Search of effective protein phosphatases inhibitors using nanochemical approaches and evaluation of their biological activity in silico

Karpov P.A., Samofalova D.A.*, Blume Ya.B.

Institute of Food Biotechnology and Genomics, Natl. Academy of Sci. of Ukraine, Osipovskogo str., 2a, *Kyiv,* 04123, *Ukraine,*

e-mail: samofalova.dariya@gmail.com

Protein phosphorylation and dephosphorylation is an important regulatory mechanisms involved in the control of many cellular functions and, in particular, transcription, translation, cell division, lipid metabolism, gluconeogenesis, muscle contraction, etc. [1] By reversing the phosphorylation of key regulatory proteins mediated by protein kinases, phosphatases serve as an important complement to kinases and attenuate activated signal transduction pathways. [1, 2]. Considering such fundamental role, it seems appropriate to have a set of compounds that selectively inhibits individual protein phosphatases activity. [3].

Our strategic goal was structure-based design and discovery of novel selective protein phosphatase inhibitors. In total, 63 experimentally resolved structures were found in Protein Data Bank, and we created a local database, that store information on *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Saccharomyces cerevisiae* and *Enterobacteria phage lambda* protein phosphatase catalytic subunits. We performed spatial structure homology modeling of animal (12), fungi (10), plant (22), bacterial (1), protozoan (6) and viral (4) phosphatase catalytic subunits of based on selected templates. In particular, using structures of known animal protein phosphatases 1 and 2A, we performed homology modeling of respective plant protein phosphatases. Built structure models was optimized (MD) and verified (MolProbity, PROCHECK, etc.).

We have performed search for known and potential (based on structure) protein phosphatase inhibitors and build up compound libraries for High-Throughput Screening (HTS) and docking. In PDB, it was found 10 serine-threonine and 11 tyrosine-specific PP in complexes with inhibitors. Analysis of protein-ligand complexes reveal structure of binding sites, role of water molecules in a ligand binding (CCDC Relibase) and relevant pharmacophores (LigandScout).

As there are significant difference in animal and plant phosphatomes, undoubtedly there are considerable target variations. To answer this question, based on available data about human protein-ligand complexes (control), we performed an chemogenomic analysis of specificity of known phosphatase inhibitors in *Arabidopsis thaliana* and *Physcomitrella patens*. Based on joint clustering of experimental and theoretical binding sites profiles, we identify the group of plant

phosphatases forming common clades with experimentally proven (PDB) protein-ligand complexes. So, we consider selected plant proteins as the most probable targets of studied inhibitors of mammalian protein phosphatases. To identify alternative ligand targets and binding sites, we carried out the blind docking (Hex 6.3) test for each of studied catalytic subunit. Due to application of GPU (CUDA), the duration of the blind docking has been reduced by more than 10 times. The potential binding sites were checked by a flexible docking in CCDC GOLD Suite v.5.1.

These methods were applied to search for new PP1 and PP2A inhibitors. Selective PP1 and PP2A inhibitor - okadaic acid (CID 446512) was used as a reference structure. Based on PubChem and ZINK structure search, were selected 26 perspective compounds. The affinity of the ligand was confirmed by molecular docking (evaluation functions) and molecular dynamics (ΔG calculation). As a result, we selected five compounds (CID10437923, CID44288019, CID3053530, CID10930902, CID44451975), which had not previously been reported as potential PP1 and PP2A inhibitors.

Acknowledgements: This study was supported by NASU-STCU grant #5215: "Search of effective protein phosphatases inhibitors using nanochemical approaches and evaluation of their biological activity *in silico*." 2011-2012.

1. F.A. Barr, P.R. Elliott, U. Gruneberg (2011) Protein phosphatases and the regulation of mitosis, J Cell Sci, **124 (Pt 14)**: 2323-2334.
2. G.B. Moorhead, V. De Wever, G. Templeton, D. Kerk (2009) Evolution of protein phosphatases in plants and animals, *Biochem J,* **417 (2)**: 401-409.
3. L. Cui, X.-P. He, L.-X. Gao, J. Li, G.-R. Chen (2013) Click Synthesis of Triazolyl Phenylalaninyl and Tyrosinyl Derivatives as New Protein Tyrosine Phosphatase Inhibitors, *J of Heterocy Chem*, **50 (3)**: 684–688.