MS12-P01 | CRYSTALLIZATION AND STRUCTURE DETERMINATION OF ALDO-KETO REDUCTASE 1C3 IN COMPLEX WITH STEROIDAL INHIBITORS USING IN SITU PROTEOLYSIS

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Human aldo-keto reductase 1C (AKR1C) isoforms are NADPH-dependent oxidoreductases that metabolize carbonyl containing drugs and are overexpressed in many cancers, where they confer resistance to chemotherapies such as doxorubicin and abiraterone. Structural methods could facilitate design of drugs that are more resistant to AKR1C activity, or inhibitors with potential as adjuvant drugs, to improve the effectiveness of current chemotherapies. To design more effective steroidal inhibitors of AKR1C isoforms, X-ray structural data were used as templates for molecular docking simulations. Potential ligands for AKR1C isoforms were then investigated using *in vitro* biochemical assays, yeast-based assays for nuclear receptor affinity, and computational simulations. Results were correlated with anti-proliferation tests using human cancer cell lines. Based on these screening results, steroidal inhibitors of AKR1C isoforms were identified, and X-ray crystal structures of new steroidal inhibitors in complex with human aldo-keto reductase 1C3 were determined. Recombinant AKR1C3 was expressed from a pET28(a+) vector with a thrombin-cleavable N-terminal His_6-tag for structural studies. *In situ* proteolysis with thrombin was used to remove the His-tag during crystallization, resulting in improved crystal morphology and diffraction quality. Our combined results were applied to help design more potent, selective inhibitors of human aldo-keto reductase 1C3 and 1C2 isoforms.

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