Development of a fluorimetric assay to identify inhibitors of recombinant CYP2B6

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Abstract

Here we describe the validation and testing of our latest assay to investigate the inhibitory potential of 27 compounds on the catalytic activity of recombinant CYP2B6 expressed with NADPH-CYP reductase and supplemented with cytchrome b5 (product code CYP/EZ041). This is useful information to have fairly early in the drug discovery/lead optimisation process as it can differentiate between compounds which are likely to have significant effect on drug-drug interactions before a lead compound reaches the stages of conducting clinical trials. Our method describes a fluorimetric assay which is relatively quick, easy and cost-effective to perform whilst also being predictive of the end result in vivo.

Method

The substrate 7-EFC (7-ethoxy-4-trifluoromethylcoumarin) is metabolised to a fluorescent metabolite 7-HFC (7-hydroxy-4-trifluoromethylcoumarin) by CYP2B6 with an apparent Km of 1.7 ± 0.1 µM and Vmax of 3100-3400 RFU/min between 2 batches of enzyme irrespective of whether DMSO or methanol was used as solvent at a final concentration of 2% (v/v). The 7-HFC production was monitored at excitation and emission wavelengths of 330 and 353 nm, respectively using a Tecan FLx800 in fluorimeter mode. Magellan software (v6.2, Reading, Berkshire, UK), formation of metabolite was linear for approximately 20 mins but slowed down at later time-points during a 30 minutes incubation period at 37°C.