***In vitro* effect of a sirtuin inhibitor and its possible synergistic effect with apotential tubulin inhibitor on breast cancer cell lines**

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Background: Breast cancer is among the most prevalent, constituting 11.6% of global incidences and 13.12% of new diagnoses in South Africa in 2018. Owing to the high prevalence of breast cancer, there is an increasing need for anticancer drugs with high efficacy and minimal side effects. One of the most promising avenues in cancer drug development is combination therapy. In this study the *in vitro* effects of newly synthesised sirtuin (PK108-C3) and potential tubulin inhibitor (PK-92) were investigated individually and in combination.

Methods: The effects of PK-92 (0.125-10.0 µM, 48H) and PK108-C3 (2.5-10.0 µM, 48H) on cell proliferation and metabolic activity were evaluated spectrophotometrically using crystal violet staining and dimethylthiazolyl-diphenyl-tetrazolium bromide assays in both MDA-MB-231 and MCF-7 cell lines. Morphological changes were investigated using light microscopy (haematoxylin and eosin staining), and polarization-optical transmitted light differential interference contrast (PlasDIC) microscopy. Cell cycle progression, possible induction of early apoptosis (annexinV-FITC) and mitochondrial membrane potential (MMP) disruption were investigated using flow cytometry. Spectrophotometry was conducted to assess the influence of individual and combination therapy on initiator and executioner caspase-8 and -6 activities.

Results: The half maximal growth inhibitory concentration (GI_{50}) after 48 hours exposure was determined to be 0.125 µM for PK-92 & 7.0 µM for PK108-C3 for both cell lines. Data from microscopy techniques showed compromised cell density and characteristics of apoptosis, including cell shrinkage, hypercondensed DNA, membrane blebbing, as well as the presence of apoptotic bodies, in PK-92, PK108-C3 and combination-treated cells. Cell cycle progression data revealed an increase in the number of cells in sub-G_1 and G_2/M in the PK-92 and combination treated cells. MMP disruption was identified in PK-92, PK108-C3 and combination-treated cells. Annexin V-FITC analysis revealed that all treatments increased the number of apoptotic cell populations, while spectrophotometry indicated the increase of caspase-6 and caspase-8 activity.

Discussion and Conclusions: This *in vitro* study provides evidence that PK-92 and PK108-C3 have potential anti-proliferative effects and induce apoptosis in the MDA-MB-231 & MCF-7 breast cell lines. These findings provide information on newly synthesised, non-commercially available, derivatives of naturally occurring benzofuran and isoflavones contributing to the knowledge base with specific focus on alternative cancer treatment regimens.

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