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Application of benzo[a]phenoxazinium chlorides in *Candida albicans* inactivation by photodynamic therapy

Pereira, João Paulo Campos (1); Lopes, Marlene Alexandra da Silva (1); Gonçalves, Maria Sameiro Torres (2); Coutinho, Paulo José Gomes (3); Belo, Isabel Maria Pires (1)

1: Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Gualtar, 4710-057 Braga, Portugal;
2: Centre of Chemistry, University of Minho, Gualtar, 4710-057 Braga, Portugal;
3: Centre of Physics, University of Minho, Gualtar, 4710-057 Braga, Portugal

E-mail: jpc_pereira@sapo.pt

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Abstract

The photodynamic therapy is a non-invasive model with a substantial potential in antimicrobial therapy. The treatment may be a fast way to control and reduce microbial burden of localized infections that are resistant to the standard antibiotic regimens. This technique results of the interaction between photons of visible light, of appropriate wavelength, and a photosensitizer in the presence of oxygen, that combine to produce cytotoxic species[1].

Along this study, the potential photodynamic action in *Candida albicans* was assessed. Three benzo[*a*]phenoxazinium chlorides, namely *N*-(5-amino-9*H*-benzo[*a*]phenoxazin-9-ylidene)- *N*-ethylethanaminium chloride (Nile Blue, **1a**) and its derivatives, *N*-ethyl-*N*-[5-(3-hydroxypropylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]ethanaminium chloride (**1b**) and *N*-[5-(3-hydroxypropylamino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene]ethanaminium chloride (**1c**) previously synthesized[2] were used as photosensitizers. In an earlier stage, the optimized times of the photosensitizers absorption by cells were determined through absorbance and fluorescence methods. Cells were exposed to radiation at a wavelength of visible light for 20 minutes in two different ways, first they were washed in PBS before being exposed to radiation, and then they were irradiated with benzo[*a*]phenoxazine solution. The cellular viability was determined using the method of reduction XTT and colony counting.

From all compounds tested, the benzo[*a*]phenoxazine **1c** reveled to be the most effective in *Candida albicans* inactivation. An inactivation rate of 85.3% for XTT method and 67.1% for the colony counting method was obtained when 4×10^7 cells in 200 µM solution of **1c** were exposed to photodynamic therapy, without washing the cells. For all benzo[*a*]phenoxazinium chlorides studied, it was obvious that the method of irradiation without cell washing achieved the higher cell inactivation for *Candida albicans*. It was also evident that Nile Blue (**1a**) only begins its inactivation of this strain when concentrations higher than 300 µM of this benzo[*a*]phenoxazine are used to inactive 4×10^7 cells/mL. Compound **1b** originated an intermediary inactivation, with better results than Nile Blue (**1a**), but less effectiveness than **1c**. This work indicates that the Nile Blue derivatives have a great potential of clinical application for *Candida albicans* infections treatment.

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