Supporting Information for

Protein and chemical determinants of BL-1249 action and selectivity for K_{2P} channels

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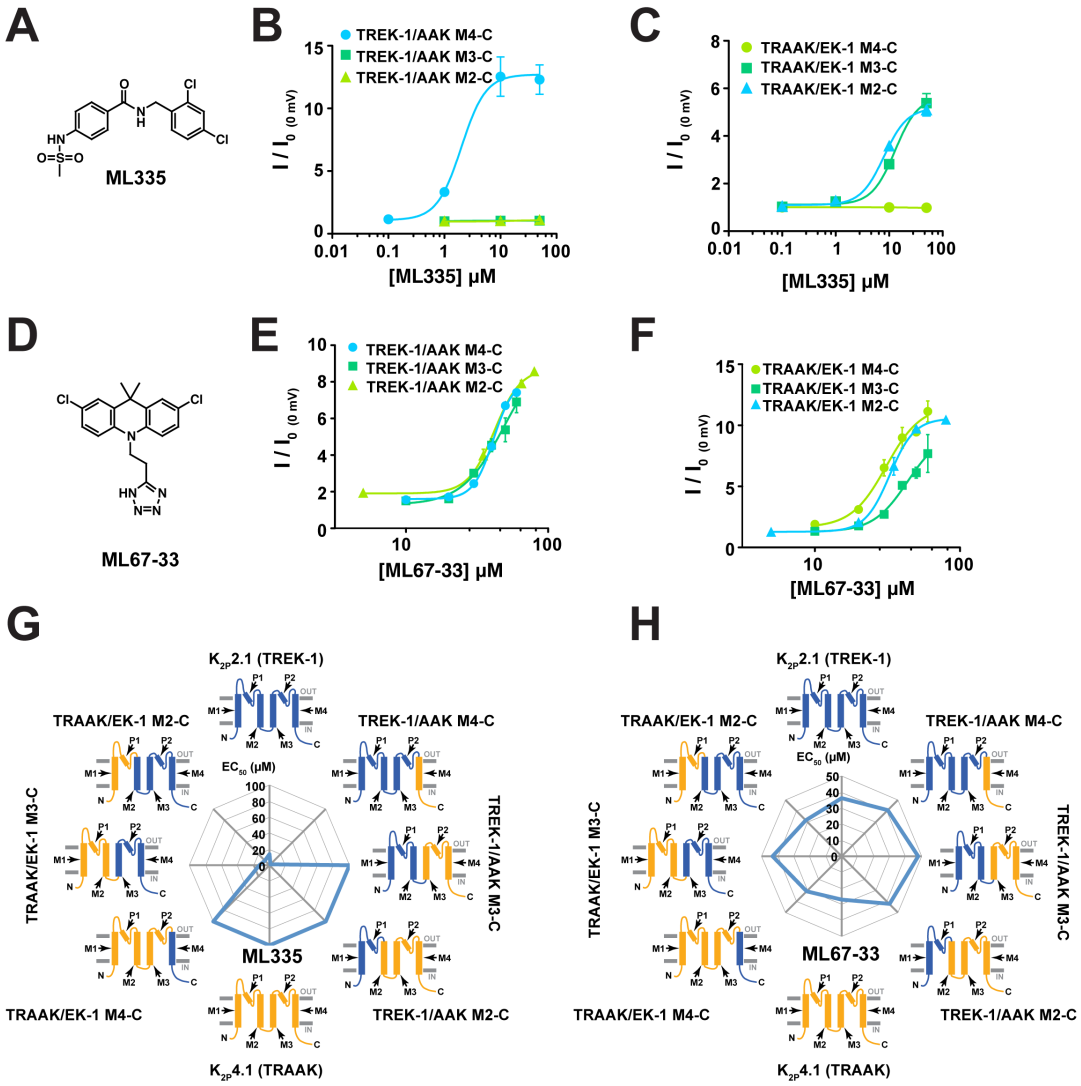

Figure S1 Responses of TREK-1/TRAAK chimeras to ML335 and ML67-33 activators. A, ML335 chemical structure. **B,** and **C,** ML335 dose-response curves for: TREK-1/AAK M4-C (light blue), TREK-1/AAK M3-C (green), and TREK-1/AAK M2-C (light green) (EC_{50} = 2.0 ± 0.6 µM, >100 µM, and >100 µM, respectively), and TRAAK/EK-1 M4-C (light green), TRAAK/EK-1 M3-C (green), and TRAAK/EK-1 M2-C (light blue) (EC_{50} = >100 µM, 13 ± 2 µM, and 8.0 ± 1.0 µM respectively). **D,** ML67-33 chemical structure. **E,** and **F,** ML67-33 dose-response curves for: TREK-1/AAK M4-C (light blue), TREK-1/AAK M3-C (green), and TREK-1/AAK M2-C (light green), (EC_{50} = 42 ± 3 µM, 49 ± 22 µM, and 42 ± 3 µM, respectively), and TRAAK/EK-1 M4-C (light green), TRAAK/EK-1 M3-C (green), TRAAK/EK-1 M2-C (light blue), (EC_{50} = 31 ± 3 µM, 44 ± 14 µM,, and 33 ± 1 µM, respectively). **G,** and **H**, Spider plot of EC_{50}s for the indicated chimeras. Cartoon schematics show channel portions from K_{2P}2.1(TREK-1) (blue) and K_{2P}4.1(TRAAK) (light orange). Error bars are s.e.m.

Figure S2

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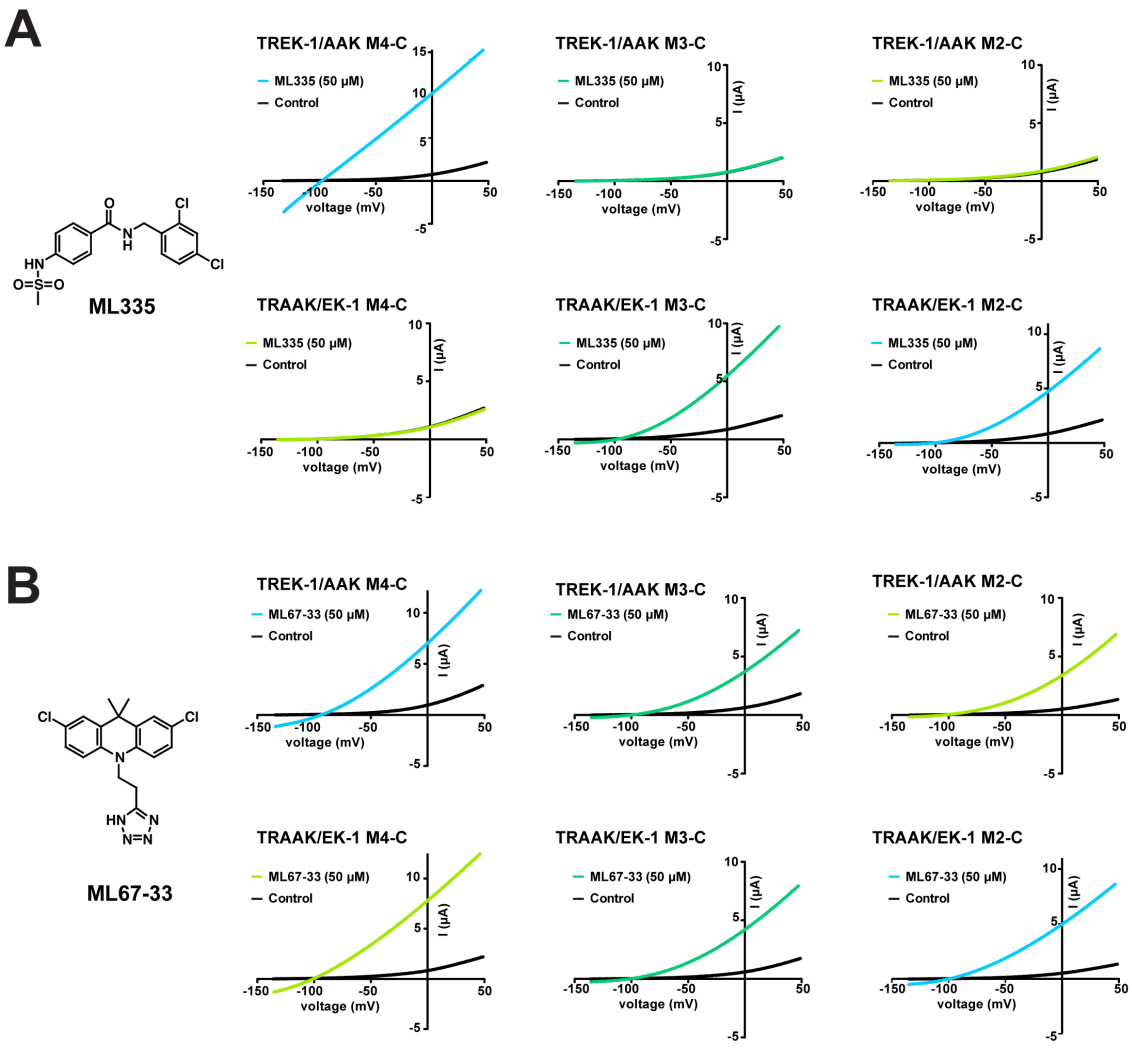


Figure S2 Exemplar responses of TREK-1/TRAAK chimeras to ML335 and ML67-33 activators. A, Chemical structure of ML335 and exemplars for responses of TREK-1/AAK M4-C (light blue), TREK-1/AAK M3-C (green), TREK-1/AAK M2-C (light green), TRAAK/EK-1 M4-C (light green), TRAAK/EK-1 M3-C (green), and TRAAK/EK-1 M2-C (light blue) to 50 µM ML335. **B,** Chemical structure of ML67-33 and exemplars for responses to 50 µM ML67-33. Colors are as in '**A**'.

Synthesis of BL-1249 analogs

General Procedures: Reactions were stirred magnetically unless otherwise indicated. Air and/or moisture sensitive reactions were carried out under an argon atmosphere in oven-dried glassware using anhydrous solvents from commercial suppliers. Air and/or moisture sensitive reagents were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa. Reaction product solutions and chromatography fractions were concentrated by rotary evaporation at room temperature at 20 Torr then at 0.5 Torr unless otherwise indicated. Thin phase chromatography was performed on EMD precoated glass-backed silica gel 60 F-254 0.25 mm plate.

Materials: All chemical reagents and solvents used were purchased from Sigma-Aldrich or Fisher Scientific. Anhydrous dichloromethane and tetrahydrofuran (EMD Drisolv) were used without further purification

The following known compounds were prepared as detailed herein, based on the reported experimental procedures as described:

Preparation of **1**: WO2014/100818; (2014) (A1) English.
Preparation of **5**: *European Journal of Organic Chemistry*, 2017(1), 203-207; 2017
Preparation of **10**: *Med. Chem. Commun.*, 2012, 3, 373-378.
Preparation of **11**: *Med. Chem. Commun.*, 2012, 3, 373-378.
Preparation of **13**: *Journal of Medicinal Chemistry,* 2001, 44 (26) 4524-4534

Synthetic Procedures

2-[(5,6,7,8-tetrahydronaphthalen-1-yl)amino]benzonitrile (1): To an oven-dried, septum-capped, 40 mL vial was added 2-amino-5,6,7,8-tetrahydronaphthalene (260 mg , 1.0 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (79 mg, 0.1 mmol, 0.1 equiv.) and cesium carbonate (589 mg, 1.8 mmol, 1.3 equiv.), along with a stirbar. 2-Bromobenzonitrile (250 mg, 1.4 mmol, 1.0 equiv.) was dissolved in dry 1,4-dioxane (8.000 ml, 93.5 mmol, 68.8 equiv.) that had been degassed with dry nitrogen. The resulting solution was transferred to the vial with the other reagents, sealed, and the reaction mixture bubbled with dry nitrogen for 1 minute (with a needle used as a nitrogen outlet, this measure serves to purge the sealed vessel with inert gas). The reaction mixture was then stirred under dry nitrogen for 3 hours at 90°C, at which time TLC (10% EA/hexane) showed a new spot (UV_{254}) with $R_f \sim 0.6$. The reaction mixture was filtered through celite and concentrated to afford a residue that was dissolved in CH_2Cl_2 and loaded on a 40 g Silicycle cartridge, eluting with a gradient of 0-10% ethyl acetate in hexane. The fractions containing product were collected and concentrated to afford the product (208 mg, 1.1 mmol). (79%) ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.50 (dd, *J*=7.72, 1.70 Hz, 1 H), 7.34 (ddd, *J*=8.67, 7.25, 1.60 Hz, 1 H), 7.09 - 7.22 (m, 2 H), 6.97 - 7.05 (m, 1 H), 6.75 - 6.90 (m, 2 H),) 6.11 (s, 1 H), 2.78 - 2.93 (m, 2 H) 2.58 - 2.74 (m, 2 H) 1.74 - 1.92 (m, 4 H); LCMS (ESI) *m/z* $[M+H]^+$249.2, 250.2

2-[(5,6,7,8-tetrahydronaphthalen-1-yl)amino]benzoic acid (BL-1249 acid) and **2-[(5,6,7,8-tetrahydronaphthalen-1-yl)amino]benzamide (BL-1249 amide).** Intermediate [(5,6,7,8-tetrahydronaphthalen-1-yl)amino]benzonitrile (compound **1**, 31 mg, 0.1 mmol, 1.0 equiv.) was placed in a 4 mL screw-cap vial with a stirbar. This was dissolved in dimethyl sulfoxide (200 µL) and potassium hydroxide (167 µL, 1.0 mmol, 8.2 equiv.) was added. Finally, water (633 µL) was added to bring the final volume up to ~1 mL. The reaction mixture was capped and stirred at 105°C for 18 hours. After cooling, 6M HCl (~0.2 mL) was added slowly to bring the pH to zero. The reaction mixture was diluted with water and extracted twice with ethyl acetate. The combined organic layers were washed twice with brine then dried with sodium sulfate, filtered

and concentrated to afford the crude product. This was dissolved in a small amount of dichloromethane, loaded on a 4g Silicycle silica cartridge pre-equilibrated with hexane, and eluted with the following gradient: 3 column volumes (CV) hexane, then 0-100% EtOAc/hexanes over 12 CV. The fractions containing acid and amide products were collected and concentrated to afford **BL-1249 acid** and **BL-1249 amide**.

2-[(5,6,7,8-tetrahydronaphthalen-1-yl)amino]benzoic acid (3 mg, 0.012 mmol, 10%), **(BL-1249 acid).** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.04 (dd, *J*=8.10, 1.51 Hz, 1 H) 7.31 (ddd, *J*=8.67, 6.97, 1.70 Hz, 1 H) 7.08 - 7.22 (m, 2 H) 6.88 - 7.00 (m, 2 H) 6.71 (ddd, *J*=8.10, 7.06, 1.04 Hz, 1 H) 2.84 (br d, *J*=6.03 Hz, 2 H) 2.67 (br d, *J*=5.84 Hz, 2 H) 1.73 - 1.88 (m, 4 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 173.28 (s, 1 C) 149.76 (s, 1 C) 139.04 (s, 1 C) 138.33 (s, 1 C) 135.17 (s, 1 C) 132.46 (s, 1 C) 132.36 (s, 1 C) 126.03 (s, 1 C) 125.70 (s, 1 C) 121.77 (s, 1 C) 116.36 (s, 1 C) 113.87 (s, 1 C) 109.62 (s, 1 C) 29.86 (s, 1 C) 24.89 (s, 1 C) 22.98 (s, 1 C) 22.74 (s, 1 C); LCMS, $[M+H]^+$ = 268.2; LCMS, ESI$^-$ $[M-H]^-$ = 266.9

2-[(5,6,7,8-tetrahydronaphthalen-1-yl)amino]benzamide (16 mg, 0.060 mmol, 49%) **(BL-1249 amide).** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.37 (s, 1 H) 7.47 (dd, *J*=7.91, 1.51 Hz, 1 H) 7.06 - 7.30 (m, 4 H) 6.87 (d, *J*=7.16 Hz, 1 H) 6.71 (ddd, *J*=7.96, 7.02, 1.22 Hz, 1 H) 5.92 (br s, 2 H) 2.81 (t, *J*=5.84 Hz, 2 H) 2.69 (t, *J*=5.93 Hz, 2 H) 1.66 - 1.88 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 172.00 (s, 1 C) 147.41 (s, 1 C) 139.27 (s, 1 C) 138.89 (s, 1 C) 133.00 (s, 1 C) 130.82 (s, 1 C) 128.26 (s, 1 C) 125.51 (s, 1 C) 124.68 (s, 1 C) 119.26 (s, 1 C) 116.76 (s, 1 C) 115.23 (s, 1 C) 115.03 (s, 1 C) 77.48 (s, 1 C) 77.06 (s, 1 C) 76.63 (s, 1 C) 29.99 (s, 1 C) 24.85 (s, 1 C) 23.07 (s, 1 C) 22.80 (s, 1 C); LCMS, $[M+H]^+$ = 250.2 (m-H_2O + 1)

N-phenyl-2-(1H-1,2,3,4-tetrazol-5-yl)aniline (BL-1249-Ph): A septum capped 40 mL vial was charged with 2-(phenylamino)benzonitrile (compound **2**, 35 mg, 0.2 mmol, 1.0 equiv.), sodium azide (38 mg, 0.6 mmol, 3.3 equiv.) and ammonium chloride (32 mg, 0.6 mmol, 3.6 equiv.). Dry DMF (1.5 mL) was added and the tube was flushed with dry nitrogen and sealed. The tube was then heated at 120°C overnight with stirring. The reaction mixture was cooled, diluted with water, and the pH of the solution determined to be ~5. The slurry was

extracted with ethyl acetate once, then twice with 20% MeOH in CH_2Cl_2, and the combined organic layer washed with brine, dried with sodium sulfate, filtered, and concentrated. The crude residue was loaded atop a 4 g column of silica gel and eluted with 0 to 10% methanol in CH_2Cl_2. The fractions containing product were collected and concentrated to afford the title compound (12.9 mg, 31%). ^{1}H NMR (300 MHz, METHANOL-*d*$_4$) d ppm 7.80 (dd, *J*=7.44, 0.85 Hz, 1 H) 7.27 - 7.42 (m, 4 H) 7.18 - 7.25 (m, 2 H) 7.00 - 7.08 (m, 1 H) 6.94 (ddd, *J*=8.01, 6.12, 2.07 Hz, 1 H); LCMS (ESI) *m/z* $[M+H]^+$238.2

2-bromo-N-phenylaniline (5). To a solution of commercially available *o*-bromoaniline (**4**, 4.32g, 2.84 mL, 25 mmol) in MeCN (30 mL), was added silylaryl triflate (**3**, 5.0g, 4.0 mL, 16.8 mmol) and 2 equivalents of CsF (7.6 g, 50 mmol). The reaction mixture was allowed to stir at room temperature and monitored by TLC. When judged complete, the reaction mixture was washed with brine (20 mL) and extracted with ether (3x30 mL). The combined ether fractions were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (Hex:EtOAc 10-40%) to afford the desired product as a colorless oil (3.83 g, 92%). ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 7.52 - 7.62 (m, 1 H) 7.32 - 7.42 (m, 2 H) 7.26 - 7.32 (m, 1 H) 7.15 - 7.25 (m, 2 H) 7.03 - 7.13 (m, 1 H) 6.73 - 6.84 (m, 1 H) 6.12 (br. s., 1 H); LCMS (ESI^+) m/z 249 ($M+H^+$).

Tert-butyl N-(2-bromophenyl)-N-phenylcarbamate (6): To a solution of 2-bromo-N-phenylaniline (**5**, 3.84 g, 15.4 mmol) in THF (20 mL), was added a solution of Boc_2O (5.05g, 23.1 mmol) in THF (5 mL) along with a catalytic amount of DMAP. The reaction mixture was stirred for 3 hours at 40°C until reaction was judged complete by LC/MS analysis. Excess DMAP was then added and the reaction mixture stirred for another 30 min, in an attempt to decompose the excess Boc_2O. Water (100 mL) was then added to mixture, which was extracted with EtOAc (3x30 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude oil was purified using silica gel column chromatography (Hex:EtOAc/0-50%) to afford the desired product (4.38 g, 81.6% yield) as a colorless oil that solidified. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ ppm 7.69 - 7.79 (m, 1 H) 7.42

(dd, *J*=4.14, 0.94 Hz, 2 H) 7.20 - 7.37 (m, 5 H) 7.09 - 7.20 (m, 1 H) 1.36 (s, 9 H); (ESI^+) m/z 349 ($M+H^+$).

1-phenylspiro[3,1-benzoxazine-4,1'-cyclohexane]-2-one (7): A flame-dried flask charged with *t*-butyl N-(2-bromophenyl)-N-phenylcarbamate (**6**, 1.68 g, 4.8 mmol, 1.0 equiv.) and dry THF (12 mL) under argon and cooled to -78°C. Tert-butyllithium (5.681 mL, 9.7 mmol, 2.0 equiv.) was added dropwise over 5 min. The reaction was stirred at -78°C for 5 min and treated dropwise with a solution of cyclohexanone (711 mg, 748 mL, 7.2 mmol, 1.5 equiv.) in 3 mL dry THF. The reaction mixture was stirred for 5 min and then quenched at -78°C with 30 mL water. The mixture was warmed to room temperature and partitioned between ethyl acetate and water. The layers were separated and the aqueous phase extracted twice more with ethyl acetate. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude residue was purified on a 40g Silicycle column eluting with 0 to 60% EtOAc/hexane to afford 1-phenyl-1,2-dihydrospiro[3,1-benzoxazine-4,1'-cyclohexane]-2-one **(7)** (1.072 g, 3.7 mmol, 76%)**.** ^1H NMR (300 MHz, DMSO-d_6) δ ppm 7.45 - 7.63 (m, 3 H) 7.33 - 7.45 (m, 3 H) 7.05 - 7.24 (m, 2 H) 6.20 (d, *J*=7.72 Hz, 1 H) 2.09 - 2.22 (m, 2 H) 1.86 - 2.02 (m, 2 H) 1.71 - 1.78 (m, 2 H) 1.59 - 1.85 (m, 4 H) 1.27 - 1.46 (m, 1 H); LCMS (ESI^+) m/z 294.0 ($M+H^+$)

1-[2-(phenylamino)phenyl]cyclohexan-1-ol (8): To a solution of 1-phenylspiro[3,1-benzoxazine-4,1'-cyclohexane]-2-one (**7**, 1.50 g, 5.11 mmol) in EtOH (5.0 mL) was added 3 equivalents of 5M NaOH (3.07 mL). The reaction mixture was heated to reflux for 8 hours, cooled, and stirred at room temperature overnight. The reaction mixture was then poured into ice/water and 0.5M HCl was added until the solution tested at pH ~7. This solution was then extracted with CH_2Cl_2 (2x20mL), dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified on a 25g Silicycle cartridge eluting with 0 to 40% EtOAc/hexanes over 12 column volumes to afford the title compound (1.03g, 3.86 mmol, 75%). ^1H NMR (300 MHz, DMSO-d_6) δ ppm 8.57 (s, 1 H) 7.07 - 7.37 (m, 4 H) 6.98 (d, *J*=8.48 Hz, 2 H) 6.72 - 6.93

(m, 2 H) 5.54 (s, 1 H) 1.90 - 2.11 (m, 3 H) 1.56 - 1.84 (m, 4 H) 1.48 (d, *J*=12.62 Hz, 2 H) 1.17 (t, *J*=7.16 Hz, 2 H) LC/MS (ESI^+) m/z 268.0 ($M+H^+$)

10H-spiro[acridine-9,1'-cyclohexane] (9): A mixture of 1-[2-(phenylamino)phenyl]cyclohexan-1-ol (**8**, 749 mg, 2.80 mmol) in 85% phosphoric acid (12 mL) was stirred at room temperature for 4 hours, at which time the reaction was judged complete by TLC. The reaction mixture was poured into water and the product was extracted with EtOAc (2x20mL). The combined organic phases were then dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified using silica gel column chromatography (HexEtOAc/ 0-40%) over 12 column volume on a 40g Silicycle cartridge. As this insoluble intermediate precipitated in the lines of the Biotage flash chromatography system, it is strongly advised that manual silica gel flash chromatography be used to purify this intermediate. The fractions containing product were collected and concentrated to afford the title compound (534 mg, 77% yield) as an oil that solidified upon standing. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 8.92 (s, 1 H) 7.40 - 7.46 (m, 2 H) 7.04 - 7.11 (m, 2 H) 6.94 (dd, *J*=7.91, 1.34 Hz, 2 H) 6.89 (td, *J*=7.49, 1.34 Hz, 2 H) 1.82 - 1.93 (m, 4 H) 1.52 - 1.61 (m, 4 H) 1.48 (d, *J*=4.63 Hz, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 140.99 (s, 1 C) 128.27 (s, 1 C) 125.90 (s, 1 C) 124.79 (s, 1 C) 119.80 (s, 1 C) 113.97 (s, 1 C) 38.84 (s, 1 C) 32.70 (s, 1 C) 25.83 (s, 1 C) 22.77 (s, 1 C) LCMS (ESI^+) m/z 250.1 ($M+H^+$)

10H-spiro[acridine-9,1'-cyclohexane]-5-carbaldehyde (10): 10H-spiro[acridine-9,1'-cyclohexane] (**9**, 500 mg, 2.01 mmol) in diethyl ether (10 ml) was cooled to -78 ^{o} C and treated with n-butyl lithium (2.5 M, 2.41 mL, 6.02 mmol) and then allowed to warm to room temperature and stirred for four days. DMF (0.232 mL, 3.01 mmol) was added and the reaction mixture was stirred for an additional 24 hours, after which the reaction mixture was quenched with excess 0.5 M aqueous HCl. The solution was extracted with ether and the combined organics were washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude product was purified on a 40 g Silicycle cartridge eluting with 0-10 % EtOAc/hexanes. To further purify the product and separate it from residual starting material, the product obtained

from chromatography was triturated with hexane, with the product predominantly in the hexane phase. TLC and ^{1}H NMR showed that the product was still impure, and it was therefore carried onto the next step without further purification. For the crude product: ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 10.58 (br. s., 1 H), 10.01 (s, 1 H) 7.73 - 7.82 (m, 1 H), 7.54 - 7.62 (m, 1 H), 7.50 (dd, *J*=7.54, 1.32 Hz, 1 H) 6.99 -7.27 (m, 4 H), 6.87 (dd, *J*=7.72, 1.13 Hz, 1 H), 6.33 (br. s., 1 H) , 1.98 - 2.17 (m, 4 H) 1.52 - 1.83 (m, 6 H)

10H-spiro[acridine-9,1'-cyclohexane]-4-carboxylic acid (11): The crude 10H-spiro[acridine-9,1'-cyclohexane]-5-carbaldehyde (**10**, 453 mg, 1.63 mmol) from the previous step was dissolved in t-BuOH:water (4:1, 90 mL) and treated with NaH_2PO_4 hydrate (1.80 g, 13.1 mmol), 2-methyl-2-butene (1.73 mL, 6.54 mmol) and $NaClO_2$ (448 mg, 6.54 mmol), and then stirred overnight. The organics were then removed by rotary evaporation and the remaining aqueous mixture was partitioned between ether and 2 M aq. NaOH. The ether layer was separated and the aqueous layer was acidified with aqueous HCl to pH ~2 forming a milky solution that was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude product was purified on a 12g Silicycle column eluting with 0-10% MeOH/CH_2Cl_2 to afford the product (77.8 mg, 21.5 % over 2 steps). ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.47 - 1.81 (m, 6 H) 1.96 - 2.16 (m, 4 H) 6.92 - 7.11 (m, 3 H) 7.15 - 7.25 (m, 1 H) 7.47 - 7.59 (m, 1H) 7.71 - 7.80 (m, 1 H) 8.01 (dd, *J*=7.91, 1.32 Hz, 1 H) 10.10 (s, 1 H) ^{13}C NMR (CHLOROFORM-*d* 75 MHz,) δ ppm 23.19 (s, 1 C) 26.33 (s, 1 C) 33.27 (s, 1 C) 39.65 (s, 1 C) 109.87 (s, 1 C) 115.55 (s, 1 C) 119.04 (s, 1 C) 121.70 (s, 1 C) 124.90 (s, 1 C) 126.39 (s, 1 C) 129.33 (s, 1 C) 130.84 (s, 1 C) 131.78 (s, 1 C) 138.91 (s, 1 C) 144.58 (s, 1 C) 174.07 (s, 1 C)

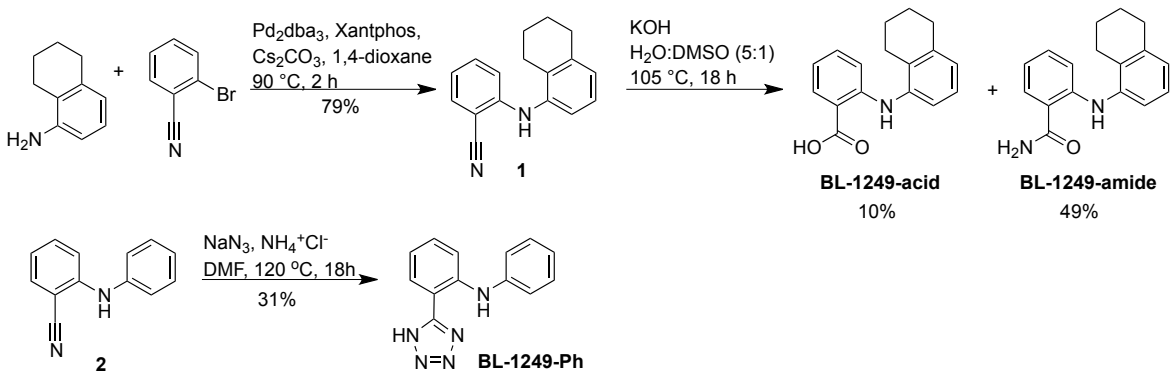
10H-spiro[acridine-9,1'-cyclohexane]-5-carboxamide (12): The intermediate 10H-spiro[acridine-9,1'-cyclohexane]-4-carboxylic acid (**11**, 58.7 mg, 0.200 mmol) was dissolved in THF (3 mL) and the mixture treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (115 mg, 0.600 mmol), 1-hydroxybenzotriazole hydrate (92 mg, 0.600 mmol), ammonium chloride (49.5 mg, 1.00 mmol), and trimethylamine (0.139 mL, 5 mmol). The

reaction mixture was stirred for 18 hours at room temperature. The solvent was evaporated and the residue dissolved in ethyl acetate and washed with half saturated aqueous $NaHCO_3$. The organic phase was washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude residue was purified on a 12 g Silicycle cartridge eluting with 0-100% EtOAc/hexanes to afford the product (41.2 mg, 0.141 mmol, 70%). ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 10.50 (s, 1 H) 7.66 (d, *J*=7.72 Hz, 1 H) 7.48 - 7.55 (m, 1 H) 7.36 (dd, *J*=7.91, 0.94 Hz, 1 H) 7.12 - 7.21 (m, 1 H) 7.02 (td, *J*=7.54, 1.32 Hz, 1 H) 6.88 - 6.99 (m, 2 H) 6.07 (br. s., 2 H) 1.95 - 2.06 (m, 3 H) 1.47 - 1.76 (m, 5 H) ^{13}C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 172.00 (s, 1 C) 143.19 (s, 1 C) 139.44 (s, 1 C) 131.28 (s, 1 C) 129.53 (s, 1 C) 128.92 (s, 1 C) 126.23 (s, 1 C) 124.84 (s, 1 C) 124.69 (s, 1 C) 121.11 (s, 1 C) 118.70 (s, 1 C) 115.34 (s, 1 C) 113.36 (s, 1 C), (s, 1 C) 39.63 (s, 1 C) 33.00 (s, 1 C) 26.31 (s, 1 C) 23.15 (s, 1 C); LCMS, ESI$^+$ $[M+H]^+$: 292.6

10H-spiro[acridine-9,1'-cyclohexane]-5-carbonitrile (13) To dry DMF (~1 mL) at 0 ^{o}C, oxalyl chloride (0.016 mL, 0.19 mmol) was slowly added via syringe. The mixture was stirred for five minutes and then dry pyridine (0.034 mL, 0.38 mmol) was added, followed by a solution of 10H-spiro[acridine-9,1'-cyclohexane]-5-carboxamide (**12**, 27.6 mg, 0.094 mmol) dissolved in dry DMF (0.75 ml). The reaction was allowed to proceed in the ice bath until the reaction was complete as judged by TLC. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous lithium chloride. The lithium chloride solution was extracted again with ethyl acetate. The combined organics were washed with brine, dried over sodium sulfate, filtered and concentrated. The crude product was purified on a 12 g Silicycle cartridge eluting with 0-20% EtOAc/hexanes to afford the title compound (18.4 mg, 0.067 mmol, 71%). ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 7.69 - 7.76 (m, 1 H) 7.51 - 7.58 (m, 1 H) 7.40 (dd, *J*=7.72, 1.13 Hz, 1 H) 7.21 (td, *J*=7.54, 1.32 Hz, 1 H) 7.09 (td, *J*=7.54, 1.32 Hz, 1 H) 6.93 - 7.04 (m, 3 H) 1.91 - 2.10 (m, 4 H) 1.49 - 1.76 (m, 6 H) ^{13}C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 143.12 (s, 1 C) 138.40 (s, 1 C) 130.62 (s, 1 C) 130.24 (s, 1 C) 129.29 (s, 1 C) 129.11 (s, 1 C) 126.65 (s, 1 C) 125.25 (s, 1 C) 122.28 (s, 1 C) 120.42 (s, 1 C) 117.41 (s, 1 C) 114.94 (s, 1 C) 96.17 (s, 1 C), 39.74 (s, 1 C) 33.18 (s, 1 C) 26.04 (s, 1 C) 23.03 (s, 1 C), LCMS, ESI$^+$ $[M+H]^+$: 275.5, 276.5

5-(1H-1,2,3,4-tetrazol-5-yl)-10H-spiro[acridine-9,1'-cyclohexane (BL-1249 tricycle) A sealed septum-capped vessel was charged with 10H-spiro[acridine-9,1'-cyclohexane]-5-carbonitrile (**13**, 16.5 mg, 0.060 mmol), sodium azide (13 mg, 0.2 mmol, 3.3 equiv.) and ammonium chloride (11 mg, 0.2 mmol, 3.6 equiv.). Dry DMF (1.5 mL) was added and the tube was flushed with dry nitrogen and sealed. The tube was then heated at 120°C 18 hrs with stirring in an aluminum heating block. The reaction mixture was cooled to room temperature, diluted with water, and the pH was adjusted to ~5 by addition of a few drops 1N HCl. The slurry was extracted with ethyl acetate once, then twice with 20% MeOH in CH_2Cl_2, and the organic layer washed with brine, dried with sodium sulfate, filtered, and concentrated. The residue was purified on a 12g Silicycle cartridge eluting with 0-10% MeOH/CH_2Cl_2 over 12 CV. After collecting and concentrating the relevant column fractions, the product contained residual DMF. The product was therefore dissolved in CH_2Cl_2, extracted with brine, dried with sodium sulfate, filtered and concentrated to afford the product (10 mg, 0.032 mmol, 53 %), ^{1}H NMR (400 MHz, METHANOL-*d*4) δ ppm 7.70 - 7.75 (m, 1 H) 7.69 (dd, J=7.79, 1.22 Hz, 1 H) 7.52 (dd, J=7.67, 0.85 Hz, 1 H) 7.06 - 7.17 (m, 2 H) 6.97 - 7.03 (m, 2 H) 1.96 - 2.09 (m, 4 H) 1.48 - 1.71 (m, 6 H); ^{13}C NMR (100 MHz, METHANOL-*d*4) δ ppm 24.44 (s, 1 C) 27.59 (s, 1 C) 34.31 (s, 1 C) 41.20 (s, 1 C) 108.12 (s, 1 C) 116.31 (s, 1 C) 121.22 (s, 1 C) 122.62 (s, 1 C) 126.19 (s, 1 C) 126.24 (s, 1 C) 127.60 (s, 1 C) 129.58 (s, 1 C) 130.40 (s, 1 C) 132.73 (s, 1 C) 141.08 (s, 1 C) 141.47 (s, 1 C) 157.17 (s, 1 C)

Scheme 1: BL-1249-acid, BL-1249-amide, BL-1249-Ph



Scheme 2: BL-1249-tricycle