Electronic Supplementary Material (ESI) for RSC Advances.

Cisplatin Combination Drugs Induce Autophagy on HeLa Cells and Interact with HSA via Electrostatic Binding Affinity†

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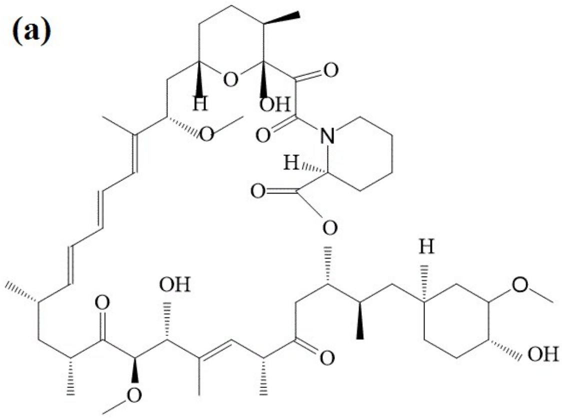
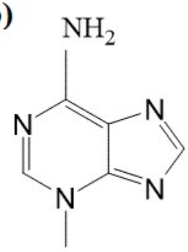
(a)



(b)



(c)

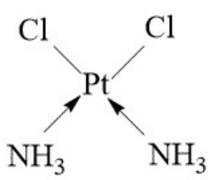


Fig. S1 The structure of (a) RAPA, (b) 3-MA and (c) cisplatin.

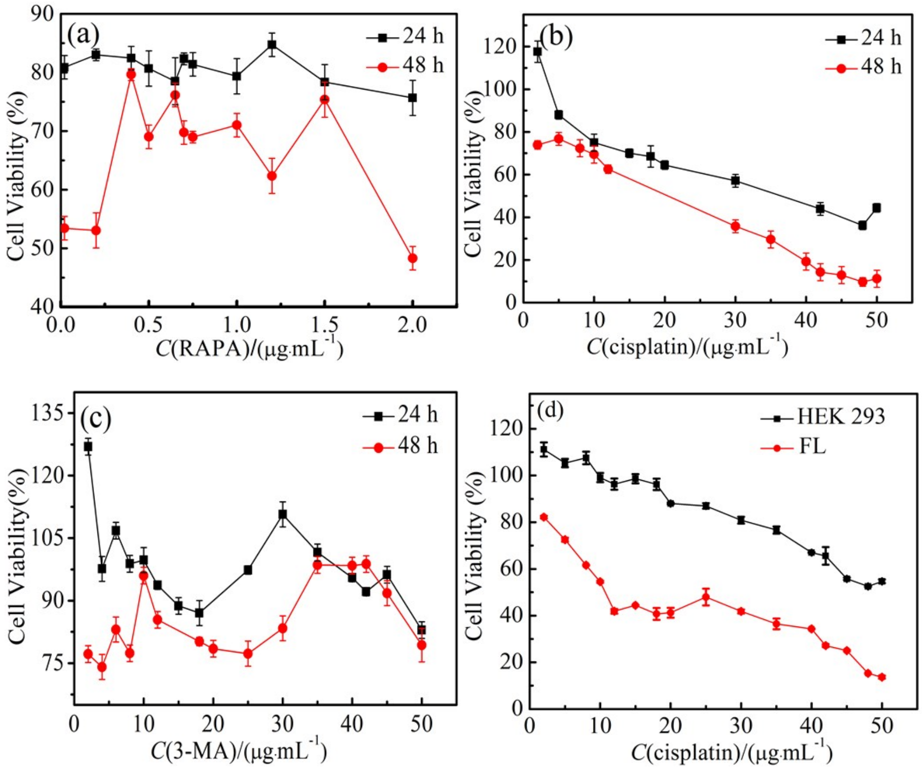


Fig. S2 Cell viability of HeLa cells exposed to drugs with different concentrations (C) for 24 h or 48 h. (a) RAPA; (b) cisplatin; (c) 3-MA. Cell viability of HEK 293 cells and FL cells exposed to (d) cisplatin for 24 h. Data are presented as mean ± SD of five independent results.

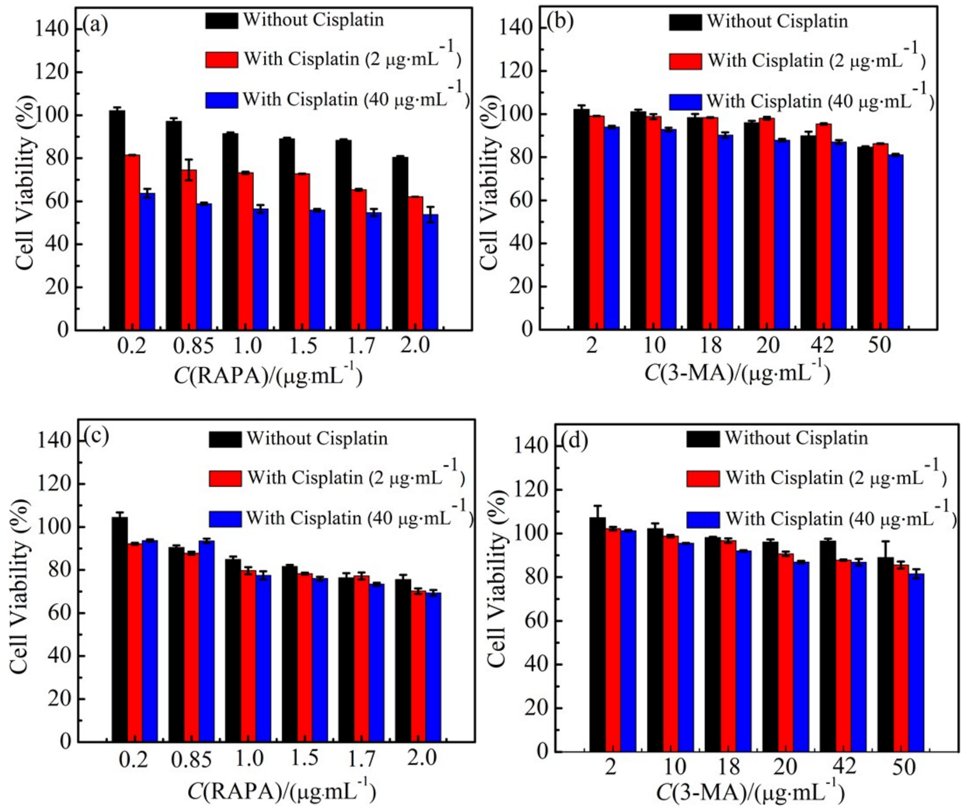


Fig. S3 Cell viability of (a, b) HEK 293 cells and (c, d) FL cells exposed to cisplatin combination drugs for 24 h. (a, c) RAPA combination drugs, cisplatin wt% ranging from 90.00 to 50.00 (cisplatin 2.0 μg·mL^{-1}+ RAPA ranging from 0.2 μg·mL^{-1} to 2.0 μg·mL^{-1}); (b, d) 3-MA combination drugs, cisplatin wt% ranging from 50.00 to 3.846 (cisplatin 2.0 μg·mL^{-1}+ 3-MA ranging from 2.0 μg·mL^{-1} to 50 μg·mL^{-1}).

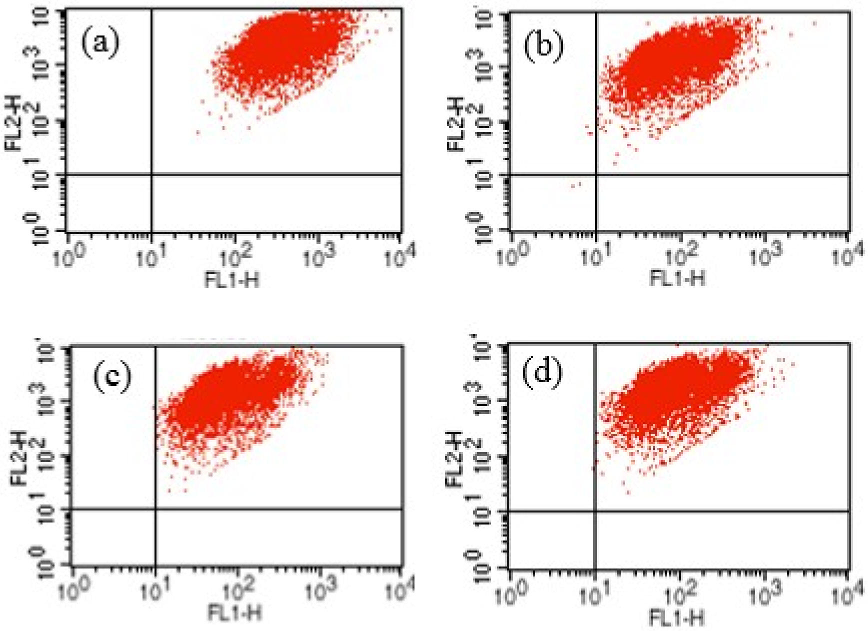


Fig. S4 The fluorescence lights of green and red on HeLa cells treated with RAPA. (a) control; (b) 0.02 μg·mL^{-1}; (c) 0.2 μg·mL^{-1}; (d) 1.0 μg·mL^{-1}.

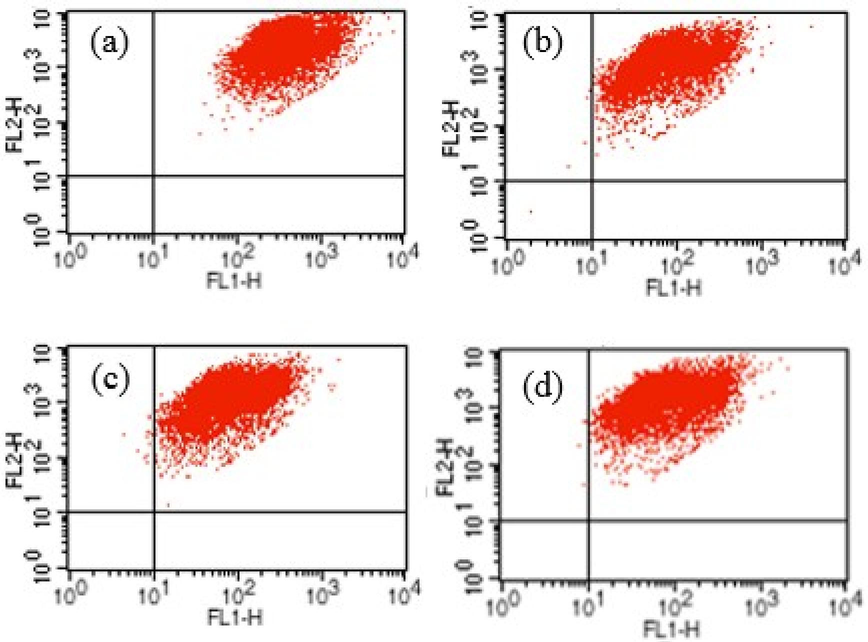


Fig. S5 The fluorescence lights of green and red on HeLa cells treated with cisplatin (2 μg·mL^{-1}) combined with RAPA .(a) control; (b) 0.02 μg·mL^{-1}; (c) 0.2 μg·mL^{-1}; (d) 1.0 μg·mL^{-1}.

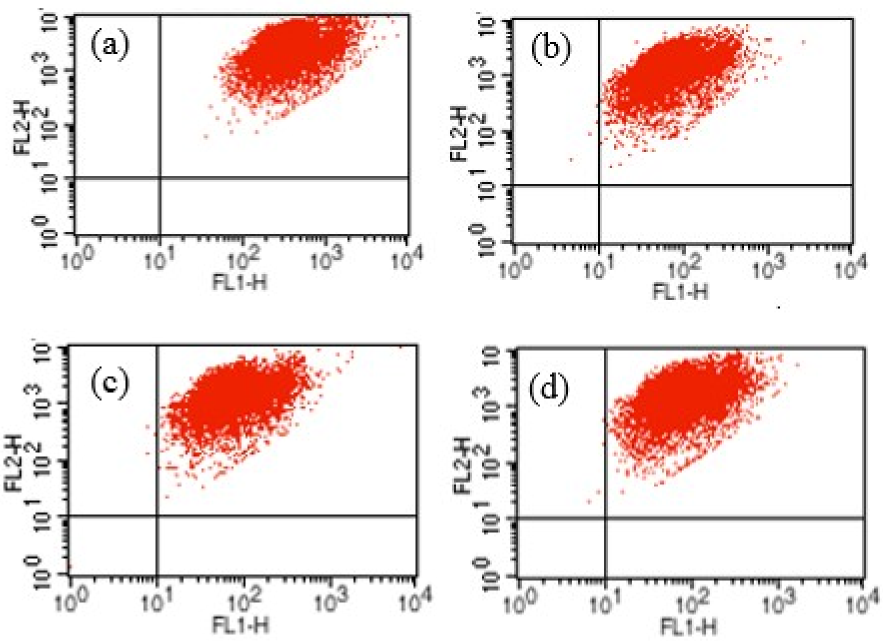


Fig. S6 The fluorescence lights of green and red on HeLa cells treated with 3-MA. (a) control; (b) 0.2 μg·mL^{-1}; (c) 2.0 μg·mL^{-1}; (d) 20 μg·mL^{-1}.

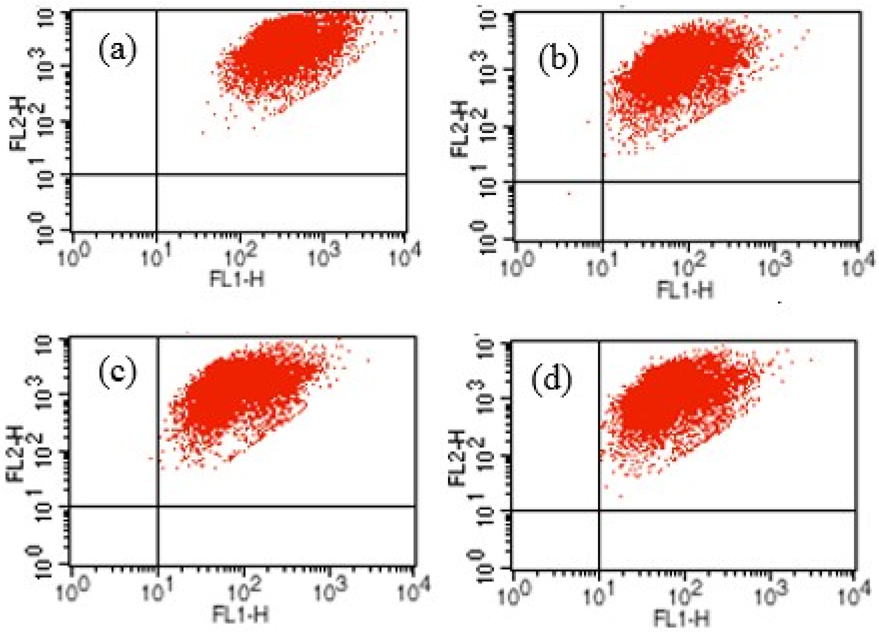


Fig. S7 The fluorescence lights of green and red on HeLa cells treated with cisplatin (2 μg·mL^{-1}) combined with 3-MA. (a) control; (b) 0.2 μg·mL^{-1}; (c) 2.0 μg·mL^{-1}; (d) 20 μg·mL^{-1}.

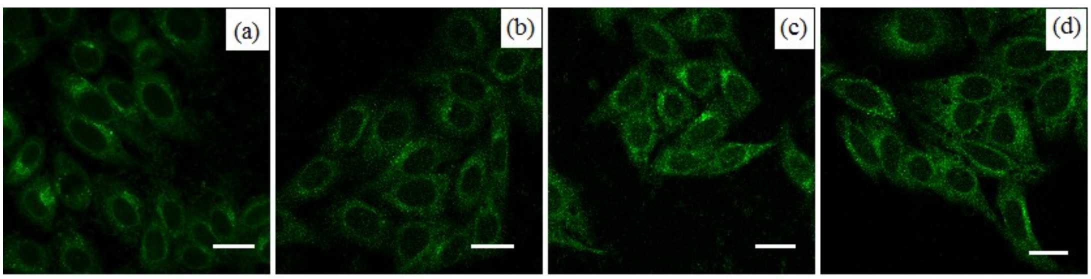


Fig. S8 Autophagic vesicles in HeLa cells exposed to RAPA from MDC staining assay observed under confocal microscopy (sacle bars, 20 μm). (a) control; (b) 0.02 μg·mL^{-1}; (c) 0.2 μg·mL^{-1}; (d) 1.0 μg·mL^{-1}.

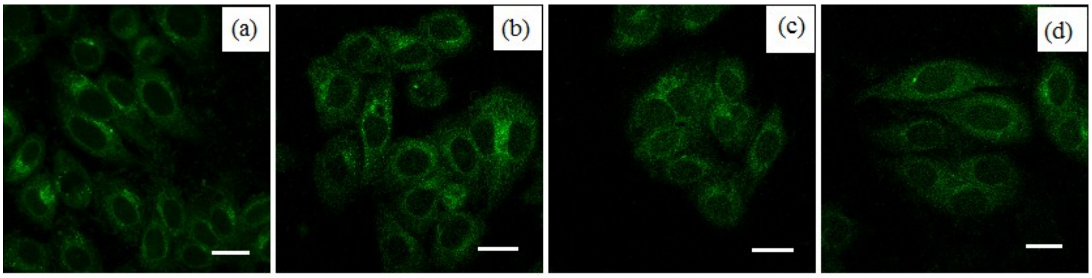


Fig. S9 Autophagic vesicles in HeLa cells exposed to 3-MA from MDC staining assay observed under confocal microscopy (sacle bars, 20 μm). (a) control; (b) 0.2 μg·mL^{-1}; (c) 2.0 μg·mL^{-1}; (d) 20 μg·mL^{-1}.

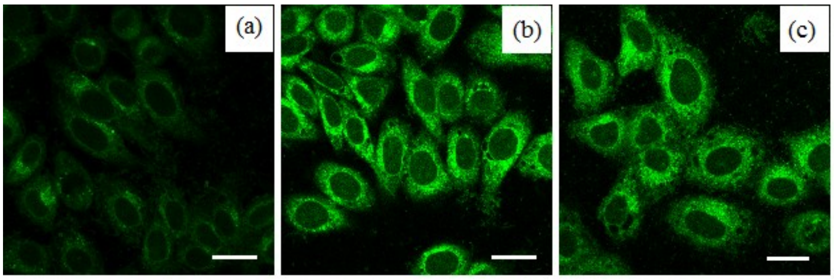


Fig. S10 Autophagic vesicles in HeLa cells exposed to cisplatin from MDC staining assay observed under confocal microscopy (sacle bars, 20 μm). (a) control; (b) 2 μg·mL^{-1}; (c) 10 μg·mL^{-1}.

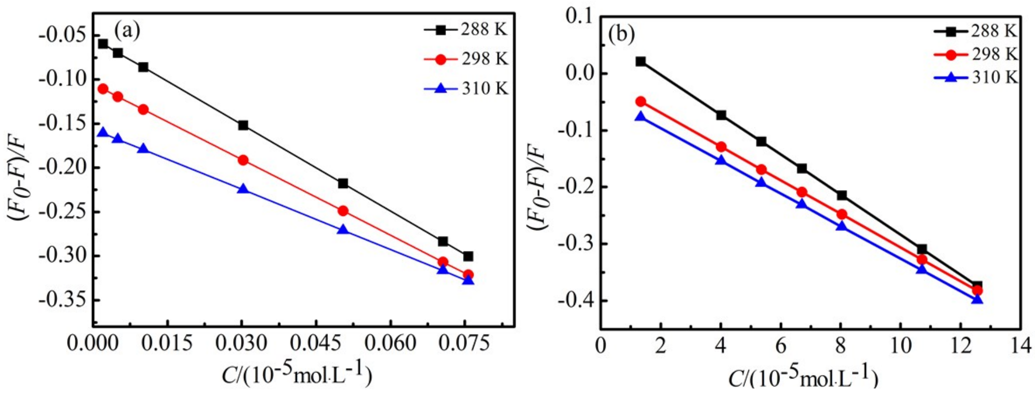


Fig. S11 Stern-Volme curves of the binding of cisplatin combination drugs to HSA, (a) RAPA combination drugs, (b) 3-MA combination drugs.

Table S1. CI values for HeLa cell lines treated with ciaplatin combination drugs.

Cisplatin (μg·mL^{-1})	3-MA (μg·mL^{-1})	CI	Cisplatin (μg·mL^{-1})	RAPA (μg·mL^{-1})	CI
2.00	2.00	2.09	2.00	0.20	0.18

2.00	10.00	3.52	2.00	0.85	0.16
2.00	18.00	3.51	2.00	1.00	0.12
2.00	20.00	2.57	2.00	1.50	0.11
2.00	42.00	1.75	2.00	1.70	0.10
2.00	50.00	1.19	2.00	2.00	0.01
40.00	2.00	5.68	40.00	0.20	3.10
40.00	10.00	4.29	40.00	0.85	2.65
40.00	18.00	3.24	40.00	1.00	1.34
40.00	20.00	2.51	40.00	1.50	1.32
40.00	42.00	4.32	40.00	1.70	1.24
40.00	50.00	2.97	40.00	2.00	1.12

Table S2 Stern-Volmer quenching constants of cisplatin combination drugs binding to HSA and the free-energy change (ΔG^o), enthalpy change (ΔH^o) and the entropy change (ΔS^o)

Drugs	$K_q\times10^{12}$ ($M^{-1}s^{-1}$)	$K_{sv}\times10^3$ (M^{-1})	T (K)	ΔH^o ($kJ\cdot mol^{-1}$)	ΔG^o ($kJ\cdot mol^{-1}$)	ΔS^o ($J\cdot mol^{-1}\cdot K^{-1}$)
3-MA+Cisplatin			288		-20.23	
	0.30	2.97	298	-13.71	-20.50	22.61
			310		-20.72	
RAPA+Cisplatin			288		-31.25	
	28.59	285.88	298	-10.06	-31.13	73.56
			310		-32.86	

Table S3 α-helical content of HSA determined from CD at 298 K in the presence of cisplatin (2 $\mu g\cdot mL^{-1}$) combination drugs.

RAPA + Cisplatin		3-MA + Cisplatin	
RAPA Concentration ($\mu g \cdot mL^{-1}$)	α-Helix (%)	3-MA Concentration ($\mu g \cdot mL^{-1}$)	α-Helix (%)
0.02	96.09	2.00	81.11
0.05	87.19	6.00	64.34
0.10	86.72	8.00	64.15
0.30	77.64	10.00	61.09
0.50	76.54	12.00	48.64
0.70	65.76	16.00	45.07
0.75	58.27	18.75	36.47

Table S4 A table for literatures about combination drugs.

Combination Drugs		Title	Year	Web Site
Component A	Component B			
cisplatin	rapamycin	Co-delivery of Cisplatin and Rapamycin for Enhanced Anticancer Therapy through Synergistic Effects and Microenvironment Modulation	2014	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4046782/
cisplatin	3-methyladenine	Concomitant inhibition of AKT and autophagy is required for efficient cisplatin-induced apoptosis of metastatic skin carcinoma	2010	http://onlinelibrary.wiley.com/doi/10.1002/ijc.25300/abstract;jsessionid=0056AD6F0A976ADFBBD908D4E57E45BA.f04t03
cisplatin	rapamycin	Combined treatment with cisplatin and sirolimus to enhance cell death in human mesothelioma	2010	http://www.sciencedirect.com/science/article/pii/S0022522309009374
cisplatin	rapamycin	Rapamycin enhances chemotherapy-induced cytotoxicity by inhibiting the expressions of TS and ERK in gastric cancer cells	2010	http://onlinelibrary.wiley.com/doi/10.1002/ijc.24990/abstract
cisplatin	rapamycin	Synergistic Effect of Rapamycin and Cisplatin in Endometrial Cancer Cells	2009	http://onlinelibrary.wiley.com/doi/10.1002/cncr.24431/abstract
cisplatin	rapamycin	Effect of combination of rapamycin and cisplatin on human cervical carcinoma Hela cells	2012	http://www.termedia.pl/Journal/-3/Streszczenie-19954
cisplatin	3-methyladenine	Inhibition of autophagy using 3-methyladenine increases cisplatin-induced apoptosis by increasing endoplasmic reticulum stress in U251 human glioma cells	2015	https://www.spandidos-publications.com/10.3892/mmr.2015.3588
cisplatin	3-methyladenine	ER stress and autophagy are involved in the apoptosis induced by cisplatin in human lung cancer cells	2016	https://www.spandidos-publications.com/10.3892/or.2016.4680
cisplatin	3-methyladenine	Inhibition of autophagy enhances cisplatin-induced apoptosis in the MG63 human osteosarcoma cell line	2015	https://www.spandidos-publications.com/10.3892/ol.2015.3692
oblongifolin C	guttiferone K	Oblongifolin C and guttiferone K extracted from Garcinia yunnanensis fruit synergistically induce apoptosis in human colorectal cancer cells in vitro	2017	http://www.nature.com/aps/journal/v38/n2/full/aps2016101a.html
sanguisorba officinalis L	5-fluorouracil	Sanguisorba officinalis L synergistically enhanced 5-fluorouracil cytotoxicity in colorectal cancer cells by promoting a reactive oxygen species-mediated, mitochondria-caspase-dependent apoptotic pathway	2016	http://www.nature.com/articles/srep34245
FK866	gemcitabine 5-Fluorouracil oxaliplatin	A pancreatic ductal adenocarcinoma subpopulation is sensitive to FK866, an inhibitor of NAMPT	2016	http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget&page=article&op=view&path%5b%5d=10776
chloroquine	primaquine	Anti-malarial Drugs Primaquine and Chloroquine Have Different Sensitization Effects with Anti-mitoticDrugs in Resistant Cancer Cells	2016	http://apps.webofknowledge.com/full_record.do?product=WOS&search_mode=GeneralSearch&qid=21&SID=2Cag6jaoGk8HQtqkWs7&page=1&doc=1
4-hydroxytamoxifen	isothiocyanates	Sensitization of estrogen receptor-positive breast cancer cell lines to 4-hydroxytamoxifen by isothiocyanates present in cruciferous plants	2015	https://link.springer.com/article/10.1007%2Fs00394-015-0930-1
resveratrol	doxorubicin	Resveratrol-induced autophagy promotes survival and attenuates doxorubicin-induced cardiotoxicity	2016	http://www.sciencedirect.com/science/article/pii/S1567576916300030
YC-1	3-methyladenine	Paxlitaxel induces apoptosis accompanied by protective autophagy in osteosarcoma cells through hypoxia-inducible factor-1 alpha pathway	2015	https://www.spandidos-publications.com/10.3892/mmr.2015.3860
actinomycin D	nutlin-3a	Actinomycin D and nutlin-3a synergistically promote phosphorylation of p53 on serine 46 in cancer cell lines of different origin	2015	http://www.sciencedirect.com/science/article/pii/S0898656815001539
salinomycin	valproate	HDAC inhibitors enhance the lethality of low dose salinomycin in parental and stem-like GBM cells	2014	http://www.tandfonline.com/doi/abs/10.4161/cbt.27309
primaquine	mefloquine	Co-treatment with the anti-malarial drugs mefloquine and primaquine highly sensitizes drug-resistant cancer cells by increasing P-gp inhibition	2013	http://www.sciencedirect.com/science/article/pii/S0006291X13017762
isobavachalcone	chloroquine	Autophagy inhibition enhances isobavachalcone-induced cell death in multiple myeloma cells	2012	https://www.spandidos-publications.com/10.3892/ijmm.2012.1066
resveratrol	cisplatin carboplatin	Resveratrol induces SIRT1-and energy-stress-independent inhibition of tumor cell regrowth after low-dose platinum treatment	2011	https://link.springer.com/article/10.1007%2Fs00280-011-1640-x
sorafenib	doxorubicin	Inhibition of doxorubicin-induced autophagy in hepatocellular carcinoma Hep3B cells by sorafenib - the role of extracellular signal-regulated kinase counteraction	2011	http://onlinelibrary.wiley.com/doi/10.1111/j.1742-4658.2011.08271.x/abstract
quinacrine	TRAIL	Quinacrine sensitizes hepatocellular carcinoma cells to TRAIL and chemotherapeutic agents	2011	http://www.tandfonline.com/doi/abs/10.4161/cbt.12.3.17033
curcumin	tamoxifen	Chemo-resistant melanoma sensitized by tamoxifen to low dose curcumin treatment through induction of apoptosis and autophagy	2011	http://apps.webofknowledge.com/full_record.do?product=WOS&search_mode=GeneralSearch&qid=37&SID=1ENs5qDEKTH9r12PupO&page=2&doc=17&cacheurlFromRightClick=no