Experimental and computational investigation of affinity and selectivity factors in CYP2D6 and CYP3A4 mediated metabolism

BRITTA BONN

GÖTEBORGS UNIVERSITET

Department of Chemistry
University of Gothenburg
Göteborg, Sweden
2010

för avläggande av filosofie doktorsexamen i kemi som, med medgivande av Institutionen för Kemi, Göteborgs Universitet, kommer att försvaras offentligt fredagen den 24 september kl 9.15 i föreläsningssal KB, Kemigården 4, Göteborgs Universitet och Chalmers Tekniska Högskola. Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent är Dr Amit Kalgutkar, Research Fellow at Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research & Development, Groton, CT, USA.

Abstract

The assessment of ADME properties and metabolic behavior of a drug is central in drug discovery and drug design. The main target for studies of metabolic properties is the Cytochrome P450 (CYP), which is responsible for the metabolism of a majority of drugs on the market and consequently also involved in many drug-drug interactions. Examples of information and tools that could guide drug design towards favorable metabolic properties are structural information of the CYPs, affinity and selectivity towards the CYPs and the site of metabolism (SOM). The present work was initiated to use experimental and computational tools to study the affinity and selectivity for two of the most important isoenzymes, CYP2D6 and CYP3A4, and highlight the benefit of combining different approaches and assays to understand the metabolic properties of a drug.

A novel computational approach resulting in an assessment of enzyme-ligand interaction patterns were successfully used for a comparison of different 3D-structures in order to highlight discriminative amino acid residues. This method could also offer an identification of important interactions when understanding affinity and selectivity for an enzyme. In order to explore affinity and selectivity factors in CYP3A4 and CYP2D6 mediated metabolism two compounds were selected for an experimental evaluation of the catalytic properties of the enzyme. Based on the results from different *in vitro* assays it could be concluded that CYP3A4 was more unselective, producing metabolites as a result of orientations presenting many different parts of the molecules to the heme. CYP2D6 on the other hand showed more restricted binding modes. The combined information from inhibition studies and metabolite identification also gave indications on productive and non-productive binding modes in the two enzymes. With further exploration of CYP2D6, and its pharmacophore, *N*-dealkylation and the effect of blocking the SOM in CYP2D6 substrates were studied. These results were in agreement with the previously stated pharmacophore and also showed that the SOM for these substrates could be successfully assigned with a ligand-based approach, which could also be used to assign selectivity for CYP2D6.

Key information, in designing compounds towards preferable metabolic properties, is obtained from metabolite identification or SOM determinations. I order to enhance metabolite identification a semi-automated software was tested that assigned the metabolite structure from MS raw data with high success rate. This could consequently be beneficial for drug design in that it enables a high throughput of metabolite identification data. During the course of these work important aspects to consider in drug design has been observed, e.g. improving metabolic stability by blocking soft spots tend to result in CYP inhibition. Instead of blocking soft spots the affinity for an enzyme could be diminished and this could be guided by the computational approach used in the first project. In summary, the combined knowledge from *in vitro* and *in silico* tools could be beneficial for the understanding of the metabolic behavior of a drug.

Keywords: Cytochrome P450, *in vitro* metabolism, inhibition, biotransformation, computational modeling, drug design