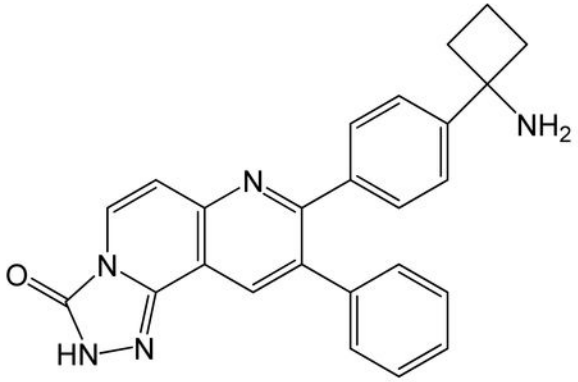
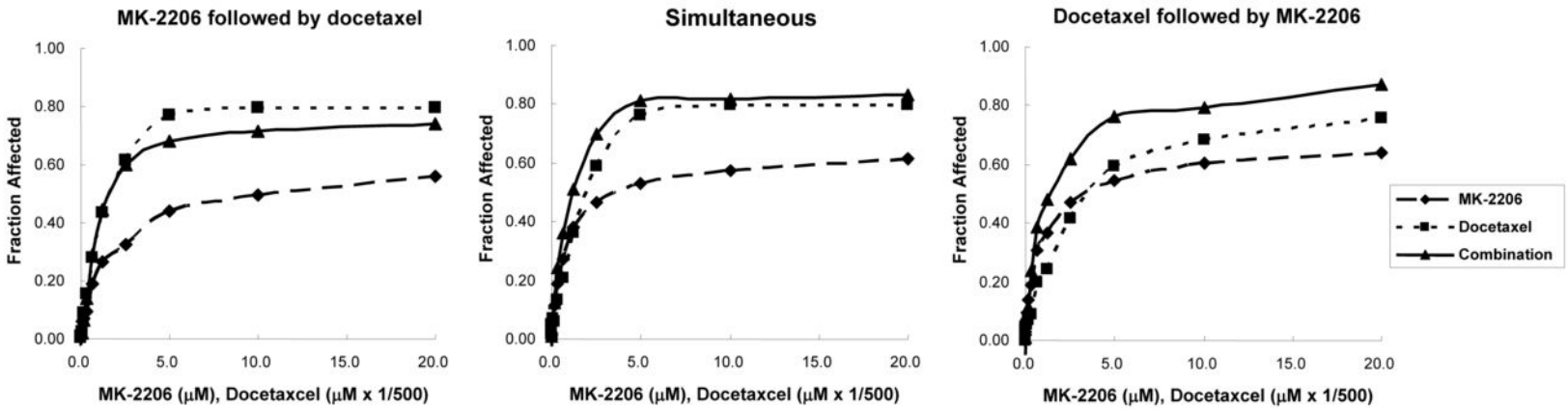
Hirai et al, Supplementary Figure 1



Chemical structure of MK-2206.

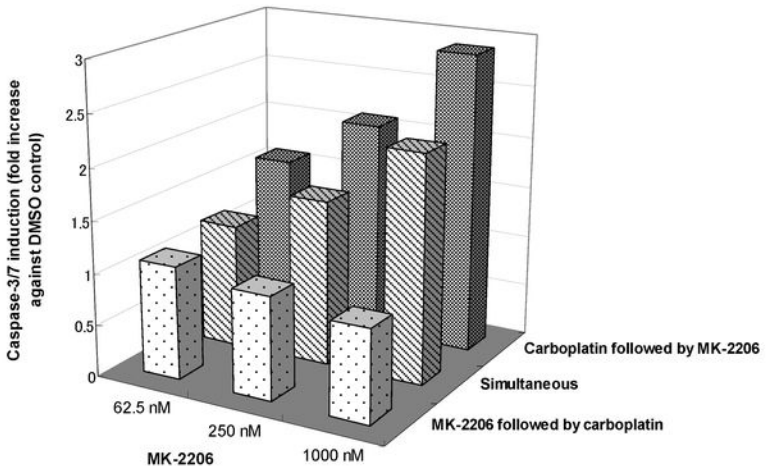
Hirai et al, Supplementary Figure 2



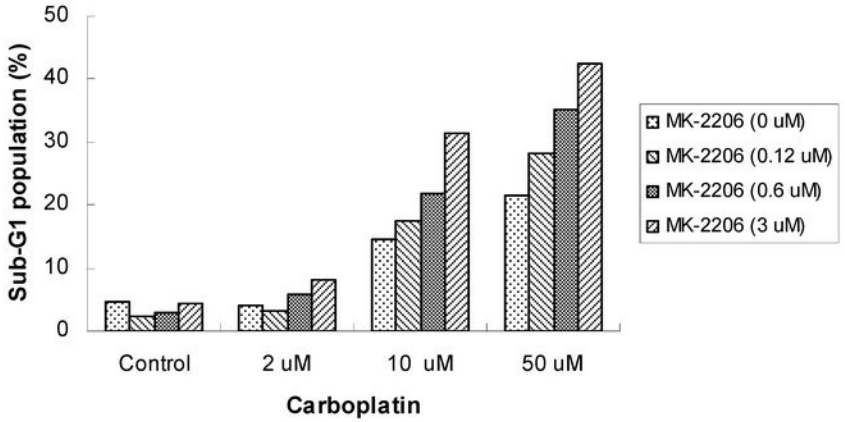
Sequence-dependent synergistic cell growth inhibition by MK-2206 and docetaxel in BT-474 cells.
Cells were treated with MK-2206 (0.01-20 μM) alone, docetaxel (0.02-40 nM) alone, or a combination of both agents with three different treatment schedules (left panel, MK-2206 first then docetaxel; middle, simultaneous; and right, docetaxel first then MK-2206). Cell viability was determined by measuring cellular ATP concentration with CellTiter-Glo reagents. Treatment combinations and sequences are described in Materials and Methods. Results are the median of two independent experiments.

Hirai et al, Supplementary Figure 3

(A)



(B)



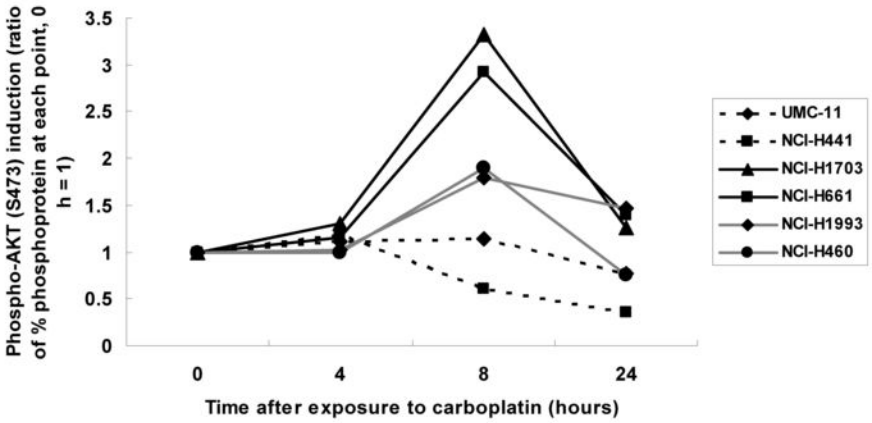
MK-2206 enhanced cell death in combination with carboplatin.

(A) Caspase-3/7 induction assay. A2780 cells were treated with MK-2206 (0.0625, 0.25, and 1 μM) in combination with 8 μM of carboplatin. The induction of caspase-3/7 was measured for 3 different treatment schedules: MK-2206 alone for 24 hours followed by carboplatin for 24 hours, both MK-2206 and carboplatin simultaneously for 48 hours, and 24 hours with carboplatin alone followed by MK-2206 for 24 hours. Results are shown as fold caspase-3/7 induction against vehicle (DMSO)-treated control.

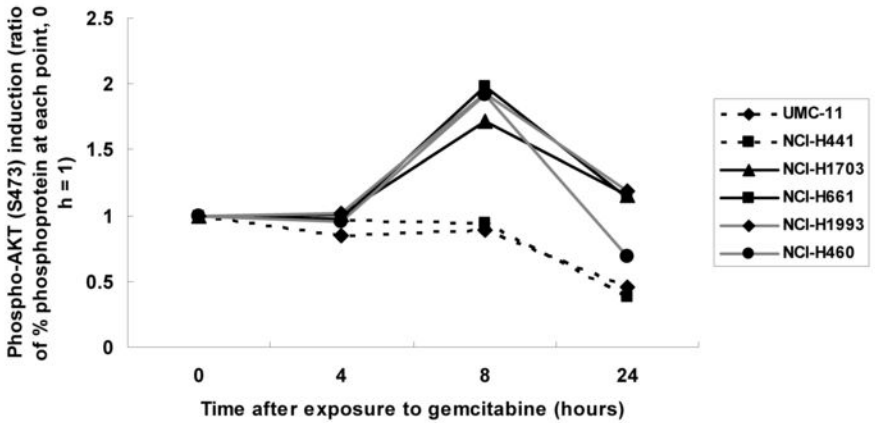
(B) FACS subG1 assay. A2780 cells were simultaneously treated with MK-2206 (0.12, 0.6, and 3 μM) in combination with carboplatin (2, 10, and 50 μM) for 72 hours. The cells were harvested, fixed and stained with propidium iodide. The proportion of cells with a fractional DNA content (sub-G1 phase) was determined by flow cytometry. Data are means from two separate experiments.

Hirai et al, Supplementary Figure 4

(A)



(B)



Induction of Akt phosphorylation by carboplatin or gemcitabine in six NSCLC cell lines.
Cells were treated with carboplatin (A) or gemcitabine (B) at the concentration needed to yield 50% growth inhibition (IC50) of each cell line for the indicated times (0, 4, 8, and 24 hours). Cell lysates were subjected to a quantitative pAkt/total Akt assay (MSD). The percentage of phosphoprotein was calculated according to the manufacturer's instruction, and the induction ratio of Akt phosphoprotein at each time point was determined by dividing with the amount of phosphoprotein at 0 hours.

Hirai et al, Supplementary Table 1

Title; Combination index and caspase-3/7 induction in combination of MK-2206 with carboplatin or gemcitabine in six NSCLC cell lines

Cell line	Combination index	Caspase-3/7
	Minimal value between ED_{25} and ED_{90}	Maximum fold induction at tested concentration
(A) Carboplatin		
NCI-H460	0.30	1.4
NCI-H1993	0.44	1.2
NCI-H661	0.37	2.0
NCI-H1703	0.53	1.3
NCI-H441	0.46	2.3
UMC-11	1.36	1.1
(B) Gemcitabine		
NCI-H460	0.33	1.6
NCI-H1993	0.14	1.2
NCI-H661	0.53	1.3
NCI-H1703	0.43	1.5
NCI-H441	0.37	2.3
UMC-11	0.53	1.2

NOTE: Cells were simultaneously treated for 72 hours with MK-2206 and carboplatin or gemcitabine at constant concentration ratios spanning the IC50 dose of each agent. The CI values were determined by the same procedure described in Table 1, and the minimal CI value at the affected fractions between ED25 and ED90 is shown. Caspase-3/7 induction was determined after exposure for 24 hours or 48 hours with MK-2206 and chemotherapeutic agents, and the maximum rate of fold induction is shown.