Materials and Methods for:

The Complex Role of the Triphenylmethyl Motif in Anti-Cancer Compounds

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Materials and Methods

General

Dry acetonitrile, benzene, dichloromethane, and tetrahydrofuran were obtained by passing over activated alumina columns or molecular sieves in a commercial solvent purification system (Innovative Technologies). Potassium osmate, 4-methylmorpholine *N*-oxide, 1 M boron tribromide (BBr_3) in CH_2Cl_2, triphenylmethanol, triphenylmethyl chloride, 4-methoxytriphenylmethyl chloride, 4,4'-dimethoxytriphenylmethyl chloride, 4,4',4''-trimethoxytriphenylmethyl chloride, S-trityl-L-cysteine, trimethyl phosphite, triethyl phosphite, tributyl phosphite, phosphorous trichloride, clotrimazole, sodium hydride (60% dispersion in mineral oil), and sulforhodamine B sodium salt were obtained from Sigma Aldrich. 4-Methoxytriphenylmethanol and 4,4',4''-trimethyltriphenylmethanol were purchased from Alfa Aesar. 2-Chlorophenyldiphenylmethyl chloride was obtained from TCI America. For biological assays, paclitaxel, nocodazole, propidium iodide, calcium ionophore A23187, phenazine methosulfate, monoclonal anti-α-tubulin−FITC antibody, Ribonuclease A (from bovine pancreas), α-chymotrypsin (from bovine pancrease type II), N-Succinyl-Ala-Ala-Pro-Phe p-nitroamide and Glucose-6-phosphate Dehydrogenase from baker's yeast were purchased from Sigma Aldrich. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt was obtained from Promega.

Compound Analysis.

All NMR experiments were recorded either in $CDCl_3$ (Sigma), DMSO-*d6* (Cambridge Isotope Laboratories) or Acetone-*d6* (Sigma) on a Varian Unity 400 MHz or 500 MHz spectrometer with residual undeuterated solvent as the internal reference. Chemical shift, δ (ppm); coupling constants, *J* (Hz); multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet); and integration are reported. High-resolution mass spectral data was recorded on a Micromass Q-Tof Ultima hybrid quadrupole/time-of-flight ESI mass spectrometer at the University of Illinois Mass Spectrometry Laboratory. All melting points are uncorrected.

Synthesis of dialkyl phosphonates by the Arbuzov reaction (Scheme 1).

The phosphonates were prepared analogous to the procedure reported by Shi *et al.*[1]

General procedure: In a 40 mL reaction flask charged with dry benzene (2 mL) under a N_2 atmosphere, was added the appropriate triphenylmethyl chloride (1 mmol). The trialkylphosphite (1.5-2.0 equiv.) was added and the reaction was refluxed for 2 h after which the solvent and excess trialkylphosphite were removed *in vacuo*. The residue was recrystallized from methanol or purified by column chromatography on silica (1:1 hexanes/ethyl acetate) to yield the desired product. The following products were obtained by this method:

TPMP-I-2

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.30 (m, 15H); 3.57 (d, 6H, *J*=10.6Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 141.23; 130.55; 127.90; 127.02; 62.90 (*J*=136.37Hz); 53.81.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 29.67.

HRMS (ESI): found: 353.1322 (M+H); calculated for $C_{21}H_{22}O_3P$: 353.1307.

IR (CH_2Cl_2, cm^{-1}): 3058; 2952; 1492; 1444; 1241; 1054; 1026; 737; 700.

m.p.: 153-154.5 °C.

Description: white solid.

TPMP-I-3

NMR ^{1}H (400 MHz, $CDCl_3$) δ ppm: 7.29 (m, 15H); 4.01 (pd, 2H, *J*=7.1Hz, *J*=10.2Hz); 3.82 (qdd, 2H, *J*=7.1Hz, *J*=8.5Hz, *J*=10.2Hz); 1.09 (t, 6H, *J*=7.1Hz).

NMR ^{13}C (101 MHz, $CDCl_3$) δ ppm: 141.58; 130.62; 127.76; 126.84; 63.29; 62.89 (*J*=135.99Hz); 16.163.

NMR ^{31}P (162 MHz, $CDCl_3$) δ ppm: 27.065.

HRMS (ESI): found: 381.1622 (M+H); calculated for $C_{23}H_{26}O_3P$: 381.1620.

IR (CH_2Cl_2, cm^{-1}): 3054; 2986; 1265; 1050; 747; 704.

m.p.: 118.5-119.5 °C.

Description: white solid.

TPMP-I-6

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.36 (m, 6H); 7.29 (m, 9H); 3.98 (qd, 2H, *J*=6.5Hz, *J*=10.1Hz); 3.77 (qd, 2H, *J*=6.7Hz, *J*=10.1Hz); 1.42 (m, 4H); 1.20 (m, 4H); 0.83 (t, 6H, *J*=7.4Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 141.49; 130.58; 127.65; 126.70; 66.81; 62.86 (*J*=136.27Hz); 32.10; 18.49; 13.39.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 26.90.

HRMS (ESI): found: 437.2244 (M+H); calculated for $C_{27}H_{34}O_3P$: 437.2246.

IR (CH_2Cl_2, cm^{-1}): 3059; 2961; 2237; 1599; 1493; 1446; 1238; 1029; 980; 909; 733; 700

m.p.: 110.5-111.5 °C.

Description: white solid.

TPMP-III-1

NMR ^{1}H (500 MHz, Acetone-*d6*) δ ppm: 7.30 (m, 10H); 7.18 (dd, 2H, J1=1.9Hz, J2=9.1Hz); 6.87 (dd, 2H, J1=0.7Hz, J2=9.1Hz); 3.80 (s, 3H); 3.55 (d, 6H, J1=10.6Hz).

NMR ^{13}C (126 MHz, Acetone-*d6*) δ ppm: 149.70; 133.23; 124.47; 122.73; 121.61; 118.83; 117.96; 104.12; 53.62; 52.53; 45.65; 44.04.

NMR ^{31}P (202 MHz, Acetone-*d6*) δ ppm: 29.61.

HRMS (ESI): found: 383.1424 (M+H); calculated for $C_{22}H_{24}O_4P$: 383.1412.

IR (CH_2Cl_2, cm^{-1}): 3056; 2953; 2848; 1607; 1579; 1511; 1463; 1445; 1293; 1250; 1187; 1053; 1028; 818; 742; 701.

m.p.: 127-128 °C.

Description: white solid.

TPMP-III-2

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.33 (m, 5H); 7.23 (d, 4H, J=7.7Hz); 6.84 (d, 1H, J=8.7Hz); 3.81 (s, 6H); 3.58 (d, 6H, J=10.5Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 158.29; 141.81; 133.43; 131.66; 130.41; 127.87; 126.91; 113.17; 61.41 (*J*=136.8 Hz); 55.14; 53.76.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 30.100.

HRMS (ESI): found: 413.1521 (M+H); calculated for $C_{23}H_{26}O_5P$: 413.1518.

IR (CH_2Cl_2, cm^{-1}): 3005; 2955; 2839; 2253; 1510; 1252; 1034; 908; 731; 649

m.p.: 137-139 °C.

Description: white solid.

TPMP-III-3

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.35 (m, 3H); 7.26 (m, 6H); 6.82 (d, 4H, *J*=8.9Hz); 4.02 (m, 2H); 3.83 (m, 2H); 3.78 (s, 6H); 1.11 (t, 6H, *J*=7.1Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 158.10; 142.01; 133.66; 131.57; 130.43; 128.16; 127.62; 126.63; 112.93; 62.98; 61.31(*J*=136.70 Hz); 55.00; 16.07;

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 27.568.

HRMS (ESI): found: 441.1832 (M+H); calculated for $C_{25}H_{30}O_5P$: 441.1831.

IR (CH_2Cl_2, cm^{-1}): 3054; 2985; 1509; 1265; 1034; 737; 704.

m.p.: 85-86 °C.

Description: white solid.

TPMP-III-4

NMR ^{1}H (500 MHz, Acetone-*d6*) δ ppm: 7.19 (dd, 6H, *J*=1.9Hz, *J*=8.9Hz); 6.86 (d, 6H, *J*=8.9Hz); 3.79 (m, 9H); 3.54 (d, 6H, *J*=10.5Hz).

NMR ^{13}C (126 MHz, Acetone-*d6*) δ ppm: 160.37; 135.90; 133.37; 114.80; 56.44; 54.77; 54.71;

NMR ^{31}P (202 MHz, Acetone-*d6*) δ ppm: 30.08.

HRMS (ESI): found: 443.1643 (M+H); calculated for $C_{24}H_{28}O_6P$: 443.1624.

IR (CH_2Cl_2, cm^{-1}): 3053; 2954; 2838; 1607; 1508; 1253; 1033; 821; 737.

m.p.: 164-165 °C.

Description: white solid.

TPMP-III-5

NMR ^{1}H (400 MHz, $CDCl_3$) δ ppm: 7.24 (m, 6H); 6.81 (m, 6H); 4.00 (m, 2H); 3.81 (m, 2H); 3.80 (m, 9H); 1.11 (t, 6H, *J*=7.1Hz).

NMR ^{13}C (101 MHz, $CDCl_3$) δ ppm: 157.94; 133.84; 131.41; 112.77; 62.88; 60.96; 59.87 (*J*=137.1Hz); 54.86; 16.04.

NMR ^{31}P (162 MHz, $CDCl_3$) δ ppm: 27.75.

HRMS (ESI): found: 471.1941 (M+H); calculated for $C_{26}H_{32}O_6P$: 471.1937.

IR (CH_2Cl_2, cm^{-1}): 2983; 1607; 1508; 1265; 1035; 738.

m.p.: 143-144 °C.

Description: white solid.

TPMP-III-6

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 8.28 (d, 1H, *J*=7.8Hz); 7.58 (dd, 4H, *J*=1.6Hz, *J*=7.4Hz); 7.39 (m, 1H); 7.30 (m, 8H); 3.40 (d, 6H, *J*=10.5Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 139.53; 137.01; 136.29; 133.00; 132.27; 130.71; 128.61; 127.548; 126.96; 126.38; 62.84 (*J*=133.10); 54.16.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 27.68.

HRMS (ESI): found: 387.0914 (M+H); calculated for $C_{21}H_{21}O_3PCl$: 387.0917.

IR (CH_2Cl_2, cm^{-1}): 3056; 2954; 2851; 1493; 1265; 1241; 1047; 822; 736; 705.

m.p.: 125-127 °C.

Description: white solid.

Synthesis of TPMP-III-7

TPMP-III-7 was synthesized according to a published procedure.[2]

NMR ^{1}H (500 MHz, DMSO-*d6*) δ ppm: 9.46 (s, 2H); 7.30 (m, 2H); 7.26 (m, 1H); 7.17 (d, 2H, *J*=7.6Hz); 6.94 (d, 4H, *J*=8.3Hz); 6.69 (d, 4H, *J*=8.6Hz); 3.47 (d, 6H, *J*=10.5Hz).

NMR ^{13}C (126 MHz, DMSO-*d6*) δ ppm: 156.00; 142.13; 131.45; 131.07; 129.84; 127.71; 126.66; 114.51; 60.60 (*J*=136.9Hz); 53.30.

NMR ^{31}P (202 MHz, DMSO-*d6*) δ ppm: 30.35.

HRMS (ESI): found: 385.1213 (M+H); calculated for $C_{21}H_{22}O_5P$: 385.1205.

IR (thin film, cm^{-1}): 3221 (b); 2954; 1510; 1215; 1180; 1053; 1023; 826; 702.

Description: white solid.

Synthesis of TPMP-III-8 (Scheme 2 in manuscript text)

To a 25 mL round bottom flask containing **TPMP-III-4** (100 mg, 0.226 mmol) dissolved in dry CH_2Cl_2 (1 mL) at -78 ^{o}C under a nitrogen atmosphere, BBr_3 (0.9 mL of 1 M BBr_3 in CH_2Cl_2, 0.9 mmol, 4 equiv.) was added dropwise. The reaction was warmed to 25 ºC and stirred for 25 h, after which 5 mL of water was added and reaction stirred for 1 h. The insoluble creamy yellow precipitate was filtered and washed with cold water. The precipitate was dissolved in the minimum amount of 50:50 methanol/ethyl acetate and purified by column chromatography on silica using 10:90 methanol/ethyl acetate as the eluent to yield **TPMP-III-8** (40 mg, 44% yield) as an orange solid.

NMR ^{1}H (500 MHz, DMSO-*d6*) δ ppm: 9.43 (s, 3H); 6.93 (d, 6H, *J*=7.8Hz); 6.67 (d, 6H, *J*=8.8Hz); 3.45 (d, 6H, *J*=10.4Hz).

NMR ^{13}C (126 MHz, DMSO-*d6*) δ ppm: 155.87; 131.97; 130.95; 114.41; 59.84 (*J*=137.0Hz); 53.19.

NMR ^{31}P (202 MHz, DMSO-*d6*) δ ppm: 30.79.

HRMS (ESI): found: 401.1165 (M+H); calculated for $C_{21}H_{22}O_6P$: 401.1154.

IR (thin film, cm^{-1}): 3164 (b); 1509; 1274; 1179; 1050; 1024; 825; 758.

Description: orange solid.

Synthesis of TPMP-II-1 (Scheme 3 in manuscript text)

Triphenylmethanol **10** (5 g, 19.206 mmol) was dissolved in a minimum volume of dry acetonitrile and the solution was placed in a 50 mL round bottom flask under a N_2 atmosphere. The flask was cooled in an ice bath and phosphorus trichloride (1.85 mL, 2.9 g, 21.126 mmol, 1.1 equiv.) was cautiously added dropwise. The reaction was stirred at 25 ºC until the yellow color dissipated. The reaction was cooled to 0 ºC and the white solid was filtered, washed with 1 M NH_4CO_3 (20 mL), water (20 mL), and petroleum ether (20 mL), and dried *in vacuo* to yield triphenylphosphonyl dichloride (6.59 g, 95% yield) as a white solid.

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.40 (m, 15H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 138.85; 130.70; 128.41; 75.68 (*J*=85.09).

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 60.20.

HRMS (ESI): found: 383.0145 ($M+Na^+$); calculated for $C_{19}H_{15}Cl_2OPNa$: 383.0135.

IR (CH_2Cl_2, cm^{-1}): 3054; 2987; 2305; 1265; 896; 743; 705

m.p.: 179-182°C.

Description: white solid.

Synthesis of 13. (Scheme 4 in manuscript text)

In a 40 mL reaction vessel under a N_2 atmosphere was placed 4-methoxytriphenylmethanol **11** (0.826 g, 2.845 mmol). Phosphorous trichloride (4 mL, 45.8 mmol, 16.1 equiv.) was added drop wise and the reaction stirred vigorously at room temperature for 2 h after which the reaction was decomposed

on crushed ice. The resulting precipitate was filtered, washed with 1 M NH_4CO_3 (20 mL), water (20 mL), petroleum ether (20 mL) and dried *in vacuo* to yield 4-methoxytriphenylmethylphosphonyl dichloride **13** (1.0 g, 90% yield) as an orange solid. The crude material was used in the synthesis of **TPMP-III-9** without further purification.

Synthesis of 14. (Scheme 4 in manuscript text)

The phosphonyl dichloride **14** was synthesized using the procedure analogous to the synthesis of **13** employing 4,4',4''-trimethyltriphenylmethanol **12** (1.0 g, 3.307 mmol) to yield 4,4',4''-trimethyltriphenylphosphonyl dichloride **14** (1.30 g, 97% yield) as a yellow solid. The crude material was used in the synthesis of **TPMP-III-10** without further purification.

Synthesis of phosphonates from triphenylmethylphosphonyl dichloride (Scheme 3 in manuscript text)

General procedure: The synthesis of phosphonates from triphenylmethylphosphonyl dichloride was performed on a 0.5 mmol scale. In a dry microwave irradiation vial, the appropriate alcohol (3.0 equiv.) was dissolved in 4 mL of THF and cooled to 0-5 ºC. Sodium hydride (2.0-2.2 equiv. of 60% dispersion in mineral oil) was added to form the alkoxide *in situ*. The solution was stirred for 30 min at which time hydrogen evolution had ceased. Triphenylmethylphosphonyl dichloride (0.5 mmol, 1 equivalent) was added as a solid and the reaction irradiated with microwaves (150 W power) at 110 ºC for 30-40 min. Upon completion of the reaction (as judged by TLC) the solvent was removed *in vacuo*. Water (5 mL) and CH_2Cl_2 (5 mL) was added and the organic layer isolated, dried over anhydrous $MgSO_4$, and solvent removed *in vacuo*. The isolated material was subjected to column chromatography on silica using 1:1 ethyl acetate/hexane as the eluent. The following products were synthesized using the procedure described:

TPMP-I-4

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.33 (m, 15H); 4.32 (m, 2H); 3.81 (m, 2H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 139.49; 130.37; 128.30; 127.71; 122.39; 63.45 (*J*=135.17Hz); 62.87.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 29.63.

HRMS (ESI): found: 489.1063 (M+H); calculated for $C_{23}H_{20}F_6O_3P$: 489.1054.

IR (CH_2Cl_2, cm^{-1}): 3062; 2968; 2250; 1599; 1492; 1290; 1249; 1173; 1100; 962; 909; 734; 700.

m.p.: 125-126 °C.

Description: white solid.

TPMP-I-5

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.30 (m, 15H); 3.90 (qd, 2H, *J*=6.5Hz, *J*=10.0Hz); 3.70 (qd, 2H, *J*=6.6Hz, *J*=9.9Hz); 1.44 (m, 4H); 0.75 (t, 6H, *J*=7.4Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 141.65; 130.71; 127.76; 126.80; 68.71; 63.00 (*J*=136.40Hz); 23.65; 10.01.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 26.80.

HRMS (ESI): found: 409.1935 (M+H); calculated for $C_{25}H_{30}O_3P$: 409.1933.

IR (CH_2Cl_2, cm^{-1}): 2973; 2253; 1471; 1282; 1235; 997; 909; 732; 650

m.p.: 106.5-108 °C.

Description: white solid.

TPMP-I-7

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.30 (m, 15H); 4.72 (s, 2H); 4.58 (s, 2H).

4.04 (ddd, 2H, *J*=6.7Hz, *J*=10.2Hz, *J*=13.4Hz); 3.83 (qd, 2H, *J*=6.9Hz, *J*=10.1Hz); 2.10 (m, 4H); 1.60 (s, 6H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 141.38; 130.75; 130.69; 127.80; 126.90; 112.42; 65.34; 63.03 (*J*=135.85Hz); 38.28; 22.22.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 27.03.

HRMS (ESI): found: 461.2245 (M+H); calculated for $C_{29}H_{34}O_3P$: 461.2246.

IR (CH_2Cl_2, cm^{-1}): 3054; 2928; 2306; 1492; 1445; 1265; 1036; 896; 739; 703

Description: yellow oil

TPMP-I-8

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.31 (m, 15H); 4.65 (m, 2H); 4.32 (m, 2H); 2.47 (m, 2H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 140.51; 130.51; 128.03; 127.22; 77.86; 75.57; 63.14 (*J*=135.75Hz); 54.51.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 29.581.

HRMS (ESI): found: 401.1310 (M+H); calculated for $C_{25}H_{22}O_3P$: 401.1307.

IR (CH_2Cl_2, cm^{-1}): 3303; 3054; 2987; 2305; 1422; 1265; 896; 740; 705

m.p.: 121.5-122.5 °C.

Description: white solid.

TPMP-I-9

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.30 (m, 15H); 4.59 (ddd, 2H, *J*=2.2Hz, *J*=8.3Hz, *J*=14.9Hz); 4.29 (ddd, 2H, *J*=2.2Hz, *J*=10.6Hz, *J*=14.9Hz); 1.81 (s, 6H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 140.95; 130.65; 127.92; 127.01; 83.65; 73.76; 63.15 (*J*=134.85Hz); 55.32; 3.70.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 28.98.

HRMS (ESI): found: 429.1629 (M+H); calculated for $C_{27}H_{26}O_3P$: 429.1620.

IR (CH_2Cl_2, cm^{-1}): 3058; 2920; 2241; 1599; 1493; 1445; 1240; 1026; 970; 732; 700

m.p.: 146-147 °C.

Description: white solid.

TPMP-I-10

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.33 (m, 15H); 5.72 (ddd, 2H, *J*=5.3Hz, *J*=10.6Hz, *J*=22.1Hz); 5.10 (m, 4H); 4.51 (m, 2H); 4.27 (tddd, 2H, *J*=1.5Hz, *J*=5.2Hz, *J*=8.1Hz, *J*=13.3Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 141.22; 132.74; 130.58; 127.79; 126.89; 117.18; 67.36; 63.04 (*J*=136.13Hz).

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 27.84.

HRMS (ESI): found: 405.1610 (M+H); calculated for $C_{25}H_{26}O_3P$: 405.1620.

IR (CH_2Cl_2, cm^{-1}): 3058; 2945; 2887; 2240 1599; 1492; 1444; 1239; 1030; 931; 732; 700

m.p.: 86.5-87.5 °C.

Description: white solid.

Synthesis of TPMP-I-12 (Scheme 5 in manuscript text)

A procedure analogous to the synthesis of phosphonates using **TPMP-II-1** was employed with the exception that 1.1 equivalents of 1,2-ethanediol was used with 2.2 equivalents of sodium hydride to yield **TPMP-I-12**.

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.45 (m, 6H); 7.32 (m, 9H); 4.30 (m, 2H); 3.43 (m, 2H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 140.32; 130.56; 128.15; 127.40; 66.51; 63.83 (*J*=125.43Hz).

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 47.55.

HRMS (ESI): found: 351.1159 (M+H); calculated for $C_{21}H_{20}O_3P$: 351.1150.

IR (CH_2Cl_2, cm^{-1}): 2913; 2251; 1496; 1447; 1257; 1045; 908; 819; 733; 650

m.p.: 190-192 °C.

Description: white solid.

Synthesis of TPMP-I-11 (Scheme 6 in manuscript text)

In a 25 mL round bottom flask, **TPMP-I-10** (79 mg, 0.1953 mmol) was dissolved in 2:1 mixture of *t*-butanol/water (2 mL). Potassium osmate (3.6 mg, 5 mol%) and 4-methylmorpholine *N*-oxide (63.4 mg, 0.469 mmol, 2.4 equiv.) was added and the reaction stirred at 25 ºC for 18 h. The solvent was

removed *in vacuo*, and water (5 mL) was added and the flask subsequently cooled to 0 ºC to precipitate the product. The precipitate was filtered, washed with ice-cold water (10 mL) and dried *in vacuo* to yield **TPMP-I-11** (63.7 mg, 69.0%) as a white solid.

TPMP-I-11

NMR ^{1}H (500 MHz, DMSO-*d6*) δ ppm: 7.31 (m, 9H); 7.22 (m, 6H); 4.84 (m, 2H); 4.53 (td, 2H, J=5.6Hz, J=8.7Hz); 3.87 (m, 1H); 3.60 (m, 2H); 3.43 (td, 2H, J=4.9Hz, J=9.7Hz); 3.15 (m, 4H);

NMR ^{13}C (126 MHz, DMSO-*d6*) δ ppm: 141.22; 130.08; 127.81; 126.90; 70.08; 67.89; 62.28 (J=136.97Hz); 61.74;

NMR ^{31}P (202 MHz, DMSO-*d6*) δ ppm: 27.32;

HRMS (ESI): found: 473.1725 (M+H); calculated for $C_{25}H_{30}O_7P$: 473.1729

IR (CH_2Cl_2, cm^{-1}): 3200 (b) 3155; 2253; 1448; 1382; 908; 734; 650

m.p.: 210-213 °C

Description: white solid.

Synthesis of TPMP-I-1

TPMP-I-1 was synthesized according to a published procedure.[3]

Synthesis of phosphonochloridates from triphenylmethylphosphonyl dichloride (Scheme 4 in manuscript text)

General procedure: A 40 mL reaction vessel under a N_2 atmosphere was charged with 5 mL dry THF and the appropriate triphenylmethylphosphonyl dichloride (0.5-1.0 mmol) and alcohol (1.3 equiv.) was added. Sodium hydride (1.3 equiv. of 60% dispersion in mineral oil) was added and the reaction was refluxed for 4 h. The solvent was removed *in vacuo*, and CH_2Cl_2 (15 mL) and water (15 mL) were added to the vessel. The organic layer was isolated, dried over anhydrous $MgSO_4$, and the solvent

removed *in vacuo*. The resulting residue was subjected to column chromatography on silica (1:3 ethyl acetate/ hexanes). The following products were obtained by this method:

TPMP-II-2

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.34 (m, 15H); 4.83 (m, 1H); 4.67 (m, 1H); 2.53 (t, 1H, *J*=2.5Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 139.66; 130.65; 128.21; 127.78; 76.74; 76.48; 68.88 (*J*=112.71Hz); 54.97.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 47.64.

HRMS (ESI): found: 381.0821 (M+H); calculated for $C_{22}H_{19}ClO_2P$: 381.0811.

IR (CH_2Cl_2, cm^{-1}): 3308; 3063; 2252; 1598; 1493; 1446; 1254; 1024; 908; 734; 700

m.p.: 133-134 °C.

Description: white solid.

TPMP-II-3

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.35 (m, 15H); 4.80 (m, 1H); 4.66 (m, 1H); 1.83 (t, 3H, *J*=2.4Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 139.85; 130.67; 128.12; 127.65; 85.28; 72.29; 68.81 (*J*=113.32Hz); 56.14; 3.71.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 47.08.

HRMS (ESI): found: 395.0965 (M+H); calculated for $C_{23}H_{21}ClO_2P$: 395.0968.

IR (CH_2Cl_2, cm^{-1}): 3059; 2245; 1597; 1492; 1445; 1255; 1011; 971; 910; 732; 699

m.p.: 115.5-117 °C.

Description: white solid.

TPMP-III-9

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.36 (m, 4H); 7.33 (m, 6H); 7.26 (dd, 2H, *J*=2.0Hz, *J*=8.8Hz); 4.75 (dddd, 2H, *J*=2.5Hz, *J*=10.9Hz, *J*=14.4Hz, *J*=15.4Hz); 3.81 (m, 3H); 2.54 (t, 1H, *J*=2.5Hz).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 158.87; 139.98; 131.85; 131.42; 130.54; 128.17; 127.69; 113.46; 76.71; 76.53; 68.25 (*J*=112.44 Hz); 55.17; 54.91.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 48.00.

HRMS (ESI): found: 428.1184 ($M+NH_4^+$); calculated for $C_{23}H_{24}NClO_3P$: 428.1182.

IR (CH_2Cl_2, cm^{-1}): 3297; 3058; 2838; 1511; 1255; 1188; 1020; 985; 737; 700

m.p.: 101-103 °C.

Description: white solid.

TPMP-III-10

NMR ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.25 (m, 6H); 7.14 (d, 6H, *J*=8.2Hz); 4.75 (dddd, 2H, *J*=2.5Hz, *J*=11.0Hz, *J*=14.5Hz, *J*=15.4Hz); 2.54 (t, 1H, *J*=2.5Hz); 2.36 (m, 9H).

NMR ^{13}C (126 MHz, $CDCl_3$) δ ppm: 137.36; 136.85; 130.44; 128.82; 76.65; 76.584; 67.99 (*J*=111.96 Hz); 54.81; 20.96.

NMR ^{31}P (202 MHz, $CDCl_3$) δ ppm: 48.29.

HRMS (ESI): found: 440.1549 ($M+NH_4^+$); calculated for $C_{25}H_{28}NClO_2P$: 440.1546.

IR (CH_2Cl_2, cm^{-1}): 3300; 3238; 3029; 2922; 2130; 1510; 1255; 1196; 1020; 986; 810; 736.

m.p.: 119-120 °C.

Description: white solid.

Cell Culture Conditions

All cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and incubated at 37 ºC in a 5% CO_2, 95% humidity atmosphere.

Sulforhodamine B assay

UACC-62, SK-MEL-5, SKNSH, IGROV-1 or MCF-7 (3000 cells in 198 μL of RPMI 1640 media) cells were placed into each well of a 96-well plate. Compound stock solutions in 100% ethanol

were added to the wells in triplicate such that the final concentrations of compound ranged between 0.001-100 μM. The plates were incubated in a 37 ºC, 5% CO_2 incubator for 72 h. The media was removed from the plate and ice-cold 10% w/v trichloroacetic acid (200 μL) was added and the plates incubated at 4 ^{o}C for 1 hour. The trichloroacetic acid was removed, and the plates washed with 200 μL of deionized water 5 times. Sulforhodamine B (200 μL of 0.04% sodium salt dissolved in 1% acetic acid) was added and the plates incubated at room temperature for 30 min. The plates were washed 5 times with 1% acetic acid. The dye was released by the addition of tris-base (200 μL of 10 mM solution) and absorbance of each well was measured at 510 nm on a Molecular Devices SpectraMax 384 plus plate reader after 30 min incubation at room temperature.

MTS Assay

HL-60, U-937, or PC-12 cells in RPMI 1640 media were added to 96-well plates (99 μL containing 1 x 10^4 cells). Ethanol solutions of compounds were added in triplicate (1 μL to each well) to achieve concentrations ranging between 0.005-100 μM. The cells were incubated in a 37 ºC, 5% CO_2, 95% humidity incubator for 24 hours. A solution containing the soluble tetrazolium salt ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and the electron coupling reagent, phenazine methosulfate (PMS) was prepared according to the manufacturer's instructions (Promega) and 20 μL of this solution was added to each well. The plates were incubated at 37 ºC for 15-30 min and then read at 490 nm on a Molecular Devices SpectraMax 384 plus plate reader.

Cell Cycle Arrest Data

HL-60 cells (2 mL of 1 x 10^6 cells/mL) were treated with 20 μL ethanol stocks of the various compounds to achieve a final concentration of 20 or 50 μM. The cells were incubated at 37 ºC for 12 hours. The cells were centrifuged (400*g* for 5 min), washed with PBS (2 mL), and fixed by the addition of ice-cold 70% ethanol (1 mL) with gentle vortexing. The samples were placed in a 4 ºC fridge

overnight. The fixed cells were centrifuged (1000*g* for 5 min), washed with PBS (1 mL), and RNase treated (50 µL of 0.14 mg/mL RNase A in PBS) for 2 hours at 4 ºC. Propidium iodide (400 µL of 50 µg/mL in PBS) was added to the samples and the DNA content was measured on a Benton Dickinson LSR II cell flow cytometer. Cell cycle population distribution was analyzed using the software FCS Express.

Kinesin Eg5 Inhibition by Confocal Microscopy

HeLa cells were grown to 90% confluency on 22 mm square cover slips and treated with either DMSO vehicle, 30 µM **STLC** or **3B** for 16 h in RPMI 1640 media such that the DMSO did not exceed 1%. The cells were washed with PBS, fixed using 1-2% glutaraldehyde, quenching with freshly made 1 mg/mL $NaBH_4$ for 1 min. This was repeated 3 times. Cells were made permeable with 0.5% Triton X-100 for 10 min and rinsed with 0.1% Triton X-100 three times. The FITC anti-α-tubulin (Sigma) was added as a 1:50 dilution and the cells incubated for 1 h. The cells were blocked in goat serum containing 100 µg/mL RNase A for 1 hr. Propidium iodide was added in the last 15 min to achieve a final conc. of 50 µg/mL. The cells were washed 3 times with TBS buffer, drained, mounted onto microscope slides using 10 µL of mounting media. The cover slips were sealed and the samples visualized immediately on a Zeiss LSM 510 scanning confocal laser microscope.

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 and 1 mM GTP) was placed in the wells of a 384-well plate. Compound (1 µL of 0.5 mM in DMSO) was added to each well to achieve a final drug concentration of 10 µM. Tubulin (10 µL of 15 mg/mL tubulin in ice-cold 500 mM K-PIPES, 0.5 mM $MgCl_2$, pH 6.9 buffer) was added to each well. The plate was placed immediately in a Molecular Devices SpectraMax 384 plus plate reader preheated to 37 ºC and the progress of the polymerization monitored at 340 nm for 60 min. The microtubule

stabilizer and destabilizer, paclitaxel and nocodazole respectively were used as controls at a final concentration of 10 μM.

Assessment of Mitochondrial-bound Hexokinase Activity

Mitochondrial-bound hexokinase activity was assessed by a method previously described with minor modifications.[4, 5] In 10 cm diameter Petri dishes, murine melanoma B16-F10 cells were cultured to confluency. The cells were washed with PBS and incubated for 2 hours at 37 ºC in the presence of 10 mL PBS (pH 7.4) containing 5 mM glucose and 15 μM of the triphenylmethyl-containing compound. The cells were isolated using a scraper, centrifuged at 270*g* for 10 min and resuspended in 1.2 mL of ice-cold sucrose buffer (250 mM sucrose, 20 mM Tris, 1 mM EGTA pH 7.4). The cells were lysed for 90 seconds using a mechanical dounce homogenizer. The lysate was centrifuged at 1500*g* for 10 min to pellet debris and 900 μL of the supernatant was isolated and centrifuged at 27000*g* at 4 ºC for 15 min to isolate the mitochondria-rich pellet which was resuspended in 100μL of sucrose buffer. The protein concentration was determined by the BCA titration method and the samples were normalized to achieve a final protein concentration between 1.2-1.8 mg/mL. To determine the hexokinase activity of the mitochondria-rich fraction, 10 μL of the mitochondria-rich fraction was added to 180 μL of assay buffer (50 mM triethanolamine, 7.5 mM $MgCl_2$, 0.5 mM EGTA, 11 mM mercaptoethanol, 4 mM glucose, 0.5 mg/mL $NADP^+$, 0.5 U/mL Glucose-6-phosphate dehydrogenase at pH 8.5) in a 96-well plate. After the addition of ATP to a final concentration of 6.6 mM, the initial velocity of NADPH production was monitored at 340 nm using a Molecular Devices SpectraMax 384 plus plate reader. Controls were conducted in the absence of ATP and subtracted. Mitochondrial hexokinase activity for compound-treated cells was calculated as a percentage of the activity of vehicle-treated cells and four independent experiments were performed.

Gardos Channel Inhibition

Gardos channel inhibition was assessed by the method of Brugnara with minor modifications.[6] Sodium heparinized whole human blood (Bioreclamation inc.) was diluted 1:1 in modified flux buffer (MFB)- 140 mM NaCl, 5 mM KCl, 10 mM Tris-base, 0.1 mM EGTA, pH 7.4. The cells were centrifuged at 400*g* for 10 min, washed 3 times with 20 mL MFB and resuspended in MFB in a total volume of 10 mL. Rubdium-86 chloride (Perkin-Elmer) was added to achieve a final concentration of 5 μCi/mL. The cells were incubated at 37 ºC for 2 hours after which they were washed four times with 40 mL of chilled MFB and resuspended to a total volume of 10 mL. The loaded cells (100 μL) were then added to 100 μL MFB containing compound in a 96-well plate and incubated in the presence of compound or vehicle for 10 min at room temperature. To initiate ^{86}Rb efflux, 2 mM $CaCl_2$ and 5 μM of the calcium ionophore A23187 were added with mixing and after a 10 min incubation at room temperature the cells were centrifuged at 3000*g* for 5 min. The supernatant was collected and analyzed for Rb^{86} content using a LS6500 Beckman liquid scintillation counter. Three independent experiments were conducted, averaged and IC_{50} values were determined using the software TableCurve 2D.

In Vitro Chymotrypsin Inhibition Assay

In a 96-well plate, 80 μL of 0.1 mg/mL of α-chymotrypsin (from bovine pancrease type II, Aldrich), in assay buffer (100 mM NaCl, 100 mM Tris-HCl, 1 mM $CaCl_2$, pH 7.2) was incubated in the presence of various concentrations (0.01-100 μM) of the triphenylmethyl-containing compounds for 40 min at room temperature. Chymotrypsin substrate N-Succinyl-Ala-Ala-Pro-Phe p-nitroamide (Aldrich) was added (20 μL of 1 mg/mL) and the room temperature kinetics of p-nitroaniline formation was at 410 nm using a Molecular Devices SpectraMax 384 plus plate reader. Chymotrypsin activity was determined using initial velocities where a velocity of zero represented 100% chymotrypsin inhibition and chymotrypsin activity in the presence of 1% ethanol vehicle only was regarded as 0% inhibition. The IC_{50} values were determined using the software TableCurve 2D.

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