Project is focused on the histamine H_4 receptor (H_4R), the latest discovered member of the histamine receptor family. Histamine receptors (H_1 H_2 H_3 H_4) are important therapeutic approaches for drug discovery. On the pharmaceutical market there are drugs that interact with H_1 receptor (ex. in therapy of allergy) H_2 receptor (treatment of diseases connected with the hyperacidity in stomach) and H_3 receptor (narcolepsy).

Substances that interact with **histamine H_4 receptor** are still far from the market but they are interesting for researchers. Due to H_4R presence in human body (on the cells of the **immune system**, **nervous system** and some **cancer cells**) substances that can interact with the H_4R can be considered as possible future drugs for **immunological disorders**, **neurological disorders** and **cancer**.

The main role of the receptors is transduction the information from outside of the cell to inside after a binding of a particular recognizable structure, called ligand to the receptor. The signal transduction is a first step of the response of the cell. Cellular receptors are one of the main approaches of drugs that are now on the market, and approximately 30-50% of all drugs are ligands of the receptors from the group called seven transmembrane receptors (7TM). These receptors are coupled with intercellular proteins (such as G-protein, β-arrestins, kinases) that are responsible for signal transduction into the cell. Histamine receptors belong to this group of receptors.

It is known that the youngest of the histamine receptor family – H_4R can transduct the signal by two independent pathways: by G protein or by recruitment of β-arrestin 2. What is more, some of the compounds can activate these pathways in the different way (for example activate one of them and deactivate second in the same time). This is called **functional selectivity.** Functional selectivity is an interesting feature of the receptors. It complicates the drug discovery (because more test are necessary) but also gives opportunity to develop the chemical substance that can activate signal transduction pathways in more selective way what leads to less side effects and higher activity of potential drugs.

Because the scientific word suggest, that is no longer sufficient to check the influence of substance only on the one signalling pathway the aim of this project is:

- Optimise the assay that can be used in determination of β-arrestin 2 activity of histamine H_4 receptor ligands.
- Adopt and validate the innovative technique for monitoring ERK1/2 activation in response to histamine H_4 receptor stimulation by employing fluorescently labelled antibodies and signal detection with use of flow cytometry
- Determine the affinity of new compounds to the H_4R by radioligand binding studies
- Evaluate a group of varied H_4R ligands for functional selectivity using three intercellular second messengers: cAMP (G protein dependent), β-arrestin (G-protein independent) and 1/2ERK (dependent on both signal transduction pathways)
- Finding the relationship between elements of the chemical structures of the ligands and they influence on the signal transduction pathways.

Knowledge of the structure-activity relationship in the H_4R ligands can help in development of the new structures, that can act selectively toward pathways. These development can improve the activity of compounds and/or decrease the side effects of those ligands. Functional selectivity (or biased signalling) is new and interesting area where are still many subjects to investigate.

Methods:

During the project modern *in vitro* techniques common in drug development will be used (e.g **radioligand binding studies** or techniques that use **fluorescence resonance energy transfer** (FRET) to measure the level of cAMP or β-arrestin recruitment.

It is also planned to adopt and validate the innovative technique for monitoring ERK1/2 activation by employing fluorescently labelled antibodies and signal detection with use of flow cytometry.

Obtained results will be published in prestigious scientific journals and will be presented during conferences (such as European Histamine Research Society meeting, Polish Histamine Research Society meeting).