Supplementary Data

Supplementary Figure Legends

Supplementary Figure 1 - Structures of the 6-aminopyrimidine PI3K/mTOR/CDK2 inhibitors evaluated

Supplementary Figure 2 - Effect of increasing concentrations of 1 on the sub-G1 fraction accumulation in HT29 cells

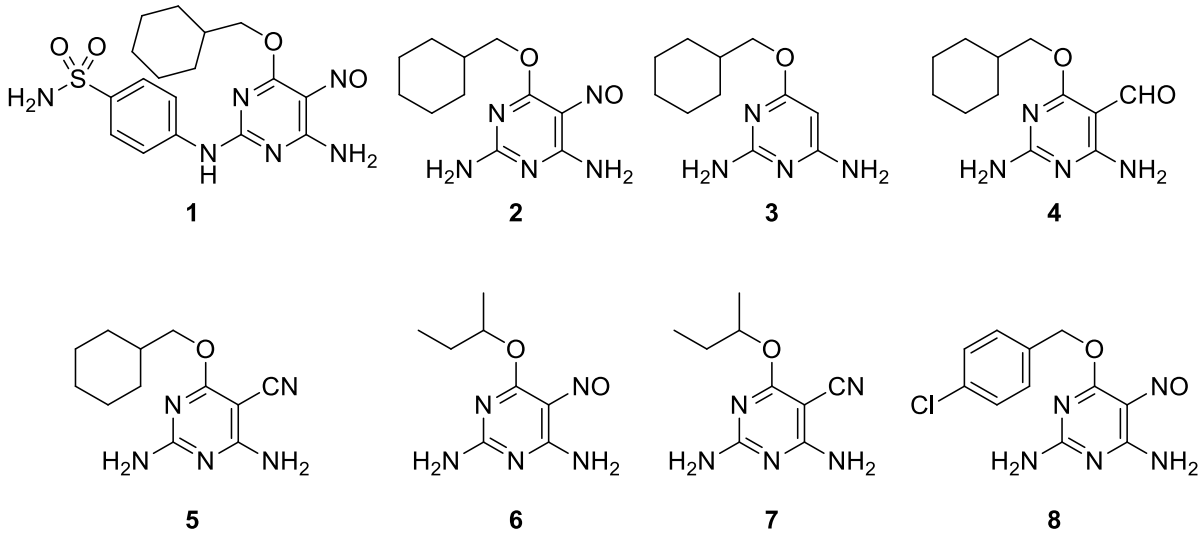
HT29 cells were treated with the concentrations of **1** shown for 24 hours and analysed as described for Figure 2.

Supplementary Figure 3 - Effects of 1 and 5 on the phosphorylation of rS6, AKT and Rb in HT29 cells

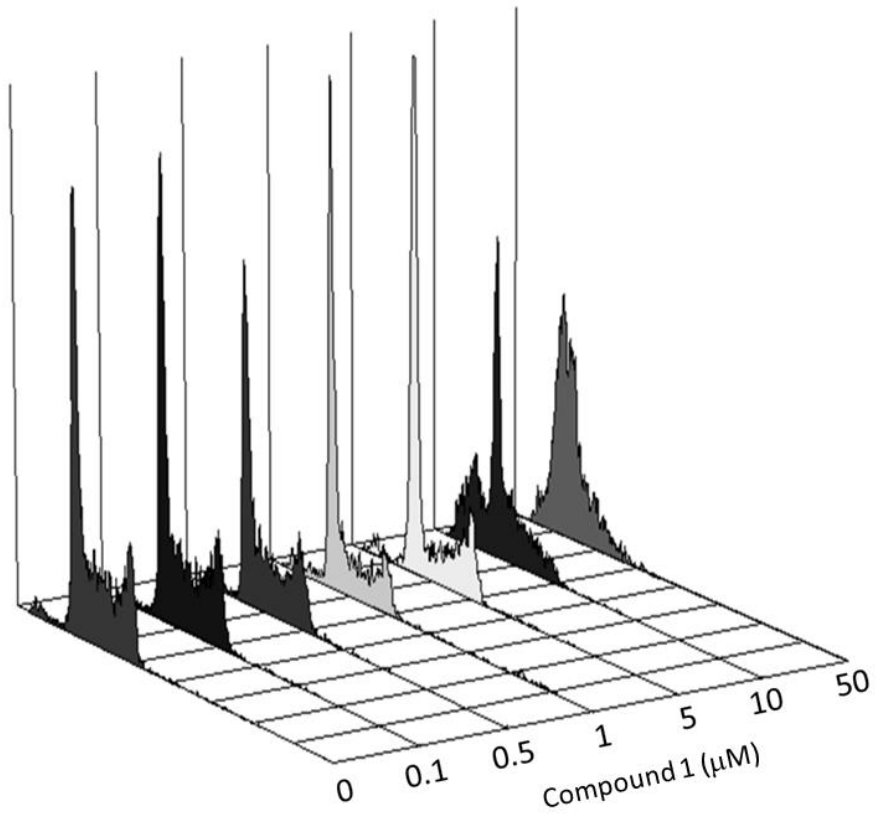
HT29 cells were treated and analysed as described for Figure 4.

Supplementary Figures

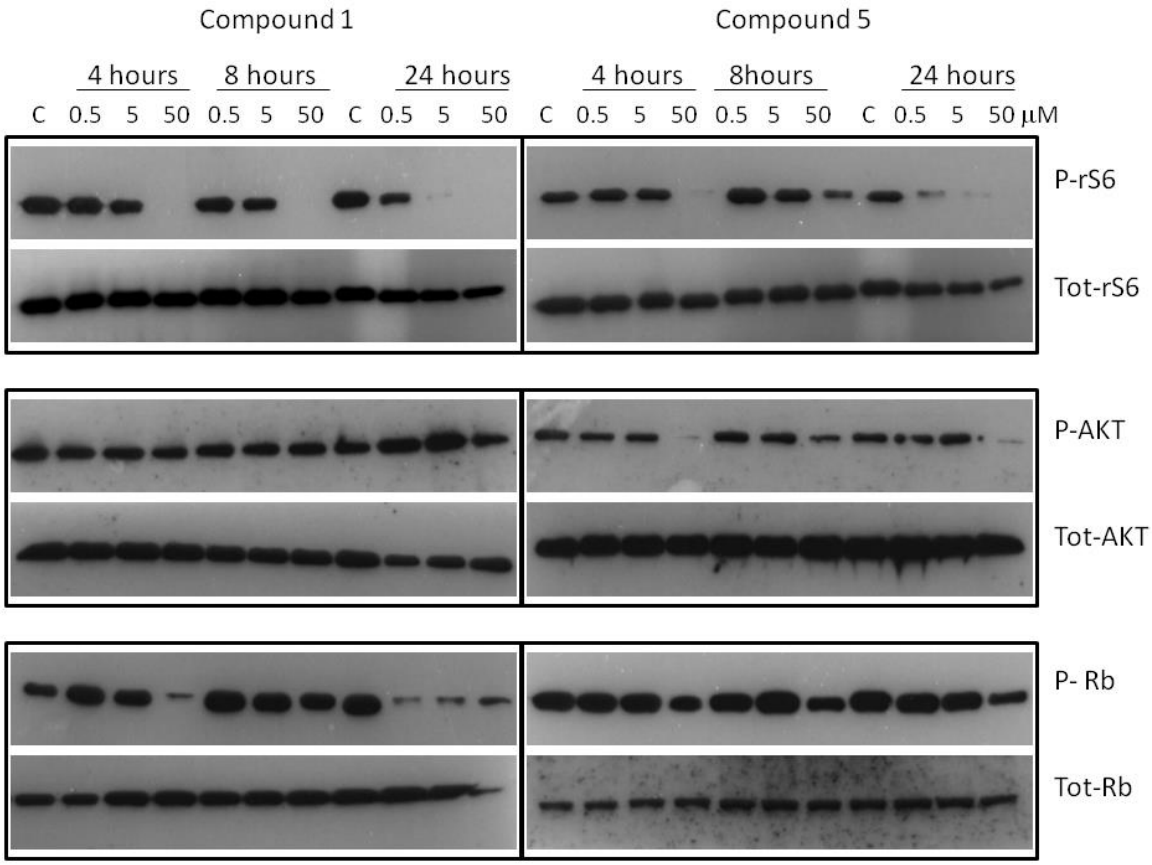
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Material – Synthesis of 7 and 8

6-(4-Chlorobenzyloxy)pyrimidine-2,4-diamine (**1**)

To a suspension of NaH (0.10 g, 4.15 mmol) in THF (2 mL) was added 4-chlorobenzyl alcohol (0.98 g, 6.90 mmol). The mixture was stirred for 2 h at room temperature, whereupon a solution of 2,6-diamino-4-chloropyrimidine (0.20 g, 1.38 mmol) in THF (2 mL) was added. The reaction mixture was heated under microwave irradiation for 20 min at 120 °C. The solvent was evaporated under reduced pressure, and the residual oil was purified by chromatography on silica, employing EtOAc:MeOH (9:1) as eluent, to furnish the title compound as a white solid (0.27 g, 77%); m.p. 129-131 °C; ν_{max}/cm^{-1} 3311, 1625, 1563; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.47 (2H, br s, D_2O exch, NH_2), 4.66 (2H, br s, D_2O exch, NH_2), 5.17 (2H, s, CH_2O), 5.23 (1H, s, H-5), 7.26-7.29 (4H, m, Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ 66.8, 79.3, 128.9, 129.5, 134.0, 136.2, 163.0, 165.9, 171.4; HRMS *m/z* calcd for $C_{11}H_{11}ClN_4O$ $[M+H]^+$ 251.0694, found 251.0693.

6-(4-Chlorobenzyloxy)-5-nitrosopyrimidine-2,4-diamine (**2**)

A solution of **1** (0.05 g, 0.20 mmol) in acetic acid (30% aqueous, 1 mL) was stirred at 80 °C, and sodium nitrite (0.018 g, 0.26 mmol) was cautiously added. The resulting solution was stirred for a further 20 min at 80 °C, and the solid that deposited on cooling was collected by filtration and recrystallised from methanol to afford the nitrosopyrimidine **2** as a purple solid (0.053 g, 96%); m.p. 253-254 °C; IR ν_{max}/cm^{-1} 3057, 1632, 1574; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.56 (2H, s, OCH_2), 7.53 (2H, d, *J* = 8.5 Hz, H-2' and H-6'), 2.58 (2H, d, *J* = 8.5 Hz, H-3' and H-

5'), 7.89 (2H, br s, D_2O exch, NH_2), 8.06 (1H, br s, D_2O exch, NH_2), 10.04 (1H, br s, D_2O exch, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 65.6, 85.9, 129.1, 130.6, 135.0, 136.3, 164.0, 166.3, 172.6; MS (ES+) *m/z* 280.2 $[M(^{35}Cl)+H]^+$, 282.2 $[M(^{37}Cl)+H]^+$; HRMS *m/z* calcd for $C_{11}H_{10}ClN_5O_2$ $[M+H]^+$ 280.0596 (^{35}Cl), found 280.0592.

5-Bromo-4-*sec*-butoxypyrimidine-2,6-diamine (**3**)

To a solution of 4-*sec*-butoxypyrimidine-2,6-diamine (28) (0.25 g, 1.37 mmol) in acetic acid (5 mL), was added *N*-bromosuccinimide (0.25 g, 1.39 mmol). After stirring at room temperature for 15 min, the reaction mixture was diluted with water (10 mL), neutralised with aqueous NaOH solution (2.5 M), and extracted with EtOAc (3 x 30 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was evaporated *in vacuo* to give the product as a cream solid (0.26 g, 72%), which was used without further purification; m.p. 103-104 °C; IR ν_{max}/cm^{-1} 3451, 3411, 3357, 1043; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 1.20 (3H, d, *J* = 6.2 Hz, $OCHCH_3$), 1.53-1.59 (2H, m, CH_2CH_3), 4.97-5.07 (1H, m, OCH), 6.06 (2H, s, NH_2), 6.26 (2H, s, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 9.6 (CH_3CH_2), 19.8 (CH_3CH), 28.9 (CH_3CH_2), 73.1 (OCH), 161.5 (CNH_2), 162.3 (CNH_2), 165.1 (CO); HRMS *m/z* calcd for $C_8H_{14}BrN_4O$ $[M+H]^+$ 261.0346 (^{79}Br) , found 261.0343.

***N, N'*-(5-Bromo-4-*sec*-butoxypyrimidine-2,6-diyl)diacetamide** (**4**)

A solution of pyrimidine **3** (2.38 g, 9.65 mmol) in acetic acid (23 mL, 405 mmol) and acetic anhydride (58 mL, 1.01 mol) was heated at 170 °C for 40 h. After cooling, water (60 mL) was cautiously added dropwise and the solution was

basified to pH 10 with concentrated aqueous ammonia solution. The beige solid that precipitated was collected by filtration, redissolved in DCM (30 mL) and the solution was dried (Na_2SO_4) and concentrated *in vacuo*. The residual solid was purified by chromatography on silica, employing EtOAc as eluent, to afford the title compound as a white solid (0.86 g, 26%); m.p.139-142 ^oC; IR ν_{max}/cm^{-1} 3215, 2974, 1677, 1571, 1311; ^1H NMR (300 MHz DMSO-d_6): δ 0.92 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.32 (3H, d, J = 6.1 Hz, $CHCH_3$), 1.65-1.74 (2H, m, CH_2CH_3), 2.18 (3H, s, $COCH_3$), 2.21 (3H, s, $COCH_3$), 5.15-5.19 (1H, m, $OCHCH_3$), 9.89 (1H, s, NH), 10.4 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 9.4 (CH_3CH_2), 19.0 (CH_3CH), 23.7 ($COCH_3$), 24.7 ($COCH_3$), 28.2 (CH_3CH_2), 75.9 (CH_3CHO), 90.3 (C^5), 154.8 (C^2), 157 (C^6), 166.1 (C-O), 168.9 (C=O), 169.2 (C=O).

2,6-Diamino-4-*sec*-butoxypyrimidine-5-carbonitrile (**5**)

To a solution of **4** (0.30 g, 0.87 mmol) in DMF (5 mL) was added CuCN (0.10 g, 1.1 mmol) and the mixture was stirred at 120 ^oC for 6 h. After cooling, 1,2-diaminoethane (5 mL, 75 mmol) was added and the solution was stirred at room temperature for a further 4 h, filtered (Celite), and diluted with water (30 mL). The filtrate was extracted with ethyl acetate (3 x 50 mL), and the combined organic layers were washed with brine (2 x 100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Recrystallisation from methanol-petrol gave the title compound as a white solid (0.093 g, 51%); m.p. 166-167 ^oC; IR ν_{max}/cm^{-1} 3380, 3125, 2199, 1660, 1628, 1572; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.23 (3H, d, J = 6.0 Hz, $CHCH_3$), 1.54-1.66 (2H, m, CH_2CH_3), 5.07-5.16 (1H, m, $CHCH_3$), 6.83 (2H, s, N^1H_2), 6.89 (2H, s, N^2H_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 9.6 (CH_3CH_2), 19.6 (CH_3CH), 28.8 (CH_3CH_2), 73.6 (OCH), 116.1 (CN),

163.6 (CNH_2), 166.4 (CNH_2), 171.3 (CO); HRMS *m/z* calcd for $C_9H_{15}N_5O$ 208.1193 $[M+H]^+$, found 208.1191^+.