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

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Introduction

Many computational methods have been developed that predict the regioselectivity of metabolism by drug metabolising isoforms of the Cytochrome P450 class of enzymes (P450) [1-5]. Here we describe recent developments to a method for predicting P450 metabolism that combines quantum mechanical (QM) simulations to estimate the reactivity of potential sites of metabolism on a compound with a ligand-based approach to account for the effects of orientation and steric constraints due to the binding pockets of different P450 isoforms.

While valuable, predicting the relative proportion of metabolite formation at different sites on a compound is only a partial solution to designing more stable compounds. The advantage of a quantum mechanical approach is that it provides a quantitative estimate of the reactivity of each site, from which additional information can be derived regarding the vulnerability of each site to metabolism in absolute terms. One such measurement is the site lability, as calculated by StarDrop™ [6], which is a measure of the efficiency of the product formation step. This is an important factor influencing the rate of metabolism and we will illustrate how this provides valuable guidance regarding the potential to redesign compounds to overcome issues due to rapid P450.

Methods

To accurately predict the regioselectivity of metabolism by Cytochrome P450, the relative rates of product formation at each potential site of metabolism (SOM) on a molecule must be calculated. The models in StarDrop achieve this by estimating the activation energy of the rate-limiting step of product formation. Models have previously been developed for hydrogen abstraction-mediated reactions leading to aliphatic carbon hydroxylation, N- and O-dealkylation and direct oxidations leading to aromatic carbon and sulphur oxidation. Recently an additional model has been added to predict N-oxidation and -hydroxylation reactions.

The intrinsic activation energy for each potential aliphatic or aromatic carbon site of metabolism is calculated using AM1 QM simulations [4]. These are corrected for known systematic errors, previously identified with computationally intensive *ab inito* calculations, to improve the accuracy of prediction and achieve a good balance of speed and accuracy. In the case of less common reaction pathways, such as sulphur and nitrogen oxidation the reaction barrier is estimated from *ab initio* QM calculations for specific atom types identified by SMARTS patterns. For N-oxidation and -hydroxylation, the activation energies published by Rydberg *et al.* [2] were transformed onto the energy scale of the StarDrop models using a linear relationship identified by the correlation of calculated energies for H-abstraction mediated reactions (R^2=0.8).

These 'electronic' activation energies are then modified for the effects of steric hindrance and orientation in the active site of the binding site for the relevant P450. These corrections are made using ligand-based models, fitted to a set of substrates of the relevant enzyme with known metabolite profiles.

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) can, in turn, be estimated and hence the proportion of metabolism at that site (R_i) (see Box 1).

To estimate the vulnerability of each site on an absolute scale, the reaction rates for product formation must be compared with a fixed baseline. For P450 metabolism, a baseline is provided by the rate of decoupling to form water and inactivated haem (k_w), as illustrated in Figure 1. From this, the site lability (L_i) can be calculated that reflects the efficiency of product formation at that site. Finally, the site labilities can be combined to calculate the 'composite site lability' (CSL), reflecting the overall efficiency of product formation for the molecule. Details are provided in Box 1.

Note that, while the CSL is an important factor in determining the rate of metabolism, other factors will influence the overall rate, including the substrate binding affinity and rates of oxidation decoupling to form peroxide. However, CSL can be used as a descriptor in a local model, along with other descriptors related to affinity, to predict the rate of metabolism for a series of related molecules [7].

$$k_i \propto e^{-\frac{E_{ai}}{kT}} \qquad R_i(\%) = \frac{k_i}{\sum_{\text{all sites}} k_j} \times 100$$

$$L_i = \frac{k_i}{k_w + k_i} \qquad CSL = \frac{\sum_{\text{all sites}} k_i}{k_w + \sum_{\text{all sites}} k_i}$$

Box 1. Equations for parameters calculated by StarDrop P450 models.

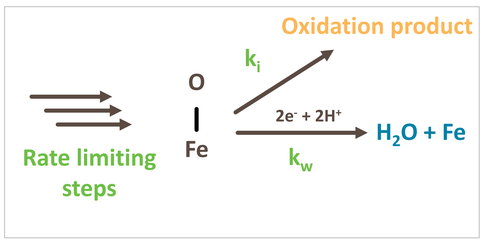

Figure 1. Reaction scheme showing competition between product formation and decoupling to form water and inactivated haem in the product formation step of the catalytic cycle.

Results

We compared the accuracy of calculations performed with the P450 metabolism models in StarDrop 5.0 (which do predict N-oxidation and -hydroxylation) with our new models (StarDrop 5.1), which include N-oxidation and -hydroxylation pathways, on independent test sets for CYP3A4, CYP2D6 and CYP2C9 metabolism.

Isoform	N	StarDrop 5.0 Models			StarDrop 5.1 Models		
		Top-2	Top-3	All sites	Top-2	Top-3	All sites
CYP3A4	273	73%	80%	56%	74%	81%	58%
CYP2D6	200	71%	81%	70%	74%	82%	72%
CYP2C9	153	73%	80%	67%	77%	84%	73%

Table 1. Results for the P450 models implemented in StarDrop 5.0 and StarDrop 5.1 on independent test sets. "Top-N" is the percentage of compounds for which the at least one site was observed to be metabolised in the top N predicted sites. "All sites" shows the percentage of sites for which all observed sites were predicted in the top 3 or to account for >10% of metabolism.

As N-oxidation and -hydroxylation reactions account for only ~5% [5] of observed P450-mediated reactions, we would only expect a small improvement in overall accuracy. However, it is important to confirm that the introduction of a new pathway does not lead to a reduction in correct prediction for other products.

With regard to N-oxidation and -hydroxylation, 46% (12/26) were predicted (top-3) for CYP3A4, 64% (9/14) for CYP2D6 and 75% (9/12) for CYP2C9.

Site Lability

The site lability provides useful information to guide the redesign of compounds to improve their metabolic stability. For example, Figure 2(a) shows the predicted regioselectivity of metabolism and site lability for the dibenzoxazepine Loxapine. This indicates that metabolism on the methyl group C23 will account for ~90% of observed metabolism and that this is a highly labile site. Blocking or removing this site is likely to significantly improve the metabolic stability, as reflected by the lower CSL, resulting in the drug Amoxapine (Figure 2(b)).

Similarly, the predicted regioselectivity of metabolism of Metaclopromide, shown in Figure 3, suggests that metabolism at the C2 and C4 positions will also account for 80% of observed metabolism. However, removing these sites is unlikely to have a significant effect on the rate of metabolism, as there are other highly labile sites and hence metabolic switching is likely to occur with little impact on the overall rate of metabolism'

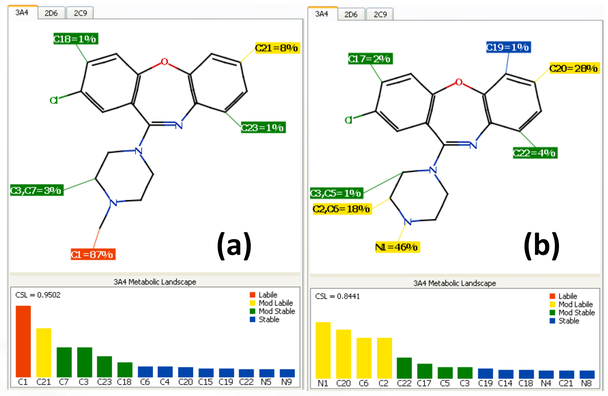

Figure 2. Predicted CYP3A4 metabolism of Loxapine (a) and Amoxapine (b). The predicted regioselectivity is shown above for each and the site liability is indicated by the 'metabolic landscape' below. The reduction in CSL suggests amoxapine will have improved metabolic stability relative to loxapine.

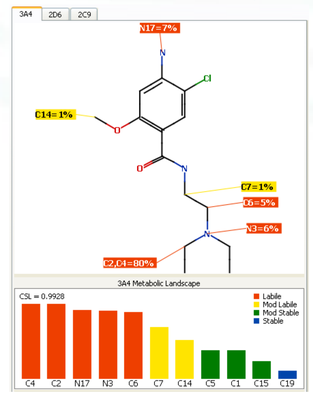

Figure 2. Predicted CYP3A4 metabolism of Metaclopromide. The large number of highly labile sites suggests that it will be necessary to block multiple sites in order to significantly improve the metabolic stability.

Conclusion

We have described models of P450 metabolism based on a combination of QM simulations, steric and orientation effects. We have shown that the addition of a model of N-oxidation and N-hydroxylation pathways has improved the accuracy of previous models and illustrated how site lability can provide additional information to guide the redesign of compounds with improved metabolic stability.

References

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