3-bromopyruvate (3-BP), a small alkylating molecule is a lactate and pyruvate analogue and it is a potent anticancer drug. 3-BP inhibits mitochondrial and glycolytic ATP production in malignant tumours that are submitted to the 'Warburg effects' characterized by increased glycolysis and abundant production of lactate in the presence of oxygen. Anticancer property of 3-BP was identified in the laboratory of P.L. Pedersen in 2000 and it's killing efficacy toward liver cancer cells has been demonstrated by Y.H. Ko in animals and humans with no apparent side effects. Research performed in Department of Genetics, Institute of Genetics and Microbiology at the University of Wroclaw shown a lack of mutagenic properties of this compound. Moreover, analysis carried indicate that 3-BP is not a substrate of the efflux pumps involved in the Pleiotropic Drug Resistance (PDR) network which confers resistance to many anticancer and antifungal drugs.
We have investigated 113 different fungal strains toward the susceptibility to 3-BP. The most sensitive strain was *Cryptococcus neoformans.* Among the genus of *Cryptococcus* only *C. neoformans* and *C. gattii* are pathogens. Systemic infections caused by pathogenic species of the genus *Cryptococcus* are an increasingly serious threat especially for people with impaired immunity. The population of patients with HIV / AIDS, after organ transplant and during chemotherapy systematically increasing. As a consequence the problem of cryptococcosis is increasingly important and widespread. Increasingly, a special attention is drawn to the lack of therapeutic options for the infections caused by multidrug-resistant strains. Most commonly used are azole drugs (for which we observe an increasing percentage of strains with acquired resistance) and highly nephro- and hepatotoxic amphotericin B. All this information and our preliminary studies led us to investigate the antifungal properties of 3-BP. The research we conducted showed that 3-BP is a strong anticryptococcal agent.

The main objective of this project is to understand the molecular reasons for the high sensitivity of pathogenic fungi *Cryptococcus spp.* to the action of new potential anticancer and antifungal drug 3-bromopyruvate (3-BP). In the first stage of the study I will compare the physiology of strains belonging to the species *Cryptococcus.* In order to verify whether there is a correlation between the sensitivity of the various species to 3-BP and the rate of accumulation of this compound in the cells, I will examine the kinetics of transport using [^{14}C]-labeled 3-BP. Another important factor determining a differentiated sensitivity of fungi is a various level of intracellular glutathione (GSH). Thus, I will compare a natural level of GSH in the cells of analyzed fungal species. In addition, I will examine the changes in the level of glutathione in the *Cryptococcus spp*. cells under the influence of incubation in the presence of 3-BP. At the same time will be controlled the viability of cells in each time-point. Next, very important objective of the project is to identify the transporters for 3-BP and other short-chain carboxylic acids in the *Cryptococcus spp.* cells. First of all, I will make *in silico* reaserch in order to identify the genes encoding potential carboxylic acid transporters. I will focus on genes which exhibit conservative amino acid construction as all the transporters from the Jen1 group. After that, I will determine the changes in the selected genes expression under the influence of 3-BP. The next step will be to conduct the heterologous expression of analyzed genes in *S. cerevisiae* Δ*jen1* Δ*ady2* cells using the P416-GPD plasmid. This strain has deletions of two genes encoding the major yeast transporters for short-chain carboxylc acids. Therefore, the subsequent examination of the 3-BP and other carboxylic acids transport kinetics in the obtained transformants cells will verify the functions of the inserted genes. Furthermore, the presence or absence of transporters of analyzed compounds may determine the susceptibility of various strains to the fungicides (eg 3-BP).

The project provides new and fundamental information on the mechanisms of cellular responses of *Cryptococcus spp.* strains to the action of 3-bromopyruvate. Understanding the changes occurring in the cells of the tested strains will be a valuable element that allows a better understanding of their physiology and genetics, and may form the basis for future research aimed at creating new drugs and strategies for cryptococcosis treatment in humans. In addition, the experiments planned in the project will allow us to verify the function and characterize the substrates of selected membrane pumps previously defined as "potential transporters for carboxylic acids" in the *Cryptococcus spp.* cells.