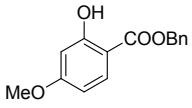
SUPPORTING INFORMATION

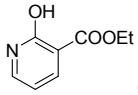
CHEMISTRY

General methods. All solvents and reagents were purchased from commercial sources and used without further purification. The compounds were spotted on silica TLC plates (Merck, Si_{60}, F254), visualized under UV-light at 254 nm or iodine over silica. Purification of the compounds for biological tests was performed on a Waters 2767 system equipped with a photodiode array and an ESI mass spectrometer using a XBridge Prep C18 (5 µm, 19 mm × 100 mm) column, equipped with an XBridge Prep C18 guard column (5 µm, 19 mm × 10 mm). The mobile phase consisted of water plus 0.1% formic acid (solvent A) and methanol plus 0.1% formic acid (solvent B), with an elution method of 0-1 min 5% B, 1-10 min 5%-95% B, 10-12 min 95%, 12-13 min 95%-5% B, 13-18 min 5% at a flow rate of 20 mL/min. The purity of these compounds was verified by analytical LC-MS on the same machine, but using an X-Bridge C18 column (5 µM, 4.6 x 100 mM) equipped with an XBridge C18 guard column (5 µm, 4.6 mm × 20 mm) and a flow rate of 1.2 mL/min instead. Purity of tested compounds was ≥ 95%. One-dimensional ^{1}H-spectra were recorded on a Bruker AV instrument at 400 MHz. Chemical shifts are reported in ppm. High-resolution mass spectra were obtained from the Mass Spectrometry Service of Department of Chemistry, Imperial College London.

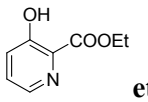


benzyl 2-hydroxy-4-methoxybenzoate (9a). A mixture of 2-hydroxy-4-methoxybenzoic acid (168 mg, 1 mmol), benzylbromide (125 µL, 1.05 mmol) and potassium carbonate (276 mg, 2 mmol) in DMF (2 mL) was stirred at room temperature for 2 hours. The

reaction mixture was diluted with ethyl acetate (20 mL) and the organic phase washed with 20 mL water, then with brine (20 mL). The organic phase was dried over Na_2SO_4 and the organic solvent was removed under reduced pressure. The resulting residue was purified by column chromatography over silica gel to afford the title compound as a white solid (220 mg, yield: 86%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 11.00 (s, 1H),7.82 (d, J=7.6 Hz, 1H), 7.50-7.33 (m, 5H), 6.48 (dd, J=7.6, 2.4 Hz, 1H), 6.45 (d, J=2.4 Hz, 1H), 5.38 (s, 2H), 3.85 (s, 3H). The synthesis procedure to afford **4a**-**6a**, **8a**-**16a** is similar to **9a**.

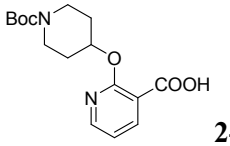


ethyl 2-hydroxynicotinate (7a). 2-hydroxynicotinic acid (139 mg, 1 mmol) was dissolved in thionyl chloride (1 mL) and allowed to heat at 90 ºC for 1 hour. The reaction mixture was then cooled to room temperature, concentrated under vacuum, and treated with ethanol (5 mL) for another 1 hour. After the reaction went to completion, the resulting mixture was then concentrated to afford the title compound as a light yellow solid (158 mg, yield: 95%), without further purification.^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.39 (d, J=7.2 Hz, 1H), 8.05 (d, J=7.2 Hz, 1H), 6.67 (t. J=7.2 Hz, 1H), 4.44 (q, J=6.8 Hz, 2H), 1.43 (t, J=6.8 Hz, 3H).

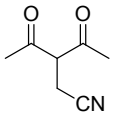


ethyl 3-hydroxypicolinate (17a). 3-hydroxypicolinic acid (139 mg, 1 mmol) was suspended in a mixture of ethanol (4 mL) and benzene (2 mL). Sulfuric acid (300 μL) was added and the reaction mixture was heated at reflux with azeotropic removal of water via Dean-Stark trap. After the reaction was complete, the organics were removed *in vacuo*. The

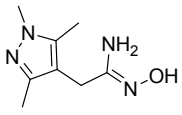
residue was dissolved in water, basified with sodium hydroxide, and extracted into ethyl acetate. The ethyl acetate layer was dried over MgSO4, concentrated *in vacuo* to give the title compound as a light-yellow solid (116 mg, 70%), without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 10.78 (s, 1H), 8.30 (s, 1H), 7.49-7.35 (m, 2H), 4.54 (q, J=7.2 Hz, 2H), 1.49 (t, J=7.2 Hz, 3H).



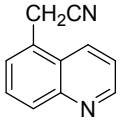
2-(1-(*t*-butoxycarbonyl)piperidin-4-yloxy)nicotinic acid (7b). To a stirred solution of **7a** (158 mg, 0.95 mmol), *t*-Butyl 4-hydroxypiperidine-1-carboxylate (484 mg, 2.38 mmol) and triphenylphosphine (630 mg, 2.38 mmol) in anhydrous THF (4 mL), DIAD (459 μL, 2.38 mmol) was added dropwise at room temperature. The resulting mixture was stirred at room temperature for 4 hours, and then concentrated *in vacuo*. The residue was re-dissolved in a mixture of 4N NaOH (1.2 mL) and MeOH (5 mL) and the mixture was allowed to heat at 50 ºC for another 2 hours. After the reaction was complete, the mixture was partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was then treated with 6N HCl to pH 3 and the free carboxylic acid was extracted into ethyl acetate (2 x 15 mL). After drying over $MgSO_4$, removal of organic solvent gave the title compound as colorless oil (307 mg, quantitative yield). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.47 (dd, J=7.6, 2.0 Hz, 1H), 8.37 (dd, J=4.8, 2.0 Hz, 1H), 7.12 (dd, J=7.6, 4.8 Hz, 1H), 5.59 (m, 1H), 3.91-3.81 (m, 2H), 3.40-3.29 (m, 2H), 2.17-2.08 (m, 2H), 1.92-1.79 (m, 2H), 1.50 (s, 9H). The synthesis procedure to afford **4b**-**6b**, **8b**-**17b** is similar to **7b**.



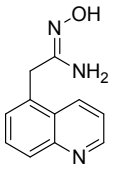
3-acetyl-4-oxopentanenitrile (25a). NaH (900 mg, 60% in oil, 22.5 mmol) was added slowly to a stirred solution of acetyl acetone (1.50 mL, 15.0 mmol) in dry THF (15 mL) at room temperature. The mixture was stirred for 1 hour and then bromoacetonitrile (1.26 mL, 18.0 mmol) was added dropwise and the resulting solution was stirred for another 4 hours. The excess NaH was cautiously quenched with 50 mL of water, followed by the extraction into ethyl acetate (3 x 30 mL). The combined ethyl acetate layers were dried over Na_2SO_4 and the organic solvent was removed under reduced pressure. The resulting residue was purified by column chromatography over silica gel to afford the title compound as yellow liquid (835 mg, yield: 40%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.06 (t, J=7.2 Hz, 1H), 2.84 (d, J=7.2 Hz, 2H), 2.36 (s, 6H).



***N′*-hydroxy-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)acetimidamide (25b)**. To a solution of **25a** (139 mg, 1 mmol) in MeOH (3 mL), methylhydrazine (54 µL, 1.05 mmol) was added. The mixture was stirred at 65 ºC for 4 hours, followed by the addition 50% hydroxylamine (50% wt in H_2O, 324 µL, 4 mmol). The resulting mixture was kept at 65ºC for another 6 hours. After the reaction went to completion, the solution was concentrated to afford the title compound as an orange solid (173 mg, yield: 95%) without further purification. LC-MS purity: 98%.



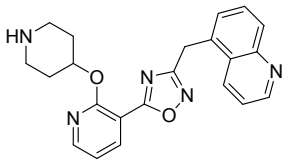
2-(quinolin-5-yl)acetonitrile (30a). $NaBH_4$ (4.35 mmol) was added to a mixture of quinoline-5-carbaldehyde (683 mg, 4.35 mmol) in MeOH (5 mL) at 0 ºC and stirred for 8 hours at room temperature. The reaction was quenched by ice pieces and concentrated under reduced pressure. The residue was suspended in water (30 mL) and extracted into ethyl acetate (2 x 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The resulting residue was re-dissolved in a solution of Et_3N (1.21 mL, 8.70 mmol) and DCM (5 mL), followed by the addition of methanesulfonyl chloride (355 µL, 4.57 mmol) at 0 ºC. After the reaction was complete, the reaction was quenched by ice pieces and concentrated under reduced pressure. The residue was suspended in water (30 mL) and extracted into ethyl acetate (2 x 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The above residue was dissolved in DMSO (4 mL) and NaCN (256 mg, 5.22 mmol) was added. The mixture was stirred at 60 ºC for 1 hr. EtOAc (50 mL) was added to the reaction mixture, and the organic layer was washed successively with water (50 mL) and brine (50 mL), and dried over $MgSO_4$. The resulting residue was purified by column chromatography over silica gel to afford the title compound as an off-white solid (157 mg, yield: 22%). LC-MS purity: 97%.



***N′*-hydroxy-2-(quinolin-5-yl)acetimidamide (30b)**. A solution of **30a** (100 mg, 0.60 mmol) in MeOH (4 mL) was added 50% hydroxylamine (50% wt in H_2O, 150 µL, 2.40 mmol). The resulting mixture was kept at 65ºC for another 6 hours. After the reaction went to

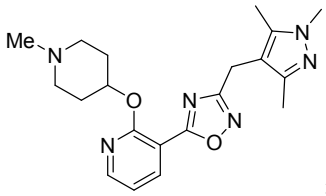
completion, the solution was concentrated to afford the title compound as an off-white solid (117 mg, yield: 97%) without further purification. LC-MS purity: 95%.

Prototypical procedure for oxadiazole formation.

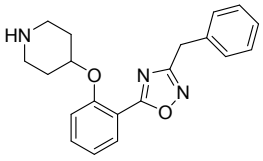


5-(2-(piperidin-4-yloxy)pyridin-3-yl)-3-(quinolin-5-ylmethyl)-1,2,4-oxadiazole (30). A mixture of **7b** (64 mg, 0.2 mmol), EDCI (42 mg, 0.22mmol), HOBt (35 mg, 0.26mmol) in anhydrous acetonitrile (3 mL) was stirred at room temperature for 30 minutes, and then treated with **30b** (43 mg, 0.21 mmol) and DIPEA (70 μL, 0.40 mmol). The resulting mixture was further stirred at room temperature for 12 hours. After that, the solution was evaporated to dryness *in vacuo*. The residue was treated with 0.5 N NaOH (20 mL) and left for another 0.5 hr, followed by the extraction into ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the *N*-Boc precursor without further purification. The above residue was re-dissolved in a solution of 200 μL TFA and DCM (2 mL). The mixture was stirred at room temperature for 2 hours. The reaction mixture was evaporated under pressure to dryness, which was further purified by preparative LC-MS to give the title compound as light yellow oil (16.5 mg, yield: 20%). ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.89 (dd, J=7.6, 1.6 Hz, 1H), 8.74 (d, J=8.0 Hz, 1H), 8.43 (dd, J=7.6, 1.6 Hz, 1H), 8.39 (dd, J=4.8, 1.6 Hz, 1H), 8.02 (d, J=8.0 Hz, 1H), 7.79 (t, J=7.6 Hz, 1H), 7.70 (d, J=7.6 Hz, 1H), 7.62 (dd, J=8.0, 4.8 Hz, 1H), 5.62 (m, 1H), 4.70 (s, 2H), 3.49-3.36 (m, 2H), 3.30-3.21 (m, 2H), 2.25-2.08 (m, 4H). Calculated exact mass for the protonated molecule ($C_{22}H_{21}N_5O_2$): 388.1773; measured accurate mass (ESI): 388.1790.

Prototypical procedure for reductive-amination.



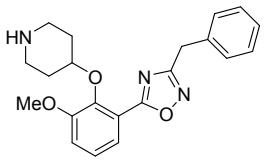
5-(2-(1-methylpiperidin-4-yloxy)pyridin-3-yl)-3-((1,3,5-trimethyl-1H-pyrazol-4-yl)methyl)-1,2,4-oxadiazole (26). A mixture of **25** (18 mg, 0.05 mmol), formaldehyde (37% aqueous, 12.5 µL. 0.15 mmol), acetic acid (17µL, 0.30 mmol) in MeOH (1 mL) was stirred at room temperature for 1 hour, followed by the addition of sodium triacetoxyborohydride (54 mg, 0.25 mmol). The resulting mixture was further stirred at room temperature overnight. After that, the mixture was diluted with ethyl acetate and sequentially washed with 0.2 M NaOH and brine (each 20 mL). The reaction mixture was evaporated under pressure to dryness, which was further purified by preparative LC-MS to give the title compound as light yellow oil (14 mg, 78% yield). ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.44 (dd, J=7.6, 1.8 Hz, 1H), 8.40 (dd, J=5.2, 1.8 Hz, 1H), 7.18 (dd, J=7.6, 5.2 Hz, 1H), 5.60-5.50 (m, 1H), 3.93 (s, 2H), 3.71 (s, 3H), 3.23-3.01 (m, 4H), 2.68 (s, 3H), 2.29 (s, 3H), 2.24 (s, 2H), 2.21-2.09 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{27}N_6O_2$): 383.2195; measured accurate mass (ESI): 383.2207.



3-benzyl-5-(2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (3)

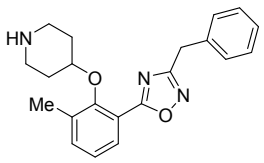
General oxadiazole formation was followed to give the title compound as a white solid in 29% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.07 (dd, J=7.6, 1.6 Hz, 1H), 7.52 (t, J=5.2 Hz, 1H),

7.48-7.26 (m, 5H), 7.07 (t, J=7.6 Hz, 2H), 4.74-4.71 (m, 1H), 4.17 (s, 2H), 3.50-3.45 (m, 2H), 3.20-3.15 (m, 2H), 2.00-1.91 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{22}N_3O_2$): 336.1712; measured accurate mass (ESI): 336.1697.



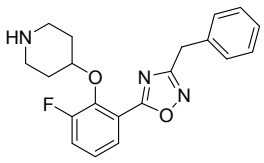
3-benzyl-5-(3-methoxy-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (4)

General oxadiazole formation was followed to give the title compound as yellow oil in 36% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.58 (dd, J=7.6, 1.6 Hz, 1H), 7.39-7.24 (m, 7H), 4.48-4.42 (m, 1H), 4.17 (s, 2H), 3.92 (s, 3H), 3.45-3.38 (m, 2H), 3.08-3.02 (m, 2H), 2.09-2.00 (m, 2H), 1.98-1.88 (m, 2H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_3$): 366.1818; measured accurate mass (ESI): 366.1803.



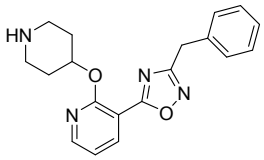
3-benzyl-5-(3-methyl-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (5)

General oxadiazole formation was followed to give the title compound as yellow oil in 47% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.83 (d, J=7.6 Hz, 1H), 7.53 (d, J=7.6 Hz, 1H), 7.42-7.32 (m, 4H), 7.27-7.26 (m, 1H), 7.24-7.22 (m, 1H), 4.17 (s, 2H), 4.06-3.98 (m, 1H), 3.31-3.25 (m, 2H), 2.86-2.79 (m, 2H), 2.38 (s, 3H), 2.03-1.93 (m, 2H), 1.91-1.77 (m, 2H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_2$): 350.1869; measured accurate mass (ESI): 350.1867.



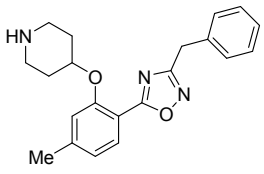
3-benzyl-5-(3-fluoro-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (6)

General oxadiazole formation was followed to give the title compound as light yellow oil in 48% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.91-7.84 (m, 1H), 7.54-7.45 (m, 1H), 7.41-7.24 (m, 6H), 4.58-4.50 (m, 1H), 4.19 (s, 2H), 3.48-3.38 (m, 2H), 3.13-3.02 (m, 2H), 2.14-1.97 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{21}N_3O_2F$): 354.1618; measured accurate mass (ESI): 354.1603.



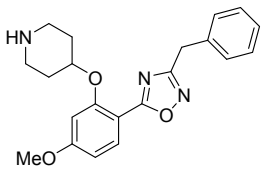
3-benzyl-5-(2-(piperidin-4-yloxy)pyridin-3-yl)-1,2,4-oxadiazole (7)

General oxadiazole formation was followed to give the title compound as light yellow oil in 46% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.46 (dd, J=8.0, 2.0 Hz, 1H), 8.41 (dd, J=4.8, 2.0 Hz, 1H), 7.40-7.24 (m, 5H), 7.19 (dd, J=8.0, 4.8 Hz, 1H), 5.69-5.62 (m, 1H), 4.18 (s, 2H), 3.52-3.42 (m, 2H), 3.32-3.24 (m, 2H), 2.24-2.14 (m, 4H). Calculated exact mass for the protonated molecule ($C_{19}H_{21}N_4O_2$): 337.1665; measured accurate mass (ESI): 337.1658.



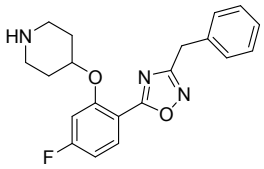
3-benzyl-5-(4-methyl-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (8)

General oxadiazole formation was followed to give the title compound as an off-white solid in 56% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.96 (d, J=8.0 Hz, 1H), 7.43-7.31 (m, 4H), 7.28-7.22 (m, 1H), 6.93 (d, J=8.0 Hz, 1H), 6.82 (s, 1H), 4.89-4.80 (m, 1H), 4.17 (s, 2H), 3.53-3.38 (m, 2H), 3.22-3.12 (m, 2H), 2.42 (s, 3H), 2.29-2.19 (m, 2H), 2.13-2.06 (m, 2H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_2$): 350.1869; measured accurate mass (ESI): 350.1874.



3-benzyl-5-(4-methoxy-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (9)

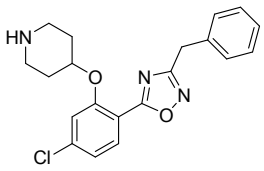
General oxadiazole formation was followed to give the title compound as yellow oil in 37% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.05 (d, J=8.8 Hz, 1H), 7.41-7.34 (m, 4H), 7.29-7.24 (m, 1H), 6.65 (dd, J=8.8, 2.0 Hz, 1H), 6.52 (d, J=2.0 Hz, 1H), 4.86-4.78 (m, 1H), 4.17 (s, 2H), 3.89 (s, 3H), 3.51-3.40 (m, 2H), 3.25-3.12 (m, 2H), 2.30-2.20 (m, 2H), 2.15-2.07 (m, 2H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_3$): 366.1818; measured accurate mass (ESI): 366.1803.



3-benzyl-5-(4-fluoro-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole

(10)

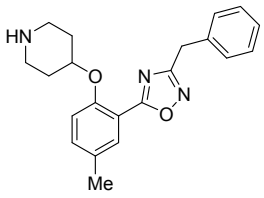
General oxadiazole formation was followed to give the title compound as yellow oil in 31% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.13-8.09 (m, 1H), 7.39-7.24 (m, 5H), 7.18-7.15 (m, 1H), 6.96-6.91 (m, 1H), 5.04-4.95 (m, 1H), 4.16 (s, 2H), 3.51-3.40 (m, 2H), 3.27-3.18 (m, 2H), 2.20-2.09 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{21}N_3O_2F$): 354.1618; measured accurate mass (ESI): 354.1612.



3-benzyl-5-(4-chloro-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole

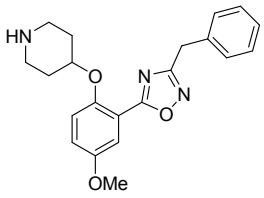
(11)

General oxadiazole formation was followed to give the title compound as yellow oil in 70% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.03 (d, J=8.8 Hz, 1H), 7.43-7.25 (m, 5H), 7.11 (dd, J=8.8, 2.0 Hz, 1H), 7.02 (d, J=2.0 Hz, 1H), 4.88-4.80 (m, 1H), 4.17 (s, 2H), 3.49-3.42 (m, 2H), 3.25-3.14 (m, 2H), 2.32-2.20 (m, 2H), 2.17-2.06 (m, 2H). Calculated exact mass for the protonated molecule ($C_{20}H_{21}N_3O_2Cl$): 370.1322; measured accurate mass (ESI): 370.1326.



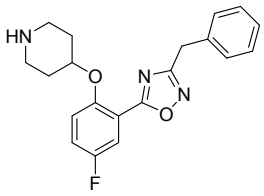
3-benzyl-5-(5-methyl-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (12)

General oxadiazole formation was followed to give the title compound as light yellow oil in 47% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.82 (d, J=2.0 Hz, 1H), 7.40 (dd, J=8.0, 2.0 Hz, 1H), 7.39-7.20 (m, 5H), 7.14 (d, J=8.0 Hz, 1H), 4.94-4.87 (m, 1H), 4.13 (s, 2H), 3.46-3.37 (m, 2H), 3.22-3.12 (m, 2H), 2.32 (s, 3H), 2.13-2.03 (m, 4H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_2$): 350.1869; measured accurate mass (ESI): 350.1855.



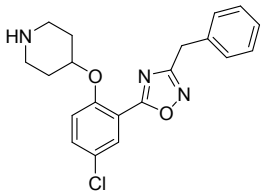
3-benzyl-5-(5-methoxy-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (13)

General oxadiazole formation was followed to give the title compound as yellow oil in 38% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.54 (d, J=2.8 Hz, 1H), 7.38-7.16 (m, 7H), 4.82-4.76 (m, 1H), 4.15 (s, 2H), 3.81 (s, 3H), 3.48-3.38 (m, 2H), 3.22-3.11 (m, 2H), 2.14-2.00 (m, 4H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_3$): 366.1818; measured accurate mass (ESI): 366.1802.



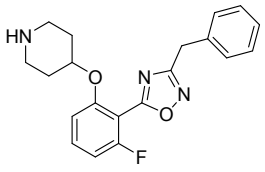
3-benzyl-5-(5-fluoro-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (14)

General oxadiazole formation was followed to give the title compound as light yellow oil in 63% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.78 (dd, J=8.8, 3.2 Hz, 1H), 7.43-7.23 (m, 7H), 4.80-4.74 (m, 1H), 4.18 (s, 2H), 3.51-3.39 (m, 2H), 3.27-3.15 (m, 2H), 2.19-2.05 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{21}N_3O_2F$): 354.1618; measured accurate mass (ESI): 354.1613.



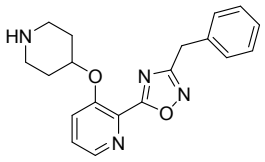
3-benzyl-5-(5-chloro-2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (15)

General oxadiazole formation was followed to give the title compound as an off-white solid in 57% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.03 (d, J=2.8 Hz, 1H), 7.60 (dd, J=9.2, 2.8 Hz, 1H), 7.42-7.25 (m, 6H), 5.02-4.96 (m, 1H), 4.18 (s, 2H), 3.50-3.39 (m, 2H), 3.28-3.17 (m, 2H), 2.20-2.08 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{21}N_3O_2Cl$): 370.1322; measured accurate mass (ESI): 370.1311.



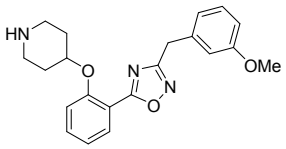
3-benzyl-5-(2-fluoro-6-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (16)

General oxadiazole formation was followed to give the title compound as yellow oil in 10% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.67-7.58 (m, 1H), 7.40-7.24 (m, 5H), 7.10 (d, J=8.8 Hz, 1H), 6.97 (t, J=8.8 Hz, 1H), 4.67-4.52 (m, 1H), 4.20 (s, 2H), 3.25-3.16 (m, 2H), 3.13-3.05 (m, 2H), 2.13-1.95 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{21}N_3O_2F$): 354.1618; measured accurate mass (ESI): 354.1622.



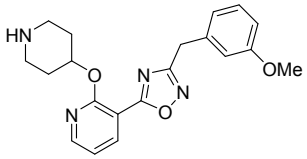
3-benzyl-5-(3-(piperidin-4-yloxy)pyridin-2-yl)-1,2,4-oxadiazole (17)

General oxadiazole formation was followed to give the title compound as a light yellow solid in 27% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.38 (dd, J=4.8, 1.2 Hz, 1H), 7.84 (dd, J=8.4, 1.2 Hz, 1H), 7.66 (dd, J=8.4, 4.8 Hz, 1H), 7.42-7.24 (m, 5H), 5.08-5.02 (m, 1H), 4.23 (s, 2H), 3.49-3.38 (m, 2H), 3.26-3.15 (m, 2H), 2.19-2.07 (m, 4H). Calculated exact mass for the protonated molecule ($C_{19}H_{21}N_4O_2$): 337.1665; measured accurate mass (ESI): 337.1659.



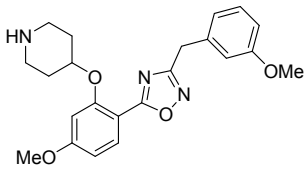
3-(3-methoxybenzyl)-5-(2-(piperidin-4-yloxy)phenyl)-1,2,4-oxadiazole (18)

General oxadiazole formation was followed to give the title compound as an off-white solid in 28% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, J=8.0 Hz, 1H), 7.55 (t, J=8.0 Hz, 1H), 7.28 (t, J=8.0 Hz, 2H), 7.14 (t, J=8.0 Hz, 1H), 7.06-6.96 (m, 3H), 6.84 (d, J=8.0 Hz, 1H), 4.91 (s, 1H), 4.18 (s, 2H), 3.55-3.50 (m, 2H), 3.22-3.19 (m, 2H), 2.27-2.24 (m, 2H), 2.16-2.13 (m, 2H). Calculated exact mass for the protonated molecule ($C_{21}H_{24}N_3O_3$): 366.1818; measured accurate mass (ESI): 366.1801.



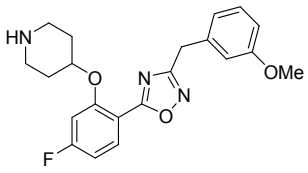
3-(3-methoxybenzyl)-5-(2-(piperidin-4-yloxy)pyridin-3-yl)-1,2,4-oxadiazole (19)

General oxadiazole formation was followed to give the title compound as yellow oil in 43% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.46 (dd, J=8.0, 2.0 Hz, 1H), 8.41 (dd, J=5.2, 2.0 Hz, 1H), 7.26 (t, J=8.0 Hz, 1H), 7.19 (dd, J=8.0, 5.2 Hz, 1H), 6.97-6.90 (m, 2H), 6.84 (dd, J=8.0, 2.4 Hz, 1H), 5.69-5.62 (m, 1H), 4.14 (s, 2H), 3.80 (s, 3H), 3.53-3.42 (m, 2H), 3.31-3.24 (m, 2H), 2.26-2.12 (m, 4H). Calculated exact mass for the protonated molecule ($C_{20}H_{23}N_4O_3$): 367.1770; measured accurate mass (ESI): 367.1770.



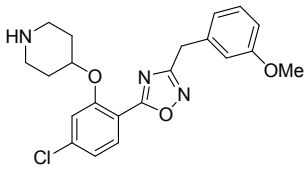
5-(4-methoxy-2-(piperidin-4-yloxy)phenyl)-3-(3-methoxybenzyl)-1,2,4-oxadiazole (20)

General oxadiazole formation was followed to give the title compound as yellow oil in 20% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.04 (d, J=8.8 Hz, 1H), 7.27-7.22 (m, 1H), 6.99-6.81 (m, 3H), 6.64 (dd, J=8.8, 2.4 Hz, 1H), 6.52 (d, J=2.4 Hz, 1H), 4.85-4.78 (m, 1H), 4.13 (s, 2H), 3.88 (s, 3H), 3.81 (s, 3H), 3.47-3.36 (m, 2H), 3.22-3.11 (m, 2H), 2.28-2.02 (m, 4H). Calculated exact mass for the protonated molecule ($C_{22}H_{26}N_3O_4$): 396.1923; measured accurate mass (ESI): 396.1919.



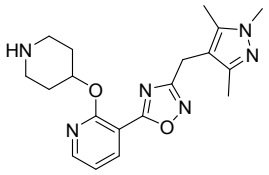
5-(4-fluoro-2-(piperidin-4-yloxy)phenyl)-3-(3-methoxybenzyl)-1,2,4-oxadiazole (21)

General oxadiazole formation was followed to give the title compound as a yellow solid in 44% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.11 (dd, J=8.4, 6.8 Hz, 1H), 7.26 (t, J=8.0 Hz, 1H), 7.16 (dd, J=6.8, 2.4 Hz, 1H), 6.97-6.89 (m, 3H), 6.84 (dd, J=8.4, 2.4 Hz, 1H), 5.03-4.98 (m, 1H), 4.13 (s, 2H), 3.79 (s, 3H), 3.53-3.42 (m, 2H), 3.29-3.19 (m, 2H), 2.22-2.09 (m, 4H). Calculated exact mass for the protonated molecule ($C_{21}H_{23}N_3O_3F$): 384.1723; measured accurate mass (ESI): 384.1723.



5-(4-chloro-2-(piperidin-4-yloxy)phenyl)-3-(3-methoxybenzyl)-1,2,4-oxadiazole (22)

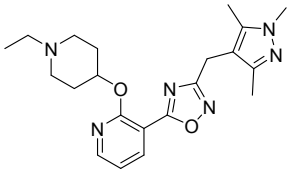
General oxadiazole formation was followed to give the title compound as yellow oil in 56% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.04 (d, J=8.4 Hz, 1H), 7.39 (d, J=1.6 Hz, 1H), 7.26 (t, J=8.0 Hz, 1H), 7.19 (dd, J=8.4, 1.6 Hz, 1H), 6.97-6.88 (m, 2H), 6.84 (dd, J=8.0, 1.6 Hz, 1H), 5.05-4.98 (m, 1H), 4.13 (s, 2H), 3.79 (s, 3H), 3.52-3.41 (m, 2H), 3.28-3.18 (m, 2H), 2.20-2.08 (m, 4H). Calculated exact mass for the protonated molecule ($C_{21}H_{23}N_3O_3Cl$): 400.1428; measured accurate mass (ESI): 400.1428.



5-(2-(piperidin-4-yloxy)pyridin-3-yl)-3-((1,3,5-trimethyl-1H-pyrazol-4-yl)methyl)-1,2,4-oxadiazole (25)

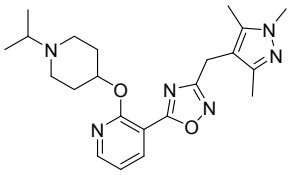
General oxadiazole formation was followed to give the title compound as light yellow oil in 56% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.46 (dd, J=8.0, 2.0 Hz, 1H), 8.42 (dd, J=5.2, 2.0 Hz, 1H), 7.20 (dd, J=8.0, 5.2 Hz, 1H), 5.71-5.62 (m, 1H), 3.93 (s, 2H), 3.72 (s, 3H), 3.54-3.42 (m, 2H), 3.36-3.28 (m, 2H), 2.30 (s, 3H), 2.22 (s, 3H), 2.28-2.18 (m, 4H). Calculated exact

mass for the protonated molecule ($C_{19}H_{25}N_6O_2$): 369.2039; measured accurate mass (ESI): 369.2053.



5-(2-(1-ethylpiperidin-4-yloxy)pyridin-3-yl)-3-((1,3,5-trimethyl-1H-pyrazol-4-yl)methyl)-1,2,4-oxadiazole (27)

General reductive-amination was followed to give the title compound as light yellow oil in 76% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.47 (dd, J=8.0, 1.6 Hz, 1H), 8.42 (dd, J=5.2, 1.6 Hz, 1H), 7.21 (dd, J=8.0, 5.2 Hz, 1H), 5.70-5.58 (m, 1H), 3.93 (s, 2H), 3.71 (s, 3H), 3.48-3.35 (m, 4H), 3.18 (q, J=7.2 Hz, 2H), 2.35-2.22 (m, 4H), 2.28 (s, 3H), 2.21 (s, 3H), 1.39 (t, J=7.2 Hz, 3H). Calculated exact mass for the protonated molecule ($C_{21}H_{29}N_6O_2$): 397.2352; measured accurate mass (ESI): 397.2356.



5-(2-(1-isopropylpiperidin-4-yloxy)pyridin-3-yl)-3-((1,3,5-trimethyl-1H-pyrazol-4-yl)methyl)-1,2,4-oxadiazole (28)

General reductive-amination was followed to give the title compound as light yellow oil in 68% yield. ^{1}H-NMR (CD_3OD, 400 MHz): δ8.47 (dd, J=8.0, 2.0 Hz, 1H), 8.42 (dd, J=4.8, 2.0 Hz,

1H), 7.21 (dd, J=8.0, 4.8 Hz, 1H), 5.71-5.61 (m, 1H), 4.69-4.60 (m, 1H), 3.92 (s, 3H), 3.71 (s, 3H), 3.54-3.39 (m, 2H), 3.36-3.20 (m, 2H), 2.33-2.15 (m, 4H), 2.29 (s, 3H), 2.20 (s, 3H), 1.38 (d, J=6.4 Hz, 6H). Calculated exact mass for the protonated molecule ($C_{22}H_{31}N_6O_2$): 411.2508; measured accurate mass (ESI): 411.2504.

BIOLOGY

Enzyme inhibition assay. All IC_{50} determinations were carried out using a 7-diethylamine-3-(4'maleimidylphenyl)-4-methylcoumarin (CPM) fluorescence assay, as described previously for PvNMT[1] and HsNMT1.[2] IC_{50} of an inhibitor was calculated by a nonlinear regression analysis using GraFit 7.0.1 version (Erithacus Software Limited, UK). The values are the mean value of two determinations; standard deviation is within 20% of the IC_{50} unless otherwise specified.

K_i values quoted are the K_i calculated from the experimentally determined IC_{50} values, the substrate concentration ([S]) and the Michaelis-Menten constant (K_m) as described by the Cheng-Prusoff equation.[3]

Equation 1. Cheng-Prusoff Equation for Determination of K_i from IC_{50}.

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

K_m values of peptide substrates were determined as described previously:[2] 3.64 μM for PfNMT, 3.29 μM for HsNMT1 and 5.71 μM for PvNMT. For example, inhibitor **30** had an experimentally determined PfNMT IC_{50} of 0.0035μM. The Michaelis Constant (K_m) was 3.64 μM and the substrate concentration was 4.0μM, resulting in a K_i of 0.0017μM.

***Plasmodium falciparum* (3D7) viability assay (SyBr green assay).** Synchronous *Pf* (3D7) late stage trophozoites at 33-36 h were used. Red blood cells used for the assay were centrifuged to remove the buffy coat and washed twice in Roswell Park Memorial Institute (RPMI) 1640 medium so that no white blood cells were present. The culture medium contained RPMI 1640 with 5 g/L Albumax, 0.025 g/L gentamycin, and 0.292 g/L L-glutamine.Sterile 96 well black tissue culture plates were used routinely for every assay. Each well (in total 100 µL, 0.5% DMSO) contained synchronous cultures of late trophozoite-stage parasites (0.1 – 0.2% parasitemia and 2% hematocrit) and variable concentrations of an inhibitor. Chloroquine was used as a standard. Two sets of control were used in duplicate wells, one set with no added test compound and one with uninfected red blood cells (RBC). The plates were incubated at 37 °C for 48 hours in a gas chamber flushed with 5% CO_2, 5% O_2, and 90% N_2. After that, the supernatants were taken out from each well and replaced with fresh drug and incubated for a further 48 hours in the same manner. At the end of the 96-hour incubation, 25µL of SYBR Green I dye (SYBR Green I nucleic acid gel stain 10000x, in DMSO, from Invitrogen) in lysis buffer (1µL dye to 1 mL lysis buffer) was added to each well and stored overnight at -20 °C. The lysis buffer contained Tris (20 mM, pH 8.0), EDTA (2 mM), saponin (0.16%) and Triton X-100 (1.6% v/v). Plates were warmed to room temperature and the fluorescence was measured at 485 nm. Fluorescence intensity unit was converted to percentage (%) of growth as follows: % growth = [(culture under inhibitor) – (uninfected RBC)] / (culture with no inhibitor) – (uninfected RBC) x 100%. EC_{50} of an inhibitor was calculated by a nonlinear regression analysis using GraFit 7.0.1 version (Erithacus Software Limited, UK). All assays were carried out in duplicate.

HepG2 toxicity assay (MTS assays). 50 µL HepG2 (1 x 10^5 / well of 96-well plate) in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS) were incubated at 37 °C and 10% CO_2 for 24 hours. For each well, the cells were treated with 100 µL of an inhibitor with varied concentrations in the same medium. The resulting cells were incubated under the same condition for another 48 hours. 20 µL of MTS/PMS solution (2 mg/ml of MTS and 0.046 mg/mL of PMS in PBS buffer) was added to each well and the resulting mixture was incubated under the same condition for additional 3.5 hours prior to measuring the fluorescence at 490 nm. Cells with no test compound were used as a positive control while cells treated with puromycin, a highly active inhibitor against HepG2, were used as a negative control. Fluorescence intensity unit was converted to percentage (%) of growth as follows: % growth = [(cell with inhibitor) – (cell with puromycin)] / (culture with no inhibitor) – (cell with puromycin) x 100%. LD_{50} of an inhibitor was calculated by a nonlinear regression analysis using GraFit 7.0.1 version (Erithacus Software Limited, UK). All assays were carried out in triplicate.

Crystallography. Crystals of the ternary complex of the non-hydrolysable co-factor and compound bound to PvNMT were obtained as described previously.[4] X-ray diffraction data were collected on synchrotron beamlines at Diamond Light Source, Harwell, UK, and processed using XDS and SCALA implemented within *xia2*.

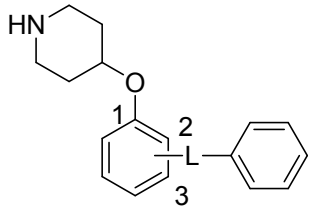
Structure refinement was by maximum likelihood methods implemented in REFMAC5 using the protein chains of 4A95.pdb[5] as a starting model, interspersed with cycles of model

building and adjustment using COOT. A summary of data collection and refinement statistics is in Supporting Information.

The coordinates and structure factor files have been deposited in the Protein Data Bank under the accession codes 4UFV (PvNMT-NHM-**18**), 4UFW (PvNMT-NHM-**22**) and 4UFX (PvNMT-NHM-**19**).

SUPPLEMENTARY TABLES

Table S1. Investigation of optimal side chain position and linkage group



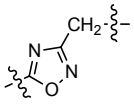
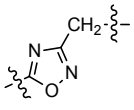
Side chain position	L	Ki (μM) PfNMT	Ki (μM) HsNMT1	Side chain position	L	Ki (μM) PfNMT	Ki (μM) HsNMT1
1,2	-$COOCH_2$-	>100	>100	1,2		1.4	33
1,3	-$COOCH_2$-	>100	>100	1,3		2.6	18

Table S2. A summary of data collection and refinement statistics

	PvNMT-NHM-**18**	PvNMT-NHM-**22**	PvNMT-NHM-**19**
PDB accession code	**4UFV**	**4UFW**	**4UFX**
Cell dimensions *a*, *b*, *c*	57.48, 118.99, 177.74	57.52, 121.91, 178.80	57.32, 118.87, 174.87
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Data collection			
Beamline / Wavelength	DLS i04 / 0.9795	DLS i24 / 0.9784	DLS i24 / 0.9784
Detector type	ADSC Q315 CCD	CMOS Pilatus 6M	CMOS Pilatus 6M
Images x oscillation (°)	450 x 0.4	1800 x 0.1	1800 x 0.1
Resolution (Å)	99–1.75 (1.84–1.75) [a]	31–1.50 (1.58–1.50)	98–1.49 (1.52–1.49)
R_{sym} (%) [b]	12.9 (59.7)	12.9 (71.4)	9.8 (42.0)
$I/\sigma I$	12.0 (2.3)	8.4 (1.7)	7.3 (1.8)
Completeness (%)	98.2 (91.7)	99.2 (96.2)	99.9 (98.9)
Redundancy	3.5 (2.6)	5.9 (4.0)	4.5 (2.7)
Refinement			
No. unique reflections	121366	199533	195189
R_{work} / R_{free} [c]	18.6 / 23.7	21.4 / 26.1	15.8 / 19.7
No. atoms	11346	11389	11935
Protein	9874	9850	10056
Ligand	81	84	81
Co-factor	192	192	192
Water	1167	1240	1567
B-factors (Å^2)			
All atoms	11.5	17.0	14.3
Protein	10.5	15.9	12.8
Ligand	11.9	25.7	13.3
Co-factor	7.5	12.2	9.5
Water	18.8	25.0	24.6
R.m.s. deviations [d]			
Bond lengths (Å)	0.020	0.021	0.023
Bond angles (°)	2.030	2.106	2.283

[a]Highest resolution shell is shown in parentheses.

[b]$R_{sym} = \Sigma_{\mathbf{h}}\Sigma_l |I_{\mathbf{h}l} - \langle I_{\mathbf{h}}\rangle| / \Sigma_{\mathbf{h}}\Sigma_l \langle I_{\mathbf{h}}\rangle$, where I_l is the l^{th} observation of reflection h and $\langle I_{\mathbf{h}}\rangle$ is the weighted average intensity for all observations l of reflection h.

[c] $R_{work} = \sum||F_o| - |F_c||/\sum|F_o|$ where F_o and F_c are the observed and calculated structure factor amplitudes, respectively.

R_{free} is the R_{cryst} calculated with 5% of the reflections omitted from refinement.

[d] Root-mean-square deviation of bond lengths or bond angles from ideal geometry.

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