Synthesis and Evaluation of Three Small-Molecule Inhibitors of Calcium Oxalate Crystallization for Hyperoxaluria

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Calcium oxalate (CaOx) crystallization is the primary cause of urolithiasis, accounting for nearly 80% of all kidney stone cases. Potassium citrate remains the standard pharmacological therapy for recurrent kidney stones associated with hyperoxaluria. However, its low potency requires high doses to achieve some therapeutic efficacy, increasing the risk of adverse side effects and disruption of calcium homeostasis. To develop more effective therapeutics with fewer side effects, novel small molecules are being designed, synthesized, and evaluated as potent inhibitors of CaOx crystallization in our laboratory. In this SURF project, three compounds were synthesized in 4 steps, in 4–7% overall yields. Purification was performed using extraction, normal- and reversed-phase chromatography, and structures were confirmed by ^{1}H-NMR, ^{13}C-NMR, and LC-MS. The ability of target compounds to inhibit CaOx crystallization was measured by incubating a supersaturated calcium oxalate solution in varying concentrations of test compounds for 72 h at 20 ºC in HEPES buffer (pH 6.8). After incubation, oxalate concentrations in the supernatant after centrifugation were quantified using an oxalate assay kit. Dose-response curves generated by plotting free oxalate concentrations vs inhibitor concentrations were used to derive EC_{50} values. Two of the three compounds were tested and demonstrated concentration-dependent inhibition of CaOx crystallization with EC_{50} of 308±24 nM for LH1568 and 27±2 nM for LH1599, which are 690- and 7,900-fold more potent than potassium citrate (EC_{50} = 212±23 µM), respectively. These results help identify leads for further optimization as potential therapeutics to prevent CaOx kidney stone formation in patients with hyperoxaluria. Supported by NIH R25ES020721.