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The review of Katarzyna Niedźwiecka's doctoral dissertation "Molecular mechanisms of 3-bromopyruvate activity in multiple myeloma cells and pathogenic microorganisms of the genus Cryptococcus and Prototheca" performed at the Institute of Genetics and Microbiology, Faculty of Biological Sciences, University of Wroclaw, under the supervision of prof. dr hab. Stanisław Ułaszewski.

The doctoral (PhD) dissertation of Katarzyna Niedźwiecka entitled "Molecular mechanisms of 3-bromopyruvate activity in multiple myeloma cells and pathogenic microorganisms of the genus Cryptococcus and Prototheca" presents results of her PhD study. This PhD study contributed for the publication of four manuscripts in relevant journals in this field with IF (Impact Factor 2.2 – 5.0), one of them as first author.

The dissertation is focused on an up-to-date topic, giving new insights on the molecular action and response mechanisms of the antifungal and anticancer molecule 3-Bromopyruvate (3BP) in fungi and algae pathogens, as well as in a multiple myeloma cell line. The presented dissertation has clearly formulated aims and hypothesis, it is generically written in very good English, very carefully and rigorously.

An exhaustive introduction detailing the response mechanisms of eukaryotes towards xenobiotics is given, focusing particularly on oxidative stress and glutathione antioxidant effects. Moreover, an updated overview about 3BP applications as antifungal and anticancer is presented along with a description of all model organism used in this thesis. This introduction chapter gives a good background and support to understand the next sections of the thesis, namely the experimental design adopted. It is well written and with references to recent bibliography

All the methodologies used in this work are allocated in one single section after introduction, which makes easy to preview and anticipate the experimental design, as well as to better assimilate the outcomes of the several experiments that were made. It is well written and detailed enough to reproduce the experiments described.

Regarding the results section, it was reported that 3BP induces oxidative stress in *C. neoformans* and multiple myeloma (MM) cells. To protect against this phenomenon, during incubation in the presence of 3BP, the overexpression of superoxide dismutase and catalase (CAT) was observed. Reduced concentration of this glutathione in MM, *Cryptococcus spp.* and *Prototheca spp.* cells was observed under 3BP action. 3BP also increases the expression of the

gene encoding glutathione S-transferase (GST), which is responsible for the GSH-3BP conjugation process to inactivate the compound. The stimulation of free radical generation and decrease in GSH levels resulted in the initiation of apoptosis and necrosis in multiple myeloma and *C. neoformans*.

A strong synergistic effect of 3BP with amphotericin B (AmB) action in cells of *C. neoformans* was found. These results can provide a basis for further research leading to minimizing the AmB doses used to treat systemic fungal infections Cytotoxicity tests using the larvae of *Galleria mellonella*, allow to determine a safe therapeutic dose of 3BP.

The difference in the rate of 3BP transport into the cells led to disparities in the susceptibility of various species of fungi. The most resistant strains showed the lowest value V_{max} and vice versa. The uptake assays by cells of *Prototheca spp.,* showed that in this case the 3BP uptake occurs by simple diffusion.

The heterologous expression of *CNAG_04704* in the *S. cerevisiae Δjen1 Δady2* cells displayed enhanced sensitivity to 3BP. Sensitivity tests carried out on the strain of *C. neoformans* with a deletion in *CNAG_04704* definitively confirmed that the Cn04 protein is responsible for 3BP uptake. The analysis of 3BP transport into cells of deletion mutants, confirmed the tested protein's functionality.

Research carried out in the framework of this doctoral dissertation provided new information on the mechanisms of cellular responses to the action of 3BP. They also may form the basis for further research aimed at creating new drugs and strategies for cryptococcosis, protothecosis and cancer treatment in humans.

The aims of the dissertation were fulfilled under rigorous conditions of scientific experiment. The dissertation presents original results, which Katarzyna Niedźwiecka published in respected research journals with IF and rigorous peer reviews.

I have a pleasure to apply to the Council of the Faculty of Biological Sciences, University of Wroclaw to accept this dissertation. In my opinion, Katarzyna Niedźwiecka deserves for PhD degree with the distinction due to the high substantive value of her thesis.

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