Antiparasitic properties and molecular target identification of new thiosemicarbazide and thiazolidinone derivates in *Toxoplasma gondii* invasions

Toxoplasma gondii (*T. gondii*) is an obligatory intracellular parasite which belongs to *Apicomplexa* phylum, that possesses the ability to infect a wide spectrum of warm-blooded animals including humans (25-30% of population is infected). Toxoplasmosis caused by *T. gondii* is a parasitosis dangerous especially for pregnant women due to the risk of congenital infection. Moreover, *T. gondii* invasion may lead to brain damage and even death in immunocompromised individuals. In *T. gondii* life cycle we can identify three infective stages: tachyzoites, bradyzoites and sporozoites. The main route of parasite transmission to humans involves ingestion of either raw or underprepared meat containing tissue cysts or water and vegetables contaminated with soil containing oocysts. Additionally, people can become infected horizontally (iatrogenic) *via* blood transfusion or organ transplantation and vertically from mother to fetus *via* placenta. Besides, the parasite is responsible for livestock infections. Farm animals, also these bred for human consumption, can acquire *T. gondii* infection through ingestion of sporulated oocysts with water or plants. After the release from tissue cysts and oocysts, which takes place in the intestines, sporozoites and bradyzoites, respectively, transform into tachyzoites and this process marks the beginning of the acute phase of parasite invasion. Tachyzoites under the pressure of developed immunity convert into slow-dividing bradyzoites enclosed within tissue cysts localized in various tissues e.g. neural or/and muscle. The only known definitive hosts of *T. gondii* are members of the *Felidae* family. Cats become infected mainly through predation of intermediate hosts with latent parasite invasion. The parasite's sexual replication takes place in the intestines and results in formation of oocysts. Cats shed large numbers of unsporulated oocysts with feces, for 1-3 weeks. In the environment oocysts sporulate within 1-5 days and become infective.

It is worth to underline that currently, the only effective mean of preventing *T. gondii* infection is a preventive healthcare, especially raising the awareness of future mothers and early diagnosis of pregnant women, and new-borns. An efficient method of complete elimination of the parasite from an infected organism has not yet been developed. Many drugs are used in the treatment of toxoplasmosis, mostly from the group of folic acid antagonists, e.g. trimethoprim or/and sulfadiazine. They act mainly on fast proliferating tachyzoites, and thus they only reduce the level of tissue damage during the acute phase of infection. Additionally, the used medications cause a number of side effects, such as: allergic reactions, leucopoenia, anaemia, thrombocytopenia, cardiac arrhythmia, gastrointestinal and skin pigmentation disorders, thus the search for less toxic and more selective drugs continues. The *T. gondii* drug resistance to commonly used medications is more and more frequently observed and unfortunately molecular mechanism underlying this phenomenon is still unknown. For that purpose, in collaboration with Department of Organic Chemistry, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, new thiosemicarbazide and thiazolidinone derivatives with proven, in preliminary experiments, ability to inhibit penetration and proliferation of *T. gondii* and low toxicity to host cells, were synthetized as potential candidates for future anti-*Toxoplasma* agents.

At present numerous scientific and clinical centres conduct research aiming at developing more effective methods of prevention and treatment of toxoplasmosis, thus, a few thiosemicarbazide and thiazolidinone derivatives, were discovered, however to date the mechanism of molecular action of these compounds was not elucidated (identified).

To resolve the therapeutic problem in the treatment of toxoplasmosis we propose this interdisciplinary research project, which combines biological *in vitro*, *in silico* (bioinformatics), genetic (use of CRISPR/Cas9 method) and molecular studies (recombinant proteins). The main goal of this project is to determine a molecular target(s) for selected new compounds from thiosemicarbazide and thiazolidinone derivatives which possess the most potent anti-*T. gondii* activity.