1. Ph.D. PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

The search for novel antimicrobial compounds targeting guanylate kinase from *Mycobacterium tuberculosis*

1.1. Project goals

The aim of this project will be the establishment of a quantitative structure-activity relationship model (QSAR). The QSAR model will be an indispensable tool for the future rational design of new regulators based on the structural data.

1.2. Outline

Tuberculosis (TB) is a severe and chronic infectious disease that most commonly affects the lungs. According to the World Health Organization, 1.5 million people died of TB in 2020. Worldwide, Tuberculosis is the 13th leading cause of death. The causative agent of TB is a pathogenic bacterium called *Mycobacterium tuberculosis*. One of the research strategies to combat TB is to identify new targets and more effective drugs [1]. Nucleotide metabolism enzymes are responsible for the production of nucleotide triphosphates, which are involved in the synthesis of RNA and DNA, as well as are the energy source for cellular processes and are involved in signaling pathways. It has been shown, that these enzymes play a key role in *Mycobacterium tuberculosis* and have begun to be investigated as potential antibacterial targets [2]. One of the enzymes responsible for converting nucleotides in the bacteria *Mycobacterium tuberculosis* is guanylate kinase (GK, EC 2.7.4.8). It is essential in the nucleotide biosynthetic pathway because it catalyzes the conversion of GMP to GDP by transferring the phosphoryl group from ATP to GMP [3]. Therefore, it seems justified to search for compounds influencing the catalytic activity of guanylate kinase from *Mycobacterium tuberculosis* (GK_{MT}). The inhibition of this enzyme can be a promising therapeutic strategy in the treatment of tuberculosis. The proposed project is planned to investigate enzyme-ligand interactions using fluorescence spectroscopy and determined the inhibitory efficiency of substrate analogs with GK_{MT} by performing the enzyme activity assay (HPLC). Obtained data (IC_{50}, K_i) will constitute the basis for the QSAR analysis (quantitative structure-activity relationship), which is necessary for the rational design of new regulators based on the structural data.

1.3. Work plan

1. Overproduction of guanylate kinase from *Mycobacterium Tuberculosis* (GK_{MT}) in a bacterial system, its purification and characterization.
2. Investigation of the enzyme-ligand interaction by fluorescence spectroscopy, determination of the binding constant.
3. Determination of the regulatory effects of substrate analogs on the activity of GK_{MT} using the HPLC method, determination of the IC_{50} value.
4. Geometry optimization of the chemical structure of substrate analogs.
5. Calculation of molecular descriptors. Building and validating QSAR models using various chemometric tools. Interpretation of the received QSAR models.

1.4. Literature

[1] Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science (2005), 307, 223–227, doi: 10.1126/science.1106753.

[2] Beck BJ, Huelsmeyer M, Paul S, Downs DM. A mutation in the essential gene gmk (encoding guanylate kinase) generates a requirement for adenine at low temperature in Salmonella enterica. J Bacteriol (2003), 185, 6732–6735, doi: 10.1128/JB.185.22.6732–6735.2003.

[3] Hible G, Christova P, Renault L, Seclaman E, Thompson A, Girard E, Munier-Lehmann H, Cherfils J. Unique GMP-Binding Site in Mycobacterium tuberculosis Guanosine Monophosphate Kinase. Proteins (2006), 62, 489–500, doi: 10.1002/prot.20662.

1.5. Required initial knowledge and skills of the Ph.D. candidate

- knowledge about basic chemistry and biochemistry
- basic laboratory skills
- interest in issues from the borderline of chemistry and medicine
- analytical and critical thinking
- hard-working person and eager to learn
- ability to work in a team

1.6. Expected development of the Ph.D. candidate's knowledge and skills

- ability to organize work in the laboratory
- ability to solve research problems
- ability to produce recombinant protein
- ability to operate the system for high-performance liquid chromatography (HPLC)
- ability to the presentation of results in oral and written form