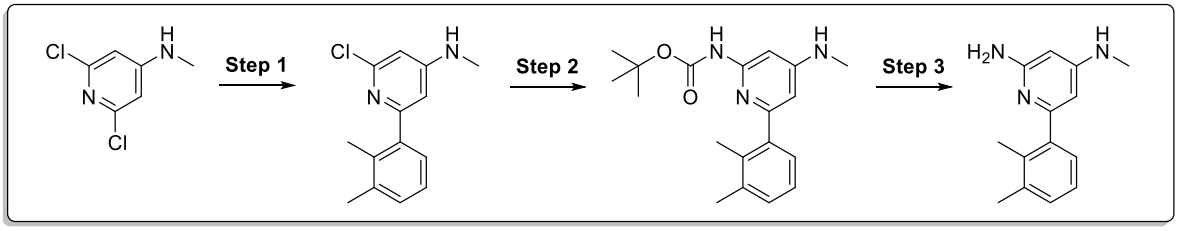
General Methods All final compounds were synthesized at Gilead Sciences, Inc (Foster City, CA, USA and Branford, CT, USA). Commercial solvents and reagents were used as received without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400-MR (400 MHz) or Varian Mercury Plus (300 MHz) spectrometers in the specified deuterated solvent. Preparative normal phase chromatography was performed on a Yamazen W-Prep 2XY instrument using pre-packed UNIVERSAL silica gel columns. Alternatively, an ISCO Combiflash Companion purification system with RediSep Rf prepacked silica gel cartridges supplied by Teledyne Isco was also used for purification of intermediates and final compounds. Preparative reverse phase high-pressure liquid chromatography (HPLC) was performed on a Varian Prostar system using a Gemini C18 110 Å column (100 x 30 mm, 5µm) at 21 °C, with a 20-98% gradient of acetonitrile and 0.1% hydrochloric acid in water, at a 20 mL/min flow rate over 20 minutes with UV detection at 254 nm. LC/MS analysis was performed on an Agilent 1200 HPLC instrument in-line with an Agilent G6120A single quadrupole mass spectrometer (MS) equiped with an API electrospray source with positive mode ionization ($[M + H]^+$). The analytical method consisted of an Agilent Zorbax Eclipse XDB-C18 column (4.6 x 20 mm, 3.5 µm), 2-95% gradient of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroacetic acid in water, at a 2.0 mL/min flow rate over 3.5 minutes. For compounds synthesized outside Foster City, LC/MS analysis was performed on a Waters SQD (Model F085QD294W) with electrospray ionization in the positive mode. The analytical method consisted of an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm), 25-75% gradient of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroactic acid in water, at a 0.8 mL/min flow rate over 1.75 minutes. High-resolution mass spectrometry (HRMS) was performed on an Agilent Infinity II 1290 HPLC system in-line with a Thermo Electron Orbitrap Elite instrument (positive mode, scan range 250-1000 mass units). Chromatography was performed on a Waters Acquity UPLC BEH C18 130 Å column (2.1 x 100 mm, 1.7 µm) at 40 °C, with a 5-90% gradient of 0.1% formic acid in acetonitrile and 0.1% formic acid in water, at a 0.8 mL/min flow rate over 8.5 minutes with UV detection at 190-400 nm. Purities of the final compounds were determined using an Agilent Infinity II 1290 HPLC system, a Phenomenex Kinetex C18 100 Å column (4.6 x 100 mm, 2.8 µm) at room temperature, with a 2-98% gradient of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroacetic acid in water, at a 1.5 mL/min flow rate over 8.5 minutes with UV detection at 254 nm. All final compounds were lyophilized.

Synthetic Procedures and Characterization



Compound 1: 6-(2,3-dimethylphenyl)-*N*^{4}-methylpyrimidine-2,4-diamine

A microwave vial was charged with 6-chloro-N^4-methylpyrimidine-2,4-diamine (50.0 mg, 0.315 mmol), 2,3-dimethylphenylboronic acid (47.0 mg, 0.315 mmol), tetrakis(triphenylphosphine)palladium(0) (36.4 mg, 0.032 mmol) and cesium carbonate (309 mg, 0.945 mmol). The vessel was purged with nitrogen. A solution of 1,4-dioxane/water (2:1, 3.0 mL) was degassed under argon and was added to the solid reagents. The vial was sealed and heated at 140 °C for 15 minutes. The reaction was cooled to room temperature, diluted with saturated $NH_4Cl_{(aq)}$ and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel chromatography (MeOH/DCM) to afford the desired product as a colorless solid (20.0 mg, 28%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.18 – 6.96 (m, 2H), 5.95 (s, 2H), 5.65 (s, 1H), 2.74 (d, J = 4.7 Hz, 3H), 2.24 (s, 3H), 2.13 (s, 3H). LCMS-ESI$^+$ (m/z): $[M+H]^+$ calcd for $C_{13}H_{16}N_4$ 229.15; found 229.21.

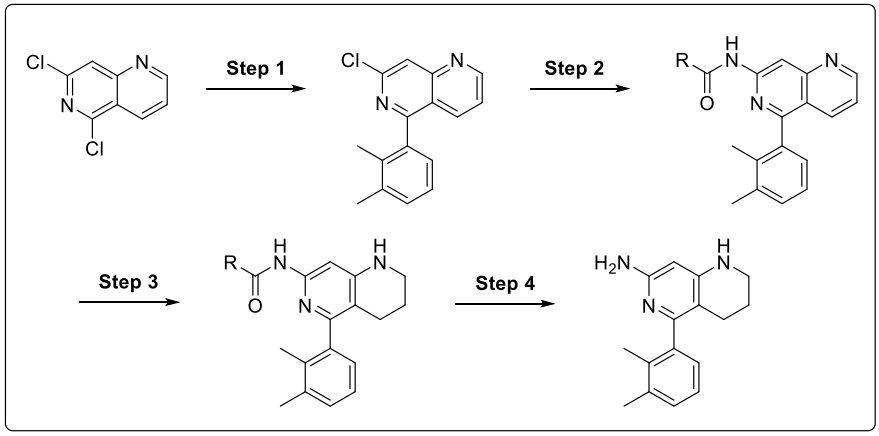


Compound 2: 6-(2,3-dimethylphenyl)-*N*^{4}-methylpyridine-2,4-diamine

Following **Step 2** of the synthesis described to prepare **Compound 6**, using 2-chloro-6-(2,3-dimethylphenyl)-*N*-methylpyridin-4-amine (103 mg, 0.417 mmol) and *tert*-butyl carbamate (245 mg, 2.09 mmol), afforded *tert*-butyl (6-(2,3-dimethylphenyl)-4-(methylamino)pyridin-2-yl)carbamate after silica gel chromatography (5-45% EtOAc/hexanes) (40.0 mg, 29%).

Step 3: A solution of (6-(2,3-dimethylphenyl)-4-(methylamino)pyridin-2-yl)carbamate (40.0 mg, 0.122 mmol) in DCM (3.0 mL) and TFA (1.0 mL) was stirred for 4 hours. The reaction was concentrated and the residue was purified by reverse phase chromatography to afford 6-(2,3-dimethylphenyl)-N^4-methylpyridine-2,4-diamine as a colorless solid (7.9 mg, 25%, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H), 7.94 – 7.85 (m, 1H), 7.35 (dd, J = 7.8, 1.5 Hz, 1H), 7.28 – 7.14 (m, 2H), 7.06 (s, 1H), 6.07 (s, 1H), 5.66 (s, 1H), 2.78 (d, J = 4.7 Hz, 3H), 2.31 (s, 3H), 2.15 (s, 3H). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{14}H_{17}N_3$ 228.2; found 228.1. HPLC purity: 100%.

Scheme S1. General synthesis for **Compounds 3**, **4** and **5**.



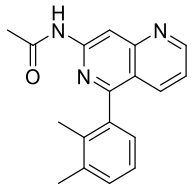
Compound 3: 5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-amine

Step 1: A vial was charged with 5,7-dichloro-1,6-naphthyridine (200 mg, 1.00 mmol), 2,3-dimethylphenylboronic acid (166 mg, 1.11 mmol), cesium carbonate (982 mg, 3.01 mmol) 1,4-dioxane (2.0 mL) and water (1.0 mL). The reaction was degassed with nitrogen for 10 minutes, then PEPPSI-IPr (68.5 mg, 0.100 mmol) was added. The vial was sealed and heated at 100 °C for 60 minutes. The reaction was cooled to room temperature, diluted with saturated $NH_4Cl_{(aq)}$ and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel chromatography (5-50% EtOAc/hexanes) to afford 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (220 mg, 82% yield). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{16}H_{13}ClN_2$ 269.1; found 269.1.

Step 2: A vial was charged with 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (90.0 mg, 0.335 mmol), *tert*-butyl carbamate (196 mg, 1.67 mmol), cesium carbonate (327 mg, 1.00 mmol), *t*-butyl-Xantphos (16.7 mg, 0.034 mmol) and 1,4-dioxane (1.7 mL). The reaction mixture was sparged with nitrogen to degas. After 10 min, tris(dibenzylideneacetone) dipalladium(0) (15.3 mg, 0.017 mmol) was added and the reaction was heated at 80 º C for 18 h. The mixture was filtered over celite, concentrated and purified by silica gel chromatography (5 – 40% EtOAc/hexanes) to afford *tert*-butyl (5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)carbamate (95 mg, 81% yield). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_3O_2$ 350.2; found 350.2.

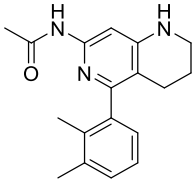
Step 3: A flask was charged with *tert*-butyl (5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)carbamate (151 mg, 0.432 mmol), EtOH (5.0 mL) and Pd / C (10 wt. %, 30.0 mg). The flask was purged with nitrogen / vacuum (3x), fitted with a balloon of hydrogen (1 atm), purged with hydrogen / vacuum (3x) and the mixture was stirred under 1 atm of hydrogen for 18 h. LCMS showed the reduction was incomplete. An additional 36 mg of Pd / C was added and the system was purged according to the procedure outlined above. The mixture was stirred at RT under 1 atm of hydrogen for 18 h. The mixture was filtered over celite, concentrated and used crude in the next reaction. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{27}N_3O_2$ 354.2; found 354.2.

Step 4: A flask was charged with *tert*-butyl (5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)carbamate (122 mg, 0.345 mmol), DCM (3.0 mL) and TFA (1.0 mL). The solution was stirred at RT for 5 h, concentrated and purified by reverse phase chromatography (2-50% ACN/water with 0.1% HCl) to afford 5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-amine as a light yellow solid (12.3 mg, 12% yield, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 8.20 (s, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.58 (bs, 2H), 5.78 (d, *J* = 1.9 Hz, 1H), 3.32 – 3.16 (m, *J* = 5.3 Hz, 2H), 2.31 (s, 3H), 2.23 – 1.99 (m, 5H), 1.78 – 1.56 (m, *J* = 7.2 Hz, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{19}N_3$ 254.2; found 254.1. HPLC purity: 100%.



Compound 4: *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)acetamide

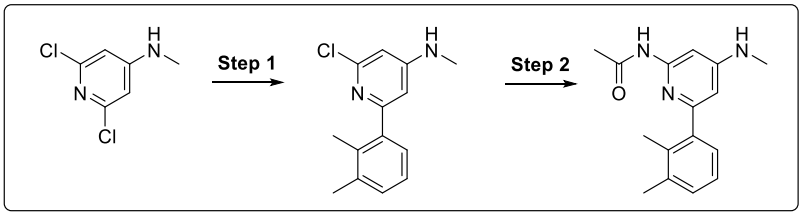
Following **Step 2** of the synthesis described to prepare **Compound 3**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (270 mg, 1.00 mmol) and acetamide (297 mg, 5.02 mmol), the crude product was purified by silica gel chromatography to afford *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)acetamide (293 mg, 45%). This product was used for the synthesis of **Compound 5**, and since the reaction was incomplete, **Compound 4** was isolated and characterized after reverse phase chromatography.



Compound 5: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)acetamide

Following **Step 3** of the synthesis described to prepare **Compound 3**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)acetamide (132 mg, 0.453 mmol) and Pd / C (10 wt. %, 13.0 mg), the reaction was stirred for 18 h under 1 atm of hydrogen. An additional 20 mg of Pd / C was added and the mixture was stirred under hydrogen for 18 h. The reaction was incomplete, thus **Compound 4** and **Compound 5** were purified by reverse phase chromatography (2-50% ACN/water with 0.1% HCl) and isolated to afford *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)acetamide (slow eluting, 13.8 mg, 9%, HCl salt) and *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)acetamide (fast eluting, 21.8 mg, 15%, HCl salt). **Compound 4** ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 9.09 (d, J = 4.5 Hz, 1H), 8.63 (s, 1H), 7.91 (d, J = 7.9 Hz, 1H), 7.56 – 7.46 (m, 1H), 7.36 (d, J = 7.4 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 7.15 (d, J = 7.4 Hz, 1H), 2.35 (s, 3H), 2.16 (s, 3H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.95, 162.16, 153.00, 150.35, 148.91, 139.09, 137.16, 136.38, 134.41, 130.41, 127.23, 125.32, 121.31, 119.80, 103.47, 24.03, 19.95, 16.48. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{17}N_3O$

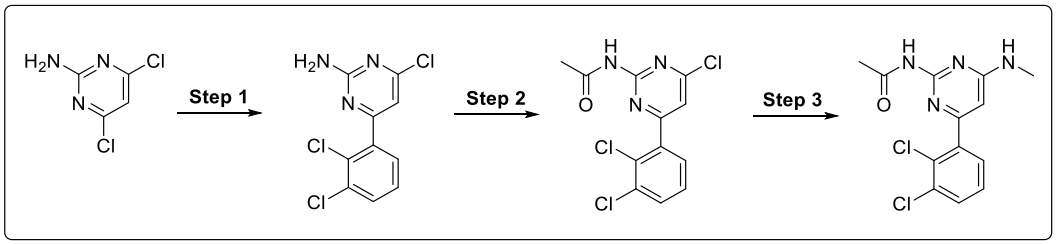
292.1450; found 292.1445. HPLC purity: 98.4%. **Compound 5** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.41 (s, 1H), 9.00 (s, 1H), 7.38 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 7.6 Hz, 1H), 7.16 (d, J = 7.5 Hz, 1H), 6.85 (s, 1H), 3.37-3.30 (m, 2H), 2.32 (s, 3H), 2.30 – 2.14 (m, 2H), 2.13 (s, 3H), 2.05 (s, 3H), 1.80 – 1.64 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.88, 156.42, 144.72, 143.82, 137.68, 134.63, 131.53, 131.50, 126.54, 126.03, 111.46, 93.63, 40.24, 24.00, 22.29, 19.91, 18.88, 16.00. LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{18}H_{21}N_3O$ 296.1763; found 296.1759. HPLC purity: 100%.



Compound 6: *N*-(6-(2,3-dimethylphenyl)-4-(methylamino)pyridin-2-yl)acetamide

Step 1: Following **Step 1** of the synthesis described to prepare **Compound 7**, using 2,6-dichloro-*N*-methyl-pyridin-4-amine (150 mg, 0.847 mmol) and 2,3-dimethylphenylboronic acid (140 mg, 0.932 mmol), the crude product was purified by silica gel chromatography (10-40% EtOAc/hexanes) to afford 2-chloro-6-(2,3-dimethylphenyl)-*N*-methylpyridin-4-amine (97.0 mg, 46%).

Step 2: A 10 mL vial was charged with 2-chloro-6-(2,3-dimethylphenyl)-*N*-methylpyridin-4-amine (97.0 mg, 0.393 mmol), acetamide (116 mg, 1.97 mmol), Xantphos (19.6 mg, 0.039 mmol) and cesium carbonate (384 mg, 1.18 mmol). 1,4-Dioxane (2.0 mL) was added and the mixture was degassed with nitrogen for 10 minutes. $Pd_2(dba)_3$ (18.0 mg, 0.020 mmol) was added, the vial was sealed then heated at 100 °C for 12 h. The reaction was cooled to room temperature, diluted with EtOAc and was filtered. The filtrate was concentrated and the crude product was purified by reverse phase chromatography to afford *N*-(6-(2,3-dimethylphenyl)-4-(methylamino)pyridin-2-yl)acetamide as a colorless solid (16 mg, 13%, HCl salt). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.42 – 7.34 (m, 1H), 7.32 – 7.21 (m, 2H), 6.59 (s, 1H), 6.31 (bs, 1H), 2.98 (s, 3H), 2.38 (s, 3H), 2.27 (s, 3H), 2.24 (s, 3H). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{16}H_{19}N_3O$ 270.2; found 270.0. HPLC purity: 100%.



Compound 7: *N*-(4-(2,3-dichlorophenyl)-6-(methylamino)pyrimidin-2-yl)acetamide

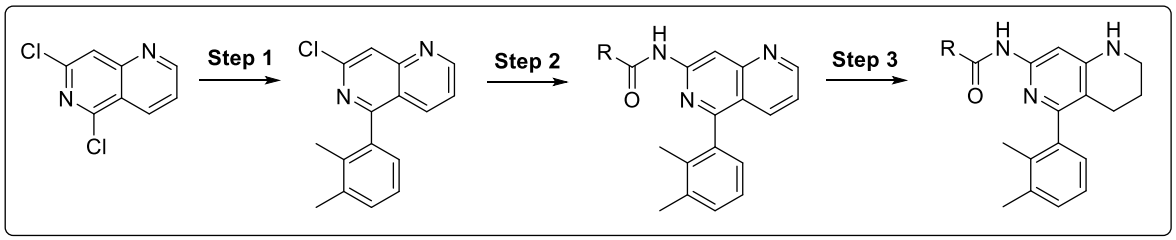
Step 1: A 20 mL vial was charged with 4,6-dichloropyrimidin-2-amine (200 mg, 1.22 mmol), 2,3-dichlorophenylboronic acid (256 mg, 1.34 mmol), tetrakis(triphenylphosphine)palladium(0) (70.5 mg, 0.061 mmol) and potassium carbonate (506 mg, 3.66 mmol). The vessel was purged with nitrogen. A solution of 1,4-dioxane/water (2:1, 9.0 mL) was degassed under argon and was added to the solid reagents. The vial was sealed and heated at 80 °C for 30 minutes. The reaction was cooled to room temperature, diluted with saturated $NH_4Cl_{(aq)}$ and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was diluted in DCM and filtered to afford the desired product as a colorless solid (185 mg, 55%). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{10}H_6Cl_3N_3$ 274.0; found 273.9.

Step 2: A 10 mL vial was charged with 4-chloro-6-(2,3-dichlorophenyl)pyrimidin-2-amine (185 mg, 0.674 mmol) and acetic anhydride (2.5 mL). The mixture was heated at 120 °C for 18 h. The reaction was concentrated, diluted with saturated $NaHCO_{3\,(aq)}$ and extracted with DCM (2x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was used in the subsequent reaction without further purification. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{12}H_8Cl_3N_3O$ 316.0; found 315.9.

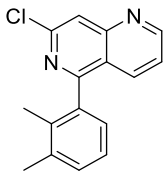
Step 3: A 10 mL vial was charged with crude *N*-(4-chloro-6-(2,3-dichlorophenyl)pyrimidin-2-yl)acetamide (213 mg, 0.674 mmol) and methylamine (2M in MeOH, 3.0 mL). The reaction was sealed and heated at 60 °C for 15 minutes. The reaction was cooled to room temperature, the solids were filtered and the filtrate was concentrated. The crude product was purified by reverse phase chromatography to afford *N*-(4-(2,3-dichlorophenyl)-6-(methylamino)pyrimidin-2-yl)acetamide as a solid HCl salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (bs, 1H), 9.82 (bs, 1H), 7.87 (dd, *J* = 5.6, 4.1 Hz, 1H), 7.62-7.51 (m, 2H), 6.64 (s, 1H), 2.99 (d, *J* = 4.7 Hz, 3H),

2.26 (s, 3H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{12}Cl_2N_4O$ 311.1; found 311.0. HPLC purity: 97.7%.

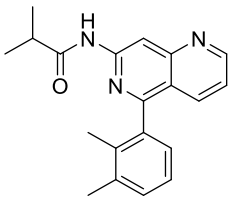
Scheme S2. General synthesis of tetrahydronaphthyridines **8-17**.



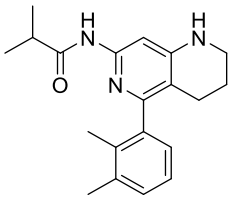
Compound 10: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)isobutyramide



Step 1: A 250 mL flask was charged with (2,3-dimethylphenyl)boronic acid (830 mg, 5.53 mmol), potassium carbonate (2.10 g, 15.2 mmol), 5,7-dichloro-1,6-naphthyridine (1.00 g, 5.02 mmol), water (6.5 mL) and 1,4-dioxane (14.0 mL). The mixture was sparged with nitrogen for 10 min to degas. After 10 min, tetrakis(triphenylphosphine)palladium(0) (290 mg, 0.251 mmol) was added and the reaction was heated to 80 °C for 40 min. The reaction was cooled to room temperature, diluted with saturated $NH_4Cl_{(aq)}$ and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified by silica gel chromatography (5-50% EtOAc/hexanes) to afford the desired product (1.30 g, 96%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (dd, J = 4.2, 1.7 Hz, 1H), 8.11 (d, J = 0.9 Hz, 1H), 7.93 (ddd, J = 8.5, 1.7, 1.0 Hz, 1H), 7.64 (dd, J = 8.5, 4.2 Hz, 1H), 7.41 – 7.34 (m, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.16 (dd, J = 7.6, 1.4 Hz, 1H), 2.36 (s, 3H), 1.89 (s, 3H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{13}ClN_2$ 269.1; found 269.1.

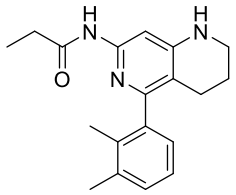


Step 2: A pressure tube was charged with isobutyramide (132 mg, 1.52 mmol), cesium carbonate (495 mg, 1.52 mmol), *t*-butyl-Xantphos (25.2 mg, 0.051 mmol), 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (136 mg, 0.506 mmol) and 1,4-dioxane (1.70 mL). The reaction mixture was sparged with nitrogen to degas. After 10 min, tris(dibenzylideneacetone) dipalladium (0) (23.2 mg, 0.025 mmol) was added and the reaction was heated to 100 º C for 16 h. The mixture was cooled to room temperature and diluted with H_2O and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified by silica gel chromatography (0–50% EtOAc/hexanes) to afford the desired product (77 mg, 48% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.77 (s, 1H), 9.01 (dt, *J* = 4.3, 1.9 Hz, 1H), 8.60 (d, *J* = 2.0 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.46 – 7.37 (m, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.23 (t, *J* = 7.5 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 2.80 (p, *J* = 6.9 Hz, 1H), 2.33 (d, *J* = 2.2 Hz, 3H), 1.86 (s, 3H), 1.11 (dd, *J* = 6.8, 2.2 Hz, 6H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{21}N_3O$ 320.2; found 320.2.



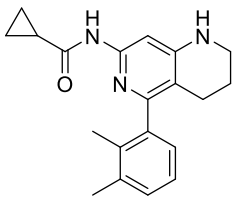
Step 3: A 250 mL Parr Shaker vessel was charged with *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)isobutyramide (70.0 mg, 0.219 mmol) and EtOH (4.4 mL). Hydrochloric acid solution (110 µL, 4M in 1,4-dioxane) and PtO_2 (24.9 mg, 0.110 mmol) were added and vessel was put on the shaker apparatus under hydrogen gas (40 psi) and shaken for 15 minutes at room temperature. The reaction residue was diluted with H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified by silica gel chromatography (0–5% MeOH/CH_2Cl_2) to afford

the desired product (77 mg, 56% yield). ^{1}H NMR (300 MHz, DMSO-d6) δ 9.82 (s, 1H), 7.24 (s, 1H), 7.18 – 7.04 (m, 2H), 6.89 (dd, J = 7.1, 1.9 Hz, 1H), 6.73 (s, 1H), 3.22 – 3.10 (m, 2H), 2.64 (p, J = 6.8 Hz, 1H), 2.26 (s, 3H), 2.23 – 2.04 (m, 1H), 1.94 (s, 3H), 1.67 (s, 2H), 1.03 (d, J = 6.8 Hz, 6H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{25}N_3O$ 324.2 found 324.2.



Compound 8: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)isobutyramide

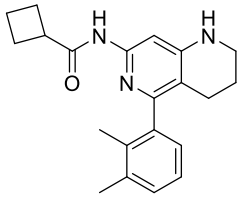
Following **Step 2** of the synthesis described to prepare **Compound 8**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (246 mg, 0.915 mmol) and propanamide (335 mg, 4.58 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)propionamide (185 mg, 66%). Following Step 3 of the synthesis described to prepare **Compound 8**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)propionamide (174 mg, 0.570 mmol) and PtO_2 (84.1 mg, 0.370 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)isobutyramide (54 mg, 31%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (s, 1H), 7.22 (s, 1H), 7.14 – 6.99 (m, 2H), 6.91 – 6.82 (m, 1H), 6.73 (s, 1H), 3.18 – 3.10 (m, 2H), 2.30 - 2.21 (m, 5H), 2.18 – 2.00 (m, 2H), 1.91 (s, 3H), 1.72 - 1.58 (m, 2H), 1.00 (t, *J* = 7.6 Hz, 3H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{23}N_3O$ 310.2; found 310.4. HPLC purity: 100%.



Compound 9: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)cyclopropanecarboxamide

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (178 mg, 0.662 mmol) and cyclopropanecarboxamide (169 mg, 1.99 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)cyclopropanecarboxamide (161 mg, 77%).

Following **Step 3** of the synthesis described to prepare **Compound 10**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)cyclopropanecarboxamide (149 mg, 0.469 mmol) and PtO_2 (85.3 mg, 0.376 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)cyclopropanecarboxamide (30 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.22 (s, 1H), 7.23 – 7.02 (m, 3H), 6.91 (d, J = 7.1 Hz, 1H), 6.73 (s, 1H), 3.17 (d, J = 5.8 Hz, 2H), 2.26 (s, 3H), 2.14 (dq, J = 16.0, 9.8, 8.2 Hz, 1H), 1.94 (s, 3H), 1.90 – 1.82 (m, 1H), 1.67 (s, 2H), 1.28 – 1.04 (m, 1H), 0.81 – 0.64 (m, 4H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{23}N_3O$ 322.1919; found 322.1915. HPLC purity: 95.7%.

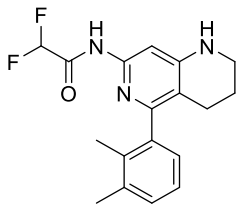


Compound 11: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)cyclobutanecarboxamide

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (198 mg, 0.737 mmol) and cyclobutanecarboxamide (219 mg, 2.21 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)cyclobutanecarboxamide (200 mg, 82%).

Following **Step 3** of the synthesis described to prepare **Compound 10**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)cyclobutanecarboxamide (198 mg, 0.603 mmol) and PtO_2 (82.2 mg, 0.362 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)cyclobutanecarboxamide (104 mg, 51%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.68 (s, 1H), 7.24 (s, 1H), 7.14 – 7.01 (m, 2H), 6.91 – 6.83 (m, 1H), 6.73 (s, 1H), 3.24 (t, J = 8.3 Hz, 1H), 3.20 - 3.10 (m, 2H), 2.24 (s, 3H), 2.20 – 2.07 (m, 4H), 2.06 – 1.94 (m, 2H), 1.91 (s,

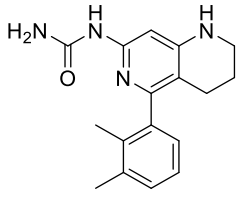
3H), 1.88 – 1.80 (m, 1H), 1.81 - 1.70 (m, 1H), 1.70 - 1.58 (m, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{25}N_3O$ 336.2; found 336.2. HPLC purity: 100%.



Compound 12: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)-2,2-difluoroacetamide

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (242 mg, 0.900 mmol) and 2,2-difluoroacetamide (428 mg, 4.50 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)-2,2-difluoroacetamide (92 mg, 31%).

Following **Step 3** of the synthesis described to prepare **Compound 10**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)-2,2-difluoroacetamide (72.0 mg, 0.220 mmol) and PtO_2 (40.0 mg, 0.176 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)-2,2-difluoroacetamide (55 mg, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.84 (s, 1H), 7.20 (s, 1H), 7.18 – 7.06 (m, 2H), 6.99 (s, 1H), 6.92 (d, J = 7.3 Hz, 1H), 6.26 (t, J = 53.9 Hz, 1H), 3.23 – 3.14 (m, 2H), 2.27 (s, 3H), 2.24 – 2.05 (m, 2H), 1.94 (s, 3H), 1.77 – 1.61 (m, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{19}F_2N_3O$ 332.2; found 333.2. HPLC purity: 100%.

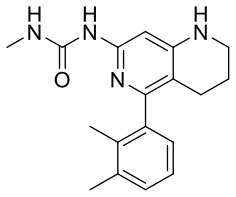


Compound 13: 1-(5-(2,3-Dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)urea

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (161 mg, 0.599 mmol), urea (72.0 mg, 1.20 mmol) and *t*-

butylBrettPhos (29.0 mg, 0.060 mmol), afforded 1-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)urea (59 mg, 34%).

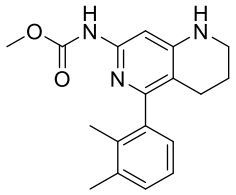
Following **Step 3** of the synthesis described to prepare **Compound 10**, using 1-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)urea (275 mg, 0.941 mmol) and PtO_2 (64.1 mg, 0.282 mmol), afforded 1-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)urea (80 mg, 29%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.18 – 7.03 (m, 2H), 6.92 (d, J = 7.2 Hz, 1H), 6.75 (s, 1H), 6.24 (s, 1H), 3.14 (s, 2H), 2.26 (s, 3H), 2.11 (ddt, J = 22.0, 15.8, 7.8 Hz, 2H), 1.96 (s, 3H), 1.73 – 1.56 (m, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{20}N_4O$ 297.2; found 297.0. HPLC purity: 100%.



Compound 14: 1-(5-(2,3-Dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)-3-methylurea

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (236 mg, 0.878 mmol) and *N*-methylurea (325 mg, 4.39 mmol), afforded 1-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)-3-methylurea (81 mg, 30%).

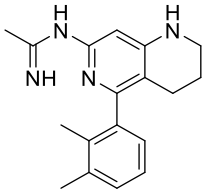
Following Step 3 of the synthesis described to prepare **Compound 10**, using 1-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)-3-methylurea (160 mg, 0.522 mmol) and PtO_2 (77.1 mg, 0.339 mmol), afforded 1-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)urea (80 mg, 29%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.40 (s, 1H), 7.18 – 7.03 (m, 2H), 6.92 (d, J = 7.2 Hz, 1H), 6.75 (s, 1H), 6.22 (s, 1H), 3.14 (s, 2H), 2.26 (d, J = 7.2 Hz, 3H), 2.30 (s, 3H), 2.25 - 2.01 (m, 2H), 1.96 (s, 3H), 1.72 – 1.59 (m, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{22}N_4O$ 311.2; found 311.2. HPLC purity: 95.6%.



Compound 15: Methyl (5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)carbamate

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (198 mg, 0.737 mmol) and methyl carbamate (166 mg, 2.21 mmol), afforded methyl (5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)carbamate (183 mg, 81%).

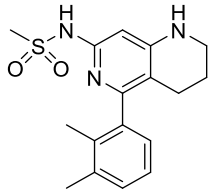
Following **Step 3** of the synthesis described to prepare **Compound 10**, using methyl (5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)carbamate (168 mg, 0.547 mmol) and PtO_2 (99.3 mg, 0.437 mmol), afforded methyl (5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)carbamate (55 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.46 (s, 1H), 7.16 – 7.03 (m, 2H), 6.95 (s, 1H), 6.88 (dd, J = 7.3, 1.8 Hz, 1H), 6.76 (s, 1H), 3.60 (s, 3H), 3.22 – 3.11 (m, 2H), 2.25 (s, 3H), 2.22 – 2.02 (m, 1H), 1.92 (s, 3H), 1.66 (d, J = 5.5 Hz, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{21}N_3O_2$ 312.2; found 312.2. HPLC purity: 100%.



Compound 16: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)acetimidamide

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (320 mg, 1.19 mmol) and acetamidine hydrochloride (124 mg, 1.31 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)acetimidamide (68 mg, 20%).

Following **Step 3** of the synthesis described to prepare **Compound 10**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)acetimidamide (70.0 mg, 0.241 mmol) and PtO_2 (32.8 mg, 0.145 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)acetimidamide (40 mg, 56%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (s, 1H), 11.38 (s, 1H), 10.02 (s, 1H), 7.41 (s, 1H), 7.15 (dt, J = 14.8, 7.4 Hz, 2H), 6.95 (d, J = 7.4 Hz, 1H), 6.30 (s, 1H), 3.20 (s, 2H), 2.31 (s, 3H), 2.27 (s, 3H), 2.17 (dt, J = 12.1, 6.4 Hz, 1H), 1.94 (s, 3H), 1.66 (d, J = 5.7 Hz, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{22}N_4$ 295.2; found 295.2. HPLC purity: 100%.

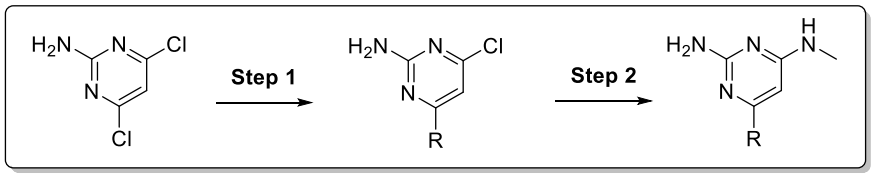


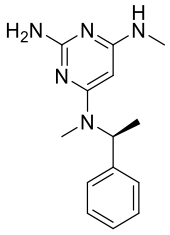
Compound 17: *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)methanesulfonamide

Following **Step 2** of the synthesis described to prepare **Compound 10**, using 7-chloro-5-(2,3-dimethylphenyl)-1,6-naphthyridine (218 mg, 0.811 mmol) and methanesulfonamide (386 mg, 4.06 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)methanesulfonamide (77 mg, 29%).

Following **Step 3** of the synthesis described to prepare **Compound 10**, using *N*-(5-(2,3-dimethylphenyl)-1,6-naphthyridin-7-yl)methanesulfonamide (59.0 mg, 0.180 mmol) and PtO_2 (32.7 mg, 0.144 mmol), afforded *N*-(5-(2,3-dimethylphenyl)-1,2,3,4-tetrahydro-1,6-naphthyridin-7-yl)methanesulfonamide (25 mg, 42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.29 (s, 1H), 7.76 (s, 1H), 7.25 (d, J = 7.4 Hz, 1H), 7.16 (t, J = 7.5 Hz, 1H), 7.00 (d, J = 7.5 Hz, 1H), 6.21 (s, 1H), 3.19 (s, 3H), 2.73 (s, 3H), 2.28 (s, 3H), 2.07 (s, 2H), 2.01 (s, 4H), 1.64 (s, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{21}N_3O_2S$ 332.1; found 332.2. HPLC purity: 100%.

Scheme S3. General synthesis of **18-30** with *N*- and *O*-linked alkyl and aryl groups.

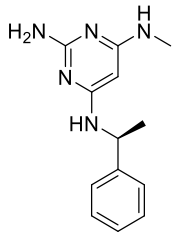




Compound 25: (*S*)-N^4,N^6-dimethyl-N^4-(1-phenylethyl)pyrimidine-2,4,6-triamine

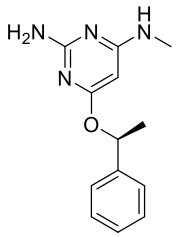
Step 1: A 10 mL microwave vial was charged with 4,6-dichloropyrimidin-2-amine (200 mg, 1.22 mmol), ethanol (2.0 ml), tetrahydrofuran (2.0 mL), *N*,*N*-diisopropylethylamine (319 µL, 1.83 mmol), and (*S*)-*N*-methyl-1-phenylethanamine (178 µL, 1.22 mmol). The vial was sealed and heated at 120 °C for 25 minutes. The reaction was cooled to room temperature, diluted with EtOAc, washed with water and brine, dried over sodium sulfate, filtered and concentrated. The crude product was purified by normal phase chromatography (0-12% methanol in dichloromethane) to afford a white solid (290 mg, 91% yield).

Step 2: A 10 mL microwave vial was charged with (*S*)-6-chloro-N^4-methyl-N^4-(1-phenylethyl)pyrimidine-2,4-diamine (150 mg, 0.571 mmol) and methylamine (3.0 mL, 33% in ethanol) was added. The vial was sealed and heated to 140 °C for 10 min in a microwave reactor. The crude reaction was concentrated, diluted with ethyl acetate, washed with water then brine, dried over sodium sulfate, filtered and concentrated. The crude product was purified by normal phase chromatography (0-10 % methanol in dichloromethane) to afford the title compound as white solid (25 mg, 17% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40 – 7.26 (m, 2H), 7.26 – 7.11 (m, 3H), 6.27 – 6.00 (m, 2H), 5.56 (s, 2H), 4.87 (s, 1H), 2.67 (d, J = 4.9 Hz, 3H), 1.42 (d, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.04, 163.25, 162.28, 142.56, 128.22, 126.68, 126.51, 72.02, 50.07, 29.23, 27.66, 16.38. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{19}N_5$ 258.1718; found 258.1713. HPLC purity: 100%.



Compound 24: (*S*)-*N*^{4}-methyl-*N*^{6}-(1-phenylethyl)pyrimidine-2,4,6-triamine

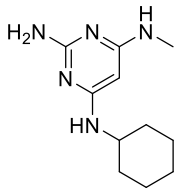
Following **Step 1** of the synthesis described to prepare **Compound 25** using 4,6-dichloropyrimidin-2-amine (200 mg, 1.22 mmol), (*S*)-1-phenylethanamine (148 mg, 1.22 mmol), and *N*,*N*-diisopropylethylamine (319 µL, 1.83 mmol), (*S*)-6-chloro-*N*^{4}-(1-phenylethyl)pyrimidine-2,4-diamine was generated without isolation. Methylamine (610 µL, 4.88 mmol, 33% in EtOH) was added directly to the crude reaction, which was heated at 170 °C for 30 minutes then 180 °C for another 30 minutes, and isolated as described in **Step 2** to prepare **Compound 25**. The title compound was isolated as a white solid (38 mg, 13%). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 7.42 – 7.21 (m, 4H), 7.21 – 7.00 (m, 1H), 6.46 (d, J = 8.3 Hz, 1H), 5.89 (d, J = 5.1 Hz, 1H), 5.28 (s, 2H), 4.70 (s, 1H), 2.56 (d, J = 4.8 Hz, 3H), 1.33 (d, J = 7.0 Hz, 3H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{13}H_{17}N_5$ 244.15; found 244.60. HPLC purity: 100%.



Compound 23: (*S*)-*N*^{4}-methyl-6-(1-phenylethoxy)pyrimidine-2,4-diamine

Step 1: To a solution of (*S*)-1-phenylethanol (552 µl, 4.57 mmol) in 2-MeTHF (10.0 mL) was added sodium hydride (60 % dispersion in mineral oil, 183 mg, 4.57 mmol) and stirred for 20 min at RT. To this solution was added 4,6-dichloropyrimidin-2-amine (500 mg, 3.05 mmol) and the mixture was heated at 80 °C for 4 h. The reaction was cooled to room temperature, diluted with EtOAc, washed with water then brine, dried over sodium sulfate, filtered and concentrated. The crude product was purified by normal phase chromatography (0-25% ethyl acetate in hexanes) to afford (*S*)-4-chloro-6-(1-phenylethoxy)pyrimidin-2-amine (370 mg, 49%) as a white solid.

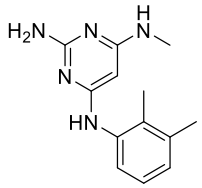
Following **Step 2** of the synthesis described to prepare **Compound 25** using (*S*)-4-chloro-6-(1-phenylethoxy)pyrimidin-2-amine (150 mg, 0.601 mmol) and methylamine (3.0 mL, 33% in ethanol) afforded (102 mg, 70%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.47 – 7.12 (m, 5H), 6.40 (d, J = 5.4 Hz, 1H), 6.05 (q, J = 6.5 Hz, 1H), 5.84 (s, 2H), 5.02 (s, 1H), 2.63 (d, J = 4.8 Hz, 3H), 1.46 (d, J = 6.6 Hz, 3H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{13}H_{16}N_4O$ 245.13; found 245.17. HPLC purity: 100%.



Compound 22: *N*^{4}-cyclohexyl-*N*^{6}-methylpyrimidine-2,4,6-triamine

A microwave vial was charged with 4,6-dichloropyrimidin-2-amine (200 mg, 1.22 mmol), ethanol (2.0 mL), triethylamine (255 µL, 1.83 mmol), cyclohexylamine (140 µL, 1.22 mmol) and the reaction was heated in a microwave reactor for 20 min at 80 °C, then 20 min at 90 °C. The reaction was concentrated, diluted with water, extracted with EtOAc, dried over $MgSO_4$, filtered, concentrated and the residue was used in the subsequent step without further purification.

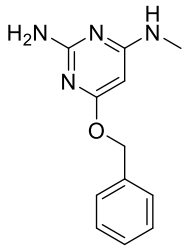
Following **Step 2** of the synthesis described to prepare **Compound 25** using 6-chloro-*N*^{4}-cyclohexylpyrimidine-2,4-diamine (100 mg, 0.441 mmol), methylamine (33% in ethanol, 2.0 mL) and heating for 30 min at 140 °C afforded *N*^{4}-cyclohexyl-*N*^{6}-methylpyrimidine-2,4,6-triamine (67.0 mg, 69%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.84 (t, J = 6.8 Hz, 2H), 5.30 (s, 2H), 4.73 (s, 1H), 3.50 (s, 1H), 3.30 (s, 1H), 2.60 (d, J = 4.9 Hz, 3H), 1.79 (d, J = 11.9 Hz, 2H), 1.66 (d, J = 12.6 Hz, 1H), 1.54 (d, J = 12.4 Hz, 1H), 1.38 – 0.98 (m, 5H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{11}H_{19}N_5$ 222.16; found 222.60. HPLC purity: 89.3%.



Compound 21: N^4-(2,3-dimethylphenyl)-N^6-methylpyrimidine-2,4,6-triamine

A 250 mL flask was charged with 4,6-dichloropyrimidin-2-amine (500 mg, 3.05 mmol), water (25.0 mL), *i*PrOH (5.0 mL) and 2,3-dimethylaniline (369 mg, 3.05 mmol). The reaction was heated at 90 °C for 18 h, then at 100 °C for 6 h. The reaction was cooled to RT, poured into cold water and the solid was collected by filtration. The cake was washed with water, *i*PrOH and hexanes and subsequently dried under vacuum to afford 6-chloro-N^4-(2,3-dimethylphenyl)pyrimidine-2,4-diamine as a colorless solid.

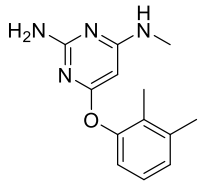
Following **Step 2** of the synthesis described to prepare **Compound 2** using 6-chloro-N^4-(2,3-dimethylphenyl)pyrimidine-2,4-diamine (50.0 mg, 0.201 mmol), methylamine (2.0M in THF, 600 µL) and heating at 190 °C for 40 minutes afforded N^4-(2,3-dimethylphenyl)-N^6-methylpyrimidine-2,4,6-triamine (29.0 mg, 59%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.12 – 6.84 (m, 3H), 6.27 (s, 1H), 5.66 (s, 2H), 4.72 (s, 1H), 2.60 (d, J = 4.7 Hz, 3H), 2.22 (s, 3H), 2.04 (s, 3H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{13}H_{17}N_5$ 244.15; found 244.02.; found 222.60. HPLC purity: 100%.



Compound 20: 6-(Benzyloxy)-N^4-methylpyrimidine-2,4-diamine

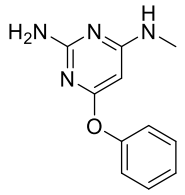
To an oven dried pressure flask was added benzyl alcohol (568 µL, 0.568 mmol) and DMSO (2.0 mL) followed by sodium hydride (60 % dispersion in mineral oil, 14.7 mg, 0.369 mmol). The mixture was stirred for 20 minutes at RT and then 6-chloro-N^4- methylpyrimidine-2,4-diamine (45.0 mg, 0.284 mmol) was added. The tube was sealed and heated at 90 °C for 5 h. The mixture was cooled to RT, diluted with water and extracted with ethyl acetate (3x). The

combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified on silica gel (EtOAc/hexanes) to afford 6-(benzyloxy)-N^4-methylpyrimidine-2,4-diamine (19 mg, 29%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.49 – 7.14 (m, 5H), 6.44 (s, 1H), 5.93 (s, 2H), 5.19 (s, 2H), 5.05 (s, 1H), 2.66 (d, J = 4.8 Hz, 3H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{12}H_{14}N_4O$ 231.12; found 231.36. HPLC purity: 100%.



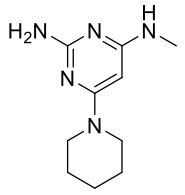
Compound 19: 6-(2,3-Dimethylphenoxy)-N^4-methylpyrimidine-2,4-diamine: Following Step 1 of the synthesis described to prepare **Compound 23** using 4,6-dichloropyrimidin-2-amine (500 mg, 3.05 mmol), 2,3-dimethylphenol (372 mg, 3.05 mmol) and potassium carbonate (632 mg, 4.57 mmol), afforded 4-chloro-6-(2,3-dimethylphenoxy)pyrimidin-2-amine (630 mg, 83%).

Following **Step 2** of the synthesis described to prepare **Compound 23** using 4-chloro-6-(2,3-dimethylphenoxy)pyrimidin-2-amine (50.0 mg, 0.200 mmol), methylamine (2.0M in THF, 600 µL) and *N*,*N*-diisopropylethylamine (0.119 mL, 0.681 mmol), afforded 6-(2,3-dimethylphenoxy)-N^4-methylpyrimidine-2,4-diamine (25 mg, 51%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.07 (t, J = 7.7 Hz, 1H), 6.99 (d, J = 7.4 Hz, 1H), 6.89 – 6.76 (m, 1H), 6.62 (s, 1H), 5.98 (s, 2H), 4.90 (s, 1H), 2.65 (d, J = 4.7 Hz, 3H), 2.23 (s, 3H), 1.97 (s, 3H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{13}H_{16}N_4O$ 245.13; found 245.01. HPLC purity: 100%.



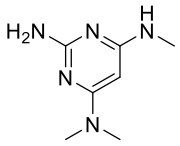
Compound 18: ***N*^{4}-methyl-6-phenoxypyrimidine-2,4-diamine** Following Step 1 of the synthesis described to prepare **Compound 23** using 4,6-dichloropyrimidin-2-amine (500 mg, 3.05 mmol), phenol (0.287 g 3.05 mmol) and potassium carbonate (0.632, 4.57 mmol), afforded 4-chloro-6-(2,3-dimethylphenoxy)pyrimidin-2-amine (420 mg, 62%).

Following **Step 2** of the synthesis described to prepare **Compound 23** using 4-chloro-6-(2,3-dimethylphenoxy)pyrimidin-2-amine (100 mg, 0.451 mmol), methylamine (2.0M in THF, 1.40 mL) and *N*,*N*-diisopropylethylamine (267 µL, 1.53 mmol), afforded 6-phenoxy-*N*^{4}-methylpyrimidine-2,4-diamine (50 mg, 51%). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 7.41 – 7.31 (m, 2H), 7.12-7.19 (m, 1H), 7.09 – 7.00 (m, 2H), 6.69 (s, 1H), 6.02 (s, 2H), 5.03 (s, 1H), 2.67 (d, J = 4.7 Hz, 3H). LCMS-ESI+ (m/z): [M+H]+ calcd for $C_{11}H_{12}N_4O$ 217.10; found 216.93. HPLC purity: 100%.



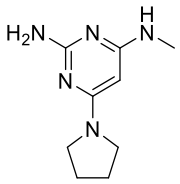
Compound 26: ***N*^{4}-methyl-6-(piperidin-1-yl)pyrimidine-2,4-diamine**

A microwave vial was charged with 6-chloro-*N*^{4}-methylpyrimidine-2,4-diamine (50.0 mg, 0.315 mmol), piperidine (155 µL, 1.58 mmol), *N*,*N*-diisopropylamine (275 µL, 1.58 mmol) and methanol (1.0 mL). The reaction was heated in a microwave reactor for 40 min at 160 °C. The reaction was concentrated and the residue was purified by silica gel chromatography (0-5% MeOH/DCM) to afford the title compound as a colorless solid (65 mg, quant.). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 11.24 (s, 1H), 6.86 (s, 1H), 6.55 (s, 2H), 5.12 (s, 1H), 3.50 (t, *J* = 5.3 Hz, 4H), 2.74 (d, *J* = 4.8 Hz, 3H), 1.60 (q, *J* = 6.3, 5.1 Hz, 2H), 1.49 (dp, *J* = 8.3, 4.9, 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 161.34, 158.33, 71.55, 45.01, 27.93, 25.16, 24.18. LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{10}H_{17}N_5$ 208.1562; found 208.1556. HPLC purity: 100%.



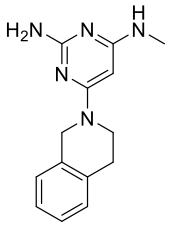
Compound 27: *N*^{4},*N*^{4},*N*^{6}-trimethylpyrimidine-2,4,6-triamine

A microwave vial was charged with 6-chloro-*N*^{4}-methylpyrimidine-2,4-diamine (50.0 mg, 0.315 mmol), dimethylamine hydrochloride (129 mg, 1.58 mmol), *N*,*N*-diisopropylamine (549 µL, 3.15 mmol) and ethanol (1.0 mL). The reaction was heated in a microwave reactor for 40 min at 160 °C. The reaction was concentrated and the residue was purified by silica gel chromatography (0-5% MeOH/DCM) to afford the title compound as a colorless solid (29.2 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.00 (d, J = 5.1 Hz, 1H), 5.38 (s, 2H), 4.82 (s, 1H), 2.86 (s, 6H), 2.65 (d, J = 4.9 Hz, 3H). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_7H_{13}N_5$ 168.1; found 167.9. HPLC purity: 100%.



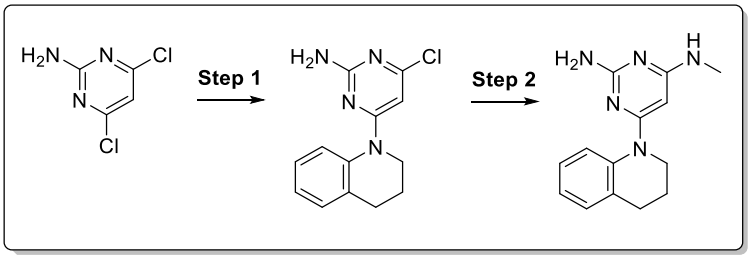
Compound 28: *N*^{4}-methyl-6-(pyrrolidin-1-yl)pyrimidine-2,4-diamine

Following the synthesis described to prepare **Compound 27** using 6-chloro-*N*^{4}-methylpyrimidine-2,4-diamine (50.0 mg, 0.315 mmol) and pyrrolidine (263 µL, 3.15 mmol), the resulting colorless precipitate was filtered, washed with EtOH (2 x 1.0 mL) and dried under vacuum to afford the tite compound as a colorless solid (36 mg, 59%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.96 (d, J = 5.1 Hz, 1H), 5.37 (s, 2H), 4.69 (s, 1H), 3.30 – 3.20 (m, 3H), 2.66 (d, J = 4.9 Hz, 3H), 1.91 – 1.76 (m, 4H). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_9H_{15}N_5$ 194.1; found 194.1. HPLC purity: 100%.



Compound 29: 6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-N^4-methylpyrimidine-2,4-diamine

Following the synthesis described to prepare **Compound 26** using 6-chloro-*N*^{4}-methylpyrimidine-2,4-diamine (50.0 mg, 0.315 mmol) and 1,2,3,4-tetrahydroisoquinoline (197 µL, 1.58 mmol), the title compound was isolated as a yellow solid (44 mg, 54%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.18 (d, *J* = 1.1 Hz, 4H), 6.43 (s, 1H), 5.95 (s, 2H), 5.10 (s, 1H), 4.61 (s, 2H), 3.72 (t, *J* = 5.9 Hz, 2H), 2.83 (t, *J* = 5.9 Hz, 2H), 2.73 (d, *J* = 4.8 Hz, 3H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{17}N_5$ 256.2; found 256.6. HPLC purity: 100%.



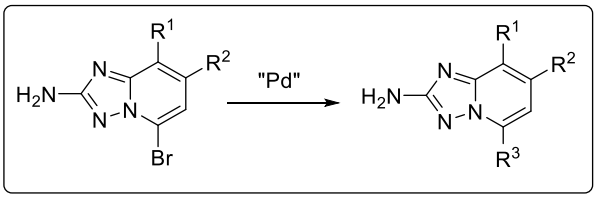
Compound 30: 6-(3,4-dihydroquinolin-1(2*H*)-yl)-*N*^{4}-methylpyrimidine-2,4-diamine

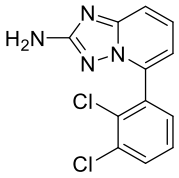
Step 1: A vial was charged with 4,6-dichloropyrimidin-2-amine (500 mg, 3.05 mmol), 1,2,3,4-tetrahydroquinoline (446 µL, 3.05 mmol), Xantphos (353 mg, 0.610 mmol), cesium carbonate (2.98 g, 9.15 mmol) and 1,4-dioxane (5.0 mL). The reaction was degassed with nitrogen for 10 minutes, then $Pd_2(dba)_3$ (140 mg, 0.152 mmol) was added. The vial was sealed and heated at 140 °C for 18 h. The reaction was diluted with EtOAc, washed with saturated $NaHCO_{3\,(aq)}$ (3x), brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc/hexanes) to afford a beige solid (68 mg, 9%).

Step 2: Following **Step 2** of the synthesis described to prepare **Compound 25** using 4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)pyrimidin-2-amine (40.0 mg, 0.153 mmol), methylamine (33% in ethanol, 1.50 mL) and heating for 40 min at 160 °C afforded the title compound as a white solid (30.9 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.15 – 6.99 (m, 2H), 6.89 (td, *J* = 7.4, 1.3 Hz, 1H), 6.29 (d, *J* = 5.1 Hz, 1H), 5.74 – 5.63 (m, 2H), 5.32

(s, 1H), 3.83 – 3.65 (m, 2H), 2.69 (t, *J* = 6.6 Hz, 2H), 2.63 (d, *J* = 4.8 Hz, 3H), 1.89 – 1.67 (m, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{17}N_5$ 256.2; found 256.4. HPLC purity: 96%.

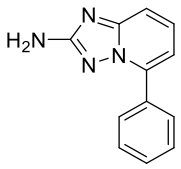
Scheme S4. General synthesis of triazolopyridines **31**, **32**, **33** and **34**.





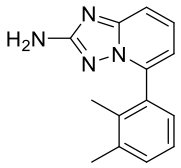
Compound 32: 5-(2,3-dichlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine

A vial was charged with 5-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (910 mg, 4.27 mmol), 2,3-dichlorophenylboronic acid (897 mg, 4.70 mmol), cesium carbonate (4.18 g, 12.8 mmol), 1,4-dioxane (15.0 mL) and water (7.5 mL). The reaction was degassed with nitrogen for 10 minutes, then PEPPSI-IPr (291 mg, 0.427 mmol) was added. The vial was sealed and heated at 100 °C for 60 minutes. The reaction was cooled to room temperature, diluted with saturated $NH_4Cl_{(aq)}$ and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel chromatography (100% EtOAc to 5% MeOH/EtOAc) to afford a colorless solid (940 mg, 79% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.81 (d, *J* = 6.6 Hz, 1H), 7.60 – 7.49 (m, 3H), 7.45 (d, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 7.1 Hz, 1H), 6.07 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 165.96, 150.53, 135.72, 134.66, 132.02, 131.50, 131.24, 130.22, 128.57 (2 carbons, confirmed by HMQC), 112.30, 112.11. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{12}H_8Cl_2N_4$ 279.0204; found 279.0204. HPLC purity: 100%.



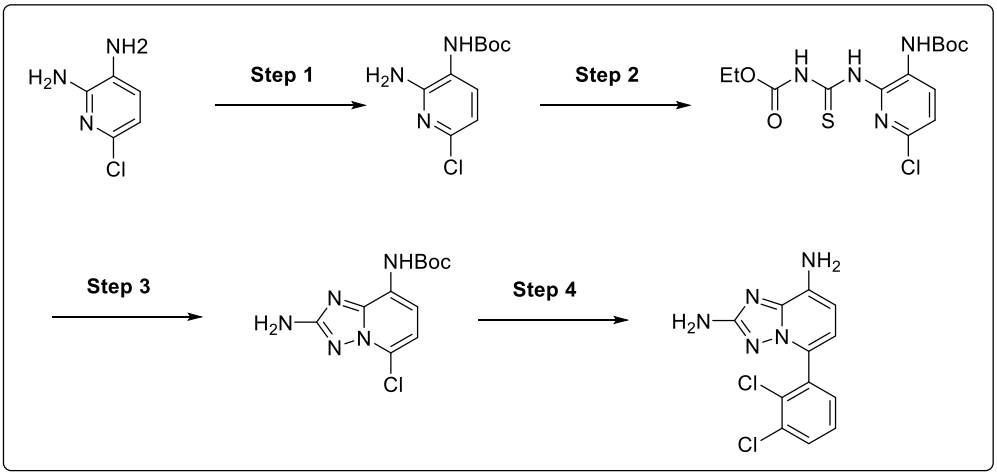
Compound 31: 5-Phenyl-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine

Following the synthetic procedure described for **Compound 32**, using 5-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (75.0 mg, 0.352 mmol) and phenylboronic acid (47.2 mg, 0.387 mmol), the crude reaction was diluted with EtOAc, filtered and purified by reverse phase chromatography to afford 5-phenyl-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine as a colorless solid (39.2 mg, 45%, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 – 7.88 (m, 2H), 7.83 (t, J = 8.2 Hz, 1H), 7.64 – 7.53 (m, 4H), 7.36 (d, J = 7.5 Hz, 1H), missing -N$\underline{H_2}$. LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{12}H_{10}N_4$ 211.1; found 211.1. HPLC purity: 100%.



Compound 33: 5-(2,3-Dimethylphenyl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine

Following the synthetic procedure described for **Compound 32**, using 5-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (75.0 mg, 0.352 mmol) and 2,3-dimethylphenylboronic acid (58.1 mg, 0.387 mmol), the crude reaction was diluted with EtOAc, filtered and purified by reverse phase chromatography to afford 5-(2,3-dimethylphenyl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine as a colorless solid (30.2 mg, 31%, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (dd, J = 8.7, 7.4 Hz, 1H), 7.62 (dd, J = 8.8, 1.3 Hz, 1H), 7.34 (d, J = 7.4 Hz, 1H), 7.24 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 6.8 Hz, 1H), 7.13 (d, J = 7.4 Hz, 1H), 2.31 (s, 3H), 1.94 (s, 3H), missing -N$\underline{H_2}$. LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{14}H_{14}N_4$ 239.1; found 239.0. HPLC purity: 100%.



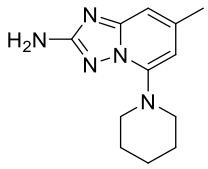
Compound 34: 5-(2,3-Dichlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyridine-2,8-diamine

Step 1: To solution of 6-chloropyridine-2,3-diamine (500 mg, 3.48 mmol) in THF (17.5 mL) was added di-*tert*-butyl dicarbonate (874 mg, 4.00 mmol). The solution was gently heated at 45 °C for 18 h. The reaction was concentrated, triturated with ether and filtered to afford *tert*-butyl (2-amino-6-chloropyridin-3-yl)carbamate as a grey solid (396 mg, 47%). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{14}ClN_3O_2$ 244.1; found 244.0.

Step 2: To a mixture of *tert*-butyl (2-amino-6-chloropyridin-3-yl)carbamate (396 mg, 1.63 mmol) in DCM (5.0 mL) was added ethoxycarbonyl isothiocyanate (0.200 mL, 1.70 mmol). The mixture was stirred at room temperature for 18 h. The reaction was concentrated and was used without further purification. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{19}ClN_4O_4S$ 375.0; found 375.0.

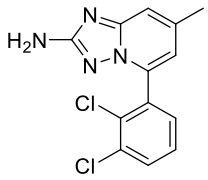
Step 3: A 250 mL round bottom flask was charged with hydroxylamine hydrochloride (565 mg, 8.13 mmol), DIPEA (849 µL, 4.88 mmol) and ethanol (18.0 mL). The mixture was stirred for 5 minutes, then ethyl *N*-[[3-(*tert*-butoxycarbonylamino)-6-chloro-2-pyridyl]carbamothioyl]carbamate (609 mg, 1.63 mmol) was added and the mixture was stirred for an additional 10 minutes. The flask was fitted with a reflux condenser and the reaction was refluxed at 80 °C for 2.5 h. The reaction mixture was concentrated, diluted with DCM and filtered to remove precipitated salts. The filtrate was dry loaded onto SiO_2 and purified by silica gel chromatography (10-60% EtOAc/hexanes) to afford a colorless solid. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{11}H_{14}ClN_5O_2$ 284.1; found 284.0.

Step 4: A 10 mL vial was charged with *tert*-butyl (2-amino-5-chloro-[1,2,4]triazolo[1,5-*a*]pyridin-8-yl)carbamate (60.0 mg, 0.352 mmol), 2,3-dichlorophenylboronic acid (44.4 mg, 0.233 mmol), cesium carbonate (207 mg, 0.634 mmol), 1,4-dioxane (2.0 mL) and water (1.0 mL). The reaction was degassed with nitrogen for 10 minutes, then PEPPSI-IPr (14.4 mg, 0.021 mmol) was added. The vial was sealed and heated at 100 °C for 2 h. Traces of desired product were observed. An additional 10 mg of catalyst was added and the reaction was heated at 120 °C for 10 h, during which the Boc protecting group underwent hydrolysis. The reaction was filtered over celite, the cake was rinsed with EtOAc and concentrated. The crude product was purified by reverse phase chromatography (2-50% ACN/water with 0.1% HCl) and lyophilized to afford 5-(2,3-dichlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyridine-2,8-diamine as a beige solid (3.7 mg, 5% yield, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 – 7.68 (m, 1H), 7.56 – 7.42 (m, 2H), 6.76 (d, J = 8.2 Hz, 1H), 6.66 (d, J = 7.9 Hz, 1H), 3.81 (bs, -N<u>H$_2$</u>). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{12}H_9Cl_2N_5$ 294.0; found 293.9. HPLC purity: 96.0%.



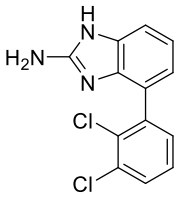
Compound 35: 7-Methyl-5-(piperidin-1-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine

A 10 mL vial was charged with 5-chloro-7-methyl-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (50.0 mg, 0.274 mmol), potassium carbonate (75.7 mg, 0.548 mmol) and piperidine (1.0 mL). The reaction was sealed and heated at 100 °C for 1 hour. The mixture was cooled to room temperature, concentrated and purified by reverse phase chromatography (ACN/water with 0.1% HCl) to afford 7-methyl-5-(piperidin-1-yl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine as a beige solid (25.1 mg, 34% yield, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (s, 2H), 7.03 (d, J = 1.6 Hz, 1H), 6.69 (d, J = 1.5 Hz, 1H), 3.49 – 3.27 (m, 4H), 2.43 (s, 3H), 1.60-1.71 (m, 6H). LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{12}H_{17}N_5$ 232.2; found 232.1. HPLC purity: 100%.



Compound 36: 5-(2,3-Dichlorophenyl)-7-methyl-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine

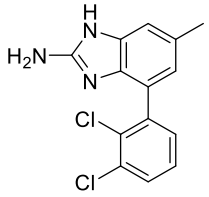
Following the synthetic procedure described for **Compound 32**, using 5-chloro-7-methyl-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (25.0 mg, 0.137 mmol), 2,3-dichlorophenylboronic acid and heating the reaction at 100 °C for 10 h, the crude reaction was diluted with EtOAc, filtered and purified by reverse phase chromatography to afford 5-(2,3-dichlorophenyl)-7-methyl-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine as a colorless solid (5.9 mg, 13%, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (m, 1H), 7.58 – 7.52 (m, 2H), 7.42 (bs, 1H), 7.06 (bs, 1H), 2.47 (s, 3H), missing -N<u>H</u>$_2$. LCMS-ESI$^+$ (*m/z*): [M+H]$^+$ calcd for $C_{13}H_{10}Cl_2N_4$ 293.0; found 292.9. HPLC purity: 100%.



Compound 37: 4-(2,3-Dichlorophenyl)-1*H*-benzo[*d*]imidazol-2-amine

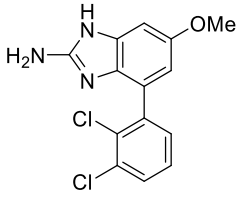
To a 10 mL vial was charged 4-bromo-6-methoxy-1*H*-benzimidazol-2-amine (200 mg, 0.943 mmol), 2,3-dichlorophenylboronic acid (225 mg, 1.18 mmol), cesium carbonate (922 mg, 2.83 mmol), 1,4-dioxane (4.0 mL) and water (2.0 mL). The reaction was degassed with nitrogen for 10 minutes, followed by the addition of tetrakis(triphenylphosphine)palladium(0) (33.4 mg, 0.094 mmol). The reaction vial was sealed and heated at 85 °C for 3 h. The mixture was cooled to room temperature, diluted with water, extracted with EtOAc (3x), washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by reverse phase chromatography (ACN/water with 0.1% HCl) to afford a pink solid (36.6 mg, 8% yield, HCl salt). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 8.15 (s, 2H), 7.78 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 7.43 (dt, *J* = 7.7, 1.5 Hz, 2H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.13 (dd, *J* = 7.7, 1.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.04, 137.60, 132.26, 130.86, 130.58,

130.49, 129.91, 128.53, 127.73, 123.99, 123.18, 122.62, 111.57. LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{13}H_9Cl_2N_3$ 278.0252; found 278.0252. HPLC purity: 98.4%.



Compound 38: 4-(2,3-Dichlorophenyl)-6-methyl-1*H*-benzo[*d*]imidazol-2-amine

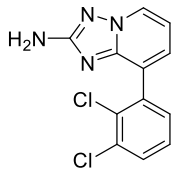
A 10 mL vial was charged with 4-bromo-6-methyl-1*H*-benzo[*d*]imidazol-2-amine (100 mg, 0.442 mmol), 2,3-dichlorophenylboronic acid (109 mg, 0.571 mmol), cesium carbonate (424 mg, 1.30 mmol), 1,4-dioxane (5.0 mL) and water (1.0 mL). The reaction was degassed with nitrogen for 10 minutes, followed by the addition of tetrakis(triphenylphosphine)palladium(0) (15.4 mg, 0.043 mmol). The reaction vial was sealed and heated at 85 °C for 3 h. The mixture was cooled to room temperature, concentrated and purified by reverse phase chromatography (ACN/water with 0.1% TFA) to afford 4-(2,3-dichlorophenyl)-6-methoxy-1*H*-benzo[*d*]imidazol-2-amine as a light yellow solid (48.6 mg, 38% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.63 (s, 1H), 7.60 (dd, J = 6.7, 2.9 Hz, 1H), 7.45 – 7.26 (m, 2H), 6.94 (dd, J = 1.6, 0.8 Hz, 1H), 6.61 (s, 1H), 6.02 (s, 2H), 2.33 (d, J = 0.7 Hz, 3H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{11}Cl_2N_3$ 292.04; found 292.03. HPLC purity: 100 %.



Compound 39: 4-(2,3-Dichlorophenyl)-6-methoxy-1*H*-benzo[*d*]imidazol-2-amine

To a 10 mL vial was charged 4-bromo-6-methoxy-1*H*-benzimidazol-2-amine (105 mg, 0.434 mmol), 2,3-dichlorophenylboronic acid (103 mg, 0.542 mmol), cesium carbonate (424 mg, 1.30 mmol) 1,4-dioxane (2.00 mL) and water (2.00 mL). The reaction was degassed with nitrogen for 10 minutes, followed by the addition of tetrakis(triphenylphosphine)palladium(0) (15.4 mg, 0.043 mmol). The reaction vial was sealed and heated at 90 °C for 3 h. The mixture was cooled

to room temperature, concentrated and purified by reverse phase chromatography (ACN/water with 0.1% HCl) to afford 4-(2,3-dichlorophenyl)-6-methoxy-1*H*-benzo[*d*]imidazol-2-amine as a light yellow solid (9.1 mg, 6% yield, HCl salt). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{11}Cl_2N_3O$ 308.04; found 308.00. HPLC purity: 100%.



Compound 40: 8-(2,3-Dichlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine

Following the synthetic procedure described for **Compound 37**, using 8-bromo-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine (120.0 mg, 0.563 mmol), 2,3-dichlorophenylboronic acid (107 mg, 0.563 mmol) and heating the reaction at 95 °C for 3 h. The mixture was cooled to room temperature, diluted with water, extracted with EtOAc (3x), washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel chromatography (MeOH / DCM) to afford 8-(2,3-dichlorophenyl)-[1,2,4]triazolo[1,5-*a*]pyridin-2-amine as a solid (130 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (dd, J = 6.9, 2.7 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.65 – 7.49 (m, 3H), 7.15 (d, J = 7.4 Hz, 1H), 6.25 (s, 2H). LCMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{12}H_8Cl_2N_4$ 279.01; found 279.05. HPLC purity: 100%.

Crystallography

MTH1 protein expression and purification pet28a-6HIS-MTH1 was generated by ligating human MTH1 between the Nde1 and Xho1 sites of pet28a to generate MTH1 preceded by a HIS tag and a thrombin cleavage site. 6HIS-MTH1 was expressed in BL21(DE3) cells (New England Biolabs) . Cells were grown in LB media at 37oC and expression was induced with 0.5 mM IPTG for 12 h at 18oC. Cells were lysed in Buffer A (50 mM TRIS pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% Glycerol, 5 mM Imidazole pH 7.5) and centrifuged at 47000xg for 45 minutes. The supernatant, containing soluble 6HIS-MTH1, was applied to a 5 ml Ni-NTA equilibrated in Buffer A. The column was washed with Buffer A supplemented with 20 mM Imidazole and eluted with Buffer A supplemented with 300 mM Imidazole. Fractions containing 6HIS-MTH1 were incubated with thrombin (2 U/mg 6HIS MTH1) and the cleaved MTH1 protein was further purified by size exclusion chromatography in Buffer B (20 mM TRIS pH 7.5, 150 mM NaCl, 2 mM TCEP, 5% Glycerol). Protein was judged >95% pure by SDS-PAGE and was concentrated to 8mg/ml in a final buffer solution contained 20mM Tris pH 7.5, 150mM NaCl, 5% glycerol, 2mM TCEP.

Crystallization and data collection Co-crystals of the MTH1 complex with inhibitors were grown at 20 °C by vapor diffusion over a reservoir solution containing 30% PEG 6000, 0.1M sodium acetate pH 4.0, 0.2M lithium sulfate. Protein and reservoir solutions were mixed at 1:1 or 1:2 ratios for a final volume of 2-3µL. Prior to cryocooling in liquid nitrogen, 20% glycerol was added in addition to the mother liquor components. X-ray diffraction data were collected on a Rigaku MM007 rotating anode or at The Advanced Light Source beamline 5.0.1 (**Table S1**) at a temperature of 100 K and processed with HKL2000[1].

Structure determination and refinement The structures of MTH1 were determined by molecular replacement with the refinement package Phenix[2] using the starting model PDB code 3ZR0. Rigid body refinement, simulated annealing, energy minimization, and B-factor refinement were additionally performed with Phenix. Model building was carried out by the molecular graphics program Coot[3].

Table S1. Data collection and refinement statistics for X-ray structures of **Compounds 5**, **4** and **32** (PDB codes 6US2, 6US3 and 6US4 respectively).

	5	**4**	**32**
Wavelength (Å)	1.54178	0.97741	1.54178
Space Group	$P22_12_1$	$P22_12_1$	$P22_12_1$
Unit Cell (a, b, c in Å)	36.3, 60.0, 66.7	36.3, 59.9, 66.5	36.2, 60.4, 66.3
Resolution (Å)	50-1.80 (1.83-1.80)	50-1.47 (1.50-1.47)	50-1.95 (1.98-1.95)
No. of reflections	52,099	123,711	36,387
No. unique	14,073	25,250	11,103
I/σ	15.6 (2.3)	23.8 (2.7)	13.0 (2.1)
R_{merge}[a] (%)	8.4 (50.8)	5.3 (51.4)	8.0 (52.6)
Completeness (%)	99.9 (100.0)	99.4 (99.8)	99.7 (100.0)
Refinement Statistics			
Resolution (Å)	32-1.80	44.5-1.47	31.8-1.95
No. reflections (F≥0)	13,275	23,974	10,486
R-factor[b]	18.0	18.6	17.7
R-free[b]	23.7	22.1	23.8
RMS bond lengths (Å)	0.007	0.006	0.006
RMS bond angles (°)	1.12	1.12	1.08

[a] $R_{merge} = [\sum h \sum i|Ih - Ihi|/\sum h \sum iIhi]$ where Ih is the mean of Ihi observations of reflection h. Numbers in parenthesis represent highest resolution shell.

[b] *R*-factor and *R*-free = $\sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$ x 100 for 90% of recorded data (*R*-factor) or 10% of data (*R*-free).

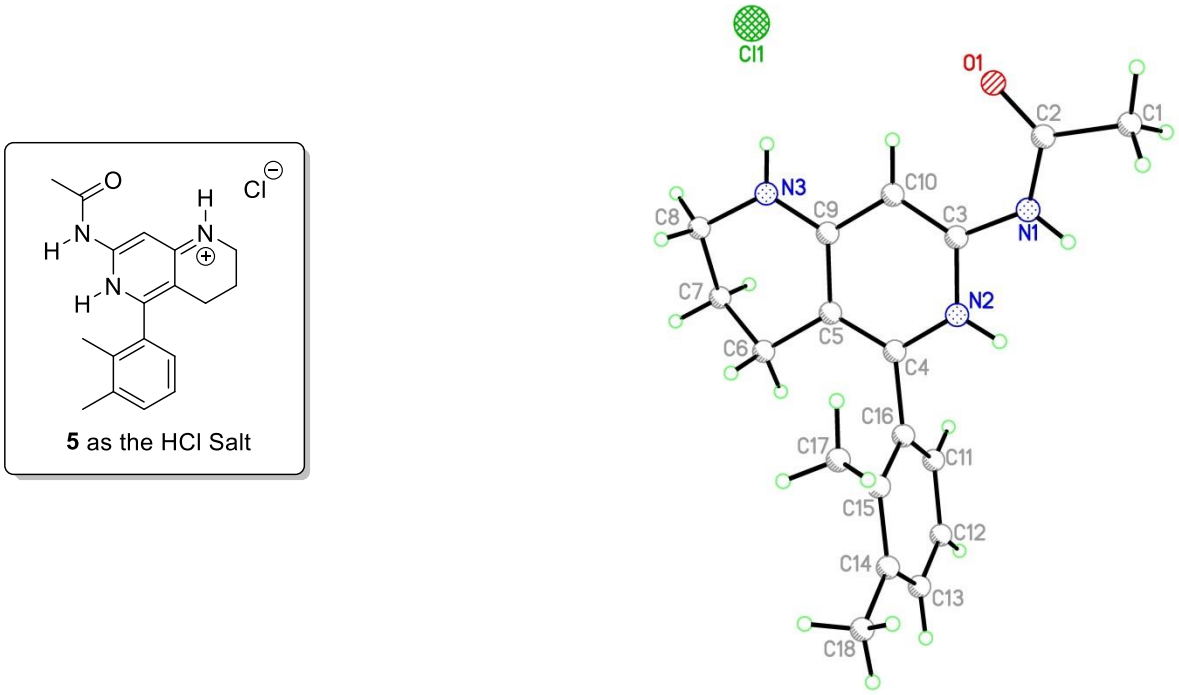


Figure S1. Small molecule crystal structure of **Compound 5** as the HCl salt.

Table S2. Crystal data and structure refinement for **Compound 5**.

Identification code	**Compound 5**	
Empirical formula	C36 H44 Cl2 N6 O2	
Formula weight	663.67	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 7.8474(9) Å	α= 90°.
	b = 37.686(4) Å	β= 95.321(3)°.
	c = 11.5046(13) Å	γ = 90°.
Volume	3387.7(7) $Å^3$	
Z	4	
Density (calculated)	1.301 Mg/m^3	
Absorption coefficient	0.234 mm^{-1}	
F(000)	1408	
Crystal size	0.300 x 0.200 x 0.080 mm^3	
Theta range for data collection	2.081 to 26.784°.	
Index ranges	-8<=h<=9, -47<=k<=47, -14<=l<=14	
Reflections collected	24783	
Independent reflections	7135 [R(int) = 0.0416]	
Completeness to theta = 25.000°	99.0 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7135 / 0 / 424	
Goodness-of-fit on F^2	1.061	
Final R indices [I>2sigma(I)]	R1 = 0.0732, wR2 = 0.1770	
R indices (all data)	R1 = 0.0894, wR2 = 0.1858	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.712 and -0.572 $e.Å^{-3}$	

Table S3. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **Compound 5**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Cl(1)	-3790(1)	464(1)	2271(1)	30(1)
Cl(2)	3495(1)	2006(1)	5234(1)	31(1)
O(1)	-2369(3)	1550(1)	3146(2)	29(1)
O(2)	10600(3)	542(1)	9548(2)	30(1)
N(1)	370(3)	1627(1)	3937(2)	22(1)
N(2)	2455(4)	1237(1)	4652(2)	24(1)
N(3)	92(4)	363(1)	3156(2)	24(1)
N(4)	12823(3)	689(1)	10878(2)	22(1)
N(5)	11178(5)	1187(1)	10302(3)	46(1)
N(6)	14376(4)	1849(1)	12267(2)	23(1)
C(1)	-1509(5)	2137(1)	3729(3)	32(1)
C(2)	-1231(4)	1744(1)	3571(3)	22(1)
C(3)	936(4)	1275(1)	3983(3)	21(1)
C(4)	3197(4)	913(1)	4847(3)	25(1)
C(5)	2460(4)	614(1)	4363(3)	24(1)
C(6)	3238(5)	253(1)	4582(3)	27(1)
C(7)	2675(4)	3(1)	3578(3)	27(1)
C(8)	743(4)	0(1)	3331(3)	27(1)
C(9)	884(4)	647(1)	3643(3)	21(1)
C(10)	131(4)	988(1)	3456(3)	21(1)
C(11)	6368(5)	978(1)	5241(3)	31(1)
C(12)	7852(5)	978(1)	6013(3)	35(1)
C(13)	7714(5)	922(1)	7188(3)	31(1)
C(14)	6195(5)	858(1)	7645(3)	28(1)
C(15)	4677(5)	854(1)	6860(3)	28(1)
C(16)	4814(5)	915(1)	5673(3)	27(1)
C(17)	3015(5)	769(1)	7325(3)	40(1)
C(18)	6096(5)	799(1)	8920(3)	36(1)
C(19)	12517(4)	80(1)	10227(3)	25(1)
C(20)	11876(4)	453(1)	10182(3)	22(1)

C(21)	12552(4)	1050(1)	10942(3)	23(1)
C(22)	10835(6)	1542(1)	10294(4)	54(1)
C(23)	11862(5)	1772(1)	10939(3)	35(1)
C(24)	11542(5)	2168(1)	10930(4)	38(1)
C(25)	12296(5)	2332(1)	12077(3)	29(1)
C(26)	14165(4)	2234(1)	12308(3)	26(1)
C(27)	13324(4)	1634(1)	11637(3)	21(1)
C(28)	13636(4)	1265(1)	11622(3)	20(1)
C(29)	7566(5)	1685(1)	10088(2)	36(2)
C(30)	6148(4)	1802(1)	9379(3)	37(2)
C(31)	6305(4)	1888(1)	8219(3)	27(1)
C(32)	7880(5)	1855(1)	7767(2)	25(1)
C(33)	9298(4)	1738(1)	8476(3)	24(1)
C(34)	9141(4)	1652(1)	9637(3)	22(1)
C(35)	10973(12)	1685(2)	7950(5)	39(2)
C(36)	8001(9)	1952(2)	6500(5)	38(2)
C(29')	10448(6)	1748(2)	8027(6)	40(5)
C(30')	9299(8)	1861(2)	7110(5)	35(2)
C(31')	7575(8)	1898(2)	7273(5)	20(2)
C(32')	7000(6)	1822(2)	8352(6)	28(3)
C(33')	8150(8)	1708(2)	9269(4)	26(2)
C(34')	9874(7)	1671(2)	9106(5)	18(2)
C(35')	7570(20)	1662(4)	10472(13)	40(3)
C(36')	5137(17)	1863(3)	8506(11)	48(3)

Table S4. Bond lengths [Å] and angles [°] for **Compound 5**.

O(1)-C(2)	1.220(4)	C(15)-C(16)	1.398(5)
O(2)-C(20)	1.228(4)	C(15)-C(17)	1.490(5)
N(1)-C(2)	1.361(4)	C(19)-C(20)	1.493(4)
N(1)-C(3)	1.398(4)	C(21)-C(28)	1.368(4)
N(2)-C(4)	1.360(4)	C(22)-C(23)	1.356(5)
N(2)-C(3)	1.365(4)	C(22)-C(34)	1.525(5)
N(3)-C(9)	1.335(4)	C(22)-C(34')	1.575(6)
N(3)-C(8)	1.467(4)	C(23)-C(27)	1.436(5)
N(4)-C(20)	1.369(4)	C(23)-C(24)	1.513(5)
N(4)-C(21)	1.380(4)	C(24)-C(25)	1.525(5)
N(5)-C(21)	1.350(4)	C(25)-C(26)	1.511(5)
N(5)-C(22)	1.366(5)	C(27)-C(28)	1.410(4)
N(6)-C(27)	1.325(4)	C(29)-C(30)	1.3900
N(6)-C(26)	1.460(4)	C(29)-C(34)	1.3900
C(1)-C(2)	1.510(4)	C(30)-C(31)	1.3900
C(3)-C(10)	1.364(4)	C(31)-C(32)	1.3900
C(4)-C(5)	1.362(4)	C(32)-C(33)	1.3900
C(4)-C(16)	1.513(5)	C(32)-C(36)	1.514(6)
C(5)-C(9)	1.429(4)	C(33)-C(34)	1.3900
C(5)-C(6)	1.505(4)	C(33)-C(35)	1.510(10)
C(6)-C(7)	1.523(4)	C(29')-C(30')	1.3900
C(7)-C(8)	1.516(5)	C(29')-C(34')	1.3900
C(9)-C(10)	1.423(4)	C(30')-C(31')	1.3900
C(11)-C(16)	1.380(5)	C(31')-C(32')	1.3900
C(11)-C(12)	1.397(5)	C(32')-C(33')	1.3900
C(12)-C(13)	1.383(5)	C(32')-C(36')	1.497(14)
C(13)-C(14)	1.368(5)	C(33')-C(34')	1.3900
C(14)-C(15)	1.427(5)	C(33')-C(35')	1.507(15)
C(14)-C(18)	1.493(5)		
C(2)-N(1)-C(3)	126.9(3)	C(27)-N(6)-C(26)	124.0(3)
C(4)-N(2)-C(3)	121.6(3)	O(1)-C(2)-N(1)	123.4(3)
C(9)-N(3)-C(8)	123.2(3)	O(1)-C(2)-C(1)	121.7(3)
C(20)-N(4)-C(21)	126.4(3)	N(1)-C(2)-C(1)	114.9(3)
C(21)-N(5)-C(22)	121.7(3)	C(10)-C(3)-N(2)	120.8(3)

C(10)-C(3)-N(1)	127.0(3)	C(23)-C(22)-C(34')	119.4(4)
N(2)-C(3)-N(1)	112.2(3)	N(5)-C(22)-C(34')	112.7(4)
N(2)-C(4)-C(5)	121.0(3)	C(22)-C(23)-C(27)	118.5(3)
N(2)-C(4)-C(16)	114.9(3)	C(22)-C(23)-C(24)	122.4(3)
C(5)-C(4)-C(16)	124.0(3)	C(27)-C(23)-C(24)	119.1(3)
C(4)-C(5)-C(9)	118.5(3)	C(23)-C(24)-C(25)	110.0(3)
C(4)-C(5)-C(6)	122.0(3)	C(26)-C(25)-C(24)	110.3(3)
C(9)-C(5)-C(6)	119.4(3)	N(6)-C(26)-C(25)	110.3(3)
C(5)-C(6)-C(7)	110.3(3)	N(6)-C(27)-C(28)	120.7(3)
C(8)-C(7)-C(6)	111.2(3)	N(6)-C(27)-C(23)	120.5(3)
N(3)-C(8)-C(7)	110.3(3)	C(28)-C(27)-C(23)	118.8(3)
N(3)-C(9)-C(10)	119.4(3)	C(21)-C(28)-C(27)	119.4(3)
N(3)-C(9)-C(5)	121.2(3)	C(30)-C(29)-C(34)	120.0
C(10)-C(9)-C(5)	119.4(3)	C(29)-C(30)-C(31)	120.0
C(3)-C(10)-C(9)	118.6(3)	C(30)-C(31)-C(32)	120.0
C(16)-C(11)-C(12)	118.9(3)	C(31)-C(32)-C(33)	120.0
C(13)-C(12)-C(11)	119.0(4)	C(31)-C(32)-C(36)	118.4(3)
C(14)-C(13)-C(12)	123.5(4)	C(33)-C(32)-C(36)	121.6(3)
C(13)-C(14)-C(15)	117.7(3)	C(34)-C(33)-C(32)	120.0
C(13)-C(14)-C(18)	122.0(3)	C(34)-C(33)-C(35)	120.8(3)
C(15)-C(14)-C(18)	120.2(3)	C(32)-C(33)-C(35)	119.1(3)
C(16)-C(15)-C(14)	118.7(3)	C(33)-C(34)-C(29)	120.0
C(16)-C(15)-C(17)	122.4(3)	C(33)-C(34)-C(22)	112.9(3)
C(14)-C(15)-C(17)	118.8(3)	C(29)-C(34)-C(22)	127.1(3)
C(11)-C(16)-C(15)	122.0(3)	C(30')-C(29')-C(34')	120.0
C(11)-C(16)-C(4)	119.5(3)	C(29')-C(30')-C(31')	120.0
C(15)-C(16)-C(4)	118.5(3)	C(30')-C(31')-C(32')	120.0
O(2)-C(20)-N(4)	122.7(3)	C(33')-C(32')-C(31')	120.0
O(2)-C(20)-C(19)	122.0(3)	C(33')-C(32')-C(36')	121.0(6)
N(4)-C(20)-C(19)	115.4(3)	C(31')-C(32')-C(36')	119.0(6)
N(5)-C(21)-C(28)	120.5(3)	C(32')-C(33')-C(34')	120.0
N(5)-C(21)-N(4)	117.9(3)	C(32')-C(33')-C(35')	120.1(7)
C(28)-C(21)-N(4)	121.6(3)	C(34')-C(33')-C(35')	119.6(7)
C(23)-C(22)-N(5)	121.1(3)	C(33')-C(34')-C(29')	120.0
C(23)-C(22)-C(34)	123.0(3)	C(33')-C(34')-C(22)	107.9(4)
N(5)-C(22)-C(34)	115.6(3)	C(29')-C(34')-C(22)	132.1(4)

Table S5. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **Compound 5**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$.

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Cl(1)	20(1)	35(1)	36(1)	-7(1)	-4(1)	3(1)
Cl(2)	35(1)	24(1)	32(1)	-5(1)	-9(1)	1(1)
O(1)	26(1)	24(1)	35(1)	2(1)	-9(1)	4(1)
O(2)	29(1)	25(1)	32(1)	-1(1)	-11(1)	-2(1)
N(1)	20(1)	18(1)	29(1)	0(1)	-1(1)	1(1)
N(2)	27(2)	18(1)	26(1)	-2(1)	-8(1)	3(1)
N(3)	20(1)	22(1)	29(1)	-3(1)	-5(1)	2(1)
N(4)	21(1)	19(1)	24(1)	1(1)	-4(1)	0(1)
N(5)	54(2)	19(1)	57(2)	-4(1)	-37(2)	2(1)
N(6)	22(2)	19(1)	25(1)	-1(1)	-6(1)	2(1)
C(1)	28(2)	24(2)	43(2)	-2(1)	-2(2)	5(1)
C(2)	24(2)	24(2)	18(2)	5(1)	1(1)	5(1)
C(3)	20(2)	23(2)	18(1)	2(1)	1(1)	3(1)
C(4)	26(2)	22(2)	26(2)	2(1)	-3(1)	3(1)
C(5)	24(2)	23(2)	24(2)	-1(1)	-5(1)	3(1)
C(6)	28(2)	20(2)	31(2)	-1(1)	-8(1)	4(1)
C(7)	24(2)	21(2)	36(2)	-5(1)	-6(1)	3(1)
C(8)	27(2)	20(2)	33(2)	-4(1)	-2(1)	0(1)
C(9)	21(2)	23(2)	19(2)	-2(1)	2(1)	1(1)
C(10)	18(2)	25(2)	19(2)	0(1)	-1(1)	2(1)
C(11)	28(2)	41(2)	24(2)	2(1)	-1(1)	-6(2)
C(12)	29(2)	44(2)	32(2)	6(2)	1(2)	-7(2)
C(13)	41(2)	24(2)	27(2)	-1(1)	-2(2)	-1(1)
C(14)	33(2)	20(2)	31(2)	-6(1)	2(2)	-1(1)
C(15)	29(2)	23(2)	33(2)	-2(1)	4(2)	5(1)
C(16)	35(2)	18(2)	28(2)	-1(1)	-3(2)	4(1)
C(17)	40(2)	55(2)	26(2)	-1(2)	-1(2)	0(2)
C(18)	42(2)	39(2)	27(2)	-1(2)	2(2)	7(2)
C(19)	24(2)	22(2)	30(2)	-4(1)	-1(1)	-1(1)
C(20)	23(2)	22(2)	21(2)	0(1)	2(1)	-4(1)
C(21)	25(2)	21(2)	22(2)	2(1)	-3(1)	0(1)

C(22)	65(3)	21(2)	65(3)	-1(2)	-46(2)	6(2)
C(23)	41(2)	18(2)	43(2)	0(1)	-18(2)	3(1)
C(24)	39(2)	20(2)	52(2)	0(2)	-19(2)	3(1)
C(25)	29(2)	19(2)	37(2)	1(1)	-2(2)	4(1)
C(26)	27(2)	18(2)	32(2)	-2(1)	-4(1)	-1(1)
C(27)	23(2)	21(2)	18(1)	1(1)	0(1)	-1(1)
C(28)	21(2)	20(1)	18(1)	2(1)	-1(1)	2(1)
C(29)	21(3)	61(4)	27(4)	2(3)	2(3)	6(3)
C(30)	25(3)	56(4)	28(3)	7(3)	-2(2)	9(3)
C(31)	28(4)	28(3)	24(3)	3(2)	-2(2)	2(2)
C(32)	36(3)	23(3)	16(3)	4(2)	-1(2)	-1(2)
C(33)	31(3)	21(2)	22(3)	5(2)	7(2)	4(2)
C(34)	22(3)	21(2)	21(3)	3(2)	-1(2)	1(2)
C(35)	45(4)	50(4)	24(3)	16(3)	5(3)	1(4)
C(36)	40(4)	48(4)	27(3)	15(3)	-1(3)	6(3)

Table S6. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3) for **Compound 5**.

	x	y	z	U(eq)
H(1A)	1129	1790	4170	27
H(2A)	2967	1426	4965	29
H(3A)	-867	395	2709	29
H(4A)	13690	602	11330	26
H(5A)	10486	1044	9879	55
H(6C)	15255	1755	12687	27
H(1B)	-2692	2197	3452	48
H(1C)	-722	2270	3280	48
H(1D)	-1296	2197	4558	48
H(6A)	2877	155	5320	32
H(6B)	4502	273	4663	32
H(7A)	3198	80	2868	33
H(7B)	3083	-240	3776	33
H(8A)	418	-143	2623	32
H(8B)	224	-111	3994	32
H(10A)	-910	1016	2973	25
H(11A)	6429	1020	4432	37
H(12A)	8940	1016	5735	42
H(13A)	8730	927	7707	37
H(17A)	2094	778	6689	61
H(17B)	2786	942	7926	61
H(17C)	3071	530	7667	61
H(18A)	7248	810	9327	54
H(18B)	5592	566	9042	54
H(18C)	5381	984	9228	54
H(19A)	11765	-68	9699	38
H(19B)	12523	-11	11026	38
H(19C)	13681	73	9985	38
H(24A)	10295	2215	10822	46
H(24B)	12073	2278	10271	46

H(25A)	12178	2593	12038	34
H(25B)	11661	2245	12725	34
H(26A)	14825	2346	11714	31
H(26B)	14616	2323	13085	31
H(28A)	14591	1168	12081	24
H(29)	7459	1626	10882	44
H(30)	5071	1824	9688	44
H(31)	5335	1968	7734	32
H(35A)	10843	1757	7129	59
H(35B)	11300	1434	8005	59
H(35C)	11863	1829	8376	59
H(36A)	9177	1916	6303	58
H(36B)	7683	2202	6377	58
H(36C)	7222	1802	6000	58
H(29')	11627	1722	7915	48
H(30')	9691	1913	6372	42
H(31')	6789	1975	6646	24
H(35D)	8531	1581	11009	60
H(35E)	6645	1487	10444	60
H(35F)	7150	1890	10745	60
H(36D)	4531	1947	7773	72
H(36E)	4995	2036	9125	72
H(36F)	4664	1634	8719	72

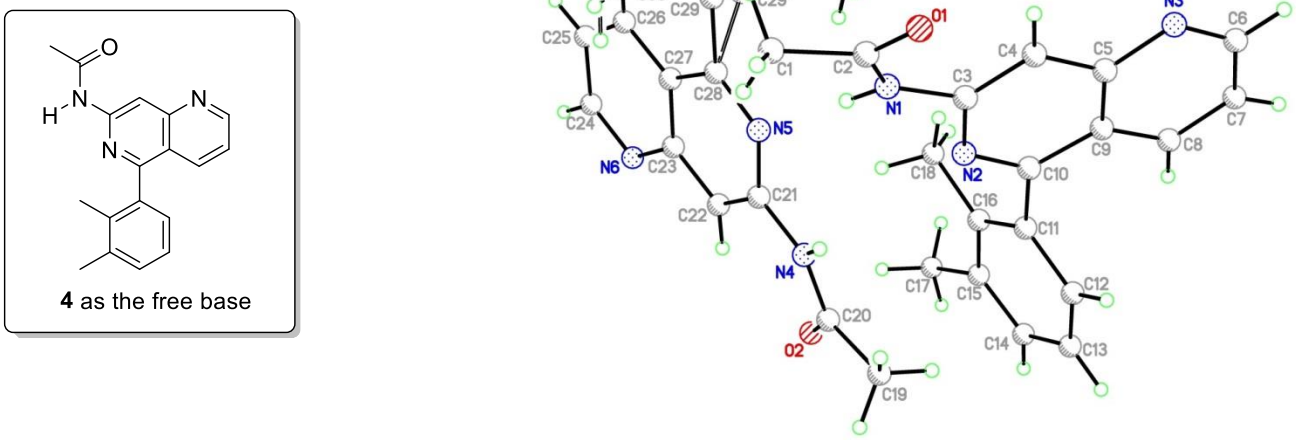


Figure S2. Small molecule crystal structure of **Compound 4** as the free base.

Table S7. Crystal data and structure refinement for **Compound 4**.

Identification code	**Compound 4**	
Empirical formula	C18 H17 N3 O	
Formula weight	291.34	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 13.0298(4) Å	α= 90°.
	b = 19.6452(6) Å	β= 116.678(2)°.
	c = 13.2305(4) Å	γ = 90°.
Volume	3026.12(17) $Å^3$	
Z, Z'	8, 2	
Density (calculated)	1.279 Mg/m^3	
Absorption coefficient	0.082 mm^{-1}	
F(000)	1232	
Crystal size	0.220 x 0.150 x 0.080 mm^3	
Theta range for data collection	2.033 to 26.367°.	
Index ranges	-13<=h<=13, -19<=k<=19, -13<=l<=13	
Reflections collected	17654	
Independent reflections	6141 [R(int) = ?]	
Completeness to theta = 26.000°	99.7 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6141 / 0 / 454	
Goodness-of-fit on F^2	1.045	
Final R indices [I>2sigma(I)]	R1 = 0.0577, wR2 = 0.1279	
R indices (all data)	R1 = 0.0864, wR2 = 0.1384	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.409 and -0.310 $e.Å^{-3}$	

Table S8. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **Compound 4**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	8771(1)	3380(1)	3826(1)	45(1)
O(2)	4580(1)	2288(1)	6690(2)	42(1)
N(1)	7731(1)	3414(1)	4820(1)	22(1)
N(2)	5805(1)	3560(1)	4244(1)	21(1)
N(3)	5466(2)	4821(1)	1573(2)	36(1)
N(4)	6103(2)	2497(1)	6325(2)	32(1)
N(5)	7667(2)	3199(1)	7149(1)	29(1)
N(6)	7387(2)	3267(1)	10169(2)	37(1)
C(1)	9641(2)	2941(1)	5701(2)	28(1)
C(2)	8684(2)	3265(1)	4690(2)	26(1)
C(3)	6706(2)	3708(1)	4023(2)	20(1)
C(4)	6619(2)	4109(1)	3137(2)	24(1)
C(5)	5543(2)	4392(1)	2431(2)	25(1)
C(6)	4451(2)	5089(1)	945(2)	42(1)
C(7)	3461(2)	4971(1)	1081(2)	39(1)
C(8)	3525(2)	4549(1)	1925(2)	31(1)
C(9)	4594(2)	4248(1)	2635(2)	22(1)
C(10)	4782(2)	3810(1)	3573(2)	21(1)
C(11)	3801(2)	3608(1)	3796(2)	27(1)
C(12)	2895(2)	3238(1)	2939(2)	34(1)
C(13)	1967(2)	3042(1)	3112(2)	44(1)
C(14)	1939(2)	3207(1)	4118(2)	44(1)
C(15)	2818(2)	3557(1)	4977(2)	38(1)
C(16)	3770(2)	3768(1)	4811(2)	29(1)
C(17)	2777(3)	3709(2)	6070(3)	55(1)
C(18)	4689(2)	4178(1)	5709(2)	32(1)
C(19)	4503(2)	1852(1)	4971(2)	45(1)
C(20)	5048(2)	2228(1)	6074(2)	33(1)
C(21)	6802(2)	2876(1)	7274(2)	26(1)
C(22)	6662(2)	2913(1)	8244(2)	28(1)

C(23)	7481(2)	3270(1)	9168(2)	29(1)
C(24)	8195(2)	3598(1)	11035(2)	44(1)
C(25)	9105(2)	3959(1)	10997(2)	46(1)
C(26)	9204(2)	3967(1)	10018(2)	38(1)
C(27)	8390(2)	3608(1)	9068(2)	29(1)
C(28)	8421(2)	3558(1)	8007(2)	31(1)
C(29')	9073(5)	4078(3)	7625(5)	25(3)
C(30')	8552(4)	4567(3)	6790(5)	20(2)
C(31')	9213(5)	4963(3)	6433(4)	36(2)
C(32')	10395(5)	4869(3)	6912(6)	29(3)
C(33')	10916(4)	4380(4)	7747(5)	27(3)
C(34')	10256(6)	3984(3)	8103(4)	25(2)
C(35')	12205(7)	4309(4)	8252(7)	38(2)
C(36')	10832(8)	3426(5)	8970(7)	28(2)
C(29)	9409(2)	3877(1)	7881(2)	27(1)
C(30)	10523(2)	3635(1)	8488(2)	39(1)
C(31)	11415(2)	3927(1)	8335(2)	50(1)
C(32)	11194(2)	4461(2)	7576(3)	42(2)
C(33)	10079(2)	4703(1)	6969(3)	36(1)
C(34)	9187(2)	4411(1)	7122(2)	26(1)
C(35)	9851(4)	5266(2)	6123(3)	52(1)
C(36)	8006(3)	4730(2)	6532(3)	27(1)

Table S9. Bond lengths [Å] and angles [°] for **Compound 4**.

O(1)-C(2)	1.220(3)	C(15)-C(17)	1.500(3)
O(2)-C(20)	1.223(3)	C(16)-C(18)	1.490(3)
N(1)-C(2)	1.360(3)	C(19)-C(20)	1.499(3)
N(1)-C(3)	1.402(3)	C(21)-C(22)	1.377(3)
N(2)-C(10)	1.320(3)	C(22)-C(23)	1.398(3)
N(2)-C(3)	1.363(2)	C(23)-C(27)	1.414(3)
N(3)-C(6)	1.316(3)	C(24)-C(25)	1.403(4)
N(3)-C(5)	1.381(3)	C(25)-C(26)	1.358(3)
N(4)-C(20)	1.368(3)	C(26)-C(27)	1.416(3)
N(4)-C(21)	1.390(3)	C(27)-C(28)	1.426(3)
N(5)-C(28)	1.322(3)	C(28)-C(29)	1.507(3)
N(5)-C(21)	1.367(3)	C(28)-C(29')	1.551(4)
N(6)-C(24)	1.326(3)	C(29')-C(30')	1.3900
N(6)-C(23)	1.384(3)	C(29')-C(34')	1.3900
C(1)-C(2)	1.501(3)	C(30')-C(31')	1.3900
C(3)-C(4)	1.374(3)	C(31')-C(32')	1.3900
C(4)-C(5)	1.405(3)	C(32')-C(33')	1.3900
C(5)-C(9)	1.407(3)	C(33')-C(34')	1.3900
C(6)-C(7)	1.400(4)	C(33')-C(35')	1.509(9)
C(7)-C(8)	1.363(3)	C(34')-C(36')	1.519(9)
C(8)-C(9)	1.414(3)	C(29)-C(30)	1.3900
C(9)-C(10)	1.438(3)	C(29)-C(34)	1.3900
C(10)-C(11)	1.489(3)	C(30)-C(31)	1.3900
C(11)-C(16)	1.397(3)	C(31)-C(32)	1.3900
C(11)-C(12)	1.417(3)	C(32)-C(33)	1.3900
C(12)-C(13)	1.382(3)	C(33)-C(34)	1.3900
C(13)-C(14)	1.386(4)	C(33)-C(35)	1.505(5)
C(14)-C(15)	1.381(4)	C(34)-C(36)	1.514(4)
C(15)-C(16)	1.415(3)		
C(2)-N(1)-C(3)	127.54(17)	C(24)-N(6)-C(23)	116.6(2)
C(10)-N(2)-C(3)	118.84(17)	O(1)-C(2)-N(1)	123.6(2)
C(6)-N(3)-C(5)	116.3(2)	O(1)-C(2)-C(1)	121.7(2)
C(20)-N(4)-C(21)	127.31(19)	N(1)-C(2)-C(1)	114.78(18)
C(28)-N(5)-C(21)	118.64(18)	N(2)-C(3)-C(4)	123.88(18)

N(2)-C(3)-N(1)	111.82(16)	C(22)-C(23)-C(27)	119.76(18)
C(4)-C(3)-N(1)	124.29(18)	N(6)-C(24)-C(25)	124.9(2)
C(3)-C(4)-C(5)	117.96(19)	C(26)-C(25)-C(24)	118.9(2)
N(3)-C(5)-C(4)	118.03(19)	C(25)-C(26)-C(27)	119.1(3)
N(3)-C(5)-C(9)	122.41(19)	C(23)-C(27)-C(26)	118.4(2)
C(4)-C(5)-C(9)	119.55(18)	C(23)-C(27)-C(28)	117.06(19)
N(3)-C(6)-C(7)	125.2(2)	C(26)-C(27)-C(28)	124.5(2)
C(8)-C(7)-C(6)	119.0(2)	N(5)-C(28)-C(27)	122.9(2)
C(7)-C(8)-C(9)	118.6(2)	N(5)-C(28)-C(29)	118.0(2)
C(5)-C(9)-C(8)	118.42(19)	C(27)-C(28)-C(29)	118.9(2)
C(5)-C(9)-C(10)	117.55(18)	N(5)-C(28)-C(29')	112.1(3)
C(8)-C(9)-C(10)	124.02(19)	C(27)-C(28)-C(29')	122.6(3)
N(2)-C(10)-C(9)	122.20(18)	C(30')-C(29')-C(34')	120.0
N(2)-C(10)-C(11)	117.68(17)	C(30')-C(29')-C(28)	124.8(4)
C(9)-C(10)-C(11)	120.10(18)	C(34')-C(29')-C(28)	115.0(4)
C(16)-C(11)-C(12)	120.7(2)	C(29')-C(30')-C(31')	120.0
C(16)-C(11)-C(10)	121.99(19)	C(30')-C(31')-C(32')	120.0
C(12)-C(11)-C(10)	117.30(19)	C(33')-C(32')-C(31')	120.0
C(13)-C(12)-C(11)	119.1(2)	C(32')-C(33')-C(34')	120.0
C(12)-C(13)-C(14)	119.8(2)	C(32')-C(33')-C(35')	117.8(5)
C(15)-C(14)-C(13)	122.5(2)	C(34')-C(33')-C(35')	122.2(5)
C(14)-C(15)-C(16)	118.6(2)	C(33')-C(34')-C(29')	120.0
C(14)-C(15)-C(17)	121.2(2)	C(33')-C(34')-C(36')	119.2(6)
C(16)-C(15)-C(17)	120.3(2)	C(29')-C(34')-C(36')	120.7(6)
C(11)-C(16)-C(15)	119.3(2)	C(30)-C(29)-C(34)	120.0
C(11)-C(16)-C(18)	121.86(19)	C(30)-C(29)-C(28)	121.11(19)
C(15)-C(16)-C(18)	118.7(2)	C(34)-C(29)-C(28)	118.9(2)
O(2)-C(20)-N(4)	123.2(2)	C(29)-C(30)-C(31)	120.0
O(2)-C(20)-C(19)	122.4(2)	C(32)-C(31)-C(30)	120.0
N(4)-C(20)-C(19)	114.4(2)	C(31)-C(32)-C(33)	120.0
N(5)-C(21)-C(22)	123.3(2)	C(34)-C(33)-C(32)	120.0
N(5)-C(21)-N(4)	112.41(17)	C(34)-C(33)-C(35)	120.8(2)
C(22)-C(21)-N(4)	124.3(2)	C(32)-C(33)-C(35)	119.2(2)
C(21)-C(22)-C(23)	118.2(2)	C(33)-C(34)-C(29)	120.0
N(6)-C(23)-C(22)	118.3(2)	C(33)-C(34)-C(36)	118.5(3)
N(6)-C(23)-C(27)	121.9(2)	C(29)-C(34)-C(36)	121.2(3)

Table S10. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **Compound 4**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$.

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	37(1)	76(1)	32(1)	10(1)	24(1)	7(1)
O(2)	35(1)	46(1)	49(1)	17(1)	23(1)	1(1)
N(1)	24(1)	27(1)	16(1)	-2(1)	11(1)	-4(1)
N(2)	26(1)	22(1)	19(1)	-2(1)	13(1)	-2(1)
N(3)	56(1)	32(1)	31(1)	12(1)	29(1)	10(1)
N(4)	36(1)	42(1)	22(1)	0(1)	17(1)	-14(1)
N(5)	34(1)	35(1)	19(1)	-4(1)	14(1)	-10(1)
N(6)	62(1)	33(1)	25(1)	7(1)	28(1)	19(1)
C(1)	24(1)	32(1)	29(1)	-6(1)	14(1)	-4(1)
C(2)	28(1)	30(1)	25(1)	-5(1)	15(1)	-7(1)
C(3)	26(1)	20(1)	17(1)	-5(1)	11(1)	-4(1)
C(4)	30(1)	24(1)	24(1)	-2(1)	18(1)	-5(1)
C(5)	39(1)	21(1)	20(1)	-3(1)	18(1)	0(1)
C(6)	67(2)	39(1)	31(1)	17(1)	33(1)	21(1)
C(7)	53(2)	40(1)	29(1)	10(1)	22(1)	23(1)
C(8)	39(1)	30(1)	27(1)	1(1)	18(1)	9(1)
C(9)	33(1)	18(1)	18(1)	-3(1)	12(1)	1(1)
C(10)	26(1)	18(1)	21(1)	-4(1)	11(1)	-1(1)
C(11)	28(1)	22(1)	30(1)	5(1)	13(1)	5(1)
C(12)	31(1)	30(1)	39(1)	1(1)	15(1)	0(1)
C(13)	31(1)	42(1)	51(2)	-10(1)	12(1)	-7(1)
C(14)	39(1)	44(2)	58(2)	-7(1)	31(1)	-1(1)
C(15)	40(1)	32(1)	50(2)	0(1)	29(1)	-4(1)
C(16)	34(1)	24(1)	32(1)	2(1)	17(1)	2(1)
C(17)	63(2)	60(2)	69(2)	-13(2)	52(2)	-13(1)
C(18)	40(1)	31(1)	31(1)	-2(1)	22(1)	-2(1)
C(19)	40(1)	45(2)	40(1)	4(1)	9(1)	-20(1)
C(20)	32(1)	32(1)	30(1)	13(1)	10(1)	-6(1)
C(21)	32(1)	28(1)	20(1)	3(1)	13(1)	-2(1)
C(22)	36(1)	26(1)	27(1)	8(1)	20(1)	7(1)
C(23)	45(1)	25(1)	20(1)	4(1)	18(1)	14(1)

C(24)	73(2)	42(1)	18(1)	1(1)	21(1)	26(1)
C(25)	59(2)	46(2)	23(1)	-7(1)	11(1)	18(1)
C(26)	44(1)	38(1)	26(1)	-9(1)	10(1)	8(1)
C(27)	36(1)	29(1)	20(1)	-1(1)	11(1)	8(1)
C(28)	36(1)	34(1)	24(1)	-6(1)	15(1)	-4(1)
C(29')	20(5)	43(6)	10(4)	-9(4)	5(4)	0(4)
C(30')	28(6)	13(4)	28(6)	-2(4)	21(6)	4(4)
C(31')	54(6)	29(4)	21(4)	0(3)	14(4)	-9(4)
C(32')	25(5)	39(6)	29(5)	-7(4)	16(4)	-5(5)
C(33')	38(6)	24(5)	33(5)	-13(4)	28(5)	-27(4)
C(34')	41(6)	25(5)	12(4)	-6(3)	14(4)	-3(4)
C(35')	34(5)	42(5)	38(5)	-6(4)	16(4)	-8(4)
C(36')	36(6)	26(5)	22(5)	1(4)	13(4)	8(4)
C(29)	20(2)	32(2)	25(2)	-13(2)	6(2)	-1(2)
C(30)	34(3)	36(3)	36(3)	-8(2)	5(2)	3(2)
C(31)	17(2)	54(3)	67(3)	-29(2)	8(2)	-2(2)
C(32)	36(3)	34(2)	70(3)	-23(2)	34(2)	-20(2)
C(33)	30(3)	39(2)	41(2)	-21(2)	19(2)	-13(2)
C(34)	25(2)	27(2)	29(2)	-11(2)	14(2)	-1(2)
C(35)	62(3)	50(2)	54(3)	-13(2)	36(2)	-27(2)
C(36)	29(2)	22(2)	29(2)	-1(2)	14(2)	2(2)

Table S11. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **Compound 4**.

	x	y	z	U(eq)
H(1D)	7761	3315	5482	26
H(4A)	6370	2422	5830	38
H(1A)	10012	2591	5450	42
H(1B)	9330	2731	6178	42
H(1C)	10206	3288	6137	42
H(4)	7268	4191	3005	29
H(6)	4384	5386	351	50
H(7)	2754	5182	593	47
H(8)	2865	4460	2034	37
H(12)	2924	3125	2254	40
H(13)	1351	2795	2544	53
H(14)	1290	3075	4220	53
H(17A)	2035	3564	6017	83
H(17B)	2874	4199	6220	83
H(17C)	3396	3463	6688	83
H(18A)	5279	4292	5471	48
H(18B)	5035	3914	6412	48
H(18C)	4356	4598	5836	48
H(19A)	4238	1405	5088	68
H(19B)	5067	1791	4678	68
H(19C)	3847	2113	4426	68
H(22)	6026	2702	8284	33
H(24)	8153	3589	11734	53
H(25)	9645	4194	11646	55
H(26)	9810	4211	9971	46
H(30')	7744	4631	6463	24
H(31')	8856	5297	5863	43
H(32')	10846	5139	6668	35
H(35A)	12515	4666	7951	57
H(35B)	12399	3861	8057	57

H(35C)	12537	4352	9076	57
H(36A)	11379	3181	8783	42
H(36B)	10248	3109	8963	42
H(36C)	11240	3630	9724	42
H(30)	10675	3270	9007	47
H(31)	12177	3761	8750	60
H(32)	11803	4660	7472	51
H(35D)	9293	5110	5372	78
H(35E)	10569	5392	6102	78
H(35F)	9542	5663	6344	78
H(36D)	8056	5214	6730	40
H(36E)	7485	4500	6771	40
H(36F)	7713	4684	5711	40

Table S12. Kinase selectivity using KINOME*scan*™ profiling services by DiscoveRx. Compounds were tested at 10 μM in a 97 kinase panel. Selectivity scores for **5**, **32**, **37** and **25** were respectively S(35) = 0, 0.011, 0.011 and 0.011.

Compound		**5**	**32**	**37**	**25**
DiscoveRx Gene Symbol	Entrez Gene Symbol	% Control	% Control	% Control	% Control
ABL1(E255K)-phosphorylated	ABL1	100	96	100	100
ABL1(T315I)-phosphorylated	ABL1	99	92	100	100
ABL1-nonphosphorylated	ABL1	88	85	100	100
ABL1-phosphorylated	ABL1	83	64	100	100
ACVR1B	ACVR1B	90	79	91	98
ADCK3	CABC1	100	90	99	100
AKT1	AKT1	100	86	100	89
AKT2	AKT2	89	88	92	97
ALK	ALK	81	93	100	100
AURKA	AURKA	92	91	100	100
AURKB	AURKB	93	94	83	100
AXL	AXL	87	92	96	94
BMPR2	BMPR2	92	77	94	96
BRAF	BRAF	100	100	100	96
BRAF(V600E)	BRAF	92	83	98	93
BTK	BTK	100	85	100	100
CDK11	CDK19	100	89	100	100
CDK2	CDK2	93	87	89	95
CDK3	CDK3	90	90	89	89
CDK7	CDK7	86	71	100	100
CDK9	CDK9	96	90	100	100
CHEK1	CHEK1	89	94	100	65
CSF1R	CSF1R	97	86	98	100
CSNK1D	CSNK1D	89	84	94	100
CSNK1G2	CSNK1G2	100	100	85	100
DCAMKL1	DCLK1	82	80	100	100
DYRK1B	DYRK1B	100	82	39	27
EGFR	EGFR	100	99	77	95
EGFR(L858R)	EGFR	100	97	89	93
EPHA2	EPHA2	97	78	100	100
ERBB2	ERBB2	98	91	100	100
ERBB4	ERBB4	100	77	96	100
ERK1	MAPK3	100	89	82	100
FAK	PTK2	88	84	95	100
FGFR2	FGFR2	100	92	100	100
FGFR3	FGFR3	100	84	100	100

FLT3	FLT3	84	89	97	99
GSK3B	GSK3B	85	88	100	100
IGF1R	IGF1R	94	84	76	100
IKK-alpha	CHUK	100	99	100	100
IKK-beta	IKBKB	100	100	91	100
INSR	INSR	80	55	100	100
JAK2(JH1domain-catalytic)	JAK2	86	30	99	100
JAK3(JH1domain-catalytic)	JAK3	99	48	100	99
JNK1	MAPK8	100	37	85	100
JNK2	MAPK9	92	36	83	97
JNK3	MAPK10	100	55	94	100
KIT	KIT	92	86	87	81
KIT(D816V)	KIT	100	86	100	100
KIT(V559D,T670I)	KIT	95	76	100	97
LKB1	STK11	86	91	93	59
MAP3K4	MAP3K4	77	51	94	99
MAPKAPK2	MAPKAPK2	92	79	73	93
MARK3	MARK3	97	94	100	66
MEK1	MAP2K1	94	80	100	100
MEK2	MAP2K2	100	87	100	100
MET	MET	95	92	96	96
MKNK1	MKNK1	82	56	100	100
MKNK2	MKNK2	100	88	72	100
MLK1	MAP3K9	86	73	90	89
p38-alpha	MAPK14	98	87	100	82
p38-beta	MAPK11	100	91	98	100
PAK1	PAK1	69	100	100	87
PAK2	PAK2	94	71	99	89
PAK4	PAK4	82	93	97	96
PCTK1	CDK16	95	86	100	100
PDGFRA	PDGFRA	86	85	100	100
PDGFRB	PDGFRB	89	87	85	81
PDPK1	PDPK1	87	81	84	100
PIK3C2B	PIK3C2B	96	85	100	100
PIK3CA	PIK3CA	91	80	99	99
PIK3CG	PIK3CG	100	43	98	100
PIM1	PIM1	86	88	83	94
PIM2	PIM2	100	71	80	96
PIM3	PIM3	79	93	99	100
PKAC-alpha	PRKACA	90	83	71	100
PLK1	PLK1	97	83	87	97

PLK3	PLK3	86	89	100	100
PLK4	PLK4	82	72	100	100
PRKCE	PRKCE	97	87	98	100
RAF1	RAF1	100	98	100	88
RET	RET	87	89	95	90
RIOK2	RIOK2	100	66	17	49
ROCK2	ROCK2	100	94	99	100
RSK2(Kin.Dom.1-*N*-terminal)	RPS6KA3	94	81	100	97
SNARK	NUAK2	100	57	100	100
SRC	SRC	95	94	83	100
SRPK3	SRPK3	90	72	88	85
TGFBR1	TGFBR1	100	97	97	79
TIE2	TEK	94	86	100	92
TRKA	NTRK1	85	71	86	84
TSSK1B	TSSK1B	83	87	100	94
TYK2(JH1domain-catalytic)	TYK2	76	39	96	100
ULK2	ULK2	94	81	100	100
VEGFR2	KDR	89	77	100	100
YANK3	STK32C	100	100	73	86
ZAP70	ZAP70	100	89	100	100

MTH1 Biochemical Assay Activity of the MTH1 enzyme was assessed by detecting the inorganic pyrophosphate generated when the nucleoside triphosphate substrate, 8-oxo-dGTP, is hydrolysed. All concentrations are final unless noted otherwise. The compounds were serial diluted from a 10 mM DMSO stock and all reactions contained a final concentration of 1% DMSO. The general reaction buffer contained 100 mM Tris HCl (pH 7.5), 40 mM NaCl, 10 mM $Mg(OAc)_2$, 2 mM DTT, 0.005% Tween 20, and 0.01% BSA. The testing compounds were pre-incubated with 0.3 nM full-length recombinant MTH1 for 30 minutes at room temperature (RT) in the reaction buffer. The reaction was initiated by the addition of substrate at 2x K_m (final concentration of 20 μM for 8-oxo-dGTP and 10 μM for 2-OH-dATP), followed immediately by the addition of 2X PPiLight™ inorganic pyrophosphate kit (Lonza, Basel, Switzerland) for phosphate detection. The reaction was incubated for 3 hours at RT before the luminescence signal was measured on an Envision plate reader (Perkin Elmer, Waltham MA). Data analysis was completed using Prism (v7.0, GraphPad, La Jolla, CA). The K_i value for **5** was measured under conditions similar to the ones described above, however 0.05 nM MTH1 and 20 μM 8-oxo-dGTP (2 x K_m) were used. The reaction was incubated for 1 hour and the linear portion of

the reaction was used to derive the rates of product formation. The apparent K_i, $K_{i(app)}$, was calculated using Morrison tight-binding equation with DynaFit software. The K_i value was then calculated from $K_{i(app)}$ using equation $K_{i(app)} = K_i(1+[S]/K_m) = 3K_i$, based on the specific assay conditions. For **5**, the $K_{i(app)}$ value was determined to be 5.2 ± 0.9 pM with 95% confidence interval of 3.4-7.3 pM, and the K_i value was determined to be 1.7 pM with 95% confidence interval of 1.1-2.4 pM.

Table S13. MTH1 biochemical potencies of compounds **1-40** and literature compounds (IC_{50} geometric mean, standard of the mean (S.E.M.) and biological replicates (*n*)).

Compound	MTH1 IC_{50} (S.E.M.) (nM)	*n*	Compound	MTH1 IC_{50} (S.E.M.) (nM)	*n*
1	7.2	1	**21**	40	1
2	9077	1	**22**	12	1
3	952	1	**23**	0.70	1
4	81	1	**24**	5.6	1
5	0.043[a]	1	**25**	0.49	1
6	0.33 (0.31)	2	**26**	0.80 (0.20)	2
7	125	1	**27**	51	1
8	0.17 (0.002)	2	**28**	3.7	1
9	0.06	1	**29**	0.82 (0.04)	2
10	0.61	1	**30**	1.1 (0.21)	2
11	<0.05	1	**31**	936	1
12	0.11 (0.04)	2	**32**	13 (1.4)	20
13	<0.05	1	**33**	40	1
14	<0.05	1	**34**	207	1
15	0.15	1	**35**	946	1
16	62	1	**36**	4.1	1
17	773	1	**37**	15	1
18	6.8	1	**38**	20	1
19	16	1	**39**	13	1
20	26	1	**40**	467	1
TH287	4.1 (0.35)	19	**TH588**	26 (0.34)	19
SCH51344	421	1	**(*S*)-crizotinib**	366 (0.34)	19

[a]For compound **5**, the IC_{50} was determined using 50 pM of MTH1 enzyme.

Cell Viability Assay U2OS cells were seeded in a 96-well tissue culture plate at a density of 2000 cells/well in 100 µL DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100mg/mL streptomycin (Gibco, Life Technologies, Carlsbad, CA) and treated in triplicate with

a titration of compounds for 72 h in a humidified atmosphere of 5% CO_2, 95% air at 37 °C. Viability was assessed using CellTiter-Glo Luminescent Cell Viability Assay (Promega Corp., Madison, WI) and read on a Synergy 4 plate reader (BioTek, Winooski, VT). Data was plotted as percent vehicle (DMSO) control.

p53 Pathway Activation in U2OS Cells using Peggy Sue™ Simple Western U2OS cells were grown overnight in DMEM medium supplemented with 10% FBS and treated the following morning with 1µg/ml mitoxantrone, 5 µM **TH287**, 5µM **TH588** or 5 µM **5** for 4 h or 24 h. Cells were harvested in lysis buffer (Cell Signaling Technology) containing: Protease Inhibitor Cocktail (Roche Diagnostics Corp), and phosphatase inhibitor sets 1 and 2 (EMD Millipore). Following 10 minutes on ice, cell lysates were cleared by centrifugation at 12,500 rpm for 10 minutes at 4 °C. Lysates were analyzed by Simple Western using Peggy Sue™ (ProteinSimple, San Jose, CA; referred to in the text as Simple Western). Data was processed using Compass software (ProteinSimple). The following antibodies were purchased from Cell Signaling Technology (Danvers, MA): p-p53 (S15) (#9286 mouse monoclonal), actin (#4967 rabbit polyclonal).

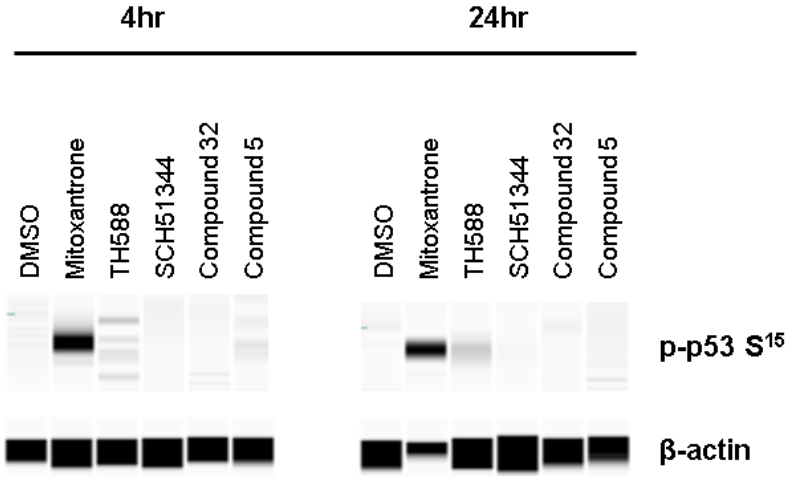


Figure S3. Expression of p-p53 and β-actin in U2OS cells treated with **TH588**, **SCH51344**, **Compound 32** and **Compound 5** at 5 µM and mitoxantrone at 2.25 µM, measured using Peggy Sue™ Western blot.

Immunostaining U2OS cells were cultured in 8-well slide (MilliporeSigma, Millicell EZ Slides, Cat#PEZGS0816), treated with inhibitors and fixed in ice-cold methanol following by washing in Dulbecco's Phosphate-Buffered Salt Solution (DPBS) (Corning) and blocking in DPBS with 10% HyClone Fetal Bovine Serum (FBS) (MilliporeSigma) and 0.1% Triton X-100 (MilliporeSigma) for 80 minutes. Anti-phospho-histone H2AX (Ser139) mouse monoclonal antibody (MilliporeSigma, 05-636-I, clone JBW301) was applied at concentration of 2.5 µg/mL in DPBS containing 1% FBS. Donkey polyclonal anti-mouse IgG (H+L) antibody conjugated with Alexa Fluor 488 (ThermoFisher Scientific A21202, 2 µg/mL) was used as the secondary antibody. The coverslips with stained cells were mounted on the glass microscopic slides (VWR International, Radnor, PA) with a drop of mounting medium Vectashield H-1300 (Vector Laboratories, Burlingame, CA) containing DNA dye propidium iodide.

Confocal imaging The samples were imaged with confocal laser scanning microscope LSM 5 PASCAL (Carl Zeiss, Germany) equipped with a Zeiss Plan-Apochromat oil immersion objective (40x magnification, 1.4 numerical aperture). The fluorophores were excited at 488 nm (Alexa Fluor 488) and 633 nm (propidium iodide). The fluorescence was detected using band-pass filter 505-600 nm for Alexa Fluor 488 and long-pass filter >650 nm for propidium iodide. The images were analyzed by manual counting of the phospho-Histone H2A.X (γH2A.X)-positive foci in individual nuclei.

Intracellular endogenous nucleotide concentration measurement in U2OS cells

MTH1 shRNA Knockdown Lentiviral transduction particles containing Mission shMTH1.GFP and shControl.GFP constructs (Millipore Sigma) were obtained to induce MTH1 knockdown: shMTH1-2 (TRCN0000288947): 5’ CCTGAGCTCATGGACGTGCAT 3’ shMTH1-3 (TRCN0000050132): 5’ CGAGTTCTCCTGGGCATGAAA 3’ as previously described (Patel, A., MTH1 Oncogene 2015). U20S and SW480 cell lines, purchased from the American Type Culture Collection (Manassas, VA), were transduced and then selected in geneticin containing media (10% FBS, 100 U/mL penicillin and 100 ug/mL streptomycin (Gibco, Life Technologies, Carlsbad, CA)). Geneticin selected tumor cells were then sorted for GFP+ expression by fluorescence activated cell sorting and analyzed for MTH1 expression.

Cells Cells were cultured in T175 Vented Flask (Corning, Kennebunk, ME) in Dulbecco's Modified Eagle Medium (DMEM; Sigma) with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO) +/- 400µg/mL G418 to maintain the selection. Approximately 10 million cells were cultured in each T175 flasks. At 48 hours post-incubation of MTH1 inhibitor **37**, extracellular media was removed, cells were trypsinized (Sigma-Aldrich) and combined into 15 mL conical tube, and then washed twice with 4 mL of ice-cold 0.9% normal saline. The cell pellets were quenched with 1 mL ice-cold 70% methanol containing 500 nM 2-chloro-adenosine-5'-triphosphate (Sigma-Aldrich) as an internal standard. Samples were stored overnight at -20°C to facilitate nucleotide extraction, centrifuged at 15,000 x *g* for 15 minutes and then supernatant was transferred to clean tubes for drying in a MiVac Duo concentrator (Genevac, Gardiner, NY). Dried samples were then combined and reconstituted in 1mM ammonium phosphate buffer (pH 7.4) for analysis by LC-MS/MS.

LC−MS/MS Instrumentation Cell lysates were analyzed using a HTS PAL autosampler with cooled sample storage stacks set at 10°C (Leap Technologies, Carrboro, NC) and an LC-20AD ternary pump system (Shimadzu Scientific Instruments, Columbia, MD). HPLC system was coupled to a Sciex API-5000 mass spectrometer (Applied Biosystems, Foster City, CA). Mass spectrometry was performed in positive-ion mode and using a multiple reaction monitoring mode (MRM). The standard stock solution of each analytes, 8-oxo-dGTP was purchased from TriLink Biotechnologies (San Diego, CA), 8-oxo-rGTP was purchased from Jena Biosciences (Jena, Germany), and dGTP and rGTP were purchased from Sigma-Aldrich. Analytes were separated using a 50 x 2 mm x 2.5 µ Luna C18(2) HST column (Phenomenex, Torrance, CA). A multi-stage linear gradient from 10% (Mobile Phase A) to 50% acetonitrile (Mobile Phase B) in a mobile phase containing 3 mM ammonium formate (pH 5.0) with 10 mM dimethylhexylamine at a flow rate of 0.15 mL/min was used to elute the analytes. Analytes were quantified using a 7 point standard curve prepared in cell extract from untreated cells.

---

[1] Otwinowski, Z. M., & Minor W., Processing of X-ray Diffraction Data Collection in Oscillation Mode. In *Methods in Enzymology*, Sweet, C. W. C. J. R. M., Ed. Academic Press: New York City, 1997; Vol. 276, pp 307-326.

[2] Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H., PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta crystallographica* **2010,** *66* (Pt 2), 213-21.

[3] Emsley, P.; Cowtan, K., Coot: model-building tools for molecular graphics. *Acta crystallographica* **2004,** *60* (Pt 12 Pt 1), 2126-32.