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Photodynamic properties and photoinactivation of *Candida albicans* mediated by brominated derivatives of triarylmethane and phenothiazinium dyes

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Summary The photodynamic activity of brominated derivatives of New Fuchsin and Azure B was studied in solution and in cell suspensions of *Candida albicans*. The spectroscopic and photodynamic properties of these photosensitizers were compared with those of Crystal Violet and Azure B, which represent active photosensitizer related to each family of compounds. Triarylmethane derivatives absorb intensely with a band centered at ~570 nm, while the phenothiazinium dyes at ~650 nm. Photooxidation of 9,10-dimethylanthracene was observed using phenothiazinium compounds indicating the formation of singlet molecular oxygen, while it was not detected using triarylmethane agents. However, triarylmethane dyes were able to photooxidize L-tryptophan. In yeast cell suspensions, the photosensitized inactivation of *C. albicans* increases with photosensitizer concentration, causing a ~5 log decrease of cell survival, when the cultures are treated with 20 μM of Crystal Violet and irradiated for 60 min. Under these conditions, the photodynamic activity of 50 μM Azure B induced a ~3 log decrease of cell survival. Studies of photodynamic action mechanism indicated that photoinactivation of *C. albicans* cells induced by triarylmethane compounds involves mainly type I photoprocess. Although, phenothiazinium derivatives produce singlet molecular oxygen, a contribution of other reactive oxygen species cannot be discarded in the photoinactivation of *C. albicans*.

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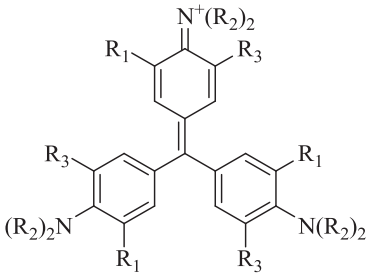
Introduction

The commensal member of the human microflora *Candida albicans* is the most important human fungal opportunistic pathogen, which can cause diseases from superficial mucosal infections to life-threatening systemic disorders [1,2]. Oral candidiasis represents an important infection in patients that are treated with chemotherapy and radiotherapy for cancer and in immunocompromised patients due to HIV infection and AIDS. Also, antimicrobial resistance is a growing problem that complicates the treatment of important nosocomial and community-acquired infections. In the last years, resistance of *C. albicans* is increasing against traditional antifungal azole derivatives [3,4]. Thus, it is imperative to develop alternative therapies for the treatment of candidiasis. A promising modality is photodynamic inactivation (PDI) of microbes with positively charged photosensitizing agents, which appears to be an interesting approach especially for the treatment of fungal infections [5,6]. PDI utilizes visible light to activate a photosensitizer, which can react with biomolecules from its direct environment by electron or hydrogen transfer, leading to the production of radicals (type I reaction). Also, it can transfer its energy to oxygen, generating the highly reactive singlet molecular oxygen, $O_2(^1\Delta_g)$ (type II reaction). Both pathways can produce reactive oxygen species that react with biomolecules conducing to cell damages and microbial inactivation [6].

Several exogenous photosensitizers have been investigated to photoinactivate fungal cells [7]. Phenothiazinium-based photosensitizers are generally cationic molecules with a core structure composed of planar tricyclic aromatic systems, which functions as the chromophore of these compounds [8]. Methylene blue (MB) is the best characterized of the phenothiazinium derivatives. Cells of *C. albicans* were photoinactivated *in vitro* by toluidine blue and thionine [9]. The higher microbial reduction was observed with toluidine blue. Moreover, photoinactivation associated with MB was able to eradicate *C. albicans* from the oral cavity of mice that were previously inoculated with this microorganism [10]. Photodynamic effect of MB presented fungicide effect on different species of *Candida* genus [11]. Therefore, the preparation and evaluation of close MB analogs like phototherapeutic agents are relatively straightforward.

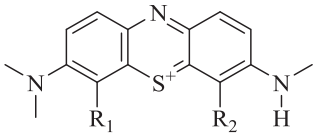
On the other hand, the number of triarylmethane derivatives tested for photodynamic activity is relatively low in comparison to other chromophoric types. Crystal Violet (CV) has been screened against a wide range of microorganisms. It has been shown that the activity of CV against bacteria and yeasts is significantly increased on illumination [9,12]. Also, cationic triarylmethanes represent a class of photosensitizers whose phototoxic effects can be developed at the mitochondrial level [13]. Thus, triarylmethane dyes display structural features that can be conveniently used as guidelines for the development of new mitochondria-targeted drugs for a variety of photochemotherapeutic applications [14,15].

The introduction of bromine atoms into the chromophoric system of brominated triarylmethane and phenothiazinium dyes produced an increase in the lipophilicity. Moreover, the *in vitro* photodynamic activity against LM-2 murine mammary carcinoma cells demonstrated that, as the degree of bromination increased, the phototoxicity remained unchanged or decreased. The lower efficiency to inactivate cultured tumor cells may be attributed to the formation of the colorless carbinol pseudobase and aggregation effects for triarylmethane and phenothiazinium dyes, respectively [16].



Photosensitizer	R_1	R_2	R_3
NF	H	H	CH_3
$NFBr_3$	Br	H	CH_3
CV	H	CH_3	H

Scheme 1 Molecular structures of triarylmethane derivatives.



Photosensitizer	R_1	R_2
AzB	H	H
AzBBr	Br	H
$AzBBr_2$	Br	Br

Scheme 2 Molecular structures of phenothiazinium dyes.

In the present work, we compared the photodynamic activity induced by brominated derivatives of triarylmethane (Scheme 1) and phenothiazinium (Scheme 2) dyes in solution bearing photooxidizable substrates. Also, the susceptibility of *C. albicans* to the phototoxic effect produced by these photosensitizers was analyzed in cellular suspensions. The behavior of the brominated compounds was compared with those obtained for New Fuchsin (NF) and Azure B (AzB), which represent active photosensitizer related to each family of compounds. On the other hand, to obtain insight about the photodynamic mechanism, the photoinactivation of *C. albicans* was evaluated in presence of sodium azide and mannitol. This investigation provides information that can be used to establish conditions for the photoinactivation of yeast cells sensitized by these compounds.

Materials and methods

General

Absorption and fluorescence spectra were performed on a Shimadzu UV-2401PC spectrometer and on a Spex FluoroMax fluorometer, respectively. Irradiation experiments were carried out with a Novamat 130 AF slide projector (Braun Photo Technik, Nürnberg, Germany) equipped with a 150 W lamp. The light was filtered through a 2.5 cm glass cuvette filled with water to absorb heat. Irradiation of the cultures was performed as previously described [17]. Experiments were carried out at room temperature and there was no heating during irradiation. A wavelength range between 350 and 800 nm was selected by optical filters. The light fluence rate at the treatment site was 40 mW/cm^2 (Radiometer Laser Mate-Q, Coherent, Santa Clara, CA, USA). Irradiation in solution was performed using a wavelength range of 455—800 nm (GG455 cut-off filter) [18]. All the chemicals from Aldrich (Milwaukee, WI, USA) were used without further purification. Ultrapure water was obtained from Labconco (Kansas, MO, USA) equipment model 90901-01.

Photosensitizers

NF, CV and AzB were purchased from Aldrich. NF was purified by a methodology previously described [19]. $NFBr_3$, AzBBr and $AzBBr_2$ were synthesized as previously described [16]. A photosensitizer stock solution (~0.5 mM) was prepared by dissolution in 1 mL of water.

Steady state photolysis

Solutions of 9,10-dimethylanthracene (DMA, 35 μM) and photosensitizer (absorbance 0.3) in *N*,*N*-dimethylformamide (DMF, 2 mL) were irradiated in 1 cm quartz cuvettes. The kinetics of DMA photooxidation were studied by following the decrease of the absorbance (A) at λ_{max} = 378 nm. Photooxidation of DMA was also used to determine $O_2(^1\Delta_g)$ quantum yield (Φ_Δ) by the photosensitizers [20]. MB was used as a reference (Φ_Δ = 0.52), which was used to calculate a value of Φ_Δ = 0.22 for AzB [8,21]. Measurements of the sample and reference under the same conditions afforded Φ_Δ for photosensitizers by direct comparison of the slopes in the linear region of the plots. The studies in the presence of L-tryptophan (Trp, 25 μM, 2 mL DMF) were performed following the decrease of the fluorescence intensity (F) at λ = 350 nm. The Trp fluorescence was excited by 290 nm light. The observed rate constants (k_{obs}) were obtained by a linear least-squares fit of the semilogarithmic plot of $\ln A_0/A$ *vs* time for DMA and $\ln F_0/F$ *vs* time for Trp.

Microorganism and growth conditions

The strain of *C. albicans* PC31 recovered from human skin lesion was previously characterized [22]. Identification of the yeast isolates to species level was done using the API 20 C AUX (BioMérieux, Marcy l'Etoile, France) system of carbohydrate assimilation profiles. Yeast was grown aerobically overnight in Sabouraud (Britania, Buenos Aires, Argentina) broth (4 mL) at 37 °C to stationary phase. Cells were harvested by centrifugation of broth cultures (3000 rpm for 15 min) and re-suspended in 4 mL of 10 mM phosphate-buffered saline (PBS, pH 7.0), corresponding to ~10^7 colony forming units (CFU)/mL. The cells were appropriately diluted to obtain ~10^6 CFU/mL in PBS. Viable *C. albicans* cells were monitored and the number of CFU was determined on Sabouraud agar plates after ~48 h incubation at 37 °C. Each cell suspension was plated in triplicate.

Photosensitized inactivation of *C. albicans* cells in PBS

Cellular suspensions of *C. albicans* (~10^6 CFU/mL) in PBS were incubated with the corresponding photosensitizer concentration for 30 min in the dark at 37 °C. In all cases, 2 mL of the cell suspensions in Pyrex brand culture tubes (13 mm × 100 mm) were used and the photosensitizer was added from the corresponding stock solution. Then, the cultures were exposed to visible light using the equipment described above for different time intervals. Sodium azide (100 mM) or mannitol (100 mM) were added to yeast suspensions from 2.5 M stock solutions in water and the cells were incubated for 30 min at 37 °C in dark previous to the treatment with the photosensitizer. Cell suspensions were serially diluted with PBS and the number of colonies formed was counted. In all cases, control experiments were carried out in the absence of photosensitizer with cellular suspensions irradiated and in the dark and in the presence of photosensitizer in the dark. Each experiment was repeated separately three times.

Results and discussion

Visible absorption spectroscopy

Fig. 1A shows the absorption spectra of triarylmethane derivatives in DMF. The values of absorption maxima are summarized in Table 1. The bromine substitution of NF leads to a bathochromic shift in the wavelength of maximum absorption. The red shift for $NFBr_3$ was ~14 nm with respect to NF. Similar behavior was previously observed with bromine derivatives of Rhodamine-123 in methanol [23]. The absorption spectra of phenothiazinium dyes showed an absorption band centered at 645 nm in DMF (Fig. 1B). The normal range for conventional phenothiazinium salts is 590—680 nm in water. Extending the absorption beyond this requires functionalization outside the auxochromic moieties [8].

Photodynamic properties

Photooxidation of DMA sensitized by triarylmethane compounds was analyzed in DMF under aerobic condition. It is known that DMA quenches $O_2(^1\Delta_g)$ exclusively by chemical reaction [20]. However, under these experimental conditions the decomposition of DMA was not observed indicating negligible production of $O_2(^1\Delta_g)$ mediated by triarylmethanes. Therefore, the amino acid Trp was used as a substrate model for the compounds of biological interest

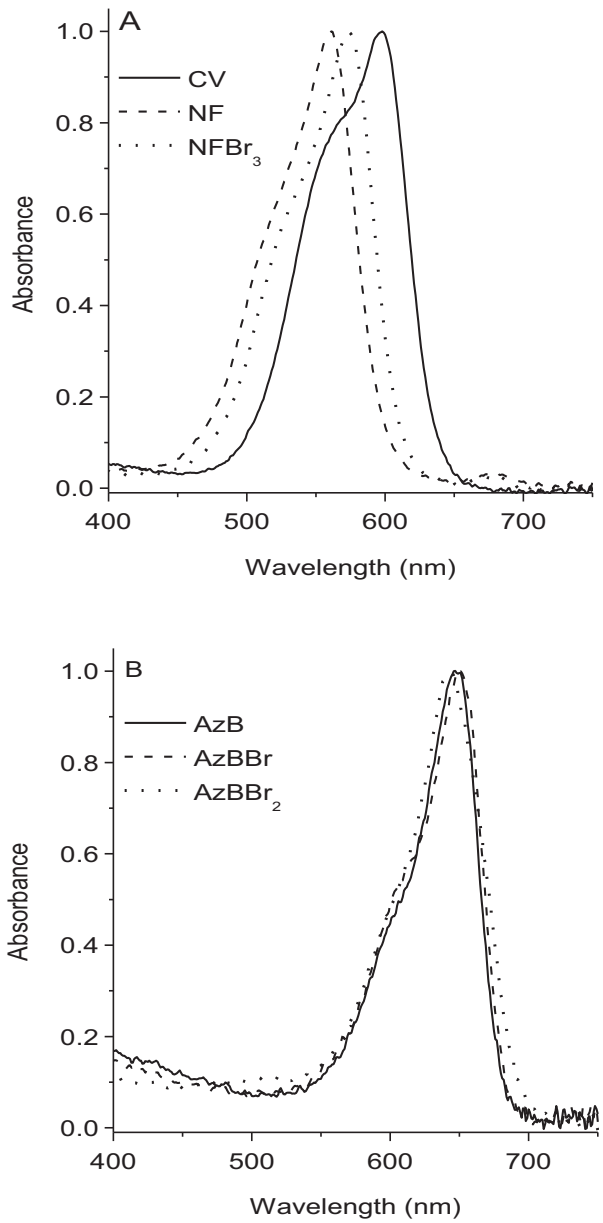


Fig. 1 Absorption spectra of triarylmethane (A) and phenothiazinium (B) dyes in DMF.

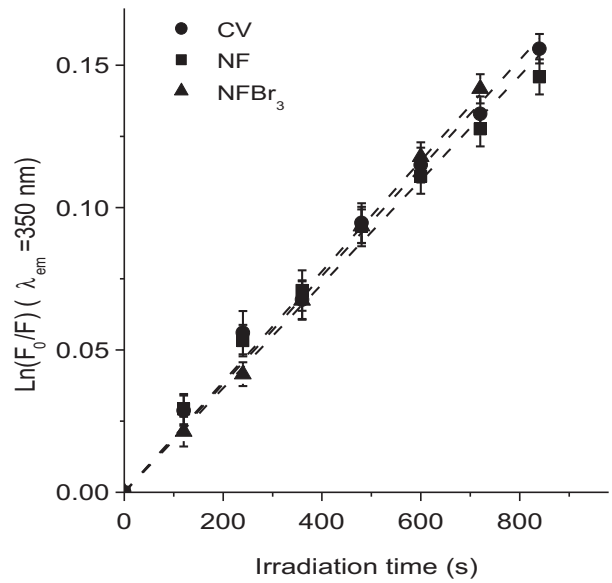


Fig. 2 First-order plots for the photooxidation of Trp (20 μM) photosensitized by CV (●), NF (■) and $NFBr_3$ (▲) in DMF. Values represent mean ± standard deviation of three separate experiments.

that can be potential targets of triarylmethanes photodynamic action. In general, this substrate can be efficiently photooxidized by both type I and type II reaction mechanisms [24]. The photoprocess follows first-order kinetics with respect to Trp concentration, as shown in Fig. 2. From the plots in Fig. 2, the values of the k_{obs}^{Trp} were calculated for Trp decomposition. The results in Table 1 indicate a slightly higher k_{obs}^{Trp} value for the reaction sensitized by $NFBr_3$.

The basic propeller structure of triarylmethanes means that, in the simple derivatives at least, the energy accrued from photoexcitation can be dissipated easily *via* internal conversion. This makes it difficult to examine parameters such as $O_2(^1\Delta_g)$ efficiencies. However, binding and rigidification of the structure in the biological medium can promote the photosensitization route [25]. For example, the triarylmethane derivatives CV and Victoria blue BO produce no measurable singlet oxygen in standard assays; however, both compounds are efficient photosensitizers in cellular media [26].

Also, the DMA reaction was evaluated in the presence of phenothiazinium dyes (Fig. 3). From first-order kinetic

Table 1 Absorption maxima (λ_{max}), kinetic parameters (k_{obs}) for the photooxidation reaction of substrates and singlet molecular oxygen quantum yield (Φ_Δ) of photosensitizers in DMF.

Photosensitizer	λ_{max} (nm)	k_{obs}^{DMA} (s^{-1})	Φ_Δ	k_{obs}^{Trp} (s^{-1})
NF	560	ND[a]	–	$(1.83 \pm 0.05) \times 10^{-4}$
$NFBr_3$	574	ND[a]	–	$(1.94 \pm 0.02) \times 10^{-4}$
CV	598	ND[a]	–	$(1.90 \pm 0.04) \times 10^{-4}$
AzB	648	$(5.21 \pm 0.05) \times 10^{-4}$	0.22 ± 0.03	
AzBBr	650	$(1.19 \pm 0.02) \times 10^{-3}$	0.50 ± 0.05	
$AzBBr_2$	643	$(1.17 \pm 0.02) \times 10^{-3}$	0.49 ± 0.05	

[a] Not detected.

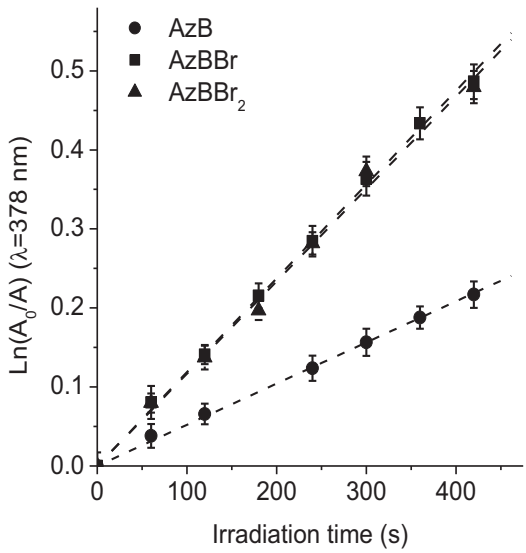


Fig. 3 First-order plots for the photooxidation of DMA (35 μM) photosensitized by AzB (●), AzBBr (■) and $AzBBr_2$ (▲) in DMF. Values represent mean ± standard deviation of three separate experiments.

plots of the DMA absorption at 378 nm with time the values of the observed rate constant (k_{obs}^{DMA}) were calculated (Table 1). As can be observed, both brominated phenothiazinium compounds photosensitized the decomposition of DMA with similar rates. Photooxidation of DMA was used as a method to evaluate the ability of the photosensitizers to produce $O_2(^1\Delta_g)$ in solution [27]. Thus, the quantum yield of $O_2(^1\Delta_g)$ production (Φ_Δ) were calculated by comparing the slope for the phenothiazinium derivatives from the plots shown in Fig. 3. The results showed higher values of Φ_Δ for AzBBr and $AzBBr_2$ in comparison with AzB. This is an important photosensitizer property because the reactions induced by $O_2(^1\Delta_g)$ are believe to be the mainly cause of cellular damage photosensitized by phenothiazinium under aerobic conditions. However, the biological microenvironment of the photosensitizer can produce changes in the photophysics of the phenothiazinium dyes determined in DMF. Therefore, there are limitations to predict the photodynamic efficiency of the photosensitizers in cells founded only on studies in solution.

Photoinactivation of *C. albicans* cells

Photoinactivation of *C. albicans* was studied in PBS cell suspensions treated with different concentrations of triarylmethane (Fig. 4) and phenothiazinium dyes (Fig. 5) derivatives for 30 min at 37 °C in dark and irradiated with visible light. Control experiments showed that the viability of *C. albicans* was unaffected by only illumination. Triarylmethane derivatives were not toxic in dark to highest concentration used (20 μM). Also, AzB and AzBBr were not toxic in dark using 50 μM. However, cell viability was not detected with that of 50 μM $AzBBr_2$ without irradiation. Therefore, 10 μM $AzBBr_2$ was used, which was not toxic in dark. Thus, the cell mortality obtained after irradiation of the cultures treated with the agent was due to the photosensitization effect in the presence of visible light.

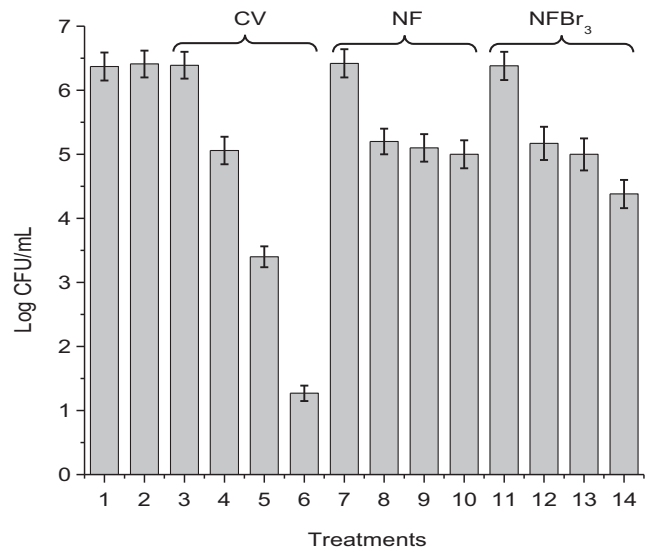


Fig. 4 Survival of *C. albicans* cells (10^6 CFU/mL) incubated with different concentrations of triarylmethane derivatives for 30 min at 37 °C in dark and exposed to visible light for different irradiation times: (1) control culture in dark; (2) control culture irradiated; (3, 7 and 11) control culture treated with 20 μM photosensitizer and keeping in dark; (4, 8 and 12) culture treated with 5 μM photosensitizer and 30 min irradiation; (5, 9 and 13) culture treated with 10 μM photosensitizer and 60 min irradiation; (6, 10 and 14) culture treated with 20 μM photosensitizer and 60 min irradiation. Values represent mean ± standard deviation of three separate experiments.

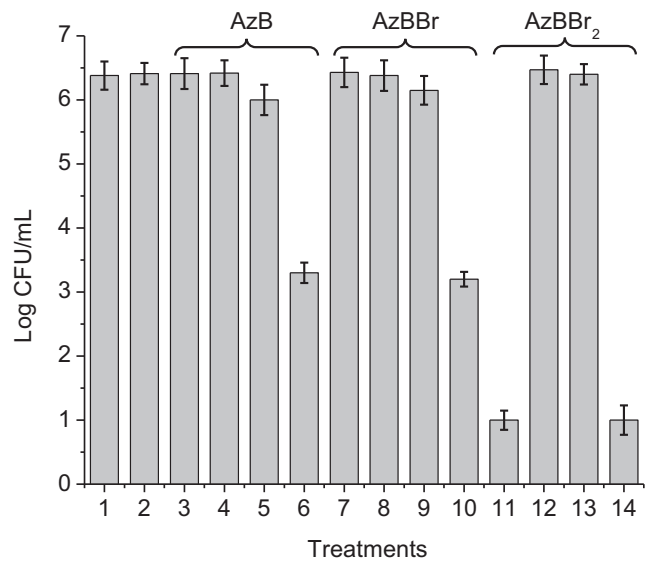


Fig. 5 Survival of *C. albicans* cells (10^6 CFU/mL) incubated with different concentrations of phenothiazinium dyes for 30 min at 37 °C in dark and exposed to visible light for different irradiation times: (1) control culture in dark; (2) control culture irradiated; (3, 7 and 11) control culture treated with 50 μM photosensitizer and keeping in dark; (4, 8 and 12) culture treated with 5 μM photosensitizer and 30 min irradiation; (5, 9 and 13) culture treated with 10 μM photosensitizer and 60 min irradiation; (6, 10 and 14) culture treated with 50 μM photosensitizer and 60 min irradiation. Values represent mean ± standard deviation of three separate experiments.

The viability of *C. albicans* cells was dependent upon photosensitizer concentrations and irradiation times used in the treatment (Figs. 4 and 5). Mainly for CV and AzB, an increase in the photosensitizer concentrations or irradiation periods was accompanied by an enhancement in the PDI efficiency. As can be observed in Fig. 4, the *C. albicans* cells were rapidly photoinactivated when the cultures treated with 20 μM CV were exposed to 60 min visible light. This photosensitizer exhibited a photosensitizing activity causing a ~5 log decrease of cell survival. This result signifies a value greater than 99.999% of cellular inactivation. When the cultures were treated with 10 μM CV and 60 min irradiation was sufficient to produce a ~2.7 log decrease of *C. albicans* viability. Photocytotoxic activity induced by NF was little effective (1 log decrease) even after 60 min irradiation, while the photoinactivation induced by 20 μM $NFBr_3$ produced a ~2 log decrease in cell viability after 60 min irradiation. On the other hand, treatment with 50 μM AzB or AzBBr was necessary to produce a ~3 log (99.9%) decrease of *C. albicans* cell viability after 60 min irradiation (Fig. 5). In contrast, no significant photoinactivation was found for cultures treated with 10 μM $AzBBr_2$ even at the longest time of irradiation used and 50 μM $AzBBr_2$ was toxic in dark (Fig. 5, lines 11 and 14).

Fungal cell walls have a relatively thick layer of beta-glucan and chitin, which present a permeability barrier intermediate between Gram-positive and Gram-negative bacteria [28]. Thus, the number and distribution of cationic groups on the photosensitizer play an important role in the inactivation of yeasts and Gram-negative bacteria [22,29]. In previous studies using the same strain of *C. albicans*, it was demonstrate that tetracationic porphyrins, such as 5,10,15,20-tetrakis(4-*N*,*N*,*N*-trimethylammonium phenyl)porphyrin ($TMAP^{4+}$) and 5,10,15,20-tetra(4-*N*-methylpyridyl)porphyrin ($TMPyP^{4+}$), were effective photosensitizers to eradicate fungal cells [22,30]. *C. albicans* treated with 5 μM porphyrin and 30 min irradiation exhibited caused over 5 log decrease of cell survival. Therefore, the lower PDI observed for triarylmethane and phenothiazinium derivatives in comparison with tetrapyrrolic macrocycles can be associated with a different distribution of agents in the cell due mainly to the number and location of the positive charge on the photosensitizer.

Mechanistic insight of the *C. albicans* photoinactivation

The effect of two suppressors, sodium azide and mannitol, were investigated with the purpose of clarify the photodynamic mechanism involved in the photosensitized inactivation of *C. albicans* cells by triarylmethane and phenothiazinium compounds. To observe the effects of these agents, only the most active photosensitizers studied in this work were selected (CV, $NFBr_3$, AzB and AzBBr).

Sodium azide is a quencher of intracellular $O_2(^1\Delta_g)$ but it also can deactivate compounds in their triplet excited state, thus preventing both type I and type II photoprocesses [31]. First, PDI of *C. albicans* was performed in the presence of 100 mM azide ion, this concentration was not toxic under irradiation without photosensitizer (Figs. 6 and 7, line 3).

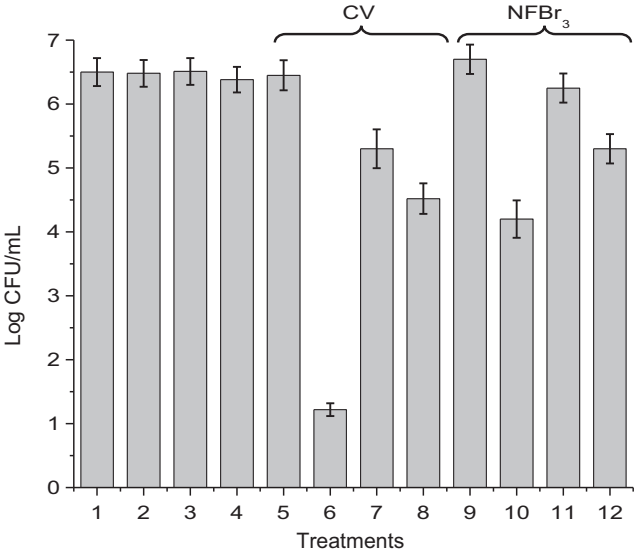


Fig. 6 Survival of *C. albicans* (~10^6 CFU/mL) incubated with 20 μM triarylmethane derivatives in dark for 60 min at 37 °C and exposed to visible light for 60 min; (1) control culture in dark; (2) control culture irradiated; (3) control culture containing 100 mM sodium azide and irradiated; (4) control culture containing 100 mM mannitol and irradiated; (5 and 9) control culture treated with photosensitizer keeping in dark; (6 and 10) culture treated with photosensitizer and irradiated; (7 and 11) culture treated with photosensitizer containing 100 mM sodium azide and irradiated; (8 and 12) culture treated with photosensitizer containing 100 mM mannitol and irradiated. Values represent mean ± standard deviation of three separate experiments.

The resulting photoinactivation was greatly affected by the azide ion, the addition of 100 mM sodium azide produced a high reduction in the inactivation of *C. albicans* mediated by triarylmethane (Fig. 6, lines 7 and 11) and phenothiazinium (Fig. 7, lines 7 and 11) dyes. Under this condition, no more than a 1–1.5 log decrease was observed in the survival of the yeast treated with photosensitizer after 60 min irradiation. In general, sodium azide is employed to quench $O_2(^1\Delta_g)$ during photoinactivation, especially when PDI is used to kill bacteria in suspension [32,33]. However, it was also observed that addition of sodium azide (100 μM or 10 mM) to Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* incubated with MB and illuminated with red light produced significantly increased bacterial killing (1–3 logs), rather than the expected protection from killing [34]. It was proposed that $N_3^{\bullet}$ was formed and bacteria were killed even in the absence of oxygen, suggesting the direct one-electron oxidation of azide anion by photoexcited MB. In the present investigation, a higher concentration was used in the cultures and the presence of azide ion produced a protective effect on *C. albicans*, possibly predominating the quenching of photocytotoxic species.

On the other hand, mannitol acts as scavenger of the superoxide anion radical and hydroxyl radical [35]. The addition of 100 mM mannitol was not cytotoxic for irradiated *C. albicans* cells without photosensitizer (Figs. 6 and 7, line 4). PDI of *C. albicans* photosensitized by CV and $NFBr_3$ was highly reduced (~3 log) in the presence of mannitol after 60 min of irradiation (Fig. 6, lines 8 and 12).

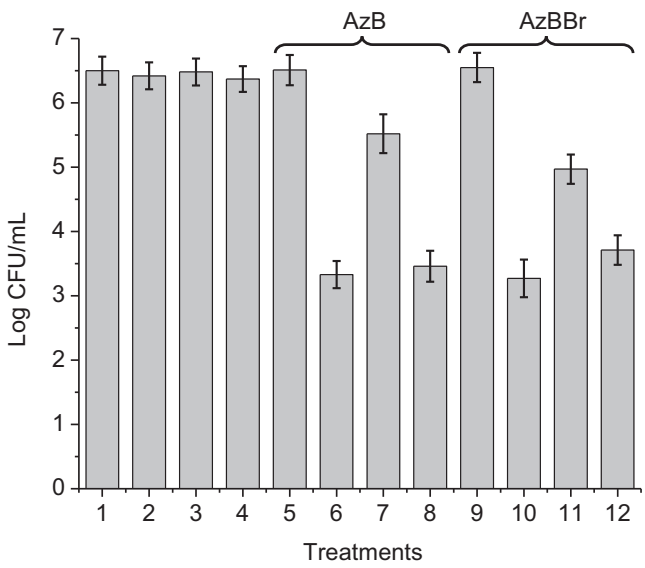


Fig. 7 Survival of *C. albicans* (~10^6 CFU/mL) incubated with 50 μM phenothiazinium dyes in dark for 30 min at 37 °C and exposed to visible light for 60 min; (1) control culture in dark; (2) control culture irradiated; (3) control culture containing 100 mM sodium azide and irradiated; (4) control culture containing 100 mM mannitol and irradiated; (5 and 9) control culture treated with photosensitizer keeping in dark; (6 and 10) culture treated with photosensitizer and irradiated; (7 and 11) culture treated with photosensitizer containing 100 mM sodium azide and irradiated; (8 and 12) culture treated with photosensitizer containing 100 mM mannitol and irradiated. Values represent mean ± standard deviation of three separate experiments.

Cytophototoxic efficacies mediated by phenothiazinium dyes were less affected (~0.5 log) when mannitol was added to *C. albicans* cells (Fig. 7, lines 8 and 12). Therefore, the involvement of $O_2(^1\Delta_g)$ can be a reactive oxygen species involved in the photodamage of *C. albicans* cells induced by phenothiazinium derivatives. However, although small, a photoprotective effect was found with AzB and AzBBr, indicating some contribution of type I reactions in the inactivation process. A similar behavior was previously observed for $TMAP^{4+}$ [33]. Studies of the photodynamic mechanism of action on *C. albicans* cells indicated that this cationic porphyrin appears to act as photosensitizers mainly *via* the intermediacy of $O_2(^1\Delta_g)$ with some contribution of type I photoprocess.

PDI using triarylmethane derivatives indicated that phototoxicity efficacies were significantly diminished when mannitol was added to *C. albicans* cells. Thus, the photoprotective effect produced by mannitol was indicative of a contribution of type I reactions in the inactivation process photosensitized by CV and $NFBr_3$. The mechanism of photoinduced damage of bovine serum albumin mediated by triarylmethane photosensitizers is known to be primarily controlled by the photosensitization mechanism type I, rather than by the $O_2(^1\Delta_g)$ pathway [25]. Also, it was observed that the mechanism of hexokinase and DNA damage photosensitized by CV does not require the involvement of molecular oxygen to operate [14]. Thus, based on previously reported on the photochemical and photosensitization properties of these triarylmethane dyes, it is reasonable to infer that their phototoxic effects rely on substantial contribution arising from the type I photosensitization mechanism [36].

Conclusions

Two families of photosensitizers derived from triarylmethane and phenothiazinium dyes were compared as agents to inactivate *C. albicans* cells. Spectroscopic and photodynamic properties in solution indicate that these compounds are candidate agents to produce photocytotoxicity in biological media. *In vitro* studies showed that *C. albicans* cellular suspensions in PBS treated with CV were efficiently photoinactivated, whereas a minor effect was found for NF and $NFBr_3$. An increase in the cellular photodamage was obtained enhancing the photosensitizer concentration. In the other hand, a higher concentration of Azure B derivatives was necessary to inactivate *C. albicans* cells suspensions. Consequently, under these conditions CV was more effective than Azure B to photoinactivate yeast cells. Studies of photodynamic mechanism of action showed that photoprotection was found using sodium azide as type II scavengers. Also, phototoxic efficacy of CV and $NFBr_3$ was reduced when mannitol was used as a type I scavenger. Thus, a contribution of type I process can be involved in the killing of microbial cells photosensitized by CV and $NFBr_3$. The photoinactivation of *C. albicans* mediated by phenothiazinium dyes was less affected in the presence of mannitol than those treated with triarylmethanes. Therefore, even when the phenothiazinium dyes can produce $O_2(^1\Delta_g)$ in solution, the participation of other active oxygen species can be present in the photoinactivation of *C. albicans* cells.

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