SUPPORTING INFORMATION

Antiparasitic Lead Discovery: Towards Optimization of a Chemotype with Activity Against Multiple Protozoan Parasites.

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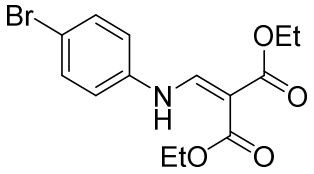
Table of contents	

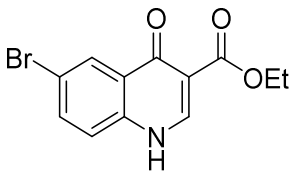
CHEMICAL SYNTHESIS AND CHARACTERIZATION

General Methods

Unless otherwise noted, reagents were obtained from Sigma-Aldrich, Inc. (St. Louis, MO), Fisher Scientific, Frontier Scientific Services, Inc. (Newark, DE), or Matrix Scientific (Columbia, SC) and used as received. Reaction solvents were dried by passage through alumina columns on a purification system manufactured by Innovative Technology (Newburyport, MA). Microwave reactions were performed using a Biotage Initiator-8 instrument. NMR spectra were obtained with Varian NMR systems, operating at 400 or 500 MHz for ^{1}H acquisitions as noted. LCMS analysis was performed using a Waters Alliance reverse-phase HPLC, with single-wavelength UV–visible detector and LCT Premier time-of-flight mass spectrometer (electrospray ionization). All newly synthesized compounds that were submitted for biological testing were deemed >95% pure by LCMS analysis (UV and ESI-MS detection) prior to submission for biological testing. Preparative LCMS was performed on a Waters Fraction Lynx system with a Waters MicroMass ZQ mass spectrometer (electrospray ionization) and a single-wavelength UV–visible detector, using acetonitrile/H_2O gradients with 0.1% formic acid. Fractions were collected on the basis of triggering using UV and mass detection. Yields reported for products obtained by preparative HPLC represent the amount of pure material isolated; impure fractions were not repurified.

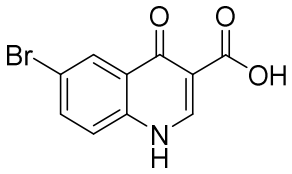


Diethyl 2-(((4-bromophenyl)amino)methylene)malonate (S1) In a 25 mL round bottom flask was added 4-bromoaniline (4.0 g, 23 mmol) and diethyl 2-(ethoxymethylene)malonate (4.7 mL, 23 mmol). The mixture was stirred at room temperature until homogeneous then heated to 100 °C where it was held for 2.5 hours. The mixture was cooled and the product was triturated with hexanes. The precipitate was filtered, washed with hexanes, and dried under a vacuum overnight to yield **S1** as a fluffy white solid in 94% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.00 (d, *J*=13.7 Hz, 1 H), 8.46 (d, *J*=13.7 Hz, 1 H), 7.48 (d, *J*=8.8 Hz, 2 H), 7.02 (d, *J*=8.8 Hz, 2 H), 4.28 (dq, *J*=28.3, 6.8 Hz, 4 H), 1.36 (dt, *J*=24.9, 7.3 Hz, 6 H). LCMS found 342.0 $[M+H]^+$; calculated 342.0 for $C_{14}H_{17}BrNO_4$.

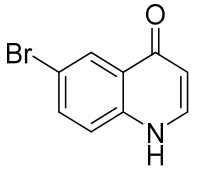


Ethyl 6-bromo-4-oxo-1,4-dihydroquinoline-3-carboxylate (S2) In a 100 mL three neck flask equipped with a stir bar was added diethyl 2-(((4-

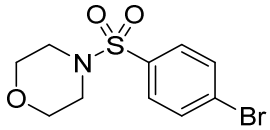
bromophenyl)amino)methylene)malonate (7.48 g, 21.9 mmol) and diphenyl ether (30 mL). The mixture was heated to reflux with stirring for 1 hour. A gentle stream of nitrogen was applied through a side neck to aid the removal of ethanol once a reflux was reached. The mixture was cooled to room temperature and diluted with hexanes (100 mL). The precipitate was vacuum filtered, washed with hexanes (2 x 50 mL), and dried under a vacuum overnight to yield **S2** as an off-white powder in 80% yield. *Insufficient solubility to obtain NMR. LCMS found 296.0 $[M+H]^+$; calculated 296.0 for $C_{12}H_{11}BrNO_3$.



6-Bromo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (S3) In a 100 mL round bottom flask equipped with a stir bar was added ethyl 6-bromo-4-oxo-1,4-dihydroquinoline-3-carboxylate (5.16 g, 17.4 mmol) and aqueous sodium hydroxide (2.5M, 35 mL). The suspension was heated to reflux with stirring for 3 hours. The solution was cooled to room temperature and acidified with aq. HCl to ~pH2 (3M, 80 mL). The precipitate was filtered, washed with water (2 x 30 mL), and dried under a vacuum to yield **S3** as a light tan colored solid in 96% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.97 (br. s., 1 H), 13.52 (br. s., 1 H), 8.92 (s, 1 H), 8.31 (d, *J*=2.5 Hz, 1 H), 8.01 (dd, *J*=8.8, 2.2 Hz, 1 H), 7.75 (d, *J*=9.1 Hz, 1 H). LCMS found 267.9 $[M+H]^+$; calculated 268.0 for $C_{10}H_7BrNO_3$.

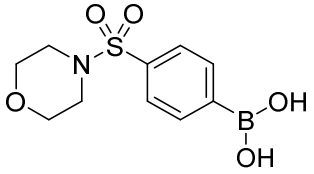


6-Bromoquinolin-4(1H)-one (10) In a 50 mL three-neck flask equipped with a stir bar was added 6-bromo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (320 mg, 1.19 mmol) and diphenyl ether (20 mL). The mixture was stirred vigorously and heated to reflux for 1 hour. The mixture was cooled to room temperature and hexanes (100 mL) was added. The precipitate was filtered, washed with hexanes (2 x 50 mL), and dried under a vacuum overnight to yield **10** as a fluffy light brown solid in 96% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.92 (br. s., 1 H), 8.16 (d, *J*=2.4 Hz, 1 H), 7.95 (d, *J*=6.8 Hz, 1 H), 7.79 (dd, *J*=8.8, 2.4 Hz, 1 H), 7.52 (d, *J*=8.8 Hz, 1 H), 6.08 (d, *J*=7.3 Hz, 1 H). LCMS found 223.9 $[M+H]^+$; calculated 224.0 for C_9H_7BrNO.

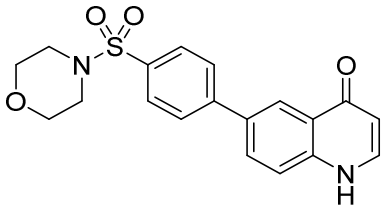


4-((4-Bromophenyl)sulfonyl)morpholine (8) In a 100 mL round bottom flask was added 4-bromobenzene-1-sulfonyl chloride (5.00 g, 19.6 mmol), tetrahydrofuran (49

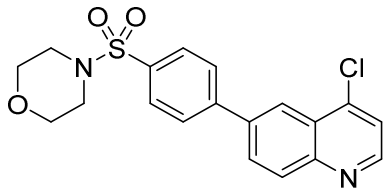
mL), and morpholine (1.8 mL, 21 mmol). The mixture was stirred at room temperature overnight. The mixture was neutralized with sat. aq. $NaHCO_3$ (20 mL) and extracted with ethyl acetate (3 x 60 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (60 mL), washed with brine (40 mL), dried over Na_2SO_4, and concentrated to yield **8** as a white solid in 94% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J*=8.8 Hz, 2 H), 7.62 (d, *J*=8.8 Hz, 2 H), 3.72 - 3.78 (m, 4 H), 2.96 - 3.04 (m, 4 H). LCMS found 306.0 $[M+H]^+$; calculated 306.0 for $C_{10}H_{13}BrNO_3S$.



(4-(Morpholinosulfonyl)phenyl)boronic acid (9) In a flame dried 100 mL flask equipped with a stir bar was added 4-((4-bromophenyl)sulfonyl)morpholine (5.2 g, 17 mmol). The flask was sealed with a septum and vacuum purged with nitrogen (3x) before adding dry THF (40 mL) with a syringe. The mixture was kept under an atmosphere of nitrogen, cooled to -78 °C, and *n*-butyllithium (2.2M in hexanes, 8.5 mL, 19 mmol) was added dropwise. The solution was allowed to stir for 1 hour before the dropwise addition of tri*iso*propyl borate (20 mL, 86 mmol). The mixture was allowed to warm to room temperature and stirred overnight. The mixture was quenched with water (120 mL), basified with 3M aq. NaOH to ~pH 12 and washed with ethyl acetate (100 mL). The layers were separated and the aqueous was acidified with 3M aq. HCl to ~pH 4 and extracted with ethyl acetate (3 x 130 mL). The combined organic layers were washed with brine (80 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude was then recrystallized with 6:1 water/THF to give **9** as short white needles in 50% yield.



6-(4-(Morpholinosulfonyl)phenyl)quinolin-4(1H)-one (11) A mixture of 6-bromoquinolin-4(1H)-one (302 mg, 1.35 mmol), (4-(morpholinosulfonyl)phenyl)boronic acid (373 mg, 1.38 mmol), triethylamine (0.57 mL, 4.1 mmol), and bis(triphenylphosphine)palladium(II) chloride (10 mg, 0.014 mmol) in 1:1 water/ethanol (13 mL) was refluxed for 2 hours. The mixture was cooled to room temperature, the ethanol was removed under reduced pressure, and the precipitate was filtered to yield **11** as an off-white solid in 87% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.92 (br. s., 1 H), 8.43 (d, *J*=2.0 Hz, 1 H), 8.08 (dd, *J*=8.5, 2.2 Hz, 1 H), 8.03 (d, *J*=8.8 Hz, 2 H), 7.96 (d, *J*=7.3 Hz, 1 H), 7.83 (d, *J*=8.8 Hz, 2 H), 7.69 (d, *J*=8.8 Hz, 1 H), 6.10 (d, *J*=7.3 Hz, 1 H), 3.61 - 3.69 (m, 4 H), 2.88 - 2.95 (m, 4 H). LCMS found 371.1 $[M+H]^+$; calculated 371.1 for $C_{19}H_{19}N_2O_4S$.



4-((4-(4-Chloroquinolin-6-yl)phenyl)sulfonyl)morpholine (12) A mixture of 6-(4-(morpholinosulfonyl)phenyl)quinolin-4(1H)-one (582 mg, 1.57 mmol) and Phosphorus oxychloride (5.0 mL, 54 mmol) was refluxed for 2 hours, cooled to room temperature, and the excess phosphorus oxychloride was removed by vacuum distillation. The remaining crude was cooled in an ice-water bath, diluted with dichloromethane (80 mL), and quenched with the dropwise addition of sat. aq. $NaHCO_3$ (100 mL). The layers were separated and the aqueous was extracted with dichloromethane (2 x 80 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (50 mL), washed with brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was then purified by flash column chromatography using a gradient of 5-20% ethyl acetate in dichloromethane to yield **12** as an off-white crystalline solid in 99% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.84 (d, *J*=4.4 Hz, 1 H), 8.46 (d, *J*=2.0 Hz, 1 H), 8.27 (d, *J*=8.8 Hz, 1 H), 8.04 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.88 - 7.95 (m, 4 H), 7.58 (d, *J*=4.9 Hz, 1 H), 3.75 - 3.82 (m, 4 H), 3.05 - 3.13 (m, 4 H). LCMS found 389.1 $[M+H]^+$; calculated 389.1 for $C_{19}H_{18}ClN_2O_3S$.

General procedure A for amination of 12:

A solution of 4-((4-(4-chloroquinolin-6-yl)phenyl)sulfonyl)morpholine (39 mg, 0.100 mmol) and *para*-toluenesulfonic acid monohydrate (19 mg, 0.10 mmol) in dry DMSO (1.0 mL) was added to a flame dried 8 mL vial containing the appropriate amine (0.11 mmol). The vial was capped and the contents heated to 80 °C for 24 hours. The mixture was cooled to room temperature, filtered (0.45 µm, cellulose), and purified by prep HPLC (C-18, 50 x 30 mm) with a gradient of 5-30% acetonitrile in water. Ethyl acetate (40 mL) and aq. NaOH (1M, 20 mL) were added to the combined product containing fractions and the layers were separated. The organic layer was washed with brine (15 mL), dried over Na_2SO_4, and concentrated.

General procedure B for amination of 12:

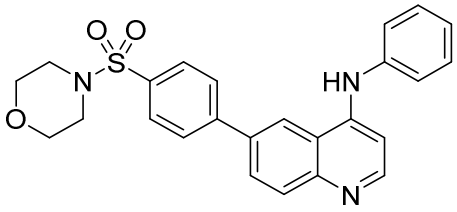
To a flame dried 8 mL vial equipped with a stir bar was added 4-((4-(4-chloroquinolin-6-yl)phenyl)sulfonyl)morpholine (39 mg, 0.10 mmol), the appropriate amine (0.20 mmol), cesium carbonate (65 mg, 0.20 mmol), $Pd_2(dba)_3$ (5 mg, 5 µmol, 5 mol%), and xantphos (3 mg, 5 µmol, 5 mol%). The vial was capped, vacuum purged with nitrogen (3x), and 1,4-dioxane (2.0 ml) was added via syringe. The mixture was refluxed under an atmosphere of nitrogen for 24 hours. The mixture was cooled to room temperature, poured over water (6 mL), and extracted with ethyl acetate (3 x 7 mL). The combined organic layers were washed with water (5 mL), washed with brine (3 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was then purified by flash column chromatography and prep HPLC as necessary.

General procedure C for amination of 12:

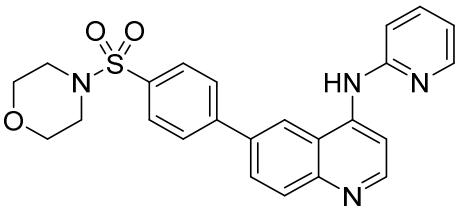
To a flame dried 8 mL vial equipped with a stir bar was added 4-((4-(4-chloroquinolin-6-yl)phenyl)sulfonyl)morpholine (39 mg, 0.10 mmol), the appropriate amine (0.20 mmol), potassium *tert*-butoxide (23 mg, 0.20 mmol), $Pd_2(dba)_3$ (5 mg, 5 µmol, 5 mol%), and xantphos (3 mg, 5 µmol, 5 mol%). The vial was capped, vacuum purged with nitrogen (3x), and 1,4-dioxane (2.0 ml) was added via syringe. The mixture was refluxed under an atmosphere of nitrogen for 24 hours. The mixture was cooled to room temperature, poured over water (6 mL), and extracted with ethyl acetate (3 x 7 mL). The combined organic layers were washed with water (5 mL), washed with brine (3 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was then purified by flash column chromatography and prep HPLC as necessary.

General procedure D for the amination of 12:

Sodium hydride (60 wt%, 16 mg, 0.40 mmol) was added to a solution of the appropriate amine (0.20 mmol) in dry DMF (1.0 mL) at 0 °C. The mixture was stirred for 10 minutes before the slow addition of a solution of 4-((4-(4-chloroquinolin-6-yl)phenyl)sulfonyl)morpholine (39 mg, 0.100 mmol) in dry 1,4-dioxane (2.0 mL). The mixture was heated to 100 °C for 1 hour, cooled to room temperature, and quenched with sat. aq. NH_4Cl (3 mL) added dropwise. Water (6 mL) was added and the mixture was extracted with ethyl acetate (3 x 6 mL). The combined organic layers were washed with water (3 x 4 mL), washed with brine (4 mL), dried over anhydrous Na_2SO_4, concentrated, and purified by flash column chromatography and prep HPLC as necessary.

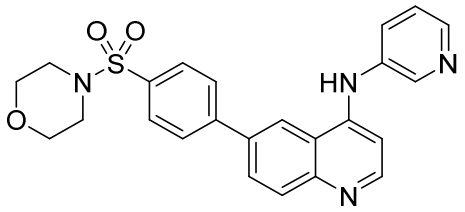


6-(4-(Morpholinosulfonyl)phenyl)-N-phenylquinolin-4-amine (13a) General procedure A, isolated as a yellow solid in 36% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.21 (br. s., 1 H), 8.82 (d, *J*=2.0 Hz, 1 H), 8.48 (d, *J*=4.9 Hz, 1 H), 8.18 (d, *J*=8.3 Hz, 2 H), 8.11 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.99 (d, *J*=8.8 Hz, 1 H), 7.88 (d, *J*=8.3 Hz, 2 H), 7.46 (t, *J*=8.3 Hz, 2 H), 7.40 (d, *J*=7.3 Hz, 2 H), 7.19 (t, *J*=7.3 Hz, 1 H), 6.96 (d, *J*=5.4 Hz, 1 H), 3.61 - 3.69 (m, 4 H), 2.89 - 2.97 (m, 4 H). LC/MS found 446.1 $[M+H]^+$; calculated 446.2 for $C_{25}H_{24}N_3O_3S$.

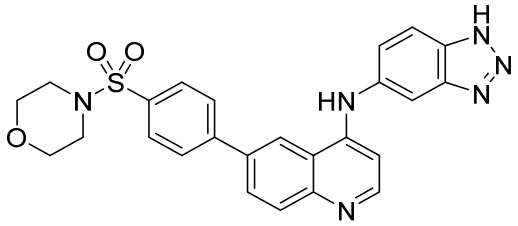


6-(4-(Morpholinosulfonyl)phenyl)-N-(pyridin-2-yl)quinolin-4-amine (13b) General procedure A, isolated as a yellow solid in 9% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.61 (s, 1 H), 8.85 (d, *J*=2.0 Hz, 1 H), 8.69 (d, *J*=5.4 Hz, 1 H), 8.34 - 8.39 (m, 2 H),

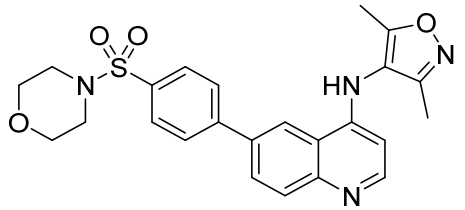
8.19 (d, *J*=8.8 Hz, 2 H), 8.12 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.05 (d, *J*=8.3 Hz, 1 H), 7.90 (d, *J*=8.3 Hz, 2 H), 7.79 (td, *J*=7.8, 2.0 Hz, 1 H), 7.37 (d, *J*=8.3 Hz, 1 H), 7.03 (dd, *J*=7.1, 5.1 Hz, 1 H), 3.62 - 3.70 (m, 4 H), 2.90 - 2.97 (m, 4 H). LC/MS found 447.1 $[M+H]^+$; calculated 447.1 for $C_{24}H_{23}N_4O_3S$.



6-(4-(Morpholinosulfonyl)phenyl)-N-(pyridin-3-yl)quinolin-4-amine (13) General procedure A, isolated as a tan colored solid in 22% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.28 (s, 1 H), 8.80 (d, *J*=2.0 Hz, 1 H), 8.66 (d, *J*=2.4 Hz, 1 H), 8.53 (d, *J*=5.4 Hz, 1 H), 8.38 (dd, *J*=4.9, 1.0 Hz, 1 H), 8.18 (d, *J*=8.3 Hz, 2 H), 8.14 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.03 (d, *J*=8.3 Hz, 1 H), 7.89 (d, *J*=8.3 Hz, 2 H), 7.84 (d, *J*=8.3 Hz, 1 H), 7.47 (dd, *J*=8.1, 4.6 Hz, 1 H), 6.99 (d, *J*=5.4 Hz, 1 H), 3.63 - 3.69 (m, 4 H), 2.89 - 2.97 (m, 4 H). LC/MS found 447.1 $[M+H]^+$; calculated 447.1 for $C_{24}H_{23}N_4O_3S$.

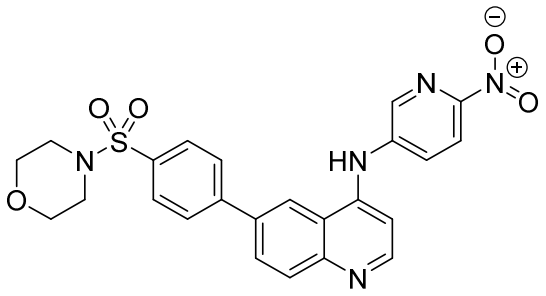


N-(1H-Benzo[d][1,2,3]triazol-5-yl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13d) General procedure A, neutralized with sat. aq. $NaHCO_3$ instead of aq. NaOH, isolated as a light yellow solid in 51% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.27 (s, 1 H), 8.92 (d, *J*=1.5 Hz, 1 H), 8.38 (d, *J*=5.4 Hz, 1 H), 8.22 (d, *J*=8.8 Hz, 2 H), 8.10 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.95 (d, *J*=8.8 Hz, 1 H), 7.87 (d, *J*=8.8 Hz, 2 H), 7.75 (d, *J*=8.8 Hz, 1 H), 7.60 (d, *J*=1.5 Hz, 1 H), 7.02 (dd, *J*=8.5, 1.7 Hz, 1 H), 6.74 (d, *J*=5.4 Hz, 1 H), 3.62 - 3.70 (m, 4 H), 2.90 - 2.97 (m, 4 H). LC/MS found 487.1 $[M+H]^+$; calculated 487.2 for $C_{25}H_{23}N_6O_3S$.

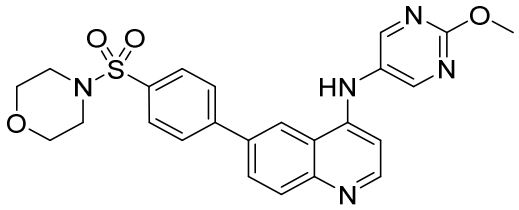


3,5-Dimethyl-N-(6-(4-(morpholinosulfonyl)phenyl)quinolin-4-yl)isoxazol-4-amine (13e) General procedure A, isolated as a white solid in 41% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.82 (d, *J*=2.0 Hz, 1 H), 8.70 (s, 1 H), 8.45 (d, *J*=5.4 Hz, 1 H), 8.17 (d, *J*=8.3 Hz, 2 H), 8.12 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.00 (d, *J*=8.8 Hz, 1 H), 7.89 (d, *J*=8.3 Hz,

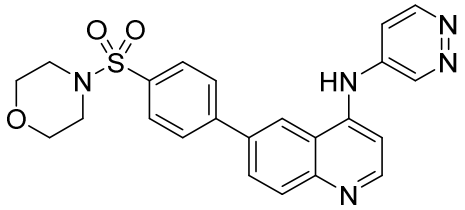
2 H), 6.22 (d, *J*=5.4 Hz, 1 H), 3.63 - 3.69 (m, 4 H), 2.89 - 2.97 (m, 4 H), 2.32 (s, 3 H), 2.10 (s, 3 H). LC/MS found 465.1 $[M+H]^+$; calculated 465.2 for $C_{24}H_{25}N_4O_4S$.



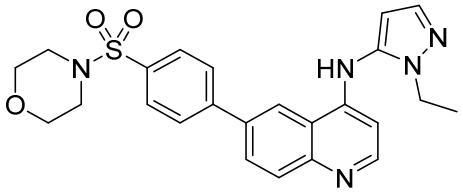
6-(4-(Morpholinosulfonyl)phenyl)-N-(6-nitropyridin-3-yl)quinolin-4-amine (13f) General procedure A, isolated as an orange solid in 15% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.94 (s, 1 H), 8.75 (d, *J*=5.4 Hz, 1 H), 8.72 (d, *J*=2.0 Hz, 1 H), 8.63 (d, *J*=2.0 Hz, 1 H), 8.34 (d, *J*=8.8 Hz, 1 H), 8.09 - 8.23 (m, 4 H), 8.04 (dd, *J*=9.0, 2.2 Hz, 1 H), 7.90 (d, *J*=8.3 Hz, 2 H), 7.51 (d, *J*=4.9 Hz, 1 H), 3.63 - 3.69 (m, 4 H), 2.89 - 2.96 (m, 4 H). LC/MS found 492.1 $[M+H]^+$; calculated 492.1 for $C_{24}H_{22}N_5O_5S$.



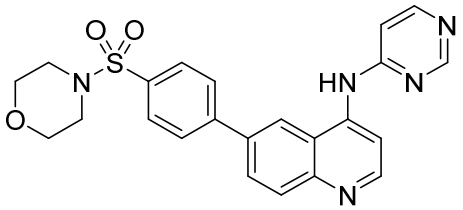
N-(2-Methoxypyrimidin-5-yl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13g) General procedure A, isolated as a cream colored solid in 15% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.17 (br. s., 1 H), 8.81 (s, 1 H), 8.71 (s, 2 H), 8.47 (d, *J*=4.9 Hz, 1 H), 8.17 (d, *J*=8.3 Hz, 2 H), 8.13 (dd, *J*=8.8, 1.5 Hz, 1 H), 8.01 (d, *J*=8.3 Hz, 1 H), 7.89 (d, *J*=8.3 Hz, 2 H), 6.70 (d, *J*=4.9 Hz, 1 H), 3.97 (s, 3 H), 3.63 - 3.68 (m, 4 H), 2.91 - 2.96 (m, 4 H). LC/MS found 478.1 $[M+H]^+$; calculated 478.2 for $C_{24}H_{24}N_5O_4S$.



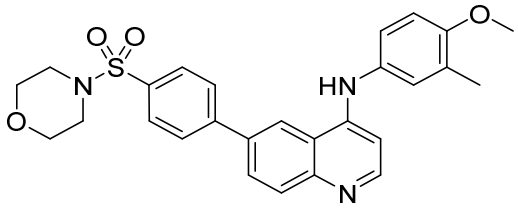
6-(4-(Morpholinosulfonyl)phenyl)-N-(pyridazin-4-yl)quinolin-4-amine (13h) General procedure A, isolated as a cream colored solid in 14% yield. ^{1}H NMR (500 MHz, 1:1 $CDCl_3$/CD_3OD) δ ppm 9.18 (d, *J*=4.4 Hz, 1 H), 9.05 (d, *J*=7.8 Hz, 1 H), 8.82 (d, *J*=2.9 Hz, 1 H), 8.42 (d, *J*=8.8 Hz, 1 H), 8.25 (dd, *J*=8.8, 1.5 Hz, 1 H), 8.06 (d, *J*=1.0 Hz, 1 H), 7.95 (d, *J*=8.8 Hz, 2 H), 7.91 (d, *J*=8.3 Hz, 2 H), 7.84 (d, *J*=4.9 Hz, 1 H), 7.46 (dd, *J*=7.3, 2.9 Hz, 1 H), 3.76 - 3.81 (m, 4 H), 3.05 - 3.10 (m, 4 H). LC/MS found 448.1 $[M+H]^+$; calculated 448.1 for $C_{23}H_{22}N_5O_3S$.



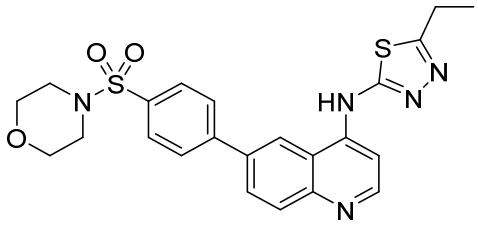
N-(1-Ethyl-1H-pyrazol-5-yl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13i) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-30% CH_3CN in H_2O, isolated as an orange-yellow solid in 65% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.88 (d, *J*=1.0 Hz, 1 H), 8.42 (br. s., 1 H), 8.09 - 8.19 (m, 4 H), 7.99 (d, *J*=8.8 Hz, 1 H), 7.88 (d, *J*=8.3 Hz, 2 H), 7.57 (d, *J*=2.0 Hz, 1 H), 6.33 (d, *J*=5.4 Hz, 1 H), 6.25 (d, *J*=1.5 Hz, 1 H), 4.01 (q, *J*=7.2 Hz, 2 H), 3.62 - 3.69 (m, 4 H), 2.90 - 2.97 (m, 4 H), 1.29 (t, *J*=7.3 Hz, 3 H). LC/MS found 464.1 $[M+H]^+$; calculated 464.2 for $C_{24}H_{26}N_5O_3S$.



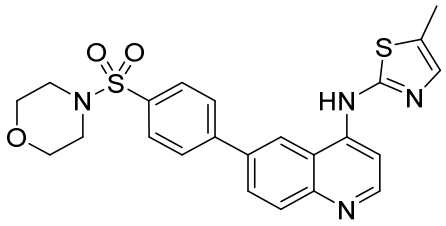
6-(4-(Morpholinosulfonyl)phenyl)-N-(pyrimidin-4-yl)quinolin-4-amine (13j) General procedure C, FCC: 1-10% CH_3OH in CH_2Cl_2 isolated as a pale yellow solid in 85% yield. ^1H NMR (399 MHz, DMSO-d_6) δ ppm 10.05 (s, 1 H), 8.79 - 8.85 (m, 2 H), 8.77 (d, *J*=2.2 Hz, 1 H), 8.52 (d, *J*=5.9 Hz, 1 H), 8.38 (d, *J*=5.1 Hz, 1 H), 8.14 - 8.21 (m, 3 H), 8.12 (d, *J*=8.1 Hz, 1 H), 7.91 (d, *J*=8.8 Hz, 2 H), 7.30 (d, *J*=5.9 Hz, 1 H), 3.63 - 3.70 (m, 4 H), 2.90 - 2.97 (m, 4 H). LC/MS found 448.1 $[M+H]^+$; calculated 448.1 for $C_{23}H_{22}N_5O_3S$.



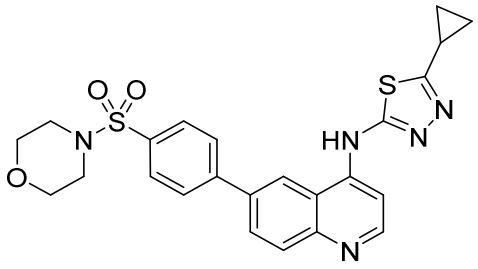
N-(4-Methoxy-3-methylphenyl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13k) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-30% CH_3CN in H_2O, isolated as a bright yellow solid in 81% yield. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 8.92 (s, 1 H), 8.81 (br. s., 1 H), 7.99 (d, *J*=8.8 Hz, 1 H), 7.94 (d, *J*=6.3 Hz, 1 H), 7.91 (d, *J*=8.3 Hz, 2 H), 7.77 (d, *J*=8.3 Hz, 1 H), 7.72 (d, *J*=8.3 Hz, 2 H), 7.08 - 7.19 (m, 2 H), 6.79 (d, *J*=8.3 Hz, 1 H), 6.50 (d, *J*=6.3 Hz, 1 H), 3.80 (s, 3 H), 3.64 - 3.73 (m, 4 H), 2.96 (br. s., 4 H), 2.15 (s, 3 H). LC/MS found 490.1 $[M+H]^+$; calculated 490.2 for $C_{27}H_{28}N_3O_4S$.



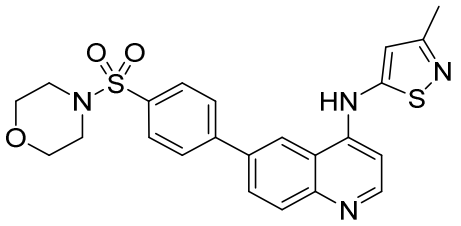
5-Ethyl-N-(6-(4-(morpholinosulfonyl)phenyl)quinolin-4-yl)-1,3,4-thiadiazol-2-amine (13l) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-95% CH_3CN in H_2O, isolated as an intense yellow solid in 46% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 12.74 (br. s., 1 H), 9.11 (d, *J*=1.5 Hz, 1 H), 8.73 (d, *J*=5.9 Hz, 1 H), 8.41 (d, *J*=5.9 Hz, 1 H), 8.26 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.22 (d, *J*=8.3 Hz, 2 H), 8.10 (d, *J*=8.8 Hz, 1 H), 7.89 (d, *J*=8.8 Hz, 2 H), 3.61 - 3.69 (m, 4 H), 3.03 (q, *J*=7.3 Hz, 2 H), 2.90 - 2.97 (m, 4 H), 1.33 (t, *J*=7.6 Hz, 3 H). LC/MS found 482.1 $[M+H]^+$; calculated 482.1 for $C_{23}H_{24}N_5O_3S_2$.



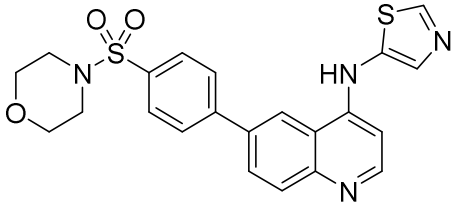
5-Methyl-N-(6-(4-(morpholinosulfonyl)phenyl)quinolin-4-yl)thiazol-2-amine (13m) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-95% CH_3CN in H_2O, isolated as an orange solid in 90% yield. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 8.70 (d, *J*=5.4 Hz, 1 H), 8.58 (d, *J*=1.5 Hz, 1 H), 8.28 (d, *J*=5.4 Hz, 1 H), 8.07 (d, *J*=8.8 Hz, 1 H), 7.80 (dd, *J*=8.5, 1.7 Hz, 1 H), 7.70 (d, *J*=8.3 Hz, 2 H), 7.65 (d, *J*=8.3 Hz, 2 H), 7.06 (d, *J*=1.5 Hz, 1 H), 3.65 - 3.72 (m, 4 H), 2.91 - 2.99 (m, 4 H), 2.36 (d, *J*=1.0 Hz, 3 H). LC/MS found 467.1 $[M+H]^+$; calculated 467.1 for $C_{23}H_{23}N_4O_3S_2$.



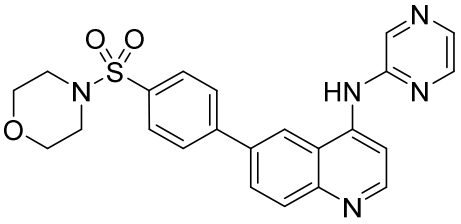
5-Cyclopropyl-N-(6-(4-(morpholinosulfonyl)phenyl)quinolin-4-yl)-1,3,4-thiadiazol-2-amine (13n) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-95% CH_3CN in H_2O, isolated as a light yellow solid in 18% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 9.19 (br. s., 1 H), 8.71 (br. s., 1 H), 8.38 (br. s., 1 H), 8.23 - 8.30 (m, 3 H), 8.12 (d, *J*=8.8 Hz, 1 H), 7.89 (d, *J*=8.8 Hz, 2 H), 3.62 - 3.69 (m, 4 H), 2.90 - 2.97 (m, 4 H), 2.44 (tt, *J*=8.3, 4.9 Hz, 1 H), 1.15 - 1.20 (m, 2 H), 0.99 - 1.04 (m, 2 H). LC/MS found 494.1 $[M+H]^+$; calculated 494.1 for $C_{24}H_{24}N_5O_3S_2$.



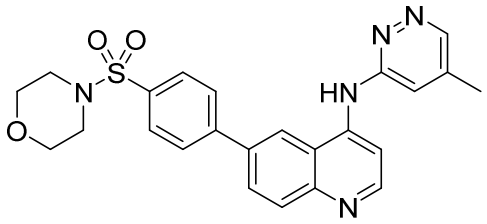
3-Methyl-N-(6-(4-(morpholinosulfonyl)phenyl)quinolin-4-yl)isothiazol-5-amine (13o) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-95% CH_3CN in H_2O, isolated as a deep yellow solid in 73% yield. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 8.68 (d, *J*=5.9 Hz, 1 H), 8.56 (s, 1 H), 8.09 (d, *J*=8.3 Hz, 1 H), 7.82 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.70 (d, *J*=8.3 Hz, 2 H), 7.62 (d, *J*=8.3 Hz, 2 H), 7.31 (d, *J*=5.4 Hz, 1 H), 7.04 (s, 1 H), 3.65 - 3.72 (m, 4 H), 2.91 - 2.99 (m, 4 H), 2.45 (s, 3 H). LC/MS found 467.1 $[M+H]^+$; calculated 467.1 for $C_{23}H_{23}N_4O_3S_2$.



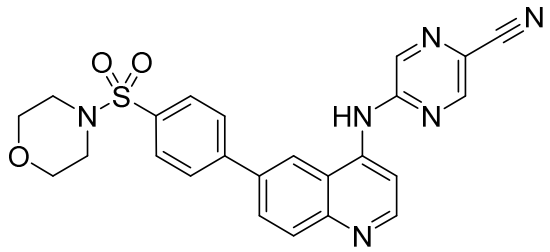
N-(6-(4-(Morpholinosulfonyl)phenyl)quinolin-4-yl)thiazol-5-amine (13p) General procedure B, FCC: 1-10% CH_3OH in CH_2Cl_2, then prep HPLC 5-95% CH_3CN in H_2O, isolated as a yellow solid in 18% yield. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 8.56 (d, *J*=4.9 Hz, 1 H), 8.38 (d, *J*=2.0 Hz, 1 H), 8.27 (d, *J*=8.8 Hz, 1 H), 8.00 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.94 (s, 1 H), 7.86 - 7.92 (m, 4 H), 7.49 (s, 1 H), 6.97 (d, *J*=4.9 Hz, 1 H), 3.75 - 3.81 (m, 4 H), 3.05 - 3.12 (m, 4 H). LC/MS found 453.1 $[M+H]^+$; calculated 453.1 for $C_{22}H_{21}N_4O_3S_2$.



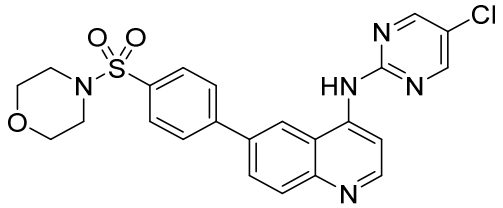
6-(4-(Morpholinosulfonyl)phenyl)-N-(pyrazin-2-yl)quinolin-4-amine (13q) General procedure D, FCC: 1-5% CH_3OH in CH_2Cl_2, then prep HPLC 5-30% CH_3CN in H_2O, isolated as a yellow solid in 45% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 10.40 (br. s., 1 H), 9.00 (d, *J*=1.5 Hz, 1 H), 8.85 (d, *J*=1.0 Hz, 1 H), 8.78 (d, *J*=5.9 Hz, 1 H), 8.38 - 8.42 (m, 2 H), 8.28 (d, *J*=2.9 Hz, 1 H), 8.20 - 8.25 (m, 3 H), 8.13 (d, *J*=8.8 Hz, 1 H), 7.90 (d, *J*=8.3 Hz, 2 H), 3.63 - 3.70 (m, 4 H), 2.89 - 2.98 (m, 4 H). LC/MS found 448.1 $[M+H]^+$; calculated 448.1 for $C_{23}H_{22}N_5O_3S$.



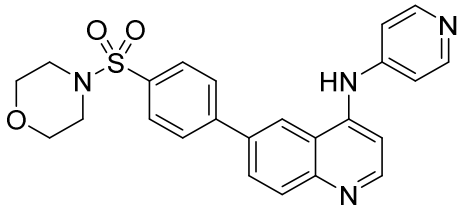
N-(5-Methylpyridazin-3-yl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13r) General procedure D, FCC: 20-80% ethyl acetate in CH_2Cl_2, then prep HPLC 5-30% CH_3CN in H_2O, isolated as a light yellow solid in 22% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.38 (br. s., 1 H), 9.40 (br. s., 1 H), 9.04 (s, 1 H), 8.90 (d, *J*=6.8 Hz, 1 H), 8.61 (br. s., 1 H), 8.50 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.27 - 8.35 (m, 3 H), 7.88 - 7.96 (m, 3 H), 3.62 - 3.71 (m, 4 H), 2.90 - 2.98 (m, 4 H), 2.42 (s, 3 H). LC/MS found 462.1 $[M+H]^+$; calculated 462.2 for $C_{24}H_{24}N_5O_3S$.



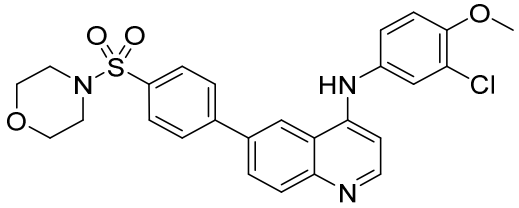
5-((6-(4-(Morpholinosulfonyl)phenyl)quinolin-4-yl)amino)pyrazine-2-carbonitrile (13s) General procedure D, FCC: 30-100% ethyl acetate in hexanes, then prep HPLC 5-30% CH_3CN in H_2O, isolated as a yellow solid in 13% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.21 (br. s., 1 H), 9.07 (s, 1 H), 8.94 - 9.00 (m, 3 H), 8.55 (d, *J*=5.9 Hz, 1 H), 8.37 (d, *J*=8.8 Hz, 1 H), 8.22 - 8.27 (m, 3 H), 7.93 (d, *J*=8.8 Hz, 2 H), 3.63 - 3.69 (m, 4 H), 2.90 - 2.97 (m, 4 H). LC/MS found 473.1 $[M+H]^+$; calculated 473.1 for $C_{24}H_{21}N_6O_3S_2$.



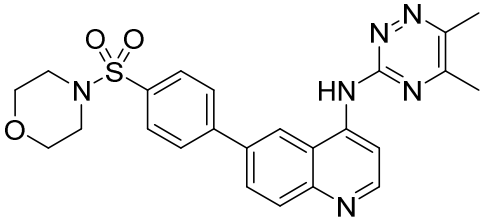
N-(5-Chloropyrimidin-2-yl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13t) General procedure D, FCC: 20-80% ethyl acetate in CH_2Cl_2, then prep HPLC 25% CH_3CN in H_2O, isolated as a yellow solid in 12% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.54 (br. s., 1 H), 9.25 (s, 1 H), 8.98 (d, *J*=6.3 Hz, 1 H), 8.96 (s, 2 H), 8.69 (d, *J*=6.3 Hz, 1 H), 8.45 (dd, *J*=8.8, 1.5 Hz, 1 H), 8.26 - 8.31 (m, 3 H), 7.93 (d, *J*=8.3 Hz, 2 H), 3.63 - 3.69 (m, 4 H), 2.92 - 2.98 (m, 4 H). LC/MS found 482.1 $[M+H]^+$; calculated 482.1 for $C_{23}H_{21}ClN_5O_3S$.



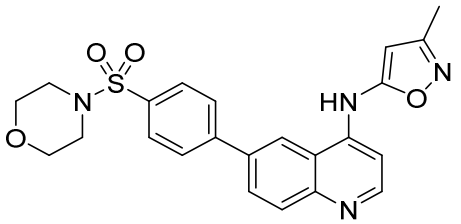
6-(4-(Morpholinosulfonyl)phenyl)-N-(pyridin-4-yl)quinolin-4-amine (13u) General procedure C, FCC: 1-10% CH_3OH in CH_2Cl_2, isolated as a bright yellow solid in 45% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.50 (s, 1 H), 8.67 - 8.73 (m, 2 H), 8.44 (d, *J*=5.4 Hz, 2 H), 8.13 - 8.20 (m, 3 H), 8.09 (d, *J*=8.3 Hz, 1 H), 7.89 (d, *J*=8.3 Hz, 2 H), 7.45 (d, *J*=5.4 Hz, 1 H), 7.33 (d, *J*=6.3 Hz, 2 H), 3.62 - 3.69 (m, 4 H), 2.88 - 2.95 (m, 4 H). LC/MS found 447.2 $[M+H]^+$; calculated 447.1 for $C_{24}H_{23}N_4O_3S$.



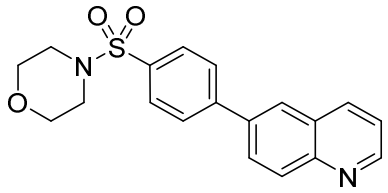
N-(3-Chloro-4-methoxyphenyl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13v) General procedure C, FCC: 1-10% CH_3OH in CH_2Cl_2, isolated as a light brown solid in 48% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.18 (br. s., 1 H), 8.80 (d, *J*=2.0 Hz, 1 H), 8.47 (d, *J*=5.4 Hz, 1 H), 8.17 (d, *J*=8.8 Hz, 2 H), 8.12 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.98 (d, *J*=8.8 Hz, 1 H), 7.88 (d, *J*=8.3 Hz, 2 H), 7.45 (d, *J*=2.4 Hz, 1 H), 7.37 (dd, *J*=8.8, 2.9 Hz, 1 H), 7.25 (d, *J*=8.8 Hz, 1 H), 6.78 (d, *J*=5.4 Hz, 1 H), 3.90 (s, 3 H), 3.62 - 3.69 (m, 4 H), 2.89 - 2.97 (m, 4 H). LC/MS found 510.2 $[M+H]^+$; calculated 510.1 for $C_{26}H_{25}ClN_3O_4S$.



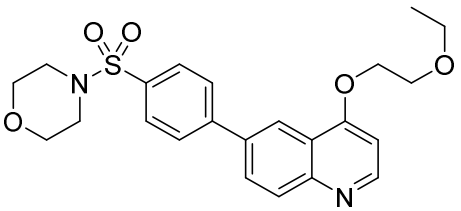
N-(5,6-Dimethyl-1,2,4-triazin-3-yl)-6-(4-(morpholinosulfonyl)phenyl)quinolin-4-amine (13w) General procedure C, FCC: FCC: 1-10% CH_3OH in CH_2Cl_2, isolated as a pale yellow solid in 65% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.55 (s, 1 H), 9.00 (d, *J*=2.0 Hz, 1 H), 8.80 (d, *J*=5.4 Hz, 1 H), 8.40 (d, *J*=5.4 Hz, 1 H), 8.27 (d, *J*=8.3 Hz, 2 H), 8.17 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.09 (d, *J*=8.8 Hz, 1 H), 7.89 (d, *J*=8.3 Hz, 2 H), 3.63 - 3.69 (m, 4 H), 2.90 - 2.97 (m, 4 H), 2.58 (s, 3 H), 2.53 (s, 3 H). LC/MS found 477.2 $[M+H]^+$; calculated 477.2 for $C_{24}H_{25}N_6O_3S$.



3-Methyl-N-(6-(4-(morpholinosulfonyl)phenyl)quinolin-4-yl)isoxazol-5-amine (13x) General procedure D, FCC: 1-5% CH_3OH in ethyl acetate, isolated as a bright yellow solid in 76% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.69 (br. s., 1 H), 8.62 (d, *J*=5.9 Hz, 1 H), 8.21 (d, *J*=8.8 Hz, 1 H), 7.92 (dd, *J*=8.8, 1.5 Hz, 1 H), 7.85 - 7.89 (m, 3 H), 7.70 (d, *J*=8.3 Hz, 2 H), 7.46 (d, *J*=5.9 Hz, 1 H), 6.05 (s, 1 H), 3.69 - 3.75 (m, 4 H), 2.95 - 3.02 (m, 4 H), 2.33 (s, 3 H). LC/MS found 451.2 $[M+H]^+$; calculated 451.1 for $C_{23}H_{23}N_4O_4S$.



4-((4-(Quinolin-6-yl)phenyl)sulfonyl)morpholine (13y) Isolated as a side product from the reaction mixture of **13p** as an off- white solid in 20% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.99 (dd, *J*=4.2, 1.7 Hz, 1 H), 8.55 (d, *J*=7.3 Hz, 1 H), 8.47 (d, *J*=2.0 Hz, 1 H), 8.22 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.18 (d, *J*=8.8 Hz, 1 H), 8.14 (d, *J*=8.8 Hz, 2 H), 7.89 (d, *J*=8.3 Hz, 2 H), 7.66 (dd, *J*=8.3, 4.4 Hz, 1 H), 3.62 - 3.69 (m, 4 H), 2.90 - 2.98 (m, 4 H). LC/MS found 355.1 $[M+H]^+$; calculated 355.1 for $C_{19}H_{19}N_2O_3S$.



4-((4-(4-(2-Ethoxyethoxy)quinolin-6-yl)phenyl)sulfonyl)morpholine (13z) Isolated as a side product from the reaction mixture of **13r** as a white solid in 7% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.77 (d, *J*=5.4 Hz, 1 H), 8.43 (d, *J*=2.0 Hz, 1 H), 8.17 (s, 1 H), 8.14 (dd, *J*=8.8, 2.0 Hz, 1 H), 8.04 - 8.10 (m, 3 H), 7.88 (d, *J*=8.8 Hz, 2 H), 7.12 (d, *J*=4.9 Hz, 1 H), 4.42 - 4.45 (m, 2 H), 3.87 - 3.91 (m, 2 H), 3.63 - 3.69 (m, 4 H), 3.58 (q, *J*=7.0 Hz, 2 H), 2.90 - 2.96 (m, 4 H), 1.15 (t, *J*=7.1 Hz, 3 H). LC/MS found 443.2 $[M+H]^+$; calculated 443.2 for $C_{23}H_{27}NO_5S$.

ANTIMALARIAL & CYTOTOXICITY DATA

Table S1. Multi-strain malaria activity and host cell toxicity of amine replacement analogs

Entry	*P. fal* D6 EC_{50} (μM)[a]	*P. fal* W2 EC_{50} (μM)[a]	*P. fal* C235 EC_{50} (μM)[a]	NIH 3T3 TC_{50} (μM)	HepG2 TC_{50} (μM)
3	0.64	1.7	1.3	>20	>5
13a	0.19	1.5	0.26	>20	>20
13b	0.54	1.5	0.67	>20	>20
13c	0.49	1	0.73	>20	>20
13d	0.18	0.63	0.18	>20	>20
13e	>20	>20	>20	>20	>20
13f	0.39	0.68	0.56	>20	>20
13g	0.76	1.6	0.97	>20	>20
13h	17	17	>20	>20	>20
13i	3.4	3.2	4.1	>20	>20
13j	8.6	>20	>20	>20	>20
13k	0.26	1.2	0.25	15	9
13l	0.89	1.9	1.2	>20	>20
13m	0.1	0.37	0.09	>20	>20
13n	0.59	1.4	0.86	>20	>20
13o	0.43	0.72	0.47	>20	>20
13p	7	5.6	>20	>20	>20
13q	4.6	>20	>20	>20	>20
13r	1.2	2.8	1.6	>20	>20
13s	0.55	0.82	0.6	>20	>20
13t	1	5.5[b]	1.5	>20	>20
13u	0.27	0.93	0.54	>20	>20
13v	0.11	0.54	0.16	>20	>20
13w	0.24	1.3	0.68	>20	>20
13x	2.2	2.3	2.7	>20	>20
13y	>20	>20	>20	>20	>20
13z	3.9	5.1	13	-	>20

[a] r^2 values >0.9

IN VITRO ADME PROPERTIES & COMPOUND REGISTRY NUMBERS

Table S2. Physicochemical properties of amine replacement analogs

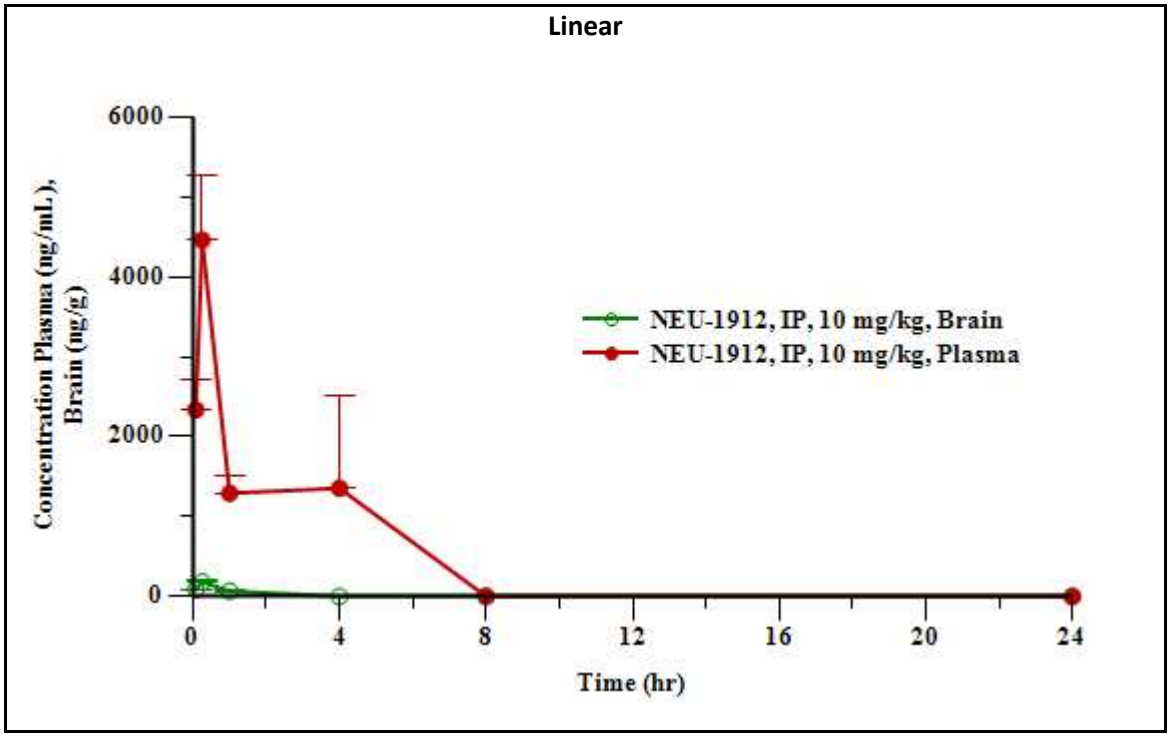
#	NEU Code	MW	clogP	Calc. pKa	PSA (Å²)	Aq. Sol (μM)	Human PPB (%)	HLM CL_{int} (μL/min/mg)	Rat Hep CL_{int} (μL/min/10^6)
3	NEU-1045	569.6	5.76	7.25	80.8	2	>99.9	98	19
13a	NEU-1903	445.5	4.05	7.24	71.5	12	98.8	71.3	121
13b	NEU-1904	446.5	3.43	6.98	84.4	-	-	-	-
13c	NEU-1905	446.5	2.84	7.20	84.4	9	94.7	86.3	53.8
13d	NEU-1906	486.5	3.28	7.23	113.1	5	96.6	52	17.6
13e	NEU-1907	464.5	2.75	7.11	97.6	2	90.8	78.2	50.5
13f	NEU-1908	491.5	3.37	7.19	130.2	4	97.6	106	47.6
13g	NEU-1909	477.5	2.65	7.15	106.5	-	-	-	-
13h	NEU-1910	447.5	1.86	7.19	97.3	661	58.0	-	7.8
13i	NEU-1911	463.6	2.93	7.04	89.4	5	94.2	134	60.1
13j	NEU-1912	447.5	2.72	6.96	97.3	1.4	95.6	207.5	31.5
13k	NEU-1913	489.6	4.41	7.25	80.8	0.1	99.5	131	147
13l	NEU-1914	481.6	3.34	6.73	97.3	< 0.1	98.2	59.3	42.2
13m	NEU-1915	466.6	4.04	6.80	84.4	21	99.6	76.3	63.3
13n	NEU-1916	493.6	3.42	6.73	97.3	-	-	-	-
13o	NEU-1917	466.6	3.32	7.02	84.4	< 1	99.3	115.4	26.9
13p	NEU-1918	452.5	2.88	7.02	84.4	13	96.2	29.5	14.4
13q	NEU-1919	447.5	2.21	6.92	97.3	1	98.4	115	48.0
13r	NEU-1920	461.5	2.96	6.91	97.3	1	95.0	126	43.2
13s	NEU-1921	472.5	2.45	6.92	121.1	-	-	-	-
13t	NEU-1923	482.0	3.41	6.68	97.3	1	99.6	113	41.5
13u	NEU-1949	446.5	2.84	8.03	84.4	7	94.1	82.4	43.7
13v	NEU-1950	510.0	4.50	7.24	80.8	1	99.7	102	81.4
13w	NEU-1951	476.6	2.09	6.62	110.2	2	97.6	114	50.9
13x	NEU-1952	450.5	2.64	6.81	97.6	3	98.8	69.8	26.3
13y	NEU-1924	354.4	2.61	4.43	59.5	18	92.9	13.3	-
13z	NEU-2084	442.5	2.77	5.77	78.0	-	-	> 300	288.8

PHARMACOKINETICS EXPERIMENTS

Table S3. Plasma and brain exposure parameters of NEU-1912

Dose (mg/kg)	Route	Matrix	T_{max} (hr)	C_{max} (ng/mL)	C_{max} ratio	AUC_{last} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)	Brain to plasma exposure ratio
10	I.P.	Plasma	0.25	4458.80	0.04	9510.01	9515.02	0.01
		Brain*	0.25	181.27		119.56	NC	

Figure S1. Mean plasma and brain concentration-time profiles of NEU-1912 following a single intraperitoneal administration in female BALB/c mice (Dose: 10 mg/kg)



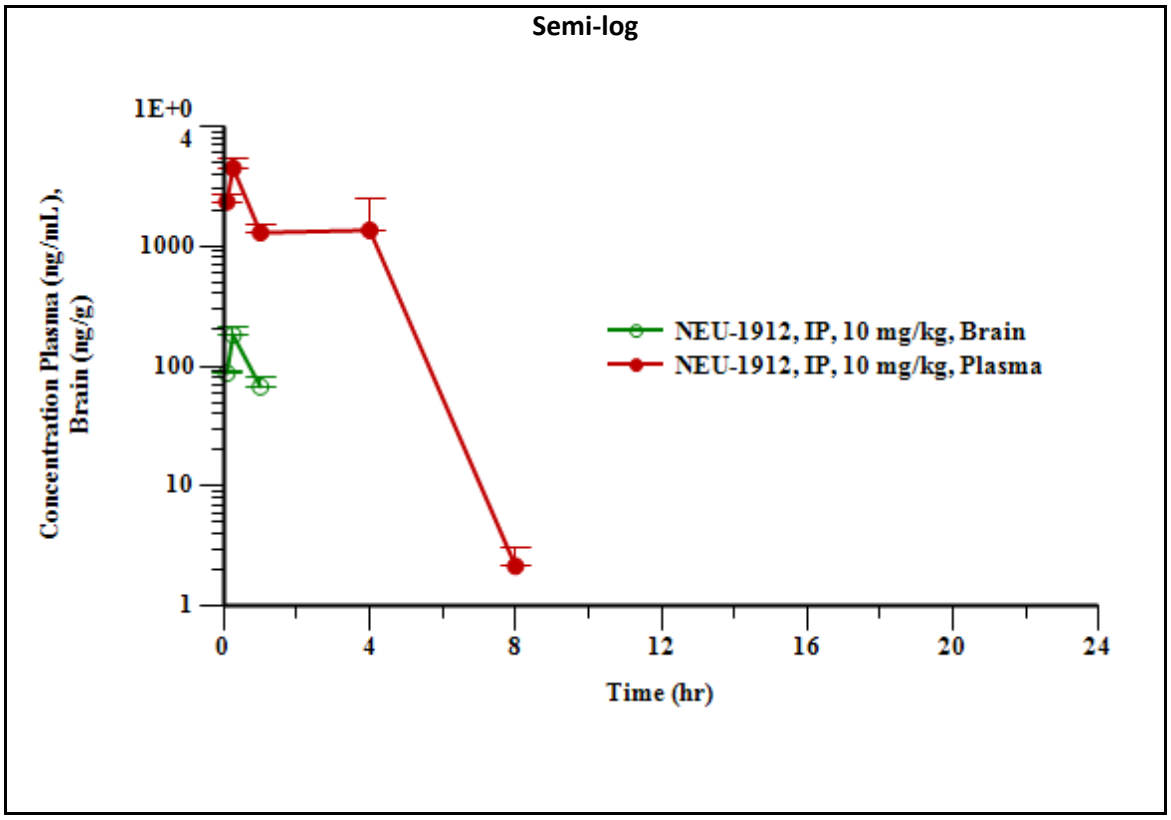


Table S4. Individual plasma concentration (ng/mL)-time data of NEU-1912 following a single intraperitoneal administration in female BALB/c mice (Dose: 10 mg/kg)

Mouse ID	Plasma Conc. (ng/mL) Time (hr)					
	0.08	**0.25**	**1**	**4**	**8**	**24**
1	1985.04					
2	2272.87					
3	2747.54					
4		3564.67				
5		5093.49				
6		4718.24				
7			1048.90			
8			1375.09			
9			1453.77			
10				2199.49		
11				1835.07		
12				27.57		
13					2.85	

14					2.56	
15					1.07	
16						0.00
17						0.00
18						0.00
Mean	**2335.15**	**4458.80**	**1292.59**	**1354.04**	**2.16**	**0.00**
SD	385.05	796.75	214.67	1163.12	0.96	NA
CV%	16.49	17.87	16.61	85.90	44.21	NA

LLOQ: 1.04 ng/mL; NA: not applicable

Table S5. Individual brain concentration (ng/mL)-time data of NEU-1912 following a single intraperitoneal administration in female BALB/c mice (Dose: 10 mg/kg)

Mouse ID	Brain Conc. (ng/mL) Time (hr)					
	0.08	**0.25**	**1**	**4**	**8**	**24**
1	30.29					
2	27.97					
3	29.12					
4		69.56				
5		50.04				
6		61.67				
7			23.23			
8			27.06			
9			17.05			
10				0.00		
11				0.00		
12				0.00		
13					0.00	
14					0.00	

15					0.00	
16						0.00
17						0.00
18						0.00
Mean	**29.13**	**60.42**	**22.45**	**0.00**	**0.00**	**0.00**
SD	1.16	9.82	5.05	NA	NA	NA
CV%	3.98	16.25	22.50	NA	NA	NA

LLOQ – 2.08 ng/mL; NA: not applicable;

No peaks observed in LC-MS/MS chromatograms and plasma concentrations below LLOQ were replaced with zero for PK analysis.

Table S6. Individual brain concentration (ng/g)-time data of NEU-1912 following a single intraperitoneal administration in female BALB/c mice (Dose: 10 mg/kg)

Mouse ID	**[c]Brain Conc. (ng/g) Time (hr)**					
	0.08	**0.25**	**1**	**4**	**8**	**24**
1	90.87					
2	83.91					
3	87.36					
4		208.68				
5		150.12				
6		185.01				
7			69.69			
8			81.18			
9			51.15			
10				0.00		
11				0.00		
12				0.00		
13					0.00	
14					0.00	

15					0.00	
16						0.00
17						0.00
18						0.00
Mean	**87.38**	**181.27**	**67.34**	**0.00**	**0.00**	**0.00**
SD	3.48	29.46	15.15	NA	NA	NA
CV%	3.98	16.25	22.50	NA	NA	NA

LLOQ -2.08 ng/mL; NA: not applicable;

c - The density of brain homogenate was considered as 1 which is equivalent to plasma density (1)

CELL ASSAY PROTOCOLS

The *T. brucei* and *T. cruzi* assays were performed at the 'Anti-Infectives Screening Core', New York University School of Medicine. http://ocs.med.nyu.edu/anti-infectives-screening

Trypanosoma brucei. In a 96-well plate, compounds were added at the specified concentrations in 100 µL of HMI-9 medium per well. To each well, 100 µL of parasites (*T. brucei brucei* strain 427) in HMI-9 media were added at a concentration of $5x10^4$ cells/mL ($5x10^3$ cells/well) and incubated at 37°C, 5% CO_2 for 48 h. Following incubation, 20 µL of PrestoBlue® were added to each well and incubated for additional 4 h. Fluorescence was read at 530 nm excitation and 590 nm emission. Suramin at 100 µM was used as positive control and reference for calculation of IC_{50}.

Trypanosoma cruzi. In a 96-well plate, 100 µL 3T3 cells in DMEM without phenol red supplemented with 2% FBS were added to each well at $5x10^5$ cells/mL ($5x10^4$ cells/well). Cells were incubated for 2 h to allow for attachment. Compounds were then added at the specified concentrations. To each well, 100 µL of parasites (*T. cruzi* Tulahuen strain expressing β-galactosidase) in the aforementioned media were added at a concentration of $5x10^5$ cells/mL ($5x10^4$ cells/well) and incubated at 37°C, 5% CO_2 for 96 h. Following incubation, 50 µL of 500 µM chlorophenol Red-β-D-galactopyranoside (CPRG) in PBS with 0.5% NP40 was added to each well and incubated at 37°C, 5% CO_2 for 4 h. Absorbance was read at 590- 595 nm. Amphotericin B at 4 µM was used as positive control and reference for calculation of IC_{50}.

***Leishmania major* promastigotes**. The promastigote assay is a microtiter plate drug sensitivity prescreen assay used to determine antileishmanial activity of candidate drugs against promastigote forms of *L. major*. Alamar Blue, or resazurin, is a non-fluorescent indicator dye that is converted to bright red– fluorescent resorufin via the activity of mitochondrial reductases. As the activity of mitochondrial reductases is reduced in cells that are dead or dying, and the intensity of the fluorescence is relative to cell number, a test compound that kills or inhibits growth of a particular target cell, such as *L. major*, will result in a lower production of fluorescent signal. This simple assay is widely used for both toxicity and proliferation assays, and it was adapted for use in Leishmania drug discovery as shown by Sharlow.[1] Predosed microtiter drug plates for use in the promastigote drug prescreen assay were produced using sterile 384-well black optical bottom tissue culture plates. Candidate drugs were diluted in dimethyl sulfoxide in four 96-well plates to either 10,000 ng/mL or 1,000 ng/mL, and 4.25 µL of diluted drug was subsequently dispensed into each well of a 384-well plate. Duplicate 384 well plates were made at each test concentration (10,000 ng/mL and 1,000 ng/mL) and amphotericin B was used as a batch control. The Tecan EVO Freedom liquid handling system (Tecan US, Inc., Durham, NC) was used to produce all drug assay plates and conduct all pipetting operations for this assay. *L. major* parasites were cultured in Schneider's medium supplemented with 20% heat inactivated FBS. Promastigotes in early log growth phase were harvested from culture, counted, suspended at $1.32x10^5$ cells/mL and 5,000 promastigotes were dispensed into each well of the 384 well plate in a volume of 38.8 µL. The 384 well plates were subsequently incubated at 28°C for 44 hours. 8.4 µL of Alamar Blue was added to each well, the plates were subsequently

incubated at 28°C and 5% CO_2 for 4 hours and then examined for the relative fluorescence units (RFU) per well using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation set at 560 nm and emission set at 590 nm. The relative fluorescence from each well was used to determine the percent growth inhibition of each candidate compound tested. Compounds with 50% or greater inhibition of growth were selected for further analysis to determine IC_{50} values.

***Leishmania major* amastigotes**.[2] The intracellular amastigote assay is a microtiter plate drug sensitivity assay that uses the activity of luciferase as a measure of proliferation of luciferase-expressing *L. major* parasites developing intracellularly inside RAW 264.7 macrophages in the presence of antileishmanial drugs or experimental compounds. As the activity of luciferase and its associated luminescence after addition of the substrate, luciferin, is relative to parasite growth, a test compound that inhibits the growth of the parasite will result in a lower luminescence. The luciferase-expressing *L. major* parasite used in this assay is genetically modified by adding the luciferase coding region to the pLEXSY-hyg2 vector (Jena Biosciences). The luciferase expression construct was created by digesting the luciferase coding region (1.66kbp) of pGL3-Basic (Promega) by using two restriction enzymes, NcoI/EagI, followed by electrophoretic separation of the luciferase coding region on a 1% agarose gel. The luciferase coding region was then ligated into pLEXSY-hyg2 vector (Jena Biosciences) which had previously been digested with NcoI/NotI. The vector was linearized with SwaI and subsequently gel purified prior to transfection into *L. major* parasites. Transfections were carried out by electroporation at 480V, 13 Ω, and 500 µF (0.4 mL of $1x10^8$ parasites/mL, and 0.1 mL of 10 µg DNA). Selection for transfectants was then carried out using hygromycin B (100 µg/mL). RAW 264.7 macrophages were cultured in DMEM media supplemented with heat-inactivated 10% FBS. To begin the assay, macrophages were harvested from culture by removing all spent media, adding in 10 mL fresh media, scraping cells, and counted using Trypan Blue. The cells were resuspended in DMEM/10% HIFBS media at $2.0x10^5$ cells/mL, and then dispensed in a volume of 50 µL to yield a final concentration of 10,000 macrophages/well in 384 well tissue-culture treated sterile white plates using a Tecan EVO Freedom robotics system. The plates were then incubated at 37 °C in 5% CO_2 atmosphere for 24 hours. After incubation, the media was removed from each well using the Tecan EVO Freedom robot, and *L. major* promastigotes were added to each well and allowed to invade the RAW macrophages. Promastigotes were cultured in Schneider's medium supplemented with 20% heat inactivated FBS. Metacyclic stage promastigotes provide the best invasion for this assay, and cultures should be grown to increase the percentage of metacyclic promastigotes for best results. Promastigotes were harvested from culture, counted, suspended at $2x10^6$ promastigotes/mL in DMEM/HIFBS media, and 100,000 promastigotes were dispensed per well in a volume of 50 µL. After overnight incubation, the media was removed from each well using the Tecan Freedom EVO robot, and each well was subsequently washed three times with 40 µL of fresh DMEM/HIFBS medium to remove all extracellular promastigotes. After the third wash, 69.2 µL of DMEM/HIFBS medium was added to each well using the Tecan EVO Freedom robot. Drug plates were prepared with the Tecan EVO Freedom using sterile 96 well plates containing twelve duplicate two-fold serial dilutions of each test compound suspended in DMSO. 7.8 µL of diluted test compound was added to the 69.2 µL of media present in each well providing

a 10-fold final dilution of compound. The final concentration range tested was 0.5 to 10,000 ng/mL for all assays. The plates were next incubated at 37 °C and 5% CO_2 for 96 hours. After 96 hours of incubation, 7.5 µL of a luciferin solution (Caliper Life Science) diluted to 150 µg/mL was added to each well, and the plates were incubated for 30 minutes at 37 °C, in the dark. Each plate was read using an Infinite M200 plate reader. The 50% inhibitory concentrations (IC_{50}) were then generated for each dose response test using GraphPad Prism (GraphPad Software Inc., San Diego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

***Plasmodium falciparum* assay**. The SYBR Green fluorescence (MSF) Assay is a microtiter plate drug sensitivity assay that uses the presence of malarial DNA as a measure of parasitic proliferation in the S35 presence of antimalarial drugs or experimental compounds. As the intercalation of SYBR Green I dye and its resulting fluorescence is relative to parasite growth, a test compound that inhibits the growth of the parasite will result in a lower fluorescence. D6 (CDC/Sierra Leone), TM91C235 (WRAIR, Thailand), and W2 (CDC/Indochina III) laboratory strains of *P. falciparum* were used for each drug sensitivity assessment. The parasite strains were maintained continuously in long-term cultures as previously.[3] Predosed microtiter drug plates for use in the MSF assay were produced using sterile 384-well black optical bottom tissue culture plates containing duplicate or quadruplicate 12 two-fold serial dilutions of each test compound suspended in dimethyl sulfoxide. The final concentration range tested was 0.5 – 10000 ng/mL for all assays. Predosed plates were stored at 4 °C until used, not to exceed five days. No difference was seen in drug sensitivity determinations between stored or fresh drug assay plates (data not shown). A batch control plate using Chloroquine (Sigma-Aldrich) at a final concentration of 2000 ng/mL was used to validate each assay run. The Tecan Freedom Evo liquid handling system (Tecan US, Inc., Durham, NC) was used to produce all drug assay plates. Based on modifications of previously described methods,[3-6] *P. falciparum* strains in late-ring or early-trophozoite stages were cultured in the predosed 384-well microtiter drug assay plates in 38 µL culture volume per well at a starting parasitemia of 0.3% and a hematocrit of 2%. The cultures were then incubated at 37 °C within a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2, for 72 hours. Lysis buffer (38 µL per well), consisting of 20 mM Tris HCl, 5mM EDTA, 1.6% Triton X, 0.016% saponin, and SYBR green I dye at a 20x concentration was then added to the assay plates for a final SYBR Green concentration of 10x. The Tecan Freedom Evo liquid handling system was used to dispense malaria cell culture and lysis buffer. The plates were then incubated in the dark at room temperature for 24 hours and examined for the relative fluorescence units (RFU) per well using the Tecan Genios Plus (Tecan US, Inc., Durham, NC). Each drug concentration was transformed into Log[X] and plotted against the RFU values. The 50% and 90% inhibitory concentrations (IC_{50} and IC_{90}, respectively) were then generated with GraphPad Prism (GraphPad Software Inc., SanDiego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

NIH 3T3 Cell Toxicity Assay. In a 96-well plate, 100 µL 3T3 cells in DMEM without phenol red supplemented with 2% FBS were added to each well at $5x10^5$ cells/mL ($5x10^4$ cells/well). Cells were incubated for 2 h to allow for attachment. Compounds were then added at the specified concentrations. Plates were incubated at 37 °C, 5%

CO_2 for 96 h. Following incubation, 10 µL of PrestoBlue® were added to each well and incubated for additional 4 h. Fluorescence was read at 530 nm excitation and 590 nm emission.

HepG2 Toxicity Assay.[7] The MTT counter screen assay is an in vitro microtiter toxicology test used to determine toxicity of drug compounds against the primary target of interest, such as a HepG2 liver cell, determine the relative toxicity of one compound versus another within a chemical series, aid in determining the toxicology structure relationship within a chemical series, and prioritize compounds for advancement for in vivo studies. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a monotetrazolium salt that is reduced by mitochondria reductases to create a formazan dye, which has a purple color. As the activity of mitochondrial reductases is reduced in cells that are dead or dying, and the intensity of the purple color is relative to cell number and metabolism, a test compound that is toxic to a particular target cell, such as the HepG2 liver cell, will result in a lower production of formazan dye and reduced purple color. This simple colorimetric test is widely used for both toxicity and proliferation assays. The MTT counterscreen is used in concert with the malaria inhibition of liver stage development S36 assay (ILSDA) as a toxicity counter screen. Interpretation of the IC_{50} determination of potentially malaria liver stage active compounds requires a toxicity test to ensure that the activity observed is specific to malaria and not simply toxicity directed against a HepG2 cell. The 384 well MTT cytotoxicity assay is a modification of the MTT method described by Ferrari[7] optimized for 384 well throughput. The HepG2 target cells for this assay were cultured as follows: HepG2 cells were cultured in complete Minimal Essential Medium prepared by supplementing MEM with 0.19% sodium bicarbonate, 10% heat inactivated FBS, 2 mM L-glutamine, 0.1 mM MEM non-essential amino, 0.009 mg/mL insulin, 1.76 mg/mL bovine serum albumin, 20 units/mL penicillin–streptomycin, and 0.05 mg/mL gentamycin. HepG2 cells cultured in complete MEM were first washed with 1X Hank's Balanced Salt Solution (Invitrogen #14175-095), trypsonized using a 0.25% trypsin/EDTA solution, assessed for viability using trypan blue, and resuspended at 250,000 cells/mL. Using a Tecan EVO Freedom robot, 38.3 µL of cell suspension were added to each well of clear, cell culture-treated 384-well microtiter plates for a final concentration of 9570 liver cells per well, and plated cells were incubated overnight in 5% CO_2 at 37 °C. Drug plates were prepared with the Tecan EVO Freedom using sterile 96 well plates containing twelve duplicate 1.6-fold serial dilutions of each test compound suspended in DMSO. 4.25 µL of diluted test compound was then added to the 38.3 µL of media in each well providing a 10-fold final dilution of compound. Compounds were tested from a range of 57 ng/mL to 10,000 ng/mL for all assays. Mefloquine was used as a plate control for all assays with a concentration ranging from 113 ng/mL to 20,000 ng/mL. After a 48 hour incubation period, 8 µL of a 1.5 mg/mL solution of MTT diluted in complete MEM media was added to each well. All plates were subsequently incubated in the dark for 1 hour at room temperature. After incubation, the media and drugs in each well was removed by shaking the plate over sink, and the plates were left to dry in a fume hood for 15 minutes. Next, 30 µL of isopropanol acidified by addition of HCl at a final concentration of 0.36% was added to dissolve the formazan dye crystals created by reduction of MTT. Plates are put on a 3-D rotator for 15-30 minutes. Absorbance was determined in all wells using a Tecan iControl 1.6 Infinite plate reader. The 50%

inhibitory concentrations (IC_{50}) were then generated for each toxicity dose response test using GraphPad Prism (GraphPad Software Inc., SanDiego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

PHARMACOKINETIC EXPERIMENT PROTOCOLS

Animal Welfare. All procedures of the present study were performed at Sai Life Sciences, Pune, India, and were in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study (IAEC Protocol NoFB-14-077)

Experimental procedures. A group of eighteen female mice was administered with NEU-1912 solution formulation at a dose of 10 mg/kg. Blood samples were collected at 0.08, 0.25, 1, 4, 8 and 24 hr. Blood was collected from set of three mice at each time point in labeled micro centrifuge tube containing K_2EDTA as anticoagulant. Plasma samples were separated by centrifugation of whole blood and stored below -70ºC until bioanalysis. After collecting blood samples, mice were humanely euthanized by CO_2 asphyxiation and brain was collected at 0.08, 0.25, 1, 4, 8 and 24 hr post dose. Following collection, the brain samples were washed in ice-cold phosphate buffer saline (pH 7.4), gently dried on filter paper, weighed and placed in polypropylene tubes. Further brain samples were homogenized using phosphate buffer saline pH 7.4 and the total homogenate volume was thrice the brain weight. The samples were then stored below -70ºC until bioanalysis. All samples were processed for analysis by protein precipitation using acetonitrile and analyzed with fit-for-purpose LC-MS/MS method (LLOQ = 1.04 ng/mL for plasma and 2.08 ng/mL for brain). Pharmacokinetic parameters were calculated using the non-compartmental analysis tool of Phoenix WinNonlin ® Enterprise software (version 6.3).

Healthy female BALB/c mice (8-12 weeks old) weighing between 20 to 35 g were procured from In vivo biosciences, Bengaluru, India. Maximum three mice were housed in each polycarbonate cage. Temperature and humidity were maintained at 22 ± 3ºC and 40-70%, respectively and illumination was controlled to give a sequence of 12 hr light and 12 hr dark cycle. The temperature and humidity were recorded by auto-controlled data logger system. Animals were provided laboratory rodent diet (Vetcare India Pvt. Ltd, Bengaluru) *ad libitum* and were provided fresh reverse osmosis water treated with UV light *ad libitum*.

Eighteen female BALB/c mice were weighed and administered by intraperitoneal route with NEU-1912 solution formulation at a dose of 10 mg/kg. The dosing volume administered for was 10 mL/kg.

Sample preparation. Accurately weighed NEU-1912 (7.45 mg) was taken into a labeled bottle to this 0.372 mL of NMP, 0.372 mL of solutol HS, 6.705 mL of normal

saline was added to bottle. The bottle was vortexed for 2 minutes after each addition and the final solution formulation was sonicated for 2 minutes to obtain a clear solution.

Sample Collection. Blood samples were collected from a set of three mice at each time point at 0.08, 0.25, 1, 4, 8 and 24 hr. The blood samples (approximately 60 µL) were collected from the retro-orbital plexus into labeled tubes, containing K_2EDTA solution, as an anticoagulant. Plasma was harvested from the blood by centrifugation at 4000 rpm for 10 min at 4 ± 2ºC and stored below -70ºC until bioanalysis. After collecting the blood samples, mice were humanely euthanized by CO_2 asphyxiation and brain was collected at 0.08, 0.25, 1, 4, 8 and 24 hr. Following collection, the brain samples were washed by dipping in 20 mL of ice-cold phosphate buffer saline (pH 7.4), dried gently on a filter paper, weighed and placed in polypropylene tubes. Further brain samples were homogenized using ice-cold phosphate buffer saline (pH 7.4) and the total homogenate volume was thrice the brain weight. The brain samples were stored at -70ºC until bioanalysis.

Data analysis. Non-Compartmental-Analysis tool of Phoenix WinNonlin® (Version 6.3) was used to assess the pharmacokinetic parameters. Peak plasma concentrations (C_{max}) and time for the peak plasma concentrations (T_{max}) were the observed values. The areas under the concentration time curve (AUC_{last} and AUC_{inf}) were calculated by linear trapezoidal rule. The terminal elimination rate constant, ke was determined by regression analysis of the linear terminal portion of the log plasma concentration-time curve. The terminal half-life ($T_{1/2}$) was estimated as 0.693/ke.

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