The common feature of neurodegenerative diseases (ND) is a deposition of toxic extracellular or intracellular protein aggregates: β-amyloid and neurofibrillary tangles in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD) or superoxide dismutase 1 in amyotrophic lateral sclerosis (ALS). These proteins activate microglia – the immune cells of the central nervous system. Microglia are responsible for inflammation-mediated neurotoxicity or neurodegenerative repair, depending on the activated state/ phenotype (M1 or M2). The first M1 is classically activated microglia that depict pro-inflammatory phenotype and is characterized by the release of proinflammatory cytokines. The second one, M2 is alternatively activated microglia that depict anti-inflammatory phenotype and is associated with neurogenesis and anti-inflammatory effects. Modulation of microglial phenotypes appears as an attractive potential therapeutic approach for the treatment of neuroinflammation. Especially now, what is very important, when the growing interest appears to consider microglia involvement in progression of SARS-CoV-2 induction of neuroinflammation and as mediators of neurological damage.

This project aims to **extend knowledge** on **the potential neuroprotective mechanism** of **GPR18 receptor ligands.** The GPR18 receptor is an orphan receptor. Orphan receptors are receptors for which neither their function and role under pathophysiological conditions were described nor endogenous ligands have been identified. They may represent new biological targets, and as such, they bear the valuable potential of a new mechanism of therapeutic intervention. Since GPR18 is reported to be activated by exogenous phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) therefore it is postulated to be a part of the endocannabinoid system (ECS). ECS is engaged in several physiological processes among other immune system functions and neuroprotection. Expression and distribution of GPR18 in cells and tissues of the immune system suggests its involvement in several biological processes such as modulation of immune central nervous system functions, inflammation and neuroprotection. In this project, it is planned to estimate the role of GPR18 ligands in activity and M1/M2 microglial polarization, ability to induce microglia to M2 phenotype and to select the most active structures in that activity. As pharmacological tools will be used GPR18 ligands. Our research team in cooperation with prof. Christa Mueller research group from the Pharmaceutical Institute University of Bonn performed pioneering works and obtained the world's first GPR18 antagonists and agonists which were shown to present anti-inflammatory activity *in vitro* and in animal models of intestinal inflammation.

The most important tasks for this project are: searching for more active and selective (than so far described) ligands of the GPR18 receptor and assessment of the role of GPR18 in microglia and inflammatory modulation. New designed compounds with potential activity against GPR18 will be synthesized and then their activity against GPR18 and selectivity at other similar receptors will be examined. The new or previously obtained most active/selective GPR18 ligands will be *in vitro* tested on BV-2 mouse microglial cell line with the confirmed expression of GPR18. The effects of GPR18 ligands on microglial inflammatory responses will be examined: GPR18 expression in resting and activated microglia, assessment of pro-inflammatory and anti-inflammatory mediators, determination of the phenotype of microglia and signalling from microglia to neuron in *in vitro* model of neurodegenerative diseases. As it was reported that some different characteristics exist between immortalized BV-2 cells and primary microglia it is planned to use both murine microglial BV-2 cells and murine primary microglia to compare the effects of GPR18 ligands on microglial inflammatory responses. Primary microglial cell cultures share the functional characteristics with endogenous cells (such as secreted factors, and cell surface markers) therefore are widely used in studies concerning neuroinflammation. Physicochemical properties of selected for *in vitro* tests GPR18 ligands will be examined on important for CNS active compounds features: ability to penetrate biological membranes, neurotoxicity and metabolic stability. The project will be performed in cooperation with scientists from Institute of Pharmacology Polish Academy of Sciences in Kraków - primary cell cultures isolation and GPR18 ligands influence on them examination, and with Pharmaceutical Institute University of Bonn - evaluation of obtained compounds activity and selectivity at orphan GPR18, GPR55, cannabinoid CB1, CB2 receptors and adenosine A1, A2A, A2B, A3 receptors- as the structure of GPR18 agonists suggests their possibility to interact with ARs.

Realization of the presented project is an opportunity to gain **the new more potent and selective GPR18 ligands useful as pharmacological tools**, to gain a deeper and more detailed understanding of orphan GPR18 receptor role in **modulation of microglia and neuroinflammation**. It can help to design and obtain compounds with valuable anti-neuroinflammatory functions, important in the innovative therapeutic strategies in such widespread pathologies as neurodegenerative diseases.