BSEP, MRP, and DILI…
Just a bad hand at Scrabble?

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Introduction

Optibrium™, as part of the European HeCaToS project, has developed models to predict a compound's inhibition of the transporters Bile Salt Efflux Pump (BSEP) and Multi-Drug Resistance Protein-4 (MRP4) in an attempt to predict the likelihood of that compound causing cholestatic Drug-Induce Liver Injury (DILI). The association of Interhepatic Cholestasis (IC) type 2 with BSEP malfunction has been drawn from the observed failure to excrete bile acids and supported by the inherited genetic mutations in the ABCB11 gene. However recent literature [1,2] has indicated that contributions from other transporters, such as MRP4, are also implicated in familial IC.

Here we present QSAR models to predict the classification of compounds into 'inhibitors' or 'non-inhibitors' of BSEP and of MRP4 based on the activity data and definitions of inhibitor/non inhibitor provided in the literature [1,2].

Our models demonstrate good predictive success for their respective target endpoints and are superior to those presented in the literature [2]. Unfortunately, they do not show good predictive ability when considering the cholestatic toxicity endpoint; an inability that is due to a poor underlying relationship between the disease and experimentally measured activity at these transporter targets.

Method and Results

We have performed QSAR experiments on literature data sets for BSEP inhibition (257 compounds) and MRP4 inhibition (86 compounds) using the StarDrop Auto-Modeller™ software [3]. Our predictions for BSEP and MRP4 activity were used to predict the cholestatic potential of an 88 compound data set of cholestatic and non-cholestatic compounds [1]. The models were built using descriptors comprising whole molecule and 2D SMARTS-based properties. The best models, presented below, used Random Forest and Gaussian process methodologies but other classification methods, such as Decision Trees, were tried for both endpoints.

	MRP4 (T/F) > 20% inhib @ 100uM	BSEP (T/F) IC_{50} <= 135uM
Training set	57 (34/23)	171 (43/128)
Test set	29 (17/12)	85 (22/63)

Figure 1: The split of data between inhibitors (True) and non-inhibitors (False) for the two endpoints and between the training and test sets

BSEP and MRP4 Models

BSEP model – Gaussian process classifier – Test set performance (Results for models in [2] shown in {})
Accuracy 89% {83%}, Kappa statistic = 0.71, MCC[5] = 0.71 {0.58}, Area Under Curve = 0.94 {0.87}

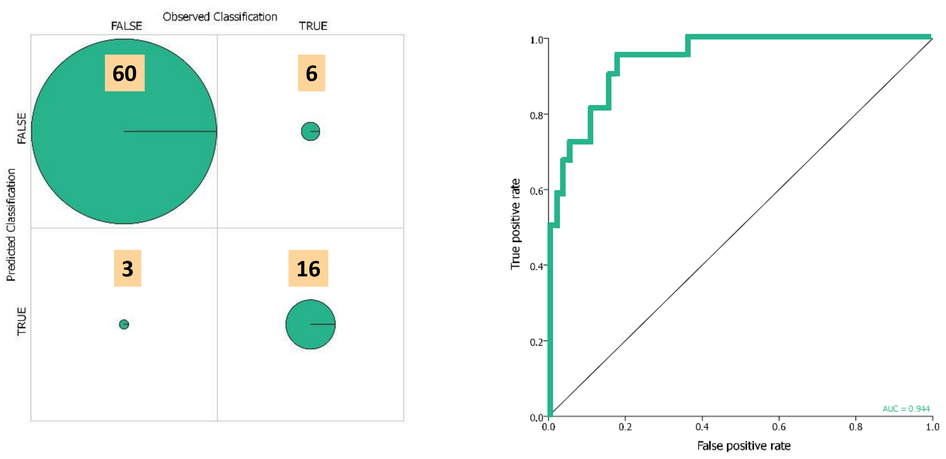

Figure 2: Confusion matrix showing the distribution of test compounds in the predicted versus actual categories and Receiver Operating Characteristic plot showing the true positive versus false positive rates as the criterion varies.

MRP4 model – Random Forest classifier – Test set performance (Results for models in [2] shown in {})
Accuracy 83% {66%}, Kappa statistic = 0.63, MCC[5] = 0.65 {0.42}, Area Under Curve = 0.86 {0.84}

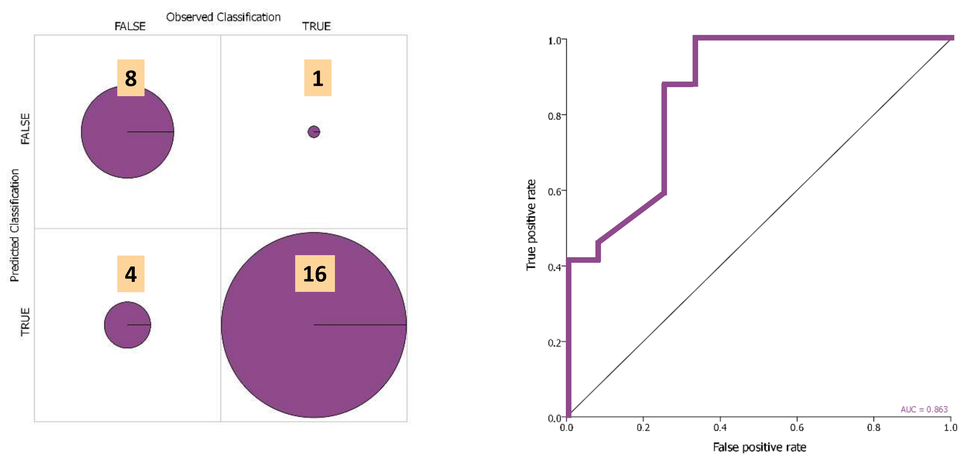

Figure 3: Confusion matrix showing the distribution of test compounds in the predicted versus actual categories and Receiver Operating Characteristic plot showing the true positive versus false positive rates as the criterion varies.

Cholestasis Models

Prediction of cholestasis based on BSEP and MRP4 predicted activity

The predictions for MRP4 and BSEP inhibitory class membership generated for the 88 compound data set are compared with their actual cholestatic activity. The kappa values for these relationships, and hence the predictive abilities of these models for cholestasis, are poor but not significantly worse than the use of the experimental data, which is detailed below in Figure 5.

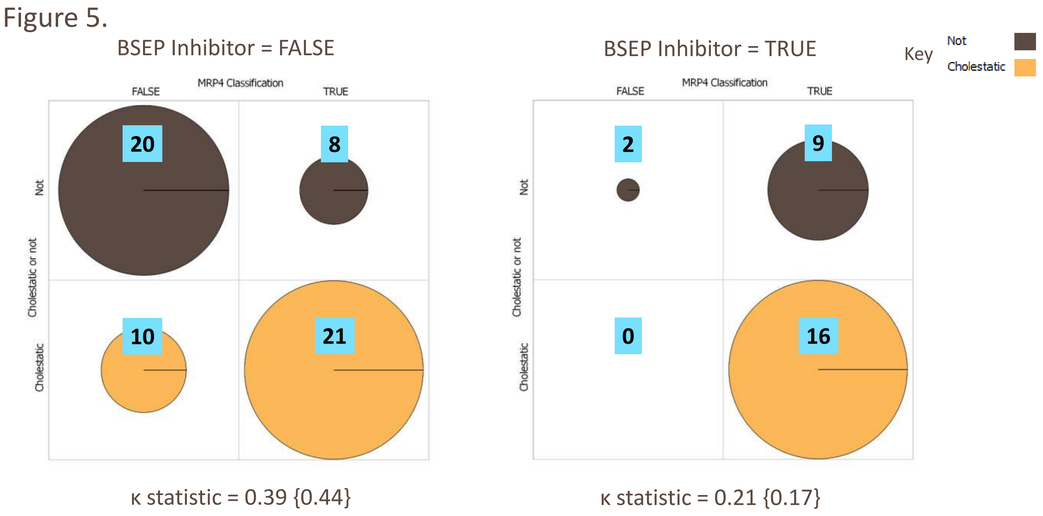

Figure 4: Confusion matrix showing the distribution of compounds using the predicted BSEP and MRP4 categorisation versus the actual cholestatic categorisation. (Kappa values from using the experimental data in {})

Experimental data for cholestasis with respect to experimental BSEP/MRP4 inhibition

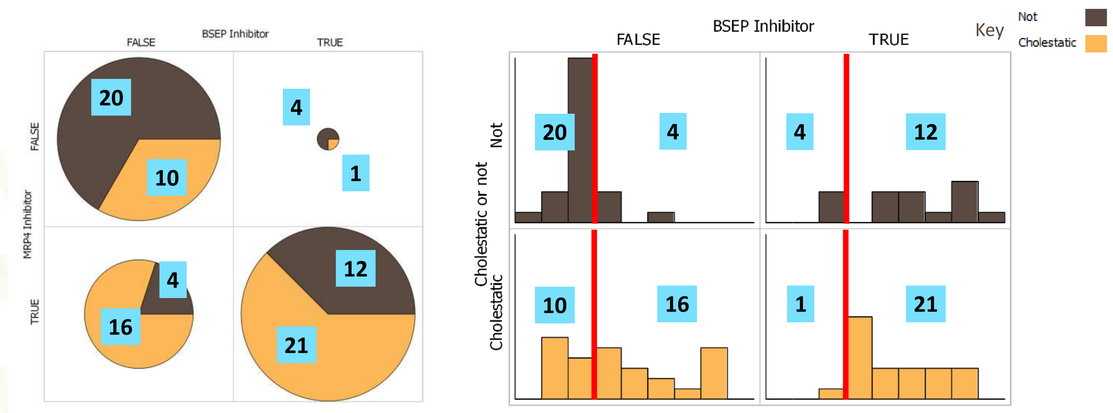

Figure 5: Distribution matrix of BSEP and MRP inhibition categorisation coloured by the fraction of cholestatic compounds in each section. The histograms show the distribution of %inhibition values for the original BSEP categorisation versus cholestatic behaviour with the classification boundary of 20% shown in red.

The choice of classification boundary of >20% inhibition at 100µM for MRP4, specified in [1], gave a false view of the importance of MRP4. The original work was looking to find reasons to rescue the false negative cholestatic compounds in the BSEP non-inhibitor group.

Discussion

There is a fair degree of overlap between inhibitory activity at BSEP and MRP4, so the inclusion of MRP4 inhibition will only be incremental in the prediction of cholestatic activity. Our analysis shows that whilst the inclusion of MRP4 may help to reduce the false negative predictions for cholestasis from measurements of BSEP inhibition alone, the activity at neither target is sufficiently predictive of the cholestasitic activity of a compound.

A recent (2014) Takeda GPR40 agonist shown in Figure 6, developed for type-2 diabetes, was terminated in Phase III due to DILI [4]. A recent paper includes the compound's activity at rat transporters that include BSEP, MRP2 (also canine), NTCP, OATP1B1, OATP1B3 with the possibility that it could have activity at other transporters. Hence while there may be a *statistically* significant relationship between BSEP/MRP4 inhibition and IC, it does not appear to be sufficiently predictive of cholestatic toxicity and models using a much greater spread of transporter targets may be required. We have built models for cholestatic activity using the 88 compound literature [1] data set but there are limited opportunities to test the general predictivity of the model due to the small amount of publically available data.

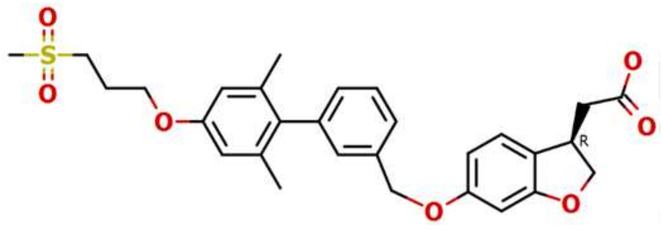

Figure 6: Takeda GPR40 agonist Fasiglifam (TAK-875)

References

[1] Köck et al. Drug Metab. Dispos. (2014) 42 pp. 665-674
[2] Welch et al. Drug Metab. Dispos. (2015) 43 pp. 725-734
[3] StarDrop, version 6.2, Optibrium Ltd, Cambridge, UK. www.optibrium.com/stardrop
[4] Li et al. Drug Metab. Dispos. (2015) 43 pp. 1751-1759
[5] MCC = Matthews Correlation Coefficient
Where MCC = ((TN*TP)-(FP*FN)) / sqrt((TP+FP)*(TP+FN)*(TN+FP)*(TN+FN))
TP=True Positive, TN=True Negative, FP=False Positive, FN=False Negative, sqrt=square root

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