The UGT glucuronosyltransferase (UGT) family of enzymes are involved in the metabolism of various compounds in the body. These enzymes transfer a hydrophilic glucuronic acid moiety to their substrates, rendering them more water soluble and suitable for excretion. The UGTs act on various endogenous substrates, such as drugs, such as diclofenac, morphine, and xenobiotics. The function of these enzymes is essential for the clearance of drugs and other toxins from the body and alteration of UGT activity could potentially cause drug-drug interactions in vivo. In an increasing interest in the activity of these enzymes and their involvement in drug metabolism. The current methods for assessing UGT activity utilize microsomes via long incubation period of several hours, which are not amenable to high-throughput screening applications for UGT inhibitors or activators. Here we present a new bioluminescent assay system for measuring UGT activity in vitro. Our assay does not involve any protein precipitation or chromatographic steps and is easily performed in a 96-well plate format. We have shown the ability of our assay to detect both enzyme inhibition and enzyme induction with various compounds, such as diclofenac and valproate. The UGT inhibition assay was successfully applied to human liver microsomes and recombinant UGT activities from human, mouse, rat, and zebrafish liver microsomes, as well as mouse and rat liver microsomes. We also observe some compounds known to inhibit numerous isoforms (diclofenac) and we validated published data showing that some isoforms are inhibited by a similar magnitude. We were surprised to find that compounds that are relatively unaffected. Assay variability, as measured by Z' values, have been calculated for UGT 1A1 (Z' = 0.83) and UGT 2B7 (Z' = 0.67). Our new assay format could greatly increase the throughput for assessing UGT activity and evaluate efficient screening of UGT inhibitors against compound libraries.

Effect of Diclofenac on UGT Supersomes™

UGT inhibition assays were carried out for 1-2 hours at 37°C under standard reaction conditions with increasing amounts of the HIV protease inhibitor ritonavir. Supersomes™ were present in the reaction at 0.2 mg/mL. 1A1 and 2B7 Supersomes were assayed with 50 µM substrate #1 and 1A4 Supersomes were assayed with 25 µM substrate #2.

Effect of Ritonavir on UGT Supersomes™

Literature reports an IC50 value of 3.6 µM for 1A1 using trifluoperazine as the substrate, and an IC50 value of 15.6 µM for 1A1 using ibuprofen as the substrate and no significant inhibition of 2B7 (IC50 > 100 µM) using 7-hydroxy-4-trifluoromethyl coumarin as the substrate.

UGT Activity with Tissue Microsomes

Tissue microsomes (0.1 mg/mL) and Supersomes™ (0.2 mg/mL) were assayed against substrates #1 (1A1 and 2B7) and #2 (1A4) at 37°C for 15 minutes. Assays were performed with Promega GentleMacs Automated Nucleic Acid Purification System.

Conclusions

- This assay can be used to detect the activity of many of the UGT isozymes in a multiwell format.
- This assay can be used with recombinant UGT enzymes or tissue microsomes (ie, human liver).
- The small reaction size (40 µL in a 96 well plate) saves the researcher money on costly microbial enzyme prep, UDPGA, and test compounds.
- Unlike conventional UGT assay methods, this assay lacks protein precipitation, centrifugation, and chromatography steps, making it amenable to HTS.

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