In vitro effect of a sirtuin inhibitor and its possible synergistic effect with a potential tubulin inhibitor on breast cancer cell line

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INTRODUCTION

Breast cancer is among the most prevalent,[1] constituting 11.6% of global incidences and 13.12% of new diagnoses in South Africa in 2018.[2] Due to low bioavailability, high dose requirements and undesirable side effects,[3,4] there is increased motivation to develop new chemotherapeutics, treatment regimens and delivery systems.[4] One of the most promising avenues in cancer drug development is combination therapy, which is employed to improve the efficacy of single agent treatments.[4] 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

Materials

- PK-92 and PK108 are not commercially available. The compounds were synthesised by Dr Selepe's group from the Department of Chemistry (University of Pretoria, South Africa).
- Estrogen receptor positive breast epithelial cell line (MCF-7) was supplied by Highveld Biological (Pty) Ltd. (Sandringham, South Africa).
- Dimethyl sulfoxide (DMSO) (<0.05%, V/V) was used as vehicle control. Commercially available Sirtinol and the non-commercially available EMBS were used as positive controls for sirtuin and tubulin inhibition, respectively.

Methods

- Spectrophotometry (crystal violet staining assay) was used to investigate the effects of PK-92 and PK108-C3 on cell proliferation. The dimethylthiazolyl-diphenyl-tetrazolium bromide (MTT) assay was used to measure cellular metabolic activity as an indicator of cell viability.
- Polarization-optical differential interference contrast microscopy (PlasDIC) was employed to view changes in cell morphology. Quantitative data for the mitotic indices was acquired by light microscopy (haematoxylin and eosin (H&E) staining).
- Cell cycle progression, possible induction of apoptosis (annexin V-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 GI_{50} after 48 hours exposure was determined, through crystal violet and MTT, 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 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.

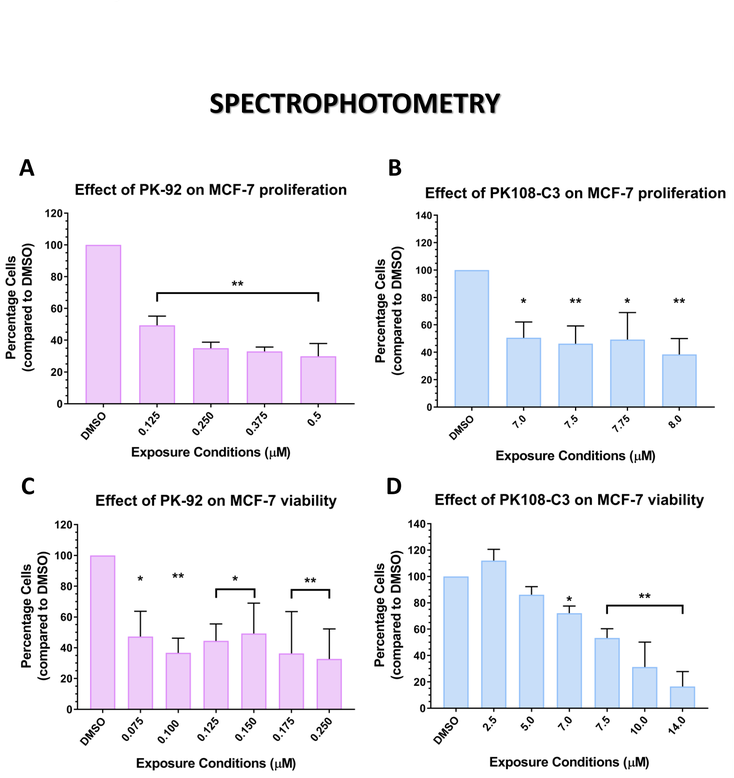

Fig 1. Cell proliferation and viability graphs of the effect(s) of 48 h exposure to PK-92 (A&C) and PK108-C3 (B&D) on MCF-7 cells. The GI50 after 48 hours was determined to be 0.125 µM for PK-92 (A) & 7.0 µM for PK108-C3 (B) using crystal violet staining. The MTT assay also showed reduced viability of MCF-7 cells following 48 h exposure.

Table 1. GI values at 48H for PK-92 and PK108-C3 combinations.

Cell line	PK-92 (GI_{50})	PK108-C3 (GI_{50})	Combination GI_{50}
MCF-7	0.125 µM	7.0 µM	PK-92: 0.10 µM PK108-C3: 4.2 µM

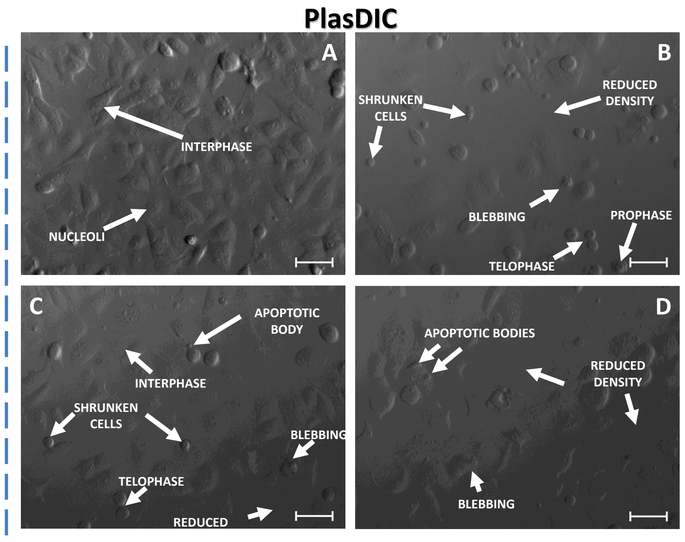

Fig 2. PlasDIC photos of MCF-7 cells after 48 h exposure to vehicle-treated control (A), PK-92 (B), PK108-C3 (C) and the combination (D) (scale bar indicates 200µm). Data showed compromised cell density and characteristics of apoptosis in PK-92, PK108-C3 and combination-treated cells.

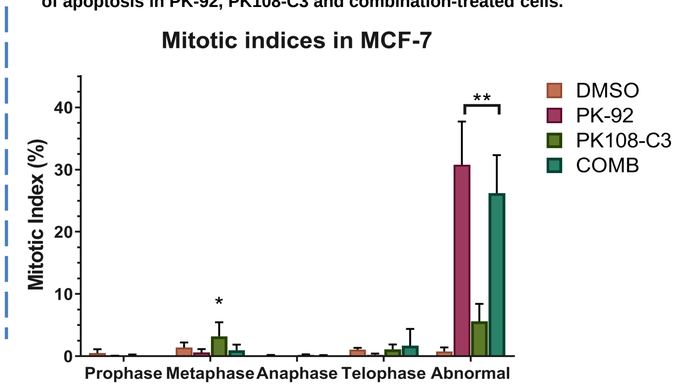

Fig 3. Mitotic indices of MCF-7 cells after 48 h exposure to PK-92, PK108-C3, combination treatment and DMSO. PK92, PK108-C3 and combination-treated cells showed significant increase in the presence of abnormal cells.

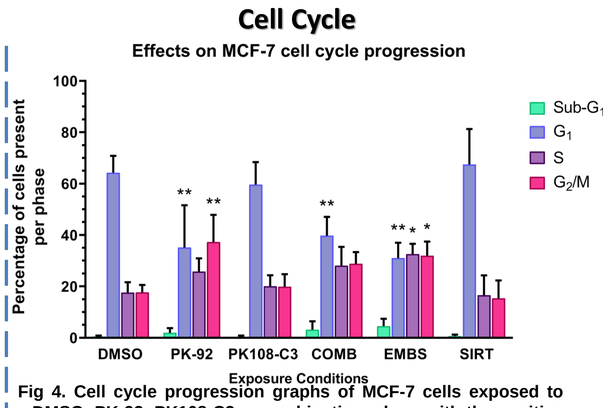

Fig 4. Cell cycle progression graphs of MCF-7 cells exposed to DMSO, PK-92, PK108-C3, a combination, along with the positive controls EMBS and sirtinol. Exposure to PK-92 and the combination significantly increased cells in G2/M, similarly to EMBS

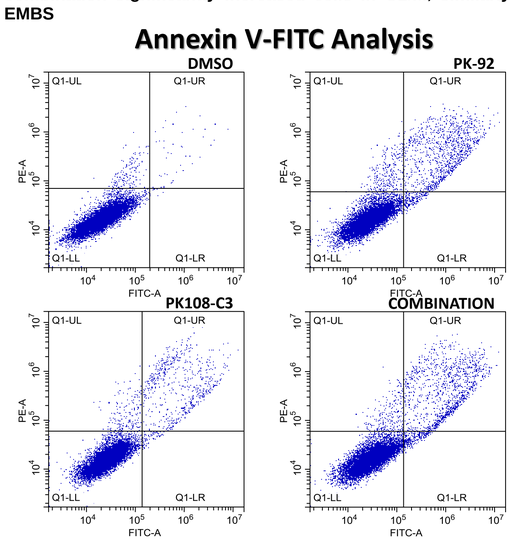

Fig 5. Apoptosis induction of MCF-7 cells. PK108-C3 exposure caused an increase in the number of cells in late apoptosis, while PK-92 and the combination treatment resulted in an increase in the number of cell in early apoptotic and in late apoptosis.

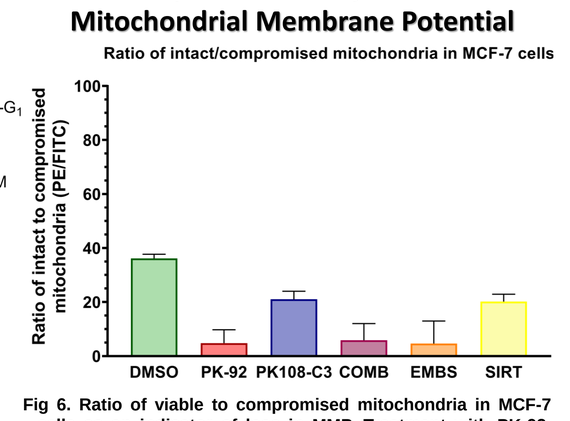

Fig 6. Ratio of viable to compromised mitochondria in MCF-7 cells as an indicator of loss in MMP. Treatment with PK-92, PK108-C3 and their combination increased MMP loss, resulting in lower ratios when compared to DMSO, similarly to their respective controls EMBS and Sirtinol. MMP disruption was identified in PK-92, PK108-C3 and combination-treated cells.

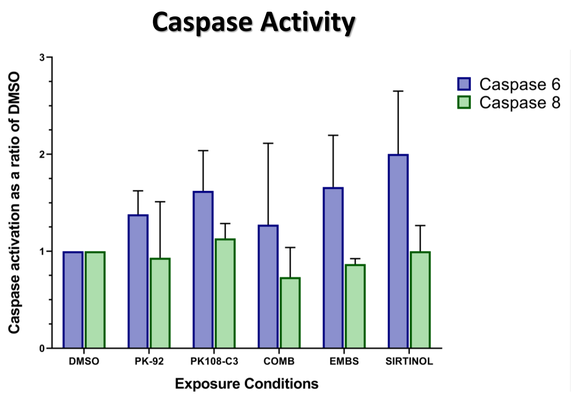

Fig 7. Caspase activation graphs of the effect(s) of 48 h exposure to PK-92, PK108-C3, the combination with their respective controls EMBS and Sirtinol. PK108-C3 resulted in an increase in both caspase-6 and -8 activity in MCF-7 cells.

DISCUSSION

This *in vitro* study provides evidence that PK-92 and PK108-C3 have potential anti-proliferative effects on MCF-7 breast cells. At the identified GI_{50}, PK-92 and PK108-C3 presented markers of apoptosis including cell shrinkage, hypercondensed DNA, membrane blebbing, as well as the presence of apoptotic bodies in PlasDIC images. Quantitative analysis of H&E images revealed a significant increase in the presence of metaphase cells following PK108-C3 exposure. Cell cycle analysis revealed an increase of cells present in G_2/M, representative of a mitotic block, following PK-92 and combination treatment. Both PK-92 and PK108-C3 result in the induction of apoptosis, evidenced by the increased annexin V-FITC binding. The three treatments resulted in an increase in compromised mitochondria, with reduced MMPs. The findings suggest that the observed cell death, and resulting decrease in cell density, is of an apoptotic origin. 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. Future studies will be conducted to further investigate the mechanism of action(s) of these compounds.

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