Supplemental information

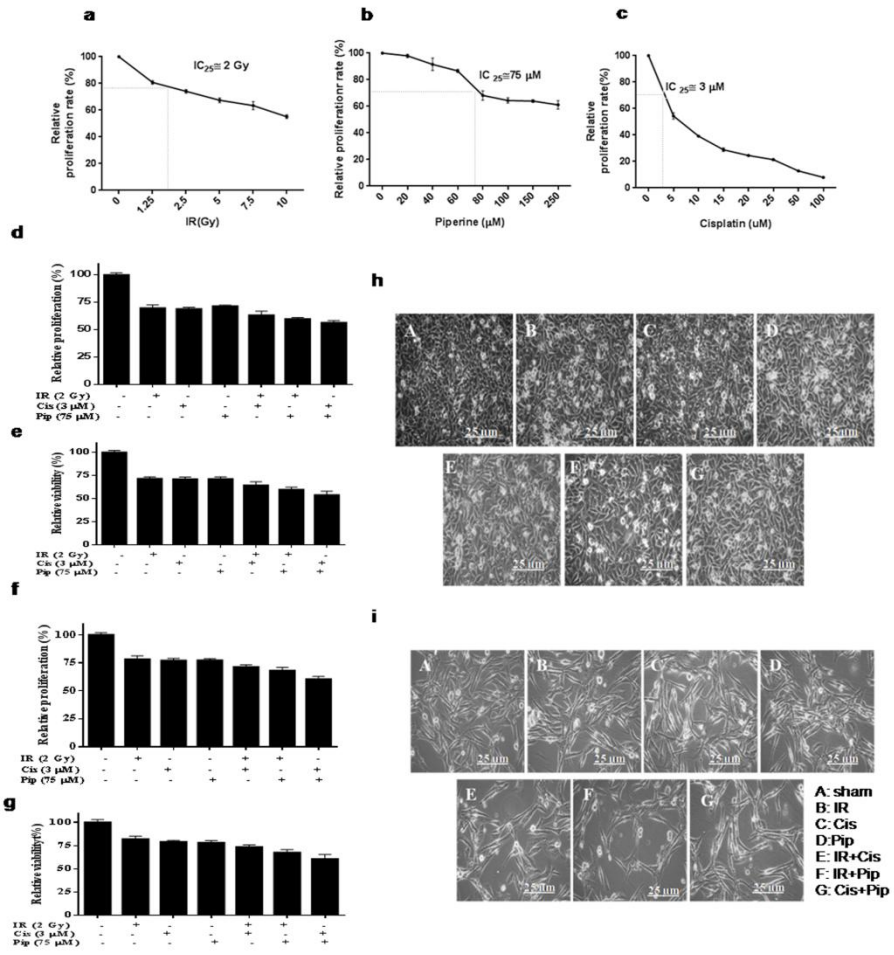


Figure S1: a) Dose optimization of γ- radiation **b**) Dose optimization of piperine. Dimethyl sulfoxide (DMSO) treated cells were used as vehicle control.**c)** Dose optimization of cisplatin. **d)** and **e)** Relative cell proliferation assay of the co-treatment by MTT and Trypan blue dye exclusion assay in MDA-MB-468 and **f)** and **g**) MDA-MB-231 cells. Data represent mean ± SD, n=3 **h)** Representative microscopic images of the MDA-MB-468 and **i)** MDA-MB-231 cells following the co-treatment.

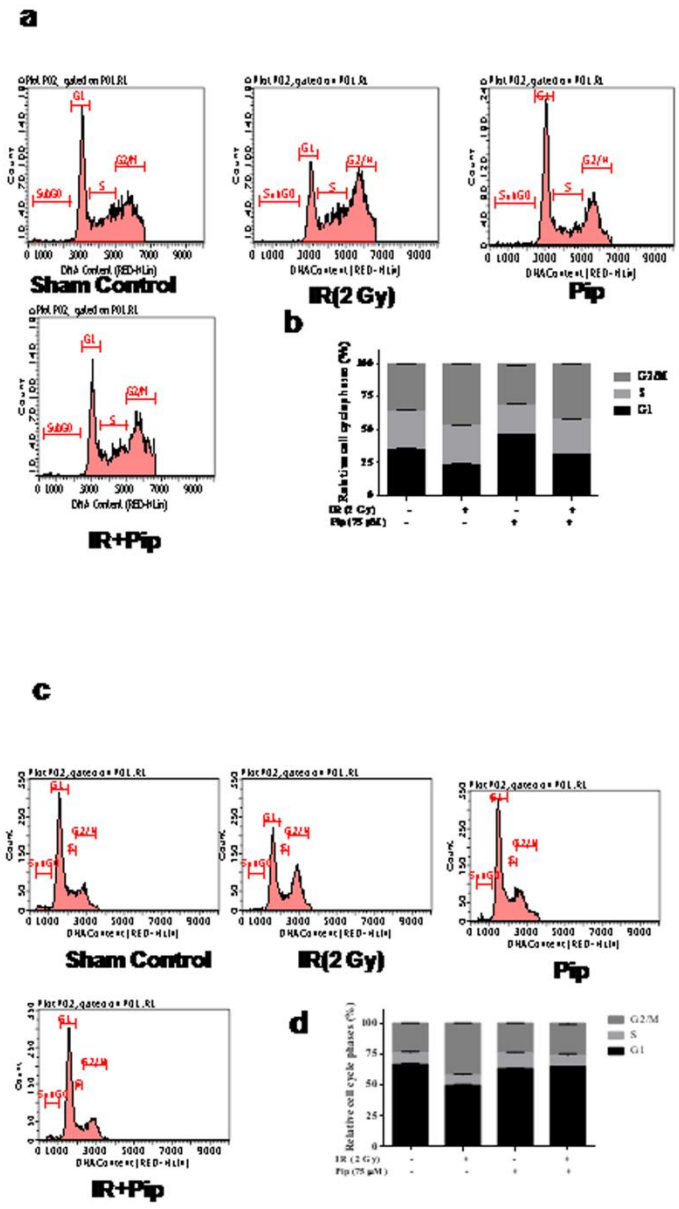


Figure S2: a) and **c)** Histogram representation of the cell cycle distribution, G1, S, and G2/ M phases following co-treatment of piperine and radiation on MDA-MB-468 and MDA-MB-231 cells respectively. **b)** and **d**) Quantification of the cells at different phases of the cell cycle is presented by bar diagram in MDA-MB-468 and MDA-MB-231 cells respectively as the Mean ± SD, n=3.

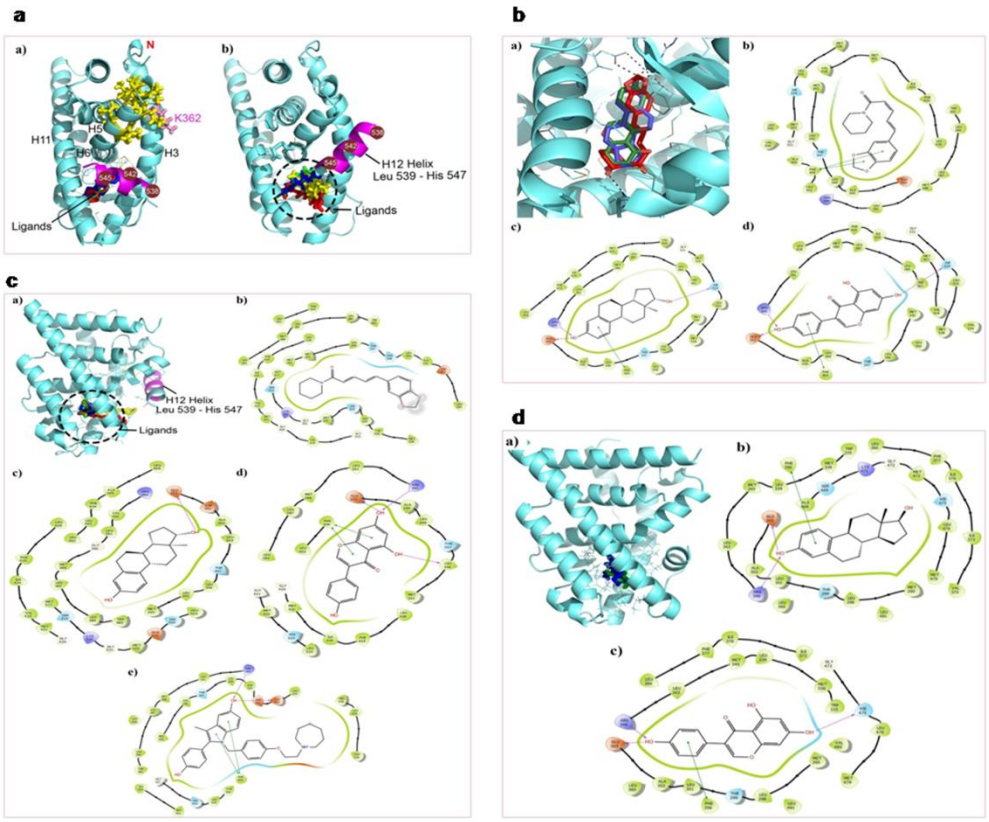
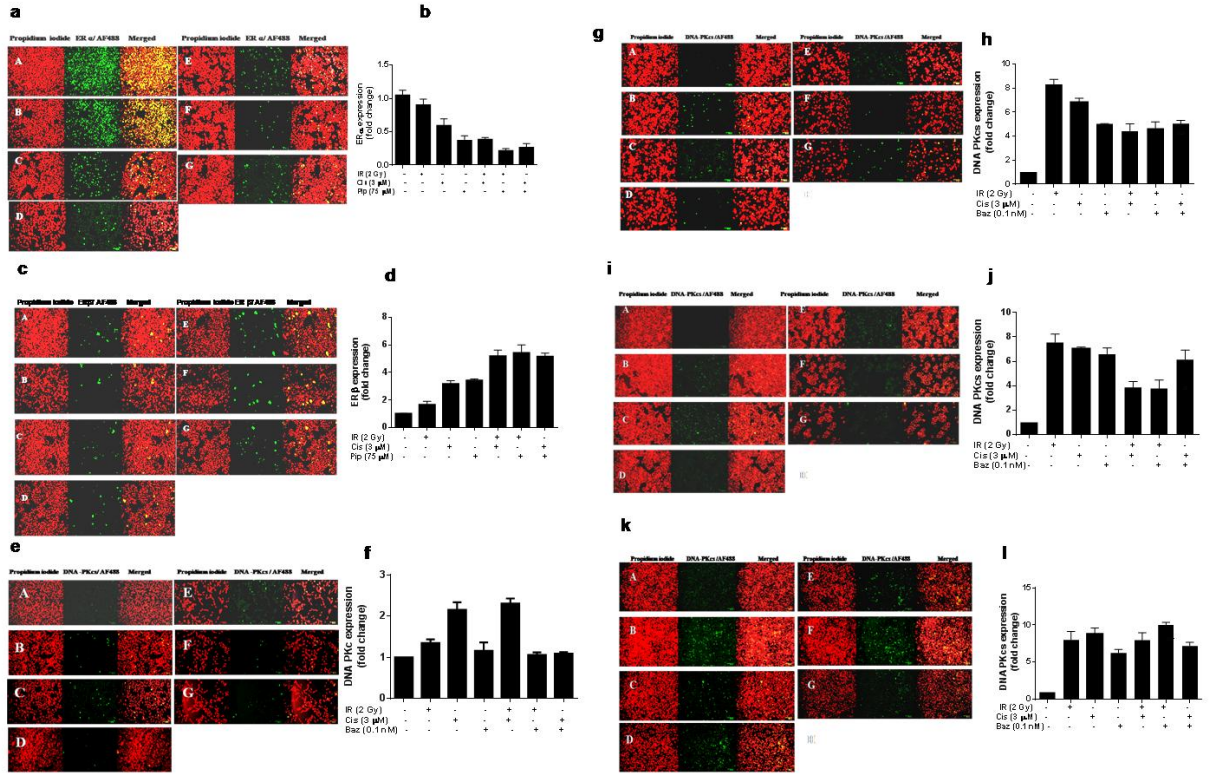


Figure S3: a) Comparison of 1X7R a) vs 1ERR b) to demonstrate piperine shows both agonistic and antagonistic conformation. **b)** Structures of piperine (red), estradiol (blue), and genistein (green) were superimposed in the active site cavity of estrogen receptor α (PDB ID 1X7R) to demonstrate agonistic conformation. Ligand interaction diagram of b) piperine, c) estradiol, and d) genistein with the receptor .**c)** Structures of piperine (red), estradiol (blue), genistein (green), and bazedoxifene (yellow) were superimposed in the active site cavity of an antagonistic conformation of estrogen receptor α(PDB ID: 1ERR) to demonstrate antgonistic conformation. Ligand interaction diagram of b) piperine, c) estradiol, d) genistein, and e) bazedoxifene with the receptor. Bazedoxifene adopted only antagonistic conformation within the ligand-binding domain (LBD) of ERα. **d)** Ligand interaction diagram of b) estradiol (blue), c) and genisteine (green) with the active site residues of estrogen receptor β PDB ID: IX7J. Piperine failed to interact with estrogen receptor β.



ERα and anti-ERβ antibodies showing nuclear localization of ERβ after co-treatment in MCF7 cells. Goat Anti-Rabbit IgG H and L (Alexa Fluor 488) secondary antibody was used for probing. Nuclei (red) were stained with propidium iodide. **b)** and **d)** Bar diagram presents the relative expression of anti-ERα and ERβ positive cells as the mean ± SD, n=3. **e), g), i),** and **k)** Representative images of the immunofluorescence analysis using DNA-PKCs antibody showing expression pattern after co-treatment with bazedoxifene in estrogen receptor-positive MCF7, The estrogen receptor-negative cell line such as MDA-MB 468 and MDA-MB-231 cells and estrogen non-responsive cell line such as A549 cells respectively. **f), h), j),** and **l)** Bar diagram presents the relative DNA-PKcs expression (mean ± SD, n=3), in estrogen receptor-positive cell line, MCF7, the estrogen receptor-negative cell lines such as MDA-MB-468 and MDA-MB-231 and estrogen non responsive cell line, A549 respectively.

Table S1: Piperine and AHR match datasheet pharmacophore with established estrogen receptor agonists and antagonists

Ligand Name	Matched Ligand Sites	Site Score	Fitness
Piperine	A(2) H(5) R(6)	0.7655	1.8174
Bazedoxifene	A(3) H(6) R(11)	0.7237	1.9449
Clomifene	A(1) H(2) R(6)	0.5895	2.3755
Cyclofenil	A(2) H(5) R(7)	0.7894	2.263
Estradiol	A(2) H(7) R(9)	0.9041	2.1693
Lazofixifene	A(1) H(5) R(7)	0.8938	2.3205
Ormeloxifene	A(2) H(8) R(11)	0.8976	2.2986
Ospemifene	A(1) H(5) R(6)	0.9943	2.8176
Raloxifene	A(2) H(7) R(9)	0.5094	1.595
Tamoxifene	A(1) H(4) R(5)	1	3
Toremifene	A(1) H(5) R(6)	1	2.8862
Torifoliol	A(5) H(9) R(12)	0.5122	1.4741

Where A: Hydrogen bond acceptor, H: Hydrophobic/ Non-polar groups, R: Aromatic ring

Table S2: Piperine and AAR match datasheet pharmacophores with established estrogen receptor agonists and antagonists

Ligand Name	Matched Ligand Sites	Site Score	Fitness
Piperine	A(3) A(2) R(6)	0.6797	1.8252
Bazedoxifene	A(2) A(3) R(11)	1	3
Coumestrol	A(4) A(5) R(11)	0.9282	2.3867
Diadzein	A(3) A(4) R(9)	0.817	2.252
Estradiol	A(1) A(2) R(9)	0.6551	1.8635
Formononetin	A(4) A(1) R(9)	0.817	2.2491
Genistein	A(4) A(5) R(11)	0.817	2.2435
Repensol	A(5) A(6) R(13)	0.9283	2.3809
Torifoliol	A(1) A(6) R(13)	0.9283	2.3791

Where A: Hydrogen bond acceptors, R: Aromatic ring