Supporting Information

Discovery of Potent and Selective Leads Against *Toxoplasma gondii* Dihydrofolate Reductase Via Structure-Based Design

Matthew E. Welsch[†€], Jian Zhou[‡], Yueqiang Gao[‡], Yunqing Yan[‡], Gene Porter[§], Gautam Agnihotri[§], Yingjie Li[‡], Henry Lu[‡], Zhongguo Chen[‡], Stephen B. Thomas[†*]

[†]Turing Pharmaceuticals AG, Research & Development, 1177 Avenue of the Americas, 39th Floor, New York, New York 10036

[‡]WuXi AppTec, International Discovery Service Unit & Research Service Division, 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, China

[§]WuXi AppTec, In Vitro Biology US, 107 Morgan Lane, Plainsborough, NJ 08536

*To whom correspondence should be addressed: E-mail: stephen@turingpharma.com

[€]Present address: Atoms2Applications, Jersey City, NJ 07306

Table of Contents

Computational Experimental……..……...…S2

Figure S1. Combinatorial Library Design…….……..…S2

Figure S2. Representative Ligand Docking Poses……..S3

Biology Experimental……...……...…..S4

Chemistry Experimental……....S6

Crystallization & Structural Determination Experimental……..……...…..….S45

Computational Experimental

*Materials and Methods***.** All molecular modeling, solvation analyses, combinatorial library generation, physiochemical property calculations, and protein alignments we performed using Molecular Operating Environment (MOE), Chemical Computing Group (CCG) Inc. Computational experiments were performed using crystallographic data published in the RCSB Protein Data Bank (PDB codes 4KYA and 4M6K). Molecular docking studies were performed using FlexX, BioSolveIT Inc., under default parameters.

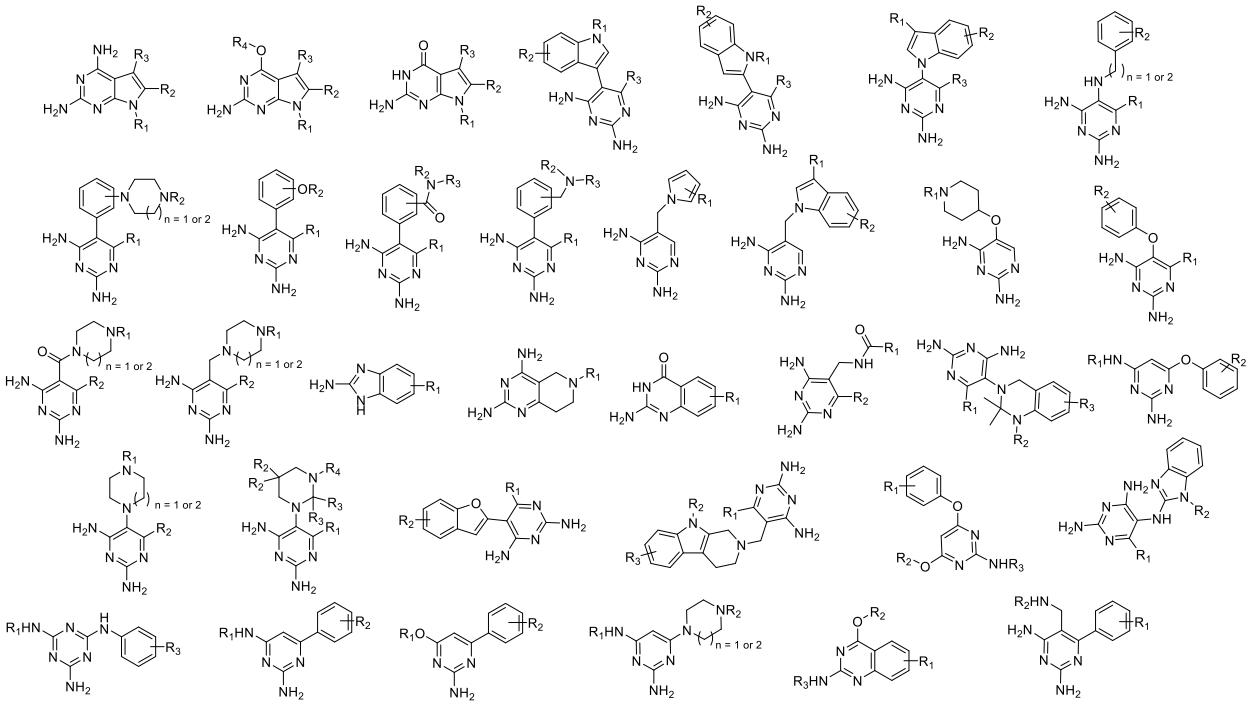


Figure S1. Combinatorial Library Design. Structures shown indicate the points of diversity (R_n) defined for combinatorial enumeration (built using MOE, Chemical Computing Group Inc.).

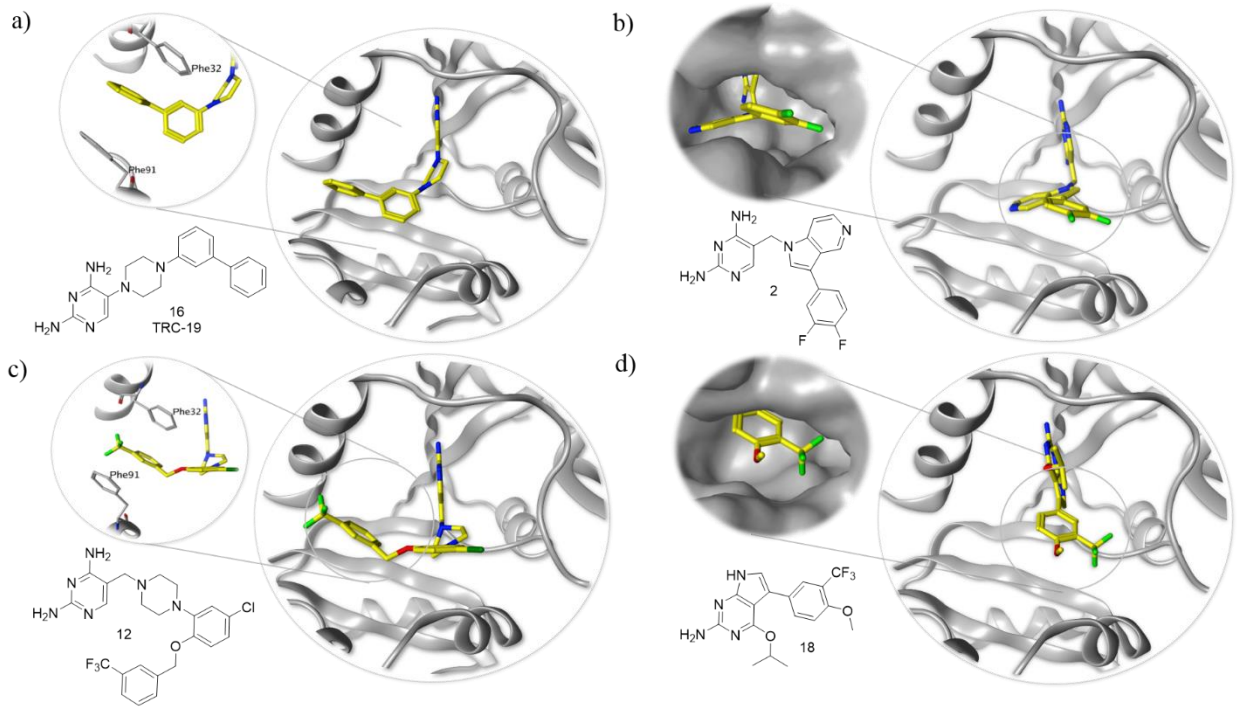


Figure S2. Representative Ligand Docking Poses**.** (a) (c) Docking poses of **16** and **12** (yellow) into TgDHFR (grey, PDB: 4KYA) with magnification indicating desired pi-stacking interaction with F32 and F91. (b) (d) Docking poses of **12** and **18** (yellow) into TgDHFR with magnification indicating occupancy of the G22 cavity in a molecular surface representation. Molecular docking performed using FlexX, BioSolveIT Inc.

Biology Experimental

Protein expression and purification

T. gondii TS-DHFR was sub-cloned into a PET15b plasmid and transformed into *Escherichia coli* BL21 competent cells. Overnight cultured bacteria were inoculated into a 1 L LB culture media at a ratio of 1:100 at 37 °C. Upon reaching an OD_{600nm} of 0.7, protein expression was induced with 0.5 mM isopropyl β-D-thiogalactoside at 16 °C overnight. Cells were then pelleted (~4.6 g) and re-suspended in buffer A (25mM Tris-HCl, pH 7.3, 100 mM NaCl, 1mM EDTA), before lysis by sonication. MTX agarose beads (~1 mL) were added to the lysate, and the beads were subsequently washed 2X with buffer A (~10 mL) and 1X with buffer B (~10 mL - 25mMTris-HCl, pH 7.3, 1M KCl, 1mM EDTA) prior to elution with buffer C (~6 mL, 25mM Tris-HCl, 10mM DTT, 10% glycerol, 2mM H2F). The eluent containing the purified enzyme was then concentrated and the protein was transferred, using a PD-MiniTrap G-25 column, to the final storage buffer (25mMTris-HCl, 10mM DTT, 10% glycerol).

The protein expression and purification protocol was adapted from a previously published procedure[1].

Purified hDHFR was obtained commercially from Sigma Aldrich; Dihydrofolate Reductase human (Sigma D6566).

Diaphorase-coupled assay for DHFR activity

Compounds were added as solutions of DMSO at 100X the desired concentration to purified enzyme (1 μg/mL) suspended in assay buffer (150 mM KCl, 8.9mM ß-mercaptoethanol in 40.0mM sodium phosphate at pH 7.4) in 384-well format (corning 3573). Following a 15 min incubation at 25 °C, solutions of NADPH (1.6 μM, Sigma N7505) and DHF (10 μM, Sigma D7006) in assay buffer were added sequentially. The plate was then incubated at 25 °C for an additional 60 min prior to the addition of diaphorase (10 U/mL) and resazurin (5 μM, Sigma R7017). After a final 10 min incubation at 25 °C, fluorescence was detected using an EnVision plate reader ($531nm^{Ex}$/$590\ nm^{Em}$).

The enzymatic assay protocol was adapted from a previously published procedure[2].

IC_{50} values were determined from the raw fluorometric data by non-linear regression using Graphpad Prism.

Methotrexate control was obtained commercially (Sigma M9929).

1. Sharma, H.; Landau, M. J.; Vargo, M. A.; Spasov, K. A.; Anderson, K. S., First three-dimensional structure of Toxoplasma gondii thymidylate synthase-dihydrofolate reductase: insights for catalysis, interdomain interactions, and substrate channeling. *Biochemistry* **2013,** *52* (41), 7305-17.
2. Kumar, A.; Zhang, M.; Zhu, L.; Liao, R. P.; Mutai, C.; Hafsat, S.; Sherman, D. R.; Wang, M. W., High-throughput screening and sensitized bacteria identify an M. tuberculosis dihydrofolate reductase inhibitor with whole cell activity. *PLoS One* **2012,** *7* (6), e39961.

Chemistry Experimental

General. Reagents and solvents that were commercially available were used without purification unless otherwise noted. NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm downfield with TMS as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), coupling constant (Hz), integration. Samples were purified either on silica gel columns or via preparative HPLC. LCMS spectra were taken on a quadrupole Shimadzu LCMS 2010 (column: sepax ODS 50×2.0 mm, 5 um) or Agilent 1200 HPLC, 1956 MSD (column: Shim-pack XR-ODS 30×3.0 mm, 2.2 um) operating in ES (+) ionization mode at a flow rate of 1.0 to 1.2 mL/min with UV detection at 220 nm and column temperature at 40 °C. HRMS spectra were taken on an Agilent 6210 TOF Mass Spectrometer in ES (+) ionization mode. Elemental analyses were performed on a Vario EL Elementar Analysensysteme.

Abbreviations: DME = Dimethyl Ether, TEA = Triethylamine, DMF = Dimethyl Formamide, ACN = Acetonitrile, DMSO = Dimethylsulfoxide, THF = Tetrahydrofuran, DCM = Dichloromethane

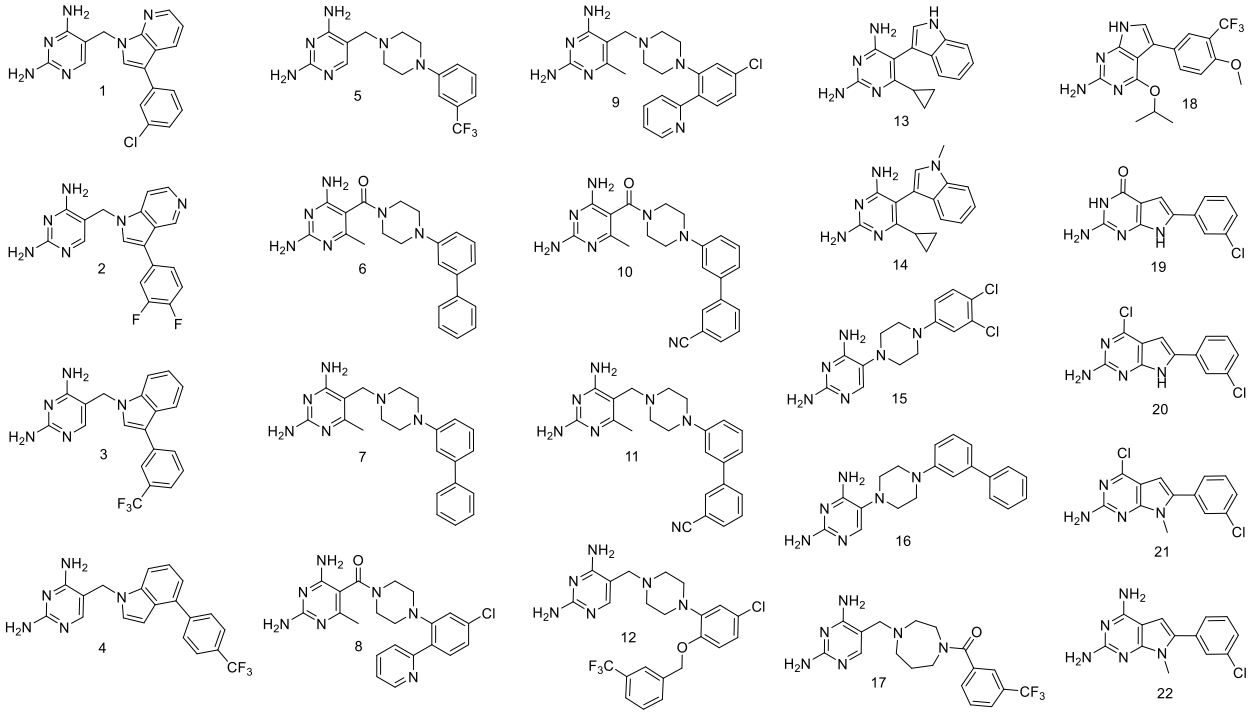
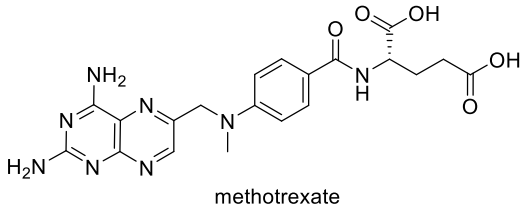
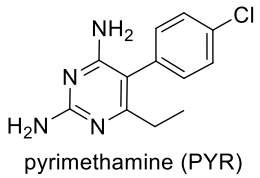


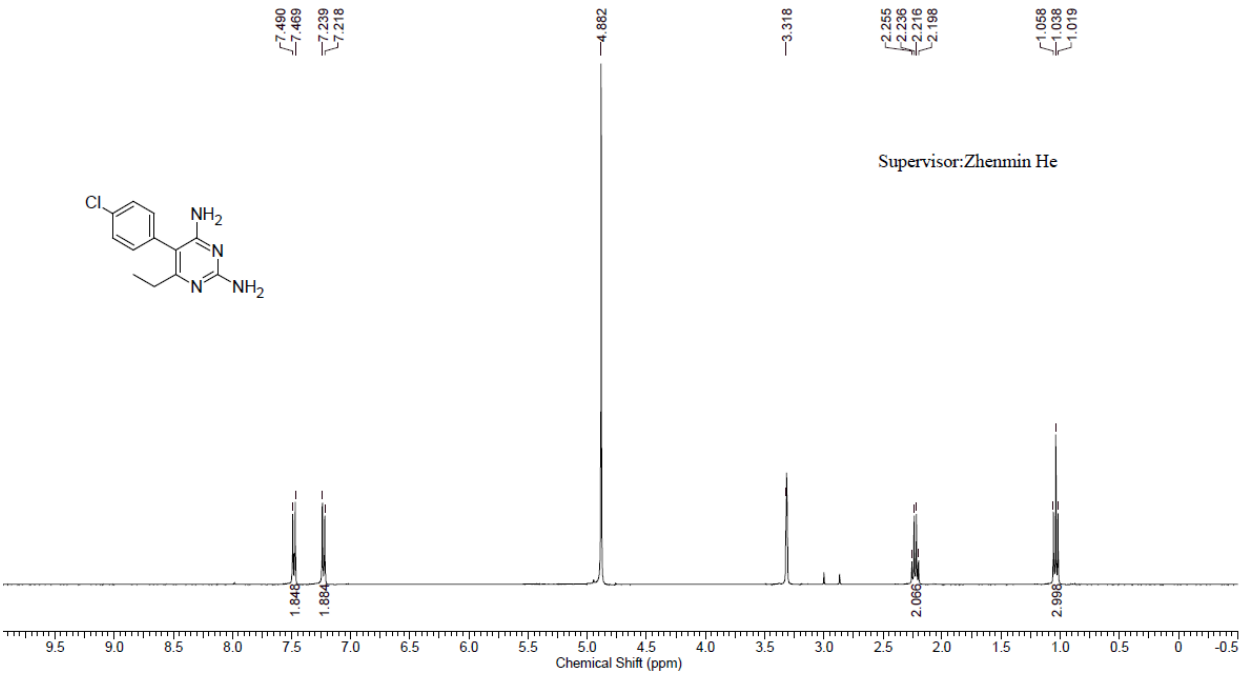
Figure S2. Compounds synthesized for initial screening efforts. 22 compounds representing a diverse set of pharmacophoric series.

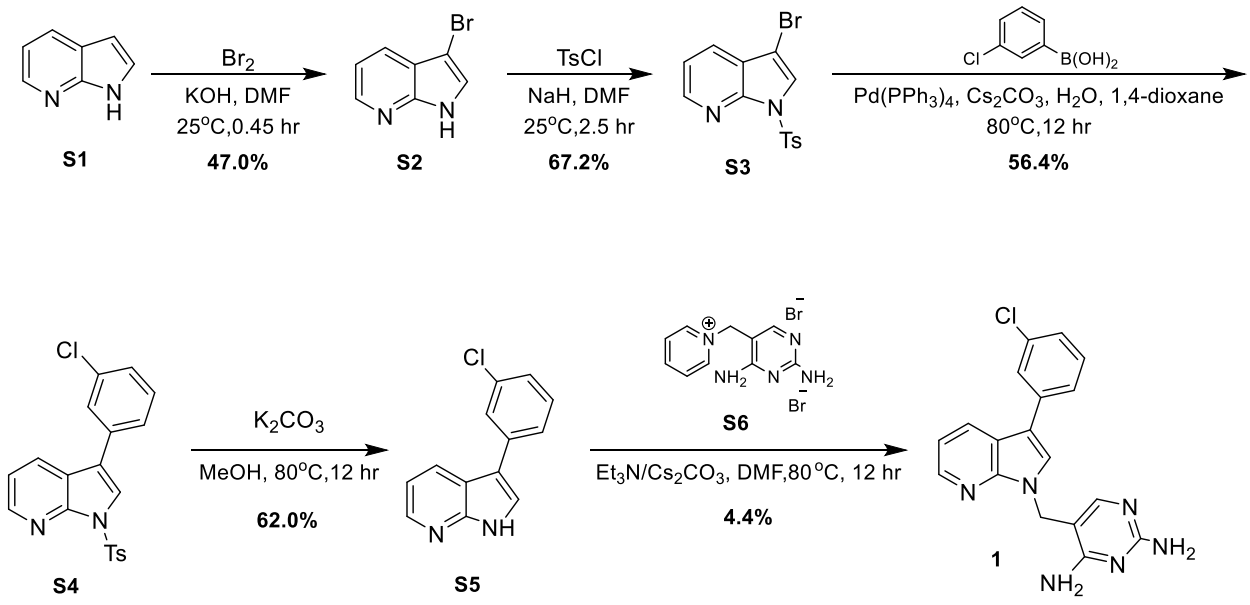


Methotrexate was obtained commercially from Sigma Aldrich (M9929). **LCMS**: (ESI+): m/z 455.1 $(M+1)^+$, Rt: 1.44 min (>99% purity).



Pyrimethamine was obtained commercially from Sigma Aldrich (1589007). **LCMS**: (ESI+): m/z 249.1 $(M+1)^+$, Rt: 1.96 min (>99% purity).





3-bromo-1H-pyrrolo[2,3-b]pyridine (S2). A solution of molecular bromine (6.7 g, 42.3 mmol, 2.1 mL, 1.0 eq) in DMF (150 mL) was dropped into a solution of 7-azaindole (**S1**) (5.0 g, 42.3 mmol, 1.0 eq) and KOH (2.3 g, 42.3 mmol. 1.0 eq) in DMF (150.0 mL) at 25 °C and stirred for 0.45 hr. The reaction mixture was extracted with EtOAc (100.0 mL*3). The combined organic layers were washed with brine (100.0 mL*3) and concentrated under reduced pressure to give gray

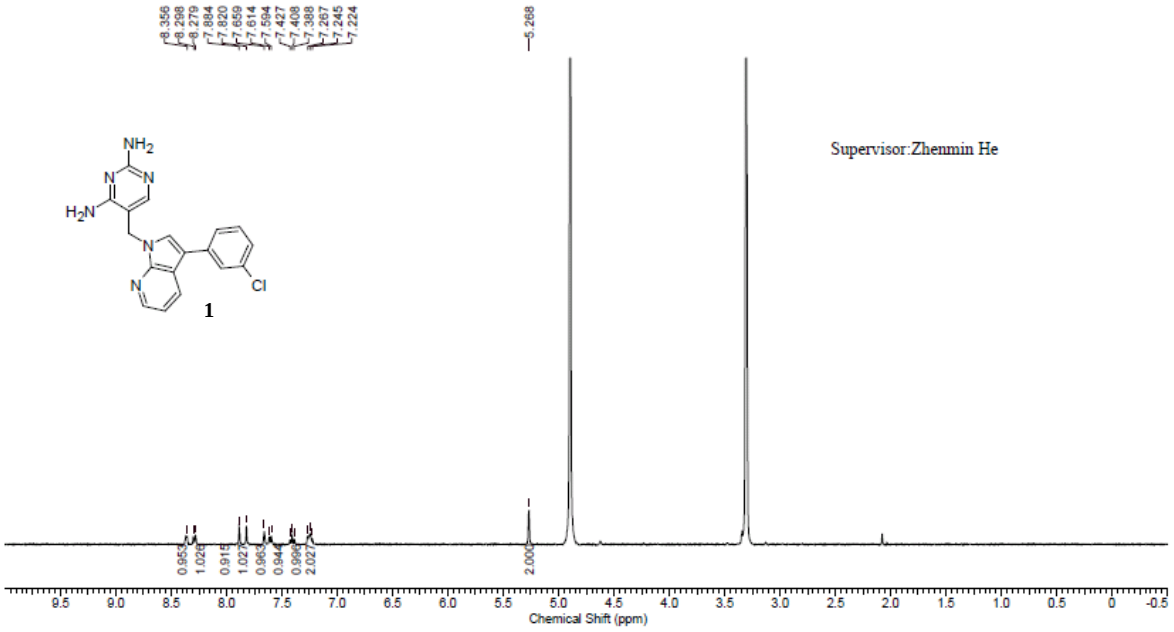
oil. The residue was purified by column chromatography (SiO_2, Et_2O/EtOAc from 1:1 to 5:1). Compound **S2** (4.5 g, 20.3 mmol, 47.0% yield) was obtained as a gray solid. **LCMS** (ESI+): m/z 197.0/199.0 $(M+1)^+$, Rt: 0.59 min. (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.6 min)

3-bromo-1-tosyl-1H-pyrrolo[2,3-b]pyridine (S3). To a mixture of **S2** (1.0 g, 5.0 mmol, 1.0 eq) and NaH (243.6 mg, 10.1 mmol, 2.0 eq) in DMF (10.0 mL) was added 4-methylbenzenesulfonyl chloride (967.6 mg, 5.0 mmol, 1.0 eq) in one portion at 2 5°C under N_2. The mixture was stirred at 25 °C for 2.5 hr. LCMS showed desired MS. The reaction mixture was extracted with ethyl acetate 30.0 mL (10.0 mL*3) and H_2O (10.0 mL). The combined organic layers were washed with saturated brine (10.0 mL*3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Et_2O/EtOAc from 20:1 to 5:1). **S3** (1.2 g, 3.4 mmol, 67.2% yield) was obtained as a white solid. LCMS (ESI+): m/z 351.0/353.0 $(M+1)^+$, Rt: 0.87 min. (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.6 min)

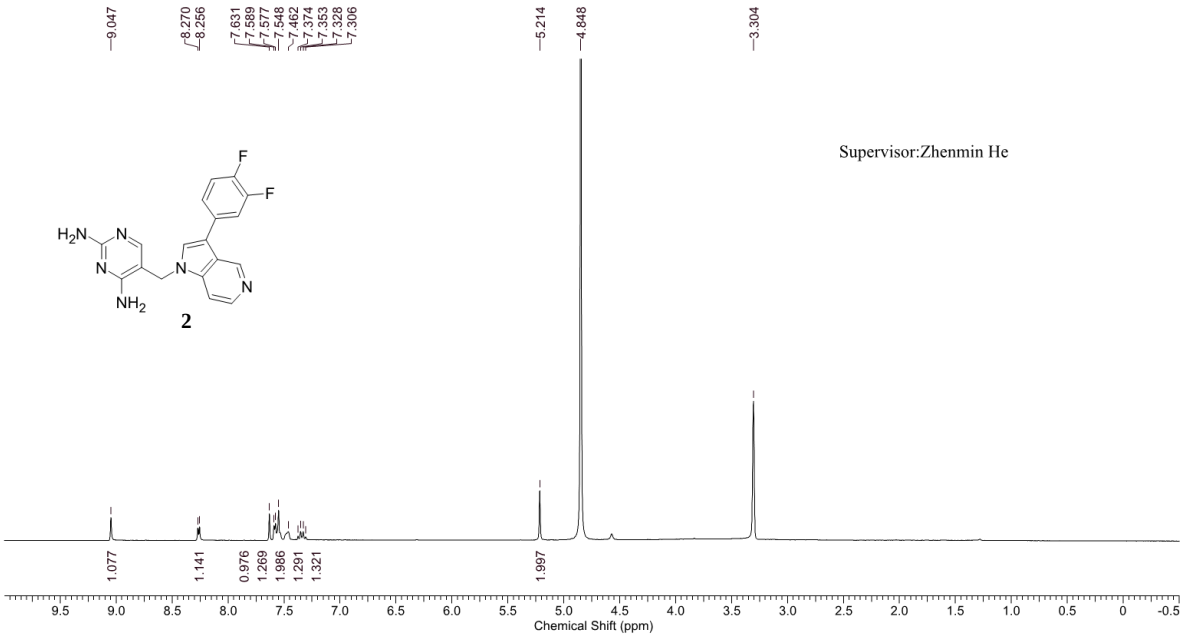
3-(3-chlorophenyl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (S4). To a mixture of **S3** (1.2 g, 3.4 mmol, 1.0 eq) and (3-chlorophenyl) boronic acid (534.2 mg, 3.4 mmol, 1.0 eq) in 1,4-dioxane (20.0 mL) and H_2O (5.0 mL) was added Cs_2CO_3 (1.6 g, 5.1 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (197.4 mg, 0.17 mmol, 0.02 eq) in one portion at 80°C under N_2. The mixture was stirred at 80°C for 12 hr. The residue was purified by column chromatography (SiO_2, Et_2O/EtOAc from 30:1 to 10:1). **S4** (1.0 g, 1.9 mmol, 56.4% yield) was obtained as a green solid. LCMS (ESI-): m/z 383.0 $(M+1)^+$, Rt: 0.94 min. (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.6 min)

3-(3-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine (S5). To a mixture of **S4** (1.0 g, 2.6 mmol, 1.0 eq) in MeOH (20.0 mL) was added K_2CO_3 (721.9 mg, 5.2 mmol, 2.0 eq) in one portion at 80°C under N_2. The mixture was stirred at 80°C for 12 hr. LCMS showed the desired MS. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Et_2O/EtOAc from 7:1 to 3:1). **S5** (400.0 mg, 1.62 mmol, 62.0 % yield) was obtained as a white solid. LCMS (ESI+): m/z 228.9 $(M+1)^+$, Rt: 1.38 min (H_2O:ACN 90/10 @ 0.01 min to 10/90 @ 2.0 min)

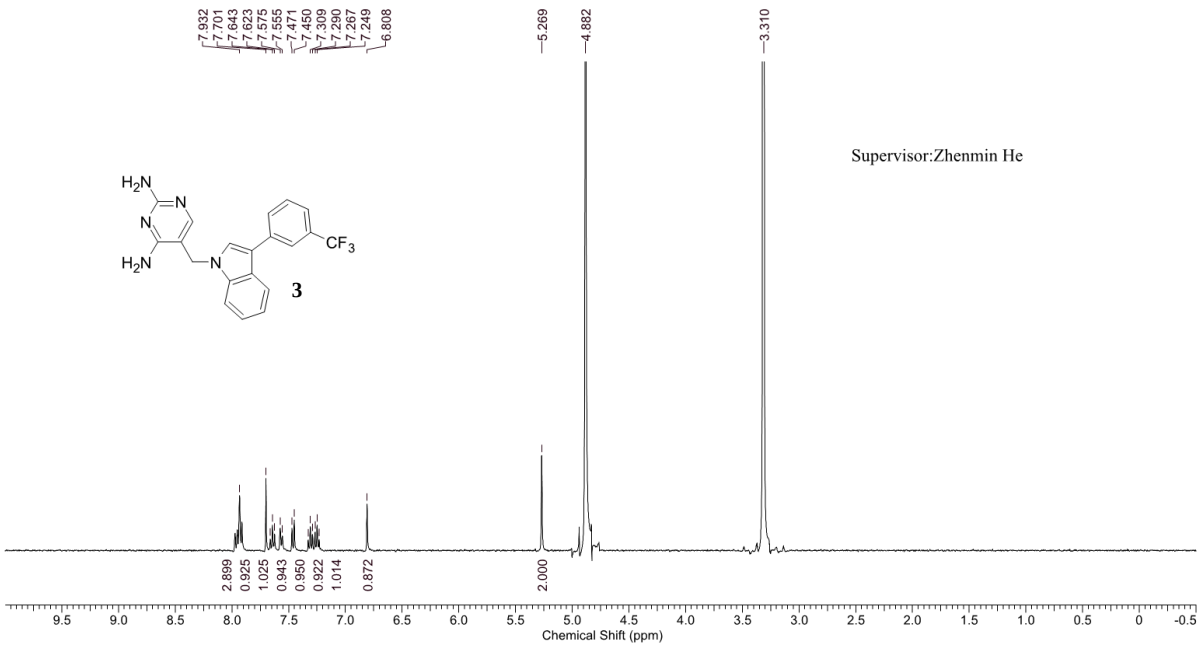
5-((3-(3-chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)methyl)pyrimidine-2,4-diamine (1). To a mixture of **S5** (300.0 mg, 1.3 mmol, 1.0 eq) and **S6** (475.6 mg, 1.3 mmol, 1.0 eq) in DMF (30.0 mL) was added Cs_2CO_3 (1.7 g, 5.2 mmol, 4.0 eq) and TEA (530.2 mg, 5.2 mmol, 726.3 uL) in one portion at 80 °C under N_2. The mixture was stirred at 80 °C for 12 hr. The reaction mixture was extracted with EtOAc (20 mL * 3) and H_2O (20 mL). The combined organic layers were washed with brine (20 mL*3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The solution was purified by prep-HPLC (basic condition). **1** (20.0 mg, 0.06 mmol, 4.4% yield, >95% purity) was obtained as a white solid. **^{1}H NMR** (MeOD, 400MHz) δ = 8.35 (br s, 1H), 8.28 (br d, J = 7.6 Hz, 1H), 7.88 (s, 1H), 7.82 (s, 1H), 7.66 (s, 1H), 7.60 (br d, J = 8.0 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.26 - 7.22 (m, 2H), 5.27 (s, 2H). **LCMS** (ESI+): m/z 351.0 $(M+1)^+$ (expected 351.1), Rt: 2.67 min (H_2O:ACN 99/1 @ 0.01 min to 1/99 @ 4.5 min)



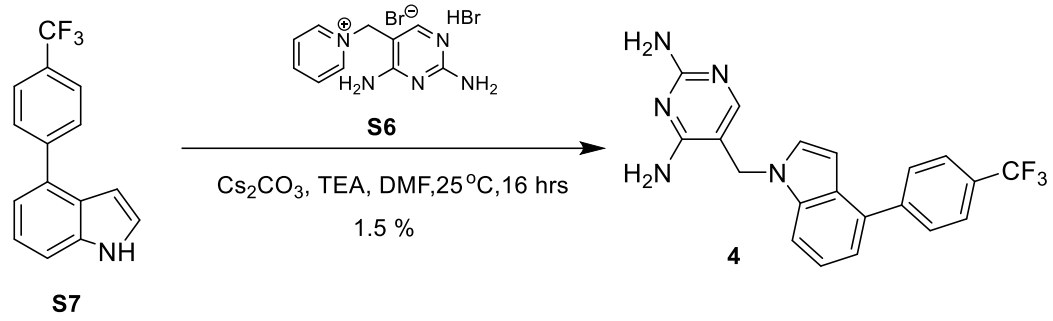
Compounds **2** and **3** were prepared in a manner analogous to **1**, with Pd-catalyzed Suzuki coupling of 3,4-difluoro and 3-trifluoromethyl substituted arylboronic acids respectively to intermediate **S3**.



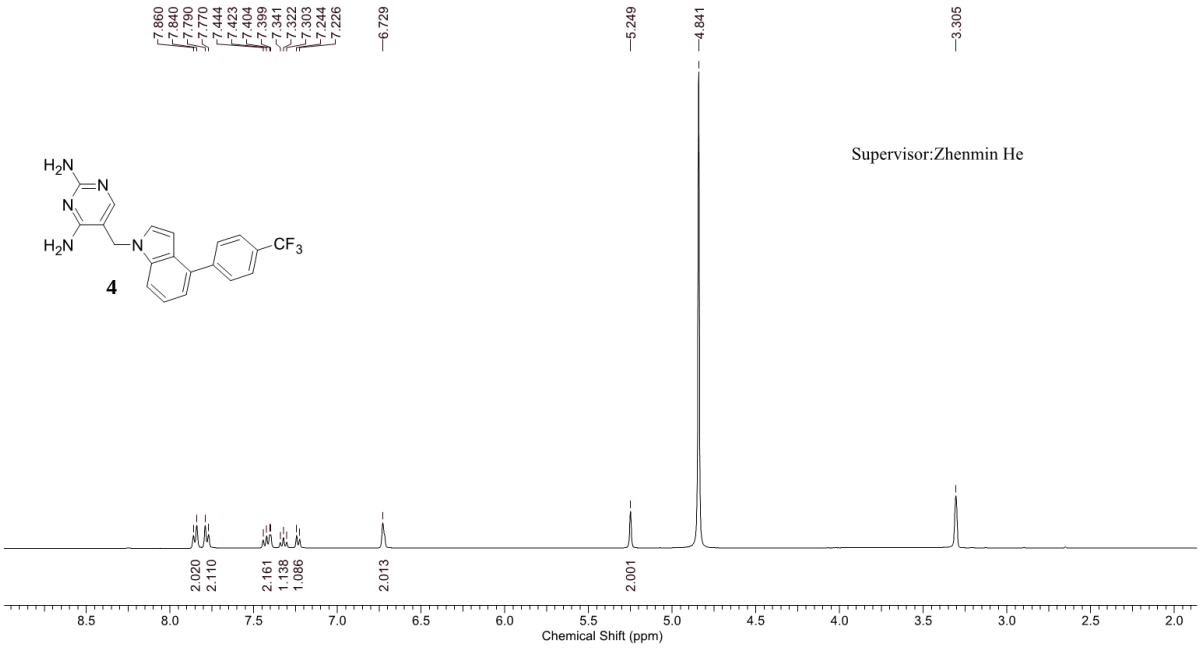
^{1}H NMR (MeOD, 400MHz) δ = 9.05 (s, 1H), 8.26 (d, *J* = 5.9 Hz, 1H), 7.63 (s, 1H), 7.58 (d, *J* = 5.1 Hz, 1H), 7.55 (s, 1H), 7.46 (br. s., 1H), 7.39 - 7.30 (m, 1H), 5.21 (s, 2H). **LCMS** (ESI+): m/z 353.0 $(M+1)^+$ (expected 353.1), Rt: 1.97 Min. (H_2O:ACN 99/1 @ 0.01 min to 1/99 @ 3.85 min), >97% purity.

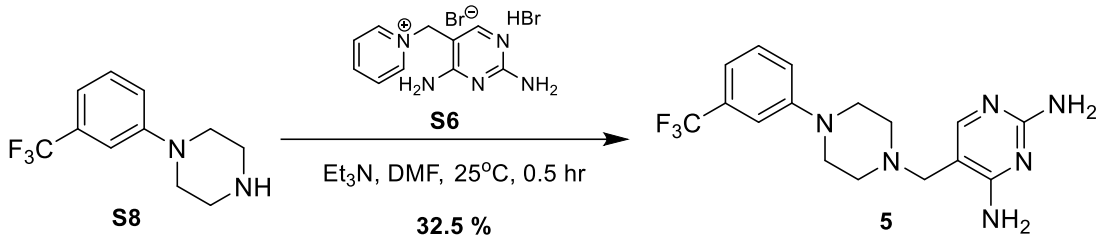


^{1}H NMR (MeOD, 400MHz) δ = 5.27 (s, 2H) 6.81 (s, 1H) 7.23 - 7.28 (m, 1H) 7.28 - 7.34 (m, 1H) 7.46 (d, *J*=8.38 Hz, 1H) 7.55 - 7.59 (m, 1H) 7.61 - 7.67 (m, 1H) 7.70 (s, 1H) 7.90 - 7.98 (m, 3H) **LCMS** (ESI+): m/z 384.0 $(M+1)^+$ (expected 385.1), Rt: 2.87 min. (H_2O:ACN 99/1 @ 0.01 min to 1/99 @ 3.85 min), >99% purity.

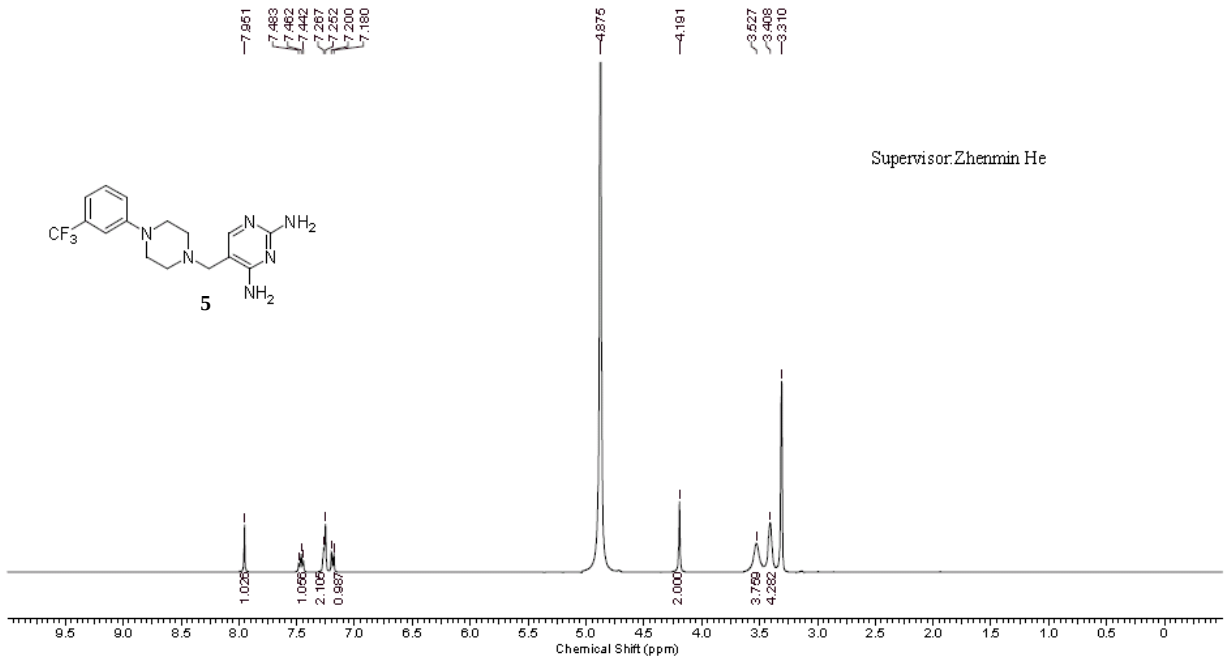


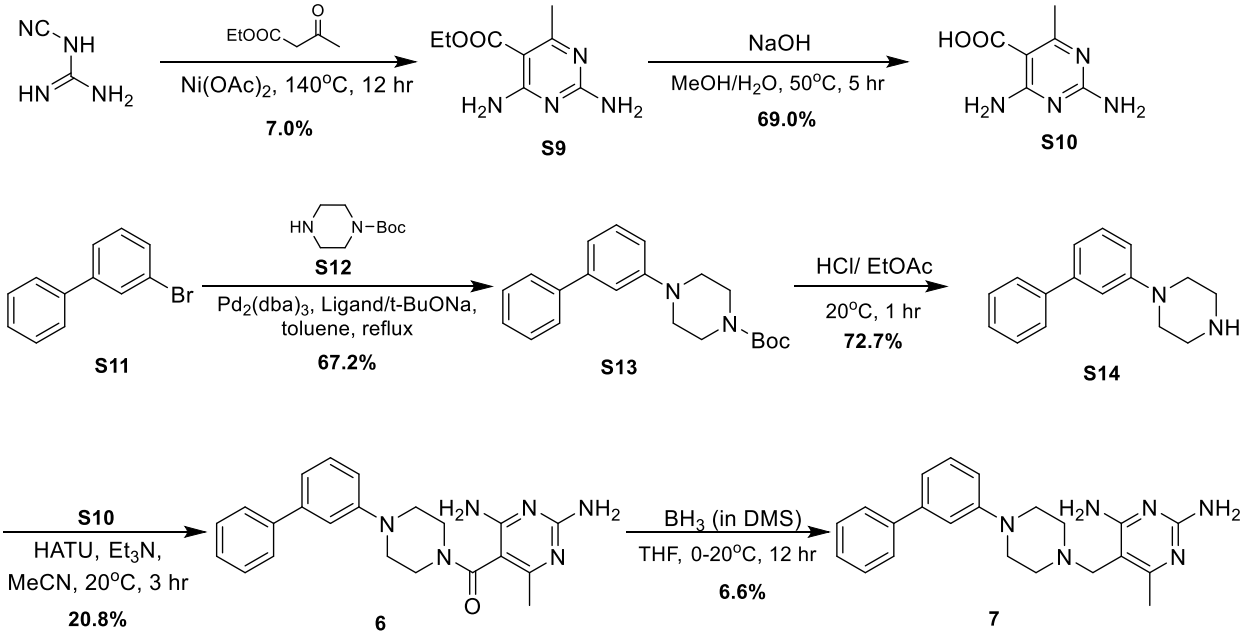
5-((4-(4-(trifluoromethyl)phenyl)-1H-indol-1-yl)methyl)pyrimidine-2,4-diamine (4). To a solution of 4-(4-(trifluoromethyl)phenyl)-1H-indole (**S7**) (130.0 mg, 0.50 mmol, 1.0 eq) in DMF (3.0 mL) was added TEA (0.27 mL, 2.0 mmol, 4.0 eq), Cs_2CO_3 (648.5 mg, 2.0 mmol) and **S6** (180.6 mg, 0.50 mmol, 1.0 eq). The mixture was stirred at 25 °C for 16 hr. The reaction mixture was concentrated under reduced pressure. The obtained residue was diluted with H_2O (50 mL) and extracted with EtOAc (50 mL*3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was purified by prep-HPLC to give **4** (3.0 mg, 0.008 mmol, 1.5% yield, >99% purity) as white solid. **^{1}H NMR** (MeOD, 400MHz) d = 7.90 - 7.81 (m, 2H), 7.81 - 7.75 (m, 2H), 7.48 - 7.38 (m, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.23 (d, *J* = 7.4 Hz, 1H), 6.73 (br. s., 2H), 5.25 (s, 2H). **LCMS** (ESI+): m/z 384.0 $(M+1)^+$ (expected 384.1), Rt: 2.859 min. (H_20:ACN 99/1 @ 0.01 min to 1/99 @ 3.85 min)





5-((4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl)pyrimidine-2,4-diamine (5). 1-(3-(trifluoromethyl)phenyl)piperazine (**S8**) (300.0 mg, 1.3 mmol, 0.244 mL, 1.0 eq) was added to the solution of **S6** (471.9 mg, 1.3 mmol, 1.0 eq) and TEA (657.7 mg, 6.5 mmol, 6.5 eq) in DMF (5.0 mL). The mixture was stirred at 25 °C for 0.5 hr. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure to give the crude product. The crude product was purified by prep-HPLC to give **5** (0.15 g, 32.5 % yield, >99% purity) as a white solid. **^{1}H NMR** (MeOD, 400MHz) δ = 7.95 (s, 1H), 7.46 (t, *J* = 8.1Hz, 1H), 7.23 - 7.29 (m, 2H), 7.19 (d, *J* = 7.9 Hz, 1H), 4.19 (s, 2H), 3.53 (br.s., 4H), 3.41 (br.s., 4H). **LCMS** (ESI+): m/z 353.1 $(M+1)^+$ (expected 353.2), Rt: 0.7 min. (H_2O:ACN 99/1 @ 0.01 min to 1/99 @ 3.85 min)

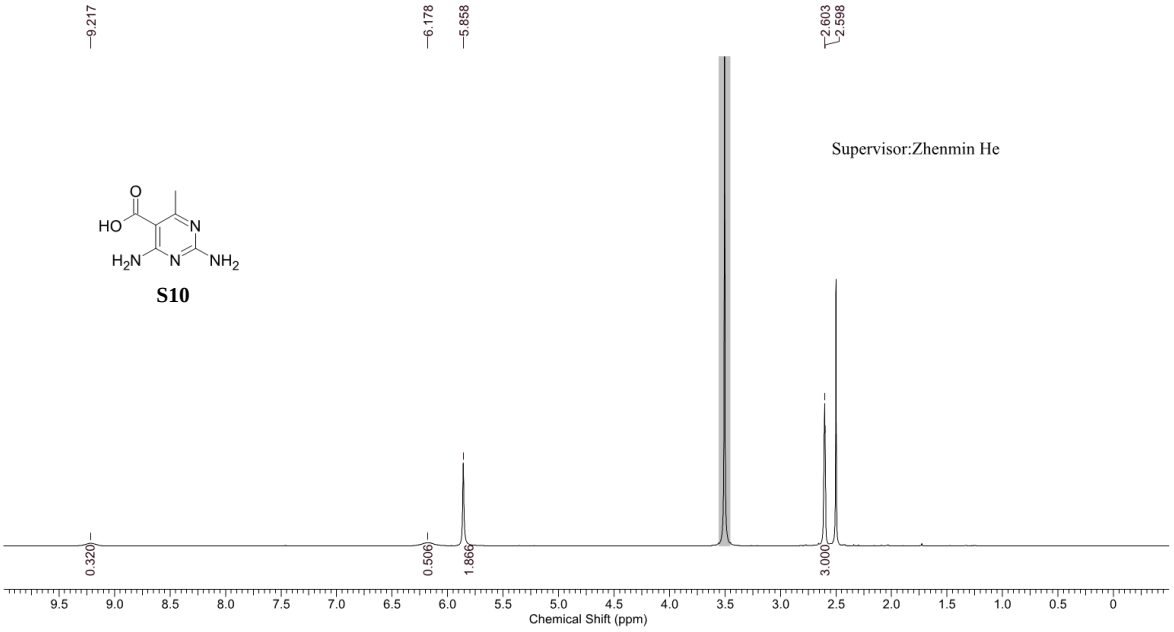




ethyl 2,4-diamino-6-methylpyrimidine-5-carboxylate (S9). A mixture of cyanoguanidine (30.0 g, 356.8 mmol, 1.0 eq) and $Ni(OAc)_2$ (1.00 g) were added to EtOAc (46.4 g, 356.8 mmol, 45.0 mL, 1.0 eq), and the resulting mixture was stirred at 140 °C for 12 hr under N_2. The mixture was allowed to cool to room temperature, concentrated under vacuum and then purified by column

(SiO_2, Et_2O/EtOAc from 1:1 to 0:1) to give **S9** (5.2 g, 26.5 mmol, 7.0% yield) as a faint yellow solid. LCMS (ESI+): m/z 197.1 $(M+1)^+$, Rt: 0.19 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

2,4-diamino-6-methylpyrimidine-5-carboxylic acid (S10). A mixture of **S9** (3.0 g, 15.3 mmol, 1.0 eq) and NaOH (1.8 g, 45.9 mmol, 3.0 eq) in methanol (60.0 mL) and H_2O (60 mL) was stirred at 50°C for 5 hr. Solvent was removed under vacuum and the mixture was adjusted to pH = 3.5 with *aq*. HCl (6 M). The solid was collected by filtration to give **S10** (1.8 g, 10.7 mmol, 69.0% yield) as a pale solid. **^{1}H NMR** (DMSO, 400MHz) δ = 9.22 (br. s., 1H), 6.18 (br. s., 1H), 5.86 (s, 2H), 2.60 (d, J=1.8 Hz, 3H). **LCMS** (ESI+): m/z 168.9 $(M+1)^+$, Rt: 0.27 min (H_2O:ACN 90/10 @ 0.01 min to 10/90 @ 1.66 min)

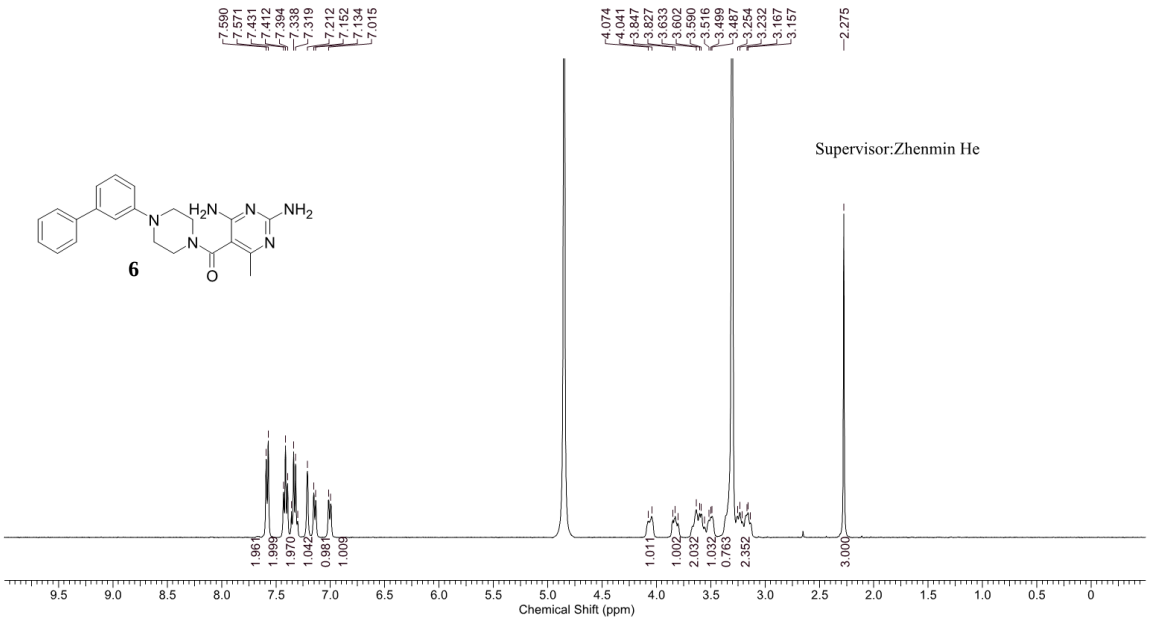


***tert*-butyl 4-([1,1'-biphenyl]-3-yl)piperazine-1-carboxylate (S13).** 3-phenylbromobenzene (**S11**) (2.0 g, 8.5 mmol, 1.4 mL, 1.0 eq) was added to a solution of sodium *tert*-butoxide (989.4 mg, 10.3 mmol, 1.2 eq) and $Pd_2(dba)_3$ (157.1 mg, 0.17 mmol, 0.02 eq) in toluene (20.00 mL). Subsequently, [1,1'-biphenyl]-2-yldicyclohexylphosphine (481.1 mg, 1.3 mmol, 0.16 eq) and 1-Boc-piperazine (**S12**) (1.6 g, 8.5 mmol, 1.0 eq) were added to the above mixture at 25°C. The resulting solution was stirred for 16 hr at 100°C under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to give a brown residue, which was purified by column (SiO_2,

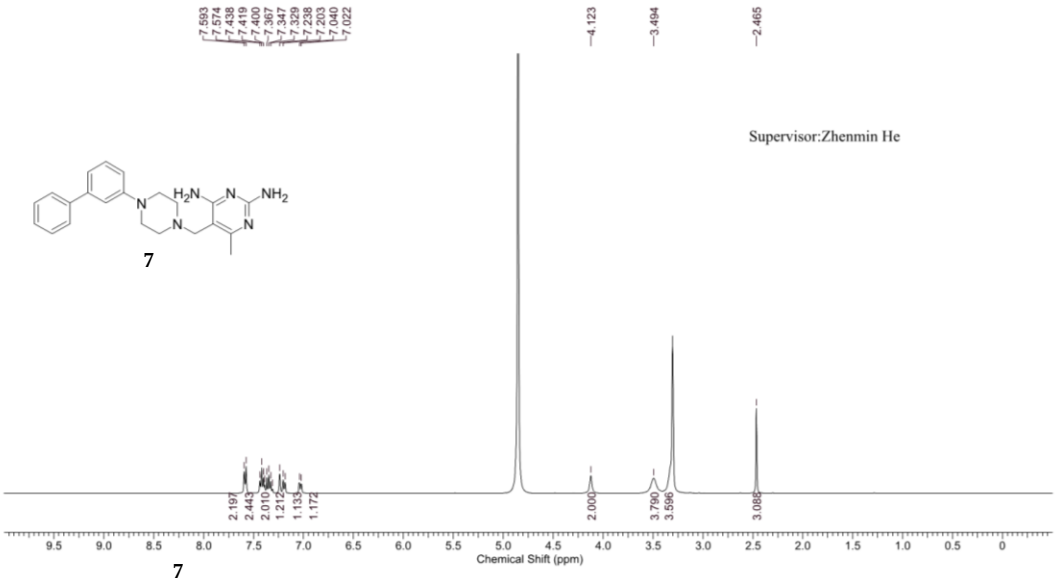
Et_2O/EtOAc from 10:1 to 5:1) to give **S13** (2.20 g, 67%) as a white solid. LCMS (ESI+): m/z 339.1 $(M+1)^+$, Rt: 1.72 min (H_2O:ACN 90/10 @ 0.01 min to 10/90 @ 1.66 min)

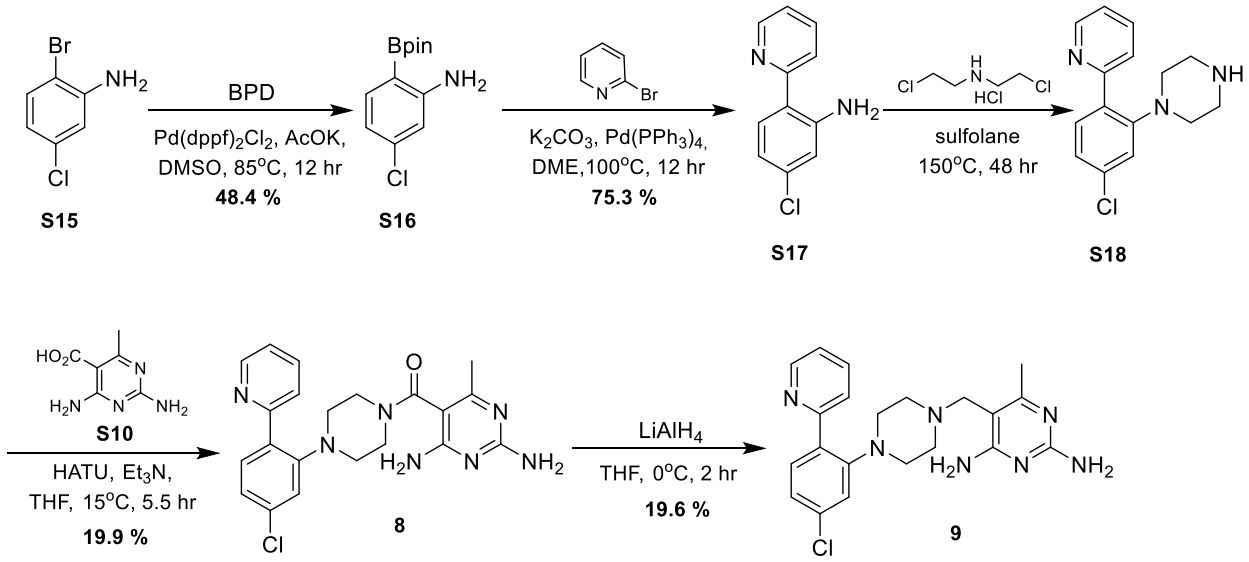
1-([1,1'-biphenyl]-3-yl)piperazine (S14). S13 (2.2 g, 6.5 mmol, 1.0 eq) was added to a solution of HCl/EtOAc (4 M, 73.3 mL, 45.1 eq) at 20 °C. The solution was stirred at 20 °C for 1 hr. The reaction mixture was concentrated under reduced pressure to give **S14** (1.50 g, 73%) as a gray solid. LCMS (ESI+): m/z 239.1 $(M+1)^+$, Rt: 0.65 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.1 min)

(4-([1,1'-biphenyl]-3-yl)piperazin-1-yl)(2,4-diamino-6-methylpyrimidin-5-yl)methanone (6). A mixture of **S14** (100.0 mg, 0.42 mmol, 1.0 eq), **S10** (70.5 mg, 0.42 mmol, 1.00 eq), TEA (0.06 mL, 0.42 mmol, 1.0 eq) and HATU (240 mg, 0.63 mmol, 1.50 eq) in acetonitrile (50.0 mL) was degassed and purged with N_2 three times, and then the mixture was stirred at 20 °C for 3 hr under N_2 atmosphere. Solvent was removed under vacuum to give a residue, which was purified by prep-HPLC to give **6** (100.0 mg, 0.26 mmol, 62% yield, >97% purity) as a white solid. **^{1}H NMR** (MeOD, 400MHz) δ = 7.58 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.37 - 7.28 (m, 2H), 7.21 (br. s., 1H), 7.14 (d, J = 7.5 Hz, 1H), 7.01 (d, J = 7.1 Hz, 1H), 4.06 (d, J = 13.2 Hz, 1H), 3.88 - 3.77 (m, 1H), 3.69 - 3.54 (m, 2H), 3.54 - 3.46 (m, 1H), 3.39 - 3.34 (m, 1H), 3.27 - 3.10 (m, 2H), 2.28 (s, 3H) **LCMS** (ESI+): m/z 389.1 $(M+1)^+$ (expected 389.2), Rt: 2.63 min (H_2O:ACN 99/1 @ 0.01 min to 1/99 @ 3.85 min)



5-((4-([1,1'-biphenyl]-3-yl)piperazin-1-yl)methyl)-6-methylpyrimidine-2,4-diamine (7). A mixture of **6** (300.0 mg, 0.77 mmol, 1.0 eq), borane dimethyl sulfide complex (10 M, 386.0 uL, 5.0 eq) in THF (10.0 mL) was degassed and purged with N_2 three times at 0 °C. The mixture was stirred at 20 °C for 12 hr under N_2 atmosphere. Methanol (5.0 ml) was added to the reaction mixture and the mixture solution was stirred at 70 °C for 2 hr. The volatile components were removed under vacuum to give the crude product. The crude product was purified by prep-HPLC to give **7** (19.3 mg, 0.052 mmol, 6.6% yield, >98% purity) as a white solid. **^{1}H NMR** (DMSO, 400 MHz) δ = 7.58 (d, J = 7.5 Hz, 2H), 7.44 - 7.38 (m, 2H), 7.38 - 7.30 (m, 2H), 7.24 (s, 1H), 7.19 (d, J = 7.5 Hz, 1H), 7.03 (d, J = 7.1 Hz, 1H), 4.12 (br. s., 2H), 3.49 (br. s., 4H), 3.37 - 3.32 (m, 4H), 2.46 (s, 3H) **LCMS** (ESI+): m/z 375.1 $(M+1)^+$ (expected 375.2), Rt: 2.39 min (H_2O:ACN 99/1 @ 0.01 min to 1/99 @ 3.85 min)



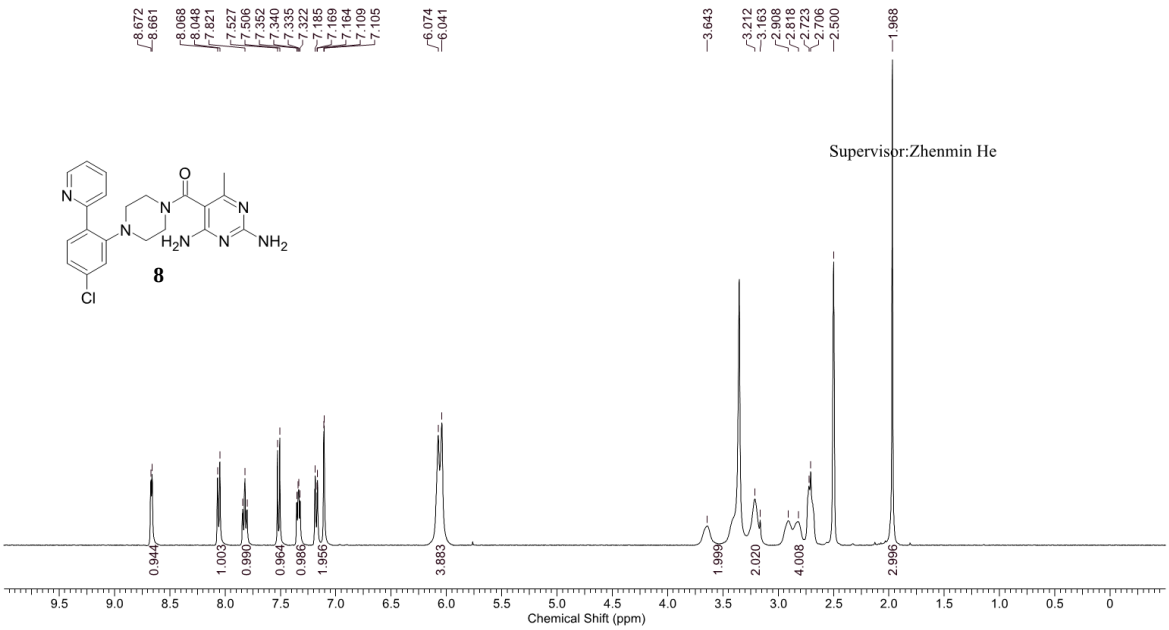


5-chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (S16). To a solution of 2-bromo-5-chloroaniline (**S15**) (10.0 g, 48.4 mmol, 1.0 eq) in DMSO (50.0 mL) was added K_2CO_3 (9.5 g, 96.8 mmol, 2.0 eq), $Pd(dppf)Cl_2.CH_2Cl_2$ (1.9 g, 2.4 mmol, 0.02 eq) and bis(pinacolato)diboron (18.4 g, 72.6 mmol, 1.5 equiv). The mixture was stirred at 85 °C for 12 hr under N_2. The reaction mixture was poured into water (150 mL) and extracted with EtOAc (150 mL * 3). The organic layers were combined, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by column (SiO_2, Et_2O/EtOAc from 30:1 to 10:1) to give S**16** (6.0 g, 48.4% yield) as a white solid. LCMS (ESI+): m/z 254.1 $(M+1)^+$, Rt: 2.04 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.5 min)

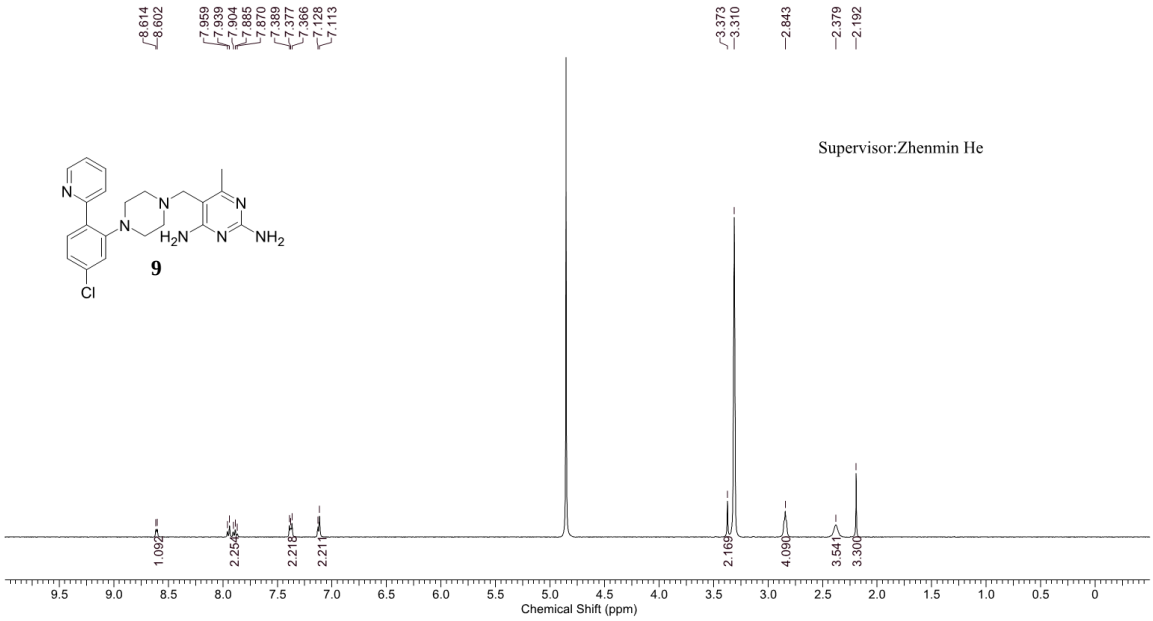
5-chloro-2-(pyridin-2-yl)aniline (S17). To a solution of **S16** (3.0 g, 11.8 mmol) in H_2O (15.0 mL) and DME (15.0 mL) was added K_2CO_3 (2.6 g, 19.4 mmol), 2-bromopyridine (2.0 g, 12.9 mmol, 1.2 mL) and $Pd(PPh_3)_4$ (749.6 mg, 0.65 mmol). The mixture was stirred at 100 °C for 12 hr. The reaction mixture was poured into water (100 mL) and extracted with EtOAc (90 mL * 3). The organic layers were combined, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by column chromatography (SiO_2, Et_2O/EtOAc from 50:1 to 5:1) to give **S17** (2.0 g, 75.3 % yield) was obtained as a yellow solid. LCMS (ESI+): m/z 205.0 $(M+1)^+$, Rt: 1.67 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.5 min)

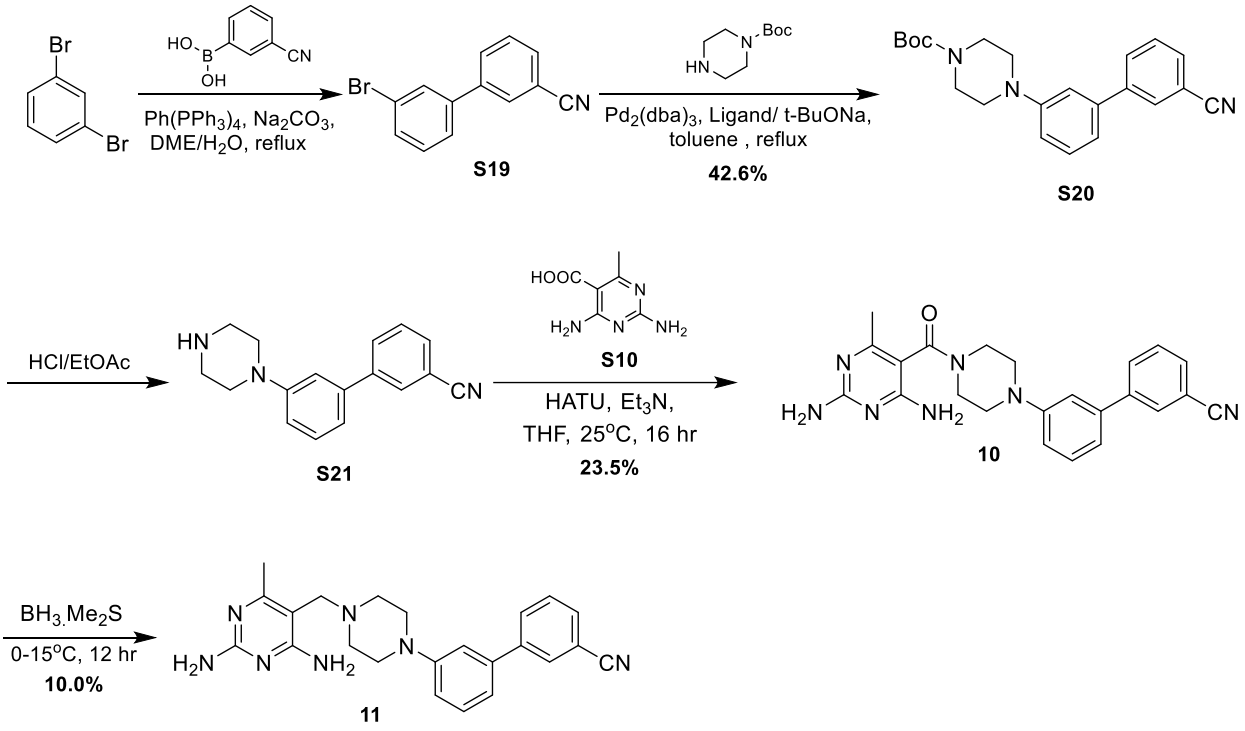
1-(5-chloro-2-(pyridin-2-yl)phenyl)piperazine (S18). To a solution of **S17** (1.5 g, 7.3 mmol, 1.0 eq) in sulfolane (20.0 mL) was added 2-chloro-N-(2-chloroethyl)ethanamine hydrochloride (1.7 g, 9.5 mmol, 1.2 eq). The mixture was stirred at 150 °C for 48 hr. The reaction mixture was cooled to 45 °C and diluted with acetone (50 mL) and further cooled to 0 °C. The mixture was maintained at 0 °C to precipitate the desired product, then subsequently filtered under N_2 and washed with chilled acetone to give **S18** (400.0 mg, crude) as a dark, oily liquid. **LCMS** (ESI+): m/z 274.1 $(M+H)^+$, Rt: 0.58 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.5 min)

(4-(5-chloro-2-(pyridin-2-yl)phenyl)piperazin-1-yl)(2,4-diamino-6-methylpyrimidin-5-yl)methanone (8). A mixture of **S18** (350.0 mg, 1.2 mmol, 1.0 eq), **S10** (60.2 mg, 0.36 mmol, 0.3 eq) and TEA (388.5 mg, 3.8 mmol, 0.532 mL) was dissolved in THF (10.0 mL) and stirred for 0.5 hr under N_2, then O-(7-azabenzotramethyluronium hexafluorophosphate (730.0 mg, 1.9 mmol, 1.5 eq) was added to the solution. The mixture was stirred at 15 °C for another 5 hr under N_2. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (20 mL * 3). The organic layers were combined, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by prep-HPLC to give **8** (110.0 mg, 19.9 % yield, >98% purity) was obtained as a white solid. **^{1}H NMR** (DMSO, 400 MHz) δ = 8.67 (d, *J*=4.41 Hz, 1H) 8.06 (d, *J*=7.94 Hz, 1H) 7.76 - 7.86 (m, 1H) 7.52 (d, *J*=8.38 Hz, 1H) 7.34 (dd, *J*=6.84, 5.07 Hz, 1H) 7.04 - 7.21 (m, 2H) 6.06 (d, *J*=13.23 Hz, 4H) 3.64 (br. s., 2H) 3.12 - 3.30 (m, 2H) 2.63 - 3.00 (m, 4H) 1.97 (s, 3H). **LCMS** (ESI+): m/z 424.1 $(M+1)^+$ (expected 424.2), Rt: 1.99 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.4 min)



5-((4-(5-chloro-2-(pyridin-2-yl)phenyl)piperazin-1-yl)methyl)-6-methylpyrimidine-2,4-diamine (9). To a solution of **8** (70.0 mg, 0.17 mmol, 1.0 eq) in THF (2.0 mL) was added $LiAlH_4$ (6.2 mg, 0.17 mmol, 1.0 eq) at 0 °C for 2 hr. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (30 mL * 3). The organic layers were combined, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by prep-HPLC and **9** (13.4 mg, 19.6 % yield, >99% purity) was obtained as a white solid. **^{1}H NMR** (MeOD, 400 MHz) δ = 8.61 (d, *J*=4.85 Hz, 1H) 7.82 - 7.98 (m, 2H) 7.32 - 7.42 (m, 2H) 7.03 - 7.16 (m, 2H) 3.37 (s, 2H) 2.84 (br. s., 4H) 2.38 (br. s., 4H) 2.19 (s, 3H). **LCMS** (ESI+): m/z 410.2 $(M+1)^+$ (expected 410.2), Rt: 1.01 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.4 min)





3'-bromo-[1,1'-biphenyl]-3-carbonitrile (S19). To a solution of 1,3-dibromobenzene (2.0 g, 8.5 mmol, 1.0 mL, 1.0 eq), (3-cyanophenyl)boronic acid (1.1 g, 7.6 mmol, 0.9 eq) and $Pd(PPh_3)_4$ (196.0 mg, 0.17 mmol, 0.02 eq) in DME (30.0 mL) was added an aqueous solution of Na_2CO_3 (2 M, 8.5 mL). The mixture was stirred under N_2 atmosphere at 85 °C for 16 hr. The reaction mixture

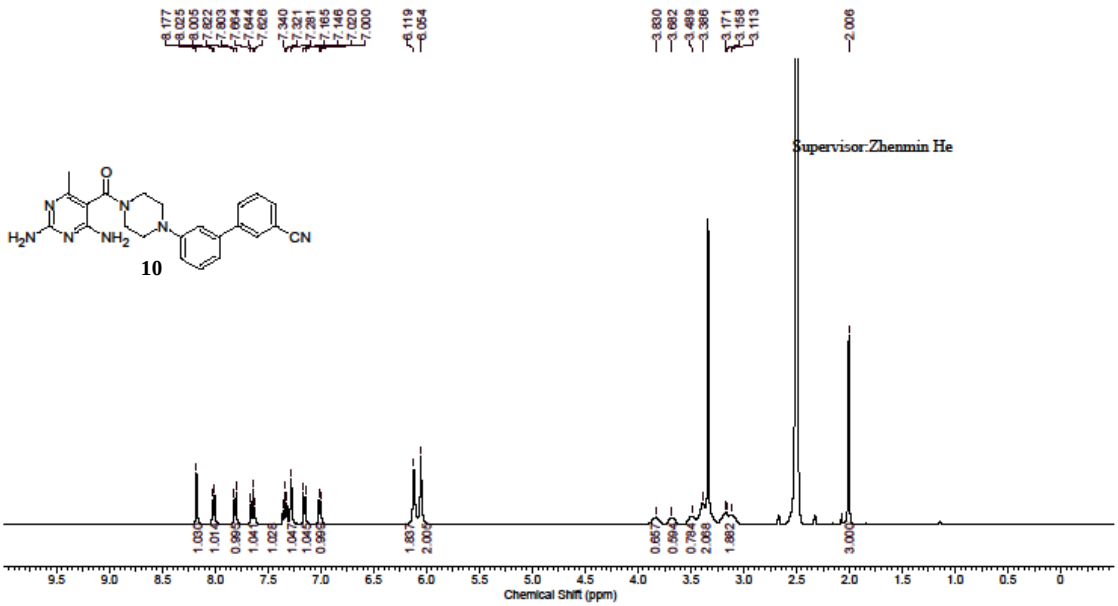
was concentrated under vacuum. The obtained residue was extracted with EtOAc (30 mL*3) and H_2O (30 mL). Then the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **S19** (2.3 g, crude) as yellow oil. It was used directly for the next step without further purification.

***tert*-butyl 4-(3'-cyano-[1,1'-biphenyl]-3-yl)piperazine-1-carboxylate (S20).** To a solution of **S19** (1.5 g, 5.8 mmol) and *tert*-butyl piperazine-1-carboxylate (1.1 g, 5.8 mmol) in toluene (15.0 mL) was added dicyclohexyl-(2-Phenylphenyl) phosphane (325.8 mg, 0.93 mmol), sodium 2-methylpropan-2-olate (670.0 mg, 7.0 mmol) and $Pd_2(dba)_3$ (106.4 mg, 0.12 mmol). The mixture was stirred at 100 °C for 12 hr under N_2 atmosphere. The reaction mixture was concentrated under vacuum. The obtained residue was purified by column chromatography (SiO_2, Et_2O/EtOAc 15:1) to get **S20** (900.0 mg, 2.4 mmol, 42.6% yield) as a yellow oil. **LCMS** (ESI+): m/z 364.2 $(M+1)^+$, Rt: 0.91 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

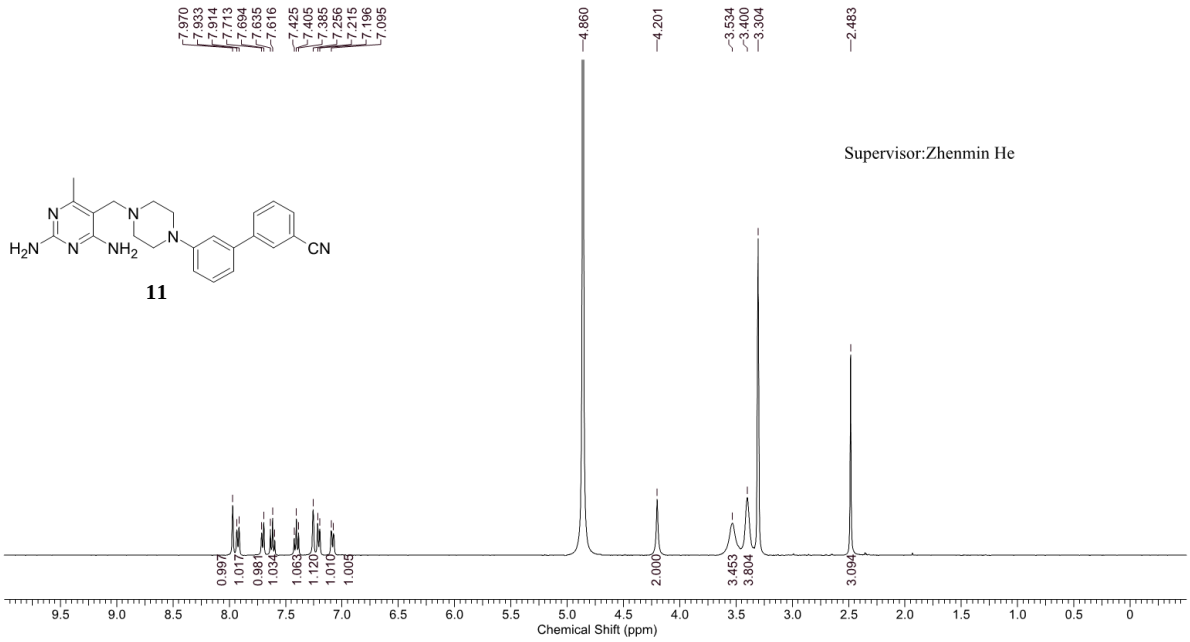
3'-(piperazin-1-yl)-[1,1'-biphenyl]-3-carbonitrile (S21). S20 (900.0 mg, 2.5 mmol) was added in a solution of HCl in EtOAc (2.5 mmol, 4 M, 20.0 mL). The mixture was stirred at 25 °C for 12 hr. The reaction mixture was concentrated and gave **S21** (700.0 mg, crude) as yellow solid. It was used directly for the next step without further purification. **LCMS** (ESI+): m/z 264.1 $(M+1)^+$, Rt: 0.65 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

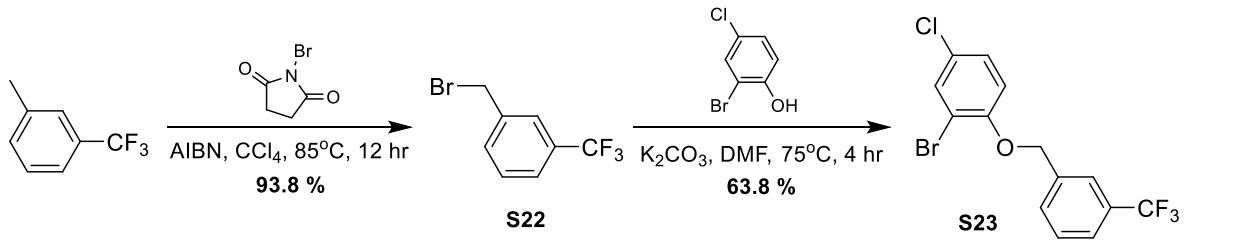
3'-(4-(2,4-diamino-6-methylpyrimidine-5-carbonyl)piperazin-1-yl)-[1,1'-biphenyl]-3-carbonitrile (10). To a solution of **S21** (250.0 mg, 0.95 mmol) and **S10** (159.6 mg, 0.95 mmol, 1.0 eq) in THF (10.0 mL) was added HATU (1.4 g, 3.80 mmol, 4.0 eq) and Et_3N (960.6 mg, 9.5 mmol, 10.0 eq). The mixture was stirred at 25 °C for 16 hr. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (100 mL*3). The combined organic layers were concentrated under reduced pressure and gave **10** (185.0 mg, 0.45 mmol, 23.5% yield, >95% purity) as light pink solid which was further purified by prep-HPLC. **^{1}H NMR** (DMSO, 400 MHz,) δ = 8.18 (s, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 7.4 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.37 - 7.30 (m, 1H), 7.28 (br. s., 1H), 7.16 (d, *J* = 7.4 Hz, 1H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.12 (br. s., 2H), 6.05 (br. s., 2H), 3.83 (br. s., 1H), 3.68 (br. s., 1H), 3.49 (br. s., 1H), 3.39 (br. s., 2H), 3.24 - 3.04

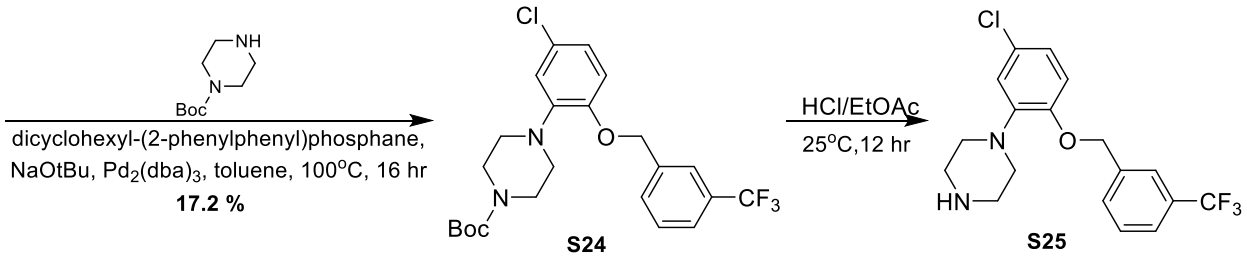
(m, 2H), 2.01 (s, 3H). **LCMS** (ESI+): m/z 414.2 $(M+1)^+$ (expected 414.2), Rt: 2.20 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.4 min)

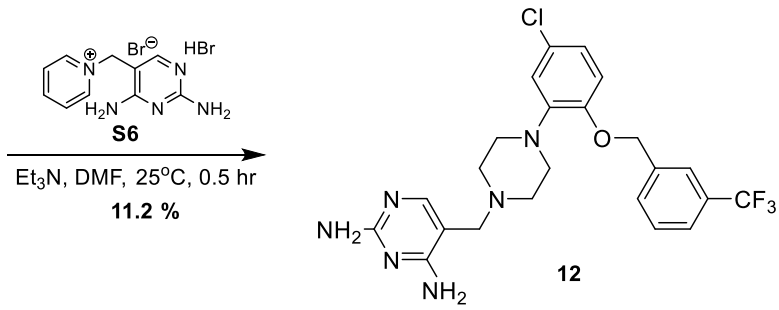


3'-(4-((2,4-diamino-6-methylpyrimidin-5-yl)methyl)piperazin-1-yl)-[1,1'-biphenyl]-3-carbonitrile (11). A mixture of **10** (100.00 mg, 0.24 mmol, 1.0 eq), BH_3-Me_2S (10 M, 72.56 uL, 3.0 eq) in THF (10.00 mL) was degassed and purged with nitrogen for 3 times at 0 °C, and then the mixture was stirred at 15 °C for 12 hr. Methanol (5 mL) was added to the mixture, and the solution was stirred at 70 °C for 2 hr. Removal of solvent under vacuum gave the crude product, which was purified by prep-HPLC to give **11** (9.7 mg, 0.024 mmol, 10.0% yield, >99% purity) as a white solid. ^{1}H NMR (DMSO, 400 MHz,) δ = 7.97 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.65 - 7.58 (m, 1H), 7.44 - 7.37 (m, 1H), 7.26 (s, 1H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 1H), 4.20 (br. s., 2H), 3.53 (br. s., 3H), 3.40 (br. s., 4H), 2.48 (s, 3H). **LCMS** (ESI+): m/z 400.1$(M+1)^+$ (expected 400.2), Rt: 2.30 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.4 min)


7.970
7.933
7.914
7.713
7.694
7.635
7.616
7.425
7.405
7.385
7.256
7.215
7.196
7.095
4.860
4.201
3.534
3.400
3.304
2.483
Supervisor:Zhenmin He
11
0.997
1.017
0.981
1.034
1.063
1.120
1.010
1.005
2.000
3.453
3.804
3.094
Chemical Shift (ppm)


AIBN, CCl4, 85°C, 12 hr
93.8 %
S22
K2CO3, DMF, 75°C, 4 hr
63.8 %
S23


Boc
dicyclohexyl-(2-phenylphenyl)phosphane,
NaOtBu, Pd2(dba)3, toluene, 100°C, 16 hr
17.2 %
S24
HCl/EtOAc
25°C,12 hr
S25


S6
Et3N, DMF, 25°C, 0.5 hr
11.2 %
12

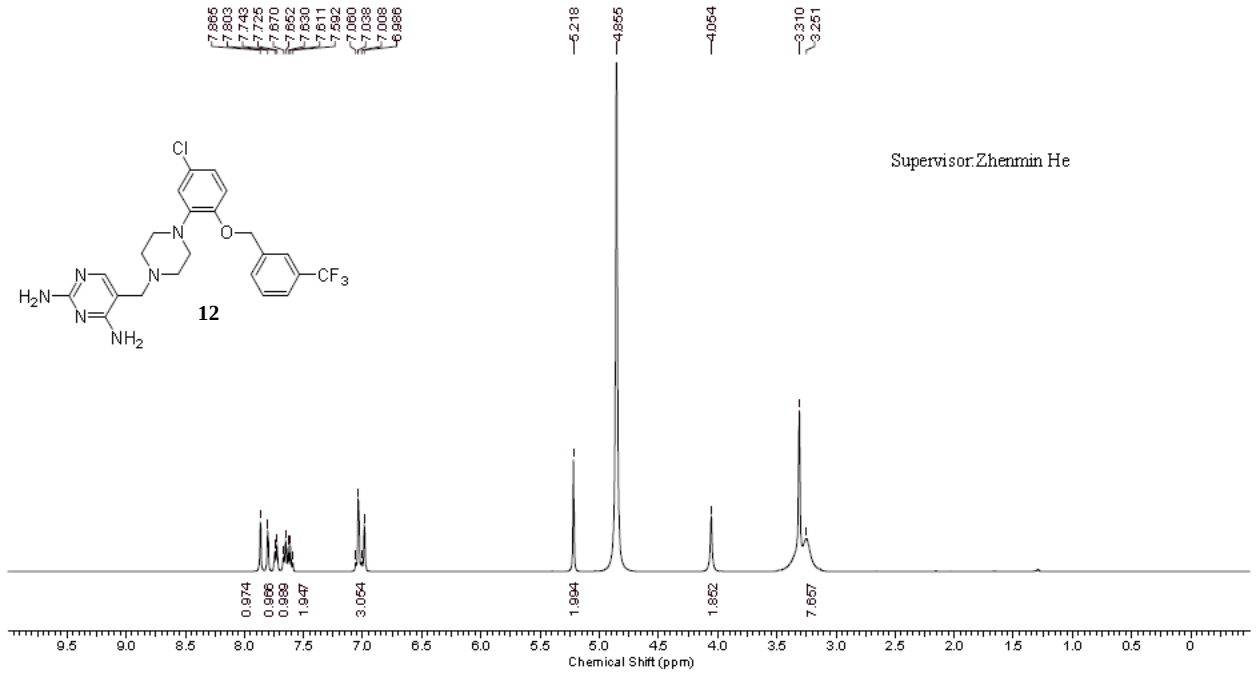
1-(bromomethyl)-3-(trifluoromethyl)benzene (S22). To a solution of 3-trifluoromethyltoluene (5.0 g, 31.2 mmol, 1.0 eq) in CCl_4 (20.0 mL) was added 1-bromopyrrolidine-2,5-dione (5.5 g, 31.2 mmol, 1.0 eq) and 2,2'-azo bisisobutyronitrile (5.1 g, 31.2 mmol, 1.0 eq). The mixture was stirred at 85 °C for 12 hr. The reaction mixture was poured into H_2O (100 mL) and the aqueous phase was extracted with DCM (100 mL * 2). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure to give **S22** (7.0 g, 29.2 mmol, 93.8 % yield).

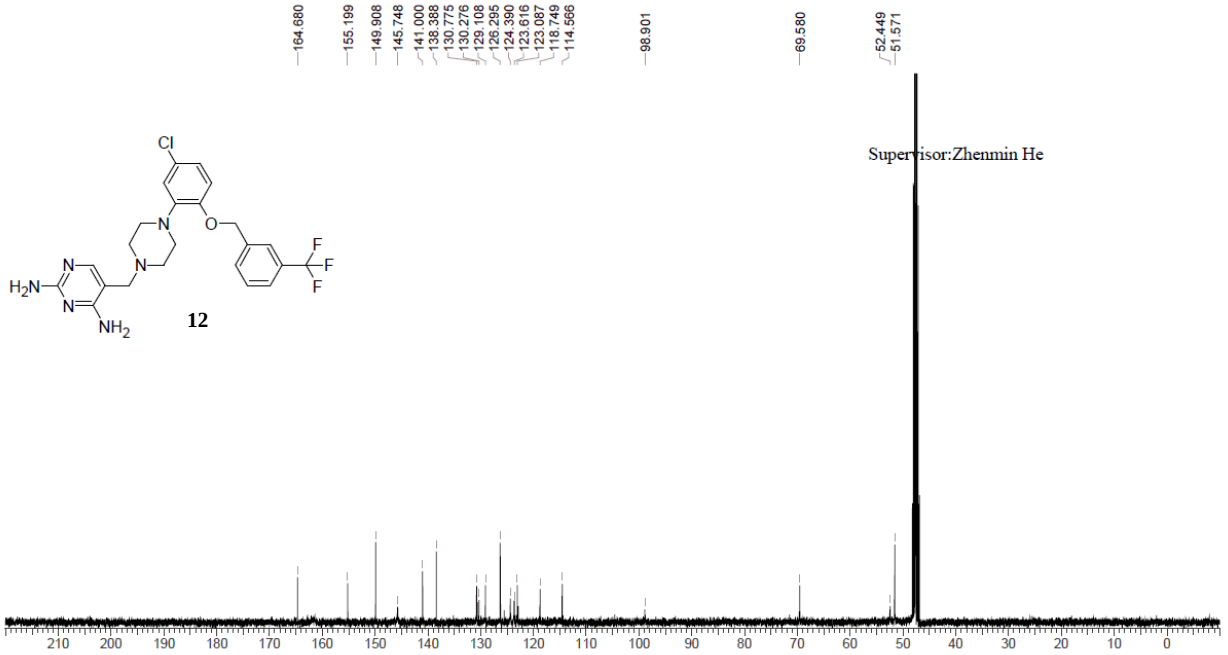
2-bromo-4-chloro-1-((3-(trifluoromethyl)benzyl)oxy)benzene (S23). To a solution of **S22** (4.0 g, 19.2 mmol, 1.0 eq) in DMF (40.0 mL) was added K_2CO_3 (5.3 g, 38.5 mmol, 2.0 eq) and 1-(bromomethyl)-3-(trifluoromethyl)benzene (5.0 g, 20.9 mmol, 3.1 mL, 1.1 eq). The mixture was stirred at 75 °C for 4 hr, then poured into H_2O (150 mL) and extracted with EtOAc (150 mL * 2). The combined organic layers were washed with brine, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by column (SiO_2, Et_2O 100%) to give **S23** (4.5 g, 12.3 mmol, 63.8 % yield).

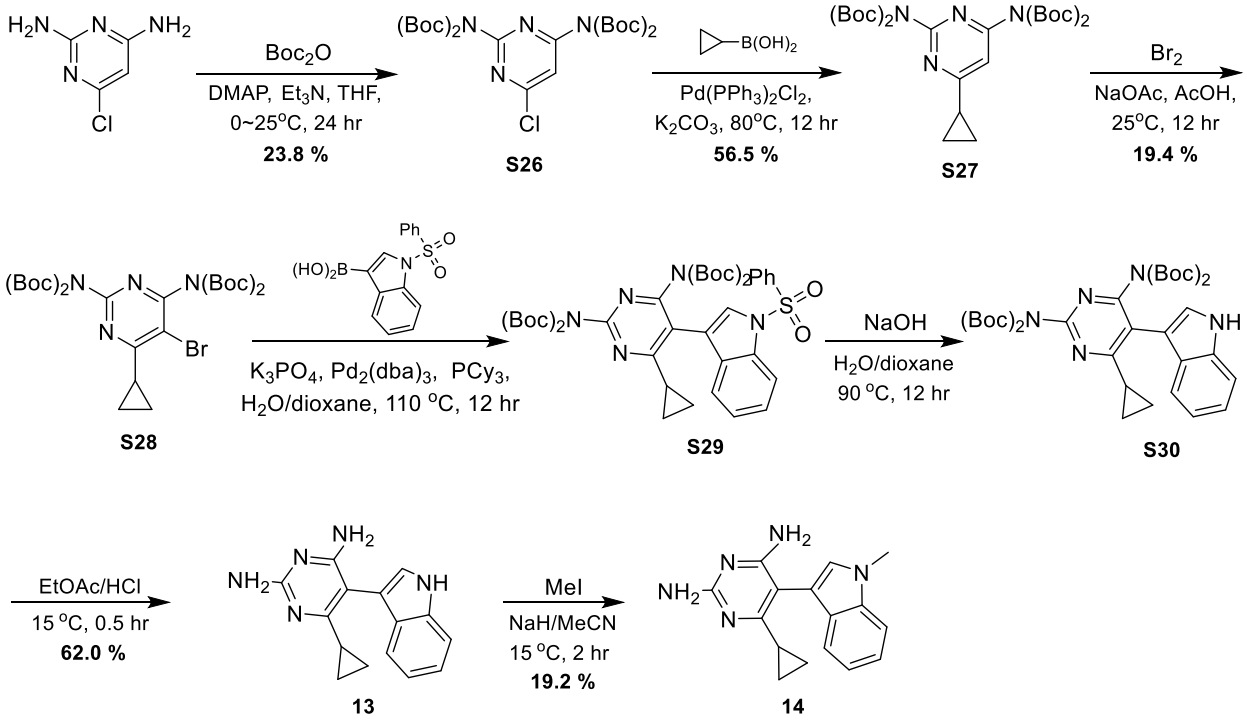
***tert*-butyl 4-(5-chloro-2-((3-(trifluoromethyl)benzyl)oxy)phenyl)piperazine-1-carboxylate (S24).** To a solution of **S23** (4.5 g, 12.3 mmol, 1.0 eq) in toluene (10.0 mL) was added NaO*t*Bu (1.4 g, 14.7 mmol, 1.1 eq), *tert*-butyl piperazine-1-carboxylate (2.2 g, 12.3 mmol, 1.0 eq), dicyclohexyl-(2-phenylphenyl)phosphane (690.2 mg, 1.9 mmol, 0.12 eq) and $Pd_2(dba)_3$ (225.4 mg, 0.25 mmol, 0.02 eq). The mixture was stirred at 100 °C for 16 hr. The reaction mixture was poured into H_2O (150 mL) and extracted with EtOAc (150 mL * 2). The combined organic layers were washed with brine, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by column (SiO_2, Et_2O/EtOAc from 1:0 to 20:1) to give **S24** (1.0 g, 2.1 mmol, 17.2 % yield).

1-(5-chloro-2-((3-(trifluoromethyl)benzyl)oxy)phenyl)piperazine (S25). S24 (1.0 g, 2.1 mmol) was added to a solution of HCl/EtOAc (4 M, 20.0 mL) and stirred at 25 °C for 12 hr. The mixture was evaporated under reduced pressure to give **S25** (0.60 g, 1.6 mmol, 77% yield). **LCMS** (ESI+): m/z 371.1 $(M+1)^+$, Rt: 0.71 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

5-((4-(5-chloro-2-((3-(trifluoromethyl)benzyl)oxy)phenyl)piperazin-1-yl)methyl)pyrimidine-2,4-diamine (12). To a solution of **S25** (0.20 g, 0.54 mmol, 1.0 eq) in DMF (2.0 mL) was added **S6** (0.200 g, 0.54 mmol, 1.0 eq) and TEA (0.27 g, 2.7 mmol, 0.38 mL, 5.0 eq). The mixture was stirred at 25 °C for 0.5 hr. The solvent was evaporated under reduced pressure and the residue was purified by prep-HPLC and lyophilized to give **12** (30.0 mg, 0.06 mmol, 11.2 % yield, >99% purity).**^{1}H NMR** (MeOD, 400MHz) δ = 7.86 (s, 1H) 7.80 (s, 1H) 7.73 (d, *J*=7.06 Hz, 1H) 7.58 - 7.69 (m, 2H) 6.96 - 7.08 (m, 3H) 5.22 (s, 2H) 4.05 (br. s., 2H) 3.12 - 3.32 (m, 8H); **^{13}C NMR** (MeOD,400MHz) δ = 164.68, 155.20, 149.91, 145.75, 141.00, 138.39, 130.78, 130.28, 129.11, 126.30, 124.39, 123.62, 123.09, 118.75, 114.57, 98.90, 69.58, 52.45, 51.57; **LCMS** (ESI+): m/z 493.1 $(M+1)^+$ (expected 493.2), Rt: 2.63 min (H_2O:ACN 99/1 @ 0.01 min to 0.100 @ 3.85 min); **HRMS** m/z: $(M+1)^+$ calcd for C23H24ClF3N6O, 493.17; found, 493.1756.







di-*tert*-butyl(6-chloropyrimidine-2,4-diyl)bis((*tert*-butoxycarbonyl)carbamate) (S26). A mixture of 6-chloro-2,4-diaminopyrimidine (5.0 g, 34.5 mmol, 3.3 eq) and dimethylaminopyridine (1.2 g, 10.3 mmol, 1.0 eq) was added to THF (50.0 mL) at 0 °C. Boc_2O (63.7 g, 276.7 mmol, xs)

and TEA (10.5 g, 103.7 mmol, 14.3 mL, 10.0 eq) were then added to the mixture. The solution was stirred at 25°C for 24 hr. The reaction mixture was poured into H^2O (120 mL) and extracted with EtOAc (150 mL*2). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by column chromatography (R_f = 0.70, Et_2O/EtOAc from 1:0 to 10:1) to give **S26** (4.5 g, 23.8 % yield) as a light yellow solid. **LCMS** (ESI+): m/z 545.2 $(M+1)^+$, Rt: 1.06 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

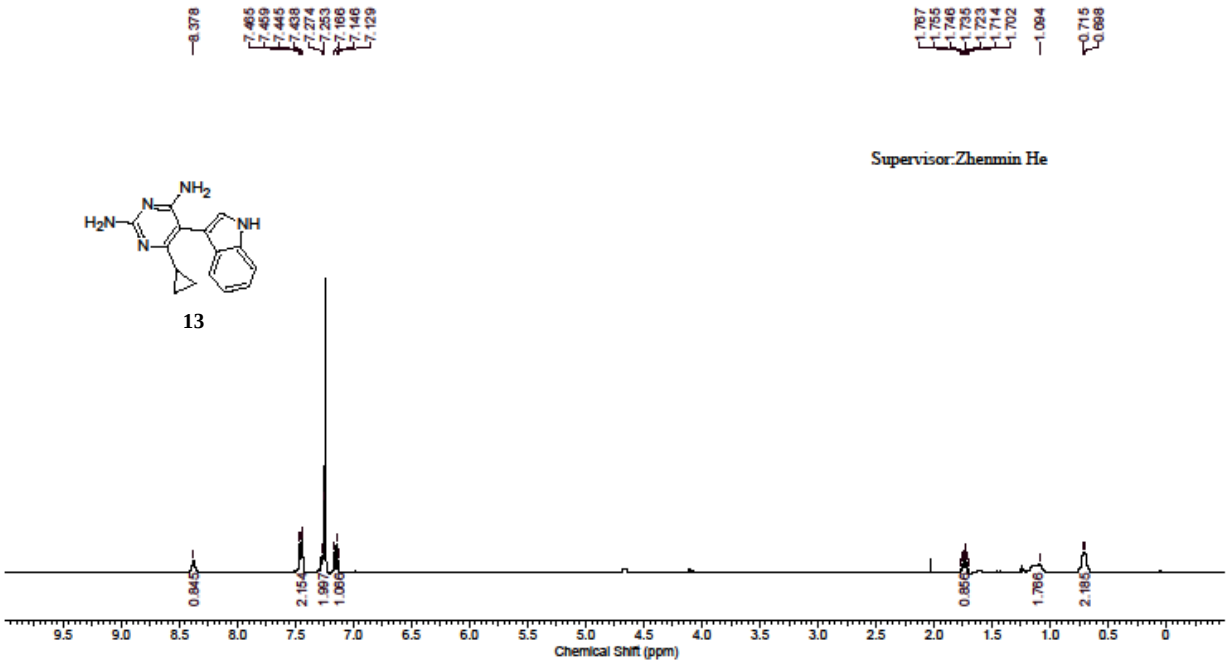
di-*tert*-butyl(6-cyclopropylpyrimidine-2,4-diyl)bis((*tert*-butoxycarbonyl)carbamate) (S27). To a solution of **S26** (3.5 g, 6.4 mmol, 1.0 eq) in H_2O (10.0 mL) and dioxane (50.0 ml) was added cyclopropylboronic acid (827.4 mg, 9.6 mmol, 1.5 eq), K_2CO_3 (1.7 g, 12.8 mmol, 2.0 eq) and $Pd(PPh_3)_2Cl_2$ (901.4 mg, 1.2 mmol, 0.2 eq). Then the mixture was stirred at 80 °C for 12 hours under N_2 atmosphere. The reaction solution was filtered and the filtrate was poured into H_2O (90 mL) and extracted with EtOAc (100 mL * 2). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by column chromatography (R_f = 0.6, Et_2O/EtOAc from 1:0 to 10: 1) to give **S27** (2.0 g, yield 56.5 %) as a yellow solid. **LCMS** (ESI+): m/z 551.2 $(M+1)^+$, Rt: 0.33 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

di-*tert*-butyl(5-bromo-6-cyclopropylpyrimidine-2,4-diyl)bis((*tert*-butoxycarbonyl)carbamate) (S28). To a solution of **S27** (1.8 g, 3.2 mmol, 1.0 eq) in AcOH (20.0 mL) was added NaOAc (938.5 mg, 11.4 mmol, 3.5 eq) and Br_2 (561.6 mg, 3.2 mmol, 1.0 eq). The mixture was stirred at 25 °C for 12 hr. The reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (100 mL * 2). The combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. The crude product was purified by prep-HPLC to give **S28** (0.4 g, 19.4 % yield) as a white solid. **LCMS** (ESI+): m/z 629.1 $(M+1)^+$, Rt: 2.45 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

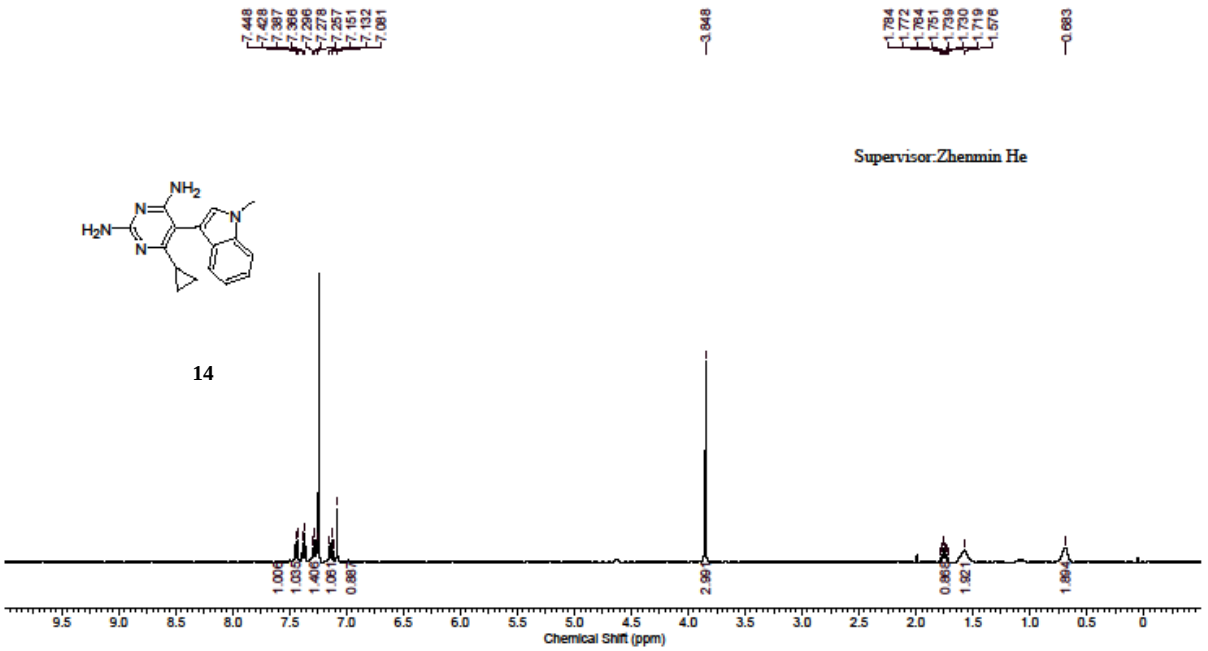
di-*tert*-butyl(6-cyclopropyl-5-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidine-2,4-diyl)bis((*tert*-butoxycarbonyl)carbamate) (S29). To a solution of **S28** (130.0 mg, 0.21 mmol, 1.0 eq) in dioxane (1.8 mL) and H_2O (0.6 mL) was added [1-(benzenesulfonyl)indol-3-yl]boronic acid (136.8 mg, 0.45 mmol, 2.0 eq), K_3PO_4 (149.0 mg, 0.70 mmol, 3.5 eq), PCy_3 (2.9 mg, 0.01 mmol, 3.3 uL, 0.05 eq) and $Pd_2(dba)_3$ (9.4 mg, 0.01 mmol, 0.05 eq). The mixture was stirred at 110 °C for 12 hr. The reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (25 mL * 2). The organic layers were combined, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. **S29** (0.4 g, crude) was obtained as a deep yellow oily liquid. **LCMS** (ESI+): m/z 506.2 $(M+1)^+$, Rt: 1.13 min. (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

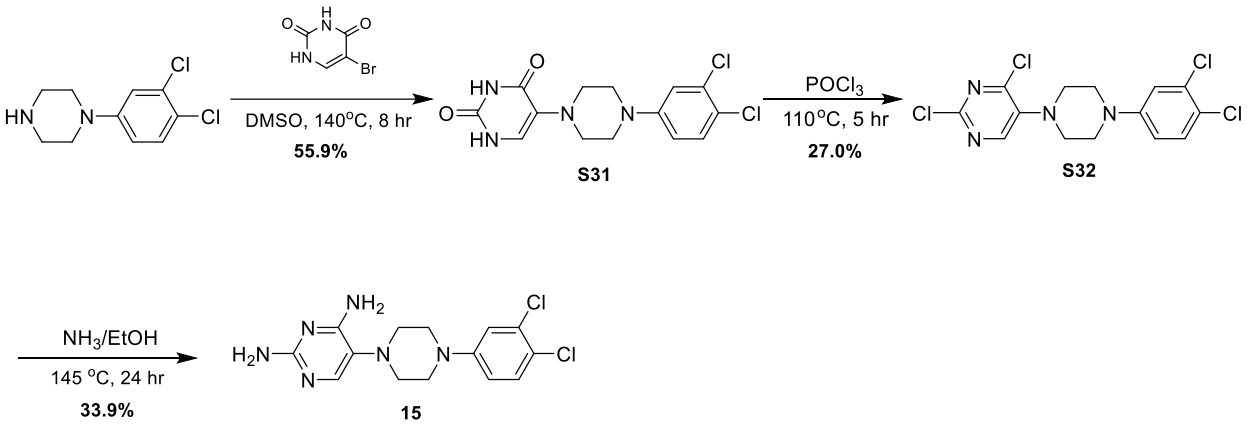
di-*tert*-butyl(6-cyclopropyl-5-(1H-indol-3-yl)pyrimidine-2,4-diyl)bis((*tert*-butoxycarbonyl)carbamate) (S30). S29 (400.0 mg, 0.50 mmol) was added into a solution of dioxane (10.0 mL) and NaOH (10.0 mL). The mixture was stirred at 90 °C for 12 hr. The reaction mixture was poured into H_2O (50 mL) and extracted with EtOAc (65 mL * 2). The organic layers were combined, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product. **S30** (800.0 mg, crude) was obtained as a deep yellow solid. **LCMS** (ESI+): m/z 266.1 $(M+H)^+$, Rt: 1.23 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

6-cyclopropyl-5-(1H-indol-3-yl)pyrimidine-2,4-diamine (13). S30 (800.0 mg, 1.20 mmol) was added into HCl/EtOAc (10.0 mL). The mixture was stirred at 15 °C for 0.5 hr. The reaction mixture was concentrated under reduced pressure to remove he solvent. The crude product was purified by prep-HPLC to give **13** (200.0 mg, 62.0 % yield, >98% purity) as a white solid. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ = 8.40 (br. s., 1H) 7.48 (dd, *J*=8.16, 2.87 Hz, 2H) 7.28 - 7.33 (m, 2H) 7.14 - 7.21 (m, 1H) 1.72 - 1.80 (m, 1H) 1.12 (br. s., 2H) 0.73 (d, *J*=7.06 Hz, 2H). **LCMS** (ESI+): m/z 266.1 $(M+1)^+$ (expected 266.1), Rt: 2.18 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.40 min)



6-cyclopropyl-5-(1-methyl-1H-indol-3-yl)pyrimidine-2,4-diamine (14). To a solution of **13** (150.0 mg, 0.57 mmol) in ACN (20.0 mL) was added NaH (45.2 mg, 1.1 mmol, 60 % purity) at 15°C and stirred for 0.5 hr, CH_3I (0.1 M, 5.6 mL) was added. The mixture was stirred at 15 °C for 1.5 hr. The reaction mixture was concentrated under reduced pressure to remove solvent. The crude product was purified by prep-HPLC to give **14** (31.0 mg, 19.2 % yield, >98% purity) as a white solid. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ = 7.46 (d, *J*=7.94 Hz, 1H) 7.40 (d, *J*=8.38 Hz, 1H) 7.28 - 7.34 (m, 1H) 7.13 - 7.19 (m, 1H) 7.11 (s, 1H) 3.87 (s, 3H) 1.72 - 1.84 (m, 1H) 1.60 (br. s., 2H) 0.71 (br. s., 2H). **LCMS** (ESI+): m/z 280.1 $(M+1)^+$ (expected 280.2), Rt: 2.33 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.40 min)

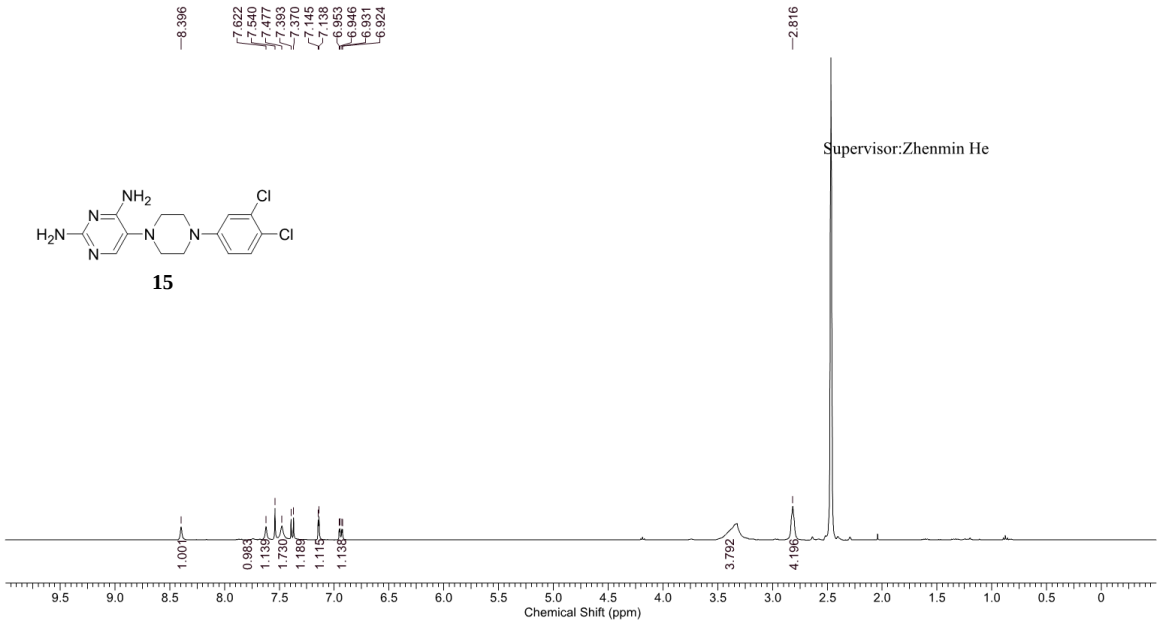




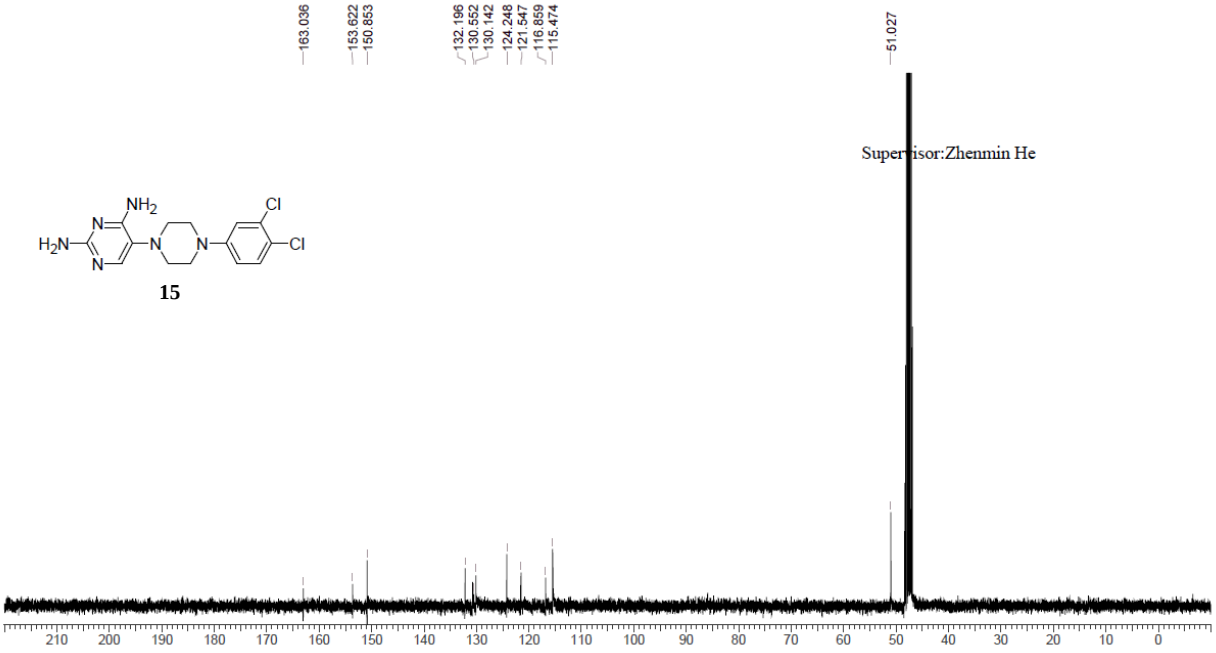
5-(4-(3,4-dichlorophenyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (S31). To a mixture of 1-(3,4-dichlorophenyl)piperazine (250.0 mg, 1.3 mmol, 1.0 eq) and 5-bromouracil (302.5 mg, 1.3 mmol, 1.0 eq) in DMSO (5.0 mL) was added KF (91.2 mg, 1.5 mmol, 36.8uL, 1.2 eq). The resulting mixture was stirred and heated to 140 ˚C for 8 hr. The reaction was allowed to cool to room temperature and poured into H_2O, filtered and collected the gray solid. The gray solid was washed with the 100 mL Et_2O/EtOAc (1:1) to give **S31** (1.0 g, 2.9 mmol, 55.9% yield) as a gray solid. **LCMS** (ESI+): m/z 340.9 $(M+1)^+$, RT: 1.15 min (H_2O:ACN 90/10 @ 0.01 min to 10/90 @ 1.65 min)

2,4-dichloro-5-(4-(3,4-dichlorophenyl)piperazin-1-yl)pyrimidine (S32). A mixture of **S31** (1.0 g, 2.9 mmol, 1.0 eq) and $POCl_3$ (8.9 g, 58.6 mmol, 5.4 mL, 20.0 eq) was degassed and purged with N_2 three times, followed by stirring at 110 °C for 5 hr under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to give a black residue. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (50 mL*2). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column (SiO_2, Et_2O/EtOAc, 1:5) to give **S32** (300.0 mg, 0.79 mmol, 27.0% yield) as a yellow solid. **LCMS** (ESI+): m/z 377.0, 379.0, 381.0 $(M+1, M+3, M+4)^+$, RT: 0.98 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

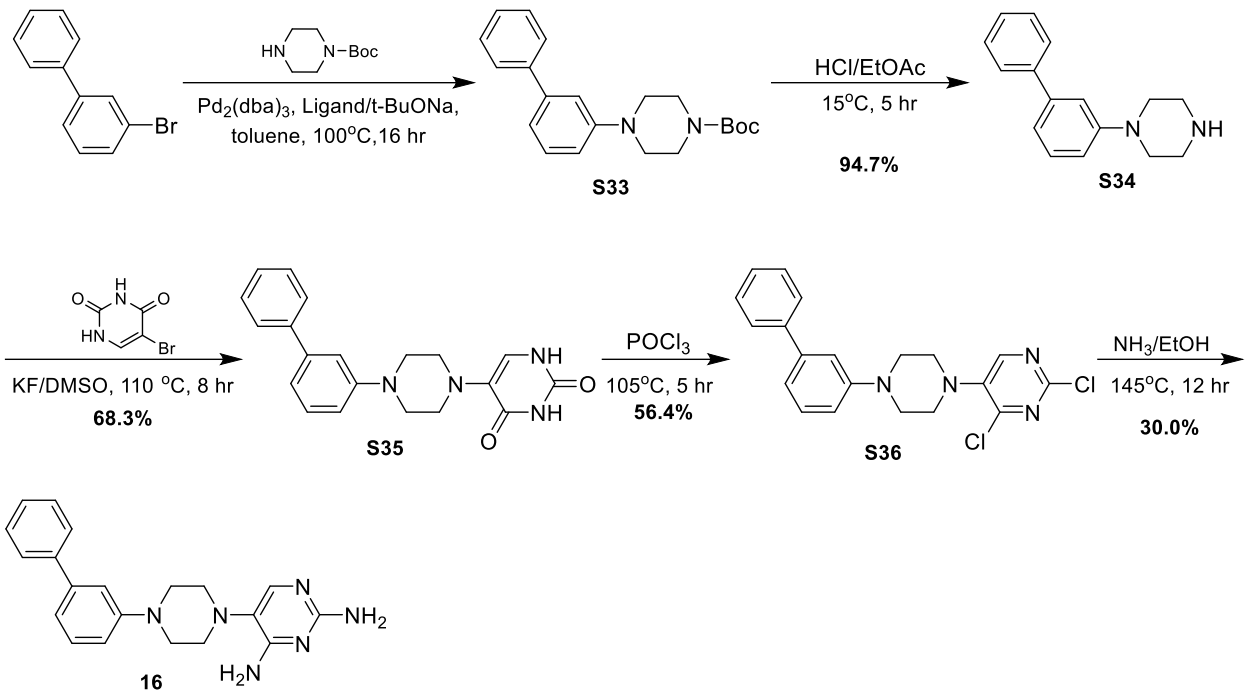
5-(4-(3,4-dichlorophenyl)piperazin-1-yl)pyrimidine-2,4-diamine (15). A mixture of **S32** (200.0 mg, 0.53 mmol, 1.0 eq), and NH_3/EtOH (15.0 mL, 0.53 mmol, 1.0 eq) was added to a steel bomb. The mixture was stirred at 145 °C for 24 hr. The suspension was cooled to room temperature and concentrated under reduced pressure to give a brown residue. The residue was purified by prep-HPLC to give **15** (61.0 mg, 0.18 mmol, 33.9% yield, >96% purity) as a faint yellow solid. **^{1}H NMR** (DMSO, 400MHz) δ = 8.40 (br.s., 1H), 7.62 (br.s., 1H), 7.54 (s, 1H), 7.48 (br.s., 2H), 7.38 (d, *J* = 9.3 Hz, 1H), 7.14 (d, *J* = 2.6 Hz, 1H), 6.94 (dd, *J* = 2.6, 8.8 Hz, 1H), 3.45 - 3.34 (m, 4H), 2.82 (br.s., 4H); **^{13}C NMR** (MeOD, 400 MHz) δ = 163.04, 153.62, 150.85, 132.20, 130.55, 130.14, 124.25, 121.55, 116.86, 115.47, 51.03; **LCMS** (ESI+): m/z 339.0 $(M+1)^+$ (expected 339.1), RT: 1.25 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.40 min) **HRMS** m/z: $(M+1)^+$ calcd for C14H16Cl2N6, 339.08; found 339.0910


8.396
7.622
7.540
7.477
7.393
7.370
7.145
7.138
6.953
6.946
6.931
6.924
2.816
Supervisor:Zhenmin He
NH2
N
H2N
N
N
N
Cl
Cl
1.001
0.983
1.139
1.730
1.189
1.115
1.138
3.792
4.196
Chemical Shift (ppm)

15


163.036
153.622
150.853
132.196
130.552
130.142
124.248
121.547
116.859
115.474
51.027
Supervisor:Zhenmin He
NH2
N
H2N
N
N
N
Cl
Cl

15



***tert*-butyl 4-([1,1'-biphenyl]-3-yl)piperazine-1-carboxylate (S33).** 3-phenyl-bromobenzene (10.0 g, 42.9 mmol, 7.1 mL, 1.0 eq) was added to a solution of sodium 2-methylpropan-2-olate (4.9 g, 51.4 mmol, 1.2 eq) and $Pd_2(dba)_3$ (785.6 mg, 0.86 mmol, 0.02 eq) in toluene (100.0 mL). Then [1,1'-biphenyl]-2-yldicyclohexylphosphine (2.4 g, 6.8 mmol, 0.16 eq) and *tert*-butyl piperazine-1-carboxylate (7.9 g, 42.9 mmol, 1.0 eq) was added to the above mixture at 25 °C. After degassing with N_2 for three times, the resulting solution was stirred for 16 hr at 100 °C under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to give a brown residue which was purified by column (SiO_2, Et_2O/EtOAc from 10:1 to 5:1) to give **S33** (7.0 g, 48.3% yield) as a white solid. **LCMS** (ESI+): m/z 239.1 $(M+1)^+$, Rt: 2.19 min (H_2O:ACN 90/10 @ 0.01 min to 10/90 @ 2.00 min)

1-([1,1'-biphenyl]-3-yl)piperazine (S34). S33 (3.0 g, 8.8 mmol, 1.0 eq) was added to a solution of HCl in MeOH (4 M, 30.0 mL, 13.5 eq) and stirred at 15 °C for 5 hr. The solution was filtered and the remaining solid was dissolved in a solution of K_2CO_3 (2M, 50 mL), stirred vigorously for several minutes and extracted with EtOAc (150 mL*2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give **S34** (2.0 g, 8.3

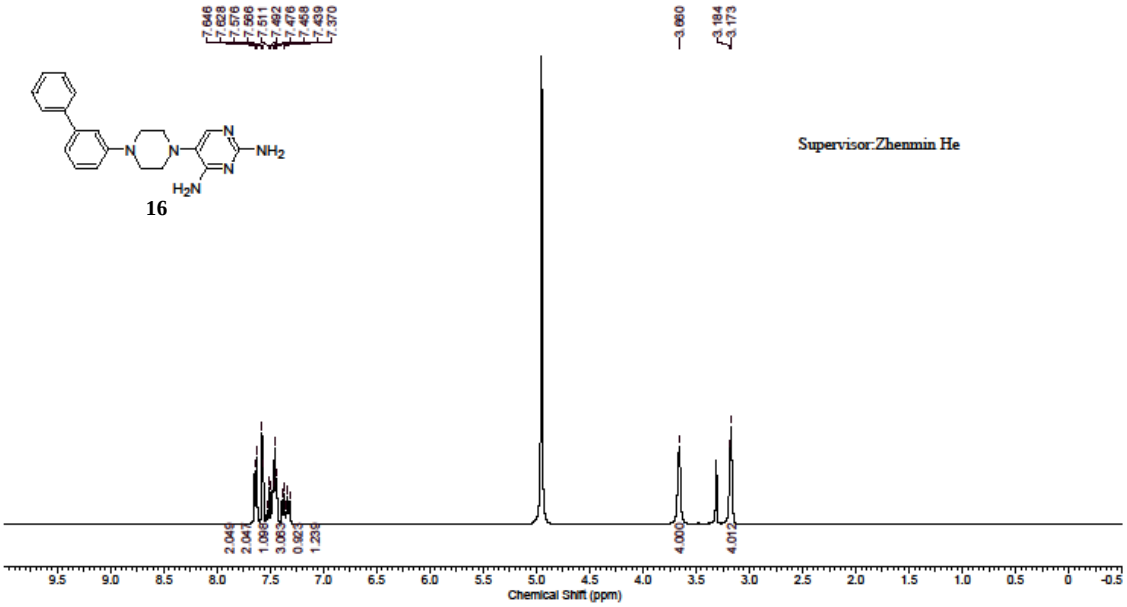
mmol, 94.7% yield) as a yellow oil. LCMS (ESI+): m/z 239.1 $(M+1)^+$, Rt: 2.19 min (H_2O:ACN 90/10 @ 0.01 min to 20/80 @ 2.48 min)

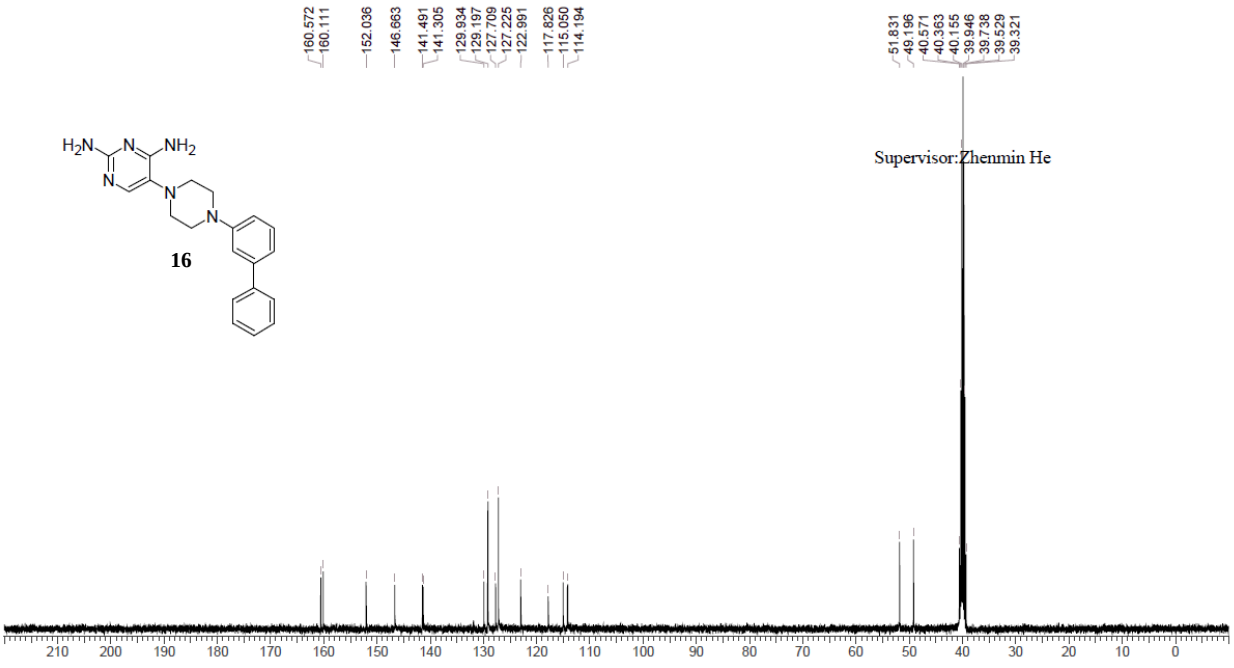
5-(4-([1,1'-biphenyl]-3-yl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (S35). To a mixture of 5-bromouracil (400.6 mg, 2.1 mmol, 1.0 eq) and **S34** (500.0 mg, 2.1 mmol, 1.0 eq) in DMSO (10.00 mL) was added potassium fluoride (182.8 mg, 3.15 mmol, 1.5 eq). The resulting mixture was stirred and heated to 110 °C for 8 hours. The suspension was cooled to room temperature and poured the mixture into water, filtered and collected the gray solid by suction. The gray solid was washed with the mixture solvents (100 mL, Ethyl acetate: Petroleum ether = 1:1) to give **S35** (500.0 mg, 1.4 mmol, 68.3% yield) as a gray solid. **LCMS** (ESI+): m/z 349.1 $(M+1)^+$, Rt: 2.16 min (H_2O:ACN 90/10 @ 0.01 min to 20/80 @ 2.48 min)

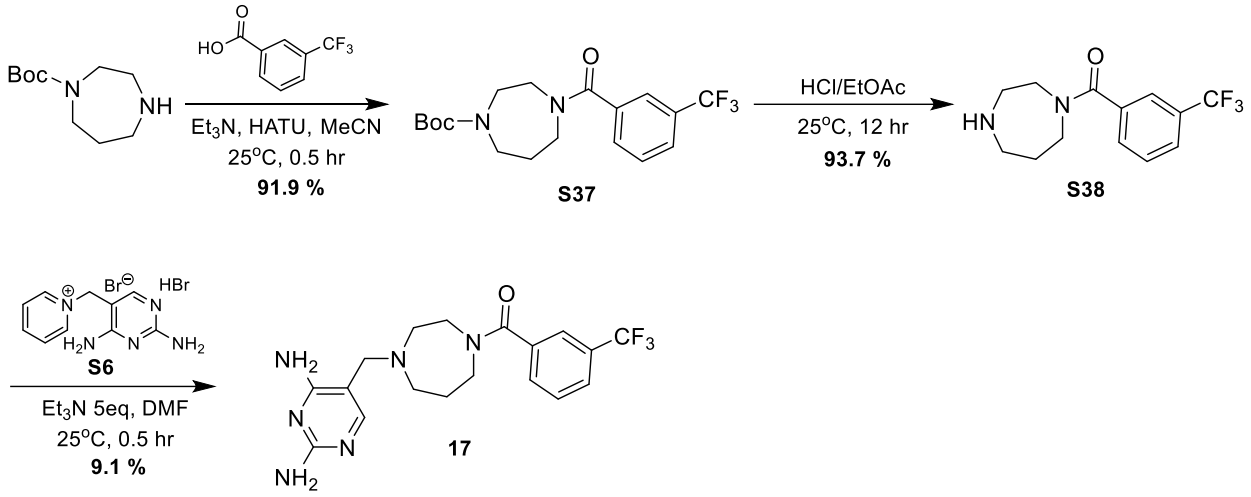
5-(4-([1,1'-biphenyl]-3-yl)piperazin-1-yl)-2,4-dichloropyrimidine (S36). A mixture of **S35** (400.0 mg, 1.1 mmol, 1.0 eq) in $POCl_3$ (26.3 g, 171.6 mmol, 15.9 mL, 149.4 eq) was degassed and purged with N_2 three times, and then the mixture was stirred at 105 °C for 5 hr under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to give a black residue. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (50 mL * 2). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **S36** (250.0 mg, 0.65 mmol, 56.4% yield) as a yellow solid. **LCMS** (ESI+): m/z 385.1 $(M+1)^+$, Rt: 2.24 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

5-(4-([1,1'-biphenyl]-3-yl)piperazin-1-yl)pyrimidine-2,4-diamine (16). A mixture solution of **S36** (100.0 mg, 0.26 mmol, 1.0 *eq*) in NH_3/EtOH (10 mL) was added to a steel bomb. The mixture was stirred at 145 °C for 12 hr. The suspension was cooled to room temperature and concentrated under reduced pressure to give a brown residue. The residue was purified by Prep-HPLC (TFA condition) to give **16** (86.5 mg, 249.6 umol, 30.0% yield, >99% purity) as white solid. **^{1}H NMR** (MeOD, 400 MHz) δ = 7.64 (d, J = 7.4 Hz, 2H), 7.59-7.55 (m, 2H), 7.54-7.49 (m, 1H), 7.46 (t, J = 7.4 Hz, 3H), 7.38 (d, J = 7.4 Hz, 1H), 7.36-7.30 (m, 1H), 3.66 (br.s., 4H), 3.18 (d, J = 4.3 Hz, 4H). **^{13}C NMR** (MeOD, 400 MHz) δ = 160.57, 160.11, 152.04, 146.66, 141.49, 141.31, 129.93, 129.20, 127.71, 127.23, 122.99, 117.83, 115.05, 114.19, 51.83, 49.20; **LCMS** (ESI+): m/z

347.1 $(M+1)^+$ (expected 347.2), Rt: 2.60 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 3.0 min) **HRMS** m/z: $(M+1)^+$ calcd for C20H22N6, 347.19 ; found, 347.2006; **Elemental**: $C_{20}H_{22}N_6 \cdot$ 0.1TFA, C: 67.92%, H: 6.26%, N: 23.33% (expected C: 67.80%, H: 6.23%, N: 23.49%)





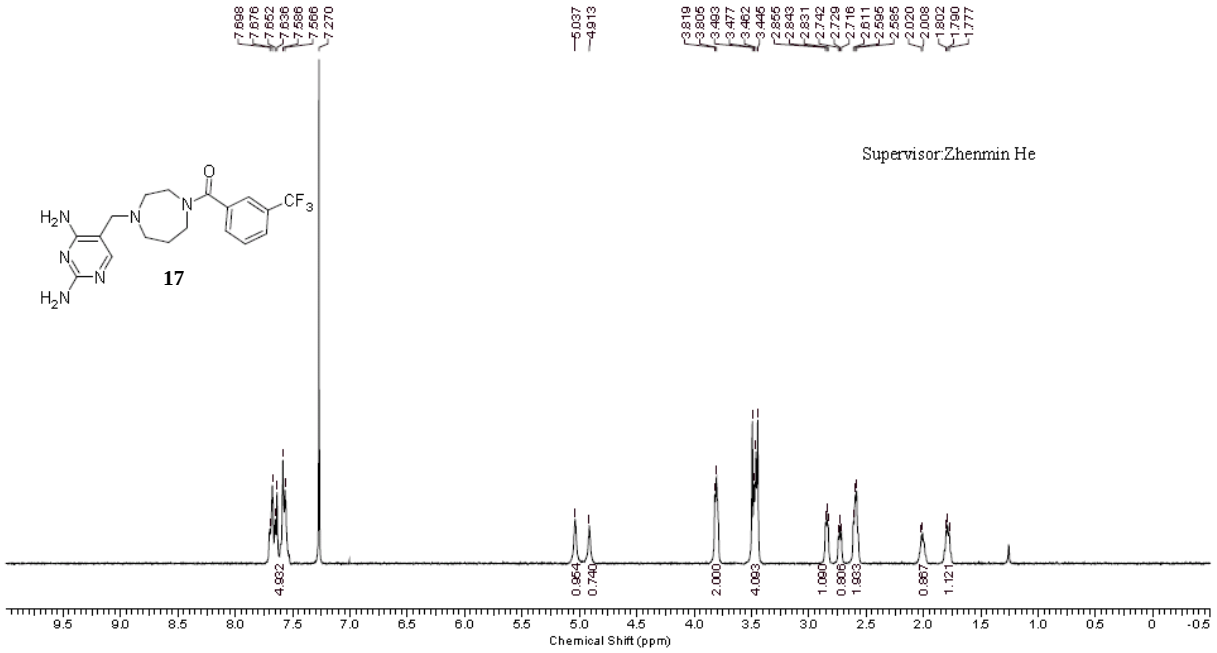


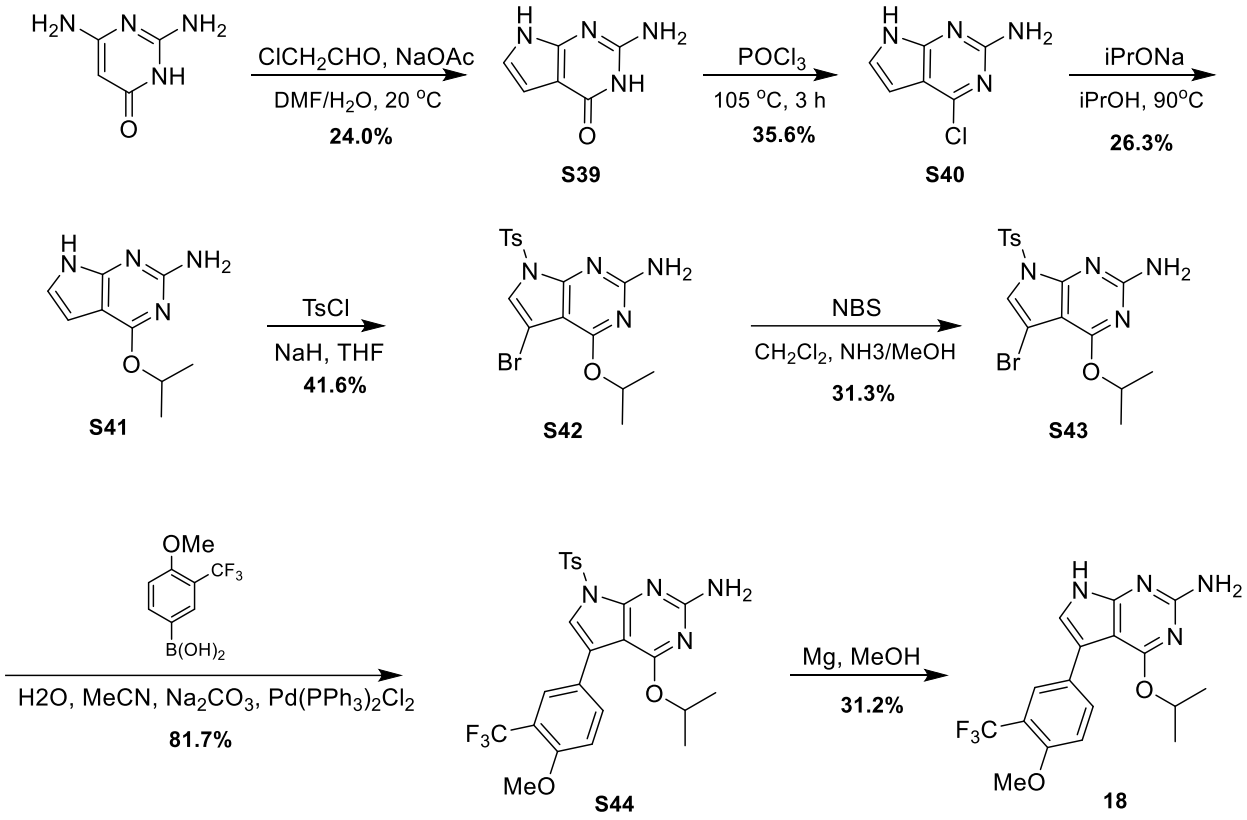
***tert*-butyl 4-(3-(trifluoromethyl)benzoyl)-1,4-diazepane-1-carboxylate (S37).** To a mixture of 1-Boc-homopiperazine (1.0 g, 5.2 mmol, 1.0 eq) and 3-trifluoromethylbenzoic acid (1.1 g, 5.7 mmol, 1.2 eq) in ACN (20.0 mL) was added TEA (1.0 g, 10.5 mmol, 1.4 mL, 2.0 eq) and O-(7-azabenzotramethyluronium hexafluorophosphate (3.0 g, 7.8 mmol, 1.5 eq). The mixture was stirred at 25 °C for 0.5 hr. The reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (100 mL * 3). The combined organic layers were washed with brine, dried with Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by column (SiO_2, Et_2O/EtOAc from 2:1 to 5:1) to give **S37** (1.78g g, 4.8 mmol, 91.9 % yield). **LCMS** (ESI+): m/z 317.1 $(M+1)^+$, Rt: 0.80 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

(1,4-diazepan-1-yl)(3-(trifluoromethyl)phenyl)methanone (S38). S37 (5.4 g, 14.5 mmol, 1.0 eq) was stirred in a solution of HCl/EtOAc (14.5 mmol HCl, 1.0 eq, 100.0 mL total) at 25 °C for 12 hr. The mixture was evaporated under reduced pressure to give **S38** (3.7 g, 13.6 mmol, 93.7 % yield). **LCMS** (ESI+): m/z 273.1 $(M+1)^+$, Rt: 0.24 min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

(4-((2,4-diaminopyrimidin-5-yl)methyl)-1,4-diazepan-1-yl)(3-(trifluoromethyl)phenyl)methanone (17). To a solution of **S38** (300.0 mg, 1.1 mmol, 1.0 eq) in DMF (5.0 mL) was added **S6** (399.3 mg, 1.1 mmol, 1.0 eq) and TEA (556.5 mg, 5.5 mmol, 762.3 uL, 5.0 eq). The mixture was stirred at 25 °C for 0.5 hr. Solvent was removed under reduced

pressure and the crude product was purified by prep-HPLC to afford **17** (40.0 mg, 0.1 mmol 9.1% yield, >99% purity). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.51 - 7.73 (m, 5H) 5.04 (br. s., 1H) 4.91 (br. s., 1H) 3.81 (d, *J*=5.73 Hz, 2H) 3.39 - 3.56 (m, 4H) 2.81 - 2.88 (m, 1H) 2.70 - 2.76 (m, 1H) 2.55 - 2.63 (m, 2H) 2.01 (d, *J*=4.85 Hz, 1H) 1.74 - 1.83 (m, 1H) **LCMS** (ESI+): m/z 395.0 $(M+1)^+$ (expected 395.2), Rt: 1.99 min (H_2O:ACN 99/1 @ 0.01 min to 0/100 @ 3.85 min)





2-amino-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (S39). To a mixture of 2,4-diamino-6-hydroxypyrimidine (20.0 g, 158.5 mmol, 1.0 eq) and 2-chloroacetaldehyde (31.1 g, 158.5 mmol, 25.5 mL, 1.0 eq) in DMF (240.0 mL) was added NaOAc (13.0 g, 158.5 mmol, 1.0 eq) at 20°C. The mixture was stirred at 20 °C for 12 hr. The reaction mixture was concentrated under reduced pressure to give a residue. Toluene (20 mL *3) was added to the mixture and concentrated under reduced pressure to give **S39** (6.5 g, 38.1 mmol, 24.0% yield) was obtained as a white solid. **LCMS** (ESI+): m/z 151.1 $(M+1)^+$, Rt: 0.07min (H_2O:ACN 95/5 @ 0.01 min to 5/95 @ 1.15 min)

4-chloro-7H-pyrrolo[2,3-d]pyrimidin-2-amine (S40). To **S39** (5.0 g, 33.3 mmol, 1.0 eq) was added $POCl_3$ (50.0 mL) at 20 °C under N_2. The mixture was stirred at 105 °C for 3 hr. The $POCl_3$ was evaporated and the residue was quenched by slowly pouring on ice with stirring followed by basification with 50% aqueous NaOH to reach pH of 6-7. The solid that had formed was collected by vacuum filtration and the filtrate was extracted further with EtOAc/MeOH (70/30 * 2) to give a crude residue. The residue was purified by column chromatography (SiO_2, EtOAc 100%). **S40**

(2.0 g, 11.8 mmol, 35.6% yield) was obtained as a yellow solid. **LCMS** (ESI+): m/z 169.1 $(M+1)^+$, Rt: 0.96 min (H_2O:ACN 90/10 @ 0.01 min to 20/80 @ 1.55 min)

4-isopropoxy-7H-pyrrolo[2,3-d]pyrimidin-2-amine (S41). S40 (1.0 g, 5.9 mmol, 1.0 eq) was added to isopropoxysodium (2.4 g, 29.6 mmol, 5.0 eq) at 15 °C under N_2. The mixture was stirred at 90 °C for 24 hr. The reaction mixture was concentrated under reduced pressure to remove propan-2-ol (10.0 mL). The residue was diluted with MeOH (10 mL) and filtered. The combined organic layers were concentrated under reduced pressure to give a **S41** (300.0 mg, 1.5 mmol, 26.3% yield) as a brown oil. **LCMS** (ESI+): m/z 193.0 $(M+1)^+$, Rt: 0.83 min (H_2O:ACN 90/10 @ 0.01 min to 10/90 @ 1.65 min)

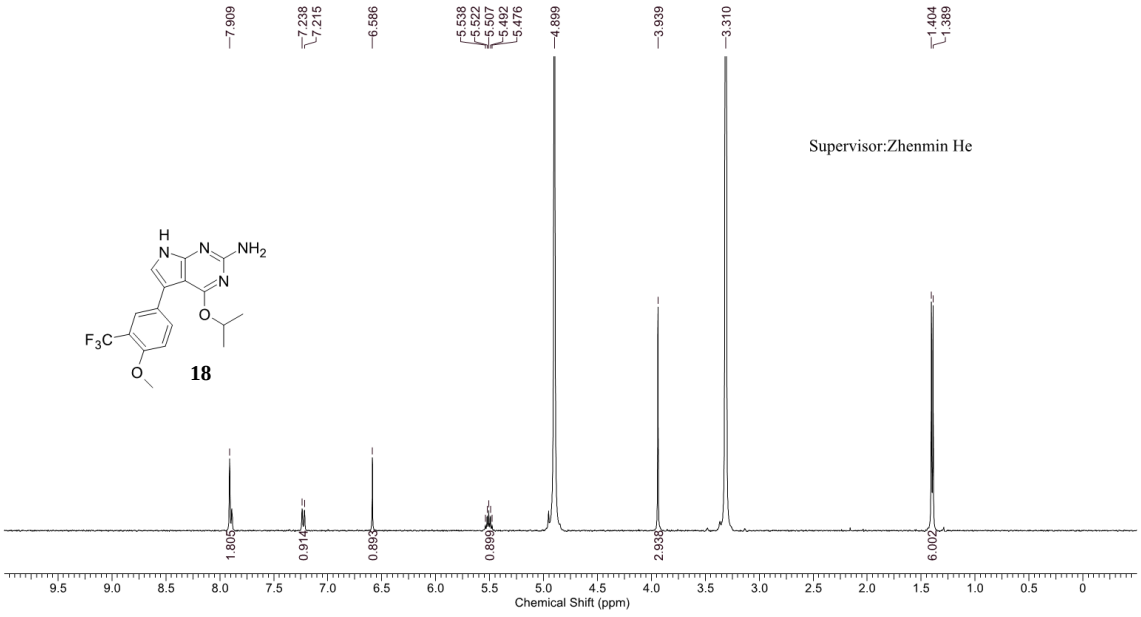
5-bromo-4-isopropoxy-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (S42). To a mixture of **S41** (200.0 mg, 1.0 mmol, 1.0 eq) and NaH (57.4 mg, 2.3 mmol, 2.3 eq) in tetrahydrofuran (3.0 mL) was added 4-methylbenzene-1-sulfonyl chloride (416.5 mg, 2.1 mmol, 2.1 eq) in one portion at 15 °C under N_2. The mixture was stirred at 15 °C for 12 hr. LCMS showed the desired MS. The residue was purified by prep-HPLC to give **S42** (150.0 mg, 0.43 mmol, 41.6% yield) as a white solid. **LCMS** (ESI+): m/z 347.1 $(M+1)^+$, Rt: 2.85 min (H_2O:ACN 90/10 @ 0.01 min to 20/80 @ 3.50 min)

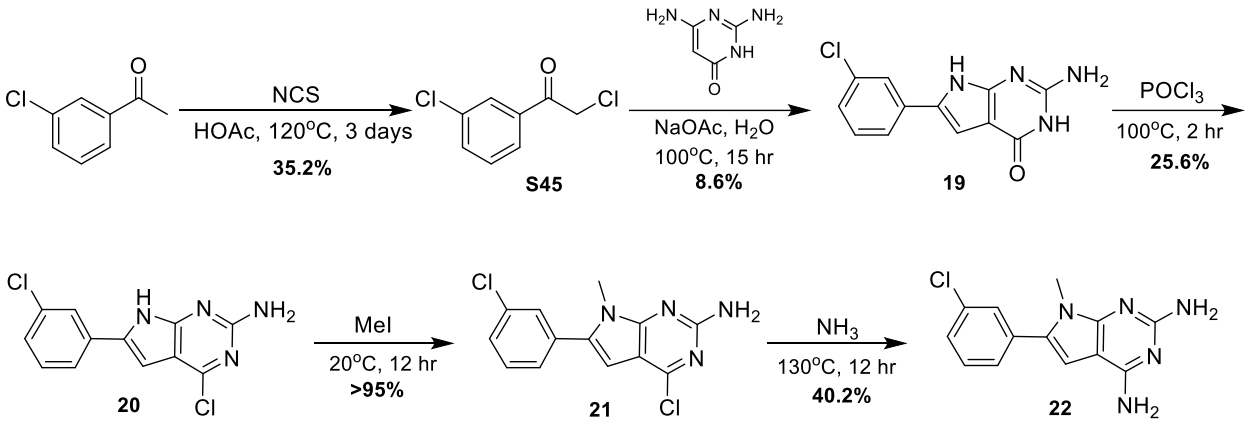
5-bromo-4-isopropoxy-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (S43). To a mixture of **S42** (65.0 mg, 0.19 mmol, 1.0 eq) in CH_2Cl_2 (4.0 mL) was added N-bromosuccinimide (40.0 mg, 0.23 mmol, 1.2 eq). The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, 10:1 DCM/MeOH). **S43** (50.0 mg, 117.5 umol, 31.3% yield) was obtained as a white solid. **LCMS** (ESI+): m/z 425.0/427.0 $(M+1/M+3)^+$, Rt: 2.10 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

4-isopropoxy-5-(4-methoxy-3-(trifluoromethyl)phenyl)-7-tosyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (S44). S43 (10.0 mg, 23.5 umol, 1.0 eq), 3-trifluoromethyl-4-methoxyboronic acid (7.2 mg, 32.9 umol, 1.4 eq), Na_2CO_3 (7.4 mg, 70.5 umol, 3.0 eq) and bis(triphenylphosphine)palladium(II) dichloride (1.6 mg, 2.3 umol, 0.1 eq) were taken up into a

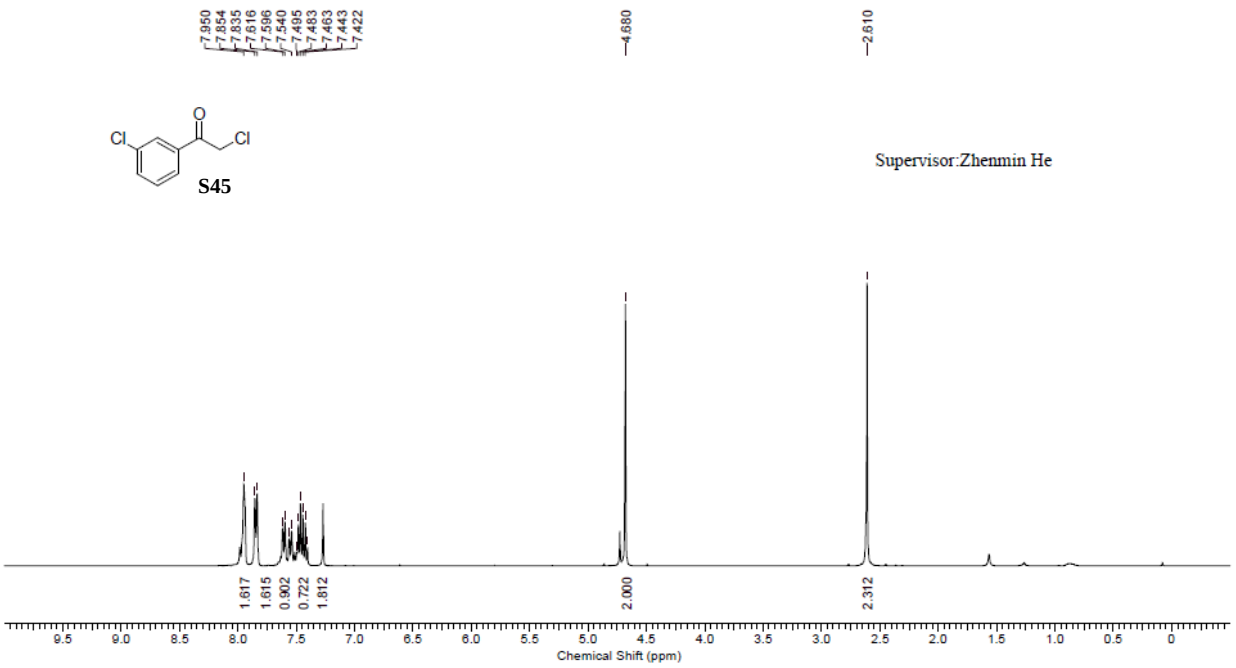
microwave tube in ACN (1.0 mL) and H_2O (1.0 mL) at 15 °C. The sealed tube was heated at 110 °C for 2 hr under microwave radiation. The reaction mixture was allowed to cool and concentrated under reduced pressure to give **S44** (10.0 mg, 19.2 umol, 81.7% yield) as a white solid. **LCMS** (ESI+): m/z 521.1 $(M+1)^+$, Rt: 2.25 min (H_2O:ACN 70/30 @ 0.01 min to 10/90 @ 2.50 min)

4-isopropoxy-5-(4-methoxy-3-(trifluoromethyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (18). To a mixture of **S44** (10.0 mg, 19.2 umol, 1.0 eq) in methyl alcohol (1.0 mL) was added Mg (4.6 mg, 192.1 umol, 10.0 eq) in one portion at 15 °C under N_2. The mixture was stirred at 70°C for 1 hour. LCMS showed the desired MS. The residue was purified by prep-HPLC (neutral condition). **18** (2.2 mg, 6.0 umol, 31.2% yield, >94% purity) was obtained as a white solid. **^{1}H NMR** (MeOD, 400 MHz) δ = 7.91 (s, 2H) 7.23 (d, J = 9.26 Hz, 1H) 6.59 (s, 1 H) 5.51 (dt, J = 12.35, 6.17 Hz, 1H) 3.94 (s, 3H) 1.40 (d, J = 6.17 Hz, 6H) **LCMS** (ESI+): m/z 367.1 $(M+1)^+$ (expected 367.1), Rt: 3.11 min (H_2O:ACN 85/15 @ 0.01 min to 0/100 @ 3.85 min)

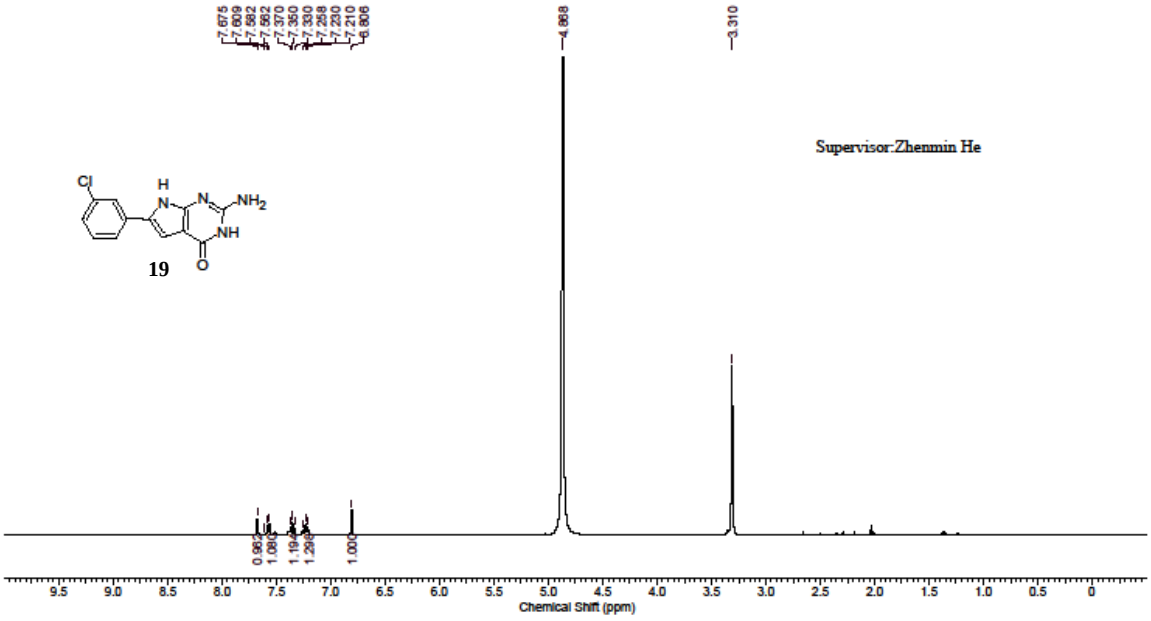




2-chloro-1-(3-chlorophenyl)ethan-1-one (S45). To a mixture of 3-chloroacetophenone (20.0 g, 129.4 mmol, 16.7 mL, 1.0 eq) in HOAc (40.0 mL) was added NCS (20.0 g, 150.1 mmol, 1.2 eq) and benzoyl benzenecarboperoxoate (199.9 mg, 0.83 mmol, 0.006 eq). The mixture was stirred for 3 days at 120 °C. The reaction mixture was then poured into 100 mL of the aqueous $NaSO_3$ and extracted with EtOAc (50 mL*3), the organic phase was dried over Na_2SO_4 then concentrated to give a residue. The residue was purified by column chromatography (SiO_2, Et_2O/EtOAc from 50:1 to 30:1. **S45** (9.2 g, 26.8 mmol, 20.7% yield, 55% purity) was obtained as red liquid. **^{1}H NMR** ($CDCl_3$, 400MHz) δ = 7.95 (br. s., 2H), 7.84 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.9 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.50 - 7.38 (m, 2H), 4.68 (s, 2H), 2.61 (s, 2H).

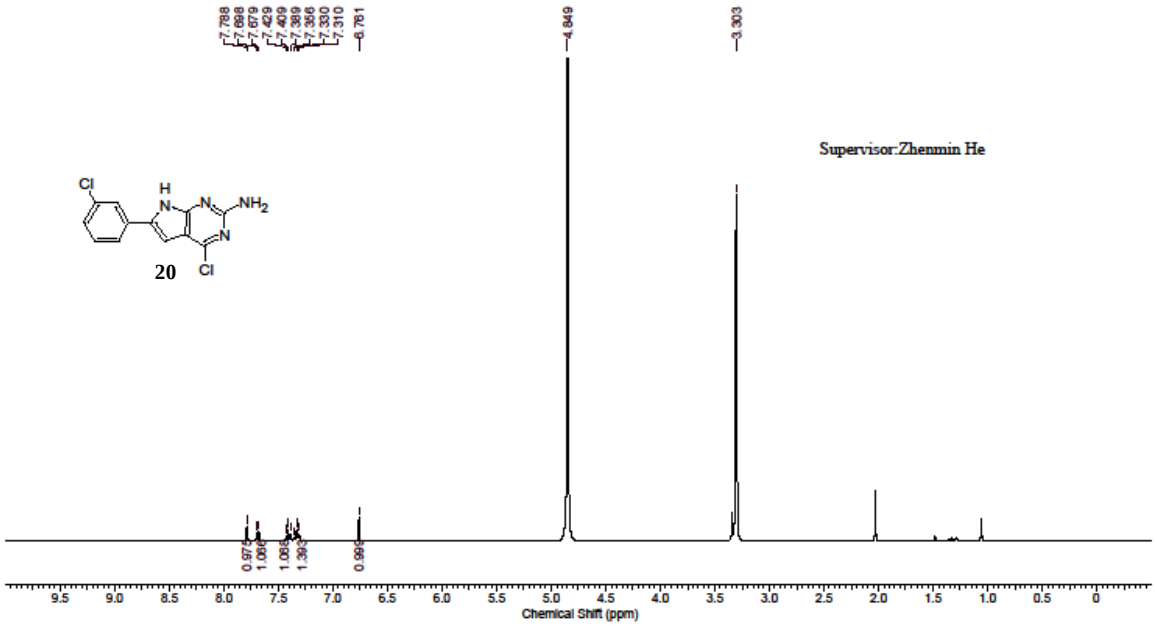


2-amino-6-(3-chlorophenyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (19). A suspension of **S45** (2.4 g, 19.0 mmol, 1.00 eq) in H_2O (80.0 mL) was treated with NaOAc (1.6 g, 19.0 mmol, 1.0 eq) and heated to 100 °C. Then 2,4-diamino-6-hydroxypyrimidine (7.9 g, 22.8 mmol, 1.2 eq) was added dropwise to the reaction mixture. The reaction mixture was stirred at 100°C for about 16 hr. The mixture was poured into 50 mL of H_2O and filtered. The solid was washed with 50 mL of acetone and purified by prep-HPLC to give **19** (450.0 mg, 1.6 mmol, 8.6% yield, >92% purity) as a yellow solid. **^{1}H NMR** (MeOD, 400MHz) δ = 7.67 (s, 1H), 7.62 - 7.54 (m, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.26 - 7.18 (m, 1H), 6.81 (s, 1H). **LCMS** (ESI+): m/z 261.0 $(M+1)^+$ (expected 261.1), Rt: 2.61 min (H_2O:ACN 99/1 @ 0.01 min to 10/90 @ 3.49 min)

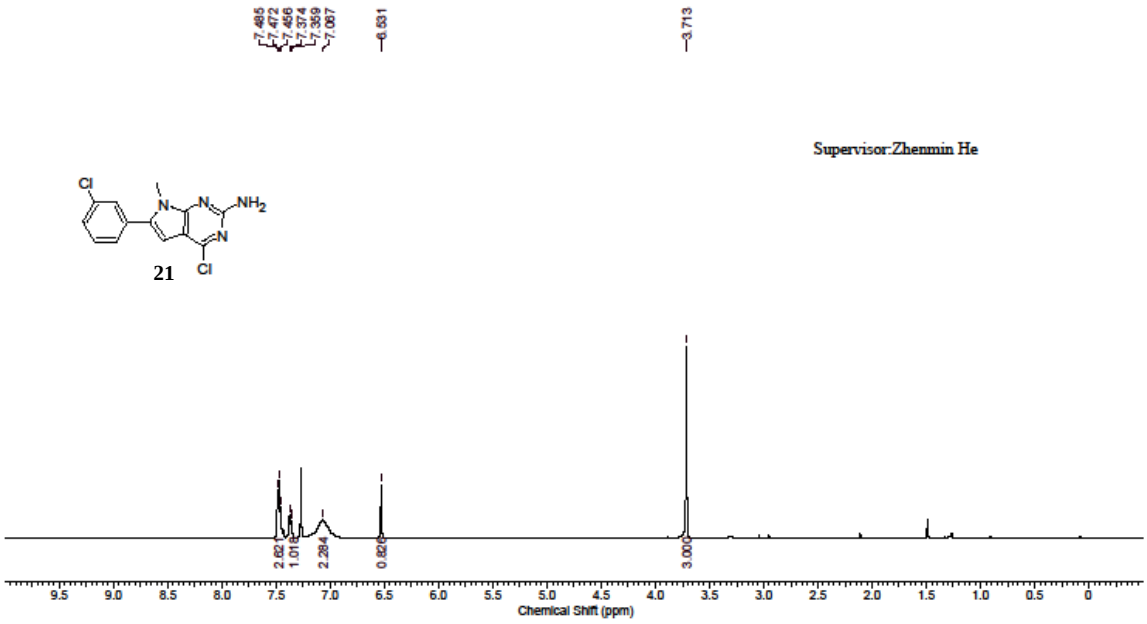


4-chloro-6-(3-chlorophenyl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (20). A mixture of **19** (100.0 mg, 0.38 mmol, 1.0 eq) and $POCl_3$ (24.7 g, 161.4 mmol, 15.0 mL, 420.7 eq) was degassed and purged with N_2 three times before the mixture was stirred at 100 °C for 2 hr. The reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure. The remaining residue was cooled to 0 °C and then adjusted to pH = 9 with NaOH (5 M). The residue was diluted with H_2O (5 mL) and extracted with EtOAc (10 mL* 2). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC to give **20** (27.0 mg, 0.10 mmol, 25.6% yield, >93% purity) as a yellow solid. **^{1}H NMR** (MeOD, 400MHz) δ = 7.79 (s, 1H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.44 - 7.38 (m, 1H), 7.36 - 7.30 (m,

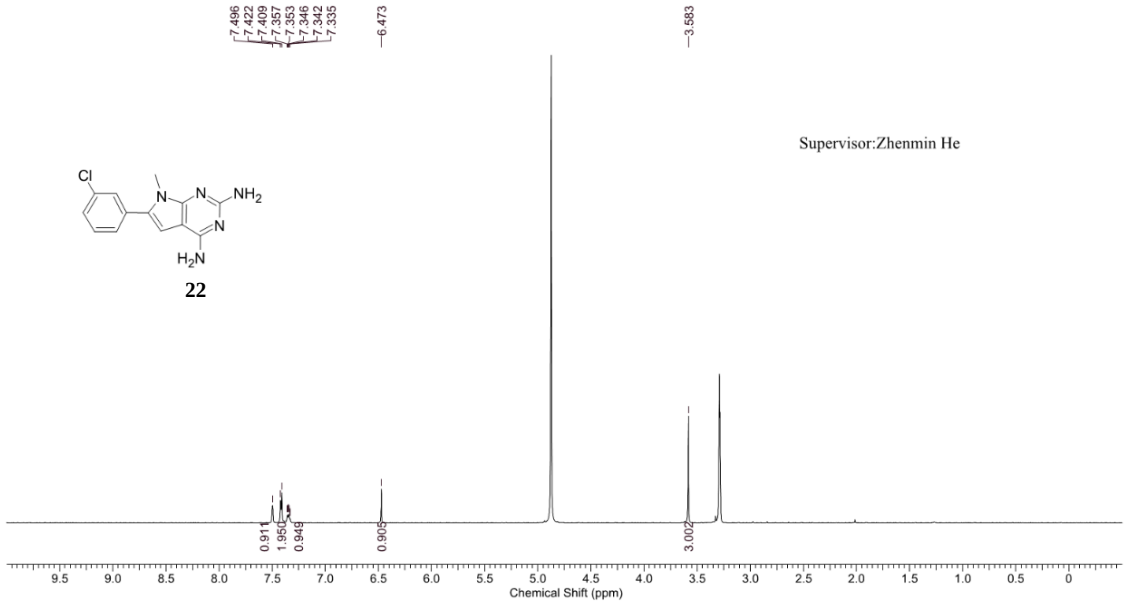
1H), 6.76 (s, 1H). **LCMS** (ESI+): m/z 278.8, 280.8 $(M+1,+3)^+$ (expected 279.0), Rt: 3.20 min (H_2O:ACN 99/1 @ 0.01 min to 0/100 @ 3.85 min)



4-chloro-6-(3-chlorophenyl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (21). A stirred suspension of **20** (30.0 mg, 0.11 mmol, 1.0 eq) in dry ACN (5.0 mL) under argon was treated with NaH (4.3 mg, 0.11 mmol, 60% purity, 1.0 eq). After 30 min, MeI (0.1 M, 1.0 mL, 1.0 eq) was added to the mixture and stirred for 12 hr. Solvent was removed and the residue was purified by prep-HPLC to give **21** (30.0 mg, 0.10 mmol, >95% yield, >94% purity) as a faint yellow solid. **^{1}H NMR** (CDCl3 400MHz) δ = 7.51 - 7.44 (m, 3H), 7.37 (d, J = 5.7 Hz, 1H), 7.07 (br. s., 2H), 6.53 (s, 1H), 3.71 (s, 3H). **LCMS** (ESI+): m/z 292.8, 294.8 $(M+1,+3)^+$ (expected 293.0), Rt: 3.11 min (H_2O:ACN 99/1 @ 0.01 min to 0/100 @ 3.85 min)



6-(3-chlorophenyl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamine (22). A mixture of **21** (20.0 mg, 68.2 umol, 1.0 eq) and NH_3/EtOH (68.2 umol, 5.0 mL, 1.0 eq) was sealed in a 50 mL facility. The mixture was stirred at 130 °C for 12 hr. Removal of the solvent gave a residue that was purified by prep-HPLC to give **22** (7.50 mg, 27.40 umol, 40.2% yield, >97% purity) as a faint yellow solid. **^{1}H NMR** (MeOD, 400MHz) δ = 7.50 (s, 1H), 7.42 (d, J = 5.3 Hz, 2H), 7.37 - 7.31 (m, 1H), 6.47 (s, 1H), 3.58 (s, 3H) **LCMS** (ESI+): m/z 274.0 $(M+1)^+$ (expected 274.1), Rt: 2.73 min (H_2O:ACN 99/1 @ 0.01 min to 0/100 @ 3.85 min)



Crystallization & Structural Determination Experimental

*Crystallization and Structure Determination of TS-DHFR complexed with **15***

The loop truncated form of *Toxoplasma gondii* TS-DHFR was expressed and purified as described previously[1]. TS-DHFR was overexpressed in *E. coli* BL21 cells after induction with IPTG, and purified by an affinity column of methotrexate bound to agarose followed by size exclusion chromatography. The protein was concentrated to 13 mg/ml in the final buffer (25mM Tris-HCl pH 7.3, 10mM DTT, 10% glycerol). The ligands NADPH, dUMP, N10-propargyl-5,8-dideazafolate (PDDF) and compound **15** were added to a final concentration of 500μM. Vapor diffusion crystallization was setup as 1 μL + 1 μL (enzyme + well solution) sitting drops with well solution containing 18% PEG 3350 and 0.2M Sodium Citrate dibasic. The crystals were cryoprotected in mother liquor containing ethylene glycol and frozen in stepwise transfers into liquid nitrogen. Data were collected at beamline 17U at Shanghai Synchrotron Radiation Facility. Initial processing of X-ray data was accomplished using HKL2000[2]. The loop truncated model (PDB accession code 4EIL) was used as the search model for molecular replacement by using PHASER[3]. Refinement of the structure was carried out by REFMAC[4]. The ligands and their topology files were generated by the JLigand and manual adjustments to model and the ligands were made in COOT[5]. Figures were generated using MOE, from CCG, Inc.

X-ray Diffraction Data for Compound 15	
Data collection	
Resolution (Å)	50.0-3.12
Space group	P212121
a	51.277
b	143.983
c	170.214
No. of total reflections	91516
No. of unique reflections	21429
Completeness (%)	92.4 (95.0)
$I/\sigma(I)$	11.4 (3.0)
Redundancy	4.3 (4.3)
R_{merge} (%)	12.7 (55.0)
Refinement	
R_{work}/R_{free} (%)	23.45/28.04
RMS bond/angel	0.005/1.066

1. Johnson, E. F.; Hinz, W.; Atrey, C. E.; Maley, F.; Anderson, K. S. Mechanistic Characterization of *Toxoplasma gondii* Thymidyalte Synthase (TS-DHFR)-Dihydrofolate Reductase. Evidence for a TS Intermediate and TS Half-Sites Reactivity. *J. Biol. Chem.* **2002**, *277*, 43126−43136.

2. Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods Enzymol. Macromol. Crystallogr.* **1997**, *276*, 307−326.

3. Collaborative Computational Project, Number 4. The CCP4 Suite: Programs for Protein Crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **1994**, *50*, 760−763.

4. Storoni, L. C.; McCoy, A. J.; Read, R. J. Likelihood-Enhanced Fast Rotation Functions. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 432−438.

5. Emsley, P.; Cowtan, K. Coot: Model-Building Tools for Molecular Graphics. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2126−2132.