Alicja Aleksandra Gawalska

Computer-aided search for multi-target-directed ligands blocking PDE4B, PDE8A and TRPA1 with potential application in the pharmacotherapy of asthma and COPD

Abstract

In the present time, chronic respiratory diseases constitute a serious and challenging problem facing modern science and healthcare. 650 million people suffer from the chronic obstructive pulmonary disease (COPD) worldwide and over 330 million contend with asthma. In the epidemic of SARS-COV-2 causing COVID-19, where COPD was a state of increased risk of severe course, the problem of effective treatment of these diseases appears to be even more alarming. The currently available therapeutic strategies for asthma and COPD have anti-inflammatory and bronchodilatory effects, which, however, do not cover all the most important pathological processes in the course of both diseases. Therefore, there is an urgent need to develop possible, comprehensive solutions.

The multifactorial nature of diseases provides the logical foundation for the development of an innovative drug design strategy based on multi-target-directed ligands (MTDL), which gives the potential to broaden the spectrum of therapy and achieve a cumulatively stronger therapeutic effect. A possible solution could be rationally designed MTDLs combining PDE4B and PDE8A inhibition with TRPA1 ion channel antagonism. PDE4B, PDE8A, and TRPA1 are expressed simultaneously in many cells relevant to the pathogenesis of chronic respiratory diseases. Their complementary and synergistic anti-inflammatory and bronchodilatory effects suggest a cumulative, more potent therapeutic effect. The additional anti-remodeling effect achieved by inhibiting PDE8A allows for broadening the spectrum of action of the proposed multifunctional ligands, giving an opportunity for an effective and multi-level therapeutic approach.

This doctoral dissertation aimed to propose and validate a strategy using computer molecular modeling techniques in the search for multi-target-directed ligands that block phosphodiesterases 4B, 8A, and the TRPA1 ion channel, and to select novel chemotypes for MTDLs.

The research was conducted in two ways using the approach based on the structures of biological targets and based on the structures of ligands.

For the structure-based approach, structural models were appropriately prepared for each of the biological targets that demonstrated the binding mode of the reference inhibitors and antagonists in individual active pockets. For the TRPA1 ion channel, a new binding pocket was predicted and a potential binding mode for its antagonist was proposed. Due to the presence of two binding sites where TRPA1 antagonists could interact, two models based on experimental structures of the ion channel, deposited in the PDB (Protein Data Bank) database, were also used in the study. In the case of PDE8A, a possible binding mode of its most active inhibitor was presented, which was proposed based on available literature data. The PDE4B model, developed on the basis of the available crystal structures, also showed the interaction mode of the selective ligand characterized by the highest inhibitory activity. Optimization of the protein model, docking reference inhibitors by the induced-fit method, retrospective virtual screening, and molecular dynamics simulations were used to prepare and verify all structural models. The analysis of MD simulations contributed also to the generation of specific pharmacophore models, the so-called dynophores. The developed pharmacophores and structural models were applied

to perform a two-stage process of virtual screening of 4,126,936 ligands: matching to pharmacophore hypotheses and docking of ligand groups selected in the first stage.

In the ligand-based approach, the conducted studies consisted of the development of empirical regression models using machine learning, which aimed to predict the value of pIC_{50} inhibitory activity. These models were trained on molecular descriptors describing the structures of known ligands of biological targets. In the process of virtual screening, their use will allow the selection of potentially effective inhibitors and provide information about the presumed IC_{50} value.

Candidates for multi-target-directed ligands selected in both approaches were analyzed in terms of assigned values of individual scoring functions and their physicochemical properties and ADME parameters were assessed. As a result, 38 potential candidates were finally selected, 12 of which were purchased and tested for their inhibitory activity against TRPA1, PDE4B, and PDE8A in *in vitro* assays.

The research conducted as part of the doctoral dissertation contributed to obtaining structural models of PDE8A, PDE4B, and TRPA1, pharmacophore models, and empirical models that allowed to expand knowledge about biological targets and the most important features that determine the inhibitory effect of their inhibitors/antagonists. A great achievement was the identification of both the binding site and interaction mode of the antagonists in the TRPA1 channel. It was later confirmed by experimental structures published in the PDB database, proving the effectiveness of the used procedure. The developed strategy for the search for multitarget ligands showed also the effectiveness of the use of various molecular modeling methods in order to select new candidates for multitarget ligands. The developed strategy for searching for multi-target ligands showed the effectiveness of using various molecular modeling methods to select ligands with confirmed inhibitory activity on single biological targets, providing a solid foundation for their further optimization towards multi-target inhibitory activity, which in the future may improve the quality of life of millions of patients including those suffering from asthma and chronic obstructive pulmonary disease.