Predicting Regioselectivity and Lability of Cytochrome P450 Metabolism using Quantum Mechanical Simulations

Jonathan Tyzack, Nicholas Foster, Peter Hunt, Matthew Segall
Optibrium Ltd, Cambridge, UK

Introduction

Optibrium™, as part of the European HeCaTos project, has developed enhancements to its P450 module within StarDrop™[1]. These include the modelling of epoxidation pathways and the capability to model the xenobiotic metabolism of an additional 4 P450 isoforms: 1A2, 2C19, 2E1 and 2C8.

The goal of the HeCaTos project is to develop integrative approaches towards highly predictive human safety assessment. The prediction of xenobiotic metabolism is an important step in this process, giving the ability to identify potential toxic metabolites. The new capability to model the formation of reactive epoxide metabolites is a vital part in this process and coupled with our new isoform specific models it enables a more complete picture of xenobiotic metabolism to be developed.

Methods

Many computational methods have been developed that predict the regioselectivity of drug metabolism by Cytochrome P450 enzymes (P450) [2-6]. The advantage of a quantum mechanical (QM) approach is that it provides a quantitative estimate of the reactivity of each potential site of metabolism (SOM), allowing the vulnerability of each SOM to be assessed in absolute terms.

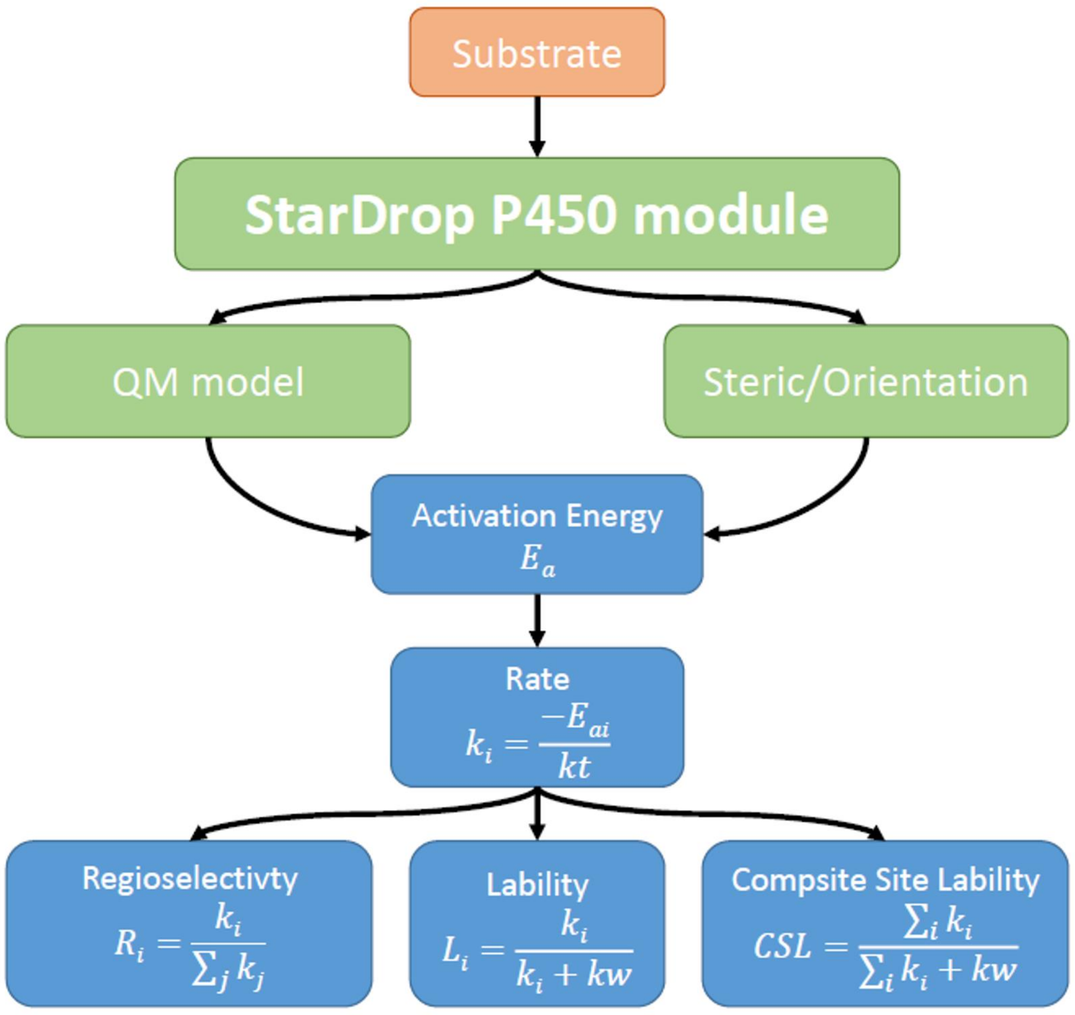

Figure 1: StarDrop methodology.

The P450 module within StarDrop combines semi-empirical AM1 QM simulations [2], to estimate the reactivity of each potential SOM, with a ligand-based approach to model orientation and steric constraints imposed by the different shaped binding pockets of the P450 isoforms. The results of these calculations are estimates of the activation energies E_{ai} for each site i, from which the relative rates of product formation k_i and the proportion of metabolism at that site R_i can be calculated.

To estimate the vulnerability of each potential SOM on an absolute scale the reaction rates for product formation are compared with k_w the rate of P450 decoupling to form water and inactivated haem. The site lability of atom i, L_i, is a measure of the efficiency of product formation at that site and these can be combined to calculate the 'Composite Site Lability' (CSL) that reflects the overall efficiency of product formation for the molecule.

Refinements to the modelling include the correction of the AM1 simulations for known systematic errors, identified with computationally intensive *ab initio* calculations. In the case of less common reaction pathways, such as sulphur and nitrogen oxidation, the activation energy is estimated by matching on SMARTS patterns calibrated from performing *ab initio* QM calculations on a series of reference molecules [5].

A rigorous literature review has also been performed to build enlarged and enhanced data sets of P450 substrates for each of the 7 isoforms where the SOM has been identified experimentally. This has enabled new steric and orientation models to be created for the additional P450 isoforms and refresh those for 3A4, 2D6 and 2C9.

Results

The predictive performance of the models on independent test sets is presented in **Figure 2** where a SOM is identified in the top 2 predictions for over 80% of the test data. The performance of the 3A4 model, the isoform responsible for the majority of hepatic clearance, is particularly favourable in comparison to SMARTCyp.

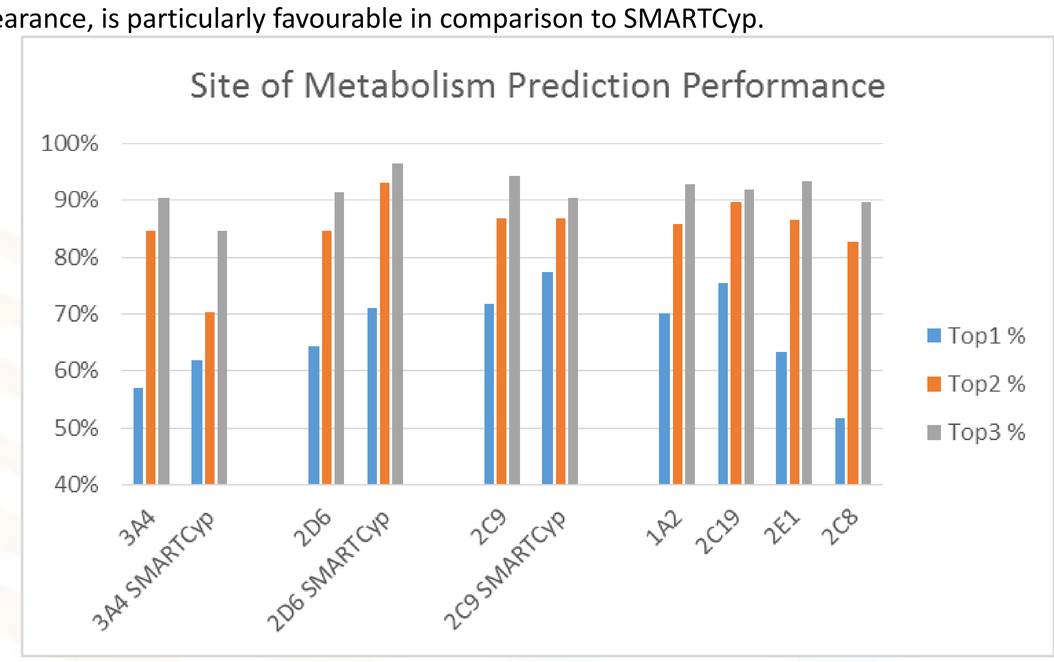

Figure 2: Predictive performance of new models with SMARTCyp comparatives where available.

Site Lability

The site lability provides useful information to guide the redesign of compounds to improve their metabolic stability. For example, **Figure 3** shows the predicted regioselectivity of metabolism and site lability for quazepam and 2-oxo-quazepam. This indicates that metabolism on sulphur S12 will account for ~98% of observed metabolism and that this is a moderately labile site. Blocking or removing this site is likely to significantly improve the metabolic stability, as reflected by the lower CSL for 2-oxo-quazepam, where C10 is now predicted to account for ~48% of observed metabolism.

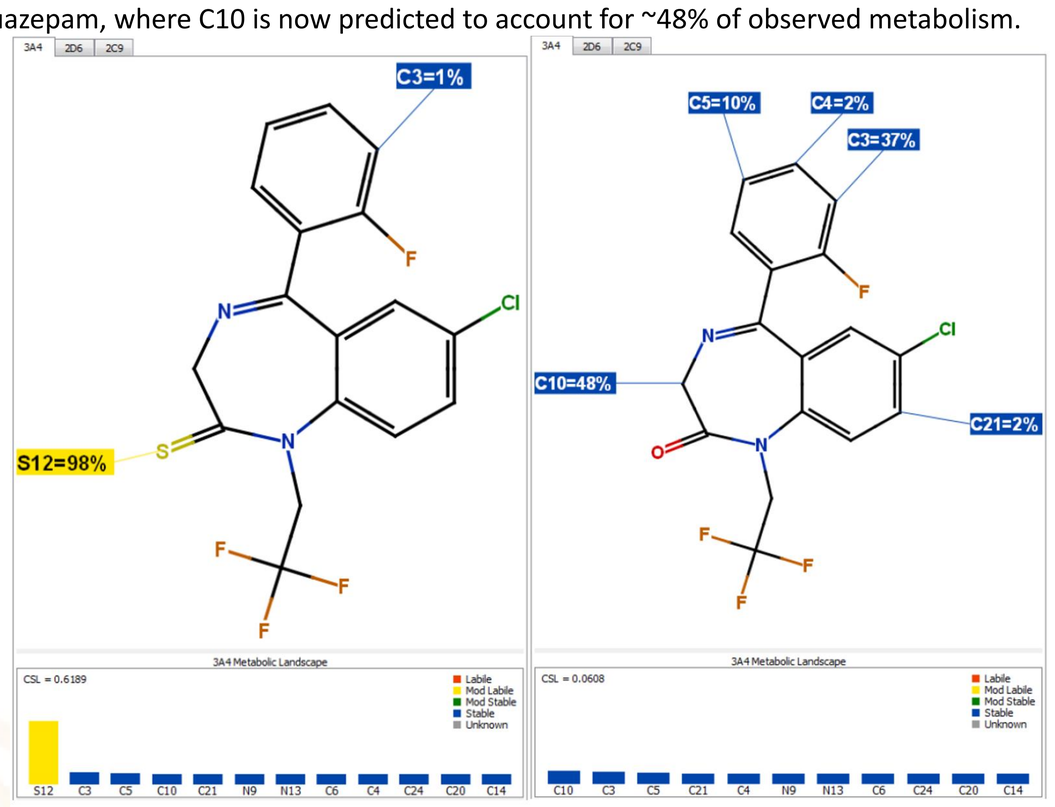

Figure 3: Predicted 3A4 metabolism of quazepam and 2-oxo-quazepam. The reduction in CSL suggests 2-oxo-quazepam will have improved metabolic stability relative to quazepam.

Conclusions

The enhancements to the P450 metabolism module in StarDrop have been presented. These include the modelling of epoxidation pathways, which are of particular interest from a toxicological perspective, and the curation of new isoform specific models enabled by the generation of large datasets.

Next steps include using the isoform specific datasets to develop models that predict which isoforms are likely to be involved in the metabolism of a particular xenobiotic.

References

[1] StarDrop, Optibrium Ltd, Cambridge, UK
[2] J.P. Jones et al. (2002) Drug Metab. Dispos. 30(1): 7-12
[3] G. Cruciani et al. (2005) J. Med. Chem. 48(22): 6970-6979
[4] M. Hennemann et al. (2009) ChemMedChem 4(4): 657-669
[5] P. Rydberg et al. (2010) ACS Med. Chem. Lett. 1(3): 96-100
[6] J. Zaretzki et al. (2011) J. Chem. Inf. Model. 51(7): 1667-1689

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under the grant agreement nº 602156