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Structure Activity Relationship Studies of Novel Diarylpentanoid Analogs Targeting The Androgen Receptor in Prostate Cancer Cells

Haili Coffin
Chapman University, coffi106@mail.chapman.edu

Marco Bisoffi
Chapman University, bisoffi@chapman.edu

Structure Activity Relationship Studies of Novel Diarylpentanoid Analogs Targeting The Androgen Receptor in Prostate Cancer Cells

Haili Coffin and Dr. Marco Bisoffi

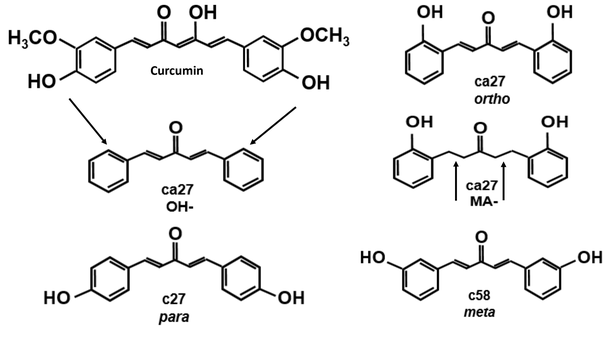
Chapman University, Schmid College of Science and Technology, Biochemistry and Molecular Biology, Orange, CA

Introduction

- Prostate cancer is the second most common cancer in American men with an incidence and mortality of approximately 240,000 and 30,000 men, accordingly.
- Curcumin, a natural phytochemical of the plant *Curcuma longa* and an ingredient in the spice Turmeric, has been shown to inhibit prostate cancer cell growth and has inspired the synthesis of synthetic analogs, including a vast number of structurally diverse diarylpentanoids.
- Prostate cancer progression relies strongly on the activation of the androgen receptor (AR) signaling pathway by its natural ligand dihydrotestosterone.
- The curcumin analog ca27 has been previously shown to down-regulate AR expression via an unknown mechanism of action.
- The purpose of the present work was to conduct functional structure activity relationship (SAR) studies for analogs of ca27 to determine the moiety of ca27 responsible for AR down-regulation.

Hypothesis and Objectives

- We hypothesized that the presence and position of the hydroxyl groups of ca27, as well as the Michael acceptors (α-β unsaturated carbonyl) are important for AR down-regulation.
- Our studies aim at identifying active pharmacophores of diarylpentanoids that down-regulate AR expression. When targeted to prostate cancer cells, this could lead to the development of novel therapeutics against castration resistant prostate cancer.
- The following diarylpentanoid compounds were used. They differ in the length of the alkenyl space between aryl rings, the nature and position of hydroxyl side groups, and the presence of Michael acceptors.



Experimental Methods

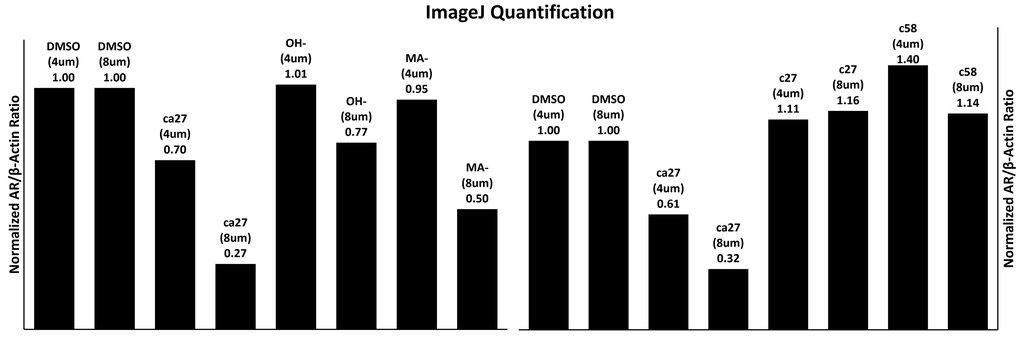
Cells: Human LNCaP prostate cancer cells were cultured at 37°C in humidified air containing 5% CO_2.
Drug Treatment: Cells were seeded at 150,000 cells per well in a 12-well plate and grown for 48 hours to ~90% confluency. Cells were treated for 16 hours with 4μM and 8μM drug final concentrations. Cells were lysed with Triton X100, spun down, and whole cell lysate was transferred into new Eppendorf tubes.
Bradford Assay: Protein concentration was determined by Bradford reagent using BSA (bovine serum albumin) as a standard. Absorbance was measured at 595 nm.
Western Blot & Image J analysis: Detection of AR protein expression was determined by Western blot using specific antibodies. AR signal intensity was normalized to β-actin. Signal intensities were determined using densitometric analysis of digitized images using ImageJ software.
Cell imaging. The dose-dependent effect of ca27 and its analogs was visualized by bright field light microscopy (transmitted light).

Results

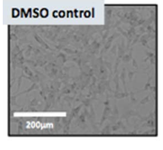
Western Blot Analysis

Levels of Androgen Receptor (AR) in LNCaP prostate cancer cells treated with ca27 analogs. β-Actin is used as the control. From left to right dose dependency is shown with 4um of each compound followed by 8um.

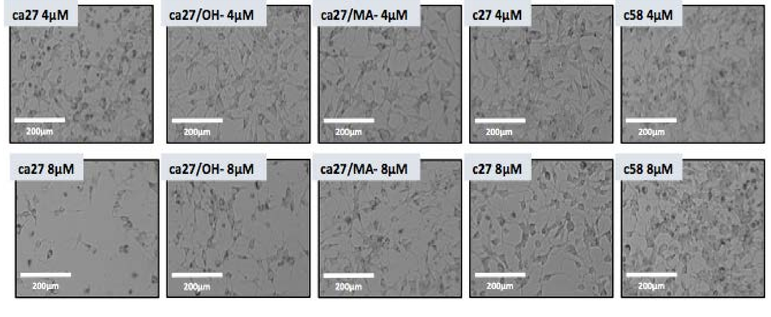
ImageJ Quantification



Quantification of Western blot analysis using Image J shows the numerical values of AR present in LNCaP cells after treatment with 4um and 8um ca27 analogs. DMSO serves as a negative control and ca27 as a positive control. The ratio's were normalized over the housekeeping gene β-Actin.



LNCaP cells imaged using bright field microscopy to visually depict morphological changes (toxicity, cell death) induced by the curcumin analogs.



Conclusions

Our results show that:

- Diarylpentanoid analogs of the natural product curcumin (a diarylheptanoid), especially ca27, are potent dose-dependent down-regulators of the androgen receptor.
- The hydroxyl groups are stronger contributors to AR down-regulation than the Michael acceptors.
- The position of the hydroxyl groups is important for the observed AR down-regulation. The ortho position is more potent than the meta or para position.
- The observations on the AR down-regulation are mimicked by the effects of the curcumin and ca27 analogs on cell morphology. Ca27 is the most cytotoxic compound and induces cell death.

Future Research

- Optimize assay design to reduce variation.
- Expand investigations to various cancer cell types, including pancreatic and breast cancer cell models.
- Determine the mechanism of action of the diarylpentanoid compounds.

Significance

- AR is a therapeutic target against prostate (and other) cancer cells.
- Diarylpentanoid analogs of curcumin may lead to the identification of lead pharmacophores that lend themselves to further drug design for the chemoprevention and/or treatment of solid tumors, including prostate cancer.

Acknowledgements

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