Mugla Journal of Science and Technology

IN VITRO EVALUATION OF THE EFFECTIVENESS OF DIFFERENT BODIPY DYES AS PHOTOSENSITIZER IN METHICILLIN-RESISTANT Staphylococcus aureus TREATMENT

Rukiye BORAN*, Vocational School of Health Service, Aksaray University, Turkey, rukiyeboran@aksaray.edu.tr
https://orcid.org/0000-0003-2395-2445
Melek PAMUK ALGI*, Health Services Vocational School & ASUBTAM Memduh Bilmez BioNano Tech Lab., Aksaray University, Aksaray, Turkey, melekalgi@aksaray.edu.tr
https://orcid.org/0000-0001-5863-3976
Aysel UGUR, Department of Basic Sciences, Faculty of Dentistry, Gazi University, Ankara, Turkey, ayselugur@hotmail.com
https://orcid.org/0000-0002-5188-1106

Received: 28.06.2018, Accepted: 09.10.2018
*Corresponding author
Research Article
DOI: 10.22531/muglajsci.448768

Abstract

The antibiotic period is now ending and the probability of discovering new classes of antibiotics is considerably low. It is required to find out alternative antimicrobial technologies that bacteria will not be able to develop resistance, and that will be equally effective regardless of the current resistance situation. In this regard, we investigated antimicrobial photodynamic inactivation effects of three boradiazaindacenes (BODIPYs) 1–3 against methicillin-resistant Staphylococcus aureus (MRSA). BODIPYs 1–3 with different substituents at the meso position (-NMe_2, -NO_2 and -Br, respectively) were synthesized. The photodynamic inactivation effects of BODIPYs 1–3 were tested against one broad spectrum antibiotic resistant bacterial model strain, a clinically described MRSA. In particular BODIPY 2 was found more effective when compared to the others at 25, 50 and 100 nM concentrations. BODIPYs 1–3 did not show any toxic effect in the dark at given concentrations. In addition, a high degree of photodynamic inactivation were detected with 2 and 3 by irradiation at 6.66 – 8.88 J/cm² light doses, while the efficiency of 1 was not significantly affected from illumination times. The results indicate that BODIPYs, especially nitro group BODIPY 2, can be used in the photodynamic inactivation of MRSA at nanomolar concentrations and low energy doses.

Keywords: BODIPY, photosensitizer, photodynamic inactivation, *Staphylococcus aureus*

FARKLI BODIPY BOYALARININ METİSİLİN DİRENÇLİ *Staphylococcus aureus* TEDAVİSİNDE FOTOSENSİTİZER OLARAK ETKİNLİĞİNİN İN VİTRO DEĞERLENDİRİLMESİ

Öz

Antibiyotik dönemi günümüzde sona eriyor ve yeni antibiyotik sınıflarını keşfetme olasılığı oldukça düşüktür. Bakterilerin direnç geliştiremeyeceği ve mevcut direnç durumundan bağımsız olarak eşit derecede etkili olacak alternatif antimikrobiyal teknolojileri bulmak gerekmektedir. Bu bağlamda, üç boradiazaindacene'nin (BODIPY) (1–3) metisiline dirençli Staphylococcus aureus (MRSA) 'a karşı antimikrobiyal fotodinamik inaktivasyon etkilerini araştırdık. Mezo pozisyonunda farklı sübstitüentlere (sırasıyla -NMe_2, -NO_2 ve -Br) sahip BODIPY 1–3 sentezlendi. BODIPY 1–3'ün fotodinamik inaktivasyon etkileri, geniş spektrumlu antibiyotik dirençli bir bakteriyel model suşuna karşı test edildi, klinik olarak tanımlanmış bir MRSA. Özellikle 25, 50 ve 100 nM konsantrasyonlarda diğerleri ile karşılaştırıldığında özellikle BODIPY 2'nin diğerlerine göre daha etkili olduğu bulunmuştur. BODIPY 1–3, verilen konsantrasyonlarda karanlıkta herhangi bir toksik etki göstermemişlerdir. Ek olarak, 1'in etkinliği aydınlatma sürelerinden önemli ölçüde etkilenmezken, 6.66 – 8.88 J/cm² ışık dozlarındaki ışınlanmada 2 ve 3'te yüksek derecede fotodinamik inaktivasyon belirlenmiştir. Sonuçlar göstermektedir ki, BODIPY'ler, özellikle nitro grup BODIPY 2, nanomolar konsantrasyonlarda ve düşük enerji dozlarında MRSA'nın fotodinamik inaktivasyonunda kullanılabilir.

Anahtar Kelimeler: BODIPY, fotosensitizer, fotodinamik inaktivasyon, *Staphylococcus*

Cite

Boran, R., Pamuk Algı, M., Uğur, A., (2018). "In vitro evaluation of the effectiveness of different BODIPY dyes as photosensitizer in methicillin-resistant *Staphylococcus aureus* treatment", Mugla Journal of Science and Technology, 4(2), 191-197.

1. Introduction

The use of antibiotics to eliminate bacteria represents one of the most important medical inventions of mankind [1]. However, antimicrobial resistance is a global threat to public health, and it will be one of the biggest concerns for the next millennium [2]. It is imperative to act strongly around the world to avert this situation that entails an ever-increasing health and economic burden [3]

One of the epidemiologically accepted resistant bacteria with high clinical efficacy is Gram-positive pathogen *Staphylococcus aureus* [3]. *S. aureus* is a major reason of infection in hospitalized patients. It is reported in 58% of patients, and usually accounts for a large portion of the etiology of infection [4-6]. This pathogen is the most important cause of pneumonia, bloodstream infections, uncomplicated skin and soft tissue suppurative infections as well as endocarditis and osteomyelitis [7, 8]. Methicillin-resistant *S. aureus* (MRSA) infections are undoubtedly one of the world's most considerable global health problems. In a report by the European Center for Disease Prevention and Control, there was a large variation ranging from 0 to 57.2% among *S. aureus* isolates in terms of MRSA percentages between European countries in 2015 [6]. MRSA isolates have a wide variety of antibiotic resistance genes, and these strains resistant to almost all antibiotic classes such as β-lactam antibiotics, tetracyclines, sulphonamides, aminoglycosides, macrolides, aminocyclitols, phenicols, lincosamides, streptogramins, pleuromutilins, fusidic acid, mupirocin, oxazolidinones, diaminopyrimidines, glycopeptides [9, 10].

There is a positive relationship between antimicrobial resistance and antibiotic consumption, and the emergences of multiple-drug resistance in microorganisms have created new difficulties for researchers [11]. For this reason, alternative strategies have to be developed [12].

Photodynamic inactivation (PDI) is accepted as a possible alternative to the treatment of localized bacterial infections in response to the problem of antibiotic resistance [13, 14]. The treatment protocol involves light, molecular oxygen and a photosensitizer (PS) [13, 15]. PDI involves killing of organisms by light in the presence of a photosensitizing agent [13]. Highly reactive oxygen species (ROS), formed as a result of photo-activation of a light-sensitizing material in the action region of the appropriate wavelength by light, cause complex chemical, biological and physiological reactions and, consequently, cell destruction [16-18]. PDI ensures considerable advantages over other available antimicrobial treatments. It is applicable to all-bacteria regardless of their antibiotic resistance; it acts more rapidly against microorganisms than antimicrobials [19]. In addition, unlike antibiotics, it does not induce resistance patterns in bacteria after multiple treatments, and singlet oxygen (or ROS) that are released can interact with different metabolic pathways and several cell structures in microbial cells [20, 21].

Boradiazaindacene dyes (BODIPYs) represent an important class of fluorescent dyes. They have unique photophysical properties such as good chemical, thermal and photostability, high molar absorption coefficients narrow emission bandwidths and high fluorescence quantum yields [22]. BODIPYs have been used as sensors to detect metal cations, anions and ROS [23]. Furthermore, they are perfect candidates for biological labeling and fluorescent switches [24]. Recently, it was shown that BODIPY derivatives can be designed to produce ROS (e.g. singlet oxygen) and used as photodynamic therapy agents [23, 25]. In recent years, 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) derivatives have been proposed as phototherapeutic agents for the photodynamic inactivation of pathogenic microorganisms [21, 26, 27].

In line with the explanations above, we investigated the *in vitro* antimicrobial effect of BODIPY dyes 1–3 against MRSA. We have chosen three different BODIPY derivatives 1–3, which have different substituents on the phenyl ring. BODIPY dye 1 has electron donating dimethyl amino group, BODIPY dye 2 [28] has electron withdrawing nitro group and BODIPY dye 3 [25] has Bromine atom on the benzene ring. These compounds were chosen in order to see the effect of substituents to the antimicrobial activity. All of the BODIPY dyes 1–3 have bromine atoms on the BODIPY core, which could increase antimicrobial activity by inducing the formation of ROS through heavy atom effect.

2. Material Methods

2.1. Bacterial strain and culture conditions

S. aureus ATCC43300 was provided from American Type Culture Collection (ATCC). The stock cultures were kept at – 80 °C in nutrient broth (NB) containing 20% (v/v) glycerol. Prior to experiment, the cells were subcultured in Mueller Hinton Broth (MHB).

2.2. Photosensitisers

We used three BODIPYs **1**–**3** (Fig. 1). BODIPY **2** [28] and **3** [25] were previously synthesized by using the reported literature procedures (Fig. 2).

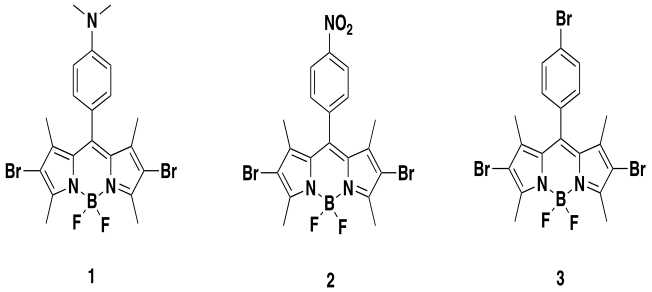

Figure 1. Structures of BODIPY dyes 1–3.

2.3. Synthesis of BODIPY 1

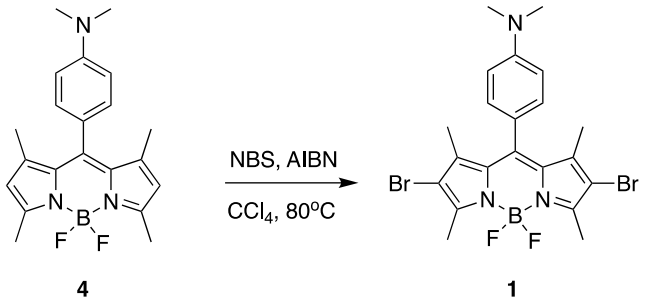

Figure 2. Synthesis of BODIPY 1.

Compound 4 [29] (0.367 g, 1.0 mmol), AIBN (0.355 g, 2.16 mmol), and NBS (0.385 g, 2.16 mmol) were refluxed in CCl_4 (25 mL) until all the starting materials were consumed (TLC). Crude product was then concentrated under vacuum, and purified by column chromatography with hexane-CH_2Cl_2 (1:1, v/v) as eluent to give 1. 0.50 g, 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: 6.95 (d, J = 8 Hz, 2H), 6.72 (d, J = 8 Hz, 2 H), 2.97 (s, 6 H), 2.53 (s, 6 H), 1.42 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm: 153.1, 150.9, 140.7, 133.4, 128.7, 127.6, 120.6, 112.3, 44.0, 40.2, 14.3.

Stock solutions of BODIPYs were prepared in dimethylsulfoxide (DMSO) at a concentration of 1 mM, filtered through a sterile 0.22 µm filter membrane and stored at 4 ºC in the dark condition before use.

2.4. Photodynamic inactivation of *S. aureus*

S. aureus strain was grown in the NB for 12 to 16 h at 37 °C and then diluted in fresh MHB to get a cell density of ~ 10^5 – 10^6 CFU/mL^{-1}. Two-fold serial dilutions of the BODIPYs were prepared in the MHB. BODIPY solutions were added to provide a desired concentration for a 2 mL cell suspension. After 10 min of pre-incubation in the dark at 37 °C, the reaction mixtures were illuminated to cool white light (400 – 800 nm) and then incubated at 37 °C for 12 h in the dark. After incubation optical density was determined at 600 nm.

In the study, two different sets of experiments were carried out: (a) PS concentrations were increased (3.125 – 100 nM) at a constant light dose (4.44 J/cm^2); (b) light dose was increased (2.22 – 8.86 J/cm^2) in the constant PS concentration (100 nM).

Three different test groups were used as controls: bacterial samples were incubated in the presence of PS and not irradiated (+ PS, – L), bacterial samples were incubated in the absence of PS and irradiated with light (– PS, + L), and bacterial samples were incubated in the absence of PS and not irradiated (– PS, – L).

2.5. Statistical analysis

The experiments were repeated at least three times. The results were presented as the average and standard deviation. The data was analyzed for statistical significance using analysis of variance (ANOVA).

3. Results

In our previous studies, we reported the synthesis and characterization of BODIPYs 2 [28] and 3 [25]. In this study, we also synthesized a novel BODIPY 1 in addition to the previously synthesized BODIPYs 2–3. All of the BODIPY dyes 1–3 are charge neutral and they have different substituents; 1 has dimethyl amino group, 2 has nitro group and 3 has bromine on the phenyl ring (Fig. 1). In this study, the photo-activation experiments were carried out with the use of increasing BODIPY dye concentrations (in the range of 3.125 – 100 nM) at a constant energy dose (4.44 J/cm^2) (Fig. 3) or using a constant PS concentration (100 nM) at increasing energy doses (2.22 to 8.88 J/cm^2) (Fig. 4). In addition, various control experiments have been conducted in the presence and absence of BODIPY dyes or light.

The bacterium was pre-incubated with BODIPYs in the dark conditions for 10 min at a concentration of 3.125 to 100 nM, after which the bacterium was illuminated with white light (400 – 800 nm, 4.44 J/cm^2). It has been determined that the concentrations of 3.125 and 6.25 nM did not affect cell viability. However, at 12.5 nM concentration of 1, almost 51 ± 0.06% the bacteria lost the ability to proliferate after illumination. At the same concentration of 3, there was no significant efficacy on cell growth. Similarly, in the presence of 25 nM 1 and 2, 61 ± 0.01% and 71 ± 0.08% decrease was observed on cell survival, respectively. The photodynamic activity of 1, 2 and 3 at 50 nM concentrations against *S. aureus* was significant: 77 ± 0.17%, 93.43 ± 0.01% and 46 ±0.01% of the bacterial growth were inhibited. Treating *S. aureus* with 1 and 3 at 100 nM, cell viability was decreased almost 80 ± 0.2%, while in the presence of 2 96 ± 0.04% decrease was observed on cell viability (Fig. 3). The bacterial grown was not affected by irradiation without BODIPYs (– PS, + L) nor to cells treated with BODIPYs in the dark conditions (+ PS, – L) (Fig. 3). These results clearly indicate that the generation of ROS is the main cause of bacterial grown inhibition, rather than the toxicity of BODIPYs 1–3.

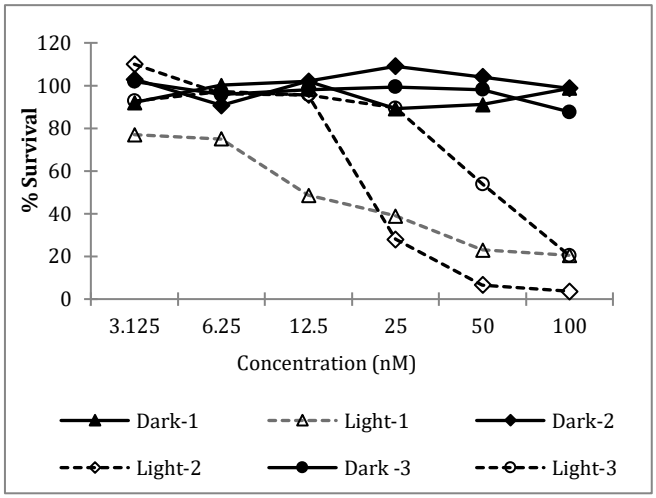

Figure 3. Effect of increasing concentration of BODIPYs 1–3 on the photodynamic inactivation of *S. aureus*; energy dose: 4.44 J/cm^2

In addition, the light intensity was increased from 2.22 J/cm^2 to 8.88 J/cm^2 in the presence of 100 nM dose of BODIPYs 1–3. Light treatment of cells in the absence of

BODIPYs (– PS, + L) had no significant effect on bacteria viability (Fig. 4). Also, cell survival was not affected by the presence of BODIPYs in the dark conditions (+ PS, – L) (Fig. 3). However, increasing the energy dose from 2.22 J/cm^2 to 8.88 J/cm^2 in presence of BODIPYs resulted reduce in cell survival (Fig. 4). Treating with 1, 2 and 3 at 100 nM, a reduction in the viable cell numbers to 37.5 ± 0.07%, 13.8 ± 0.37% and 30.6 ± 0.19% were observed at 2.22 J/cm^2 energy dose, respectively. However, after irradiation with light at 2.22 J/cm^2, 85 ± 0.31% cell viability was observed in the absence of BODIPYs. Irradiating with 4.44 J/cm^2 light caused a 77.7 ± 0.10%, 93.6 ± 0.07% and 72.6 ± 0.43% decline of viable cell treated with 1, 2 and 3, respectively ($P < 0.01$). However, at the same light dose almost 16 ±0.01% decrease was observed on cell survival in the absence of BODIPYs. In the presence of 1, 2 and 3, 83 ± 0.13%, 95 ± 0.16% and 93 ± 0.05% decrease were observed on cell survival at 6.66 J/cm^2, respectively. When *S. aureus* was treated with 1–3 at 8.88 J/cm^2 energy dose, cell viability was decreased to 89 ± 0.21%, 95 ± 0.05% and 93 ± 0.08%, respectively.

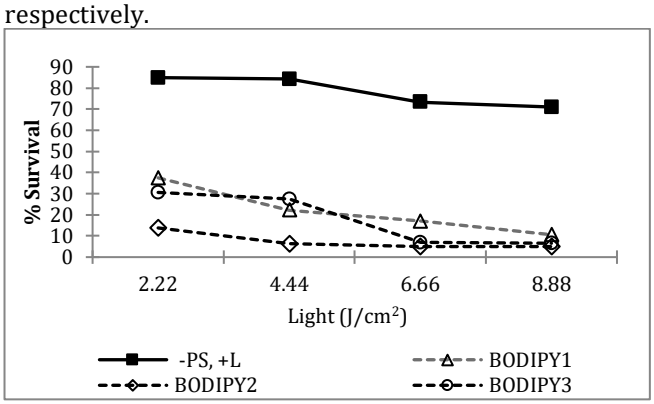

Figure 4. Effect of increasing energy doses in the presence of 100 nM BODIPYs 1–3 on the photodynamic inactivation of *S. aureus.*

4. Discussion

In this study, MRSA was chosen as a model bacterium to prove the efficacy of using BODIPYs as PS. MRSA is the most common antibacterial resistant organism identified in health care facilities [30]. Compared to methicillin-sensitive *S. aureus*, MRSA has a higher rate of infection, morbidity and mortality [31]. Studies have shown that *S. aureus* isolates have a wide variety of antibiotic resistance genes. Until now, approximately sixty different antibiotic resistance genes have been found in these bacteria [10].

Therefore, there is a need to develop new effective antimicrobial treatment therapies. Some of the proposals made in this respect are killing factors, bacteriocins, quorum quenching, phage therapy and photosensitized inactivation of microorganisms [11]. The photosensitized inactivation of microorganisms involves a combination of light and a photosensitising drug. The irradiation with visible light of photosensitising drug stimulates PS and this generates ROS to be released. These released reactive species react with biological molecules in the cell, resulting in loss of biological functionality and ultimately cell death [32]. Recently, BODIPYs have been suggested as potential PS for the killing of pathogenic microbial cells [1, 26, 27].

BODIPY dyes 1–3 with different substituents (-NMe_2, -NO_2 and -Br, respectively) at the meso position were synthesized starting from 2,4-dimethylpyrrole and corresponding aldehydes after oxidation with 2,3-Dichloro-5,6-Dicyanobenzoquinone (DDQ) followed by complexation with boron trifluoride etherate (BF_3OEt_2) (Fig. 1). All BODIPY dyes 1–3 have bromine atoms on the BODIPY core, which would induce the formation of ROS through the heavy atom effect.

It was envisaged that these BODIPYs 1–3 were compatible with antimicrobial PDI as promising PS candidates, since they would; i- have a low level of dark toxicity, and ii- be able to kill microbial cells at relatively low concentrations after illumination with light.

The cationic amphiphilic PSs have shown many applications for various biological systems [33]. The positive charge on the PS molecule promotes a strong electrostatic interaction with the negatively charged regions of the complex *outer* barrier structure of Gram-negative bacteria, which enhances the effectiveness of photoinactivation processes [34]. However, anionic and neutral PSs generally inactivate Gram-positive bacteria cells and fungal strains [12].

High sensitivities of Gram-positive bacterial and fungal strains to anionic or neutral PS are clarified by their physiology. Because, in yeast and Gram-positive bacteria, the cytoplasmic membrane is surrounded by beta-glucan and chitin layer or, a porous peptidoglycan and lipoteicoic acid, respectively. Both of these structures allow non-cationic PSs to pass through of membrane [34, 35].

In this study, three neutral BODIPY derivatives 1–3 were used to demonstrate photosensitized inactivation of *S. aureus*. Bacterial growth reductions were about 77 ± 0.17%, 93.43 ± 0.01% and 46 ± 0.01% in the BODIPY 1, 2 and 3 at 50 nM concentrations, and almost 80 ± 0.2%, 96 ± 0.04% and 80 ± 0.03% bacterial growth reductions were observed in the BODIPYs 1, 2 and 3 at 100 nM concentrations, respectively (Fig. 3). The results obtained pointed out that BODIPY 2 showed better antibacterial effects at the 25 – 100 nM concentrations than the parent BODIPYs 1–3 ($P < 0.05$).

The antimicrobial PDI susceptibility depends not only on the PS concentration but also to irradiation energy [26, 36].Our results indicated that 100 mM concentrations of 2 and 3 showed better antibacterial effects at the 2.22 – 8.88 J/cm^2 irradiation energy when compared to that of dimethyl amino group BODIPY 1. In addition, BODIPY 2 was found to be more efficient than 1 and 3 from 2.22 J/cm^2 to 8.88 J/cm^2 energy ($P < 0.01$). However, light treatment of cells in the absence of BODIPYs (– PS, + L) had no significant effect on bacteria viability (Fig. 4). Also, cell survival was not affected by the presence of BODIPYs in the dark conditions (+ PS, – L).

There are various studies applied PDI using BODIPYs on different pathogenic microorganisms. These studies

revealed that high rates of reduction in the cell survivals. However, the main advantages of BODIPYs 1–3 are able to kill microbial cells at relatively low concentrations (nM) and low energy doses (2.22 J/cm^2 to 8.88 J/cm^2) when compared to the other studies [12, 21, 26, 27].

In a study, Orlandi et al. [26] reported that PDI combines the use of 2,6-diiodo-1,3,5,7-tetramethyl-8-(N-benzyl-4-pyridyl)-4,4′-difluoroboradiazaindacene PS against *Pseudomonas aeruginosa*. In the study, 7 log unit decrease of cell viability was detected when applied at 2.5 μM with 171 J/cm^{-2} energy dose. In another study, photodynamic inactivation of cationic BODIPYs were researched on *Escherichia coli* and *S. aureus.*A reduction of > 5 log in the viable cells of *S. aureus* was detected when using 1 μM BODIPYs and 5 min irradiation while in *E. coli*, these BODIPYs caused ~ 2.5 log inhibition in the survival when used 5 μM PS and 15 min irradiation [27]. Similarly the antimicrobial effects of two iodinated and cationic BODIPYs were also determined against *E. coli* and *Staphylococcus xylosus*. In the study, the cationic methylated BODIPY was more efficient than the cationic benzylated BODIPY against *E. coli* and *S. xylosus. S. xylosus* cell number decreased from 3 to 7 log units when light dose was increased from 2.76 to 11.04 J/cm^2 in the presence of cationic methylated BODIPY at 0.1 μM concentrations [21].

Antimicrobial effect of 2, 6-diiodo-1,3,5,7-tetramethyl-8-(*N*-methyl-4-pyridyl)-4,4′-difluoroboradiazaindacene was examined against drug-resistant bacteria [37]. After irradiation with visible light (1 J/cm^2), 5 – 6 log reduction were found in the cell survival of *S. aureus*, MRSA and vancomycin-resistant *Enterococcus faecium* when using 0.1 mM BODIPY. After 1 J/cm^2 irradiation, 4 – 5 log inhibition were obtained in the cell survivals of *Klebsiella pneumoniae* (1 mM), *P. aeruginosa* (0.5 mM), *Acinetobacter baumannii* (0.25 mM) and multidrug-resistant *A. baumannii* (0.1 mM). Frimannsson et al. [12] investigated the PDI application of the brominated azaBODIPY against *E. coli* and *S. aureus.* In study, increasing the concentration of 5 μg/mL to 6 μg/mL dose of azaBODIPY with 16 J/cm^2 energy dose led to a > 99.9999% and > 99.9% eradication of *S. aureus* and a MRSA strain, respectively. In similar treatments a 99.99% decrease was obtained in the cell survival of *E. coli*. In another study, the photodynamic effects of BODIPYs were researched on the cell of *S. aureus* and *E. coli*. After 15 min irradiation with visible light (150 W lamp), BODIPYs achieved complete eradication in *S. aureus* cell when using 1 μM PSs. Similar results were obtained in the *E. coli* viability when using a BODIPY at 5 μM [38].

5. Conclusion

In conclusion, it was found that these BODIPYs 1–3 were compatible with antimicrobial PDI and they are promising PS candidates, since they have a low level of dark toxicity, and they are able to kill microbial cells at relatively low concentrations and low fluences of light. On the basis of these results, it can be pointed out that BODIPYs 1–3 bearing bromine atoms on the BODIPY core induce the formation of ROS efficiently. Especially, BODIPY 2 is the most promising candidate among the BODIPYs 1–3. It is reasonable to assume that nitro group in BODIPY 2, might play a critical role in antimicrobial PDI due to its strong electron withdrawing nature. However, BODIPYs 1–3 represent great potential as novel antimicrobial photodynamic therapeutic agents that are capable of eradicating of MRSA at nanomolar level doses and short illumination times. Hence, BODIPYs 1–3 could be used for the photoinactivation of Gram-positive bacteria such as MRSA.

6. References

[1]. Durantini, A.M., Heredia, D.A., Durantini, J.E., Durantini, E.N., "BODIPYs to the rescue: Potential applications in photodynamic inactivation" *Eur J Med Chem,* 144, 651-661, 2018.

[2]. Lawrence, R. and Jeyakumar, E., "Antimicrobial Resistance: A Cause for Global Concern" *BMC Proc*, 7, 3., 2-14, 2013.

[3]. Theuretzbacher, U., "Global antibacterial resistance: The never-ending story" *J Glob Antimicrob Re*, 1, 2., 63-69, 2013.

[4]. Acton, D.S., Plat-Sinnige, M.J., van Wamel, W., de Groot, N., van Belkum, A., "Intestinal carriage of Staphylococcus aureus: how does its frequency compare with that of nasal carriage and what is its clinical impact?" *Eur J Clin Microbiol Infect Dis*, 28, 2., 115-127, 2009.

[5]. Knox, J., Uhlemann, A-C., Lowy, F.D., "*Staphylococcus aureus* infections: transmission within households and the community" *Trends Microbiol*, 23, 7., 437-444, 2015.

[6]. Pomorska-Wesołowska, M., Chmielarczyk, A., Chlebowicz, M., Ziółkowski, G., Szczypta, A., Natkaniec, J., Romaniszyn, D., Pobiega, M., Dzikowska, M., Krawczyk, L., Kozioł J, Wójkowska-Mach J. "Virulence and antimicrobial resistance of *Staphylococcus aureus* isolated from bloodstream infections and pneumonia in Southern Poland" *J Glob Antimicrob Re*, 11, 100-104, 2017.

[7]. Shinefield, H., Black, S., Fattom, A., Horwith, G., Rasgon, S., Ordonez, J., Yeoh, H., Law, D., Robbins, J.B., Schneerson, R., Muenz L, Fuller S, Johnson J, Fireman B, Alcorn H, Naso R., "Use of *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis" *N Engl J Med,* 346, 491-496, 2002.

[8]. Edelsberg, J., Weycker, D., Barron, R., Li, X., Wu, H., Oster, G., Badre, S., Langeberg, W.J., Weber, D.J., "Prevalence of antibiotic resistance in US hospitals" *Diagn Microbiol Infect Dis*, 78, 3., 255-262, 2014.

[9]. Mohammad, H., Narasimha Reddy, P.V., Monteleone, D., Mayhoub, A.S., Cushman, M., Seleem, M.N., "Synthesis and antibacterial evaluation of a novel series of synthetic phenylthiazole compounds against methicillin-resistant *Staphylococcus aureus* (MRSA)"*Eur J Med Chem,* 94, 306-316, 2015.

[10]. Feßler, A.T., Li, J., Kadlec, K., Wang, Y., Schwarz, S. (Fetsch, A. editor) *Antimicrobial Resistance*

Properties of Staphylococcus aureus. Elsevier; Cambridge USA, 2018.

[11]. Nigam, A., Gupta, D., Sharma, A., "Treatment of infectious disease: beyond antibiotics" *Microbiol Res*, 169, 9-10., 643-651, 2014.

[12]. Frimannsson, D.O., Grossi, M., Murtagh, J., Paradisi, F., O'Shea, D.F., "Light induced antimicrobial properties of a brominated boron difluoride (BF2) chelated tetraarylazadipyrromethene photosensitizer" *J Med Chem*, 53, 20., 7337-7343, 2010.

[13]. Hajim, K.I., Salih, D.S., Rassam, Y.Z., "Laser light combined with a photosensitizer may eliminate methicillin-resistant strains of *Staphylococcus aureus*" *Lasers in Medical Science*, 25, 743–748, 2010.

[14]. Kashef, N.,Abadi, G.R.S., Djavid, G.E., "Phototoxicity of phenothiazinium dyes against methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Escherichia coli*" *Photodiagnosis and Photodynamic Therapy*, 9, 11-15, 2012.

[15]. Kilic, B., Yesilgul, N., Polat, V., Gercek, Z., Akkaya, E.U., "Bodipy-based photosensitizers with long alkyl tails at the meso position: efficient singlet oxygen generation in Cremophor-EL micelles" *Tetrahedron Lett,* 57, 12., 1317-1320, 2016.

[16]. Nishiyama, N., Morimoto, Y., Jang, W.D., Kataoka, K., "Design and development of dendrimer photosensitizer-incorporated polymeric micelles for enhanced photodynamic therapy" *Adv Drug Deliv Rev*, 61, 4., 327-338, 2009.

[17]. Rossetti, F.C., Lopez, L.B., Carollo, A.R., Thomazini, J.A., Tedesco, A.C., Bentley, M.V., "A delivery system to avoid self-aggregation and to improve in vitro and in vivo skin delivery of a phthalocyanine derivative used in the photodynamic therapy" *J Control Release*, 155, 3., 400-408, 2011.

[18]. Pamuk Algi, M., "A highly selective dual channel hypochlorite probe based on fluorescein and 1, 10-phenanthroline" *Tetrahedron,* 72, 12., 1558-1565, 2016.

[19]. Rosa, L.P., Cristina da Silva, F., Nader, S.A., Meira, G.A., Viana, M.S., "Antimicrobial photodynamic inactivation of *Staphylococcus aureus* biofilms in bone specimens using methylene blue, toluidine blue ortho and malachite green: An in vitro study" *Arch Oral Biol,* 60, 5., 675-680, 2015.

[20]. Schastak, S., Ziganshyna, S., Gitter, B., Wiedemann, P., Claudepierre, T., "Efficient Pho-todynamic therapy against gram-positive and gram-negative bacteria using THPTS, a cationic photosensitizer excited by infrared wavelength" *Plos One*, 5, 7., 11674, 2010.

[21]. Caruso, E., Banfi, S., Barbieri, P., Leva, B., Orlandi, V.T., "Synthesis and antibacterial activity of novel cationic BODIPY photosensitizers" *JPPB*, 114, 44-51, 2012.

[22]. Karakaya, S. and Algi, F., "A novel dual channel responsive zinc (II) probe" *Tetrahedron Lett,* 55, 40., 5555-5559, 2014.

[23]. Benstead, M., Mehl, G.H., Boyle, R.W., "4,4′-Difluoro-4-bora-3a,4a-diaza-*s*-indacenes (BODIPYs) as components of novel light active materials" *Tetrahedron,* 67, 20., 3573-3601, 2011.

[24]. Pamuk, M. and Algi, F., "Synthesis of a novel on/off fluorescent cadmium (II) probe" *Tetrahedron Lett*, 53, 51., 7010-7012, 2012.

[25]. Pamuk Algi, M., Tirkes, S., Ertan, S., Cansu Ergun, E.G., Cihaner, A., Algi, F., "Design and synthesis of new 4, 4′-difluoro-4-bora-3a, 4a-diaza-s-indacene based electrochromic polymers" *Electrochimica Acta*, 109, 766-774, 2013.

[26]. Orlandi, V.T., Rybtke, Caruso, E., Banfi, S., Tolker-Nielsen, T., Barbieri, P., "Antimicrobial and anti-biofilm effect of a novel BODIPY photosensitizer against *Pseudomonas aeruginosa* PAO1" *Biofouling,* 30, 8., 883-891, 2014.

[27]. Agazzi, M.L., Ballatore, M.B., Reynoso, E., Quiroga, E.D., Durantini, E.N., "Synthesis, spectroscopic properties and photodynamic activity of two cationic BODIPY derivatives with application in the photoinactivation of microorganisms" *Eur J Med Chem*, 126, 110-121, 2017.

[28]. Cihaner, A. and Algi, F., "Synthesis and properties of 4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacene (BODIPY)-based conducting copolymers" *React Funct Polym*, 69, 1., 62-67, 2009.

[29]. Kollmannsberger, M., Rurack, K., Resch-Genger, U., Daub, J., "Ultrafast Charge Transfer in Amino-Substituted Boron Dipyrromethene Dyes and Its Inhibition by Cation Complexation: A New Design Concept for Highly Sensitive Fluorescent Probes" *J Phys Chem A*, 102, 50., 10211-10220, 1998.

[30]. Uematsu, H., Yamashita, K., Mizuno, S., Kunisawa, S., Shibayama, K., Imanaka, Y., "Effect of methicillin-resistant *Staphylococcus aureus* in Japan" *Am J Infect Control,* 0196, 30468-30471, 2018.

[31]. Cosgrove, S.E., Sakoulas, G., Perencevich, E.N., Schwaber, M.J., Karchmer, A.W., Carmeli, Y., "Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: A meta-analysis" *Clin Infect Dis*, 36, 1., 53-59, 2003.

[32]. Denis, T.G., Dai, T., Izikson, L., Astrakas, C., Anderson, R.R., Hamblin, M.R., Tegos, G.P., "All you need is light: antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease" *Virulence,* 2, 6., 509-520, 2011.

[33]. Abrahamse, H. and Hamblin, M.R., "New photosensitizers for photodynamic therapy" *Biochem J*, 473, 4., 347-364, 2016.

[34]. Alves, E., Faustino, M.A., Neves, M.G., Cunha, A., Tome, J., Almeida, A., "An insight on bacterial cellular targets of photodynamic inactivation" *Fut Med Chem*, 6, 2., 141-164, 2014.

[35]. Huang, L., Dai, T., Hamblin, M.R., "Antimicrobial photodynamic inactivation and photodynamic therapy for infections" *Methods Mol Biol*, 635, 155-173, 2010.
[36]. Sabbahi, S., Ben Ayed, L., Boudabbous, A., "Cationic, anionic and neutral dyes: effects of photosensitizing properties and experimental conditions on the photodynamic inactivation of pathogenic bacteria" *J Water Health,* 11, 4., 590-599, 2013.
[37]. Carpenter, B.L., Situ, X., Scholle, F., Bartelmess, J., Weare, W.W., Ghiladi, R.A., "Antiviral, antifungal and antibacterial activities of a BODIPY-Based photosensitizer" *Molecules*, 20, 6., 10604-10621, 2015.
[38]. Reynoso, R., Quiroga, E.D., Agazzi, M.L., Ballatore, M.B., Bertolotti, S.G., Durantini, E.N., "Photodynamic inactivation of microorganisms sensitized by cationic BODIPY derivatives potentiated by potassium iodide" *Photochem Photobiol Sci,* 16, 10., 1524-1536, 2017.