SCREENING OF HUMAN SULFOTRANSFERASE FORM-SELECTIVE INHIBITORS SUITABLE FOR METABOLIC REACTION PHENOTYPING STUDIES

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Identification of the enzyme(s) responsible for biotransformation of a new chemical entity (NCE) is a necessary and critical step in the drug discovery and development process. The results of these studies, usually referred to as in vitro metabolic reaction phenotyping studies, are used to estimate the potential of the NCE to be involved in drug–drug interaction via enzyme inhibition, and to predict the possible impact of induction and genetic polymorphisms of drug-metabolizing enzymes on NCE clearance. Though historically the focus of reaction phenotyping studies has been on cytochrome P450s (CYPs), there is an increasing effort in recent years to characterize drug biotransformation catalyzed by non-CYP enzymes, including sulfotransferases (SULTs). The most relevant and commonly used approach for CYP reaction phenotyping inhibition experiments is the use of enzyme form-selective low molecular weight inhibitors (Zientek and Youdim, 2015). Unfortunately, there is no panel of well-characterized SULT form-selective chemicals. Thus, we aimed at identifying through in silico and in vitro studies selective inhibitors of four major drug metabolizing human cytosolic SULTs (i.e., SULT1A1, SULT1A3, SULT1E1, and SULT2A1) as reagents for metabolic reaction phenotyping assays (James and Ambadapadi, 2013). In a first run of in silico docking-based experiments, 85 compounds extracted from the FDA Approved Drug database (DrugBank ver. 5.0: https://www.drugbank.ca/) have been evaluated as ligands of the above-mentioned SULTs. This screening led to the identification of 12 prospective SULT ligands, which were then investigated in vitro for inhibitory activity towards human recombinant SULTs using the probe substrates 4-nitrophenol (SULT1A1), dopamine (SULT1A3), 17β-estradiol (SULT1E1), and dehydroepiandrosterone (DHEA; SULT2A1). Identification and quantitation of probe substrates' metabolites was accomplished using HPLC with diode-array detection (4-nitrophenyl sulfate) or LC-MS/MS (dopamine 4-O-sulfate; 17β-estradiol sulfate; and DHEA sulfate). Three out of the twelve in vitro screened compounds exhibited remarkable inhibitory activity towards one or more of the SULT enzymes studied. In particular, nitroxoline was a highly potent inhibitor of SULT1A1-mediated 4-nitrophenol sulfation (IC50 < 0.1 μM), and exhibited a high level of enzyme form selectivity (less than 55% inhibition of SULT1A3, SULT1E1, and SULT2A1 at 50 μM). Moreover, ivacaftor and pirlindole demonstrated potent inhibitory activity (IC50 values in the low micromolar range) towards SULT1A1, and SULT1A3, respectively.

The suitability of the identified compounds as tools for SULT reaction phenotyping is currently being evaluated through testing of their stability in human liver and intestinal cytosolic fractions.

References:

James MO and Ambadapadi S (2013). Interactions of cytosolic sulfotransferases with xenobiotics. Drug Metab Rev; 45:401-14.

Zientek MA and Youdin K (2015). Reaction phenotyping: advances in the experimental strategies used to characterize the contribution of drug-metabolizing enzymes. Drug Metab Dispos; 43:163-81.