Treatment of cancer diseases is a huge challenge for practicing physicians and scientists who develop new effective therapies. The main objective of innovative medicine is to prevent new cancer diseases, and to reduce the mortality rate of cancer patients. To accomplish these goals it is necessary to develop improved diagnostic methods and effective drugs. The effectiveness of the new drug is not the sole criterion of its usefulness in medicine. Researchers developing them have also to focus on reducing the side effects of its use. Many of the currently used anti-cancer drugs has low specificity toward the target cancer cells or high toxicity. Consequently, destruction of normal cells is observed and the treatment is accompanied by severe side effects. For this reason, the development of new drugs is essential. Still ongoing research aimed at understanding the differences between normal and cancer cells. Some of the differences relate to the increased activity of certain enzymes, or increased activity/expression of membrane receptors on the surface of tumor cells. Finding compounds that reduce the activity important for the development of cancer enzymes, or affecting receptor activity can lead to the development of new effective chemotherapeutic agents.

Our project is aimed at finding new chemicals with anticancer activity, acting as antagonists of AMPA ionotropic glutamate receptors (iGluRs). Ionotropic receptors, perform the functions of ion channels for Ca2+ and Na+. iGluRs significance in the metabolism and proliferation of tumor cells was confirmed experimentally. By silencing the expression of gene encoding the GluR1 it was shown that activity of iGluR receptors is necessary for the proliferation and migration of tumor cells. Further studies have shown that binding of GluR1 receptors to the surface of tumor cells is regulated by the phosphorylation dependent on casein kinase CK2, the increased activity of which in the cells of many types of cancer are described in numerous publications. These facts prompted us to undertake research on the simultaneous use of known kinase CK2 inhibitors and new glutamate receptor antagonists whose synthesis method will be work out.

To gain this goal, we invented compounds 1-3, shown in Figure 1. The compounds are derivatives of the natural nucleoamino acid - willardiine. Effectiveness of our compounds will be determined on cells of the selected malignant brain cancers (glioblastona and astrocytoma) as well as on cell of lung cancer. Moreover, an effect of our compounds on binding properties of a natural ligand of the AMPA receptors, i.e. α-amino-3-hydroxy-5-methyl-4-isooxazolepropionic acid, will be examined. According to the literature data, willardiine derivatives are a relatively narrow class of compounds. To the best of our knowledge, anticancer activity of willardiine derivatives has not been examined. The compounds 1-3 designed for the purpose of this project have not been described in the literature. The development of effective methods for the synthesis of compounds 1-3 is our first task. Executing this task, we will focus on: utilization of easy available substrates, limitation of a number of the reaction steps, and synthesizing of the intermediate compounds in high yields. Structures of both intermediates and final compounds 1-3 will be solved by modern spectroscopic techniques (e.q. 2D NMR methods). Results of our synthetic studies would broaden knowledge on reactivity and spectroscopic properties of valuable organic or inorganic compounds, i.e. new derivatives of 1,2,3-triazolo-carboxylic acids, ferrocenyl-amino acids, respectively, as well as of the target willardiine congeners featuring these structural moieties.

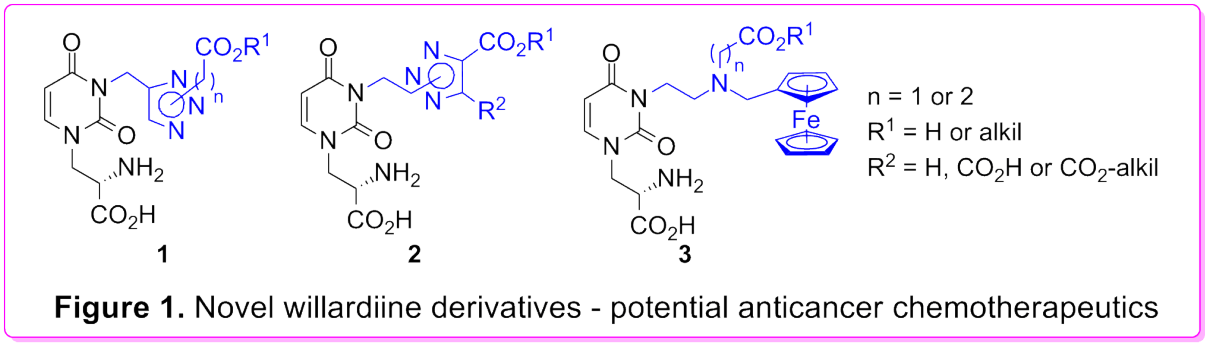

Figure 1. Novel willardiine derivatives - potential anticancer chemotherapeutics

Biological studies will involve determining the degree of anti-tumor activity of newly synthesized substances, in particular:

1. to determine the effect of test compounds on proliferation of cancer cells at different time intervals (24-96 hours). To achieve this, in addition to the MTT test based on the measurement of the metabolic activity of cells, and evaluating the cytotoxicity test compounds, will be executed test to determine the incorporation of bromodeoxyuridine (BrdUrd) into DNA as a typical test for evaluating a degree of proliferation of tumor cells;
2. to assess the degree of apoptosis in cancer cells - one of the basic mechanisms of cytotoxicity of chemotherapeutic agents, the colorimetric assay ELISA - Cell Death Detection ELISAPLUS kit (Roche) using a flow cytometer (FACS Calibur), and the classic caspase 3 and 7 activation tests, with specific antibodies recognizing active forms of these caspases - Western blot technique, will be performed.

Recombinant receptors GluR1 and GluR2, obtained in the bacteria E. coli in the form of a truncated constructs HS1S2 will also be used for the studies. HS1S2 construct is composed of two polypeptide segments forming after solubilization of the inclusion body soluble ligand binding domain (glutamine or AMPA). Full versions of the receptors will be overexpressed in insect cells (Sf9) or mammalian cells. In this method, isolating a membrane fraction containing the receptors or overexpressed protein will be purified using affinity chromatography.

Impact of new willardiine derivatives on the binding of AMPA receptors will be examined by isotopic assay. We will also examine the surface expression of a receptor GluR1 and GluR2 in cells treated and untreated with AMPA antagonists and inhibitors of CK2 using the method of immunodetection, and confocal microscopy.

The scientific contribution of Faculty of Chemistry students, Warsaw University of Technology represents the added value of our project. Experimental practice acquired by the students during execution of this grant will enrich their personal background and will strengthen their position on the job market.