Results of the STSM by **Aleksandar Cvetkovski**

The main goal in the submitted Research Proposal for my STSM, the Molecular Modeling on Drug docking for calculation affinity/ binding constants of antiparasitic drugs from a specific pharmacotherapeutic group with their reliable targets (e.g. receptor, enzymes) for pharmacological effect of drug action based on molecular structure-properties-activity relationship, has been carried out during my STSM mission.

The objective underlined in the submitted Research Proposal for my STSM has remained the same, but because both I and my host, Dr. Alfonso Garsia-Sosa, are computational chemists, for realization of our proposed objective, in the meantime, after the approval of the Research Proposal, we established cooperation with colleagues from Universities in Glasgow (UK) and Genth (Belgium), both members of CA21111, who provided us with their compound library of antiparasitic drugs tested on cytotoxicity and genes of the parasite strains used in theirs *in vitro* testing. Therefore, apart from the change in type of antiparasitic drug compounds and reliable targets outlined in the submitted Research proposal and that one's what we have carried out in our research during my STSM, the research methodology remains the same.

The final goal of our research relates justification by utilizing Molecular Modeling (Drug-Docking technology) why many nucleoside drugs have much stronger activity against Trypanosoma brucei than against Leishmania species that was revealed during *in vivo* testing of cytotoxicity.

Fore realization of this aim the applied Molecular Modeling technology encompasses following steps:

- Revealing the amino acid sequences from the three genes, two of them relate to Trypanosoma brucei (T.b) and one on Leischmania Mexicana (L.mex) by utilizing their accession numbers deposited in TriTrypDB database and that all relate to expression of adenosine kinase (AK) enzyme in each of these parasite mutant strain lines for the former and null cell line for the later
- the PDB (Protein Data Bank)
- Generating a AK structures based on amino acid sequences for two mutants of T.brucei and for L.mexicana in AlphaFold software package.
- Optimizing three structures toward superimposition with referent structure from PDB based on the statistical metrics using the software package PyMOL.
- Adapting the drug compounds with nucleotide type of molecular structures from the compound library with 24 selected drug compounds for testing activity to Aks (PubChem database)
- Molecular docking has been performed at GNINA (Machine learning software package) generating the sets of intermolecular interactions between each of 24 drug compounds and with each of two T.b AK and L.mex AK, respectively.
- Analyses of intermolecular interactions in binding site of AK imply to difference in binding affinity between AK from T.brucei and L.mexicana for the same drug model from nucleotide type of drugs.

21. Molecular modelling on antiparasitic nucleoside drugs: revealing mechanism of drug action

Cvetkovski A.[a], García-Sosa T.A. [b], De-Koning H [c].

[a] University "Goce Delcev" Stip Faculty of medical science Krste Misirkov b.b. P. fax. 201, 2000 Stip Country: Republic of N. Macedonia
[b] Institute of Chemistry, University of Tartu Ravila 14a 50411Tartu, Estonia
[c] School of Infection & Immunity, University of Glasgow, UK

e-mail: aleksandar.cvetkovski@ugd.edu.mk

Adenosine kinase (ADK; EC 2.7.1.20) is phosphotransferase type of enzyme that converts the purine ribonucleoside adenosine into 5'-adenosine-monophosphate.

$$\text{Adenosine} + \text{ATP} \xrightarrow{Mg^{2+}} \text{5'-adenosine-monophosphate} + \text{ADP}$$

ADK is an upstream regulator of adenosine, and thus affects its extremely short plasma half-life (<1 s). Inhibition of adenosine kinase results in increased intracellular adenosine which passes out of the cell via passive diffusion or via nucleoside transporter(s) to activate nearby cell-surface adenosine receptors. Consequently, ADK inhibition plays role as an alternative mechanism for activation of adenosine receptors and production of adenosine-associated potential inhibitors. Parasitic protozoa are incapable of *de novo* synthesis of the purine rings making themselves obliged to utilize a unique series of purine salvage enzymes to scavenge host and exogenous purines for their own growth and reproduction. Biophysical and biochemical characteristics of ADK isolated form *Leishmania Donovan* are different then ADK from other eukaryotic sources regarding to the mode of action.

Referring to the differences in purine salvage between *T. brucei* and *Leishmania* due to adenosine kinase is not subject to substrate inhibition, and it has a comparably low affinity for adenosine (K_m = 33 μM) making the major route of salvage to occur via cleavage to adenine, which is deaminated by adenine amidotransferase, a *Leishmania*-specific enzyme that does not exist in other studied trypanosomatids or in mammalian cells, we present results from *in silico* molecular modelling based on docking study for testing binding affinity of antiparasitic nucleoside drugs such tubercidin and other lead compounds testing the hypothesis whether ADK of Leishmania is less effective in phosphorylation reaction of nucleoside analogues then *Tb*ADK.

[1] Datta AK, Bhaumik D, Chatterjee R. Isolation and characterization of adenosine kinase from Leishmania donovani. J Biol Chem. 1987.
[2] Vodnala M, Fijolek A, Rofougaran R, Mosimann M, Mäser P, Hofer A. Adenosine kinase mediates high affinity adenosine salvage in Trypanosoma brucei. J Biol Chem. 2008.