TOWARDS NEW 5-HT7R LIGANDS WITH IMPROVED METABOLIC STABILITY – SYNTHESIS OF LP-211 DERIVATIVES AND THEIR COMPREHENSIVE EVALUATION *IN SILICO* AND *IN VITRO*

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Background

Serotonin receptor 5-HT_7 belongs to the aminergic family of G protein-coupled receptors. It constitutes an important drug target, and compounds modulating its activity play an important role not only in the treatment of depression, cognitive disorders, anxiety and Alzheimer's disease, but flawed signal transduction within this receptor subtype is also associated with disorders of gastrointestinal peristaltic activity, cardiovascular reflexes, inflammation or epilepsy. Up to date, there are several groups of 5-HT_7R ligands (Figure 1). The most important include long chain arylpiperazine derivatives (a), aporphine derivatives (b) ergoline derivatives (c), tetralin derivatives (d), piperidine derivatives (e) or tricyclic compounds (f, e.g., dibenzothiapine, thioxanthene, or phenothiazine derivatives).

The complex process of designing new compounds with potential biological activity is based not only on the provision of the activity towards the desired set of receptors, but the compounds should also have favourable physicochemical and pharmacokinetic properties. Metabolic stability is very important parameter, as compound should not be metabolized before triggering the desired biological response.

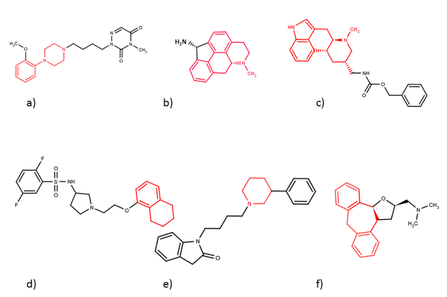


Figure 1. Example of cores of 5-HT_7R ligands.

Aim of the study

A series of 30 long chain arylpiperazine analogs of the selective 5-HT_7R agonist LP-211[1] was synthesized and evaluated *in vitro* (Figure 2). The compounds were designed with the aim to enhance stability towards microsomal oxidative metabolism. In order to facilitate and support the design of new stable 5-HT_7R ligands, *in silico* model for the metabolic stability evaluation was developed, based on the Support Vector Machines algorithm.[2]

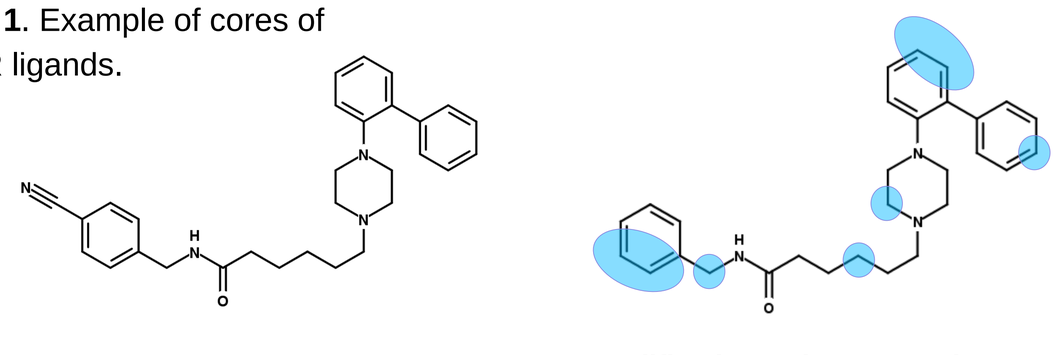


Figure 2. Structure of the reference compound LP-211 and modifications introduced in the newly synthesized derivatives.

Biological activity of the synthesized compounds

All newly synthesized compounds were tested for their affinity for 5-HT_7R and selectivity over serotonin 5-HT_{1A}, 5-HT_6, dopamine D_2, and adrenergic α_1 receptors. Biological experiments indicated, that the activity towards 5-HT_7 receptor was preserved (significant loss of activity was observed only for one compound); also the affinities towards other receptors considered were similar in comparison with LP-211; therefore they all possessed similar selectivity profile.

Metabolic stability of the synthesized compounds

The compounds were evaluated in terms of their metabolic stability in *in vitro* experiments using liver microsomes - 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. Then, compounds characterized by > 20% recovery were further examined by evaluating their $t_{1/2}$ and intrinsic clearance (Cl_{int}) – predictors of *in vivo* hepatic clearance. The results of these tests are presented in Table 1.

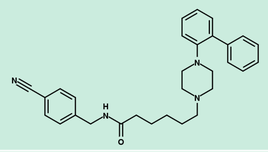
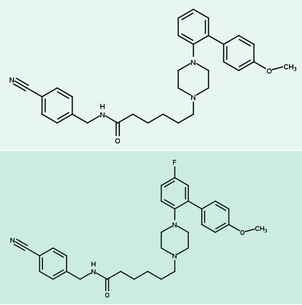
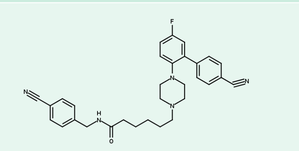
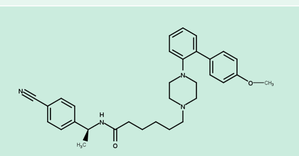
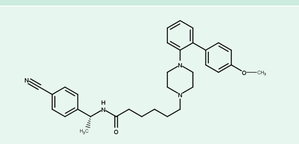
In silico model of compounds metabolic stability

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 3).

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).[3] 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 4).

The proposed computational 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 (Figure 5). Metabolic stability is the result of the susceptibility to and the rate of many simultaneously occurring metabolic reactions and thus, the accurate prediction can be very challenging.

Table 1. Half-life and intrinsic clearance of selected compounds.

Compound structure	$t_{1/2}$ (min)	CL_{int} (µL/min/mg)
	15	45.9
	18	37.9
	45	16.1
	26	26.3
	29	24.5
	23	30.1

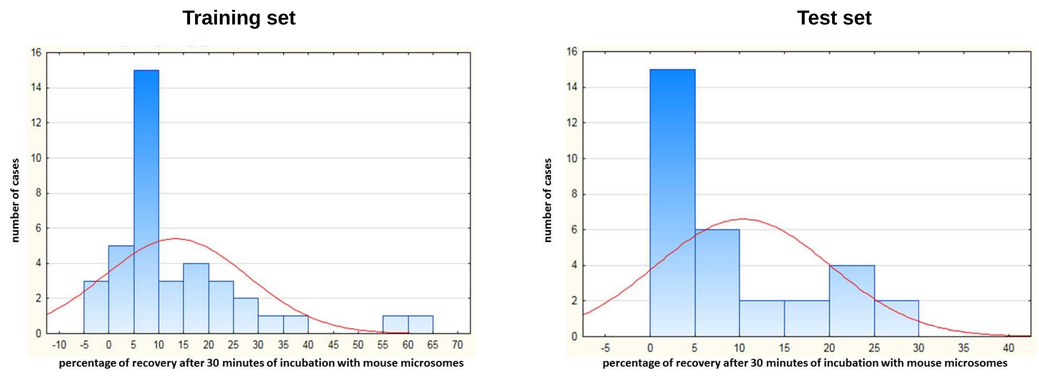


Figure 3. Histograms of the percentage of recovery values for the training and test set.

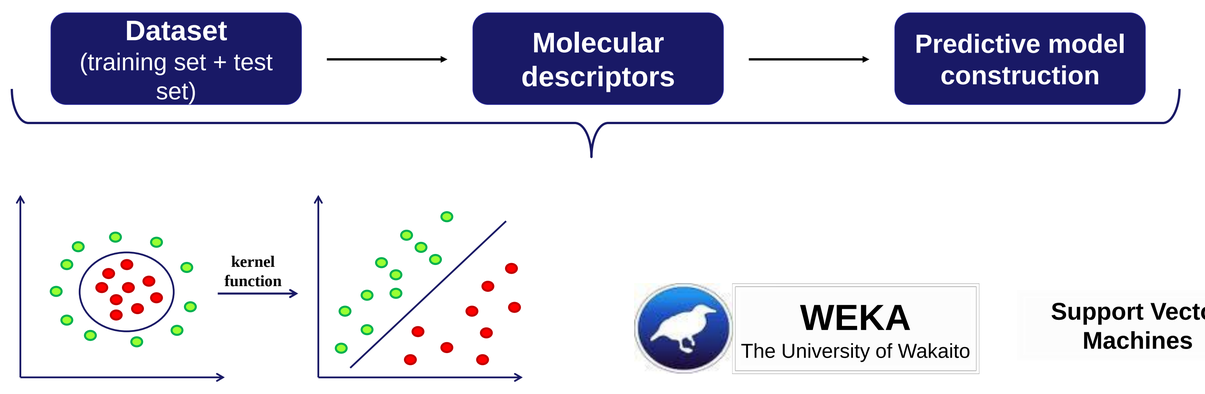


Figure 4. Scheme of the construction of the *in silico* model for metabolic stability predictions.

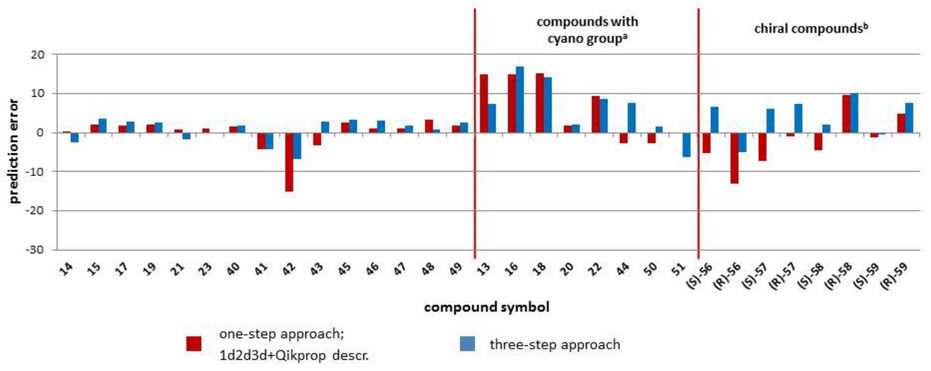


Figure 5. Error bars for one-step and three-step approach. Red lines indicate the division of compounds for particular groups in the three-step approach.
[a]Compounds predicted in the second step in the three-step approach; [b]compounds predicted in the third step in the three-step approach.

References

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[2] 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**, in press
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Acknowledgments

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