Supplementary file 1

Table S1. Co-treatment of PTX and SNPs against MDA-MB 231: A) Experimental range and level of SNPs and PTX concentrations. B) RSM to study the synergistic effect of SNPs and PTX on MDA-MB 231 cell line viability. C) Analysis of variance for response surface 2FI Model. D) Optimum values of SNPs and PTX in coded and none-coded forms.

A

Variables	Symbol coded	Range and level		
		−1	0	+1
paclitaxel (μM)	X_1	0.01	1.505	3
SNP (μM)	X_2	1	4	7

C

Source	Sum of Squares	df	Mean Square	F value	p-value
X_1	1230.998	1	1230.998	32.784	0.0003
X_2	1037.899	1	1037.899	27.642	0.0005
$X_1 X_2$	272.250	1	272.250	7.250	0.0247

D

Variable	X_1	X_2	Cell viability
Coded	-0.318	0.583	50
Non coded	1.03 (μM)	5.75 (μM)	50

B

Comment	X_1	X_2	Cell viability (%)
Center	0	0	52
Center	0	0	51
Center	0	0	53
Center	0	0	52
Center	0	0	52
Axial	0	-1.02	58
Axial	-1.02	0	66
Axial	1.02	0	47
Axial	0	1.02	46
Fact	1	-1	50
Fact	1	1	33
Fact	-1	-1	100
Fact	-1	1	50

The obtained data in B, last column, were analyzed by Design Expert Software. The coefficients of a regression equation were calculated with R^2 of 0.88:

$$Y = 97.58090 - 16.91972 X_1 - 7.14373 X_2 + 1.83946 X_1 X_2$$

Where, Y is the percent of MDA-MB 231 cells viability, X_1 and X_2 are the concentration (μM) of PTX and SNPs, respectively. According to this equation, in addition to the linear effects of X_1 and X_2, the interaction effect of X_1 and X_2 is also effective on cell viability (P < 0.05).

Table S2. Optimization of SNPs-PTX@BSANPs preparation. A) Experimental range and level of BSA, BSA-Drug and pH, B) Experimental design through the RSM to study optimization of the variables based on NPs size , C) Analysis of variance for Response Surface-Reduced Quadratic Model. D) Optimum values of BSA, BSA-Drug and pH in coded and none coded forms.

A

Variables	Symbol coded	Range and level		
		−1	0	+1
BSA (%)	A	1	3	5
BSA:Drug (µM: µM)	B	1.5	5.25	9
pH	C	5.40	6.40	7.40

B

Comment	BSA% (A)	BSA: SNPs-PTX@BSANPs (B)	pH (C)	Size (nm)
Center	0	0	0	58
Center	0	0	0	58
Center	0	0	0	58.1
Center	0	0	0	58.1
Center	0	0	0	58
Center	0	0	0	58.6
Axial	0	-1.3	0	106.4
Axial	0	0	-1.3	58
Axial	0	1.3	0	70
Axial	0	0	1.3	60
Axial	-1.3	0	0	60
Axial	1.3	0	0	131.7
Fact	-1	1	1	50.9
Fact	-1	-1	1	80.1
Fact	1	-1	1	130
Fact	1	1	-1	105
Fact	1	-1	-1	130
Fact	-1	1	-1	65.91
Fact	1	1	1	110
Fact	-1	-1	-1	80

C

Source	Sum of Squares	df	Mean Square	F value	p-value
A	7460.547	1	7460.547	603.3909	< 0.0001
B	1618.016	1	1618.016	130.8612	< 0.0001
A^2	2977.024	1	2977.024	240.7745	< 0.0001
B^2	1827.456	1	1827.456	147.8002	< 0.0001

D

| Variable	A	B	C	Size
Coded	-0.79	+0.315	+0.88	50
Non coded	1.42	6.43	7.28	50

The obtained data in B-last column, were analyzed by design expert software. The coefficients of the regression equation were calculated according to the following equation with R^2 of 0.98:

$$Z = 114.07771 - 17.84403\,A - 15.10180\,B + 5.09986\,A^2 + 1.13655\,B^2$$

Where, Z is the size of NPs, A is the percent of BSA and B is the ratio of BSA to anticancer agents. In addition to A and B, the square of A and B also affect the size of NPs (P < 0.05). However, the effect of pH was not significant..
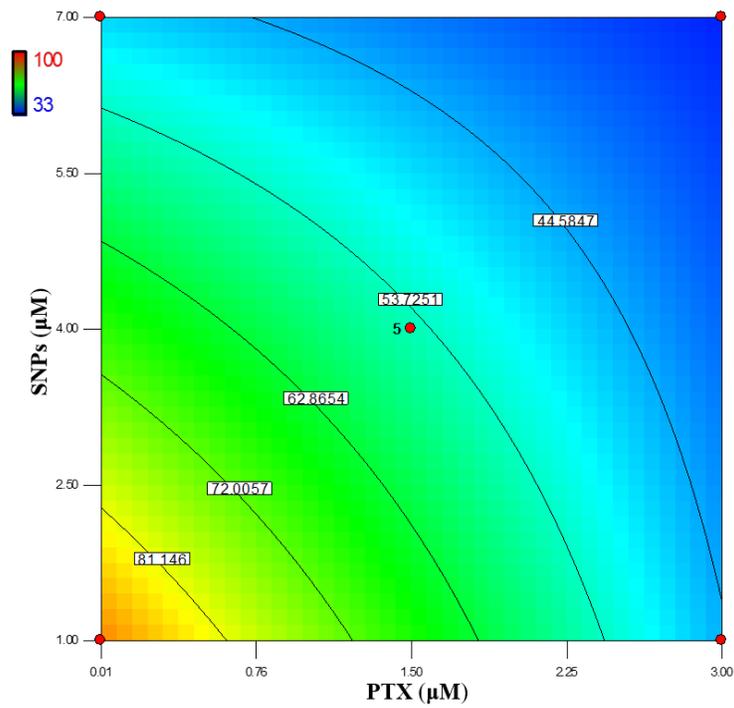
Figure S1. Two-dimensional contour plots of cell viability of MDA-MB 231 (1×10^4 cell/ml) treated with different concentrations of PTX (0.01 to 3 µM) and SNPs (1 to 7 µM) simultaneously. \The sign ● designate the designed points. The color change from red to blue indicates reducing the cell viability.
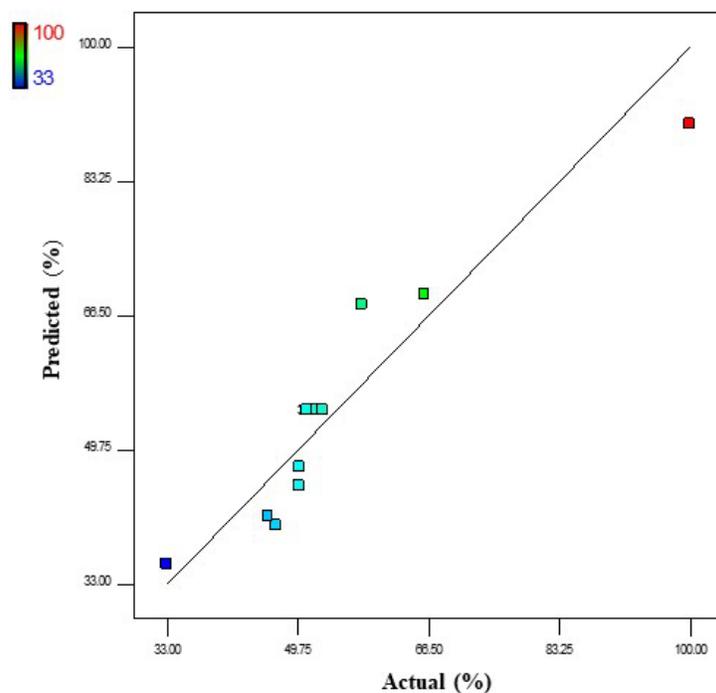
Figure S2. Correlation between the calculated and experimental values of cell viability in synergistic system of PTX and SNPs. The color change from red to blue indicates reducing the cell viability.
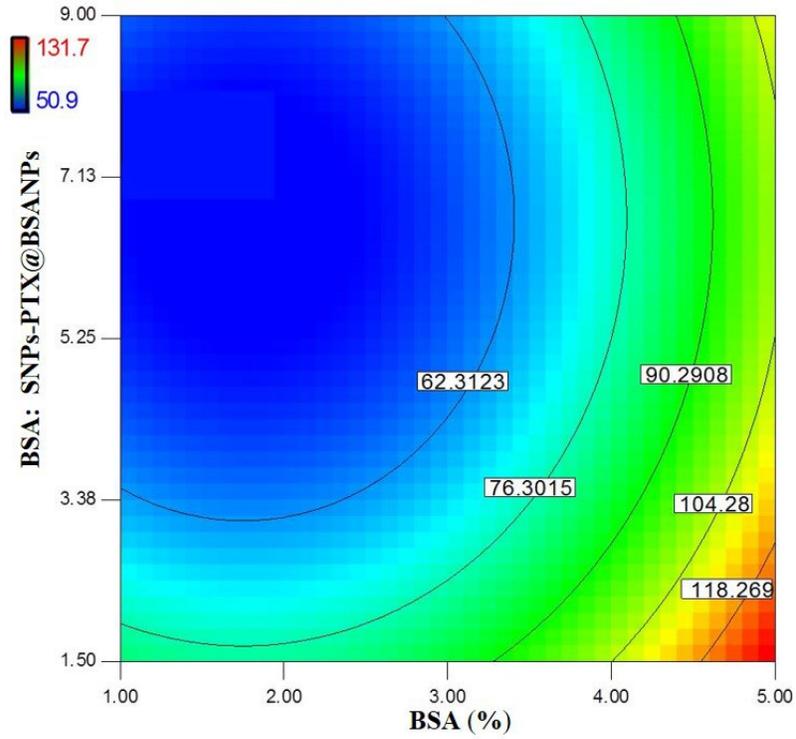
Figure S3. Two-dimensional contour plots of the effect of BSA percent (horizontal axis) and ratio of BSA: Drug (Vertical axis) on size of SNPs-PTX@BSANPs. The color change from red to blue indicates reducing the diameter from 131.7 to 50.9 nm.
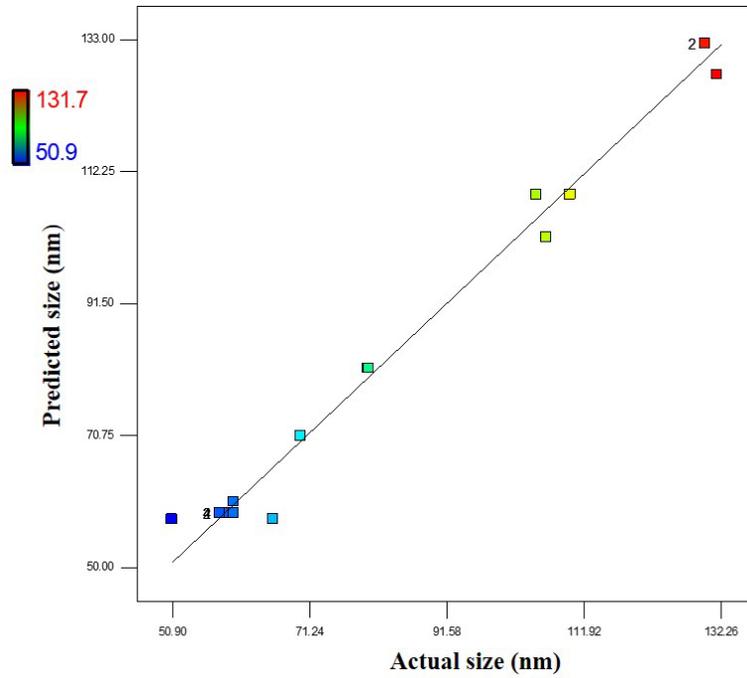
Figure S4. Correlation between calculated and experimental values of size of NPs in optimization of SNPs-PTX@BSANPs preparation. The color change from red to blue indicates reducing the size of NPs.