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SUPPORTING INFORMATION

Repurposing human Aurora kinase inhibitors as leads for antiprotozoan drug discovery.

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SYNTHETIC CHEMISTRY

General Procedures. All starting materials were obtained commercially from Aldrich, Inc, or Fisher Scientific and were used without further purification. Reaction solvents were purified by passage through alumina columns on a purification system manufactured by Innovative Technology (Newburyport, MA). NMR spectra were obtained on Varian NMR systems, operating at 400 MHz or 500 MHz for ^{1}H acquisitions.

Analytical and purification. Preparative HPLC purification was performed using a FractionLynx system (Waters, Inc) consisting of a Waters 2525 binary pump, Waters 2767 sample manager, Waters 2489 UV/Visible detector, Waters 2x515 pumps, Waters pump control, Waters column fluidics organizer, MicroMass ZQ mass detector using electrospray ionization. Compound detection and mass-directed collection utilized UV: λ=254 nm and ESI+ mode. The Columns utilized were Waters SymmetryC8 30x50mm, 5µm or Waters Xbridge OBD RP18 30x50mm, 5µm, running A:B =H_2O/acetonitrile gradients (0.1% v/v formic acid). Compounds were analyzed using HPLC-MS, Waters e2795 Alliance HPLC separation module, Waters 2489 UV/visible detector, Waters LCT premier time-of-flight mass spectrometer (electrospray ionization). Columns used were Waters SunFire C18 4.6x50mm, 3.5µm or Waters SunFire C8 4.6x50mm, 3.5µm; running A:B = H_2O/acetonitrile gradients (0.1% v/v formic acid). Yields reported for compounds isolated by preparative HPLC purification corresponding to the purest fraction collected, which does not include the mass of impure fractions.

Synthesis.

(Z)-1-acetyl-3-(methoxy(phenyl)methylene)indolin-2-one (6a)

To a solution of 1-acetylindolin-2-one (**4**, 0.5 g, 2.85 mmol) in acetic anhydride (2.69 mL, 28.5 mmol) was added (trimethoxymethyl)benzene (1.225 mL, 7.14 mmol) at 25 °C and stirred for 3 h at 150 °C. After LCMS analysis, volatile substances were evaporated off under reduced pressure to afford crude product. The residue was purified via Biotage (0-100% EtOAc-Hexane

afforded pure (Z)-1-acetyl-3-(methoxy(phenyl)methylene)indolin-2-one (**6a**, 0.29 g, 34.6% yield). LCMS: 294.0 $(M+H)^+$

(Z)-1-acetyl-3-(methoxy(phenyl)methylene)-5-nitroindolin-2-one (6b)[1] To a solution of 5-nitroindolin-2-one (**5**, 1 g, 5.61 mmol) in acetic anhydride (11.46 g, 112 mmol) was added (trimethoxymethyl)benzene (3.07 g, 16.84 mmol) at 25 °C. The reaction mixture was stirred at 140 °C for 5 h. After LCMS analysis, organic volatile materials were evaporated off. The residue was purified via Biotage flash column chromatography (0-100% EtOAc-Hexanes) to afford (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-5-nitroindolin-2-one (**6b**, 0.86 g, 2.54 mmol, 45.3 % yield). LCMS: 339.0 $(M+H)^+$.

(Z)-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)-indolin-2-one (7a, NEU-507). A solution of (Z)-1-acetyl-3-(methoxy(phenyl)methylene)indolin-2-one (**6a**, 0.10 g, 0.341 mmol) and 4-(piperidin-1-ylmethyl)aniline (0.078 g, 0.409 mmol) in N,N-dimethylformamide (DMF) (0.5 mL) was stirred at 120 °C for 3h. N-deacylation was achieved by adding 2 M solution of sodium hydroxide (0.35 mL, 0.7 mmol) in MeOH (5 mL) to the reaction mixture and stirred at 25 °C for next 5 h. Desired product formation was confirmed by LCMS. Volatile substances were evaporated off, the residue was purified via Biotage flash column chromatography (20-100% EtOAc-Hexanes to afford (Z)-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)-indolin-2-one (**7a**, 47 mg, 0.115 mmol, 33.7 % yield) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 10.70 (s, 1H), 7.51 - 7.65 (m, 3H), 7.42 - 7.51 (m, 2H), 7.03 (d, *J* = 8.06 Hz, 2H), 6.80 - 6.92 (m, 2H), 6.73 (d, *J* = 7.33 Hz, 2H), 6.49 - 6.57 (m, 1H), 5.71 - 5.80 (m, 1H), 3.26 (br. s., 2H), 2.21 (br. s., 4H), 1.43 (br. s., 4H), 1.34 (br. s., 2H); LCMS: 410.2022 $(M+H)^+$.

(Z)-1-acetyl-5-nitro-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (7b, NEU-508). To a solution of (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-5-nitroindolin-2-one (**6b**, 0.8 g, 2.365 mmol) DMF (3.66 mL, 47.3 mmol) was added 4-(piperidin-1-ylmethyl)aniline (0.450 g, 2.365 mmol) at 25 °C and stirred at 120 °C for 90 min. After completion of the reaction (LCMS), the residue was triturated with MeOH. The resulting solid

was filtered through a Buchner funnel, washed with cold MeOH, and vacuum dried to afford (Z)-1-acetyl-5-nitro-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**7b**, 0.97 g, 1.953 mmol, 83 % yield) as light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.06 (s, 1H), 8.38 (d, *J* = 8.79 Hz, 1H), 7.92 (dd, *J* = 2.20, 8.79 Hz, 1H), 7.62 - 7.66 (m, 1H), 7.54 - 7.60 (m, 2H), 7.36 - 7.41 (m, 2H), 7.15 (d, *J* = 8.06 Hz, 2H), 6.80 (d, *J* = 8.79 Hz, 2H), 6.65 (d, *J* = 2.20 Hz, 1H), 3.41 (br. s., 2H), 2.85 (s, 3H), 2.35 (br. s., 4H), 1.52 - 1.81 (m, 4H), 1.43 (br. s., 2H); LCMS: 497.1$(M+H)^+$.

(Z)-1-acetyl-5-amino-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (8, NEU-509). To a solution of (Z)-1-acetyl-5-nitro-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**7b**, 0.57 g, 1.148 mmol) in MeOH (7.36 g, 230 mmol) and water (1.034 g, 57.4 mmol) was added ammonium chloride (0.553 g, 10.33 mmol). Small portions of zinc (0.451 g, 6.89 mmol) were added to the stirring reaction mixture at 25 °C. [Caution: Exotherm, gas evaluation] Reaction mixture was stirred for next 6 h. Volatile substances were evaporated off. To the residues, 25 mL of MeOH:DCM (1:1) was added and triturated for 10 min., inorganic residues were filtered off. The filtrate was evaporated and the residue was purified via Biotage flash column chromatography (MeOH:DCM 0-10%) afforded (Z)-1-acetyl-5-amino-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**8**, 0.32 g, 0.686 mmol, 59.7 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (s, 1H), 7.82 (d, *J* = 8.79 Hz, 1H), 7.50 - 7.63 (m, 3H), 7.44 (d, *J* = 7.33 Hz, 2H), 7.28 (d, *J* = 7.33 Hz, 2H), 6.87 (d, *J* = 8.06 Hz, 2H), 6.26 (d, *J* = 8.06 Hz, 1H), 5.11 (s, 1H), 4.05 (br. s., 1H), 2.72 (br. s., 4H), 2.65 (s, 3H), 1.66 (br. s., 6H); LCMS: 467.2 $(M+H)^+$.

(Z)-5-amino-3-(phenyl((4-(piperidin-1-ylmethyl)phenyl)amino)methylene)indolin-2-one (10, NEU-523). To a solution of (Z)-1-acetyl-5-amino-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**8**, 25 mg, 0.054 mmol) in MeOH (0.4 mL) was added 2M sodium hydroxide (0.027 mL, 0.054 mmol) at 25 °C. Reaction mixture was stirred for next 12 h, LCMS confirmed the product formation. Product was purified by preparative HPLC

afforded (Z)-5-amino-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**10**, 16.2 mg, 0.038 mmol, 71.2 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, 1H), 10.24 (s, 1H), 7.48 - 7.56 (m, 3H), 7.36 - 7.45 (m, 2H), 6.99 (d, *J* = 7.33 Hz, 2H), 6.64 (d, *J* = 7.33 Hz, 2H), 6.52 (d, *J* = 8.06 Hz, 1H), 6.20 (dd, *J* = 2.20, 8.06 Hz, 1H), 5.21 (s, 1H), 4.09 (br. s., 2H), 3.24 (s, 2H), 2.20 (br. s., 4H), 1.21 - 1.50 (m, 6H); LCMS: 425.4 $(M+H)^+$

(Z)-N-(2-oxo-3-(phenyl((4-(piperidin-1-ylmethyl)phenyl)amino)methylene)indolin-5-yl)methanesulfonamide (11a, NEU-521). The title compound was synthesized using same procedure of (Z)-N-(2-oxo-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-5-yl)benzenesulfonamide (**11b**). Instead of benzene sulfonyl chloride, methane sulfonyl chloride was used to afford (Z)-N-(2-oxo-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-5-yl)methanesulfonamide (**11a**, 6.5 mg, 0.013 mmol, 15.08 % yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 10.69 (s, 1H), 8.96 (s, 1H), 7.47 - 7.58 (m, 3H), 7.39 - 7.46 (m, 2H), 7.02 (d, *J* = 8.79 Hz, 2H), 6.67 - 6.80 (m, 4H), 5.83 (d, *J* = 1.47 Hz, 1H), 3.26 (s, 2H), 2.65 (s, 3H), 2.20 (br. s., 4H), 1.37 - 1.49 (m, 4H), 1.33 (d, *J* = 4.40 Hz, 2H); LCMS: 503.2 $(M+H)^+$.

(Z)-N-(2-oxo-3-(phenyl((4-(piperidin-1-ylmethyl)phenyl)amino)methylene)indolin-5-yl)benzenesulfonamide (11b, NEU-511). To the solution of (Z)-1-acetyl-5-amino-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**8**, 0.04 g, 0.086 mmol) in DMF (0.25 mL) was added triethylamine (0.036 mL, 0.257 mmol) followed by benzenesulfonyl chloride (0.013 mL, 0.103 mmol) at 0 °C. Reaction mixture was stirred for next 16 h at 25 °C. LCMS confirmed the formation of sulfonamide **9**. The hydrolysis of acetyl group was performed by treating reaction with 2M aqueous solution of sodium hydroxide (0.257 mL, 0.514 mmol) in MeOH (0.400 mL). Reaction mixture was stirred at 25 °C for next 18 h. Final product formation was confirmed by LCMS. Organic volatile substances were evaporated off under reduced pressure and crude reaction mass was loaded on Biotage column (DCM prime before loading sample MeOH-DCM: gradient 0-25%). The most purified fractions were combined, evaporated

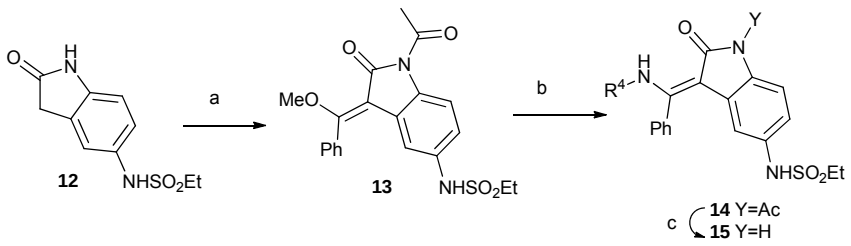
and re purified by Prep. HPLC afforded (Z)-N-(2-oxo-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-5-yl)benzenesulfonamide (**11b**, 17.8 mg, 0.032 mmol, 36.8 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, 1H), 10.66 (br. s., 1H), 9.54 (s, 1H), 7.23 - 7.73 (m, 10H), 6.61 - 6.84 (m, 3H), 6.55 (d, *J* = 6.60 Hz, 1H), 5.84 (br. s., 1H), 4.0 (br. s., 2H), 3.17 (br. s., 2H), 2.22 (br. s., 2H), 1.19 - 1.89 (m, 8H); LCMS: 565.3 $(M+H)^+$.

(Z)-N-(2-oxo-3-(phenyl((4-(piperidin-1-ylmethyl)phenyl)amino)methylene)indolin-5-yl)acetamide (11c, NEU-522) To the solution of (Z)-1-acetyl-5-amino-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-2-one (**8**, 25 mg, 0.054 mmol) in DMF (0.2 mL) was added triethylamine (0.022 mL, 0.161 mmol) followed by acetyl chloride (5.71 µl, 0.080 mmol) at 0 °C. Reaction mixture was stirred for next 12 h at 25 °C. LCMS confirmed the product formation. The hydrolysis of oxindole ring N-acetyl group was performed by treating reaction with 2M aqueous solution of sodium hydroxide (0.027 mL, 0.054 mmol) in MeOH (0.400 mL). Reaction mixture was stirred at 25 °C for next 18 h. LCMS confirmed the product formation. Volatile substances were distilled off under reduced pressure, the residue was purified via prep HPLC, 8 min run afforded (Z)-N-(2-oxo-3-(phenyl(4-(piperidin-1-ylmethyl)phenylamino)methylene)indolin-5-yl)acetamide (6 mg, 0.013 mmol, 24.00 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 10.66 (s, 1H), 9.35 (s, 1H), 7.54-7.57 (m, , 3H), 7.45 (d, *J* = 7.33 Hz, 2H), 7.24 (br. s., 2H), 7.03 (d, *J* = 8.06 Hz, 1H), 6.69 - 6.77 (m, 4H), 6.38 (br. s., 1H), 4.09 (br. s., 2H), 3.30 (br. s., 2H), 3.18 (br. s., 2H), 2.73 (br. s., 2H), 1.84 (s, 3H), 1.55 - 1.80 (m, 2H); LCMS: 468.0 $(M+2)^+$.

N-(2-oxoindolin-5-yl)ethanesulfonamide (12)

To a solution of 5-nitroindolin-2-one (8.5 g, 47.7 mmol) in MeOH (87 mL, 2147 mmol) and water (8.60 mL, 477 mmol) was added ammonium chloride (7.66 g, 143 mmol). Reflux condencer was fixed to RBF and at 25 °C to the reaction mixture added zinc (6.24 g, 95 mmol) in portions [Caution: Exothermic, vigrous gas evaluation]. Continued stirring for next 6 h, after completion of the reaction, volatile substances were evaporated off under reduced pressure to ensure no

trace of water present in the reaction mass. The solid mass was triturated with 15 mL of DMF, warmed it to 50 °C to dissolve maximum amount of product and solid inorganic salts and residues were filtered off, washed with 2 mL of DMF. In RBF to the combine DMF filtrate, added triethylamine (6.65 mL, 47.7 mmol) followed by ethanesulfonyl chloride (4.52 mL, 47.7 mmol) at 0 °C. Reaction mixture was stirred at 25 °C for 12 h. LCMS confirmed the product formation. Volatile substances were evaporated off under reduced pressure and the residue was purified via flash column chromatography (silica gel 230-400 mesh size, 50% to 100% EtOAc - Hexane, Large glass column) afforded N-(2-oxoindolin-5-yl)ethanesulfonamide (**12**, 2.62 g, 10.90 mmol, 22.85 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.43 (s, 1H), 7.08 (s, 1H), 7.01 (d, *J* = 10.26 Hz, 1H), 6.74 (d, *J* = 8.06 Hz, 1H), 3.45 (s, 2H), 2.96 (q, *J* = 7.33 Hz, 2H), 1.17 (t, *J* = 7.33 Hz, 3H); LCMS: 241.0 $(M+H)^+$.



Scheme for general procedure A: *Reagents and conditions.* (a) $PhC(OCH_3)_3$, Ac_2O; (b) R-NH_2, DMF; (c) NaOH, MeOH.

General procedure A:

(Z)-N-(1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide (13). A solution of N-(2-oxoindolin-5-yl)ethanesulfonamide (**12**, 0.75 g, 3.12 mmol)and (trimethoxymethyl)benzene (2.68 mL, 15.61 mmol) in acetic anhydride (7.5 mL, 78 mmol) was stirred at 140 °C for 5 h. Reaction progress was monitored by LCMS (M+H)+ : 443.1. Volatile organic substances were evaporated off under reduced pressure at 130 °C to afford dark brown crude mass. The residue was purified via Biotage flash column

chromatography (0-100% EtOAc-Hexanes) to afford 0.4 g of mixture of mono and di N-Acyl products. This mixture was used as such without further purification for next reaction.

To a solution of (Z)-N-(1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide (**13**, 50 mg, 0.113 mmol) in DMF (0.25 mL) was added respective amine (0.226 mmol). Reaction mixture was stirred at 100 °C for 5 h. LCMS confirmed the formation of **14**. After completion of the reaction, N-deacylation was achieved by adding 2 M solution of sodium hydroxide (0.113 mL, 0.226 mmol) in MeOH (0.5 mL) to the reaction mixture and stirred at 25 °C for next 18 h. Desired product formation of **15** was confirmed by LCMS. Organic volatile substances were evaporated off under reduced pressure and crude reaction mass was loaded on Biotage column (DCM prime before loading, eluted in 0-25% MeOH-DCM). In case where the fractions were not pure enough, most purified fractions were combined, evaporated and re purified by Prep. HPLC.

(Z)-N-(3-(((1-methylpiperidin-4-yl)amino)(phenyl)methylene)-2-oxoindolin-5-yl)ethanesulfonamide formate salt (15a, NEU-516). Prepared using General Procedure A. Yield: 17%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 10.39 (d, *J* = 8.79 Hz, 1H), 8.93 (s, 1H), 8.16 (s, 1H), 7.57 - 7.66 (m, 3H), 7.39 - 7.46 (m, 2H), 6.70 - 6.74 (m, 1H), 6.62 - 6.67 (m, 1H), 5.49 (s, 1H), 3.06 (br. s., 2H), 2.57 - 2.73 (m, 3H), 2.14 (s, 3H), 1.91 (br. s., 2H), 1.75 (d, *J* = 10.99 Hz, 2H), 1.53 (q, *J* = 10.26 Hz, 2H), 1.05 (t, *J* = 7.33 Hz, 3H); LCMS: 441.2 $(M+H)^+$.

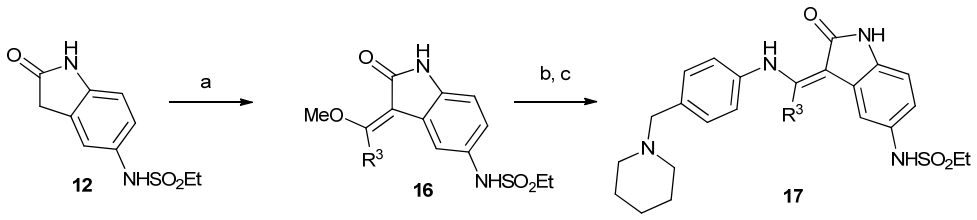
(Z)-N-(2-oxo-3-(phenyl(phenylamino)methylene)indolin-5-yl)ethanesulfonamide (15b, NEU-518). Prepared using General Procedure A. Yield: 38%;^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 10.58 (br. s., 1H), 9.04 (br. s., 1H), 7.47 - 7.57 (m, 3H), 7.39 - 7.46 (m, 2H), 7.12 (t, *J* = 7.69 Hz, 2H), 6.92 - 7.01 (m, 1H), 6.67 - 6.80 (m, 4H), 5.76 (s, 1H), 2.61 (q, *J* = 6.84 Hz, 2H), 1.02 (t, *J* = 7.33 Hz, 3H); LCMS: 420.1 $(M+H)^+$.

(Z)-N-(2-oxo-3-(phenyl(p-tolylamino)methylene)indolin-5-yl)ethanesulfonamide (15c, NEU-519). Prepared using General Procedure A. Yield: 43%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 10.35 (br. s., 1H), 7.44 - 7.56 (m, 3H), 7.39 (m, 3H), 6.91 (d, *J* = 8.06 Hz, 2H),

6.54 - 6.67 (m, 4H), 5.62 (br. s., 1H), 2.57 (q, *J* = 6.84 Hz, 2H in DMSO-d_6 peak), 2.14 (s, 3H), 0.97 (t, *J* = 7.33 Hz, 3H); LCMS: 434.1 $(M+H)^+$.

(Z)-N-(3-(((4-(morpholinomethyl)phenyl)amino)(phenyl)methylene)-2-oxoindolin-5-yl)ethanesulfonamide (15d, NEU-510). Prepared using General Procedure A. Yield: 17%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 10.70 (s, 1H), 9.04 (br. s., 1H), 7.38 - 7.60 (m, 5H), 7.05 (d, *J* = 8.06 Hz, 2H), 6.66 - 6.81 (m, 4H), 5.83 (d, *J* = 2.20 Hz, 1H), 3.51 (t, *J* = 4.40 Hz, 4H), 3.31 (s, 2H), 2.70 (q, *J* = 7.33 Hz, 2H), 2.24 (br. s., 4H), 1.06 (t, *J* = 7.33 Hz, 3H); LCMS: 519.2001 $(M+H)^+$.

(Z)-N-(2-oxo-3-(phenyl((4-(piperidin-1-yl)phenyl)amino)methylene)indolin-5-yl)ethanesulfonamide (15e, NEU-515). Prepared using General Procedure A. Yield: 16%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 10.62 (s, 1H), 8.99 (s, 1H), 7.45 - 7.59 (m, 3H), 7.34 - 7.45 (m, 2H), 6.74 - 6.83 (m, 1H), 6.58 - 6.84 (m, 5H), 5.76 (s, 1H), 3.00 (br. s., 4H), 2.69 (q, *J* = 7.33 Hz, 2H), 1.40 - 1.60 (m, 6H), 1.05 (t, *J* = 7.33 Hz, 3H); LCMS: 503.2 $(M+H)^+$.



Scheme for General procedure B: *Reagents and conditions.* (a) $R^3C(OCH_3)_3$, Ac_2O; (b) 4-(piperidin-1-ylmethyl)aniline, DMF; (c) NaOH, MeOH..

General procedure B. To a 8 mL glass vial (combi block) weighed N-(2-oxoindolin-5-yl)ethanesulfonamide (**12**, 0.125 g, 0.520 mmol) and trialkoxyalkane/arene (2.60 mmol). To the reaction mixture added acetic anhydride (1 mL, 10.60 mmol) and stirred at 130 °C for 18 h. LCMS confirmed the product formation. Volatile organic substances were evaporated off under reduced pressure at 130 °C afforded crude (Z)-N-(1-acetyl-3-(1-(alkoxy) aryl or alkylidene)-2-

oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide which was used without further purification immediately.

To a solution of (Z)-N-(1-acetyl-3-(1-(alkoxy) aryl or alkylidene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide (0.45 mmol) in DMF (0.4 mL) was added 4-(piperidin-1-ylmethyl)aniline (0.675 mmol, 1.5 eq). Reaction mixture was stirred at 100 °C for 6 h. LCMS confirmed the product formation (mono and di N-acyl product). The hydrolysis of acetyl group was performed by treating reaction with 2M aqueous solution of sodium hydroxide (0.9 mmol, 2 eq) in MeOH (0.4 mL). Reaction mixture was stirred at 25 °C for next 18 h.

Final de acylated product formation was confirmed by LCMS. Organic volatile substances were evaporated off under reduced pressure and crude reaction mass was purified via flash column chromatography (MeOH-DCM 0-25%) and/or Preparative HPLC afforded purest fractions.

N-(2-oxo-3-(((4-(piperidin-1-ylmethyl)phenyl)amino)methylene)indolin-5-yl)ethanesulfonamide (17a, NEU-520). Prepared using General Procedure B. A solution of N-(2-oxoindolin-5-yl)ethanesulfonamide (**12**, 0.1 g, 0.416 mmol), triethoxymethane (0.692 mL, 4.16 mmol) in acetic anhydride (1 mL, 10.60 mmol) was stirred at 130 °C for 7 h. Title compound was prepared following general procedure B afforded crude (Z)-N-(1-acetyl-3-(ethoxymethylene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide Second step yield 38.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (d, *J* = 12.46 Hz, 1H), 10.48 (s, 1H), 9.32 (s, 1H), 8.62 (d, *J* = 12.46 Hz, 1H), 7.46 (s, 1H), 7.21 - 7.38 (m, 4H), 6.75 - 6.87 (m, 2H), 3.37 (s, 2H), 2.98 (q, *J* = 7.33 Hz, 2H), 2.29 (br. s., 4H), 1.33 - 1.54 (m, 6H), 1.22 (t, *J* = 7.33 Hz, 3H); LCMS: 441.3 $(M+H)^{+}$.

N-(2-oxo-3-(1-((4-(piperidin-1-ylmethyl)phenyl)amino)ethylidene)indolin-5-yl)ethanesulfonamide (17b, NEU-512) Prepared following general procedure B. 1,1,1-triethoxyethane (0.474 mL, 2.60 mmol) was used for first step resulted crude (Z)-N-(1-acetyl-3-(1-ethoxyethylidene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide.

Second step yield 4.22%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.20 (s, 1H), 10.55 (s, 1H), 9.32 (s, 1H), 8.15 (s, 1H), 7.35 (d, *J* = 8.06 Hz, 2H), 7.19 - 7.31 (m, 2H), 6.79 - 6.92 (m, 2H), 3.51 (s, 2H), 2.96 (q, *J* = 7.33 Hz, 2H), 2.46 (s, 3H), 2.30 - 2.44 (m, 4H), 1.34 - 1.58 (m, 7H), 1.18 (t, *J* = 7.33 Hz, 3H); LCMS: 455.2 $(M+H)^+$.

(Z)-N-(2-oxo-3-(1-((4-(piperidin-1-ylmethyl)phenyl)amino)propylidene)indolin-5-yl)ethanesulfonamide (17c, NEU-514) Prepared following general procedure B. 1,1,1-trimethoxypropane (0.370 mL, 2.60 mmol) was used for first step resulted crude (Z)-N-(1-acetyl-3-(1-methoxypropylidene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide. Second step yield 4.01%; ^{1}H NMR (400 MHz, DMSO-d_6) δ□□12.16 (s, 1H), 10.55 (s, 1H), 9.32 (s, 1H), 7.36 (d, *J* = 7.33 Hz, 2H), 7.19 - 7.28 (m, 3H), 6.78 - 6.91 (m, 2H), 3.44 (br. s., 2H), 2.95 (q, *J* = 7.33 Hz, 2H), 2.71 (m, 2H), 2.33 (br. s., 4H), 1.30 - 1.59 (m, 6H), 1.09 - 1.29 (m, 6H); LCMS: 470.2 $(M+2H)^+$.

(Z)-N-(2-oxo-3-(1-((4-(piperidin-1-ylmethyl)phenyl)amino)pentylidene)indolin-5-yl)ethanesulfonamide (17d, NEU-513). Prepared following general procedure B. 1,1,1-trimethoxypentane (0.448 mL, 2.60 mmol) was used for first step resulted crude (Z)-N-(1-acetyl-3-(1-methoxypentylidene)-2-oxoindolin-5-yl)-N-(ethylsulfonyl)acetamide. Second step yield 4.01%; ^{1}H NMR (400 MHz, DMSO-d_6) δ□ 12.18 (s, 1H), 10.55 (s, 1H), 9.38 (s, 1H), 7.36 (d, *J* = 7.33 Hz, 2H), 7.23 (d, *J* = 8.06 Hz, 2H), 7.17 (s, 1H), 6.79 - 6.91 (m, 2H), 3.34 - 3.55 (m, 2H), 2.94 (q, *J* = 7.33 Hz, 2H), 2.62 - 2.76 (m, 2H), 2.31 (br. s., 4H), 1.43 - 1.64 m, 6H), 1.28 - 1.42 (m, 4H), 1.17 (t, *J* = 7.33 Hz, 3H), 0.76 (t, *J* = 7.33 Hz, 3H); LCMS: 497.3 $(M+H)^+$.

CELL ASSAY PROTOCOLS

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

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

Trypanosoma cruzi. In a 96-well plate, 100 µL 3T3 cells in DMEM without phenol red supplemented with 2% FBS were added to each well at $5x10^5$ cells/mL ($5x10^4$ cells/well). Cells were incubated for 2 h to allow for attachment. Compounds were then added at the specified concentrations. To each well, 100 µL of parasites (*T. cruzi* expressing β-galactosidase) in the aforementioned media were added at a concentration of $5x10^5$ cells/mL ($5x10^4$ cells/well) and incubated at 37°C, 5% CO_2 for 96 h. Following incubation, 50 µL of 500 µM chlorophenol Red-β-D-galactopyranoside (CPRG) in PBS with 0.5% IGEPAL CA-630 was added to each well and incubated at 37°C, 5% CO_2 for 4 hours. Absorbance was read at 590-595 nm.

***Leishmania major* promastigotes**. The promastigote assay is a microtiter plate drug sensitivity prescreen assay used to determine antileishmanial activity of candidate drugs against promastigote forms of *L. major*. Alamar Blue, or resazurin, is a non-fluorescent indicator dye that is converted to bright red–fluorescent resorufin via the activity of mitochondrial reductases. As the activity of mitochondrial reductases is reduced in cells that are dead or dying, and the intensity of the fluorescence is relative to cell number, a test compound that kills or inhibits

growth of a particular target cell, such as *L. major*, will result in a lower production of fluorescent signal. This simple assay is widely used for both toxicity and proliferation assays, and it was adapted for use in Leishmania drug discovery as shown by Sharlow.[2] Pre-dosed microtiter drug plates for use in the promastigote drug prescreen assay were produced using sterile 384-well black optical bottom tissue culture plates. Candidate drugs were diluted in dimethyl sulfoxide in four 96-well plates to either 10,000 ng/ml or 1,000 ng/ml, and 4.25 µLof diluted drug was subsequently dispensed into each well of a 384-well plate. Duplicate 384 well plates were made at each test concentration (10,000 ng/mL and 1,000 ng/mL) and amphotericin B was used as a batch control. The Tecan EVO Freedom liquid handling system (Tecan US, Inc., Durham, NC) was used to produce all drug assay plates and conduct all pipetting operations for this assay. *L. major* parasites were cultured in Schneider's medium supplemented with 20% heat inactivated FBS. Promastigotes in early log growth phase were harvested from culture, counted, suspended at $1.32x10^5$ cells/mL and 5,000 promastigotes were dispensed into each well of the 384 well plate in a volume of 38.8 µL. The 384 well plates were subsequently incubated at 28°C for 44 hours. 8.4µL of Alamar Blue was added to each well, the plates were subsequently incubated at 28°C and 5% CO_2 for 4 hours and then examined for the relative fluorescence units (RFU) per well using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation set at 560 nm and emission set at 590 nm. The relative fluorescence from each well was used to determine the percent growth inhibition of each candidate compound tested. Compounds with 50% or greater inhibition of growth were selected for further analysis to determine IC_{50} values.

***Leishmania major* amastigotes**.[3] The intracellular amastigote assay is a microtiter plate drug sensitivity assay that uses the activity of luciferase as a measure of proliferation of luciferase-expressing *L. major* parasites developing intracellularly inside RAW 264.7 macrophages in the presence of antileishmanial drugs or experimental compounds. As the activity of luciferase and its associated luminescence after addition of the substrate, luciferin, is relative to parasite

growth, a test compound that inhibits the growth of the parasite will result in a lower luminescence. The luciferase-expressing *L. major* parasite used in this assay is genetically modified by adding the luciferase coding region to the pLEXSY-hyg2 vector (Jena Biosciences). The luciferase expression construct was created by digesting the luciferase coding region (1.66kbp) of pGL3-Basic (Promega) by using two restriction enzymes, NcoI/EagI, followed by electrophoretic separation of the luciferase coding region on a 1% agarose gel. The luciferase coding region was then ligated into pLEXSY-hyg2 vector (Jena Biosciences) which had previously been digested with NcoI/NotI. The vector was linearized with SwaI and subsequently gel purified prior to transfection into *L. major* parasites. Transfections were carried out by electroporation at 480V, 13 Ω, and 500μF (0.4mL of $1x10^8$parasites/mL, and 0.1mL of 10μg DNA). Selection for transfectants was then carried out using hygromycin B (100μg/mL). RAW 264.7 macrophages were cultured in DMEM media supplemented with heat-inactivated 10% FBS. To begin the assay, macrophages were harvested from culture by removing all spent media, adding in 10mL fresh media, scraping cells, and counted using Trypan Blue. The cells were resuspended in DMEM/10% HIFBS media at $2.0x10^5$ cells/mL, and then dispensed in a volume of 50 μL to yield a final concentration of 10,000 macrophages/well in 384 well tissue-culture treated sterile white plates using a Tecan EVO Freedom robotics system. The plates were then incubated at 37^o C in 5% CO_2 atmosphere for 24 hours. After incubation, the media was removed from each well using the Tecan EVO Freedom robot, and *L. major* promastigotes were added to each well and allowed to invade the RAW macrophages. Promastigotes were cultured in Schneider's medium supplemented with 20% heat inactivated FBS. Metacyclic stage promastigotes provide the best invasion for this assay, and cultures should be grown to increase the percentage of metacyclic promastigotes for best results. Promastigotes were harvested from culture, counted, suspended at $2x10^6$ promastigotes/mL in DMEM/HIFBS media, and 100,000 promastigotes were dispensed per well in a volume of 50 μL. After overnight incubation, the media was removed from each well using the Tecan Freedom EVO robot, and

each well was subsequently washed three times with 40 µL of fresh DMEM/HIFBS medium to remove all extracellular promastigotes. After the third wash, 69.2µL of DMEM/HIFBS medium was added to each well using the Tecan EVO Freedom robot. Drug plates were prepared with the Tecan EVO Freedom using sterile 96 well plates containing twelve duplicate two-fold serial dilutions of each test compound suspended in DMSO. 7.8 µL of diluted test compound was added to the 69.2 µL of media present in each well providing a 10 fold final dilution of compound. The final concentration range tested was 0.5 to 10,000 ng/ml for all assays. The plates were next incubated at 37°C and 5% CO_2 for 96 hours. After 96 hours of incubation, 7.5 µL of a luciferin solution (Caliper Life Science) diluted to 150µg/mL was added to each well, and the plates were incubated for 30 minutes at 37°C, in the dark. Each plate was read using a Infinite M200 plate reader. The 50% inhibitory concentrations (IC_{50}s) were then generated for each dose response test using GraphPad Prism (GraphPad Software Inc., San Diego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

***Plasmodium falciparum* assay.** The SYBR Green fluorescence (MSF) Assay is a microtiter plate drug sensitivity assay that uses the presence of malarial DNA as a measure of parasitic proliferation in the presence of antimalarial drugs or experimental compounds. As the intercalation of SYBR Green I dye and its resulting fluorescence is relative to parasite growth, a test compound that inhibits the growth of the parasite will result in a lower fluorescence. D6 (CDC/Sierra Leone), TM91C235 (WRAIR, Thailand), and W2 (CDC/Indochina III) laboratory strains of *P. falciparum* were used for each drug sensitivity assessment. The parasite strains were maintained continuously in long-term cultures as previously.[4] Pre-dosed microtiter drug plates for use in the MSF assay were produced using sterile 384-well black optical bottom tissue culture plates containing duplicate or quadruplicate 12 two-fold serial dilutions of each test compound suspended in dimethyl sulfoxide. The final concentration range tested was 0.5 – 10000 ng/ml for all assays. Predosed plates were stored at 4°C until used, not to exceed five

days. No difference was seen in drug sensitivity determinations between stored or fresh drug assay plates (data not shown). A batch control plate using Chloroquine (Sigma-Aldrich) at a final concentration of 2000 ng/ml was used to validate each assay run. The Tecan Freedom Evo liquid handling system (Tecan US, Inc., Durham, NC) was used to produce all drug assay plates. Based on modifications of previously described methods by Plouffe and Johnson,[4, 5] *P. falciparum* strains in late-ring or early-trophozoite stages were cultured in the predosed 384-well microtiter drug assay plates in 38 µl culture volume per well at a starting parasitemia of 0.3% and a hematocrit of 2%. The cultures were then incubated at 37°C within a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2, for 72 hours. Lysis buffer (38 µl per well), consisting of 20mM Tris HCl, 5mM EDTA, 1.6% Triton X, 0.016% saponin, and SYBR green I dye at a 20x concentration was then added to the assay plates for a final SYBR Green concentration of 10x. The Tecan Freedom Evo liquid handling system was used to dispense malaria cell culture and lysis buffer. The plates were then incubated in the dark at room temperature for 24 hours and examined for the relative fluorescence units (RFU) per well using the Tecan Genios Plus (Tecan US, Inc., Durham, NC). Each drug concentration was transformed into Log[X] and plotted against the RFU values. The 50% and 90% inhibitory concentrations (IC50s and IC90s, respectively) were then generated with GraphPad Prism (GraphPad Software Inc., SanDiego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

HepG2 Toxicity Assay.[6] The MTT counter screen assay is an *in vitro* microtiter toxicology test used to determine toxicity of drug compounds against the primary target of interest, such as a HepG2 liver cell, determine the relative toxicity of one compound versus another within a chemical series, aid in determining the toxicology structure relationship within a chemical series, and prioritize compounds for advancement for *in vivo* studies. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a monotetrazolium salt that is reduced by mitochondria

reductases to create a formazan dye, which has a purple color. As the activity of mitochondrial reductases is reduced in cells that are dead or dying, and the intensity of the purple color is relative to cell number and metabolism, a test compound that is toxic to a particular target cell, such as the HepG2 liver cell, will result in a lower production of formazan dye and reduced purple color. This simple colorimetric test is widely used for both toxicity and proliferation assays. The MTT counterscreen is used in concert with the malaria inhibition of liver stage development assay (ILSDA) as a toxicity counter screen. Interpretation of the IC_{50} determination of potentially malaria liver stage active compounds requires a toxicity test to insure that the activity observed is specific to malaria and not simply toxicity directed against a HepG2 cell. The 384 well MTT cytotoxicity assay is a modification of the MTT method described by Ferrari[6] optimized for 384 well throughput. The HepG2 target cells for this assay were cultured as follows: HepG2 cells were cultured in complete Minimal Essential Medium prepared by supplementing MEM with 0.19% sodium bicarbonate, 10% heat inactivated FBS, 2 mM L-glutamine, 0.1 mM MEM non-essential amino, 0.009 mg/ml insulin, 1.76 mg/ml bovine serum albumin, 20 units/ml penicillin–streptomycin, and 0.05 mg/ml gentamycin. HepG2 cells cultured in complete MEM were first washed with 1X Hank's Balanced Salt Solution (Invitrogen #14175-095), trypsonized using a 0.25% trypsin/EDTA solution, assessed for viability using trypan blue, and resuspended at 250,000 cells/ml. Using a Tecan EVO Freedom robot, 38.3 µL of cell suspension were added to each well of clear, cell culture-treated 384-well microtiter plates for a final concentration of 9570 liver cells per well, and plated cells were incubated overnight in 5% CO_2 at 37^{o}C. Drug plates were prepared with the Tecan EVO Freedom using sterile 96 well plates containing twelve duplicate 1.6-fold serial dilutions of each test compound suspended in DMSO. 4.25 µL of diluted test compound was then added to the 38.3 µL of media in each well providing a 10 fold final dilution of compound. Compounds were tested from a range of 57 ng/ml to 10,000 ng/ml for all assays. Mefloquine was used as a plate control for all assays with a concentration ranging from 113 ng/ml to 20,000 ng/ml. After a 48 hour incubation period, 8 µL of

a 1.5 mg/ml solution of MTT diluted in complete MEM media was added to each well. All plates were subsequently incubated in the dark for 1 hour at room temperature. After incubation, the media and drugs in each well was removed by shaking the plate over sink, and the plates were left to dry in a fume hood for 15 minutes. Next, 30 µL of isopropanol acidified by addition of HCl at a final concentration of 0.36% was added to dissolve the formazan dye crystals created by reduction of MTT. Plates are put on a 3-D rotator for 15-30 minutes. Absorbance was determined in all wells using a Tecan iControl 1.6 Infinite plate reader. The 50% inhibitory concentrations (IC50s) were then generated for each toxicity dose response test using GraphPad Prism (GraphPad Software Inc., SanDiego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

SCREENING DATA

Table S1. Table 2 from the manuscript is recapitulated here with NEU registry numbers. This information will assist searching of these compounds within the shared data set at http://www.collaborativedrug.com

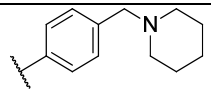
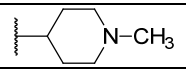
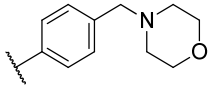
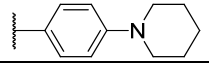
						EC_{50} (μM)					TC_{50} (μM)
Cmpd	NEU #	R^1	R^2	R^3	R^4	*T brucei*	*T. cruzi*	*L. major* Promastig	*L. major* Amastig	*P. falciparum*	HepG2
1	NEU517	$NHSO_2Et$	H	Ph		0.06	39	0.12	2.37	0.01	<0.2
7a	NEU507	H	H	Ph		0.07	25	0.77	>4	0.230	> 6.0
7b	NEU508	NO_2	Ac	Ph		0.13	3	1.08	2.04	0.275	> 6.0
8	NEU509	NH_2	Ac	Ph		3.25	45	>4	>4	0.218	1.81
10	NEU523	NH_2	H	Ph		1.16	>50	0.79	>4	0.199	> 6.0
11a	NEU521	$NHSO_2Me$	H	Ph		0.02	>50	0.39	>4	0.052	0.2
11b	NEU511	$NHSO_2Ph$	H	Ph		0.01	16	0.85	1.91	0.107	3.29
11c	NEU522	NHAc	H	Ph		0.15	>50	1.52	>4	0.068	> 6.0
17a	NEU520	$NHSO_2Et$	H	H		1.32	>50	2.77	>4	0.241	5
17b	NEU512	$NHSO_2Et$	H	Me		3.36	>50	>4	>4	1.364	> 6.0
17c	NEU514	$NHSO_2Et$	H	Et		1.79	>50	2.8	>4	0.233	> 6.0
17d	NEU513	$NHSO_2Et$	H	*n*-Bu		1.67	>50	2.6	>4	0.167	> 6.0
15a	NEU516	$NHSO_2Et$	H	Ph		>50	>50	>4	>4	>15	>10.0
15b	NEU518	$NHSO_2Et$	H	Ph	Ph	1.44	>50	>4	>4	0.109	4.7
15c	NEU519	$NHSO_2Et$	H	Ph	p-Tol	0.37	>50	2.6	>4	1.607	3.1
15d	NEU510	$NHSO_2Et$	H	Ph		0.1	>50	0.94	1.68	0.054	1.31
15e	NEU515	$NHSO_2Et$	H	Ph		1.53	>50	0.62	>4	0.595	> 6.0

Table S2. Screening of compounds against resistant strains of *Plasmodium falciparum.*

		***Plasmodium falciparum* EC_{50} (uM)**			
Cmpd	**NEU#**	**D6**	**W2**	**C235**	**C2B**
7a	NEU507	0.23	0.273	0.227	0.16
7b	NEU508	0.275	0.307	0.271	0.249
8	NEU509	0.218	0.241	0.243	0.21
15d	NEU510	0.054	0.077	0.031	nd[a]
11b	NEU511	0.107	0.144	0.12	0.082
17b	NEU512	1.364	1.255	1.189	1.062
17d	NEU513	0.167	0.217	0.17	0.148
17c	NEU514	0.233	0.347	0.255	0.143
15e	NEU515	0.595	0.832	0.647	0.735
15a	NEU516	> 15.0	> 15.0	> 15.0	> 15.0
1	NEU517	0.01	0.021	0.01	0.014
15b	NEU518	0.109	0.143	0.118	0.117
15c	NEU519	1.607	2.49	1.635	1.571
17a	NEU520	0.241	0.273	0.242	0.173
11a	NEU521	0.052	0.086	0.058	nd[a]
11c	NEU522	0.068	0.088	0.074	0.056
10	NEU523	0.199	0.228	0.176	0.175

[a]Not determined.

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