SUPPORTING INFORMATION

From cells, to mice, to target: Characterization of NEU-1053 (SB-443342) and its analogs for treatment of human African trypanosomiasis.

William G. Devine,[a] Rosario Diaz-Gonzalez,[b] Gloria Ceballos-Perez,[b] Domingo Rojas,[b] Takashi Satoh,[a] Westley Tear,[a] Ranae M. Ranade,[c] Ximena Barros-Alvarez,[d] Wim G. Hol,[d] Frederick S. Buckner,[c] Miguel Navarro,[b] and Michael P. Pollastri[a *]

[a]Department of Chemistry & Chemical Biology, Northeastern University 360 Huntington Avenue, Boston, MA USA; [b]Instituto de Parasitología y Biomedicina "López-Neyra", Granada 18100 Spain; Departments of [c]Medicine, and [d]Biochemistry, University of Washington, Seattle, WA 98195 USA; [e]Laboratorio de Enzimología de Parásitos, Facultad de Ciencias, Universidad de los Andes, Mérida, Venezuela.

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CHEMICAL SYNTHESIS

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 Initiatior-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.

General Reduction Procedure A: Lithium aluminum hydride (2.2-4.4 eq.) was added to a solution of the appropriate ester (1 eq.) in dry THF (0.1M) cooled to 0 °C under an atmosphere of N_2. After 30 minutes the mixture was warmed to room temperature and stirred a further 1.5 hours. The mixture was cooled back down to 0 °C and quenched by the dropwise additions of water (8.5 eq.), 3M aq. NaOH (0.5 eq.), and water (25.5 eq.). The mixture was warmed to room temperature and stirred vigorously for 30 minutes before the addition of $MgSO_4$. Stirring was continued for an additional 15 minutes before the mixture was filtered through a pad of diatomaceous earth. The filtrate was concentrated and the residue was purified by flash column chromatography.

General Reduction Procedure B: Di*iso*-butylaluminum hydride (1.2M in toluene, 2.2-4.4 eq.) was added to a solution of the appropriate ester (1 eq.) in dry THF (0.1M) at 0 °C under N_2. The mixture was warmed to room temperature, stirred for 1 hour, cooled back down to 0 °C, and quenched with ethyl acetate. Saturated potassium sodium tartrate was added and the mixture was stirred vigorously overnight. The layers were separated and the aqueous was extracted with ethyl acetate (3x). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography.

General Oxidation Procedure C: Freshly prepared manganese dioxide (15 eq.) was added to a solution of the appropriate alcohol (1 eq.) in dry CH_2Cl_2 (0.05M) or THF (0.1M) and the mixture

was stirred at room temperature for 2 hours. The mixture was filtered through a pad of diatomaceous earth and the filtrate concentrated. The residue was purified by flash column chromatography.

General Amination Procedure D: 2-Chloro-1*H*-benzo[*d*]imidazole (1 eq.) was added to a solution of mono-boc protected diamine (2 eq.) in toluene (0.2M) in microwave vial. The vial was sealed with a septum and the contents were irradiated to 150 °C where the temperature was modulated for 6 hours with stirring. After cooling to room temperature the mixture was diluted with ethyl acetate (150 mL), washed with sat. aq. $NaHCO_3$ (100 mL), washed with water (100 mL), washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography.

General Amination Procedure E: 2-Chloro-1*H*-benzo[*d*]imidazole (1 eq.) was added to a solution of diamine (5 eq.) in xylenes (0.2M) in microwave vial. The vial was sealed with a septum and the contents were irradiated to 200 °C where the temperature was modulated for 15 minutes with stirring. After cooling to room temperature the mixture was diluted with ethyl acetate (100 mL), washed with sat. aq. $NaHCO_3$ (50 mL), washed with water (50 mL), washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography.

General de-Boc Procedure F:

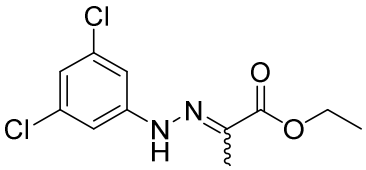
Trifluoroacetic acid (10 eq.) was added to a suspension of the appropriate Boc protected amine in CH_2Cl_2 (0.05M) and the resulting solution was stirred overnight. The mixture was concentrated under reduced pressure and the product was used without any further purification.

General Reductive Amination Procedure G:

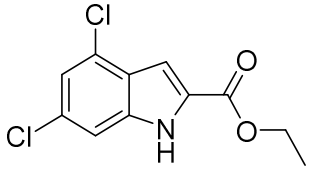
Sodium cyanoborohydride (4 eq.) was added to a solution of the appropriate aldehyde (1 eq.), the appropriate amine (1.1 eq.), and potassium acetate (4 eq.) in CH_3OH (0.1M). The mixture was stirred at room temperature overnight then poured over water (40 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with aq. NaOH (1M, 30 mL), washed with water (30 mL), washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography.

General Reductive Amination Procedure H:

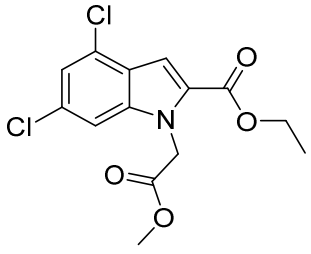
Glacial acetic acid (1 drop) followed by sodium cyanoborohydride (4 eq.) were added to a solution of the appropriate aldehyde (1 eq.) and appropriate amine (1.3 eq.) in CH_3OH (0.1M). The mixture was stirred at room temperature overnight, quenched with sat. aq. $NaHCO_3$ (2 mL), poured in to water (10 mL) and extracted with ethyl acetate (3 x 8 mL). The combined organic layers were washed with aq. NaOH (1M, 5 mL), washed with brine (5 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography.



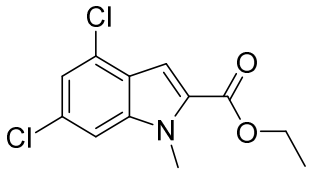
Ethyl 2-(2-(3,5-dichlorophenyl)hydrazono)propanoate (3) Ethyl pyruvate (8.9 mL, 80 mmol) was added to a solution of (3,5-dichlorophenyl)hydrazine·HCl (17.0 g, 80 mmol) in ethanol (160 mL) and the mixture was refluxed for 1 hour. After cooling to room temperature the solvent removed *in vacuo* and the crude was purified by flash column chromatography using a gradient of 5-15% ethyl acetate in hexanes. **3** was collected as a mixture of *E* and *Z* isomers that re-equilibrated on standing at room temperature. *Z*-Isomer (major): TLC (10% ethyl acetate in hexanes) R_f = 0.67. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 12.03 (s, 1 H), 7.06 (d, *J*=1.5 Hz, 2 H), 6.89 (t, *J*=1.7 Hz, 1 H), 4.29 (q, *J*=7.3 Hz, 2 H), 2.17 (s, 3 H), 1.37 (t, *J*=7.3 Hz, 3 H). LC/MS found $[M+H]^+$. *E*-Isomer (minor): TLC (10% ethyl acetate in hexanes) R_f = 0.17. ^{1}H NMR (500 MHz, CHLOROFORM-*d*) d ppm 7.62 (s, 1 H), 7.11 (d, *J*=2.0 Hz, 2 H), 6.95 (t, *J*=1.7 Hz, 1 H), 4.34 (q, *J*=7.0 Hz, 2 H), 2.12 (s, 3 H), 1.40 (t, *J*=7.3 Hz, 3 H). LC/MS found 275-1 $[M+H]^+$.



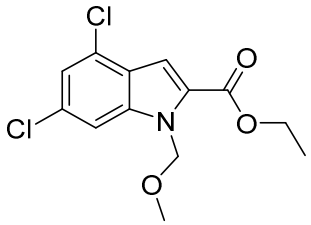
Ethyl 4,6-dichloro-1*H*-indole-2-carboxylate (4) In a 600 mL beaker was slowly dissolved ethyl 2-(2-(3,5-dichlorophenyl)hydrazono)propanoate (18.5 g, 67.2 mmol) in polyphosphoric acid (80 ml) at 70 °C with mechanical stirring. The mixture was raised to 130 °C where it was held for 10 minutes, then cooled in an ice-water bath and slowly quenched with ice. Ethyl acetate (500 mL) was added and the layers were separated. The aqueous layer was neutralized with NaOH pellets and extracted with ethyl acetate (2 x 300 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (200 mL), washed with brine (200 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was recrystallized from ethanol/water (4.5:1, 220 mL) to give **4** as a pale tan colored solid in 74% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.37. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.41 (br. s., 1 H), 7.45 (s, 1 H), 7.28 (d, *J*=1.5 Hz, 1 H), 7.11 (d, *J*=2.0 Hz, 1 H), 4.36 (q, *J*=7.0 Hz, 2 H), 1.35 (t, *J*=7.1 Hz, 3 H). LC/MS found 258.0 $[M+H]^+$.



Ethyl 4,6-dichloro-1-(2-methoxy-2-oxoethyl)-1*H*-indole-2-carboxylate (5a) Potassium carbonate (604 mg, 4.4 mmol) was stirred in dry DMF (10 mL) for 1 hour prior to the addition of ethyl 4,6-dichloro-1H-indole-2-carboxylate (1.00 g, 3.9 mmol). After 1 hour methyl 2-bromoacetate (0.41 mL, 4.3 mmol) was added and the resulting mixture was stirred overnight. The mixture was then poured over water (100 mL) and extracted with 1:1 ethyl acetate/hexanes (3 x 100 mL). The combined organic layers were washed with water (2 x 100 mL) and brine (80 mL), dried over anhydrous Na_2SO_4, concentrated on to silica, and purified by flash column chromatography using a gradient of 5-20% ethyl acetate in hexanes to give **5a** as a white solid in 97% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.28. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.42 (s, 1 H), 7.19 - 7.22 (m, 2 H), 5.27 (s, 2 H), 4.38 (q, *J*=7.3 Hz, 2 H), 3.78 (s, 3 H), 1.41 (t, *J*=7.1 Hz, 3 H). LC/MS found 330.1 $[M+H]^+$.

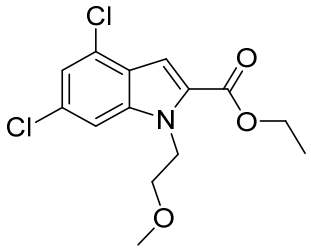


Ethyl 4,6-dichloro-1-methyl-1*H*-indole-2-carboxylate (5b) A solution of ethyl 4,6-dichloro-1*H*-indole-2-carboxylate (103 mg, 0.40 mmol) in dry DMF (2 mL) was added to a slurry of sodium hydride (60 wt%, 24 mg, 0.60 mmol) in dry DMF (2 mL) cooled to 0 °C. The mixture was stirred for 30 minutes before the addition of methyl iodide (0.08 mL, 1.28 mmol). The mixture stirred an additional 1 hour at 0 °C, warmed to room temperature, and stirred for 3 hours. The mixture was quenched with sat. aq. NH_4Cl (10 mL) and extracted with 1:1 hexanes/ethyl acetate (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL), washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 0-10% ethyl acetate in hexanes to give **5b** as a white solid in 88% yield. TLC (5% ethyl acetate in hexanes): R_f = 0.28, fluoresces. ^{1}H NMR (399 MHz, $CDCl_3$) δ ppm 7.34 (s, 1 H), 7.30 (s, 1 H), 7.17 (s, 1 H), 4.40 (q, *J*=7.1 Hz, 2 H), 4.05 (s, 3 H), 1.43 (t, *J*=7.3 Hz, 3 H). LC/MS found 272.0 $[M+H]^+$.

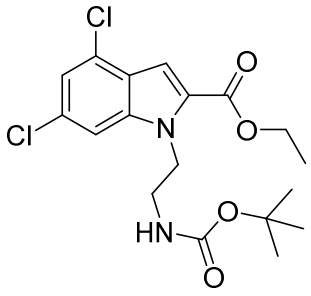


Ethyl 4,6-dichloro-1-(methoxymethyl)-1*H*-indole-2-carboxylate (5c) A solution of ethyl 4,6-dichloro-1*H*-indole-2-carboxylate (103 mg, 0.40 mmol) in dry DMF (2 mL) was added to a slurry of sodium hydride (60 wt%, 24 mg, 0.60 mmol) in dry DMF (2 mL) cooled to 0 °C. The mixture was stirred for 30 minutes before the addition of chloromethyl ethyl ether (0.12 mL, 1.28 mmol). The mixture stirred an additional 1 hour at 0 °C, warmed to room temperature, and

stirred for 3 hours. The mixture was quenched with sat. aq. NH_4Cl (10 mL) and extracted with 1:1 hexanes/ethyl acetate (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL), washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 0-10% ethyl acetate in hexanes to give **5c** as a white solid in 80% yield. TLC (5% ethyl acetate in hexanes): R_f = 0.25. ^{1}H NMR (399 MHz, $CDCl_3$) δ ppm 7.48 (s, 1 H), 7.40 (s, 1 H), 7.21 (d, *J*=1.5 Hz, 1 H), 5.94 (s, 2 H), 4.41 (q, *J*=7.3 Hz, 2 H), 3.30 (s, 3 H), 1.44 (t, *J*=7.3 Hz, 3 H). LC/MS found 324.0 $[M+Na]^+$.

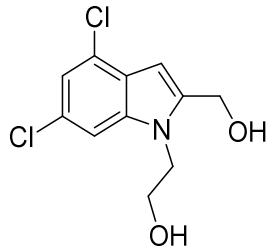


Ethyl 4,6-dichloro-1-(2-methoxyethyl)-1*H*-indole-2-carboxylate (5d) A solution of ethyl 4,6-dichloro-1*H*-indole-2-carboxylate (103 mg, 0.40 mmol) in dry DMF (2 mL) was added to a slurry of sodium hydride (60 wt%, 24 mg, 0.60 mmol) in dry DMF (2 mL) cooled to 0 °C. The mixture was stirred for 30 minutes before the addition of 1-bromo-2-methoxyethane (0.12 mL, 1.26 mmol). The mixture stirred an additional 1 hour at 0 °C, warmed to room temperature, and stirred for 18 hours. The mixture was quenched with sat. aq. NH_4Cl (10 mL) and extracted with 1:1 hexanes/ethyl acetate (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL), washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 0-10% ethyl acetate in hexanes to give **5d** as an off-white solid in 60% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.35. ^{1}H NMR (399 MHz, $CDCl_3$) δ ppm 7.42 (s, 1 H), 7.36 (s, 1 H), 7.16 (d, *J*=1.5 Hz, 1 H), 4.68 (t, *J*=5.5 Hz, 2 H), 4.39 (q, *J*=7.3 Hz, 2 H), 3.72 (t, *J*=5.5 Hz, 2 H), 3.28 (s, 3 H), 1.43 (t, *J*=7.3 Hz, 3 H). LC/MS found 346.1 $[M+H]^+$.

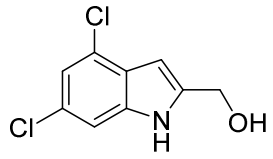


Ethyl 1-(2-((*tert*-butoxycarbonyl)amino)ethyl)-4,6-dichloro-1*H*-indole-2-carboxylate (5e) Potassium carbonate (84 mg, 0.61 mmol) was added to a solution of ethyl 4,6-dichloro-1*H*-indole-2-carboxylate (102 mg, 0.40 mmol) in dry DMF (4 mL) and the mixture was stirred for 15 minutes at room temperature. 2-((*tert*-Butoxycarbonyl)amino)ethyl 4-methylbenzenesulfonate

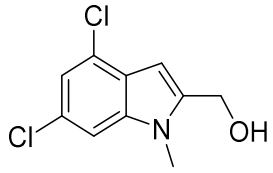
(188 mg, 0.60 mmol) was then added and the mixture was stirred overnight. The mixture was then poured over water (30 mL) and extracted with 1:1 hexanes/ethyl acetate (3 x 25 mL). The combined organic layers were washed with water (2 x 30 mL), washed with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated on to silica. The crude was then purified by flash column chromatography using a gradient of 10-20% ethyl acetate in hexanes to give **5e** as a white solid in 69% yield. TLC (20% ethyl acetate in hexanes): R_f = 0.39. ^{1}H NMR (399 MHz, $CDCl_3$) δ ppm 7.38 (s, 2 H), 7.17 (d, *J*=1.5 Hz, 1 H), 4.64 (t, *J*=5.9 Hz, 3 H), 4.39 (q, *J*=6.8 Hz, 2 H), 3.54 (q, *J*=5.9 Hz, 2 H), 1.37 - 1.46 (m, 12 H). LC/MS found 401.0 $[M+H]^+$.



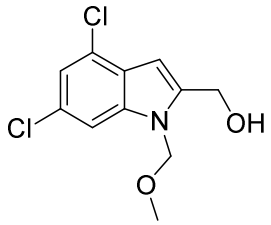
2-(4,6-Dichloro-2-(hydroxymethyl)-1*H*-indol-1-yl)ethan-1-ol (6a) General procedure A (91% yield) or general procedure B (85% yield). Off-white solid. FCC: 30-70% ethyl acetate in hexanes. TLC (50% ethyl acetate in hexanes): R_f = 0.29. ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 7.61 (s, 1 H), 7.15 (d, *J*=1.5 Hz, 1 H), 6.43 (s, 1 H), 5.35 (t, *J*=5.9 Hz, 1 H), 4.93 (t, *J*=5.5 Hz, 1 H), 4.67 (d, *J*=5.1 Hz, 2 H), 4.27 (t, *J*=5.5 Hz, 2 H), 3.68 (q, *J*=5.5 Hz, 2 H). LC/MS found 260.0 $[M+H]^+$.



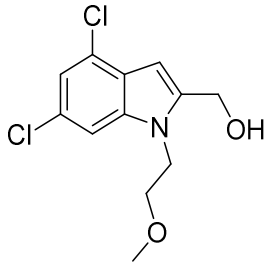
(4,6-Dichloro-1*H*-indol-2-yl)methanol (6b) General procedure A, FCC: 20-50% ethyl acetate in hexanes. Collected as an off-white solid in 93% yield. TLC (40% ethyl acetate in hexanes): R_f = 0.29. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.54 (br. s., 1 H), 7.35 (s, 1 H), 7.10 (d, *J*=2.0 Hz, 1 H), 6.33 (d, *J*=1.0 Hz, 1 H), 5.39 (br. s., 1 H), 4.62 (s, 2 H). LC/MS found 216.0 $[M+H]^+$.



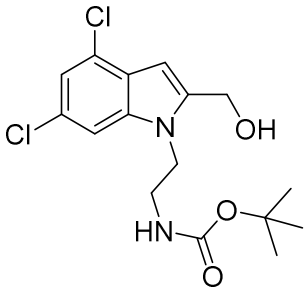
(4,6-Dichloro-1-methyl-1*H*-indol-2-yl)methanol (6c) General procedure A, FCC: 20-50% ethyl acetate in hexanes. Collected as a pale yellow solid in 89% yield. TLC (40% ethyl acetate in hexanes): R_f = 0.30. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.21 (d, *J*=1.0 Hz, 1 H), 7.11 (d, *J*=1.0 Hz, 1 H), 6.52 (s, 1 H), 4.80 (s, 2 H), 3.76 (s, 3 H). LC/MS found 230.0 $[M+H]^+$.



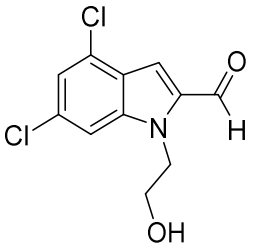
(4,6-Dichloro-1-(methoxymethyl)-1*H*-indol-2-yl)methanol (6d) General procedure A, FCC: 20-50% ethyl acetate in hexanes. Collected as a pale yellow solid in 87% yield. TLC (40% ethyl acetate in hexanes): R_f = 0.39. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.36 (s, 1 H), 7.16 (d, *J*=2.0 Hz, 1 H), 6.61 (s, 1 H), 5.51 (s, 2 H), 4.82 (s, 2 H), 3.29 (s, 3 H), 2.26 (br. s., 1 H). LC/MS found 260.0 $[M+H]^+$.



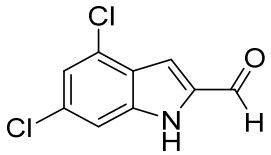
(4,6-Dichloro-1-(2-methoxyethyl)-1*H*-indol-2-yl)methanol (6e) General procedure A, FCC: 20-50% ethyl acetate in hexanes. Collected as a pale green oil in 96% yield. TLC (40% ethyl acetate in hexanes): R_f = 0.31. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.18 (s, 1 H), 7.13 (d, *J*=2.0 Hz, 1 H), 6.56 (s, 1 H), 4.72 (s, 2 H), 4.33 (t, *J*=4.9 Hz, 2 H), 3.73 (t, *J*=4.9 Hz, 2 H), 3.30 (s, 3 H). LC/MS found 274.1 $[M+H]^+$.



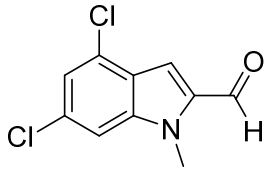
***tert*-Butyl (2-(4,6-dichloro-2-(hydroxymethyl)-1*H*-indol-1-yl)ethyl)carbamate (6f)** General procedure B, FCC: 30-80% ethyl acetate in hexanes. Collected as an yellow solid in 64% yield. TLC (30% ethyl acetate in hexanes): R_f = 0.43. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.25 (s, 1 H), 7.11 (d, *J*=1.5 Hz, 1 H), 6.56 (s, 1 H), 4.85 (br. s., 1 H), 4.79 (s, 2 H), 4.27 (t, *J*=6.3 Hz, 2 H), 3.52 (q, *J*=6.3 Hz, 2 H), 1.68 (br. s., 1 H), 1.38 (s, 9 H). LC/MS found 359.0 $[M+H]^+$.



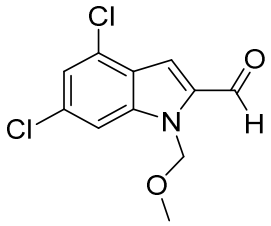
4,6-Dichloro-1-(2-hydroxyethyl)-1*H*-indole-2-carbaldehyde (7a) General procedure C with THF. Off-white solid. FCC: 0-20% ethyl acetate in CH_2Cl_2. TLC (10% ethyl acetate in CH_2Cl_2): R_f = 0.40. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.96 (s, 1 H), 7.83 (d, *J*=1.0 Hz, 1 H), 7.52 (d, *J*=1.0 Hz, 1 H), 7.36 (d, *J*=2.0 Hz, 1 H), 4.82 (t, *J*=5.4 Hz, 1 H), 4.59 (t, *J*=5.4 Hz, 2 H), 3.67 (q, *J*=5.7 Hz, 2 H). LC/MS found 258.0 $[M+H]^+$.



4,6-Dichloro-1*H*-indole-2-carbaldehyde (7b) General Procedure C, FCC: 5-20% ethyl acetate in hexanes. Collected as a white solid in 58% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.23. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.47 (br. s., 1 H), 9.90 (s, 1 H), 7.45 - 7.47 (m, 2 H), 7.33 (d, *J*=1.5 Hz, 1 H). LC/MS found 213.9 $[M+H]^+$.

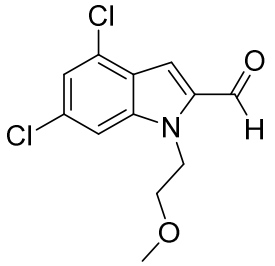


4,6-Dichloro-1-methyl-1*H*-indole-2-carbaldehyde (7c) General Procedure C, FCC: 0-10% ethyl acetate in hexanes. Collected as a white solid in 50% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.39. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 9.91 (s, 1 H), 7.31 - 7.34 (m, 2 H), 7.21 (d, *J*=1.5 Hz, 1 H), 4.08 (s, 3 H). LC/MS found 228.0 $[M+H]^+$.

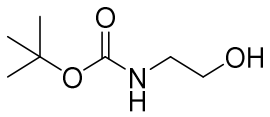


4,6-Dichloro-1-(methoxymethyl)-1*H*-indole-2-carbaldehyde (7d) General Procedure C, FCC: 0-10% ethyl acetate in hexanes. Collected as a white solid in 51% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.37. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 9.92 (s, 1 H), 7.51 (s, 1 H), 7.39 (d,

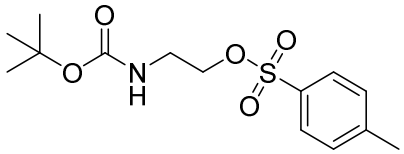
J=1.0 Hz, 1 H), 7.26 (d, *J*=2.0 Hz, 1 H), 5.94 (s, 2 H), 3.31 (s, 3 H). LC/MS found 258.0 $[M+H]^+$.



4,6-Dichloro-1-(2-methoxyethyl)-1*H*-indole-2-carbaldehyde (7e) General Procedure C, FCC: 0-15% ethyl acetate in hexanes. Collected as a white solid in 46% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.29. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 9.90 (s, 1 H), 7.44 (s, 0 H), 7.36 (d, *J*=1.0 Hz, 1 H), 7.20 (d, *J*=2.0 Hz, 1 H), 4.66 (t, *J*=5.4 Hz, 2 H), 3.72 (t, *J*=5.4 Hz, 2 H), 3.27 (s, 3 H). LC/MS found 272.0 $[M+H]^+$.

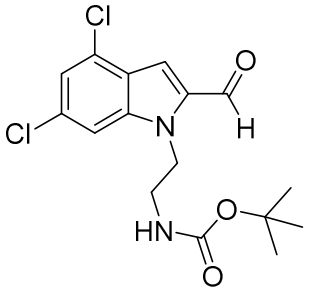


***tert*-Butyl (2-hydroxyethyl)carbamate (S1)** To a solution of ethanolamine (185 mg, 3.0 mmol) in dry CH_2Cl_2 (9 mL) at 0 °C was added triethylamine (0.63 mL, 4.5 mmol), and Boc_2O (656 mg, 3.0 mmol). The mixture was gradually allowed to warm to room temperature and stirred overnight. Sat. aq. NH_4Cl (20 mL) was added and the mixture was extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated to give **S1** as a colorless solid in 100% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.98 (br. s., 1 H), 3.71 (br. s., 2 H), 3.29 (q, *J*=5.2 Hz, 2 H), 2.50 (br. s., 1 H), 1.45 (s, 9 H). LC/MS found 161.9 $[M+H]^+$.

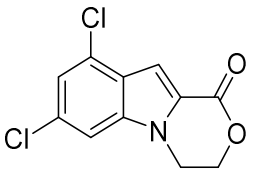


2-((*tert*-Butoxycarbonyl)amino)ethyl 4-methylbenzenesulfonate (S2) To a solution of *tert*-butyl (2-hydroxyethyl)carbamate (476 mg, 3.0 mmol) in CH_2Cl_2 (10 mL) cooled to 0 °C was added tosyl chloride (843 mg, 4.4 mmol) and triethylamine (0.82 mL, 5.9 mmol). The mixture was stirred for 10 minutes at 0 °C, warmed to room temperature, and stirred a further 2 hours. The mixture was then concentrated and purified by flash column chromatography using a gradient of 10-30% ethyl acetate in hexanes to give **S2** as a colorless oil in 81% yield. TLC (30% ethyl acetate in hexanes): R_f = 0.40. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.79 (d, *J*=8.3 Hz, 2 H),

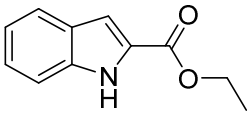
7.36 (d, *J*=8.3 Hz, 2 H), 4.85 (br. s., 1 H), 4.07 (t, *J*=5.1 Hz, 2 H), 3.38 (q, *J*=4.9 Hz, 2 H), 2.45 (s, 3 H), 1.41 (s, 9 H). LC/MS found 316.1 $[M+H]^+$.



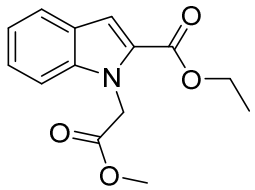
***tert*-Butyl (2-(4,6-dichloro-2-formyl-1*H*-indol-1-yl)ethyl)carbamate (7f)** General Procedure C, FCC: 10-20% ethyl acetate in hexanes. Collected as a off-white solid in 71% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 9.89 (s, 1 H), 7.42 (s, 1 H), 7.38 (s, 1 H), 7.21 (d, *J*=1.5 Hz, 1 H), 4.62 (t, *J*=5.9 Hz, 3 H), 3.51 (q, *J*=6.0 Hz, 2 H), 1.40 (s, 9 H). LC/MS found 357.0 $[M+H]^+$.



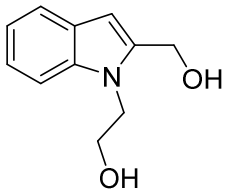
7,9-dichloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]indol-1-one (8) General procedure C with THF, isolated as a side product from the reaction mixture as a white solid in 20% yield. TLC (10% ethyl acetate in dichloromethane): R_f = 0.79. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.87 (dd, *J*=2.0, 1.0 Hz, 1 H), 7.39 (d, *J*=2.0 Hz, 1 H), 7.30 (d, *J*=1.0 Hz, 1 H), 4.79 (t, *J*=5.4 Hz, 1 H), 4.49 (t, *J*=5.4 Hz, 1 H). LC/MS found 255.9 $[M+H]^+$.



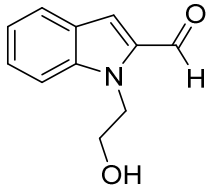
Ethyl 1*H*-indole-2-carboxylate (10) Conc. sulfuric acid (1 mL, 18.8 mmol) was added to a solution of 1*H*-indole-2-carboxylic acid (1.00 g, 6.2 mmol) in ethanol (12.5 mL) and the mixture was refluxed overnight. The mixture was cooled to room temperature, diluted with water (80 mL), bacified to pH 12 with 1M aq. NaOH, and extracted with ethyl acetate (3 x 70 mL). The combined organic layers were washed with 1M aq. NaOH (40 mL), washed with water (40 mL), washed with brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated to give **10** as a brown solid in 86% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.29. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.86 (br. s., 1 H), 7.70 (d, *J*=7.8 Hz, 1 H), 7.44 (d, *J*=8.3 Hz, 1 H), 7.34 (t, *J*=7.8 Hz, 1 H), 7.24 (s, 1 H), 7.17 (t, *J*=7.6 Hz, 1 H), 4.42 (q, *J*=7.3 Hz, 2 H), 1.43 (t, *J*=7.1 Hz, 3 H). LC/MS found 190.1 $[M+H]^+$.



Ethyl 1-(2-methoxy-2-oxoethyl)-1*H*-indole-2-carboxylate (11) Potassium carbonate (738 mg, 5.34 mmol) was added to a solution of ethyl 1*H*-indole-2-carboxylate (1.01 g, 5.34 mmol) in dry DMF (14 mL). The mixture was stirred at room temperature under an atmosphere of nitrogen for 30 minutes. Methyl bromoacetate (0.50 mL, 5.43 mmol) was then added and the mixture was stirred at room temperature for an additional 3 hours. The mixture was poured over water (70 mL) and extracted with a 1:1 mixture of ethyl acetate/hexanes (3 x 70 mL). The combined organic layers were washed with water (70 mL), washed with brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 5-20% ethyl acetate in hexanes to give **11** as a pale yellow oil in 44% yield. TLC (10% ethyl acetate in hexanes): R_f = 0.21. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.71 (d, *J*=7.8 Hz, 1 H), 7.39 (s, 1 H), 7.37 (d, *J*=7.3 Hz, 1 H), 7.29 (d, *J*=7.8 Hz, 1 H), 7.19 (t, *J*=7.8 Hz, 1 H), 5.34 (s, 2 H), 4.37 (q, *J*=7.3 Hz, 2 H), 3.76 (s, 3 H), 1.40 (t, *J*=7.3 Hz, 3 H). LC/MS found 262.1 $[M+H]^+$.

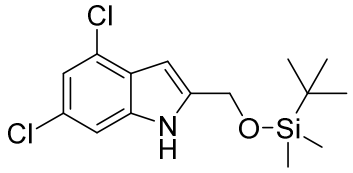


2-(2-(Hydroxymethyl)-1*H*-indol-1-yl)ethan-1-ol (12) General procedure A, FCC 50-80% ethyl acetate in hexanes. Collected as an off-white solid in 76% yield. TLC (70% ethyl acetate in hexanes): R_f = 0.32. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.48 (d, *J*=7.8 Hz, 1 H), 7.42 (d, *J*=8.3 Hz, 1 H), 7.09 (ddd, *J*=8.2, 7.0, 1.0 Hz, 1 H), 6.98 (t, *J*=7.8 Hz, 1 H), 6.34 (s, 1 H), 5.21 (t, *J*=5.4 Hz, 1 H), 4.96 (t, *J*=5.1 Hz, 1 H), 4.66 (d, *J*=5.4 Hz, 2 H), 4.27 (t, *J*=6.1 Hz, 2 H), 3.69 (q, *J*=5.9 Hz, 2 H). LC/MS found 192.1 $[M+H]^+$.

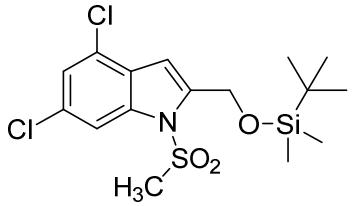


1-(2-Hydroxyethyl)-1*H*-indole-2-carbaldehyde (13) General Procedure C, FCC 10-40% ethyl acetate in hexanes. Collected as an off-white solid in 78% yield. TLC (50% ethyl acetate in hexanes): R_f = 0.44. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.91 (s, 1 H), 7.75 (d, *J*=8.3 Hz, 1 H), 7.64 (d, *J*=9.3 Hz, 1 H), 7.46 (s, 1 H), 7.39 (ddd, *J*=8.5, 7.1, 1.0 Hz, 1 H), 7.15 (t, *J*=7.8 Hz, 1

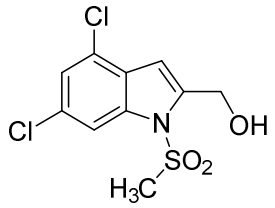
H), 4.83 (t, *J*=5.4 Hz, 1 H), 4.58 (t, *J*=5.9 Hz, 2 H), 3.67 (q, *J*=5.9 Hz, 2 H). LC/MS found 190.1 $[M+H]^+$.



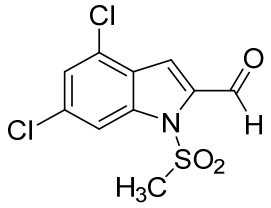
2-(((*tert*-Butyldimethylsilyl)oxy)methyl)-4,6-dichloro-1*H*-indole (14) To a stirred solution of (4,6-dichloro-1*H*-indol-2-yl)methanol (540 mg, 2.5 mmol) and 1*H*-imidazole (286 mg, 4.2 mmol) in CH_2Cl_2 (10 mL) was added *tert*-butylchlorodimethylsilane (420 mg, 2.8 mmol) and the resulting mixture was stirred for 1 hour. The mixture was diluted with dichloromethane (100 mL), washed with water (100 mL), washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 0-10% ethyl acetate in hexanes to give **14** as a white solid in 96% yield. TLC (5% ethyl acetate in hexanes): R_f = 0.34. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.42 (br. s., 1 H), 7.28 (s, 1 H), 7.11 (s, 1 H), 6.38 (s, 1 H), 4.87 (s, 2 H), 0.95 (s, 9 H), 0.13 (s, 6 H).



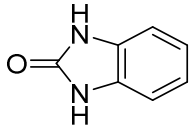
2-(((*tert*-Butyldimethylsilyl)oxy)methyl)-4,6-dichloro-1-(methylsulfonyl)-1*H*-indole (15) Sodium hydride (60 wt% dispersion in oil, 20 mg, 0.50 mmol) was added to a solution of 2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4,6-dichloro-1*H*-indole (100 mg, 0.30 mmol) in dry DMF (3.0 mL) and the mixture was stirred for 30 minutes at room temperature under nitrogen. Methanesulfonyl chloride (0.07 mL, 0.91 mmol) was then added and the mixture was stirred for 1 hour. The mixture was poured over water (8 mL) and extracted with 1:1 hexanes/ethyl acetate (3 x 8 mL). The combined organic layers were washed with water (3 x 8 mL), washed with brine (5 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 10-50% ethyl acetate in hexanes to give **15** as an off-white solid in 9% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.99 (s, 1 H), 7.31 (d, *J*=2.0 Hz, 1 H), 6.75 (s, 1 H), 4.96 (s, 2 H), 3.26 (s, 3 H), 0.96 (s, 9 H), 0.17 (s, 6 H). Does not ionize by ESI LC/MS.



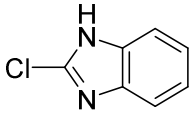
(4,6-Dichloro-1-(methylsulfonyl)-1*H*-indol-2-yl)methanol (16) Collected as a side product from WGD2-077 in 31% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.95 (d, *J*=1.0 Hz, 1 H), 7.32 (d, *J*=2.0 Hz, 1 H), 6.76 (s, 1 H), 4.90 (d, *J*=2.4 Hz, 2 H), 3.25 (s, 3 H), 2.77 (br. s., 1 H). LC/MS found 294.0 $[M+H]^+$.



4,6-Dichloro-1-(methylsulfonyl)-1*H*-indole-2-carbaldehyde (17) General Procedure C, FCC: 10-30% ethyl acetate in hexanes. Collected as a tan colored solid in 54% yield. TLC (20% ethyl acetate in hexanes): R_f = 0.42. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.95 (d, *J*=1.0 Hz, 1 H), 7.32 (d, *J*=2.0 Hz, 1 H), 6.76 (s, 1 H), 4.90 (d, *J*=2.4 Hz, 2 H), 3.25 (s, 3 H), 2.77 (br. s., 1 H). LC/MS found 291.9 $[M+H]^+$.

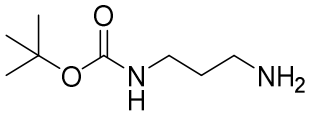


1,3-Dihydro-2*H*-benzo[*d*]imidazol-2-one (19) A mixture of *o*-phenylenediamine (10.8 g, 100 mmol) and urea (110 mmol) in ethylene glycol (20 mL) was heated to 140 °C for 1 hour, then 170 °C for 7 hours. The mixture was cooled to ~50 °C, ethanol (20 mL) was added, and the mixture was stirred for an additional 10 minutes. Water (20 mL) was then added and the resulting precipitate was vacuum filtered and washed with ethanol and water to give **19** as a shimmering white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.57 (s, 2 H), 6.91 (s, 4 H). LC/MS found 135.1 $[M+H]^+$.

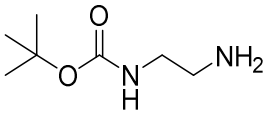


2-Chloro-1*H*-benzo[*d*]imidazole (20) 1,3-Dihydro-2*H*-benzo[*d*]imidazol-2-one (10.9 g, 81 mmol) was added slowly to phosphorus oxychloride (38 mL, 408 mmol) cooled to 0 °C. The mixture was refluxed for 2 hours, cooled to room temperature and concentrated by vacuum distillation. The remaining residue was taken up in dichloromethane (500 mL), cooled to 0 °C, and quenched by dropwise addition of sat. aq. $NaHCO_3$ (200 mL). The mixture was allowed to stir for 1 hour then warmed to room temperature and stirred an additional hour. The layers were separated and the aqueous was extracted with dichloromethane (4 x 250 mL). The combined organic layers were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography with a gradient of 0-

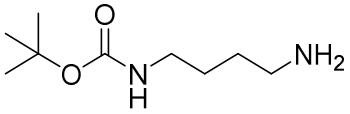
10% CH_3OH in CH_2Cl_2. to give **20** as a beige solid. TLC (30% ethyl acetate in hexanes): R_f = 0.45, streaks. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.23 (br. s., 1 H), 7.51 (br. s., 2 H), 7.20 - 7.24 (m, 2 H). LC/MS found 153.0 $[M+H]^+$.



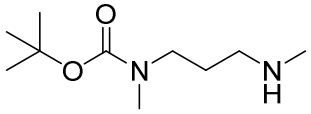
***tert*-Butyl (3-aminopropyl)carbamate (22a)** A solution of Boc_2O (13.1 g, 60 mmol) in $CHCl_3$ (120 mL) was added dropwise over 2 hours to a solution of 1,3-diaminopropane (25 mL, 300 mmol) in $CHCl_3$ (240 mL) cooled to 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. After which time the precipitate was filtered off and the filtrate was concentrated. The residue was dissolved in ethyl acetate (300 mL), washed with half sat. aq. $NaHCO_3$ (3 x 100 mL), dried over anhydrous Na_2SO_4, and concentrated to give **22a** as a colorless oil in 69% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.95 (br. s., 1 H), 3.18 (m, *J*=6.3, 6.3, 6.3 Hz, 2 H), 2.75 (t, *J*=6.6 Hz, 2 H), 1.60 (quin, *J*=6.6 Hz, 2 H), 1.43 (s, 9 H), 1.30 (s, 2 H).



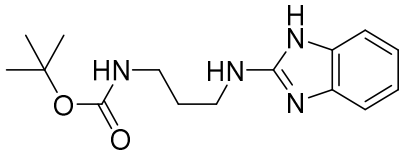
***tert*-Butyl (2-aminoethyl)carbamate (22b)** A solution of Boc_2O (1.72 mL, 7.4 mmol) in CH_2Cl_2 (110 mL) was added dropwise to a vigorously stirred solution of ethylenediamine (3.0 mL, 44 mmol) in CH_2Cl_2 (14 mL) over a period of 7 hours at room temperature. The reaction was stirred a further 24 hours before the mixture was evaporated *in vacuo* to leave a crude oily residue, to which aqueous Na_2CO_3 (2M, 80 mL) was added and the aqueous layer was separated and extracted with dichloromethane (2 x 80 mL). The combined organic extracts were evaporated *in vacuo* to give **22b** as a colorless viscous liquid in 88% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.93 (br. s., 1 H), 3.18 (q, *J*=5.7 Hz, 2 H), 2.81 (t, *J*=5.9 Hz, 2 H), 1.50 (s, 2 H), 1.44 (s, 9 H).



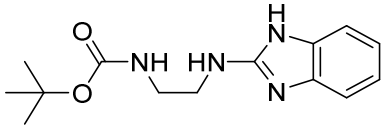
***tert*-Butyl (4-aminobutyl)carbamate (22c)** A solution of Boc_2O (282 mg, 1.29 mmol) in $CHCl_3$ (24 mL) was added dropwise to a vigorously stirred solution of butane-1,4-diamine (559 mg, 6.34 mmol) in $CHCl_3$ (8 mL) over a period of 2 hours at room temperature. The reaction was stirred a further 24 hours before the mixture was diluted with CH_2Cl_2 (60 mL), washed with water (2 x 50 mL), washed with brine (30 mL), and evaporated *in vacuo* to give **22c** as a colorless viscous liquid in 91% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.68 (br. s., 1 H), 3.13 (q, *J*=6.6 Hz, 2 H), 2.74 (t, *J*=6.6 Hz, 2 H), 1.94 (br. s., 2 H), 1.48 - 1.56 (m, 4 H), 1.44 (s, 9 H).



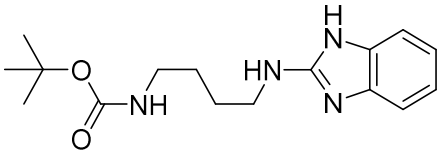
***tert*-Butyl methyl(3-(methylamino)propyl)carbamate (22d)** To a stirred solution of N^1,N^3-dimethylpropane-1,3-diamine (3.0 mL, 24 mmol) in CH_2Cl_2 (80 mL) was added a solution of Boc_2O (1.05 g, 4.81 mmol) in CH_2Cl_2 (16 mL) dropwise over 15 minutes at room temperature and the mixture was stirred overnight. The mixture was filtered and the filtrate was concentrated. The crude was taken up in ethyl acetate (300 mL) and washed with half saturated brine (3 x 100 mL). The organic layer was dried over Na_2SO_4 and concentrated to give **22d** as a yellow oil in 86% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.27 (br. s., 2 H), 2.83 (br. s., 3 H), 2.56 (t, *J*=6.8 Hz, 2 H), 2.42 (s, 3 H), 1.85 (br. s., 1 H), 1.70 (br. s., 2 H), 1.44 (s, 9 H).



***tert*-Butyl (3-((1*H*-benzo[d]imidazol-2-yl)amino)propyl)carbamate (23a)** General Procedure D, FCC: 10-50% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a white solid in 59% yield. TLC (5% CH_3OH in ethyl acetate): R_f = 0.34. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.72 (br. s., 1 H), 7.10 (d, *J*=8.8 Hz, 2 H), 6.93 (t, *J*=5.6 Hz, 1 H), 6.84 (br. s., 2 H), 6.49 (t, *J*=5.9 Hz, 1 H), 3.26 (q, *J*=6.5 Hz, 2 H), 3.00 (q, *J*=6.5 Hz, 2 H), 1.66 (quin, *J*=6.5 Hz, 2 H), 1.38 (s, 9 H). LC/MS found 291.2 $[M+H]^+$.

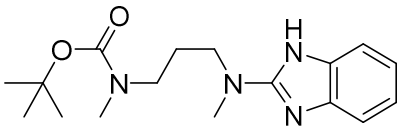


***tert*-Butyl (2-((1*H*-benzo[*d*]imidazol-2-yl)amino)ethyl)carbamate (23b)** General Procedure D, FCC: 30-700% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a cream colored solid in 59% yield. TLC (70% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.32. ^{1}H NMR (399 MHz, Acetone-d_6) δ ppm 7.16 (dd, *J*=5.5, 3.3 Hz, 2 H), 6.89 (dd, *J*=5.5, 3.3 Hz, 2 H), 6.44 (br. s., 1 H), 6.14 (br. s., 1 H), 3.53 (t, *J*=5.9 Hz, 2 H), 3.33 (q, *J*=5.9 Hz, 2 H), 1.38 (s, 9 H). LC/MS found 277.2 $[M+H]^+$.

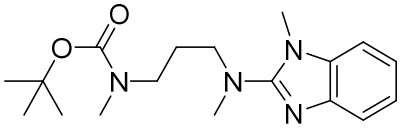


***tert*-Butyl (4-((1*H*-benzo[*d*]imidazol-2-yl)amino)butyl)carbamate (23c)** General Procedure D, FCC: 30-70% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a tan colored solid in

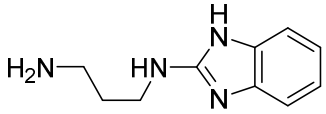
69% yield. TLC (70% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.31. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.66 (br. s., 1 H), 7.07 - 7.12 (m, 2 H), 6.79 - 6.87 (m, 3 H), 6.52 (t, *J*=5.6 Hz, 1 H), 3.24 (q, *J*=6.6 Hz, 2 H), 2.94 (q, *J*=6.5 Hz, 2 H), 1.48 - 1.57 (m, 2 H), 1.40 - 1.47 (m, 2 H), 1.37 (s, 9 H). LC/MS found 305.2 $[M+H]^+$.



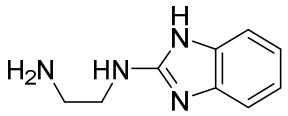
***tert*-Butyl (3-((1*H*-benzo[*d*]imidazol-2-yl)(methyl)amino)propyl)(methyl)carbamate (23d)** General Procedure D, FCC: 10-40% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as an off-white solid in 80% yield. TLC (30% acetone in CH_2Cl_2): R_f = 0.29. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.13 (br. s., 1 H), 7.14 (d, *J*=8.8 Hz, 2 H), 6.87 (br. s., 2 H), 3.44 (t, *J*=7.1 Hz, 2 H), 3.19 (t, *J*=7.3 Hz, 2 H), 3.03 (s, 3 H), 2.78 (br. s., 3 H), 1.78 (br. s., 2 H), 1.37 (br. s., 9 H). LC/MS found 319.2 $[M+H]^+$.



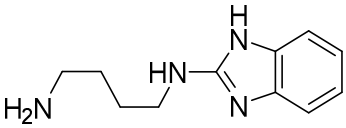
***tert*-Butyl methyl(3-(methyl(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)carbamate (23e)** Sodium hydride (15 mg, 60 wt%, 0.38 mmol) was added to a solution of *tert*-butyl (3-((1*H*-benzo[*d*]imidazol-2-yl)(methyl)amino)propyl)(methyl)carbamate in dry DMF (3.0 mL). The mixture was stirred under nitrogen for 20 minutes before a solution of iodomethane in dry DMF (0.8M, 0.46 mL, 0.37 mmol) was added. After 1 hour the mixture was diluted with ethyl acetate (80 mL), washed with water (3 x 60 mL), washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 50-100% ethyl acetate in hexanes to give **23e** as a pale yellow oil in 87% yield. TLC (ethyl acetate): R_f = 0.43. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.55 - 7.61 (m, 1 H), 7.13 - 7.21 (m, 3 H), 3.62 (s, 3 H), 3.23 - 3.34 (m, 4 H), 2.98 (s, 3 H), 2.83 (br. s., 3 H), 1.88 (quin, *J*=7.3 Hz, 2 H), 1.44 (s, 9 H). LC/MS found 333.1 $[M+H]^+$.



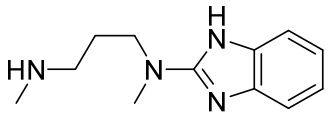
***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-yl)propane-1,3-diamine (24a)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 99% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.17 (br. s., 2 H), 9.30 (t, *J*=5.6 Hz, 1 H), 7.91 (br. s., 3 H), 7.37 - 7.44 (m, 2 H), 7.20 - 7.26 (m, 2 H), 3.48 (q, *J*=6.3 Hz, 2 H), 2.86 - 2.97 (m, 2 H), 1.91 (quin, *J*=7.4 Hz, 2 H). LC/MS found 191.1 $[M+H]^+$.



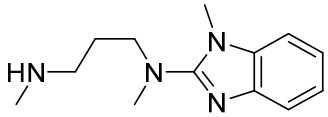
***N*^{1}-(1*H*-benzo[*d*]imidazol-2-yl)ethane-1,2-diamine (24b)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 94% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.21 (br. s., 2 H), 9.28 (t, *J*=5.4 Hz, 1 H), 8.06 (br. s., 3 H), 7.39 - 7.44 (m, 2 H), 7.22 - 7.27 (m, 2 H), 3.66 (q, *J*=5.7 Hz, 2 H), 3.13 (br. s., 2 H). LC/MS found 177.1 $[M+H]^+$.



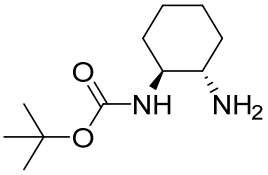
***N*^{1}-(1*H*-benzo[*d*]imidazol-2-yl)butane-1,4-diamine (24c)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 99% yield. ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 12.90 (br. s., 2 H), 9.17 (t, *J*=5.6 Hz, 1 H), 7.77 (br. s., 3 H), 7.35 - 7.42 (m, 2 H), 7.20 - 7.27 (m, 2 H), 3.40 (q, *J*=6.2 Hz, 2 H), 2.84 (q, *J*=6.2 Hz, 2 H), 1.56 - 1.70 (m, 4 H). LC/MS found 205.0 $[M+H]^+$.



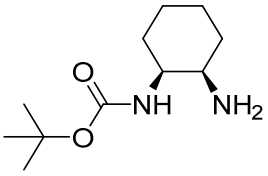
***N*^{1}-(1*H*-benzo[*d*]imidazol-2-yl)-*N*^{1},*N*^{3}-dimethylpropane-1,3-diamine (24d)** General Procedure F: Collected as the 2·TFA salt as an yellow solid in 100% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.38 - 7.43 (m, 2 H), 7.26 - 7.32 (m, 2 H), 3.70 (t, *J*=7.6 Hz, 2 H), 3.30 (s, 3 H), 3.10 - 3.17 (m, 2 H), 2.74 (s, 3 H), 2.14 (dt, *J*=15.6, 7.6 Hz, 2 H). LC/MS found 219.2 $[M+H]^+$.



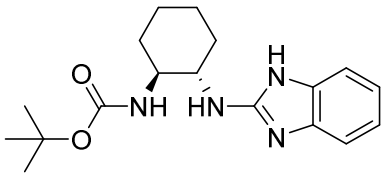
***N*^{1},*N*^{3}-Dimethyl-*N*^{1}-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)propane-1,3-diamine (24e)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 100% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.52 - 7.60 (m, 1 H), 7.46 - 7.52 (m, 1 H), 7.36 - 7.45 (m, 2 H), 3.88 (s, 3 H), 3.69 (t, *J*=7.6 Hz, 2 H), 3.35 (s, 3 H), 3.13 (t, *J*=7.6 Hz, 2 H), 2.74 (s, 3 H), 2.19 (quin, *J*=7.6 Hz, 2 H). LC/MS found 233.0 $[M+H]^+$.



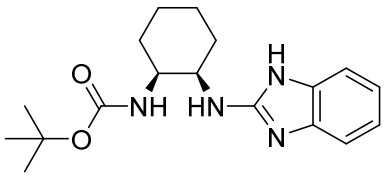
***trans-tert*-Butyl (2-aminocyclohexyl)carbamate (26a)** A solution of Boc_2O (505 mg, 2.3 mmol) in CH_2Cl_2 (6.5 mL) was added dropwise to a solution of *trans*-1,2-diaminocyclohexane (794 mg, 7.0 mmol) in CH_2Cl_2 (6.5 mL) cooled to 0 °C over 30 minutes. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was poured over water (50 mL), the aqueous was acidified to pH 5 with aq. 1M HCl, and the di-boc side product was extracted with CH_2Cl_2 (3 x 30 mL). The aqueous was then basified to pH 11 with aq. 1M aq. NaOH and extracted with ethyl acetate (3 x 40 mL). The combined ethyl acetate layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated to give **26a** as a white solid in 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.45 (br. s., 1 H), 3.13 (d, *J*=7.8 Hz, 1 H), 2.32 (td, *J*=10.3, 3.9 Hz, 1 H), 1.91 - 2.04 (m, 2 H), 1.71 (dt, *J*=11.8, 2.4 Hz, 2 H), 1.45 (s, 9 H), 1.39 (br. s., 2 H), 1.04 - 1.35 (m, 4 H). LC/MS found 215.1 $[M+H]^+$.



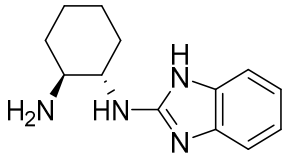
***cis-tert*-Butyl (2-aminocyclohexyl)carbamate (26b)** A solution of Boc_2O (505 mg, 2.3 mmol) in CH_2Cl_2 (6.5 mL) was added dropwise to a solution of *trans*-1,2-diaminocyclohexane (794 mg, 7.0 mmol) in CH_2Cl_2 (6.5 mL) cooled to 0 °C over 30 minutes. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was poured over water (50 mL), the aqueous was acidified to pH 5 with aq. 1M HCl, and the di-boc side product was extracted with CH_2Cl_2 (3 x 30 mL). The aqueous was then basified to pH 11 with aq. 1M aq. NaOH and extracted with ethyl acetate (3 x 40 mL). The combined ethyl acetate layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated to give **26b** as an off-white solid in quantitative conversion. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.99 (br. s., 1 H), 3.60 (br. s., 1 H), 2.96 - 3.06 (m, 1 H), 2.80 - 2.89 (m, 1 H), 1.29 - 1.65 (m, 16 H), 1.22 (br. s., 2 H). LC/MS found 215.1 $[M+H]^+$.



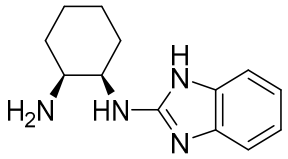
***trans-tert*-Butyl (2-((1*H*-benzo[*d*]imidazol-2-yl)amino)cyclohexyl)carbamate (27a)** General Procedure D, FCC: 10-40% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH, then 1% CH_3OH in ethyl acetate. Collected as a light brown solid in 14% yield. TLC (5% CH_3OH in ethyl acetate): R_f = 0.57. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.26 (br. s., 2 H), 6.97 - 7.05 (m, 2 H), 5.92 (br. s., 1 H), 5.03 - 5.21 (m, 1 H), 3.74 (br. s., 1 H), 3.44 (br. s., 1 H), 2.12 - 2.25 (m, 1 H), 1.91 - 2.03 (m, 1 H), 1.67 (br. s., 2 H), 1.19 - 1.41 (m, 14 H). LC/MS found 331.2 $[M+H]^+$.



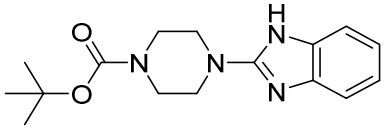
***cis-tert*-Butyl (2-((1*H*-benzo[*d*]imidazol-2-yl)amino)cyclohexyl)carbamate (27b)** General Procedure D, FCC: 1% CH_3OH in CH_2Cl_2. Collected as a pale yellow solid in 14% yield. TLC (10% CH_3OH in ethyl acetate): R_f = 0.53 ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.24 (dd, *J*=5.6, 3.2 Hz, 2 H), 7.06 (dd, *J*=5.9, 3.4 Hz, 2 H), 6.11 (br. s., 1 H), 4.97 (br. s., 1 H), 4.54 (br. s., 1 H), 4.22 (br. s., 1 H), 3.68 - 3.79 (m, 1 H), 1.23 - 1.84 (m, 17 H). LC/MS found 331.2 $[M+H]^+$.



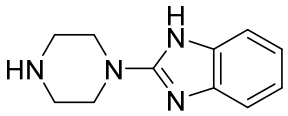
***trans*-N^1-(1*H*-benzo[*d*]imidazol-2-yl)cyclohexane-1,2-diamine (28a)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 91% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.38 - 7.43 (m, 2 H), 7.27 - 7.32 (m, 2 H), 3.79 (td, *J*=10.6, 4.2 Hz, 1 H), 3.27 (td, *J*=11.0, 3.7 Hz, 1 H), 2.21 (dt, *J*=12.6, 3.5 Hz, 2 H), 1.83 - 1.93 (m, 2 H), 1.40 - 1.64 (m, 4 H). LC/MS found 231.1 $[M+H]^+$.



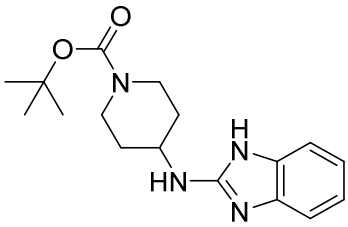
***cis*-N^1-(1*H*-benzo[*d*]imidazol-2-yl)cyclohexane-1,2-diamine (28b)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 93% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.40 - 7.46 (m, 2 H), 7.27 - 7.32 (m, 2 H), 4.21 (dt, *J*=6.6, 3.5 Hz, 1 H), 3.68 (td, *J*=6.5, 3.7 Hz, 1 H), 1.84 - 2.02 (m, 4 H), 1.67 - 1.83 (m, 2 H), 1.50 - 1.66 (m, 2 H). LC/MS found 231.1 $[M+H]^+$.



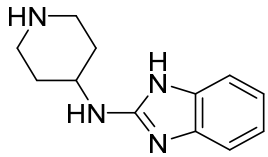
***tert*-Butyl 4-(1*H*-benzo[*d*]imidazol-2-yl)piperazine-1-carboxylate (30)** General Procedure D, FCC: 5-30% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as an off-white solid in 80% yield. TLC (20% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.33. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.35 (dd, *J*=6.1, 3.2 Hz, 2 H), 7.09 (dd, *J*=5.9, 2.9 Hz, 2 H), 3.58 (s, 8 H), 1.49 (s, 9 H). LC/MS found 303.2 $[M+H]^+$.



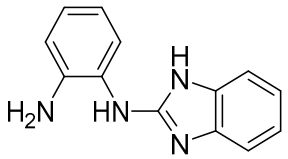
2-(piperazin-1-yl)-1*H*-benzo[*d*]imidazole (31) General Procedure F: Collected as the 2·TFA salt as an off-white solid in 99% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.49 (br. s., 1 H), 9.36 (br. s., 2 H), 7.42 - 7.49 (m, 2 H), 7.24 - 7.31 (m, 2 H), 3.82 - 3.95 (m, 4 H), 3.31 - 3.42 (m, 4 H). LC/MS found 203.0 $[M+H]^+$.



***tert*-Butyl 4-((1*H*-benzo[*d*]imidazol-2-yl)amino)piperidine-1-carboxylate (33)** General Procedure D, FCC: 10-40% acetone in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a light brown solid in 18% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.45. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.11 - 7.17 (m, 2 H), 6.86 - 6.91 (m, 2 H), 6.80 (d, *J*=5.4 Hz, 1 H), 3.91 (d, *J*=11.7 Hz, 2 H), 3.75 (qt, *J*=7.2, 3.5 Hz, 1 H), 2.88 (br. s., 2 H), 1.93 (dd, *J*=12.5, 3.2 Hz, 2 H), 1.41 (s, 9 H), 1.35 (qd, *J*=11.0, 3.2 Hz, 2 H). LC/MS found 317.2 $[M+H]^+$.

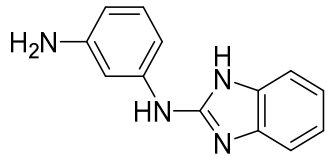


***N*-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2-amine (34)** General Procedure F: Collected as the 2·TFA salt as an off-white solid in 94% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.36 - 7.42 (m, 2 H), 7.25 - 7.30 (m, 2 H), 3.90 - 4.00 (m, 1 H), 3.54 (dt, *J*=13.2, 3.4 Hz, 2 H), 3.18 (td, *J*=12.2, 2.9 Hz, 2 H), 2.32 (dd, *J*=14.2, 2.9 Hz, 2 H), 1.88 - 1.99 (m, 2 H). LC/MS found 217.0 $[M+H]^+$.

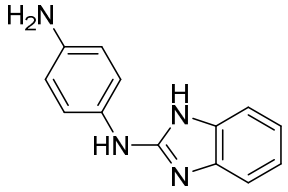


***N*^{1}-(1*H*-benzo[*d*]imidazol-2-yl)benzene-1,2-diamine (36a)** General Procedure E, FCC: 1-10% CH_3OH in CH_2Cl_2. Collected as a dark brown solid in 70% yield with 95% purity (5% di-addition side product). TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.45. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.56 (dd, *J*=7.8, 1.5 Hz, 1 H), 7.20 - 7.25 (m, 2 H), 6.93 - 6.96 (m, 2 H), 6.87 (td,

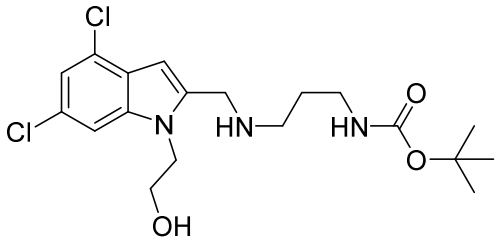
J=7.8, 1.5 Hz, 1 H), 6.77 (dd, *J*=7.8, 1.5 Hz, 1 H), 6.63 (td, *J*=7.8, 1.5 Hz, 1 H). LC/MS found 225.1 $[M+H]^+$.



***N*^{1}-(1*H*-benzo[*d*]imidazol-2-yl)benzene-1,3-diamine (36b)** General Procedure E, FCC: 1-10% CH_3OH in CH_2Cl_2. Collected as a dark brown solid in 86% yield with 95% purity (5% di-addition side product). TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.36. ^{1}H NMR (500 MHz, DMSO-d_6) d ppm 10.74 (br. s., 1 H), 9.07 (br. s., 1 H), 7.23 - 7.30 (m, 2 H), 6.89 - 7.01 (m, 4 H), 6.78 (dd, *J*=8.1, 1.2 Hz, 1 H), 6.16 (dd, *J*=8.1, 1.2 Hz, 1 H), 5.06 (br. s., 2 H). LC/MS found 225.1 $[M+H]^+$.

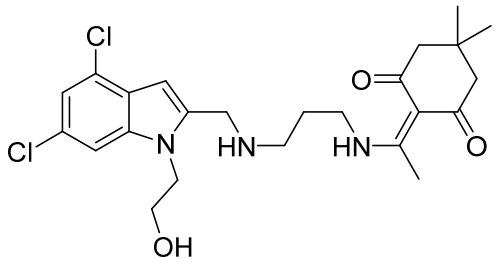


***N*^{1}-(1*H*-benzo[*d*]imidazol-2-yl)benzene-1,4-diamine (36c)** General Procedure E, FCC: 5-15% CH_3OH in CH_2Cl_2. Collected as a deep, dark red solid in 67% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.21. ^{1}H NMR (500 MHz, DMSO-d_6) d ppm 8.90 (br. s., 1 H), 7.30 (d, *J*=8.3 Hz, 2 H), 7.18 - 7.23 (m, 2 H), 6.90 - 6.95 (m, 2 H), 6.57 (d, *J*=8.3 Hz, 2 H), 3.17 (s, 1 H). LC/MS found 225.0 $[M+H]^+$.

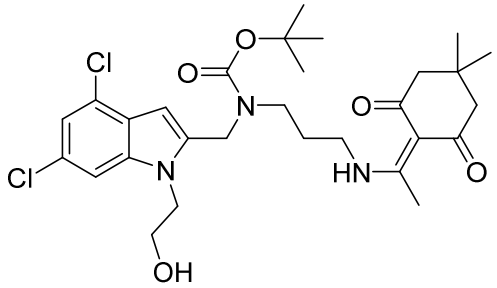


***tert*-Butyl (3-(((4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)amino)propyl)carbamate (40)** To a solution of **22a** (37 mg, 0.2 mmol) and acetic acid (600 µL, 10 mmol) in CH_3OH (2 mL) was added **7a** (50 mg, 0.19 mmol). The mixture was stirred for 30 minutes at room temperature before the addition of a solution of $NaBH_3CN$ (17 mg, 0.27 mmol) in CH_3OH (1 mL). The resulting mixture was stirred at room temperature for 12 hours, concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (20 mL) and sat. aq. $NaHCO_3$ (20 mL). The layers were separated and the aqueous was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography using a gradient of 0-10% CH_3OH in CH_2Cl_2 to give

40 in 67% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.15 (s, 1 H), 7.10 (s, 1 H), 6.49 (s, 1 H), 4.84 (t, *J*=5.9 Hz, 1 H), 4.26 (t, *J*=4.4 Hz, 2 H), 3.88 - 3.93 (m, 2 H), 3.87 (s, 2 H), 3.15 (q, *J*=5.9 Hz, 2 H), 2.70 (t, *J*=6.6 Hz, 2 H), 1.65 (quin, *J*=6.6 Hz, 2 H), 1.41 (s, 9 H). LC/MS found 416.0 $[M+H]^+$.

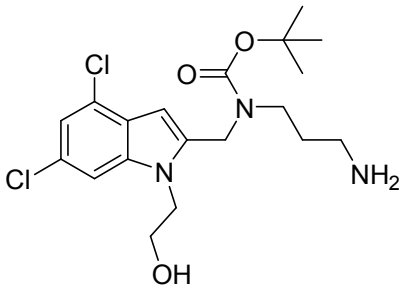


2-(1-((3-(((4,6-Dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)amino)propyl)amino)ethylidene)-5,5-dimethylcyclohexane-1,3-dione (41) To a solution of **40** (54 mg, 0.13 mmol) in THF (2 mL) was added aq. HCl (3M, 0.5 mL, 1.5 mmol). The mixture was stirred at room temperature for 1 hour, then heated to 80 °C for 1 hour. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude was suspended in CH_2Cl_2 (5 mL) and CH_3OH (0.2 mL) and *i*-Pr_2NEt (90 μL, 0.5 mmol) followed by DDE-OH (23 mg, 0.13 mmol) were added. The mixture was stirred at room temperature for 12 hours, then concentrated *in vacuo*, and the residue was purified by flash column chromatography using a gradient of 0-10% CH_3OH in CH_2Cl_2 to give **41** in 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.17 (s, 1 H), 7.10 (s, 1 H), 6.49 (s, 1 H), 4.28 (t, *J*=4.6 Hz, 2 H), 3.92 (s, 2 H), 3.90 (t, *J*=4.9 Hz, 2 H), 3.44 (q, *J*=6.2 Hz, 2 H), 2.82 (t, *J*=7.1 Hz, 2 H), 2.51 (s, 3 H), 2.31 (s, 4 H), 1.86 (quin, *J*=6.8 Hz, 2 H), 1.00 (s, 6 H). LC/MS found 480.0 $[M+H]^+$.

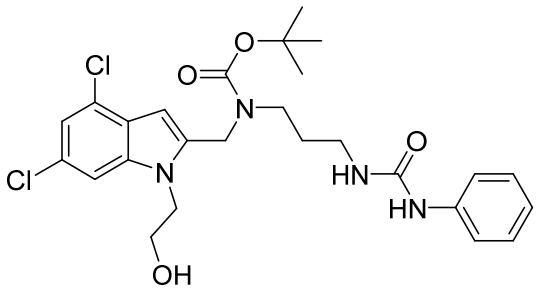


***tert*-Butyl ((4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)(3-((1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl)amino)propyl)carbamate (42)** To a stirred solution of **41** (60 mg, 0.12 mmol) in 9:1 CH_3CN/H_2O (5 mL) was added K_2CO_3 (17 mg, 0.12 mmol) followed by Boc_2O (27 mg, 0.12 mmol). The mixture was stirred at room temperature for 12 hours, then poured into ethyl acetate (40 mL) and H_2O (26 mL). The layers were separated and the organic layer was washed with brine (25 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was purified by flash column chromatography using a gradient of 0-10% CH_3OH in CH_2Cl_2 to give **42** in 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.23 (s, 1 H), 7.07 (s, 1 H), 6.41 (br s, 1 H), 4.69 (s, 2 H), 4.20 (t, *J*=4.9 Hz, 2 H), 3.85 (br s, 2 H), 3.27 - 3.38 (m, 4 H), 2.43

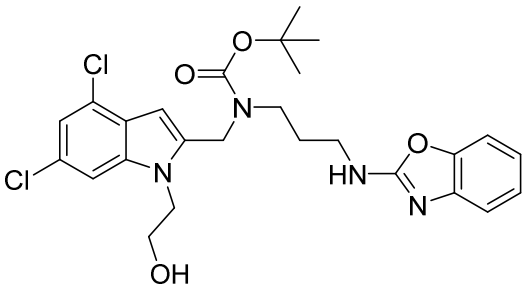
(s, 3 H), 2.26 (s, 4 H), 1.83 - 1.91 (m, 2 H), 1.47 (s, 9 H), 0.97 (s, 6 H). LC/MS found 580.0 $[M+H]^+$.



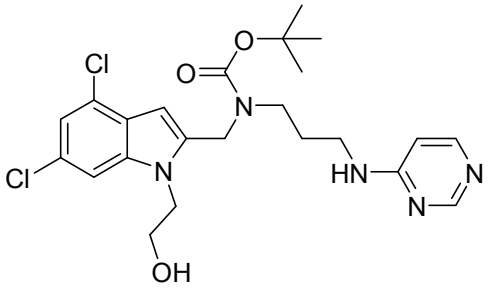
***tert*-Butyl (3-aminopropyl)((4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)carbamate (43)** To a solution of **42** (15 mg, 0.03 mmol) in CH_3CN (1 mL) was added hydrazine monohydrate (63 µL, 0.13 mmol) and the mixture was heated to 45 °C for 3 hours. The mixture was cooled to room temperature and concentrated *in vacuo* to give crude **43** which was used without further purification in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm LC/MS found 416.1 $[M+H]^+$.



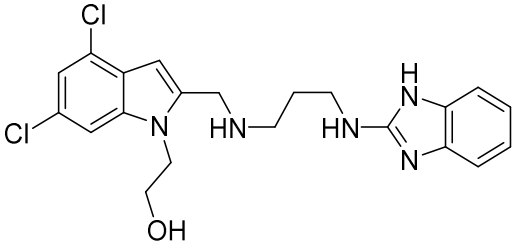
***tert*-Butyl ((4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)(3-(3-phenylureido)propyl)carbamate (44a)** To a solution of **43** (16 mg, 0.04 mmol) in CH_3CN (1 mL) was added phenyl isocyanate (4.5 µL, 0.04 mmol). The mixture was stirred at room temperature for 12 hours, then concentrated *in vacuo*. The residue was purified by flash column chromatography using a gradient of 0-10% CH_3OH in CH_2Cl_2 to give **44a** in 51 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.19 - 7.27 (m, 6 H), 7.09 (s, 1 H), 7.03 (t, *J*=6.8 Hz, 1 H), 6.69 (s, 1 H), 6.40 (br s, 1 H), 4.62 (s, 2 H), 4.13 - 4.20 (m, 2 H), 3.82 (br s, 2 H), 3.21 - 3.29 (m, 2 H), 3.05 - 3.15 (m, 2 H), 1.74 (br s, 1 H), 1.59 (q, *J*=6.3 Hz, 2 H), 1.45 (s, 9 H). LC/MS found 535.0 $[M+H]^+$.



***tert*-Butyl (3-(benzo[*d*]oxazol-2-ylamino)propyl)((4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)carbamate (44b)** To a stirred solution of **43** (16 mg, 0.04 mmol) and *i*-Pr_2NEt (10 µL, 0.06 mmol) in CH_3CN (1 mL) was added 2-chlorobenzoxazole (7 mg, 0.05 mmol). The mixture was heated to 80 °C for 12 hours, cooled to room temperature, poured over H_2O (15 mL), and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography using a gradient of 0-10% CH_3OH in CH_2Cl_2 to give **44b** in 58% yield. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 7.24 - 7.31 (m, 2 H), 7.21 (d, *J*=7.8 Hz, 1 H), 7.15 (t, *J*=7.6 Hz, 1 H), 7.07 (s, 1 H), 7.02 (t, *J*=7.8 Hz, 1 H), 6.45 (s, 1 H), 4.69 (s, 2 H), 4.18-4.30 (m, 2 H), 3.91 (t, *J*=5.1 Hz, 2 H), 3.27 - 3.47 (m, 4 H), 1.70 - 1.92 (m, 2 H), 1.51 (s, 9 H). LC/MS found 533.0 $[M+H]^+$.

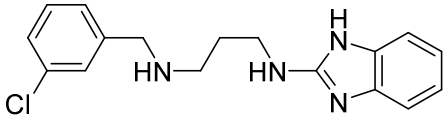


***tert*-Butyl ((4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)(3-(pyrimidin-4-ylamino)propyl)carbamate (44c)** To a stirred solution of **43** (11 mg, 0.03 mmol) and *i*-Pr_2NEt (10 µL, 0.06 mmol) in CH_3CN (1 mL) was added 4-chloropyrimidine (5 mg, 0.03 mmol). The mixture was stirred at room temperature for 12 hours, then heated under microwave irradiation at 120 °C for 30 minutes, then to 150 °C for 2 hours. The mixture was cooled to room temperature, poured over H_2O (15 mL), and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography using a gradient of 0-10% CH_3OH in CH_2Cl_2 to give **44c** in 45% yield. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 8.46 (s, 1 H), 8.07 (s, 1 H), 7.25 (s, 1 H), 7.11 (s, 1 H), 6.43 (s, 1 H), 6.22 (br s, 1 H), 4.69 (s, 2 H), 4.24 (t, *J*=4.4 Hz, 2 H), 3.90 (t, *J*=4.9 Hz, 2 H), 3.30 - 3.39 (m, 2 H), 3.25 (br s, 2 H), 1.69 - 1.79 (m, 2 H), 1.50 (s, 9 H). LC/MS found 494.0 $[M+H]^+$.

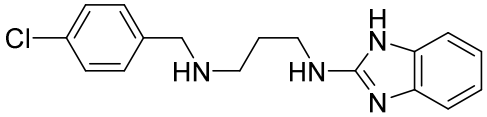


2-(2-(((3-((1*H*-Benzo[*d*]imidazol-2-yl)amino)propyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (1, NEU-1053) General procedure F, FCC: 0-20% CH_3OH in ethyl acetate. Collected as a white solid in 69% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.33. ^1H NMR (500 MHz, CD_3OD) δ ppm 7.43 (s, 1 H), 7.13 - 7.18 (m, 2 H), 7.08 (d, *J*=2.0 Hz, 1 H), 6.94 - 6.98 (m,

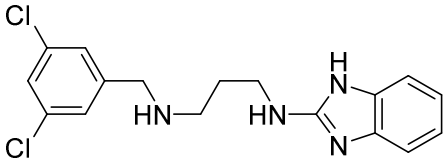
2 H), 6.57 (s, 1 H), 4.34 (t, *J*=5.1 Hz, 2 H), 4.06 (s, 2 H), 3.86 (t, *J*=5.1 Hz, 2 H), 3.44 (t, *J*=6.6 Hz, 2 H), 2.86 (t, *J*=7.1 Hz, 2 H), 1.89 (quin, *J*=7.0 Hz, 2 H). LC/MS found 431.9 $[M+H]^+$.



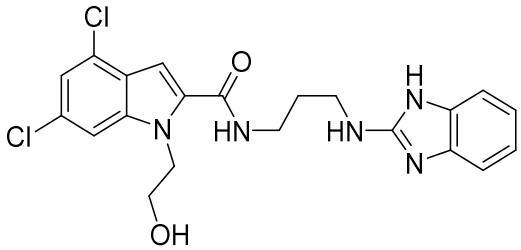
N^1-(1*H*-benzo[*d*]imidazol-2-yl)-N^3-(3-chlorobenzyl)propane-1,3-diamine (38a, NEU-2185) General procedure G, FCC: 0-10% CH_3OH with 10% NH_4OH in CH_2Cl_2. Collected in 82% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.40 (s, 1 H), 7.30-7.24 (m, 1 H), 7.23-7.16 (m, 1 H), 7.13-6.78 (m, 1 H), 3.89-3.39 (m, 1 H), 2.71 (t, *J*=7.08 Hz, 1 H), 1.88 (quin, *J*=7.0 Hz, 1 H). LC/MS found 315.1 $[M+H]^+$.



N^1-(1*H*-benzo[*d*]imidazol-2-yl)-N^3-(4-chlorobenzyl)propane-1,3-diamine (38b, NEU-2186) General Procedure H, FCC: 0-10% CH_3OH with 10% NH_4OH in CH_2Cl_2. Collected in 76% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.33-7.26 (m, 4H), 7.21-7.14 (m, 2 H), 7.04-6.90 (m, 2H), 3.75 (s, 2H), 3.44 (t, *J*=6.8 Hz, 2H), 2.70 (t, *J*=7.1 Hz, 2H), 1.88 (quin, *J*=7.0 Hz, 2H). LC/MS found 315.1 $[M+H]^+$.

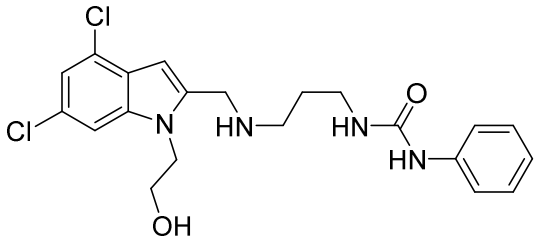


N^1-(1*H*-benzo[*d*]imidazol-2-yl)-N^3-(3,5-dichlorobenzyl)propane-1,3-diamine (38c, NEU-2172) General Procedure H, FCC: 0-10% CH_3OH with 10% NH_4OH in CH_2Cl_2. Collected in 81% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.37-7.31 (m, 3H), 7.21, 7.14 (m, 2H), 7.00-6.94 (m, 2H), 3.75 (s, 2H), 3.44 (t, *J*=6.6 Hz, 2 H), 2.71 (t, *J*=7.1 Hz, 2H), 1.88 (quin, *J*=7.0 Hz, 2H). LC/MS found 349.0 $[M+H]^+$.

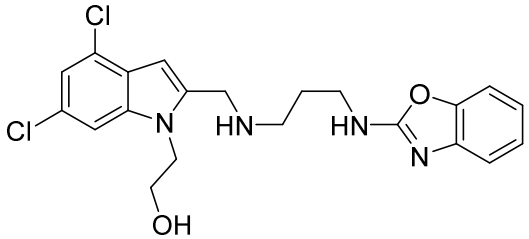


***N*-(3-((1*H*-Benzo[*d*]imidazol-2-yl)amino)propyl)-4,6-dichloro-1-(2-hydroxyethyl)-1*H*-indole-2-carboxamide (39, NEU-2128)** A 0.5-2.0 mL flame dried microwave vial equipped

with a stir bar was charged with 7,9-dichloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]indol-1-one (89 mg, 0.35 mmol) and *N*1-(1*H*-benzo[*d*]imidazol-2-yl)propane-1,3-diamine (136 mg, 0.72 mmol). The vial was sealed with a septum and dry DMF (1.8 mL) was added via syringe. The mixture was irradiated to 150 °C were the temperature was modulated for 1 hour. After cooling to room temperature the mixture was poured over water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with water (3 x 20 mL), washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 5-15% CH_3OH in CH_2Cl_2 to give **39** as a white solid in 96% yield. TLC (10% CH_3OH in CH_2Cl_2): $R_f = 0.31$. ^1H NMR (500 MHz, CD_3OD) δ ppm 7.57 (s, 1 H), 7.18 - 7.23 (m, 2 H), 7.16 (d, *J*=1.5 Hz, 1 H), 7.15 (s, 1 H), 6.96 - 7.01 (m, 2 H), 4.61 (t, *J*=5.4 Hz, 2 H), 3.86 (t, *J*=5.4 Hz, 2 H), 3.50 (dt, *J*=11.0, 6.5 Hz, 4 H), 1.96 (quin, *J*=6.7 Hz, 2 H). LC/MS found 446.0 $[M+H]^+$.

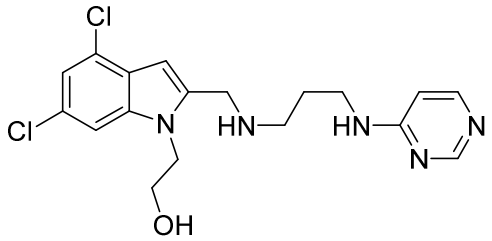


1-(3-(((4,6-Dichloro-1-(2-hydroxyethyl)-1*H*-indol-2-yl)methyl)amino)propyl)-3-phenylurea (45a, NEU-2176) To **44a** (11 mg, 0.02 mmol) was added HCl in 1,4-dioxane (4M, 0.2 mL, 0.8 mmol) and the mixture was stirred at room temperature for 6 hours. The mixture was concentrated *in vacuo* and the residue was taken up in sat. aq. $NaHCO_3$ (15 mL) and CH_2Cl_2 (15 mL). The layers were separated and the aqueous was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC using 10% CH_3OH (10% NH_4OH) in CH_2Cl_2 to give **45a** in 47% yield. ^1H NMR (500 MHz, CD_3OD) δ ppm 7.45 (s, 1H), 7.36-7.30 (m, 2H), 7.28-7.21 (m, 2H), 7.10 (d, *J*=1.5 Hz, 1H), 6.99 (t, *J*=7.3 Hz, 1H), 6.59 (s, 1H), 4.38 (t, *J*=5.1 Hz, 2H), 4.09-4.03 (m, 2H), 3.88 (t, *J*=5.1 Hz, 2H), 3.29 (t, *J*=6.6 Hz, 2H), 2.81 (t, *J*=7.1 Hz, 2H), 1.79 (quin, *J*=7.0 Hz, 2H). LC/MS found 435.0 $[M+H]^+$.

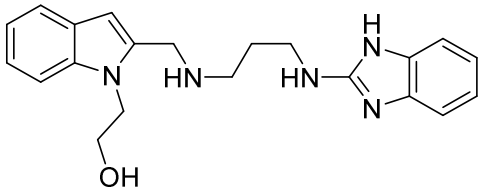


2-(2-(((3-(Benzo[*d*]oxazol-2-ylamino)propyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (45b, NEU-2175) To **44b** (11 mg, 0.02 mmol) was added HCl in 1,4-dioxane (4M, 0.2 mL, 0.8 mmol) and the mixture was stirred at room temperature for 6 hours. The mixture was

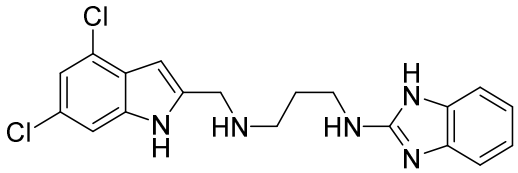
concentrated *in vacuo* and the residue was taken up in sat. aq. $NaHCO_3$ (15 mL) and CH_2Cl_2 (15 mL). The layers were separated and the aqueous was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC using 10% CH_3OH (10% NH_4OH) in CH_2Cl_2 to give **45b** in 36% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.42 (s, 1H), 7.26 (t, *J*=6.8 Hz, 2H), 7.16 (t, *J*=7.6 Hz, 1H), 7.10-7.01 (m, 2H), 6.53 (s, 1H), 4.36 (t, *J*=5.1 Hz, 2H), 4.02-3.96 (m, 2H), 3.88 (t, *J*=5.1 Hz, 2H), 3.47 (t, *J*=6.84 Hz, 2H), 2.80 (t, *J*=7.3 Hz, 2H), 1.96-1.84 (m, 2H). LC/MS found 433.1 $[M+H]^+$.



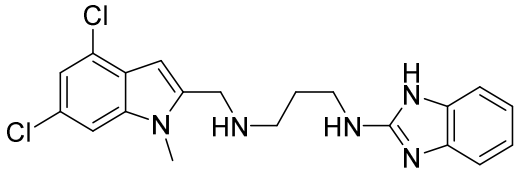
2-(4,6-Dichloro-2-(((3-(pyrimidin-4-ylamino)propyl)amino)methyl)-1*H*-indol-1-yl)ethan-1-ol (45c, NEU-2173) To **44c** (11 mg, 0.02 mmol) was added HCl in 1,4-dioxane (4M, 0.2 mL, 0.8 mmol) and the mixture was stirred at room temperature for 6 hours. The mixture was concentrated *in vacuo* and the residue was taken up in sat. aq. $NaHCO_3$ (15 mL) and CH_2Cl_2 (15 mL). The layers were separated and the aqueous was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC using 10% CH_3OH (10% NH_4OH) in CH_2Cl_2 to give **45c** in 56% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.38 (br. s., 1H), 7.99 (br. s., 1H), 7.49 (s, 1H), 7.13 (d, *J*=1.5 Hz, 1H), 6.62 (s, 1H), 6.51 (d, *J*=5.9 Hz, 1H), 4.41 (t, *J*=4.9 Hz, 2H), 4.16 (br. s., 2H), 3.90 (t, *J*=5.1 Hz, 2H), 3.54-3.42 (m, 2H), 2.90 (t, *J*=7.1 Hz, 2H), 1.89 (quin, *J*=7.0 Hz, 2H). LC/MS found 394.0 $[M+H]^+$.



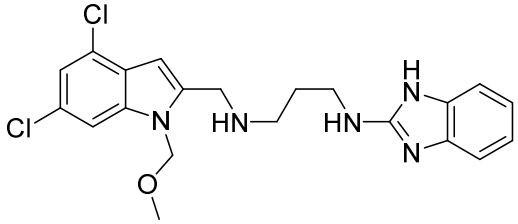
2-(2-(((3-((1*H*-Benzo[*d*]imidazol-2-yl)amino)propyl)amino)methyl)-1*H*-indol-1-yl)ethan-1-ol (46, NEU-2057) General procedure F, FCC: 5-15% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a light yellow solid in 57% yield. TLC (10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.26. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.57 (d, *J*=7.8 Hz, 1 H), 7.50 (d, *J*=8.3 Hz, 1 H), 7.19 (t, *J*=7.3 Hz, 1 H), 7.10 - 7.15 (m, 2 H), 7.07 (t, *J*=7.8 Hz, 1 H), 6.89 - 6.96 (m, 2 H), 6.69 (s, 1 H), 4.43 (s, 2 H), 4.38 (t, *J*=4.9 Hz, 2 H), 3.71 (t, *J*=4.9 Hz, 2 H), 3.42 (t, *J*=6.3 Hz, 2 H), 3.05 (t, *J*=7.1 Hz, 2 H), 1.95 (quin, *J*=6.8 Hz, 2 H). LC/MS found 364.2 $[M+H]^+$.



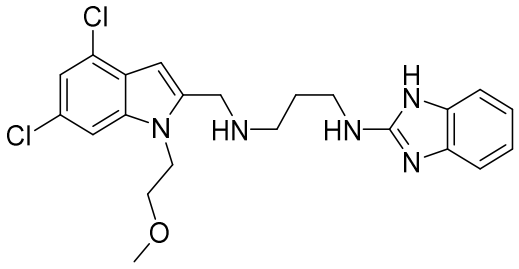
***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*^{3}-((4,6-dichloro-1*H*-indol-2-yl)methyl)propane-1,3-diamine (47a, NEU-2054)** General procedure F, FCC: 5-15% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as an off-white solid in 70% yield. TLC (10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.21 ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.69 (br. s., 1 H), 7.41 (s, 1 H), 7.14 (d, *J*=1.5 Hz, 1 H), 7.04 - 7.10 (m, 2 H), 6.81 - 6.88 (m, 2 H), 6.50 (s, 1 H), 4.04 (s, 2 H), 3.38 (t, *J*=6.6 Hz, 2 H), 2.76 (t, *J*=6.8 Hz, 2 H), 1.83 (quin, *J*=6.7 Hz, 2 H). LC/MS found 388.1 $[M+H]^+$.



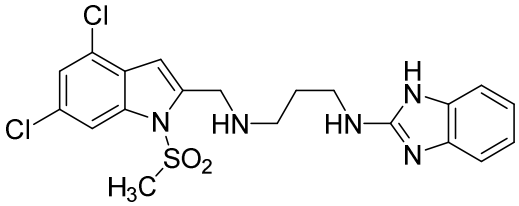
***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*^{3}-((4,6-dichloro-1-methyl-1*H*-indol-2-yl)methyl)propane-1,3-diamine (47b, NEU-2055)** General procedure F, FCC: 5-10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as an opaque solid in 90% yield. TLC (10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.42. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.60 (s, 1 H), 7.15 (d, *J*=2.0 Hz, 1 H), 7.04 - 7.11 (m, 2 H), 6.81 - 6.87 (m, 2 H), 6.66 (br. s., 1 H), 6.48 (s, 1 H), 3.94 (s, 2 H), 3.76 (s, 3 H), 3.36 (t, *J*=6.8 Hz, 2 H), 2.70 (t, *J*=6.8 Hz, 2 H), 1.78 (quin, *J*=6.7 Hz, 2 H). LC/MS found 402.1 $[M+H]^+$.



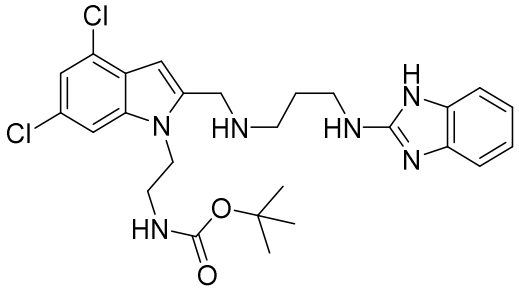
***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*^{3}-((4,6-dichloro-1-(methoxymethyl)-1*H*-indol-2-yl)methyl)propane-1,3-diamine (47c, NEU-2056)** General procedure F, FCC: 5-10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as an opaque solid in 66% yield. TLC (10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.42. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.78 (s, 1 H), 7.24 (d, *J*=2.0 Hz, 1 H), 7.03 - 7.10 (m, 2 H), 6.81 - 6.88 (m, 2 H), 6.63 (s, 1 H), 5.62 (s, 2 H), 4.05 (s, 2 H), 3.37 (t, *J*=6.8 Hz, 2 H), 3.15 (s, 3 H), 2.75 (t, *J*=6.8 Hz, 2 H), 1.80 (quin, *J*=6.7 Hz, 2 H). LC/MS found 432.2 $[M+H]^+$.



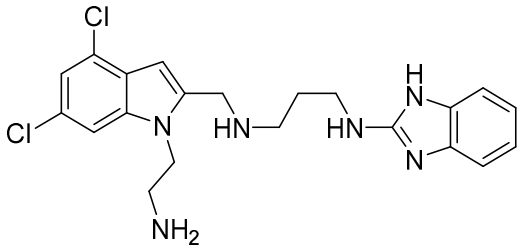
***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*^{3}-((4,6-dichloro-1-(2-methoxyethyl)-1*H*-indol-2-yl)methyl)propane-1,3-diamine (47d, NEU-2063)** General procedure F, FCC: 5-10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a cream colored solid in 75% yield. TLC (10% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH): R_f = 0.38. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.61 (s, 1 H), 7.16 (d, *J*=1.5 Hz, 1 H), 7.05 - 7.10 (m, 2 H), 6.81 - 6.87 (m, 2 H), 6.49 (s, 1 H), 4.43 (t, *J*=5.1 Hz, 2 H), 3.96 (s, 2 H), 3.59 (t, *J*=5.4 Hz, 2 H), 3.36 (t, *J*=6.8 Hz, 2 H), 3.15 (s, 3 H), 2.70 (t, *J*=6.8 Hz, 2 H), 1.77 (quin, *J*=6.7 Hz, 2 H). LC/MS found 446.2 $[M+H]^+$.



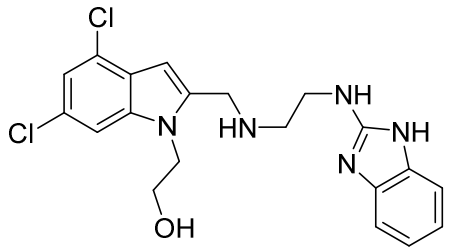
***N*^{1}-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*^{3}-((4,6-dichloro-1-(methylsulfonyl)-1*H*-indol-2-yl)methyl)propane-1,3-diamine (47e, NEU-2109)** General procedure F, FCC: 5-20% CH_3OH in ethyl acetate. Collected as an off-white solid in 71% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.95 (s, 1 H), 7.31 (s, 1 H), 7.18 - 7.24 (m, 2 H), 7.02 - 7.08 (m, 2 H), 6.80 (s, 1 H), 4.12 (s, 2 H), 3.47 (t, *J*=6.8 Hz, 2 H), 3.30 (s, 3 H), 2.81 (t, *J*=6.8 Hz, 2 H), 1.91 (quin, *J*=6.8 Hz, 2 H). LC/MS found 466.0 $[M+H]^+$.



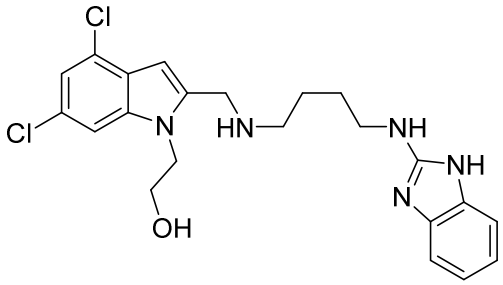
***tert*-Butyl (2-(2-(((3-((1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethyl)carbamate (47f, NEU-2110)** General procedure F, FCC: 5-20% CH_3OH in ethyl acetate. Collected as a white solid in 90% yield. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.34 (s, 1 H), 7.18 (dd, *J*=5.9, 2.9 Hz, 2 H), 7.06 (s, 1 H), 7.01 (dd, *J*=5.9, 2.9 Hz, 2 H), 6.54 (s, 1 H), 4.22 (t, *J*=6.1 Hz, 2 H), 3.97 (s, 2 H), 3.49 (t, *J*=6.6 Hz, 2 H), 3.38 (t, *J*=6.0 Hz, 2 H), 2.86 (t, *J*=6.6 Hz, 2 H), 1.93 (quin, *J*=6.7 Hz, 2 H), 1.35 (s, 9 H). LC/MS found 531.1 $[M+H]^+$.



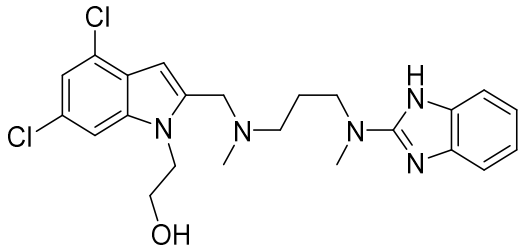
N^1-((1-(2-Aminoethyl)-4,6-dichloro-1*H*-indol-2-yl)methyl)-N^3-(1*H*-benzo[*d*]imidazol-2-yl)propane-1,3-diamine (47g, NEU-2111) Trifluoroacetic acid (0.14 mL, 1.83 mmol) was added to a suspension of *tert*-butyl (2-(2-(((3-((1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethyl)carbamate (37 mg, 0.07 mmol) in dry CH_2Cl_2 (1.4 mL). The mixture was stirred at room temperature overnight, concentrated, and redissolved in ethyl acetate (30 mL). The mixture was washed with sat. aq. $NaHCO_3$ (2 x 10 mL), washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using a gradient of 10-30% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH to give **47g** as a light yellow solid in 50% yield. TLC (15% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH.): R_f = 0.21. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.44 (s, 1 H), 7.11 - 7.18 (m, 2 H), 7.05 (d, *J*=1.5 Hz, 1 H), 6.91 - 6.97 (m, 2 H), 6.52 (s, 1 H), 4.20 (t, *J*=7.1 Hz, 2 H), 3.94 (s, 2 H), 3.44 (t, *J*=6.8 Hz, 2 H), 2.93 (t, *J*=6.8 Hz, 2 H), 2.80 (t, *J*=7.1 Hz, 2 H), 1.88 (quin, *J*=7.0 Hz, 2 H). LC/MS found 431.0 $[M+H]^+$.



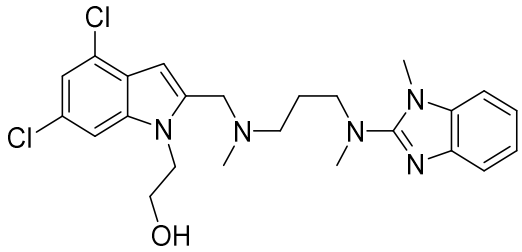
2-(2-(((2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)ethyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48a, NEU-2062) General procedure F, FCC: 5-20% CH_3OH in CH_3Cl. Collected as a yellow solid in 75% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.30. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.65 (d, *J*=1.0 Hz, 1 H), 7.17 (d, *J*=1.5 Hz, 1 H), 7.14 (dd, *J*=5.9, 2.9 Hz, 2 H), 6.89 (dd, *J*=5.9, 3.4 Hz, 2 H), 6.55 (s, 1 H), 4.35 (t, *J*=5.1 Hz, 2 H), 4.12 (s, 2 H), 3.69 (t, *J*=5.1 Hz, 2 H), 3.48 (t, *J*=5.9 Hz, 2 H), 2.93 (t, *J*=5.9 Hz, 2 H). LC/MS found 418.1 $[M+H]^+$.



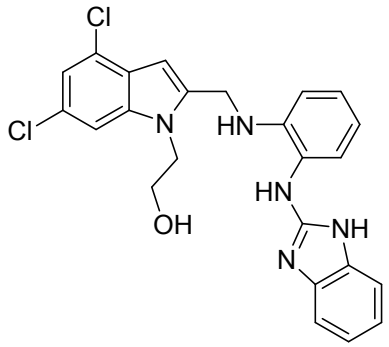
2-(2-(((4-((1*H*-Benzo[*d*]imidazol-2-yl)amino)butyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48b, NEU-2064) General procedure F, FCC: 5-20% CH_3OH in CH_2Cl_2 with 0.1% conc. aq. NH_4OH. Collected as a yellow solid in 35% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.18. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 7.68 (d, *J*=1.0 Hz, 1 H), 7.19 (d, *J*=2.0 Hz, 1 H), 7.09 - 7.13 (m, 2 H), 6.84 - 6.89 (m, 2 H), 6.62 (s, 1 H), 4.37 (t, *J*=5.1 Hz, 2 H), 4.19 (s, 2 H), 3.70 (t, *J*=5.1 Hz, 2 H), 3.27 (t, *J*=6.3 Hz, 2 H), 2.82 (t, *J*=6.3 Hz, 2 H), 1.56 - 1.67 (m, 4 H). LC/MS found 446.2 $[M+H]^+$.



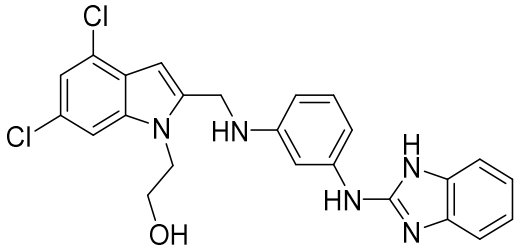
2-(2-(((3-((1*H*-Benzo[*d*]imidazol-2-yl)(methyl)amino)propyl)(methyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48c, NEU-2061) General procedure F, FCC: 1-15% CH_3OH in ethyl acetate. Collected as a light yellow solid in 36% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 11.13 (br. s., 1 H), 7.61 (s, 1 H), 7.08 - 7.17 (m, 3 H), 6.86 (br. s., 2 H), 6.43 (s, 1 H), 4.38 (t, *J*=5.4 Hz, 2 H), 3.65 - 3.74 (m, 4 H), 3.47 (t, *J*=7.3 Hz, 2 H), 3.00 (s, 3 H), 2.45 (t, *J*=7.1 Hz, 2 H), 2.15 (s, 3 H), 1.78 (quin, *J*=7.2 Hz, 2 H). LC/MS found 460.2 $[M+H]^+$.



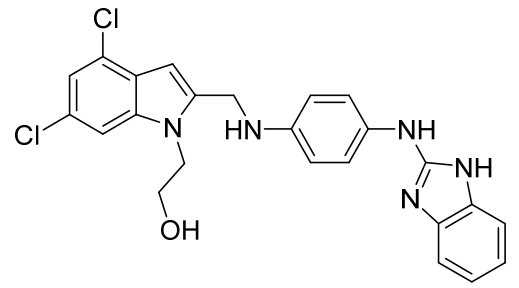
2-(4,6-Dichloro-2-((methyl(3-(methyl(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)amino)methyl)-1*H*-indol-1-yl)ethan-1-ol (48d, NEU-2104) General procedure F, FCC: 0-20% CH_3OH in ethyl acetate. Collected as a colorless semi-solid in 28% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 7.53 (s, 1 H), 7.31 - 7.36 (m, 1 H), 7.23 - 7.28 (m, 1 H), 7.14 (d, *J*=2.0 Hz, 1 H), 7.02 - 7.07 (m, 2 H), 6.40 (s, 1 H), 4.30 (t, *J*=5.6 Hz, 2 H), 3.62 - 3.68 (m, 4 H), 3.48 (s, 3 H), 3.24 (t, *J*=7.3 Hz, 2 H), 2.86 (s, 3 H), 2.43 (t, *J*=6.8 Hz, 2 H), 2.13 (s, 3 H), 1.78 (quin, *J*=7.1 Hz, 2 H). LC/MS found 474.0 $[M+H]^+$.



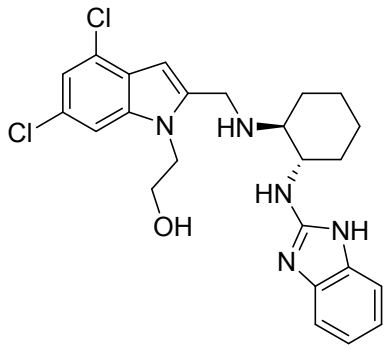
2-(2-(((2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)phenyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48e, NEU-2060) General procedure G, FCC: 0-10% CH_3OH in CH_2Cl_2. Collected as a brown solid in 39% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.54. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 10.97 (br. s., 1 H), 8.38 (br. s., 1 H), 7.62 (d, *J*=1.0 Hz, 1 H), 7.53 (dd, *J*=7.8, 1.5 Hz, 1 H), 7.19 - 7.26 (m, 2 H), 7.13 (d, *J*=1.5 Hz, 1 H), 6.90 - 6.99 (m, 3 H), 6.79 (dd, *J*=8.3, 1.5 Hz, 1 H), 6.70 (td, *J*=7.6, 1.0 Hz, 1 H), 6.53 (s, 1 H), 5.68 (br. s., 1 H), 5.00 (br. s., 1 H), 4.60 (d, *J*=3.9 Hz, 2 H), 4.31 (t, *J*=5.1 Hz, 2 H), 3.70 (br. s., 2 H). LC/MS found 466.1 $[M+H]^+$.



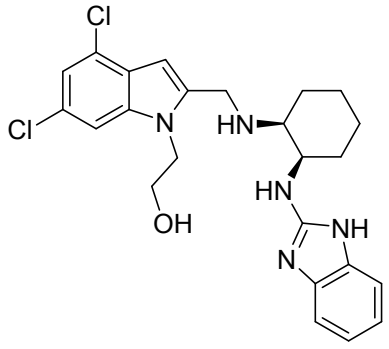
2-(2-(((3-((1*H*-Benzo[*d*]imidazol-2-yl)amino)phenyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48f, NEU-2059) General procedure G, FCC: 0-10% CH_3OH in CH_2Cl_2. Collected as a pale yellow solid in 50% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.47. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 10.75 (s, 1 H), 9.14 (s, 1 H), 7.62 (s, 1 H), 7.31 (d, *J*=6.8 Hz, 1 H), 7.26 (d, *J*=6.8 Hz, 1 H), 7.15 (t, *J*=2.0 Hz, 1 H), 7.13 (d, *J*=2.0 Hz, 1 H), 6.89 - 7.03 (m, 4 H), 6.49 (s, 1 H), 6.22 - 6.31 (m, 2 H), 5.03 (t, *J*=5.4 Hz, 1 H), 4.52 (d, *J*=5.9 Hz, 2 H), 4.33 (t, *J*=5.1 Hz, 2 H), 3.73 (q, *J*=5.2 Hz, 2 H). LC/MS found 466.1 $[M+H]^+$.



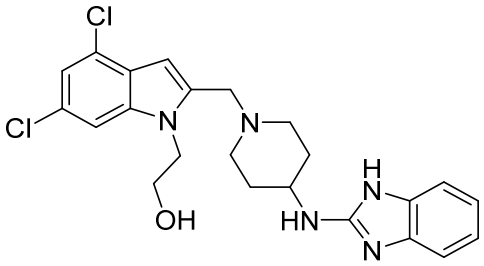
2-(2-(((4-((1*H*-Benzo[*d*]imidazol-2-yl)amino)phenyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48g, NEU-2058) General procedure G, FCC: 5-10% CH_3OH in CH_3Cl. Collected as a brown solid in 46% yield. TLC (10% CH_3OH in CH_2Cl_2): R_f = 0.42. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.76 (br. s., 1 H), 8.90 (br. s., 1 H), 7.62 (d, *J*=1.0 Hz, 1 H), 7.41 (d, *J*=8.8 Hz, 2 H), 7.18 - 7.24 (m, 2 H), 7.14 (d, *J*=2.0 Hz, 1 H), 6.89 - 6.95 (m, 2 H), 6.70 (d, *J*=8.8 Hz, 2 H), 6.43 (s, 1 H), 5.94 (br. s., 1 H), 5.03 (br. s., 1 H), 4.50 (d, *J*=3.4 Hz, 2 H), 4.32 (t, *J*=5.1 Hz, 2 H), 3.72 (br. s., 2 H). LC/MS found 466.1 $[M+H]^+$.



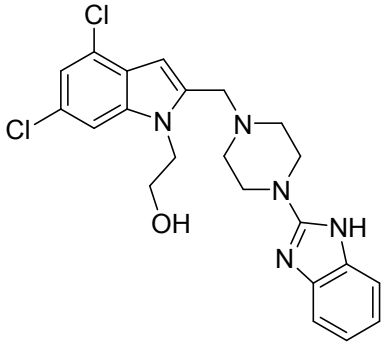
***trans*-2-(2-(((2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)cyclohexyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48h, NEU-2107)** General procedure F, FCC: 0-5% CH_3OH in ethyl acetate. Collected as an off-white solid in 82% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.51 (d, *J*=1.0 Hz, 1 H), 7.11 - 7.16 (m, 2 H), 7.09 (d, *J*=1.5 Hz, 1 H), 6.87 - 6.92 (m, 2 H), 6.34 (s, 1 H), 4.22 (t, *J*=5.1 Hz, 2 H), 4.10 (d, *J*=14.6 Hz, 1 H), 3.95 (d, *J*=14.6 Hz, 1 H), 3.60 (t, *J*=5.4 Hz, 2 H), 3.52 - 3.57 (m, 1 H), 2.64 (t, *J*=9.8 Hz, 1 H), 2.11 (d, *J*=10.3 Hz, 1 H), 1.98 - 2.05 (m, 1 H), 1.62 - 1.74 (m, 2 H), 1.17 - 1.38 (m, 4 H). LC/MS found 472.1 $[M+H]^+$.



***cis*-2-(2-(((2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)cyclohexyl)amino)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48i, NEU-2108)** General procedure F, FCC: 0-5% CH_3OH in ethyl acetate, then by prep HPLC with a gradient of 5-30% CH_3CN in H_2O. Collected as an off-white solid in 27% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.50 (s, 1 H), 7.10 - 7.16 (m, 2 H), 7.06 (d, *J*=2.0 Hz, 1 H), 6.84 - 6.90 (m, 2 H), 6.38 (s, 1 H), 4.17 - 4.30 (m, 2 H), 4.05 (br. s., 0 H), 3.89 - 3.98 (m, 2 H), 3.60 (td, *J*=5.2, 2.2 Hz, 2 H), 2.87 - 2.94 (m, 1 H), 1.74 - 1.84 (m, 1 H), 1.46 - 1.68 (m, 5 H), 1.25 - 1.42 (m, 2 H), 1.20 (s, 1 H). LC/MS found 472.1 $[M+H]^+$.



2-(2-((4-((1*H*-Benzo[*d*]imidazol-2-yl)amino)piperidin-1-yl)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48j, NEU-2106) General procedure F, FCC: 0-5% CH_3OH in ethyl acetate. Collected as a bright yellow solid in 42% yield. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 7.58 (d, *J*=1.0 Hz, 1 H), 7.14 (d, *J*=1.5 Hz, 1 H), 7.09 - 7.13 (m, 2 H), 6.86 (dd, *J*=5.6, 3.2 Hz, 2 H), 6.43 (s, 1 H), 4.33 (t, *J*=5.4 Hz, 2 H), 3.70 (t, *J*=5.4 Hz, 2 H), 3.59 (m, *J*=4.4 Hz, 1 H), 3.15 (s, 2 H), 2.84 (d, *J*=11.2 Hz, 2 H), 2.17 (t, *J*=11.0 Hz, 2 H), 1.92 (d, *J*=10.3 Hz, 2 H), 1.43 (q, *J*=10.3 Hz, 2 H). LC/MS found 458.0 $[M+H]^+$.



2-(2-((4-(1*H*-Benzo[*d*]imidazol-2-yl)piperazin-1-yl)methyl)-4,6-dichloro-1*H*-indol-1-yl)ethan-1-ol (48k, NEU-2105) General procedure F, FCC: 0-10% CH_3OH in ethyl acetate. Collected as a white solid in 48% yield. TLC (2% CH_3OH in ethyl acetate): R_f = 0.44. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 11.38 (br. s., 1 H), 7.61 - 7.65 (m, 1 H), 7.12 - 7.24 (m, 3 H), 6.92 (br. s., 2 H), 6.46 (s, 1 H), 5.07 (br. s., 1 H), 4.38 (t, *J*=5.4 Hz, 2 H), 3.69 - 3.78 (m, 4 H), 3.48 (br. s., 4 H), 2.56 (t, *J*=4.6 Hz, 4 H). LC/MS found 444.0 $[M+H]^+$.

PHARMACOKINETIC EXPERIMENTS

Table S1. Plasma and brain exposure parameters of NEU-1053

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	507.20	0.08	2225.48	2375.63	0.39
		Brain*	8.00	40.97		869.68	NC	

Figure S1. Mean plasma and brain concentration-time profiles of NEU-1053 following a single intraperitoneal administration in female BALB/c mice (Dose: 10 mg/kg).

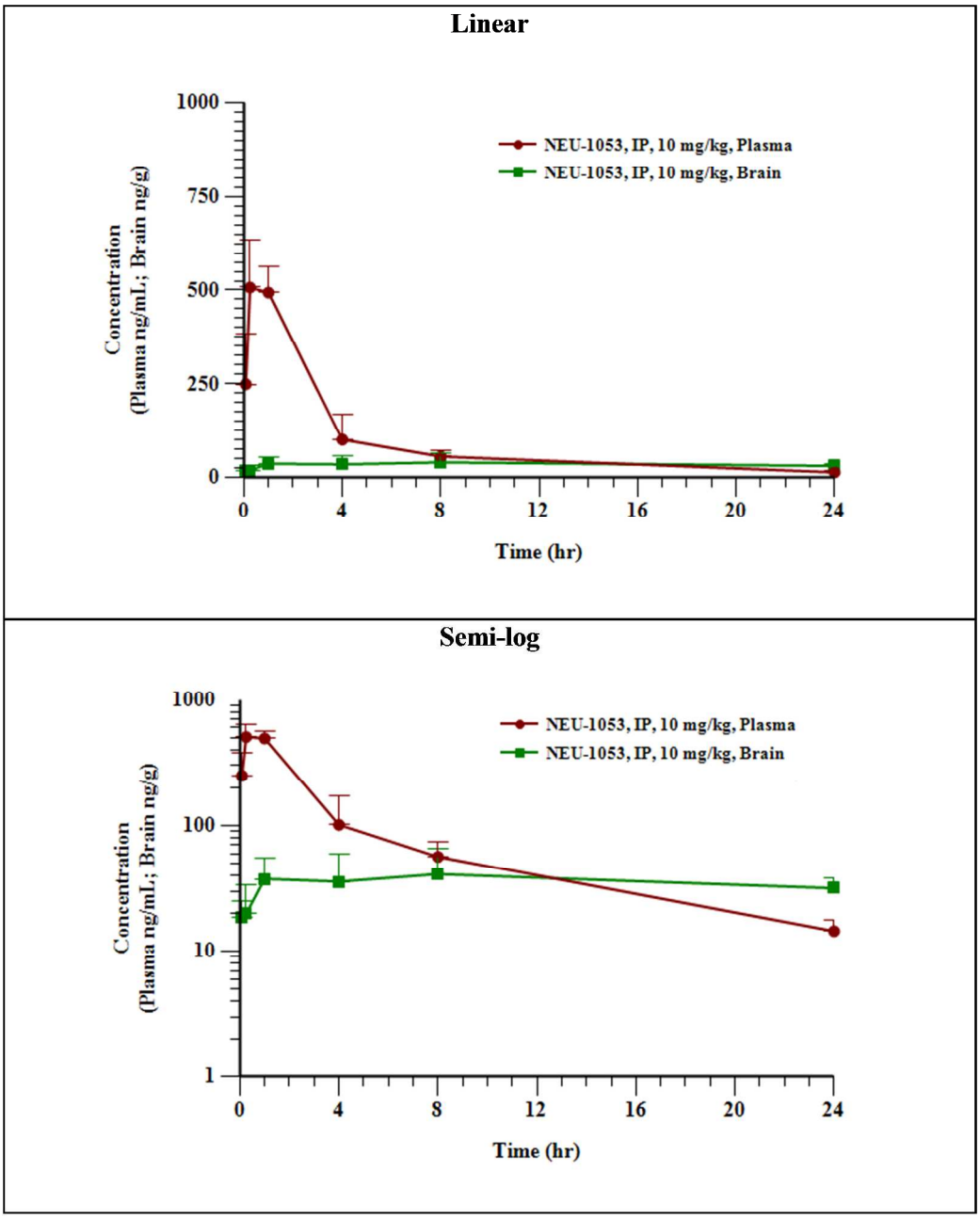


Table S2. Individual plasma concentration (ng/mL)-time data of NEU-1053 following a single intraperitoneal administration in female BALB/c mice (Dose: 10 mg/kg)

Mouse ID	Plasma Conc. (ng/mL) Time (hr)					
	0.08	**0.25**	**1**	**4**	**8**	**24**
1	263.75					
2	374.33					
3	110.35					
4		617.53				
5		535.12				
6		368.94				
7			455.01			
8			453.39			
9			572.44			
10				27.65		
11				113.20		
12				163.50		
13					57.83	
14					72.59	
15					39.03	
16						18.12
17						12.01
18						13.20
Mean	**249.48**	**507.20**	**493.61**	**101.45**	**56.48**	**14.44**
SD	132.57	126.63	68.27	68.68	16.82	3.24
CV%	53.10	25.00	13.80	67.70	29.80	22.40

LLOQ: 2.01 ng/mL

Table S3. Individual brain concentration (ng/g)-time data of NEU-1053 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	18.66					
2	25.17					
3	11.40					
4		16.41				
5		34.98				
6		8.19				
7			48.30			
8			16.92			
9			47.49			
10				11.19		
11				37.83		
12				58.17		
13					66.12	
14					41.31	
15					15.48	
16						33.69
17						36.84
18						24.36
Mean	**18.41**	**19.86**	**37.57**	**35.73**	**40.97**	**31.63**
SD	6.89	13.72	17.89	23.56	25.32	6.49
CV%	37.40	69.10	47.60	65.90	61.80	20.50

LLOQ 2.01 ng/mL; NA: not applicable;

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

Table S-4. Mice without parasitemia in a mouse model of CNS infection with *T. brucei* treated with NEU-1053.

	NEU-1053 (parasites/mL)				
Day/Mouse	1	2	3	4	5
0	1.00E+04	1.00E+04	1.00E+04	1.00E+04	1.00E+04
14	3.43E+08	5.00E+06	3.20E+07	3.50E+07	1.80E+08
28	N.D.	2.50E+05	1.50E+05	N.D.	N.D.
35	N.D.	2.80E+07	N.D.	N.D.	N.D.
39	3.80E+07	1.25E+08	3.60E+07	2.05E+07	3.55E+08
43	X	X	X	X	X

Dose: IP 20 mg/kg/day on days 21-25

N. D.: No detectable parasitemia (<125.000 p/mL blood)

	Berenil (parasites/mL)				
Day/Mouse	1	2	3	4	5
0	1.00E+04	1.00E+04	1.00E+04	1.00E+04	1.00E+04
14	1.78E+08	3.98E+08	4.75E+07	5.00E+06	1.50E+07
28	N.D.	N.D.	N.D.	N.D.	N.D.
35	N.D.	N.D.	N.D.	N.D.	N.D.
39	N.D.	N.D.	N.D.	N.D.	N.D.
43	N.D.	8.00E+08	8.40E+08	N.D.	N.D.

Dose: IP 40 mg/kg on day 21

N. D.: No detectable parasitemia (<125.000 p/mL blood)

CELLULAR ASSAYS

Strains and media

Bloodstream *Trypanosoma brucei brucei* Lister 427 was the selected strain to perform the HTS experiments and the profiling assay. Cell strain was cultured in Hirumi's modified Iscove's medium (HMI-9), supplemented with 10% heat-inactivated FBS, at 37 ºC and 5% CO_2 in T-25 vented flask (Corning®).

Bloodstreams *Trypanosoma brucei brucei* GVR35 was the selected strain for chronic animal model. Cell strain was maintained through mice passage when required.

Determination of EC_{50}

For dose-response experiments, serial compound dilutions of analogues were plotted against compound concentration. Dose response starting at 10 mM and with 3-fold dilutions for 10 points, were made in 96-well transparent Nunclon plates in 100% DMSO. 4 μL per well from those masterplates were dispensed in a new plate and 96 μL of HMI-9 per well was added, generating a 4% DMSO intermediate plate.

Mid-log growing cultures of *Trypanosoma brucei brucei* were diluted to a working cell density of 2,750 cells/mL and 90 μL/well were dispensed in 96-well assay plate. 10 μL/well from fresh made intermediate plates were added; final top concentration for compounds was 40 μM in 0.4% DMSO per well.

Assay plates were incubated for 72h at 37 ºC and 5% CO_2; four hours prior finishing incubation, 20 uL of a 440 μM resazurin solution in prewarmed HMI-9 was added to each well followed of 4 hours further incubation. At the end of incubation, obtained fluorescence was read in an Infinite F200 plate reader (Tecan) at 550 nm (excitation filter) and 590 mn (emission filter). A 4-parameter equation was used to fit the dose-response curves and determination of EC_{50} as concentration that reduces 50% viability compared to solvent-treated control by SigmaPlot® 13.0 software. Assays were performed in duplicate at least two times, to achieve a minimal n=3 per dose response.

Rate of Action assays.

Serial compound dilution of selected hits from single concentration screening assay, starting at 10 mM and with 3-fold dilutions for 10 points, were made in masterplates. Intermediate plates were prepared as previously described.

Mid-log *T. brucei brucei* cultures were diluted to the required cell density, according to the different incubation time points described, and 90 μL per well were dispensed in final assay plates Nunclon 96-well Solid White: 10 μL of intermediate plates were added to each well to

achieve a final assay top concentration of 40 μM, and 4 sets of assay plates were arranged to assay in order to be sequentially stopped at their correspondent time point. Top and bottom rows were dismissed for compound assay, to reduce evaporation effects.

Plates were incubated at 37 ºC and 5%CO_2 for the indicated time points; incubation was stopped by addition of 10 μL of Cell Titer Glo reagent (Promega), and after shaking the plates were incubated at room temperature for 10 min, to allow the signal to settle. Plate luminescence was read on an Infinite F200 plate reader (Tecan), and raw data were processed and analyzed as previously described.

MRC5-SV2 cell culture and growth assays

MRC5-SV2 cell line (SV40-transformed human lung fibroblast cell line) was cultured in DMEM medium supplemented with 10% heat-inactivated FBS at 37 ºC and 5% CO_2 in T-75 vented flask (Corning®).

Cytotoxicity assay

Dose response starting at 10 mM with 3-fold dilutions for 5 points was made in 96-well transparent master plates. Intermediate plates were made as described, adding 95 μL of DMEM complete media to 5 μL of compound per well setting a 5% DMSO amount.

Log-phase MRC5 cells were removed from a T-75 TC flask using TrypLE® Express (Thermo) and dispersed by gentle pipetting. Cell density was adjusted to working concentration in prewarmed DMEM medium: 25,000 cells in 90 μL of culture were plated in 96-well transparent Nunclon plates and let to settle for 24 hours at 37 ºC and 5% CO_2.

After settling incubation, 10 μL of fresh made intermediate plate were added per well: final top concentration for compounds was 50 μM in 0.5% DMSO per well. Plates were incubated for 48h at 37 ºC and 5% CO_2. 4 hours prior fluorescence measure, 20 μL of 500 μM resazurin solution was added. Fluorescence was read in Infinite F200 plate reader (Tecan) at 550 nm (excitation filter) and 590 nm (emission filter).

A 4-parameter equation was used to fit the dose-response curves and determination of EC_{50} as concentration that reduces 50% viability compared to solvent-treated by SigmaPlot ® 13.0 software. Assays were performed in duplicate at least two times, to achieve a minimal n=3 per dose response.

ANIMAL EFFICACY EVALUATION

Animals and ethical statement

Experimental procedures, Ref. Code Ethics Committee of Animal Experiment MNC.2/2015 was reviewed and evaluated by the internal Ethical Committee on Animal Experimentation (CEEA) of the Instituto de Parasitología y Biomedicina ‘‘López Neyra’’ and the institutional Commission on Bioethics at the Spanish National Research Council (CSIC), and approved by Spanish authorities, as stipulated by Spanish and European laws. All animal experiments were addressed with the maximum ethical consideration and respect to the animals, minimizing the pain and using standardized and approved procedures according to the Law and as recommended by FELASA.

Mouse model of CNS infection

Female NMRI mice were obtained from Charles River Laboratories and were kept under the standard conditions in a conventional room at 20–24 °C with a 12/12-h light/dark cycle. The animals were provided with sterilized water and commercial pellets *ad libitum*. Infection was performed by I.P. injection of 10^4 bloodstream forms in 0.2 mL TDB glucose of *T. brucei brucei* (GVR25 strain) from a stock of cryopreserved stabilates containing 10 % glycerol. Fourteen days later, infected animals with confirmed parasitemia were divided into two equal groups: Control (infected mice treated with a single dose of 40 mg/kg of body weight of diminazene aceturate (Berenil) and then treated with the vehicle DMSO in PBS (Sigma-Aldrich); and drug-treated (infected mice treated with 2 doses of 10 mg/kg per day (20 mg/kg/day)). For treatment, NEU-1053 from a stock solution (30 mg/mL) diluted in DMSO, was prepared in PBS and then heated at 50 °C for 10 min to solubilize. Five mice in each group were used in this assay. Drug-treated mice received a 0.2 mL I.P. injection at the 21^{st} day from infection during 5 consecutive days. After a 2 day hiatus, drug was administered for additional 5 days. Parasitemia was individually checked by direct microscopic counting of parasites in a Neubauer chamber using 2 µL of blood from infected mice tail, diluted in 100 µL of TDB glucose. Parasitaemia of all mice were checked twice a week by tail blood examination and thereafter mice with parasitaemia relapses were euthanized and the day of parasitaemia relapse was recorded.

TARGET ID EXPERIMENTS

Methods:

Potency shift in mutant parasites

Wild-type and 1433-resistant *T. brucei brucei* [1] were cultured as previously described. [1, 2] Test compounds were assayed in a growth inhibition assay against both a wild-type and a 1433-resistant clone as previously described.[1, 3]

Biochemical assay

Test compound were assayed against *T. brucei* methionly-tRNA synthetase (*T. brucei* MetRS) using an ATP-depletion chemiluminescence assay in a 96-well format as previously described.[4] Test compounds (assayed in triplicate using 12 serial three dilutions starting at 10 μM) were pre-incubated for 15 minutes at room temperature with 200 μg/mL bulk *E. coli* tRNA (Sigma-Aldrich or the equivalent activity of Roche), 50 nM *T. brucei* MetRS, 0.1 U/mL pyrophosphatase, 0.2 mM spermine, 0.1 mg/mL bovine serum albumin, 2.5 mM dithiothreitol, 25 mM HEPES-KOH pH 7.9, 10 mM $MgCl_2$, 50 mM KCl, and 2% DMSO. The reaction was started with the addition of 100 nM ATP and 32 μM L-methionine and incubated at room temperature for 120 minutes in a total assay volume of 50 μL/well. Then 50 uL/well of Kinase-Glo® (Promega) was added and the relative light units (RLU) were quantified on the EnVision plate reader (PerkinElmer).

$$\text{Percent inhibition} = 100 \times \frac{\text{(test compound – AVG low control)}}{\text{(AVG high control – AVG low control)}}$$

Where low control is everything except test compounds and high control is everything except test compounds and L-methionine.

IC_{50} values were calculated by non-linear regression, sigmoidal-dose response, in Prism 3.0. The lower end of accurate detection for this assay is 25 nM corresponding to half the enzyme concentration of 50 nM used in the assay.

X-RAY CRYSTALLOGRAPHY

Protein expression and purification for crystallography

T. brucei MetRS was expressed and purified following procedures reported earlier [Koh *et al*., 2012].[5] For crystallization, truncated TbruMetRS (237–773), as previously cloned into the AVA0421 vector with the surface residues $_{452}$KKE$_{454}$ changed into ARA, was used. The mutated residues are remote from the active site and their modification is critical for crystallization. TbruMetRS was expressed in *E. coli* and purified by a Ni-NTA affinity chromatography followed by overnight cleavage at 4°C of the N-terminal His_6-tag using N-terminally His_6-tagged 3C protease. The cleaved TbruMetRS was purified from the 3C protease by a second Ni-NTA step followed by size-exclusion chromatography on a Superdex 75 column (Amersham Pharmacia Biotech) using SEC buffer (25 mM HEPES at pH 7.0, 500 mM NaCl, 2 mM DTT, 5% glycerol, 0.025% NaN_3) with 10 mM L-methionine. Purified TbruMetRS retained five residues of the 3C protease cleavage site (GPGSM) at the N-terminus.

Protein crystallization and crystal soaking

The truncated TbruMetRS surface mutant was crystallized essentially as previously reported [Koh *et al*., 2012].[5] Protein crystals were obtained after 1-2 days at room temperature by vapor diffusion using sitting drops equilibrated against a reservoir containing 2.1 M $(NH_4)_2SO_4$, 0.2 M NaCl and 0.1 M sodium cacodylate pH 6.2. The drops consisted of a mixture of 1 µL protein at 10 mg/mL with 10 mM L-methionine (critical for crystallization) plus 1 mM tris(2-carboxyethyl)phosphine and 1 µL of reservoir solution. To obtain TbruMetRS•NEU-0001053 complexes, TbruMetRS•Met crystals were soaked in a cryo-solution containing the inhibitor as described before [Koh *et al*., 2012].[5] To avoid their otherwise imminent disintegration upon soaking, crystals were soaked for a maximum of 20 seconds in a 10 µL solution obtained by mixing 1 µL of 20 mM NEU-0001053 in 20% DMSO, 4 µL reservoir solution and 5 µL 60% glycerol in SEC buffer, and were immediately flash frozen in liquid nitrogen.

Data Collection and Structure Determination

Data were collected under cryogenic conditions at Stanford Synchrotron Radiation Lightsource (SSRL) beamline 12-2 at a wavelength of 1 Å. The data was processed with HKL2000 (Table S1).[6] Phase determination was done by molecular replacement with Phaser[7]using previously reported structures of TbruMetRS.[8] Coot[9] and REFMAC5[10] were used in iterated building, rebuilding and refinement of the structure. The Grade web server[11] was used in the generation of the refinement restraints for the ligands. The structure determination progress was validated throughout the process with the structure validation server MolProbity.[12] The final crystallographic refinement statistics are given in Table S1. Figures were created and rendered with Pymol [Schrödinger, LLC]. Coordinates and structure factors for TbruMetRS in complex with compound NEU-0001053 have been deposited in the Protein Data Bank under PDB ID: 5EEJ.

Table S5. Crystallographic data collection and refinement statistics.

Compound	**NEU-0100053**
PDB ID	**5EEJ**
Data collection	
Space group	$P\ 2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	88.0, 106.3, 207.3
Resolution (Å)	48.4 – 2.60 (2.67 – 2.60)
R_{merge}	0.223 (1.822)
R_{pim}	0.099 (0.857)
Observed reflections	357029 (22979)
Unique reflections	60480 (4352)
Mean $I / \sigma I$	5.5 (1.2)
Multiplicity	5.9 (5.3)
Completeness (%)	99.7 (98.3)
$CC_{1/2}$	0.989 (0.363)
Refinement	
Resolution (Å)	48.4 – 2.60
Reflections used	57423
R_{work} / R_{free}	0.217 (0.219)
Number of atoms	
Protein	8094
Met	9
Other solvent ligands	11
Water	205
Number of residues	1027
Average *B*-factors (Å^2)	
Protein	60.1
Met	46.6
Other solvent ligands	64.1
Water	46.7
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.35
Ramachandran plot[#]	
Favored (%)	97
Outlier (%)	0
Compound	
Number of atoms	29
Average *B*-factors (Å^2)	60.0
LLDF[@]	0.26
RSR[^]	0.15

Values in parentheses are for highest-resolution shell

[#]Ramachandran Plot statistics as reported by the wwPDB validation report

[@]Local ligand density fit as reported by the wwPDB validation report[13121110101098]

[^]Real space R value as reported by the wwPDB validation report

Table S5. Compound summary data table

Registry Number	Manuscript number	*T. brucei* EC_{50} (uM)	*T. brucei* EC_{50} (SD)	MRC5-SV2 TC_{50} (uM)	MRC5-SV2 TC_{50} (SD)
NEU-0001053	**1**	0.001		11.9	
NEU-0002128	**39**	0.500	0.102	18.0	1.1
NEU-0002057	**46**	0.075	0.000	27.3	9.3
NEU-0002185	**38a**	0.150	0.010	22.5	2.6
NEU-0002186	**38b**	0.963	0.195	22.2	2.1
NEU-0002172	**38c**	0.016	0.002	21.9	1.5
NEU-0002176	**45a**	0.002	0.000	14.2	2.8
NEU-0002175	**45b**	0.790	0.140	26.3	2.3
NEU-0002173	**45c**	0.270	0.100	> 50	
NEU-0002054	**47a**	0.009	0.002	8.8	2.6
NEU-0002055	**47b**	0.005	0.001	9.8	2.1
NEU-0002056	**47c**	0.008	0.001	7.4	0.6
NEU-0002063	**47d**	0.619	0.068	10.2	1.9
NEU-0002109	**47e**	0.339	0.098	1.4	2.6
NEU-0002110	**47f**	0.788	0.199	8.1	2.3
NEU-0002111	**47g**	0.200	0.062	8.4	1.5
NEU-0002062	48a	0.005	0.001	25.9	8.8
NEU-0002064	**48b**	0.005	0.004	11.1	2.6
NEU-0002061	**48c**	0.977	0.274	22.4	2.4
NEU-0002104	**48d**	2.214	0.083	10.2	1.0
NEU-0002060	**48e**	0.422	0.124	25.2	2.2
NEU-0002059	**48f**	0.960	0.305	21.5	2.0
NEU-0002058	**48g**	5.549	0.553	23.3	0.5
NEU-0002107	**48h**	0.344	0.083	17.2	1.8
NEU-0002108	**48i**	0.683	0.151	14.2	2.3
NEU-0002106	**48j**	0.401	0.089	11.9	1.3
NEU-0002105	**48k**	> 5		> 50	

Table S6. Compound SMILES strings.

Registry Number	Cmpd	SMILES
NEU-0001053	1	OCCN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002128	39	OCCN1C(=CC2=C(Cl)C=C(Cl)C=C12)C(=O)NCCCNC1=NC2=CC=CC=C2N1
NEU-0002057	46	OCCN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=CC=CC=C12
NEU-0002185	38a	ClC1=CC=CC(CNCCCNC2=NC3=CC=CC=C3N2)=C1
NEU-0002186	38b	ClC1=CC=C(CNCCCNC2=NC3=CC=CC=C3N2)C=C1
NEU-0002172	38c	ClC1=CC(CNCCCNC2=NC3=CC=CC=C3N2)=CC(Cl)=C1
NEU-0002176	45a	OCCN1C(CNCCCNC(=O)NC2=CC=CC=C2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002175	45b	OCCN1C(CNCCCNC2=NC3=CC=CC=C3O2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002173	45c	OCCN1C(CNCCCNC2=CC=NC=N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002054	47a	ClC1=CC(Cl)=C2C=C(CNCCCNC3=NC4=CC=CC=C4N3)NC2=C1
NEU-0002055	47b	CN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002056	47c	COCN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002063	47d	COCCN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002109	47e	CS(=O)(=O)N1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002110	47f	CC(C)(C)OC(=O)NCCN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002111	47g	NCCN1C(CNCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002062	48a	OCCN1C(CNCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002064	48b	OCCN1C(CNCCCCNC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002061	48c	CN(CCCN(C)C1=NC2=CC=CC=C2N1)CC1=CC2=C(Cl)C=C(Cl)C=C2N1CCO
NEU-0002104	48d	CN(CCCN(C)C1=NC2=CC=CC=C2N1C)CC1=CC2=C(Cl)C=C(Cl)C=C2N1CCO
NEU-0002060	48e	OCCN1C(CNC2=CC=CC=C2NC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002059	48f	OCCN1C(CNC2=CC=CC(NC3=NC4=CC=CC=C4N3)=C2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002058	48g	OCCN1C(CNC2=CC=C(NC3=NC4=CC=CC=C4N3)C=C2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002107	48h	OCCN1C(CN[C@H]2CCCC[C@@H]2NC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002108	48i	OCCN1C(CN[C@H]2CCCC[C@H]2NC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002106	48j	OCCN1C(CN2CCC(CC2)NC2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12
NEU-0002105	48k	OCCN1C(CN2CCN(CC2)C2=NC3=CC=CC=C3N2)=CC2=C(Cl)C=C(Cl)C=C12

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