European Journal of Medicinal Chemistry 144 (2018) 651–661

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Review article

BODIPYs to the rescue: Potential applications in photodynamic inactivation

Andrés M. Durantini [1], Daniel A. Heredia [1], Javier E. Durantini [1], Edgardo N. Durantini*

Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601, X5804BYA Río Cuarto, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 15 August 2017
Received in revised form
6 December 2017
Accepted 18 December 2017
Available online 19 December 2017

Keywords:
BODIPY
Photosensitizer
Photoinactivation
Singlet oxygen
Microorganisms

ABSTRACT

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives have been proposed in several potential biomedical applications. BODIPYs absorb strongly in blue-green region with high fluorescence emission, properties that convert them in effective fluorophores in the field of biological labeling. However, BODIPY structures can be conveniently modified by heavy atoms substitution to obtain photosensitizers with applications in photodynamic therapy. Also, external heavy atoms effect can be used to increase the photodynamic activity of these compounds. In recent years, BODIPYs have been proposed as phototherapeutic agents for the photodynamic inactivation of microorganisms. Therefore, BODIPY structures need to be optimized to produce an efficient photocytotoxic activity. In this way, amphiphilic cationic BODIPYs can selectively bind to microbial cells, inducing an effective photokilling of pathogenic microbial cells. This review summarizes the attributes of BODIPY derivatives for applications as antimicrobial photosensitizing agents.

Contents

1. BODIPY derivatives with biological applications

The basic structure of 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) consists in two pyrrole units linked by a methine bridge and a BF_2 group that connect both pyrrolic nitrogens (Fig. 1) [1,2]. The central carbon of BODIPY is denoted the *meso* position, α-positions are adjacent to the nitrogen atoms, while the others are β-positions, which are located in 8, 5-3 and 1-2-6-7 according to IPUAC nomenclature, respectively.

Some of the most important properties of BODIPYs involve high absorption and fluorescence emission in the visible range, low generation of excited triplet state, photochemical stability, chemically robustness and good solubility in organic solvents [1,3]. These complexes are stable at physiological pH, which combined with a low toxicity make them excellent probes for use in biological systems. Thus, BODIPYs have received substantial interest as fluorophores in bioimaging, biological labeling and fluorescence assays

* Corresponding author.
E-mail address: edurantini@exa.unrc.edu.ar (E.N. Durantini).
[1] AMD, DAH and JED contributed equally to the manuscript.

https://doi.org/10.1016/j.ejmech.2017.12.068
0223-5234/

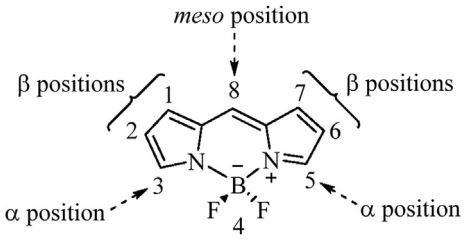

Fig. 1. Chemical structure and IUPAC numbering system of BODIPYs.

[4–9]. Also, BODIPYs have been proposed as light-harvesting antennas to improve the absorption of different chromophores [10–12].

The versatility of the synthetic pathways to obtain BODIPYs allows manipulating different strategies to find an adequate relation between the structure and the desired spectroscopic and photophysical characteristics [13,14]. Thus, BODIPY structures have been modified to reduce fluorescence and increase singlet-to-triplet intersystem crossing for applications in photodynamic therapy (PDT) [15–17]. Spin-coupling to heavy atoms is a frequent modification employed to enhance triplet state formation by halogenation reactions [18]. Therefore, the BODIPY fluorophore can be changed into a photosensitizer (PS) by attaching heavy atoms directly on the *s*-indacene ring [19]. This effect produces a long-lived electronically excited triplet state able to produce efficiently reactive oxygen species (ROS).

In the last years, BODIPYs have been proposed as PS with potential applications in killing microbial cells [20–27]. Moreover, the rigid and extended π conjugation of the BODIPY structure makes it a good candidate to be used for bactericidal application in deep tissues since red light can penetrate dipper [28,29]. Thus, a key factor to improve the efficacy of photoinactivation of microorganisms mediated by BODIPYs is the development of suitable molecular structures with appropriated photophysical and biological properties [30]. Therefore, this review deals with the evolution of these PSs with potential applications in photokilling of microorganisms.

2. Photodynamic inactivation of microorganisms

The use of antibiotics to selectively eliminate microorganisms has been one of the most important advances in medicine. However, the considerable increase in multi-drug resistance in microbes has presented an immediate challenge for researchers [31]. Among other causes, this is owing to inappropriate prescriptions of antibiotics, the demand in prophylaxis, the systemic use and the failure of patients to complete the treatments. The best epidemiologically recognized resistance with high clinical influence involves the Gram-positive pathogen *Staphylococcus aureus* [32]. Moreover, the global appearance of multidrug-resistant Gram-negative bacteria is an increasing risk to antibiotic therapy [33]. The antibiotic resistance can significantly be increased by the chromosomally encoded drug efflux mechanisms, which are specific of these bacteria. In particular, *Escherichia coli* can cause severe hospital-acquired infections and have developed resistance to most available antibiotics. Not only bacteria have developed resistance, but also systemic fungal infections are a very difficult task. The antifungal drug resistance is an important factor causal of therapeutic failure [34]. Thus, *Candida albicans* can produce diseases that involve from superficial mucosal infections to systemic disorders [35–37]. Therefore, it is necessary the development of novel alternative antimicrobial therapies. Some proposals cover phage therapy, bacteriocins, killing factors, quorum quenching and photosensitized inactivation of microbes [31]. The last one involves the photodynamic inactivation (PDI), which has been projected as an interesting alternative to eradicate microorganisms [38,39]. Mostly, PDI is based on the addition of a PS that is rapidly bound to microbial cells (Fig. 2). The subsequent irradiation with visible light induces PS excitation, which in presence of oxygen produces ROS. These reactive species react with the surrounding biomolecules in the cell leading to a loss of biological functionality and consequent cell death [40].

A wide variety of PSs have been efficiently used to photokilling Gram-positive bacteria. However, neutral or negatively charged PSs are very little active to inactivate Gram-negative bacteria [38]. The presence of a highly organized outer membrane (OM) has been considered as the cause of resistance to the action of PSs [41,42]. Gram-positive (Fig. 3A) and Gram-negative (Fig. 3B) bacteria mainly differ in the composition of their outer envelope. More specifically, the cell envelope of Gram-positive bacteria is formed by two layers, the cytoplasmic membrane and a thick peptidoglycan layer containing teichoic acid. This network does not represent a permeability barrier because it is relatively porous. In contrast, Gram-negative bacteria are enveloped by a highly complex multilayered structure that consists of a peptidoglycan layer to which the OM is attached. The additional membrane layer in the cell wall architecture, which is located outside the peptidoglycan layer, presents an asymmetric lipid structure formed by negatively charged lipopolysaccharides (LPS), lipoproteins and proteins with porin function. Molecules of LPS yield a polyanionic external surface, which is partially neutralized by divalent cations Mg^{2+} and Ca^{2+}. Thus, cationic groups on the PS structures produce a tight electrostatic interaction with negatively charged sites at the outer surface of the Gram-negative bacteria. This effect increases the photocytotoxicity mediated by the PSs [43]. On the other hand, the fungal wall is composed by β-glucans, chitin and mannoproteins, which confer rigidity and morphology to the cells (Fig. 3C) [44,45]. The layer of β-glucan and chitin provide a permeability barrier, which resulting in some resistance to the photoinactivation process. The cell envelope of fungi produces a permeability intermediate between Gram-positive and Gram-negative bacteria [46]. Therefore, the photosensitized processes are more significant when agents can penetrate into the inner cell area. Experimental studies have indicated that yeasts can be effectively photokilled *in vitro* by different PSs [47,48]. Consequently, the development of appropriate PSs is very important to improve the efficacy of PDI. Several potential specific compounds have been proposed for the photokilling of different microorganisms [49,50]. In this sense, structural modification of BODIPY core can be used to obtain strong candidates for better PSs.

3. BODIPY structures for applications in PDI

The BODIPY structure can be easily handled due to their good solubility in most organic solvents and the feasibility of purification by chromatographic techniques [13,14]. The *meso*, *α* and *β* positions of BODIPYs can be functionalized and modified synthetically in order to achieve the desired solubility and photochemical properties [51–53]. In the last years, several review articles have discussed the synthetic aspect and modification of the BODIPY core [13,54]. However, the aim of this section is to analyze the main structure features to obtain BODIPY derivatives with potential applications in PDI.

An unlimited number of derivatives can be designed and prepared without excessive synthetic efforts. However, only a few studies exhibit the real application of BODIPYs as antimicrobial photosensitizers. O'Shea and co-workers modified the structure of BF_2-chelated tetraarylazadipyrromethene for PDI of

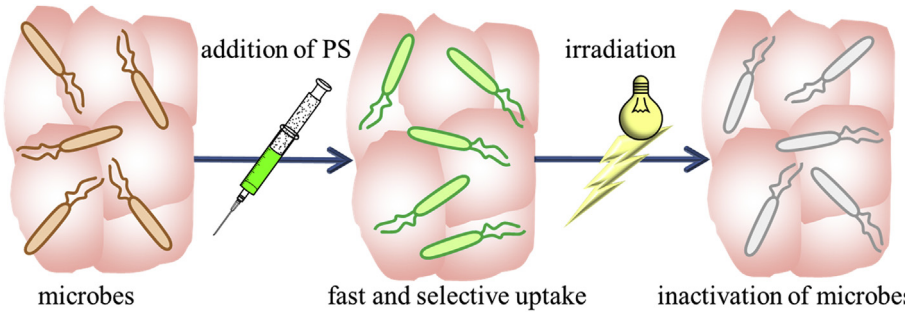

Fig. 2. Schematic representations of the photodynamic inactivation (PDI) of microorganisms.

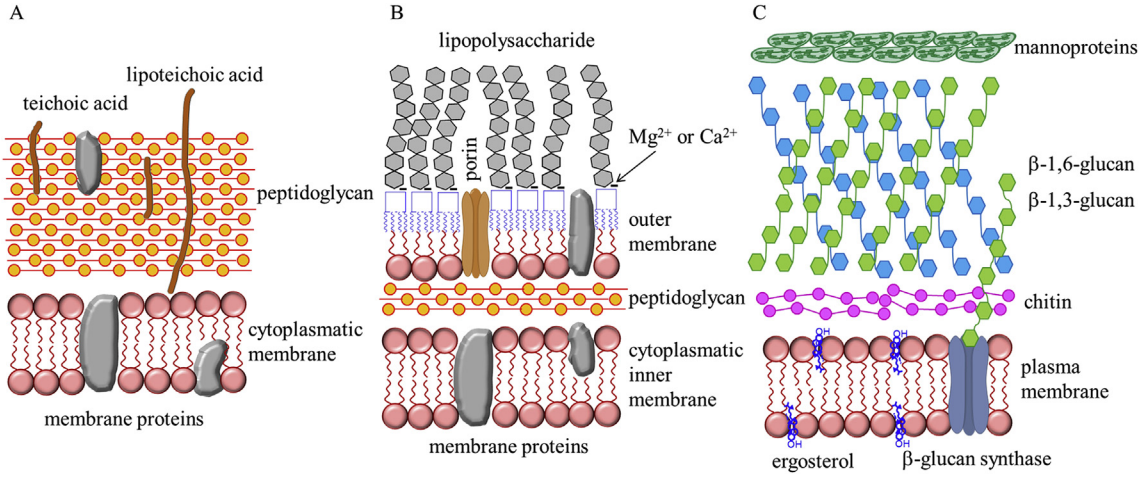

Fig. 3. Schematic representations of the cell envelope of (A) Gram-positive, (B) Gram-negative bacteria and (C) yeasts.

microorganisms [20]. They envisaged a design that involved the substitution of the PS with cationic quaternary ammonium salt functional groups (Fig. 4). Bis-ammonium salt can impart sufficient water solubility without the requirement of additional solubilizing agents and the amphiphilic nature of the PS can promote microbial cellular uptake. The inclusion of bromine atoms at the β-pyrrole positions of **1** leads to elevated ROS generation. Parallel to **1**, the structurally related nonbrominated analogue **2** was used for examination of the cellular uptake and for fluorescence imaging because of its expected superior fluorescence emission.

Caruso *et* al. proposed two cationic BODIPY derivatives **3** and **4** as PSs to inactivate bacteria (Fig. 5) [20]. These BODIPYs are both characterized by the presence of one pyridinium cationic group on position 8 and two iodine atoms at the 2,6-positions of the dipyrrolylmethene structure. Thus, these structures ensure solubility in 1/1 water/organic solvent mixture and a good ROS formation rate [20]. Both PSs differ in the moiety attached on the nitrogen atom of the pyridine, whereas **3** carries a methyl group and compound **4** has a benzyl group.

In 2015, Rice *et* al. reported the synthesis of two structurally related BODIPYs **5** and **6**, which were conjugates of a zinc(II)-dipicolylamine (Fig. 6) [24]. Thus, two BODIPYs analogues were obtained, a fluorescent probe agent **5** and a PS analogue **6**. Both compounds have intrinsic positive charges achieved by their complexation with Zn(II), which improved the binding of the PS agents to the microbial cells. The main difference lies in the fact that **6** have halogen atoms at the 2,6- positions of the BODIPY core that confers it a good generation of ROS, while **5** can be used as fluorescent probe.

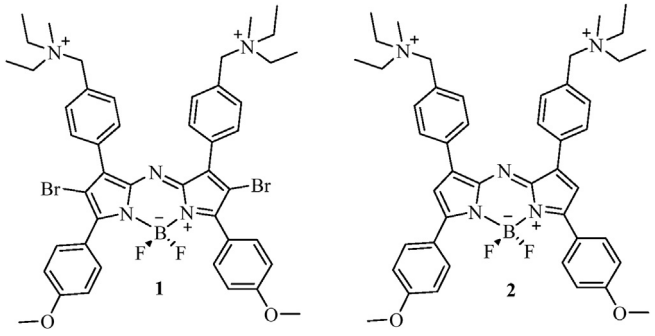

Fig. 4. Structures of BODIPYs **1** and **2.**

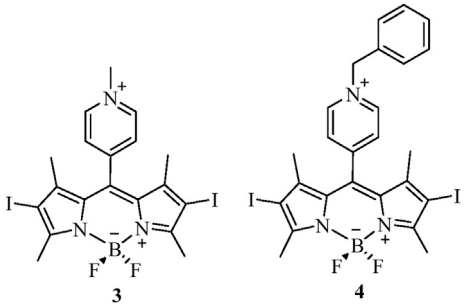

Fig. 5. Structures of BODIPYs **3** and **4.**

Lately, Durantini *et* al. disclosed the preparation of the BODIPYs **7** (Fig. 7) [25]. Compound **7** is based on a two segment molecule, a trap and a PS. The PS moiety consists of a Br-substituted BODIPY dye. The decoration of the heterocyclic skeleton with bromine substituents ensured rapid intersystem crossing from the singlet to the triplet excited state, favoring the formation of ROS. The trap segment is consisted by the chromanol ring of α-tocopherol, the most potent antioxidant found in the nature. The combination of an antioxidant with a potential ROS producer initially dormant, enables the autocatalytic activation of singlet molecular oxygen, $O_2(^1\Delta_g)$, sensitization. Thus, ROS-mediated reaction of chromanol moiety generates **8**, which provide a chemical cue for the spatiotemporal control of $O_2(^1\Delta_g)$ production. Moreover, BODIPYs based PSs **9** and **10** (Fig. 8) were synthetized featuring an acetoxymethyl substituent at the *meso*-position [26]. These PSs showed improved photostability against $O_2(^1\Delta_g)$, when compared to a BODIPY lacking the acetoxymethyl group.

Recently, Agazzi *et* al. reported the synthesis of two cationic BODIPYs **11** and **12** (Fig. 9) [27]. The presence of cationic substituents can modulate the amphiphilic character of these compounds, which increases the solubility of BODIPYs in the biological environment. This effect also enhances the binding and penetration into the microbial cells. Compound **11** contains a *N*,*N*,*N*-trimethylamino substituent directly attached to the phenylene unit and four methyl groups at the 1,3,5 and 7 positions of the *s*-indacene ring. These β-substitutions impact on the rotation of the *meso*-phenyl ring. In contrast, in compound **12** the cationic center is isolated from the BODIPY structure by an aliphatic chain. Thus, this charge has negligible effect on the electronic density of the BODIPY core. This helps to retain the consistency of the photophysical properties of the PS [55]. Also, the propoxy bridge provides a higher mobility of the charge, which could facilitate the interaction with the cell envelope of microorganisms.

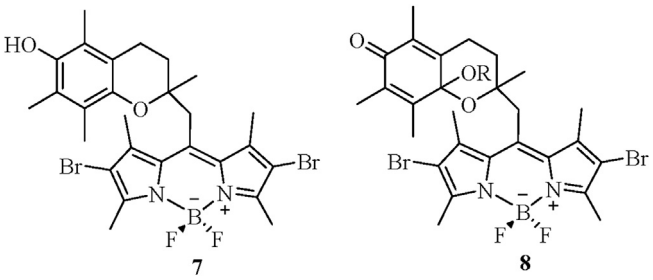

Fig. 7. Structures of BODIPYs **7** and **8.**

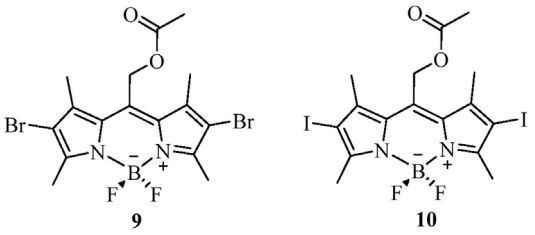

Fig. 8. Structures of BODIPYs **9** and **10.**

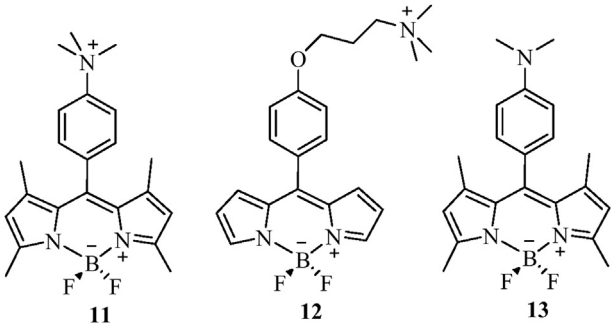

Fig. 9. Structures of BODIPYs **11**–**13.**

4. Absorption and fluorescence spectroscopic properties of BODIPYs

The spectroscopic properties of BODIPYs **1**–**12** are compiled in Table 1. The main absorption band of BODIPYs was attributed to the 0-0 vibrational band of a strong $S_0 \rightarrow S_1$ transitions with a high molar extinction coefficients (~$10^5\ M^{-1}\ cm^{-1}$) [56,57]. The absorption spectra of BF_2-chelated tetraarylazadipyrromethenes **1** and **2** showed wavelengths of maximum absorbance shifted to the red end of the spectrum by ~200 nm respect to non-substituted BODIPY core [20]. The effect of iodine atoms at the 2,6-positions in compounds **3**, **4**, **6** and **10** produced a bathochromic shift of ~50 nm [21,24,26]. This shift was also observed with bromine atoms in compounds **7** and **9** but the effect was less pronounced that for

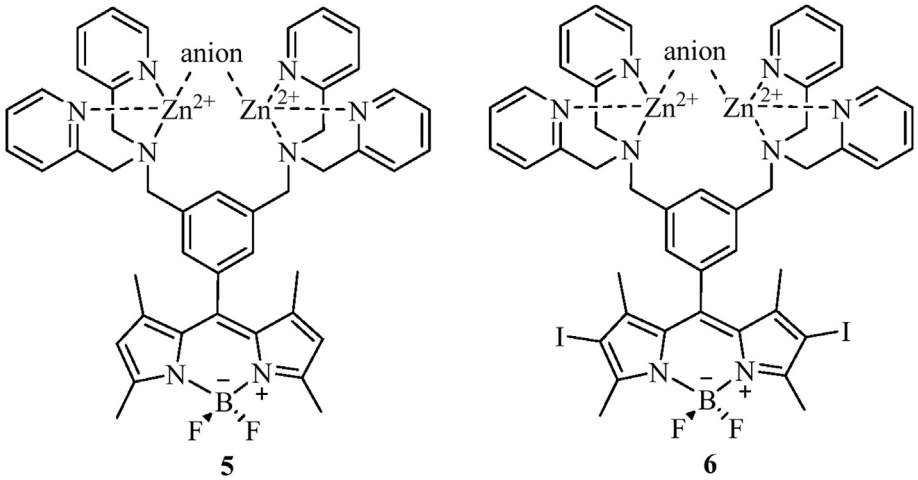

Fig. 6. Structures of BODIPYs **5** and **6.**

iodine atoms [25,26]. The absorption spectra of **11** and **12** are typical of *meso*-substituted BODIPYs [58,59]. In the absence of structural modifications, the theoretical calculations of these BODIPYs predict that the lowest-lying $S_0 \rightarrow S_1$ excitation is almost exclusively associated with the HOMO → LUMO transition of the π -system (Fig. 10) [60,61].

The absorption and fluorescence emission spectra of BODIPYs are almost mirror images of each other, which disclose that the emitting and absorbing species are similar [1]. This emission band was assigned to the 0-0 vibrational band of the $S_1 \rightarrow S_0$ electronic transition. According with their absorption spectra, BF_2-chelated tetraarylazadipyrromethenes **1** and **2** showed red fluorescence, while BODIPYs **3**–**12** produced green fluorescence emission (Table 1). Fluorescence quantum yield (Φ_F) decreases in brominated compounds **1** and **9** when compared with the unsubstituted molecules [20,26]. A considerable drop in the Φ_F values was observed in compounds **3**, **4**, **6** and **10**, which are substituted by iodine atoms [21,24,26]. This decrease in Φ_F was attributed to the heavy atom effect that facilitates intersystem crossing (ISC) processes. Similar low fluorescence was found with brominated compound **7**, but here the low fluorescence is mainly due to photoinduced electron transfer (PeT) [25]. Moreover, the Φ_F value for BODIPY **11** was similar to that previously reported for its homologue protonated **13** (Fig. 9) [62]. At neutral pH, the amine group acts as an electron donor and the BODIPY core acts as an acceptor producing a reductive-PeT (a-PeT, Fig. 11) [3,63–67]. Thus, methylation of amine group in BODIPY **11** avoids a-PeT and the fluorescence considerably increases in this fluorophore. The low fluorescence observed in BODIPY **12** can be due to a-PeT (Fig. 11) and the free rotation of the *meso*-substituents [68,69].

5. Photodynamic activity of BODIPYs

Essentially, PDI requires the interaction of a PS, light and molecular oxygen [39]. The excitation of PS ground state (S_0) with visible light leads to the population of the singlet excited state (S_1) (Fig. 12). The lifetime of this intermediate state is relatively short (bellow the nanosecond). In addition to losing energy by fluorescence emission or internal conversion, the S_1 can undergo rapidly ISC to the long-lived (tens to hundreds of microseconds) excited triplet state (T_1). In presence of molecular oxygen, the T_1 can produce ROS. This process can involve energy transfer to form $O_2(^1\Delta_g)$ (type II pathway) or electron transfer to generate superoxide anion radical ($O_2^{\bullet -}$) (type I pathway) [70]. Also, $O_2^{\bullet -}$ can produce hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) [71,72]. The electron transfer process (type I) mainly occurs in a polar microenvironment and in the presence of reducing agents [73,74].

Table 1
Photophysical properties of BODIPY derivatives.

BODIPY	λ_{max}^{Abs} (nm)	λ_{max}^{em} (nm)	Φ_F	Φ_Δ
1[a]	681	722	0.10	0.47[b]
2[a]	702	732	0.22	–
3[c]	546	590	0.09	0.15[d]
4[c]	548	595	0.18	0.06[d]
5[e]	499	520	0.53	–
6[e]	542	–	<0.01	0.57
7[f]	532	557	≤0.008	–
8[f]	–	–	–	0.68
9[g]	543	562	0.14	0.84
10[g]	550	572	0.02	0.98
11[h]	502	513	0.29	0.07
12[h]	498	512	0.03	0.03

[a] From Ref. [20].
[b] From Ref. [20] considering $\Phi_\Delta = 0.52$ for methylene blue according to ref. [75].
[c] From Ref. [20].
[d] From Ref. [20] considering $\Phi_\Delta = 0.76$ for Rose Bengal according to ref. [76].
[e] From Ref. [24].
[f] From Ref. [25].
[g] From Ref. [26].
[h] From Ref. [27].

Nagano and coworkers was the first to decorate a BODIPY core with heavy atoms, iodine in this case [19]. However, there is no evidence of the use of this PS in photodynamic inactivation of bacteria. O'Shea and coworkers examined the PDI capability of an aza-BODIPY analog presenting a bis-cationic structure [20]. Thus, $O_2(^1\Delta_g)$ production sensitized by compounds **1** and **2** was investigated in presence of 1,3-diphenylbenzofuran (DPBF) a well-known $O_2(^1\Delta_g)$ scavenger. The efficiency to generate $O_2(^1\Delta_g)$ by **1** was in par with that of methylene blue (MB) mainly due to the presence of two heavy atoms [75]. This heavy-atom effect indicates the potential use of **1** as a photodynamic agent and **2** as a near-infrared fluorescence imaging probe. It was Caruso *et* al. who paved the way for the PDI of microorganism using modified BODIPY dyes **3** and **4** as PS [20]. The generation of $O_2(^1\Delta_g)$ was monitored following the disappearance of DPBF. The results were reported as relative $O_2(^1\Delta_g)$ generation rates normalized with respect to Rose Bengal (RB). The quantum yield of $O_2(^1\Delta_g)$ generation (Φ_Δ) for compounds **3** and **4** (Table 1) were estimated, considering a $O_2(^1\Delta_g)$ quantum yield of 0.76 for RB in isopropanol according to Schmidt et al. [76]. The presence of the cationic group in compounds **3** and **4** decreases the generation of $O_2(^1\Delta_g)$. The methylated derivative **3** is about ten times less effective than its uncharged analogue and twice more roductive than the benzylated compound **4**. Following the halogenated cationic BODIPYs saga, Rise et al. designed and synthetized a BODIPY core adorned with iodine atoms at the 2,6-positions and bearing a zinc(II)-dipicolylamine coordination complex at the *meso* position [24]. Thus, compound **6** yielded a $O_2(^1\Delta_g)$ generation that lines up with MB with good photostability.

As mentioned above, Durantini et al. developed a two segment PS-trap molecule, combining the concepts of autocatalysis and activatibility, with a quenching mechanism that relies on PeT. Upon oxidation of the chromanol moiety by ROS the initially dormant PS **7** starts generating $O_2(^1\Delta_g)$ in an autocatalytic manner, achieving a high yield of $O_2(^1\Delta_g)$ production (**8**, Table 1). They also prepared a set of BODIPY dyes bearing heavy atoms such as bromine and iodine at the 2,6-positions (compounds **9** and **10**, Table 1) [25]. These compounds exhibited a triplet state lifetime up to 60 μs and a $O_2(^1\Delta_g)$ quantum yield close to unity determined directly from $O_2(^1\Delta_g)$ phosphorescence studies. Following this work, Lincoln et al. demonstrated the superior photostability and resilience toward $O_2(^1\Delta_g)$ itself of PSs **9** and **10** [26].

However, halogenation of the BODIPY core is not the only pathway toward triplet sensitization. Agazzi *et* al. evaluated two cationic BODIPY **11** and **12**, which induce photosensitized oxidation of DPBF with low yields of $O_2(^1\Delta_g)$ (Table 1) [27]. Nevertheless, the photodynamic activity increases in a microheterogenic medium formed by AOT reverse micelles. Localization of BODIPYs in the micellar interface decrease the vibrational decay, favoring the photosensitization of $O_2(^1\Delta_g)$. Moreover, both BODIPYs were able to produce a rapid photooxidation of L-tryptophan (Trp). A significant contribution of type I mechanism was found for photodecomposition of Trp by BODIPYs **11** and **12**. Moreover, the addition of KI increases the photoinduced oxidation of Trp. Iodide anions can increase the formation of triplet excited state by external heavy-atom effect [77,78]. Moreover, the formation of reactive iodine species can be formed in this system.

6. BODIPYs for antimicrobial photoinactivation

This section summarizes and highlights the antimicrobial

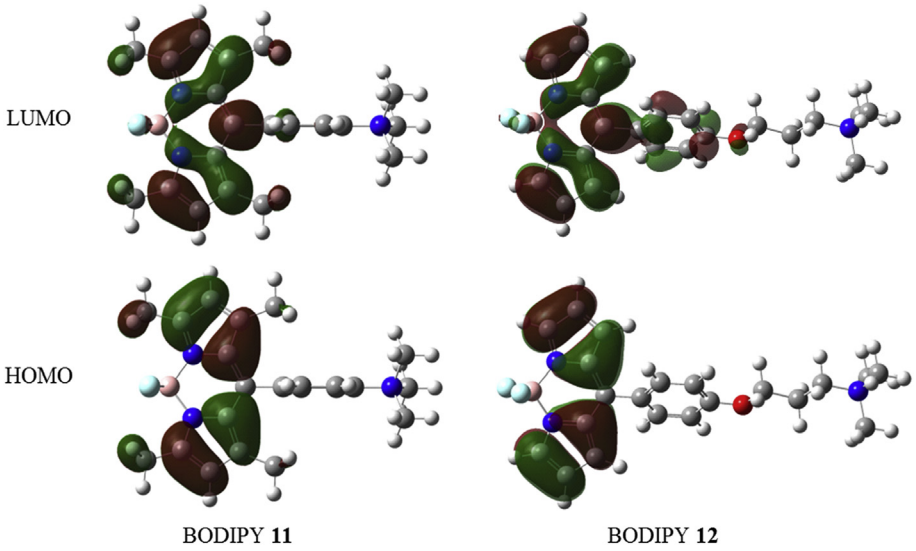

Fig. 10. The calculated HOMO, LUMO, difference density and transition density of BODIPYs **11** and **12**. Calculated by DFT at the B3LYP/6-31G(d) level using Gaussian 09.

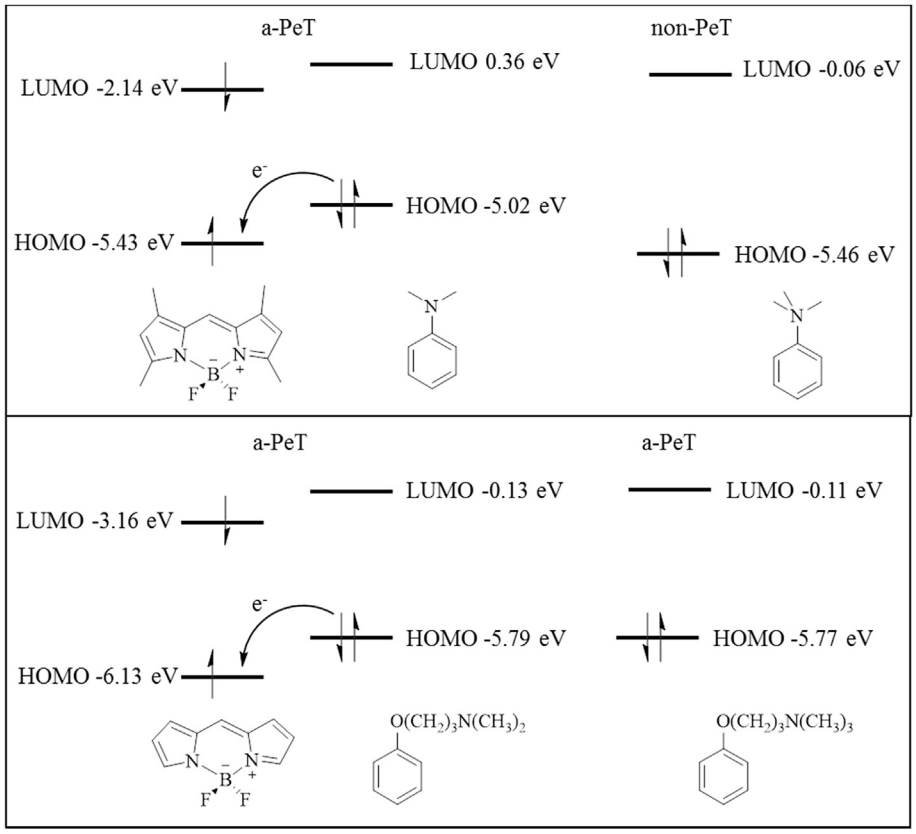

Fig. 11. Schematic molecular orbital diagram of HOMO and LUMO energy levels of exited state of BODIPY core and grown state of benzene moiety for compounds **11** and **12** and their uncharged models. BODIPY derivatives were divided into two parts and calculated by DFT at the B3LYP/6-31G(d) level using Gaussian 09.

photoinactivation capability of BODIPYs based PSs obtained by several research groups. A bis-cationic BF_2-chelated tetraarylazadipyrromethene **1** was proposed as PS with a broad potential as antimicrobial photodynamic therapeutic agent antimicrobial. Compound **1** was capable of eradicating several pathogens [20]. Photodynamic activity was evaluated in *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. coli* and *C. albicans* strains. A rapid 10 min uptake of this compound class into bacterial and fungal strains was demonstrated using a related nonbrominated fluorescent analogue **2**. Light activation with a dose of 16 J/cm^2 and 4.3 μM **1** resulted in a 6.8 and 3.4 log reduction of *S. aureus* and MRSA, respectively (Table 2). Moreover, increasing the light dose to 75 J/cm^2, it was observed 3.6 and 5.7 log decreases in viable cell numbers achievable for *E. coli* and *C. albicans*, respectively (Table 2).

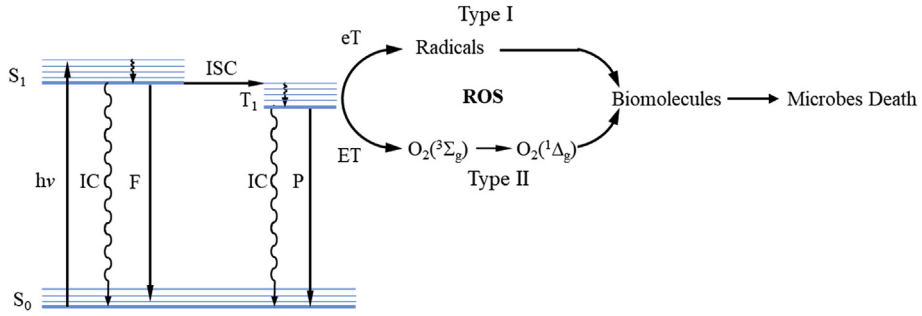

Fig. 12. Representation of type I (electron transfer, eT) and type II (energy transfer, ET) mechanisms after photoexcitation.

Therefore, PS **1** was highly phototoxic to various strains of pathogens, including the Gram-negative strain that is generally more resistant to the effects of photodynamic antibacterial therapy.

The antimicrobial capacity of two cationic BODIPYs **3** and **4** was also assessed against the Gram-positive *Staphylococcus xylosus* and the Gram-negative *E. coli* [20]. Even when there is a small structural modification between **3** and **4**, a significant difference in the photoinactivation efficacy was observed against these microorganisms. Compound **3**, bearing a methyl group attached to the quaternary amine of the pyridine ring, was more effective than the benzylated one **4**. Looking forward into these results, the author decided to perform a deeper examination, evaluating only the antibacterial activity of compound **3**. After irradiation with green light at 1.38 J/cm^2, the therapeutic treatment was very effective throwing values above 6 log units at low concentrations, particularly for *S. xylosus* where the concentration was 0.5 μM (Table 2). However, the concentration necessary to eliminate *E. coli* was one order of magnitude higher, indicating that the phototoxic activity of BODIPY **3** depends on its concentration and on light dose. Thus, these experimental conditions can be controlled to produce the eradication of the microorganisms.

Table 2
PDI of microorganisms mediated by BODIPY derivatives.

BODIPYs	Microbe	[PS] (μM)	Cell density (CFU/mL)	PDI
1	*S. aureus*	4.3	10^8	6.8 log[a]
1	*S. aureus* (MRSA)	4.3	10^8	3.4 log[a]
1	*E. coli*	4.3	10^8	3.6 log[a]
1	*C. albicans*	4.3	10^8	5.7 log[a]
3	*S. xylosus*	0.5	10^8	6 log[b]
3	*E. coli*	5.0	10^8	6 log[b]
3	*P. aeruginosa*	2.5	10^8	7 log[c]
6	*E. coli*	10.0	10^6	3.5 log[d]
6	*S. aureus*	10.0	10^6	4.5 log[d]
6	*K. pneumoniae*	10.0	10^6	2.2 log[d]
6	*B. thuriengiensis*	10.0	10^6	4.5 log[d]
7	*E. coli*	10.0	10^6	5.5 log[e]
8	*E. coli*	10.0	10^6	5.5 log[e]
9	*E. coli*	10.0	10^6	5.5 log[f]
10	*E. coli*	10.0	10^6	5.5 log[f]
11	*S. aureus*	1.0	10^8	5.0 log[g] (5.0)[h]
11	*E. coli*	5.0	10^8	2.5 log[g] (3.5)[h]
11	*C. albicans*	5.0	10^6	3.7 log[g] (5.5)[h]
12	*S. aureus*	1.0	10^8	4.5 log[g] (4.5)[h]
12	*E. coli*	5.0	10^8	2.5 log[g] (6.5)[h]
12	*C. albicans*	5.0	10^6	0.9 log[g] (3.5)[h]

[a] From Ref. [20] (irradiation with 690 nm light, *S. aureus* 16 J/cm^2, *E. coli* and *C. albicans* 75 J/cm^2).
[b] From Ref. [20] (irradiation with a green LED device, 1.38 J/cm^2).
[c] From Ref. [22] (irradiation with a halogen lamp, 171 J/cm^2).
[d] From Ref. [24] (irradiation with green light, 180 J/cm^2).
[e] From Ref. [25] (ROS-stressed bacteria, irradiation with 520 nm light, 9.36 J/cm^2).
[f] From Ref. [26] (irradiation with 520 nm light, 3.6 J/cm^2).
[g] From Ref. [27] (irradiated with visible light, *S. aureus* 21 J/cm^2, *E. coli* 63 J/cm^2, *C. albicans* 126 J/cm^2).
[h] From Ref. [84] (idem to g in the presence of 50 mM KI).

Furthermore, Orlandi *et* al. decided to keep working with BODIPY **3** and in a later publication they found that this PS was also effective against bacterial suspensions of *Pseudomonas aeruginosa*, responsible of several respiratory infections. Irradiating the antimicrobial agent with 171 J/cm^2 from a halogen lamp caused a 7 log unit reduction of viable cells treated with 2.5 μM BODIPY **3** [22]. In contrast, in oxygen deficient environment the decrease was less than half (~3 log reduction). The photokilling activity of BODIPY **3** was also evaluated against *P. aeruginosa* biofilms grown in flow-cells and microtiter trays. In a static biofilm model, after PDI no biomass depletion was detected independently of the BODIPY **3** concentration (2.5–80 μM). On the contrary, 24 h after the treatment a significant biomass reduction was determined in samples exposed to BODIPY **3** (≥5 μM) and irradiation. A decrease in cell viability due to PDI was observed with high BODIPY **3** concentration (≥20 μM). On the other hand, no effect was found when PDI was applied to biofilms grown in flow-cells with 40 μM BODIPY **3** and a light intensity of 171 J/cm^2. However, a substantial increase in propidium iodide stained dead cells in the flowcell-grown biofilms was found using 513 J/cm^2. Thus, this result suggested that the treatment mediated by compound **3** has a biocidal effect against bacterial biofilm cells.

Antimicrobial PDI sensitized by BODIPY **3** was investigated against drug-resistant bacteria, pathogenic yeast and model viruses [23]. After irradiation with visible light (400–700 nm, 118 J/cm^2), it was observed 5–6 log decrease in cell survival for *S. aureus* (0.1 μM), methicillin-resistant *S. aureus* (0.1 μM) and vancomycin-resistant *Enterococcus faecium* (0.1 μM); a 4–5 log unit reduction for *Acinetobacter baumannii* (0.25 μM), multidrug resistant *A. baumannii* (0.1 μM), *Pseudomonas aeruginosa* (0.5 μM), and *Klebsiella pneumoniae* (1 μM); and a 3 log unit reduction for *Mycobacterium smegmatis* mc^{2}155. Moreover, a 5 log unit reduction in cell survival was found for *C. albicans* (1 μM) and *Cryptococcus neoformans* (0.5 μM), and a 3 log unit reduction was found for *Candida glabrata* (1 μM). Also, cell viability was decreased by 6 log in dengue 1 (0.1 μM), by 5 log (0.5 μM) in vesicular stomatitis virus, and by 2 log (5 μM) in human adenovirus-5. In general, BODIPY **3** was more active over the entire range of microbes studied than methylene blue or 5,10,15,20-tetrakis(4-*N*-methylpyridyl) porphyrin. Therefore, this study remarks the capacity of the BODIPYs as PSs for a broad spectrum of potential PDI applications.

Two structurally related optical probes BODIPYs **5** and **6** were studied in different microorganisms [24]. These compounds are conjugates of a zinc(II)-dipicolylamine targeting unit and a BODIPY chromophore. mSeek **5** was a microbial targeted fluorescent imaging agent, while mDestroy **6** was a $O_2(^1\Delta_g)$ photosensitizing analogue. Fluorescence imaging and detection studies of mSeek **5** were examined in bacterial strains of *E. coli*, *S. aureus*, *K. pneumonia*

and *B. thuringiensis* vegetative cells and purified spores. The fluorescent probe, mSeek **5**, was not phototoxic and enabled detection of all tested bacteria. The PS analogue, mDestroy **6**, inactivated 99–99.99% of bacterial samples (Table 2) and selectively killed bacterial cells in the presence of mammalian cells. However, mDestroy **6** was ineffective against *B. thuringiensis* spores. The results demonstrate a new two-probe strategy that can be used to optimize PDI in the treatments of bacterial infections.

On the other hand, a BODIPY derivative was designed to obtain a dormant $O_2(^1\Delta_g)$ PS that can be activated upon its reaction with reactive ROS [25]. This structure was successfully tested via the PDI of a ROS stressed *E. coli* strain. Thus, compound **7** was evaluated toward the selective photoinactivation of ROS-stressed *vs* regular bacteria. Cells were stressed using hydrogen peroxide, known to stimulate ROS production in Gram-negative cells. A drastic drop in colony forming units was recorded following the combined action of compound **7** and irradiation for stressed cells (Table 2). No inactivation was observed in turn for nonstressed healthy cells. A control experiment where cells were treated with preactivated **7** (compound **8**, Fig. 7) and light but no hydrogen peroxide also showed a drastic drop in CFU. The usefulness of this approach to selectively photoactivate the production of $O_2(^1\Delta_g)$ in ROS stressed *vs* regular cells was successfully tested via the photodynamic inactivation of a ROS stressed Gram negative *E. coli* strain. Also, BODIPYs **9** and **10** were evaluated for PDI against *E. coli* bacteria [26]. The results showed no bactericidal activity at 5 μM during 30 min irradiation period. At a slightly higher concentration (7.5 μM) no significant increase of cell lethality was observed. The photodynamic minimal bactericidal concentration was reached at 10 μM (Table 2). BODIPYs **9** and **10** were similarly effective to photoinactivate *E. coli*.

The photokilling activity sensitized by BODIPYs **11** and **12** were studied on *S. aureus*, *E. coli* and *C. albicans* [27]. *In vitro* experiments indicated that these BODIPYs were rapidly bound to microbial cells at short incubation periods (<5 min). The uptake of PSs by microbial cells is an important feature for the efficacy of photoinactivation [79,80]. Also, fluorescence microscopy images showed the green emission of the BODIPYs bound to microbial cells (Fig. 13). As mentioned above, the Φ_F value in solution of BODIPY **12** was about one order of magnitude lower than BODIPY **11**. However, the fluorescence intensity of BODIPYs **11** and **12** bound to the cells did not show differences between both BODIPYs (Fig. 13). This behavior may occurs because the reduction of the rotation substituent group in environment more viscous, as surrounding the microbial cell [81]. In general, the PS affinity is consequently accompanied by an increase in the photocytotoxic activity [82,83]. Similar photoinactivation activity was found for both BODIPYs in bacteria (Table 2). *S. aureus* cell suspensions treated with 1 μM BODIPYs produced an over 4.5 log decrease in the viability after 5 min irradiation. Under these conditions, complete eradication was found after 15 min irradiation with compounds **11** or **12**. A reduction of 2.5 log in the *E. coli* viability was found using 5 μM PSs and 15 min irradiation. A decrease of 4.5 log was observed for both BODIPYs after 30 min irradiation [84]. As expected, due to the nature of the envelope of Gram-negative bacteria, it was more difficult to inactivate *E. coli* than *S. aureus*. Furthermore, treatment of *C. albicans* with BODIPY **11** was significantly more effective than **12**. Yeast cell suspensions incubated with 5 μM BODIPY **11** produced a decrease of ~4 log after 30 min irradiation. In contrast, the photoinactivation remained low (<1 log) using BODIPY **12** even at the time of longer irradiation. Moreover, the heavy atom iodine was externally incorporated as an inert salt [84]. A smaller effect was observed in *S. aureus* because these PSs alone were already very effective (Table 2). In *E. coli* and *C. albicans*, the effect of KI produced an increase in photokilling. Photoinactivation of yeast cells treated

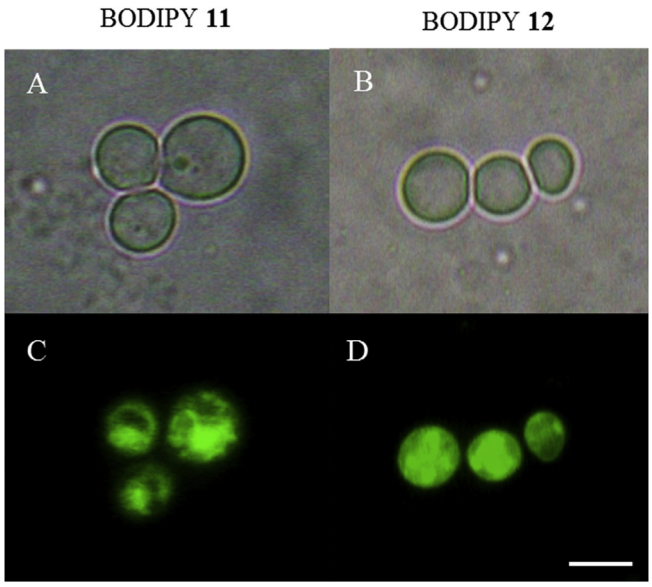

Fig. 13. Microscopic observations of *C. albicans* cells treated with BODIPY **11** or **12**, (A) and (B) cells under bright field, (C) and (D) fluorescence images (100×microscope objective, scala bar 4 μm).

with KI and BODIPYs **11** or **12** produced a 5.5 log and 3.5 log decrease after 30 min irradiation, respectively. The addition of KI was also used to increase the photoinactivation of microorganisms mediated by effective photosensitizers, such as MB and fullerene derivatives [85,86]. However, this salt can be added to enhance the photokilling of microbial cells mediated by BODIPYs that have a low production of $O_2(^1\Delta_g)$. This potentiation in the presence of the inorganic salt may be mainly due to the increase in the formation of excited triplet states of BODIPYs by intersystem crossing through the effect of the external heavy atom. Thus, an increase in ROS production may be expected together with generation of reactive iodine species, which enhance the cell damage. In aqueous media, the reaction of iodide anions and $O_2(^1\Delta_g)$ results in the production of triiodide anions (I_3^-) [87,88]. In this process hydrogen peroxide (H_2O_2) is formed, which can react further with iodide anions to generate molecular iodine (I_2). In presence of an excess of iodide anions, the iodine atoms produces I_3^-. In this system, other short-lived reactive iodine species, such as iodine atoms (I•) and iodine radical anions ($I_2^{\bullet-}$) could also be generated [89,90]. The I• formed in the above process can react with I^- to produce $I_2^{\bullet-}$. The $I_2^{\bullet}$ intermediates are unstable and are involved in disproportionation reaction, yielding I_3^- and I^- [91]. Therefore, the interaction of the ROS and KI during light exposure, biocidal I_2 or I_3^- can be formed improving bacterial inactivation [92]. Therefore, KI can be also used to enhance the photokilling of microorganisms induced by BODIPYs that have a low production of $O_2(^1\Delta_g)$.

At the present, there has been a very large number of natural and synthetic PSs with interesting applications in PDI of microorganisms [30,38]. However, several factors need to be considered when trying to compare the relative activity of completely different PS structures in PDI. A recent review of Hamblin and co-workers contains an interesting compilation of PDI with different PSs [30]. The most well-known class of relevant PSs is tetrapyrrolic macrocycle derivatives [49]. In particular, BODIPYs can be considered as dipyrrolic compounds, maybe similar to half a porphyrin. Thus, the photodynamic activity mediated by BODIPYs **11** or **12** can be directly compared with those of other cationic PSs under similar experimental conditions and using the same microbial strain. In presence of 50 mM KI, the photoinactivation sensitized by BODIPY

11 was higher than that produced by 5,10,15,20-tetrakis[4-(3-*N*,*N*-dimethylaminopropoxy)phenyl]porphyrin (TAPP) in *S. aureus* and *C. albicans*, while the photokilling effect was similar in *E. coli* [46]. However, BODIPY **12** was more effective to inactivate *E. coli* reaching a value higher than 6 log decrease, which was similar to that obtained with 5,10,15,20-tetrakis[4-(3-*N*,*N*-dimethylaminopropoxy)phenyl]chlorin (TAPC). Also, BODIPY **11** was as effective as TAPC to inactivate *C. albicans* [83]. Likewise, these both BODIPYs were more photoactive in inactivating *S. aureus* than *N*,*N*-dimethyl-2-[4-(3-*N*,*N*,*N*-trimethylammoniopropoxy)phenyl]fulleropyrrolidinium (DPC_{60}^{2+}), which contain a similar substituent than BODIPY **12** [73,74].

7. Conclusions and future directions

The organic synthesis offers an enormous versatility in the design of new BODIPYs derivatives with defined and optimized properties for the required application in PDI. In this context, an unlimited number of derivatives can be proposed and prepared without excessive synthetic efforts. However, only a few studies exhibit real application of BODIPYs as antimicrobial PSs. This can be explained by taking into account that most of the derivatives reported have been prepared thinking in others applications and do not have suitable characteristics for their use in PDI. A key factor to improve the efficacy of BODIPY derivatives in PDI involves optimizing the light absorption in the visible region, a long-lived electronically excited triplet state to efficiently produce ROS in a short period of illumination time, and decrease the lipophilicity of the resulting of the *s*-indacene ring for a better transport in biological fluids and cell penetration.

After an analysis of the literature, we can summarize some guidelines that should be considered to apply BODIPYs in PDI. These PSs have high extinction coefficients in the visible region with absorption maxima at ~500 nm. However, red light activation can be required in those situations to obtain deep light penetration into tissue. Several strategies can be used to achieve the required electronic transitions in this range (650–800 nm). In these cases, the α positions of both pyrrole units can be substituted to extend the conjugation of the π system of the *s*-indacene core. In that sense, the Knoevenagel condensation has been employed to incorporate styryl moieties. This can be used together with the expansion of the BODIPY core via fused aromatic rings, which ensure a significant bathochromic shift. Other strategies involve the introduction of electron donating groups in the α positions, the modification of the *meso* position introducing a nitrogen atom, which affords aza-BODIPY derivatives and the attachment of aryl groups or heterocycles to extend the conjugation. These modification strategies generate a bathochromic effect by decreasing the energy gap between the HOMO and LUMO of the product.

Another disadvantage of these heterocycles is their high fluorescence quantum yields that hinders the generation of ROS. An attractive way of favoring the ISC is by decorating the boron dipyrromethene core with heavy atoms. This strategy enhances the transition stage from singlet to the triplet excited state and, subsequently, it improves the production of ROS. Therefore, the introduction of halogen atoms directly attached to both pyrrole units, most commonly bromine and iodine, is the most frequently used choice for this propose. On the other hand, the yield of triplet excited state of halogen-free BODIPY can be favoured by external addition of the heavy atoms. Also, the formation of BODIPY dimers can be used to increase the formation of exited triplet state. Another possibility is the formation of dyads, in which the structure of the BODIPY can act as a light-harvesting antenna. Thus, switch between a high fluorescence intensity of BODIPY for diagnostic imaging and efficient photodynamic effect for antimicrobial activity can be a very interesting approach in the future.

The third characteristic which limits the application of BODIPYs in PDI refers to their low aqueous solubility and poor membrane permeability. However, this is a less difficult problem, since the versatile core of the BODIPYs can be subjected to the simple synthetic procedures to enhance its hydrophilicity. In addition, BODIPY derivatives can be designed to have an amphiphilic nature. The combination of the hydrophobic core and intrinsic positive charges (or cationic center precursors) synthetically added in the structure of the PS, results in the formation of an axis of intramolecular polarity. This effect facilitates the penetration of the cell membrane and produces a better accumulation of the sensitizer in subcellular compartments, improving the effectiveness of the photoinactivation. The cationic centers built in these molecules also increased their amphiphilic character, improving the binding of the PS to the microbial cells.

As demonstrated by the investigations to date discussed in this review, BODIPY derivatives can be used as effective PSs to eradicate a broad spectrum of microorganisms that threaten the global population health. Moreover, these compounds could be used in diagnostic or therapy, controlling the balance between the fluorescence emission and the photodynamic activity. There is still much work to be done in the search for ideal PSs with applications in PDI. However, the bases of the studies are underway and new investigations should be carried out to obtain more information about the mechanisms of photodynamic action, the site of cell damage and experiments *in vivo* using infection models.

Acknowledgements

Authors are grateful to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina. A.M.D., D.A.H., J.E.D. and E.N.D. are Scientific Members of CONICET.

References

[1] A. Treibs, F.H. Kreuzer, Difluorboryl-komplexe von di- and tripyrrylmethenen, Justus Liebigs Ann. Chem. 718 (1968) 208–223.
[2] F. López Arbeloa, J. Bañuelos, V. Martínez, T. Arbeloa, I. López Arbelos, Structural, photophysical and lasing properties of pyrromethene dyes, Inter. Rev. Phys. Chem. 24 (2005) 339–374.
[3] G. Ulrich, R. Ziessel, A. Harriman, The chemistry of fluorescent bodipy dyes: versatility unsurpassed, Angew. Chem. Int. Ed. Engl. 47 (2008) 1184–1201.
[4] F. Bergström, I. Mikhalyov, P. Hägglöf, R. Wortmann, T. Ny, L.B.-Å. Johansson, Dimers of dipyrromethene boron difluoride (BODIPY) with light spectroscopic applications in chemistry and biology, J. Am. Chem. Soc. 124 (2002) 196–204.
[5] N. Boens, V. Leen, W. Dehaen, Fluorescent indicators based on BODIPY, Chem. Soc. Rev. 41 (2012) 1130–1172.
[6] H. Zhu, J. Fan, J. Wang, H. Mu, X. Peng, An "Enhanced PET"-based fluorescent probe with ultrasensitivity for imaging basal and elesclomol-induced HClO in cancer cells, J. Am. Chem. Soc. 136 (2014) 12820–12823.
[7] T. Gayathri, A.K. Barui, S. Prashanthi, C.R. Patra, S.P. Singh, *meso*-Substituted BODIPY fluorescent probes for cellular bio-imaging and anticancer activity, RSC Adv 4 (2014) 47409–47413.
[8] Y. Chang, S. Zhou, E. Li, W. Zhao, Y. Ji, X. Wen, H. Sun, H. Yuan, Fragment-based discovery of novel pentacyclic triterpenoid derivatives as cholesteryl ester transfer protein inhibitors, Eur. J. Med. Chem. 126 (2017) 143–153.
[9] M. Blaess, N. Bibak, R.A. Claus, M. Kohl, G.A. Bonaterra, R. Kinscherf, S. Laufer, H.-P. Deigner, NB 06: from a simple lysosomotropic aSMase inhibitor to tools for elucidating the role of lysosomes in signaling apoptosis and LPS-induced inflammation, Eur. J. Med. Chem. (2017), https://doi.org/10.1016/j.ejmech.2017.09.021.
[10] T.K. Khan, M. Broring, S. Mathur, M. Ravikanth, Boron dipyrrin-porphyrin conjugates, Coord. Chem. Rev. 257 (2013) 2348–2387.
[11] M. Benstead, G.H. Mehl, R.W. Boyle, 4,4′-Difluoro-4-bora-3a,4a-diaza-*s*-indacenes (BODIPYs) as components of novel light active materials, Tetrahedron 67 (2011) 3573–3601.
[12] A. Bessette, G.S. Hanan, Design, synthesis and photophysical studies of dipyrromethene-based materials: insights into their applications in organic photovoltaic devices, Chem. Soc. Rev. 43 (2014) 3342–3405.
[13] A. Loudet, K. Burgess, BODIPY dyes and their derivatives: syntheses and spectroscopic properties, Chem. Rev. 107 (2007) 4891–4932.
[14] T.E. Wood, A. Thompson, Advances in the chemistry of dipyrrins and their complexes, Chem. Rev. 107 (2007) 1831–1861.

[15] A. Kamkaew, S.H. Lim, H.B. Lee, L.V. Kiew, L.Y. Chung, K. Burgess, BODIPY dyes in photodynamic therapy, Chem. Soc. Rev. 42 (2013) 77–88.
[16] S.G. Awuah, Y. You, Boron dipyrromethene (BODIPY)-based photosensitizers for photodynamic therapy, RSC Adv 2 (2012) 11169–11183.
[17] M. Dichiara, O. Prezzavento, A. Marrazzo, V. Pittalà, L. Salerno, A. Rescifina, E. Amata, Recent advances in drug discovery of phototherapeutic non-porphyrinic anticancer agents, Eur. J. Med. Chem. (2017), https://doi.org/10.1016/j.ejmech.2017.08.070.
[18] J. Zhao, K. Xu, W. Yang, Z. Wang, F. Zhong, The triplet excited state of Bodipy: formation, modulation and application, Chem. Soc. Rev. 44 (2015) 8904–8939.
[19] T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa, T. Nagano, Highly efficient and photostable photosensitizer based on BODIPY chromophore, J. Am. Chem. Soc. 127 (2005) 12162–12163.
[20] D.O. Frimannsson, M. Grossi, J. Murtagh, F. Paradisi, D.F. O'Shea, Light induced antimicrobial properties of a brominated boron difluoride (BF_2) chelated tetraarylazadipyrromethene photosensitizer, J. Med. Chem. 53 (2010) 7337–7343.
[21] E. Caruso, S. Banfi, P. Barbieri, B. Leva, V.T. Orlandi, Synthesis and antibacterial activity of novel cationic BODIPY photosensitizers, J. Photochem. Photobiol. B Biol. 114 (2012) 44–51.
[22] V.T. Orlandi, M. Rybtke, E. Caruso, S. Banfi, T. Tolker-Nielsen, P. Barbieria, Antimicrobial and anti-biofilm effect of a novel BODIPY photosensitizer against *Pseudomonas aeruginosa* PAO1, Biofouling 30 (2014) 883–891.
[23] B.L. Carpenter, X. Situ, F. Scholle, J. Bartelmess, W.W. Weare, R.A. Ghiladi, Antiviral, antifungal and antibacterial activities of a BODIPY-based photosensitizer, Molecules 20 (2015) 10604–10621.
[24] D.R. Rice, H. Gan, B.D. Smith, Bacterial imaging and photodynamic inactivation using zinc(II)-dipicolylamine BODIPY conjugates, Photochem. Photobiol. Sci. 14 (2015) 1271–1281.
[25] A.M. Durantini, L.E. Greene, R. Lincoln, S.R. Martínez, G. Cosa, Reactive oxygen species mediated activation of a dormant singlet oxygen photosensitizer: from autocatalytic singlet oxygen amplification to chemicontrolled photodynamic therapy, J. Am. Chem. Soc. 138 (2016) 1215–1225.
[26] R. Lincoln, A.M. Durantini, L.E. Greene, S.R. Martínez, R. Knox, M.C. Becerra, G. Cosa, *meso*-Acetoxymethyl BODIPY dyes for photodynamic therapy: improved photostability of singlet oxygen photosensitizers, Photochem. Photobiol. Sci. 16 (2017) 178–184.
[27] M.L. Agazzi, M.B. Ballatore, E. Reynoso, E.D. Quiroga, E.N. Durantini, Synthesis, spectroscopic properties and photodynamic activity of two cationic BODIPY derivatives with application in the photoinactivation of microorganisms, Eur. J. Med. Chem. 126 (2017) 116–121.
[28] Z. Dost, S. Atilgan, E.U. Akkaya, Distyryl-boradiazaindacenes: facile synthesis of novel near IR emitting fluorophores, Tetrahedron 62 (2006) 8484–8488.
[29] J. Chen, A. Burghart, A. Derecskei-Kovacs, K. Burgess, 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes modified for extended conjugation and restricted bond rotations, J. Org. Chem. 65 (2000) 2900–2906.
[30] R. Yin, M.R. Hamblin, Antimicrobial photosensitizers: drug discovery under the spotlight, Curr. Med. Chem. 22 (2015) 2159–2185.
[31] A. Nigam, D. Gupta, A. Sharma, Treatment of infectious disease: beyond antibiotics, Microbiol. Res. 169 (2014) 643–651.
[32] U. Theuretzbacher, Global antibacterial resistance: the never-ending story, J. Glob. Antimicrob. Resist. 1 (2013) 63–69.
[33] X.-Z. Li, P. Plésiat, H. Nikaido, The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria, Clin. Microbiol. Rev. 28 (2015) 337–418.
[34] G. Criseo, F. Scordino, O. Romeo, Current methods for identifying clinically important cryptic *Candida* species, J. Microbiol. Meth. 111 (2015) 50–56.
[35] G. Morace, F. Perdoni, E. Borghi, Antifungal drug resistance in *Candida* species, J. Glob. Antimicrob. Resist 2 (2014) 254–259.
[36] E.N. Durantini, New insights into the antimicrobial blue light inactivation of *Candida albicans*, Virulence 7 (2016) 493–494.
[37] Y. Tong, J. Tang, *Candida albicans* infection and intestinal immunity, Microbiol. Res. 198 (2017) 27–35.
[38] E. Alves, M.A. Faustino, M.G. Neves, A. Cunha, J. Tome, A. Almeida, An insight on bacterial cellular targets of photodynamic inactivation, Fut. Med. Chem. 6 (2014) 141–164.
[39] M.R. Hamblin, Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes, Curr. Opin. Microbiol. 33 (2016) 67–73.
[40] T. G.St Denis, T. Dai, L. Izikson, C. Astrakas, R.R. Anderson, M.R. Hamblin, G.P. Tegos, All you need is light: antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease, Virulence 2 (2011) 509–520.
[41] S.A.G. Lambrechts, M.C.G. Aalders, D.H. Langeveld-Klerks, Y. Khayali, J.W.M. Lagerberg, Effect of monovalent and divalent cations on the photoinactivation of bacteria with *meso*-substituted cationic porphyrins, Photochem. Photobiol. 79 (2004) 297–302.
[42] M. Salmon-Divon, Y. Nitzan, Z. Malik, Mechanistic aspect of *Escherichia coli* photodynamic inactivation by cationic tetra-*meso*(*N*-methylpyridyl)porphine, Photochem. Photobiol. Sci. 203 (2004) 423–429.
[43] E.N. Durantini, Photodynamic inactivation of bacteria, Curr. Bioact. Compd. 2 (2006) 127–142.
[44] T. Dai, Y.-Y. Huang, M.R. Hamblin, Photodynamic therapy for localized infections-State of the art, Photodiagn. Photodyn. Ther. 6 (2009) 170–188.
[45] N.A.R. Gow, B. Hube, Importance of the *Candida albicans* cell wall during commensalism and infection, Curr. Opin. Microbiol. 15 (2012) 406–412.
[46] D.D. Ferreyra, E. Reynoso, P. Cordero, M.B. Spesia, M.G. Alvarez, M.E. Milanesio, E.N. Durantini, Synthesis and properties of 5,10,15,20-tetrakis [4-(3-*N*,*N*-dimethylaminopropoxy)phenyl]chlorin as potential broad-spectrum antimicrobial photosensitizers, J. Photochem. Photobiol. B Biol. 158 (2016) 243–251.
[47] P.G. Calzavara-Pinton, M. Venturini, R. Sala, A comprehensive overview of photodynamic therapy in the treatment of superficial fungal infections of the skin, J. Photochem. Photobiol. B Biol. 78 (2005) 1–6.
[48] F. Pereira Gonzales, T. Maisch, Photodynamic inactivation for controlling *Candida albicans* infections, Fungal Biol. 116 (2012) 1–10.
[49] E. Alves, M.A.F. Faustino, M.G.P.M.S. Neves, Â. Cunha, H. Nadais, A. Almeida, Potential applications of porphyrins in photodynamic inactivation beyond the medical scope, J. Photochem. Photobiol. C Photochem. Rev. 22 (2015) 34–57.
[50] A. Martinez de Pinillos Bayona, P. Mroz, C. Thunshelle, M.R. Hamblin, Design features for optimization of tetrapyrrole macrocycles as antimicrobial and anticancer photosensitizers, Chem. Biol. Drug. Des 89 (2017) 192–206.
[51] L. Jiao, C. Yu, J. Li, Z. Wang, M. Wu, E. Hao, β-Formyl-BODIPYs from the Vilsmeier-Haack reaction, J. Org. Chem. 74 (2009) 7525–7528.
[52] Y.-C. Lai, S.-Y. Su, C.-C. Chang, Special reactive oxygen species generation by a highly photostable BODIPY-based photosensitizer for selective photodynamic therapy, ACS Appl. Mater. Interfaces 5 (2013) 12935–12943.
[53] M. Laine, N.A. Barbosa, A. Kochel, B. Osiecka, G. Szewczyk, T. Sarna, P. Ziółkowski, R. Wieczorek, A. Filarowski, Synthesis, structural, spectroscopic, computational and cytotoxicstudies of BODIPY dyes, Sens. Actuators B 238 (2017) 548–555.
[54] N. Boens, B. Verbelen, W. Dehaen, Postfunctionalization of the BODIPY core: synthesis and spectroscopy, Eur. J. Org Chem. 30 (2015) 6577–6595.
[55] S.J. Mora, M.E. Milanesio, E.N. Durantini, Spectroscopic and photodynamic properties of 5,10,15,20-tetrakis[4-(3-*N*,*N*-dimethylaminopropoxy)phenyl] porphyrin and its tetracationic derivative in different media, J. Photochem. Photobiol. A: Chem. 270 (2013) 75–84.
[56] M. Baruah, W. Qin, C. Flors, J. Hofkens, R.A.L. Vallée, D. Beljonne, M. Van der Auweraer, W.M. De Borggraeve, N. Boens, Solvent and pH dependent fluorescent properties of a dimethylaminostyryl borondipyrromethene dye in solution, J. Phys. Chem. A 110 (2006) 5998–6009.
[57] N. Shivran, M. Tyagi, S. Mula, P. Gupta, B. Saha, B.S. Patro, S. Chattopadhyay, Syntheses and photodynamic activity of some glucose-conjugated BODIPY dyes, Eur. J. Med. Chem. 122((2016) 352–365.
[58] Q. Zheng, G. Xu, P.N. Prasad, Conformationally restricted dipyrromethene boron difluoride (BODIPY) dyes: highly fluorescent, multicolored probes for cellular imaging, Chem. Eur J. 14 (2008) 5812–5819.
[59] A. Schmitt, B. Hinkeldey, M. Wild, G. Jung, Synthesis of the core compound of the BODIPY dye class: 4,4′-difluoro-4-bora-(3a,4a)-diaza-s-indacene, J. Fluoresc. 19 (2009) 755–758.
[60] H. Lu, J. Mack, T. Nyokong, N. Kobayashi, Z. Shen, Optically active BODIPYs, Coord. Chem. Rev. 318 (2016) 1–15.
[61] W. Wu, H. Guo, W. Wu, S. Ji, J. Zhao, Organic triplet sensitizer library derived from a single chromophore (BODIPY) with long-lived triplet excited state for triplet-triplet annihilation based upconversion, J. Org. Chem. 76 (2011) 7056–7064.
[62] S. Hoogendoorn, A.E.M. Blom, L.I. Willems, G.A. van der Marel, H.S. Overkleeft, Synthesis of pH-activatable red fluorescent BODIPY dyes with distinct functionalities, Org. Lett. 13 (2011) 5656–5659.
[63] M.-N. Belzile, R. Godin, A.M. Durantini, G. Cosa, Monitoring chemical and biological electron transfer reactions with a fluorogenic vitamin K analogue probe, J. Am. Chem. Soc. 138 (2016) 16388–16397.
[64] J.-J. Shie, Y.-C. Liu, Y.-M. Lee, C. Lim, J.-M. Fang, C.-H. Wong, An azido-BODIPY probe for glycosylation: initiation of strong fluorescence upon triazole formation, J. Am. Chem. Soc. 136 (2014) 9953–9961.
[65] W. Qin, M. Baruah, M. Van der Auweraer, F.C. De Schryver, N. Boens, Photophysical properties of borondipyrromethene analogues in solution, J. Phys. Chem. A 109 (2005) 7371–7384.
[66] T. Ueno, Y. Urano, H. Kojima, T. Nagano, mechanism-based molecular design of highly selective fluorescence probes for nitrative stress, J. Am. Chem. Soc. 128 (2006) 10640–10641.
[67] H. Sunahara, Y. Urano, H. Kojima, T. Nagano, Design and synthesis of a library of BODIPY-based environmental polarity sensors utilizing photoinduced electron-transfer-controlled fluorescence on/off switching, J. Am. Chrm. Soc. 129 (2007) 5597–5604.
[68] G.J. Hedley, A. Ruseckas, A. Harriman, D.W. Samuel, Conformational effects on the dynamics of internal conversion in boron dipyrromethene dyes in solution, Angew. Chem. Int. Ed. 50 (2011) 6634–6637.
[69] M. Zhang, E. Hao, J. Zhou, C. Yu, G. Bai, F. Wang, L. Jiao, Synthesis of pyrrolyldipyrrinato BF_2 complexes by oxidative nucleophilic substitution of boron dipyrromethene with pyrrole, Org. Biomol. Chem. 10 (2012) 2139–2145.
[70] P.R. Ogilby, Singlet oxygen: there is still something new under the sun, and it is better than ever, Photochem. Photobiol. Sci. 9 (2010) 1543–1560.
[71] T. Dai, B.B. Fuchs, J.J. Coleman, R.A. Prates, C. Astrakas, T.G. St Denis, M.S. Ribeiro, E. Mylonakis, M.R. Hamblin, G.P. Tegos, Concepts and principles of photodynamic therapy as an alternative antifungal discovery platform, Front. Microbiol. 3 (2012) 1–16.
[72] L. Costa, M.A.F. Faustino, M.G.P.M.S. Neves, Â. Cunha, A. Almeida, Photodynamic inactivation of mammalian viruses and bacteriophages, Viruses 4 (2012) 1034–1074.
[73] M.L. Agazzi, M.B. Spesia, N.S. Gsponer, M.E. Milanesio, E.N. Durantini,

Synthesis, spectroscopic properties and photodynamic activity of a fulleropyrrolidine bearing a basic amino group and its dicationic analog against *Staphylococcus aureus*, J. Photochem. Photobiol. A: Chem. 310 (2015) 171–179.
[74] N.S. Gsponer, M.L. Agazzi, M.B. Spesia, E.N. Durantini, Approaches to unravel pathways of reactive oxygen species in the photoinactivation of bacteria induced by a dicationic fulleropyrrolidinium derivative, Methodos 109 (2016) 167–174.
[75] M.C. DeRosa, R.J. Crutchley, Photosensitized singlet oxygen and its applications, Coord. Chem. Rev. 233–234 (2002) 351–371.
[76] R. Schmidt, E. Afshari, Effect of solvent on the phosphorescence rate constant of singlet molecular oxygen ($^1\Delta_g$), J. Phys. Chem. 94 (1990) 4377–4378.
[77] J. Widengren, P. Schwille, Characterization of photoinduced isomerization and back-isomerization of the cyanine dye Cy5 by fluorescence correlation spectroscopy, J. Phys. Chem. A 104 (2000) 6416–6428.
[78] A. Chmyrov, T. Sanden, J. Widengren, Iodide as a fluorescence quencher and promoters-mechanisms and possible implications, J. Phys. Chem. B 114 (2010) 11282–11291.
[79] S. George, M.R. Hamblin, A. Kishen, Uptake pathways of anionic and cationic photosensitizers into bacteria, Photochem. Photobiol. Sci. 8 (2009) 788–795.
[80] M.A. Di Palma, M.G. Alvarez, A.L. Ochoa, M.E. Milanesio, E.N. Durantini, Optimization of cellular uptake of zinc(II) 2,9,16,23-tetrakis[4-(*N*-methylpyridyloxy)]phthalocyanine for maximal photoinactivation of *Candida albicans*, Fungal Biol. 117 (2013) 744–751.
[81] L. Beney, P. Gervais, Influence of the fluidity of the membrane on the response of the microorganisms to environmental stresses, Appl. Microbiol. Biotechnol. 57 (2001) 34–42.
[82] D.A. Caminos, M.B. Spesia, E.N. Durantini, Photodynamic inactivation of *Escherichia coli* by novel *meso*-substituted porphyrins by 4-(3-*N*,*N*,*N*-trimethylammoniumpropoxy)phenyl and 4-(trifluoromethyl)phenyl groups, Photochem. Photobiol. Sci. 5 (2006) 56–65.
[83] E.D. Quiroga, S.J. Mora, M.G. Alvarez, E.N. Durantini, Photodynamic inactivation of Candida albicans by a tetracationic tentacle porphyrin and its analogue without intrinsic charges in presence of fluconazole, Photodiagn. Photodyn. Ther. 13 (2016) 334–340.
[84] E. Reynoso, E.D. Quiroga, M.L. Agazzi, M.B. Ballatore, S.G. Bertolotti, E.N. Durantini, Photodynamic inactivation of microorganisms sensitized by cationic BODIPY derivatives potentiated by potassium iodide, Photochem. Photobiol. Sci. 16 (2017) 1524–1536.
[85] D. Vecchio, A. Gupta, L. Huang, G. Landi, P. Avci, A. Rodas, M.R. Hamblin, Bacterial photodynamic inactivation mediated by methylene blue and red light is enhanced by synergistic effect of potassium iodide, Antimicrob. Agents Chemother. 59 (2015) 5203–5212.
[86] Y. Zhang, T. Dai, M. Wang, D. Vecchio, L.Y. Chiang, M.R. Hamblin, Potentiation of antimicrobial photodynamic inactivation mediated by a cationic fullerene by added iodide: in vitro and in vivo studies, Nanomedicine 10 (2015) 603–614.
[87] J. Mosinger, M. Janošková, K. Lang, P. Kubát, Light-induced aggregation of cationic porphyrins, J. Photochem. Photobiol. A: Chem. 181 (2006) 283–289.
[88] A. Felgenträger, T. Maisch, A. Späth, J.A. Schröderc, W. Bäumlera, Singlet oxygen generation in porphyrin-doped polymeric surface coating enables antimicrobial effects on Staphylococcus aureus, Phys. Chem. Chem. Phys. 16 (2014) 20598–20607.
[89] J.M. Gardner, M. Abrahamsson, B.H. Farnum, G.J. Meyer, Visible light generation of iodine atoms and I-I bonds: sensitized I^- oxidation and I_3 photodissociation, J. Am. Chem. Soc. 131 (2009) 16206–16214.
[90] G. Boschloo, A. Hagfeldt, Characteristics of the iodide/triiodide redox mediator in dye-sensitized solar cells, Acc. Chem. Res. 42 (2009) 1819–1826.
[91] J.G. Rowley, B.H. Farnum, S. Ardo, G.J. Meyer, Iodide chemistry in dye-sensitized solar cells: making and breaking I-I bonds for solar energy conversion, J. Phys. Chem. Lett. 1 (2010) 3132–3140.
[92] M.A. Atemnkeng, J. Plaizier-Vercammen, A. Schuermans, Comparison of free and bound iodine and iodide species as a function of the dilution of three commercial povidone-iodine formulations and their microbicidal activity, Int. J. Pharm. 317 (2006) 161–166.