Novel synthetic naphthoquinone-derived compounds can inhibit cell viability by blocking cell cycle progression and inducing cell death in metastatic prostate cancer cell lines

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INTRODUCTION

Patients presenting metastatic prostate cancer (PCa) generally acquire resistance to androgen deprivation and docetaxel (DXT) therapies. New approaches have been tested in order to propose innovative therapeutic approaches, including testing of novel products derived from natural compounds or their combination with current chemotherapeutic drugs used at first line treatment in PCa.

OBJECTIVES

- Evaluate the cytotoxic effect of novel synthetic naphthoquinone-derived compounds in metastatic prostate cancer cells lines
- Investigate the cellular and molecular mechanisms by which compounds the inhibit cell viability

METHODOLOGY

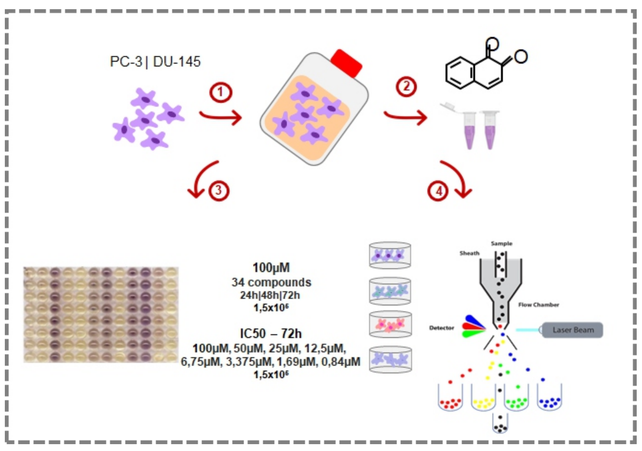

Figure 01. Methodology. 1) PC-3 and DU-145 metastatic prostate cancer cells were cultured in RPMI-1640 medium supplemented with 10% SFB + 1% P/S; 2) The naphthoquinone derived compounds were resuspended in DMSO and diluted in PBS; 3) Cell lines were plated in 96 well plates for 24h until the next day. Cell viability in response to 100µM of each compound was tested after 24h, 48h and 72h by using MTT assays. IC50 values of each compound was selected by plating cells in 6-well plates for 24h until the next day using concentration ranges from 0,84 µM of 100 µM. By using the concentrations that induced higher inhibition on cell viability, we the performed cell cycle analysis and apoptosis assays.

Table 01. Structural and molecular formula of novel synthetic naphthoquinone-derived compounds. We evaluated cytotoxic effect of 34 synthetic distinct naphthoquinone-derived compounds, classified into bis-quinones (n=3), thiols-quinones (n=3), mannich bases (n=18) and coumarin thiols (n=10) over PC-3 and DU-145 metastatic prostate cancer cells lines by using 3-(4,5-dimethylthiazol -2-yl)-2,5-di-phenyl tetrazolium bromide (MTT) assays. Propidium iodide staining for simultaneous cell cycle analysis and apoptosis determination have been performed.

SYNTHETIC NAPHTOQUINONE-DERIVED		
Name	**Molecular Formula**	**Chemical Structure**
MANNICH BASES		
RC 01	$C_{21}H_{19}NO_4$	
RC 02	$C_{21}H_{18}N_2O_6$	
RC 03	$C_{21}H_{18}NO_5$	
RC 04	$C_{13}H_{13}NO_3$	
RC 05	$C_{17}H_{12}O_3S$	
RC 06	$C_{18}H_{14}O_3S$	
RC 07	$C_{15}H_{15}NO_4$	
RC 08	$C_{16}H_{17}NO_3$	
RC 09	$C_{17}H_{19}NO_3$	
RCE-11	$C_{24}H_{19}NO_3$	
RCE-12	$C_{24}H_{18}BrNO_3$	
RCE-13	$C_{24}H_{19}NO_4$	
RCE-14	$C_{19}H_{17}NO_3$	
RCE-15	$C_{20}H_{19}NO_3$	
RCE-16	$C_{24}H_{18}N_2O_5$	
RCE-17	$C_{24}H_{18}ClNO_3$	
RCE-18	$C_{24}H_{18}FNO_3$	
RCE-19	$C_{21}H_{21}NO_3$	

SYNTHETIC NAPHTOQUINONE-DERIVED		
Name	**Molecular Formula**	**Chemical Structure**
COUMARIN THIOLS		
CA-02	$C_{17}H_{14}O_3S$	
CA-05	$C_{16}H_{11}ClO_3S$	
CA-06	$C_{17}H_{14}O_4S$	
CA-07	$C_{16}H_{11}FO_3S$	
CA-08	$C_{15}H_{12}O_4S$	
CA-09	$C_{16}H_{12}O_4S$	
CA-10	$C_{17}H_{14}O_3S_2$	
CA-12	$C_{16}H_{12}O_3S$	
CA-13	$C_{20}H_{14}O_3S$	
CA-16	$C_{16}H_{18}O_3S$	
BIS-QUINONES		
RC 10	$C_{21}H_{12}O_7$	
RCDFC	$C_{21}H_{12}O_6$	
RCDOH	$C_{21}H_{12}O_8$	
THIOLS-QUINONES		
4F-Tiol	$C_{20}H_{15}FO_3S$	
Fenil Sulfona	$C_{20}H_{16}O_5S$	
3-CH3-Tiol	$C_{21}H_{18}O_3S$	

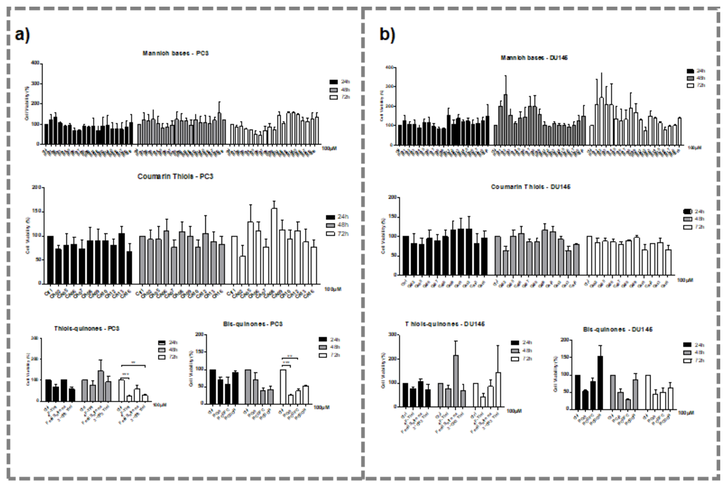

Figure 02. Cell viability of metastatic prostate cancer cells lines in response to novel synthetic naphthoquinone-derived compounds . MTT assays using a) PC-3 and b) DU145 metastatic cell lines have been performed using 100 µM of each compound, which are classified into 4 families: Mannich bases, coumarin thiols, thiols-quinones and bis-quinones. Among the 34 compounds tested, 4 of them (2 mannich bases and 2 thiols-quinone) promoted higher inhibition on PC-3 and DU-145 cells. The results are represented as mean ± standard deviation (SD). Statistical analysis was performed using t-test (*p<0.01, **p<0.001, ***p<0.0001).

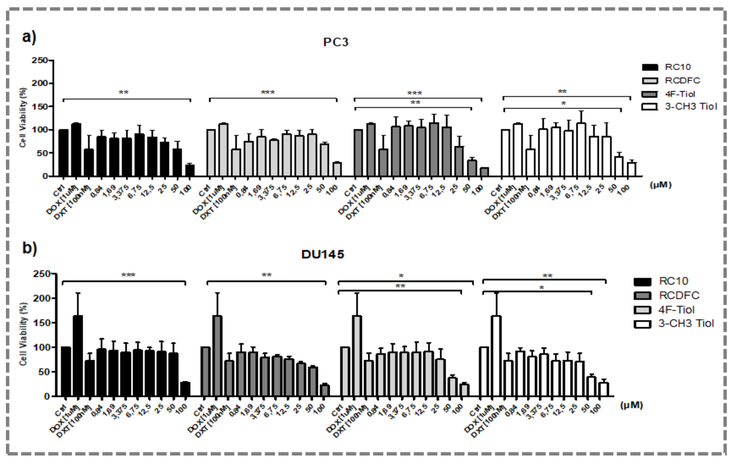

Figure 03. IC50 analysis of of 4 novel synthetic naphthoquinone-derived compounds metastatic prostate cancer cells lines. The analysis was performed using concentrations ranging from 0,84 µM to 100 µM of each compound. Two of these compounds are mannich bases, while the other 2 correspond to thiols. a) PC-3, and b) DU145 metastatic cell lines. In both cell lines, we observed a decreased cell viability using 100 µM of both RC10 and RCDFC compounds and 100 µM and 50 µM of 4F-tiol and 3-CH3 tiol, respectively. Based on obtained results, we choose these compound concentrations to evaluate cell cycle and apoptosis. The results are represented as mean ± standard deviation (SD). Statistical analysis was performed using t-test (*p<0.01, **p<0.001, ***p<0.0001).

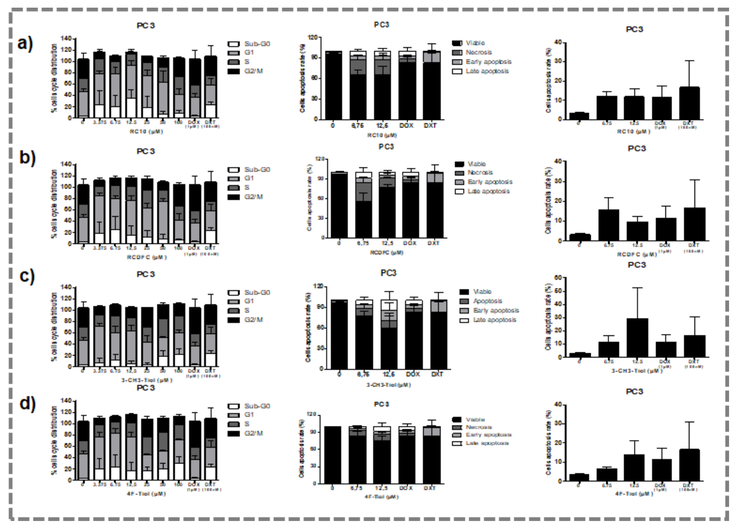

Figure 04. Cell cycle and apoptosis analysis of metastatic prostate cancer cells lines in response to 4 novel synthetic naphthoquinone-derived compounds. Cell cycle analysis was performed using compound concentrations exhibiting higher inhibition on cell viability (50 µM, 25 µM, 12,5 µM, 6,75 µM and 3,375 µM). Apoptosis analysis were been performed using the 2 compound concentrations (6,75 µM and 12,5 µM), which promoted higher cell accumulation in Sub-G0/G1 phase of cell cycle. a) RC10, b) RCDFC, c) 3-CH3-Tiol, d) 4F-Tiol. In cell cycle analysis 12,5 µM and 6,75 µM of each compound promoted higher accumulation of cells in Sub-G0/G1 phase of cell cycle. In these concentrations there were only ~10% apoptotic cells. The results are represented as mean ± standard deviation (SD).

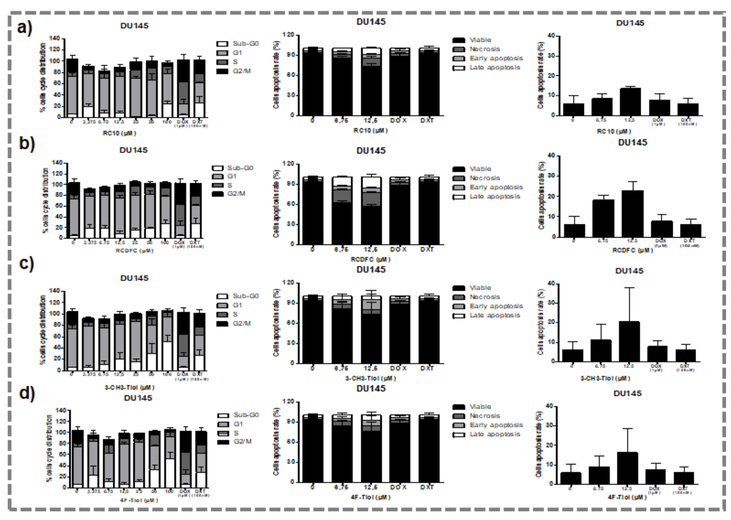

Figure 05. Cell cycle and apoptosis analysis of DU145 cancer cell line in response to 4 novel synthetic naphthoquinone-derived compounds. Cell cycle analysis was performed using compound concentrations promoting higher inhibition on cell viability according IC50 analysis (50 µM, 25 µM, 12,5 µM, 6,75 µM and 3,375 µM) of each compound. In apoptosis analysis, we used 2 **compound** concentrations that promoted higher accumulation of cells in Sub-G0/G1 of cell cycle. a) RC10, b) RCDFC, c) 3-CH3-Tiol, d) 4F-Tiol. In cell cycle analysis, we found that 12,5 µM and 6,75 µM of most of compounds promoted a higher accumulation of cells in Sub-G0/G1 phase and ~10% of apoptotic cells. . The results are represented as mean ± standard deviation (SD).

CONCLUSIONS

Altogether, our data evidence the characterization of 4 novel naphthoquinone-derived compounds pertaining to bis-quinones and thiols-quinones class, displaying cytotoxic effect over PC3 and DU145 metastatic prostate cancer cells, mainly by blocking cell cycle progression. Further studies should better investigate the mechanisms and signaling pathways by which these compounds inhibit PCa cell viability and how it could behave in combination to currently used chemotherapy, such as DXT.

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