ACD/ADME Suite

P450 Regioselectivity

Evaluation study

1. Introduction

Objectives of the Study

The purposes of this study were:

- To compare P450 regioselectivity predictions from ACD/ADME Suite with those of other regioselectivity predictors, MetaSite (www.moldiscovery.com) and StarDrop (www.optibrium.com).
- To evaluate P450 regioselectivity predictions generated by ACD/ADME Suite using compounds which were not included in the training sets used to develop the models.

Description of ACD/ADME Suite P450 Regioselectivity Module

The QSAR models for Regioselectivity of metabolism in human liver microsomes are based on a dataset with >900 compounds. For each compound in the training set, every atom was marked as either a site of metabolism site (1) or not (0) and five separate probabilistic models were developed for 5 most common reactions (N-dealkylation, O-dealkylation, aliphatic hydroxylation, aromatic hydroxylation, S-oxidation).

The recently developed GALAS (*Global, Adjusted Locally According to Similarity*) modeling technique was used to build the regioselectivity model [1]. A key feature of GALAS methodology is the ability to provide an estimate of prediction reliability—a measure of whether the analyzed metabolism site falls within the model applicability domain.

Reliability estimation. Each prediction produced by a GALAS model is supplemented by a calculated Reliability Index (RI). RI values ranging from 0 to 1 provide a quantitative estimate of the prediction accuracy. Calculated RI for any atom of the compound depends on the following aspects:

- Similarity to the training set atoms. If there are no similar atoms in the training set that was used to derive the algorithm, no reliable predictions can be made.
- Data consistency. The quality of predictions is generally lower if experimental data for similar atoms are inconsistent.

The ACD/ADME Suite P450 Regioselectivity module predicts the possibility for each atom in the compound of interest to be a metabolism site in human liver microsomes. The results are reported as a calculated score of metabolism, supplemented by the RI. Atoms are colored-coded according to score values (red – possible site of metabolism, score >0.6; green – confident prediction of the non-metabolized atom, score <0.4; grey – inconclusive predictions, score between 0.4 and 0.6) to easily visualize potentially active sites.

The main predictor in ACD/ADME Suite P450 Regioselectivity module is Human Liver Microsomes Regioselectivity. It predicts possible metabolism sites for overall metabolism in human liver microsomes. This module is provided together with 5 supplementary modules, which predict possible metabolism sites for 5 most significant human liver enzymes (CYP3A4, CYP2D6, CYP2C9, CYP1A2, and CYP2C19).

2. Comparison with Competitive Software

2.1 Methods of comparison

The comparison of ACD/ADME Suite Regioselectivity predictions with competitive products was based on results reported in a poster by V. Sashi Gopaul et al., presented at the ISSX conference [2] (Comparison using standard cytochrome P450 substrates), and in an article by Markus Trunzer et al. [3] (Comparison using data reported by Trunzer et al.).

It is important to note, that carrying out fair comparisons between different software programs is difficult without knowing whether the data set studied existed as part of the training sets of any of the models under study. Most of the compounds used in the published comparison and evaluation studies are standard substrates of the corresponding cytochrome P450 enzymes. As a result the comparison of

ACD/ADME Suite predictions with results reported in poster [2] can be interpreted as the ability of the program to recognize the standard substrates of cytochrome P450 enzymes.

For the second comparison, again most of the compounds studied were part of the ACD/ADME Suite training set. Since there were also some compounds *not* included in the training set in this comparison, their predictions will be analyzed more detail.

2.2 Comparison using standard cytochrome P450 substrates

The poster [2] gives the results of predictions for separate enzymes (CYP3A4, CYP2D6 and CYP2C9). Most of the reported compounds are included in the training set of ACD/ADME Suite Regioselectivity models and the similarity between results suggests that the same compounds also exist in the training set of StarDrop.

In the tables below, the prediction result was given three points if its most likely site of metabolism was ranked first, two points if second, one point if third. The last row gives the total sum of points for all programs.

Table 1. Prediction of CYP3A4 metabolism sites.

	MetaSite*			**StarDrop***			**ADME Suite**		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Testosterone	3			3			3		
Progesterone	3			3				2	
Ethinylestradiol	3			3			3		
Midazolam		2		3			3		
Phenacetin	3				2		3		
Nifedipine			1				3		
Verapamil		2			2		3		
Aflatoxin			1		2				
Erythromycin		2		3			3		
Etoposide	3			3			3		
Tamoxifen		2		3			3		
Amitriptyline	3			3			3		
			28			30			32

*The results for MetaSite and StarDrop are reported in poster [2].

Table 2. Prediction of CYP2D6 metabolism sites.

	MetaSite*			StarDrop*			ADME Suite		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Bufuralol	3			3			3		
Propranolol					2		3		
Metoprolol	3			3			3		
Debrisoquine			1	3					
Imipramine				3			3		
MPTP	3					1	3		
Dextromethorphan	3			3			3		
Codeine	3			3			3		
Desipramine	3			3			3		
			19			24			24

*The results for MetaSite and StarDrop are reported in poster [2].

Table 3. Prediction of CYP2C9 metabolism sites.

	MetaSite*			StarDrop*			ADME Suite		
CYP2C9	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Naproxen	3			3			3		
Diclofenac	3			3			3		
Tolbutamide		2		3			3		
Tienilic Acid							3		
Mefenamic Acid	3			3				2	
Flurbiprofen	3			3			3		
Piroxicam				3			3		
Lornoxicam				3			3		
Warfarin		2		3				2	
			16			24			25

*The results for MetaSite and StarDrop are reported in poster [2].

Based on the results presented, the predictions of ACD/ADME Suite for standard cytochrome P450 substrates are better than those of either MetaSite or StarDrop. The superior ability to predict sites of metabolism for standard cytochrome P450 substrates reflects that the model was developed using a large training set, resulting in a larger model applicability domain than the other products.

2.3 Comparison using data reported by Trunzer et al.

The second comparison, between ACD/ADME Suite and MetaSite regioselectivity predictions, is based on the results reported by Trunzer et al. [3], who studied MetaSite predictions for 18 compounds. Compounds that are not present in the ACD/ADME Suite training set are marked by an asterisk in the table below, and predictions for these compounds are analyzed in greater detail.

The exact metabolism sites were not reported in the article except for 4 of the compounds. Therefore, metabolism sites were identified by analyzing the scientific literature (when possible) or by marking the only possible atom for dealkylation.

Table 4. The comparison between ACD/ADME Suite and MetaSite according to data reported by Trunzer et al. [3]

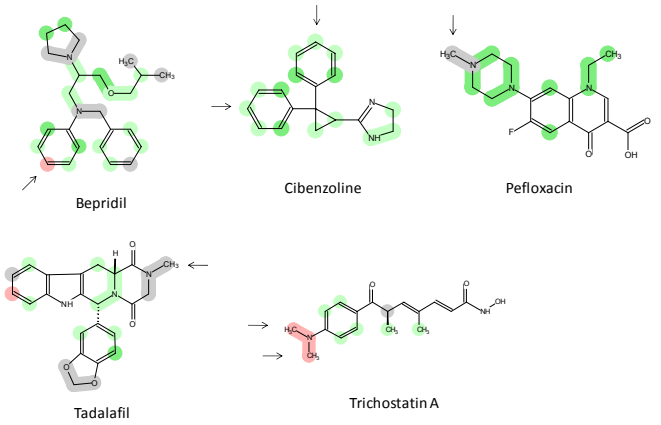
			MetaSite	ADME Suite		
Name	ΔMW	Reaction	Rank	Rank	Score	RI
Acenocoumarol	16	Hydroxylation	2	1	0,89	0,75
Bepridil*	16	Hydroxylation	6	1	0,65	0,36
Carvedilol	16	Hydroxylation	4	2	0,88	0,81
Chlorpromazine	16	Hydroxylation	4	2	0,84	0,81
Cibenzoline*	16	Hydroxylation	1	4	0,33	0,49
Citalopram	-14	Dealkylation	1	1	0,88	0,73
Kaempferol	16	Hydroxylation	1	1	0,92	0,88
Ketamine	-14	Dealkylation	6	1	0,68	0,61
Maprotiline	-14	Dealkylation	2	1	0,83	0,62
Mequitazine	16	Hydroxylation	1	1	0,76	0,61
Midazolam	16	Hydroxylation	1	1	0,95	0,91
Pefloxacin*	-14	Dealkylation	2	1	0,53	0,39
Propranolol	16	Hydroxylation	3	1	0,96	0,95
Tadalafil*	-14	Dealkylation	1	2	0,59	0,25
Toremifene	-14	Dealkylation	3	1	0,95	0,93
Trichostatin-A*	-14	Dealkylation	1	1	0,61	0,36
Tropisetron	-14	Dealkylation	1	1	0,96	0,79
Venlafaxine	-14	Dealkylation	1	1	0,93	0,81
		% with rank 1:	50%	78%		

* Compound was not present in ACD/ADME Suite Regioselectivity training set

In this comparison ACD/ADME Suite performed better than MetaSite predictions as indicated by the percentage of compounds where the major site of metabolism was ranked first by the software and because metabolism sites of most compounds obtain higher rank in ACD/ADME Suite than in MetaSite.

Moreover, in this case five compounds (Bepridil, Cibenzoline, Pefloxacin, Tadalafil, and Trichostatin-A) can be used for more fair evaluation since they were not part of the training set used to develop ACD/ADME Suite Regioselectivity models. The figure below shows the prediction for these compounds. Arrows indicate experimental metabolism sites.

Figure 1. ACD/ADME Suite predictions for Bepridil, Cibenzoline, Pefloxacin, Tadalafil, and Trichostatin-A.



Three of five compounds (Bepridil, Pefloxacin, Trichostatin-A) have an experimental site of metabolism ranked 1st by ACD/ADME Suite Human Liver Microsomes Regioselectivity module. The experimental site of metabolism for Tadalafil was ranked 2nd by the software. It is important to note, that in all these cases

the predicted score is higher than 0.5. The predictions for Cibenzoline were less accurate due to lack of similar sites of metabolism in the training set.

When considering predictions for only these five compounds, ACD/ADME Suite Regioselectivity module performed better than MetaSite. As a matter of interest, a further study was conducted predicting regioselectivity for novel compounds using ACD/ADME Suite.

3. Evaluation Using New Experimental Data

3.1 Validation dataset

An evaluation of ACD/Labs Regioselectivity module was performed using an external test set containing 38 compounds. The experimental data for those compounds were collected from newly published articles. None of the 38 compounds were used in the development of the ACD/Labs model.

3.2 Evaluation Results

About 80% of predicted metabolism sites generated by ACD/ADME Suite were also observed experimentally. Analyzing the results for individual compounds, the predictions could be divided into four classes according to their quality; ‘excellent’, ‘good’, ‘satisfactory’, and ’unsatisfactory‘.

The prediction for a compound was marked as ‘excellent’ when the software produced scores of >0.5 for all experimentally determined metabolism sites and the atom ranked 1st by the software was an experimentally determined 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.

Greater than 60% of the compounds in the validation set produced ‘excellent’ or ‘good’ predictions (18 excellent, 5 good). The figures below illustrate ACD/ADME Suite P450 Regioselectivity predictions color-coded as described above, and arrows indicate experimentally determined metabolism sites.

Figure 2. Examples of “excellent” prediction.

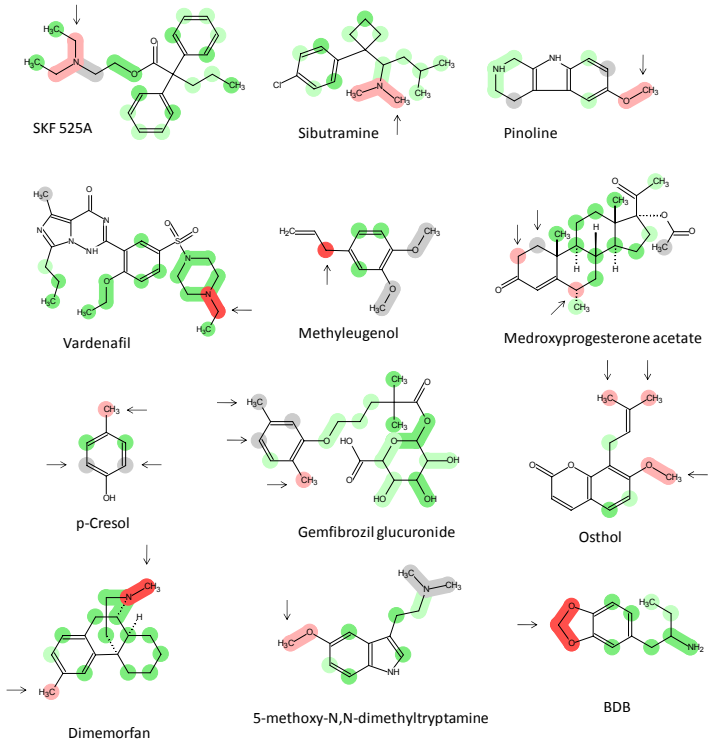
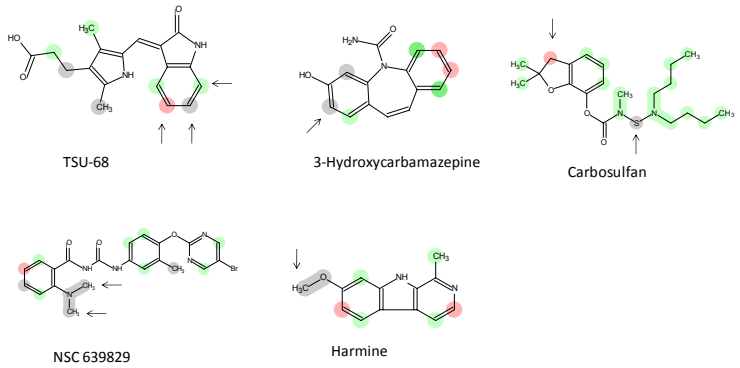


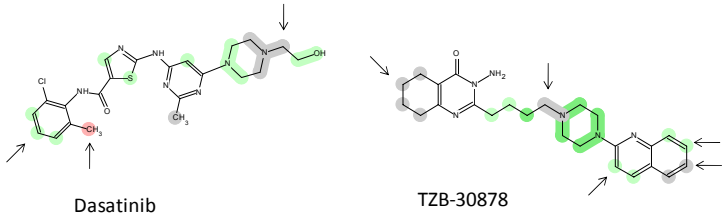
Figure 3. Examples of “good” prediction.



As it can be seen from the above figures, all types of reactions are represented among compounds with “Excellent” and “Good” predictions. Some of these compounds are from well-known drug classes (such as steroids, opioids, PDE5 inhibitors), which are well represented in the training sets, but there are also compounds belonging to novel classes.

When less than a half of experimentally determined metabolism sites were predicted by the software to be sites of metabolism with scores >0.5, the prediction was labeled ’satisfactory‘. When the only experimentally determined metabolism site was ranked as one of three most probable metabolism sites by the software, but the score was less than 0.5, the prediction was also labeled ’satisfactory‘. 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.

Figure 4. Examples of ’satisfactory’ prediction.



For the remaining compounds, predictions failed to identify experimental sites of metabolism and thus were labeled ‘unsatisfactory’. In these cases the predicted scores were <0.5 and the experimental metabolism site was not one of the top three most likely sites. No metabolism sites were predicted for only 6 compounds (“unsatisfactory” results); however, 4 of them are natural compounds, such as bile acids or secondary metabolites. There were no similar metabolism sites in the training set for these structures. Moreover, natural compounds often contain atypical sites of metabolism which are difficult to predict, thus the predictions are still in agreement with the general trends of cytochrome P450 reactivity of drug-like chemicals.

The table below lists all compounds in the validation set with the evaluation of predictions, and references from which the experimental data were taken.

Table 5. Evaluation of ADME Suite Regioselectivity prediction for external validation set compounds.

Name	References	Evaluation of prediction
SKF 525A	[4]	Excellent
SKF8742	[4]	Excellent
Sibutramine	[5]	Excellent
Sibutramine metabolite M1	[5]	Excellent
Pinoline	[6]	Excellent
Vardenafil	[7]	Excellent
R-125528	[8]	Excellent
Methyleugenol	[9,10]	Excellent
Medroxyprogesterone acetate	[11]	Excellent
p-Cresol	[12]	Excellent
Gemfibrozil glucuronide	[13]	Excellent
2-nitroanisole	[14]	Excellent
Trabectedin	[15]	Excellent
Osthol	[16]	Excellent
Dimemorfan	[17]	Excellent
5-methoxy-N,N-dimethyltryptamine	[18]	Excellent
BDB	[19]	Excellent
Harmaline	[20]	Excellent
Harmine	[20]	Good
TSU-68	[21]	Good
NSC 639829	[22]	Good
Carbosulfan	[23,24]	Good
3-Hydroxycarbamazepine	[25]	Good
2-Hydroxycarbamazepine	[25]	Satisfactory
Dasatinib	[26]	Satisfactory
Voreloxin	[27]	Satisfactory
TZB-30878	[28]	Satisfactory
Pactimibe	[8]	Satisfactory
Tanespimycin	[29]	Satisfactory
Fluticasone	[30]	Satisfactory
CP-533,536	[31]	Satisfactory
Piperacillin	[32]	Satisfactory
Flu-1	[33,34]	Unsatisfactory
Cholic acid	[35]	Unsatisfactory
Chenodeoxycholic acid	[35]	Unsatisfactory
Sanguinarine	[36]	Unsatisfactory
Zearalenone	[37]	Unsatisfactory
Zearalenol	[38]	Unsatisfactory

4. Conclusions

The results presented above show that ACD/ADME Suite P450 Regioselectivity module can be used to predict the most likely metabolism sites for drug-like compounds.

The prediction for standard cytochrome P450 substrates was shown to be better than the predictions of MetaSite and StarDrop programs. Moreover, when comparing the predictions for five novel compounds, the results of ACD/ADME Suite were better than those of MetaSite.

About 80% of ACD/ADME Suite predicted metabolism sites were also observed experimentally, this result is comparable to 85% reported by MetaSite authors. Moreover, most of the validation set compounds had excellent, good or at least satisfactory results (32 compounds of 38, >80%), better than the prediction results reported by authors of StarDrop.

ACD/ADME Suite provides predictions with the Reliability Index (RI) – an indicator of the quality of the prediction. For the compounds of well-known drug classes obtaining 'excellent' predictions, the RI were high, and for novel drugs and compounds which resulted in incorrect predictions, the RI were low. Therefore, the RI is a valuable tool to identify the compounds for which confident predictions cannot be made.

References

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