Cytosine-based TET Enzyme Inhibitors

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TET Enzyme Computational Models. A solved crystal structure of human TET2 bound to DNA (PDB: 4NM6) was used in the Molecular Operating Environment (MOE) software for all computational analyses.[1] A homology model of human TET1 was then produced by aligning its relevant primary sequence with that of TET2 (Figure S1), and then substituting the linear amino acid sequence with an induced fit around the N-oxalylglycine – Fe – methylated dsDNA complex using the Amber 10 EHT force field in the MOE software package. TET2 was crystalized, bound to dsDNA, with N-oxalylglycine, a pan inhibitor of KG-dependent dioxygenase. For both TET1 and TET2 models the nitrogen in N-oxalylglycine, which binds to the KG co-factor site and chelates the catalytic Fe center, was then converted to an sp^3 hybridized carbon to produce KG. Then, the dsDNA was removed from the model and the bound 5mC in the active site was used as the starting pose for all cytosine-based inhibitors.

Inhibitor Docking. Analogs of 5-chlorocytosine were generated using the molecule builder feature in the MOE software, and based on the binding position of 5mC in the crystal structure. Each compound was first allowed to minimize to its lowest energy conformation within the pocket in the Amber 10 EHT force field, while all other protein atoms were fixed. Then, a systematic conformational search was run for each compound, and each rotatable bond in R_2 was rotated to generate a library of low energy conformers. Each member of this library was then docked using the docking function in MOE and scored using the London ΔG function to obtain a score for free energy of binding. No placement methodology was used, but the induced fit function was employed as the refinement methodology, while allowing protein atoms of residues within 4.5 Å of the inhibitor free to minimize torsional strain and maximize interactions.

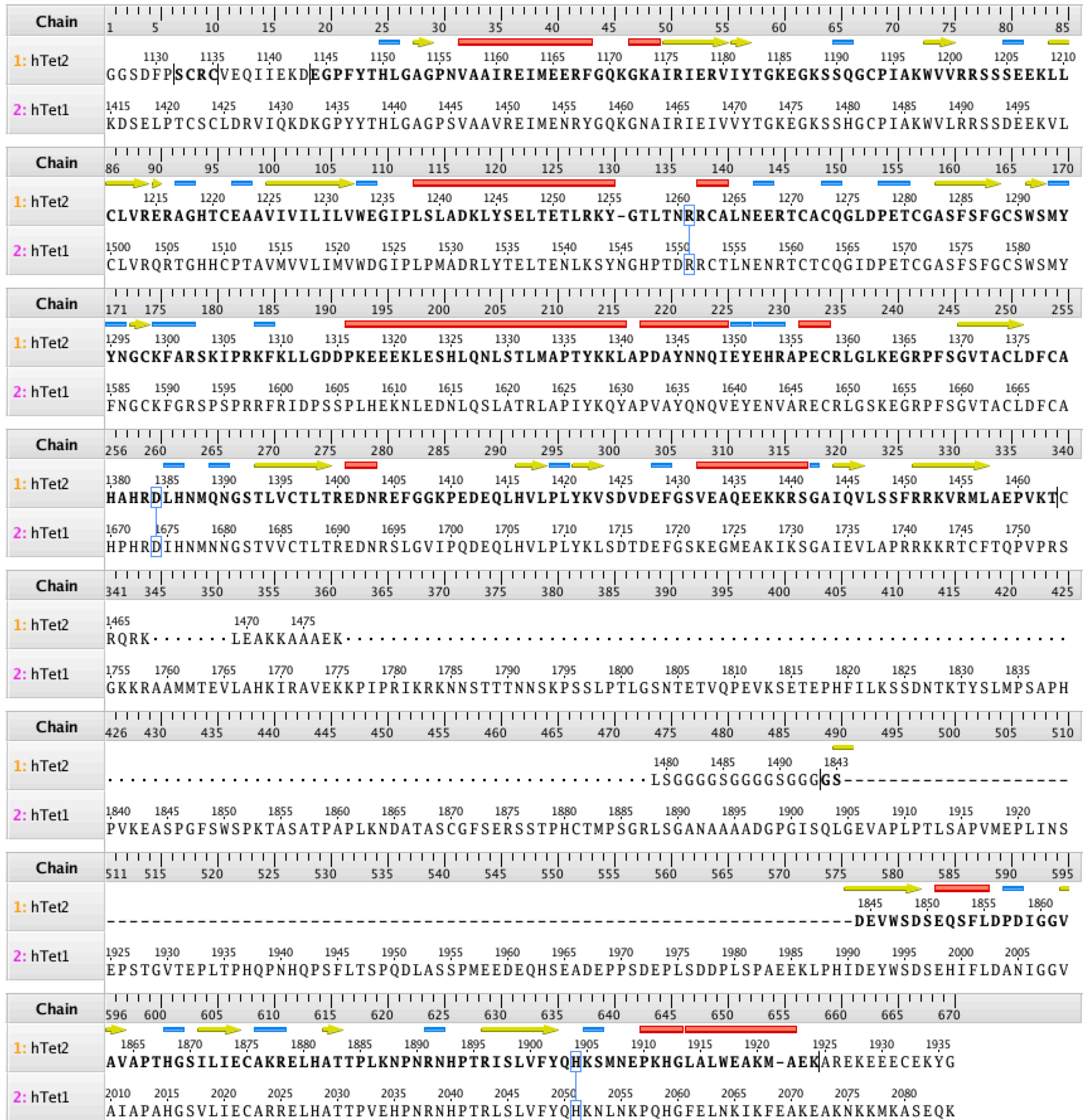


Figure S1. Sequence alignment of TET1 and TET2.

Chemiluminescence ELISA. Procedure adapted from manual (Bioscience; TET1: 50651, TET2: 50652). Prepare TBST buffer (1X TBS, pH 8.0, containing 0.05% Tween-20). Dilute 4.0X TET Assay Buffer (TAB) to 1.5X TAB and 1.0X TAB evenly with diluted water. Thaw and dilute (5.0 ng/µl for TET1 and 10 ng/µl for TET2) TET enzyme from kit with 1.0X TAB. Dilute primary antibody 100-fold with blocking buffer. Diluted secondary antibody 1000-fold with blocking buffer. Dilute DMSO inhibitor solutions with 1.0X TAB to wanted concentration (ensure solutions are 5% DMSO). To 96-well plate provided, add 200 µl TBST buffer to each well and incubate at room temperature for 15 min. Remove TBST buffer and add 20 µl 1.5X TAB, 10 µl inhibitor solution, 20 µl diluted TET to each well. For controls, add 10 µl 5% DMSO solution and 20 µl 1.0X TAB. Incubate at room temperature for 2 h. Remove reaction solution and wash 3X with TBST buffer (200, 200, and 100 µl). Add 100 µl blocking buffer 53 µl diluted primary antibody and shake at room temperature for 1 h. Remove diluted primary antibody and wash 3X with TBST buffer (200, 200, and 100 µl). Add 100 µl blocking buffer to each well and shake at room temperature for 10 min. Remove blocking buffer. Add 100 µl diluted secondary antibody. Shake at room temperature for 30 min. Remove diluted secondary antibody and wash 3X with TBST buffer (200, 200, and 100 µl). Add 100 µl blocking buffer to each well and shake at room temperature for 10 min. Remove blocking buffer. Combine horseradish peroxidase (HRP) substrate A and HRP substrate B at 1:1 ratio. Add 100 µl of HRP solution to each well. Immediately, read chemiluminescence (BioTek Synergy 2 plate reader).

Neuronal Cell Assay

Cell culture

HT22 cells were provided by David Schubert at the Salk Institute (San Diego, CA). Cells were cultured in Dulbecco's Modified Eagle Medium (Life Technologies, Carlsbad, CA) supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Carlsbad, CA) at 37˚C and 5% CO_2. HT22 cells were kept at 50-70% confluency and were passaged twice a week. Briefly, culture medium was removed and replaced by 0.05% trypsin (Life Technologies, Carlsbad, CA). The cells were incubated with trypsin for 5 minutes and 1.5 x volume of culture medium was added to the cell-trypsin suspension. Finally, cells were added at a ratio of 1:10 to fresh culture medium in 35 mm dishes to be used for experiments. Cultured HT22 cells were treated with prepared solutions of Bobcat339 and Bobcat212. 22 μl of compound in DMSO was added to dishes containing 2.2 ml of cell medium, resulting in a 10 μM final concentration of inhibitor and an overall 1% DMSO concentration. Higher concentrations of Bobcat339 suffered from insufficient solubility. Cells were incubated at 37˚C and 5% CO_2 for 24 hours.

DNA extraction

Procedure adapted from manual (Qiagen; 69504). Remove culture medium from dishes. Add 180 μl buffer ATL to each dish and scrape. Transfer liquid to 1.5 ml microcentrifuge tube. For each sample, add 20 μl proteinase K and immediately mix by pulse vortex. Incubate overnight at 56˚C. After incubation, remove from incubator and vortex immediately for 15 seconds. Add 4 μl RNase A to each tube and vortex immediately. Let incubate for 2 mins at RT on bench top. To each sample, add 200 μl Buffer AL and mix thoroughly by vortexing. Add 200 μl ethanol (100%). Immediately mix by vortexing. Pipet each sample mixture into a DNeasy spin column placed in a

2 ml collection tube. Centrifuge at 6000 x g (6000 rcf) for 1 minute. Discard the flow-through and collection tubes. Place each spin column in a new 2 ml collection tube, add 600 µl Buffer AW1, and centrifuge for 1 minute at 6,000 x g. Discard the flow-through and collection tubes. Place the spin column in a new 2 ml collection tube, add 600 µl Buffer AW2, and centrifuge for 3 minutes at 18,213 x g (18,213 rcf). Discard the flow-through and collection tubes, place spin column in new 2 ml collection tube, and centrifuge for another 3 minutes at 18,213 x g (18,213 rcf). Place spin column into final full-description labeled 1.5 mL capped centrifuge tube. Add 22 µl DNase/RNase free water to each spin column as elution buffer and incubate on the benchtop at room temp for 15 minutes. Centrifuge for one minute at 6,000 x g (6,000 rcf = 6,000 x g) and discard spin column. DNA concentrations were determined using a NanoDrop spectrophotometer and samples stored at -20˚C.

MethylFlash Global DNA Hydroxymethylation (5-hmC) ELISA Easy Kit (Colorimetric)

Procedure adapted from manual (Epigentek: P-1032-48). Prepare Dilute Wash Buffer (1X Wash Buffer) by adding 13 ml of 10X Wash Buffer to 117 ml distilled water and adjusting pH 10 7.2-7.5. 100 µl of binding solution was to each well followed by 100 ng of extracted sample DNA or known standards, then incubated at 37˚C for 1 hour. Prepare 5-hmC Detection Complex Solution during the last 10 minutes of incubation by adding 1 µl hmAb, Signal Indicator, and Enhancer Solution per ml of Diluted WB (4-5 ml). After 1-hour incubation is complete, remove binding solution from each well and wash each well with 150 µl of diluted WB three times. After washing, add 50 µl of 5-hmC Detection Complex Solution to each well, mix by gently shaking the plate, then cover and incubate at room temperature for 50 minutes. After incubation, remove antibody solution from each well and wash each well with 150 µl each time for five times. After washing,

add 100 µl of Developer Solution to each well column-wise so that replicates are developed at the same time. Incubate for 3-5 minutes or until the solution in the 1% PC wells turn dark blue. Stop the reaction by adding 100 µl of Stop Solution to each well column-wise. Incubate for 2 minutes, then read absorbance at 450 nm (BioTek Synergy 2 platereader).

General Synthetic Materials and Methods. All nonaqueous reactions were conducted in oven and flame-dried glassware under nitrogen atmosphere with dry solvents and magnetic stirring. The nitrogen was dried by passing through a tube of Drierite. Dichloromethane (CH_2Cl_2 or DCM), methanol (MeOH), anhydrous tetrahydrofuran (THF), ethyl acetate (EtOAc), and dimethyl sulfoxide (DMSO) were purchased from Aldrich Chemicals or Fisher Scientific and used as received. All other reagents were purchased from Acros Chemicals, Aldrich Chemicals, and Bachem. Reactions were monitored by thin layer chromatography (TLC) using 0.25 mm Whatman precoated silica gel plates. Column chromatography was performed with the indicated solvents and Dynamic Absorbents silica gel (particle size 0.023 – 0.040 mm). Proton (1H) and carbon (13C) NMR spectra were 54 recorded on Bruker Avance 400 at 300 K. Chemical shifts are reported in ppm (δ) values relative to DMSO-d6 (δ 2.50 for proton and δ 39.5 for carbon NMR). TLC plates were stained with Seebach's Dip – 25 mL concentrated sulfuric acid was added dropwise to a solution of 25 g phosphomolybdic acid and 7.5 g cerium (IV) sulfate in 479 mL water.

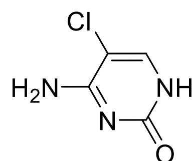
General Procedure A: Cytosine and Boronic Acid Coupling. To a 0.5 M solution of Cytosine/chlorocytosine (1.0 eq.) in a 3:1 mixture (by volume) of MeOH:H_2O was added the boronic acid (1.5 eq.) and $Cu(OAc)_2$ (1.0 eq.) while stirring. TMEDA (2.0 eq.) was then added dropwise. The reaction was stirred for 4 h at room temperature and monitored by TLC. Then, the reaction was evaporated to dryness and purified by column chromatography.

Liquid Chromatography and Mass Spectrometry for Evaluation of Chemical Purity. Compounds submitted for biological evaluation were determined to be > 95% pure by LCMS evaluation performed by the Mass Spectrometry Laboratory in the School of Chemical Sciences

at the University of Illinois Urbana-Champagne (Urbana, IL). High performance liquid chromatography – mass spectrometry (LCMS) was carried out using an Agilent 2.1x50mm C-18 column and a Micromass Q-tof Ultima mass spectrometer. Mobile phase A consisted of HPLC grade H_2O with 0.01% Formic Acid; mobile phase B consisted of MeCN with 0.01% Formic Acid. LCMS identification and purity utilized a binary gradient starting with 90% A and 10% B and linearly increasing to 100% B over the course of 6 min, followed by an isocratic flow of 100% B for an additional 3 min. A flow rate of 0.5 mL / min was maintained throughout the HPLC method. The purity of all products was determined by integration of the total ion count (TIC) spectra and integration of the ultraviolet (UV) spectra at 214 nm. Retention times are abbreviated t_R; mass to charge ratios are abbreviated as m/z.

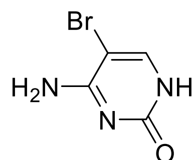
Characterization and Synthetic Procedure

5-chloro-cytosine



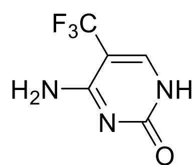
To a 0.6 M solution of Cytosine (1.0 eq.) in glacial acetic acid was added N-chlorosuccinimide (1.1 eq.) and heated for 4 hours at 70 °C. Acetic acid was evaporated under low pressure. The crude solid was diluted with 60 mL of distilled water and sodium bicarbonate slowly added until the solution was pH 9. The solid was then filtered under vacuum. 84.4%. White solid. ^{1}H NMR (400 MHz, DMSO) δ 11.00 (brs, 1H), 7.85 (s, 1H), 7.20 (brs, 2H). ^{13}C NMR (400 MHz, DMSO) δ 162.22, 156.94, 143.63, 97.99.

5-bromo-cytosine



To a 0.6 M solution of Cytosine (1.0 eq.) in glacial acetic acid was added N-bromosuccinimide (1.1 eq.) and heated for 4 hours at 70 °C. Acetic acid was evaporated under low pressure. The crude solid was diluted with 60 mL of distilled water and sodium bicarbonate slowly added until the solution was pH 9. The solid was then filtered under vacuum. 65.5%. White solid. ^{1}H NMR (400 MHz, DMSO) δ 11.00 (brs, 1H), 7.75 (s, 1H), 7.10 (brs, 2H). ^{13}C NMR (400 MHz, DMSO) δ 162.67, 156.66, 145.47, 85.17.

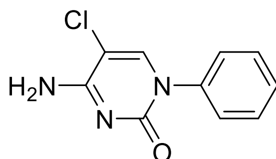
5-trifluoromethyl-cytosine



To a 0.4 M solution of cytosine (1.0 eq.) in DMSO was added iron (II) sulfate (1.0 eq.), hydrogen peroxide (0.26 eq.), sulfuric acid (0.01 eq.), and trifluoromethyl iodide in excess (via balloon). The reaction was then stirred at r.t. for 2 hours, then the pH of the reaction was to 8-9 using saturated sodium bicarbonate. The reaction was then filtered and the eluent purified by column chromatography (4:1 DCM to MeOH). 36%. White solid. ^{1}H NMR (400 MHz, DMSO) δ 7.95 (s,

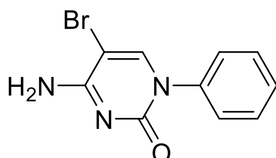
1H), 7.85 (brs, 1H), 6.95 (brs, 2H). ^{13}C NMR (400 MHz, DMSO) δ 161.5, 156, 145, 124, 94.3 (q, CF_3).

4-amino-5-chloro-1-phenylpyrimidin-2(1H)-one (**Bobcat216**)



General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and phenylboronic acid (1.5 eq.) to yield title compound. 70%. White powder. R_f = 0.55 (8.3% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 11.95 (s, 1H), 8.07 (s, 1H), 7.43 (m, 5H), 7.31 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.40, 153.88, 144.18, 141.01, 129.81, 129.25, 128.19, 127.17. LCMS: t_R = 3.13; m/z = 380.3. HRMS m/z calc. for $C_{10}H_8N_3OCl$ (M+H), 222.0434; found, 222.0432.

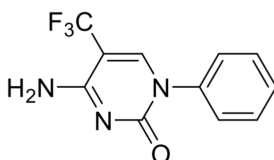
4-amino-5-bromo-1-phenylpyrimidin-2(1H)-one (**Bobcat371**)



General Procedure A was used to couple bromocytosine (380 mg, 2.0 mmol) and phenylboronic acid (1.5 eq.) to yield title compound. 74%. R_f = 0.60 (10% MeOH in DCM; Seebach's Dip). ^{1}H

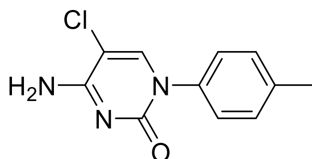
NMR (400 MHz, DMSO) δ 8.10 (s, 1H), 8.05-7.87 (brs, 1H), 7.50-7.35 (m, 5H), 7.15-7.07 (brs, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.89, 154.10, 146.74, 140.77, 129.26, 128.21, 127.16, 86.98. LCMS: t_R = 3.31; m/z = 266.0. HRMS m/z calc. for $C_{10}H_8N_3OBr$ (M+H), 265.9929; found, 265.9929.

4-amino-1-phenyl-5-(trifluoromethyl)pyrimidin-2(1H)-one (**Bobcat212**)



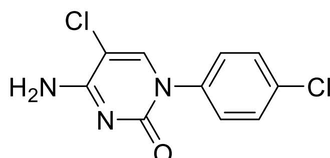
General procedure A was used to couple trifluorocytosine (43 mg, 0.24 mmol) and phenylboronic acid (1.5 eq.) to yield title compound. 25%. White powder. R_f = 0.65 (8.3% MeOH in DCM; UV active). ^{1}H NMR (400 MHz, DMSO) δ 8.23 (d, 1H), 8.01 (s, 1H), 7.45 (m, 5H), 7.16 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 172.49, 161.03, 154.00, 148.21, 140.53, 129.30, 128.59, 127.35, 95.33 (q, CF_3). LCMS: t_R = 3.85; m/z = 256.1. HRMS m/z calc. for $C_{11}H_8N_3OF_3$ (M+H), 256.0598; found, 256.0694.

4-amino-5-chloro-1-(p-tolyl)pyrimidin-2(1H)-one (**Bobcat308**)



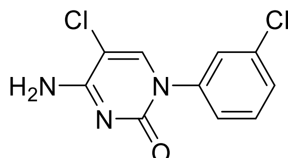
General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and p-tolylboronic acid (1.5 eq.) to yield title compound. 78%. White powder. R_f = 0.55 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.25 (m, 1H), 7.42 (m, 5H), 5.83 (m, 1H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 162.42, 154.15, 144.19, 138.37, 137.66, 129.68, 126.85, 55.38, 21.08. LCMS: t_R = 3.90; m/z = 236.1. HRMS m/z calc. for $C_{11}H_{10}N_3OCl$ (M+H), 236.0591; found, 236.0589.

4-amino-5-chloro-1-(4-chlorophenyl)pyrimidin-2(1H)-one (**Bobcat218**)



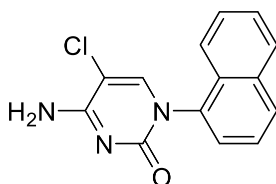
General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and 4-chloroboronic acid (1.5 eq.) to yield title compound. 47%. White powder. R_f = 0.5 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.08 (s, 1H), 8.01 (s, 1H), 7.48 (m, 4H), 7.35 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.43, 153.82, 144.00, 139.58, 132.61, 129.56, 129.15, 129.09. LCMS: t_R = 4.03; m/z = 256.0. HRMS m/z calc. for $C_{10}H_7N_3OCl_2$ (M+H), 256.0044; found, 256.0040.

4-amino-5-chloro-1-(3-chlorophenyl)pyrimidin-2(1H)-one (**Bobcat205**)



General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and 3-chlorophenylboronic acid (1.5 eq.) to yield title compound. 41%. White powder. R_f = 0.8 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.13 (s, 2H), 7.55 (s, 1H), 7.42 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 162.54, 153.64, 143.95, 142.04, 133.28, 130.79, 128.19, 127.41, 126.07. LCMS: t_R = 4.01; m/z = 256.0. HRMS m/z calc. for $C_{10}H_7N_3OCl_2$ (M+H), 256.0044; found, 256.0041.

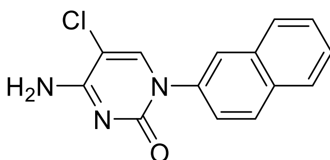
4-amino-5-chloro-1-(naphthalen-1-yl)pyrimidin-2(1H)-one (**Bobcat374**)



General procedure A was used to couple chlorocytosine (291 mg, 2.0 mmol) and naphthalene-1-ylboronic acid (1.5 eq.) to yield title compound. 34%. R_f = 0.5 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.00-7.95 (m, 2H), 7.70-7.63 (m, 1H), 7.61-7.52 (m, 4H), 7.48 (dd, J=7.3, 1H), 7.28 (s, 1H), 5.92-5.77 (brs, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.80,

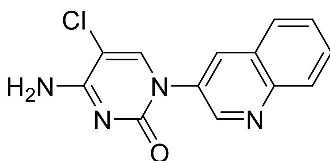
154.49, 145.11, 137.57, 134.20, 130.02, 129.33, 128.70, 128.68, 127.63, 126.99, 126.16, 126.09, 122.97. LCMS: t_R = 4.37; m/z = 272.1. HRMS m/z calc. for $C_{14}H_{10}N_3OCl$ (M+H), 272.0591; found, 272.0587.

4-amino-5-chloro-1-(naphthalen-2-yl)pyrimidin-2(1H)-one (**Bobcat330**)



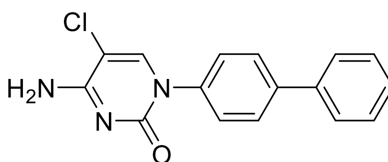
General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and naphthalene-2-ylboronic acid (1.5 eq.) to yield title compound. 54%. R_f = 0.5 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.21 (s, 1H), 8.05 (s, 1H), 7.96 (m, 4H), 7.57 (m, 3H), 7.39 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 163.53, 154.16, 144.40, 138.57, 133.30, 132.42, 128.59, 128.38, 128.01, 127.10, 127.07, 125.80, 125.05. LCMS: t_R = 4.58; m/z = 272.1. HRMS m/z calc. for $C_{14}H_{10}N_3OCl$ (M+H), 272.0591; found, 272.0584.

4-amino-5-chloro-1-(quinolin-3-yl)pyrimidin-2(1H)-one (**Bobcat211**)



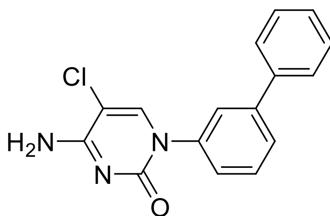
General procedure A was used to couple chlorocytosine (113.5 mg, 0.78 mmol) and 3-quinoline boronic acid pinacol ester (1.5 eq.) to yield title compound. 41%. White powder. R_f = 0.55 (8.3% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.94 (d, 1H), 8.46 (d, 1H), 8.31 (s, 1H), 8.06 (m, 3H), 7.83(ddd, 1H), 7.48 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.71, 154.13, 149.81, 146.53, 144.15, 134.38, 132.63, 130.49, 129.10, 128.68, 127.79, 127.72, 100.39. LCMS: t_R = 3.45; m/z = 273.1. HRMS m/z calc. for $C_{13}H_9N_4OCl$ (M+H), 273.0543; found, 273.0539.

1-([1,1'-biphenyl]-4-yl)-4-amino-5-chloropyrimidin-2(1H)-one (**Bobcat219**)



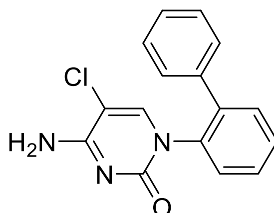
General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and 4-biphenylboronic acid (1.5 eq.) to yield title compound. 72%. White powder. R_f = 0.65 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.13 (s, 1H), 7.94 (d, 1H), 7.72 (m, 4H), 7.49 (m, 4H), 7.41 (m, 1H), 7.35 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.40, 154.00, 144.18, 140.10, 139.99, 139.75, 129.49, 128.19, 127.64, 127.47. 127.22. LCMS: t_R = 5.15; m/z = 298.1. HRMS m/z calc. for $C_{16}H_{12}N_3OCl$ (M+H), 298.0747; found, 298.0746.

1-([1,1'-biphenyl]-3-yl)-4-amino-5-chloropyrimidin-2(1H)-one (**Bobcat339**)



General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and 2-biphenylboronic acid (1.5 eq.) to yield title compound. 79%. Green powder. $R_f = 0.50$ (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 8.20 (s, 1H), 8.03 (s, 1H), 7.70 (m, 4H), 7.44 (m, 6H). ^{13}C NMR (100 MHz, DMSO) δ 162.50, 153.98, 144.33, 141.45, 141.38, 139.67, 129.74, 129.43, 128.80, 128.28, 127.28, 126.34, 126.22, 125.36. LCMS: $t_R = 5.09$; m/z = 298.1. HRMS m/z calc. for $C_{16}H_{12}N_3OCl$ (M+H), 298.0747; found, 298.0749.

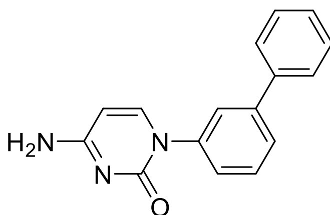
1-([1,1'-biphenyl]-2-yl)-4-amino-5-chloropyrimidin-2(1H)-one (**Bobcat337**)



General procedure A was used to couple chlorocytosine (250 mg, 1.72 mmol) and 2-biphenylboronic acid (1.5 eq.) to yield title compound. 9%. White powder. $R_f = 0.45$ (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 11.95 (s, 1H), 7.82 (s, 1H), 7.35 (m, 9H),

7.18 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 162.24, 154.55, 144.71, 139.68, 138.50, 138.44, 130.99, 129.44, 1.29.36, 128.92, 128.80, 127.96. LCMS: t_R = 4.72; m/z = 298.1. HRMS m/z calc. for $C_{16}H_{12}N_3OCl$ (M+H), 298.0747; found, 298.0750.

1-([1,1'-biphenyl]-3-yl)-4-aminopyrimidin-2(1H)-one (**Bobcat222**)



General procedure A was used to couple cytosine (250 mg, 2.25 mmol) and 3-biphenylboronic acid (1.5 eq.) to yield title compound. 88%. White powder. R_f = 0.5 (10% MeOH in DCM; Seebach's Dip). ^{1}H NMR (400 MHz, DMSO) δ 7.72 (m, 3H), 7.65 (m, 2H), 7.51 (m, 3H), 7.38 (m, 2H), 7.28 (d, 2H), 5.82 (d, 1H). ^{13}C NMR (100 MHz, DMSO) δ 166.63, 155.37, 146.29, 142.35, 141.34, 139.77, 129.77, 129.45, 128.25, 127.27, 126.05, 125.96, 125.24. LCMS: t_R = 4.21; m/z = 264.1. HRMS m/z calc. for $C_{16}H_{13}N_3O$ (M+H), 264.1137; found, 264.1133.

1. Hu, L.; Li, Z.; Cheng, J.; Rao, Q.; Gong, W.; Liu, M.; Shi, Y. G.; Zhu, J.; Wang, P.; Xu, Y., Crystal structure of TET2-DNA complex: insight into TET-mediated 5mC oxidation. *Cell* **2013,** *155* (7), 1545-1555.