A BENZOTHIAZOLE DERIVATIVE (5g) INDUCES DNA DAMAGE AND POTENT G2/M ARREST IN CANCER CELLS

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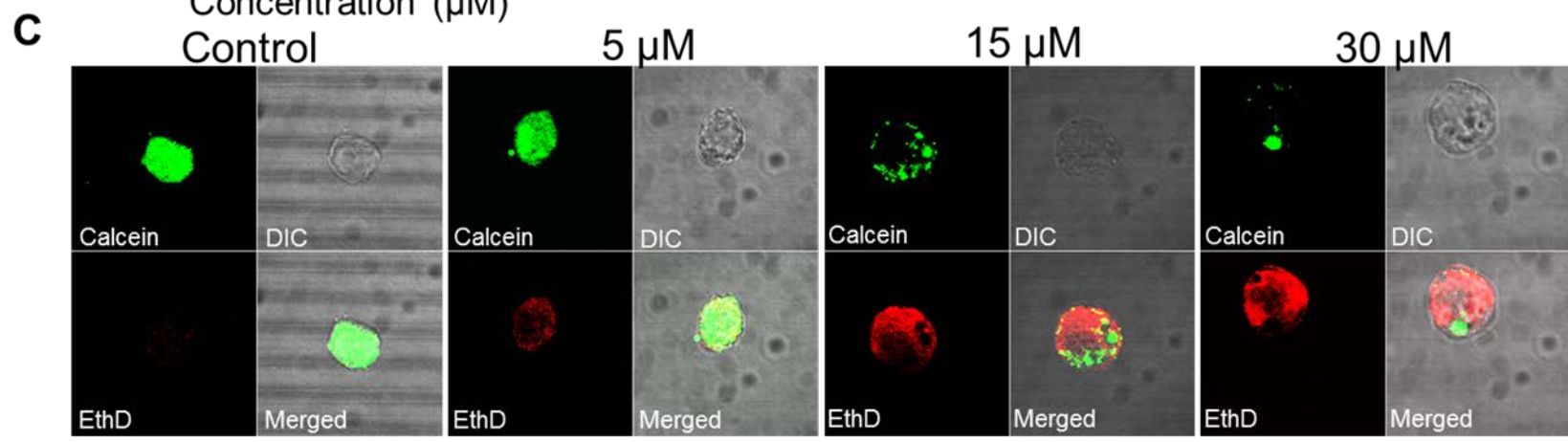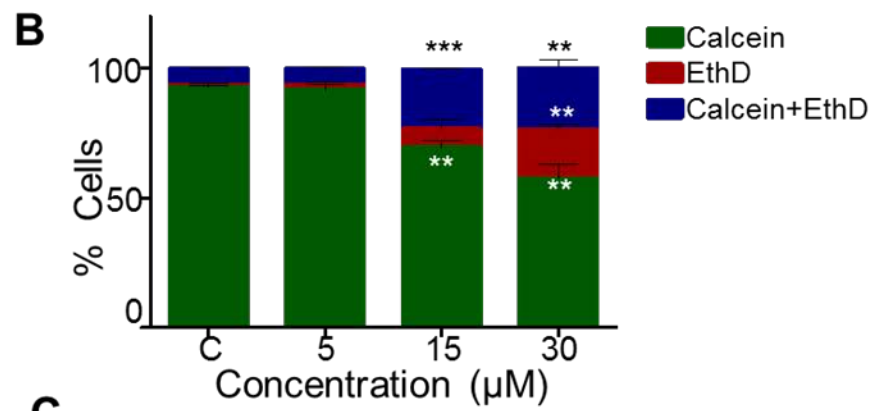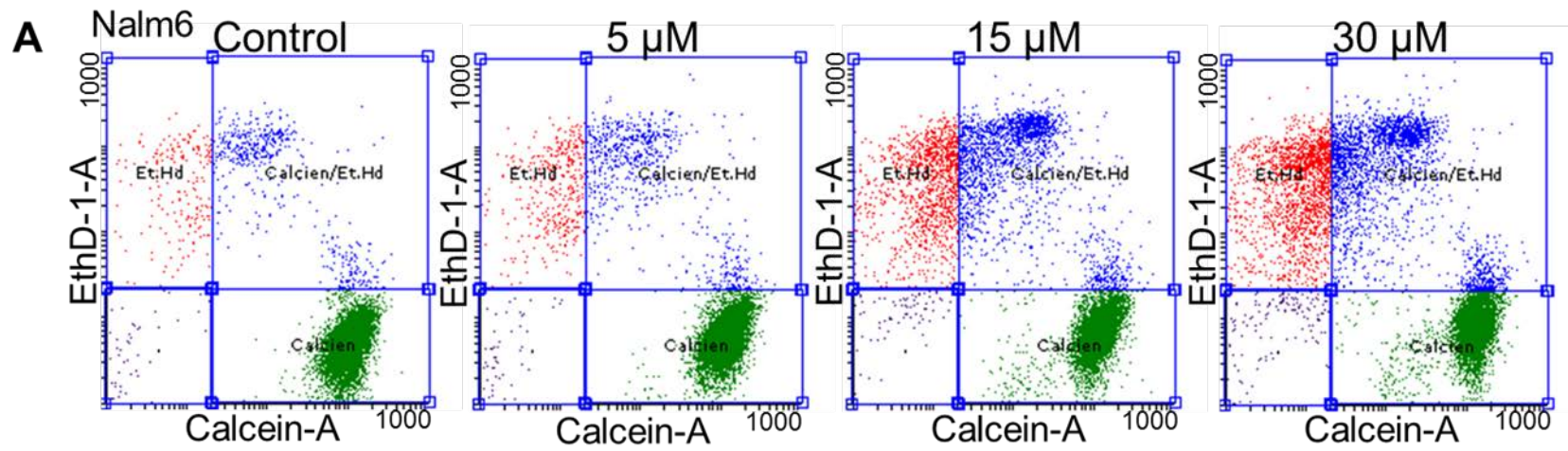
Supplementary Figure legends

Supplementary Figure 1. Live dead cell assay of 5g-treated Nalm6 cells. Nalm6 cells were treated with 5g (0, 5, 15 and 30 μM; 48 h), stained with Calcein-AM and Ethidium Homodimer following which samples were subjected to FACS and confocal microscopy. **A-C.** Dot plots showing 5g-treated Nalm6 cells (A). Cells which are positive for Calcein-AM, Ethidium homodimer and both Calcein-AM/Ethidium Homodimer were quantified and represented in bar diagram (B). The staining was further confirmed using confocal microscopy and representative images are presented with 100x magnification (C). Statistical significance was calculated using student t-test and significance was shown if the p-value was equal to or less than 0.05 (0.05*, 0.005**, 0.0005***).
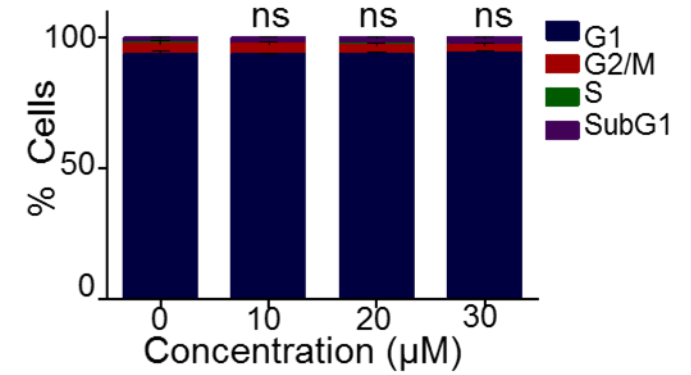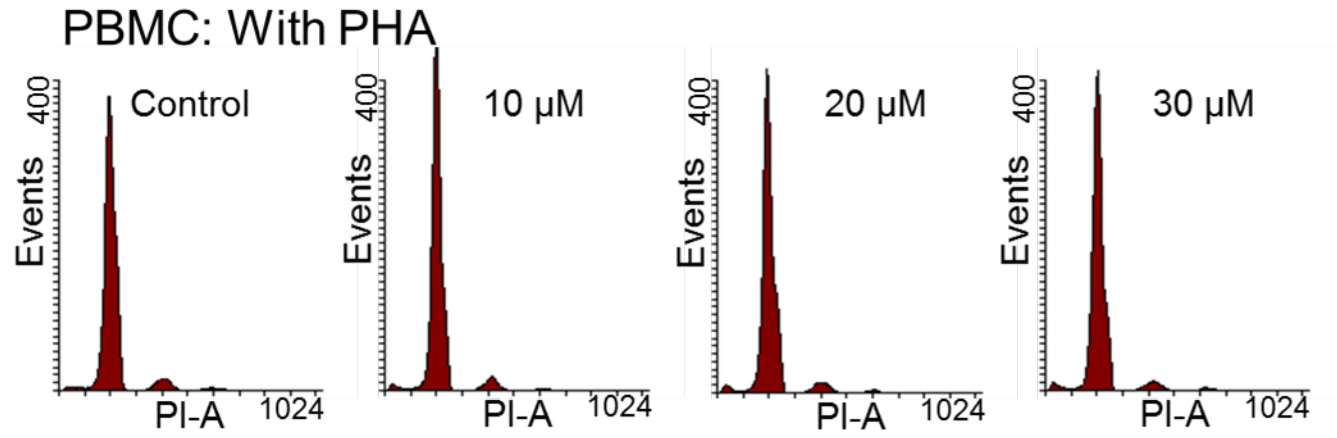
Supplementary Figure 2. Cell cycle analysis of 5g treated PBMCs. PBMCs were treated with different concentrations of 5g (0, 10, 20 and 30 μM; 24 h) in presence of PHA and subjected to FACS analysis. In each case, quantification of different phases of cell cycle was carried out and presented as bar diagram (n=2). Statistical significance was calculated in G2/M population cells using student t-test and significance was shown if the p-value was equal to or less than 0.05 (0.05*, 0.005**, 0.0005***).

Supplementary Figure 3. Cell cycle analysis following treatment with 5g in Nalm6 cells, after different time points. 5g treated Nalm6 cells (0, 1, 5, 10 and 15 μM) were harvested at different time points and processed for cell cycle analysis. **A-C.** Histograms showing effect of 5g on Nalm6 cells at 12, 36 and 48 h after treatment. Quantification of different phase of cell cycle was carried out and presented as bar diagram for respective time points (n=2). Statistical significance was calculated in G2/M population cells using student t-test and significance was shown if the p-value was equal to or less than 0.05 (0.05*, 0.005**, 0.0005***).
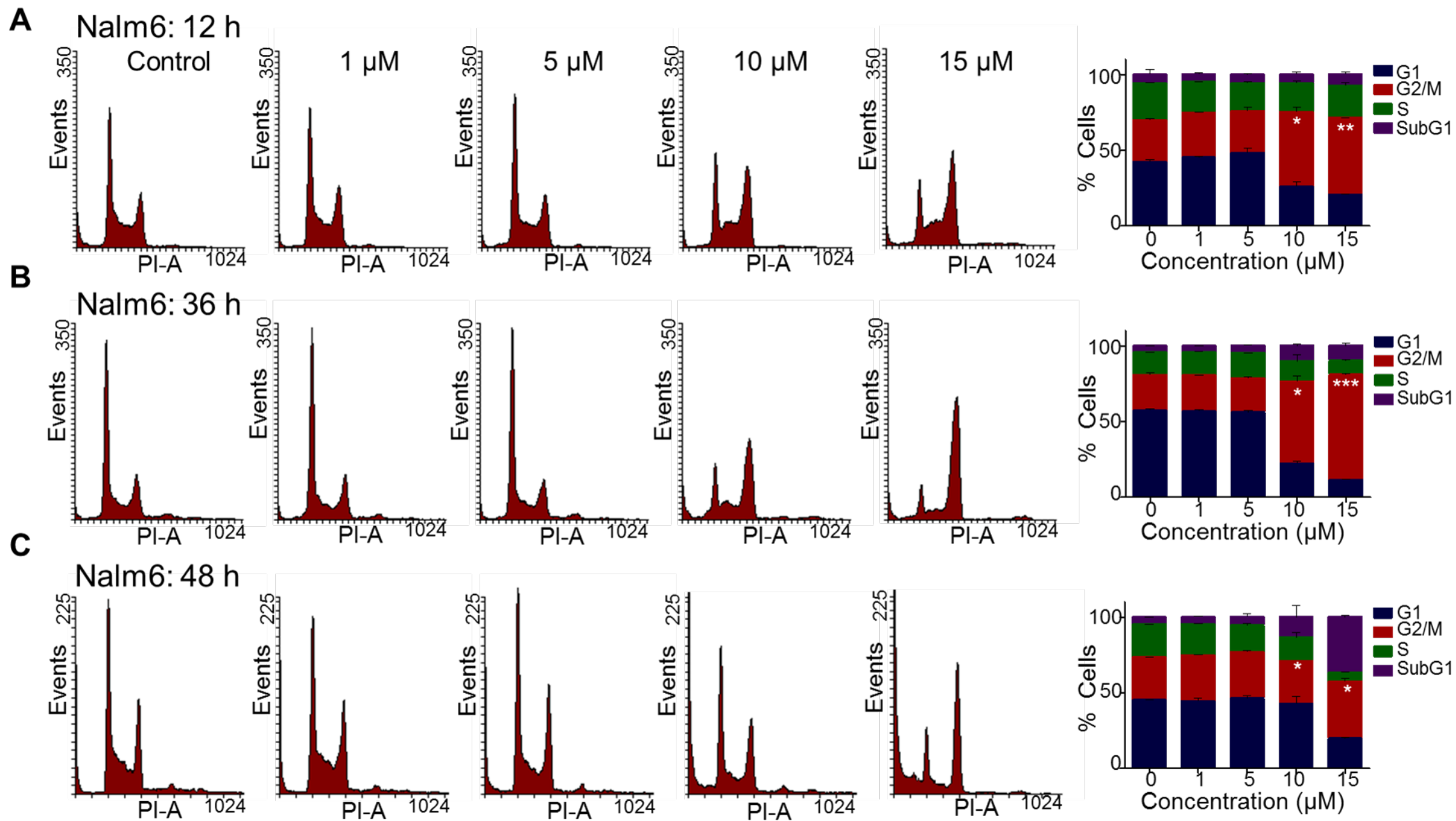
Supplementary Figure 4. Molecular docking studies to assess the binding potential of 5g to Cyclin Dependent Kinases (CDK1 and CDK2). Docked poses of 5g with two of its potential
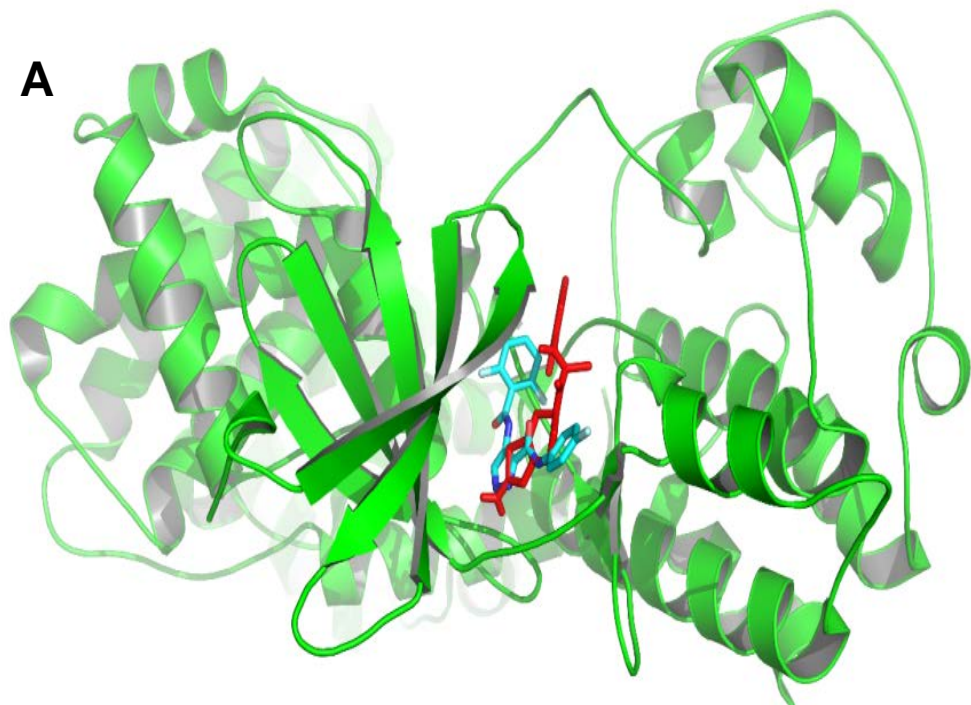
targets (based on higher docking scores), CDK1 (A) and CDK2 (B). Original ligand LZ9 was shown in 'cyan' and docked pose of 5g was shown in red colour. Image depicting important amino acid interactions with 5g is also presented as a separate panel below.

A Nalm6

Control | 5 µM | 15 µM | 30 µM

(Flow cytometry plots: EthD-1-A vs Calcein-A, with gates labeled Et.Hd, Calcein/Et.Hd, and Calcien)

B

% Cells vs Concentration (µM): C, 5, 15, 30

Legend: Calcein (green), EthD (red), Calcein+EthD (blue)

*** ** over 15 and 30 bars; ** annotations within bars

C

Control | 5 µM | 15 µM | 30 µM

Calcein | DIC
EthD | Merged

Suppl. Figure 1

PBMC: With PHA

Suppl. Figure 2

A Nalm6: 12 h

B Nalm6: 36 h

C Nalm6: 48 h

Suppl. Figure 3

A

ILE-10

LEU-83

GLU-81

B

LEU-83

ASP-145

Suppl. Figure 4