Supplementary Information

Identification of 2,4-Diamino-6,7-dimethoxyquinoline derivatives as G9a Inhibitors

Nitipol Srimongkolpithak,[a,b] Sandeep Sundriyal[a] Fengling Li[c], Masoud Vedadi[c] and Matthew J. Fuchter[a*]

[a] *Department of Chemistry, Imperial College London, London SW7 2AZ, UK. E-mail: m. fuchter@imperial.ac.uk; Fax: +44(0)2075945805; Tel: +44(0)2075945815*

[b] *Institute of Chemical Biology, Imperial College London, London SW7 2AZ, UK*

[c] *Structural Genomics Consortium, University of Toronto, Toronto, Ontario M5G 1L7*

Computational experiments.

Experimental:

All calculations were performed on a Windows 7.0 based Workstation using the Maestro 9.7 graphical user interface (GUI) of the Schrodinger® software suite (2014-1 release).[1]

Protein preparation: The X-ray structure of G9a (Chain A of PDB 3K5K)[2] was downloaded from the protein data bank and prepared using the 'Protein Preparation Wizard'. All water molecules and ions were deleted; atom types and bond orders were corrected, and the hydrogen atoms were reassigned after deleting the original ones. Missing side chains were added using the Prime 3.5 program.[3] The protonation states of acidic/basic amino acids were adjusted for pH 7.0. Restrained minimization of the protein was performed employing the OPLS-2005 forcefields[4] with the convergence criteria of RMSD of 0.3 Angstroms for heavy atoms.

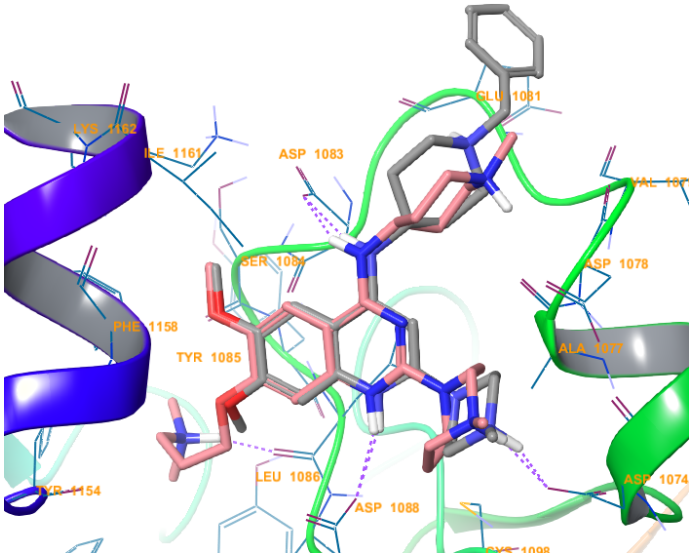
Ligand preparation: All molecules were drawn in ChemDraw Pro 13.0® and prepared using the LigPrep 2.9 program.[5] Epik 2.7, implemented in the Schrodinger suite, was used to generate energetically accessible protonation states. Non-protonated states of all molecules were also retained.[6-8] No tautomeric forms were generated for the ligands.

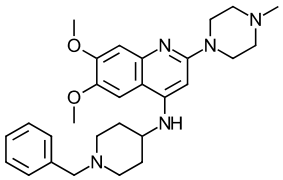
Docking: A receptor grid was generated using the centroid of the co-crystallized ligand with default settings for the size of the enclosing box. The hydroxyl and thiol groups of Tyr1067 and Cys1098, respectively, were allowed to rotate due to their vicinity to the co-crystallized ligand. All other default settings were used.

The ligands were docked into the prepared protein using the Glide 6.2 program[9-11] implemented in Schrodinger using both standard (SP) and extra precision (XP) modes.[12] No constraints were applied to the docking. Ligands were sampled throughout the docking, including ring conformations. Amide bonds were penalized in their nonplanar conformation. Epik state penalties were added to the final score. A maximum of 10 poses per ligand were allowed and post-docking minimization was also allowed. The number of poses identified for some ligands was more than 10, depending upon the number of protonated forms. All poses were individually visualized using the Pose Viewer and compared with UNC0224. A pose was classified as 'desired' or 'expected' when the N1 nitrogen and 4-amino group of the docked molecule overlaid the corresponding atoms of UNC0224. When more than one 'desired' pose was identified for a molecule the highest SP or XP score was reported.

pKa calculations: The calculated pKa of all ligands in their neutral state were generated using Epik 2.7 program[6] implemented in Schrodinger. Water was selected as the solvent model and 'Sequential pKa' mode at pH 7.0 was used.

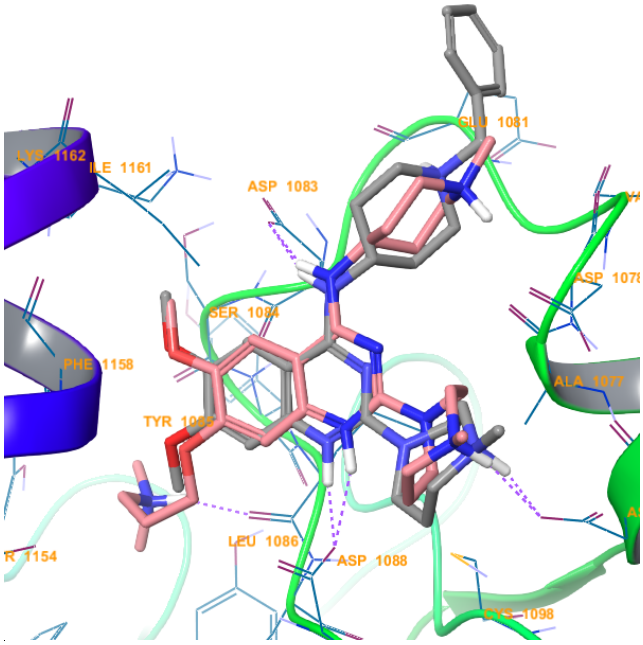
Fig. 2 was generated using freely available Discovery Studio 4.0 Visulalizer (Accelrys)®[13] while Fig. 3 and Fig. S1-S4 were generated using Maestro 9.7.

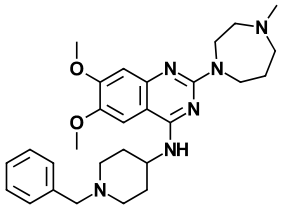




Compound **42**

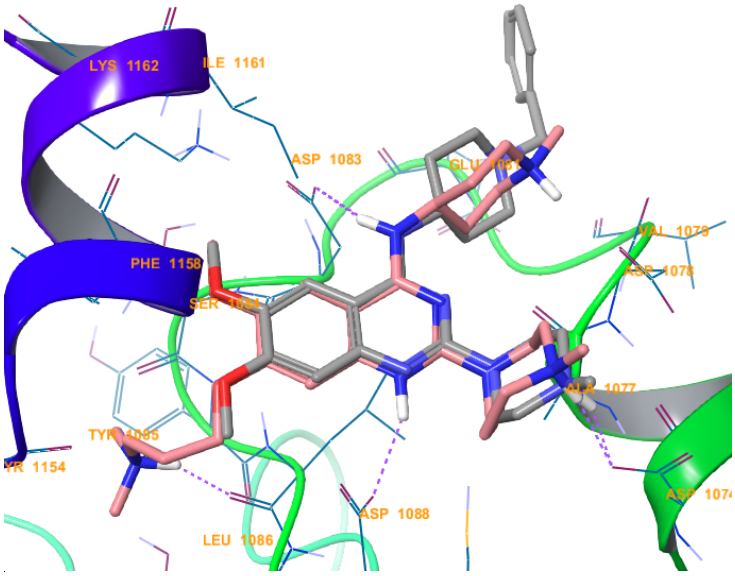
Figure S1: The top docking pose of quinoline analogue **42** (grey sticks) overlaid with UNC0224 (pink sticks; PDB 3K5K) in the G9a substrate binding pocket. Purple dashed lines display H-bonds. As expected, protonated N-1 and 4-amino moiety of **42** are shown to interact with Asp1088 and Asp1083, respectively.

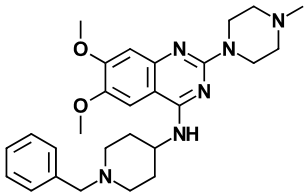




BIX-01294

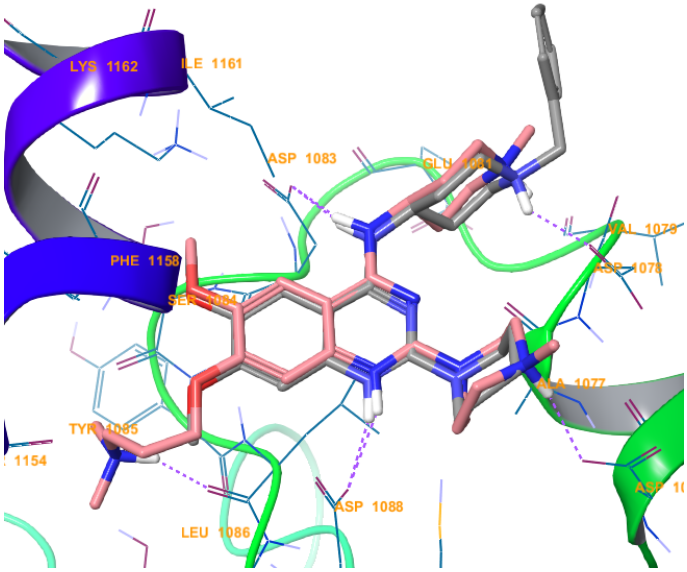
Figure S2: The top docking pose of BIX-01294 **1** (grey sticks) overlaid with UNC0224 (pink sticks; PDB 3K5K) in the G9a substrate binding pocket.

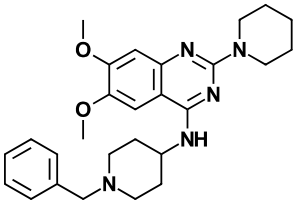




Compound **2**

Figure S3: The top docking pose of **2** (grey sticks) overlaid with UNC0224 (pink sticks; PDB 3K5K) in the G9a substrate binding pocket.





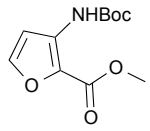
Compound **3**

Figure S4: The top docking pose of **3** (grey sticks) overlaid with UNC0224 (pink sticks; PDB 3K5K) in the G9a substrate binding pocket.

Analogue Synthesis

All dry and anhydrous reaction solvents were obtained from commercial suppliers or were freshly distilled from calcium hydride (dichloromethane, toluene, triethylamine). All reagents and other solvents were obtained from commercial suppliers. All reactions were performed under an atmosphere of dry nitrogen unless otherwise stated. Flash column chromatography was carried out using Merck Kiesegel 60 silica gel (230-400 mesh, 0.040 - 0.063 mm). Thin- layer chromatography (TLC) was performed on aluminium plates using Merck Kiesegel 60 F254 (230-400 mesh) fluorescent treated silica which were visualised under ultraviolet light (254 nm), or by staining with ninhydrin solution or others as appropriate. Preparative TLC plates were purchased from Analtec, inc.
All ^{1}H were recorded using a Bruker 400 MHz spectrometer. Chemical shifts (δ) are quoted in units of parts per million (ppm) downfield from tetramethylsilane and are referenced to a residual solvent peak. Coupling constants (*J*) are given in Hertz (Hz). High and low resolution mass spectra were recorded on Micromass Platform II and Micromass AutoSpec-Q spectrometers. All compounds tested *in vitro* were found to be pure (>95%) using LCMS. LCMS gradient: From 95:5, A:B to 5:95, A:B over 18 minutes, where A is water (0.1% formic acid), and B is methanol. Column : XBridge C18 columns with dimensions 4.6 mm x 100 mm.

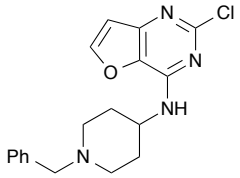
Compound **7**[14]



A solution of *ter*t-butyl-furan-3-ylcarbamate (2.8 g, 16.8 mmol) and TMEDA (6.27 mL, 41.86 mmol) in dried THF (70 mL) was cooled to -78 ^{o}C and then *n*-BuLi was added over 10 min. The solution was allowed to warm to 40 ^{o}C for 2h. Subsequently, methylchloroformate (1.95 mL, 25.1 mmol) was added and the mixture was warmed to 0 ^{o}C for 45 min. The mixture was diluted with 30 mL of a saturated ammonium chloride solution. The organic layer was washed with brine (50 mL x 3), dried with magnesium sulfate and concentrated under reduced pressure. The crude oil was purified by column chromatography to obtain a product **7** as yellow oil (1.7 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (br s, 1H), 7.39 (m, 1H), 7.24 (br s, 1H), 3.94 (s, 3H), 1.51 (s, 9H); HRMS (ESI) 241.0946 $[M]^+$ $C_{11}H_{15}NO_5$ requires 241.0950.

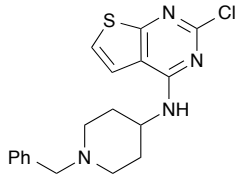
General procedure **A**
The required 4-amino-benzylpiperidine derivative (1.5 eq) and DIPEA (1.5 eq) were added to a suspension of the 2,4-dichloro-fused-pyrimidine (1 eq) in THF. The mixture was stirred for 24 h at room temperature. The mixture was concentrated and purified by column chromatography (0-5% methanol in dichloromethane) to afford the product.

Compound **11a**



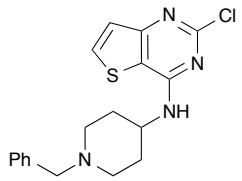
Following general procedure **A** (3.2 mmol scale), the product **11a** was obtained as a yellow solid (410 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.73 (br s, 1H), 7.26-7.20 (m, 5H), 6.78 (d, *J* = 2.4, 1H), 5.25 (br s, 1H), 4.23-4.14 (m, 1H), 3.56 (s, 2H), 2.89 (d, *J* = 11.6 Hz, 2H), 2.24 (t, *J* = 6.0 Hz, 2H), 2.08 (d, *J* = 11.6 Hz, 2H), 1.68-1.59 (m, 2H); HRMS (ES) 343.1321 $[M+H]^+$ $C_{18}H_{20}N_4OCl$ requires 343.1326.

Compound **11b**



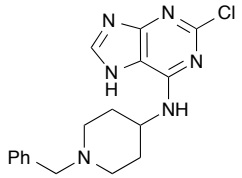
Following general procedure **A** (5 mmol scale), the product **11b** was obtained as a yellow solid, 576 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.74 (d, *J* = 5.6 Hz, 1H), 7.36-7.26 (m, 6H), 4.97 (br s, 1H), 4.28-4.19 (m, 1H), 3.56 (s, 2H), 2.90 (d, *J* =12.0 Hz, 2H), 2.25 (dt, *J* =12, 4Hz, 2H), 2.13(d, *J* = 12Hz, 2H), 1.68-1.65 (m, 2H); HRMS (ES) 359.1096 $[M+H]^+$ $C_{18}H_{20}N_4SCl$ requires 359.1097.

Compound **11c**



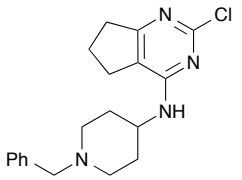
Following general procedure **A** (5 mmol scale), the product **11c** was obtained as a pale yellow solid (1.2 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.35-7.26 (m, 6H), 7.12 (d, *J* = 6.0 Hz, 1H), 5.6 (d, *J* = 8.0 Hz, 1H), 4.29-4.19 (m, 1H), 3.56 (s, 2H), 2.90 (d, *J* = 12.0 Hz, 2H), 2.25 (t, *J* = 12, 2H), 2.13 (d, *J* =12Hz, 2H), 1.66-1.62 (m, 2H); HRMS (ES) 359.1104 $[M+H]^+$ $C_{18}H_{20}N_4SCl$ requires 359.1097.

Compound **11d**



A solution of the **10e** (1.3 g, 6.95 mmol) and triethylamine (1.28 mL, 7.64 mmol) in 15 mL of *n*-butanol was heated and stirred in a sealed tube overnight. The resulting mixture was concentrated and dissolved in DCM (25 mL). The organic layer was washed with water (25 mL x 3), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (0-5% 7 M ammonia in methanol) to afford the product **11d** as a white solid (664 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$ + MeOD) 7.79 (s, 1H), 7.32-7.22 (m, 5H), 4.12 (s, 2H), 3.31 (m, 1H), 2.85 (m, 2H), 2.24 (t, *J* = 12 Hz, 2H), 1.65 (dd, *J* = 12.4 Hz, 2H), 1.64 (m, 2H); HRMS (ES) 343.1431 $[M+H]^+$ $C_{17}H_{20}N_6Cl$ requires 343.1438.

Compound **11e**

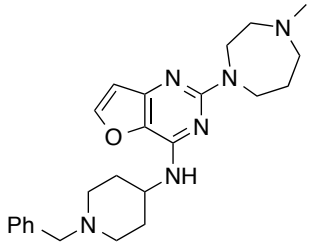


A mixture of **10e** (0.344g, 1.82 mmol), 1-benzyl-4-piperidylamine (0.296 mL, 1.46 mmol) and triethylamine (0.63 mL, 4.55 mmol) was stirred in THF for 20 h at room temperature. The reaction mixture was concentrated and purified by column chromatography (0-5%, 7 M ammonia methanol in dichloromethane). The title compound **11e** was obtained as a white solid (0.244 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.25 (m, 5H), 4.33 (s, 1H, br), 4-09-4.01 (m, 1H), 3.53 (s, 2H), 2.88-2.81 (m, 4H), 2.60 (t, *J* = 7.4 Hz, 2H), 2.23-2.00 (m, 6H), 1.50 (qd, *J* = 11.8, 3.7 Hz, 2H); HRMS (ESI) 343.1703 $[M+H]^+$ $C_{19}H_{24}N_4C$ requires 343.1689.

General procedure **B**

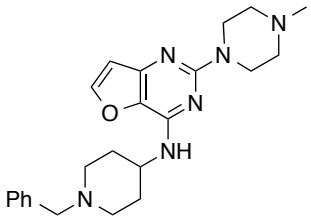
A mixture of the prepared 4-substituted pyrimidine (**11a**-**11e**) and a secondary amine (selected from 1-methylhomopiperazine, 1-methylpiperazine, piperidine or 1-(2-pyridyl)piperazine) was heated at 185^o C (neat) in a microwave for 30 min. The resultant mixture was purified by column chromatography to give the desired product.

Compound **12**



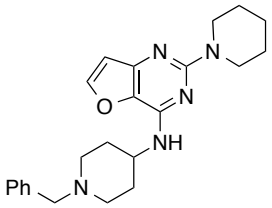
Following general procedure **B** using **11a** (50 mg, 0.15 mmol), and 1-methylhomopiperazine (0.014 mL, 1.5 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **12** as a white solid, (64 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 2.0 Hz, 1H), 7.35-7.25 (m, 5H), 6.61 (d, *J* = 2.4, 1H), 4.80 (d, *J* = 7.6 Hz, 1H), 4.18-4.04 (m, 1H), 3.95 (t, *J* = 4.4 Hz, 2H), 3.83 (t, *J* = 6.0 Hz, 2H), 3,53 (s, 2H), 2.89 (d, *J* = 12.0 Hz, 2H), 2.70 (t, *J* = 4.4 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H) 2.38 (s, 3H), 2.20 (dt, *J* = 10.8, 1.6 Hz, 2H), 2.11 (dd, *J* = 12.8, 3.2 Hz, 2H), 2.04-1.98 (m, 2H), 1.63-1.60 (m, 2H); HRMS (ES) 421.2721 $[M+H]^+$ $C_{24}H_{33}N_6O$ requires 421.2716.

Compound **13**



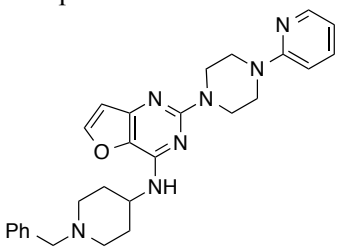
Following general procedure **B** using **11a** (50 mg, 0.15 mmol), and 1-methylpiperazine (0.016 mL, 1.5 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **13** as a white solid (61 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 2.0 Hz, 1H), 7.34-7.26 (m, 5H), 6.61 (d, *J* = 2.4, 1H), 5.25 (d, *J* = 8.0 Hz, 1H), 4.12-4.04 (m, 1H), 3.80 (t, *J* = 4.8 Hz, 4H), 3.55 (s, 2H), 2.89 (d, *J* = 12.0 Hz, 2H), 2.50 (t, *J* = 4.8 Hz, 4H), 2.35 (s, 3H), 2.21 (dt, *J* = 11.2, 1.6 Hz, 2H), 2.09 (dd, *J* = 12.8, 2.8 Hz, 2H), 1.65-1.61 (m, 2H); HRMS (ES) 407.2593 $[M+H]^+$ $C_{23}H_{31}N_6O$ requires 407.2559.

Compound **14**



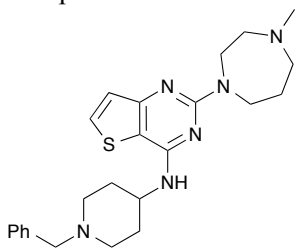
Following general procedure **B** using **11a** (50 mg, 0.15 mmol), and piperidine (0.011 mL, 1.5 mmol). the title compound was obtained and purified by column chromatography (0-4 % 7 M ammonia methanol in dichloromethane). This gave **14** as a white solid (21 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 2.0 Hz, 1H), 7.36-7.28 (m, 5H), 6.62 (d, *J* = 2.4, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 4.12-4.05 (m, 1H), 3.74 (br s, 4H), 3.58 (s, 2H), 2.91 (d, *J* = 12.0 Hz, 2H), 2.24 (t, *J* = 11.2, 2H), 2.12 (d, *J* = 11.2 Hz, 2H), 1.65-163 (m, 8H); HRMS (ES) 392.2460 $[M+H]^+$ $C_{23}H_{30}N_5O$ requires 392.2450.

Compound **15**



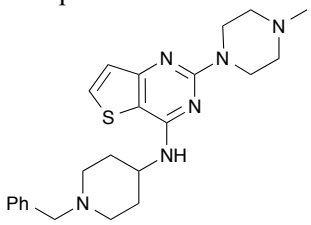
Following general procedure **B** using **11a** (40 mg, 0.12 mmol), and 1-(2-pyridyl)piperazine (0.011 mL, 1.5 mmol), the title compound was obtained and purified by column chromatography (0-3 % 7 M ammonia methanol in dichloromethane). This gave **15** as a white solid (50 mg, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) 8.25 (d, *J* =7.2 Hz, 1H), 7.80(d, *J* =2.0 Hz, 1H), 7.53-7.49 (m, 1H), 7.36-7.28 (m, 5H), 6.71 (d, *J* = 8.4 Hz, 1H), 6.67-6.64 (m, 2H), 4.86(d, *J* = 8.0 Hz, 1H), 4.15-4.06 (m, 1H), 3.91(t, *J* = 6.0 Hz, 4H), 3.65(t, *J* = 6.0 Hz, 4H), 3.58 (s, 2H), 2.91 (d, *J* = 12.0 Hz, 2H), 2.23 (t, *J* = 10.4, 2H), 2.12 (d, *J* = 10.4 Hz, 2H), 1.68-1.59 (m, 2H); HRMS (ES) 470.2655 $[M+H]^+$ $C_{27}H_{32}N_7O$ requires 470.2668.

Compound **16**



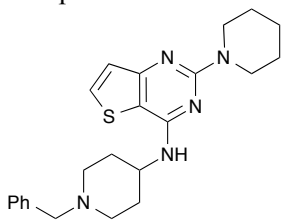
Following general procedure **B** using **11c** (100 mg, 0.27 mmol), and 1-methylhomopiperazine (0.032 mL, 2.7 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **16** as a white solid, (42 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.50 (d, *J* = 5.6 Hz, 1H), 7.34 - 7.33 (m, 4H), 7.29 - 7.24 (m, 1H), 7.14 (d, *J* =5.6 Hz, 1H), 4.53(d, *J* = 6.8 Hz, 1H), 4.13-4.04 (m, 1H), 3.96 (t, *J* = 4.8 Hz, 2H), 3.85 (t, *J* = 6.4 Hz, 2H), 3.54 (s, 2H), 2.89 (d, *J* = 12.0 Hz, 2H), 2.70 (t, *J* = 4.8 Hz, 2H), 2.57 (t, *J* = 6.4 Hz, 2H), 2.37 (s, 3H), 2.21- 2.10 (m, 4H), 2.04 - 1.98 (m, 2H), 1.62-158 (m, 2H); HRMS (ES) 437.2493 $[M+H]^+$ $C_{24}H_{33}N_6S$ requires 437.2484.

Compound **17**



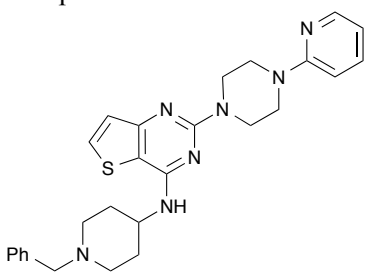
Following general procedure **B** using **11c** (178 mg, 0.50 mmol), and 1-methylpiperazine (0.029 mL, 2.5 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **17** as a yellow solid (186 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.53 (d, J = 5.6 Hz, 1H), 7.35-7.27 (m, 5H), 7.14 (d, J = 5.6 Hz, 1H), 4.58 (d, J = 7.2 Hz, 1H), 4.15-4.04 (m, 1H), 3.86 (t, J = 4.8 Hz, 4H), 3.57 (s, 2H), 2.91 (d, J = 11.6 Hz, 2H), 2.52 (t, J = 4.8 Hz, 4H), 2.37 (s, 3H), 2.22 (t, J = 10.0, 2H), 2.12 (d, J = 10.0 Hz, 2H), 1.65-1.62 (m, 2H); HRMS (ES) 423.2326 $[M+H]^+$ $C_{23}H_{31}N_6S$ requires 423.2331.

Compound **18**



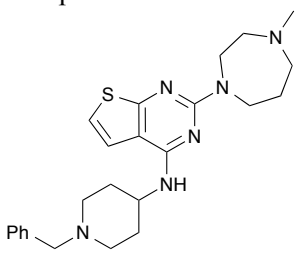
Following general procedure **B** using **11c** (100 mg, 0.27 mmol), and piperidine (0.032 mL, 2.7 mmol), the title compound was obtained and purified by column chromatography (0-4 % 7 M ammonia methanol in dichloromethane). This gave **18** as a yellow solid (42 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.52 (d, J = 5.6 Hz, 1H), 7.36-7.28 (m, 5H), 7.13 (d, J = 5.6 Hz, 1H), 4.52 (d, J = 7.2 Hz, 1H), 4.18-4.08 (m, 1H), 3.78 (t, J =4.4 Hz, 4H), 3.59 (s, 2H), 2.91 (d, J = 12.0 Hz, 2H), 2.52 (t, J = 11.6 Hz, 2H), 2.13 (d, J = 12.0 Hz, 2H), 1.68-1.55 (m, 8H); MS (ESI) 408.47 $[M+H]^+$ $C_{23}H_{30}N_5S$ requires 408.22.

Compound **19**



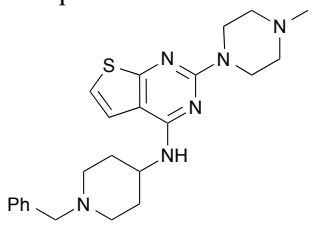
Following general procedure **B** using **11c** (100 mg, 0.21 mmol), and 1-(2-pyridyl)piperazine (0.016 mL, 1.05 mmol), the title compound was obtained and purified by column chromatography (0-3 % 7 M ammonia methanol in dichloromethane). This gave **19** as a pale yellow solid (85 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) 8.22 (d, J = 3.6 Hz, 1H), 7.55-7.53 (m, 2H), 7.36-7.16 (m, 5H), 7.16 (d, J = 5.2 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.64-6.63 (m, 1H), 4.71 (d, J = 7.2 Hz, 1H), 4.19-4.11 (m, 1H), 3.96 (t, J = 4.8 Hz, 4H), 3.65 (t, J = 4.8 Hz, 4H), 3.59 (s, 2H), 2.94 (d, J = 12.0 Hz, 2H), 2.24 (t, J = 11.2 Hz, 2H), 2.14 (d, J = 12.0 Hz, 2H), 1.67-1.63 (m, 2H); HRMS (ES) 486.2488 $[M+H]^+$ $C_{27}H_{32}N_7S$ requires 486.2480.

Compound **20**



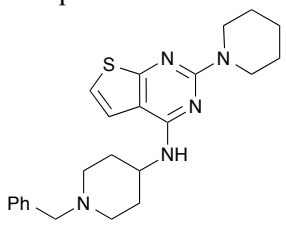
Following general procedure **B** using **11b** (50 mg, 0.13 mmol), and 1-methylhomopiperazine (0.016 mL, 1.3 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **20** as a white solid, (54 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.35-7.25 (m, 5H), 6.91 (d, J = 6.0 Hz, 1H), 6.75 (d, J = 6.0 Hz, 1H), 4.8 (br s, 1H), 4.11 - 4.02 (m, 1H), 3.95 (t, J = 4.8 Hz, 2H), 3.85 (t, J = 6.4 Hz, 2H), 3.58 (s, 2H), 2.89 (d, J =12.0 Hz, 2H), 2.69 (t, J = 4.8 Hz, 2H), 2.56 (t, J = 6.4 Hz, 2H), 2.37 (s, 3H), 2.19 (t, J = 15.6 Hz, 2H), 2.11 (d, J =15.6 Hz, 2H), 2.00 (d, J = 5.2 Hz, 2H), 1.61-1.57 (m, 2H); HRMS (ES) 437.2487 $[M+H]^+$ $C_{24}H_{33}N_6S$ requires 437.2487.

Compound **21**



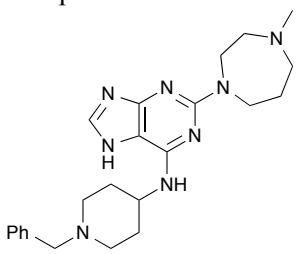
Following general procedure **B** using **11b** (100 mg, 0.27 mmol), and 1-methypiperazine (0.015 mL, 1.39 mmol), the title compound was obtained and purified by column chromatography (0-9 % 7 M ammonia methanol in dichloromethane). This gave **21** as a pale yellow solid (105 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.35-7.25 (m, 5H), 6.94 (d, J = 5.6 Hz, 1H), 6.79 (d, J = 5.6 Hz, 1H), 4.92 (d, J = 7.2Hz, 1H), 4.14-4.05 (m, 1H), 3.86 (t, J = 4.4 Hz, 4H), 3.57 (s, 2H), 2.91 (d, J = 11.6 Hz, 2H), 2.50 (t, J = 4.8 Hz, 4H), 2.36 (s, 3H), 2.23 (t, J = 11.2, 2H), 2.12 (d, J = 9.6 Hz, 2H), 1.64-1.60 (m, 2H); MS (ESI) 423.49 $[M+H]^+$ $C_{23}H_{31}N_6S$ requires 422.23.

Compound **22**



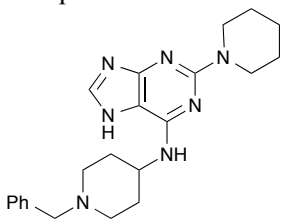
Following general procedure **B** using **11b** (100 mg, 0.27 mmol), and piperidine (0.013 mL, 1.39 mmol), the title compound was purified by column chromatography (0-4 % 7 M ammonia methanol in dichloromethane). This gave **22** as a yellow solid (81 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.36-7.27 (m, 5H), 6.93 (d, J = 6.0 Hz, 1H), 6.75 (d, J = 6.0 Hz, 1H), 4.86 (d, J = 7.2 Hz, 1H), 4.16-4.15 (m, 1H), 3.80 (t, J = 5.6 Hz, 4H), 3.57 (s, 2H), 2.91 (d, J = 12.0 Hz, 2H), 2.23 (t, J = 11.2 Hz, 2H), 2.13 (d, J = 12.0 Hz, 2H), 1.67-1.58 (m, 8H); MS (ESI) 408.47 $[M+H]^+$ $C_{23}H_{30}N_5S$ requires 407.21.

Compound **23**



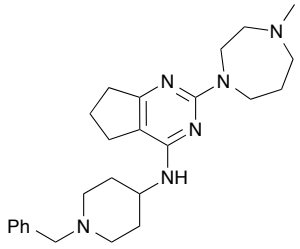
Following general procedure **B** using **11d** (100 mg, 0.29 mmol), and 1-methylhomopiperazine (0.036 mL, 2.9 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **23** as a white solid, (41 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.52 (s, 1H), 7.37-7.27 (m, 5H), 5.59 (br s, 1H), 4.09 (br s, 1H), 4.01-3.98 (m, 2H), 3.86 (t, J = 8 Hz, 2H), 3.65 (s, 2H), 2.93-2.88 (m, 2H), 2.79-2.76 (m, 2H), 2.66-2.63 (m, 2H), 2.42 (s, 3H), 2.23-2.21 (m, 2H), 2.10-2.07 (m, 4H), 1.71-1.62 (m, 2H); MS (ESI) 421.47 $[M+H]^+$ $C_{23}H_{33}N_8$ requires 421.28.

Compound **24**



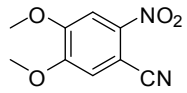
Following general procedure **B** using **11d** (80 mg, 0.24 mmol), and piperidine (0.023 mL, 2.4 mmol), the title compound was obtained and purified by column chromatography (0-5 % 7 M ammonia methanol in dichloromethane). This gave **24** as a white solid, (22 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.56 (s, 1H), 7.31-7.27 (m, 5H), 5.62 (br s, 1H), 4.14 (br s, 1H), 3.80 (t, J = 8 Hz, 4H), 3.56 (s, 2H), 2.91-2.87(m, 2H), 2.28-2.22 (m, 2H), 2.14-2.10 (m, 2H), 1.70-1.64 (m, 8H); MS (ESI) 392.42 $[M+H]^+$ $C_{22}H_{30}N_7$ requires 392.26.

Compound **25**



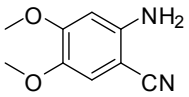
A mixture of **11e** (17 mg, 0.049 mmol) and 1-methylpiperazine (0.055 mL, 0.49 mmol) in toluene was heated at 130 ^{o}C using microwave irradiation for 1 hour. The reaction mixture was concentrated and purified by silica gel chromatography (2-5%, 7 M ammonia methanol in dichloromethane). The title compound **25** was obtained as a yellow solid (8 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.25 (m, 5H), 4.00-3.87 (m, 3H), 3.77 (t, J = 6.4 Hz, 2H), 3.52 (s, 2H), 2.86-2.83 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.66-2.63 (m, 2H), 2.55-2.49 (m, 4H), 2.38-2.35 (m, 4H), 2.17-2.11 (m, 2H), 2.05-1.93 (m, 6H), 1.50 (td, J = 14.3, 3.7 Hz, 2H); HRMS (ES) 421.3089 $[M+H]^+$ $C_{25}H_{37}N_6$ requires 421.3080.

4,5-Dimethoxy-2-nitrobenzonitrile (intermediate for compound **39a/b)**



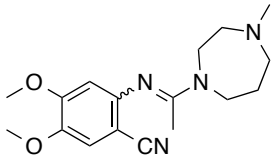
The commercially available 3-4 dimethoxybenzonitrile **38** (16.3 g, 0.1 mol) was dissolved in acetic anhydride (130 mL) at 0 ^{o}C. Excess of nitric acid (36.5 mL) was continuously added to the mixture over 15 min. Then, the reaction was allowed to room temperature and stirred overnight. The mixture was poured into ice-cooled water (1 L) and the resulting precipitate was filtered, washed with water (1 L) and dried under vacuum to afford the title product as a yellow solid in quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) 7.80 (s, 1H), 7.23 (s, 1H), 4.04 (s, 3H), 4.03 (s, 3H); HRMS (ES) 208.0416 $[M+H]^{+}$ $C_9H_8N_2O_4$ requires 208.0489.

2-Amino-4,5-dimethoxybenzonitrile (intermediate for compound **39a/b**)



To a solution of 4,5-dimethoxy-2-nitrobenzonitrile (30.5 g, 146 mmol) in dichloromethane (250 mL), tetrabutylammonium bromide (10.40 g, 36.8 mmol) was added, followed by the slow addition of a solution of sodium dithionite (100 g, 576 mmol) in water (250 mL). The mixture was stirred overnight at room temperature and then basified to pH 9 using 2 N sodium hydroxide. The aqueous layer was extracted with dichloromethane (250 mL x 3). The organic layer was concentrated to 100 mL. 4 M HCl in dioxane then was added slowly until precipitation occurred. The resulting solid was collected by filtration and re-dissolved in 2 N sodium hydroxide (200 mL). Finally, the aqueous layer was extracted again with dichloromethane (200 mL x 3), dried over magnesium sulphate and concentrated under reduced pressure to afford the title product as a fine yellow solid (14.5 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) 6.76 (s, 1H), 6.26 (s, 1H), 4.30 (br s, 2H), 3.85 (s, 3H), 3.78 (s, 3H); MS (ESI) 179.10 $[M+H]^{+}$ $C_9H_{10}N_2O_2$ requires 179.05.

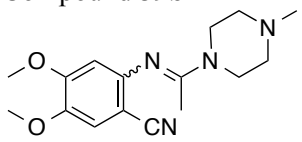
Compound **39a**



A stirred solution of 2-amino-4,5-dimethoxybenzonitrile (1.6 g, 9.14 mmol) in triethylorthoacetate (5.4 mL, 28.6 mmol) was heated at 150 ^{o}C under slightly reduced pressure for 6 h. After the reaction mixture was allowed to cool to room temperature, the mixture was concentrated and left under a high-vacuum for 48 h to afford the intermediate *N*-2-cyano-4,5-dimethoxyphenylacetimidate as a brown solid (2.16 g, 96%) that was used in the next step without further purification. HRMS (ES) 249.1240 $[M+H]^{+}$ $C_{13}H_{16}N_2O_3$ requires 249.1239. *p*-Toluenesulfonic acid (20 mg) was added into a solution of the intermediate ethyl *N*-2-cyano-4,5-dimethoxyphenylacetimidate (3.0 g, 12.0 mmol) in 1-methylhomopiperazine (4.0 mL, 24.0 mmol). The reaction mixture was heated at 100 ^{o}C for 24 h. The reaction then was allowed to cool to room temperature and dichloromethane (30 mL) was added. The organic layer was extracted with 2 N HCl (30 mL x 3) and the combined aqueous layer was basified by adding sodium carbonate powder until pH 10 was reached. The aqueous layer was extracted again with dichloromethane (100 mL x 3), dried with magnesium sulphate, and concentrated under reduced pressure. The residue was purified by column chromatography (0-10% methanol in dichloromethane) to afford the title

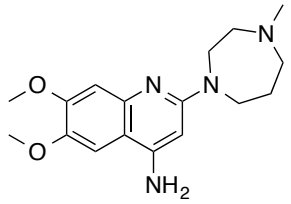
product **39a** as yellow oil (1.5 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.34 (s, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.80-3.63 (m, 4H), 2.73 (br s, 2H), 2.68-2.65 (m, 2H), 2.43 (s, 3H), 2.01-1.98 (m, 2H), 1.96 (s, 3H); HRMS (ES) 317.1985 $[M+H]^+$ $C_{17}H_{25}N_4O_2$ requires 317.1978.

Compound **39b**



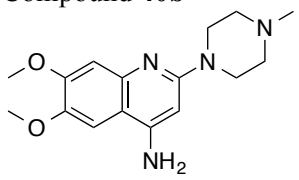
p-Toluenesulfonic acid (15 mg) was added into a solution of the intermediate ethyl *N*-2-cyano-4,5-dimethoxyphenylacetimidate (2.0 g, 8.0 mmol) in 1-methylpiperazine (1.97 mL, 16.0 mmol). The reaction mixture was heated at 100 ^{o}C for 24 h. The reaction was allowed to cool to room temperature and dichloromethane (30 mL) was added. The organic layer was extracted with 2 N HCl (30 mL x 3) and the combined aqueous layer was basified by adding sodium carbonate powder until pH 8. The aqueous layer was extracted again with dichloromethane (100 mL x 3), dried over magnesium sulphate, concentrated under reduced pressure. The residue was purified by column chromatography (0-4% 7 N ammonia methanol in dichloromethane) to afford the title product **39b** as yellow oil (990 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.34 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.65 (t, J = 8 Hz, 4H), 2.49 (t, J = 8 Hz, 4H), 2.32 (s, 3H), 1.94 (s, 3H); HRMS (ES) 303.1827 $[M+H]^+$ $C_{16}H_{23}N_4O_2$ requires 303.1821.

Compound **40a**



A solution of **39a** (1.4 g, 4.43 mmol) and zinc chloride (3.61 g, 26.58 mmol) in dimethylacetamide (40 mL) was heated at reflux for 36 h. The reaction mixture was cooled down to room temperature and diethyl ether (400 mL x 2) was added to the mixture and it was stirred for 10 min. The supernatant was discarded each time. The tar residue was treated with 2 N sodium hydroxide (200 mL) and the mixture was stirred at room temperature for 10 min. The resulting suspension was extracted by dichloromethane (100 mL x 3). The combined organic layer was dried with magnesium sulphate and concentrated under reduce pressure. The residue was purified by column chromatography (0-12% 7M ammonia methanol in dichloromethane) to afford the title compound **40a** as a yellow crystalline solid (456 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (br s, 1H), 6.85 (s, 1H), 5.99 (s, 1H), 4.33 (br s, 2H), 4.00 (s, 3H), 3.96 (s, 3H), 3.95-3.92 (m, 2H), 3.75 (t, J = 4 Hz, 2H), 2.74 (m, 2H), 2.62-2.58 (m, 2H), 2.40 (s, 3H), 2.17-2.13 (m, 2H); HRMS (ES) 317.1992 $[M+H]^+$ $C_{17}H_{25}N_4O_2$ requires 317.1978.

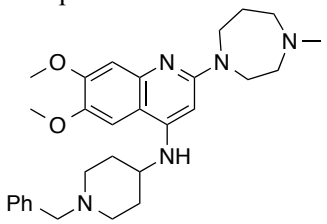
Compound **40b**



A solution of **39b** (800 mg, 3.04 mmol) and zinc chloride (2.48 g, 18.24 mmol) in dimethylacetamide

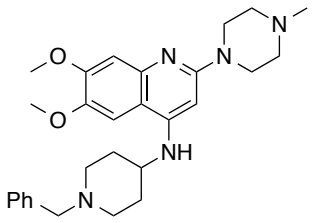
(40 mL) was heated at reflux for 24 h. After the reaction was cooled down to the room temperature and diethyl ether (100 mL x 2) was added to the mixture and it was stirred for 10 min. The supernatant was discarded each time. The tar residue was treated with 2 N sodium hydroxide (100 mL) and the mixture was stirred at room temperature for 10 min. The resulting suspension was extracted by dichloromethane (100 mL x 3). The combined organic layer was dried over magnesium sulphate and concentrated under reduce pressure. The residue was purified by column chromatography (0-5% 7 M ammonia methanol in dichloromethane) to afford the title compound **40b** as a yellow crystalline solid (330 mg, 36%). ^{1}H NMR (400 MHz, MeOD) δ 7.27 (s, 1H), 7.04 (s, 1H), 6.15 (s, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.56 (t, *J* = 8 Hz, 4H), 2.58 (t, *J* = 8, 4H), 2.35 (s, 3H); HRMS (ES) 303.1828 $[M+H]^+$ $C_{16}H_{23}N_4O_2$ requires 303.1821.

Compound **41**



A mixture of **40a** (166 mg, 0.51 mmol), 1- benzyl-4-piperidone (94 µL, 0.56 mmol) and acetic acid (15 µL) in toluene (10 mL) was refluxed using Dean-Stark apparatus for 2 days. The mixture was then concentrated under reduced pressure andtetrahydrofuran (10 mL) and sodium triacetoxyborohydride (215 mg, 1.02 mmol) was added. The reaction mixture was heated at reflux for 2 days. After the reaction was allowed to cool to room temperature, it was concentrated under reduced pressure. The residue was suspended in 2 N sodium hydroxide (25 mL) and the aqueous layer was extracted with dichloromethane. The combined organic layer was dried with magnesium sulphate and concentrated. The resulting residue was purified by silica column chromatography (0-4% 7 M ammonia methanol in dichloromethane) to give a mixture of starting material and the title product hat was further purified by preparative TLC (1% ammonia methanol in dichloromethane, repeated until R_f ~0.5) to afford the final compound **41** as white solid (52 mg, 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5H), 7.11 (br s, 1H), 6.76 (s, 1H), 5.77 (s, 1H), 4.36 (br s, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.95-3.93 (m, 2H), 3.58 (t, *J* = 6 Hz, 2H), 3.58 (s, 2H), 3.52-3.51 (m, 1H), 2.91 (d, *J* = 12.0 Hz, 2H), 2.75 (t, *J* = 4.4 Hz, 2H), 2.60 (t, *J* = 5.2 Hz, 2H), 2.40 (s, 3H), 2.25 (t, *J* =10.4 Hz, 2H), 2.16(d, *J* =12.0 Hz, 2H), 2.05(m, 2H), 1.70-16.7 (m, 2H); HRMS (ES) 490.3186 $[M+H]^+$ $C_{29}H_{40}N_5O_2$ requires 409.32182.

Compound **42**



A mixture of **40b** (90 mg, 0.30 mmol), 1- benzyl-4-piperidone (55 µL, 0.30 mmol) and acetic acid (5 µL) in toluene (10 mL) was refluxed using Dean-Stark apparatus for 4 days. The mixture then was concentrated under reduced pressure and tetrahydrofuran (10 mL) and sodium borohydride (67.6 mg, 1.8 mmol) was added. The resultant reaction mixture was heated at reflux for 1 day. After the reaction was allowed to cool to room temperature, it was concentrated under reduced pressure. The residue was suspended in 2 N sodium hydroxide (25 mL) and the aqueous layer was extracted with dichloromethane. The combined organic layer was dried over magnesium sulphate and concentrated. The resulting residue was repeatedly purified by silica column chromatography (0-3% 7 M ammonia methanol in dichloromethane) to afford the title product **42** as yellow solid (14 mg, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.27 (m, 5H), 7.15 (br s, 1H), 6.75 (s, 1H), 5.89 (br s, 1H), 4.34 (br s, 1H),

3.97 (s, 3H), 3.95 (s, 3H), 3.65-3.64 (m, 4H), 3.57 (s, 2H), 3.53-3.49 (m, 1H), 2.90 (d, J = 12.0 Hz, 2H), 2.56 (t, J = 4.8 Hz, 4H), 2.35 (s, 3H), 2.23 (dt, J = 11.6, 2.4 Hz, 2H), 2.14 (dd, J = 11.6, 4 Hz, 2H), 1.67-1.65 (m, 2H); HRMS (ES) 476.3042 $[M+H]^+$ $C_{28}H_{38}N_5O_2$ requires 476.3026.

Table S1. Selectivity Data of BIX-01294, 41 and 42.

Protein	Activity (%)								
	BIX-01294 (µM)			Compound 41			Compound 42		
	50	10	1	50	10	1	50	10	1
G9a	6	6	11	5	4	6	5	6	7
EHMT1 (GLP)	2	1	3	1	1	2	1	1	2
SUV39H2	83	98	102	74	95	107	85	105	116
PRDM9	58	89	95	67	89	104	54	87	90
SETD7	114	106	104	106	106	106	109	105	107
MLL1	78	96	101	73	93	99	89	92	103
SETD8	101	108	106	97	98	99	100	84	104
SUV420H1	80	105	108	69	92	97	86	93	101
SUV420H2	84	100	107	94	104	107	101	99	106
PRMT1	103	104	99	100	98	103	105	97	102
PRMT3	83	99	94	77	100	99	95	98	103
PRMT5	99	95	98	103	101	104	101	97	102
PRMT6	76	99	100	40	116	100	71	93	99
PRMT7	100	100	95	99	109	101	101	94	98
PRMT8	81	92	118	46	97	95	69	88	100
DOT1L	112	113	103	122	116	90	109	105	101
EZH1	76	95	99	69	100	103	93	101	108
EZH2	35	76	97	25	72	93	55	88	101
SMYD2	65	94	106	59	95	111	76	99	112
NSD1	280	169	108	567	284	131	447	208	112
NSD2	293	161	111	615	227	119	361	180	106
NSD3	248	157	110	701	285	121	567	208	109
SETD2	6	67	94	1	44	94	22	77	104
DNMT1	126	105	105	140	130	99	137	115	110

Selectivity Assay Methods:

Effects of BIX-01294, compound **41** and **42** on methyltransferase activity of G9a, EHMT1, SUV39H2, SUV420H1, SUV420H2, SETD7, SETD8, SETD2, MLL1 complex, EZH1 complex EZH2 complex, PRMT1, PRMT3, PRMT5-MEP50 complex, PRMT6, PRMT7, PRMT8, SMYD2, DNMT1, PRDM9, was assessed by monitoring the incorporation of tritium-labeled methyl group to lysine or arginine residues of peptide substrates using Scintillation Proximity Assay (SPA) (PMID: 25032507). Assays were performed in a 10 µl reaction mixture containing ^{3}H-SAM (Cat.# NET155V250UC; Perkin Elmer; www.perkinelmer.com) at substrate concentrations close to K_m values for each enzyme. Three compound concentrations (1, 10 and 50 µM) were used in all selectivity assays. To stop the enzymatic reactions, 10 µl of 7.5 M Guanidine hydrochloride was added, followed by 180 µl of buffer (20 mM Tris, pH 8.0), mixed and then transferred to a 96-well FlashPlate (Cat.# SMP103; Perkin Elmer; www.perkinelmer.com). After mixing, the reaction mixtures in Flash plates were incubated for 1 hour and the CPM were measured using Topcount plate reader (Perkin Elmer, www.perkinelmer.com). The CPM counts in the absence of compound for

each data set were defined as 100% activity. In the absence of the enzyme, the CPM counts in each data set were defined as background (0%).

For DNMT1 the dsDNA substrate was prepared by annealing two complementary strands (biotintlated forward strand: B-GAGCCCGTAAGCCCGTTCAGGTCG and reverse strand: CGACCTGAACGGGCTTACGGGCTC), synthesized by Eurofins MWG Operon.

For DOT1L, NSD1, NSD2, and NSD3 a filter-based assay was used. In this assay, 10 µl of reaction mixtures were incubated at RT for 1 hour, 60 µl of 10% TCA was added, mixed and transferred to filter-plates (Millipore; cat.# MSFBN6B10; www.millipore.com). Plates were centrifuged at 2000 rpm (Allegra X-15R - Beckman Coulter, Inc.) for 2 min followed by 2 additional 10% TCA wash and one ethanol wash (180 µl) followed by centrifugation. Plates were dried and 20 µl MicroO (MicroScint-O; Cat.# 6013611, Perkin Elmer; www.perkinelmer.com) was added to each well, centrifuged and removed. 20 µl of MicroO was added again and CPM was measured using Topcount plate reader.

pKa Measurements:

All pKa measurements were conducted by Sirius Analytical Ltd. Briefly, the samples were initially investigated using a Fast-UV titration from pH 2 – 12 at concentrations of 31 – 19 µM under aqueous conditions. No precipitation or decomposition of the sample was observed and three approximate pKas were observed for each sample. The samples were subsequently titrated in a triple titration from pH 2 to pH 12 at concentrations of 32 – 23 µM under aqueous conditions. No precipitation or decomposition of the sample was observed and three pKas were determined from spectroscopic data for each compound.

Cmpd	Measured pKa	T/°C	Ionic Environment	Experimental method	Calculated pKa[1]	Assignment based on calculated pKa and UV-vis data (Fig S5 and S6)
1 (BIX-01294)	6.94 ± 0.01	25.0	0.15 M KCl	UV-metric	8.13 ± 2.22	N-1
	8.24 ± 0.05	25.0	0.15 M KCl	UV-metric	8.51 ± 0.78	homo piperazine nitrogen
	9.22 ± 0.04	25.0	0.15 M KCl	UV-metric	10.35 ± 0.78	benzylpiperidine nitrogen
41	7.14 ± 0.01	25.0	0.15 M KCl	UV-metric	8.18 ± 0.78	homo piperazine nitrogen
	8.05 ± 0.02	25.0	0.15 M KCl	UV-metric	10.35 ± 0.78	benzylpiperidine nitrogen
	9.50 ± 0.01	25.0	0.15 M KCl	UV-metric	10.57 ± 2.22	N-1

[1]'Sequential' pKa value were calculated at pH 7.0, with water as the solvent model, using the Epik 2.7 program implemented in Schrodinger (see above).

Mean pKa result

pKa	Std Dev	Ionic strength	Temperature
6.94	0.012	0.165 M	25.0℃
8.24	0.054	0.165 M	25.0℃
9.22	0.044	0.165 M	25.0℃

Mean pKa individual results

Titration	Direction	Ionic strength	Temperature	Chi Squared	pKa 1	pKa 2	pKa 3
14H-11007 Points 4 to 47	Up	0.153 M	25.0℃	0.2508 ☑	6.93 ☑	8.21 ☑	9.18
14H-11007 Points 49 to 94	Up	0.165 M	25.0℃	0.3107 ☑	6.95 ☑	8.22 ☑	9.21
14H-11007 Points 96 to 142	Up	0.176 M	25.0℃	0.3752 ☑	6.95 ☑	8.31 ☑	9.27

Graphs

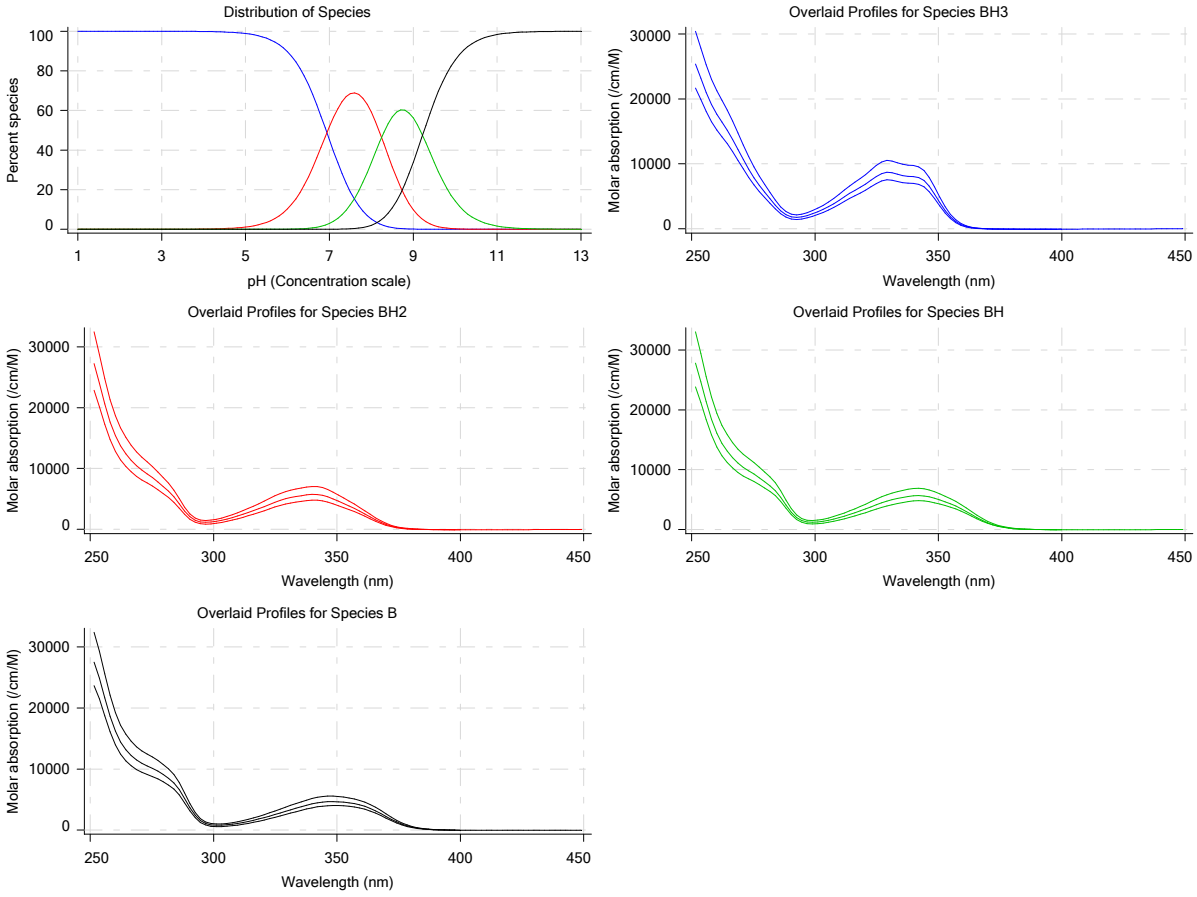


Figure S5: pKa determination of BIX-01294 (**1**) by UV-vis, determined using three independent measurements. 'B' corresponds to the free base species and 'BH', 'BH2' and 'BH3' to the mono-, di-, and tri-protonated species respectively. The largest spectral change, assigned to protonation/deprotontation at N-1, is observed upon moving from BH2 to BH3.

Mean pKa result

pKa	Std Dev	Ionic strength	Temperature
7.14	0.010	0.165 M	25.0℃
8.05	0.016	0.165 M	25.0℃
9.50	0.012	0.165 M	25.0℃

Mean pKa individual results

Titration	Direction	Ionic strength	Temperature	Chi Squared	pKa 1	pKa 2	pKa 3
14H-11008 Points 4 to 49	Up	0.153 M	25.0℃	0.0802 ☑	7.13 ☑	8.03 ☑	9.49
14H-11008 Points 51 to 98	Up	0.165 M	25.0℃	0.1471 ☑	7.13 ☑	8.05 ☑	9.50
14H-11008 Points 100 to 147	Up	0.175 M	25.0℃	0.1561 ☑	7.15 ☑	8.07 ☑	9.51

Graphs

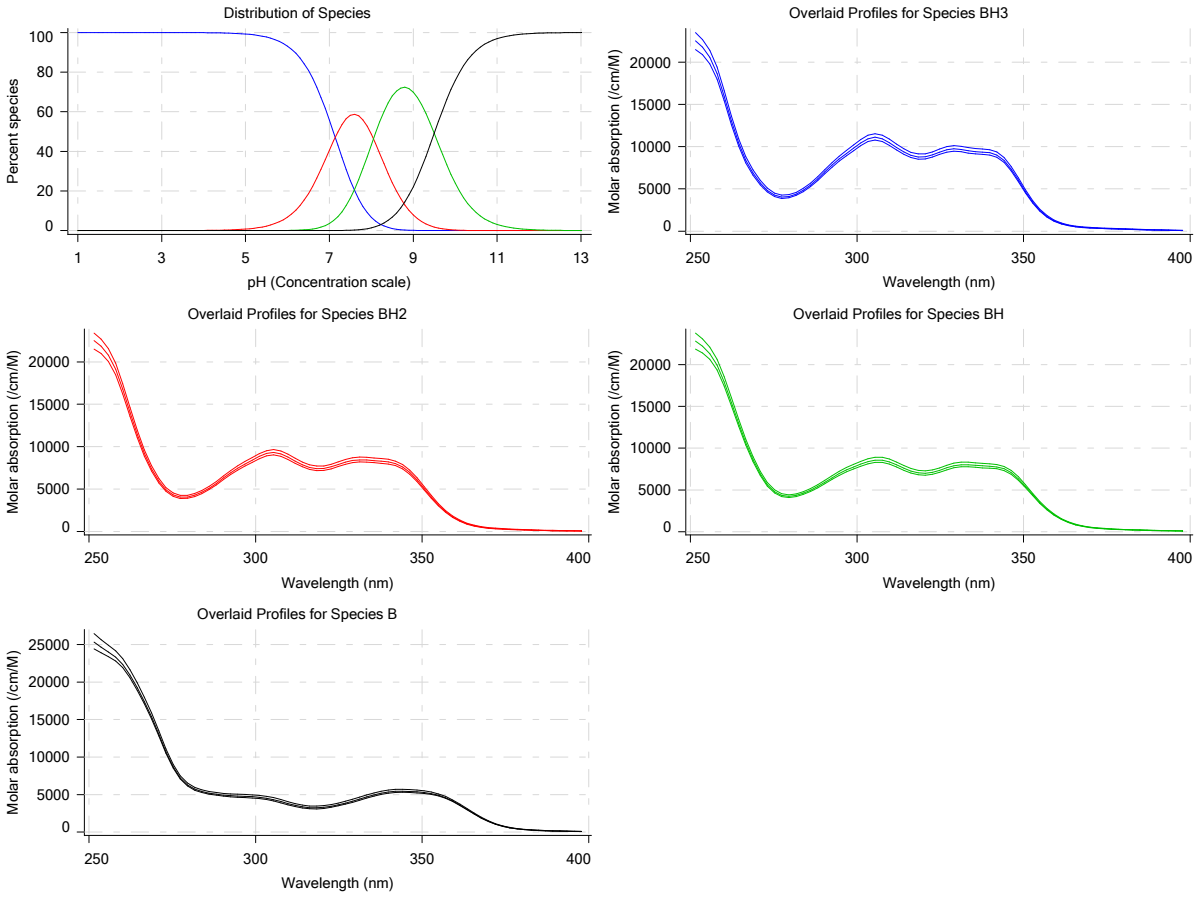


Figure S6: pKa determination of quinoline **41** by UV-vis, determined using three independent measurements. 'B' corresponds to the free base species and 'BH', 'BH2' and 'BH3' to the mono-, di-, and tri-protonated species respectively. The largest spectral change, assigned to protonation/deprotontation at N-1, is observed upon moving from B to BH.

References

1. *Schrödinger Release 2014-1*: Maestro, version 9.7, Schrödinger, LLC, New York, NY, **2014**.
2. F. Liu, X. Chen, A. Allali-Hassani, A. M. Quinn, G. A. Wasney, A. Dong, D. Barsyte, I. Kozieradzki, G. Senisterra, I. Chau, A. Siarheyeva, D. B. Kireev, A. Jadhav, J. M. Herold, S. V. Frye, C. H. Arrowsmith, P. J. Brown, A. Simeonov, M. Vedadi and J. Jin, *J. Med. Chem.*, 2009, **52**, 7950-7953.
3. *Schrödinger Release 2014-1*: Prime, version 3.5, Schrödinger, LLC, New York, NY, **2014**.
4. W. L. Jorgensen, D. S. Maxwell and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1996, **118**, 11225-11236.
5. *Schrödinger Release 2014-1*: LigPrep, version 2.9, Schrödinger, LLC, New York, NY, **2014**.
6. *Schrödinger Release 2014-1*: Epik, version 2.7, Schrödinger, LLC, New York, NY, **2014**.
7. J. Shelley, A. Cholleti, L. Frye, J. Greenwood, M. Timlin and M. Uchimaya, *J. Comput. Aided Mol. Des.*, 2007, **21**, 681-691.
8. J. Greenwood, D. Calkins, A. Sullivan and J. Shelley, *J. Comput. Aided Mol. Des.*, 2010, **24**, 591-604.
9. *Small-Molecule Drug Discovery Suite 2014-1*: Glide, version 6.2, Schrödinger, LLC, New York, NY, **2014**
10. T. A. Halgren, R. B. Murphy, R. A. Friesner, H. S. Beard, L. L. Frye, W. T. Pollard and J. L. Banks, *J. Med. Chem.*, 2004, **47**, 1750-1759.
11. R. A. Friesner, J. L. Banks, R.B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, D. E. Shaw, P. Francis and P. S. Shenkin, *J. Med. Chem.*, 2004, **47**, 1739-1749.
12. R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin and D. T. Mainz, *J. Med. Chem.*, 2006, **49**, 6177-6196.
13. *Discovery Studio Modeling Environment*, Release 4.0, San Diego: Accelrys Software Inc., **2013**.
14. T. P. Heffron, B. Wei, A. Olivero, S. T. Staben, V. Tsui, S. Do, J. Dotson, A. J. Folkes, P. Goldsmith, R. Goldsmith, J. Gunzner, J. Lesnick, C. Lewis, S. Mathieu, J. Nonomiya, S. Shuttleworth, D. P. Sutherlin, N. C. Wan, S. Wang, C. Wiesmann and B. Y. Zhu, *J. Med. Chem.,* 2011, **54**, 7815-7833.