ABSTRACT

Metabolism is an important part of drug discovery and it is essential to predict, which structural modifications in a compound that are likely to lead to altered metabolic properties. The cytochrome P450 (CYP) enzymes are the most important enzyme in the phase I metabolism of xenobiotics. However, there are other important oxidative enzymes such as monoamine oxidase (MAO). The present work was initiated to investigate the interactions between xenobiotics and their metabolizing enzymes and from the gained knowledge decide how to optimize the metabolic properties of a compound. To achieve this the recently solved crystal structures of CYP2C9 and CYP3A4 were used. Both experimentally determined data and several computational methodologies were applied or developed in order to perform structure-based design.

Firstly, a novel protocol for virtual screening of potential lead compounds was developed, using GRID Molecular Interactions Field (MIF) derived descriptors. From this search a 15-fold concentration of the library was achieved. Another area also explored using GRID MIFs was fragment replacement, which can be one important approach to overcome problems with metabolism or inhibition. Docking solutions of the compounds containing the new fragment showed the same binding pattern as the co-complexed compound. To investigate important interactions of substrates and inhibitors with CYP2C9, docking together with site of metabolism prediction were used. The two methods predicted the site of metabolism and determined the productive docking pose of the test compounds. This information led to the design of new compounds with improved metabolic properties. If a compound shows inhibitory properties it is of interest to be able to determine if the inhibition is due to direct coordination to the heme iron (type II) or due to binding close to the heme without direct coordination (type I). Depending on the type of inhibition different approaches are needed to alter the inhibition. The structural characteristics of type II ligands were investigated by recording difference spectra, determine the IC_{50}-values and develop an *in silico* method. The set of compounds tested has considerably increased the knowledge about interactions important for binding of type II ligands in CYP3A4 and CYP2C9. The examination of MAO substrates showed that commercial human liver microsomes (HLM) display MAO-like activity. Thus, it is important to consider that other enzymes than CYPs mediate metabolism in HLM. The methods developed in this work can be valuable tools when designing compounds with improved metabolic properties. This together with the information gained from the experimental measurements may result in better decisions during the drug discovery process.

Keywords: Cytochrome P450, Structure-based design, Computational Modelling, Metabolism, Inhibition, Monoamine Oxidase

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