Supplementary data

An asymmetrically-substituted quadruplex-binding naphthalene diimide showing potent activity in pancreatic cancer models

Ahmed A Ahmed,[a] Richard Angell,[a] Sally Oxenford,[a] Jenny Worthington[b] et al, Naomi Barton,[c] Thomas G Fowler,[c] Daniel E O'Flynn,[c] Mihiro Sunose,[c] Matthew McConville,[c] Tam Vo,[d] W David Wilson,[d] Saadia A Karim,[e] Jennifer P Morton,[e,f] Stephen Neidle[a*]

[a]School of Pharmacy, University College London, London WC1N 1AX, U.K.

[b]Axis Bio Discovery Services Ltd, Coleraine, Northern Ireland BT51 3RP, U.K.

[c]Sygnature Discovery Limited, Nottingham, NG1 1GR, U.K.

[d]Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, Georgia 30303-3083, U.S.A.

[e]Cancer Research UK Beatson Institute, Glasgow, G61 1BD, U.K.

[f]Institute of Cancer Sciences, University of Glasgow, Glasgow, G61 1QH, U.K.

Structure-activity relationships

Compound **2**: 5-fold drop-off in cellular GI_{50} value. The incorporation of the amine into an amide renders it non-basic and will therefore be uncharged at physiological (assay) pH. Electrostatic interaction with the phosphates will be minimal and the benefit to binding of an extra amine will essentially be lost.

Compound **3**: 50-fold drop-off in cellular GI_{50} value. Removal of the methylene spacer relative to **11** has three consequences. It shortens the linker, rigidifies the linker and creates a dialkyl aniline (pKa ca. 2.4, Bordwell pKa table) rather than a dialkyl benzylamine (pKa ca. 7.6, Bordwell pKa table). As outlined above these can all be detrimental to binding and, therefore, the cellular IC_{50}.

Compound **4**: 37-fold drop-off in cellular GI_{50} value. The vector of the basic amine is likely the primary reason for the poor activity exhibited by compound **4**. Coming from the 2-position it will be orientated towards the core, rather than towards the phosphate backbone of the G4, preventing the electrostatic interaction hypothesised to be one of the primary drivers of binding. Additionally, there is likely steric repulsion between the pyrrolidine and naphthalene diimide core. This will likely increase the torsion angle of the aryl-aryl bond past the 30^{o} maximum for face-face interactions, losing the π-stacking. This could also result in displacement of the ND core further reduction in π-stacking interactions.

Compound **5**: 5-fold drop off in cellular GI_{50} value. As with compound 4, the vector of the amine relative to the core is significantly different and likely non-optimal for binding. It has regained some activity relative to **4** so it is possible that some interaction exists in an alternative binding mode to that suggested for **11** in figure 1 f.

Compounds **6-9**: The larger steric demand around the amine can be detrimental to the binding and, therefore, cellular activity. However, the magnitude of these changes may be too small to draw conclusions from cellular data.

Compound **10:** In addition to its increased steric demand, the piperazine has different protonation behaviour to that of the piperazine and a significantly different electrostatic profile. This could be another factor in the observed 5-fold decrease in GI_{50}.

Differences between the MIA PaCa2 xenograft model and the KPC genetic model for pancreatic cancer

In terms of genotype, Mia PaCa-2 cells are not dissimilar to the KPC model. Both carry an activating KRAS mutation, and a homozygous p53 mutation as well as wild-type SMAD4. The one difference is they also exhibit homozygous deletion of CDKN2A.[1] The greater differences between models lie in the pattern of tumour progression and microenvironment. Whilst xenograft models are relatively quick and inexpensive to perform, they lack many of the features of the human disease compared with autochthonous models such as the lack of an intact immune system in immunocompromised mice used for xenograft studies. Perhaps more importantly, the tumour microenvironment, which has a major impact on drug efficacy, is not well represented in xenograft tumours. Only KPC-type models faithfully recapitulate the full spectrum of pancreatic tumour development from pre-neoplastic PanIN lesions to aggressive locally invasive and metastatic disease. KPC tumours also develop within the same dense, complex desmoplastic stroma observed in human PDAC, and this likely accounts for the observed differences in efficacy.[2]

1. Olive, K. P. *et al*. Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* **2009**, *324*, 1457-1461.
2. Springfeld, C.; Jäger, D.; Büchler, M. W.; Strobel, O.; Hackert, T.; Palmer, D. H.; Neoptolemos, J. P. Chemotherapy for pancreatic cancer. *Presse Med*. **2019**, *48*, e159-e174.

RNA-seq data on selected tumour-suppressor genes

We have examined mRNA transcriptome data for three of the major tumour suppressor genes.

The APC (adenomatous polyposis coli) gene has 3 potential G4s (PQs) in its promoter, which is an under-representation bearing in mind that this is a large gene coding for a 312 KDa protein. The RB (retinoblastoma) gene, which codes for a 106 KDa protein, also has 3 PQs in its promoter. The P53 gene, which codes for a 53 KDa protein, has 7 PQs in its promoter.

We have examined the observed mRNA changes in these genes in terms of log2 FC fold changes:

APC: -0.019 at 6 hrs, 0.300 at 24 hrs

RB: 0.190 at 6 hrs, 0.0088 at 24 hrs

P53: 0.287 at 6 hrs, 0.055 at 24 hrs

We conclude that for these three tumour suppressor genes, no significant down regulation has taken place on SOP1812 dosing of MIA PaCa-2 cells and instead some modest mRNA up regulation was found, which presumably contributes to the overall anti-tumour activity of SOP1812.

The APC2 gene, which is related to the APC gene but plays a role in the Wnt/β-catenin pathway, is down-regulated by SOP1812 and CM03, and by contrast to APC itself, contains 38 PQs (Table 6 in the main text).

Table S1. Blood pharmacokinetic parameters

(a) in female athymic nude mice following a single intravenous (IV) administration. Three mice were in each group, and data shown for each parameter is the mean value. Standard deviations are in parentheses.

	CM03 (1 mg/kg)	**CM03 (17 mg/kg)**	**SOP1812 (0.84 mg/kg)**	**SOP1812 (2.1 mg/kg)**
$T_{1/2}$ (h)	20 (4.3)	27 (28)	61 (56)	87 (92)
Cl_{obs} (mL/min/kg)	4.0 (0.77)	9.2 (5.8)	2. (1.3)	1.7 (1.3)
Vss (L/kg)	6.1 (2.4)	10 (7.7)	7.8 (1.9)	7.5 (3.3)
Vz (L/kg)	7.3 (2.8)	14 (8.1)	8.4 (1.5)	7.6 (3.2)
C_{max} (ng/mL)	975 (558)	16820 (3120)	1661 (932)	2189 (307)
AUC_{all} (ng.h/mL)	2716 (865)	24254 (7995)	2198 (224)	6461 (2770)
AUC_{INF_obs} (ng.h/mL)	4223 (826)	39508 (21485)	8161 (6057	30382 (21082)

(b) in female rats following a single intravenous (IV) administration. Three mice were in each group, and data shown for each parameter is the mean value. Standard deviations are in parentheses.

	CM03 (15 mg/kg)	**SOP1812 (1.0 mg/kg)**
$T_{1/2}$ (h)	46(20)	36(19)
Cl_{obs} (mL/min/kg)	≤8.7*	≤2.1*
Vss (L/kg)	23(6)	5.3(2.6)
Vz (L/kg)	26(5)	5.1(2.8)
C_{max} (ng/mL)	2073(536)	142(15)
AUC_{all} (ng.h/mL)	13242(3746)	1623(685)
AUC_{INF_obs} (ng.h/mL)	20802**(11022)	2126(647)

Experimental methods

Chemistry. All chemicals, reagents, and solvents were purchased from commercial sources and used as received unless otherwise stated. Solvents were commercial HPLC grade unless dry solvent is specified, in which case the Aldrich 'Sure Seal' dry solvents were used. Column chromatography was performed on pre-packed silica (230-400 mesh, 40-63 µm) cartridges using the eluent indicated. Reverse phase chromatography was carried out using pre-packed silica cartridges (40 µm, C18).

^{1}H NMR Spectra were acquired on a Bruker Avance III spectrometer at 400 MHz or Bruker Avance III HD at 500 MHz using residual undeuterated solvent as reference.

Analytical LCMS was carried out using either acidic or basic methods as follows:

Acidic, HPLC: Waters X-Select CSH C18, 2.5 µm, 4.6 x 30 mm column eluting with a gradient of 0.1% formic acid in MeCN in 0.1% formic acid in water. The gradient from 5-95 % 0.1% Formic acid in MeCN occurred between 0.00-3.00 mins at 2.5 ml/min with a flush from 3.01-3.5 mins at 4.5 ml/min. A column re-equilibration to 5% MeCN was from 3.60-4.00 min at 2.5 ml/min. UV spectra of the eluted peaks were measured using an Agilent 1260 Infinity or Agilent 1200 VWD at 254 nm. Mass spectra were measured using an Agilent 6120 or Agilent 1956 MSD running with positive/negative switching or an Agilent 6100 MSD running in either positive or negative mode.

Basic, HPLC: Waters X-Select BEH C18, 2.5 µm, 4.6 x 30 mm column eluting with a gradient of MeCN in aqueous 10mM ammonium bicarbonate. The gradient from 5-95 % MeCN occurred between 0.00-3.00 mins at 2.5 ml/min with a flush from 3.01-3.5 mins at 4.5 ml/min. A column re-equilibration to 5% MeCN was from 3.60-4.00 mins at 2.5 ml/min. UV spectra of the eluted peaks were measured using an Agilent 1260 Infinity or Agilent 1200 VWD at 254 nm. Mass spectra were measured using an Agilent 6120 or Agilent 1956 MSD running with

positive/negative switching or an Agilent 6100 MSD running in either positive or negative mode.

Alternatively analytical UPLC/MS was carried out using either acidic or basic methods as follows:

Acidic, UPLC: Waters Acquity CSH C18, 1.7 µm, 2.1 x 30 mm column eluting with a gradient of 0.1% formic acid in MeCN in 0.1% formic acid in water. The gradient was structured with a starting point of 5% MeCN held from 0.0-0.11 mins. The gradient from 5-95% occurred between 0.11-2.15 mins with a flush from 2.15-2.56 mins. A column re-equilibration to 5% MeCN was from 2.56-2.83 mins. UV spectra of the eluted peaks were measured using an Acquity PDA and mass spectra were recorded using an Acquity QDa detector with ESI pos/neg switching.

Basic UPLC: Waters Acquity BEH C18, 1.7 µm, 2.1 x 30 mm column eluting with a gradient of MeCN in aqueous 10 mM ammonium bicarbonate. The gradient was structured with a starting point of 5% MeCN held from 0.0-0.11 minutes. The gradient from 5-95 % occurred between 0.11-2.15 mins with a flush from 2.15-2.56 mins. A column re-equilibration to 5% MeCN was from 2.56-2.83 mins. UV spectra of the eluted peaks were measured using an Acquity PDA and mass spectra were recorded using an Acquity QDa detector with ESI pos/neg switching.

Preparative HPLC was carried out using a Waters Xselect CSH C18, 5 µm, 19 x 50 mm column using either a gradient of either 0.1 % formic acid in MeCN in 0.1 % aqueous Formic Acid or a gradient of MeCN in aqueous 10 mM ammonium bicarbonate, or with a Waters Xbridge BEH C18, 5 µm, 19x50 mm column using a gradient of MeCN in aqueous 10 mM ammonium bicarbonate. Fractions were collected following detection by UV at a single wavelength measured by a variable wavelength detector on a Gilson 215 preparative HPLC or Varian PrepStar preparative HPLC; by mass and UV at a single wavelength measured by a ZQ

single quadrupole mass spectrometer, with positive and negative ion electrospray, and a dual wavelength detector on a Waters Fraction Lynx LCMS.

Assayed compounds **1**-**11** were all isolated in > 95% purity by both 1H-NMR and LCMS, unless otherwise stated.

4,9-dibromoisochromeno[6,5,4-def]isochromene-1,3,6,8-tetraone

Isochromeno[6,5,4-def]isochromene-1,3,6,8-tetraone (20 g, 74.6 mmol) was suspended in H_2SO_4 (240 mL) and 1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (42.6 g, 149 mmol) added with stirring. The mixture was heated to 80 °C for 2 h using a water scrubber to remove any bromine. The reaction was poured carefully onto ice (1 kg) and the resulting suspension further diluted with water (400 mL). The solid was filtered off washing with water (200 mL) and MeOH (100 mL). 4,9-dibromoisochromeno[6,5,4-def]isochromene-1,3,6,8-tetraone (26.2 g, 82 % yield) was isolated as a yellow solid. ^{1}H NMR in DMSO-d6 1863-10 was consistent with product structure at 99% purity. Ratio of mono:di:tri brominated products = 10:77:13. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.80 (s, 2H).

4,9-dibromo-2,7-bis(3-morpholinopropyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone

3-morpholinopropan-1-amine (7.20 ml, 49.3 mmol) was added slowly over 15 min to a slurry of 4,9-dibromoisochromeno[6,5,4-def]isochromene-1,3,6,8-tetraone (10 g, 23.5 mmol) in acetic acid (100 ml, 1747 mmol) and the resulting yellow slurry heated to 75 °C (internal temp) for 1 h. The reaction mixture was cooled to room temperature and concentrated onto silica gel. The crude product was purified by chromatography on silica gel (120 g cartridge, flush with 4 x CV of DCM, then 0-5% (0.7 M Ammonia/MeOH)/DCM) to afford the subtitle product (7.07 g, 9.38 mmol, 40.0 % yield) as a light yellow solid. ^{1}H NMR (500 MHz, Acetic

Acid-d_4) δ 8.98 (s, 2H), 4.30 (t, J = 6.5 Hz, 4H), 4.05 – 3.88 (m, 8H), 3.80 – 3.42 (m, 4H), 3.38 – 3.31 (m, 4H), 3.31 – 2.88 (m, 4H), 2.43 – 2.16 (m, 4H).

4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone

2-(pyrrolidin-1-yl)ethanamine (3.97 ml, 31.3 mmol) was added to a slurry of 4,9-dibromo-2,7-bis(3-morpholinopropyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (7.07 g, 10.4 mmol) in toluene (120 mL) and the resulting yellow slurry heated to reflux for 1 h. The reaction mixture was cooled to room temperature. The solvent was then removed and the dark red residue slurried in DCM (200 mL). The SM was removed by filtration, washing with DCM (2 x 30 mL) and the organic washings concentrated in vacuo. The dark red residue was then triturated with MeCN (100 mL) and the resulting red solid collected by filtration, washing with MeCN (3 x 25 mL) to afford the subtitled product (4.05 g, 4.84 mmol, 46.4 % yield) as a dark red solid. The product was analysed by LCMS (Waters Acquity UPLC, X-Select, Waters X-Select UPLC C18, 1.7 µm, 2.1x30mm, basic (0.1% Ammonium Bicarbonate) 3 min method, 5-95% MeCN/water): found m/z 711.5 and 713.5: (C34H43BrN6O6 (MH+) requires 711.2 & 713.2) (ES+) at 1.55 min, 85% purity (254 nm). ^{1}H NMR (500 MHz, Chloroform-d) δ 10.23 (t, J = 5.5 Hz, 1H), 8.84 (s, 1H), 8.28 (s, 1H), 4.30 – 4.23 (m, 4H), 3.78 – 3.69 (m, 2H), 3.66 – 3.52 (m, 8H), 2.98 – 2.90 (m, 2H), 2.76 – 2.62 (m, 3H), 2.51 (t, J = 7.0 Hz, 4H), 2.49 – 2.37 (m, 8H), 1.98 – 1.89 (m, 5H), 1.88 – 1.83 (m, 4H).

1-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrrolidine, 1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrrolidine, 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)piperidine, N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)cyclopentanamine, 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-

yl)benzyl)piperazine and N-ethyl-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)ethanamine were all synthesised according to the following general procedure:

A stirred solution of an appropriate bromide in MeCN (5 mL) was treated with amine. After 20-64 hr, the mixture was analysed by LCMS then evaporated. The residue was taken up in DCM (5 mL), washed with $NaHCO_3$ (10 mL, sat aq), passed through a hydrophobic frit and concentrated in vacuo. The crude products were used without further purification or characterisation.

*4-(4-(morpholinomethyl)phenyl)-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**1**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (202 mg, 0.284 mmol) and (4-(morpholinomethyl)phenyl)boronic acid (188 mg, 0.852 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.57 mL of a 2 M aq solution, 1.14 mmol) and de-gassed by evacuation and backfilling with nitrogen. S-Phos Pd G3 (6.6 mg, 8.5 µmol) was added and the mixture again de-gassed then the reaction heated to 80 ºC (block temp, pre-heated). After 18 h, the mixture cooled, diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography (12 g Buchi FlashPure, pre-adsorbed, 10-70% [9:1 (1:1 THF:DCM): 7 M NH_3 in MeOH] in (1:1 THF:DCM)). Selected product containing fractions were concentrated and taken up in MeCN (2 mL). After ~48 h, this was filtered and the solid discarded. The remaining product containing fractions were evaporated and re-slurried from *iso*-hexanes. This material was combined with the MeCN liquors from the above batch and the resultant purified by column chromatography (12 g RediSep Gold, 30-70% (9:1 DCM: 0.7 M NH_3 in MeOH) in DCM, loading in DCM). The central cut of this band was evaporated to afford the product as a bright red glassy solid

(50 mg, 22%). LCMS: found *m/z* 808.3 ($C_{45}H_{58}N_7O_7$ (MH^+) requires 808.4) @ 6.68 min. ^{1}H NMR (500 MHz, chloroform-*d*) δ 10.24 (t, *J* = 5.3 Hz, 1H), 8.50 (s, 1H), 8.33 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 4.30 (t, *J* = 7.4 Hz, 2H), 4.15 (t, *J* = 7.4 Hz, 2H), 3.83 – 3.71 (m, 6H), 3.65 – 3.59 (m, 10H), 2.95 (t, *J* = 6.4 Hz, 2H), 2.70 - 2.67 (m, 4H), 2.58 – 2.50 (m, 6H), 2.47 – 2.40 (m, 10H), 1.96 (app p, *J* = 7.1 Hz, 2H), 1.90 – 1.84 (m, 6H).

*2,7-bis(3-morpholinopropyl)-4-((2-(pyrrolidin-1-yl)ethyl)amino)-9-(4-(pyrrolidine-1-carbonyl)phenyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**2**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (201 mg, 0.282 mmol) and (4-(pyrrolidine-1-carbonyl)phenyl)boronic acid (155 mg, 0.706 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.42 mL of a 2 M aq solution, 0.84 mmol), de-gassed evacuation and backfilling with nitrogen, then treated with SPhos PdG3 (6.6 mg, 8.4 µmol), again de-gassed, and heated to 80 ºC (block temp) under nitrogen. After 16 h, the mixture was cooled, then poured onto a mixture of water (20 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (3 x 20 mL). The combined organics were evaporated onto silica and purified by column chromatography (12 g Buchi FlashPure, pre-adsorbed, 10-70% [9:1 (1:1 THF:DCM): 7 M NH_3 in MeOH] in (1:1 THF:DCM)). The center of the product band was evaporated and taken up in MeCN (2 mL). After ~100 h, the mixture was filtered and the solid discarded. The liquors were evaporated and purified by column chromatography (12 g RediSep Gold, 30-60% (9:1 DCM: 0.7 M NH_3 in MeOH) in DCM, loading in DCM). The central cut of this band was evaporated and dried in the vacuum desiccator at 40 ºC to afford the product as a bright red glassy solid (55 mg, 24%). LCMS: found *m/z* 806.2: ($C_{45}H_{56}N_7O_7$ (MH^+) requires 806.4) @ 6.38 min. ^{1}H NMR (500 MHz, methylene chloride-d_2) δ 10.29 (t, *J* = 5.1 Hz, 1H), 8.47 (s, 1H), 8.36 (s, 1H), 7.62 (t, *J* = 8.3 Hz, 2H), 7.42 (t, *J* = 8.3 Hz, 2H), 4.30 (t, *J* = 7.3 Hz, 2H), 4.14 (t, *J* = 7.3 Hz 2H), 3.76 (app q, *J* = 5.8 Hz, 2H), 3.67

(t, *J* = 6.9 Hz, 2H), 3.59 – 3.52 (m, 10H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.77 – 2.63 (m, 4H), 2.51 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.29 (m, 10H), 2.08 – 1.79 (m, 12H).

*2,7-bis(3-morpholinopropyl)-4-((2-(pyrrolidin-1-yl)ethyl)amino)-9-(4-(pyrrolidin-1-yl)phenyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**3**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (200 mg, 0.281 mmol) and (4-(pyrrolidin-1-yl)phenyl)boronic acid hydrochloride (192 mg, 0.843 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.84 mL of a 2 M aq solution, 1.68 mmol) then de-gassed by purging with nitrogen. S-Phos Pd G3 (6.6 mg, 8.4 µmol) was charged, the mixture again de-gassed, then heated to 80 ºC (block temp). After 18 h, the mixture cooled then poured into diluted $NaHCO_3$ (10 mL sat aq + 10 mL water) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g Redi-Sep Gold, 15-55% (9:1 DCM: 0.7 M NH_3 in MeOH) in DCM, loading in DCM) gave the product as a deep blue glassy solid (130 mg, 59%). LCMS: found *m/z* 778.3: ($C_{44}H_{56}N_7O_6$ (MH^+) requires 778.4) @ 8.74 min. ^{1}H NMR (500 MHz, methylene chloride-d_2) δ 10.15 (t, *J* = 5.1 Hz, 1H), 8.51 (s, 1H), 8.29 (s, 1H), 7.28 (t, *J* = 8.6 Hz, 2H), 6.66 (t, *J* = 8.6 Hz, 2H), 4.28 (t, *J* = 7.4 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 3.73 (q, *J* = 5.9 Hz, 2H), 3.58 – 3.55 (m, 8H), 3.44 – 3.38 (m, 4H), 2.94 (t, *J* = 6.2 Hz, 2H), 2.70 – 2.64 (m, 4H), 2.50 (t, *J* = 6.8 Hz, 2H), 2.46 – 2.39 (m, 10H), 2.13 – 2.04 (m, 4H), 1.93 (p, *J* = 7.0 Hz, 2H), 1.89 – 1.83 (m, 6H).

*2,7-bis(3-morpholinopropyl)-4-((2-(pyrrolidin-1-yl)ethyl)amino)-9-(2-(pyrrolidin-1-ylmethyl)phenyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**4**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (144 mg, 0.202

mmol) and 1-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrrolidine (174 mg, 0.607 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.41 mL of a 2 M aq solution, 0.82 mmol) and de-gassed. S-Phos Pd G3 (4.7 mg, 6.1 µmol) was charged, the mixture again de-gassed then heated to 80 ºC (block temp). After 16 h, LCMS analysis showed poor conversion and the mixture was treated with additional 1-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrrolidine (174 mg, 0.607 mmol), de-gassed, then treated with additional S-Phos Pd G3 (4.7 mg, 6.1 µmol), de-gassed and heated for a further 24 h. The mixture cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g RediSep Gold, 30-60% (9:1 DCM: 0.7 M NH_3 in MeOH) in DCM, loading in DCM) gave the desired product as a bright red glassy solid (40 mg, 25%). LCMS: found *m/z* 792.3: ($C_{45}H_{58}N_7O_6$ (MH^+) requires 792.4) @ 7.17 min. ^{1}H NMR (500 MHz, methylene chloride-d_2) δ 10.25 (t, *J* = 5.1 Hz, 1H), 8.42 (s, 1H), 8.33 (s, 1H), 7.52 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.42 (td, *J* = 7.5, 1.5 Hz, 1H), 7.38 (td, *J* = 7.4, 1.5 Hz, 1H), 7.18 (dd, *J* = 7.4, 1.5 Hz, 1H), 4.30 (t, *J* = 7.3 Hz, 2H), 4.08 (td, *J* = 7.4, 2.2 Hz, 2H), 3.76 (q, *J* = 5.9 Hz, 2H), 3.57 – 3.52 (dt, *J* = 15.8, 4.7 Hz, 8H), 3.30 (d, *J* = 13.3 Hz, 1H), 3.17 (d, *J* = 13.3 Hz, 1H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.70 – 2.66 (m, 4H), 2.51 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.28 (m, 10H), 2.25 – 2.16 (m, 4H), 1.94 (app p, *J* = 6.9 Hz, 2H), 1.91 – 1.76 (m, 6H), 1.58 – 1.56 (m, 4H).

*2,7-bis(3-morpholinopropyl)-4-((2-(pyrrolidin-1-yl)ethyl)amino)-9-(3-(pyrrolidin-1-ylmethyl)phenyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**5**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (149 mg, 0.209 mmol) and 1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrrolidine (180 mg, 0.628 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.42 mL of a 2 M aq

solution, 0.84 mmol) and de-gassed. S-Phos Pd G3 (4.9 mg, 6.3 µmol) was charged, the mixture again de-gassed and the whole heated to 80 ºC. After 16 h, the mixture cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g RediSep Gold, 30-60% (9:1 DCM: 0.7 M NH_3 in MeOH) in DCM, loading in DCM).The resulting residue was further purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water), loading in DMSO). The resulting residue was re-purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water, loading in DMSO) to afford the product as a bright red glassy solid (16 mg, 10%). LCMS: found *m/z* 792.4: ($C_{45}H_{58}N_7O_6$ (MH^+) requires 792.4) @ 6.42 min. ^{1}H NMR (500 MHz, Methylene Chloride-d_2) δ 10.26 (t, *J* = 5.5 Hz, 1H), 8.47 (s, 1H), 8.35 (s, 1H), 7.47 – 7.37 (m, 2H), 7.35 (br s, 1H), 7.26 (dt, *J* = 7.2, 1.7 Hz, 1H), 4.30 (t, *J* = 7.4, 2H), 4.13 (t, *J* = 7.4 Hz, 2H), 3.82 – 3.65 (m, 4H), 3.52 – 3.58 (m, 8H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.69 – 2.66 (m, 4H), 2.61 – 2.30 (m, 16H), 1.93 (p, *J* = 6.9 Hz, 2H), 1.89 – 1.75 (m, 10H).

*2,7-bis(3-morpholinopropyl)-4-(4-(piperidin-1-ylmethyl)phenyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (***6***)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (155 mg, 0.218 mmol) and 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)piperidine (197 mg, 0.653 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.44 µL of a 2 M aq solution, 0.88 mmol) and de-gassed. S-Phos Pd G3 (5.1 mg, 6.5 µmol) was charged, the mixture again de-gassed and the reaction heated to 80 ºC. After 16 h, the mixture was cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g RediSep Gold, 30-60% (9:1 DCM: 0.7 M NH_3 in MeOH) in DCM, loading in DCM) gave

product in moderate purity. The residue was purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water, loading in DMSO) to afford the product as a bright red glassy solid (61 mg, 35%). LCMS: found *m/z* 806.3: ($C_{46}H_{60}N_7O_6$ (MH^+) requires 806.5) @ 7.38 min. ^{1}H NMR (500 MHz, Methylene Chloride-d_2) δ 10.25 (t, *J* = 5.1 Hz, 1H), 8.47 (s, 1H), 8.34 (s, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 4.29 (t, *J* = 7.4 Hz, 2H), 4.14 (t, *J* = 7.3 Hz, 2H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.64 – 3.50 (m, 10H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.69 – 2.66 (m, 4H), 2.56 – 2.29 (m, 16H), 1.93 (p, *J* = 7.0 Hz, 2H), 1.89 – 1.82 (m, 6H), 1.68 – 1.62 (m, 4H), 1.53 – 1.49 (m, 2H).

4-(4-((diethylamino)methyl)phenyl)-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (7)

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (155 mg, 0.218 mmol) and N-ethyl-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)ethanamine (189 mg, 0.653 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.44 mL of a 2 M aq solution, 0.88 mmol) and de-gassed. S-Phos Pd G3 (5.1 mg, 6.5 µmol) was charged, the mixture again de-gassed and the whole heated to 80 ºC. After 16 h, the mixture was cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g BuchiFlashPure, 30-60% (9:1 DCM: 1.4 M NH_3 in MeOH) in DCM, loading in DCM) gave product in moderate purity.The residue was purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water, loading in DMSO) to afford the product as a bright red glassy solid (81 mg, 47%). LCMS: found *m/z* 794.2: ($C_{45}H_{60}N_7O_6$ (MH^+) requires 794.5) @ 6.93 min. ^{1}H NMR (500 MHz, Methylene Chloride-d_2) δ 10.24 (t, *J* = 5.2 Hz, 1H), 8.46 (s, 1H), 8.32 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 4.28 (t, *J* = 7.4 Hz, 2H), 4.13 (t, *J* = 7.4 Hz, 2H), 3.79 – 3.71

(m, 2H), 3.69 (s, 2H), 3.58 – 3.53 (m, 8H), 2.94 (t, *J* = 6.2 Hz, 2H), 2.69 – 2.66 (m, 4H), 2.61 (q, *J* = 7.1 Hz, 4H), 2.50 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.30 (m, 10H), 1.93 (p, *J* = 6.9 Hz, 2H), 1.88 – 1.82 (m, 6H), 1.12 (t, *J* = 7.1 Hz, 6H).

*4-(4-((cyclopentylamino)methyl)phenyl)-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**8**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (153 mg, 0.215 mmol) and N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)cyclopentanamine (194 mg, 0.645 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.43 mL of a 2 M aq solution, 0.86 mmol) and de-gassed. S-Phos Pd G3 (5.0 mg, 6.5 µmol) was charged, the mixture again de-gassed and heated to 80 ºC. After 16 h, the mixture was cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g BuchiFlashPure, 30-60% (9:1 DCM: 1.4 M NH_3 in MeOH) in DCM, loading in DCM) gave product in moderate purity. The residue was purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water, loading in DMSO) to afford the product as a bright red glassy solid (29 mg, 17%). LCMS: found *m/z* 806.4: ($C_{46}H_{60}N_7O_6$ (MH^+) requires 806.5) @ 6.57 min. ^{1}H NMR (500 MHz, Methylene Chloride-d_2) δ 10.25 (t, *J* = 5.1 Hz, 1H), 8.46 (s, 1H), 8.34 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 4.29 (t, *J* = 7.3 Hz, 2H), 4.13 (t, *J* = 7.4, Hz, 2H), 3.88 (s, 2H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.58 – 3.53 (m, 8H), 3.23 (p, *J* = 6.4 Hz, 1H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.69 – 2.66 (m, 4H), 2.50 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.30 (m, 10H), 1.96 -1.89 (m, 4H), 1.88 – 1.80 (m, 6H), 1.79 – 1.72 (m, 2H), 1.65 – 1.58 (m, 2H), 1.51 – 1.42 (m, 2H), CH_2N**H**CH not observed.

*4-(4-(azepan-1-ylmethyl)phenyl)-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**9**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (223 mg, 0.313 mmol) and 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)azepane (296 mg, 0.940 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.63 mL of a 2 M aq solution, 1.26 mmol) and de-gassed. S-Phos Pd G3 (7.3 mg, 9.4 µmol) was charged, the mixture again de-gassed and heated to 80 ºC. After 16 h, the mixture was cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g Buchi FlashPure, 30-60% (9:1 DCM: 1.4 M NH_3 in MeOH) in DCM, loading in DCM) gave product in moderate purity. The residue was purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water, loading in DMSO) to afford the product as a bright red glassy solid (130 mg, 51%). LCMS: found *m/z* 820.3: ($C_{47}H_{62}N_7O_6$ (MH^+) requires 820.5) @ 7.69 min. ^{1}H NMR (500 MHz, methylene chloride-d_2) δ 10.25 (t, *J* = 5.1 Hz, 1H), 8.47 (s, 1H), 8.34 (s, 1H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 4.29 (t, *J* = 7.4 Hz, 2H), 4.14 (t, *J* = 7.3 Hz, 2H), 3.82 – 3.70 (m, 4H), 3.57 – 3.53 (m, 8H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.78 – 2.61 (m, 8H), 2.50 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.30 (m, 10H), 1.93 (p, *J* = 7.0 Hz, 2H), 1.88 – 1.82 (m, 6H), 1.73 – 1.68 (m, 8H).

*2,7-bis(3-morpholinopropyl)-4-((2-(pyrrolidin-1-yl)ethyl)amino)-9-(4-(1,4-benzylpiperazine)phenyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**10**)*

A stirred mixture of 4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (213 mg, 0.299 mmol) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)piperazine (284 mg, 0.898 mmol) in dioxane (4 mL) was treated with potassium carbonate (0.60 mL of a 2 M aq solution, 1.20 mmol) and de-gassed by evacuation and backfilling with nitrogen. S-Phos Pd G3 (7.0 mg, 9.0 µmol) was charged, the mixture again de-gassed and heated to 80

ºC. After 16 h, the mixture cooled then diluted with water (10 mL) and sat aq $NaHCO_3$ (10 mL) and extracted with DCM (2 x 20 mL). The combined organics were dried over Na_2SO_4 and evaporated. Column chromatography (12 g BuchiFlashPure, 30-60% (9:1 DCM: 3.5 M NH_3 in MeOH) in DCM, loading in DCM) gave product in moderate purity. The residue was purified by reverse phase column chromatography (12 g Reveleris C-18, 75-100% (70 mM NH_3 in MeOH) in water, loading in DMSO) to afford the product as a bright red glassy solid (111 mg, 45%) LCMS: found *m/z* 821.2: ($C_{46}H_{61}N_8O_6$ (MH^+) requires 821.5) @ 6.06 min. ^{1}H NMR (500 MHz, methylene chloride-d_2) δ 10.25 (t, *J* = 5.1 Hz, 1H), 8.46 (s, 1H), 8.34 (s, 1H), 7.43 (d, J = 8.1 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 4.29 (t, *J* = 7.4 Hz, 2H), 4.13 (t, *J* = 7.3 Hz, 2H), 3.75 (q, *J* = 5.9 Hz, 2H), 3.62 (s, 2H), 3.57 – 3.53 (m, 8H), 2.95 (t, *J* = 6.2 Hz, 2H), 2.77 – 2.20 (m, 27H), 1.94 (q, *J* = 7.1 Hz, 2H), 1.89 – 1.80 (m, 6H).

*2,7-bis(3-morpholinopropyl)-4-((2-(pyrrolidin-1-yl)ethyl)amino)-9-(4-(pyrrolidin-1-ylmethyl)phenyl)benzo[lmn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetraone (**11**: SOP1812)*

4-bromo-2,7-bis(3-morpholinopropyl)-9-((2-(pyrrolidin-1-yl)ethyl)amino) benzo[lmn][3,8] phenanthroline-1,3,6,8(2H,7H)-tetraone (100 mg, 0.141 mmol), 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrrolidine (121 mg, 0.422 mmol) and $Pd(Ph_3P)_4$ (8.12 mg, 7.03 μmol) were dissolved in THF/2 M K_2CO_3 (3:1, 2 mL) and degassed by evacuation and backfilling with nitrogen three times. The mixture was heated (70 °C block temperature) with stirring for 3 h. The reaction was cooled, diluted with DCM (15 mL), washed with water (15 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The crude product was purified by preparative HPLC, Basic, 20-50 MeCN in water to afford the title compound (7.1 mg, 8.34 μmol, 6 % yield) as a dark red solid. LCMS: found: m/z 792.4 ($C_{45}H_{57}N_7O_6$ (MH+) requires 792.4) @ 4.00 min. ^{1}H NMR (400 MHz, Chloroform-d) δ 10.24 (t, J = 5.3 Hz, 1H), 8.48 (s, 1H), 8.32 (s, 1H), 7.52 (d, J = 7.9 Hz, 2H), 7.38 - 7.29 (m, 2H), 4.34 - 4.25 (m, 2H), 4.21 - 4.07 (m, 2H), 3.87 (s, 2H), 3.75 (q, J = 6.2 Hz, 2H), 3.62 (dt, J = 16.8, 4.7 Hz, 8H), 2.95

(t, J = 6.5 Hz, 2H), 2.78 (s, 4H), 2.71 - 2.64 (m, 4H), 2.53 (t, J = 7.0 Hz, 2H), 2.50 - 2.35 (m, 10H), 2.02 - 1.78 (m, 12H).

Biophysical studies. The experimental details for the surface plasmon resonance studies have been recently described in detail.[1]

Cell culture studies.

SRB assays. Cell lines (MIA PaCa-2, PANC-1, BxPC-3 and Capan-1) were purchased from ATCC (cat #: CRL-1420, CRL-1469, CRL-1687 and HTB-79). The former two cell lines were maintained in DMEM and the latter two cell lines in RPMI-1640 and IMEM, respectively. All media were supplemented with 10 % foetal bovine serum (FBS) (ThermoFisher, cat #: 10270106), 2 mM L-glutamine (Sigma-Aldrich, cat #: D6429), 0.1 mg/ml streptomycin and 100 U/ml penicillin (Sigma-Aldrich, cat #: P4333). Specifically, MIA PaCa-2 medium was also supplemented with 2.5 % horse serum (ThermoFisher, cat #: 16050130) and Capan-1 medium with extra 10% FBS to make 20 % in total. Cell lines were maintained at 37 °C, 5% CO_2 and passaged or their media were changed every 2 – 3 days. The cell lines were routinely tested to ensure that they were mycoplasma-free by a RT-qPCR-based method.

Drugs were dissolved in PBS and for some compounds the addition of a few drops of 0.1 M HCl helped to fully solubilise them. Stocks of 10 mM were prepared and filtered through 0.22 µm pore-size filter units before addition to appropriate cell line media. Cellular growth inhibition after 96 h incubation was measured using the sulforhodamine B (SRB) assay in 96-well plates as described previously.[2] 50% growth inhibitory concentrations (GI_{50}) were determined by taking the mean absorbance at 540 nm for each drug concentration expressed as a percentage of the absorbance of untreated control wells. At least three independent experiments were performed in triplicate and then the mean and SD values were calculated.

MTS assays. The CellTiter 96® AQueous One Solution Cell Proliferation Assay (Invitrogen) is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The CellTiter 96® AQueous One Solution Reagent contains the tetrazolium compound MTS and an electron coupling reagent (phenazine ethosulfate; PES). PES has enhanced chemical stability, which allows it to be combined with MTS to form a stable solution. The MTS tetrazolium compound (Owen's reagent) is bioreduced by cells into a coloured formazan product that is soluble in tissue culture medium. Assays were performed by adding a small amount of the CellTiter 96® AQueous One Solution Reagent directly to culture wells, incubating for 1–4 h and then recording the absorbance at 490 nm with a 96-well plate reader. The quantity of formazan product as measured by absorbance at 490 nm is directly proportional to the number of living cells in culture. The kit was used as per the manufacturer's instructions. After 96 h incubation with each example compound in MIA PaCa-2 cells, the cell proliferation of each sample was measured using the MTS Cell Titre 96 Aqueous One Solution Cell Proliferation Assay (Promega Ltd). The percentage of inhibition was calculated against the mean of the DMSO treated control samples.

Immunofluorescence staining of G-quadruplex. The BG4 antibody (MABE917) was purchased from MerckMillipore UK. It was supplied as purified (by Immobilized Metal Affinity Chromatography) monoclonal antibody in PBS with 0.05 % sodium azide. PANC-1 cells were used instead of MIA PaCa-2 because they attach and spread better to plate surface. PANC-1 cells were seeded in duplicate to have about 60% confluency and allowed to attach for 24 h in a CoverWell™ Incubation Chamber Gasket (ThermoFisher cat #: c18156. Then cells were treated with CM03 and SOP1812 for 6 and 24 h at various concentrations (400 nM for CM03 and 100, 400 and 800 nM for SOP1812). The GI_{50} values for CM03 and SOP1812 are 15.6 nM and 1.4 nM, respectively, after 4 days (96 h) of treatment. 400 nM CM03 and 100 nM SOP1812 was used in visualization experiment because shorter treatment times were used, 6 h and 24 h.

The idea was to choose the lowest concentrations of CM03 and SOP1812 that can achieve minimal cell death (10%) after 6 and 24 h treatment, in order to detect the number of BG4 foci before cells advance to apoptosis. The chosen concentration for CM03 had been previously shown to cause statistically significant changes in numbers of BG4 foci. The concentration of SOP1812 was further increased (400 nM and 800 nM) to examine dose-dependent effects.

Cells were fixed with 4% paraformaldehyde and immunofluorescence stained for BG4 as per the manufacturer's recommended procedures and as previously described.[3] The concentration of BG4 antibody was adjusted in order to obtain optimal signal-to-noise images of the fluorescent foci, with a 1:2000 dilution in 0.5% goat serum + PBST (phosphate-buffered saline with Tween® detergent) providing the best images. Z-stack images were captured using the multiphoton SP8 confocal microscope (Leica Microsystems, UK) at UCL Confocal Imaging Facility. Images were captured with 63× 1.4 NA oil immersion objective. The fluorescent signals were sequentially acquired using the HyD detector, BG4: 500 nm – 570 nm. Z-stacks were performed at 0.3 μm intervals to depths of approximately 10 – 20 μm. Z-projections were processed using Leica software and images were saved in tif format. Deconvolusion of images was done using Huygens software (Scientific Volume Imaging). Numbers of BG4 foci were quantitated using the Imaris software package (https://imaris.oxinst.com/). Data represent mean ± SEM of three independent experiments, * P <0.05, obtained from Student's t-test in which treated was compared to untreated/control.

Pharmacokinetic and associated studies. *Using tumour-free mice.* A total of 12 female athymic nude mice aged 5-7 weeks, weighing approximately 28-35 gm were used for the study (animals were purchased from Charles River Inc). Mice were housed in IVC cages (up to 5 per cage) with individual animals identified by tail mark. The holding room was maintained under standard conditions: 20-24°C, 40-70% humidity and a 12 h light/dark cycle. Animals had access to food and water *ad libitum*.

Animals received a single dose of treatment. Individual dose was calculated from the bodyweight recorded on the day of dosing. At the time points 0.05, 0.25, 0.5, 1, 2, 4, 8 and 24 h post dose, whole blood samples were collected from the lateral tail vein into corning tubes containing EDTA (1:1 dilution). At 4 and 24 h post dose, whole blood was also collected and plasma prepared (1:1 dilution ultrapure water). Samples were stored at -80 °C before being transported for bioanalysis on dry ice.

Dosing solutions were freshly prepared as follows:

CM03: For 15 mg/kg dose – 2.5 mg compound was weighed and 833 µl of acidified PBS (pH 6, a few drops of 0.1 mM HCl) was added. The dosing matrix was a red solution at a final concentration of 3 mg/ml.

For 1 mg/kg dose - 50 µl of the 15mg/kg dosing solution was added to 700 µl of acidified PBS. The dosing matrix was a pale red solution at a final concentration of 0.2 mg/ml.

SOP1812: For 2 mg/kg dose – 1 mg compound was weighed and 2.5 ml of acidified PBS (pH 6, a few drops of 0.1 mM HCl was added). The dosing matrix was a red solution at a final concentration of 0.4 mg/ml.

For 1 mg/kg dose – 1.25 ml of the 2 mg/kg dosing solution was added to 1.25 ml of acidified PBS. The dosing matrix was a red solution at a final concentration of 0.2 mg/ml.

Whole blood (20 µl) was collected at each time point by lateral tail vein bleed and was placed into Corning tubes containing EDTA (1:1 dilution) to prevent clotting. Approximately 70 µl of whole blood was collected at the 4 h and 24 h time points. 20 µl was diluted 1:1 with EDTA as above, while the remaining blood was placed into a tube coated with K2-EDTA and centrifuged to obtain plasma. The plasma was diluted 1:1 with ultrapure water and stored at -80 °C prior to transportation for bioanalysis on dry ice.

Xenograft studies. *MIA PaCa-2 xenograft model.* All animal experiments in this section were performed at AXISBIO Discovery Services, Northern Ireland in accordance with the UK Home Office Animals Scientific Procedures Act 1986 and the United Kingdom Co-ordinating Committee on Cancer Research Guidelines for the Welfare and Use of Animals in Cancer Research[4] and with the approval of the AXISBIO Animal Ethics Committee. Mice had access to food and water *ad libitum*.

The maximum tolerated dose (MTD) studies of CM03 and SOP1812 were performed in female athymic nude mice. The MTD of a single dose administered intravenously (IV) was examined and repeat dosing was performed per week at different concentrations of CM03 (10, 20, 30, 35, 40, 45, 50 and 60 mg/kg) and SOP1812 (20, 25 and 30 mg/kg), and with two mice per group. Compounds CM03 and SOP1812 were administered in acidified PBS (pH 6; plus a few drops of 0.1 mM HCl if needed) and gemcitabine was administered in sterile saline solution. Mice were monitored visually for signs of adverse effects, over periods of up to 72 h. The MTD was judged to have been reached when all mice in a particular dose cohort survived in the absence of any observed ill-effects, for the period of the experiment.

For therapy studies, female athymic nude mice (2-3 months old, weighing 20−25 g) were injected subcutaneously with 10^7 MIA PaCa-2 cells in Matrigel in the right flank. When the tumours were established (approx. 13 days, mean size 0.05 cm^3), the mice were randomly assigned into six treatment groups with eight mice/group. All compounds were administered IV. Tumour size was measured 3-times weekly by calliper using the π-based ellipsoid volume formula (length × width × height × π/6), and the mice were also weighed at the same time. Animals were examined daily for any signs of distress of toxicity from the treatments.

Group 1: 15 mg/kg of gemcitabine (Sigma), twice weekly dose.

Group 2: 10 mg/kg of CM03, twice weekly dose.

Group 3: 15 mg/kg of CM03, twice weekly dose.

Group 4: 1.0 mg/kg of SOP1812, twice weekly dose.

Group 5: 1.0 mg/kg of SOP1812, once weekly dose.

Group 6: saline only, twice weekly dose.

The xenograft data were analysed using Student's t test (GraphPad Inc.). Responses were taken to be significant for those with probabilities (*P*) less than 5% ($P< 0.05$). Mice were culled if there was any sign of tumour ulceration, if tumour volume exceeded 1.5 cm^3, or if a weight loss over 20% of initial body weight was observed.

Tumour volumes and animal weights were taken twice weekly. Tumour dimensions were measured by digital caliper and data including individual and mean estimated tumour volumes (Mean TV ± SEM) recorded for each group; tumour volume was calculated using the formula: TV= $width^2$ x length x 0.52. Animals were observed daily for signs of toxicity.

Quantification of expression protein within tumour tissue was undertaken, using tissue taken from control and treated animals sacrificed at day 28 of the xenograft experiment. Protein was extracted from all tumour samples and used to quantify the expression of three proteins using Western blotting, MAPK11, hTERT and PARP. GAPDH was used as a house-keeping gene. Densitometric quantitation was performed to measure protein level, normalised to GAPDH and then statistical significance was analysed using Student's t test (GraphPad Inc.).

KPC mouse model. KPC (*Pdx1-Cre; LSL-Kras$^{G12D/+}$; LSL-Trp53$^{R172H/+}$*) mice have been described previously.[5] Mice on a mixed background were bred in-house at the Beatson Laboratories and maintained in conventional caging with environmental enrichment, access to standard chow and water *ad libitum*. Genotyping was performed by Transnetyx (Cordoba, TN, USA). Mice were monitored 3 times weekly and when a diagnosis of pancreatic cancer

was made by abdominal palpation, and confirmed by ultrasound imaging, mice of both sexes were recruited onto study and treated with either 1.0 mg/kg of SOP1812, PBS vehicle control IV, weekly or 100 mg/kg Gemcitabine I.P. twice weekly.. There were 6 mice per group. Mice were culled by Schedule 1 method, as per Institutional guidelines, when exhibiting moderate symptoms of PDAC (swollen abdomen, loss of body conditioning resembling cachexia, reduced mobility). Statistical assessment of survival from start of treatment was carried out by Kaplan-Meier and Log-Rank analysis. All animal experiments were performed under a UK Home Office licence and approved by the University of Glasgow Animal Welfare and Ethical Review Board.

RNA-seq studies. RNA-seq analyses of exposure to SOP1812 were undertaken with MIA PaCa-2 cells using procedures similar to those previously used.[6]

Cell culture. MIA PaCa-2 cells from ATCC (cat # CRL-1420) were maintained in DMEM supplemented with 10% FBS, 2.5% horse serum, 2 mM L-glutamine and antibiotics (streptomycin and penicillin) in a humidified incubator containing 5% CO_2 and at 37 °C as mentioned before in cell culture studies section. The cell line was confirmed mycoplasma-free by RT-qPCR method.

Determination of SOP1812 dosage for RNA-Seq. The MIA PaCa-2 cell line was seeded overnight at 2 x 10^3 cells/well in 96-well plates in triplicate. Following day, cells were treated with 0 - 1 μM SOP1812 (stock 1 mM in PBS) for 24 h. SRB assay was performed as described before to obtain dose-response curve. The concentration with the least cytotoxic effect (~10% cell death) was chosen for RNA-seq experiment.

RNA-seq. MIA PaCa-2 cells (2.5×10^6 cells for 6 h and 1.0×10^6 cells for 24 h) were seeded overnight in 100 mm plates. The following day, cells were treated with 40 nM SOP1812 for 6 and 24 h. Total RNA was extracted using Qiagen RNeasy Mini Kit (Qiagen, cat #: 74104) with on-column DNase1 digestion (Qiagen, cat #: 79254) following the manufacturer's instructions.

RNA quality (RIN > 7.0) was checked with an Aligent 2100 Bioanalyser RNA 6000 Nano Chip and RNA concentration was quantified using a Qubit® fluorometer (ThermoFisher) and Qubit® RNA HS Assay Kit (ThermoFisher, cat #: Q32852). RNA-seq libraries were then generated using the KAPA mRNA Library HyperPrep Kit for Illumina® following the manufacturer's instructions and sequenced using an Illumina NextSeq 500 instrument (undertaken at the UCL Genomics Facility).

RNA-Seq Analysis. The sequencing data are available in the GEO public functional genomics data repository (https://www.ncbi.nlm.nih.gov/geo/), as GSE151741. RNA-seq data for the effect of CM03 and gemcitabine compounds on MIA PaCa-2 cells was re-used from our previous study for comparison purposes during the analyses (GEO accession GSE105083).[6]

The run data were demultiplexed and converted to FASTQ files using Illumina's bcl2fastq Conversion Software (v2.19). The adapter contamination and poor quality sequences were removed from FASTQ files using the program Trimmomatic (v0.36).[7] Then, FASTQ files were mapped to the human reference genome GRCh38 using the RNA-seq aligner STAR (v2.5b: https://github.com/alexdobin/STAR). The JE-Suite[8] was used to estimate duplication levels, a Unique Molecule Identifier program to deduplicates, and then marking the reads that are the result of PCR amplification. Then, reads per transcript were counted using the program FeatureCounts[9] (v1.4.6p5) followed by normalisation, modelling and differential expression analysis using the SARTools (v1.3.2) package.[10]

Differentially expressed genes (DEGs) for drug treatment versus untreated were split into 4 subsets with certain $\log_2$ fold change ($\log_2$FC) and false discovery rate (FDR) with the following assignment: Down = DEGs with $\log_2$FC < -0.5 and FDR < 0.1; Down Strong = DEGs with $\log_2$FC ≤ -1 and FDR < 0.05; Up = DEGs with $\log_2$FC > 0.5 and FDR < 0.1; Strong Up = DEGs with $\log_2$FC ≥ 1 and FDR < 0.05. The KEGG signalling pathway enrichment analysis was performed using the DAVID functional annotation tool

(https://david.ncifcrf.gov/).[11] The Pathview maps tool (https://pathview.uncc.edu/)[12] was used to visualise enriched signalling pathways and show the up-regulated and down-regulated genes in the pathway.

For the numbers of putative G4 sequences in an individual gene (PQs in Table 6), the occurrence of the canonical G4 motif ($G_{\geq 3}N_{1\text{-}7}G_{\geq 3}N_{1\text{-}7}G_{\geq 3}N_{1\text{-}7}G_{\geq 3}$), was used as previously reported,[6] in gene promoters (defined for this purpose as being up to 2 kilobases upstream of the transcription start site (TSS) and 100 bases downstream) and in exons and introns.

References

1. Vo, T.; Oxenford, S.; Angell, R.; Marchetti, C.; Ohnmacht, S. A.; Wilson, W. D.; Neidle, S. Substituted naphthalenediimide compounds bind selectively to two human quadruplex structures with parallel topology. *ACS Med. Chem. Lett.* **2020**, *11*, 991-999.
2. Moore, M. J.; Schultes, C. M.; Cuesta, J.; Cuenca, F.; Gunaratnam, M.; Tanious, F. A.; Wilson, W. D.; Neidle, S. Trisubstituted acridines as G-quadruplex telomere targeting agents. Effects of extensions of the 3,6- and 9-side chains on quadruplex binding, telomerase activity, and cell proliferation. *J. Med. Chem.* **2006,** *49*, 582-599.
3. Biffi, G.; Tannahill, D.; McCafferty, J.; Balasubramanian, S. Quantitative visualization of DNA G-quadruplex structures in human cells. *Nat. Chem.* **2013,** *5*, 182-186.
4. Workman, P.; Aboagye, E. O.; Balkwill, F.; Balmain, A.; Bruder, G.; Chaplin, D. J.; Double, J. A.; Everitt, J.; Farningham, D. A.; Glennie, M. J.; Kelland, L. R.; Robinson, V.; Stratford, I. J.; Tozer, G. M.; Watson, S.; Wedge, S. R.; Eccles, S. A. Guidelines for the welfare and use of animals in cancer research. *Br. J. Cancer* **2010,** *102*, 1555-1577.
5. Hingorani, S. R.; Wang, L.; Multani, A. S.; Combs, C.; Deramaudt, T. B.; Hruban, R. H.; Rustgi, A. K.; Chang, S.; Tuveson, D. A. Trp53R172H and KrasG12D cooperate to

promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* **2005,** *7*, 469-483.

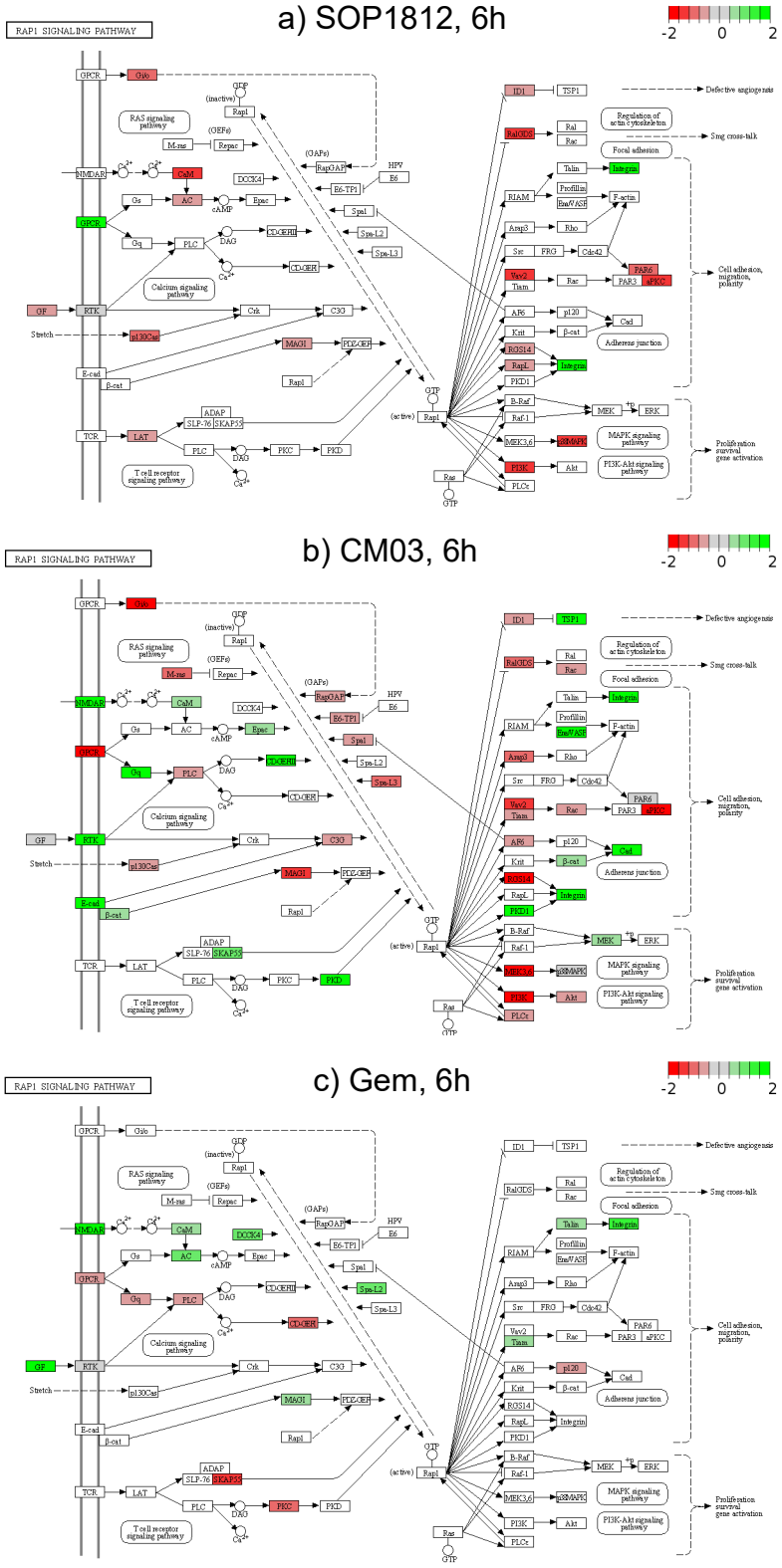
6. Marchetti, C.; Zyner, K. G.; Ohnmacht, S. A.; Robson, M.; Haider, S. M.; Morton, J. P.; Marsico, G.; Vo, T.; Laughlin-Toth, S.; Ahmed, A. A.; Di Vita, G.; Pazitna, I.; Gunaratnam, M.; Besser, R. J.; Andrade, A. C. G.; Diocou, S.; Pike, J. A.; Tannahill, D.; Pedley, R. B.; Evans, T. R. J.; Wilson, W. D.; Balasubramanian, S.; Neidle, S. Targeting multiple effector pathways in pancreatic ductal adenocarcinoma with a G-quadruplex-binding small molecule. *J. Med. Chem.* **2018,** *61*, 2500-2517.
7. Bolger, A. M.; Lohse, M.; Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114-2120.
8. Girardot, C.; Scholtalbers, J.; Sauer, S.; Su, S. Y.; Furlong, E. E. Je, a versatile suite to handle multiplexed NGS libraries with unique molecular identifiers. *BMC Bioinformatics* **2016**, *17*, 419.
9. Liao, Y.; Smyth, G. K.; Shi, W. FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **2014**, *30*, 923-930.
10. Varet, H.; Brillet-Guéguen, L.; Coppée, J.-Y.; Dillies, M. A. SARTools: A DESeq2- and EdgeR-Based R Pipeline for Comprehensive Differential Analysis of RNA-Seq Data. *PLoS One* **2016**, *11*, e0157022.
11. Huang, D. W.; Sherman, B. T.; Lempicki, R. A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. **2009**, *37*, 1-13.
12. Luo, W.; Pant, G.; Bhavnasi, Y. K.; Blanchard, S. G.; Brouwer, C. Pathview Web: user friendly pathway visualization and data integration. *Nucleic acids Res.* **2017**, *45*, W501-W508.

Figure S1. **Supplementary Figures:**

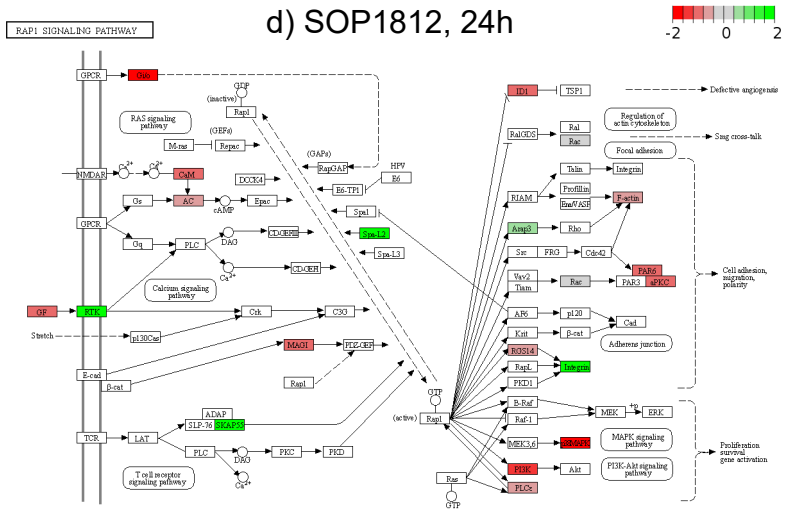
Significantly enriched KEGG pathways after 6 h or 24 h treatment of SOP1812, CM03 and gemcitabine.

MIA PaCa-2 cells were treated for 6 h and 24 h with SOP1812, CM03 and gemcitabine and then used in RNA-seq experiment. KEGG pathway enrichment analysis was performed using the complete down-regulated gene set ($Log_2FC < -0.5$ and FDR <0.1). The pathway views display significant DEGs: red = down-regulated genes ($Log_2FC < -0.5$ and FDR <0.1) and green = up-regulated genes ($Log_2FC > 0.5$ and FDR <0.1).

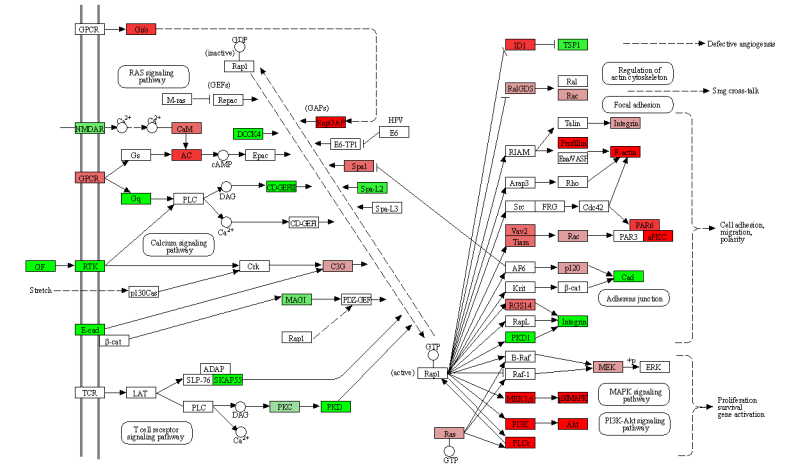
Figure S1(a) Enriched Rap1 signalling pathway



d) SOP1812, 24h



e) CM03, 24h



f) Gem, 24h

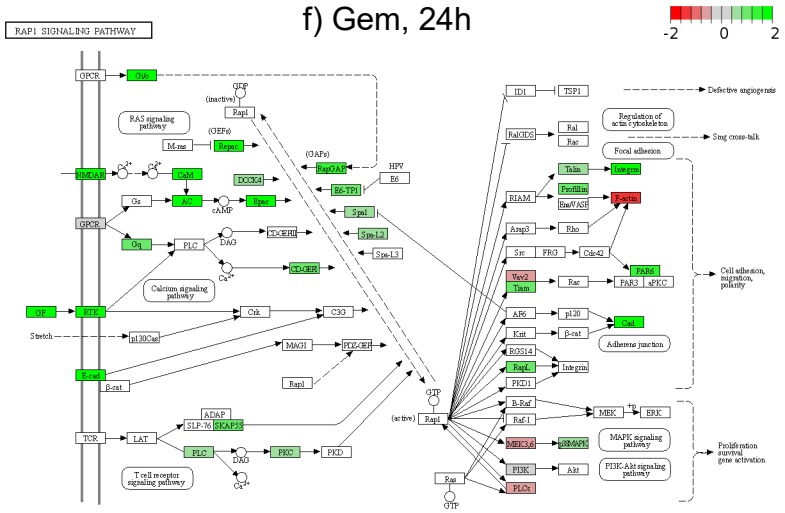


Figure S1(b) Hippo pathway

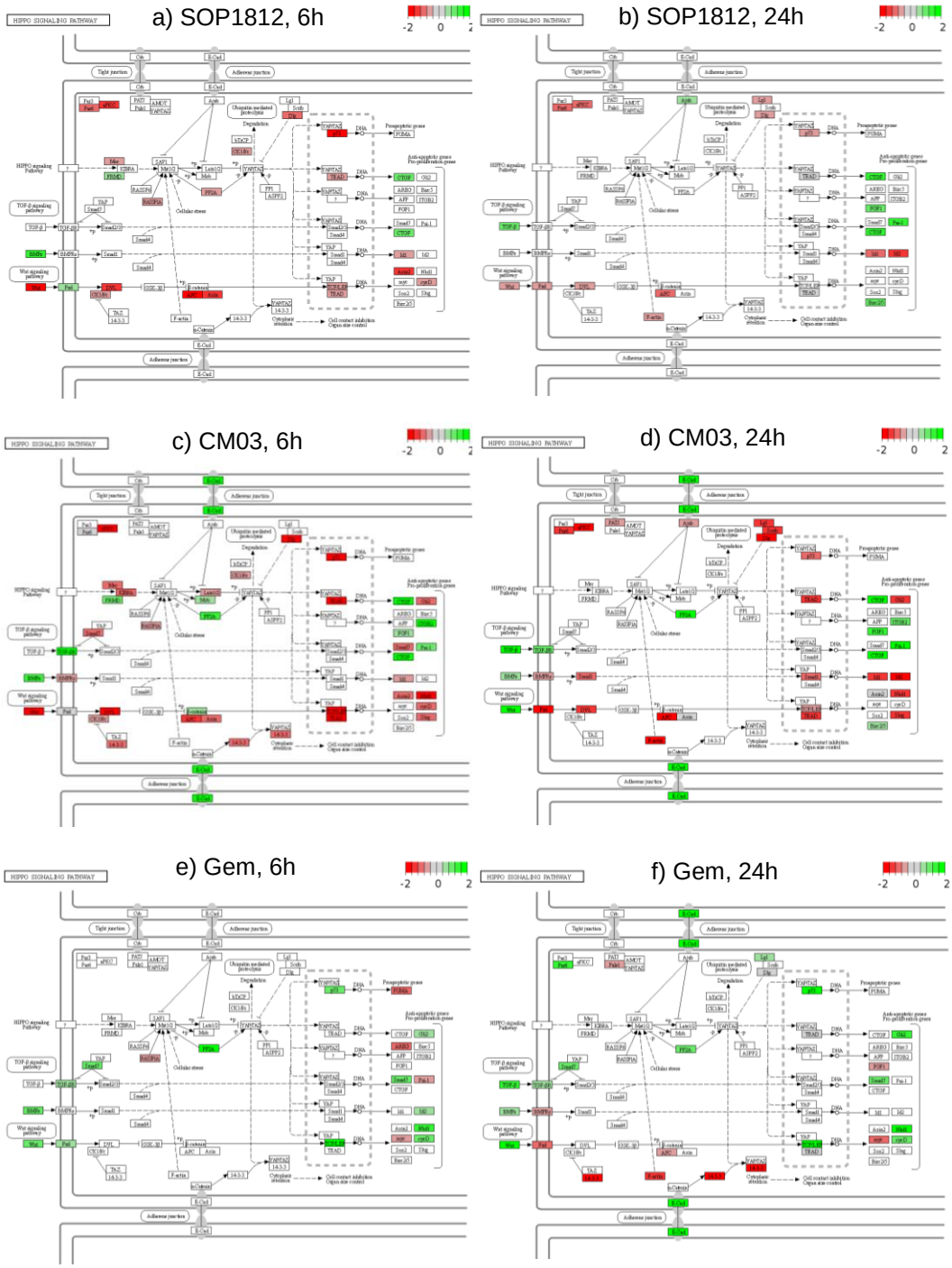
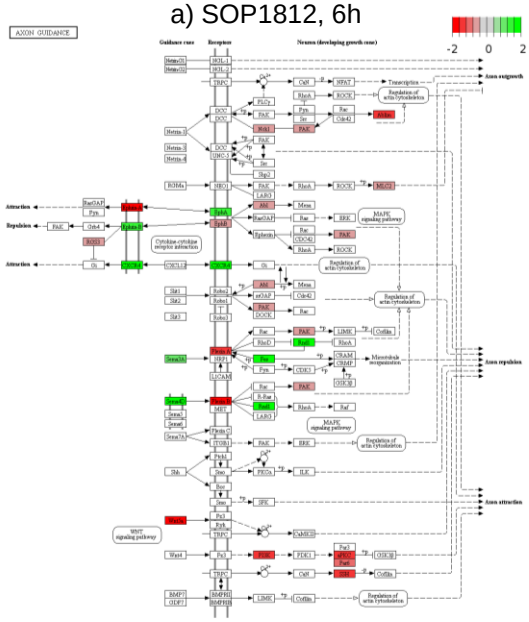
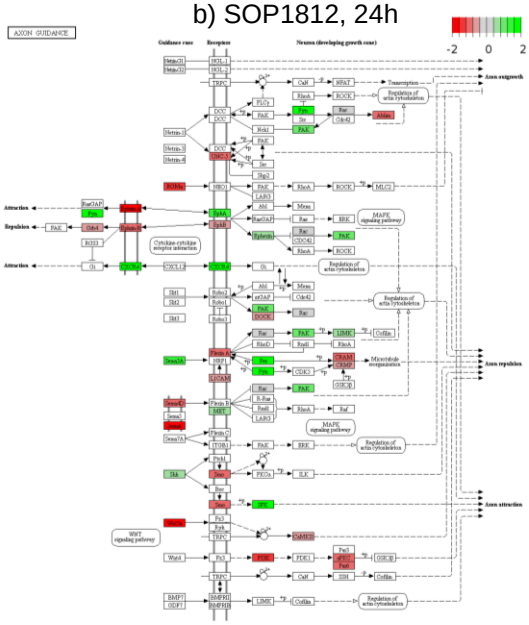


Figure 1© Axon guidance pathway

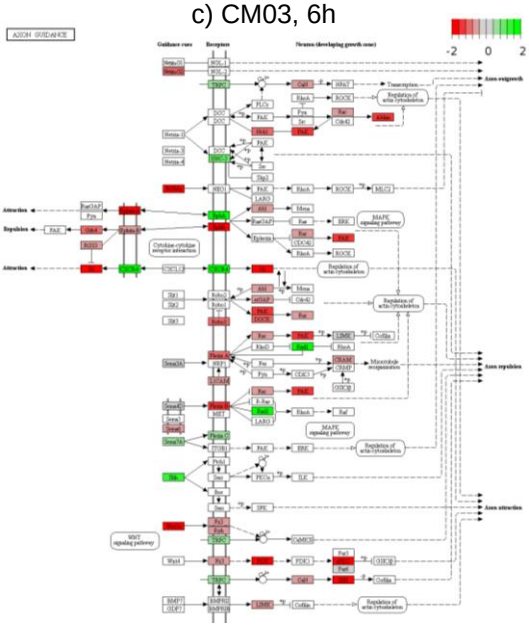
a) SOP1812, 6h



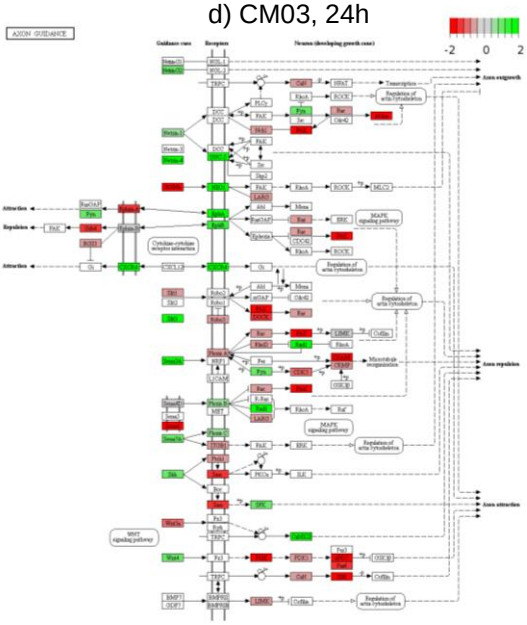
b) SOP1812, 24h



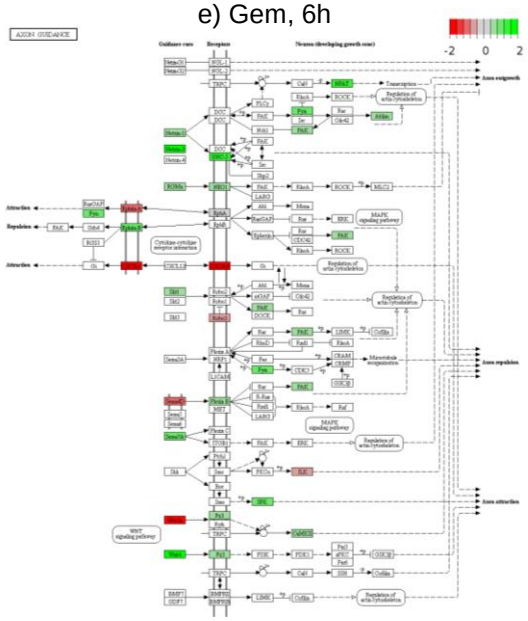
c) CM03, 6h



d) CM03, 24h



e) Gem, 6h



f) Gem, 24h

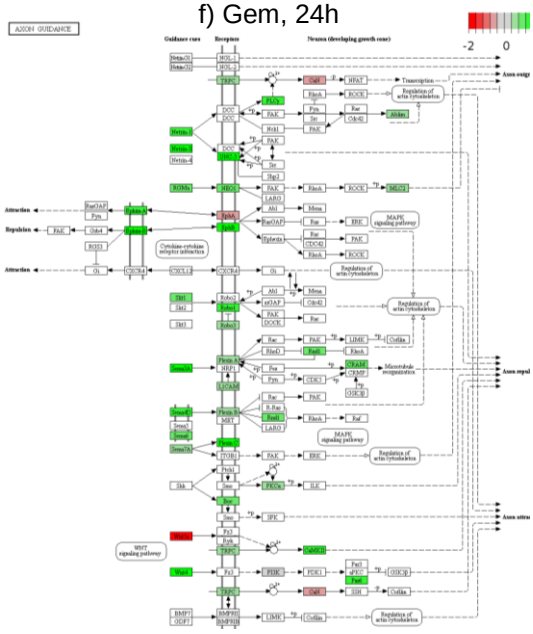


Figure S1(d) MAPK pathway