Using a set of five isogenic mutant strains, we tested the effects of a set of twelve aminoesters of fatty acids belonging to two structural groups, which have been previously classified among lysosomotropic antifungals (LA), on membrane potential and the activity of MDR pumps Pdr5p, Snq2p and Yor1p in S. cerevisiae by a newly developed assay – a combination of the diS-C3(3) method and biological tests [1]. Depending on their chemical structure and concentration, the LA displayed several effects: (a) membrane depolarization, (b) interaction with MDR pumps, and (c) membrane damage leading to cell permeabilization. Membrane depolarization was observed with nearly all LA while only three of the tested compounds interacted with the MDR pumps - a competitive inhibition was detected. The above diagnostic fluorescence method using the cationic redistribution probe diS-C3(3) can readily be employed to exclude from the group of potential inhibitors those compounds (even though they can be substrates of Pdr5p and Snq2p) that do not satisfy the necessary condition of inhibiting MDR pumps Pdr5p and Snq2p already at low concentration.