Purpose
Prostate cancer is the second leading cause of mortality among American men with the highest incidence rate of all cancers reported in the US. Conventional therapies to treat prostate cancer suffer from dose limiting toxicity and are invariably marked by therapeutic failure and by emergence of a more aggressive and fatal phenotype known as castration resistant prostate cancer (CRPC). CRPC is marked by an increase in intratumoral androgen biosynthesis, which is brought about by the upregulation of enzymes responsible for androgen synthesis. Aldo–keto reductase 1C3 (AKR1C3) is one of the steroidogenic enzymes responsible and catalyzes the downstream conversion of androgen precursors to the potent androgen receptor (AR) ligands: testosterone and 5α-dihydrotestosterone. Expression levels of AKR1C3 were found to be significantly higher in CRPC patients and the enzyme has been validated as a promising therapeutic target for the management of both androgen dependent prostate cancer and CRPC.

Acute Myeloid Leukemia (AML) affects more than 12,000 people in the US and is characterized by a differentiation arrest and proliferation of naïve precursor cells of myelocytic lineage. Chemotherapy with anthracyclines (daunorubicin, idarubicin) is the preferred clinical treatment strategy for managing AML. They are however; transformed into inactive hydroxy metabolites upon exposure to AKR1C3 that imparts resistance to these chemotherapeutics. AKR1C3 is also known to be an important regulator of myeloid cell proliferation and differentiation – important facets in leukemia’s, with the 1C3 isoform overexpressed in a range of leukemia cell lines. Also known as prostaglandin F synthase (PGFS), the enzyme catalyzes the conversion of PGD2 to 11β-PGF2α and PGF2α prostanooids that exert a pro-proliferative influence on myeloblasts and myelocytes. Such activities make AKR1C3 an attractive target for managing AML resistance and disease progression.

Methods
The structurally novel natural product baccharin, isolated from Brazilian green propolis exhibits potent AKR1C3 inhibition activity (IC50 = 100 nM) and crucially, exquisite selectivity for the AKR1C3 isoform. Baccharin however, bears metabolic hotspots in its structure and can be biotransformed into another compound drupanin with complete loss of activity. We adopted a medicinal chemistry approach to address the metabolic concerns of the baccharin structure and also elucidated a preliminary structure activity relationship (SAR) of baccharin by synthesizing various classes of analogues to improve the AKR1C3 inhibitory activity. The biological activity of all compounds was identified based on a recombinant enzyme inhibition screen. Lead compounds were identified that demonstrated a higher AKR1C3 inhibitory activity than baccharin combined with a better metabolic stability. Identified lead compounds were then screened for their cytotoxicity on androgen dependent and independent prostate cancer (CaP) cells and a panel of AML cells. A combinatorial approach was followed up to analyze the adjuvant effect of the lead compounds with etoposide and enzalutamide in AML and CaP cells respectively. The Chou-Talalay method was used to determine the degree of synergism in the combination treatment.

Results
Based on the SAR studies a novel AKR1C3 inhibitor was identified that exerted up to two fold enhanced enzyme inhibitory activity than baccharin with retention of the isoform selectivity. Lead compounds exhibited enzymatic IC50 values in the range of 66 – 326 nM, maintaining a fold selectivity ratio in the range of 109 - 510 over AKR1C2, an enzyme isoform with high homology. Based on results from a MTS cell viability assay analyzed at 72 hr treatment; lead compounds exerted a moderate cytotoxicity (IC50 = 50 μM) in LNCaP cells that exhibit low AKR1C3 levels, which was more pronounced in the VCaP cell model, expressing high AKR1C3 levels (IC50 = 20 μM). A significant synergism was observed when prostate cancer cells were pretreated with an AKR1C3 inhibitor, followed by exposure to AR antagonist enzalutamide at 5 μM concentration. Preliminary experiments show that 24 hr pretreatment of LNCaP cells with 1 μM of AKR1C3 inhibitor reduced the dose of enzalutamide required by 40-fold to exert the same cytotoxic effect. [Combination IC50 = 2.11 μM; Combination Index (CI) = 0.03; Dose Reduction Index (DRI) = 40]. In the AML cell models (HL-60 and KG1a) the lead compounds exhibited only a moderate cytotoxicity when administered alone (IC50 = 50 μM). These compounds when delivered at 0.1 μM concentration along with etoposide demonstrated a potent synergistic effect by enhancing the cytotoxicity of etoposide by up to six fold (CI = 0.16; DRI = 6.25).

Conclusion
The SAR studies performed on Baccharin, served as a platform for the synthesis of more potent and metabolically stable compounds. Inhibition of AKR1C3 has been proven to be a validated approach for the management of CRPC and AML as the enzyme is shown to be upregulated in both of these malignancies and a very high degree of synergism was observed when AKR1C3 inhibitors were combined with clinically employed chemotherapeutic agents. The inherent toxicity of AKR1C3 inhibitors were found to be low while they significantly reduced the dosing of clinical chemotherapeutics consequently palliating their adverse effects.