A new strategy to fight antibiotic resistance based on hybrids of fluoroquinolones and quaternary ammonium salts

Rapidly growing bacterial resistance to commonly used antibacterials poses one of the critical problems to public health. Antibiotic resistance arises when bacteria create mechanisms that protect them from the effects of antibiotic agents. Illnesses caused by resistant forms of microbes are more problematic and often more expensive in treatment. The patients need higher doses of antimicrobials or alternative medications which may be toxic and prolong the hospitalization. The most threatening and difficult to cure are diseases triggered by microorganisms resistant to several antimicrobials that are called multidrug-resistant (MDR). The period between 1950 and 1970, when a lot of new classes of antibiotics were discovered, was named "the golden age of antibiotics". It has become possible to treat countless medical conditions that were incurable until that time, even the fatal ones. Nevertheless, the discovery of new antibiotics has fallen since then and resistant bacteria are becoming more and more common.

Nowadays, many strategies are employed to combat antimicrobial resistance; one of them requires hybrid drugs development. A hybrid drug molecule consists of diverse bioactive fragments fused in one compound. The distinct pharmacophoric parts act against different molecular targets; therefore, there is a lower risk that bacteria will mutate and evolve defense mechanisms against multiple drug units. What is interesting, the most widely described hybrid compounds are fluoroquinolones (FQ) joined with other antibacterials. FQ are pure synthetic broad-spectrum bacteriocidals that were discovered by George Lesher in 1962. They are applied in hospital-acquired, urinary, and genital tract infections. Generally, FQ are well-tolerated; however, sometimes side effects may occur during the treatment. The most common are diarrhea, nausea, vomiting, insomnia, tendon rupture, nerves damages, and mental health problems. The molecular targets of FQ are bacterial enzymes involved in the DNA replication process. Quaternary ammonium salts (QAS) are another type of molecules that can be incorporated into hybrid drugs. They are members of cationic detergents (surfactants) and exhibit antimicrobial activity by acting as disinfectants or antistatic agents and are used in cosmetics, food, or paper industries. They serve as sanitizing agents for sterilization of intact skin before the operation or noncritical surfaces and tools. Their applications comprise veterinary products, contraception formulations, vaccine production, diagnostic testing, and nasal formulations, and fabric softeners.

In view of the above, a research program devoted to the investigation of novel hybrids combining FQ and QAS seems to be rational to undertake. The presented project pertaining to medicinal chemistry aimed at the development of a new class of antibacterial hybrid agents. Within the present proposal, we plan to design, obtain and evaluate novel low-molecular-weight molecules bearing FQ and QAS moieties. This study is based on the results of our preliminary investigations indicating that this type of compounds exhibits confirmed antibacterial activity. Our research will verify the hypothesis that the obtained molecules display a unique dual molecular mechanism of action that includes inhibition of bacterial enzymes due to the presence of FQ drug, as well as destabilization of bacterial lipid membrane structures caused by QAS. The planned approach is beneficial due to the simple and inexpensive synthesis of the planned molecules, as well as their adjustable lipophilicity crucial for the biological activity that can be changed by the introduction of various alkyl substituents into the quaternary structure. The permanent positive charge present in the QAS compounds should decrease the ability to cross the blood-brain barrier and thus reduce the adverse effects caused by FQ in the central nervous system.

The primary aim of the project involves the design and preparation of a series of novel molecules via exhaustive alkylation reactions. Subsequently, the planned work will focus on the investigation of their physicochemical properties, i.e. lipophilicity as well as affinity to phospholipids and human serum proteins since these factors have been documented to affect absorption, distribution, metabolism, and excretion of the drug, as well as its uptake and cellular accumulation. The next steps comprise a comprehensive evaluation of the bioactivity of the obtained compounds. Their ability to kill bacteria and susceptibility to resistance development will be tested against common human pathogens including antibiotic-resistant strains. Additionally, the study will also include extremely difficult to remove biofilm forms of the microorganisms. Biofilm composed of adherent cells and extracellular matrix is estimated to be involved in roughly 80% of all infections. It causes chronic and recurrent infectious states and shows significantly higher antibiotic resistance that may require even 1000-times higher antibiotic concentrations to eliminate, and hence, it represents a threat to public health mostly when colonizing biomaterials and medical devices. The cytotoxicity assessment against human and animal cell lines will be conducted in order to select the most promising compounds and confirm safety issues. Experiments in mice will be performed to assess pharmacokinetics and effectiveness *in vivo*. In the final step of the project, as well as computer modelling will be performed to rationalize the experimental findings.

The developed hybrids may constitute a new group of antibacterials and be proof of enhanced activity superior to a 1:1 combination of co-administrated drugs. The project might give insights into the synthetic chemistry of quaternization procedures as well as biological activity in terms of antibacterial action.