Mycobacterium tuberculosis (*M.tb)* was identified in 1882 by Robert Koch as a causative agent of tuberculosis (TB). Nowadays, it remains a major threat to public health with high mortality and morbidity worldwide. Every year *M.tb* is responsible for 1.5 million deaths and 10 million new TB cases. It is estimated that one-third of the human population is infected latently with tubercle bacilli, and about 5% of those people develop active disease during their lifetime. *M. tb* is an intracellular pathogen, able to survive and propagate in human professional phagocytes, namely macrophages, and its life cycle includes a long state of persistence in a latent form. Tubercle bacilli during infection are found in various metabolic phases and various ecological niches, extracellularly, and intracellularly, in early, and late granulomas and are extremely difficult to eliminate even when they show sensitivity to the first-line anti-TB drugs. In addition to tubercle bacilli, the genus *Mycobacterium* possesses 150 other mycobacterial species (nontuberculous mycobacteria, NTM) including *M. leprae*, the causative agent of leprosy, and 25 other species known to cause disease in humans. NTM species are considered an emerging threat to the immunocompromised population including patients under immunosuppressive therapy or with AIDS. NTMs are widespread in the environment and their infections are difficult to diagnose and treat. The most common infections caused by NTM strains are pulmonary infections which are hard to distinguish from TB based on clinical observations, however, NTMs are also responsible for skin and soft tissue infections. NTM has a high environmental resistance profile, thanks to their hydrophobicity which permits them to form biofilms and be aerosolized. The treatment of NTM-related diseases is largely empirical, lasts for 18 months, is very expensive, and often is associated with drug-related toxicities and side effects. TB caused by drug-sensitive strains is a fully treatable disease, however, the therapy is lasting six months and requires four drugs to be used daily. A serious problem is drug-resistant TB, the treatment of which is long-lasting (up to 18 months), very expensive and effective only in about 50% of cases, and in radical cases regresses the treatment process to the pre-antibiotic era. The rise of drug resistance among *M. tuberculosis* strains in recent years, and the phenomenon of HIV-*M.tb* coinfection, are serious public health challenges worldwide. Therefore, **the development of alternative medical strategies based on a new generation of drugs is desperately needed to effectively cure drug-resistant-TB, reduce the duration of current therapies, and minimize the toxicity, and cost of the antituberculosis agents used**. In the preliminary study, we identified six promising compounds representing three chemical categories able to kill tubercle bacilli in very low concentrations and presenting no cytotoxicity to human cells. **The main aim of this project is to develop and estimate the library of compounds effective against metabolically active *M. tb* and/or non-tuberculous pathogen, *M. abscessus*, bacilli in the latent, non-replicating stage, forming biofilms, deposited in the professional human phagocytes, namely macrophages, as well as, in the mice model of infection.** First, using various genetic approaches, we will identify the molecular targets for the selected in the preliminary study compounds within tubercle bacilli. Next, the library of optimal compounds with maximal efficiency of binding to the target molecule, for each group of compounds identified above, will be developed. The dedicated libraries of analogs of these compounds will be prepared by using the structural information, *in silico* analysis, and medicinal chemistry expertise, and the compounds will be selected among commercially available ones or synthesized by medicinal chemistry experts. The newly synthesized and commercially available compounds will be tested by phenotypic screening against *M.tb* and *M. abscessus*. The selected bactericidal compounds will be assessed against clinical strains, mature biofilms developed by mycobacteria, bacilli in non-replicating, latent stages, and bacteria deposited within human phagocytes. The cytotoxicity of the selected compounds will be also determined using liver and kidney cell lines. The most promising compounds presenting bactericidal effects against bacilli in various metabolic stages will be evaluated concerning drug metabolism and pharmacokinetics properties *in vitro* and in mice. The best drug candidates will be used to treat *M.tb* and *M. abscessus* infection in mice alone, as well as, in combination with current drugs. The potential side-effect of the drug candidates will be also assessed. **If the project is successful, it will provide new, well-characterized potential anti-tuberculosis drugs that can enter the phase of clinical trials.**