

Suppl. Fig. 1: Percentage of sub-G1 (<2N DNA) fraction (apoptotic cells) in PC-3 cells treated with increasing dose-levels of Red-Br-nos. Data are mean \pm SD (p < 0.05).



Suppl. Fig. 2: Red-Br-nos impedes cell-cycle progression by causing a G2/M arrest followed by emergence of a characteristic hypodiploid (sub-G1) DNA peak, indicative of apoptosis. Panels **A** and **B** depict cell-cycle distribution profile in a three-dimensional disposition as determined by flow-cytometry of PC-3 cells treated with 10 and 25 μ M Red-Br-nos for 0, 6, 12, 18, 24, 36 and 48h. Panel **C** is a graphical representation of the quantitation of the percent sub-G1 population at the two dose regimes (10 and 25 μ M) over the time course of treatment.



Suppl. Fig. 3: Immunoblot analysis of LC3-II expression levels in lysates from PC-3 cells treated with 0, 50 and 100 μ M Red-Br-nos for 24h. β -actin was used to ensure equal protein loading. Similar results were observed in at least two independent experiments.



Suppl. Fig. 4: Immunofluorescence micrographs showing DCF staining in tiron (ROS scavenger) and tiron + Red-Br-nos treated PC-3 cells.

Suppl. Fig. 4



Suppl. Fig. 5: Immunoblotting data for cleaved caspase-3, 7, 8, 9, and 2 using lysates from PC-3 cells treated with vehicle (0.1% DMSO), 25 μ M Red-Br-nos, 2 nm docetaxel for 48h. The blots were stripped and re-probed with anti-actin antibody to ensure equal protein loading.



Suppl. Fig. 6: Percentage of sub-G1 (<2N DNA) fraction (apoptotic cells) in PC-3 cells treated with vehicle (control), Red-Br-nos, z-vad-fmk and Red-Br-nos + z-vad-fmk. The caspase-inhibitor, z-vad-fmk, along with Red-Br-nos does not reduce sub-G1 population induced by Red-Br-nos alone. Data are mean \pm SD.



Suppl. Fig. 7: Effect of Red-Br-nos treatment over time (6, 12, 18, 24, 36, 48 and 72h exposure) on cell viability as assessed by trypan blue staining. Data are mean \pm SD (p < 0.05).





Suppl. Fig. 8: Immunoblot analysis of cleaved caspase-3 in lysates from PC-3 cells treated with vehicle (control), Red-Br-nos, autophagy inhibitor (3-MA), and Red-Br-nos + 3-MA for 48h. β -actin was used to ensure equal protein loading. Similar results were observed in at least two independent experiments.



Suppl. Fig. 9: A. Immunoblot analysis of beclin-1 in lysates from PC-3 cells treated with vehicle (control), Red-Br-nos, cyclosporin A, and Red-Br-nos + cyclosporin A for 48h. β -actin was used to ensure equal protein loading. **B.** Micrographs show immunocytochemical staining of 48h Red-Br-nos treated PC-3 cells for AIF (green), DNA (blue) and merged. Data show that there was absence of drug-induced nuclear translocation of AIF in the presence of cyclosporin A.



Suppl. Fig. 10: Immunofluorescence micrographs showing DCF staining in control and 6h drugtreated PC-3 cells. Bar-graphical quantitation of increase in mean fluorescence intensity (JC-1) in PC-3 cultures treated with DMSO (control) or Red-Br-nos for 6h. Columns, mean \pm SD (*, p<0.05).