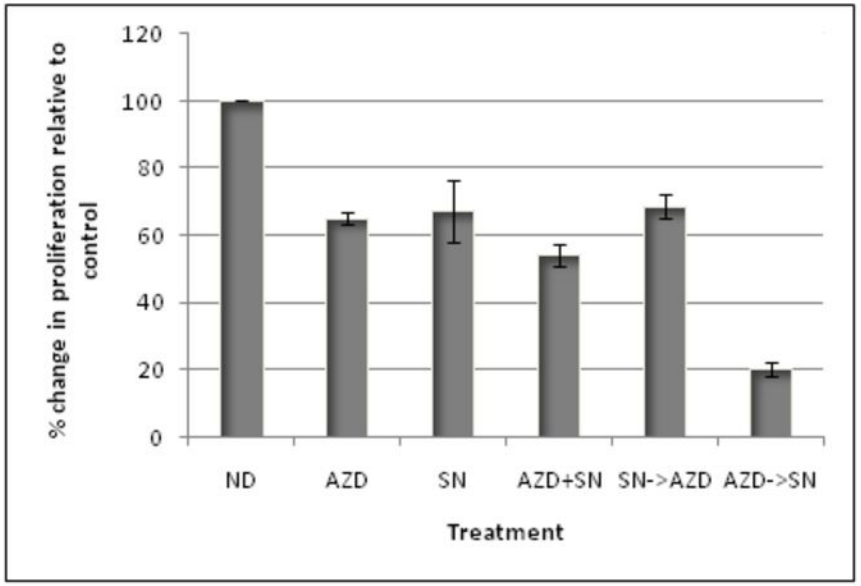
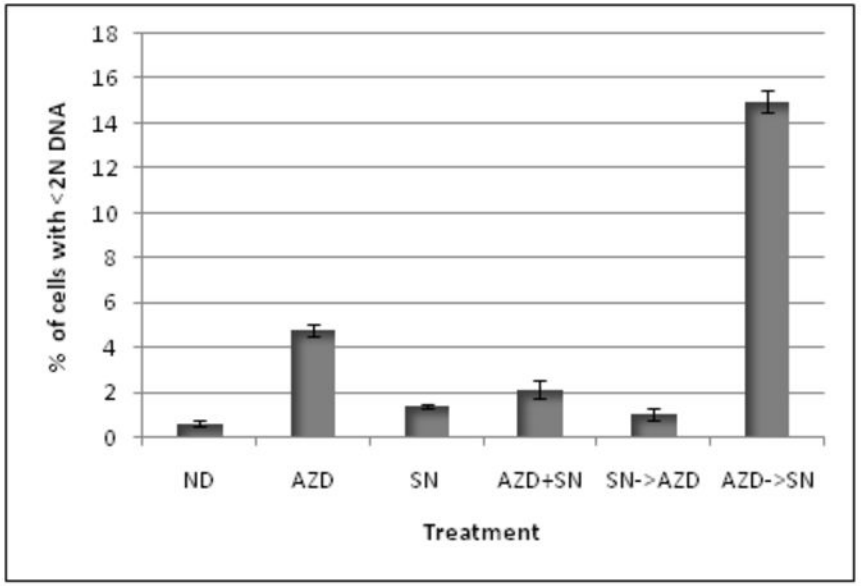
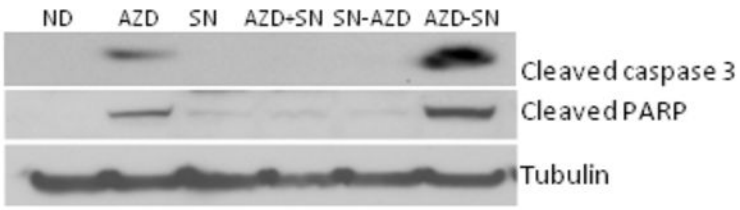
A.


% change in proliferation relative to control
120
100
80
60
40
20
0
ND
AZD
SN
AZD+SN
SN->AZD
AZD->SN
Treatment

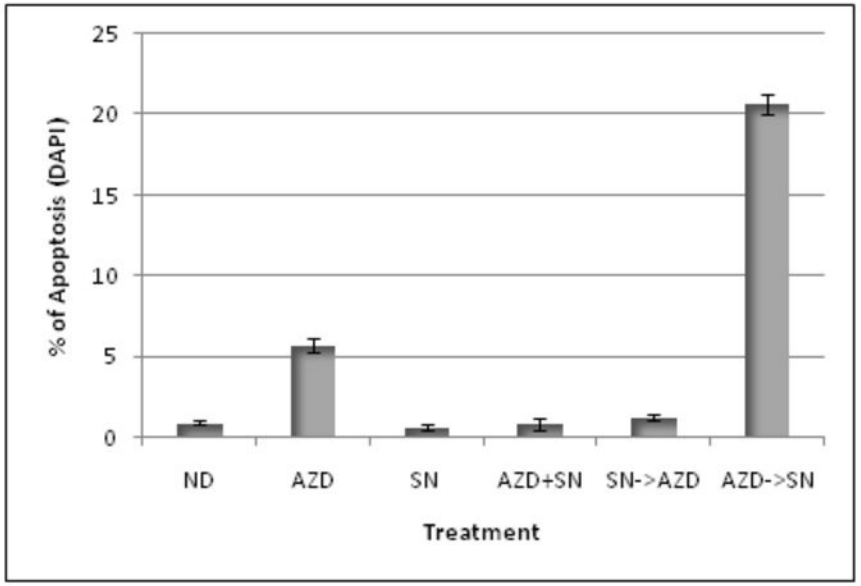
B.


% of cells with <2N DNA
18
16
14
12
10
8
6
4
2
0
ND
AZD
SN
AZD+SN
SN->AZD
AZD->SN
Treatment

C.


ND
AZD
SN
AZD+SN
SN-AZD
AZD-SN
Cleaved caspase 3
Cleaved PARP
Tubulin

D.


% of Apoptosis (DAPI)
25
20
15
10
5
0
ND
AZD
SN
AZD+SN
SN->AZD
AZD->SN
Treatment

Supplement Figure legend:

SN-38 (SN) antagonizes or enhances the effect of AZD1152-HQPA (AZD) in a sequence specific manner in Colo-205 cells. A. Colo-205 cells were treated with SN (20 nM), AZD (50 nM) or in combination and viable cell mass was measured using an assay which measures activity of dehydrogenases in the cells, showing greatest % of cell growth inhibition in AZD->SN combination compared to other conditions tested (experiments were performed in sextuplicate and the graph given is the average of three independent experiments and error bars represent 1 standard deviation). Inhibition of cell growth was significantly greater for AZD->SN than AZD, SN or SN->AZD ($p>0.0002$, 0.015, 0.0004) and for AZD+SN ($p=0.02$). B-D. AZD->SN induces apoptosis in Colo-205 cells. Colo-205 cells were treated with SN-38 or AZD1152-HQPA alone or in combination and % of apoptotic cells were determined by: flow cytometric analysis (<2N DNA) after staining with PI (B), western blot analysis for cleavage of PARP and caspase 3 of Colo-205 cell lysates (C), and quantitative fluorescent microscopy (QFM) with DAPI staining (D) after treatment with AZD and SN combinations. α-tubulin is used as a loading control. When AZD1152-HQPA was given prior to SN-38 (AZD->SN), there was an increase in the sub G1 (<2N) population, (5±1% with AZD alone to 15±1% with AZD->SN). Western blot analysis also showed an increase in PARP cleavage and caspase 3 cleavage with AZD->SN, an effect not observed with the other combinations. Examination of the DAPI stained nuclear morphology showed 6±1% with AZD alone to 20±1% with AZD->SN (p value 0.02 for AZD vs AZD->SN, 0.04 for AZD+SN vs AZD->SN and 0.01 for SN->AZD vs AZD->SN). Experiments were repeated at least 3 times and error bars represent 1 standard deviation. Western blot shown is representative of three independent experiments.