Systemic fungal infections are caused mainly by species belonging to *Aspergillus* and *Candida*. Fungi are also known as one of the most common infectious agents causing nosocomial infections. Among human fungal pathogens, *Candida albicans* plays a dominant role. This opportunistic yeast is responsible for ~ 8% of all hospital-acquired microbial infections. Particularly systemic infections caused by human pathogenic fungi in immunocompromized patients are important clinical problems. Number of patients increases, both because of AIDS epidemic, as well as the consequences of applying therapies that cause weakening of the immune system (such as steroid therapy, the use of immunosuppressants in transplant patients).

Efficacious antifungal chemotherapeutic should be characterized by fungicidal but not fungistatic mechanism of action, the widest possible spectrum of activity, the lowest mammalian toxicity and the minimum capacity to induce specific and/or multi-drug resistance. The antifungal agents currently available for the treatment of systemic fungal infections include: a polyene macrolide antibiotic amphotericin B and its lipid formulations, 5-fluorocytosine, inhibitors of ergosterol biosynthesis: fluconazole, voriconazole and itraconazole and an inhibitor of glucan synthase, caspofungin. None of these drugs meets all of the above conditions. Over the past ten years only two new antifungal drugs has been introduced into medical practice, namely voriconazole and caspofungin. In addition, only the latter has an unique, previously unexploited molecular target, an enzyme participating in biosynthesis of the crucial component of fungal cell wall. The continuing increase in the incidence of fungal infections together with the gradual rise in microbial resistance to antifungal drugs highlights the need to find novel compounds with divergent mechanisms of action.

Among 20 proteinogenic amino acids, 9 are regarded as essential for humans: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine, and histidine. Mammals acquire them from the diet to guarantee optimal growth and development, while bacteria, plants and fungi have developed own pathways of their biosynthesis. Aromatic aminotransferases Aro8p and Aro9p are probably involved in the biosynthesis of three of that amino acids (Phe, Trp and Lys) and therefore are promising molecular targets.

The main purpose of this project is determination of molecular consequences of the lack of aromatic aminotransferase activity (Aro8p and Aro9p) in *C. albicans* cells. The proposed studies should allow verification of a hypothesis whether a therapy concerning the possible inhibitors of unique fungal enzymes of aromatic amino acids biosynthesis pathway could give the opportunity to overcome fungal infection. The proposed approach results from the exogenous (Phe, Trp) or relatively exogenous (Tyr) nature of the aromatic amino acids for humans and the ability of fungal cells for its synthesis. On the other hand, there is a lack of antifungal drugs characterized by a low toxicity or broad spectrum. Key processes during infections, such as adhesion, yeast to mycelium transformation, penetration and destruction of host cells are associated with the expression of many proteins, which leads to a significant increase in the demand for amino acids. In addition, because of the likely wide substrate spectrum of Aro8p and Aro9p, these enzymes may play key roles in a number of essential for the proper functioning of fungal cells biosynthetic pathways (including aromatic amino acid biosynthesis and L-Lys).

Usefulness of these unique fungal enzymes as molecular bases for new drugs discovery will be assessed by testing virulence and survival of *C. albicans* mutant cells, lacking *ARO8* and *ARO9* genes encoding aromatic aminotransferases, performing full biochemical characterization of both enzymes and obtaining structural information necessary for the rational design of potential inhibitors. Genes disruption would be performed by SAT-FLP method. In the subsequent step, we are going to characterize mutant cells. Under the present project we will analyze the growth ability of the mutants in different media and in defined media, used routinely for cultivation of mammalian cells in tissue cultures. The mutant cells will be also characterized in order to determine its ability to yeast-to-mycelia transformation, biofilm formation, the adhesion capacity in vitro and the ability to produce secreted aspartyl proteases responsible for host cell damage. *C. albicans* mutant cells virulence will be also determined *in vitro* (in the model of mixed culture of fungal and mammalian cells) and *in vivo* in the models of murine candidiasis. Project also involves isolation, characterization, protein crystallization attempts and structural studies of both Aro8p and Aro9p. Isolation of proteins with the highest possible degree of purity exceeding 99% is a necessary condition for the possibility of obtaining crystals useful for X-ray studies. For this purpose we are going to isolate and characterize both enzymes also as His – tagged fusion proteins. We will use standard sets of crystallization solutions commercially available: e.g. from Hampton Research, Jena Biosciences. Growing of crystals will be carried out by the most common method of protein crystallization - hanging drop vapor diffusion. X-ray diffraction measurements will be carried out in cooperation with the Institute of Bioorganic Chemistry of Polish Academy of Sciences in Poznań. Isolated proteins will be characterized in terms of steady-state kinetic parameters, substrate specificity and cofactor requirements. Biochemical characteristics will include estimation of molecular weight of native enzymes and quaternary structure analysis.

The project involves research, which will be necessary and important starting point for further development and testing new therapeutic strategies. The results will provide information on the molecular consequences of blocking the aromatic amino acids biosynthetic pathway at the stage of functioning of aromatic aminotransferases Aro8p and Aro9p.