

*Supporting Information for:*

**Novel Prodrug Supramolecular Nanoparticles Capable of Rapid Mitochondrial-Targeted  
and ROS-Responsive for Pancreatic Cancer Therapy**

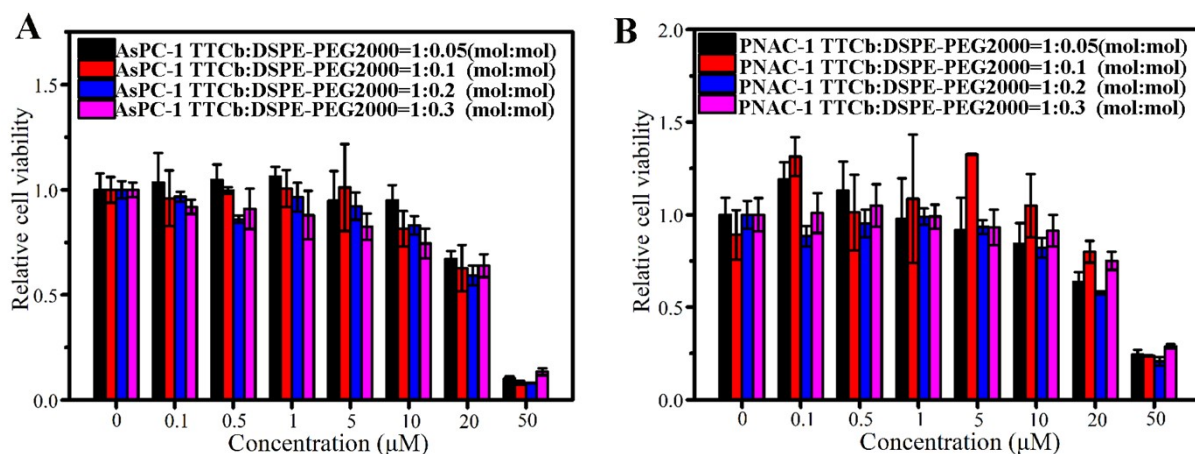
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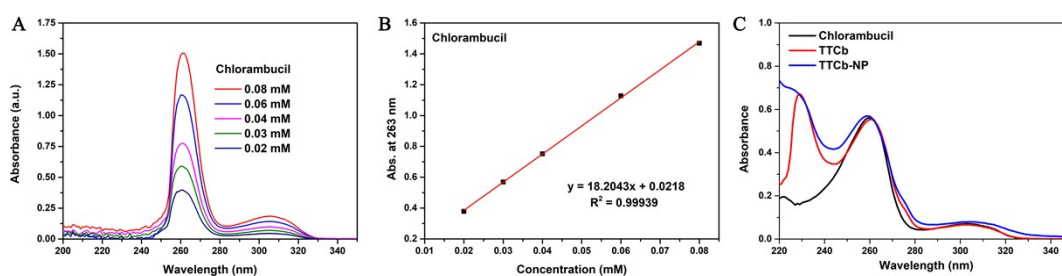
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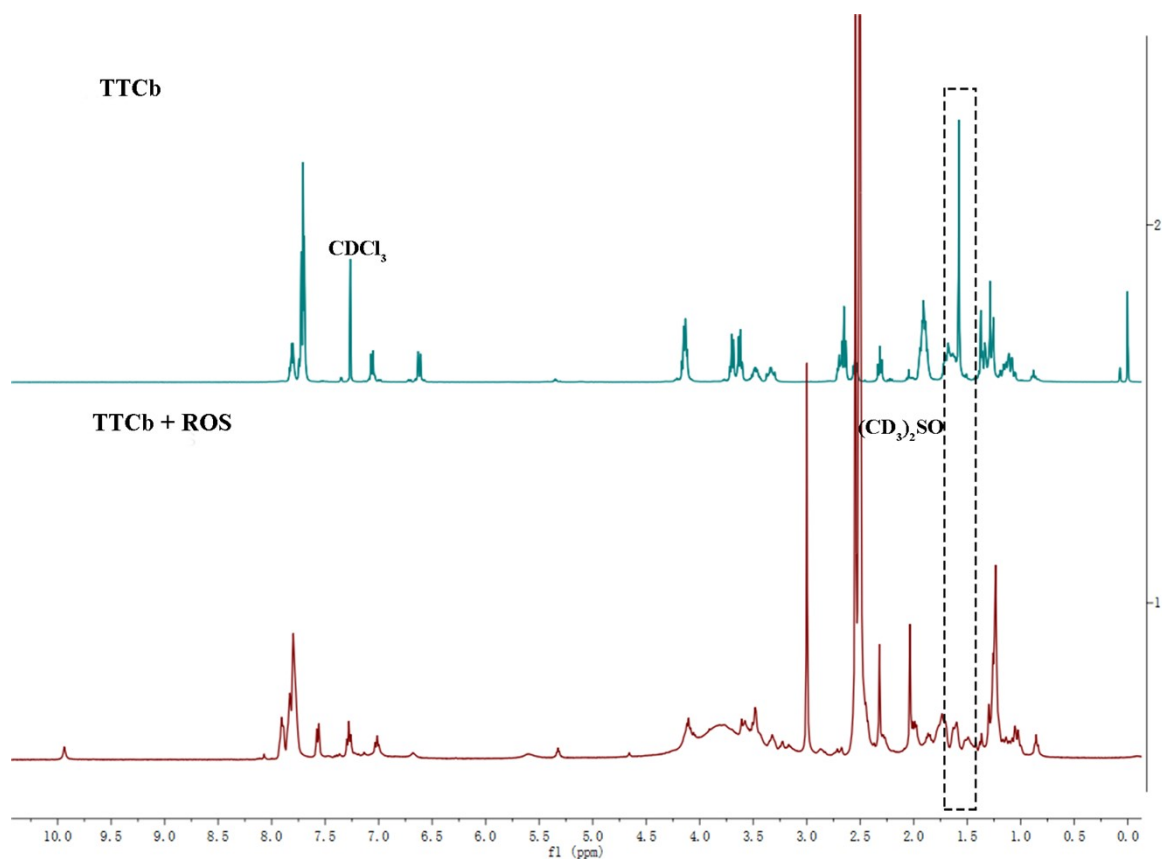
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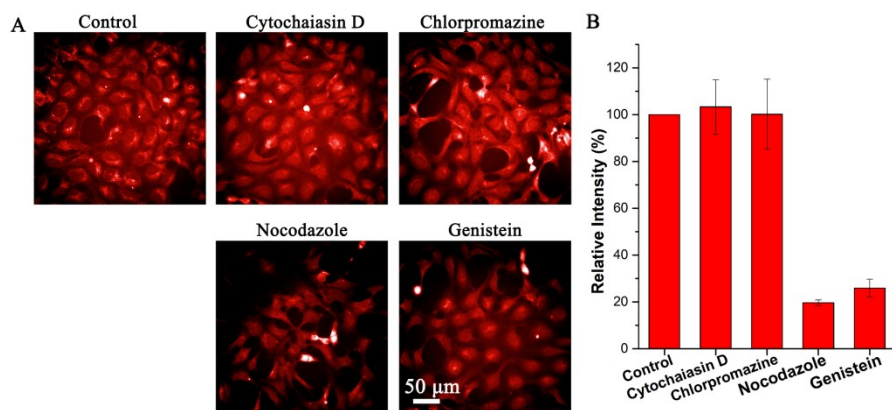
**Fig. S1** *In vitro* cytotoxicity of TTCb-NPs constructed with different molar ratios of TTCb : DSPE-PEG2000 in AsPC-1 (A) and PNAC-1 (B) cells treated with various concentrations for 48 h. Data represent mean  $\pm$  SD (n = 3).



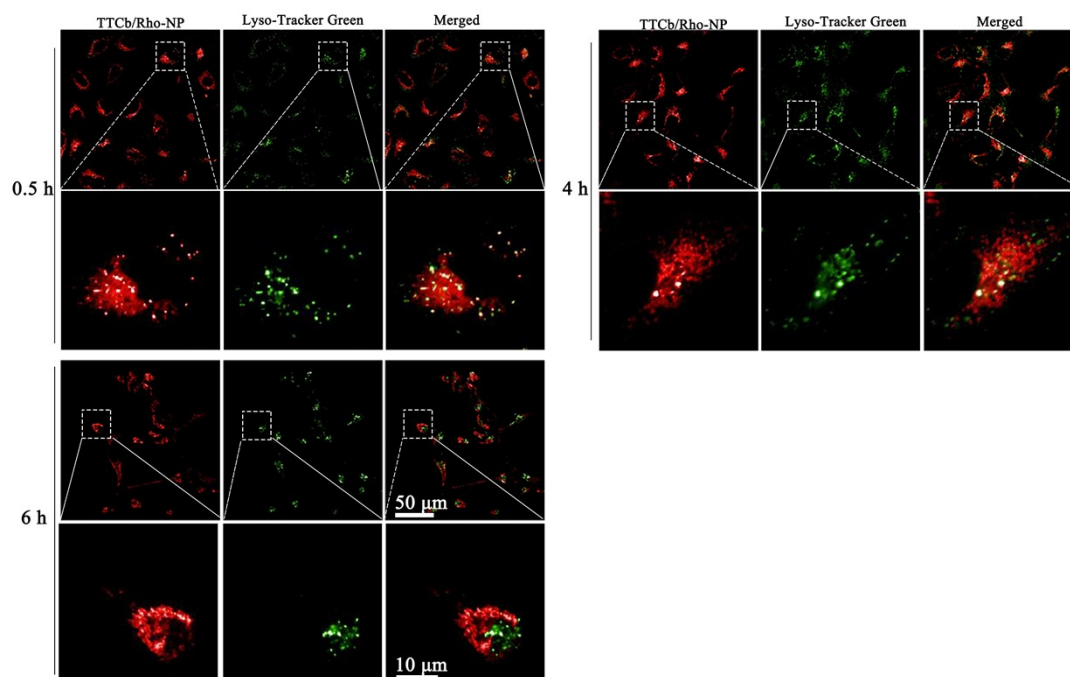
**Fig. S2** (A) UV-vis-NIR spectra of chlorambucil at different concentrations. (B) The calibration curve of chlorambucil at 263 nm. (C) UV-vis-NIR spectra of chlorambucil, TTCb, TTCb-NP.



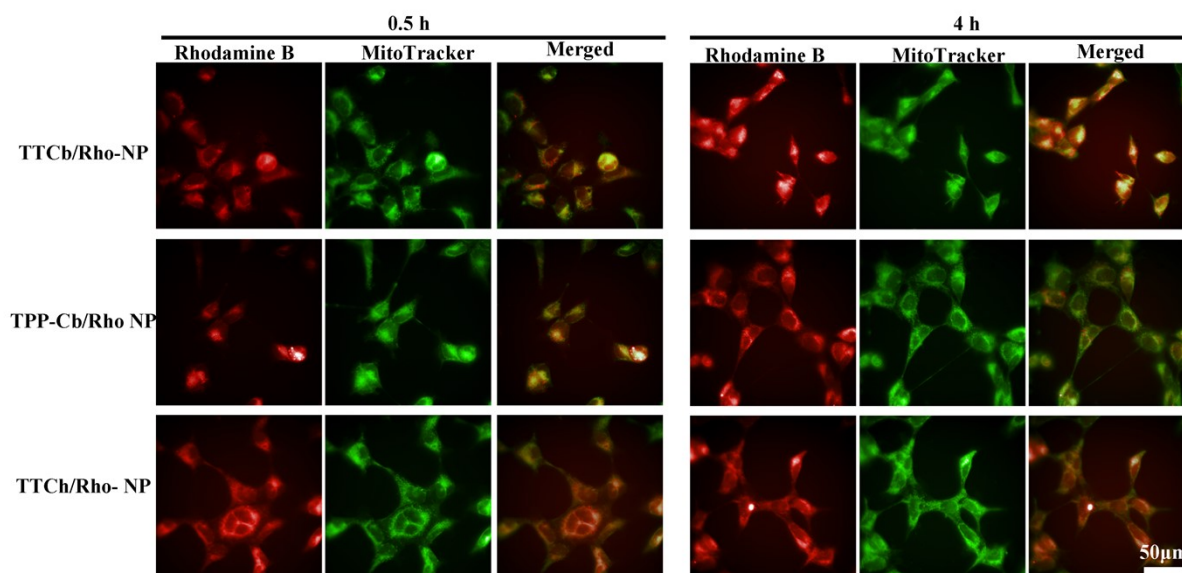
**Fig. S3** The <sup>1</sup>H NMR spectra of **TTCb** before/after treating with 200 mM H<sub>2</sub>O<sub>2</sub>.



**Fig. S4** Images of the BxPC3 cells treated with **TTCb/Rho-NP** in the presence of various endocytosis inhibitors. The images were captured by high content analysis system-operetta CLSTM (A). The Relative intensity of rhodamine fluorescent in the images was calculated by the imageJ software (B).

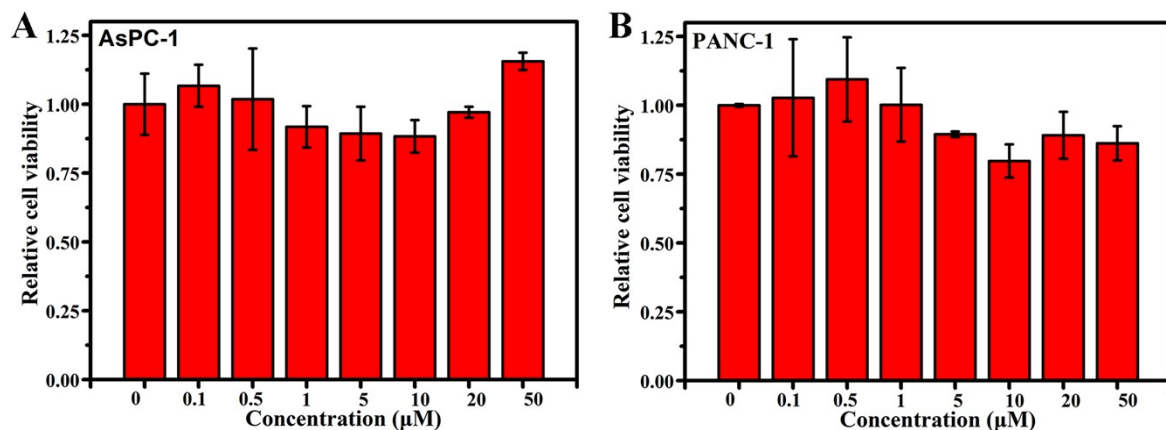


**Fig. S5** Endosomal escape behaviors of **TTCb/Rho-NPs** in vitro: Images of the BxPC3 cells treated with **TTCb/Rho-NPs** at the concentration of 10  $\mu\text{M}$  for 0.5, 4 and 6 h, captured by high content analysis system-operetta CLS<sup>TM</sup>. For each row, from left to right: **TTCb/Rho-NPs** (red, 546 nm excitations); lysosomes and acidic late endosomes stained by LysoTracker Green (green, 504 nm excitation); merged image.

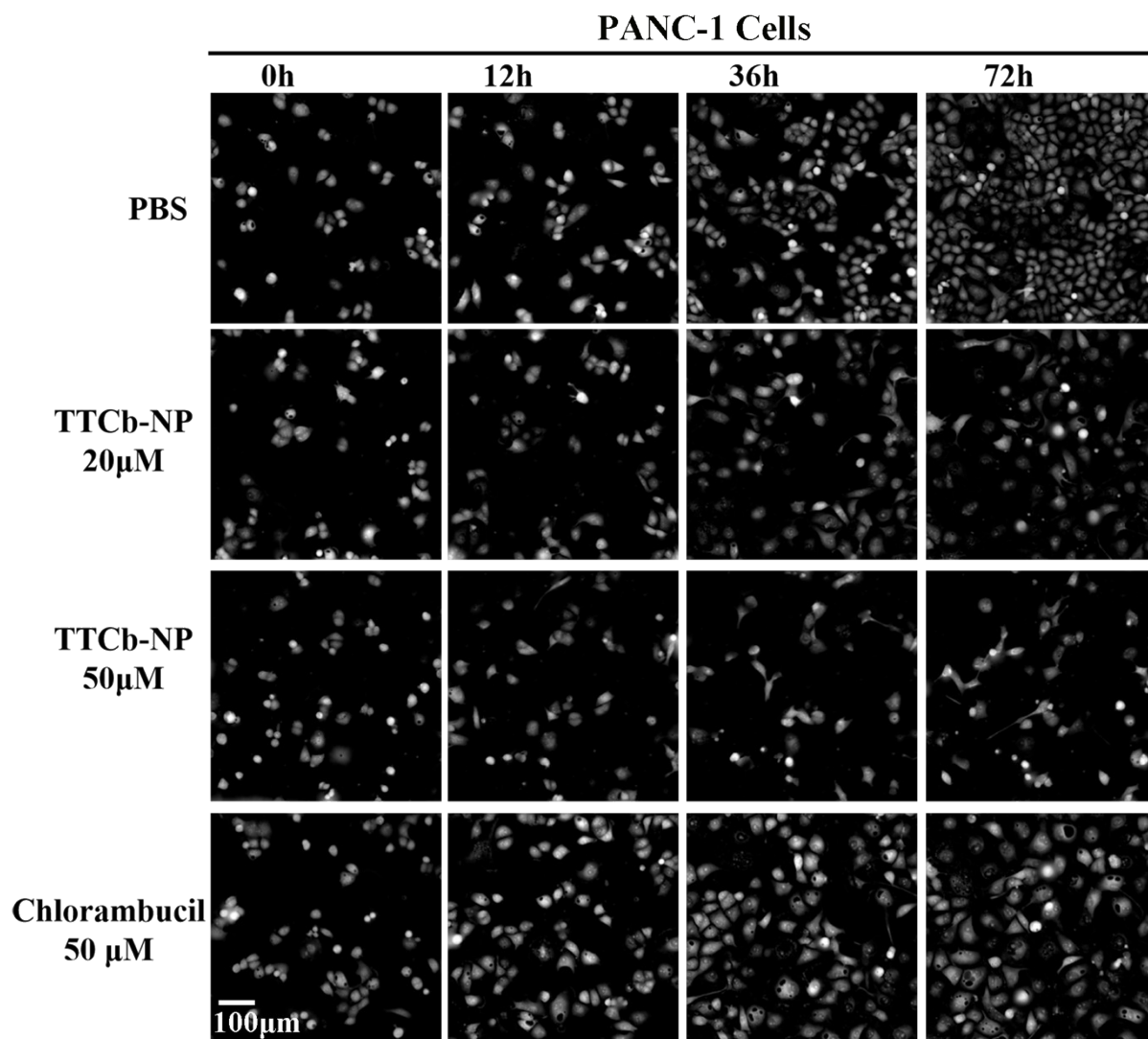


**Fig. S6** Colocalization study of **TTCb/Rho**, **TPP-Cb/Rho** and **TTCh/Rho** NPs with mitochondria. Images of the BxPC3 cells treated with different NPs at the concentration of 20  $\mu\text{M}$  for 0.5 h and 4 h captured by high content analysis system-operetta CLS<sup>TM</sup>. For each row, from left to right: Rhodamine B (546 nm excitations); mitochondria stained by MitoTracker Green (488 nm excitation); merged image. Scale bars: 50

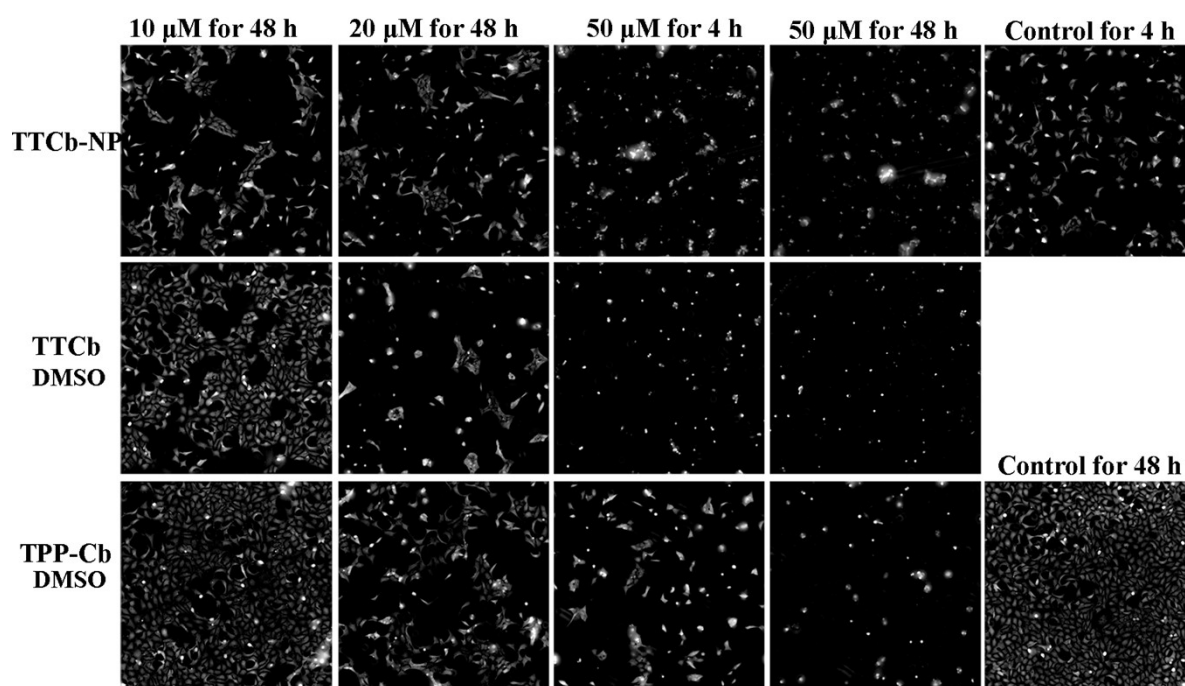
$\mu\text{m}$ .



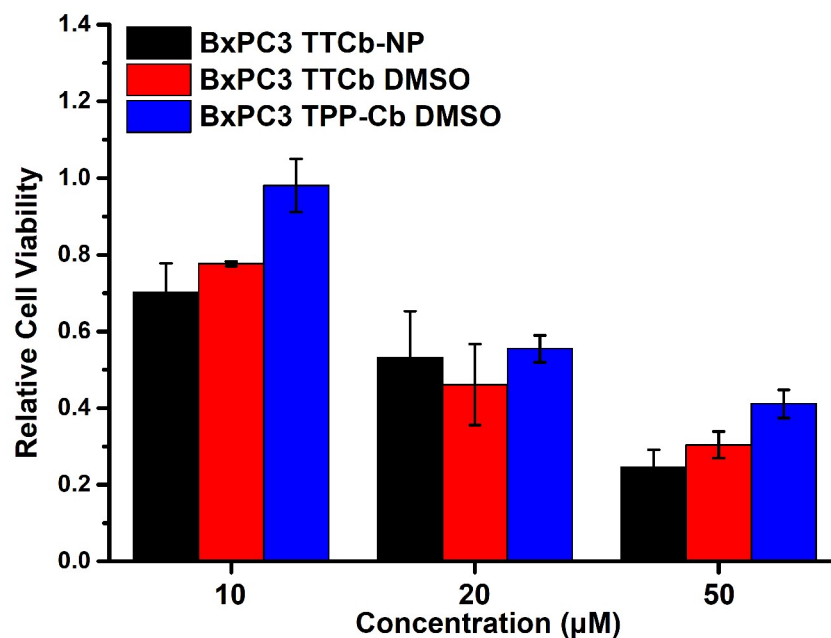
**Fig. S7** Cell viability of AsPC-1 (A) and PANC-1 (B) cells after being treated with different concentration of TTCh-NPs for 48 h. Data represent mean  $\pm$  SD (n = 3).



**Fig. S8** Digital phase contrast images of PANC-1 cells at different times after different treatment. Digital phase contrast images were captured by high content analysis system-operetta CLS<sup>TM</sup>. Scale bar: 100  $\mu\text{m}$ .



**Fig. S9** Digital phase contrast images of BxPC3 cells treated with **TTCb-NPs**, **TTCb** (dissolved in DMSO) and **TPP-Cb** (dissolved in DMSO) at various concentrations after 4 h or 48 h were captured by high content analysis system-operetta CLS™.



**Fig. S10** *In vitro* cytotoxicity of **TTCb-NPs**, **TTCb** (dissolved in DMSO) and **TPP-Cb** (dissolved in DMSO) at various concentrations against BxPC3 cells after 48 h incubation.

Copies of all  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra.

