

One of the main reasons for the treatment failure of infections caused by pathogenic microorganisms is the overexpressing of efflux membrane proteins, which actively remove drugs from cells, leading to a phenomenon called multidrug resistance MDR. In this work, we focused on the functional characterization of the MDR pump Pdr5p in the yeast *Saccharomyces cerevisiae*. We have verified that diS-C₃(3) fluorescence method can be used to determine the binding sites where the substrates bind in the binding pocket of the pump ScPdr5p. We focused on the study of the ScPdr5p binding pocket using triazole derivatives: ravuconazole, voriconazole and fluconazole. Using disc diffusion assay, we showed that all three studied triazoles are substrates of the pump ScPdr5p. We have found that these structural analogs have a significantly different effect on the inhibition of the potentiometric fluorescent probe diS-C₃(3) transport by the pump ScPdr5p, and also that ravuconazole and voriconazole compete with each other for transport by the pump ScPdr5p. We have used a fluorescent approach to study the binding of azoles to the binding pocket of pump ScPdr5p using benchmark substrates, that bind selectively to only one binding site in the binding pocket of the pump ScPdr5p, and we have supported the hypothesis that ravuconazole and voriconazole bind to more than one binding site.