

## **Targeting enzymes of the parasite's purine salvage pathway as a new approach to combat *H. pylori* infections, including those caused by strains resistant to available antibiotics**

The bacterium *Helicobacter pylori*, discovered about 40 years ago, colonizing the stomach wall, causes serious health problems around the world. It is estimated that more than half of the world's population is infected with *H. pylori*, and in some countries or certain age groups even 80%. Many strains of *H. pylori* cause asymptomatic infections, but about 10-20% of infected people develop diseases such as chronic gastritis, peptic ulcer, duodenal ulcer, and about 1% of patients suffer from stomach cancer or MALT lymphoma. Therefore, the *H. pylori* bacterium is considered to be one of the most dangerous human pathogens.

A vaccine against *H. pylori* infection is not available, and existing treatments, although consisting of at least two, and more often three or four, drugs, are ineffective in about 20% of cases due to the antibiotic resistance of many strains of bacteria. Consequently, new approaches to the eradication of *H. pylori* are needed that will employ different molecular targets and molecular mechanisms of action than those used by current drugs. This project is devoted to such research.

Some organisms, including some pathogens are not able to synthesize *de novo* "building blocks" necessary for the construction of DNA and RNA nucleic acids. These "building blocks" are purine and pyrimidine nucleic bases and their nucleosides and nucleotides. Therefore development of such organisms depends entirely on the metabolic route known as the salvage pathways in which these "building blocks" are recovered from the metabolic "waste". Blocking such a pathway therefore deprives such organisms of the ability to synthesize DNA and RNA, and thus should inhibit their multiplication. Relatively recently, *H. pylori* was discovered to be an organism that is unable to *de novo* synthesize DNA and RNA purine "building blocks", making enzymes belonging to the purine salvage pathway a promising new molecular target for combating this bacterium.

In our previous project, using genetic engineering methods we obtained two enzymes from the *H. pylori* purine salvage pathway, purine nucleoside phosphorylase (PNP) and adenylosuccinate synthetase (AdSS). Using various biophysical and biochemical methods, we examined the properties of PNP and AdSS at the molecular level and identified chemical compounds (so-called inhibitors) that inhibit the action of these enzymes. We then showed that the presence of some of these compounds in *H. pylori* bacterial cultures inhibited the multiplication of bacteria. We have thus shown that inhibitors of PNP and AdSS are good drug candidates against *H. pylori*.

In the current project, we want to take a step forward and optimize the interactions of these inhibitors with the target enzymes, PNP and AdSS, in order to lower the amount of the inhibitor required to subside to prevent bacterial proliferation. This is important because the need to administer a large amount of such an inhibitor may prevent it from undergoing clinical trials due to unwanted side effects. We will also check the influence of these compounds on *H. pylori* strains resistant to known antibiotics currently used in anti-*H. pylori* therapies. As mentioned above, all treatments for eradication of *H. pylori* consist of at least two drugs. The new drugs will most likely also be administered as part of the therapy in conjunction with other drugs. Therefore, we will also check the effect on *H. pylori* bacterial cultures of various combinations of drugs currently used to combat *H. pylori*, in which one of the components will be replaced by a PNP or AdSS inhibitor from among those identified in our projects.

Therefore, if the goals of the project are achieved, the PNP and AdSS inhibitors may in the future become a new type of drugs to combat *H. pylori* infections, operating on the basis of a different molecular mechanism than those already known and used today. This will not only reduce the use of currently administrated antibiotics, but will also help in the fight against *H. pylori* strains resistant to currently available therapies.