A novel pyrazolo[1,5-*a*]pyrimidine is a potent inhibitor of cyclin-dependent protein kinases 1, 2 and 9, which demonstrates anti-tumor effects in human tumor xenografts following oral administration

Dean A. Heathcote[1], Hetal Patel[1], Sebastian H. B. Kroll[2], Pascale Hazel[3], Manikandan Periyasamy[1], Mary Alikian[1], Seshu K. Kanneganti[1], Ashutosh S. Jogalekar[4], Bodo Scheiper[2], Jan Brackow[2], Alekasandra Siwicka[2], Robert D. M. Pace[2], Matthew J. Fuchter[2], James P. Snyder[4], Dennis C. Liotta[4], Paul. S. Freemont[3], Eric O. Aboagye[1], R. Charles Coombes[1], Anthony G. M. Barrett[2], Simak Ali[1*]

[1]Dept of Oncology, Imperial College London, London W12 0NN, England; [2]Department of Chemistry, Imperial College London, London SW7 2AZ, England; [3]Division of Molecular Biosciences, Imperial College London, London SW7 2AZ, England; [4]Department of Chemistry, Emory University, Atlanta GA30322, USA

Supporting Information

Synthetic Methods for 4a – 4j

(*R*)-*tert*-butyl benzyl(5-((1-((*tert*-butyldimethylsilyl)oxy)butan-2-yl)amino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3a

(*R*)-*tert*-butyl benzyl(5-((1-((*tert*-butyldimethylsilyl)oxy)but-3-en-2-yl)amino)-3-*iso*-propyl-pyrazolo[1,5-a]pyrimidin-7-yl)carbamate **3f** (19.0 mg 0.034 mmol) was dissolved in EtOAc (3.0 mL) and Pd/C (10 wt %, 3.0 mg) was added. The resulting suspension was vigorously stirred under a hydrogen atmosphere for 12 h. The mixture was filtered through celite, concentrated *in vacuo* and the crude product was chromatographed (hexanes : EtOAc 4 : 1) to yield carbamate **3a** (18.0 mg, 93%): $[\alpha]_D$ (c 0.90, CH_2Cl_2): + 41.8. IR (neat) 3372, 1721, 1701, 1518, 1391, 1252, 1157, 837, 776, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (s, 1H), 7.29 (m, 5H), 5.67 (s, 1H), 4.95 (m, 2H), 4.69 (m, 1H), 3.94 (m, 1H), 3.69 (m, 2H), 3.10 (m, 1H), 1.69 - 1.27 (m, 17H), 0.90 (m, 12H), 0.05 (s, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 154.3, 146.3, 142.7, 141.4, 137.8, 128.4, 128.0, 127.4, 113.1, 97.4, 82.0, 63.7, 53.6, 51.4, 28.1, 25.9, 24.3, 23.9, 23.2, 10.6, -5.4; MS *m/z* (CI) 568 $(M + H)^+$. HRMS (CI) calc. for $C_{31}H_{50}N_5O_3Si$: 568.3683; Found: 568.3677.

(*R*)-2-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)butan-1-ol 4a

Synthesized according to the general procedure for acidic deprotection. **3a** (17.0 mg, 0.030 mmol) in HCl/MeOH (3.0 mL). Yield **4a** (10.0 mg, 0.028 mmol, 94 %): $[\alpha]_D$ (c 0.50, CH_2Cl_2): + 47.4. IR (neat) 3305, 1638, 1580, 1444, 1222 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (s, 1H), 7.35 (m, 5H), 6.47 (m, 1H), 5.04 (m, 1H), 4.48 (m, 3H), 3.86 (m, 2H), 3.56 (m, 1H), 3.11 (m, 1H), 1.55 (m, 2H), 1.31 (m, 7H), 0.98 (m, 3H); ^{13}C ($CDCl_3$, 75 MHz) δ 140.7, 128.9, 127.9, 127.1, 83.7, 72.9, 68.4, 56.9, 46.1, 25.0, 23.6, 23.4; MS *m/z* (CI) 354 $(M + H)^+$. HRMS (CI) calc. for $C_{20}H_{28}N_5O$: 354.2294; Found: 354.2302.

***tert*-butyl benzyl(3-*iso*-propyl-5-(iso-propylamino)pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3b**

Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (50 mg, 0.12 mmol), Pd_2dba_3 (6.0 mg, 0.006 mmol), BINAP (12.0 mg, 0.020 mmol), sodium *tert*-butoxide (17.0 mg, 0.17 mmol), *iso*-propylamine (17.0 μL, 0.17 mmol), PhMe (0.50 mL) yielded **3b** (39.0 mg, 0.092 mmol, 77 %). Purification EtOAc : hexanes (1 : 10): IR (neat) 3361, 1719, 1698, 1644, 1580, 1520, 1158 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (s, 1H), 7.26 (m, 5H), 5.66 (s, 1H), 4.93 (s, 2H), 4.52 (m, 1H), 4.03 (m, 1H), 3.11 (hept, *J* = 6.8 Hz, 1H), 1.41 (s, 9H), 1.33 (d, *J* = 6.8 Hz, 6H), 1.16 (d, *J* = 6.8 Hz 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 154.0, 146.3, 141.5, 137.8, 128.4,

127.9, 127.4, 113.1, 97.0, 82.1, 51.3, 43.0, 28.0, 23.8, 23.2, 22.6; MS *m/z* (CI) 424 $(M + H)^+$. HRMS (CI) calc. for $C_{24}H_{34}N_5O_2$: 424.2713; Found: 424.2706.

N^7-benzyl-N^5,3-di-*iso*-propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine 4b

Synthesized according to the general procedure for acidic deprotection. **3b** (39.0 mg, 0.013 mmol) in HCl/MeOH (5.0 mL). Yield **4b** (29.0 mg, 0.089 mmol, 97 %); IR (neat) 3263, 1634, 1578, 1441, 1220 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (s, 1H), 7.33 (m, 5H), 6.51 (s, 1H), 5.01 (s, 1H), 4.47 (m, 3H), 3.93 (m, 1H), 3.10 (m, 1H), 1.31 (m, 6H), 1.18 (m, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 156.0, 146.7, 140.6, 136.9, 128.8, 127.7, 127.1, 112.4, 72.2, 46.1, 43.1, 23.7, 23.3, 22.9; MS *m/z* (CI) 324 $(M + H)^+$; HRMS (CI) calc. for $C_{19}H_{26}N_5$: 324.2188; Found: 324.2187.

***tert*-butyl benzyl(5-((6-((tert-butoxycarbonyl)amino)hexyl)amino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3c**

Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (50 mg, 0.12 mmol), Pd_2dba_3 (6.0 mg, 0.006 mmol), BINAP (12.0 mg, 0.020 mmol), sodium *tert*-butoxide (17.0 mg, 0.17 mmol), *tert*-butyl (6-aminohexyl)carbamate (32.0 mg, 0.15 mmol), PhMe (0.50 mL) yield **3c** (35.0 mg, 0.055 mmol, 52 %). Purification EtOAc : hexanes (1 : 4): IR (neat) 3356, 1713, 1694, 1645, 1524, 1366, 1161 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (s, 1H), 7.27 (m, 5H), 5.68 (s, 1H), 4.93 (m, 2H), 4.78 (m, 1H), 4.56 (m, 1H), 3.30 (m, 2H), 3.12 (m, 3H), 1.44 - 1.32 (m, 32H); ^{13}C ($CDCl_3$, 75 MHz) δ 156.0, 154.8, 153.6, 146.3, 142.7, 141.4, 137.8, 128.4, 127.9, 127.4, 113.1, 97.0, 82.0, 79.1, 51.4, 40.3, 30.0, 29.1, 28.4, 28.0, 26.4, 26.3, 23.8, 23.2; MS *m/z* (CI) 581 $(M + H)^+$; HRMS (CI) calc. for $C_{32}H_{49}N_6O_4$: 581.3815; Found: 581.3802.

N^5-(6-aminohexyl)-N^7-benzyl-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine 4c

Synthesized according to the general procedure for acidic deprotection. **3c** (32.0 mg, 0.055 mmol) in HCl/MeOH (5.0 mL). Yield **4c** (13.0 mg, 0.034 mmol, 62 %): IR (neat) 3368, 3282, 2926, 2857, 1637, 1578, 1443, 1224 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (s, 1H), 7.34 (m, 5H), 6.42 (s, 1H), 5.04 (m, 1H), 4.59 (m, 1H), 4.49 (m, 2H), 3.28 (m, 2H), 3.10 (hept, *J* = 6.8 Hz, 1H), 2.72 (m, 2H), 2.41 (m, 2H), 1.55 (m, 4H), 1.48 (m, 4H), 1.30 (d, *J* = 6.8 Hz, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 156.9, 146.7, 140.6, 136.9, 128.8, 127.8, 127.2, 112.4, 71.9, 46.1, 41.8, 32.9, 29.6, 26.8, 26.6, 23.7, 23.4; MS *m/z* (CI) 381 $(M + H)^+$; HRMS (CI) calc. for $C_{22}H_{33}N_6$: 381.2767; Found: 381.2767.

***tert*-butyl benzyl(5-((*tert*-butoxycarbonyl)(6-cyanohexyl)amino)-3-*iso*-propylpyrazolo[1,5-a]pyrimidin-7-yl)carbamate 3d**

Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (244 mg, 0.61 mmol), Pd_2dba_3 (27.0 mg, 0.029 mmol), BINAP (56.0 mg, 0.090 mmol), sodium *tert*-butoxide (88.0 mg, 0.92 mmol), *tert*-butyl 6-cyanohexylcarbamate (178 mg, 0.73 mmol), PhMe (1.2 mL) yield **3d** (170.0 mg, 0.29 mmol, 47 %). Purification EtOAc : hexanes (1 : 4): IR 3377, 1718, 1644 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δH 7.89 (s, 1H), 7.20 - 7.32 (m, 5H), 7.17 (s, 1H), 5.00 (br s, 2H), 4.02 (m, 2H), 3.18 (hept, *J* = 6.9 Hz, 2H), 2.32 (t, *J* = 7.1 Hz, 2H), 1.55 - 1.71 (m, 4H), 1.42 - 1.57 (m, 11H), 1.27 - 1.44 (m, 17H); ^{13}C (100 MHz, $CDCl_3$) δC; 153.3, 151.5, 144.5, 142.9, 141.8, 137.5, 128.9, 127.8, 127.4, 119.7, 116.1, 102.2, 82.3, 82.0, 51.8, 46.6, 28.4, 28.2, 28.0, 26.2, 25.3, 24.2, 23.1, 17.1; HRMS (CI) calc. for $C_{33}H_{47}N_6O_4$: 591.366; Found: 591.365.

7-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)heptanenitrile 4d

Synthesized according to the general procedure for acidic deprotection. **3d** (95.0 mg, 0.16 mmol) in HCl/MeOH (5.0 mL). Yield **4d** (32.0 mg, 0.081 mmol, 51 %): IR (neat) 3377 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δH 7.67 (s, 1H), 7.24 - 7.41 (m, 5H), 6.54 (br s, 1H), 5.04 (s, 1H), 4.61 (br s, 1H), 4.50 (d, *J* = 7.2 Hz, 2H), 3.33 (q, *J* 6.9 Hz, 2H), 3.19 (hept, *J* = 6.9 Hz, 1H), 2.34 (t, *J* = 7.2 Hz, 2H), 1.54 - 1.76 (m, 4H), 1.37 - 1.54 (m, 4H), 1.33 (d, *J* = 6.9 Hz, 6H); ^{13}C (100 MHz, $CDCl_3$) δC 156.7, 146.7, 145.4, 140.7, 136.8, 128.9, 127.8, 127.2, 119.7, 112.5, 71.9, 46.1, 41.6, 29.3, 28.4, 26.2, 25.2, 23.7, 23.4, 17.1; MS (CI): *m/z* 391 $(M + H)^+$; HRMS (CI) calc. for $C_{23}H_{31}N_6$: 391.2610; Found: 391.2610.

7-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)heptanimidamide 4e

Ammonium chloride (6.2 mg, 0.11 mmol) was suspended in PhMe (1.0 mL) and cooled to 0 ˚C. Me_3Al (2.0 M hexane, 55 μL, 0.11 mmol) was added and the mixture stirred at room temperature for 1 h. Nitrile **4d** (9.0 mg, 0.023 mmol) in PhMe (0.50 mL) was added and the mixture heated to 80 ˚C for 14 h. The mixture was cooled and MeOH(10 mL) was added and stirring continued for a further 2 h. The mixture was filtered through Celite and concentrated *in vacuo*. Chromatography (EtOAc : hexanes : Et_3N 30 : 70 : 1) gave amide **4e** (5.9 mg, 0.014 mmol, 63 %): IR (neat) 3377, 1610 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δH 7.66 (s, 1H), 7.28 (m, 5H), 6.40 (m, 1H), 5.03 (s, 1H), 4.51 (d, *J* = 6.9 Hz, 2H), 3.30 (q, *J* = 6.9 Hz, 2H), 3.10 (hept, *J* = 6.9 Hz, 1H), 2.34 (t, *J* = 6.9 Hz, 2H), 1.45 - 1.70 (m, 12H), 1.31 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δC 156.7, 146.8, 140.7, 136.7, 128.9, 127.8, 127.2, 119.7, 112.5, 71.8, 46.1, 41.6, 29.6, 29.3, 28.4, 26.2, 25.2, 23.6, 23.3, 17.1; MS (CI) *m/z* 408 $(M + H)^+$; HRMS (CI) calc. for $C_{23}H_{34}N_7$: 408.2870; Found: 408.3119.

(*R*)-2-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-a]pyrimidin-5-yl)amino)but-3-en-1-ol 4f

Synthesized according to the general procedure for acidic deprotection. **3f** (19.0 mg, 0.034 mmol), HCl/MeOH (5.0 mL). Yield **4f** (10.0 mg, 0.028 mmol, 84 %): $[\alpha]_D$ (c 0.50, CH_2Cl_2): + 33.2; IR (neat) 3285, 2956, 2924, 1636, 1580, 1444, 1064 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.66 (s, 1H), 7.34 (m, 5H), 6.51 (s, 1H), 5.88 (m, 1H), 5.24 (m, 2H), 5.06 (m, 1H), 4.78 (m, 1H), 4.54 - 4.47 (m, 3H), 3.84 (m, 1H), 3.70 (m, 1H), 3.09 (hept, *J* = 6.8 Hz, 1H), 1.30 (d, *J* = 6.8 Hz, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 156.6, 146.8, 140.8, 136.6, 135.6, 128.9, 127.9, 127.1, 117.1, 113.2, 72.9, 67.6, 57.2, 46.1, 23.6, 23.3; MS *m/z* (CI) 352 $(M + H)^+$; HRMS (CI) calc. for $C_{20}H_{26}N_5O$: 352.2137; Found: 352.2143.

(*R*)-*tert*-butyl benzyl(5-((2,2,3,3,15,15-hexamethyl-13-oxo-4,14-dioxa-12-aza-3-silahexadecan-6-yl)amino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3g

Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (51.0 mg, 0.13 mmol), Pd_2dba_3 (6.0 mg, 0.0065 mmol), BINAP (11.0 mg, 0.018 mmol), sodium *tert*-butoxide (19.0 mg, 0.20 mmol), (*R*)-*tert*-butyl (6-amino-7-((*tert*-butyldimethylsilyl)oxy)heptyl)carbamate (50.0 mg, 0.14 mmol), PhMe (0.60 mL); yield **3g** (30.0 mg, 0.041 mmol, 32 %). Purification: EtOAc : hexanes (9 : 1): $[\alpha]_D$ (c 1.45, CH_2Cl_2): + 16.1; IR (neat) 1721, 1711, 1692, 1642, 1516, 1367, 1159 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.35 (m, 5H), 5.75 (s, 1H), 5.05 (m, 2H), 4.77 (m, 1H), 4.59 (m, 1H), 4.12 (m, 1H), 4.03 (m, 1H), 3.75 (m, 2H), 3.17 (m, 3H), 1.52 - 1.40 (m, 31H), 0.96 (s, 9H), 0.12 (s, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 154.2, 153.6, 147.9, 146.3, 142.7, 141.4, 137.8, 128.4, 127.9, 127.8, 127.4, 113.1, 97.5, 82.0, 64.1, 51.9, 51.4, 31.3, 30.0, 28.4, 28.2, 28.1, 27.9, 26.7, 25.9, 25.7, 23.8, 23.2, 23.1, 18.3, -5.4.

(*R*)-7-amino-2-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)heptan-1-ol 4g

Synthesized according to the general procedure for acidic deprotection. **3g** (31.0 mg, 0.041 mmol), HCl/MeOH (5.0 mL). Yield **4g** (10.0 mg, 0.024 mmol, 59 %): $[\alpha]_D$ (c 0.50, CH_2Cl_2): + 32.0. IR (neat) 3281, 1638, 1578, 1443 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (s, 1H), 7.35 (m, 5H), 6.47 (m, 1H), 5.04 (s, 1H), 4.56 - 4.47 (m, 3H), 3.94 (m, 1H), 3.79 (m, 1H), 3.58 (m, 1H), 3.08 (hept, *J* = 6.8 Hz, 1H), 2.67 (m, 2H), 1.57 - 1.28 (m, 17H); ^{13}C ($CDCl_3$, 75 MHz) δ 157.0, 146.8, 144.4, 140.7, 136.6, 128.9, 127.9, 127.1, 113.1, 72.9, 68.5, 55.1, 46.1, 42.1, 33.5, 32.0, 26.8, 26.2, 23.6, 23.3; MS *m/z* (CI) 411 $(M + H)^+$; HRMS (CI) calc. for $C_{23}H_{35}N_6O$: 411.2872; Found: 411.2871.

(*R*)-*tert*-butyl benzyl(5-((2,2,3,3,16,16-hexamethyl-14-oxo-4,15-dioxa-13-aza-3-silaheptadecan-6-yl)amino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3h

Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (20.0 mg, 0.050 mmol), Pd_2dba_3 (2.0 mg, 0.0022 mmol), BINAP (5.0 mg, 0.0082 mmol), sodium *tert*-butoxide (7.0 mg, 0.072 mmol), (*R*)-*tert*-butyl (7-amino-8-((*tert*-butyldimethylsilyl)oxy)octyl)carbamate (22.0 mg, 0.059 mmol), PhMe (0.50 mL) yield **3h** (16.0 mg, 0.022 mmol, 43 %). Purification EtOAc : hexanes (9 : 1): $[\alpha]_D$ (c 1.4, CH_2Cl_2): + 14.1. IR (neat) 3371, 1720, 1712, 1642, 1517, 1367, 1252, 1159, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.36 (m, 5H), 5.74 (s, 1H), 5.01 (m, 2H), 4.75 (m, 1H), 4.58 (m, 1H), 4.04 (m, 1H), 3.74 (m, 1H), 3.17 (m, 2H), 1.52 - 1.40 (m, 35H), 0.96 (s, 9H), 0.09 (s, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 154.2, 142.7, 141.4, 137.8, 128.4, 127.9, 127.8, 127.7, 127.4, 127.1, 113.1, 84.4, 82.0, 64.1, 51.4, 31.3, 30.0, 29.3, 28.4, 28.2, 28.1, 27.9, 26.7, 26.0, 25.9, 23.8, 23.2, 23.1, -5.4.

(*R*)-8-amino-2-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)octan-1-ol 4h

Synthesized according to the general procedure for acidic deprotection. **3h** (28.0 mg, 0.038 mmol), HCl/MeOH (5.0 mL). Yield **4h** (7.0 mg, 0.016 mmol, 43 %): $[\alpha]_D$ (c 0.30, CH_2Cl_2): + 51.7; IR (neat) 3287, 1632, 1579, 1443 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (s, 1H), 7.35 (m, 5H), 6.45 (m, 1H), 5.04 (s, 1H), 4.48 (m, 3H), 3.94 (m, 1H), 3.79 (m, 1H), 3.58 (m, 1H), 3.09 (m, 1H), 2.65 (m, 2H), 1.56 - 1.28 (m, 19H); ^{13}C ($CDCl_3$, 75 MHz) δ 140.8, 136.6, 133.9, 128.9, 127.9, 127.1, 72.9, 68.7, 46.1, 42.2, 33.7, 32.0, 30.4, 29.5, 29.4, 26.7, 26.3, 23.6, 23.3; MS *m/z* (CI) 426 $(M + H)^+$.

(*R*)-*tert*-butyl benzyl(5-((1-hydroxy-8-(pyridin-2-yl)octan-2-yl)amino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3i

Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (62.0 mg, 0.16 mmol), Pd_2dba_3 (7.0 mg, 0.0098 mmol), BINAP (15.0 mg, 0.024 mmol), sodium *tert*-butoxide (23.0 mg, 0.24 mmol), (*R*)-1-((*tert*-butyldimethylsilyl)oxy)-8-(pyridin-2-yl)octan-2-amine (59.0 mg, 0.18 mmol), PhMe (0.75 mL) yield **3i** (24.0 mg, 0.0040 mmol, 26 %). PurificationEtOAc : hexanes (1 : 2): $[\alpha]_D$ + 17.5 (*c* 1.20 CH_2Cl_2); IR (neat) 3368, 1721, 1638, 1540, 1519, 1455, 1427 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.53 (m, 1H), 7.86 (s, 1H), 7.75 (s, 1H), 7.65 (m, 1H), 7.27 (m, 5H), 7.17 (m, 1H), 6.06 (s, 1H), 4.99 (m, 2H), 4.47 (m, 2H), 3.12 (m, 1H), 2.84 (m, 2H), 1.69 (m, 2H), 1.40 (s, 9H), 1.33 (m, 15H); ^{13}C (CDCl3, 100 MHz) δ 160.4, 154.0, 153.6, 144.3, 141.8, 141.6, 128.5, 128.5, 127.8, 127.7, 123.2, 121.3, 115.6, 113.3, 97.5, 82.5, 67.7, 51.5, 31.6, 29.7, 29.7, 29.2, 28.0, 28.0, 25.9, 23.9, 23.8, 23.1.

(*R*)-2-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)-8-(pyridin-2-yl)octan-1-ol 4i

Synthesized according to the general procedure for acidic deprotection. **3i** (22.0 mg, 0.038 mmol), HCl/MeOH (5.0 mL). Yield **4i** (11.0 mg, 0.023 mmol, 61 %): $[\alpha]_D$ (*c* 0.55, CH_2Cl_2): + 17.3; IR (neat) 3543, 3392, 1633, 1634, 1580 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.52 (d, *J* = 4 Hz, 1H), 7.73 (s, 1H), 7.66 (s, 1H), 7.59 (m, 1H), 7.35 (m, 5H), 7.12 (m, 1H), 5.38 (s, 1H), 5.00 (s, 1H), 4.49 (m, 3H), 4.24 (m, 2H), 3.13 (hept, *J* = 6.8 Hz, 1H), 2.78 (m, 2H), 1.32 (m, 16H); ^{13}C (CDCl3, 100 MHz) δ 162.3, 147.7, 143.8, 141.0, 136.5, 136.3, 128.9, 128.0, 127.4, 127.2, 120.9, 114.7, 112.5, 73.9, 67.7, 46.2, 46.1, 38.3, 31.9, 29.7, 28.0, 26.0, 23.9, 23.3.

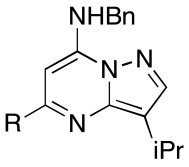
***tert*-butyl benzyl(5-((2-(2-((tert-butyldimethylsilyl)oxy)ethoxy)ethyl)amino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 3j**

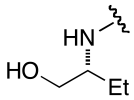
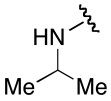
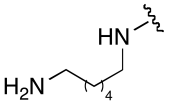
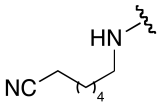
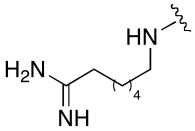
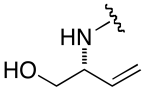
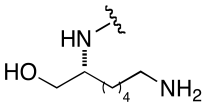
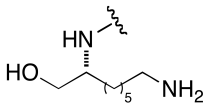
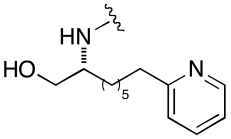
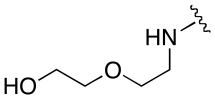
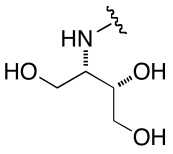
Synthesized according to the general procedure for Buchwald-Hartwig coupling, **2** (100 mg, 0.25 mmol), Pd_2dba_3 (11.0 mg, 0.012 mmol), BINAP (23.0 mg, 0.037 mmol), sodium *tert*-butoxide (36.0 mg, 0.36 mmol), 2-(2-((*tert*-butyldimethylsilyl)oxy)ethoxy)ethanamine (66.0 mg, 0.30 mmol), PhMe (0.75 mL) yielded **3j** (84.0 mg, 0.14 mmol, 58%). Purification EtOAc : hexanes (1 : 4): IR (neat) 3374, 1721, 1644, 1582, 1524 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.75 (s, 1H), 7.26 (m, 5H), 5.68 (s, 1H), 5.05 - 4.93 (m, 3H), 3.78 - 3.53 (m, 9H), 3.13 (hept, *J* = 6.8 Hz, 1H), 1.40 (s, 9H), 1.33 (d, *J* = 6.8 Hz, 6H), 0.07 (s, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 154.4, 153.5, 146.2, 142.6, 141.4, 137.7, 128.4, 127.8, 127.4, 113.3, 97.4, 82.0, 72.4, 69.4, 62.6, 51.3, 41.0, 28.0, 25.9, 23.8, 23.2, 23.1, 18.3; MS *m/z* (CI) 584 $(M + H)^+$; HRMS (CI) calc. for $C_{31}H_{50}N_5O_4Si$: 584.3632; Found: 584.3626.

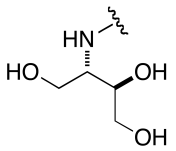
2-(2-((7-(benzylamino)-3-*iso*-propylpyrazolo[1,5-*a*]pyrimidin-5-yl)amino)ethoxy)ethanol 4j

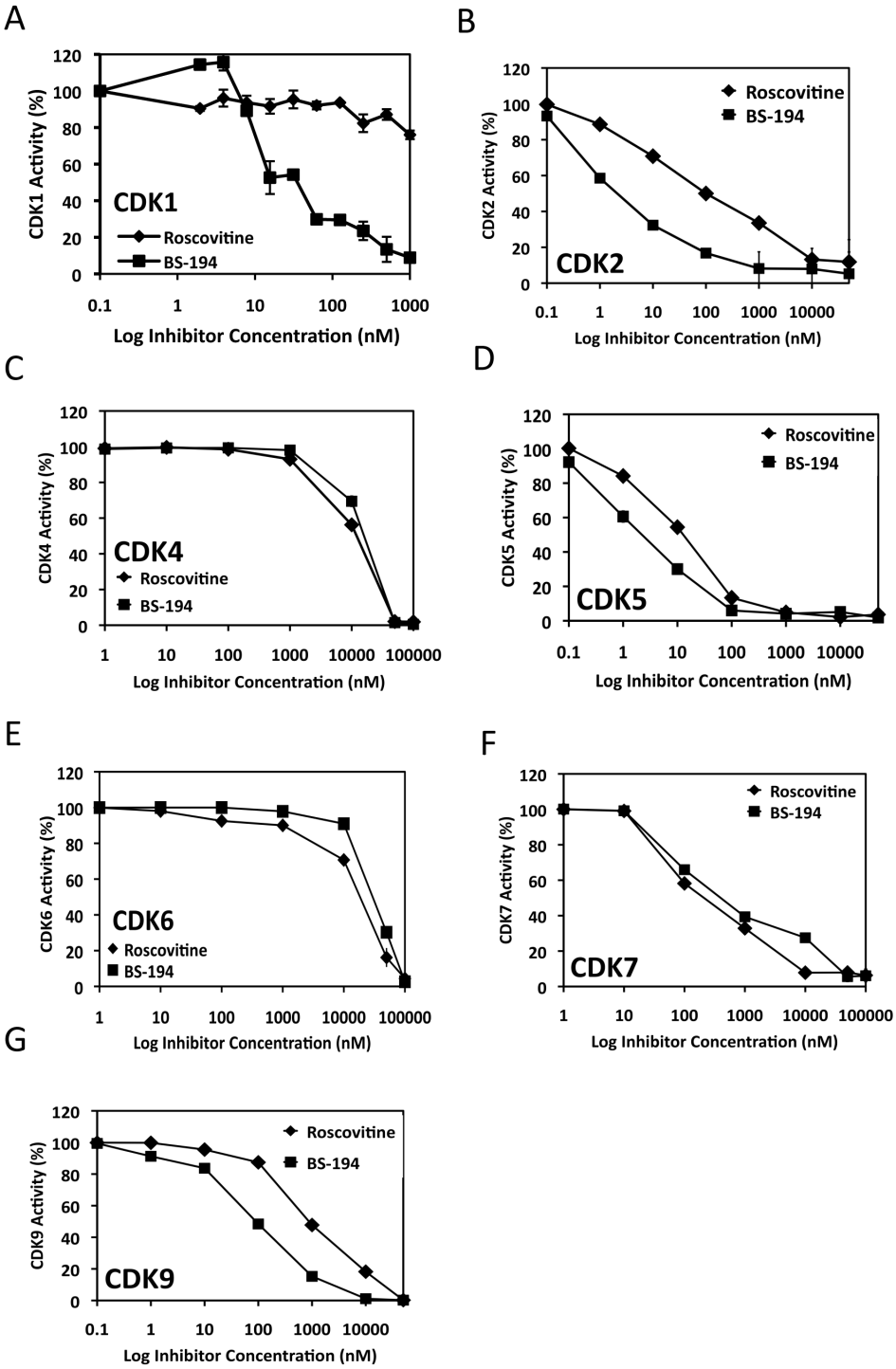
Synthesized according to the general procedure for acidic deprotection. **3j** (20.0 mg, 0.034 mmol), HCl/MeOH (5.0 mL). Yield **4j** (12.0 mg, 0.032 mmol, 96 %): IR (neat) 3334, 1637, 1578 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.66 (s, 1H), 7.33 (m, 5H), 6.44 (s, 1H), 5.03 (m, 2H), 4.44 (m, 2H), 3.73 - 3.58 (m, 9H), 3.11 (hept, *J* = 6.8 Hz, 1H), 1.32 (d, *J* = 6.8 Hz, 6H); ^{13}C ($CDCl_3$, 75 MHz) δ 156.5, 146.7, 140.7, 136.8, 128.8, 127.8, 127.2, 112.8, 72.6, 72.2, 69.9, 61.7, 46.1, 41.3, 23.7, 23.3; MS *m/z* (CI) 370 $(M + H)^+$; HRMS (CI) calc. for $C_{20}H_{28}N_5O_2$: 370.2243; Found: 370.2241.

Supplementary Table 1. CDK Inihibition by Compound Series. Kinase assays were performed using each compound at a concentration of 1 μmol/L

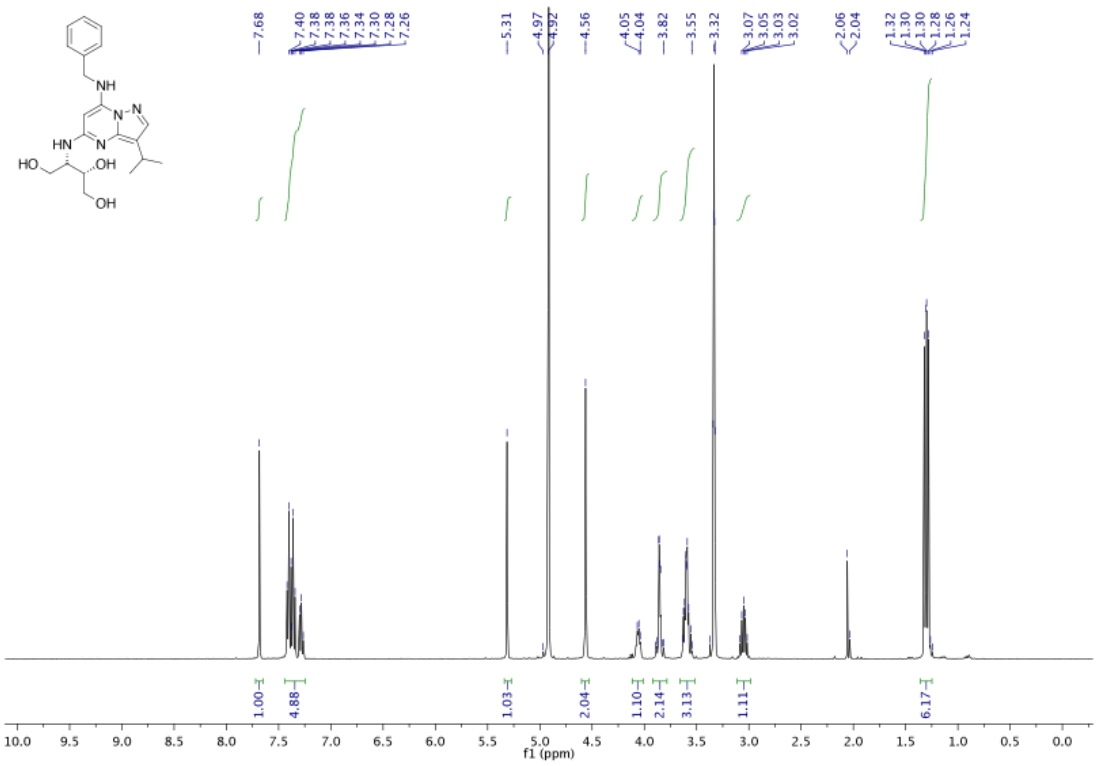


Compound	R	Kinase Inhibition in vitro (% Inhibition) CDK2	CDK4	CDK5	CDK7	CDK9	MCF-7 Growth Inhibition GI_{50} (μmol/L)
4a		0	42	46	60	15	8.5
4b		45	25	16	25	7	20
4c		10	1	8	83	9	21
4d		48	1	6	ND	3	35
4e		91	2	7	ND	1	28
4f		5	23	17	20	0	8.5
4g		14	25	16	26	12	17
4h		39	23	20	19	15	21
4i		16	2	11	ND	0	87
4j		12	17	10	75	10	40
4k		24	0	86	61	46	0.3

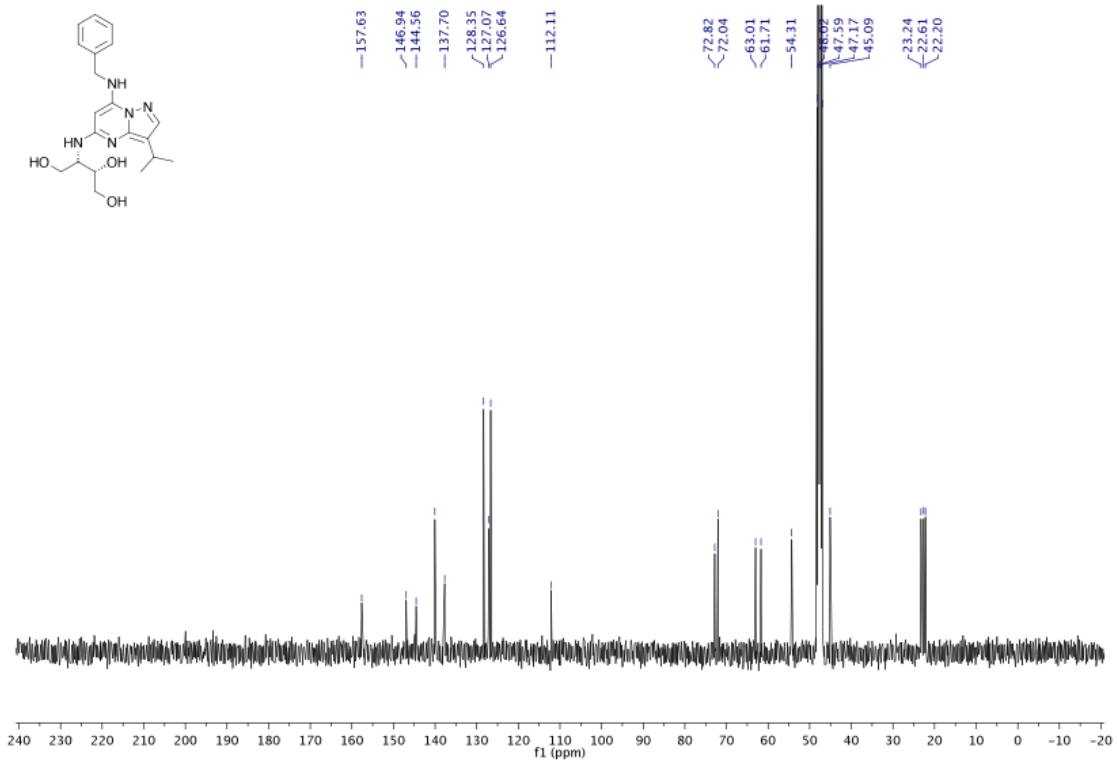
4l		34	29	74	19	43	0.5



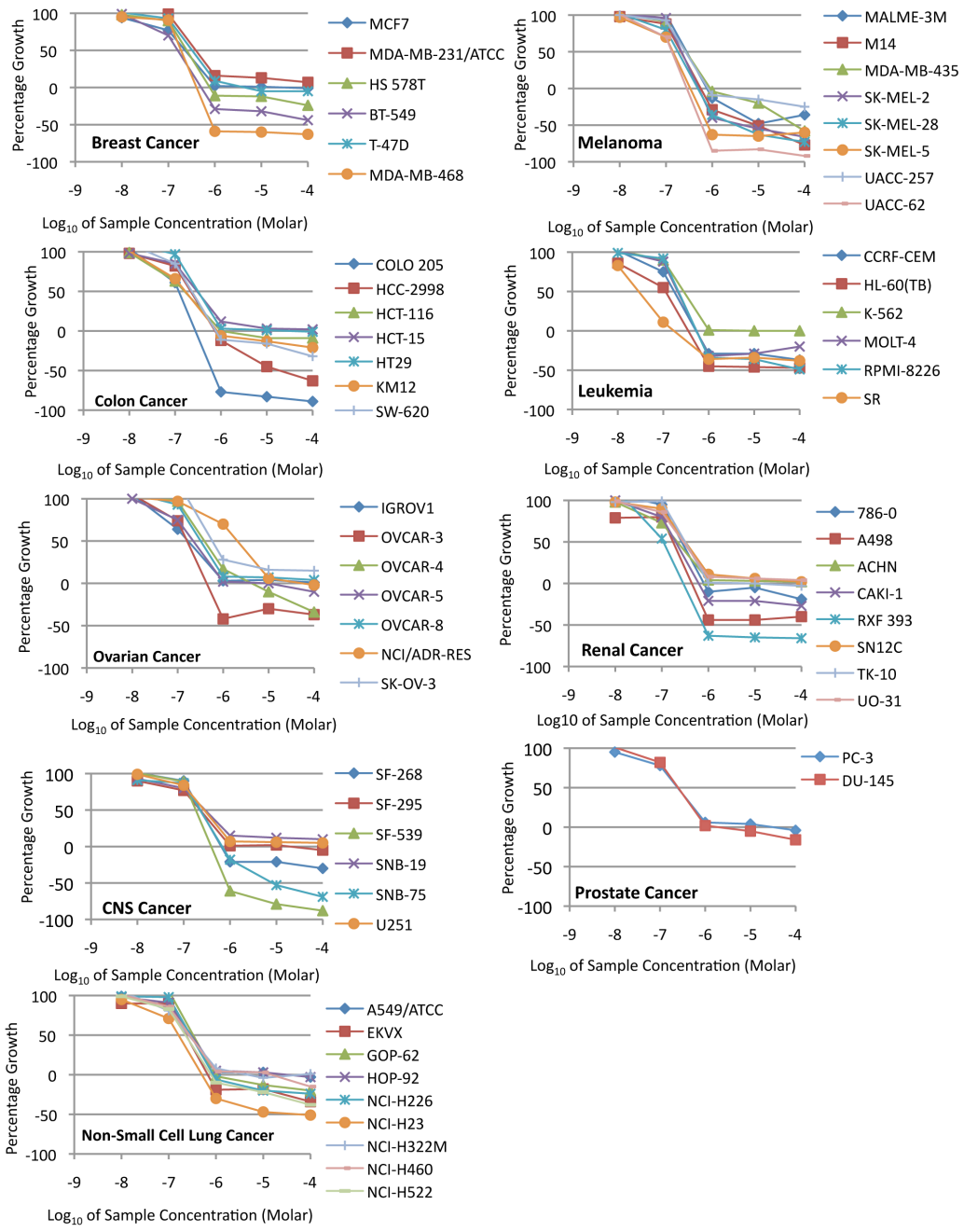
Supplemental Figure 1. The in vitro kinase inhibition profiles of 4k and Roscovitine. (A-G) In vitro kinase assays were carried out in the presence of increasing concentrations of **4k** or Roscovitine (as described in Materials and Methods). The line graphs show inhibition of kinase activity as percentage relative to the vehicle treatment alone. The graphs show the means of 3 experiments, with error bars depicting the standard errors of the mean. IC_{50} values generated from these data are shown in Table 1.



Supplemental Figure 2. ^{1}H NMR Spectrum of **4k** (400 MHz, CD_3OD).



Supplemental Figure 3. ^{13}C NMR Spectrum of **4k** (100 MHz, CD_3OD).



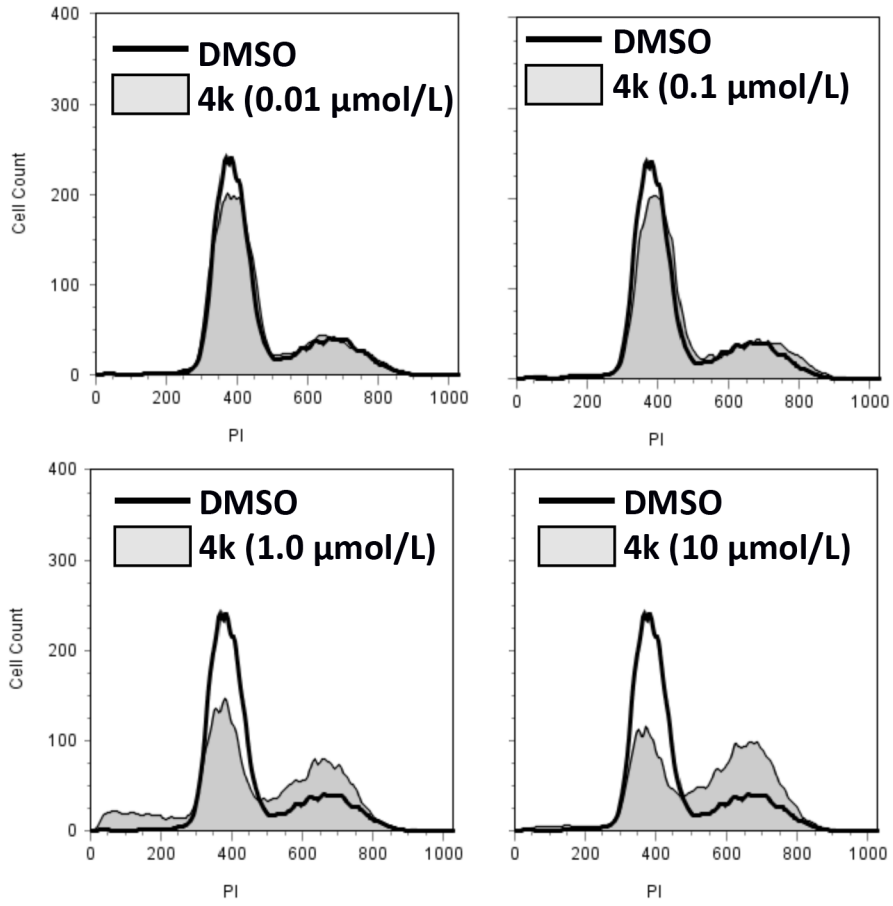
Supplemental Figure 4. Shown is the growth inhibition obtained for **4k** for the NCI 60 cancer cell line panel, summarised in Figure 1B, expanded to more clearly show the cell lines according to cancer type. The assays were carried out through the Developmental Therapeutics Program at the NCI (http://dtp.nci.nih.gov) using the SRB Assay, as described in the text. The actual GI_{50} values for each line are given in Supplemental Table 1.

Supplemental Table 1. Growth inhibition by **4k** in the NCI 60 cancer cell line panel

Panel/Cell Line	GI_{50}	TGI	LC_{50}
Leukemia			
CCRF-CEM	1.73E-07	5.23E-07	>1.00E-4
HL-60(TB)	1.12E-07	3.56E-07	>1.00E-4
K-562	2.08E-04	>1.00E-4	>1.00E-4
MOLT-4	2.09E-07	5.45E-07	>1.00E-4
RPMI-8226	2.16E-07	5.36E-07	>1.00E-4
SR	2.86E-08	1.73E-07	>1.00E-4
Non-Small Cell Lung Cancer			
A549/ATCC	2.75E-07	3.29E-05	>1.00E-4
EKVX	2.33E-07	6.73E-07	>1.00E-4
GOP-62	3.29E-07	9.59E-07	>1.00E-4
HOP-92	2.99E-07	3.42E-05	>1.00E-4
NCI-H226	2.90E-07	8.75E-07	>1.00E-4
NCI-H23	1.61E-07	5.02E-07	4.47E-05
NCI-H322M	2.64E-07	.	>1.00E-4
NCI-H460	2.76E-07	1.48E-05	>1.00E-4
NCI-H522	2.27E-07	7.76E-05	>1.00E-4
Colon Cancer			
COLO 205	1.20E-07	2.77E-07	6.37E-07
HCC-2998	2.21E-07	7.47E-07	1.91E-05
HCT-116	1.59E-07	9.83E-07	>1.00E-4
HCT-15	3.03E-07	>1.00E-4	>1.00E-4
HT29	3.19E-07	3.82E-05	>1.00E-4
KM12	1.67E-07	8.28E-07	>1.00E-4
SW-620	2.33E-07	7.66E-07	>1.00E-4
Panel/Cell Line			
CNS Cancer	GI_{50}	TGI	LC_{50}
SF-268	2.28E-07	6.45E-07	>1.00E-4
SF-295	2.27E-07	1.88E-05	>1.00E-4
SF-539	1.83E-07	3.94E-07	8.50E-07
SNB-19	2.90E-07	>1.00E-4	>1.00E-4
SNB-75	2.24E-07	6.71E-07	8.34E-06
U251	2.73E-07	>1.00E-4	>1.00E-4
Melanoma			
LOX IMVI	2.39E-07	>1.00E-4	>1.00E-4
MALME-3M	2.78E-07	7.62E-07	>1.00E-4
M14	2.10E-07	5.63E-07	9.23E-06
MDA-MB-435	2.70E-07	9.10E-07	6.28E-05
SK-MEL-2	2.18E-07	5.08E-07	4.58E-06
SK-MEL-28	1.80E-07	4.87E-07	3.27E-06
SK-MEL-5	1.42E-07	3.38E-07	8.03E-07
UACC-257	2.64E-07	8.10E-07	>1.00E-4
UACC-62	1.34E-07	2.82E-07	5.91E-07
Ovarian Cancer			
IGROV1	1.68E-07	>1.00E-4	>1.00E-4
OVCAR-3	1.60E-07	4.34E-07	>1.00E-4
OVCAR-4	3.90E-07	4.27E-06	>1.00E-4

OVCAR-5	2.19E-07	8.36E-06	>1.00E-4
OVCAR-8	3.21E-07	>1.00E-4	>1.00E-4
NCI/ADR-RES	2.03E-06	5.68E-05	>1.00E-4
SK-OV-3	5.70E-07	>1.00E-4	>1.00E-4

Panel/Cancer Cell Line	GI_{50}	TGI	LC_{50}
Renal Cancer			
786-0	2.68E-07	8.02E-07	>1.00E-4
A498	1.75E-07	4.43E-07	>1.00E-4
ACHN	2.14E-07	>1.00E-4	>1.00E-4
CAKI-1	1.98E-07	6.17E-07	>1.00E-4
RXF 393	1.09E-07	2.92E-07	7.81E-07
SN12C	3.20E-07	>1.00E-4	>1.00E-4
TK-10	3.19E-07	1.07E-05	>1.00E-4
UO-31	2.89E-07	>1.00E-4	>1.00E-4
Prostate Cancer			
PC-3	2.44E-07	3.18E-05	>1.00E-4
DU-145	2.49E-07	1.87E-06	>1.00E-4
Breast Cancer			
MCF7	2.28E-07	2.99E-05	>1.00E-4
MDA-MB-231/ATCC	3.88E-07	>1.00E-4	>1.00E-4
HS 578T	2.48E-07	7.74E-07	>1.00E-4
BT-549	1.60E-07	5.13E-07	>1.00E-4
T-47D	3.27E-07	4.28E-06	>1.00E-4
MDA-MB-468	1.88E-07	4.06E-07	8.77E-07



Supplemental Figure 5. 4k treatment of HCT116 cells leads to G1 arrest at and apoptosis. HCT116 cells were treated with **4k** at the concentrations shown, or with vehicle (DMSO) for 24 hours, prior to fixation, staining with propidium iodide (PI) and flow cytometric analysis. The results of a representative experiment are shown, with the mean of three independent experiments summarized in Figure 3.