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Overview

- The purpose of this study was to develop a high content method to rapidly screen compounds (10 compounds at three concentrations with an additional positive control) for CYP3A4 induction (can be used for other CYPs as well).
- A single donor of primary human hepatocytes was evaluated for CYP3A4 inducibility after a 24-hour and a 48-hour culture adaptation period.
- Automated RNA isolation and qPCR were used to determine CYP3A4 mRNA expression levels.
- Negative and positive controls for CYP induction, including prototypical CYP3A4 inducers, responded appropriately as either non-inducing or inducing, respectively, with a 24-hour culture adaptation period followed by a 24-hour treatment period.
- A 48-hour culture adaptation period was not required to determine which compounds caused CYP3A4 mRNA induction.

Introduction

Cytochrome P450 (CYP) enzymes play an important role in the oxidative metabolism of many drugs. Consequently, inhibition or induction of these enzymes by perpetrator drugs can result in alterations in the clearance of a victim drug that is metabolized by CYP pathways (*i.e.* drug-drug interactions; DDIs) [1]. The induction of CYP enzymes, which results in elevated CYP expression levels, can lead to an increase in the clearance of a victim drug resulting in potential loss of drug efficacy [2]. Thus, characterizing a new drug's CYP induction potential early in drug development can lead to better and safer drug design. In the present study, we developed a rapid CYP induction screen evaluating up to 10 compounds in one assay (at three concentrations, with an additional positive control) with the fold change of CYP3A4 mRNA expression levels measured as an endpoint.

Materials & Methods

Chemicals and test systems

All chemicals used for treatments were purchased from Sigma-Aldrich (St. Louis, MO) or Toronto Research Chemicals (Toronto, ON, Canada) and were of analytical grade. The sources of all other reagents have been described previously [3]. Cryopreserved human hepatocytes (HC5-27) from a single donor were prepared from a non-transplantable liver and characterized at XenoTech, LLC (Lenexa, KS) as described previously [4, 5].

In vitro CYP induction screen

The typical workflow for the CYP induction screen is shown in **Figure 1**.

Treatments: Cryopreserved human hepatocytes from a non-transplantable single human liver donor were seeded and cultured in a collagen-Matrigel sandwich on 96-well plates at $37 \pm 1^\circ\text{C}$ (with $95 \pm 5\%$ humidity and $5 \pm 1\%$ CO_2). Cells were allowed to adapt in culture for 24 hours or 48 hours after seeding and overlay, followed by a single treatment for 24 hours, in triplicate, with carbamazepine (1, 10, 100 μM), clobazam (1, 10, 50 μM), flumazenil (1, 10, 25 μM , non-inducer), nifedipine (1, 10, 100 μM), omeprazole (1, 10, 50 μM), phenytoin (1, 10, 40 μM), pioglitazone (1, 10, 50 μM), pleconaril (1, 10, 50 μM), rifampin (1, 5, 10, 20 μM), ritonavir (1, 10, 20 μM), rosiglitazone (1, 10, 50 μM), DMSO (0.1 % v/v; vehicle), or rifampin (up to 20 μM ; positive control) in supplemented Modified Chee's Medium (MCM+). This was repeated up to $n = 4$.

RNA isolation and qPCR: Twenty-four hours after treatment, cells were harvested with Buffer RLT (Qiagen) with β -mercaptoethanol for isolation on the KingFisher Flex (KFF; Thermo Scientific) with the MagMAX-96 for Microarrays kit (Ambion). RNA was quantified with the NanoDrop 8000 UV-Vis Spectrophotometer (Thermo Scientific). Reverse transcription was performed with the High

Capacity cDNA Reverse Transcription kit (Life Technologies) on the 7900HT Real-Time PCR System (AB7900; Applied Biosystems). qRT-PCR was performed in triplicate on the AB7900 and data were analyzed by the $\Delta\Delta\text{Ct}$ method (Applied Biosystems User Bulletin #2). Relative quantification measured change relative to the DMSO and normalized to the endogenous control (GAPDH) for CYP3A4 mRNA expression. Data were reported through Galileo LIMS (Thermo Scientific) and Crystal reports 2013 (SAP Business Objects).

Results

Data were collected from two culture conditions; either with a 24-hour or a 48-hour adaptation period after hepatocytes were initially seeded, overlaid and treated with various compounds for 24 hours. The results (shown in **Figure 2** and **3**) demonstrated robust concentration-dependent CYP3A4 mRNA induction. The highest level of induction for each compound was observed at the highest concentration tested (with the exception of nifedipine and ritonavir which plateaued at $\leq 10\ \mu\text{M}$). As expected, the non-inducer, flumazenil showed little to no induction and served as a negative control. CYP3A4 mRNA fold changes for each compound were substantially greater after a 24-hour adaptation period (**Figure 2**) compared to plates with a 48-hour adaptation period (**Figure 3**). This is likely due to cellular normalization of CYP transcript levels over time. However with both adaptation periods, concentration-dependent induction of all prototypical inducers was observed, indicating that an additional 24-hour culture adaptation period (*i.e.* 48-hour adaptation) is not required to identify potential CYP3A4 inducers.

Figure 1. Workflow for CYP induction screening studies

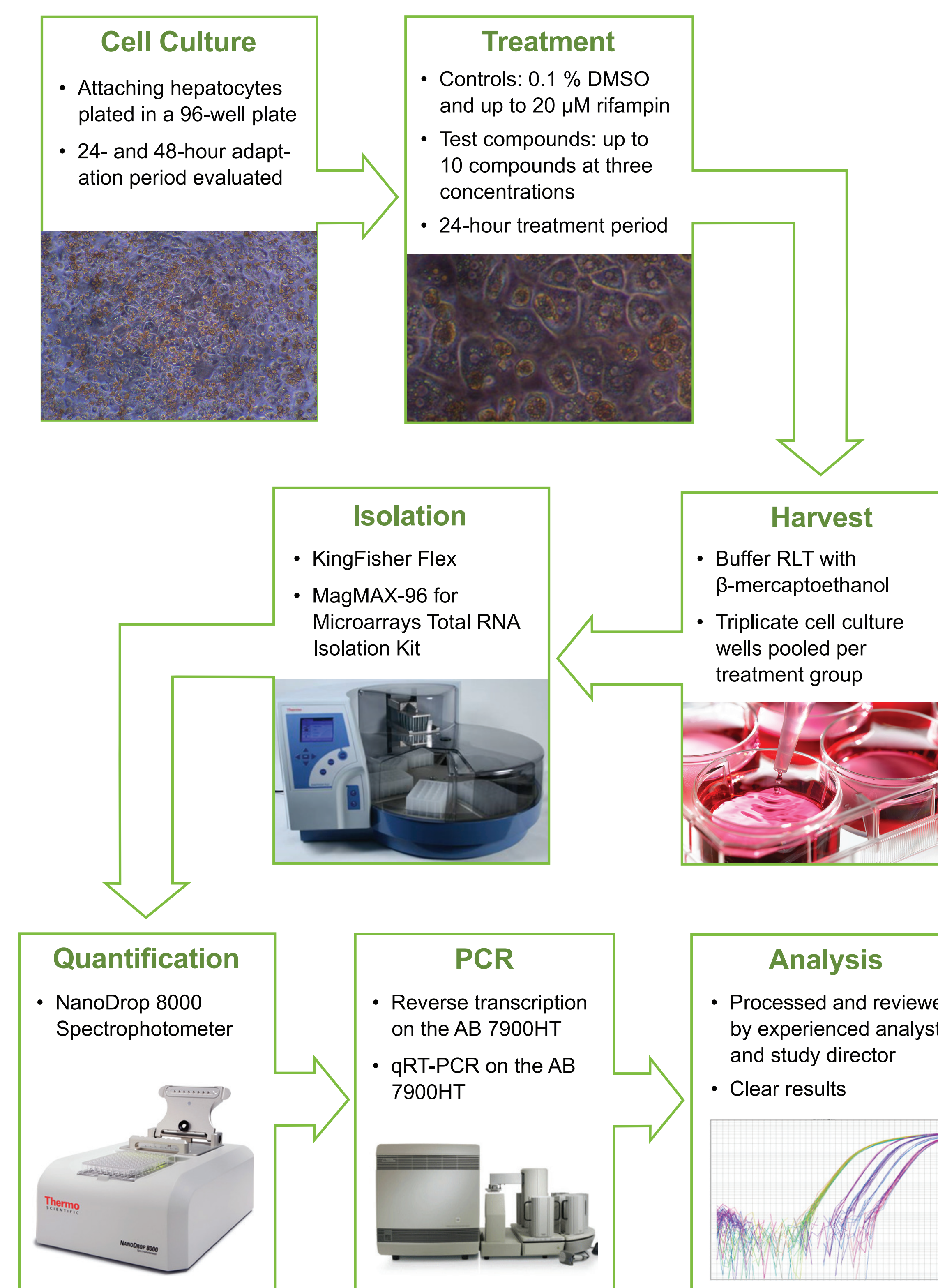


Figure 2. Mean fold induction of CYP3A4 mRNA by a panel of known inducers with a 24-hour culture adaptation period followed by a 24-hour treatment period

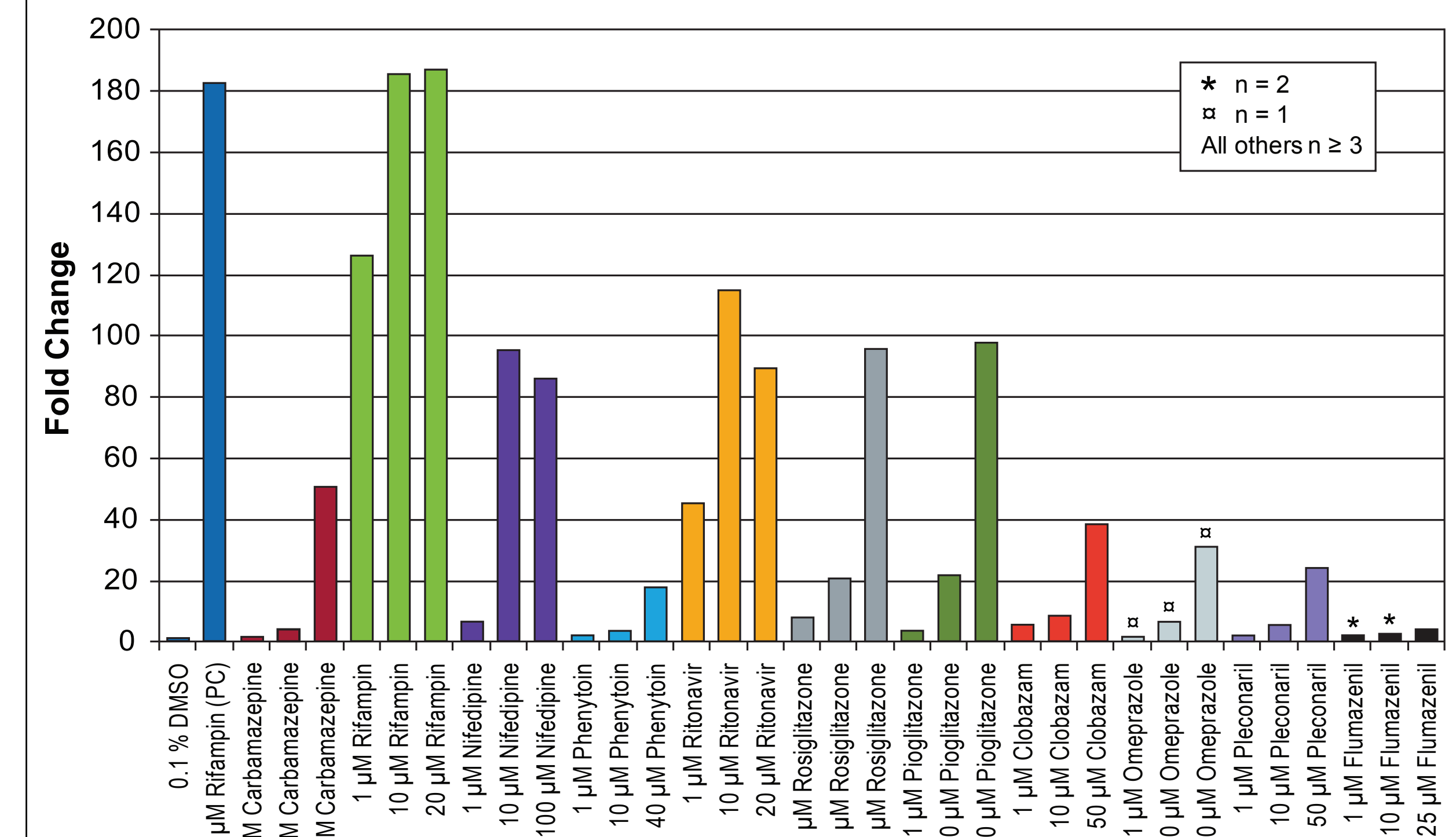
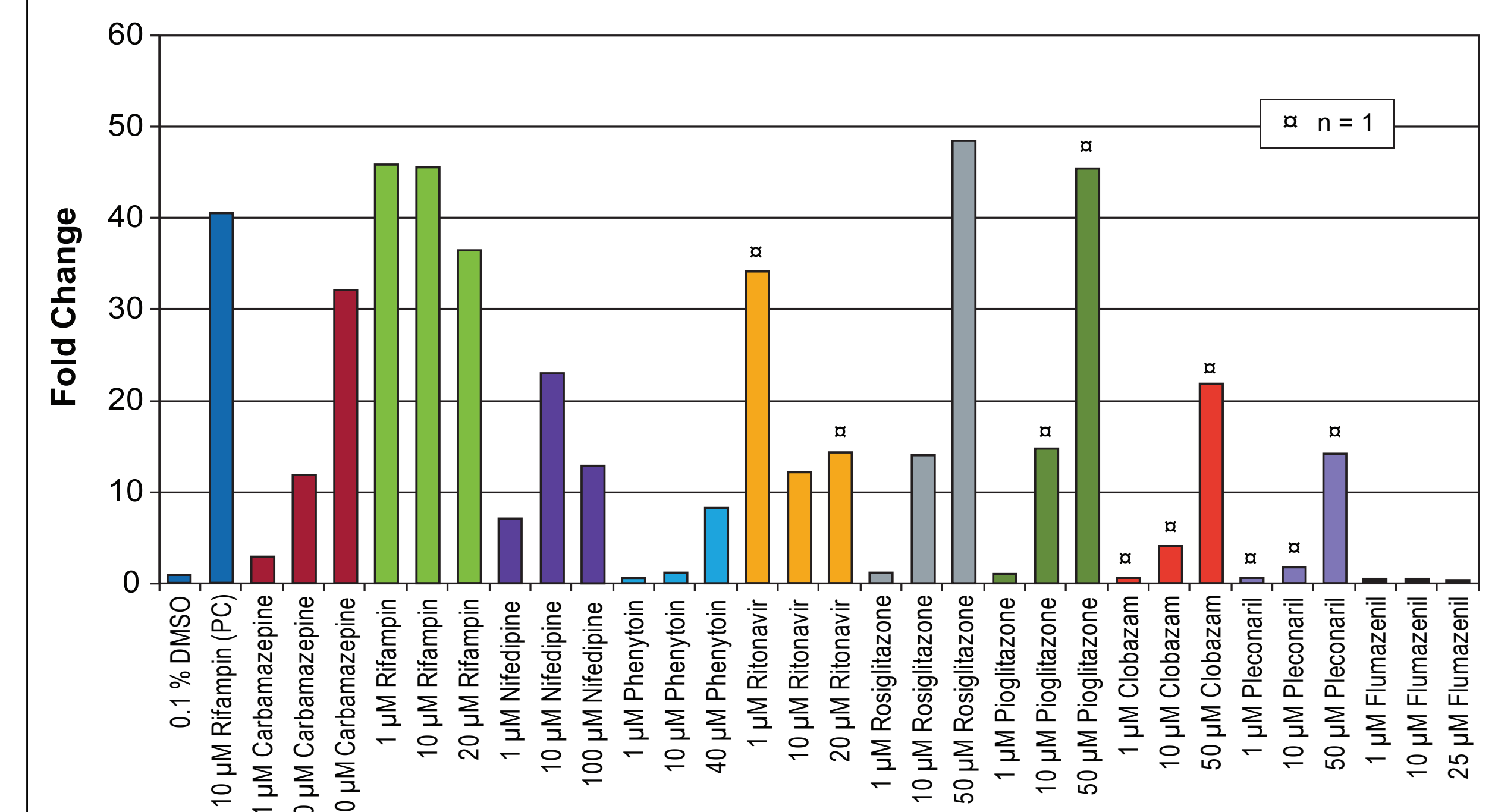


Figure 3. Mean fold induction of CYP3A4 mRNA by a panel of known inducers with a 48-hour culture adaptation period followed by a 24-hour treatment period



Conclusions

- A 24-hour culture adaptation period was sufficient to screen for a potential CYP3A4 mRNA inducer. Concentration-dependent induction was observed with all known CYP3A4 inducers, demonstrating successful assay performance.
- Overall, the results of the assay with the compounds tested were as expected, demonstrating the utility of this screen to rapidly and efficiently obtain CYP induction data early in the drug development process.

References

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