MULTIDISCIPLINARY CHARACTERIZATION OF MOLECULAR INTERACTIONS BETWEEN NICOTINIC ACETYLCHOLINE RECEPTOR AND ITS LIGANDS

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Nicotinic Acetylcholine Receptor (nAChR) is a pentameric ligand gated ion channel found at the neuronal and nerve/muscle synapses. The broad range of various CNS drugs interact *directly* with the neurotransmitter binding sites or *allosterically* on several other binding domains of the receptor modulating its synaptic activity. Fast and reliable methods of measuring and characterization of this modulation is of great interest in CNS pharmacology and drug discovery.

New methods to characterize experimentally and theoretically the interactions between the receptor and various classes of drugs will be presented:

Bio-affinity chromatography technique, where the membranes containing nAChR were immobilized on the chromatographic stationary phase, can be used to describe the affinity of small ligands towards the receptor and the kinetics of this process. We used this method in fast screening of the series of constrained nicotine analogs and the method was able to successfully sort out the compounds with pronounced activity on nAChR. High agonistic activities were further confirmed in regular functional assays for these compounds.

Bioaffinity chromatography was also employed to determine the affinity of non-competitive (allosteric) inhibitors (NCIs) towards the receptor. This application is particularly important since other methods are hardly applicable to characterize the strength of binding for this class of ligands. The data collected for the series of non-competitive inhibitors was used to generate QSAR models describing the affinity of ligands towards the internal channel domain of nAChR.

Computational modeling techniques were used to elucidate the molecular mechanism of non-competitive inhibition. The inner surface of the nAChR channel is regarded as the most common active site for the binding and the inhibition of the ion flux by NCIs. The molecular model of the channel domain was generated by homology modeling. Molecular docking procedure was elaborated and the series of ligands-active site molecular complexes were developed. Free energies of binding (ΔG) obtained in these simulations were found to be strongly correlated with ligands affinities measured in affinity chromatography experiments.

The results of the computational simulations and QSAR modeling suggested the alternative mechanism of blocking action for non-competitive inhibitors of ion channels. Both experimental procedures and theoretical modeling can be employed for fast screening of pharmacological modulation of nAChR by CNS drugs and new drug candidates. Various subtypes of the receptor are of increasing interest for medicinal chemists as potential drug targets and presented methods of ligand characterization can be used in drug discovery and design.