Combining Luminescence-based CYP Inhibition Assays and Simple, Robust Instrumentation for Use in Automated Cytochrome P450 Profiling

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Abstract

Most small molecule drugs are metabolized predominantly in the liver by cytochrome P450 (CYP) enzymes, particularly CYP isoforms 3A4, 2C9 and 2D6. It is important to assess metabolism for appropriate dosing, but also for establishing metabolism-related drug-drug interactions where one drug may inhibit the metabolism of another leading to possible toxic effects. While the gold standard method for in vitro determination of lead compound inhibition of CYP isoforms involves monitoring the metabolism of drug substrates by human liver microsomes or primary hepatocytes using LC-MS/MS, the use of recombinant CYP isoforms with optical readouts based on labeled drug substrates specific to the isofrom is gaining favor as a low cost, higher throughput alternative that can assess metabolic profiles of leads earlier in the drug discovery process. One desired component of this change is the ability to profile compounds against multiple CYP enzymes using the same basic procedure. The second is an easy, yet dependable way to dilute compounds that will create accurate titration curves.

Here we demonstrate the automation of the profiling process, from compound titration through assay component transfer, using simple, yet robust instrumentation. IC50s of small molecule drugs were determined using recombinant CYP isoforms 3A4, 2C9 and 2D6 as well as luminesogenic substrates specific to each. Compounds were profiled against all three isoforms on the same 384-well assay plate to demonstrate the ease of this combined procedure. The combination of chemistry and instrumentation creates an ideal solution for high-throughput cytochrome P450 profiling of lead compounds in drug discovery campaigns.

Introduction

The determination of effects that lead compounds have on cytochrome P450 (CYP) enzymes is an important part of ADMET/tox testing. Current methods involve running one, or multiple compounds, in dose response format against a single CYP enzyme. This process is time consuming, and can lead to data set variability due to assay and day-to-day laboratory variability.

The automated assay shown here eliminates these concerns and provides the ability to profile multiple compounds against the CYP2C9, -2D6, and -3A4 isoforms on the same 384-well assay plate using one simple process.

P450-Glo™ Luminescent CYP450 Assay

A serial 12-point 1:4 titration method was set up on the Precision to be used for compound dilution. The titration method’s linearity was tested using ATP in 1% 1D50, beginning with a top concentration of 100 µM. 5 µL transfers were performed in quadruplicate for each point in the titration to the 384-well microplate. An ATP determination of lead compound in vitro.

Precision Compound Titration Method

A 0-100 µM ATP Titration

Final testing of the automated P450-Glo assays involved profiling test compounds against the three CYP450 isoforms. Two compounds were tested on a single assay plate using the three validated P450-Glo assays.

Conclusions

1. The Precision™ Microplate Pipetting System combines an 8-channel pipetting head and an 8-channel bulk reagent dispenser in one instrument. The instrument was used to serially titrate compounds across a 96-well polypropylene plate, as well as dispense all assay components to the 384-well assay plates.

2. The Synergy™ Mx Monochromator-based Multi Mode Microplate Reader incorporates a quadruple monochromator system along with a dedicated optical system, separate from the fluorescence optics, for high-performance luminescence detection. The instrument was used to detect the luminescent P450-Glo™ signal from each assay well.

3. Precision® Microplate Pipetting System is an ideal solution for high-density, automated cytochrome P450 profiling.

As results are brought together, the effects a compound will have on the different CYP enzymes become apparent, or how an individual enzyme will be affected by each lead compound. An example is the antifungal drug miconazole. This compound is a well known inhibitor of CYP2C9(25). However, as Figure 6 shows, it is also a potent inhibitor of CYP3A4 and -2D6, which also agrees with the literature(26).

4. The combination of BioTek’s instrumentation, and Promega’s P450-Glo Screening Systems create an ideal solution for high-density, automated cytochrome P450 profiling.