Supplementary information:

Synergistic Action of Benzyl Isothiocyanate and Sorafenib in a Nanoparticle Delivery System for Enhanced Triple-Negative Breast Cancer Treatment

Qi Wang1*†, Nan Cheng1†, Wei Wang1, Yongping Bao1*

1 Norwich Medical school, University of East Anglia, NR4 7UQ, United Kingdom

* Correspondence: QW: q.wang1@uea.ac.uk, YPB: y.bao@uea.ac.uk

† These two authors contributed equally to this work

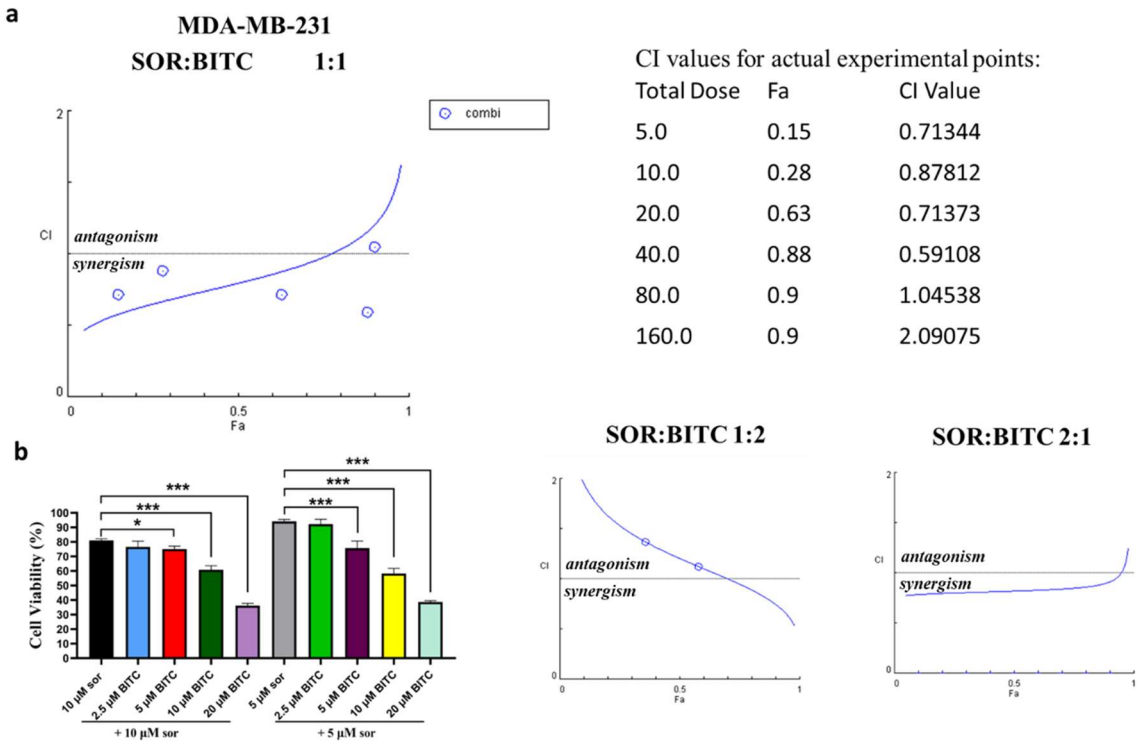

Figure S1. Chou-Talalay's Combinational Index (CI) was calculated using cell viability assay value in MDA-MB-231 cells treated with BITC and SOR combinations. (a) Combination Index Plot of BITC and SOR combination treatment. (Total Dose: dose of BITC plus dose of SOR; Fa: average effect values; CI value: combinational index value. CI values for synergism is 0-1, and for antagonism is 1-∞) (b) Cell viability of MDA-MB-231 cells was measured using MTT assays 24 hours after BITC and SOR treatment.

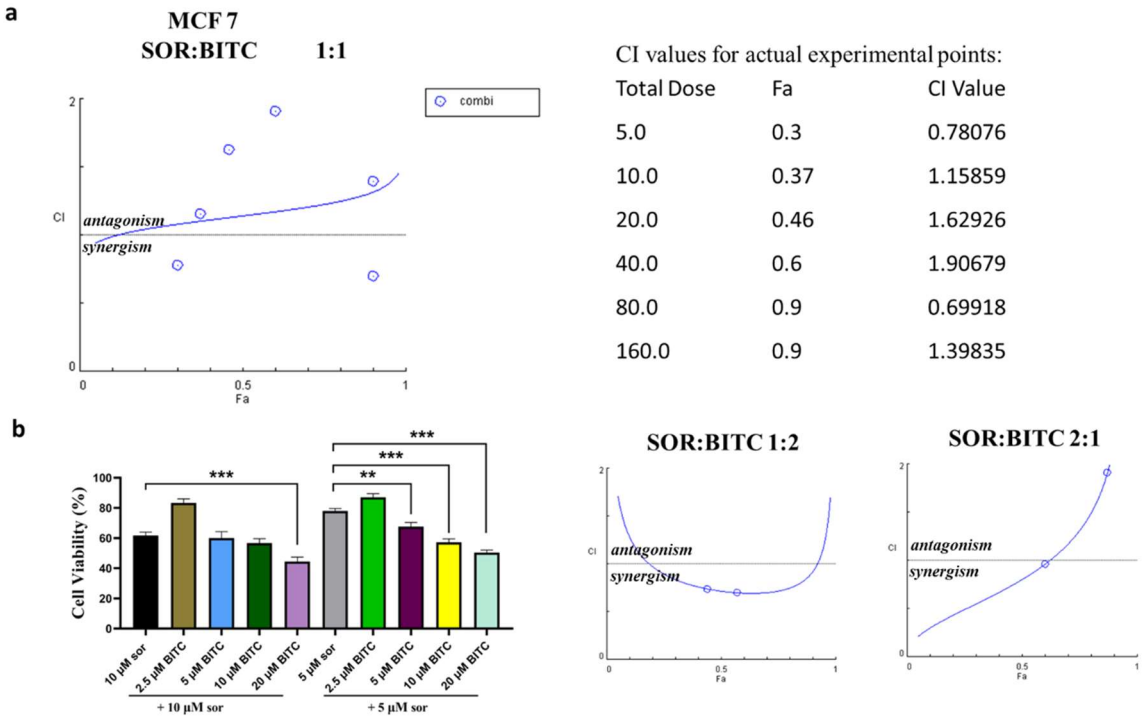

Figure S2. Chou-Talalay's Combinational Index (CI) was calculated using cell viability assay value in MCF-7 cells treated with BITC and SOR combinations. (a) Combination Index Plot of BITC and SOR combination treatment. (Total Dose: dose of BITC plus dose of SOR; Fa: average effect values; CI value: combinational index value. CI values for synergism is 0-1, and for antagonism is 1-∞) (b) Cell viability of MCF-7 cells was measured using MTT assays 24 hours after BITC and SOR treatment.

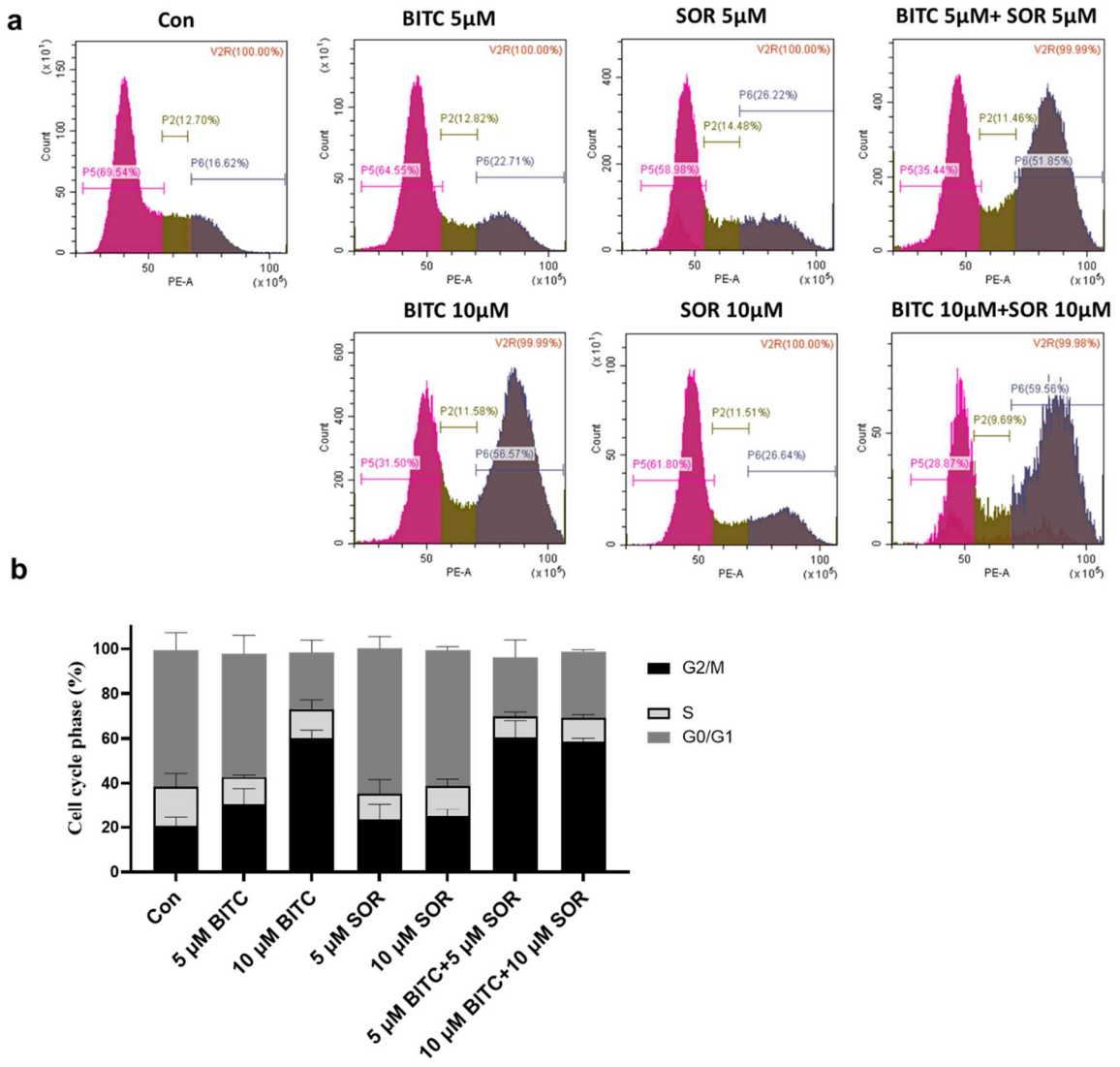

Figure S3. Effects of BITC and SOR combined treatment on MCF-7 cell cycle. (a) Flow cytometry analysis of the cell cycle distribution after 24 hours of treatment with BITC and/or SOR. (b) Quantitative evaluation of cells arrested at G2. Data are presented as means ± SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001.

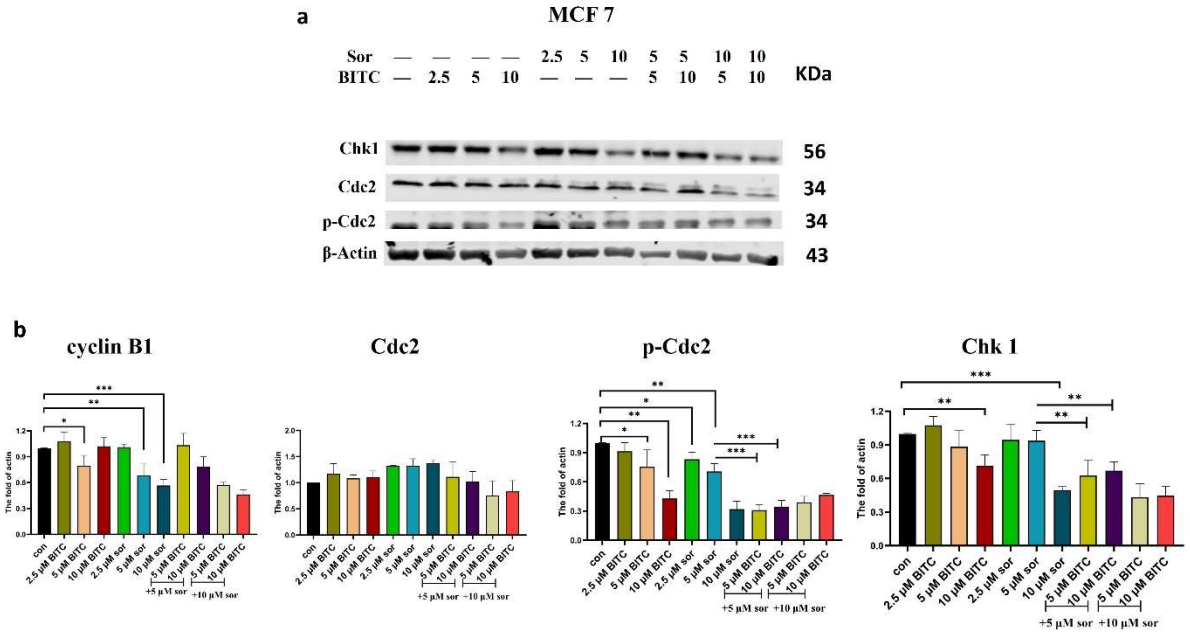

Figure S4. Effects of BITC and SOR treatment on G2 phase protein expression in MCF-7 cells. 24 hours after treatment, the expression of cyclin B1, Chk1, Cdc2, and p-Cdc2 proteins was analyzed by Western blot. Expression of β-Actin served as a loading control. Band densities were normalized against β-actin, and results were expressed as fold changes relative to controls. Data are presented as means ± SD (n = 3). *P < 0.05, **P < 0.01.

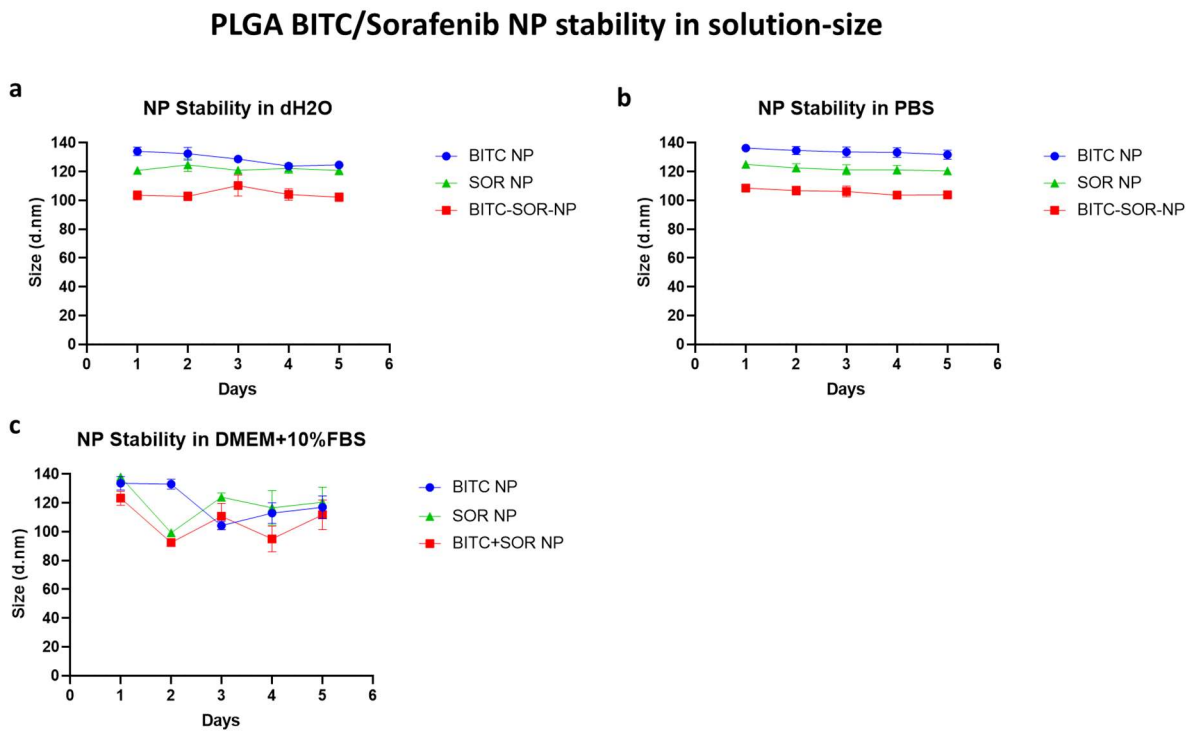

Figure S5. BITC and SOR encapsulated nanoparticle size stability in various solutions. (a) dH_2O, (b) PBS, (c) DMEM+10%FBS. The size of nanoparticles was measured by dynamic light scattering.

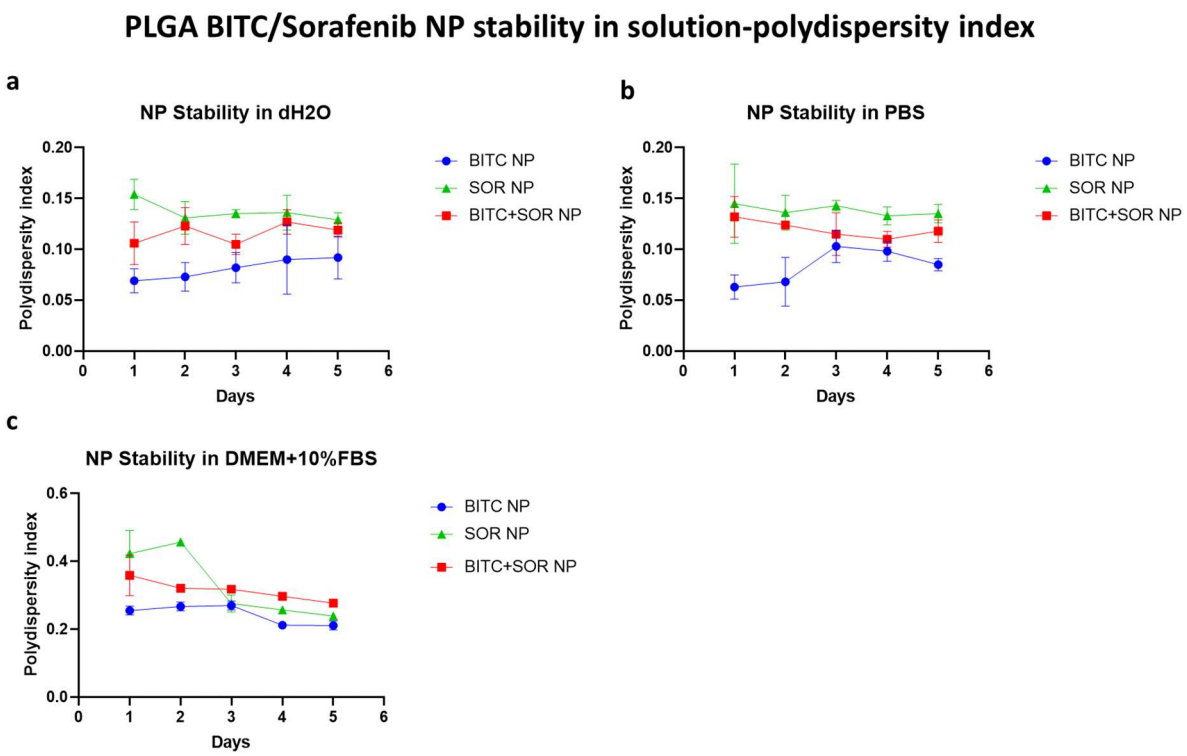

Figure S6. BITC and SOR encapsulated nanoparticle Polydispersity index (PDI) stability in various solutions. (a) dH_2O, (b) PBS, (c) DMEM+10%FBS. The PDI of nanoparticles was measured by dynamic light scattering.

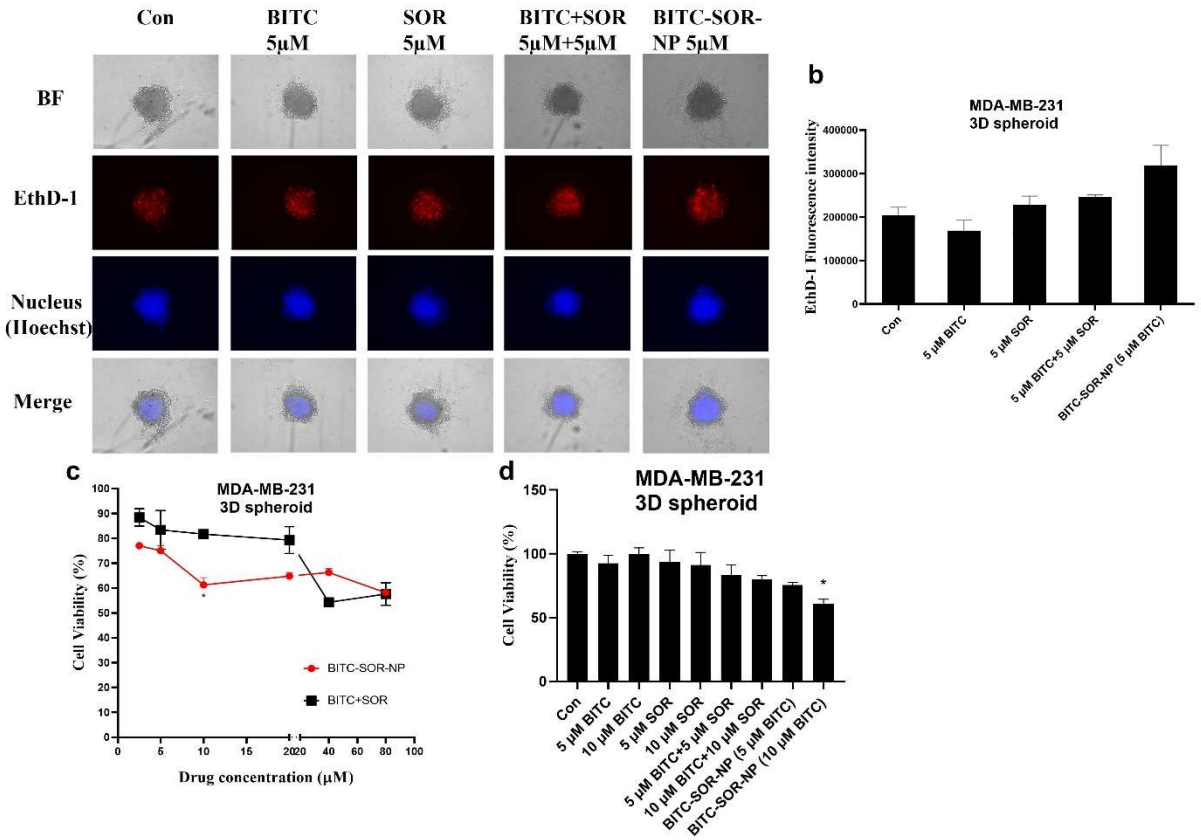

Figure S7. Effects of BITC-SOR-NPs treatment on 3D spheroid of MDA-MB-231 spheroids. (a) Cytotoxicity of MDA-MB-231 spheroids after BITC and/or SOR free drugs or encapsulated NP treatments for 4 days. Representative images of MDA-MB-231 spheroids were captured using EVOS M5000 microscope under 10x magnification brightfield image, nuclear (blue Hoechst dye staining) and dead (red EthD-1 dye staining) cell population. (b) Fluorescence intensity of EthD-1 dye staining on MDA-MB-231 spheroids after BITC and/or SOR treatments. (c) and (d) Cell viability study of MDA-MB-231 spheroids after 48h treatments of BITC-SOR-NPs and free drug combination. Data are presented as means ± SD (n = 3). *P < 0.05.

Table S1: Table of PLGA BITC/Sorafenib NP characteristics

	Size (d.nm)	Polydispersity Index (PdI)	BITC Encapsulation efficiency (BITC-EE%)	Sorafenib Encapsulation efficiency (Sor-EE%)	Zeta potential (mV)
BITC NP	136.3±1.4	0.063±0.012	66.2±6.0	NA	-12.0±0.4
SOR NP	125.0±0.8	0.145±0.039	NA	42.6±2.1	-12.1±0.1
BITC-SOR-NP	108.6±1.9	0.132±0.020	24.0±1.5	38.4±6.3	-7.6±0.3
ENP[+]	93.8±0.7	0.137±0.036	NA	NA	-5.0±0.7

+ ENP: empty NP without drug encapsulation