The development of modern pharmacotherapy has resulted in the introduction of many effective therapies for illnesses previously considered incurable. Huge resources have been invested in the fight against diseases, the treatment of which is the huge need of humanity, such as: cancer, diseases of the cardiovascular system and autoimmune diseases. In recent years, searching for effective drugs for diseases related to the central nervous system has become more difficult. Meanwhile, the need here is growing: more and more people suffer from depression, anxiety attacks, Parkinson's disease or one of the many types of epilepsy. Worse still, some of them like Alzheimer's and Huntington's disease remain incurable. All these diseases are undoubtedly a difficult area of research, and the development of new drugs related to the central nervous system is a great challenge that we take up with this project. We believe that the basis of success in searching for new therapies, not only for the central nervous system, is discovering and exploring new biological targets. We focused our attention on a group of transporters related to one of the most important molecules in the body, i.e. γ-aminobutyric acid (GABA), which is a neurotransmitter that inhibits stimulation of the nerve cells. GABA transporters (GAT) inactivate GABA, that is located in the synaptic cleft by its absorption into the nerve cell where it is metabolised. Currently, four GAT subtypes are known (GAT1, BGT1, GAT2 and GAT3), but two of them, GAT1 and GAT3, are clearly dominant in the central nervous system. Modern science already knows a lot about GAT1, its location, functionality and the therapeutic effects of its inhibition. These effects were so advantageous that allow introduced GAT1 inhibitor tiagabine as an anti-epileptic drug to the therapy. In turn, knowledge about GAT3 is still relatively poor and it remains, a poorly explored biological target `ando also a great challenge for modern medical chemistry that we are facing. Some knowledge we currently possessing shows, that there are large differences in distribution and functionality between GAT 1 and GAT 3. Therefore the benefits of blocking it may be different than in case of GAT1 inhibition. In the presented project, we want to enable the understanding of the therapeutic function and potential of GAT3 by creating development of pharmacological tools that are its potent and selective inhibitors. In addition, we would like to test their effectiveness in selected, most probable animal models of central nervous system diseases.

In a framework of preliminary study, we performed a biological screening of selected compounds from our in-house library and found a selective over other subtypes GAT3 inhibitor. Its activity was so high that it exceeds all known selective GAT3 inhibitors, but still too weak to be effective pharmacological tool that may become the basis for future therapies. Undoubtedly, this compound is an excellent starting point for designing new GAT3 inhibitors and introducing some beneficial modifications, which is certainly a great advantage of our project and minimizes the risk. In the next stage of the project, we intend to obtain the designed compounds by using organic synthesis methods, which will then be tested for their ability to inhibit GATs *in vitro* (outside of a living organism). Thanks to this, we will get a full inhibitory profile for our compounds and information about their activities against GAT3 and selectivity to other subtypes. The next task will be an *in vitro* evaluation of pharmacokinetic and toxic properties of the most active and selective compounds. We will study the ability to cross biological membranes, which is very important in terms of CNS-penetration and targeting GAT3, metabolic stability and the potential for drug-drug interactions. As part of the initial safety assessment, we will also determine the cytotoxicity and hepatotoxicity of the compounds and lack of serious interaction with anti-targets, i.e. that are the main source of adverse effects. At this stage, we will select the five most promising compounds for which we will start *in vivo* studies. For this purpose, we will re-synthesize them in the amount and purity appropriate for such research, and then perform pharmacokinetic tests on mice to assess their distribution in the organism. Among tested compounds we will select 1 or 2 to perform pharmacodynamics *in vivo* tests, to assess the therapeutic potential of selective GAT3 inhibitors towards the most likely directions of their action. To check the beneficial effects of the compounds we selected some animal models of: epilepsy, neuropathic pain and anxiety due to their clear connection with GABAergi system disorder in etiopathogenesis.

The expected outcome of the project will be the development of selective GAT3 inhibitors, which will allow to effectively study this transporter and above all, indicate the possibility of using its inhibitors in the treatment of certain diseases of the central nervous system.