Inhibitors of AKR1C3 as Novel Therapeutics for the Treatment of Castration Resistant Prostate Cancer (CRPC) and Acute Myeloid Leukemia (AML)

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Purpose

Aldo–Keto Reductase 1C3 (AKR1C3) is an oxidoreductase that catalyzes the downstream conversion of androgen precursors to the potent androgen receptor (AR) ligands: testosterone and 5α-dihydrotestosterone. Also known as prostaglandin (PG) F synthase, AKR1C3 catalyzes the conversion of PGD2 to 11β-PGF2α and PGF2α prostanoids and hence acts as an important regulator of myeloid cell proliferation and differentiation. This dual enzymatic action makes AKR1C3 responsible for the pathogenesis and progression of Castration Resistant Prostate Cancer (CRPC) and Acute Myeloid Leukemia (AML) (figure 1). AKR1C3 also mediates resistance to clinical chemotherapeutics including enzalutamide and the anthracyclines. Such activities make AKR1C3 an attractive target for managing CRPC and AML disease progression as well as therapeutic resistance. Closely related isoforms AKR1C1 and 1C2 share high homology to 1C3 and carry out normal steroid metabolism, hence inhibition of these is undesirable. Baccharin, a component of Brazilian propolis was isolated as a natural hit compound exhibiting potent inhibitory activity and selectivity towards AKR1C3 isoform. However, the presence of an ester side chain makes it labile to hydrolysis with complete abrogation of AKR1C3 inhibitory activity.

Methods

We conducted a detailed structure-activity relationship (SAR) study on the baccharin structural scaffold to identify metabolically stable, potent and highly selective compounds as AKR1C3 inhibitors. The biological activity and isoform selectivity of all compounds was identified based on a recombinant enzyme inhibition screen. Lead compounds were evaluated for cytotoxic effect alone and in combination with clinical chemotherapeutics in a variety of CRPC and AML cell lines using MTS assay. The Chou-Talalay method was used to determine the degree of synergism in the combination treatments. Metabolic stability of baccharin and lead compounds was determined by exposure to human S9 fraction and analysis by an LC-MS based method.

Results

Based on the SAR studies (figure 2), a novel library of AKR1C3 inhibitors was identified that exert potent inhibitory activity and exquisite selectivity for the AKR1C3 isoform. Up to two-fold enhancement in the enzyme inhibitory activity and six-fold increase in selectivity over baccharin was achieved. Our lead compound, exhibiting >2800 fold selectivity toward AKR1C3 inhibition is the most selective AKR1C3 inhibitor ever discovered. An increase in metabolic half-life to 240 min was observed for the lead compound as compared to baccharin that was readily hydrolyzed. Pretreatment with AKR1C3 inhibitors sensitized resistant 22Rv1 CRPC cells towards enzalutamide cytotoxicity, reducing the effective chemotherapeutic dosing by >100-fold. Similarly, a very high degree of drug synergism was also observed in LNCaP cells. Reduction in AKR1C3 expression among 22Rv1 prostate cancer cells was noted after inhibitor treatments by immunofluorescence analyses that were confirmed by western blotting. A concomitant reduction in androgen receptor (AR) levels was observed with an increase in c-caspase 3 and c-PARP suggesting apoptosis. In addition, a reduction in prostate specific antigen (PSA) biomarker levels was observed after treatment with inhibitor and even more so in combination treatments, indicative of therapeutic drug effects. In AML cell models (HL-60 and KG1a) similar synergistic effects were observed upon combination treatment with AKR1C3 inhibitors, reducing the dosing of etoposide, daunorubicin (an anthracycline) and cytarabine by up to six, ten and eighteen-fold respectively.

Conclusion

Utilizing medicinal chemistry techniques and SAR studies, a library of approximately seventy compounds have been synthesized to date as AKR1C3 inhibitors. Lead compounds exhibiting a greater activity and isoform selectivity than baccharin along with ‘drug-like’ properties were identified that exerted very high synergistic action with clinical chemotherapeutics. Since AKR1C3 enzymatic activity contributes to cancer pathogenesis as well as chemotherapeutic resistance use of such small molecule inhibitors represents a promising approach to overcome therapeutic resistance, reduce severe toxicity associated with the use of chemotherapy drugs and consequently improve patient survival.