Prediction of Non-Specific Hepatic Microsomal Binding from Readily Available Physicochemical Properties

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INTRODUCTION

An important aspect of the extrapolation of *in vivo* drug clearance from data obtained with liver or recombinantly expressed microsomal systems is the knowledge of unbound drug concentration in the *in vitro* system[1]. In particular, for lipophilic compounds the prediction of clearance can be improved considerably by accounting for non-specific microsomal binding of substrate, expressed as the unbound drug fraction in a microsomal incubation (fu_{mic}). Thus, it is of interest to be able to predict fu_{mic} using *in silico* models. Here we describe the prediction of hepatic fu_{mic} based upon readily available physicochemical properties *viz.* acid-base-neutral class, ionisation state and $logP_{o:w}$. The results obtained represent a significant improvement on a previously reported model for the prediction of fu_{mic} from physicochemical properties[2].

AIM & OBJECTIVES

- To build robust models for the prediction of fu_{mic} from physico-chemical properties.
- To assess the performance of previously reported models for the prediction of fu_{mic} from physico-chemical properties.

DATA COLLECTION

- A large, diverse, unpublished dataset (n = 135 different drugs) was collected from Simcyp Consortium members (www.simcyp.com). This was complemented by other data from the literature or unpublished data from academia (n = 75); bringing the total number of drugs (after consolidation and cleaning) to 156.
- Proprietary data was "anonymised" (chemical structure was not known)
- The experimental data comprised fu_{mic} measurements, typically at or around 1 µM (see also assumptions section), with human or rat liver microsomes (where comparison was possible there was no significant difference between human and rat fu_{mic} values).
- Experimental or predicted pK_a and $logP_{o:w}$ values were used as covariates.
- fu_{mic} values were adjusted to a microsomal protein concentration of 1 mg/ml using Equation 1 before building the models.

ASSUMPTIONS AND METHODS

- Microsomal binding is non-specific.
- The system is a non-saturable microsome-buffer phase equilibrium which can be expressed in the form of a partition coefficient (K_{mic}) where:

$$K_{mic} = (1 - fu_{mic}) / fu_{mic}$$

- fu_{mic} values at any given protein concentration (mg/ml) can be adjusted to fu_{mic} at 1 mg/ml protein concentration using the method of Austin *et al*[2]:

$$fu_2 = 1 \Big/ \left(\frac{C_{mic,2}}{C_{mic,1}} \left(\frac{1 - fu_1}{fu_1} \right) + 1 \right) \qquad (1)$$

- Compounds were divided into basic, neutral and acidic classes
- For each of these classes separate models were built for $logK_{mic}$ *vs* $logP_{o:w}$
- All models were validated using repeated leave-many-out crossvalidation (≡ multiple random test sets)

RESULTS

- For each ionisation state class a strong correlation of $logK_{mic}$ with $logP_{o:w}$ was obtained[3] :

Predominantly ionised bases: $logK_{mic} = 0.58 \times logP - 2.02$ [$r^2 = 0.73$] ; *Predominantly neutral compounds:* $logK_{mic} = 0.46 \times logP - 1.51$ [$r^2 = 0.61$]

Predominantly ionised acids: $logK_{mic} = 0.20 \times logP - 1.54$ [$r^2 = 0.64$]

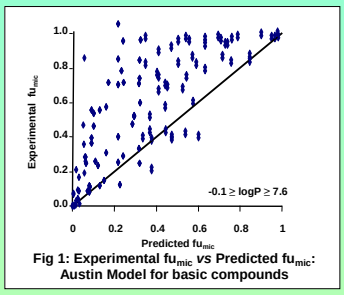

Fig 1: Experimental fu_{mic} *vs* Predicted fu_{mic}: Austin Model for basic compounds

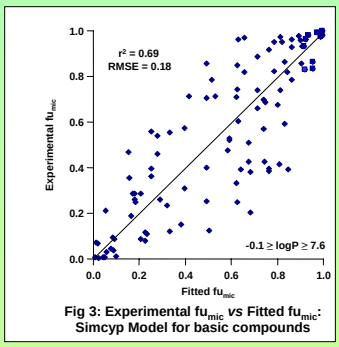

Fig 3: Experimental fu_{mic} *vs* Fitted fu_{mic}: Simcyp Model for basic compounds

Comparison of the Simcyp Models with Previously Reported Models

The Simcyp Models use logP alone as a covariate of fu_{mic} while other models have used "logP|D" (*i.e.,* logP for bases and neutrals or logD for acids):

***Austin Model*[2]** [n = 56]**:** $logK_{mic} = 0.56 \times logP|D - 1.41$

***Hallifax Model*[4]** [n = 92]**:** $logK_{mic} = 0.072 \times logP|D^2 + 0.067 \times logP|D - 1.126$

The Austin Model tends to under-predict the fu_{mic} of basic (Fig 1) and neutral (Fig 2) compounds compared to the Simcyp Model (Figs 2 & 3) while the Hallifax model provides similar predictive accuracy to that of the Simcyp Model. However, the predictions of the Hallifax model for acids are much poorer than those of the other models (Fig 4).

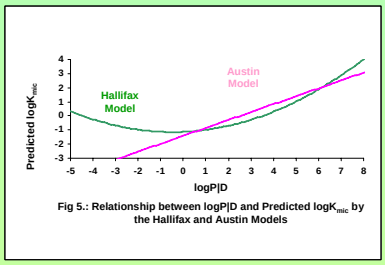

Fig 5.: Relationship between logP|D and Predicted $logK_{mic}$ by the Hallifax and Austin Models

Furthermore, the non-linear form of the Hallifax Model (Fig 5) means that where logP|D < ~0 $logK_{mic}$ increases with decreasing logP|D; a relationship that lacks experimental support[5].

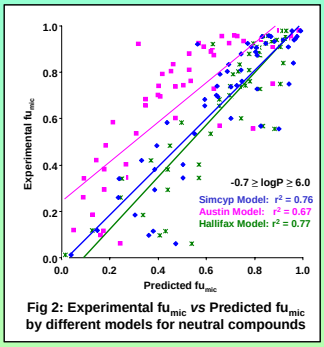

Fig 2: Experimental fu_{mic} *vs* Predicted fu_{mic} by different models for neutral compounds

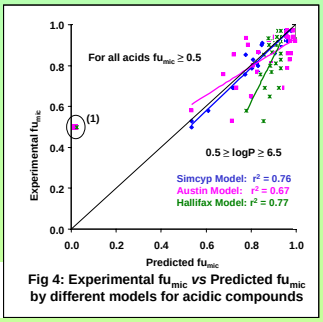

Fig 4: Experimental fu_{mic} *vs* Predicted fu_{mic} by different models for acidic compounds

(1) Outlier excluded from r^2 scores and trend lines for Austin and Hallifax Models.

CONCLUSION

A large dataset, collected from Simcyp Industrial Consortium members and the literature, has enabled both the assessment of previously published models for the prediction of fu_{mic} and the development of new more predictive models. Based on the comparisons made using this dataset, the Simcyp Model appears to perform significantly better than the Austin Model. The Hallifax Model, while performing well for basic and neutral compounds, performs poorly with acidic compounds and its non-linear form lacks experimental support. The variance in fu_{mic} not explained by logP alone can in part be attributed to the often significant intra- and inter-laboratory experimental measurement differences. Information on structural attributes of the compounds may help to explain some of the residual variability, but would add complexity and detract from practicality.

REFERENCES

1. Grime & Riley (2006) *Curr Drug Metab* **7:**251-264; **2.** Austin *et al.* (2002) *Drug Metab Dispos* **30:**1497-1503; **3.** Turner et al. manuscript in preparation; **4**. Hallifax & Houston (2006) *Drug Metab Dispos* **34:**724-726; **5.** Austin *et al.* (2006) *Drug Metab Dispos* **34:**727.