

Quinolinylnyl pyrimidines: potent inhibitors of NDH-2 as a novel class of anti-TB agents

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SUPPORTING INFORMATION

Experimental Section:

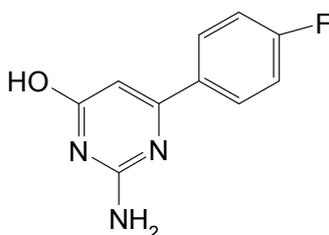
All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Sigma Aldrich Chemical Co. or Fisher Scientific. Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). General methods of purification of compounds involved the use of silica cartridges purchased from Grace Purification systems. and/or recrystallization. The reactions were monitored by TLC on precoated Merck 60 F254 silica gel plates and visualized using UV light (254 nm). All compounds were analyzed for purity by HPLC and characterized by ¹H and ¹³C NMR using Bruker 300MHz NMR and/or Bruker 400 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform δ 7.26 and δ 77.23, methanol δ 3.31 and δ 49.00, DMSO-d₆ δ 3.33 and coupling constants (J) are reported in hertz (Hz) (where, s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt – triple triplet, q = quartet, m = multiplet) and analyzed using ACD NMR data processing. Mass spectra values are reported as m/z .

All reactions were conducted under Nitrogen unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator.

Abbreviations: NMP= N-methyl Pyrrolidine; HCl= hydrochloric acid; DMF= dimethylformamide; NaH= sodium hydride.

Preparation of Key Intermediates and Final Products:

2-amino-6-(4-fluorophenyl)pyrimidin-4-ol:

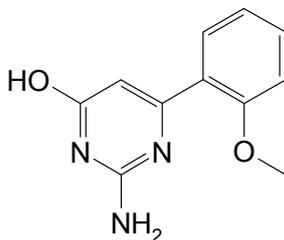


In a 2 neck 100ml round bottom flask fitted with a refluxed condensor and an addition funnel were added oven activated molecular sieves under stream of nitrogen. NaH (0.586 g, 14.66 mmol) was added to the flask at room temperature followed by 20ml of dry DMF. It was stirred to get a suspension of solids. A solution of Guanidine hydrochloride (0.7 g, 7.33 mmol) in dry DMF(20ml) was taken in the addition funnel and was added drop wise into the reaction mixture over 15 minutes. It was then stirred for 30 minutes at room temperature. Ethyl 3-(4-fluorophenyl)-3-oxopropanoate (1.541 g, 7.33 mmol) was dissolved in dry DMF(20 ml) and was added to the addition funnel and it was added to the reaction mixture drop wise over 15 minutes. The mixture was then heated at reflux overnight. The reaction mixture turned into green and then orange colour solution after heating. The reaction mixture was filtered hot to get a heterogeneous mixture. water(25ml) was added to it to get a solid product which was filtered and then washed with hexane to get product 2-amino-6-(4-fluorophenyl)pyrimidin-4-ol (0.557 g, 2.71 mmol,37.0 %)

$^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ ppm 6.05 (s, 1 H) 6.98 (br. s., 2 H) 7.20 - 7.29 (m, 2 H) 7.41 (br. s., 1 H) 8.00 (dd, $J=8.76, 5.75$ Hz, 2 H) 8.10 (br. s., 1 H)

$m/z = 206.1$ (M+1)

2-amino-6-(2-methoxyphenyl)pyrimidin-4-ol:

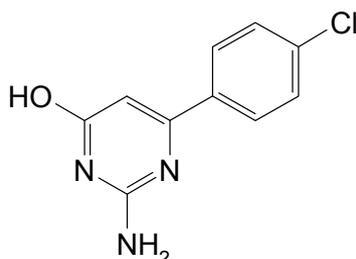


In a 2 neck 100ml round bottom flask fitted with a refluxed condensor and an addition funnel were added oven activated molecular sieves under stream of nitrogen. NaH (0.163 g, 4.08 mmol) was added to the flask at room temperature followed by 10ml of dry DMF. It was stirred to get a suspension of solids. A solution of Guanidine hydrochloride (0.195 g, 2.04 mmol) in dry DMF(10ml) was taken in the addition funnel and was added drop wise into the reaction mixture over 15 minutes. It was then stirred for 30 minutes at room temperature. Ethyl 3-(2-methoxyphenyl)-3-oxopropanoate (0.397 mL, 2.04 mmol) was dissolved in dry DMF(10 ml) and was added to the addition funnel and it was added to the reaction mixture drop wise over 15 minutes. The mixture was then heated at reflux overnight. The reaction mixture turned into green and then orange colour solution after heating. The reaction mixture was filtered hot to get a heterogeneous mixture. water(10ml) was added to it to get a solid product which was filtered and then washed with hexane to get product 2-amino-6-(2-methoxyphenyl)pyrimidin-4-ol (0.181 g, 0.83 mmol, 40.8 %).

^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ ppm 3.82 (s, 3 H) 6.09 (s, 1 H) 6.51 (br. s., 2 H) 7.00 (t, $J=7.44$ Hz, 1 H) 7.09 (d, $J=8.48$ Hz, 1 H) 7.33 - 7.43 (m, 1 H) 7.75 (d, $J=7.72$ Hz, 1 H) 10.82 (br. s., 1 H)

$m/z = 218.1$ (M+1)

2-amino-6-(4-chlorophenyl)pyrimidin-4-ol:

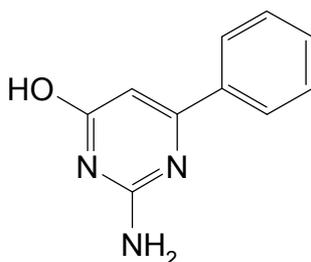


In a 2 neck 100ml round bottom flask fitted with a refluxed condenser and an addition funnel were added oven activated molecular sieves under stream of nitrogen. NaH (0.168 g, 4.19 mmol) was added to the flask at room temperature followed by 10ml of dry DMF. It was stirred to get a suspension of solids. A solution of Guanidine hydrochloride (0.2g, 2.09 mmol) in dry DMF(10ml) was taken in the addition funnel and was added drop wise into the reaction mixture over 15 minutes. It was then stirred for 30 minutes at room temperature. Ethyl 3-(4-chlorophenyl)-3-oxopropanoate (0.390 mL, 2.09 mmol) was dissolved in dry DMF(10 ml) and was added to the addition funnel and it was added to the reaction mixture drop wise over 15 minutes. The mixture was then heated at reflux overnight. The reaction mixture turned into green and then orange colour solution after heating. The reaction mixture was filtered hot to get a heterogeneous mixture. water(10ml) was added to it to get a solid product which was filtered and then washed with hexane to get product 2-amino-6-(4-chlorophenyl)pyrimidin-4-ol (0.100 g, 0.45 mmol, 21.54 %).

^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ ppm 5.96 (s, 1 H) 6.63 (br. s., 2 H) 7.23 - 7.35 (m, 2 H) 7.41 - 7.52 (m, 1 H) 7.88 (t, $J=7.16$ Hz, 1 H) 10.93 (br. s., 1 H)

$m/z = 222.0$ (M+1)

2-amino-6-phenylpyrimidin-4-ol:

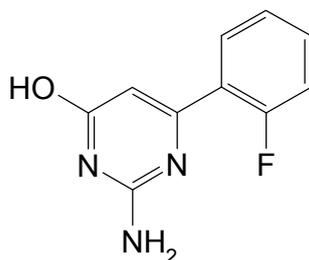


In a 2 neck 100ml round bottom flask fitted with a refluxed condensor and an addition funnel were added oven activated molecular sieves under stream of nitrogen. NaH (0.419 g, 10.47 mmol) was added to the flask at room temperature followed by 10ml of dry DMF. It was stirred to get a suspension of solids. A solution of Guanidine hydrochloride (0.5 g, 5.24 mmol) in dry DMF(10ml) was taken in the addition funnel and was added drop wise into the reaction mixture over 15 minutes. It was then stirred for 30 minutes at room temperature. Ethyl 3-oxo-3-phenylpropanoate (0.901 mL, 5.24 mmol) was dissolved in dry DMF(10 ml) and was added to the addition funnel and it was added to the reaction mixture drop wise over 15 minutes. The mixture was then heated at reflux overnight. The reaction mixture turned into green and then orange colour solution after heating. The reaction mixture was filtered hot to get a heterogeneous mixture. water(10ml) was added to it to get a solid product which was filtered and then washed with hexane to get product 2-amino-6-phenylpyrimidin-4-ol (0.410 g, 2.19mmol, 41.8 %).

$^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ ppm 6.09 (s, 1 H) 6.63 (br. s., 2 H) 7.37 - 7.50 (m, 3 H) 7.84 - 8.01 (m, 2 H) 10.95 (br. s., 1 H)

$m/z = 188.1$ (M+1)

2-amino-6-(2-fluorophenyl)pyrimidin-4-ol:

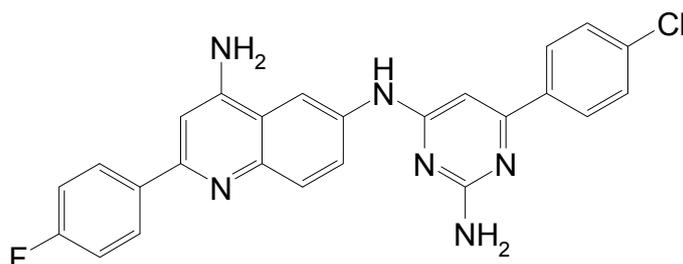


In a 2 neck 100ml round bottom flask fitted with a refluxed condensor and an addition funnel were added oven activated molecular sieves under stream of nitrogen. NaH (0.168 g, 4.19 mmol) was added to the flask at room temperature followed by 10ml of dry DMF. It was stirred to get a suspension of solids. A solution of Guanidine hydrochloride (0.2 g, 2.09 mmol) in dry DMF(10ml) was taken in the addition funnel and was added drop wise into the reaction mixture over 15 minutes. It was then stirred for 30 minutes at room temperature. ethyl 3-(2-fluorophenyl)-3-oxopropanoate (0.440 g, 2.09 mmol) was dissolved in dry DMF(10 ml) and was added to the addition funnel and it was added to the reaction mixture drop wise over 15 minutes. The mixture was then heated at reflux overnight. The reaction mixture turned into green and then orange colour solution after heating. The reaction mixture was filtered hot to get a heterogeneous mixture. water(10ml) was added to it to get a solid product which was filtered and then washed with hexane to get product 2-amino-6-(2-fluorophenyl)pyrimidin-4-ol (0.310 g, 1.51 mmol, 72.1 %).

^1H NMR (300 MHz, DMSO- d_6) δ ppm 5.96 (s, 1 H) 6.63 (br. s., 2 H) 7.21 - 7.33 (m, 2 H) 7.42 - 7.52 (m, 1 H) 7.88 (t, $J=7.06$ Hz, 1 H) 10.94 (br. s., 1 H)

$m/z = 206.0$ (M+1)

N6-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (13a):



A mixture of compound **5** 2-amino-6-(4-chlorophenyl)pyrimidin-4-ol (80 mg, 0.36 mmol), N-phenyltrifluoromethanesulphonamide (142 mg, 0.40 mmol), and triethylamine (0.050 mL, 0.36 mmol) were taken in dry NMP (1 mL) in a 25 ml round bottom flask to give a brown solution. The mixture was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (91 mg, 0.36 mmol) was dissolved in N-methyl-2-pyrrolidinone (0.5 mL) and added to the above reaction followed by 4 mole equivalents (0.361 mL, 1.44 mmol) of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon solid formed after cooling. The solid was filtered and washed with methanol (20 mL) to get N6-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (30.0 mg, 0.07 mmol, 18.19 %).

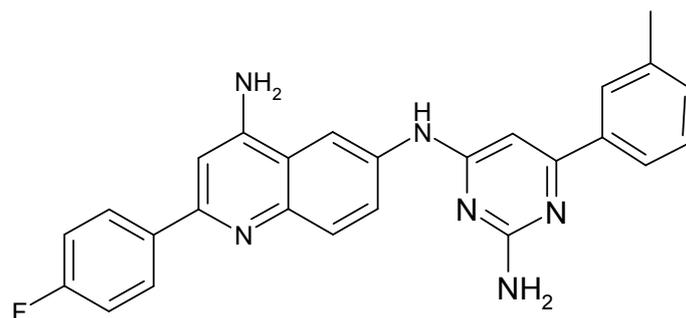
^1H NMR (300 MHz, DMSO- d_6) δ ppm 6.79 (br. s., 1 H) 7.06 (s, 1 H) 7.56 (t, $J=8.48$ Hz, 3 H) 7.70 (d, $J=7.54$ Hz, 3 H) 7.95 (d, $J=7.91$ Hz, 2 H) 8.01 (br. s., 3 H) 8.14 (s, 2 H) 8.09 (s, 1 H) 8.98 (br. s., 1 H) 13.65 (br. s., 1 H)

$m/z = 457.1$ (M+1)

Purity 95%, HPLC tR = 6.1 min.

HRMS (EI), M+1 calcd. for $\text{C}_{25}\text{H}_{18}\text{ClFN}_6$, 457.1344; found 457.1332

Preparation of N6-(2-amino-6-m-tolylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (13b).



In a 25 mL round-bottomed flask A mixture of 2-amino-6-m-tolylpyrimidin-4-ol (0.072 g, 0.36 mmol), triethylamine (0.050 mL, 0.36 mmol), N-phenyl-bis(trifluoromethanesulfonamide) (0.127 g, 0.36 mmol) in N-methyl-2-pyrrolidinone (1.5 mL) under nitrogen was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (90 mg, 0.36 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 75 mg (48.4 %).

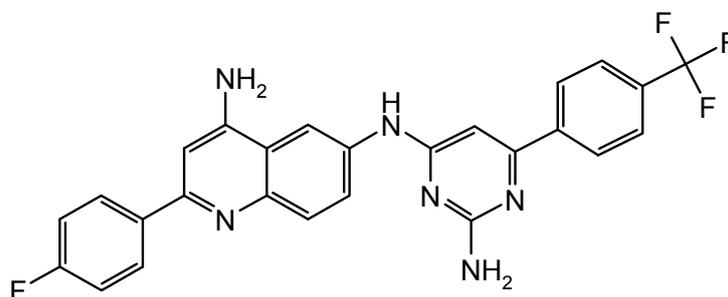
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 2.44 (s, 3 H) 6.89 (s, 1 H) 7.09 (s, 1 H) 7.47 - 7.59 (m, 5 H) 7.71 - 7.78 (m, 2 H) 8.03 (dd, *J*=9.04, 5.27 Hz, 2 H) 8.09 - 8.16 (m, 1 H) 8.19 - 8.25 (m, 1 H) 8.98 (br. s., 2 H) 11.35 (br. s., 1 H) 13.75 (br. s., 1 H).

m/z =437 (M+1)

Purity 95%, HPLC *t*_R = 5.4 min.

HRMS (EI), M+1 calcd. for C₂₆H₂₁FN₆, 437.188447; found 437.1874

Preparation of N6-(2-amino-6-(4-(trifluoromethyl)phenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (13c).



In a 25 mL round-bottomed flask A mixture of 2-amino-6-(4-(trifluoromethyl)phenyl)pyrimidin-4-ol (50 mg, 0.20 mmol), N-phenyl-bis(trifluoromethanesulfonamide) (70.0 mg, 0.20 mmol), and triethylamine (0.027 mL, 0.20 mmol) in N-methyl-2-pyrrolidinone (1.5 mL) under nitrogen was

heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (49.6 mg, 0.20 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 30 mg (31.2 %).

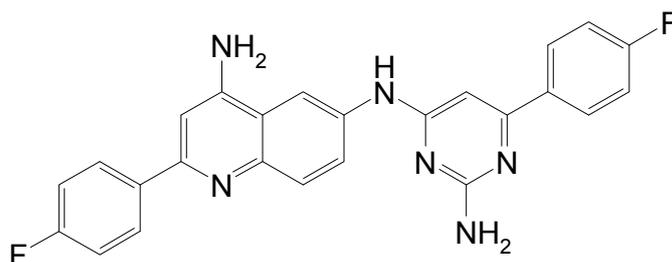
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.87 (br. s., 1 H) 7.06 (s, 1 H) 7.53 - 7.61 (m, 2 H) 7.98 - 8.05 (m, 4 H) 8.09 - 8.20 (m, 3 H) 8.69 (br. s., 1 H) 9.01 (br. s., 1 H) 9.10 (br. s., 1 H) 13.70 (br. s., 1 H).

m/z = 491 (M+1)

Purity 96%, HPLC tR = 5.9 min.

HRMS (EI), M+1 calcd. for C₂₆H₁₈F₄N₆, 491.160731; found 491.1624

N6-(2-amino-6-(4-fluorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13d):



A mixture of compound **2** 2-amino-6-(4-fluorophenyl)pyrimidin-4-ol (100 mg, 0.49 mmol), N-phenyltrifluoromethanesulphonamide (174 mg, 0.49 mmol), and triethylamine (0.068 mL, 0.49 mmol) were taken in dry NMP (0.5 mL) in a 25 ml round bottom flask to give a brown solution. The mixture was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (123 mg, 0.49 mmol) was dissolved in N-methyl-2-pyrrolidinone (1.5 mL) and added to the above reaction followed by 4 mole equivalents(0.487 mL, 1.95 mmol) of HCl (4M in Dioxane).The mixture was heated to 80 °C for 10h where upon solid formed after cooling. The solid was filtered and washed with methanol (50ml) to get N6-(2-amino-6-(4-fluorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (30.0 mg, 0.07 mmol ,13.98 %).

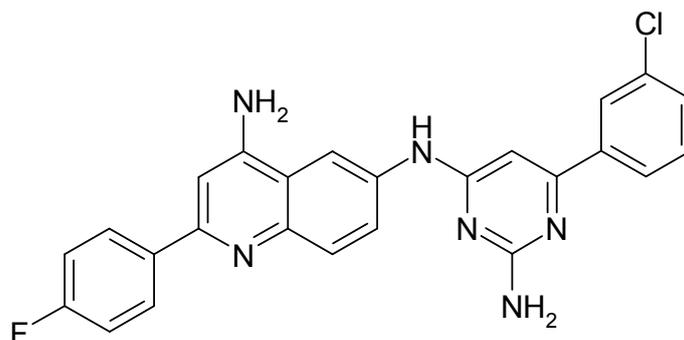
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.74 (br. s., 1 H) 7.05 (s, 1 H) 7.39 - 7.52 (m, 2 H) 7.56 (t, *J*=8.76 Hz, 3 H) 8.00 (td, *J*=8.76, 5.46 Hz, 5 H) 8.13 (s, 2 H) 8.09 (s, 1 H) 8.97 (br. s., 1 H) 13.63 (br. s., 1 H)

m/z = 441.1 (M+1)

Purity 93%, HPLC t_R = 5.9 min.

HRMS (EI), M+1 calcd. for C₂₅H₁₈F₂N₆, 441.1639; found 441.1652

Preparation of N6-(2-amino-6-(3-chlorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13e):



In a 25 mL round-bottomed flask a mixture of 2-amino-6-(3-chlorophenyl)pyrimidin-4-ol (0.1 g, 0.45 mmol), N-phenyl-bis(trifluoromethanesulfonamide) (0.161 g, 0.45 mmol) and triethylamine (0.063 mL, 0.45 mmol) in N-methyl-2-pyrrolidinone (1.5 mL) under nitrogen was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (114 mg, 0.45 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 80 mg (38.8 %).

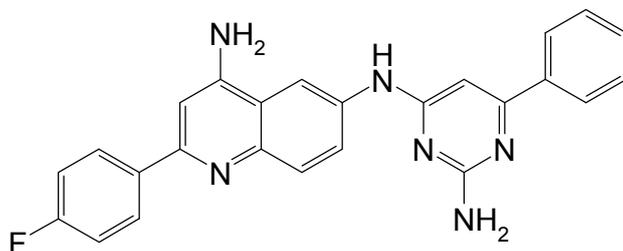
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.83 (s, 1 H) 7.07 (s, 1 H) 7.56 (t, *J*=8.85 Hz, 3 H) 7.62 - 7.73 (m, 3 H) 7.87 (dt, *J*=7.30, 1.53 Hz, 1 H) 7.99 - 8.04 (m, 3 H) 8.09 (d, *J*=11.49 Hz, 1 H) 8.13- 8.20 (m, 1 H) 8.98 (d, *J*=2.07 Hz, 2 H) 13.66 (br. s., 1H).

m/z = 457(M+1)

Purity 95%, HPLC t_R = 6.1 min.

HRMS (EI), M+1 calcd. for C₂₅H₁₈ClFN₇, 457.134375; found 457.1330

N6-(2-amino-6-phenylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13f):



A mixture of compound **8** 2-amino-6-phenylpyrimidin-4-ol (80 mg, 0.43 mmol), N-phenyltrifluoromethanesulphonamide (168 mg, 0.47 mmol), and triethylamine (0.060 mL, 0.43 mmol) were taken in dry NMP (1mL) in a 25 ml round bottom flask to give a brown solution. The mixture was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (108 mg, 0.43 mmol) was dissolved in N-methyl-2-pyrrolidinone (1 mL) and added to the above reaction followed by 4 mole equivalents (0.122ml, 1.71 mmol) of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon solid formed after cooling. The solid was filtered and washed with methanol (50ml) to get N6-(2-amino-6-phenylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (60.0 mg, 0.14 mmol 33.2 %).

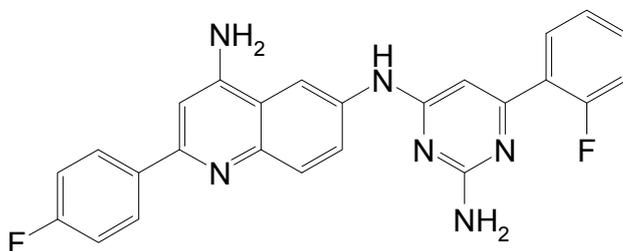
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.89 (s, 1 H) 7.08 (s, 1 H) 7.56 (t, *J*=8.76 Hz, 2 H) 7.62 - 7.70 (m, 3 H) 7.89 - 7.96 (m, 2 H) 8.03 (dd, *J*=8.57, 5.37 Hz, 2 H) 8.13 (d, *J*=9.61 Hz, 1 H) 8.21 (d, *J*=9.23 Hz, 1 H) 8.79 (br. s., 1 H) 8.98 (br. s., 2 H) 9.13 (br. s., 1 H) 11.34 (br. s., 1 H) 13.22 (br. s., 1 H) 13.73 (br. s., 1 H)

m/z = 423.1 (M+1)

Purity 95%, HPLC *t*_R =5.8 min.

HRMS (EI), M+1 calcd. for C₂₅H₁₉FN₆, 423.1733; found 423.1744

N6-(2-amino-6-(2-fluorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13g):



A mixture of compound **9** 2-amino-6-(2-fluorophenyl)pyrimidin-4-ol (80 mg, 0.39 mmol), N-phenyltrifluoromethanesulphonamide (153 mg, 0.43 mmol), and triethylamine (0.054 mL, 0.39 mmol) were taken in dry NMP (1mL) in a 25 ml round bottom flask to give a brown solution. The mixture was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (99 mg, 0.39

mmol) was dissolved in N-methyl-2-pyrrolidinone (1 mL) and added to the above reaction followed by 4 mole equivalents (0.122ml, 1.56 mmol) of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon solid formed after cooling. The solid was filtered and washed with methanol (20ml) to get N6-(2-amino-6-(2-fluorophenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (40.0 mg, 0.09 mmol, 23.29 %)

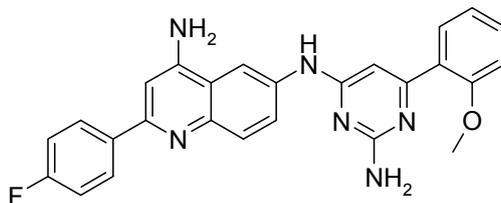
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.82 (s, 1 H) 7.09 (s, 1 H) 7.40 - 7.60 (m, 5 H) 7.69 (d, *J*=5.46 Hz, 1 H) 7.90 (t, *J*=7.44 Hz, 2 H) 8.03 (dd, *J*=8.38, 5.37 Hz, 2 H) 8.11 (d, *J*=8.48 Hz, 1 H) 8.21 (d, *J*=9.04 Hz, 1 H) 8.99 (br. s., 2 H) 11.22 (br. s., 1 H) 13.73 (br. s., 1 H)

m/z = 441.1 (M+1)

Purity 97%, HPLC *t*_R = 5.8 min.

HRMS (EI), M+1 calcd. for C₂₅H₁₈F₂N₆, 441.1639; found 441.1647

N6-(2-amino-6-(2-methoxyphenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13h):



A mixture of compound **3** 2-amino-6-(2-methoxyphenyl)pyrimidin-4-ol (0.081 g, 0.37 mmol), N-phenyltrifluoromethanesulphonamide (0.147 g, 0.41 mmol), and triethylamine (0.052 mL, 0.37 mmol) were taken in dry NMP (0.5mL) in a 25 ml round bottom flask to give a brown solution. The mixture was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (0.094 g, 0.37 mmol) was dissolved in N-methyl-2-pyrrolidinone (1.5 mL) and added to the above reaction followed by 4 mole equivalents ((0.6ml, 1.49 mmol) of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon solid formed after cooling. The solid was filtered and washed with methanol (10ml) to get N6-(2-amino-6-(2-methoxyphenyl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (0.030 g, 0.07mmol, 17.78 %).

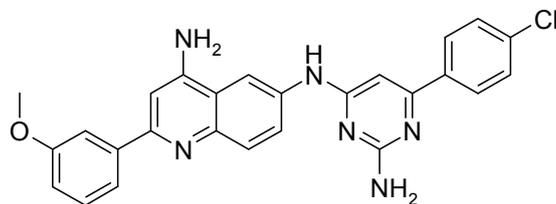
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 3.93 (br. s., 3 H) 6.80 (br. s., 1 H) 7.09 (br. s., 1 H) 7.18 (br. s., 1 H) 7.31 (br. s., 1 H) 7.48 - 7.74 (m, 4 H) 8.03 (br. s., 2 H) 8.20 (s, 2 H) 8.13 (s, 1 H) 8.96 (br. s., 2 H) 11.28 (br. s., 1 H) 12.71 (br. s., 1 H) 13.74 (br. s., 1 H).

m/z = 453.1 (M+1)

Purity 98%, HPLC *t*_R = 5.7 min.

HRMS (EI), M+1 calcd. for C₂₆H₂₁FN₆O, 453.1839; found 453.1846

Preparation of N6-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)-2-(3-methoxyphenyl)quinoline-4,6-diamine (13i).



A mixture of compound 2-amino-6-(4-chlorophenyl)pyrimidin-4-ol (80 mg, 0.36 mmol), N-phenyltrifluoromethanesulphonamide (142 mg, 0.40 mmol), and triethylamine (0.050 mL, 0.36 mmol) were taken in dry NMP (1mL) in a 25 ml round bottom flask to give a brown solution. The mixture was heated to 60°C for 2h. 2-(3-methoxyphenyl)quinoline-4,6-diamine (0.096 g, 0.36 mmol) was dissolved in N-methyl-2-pyrrolidinone (1.5 mL) and added to the above reaction followed by 4 mole equivalents ((0.6ml, 1.49 mmol) of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon solid formed after cooling. The solid was filtered and washed with methanol (10ml) to get N6-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)-2-(3-methoxyphenyl)quinoline-4,6-diamine (0.033 g, 0.073 mmol, 20.09 %).

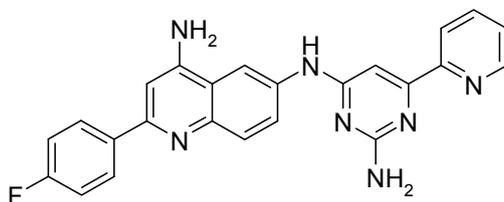
¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 3.91 (s, 3 H) 6.77 (br. s., 1 H) 7.09 (s, 1 H) 7.24 - 7.33 (m, 1 H) 7.46 - 7.53 (m, 2 H) 7.63 (t, *J*=7.95 Hz, 1 H) 7.73 (br. s., 2 H) 7.93 (d, *J*=8.56 Hz, 2 H) 8.08 (br. s., 1 H) 8.16 (d, *J*=8.56 Hz, 1 H) 8.65 (br. s., 1 H) 8.99 (br. s., 2 H).

m/z = 470 (M+1).

Purity 99 %, HPLC *t*_R = 6.0 min.

HRMS (EI), M+1 calcd. for C₂₆H₂₁ClN₆O, 469.1538 found 469.1569.

Preparation of N6-(2-amino-6-(pyridin-2-yl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine:(13j)



In a 25 mL round-bottomed flask A mixture of 2-amino-6-(pyridin-2-yl)pyrimidin-4-ol (0.080 g, 0.43 mmol), triethylamine (0.059 mL, 0.43 mmol), N-phenyl-bis(trifluoromethanesulphonamide) (0.167 g, 0.47 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C

for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (108 mg, 0.43 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 70 mg (38.9 %).

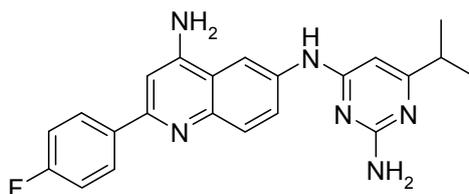
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 7.09 (s, 1 H) 7.30 (s, 1 H) 7.55 (t, *J*=8.76 Hz, 3 H) 7.68 - 7.75 (m, 1H) 8.03 (dd, *J*=8.85, 5.27 Hz, 2 H) 8.11 - 8.24 (m, 5 H) 8.85 (d, *J*=5.84 Hz, 1 H) 8.96 (br. s., 1 H).

m/z = 424 (M+1)

Purity 95%, HPLC *t_R* = 5.36 min.

HRMS (EI), M+1 calcd. for C₂₄H₁₈FN₇, 424.168596; found 424.1698

Preparation of N₆-(2-amino-6-isopropylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine:(13k)



In a 25 mL round-bottomed flask A mixture of 2-amino-6-isopropylpyrimidin-4-ol (0.054 g, 0.35 mmol), triethylamine (0.050 mL, 0.36 mmol), and N-phenyl-bis(trifluoromethanesulfonamide) (0.127 g, 0.36 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (0.09 g, 0.36 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 50 mg (36.2 %).

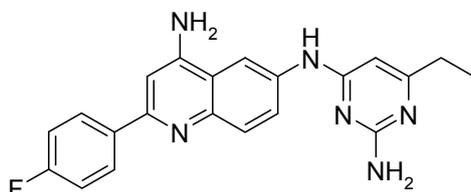
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.29 (d, *J*=6.78 Hz, 6 H) 2.91 (ddd, *J*=13.75, 7.16, 6.97 Hz, 1 H) 6.38 (s, 1 H) 7.08 (s, 1 H) 7.55 (t, *J*=8.85 Hz, 2 H) 7.96 - 8.11 (m, 3 H) 8.16- 8.24 (m, 1 H) 8.91 (br. s., 1 H) 11.15 (br. s., 1 H) 13.04 (br. s., 1 H) 13.72 (br. s., 1 H).

m/z = 389 (M+1)

Purity 99%, HPLC *t_R* = 7.08 min.

HRMS (EI), M+1 calcd. for C₂₂H₂₁FN₆, 389.188997; found 389.1897

Preparation of N6-(2-amino-6-ethylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine:(13l)



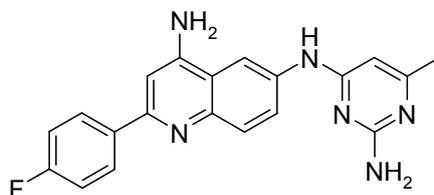
In a 25 mL round-bottomed flask A mixture of 2-amino-6-ethylpyrimidin-4-ol (0.049 g, 0.36 mmol), triethylamine (0.050 mL, 0.36 mmol) and N-phenyl-bis(trifluoromethanesulfonamide) (0.127 g, 0.36 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (0.09 g, 0.36 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 50 mg (37.1 %).

¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 1.25 (t, *J*=7.44 Hz, 3 H) 2.59 - 2.72 (m, 2 H) 6.36 (s, 1 H) 7.07 (s, 1 H) 7.48 - 7.64 (m, 2 H) 7.97 - 8.11 (m, 3 H) 8.14 - 8.24 (m, 1 H) 8.90 (br. s., 3 H) 11.07 (br. s., 1 H), 12.95 (br. s., 1 H) 13.70 (br. s., 1 H).

m/z = 375(M+1)

Purity 99%, HPLC *t_R* = 6.75 min.

Compound 17: Preparation of N6-(2-amino-6-methylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13m):



A mixture of 4-chloro-6-methylpyrimidin-2-amine (0.085 g, 0.59 mmol) and 2-(4-fluorophenyl)quinoline-4,6-diamine (0.075 g, 0.30 mmol) were taken in a thermal reactor and NMP (3 mL) and 2 drops of conc. HCl were added to it. It was heated to 100°C overnight under nitrogen. The reaction was cooled, A precipitate was formed which was filtered and washed several times with hexane to get a light yellow solid N6-(2-amino-6-methylpyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine (0.075 g, 0.21 mmol, 70.3 %).

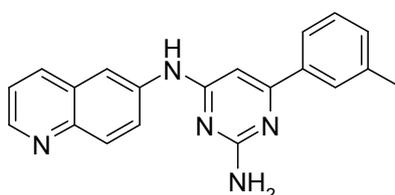
^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ ppm 2.35 (s, 3 H) 6.34 (s, 1 H) 7.07 (s, 1 H) 7.56 (t, $J=8.76$ Hz, 2 H) 8.02 (dd, $J=8.67, 5.27$ Hz, 2 H) 8.07 (d, $J=10.36$ Hz, 1 H) 8.15 - 8.21 (m, 1 H) 8.72 (br. s., 1 H) 8.89 (br. s., 1 H) 9.13 (br. s., 1 H) 11.05 - 11.17 (m, 1 H) 13.00 (br. s., 1 H) 13.72 (br. s., 1 H)

$m/z = 361.1$ (M+1).

Purity 98%, HPLC $t_R = 6.3$ min.

HRMS (EI), M+1 calcd. for $\text{C}_{20}\text{H}_{17}\text{FN}_6$, 361.1577; found 361.1586

Compound 18: Preparation of N4-(quinolin-6-yl)-6-m-tolylpyrimidine-2,4-diamine:(13n).



In a 25 mL round-bottomed flask a mixture of 2-amino-6-m-tolylpyrimidin-4-ol (0.070 g, 0.35 mmol), triethylamine (0.048 mL, 0.35 mmol) and N-phenyl-bis(trifluoromethanesulfonamide) (0.124 g, 0.35 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C for 2h. quinolin-6-amine (0.05 g, 0.35 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80°C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 50 mg (44 %).

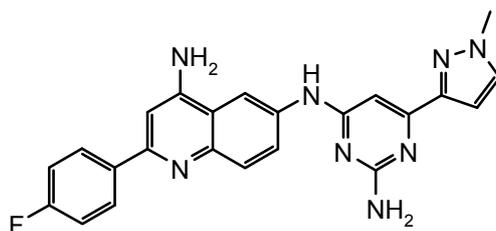
^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ ppm 2.45 (s, 3 H) 6.73 (s, 1 H) 7.48 - 7.59 (m, 3 H) 7.63 - 7.72 (m, 3 H) 8.11 (t, $J=9.03$ Hz, 2 H) 8.50 (d, $J=8.03$ Hz, 1 H) 8.80 (br. s., 1 H) 8.94 (d, $J=3.01$ Hz, 1 H) 11.09 (br. s., 3 H) 12.77 (br. s., 3 H).

$m/z = 328$ (M+1)

Purity 99%, HPLC $t_R = 5.40$ min.

HRMS (EI), M+1 calcd. for $\text{C}_{20}\text{H}_{17}\text{N}_5$, 328.15622; found 328.1570

Compound 19: Preparation of N6-(2-amino-6-(1-methyl-1H-pyrazol-3-yl)pyrimidin-4-yl)-2-(4-fluorophenyl)quinoline-4,6-diamine(13o):



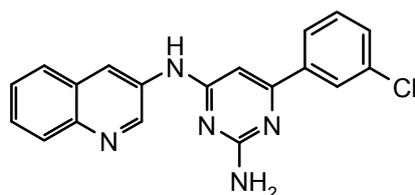
In a 25 mL round-bottomed flask A mixture of 2-amino-6-(1-methyl-1H-pyrazol-3-yl)pyrimidin-4-ol (67.9 mg, 0.36 mmol), triethylamine (0.050 mL, 0.36 mmol), N-phenyl-bis(trifluoromethanesulfonamide) (140 mg, 0.39 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C for 2h. 2-(4-fluorophenyl)quinoline-4,6-diamine (90 mg, 0.36 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 50 mg (33 %).

¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 4.01 (s, 3 H) 6.87 (s, 1 H) 7.03 (s, 1 H) 7.07 (s, 1 H) 7.50 - 7.61 (m, 3 H) 7.97 - 8.05 (m, 3 H) 8.08 (d, *J*=8.10 Hz, 1 H) 8.14 - 8.20 (m, 1 H) 8.92 (br. s., 2 H) 13.66 (br. s., 1 H).

m/z = 427(M+1)

Purity 99%, HPLC *t_R* = 5.06 min.

Compound 20: Preparation of 6-(3-chlorophenyl)-N4-(quinolin-3-yl)pyrimidine-2,4-diamine;(14)



In a 25 mL round-bottomed flask A mixture 2-amino-6-(3-chlorophenyl)pyrimidin-4-ol (0.111 g, 0.50 mmol) triethylamine (0.069 mL, 0.50 mmol) and N-phenyl-bis(trifluoromethanesulfonamide) (0.197 g, 0.55 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C for 2h. quinolin-3-amine (0.072 g, 0.50 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a yellow solid. 75 mg (43.1 %).

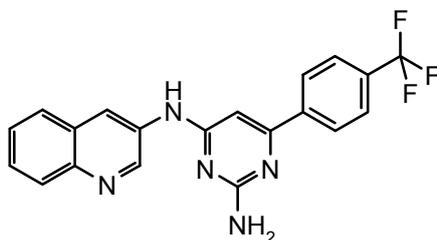
¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.83 (s, 1 H) 7.63 - 7.78 (m, 4 H) 7.87 (dt, *J*=7.49, 1.53 Hz, 1 H), 7.98 - 8.07 (m, 3 H) 8.98 (br. s., 1 H) 9.17 (d, *J*=2.45 Hz, 1 H) 11.20 (br. s., 1 H).

m/z = 348 (M+1)

Purity 98%, HPLC *t_R* = 5.82 min

HRMS (EI), M+1 calcd. for C₁₉H₁₄ClN₅, 348.101598; found 348.1024

Preparation of N4-(quinolin-3-yl)-6-(4-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine: (15)



In a 25 mL round-bottomed flask a mixture 2-amino-6-(4-(trifluoromethyl)phenyl)pyrimidin-4-ol (0.111 g, 0.43 mmol), N-phenyl-bis(trifluoromethanesulfonamide) (0.155 g, 0.43 mmol), and triethylamine (0.061 mL, 0.43 mmol) in N-methyl-2-pyrrolidinone (1.0 mL) under nitrogen was heated to 60°C for 2h. quinolin-3-amine (0.063 g, 0.43 mmol) was dissolved in N-methyl-2-pyrrolidinone (1ml) and added to the above reaction followed by 4 mole equivalents of HCl (4M in Dioxane). The mixture was heated to 80 °C for 10h where upon a solid formed. After cooling to room temperature, methanol (10 ml) was added solid precipitates out. The resulting solid was filtered and washed with methanol and dried as a white solid. 75 mg (45.2 %).

¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 6.72 (s, 1 H) 7.62 - 7.79 (m, 2 H) 7.92 - 8.07 (m, 6 H) 9.00 (d, *J*=2.64 Hz, 1 H) 9.08 (d, *J*=2.45 Hz, 1 H).

m/z= 382 (M+1)

Purity 99%, HPLC *t_R* = 5.98 min

HRMS (EI), M+1 calcd. for C₂₀H₁₄F₃N₅, 382.127954; found 382.1293

Preparation of N-(2,6-dimethylpyrimidin-4-yl)-2-methylquinolin-6-amine: (16).



In a Biotage microwave vial 5 mL a mixture of 2,6-dimethylpyrimidin-4-amine (0.1 g, 0.81 mmol), 6-chloro-2-methylquinoline (0.131 g, 0.74 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.034 g, 0.04 mmol), BINAP (0.046 g, 0.07 mmol) and potassium tert-butoxide (0.096 g, 0.81 mmol) was added under nitrogen in DMF 2 ml. The reaction mixture was irradiated to microwave conditions for 30 min at 130 ° C. The reaction mixture was concentrated under *vacuo*. A solid was obtained. The solid was purified by reverse phase chromatography as a white solid. 9 mg (4.6 %).

¹H NMR (300 MHz, DMSO-*d*₆) δ ppm 2.29 (s, 3 H) 2.48 (s, 4 H) 2.63 (s, 2 H) 6.55 (s, 1 H) 7.35 (d, *J*=8.48 Hz, 1 H) 7.86 (d, *J*=1.70 Hz, 2 H) 8.13 (d, *J*=8.29 Hz, 1 H) 8.30 (t, *J*=1.41 Hz, 1 H) 9.50 (s, 1H).

m/z = 265 (M+1)

Purity 99%, HPLC *t_R* = 4.62 min

HRMS (EI), M+1 calcd. for C₁₆H₁₆N₄, 265.145321; found 265.1462

Enzyme assay

The standard reaction buffer contained 50 mM Hepes buffer pH 7.5, 0.008% Brij-35 and 25 mM NaCl and. Reaction was monitored at 340 nm by following the depletion of NADH. NADH was used at 200uM concentration. The reaction was initiated by adding menaquinone and was monitored spectrophotometrically by following the absorbance changes at 340 nm at 25 °C after 60 min in a SpectraMax (Molecular Devices, Sunnyvale, CA) spectrophotometer. The specific activities were calculated using an extinction coefficient of 6700 M⁻¹ cm⁻¹ for NADH at 340 nm.

Enzyme purification protocol

Mtu NDH2 with c-term His-tag was expressed in *Msm* (IPTG inducible vector developed in AZ). Reported protocol (*Yano et al, J. Biol. Chem.,2006, 281, 11456-11463*) was used to further optimize growth conditions in order to improve cell mass. Purification of protein was also optimized using various detergents (0.5% for extraction from the membrane fraction followed by 0.25% during purification). Extraction of NDH2 protein was optimized which improved the yield of active protein. The purification protocol used is as follows: 1) Cell disruption done by French press followed by sonication, cell lysate centrifuged at 6k for 30 mins, supernatant ultra

centrifuged 1,50,000xg for 1hr 2) Re-suspended in extraction-buffer with 0.4% CHAPS at protein (4mg/ml) followed by sonication for 1 min and then gently stirred at 4⁰ C for 1 hr 3) Ultra centrifugation for 1,50,000xg for 1hr 4) Supernatant incubated with Talon resin for 1hr 5) Washed with 0.18% CHAPS + 10mM imidazole, followed by 0.18% CHAPS + 50mM imidazole 6) Eluted with 0.18% CHAPS + 200mM imidazole 7) Dialyzed overnight- 100mM potassium phosphate buffer, pH 6.8 containing 40% glycerol.

MABA/Resazurin based MIC determination

Two hundred micro liters of sterile deionized water is added to all outer-perimeter wells of sterile 96-well plates (Tarsons) to minimize evaporation of the medium in the test wells during incubation. The wells in rows B to G in columns 2 to 10 receive 4 micro liters of the different drug (compound) concentrations (previously diluted in 100% DMSO) starting either from 128, 64 or 32 micrograms per ml with the help of a liquid handling system. Final drug concentration range is as follows: Isoniazid (INH), 0.03 to 4.0 ug/ml; and compounds, 0.5 to 128 mg/ml. A multichannel pipette is used to dispense, 200 micro liters of *M. tuberculosis* inoculum into the wells (rows B to G, columns 2 to 10). Thus, the wells in column 11 served as drug-free (inoculum-only) and in column 12 as medium controls. The plates were packed in gas permeable polythene bags and were incubated at 37°C for 5 days. Forty micro liters of a freshly prepared 1:1 mixture of 10X Alamar Blue (MABA from Accumed International, Westlake, Ohio), or Resazurin (20mg/100ml) reagent and 10% Tween 80 was added to well B11. The plates were incubated at 37°C for 24 h. If well B11 turned pink, the reagent mixture was added to all wells in the micro plate (if the well remained blue, the reagent mixture would be added to another control well and the result would be read on the following day). The micro plates were incubated for an additional 24 h at 37°C, and the colors of all wells were recorded. If the wells remain blue, it is interpreted as no growth/inhibition, and a change to pink color was scored as growth/no inhibition. A few wells appeared violet after 24 h of incubation, but they invariably changed to pink after another day of incubation and thus were scored as growth (while the adjacent blue wells remained blue). The addition of the reagent is done for only one plate which is then read in a spectrophotometer at two different wavelengths – 575 & 610 nm. The MIC is calculated from these readings using an Activity Base template.

Solubility Assay

1. Compounds are dispensed in 96 well plate – 100ul at 10mM in 96 Half Deep Axygen-A1196
2. Glyburide is used as QC standard
3. 0.1M phosphate buffer is prepared: Weigh out 11.4g Na₂HPO₄ (sodium phosphate dibasic, FW141.96) and 2.72g NaH₂PO₄ (sodium phosphate monobasic, FW137.99). QS to 1L with dH₂O; adjust pH to 7.4 with dilute NaOH or phosphoric acid as needed. Stored in refrigerator
4. Compounds are diluted as given below:
 - A. 100ul 10mM cmpd diluted with 150ul DMSO in compound plate (1:4 dil.)
 - B. 70ul 4mM cmpd + 210ul DMSO to make 1mM (1:4 dil.)
 - C. 100ul 4mM cmpd transferred to assay plate
 - D. 45ul 1mM cmpd + 855ul 40:60 ACN/water to make 50ulM (1:20 dil.)
 - E. 50ul 50ulM cmpd + 450ul 40:60 ACN/water to make 5ulM (1:10 dil.)
 - F. 100ul 5ulM cmpd + 400ul 40:60 ACN/water to make 1ulM (1:5 dil.)
5. Samples are dried in Genevac for 4 hrs
6. Stir stix are added to all tubes in plate
7. 800ul of buffer is added to all tubes.
8. Plates are stir covered for 24 hours on Eppendorf Thermomix R at 750rpm at 25C.
9. Compounds are dispensed for UV and MS analysis

Plasma protein binding assay

Protein binding is measured using the equilibrium dialysis technique. Compound is added to 10% plasma giving a concentration of 20 μM and dialysed with isotonic buffer for 18 hours at 37°C. The plasma and buffer solutions are analysed using generic LCUVMS and the first apparent binding constant for the compound derived. The binding constant is then used to determine the % free in 100% plasma.

Metabolic stability assay (mouse microsomal Clint)

These experiments are usually performed manually although a robotic liquid handling system can also be used. The compounds to be assayed are added from stock solution of 100 μM in

microsomal buffer [1.96 g of Tris-HCl (50mM), 1.28g of KCl (69mM) and 0.50 g of MgCl₂ (10mM) was weighed and dissolved in 250ml of HPLC grade water and the pH adjusted to 7.40 with 1M KOH] diluted from a 5 mM or 1mg/ml stock solubilised in an appropriate solvent like Acetonitrile (containing no more than 10 % DMSO v/v) to a suspension of microsomal protein in the microsomal buffer. After a 30 second pre-incubation at 37°C, the cofactor NADPH is added so that the initial reaction conditions are 10 µM substrate, 1mg/ml microsomal protein and 2mg/ml NADPH in a total reaction volume of 500 µL. At appropriate time points (eg. 0, 5, 10, 20 and 30 min), an aliquot of 85 ul is taken from the incubation and added to equal volumes of an appropriate organic solvent (e.g. acetonitrile) to terminate the reactions and denature the microsomal enzymes. Control incubations may also be conducted from which NADPH or microsomes have been omitted. Once the incubations have been quenched, the samples are vortexed and then centrifuged for 15 min at 13000 rpm. 150 µL of the supernatant is further diluted by adding 150 µL HPLC grade water analysed by HPLC using the method developed for the particular project or the probe substrates of interest.