A WINDING ROAD OF METABOLIC STABILITY PREDICTIONS

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Background

The complex process of the development of new potential drugs is based not only on the provision of the activity towards the desired set of receptors, but also on establishing favourable physicochemical and pharmacokinetic properties, metabolic stability and a lack of toxicity – the parameters that disqualify compounds from further consideration despite a preferential activity profile. Even though there is an undeniable desire of construction of the tools for metabolic stability evaluation, the extreme complexity of the this phenomenon renders it very difficult to obtain high accuracy of predictions.

Aim of the study

The objective of this study was to design selective 5-HT$_7$R agonists with enhances stability towards microsomal oxidative metabolism pathway. A series of 30 long chain arylpiperazine analogs of LP-211 was synthesized and evaluated in vitro (Figure 1).[1] The obtained data on the metabolic stability of the evaluated compounds were used for comparison of the efficiency of predictions of stability by various online tools and the in house methodology based on machine learning methods and the hybrid representation of compounds.

Figure 1. Structure of the reference compound LP-211 and modifications introduced in the newly synthesized derivatives.

Results of *in vitro* experiments

The 5-HT$_7$R activity was confirmed for all the newly synthesized compounds. Metabolic stability was evaluated in two ways: at first, in the form of the percentage of the parent compound that is recovered after 30 min. incubation with microsomes in the presence of a NADPH regenerating system (%rec$_{30min}$.); then, selected compounds were characterized by their half-life time (t1/2) and intrinsic clearance (Cl$_{int}$) – predictors of in vivo hepatic clearance.

MetaPrint2D/pK$_a$ evaluation

The possible sites of compounds metabolism were detected by an online tool – MetaPrint2D. The colored spots correspond with the NOR (normalized occurrence ratio) values – high, indicate increased probability of metabolism occurring at a particular site, lower values correspond with lower likelihood of metabolism at particular point.
Additionally, pK$_a$ analysis was carried out using InstantJChem.
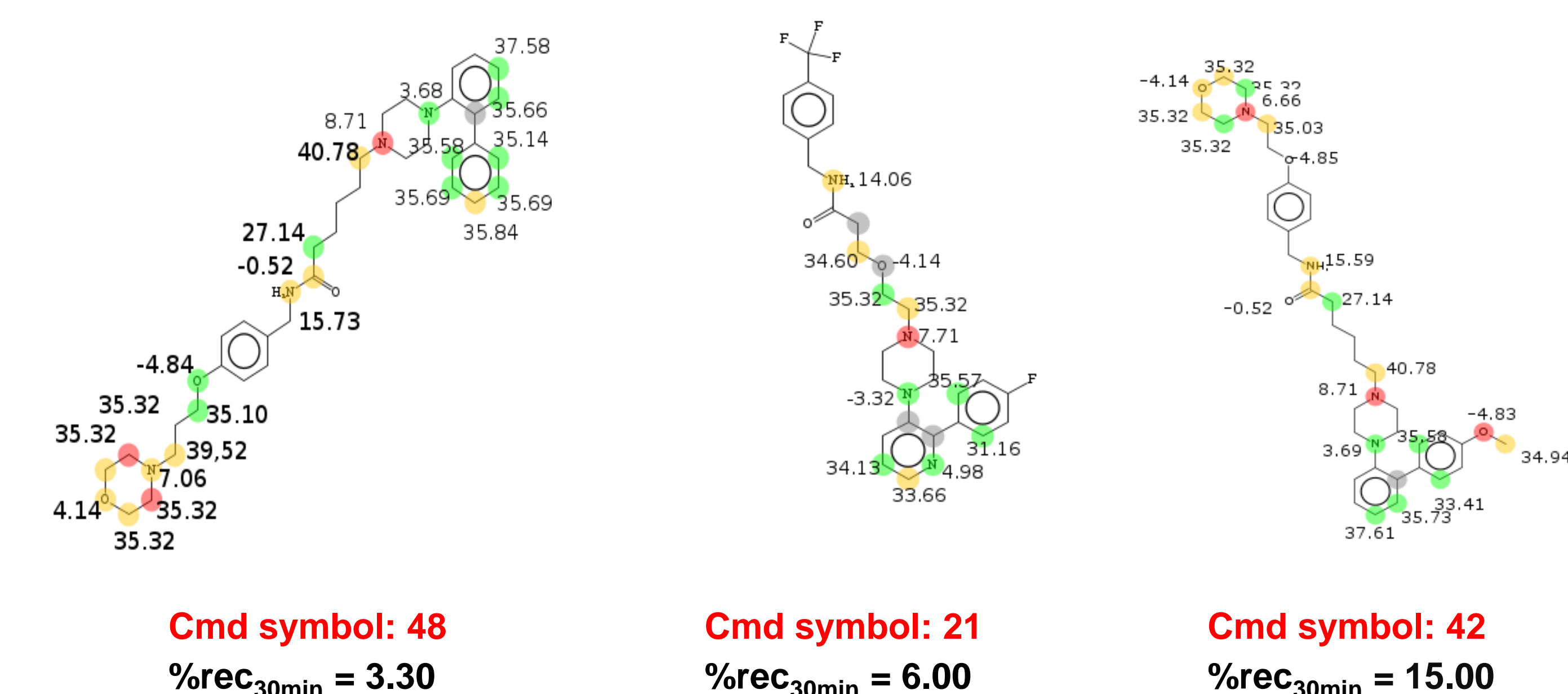The results for example compounds are presented below (Figure 2).

Cmd symbol: 48
%rec$_{30min}$ = 3.30

Cmd symbol: 21
%rec$_{30min}$ = 6.00

Cmd symbol: 42
%rec$_{30min}$ = 15.00

Figure 2. Analysis of pK$_a$ and indication of possible metabolic hotspots by the MetaPrint2D software. Red – high; orange – medium; green – low probability of a compound being metabolized at a particular spot.

ADMET Predictor, SMARTCyp & docking

The ADMET Predictor software[2] allowed the assessment of the possibility that the compound will be metabolized by specific cytochrome subtype. The analyzed compounds were predicted to be substrates to CYP3A4 and CYP2D6. In order to examine the possible interactions with the enzymes, the docking studies to the respective crystal structures were performed (Glide docking software, crystal structures: 1W0G (3A4), 3QM4 (2D6)).
Additionally, the most probable metabolic hotspots for CYP2D6 were detected using the SMARTCyp server (CYP A4 is not supported).[3]
The results of SMARTCyp predictions are presented in Figure 3, and of docking results in Figure 4.
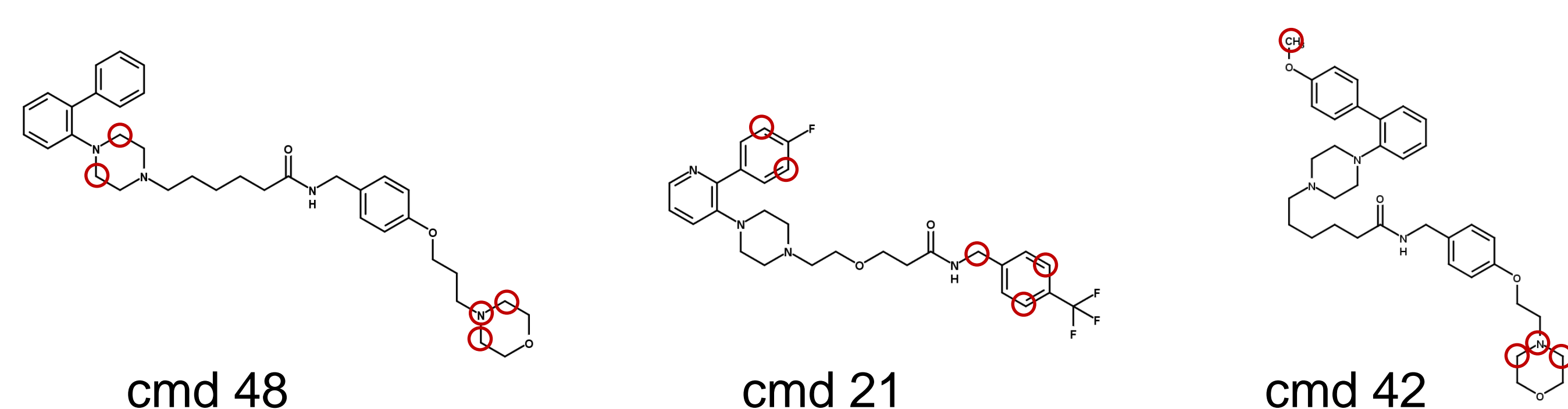

cmd 48   cmd 21   cmd 42

Figure 3. The most possible hotspots for metabolism via CYP2D6 indicated by SMARTCyp.
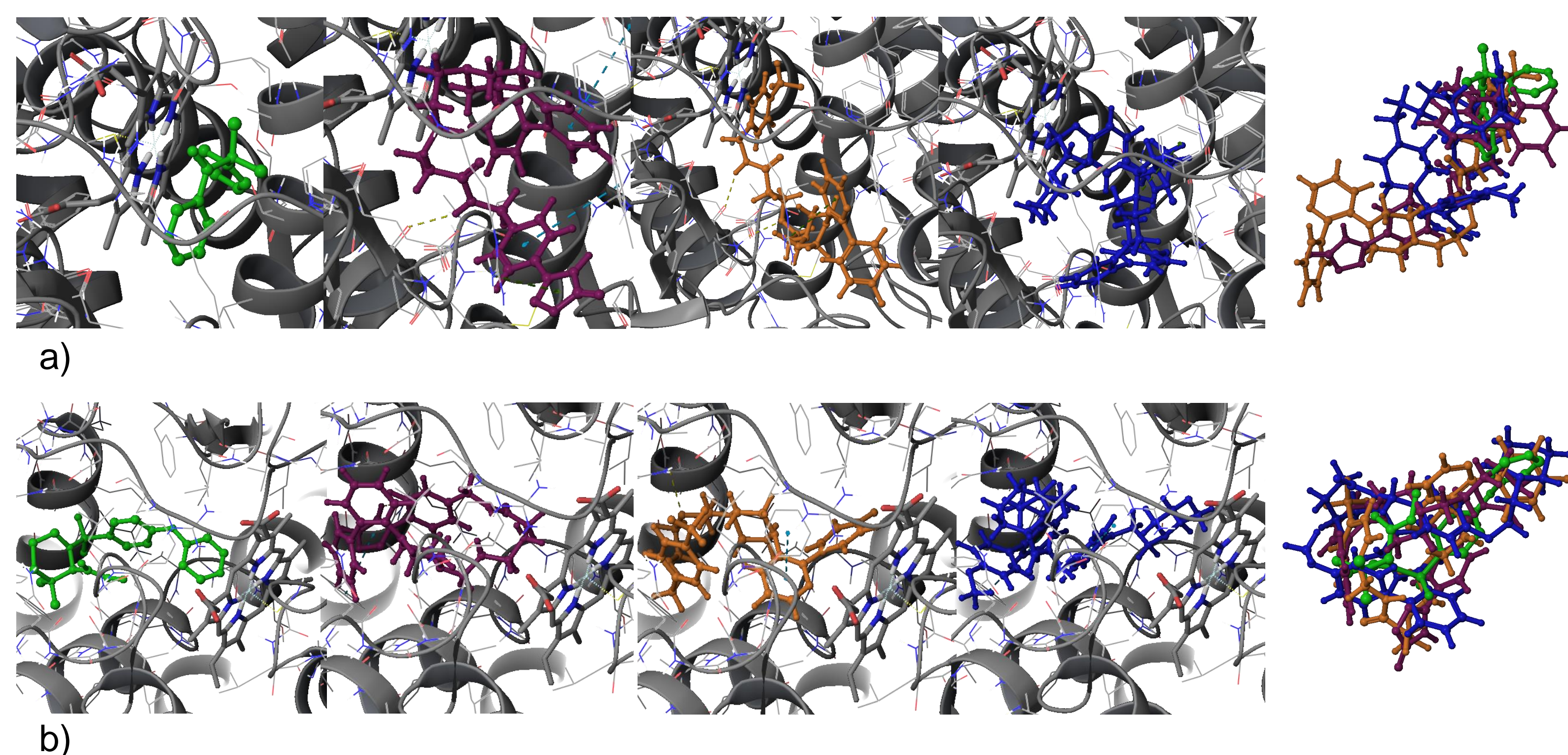

Figure 4. Docking results to a) CYP3A4 and b) CYP2D6 crystal structures. Green – crystallized ligand; maroon – cmd 48, orange - .cmd 21, blue – cmd 42.

In-house experiments

Additionally, the metabolic stability evaluation of the selected sets of compounds was performed via ligand-based approach on the basis of the metabolic stability data from the internal database of the Faculty of Pharmacy University of Bari that formed the training set, whereas the newly synthesized compounds constituted the test set (Figure 5).
The compounds were characterized with the hybrid representation forged from one-, two- and three-dimensional molecular descriptors (generated by PaDEL-Descriptor and QikProp), and the predictive model was constructed on the basis of the Support Vector Machine (SVM) algorithm adjusted to solving the regression problems (SMOreg). Experiments were conducted in the leave-one-compound-out configuration:the model was constructed on the largest possible dataset, without one compound, forming the test set in one step or after division of the test set into three parts, constituting an extension of the training set after each stage.(Figure 6).
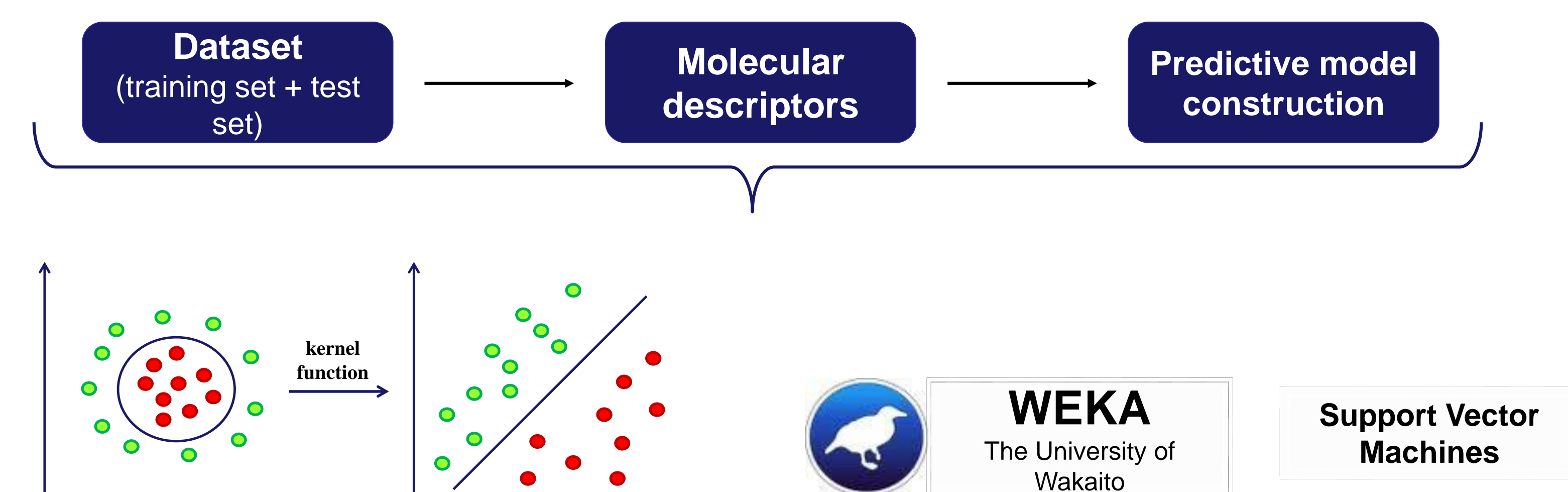

Figure 5. A scheme of the construction of the *in silico* model for predicting the metabolic stability.
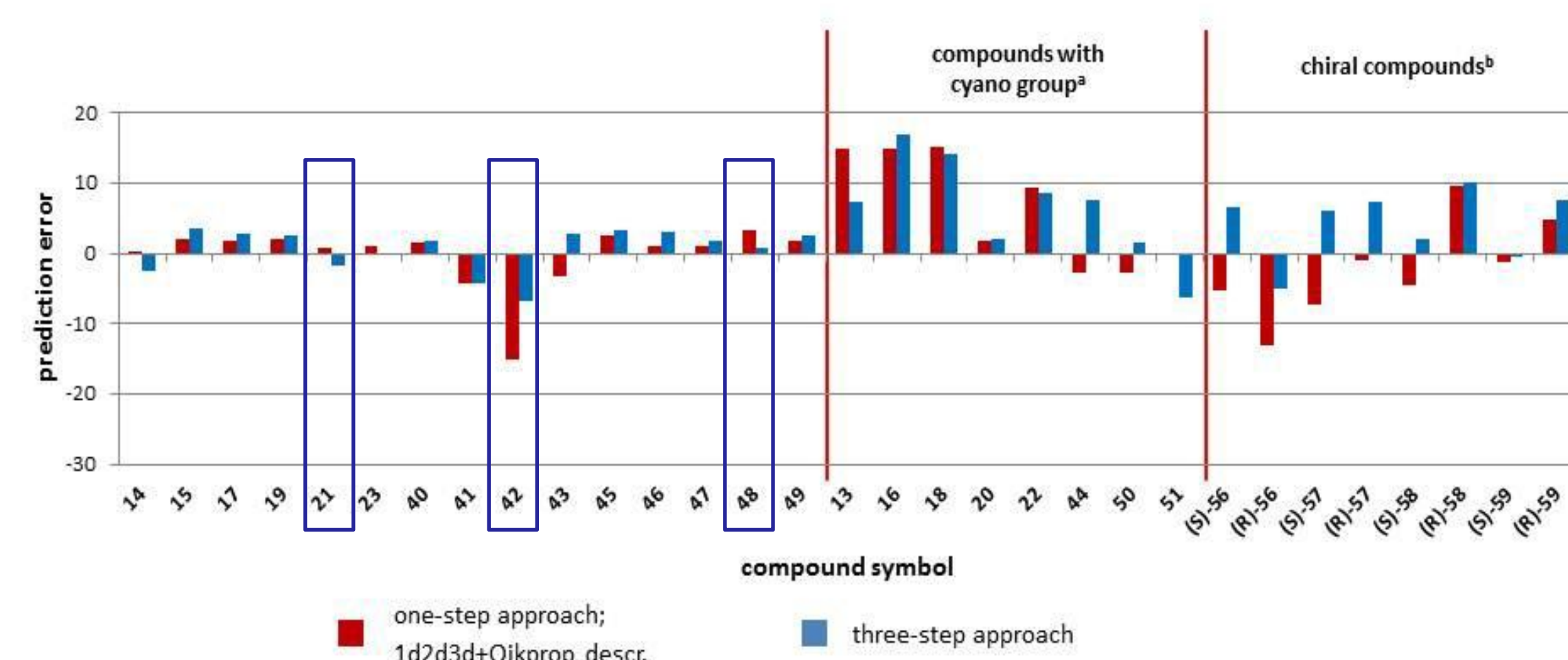

Figure 6. Error bars for one-step and three-step approach. Red lines indicate the division of compounds for particular groups in the three-step approach.
[a]Compounds predicted in the second step in the three-step approach; [b]compounds predicted in the third step in the three-step approach.

Conclusions

The obtained results, or rather high differences in the outcome provided by various tools, indicate the extreme complexity of metabolic stability problem, and therefore the difficulties in the *in silico* evaluation of this parameter. There is no explicit correlation between such parameters as logP or pK$_a$ and metabolic stability; there is also no consistency between the indication of metabolic hotspots by various available tools. The docking results are also difficult for interpretation, although the difference between the docking pose of **42** to CYP3A4 (the least stable compound out of the presented examples) and the remaining compounds is significant. The proposed ligand-based approach allowed to build a model that, in a given chemical space, is able to describe and quantitatively predict the metabolic stability of tested compounds. Metabolic stability is the result of many simultaneously occurring metabolic reactions and thus, its accurate prediction can be very challenging.

References

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[3] Rydberg, P.; Gloriam, D. E.; Zaretzki, J.; Breneman, C.; Olsen, L., *ACS Med. Chem. Lett.* **2010**, *1*, 96-100.

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