THE SERVICE OF *IN SILICO* METHODS IN THE DEVELOPMENT OF METABOLICALLY STABLE LIGANDS

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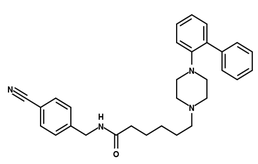
Background

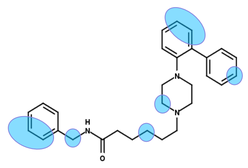
The complex process of development of new potential drugs is based not only on the provision of the activity towards the desired set of receptors, but the compounds should also possess favourable physicochemical and pharmacokinetic properties, metabolic stability and a lack of toxicity – the parameters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

Aim of the study

A series of 30 long chain arylpiperazine analogs of the selective 5-HT_7R agonist LP-211 was synthesized and evaluated *in vitro* (Figure 1).[1] The compounds were designed with the aim to enhance stability towards microsomal oxidative metabolism. 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.



LP-211



Modifications of compounds structures

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

Results of *in vitro* experiments

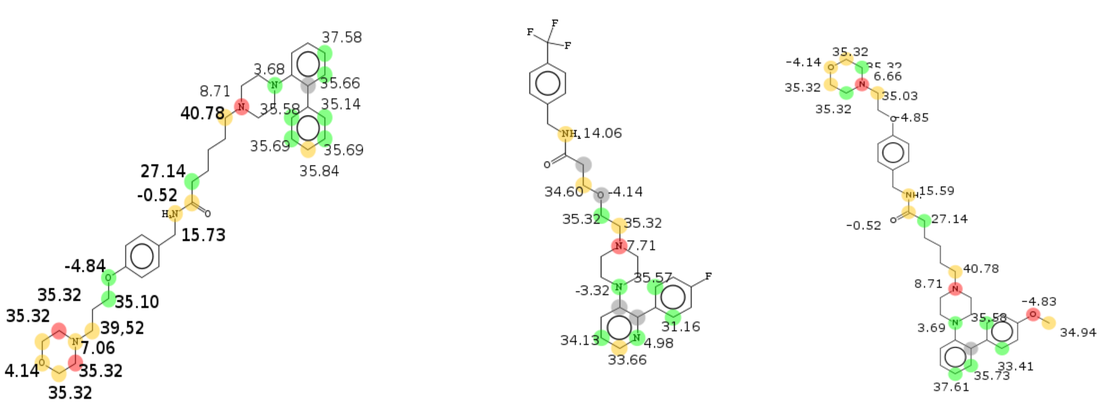
The 5-HT_7R activity was confirmed for all the newly synthesized compounds. Metabolic stability was evaluated in two ways: at first, in the form of the percentage of the parent compound that is recovered after 30 min. incubation with microsomes in the presence of a NADPH regenerating system (%$rec_{30min.}$); 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 (it was performed in InstantJChem).

The results for some 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

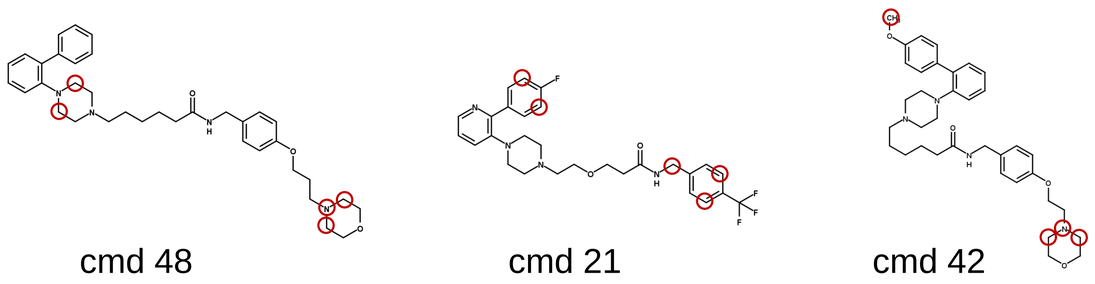
Figure 2. Analysis of pK_a and indication of possible metabolic hotspots by the MetaPrint2D software. Red – high; orange – medium; green – low probability of a compound being metabolized at a particular spot.

ADMET Predictor, SMARTCyp & docking

The ADMET Predictor software[2] allowed for the prediction of the possibility that the compound will be metabolized by particular cytochrome subtype. The analyzed compounds were predicted to be substrates to CYP3A4 and CYP2D6. In order to examine the possible interactions of compounds with the enzymes responsible for their metabolism, the docking studies to the respective crystal structures were performed (Glide docking software, crystal structures: 1W0G (3A4), 3QM4 (2D6)).

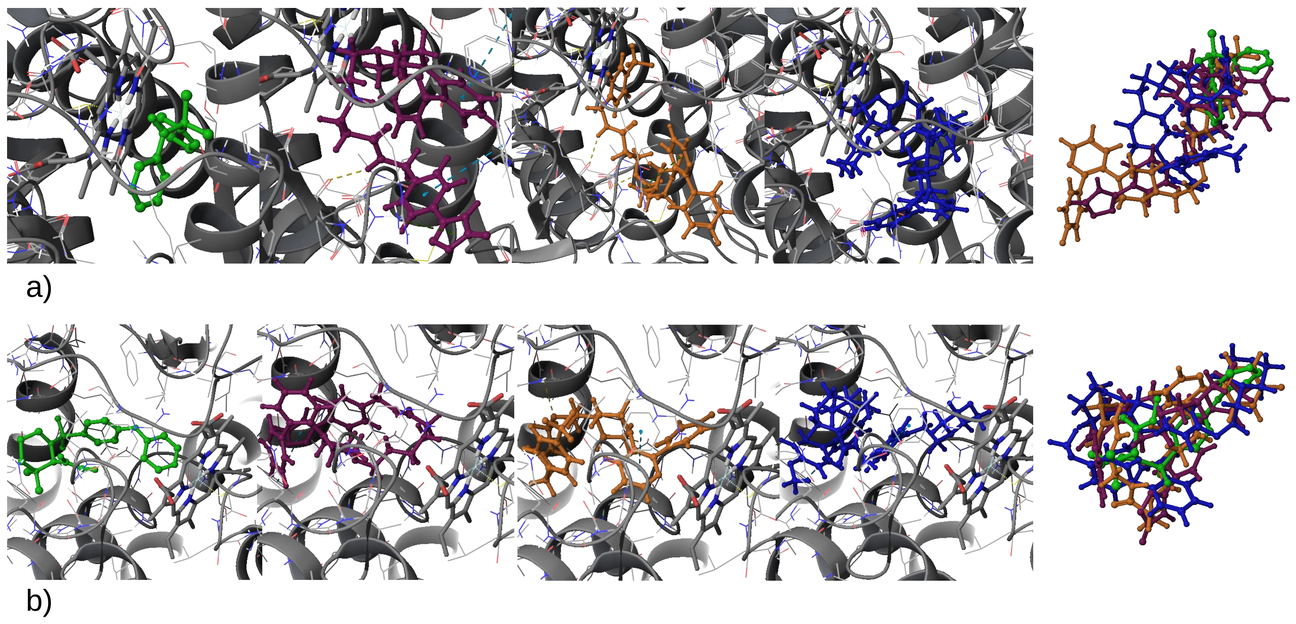
Additionally, the most possible metabolic hotspots for CYP2D6 were detected using the SMARTCyp server (CYP A4 is not supported).[3]

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.



a)

b)

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 syntesized compounds constituted the test set (Figure 5).

The compounds were characterized with the hybrid representation constituted of 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 that is the model was constructed on the largest possible dataset, without one compound, constituting in the particular iteration 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).

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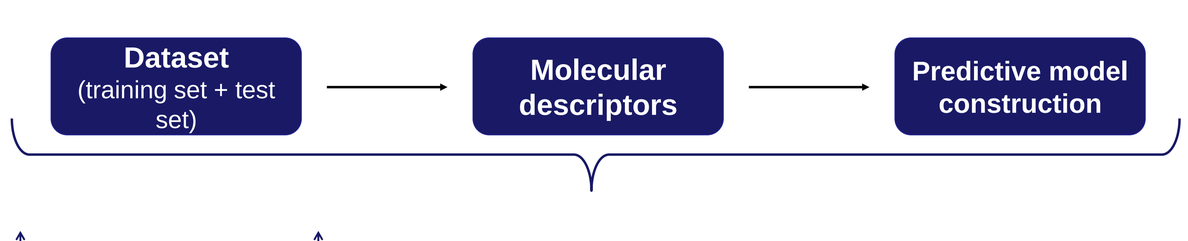

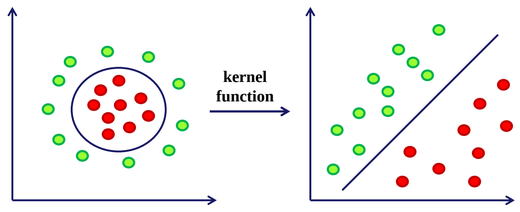

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Support Vector Machines

Figure 5. Scheme of the construction of the *in silico* model for metabolic stability predictions.

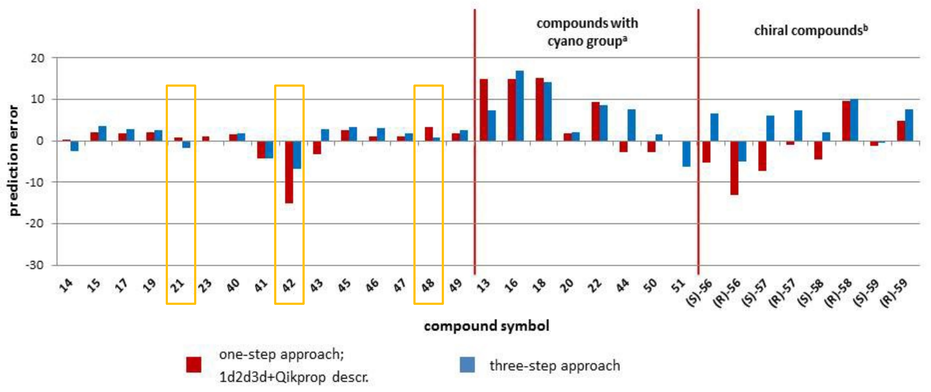

Figure 6. Error bars for one-step and three-step approach. Red lines indicate the division of compounds for particular groups in the three-step approach.

[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 simple 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 tricky 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 our compounds.

Metabolic stability is the result of many simultaneously occurring metabolic reactions and thus, its accurate prediction can be very challenging.

References

[1] Lacivita, E.; Podlewska, S.; Speranza, L.; Niso, M.; Satała, G.; Perrone, R.; Perrone-Capano, C.; Bojarski, A. J.; Leopoldo, M; *Eur. J. Med. Chem*; **2016**, *120*, 363-379.

[2] ADMET Predictor, Simulations Plus, Inc. (http://www.simulations-plus.com/Products.aspx?pID=13).

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

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