Supporting Information

N-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)arylamide as a New Scaffold that Provides Rapid Access to Antimicrotubule Agents: Synthesis and Evaluation of Antiproliferative Activity Against Select Cancer Cell Lines

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Contents

Chemistry: General Information

All reagents were purchased from Aldrich, Arcos, Advanced ChemTech, and Fisher and used without purification unless otherwise indicated. Tetrahydrofuran was distilled from a mixture of sodium metal and benzophenone ketyl. Acetonitrile and triethylamine were distilled from CaH_2. All other solvents were used without purification.

NMR spectra were recorded on a Varian Unityplus 300 MHz spectrometer, Varian Inova 500 MHz spectrometer, or Varian 600 MHz spectrometer at ambient temperature. Chemical shifts are indicated in δ values (ppm) from internal reference peaks (TMS ^{1}H 0.00; $CDCl_3$ ^{1}H 7.27, ^{13}C 77.00; CD_3OD ^{1}H 3.31, ^{13}C 49.15; DMSO-d6 ^{1}H 2.50, ^{13}C 39.51). Coupling constants (*J*) were measured using ACD/SpecManager Version 10 and are reported in hertz (Hz) (where, s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt = triple triplet, q = quartet, m = multiplet).

Infrared spectra were recorded on an FT-IR spectrometer using thin films on NaCl plates. Melting points were measured on a Thomas Hoover melting point apparatus and are uncorrected. TLC was performed with aluminum backed Merck silica gel 60 F_{254} using UV (254 nm) light, potassium permanganate stain, ceric ammonium molybdate stain, or ninhydrin for visualization.

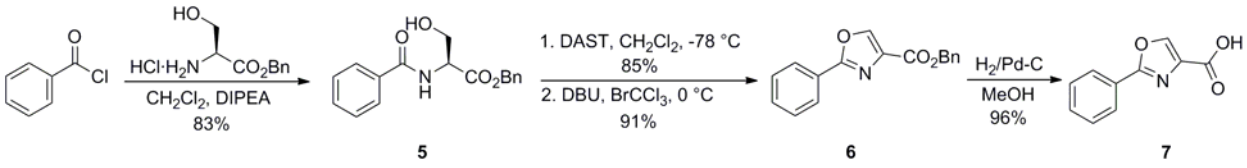
The LC/HRMS analyses were carried out on Waters ZQ instrument consisting of chromatography module Alliance HT, photodiode array detector 2996, and mass spectrometer Micromass ZQ, using a 3 x 50 mm Pro C18 YMC reverse phase column. Mobile phases: 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 minutes at 0.7 mL/min and referred in manuscript as "method A." A typical run is 10 minutes. The MS electrospray source operated at capillary voltage 3.5 kV and a desolvation temperature 300 ^{o}C. The HPLC analyses were carried out on a Waters 2695 instrument consisting of a photodiode array detector 996, using the Waters symmetry C_{18} 5 μm reverse phase column (Waters, Milford, MA, www.waters.com). Mobile phases: (a) 50% HPLC grade acetonitrile in Millipore purified water at a flow rate of 1.0 mL min^{-1} and UV diction at 254 nm. (b) 60% HPLC grade acetonitrile in Millipore purified water at a flow rate of 1.0 mL min^{-1} and UV diction at 254 nm and referred in manuscript as "method B." A typical run time was 20 minutes. All compounds are $\geq$95% pure as determined by HPLC or LC/HRMS as indicated above.

All reactions were conducted under argon gas unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. All reactions were carried out at ambient temperature (~22 °C) unless stated otherwise. Flash chromatography was performed on Sorbent Technologies silica gel 60 (32–63 μm).

Abbreviations: ACN = acetonitrile; DAST = Diethylaminosulfur trifluoride; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DIPEA = diisopropylethylamine; DMF = dimethylformamide; EDC = *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; EtOAc = ethyl acetate; HOAc = acetic acid.

Chemistry: Experimental Procedures and Analytical Data

Scheme 1. Synthesis of 2-phenyloxazole-4-carboxylic acid.



Synthesis of 2-phenyl-1,3-oxazole-4-carboxylic acid (7):

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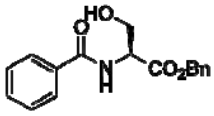
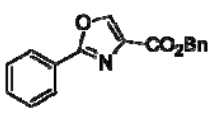


(*S*)-Benzyl 2-(benzamido)-3-hydroxypropanoate ($C_{17}H_{17}NO_4$, 5). Benzoyl chloride (4.15 g, 3.4 mL, 28.6 mmol) was dissolved in anhydrous CH_2Cl_2 (120 mL) and cooled to 0 ^oC (ice bath). L-Serine benzyl ester hydrochloride (7.2 g, 30.1 mmol) and DIPEA (9.3 g, 12.5 mL, 71.6 mmol) were added slowly and the reaction mixture was heated at reflux for 14 h. The reaction mixture was cooled and washed with 10% aqueous $NaHCO_3$, water, 0.5 N citric acid, and brine. The organic phase was separated, dried with Na_2SO_4, filtered, and concentrated *in vacuo* to give **5** as an off-white solid (7.15 g, 83%): ^1H NMR (300 MHz, $CDCl_3$) δ 7.85–7.79 (m, 2H), 7.56–7.30 (m, 7H), 7.14 (d, J = 7.1 Hz, 1H), 5.25 (s, 2H), 4.91 (td, J_1 = 7.1 Hz, J_2 = 3.5 Hz, 1H), 4.15–4.01 (m, 2H).

6



Benzyl 2-phenyloxazole-4-carboxylate ($C_{17}H_{13}NO_3$, 6). (*S*)-Benzyl 2-(benzamido)-3-hydroxypropanoate (**5**, 7.15 g, 28.5 mmol) was dissolved in CH_2Cl_2 (240 mL) and cooled to −78 °C (dry ice-acetone bath). diethylaminosulfur trifluoride (DAST, 4.4 g, 3.6 mL, 27.5 mmol) was added slowly under $Ar_{(g)}$ and stirred for 30 min. K_2CO_3 (8.9 g, 64.4 mmol) was added and the reaction mixture was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the reaction mixture was poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2x). The organic phase was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The resultant crude material was purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 stepwise elution, 1:1 to 10:1) to give (*S*)-Benzyl 4,5-dihydro-2-phenyloxazole-4-carboxylate as a white solid (5.7 g, 85%): mp 51−52 °C; TLC R_f = 0.25 (CH_2Cl_2); FT−IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 1741 (C=O), 1643 (C=N), 1188; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04–7.92 (m, 2H), 7.57–7.29 (m, 8H), 5.26 (dd, J_1 = 20.9 Hz, J_2 = 12.3 Hz, 2H), 4.99 (dd, J_1 = 10.6 Hz, J_2 = 7.9 Hz, 1H), 4.80–4.42 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.42, 163.45, 158.40, 156.25, 135.56, 135.48, 135.14, 128.67, 128.61, 128.49, 126.70, 124.19, 118.88, 118.71, 70.31, 68.83, 67.60; $[\alpha]_D^{25}$ +1.14 (c = 1.54 g/100 mL $CHCl_3$).

(*S*)-Benzyl 4,5-dihydro-2-phenyloxazole-4-carboxylate (6.27 g, 22.3 mmol) was dissolved in anhydrous CH_2Cl_2 (75 mL) and cooled to 0 °C (ice bath temperature). DBU (10 mL, 66.9 mmol) was added and followed by a slow addition of bromo-trichloromethane (6.6 mL, 66.9 mmol). After stirring for 1 h at 0 °C, the reaction was allowed to warm to room temperature. Once the reaction appeared complete by TLC analysis, the mixture was poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2x). The organic phase was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The resultant crude material was purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 stepwise elution, 1:10 to 1:1) to give **6** as a white solid (5.64 g, 91%): mp = 94–95 °C; TLC R_f = 0.37 (CH_2Cl_2); FT−IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 1741 (C=O), 1323, 1144, 1114; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.12 (dt, J_1 = 4.9 Hz, J_2 = 4.7 Hz, J_3 = 3.1 Hz, 2H), 7.52–7.31 (m, 8H), 5.41 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.49, 161.13, 143.84, 135.47, 134.32, 131.15, 128.96, 128.78, 128.57, 128.50, 128.46, 128.41, 126.86, 126.35, 66.79; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{17}H_{13}NO_3$, 280.0974; found, 280.0973.

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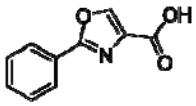
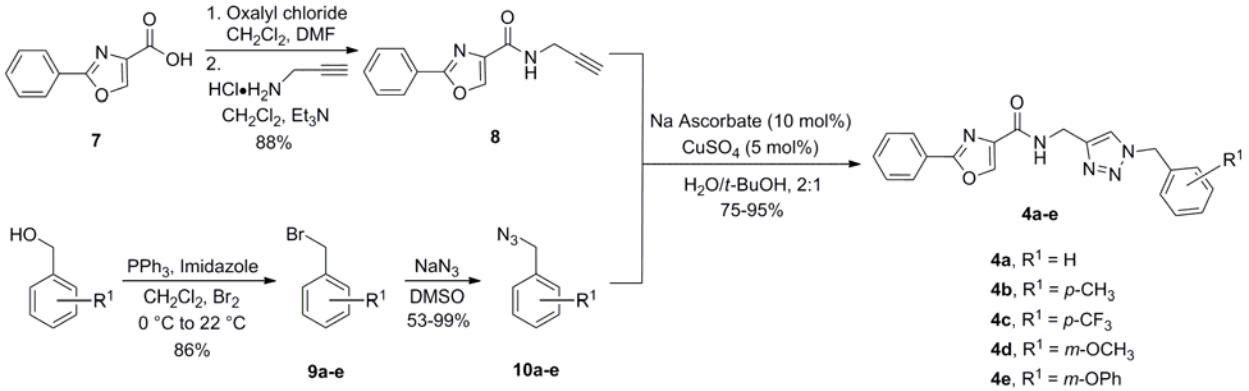


2-Phenyloxazole-4-carboxylic acid ($C_{10}H_7NO_3$, 7). Benzyl 2-phenyloxazole-4-carboxylate (**6**, 5.34 g, 19.1 mmol) was dissolved in MeOH (100 mL) and EtOAc (75 mL) and purged with bubbling $Ar_{(g)}$. Palladium on carbon (203 mg, 1.9 mmol) was added carefully. The reaction was sealed with a septum and evacuated by vacuum. Hydrogen gas was bubbled through the reaction mixture for 5 minutes while under vacuum. The reaction was stirred under hydrogen gas (1 atm) until the reaction appeared complete by TLC analysis. The reaction flask was purged with $Ar_{(g)}$, diluted with CH_2Cl_2 (100 mL), and filtered through glass filter paper to remove the palladium on carbon. The filtrate was concentrated to afford **7** as a white solid (3.46 g, 96%): mp = 207–208 ^oC; HPLC t_r = 2.0 min (method A); ^1H NMR (300 MHz, $CDCl_3$δ 8.56 (s, 1H), 8.12–8.04 (m, 2H), 7.59–7.47 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) ẟ 167.49, 161.46, 141.45, 140.11, 130.77, 128.87, 127.21, 126.39; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{10}H_8NO_3$, 190.0499; found, 190.0557.

Scheme 2. Synthesis of *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-phenyl-oxazole-carboxamide scaffold.



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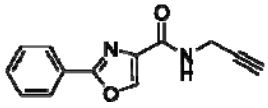
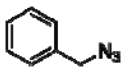


2-Phenyl-*N*-(prop-2-ynyl)oxazole-4-carboxamide ($C_{13}H_{10}N_2O_2$, 8). 2-phenyloxazole-4-carboxylic acid (**7**, 2.02 g, 10.7 mmol) was suspended in anhydrous CH_2Cl_2 (55 mL) under $Ar_{(g)}$. Oxalyl chloride (2.2 mL, 25.6 mmol) and DMF (50 μL) were added carefully to the mixture because of gas evolution. The reaction slowly turned to a light yellow homogeneous solution over 3 h. The solution was concentrated *in vacuo* to give 2-phenyloxazole-4-carbonyl chloride (2.2 grams, 100%) as an off-white solid, which was used immediately in the next reaction without characterization.

2-phenyloxazole-4-carbonyl chloride (2.20 g, 10.6 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL) under $Ar_{(g)}$ and cooled to 0 °C (ice bath). Propargyl amine hydrochloride (1.09 g, 11.8 mmol) and *N*,*N*-diisopropylethylamine (5.6 mL, 32.1 mmol) were added with stirring. The reaction was allowed to warm to room temperature. After stirring for 20 h, TLC analysis indicated completion of the reaction. The mixture was poured into a solution of 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2x). The organic layer was separated, washed with 10% aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The resultant crude material was purified by column chromatography (SiO_2, EtOAc/CH_2Cl_2 stepwise elution, 1:1 to 10:1) to give **8** as a white solid (2.13 g, 88%): mp = 123–124 °C; HPLC t_r = 3.4–3.7 min (method A); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.05–8.01 (m, 2H), 7.52–7.45 (m, 3H), 7.26 (bs, NH, 1H), 4.25 (dd, J_1 = 5.6 Hz, J_2 = 2.6 Hz, 2H), 2.29 (t, J = 2.6 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.48, 160.22, 141.02, 136.65, 131.09, 128.87, 126.57, 79.12, 71.74, 28.69; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{13}H_{10}N_2O_2$, 227.0815; found, 227.0789.

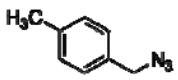
Synthezes of Azides 10a – e.

10a



(Azidomethyl)benzene ($C_7H_7N_3$, 10a). Sodium azide (4.9 g, 75.1 mmol) was dissolved in DMSO (150 mL) by stirring in a flame–dried 500 mL round bottom flask under $Ar_{(g)}$ for 12 h. A solution of benzyl bromide **9a** (7.9 mL, 68.4 mmol) was added and the reaction was stirred for 10 h. Water (200 mL) was added slowly to quench the reaction (exothermic process) and stirred until it cooled to room temperature. The mixture was poured into water (100 mL) and extracted with diethyl ether (3 x 100 mL). The organic layer was separated, washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **10a** as a clear oil (8.2 g, 91%): FT–IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 2931, 2097 (-N_3), 1496, 1456, 1256, 1203, 1078, 1029; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.35 (m, 5H), 4.37 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.23, 128.67, 128.13, 128.06, 54.59.

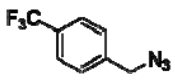
10b



1-(Azidomethyl)-4-methylbenzene ($C_8H_9N_3$, 10b). Sodium azide (102 mg, 1.56 mmol) was dissolved in DMSO (3.1 mL) by stirring in a flame–dried 25 mL round bottom flask under $Ar_{(g)}$ for 3 h. To this

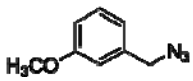
solution was added α-chloro-p-xylene (0.2 g, 1.42 mmol) and the reaction was stirred for 14 h. Water (7 mL) was added slowly to quench the reaction (exothermic process) and stirred until it cooled to room temperature. The mixture was extracted with diethyl ether (3 x 5 mL). The organic layer was separated, washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **10b** as a clear oil (208 mg, 99%): FT–IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 2923, 2097.7 ($-N_3$), 1515, 1254; ^1H NMR (300 MHz, $CDCl_3$) δ 7.24–7.17 (m, 4H), 4.29 (s, 2H), 2.36 (s, 3H).

10c



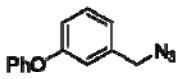
1-(Azidomethyl)-4-(trifluoromethyl)benzene ($C_8H_6F_3N_3$, 10c). Sodium azide (61 mg, 0.93 mmol) was dissolved in DMSO (1.9 mL) by stirring in a flame–dried 25 mL round bottom flask under $Ar_{(g)}$ for 3 h. To this solution was added 4-(bromomethyl)-1-(trifluoromethyl)benzene (0.2 g, 0.85 mmol) and the reaction was stirred for 14 h. Water (5 mL) was added slowly to quench the reaction (exothermic process) and stirred until it cooled to room temperature. The mixture was extracted with diethyl ether (3 x 7 mL). The organic layer was separated, washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **10c** as a clear oil (91 mg, 53%): FT–IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 2936, 2105 ($-N_3$), 1620, 1420, 1328, 1166, 1125, 1057; ^1H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 8.1 Hz, 2H), 7.49–7.41 (m, 2H), 4.43 (s, 2H).

10d



1-(Azidomethyl)-3-methoxybenzene ($C_8H_9N_3O$, 10d). Sodium azide (91 mg, 1.40 mmol) was dissolved in DMSO (2.8 mL) by stirring in a flame–dried 25 mL round bottom flask under $Ar_{(g)}$ for 3 h. To this solution was added 1-(chloromethyl)-3-methoxybenzene (0.2 g, 1.27 mmol) and the reaction was stirred for 14 h. Water (7 mL) was added slowly to quench the reaction (exothermic process) and stirred until it cooled to room temperature. The mixture was extracted with diethyl ether (3 x 7 mL). The organic layer was separated, washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **10d** as a clear oil (207 mg, 99%): FT–IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 3005, 2940, 2837, 2099 ($-N_3$), 1602, 1587, 1456, 1437, 1343, 1266, 1152, 1051; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (t, J = 7.7 Hz, 1H), 6.94–6.84 (m, 3H), 4.32 (s, 2H), 3.83 (s, 3H).

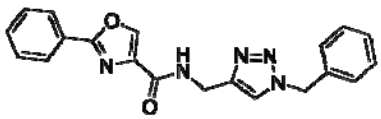
10e



1-(Azidomethyl)-3-phenoxybenzene ($C_{13}H_{11}N_3O$, 10e). Sodium azide (1.02 g, 15.5 mmol) was dissolved in DMSO (20 mL) by stirring in a flame–dried 50 mL round bottom flask under Ar(g) for 1 h. A solution of 3-phenoxybenzyl bromide **9e** (3.70 g, 14.1 mmol) in DMSO (5 mL) was added and stirred for 10 h. Water (10 mL) was added slowly to quench the reaction (exothermic process) and stirred until it cooled to room temperature. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layer was separated, washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **10e** as a clear oil (3.11 g, 98.1%): FT–IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 3040, 2111 ($-N_3$) 1583, 1484, 1445, 1341, 1231, 1211, 1162, 1142; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.31 (m, 3H), 7.14 (m, 1H), 6.95–7.09 (m, 5H), 4.32 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.77, 156.75, 137.26, 130.17, 129.83, 123.59, 122.76, 119.14, 118.47, 118.25, 54.42.

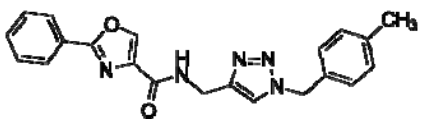
Triazoles 4a – e can be prepared by the general method described in this manuscript.

4a



***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-phenyloxazole-4-carboxamide ($C_{20}H_{17}N_5O_2$, 4a).** mp 180–182 °C; HPLC t_r = 4.4 min (method A); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.06–7.99 (m, 2H), 7.60 (bs, NH, 1H), 7.53 (s, 1H), 7.48 (dd, J_1 = 5.1 Hz, J_2 = 1.9 Hz, 3H), 7.41–7.24 (m, 5H), 5.51 (s, 2H), 4.71 (d, J = 6.1 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.46, 160.67, 144.93, 140.78, 136.90, 134.39, 131.08, 129.12, 128.89, 128.79, 128.13, 126.59, 126.46, 122.21, 54.23, 34.49; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{20}H_{18}N_5O_2$, 260.1455; found, 360.1481.

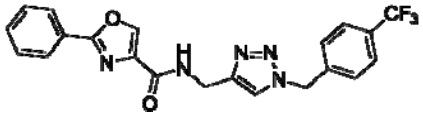
4b



***N*-((1-(4-Methylbenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-phenyloxazole-4-carboxamide ($C_{21}H_{19}N_5O_2$, 4b).** mp 178–179 °C; HPLC t_r = 7.45 min (method B); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H),

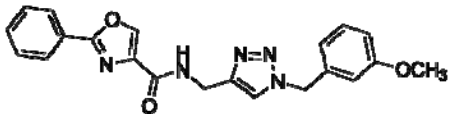
8.04–8.00 (m, 2H), 7.59 (s, 1H, NH), 7.51–7.45 (m, 4H), 7.18 (s, 4H), 5.46 (s, 2H), 4.71 (d, J = 6.1 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.73, 160.92, 145.11, 141.05, 139.02, 137.20, 131.64, 131.35, 130.06, 129.17, 128.48, 126.87, 126.76, 122.35, 54.32, 34.77, 21.43; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{21}H_{19}N_5O_2$, 374.1617; found, 374.1622.

4c



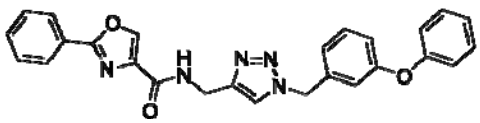
2-Phenyl-*N*-((1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)oxazole-4-carboxamide ($C_{21}H_{16}F_3N_5O_2$, 4c). mp 183–184 °C; HPLC t_r = 8.0 min (method B); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.02 (m, 2H), 7.65–7.59 (m, 3H), 7.56–7.41 (m, 3H), 7.38 (d, J = 8.1 Hz, 2H), 5.57 (s, 2H), 4.71 (d, J = 6.1 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.52, 160.75, 145.35, 140.81, 138.38, 136.86, 131.13, 130.91, 128.91, 128.25, 126.59, 126.43, 126.16, 126.13, 126.10, 124.80, 122.46, 53.51, 34.49; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{21}H_{16}F_3N_5O_2$, 428.1337; found, 428.1328.

4d



***N*-((1-(3-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-phenyloxazole-4-carboxamide ($C_{21}H_{19}N_5O_3$, 4d).** mp 131–132 °C; HPLC t_r = 7.9 min (method B); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.05–7.99 (m, 2H), 7.59 (bs, 1H, NH), 7.53 (s, 1H), 7.51–7.44 (m, 3H), 7.32–7.24 (m, 1H), 6.94–6.76 (m, 3H), 5.47 (s, 2H), 4.71 (d, J = 6.1 Hz, 2H), 3.77 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.45, 160.66, 160.05, 144.93, 140.77, 136.91, 135.82, 131.06, 130.19, 128.88, 126.59, 126.46, 122.22, 120.30, 114.24, 113.69, 55.28, 54.16, 34.50; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{21}H_{19}N_5O_3$, 390.1566; found, 390.1578.

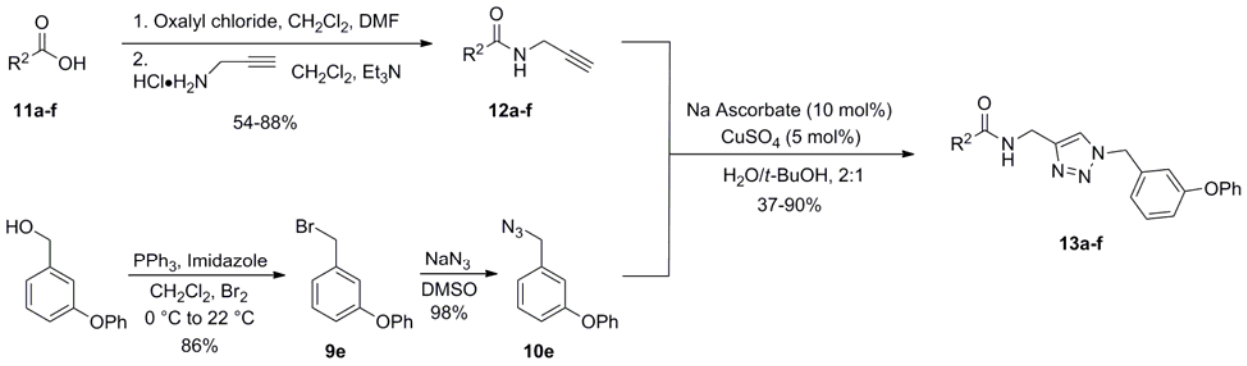
4e

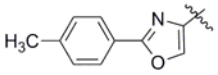
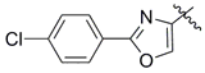
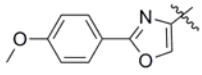


***N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-phenyloxazole-4-carboxamide ($C_{26}H_{21}N_5O_3$, 4e).** mp 134–135 °C; HPLC t_r = 8.6 min (method B); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.03 (dd, J_1 = 6.6 Hz, J_2 = 3.2 Hz, 2H), 7.60 (bs, 1H, NH), 7.54 (s, 1H), 7.48 (dd, J_1 = 5.2 Hz, J_2

=1.9 Hz, 3H), 7.40–7.27 (m, 3H), 7.19–6.88 (m, 6H), 5.47 (s, 2H), 4.72 (d, J = 6.1 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.47, 160.68, 158.05, 156.36, 145.01, 140.79, 136.90, 136.27, 131.08, 130.47, 129.87, 128.89, 126.59, 126.45, 123.81, 122.48, 122.29, 119.26, 118.58, 118.06, 53.87, 34.49; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{26}H_{21}N_5O_3$, 452.1723; found, 452.1748.

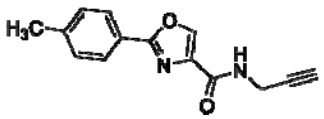
Scheme 3. Synthesis of *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)arylamide scaffold.



Compounds	(11-13)a	(11-13)b	(11-13)c	(11-13)d	(11-13)e	(11-13)f
R^2 =						

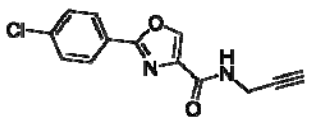
Alkynes 12a – f can all be prepared by the methods described for compound 8 in this manuscript.

12a



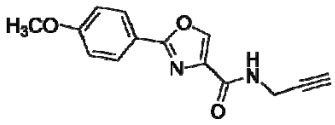
***N*-(Prop-2-ynyl)-2-*p*-tolyloxazole-4-carboxamide ($C_{14}H_{12}N_2O_2$, 12a).** mp 154–155 °C; HPLC t_r = 4.3–4.4 min (method A); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 7.9 Hz, 2H), 7.25 (bs, NH, 1H), 4.25 (dd, J_1 = 5.6 Hz, J_2 = 2.6 Hz, 2H), 2.41 (s, 1H), 2.28 (t, J = 2.6 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.73, 160.33, 141.55, 140.73, 136.54, 129.60, 126.55, 123.76, 79.16, 71.73, 28.69, 21.56; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{14}H_{12}N_2O_2$, 241.0972; found, 241.0994.

12b



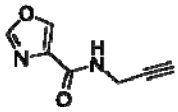
2-(4-Chlorophenyl)-*N*-(prop-2-ynyl)oxazole-4-carboxamide ($C_{13}H_9ClN_2O_2$, 12b). mp 146–147 °C; HPLC t_r = 4.6–4.7 min (method A); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.93 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.6 Hz, 2H), 7.21 (bs, NH, 1H), 4.24 (dd, J_1 = 5.6 Hz, J_2 = 2.5 Hz, 2H), 2.29 (t, J = 2.5 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.53, 160.00, 141.17, 137.29, 136.78, 129.21, 127.83, 124.87, 79.06, 71.79, 28.70; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{13}H_9ClN_2O_2$, 261.0425; found, 461.0451.

12c



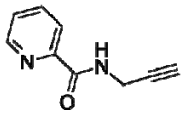
2-(4-Methoxyphenyl)-*N*-(prop-2-ynyl)oxazole-4-carboxamide ($C_{14}H_{12}N_2O_3$, 12c). mp 151–152 °C; HPLC t_r = 0.7–0.8 min (method A); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.82 (d, J = 8.5 Hz, 2H), 7.13 (bs, NH, 1H), 6.84 (d, J = 8.5 Hz, 2H), 4.15–4.07 (m, 2H), 3.72 (s, 3H), 2.15 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.82, 161.57, 160.40, 140.49, 136.44, 128.83, 128.29, 119.13, 114.26, 113.69, 79.17, 71.69, 55.37, 28.66; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{14}H_{12}N_2O_3$, 257.0921; found, 257.0930.

12d



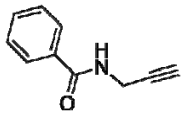
***N*-(Prop-2-ynyl)oxazole-4-carboxamide ($C_7H_6N_2O_2$, 12d).** mp 94–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.88 (s, 1H), 7.14 (bs, NH, 1H), 4.23 (m, 2H), 2.28 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.85, 150.56, 141.61, 28.73, 135.40, 78.98, 71.80; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_7H_6N_2O_2$, 151.0508; found, 151.0496.

12e



***N*-(Prop-2-ynyl)picolinamide ($C_9H_8N_2O$, 12e).** mp 75–76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (ddd, J_1 = 4.9 Hz, J_2 = 1.7 Hz, J_3 = 0.9 Hz, 1H), 8.21–8.16 (m, 1H), 7.84 (dt, J_1 = 7.7 Hz, J_2 = 1.7 Hz, 1H), 7.43 (ddd, J_1 = 7.6 Hz, J_2 = 4.8 Hz, J_3 = 1.2 Hz, 1H), 7.25 (bs, NH, 1H), 4.27 (dd, J_1 = 5.6 Hz, J_2 = 2.5 Hz, 2H), 2.26 (t, J = 2.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.00, 149.27, 148.13, 137.34, 126.38, 122.30, 79.35, 71.54, 29.09; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_9H_8N_2O$, 161.0715; found, 161.0725.

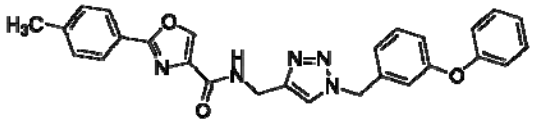
12f



***N*-(Prop-2-ynyl)benzamide ($C_{10}H_9NO$, 12f).** mp 109–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.76 (m, 2H), 7.57–7.40 (m, 3H), 6.34 (bs, NH, 1H), 4.27 (m, 2H), 2.29 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.17, 133.64, 131.72, 128.53, 127.01, 79.48, 71.74, 29.70.

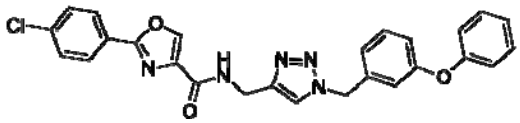
<u>Triazoles 13a – f can all be prepared by the method described in manuscript.</u>

13a



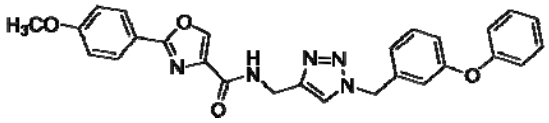
***N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-p-tolyloxazole-4-carboxamide ($C_{27}H_{23}N_5O_3$, 13a)**. HPLC t_r = 5.6–5.7 min (method A); mp 160–161 °C; FT–IR (NaCl, thin film) $\bar{\nu}_{max}$ (cm^{-1}) 1656.6, 1594.6, 1486; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.91 (d, J = 7.9 Hz, 2H), 7.60 (bs, 1H, NH), 7.54 (s, 1H), 7.25–7.36 (m, 5H), 7.12 (tt, J_1 = 7.4 Hz, J_2 = 1.1 Hz, 1H), 6.90–7.01 (m, 5H), 5.47 (s, 2H), 4.71 (d, J = 6.1 Hz, 2H), 2.41 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.73, 160.80, 158.08, 156.39, 145.04, 141.54, 140.51, 136.79, 136.30, 130.48, 129.87, 129.61, 126.57, 123.82, 123.78, 122.48, 122.28, 119.28, 118.59, 118.07, 53.87, 34.49, 21.57; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{27}H_{23}N_5O_3$, 466.1874; found, 466.1867.

13b



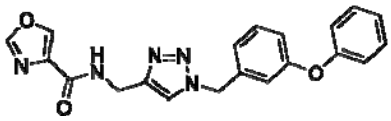
2-(4-Chlorophenyl)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)oxazole-4-carboxamide ($C_{26}H_{20}ClN_5O_3$, 13b). HPLC t_r = 9.33 min (method B); mp 172–173 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.71 (d, J = 6.2 Hz, 2H), 5.47 (s, 2H), 6.90–7.02 (m, 5H), 7.12 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 7.28–7.37 (m, 3H), 7.41–7.47 (m, 2H), 7.56 (s, 1H), 7.64 (bs, 1H), 7.91–7.98 (m, 2H), 8.22 (d, J = 1.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.44, 53.80, 118.03, 118.52, 119.20, 122.23, 122.44, 123.77, 124.88, 127.83, 129.19, 129.82, 130.42, 136.26, 137.02, 137.24, 140.92, 144.83, 156.32, 158.01, 160.44, 160.49; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{26}H_{20}ClN_5O_3$, 486.1333; found, 486.1342.

13c



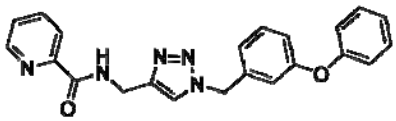
2-(4-Methoxyphenyl)-*N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)oxazole-4-carboxamide ($C_{27}H_{23}N_5O_4$, 13c). HPLC t_r = 1.1–1.2 min (method A); mp 119.1–119.4 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1 H), 7.99–7.93 (m, 2 H), 7.60 (bs, NH, 1 H), 7.55 (s, 1 H), 7.39–7.28 (m, 3 H), 7.16–7.09 (m, 1 H), 7.03–6.90 (m, 7 H), 5.47 (s, 2 H), 4.72 (d, J = 6.1 Hz, 2 H), 3.88 (s, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.83, 161.56, 160.84, 158.04, 156.36, 145.05, 140.26, 136.70, 136.29, 130.46, 129.85, 128.31, 123.79, 122.46, 122.27, 119.26, 119.18, 118.56, 118.04, 114.28, 55.40, 53.84, 34.46. HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{27}H_{23}N_5O_4$, 482.1823; found, 482.1803.

13d



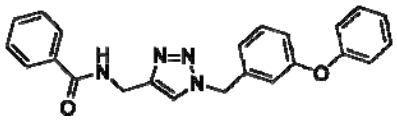
***N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)oxazole-4-carboxamide ($C_{20}H_{17}N_5O_3$, 13d).** HPLC t_r = 6.67 min (method B); m.p. 130–131°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.69 (d, J = 6.0 Hz, 2H), 5.46 (s, 2H), 6.87–7.04 (m, 4H), 7.13 (t, J = 7.4 Hz, 1H), 7.22–7.41 (m, 3H), 7.53 (s, 2H), 7.85 (d, J = 1.0 Hz, 1H), 8.23 (d, J = 0.5 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.30, 158.07, 156.37, 150.53, 144.84, 141.36, 136.28, 135.65, 130.47, 129.87, 123.82, 122.46, 122.24, 119.27, 118.58, 118.05, 53.85, 34.53; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{20}H_{17}N_5O_3$, 376.1410; found, 376.1413.

13e



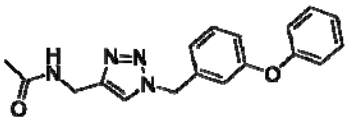
***N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)picolinamide ($C_{22}H_{19}N_5O_2$, 13e).** HPLC t_r = 7.40 min (method B); mp 98–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, J = 6.2 Hz, 2H), 5.46 (s, 2H), 6.88–7.03 (m, 4H), 7.08–7.16 (m, 1H), 7.27–7.38 (m, 3H), 7.43 (dddd, J_1 = 7.4 Hz, J_2 = 4.9 Hz, J_3 = 1.2 Hz, J_4 = 1.1 Hz, 1H), 7.53 (s, 1H), 7.79–7.90 (m, 1H), 8.18 (dq, J_1 = 7.8 Hz, J_2 = 1.1 Hz, 1H), 8.54 (dddd, J_1 = 3.9 Hz, J_2 = 1.8 Hz, J_3 = 1.0 Hz, J_4 = 0.8 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.41, 158.04, 156.37, 149.51, 148.18, 145.23, 137.28, 136.34, 130.44, 129.85, 126.29, 123.79, 122.46, 122.17, 122.14, 119.26, 118.55, 118.03, 53.82, 34.94. HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{22}H_{19}N_5O_2$, 386.1617; found, 386.1601.

13f



***N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)benzamide ($C_{23}H_{20}N_4O_2$, 13f).** HPLC t_r = 7.65 min (method B); mp 117–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70 (d, *J* = 5.7 Hz, 2H), 5.47 (s, 2H), 6.89–7.04 (m, 5H), 7.09–7.17 (m, 1H), 7.26–7.54 (m, 6H), 7.57 (s, 1H), 7.76–7.82 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 35.33, 53.89, 118.06, 118.62, 119.26, 122.32, 122.47, 123.83, 126.97, 128.58, 129.88, 130.50, 131.67, 133.90, 136.24, 144.99, 156.38, 158.09, 167.38; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{23}H_{20}N_4O_2$, 385.1665; found, 385.1655.

14



***N*-((1-(3-Phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide ($C_{18}H_{18}N_4O_2$, 14).** HPLC t_r = 6.08 min (method B); mp 118–119 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99 (s, 3H), 4.48 (d, *J* = 5.74 Hz, 2H), 5.46 (s, 2H), 6.15 (br. s., 1H), 6.89–7.03 (m, 5H), 7.14 (t, *J* = 7.4 Hz, 1H), 7.28–7.40 (m, 3H), 7.47 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 23.04, 34.81, 53.78, 118.04, 118.55, 119.20, 122.24, 122.42, 123.79, 129.85, 130.43, 136.24, 145.09, 156.34, 158.00, 170.22; HRMS–FAB (m/z) $[M+H]^+$ calcd for $C_{18}H_{19}N_4O_2$, 323.1508; found, 323.1497.

In Vitro MCF-7 Assay

In vitro activities against MCF-7 cells were determined with a crystal violet biomass reduction assay. MCF-7 cells were grown in α-MEM media supplemented with 5% fetal bovin serum and trichostatin-A antibiotic and incubated at 37 °C and 5% CO_2. After cell inoculation, the microtiter plates were incubated at 37 °C and 5% CO_2 for 24 h prior to addition of compounds. To MCF-7 cell cultures in 96-well plates (100 μL containing 2,500 cells), DMSO solutions of compounds were added in replicates of 3. The cells were incubated in a 37 ºC, 5% CO_2 for 72 h. The media was removed and the cells were stained with crystal violet, washed and air-dried. The stain was eluted with solution of Triton-X-100 and optical density was measured at 595 nm. Using at least seven absorbance measurements [well background (no cells) (T_b), vehicle control growth (C), and test growth in the presence of compound concentration levels (T_i) (≥ 5 compound concentration levels)], the percentage reduction in biomass was calculated at each of

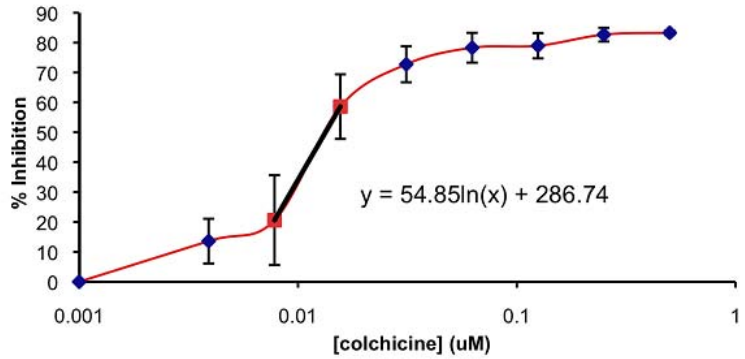
the compound concentrations levels. IC_{50} (half maximal inhibitory concentration) values were calculated from dose response curves where $[(T_i - T_b)/(C - T_b)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net cell mass (as measured by crystal violet staining) relative to the vehicle control cells during the compound incubation.

N.B. IC_{50} and GI_{50} differ in that IC_{50} is calculated with T_b (well background/no cells) and GI_{50} is calculated with T_z (cells at time zero/time of addition).

MCF-7 IC_{50} Data Summary

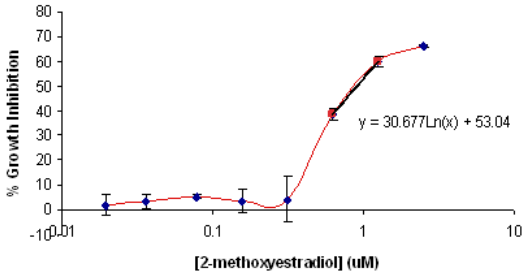
ND #	Notebook #	Compound	Trial 1	Trial 2	Trial 3	Trial 4	AVE	STDEV	MCF-7 IC50
x	x	**Colchicine**	0.013	nd	nd	nd	nd	nd	0.013
x	x	**2-methoxy-estradiol**	0.905655	0.777926	nd	nd	0.84179	0.090318	0.842 ± 0.090
ND-6492	gm7-70-1	**4a**	15.855	nd	nd	nd	nd	nd	15.9
ND-6735	RJP-1-34-3	**4b**	7.591	nd	nd	nd	nd	nd	7.59
ND-6730	RJP-I-22	**4c**	7.33	nd	nd	nd	nd	nd	7.33
ND-6731	RJP-I-23-2	**4d**	8.35	nd	nd	nd	nd	nd	8.35
ND-6732	JAS-I-191	**4e**	0.676514	0.4654	0.537256	nd	0.559723	0.107335	0.56 ± 0.11
ND-8467	JAS-I-171	**13a**	0.398593	0.307611	0.304077	0.311886	0.330542	0.04548	0.33 ± 0.045
ND-8186	JAS-I-166	**13b**	3.565396	1.047174	1.091277	nd	1.901282	1.441333	1.9 ± 1.4
ND-7887	JAS-II-1	**13c**	1.025626	0.4982	0.4695	nd	0.664	0.313	0.66 ± 0.31
ND-8505	JAS-I-190	**13d**	1.038797	0.43823	0.442965	nd	0.639997	0.345379	0.64 ± 0.35
ND-8504	JAS-I-189	**13e**	0.071149	0.0331	0.0325	nd	0.0456	0.0222	0.046 ± 0.022
ND-8506	JAS-I-188	**13f**	0.251466	0.245464	0.237373	nd	0.244768	0.007072	0.245 ± 0.007
ND-8492	JAS-I-181	**14**	7.832581	5.735645	5.498479	nd	6.355568	1.284616	6.4 ± 1.3

MCF-7 Dose-Response Curve: **Colchicine**

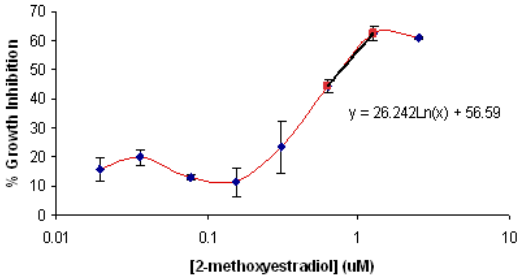


MCF-7 Dose-Response Curves: **2-methoxyestradiol**

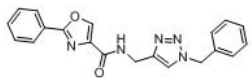
Trial 1

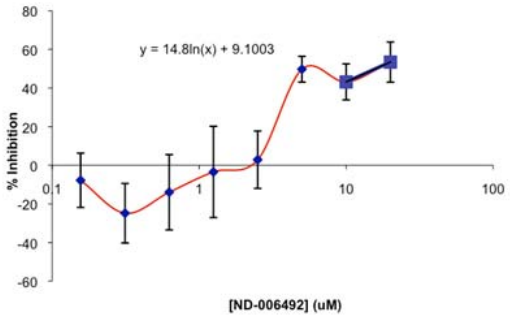


Trial 2

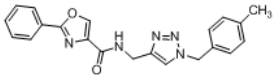


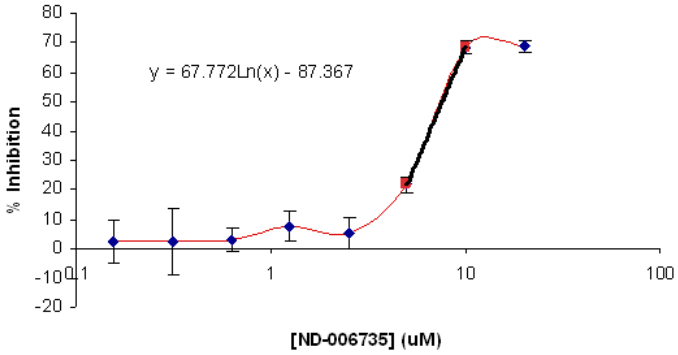
MCF-7 Dose-Response Curve: **4a** (ND-006492)



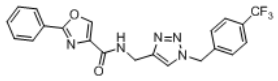


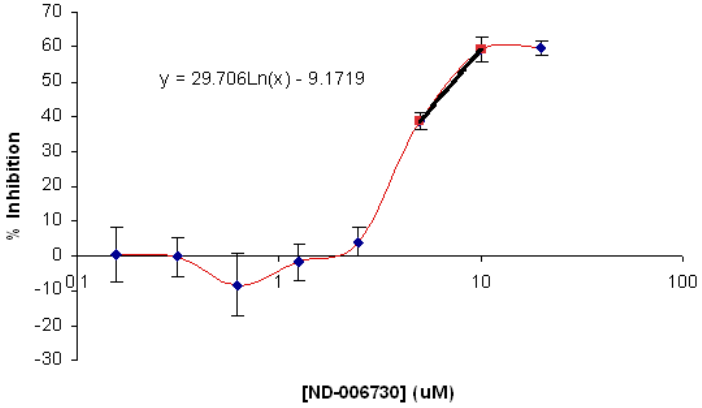
MCF-7 Dose-Response Curve: **4b** (ND-006735)



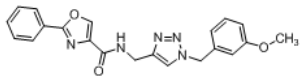


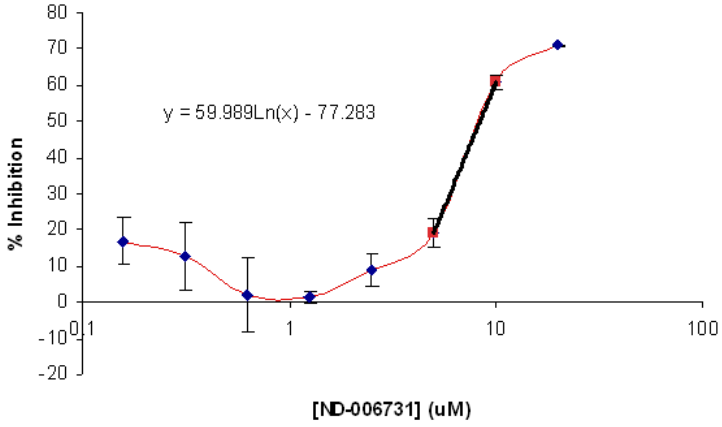
MCF-7 Dose-Response Curve: **4c** (ND-006730)





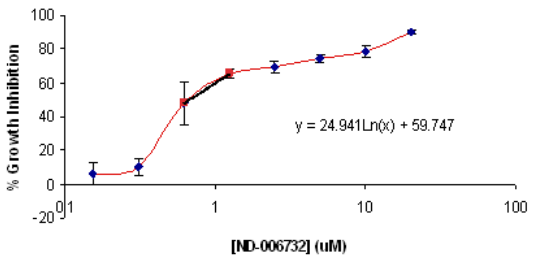
MCF-7 Dose-Response Curve: **4d** (ND-006731)



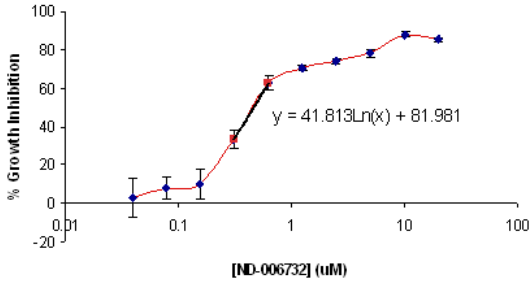


MCF-7 Dose-Response Curves: **4e** (ND-006732)

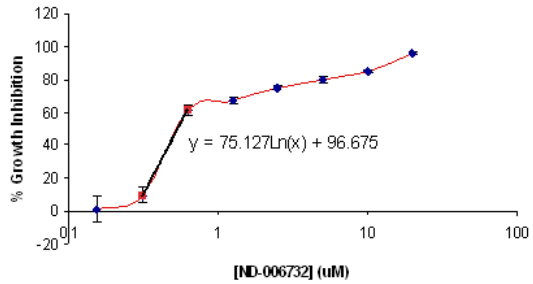
Trial 1

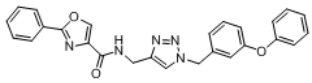


Trial 2

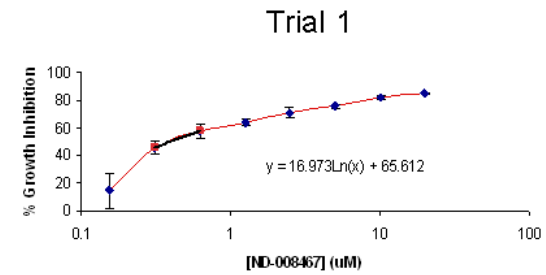


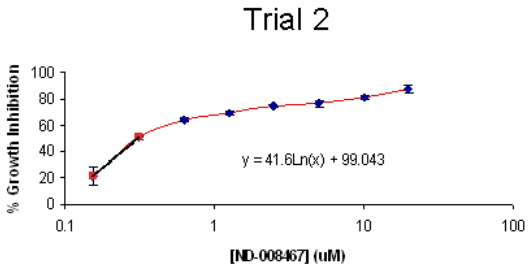
Trial 3

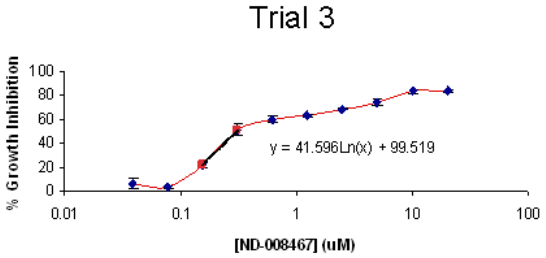


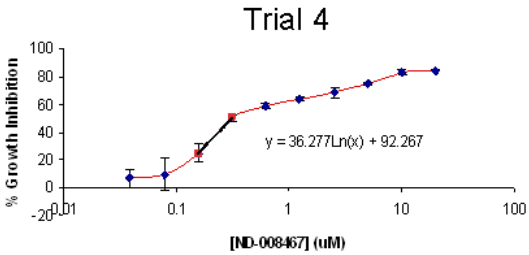


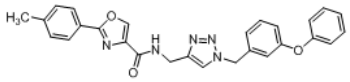
MCF-7 Dose-Response Curves: **13a** (ND-008467)





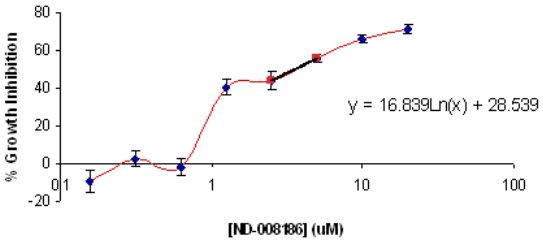




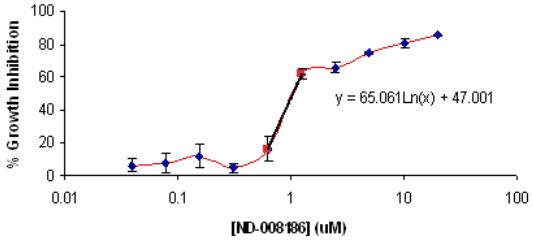


MCF-7 Dose-Response Curves: **13b** (ND-008186)

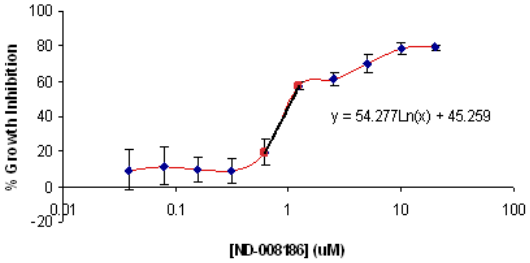
Trial 1

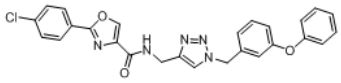


Trial 2



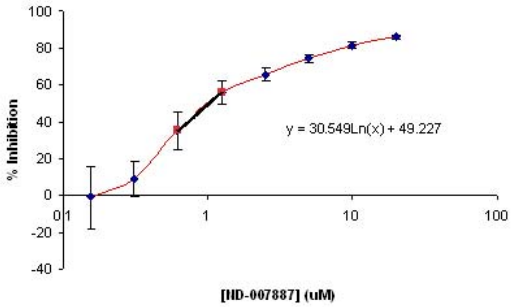
Trial 3



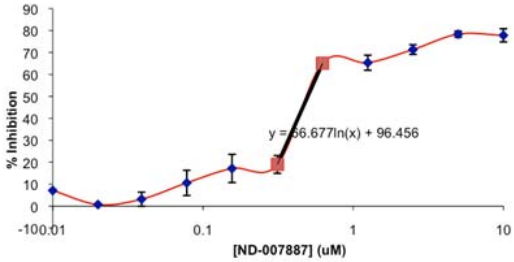


MCF-7 Dose-Response Curves: **13c** (ND-007887)

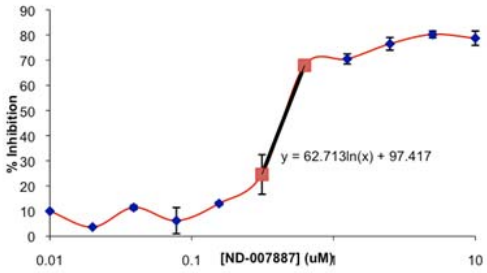
Trial 1

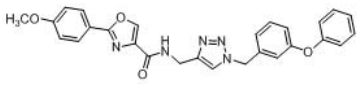


Trial 2



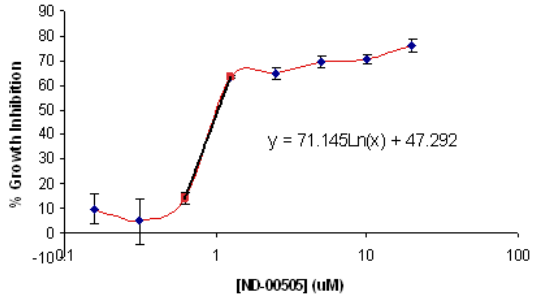
Trial 3



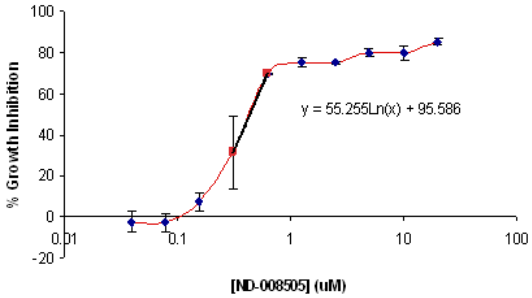


MCF-7 Dose-Response Curves: **13d** (ND-008505)

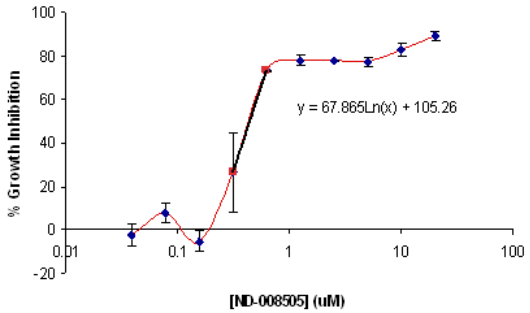
Trial 1

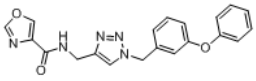


Trial 2



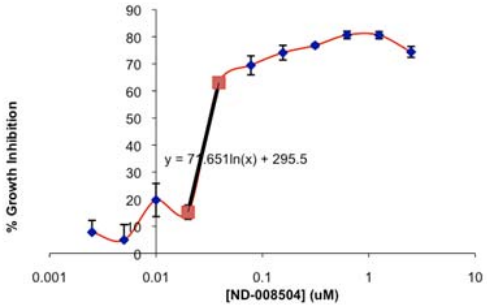
Trial 3



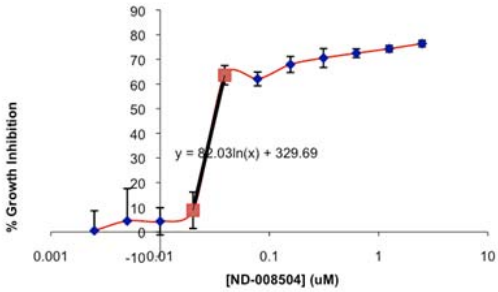


MCF-7 Dose-Response Curves: **13e** (ND-008504)

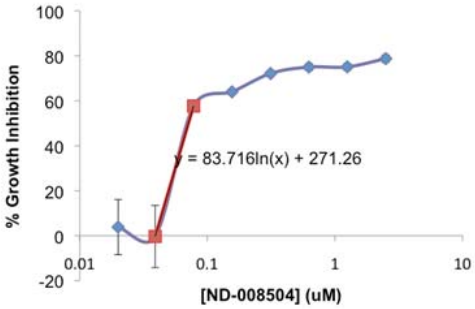
Trial 1

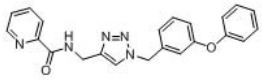


Trial 2



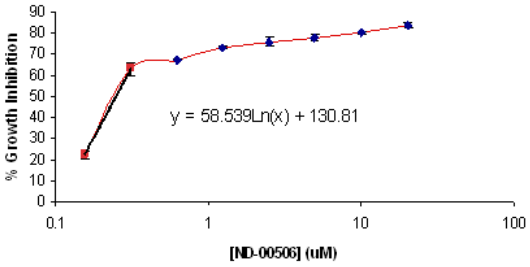
Trial 3



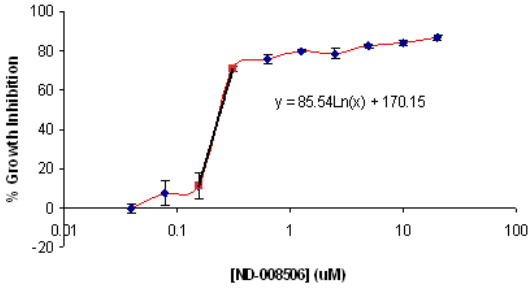


MCF-7 Dose-Response Curves: **13f** (ND-008506)

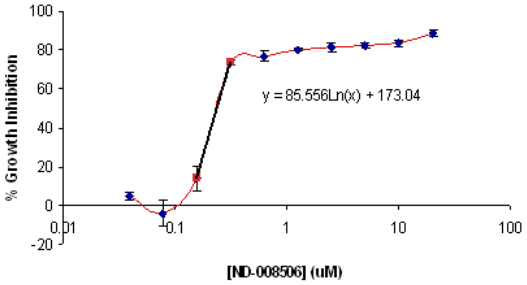
Trial 1

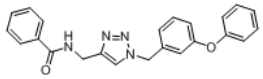


Trial 2

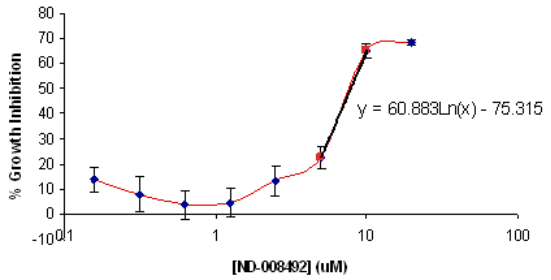


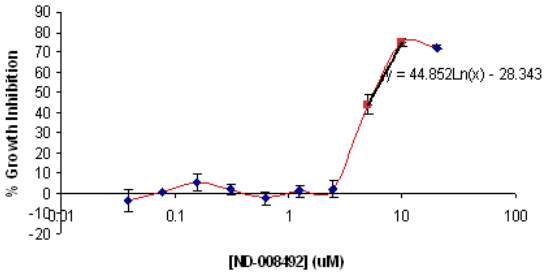
Trial 3

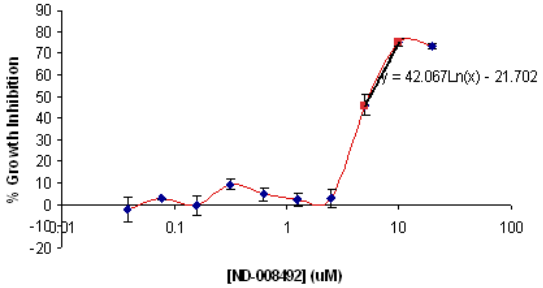


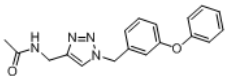


MCF-7 Dose-Response Curves: **14** (ND-008492)









In Vitro U-937 Assay

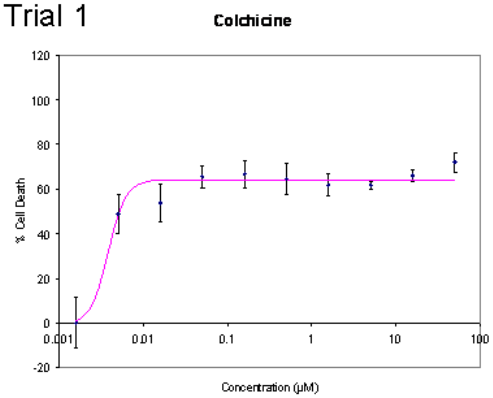
In vitro activities against U-937 cells were determined with a Sulforhodamine B biomass reduction assay. U-937 cells were grown in RPMI-1640 media supplemented with 10% fetal bovine serum (Gemini Bioproducts) and 1% Pen-Strep antibiotic and incubated at 37 ºC in a 5% CO_2, 95% humidity atmosphere. DMSO solutions of compounds were added in replicates of 5 (1 µL to each well) in 96-well plates and U-937 cells were added (199 µL containing 4 x 104 cells). The cells were incubated in a 37 ºC, 5% CO_2, 95% humidity incubator for 72 h. Ice-cold trichloroacetic acid (50 µL of 50% w/v) was added directly to the wells containing media and the cells fixed overnight at 4 ºC. The plates were washed with water 5 times and stained with sulforhodamine B (50 µL of a 0.057% solution in 1% acetic acid) at room temperature for 30 min. The plates were washed 5 times with 1% acetic acid to remove excess sulforhodamine B and the bound-dye was released by the addition of Tris-base (200 µL of 10 mM solution). The absorbance at 510 nm measured on a Molecular Devices SpectraMax 384 plus plate reader. The percentage reduction in biomass was calculated using vehicle-treated cells and wells without cells as the 100% and 0% biomass controls respectively. IC_{50} values were determined by fitting the dose response data to the logistic dose response equation in the software TableCurve 2D.

U937 IC_{50} Data Summary

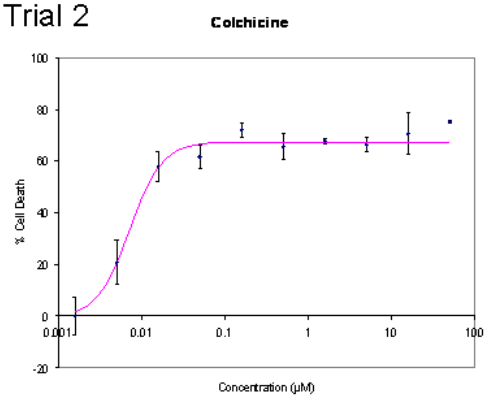
ND #	Notebook #	Compound	Trial 1	Trial 2	Trial 3	AVE	STDEV	U973 IC50
x	x	**Colchicine**	0.005	0.011	0.0067	0.008	0.003	0.008 ± 0.003
x	x	**2-methoxy-estradiol**	4	1.67	3.07	2.91	1.17	2.91 ± 1.17
ND-6732	JAS-I-191	**4e**	1.58	1.38	1.23	1.40	0.18	1.40 ± 0.18
ND-8467	JAS-I-171	**13a**	0.735	1.60	1.05	1.13	0.44	1.13 ± 0.44
ND-7887	JAS-II-1	**13c**	1.73	4.43	5.08	3.75	1.78	3.75 ± 1.78
ND-8505	JAS-I-190	**13d**	2.18	1.62	2.77	2.19	0.58	2.19 ± 0.58
ND-8504	JAS-I-189	**13e**	0.242	0.45	1.06	0.58	0.42	0.58 ± 0.42
ND-8492	JAS-I-181	**14**	50	15	18	28	19	28 ± 19

U937 Dose-Response Curves: **Colchicine**

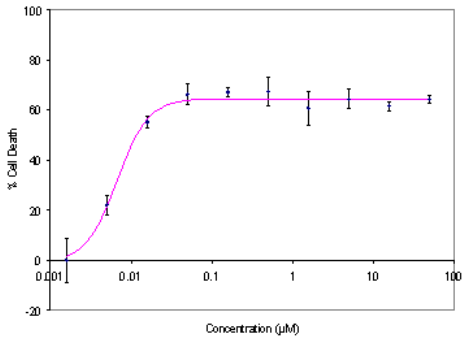
Trial 1



Trial 2

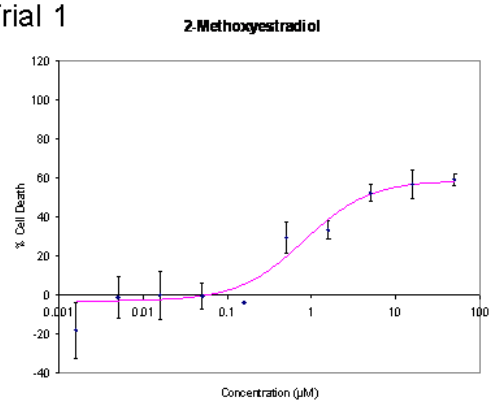


Trial 3

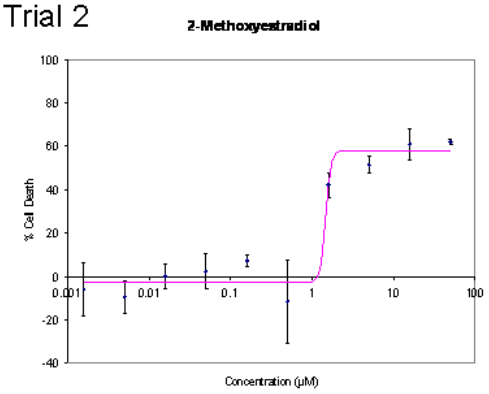


U937 Dose-Response Curves: **2-methoxyestradiol**

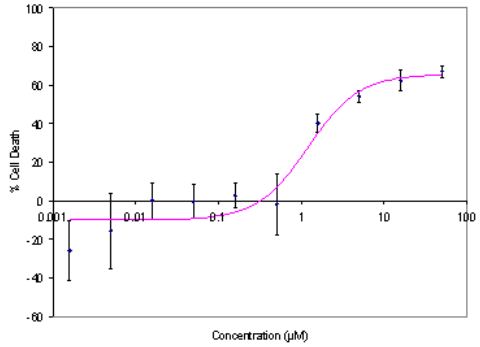
Trial 1



Trial 2

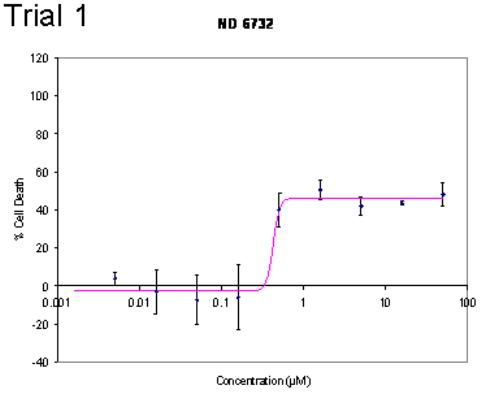


Trial 3

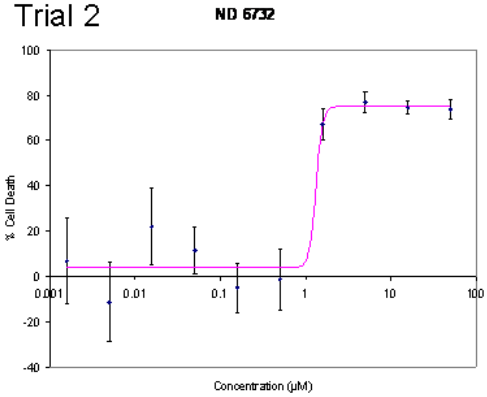


U937 Dose-Response Curves: **4e** (ND-006732)

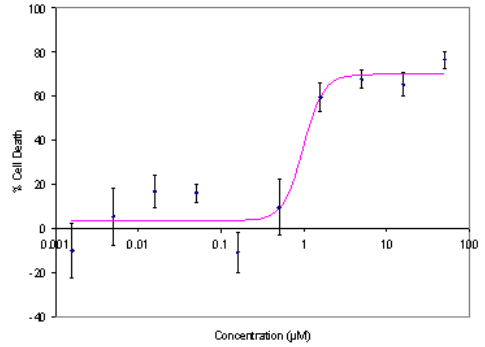
Trial 1

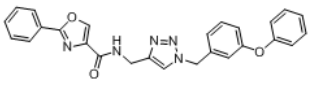


Trial 2



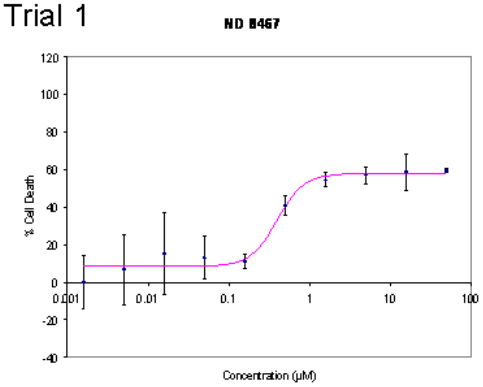
Trial 3



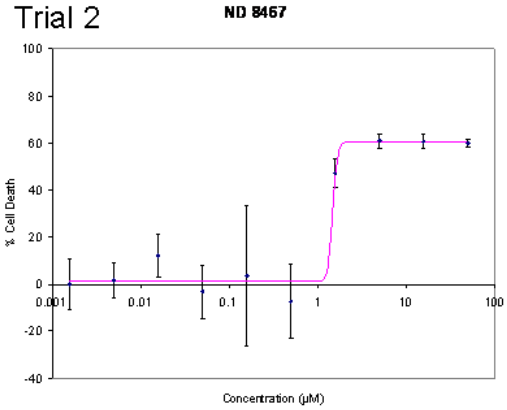


U937 Dose-Response Curves: **13a** (ND-008467)

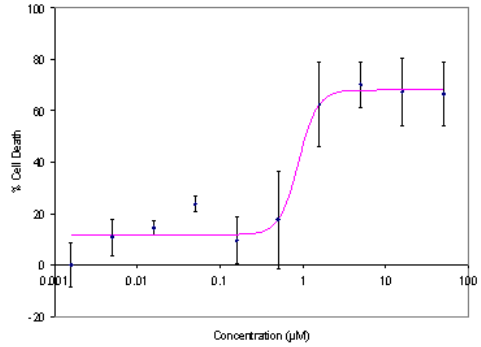
Trial 1

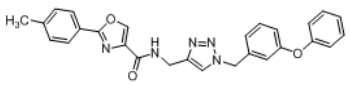


Trial 2



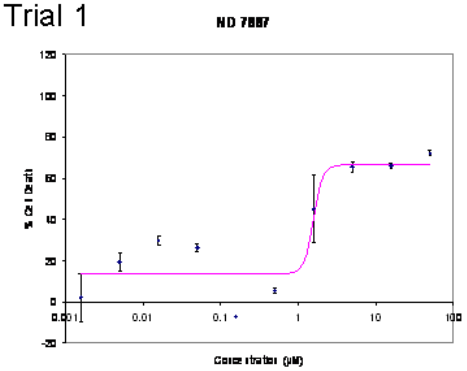
Trial 3



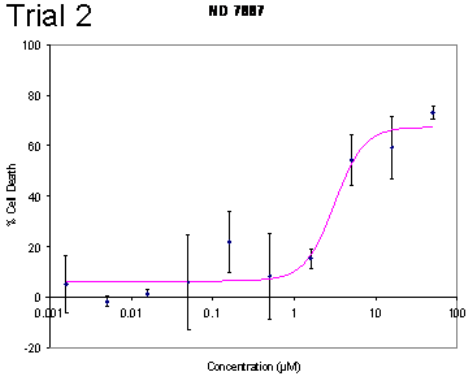


U937 Dose-Response Curves: **13c** (ND-007887)

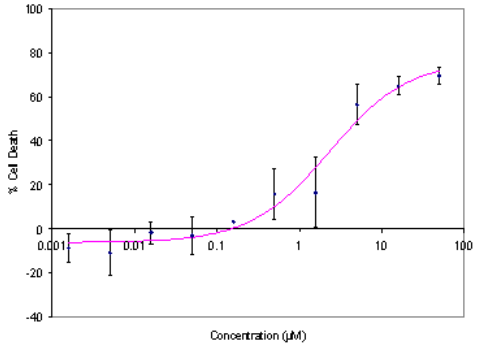
Trial 1

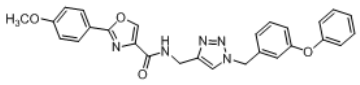


Trial 2



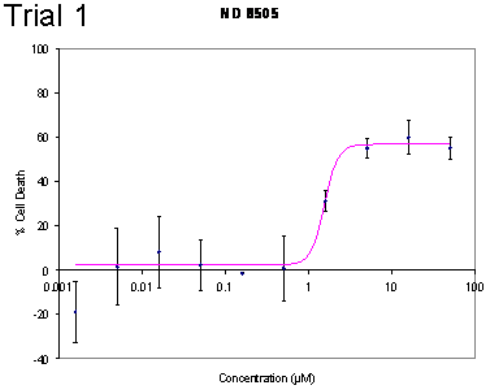
Trial 3



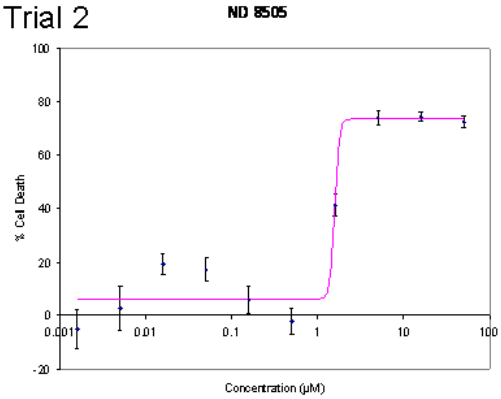


U937 Dose-Response Curves: **13d** (ND-008505)

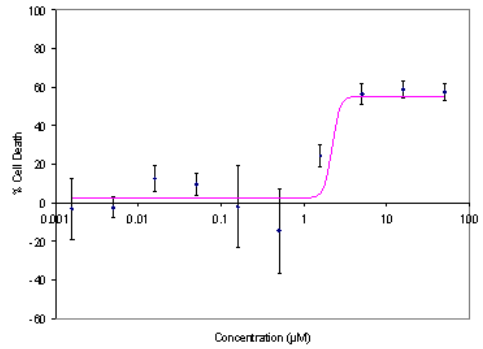
Trial 1

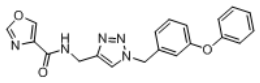


Trial 2



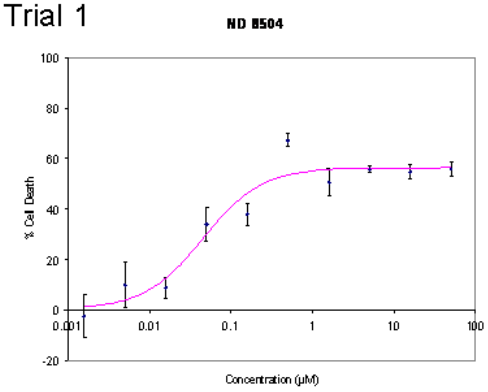
Trial 3



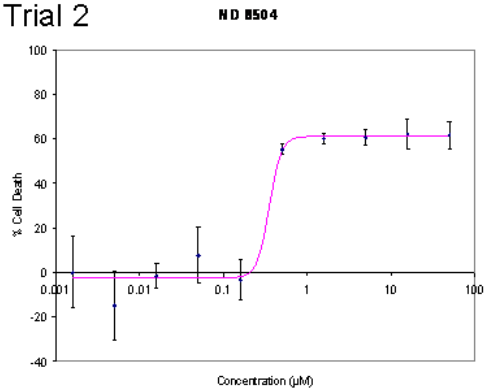


U937 Dose-Response Curves: **13e** (ND-008504)

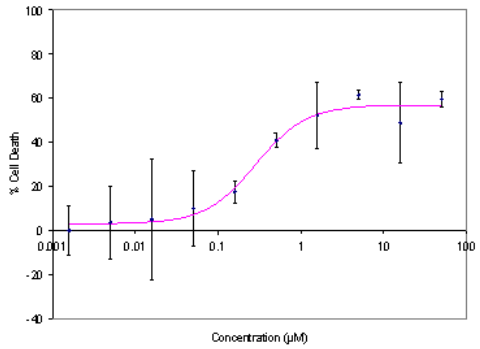
Trial 1

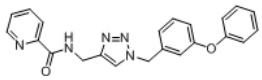


Trial 2

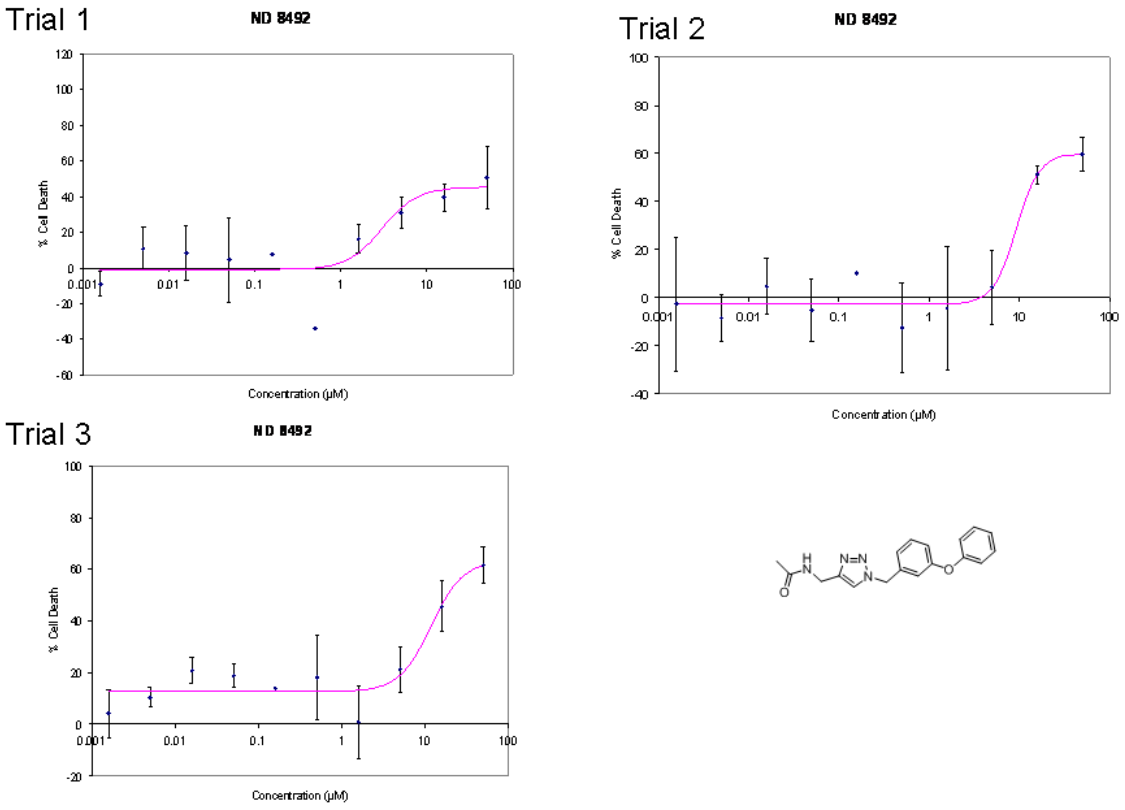


Trial 3





U937 Dose-Response Curves: **14** (ND-008492)



In Vitro NCI60 Assay

The methodology of the NCI60 in vitro cancer screen can be found on-line (http://dtp.nci.nih.gov/branches/btb/ivclsp.html) and is reproduced here:

The human tumor cell lines of the cancer screening panel are grown in RPMI–1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to

use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (T_z), control growth, (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ for concentrations for which } T_i \geq T_z$$

$$[(T_i - T_z)/T_z] \times 100 \text{ for concentrations for which } T_i < T_z.$$

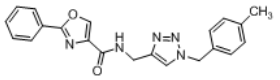
Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

COMPARE Analysis

Matrix COMPARE Analysis of Compounds 4b–e. A Matrix COMPARE analysis was performed through the Private COMPARE web site http://dtp.nci.nih.gov/compare-web-private_compare/login.do. The differential antiproliferative activity profiles (based on GI_{50}) of compounds **4b**–**e** were compared to each other through the matrix compare. The NSC numbers used to retrieve the data for this Matrix COMPARE were NSC 743295 (**4b**), NSC 744736 (**4c**), NSC 744737 (**4d**), and NSC 743294 (**4e**).

Standard COMPARE Analysis of 4e (NSC 743294). A Standard COMPARE analysis was performed through the Private COMPARE web site http://dtp.nci.nih.gov/compare-web-private_compare/login.do. Compound **4e** was tested twice in the NCI60 5-Dose Assay, so the average data (NSC_FIVE_DOSE; S743294 AVGDATA –4.0) was used in the analysis. Standard COMPARE analyses were run using all three DTP Screening Data Endpoints (GI_{50}, TGI, & LC_{50}). The configurations for each standard COMPARE analysis can be viewed in the Supporting Information (along with the results). A standard COMPARE analysis was also performed for **4b**–**d** using similar procedures (COMPARE configurations and results in Supporting Information).

COMPARE GI_{50}: **4b** (ND-006735) [NSC-743295]

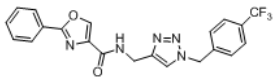


COMPARE Seed	
Namecode	Q38V-1
Vector ID	858137
Ident For Display	NSC:S743295 Endpt:GI50 Expld:0611NS42 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	GI50 - 50% growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Mimimum Standard Deviation	0.05

Job ID: 3286445683557783051

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.702	NSC:S325319 Endpt:GI50 Expld:AVGDATA hiConc:-10.0	didemnin B
2	0.691	NSC:S79037 Endpt:GI50 Expld:AVGDATA hiConc:-4.	CCNU
3	0.675	NSC:S73754 Endpt:GI50 Expld:AVGDATA hiConc:-2.6	fluorodopan
4	0.643	NSC:S132319 Endpt:GI50 Expld:AVGDATA hiConc:-2.0	indicine N-oxide
5	0.642	NSC:S95441 Endpt:GI50 Expld:AVGDATA hiConc:-3.6	methyl-CCNU

COMPARE GI_{50}: **4c** (ND-006730) [NSC-744736]

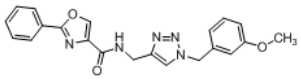


COMPARE Seed	
Namecode	Q38V-1
Vector ID	756273
Ident For Display	NSC:S744736 Endpt:GI50 Expld:0709NS10 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	GI50 - 50% growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Mimimum Standard Deviation	0.05

Job ID: 5567190687614250772

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.49	NSC:S192965 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	spirogermanium
2	0.486	NSC:S192965 Endpt:GI50 Expld:AVGDATA hiConc:-2.0	spirogermanium
3	0.469	NSC:S163501 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	AT-125 (acivicin)
4	0.455	NSC:S126771 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	dichloroallyl lawsone
5	0.454	NSC:S95441 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	methyl-CCNU

COMPARE GI_{50}: **4d** (ND-006731) [NSC-744737]

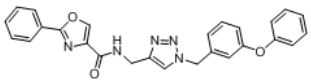


COMPARE Seed	
Namecode	Q38V-1
Vector ID	518243
Ident For Display	NSC:S744737 Endpt:GI50 Expld:0709NS10 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	GI50 - 50% growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Minimum Standard Deviation	0.05

Job ID: 1011257014195225778

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.503	NSC:S163501 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	AT-125 (acivicin)
2	0.471	NSC:S150014 Endpt:GI50 Expld:AVGDATA hiConc:-2.5	hydrazine sulfate
3	0.468	NSC:S95441 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	methyl-CCNU
4	0.459	NSC:S141537 Endpt:GI50 Expld:AVGDATA hiConc:-7.0	anguidine
5	0.455	NSC:S368390 Endpt:GI50 Expld:AVGDATA hiConc:-3.3	DUP785 (brequinar)

COMPARE GI_{50}: **4e** (ND-006732) [NSC-743294]

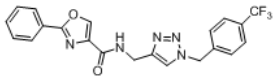


COMPARE Seed	
Namecode	Q38V-1
Vector ID	398491
Ident For Display	NSC:S743294 Endpt:GI50 Expld:AVGDATA hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	GI50 - 50% growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Minimum Standard Deviation	0.05

Job ID: 8583807729059955545

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.541	NSC:S743294 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	paclitaxel (Taxol)
2	0.534	NSC:S743294 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	maytansine
3	0.50	NSC:S743294 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	vincristine sulfate
4	0.48	NSC:S743294 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	trimetrexate
5	0.46	NSC:S743294 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	soluble Baker's Antifol

COMPARE TGI: **4c** (ND-006730) [NSC-744736]

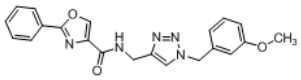


COMPARE Seed	
Namecode	Q38V-1
Vector ID	619734
Ident For Display	NSC:S744736 Endpt:TGI ExpId:0709NS10 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	TGI – total growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Minimum Standard Deviation	0.05

Job ID: 629443433769171751

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.652	NSC:S127716 Endpt:TGI ExpId:AVGDATA hiConc:-3.4	5-azadeoxycytidine
2	0.604	NSC:S125973 Endpt:TGI ExpId:AVGDATA hiConc:-6.0	paclitaxel (Taxol)
3	0.587	NSC:S284751 Endpt:TGI ExpId:AVGDATA hiConc:-3.5	8Cl-cyc-AMP
4	0.503	NSC:S293015 Endpt:TGI ExpId:AVGDATA hiConc:-4.2	flavoneacetic acid ester
5	0.472	NSC:S73754 Endpt:TGI ExpId:AVGDATA hiConc:-2.6	fluorodopan

COMPARE TGI: **4d** (ND-006731) [NSC-744737]

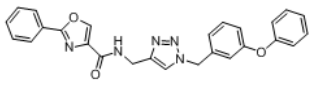


COMPARE Seed	
Namecode	Q38V-1
Vector ID	664179
Ident For Display	NSC:S744737 Endpt:TGI ExpId:0709NS10 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	TGI – total growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Minimum Standard Deviation	0.05

Job ID: 6400153564696226613

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.712	NSC:S127716 Endpt:TGI ExpId:AVGDATA hiConc:-3.4	5-azadeoxycytidine
2	0.621	NSC:S284751 Endpt:TGI ExpId:AVGDATA hiConc:-3.5	8Cl-cyc-AMP
3	0.603	NSC:S125973 Endpt:TGI ExpId:AVGDATA hiConc:-6.0	paclitaxel (Taxol)
4	0.498	NSC:S73754 Endpt:TGI ExpId:AVGDATA hiConc:-2.6	fluorodopan
5	0.481	NSC:S293015 Endpt:TGI ExpId:AVGDATA hiConc:-4.2	flavoneacetic acid ester

COMPARE TGI: **4e** (ND-006732) [NSC-743294]

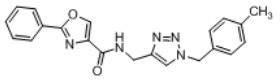


COMPARE Seed	
Namecode	Q38V-1
Vector ID	321460
Ident For Display	NSC:S743294 Endpt:TGI ExpId:AVGDATA hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	TGI – total growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Minimum Standard Deviation	0.05

Job ID: 3381429712570732660

Rank	Correlation	Target Vector ident	Target Vector Discriptor
1	0.654	NSC:S125973 Endpt:TGI ExpId:AVGDATA hiConc:-5.0	paclitaxel (Taxol)
2	0.646	NSC:S125973 Endpt:TGI ExpId:AVGDATA hiConc:-6.0	paclitaxel (Taxol)
3	0.642	NSC:S125973 Endpt:TGI ExpId:AVGDATA hiConc:-4.6	paclitaxel (Taxol)
4	0.621	NSC:S153858 Endpt:TGI ExpId:AVGDATA hiConc:-9.0	maytansine
5	0.606	NSC:S49842 Endpt:TGI ExpId:AVGDATA hiConc:-5.6	vinblastine sulfate

COMPARE TGI: **4b** (ND-006735) [NSC-743295]

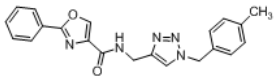


COMPARE Seed	
Namecode	Q38V-1
Vector ID	558845
Ident For Display	NSC:S743295 Endpt:TGI ExpId:0611NS42 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	TGI – total growth inhibition
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Minimum Standard Deviation	0.05

Job ID: 6448311945586022553

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	0.484	NSC:S167780 Endpt:TGI ExpId:AVGDATA hiConc:-4.0	asaley
2	0.477	NSC:S125973 Endpt:TGI ExpId:AVGDATA hiConc:-4.0	paclitaxel (Taxol)
3	0.474	NSC:S27640 Endpt:TGI ExpId:AVGDATA hiConc:-2.6	5-FUDR
4	0.438	NSC:S332598 Endpt:TGI ExpId:AVGDATA hiConc:-5.3	rhizoxin
5	0.436	NSC:S24559 Endpt:TGI ExpId:AVGDATA hiConc:-2.9	mitramycin

COMPARE LC_{50}: **4b** (ND-006735) [NSC-743295]

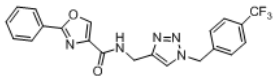


COMPARE Seed	
Namecode	Q38V-1
Vector ID	630268
Ident For Display	NSC:S743295 Endpt:LC50 Expld:0611NS42 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	LC50 – 50% cell kill
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Mimimum Standard Deviation	0.05

Job ID: 3383383595683701483

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	<0.2		
2			
3			
4			
5			

COMPARE LC_{50}: **4c** (ND-006730) [NSC-744736]

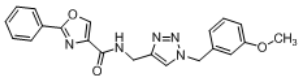


COMPARE Seed	
Namecode	Q38V-1
Vector ID	889120
Ident For Display	NSC:S744736 Endpt:LC50 Expld:0709NS10 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	LC50 – 50% cell kill
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Mimimum Standard Deviation	0.05

Job ID: 4377333757265360978

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	< 0.2		
2			
3			
4			
5			

COMPARE LC_{50}: **4d** (ND-006731) [NSC-744737]

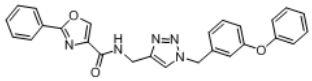


COMPARE Seed	
Namecode	Q38V-1
Vector ID	463441
Ident For Display	NSC:S744737 Endpt:LC50 Expld:0709NS10 hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	LC50 – 50% cell kill
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Mimimum Standard Deviation	0.05

Job ID: 1019476158446175237

Rank	Correlation	Target Vector NSC	Target Vector Discriptor
1	< 0.2		
2			
3			
4			
5			

COMPARE LC_{50}: **4e** (ND-006732) [NSC-743294]



COMPARE Seed	
Namecode	Q38V-1
Vector ID	369328
Ident For Display	NSC:S743294 Endpt:LC50 Expld:AVGDATA hiConc:-4.0
Parameters for Standard COMPARE	
Target Set Name	STANDARD_AGENTS
Target Set Endpoint	LC50 – 50% cell kill
Lookup Word	MJMLab
Minimum Correlation	0.2
Count Results to Return	50
Minimum Count Common Cell Lines	40
Mimimum Standard Deviation	0.05

Job ID:1401022145659285532

Rank	Correlation	Target Vector ident	Target Vector Discriptor
1	0.681	NSC:S332598 Endpt:LC50 Expld:AVGDATA hiConc:-9.0	rhizoxin
2	0.65	NSC:S332598 Endpt:LC50 Expld:AVGDATA hiConc:-4.0	rhizoxin
3	0.558	NSC:S49842 Endpt:LC50 Expld:AVGDATA hiConc:-7.6	vinblastine sulfate
4	0.545	NSC:S71851 Endpt:LC50 Expld:AVGDATA hiConc:-2.3	A-TGDR
5	0.54	NSC:S49842 Endpt:LC50 Expld:AVGDATA hiConc:-4.0	vinblastine sulfate

In Vitro Tubulin Polymerization Assay

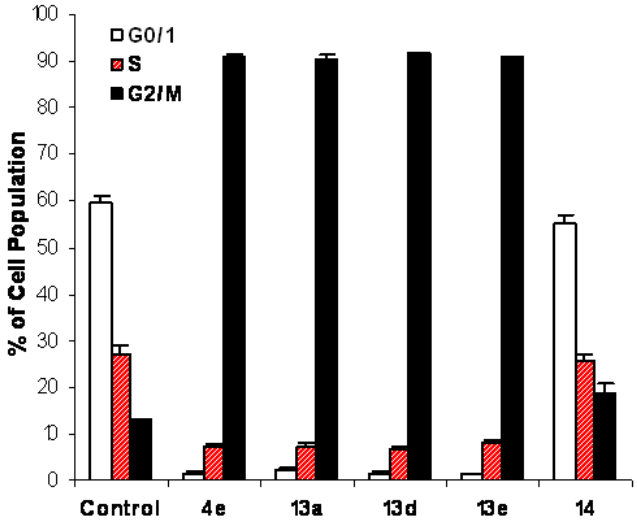
Ice-cold assay buffer (39 μL of 80 mM PIPES pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$, 5% glycerol) was placed in a 384-well plate. Compound (1 μL of 0.5 mM in DMSO) was added to achieve a final drug concentration of 10 μM. Tubulin (10 μL of 15 mg/mL in ice-cold 500 mM K–PIPES, 0.5 mM $MgCl_2$, pH 6.9 buffer) was added to each well. The plate was cooled on ice for 10 min and GTP (0.5 μL from 100 mM) stock was added using an automated multichannel pipette followed by mixing three times (mix volume = 12 μL). The plate was placed immediately in a Molecular Devices SpectraMax 384 plus plate reader pre-heated to 37 ºC and the progress of polymerization monitored at 340 nm for 30 min. The microtubule stabilizer and destabilizer, paclitaxel and nocodazole respectively were used as controls at a final concentration of 10 μM.

Cell Cycle Arrest

HeLa cells were grown in RPMI–1640 media supplemented with 10% fetal bovine serum (Gemini Bioproducts) and 1% Pen-Strep antibiotic and incubated at 37 ºC in a 5% CO_2, 95% humidity atmosphere. The HeLa cells (8 x 105 in 4 mL) were treated with 5 μM compound (0.05% DMSO final) in 6-well plates for 18 h in replicates of three. The cells were harvested, fixed in 70% ice-cold ethanol for 1 h at –20 ºC, rinsed with phosphate buffered saline (PBS pH 7.4) and RNase treated (50 μL of 0.1 mg/mL RNase A in PBS) for 2 h and subsequently stained with propidium iodide (50 μg/mL in PBS). The DNA content was measured using a Benton Dickinson LSR II cell flow cytometer with 20,000 events acquired per sample. The data was gated for single cells using propidium iodide-width vs. area as discriminators (see supporting information for raw data). Population distribution in the various phases of the cell cycle was determined using the software FCS Express version 3.

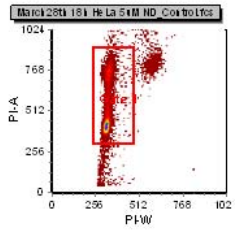
Summary of Cell Cycle Analysis Results:

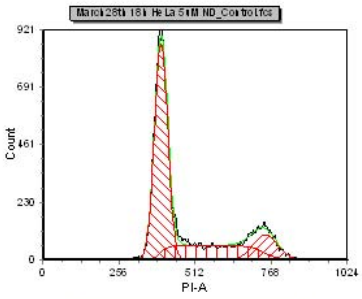
- 800,000 HeLa cells in 4mL treated with 5μM Compound (0.05% DMSO) in 6-well plates in triplicate for 18h.
- Cells were harvested, fixed in 70% ice-cold EtOH for 1hr, RNase (0.1mg/ml) treated for 2h and stained with propidium iodide (50 μg/mL) and DNA content was measured by Cell flow cytometry with 20,000 events acquired per sample. The data was gated for single cells using PI-width vs. PI-Area.



Cell Cycle Analysis Results: **Control**

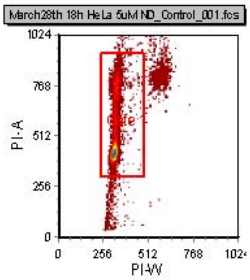
Replicate 1.

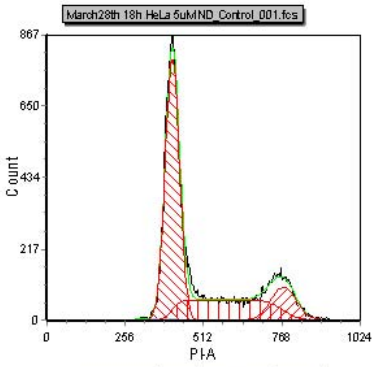




Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	61.08	13.15	25.76	1.87	100.00

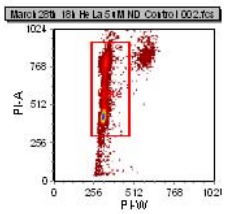
Replicate 2.

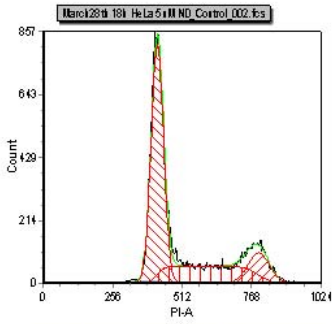




Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	58.06	13.22	28.72	1.88	100.00

Replicate 3.

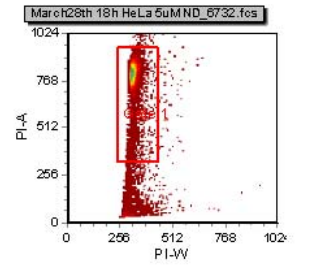


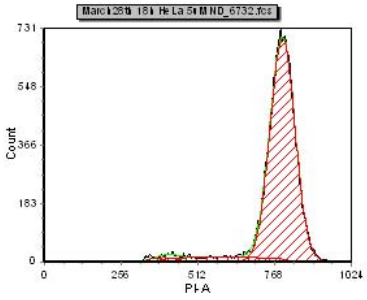


Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	59.51	13.02	27.47	1.88	100.00

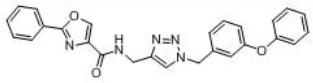
Cell Cycle Analysis Results: **4e** (ND-6732)

Replicate 1.

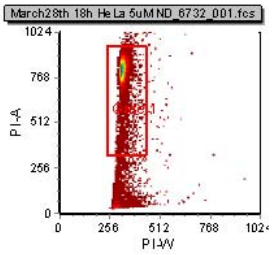


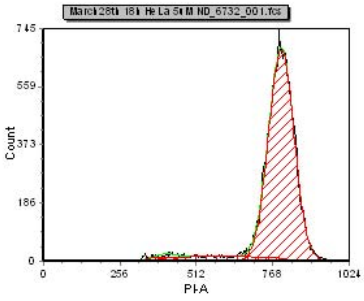


Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.70	91.34	6.96	1.98	100.00



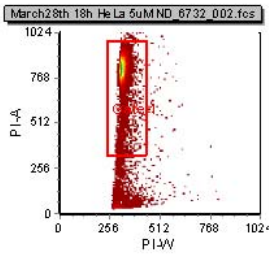
Replicate 2.

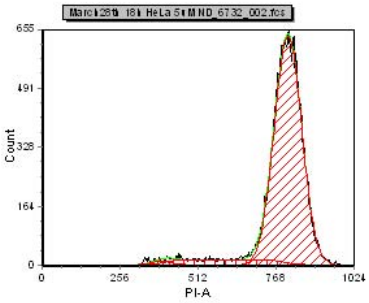




Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.62	91.28	7.10	1.99	100.00

Replicate 3.

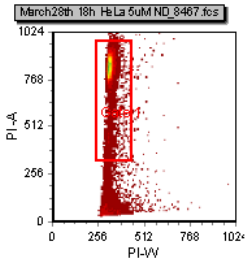


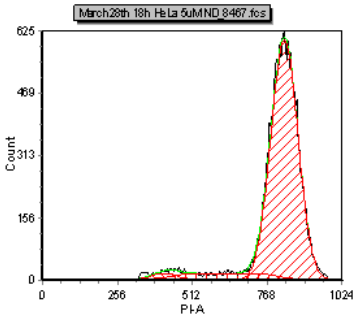


Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.39	90.66	7.95	2.05	100.00

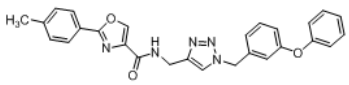
Cell Cycle Analysis Results: **13a** (ND-8467)

Replicate 1.

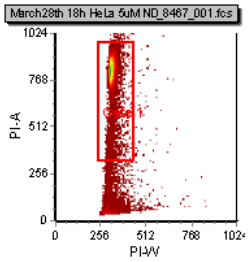


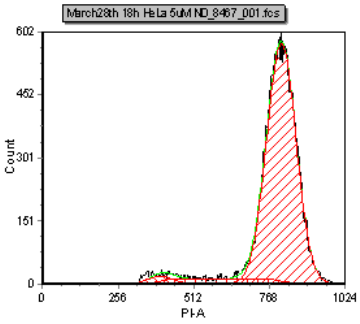


Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	2.62	88.97	8.41	1.98	100.00



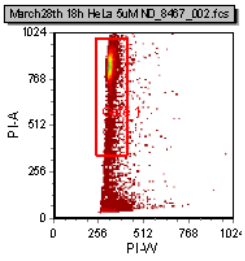
Replicate 2.

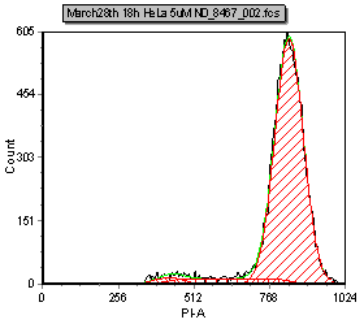




Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	2.41	91.06	6.53	2.01	100.00

Replicate 3.

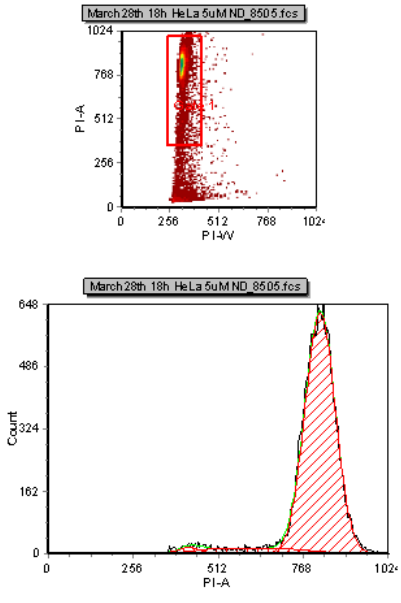




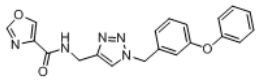
Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	2.28	90.90	6.82	1.94	100.00

Cell Cycle Analysis Results: **13d** (ND-8505)

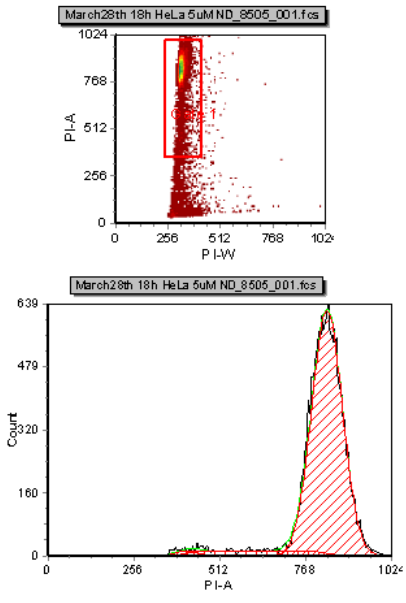
Replicate 1.



Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.76	91.84	6.41	1.93	100.00

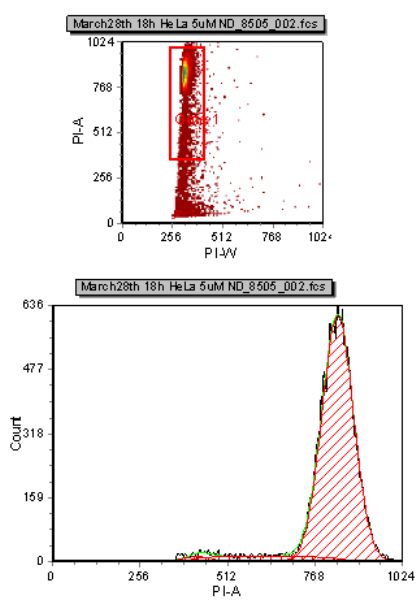


Replicate 2.



Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.25	91.85	6.90	2.00	100.00

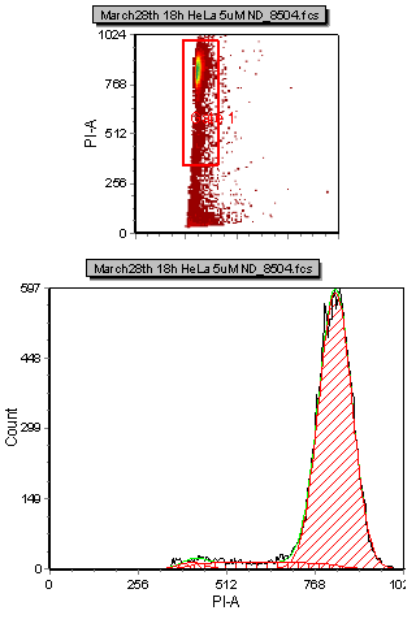
Replicate 3.



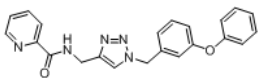
Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.56	91.41	7.03	1.97	100.00

Cell Cycle Analysis Results: **13e** (ND-8504)

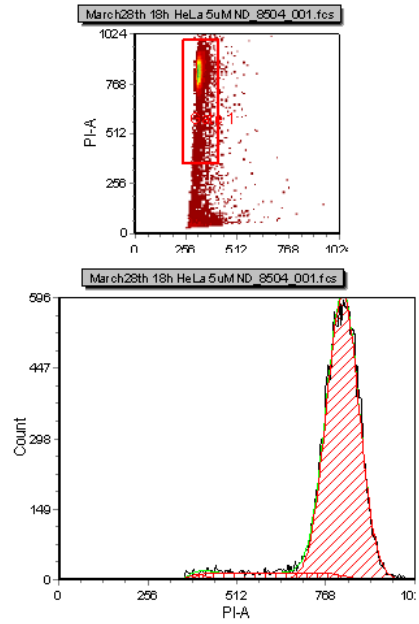
Replicate 1.



Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.61	90.80	7.59	2.00	100.00

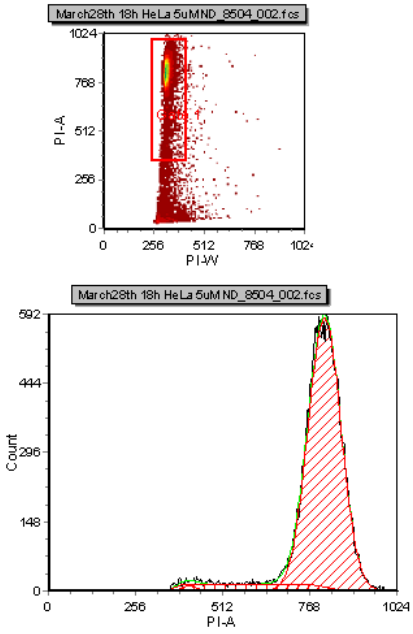


Replicate 2.

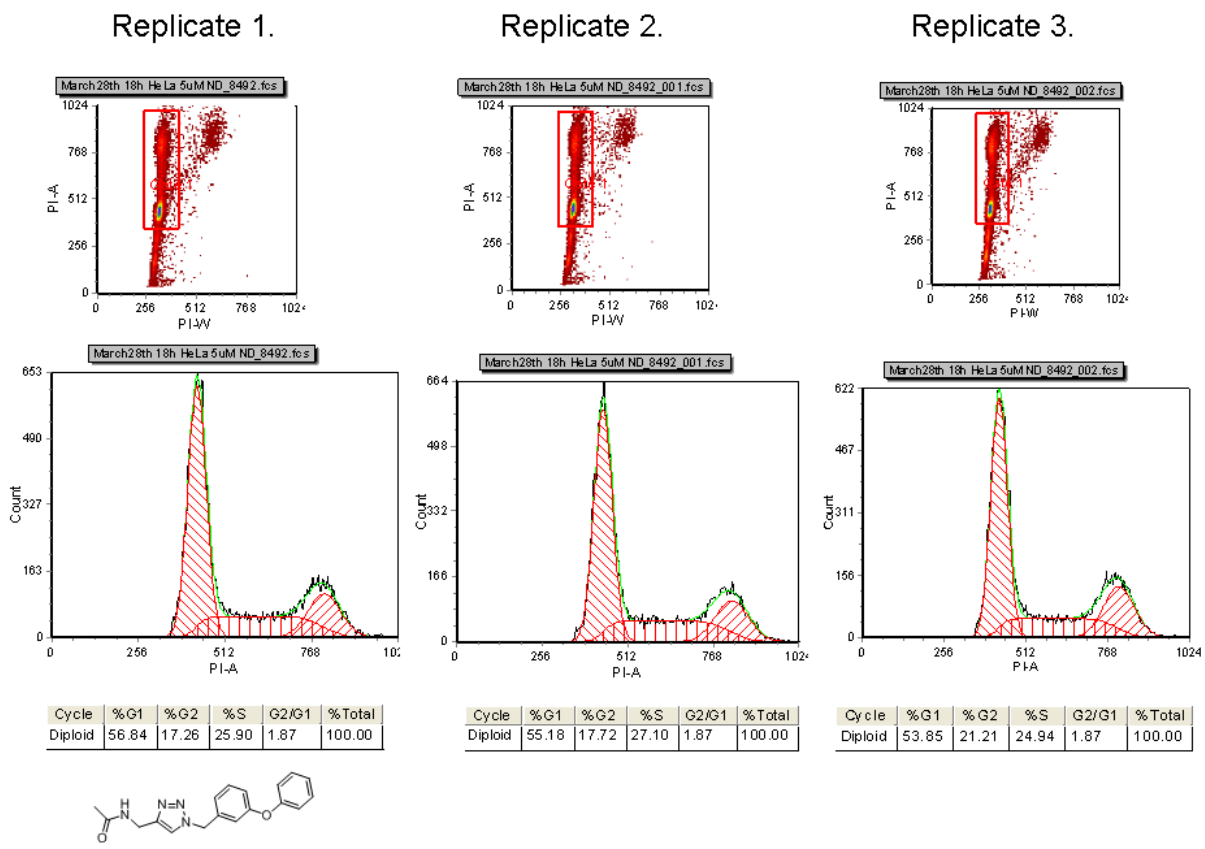


Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	0.86	90.89	8.25	2.03	100.00

Replicate 3.



Cycle	%G1	%G2	%S	G2/G1	%Total
Diploid	1.10	90.72	8.19	2.01	100.00



Confocal Microscopy

HeLa cells were grown in RPMI–1640 media supplemented with 10% fetal bovine serum (Gemini Bioproducts) and 1% Pen-Strep antibiotic and incubated at 37 ºC in a 5% CO_2, 95% humidity atmosphere. The HeLa cells (2.5 x 106 cells/mL) were placed on 22 mm Corning type 1 square cover slips and incubated for 3 h to allow the cells to attach. The cells were then incubated for 18 h in media containing DMSO vehicle or 5 µM compound (0.05% DMSO final). The media was aspirated and cells treated with microtubule stabilizing buffer (MTSB: 80 mM PIPES pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA) for 30 seconds after which glutaraldehyde was added to 0.5% final and the cells fixed for 10 min. Excess glutaraldehyde was quenched by treating the samples twice with a freshly made solution of $NaBH_4$ (1 mg/mL in PBS) for 5 min. The samples were rinsed with PBS and blocked for 10 min with antibody diluting solution (PBS pH 7.4, 0.2% Triton X–100, 2% BSA, 0.1% sodium azide). Monoclonal anti-α-tubulin mouse FITC conjugated antibody (1:50 dilution) in antibody diluting solution containing 0.5 mg/mL RNase A (Qiagen) was added and the samples incubated for 2.5 hrs and then treated for 15 min

with propidium iodide (50 μg/mL in antibody diluting solution). The samples were washed four times (5 min each) with PBS containing 0.1% Triton X-100, drained, mounted onto microscope slides using 10 μL of vectashield (Vector Technologies) mounting media. The cover slips were sealed and the samples visualized immediately on a Zeiss confocal LSM510 microscope.