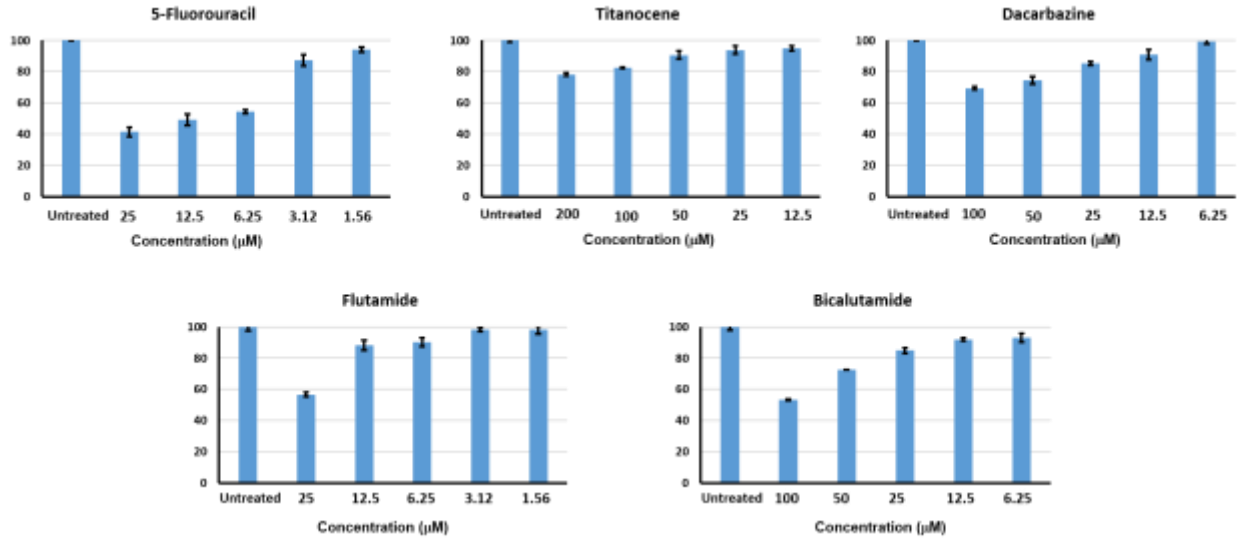
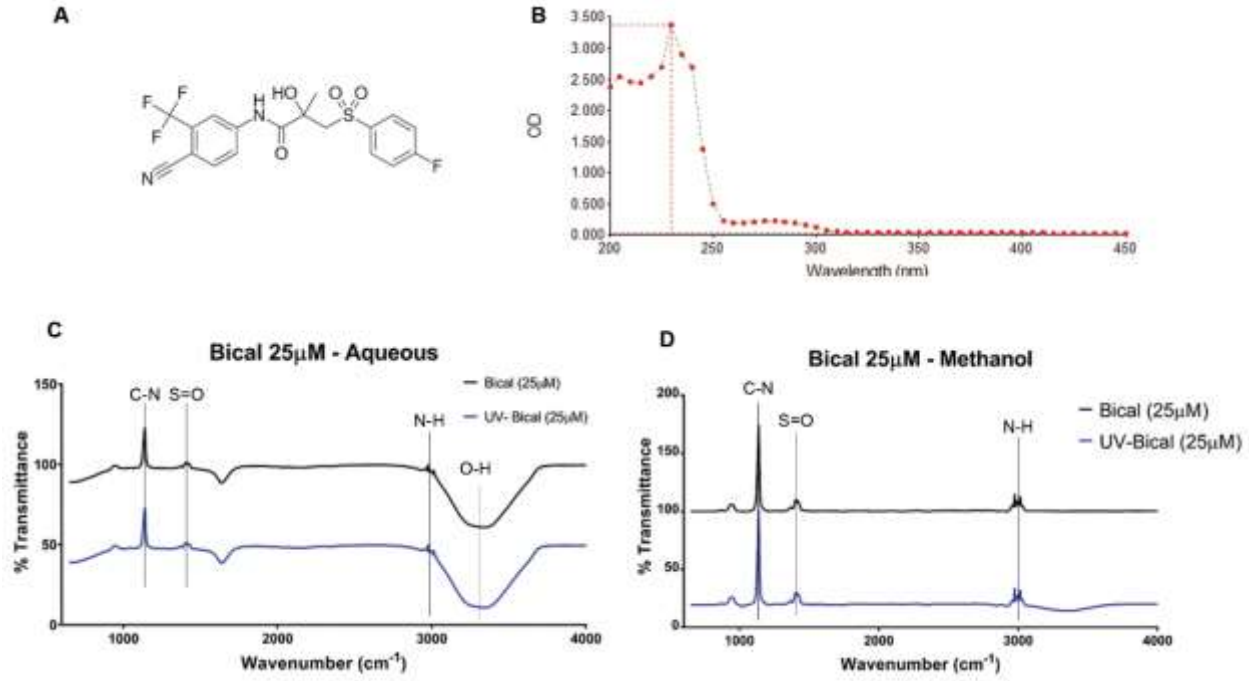
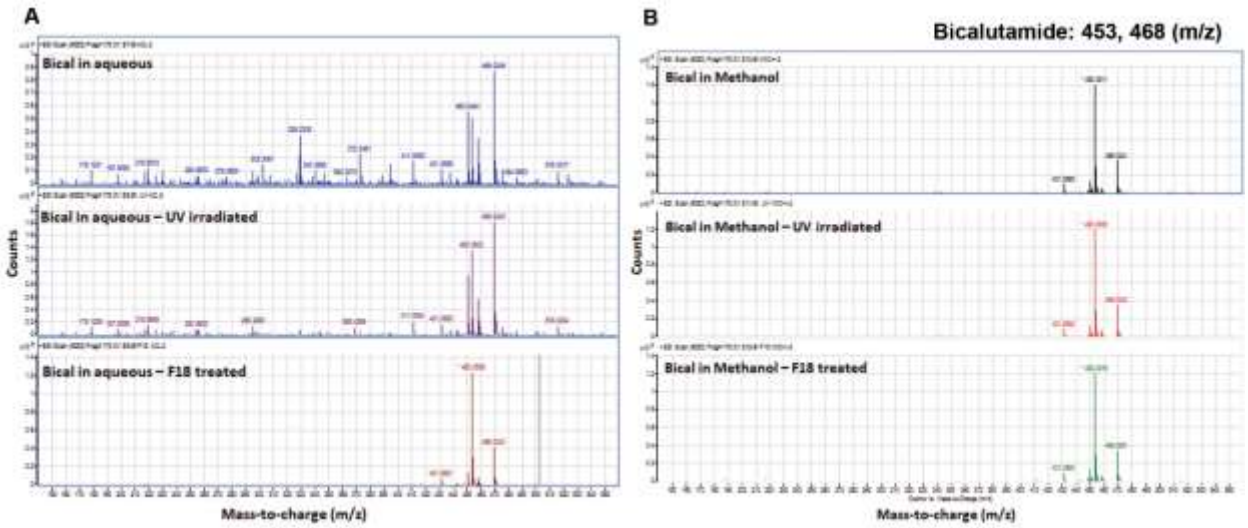


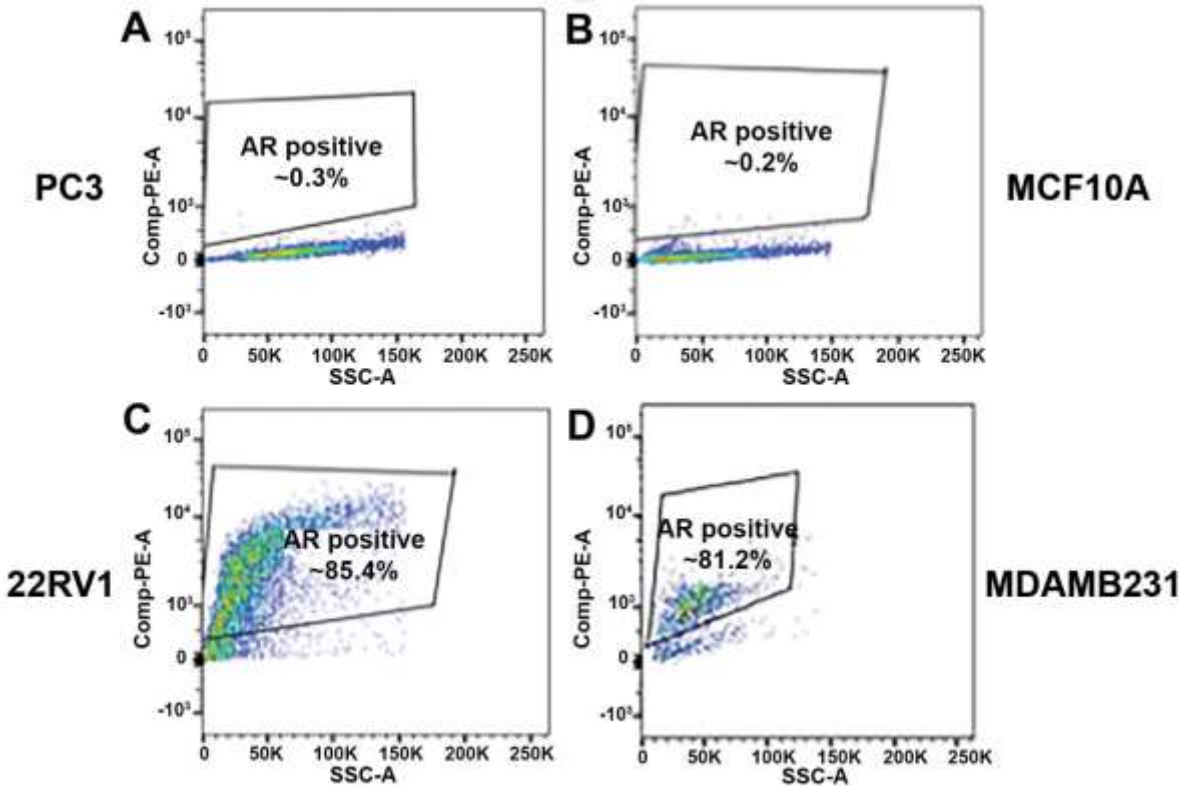
Supplemental Figure 1. Dark toxicity evaluation of 5-Fluorouracil, Titanocene, Dacarbazine, Flutamide and Bicalutamide in MDAMB231 cells. % viability of cells is measured using MTS assay after 72h incubation.



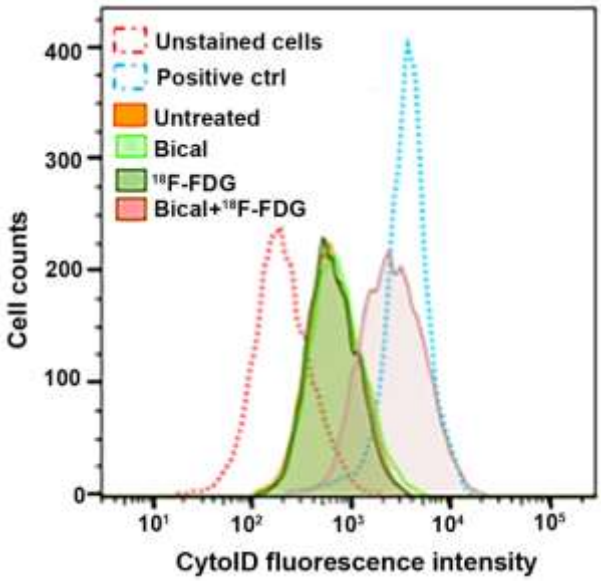
Supplemental Figure 2. (A) Chemical structure of Bicalutamide. (B) UV-vis absorption spectrum of Bicalutamide. (C) FTIR spectrum of Bical in aqueous phase showing no change in the functional groups with and without UV-radiation. (D) FTIR spectrum of Bical in methanol showing no change in the functional groups with and without UV-radiation.



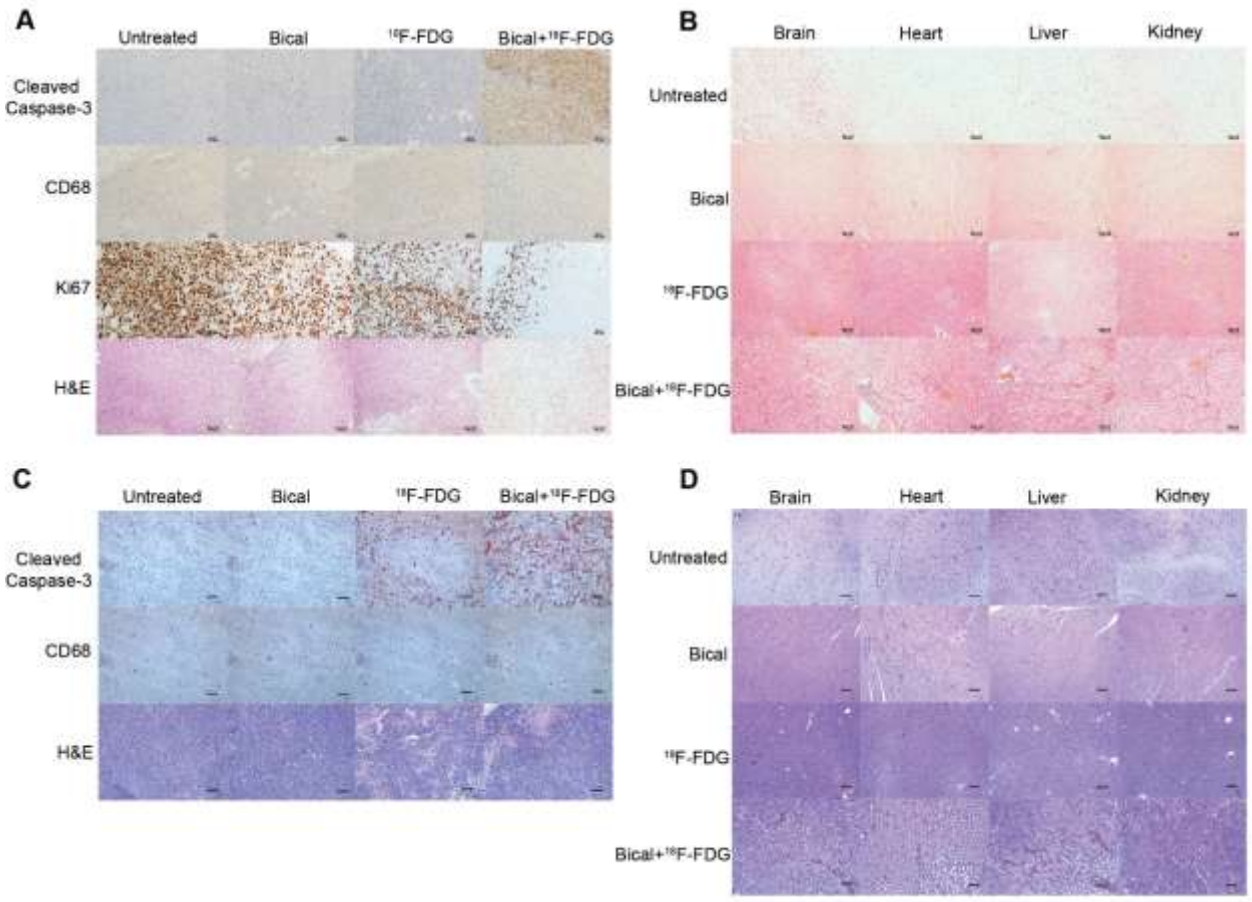
Supplemental Figure 3. (A) Mass spectrometry chromatogram of Bical in aqueous phase irradiated with UV and ^{18}F-FDG, showing no change in the molecular mass (453, 468 m/z). (B) Mass spectrometry chromatogram of Bical in methanol irradiated with UV and ^{18}F-FDG, also showing no change in the molecular mass (453, 468 m/z).



Supplemental Figure 4. Quantification of AR expression in (A) PC3 prostate cancer cells, (B) MCF10A normal breast epithelial cells, (C) 22RV1 prostate cancer cells and (D) MDAMB231 TNBC cells by Flow Cytometry.



Supplemental Figure 5. Flow cytometer analysis of Bical-^{18}F-FDG treated MDAMB231 cells undergoing autophagy. Rapamycin/Chloroquine treated cells used as a positive control.



Supplemental Figure 6. (A) Immunohistochemical staining of xenograft MDAMB231 breast tumor tissues for expression of cleaved caspase-3, measuring tumor macrophage infiltration by CD68 positive staining and Ki67 expression quantification for high proliferating cancer cells, and H&E staining. (B) H&E staining of organs harvested from MDAMB231 tumor bearing mice to confirm the absence of cancer metastasis (Score: 0 for all organs) and minimal off-target toxicity to these vital organs. (C) Immunohistochemical staining of xenograft PC3 prostate tumor tissues for evaluating induction of apoptosis using a TUNEL assay, measuring tumor macrophage infiltration by CD68 positive staining and H&E staining. (D) H&E staining of organs harvested from PC3 tumor bearing mice to confirm the absence of cancer metastasis (Score: 0 for all organs) and minimal off-target toxicity to these vital organs. Scale bar is 100μm.

ADDITIONAL METHODS

Authentication of cell lines

The cells were authenticated for Mycoplasma presence using MycoAlert mycoplasma detection kit (Lonza). Cell lines were disregarded after 20 passages from thawing.

Drug screening in combination with ^{18}F-FDG

Cells were cultured in RPMI cell growth media supplemented with 10% Fetal bovine serum and 1% Pencillin-streptomycin at 37 °C under CO_2 atmosphere. For MTS assay cells were cultured in a 96 well plate at seeding density of 10,000 cells per well overnight. Cells were seeded in triplicates at three dilutions into 96-well plates and incubated for 12–16 hours prior to treatment.

Staining cells for flow cytometry

Cells were stained with fluorochrome conjugated primary antibody: PE conjugated AR (D6F11) XP® Rabbit monoclonal antibody (purchased from Cell Signaling Technology®). Cultured cells ($1\text{-}2x10^6$) were trypsinized and stained with Zombie UV dye for live/dead analysis. The stained cells were washed several times, fixed by 4% PFA and permeabilized by incubating the cells in 90% methanol at least for 30 minutes in ice. The fixed cells were stained with PE conjugated AR (volume ratio of 50:1) with final volume of 100μL 0.5% BSA.

Cell death detection assay

Cell death detection assay was carried out by the measurement of endo- and oligo-nucleosomes that are DNA fragments formed as a result of DNA damage which eventually leads to cell death due to apoptosis. Cells were harvested in 24 well plate with a seeding density $5x10^4$ per well,

with different groups of Bical-^{18}F-FDG along with control treatments of Bical, ^{18}FDG, and untreated cells in triplicate. Cells were washed and lysed at the end of the experiment. Cytoplasmic fractions of cell lysates were separated by centrifugation which contained the damaged DNA as a result of the treatments. Detection of DNA fragments was determined by an ELISA- based technique which involves binding of nucleosomes present in the cytoplasmic fraction via their histone components to the immobilized anti-histone antibody on the microwell plate [using Roche's Cell Death Detection kit]. It was then followed by addition of anti-DNA-peroxidase (POD) which reacts with the DNA-part of the nucleosome and washed thoroughly. The amount of peroxidase retained in the immunocomplex with ABTS (2, 2'-azino-di-[3-ethylbenzthiazoline sulfonate] substrate was determined by absorbance measurement at 405nm and 490nm (as reference wavelength). DNA damage was quantified by the measurement of mono- and oligo-nucleosomes, which is directly correlated with the enrichment factor measured. The enrichment factor was calculated by the ratio of the net absorbance (A_0) of treated cells to that of control cells in a Cytation I plate reader.

H2AX phosphorylation assay

Cells were fixed using 95% ethanol, 5% acetic acid and 1% formaldehyde, followed by treatment with a blocking buffer containing 3% BSA, for one hour at 37°C. Cells were then treated with primary antibody and incubated overnight at 4°C. After proper washing, cells were further treated with detection antibody and incubated for one hour at room temperature. The final step was to incubate the cells with the LumiGLO® chemiluminescent substrate solution and measure the luminescence between 10 and 20 minutes of incubation in a Cytation I plate reader.

Apoptosis, autophagy and cell cycle analysis

MDAMB231 cells were cultured in 6-well culture plates with a seeding density of $0.3x10^6$ and subjected to the treatments with Bical and ^{18}FDG. After 48 hours, the cells were washed properly in PBS and trypsinized with 0.25% Trysin-EDTA. The cells were washed thoroughly by centrifugation at 4°C and were stained with Biolegend's Annexin V-FITC and PI staining solution (1:2) prepared in Annexin V Binding buffer. After 15 minutes of incubation at RT in the dark, the samples were analyzed in a flow cytometer. Similarly, a set of cells samples after Bical-^{18}FDG treatment were collected after trypsinization and washed thoroughly in 1X assay buffer. After 30 minutes of incubation at 37°C in the dark, stained live cells were analyzed in a flow cytometer (BD/LSRII). For cell cycle analysis, MDAMB231 cells were cultured in 6-well plates with a density of 0.3 million cells per well. After 24 hours of post-treatment with Bical and ^{18}FDG, along with their controls, the cells were washed and fixed with 70% ethanol at 4°C for a minimum of one hour. The fixed cells were then stained with RNAse (0.1mg/mL) containing DNA staining PI red dye at the concentration of 20μg/mL at room temperature for 30 minutes. The stained cells were analyzed by flow cytometry for cell cycle arrest checkpoints in response to various treatments.

Animal studies

While 0.74 MBq dose of ^{18}F-FDG in mice is equivalent to a typical human PET scan, the basal metabolic rate per gram of body weight in mice is approximately seven times greater in mice than in humans, and the need for higher spatial resolution in mouse PET imaging necessitates using higher dose in mice for diagnostic imaging. Previous studies have demonstrated that a 0.74 MBq ^{18}F-FDG injection did not cause a significant increase in DNA

damage nor did it generate an adaptive response. The high injection activities such as 7.4 MBq, as used in this study, also did not lead to residual DNA damage as any damage caused was effectively repaired (*1*).

Tumor growth was measured every two days using Vernier calipers by using the formula: $(L \times W^2)/2$ (mm^3), where L is the longest axis and W the shortest axis. Animals were maintained in an Association for the Assessment and Accreditation of Laboratory Animal Care approved facility in accordance with current regulations of the U.S. Department of Agriculture and Department of Health and Human Services. Experimental methods were approved by and in accordance with institutional guidelines established by the Institutional Animal Care and Use Committee.

REFERENCES

1. Taylor K, Lemon JA, Boreham DR. Radiation-induced DNA damage and the relative biological effectiveness of 18F-FDG in wild-type mice. *Mutagenesis.* 2014;29:279-287.