Supporting Information

Power of organic electron acceptor in modulation of intracellular mitochondrial ROS: Induces JNK and caspase dependent apoptosis of cancer cells

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Materials. Cover glass bottom dishes were purchased from SPL. Cleaved caspase antibody sampler kit, NF-κB p65 rabbit mAB, Phopho-p38 MAPK rabbit mAb, stress and apoptosis antibody sample kit and pro-apoptosis Bcl-2 family antibody sampler kit has been purchased from cell signaling technology. MitoSOX and DHE were procured form ThermoFisher. N-acetyl cysteine (NAC), MitoTempo, Ac-DEVD-pNA were purchased from cayman. BBT, LBT, CS and FAP has been synthesized and characterized as described before.[1]

Western Blot. Western blot analysis has been performed following standard protocol as reported earlier.[2]

Mitochondria complex I activity assay. Complex I activity was analysed using MitoCheck Complex I Activity Assay Kit following manufacturer's kit (Cayman chemicals).

Reference

1. Ie, Y.; Nitani, M.; Karakawa, M.; Tada, H.; Aso, Y., Air-Stable n-Type Organic Field-Effect Transistors Based on Carbonyl-Bridged Bithiazole Derivatives. *Adv. Funct. Mater.* **2010**, 20(6): 907-913.
2. Saha, A.; Mohapatra, S.; Das, G.; Jana, B.; Ghosh, S.; Bhunia, D., Cancer Cell Specific Delivery of Photosystem I Through Integrin Targeted Liposome Shows Significant Anticancer Activity. *ACS Appl. Mater. Interfaces* **2017,** *9* (1), 176-188.

Figures

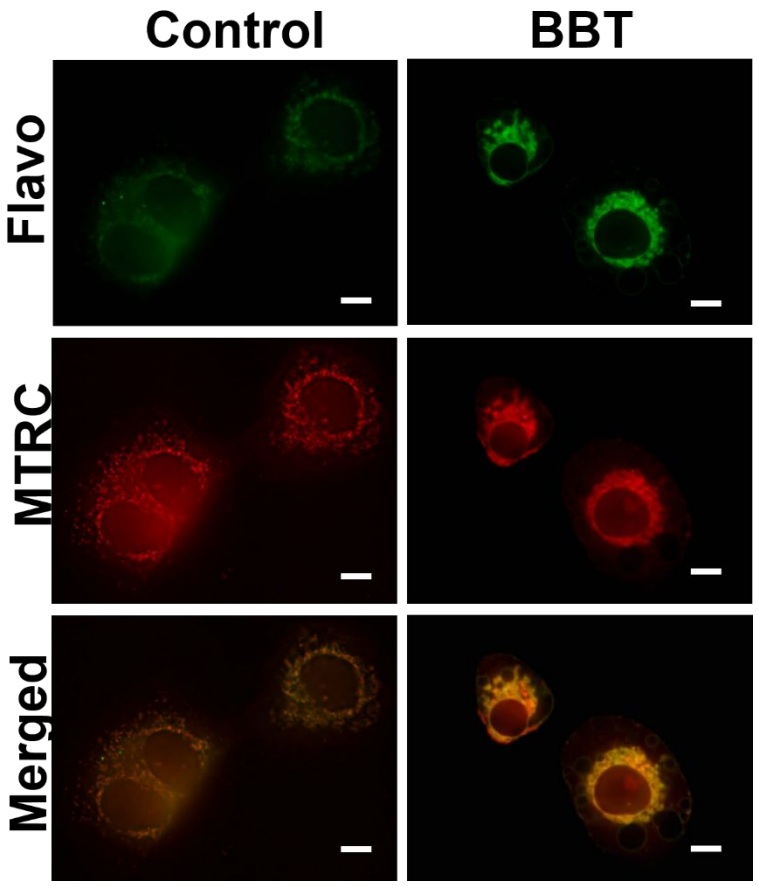


Figure S1. Microscopic images show oxidative state of flavoprotein in green fluorescence and mitochondria has been stained with MTRC (MitoTracker Red CMXRos) showing red fluorescence. Scale bars correspond to 10 μm and the Magnification is 60X.

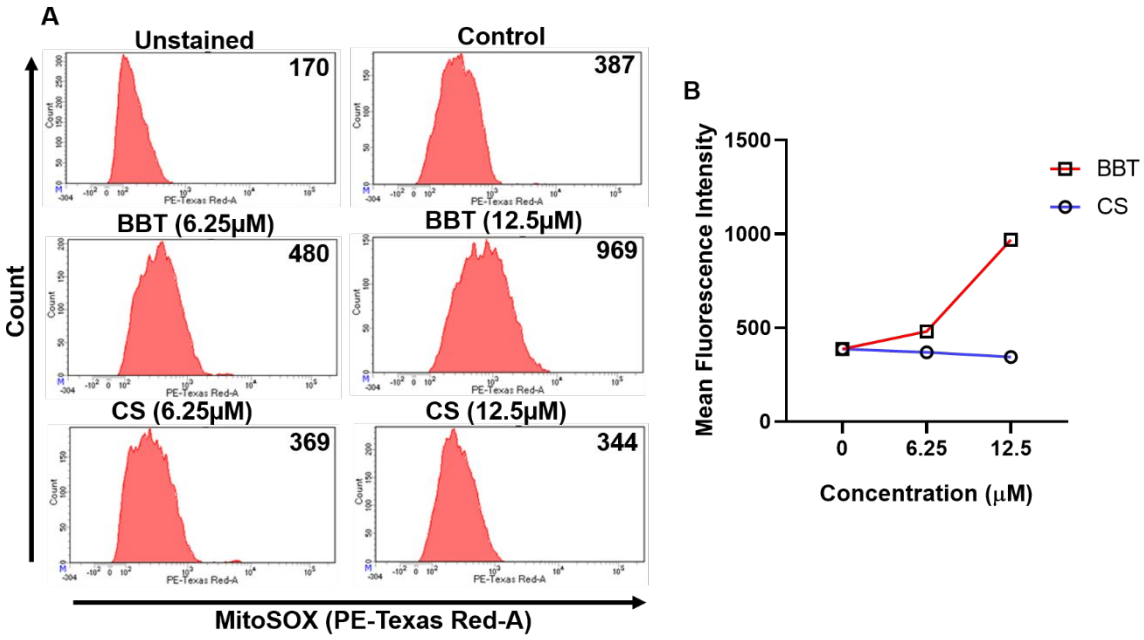


Figure S2. Histograms (A) and graph (B) represent MitoSOX assay in MCF7 cells. Cells incubated in absence (control) or presence of various concentrations of BBT and CS. The cells were subjected to MitoSOX reagent (5 μM) and fluorescence intensity from cells was assayed using FACS. MitoSOX untreated cells were considered as unstained.

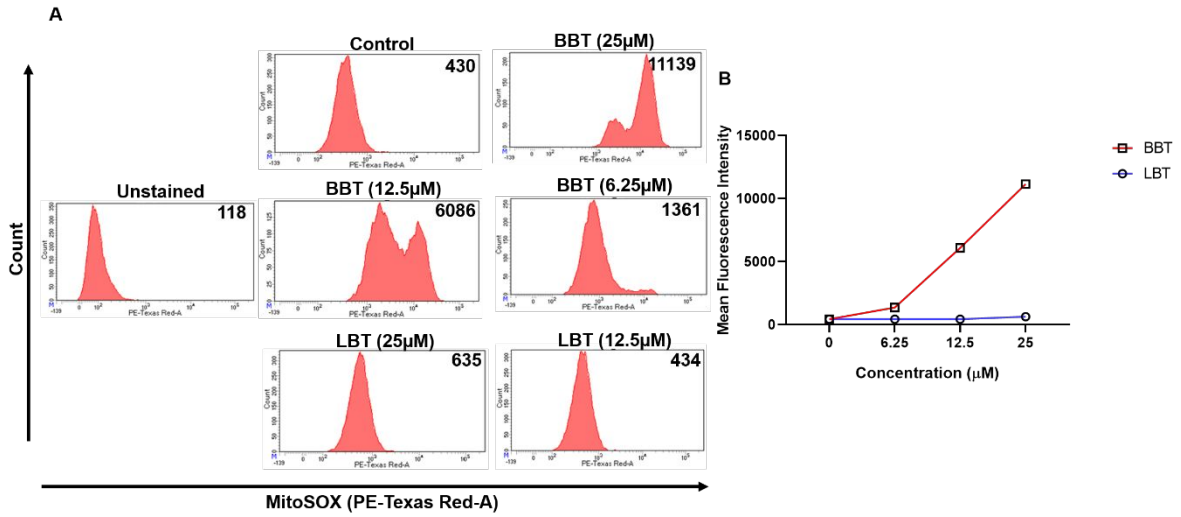


Figure S3. Histograms (A) and graph (B) represent mitochondrial ROS in HeLa cells using MitoSOX. (A)Cells incubated in absence (control) or presence of various concentrations of BBT and LBT. The cells were then subjected to MitoSOX (5 μM) reagent and fluorescence intensity from cells were assayed using FACS. MitoSOX untreated cells were considered as unstained.

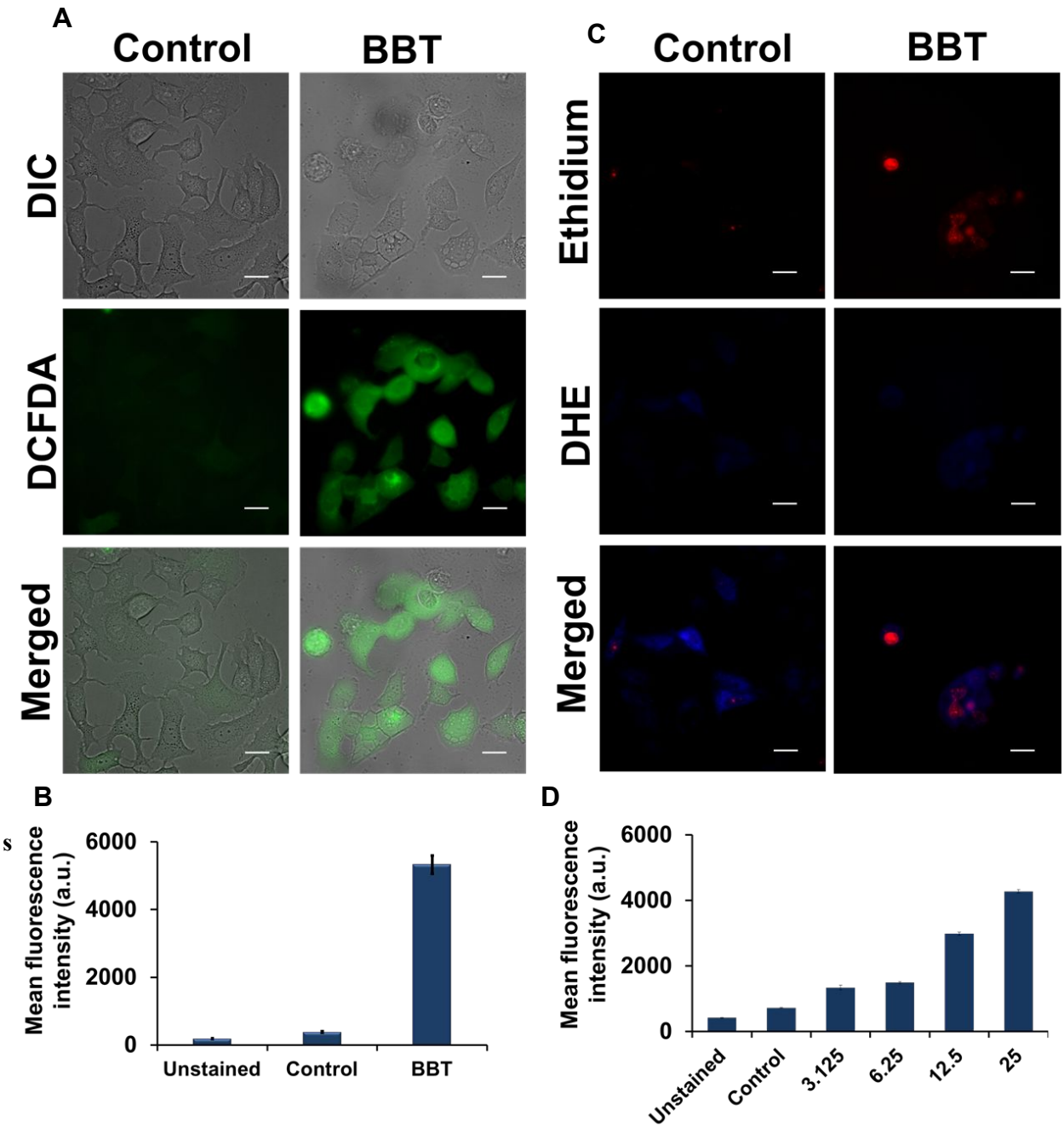


Figure S4. BBT increases cellular oxidative stress and affect cancer cell survival. Microscopic images of DCFDA assay in MCF7 cells. Cells were treated with 12.5 µM of compound BBT for 24 h and subjected to DCFDA staining. Scale bars correspond to 20 µm (A). Flow-cytometric quantification of DCFDA assay in cells after treatment of 25 µM of BBT. Data shown as means ± SD (B). Microscopic images representing DHE assay in MCF7. Cells were treated with 12.5 µM of BBT for 24 h and subjected to DHE staining. Scale bars correspond to 20 µm (C). DHE assay based flow-cytometric quantification of ROS generation in MCF7 after treatment with increasing concentration of BBT. Data shown as means ± SD (D).

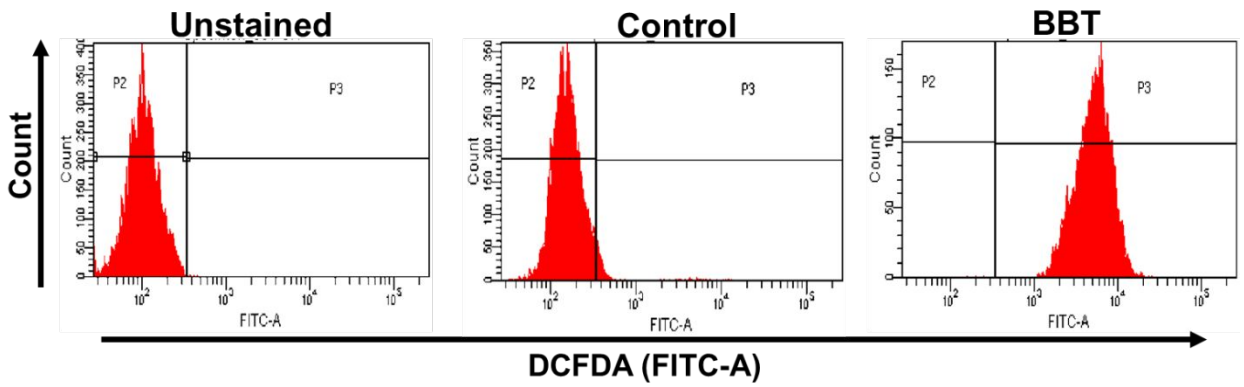


Figure S5. Histogram represents quantification of cytoplasmic ROS using DCFDA in MCF7 cells. Cells incubated in absence (control) or presence of BBT (25 µM). The cells were then subjected to DCFDA (20 µM) and fluorescence intensity from cells were assayed using FACS. DCFDA untreated cells were considered as unstained.

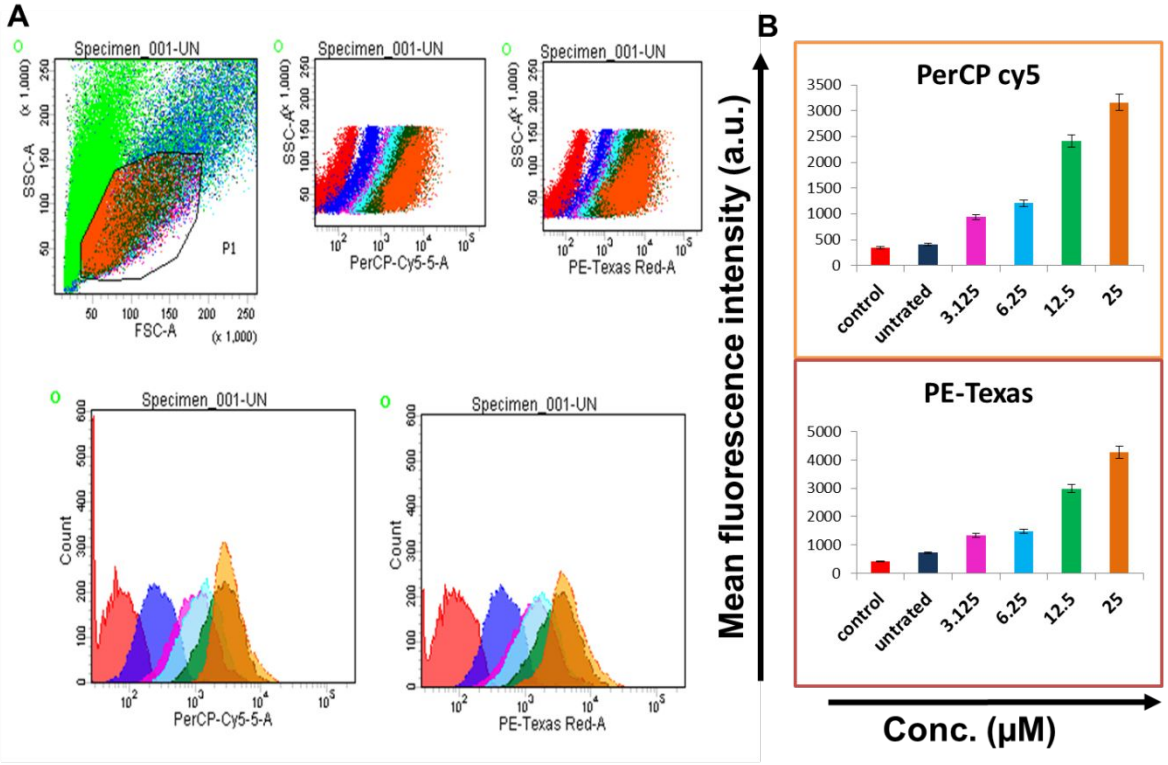


Figure S6. Histogram represents quantification of cytoplasmic ROS using DHE in MCF7 cells (A). Cells incubated in absence (control) or presence of various concentrations of BBT. Then cells were subjected to DHE (5 µM) reagent and fluorescence intensity from cells was assayed using FACS. DHE untreated cells were considered as unstained. Bar diagram representation of quantification of ROS using DHE in MCF7 (B). The colours in the Bar diagram in Panel B of the figure corresponds to the colours of the histogram in Panel A.

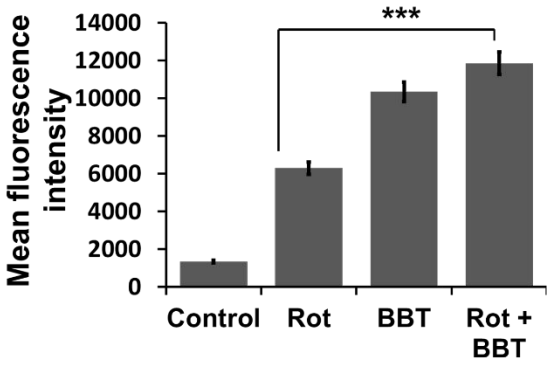


Figure S7. Analysis of mitochondrial ROS using MitoSOX dye in HeLa cells after treatment of 40 µM of Rot (rotenone) 12.5 µM of BBT and Rot with BBT using FACS. Data shown as means ± SD and has been analyzed using One-way ANOVA (***p< 0.0001).

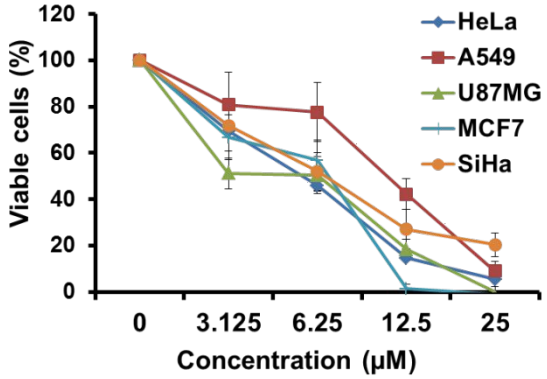


Figure S8. Cell viability curve of various cancer cells after treatment of various concentration of BBT. Data shown as means ± SD

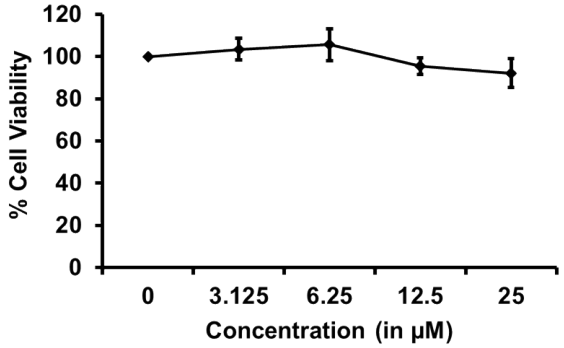
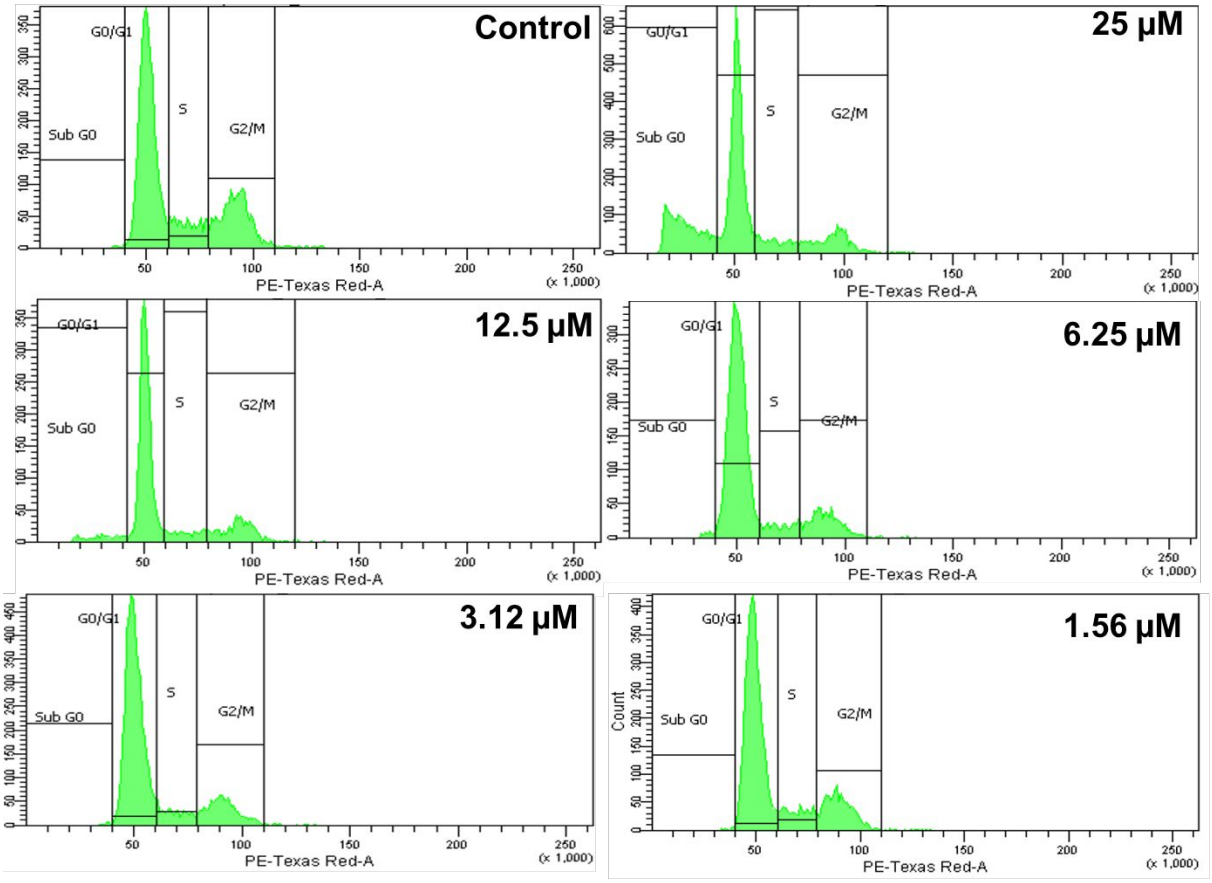


Figure S9. Cell viability curve of BBT treated WI-38 cells. Data shown as means ± SD


Control
25 μM
12.5 μM
6.25 μM
3.12 μM
1.56 μM
G0/G1
S
G2/M
Sub G0
PE-Texas Red-A
(x 1,000)
Count

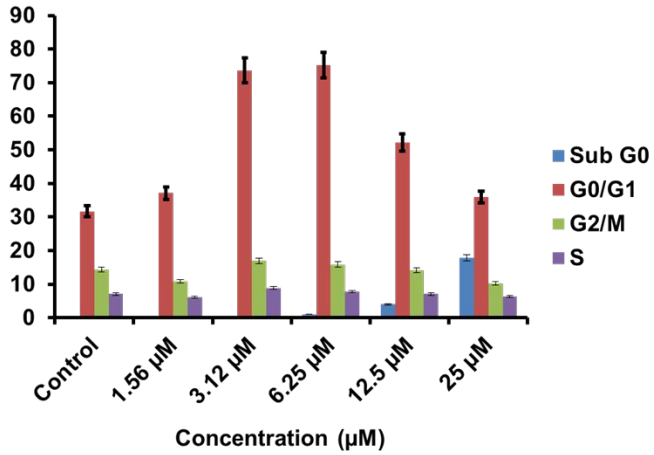

Sub G0
G0/G1
G2/M
S
Control
1.56 μM
3.12 μM
6.25 μM
12.5 μM
25 μM
Concentration (μM)

Figure S10. Bar Diagram represents the proportion of MCF-7 cells in each phase on treatment with varying doses of BBT. Data shown as means ± SD.

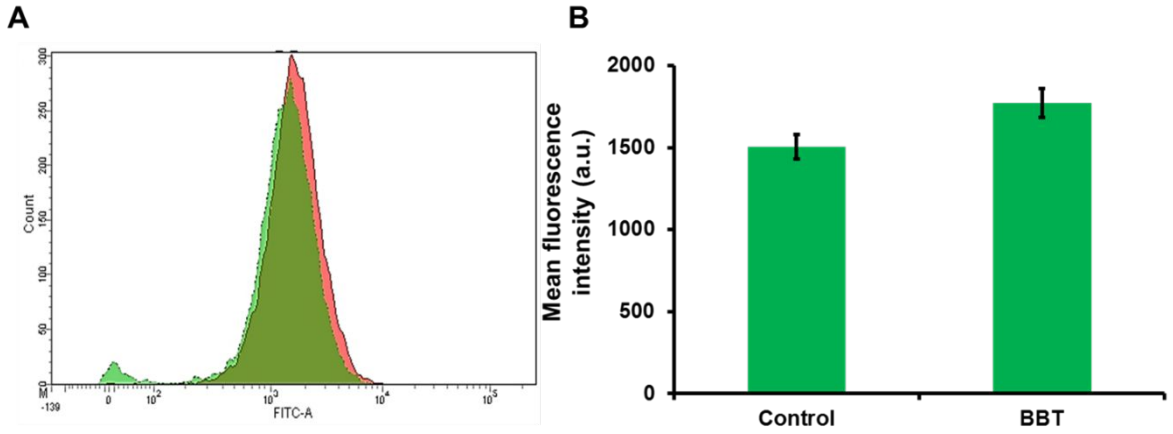


Figure S11. Histogram represents autophagic cell population using anti-LC3 (FITC) antibody by FACS in absence (Control; green histogram) and presence of 12.5 µM BBT (red histogram) (A). Bar diagram represent fluorescence intensity of LC-3 in untreated (Control) and treated (BBT) cells. (B)

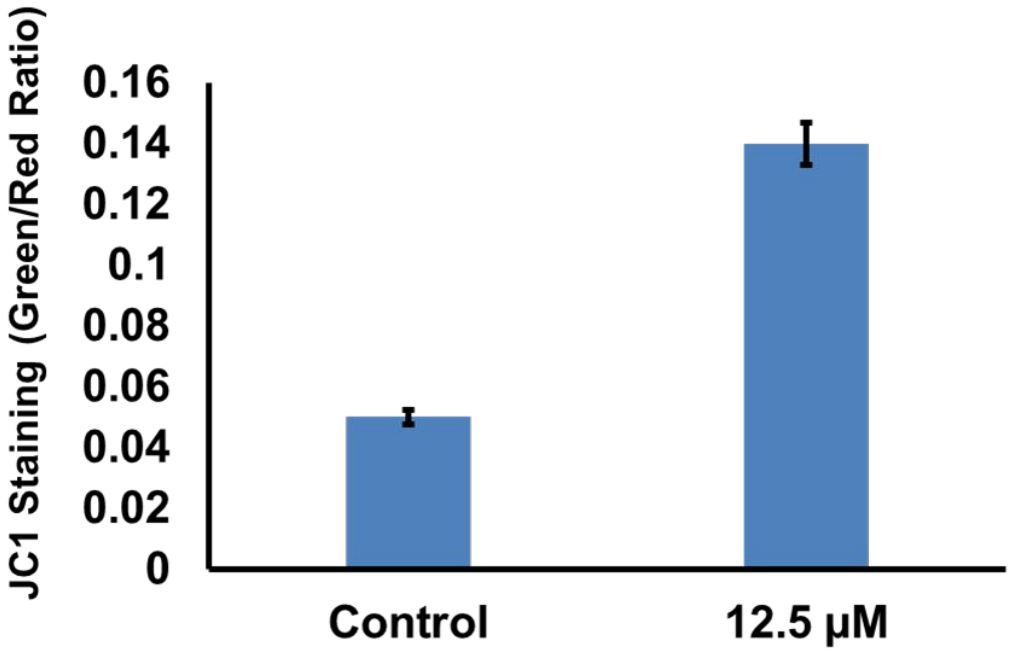


Figure S12. Bar diagram representation of the JC-1 assay measured through the Green/Red ratio of the JC-1 dye. Data shown as means ± SD.

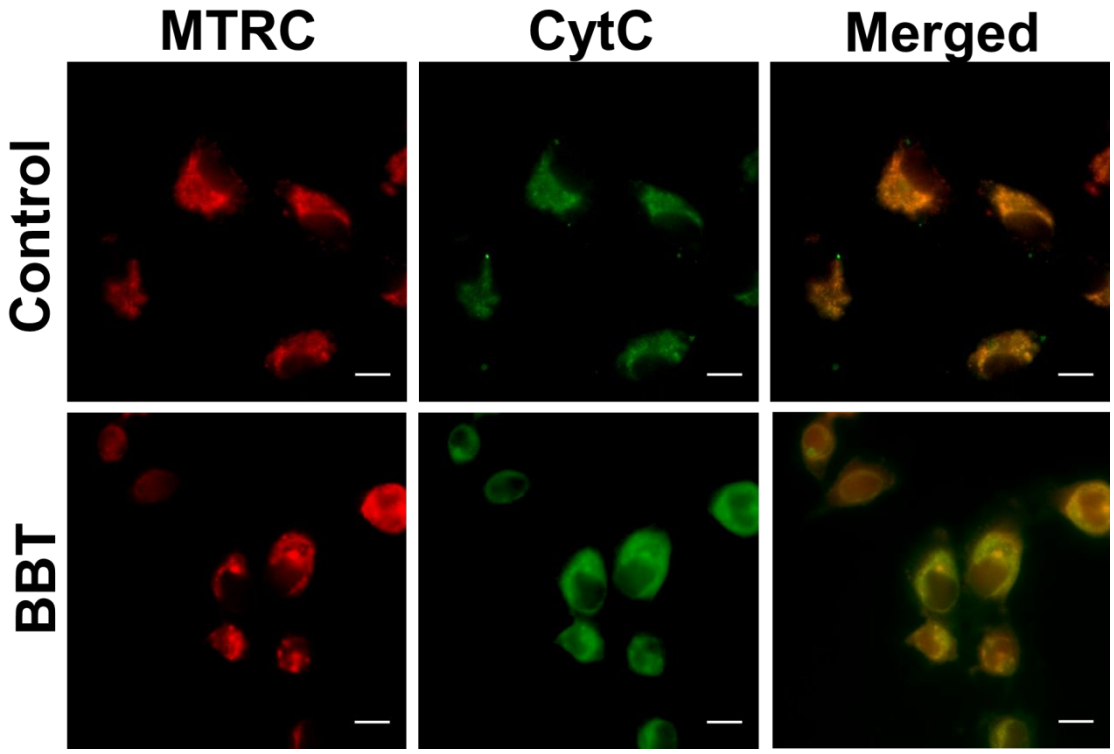


Figure S13. Microscopic images show immunolocalization of cytochrome C (CytC) in HeLa cells. Pearson's correlation coefficient R (n) is 0.865.Scale bars correspond to 20 µm

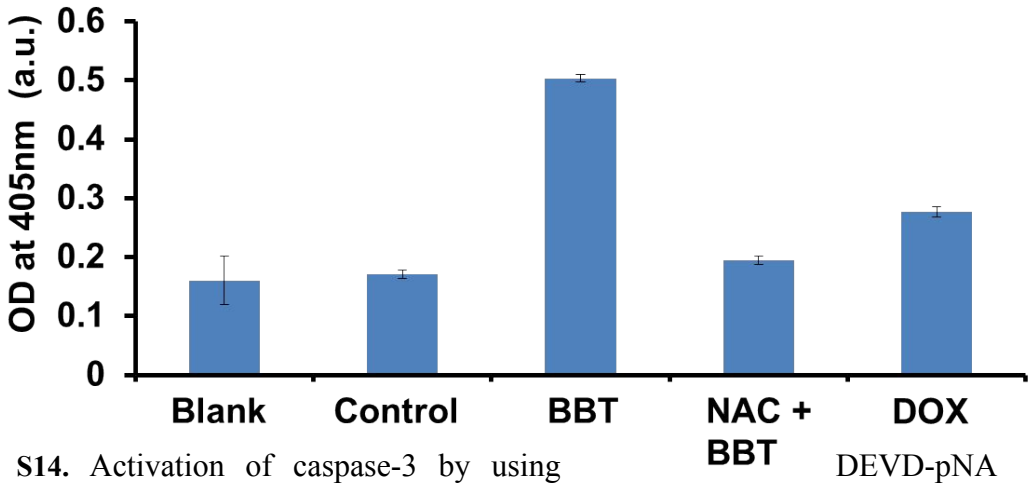


Figure S14. Activation of caspase-3 by using DEVD-pNA using spectrophotometer. Bar diagram denotes absorbance of cell lysate without substrate (blank), absorbance of DEVD-pNA with untreated (control), BBT treated (BBT), NAC with BBT treated (NAC+BBT), and Doxorubicin treated (DOX) cell lysates.

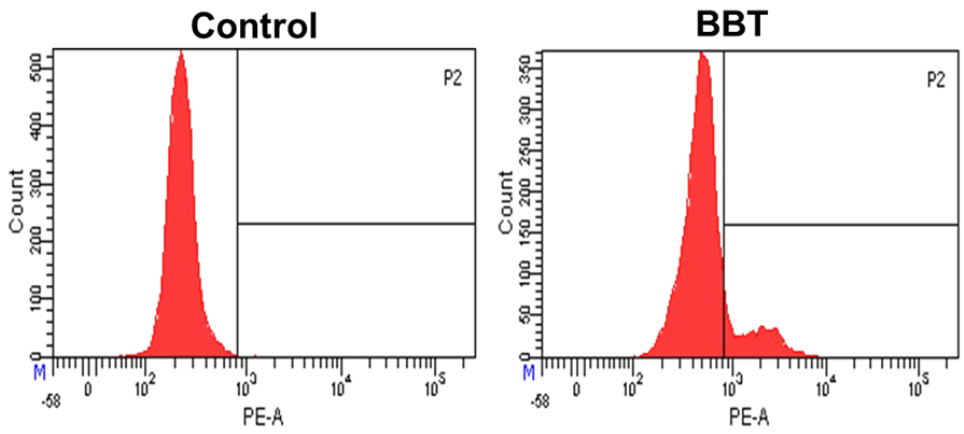


Figure S15. Cleaved caspase-3 analysis in HeLa cells before (Control) and after (12.5 μM of BBT) treatment by flow-cytometric.

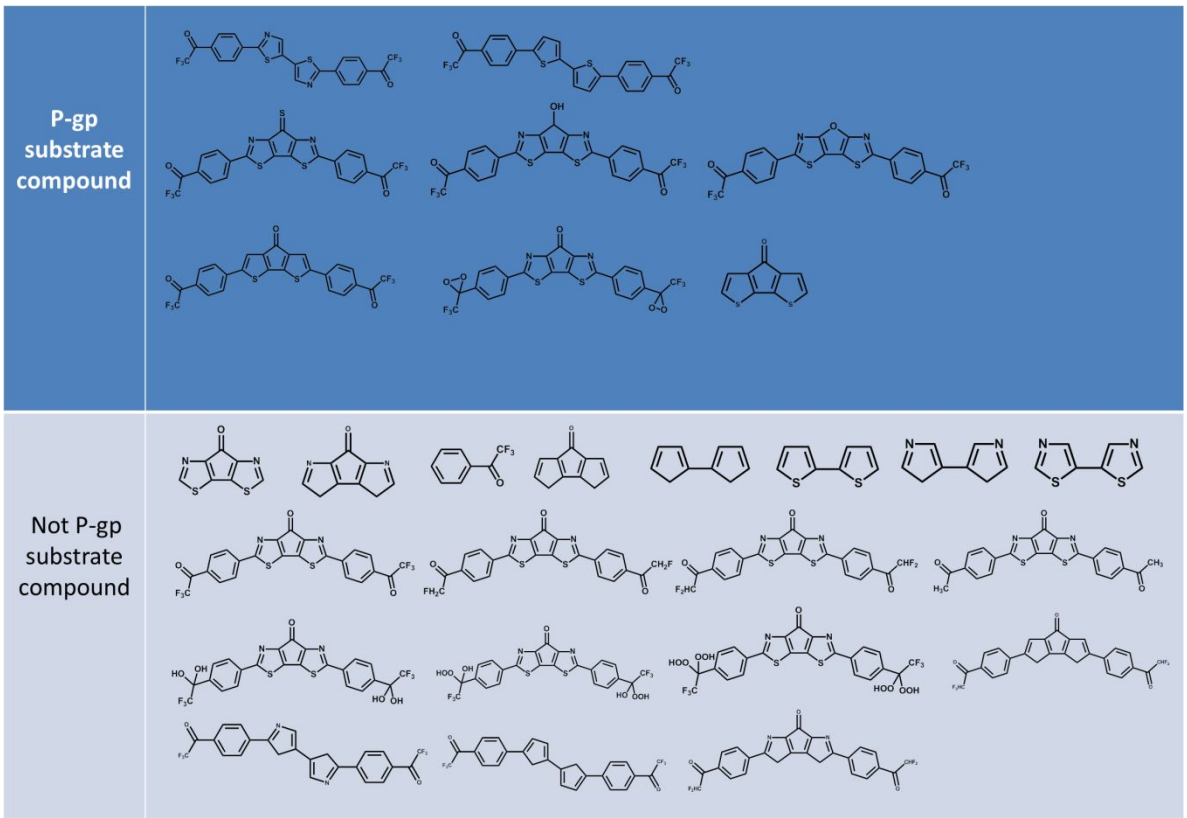


Figure S16. Classification of BBT and its analogues as P-gp substrate or non-P-gp substrate.