QSAR Model of Regioselectivity of Metabolism in Human Liver Microsomes: Development, Validation, Comparison and Adaptation to Novel Compounds

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INTRODUCTION

Analytical identification of metabolites for a drug candidate is usually a time consuming and low-throughput task which is performed only in late drug development phases. Therefore the ability to predict possible sites of human liver microsomal metabolism using *in silico* techniques would be highly beneficial for any medicinal chemist. Moreover, the available predictions of most likely metabolism sites in a molecule later on could potentially facilitate the analysis of spectroscopic data and thus ease the experimental identification of metabolites.

In this work we present QSAR models for the prediction of metabolism regioselectivity. They provide the probability to be metabolized in human liver microsomes (HLM) for every atom of the molecule and are based on a novel GALAS (**G**lobal, **A**djusted **L**ocally **A**ccording to **S**imilarity) methodology – an approach enabling the evaluation of Model Applicability Domain via the calculation of prediction Reliability Index (RI).

DATA SET

Experimental data on metabolism in human liver microsomes for 873 compounds were collected from scientific publications dealing with analytical identification of the metabolites observed after the incubation of compound with human liver microsomes or recombinant cytochrome P450 enzymes. The preparation of the data for the modeling included marking of every atom in all molecules whether it is a metabolism site and splitting the resulting database of 8606 atoms into subsets according to reaction types (see Table 1).

TABLE 1. The structure of the HLM metabolism regioselectivity dataset.

Subset	No. of compounds	No. of metabolism sites	Total No. of marked atoms
N-dealkylation	511	333	1173
O-dealkylation	488	260	1033
Aliphatic hydroxylation	723	318	2904
Aromatic hydroxylation	739	358	3341
S-oxidation	135	57	157
Total	873	1326	8606

MODEL DEVELOPMENT

Modeling procedure is briefly outlined in Figure 1. It consisted of the following main steps:

- Atom centered fragmentation for every marked atom;
- Development of a baseline model predicting the probability to be metabolized for every atom in a molecule using PLS in combination with bootstrapping;
- Correction of baseline predictions according to experimental data for 5 most similar atoms in the training set (training library) accompanied by the estimation of prediction reliability in the form of Reliability Index (RI).

The whole described process of the molecule fragmentation and subsequent statistical analysis was realized using Algorithm Builder application. More details on GALAS modeling method can be found in our recent publications [1,2].

MODELING RESULTS

As it can be seen in Table 2, the overall results prove the effectiveness of local similarity correction and the usefulness of RI in assessing the Model Applicability Domain.

TABLE 2 The validation of HLM metabolism regioselectivity model on the test sets constituting 30% of the initial sites.

		Predicted value					
		Baseline*		Similarity Corrected*		Similarity Corrected (RI>0.5)	
		Positive	Negative	Positive	Negative	Positive	Negative
Observed value	Positive	212	88	203	97	119	34
	Negative	332	1603	71	1864	22	1310

* - Unreliable predictions (RI<0.3) were not considered which led to the exclusion of 345 marked atoms (101 metabolism sites) from the initial test set.

EVALUATION OF REGIOSELECTIVITY PREDICTIONS

The predictions of regioselectivity of metabolism in human liver microsomes were evaluated using an external test set of 42 compounds. The experimental data for these compounds were collected from newly published articles. None of the compounds was used in the development of our model.

The predictions were also compared to other software packages predicting the regioselectivity of cytochrome P450 catalyzed metabolism. The detailed results of this comparison and validation of regioselectivity model can be found elsewhere [3].

A brief summary of this validation study is provided in the next section.

FIGURE 1. Outline of modeling methodology.

$$logit(p) = \sum_i a_i x_i$$

$$X = \text{Baseline } logit(p) + \Delta logit(p)$$

$$p = \frac{e^X}{1 + e^X}$$

About 80% of predicted metabolism sites were also observed experimentally. Analyzing the results for individual compounds, the predictions could be divided into four classes according to their quality:

- 'Excellent' predictions were those when the software produced scores of >0.5 for all experimentally determined metabolism sites and the atom ranked 1st was experimentally determined as a metabolism site.

- In cases where most metabolism sites were predicted with score >0.5, the prediction was marked as 'good', though for some compounds the atom ranked by the software as most probable metabolism site was experimentally not found to be metabolized.

- When less than a half of experimentally determined metabolism sites obtained scores >0.5, the prediction was labeled 'satisfactory'. If the only experimentally determined metabolism site was ranked as one of three most probable sites by the software, but the score was <0.5, the prediction was also labeled 'satisfactory'.

- If the software failed to identify metabolism sites both by score and rank, the prediction was marked as 'unsatisfactory'.
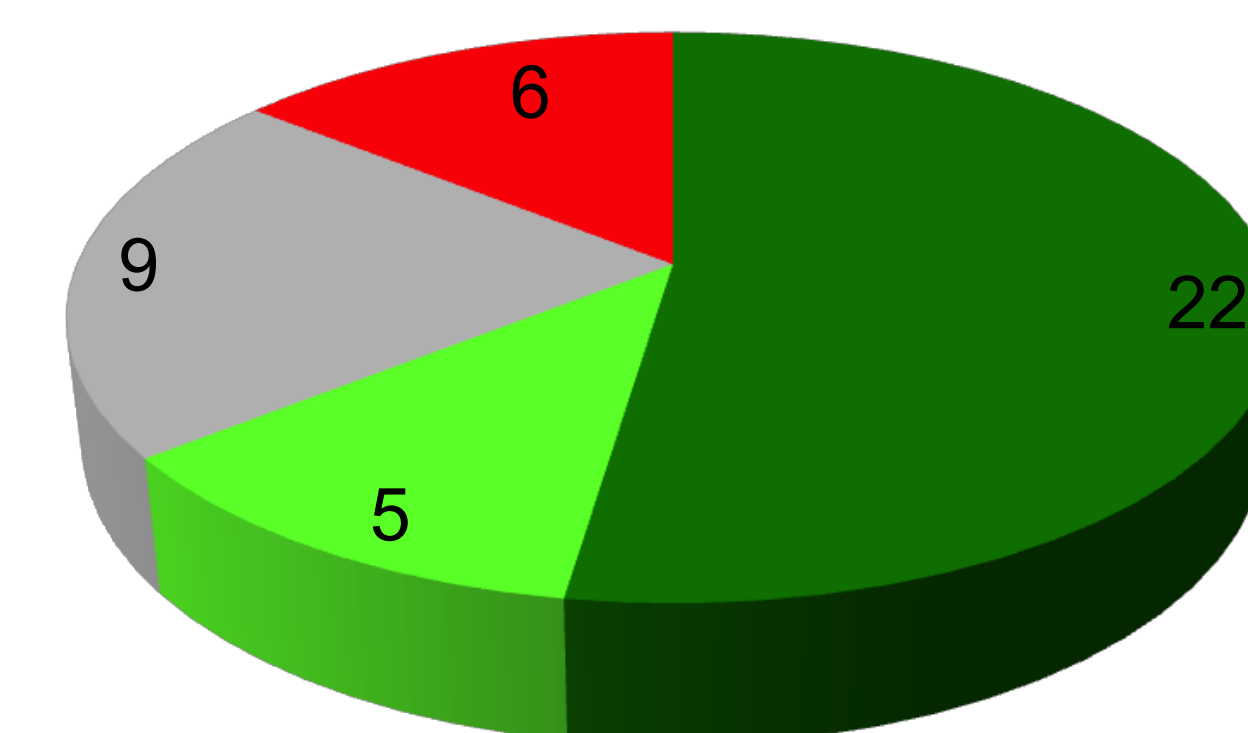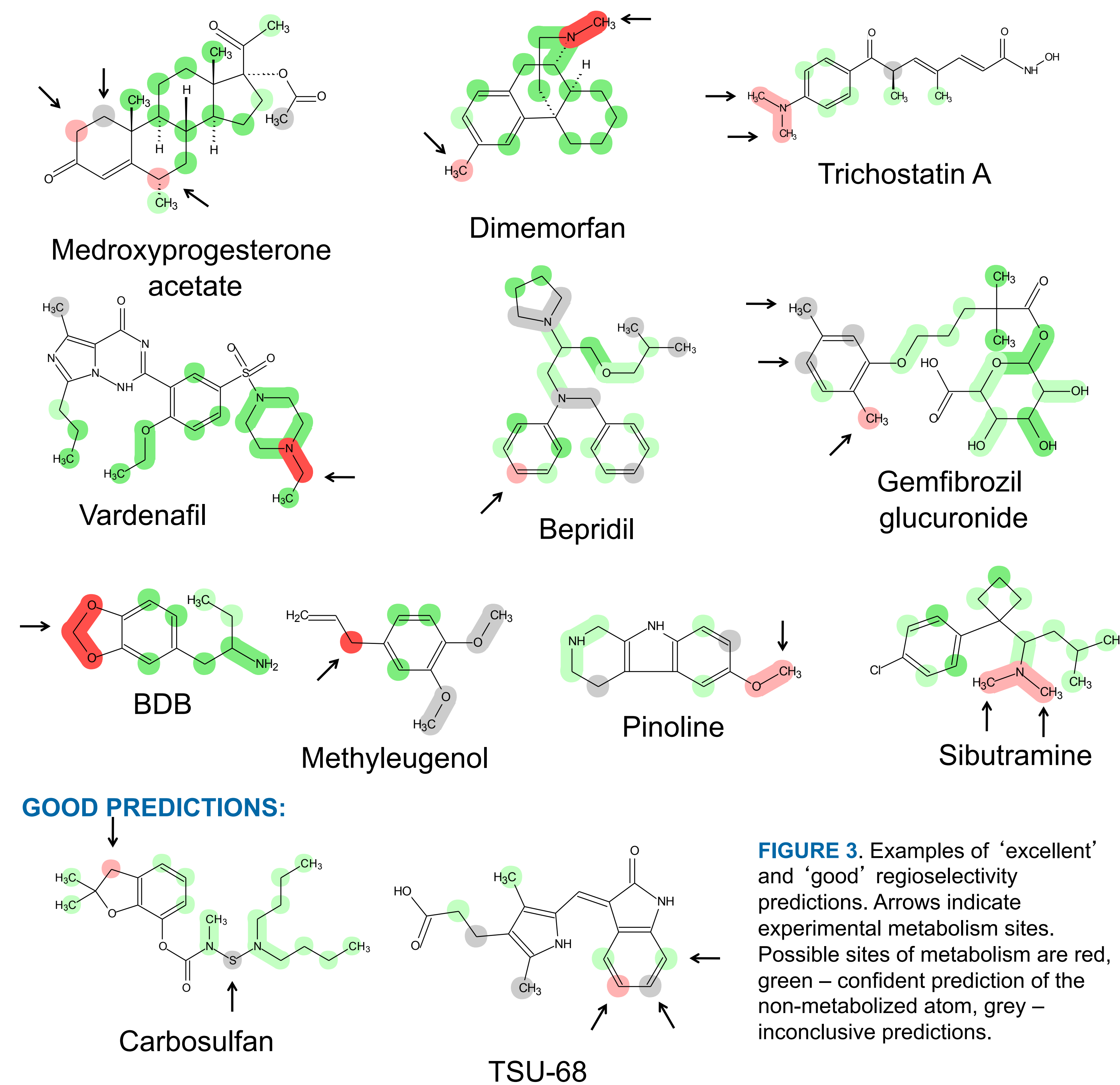

FIGURE 2. The distribution of predictions according to their quality.

Legend: ■ Excellent ■ Good ■ Satisfactory ■ Unsatisfactory

EXCELLENT PREDICTIONS:

Medroxyprogesterone acetate

Dimemorfan

Trichostatin A

Vardenafil

Bepridil

Gemfibrozil glucuronide

BDB

Methyleugenol

Pinoline

Sibutramine
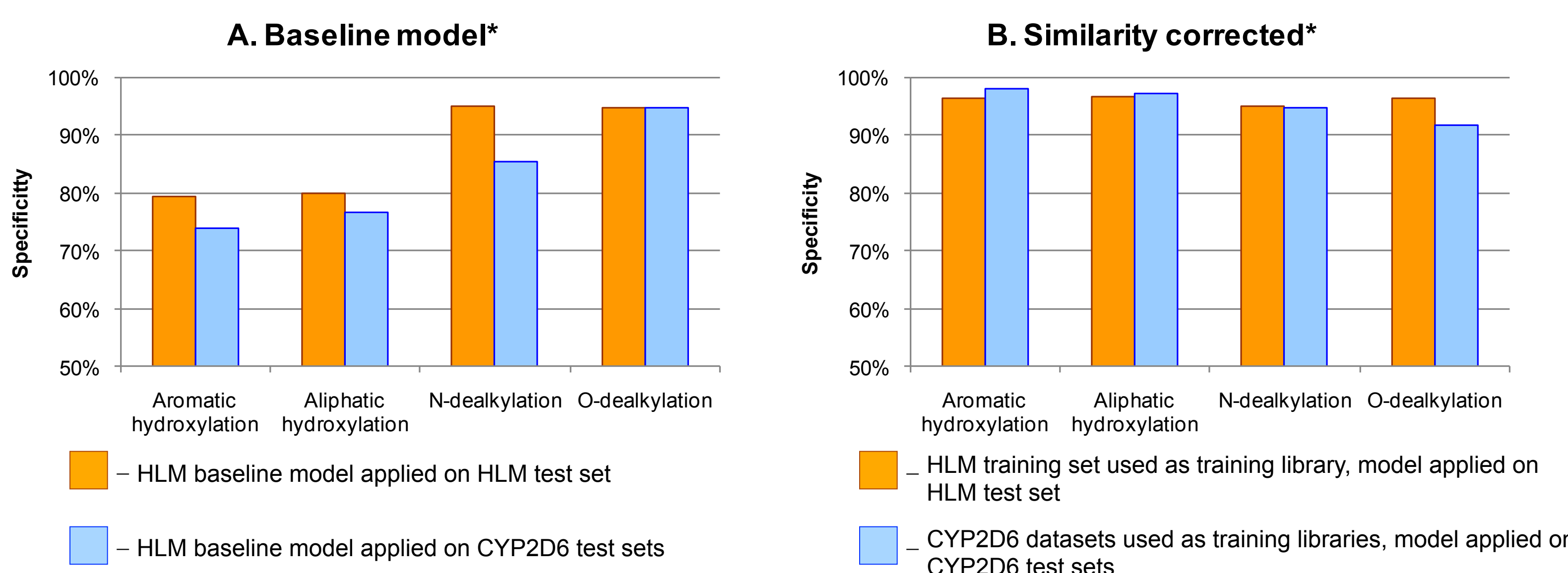
GOOD PREDICTIONS:

Carbosulfan

TSU-68

FIGURE 3. Examples of 'excellent' and 'good' regioselectivity predictions. Arrows indicate experimental metabolism sites. Possible sites of metabolism are red, green – confident prediction of the non-metabolized atom, grey – inconclusive predictions.

The overall results show reasonable agreement between predictions and experimental data. Some compounds obtaining 'excellent' or 'good' predictions are from well-known drug classes, which are well represented in the training sets, but there are also novel compounds. It is important to note, that while most of the 'satisfactory' predictions had low reliability indices, the experimentally determined metabolism sites were still predicted correctly by the software. No metabolism sites were predicted for only 6 compounds ('unsatisfactory' results). These compounds contained atypical metabolism sites, thus predictions are still in agreement with the general cytochrome P450 reactivity trends.

MODEL ADAPTATION FOR PREDICTION OF CYP2D6 SPECIFICITY

In addition to determining the metabolic profile in human liver microsomes, some projects focused on phenotyping major cytochrome P450 enzymes. The availability of *in silico* regioselectivity predictions of individual enzymes would be useful in these cases. Here we present how baseline model of human liver microsomal metabolism is adapted to the prediction of regioselectivity of CYP2D6 metabolism by means of training libraries containing experimental data for only a individual enzyme of interest.

As it was expected, the baseline HLM models produce large numbers of false positive predictions on CYP2D6 data. This is due to the fact that HLM models take into account metabolites produced by all enzymes, yet one enzyme can be only responsible for some of them. In other words, if a particular atom is metabolized by CYP2D6, it is metabolized in human liver microsomes, whereas the reverse logic is not necessarily true.

Therefore, the HLM baseline models demonstrate higher sensitivity on individual enzyme data compared to the HLM test set (not shown here), but the opposite is observed for specificity (Figure 4A). This situation noticeably changes and specificity improves after application of similarity corrections based on CYP2D6 data (Figure 4B) because the number of false positive predictions reduces.


FIGURE 4. Specificity of metabolism regioselectivity models. A – HLM baseline models, B – Similarity corrected models with corresponding training libraries.

* Only results with RI > 0.3 are shown.

Such results clearly illustrate that the GALAS modeling methodology features allow for an easy and straightforward adaptation of the resulting HLM baseline models for predictions of an individual drug metabolizing enzyme regioselectivity.

MODEL ADAPTATION TO NOVEL COMPOUND CLASS
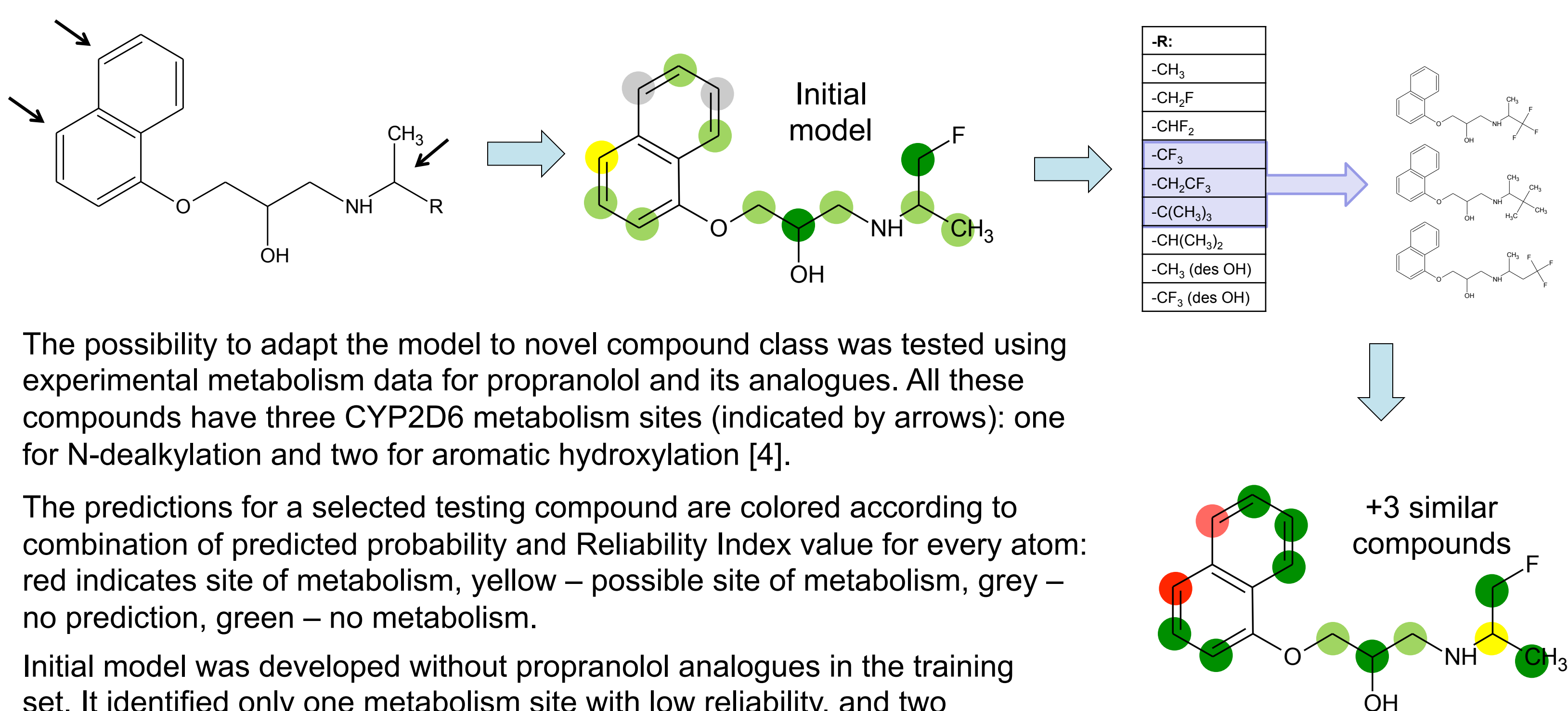
The possibility to adapt the model to novel compound class was tested using experimental metabolism data for propranolol and its analogues. All these compounds have three CYP2D6 metabolism sites (indicated by arrows): one for N-dealkylation and two for aromatic hydroxylation [4].

The predictions for a selected testing compound are colored according to combination of predicted probability and Reliability Index value for every atom: red indicates site of metabolism, yellow – possible site of metabolism, grey – no prediction, green – no metabolism.

Initial model was developed without propranolol analogues in the training set. It identified only one metabolism site with low reliability, and two atoms were predicted as inconclusive. Three randomly selected compounds have been added to the training library. After this procedure, all metabolism sites are predicted with high probability. However, the prediction for N-dealkylation site is of lower reliability, and further training is needed to increase it.

FIGURE 5. The workflow of testing the model adaptation to compounds of novel chemical class.

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