Application Note

Measuring Cytochrome P450-3A4 Inhibition Assay on Hudson Robotics' VaryScreen ADME Equipped with Berthold Technologies' Mithras

Alan H. Katz[1], Bettina Felletschin[2]

Abstract

ADME (absorption, distribution, metabolism and excretion) describes the disposition of a pharmaceutical compound within an organism influencing its performance and pharmacological activity.

Hudson Robotics developed an assay system which integrates several plate handling and liquid handling robots with the Mithras multimode reader to carry out various ADME protocols.

Here we report the results of the Cytochrome P450-3A4 Inhibition Assay.

Introduction

The VaryScreen ADME system (figure 1) includes the SOLO single channel pipettor, which carries out most of the liquid transfers in this assay. It also contains the Micro10x multichannel dispenser, which is used to add common reagents, such as DMSO and water for preparing the compounds for testing, and the Luciferin detection reagent. Plates of compounds to be tested are stored in the StackLink and transferred via tracks to the SOLO and the Micro10x. Plates are moved from the deck of the SOLO to the Mithras reader with a PlateCrane EX, which also contains stacks for storing pipette tips and completed plates. One of the decks of the SOLO contains a Teleshake nest.

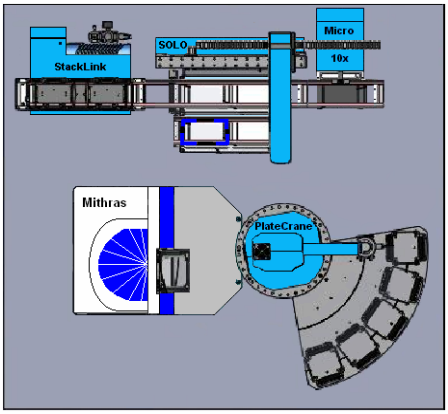

Figure 1: Schematic view of the VaryScreen ADME assay system from Hudson equipped with Mithras

The P450-GLO assay (figure 2) uses luminescence to measure the amount of Luciferin (2) present after it has been produced by the action of a Cytochrome P450 3A4 enzyme on the isopropyl acetal, Luciferin IPA (1).

1 2

Figure 2: Cytochrome P450 3A4 enzymatic reaction of Luciferin IPA (1) to Luciferin (2)

[1] Hudson Robotics, Inc, 10 Stern Avenue, Springfield, NJ 07081, akatz@hudsonrobotics.com
[2] BERTHOLD TECHNOLOGIES, Bad Wildbad, Germany, Bettina.Felletschin@Berthold.com

Mithras

The Mithras LB 940 is a multimode plate reader with a unique optical design (DOPS – Dedicated Optical Path System) to ensure optimized performance for the detection technologies implemented. These are

- Luminescence
- BRET/BRET²
- Fluorescence
- FRET
- UV/VIS absorbance
- Fluorescence polarization
- AlphaScreen®
- TRF
- HTRF®

In addition options like reagent injectors, temperature control and cooled PMT detection units are available.

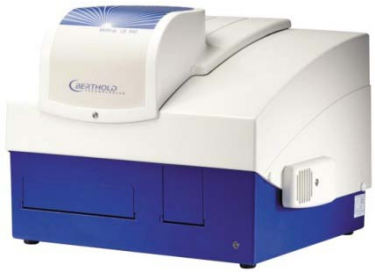

Figure 3: Mithras LB 940 multimode reader

Experimental Procedure

A test compound plate was prepared as follows: 100 µL of 100 micromolar solution of the compound in DMSO was diluted with 900 µL of Luciferin-free water. The Micro10x was used to fill each row of a 96 well plate with 50 µL of water, and the SOLO added 25 µL of the 10 micromolar compound solution to column 1 of each row. The SOLO then carried out the serial dilution by mixing and aspirating 25 µL of the mixture and dispensing it into the adjacent well. This was carried out until column 12 of the plate was reached.

A 384-well assay plate was introduced to an open position of the SOLO, and 12.5 µL aliquots of the serially diluted compound samples were transferred to the first 12 columns of each of the rows of the plate. 12.5 µL of a membrane preparation containing recombinant Cytochrome P450 3A4 is mixed with 1M potassium phosphate buffer and Luciferin IPA in Luciferin-free water was added to each well. For a positive control, 12.5 µL of the membrane preparation were mixed with 12.5 µL of water. Similarly, a negative control was created by substituting the membrane preparation with a similar membrane preparation that didn't contain enzyme.

After 10 minutes of incubation, the enzyme was activated by addition of 12.5 µL NADPH regeneration system, and the mixture stirred at room temperature for 10 minutes. A Luciferin detection reagent was then added to the mixture (50µL) and the plate was stirred for an additional 20 minutes at room temperature. The plate was then put in the Mithras and luminescence was measured.

Results

Mithras LB 940 is operated through the PC software MikroWin 2000 which can also serve as a data evaluation tool. The Advanced II version needs to be installed to carry out curve fitting calculations.

We calculated the IC 50 values (table 1) and used the curve fit option to compute the standard curves (figure 4).

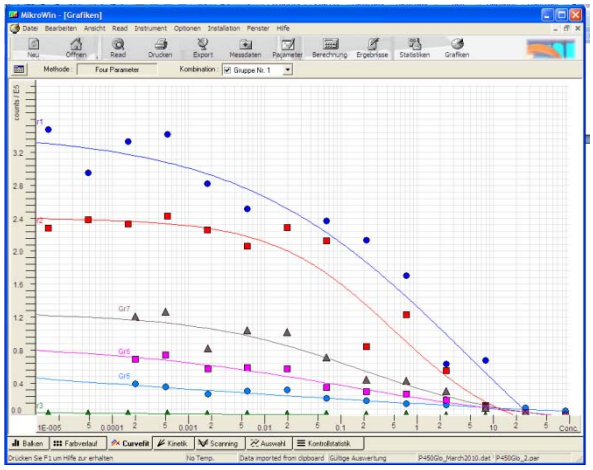

Figure 4: Compound standard curves as shown in MikroWin curve fit option

Compound	IC50
Verapamil	0.4
Diliazen	0.5
Chlortrimazole	0.07
Diisopyramide	30
Pimozide	5
Quinidine	1

Table 1: IC50 values of tested compounds

Conclusion

The VaryScreen ADME assay system, in combination with the Mithras multimode reader, is perfectly suited for preparing compound plates for the Cytochrome P450-3A4 Inhibition assay. IC50 values correlate well with those reported in the literature. The combination of VaryScreen and Mithras multimode reader gives the opportunity to perform further ADME assay protocols in future.

Material

- VaryScreen ADME assay system (Hudson Robotics)
- Mithras (Berthold Technologies)
- Cytochrome P450-GLO assay (Promega)
- 384 well plate white

Literature

- Guengerich, F.P. (2001) *Chem. Res. Toxicol.* 14, 611–50.
- Wienkers, L.C. and Hutzler, J.M. (2002) *Curr. Drug Disc*. 23–6.
- Sai, Y. et al. (2000) *Xenobiotica* 30, 327–43.